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Research Article

# Description of two new species of *Ophiocordyceps*: *O. sinocampes* and *O. cystidiata* (Ophiocordycipitaceae, Hypocreales) from typical karst landform forests in Guizhou, China

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## Abstract

Karst habitats are hotspots of diversity and endemism. Their naturally fragmented distributions across broad geographic landscapes have led to a complex array of smaller evolutionary ecosystems. Comprehensive biodiversity assessments of karst habitats have revealed that these ecosystems contain a high level of endemism. During a survey of cordycipitoid fungi in the karst region of southwest Guizhou, China, we identified and proposed two new species, based on morphology and multi-locus (nrSSU, nrLSU, *tef*, *rpb1*, and *rpb2*) phylogenetic analyses. *O. cystidiata* **sp. nov.** is characterized by gray-white to yellow fertile part, verrucose phialides, and conidia enveloped in a cystic thickened mucus sheath, distinguishing it from other species. *O. sinocampes* **sp. nov.** is distinguished by long tapering phialides with inflated bases. Phylogenetic analyses using five loci reveal that *O. cystidiata* shares a close relationship with *O. fenggangensis*, *O. musicaudata*, *O. alboperitheciata*, and *Hirsutella kuankuoshuiensis*, while *O. sinocampes* is closely related to *O. multiperitheciata* and *H. strigosa*. Herein, we completed the descriptions, illustrations and molecular phylogeny of these two new species. The host diversity of *O. sinocampes* has also been documented within the orders Lepidoptera and Coccoidea. Our research further enriches the diversity of cordycipitoid species in the typical karst landform areas of Guizhou, China.

**Key words:** Cordycipitoid fungi, invalidation, karst landform, nomenclature, phylogeny

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## Introduction

Karst landform encompasses surface and subterranean features that are shaped by the action of water on soluble rocks. Karst topography represents distinctive geological formations characterized by caves, sinkholes, and other notable features. These environments harbor diverse landforms and microclimates that foster rich biological diversity. Karst habitats are hotspots of diversity and endemism due to their distinct ecological niches, which allow for the diversification of a wide variety of species (Clements et al. 2006; Grismer et al. 2021). Numerous research expeditions of karst landforms have uncovered new species with localized distributions (Tian and Huang 2015; Huang et al. 2019; Agung et al. 2021; Qu et al. 2021), identifying karsts as hotspots of endemism and biodiversity and priorities for conservation.

Guizhou, referred to as the “Karst Province of China” and “the Karst Museum”, has an extensive karst area of 128,000 km$^2$, accounting for 73% of its total land area. This makes it the largest karst region in China, with diverse landscapes including peak forests, stone forests, canyons, valleys, enigmatic caves, and vast sinkholes (Li 2011). The karst landscapes of Guizhou exhibit remarkable diversity, featuring peak forests, stone forests, unique canyons, blind valleys, enigmatic caves, and vast sinkholes.

The genus *Ophiocordyceps* was established by Petch (1931) to classify the species with non-disarticulating ascospores and clavate asci with thickened apices. Most species in *Ophiocordyceps* possess firm, darkly pigmented stromata or subiculum, especially those with *Hirsutella* Pat. asexual morphs. Conversely, some species exhibit brightly or palely colored stromata associated with *Hymenostilbe* Petch and *Paraisaria* Samson & Brady asexual morphs (Mongkolsamrit et al. 2019). The stromata are generally tough, wiry, fibrous, or pliant. The perithecia, which can be superficial or completely immersed, are typically arranged obliquely or in an ordinal fashion. Ascospores in this genus are typically cylindrical and multiseptated, disarticulating into part-spores or remaining whole upon discharge (Sung et al. 2007; Xiao et al. 2023). Species of *Ophiocordyceps* are distributed worldwide across various forest ecosystems, including tropical and subtropical regions (Petch 1937; Kobayasi 1941; Tzean et al. 1997; Chen et al. 2013; Ban et al. 2014; Sanjuan et al. 2015; Luangsa-ard et al. 2018; Araújo et al. 2018; Mongkolsamrit et al. 2019; Zha et al. 2021). Notable species with widespread distribution include *O. nutans* (Friedrich et al. 2018). Among the most prominent species in this genus is *O. sinensis*, a well-known traditional Chinese medicine found exclusively in alpine environments of the Qinghai-Tibet Plateau (Dai et al. 2020). Another notable group within the genus, *O. unilateralis s.l.*, is famous for its ability to manipulate ants, turning them into “zombie ants” that facilitate the spread of spores (Evans et al. 2011). Recently, a newly discovered species, *O. megala*, was identified, notable for being the largest cordycipitoid fungus with a single specimen weighing 28 g, a hundred times heavier than typical *O. sinensis* (Dai et al. 2024).

In this study, we undertook a comprehensive survey of cordycipitoid fungi within the Xingyi karst landform area, spanning the Baishi Mountains, Malinghe Valley, and Wanfeng Forests. A total of 55 specimens were collected. One species infecting lepidopteran larvae in the Baishi Mountains was identified as *O. cystidiata*. Another specimen, parasitizing *Coccoidea* sp. from the Malinghe Valley, was validated and reclassified it as *O. sinocampes*. Morphological, microstructural, and multi-gene phylogenetic analyses were conducted to compare the new species with closely related taxa for classification and descriptions.

## Materials and methods

### Specimen collection

Specimens were collected from three karst landform areas in Xingyi City, Guizhou Province, China: (1) Baishi Mountains, Baiwanyao Village (25°4'12"N, 99°10'12"E), (2) Malinghe Valley (25°8'24"N, 104°57'36"E), and (3) Wanfeng Forests (24°59'24"N, 104°58'12"E).

### Fungal isolation and culture

Upon collection, specimens underwent surface sterilization with 75% ethanol for 1–3 min, followed by rinsing with sterile water. The internal sclerotia were isolated and cultured on potato dextrose agar (PDA) at 20 °C under dark conditions. All isolated strains were deposited at the Institute of Fungal Resources Collection, Guizhou University (GZAC), China.

### Microscopic morphological structure observation

Specimens collected in the field were analyzed and photographed using an Olympus SZ61 stereomicroscope. Fruiting bodies were sectioned and examined under a Leica S9E stereomicroscope. Sections of the fertile head were mounted on glass slides with a drop of lactic acid and lactophenol cotton blue, covered with a cover slip, and observed and photographed under a Leica DM2500 compound microscope for detailed measurements of perithecia, asci, peridium, apical cap, ascospores, and secondary ascospores.

The slant cultures were transferred to new PDA plates and incubated at 20 °C for three weeks for colony morphological observations. Circular agar blocks approximately 5 mm in diameter were extracted from a colony and transferred to fresh PDA plates for further colony morphological observations.

For the morphological description, microscope slide cultures were prepared by placing small pieces of mycelia on 5-mm diameter PDA medium blocks, which were then overlaid with a cover slip. Micro-morphological observations and measurements, including those of hyphae, phialides, and conidia, were conducted using an Olympus CX40 microscope.

### Scanning electron microscope observations

Electron microscopy was performed as previously described by Qu et al. (2021). For scanning electron microscopy (SEM) observations, 1-cm-wide agar blocks with hyphae were excised from PDA cultures. Samples were fixed with 4% glutaraldehyde overnight at 4 °C, washed thrice with phosphate buffer solution (PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, and 1.5 mM $KH_2PO_4$; pH 7.4), each time for 10 min. Next, the fixed hyphae and conidia underwent dehydration using a series of alcohol concentrations (50%, 70%, 90%, and 100% alcohol), each step lasting 10 min.

Subsequently, the samples were dehydrated with supercritical carbon dioxide. The gold coating was then applied to the samples prior to examination. Conidia and mucilage were visualized and photographed using a Hitachi S-3400N scanning electron microscope (Japan).

## DNA extraction, polymerase chain reaction amplification, and sequencing

Genomic DNA from both the fungus and its host was extracted using a Fungi DNA isolation Kit following the manufacturer's instructions (Transgen Bio-Tek, USA). DNA was extracted from the stroma and the surface of sclerotium sections, respectively. Additionally, genomic DNA was extracted from fungal pure cultures using 0.05–0.1 g of axenic mycelia. The concentration of the obtained genomic DNA was larger than 20 ng/μL, and this DNA was used as a template for polymerase chain reaction (PCR) amplification of target DNA fragments.

Six nuclear loci of the fungus were targeted for amplification and sequencing, namely the internal transcribed spacer (ITS), the small and large subunit ribosomal RNA (nrSSU and nrLSU, respectively), the transcription elongation factor-1 alpha (*tef*), and the largest and second largest subunits of RNA polymerase II (*rpb1* and *rpb2*, respectively). The PCR assays followed protocols described by Qu et al. (2021) and Peng et al. (2024). Detailed information regarding the primers used was provided in Suppl. material 1. PCR products were subsequently sequenced using an ABI3700 automatic sequence analyzer (Sangong, Shanghai).

## Phylogenetic analysis

For the construction of a phylogenetic tree encompassing the potential new *Ophiocordyceps* species, representative taxa were selected based on five loci: nrSSU, nrLSU, *tef*, *rpb1*, and *rpb2*. These taxa were selected from previous studies within the genus *Ophiocordyceps* (Sung et al. 2007; Ban et al. 2014; Quandt et al. 2014; Sanjuan et al. 2015; Simmons et al. 2015; Luangsa-ard et al. 2018; Mongkolsamrit et al. 2019; Fan et al. 2021; Qu et al. 2021; Peng et al. 2024) (Table 1). Sequences for each locus were retrieved from GenBank using their respective accession numbers. We combined the published data with our newly generated sequences from the present study to establish a five-locus dataset. This dataset comprised a total of 212 taxa, encompassing sequence data from nrSSU, nrLSU, *tef*, *rpb1*, and *rpb2*, aimed at capturing the diversity within *Ophiocordyceps* (Table 1). As outgroups, *Drechmeria coniospora* and *Haptocillium sinense* were selected based on Kepler et al. (2014).

The alignment of nrSSU and nrLSU sequences was performed using MAFFT (Katoh et al. 2002) with default settings. For the exon regions of *tef*, *rpb1*, and *rpb2*, alignment was conducted using codon models. The total alignment lengths for the five loci were as follows: 1060 bp for nrSSU; 968 bp for nrLSU; 936 bp for *tef*; 555 bp for *rpb1* and 936 bp for *rpb2*, resulting in a combined dataset length of 4455 bp. All five loci were integrated into a unified dataset, which was further partitioned into 11 distinct segments for analysis. This partitioning included one segment each for nrSSU and nrLSU, along with nine additional segments corresponding to the three codon positions within the protein-coding genes *tef*, *rpb1*, and *rpb2*.

The optimal partitioning scheme and evolutionary models for the 11 predefined partitions were determined using PartitionFinder2 (Lanfear et al. 2016), employing a greedy algorithm and the Akaike information criterion. The analysis yielded the following 10 partitions with their respective best-fit models: Partition 1—nrSSU: TRNEF+I+G, Partition 2—nrLSU, Partitions 3–5—*tef* codon1, codon 2 and codon 3: GTR+I+G, Partition 6—*rpb1* codon1, *rpb2* codon1: TVM+I+G; Partitions 7–9 —*rpb1* codon2, codon3 and *rpb2* codon2: GTR+I+G, and Partition 10— *rpb2* codon3: TIM+I+G.

**Table 1.** Specimens and GenBank accession numbers for nrSSU, nrLSU, *tef*, *rpb1*, and *rpb2* sequences included in phylogenetic analyses.

| Species | Voucher | Host | nrSSU | nrLSU | *tef1-a* | *rpb1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|---|
| *D. balanoides* | CBS 250.82$^{T}$ | Lepidoptera | AF339539 | AF339588 | DQ522342 | – | DQ522442 | Sung et al. 2007 |
| *D. coniospora* | ARSEF 6962 | – | collected from its genome scaffold sequences (LAYC00000000) | | | | | Zhang et al. 2016 |
| *D. gunnii* | OSC 76404 | Lepidoptera | AF339572 | AF339522 | AY489616 | AY489650 | DQ522426 | Kepler et al. 2012 |
| *D. sinense* | CBS 567.95 | – | AF339594 | AF339545 | DQ522343 | DQ522389 | DQ522443 | Sung et al. 2001 |
| *H. fusiformis* | ARSEF 5474 | Coleoptera | KM652067 | KM652110 | KM651993 | KM652033 | – | Simmons et al. 2015 |
| *H. gigantea* | ARSEF 30 | Hymenoptera | – | JX566977 | JX566980 | KM652034 | – | Simmons et al. 2015 |
| *H. guyana* | ARSEF 878 | Hemiptera:Cicadellidae | KM652068 | KM652111 | KM651994 | KM652035 | – | Simmons et al. 2015 |
| *H. illustris* | ARSEF 5539 | Hemiptera | KM652069 | KM652112 | KM651996 | KM652037 | – | Simmons et al. 2015 |
| *H. kirchneri* | ARSEF 5551 | Acari:Eriophyidae | KM652070 | KM652113 | KM651997 | – | – | Simmons et al. 2015 |
| *H. kuankuoshuiensis* | GZUIFR-2012KKS3-1 | Lepidoptera larvae | – | KY415582 | KY415590 | KY945360 | – | Qu et al. 2021 |
| *H. lecaniicola* | ARSEF 8888 | Hemiptera:Coccidae | KM652071 | KM652114 | KM651998 | KM652038 | – | Simmons et al. 2015 |
| *H. liboensis* | ARSEF 9603 | Lepidoptera: Cossidae | KM652072 | KM652115 | – | – | – | Simmons et al. 2015 |
| *H. minnesotensis* | 3608 | *Heterodera glycines* | collected from its genome scaffold sequences(JPUM00000000) | | | | | Lai et al. 2014 |
| *H. nodulosa* | ARSEF 5473 | Lepidoptera:Pyralidae | KM652074 | KM652117 | KM652000 | KM652040 | – | Simmons et al. 2015 |
| *H. radiata* | ARSEF 1369 | Diptera | KM652076 | KM652119 | KM652002 | KM652042 | – | Simmons et al. 2015 |
| *H. repens* nom. *inval.* | ARSEF 2348 | Hemiptera:Delphacidae | KM652077 | KM652120 | KM652003 | – | – | Simmons et al. 2015 |
| *H. rhossiliensis* | ARSEF 3751 | – | KM652081 | KM652124 | KM652007 | KM652046 | – | Simmons et al. 2015 |
| *H. rhossiliensis* | ARSEF 2931 | Tylenchida:Heteroderidae | KM652078 | KM652121 | KM652004 | KM652043 | – | Simmons et al. 2015 |
| *H. rhossiliensis* | ARSEF 3207 | – | KM652079 | KM652122 | KM652005 | KM652044 | – | Simmons et al. 2015 |
| *H. rhossiliensis* | ARSEF 3747 | Tylenchida:Criconematidae | KM652080 | KM652123 | KM652006 | KM652045 | – | Simmons et al. 2015 |
| *H. satumaensis* | ARSEF 996 | Lepidoptera:Pyralidae | KM652082 | KM652125 | KM652008 | KM652047 | – | Simmons et al. 2015 |
| *H. subulata* | ARSEF 2227 | Lepidoptera | KM652086 | KM652130 | KM652013 | KM652051 | – | Simmons et al. 2015 |
| *H. thompsonii* | ARSEF 256 | – | KM652090 | KM652135 | KM652018 | KM652053 | – | Simmons et al. 2015 |
| *H. thompsonii* | MTCC 3556 | – | collected from its genome scaffold sequences (APKB01000000) | | | | | |
| *H. thompsonii* | MTCC 6686 | – | collected from its genome scaffold sequence (APKU01000000) | | | | | |
| *H. versicolor* | ARSEF 1037 | Hemiptera:Membracidae | KM652102 | KM652150 | KM652029 | KM652063 | – | Simmons et al. 2015 |
| *H. vnecatrix* | ARSEF 5549 | Ixodida | KM652073 | KM652116 | KM651999 | KM652039 | – | Simmons et al. 2015 |
| *H. cryptosclerotium* | ARSEF 4517 | Hemiptera | KM652066 | KM652109 | KM651992 | KM652032 | – | Simmons et al. 2015 |
| *H. strigosa* | ARSEF 2197 | Hemiptera: Cicadellidae | KM652085 | KM652129 | KM652012 | KM652050 | – | Simmons et al. 2015 |
| *Hirsutella* sp. | NHJ 12525 | – | – | EF469078 | EF469063 | EF469092 | EF469111 | Sung et al. 2007 |
| *O. acicularis* | OSC 110987 | Coleoptera | EF468950 | EF468805 | EF468744 | EF468852 | – | Sung et al. 2007 |
| *O. acicularis* | OSC 110988 | Coleoptera | EF468951 | EF468804 | EF468745 | EF468853 | – | Sung et al. 2007 |

| Species | Voucher | Host | nrSSU | nrLSU | ***tef1-a*** | ***rpb1*** | ***rpb2*** | References |
|---|---|---|---|---|---|---|---|---|
| *O. agriota* | ARSEF 5692 | Coleoptera | DQ522540 | DQ518754 | DQ522322 | DQ522368 | DQ522418 | Kepler et al. 2012 |
| *O.alboperitheciata* | YHH 16755$^T$ | Lepidoptera | – | MT222278 | MT222279 | MT222280 | MT222281 | Fan et al. 2021 |
| *O. aphodii* | ARSEF 5498 | Coleoptera | DQ522541 | DQ518755 | DQ522323 | – | DQ522419 | Spatafora et al. 2007 |
| *O. appendiculata* | NBRC 106959 | Coleoptera | JN941729 | JN941412 | AB968578 | JN992463 | AB968540 | Ban et al. 2015 |
| *O. appendiculata* | NBRC 106960 | Coleoptera | JN941728 | JN941413 | AB968577 | JN992462 | AB968539 | Ban et al. 2015 |
| *O. araracuarensis* | HUA 186148 | – | KC610790 | KF658679 | KC610739 | KF658667 | KC610717 | Dai et al. 2024 |
| *O. arborescens* | NBRC 105890 | Cossidae; Lepidoptera | – | AB968415 | AB968573 | – | AB968535 | Ban et al. 2015 |
| *O. arborescens* | NBRC 105891 | Cossidae; Lepidoptera | – | AB968414 | AB968572 | – | AB968534 | Ban et al. 2015 |
| *O. australis* | 1348a | Hymenoptera | collected from its genome scaffold sequences(NJEU00000000) | | | | | De Bekker et al. 2017 |
| *O. australis* | Map64 | Hymenoptera | collected from its genome scaffold sequences(DAJKKO000000000) | | | | | De Bekker et al. 2017 |
| *O. bispora* | ERS1123077 | Hymenoptera | collected from its genome scaffold sequences(DAJKKO000000000) | | | | | Conlon et al. 2017 |
| *O. blakebarnesii* | MISSOU1 | – | KX713644 | – | KX713686 | KX713713 | – | Araújo et al. 2018 |
| *O. blakebarnesii* | MISSOU3 | – | KX713643 | KX713608 | KX713687 | KX713714 | – | Araújo et al. 2018 |
| *O. brunneanigra* | BCC69032 | – | – | MF614654 | MF614638 | MF614668 | MF614681 | Luangsa-ard et al. 2018 |
| *O. brunneaperitheciata* | BCC64201 | – | – | MF614658 | MF614643 | – | MF614685 | Luangsa-ard et al. 2018 |
| *O. brunneiperitheciata* | BCC 49312 | Lepidoptera | – | MF614660 | MF614642 | – | MF614686 | Luangsa-ard et al. 2018 |
| *O. brunneipunctata* | OSC 128576 | Coleoptera (Elateridae) | DQ522542 | DQ518756 | DQ522324 | DQ522369 | DQ522420 | Spatafora et al. 2007 |
| *O. camponoti-balzani* | G104 | *Camponotus balzani* | KX713660 | KX713593 | KX713703 | KX713703 | – | Araújo et al. 2018 |
| *O. camponoti bispinosi* | OBIS4 | – | KX713637 | – | KX713692 | KX713720 | – | Araújo et al. 2018 |
| *O. camponoti- femorati* | FEMO2 | – | KX713663 | KX713590 | KX713678 | KX713702 | – | Araújo et al. 2018 |
| *O. camponoti-hippocrepidis* | HIPPOC | Hemiptera | KX713655 | KX713597 | KX713673 | KX713707 | – | Araújo et al. 2018 |
| *O. camponoti leonardi* | BCC 80369 | – | collected from its genome scaffold sequences(PDHP01000000) | | | | | Kobmoo et al. 2019 |
| *O. camponoti-nidulantis* | NIDUL2 | – | KX713640 | KX713611 | KX713669 | KX713717 | – | Araújo et al. 2018 |
| *O. camponoti- rufipedis* | G108 | – | KX713659 | KX713594 | KX713679 | KX713704 | – | Araújo et al. 2018 |
| *O. camponoti- rufipedis* | Map16 | – | collected from its genome scaffold sequence(NJES00000000) | | | | | De Bekker et al. 2017 |
| *O. camponoti- saundersi* | BCC 79314 | – | collected from its genome scaffold sequences(PDHQ00000000) | | | | | Kobmoo et al. 2019 |
| *O. camponoti-renggeri* | ORENG | – | KX713634 | KX713617 | KX713671 | – | – | Araújo et al. 2018 |
| *O.* cf. *acicularis* | OSC 128580 | Coleoptera | DQ522543 | DQ518757 | DQ522326 | DQ522371 | DQ522423 | Spatafora et al. 2007 |
| *O. clavata* | NBRC 106961 | – | – | JN941414 | AB968586 | – | AB968547 | Schoch et al. 2012 |
| *O. coccidiicola* | NBRC 100682 | – | AB968391 | AB968419 | AB968583 | – | AB968545 | Ban et al. 2015 |
| *O. cochlidiicola* | HMAS 199612 | – | KJ878917 | KJ878884 | KJ878965 | KJ878998 | – | Quandt et al. 2014 |
| *O. crinalis* | GDGM 17327 | Lepidoptera | KF226253 | KF226254 | KF226256 | KF226255 | | Wang et al. 2014 |
| *O. curculionum* | OSC 151910 | – | KJ878918 | KJ878885 | | KJ878999 | – | Quandt et al. 2014 |
| ***O. cystidiata*** | **GZUIFR-2023XY-OA5** | **Hepialidae** | **PQ497594** | **PQ497634** | **–** | **PQ516632** | **PQ516636** | **This study** |

| Species | Voucher | Host | nrSSU | nrLSU | *tef1-a* | *rpb1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|---|
| ***O. cystidiata*** | **GZUIFR-2023XY-OA5C** | **Hepialidae** | **PQ497595** | **PQ497635** | **–** | **PQ516633** | **PQ516637** | **This study** |
| *O. desmidiospora* | SJS3Des | – | MH536515 | MH536514 | MN785129 | MN785131 | – | Saltamachia and Araújo 2020 |
| *O. elongata* | OSC 110989 | Lepidoptera | – | EF468808 | EF468748 | EF468856 | – | Sung et al. 2007 |
| *O. entomorrhiza* | KEW 53484 | - | EF468954 | EF468809 | EF468749 | EF468857 | EF468911 | Sung et al. 2007 |
| *O. evansii* | Ophsp 858 | Lepidoptera | EF468954 | EF468809 | EF468749 | EF468857 | – | Sanjuan et al. 2015 |
| *O. fenggangensis* | FG21042850 | Lepidoptera | OR527538 | OR527541 | OR526345 | OR526350 | OR526353 | Peng et al. 2024 |
| *O. fenggangensis* | HKAS 125848$^{T}$ | Lepidoptera | – | OR527542 | OR526346 | OR526351 | – | Peng et al. 2024 |
| *O. formicarum* | TNS F18565 | – | KJ878921 | KJ878888 | KJ878968 | KJ879002 | KJ878946 | Quandt et al. 2014 |
| *O. formosana* | MFLU:15-3888 | – | KU854951 | – | KU854949 | KU854947 | – | Li et al. 2016 |
| *O. formosana* | NTU 00035 | – | – | – | KT275192 | KT275190 | KT275191 | Wang et al. 2015a |
| *O. forquignonii* | OSC 151902 | – | KJ878912 | KJ878876 | – | KJ878991 | KJ878945 | Quandt et al. 2014 |
| *O. fulgoromorphila* | Ophara729 | – | KC610795 | KC610761 | KC610730 | KF658677 | AB968554 | Sanjuan et al. 2015 |
| *O. geometridicola* | BCC35947 | – | – | MF614647 | MF614631 | MF614664 | MF614678 | Luangsa-ard et al. 2018 |
| *O. geometridicola* | BCC79823 | – | – | MF614648 | MF614632 | MF614663 | MF614679 | Luangsa-ard et al. 2018 |
| *O. ghanensis* | Gh41 | – | KX713656 | – | KX713668 | KX713706 | – | Araújo et al. 2018 |
| *O. highlandensis* | HKAS83207 | Scarabaeoidea | KM581284 | – | – | KM581274 | KM581278 | Yang et al. 2015 |
| *O. hignlandensis* | YHH OH1301 | Melolonthidae | KR479869 | – | KR479870 | KR479872 | KR479874 | Wang et al. 2015b |
| *O. irangiensis* | OSC 128578 | Hymenoptera:ant | DQ522556 | DQ518770 | DQ522345 | DQ522391 | DQ522445 | Spatafora et al. 2007 |
| *O. karstii* | MFLU: 15-3884 | Hepialidae | KU854952 | – | KU854945 | KU854943 | – | Li et al. 2016 |
| *O. karstii* | MFLU: 15-3885 | Hepialidae | KU854953 | – | KU854946 | KU854944 | – | Li et al. 2016 |
| *O. khonkaenensis* | BCC81463 | – | MK632127 | MK632102 | MK632076 | MK632169 | MK632158 | Crous et al. 2019 |
| *O. kimflemingiae* | SC09B | – | KX713631 | – | KX713698 | KX713724 | – | Araújo et al. 2018 |
| *O. kniphofioides* | MF90 | Hymenoptera | MK874746 | MK875538 | – | MK863827 |  | Araújo et al. 2018 |
| *O. konnoana* | EFCC 7315 | Coleopteran | EF468959 | – | EF468753 | EF468861 | EF468916 | Sung et al. 2007 |
| *O. 1anpingensis* | YHOL0707 | Hepialidae | KC417459 | KC417461 | KC417463 | KC417465 | – | Chen et al. 2013 |
| *O. liangii* | HKAS 125845$^{T}$ | Lepidoptera | OR527539 | OR527543 | OR526347 | – | – | Peng et al. 2024 |
| *O. liangii* | LB22071253 | Lepidoptera | OR527540 | OR527544 | OR526348 | – | OR526354 | Peng et al. 2024 |
| *O. liangshanensis* | YFCC 8577 | Lepidoptera(Hepialidae | MT774218 | MT774225 | MT774246 | MT774232 | MT774239 | Wang et al. 2021 |
| *O. liangshanensis* | YFCC 8578 | Lepidoptera(Hepialidae) | MT774219 | MT774226 | MT774247 | MT774233 | MT774240 | Wang et al. 2021 |
| *O. lloydii* | OSC 151913 | Hymenoptera (Camponotus) | KJ878924 | KJ878891 | KJ878970 | KJ879004 | – | Quandt et al. 2014 |
| *O. 1ongissima* | NBRC 106965 | – | AB968392 | AB968420 | AB968584 | – | AB968546 | Ban et al. 2015 |
| *O. longissima* | TNS F18448 | – | KJ878925 | KJ878892 | KJ878971 | KJ879005 | – | Quandt et al. 2014 |
| *O. macroacicularis* | BCC 22918 | Lepidopter | – | MF614655 | MF614639 | MF614669 | MF614675 | Luangsa-ard et al. 2018 |
| *O. macroacicularis* | NBRC 100685 | – | AB968388 | AB968416 | AB968574 | – | AB968536 | Ban et al. 2015 |

| Species | Voucher | Host | nrSSU | nrLSU | *tef1-a* | *rpb1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|---|
| *O. macroacicularis* | NBRC 105888 | Hepialidae | AB968389 | AB968417 | AB968575 | – | AB968537 | Ban et al. 2015 |
| *O. macroacicularis* | NBRC 105889 | Hepialidae | AB968390 | AB968418 | AB968576 | – | AB968538 | Ban et al. 2015 |
| *O. macroacicularis* | TNS F18550 | – | KJ878911 | KJ878875 | KJ878959 | – | – | Quandt et al. 2014 |
| *O. megala* | YHH OMYP 1507001 | Hepialidae | NMDCN00011VK | NMDCN00011VM | NMDCN00011VO | NMDCN00011VQ | NMDCN00011VS | Dai et al. 2024 |
| *O. megala* | YFCC OMLP15079192 | Hepialidae | NMDCN00011VL | NMDCN00011VN | NMDCN00011VP | NMDCN00011VR | NMDCN00011VT | Dai et al. 2024 |
| *O. melolonthae* | Ophgrc679 | – | – | KC610768 | KC610744 | KF658666 | – | Sanjuan et al. 2015 |
| *O. melolonthae* | OSC 110993 | Coleoptera | – | – | DQ522331 | DQ522376 | – | Spatafora et al. 2007 |
| *O. monacidis* | MF74 | Hymenoptera | KX713647 | KX713605 | – | KX713712 | – | Araújo et al. 2018 |
| *O. multiperitheciata* | BCC 22861 | Lepidoptera | – | MF614656 | MF614640 | MF614670 | MF614683 | Luangsa-ard et al. 2018 |
| *O. multiperitheciata* | BCC 69008 | Lepidoptera | – | MF614657 | MF614641 | – | MF614682 | Luangsa-ard et al. 2018 |
| *O. musicaudata* | SY22072879 | Lepidoptera | – | OR527545 | OR526349 | OR526352 | – | Peng et al. 2024 |
| *O. myrmecophila* | CEM1710 | – | KJ878928 | KJ878894 | KJ878974 | KJ879008 | – | Peng et al. 2024 |
| *O. myrmecophila* | TNS 27120 | – | KJ878929 | KJ878895 | KJ878975 | KJ879009 | – | Quandt et al. 2014 |
| *O. naomipierceae* | DAWKSANT | Hymenoptera | KX713664 | KX713589 | – | KX713701 | – | Araújo et al. 2018 |
| *O. neovolkiana* | OSC 151903 | – | KJ878930 | KJ878896 | KJ878976 | – | – | Quandt et al. 2014 |
| *O. nigre1la* | EFCC 9247 | Lepidoptera | EF468963 | EF468818 | EF468758 | EF468866 | EF468920 | Sung et al. 2007 |
| *O. nooreniae* | BRIP 55363 | Hymenoptera | KX673811 | KX673810 | KX673812 | – | KX673809 | Crous et al. 2016 |
| *O. nujiangensis* | YFCC8880 | Hepialidae | ON723384 | ON723381 | ON868820 | ON868823 | ON868826 | Sun et al. 2022 |
| *O. nujiangensis* | YHH 20041 | Lepidoptera | ON723385 | ON723383 | ON868822 | ON868825 | ON868827 | Sun et al. 2022 |
| *O. nutans* | NBRC 100944 | – | JN941713 | JN941428 | AB968588 | – | AB968549 | Ban et al. 2015 |
| *O. nutans* | OSC 110994 | stink bug | DQ522549 | DQ518763 | DQ522333 | DQ522378 | – | Spatafora et al. 2007 |
| *O. ootakii* | J13 | Hymenoptera (Polyrhachis moesta) | KX713652 | KX713600 | KX713681 | KX713708 | – | Araújo et al. 2018 |
| *O. ovatospora* | YHH2206001 | – | – | OP295113 | OP313801 | OP313803 | OP313805 | Tang et al. 2022 |
| *O. ovatospora* | YFCC22069184 | – | OP295111 | OP295114 | OP313802 | OP313804 | – | Tang et al. 2022 |
| *O. pauciovoperitheciata* | BCC39781 | – | – | MF614650 | MF614635 | MF614667 | MF614671 | Luangsa-ard et al. 2018 |
| *O. pauciovoperitheciat* | BCC45562 | – | – | MF614651 | MF614634 | MF614666 | MF614674 | Luangsa-ard et al. 2018 |
| *O.polyrhachis -furcata* | BCC 54312 | – | collected from its genome scaffold sequences(LKCN00000000) | | | | | Wichadakul et al. 2015 |
| *O. ponerus* | XCH ant 03 | Hymenoptera | KY953152 | – | KY953153 | KY953154 | – | Qu et al. 2018 |
| *O. pruinosa* | NHJ 12994 | Hemiptera | EU369106 | EU369041 | EU369024 | EU369063 | EU369084 | Johnson et al. 2009 |
| *O. pseudoacicularis* | BCC49256 | Hymenoptera: ant | – | MF614645 | MF614629 | MF614662 | MF614676 | Luangsa-ard et al. 2018 |
| *O. pseudoacicularis* | BCC53843 | Hymenoptera: ant | – | MF614646 | MF614630 | MF614661 | MF614677 | Luangsa-ard et al. 2018 |
| *O. pulvinata* | TNS F 30044 | Hymenoptera: ant | GU904208 | AB721305 | GU904209 | GU904210 | – | Kepler et al. 2011 |
| *O. purpureostromata* | TNS F18430 | Coleoptera | KJ878931 | KJ878897 | KJ878977 | KJ879011 | – | Quandt et al. 2014 |

| Species | Voucher | Host | nrSSU | nrLSU | *tef1-a* | *rpb1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|---|
| *O. ramosissimum* | GZUH2012HN2 | *Endoclita* sp. (Hepialidae) | KJ028013 | – | KJ028016 | KJ028018 | – | Wen et al. 2014 |
| *O. ramosissimum* | GZUHHN8 | *Phassus nodus* | KJ028012 | – | KJ028014 | KJ028017 | – | Wen et al. 2014 |
| *O. ravenelii* | OSC 151914 | – | KJ878932 | – | KJ878978 | KJ879012 | KJ878950 | Quandt et al. 2014 |
| *O. robertsii* | UoM1 | Hepialidae | collected from its genome scaffold sequences(JAPEBV000000000) | | | | | Xu et al. 2023 |
| *O. robertsii* | UoM4 | Hepialidae | collected from its genome scaffold sequences(JAPEBW000000000) | | | | | Xu et al. 2023 |
| *O. rubiginosiperitheciata* | NBRC 100946 | – | JN941705 | JN941436 | AB968581 | JN992439 | AB968543 | Schoch et al. 2012 |
| *O. rubiginosiperitheciata* | NBRC 106966 | – | JN941704 | JN941437 | AB968582 | JN992438 | AB968544 | Schoch et al. 2012 |
| *O. salganeicola* | JPMA107 | – | MT741703 | MT741716 | MT759574 | MT759577 | – | Araujo et al. 2020 |
| *O. salganeicola* | Mori01 | – | MT741705 | MT741719 | MT759575 | MT759578 | MT759580 | Araujo et al. 2020 |
| *O. satoi* | J19 | *Polyrhachis lamellidens* | KX713650 | KX713601 | KX713684 | KX713710 | – | Araújo et al. 2018 |
| *O. sinensis* | QH06-197 | Hepialidae | JX968025 | JX968030 | JX968015 | JX968005 | JX968010 | Zhang et al. 2013 |
| *O. sinensis* | QH09-201 | Hepialidae | JX968024 | JX968029 | JX968014 | JX968004 | JX968009 | Zhang et al. 2013 |
| *O. sinensis* | XZ06-44 | Hepialidae | JX968026 | JX968031 | JX968016 | JX968006 | JX968011 | Zhang et al. 2013 |
| *O. sinensis* | YN07-8 | Hepialidae | JX968027 | JX968032 | JX968017 | JX968007 | JX968012 | Zhang et al. 2013 |
| *O. sinensis* | YN09-64 | Hepialidae | JX968028 | JX968033 | JX968018 | JX968008 | JX968013 | Zhang et al. 2013 |
| *O. sinensis* | CO18 | Hepialidae | collected from its genome scaffold sequences(ANOV00000000) | | | | | |
| *O. sinensis* | CUHK CSC2 | Hepialidae | – | HM595902 | HM595936 | HM595968 | – | Chan et al. 2011 |
| *O. sinensis* | ZJB12195 | Hepialidae | collected from its genome scaffold sequences(LWBQ01000000) | | | | | |
| ***O. sinocampes*** | **GZUIFR 2010MC-1** | **Lepidoptera** | **–** | **PQ766190** | **PQ787212** | **–** | **PQ787213** | **This study** |
| ***O. sinocampes*** | **GZUIFR-2022MLH-H1** | **Coccoidea** | **PQ497592** | **PQ497632** | **PQ516628** | **PQ516630** | **PQ516634** | **This study** |
| ***O. sinocampes*** | **GZUIFR-2022MLH-H1C** | **Coccoidea** | **PQ497593** | **PQ497633** | **PQ516629** | **PQ516631** | **PQ516635** | **This study** |
| *O. sobolifera* | KEW 78842 | Cicadidae | EF468972 | EF468828 | – | EF468875 | EF468925 | Sung et al. 2007 |
| *O. sobolifera* | NBRC 106967 | Cicadidae | AB968395 | AB968422 | AB968590 | – | AB968551 | Ban et al. 2015 |
| *O. spataforae* | MY11765 | – | – | MG831747 | MG831746 | MG831748 | MG831749 | Luangsa-ard et al. 2018 |
| *O. spataforae* | OSC 128575 | Hemiptera | EF469126 | EF469079 | EF469064 | EF469093 | EF469110 | Sung et al. 2007 |
| *O. sphecocephala* | NBRC 101416 | – | JN941698 | JN941443 | | JN992432 | – | Schoch et al. 2012 |
| *O. spicatus sp. nov.* | MFLU18-0164 | Coleoptera: Tenebrionoidea | MK863047 | MK863054 | MK860192 | – | – | Zha et al. 2021 |
| *O. stylophora* | OSC 111000 | Coleoptera (Elateridae | DQ522552 | DQ518766 | DQ522337 | DQ522382 | DQ522433 | Spatafora et al. 2007 |
| *O. stylophora* | NBRC 100947 | – | JN941694 | JN941447 | AB968579 | JN992428 | AB968541 | Schoch et al. 2012 |
| *O. stylophora* | NBRC 100948 | – | JN941693 | JN941448 | AB968580 | JN992427 | AB968542 | Schoch et al. 2012 |
| *O. stylophora* | NBRC 100949 | – | JN941692 | JN941449 | – | JN992426 | – | Schoch et al. 2012 |
| *O. stylophora* | OSC 110999 | – | EF468982 | EF468837 | EF468777 | EF468882 | EF468931 | Sung et al. 2007 |
| *O. stylophora* | OSC111000 | Coleoptera | DQ522552 | DQ518766 | DQ522337 | DQ522382 | DQ522433 | Spatafora et al. 2007 |

| Species | Voucher | Host | nrSSU | nrLSU | *tef1-a* | *rpb1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|---|
| *O. thanathonensis* | MFU16-2909 | – | – | MF850378 | MF872613 | MF872615 | – | Xiao et al. 2017 |
| *O. tiputini* | Ophsp. 11465 | – | KC610792 | KC610773 | KC610745 | KF658671 | – | Sanjuan et al. 2015 |
| *O. tricentri* | NBRC 106968 | – | AB968393 | AB968423 | AB968593 | – | AB968554 | Ban et al. 2015 |
| *O. unilateralis* | Ophuni866 | – | KC610799 | – | KC610742 | KF658674 | KC610718 | Sanjuan et al. 2015 |
| *O. unilateralis* | OSC 128574 | Hymenoptera | DQ522554 | DQ518768 | DQ522339 | DQ522385 | DQ522436 | Spatafora et al. 2007 |
| *O. unilateralis* | SC16a | – | collected from its genome scaffold sequences(LAZP02000001) | | | | | De Bekker et al. 2015 |
| *O. unilateralis* | SERI1 | *Camponotus sericeiventris* | KX713628 | KX713626 | KX713675 | KX713730 | – | Araújo et al. 2018 |
| *O. unitubercula* | YFCC HU1301 | Lepidoptera: Noctuidae | KY923213 | KY923211 | KY923215 | KY923217 | – | Wang et al. 2018 |
| *O. unitubercula* | YHH HU1301 | Lepidoptera: Noctuidae | KY923214 | KY923212 | KY923216 | KY923218 | – | Wang et al. 2018 |
| *O. variabilis* | ARSEF 5365 | Dipteran | DQ522555 | DQ518769 | DQ522340 | DQ522386 | DQ522437 | Spatafora et al. 2007 |
| *O. xuefengensis* | GZUH2012HN13 | *Phassus nodus* | KC631787 | – | KC631792 | KC631797 | – | Wen et al. 2013 |
| *O. xuefengensis* | GZUH2012HN14$^{T}$ | *Phassus nodus* | KC631789 | – | KC631793 | KC631798 | – | Wen et al. 2013 |
| *O. yakusimensis* | HMAS _199604 | Cicadidae | KJ878938 | KJ878902 | – | KJ879018 | KJ878953 | Quandt et al. 2014 |
| *Ophiocordyceps* sp1. | HKAS125843 | – | – | OQ110570 | OQ116920 | OQ116923 | – | unpublished |
| *Ophiocordyceps* sp1. | HKAS125849 | – | – | OQ110571 | OQ116921 | OQ116924 | – | unpublished |
| *Ophiocordyceps* sp1. | HKAS125850 | – | – | OQ110572 | OQ116922 | OQ116925 | – | unpublished |
| *Ophiocordyceps* sp2. | TNS 16250 | Coleoptera | KJ878942 | – | KJ878987 | KJ879021 | – | Quandt et al. 2014 |
| *Ophiocordyceps* sp2. | TNS 16252 | – | KJ878941 | KJ878906 | KJ878986 | – | – | Quandt et al. 2014 |
| *Ophiocordyceps* sp3. | NHJ 12581 | Lepidoptera | EF468973 | EF468831 | EF468775 | – | EF468930 | Sung et al. 2007 |
| *Ophiocordyceps* sp3. | NHJ 12582 | Lepidoptera | EF468975 | EF468830 | EF468771 | – | EF468926 | Sung et al. 2007 |
| *Ophiocordyceps* sp4. | OSC 110997 | – | EF468976 | – | EF468774 | EF468879 | EF468929 | Sung et al. 2007 |
| *par. amazonica* | Ophama2026 | – | KJ917562 | KJ917571 | KM411989 | KP212902 | KM411982 | Sanjuan et al. 2015 |
| *Par. blattarioides* | HUA 186093 | Blattodea | KJ917559 | KJ917570 | KM411992 | KP212910 | – | Sanjuan et al. 2015 |
| *Par. Coenomyiae* | NBRC 108993 | – | AB968384 | AB968412 | AB968570 | – | AB968532 | Ban et al. 2015 |
| *par. gracilioides* | Ophgrc934 | – | KJ917556 | – | – | KP212914 | KP212914 | Sanjuan et al. 2015 |
| *par. gracilis* | EFCC 3101 | Lepidoptera | EF468955 | EF468810 | EF468750 | EF468858 | EF468913 | Sung et al. 2007 |
| *par. gracilis* | EFCC 8572 | Lepidoptera | EF468956 | EF468811 | EF468751 | EF468859 | EF468912 | Sung et al. 2007 |
| *par. heteropoda* | NBRC 100642 | – | JN941720 | JN941421 | AB968594 | – | AB968555 | Ban et al. 2015 |
| *par. orthopterorum* | BBC88305 | Orthoptera (nymph) | – | MK332583 | MK214080 | MK214084 | – | Mongkolsamrit et al. 2019 |
| *par. phuwiangensis* | TBRC9709 | Coleoptera; Elateridae | – | MK192057 | MK214082 | MK214086 | – | Mongkolsamrit et al. 2019 |
| *par. tettigonia* | GZUHCS14062709 | Tettigoniidae sp. | KT345955 | – | KT375440 | KT375441 | – | Wen et al. 2014 |
| *par. yodhathaii* | BBH43163 | Coleoptera; Elateridae | – | MK332584 | MH211353 | MH211353 | – | Mongkolsamrit et al. 2019 |
| *Podonectria citrina* | TNSF18537 | – | – | KJ878903 | KJ878983 | – | KJ878954 | Quandt et al. 2014 |
| *Stilbella buquetii* | HMAS199617 | – | KJ878940 | KJ878905 | KJ878985 | KJ879020 | – | Quandt et al. 2014 |

The maximum likelihood phylogenetic tree was constructed using IQ-TREE (Nguyen et al. 2015) with 1000 ultrafast bootstrap replicates (Minh et al. 2013). The Shimodaira–Hasegawa-like approximate likelihood ratio test was employed to assess branch support (Guindon et al. 2010). The entire phylogenetic analysis was performed using PhyloSuite v1.2.2 (Zhang et al. 2020).

The Bayesian inference phylogenetic tree was constructed using MrBayes 3.2.6 (Ronquist et al. 2012) with a partition model. The analysis involved running two parallel Markov Chain Monte Carlo (MCMC) runs for 50,000,000 generations. The substitution model settings (lset) used the general time reversible model (nst = 6) and a gamma distribution of rate variation across sites (rates = invgamma), which was applied uniformly across all 10 partitions. To ensure the robustness of the phylogenetic inference, the initial 25% of sampled data were discarded as burn-in. The convergence of MCMC chains was monitored throughout the analysis, and the operation was stopped when the average standard deviation of split frequencies fell below 0.01, indicating convergence. Due to the extensive dataset and the time-consuming process, we employed the CIPRES Science Gateway (https://www.phylo.org/portal2/) to conduct the Bayesian phylogenetic analysis. The consensus tree was visualized and analyzed for tree topology and branch support using FigTree v.1.6 (http://tree.bio.ed.ac.uk/software/figtree/). While ITS sequences were not used to build the phylogenetic tree, they helped distinguish the relationships between the two novel taxa and closely related species.

## Results

### Phylogenetic analysis

A total of 213 taxa were classified into five well-supported clades within *Ophiocordyceps* based on the combined five-locus dataset (nrSSU, nrLSU, *tef*, *rpb1*, and *rpb2*) using maximum likelihood (ML) and Bayesian inference (BI) analyses. These clades were designated here as the *Hirsutella*-like A clade (BI = 0.999), *Hirsutella*-like B clade (BI = 1.00), *O. nutans* clade (BI = 1.00), and *O. ravenelii* clade (BI = 0.859) (Fig. 1). *O. sinocampes* was found to belong to the *Hirsutella*-like A clade, clustering within the *H. strigosa* sub-clade. It was identified as a sister species to *O. multiperitheciata*. The separate clade with high support values highlighted the distinctiveness of *O. sinocampes* from its closely related species. Similarly, *O. cystidiata* was positioned within the *Hirsutella*-like B clade, specifically clustering into the *H. gigantea* sub-clade. It was found to be a sister species to *O. fenggangensis*, *O. musicaudata*, *O. alboperitheciata*, and *H. kuankuoshuiensis*. The distinct clade formed by *O. cystidiata* with high support values underscores its differentiation from other species within the sub-clade.

### Taxonomy

***Ophiocordyceps sinocampes*** **X. Zou, Zhong S. Xu & J.J. Qu, sp. nov.**
MycoBank No: 854597
Fig. 2

**Synonym.** *Hirsutella campes* nom. invalid. X. Zou, J.J. Qu, Y.F. Han & Z.Q. Liang, Journal of Mountain Agriculture and Biology 40(6): 1–12, 2021 (in Chinese).

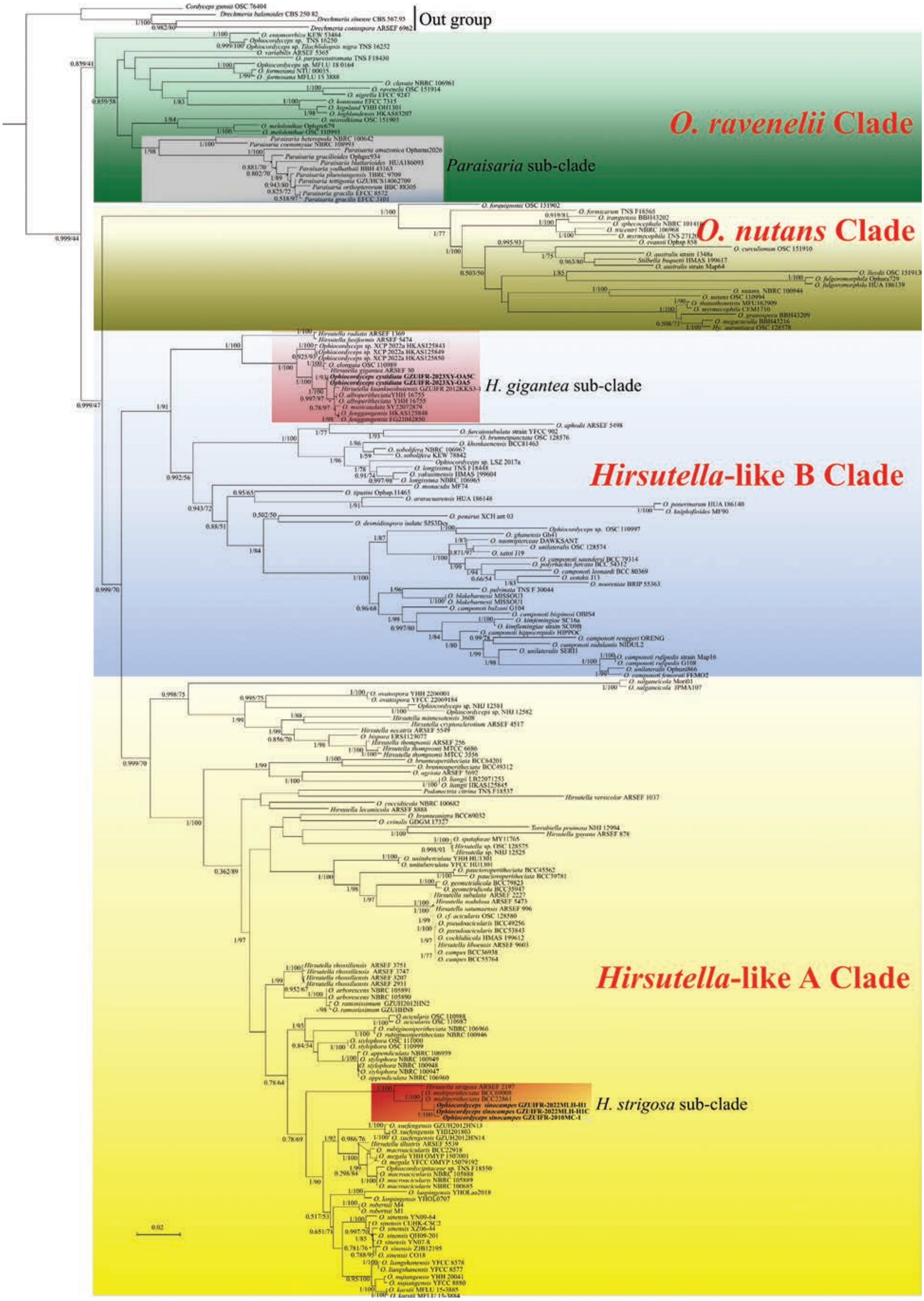

**Figure 1.** The phylogeny of *Ophiocordyceps* with emphasis on *O. sinocampes*, *O. cystidiata* and their related species based on 5-locus (nrSSU, nrLSU, *tef*, *rpb1*, and *rpb2*) datasets.

**Etymology.** The name *sinocampes* was derived from “sino,” referring to China, and “campes,” referring to the host in Latin, meaning caterpillar.

**Holotype.** GZUIFR-2010MC(Fig. 2a), China • Guizhou Province: Kuankuoshui National Nature Reserve (28°6'36"N, 107°2'24"E). The specimen was found on the larva of Lepidoptera buried in soil, collected in July 2010 by X. Zou (ex-holotype: GZUIFR-2010MC-1) (The GenBank accession number of ITS: PQ765882; nrLSU: PQ766190; tef: PQ787212; rpb2: PQ787213).

**Host.** The larvae of Lepidoptera.

**Description.** ***Stromata*:** Single, clavate, solid, lignified, yellow-brown, arising from the head of the host, 120–150 × 0.5–1.0 mm. ***Fertile part*:** Cylindrical, yellowish, about 5 cm long. ***Perithecia*:** Superficial, ovoid, 320–350 × 260–300 μm. ***Asci*:** Cylindrical, hyaline, 8-spored, 130–210 × 4–6 μm, with the apex thickened to form a hemispherical ascus cap that is, measuring 5–5.5 × 3.2–4.0 μm. ***Ascospores*:** Filiform, hyaline, irregular, multi-septate, disarticulating into secondary ascospores, 4.5–11 × 1.5–2.0 μm.

***Asexual morph*:** *Hirsutella*-like.

***Colonies*:** The colony reaches 13–18 mm in diameter after two weeks on PDA at 22 °C, appearing round with irregular swellings. The edge of the colony is fluffy, with a slight yellow protrusion in the middle and dark brown pigment secreted on the back, measuring approximately 10–15 mm in diameter. ***Hyphae*:** Hyaline, smooth-walled, septate, branched, 1.8–3.6 μm wide. ***Conidiogenous cells*:** Monophialidic, hyaline, smooth-walled, subulate, growing directly or laterally from hyphae, tapering gradually into a slender neck (21.6–38.4 μm long). The base width measures 2.4–4.8 μm, and the neck width measures 0.9–1.5 μm. ***Conidia*:** Hyaline, smooth, arising solitarily from the apex of conidiogenous cells, oval or orange-like shape, often enveloped in a mucous sheath, usually single, rarely aggregated in pairs or triplets (6–8.4 × 2.9–4.3 μm).

**Distribution.** China, Guizhou Province: Zunyi and Xingyi City.

**Additional specimens examined.** GZUIFR-2022MLH-H1 (Fig. 2l), and its pure culture GZUIFR-2022MLH-H1C, China. Guizhou Province: Malinghe Valley, Xingyi City (25°8'24"N, 104°57'36"E; altitude, 1068 m). These specimens were found on a larva of Coccoidea in soil, collected in July 2022 by Xiao Zou, Jiaojiao Qu, and Zhongshun Xu.

**Notes.** The basionym of *O. sinocampes* is *H. campes*, which was initially documented in the Journal of Mountain Agriculture and Biology (in Chinese) in 2021(Table 2). Notably, the taxonomic validity of *H. campes* is compromised due to its description being solely in Chinese, which does not meet the requisite standards set forth by the International Code of Nomenclature for algae, fungi, and plants (ICN). According to the ICN, the descriptions of new species must be provided in English or Latin (McNeill et al. 2012).

Furthermore, based on the priority under ICN, the genus *Hirsutella* has been considered as a synonym of the genus *Ophiocordyceps* (Quandt et al. 2014). Through morphological and five-gene phylogenetic analyses, it is more appropriate to assign this species to *Ophiocordyceps*. Since the name *O. campes* was already used by Tasanathai et al. (2020), we renamed our species as *O. sinocampes*.

In this study, we described the sexual stage, completing the species’ sexual and asexual stage descriptions. Additionally, a new specimen of this species was reported in the karst landform area— Malinghe Valley, Xingyi, enhancing our understanding of the species’ hosts and habitats.

**Table 2.** Morphological comparison of *O. sinocampes* and its relatives.

| Species | Host | Habitat | Stromata (mm) | Perithecium (μm) | Asci (μm) | Ascospore (μm) | Colony (mm) | Conidiogenous cells (μm) | Conidia (μm) | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *O. sinocampes* | Homoptera, Coccoidea | The karst-landform forest of Xingyi City, Guizhou Province, China. | Single, clavate, solid, lignified, yellow-brown, arising from the head of the host, 120–150 × 0.5–1.0 | 300-350 × 210-290 | 110-230 × 4-7 | 6-12 × 1.5–2.5 | reaches 13–18 in diameter after two weeks on PDA at 22 °C, appearing round with irregular swellings. fluffy, with a slight yellow protrusion in the middle and dark brown pigment secreted on the back | Monophialidic, hyaline, smooth-walled, growing directly or laterally from hyphae. | Smooth, oval or orange-like shape, often enveloped in a mucous sheath, usually single, rarely aggregated in pairs or triplets (6–8.4 × 2.9–4.3) | In this study |
| | Lepidopteran caterpillars | Kuankuoshui National Nature Reserve | – | – | – | – | villous and yellowish in the middle. The color is convex, the back secretes dark brown pigment, and the diffusion circle is large, 10–15 | The base is cylindrical or conical, 21.6 –38.4 | fusiform or orange-petaled, 6–8.4 × 2.9–4.3 | In this study; Zou et al. 2021a |
| *O. multiperitheciata* | Hepialidae | On Lepidoptera larva in the leaf litter of forest floor. | Several,cylindrical, branched dentritic, 75–110 long, 1–1.5 wide, dark brown to black | superficial, gregarious, distributing unequally on upper of the stromata, ordinal in arrangement, narrowly ovoid, brown to dark brown, 990–1200 × 350–450 | Asci hyaline, cylindrical, 8-spores, 400–600 × 6–7.5 | hyaline, filiform, 470–660 × 1.5–2.5, remaining whole after discharge, multiseptate | Colonies on PDA growing slowly, flat and velvety in the middle, attaining a diameter of 16–22 within 20 d at 20 °C. | monophialidic or polyphialidic,arising from hyphae laterally or terminally, hyaline, cylindrical to lanceolate,tapering gradually or abruptly into a long slender neck | hyaline, 1-celled, smooth walled, oval to lemon shaped, 8–14 × 5–8, embedded in a mucous sheath. | Luangsa-ard et al. 2018 |
| *H. strigosa* | Cicadellidae, Homoptera | – | – | – | – | – | – | swollen in basal portion, 4.5–7.2 × 1.4–2.5, and tapering to 0.4–0.9 wide and 6.3–14.4 overall length | Conidia Cymbiform or orange segments 8.0–12.0 × 3.0–5.0 | Petch 1939 |
| *H. shennongjiaensis* | Earwig, Dermaptera | Shennongjia Forest Area | Synnemata cylindrical, size 60.0 × 1.0–2.0, brown | – | – | – | Colonies diam. up to 24.0–32.0 after 30 d at 16 °C on PDA agar, white or brown, flat, felty, the middle light-brown part with cashmere, reverse orange yellow to light-brown | Conidiogenous cells solitary, phialides cylindrical or awllike, 14.4–26.1 or 6.3–14.4 | Conidia, hyaline,aseptate, smooth, sausage-shaped, single or double from the apex of the neck, 6.3–10.8 × 3.6–6.3 | Zou et al. 2016a |

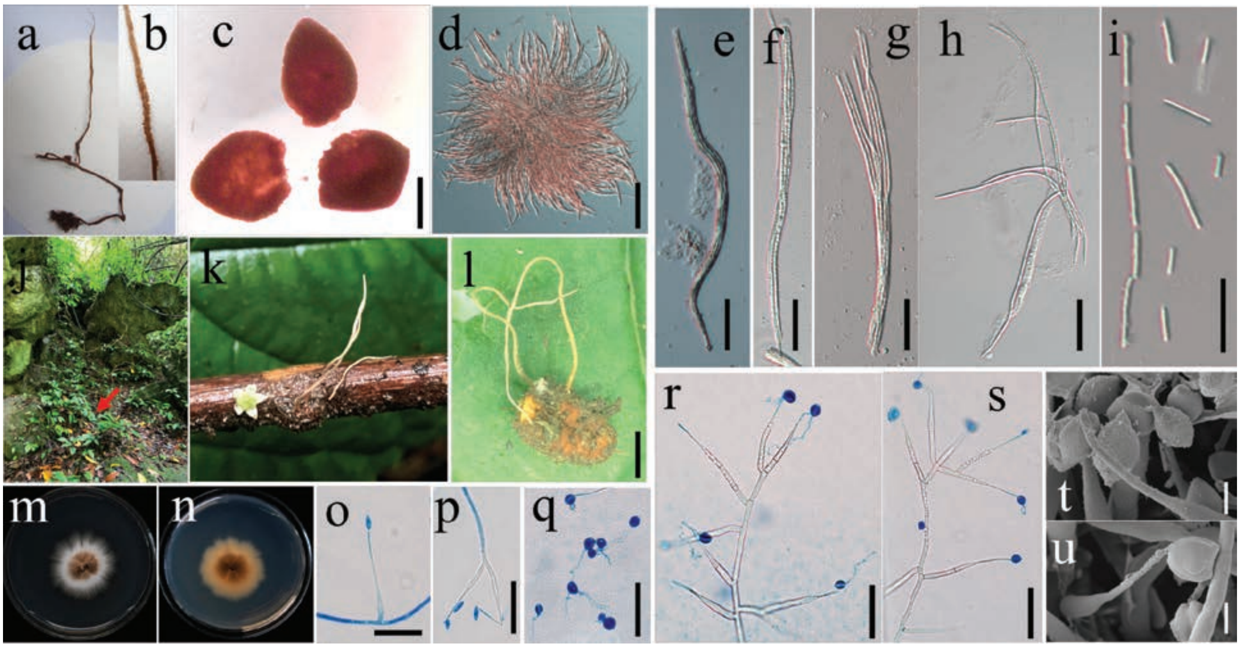

**Figure 2.** Morphological characteristics of *O. sinocampes* **a–i** morphological and micromorphological characteristics of specimen GZUIFR-2010MC **c** perithecia **d–i** ascus and ascospore **j–t** morphological characteristics of specimen GZUIFR-2022MLH-H1 and its pure culture GZUIFR-2022MLH-H1C **j–l** wild morph **m, n** fungus in culture **o–t** phialides and conidia. Scale bars: 150 μm (**c**); 50 μm (**d**); 30 μm (**e–i**); 15 μm (**o–s**); 5 μm (**t, u**).

*O. sinocampes* is closely related to *O. multiperitheciata* Tasan., Thanakitp., Khons. & Luangsa-ard (Luangsa-ard et al. 2018) and *H. strigosa* (Petch 1939). Morphologically, *O. sinocampes* is similar to *H. strigosa* due to the long and base-inflated phialides, but it differs in having tapering phialides of *O. sinocampes*.

***Ophiocordyceps cystidiata*** **X. Zou, Zhong S. Xu & Y.D. Dai, sp. nov.**
MycoBank No: 854598
Fig. 3

**Etymology.** *Cystidiata* refers to the saccate mucous sheath that envelopes the conidium.

**Holotype.** GZUIFR-2023XY-OA5 (Fig. 3b), China • Guizhou Province: Baishi Mountains, Baiwanyao Village, Xingyi City (25°4'12"N, 99°10'12"E; altitude, 1720 m). These specimens were found on a larva of Hepialidae, buried in soil, collected in July 2023 by Zhongshun Xu, Binghui Zhou, Yongdong Dai, Huiling Tian, and Xiao Zou (ex-holotype: GZUIFR-2023XY-OA5C). (The GenBank accession numbers: nrSSU, PQ497594; nrLSU, PQ497634; rpb1, PQ516632; rpb2, PQ516636).

**Host.** The larvae of Hepialidae (Lepidoptera).

**Description.** ***Stromata*:** Arising from the head the host, lignified, solitary, rarely branched, brown to yellow-brown, 60–146 mm long. ***Fertile part*:** Cylindrical, yellowish, without a sterile tip, surface spinous due to protruding ostioles, up to 18 × (0.8-) 1.44 mm.

***Perithecia*:** Immersed, ovoid to oblong-ovate, 355–434 × 178–220 μm. ***Asci*:** Cylindrical, hyaline, eight-spored ascus, 133–224 × 5–7 μm, apex thickened to form an ascus cap, hemispherical, 4.7–5.6 × 3.6–4.0 μm. ***Ascospores*:** Filiform, hyaline, irregular, multi-septate, non-disarticulating, 13.2–25.2 × 1.5–2.6 μm, with septa.

***Asexual morph*:** *Hirsutella*-like

***Colonies*:** On PDA, reaching 13–16 mm in diameter after two weeks at 20 °C, round, irregularly swollen, initially light yellow, gradually changing color with an outer layer of white, an inner layer of rose red, and a central white protrusion. The back of the colony is rose red. ***Hyphae*:** The basal hyphae are hyaline, smooth-walled, and septate; the apical hyphae are verrucose (2.5–3.3 μm wide). ***Conidiogenous cells*:** Growing from verrucose apical hyphae, monophialidic, 22–56 μm long. The base is cylindrical, with an inflated structure near the bottom, tapering gradually into a slender neck. The base width measures 2.4–3.3 μm, and the neck width measures 0.9–1.4 μm. ***Conidia*:** Arising solitarily from the apex of conidiogenous cells, fusiform or orange-like shape, usually solitary, hyaline, smooth-walled, measuring 10–12 × 2.5–3.2 μm, often enveloped in a hyaline mucous sheath (1.5–3.0 μm thick).

**Distribution and habitat.** The karst-landform forest of Xingyi City, Guizhou Province, China.

**Additional specimens examined.** GZUIFR-2023XY-OA2, 3, 6, 7, 8, 9, 10, 11 (Fig. 3f). Location: China. Guizhou Province: Baishi Mountains, Baiwanyao Village, Xingyi City (25°4'12"N, 99°10'12"E; altitude: 1710–1730 m). These specimens were found on a larva of *Hepialidae* sp. buried in soil, collected in July 2023 by Zhongshun Xu, Binghui Zhou, Yong-dong Dai, Huiling Tian, and Xiao Zou.

**Notes.** *O. cystidiata* is closely related to *O. fenggangensis* (Peng et al. 2024), *O. musicaudata* (Peng et al. 2024), *O. alboperitheciata* (Fan et al. 2021), and *H. kuankuoshuiensis* (Qu et al. 2021). Morphologically, *O. cystidiata* is similar to *O. fenggangensis* and *O. musicaudata* in the shape of the stromata, fertile part, and perithecia but it differs by its inconspicuous separate ascospores (Table 3). It also resembles *H. kuankuoshuiensis* in asexual morphology but differs in having phialides with a verrucose apex and conidia enveloped by a thickened mucous sheath.

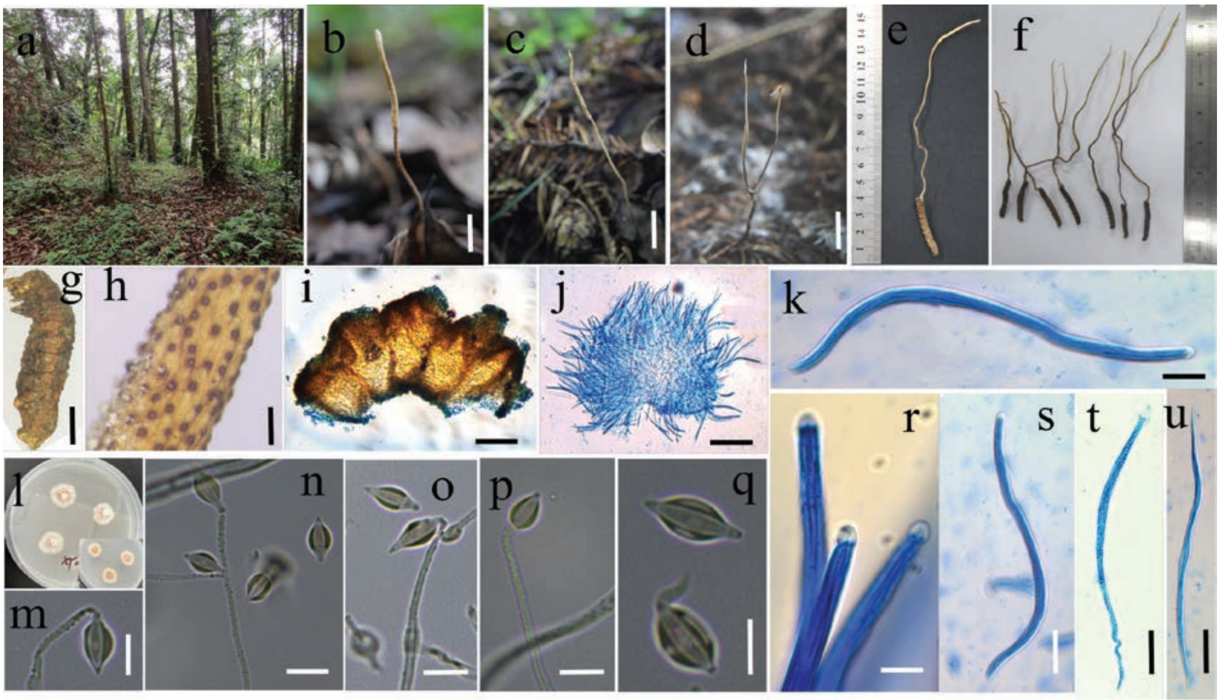

**Figure 3.** The morphological and micromorphological characteristics of *O. cystidiata* **a** wild environment of *O. cystidiata* **b–f** Wild morph **g** host **h, i** superficial perithecia **j, k, r–u** ascus and ascospore **i** colony **m–q** phialide and conidium. Scale bars: 1 cm (**g**); 1 mm (**h**); 100 μm (**i–j**); 20 μm (**k, r–u**); 5 μm (**m–q**).

**Table 3.** Morphological comparison of *O. cystidiata* and its relatives.

| Species | Host | Habitat | Stromata (mm) | Perithecium (μm) | Asci (μm) | Ascospore (μm) | Colony (mm) | Conidiogenous cells (μm) | Conidia (μm) | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *O. cystidiata* | Hepialidae sp | Karst-landform forest | Arising from the head of host, lignified, solitary, rare branched, brown to yellow-brown, 60–146 long | Immersed, ovoid to oblong-ovate, 355–434 × 178–220 | Cylindrical, hyaline, eight-spored ascus, 133–224 × 5–7, apex thickened to form ascus cap, hemispherical, 4.7–5.6 × 3.6–4.0 | Filiform, hyaline, irregular, multi-septate, non-disarticulating, 13.2–25.2 × 1.5–2.6 septa | 13–16 in diameter after two weeks at 20 °C, round, irregularly swollen, initially light yellow, gradually with an outer layer of white, an inner layer of rose red, and a central white protrusion. Rose red of back | Monophialidic, 22–56 long. The base is cylindrical, with a clearly inflated structure near the bottom, tapering gradually into a slender neck. The base 2.4–3.3 wide, and neck 0.9–1.4 wide. | Fusiform or orange-like shape, usually solitary, hyaline, smooth-walled, measuring10–12 × 2.5–3.2, often enveloped in a hyaline mucous sheath (1.5–3.0 thick). | In this study |
| *O. fenggangensis* | Lepidoptera | – | 102 × 1–1.5, solitary, cylindrical, brown to off-white | 306–496 × 134–223, immersed, off-white to yellowish, ovoid to oblong-ovate | 91–176 × 2–8, cylindrical, apex thickened | 0.3–0.7 wide, filiform, hyaline, disarticulating, secondary ascospores 2.8–6.0 × 0.3–0.7, cylindrical | – | – | – | Peng et al. 2024 |
| *O. musicaudata* | Lasiocampidae, Lepidoptera | – | 130–140 × 1–2, solitary or numerous, simple or branched, cylindrical, brown to yellowish | 260–492 × 144–314, immersed, yellowish, flask-shaped | 123–264 × 5–13, filiform, cylindrical, 8-spored, usually without thickened Apices | 114–298 × 1.5–4.0, cylindrical, Irregular multi-septate, non disarticulating | – | – | – | Peng et al. 2024 |
| *O. alboperitheciata* | Noctuidae, Lepidoptera | Buried in fallen leaves | Stromata in pairs, rigid, the stalk is smooth, unbranched, long 54–65, light brown to dark brown, with a clavate fertile part, white to light brown, 4.1–4.5 × 0.8–1.4, and a sterile tip. | Superficial, scattered or crowded, 410–550 230–320, nearly ovoid, white nearly light brown. | Asci hyaline, cylindrical, 8-spores, 144–246 × 3.5–4.7, with a hemispheric apical cap, 3.2–4.2 × 2.3–2.5 | Hyaline, cylindric, multiseptate, 0.5–0.6 diameter, with septa 1.1–1.3 apart | – | – | – | Fan et al. 2021 |
| *O. elongata* | Apalela americana (Lepidoptera) | – | The stalk is flexuose, longitudinally sulcate and twisted, 110 long, pale brown. | The perithecia are immersed, scattered or crowded, ovato conoid, size 50 × 30 mm, apex subacute, wall yellow by transmitted light. | The asci are 220 long, 8 diameter. | Cylindric,2 diameter, with septa 4–12 apart. | – | – | – | Fan et al. 2021 |
| *H. kuankuoshuiensis* | Lepidoptera sp. | – | Synnemata are single, extending from the head of insect; 86 long, dark brown and changing to brown towards the apex; no conidiation was observed | – | – | – | Centre of surface with brown dense bulges and grey-white sparse flocculent aerial hyphae. Colony margin is flat with radial groove; the back of colony appears dark brown; thickness 10–12 | Monophialidic, hyaline, borne perpendicular or at an acute angle to the subtending hyphae | Clavate, narrow fusiform or botuliform without a diaphragm, 9.9–12.6 × 2.7–4.5, single-or double-enveloped in a hyaline mucus, thickness 2.0–3.0 | Qu et al. 2021 |

## Discussion

*Ophiocordyceps sinocampes* was originally discovered on a caterpillar in the Kuankuoshui National Nature Reserve, a non-karst landform area in Guizhou. In this study, we present a new specimen parasitizing *Coccoidea* sp. (Hemiptera), a typical karst landform environment. Despite significant differences in host and ecological habitat, no distinguishing differences were observed in the asexual morph. Furthermore, the specimens were identical in their ITS, nrLSU, and *tef* sequences. The initial discrepancy in the nrLSU sequence (GenBank No. MF623040) was attributed to machine misreading. The only two mutations presented in the *rpb2* gene are synonymous: site 369 (TTG/TTA) [Leu] and site 564 (GTT/GTC) [Val] (Suppl. material 2). Based on the morphological indistinction and the highly consistent sequences across multiple loci, the evidence strongly supports that both specimens belong to the same species-*O. sinocampes*, underscoring the importance of molecular data in species identification.

Moreover, host jumping is also widely discovered in cordycipitiod fungi, such as *Beauveria*, *Metarhizium* and ant-infecting *Ophiocordyceps* (Pu et al. 2013; Lin et al. 2020; Patil et al. 2021). The discovery of the host transboundary of *O. sinocampes* provides further evidence of this phenomenon in *Ophiocordyceps* species. However, considering the differences in hosts, it is necessary to conduct omics analyses of the two strains in the future.

*O. cystidiata* is a recently identified species found in the Xingyi karst landform area. It is characterized by its gray-white to yellow fertile part, verrucose phialides, and conidia enveloped in a cystic mucous sheath. Among *Ophiocordyceps* species with *Hirsutella*-like characteristics and mucous sheaths outside the conidia, notable examples include *H. shennongjiaensis* (Zou et al. 2016a), *H. liboensis* (Zou et al. 2010), *H. nodulosa*, *H. tortricicola* (Zou et al. 2016b), and *H. kuankuoshuiensis* (Qu et al. 2021). However, the mucous sheath of *O. cystidiata* is thicker and darker, giving its entire structure the appearance of a typical lantern.

Karst landform areas represent a distinct geological feature and habitat type, making the investigation of cordycipitoid fungus biodiversity in these regions highly significant. Zhu et al. (2004) summarized 45 cordycipitoid species found in the karst areas of Guizhou, Yunnan, and Guangxi, highlighting the richness of resources in these landscapes. Chen et al. (2022) conducted a study in the Monkey-Ear Tiankeng karst region (Kaiyang, Guizhou Province), reporting 15 species, including 8 new *Cordyceps*-like fungi, which further illustrated the abundance of cordycipitoid fungi in the Karst Tiankeng. Our present study surveyed cordycipitoid resources in the typical karst landform areas of Xingyi, resulting in the identification of two new species. Karst ecosystems are more fragile than other ecosystems, with low stability and high vulnerability to disturbance. Consequently, conservation is crucial to safeguard the biodiversity and ecological functions of karst environments.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Specimen collection and fungal isolation: Zhong-Shun Xu, Hui-Ling Tian, Jiao-Jiao Qu, Yong-Dong Dai and Xiao Zou; molecular biology analysis and phylogenetic analysis: Zhong-Shun Xu, Li-Ping Deng, Hui-Ling Tian and Yong-Dong Dai; microscopy: Zhong-Shun Xu, Li-Ping Deng; original draft preparation: Zhong-Shun Xu and Yong-Dong Dai; review and editing: Zhong-Shun Xu, Hai-Yan Wang and Yong-Dong Dai, Xiao Zou; Funding: Yong-Dong Dai and Xiao Zou. All authors reviewed and approved the final manuscript.

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### Data availability

All sequences were submitted to GenBank and obtained the accession number. The alignment dataset of multigene, and the phylogenetic tree files were submitted to figshare. https://doi.org/10.6084/m9.figshare.28043432.v2.

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## Supplementary material 1

### Primer pairs for gene amplification and sequencing used in this study

Authors: Zhong-Shun Xu, Li-ping Deng, Hai-Yan Wang, Hui-Ling Tian, Jiao-Jiao Qu, Yong-dong Dai, Xiao Zou

Data type: xlsx

Link: https://doi.org/10.3897/mycokeys.114.134323.suppl1

## Supplementary material 2

### Identification and recognition of mutation in two strains of *Ophiocordyceps sinocampes*

Authors: Zhong-Shun Xu, Li-ping Deng, Hai-Yan Wang, Hui-Ling Tian, Jiao-Jiao Qu, Yong-dong Dai, Xiao Zou

Data type: png

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Research Article

# Four new species of *Erioscyphella* (Leotiomycetes, Helotiales) from southwestern China

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## Abstract

*Erioscyphella* is found across various regions and is part of the family Lachnaceae (Helotiales). It is distinguished by its white to orange disc-shaped apothecia, white to brown receptacles, and granulated hairs that contain amorphous or resinous material. These hairs lack swelling apices and crystals. Additionally, this genus is unique for its long ascospores. In the present study, we collected eight specimens from southwestern China. Morphological and phylogenetic analyses based on the combined LSU, ITS, mtSSU and *RPB2* dataset showed that our specimens represent four new species of *Erioscyphella*, including *E. ailaoensis*, *E. baimana*, *E. gelangheica* and *E. tengyueica*. Here, we provide complete morphological descriptions with illustrations and sequence data essential for future taxonomic and evolutionary research.

**Key words:** 4 novel species, Lachnaceae, morphology, phylogeny, taxonomy

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## Introduction

The monophyletic genus *Erioscyphella* belongs to the family Lachnaceae (Dennis 1954; Spooner 1987; Cantrell and Haines 1997; Guatimosim et al. 2016; Tochihara and Hosoya 2022; Wijayawardene et al. 2020, 2022; Hyde et al. 2024) and includes 22 records (Index Fungorum 2024). The original description of *Erioscyphella* by Kirschstein (1939), which lacked typification, was inaccurately characterized based on traits that lack taxonomic significance, such as filiform, colored, and pigmented ascospores, as well as lanceolate paraphyses (Korf 1978; Perić and Baral 2014). It was not until Haines (1984) selected *E. longispora*, under which *Peziza abnormis* was later synonymized, as the lectotype of *Erioscyphella* that a more accurate characterization was established. These features are now recognized as insufficient for distinguishing this genus from related taxa. This genus was initially confused with the closely related genus *Lachnum*, but detailed morphological and molecular phylogenetic studies based on LSU, ITS, mtSSU and

*RPB2* data have since clarified their distinct characteristics and proposed a new concept based on the examination of Japanese materials (Tochihara and Hosoya 2022). *Erioscyphella* is characterized by scattered and cupulate apothecia on leaves or bamboo sheaths, straight or irregularly curved, septate and granulated hairs, mostly covered by apical amorphous materials or resinous material, lanceolate or filiform paraphyses, 8-spored asci with an amyloid apical pore and fusiform to long needle-like ascospores (Kirschstein 1939; Perić and Baral 2014; Tochihara and Hosoya 2022). There are no reports of asexual morph in this genus.

The species of *Erioscyphella* are primarily distributed in temperate and tropical regions, commonly inhabiting decaying wood and plant debris. *Erioscyphella* species are distributed mainly in China and a few other countries; *E. curvispora* is described from Montenegro (Perić and Baral 2014), *E. griseibambusicola*, *E. latispora*, *E. lunata*, *E. lushanensis* and *E. subinsulae* are collected from China (Guatimosim et al. 2016; Tello and Baral 2016; Li et al. 2022; Su et al. 2023); *E. euterpes* collected from Puerto Rico (Guatimosim et al. 2016). In contrast to the above-mentioned species that show a narrower distribution, *E. abnormis* shows a worldwide distribution, especially endemic in tropical regions (Tello and Baral 2016). They play a crucial role in breaking down organic matter, thereby aiding in nutrient cycling within their ecosystems. The ecological role of *Erioscyphella* as decomposers underscores their importance in forest ecosystems (Tochihara and Hosoya 2022; Niego et al. 2023a, 2023b). Ongoing research aims to better define species boundaries and elucidate the phylogenetic relationships within the genus through morphological studies and DNA sequencing. This work aids in the precise classification and discovery of new species.

During the investigation of Leotiomycetes in southwest China (Li et al. 2022, 2024a, 2024b; Su et al. 2022, 2023; Luo et al. 2024; Thiyagaraja et al. 2024), eight collections of *Erioscyphella* were obtained. We used morphological and phylogenetic analyses based on LSU, ITS, mtSSU and *RPB2* data to confirm that these eight collections differ from all known species of *Erioscyphella*. We introduce four species to accommodate these collections. Here, we provide complete morphologies, illustrations, and their phylogenetic relationships for future taxonomic and evolutionary studies.

## Material and methods

### Specimen collection and morphological examination

We collected eight specimens from southwest China. All samples were collected from highly humid, natural broadleaf forests and protected areas with minimal human access. Altitudes were determined by the GPS device. The fruiting bodies were discovered on the surface of extremely wet, decaying wood litter. The samples were dehydrated in a dehydrator at a temperature range of 25–30 °C. After studying the morphology of the specimens and getting their genomic DNA, they were deposited at the Cryptogamic Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Facesoffungi and Index Fungorum numbers were obtained as in Jayasiri et al. (2015) and Index Fungorum (2024), respectively. All the species identifications followed Chethana et al. (2021). The morphological descriptions were submitted to the Greater Mekong Subregion database (Chaiwan et al. 2021). The dried specimens were examined with a stereomicroscope (C-PSN, Nikon, Japan) and were captured with a digital camera (Canon EOS 70D, Japan) connected to the stereomicroscope. Free-hand sections of the dried specimens were mounted in a drop of water for observing microscopic characteristics, such as apothecia, exciple, paraphyses, asci and ascospores, using a Nikon compound microscope (Nikon, Japan) equipped with a DS-Ri2 camera. In addition, the sections were pretreated with Melzer's reagent for the Iodine test (MLZ) (Tochihara and Hosoya 2022). Microstructures were measured using the Tarosoft (R) Image Frame Work program v.0.97 (Tarosoft, Thailand). The obtained measurements were presented in the format of (a–) b–c(–d), where 'a' represented the minimum value, 'd' represented the maximum value, and the range 'b–c' reflected the 90% confidence interval. The $\bar{x}$ indicated the average value of measurements. Ascospore measurements were given as [n/m/p], indicating that the n number of ascospores were measured from m ascomata of the p number of collections. Images used for figures were processed with Adobe Photoshop CS6 Extended version 13.0 × 64 (Adobe Systems, USA).

## DNA extraction, PCR amplifications and sequencing

Genomic DNA was extracted from the dried apothecia (around 50–100 mg) using a TSP101 DNA extraction kit (TSINGKE, China). Following the latest studies (Tochihara and Hosoya 2022; Su et al. 2023; Luo et al. 2024), LSU, ITS, mtSSU and *RPB2* were used for PCR amplification, using the primers LR0R/LR5 (Vilgalys and Hester 1990), ITS1F/ITS4 (White et al. 1990; Gardes and Bruns 1993), mrSSU1/mrSSU3R (Zoller et al. 1999) and f*RPB2*-5F/f*RPB2*-7cR (Liu et al. 1999), respectively. For LSU, ITS, mtSSU and *RPB2*, the total volume of PCR amplifications was 25 µL, comprising 12.5 µL 2 × PCR G013 Taq MasterMix with Dye (Applied Biological Materials, Canada), 1 µL of each primer (10 µM), 2 µL genomic DNA, and 8.5 µL of sterilized, distilled water. Amplifications of LSU, ITS and *RPB*2 were conducted under the following conditions: pre-denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 20 sec, annealing at 56 °C (LSU)/53 °C (ITS and *RPB*2) for 10 sec, elongation at 72 °C for 20 sec, and final elongation at 72 °C for 7 min. For mtSSU, the total volume of PCR amplifications was 25 µL, which comprised 21 µL 1 × PCR TSE101 Mix (TSINGKE, China), 1 µL of each primer (10 µM), and 2 µL genomic DNA. Amplifications of mtSSU were conducted under the following conditions: initial denaturation at 98 °C for 3 min, followed by 40 cycles of denaturation at 98 °C for 1 min, annealing at 52 °C for 1 min, elongation at 72 °C for 1 min, and final elongation at 72 °C for 10 min. Gel electrophoresis with 1% TAE and TSJ003 GoldView nucleic acid dye (TSINGKE, China) was used to confirm the obtained PCR products. Finally, the PCR products were sequenced at the Tsingke Biotechnology Co., Ltd., Kunming, China. Newly produced sequences were deposited in the GenBank and the accession numbers were given in Table 1.

**Table 1.** Taxa included in the phylogenetic analyses and the GenBank accession numbers of LSU, ITS, mtSSU and *RPB2* sequences.

| Species | Strain | Gene accession No. | | | | References |
|---|---|---|---|---|---|---|
| | | ITS | LSU | mtSSU | *RPB2* | |
| *Capitotricha bicolor* | TNS-F-65670 | LC424834 | LC424942 | LC533244 | LC425011 | Tochihara and Hosoya (2022) |
| *Capitotricha rubi* | TNS-F-65752 | LC438560 | LC438573 | LC533243 | LC440395 | Tochihara and Hosoya (2022) |
| *Erioscyphella abnormis* | TNS-F-16609 | AB705234 | LC533175 | LC533256 | LC533184 | Tochihara and Hosoya (2022) |
| *Erioscyphella abnormis* | TNSF38452 | LC669457 | LC533171 | LC533262 | LC533210 | Tochihara and Hosoya (2022) |
| *Erioscyphella abnormis* | TNS-F-80478 | LC424837 | LC424949 | LC533283 | - | Tochihara and Hosoya (2019) |
| *Erioscyphella abnormis* | TNS-F-46841 | LC669474 | LC533170 | LC533279 | LC533209 | Tochihara and Hosoya (2022) |
| ***Erioscyphella ailaoensis*** | **HKAS135686**$^{(T)}$ | **PQ349783** | **PQ349775** | **PQ358800** | **PQ424108** | This study |
| ***Erioscyphella ailaoensis*** | **HKAS135687** | **PQ349784** | **PQ349776** | **PQ358801** | **PQ424109** | This study |
| *Erioscyphella alba* | MFLU16-0614$^{(T)}$ | MK584965 | MK591990 | - | - | Ekanayaka et al. (2019) |
| *Erioscyphella aseptata* | MFLU16-0590$^{(T)}$ | MK584957 | MK591986 | - | MK388223 | Ekanayaka et al. (2019) |
| ***Erioscyphella baimana*** | **HKAS135697**$^{(T)}$ | **PQ349785** | **PQ349777** | **PQ358802** | **PQ424110** | This study |
| ***Erioscyphella baimana*** | **HKAS135696** | **PQ349786** | **PQ349778** | **PQ358803** | **PQ424111** | This study |
| *Erioscyphella boninensis* | TNS-F-26520$^{(T)}$ | NR185389 | LC533151 | LC533254 | LC533196 | Tochihara and Hosoya (2022) |
| *Erioscyphella brasiliensis* | MFLU16-0577b | MK584967 | MK591993 | - | - | Ekanayaka et al. (2019) |
| *Erioscyphella brasiliensis* | TNS-F-46419 | LC669456 | LC533133 | LC533278 | LC549672 | Tochihara and Hosoya (2022) |
| *Erioscyphella curvispora* | KL 381$^{(T)}$ | MH190414 | MH190415 | - | - | Perić and Baral (2014) |
| *Erioscyphella euterpes* | PR 147 | U58640 | - | - | - | Cantrell and Haines (1997) |
| *Erioscyphella fusiforme* | MFLU15-0230$^{(T)}$ | MK584948 | MK591975 | - | MK614728 | Ekanayaka et al. (2019) |
| ***Erioscyphella gelangheica*** | **HKAS135689**$^{(T)}$ | **PQ349787** | **PQ349779** | **PQ358804** | **-** | This study |
| ***Erioscyphella gelangheica*** | **HKAS135695** | **PQ349788** | **PQ349780** | **PQ358805** | **-** | This study |
| *Erioscyphella griseibambusicola* | HKAS124657 | OP451797 | OP451791 | OP451844 | OP432252 | Su et al. (2023) |
| *Erioscyphella griseibambusicola* | HKAS124656$^{(T)}$ | OP451796 | OP451790 | OP451843 | OP432251 | Su et al. (2023) |
| *Erioscyphella hainanensis* | TNS-F-35056 | LC669465 | LC533169 | LC533275 | LC533206 | Tochihara and Hosoya (2022) |
| *Erioscyphella hainanensis* | TNS-F-35049 | LC669452 | LC533168 | LC533274 | LC533205 | Tochihara and Hosoya (2022) |
| *Erioscyphella insulae* | TNS-F-26500 | LC669448 | LC533149 | LC533252 | LC533194 | Tochihara and Hosoya (2022) |
| *Erioscyphella insulae* | TNS-F-39720$^{(T)}$ | LC669451 | LC533177 | LC533261 | LC533207 | Tochihara and Hosoya (2022) |
| *Erioscyphella latispora* | HKAS124391 | OP113849 | OP113850 | - | OP715727 | Li et al. (2022) |
| *Erioscyphella latispora* | HKAS124389$^{(T)}$ | OP310823 | OP113844 | - | OP715728 | Li et al. (2022) |
| *Erioscyphella lunata* | JA-CUSSTA 8292 | KX501132 | KX501133 | - | - | Tello and Baral (2016) |
| *Erioscyphella lushanensis* | HMAS81575 | JF937582 | - | - | - | Zhao and Zhuang (2011) |
| *Erioscyphella otanii* | TNS-F-81472$^{(T)}$ | NR185393 | LC533179 | LC533286 | LC533226 | Tochihara and Hosoya (2022) |
| *Erioscyphella papillaris* | TNS-F-81272$^{(T)}$ | NR185391 | LC533161 | LC533285 | LC533204 | Tochihara and Hosoya (2022) |
| *Erioscyphella paralushanensis* | TNS-F-61920$^{(T)}$ | NR185390 | LC533141 | LC533267 | LC533220 | Tochihara and Hosoya (2022) |
| *Erioscyphella sasibrevispora* | TNS-F-80399 | LC669470 | LC533173 | LC533268 | LC533216 | Tochihara and Hosoya (2022) |
| *Erioscyphella sasibrevispora* | TNS-F-81401$^{(T)}$ | LC669472 | LC533174 | LC533269 | LC533217 | Tochihara and Hosoya (2022) |
| *Erioscyphella sclerotii* | TNS-F-26492 | LC669438 | LC533152 | LC533255 | LC533197 | Tochihara and Hosoya (2022) |
| *Erioscyphella sclerotii* | TNS-F-38480 | LC669458 | LC533134 | LC533263 | LC549673 | Tochihara and Hosoya (2022) |
| *Erioscyphella sclerotii* | MFLU 16-0569 | MK584951 | MK591980 | - | - | Ekanayaka et al. (2019) |
| *Erioscyphella sclerotii* | MFLU 18-0688 | MK584969 | MK591995 | - | - | Ekanayaka et al. (2019) |
| *Erioscyphella sinensis* | TNS-F-32161 | LC669449 | LC533167 | LC533273 | LC533219 | Tochihara and Hosoya (2022) |
| *Erioscyphella sinensis* | TNS-F-16838 | AB481280 | LC533164 | LC533235 | AB481364 | Tochihara and Hosoya (2022) |
| *Erioscyphella subinsulae* | HKAS 124659 | OP451799 | OP451793 | OP451846 | OP432254 | Su et al. (2023) |
| *Erioscyphella subinsulae* | HKAS 124660 | OP451800 | OP451794 | OP451847 | OP432255 | Su et al. (2023) |
| *Erioscyphella subinsulae* | HKAS 124661 | OP451801 | OP451795 | OP451848 | OP432256 | Su et al. (2023) |
| *Erioscyphella subinsulae* | HKAS 124658$^{(T)}$ | OP451798 | OP451792 | OP451845 | OP432253 | Su et al. (2023) |
| ***Erioscyphella tengyueica*** | **HKAS135688**$^{(T)}$ | **PQ349789** | **PQ349781** | **PQ358806** | **-** | This study |
| ***Erioscyphella tengyueica*** | **HKAS135693** | **PQ349790** | **PQ349782** | **PQ358807** | **-** | This study |
| *Lachnellula calyciformis* | TNS-F-81248 | LC438561 | LC438574 | LC533247 | LC438590 | Tochihara and Hosoya (2022) |
| *Lachnellula suecica* | TNS-F-16529 | AB481248 | LC424944 | LC533231 | AB481341 | Tochihara and Hosoya (2022) |
| *Neodasyscypha cerina* | TNS-F-65625 | LC424836 | LC424948 | LC533242 | LC425013 | Tochihara and Hosoya (2022) |

*Names in indicate the specimens from the current study. Names with $^{(T)}$ indicate type specimens and - denotes unavailable data in the GenBank.

### Phylogenetic analyses

New DNA sequences generated from forward and reverse primers were assembled using BioEdit v.7.2.5 (Hall 1999) to obtain consensus sequences. The concatenated sequences were used to search for the closer relatives in the NCBI (Johnson et al. 2008). According to the close relatives and recent studies, the newly generated sequences and some published sequences were used for the phylogenetic analyses (Table 1). *Capitotricha bicolor* (TNS-F-65670), *C. rubi* (TNS-F-65752), *Lachnellula calyciformis* (TNS-F-81248), *L. suecica* (TNS-F-16529) and *Neodasyscypha cerina* (TNS-F-65625) were used as the outgroup taxa. The phylogenetic analysis was conducted based on the datasets including reference DNA sequences and newly generated DNA sequences using OFPT (Zeng et al. 2023) with the following protocol. Datasets of each gene region were first independently aligned with 'auto' strategy (based on data size) by MAFFT (Katoh and Standley 2013) and trimmed with 'gappyout' method (based on gaps' distribution) by TrimAl (Capella-Gutiérrez et al. 2009). The best-fit nucleotide substitution model for each dataset was then selected based on the Bayesian information criterion (BIC) from twenty-two common DNA substitution models with rate heterogeneity by ModelFinder (Kalyaanamoorthy et al. 2017). Afterwards, all datasets were concatenated with partition information for the subsequent phylogenetic analyses. Maximum likelihood with 1000 replicates was performed using ultrafast bootstrap approximation (Hoang et al. 2018) with SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010) in IQ-TREE (Nguyen et al. 2015). The consensus tree was summarized based on the extended majority rule. Bayesian inference (BI) analyses were run in the CIPRES Science Gateway v.3.3 (Miller et al. 2010). The best-fit nucleotide substitution models were determined using jModelTest2 on XSEDE (2.1.6). The BI was performed in MrBayes on XSEDE v. 3.2.7a (Ronquist et al. 2012), with four simultaneous Markov chain Monte Carlo (MCMC) chains and four runs for 3,000,000 generations, with trees sampled at each 300th generation. The first 25% of trees were discarded as burn-in, and BI posterior probabilities (PP) were conducted from the remaining trees. The consensus phylograms were visualized on FigTree v. 1.4.4, and edited with Adobe Illustrator CC 2019, Adobe Systems (USA). Decisions as to whether species are new followed the polyphasic approach as recommended by Chethana et al. (2021) and Maharachchikumbura et al. (2021).

## Results

### Phylogenetic analysis

The phylogenetic analyses were based on 45 *Erioscyphella* taxa, including *C. rubi* (TNS-F-65752), *C. bicolor* (TNS-F-65670), *L. calyciformis* (TNS-F-81248), *L. suecica* (TNS-F-16529) and *N. cerina* (TNS-F- 65625) as the outgroup taxa. The alignment comprised 4 partitions and 3944 total sites (ITS: 758 bp; LSU: 1105 bp; mtSSU: 947 bp; *RPB*2: 1134 bp), with 14.359% gaps and completely undetermined characters. The ML tree has the same topology as the BI tree. The best ML tree with a final optimization likelihood of -25316.078809 is displayed in Fig. 1. In the BI analyses, the final average standard deviation of split

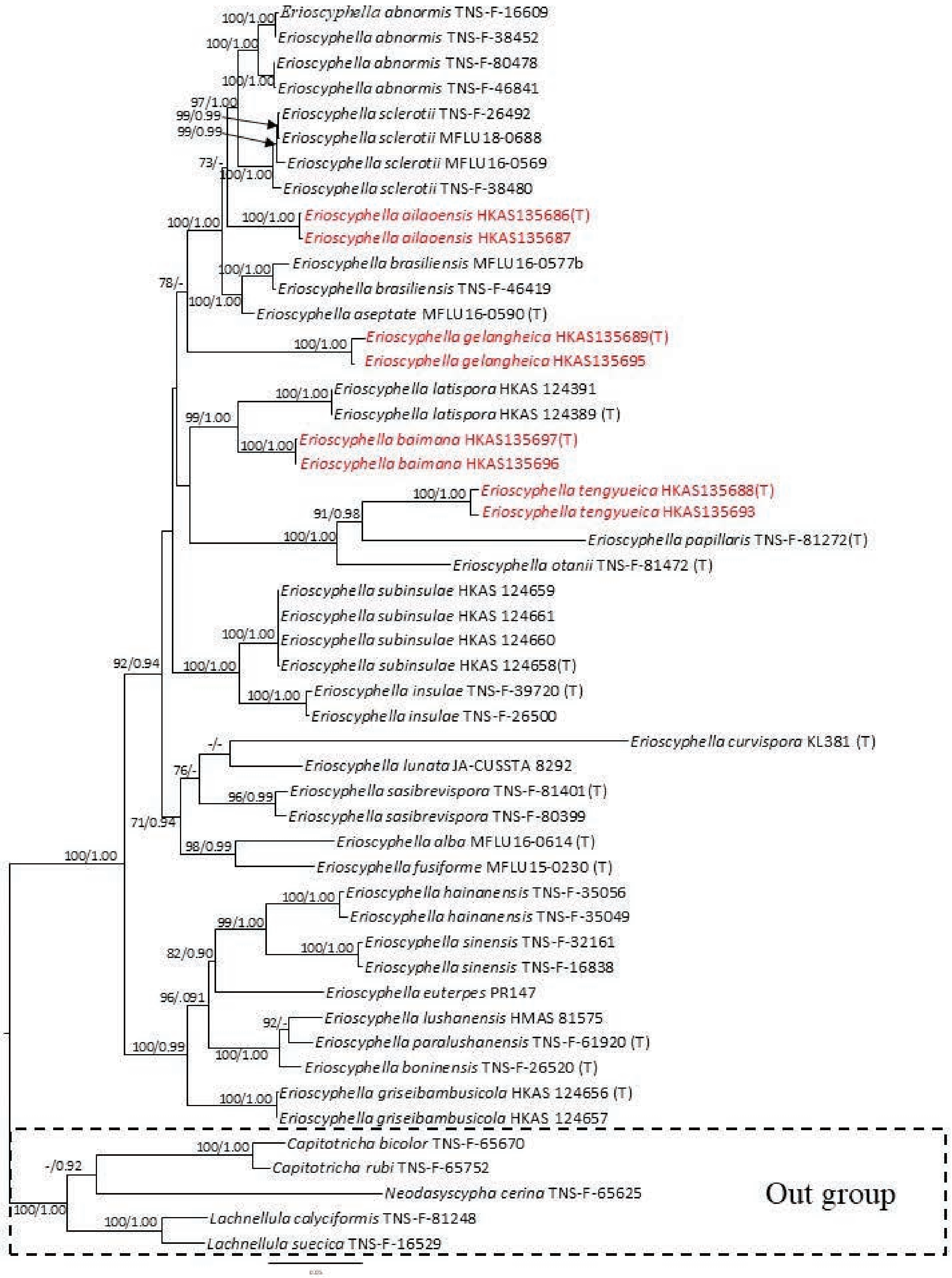

**Figure 1.** The Maximum Likelihood tree based on the combined LSU, ITS, mtSSU and *RPB2* sequence data for *Erioscyphella*. *Capitotricha bicolor* (TNS-F-65670), *C. rubi* (TNS-F-65752), *Lachnellula calyciformis* (TNS-F-81248), *L. suecica* (TNS-F-16529) and *Neodasyscypha cerina* (TNS-F-65625) are used as the outgroup taxa. The MLBP ≥ 70% and BPP ≥ 0.90 are shown at the nodes as MLBP/BPP. MLBS < 70% and BPP < 0.90 are expressed as a hyphen ("-"). Names with (T) indicate type specimens. Names in red indicate new species.

frequencies was 0.007666, which revealed convergence. In the multi-gene phylogenetic tree based on the combined ITS, LSU, mtSSU and *RPB*2 dataset, all taxa of *Erioscyphella* clustered together. *Erioscyphella ailaoensis* was sister to the clade comprising *E. sclerotii* and *E. abnormis*, with 73% maximum likelihood bootstrap (MLBP) and 0.89 Bayesian posterior probabilities (BPP) support. *Erioscyphella gelangheica* was sister to the clade comprising *E. sclerotii* and *E.abnormis*, *E. ailaoensis*, *E. brasiliensis* and *E. aseptata* with 78% MLBP and 0.88 BPP support. *Erioscyphella baimana* was sister to *E. latispora* with 99% MLBP and 1.00 BPP support. Besides, *E. tengyueica* was sister to *E. papillaris* with 91% MLBP and 0.98 BPP. The phylogenetic result showed that *Erioscyphella* species clustered together, similar to previously published studies (Su et al. 2023).

## Taxonomy

***Erioscyphella ailaoensis* L. Luo, K.D. Hyde & H.L. Su, sp. nov.**
Index Fungorum: IF902529
Facesoffungi Number: FoF16389
Fig. 2

**Etymology.** The epithet "*ailaoensis*" refers to the collection site, Ailao Mountain, where the holotype specimen was collected.

**Holotype.** HKAS135686.

**Description.** ***Saprobic*** on the dead bark. ***Sexual morph*: *Apothecia*** scattered to partly gregarious, superficial, 1–2.4 mm in diameter, 0.4–1.4 mm high when dry, discoid to cupulate, shortly stipitate, externally covered with short, white to brown hairs. ***Disc*** concave, surface slightly rough, yellow to brown. Margin flat to slightly involute, pale yellow, covered with yellow to pale brown hairs. ***Receptacle*** discoid to cupulate, yellow to pale brown, clothed entirely with short, yellow to pale brown hairs. ***Stipe*** 0.2–1 mm in diameter, 0.2–0.6 mm long when dry, cylindrical, solitary, yellow to pale brown, clothed with yellow to pale brown hairs. ***Hairs*** 22–92 × 3.0–4.3 µm ($\bar{x}$ = 51 × 3.7 µm, n = 30), clavate to cylindrical, straight to slightly curved, septate, hyaline, thick-walled, covered with hyaline granules, obtuse apex, apical amorphous or resinous material. ***Hymenium*** 120–230 µm ($\bar{x}$ = 153 µm, n = 12), concave, surface slightly rough, light yellowish brown in dry. ***Medullary excipulum*** 40–90 µm ($\bar{x}$ = 58 µm, n = 20), thin, hyaline to light yellow, thin-walled, smooth cells of ***textura oblita***, 2.1–5.9 µm ($\bar{x}$ = 3.7 µm, n = 50) in diameter. ***Ectal excipulum*** 55–80 µm ($\bar{x}$ = 65 µm, n = 20), thin-walled, smooth, light yellowish cells of ***textura prismatica*** to ***globulosa***, 2–4.9 µm ($\bar{x}$ = 3.0 µm, n = 60) in diameter. ***Paraphyses*** 100–138 × 1.6–3.9 µm ($\bar{x}$ = 117 × 2.8 µm, n = 25), longer than asci, filiform, straight to slightly curved, aseptate, hyaline, thin-walled, rough, with slightly acute apex. ***Asci*** 85–143 × (4.5–) 5.5–9.0(–9.5) µm ($\bar{x}$ = 100 × 7.3 µm, n = 34), 8-spored, unitunicate, overlapping fascicles, clavate, straight to slightly curved, inoperculate, hyaline, apically thickened wall, laterally relatively thin, slightly smooth, with an apical, non-amyloid pore and tapered ends, J- in MLZ. ***Ascospores*** (50/14/2) (43.0–)45.5–97(–101.0) × 1.4–2.4(–2.6) µm, ($\bar{x}$ = 68 × 1.9 µm), fascicled, filiform, multi-septate, thin-walled, hyaline, rough with taper, obtuse ends, without oil guttules, hyaline, slightly smooth. ***Asexual morph*:** Not observed.

**Figure 2.** *Erioscyphella ailaoensis* (HKAS135686, holotype) **a–c** dried ascomata on the bark **d** vertical section of an ascoma **e** excipulum **f** hairs **g** paraphyses **h–k** asci (**j, k** asci in MLZ) **l–o** ascospores (**l–n** ascospores in MLZ). Scale bars: 100 μm (**d**); 50 μm (**e–k**); 30 μm (**l–o**).

**Material examined.** China • Yunnan Province, Puer City, Jingdong County, Ailao Mountain, altitude 2478 m, on the decayed unidentified bark, 8 June 2022, Hongli Su, SU872 (HKAS135686, ***holotype***); China • Xizang Province, Shigatse City, altitude 1774 m, on the decayed unidentified twig, 6 July 2022, Hongli Su, SU1423 (HKAS135687, ***paratype***).

**Notes.** Our specimens, HKAS135686 and HKAS135687, were grouped as a distinct clade, separated from the clade comprising *E. abnormis* and *E. sclerotii* by 73% MLBS and 0.89 BIPP (Fig. 1). The new species exhibited morphological differences from *E. abnormis* and *E. sclerotii* by having J- apical pores, whereas the latter species have apical pores that are J- in MLZ. In contrast to the septate paraphyses of *E. abnormis*, *E. ailaoensis* has aseptate paraphyses (Han et al. 2021). Furthermore, asci and ascospores of *E. ailaoensis* are longer than those of *E. sclerotii* (Nagao 1996; Perić and Baral 2014). Therefore, *E. ailaoensis* is introduced here as a new species.

### ***Erioscyphella baimana*** **L. Luo, K.D. Hyde & Q. Zhao, sp. nov.**

Index Fungorum: IF902530
Facesoffungi Number: FoF16391
Fig. 3

**Etymology.** The epithet "*baimana*" refers to the collection site, Baima Mountain, where the holotype specimen was collected.

**Holotype.** HKAS 135697.

**Description.** ***Saprobic*** on dead twigs. ***Sexual morph*****:** ***Apothecia*** superficial, gregarious, 0.3–1.1 mm in diameter, 0.3–1.4 mm high when dry, discoid to cupulate, long stipitate, externally covered with short, white hairs. ***Disc*** concave, surface slightly smooth, yellow. ***Margin*** flat to slightly involute, white, covered with white hairs. ***Receptacle*** cupulate to discoid, white, covered entirely with short, white hairs. ***Stipe*** 0.2–0.6 mm in diameter, 0.3–1.1 mm long when dry, cylindrical, solitary, white, clothed with white hairs. ***Hairs*** 30–120 × 2.8–4.7 µm ($\bar{x}$ = 74 × 3.7 µm, n = 30), clavate to cylindrical, straight to slightly curved, aseptate, hyaline, thin-walled, covered with fine granules, obtuse apex, lacks apical amorphous. ***Hymenium*** 165–230 µm ($\bar{x}$ = 195 µm, n = 12), concave, surface slightly smooth, yellow in dry. ***Medullary excipulum*** 35–120 µm ($\bar{x}$ = 70 µm, n = 18), thin, hyaline, thin-walled cells of ***textura porrecta***, 1.3–3.8 µm ($\bar{x}$ = 2.5 µm, n = 50) in diameter. ***Ectal excipulum*** 40–120 µm ($\bar{x}$ = 68 µm, n = 18) thin, thin-walled, smooth, light yellowish cells of ***textura porrecta*** to ***oblita***, 1.9–6.3 µm ($\bar{x}$ = 4.1 µm, n = 60) in diameter. ***Paraphyses*** 95–170 × 1.2–2.5 µm ($\bar{x}$ = 140 × 1.6 µm, n = 25), longer than asci, filiform, straight to slightly curved, aseptate, hyaline, light smooth, with slightly obtuse apex. ***Asci*** 100–152 × 3.6–9.5 µm ($\bar{x}$ = 123 × 7.2 µm, n = 34), 8-spored, unitunicate, clavate, straight to slightly curved, inoperculate, hyaline, slightly smooth, with an apical, amyloid pore and rounded ends, J+ in MLZ, tapered long stipitate base. ***Ascospores*** (85/7/2) (29.5–)30.5–37.5(–40.0) × 2.2–5.0 (–5.5) µm, ($\bar{x}$ = 32.9 × 4.1 µm, n = 85), biseriate, fusoid-clavate with blunt ends, fusiform, 1–3-septate, thin-walled, hyaline, slightly smooth, tapering towards the obtuse ends, with longitudinal striations, without oil guttules. ***Asexual morph*****:** Not observed.

**Material examined.** China • Yunnan Province, Diqing City, Deqin County, Baima Mountain, altitude 3485 m, on the decayed unidentified twig, 21 July 2022, Le Luo, Ly7 (HKAS 135697, ***holotype***); • *ibid*., Le Luo, Ly26 (HKAS 135696, ***isotype***).

**Notes.** Our specimens, HKAS 135697 and HKAS 135696, were grouped in a distinct clade, separated from *E. latispora* by 99% MLBS and 1.00 BIPP (Fig. 1). Our species, *E. baimana*, morphologically differs from *E. latispora* by having aseptate hairs, ascospores without oil guttules whereas the latter species possess septate hairs, asci with rounded to subconical apex, and ascospores with four or more large guttules. Therefore, *E. baimana* is introduced here as a new species.

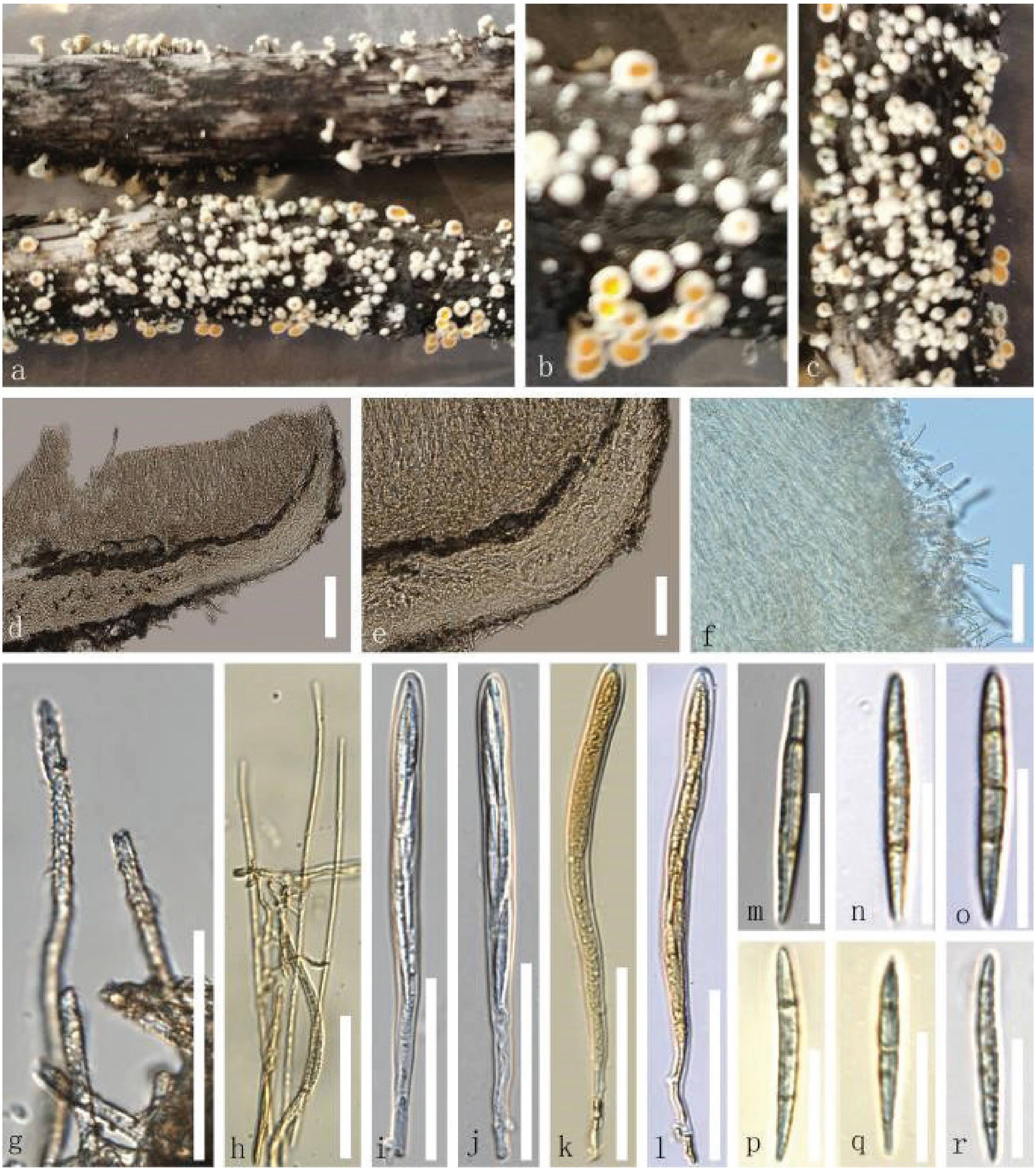

**Figure 3.** *Erioscyphella baimana* (HKAS 135697, holotype) **a–c** dried ascomata on the twig **d** a vertical section of part of an ascoma **e** excipulum **f, g** hairs **h** paraphyses **i–l** asci (**k, l** asci in MLZ) **m–r** ascospores. Scale bars: 100 μm (**d–f**); 50 μm (**g–l**); 30 μm (**m–r**).

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***Erioscyphella gelangheica*** **L. Luo, K.D. Hyde, H.L. Su & C.J.Y. Li, sp. nov.**
Index Fungorum: IF902529
Facesoffungi Number: FoF16390
Fig. 4

**Etymology.** The epithet "*gelangheica*" refers to the collection site Gelanghe township where the holotype specimen was collected.

**Holotype.** HKAS135689.

**Description.** ***Saprobic*** on dead bark. ***Sexual morph*****:** ***Apothecia*** scattered to partly gregarious, superficial, 0.24–0.5 mm in diameter, 0.35–0.5 mm high when dry, discoid to cupulate, long stipitate, externally covered with short, white to yellowish hairs. ***Discs*** concave, surface slightly rough, white to yellow. ***Margin*** flat to slightly involute, white to pale yellow, covered with white to pale yellow hairs. ***Receptacle*** discoid to cupulate, white to pale brown, clothed entirely with short, white to slightly yellow hairs. ***Stipe*** 0.06–0.18 mm in diameter, 0.14–0.3 mm long when dry, cylindrical, solitary, white to pale yellow, clothed with white to pale yellow hairs. ***Hairs*** 28–117 × 1.6–3.9 µm ($\bar{x}$ = 60 × 2.9 µm, n = 30), clavate to cylindrical, straight to slightly curved, septate, hyaline, thin-walled, covered with hyaline granules, obtuse apex. ***Hymenium*** 65–120 µm ($\bar{x}$ = 85 µm, n = 12), concave, surface slightly rough, light yellow in dry. ***Medullary excipulum*** 23.5–65 µm ($\bar{x}$ = 37 µm, n = 18), thin, hyaline to light yellow, thin-walled cells of ***textura intricata***, 1.3–3.6 µm ($\bar{x}$ = 2.2 µm, n = 50) in diameter. ***Ectal excipulum*** 20–95 µm ($\bar{x}$ = 48 µm, n = 18) thick, thin-walled, smooth, hyaline cells of ***textura porrecta*** to ***textura globulosa***, 1.6–4.2 µm ($\bar{x}$ = 2.7 µm, n = 60). ***Paraphyses*** 25–68 × 0.9–2.0 µm ($\bar{x}$ = 43 × 1.3 µm, n = 25), longer than asci, filiform, straight, aseptate, hyaline, thin-walled, rough, with slightly acute apex. ***Asci*** 35–58 × 2.2–3.60 µm ($\bar{x}$ = 46 × 3.0 µm, n = 34), 8-spored, unitunicate, clavate, straight to slightly curved, inoperculate, hyaline, wall apically thickened, laterally relatively thin, slightly smooth, with an apical, amyloid pore and tapered ends, J+ in MLZ. ***Ascospores*** (85/6/2) 6.0–8.3 × 1.2–1.8 µm, ($\bar{x}$ = 7.3 × 1.5 µm, n = 86), partially biseriate, filiform, aseptate, thin-walled, hyaline, rough with tapering towards the obtuse ends, partially oil guttules, subspherical, hyaline, slightly smooth. ***Asexual morph*****:** Not observed.

**Material examined.** China • Yunnan Province, Xishuangbanna City, Menghai County, Gelanghe township, altitude 2097 m, on the decayed unidentified bark, 6 September 2022, Cuijinyi Li, LCJY1389 (HKAS 135689, ***holotype***); • *ibid*., Hongli Su, SU1978 (HKAS 135695, ***paratype***).

**Notes.** Our specimens, HKAS 135689 and HKAS 135695, were grouped in a distinct clade, separated from the clade comprising *E. abnormis*, *E. sclerotii*, *E. ailaoensis*, *E. brasiliensis* and *E. aseptata* by 78% MLBS and 0.88 BIPP (Fig. 1). *Erioscyphella gelangheica* has shorter asci (35–58 µm vs. 41–104 µm), ascospores (6.0–8.3 µm vs. 39–81 µm), and paraphyses (25–68 µm vs. 52–123 µm) than those of *E. abnormis* (Perić and Baral 2014). *Erioscyphella gelangheica* differs from *E. sclerotii* by having long stipitate apothecia and aseptate ascospores, while *E. sclerotii* has short stipitate apothecia and 1–3-septate ascospores. Our species, *E. gelangheica*, has shorter asci (35–58 µm vs. 85–143 µm), ascospores (6.0–8.3 µm vs. 45.5–97 µm), and paraphyses (25–68 µm vs. 100–138 µm) than those of *E. ailaoensis*. Furthermore, *E. ailaoensis* has septate ascospores in contrast to the aseptate ascospores of *E. gelangheica*. Compared to long stipitate apothecia with white to pale yellow hairs and aseptate ascospores of *E. gelangheica*, *E. brasiliensis* has 0–1-septate ascospores and stipitate apothecia, with the stipe base often devoid of hairs and blue-black (Haines 1992). In addition to having aseptate ascospores, *E. gelangheica* has shorter asci (35–58 µm vs. 70–100 µm) and ascospores (6.0–8.3 µm vs. 28.5–45.6 µm) than those of *E. aseptata*, which has septate ascospores (Ekanayaka et al. 2019). Therefore, *E. gelangheica* is introduced here as a new species.

**Figure 4.** *Erioscyphella gelangheica* (HKAS 135689, holotype) **a–d** dried ascomata on the host **e** a vertical section of an ascoma **f** excipulum **g, h** hairs **i** paraphyses and asci **j–n** asci (**l–n** asci in MLZ) **o** apices of asci treated with Melzer's reagent **p**–**u** ascospores. Scale bars: 100 μm (**e**); 50 μm (**f**); 20 μm (**g–n**); 5 μm (**o–u**).

***Erioscyphella tengyueica*** **L. Luo, K.D. Hyde & C.J.Y. Li, sp. nov.**
Index Fungorum: IF902531
Facesoffungi Number: FoF16392
Fig. 5

**Etymology.** The epithet refers to the collection site of the type specimen.

**Holotype.** HKAS 135688.

**Description.** ***Saprobic*** on the dead twigs. ***Sexual morph*: *Apothecia*** superficial, scattered to partly gregarious, 0.16–0.68 mm in diameter, 0.3–0.7 mm high when dry, discoid to cupulate, shortly stipitate, externally covered with short, white hairs. ***Discs*** concave, surface slightly rough, white. ***Margin*** slightly involute, white, covered with white hairs. ***Receptacle*** cupulate, concolorous, clothed entirely with short, white hairs. ***Stipe*** 0.09–0.23 mm in diameter, 0.18–0.4 mm long when dry, cylindrical, solitary, concolorous with the receptacle, clothed with white hairs. Hairs 45–95 × 2.8–6.9 µm (x̄ = 68 × 4.8 µm, n = 10), clavate to cylindrical, straight or curved, septate, hyaline, thin-walled, less covered with hyaline granules, obtuse apex. ***Hymenium*** 65–115 µm (x̄ = 87 µm, n = 12), concave, surface slightly rough, light white in dry. ***Medullary excipulum*** 18–33 µm (x̄ = 26 µm, n = 18), thick, comprising hyaline, thin-walled, poorly developed cells of ***textura***

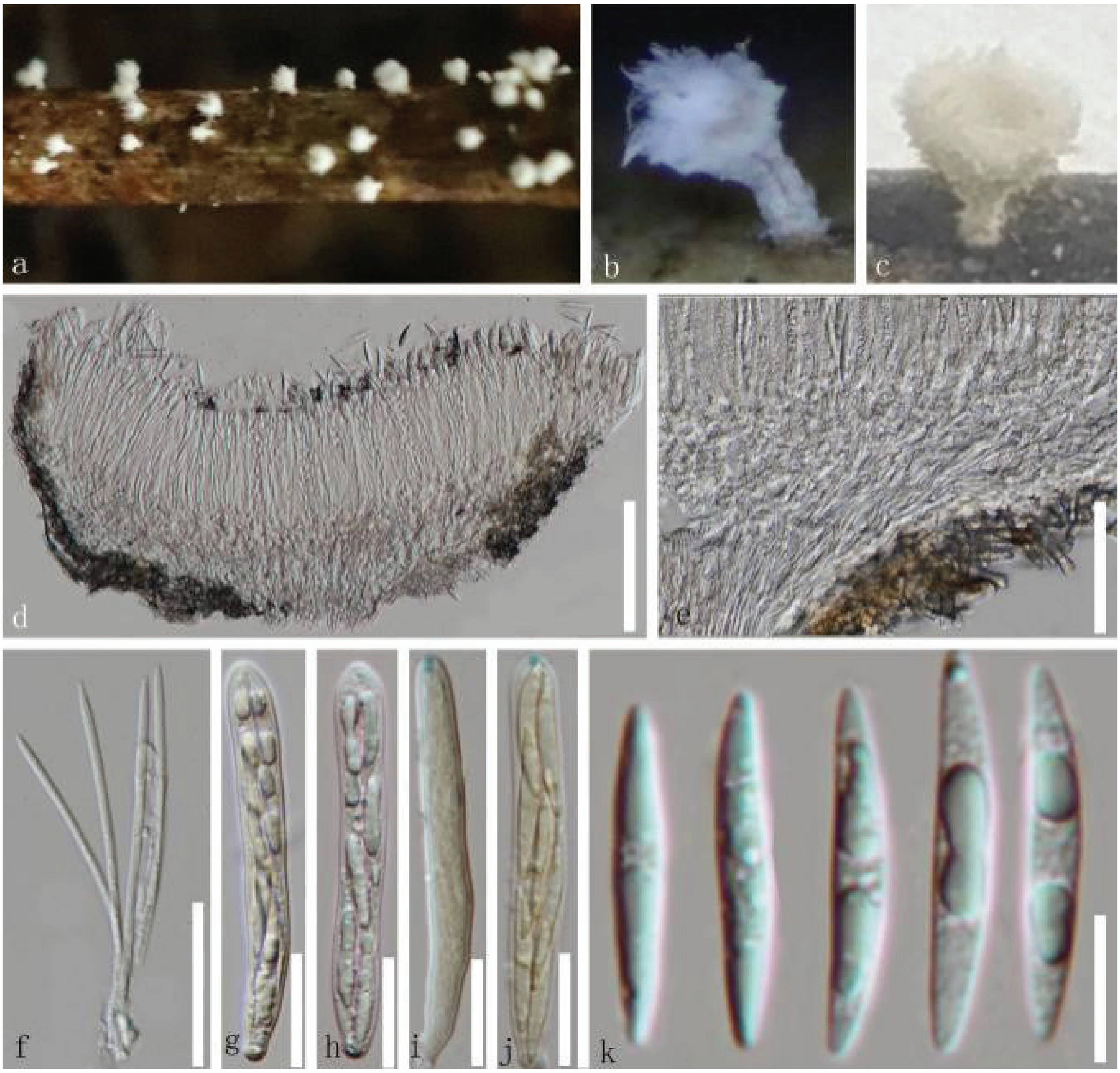

**Figure 5.** *Erioscyphella tengyueica* (HKAS 135688, holotype) **a–c** dried ascomata on the host **d** a vertical section of an ascoma **e** excipulum **f** paraphyses **g–j** asci (**i, j** asci in MLZ) **k** ascospores. Scale bars: 100 µm (**d**); 50 µm (**e, f**); 20 µm (**g–j**); 10 µm (**k**) .

***globulosa***, 1.4–3.5 µm (x̅ = 2.4 µm, n = 50) in diameter. Ectal excipulum 11–30 µm (x̅ = 21 µm, n = 18) thick, comprising thick-walled, smooth, light yellowish cells of ***textura oblita*** to ***textura porrecta***, 1.2–3.8 µm (x̅ = 2.2 µm, n = 60). ***Paraphyses*** 65–97 × 1.8–3.0 µm (x̅ = 86 × 2.5 µm, n = 25), longer than asci, filiform, straight, aseptate, hyaline, thin-walled, narrow lanceolate, smooth, less covered with hyaline granules, with slightly obtuse apex. ***Asci*** 60–80 × 6.0–9.3 µm (x̅ = 70 × 7.6 µm, n = 34), 8-spored, clavate, straight to slightly curved, inoperculate, hyaline, unitunicate, slightly smooth, with an apical, amyloid pore and rounded ends, croziers absent at the basal septum, J+ in MLZ. ***Ascospores*** (80/6/2) 25–31.5 × 1.6–5.5 µm, (x̅ = 27.7 × 3.3 µm, n = 80), overlapping biseriate, filiform, aseptate, thin-walled, hyaline, rough with tapering towards obtuse ends, filled with oil guttules or with 1–2- large oil guttules. ***Asexual morph*:** Not observed.

**Material examined.** China • Yunnan Province, Tengchong City, Tengyue Street, altitude 1983.3 m, on the decayed unidentified twig, 21 August 2022, Cuijinyi Li, LCJY1171 (HKAS 135688, ***holotype***); • *ibid*., altitude 1774 m, on the decayed unidentified twig, 18 August 2022, Le Luo, Ly255 (HKAS 135693, ***paratype***).

**Notes.** Our specimens, HKAS 135688 and HKAS 135693, were grouped into a distinct clade, separated from *E. papillaris* (TNS-F-81272) by 91% MLBS and 0.98 BIPP (Fig. 1). *Erioscyphella tengyueica* differs from *E. papillaris* by having aseptate ascospores and aseptate paraphyses, while the latter has septate paraphyses and aseptate or one-septate (rarely two-septate) ascospores. Our species differs from *E. otanii* by having longer asci (60–80 µm vs. 34–38.8 µm), longer ascospores (34–38.8 µm vs. 12.3–14.6 µm) and aseptate paraphyses, in contrast to the septate paraphyses of *E. otanii* (Tochihara and Hosoya 2022). Therefore, *E. tengyueica* is introduced here as a new species.

## Discussion

In China, the diversity of Leotiomycetes is substantial due to varied climates and ecosystems in the country (Li et al. 2022; Su et al. 2023; Guo et al. 2024; Luo et al. 2024; Zhang et al. 2024). Ongoing research continues to uncover new species and understand their roles in ecosystem functioning, highlighting the importance of preserving fungal diversity for ecological health and agricultural sustainability (Li et al. 2022; Su et al. 2023; Lestari and Chethana 2024; Luo et al. 2024).

Based on the previous research on the phylogeny, morphology and ecology of Lachnaceae (Hosoya et al. 2010), delimiting generic boundaries in Lachnaceae can no longer be defined by morphological characteristics alone (Li et al. 2022; Tochihara and Hosoya 2022; Su et al. 2023). The latest concept for *Erioscyphella* was proposed as the typical taxa characterized by the hair structures (the swollen apices, apical anamorph material/resinous material) and ascospore length (Tochihara and Hosoya 2022). This revision signifies a departure from the previously emphasized characteristics, highlighting the dynamic nature of fungal taxonomy (Tochihara and Hosoya 2022).

Despite advances in molecular techniques and the integration of morphological and ecological data, our analysis reveals persistent challenges in clarifying species boundaries. Particularly for paraphyletic members of long-spored *Lachnum* within *Erioscyphella*, the utilization of the UNITE Species Hypotheses (SH) system analysis based on ITS gene fragment, alongside traditional methods, has not provided definitive resolutions, indicating the ongoing complexity in species delineation (Tochihara and Hosoya 2022). Phylogenetic relationships in *Erioscyphella* gradually became clear as more fresh collections were identified with sequence data (Li et al. 2022; Su et al. 2023). Almost all species of *Erioscyphella* have stable phylogenetic positions with strong statistical support, except for *E. bambusina*, which lacks sequences in public databases.

Nearly all *Erioscyphella* species occupy stable phylogenetic positions supported by strong statistical evidence, except for *E. bambusina*, which lacks sequence data in public databases. We compared the morphology of *E. bambusina* with our newly established species (Dennis 1954; Su et al. 2023). *Erioscyphella bambusina* has septate, shorter ascospores, and septate paraphyses, in contrast to the multi-septate, longer ascospores, and aseptate paraphyses of *E. ailaoensis*. *Erioscyphella gelangheica* differs from *E. bambusina* by having smaller apothecia and aseptate paraphyses, while the latter has larger apothecia and septate paraphyses. *Erioscyphella baimana* has aseptate hairs and aseptate paraphyses, while *E. tengyueica* has white discs, and J- in MLZ with and without 3% KOH pretreatment. In contrast to these two, *E. bambusina* has cream to pale yellow discs, pore blued in MLZ, and septate paraphyses.

Most records of *Erioscyphella* are from the tropics (Spooner 1987; Haines and Dumont 1983; Tochihara and Hosoya 2022). Although sample collections have extended to subtropic, temperate and cold-temperate regions (Bien and Damm 2020; Tochihara and Hosoya 2022), collected samples are still scarce. Further, it was found that the lack of available sequences of these species led to Lachnaceae and Helotiaceae taxa exhibiting paraphyletic characters in the ITS-LSU phylogeny (Johnston et al. 2019; Quandt and Haelewaters 2021). Although Johnston et al. (2019) solved this issue based on phylogenetic analyses of up to 15 concatenated genes across 279 specimens, obtaining gene sequences for multiple loci for Lachnaceae taxa is still one of the critical problems.

Continued interdisciplinary research efforts are warranted to refine our understanding of fungal taxonomy within the Lachnaceae. Future studies should explore novel methodologies, such as high-throughput sequencing and ecological niche modeling, to elucidate species boundaries and evolutionary relationships more comprehensively. Additionally, comprehensive taxonomic revisions, including detailed examination of type specimens and expanded sampling, will be crucial for resolving taxonomic ambiguities and advancing fungal systematics.

In conclusion, our study contributes to the growing body of knowledge on fungal taxonomy and highlights the need for an integrated approach combining molecular, morphological, and ecological data to address the complexities inherent in delineating generic boundaries and species relationships within the Lachnaceae family.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Supervision: KWTC. Writing - original draft: LL, FA. Writing - review and editing: QZ, KWTC, KDH. Collection: LL, HLS, CJYL, VL.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# Phylogeny and phenotype of *Filobasidium* revealing three new species (Filobasidiaceae, Filobasidiales) from China

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## Abstract

The genus *Filobasidium*, a member of the family Filobasidiaceae in the order Filobasidiales, is a group of basidiomycetes with many representative species. To date, 14 species have been described and accepted in *Filobasidium*. Although some newfound species from China have recently been published, the species diversity of *Filobasidium* remains incompletely understood. Samples from various areas of China were obtained and examined to investigate the species diversity of the genus. Three new species, namely *F. pseudomali* **sp. nov.**, *F. castaneae* **sp. nov.**, and *F. qingyuanense* **sp. nov.**, were introduced based on phylogenetic analyses of the internal transcribed spacer (ITS) region and the D1/D2 domain of the large subunit (LSU) rRNA gene and the ITS sequence alone coupled with phenotypic characteristics. Full descriptions, illustrations, comparisons with similar species, and phylogenetic analyses are provided. Findings from this study substantially enrich the biodiversity of *Filobasidium* in China.

**Key words:** Basidiomycetes, phylogenetic analysis, phylloplane, taxonomy

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## Introduction

The genus *Filobasidium* was first characterized by Olive (1968) in the description of a sexual species, *F. floriforme*, resulting in the establishment of the Filobasidiaceae family. Four additional species, *F. capsuligenum* (Rodrigues de Miranda 1972), *F. uniguttulatum* (Kwon-Chung 1977), *F. elegans* (Bandoni et al. 1991), and *F. globisporum* (Bandoni et al. 1991), were later described according to phenotypic characteristics. Scorzetti et al. (2002) established a phylogeny of the genus *Filobasidium* based on the internal transcribed spacer (ITS) region and the D1/D2 domain of the large subunit (LSU) rRNA gene, and placed the genus in Filobasidiales. Five asexual *Cryptococcus* species, including *C. chernovii*, *C. magnus*, *C. oeirensis*, *C. stepposus*, and *C. wieringae*, are members of the *floriforme* clade as demonstrated by phylogenetic analyses of the small subunit (SSU), D1/D2 domain, and ITS region (Fell et al. 2000; Fonseca et al. 2000; Scorzetti et al. 2002; Golubev et al. 2006). According to the Melbourne

Code (McNeill et al. 2012), which specifies that related anamorphic and teleomorphic species can be assigned to the same genus, these five asexual *Cryptococcus* species were consequently reassigned to the genus as the new combinations, *F. chernovii*, *F. magnum*, *F. oeirense*, *F. stepposum*, and *F. wieringae*, based on multi-gene phylogeny (Liu et al. 2015a, 2015b). Correspondingly, the unique fermentative species, *F. capsuligenum*, was removed from this genus as it was located outside the *Filobasidium* clade and was closely associated with the *Piskurozyma* clade (Liu et al. 2015b). In recent years, several new species, such as *F. dingjieense*, *F. globosum*, *F. mali*, *F. mucilaginum* (Li et al. 2020), and *F. chaidanensis* (Wei et al. 2022), have been published.

*Filobasidium* species can reproduce both asexually and sexually. Through asexual reproduction, the species reproduce by budding, and some species produce pseudohyphae and/or true hyphae with clamp connections and haustorial branches (Fonseca et al. 2011; Li et al. 2020; Wei et al. 2022). Alternatively, four sexual species, *F. floriforme*, *F. elegans*, *F. globisporum*, and *F. uniguttulatum*, generate long, slender, nonseptate basidia with terminal sessile basidiospores (Kwon-Chung 2011; Liu et al. 2015b). Most of the known *Filobasidium* species can grow on L-malic, saccharic, as well as protocatechuic and p-hydroxybenzoic acids, while nitrate utilization was observed in some species. The primary ubiquinone in the *Filobasidium* species is CoQ-9 or CoQ-10 (Liu et al. 2015b).

Members of the genus *Filobasidium* have been investigated for various biotechnological applications and pathology. Most previous studies have focused on the most widely distributed species, *F. magnum* (Wei et al. 2022). For instance, as a bio-transformer, the *F. magnum* strain JD1025 can effectively convert sclareol to sclareolide (Fang et al. 2023). Strain JD1025 of *F. magnum* can metabolize nobiletin for the biosynthesis of 6- and 7-mono-demethylated nobiletin (Su et al. 2022). Endophytic strains of *F. magnum* are associated with the formation of grape flavor, acting as a candidate for wine flavor enhancement (Sayed et al. 2021). Moreover, *F. globisporum* is frequently detected in industrial-scale malting processes. It can produce extracellular β-glucanase and cellulase with a potentially positive contribution to the malt enzyme spectrum (Laitila et al. 2006). *Filobasidium magnus* and *F. unigutulatum* have been reported to exist as clinical specimens. However, only *F. magnus* can grow at the human body temperature, suggesting that it may be an opportunistic human pathogen (Fonseca et al. 2011; Aboutalebian et al. 2020; Baptista et al. 2020).

Currently, 14 species in the genus *Filobasidium* have been recorded in Mycobank [https://www.mycobank.org (accessed on 20 November 2024)]. In China, 13 *Filobasidium* species have been reported, encompassing six species initially described in China (Luo et al. 2019; Li et al. 2020; Wei et al. 2022). While some new species from China have recently been published, the diversity of *Filobasidium* remains only partially understood. In this study, seven basidiomycetous yeast strains were collected from Guangdong, Guizhou, and Henan Provinces of China. Morphological characteristics and phylogenetic analysis based on the combined ITS and LSU sequences and the ITS sequence alone revealed that these strains represent three undescribed species of *Filobasidium*. Our aim in this investigation is to employ an integrative taxonomic approach for the identification and description of these new taxa.

## Materials and methods

### Sample collection and yeast isolation

A total of 25 leaf samples were obtained from the Guangdong, Guizhou, and Henan Provinces of China. Leaf samples were stored in sterile plastic bags and kept in an icebox for 6–12 h during transfer to the laboratory. Yeast strains were isolated from leaf surfaces using the improved ballistospore-fall method, as described previously (Nakase and Takashima 1993). Vaseline was utilized to affix fresh and healthy leaves to the insides of Petri dishes filled with yeast extract-malt extract (YM) agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, and 2% agar). The YM agar plates were incubated at 20 °C until visible colonies formed. Different yeast morphotypes were chosen and purified by streaking on distinct YM agar plates. Following purification, yeast strains were suspended in 20% (v/v) glycerol and stored at −80 °C. Cultures for all obtained isolates were preserved in the Microbiology Lab at Nanyang Normal University, Henan, China. All isolates employed in this study and their origins are presented in Table 1.

### Phenotypic characterization

Morphological, physiological, and biochemical characteristics were assessed based on methods established by Kurtzman et al. (2011). Sexual processes of all strains were investigated on potato dextrose agar (PDA, 20% potato extract, 2% glucose, and 1.5% agar), corn meal (CM) agar (2.2% corn extract and 1.5% agar), and yeast carbon base supplemented with 0.01% ammonium sulfate (YCBS) agar at 17 °C for two months and observed weekly (Kwon-Chung 2011; Li et al. 2020). The inverted-plate method (do Carmo-Sousa and Phaff 1962) was employed to observe the ballistoconidium-forming activity of all yeasts following two weeks of incubation on CM agar at 17 °C. Glucose fermentation was performed in a liquid medium using Durham fermentation tubes. Carbon and nitrogen source assimilation tests were performed in a liquid medium, and starved

**Table 1.** Yeast strains and isolation sources utilized in this study.

| Strain | Source | Location | Date |
|---|---|---|---|
| *Filobasidium pseudomali* sp. nov. | | | |
| NYNU 228108$^T$ | Leaf of *Photinia* sp. | Guiyang Medicinal Botanical Garden, Guiyang, Guizhou, China | August 2022 |
| NYNU 22986 | Leaf of *Litsea cubeba* | Guiyang Medicinal Botanical Garden, Guiyang, Guizhou, China | August 2022 |
| *Filobasidium castaneae* sp. nov. | | | |
| NYNU 2111105$^T$ | Leaf of *Castanea mollissima* | Baotianman Nature Reserve, Nanyang, Henan, China | November 2021 |
| NYNU 23230 | Leaf of *Mussaenda pubescens* | Pingtang county, Buyi and Miao Autonomous Prefecture of Qian Nan, Guizhou, China | February 2023 |
| NYNU 23245 | Leaf of *Mussaenda pubescens* | Pingtang county, Buyi and Miao Autonomous Prefecture of Qian Nan, Guizhou, China | February 2023 |
| *Filobasidium qingyuanense* sp. nov. | | | |
| NYNU 223211$^T$ | Leaf of *Lespedeza formosa* | Qingyuan Mountain, Quanzhou, Guangdong, China | March 2022 |
| NYNU 23239 | Leaf of *Mussaenda pubescens* | Pingtang county, Buyi and Miao Autonomous Prefecture of Qian Nan, Guizhou, China | February 2023 |

inoculum was utilized for the nitrogen test (Kurtzman et al. 2011). Growth at different temperatures (15, 20, 25, 30, 35, and 37 °C) was characterized by growth on YM agar. Cell morphology was assessed using a Leica DM 2500 microscope (Leica Microsystems GmbH, Wetzlar, Germany) alongside a Leica DFC295 digital microscope color camera. All novel taxonomic descriptions and proposed names were deposited in the MycoBank database (http://www.mycobank.org).

## DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from yeast strains using the Ezup Column Yeast Genomic DNA Purification Kit following the manufacturer's instructions (Sangon Biotech Co., Shanghai, China). The ITS region, the D1/D2 domain of the LSU rRNA gene, the largest subunit of RNA polymerase I (*RPB1*) gene, and the second largest subunit of RNA polymerase II (*RPB2*) gene were amplified using primers ITS1/ITS4 (White et al. 1990), NL1/NL4 (Kurtzman and Robnett 1998), RPB1-Af/RPB1-Cr (Kurtzman and Robnett 2003), and RPB2-5F/RPB2-7cAR (Kurtzman and Robnett 2003), respectively. Amplification was performed in a 25 μL reaction-volume tube containing 9.5 μL of $ddH_2O$, 12.5 μL of 2 × Taq PCR Master Mix with blue dye (Sangon Biotech Co., Shanghai, China), 1 μL of DNA template, and 1 μL of each primer. PCR was conducted as described by Toome et al. (2013) for the ITS and LSU regions. For the partial *RPB1* and *RPB2* genes, we utilized a touchdown PCR protocol as previously described (Wang et al. 2014). The PCR products were purified and sequenced at Sangon Biotech Co., Ltd (Shanghai, China) using the same primers. We determined the identity and accuracy of the newly obtained sequences by comparing them to sequences found in the GenBank database and assembled them using BioEdit v. 7.1.3.0 (Hall 1999). All newly generated sequences were deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

## Phylogenetic analysis

Sequences generated in this study and those obtained from GenBank (Table 2) were used for phylogenetic analyses. Firstly, the combined dataset of the ITS and LSU regions was used to explore the phylogenetic positions of the newly studied specimens within *Filobasidium*. Secondly, the ITS sequence alone was used to further differentiate species identities within this genus. Each dataset was aligned using MAFFT v. 7.110 (Katoh and Standley 2013) with the G-INI-I option. Alignments were visualized, trimmed, and edited, where necessary, using MEGA v.7.0.26 (Kumar et al. 2016). Regarding the combined dataset of the ITS and LSU regions, each region was aligned separately, and then the alignments of the two regions were concatenated as a single alignment.

Maximum likelihood (ML) and Bayesian inference (BI) methods were utilized for phylogenetic analyses. The ML method was conducted with RAxML v. 8.2.3 using the GTRGAMMA model (Stamatakis 2014). ML bootstrap values (MLBS) were evaluated using 1,000 rapid bootstrap replicates. For the BI approach, the optimal evolutionary model for each partition was determined using ModelFinder (Kalyaanamoorthy et al. 2017). The BI method was performed with MrBayes v. 3.2.7a (Ronquist et al. 2012) in the CIPRES Science Gateway version 3.3. Six simultaneous Markov chains were performed over 50 million generations, and

**Table 2.** Taxa included in molecular phylogenetic analyses and their GenBank accession numbers. Entries in bold were newly generated for this study.

| Taxa name | Sample | GenBank accession numbers | |
|---|---|---|---|
| | | ITS | LSU D1/D2 |
| ***Filobasidium castaneae* sp. nov.** | **NYNU 2111105$^{T}$** | **OM049430** | **OM049431** |
| ***Filobasidium castaneae* sp. nov.** | **NYNU 23230** | **PP114094** | **PP114092** |
| ***Filobasidium castaneae* sp. nov.** | **NYNU 23245** | **PP114096** | **PP114097** |
| *Filobasidium chaidanensis* | CGMCC 2.6796$^{T}$ | OM417191 | OM417191 |
| *Filobasidium chernovii* | CBS 8679$^{T}$ | NR_073223 | NG_068965 |
| *Filobasidium dingjieense* | CGMCC 2.5649$^{T}$ | NR_174759 | MK050342 |
| *Filobasidium elegans* | CBS 7640$^{T}$ | AF190006 | AF181548 |
| *Filobasidium floriforme* | CBS 6241$^{T}$ | NR_119429 | NG_069409 |
| *Filobasidium globisporum* | CBS 7642$^{T}$ | NR_119453 | NG_070553 |
| *Filobasidium globosum* | CGMCC 2.5680$^{T}$ | NR_174760 | MK050344 |
| *Filobasidium magnum* | CBS 140$^{T}$ | NR_130655 | NG_069409 |
| *Filobasidium mali* | CGMCC 2.4012$^{T}$ | NR_174761 | MK050346 |
| *Filobasidium mucilaginum* | CGMCC 2.3463$^{T}$ | NR_174762 | MK050349 |
| *Filobasidium oeirensis* | CBS 8681$^{T}$ | NR_077106 | NG_070508 |
| ***Filobasidium pseudomali* sp. nov.** | **NYNU 228108$^{T}$** | **OP581930** | **OP566876** |
| ***Filobasidium pseudomali* sp. nov.** | **NYNU 22986** | **PP108743** | **PP108744** |
| ***Filobasidium qingyuanense* sp. nov.** | **NYNU 223211$^{T}$** | **OP278683** | **OP278680** |
| ***Filobasidium qingyuanense* sp. nov.** | **NYNU 23239** | **PP114093** | **PP114095** |
| *Filobasidium stepposum* | CBS 10265$^{T}$ | NR_111207 | KY107724 |
| *Filobasidium uniguttulatum* | CBS 1730$^{T}$ | NR_111070 | NG_056269 |
| *Filobasidium wieringae* | CBS 1937$^{T}$ | NR_077105 | NG_067314 |
| *Filobasidium* sp. | KBP Y-5548 | MH697755 | MH697755 |
| *Filobasidium* sp. | UFMG-CM-Y6635 | OM480729 | OM321340 |
| *'Cryptococcus'* sp. | 2 IA06 | KM246189 | KM246106 |
| *'Cryptococcus'* sp. | 2 MG34 | KM246229 | KM246145 |
| *'Cryptococcus'* sp. | 11-1115 | KM986117 | KM206723 |
| *'Cryptococcus'* sp. | RP419_8 | KX067803 | KX067803 |
| *Goffeauzyma aciditolerans* | CBS 10872$^{T}$ | NR_137808 | NG_058295 |
| *Goffeauzyma gastrica* | CBS 2288$^{T}$ | NR_111048 | NG_058296 |
| Uncultured fungus clone | OTU_812 | MH365273 | – |
| Uncultured fungus clone | – | LR880016 | – |
| Uncultured fungus clone | – | LR136377 | – |
| Uncultured fungus clone | – | LT995797 | – |

CBS, CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC, China General Microbiological Culture Collection Center, Beijing, China; NYNU, Microbiology Lab, Nanyang Normal University, Henan, China; $^{T,}$ type strain.

trees were sampled every 1,000 generation. The first 25% of the created sample trees were removed as they represent the burn-in phase of analysis. The remaining trees were employed to determine the Bayesian posterior probabilities (BPP). FigTree v. 1.4.3 was used to visualize the phylogenetic trees (Andrew 2016). Branches with bootstrap values for MLBS ≥ 50% and BPP ≥ 0.95 were considered significantly supported.

## Results

### Yeast isolation and diversity

During this study, 106 yeast strains were isolated from 25 leaf samples collected in the Guangdong, Guizhou, and Henan Provinces of China. All strains were identified to the species level based on the threshold of >99% sequence identity with the type strain of a described species in the D1/D2 domain or ITS region (Kurtzman and Robnett 1998; Fell et al. 2000; Scorzetti et al. 2002; Vu et al. 2016). A total of 95 strains present in the samples were classified as Basidiomycota belonging to 20 species in 12 genera: *Bannoa ogasawarensis*, *Bullera alba*, *Bullera mrakii*, *Bulleribasidium pseudovariabile*, *Cystobasidium pallidum*, *Derxomyces komagatae*, *Dioszegia hungarica*, *Erythrobasidium hasegawianum*, *Hannaella sinensis*, *Hannaella taiwanensis*, *Sporidiobolus metaroseus*, *Sporobolomyces carnicolor*, *Sporobolomyces roseus*, *Tilletiopsis washingtonensis*, *Vishniacozyma foliicola*, *Vishniacozyma carnescens*, *Vishniacozyma victoriae*, and three *Filobasidium* species that are not yet formally described and therefore represent new species. In addition, eleven strains belonging to Ascomycota were also obtained from these samples. The ascomycetous yeasts were found to be four known species in four genera: *Aureobasidium pullulans*, *Candida silvanorum*, *Yamadazyma scolyti*, and *Wickerhamomyces sydowiorum.* Among the 24 species identified, *Tilletiopsis washingtonensis* was the most dominant species, which occurred in six samples collected from different locations, while *Bannoa ogasawarensis*, *Bullera mrakii*, and *Yamadamyces terricola* occurred only in one sample or location.

### Phylogeny of novel yeast species

Seven specimens preliminarily identified as *Filobasidium* were studied further. ITS and LSU regions were newly generated from all these specimens (Table 2).

The combined dataset of ITS and LSU regions consisted of 29 sequences from 24 taxa, including 14 newly generated sequences (seven for ITS and seven for LSU). The final alignment included 1,124 characters (486 characters from ITS and 637 characters from LSU), of which 752 were constant, 372 were variable, 279 were parsimony-informative, and 93 were singletons. Both ML and BI methods produced similar topologies in the main lineages. The ML-derived topology, along with MLBS and BPP values above 50% and 0.95, respectively, is presented (Fig. 1). The phylogeny indicated that seven strains isolated in this study formed three highly supported groups (Fig. 1) within the genus and were distinct from other species of *Filobasidium*.

The ITS dataset consisted of 33 sequences from 24 taxa, including seven newly generated sequences. The final alignment included 486 characters, of which 267 were constant, 219 were variable, 195 were parsimony-informative, and 26 were singletons. The ML and BI methods yielded similar topologies in the main lineages. The ML-derived topology, with MLBS and BPP values above 50% and 0.95, respectively, is shown (Fig. 2). This tree demonstrated 14 known *Filobasidium* species, while the newly isolated strains formed three independent groups, consistent with the combined ITS and LSU dataset phylogeny.

Strains NYNU 228108 and NYNU 22986 had identical sequences in the D1/D2 domain and ITS region, indicating their conspecificity. In the phylogenetic

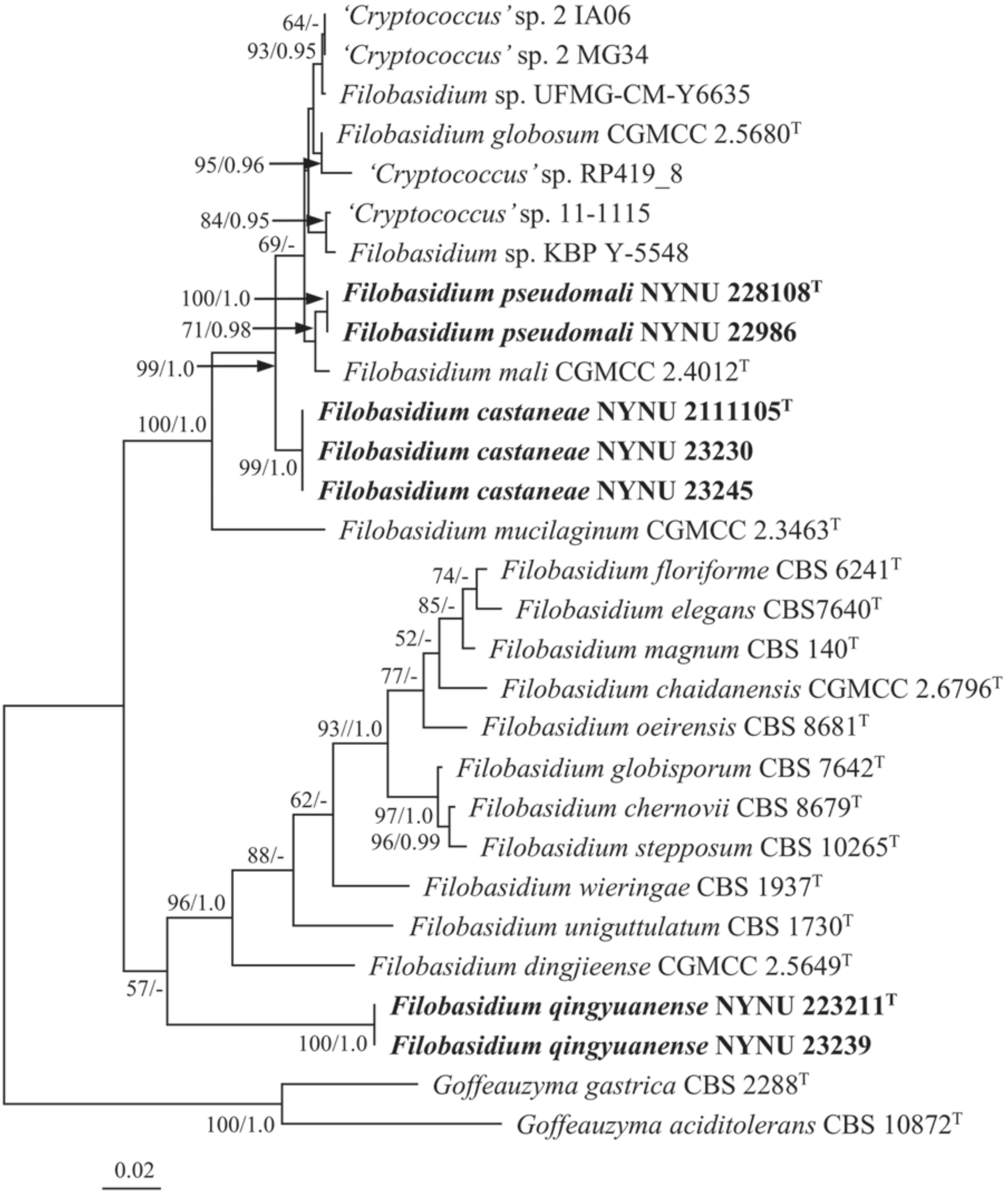

**Figure 1.** Maximum likelihood (ML) phylogenetic tree of *Filobasidium* derived from combined ITS and LSU sequence data. The tree is rooted with *Goffeauzyma gastrica* CBS 2288$^{\mathrm{T}}$ and *Goffeauzyma aciditolerans* CBS 10872$^{\mathrm{T}}$. Bootstrap values (MLBS ≥ 50% and BPP ≥ 0.95) are shown around branches. Sequences from type strains are marked with (T), and the new species are indicated in bold.

tree, two strains formed a well-supported clade grouped with *F. mali* with moderate support (Figs 1, 2). They differed from their closest relative, *F. mali*, by two nucleotide (nt) substitutions in the D1/D2 domain. However, they differed by 15 nt (~2.5%) mismatches from *F. mali* in the ITS region. Generally, the basidiomycetous yeast strains differing by two or more nucleotide substitutions in the D1/D2 domain or 1–2% nucleotide differences in the ITS region may represent different taxa (Scorzetti et al. 2002). Recently, Li et al. (2020) determined the

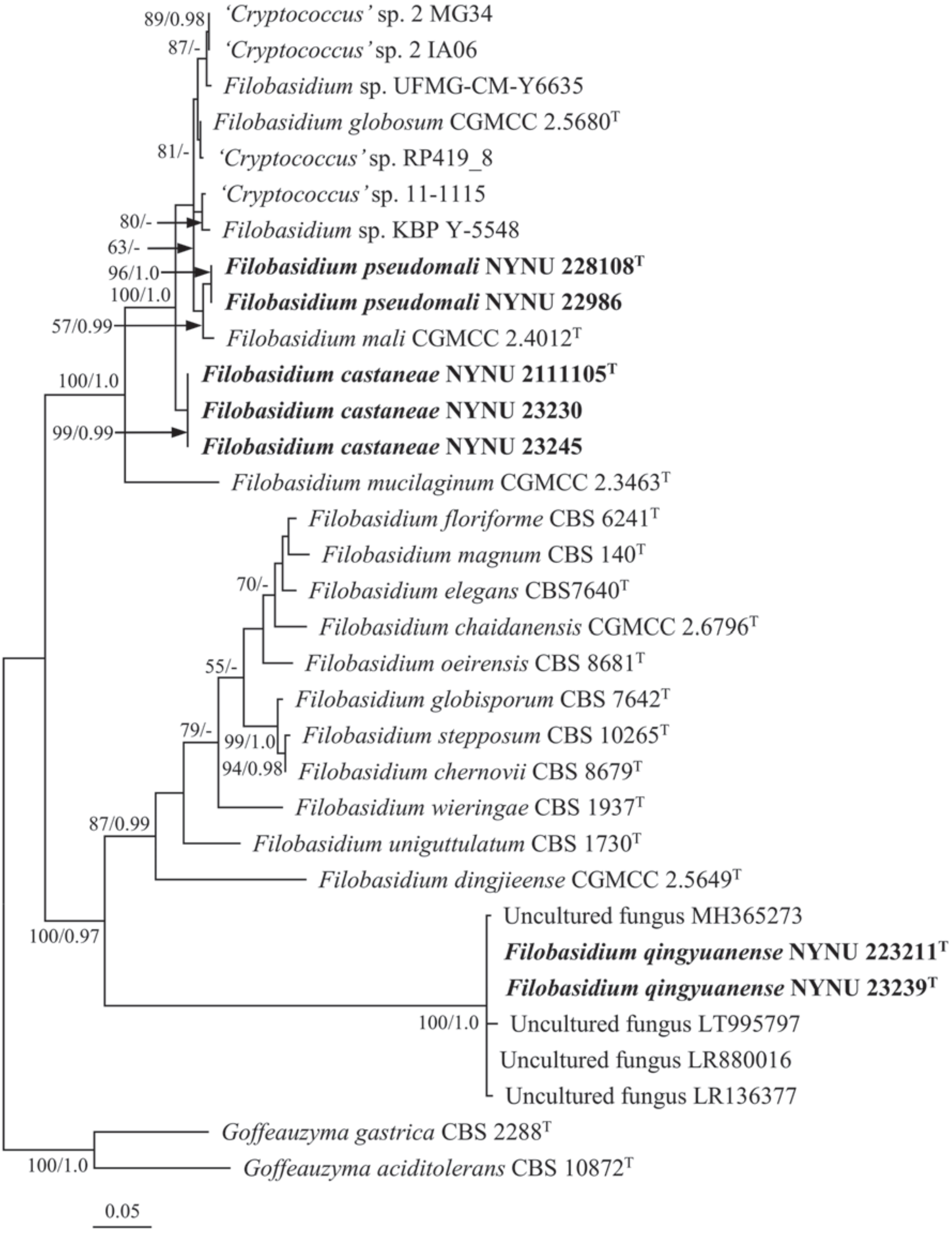

**Figure 2.** Maximum likelihood (ML) phylogenetic tree of *Filobasidium* derived from ITS sequence data. The tree is rooted with *Goffeauzyma gastrica* CBS 2288$^T$ and *Goffeauzyma aciditolerans* CBS 10872$^T$. Bootstrap values (MLBS ≥ 50% and BPP ≥ 0.95) are shown around branches. Sequences from type strains are marked with (T), and the new species are indicated in bold.

number of nucleotide variations and sequence similarities in the D1/D2 domain and ITS region among the type strains of species from 40 genera of *Agaricomycotina* and 30 genera of *Pucciniomycotina*. They demonstrated that the nucleotide variation among the strains of *Filobasidium* species is 0–21 nt (~0–3.3%)

in the D1/D2 domain and 4–106 nt (~0.7–5.8%) in the ITS region. The sequence divergences in the D1/D2 and ITS regions have raised the possibility that the two strains may represent a novel species distinct from *F. mali*. Moreover, the partial *RPB1* and *RPB2* gene sequences further confirmed the novelty of this species, as the two strains differed by 114 (~16.8%) and 143 (~12.9%) nt substitutions from *F. mali* in these regions. The sequence comparison and results of the phylogenetic analyses indicated that the strains NYNU 228108 and NYNU 22986 represent a novel species of *Filobasidium*. Therefore, the name *Filobasidium pseudomali* sp. nov. is proposed to accommodate these two strains.

Strains NYNU 2111105, NYNU 23230, and NYNU 23245 with identical sequences in the D1/D2 domain and ITS region formed a separate clade, clustering alongside *F. globosum*, *F. mali*, *F. pseudomali* sp. nov., and five unpublished strains, *Filobasidium* sp. KBP Y-5548, *Filobasidium* sp. UFMG-CM-Y6635, '*Cryptococcus*' sp. RP419_8, '*Cryptococcus*' sp. 2 IA06, and '*Cryptococcus*' sp. 2 MG34, with high support (Figs 1, 2). They differed from the above three described species and five unpublished strains by 5–7 nt (~0.8–1.2%) substitutions in the D1/D2 domain and by more than 23 nt (~3.9%) mismatches in the ITS region. Thus, these three strains represent a novel *Filobasidium* species, for which the name *Filobasidium castaneae* sp. nov. is proposed.

Strains NYNU 223211 and NYNU 23239 possessed identical sequences in the D1/D2 domain and ITS region, forming a subclade with four uncultured fungus clones (MH365273, LR880016, LR136377, and LT995797) in the tree of the ITS dataset (Fig. 2). A BLASTn search of the ITS sequences revealed that NYNU 223211 and NYNU 23239 had 99.2–100% sequence similarities with four uncultured fungus clones, which indicated that they may be conspecific. In the tree of the combined ITS and LSU dataset, strains NYNU 223211 and NYNU 23239 formed separate branches at the bottom of the *Filobasidium* clade (Fig. 1). They differed from other known *Filobasidium* species by 18 nt (~3%) substitutions in the D1/D2 domain and more than 34 nt (~9.3%) mismatches in the ITS region, suggesting that they represent a novel *Filobasidium* species. Therefore, a novel species, *Filobasidium qingyuanense*, is proposed to accommodate these two strains.

## Taxonomy

### ***Filobasidium pseudomali*** **C.Y. Cai & F.L. Hui, sp. nov.**

MycoBank No: 851823
Fig. 3A

**Etymology.** The specific epithet *pseudomali* refers to similar colony morphological and physiological characteristics to that of *Filobasidium mali*.

**Typus.** China • Guizhou Province, Guiyang City, Guiyang Medicinal Botanical Garden, in the phylloplane of *Photinia* sp., August 2022, L. Zhang and F.L. Hui, NYNU 228108 (holotype GDMCC 2.305$^T$ preserved in a metabolically inactive state in Guangdong Microbial Culture Collection Center, culture ex-type PYCC 9928 deposited in the Portuguese Yeast Culture Collection).

**Description.** On YM agar, after 7 days at 20 °C, the streak culture is gray-cream, mucoid, smooth, and glossy. The margin is entire. On YM agar, after 7 days at 20 °C, cells are globosal and ellipsoidal, 3.8–6.4 × 5.2–8.4 μm, and single, budding is polar. After 1 month at 20 °C, a ring and sediment are present. In

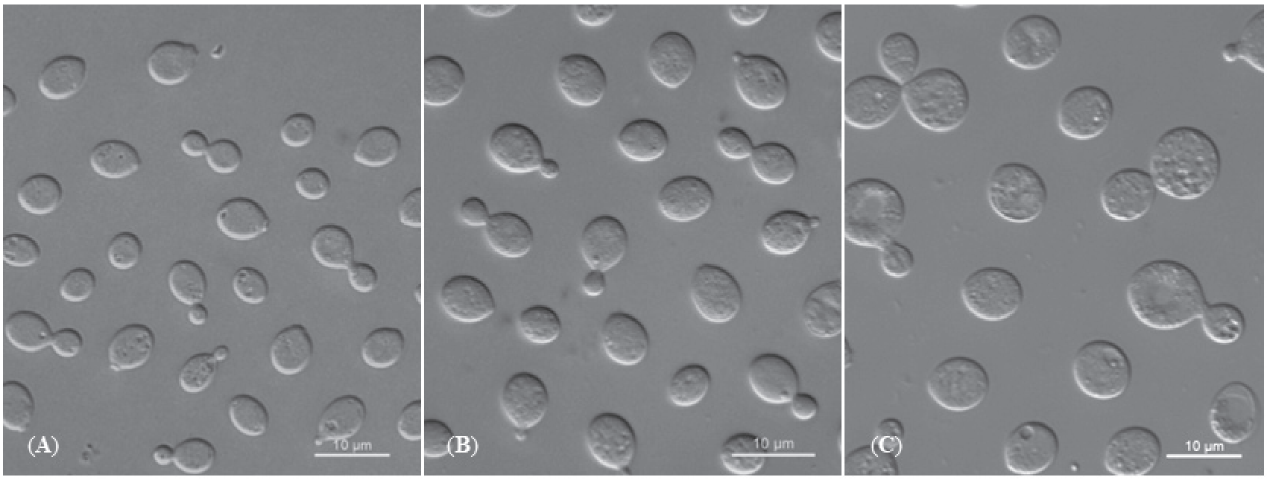

**Figure 3.** Vegetative cells of *F. pseudomali* sp. nov. NYNU 228108$^T$ (**A**), *F. castaneae* sp. nov. NYNU 2111105$^T$ (**B**), and *F. qingyuanense* sp. nov. NYNU 223211$^T$ (**C**) following growth in YM broth for 7 days at 20 °C. Scale bars: 10 µm.

Dalmau plate culture on corn meal agar, pseudohyphae are not formed. Sexual structures are not observed on PDA, CM agar, and YCBS agar for two months. Ballistoconidia are not produced. Glucose fermentation is absent. Glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, methyl-α-D-glucoside, cellobiose, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, 5-keto-D-gluconate, ethanol, ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, succinate, citrate, D-gluconate, 2-keto-D-gluconate, D-glucuronate, and glucono-1,5-lactone are assimilated as sole carbon sources. Salicin, D-ribose, methanol, glycerol, erythritol, DL-lactate, D-glucosamine, and N-acetyl-D-glucosamine are not assimilated. Nitrate, nitrite, ethylamine, and L-lysine (weak) are assimilated as sole nitrogen sources. Cadaverine is not assimilated. Maximum growth temperature is 30 °C. Growth in vitamin-free medium is positive. Growth on 50% (w/w) glucose-yeast extract agar is negative. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive.

**Additional strain examined.** China • Guizhou Province, Guiyang City, Guiyang Medicinal Botanical Garden, in the phylloplane of *Litsea cubeba*, August 2022, L. Zhang and F.L. Hui, NYNU 22986.

**GenBank accession numbers.** Holotype GDMCC 2.305$^T$ (ITS: OP581930, D1/D2: OP566876, *RBP1*: OR963293, *RBP2*: PP151258); additional strain NYNU 22986 (ITS: PP108743, D1/D2: PP108744, RBP1: PP841943, RBP2: PP151259).

**Note.** *Filobasidium pseudomali* sp. nov. can be physiologically distinguished from its closest known species, *F. mali*, by its ability to assimilate inulin and citrate and its inability to assimilate salicin and cadaverine. Additionally, *F. pseudomali* nov. can grow in a vitamin-free medium, while *F. mali* cannot (Table 3).

### ***Filobasidium castaneae* C.Y. Cai & F.L. Hui, sp. nov.**

MycoBank No: 851825
Fig. 3B

**Etymology.** The specific epithet *castaneae* refers to *Castanea*, the plant genus from which the type strain was isolated.

**Table 3.** Physiological and biochemical features differing between the new species and closely related species.

| Characteristics | 1 | 2* | 3* | 4 | 5 | 6* |
|---|---|---|---|---|---|---|
| Carbon assimilation | | | | | | |
| Inulin | + | – | + | + | + | – |
| Salicin | – | +/w | – | + | + | – |
| L-Sorbose | + | + | – | + | – | – |
| L-Rhamnose | + | +/d/w | d/w | + | + | – |
| D-Arabinose | + | v | – | - | + | – |
| D-Ribose | – | – | – | + | – | – |
| Glycerol | – | – | – | + | w | d/w |
| Ethanol | + | +/w | – | – | – | d/w |
| Ribitol | + | + | – | + | + | – |
| Galactitol | + | + | – | + | – | – |
| D-Mannitol | + | + | + | + | + | – |
| D-Glucitol | + | v | – | + | + | – |
| Citrate | + | – | – | + | + | + |
| Nitrogen assimilation | | | | | | |
| Nitrite | + | – | – | + | + | + |
| Cadaverine | – | + | + | – | – | d/w |
| L-Lysine | d | – | + | n | n | n |
| Growth tests | | | | | | |
| Growth in vitamin-free medium | + | – | + | + | + | n |
| Growth at 25 °C | + | + | + | + | + | – |
| Growth at 30 °C | + | + | – | – | + | – |

1, *F. pseudomali* sp. nov.; 2, *F. mali*; 3, *F. globosum*; 4, *F. castaneae* sp. nov.; 5, *F. qingyuanense* sp. nov.; 6, *F. dingjieense*; +, positive reaction; –, negative reaction; d, delayed positive; w, weakly positive; n, data not available. All data from this study, except * which were obtained from the original description (Li et al. 2020).

**Typus.** China • Henan Province, Nanyang City, Baotianman Nature Reserve, in the phylloplane of *Castanea mollissima*, November 2021, R.R. Jia and W.T. Hu, NYNU 2111105 (holotype CICC 33541$^T$ preserved in a metabolically inactive state in the China Centre of Industrial Culture Collection, culture ex-type JCM 35729 deposited in the Japan Collection of Microorganisms).

**Description.** On YM agar, after 7 days at 20 °C, the streak culture is gray-cream, mucoid, smooth, and glossy. The margin is entire. On YM agar, after 7 days at 20 °C, cells are globosal and ellipsoidal, 3.6–5.8 × 4.2–7.1 µm, and single, budding is polar. After 1 month at 20 °C, a ring and sediment are present. In Dalmau plate culture on corn meal agar, pseudohyphae are not formed. Sexual structures are not observed on PDA, CM agar, and YCBS agar for two months. Ballistoconidia are not produced. Glucose fermentation is absent. Glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, methyl-α-D-glucoside, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, 5-keto-D-gluconate, D-ribose, glycerol, ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, succinate, citrate, D-gluconate, N-acetyl-D-glucosamine, 2-keto-D-gluconate, D-glucuronate, and glucono-1,5-lactone are assimilated as sole carbon sources. D-Arabinose, methanol, ethanol, erythritol, DL-lactate, and D-glucosamine are not assimilated. Nitrate, nitrite, ethylamine, and L-lysine are assimilated as sole nitrogen sources. Cadaverine is not assimilated. Maximum growth temperature is 25 °C. Growth in vitamin-free medium is positive. Growth on 50% (w/w) glucose-yeast extract agar is negative. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive.

**Additional strain examined.** China • Guizhou Province, Buyi and Miao Autonomous Prefecture of Qian Nan, Pingtang County, in the phylloplane of *Mussaenda pubescens*, February 2023, D. Lu, NYNU 23230 and NYNU 23245.

**GenBank accession numbers.** Holotype CICC 33541$^T$ (ITS: OM049430, D1/D2: OM049431); additional strains NYNU 23230 (ITS: PP114094, D1/D2: PP114092) and NYNU 23245 (ITS: PP114096, D1/D2: PP114097).

**Note.** *Filobasidium castaneae* sp. nov. can be physiologically distinguished from its closely related species *F. globosum*, *F. mali*, and *F. pseudomali* sp. nov. through its ability to assimilate D-ribose and glycerol (Table 3).

### ***Filobasidium qingyuanense*** C.Y. Cai & F.L. Hui, sp. nov.

MycoBank No: 851824
Fig. 3C

**Etymology.** The specific epithet *qingyuanense* refers to the geographic origin of the type strain, Qingyuan Mountain, Quanzhou City, Guangdong Province.

**Typus.** China • Guangdong Province, Quanzhou City, Qingyuan Mountain, in the phylloplane of *Lespedeza formosa*, March 2022, W.T. Hu and S.B. Chu, NYNU 223211 (holotype GDMCC 2.309$^T$ preserved as a metabolically inactive state in the Guangdong Microbial Culture Collection Center, culture ex-type PYCC 9927 deposited in the Portuguese Yeast Culture Collection).

**Description.** On YM agar, after 7 days at 20 °C, the streak culture is gray-cream, mucoid, smooth, and glossy. The margin is entire. On YM agar, after 7 days at 20 °C, cells are globosal and ellipsoidal, 6.7–10.2 × 7.6–10.4 µm and single, budding is polar. After 1 month at 20 °C, a ring and sediment are present. In Dalmau plate culture on corn meal agar, pseudohyphae are not formed. Sexual structures are not observed on PDA, CM agar, and YCBS agar for two months. Ballistoconidia are not produced. Glucose fermentation is absent. Glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, methyl-α-D-glucoside, cellobiose, salicin, L-rhamnose, D-xylose, L-arabinose, D-arabinose, 5-keto-D-gluconate, glycerol (weak), ribitol, D-mannitol, D-glucitol, myo-inositol, succinate, citrate, D-gluconate, 2-keto-D-gluconate, D-glucuronate, and glucono-1,5-lactone are assimilated as sole carbon sources. L-Sorbose, D-ribose, methanol, ethanol, erythritol, galactitol, DL-lactate, D-glucosamine, and N-acetyl-D-glucosamine are not assimilated. Nitrate, nitrite, ethylamine, and L-lysine are assimilated as sole nitrogen sources. Cadaverine is not assimilated. Maximum growth temperature is 30 °C. Growth in vitamin-free medium is positive. Growth on 50% (w/w) glucose-yeast extract agar is negative. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is positive.

**Additional strain examined.** China • Guizhou Province, Qianxinan Buyei and Miao Autonomous Prefecture, Pingtang County, in the phylloplane of *Mussaenda pubescens*, February 2023, D. Lu, NYNU 23239.

**GenBank accession numbers.** Holotype GDMCC 2.309$^T$ (ITS: OP278683, D1/D2: OP278680); additional strain NYNU 23239 (ITS: PP114093, D1/D2: PP114095).

**Note.** *Filobasidium qingyuanense* sp. nov. can be physiologically distinguished from its closest known species, *F. dingjieense*, by its ability to assimilate inulin, raffinose, melibiose, lactose, salicin, L-rhamnose, ribitol, D-mannitol, and D-glucitol, as well as an inability to assimilate ethanol. Additionally, *F. qingyuanense* nov. can grow at 25 °C, while *F. dingjieense* cannot (Table 3).

## Discussion

The present study described three new species (*F. pseudomali* sp. nov., *F. castaneae* sp. nov., and *F. qingyuanense* sp. nov.) based on phylogenetic analyses and phenotypic characteristics. Phylogenetically, these three species fell within the *Filobasidium* clade and were separated from other known species of *Filobasidium* and each other (Figs 1, 2). In contrast, phenotypically, all three species possessed similar cell shape, colony morphology, and color, differing from the closest known species in physiological and biochemical characteristics (Table 3). Phylogenetic analyses and phenotypic characteristics documented in this study confirm the existence of these new species in China.

Since the inception of *Filobasidium* in 1968, several *Filobasidium* species have been described based on phenotype (Kwon-Chung 1977; Bandoni et al. 1991). The classification based on phenotypical features, however, was in many cases not consistent with the results obtained from phylogenetic analyses. With the development of molecular biology, ribosomal DNA gene sequencing technology has been widely employed for yeast identification. The D1/D2 domain of the LSU rRNA gene is the most commonly used molecular marker for species delimitation of *Filobasidium* through phylogenetic analysis, as revealed by Scorzetti et al. (2002) and Kwon-Chung (2011). However, strains of different *Filobasidium* species sometimes shared identical or similar D1/D2 sequences but showed distinct sequences of the ITS region (Fell et al. 2000). Scorzetti et al. (2002) suggested that both gene regions are necessary for reliable species delimitation. For example, zero to two substitutions are present in the D1/D2 domain of the ex-type strains of the closest related species within *Filobasidium*, including *F. floriforme* and *F. magnum* (zero nt difference), *F. globosum* and *F. mali* (one nt difference), and *F. floriforme* and *F. oeirense* (two nt differences) (Li et al. 2020). Likewise, *F. pseudomali*, described in this study, differed from its close relative *F. mali* by only two nt substitutions. The other gene markers, including *RPB1*, *RPB2*, and the translation elongation factor 1-alpha (*TEF1*), exhibit increased variation between these closely related, well-defined species relative to the low nucleotide differences in the D1/D2 domain (Liu et al. 2018; Li et al. 2020). Although the D1/D2 domain is still an appropriate marker to use for higher-level taxon delimitations, it is clear that this region alone is insufficient for all species delimitation in the *Filobasidium*. Therefore, the data obtained from multiple genetic markers can allow for more accurate insights into the relationships between distinct taxa within *Filobasidium*.

Members of the genus *Filobasidium* have been found in diverse substrates, especially plant materials, including flowers, leaves, and fruit. More than 50% of the described *Filobasidium* species are associated with plant materials (Olive 1968; Kwon-Chung 1977; Bandoni et al. 1991; Kemler et al. 2017; Li et al. 2020; Wei et al. 2022). Strains of *Filobasidium* species have also been isolated from soil (Bandoni et al. 1991; Hong et al. 2002; Vishniac 2006; Fonseca et al. 2011; Yurkov 2017; Yurkov 2018; Li et al. 2020; Wei et al. 2022) and glacier ice (Fonseca et al. 2011). In addition, *F. globisporum* has been recognized as a relevant yeast species for the malting processes (Laitila et al. 2006). Furthermore, several phylloplane isolates of *F. magnus* and *F. wieringae* had multiple enzymatic activities, specifically the capacity to hydrolyze gelatin, casein, carboxymethyl-cellulose, and polygalacturonic acid to varying degrees (Fonseca et al. 2011). The biotechnological relevance of these hydrolytic activities has not been assessed, but they may have ecological relevance in the decomposition of plant material. In this study, we isolated seven strains of three new *Filobasidium* species, *F. pseudomali* sp. nov., *F. castaneae* sp. nov., and *F. qingyuanense* sp. nov., in the phylloplane, which may have similar ecological roles as *F. magnus* and *F. wieringae*.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Data curation: CYC; Methodology: CYC; Molecular phylogeny: ZWX. and CYC; Writing – original draft: CYC; Writing – review and editing: QHN., FLH. All authors read and approved the final manuscript.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# Three new species and a new record of *Conocybe* section *Pilosellae* (Bolbitiaceae, Agaricales) from Jilin Province, China

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## Abstract

This study is based on the phylogenetic framework of *Conocybe* section *Pilosellae* and incorporates materials from Jilin Province. A systematic phylogenetic tree was constructed using maximum likelihood and Bayesian analyses of internal transcribed spacer region (ITS) and nuclear large subunit ribosomal DNA (nrLSU), and translation elongation factor 1-alpha (*tef1-α*) sequences. As a result, three new species were discovered in Jilin Province: *Conocybe verna*, which emerges in broad-leaved forests during spring; *C. angulispora*, characterized by angular and submitriform or slightly hexagonal basidiospores; and *C. rubrocyanea*, with basidiomata displaying a reddish hue when fresh and a bluish hue when dry. Additionally, a new record for China, *C. hexagonospora* was identified, characterized by the lack of distinct pubescence on the pileus and slightly hexagonal basidiospores, increasing the total number of species within sect. *Pilosellae* to 22. Key for sect. *Pilosellae* is provided, accompanied by morphological descriptions and line drawings for the new species and a new record for China.

**Key words:** *Conocybe* section *Pilosellae*, morphology, new taxa, phylogeny

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## Introduction

*Conocybe* Fayod belongs to the family Bolbitiaceae Singer and was established by Fayod 1889. Its taxonomic status has undergone multiple revisions and clarifications (Fries 1821; Fayod 1889; Kühner 1935; Singer 1949; Watling 1982). *Conocybe* sect. *Pilosellae* Singer, a basal group of *Conocybe*, was established by Singer in 1962 based on stipes with hairs and non-lecythiform caulocystidia (Singer 1962). The classification history has been summarized by Song and Bau (2023), and we won't go into further detail here. Please refer to the following references for more information (Kühner 1935; Singer 1949; Singer 1962; Watling 1982; Bon 1992; Arnolds 2005; Hausknecht 2005; Hausknecht and Krisai-Greilhuber 2006). Hausknecht and Krisai-Greilhuber (2006) divided sect. *Pilosellae* into two subsections, based on the size and shape of basidiospores, the presence of lecythiform caulocystidia, and habitat. The two subsections are subsect. *Pilosellae* and subsect. *Siligineae* Hauskn. & Krisai. Subsect. *Pilosellae* includes seven series: ser. *Pilosella*, ser. *Sienophylla* Hauskn. & Krisai, ser. *Anthracophila* Hauskn. & Krisai, ser. *Bispora* Hauskn. & Krisai, ser. *Microrrhiza* Hauskn. & Krisai, ser. *Inocybeoides* Hauskn. & Krisai, and ser. *Cylindracea* Hauskn. & Krisai. Subsection *Siligineae* Hauskn. & Krisai includes four series: ser. *Siliginea* Hauskn. & Krisai, ser. *Fimetaria* Hauskn. & Krisai, ser. *Murinacea* Hauskn. & Krisai, and ser. *Lenticulospora* Hauskn. & Krisai (Hausknecht and Krisai-Greilhuber 2006; Hausknecht 2009). Tóth et al. (2013) included members of sect. *Pilosellae* in a molecular phylogenetic analysis of Bolbitiaceae based on combined dataset of ITS, nrLSU, and *tef1-α* sequences, providing important reference data for subsequent phylogenetic studies of Bolbitiaceae. Species of sect. *Pilosellae* have a wide distribution and are mostly found in fertile soil and herbivore dung (Hausknecht 2009). They contain toxic substances such as psilocybin, phallotoxins, and amatoxins, and have certain pharmacological value (Griffiths et al. 2016; Johnson et al. 2017; Paul 2021).

In China, research on *Conocybe* began with Tai (1979). As of 2023, a total of 40 species of *Conocybe* have been recorded in China, including 17 species in sect. *Pilosellae* (Tai 1979; Xie et al. 1986; Li et al. 1993; Bi et al. 1994; Yuan and Sun 1995; Zhang and Mao 1995; Li and Bau 2003; Li and Azbukina 2011; Bau et al. 2014; Li et al. 2015; Wang and Tzean 2015; Bau 2016; Liu and Bau 2018; Liu 2018; Zhang 2019; Ye 2021; Song et al. 2023; Song and Bau 2023). However, it is important to note that the majority of these species are distributed in Northeast China.

Jilin Province is characterized by a temperate monsoon climate with distinct seasons. The summers are rainy and warm, while the winters are dry and cold. The region's main geographical features include mountains and plains. Forested areas are mainly concentrated in the Changbai Mountains, where diverse vegetation types, such as mixed coniferous and broadleaf forests, dominate. The region's excellent natural environment and abundant vegetation provide favorable conditions for fungal diversity. However, the number of reported species of sect. *Pilosellae* in Jilin province is significantly lower compared to Europe and North America at the same latitude. This indicates the need for further investigation and research. Based on results from this study, the number of species of sect. *Pilosellae* species in China is increased to 22 (include *C. siliginea*, collected from Henan Province).

## Materials and methods

### Samplings and morphological analyses

The specimens for this study were collected from Jilin Province, China, from 2022 to 2023. Upon discovery, photographs were taken, and information on habitat and morphological features was recorded. Subsequently, the materials were dried using silica gel desiccants, prepared as specimens, and stored at the Fungarium of Jilin Agricultural University (FJAU). To examine the microscopic structures of the specimens, they were treated with a 5% KOH solution and a 1% Congo red solution. Reacting with lamellar structures using a 25% ammonia solution (Hausknecht 2009). The observations were made using a Carl Zeiss Primo Star optical microscope from Jena, Germany. Additionally, the color of fresh or dried basidiomata was described using the color-coding system developed by the German Institute for Quality Assurance and Certification

(Reichs-Ausschuss fur Lieferbedingungen und Guetesicherung, available at https://www.ral-guetezeichen.de/), the abbreviation used in the text (RAL).

In this study, the basidiospore measurements do not include the apiculus. They are presented as '(a)b–c(d)', where 'b–c' represents the minimum at least 90% of the measured values, and 'a' and 'd' represent the extreme values. To accurately record their dimensions, the main body (excluding sterigmata or excrescences) of the basidia, cheilocystidia, caulocystidia, and pileipellis were measured if present. At least 20 were measured. The notation (n/m/p) indicates that the measurements were made on "n" randomly selected basidiospores from "m" basidiomata of "p" collections. Twenty basidiospores are measured from each basidioma. This sampling method ensures a representative measurement sample. The ratio of length divided by width, known as Q, provides a measure of the elongation of the spores. The average quotient (length/width and breadth), denoted as Qm, is calculated along with the standard deviation to provide an overall average value with variation.

## DNA extraction, PCR amplification, and sequencing

To extract total genomic DNA from the dried specimens, we followed the manufacturer's instructions and used a NuClean Plant Genomic DNA kit (ComWin Biotech, CW0531M, Taizhou, China). For amplifications, we employed the primer pairs ITS1F/ITS4 (White et al. 1990; Gardes and Bruns 1993), LR0R/LR7 (Vilgalys and Hester 1990; Moncalvo et al. 2000), and EF1-983F/EF1-2218R (Rehner and Buckley 2005) for the ITS, nrLSU, and tef1-α sequences, respectively. Polymerase chain reaction (PCR) amplification was conducted on a Bio-Rad T100TM Thermal cycler (Bio-RAD Inc., Hercules, CA, USA). In a 30 μL reaction mixture, we used the following final concentrations or total amounts: 2 μL of template DNA, 15 μL of 2× SanTaq PCR Master Mix (B532061, Sangon Biotech, Shanghai, China), 1.5 μL of each primer, and 10 μL of double-distilled water ($ddH_2O$).

The PCR protocol for ITS and nrLSU involved the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C (ITS, nrLSU) for 30 s, and extension at 72 °C for 45 s (ITS)/80 s (nrLSU). The final extension was performed at 72 °C for 10 min, followed by cooling at 4 °C indefinitely. For *tef1-α*, the touchdown PCR protocol was as follows: initial denaturation at 94 °C for 3 min, followed by 8 cycles of denaturation at 94 °C for 40 s, annealing at 60 °C for 40 s (with the temperature decreasing by 1 °C per cycle), and extension at 72 °C for 2 min. After the initial cycles, the denaturation step was repeated at 94 °C for 45 s, followed by annealing at 60 °C for 40 s and extension at 72 °C for 2 min, repeated for a total of 36 cycles. Finally, a final extension step was performed at 72 °C for 10 min and cooling at 15 °C indefinitely.

Following the PCR amplification, the products were electrophoresed on a 1% agarose gel along with known standard DNA markers. The resulting PCR products were sent for sequencing services to Sangon Biotech (Shanghai) Co., Ltd., and sequence data was obtained. To ensure the quality of the chromatograms, they were checked in BioEdit v7.2.5 (Hall 1999), ensuring that each base was of good quality. Additionally, a BLAST search was conducted using the National Center of Biotechnology Information (NCBI) database to confirm that the sequencing results matched the specimens. Finally, the sequences were submitted to GenBank.

### Phylogenetic analyses

Sequences were downloaded from GenBank (Table 1). The ITS, nrLSU, and *tef1-α* sequences were aligned using the G-INS-i algorithm with two iterative cycles only via the online Mafft tool (Katoh et al. 2019; https://mafft.cbrc.jp/alignment/server/). The resulting alignment was then manually refined and trimmed using MEGA7 (Kumar et al. 2016). To generate the concatenated alignment, PhyloSuite 1.2.2 (Zhang et al. 2020) was employed. The best-fit partition model (edge-unlinked) was selected using the BIC criterion with ModelFinder v2.2.0 (Kalyaanamoorthy et al. 2017). For maximum likelihood phylogenies, IQ-TREE was used under the Edge-linked partition model for 1000 standard bootstraps, along with the Shimodaira-Hasegawa-like approximate likelihood-ratio test, with settings based on the results from ModelFinder (Nguyen et al. 2015; Guindon et al. 2010). Bayesian inference phylogenies were inferred using MrBayes 3.2.7a under the partition model (Ronquist et al. 2012) through two parallel runs (MCMC) and 4,500,000 generations, discarding the initial 25% of the sampled data as burn-in, average standard deviation of split frequencies is 0.009. Finally, the figures were edited using iTOL (Letunic and Bork 2019), Adobe Photoshop 2021, and Adobe Illustrator 2021. The outgroup used was the *Psathyrella* species (Song and Bau 2023).

## Results

### Phylogenetic analyses

The Bayesian tree was constructed based on a combined dataset of ITS, nrLSU, and *tef1-α*, while the ML phylogenetic tree was not presented due to their similar topology. Bootstrap support values were indicated on the tree nodes. Only the data meeting the criteria of Bayesian posterior probabilities (PP ≥ 0.9) and ML bootstrap values (MLbs ≥ 70%) were retained (Fig. 1). The multi-locus dataset (ITS + nrLSU + *tef1-α*) of *Conocybe* comprised 826 bp for ITS, 1299 bp for nrLSU, and 1131 bp for *tef1-α*. The alignment included 94 sequences with 3256 columns, resulting in 1446 distinct patterns, 997 parsimony-informative sites, 316 singleton sites, and 1943 constant sites. During the construction of ML phylogenetic trees, the best-fit models, GTR+F+R4 for ITS, TIM3+F+I+I+R2 for nrLSU, and TIM2e+I+I+R4 for *tef1-α* based on the BIC. Similarly, for Bayesian phylogenetic trees, the best-fit models according to the BIC were GTR+F+I+G4 for ITS and nrLSU, and SYM+I+G4 for *tef1-α*.

In the phylogenetic tree, the newly proposed species are indicated in bold red color, while the newly recorded species is indicated in bold black color (Fig. 1). Notably, specimens FJAU65123, FJAU71654, and HMJAU64964 clustered together, forming a distinct branch and serving as sister taxa to *Conocybe muscicola* T. Bau & H.B. Song. However, their phylogenetic relationship exhibits low support, with a PP/MLbs value of 0.7/55. Similarly, FJAU65120 and FJAU65122 comprise a separate branch and act as sister taxa to *C. hexagonospora* Métrod ex Hauskn. & Enderle (FJAU71661), demonstrating a PP/MLbs value of 1/84. Furthermore, FJAU65117 and FJAU65118 form another distinct branch and serve as sister taxa to *C. ingridiae* Hauskn. and *C. ochrostriata* var. *favrei* Hauskn., with a PP/MLbs value of 1/99. We conducted a Standard Nucleotide BLAST of the ITS sequences of *Conocybe verna*

**Table 1.** Information on the DNA sequences used to reconstruct phylogenetic trees. Sequences in bold were newly generated in this study. T = holotype.

| Taxon | Voucher specimen | GenBank accession numbers | | | Origin | References |
|---|---|---|---|---|---|---|
| | | ITS | nrLSU | *tef1-α* | | |
| *Bolbitius coprophilus* | HMJAU64958 | OQ780315 | OQ758216 | – | China | Song and Bau 2023 |
| *B. coprophilus* | SZMC-NL-2640 | JX968253 | JX968370 | – | Hungary | Tóth et al. 2013 |
| *B. reticulatus* | WU30001 | JX968249 | JX968366 | JX968455 | Hungary | Tóth et al. 2013 |
| *B. subvolvatus* | WU28379 | JX968248 | JX968365 | JX968454 | Italy | Tóth et al. 2013 |
| *Conocybe alkovii* | LE262841 | JQ247196 | – | – | Russia | Malysheva 2012 |
| ***C. angulispora*** | **FJAU65120 T** | **PP501383** | **PP501393** | **PP501651** | **China** | **This study** |
| ***C. angulispora*** | **FJAU65122** | **PP501384** | **PP501394** | **PP501652** | **China** | **This study** |
| *C. anthracophila* var. *ovispora* | WU25461 | JX968237 | JX968355 | – | Italy | Tóth et al. 2013 |
| *C. antipus* | WU19791 | JX968215 | JX968332 | JX968432 | Austria | Tóth et al. 2013 |
| *C. bispora* | SZMC-NL-2573 | JX968203 | JX968320 | JX968423 | Hungary | Tóth et al. 2013 |
| *C. bisporigera* | SZMC-NL-1904 | JX968235 | JX968353 | JX968446 | Hungary | Tóth et al. 2013 |
| *C. brachypodii* | HMJAU45017 | MH141423 | – | – | China | Liu 2018 |
| *C. brunneidisca* | HMJAU45069 | OQ780317 | – | – | China | Song and Bau 2023 |
| *C. ceracea* | HMJAU64951 | OQ758110 | OQ758218 | OQ758305 | China | Song and Bau 2023 |
| *C. coniferarum* | LE313009 | KY614061 | – | – | Russia | Malysheva 2017 |
| *C. crispella* | WU27367 | JX968208 | JX968325 | JX968426 | Australia | Tóth et al. 2013 |
| *C. cylindracea* | WU20796 | JX968240 | JX968358 | JX968449 | Italy | Tóth et al. 2013 |
| *C. cylindrospora* | HMJAU42440 | MG250375 | OQ758203 | – | China | Liu and Bau 2018;<br>Song and Bau 2023 |
| *C. deliquescens* | HMJAU61998 | OP373403 | OQ758204 | OQ758292 | China | Song and Bau 2023 |
| *C. elegans* | SZMC-NL-0908 | JX968223 | JX968341 | JX968437 | Sweden | Tóth et al. 2013 |
| *C. enderlei* | WU21272 | JX968163 | JX968279 | – | Italy | Tóth et al. 2013 |
| *C. fuscimarginata* | HMJAU45033 | OQ780310 | OQ758208 | OQ758296 | China | Song and Bau 2023 |
| *C. fuscimarginata* | SZMC-NL-3668 | JX968238 | JX968356 | JX968448 | Sweden | Tóth et al. 2013 |
| *C. gigasperma* | SZMC-NL-3972 | JX968179 | JX968295 | JX968403 | Slovakia | Tóth et al. 2013 |
| *C. hausknechtii* | LE253789 | JQ247194 | – | – | Russia | Malysheva 2013 |
| ***C. hexagonospora*** | **FJAU71661** | **PP501385** | **PP501395** | **PP501653** | **China** | **This study** |
| *C. hydrophila* | HMJAU64954 | OQ758116 | OQ758232 | OQ758313 | China | Song and Bau 2023 |
| *C. incarnata* | FJAU71663 | PP501390 | PP501400 | PP501658 | China | Song and Bau 2023 |
| *C. incarnata* | WU21897 | JX968229 | JX968347 | JX968441 | Finland | Tóth et al. 2013 |
| *C. incerta* | LE313017 | KY614062 | – | – | Russia | Malysheva 2017 |
| *C. ingridiae* | WU28158 | JX968244 | JX968361 | JX968451 | Italy | Tóth et al. 2013 |
| *C. karakensis* | KTK05 | ON392730 | – | – | Pakistan | Ullah et al. 2023 |
| *C. lenticulospora* | SZMC-NL-0923 | JX968242 | JX968359 | JX968450 | Sweden | Tóth et al. 2013 |
| *C. mesospora* | HMJAU45049 | MH141419 | – | – | China | Liu 2018 |
| *C. microrrhiza* | SZMC-NL-2180 | JX968222 | JX968340 | JX968436 | Hungary | Tóth et al. 2013 |
| *C. moseri* | GLM-F40421 | MK412354 | – | – | Germany | Unpublished |
| *C. moseri* | HMJAU45075 | OQ780309 | OQ758207 | – | China | Song and Bau 2023 |
| *C. muscicola* | HMJAU64939 | OQ758113 | OQ758223 | OQ758309 | China | Song and Bau 2023 |
| *C. nigrescens* | WU27557 | JX968234 | JX968352 | JX968445 | Italy | Tóth et al. 2013 |
| *C. nitrophila* | WANG140019 | KR998384 | – | – | China | Wang and Tzean 2015 |
| *C. nitrophila* | WU20916 | JX968233 | JX968351 | JX968444 | India | Tóth et al. 2013 |
| *C. ochrostriata* var. *favrei* | WU29786 | JX968245 | JX968362 | JX968452 | Italy | Tóth et al. 2013 |
| *C. olivaceopileata* | LE313106 | KY614059 | – | – | Russia | Malysheva 2017 |
| *C. pallidospora* | WU7395 | JX968239 | JX968357 | – | Austria | Tóth et al. 2013 |
| *C. parapilosella* | 90551 | MN872706 | – | – | Spain | Siquier and Salom 2021 |
| *C. pilosella* | HMJAU45062 | OQ780305 | OQ758205 | OQ758294 | China | Song and Bau 2023 |
| *C. pilosa* | HMJAU64947 | OQ758122 | OQ758222 | OQ758307 | China | Song and Bau 2023 |

| Taxon | Voucher specimen | GenBank accession numbers | | | Origin | References |
|---|---|---|---|---|---|---|
| | | ITS | nrLSU | *tef1-α* | | |
| *C. praticola* | HMJAU64965 | OQ780303 | – | – | China | Song and Bau 2023 |
| *C. pseudocrispa* | HMJAU64946 | OQ780307 | OQ758212 | OQ758293 | China | Song and Bau 2023 |
| *C. pseudocrispa* | WU18009 | JX968230 | JX968348 | JX968442 | Austria | Tóth et al. 2013 |
| *C. pubescens* | WU20759 | JX968170 | JX968286 | JX968396 | Italy | Tóth et al. 2013 |
| *C. reniformis* | HMJAU64942 | OQ758108 | OQ758229 | OQ758311 | China | Song and Bau 2023 |
| *C. rickenii* | AH21067 | MF142238 | – | – | Spain | Siquier and Salom 2018 |
| *C. romagnesii* | HMJAU64960 | OQ780304 | – | – | China | Song and Bau 2023 |
| *C. rostellata* | SZMC-NL-2499 | JX968162 | JX968278 | JX968390 | Sweden | Tóth et al. 2013 |
| *C. rubrocyanea* | HMJAU64964 | OQ749742 | – | – | China | Song and Bau 2023 |
| ***C. rubrocyanea*** | **FJAU65123 T** | **PP501388** | **PP501398** | **PP501656** | **China** | **This study** |
| ***C. rubrocyanea*** | **FJAU71654** | **PP501389** | **PP501399** | **PP501657** | **China** | **This study** |
| *C. rufostipes* | HMJAU64937 | OQ758120 | OQ758227 | OQ758317 | China | Song and Bau 2023 |
| *C. semiglobata* | WU8794 | JX968188 | JX968304 | – | Austria | Tóth et al. 2013 |
| *C. siennophylla* | HMJAU64966 | OQ780312 | OQ758210 | OQ758297 | China | Song and Bau 2023 |
| *C. siennophylla* | SZMC-NL-1210 | JX968246 | JX968363 | JX968453 | Hungary | Tóth et al. 2013 |
| *C. siliginea* | SZMC-NL-2313 | JX968225 | JX968343 | JX968438 | Sweden | Tóth et al. 2013 |
| ***C. siliginea*** | **FJAU71664** | **PP501392** | **PP501402** | **PP501660** | **China** | **This study** |
| *C. singeriana* | HMJAU64956 | OQ780314 | OQ758214 | – | China | Song and Bau 2023 |
| *C. singeriana* | WU22129 | JX968166 | JX968282 | JX968393 | Austria | Tóth et al. 2013 |
| *C. sinobispora* | HMJAU64949 | OQ758118 | OQ758230 | OQ758315 | China | Song and Bau 2023 |
| *Conocybe* sp.1 | HMJAU44988 | OQ749737 | OQ740305 | OQ758302 | China | Song and Bau 2023 |
| *Conocybe* sp.2 | HMJAU64963 | OQ749740 | OQ740307 | OQ758304 | China | Song and Bau 2023 |
| *Conocybe* sp.3 | HMJAU64967 | OQ749741 | – | – | China | Song and Bau 2023 |
| *C. tetrasporoides* | WU17385 | JX968232 | JX968350 | – | New Zealand | Tóth et al. 2013 |
| *C. velutinomarginata* | WU28695 | JX968226 | JX968344 | JX968439 | Germany | Tóth et al. 2013 |
| ***C. velutipes*** | **FJAU71662** | **PP501391** | **PP501401** | **PP501659** | **China** | **This study** |
| *C. velutipes* | SZMC-NL-2187 | JX968228 | JX968346 | JX968440 | Hungary | Tóth et al. 2013 |
| ***C. verna*** | **FJAU65117 T** | **PP501386** | **PP501396** | **PP501654** | **China** | **This study** |
| ***C. verna*** | **FJAU65118** | **PP501387** | **PP501397** | **PP501655** | **China** | **This study** |
| *C. volvicystidiata* | LIP0001212 | KY346827 | – | – | France | Hausknecht and Broussal 2016 |
| *C. watlingii* | WU22744 | JX968172 | JX968288 | JX968398 | Finland | Tóth et al. 2013 |
| *Conocybula. coprophila* | SZMC-NL-2176 | JX968156 | JX968273 | – | Hungary | Tóth et al. 2013; Song and Bau 2024 |
| *Co. cyanopus* | WU2134 | JX968157 | JX968274 | JX968388 | Austria | Tóth et al. 2013; Song and Bau 2024 |
| *Co. smithii* | HMJAU62001 | OP373407 | OQ758215 | OQ758300 | China | Song and Bau 2023; Song and Bau 2024 |
| *Conobolbitina dasypus* | SZMC-NL-2279 | JX968152 | JX968269 | JX968385 | Hungary | Tóth et al. 2013; Song and Bau 2024 |
| *Descolea antarctica* | NZ5182 | AF325647 | – | – | USA | Peintner et al. 2001 |
| *D. quercina* | HMJAU64959 | OQ780313 | OQ758213 | OQ758299 | China | Song and Bau 2023 |
| *Pholiotina arrhenii* | SZMC-NL-2509 | JX968261 | JX968377 | – | Sweden | Tóth et al. 2013 |
| *Ph. brunnea* | SZMC-NL-1216 | JX968259 | JX968375 | JX968461 | Hungary | Tóth et al. 2013 |
| *Ph. dentatomarginata* | SZMC-NL-2921 | JX968258 | JX968374 | JX968460 | Hungary | Tóth et al. 2013 |
| *Ph. serrata* | HMJAU62006 | OP538570 | OQ758217 | OQ758301 | China | Song and Bau 2023 |
| *Ph. sulcata* | SZMC-NL-1975 | JX968153 | JX968270 | JX968386 | Hungary | Tóth et al. 2013 |
| *Ph. teneroides* | SZMC-NL-3501 | JX968264 | JX968379 | JX968465 | Slovakia | Tóth et al. 2013 |
| *Ph. utricystidiata* | WU20164 | JX968262 | JX968463 | – | Germany | Tóth et al. 2013 |
| *Ph. vexans* | SZMC-NL-3967 | JX968265 | JX968380 | JX968466 | Slovakia | Tóth et al. 2013 |
| *Psathyrella leucotephra* | SZMC-NL-1953 | FM163226 | FM160683 | FM897219 | Hungary | Nagy et al. 2011 |
| *P. piluliformis* | HMJAU37922 | MG734716 | MW413364 | MW411001 | China | Yan and Bau 2018 |

Figure 1. The phylogenetic relationships of *Conocybe* sect. *Pilosellae* in Bolbitiaceae using Bayesian inference and maximum likelihood methods based on a multi-locus dataset (ITS, nrLSU, and *tef1-α*). In the phylogenetic tree, the newly proposed species are indicated in bold red color, while the newly recorded species is indicated in bold black color, the outgroup is *Psathyrella* species, T = holotype.

(FJAU65117), *C. angulispora* (FJAU65120), and *C. rubrocyanea* (FJAU65123) against the NCBI database. The results, presented in descending order of similarity, showed that *C. verna* had a similarity of 96.7% with *Conocybe* cf. *rostellata* (SMNS-STU-F-0900917), 92.8% with *C. ingridiae* (WU28158), and 93.1% with *C. ochrostriata* var. *favrei* (WU29786). *Conocybe angulispora* exhibited a similarity of 98.2% with *C. lenticulospora* (HMJAU45069), 98.6% with *C. cylindracea* (WU20796), and 88.7% with *C. hydrophila*. The similarity between *C. rubrocyanea* and *C. muscicola* (HMJAU64939) was 95.2%, with *C. velutipes* (SZMC-NL-2187) was 94.4%, and with *C. fuscimarginata* (HMJAU45033) was 93.8%. And then based on the phylogenetic tree and morphological findings, three new species are proposed: *C. rubrocyanea* (for the clade FJAU65123, FJAU71654, and HMJAU64964), *C. angulispora* (for the clade FJAU65120 and FJAU65122), and *C. verna* (for the clade FJAU65117 and FJAU65118). While the type specimen of *C. hexagonospora* lacks sequence data, the identification of this species as a new record for China was accomplished through traditional morphology, and reference sequences have been provided to facilitate future confirmation. Finally, the branch containing *C. angulispora*, *C. hexagonospora*, *C. cylindracea* Maire & Kühner ex Singer, and *C. brunneidisca* (Murrill) Hauskn. referred to as ser. *Lenticulospora*, following the viewpoint of Hausknecht and Krisai-Greilhuber (2006) (for more detailed information, please refer to the Discussion section).

## Taxonomy

***Conocybe verna*** **T. Bau & H. B. Song, sp. nov.**
MycoBank No: 852866
Figs 2A–D, 3, 4

**Etymology.** "*verna*" refers to spring-born.

**Holotypus.** China, • Jilin Province, Tonghua City, Ji'an City, Yushan Park, 8 May 2023, 41°08'01"N, 126°10'45"E, alt. 280 m, Zheng-Qing Chen, CZQ23050801 (FJAU65117).

**Diagnosis.** The main characteristic of *Conocybe verna* includes a straight to reflexed edge of the pileus after maturity, with no surface pubescence. The basidiospores exhibit a suprahilar depression and have an oblong, subcylindrical shape with a slightly thin wall. The basidia are 2-spored.

**Description.** Basidioma mycenoid. Pileus diameter 0.5–2.5 cm, initially paraboloid, nearly hemispherical, margin deflexed, matured obtusely conical, campanulate, margin straight to reflexed. Pileus initially beige (RAL1001) to ivory (RAL1014), matured light ivory (RAL1015), powdery yellow (RAL1034) to ochre brown (RAL8001), surface hygrophanous, pubescence absent, when moist, it exhibits striae, which disappear upon slight drying, margin undulate. Context thin, ivory (RAL1014) to beige (RAL1001), no specific odor or taste. Lamellae adnexed to narrowly adnate, ventricose, crowded, unequal in length, ivory (RAL1014), powdery yellow (RAL1034) to ochre brown (RAL8001), smooth margin. Stipe 2.0–8.0 cm long, 1.0–3.0 mm thick, cylindrical, slightly thicker downward, ivory (RAL1014) to ochre brown (RAL8001), deer brown (RAL8007), surface pruinose and short pubescent, longitudinally fibrous striate, subbulbous at the base.

***Basidiospores*** (60/3/3) (10–)11–15.5(–16) × (5.5–)6–8.5(–9) μm, Q=(1.65–)1.71–2.07(–2.21), Qm = 1.86(±0.10), with a suprahilar depression, oblong, subcylindrical, wall slightly thin, containing oil droplets, germ pore diameter 0.5–2.0 μm. Basidiospores in 5% KOH solution appear ochre brown (RAL8001) to copper brown (RAL8004). Basidia (20–)21–33(–35) × (7–)8–11 μm, clavate, 2-spored, sterigmata 3–7 μm long, basidia with vacuolar contents. Cheilocystidia (16–)17–25(–26) × (6–)7–11(–13) μm, lecythiform, with capitula 3–6 μm wide. Caulocystidia ellipsoid to oblong, lageniform, long-necked lageniform, subcylindrical, clavate, narrowly utriform to utriform, fusiform, conical, nettle hair-shaped, (9–)10–50(–53) × 5–12 μm, with capilliform elements reaching up to 80 μm, among which rare lecythiform cystidia are mixed. Pileipellis hymeniform, composed of (23–)31–63(–65) × (14–)15–22(–23) μm sphaeropedunculate elements, with yellow pigments at the base. Pileocystidia absent. All structures have clamp connections. Weakly positive reaction with ammonia forming rhomboid crystals.

**Habitat.** Found singly or scattered in broad-leaved forests during spring.

**Known distribution.** Jilin Province, China.

**Additional specimens measured.** China, • Jilin Province, Tonghua City, Ji'an City, Yushan Park, 8 May 2023, 41°08'01"N, 126°10'45"E, alt. 280 m, Qian-Ru Liu, LQR23050801 (FJAU65118); • Tonghua City, Ji'an City, Jiangkou Village, 9 May 2023, 40°59'37"N, 126°03'02"E, alt. 260 m, Mu Liu, LM230509 (FJAU65119).

**Notes.** *Conocybe verna* is classified in sect. *Pilosellae* primarily due to the presence of non-lecythiform caulocystidia. The distinguishing characteristics of *C. verna* from other 2-spored species in sect. *Pilosellae* are as follows: *C. verna* differs from *C. bisporigera* (Hausknecht & Krisai) Arnolds in that the latter has a chocolate brown pileus and lentiform basidiospores (Arnolds 2003). The distinction between *C. verna* and *C. caespitosa* (Murrill) Watling is that the latter has basidiospores with a suprahilar plage and basidia measuring 19–24 μm in length, which is shorter than the basidia of *C. verna* (Hausknecht 2009). In contrast to *C. bispora* (Singer) Hauskn., *C. verna* has a pileus without distinct striations, while the basidiospores of *C. bispora* are on average 2 μm shorter (Hausknecht 1998). The distinction between *C. verna* and *C. umbellula* var. *lednicensis* lies in the latter having a striate pileus, and basidia measuring less than 20 μm in length (Hausknecht 2009). Furthermore, *C. verna* is differentiated from *C. leporina* (Velen.) Singer and *C. microrrhiza* Hauskn. by the presence of a pseudorhiza in the latter two, as well as their smaller basidiospores (Singer 1989; Hausknecht 1999). *Conocybe verna* differs from *C. inocybeoides* Watling in that the latter has a pileus with radiating striations and possesses pileocystidia (Watling 1980). Additionally, *C. verna* is distinguished from *C. velutinomarginata* Hauskn. & Zugna and *C. rickenii* (Jul. Schäff.) Kühner by the presence of capilliform pileocystidia in the latter two; *C. velutinomarginata* has a nearly spherical pileus, while *C. rickenii* has a grayish-brown pileus (Kühner 1935; Hausknecht 2009). *Conocybe verna* can be differentiated from *C. siliginea* (Fr.) Kühner by the latter's lime-colored pileus and lecythiform pileocystidia (Kühner 1935). Finally, the distinction between *C. verna* and *C. gigasperma* Enderle & Hauskn. lies in the latter's basidiospores measuring 18.3–20.1 μm in length, which are larger than those of *C. verna*, and the presence of pileocystidia (Hausknecht and Enderle 1992). *Conocybe verna* is also distinguished from *C. sinobispora* T. Bau & H.B. Song, as the latter has a striate pileus and cylindrical to lageniform pileocystidia (Song and Bau 2023).

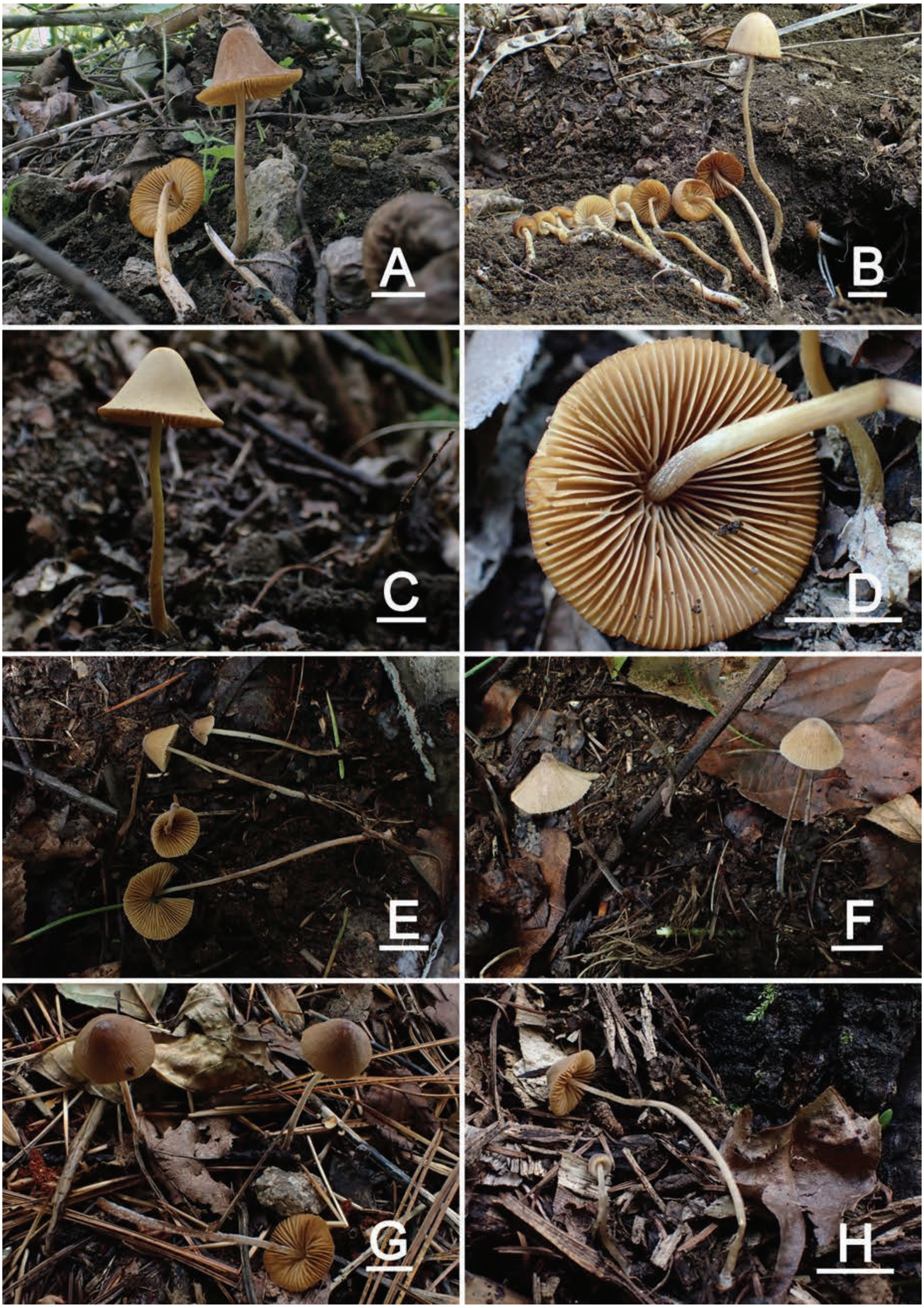

**Figure 2.** Basidiomata of *Conocybe* sect. *Pilosellae* species **A** *C. verna* (FJAU65117 T) **B** *C. verna* (FJAU65118) **C, D** *C. verna* (FJAU65119) **E, F** *C. angulispora* (FJAU65120 T) **G** *C. angulispora* (FJAU65122) **H** *C. angulispora* (FJAU65121) **I** *C. rubrocyanea* (FJAU65123 T) **J** *C. rubrocyanea* (FJAU71654) **K** *C. rubrocyanea* (FJAU71658) **L** *C. rubrocyanea* (FJAU71652) **M** *C. rubrocyanea* (FJAU71650) **N–P** *C. hexagonospora* (FJAU71661), Scale bars: 1 cm, T = holotype.

**Figure 2.** Continued.

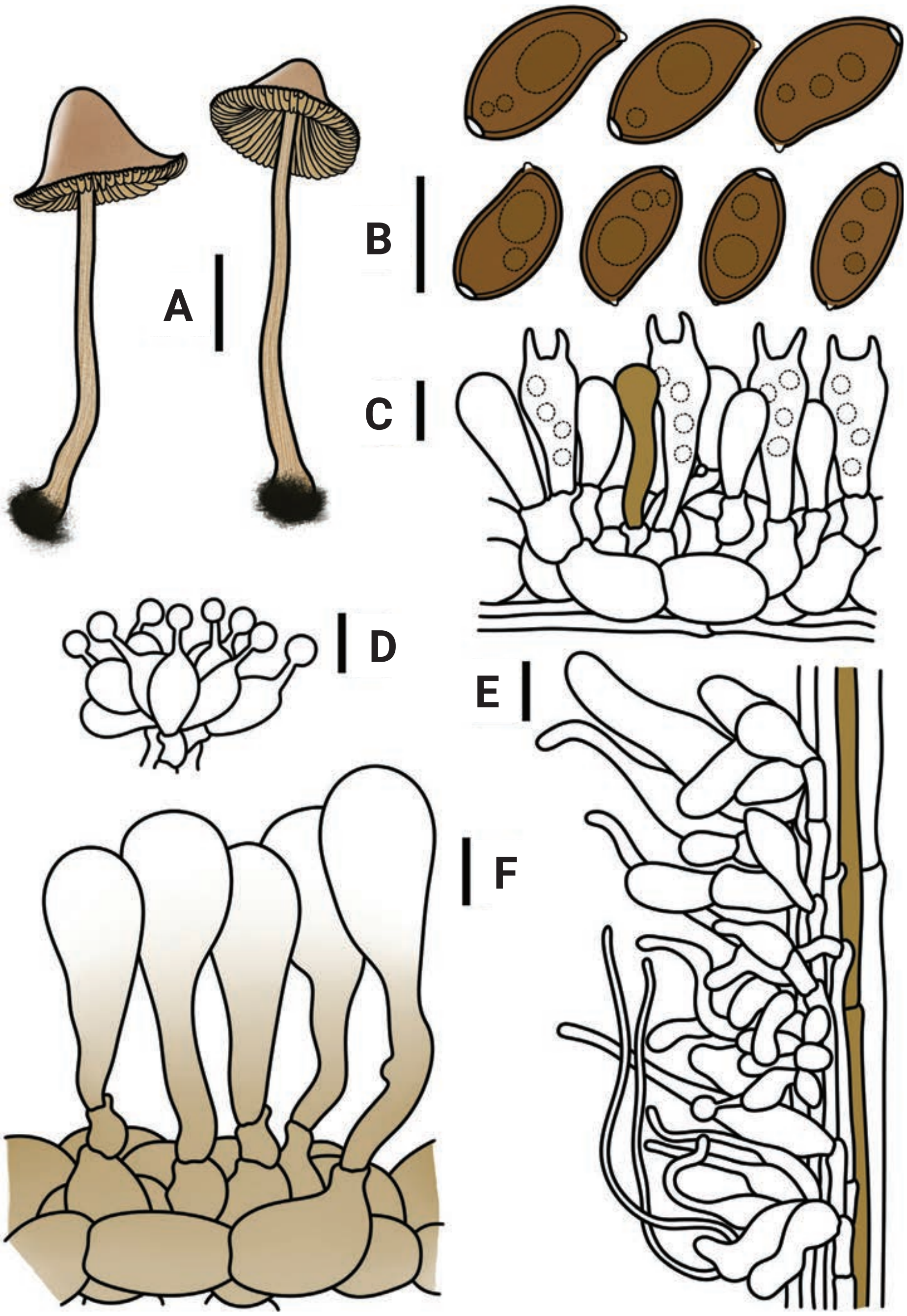

**Figure 3.** *Conocybe verna* (FJAU65117) **A** basidiomata **B** basidiospores in KOH **C** hymenium and subhymenium **D** cheilocystidia **E** stipitipellis **F** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–F**).

In terms of phylogeny, *C. verna* is closely related to *C. ingridiae* and *C. ochrostriata* var. *favrei*. However, *C. ingridiae* has a pileus with distinct striations and basidiospores measuring 9.6–10.5 µm in length, while *C. ochrostriata* var. *favrei* also has a striate pileus and possesses 4-spored basidia, making them easily distinguishable (Hausknecht 2009). Among these similar species, the following have been sequenced and are clearly separated in the phylogeny: *C. bisporigena*, *C. bispora*, *C. ingridiae*, *C. microrrhiza*, *C. velutinomarginata*, *C. rickenii*, *C. siliginea*, and *C. sinobispora*.

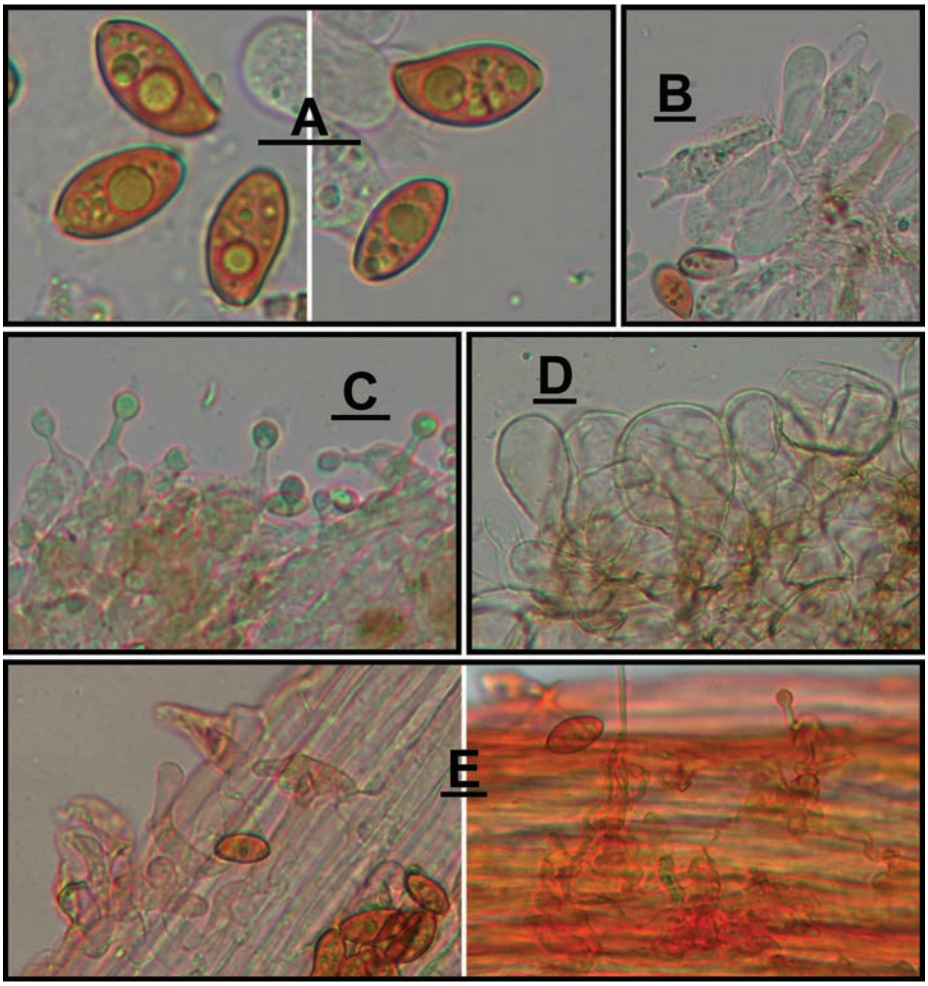

**Figure 4.** Microscopic structure images of *Conocybe verna* (FJAU65117) **A** basidiospores **B** basidia **C** cheilocystidia **D** pileipellis **E** stipitipellis. Scale bars: 10 μm (**A–E**).

***Conocybe angulispora*** **T. Bau & H. B. Song, sp. nov.**
MycoBank No: 852867
Figs 2E–H, 5, 6

**Etymology.** *"angulispora"* refers to basidiospores that are angular and submitriform or slightly hexagonal in shape.

**Holotypus.** China, • Jilin Province, Jilin City, Jiaohe City, Shansongling, 26 August 2023, 43°32'25"N, 127°02'21"E, alt. 550 m, Hong Cheng, C2382612 (FJAU65120).

**Diagnosis.** *Conocybe angulispora* basidiospores are lentiform, frontal view slightly hexagonal or submitriform, side view ellipsoid to oblong, ovoid, amygdaliform, basidia are 4(2)-spored, and pileocystidia are abundant.

**Description.** Basidioma mycenoid. Pileus diameter 0.5–2.5 cm, initially paraboloid to obtusely conical, later conical to broadly conical, edge straight, undulate. In early stages, pileus center color ranges from signal brown (RAL8002) to mahogany brown (RAL8016), with slightly lighter color at the edges, brown beige (RAL1011), sandy yellow (RAL1002) to maize yellow (RAL1001). When mature, pileus center color changes to reddish-brown (RAL8012) to mahogany

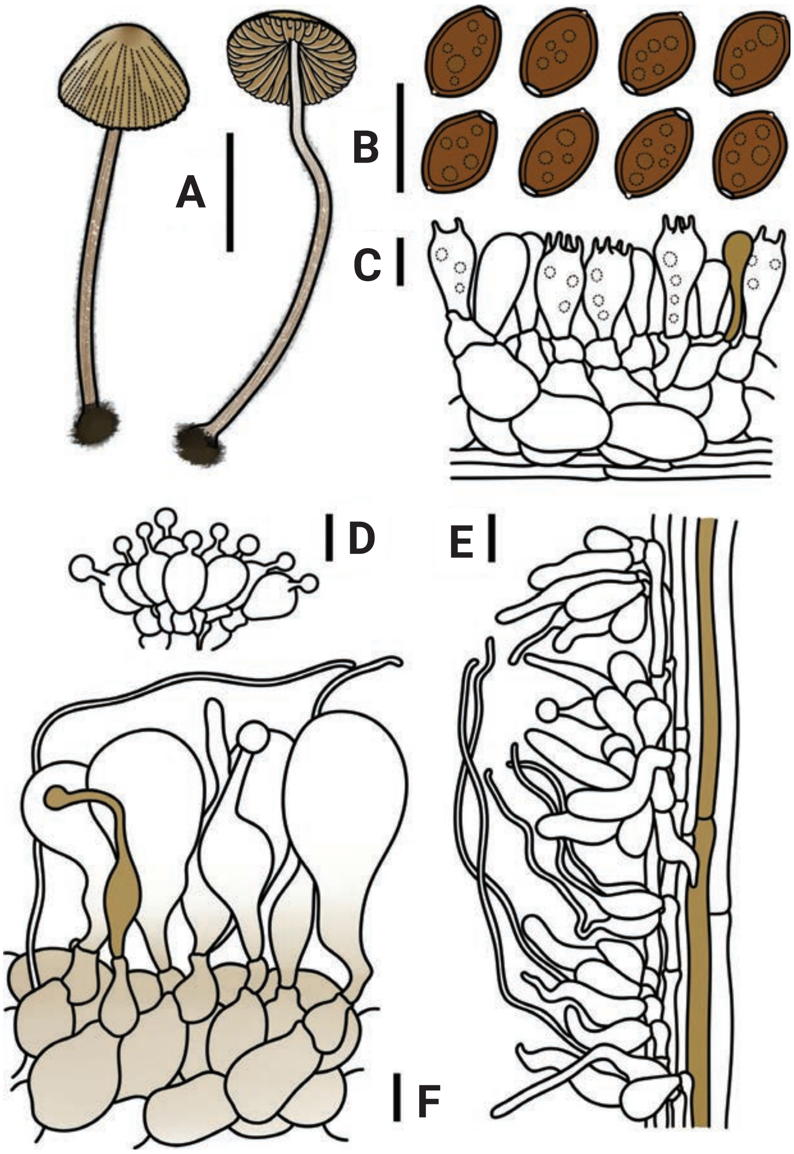

**Figure 5.** *Conocybe angulispora* (FJAU65120) **A** basidiomata **B** basidiospores in KOH **C** hymenium and subhymenium **D** cheilocystidia **E** stipitipellis **F** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–F**).

brown (RAL8016), while the edge remains brown beige (RAL1011) and ivory (RAL1014). Pileus hygrophanous, distinctly pubescent, with striations extending to the center. Context thin, ivory (RAL1014) to light ivory (RAL1015), no specific odor or taste. Lamellae adnexed to narrowly adnate, ventricose, slightly crowded, unequal in length, sandy yellow (RAL1002) to ochre brown (RAL8001), with smooth edges. Stipe length 2.5–5.0 cm, thick 1.0–2.0 mm, cylindrical, light ivory (RAL1015), sandy yellow (RAL1002) to signal brown (RAL8002), surface covered with pubescent, longitudinally fibrous striations, subbulbous base.

***Basidiospores*** (60/3/3) 8–10(–10.5) × 5.5–6.5 × (4.5–)5–6 µm, Q=(1.35–)1.39–1.76(–1.83), Qm = 1.57(±0.11), lentiform, angular and submitriform or slightly hexagonal in frontal view, ellipsoid to oblong, ovoid, or amygdaliform in side view, with partially thick walls and containing oil droplets, germ pore diameter 0.5–2.0 µm, basidiospores in 5% KOH solution ochre brown (RAL8001) to copper brown (RAL8004) in KOH. Basidia 14–24(–25) × (8–)9–11(–12) µm,

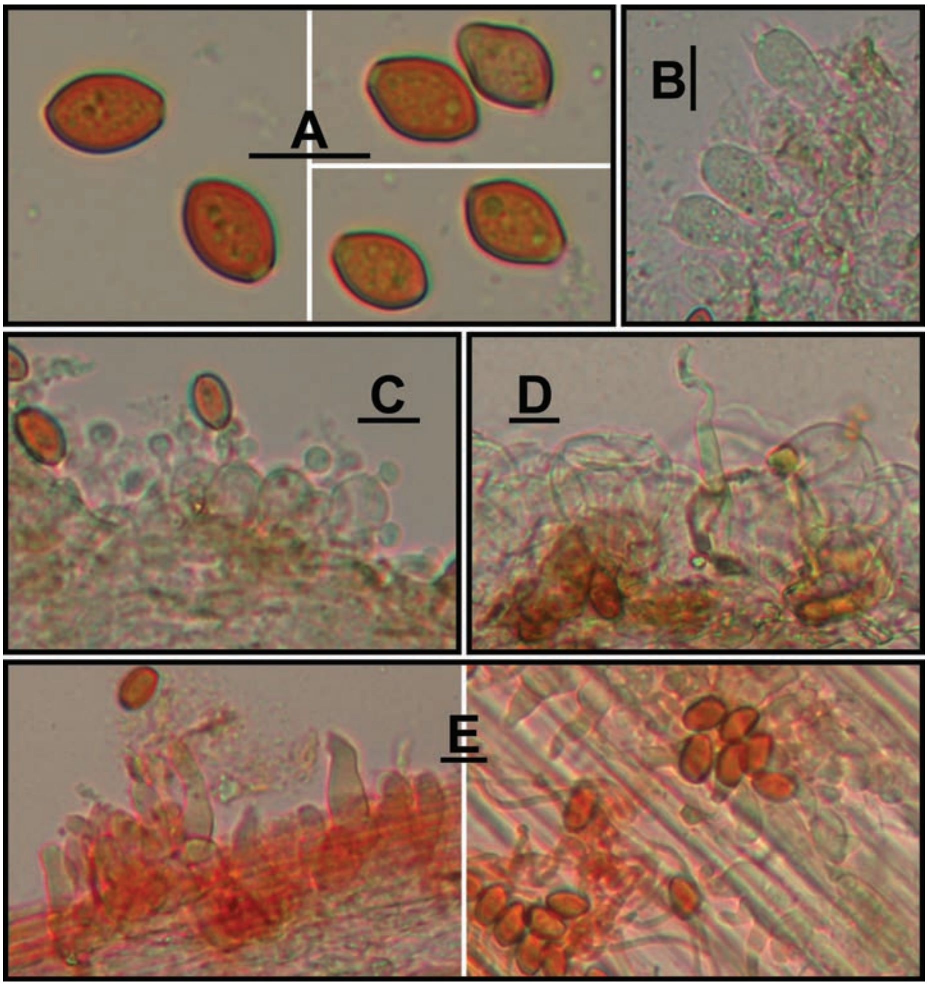

**Figure 6.** Microscopic structure images of *Conocybe angulispora* (FJAU65120) **A** basidiospores **B** basidia **C** cheilocystidia **D** pileipellis **E** stipitipellis. Scale bars: 10 µm (**A–E**).

broadly clavate to clavate, 4(2)-spored, sterigmata 2–6 µm long, basidia with vacuolar contents. Cheilocystidia 13–22 × 6–10(–11) µm, lecythiform, with capitula 3–6 µm wide. Caulocystidia ellipsoid to oblong, lageniform, long-necked lageniform, nettle hair-shaped, narrowly conical, fusiform, cylindrical, clavate, narrowly utriform to utriform, (10–)11–42(–45) × (4–)5–9 µm, capilliform cystidia can exceed 100 µm, among which rare lecythiform cystidia are mixed at the apex. Pileipellis hymeniform, composed of (25–)28–62(–66) × 15–34(–36) µm broadly clavate, spheropedunculate, and obpyriform elements, with yellow pigment at the base. Pileocystidia abundant, (22–)23–58(–60) × 5–18(–19) µm, lageniform to long-necked lageniform, lecythiform, tibiiform, and nettle hair-shaped, capilliform cystidia can exceed 100 µm. Clamp connections are rare in all tissues. Shows negative reaction with ammonia solution.

**Habitat.** In summer, they grow scattered or in groups in the humus layer of mixed forests.

**Known distribution.** Jilin Province, China.

**Additional specimens measured.** China, • Jilin Province, Jilin City, Jiaohe City, Laoyeling, 28 July 2023, 43°40'57"N, 127°11'58"E, alt. 430 m, Xia Wang, W23072815 (FJAU65121); • Jilin City, Jiaohe City, Shansongling, 26 August 2023, 43°32'09"N, 127°02'23"E, alt. 530 m, Hong Cheng, C2382621 (FJAU65122).

**Notes.** In some species of section *Pilosellae*, the frontal view of basidiospores appears slightly hexagonal, which can be easily confused with *C. angulispora*. The difference between *C. angulispora* and *C. hexagonospora* is that *C. hexagonospora* lacks distinct pubescence on the pileus and has rare pileocystidia, making it easy to differentiate (Hausknecht 1993). Additionally, the ITS sequence similarity between *C. angulispora* and *C. hexagonospora* is 91.2%. *Conocybe angulispora* can be distinguished from *C. brunneidisca* by the larger length of basidiospores in *C. brunneidisca*, which can reach 9.9–12.1 μm, and it is found in fertile grasslands or dung (Hausknecht and Contu 2007). *Conocybe angulispora* can be differentiated from *C. pulchra* (Clem.) Hauskn., Krisai & Voglmayr by the length of basidiospores, which measures 11.5–15 μm in *C. pulchra*, and *C. pulchra* lacks pileocystidia (Hausknecht et al. 2004). The difference between *C. angulispora* and *C. lentispora* Singer is that the basidiospores of *C. lentispora* are shorter than 7 μm and broadly ellipsoid in shape (Hausknecht 2005). *Conocybe angulispora* can be differentiated from *C. brunneoaurantiaca* K.A. Thomas, Hauskn. & Manim. such that *C. brunneoaurantiaca* lacks pubescence on the pileus and pileocystidia (Hausknecht 2009; Thomas et al. 2001).

### ***Conocybe rubrocyanea*** **T. Bau & H. B. Song, sp. nov.**

MycoBank No: 852868
Figs 2I–M, 7, 8

**Etymology.** " *rubrocyanea* " refers to basidiomata that have a reddish hue when fresh and a bluish hue when dry.

**Holotypus.** China, • Jilin Province, Jilin City, Jiaohe City, Shansongling, 30 July 2023, 43°32'14"N, 127°01'33"E, alt. 610 m, Shi-En Wang, E2307268 (FJAU65123).

**Diagnosis.** *Conocybe rubrocyanea*, when fresh, displays a mainly red color on the pileus, transitioning to blue upon drying. Basidiospores are lentiform, ellipsoid to oblong, frontal view near hexagonal, side view phaseoliform, cheilocystidia clavate, utriform, ellipsoid, or fusiform on one side near the edge of the pileus, and lecythiform on the side near the stipe, and some pileipellis cells contain blue lilac pigment.

**Description.** Basidioma mycenoid. Pileus diameter 0.5–2.0 cm, initially hemispherical, conical, later obtusely conical, with straight, undulate margin. When fresh, pileus salmon orange (RAL2012), antique pink (RAL3014) to rose (RAL3017), tomato red (RAL3013) to pearl ruby red (RAL3032), and when dry, it becomes slate gray (RAL7015), brown gray (RAL7013) to cobalt blue (RAL5013). Pileus hygrophanous, covered in distinct pubescence and striations that extend up to one-third towards the center. Context thin, salmon orange (RAL2012) to light ivory (RAL1015), no specific odor or taste. Lamellae ventricose, adnexed to narrowly adnate, moderately crowded, unequally long, initially light ivory (RAL1015) to ivory (RAL1014), later pastel yellow (RAL1034) to ochre-brown (RAL8001), with inconspicuous, slightly eroded edges. Stipe 2.0–8.0 cm long, 1.0–4.0 mm thick, cylindrical, clay brown (RAL8003), rose (RAL3017), antique pink (RAL3014) to pearl ruby red (RAL3032), surface pruinose and pubescent, longitudinally striate, base bulbous.

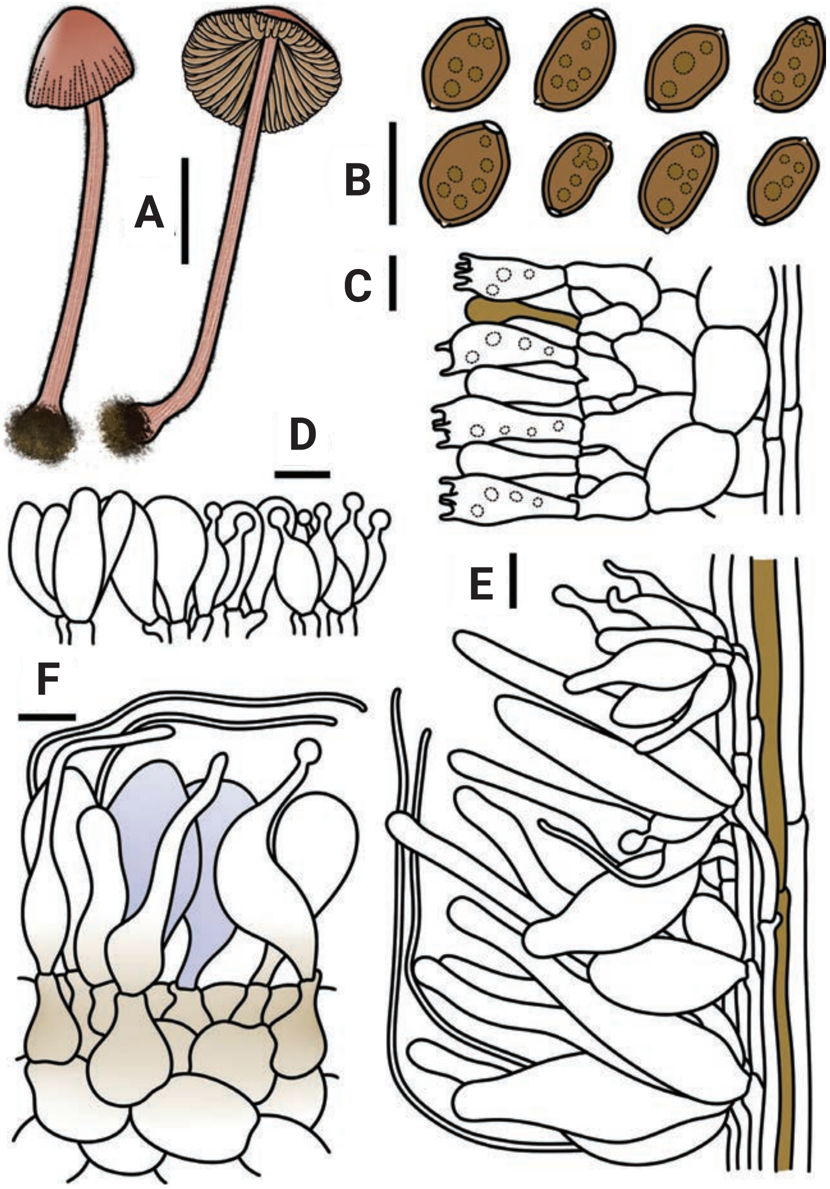

**Figure 7.** *Conocybe rubrocyanea* (FJAU65123) **A** basidiomata **B** basidiospores in KOH **C** hymenium and subhymenium **D** cheilocystidia **E** stipitipellis **F** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–F**).

***Basidiospores*** (60/3/3) 8–11.5(–12.5) × 5–7.5 × 5–6(–6.5) µm, Q=(1.33–)1.42–2.08(–2.14), Qm = 1.76(±0.17), lentiform, ellipsoid to oblong, frontal view near hexagonal, side view phaseoliform, slight constriction at center, with thick walls, containing oil droplets, germ pore diameter 0.5–2.0 µm, basidiospores in KOH solution ochre brown (RAL8001) to copper brown (RAL8004). Basidia (13–)15–26(–27) × 8–11(–12) µm, broadly clavate to clavate, 4(2)-spored, with sterigmata measuring 2–6 µm in length, basidia contain vacuolar contents. Cheilocystidia (14–)15–27(–28) × (6–)7–14(–15) µm, clavate, utriform, ellipsoid, or fusiform on one side near the edge of the pileus, and lecythiform on the side near the stipe, with capitula 3–6 µm wide. Caulocystidia elliptical to oblong, lageniform, long-necked lageniform, nettle hair-shaped, conical, fusiform, cylindrical, clavate, narrowly utriform to utriform, (10–)12–82(–85) × (5–)6–16 µm,

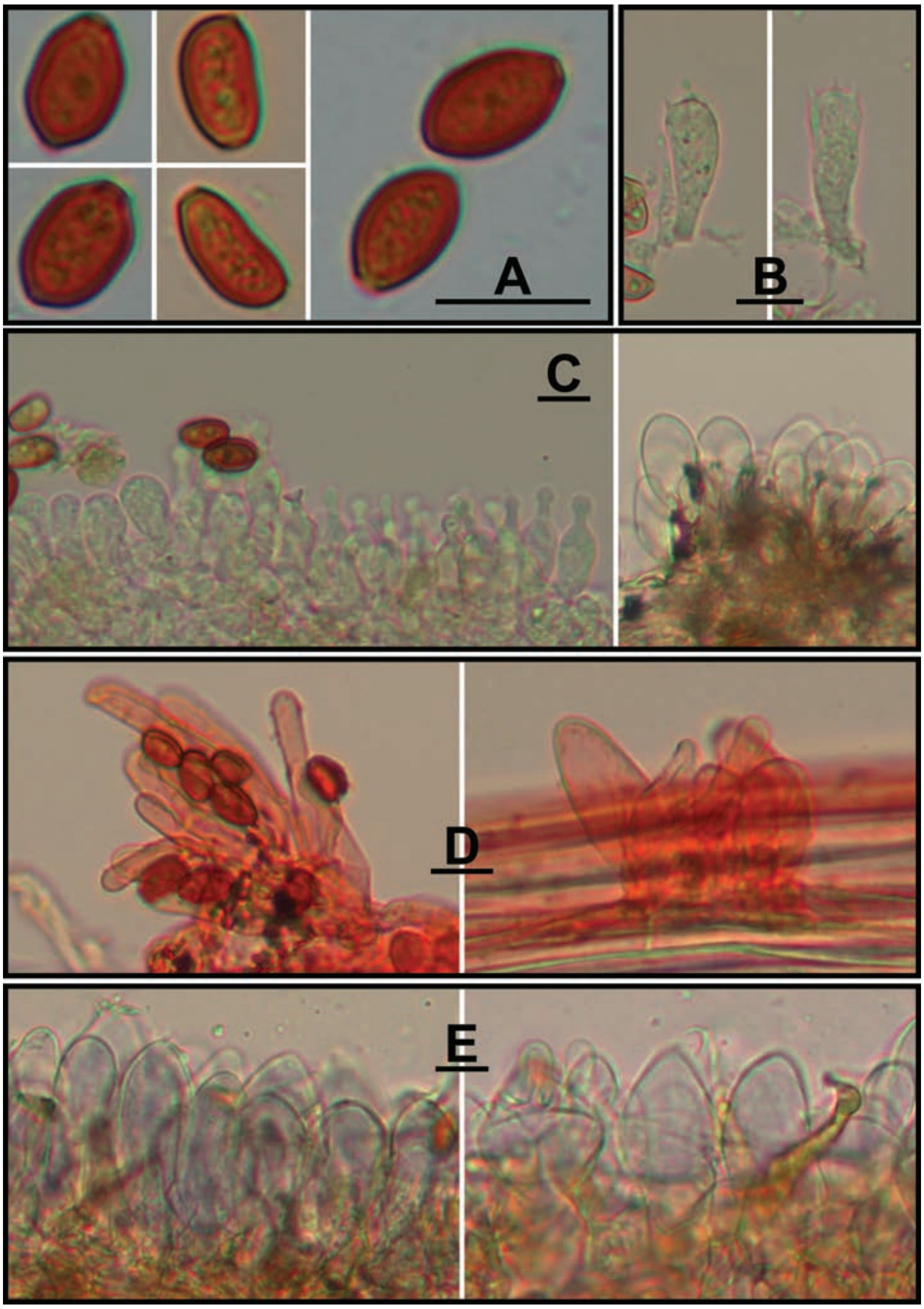

**Figure 8.** Microscopic structure images of *Conocybe rubrocyanea* (FJAU65123) **A** basidiospores, **B** basidia **C** cheilocystidia **D** stipitipellis **E** pileipellis. Scale bars: 10 µm (**A–E**).

capilliform cystidia may exceed 100 µm, with rare occurrences of lecythiform and sub-lecythiform cystidia at the apex. Pileipellis hymeniform, composed of spheropedunculate and fusiform cells (25–)27–53(–54) × (14–)15–28(–29) µm, some containing blue lilac (RAL4005) pigment, with yellow pigment at the base. Pileocystidia (21–)23–55(–60) × 4–23 µm, with long-necked lageniform, lecythiform, cylindrical, and nettle hair-shaped forms, and capilliform cystidia can exceed 100 µm. Clamp connections are rare in all tissues. It shows a positive reaction with ammonia, forming diamond-shaped crystals.

**Habitat.** Scattered or grouped in mixed forests during the summer season, on cow dung.

**Known distribution.** Jilin Province, China.

**Additional specimens measured.** China, • Jilin Province, Jilin City, Jiaohe City, Shansongling, 26 July 2022, 43°32'02"N, 127°02'36"E, alt. 580 m, Han-Bing Song, S22072618 (HMJAU64964); • Jilin City, Jiaohe City, Shansongling, 29 July 2023, 43°32'20"N, 127°03'09"E, alt. 530 m, Shi-En Wang, E2307247 (FJAU71648); • Jilin City, Jiaohe City, Shansongling, 30 July 2023, 43°32'20"N, 127°01'50"E, alt. 550 m, Shi-En Wang, Xia Wang, Si-Ying Li, W23073002 (FJAU71649), W23073003 (FJAU71650), W23073004 (FJAU71651), E2307277 (FJAU71652), L23073033 (FJAU71653); • Jilin City, Jiaohe City, Shansongling, 26 August 2023, 43°32'26"N, 127°02'23"E, alt. 550 m, Zheng-Qing Chen, Mu Liu, Hong Cheng, Q2382626 (FJAU71654), LM230864 (FJAU71655), C2382603 (FJAU71656), C2382605 (FJAU71657), C2382611 (FJAU71658), C2382615 (FJAU71659); • Jilin City, Huadian City, Redstone National Forest Park, 28 August 2023, 42°58'08"N, 127°03'36"E, alt. 430 m, Xian-Yan Zhou, Y2382804 (FJAU71660).

**Notes.** *Conocybe rubrocyanea* can be differentiated from species with near hexagonal basidiospores in sect. *Pilosellae*, such as *C. hexagonospora*, *C. brunneidisca*, *C. lentispora*, *C. brunneoaurantiaca*, *C. pulchra* and *C. angulispora*, by presence of red color tone on the pileus (Hausknecht 2009). *Conocybe rubrocyanea* is closely related to *C. incarnata* (Jul. Schäff.) Hauskn. & Arnolds and *C. muscicola*, and they are easily confused in macroscopic morphology. However, *C. incarnata* and *C. muscicola* basidiospores are not lentiform or hexagonal, and pileipellis cells lack blue lilac pigment (Arnolds and Hausknecht 2003).

### ***Conocybe hexagonospora* Métrod ex Hauskn. & Enderle**

Figs 2N–P, 9, 10

**Description.** Basidioma mycenoid. Pileus diameter 1.0–1.5 cm, obtusely conical, edge straight, undulate, center signal brown (RAL8002) to deer brown (RAL8007), fading towards the edge, brown beige (RAL1011) to ivory (RAL1014), pileus hygrophanous, smooth, striate towards the center. Context thin, ivory (RAL1014) to light ivory (RAL1015), no specific odor or taste. Lamellae adnexed to narrowly adnate, ventricose, slightly loosely, unequal in length, beige (RAL1001) to sandy yellow (RAL1002), with smooth margins. Stipe length 3.5–4.0 cm, width 0.5–1.5 mm, cylindrical, brown beige (RAL1011) to sandy yellow (RAL1002), surface pubescent, longitudinally fibrous striate, subbulbous at the base.

***Basidiospores*** (40/1/1) 7.5–9.5(–10) × 5.5–6.5 × 5–6 µm, Q=(1.32–)1.34–1.78(–1.80), Qm = 1.49(±0.11), lentiform, frontal view nearly hexagonal or submitriform, side view ellipsoid to oblong, thick-walled, containing oil droplets, germ pore diameter 0.5–1.5 µm. Basidiospores in 5% KOH solution ochre brown (RAL8001) to copper brown (RAL8004). Basidia (14–)15–21(–22) × 8–10 µm, broadly clavate to clavate, 4-spored, with sterigmata length 3–6 µm, basidia contain vacuolar contents. Cheilocystidia (13–)15–21 × 7–10(–11) µm, lecythiform, with capitula 3–6 µm wide. Caulocystidia are ellipsoid to oblong, lageniform, long-necked lageniform, nettle hair-shaped, narrowly conical, fusiform, cylindrical, clavate, narrowly utriform to utriform, measuring (20–)22–55(–57) × (5–)6–16 µm, capilliform cystidia can reach a length of 100 µm, with rare lecythiform cystidia mixed in. Pileipellis hymeniform, consists of spheropedunculate

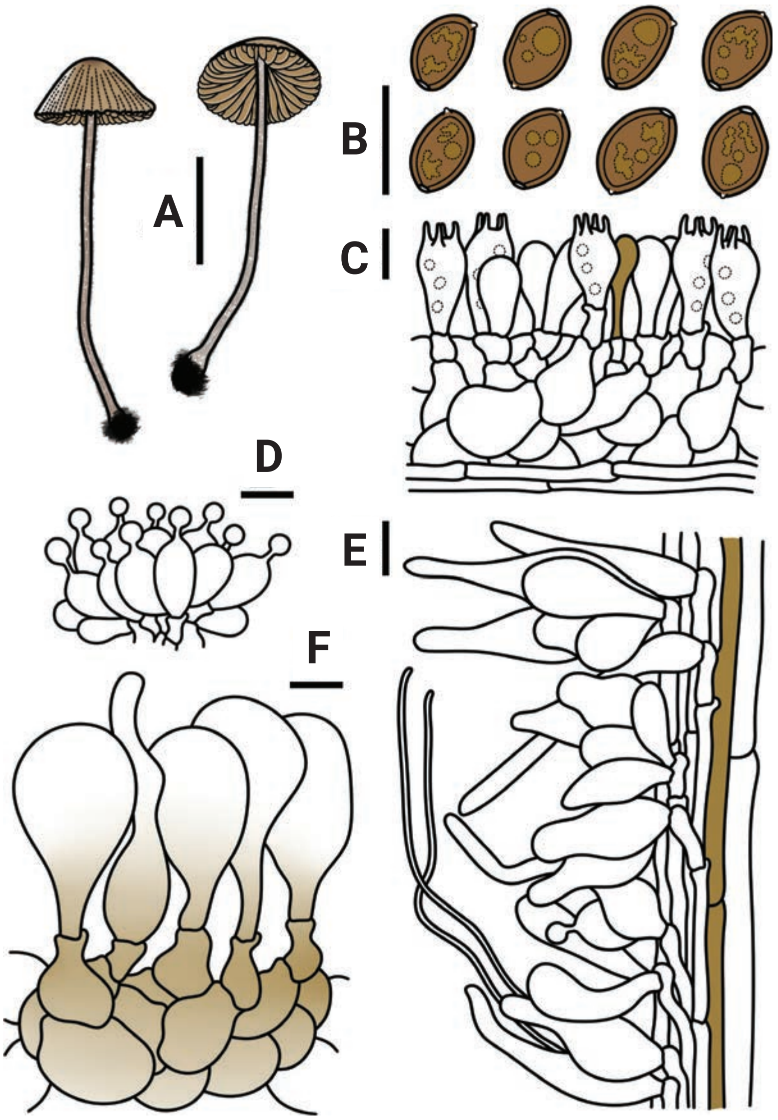

**Figure 9.** *Conocybe hexagonospora* (FJAU71661) **A** basidiomata **B** basidiospores in KOH **C** hymenium and subhymenium **D** cheilocystidia **E** stipitipellis **F** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–F**).

and obpyriform cells, 29–48(–50) × (18–)19–27(–30) µm, with yellow pigment at the base. Pileocystidia are rare and lageniform in shape. All tissues exhibit clamp connections. It shows a negative reaction to ammonia solution.

**Habitat.** Solitary in mixed forests during autumn.

**Known distribution.** Asia: China, Russia; Europe: Sweden, Finland, Latvia, Hungary, Germany, Austria (Holotype), Belgium, United Kingdom, France, Italy (Hausknecht 2009).

**Additional specimens measured.** China, • Jilin Province, Siping City, Yitong Manchu Autonomous County, 7 September 2023, 43°35'58"N, 125°12'12"E, alt. 290 m, Han-Bing Song, S23090710 (FJAU71661).

**Notes.** Although this species does not have gene sequences in the NCBI database, its macroscopic and microscopic structures are consistent with those of *C. hexagonospora*, leading to its identification as *C. hexagonospora*. There are also some species in sect. *Pilosellae* with basidiospores' shapes similar to *C. hexagonospora*, but they are distinct species, differentiated as follows:

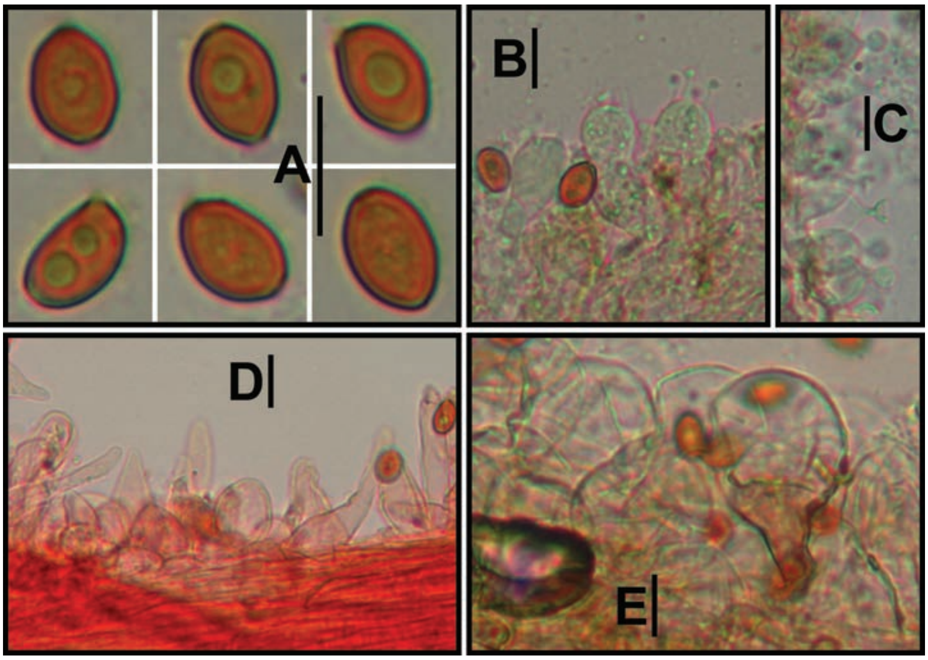

**Figure 10.** Microscopic structure images of *Conocybe hexagonospora* (FJAU71661) **A** basidiospores **B** basidia **C** cheilocystidia **D** stipitipellis **E** pileipellis. Scale bars: 10 µm (**A–E**).

The difference between *C. hexagonospora* and *C. brunneidisca* is that the latter has longer basidiospores, reaching a length of 9.9–12.1 µm, and the pileus color and habitat are also different (Hausknecht and Contu 2007). The difference between *C. hexagonospora* and *C. pulchra* is that the latter has basidiospores measuring 11.5–15 µm in length and lacks pileocystidia (Hausknecht et al. 2004). The difference between *C. hexagonospora* and *C. lentispora* is that the latter has basidiospores with a length smaller than 7 µm and are broadly ellipsoid (Hausknecht 2005). Meanwhile, the difference between *C. hexagonospora* and *C. brunneoaurantiaca* is that *C. brunneoaurantiaca* has cheilocystidia reaching up to 30 µm and lacks pileocystidia (Thomas et al. 2001). In the phylogenetic tree, *C. hexagonospora* and C. *angulispora* are sister taxa to each other, but their ITS sequence similarity is only 91%. *Conocybe angulispora* has distinct pubescence on its pileus, allowing for differentiation from *C. hexagonospora*. Of these similar species, the following are sequenced and clearly separate in the phylogeny: *C. hexagonospora*, *C. brunneidisca*, *C. angulispora*.

## Key to Chinese Species of *Conocybe* Sect. *Pilosellae*

1 2-spored .......... **2**
– 4-spored .......... **6**
2 Pileus unstriated or not distinct .......... **3**
– Pileus striated .......... **4**
3 Tibiiform pileocystidia present .......... ***C. siliginea***
– Tibiiform pileocystidia absent .......... ***C. pseudocrispa***
4 Basidiospores with suprahilar depression .......... ***C. verna***
– Basidiospores with suprahilar plage .......... **5**
5 Pileus blackish in color .......... ***C. bisporigera***
– Pileus yellowish in color .......... ***C. sinobispora***

6 Basidiospores nearly hexagonal ........ **7**
– Basidiospores not hexagonal ........ **10**
7 Pileus reddish in color ........ ***C. rubrocyanea***
– Pileus lacking a red color ........ **8**
8 Average length of basidiospores can reach 12 μm ........ ***C. brunneidisca***
– Average length of basidiospores is less than 10 μm ........ **9**
9 Pileus pubescence distinct ........ ***C. angulispora***
– Pileus pubescence absent or indistinct ........ ***C. hexagonospora***
10 Pseudorhiza present ........ ***C. incarnata***
– Pseudorhiza absent ........ **11**
11 Germ pore absent or not distinct ........ ***C. pilosella***
– Germ pore present ........ **12**
12 Basidiospores phaseoliform, reniform ........ ***C. reniformis***
– Basidiospores never phaseoliform ........ **13**
13 Pileus unstriated ........ **14**
– Pileus striated ........ **15**
14 Waxy crystals precipitate upon drying ........ ***C. ceracea***
– No crystallization occurs upon drying ........ ***C. fuscimarginata***
15 Pileus densely pubescent ........ **16**
– Pileus pubescence absent or slight ........ **17**
16 Pileus salmon orange when young ........ ***C. muscicola***
– Pileus blackish-red when young ........ ***C. pilosa***
17 Lamellae edge serrate ........ ***C. hydrophila***
– Lamellae edge not serrate ........ **18**
18 Basidiospores lentiform ........ **19**
– Basidiospores never lentiform ........ **20**
19 Length of basidiospores may exceed 15 μm, 11–16 μm ........ ***C. nitrophila***
– Length of basidiospores may be less than 10 μm, 9–13 μm ... ***C. velutipes***
20 Grows on cow dung ........ ***C. rufostipis***
– Grows in meadows ........ **21**
21 Pileus honey yellow ........ ***C. siennophylla***
– Pileus brown beige ........ ***C. moseri***

## Discussion

Building on the phylogenetic framework of Tóth et al. (2013) and Song and Bau (2023), a phylogenetic tree was reconstructed for sect. *Pilosellae* incorporating materials from Jilin province and using ITS, nrLSU, and *tef1-α*. The analysis revealed the presence of three new species and a new record for China. The new species are *C. verna*, *C. angulispora*, and *C. rubrocyanea*, while the newly recorded species is *C. hexagonospora*. *Conocybe verna* is found in spring in broad-leaved forests and has a campanulate pileus, which lacks pubescence. It has 2-spored basidia and basidiospores are with a suprahilar depression, which distinguishes it from other species in sect. *Pilosellae*. On the other hand, *C. angulispora* is found in mixed forests and has an obtusely conical pileus with distinct pubescence, and basidiospores are lentiform, angular, submitriform, or slightly hexagonal in frontal view. *Conocybe rubrocyanea* grows on cow dung, with macroscopic features similar to *C. incarnata*, but the basidiospores of *C. rubrocyanea* are lentiform in shape, and the pileipellis contains blue lilac pigment. Both dried specimens in water and KOH solution secrete blue-purple pigments. Among the three new species, *C. rubrocyanea* is a particularly unique species. Its caulocystidia predominantly exhibit a non-lecythiform shape, which aligns with the stipe type in sect. *Pilosellae*. However, its pileipellis is composed of spheropedunculate and fusiform elements, some of which are partly rostellate, characteristics that are consistent with the classification features of sect. *Obscurae* Hauskn. & Krisai (Hausknecht and Krisai-Greilhuber 2006). Currently, the sect. *Obscurae* includes only one species, *C. obscura* Watling, which is found in the Democratic Republic of the Congo in Africa. The caulocystidia of *C. obscura* also conform to the stipe type in sect. *Pilosellae*, yet it lacks molecular sequences, leaving its taxonomic position unclear. It is uncertain whether *C. obscura* clusters with *C. rubrocyanea*, especially since its basidiospores are neither lentiform nor hexagonal, making them easy to distinguish from those of *C. rubrocyanea* (Watling 1973). Although the morphological features of *C. rubrocyanea* are consistent with other species of sect. *Obscurae*, its position on the basis of the molecular phylogeny is actually within sect. *Pilosellae*. If *C. obscura* and *C. rubrocyanea* do not cluster together, this would suggest that pileipellis characteristics are not used as criteria for distinguishing sections. In this case, *C. obscura* would also belong to sect. *Pilosellae*. To resolve this issue, further research on the holotype specimen of *C. obscura* is necessary before reconsidering the classification of *C. obscura* and *C. rubrocyanea*. In this study the holotype of *C. hexagonospora* lacks a sequence, the macroscopic and microscopic structures of specimen FJAU71661 are consistent with that of *C. hexagonospora*, thus confirming FJAU71661 as *C. hexagonospora* (Hausknecht 2009). Additionally, *C. siliginea* (FJAU71664), collected from Henan Province, grows in greenhouse soil, with a lime-colored pileus and 2-spored basidia. We identified specimen FJAU71662 as *C. velutipes* based on its macroscopic and microscopic structures. Subsequently, we obtained the ITS, nrLSU, and *tef1-α* sequences of specimen FJAU71662, which are similar to those of *C. velutipes* (SZMC-NL-2187), with an ITS similarity of 99.7%, nrLSU similarity of 99.9%, and *tef1-α* similarity of 99.2%. This further supports the correctness of our traditional taxonomic identification of the species. Hausknecht and Contu (2007) has already stated that *C. lenticulospora* is a synonym of *C. brunneidisca*, but since 2007, some have continued to use *C. lenticulospora* as a species name, and it is still treated as an independent species in MycoBank and Index Fungorum. Liu (2018) described and illustrated specimen HMJAU45069 as *C. lenticulospora* Watling, with its ITS sequence showing a similarity of 99.7% to *C. lenticulospora* (SZMC-NL-0923). Therefore, we reexamined HMJAU45069, and its basidiospores measured 9.5–13.5 × 6.5–8.5 × 5.5–7.5 μm, lentiform in shape, with a nearly hexagonal or submitriform frontal view and an ellipsoid to oblong side view. The microscopic features were consistent with those of *C. brunneidisca*, supporting Hausknecht and Contu's (2007) viewpoint that *C. lenticulospora* is a synonym of *C. brunneidisca*.

Based on morphological classification, Hausknecht and Krisai-Greilhuber (2006) divided sect. *Pilosellae* into 2 subsections and 11 series, contributing significantly to this field. However, when molecular techniques and phylogenetic methods were applied to taxonomy, the correlation between morphological classification and phylogeny revealed some discrepancies. For instance, *C. cylindracea*, classified under ser. *Cylindracea*, clustered with *C. brunneidisca*, which is the type species of ser. *Lenticulospora*. Given that *C. cylindracea* has lentiform and slightly angular basidiospores, we propose placing it in ser. *Lenticulospora*, a change supported by phylogenetic analysis (Fig. 1). This finding suggests that pileus shape is not a reliable feature for series classification. Similarly, *C. angulispora* and *C. hexagonospora*, discovered in Jilin province, fit the definition of ser. *Lenticulospora*. Consequently, the branch consisting of *C. brunneidisca*, *C. cylindracea*, *C. angulispora*, and *C. hexagonospora* is now designated as ser. *Lenticulospora*, based on consistency between morphological and phylogenetic analyses. However, *C. rubrocyanea*, which possesses lentiform basidiospores and a hexagonal frontal view does not cluster with ser. *Lenticulospora*. This disparity between morphological and phylogenetic congruence is also observed in other series. For example, *C. incarnata*, belonging to ser. *Microrrhiza* due to its pseudorhiza, clusters with *C. muscicola* and *C. rubrocyanea*, making it challenging to differentiate it from other series. Although all these species share the common feature of a reddish pileus, we do not introduce it as a new series. This decision is based on the extensive description and recording of nearly 60 species within sect. *Pilosellae* by Hausknecht (2009), with many species lacking sequences. Consequently, it remains uncertain whether other species can cluster with the branch containing *C. rubrocyanea*.

This article primarily introduces three new species from Jilin province and a new record for China. Additionally, a key to differentiate the 22 species within sect. *Pilosellae* in China is provided. However, the phylogenetic positions of the series within sect. *Pilosellae* are still uncertain. To address this issue, a substantial number of specimens and sequences are required to identify stable shared characteristics for distinguishing different branches. Further in-depth research is needed to investigate this matter.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: Tolgor Bau and Han-Bing Song. Methodology: Han-Bing Song. Software: Han-Bing Song. Validation: Han-Bing Song and Tolgor Bau. Formal analysis: Han-Bing Song. Investigation: Han-Bing Song and Tolgor Bau. Resources: Han-Bing Song and Tolgor Bau. Data curation: Han-Bing Song and Tolgor Bau. Writing original draft preparation: Han-Bing Song. Writing review and editing: Han-Bing Song and Tolgor Bau. Visualization: Han-Bing Song and Tolgor Bau. Supervision: Tolgor Bau. Project administration: Tolgor Bau. Funding acquisition: Tolgor Bau.

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## Data availability

All the sequences have been deposited in GenBank (https://www.ncbi.nlm.nih.gov) and Mycobank (https://www.mycobank.org). The data presented in this study are deposited in the Zenodo repository, accession number https://doi.org/10.5281/zenodo.14836573.

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Research Article

# A new psychrophilic yeast of Kriegeriaceae (Kriegeriales) isolated from lichen in the Arctic, with the description of *Lichenia svalbardensis* gen. et sp. nov.

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## Abstract

Yeasts are an important component of the microbiome in circumpolar regions that are characterized by unique environmental conditions. However, the taxonomy of yeasts remains largely unknown in high- and low-latitude regions. Curing a field survey of yeasts in the Svalbard Archipelago, Norway, a new yeast genus in Kriegeriales was isolated from dendritic lichens. Based on the phylogeny of multiple loci (ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*), morphology, and physiological characteristics, the new genus *Lichenia* is proposed with the type species *Lichenia svalbardensis*. Additionally, 10 °C and 15 °C are the fastest growth temperatures of *L. svalbardensis*. It has low or no growth at temperatures above 20 °C, and there appears to be a morphogenetic transition from yeast to pseudohyphae or hyphae above 10 °C.

**Key words:** Kriegeriales, lichen, phylogeny, psychrophilic yeast, taxonomy

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## Introduction

Basidiomycetous yeasts comprise decomposers, symbionts, and pathogens in different ecosystems (Buzzini and Martini 2000; Nagahama 2006; Buzzini et al. 2012; Peter et al. 2017; Sampaio and Gonçalves 2017; Sannino et al. 2017). Currently, five classes of Basidiomycota (Agaricostilbomycetes, Cystobasidiomycetes, Microbotryomycetes, Tremellomycetes, and Spiculogloeomycetes) are dominated by (dimorphic) species that comprise a yeast stage (Aime et al. 2006; Bauer et al. 2006; Hibbett et al. 2007; Boekhout et al. 2011; Weiß et al. 2014; Oberwinkler 2017; Li et al. 2020; Schoutteten et al. 2023). Microbotryomycetes, the second largest class in Pucciniomycotina (Basidiomycota), contains eight orders named Curvibasidiales, Heitmaniales, Heterogastridiales, Kriegeriales, Leucosporidiales, Microbotryales, Rosettozymales, and Sporidiobolales (Aime et al. 2006, 2014; Li et al. 2020; Schoutteten et al. 2023). In older classification systems, most of these species were lumped in artificial, large, polyphyletic genera such as *Sporobolomyces*, *Rhodotorula*, and *Tremella* (Li et al. 2020; Schoutteten et al. 2023; Jiang et al. 2024). With the use of molecular phylogenies as a base

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for yeast systematics, more than 2,000 species with yeast states have been proposed to accommodate the diversity of Basidiomycetous yeasts (Wang et al. 2015b; Li et al. 2020; Boekhout et al. 2022; Schoutteten et al. 2023). In the past, the placements of many monotypic genera in Microbotryomycetes were classified as incertae sedis (e.g., *Kriegeria*, *Meredithblackwellia*, *Pseudoleucosporidium*, *Psychromyces*, *Reniforma*, *Trigonosporomyces*, and *Udeniozyma*) (Aime et al. 2006; Wang et al. 2015a, 2015b; Schoutteten et al. 2023). The family Kriegeriaceae, identified with subgloboid spindle pole bodies and simple pore septa, was recognized by Toome et al. (2013) by using a phylogeny based on the SSU, LSU, and ITS regions of the ribosomal DNA. Toome et al. (2013) found that Kriegeriaceae have an interesting morphological feature: rosette-shaped budding patterns appear in culture conditions. Later, Wang et al. (2015b) reclassified the five *Rhodotorula* species in Kriegeriaceae into *Phenoliferia* spp. and *Yamadamyces* spp. Kriegeriaceae was not always recovered as a monophyletic lineage because of the contaminant protein-coding genes (*rpb1*, *rpb2*, *tef1-α*, and *cytb*) for the type strains of *Kriegeria eriophori* (CBS 8387) and *Libkindia masarykiana* (PYCC 6886) derived from *Candida* (Ascomycota) and the missing genes for *Meredithblackwellia eburnea* (Schoutteten et al. 2023). Therefore, a robust molecular dataset that includes ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb* was important to clarify the phylogenetic position of the Kriegeriaceae and its internal relationships (Wang et al. 2015b; Masinova et al. 2017; Schoutteten et al. 2023).

Psychrophilic yeasts have been discovered in various groups of Basidiomycota, such as Cystobasidiomycetes, Microbotryomycetes, and Tremellomycetes (Margesin and Miteva 2011; Buzzini et al. 2012; Selbmann et al. 2014; Franca et al. 2016). Various species in Microbotryomycetes were described from polar regions. Perini et al. (2021) identified *Psychromyces glacialis* and *Camptobasidium arcticum* from glaciers in Greenland and Svalbard. *Cryolevonia schafbergensis*, a yeast unable to grow at 18 °C or higher temperatures, was collected from ancient permafrost and melted sea ice (Pontes et al. 2020). De Garcia et al. (2020) obtained two psychrophilic yeasts (*Cryolevonia giraudoae* and *Camptobasidium gelus*) from ice collected in cold environments. These psychrophilic yeast species in the genera *Camptobasidium*, *Glaciozyma*, *Cryolevonia*, and *Psychromyces* all cluster in Camptobasidiaceae (Schoutteten et al. 2023). Based on a phylogeny of ribosomal markers (ITS, LSU, and SSU), Toome et al. (2013) found that Camptobasidiaceae appeared as a sister lineage to Kriegeriaceae. In later analyses, the positions of Camptobasidiaceae and Kriegeriaceaea differed in phylograms based on the different datasets (protein-coding genes vs. ribosomal loci) (Wang et al. 2015a; Schoutteten et al. 2023). Currently, six genera, namely *Kriegeria*, *Kriegeriopsis*, *Libkindia*, *Meredithblackwellia*, *Phenoliferia*, and *Yamadamyces*, are recognized in Kriegeriaceae, and most of these were isolated from neotropical or temperate regions (Toome et al. 2013; Wang et al. 2015b; Masinova et al. 2017; Li et al. 2020; Diederich et al. 2022; Schoutteten et al. 2023). Species of *Kriegeria*, *Libkindia*, *Meredithblackwellia*, and *Yamadamyces* were isolated from neotropical or temperate forests in Asia, Europe, North America, or South America (Doubles and McLaughlin 1992; Golubev and Scorzetti 2010; Toome et al. 2013; Masinova et al. 2017; Li et al. 2020). *Kriegeriopsis livingstonensis* was described from Antarctica (Diederich et al. 2022). The remaining three *Phenoliferia* species were collected from glacier cryoconite, mud, and soil in Europe and identified as psychrophilic yeasts (Margesin et al. 2007).

The family Camptobasidiaceae mainly comprises psychrophilic yeasts. Psychrophilic yeasts in Kriegeriaceae require further research.

Yeasts were isolated from numerous substrates, such as fruits, soil, insects, invertebrates, seawater, and wine (Nakase 2000; Whipps et al. 2008; Boekhout et al. 2022). However, yeasts related to lichen thalli remain largely unknown because lichens are substantially undersampled (Hawksworth and Grube 2020). Yeasts in Tremellomycetes, Cystobasidiomycetes, and Microbotryomycetes have been isolated from lichen in several studies (Cernajova and Skaloud 2019; Kachalkin et al. 2024; Schoutteten et al. 2024). *Lichenozyma pisutiana* was isolated from *Cladonia* in Europe by Cernajova and Skaloud (2019) and was later reclassified to the genus *Occultifur* by Schoutteten et al. (2024). Nguyen et al. (2023) proposed *Microsporomyces cladoniophilus* associated with the thalli of *Cladonia rei* in Japan. Based on a seven-loci phylogenetic reconstruction, Schoutteten et al. (2024) introduced the genus *Millanizyma* to accommodate this species. Various lichen-inhabiting yeasts in other genera (*Colacogloea*, *Cyrenella*, *Genolevuria*, *Teunia*, *Phaeotremella*, *Piskurozyma*, and *Piskurozyma*) were introduced by Kachalkin et al. (2024). However, the taxonomy of many yeast species associated with lichen lacked in Kriegeriaceae, especially in high-latitude regions.

Svalbard is located in a freezing area inside the Arctic Circle. It has an extremely cold and dry climate, with less than 10 °C of temperature and 500 mm precipitation annually (Forland et al. 2011). Various microorganisms have been investigated in this place. Singh and Singh (2012) reported the yeast and filamentous fungi from Svalbard and identified them as *Articulospora*, *Cryptococcus*, *Mrakia*, *Phialophora*, and *Rhodotorula*. In Svalbard, two ascomycetous yeasts (*Metschnikowia bicuspidata* and *M. zobellii*) were isolated from seawater and puddles on snow/ice (Butinar et al. 2011). Although some studies investigated the mycodiversity of these islands, limited knowledge is available about the diversity and taxonomy of yeast in this region. During the investigation of fungal diversity in Svalbard, Norway (78°13'12.91"N, 15°20'6.39"E), a piece of dendritic lichen was collected and a novel taxon was subsequently isolated. This study aims to reveal the taxonomy of this isolate combining the phylogenetic, physiological and morphological characteristics.

## Materials and methods

### Collection and isolation

During the survey of microbial diversity, specimens were collected in Longyearbyen, Svalbard, Norway, with the Chinese Arctic Scientific Expedition (applications to the Governor of Svalbard for research activity have been submitted in July 2014; RiS ID: 6754). Of which, a lichen in Usneaceae (might be *Usnea sphacelata*) was collected. The whole lichen was sampled from the rock to a sterile envelope with a sterile blade. The lichen thallus was cut into small pieces and dissolved in sterile water. After grinding with magnetic beads for 15 min at 160 rpm, the microbial suspension was inoculated to plates containing different carbon sources media (cellulose, chitosan, petroleum, plastic, or xylose as the sole carbon sources). Emerging yeast colonies were transferred with a sterile bamboo skewer into a new potato dextrose agar media (PDA) plate. Plates were incubated at 10 °C for up to four weeks. Strains were deposited in the China Center for Type Culture Collection (CCTCC) and the Japan Collection of Microorganisms (JCM).

## DNA extraction and PCR amplification

After the strains were grown on PDA for four weeks, yeast cells were obtained for extraction of genomic DNA with the Plant/Fungus DNA Kit (Simgen, Hangzhou, China). Polymerase chain reactions (PCR) were conducted to amplify ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*. The primers and PCR conditions are listed in Table 1. Purified PCR products were sequenced by Wuhan Nextomics Corporation (Wuhan, Hubei Province) using the PACBIO RS II platform. Consensus sequences were obtained from DNA sequences generated by each primer combination with the software Seqman v. 9.0.4 (DNASTAR Inc., Madison, WI, United States).

## Morphological observation

To observe the morphological characters of the obtained yeasts, the strains were incubated in/on PDA (20% potato infusion, 2% glucose, 2% agar), PDB (20% potato infusion, 2% glucose), YM (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose), or YMA (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% agar) at 4 °C, 10 °C, 15 °C, and 20 °C for a month. The micromorphological features of the yeast cells were observed under an ICX41 microscope (Sunny Optical, Yuyao, China) at 1000× magnification. Over 30 yeast cells were measured to obtain the length and width. The cell culture characteristics (color, texture of colony) were recorded. To investigate the potential sexual cycles, the yeast cells were inoculated on CMA (5% corn meal infusion, 1.5% agar), MEA (5% malt extract, 2% agar), PDA, and YMA, according to Kurtzman et al. (2011). Yeast cells were incubated at 20 °C for one month.

## Phylogenetic analyses

The yeast isolate from the lichen was initially identified as Kriegeriaceae sp. based on the BLAST results in NCBI. A dataset of all currently known species in Kriegeriaceae and representative type species of other lineages in Microbotryomycetes was compiled based on recent published literature (Wang et al. 2015a, 2015b; Masinova et al. 2017; Li et al. 2020; Schoutteten et al. 2023, see Table 2). Contaminant sequences (*rpb1*, *rpb2*, *tef1-α*, and *cytb* for *Kriegeria eriophori* and *Libkindia masarykiana*) were removed from the dataset (Schoutteten et al. 2023). The compiled DNA sequence datasets of the different loci were aligned with the ClustalW algorithm in MEGA v. 6.0 (Tamura et al. 2013), after which the alignment was manually curated. The topologies between the different genetic loci were checked. The phylogenetic position of the newly discovered yeast was inferred through concatenating the alignments of the seven genetic regions (ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*) to construct the phylogenetic tree. *Pseudomicrostroma phylloplana* (CBS 8073) and *Ustilago maydis* (CBS 504.76) (Ustilaginomycotina, Basidiomycota) were used as the outgroup in the phylogenetic analyses. The maximum likelihood (ML) (Guindon et al. 2010) and Bayesian Inference (BI) analyses (Ronquist and Huelsenbeck 2003) were performed using PhyML v. 3.0 and MrBayes v. 3.1.2, respectively. FigTree v. 1.3.1 was used to show phylograms of Microbotryomycetes (Rambaut and Drummond 2010). The sequence data of *Lichenia svalbardensis* sp. nov. has been deposited in GenBank (Table 2). The concatenated seven-locus DNA sequence alignment used in this study has been deposited in TreeBASE (www.treebase.org; study ID 31855).

**Table 1.** Genes used in this study with PCR primers, primer DNA sequence, and optimal annealing temperature.

| Locus | PCR primers | Amplification primers | PCR: thermal cycles: (Annealing temp. in bold) | Reference |
|---|---|---|---|---|
| ITS | ITS1 | 5’- TCCGTAGGTGAACCTGCGG -3’ | (94 °C: 1 min, **52 °C**: 1 min, 72 °C: 1 min) × 35 cycles | White et al. 1990 |
| | ITS4 | 5’- TCCTCCGCTTATTGATATGC -3’ | | |
| LSU | NL1 | 5’- GCATATCAATAAGCGGAGGAAAAG -3’ | (94 °C: 1 min, **52 °C**: 1 min, 72 °C: 1 min) × 35 cycles | Kurtzman and Robnett 1998 |
| | NL4 | 5’- GGTCCGTGTTTCAAGACGG -3’ | | |
| SSU | NS1 | 5’- GTAGTCATATGCTTGTCTC -3’ | (94 °C: 1 min, **55 °C**: 30 s, 72 °C: 1.5 min) × 33 cycles | Sugita and Nakase 1999 |
| | NS8 | 5’- TCCGCAGGTTCACCTACGGA -3’ | | |
| *rpb1* | RPB1-Af | 5’- GARTGYCCDGGDCAYTTYGG -3’ | (94 °C: 1 min, **52 °C**: 1 min, 72 °C: 1 min) × 35 cycles | Stiller and Hall 1997 |
| | RPB1-Cr | 5’- CCNGCDATNTCRTTRTCCATRTA -3’ | | |
| *rpb2* | fRPB2-5F | 5’- GAYGAYMGWGATCAYTTYGG -3’ | (94 °C: 30 s, **55 °C**: 30 s, 72 °C: 1 min) × 40 cycles | Liu et al. 1999 |
| | fRPB2-7cR | 5’- CCCATRGCTTGYTTRCCCAT -3’ | | |
| *tef1-α* | EF1-983F | 5’- GCYCCYGGHCAYCGTGAYTTYAT -3’ | (95 °C: 15 s, **50 °C**: 20 s, 72 °C: 1 min) × 35 cycles | Rehner and Buckley 2005 |
| | EF1-1567R | 5’- ACHGTRCCRATACCACCRATCTT -3’ | | |
| *cytb* | E1M4 | 5’- TGRGGWGCWACWGTTATTACTA -3’ | (94 °C: 30 s, **49 °C**: 30 s, 72 °C: 2 min) × 35 cycles | Green et al. 2019 |
| | E2 mr4 | 5’- AGCACGTARWAYWGCRTARWAHGG -3’ | | |

**Table 2.** Strains of Microbotryomycetes used in the molecular analyses in the present study.

| Species | Strain | GenBank accession numbers | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ITS | LSU | SSU | *rpb1* | *rpb2* | *tef1-α* | *cytb* |
| *Camptobasidium arcticum* | EXF 12713H$^{T}$ | MN983248 | MK454798 | MT304813 | NA | MT260386 | MT260390 | MT260394 |
| *Camptobasidium gelus* | EXF 12745$^{T}$ | AY040665 | AY040647 | NA | NA | NA | NA | NA |
| *Colacogloea falcata* | JCM 6838$^{T}$ | AF444543 | AF075490 | AB021670 | KJ708124 | KJ708301 | KJ707943 | KJ707723 |
| *Colacogloea foliorum* | JCM 1696$^{T}$ | AF444633 | AF317804 | KJ708378 | KJ708126 | KJ708230 | KJ707941 | AB040622 |
| *Colacogloea hydrangeae* | CGMCC 2.2798$^{T}$ | MK050451 | NA | NA | MK849147 | NA | MK849017 | NA |
| *Colacogloea rhododendri* | CGMCC 2.5821$^{T}$ | MK050452 | NA | NA | MK849145 | MK849286 | MK849014 | MK848887 |
| *Curvibasidium pallidicorallinum* | CBS 9091$^{T}$ | AF444641 | AF444736 | KJ708420 | KJ708000 | KJ708167 | KJ707767 | KJ707665 |
| *Fellozyma inositophila* | JCM 5654$^{T}$ | AF444559 | AF189987 | AB021673 | KJ708136 | KJ708306 | KJ707951 | KJ707718 |
| *Glaciozyma antarctica* | JCM 9057$^{T}$ | AF444529 | AF189906 | DQ785788 | KJ708131 | KJ708182 | NA | KJ707745 |
| *Hamamotoa lignophila* | CBS 7109$^{T}$ | AF444513 | AF189943 | KJ708372 | KJ708139 | KJ708241 | KJ707953 | KJ707637 |
| *Hamamotoa singularis* | JCM 5356$^{T}$ | AF444600 | AF189996 | AB021690 | KJ708140 | KJ708336 | KJ707957 | KJ707716 |
| *Kriegeria eriophori* | CBS 8387$^{T}$ | AF444602 | NR119455 | DQ419918 | NA | NA | NA | NA |
| *Kriegeriopsis livingstonensis* | AM1149$^{T}$ | ON922980 | ON926889 | NA | NA | NA | NA | NA |
| *Kriegeriopsis livingstonensis* | AM1150 | ON922981 | ON926890 | NA | NA | NA | NA | NA |
| *Leucosporidium creatinivorum* | CBS 8620$^{T}$ | AF444629 | AF189925 | KJ708418 | KJ708036 | KJ708178 | KJ707789 | KJ707658 |
| *Leucosporidium fellii* | JCM 9887$^{T}$ | AF444508 | AF189907 | KJ708449 | KJ708030 | KJ708184 | KJ707784 | KJ707748 |
| *Leucosporidium fragarium* | CBS 6254$^{T}$ | AF444530 | AF070428 | KJ708413 | KJ708031 | KJ708179 | KJ707791 | AB040623 |
| *Leucosporidium muscorum* | CBS 6921$^{T}$ | AF444527 | AF070433 | KJ708414 | KJ708038 | KJ708180 | KJ707793 | AB040638 |
| *Leucosporidium scottii* | JCM 9052$^{T}$ | AF444495 | AF070419 | X53499 | KJ708033 | KJ708186 | KJ707788 | AB040658 |
| *Leucosporidium yakuticum* | CBS 8621$^{T}$ | AY212989 | AY213001 | KJ708419 | NA | KJ708181 | NA | KJ707659 |
| *Libkindia masarykiana* | PYCC 6886$^{T}$ | KU187885 | KU187889 | OP883947 | NA | NA | NA | NA |
| ***Lichenia svalbardensis*** | **CCTCC AY 2022006$^{T}$** | **OP866826** | **OP866960** | **OP866961** | **NA** | **OR485568** | **NA** | **NA** |
| ***Lichenia svalbardensis*** | **JCM 36172** | **PQ164714** | **PQ164717** | **PQ164721** | **NA** | **OR485569** | **NA** | **NA** |
| *Meredithblackwellia eburnea* | CBS 12589$^{T}$ | JX508799 | JX508798 | JX508797 | NA | NA | NA | NA |
| *Microbotryum violaceum* | CBS 143.21$^{T}$ | KJ708462 | KJ708462 | KJ708388 | KJ708042 | KJ708192 | KJ707811 | KJ707613 |
| *Microstroma phylloplanum* | CBS 8073$^{T}$ | AB038131 | AF190004 | AJ496258 | KP322906 | KP323063 | KP323116 | AB041051 |
| *Oberwinklerozyma dicranopteridis* | CGMCC 2.3441$^{T}$ | MK050426 | NA | NA | MK849162 | MK849300 | NA | MK848901 |
| *Oberwinklerozyma nepetae* | CGMCC 2.5824$^{T}$ | MK050427 | NA | NA | MK849254 | MK849391 | NA | MK848992 |

| Species | Strain | GenBank accession numbers | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ITS | LSU | SSU | *rpb1* | *rpb2* | *tef1-α* | *cytb* |
| *Oberwinklerozyma yarrowii* | JCM 8232$^{T}$ | AF444628 | AF189971 | AB032658 | NA | KJ708275 | KJ707938 | KJ707735 |
| *Phenoliferia glacialis* | CBS 10436$^{T}$ | EF151249 | EF151258 | KJ708381 | KJ708067 | KJ708233 | KJ707831 | KJ707597 |
| *Phenoliferia psychrophenolica* | CBS 10438$^{T}$ | EF151246 | EF151255 | KJ708382 | KJ708071 | KJ708259 | KJ707859 | KJ707598 |
| *Phenoliferia psychrophila* | CBS 10440$^{T}$ | EF151243 | EF151252 | KJ708383 | NA | KJ708260 | KJ707833 | KJ707599 |
| *Pseudohyphozyma bogoriensis* | JCM 1692$^{T}$ | AF444536 | AF189923 | KJ708363 | KJ708130 | KJ708216 | KJ707949 | AB040619 |
| *Pseudohyphozyma hydrangeae* | CGMCC 2.2796$^{T}$ | MK050443 | NA | NA | MK849126 | MK849287 | MK849015 | MK848888 |
| *Pseudohyphozyma lulangensis* | CGMCC 2.2612$^{T}$ | MK050442 | NA | NA | MK849129 | MK849270 | NA | MK848875 |
| *Pseudohyphozyma pustula* | JCM 3934$^{T}$ | AF444531 | AF189964 | KJ708361 | KJ708128 | KJ708261 | KJ707937 | AB040642 |
| *Psychromyces glacialis* | EXF 13111$^{T}$ | MK671633 | MT301949 | MT248408 | NA | MW036268 | MT260389 | MT260392 |
| *Rhodosporidiobolus azoricus* | JCM 11251$^{T}$ | AB073229 | AF321977 | AB073269 | KJ708053 | KJ708202 | KJ707813 | KJ707693 |
| *Rhodosporidiobolus fluvialis* | JCM 10311$^{T}$ | AY015432 | AF189915 | AB073272 | KJ708046 | KJ708204 | KJ707816 | KJ707679 |
| *Rhodosporidiobolus jianfalingensis* | CGMCC 2.3532$^{T}$ | MK050402 | NA | NA | MK849179 | MK849317 | MK849048 | MK848917 |
| *Rhodosporidiobolus microsporus* | JCM 6882$^{T}$ | AF444535 | AF070436 | KJ708441 | KJ708054 | KJ708284 | KJ707817 | KJ707724 |
| *Rhodosporidiobolus odoratus* | JCM 11641$^{T}$ | KJ778638 | AF387125 | KJ708427 | KJ708045 | KJ708322 | KJ707819 | KJ707694 |
| *Rhodosporidiobolus ruineniae* | JCM 1839$^{T}$ | AF444491 | AF070434 | AB021693 | KJ708052 | KJ708286 | KJ707820 | KJ707700 |
| *Rhodotorula araucariae* | JCM 3770$^{T}$ | AF444510 | AF070427 | KJ708435 | KJ708096 | KJ708209 | KJ707862 | AB041048 |
| *Rhodotorula babjevae* | JCM 9279$^{T}$ | AF444542 | AF070420 | AB073270 | NA | NA | KJ707874 | KJ707746 |
| *Rhodotorula glutinis* | JCM 8208$^{T}$ | AF444539 | AF070429 | X69853 | NA | NA | KJ707869 | AB040626 |
| *Rhodotorula graminis* | JCM 3775$^{T}$ | AF444505 | AF070431 | X83827 | KJ708093 | KJ708234 | KJ707868 | AB040628 |
| *Slooffia cresolica* | JCM 10955$^{T}$ | AF444570 | AF189926 | KJ708365 | KJ708135 | KJ708222 | KJ707942 | NA |
| *Slooffia pilatii* | JCM 9036$^{T}$ | AF444598 | AF189963 | KJ708364 | KJ708137 | KJ708256 | KJ707947 | AB040641 |
| *Sporobolomyces johnsonii* | CBS 5470$^{T}$ | AY015431 | AY015431 | AY015431 | AY015431 | AY015431 | AY015431 | AY015431 |
| *Ustilago maydis* | CBS 504.76$^{T}$ | AF453938 | AY854090 | X62396 | XM401478 | AY485636 | AY885160 | AB040663 |
| *Yamadamyces rosulatus* | CBS 10977$^{T}$ | EU872492 | EU872490 | KJ708384 | KJ708083 | KJ708263 | KJ707854 | KJ707607 |
| *Yamadamyces terricola* | CGMCC 2.5820$^{T}$ | MK050425 | NA | NA | MK849127 | MK849268 | MK848999 | MK848874 |
| *Yurkovia longicylindrica* | CGMCC 2.5603$^{T}$ | MK050441 | NA | NA | MK849218 | MK849357 | MK849084 | MK848952 |

[1] CBS: Westerdijk Fungal Biodiversity Institute (CBS-KNAW Fungal Biodiversity Centre), Utrecht, The Netherlands; CCTCC, China Center for Type Culture Collection, Wuhan, China; CGMCC, Chinese General Microbiological Culture Collection Center, Beijing, China; EXF, Microbial Culture Collection Ex of the Infrastructural Centre Mycosmo, Ljubljana, Slovenia; JCM, Japan Collection of Microorganisms, RIKEN BioResource Center, Saitama, Japan; PYCC, Portuguese Yeast Culture Collection, Caparica, Portugal; NA: not applicable. All the new isolates used in this study are in bold, and the type materials are marked with T.

## Biochemical and physiological tests

Biochemical and physiological tests were performed according to the protocols described by Kurtzman et al. (2011). All results were recorded 30 days post inoculation. The test tubes were sterilized by 1 N HCl to guarantee their cleanliness in assimilation tests. Starved cells were prepared through shaking in 1 mL of sterilized water for 7 days at 10 °C. For growth tests on carbon compounds, each tube of YNB medium containing carbon compound equal to 0.5% glucose was inoculated with starved cells, and YCB containing nitrogen compound equal to 0.0108% of nitrogen for nitrogen growth tests. Starved cells were inoculated on a vitamin-free yeast base in vitamin-free growth tests. Cell cultures were serially diluted $10/10^2/10^3/10^4/10^5$-fold, spotted onto PDA medium, and incubated for 7 days to measure the growth at various temperatures (4 °C, 10 °C, 15 °C, 20 °C, 22.5 °C, 25 °C). Tolerance of NaCl was tested with 10% NaCl concentrations (10% NaCl, 5% glucose, 0.2% $(NH4)_2SO_4$, 0.02% $MgSO_4$, 0.001% $CaCl_2$, 0.00001% $FeSO_4$, 0.15% $Na_2HPO_4$, 0.15% $K_2HPO_4$, and 2% agar). The growth in high osmotic pressure was measured in PDA plates with 50% D-Glucose. To measure the growth curve of *Lichenia svalbardensis* sp. nov., 300 μL plateau cells were inoculated to 30 mL PDB at 10 °C for 10 days. The values for optical density of yeast cells at 600 nm (OD600) were measured by using the spectrophotometer. For the hydrolysis test of urea, cells from PDA slant were incubated on Christensen's urea agar slant (0.1% peptone, 0.5% NaCl, 0.2% $(NH_4)H_2PO_4$, and 0.0012% phenol red, 2% agar) for four days. DBB reagent was applied to the surface of the culture to conduct the diazonium blue B color reaction. Three replicates were conducted for each test. The result of physiological tests has been recorded below.

## Result

### Phylogeny

The phylogenetic position of *Lichenia svalbardensis* in Microbotryomycetes was analyzed based on two datasets, namely a concatenated seven-loci dataset (SSU, ITS, LSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*) and a concatenated ITS and LSU dataset. The seven loci analyses were similar to the tree topologies of the combined analyses. The dataset consisted of 54 isolates representing 52 species and 25 genera, including two outgroup taxa (*Pseudomicrostroma phylloplana* CBS 8073 and *Ustilago maydis* CBS 504.76). The total length of the concatenated seven-locus alignment was 10,732 characters, including gaps (2,341 for SSU, 924 for ITS, 652 for LSU, 1,265 for *rpb1*, 1,722 for *rpb2*, 3,378 for *tef1-α*, and 430 for *cytb*), and 1,580 characters, including gaps (924 for ITS and 652 for LSU), for the ITS+LSU alignment. The phylogram of the concatenated dataset resulting from ML analyses was similar to the result of BI analyses. ML bootstraps (ML BS ≥ 70%) and Bayesian Posterior Probabilities (BPP ≥ 0.95) were given at the nodes in the phylograms. (Fig. 1, Suppl. material 1). The phylogenetic trees reveal that *L. svalbardensis* had a close relation with *Phenoliferia*, *Kriegeria*, *Kriegeriopsis*, *Libkindia*, *Meredithblackwellia*, and *Yamadamyces* with high support value (ML/BI = 94/1.00), which has been described below.

### Taxonomy

***Lichenia* Zeyu Tang & Fang Peng, gen. nov.**
MycoBank No: 846865

**Etymology.** The name reflects the organism that the species was isolated from, lichen.

**Type species.** *Lichenia svalbardensis* Zeyu Tang & Fang Peng

**Culture characteristics.** Colonies on PDA butyrous, white. Hyphae, pseudohyphae, and budding cells were observed. Hyphae and pseudohyphae hyaline, unbranched, white to grey, septate. Cells and budding cells hyaline, ellipsoidal, smooth, guttulate. Sexual reproduction not known.

**Notes.** In the phylogenetic trees, *Kriegeria*, *Kriegeriopsis*, *Libkindia*, *Lichenia*, *Meredithblackwellia*, *Phenoliferia*, and *Yamadamyces* were clustered in Kriegeriaceae (Fig. 1, Suppl. material1). The identity rates of ITS and LSU between *Lichenia* and other genera in Kriegeriaceae are lower than the genera thresholds of 96.31% for ITS and 97.11% for LSU (Table 3), agreeing with the taxonomic thresholds predicted by Vu et al. (2016). Therefore, we propose *Lichenia* as a new genus in Kriegeriaceae.

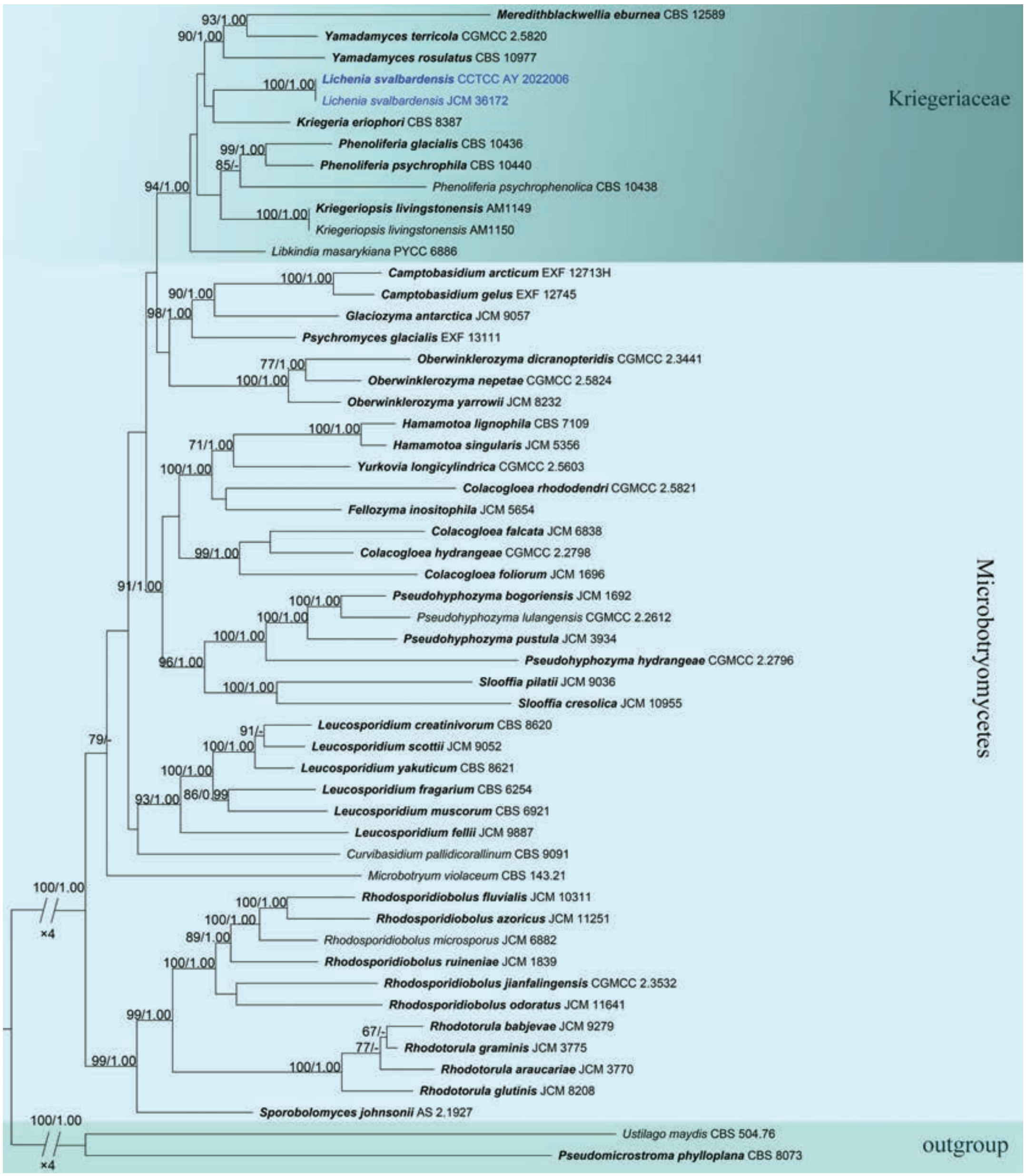

**Figure 1.** Phylogram of Microbotryomycetes resulting from a maximum likelihood analysis based on a combined matrix of ITS, LSU, SSU, *rpb1*, rpb2, *tef1-α*, and *cytb*. Numbers above the branches indicate ML bootstraps (left, ML BS ≥ 70%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.95). The tree is rooted with *Pseudomicrostroma phylloplana* CBS 8073 and *Ustilago maydis* CBS 504.76. Isolates from the present study are marked in blue, and holotype isolates are made in bold.

**Table 3.** Identity rates in ITS and LSU between *Lichenia svalbardensis* and other species in Kriegeriaceae (%).

| Species | ITS | LSU |
|---|---|---|
| ***Kriegeria eriophori*** | 88.36% | 95.87% |
| ***Kriegeriopsis livingstonensis*** | 86.60% | 96.00% |
| ***Libkindia masarykiana*** | 93.71% | 95.60% |
| ***Meredithblackwellia eburnea*** | 86.41% | 91.60% |
| ***Phenoliferia glacialis*** | 90.36% | 95.71% |
| ***Phenoliferia psychrophenolica*** | 89.38% | 95.84% |
| ***Phenoliferia psychrophila*** | 89.80% | 96.38% |
| ***Yamadamyces rosulatus*** | 89.32% | 96.05% |
| ***Yamadamyces terricola*** | 89.44% | 96.38% |

***Lichenia svalbardensis* Zeyu Tang & Fang Peng, sp. nov.**
MycoBank No: 846866
Fig. 2

**Etymology.** The name reflects the station where this species was collected, Svalbard, Norway.

**Specimens examined.** Norway, Svalbard, isolate from dendritic lichen (Usneaceae) on the rock, 78°13'12.91"N, 15°20'6.39"E, Jul. 2014, Fang Peng (holotype CCTCC AY 2022006, preserved in a metabolically inactive state; other living culture: JCM 36172).

**Culture characteristics.** On YMA and PDA plates, after 7 days and 30 days at 4 °C, cultures are smooth, butyrous, creamy-white, without hypha around the single colony (Fig. 2C); after 7 days and 30 days at 10 °C and 15 °C, cultures white to yellowish, smooth, butyrous, filamented margin, hyphae grow around the most single colony (Fig. 2D); after 7 days and 30 days at 20 °C, cultures white to yellowish, with rough surface and edge, smooth single colonies are observed seldomly (Fig. 2E).

**Micromorphology.** In YM and PD broth, yeast cells are hyaline, ellipsoidal, smooth, guttulate, 9.5–15.6 × 3.4–4.5 μm (av. = 12.6 ± 3.5 × 4.0 ± 0.8 μm, n = 30), with a gelatinous sheath (Fig. 2I–K). Budding is enteroblastic and occurs on a narrow base from each pole (Fig. 2H). After 7 days at 10 °C, pseudohyphae are formed; at 15 °C and 20 °C, numerous pseudohyphae and hyphae are formed (Fig. 2F–G), numerous yeasts forming rosettes (Fig. 2G). Sexual structures are not observed on YMA, PDA, and CMA. Ballistoconidia are not produced.

**Notes.** *Lichenia svalbardensis* was isolated from lichen in polar habitats. Numerous yeast cells of *Lichenia svalbardensis* clustered and formed rosettes. It is consistent with the morphological characteristics of Kriegeriaceae (Toome et al. 2013). In the seven loci phylogenetic analyses, *L. svalbardensis* from lichen (Usneaceae) formed a well-supported monophyletic clade, distinct from *Kriegeria eriophori*, *Libkindia masarykiana*, and *Meredithblackwellia eburnea* (Fig. 1). Morphologically, cells of *L. svalbardensis* (9.5–15.6 × 3.4–4.5 μm) are shorter than *Meredithblackwellia eburnea* (12.6–17.6 × 3.9–5.2 μm), wider than *Libkindia masarykiana* (8.5–12.0 × 2.0–3.0 μm), and shorter than *Kriegeria eriophori* (23.0–29.0 × 4.0–5.0 μm) (Doubles and McLaughlin 1992; Toome et al. 2013; Masinova et al. 2017). Therefore, we kept *L. svalbardensis* separate.

## Physiological and biochemical characteristics

Physiological characteristics of *Lichenia svalbardensis* in the current study have been measured. In detail, D-(+)-glucose, inulin, β-lactose, maltose, methyl-α-D-glucoside, D(+)-raffinose, sucrose, and D-(+)-xylose fermentation are negative. D-(+)-glucose, D-(+)-cellobiose, ethanol, D-(+)-galactose, D-gluconate, D-glucitol, β-lactose, L-(+)-arabinose, maltose, D-(+)-melibiose, D-(+)-melezitose, ribitol, D(+)-raffinose, L-rhamnose, D-(-)-ribose, D-(+)-trehalose, xylitol, citrate (weak), D-arabinose (weak), inulin (weak), DL-lactate (weak), D-mannitol (weak), D-glucosamine (delayed), and D-(+)-xylose (delayed) are assimilated as sole carbon sources. Meso-erythritol, glycerol, galactitol, myo-inositol, methyl-α-D-glucoside, L-(-)-sorbose, and sucrose are

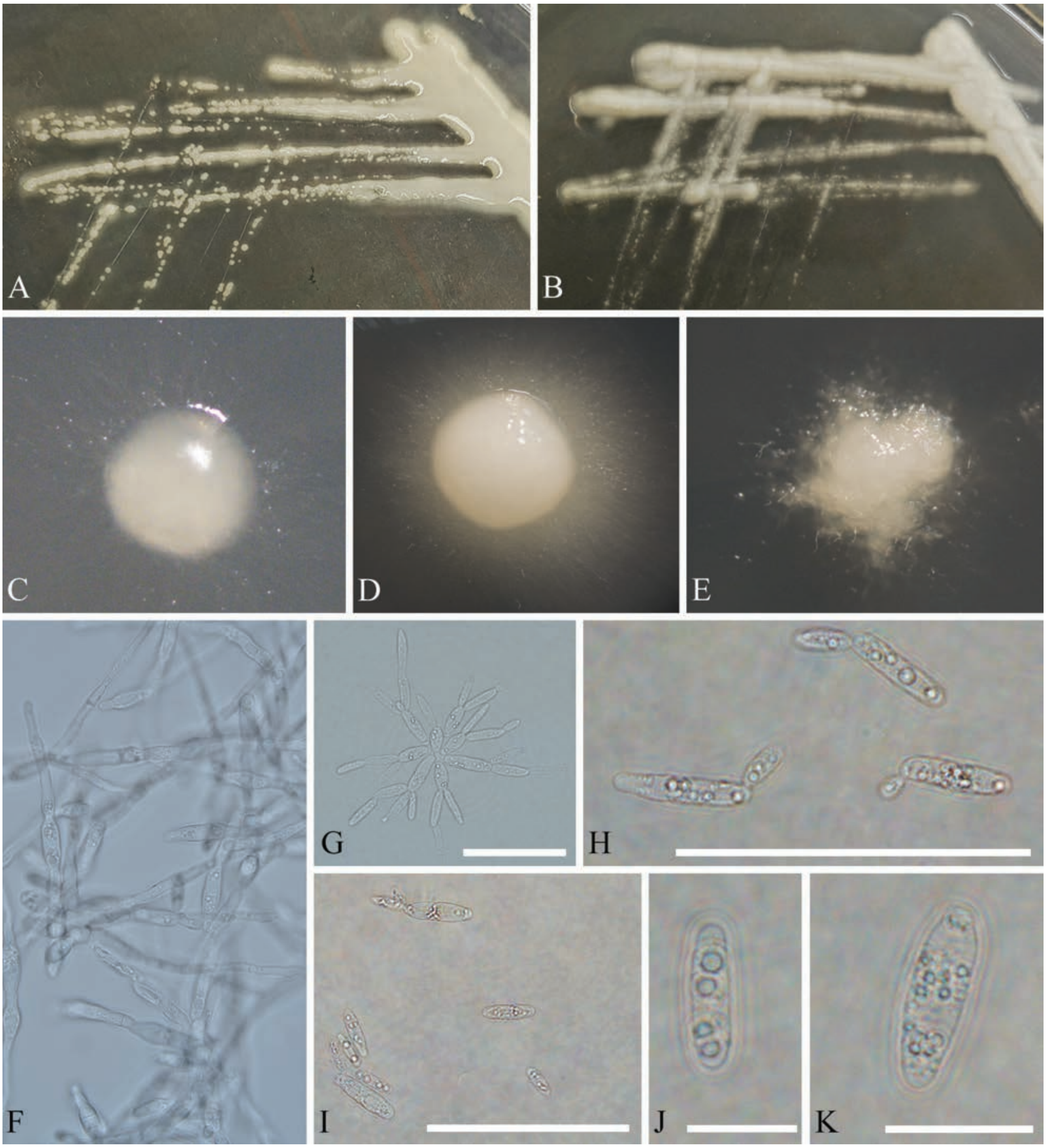

**Figure 2.** Morphology of *Lichenia svalbardensis* **A–E** cultures after incubation for 1 week **A** cultures on YMA at 10 °C **B** cultures on YMA at 20 °C **C** single colony on YMA at 4 °C **D** single colony on YMA at 10 °C **E** single colony on YMA at 20 °C **F** hyphae **G** pseudohyphae **H** apically budding yeast cells **I–K** yeast cells. Scale bars: 50 µm (**G, I**); 30 µm (**H**); 10 µm (**J–K**).

not assimilated. Ethylamine, N-acetyl-D-glucosamine, nitrate, nitrite, and creatinine (delayed) are assimilated as sole nitrogen sources. Cadaverine, D-glucosamine, and L-lysine are not assimilated. The maximum growth temperature is 20 °C. Growth in vitamin-free medium is positive. Growth on 50% (w/w) glucose yeast extract agar is negative. Growth on glucose agar with 10% NaCl is negative. Urease activity is positive. Diazonium blue B reaction is positive. Comparisons of physiological characteristics of *L. svalbardensis* and other members of Kriegeriaceae have been listed in Table 4.

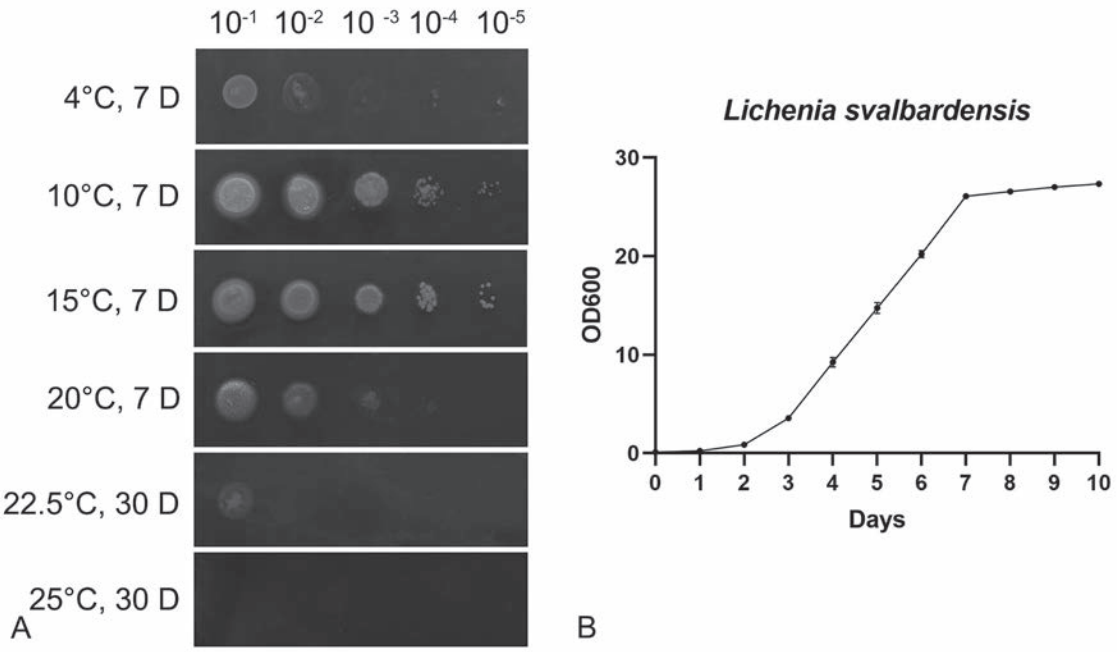

**Figure 3.** Growth of *Lichenia svalbardensis* at different temperatures **A** cell cultures spotted onto PDA medium and incubated at 4 °C, 10 °C, 15 °C, 20 °C, 22.5 °C, and 25 °C **B** growth curve of *Lichenia svalbardensis* in PBD at 10 °C.

**Table 4.** Comparison of physiological characteristics of *Lichenia svalbardensis* and other members of Kriegeriaceae and Camptobasidiaceae.

| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Carbon source** | | | | | | | | | | | | | |
| L-Sorbose | – | d | d, w | w | – | – | – | – | – | – | – | – | + |
| D-Galactose | + | + | + | – | – | – | – | – | – | – | – | + | d |
| D-Glucosamine | d | – | – | – | – | – | – | w | d, w | – | – | – | d,w |
| D-Ribose | + | + | – | + | – | – | – | – | – | – | – | – | – |
| D-Xylos | d | + | + | w | n/a | n/a | – | w | – | v | – | v | d,w |
| L-Arabinose | + | + | + | w | + | – | – | – | – | – | – | – | – |
| L-Rhamnose | + | + | – | w | + | + | – | + | – | – | – | – | – |
| Sucrose | – | + | + | + | + | + | + | + | + | + | + | v | + |
| Cellobiose | + | + | – | + | – | – | – | + | – | + | – | – | w |
| Melibiose | + | d | – | – | – | – | – | – | – | v | – | – | – |
| Melezitose | + | + | + | + | n/a | – | + | + | + | + | + | – | + |
| Lactose | + | – | – | – | – | – | – | – | – | v | – | – | – |
| Raffinose | + | – | – | – | + | + | + | – | – | d | – | – | + |
| Glycerol | – | + | + | + | – | – | – | w | w | – | + | w | – |
| myo-Inositol | – | – | – | – | – | – | – | + | – | v | – | – | – |
| DL-Lactat | w | d | – | + | – | – | – | d | – | n/a | – | n/a | n/a |
| Citrate | w | + | w | – | – | – | – | d | – | n/a | – | n/a | n/a |
| **Nitrogen source** | | | | | | | | | | | | | |
| Nitrite | + | + | – | – | – | – | – | + | – | – | + | + | + |
| Nitrate | d | + | – | – | + | + | + | + | – | + | + | + | + |
| Ethylamine | d | + | + | + | + | + | + | + | + | n/a | – | n/a | n/a |
| **Others** | | | | | | | | | | | | | |
| Existence of dimorphic stage | + | + | – | – | – | – | – | + | – | – | + | + | + |
| w/o vitamins | + | + | + | + | n/a | n/a | n/a | – | + | n/a | + | n/a | n/a |

1. *Lichenia svalbardensis*; 2. *Kriegeria eriophori*; 3. *Libkindia masarykiana*; 4. *Meredithblackwellia eburnea*; 5. *Phenoliferia glacialis*; 6. *Phenoliferia psychrophenolica*; 7. *Phenoliferia psychrophila*; 8. *Yamadamyces rosulatus*; 9. *Yamadamyces terricola*; 10. *Camptobasidium arcticum*; 11. *Cryolevonia schafbergensis*; 12. *Glaciozyma antarctica*; 13. *Psychromyces glacialis*. +. positive; –. negative; d. delayed; w. weak; v. variable (–/+/w/d); n/a = data not available.

Through examining the effect of temperature on *L. svalbardensis*, we found that this species can grow well from 4 °C to 20 °C (Fig. 3A). The fastest growth rates were observed at 10 °C and 15 °C. However, *L. svalbardensis* remained at no growth at 25 °C or higher temperatures after one month (Fig. 3A). Because pseudohyphae and hyphae were observed for a large proportion at 15 °C and 20 °C, which can influence the values for optical density. The growth curve of *L. svalbardensis* was measured at 10 °C. This species grows slowly and reaches a plateau at 7 days (Fig. 3B).

## Discussion

The present study reports a new psychrophilic yeast in the Kriegeriaceae family associated with lichen in the Arctic. The isolates in this study were identified as a new genus with *Lichenia svalbardensis* as the type species. It grows fastest at 10 °C and 15 °C. Moreover, pseudohyphae and hyphae can be observed from 10 °C to 20 °C.

Based on modern taxonomic concepts, we propose the isolate as a new genus in Kriegeriaceae. The taxonomic thresholds predicted for yeast species delimitation at the genus level were 96.31% for ITS and 97.11% for LSU recommended by Vu et al. (2016). Phylogenetically, the identity rates of ITS and LSU between *Lichenia* and other genera in Kriegeriaceae are lower than the genera thresholds (Table 3). Although *L. svalbardensis* appeared to be closely related to *Kriegeria eriophori* in the phylogenetic trees of seven loci (ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*) and two loci (ITS and LSU) combined (Fig. 1, Suppl. material 1), the identity rates of ITS (88.36% vs. 96.31%) and LSU (95.87% vs. 97.11%) between *Lichenia* and *Kriegeria* are much lower than the genera thresholds (Table 3), especially ITS. Morphologically, the yeast cells of *L. svalbardensis* (9.5–15.6 × 3.4–4.5 μm) are much shorter, significantly different from *K. eriophori* (23.0–29.0 × 4.0–5.0 μm) (Doubles and McLaughlin 1992). Moreover, *L. svalbardensis* is different from *K. eriophori* by host association and sampling location (lichen in the Arctic vs. *Scirpus atrovirens* in North America). Therefore, *Lichenia* was considered a new genus.

The phylogram of two ribosomal loci (ITS and LSU) is similar to the seven loci (ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*). All of the species in Kriegeriaceaea clustered together with high support values of ML/BI = 94/1.00 in the phylogenetic analyses of seven loci (ITS, LSU, SSU, *rpb1*, *rpb2*, *tef1-α*, and *cytb*). When contaminant genes are deleted from the dataset, *Lichenia* clusters with *Kriegeria*, *Meredithblackwellia*, and *Yamadamyces* in the two phylogenetic trees. However, the *Libkindia masarykiana* clustered with *Kriegeriopsis livingstonensis* in the phylogram of ITS and LSU, different from the phylogram of seven genes. This may be due to the influence of the missing SSU locus. For example, only ITS and LSU loci were available for *Kriegeriopsis livingstonensis*, which was obtained from lichenicolous specimens instead of cultures (Diederich et al. 2022). Additionally, the low support values between *Libkindia masarykiana* and *Kriegeriopsis livingstonensis* (ML/BI = 48/0.79) also lead to this result. Therefore, a more robust and complete molecular dataset is needed.

With only ribosomal loci (ITS, LSU, and SSU) incorporated in the analyses, Camptobasidiaceae and Kriegeriaceaea clustered as sisters in the phylogenetic tree (Toome et al. 2013). But when seven loci were used in the phylogenetic analyses, the two families clustered in different clades (Schoutteten et al. 2023). The physiological characters of the two families also showed no obvious association (Table 4). *Lichenia svalbardensis* in this study and other four species in Kriegeriaceaea (*Phenoliferia glacialis*, *P. psychrophenolica*, and *P. psychrophila*) were confirmed as psychrophilic yeasts (Margesin et al. 2007). Camptobasidiaceae mainly comprises psychrophilic yeasts (De Garcia et al. 2020; Pontes et al. 2020; Perini et al. 2021). Psychrophilia of these species in the two families indicates they may have a close genetic relationship. Due to the lack of more samples and other evidence, the relationship between Camptobasidiaceae and Kriegeriaceaea, as well as the higher systematics of Microbotryomycetes in general, need further study.

The physiological characteristics of all species in Kriegeriaceae show that lactose is assimilated as the sole carbon source and that sucrose is not assimilated for *L. svalbardensis*, which are different from other species in Kriegeriaceae (Table 4). Hence, *L. svalbardensis* can be distinguished from other species in Kriegeriaceae by its capacity to assimilate lactose and sucrose. Moreover, the result of the diazonium blue B reaction and urease activity are positive, agreeing with the characters of Basidiomycetous (Hagler and Ahaearn 1981).

Microorganisms that show no growth above 20 °C can be classified as psychrophiles (Margesin et al. 2003). Colonies of *L. svalbardensis* in the current study grew from 4 °C to 20 °C but not at 25 °C or higher temperatures after one month of incubation (Fig. 3A). Compared to colonies at 4–20 °C after one week of incubation, the colony grows at a significantly lower level at 22.5 °C after one month of incubation (Fig. 3A). Therefore, *L. svalbardensis* could be classified as a psychrophile, which may be due to *L. svalbardensis* being isolated from the polar region. Psychrophilic yeasts with various extracellular enzymatic activities (extracellular amylolytic, proteolytic, lipolytic, esterasic, pectinolytic, chitinolytic, and cellulolytic activities) were screened by Brizzio et al. (2007). These psychrophilic yeasts could be considered a potential source of industrially relevant cold-active enzymes. This implies that *L. svalbardensis* may also become a resource in cold-active industries.

One of the most prominent traits documented for yeasts is their ability to grow in different forms (e.g., *Paracoccidioides brasiliensis* and *Yarrowia lipolytica*) (Klein and Tebbets 2007; Wu et al. 2020). The morphology of yeasts can be regulated by various environmental factors (Wang et al. 2020). In this study, *L. svalbardensis* can undergo morphological changes between yeast, pseudohyphal, and hyphal forms of growth in different temperatures. Dimorphic switching is a specialized adaptation to the environment (Boyce and Andrianopoulos 2015). *Lichenia svalbardensis* was isolated from lichen. In the collaboration of photosynthetic alga or cyanobacterium, yeast can offer protection from the environment (Spribille et al. 2016). Morphological transformation of this species may be to adapt to different environments, which may contribute to lichen adapting to different temperatures. Although there is not enough evidence that *L. svalbardensis* can offer protection, physiological characteristics (cellobiose, ethanol, melezitose, and melibiose are assimilated as sole carbon sources) imply that *L. svalbardensis* may be symbiotic with photosynthetic species.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: YB, FP. Data curation: YB, ZT. Formal analysis: MS, ZT, YB. Funding acquisition: FP. Investigation: FP. Methodology: JL, YB, ZT. Project administration: FP. Resources: FP. Software: YB. Supervision: FP. Validation: FP, YB, ZT. Visualization: YB. Writing - original draft: YB, ZT. Writing - review and editing: YB, XP, FP, JH.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Phylogram of Microbotryomycetes resulting from a maximum likelihood analysis based on a combined matrix of ITS and LSU

Authors: AuthorsNames

Data type: tif

Explanation note: Numbers above the branches indicate ML bootstraps (left, ML BS ≥ 70%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.95). The tree is rooted with *Pseudomicrostroma phylloplana* CBS 8073 and *Ustilago maydis* CBS 504.76. Isolates from present study are marked in blue and holotype isolates are made in bold.

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Research Article

# Four new species of *Pichia* (Pichiales, Pichiaceae) isolated from China

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## Abstract

The genus *Pichia* belonging to the family Pichiaceae is widely distributed worldwide and has garnered significant attention due to its importance in various industries and its potential role in human infections. During our investigation of yeast diversity in China, several strains representing undescribed taxa were isolated from forests in Hainan province, Hubei province, Beijing city and a mudflat in Guangdong province. Based on phylogenetic analyses of the internal transcribed spacer (ITS) region and the D1/D2 domain of the large subunit (LSU) rRNA, these strains were identified as four new species: *Pichia kregeriana* **sp. nov.** (holotype strain CGMCC 2.7383$^T$), *P. phaffii* **sp. nov.** (holotype strain CGMCC 2.8239$^T$), *P. ureolytica* **sp. nov.** (holotype strain CGMCC 2.6825$^T$) and *P. wuzhishanensis* f.a. **sp. nov.** (holotype strain CGMCC 2.7381$^T$). The six strains of *P. ureolytica* were identified as positive for urease production. This phenomenon is extremely rare in the genus *Pichia*, as only *P. bovicola* is reported to exhibit weak urease activity.

**Key words:** New species, phylogeny, taxonomy, urease activity

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## Introduction

In the past decade, approximately 400 research papers related to *Pichia* species have been published annually according to data from Web of Science (accessed on October 31, 2024). *Pichia* species have significant applications in various industries, including biosynthesis (Zhang et al. 2023), biotransformation (Ranieri et al. 2024), alcoholic and food fermentation (Teramoto et al. 2001; Hu et al. 2022; Xiao et al. 2023), feed production (Qvirist et al. 2016), enzyme production (Tsang 2011) and epidemiology (Yadav et al. 2012). Furthermore, besides the benefits mentioned above, the species *Candida krusei*, a synonym of *Pichia kudriavzevii*, is one of the significant species, which can cause human infections even on the WHO fungal priority pathogens list (Nguyen et al. 2024).

The genus *Pichia*, which was previously classified under the order Saccharomycetales within the class Saccharomycetes, has now been transferred to the

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[*] Contributed equally to this work.

order Pichiales within the class Pichiomycetes by Groenewald et al. (2023). It was established by Hansen in 1904 with *Pichia membranifaciens* as the type species. This genus is characterized by its multilateral budding, the occasional presence of pseudohyphae (not true hyphae), and the formation of ascospores that may be hat-shaped, hemispheroidal, or spherical, with or without a ledge (Kurtzman 2011). Almost all species within *Pichia* are capable of fermenting glucose, but do not assimilate nitrate (Kurtzman 2011). In the fourth edition of "*The Yeasts, a Taxonomic Study*," Kurtzman (1998) accepted 91 species within the genus *Pichia*, which was traditionally considered polyphyletic. However, Kurtzman et al. (2008) later redefined the genus *Pichia* and clarified its phylogenetic relationships with genera such as *Issatchenkia* and *Williopsis*, using the large and small subunit (LSU and SSU) rRNA genes and the translation elongation factor-1a (*tef-1a*) gene. In the fifth edition of "*The Yeasts, a Taxonomic Study*," Kurtzman (2011) recognized 20 species within the genus *Pichia*. Since then, several new species and new combinations have been reported in the genus *Pichia* (Bhadra et al. 2008; Limtong et al. 2009; Ganter et al. 2010; Sipiczki 2012; Ren et al. 2015; Kobayashi et al. 2017; Gao et al. 2018; Groenewald et al. 2018; Angchuan et al. 2022; Opulente et al. 2023; Chai et al. 2024; Zhu et al. 2024). For example, Zhu et al. (2024) described a novel species from marine sediment and transferred eight related *Candida* species to the genus *Pichia*. Chai et al. (2024) documented *Pichia teotihuacanensis* from a sample of the Mexican alcoholic beverage Pulque. The genus currently includes a total of 41 species (Suppl. material 1).

In this study, nine yeast strains were isolated from rotten wood in Hainan province, marine sediment in Guangzhou province, and bark samples in Hubei province and Beijing city. These strains were identified as four new species of the genus *Pichia* based on phylogenetic analysis and their morphological, physiological and biochemical characteristics, which contributed to enriching the diversity of *Pichia* in China.

## Materials and methods

### Sample collection and yeast isolation

A variety of samples for yeast isolation were collected from 2021 to 2023, including marine sediments from Dongguan city in Guangdong province, rotten wood from Wuzhishan city in Hainan province and bark from Shennongjia Forest Area in Hubei province and Mentougou district in Beijing city. Samples were sealed in 50 mL sterile centrifuge tubes and transported to the laboratory immediately for storage at 4 °C. Yeasts from rotten wood and bark samples were isolated using the enrichment method described by Yu et al. (2023). The isolation of yeast from marine sediments followed the methods described by Zhu et al. (2023). All strains were suspended in 25% glycerol and stored at –80 °C for long-term preservation.

### Morphological, physiological and biochemical studies

Morphological, physiological and biochemical properties were conducted according to standard methods established by Kurtzman et al. (2011). Carbon and nitrogen assimilation tests were performed in liquid media, with starved inoculum used for nitrogen testing. Sugar fermentation was tested in a liquid medium with Durham tubes. Growth at various temperatures (25, 30, 37 and 40 °C) was determined by cultivation on YPD agar. The formation of pseudohyphae was investigated using the Dalmau plate on corn meal agar (CMA: 2.5% corn starch and 2% agar) with sterile slides to create an anaerobic environment. The potential sexual cycles of strains were investigated using CMA, potato dextrose agar (PDA: 20% potato infusion, 2% glucose, and 2% agar), yeast carbon base agar (YCB: 1.17% yeast carbon base, 2% agar) and V8 agar (10% V8 juice and 2% agar). Each test strain was inoculated separately or mixed on agar plates and incubated at 25 °C for up to two months, which was observed once every half a month.

### DNA extraction, PCR amplification and sequencing

DNA of yeast cells was extracted using the method described by Wang and Bai (2008). The ITS region and D1/D2 domain were amplified with primers ITS1/ITS4 (White et al. 1990) and NL1/NL4 (Kurtzman and Robnett 1998), respectively. PCR products were commercially sequenced by Sangon Biotech Co., LTD. (Beijing, China) and the identity and accuracy of obtained nucleotide sequences were determined by comparing them to sequences in GenBank. All newly generated sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

### Phylogenetic analyses

The sequences obtained in this study and reference sequences downloaded from GenBank (Suppl. material 1) were aligned using MAFFT v. 7 and manually improved using MEGA v. 7 where it was necessary (Katoh and Standley 2013; Kumar et al. 2016). Phylogenetic analysis based on single D1/D2 or ITS sequences was performed based on the evolutionary distance data calculated from Kimura's two parameter model using the neighbour-joining algorithm in MEGA v. 7 (Kimura 1980; Kumar et al. 2016; Lachance 2022). Maximum-likelihood phylogenetic analysis based on the concatenated D1/D2 and ITS sequences was performed using the optional model GTR+I+G determined in MEGA v. 7 (Kumar et al. 2016). The confidence levels of the clades were estimated through 1000 replicates bootstrap analysis (Felsenstein 1985).

## Results

### Phylogenetic analyses

Among the yeasts isolated from marine sediment, bark and rotten wood samples collected from different regions of China, nine yeast strains that could not be identified as any known species were selected for further taxonomic study. To determine the phylogenetic placement of the potential novel strains, phylogenetic analysis was conducted using the ITS and D1/D2 sequences of the nine strains and type strains of members in the genus *Pichia*. The phylogenetic trees indicated that these nine strains represented four new species in the *Pichia* clade (Fig. 1, Suppl. materials 2, 3).

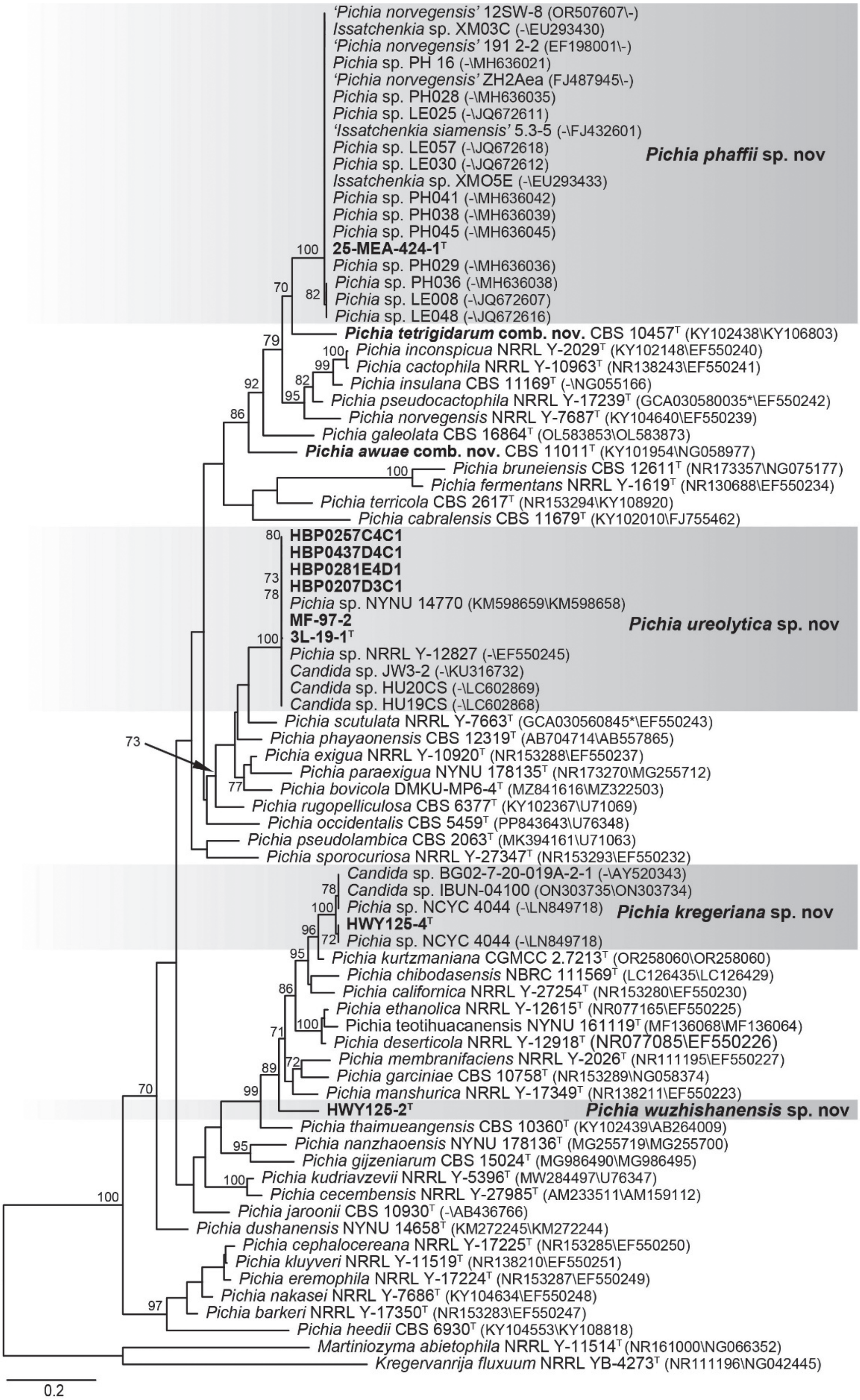

**Figure 1.** Maximum likelihood phylogenetic tree of the genus *Pichia* based on D1/D2 and ITS sequences. The species *Kregervanrija fluxuum* and *Martiniozyma abietophila* were used as the outgroup. Bootstrap values above 70% are shown on the branches. Type strains are denoted with the superscript 'T'. Strains isolated in this study and the new combinations were marked in bold. Bar, 0.2 substitutions per nucleotide position.

Strain HWY125-4 from Hainan province formed a clade together with strains BG02-7-20-019A-2-1 from the USA, IBUN-04100 from Brazil, and NCYC 4038 and NCYC 4044 from Ecuador. These five strains exhibited one nucleotide difference in the D1/D2 domain. When the ITS sequence is available for comparison, strains HWY125-4 and IBUN-04100 possessed similar ITS sequences with no more than four nucleotide mismatches. The clade represented by type strain HWY125-4 is closely related to *P. kurtzmaniana* but differed from the type strain by 15 (2.8%, 11 substitutions and four gaps) and 35 (8.7%, 14 substitutions and 21 gaps) nucleotide mismatches in the D1/D2 domain and ITS region, respectively (Fig. 1, Suppl. materials 2, 3). The result suggested that the HWY125-4 clade represented a novel species in the genus *Pichia*.

Strain 25-MEA-424-1 from Guangdong province was grouped in a distinct clade along with 14 other unidentified strains and one previously identified as *Issatchenkia siamensis* with only D1/D2 sequences available in GenBank (Suppl. material 2). The strains in this clade possess similar D1/D2 sequences with no more than one nucleotide difference (Suppl. material 2). Meanwhile, strain 25-MEA-424-1 formed a clade together with three other strains previously identified as *Pichia norvegensis* with only ITS sequences available in GenBank. These four strains in this clade possessed identical ITS sequences (Suppl. material 3). The strains in the 25-MEA-424-1 group previously named as *Issatchenkia siamensis* and *Pichia norvegensis* (Suppl. material 1, Fig. 1) were obviously misidentifications. The name *Issatchenkia siamensis* cannot be found in any global fungal name registration repositories, namely, Index Fungorum, Mycobank and Fungal Names, which is not recognized as a valid name. The group 25-MEA-424-1 differed from the closest species *Pichia tetrigidarum* comb. nov. by 31 (5.7%, 26 substitutions and five gaps) nucleotide mismatches and 61 (14.7%, 31 substitutions and 30 gaps) nucleotide mismatches in the D1/D2 domain and ITS region, respectively (Fig. 1, Suppl. materials 2, 3). These results suggested that the 25-MEA-424-1 clade represented a novel species in the genus *Pichia*.

Strains 3L-19-1 and MF-97-2 from Beijing city and HBP0207D3C1, HBP0257C4C1, HBP0281E4D1, and HBP0437D4C1 from Hubei province in this study as well as other five unidentified strains possessed similar D1/D2 sequences with no more than three nucleotide differences. Only the ITS sequence of NYNU14770 is available for comparison among the five strains. The ITS sequences of all these seven strains were similar with one nucleotide difference. The group represented by strain 3L-19-1 together with six other known species, *viz*. *P. bovicola*, *P. exigua*, *P. paraexigua*, *P. phayaonensis*, *P. rugopelliculosa*, and *P. scutulata* clustered in a branch with high support value. This group differed from the other species in this branch by 31~66 (5.8%~11.9%) nucleotide mismatches and 34~45 (9.4%~11.6%) nucleotide differences in the D1/D2 domain and ITS region, respectively. These results suggested that they represented a novel species in the genus *Pichia*.

Strain HWY125-2 from Hainan province formed a separate branch and did not cluster with any other strains (Fig. 1, Suppl. materials 2, 3). It differed from the closest species *Pichia thaimueangensis* by 35 (6.5%, 25 substitutions and 10 gaps) and 12 (5.4%, 7 substitutions and five gaps) nucleotide mismatches in the D1/D2 domain and ITS region, respectively (Fig. 1, Suppl. materials 2, 3). These results indicated that strain HWY125-2 represented a novel species in the genus *Pichia*.

## Taxonomy

***Pichia kregeriana*** **S. Hu, L.C. Guo, F.Y. Bai & P.J. Han, sp. nov.**
Fungal Names: FN 572224

**Etymology.** The species *kregeriana* (kre.ge.ri.a'na. N.L. fem. adv.) is named in honor of the late Dr. Nelly Jeanne Wilhelmina Kreger-van Rij for her work in the field of microbiology, particularly in yeast systematics and ultrastructure.

**Type.** The holotype CGMCC 2.7383 (original number = HWY125-4) was isolated from rotten wood of *Garcinia mangostana* collected from Wuzhishan city, Hainan province, China (18.902°N, 109.688°E; tropical monsoon oceanic climate) by S. Hu in August, 2023 and had been deposited in a metabolically inactive state in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. An ex-type culture had been deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan, as JCM 36906. GenBank accessions: ITS-PQ586094 and LSU-PQ586304.

**Culture characteristics.** After growth on YPD agar for 3 days at 25 °C, colonies are white, butyrous, nearly circular, lightly raised, rough and wrinkled with irregular surfaces and margins (Fig. 3A). Cells are oval-shaped or oval (3.3–4.5 × 4.9–8.7 μm) and budding is multilateral (Fig. 3B). No pseudohyphae are formed. Asci (2.4–4.6 × 5.0–5.4 um) are persistent and conjugated, typically forming two to four spherical spores within shuttle-shaped or triangular ascospores (Fig. 3C). The sexual structures were observed on YCB agar after 30 days at 25 °C. Conjugation can occur between a cell and its bud, or between independent cells.

**Physiological and biochemical characteristics.** Glucose is not fermented. Glucose, ethanol, N-acetyl-D-glucosamine, glycerol (slow), D-glucosamine (weak), inulin (weak), sucrose (weak) and succinic acid are assimilated as sole carbon sources. D-galactose, L-sorbose, erythritol, D-xylose, glucitol, D-maltose, sodium citrate dihydrate, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, L-arabinose, D-arabinose, xylitol, D-ribose, L-rhamnose, methanol, ribitol, galactitol, D-mannitol, α-methyl-D-glucoside, salicin, D-glucuronic acid, DL-lactic acid, inositol and hexadecane are not assimilated as sole carbon sources. Ethylamine hydrochloride, cadaverine dihydrochloride, L-lysine and ammonium sulfate are assimilated as sole nitrogen sources. Potassium nitrate and sodium nitrite are not assimilated as sole nitrogen sources. Growth in vitamin-free medium and on 50% (w/v) glucose are positive, while growth in 10% NaCl plus 5% glucose medium and on 60% (w/v) glucose are negative. Diazonium blue B, urease activity and production of extracellular starch-like compounds are negative. Growth on YPD agar at 30 °C is positive, but negative at 37 °C.

**Note.** *Pichia kregeriana* is physiologically differentiated from its closely related species, *Pichia kurtzmaniana*, by fermentation of glucose, growth on 60% (w/v) glucose and growth in 10% NaCl plus 5% glucose medium.

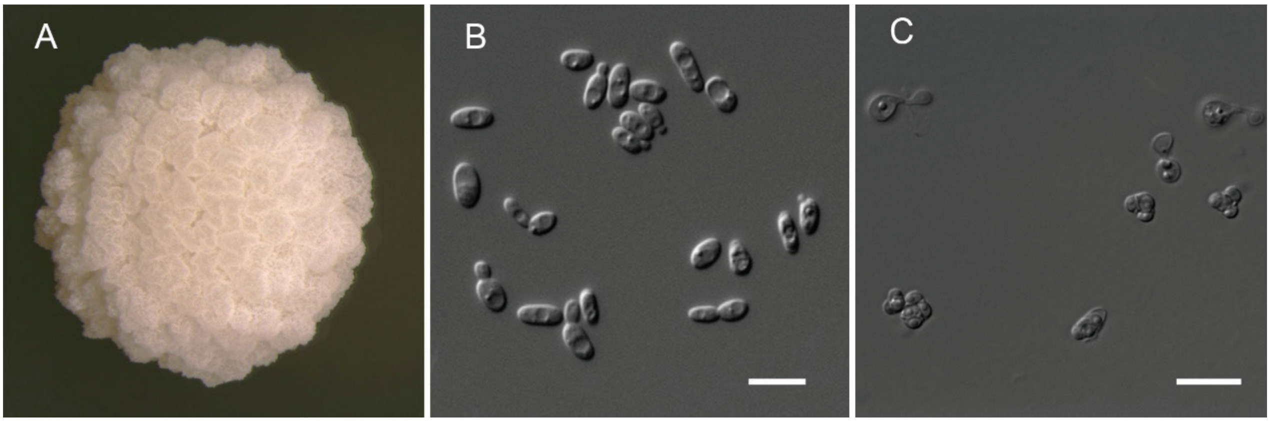

**Figure 2.** Morphology of *Pichia kregeriana* strain HWY125-4$^T$. **A** Colony on YPD agar at 25 °C after 3 days **B** vegetative cells on YPD agar at 25 °C after 3 days **C** asci and ascospores on YCB agar at 25 °C after 30 days. Scale bars: 10 µm.

***Pichia phaffii*** **H.Y. Zhu, L.C. Guo, F.Y. Bai & P.J. Han, sp. nov.**
Fungal Names: FN 572225

**Etymology.** This species *phaffii* (phaf'fi.i. N.L. gen. n.) is named in honor of the late Prof. Herman Phaff for his significant contributions to the field of microbiology, particularly in yeast systematics and ecology.

**Type.** The holotype CGMCC 2.8239 (original number = 25-MEA-424-1) was isolated from mudflat soil sediment collected from Dongguan city, Guangdong province, China (23.042°N, 113.743°E; subtropical monsoon climate) by H.Y. Zhu in July 2022 and had been deposited in a metabolically inactive state in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. GenBank accessions: ITS-PQ586092 and LSU-PQ586298.

**Culture characteristics.** After growth on YPD agar for 3 days at 25 °C, colonies are white butyrous, rough and wrinkled with irregular surfaces and margins (Fig. 2A). Cells are ovoid to elongate (2.1–3.2 × 3.3–7.1 µm) and occur singly or in pairs (Fig. 2B). Budding is multilateral and pseudohyphae are formed (Fig. 2D). Asci (2.8–3.5 × 6.2–7.1 µm) are persistent and unconjugated, typically forming two to four spherical spores within diamond-shaped ascus ascospores, although a linear arrangement of ascospores is seen as well (Fig. 2C). The sexual structures were observed on YCB agar after 30 days at 25 °C.

**Physiological and biochemical characteristics.** Glucose is not fermented. Glucose, ethanol, erythritol (weak), succinic acid, sodium citrate dihydrate (weak), D-glucosamine, inulin (weak) and DL-lactic acid are assimilated as sole carbon sources. D-galactose, L-sorbose, D-xylose, glycerol, glucitol, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, N-acetyl-D-glucosamine, L-arabinose, D-arabinose, xylitol, D-ribose, L-rhamnose, methanol, ribitol, galactitol, D-mannitol, α-methyl-D-glucoside, salicin, D-glucuronic acid, citrate acid, inositol and hexadecane are not assimilated as sole carbon sources. Ethylamine hydrochloride, cadaverine dihydrochloride, L-lysine, and ammonium sulfate are assimilated as sole nitrogen sources. Potassium nitrate and sodium nitrite are not assimilated as sole nitrogen sources. Growth in vitamin-free medium and on 50% (w/v) glucose are positive, while growth in 10% NaCl plus 5% glucose medium and on 60%

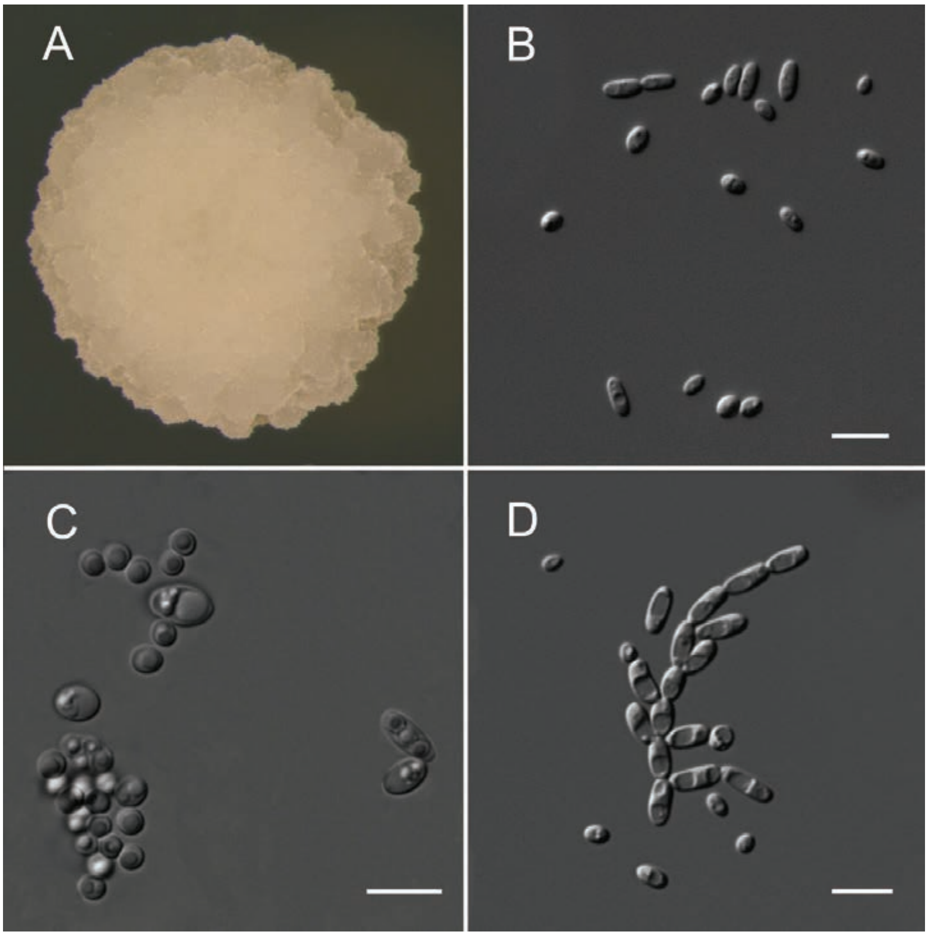

**Figure 3.** Morphology of *Pichia phaffii* strain 25-MEA-424-1$^T$. **A** Colony on YPD agar at 25 °C after 3 days **B** vegetative cells on YPD agar at 25 °C after 3 days **C** asci and ascospores on YCB agar at 25 °C after 30 days **D** pseudohyphae on YPD agar at 25 °C after 15 days. Scale bars: 10 µm.

(w/v) glucose is negative. Diazonium blue B, urease activity and production of extracellular starch-like compounds are negative. Growth on YPD agar at 30 °C is positive, but negative at 37 °C.

**Notes.** *Pichia phaffii* sp. nov. is physiologically differentiated from its closely related species *Pichia tetrigidarum* comb. nov. in that *Pichia tetrigidarum* comb. nov. can ferment D-glucose, D-galactose, sucrose, maltose, trehalose, while *Pichia phaffii* sp. nov. does not.

### ***Pichia ureolytica*** **L.C. Guo, Y.J. Shang, F.Y. Bai & P.J. Han, sp. nov.**

Fungal Names: FN 572226

**Etymology.** The specific epithet *ureolytica* (u.re.o.ly'ti.ca. L. fem. adj.) derived from "urea" (the compound that can be broken down) and "lyticus," which indicates the ability to break down or decompose urea.

**Type.** The holotype CGMCC 2.6825 (original number = 3L-19-1) was isolated from bark of *Quercus wutaishansea* collected from Mentougou district, Beijing city, China (39.866°N; 115.598°E; warm temperate semi-humid and semi-arid monsoon climate) by Y.J. Shang in August 2021 and had been deposited in a metabolically inactive state in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. The ex-type culture had been deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan, as JCM 36370. GenBank accessions: ITS-PQ586091 and LSU-PQ586297.

**Culture characteristics.** After growth on YPD agar for 3 days at 25 °C, colonies are white, circular, butyrous and smooth with entire margins (Fig. 4A). Cells are ovoid (3.5–5.0 × 3.8–5.2 μm) and occur singly or in pairs. Budding is multilateral and pseudohyphae are not formed (Fig. 4B). Asci (4.7–5.4 × 5.5–6.2 μm) are persistent, typically forming four spherical spores within a diamond-shaped or subrounded ascus. (Fig. 4C). The sexual structures were observed on YCB agar after 30 days at 25 °C.

**Physiological and biochemical characteristics.** Glucose fermentation is weak. Glucose, ethanol, succinic acid (slow/weak) and DL-lactic acid are assimilated as sole carbon sources. Galactose, L-sorbose, D-xylose, D-glucosamine, glycerol, glucitol, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, N-acetyl-D-glucosamine, L-arabinose, D-arabinose, xylitol, D-ribose, L-rhamnose, methanol, erythritol, ribitol, galactitol, D-mannitol, α-methyl-D-glucoside, salicin, D-glucuronic acid, sodium citrate dihydrate, inositol and hexadecane are not assimilated as sole carbon sources. Ethylamine hydrochloride, cadaverine dihydrochloride, L-lysine and ammonium sulfate are assimilated as sole nitrogen sources. Potassium nitrate and sodium nitrite are not assimilated as sole nitrogen sources. Growth in vitaminfree medium and on 50% and 60% (w/v) glucose are positive, while growth in 10% NaCl plus 5% glucose medium is negative. Urease activity is positive (Suppl. material 4). Diazonium blue B reaction and production of extracellular starchlike compounds are negative. Growth on YPD agar at 30 °C is positive, but negative at 37 °C.

**Notes.** *Pichia ureolytica* is physiologically differentiated from its closely related species *Pichia paraexigua* and *Pichia scutulata* in terms of urease activity; from its closely related species *Pichia bovicola* and *Pichia phayaonensis* by growth on 60% (w/v) glucose; from its closely related species *Pichia exigua* and *Pichia occidentalis* by the fact that *Pichia exigua* and *Pichia occidentalis* are capable of growth at 37 °C, whereas *Pichia ureolytica* does not show growth under the same temperature conditions.

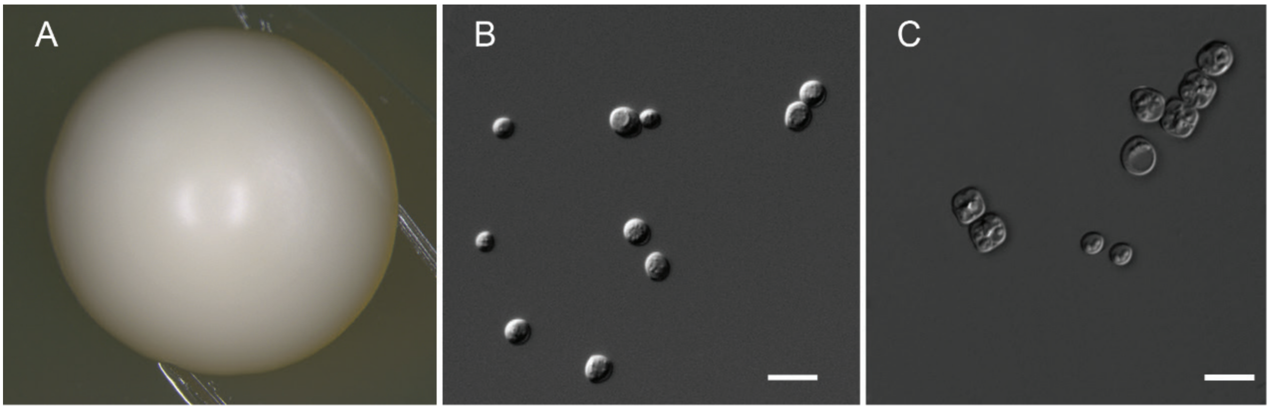

**Figure 4.** Morphology of *Pichia ureolytica* strain 3L-19-1$^T$. **A** Colony on YPD agar at 25 °C after 3 days **B** vegetative cells on YPD agar at 25 °C after 3 days **C** asci and ascospores on YCB agar at 25 °C after 30 days. Scale bars: 10 μm.

**_Pichia wuzhishanensis_ f.a. S. Hu, L.C. Guo, F.Y. Bai & P.J. Han, sp. nov.**
Fungal Names: FN 572227

**Etymology.** The specific epithet *wuzhishanensis* (wu.zhi.han'en.sis. N.L. fem. adj.) is named after the location where the type strain of the species was isolated: Wuzhishan city, Hainan province, China.

**Type.** The holotype CGMCC 2.7381 (original number = HWY125-2) was isolated from rotten wood of *Garcinia mangostana* collected from Wuzhishan city, Hainan province, China (18.902°N, 109.688°E; tropical monsoon oceanic climate) by S. Hu in August, 2023 and had been deposited in a metabolically inactive state in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. An ex-type culture had been deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan, as JCM 36905. GenBank accessions: ITS-PQ586093 and LSU-PQ586303.

**Culture characteristics.** After growth on YPD agar for 3 days at 25 °C, colonies are white, butyrous, circular and glossy, with a smooth surface and entire margins (Fig. 5A). Cells are oval-shaped or oval (2.1–4.5 × 3.1–5.7 µm) and budding is monopolar (Fig. 5B). Sexual structures were not observed in single or mixed strain cultures on CMA, PDA and V8 agar after 2 months of incubation at 25 °C.

**Physiological and biochemical characteristics.** Glucose is not fermented. Glucose, ethanol, N-acetyl-D-glucosamine, glycerol, D-glucosamine, succinic acid and DL-lactic acid (weak) are assimilated as sole carbon sources. D-galactose, L-sorbose, erythritol, D-xylose, glucitol, maltose, sodium citrate dihydrate, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, L-arabinose, D-arabinose, xylitol, D-ribose, L-rhamnose, methanol, ribitol, galactitol, D-mannitol, α-methyl-D-glucoside, salicin, D-glucuronic acid, inositol, sucrose, inulin and hexadecane are not assimilated as sole carbon sources. Ethylamine hydrochloride, cadaverine dihydrochloride, L-lysine and ammonium sulfate are assimilated as sole nitrogen sources. Sodium nitrite and potassium nitrate are not assimilated as sole nitrogen sources. Growth in vitamin-free medium and on 50% (w/v) glucose are positive, while growth in 10% NaCl plus 5% glucose medium and on 60% (w/v) glucose are negative. Diazonium blue B,

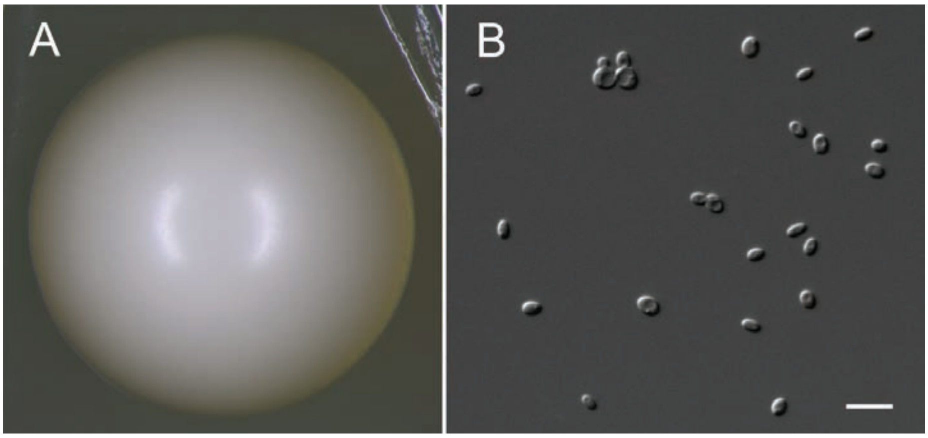

**Figure 5.** Morphology of *Pichia wuzhishanensis* strain HWY125-2$^T$. **A** Colony on YPD agar at 25 °C after 3 days **B** vegetative cells on YPD agar at 25 °C after 3 days. Scale bars: 10 µm.

urease activity and production of extracellular starch-like compounds are negative. Growth on YPD agar at 37 °C is positive, but negative at 42 °C.

**Notes.** *Pichia wuzhishanensis* is physiologically differentiated from its closely related species *Pichia thaimueangensis* by its ability to assimilate D-xylose.

### New combinations

Although the sexual state of the new species, represented by the single strain HWY125-2, was not observed on any agar medium, following the Shenzhen Code (Turland et al. 2018), we classify the new species within the genus *Pichia* and propose the name *Pichia kregeriana* sp. nov. for it. Similarly, we reassign *Candida awuae* and *Candida tetrigidarum*, which are robustly supported as members of the *Pichia* clade in the phylogenetic analyses (Fig. 1, Suppl. materials 2, 3) to the genus *Pichia*.

***Pichia awuae*** **(D.S. Nielsen, M. Jakobsen & L. Jespersen) S. Hu, L.C. Guo, F.Y. Bai & P.J. Han, comb. nov.**
Fungal Names No: FN 572228

**Basionym.** *Candida awuae* D.S. Nielsen, M. Jakobsen & L. Jespersen, International Journal of Systematic and Evolutionary Microbiology 60, 1460 (2010).

***Pichia tetrigidarum*** **(S.O. Suh, N.H. Nguyen & M. Blackw) S. Hu, L.C. Guo, F.Y. Bai & P.J. Han, comb. nov.**
Fungal Names No: FN 572229

**Basionym.** *Candida tetrigidarum* S.O. Suh, N.H. Nguyen & M. Blackw., FEMS Yeast Research 8(1): 97 (2008).

## Discussions

In the present study, nine yeast strains from eight samples were isolated from various sources in different regions of China during the collection trips for yeast diversity conducted in 2021–2023. Four novel *Pichia* species, *P. kregeriana* sp. nov., *P. phaffii* sp. nov., *P. ureolytica* sp. nov., and *P. wuzhishanensis* f.a. sp. nov., were described from these strains based on a single-segment (D1/D2 or ITS) and a two-segment (ITS and D1/D2) approach, morphological, physiological and biochemical characteristics comparison. Notably, the two new species *P. wuzhishanensis* sp. nov. and *P. kregeriana* sp. nov. were isolated from the same rotten wood sample, which suggests that there may be many unknown microorganisms including novel yeast species in the environment around us waiting to be discovered and studied.

Urea is the main nitrogen-containing product of protein metabolism and decomposition in mammals, amphibians, and certain fish. Urea is not only present in urine, but also in serum, sweat, and exocrine gland secretions in humans. It can be used as a fertilizer, animal feed, explosive, glue stabilizer, and chemical raw material. And urease increases the decomposition rate of urea by $10^{14}$ times, promoting nitrogen assimilation back to amino acids, thus playing a crucial role in the global nitrogen cycle (Loharch and Berlicki 2022). In addition, urease is a virulence factor found in many pathogenic bacteria (Cox et al. 2000; Subramaniyan et al. 2023). Basidiomycetous yeasts with few exceptions are capable of hydrolyzing urea but most of them are unable to ferment glucose, whereas ascomycetous yeasts exhibit the opposite characteristics (Moore 1980). The ascomycetous yeasts *Schizosaccharomyces pombe* and *Lipomyces* species are exceptions with positive urease activity (Kurtzman et al. 2011). In this study, the six strains isolated from China of the new species *Pichia ureolytica* sp. nov. were identified as positive for production of urease (Suppl. material 4). This phenomenon is extremely rare in the genus *Pichia*, and only *Pichia bovicola* is reported to indicate weak urease activity (Suppl. material 1).

Species of *Pichia* are ubiquitous and exist in various environments around the world (Suppl. material 1). In this study, the 19 strains representing *Pichia phaffii* sp. nov. are from various sources across different countries, such as digestive tract of insect in Brazil, mangrove and marine sediment in China, and artificial lake sediment or water in Colombia; the 11 strains representing *Pichia ureolytica* sp. nov. originate from diverse locations, including bark in China, soil in Korea, and artificial lake sediment or water in Colombia; and the 5 strains representing *Pichia kregeriana* sp. nov. are sourced from rotten wood in China and gut of insect in USA. *Pichia kurtzmaniana*, as described by Zhu et al (2024), is also found in various environments, such as soil and marine water in China, soil in Brazil, deteriorated strawberry soft drinks in the UK, and fermentation broth and black viscous substances in Japan. Apart from these mentioned countries, species of *Pichia* are also found in Mexico (Chai et al. 2024); Thailand (Limtong et al. 2009), Indonesia (Kobayashi et al. 2017), the Netherlands (Groenewald et al. 2018), India (Bhadra et al. 2007), Caribbean (Ganter et al. 2010), Indonesia (Kobayashi et al. 2017), Japan (Ninomiya et al. 2010), Spain (Flórez et al. 2010), Borneo (Sipiczki 2012). In addition to natural environments, novel *Pichia* species have been identified in fermentation environments, including alcoholic beverages and blue-veined Cabrales cheese.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

Liang-Chen Guo and Shuang Hu: investigation, methodology, visualization, data curation, writing – original draft; Hai-Yan Zhu: validation; Yu-Jie Shang, Yan-Jie Qiu, Zhang Wen, Shen-Xi Chen: investigation; Feng-Yan Bai: funding acquisition, project administration, supervision; Pei-Jie Han: conceptualization, methodology, funding acquisition, writing – review & editing.

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## Data availability

The new sequences generated in this study have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers indicated in Suppl. material 1 and released to the public. All new taxa have been registered in Fungal Names (https://nmdc.cn/fungalnames/). The type strains of the new species described have been deposited in the China General Microbiological Culture Collection Center (CGMCC) with strain numbers indicated in Suppl. material 1.

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## Supplementary material 1

### The yeast species and strains were employed in this study

Authors: Liang-Chen Guo, Shuang Hu, Hai-Yan Zhu, Yu-Jie Shang, Yan-Jie Qiu, Zhang Wen, Shen-Xi Chen, Feng-Yan Bai, Pei-Jie Han

Data type: xlsx

Link: https://doi.org/10.3897/mycokeys.114.142474.suppl1

## Supplementary material 2

### Neighbor-Joining phylogenetic tree of the genus *Pichia* based on the D1/D2 sequences

Authors: Liang-Chen Guo, Shuang Hu, Hai-Yan Zhu, Yu-Jie Shang, Yan-Jie Qiu, Zhang Wen, Shen-Xi Chen, Feng-Yan Bai, Pei-Jie Han

Data type: tif

Explanation note: Bootstrap values above 70% are shown on the branches. The species *Kregervanrija fluxuum* and *Martiniozyma abietophila* were used as the outgroup. Type strains are denoted with the superscript 'T'. Strains isolated in this study and the new combinations were marked in bold. Bars, 0.02 substitutions per nucleotide position.

Link: https://doi.org/10.3897/mycokeys.114.142474.suppl2

## Supplementary material 3

### Neighbor-Joining phylogenetic tree of the genus *Pichia* based on the ITS sequences

Authors: Liang-Chen Guo, Shuang Hu, Hai-Yan Zhu, Yu-Jie Shang, Yan-Jie Qiu, Zhang Wen, Shen-Xi Chen, Feng-Yan Bai, Pei-Jie Han

Data type: tif

Explanation note: Bootstrap values above 70% are shown on the branches. The species *Kregervanrija fluxuum* and *Martiniozyma abietophila* were used as the outgroup. Type strains are denoted with the superscript 'T'. Strains isolated in this study and the new combinations were marked in bold. Bars, 0.02 substitutions per nucleotide position.

Link: https://doi.org/10.3897/mycokeys.114.142474.suppl3

## Supplementary material 4

### Christensen agar plate for three days

Authors: Liang-Chen Guo, Shuang Hu, Hai-Yan Zhu, Yu-Jie Shang, Yan-Jie Qiu, Zhang Wen, Shen-Xi Chen, Feng-Yan Bai, Pei-Jie Han

Data type: tif

Explanation note: (a) Negative for *Saturnispora sinensis* strain CGMCC 2.6937. (b) Positive for *Vishniacozyma pseudocarnescens* strain CGMCC 2.6457. (c) Positive for *Vishniacozyma pseudodimennae* strain CGMCC 2.6790. (d) Positive for *Pichia ureolytica* 3L-19-1. (e) Positive for *Pichia ureolytica* MF-97-2. (f) Positive for *Pichia ureolytica* HBP0207D3C1. (g) Positive for *Pichia ureolytica* HBP0437D4C1. (h) Positive for *Pichia ureolytica* HBP0257C4C1. (i) Positive for *Pichia ureolytica* HBP0281E4D1.

Link: https://doi.org/10.3897/mycokeys.114.142474.suppl4

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Research Article

# Morphological characteristics and phylogenetic analyses reveal five new species of Hymenochaetales (Agaricomycetes, Basidiomycota) from southwestern China

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## Abstract

Wood-inhabiting fungi can decompose wood materials and play a crucial role in the natural world by maintaining the equilibrium of the Earth's ecosystems. In the present study, five new wood-inhabiting fungal species belonging to the order Hymenochaetales, *Hymenochaete weishanensis*, *Lyomyces albofarinaceus*, *Lyomyces albomarginatus*, *Tubulicrinis albobadius* and *Xylodon musicola*, collected from southern China, are proposed based on a combination of morphological features and molecular evidence. *Hymenochaete weishanensis* is characterized by a coriaceous, tuberculate hymenial surface, a monomitic hyphal system with simple-septate generative hyphae, and ellipsoid to narrow ellipsoid basidiospores (4.0–5.0 × 2.0–3.0 µm); *Lyomyces albofarinaceus* is characterized by pruinose hymenial surface, a monomitic hyphal system with clamped generative hyphae, and broadly ellipsoid basidiospores (6.0–7.0 × 5.0–6.0 µm); *Lyomyces albomarginatus* is characterized by the cracked hymenial surface, clamped generative hyphae, and elliposoid basidiospores (4.0–5.5 × 2.7–3.5 µm); *Tubulicrinis albobadius* is characterized by an arachnoid hymenial surface, a monomitic hyphal system with clamped generative hyphae and cylindrical to allantoid basidiospores (4.0–6.0 × 1.5–2.2 µm) and *Xylodon musicola* is characterized by an arachnoid hymenial surface, a monomitic hyphal system with clamped generative hyphae and broadly ellipsoid to globe basidiospores (4.0–5.5 × 3.5–5.0 µm). Sequences of the internal transcribed spacers (ITS) and the large subunit (nrLSU) of the nuclear ribosomal DNA (rDNA) markers of the studied samples were generated. Phylogenetic analyses were performed using maximum likelihood, maximum parsimony, and Bayesian inference methods. Full descriptions, illustrations, and phylogenetic analysis results for the five new species are provided.

**Key words:** Biodiversity, Classification, Molecular systematics, New taxa, Wood-inhabiting fungi, Yunnan Province

## Introduction

Fungi are well-known as a diverse group of microorganisms that play important roles in forest ecosystems (Phookamsak et al. 2019). Mushroom-forming fungi (Agaricomycetes) have the greatest morphological diversity and complexity of fungi (Varga et al. 2019). Wood-inhabiting fungi are essential to natural ecosystems for nutrient cycling and maintaining plant diversity (Drinkwater et al. 2017; Horwath 2017; Hyde et al. 2018; Wu et al. 2022; Guan et al. 2023; Yuan et al. 2023; Deng et al. 2024a, b; Dong et al. 2024; Yuan and Zhao 2024; Zhang et al. 2024). The order Hymenochaetales was described as a monotypic order to accommodate Hymenochaetaceae (Frey et al. 1977; Wu et al. 2022). Hymenochaetales is globally distributed in forest ecosystems, and it comprises 15 families and 84 genera, of which 19 genera have no certain position at the family level (Wu et al. 2022; Wang et al. 2023; Wang and Zhou 2024). Most of the species in Hymenochaetales are polypores and corticioid fungi, which show high morphological diversity and various trophic modes, including saprotrophs, parasites, and symbionts (Wang and Zhou 2024).

The genus *Hymenochaete* Lév. was erected in 1846 and typified by *H. rubiginosa* (Dicks.) Lév. *Hymenochaete* is characterized by annual to perennial, resupinate, effused-reflexed to pileate basidioma with smooth, tuberculate, lamellate, poroid or hydnoid hymenophores; a monomitic or dimitic hyphal system; presence of setae, and hyaline, thin-walled, narrowly cylindrical to globose basidiospores (Léger 1998; Parmasto 2001; He and Dai 2012; Li et al. 2024a). Léger (1998) wrote a worldwide monograph on *Hymenochaete*, providing a key to this genus. According to Index Fungorum (www.indexfungorum.org; accessed on 4 February 2025), the genus *Hymenochaete* has 362 registered names with 235 accepted species worldwide (Léger 1998; Parmasto 2001; Parmasto and Gilbertson 2005; He and Dai 2012; Parmasto 2012; He et al. 2017; Nie et al. 2017; Pacheco et al. 2018; Miettinen et al. 2019; Du et al. 2021a, b; Li et al. 2024a).

The genus *Lyomyces* P. Karst. was introduced by Karsten (1881) and is typified by *L. sambuci* (Pers.) P. Karst. *Lyomyces* comprises corticioid fungi characterized by thin, effused, membranaceous basidiomata that appear fragile in a dry state and show hymenial surface predominantly white or whitish. The hyphal system is monomitic, subicular hyphae thin- or somewhat thick-walled, while the cystidia are thin-walled with tapering, cylindrical, sub-capitate, or capitate apical parts. Basidia are utriform, and the basidiospores are colorless with thin to thick, smooth, or occasionally minutely warted walls (Yurchenko et al. 2024a). The members of *Lyomyces* grow on dead, still-attached, or fallen branches of angiosperms, on dead, wooden, or herbaceous stems, and occasionally on gymnosperm wood (Yurchenko et al. 2017; Chen and Zhao 2020). Molecular studies on *Lyomyces* and related genera have been carried out recently (Riebesehl and Langer 2017; Yurchenko et al. 2017; Viner et al. 2018; Riebesehl et al. 2019; Chen and Zhao 2020; Yuan et al. 2024). Riebesehl and Langer (2017) indicate that *Hyphodontia* s.l. should be divided into several genera as *Hastodontia* (Parmasto) Hjortstam & Ryvarden, *Hyphodontia* J. Erikss, *Kneiffiella* (Pers.) Gray, *Lagarobasidium* Jülich, *Lyomyces* and *Xylodon* (Pers.) Gray and thus thirty-five new combinations were proposed, including fourteen *Lyomyces* species (Dong et al. 2024). The *Lyomyces sambuci* complex was clarified based on ITS and 28S sequences analyses and four new species of *Lyomyces* were described (Yurchenko et al. 2017; Dong et al. 2024). Viner et al. (2018) studied the taxonomy of *Lagarobasidium* and *Xylodon* and indicated that twelve species clustered into the *Lyomyces* clade and then grouped with the *Xylodon* clade. Phylogenetic and morphological studies on *Lyomyces* showed that *Lyomyces* grouped with *Hastodontia*, *Hyphodontia*, *Kneiffiella*, and *Xylodon*, in which the *Lyomyces* type species *L. sambuci* was sister to *L. crustosus* (Pers.) P. Karst. formed a single lineage with high support (Riebesehl et al. 2019).

The genus *Tubulicrinis* Donk, typified by *T. glebulosus* (Fr.) Donk (Donk 1956), was a member of the corticioid fungi. They are characterized by resupinate basidiomata, firmly adnate, smooth, pruinose toporulose hymenophore, a monomitic hyphal system with clamped connections on generative hyphae and conspicuous, projecting, amyloid cystidia and small basidia, and cylindrical to allantoid or globose to ellipsoid, thin-walled, smooth, IKI– (both inamyloid and indextrinoid), acyanophilous basidiospores (Donk 1956; Bernicchia and Gorjón 2010; Dong et al. 2024). So far about 46 species have been accepted in the genus worldwide (Donk 1956; Eriksson 1958; Cunningham 1963; Oberwinkler 1966; Hayashi 1974; Ryvarden 1975; Hjortstam 1981; Hjortstam et al. 1988; Rajchenberg 2002; Sharma et al. 2015; Crous et al. 2016; Gruhn et al. 2016; He et al. 2021; Dong et al. 2024). Molecular studies in the genus *Tubulicrinis* have been carried out by Larsson et al. (2006); Dai (2011); Crous et al. (2016); and Dong et al. (2024) and indicated that two *Tubulicrinis* species, *T. gracillimu* (Ellis & Everh. ex D.P. Rogers & H.S. Jacks.) G. Cunn. and *T. subulatus* (Bourdot & Galzin) Donk, formed a monophyletic lineage and then grouped with *Coltricia* clade in Hymenochaetaceae (Larsson et al. 2006). A revised checklist of corticioid and hydnoid fungi showed that six species of *Tubulicrinis* were recorded (Dai 2011); they were nested into the Tubulicrinaceae clade, which belongs to the order Hymenochaetales (Crous et al. 2016; Dong et al. 2024). Based on morphological and molecular analysis of *Tubulicrinis*, two new species were described as *T. xantha* C.L. Zhao and *T. yunnanensis* C.L. Zhao (He et al. 2021).

The genus *Xylodon* (Pers.) Gray is typified by *X. quercinus* (Pers.) Gray (Bernicchia and Gorjón 2010; Yuan and Zhao 2024). The taxa of this genus grow on rotten gymnosperm or angiosperm trunks and stumps, bamboo, and ferns (Greslebin and Rajchenberg 2000; Kotiranta and Saarenoksa 2000; Girometta et al. 2021; Guan et al. 2023; Yuan and Zhao 2024). This genus is characterized by the resupinate or effused basidiomata with a smooth, tuberculate, grandinioid, odontioid, coralloid, irpicoid, or poroid hymenophore; a monomitic or dimitic hyphal system with clamped generative hyphae; the presence of different types of cystidia; utriform or suburniform basidia; and cylindrical to ellipsoid to globose basidiospores (Gray 1821; Bernicchia and Gorjón 2010; Zhang et al. 2024; Yuan and Zhao 2024). Based on the MycoBank database (http://www.mycobank.org, accessed on 4 February 2025 and the Index Fungorum (http://www.indexfungorum.org, accessed on 4 February 2025, 241 specific and infraspecific names are registered for *Xylodon*, of which, 134 are accepted species (Chevallier 1826; Kuntze 1898; Wu 1990, 2000, 2001, 2006; Hjortstam and Ryvarden 2007, 2009; Xiong et al. 2009, 2010; Bernicchia and Gorjón 2010; Tura et al. 2011; Dai 2012; Lee and Langer 2012; Yurchenko and Wu 2014; Zhao et al. 2014; Chen et al. 2016; Kan et al. 2017a, b; Wang and Chen 2017; Viner et al. 2018, 2021; Riebesehl et al. 2019; Shi et al. 2019; Dai et al. 2021; Luo et al. 2021a, 2022; Qu and Zhao 2022; Qu et al. 2022; Guan et al. 2023; Dong et al. 2024; Yuan et al. 2024; Yurchenko et al. 2024b; Zhang et al. 2024).

The present work describes five new species of Hymenochaetales from southwest China, based on the morphology and phylogeny. To clarify the placement and relationships of these new species, we carried out a phylogenetic and taxonomic study based on the combined ITS+nLSU and ITS only sequences analyses. Full descriptions, illustrations, and comparison of five new species with closely related taxa and phylogenetic trees showing the placement of five new species within the order Hymenochaetales are provided.

## Materials and methods

### Sample collection and herbarium specimen preparation

The fresh fruiting bodies were collected on the fallen angiosperm branches which came from Dali, Zhaotong, and Qujing of Yunnan Province, China, and the important collection information was noted (Rathnayaka et al. 2024). The samples were photographed in situ, and fresh macroscopic details were recorded. Photographs were recorded by a Nikon D7100 camera. All the photos were focus-stacked using Helicon Focus software, and macroscopic details were recorded. Specimens were dried in an electric food dehydrator at 40 °C (Hu et al. 2022; Dong et al. 2024). Once dried, the specimens were sealed in an envelope and zip-lock plastic bags and labeled (Dong et al. 2024). The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

### Morphology

The macromorphological descriptions were based on field notes and photos captured in the field and lab. The color terminology follows Petersen (1996). The micromorphological data were obtained from the dried specimens after observation under a light microscope with a magnification of 10 × 100 (Zhao et al. 2023; Dong et al. 2024). Sections were mounted in 5% KOH and 2% phloxine B dye ($C_{20}H_2Br_4Cl_4Na_2O_5$), and we also used other reagents, including Cotton Blue and Melzer's reagent to observe micromorphology following (Dong et al. 2024). To show the variation in spore sizes, 5% of measurements were excluded from each end of the range and shown in parentheses. At least thirty basidiospores from each specimen were measured. Stalks were excluded from basidia measurements, and the hilar appendage was excluded from basidiospores measurements. The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB– = acyanophilous, IKI– = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and n = a/b (number of spores (a) measured from given number (b) of specimens).

### Molecular phylogeny

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from the dried specimens according to the manufacturer's instructions. The ITS region was amplified with ITS5 and ITS4 primers (White et al. 1990). The nLSU region was amplified with the LR0R and LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, P.R. China). The newly generated sequences were deposited in NCBI GenBank (Table 1).

The sequences were aligned in MAFFT version 7 (Katoh et al. 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). A sequence of *Thelephora ganbajun* obtained from GenBank was used as an outgroup to root trees in the ITS+nLSU analysis (Fig. 1) in the order Hymenochaetales (Wang and Zhou 2024) (TreeBASE WEB submission ID 31958). Sequence of *Hydnoporia tabacina* (Sowerby) Spirin, Miettinen & K.H. Larss. obtained from GenBank was used as an outgroup to root trees in the ITS analysis in the genus *Hymenochaete* (Fig. 2) (TreeBASE WEB submission ID 31959). Sequences of *Xylodon quercinus* (Pers.) Gray and *Xylodon ramicida* Spirin & Miettinen obtained from GenBank were used as outgroups to root trees in the ITS analysis in the genus *Lyomyces* (Fig. 3) (TreeBASE WEB submission ID 31960). A sequence of *Gyroporus castaneus* (Bull.) Quél. obtained from GenBank was used as an outgroup to root trees in the ITS analysis in the genus *Tubulicrinis* (Fig. 4) (TreeBASE WEB submission ID 31961). A sequence of *Lyomyces sambuci* (Pers.) P. Karst. obtained from GenBank was used as an outgroup to root trees in the ITS analysis in the genus *Xylodon* (Fig. 5) (TreeBASE WEB submission ID 31962).

Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined three datasets following a previous study (Zhao and Wu 2017), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All of the characters were equally weighted, and gaps were treated as missing data. Using the heuristic search option with TBR branch swapping and 1,000 random sequence additions, trees were inferred. Max trees were set to 5,000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree generated. The multiple sequence alignments were also analyzed using maximum likelihood (ML) in RAxML-HPC2 on XSEDE v 8.2.8 with default parameters (Miller et al 2012). Branch support (BS) for ML analysis was determined by 1,000 bootstrap replicates.

jModelTest v2 (Darriba et al. 2012) was used to determine the best-fit evolution model for each dataset for the purposes of Bayesian inference (BI), Bayesian inference was performed using MrBayes 3.2.7a with a GTR+I+G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist et al. 2012). The first one-fourth of all the generations were discarded as burnins. The majority-rule consensus tree of all the remaining trees was calculated. Branches were considered significantly supported if they received a maximum likelihood bootstrap value (BS) of ≥ 70%, a maximum parsimony bootstrap value (BT) of ≥ 70%, or Bayesian posterior probabilities (BPP) of ≥ 0.95.

**Table 1.** List of species, specimens, and GenBank accession number of sequences used in this study. [New species is shown in bold; * type material; – is shown data without used].

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| Boletales/Gyroporaceae | *Gyroporus castaneus* | JMP 0028 | EU819468 | – | USA | Palmer et al. (2008) |
| Hymenochaetales/ Chaetoporellaceae | *Echinoporia hydnophora* | LWZ 20150802-9 | ON063639 | ON063838 | China | Wang et al. (2023) |
| | *Kneiffiella eucalypticola* | LWZ 20180509-11 | MT319410 | MT319142 | China | Wang et al. (2021b) |
| | *Kneiffiella subglobosa* | LWZ 20180416-6 | MT319413 | MT319145 | China | Wang et al. (2021b) |
| -/Hymenochaetaceae | *Basidioradulum mayi* | LWZ 20180510-18 | MN017785 | MN017792 | Australia | Wang et al. (2021a) |
| | *Basidioradulum radula* | LWZ 20201017-62 | ON063684 | ON063884 | China | Wang et al. (2023) |
| | *Coltricia abieticola* | Cui 10321 | KX364785 | KX364804 | China | Bian and Dai (2017) |
| | *Fulvoderma australe* | LWZ 20190809-39b | ON063644 | ON063843 | China | Wang et al. (2023) |
| | *Fulvoderma yunnanense* | CLZhao 10651 | OL619278 | OL619278 | China | Direct Submission |
| | *Fuscoporia gilva* | MSU653 | JF461327 | JF461327 | Thailand | Insumran et al. (2012) |
| | *Fuscoporia sinica* | LWZ 20190816-19a | ON063649 | ON427358 | China | Wang et al. (2023) |
| | *Hydnoporia tabacina* | LWZ 20210924-26a | ON063651 | ON063851 | China | Wang et al. (2023) |
| | *Hydnoporia tabacina* | He 390 | JQ279610 | – | China | He et al.(2017) |
| | *Hymenochaete acerosa* | He 338 | JQ279543 | – | China | He et al.(2017) |
| | *Hymenochaete adusta* | He 207 | JQ279523 | – | China | He et al.(2017) |
| | *Hymenochaete angustispora* | Dai 17045 | MF370592 | – | China | He et al.(2017) |
| | *Hymenochaete angustispora* | Dai 17049 | MF370593 | – | China | He et al.(2017) |
| | *Hymenochaete anomala* | He 592 | JQ279566 | – | China | He et al.(2017) |
| | *Hymenochaete asetosa* | Dai 10756 | JQ279559 | – | China | He et al.(2017) |
| | *Hymenochaete attenuata* | He 28 | JQ279526 | – | China | He et al.(2017) |
| | *Hymenochaete bambusicola* | He 4116 | KY425674 | – | China | He et al.(2017) |
| | *Hymenochaete berteroi* | He 1488 | KU975459 | – | China | He et al.(2017) |
| | *Hymenochaete biformisetosa* | He 1445 | KF908247 | – | China | Yang and He (2014) |
| | *Hymenochaete boddingii* | MEH 66068 | MN030343 | – | India | Du et al.(2021a) |
| | *Hymenochaete boddingii* | MEH 69996 | MN030341 | – | India | Du et al.(2021a) |
| | *Hymenochaete boddingii* | MEH 66150 | MN030344 | – | India | Du et al.(2021a) |
| | *Hymenochaete borbonica* | CBS 731.86 | MH862026 | – | Netherlands | Du et al.(2021a) |
| | *Hymenochaete cana* | He 1305 | KF438169 | – | China | He et al.(2017) |
| | *Hymenochaete cinnamomea* | He 755 | JQ279548 | – | China | He et al.(2017) |
| | *Hymenochaete colliculosa* | Dai 16427 | MF370595 | – | China | He et al.(2017) |
| | *Hymenochaete colliculosa* | Dai 16428 | MF370596 | – | China | He et al.(2017) |
| | *Hymenochaete colliculosa* | Dai 16429 | MF370597 | – | China | He et al.(2017) |
| | *Hymenochaete conchata* | MEH 70144 | MF373838 | – | India | Du et al.(2021a) |
| | *Hymenochaete contiformis* | He 1166 | KU975461 | – | China | He et al.(2017) |
| | *Hymenochaete cruenta* | He 766 | JQ279595 | – | China | He et al.(2017) |
| | *Hymenochaete cyclolamellata* | Cui 7393 | JQ279513 | – | China | He et al.(2017) |
| | *Hymenochaete damicornis* | URM 84261 | KC348466 | – | Brazil | Du et al.(2021a) |
| | *Hymenochaete damicornis* | URM 84263 | KC348467 | – | Brazil | Du et al.(2021a) |
| | *Hymenochaete denticulata* | He 1271 | KF438171 | – | China | He et al. (2017) |
| | *Hymenochaete dracaenicola* | Dai 22090 | MW559797 | – | China | Du et al. (2021a) |
| | *Hymenochaete dracaenicola* | Dai 22096 | MW559798 | – | China | Du et al. (2021a) |
| | *Hymenochaete duportii* | AFTOL ID666 | DQ404386 | – | USA | He et al.(2017) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Hymenochaetaceae | *Hymenochaete epichlora* | He 525 | JQ279549 | – | China | He and Dai (2012) |
| | *Hymenochaete floridea* | He 536 | JQ279597 | – | China | He and Dai (2012) |
| | *Hymenochaete fuliginosa* | He 1188 | KU975465 | – | China | Du et al.(2021a) |
| | *Hymenochaete fulva* | He 640 | JQ279565 | – | China | He and Dai (2012) |
| | *Hymenochaete huangshanensis* | He 432 | JQ279533 | – | China | He and Dai (2012) |
| | *Hymenochaete japonica* | He 245 | JQ279590 | – | China | He and Dai (2012) |
| | *Hymenochaete innexa* | He 555 | JQ279584 | – | China | He and Dai (2012) |
| | *Hymenochaete legeri* | He 960 | KU975469 | – | China | He et al.(2017) |
| | *Hymenochaete longispora* | He 217 | JQ279537 | – | China | He and Dai (2012) |
| | *Hymenochaete luteobadia* | He 8 | JQ279569 | – | China | He and Dai (2012) |
| | *Hymenochaete macrochloae* | ARAN-Fungi 7079 | MF990738 | – | Spain | Du et al.(2021a) |
| | *Hymenochaete megaspora* | He 302 | JQ279553 | – | China | He and Dai (2012) |
| | *Hymenochaete minor* | He 933 | JQ279555 | – | China | He and Dai (2012) |
| | *Hymenochaete minuscula* | He 253 | JQ279546 | – | China | He and Dai (2012) |
| | *Hymenochaete muroiana* | He 405 | JQ279542 | – | China | Du et al.(2021a) |
| | *Hymenochaete nanospora* | He 475 | JQ279531 | – | China | He and Dai (2012) |
| | *Hymenochaete ochromarginata* | He 47 | JQ279579 | – | China | He and Dai (2012) |
| | *Hymenochaete tabacina* | Dai 11635 | JQ279563 | – | China | He and Dai (2012) |
| | *Hymenochaete orientalis* | He 4601 | KY425677 | – | China | He et al.(2017) |
| | *Hymenochaete parmastoi* | He 867 | JQ780063 | – | China | He et al.(2017) |
| | *Hymenochaete paucisetigera* | Cui 7845 | JQ279560 | – | China | He and Dai (2012) |
| | *Hymenochaete quercicola* | He 373 | KU975474 | – | China | He et al.(2017) |
| | *Hymenochaete rhabarbarina* | He 280 | JQ279574 | – | China | He and Dai (2012) |
| | *Hymenochaete rheicolor* | Cui 8317 | JQ279529 | – | China | Du et al.(2021a) |
| | *Hymenochaete rhododendricola* | He 389 | JQ279577 | – | China | He and Dai (2012) |
| | *Hymenochaete rubiginosa* | He 1049 | JQ716407 | – | China | Yang et al.(2016) |
| | *Hymenochaete rufomarginata* | He 1489 | KU975477 | – | China | He et al.(2017) |
| | *Hymenochaete sharmae* | CAL 1535 | KY929017 | – | India | Du et al.(2021a) |
| | *Hymenochaete sharmae* | 66088 | MK588753 | – | India | Du et al.(2021a) |
| | *Hymenochaete sinensis* | CLZhao 26040 | OR659001 | – | China | Li et al. (2024a) |
| | *Hymenochaete sinensis* | CLZhao 26652 | PQ060540 | – | China | Li et al. (2024a) |
| | *Hymenochaete separabilis* | He 460 | JQ279572 | – | China | He and Dai (2012) |
| | *Hymenochaete spathulata* | He 685 | JQ279591 | – | China | He et al.(2017) |
| | *Hymenochaete sphaericola* | He 303 | JQ279599 | – | China | He and Dai (2012) |
| | *Hymenochaete sphaerospora* | He 715 | JQ279594 | – | China | He et al.(2017) |
| | *Hymenochaete subferruginea* | Cui 8122 | JQ279521 | – | China | Du et al.(2021a) |
| | *Hymenochaete subferruginea* | He 1598 | KU975481 | – | China | Du et al.(2021a) |
| | *Hymenochaete tasmanica* | He 449 | JQ279582 | – | China | He et al.(2017) |
| | *Hymenochaete tenuis* | He 779 | JQ279538 | – | China | Du et al.(2021a) |
| | *Hymenochaete tongbiguanensis* | He 1552 | KF908248 | – | China | He et al.(2017) |
| | *Hymenochaete tropica* | He 574 | JQ279587 | – | China | He et al.(2017) |
| | *Hymenochaete ulmicola* | He 864 | JQ780065 | – | China | He et al.(2017) |
| | *Hymenochaete unicolor* | He 468a | JQ279551 | – | China | He et al.(2017) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Hymenochaetaceae | *Hymenochaete verruculosa* | Dai 17052 | MF370594 | – | China | He et al.(2017) |
| | *Hymenochaete villosa* | He 537 | JQ279528 | – | China | He et al.(2017) |
| | ***Hymenochaete weishanensis*** | **CLZhao 22615*** | **PQ523357** | **PQ523363** | **China** | **Present study** |
| | *Hymenochaete xerantica* | LWZ 20190814-13b | ON063657 | ON063856 | China | Wang et al. (2023) |
| | *Hymenochaete yunnanensis* | He 1447 | KU975486 | – | China | He et al.(2017) |
| | *Inonotus hispidus* | LWZ 20180703-1 | ON063659 | ON063858 | China | Wang et al. (2023) |
| | *Phellinus piceicola* | LWZ 20190921-5 | ON063662 | ON063862 | China | Wang et al. (2023) |
| | *Phylloporia oreophila* | LWZ 20190811-27a | ON063665 | ON063865 | China | Wang et al. (2023) |
| | *Porodaedalea laricis* | LWZ 20190724-9 | ON063668 | ON063868 | China | Wang et al. (2023) |
| | *Sanghuangporus weigelae* | LWZ 20210623-2a | ON063671 | ON063870 | China | Wang et al. (2023) |
| | *Trichaptum biforme* | LWZ 20210919-32a | ON063701 | ON063901 | China | Wang et al. (2023) |
| | *Trichaptum fuscoviolaceum* | LWZ 20210918-5b | ON063703 | ON063903 | China | Wang et al. (2023) |
| | *Hyphodontia arguta* | 3216b | DQ873605 | DQ873605 | Sweden | Larsson et al. (2006) |
| | *Hyphodontia borbonica* | FR 0219441 | KR349240 | KR349240 | Reunion | Riebesehl and Langer (2017) |
| | *Hyphodontia pachyspora* | LWZ 20170908-5 | MT319426 | MT319160 | China | Wang et al. (2021b) |
| | *Hyphodontia pallidula* | Kotiranta_18839 | OP620785 | OP620785 | Finland | Viner et al. (2023) |
| | *Hyphodontia zhixiangii* | LWZ 20170818-13 | MT319420 | MT319151 | China | Wang er al. (2021b) |
| -/Odonticiaceae | *Leifia brevispora* | LWZ 20170820-48 | MK343470 | MK343474 | China | Liu et al. (2019) |
| | *Leifia flabelliradiata* | KG Nilsson 36270 | DQ873635 | DQ873635 | Sweden | Larsson et al. (2006) |
| | *Odonticium romellii* | KHL s. n. | DQ873639 | DQ873639 | Norway | Larsson et al. (2006) |
| -/Peniophorellaceae | *Peniophorella praetermissa* | LWZ 20180903-14 | ON063686 | ON063886 | China | Wang et al. (2023) |
| | *Peniophorella pubera* | LWZ 20210624-16b | ON063687 | ON063887 | China | Wang et al. (2023) |
| | *Peniophorella rude* | LWZ 20171026-7 | ON063688 | ON063888 | China | Wang et al. (2023) |
| | *Peniophorella subpraetermissa* | LWZ 20190816-3b | ON063689 | ON063889 | China | Wang et al. (2023) |
| -/Repetobasidiaceae | *Repetobasidium conicum* | KHL 12338 | DQ873647 | DQ873647 | USA | Larsson et al. (2006) |
| | *Repetobasidium mirificum* | FP-133558-sp | – | AY293208 | USA | Binder et al. (2005) |
| -/Resiniciaceae | *Resinicium austroasianum* | LWZ 20191208-11 | ON063691 | ON063891 | China | Wang et al. (2023) |
| | *Resinicium bicolor* | AFTOL-810 | DQ218310 | AY586709 | USA | Larsson et al. (2004) |
| | *Resinicium friabile* | LWZ 20210923-23a | ON063692 | ON427362 | China | Wang et al. (2023) |
| -/Rickenellaceae | *Rickenella danxiashanensis* | GDGM45513 | MF326424 | – | China | Zhang et al. (2018) |
| | *Rickenella fibula* | PBM 2503 | DQ241782 | MF318953 | USA | Lutzoni et al. (2004) |
| -/Rigidoporaceae | *Leucophellinus hobsonii* | Cui 6468 | KT203288 | KT203309 | China | Direct Submission |
| | *Leucophellinus irpicoides* | Yuan 2690 | KT203289 | KT203310 | China | Direct Submission |
| | *Rigidoporus cirratus* | LWZ 20170818-16 | ON427472 | ON427355 | China | Wang et al. (2023) |
| | *Rigidoporus populinus* | LWZ 20190811-39a | ON063674 | ON063874 | China | Wang et al. (2023) |
| -/Schizocorticiaceae | *Schizocorticium lenis* | LWZ 20180922-61 | ON063698 | ON063898 | China | Wang et al. (2023) |
| | *Schizocorticium magnosporum* | Wu 1510-34 | MK405351 | MK405337 | China | Wu et al. (2021) |
| | *Schizocorticium mediosporum* | LWZ 20180921-7 | ON063696 | ON063896 | China | Wang et al. (2023) |
| | *Schizocorticium mediosporum* | Chen 2456 | MK405359 | MK405345 | China | Wu et al. (2021) |
| | *Schizocorticium parvisporum* | GC 1508-127 | MK405361 | MK405347 | China | Wu et al. (2021) |
| -/Schizoporaceae | *Fasciodontia brasiliensis* | MSK-F 7245a | MK575201 | MK598734 | Brazil | Yurchenko et al. (2020) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Schizoporaceae | *Fasciodontia yunnanensis* | LWZ 20190811-50a | ON063675 | ON427360 | China | Wang et al. (2023) |
| | ***Lyomyces albofarinaceus*** | **CLZhao 33479*** | **PQ523359** | **–** | **China** | **Present study** |
| | ***Lyomyces albofarinaceus*** | **CLZhao 26661** | **PQ523360** | **–** | **China** | **Present study** |
| | *Lyomyces albopulverulentus* | CLZhao 21478 | OP730712 | – | China | Guan et al (2023) |
| | *Lyomyces allantosporus* | KAS-GEL4933 | KY800401 | – | France | Yurchenko et al. (2017) |
| | *Lyomyces allantosporus* | FR-0249548 | KY800397 | – | France | Yurchenko et al. (2017) |
| | *Lyomyces austro-occidentalis* | LWZ 20190816-40a | MZ262538 | – | China | Liu et al. (2024) |
| | *Lyomyces bambusinus* | CLZhao 4831 | MN945968 | – | China | Chen and Zhao (2020) |
| | *Lyomyces bambusinus* | CLZhao 4808 | MN945970 | – | China | Chen and Zhao (2020) |
| | *Lyomyces boquetensis* | EYu 190727-12 | PP471797 | – | Panama | Yurchenko et al. (2024a) |
| | *Lyomyces cremeus* | CLZhao 4138 | MN945974 | – | China | Chen and Zhao (2020) |
| | *Lyomyces cremeus* | CLZhao 8295 | MN945972 | – | China | Chen and Zhao (2020) |
| | *Lyomyces crustosus* | TASM:YG G39 | MF382993 | – | Uzbekistan | Gafforov et al. (2017) |
| | *Lyomyces crustosus* | LWZ 20170815-23 | MT319465 | MT319201 | China | Wang et al. (2021b) |
| | *Lyomyces crystallina* | LWZ 20190810-6b | OQ540901 | – | China | Liu et al. (2024) |
| | *Lyomyces daweishanensis* | CLZhao 18344 | OR094474 | – | China | Dong et al. (2024) |
| | *Lyomyces densiusculus* | Ryvarden 44818 | OK273853 | – | Uganda | Viner et al. (2021) |
| | *Lyomyces denudatus* | Ryvarden 19256 | ON980759 | – | Argentina | Viner and Miettinen (2022) |
| | *Lyomyces denudatus* | Ryvarden 19436 | ON980760 | – | Argentina | Viner and Miettinen (2022) |
| | *Lyomyces elaeidicola* | LWZ20180411-20 | MT319458 | – | China | Wang et al. (2021b) |
| | *Lyomyces elaeidicola* | LWZ20180411-19 | MT319457 | – | China | Wang et al. (2021b) |
| | *Lyomyces erastii* | TASM:YG 022 | MF382992 | – | Uzbekistan | Gafforov et al. (2017) |
| | *Lyomyces erastii* | 23cSAMHYP | JX857800 | – | Spain | Unpublished |
| | *Lyomyces fimbriatus* | Wu910620-7 | MK575209 | – | China | Yurchenko et al. (2020) |
| | *Lyomyces fimbriatus* | Wu911204-4 | MK575210 | – | China | Yurchenko et al. (2020) |
| | ***Lyomyces albomarginatus*** | **CLZhao 22551*** | **PQ644120** | **PQ644121** | **China** | **Present study** |
| | *Lyomyces fissuratus* | CLZhao 4352 | MW713742 | – | China | Luo et al. (2021b) |
| | *Lyomyces fissuratus* | CLZhao 4291 | MW713738 | – | China | Luo et al. (2021b) |
| | *Lyomyces fumosus* | CLZhao 8188 | MW713744 | – | China | Luo et al. (2021b) |
| | *Lyomyces gatesiae* | LWZ20180515-3 | MT319447 | – | China | Wang et al. (2021b) |
| | *Lyomyces gatesiae* | LWZ20180515-32 | MT319448 | – | China | Wang et al. (2021b) |
| | *Lyomyces granulosus* | KAS-GEL1662 | PP471799 | – | Costa Rica | Yurchenko et al. (2024a) |
| | *Lyomyces griseliniae* | KHL 12971 (GB) | DQ873651 | – | Costa Rica | Larsson et al. (2006) |
| | *Lyomyces griseliniae* | CBS:126042 | MH864057 | – | New Zealand | Vu et al. (2018) |
| | *Lyomyces guttulatus* | LWZ 20200921-29a | OQ540899 | – | China | Liu et al. (2024) |
| | *Lyomyces guttulatus* | LWZ 20190810-20b | OQ540898 | – | China | Liu et al. (2024) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Schizoporaceae | *Lyomyces hengduanensis* | CLZhao 20627 | OR793233 | – | China | Yuan and Zhao (2024) |
| | *Lyomyces hengduanensis* | CLZhao 25551 | OR658999 | – | China | Yuan and Zhao (2024) |
| | *Lyomyces incanus* | CLZhao 22813 | OR094480 | – | China | Dong et al. (2024) |
| | *Lyomyces incanus* | CLZhao 22900 | OR094481 | – | China | Dong et al. (2024) |
| | *Lyomyces juniperi* | FR-0261086 | KY081799 | – | France | Riebesehl and Langer (2017) |
| | *Lyomyces leptocystidiatus* | LWZ 20170814-14 | MT319429 | MT319163 | China | Wang et al. (2021b) |
| | *Lyomyces leptocystidiatus* | LWZ 20170818-1 | MT326514 | – | China | Wang et al. (2021b) |
| | *Lyomyces leptocystidiatus* | LWZ 20170818-2 | MT326513 | – | China | Wang et al. (2021b) |
| | *Lyomyces lincangensis* | CLZhao 22966 | OR094487 | – | China | Dong et al. (2024) |
| | *Lyomyces luteoalbus* | CLZhao 18211 | OR094485 | – | China | Dong et al. (2024) |
| | *Lyomyces luteoalbus* | CLZhao 18347 | OR094486 | – | China | Dong et al. (2024) |
| | *Lyomyces macrosporus* | CLZhao 4516 | MN945977 | – | China | Chen and Zhao (2020) |
| | *Lyomyces mascarensis* | KAS-GEL4833 | KY800399 | – | France | Yurchenko et al. (2020) |
| | *Lyomyces mascarensis* | KAS-GEL4908 | KY800400 | – | France | Yurchenko et al. (2020) |
| | *Lyomyces microfasciculatus* | CLZhao 5109 | MN954311 | – | China | Chen and Zhao (2020) |
| | *Lyomyces napoensis* | EYu 190720-18 | PP471800 | – | Ecuador | Yurchenko et al. (2024a) |
| | *Lyomyces neocrustosus* | EYu 190728-14 | PP471801 | – | Panama | Yurchenko et al. (2024a) |
| | *Lyomyces niveomarginatus* | CLZhao 16360 | PP537949 | – | China | Yuan and Zhao (2024) |
| | *Lyomyces niveus* | CLZhao 6431 | MZ262541 | – | China | Luo et al. (2021b) |
| | *Lyomyces niveus* | CLZhao 6442 | MZ262542 | – | China | Luo et al. (2021b) |
| | *Lyomyces ochraceoalbus* | CLZhao 4385 | MZ262535 | – | China | Luo et al. (2021b) |
| | *Lyomyces ochraceoalbus* | CLZhao 4725 | MZ262536 | – | China | Luo et al. (2021b) |
| | *Lyomyces oleifer* | KAS-Ec47 | PP471802 | – | Ecuador | Yurchenko et al. (2024a) |
| | *Lyomyces orarius* | EYu 190724-1 | PP471805 | – | Ecuador | Yurchenko et al. (2024a) |
| | *Lyomyces organensis* | MSK7247 | KY800403 | – | Brazil | Yurchenko et al. (2017) |
| | *Lyomyces orientalis* | GEL3376 | DQ340325 | – | Germany | Yurchenko et al. (2017) |
| | *Lyomyces pantropicus* | EYu 190727-23b | PP471808 | – | Panama | Yurchenko et al. (2024a) |
| | *Lyomyces parvus* | KAS-GEL1599 | PP471810 | – | Costa Rica | Yurchenko et al. (2024a) |
| | *Lyomyces pruni* | GEL2327 | DQ340312 | – | Germany | Larsson et al. (2006) |
| | *Lyomyces pruni* | Ryberg 021018 (GB) | DQ873624 | – | Sweden | Larsson et al. (2006) |
| | *Lyomyces punctatomarginatus* | CLZhao 22699 | OR844492 | – | China | Li et al. (2024b) |
| | *Lyomyces punctatomarginatus* | CLZhao 11629 | OR844491 | – | China | Li et al. (2024b) |
| | *Lyomyces qujingensis* | CLZhao 27462 | OR167768 | – | China | Dong et al. (2024) |
| | *Lyomyces sambuci* | KAS-JR7 | KY800402 | – | Germany | Yurchenko et al. (2017) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Schizoporaceae | *Lyomyces sambuci* | 83SAMHYP | JX857721 | – | Sweden | Yurchenko et al. (2017) |
| | *Lyomyces sambuci* | LWZ 20180905-1 | MT319444 | MT319178 | China | Wang et al. (2021b) |
| | *Lyomyces sceptrifer* | KAS-Ec661 | PP471811 | – | Ecuador | Yurchenko et al. (2024a) |
| | *Lyomyces sinensis* | CLZhao 27391 | OR167769 | – | China | Dong et al. (2024) |
| | *Lyomyces sinensis* | CLZhao 27464 | OR167770 | – | China | Dong et al. (2024) |
| | *Lyomyces subcylindricus* | EYu 190727-25 | PP471817 | – | Panama | Yurchenko et al. (2024a) |
| | *Lyomyces tasmanicus* | LWZ 20180515–17 | OQ540900 | – | China | Liu et al. (2024) |
| | *Lyomyces vietnamensis* | TNM F9073 | JX175044 | – | China | Yurchenko et al. (2017) |
| | *Lyomyces wuliangshanensis* | CLZhao 4108 | MN945980 | – | China | Chen and Zhao (2020) |
| | *Lyomyces wuliangshanensis* | CLZhao 4167 | MN945979 | – | China | Chen and Zhao (2020) |
| | *Lyomyces wumengshanensis* | CLZhao 29374 | OR803021 | – | China | Yuan and Zhao (2024) |
| | *Lyomyces wumengshanensis* | CLZhao 31486 | OR899208 | – | China | Yuan and Zhao (2024) |
| | *Lyomyces yunnanensis* | CLZhao 9375 | OP730710 | – | China | Guan et al. (2023) |
| | *Lyomyces yunnanensis* | CLZhao 10041 | OP730709 | – | China | Guan et al. (2023) |
| | *Lyomyces zhaotongensis* | CLZhao 32878 | PP537950 | – | China | Yuan et al. (2024) |
| | *Xylodon acuminatus* | Larsson 16029 | ON197552 | – | Brazil | Viner et al. (2023) |
| | *Xylodon acystidiatus* | LWZ 20180514-9 | MT319474 | – | China | Wang et al. (2021b) |
| | *Xylodon afromontanus* | O-F-904012 | OQ645463 | – | Rwanda | Yurchenko et al. (2024a) |
| | *Xylodon angustisporus* | Ryvarden 50691b | OK273831 | – | Cameroon | Viner et al. (2021) |
| | *Xylodon apacheriensis* | Canfield 180 | KY081800 | – | USA | Wang et al. (2021b) |
| | *Xylodon asiaticus* | CLZhao 10368 | OM959479 | – | China | Unpublished |
| | *Xylodon asper* | Spirin 11923 | OK273838 | – | Russia | Viner et al. (2021) |
| | *Xylodon astrocystidiatus* | TNM F24764 | NR154054 | – | USA | Yurchenko and Wu (2014) |
| | *Xylodon attenuatus* | Spirin 8775 | MH324476 | – | Russia | Wang et al. (2021b) |
| | *Xylodon australis* | LWZ 20180509-8 | MT319503 | – | China | Wang et al. (2021b) |
| | *Xylodon bambusinus* | CLZhao 9174 | MW394657 | – | China | Ma and Zhao (2021) |
| | *Xylodon bamburesupinus* | CLZhao 23088 | OR167773 | – | China | Dong et al. (2024) |
| | *Xylodon borealis* | JS 26064 | AY463429 | – | Sweden | Larsson et al. (2004) |
| | *Xylodon brevisetus* | JS 17863 | AY463428 | – | Sweden | Larsson et al. (2004) |
| | *Xylodon cremeoparinaceus* | CLZhao 23388 | PP537951 | – | China | Yuan and Zhao (2024) |
| | *Xylodon crystalliger* | KUN 2312 | NR166242 | – | USA | Viner et al. (2018) |
| | *Xylodon cymosus* | Miettinen 19606 | ON197554 | – | Finland | Viner et al. (2023) |
| | *Xylodon cystidiatus* | FR-0249200 | MH880195 | – | Germany | Wang et al. (2021b) |
| | *Xylodon damansaraensis* | LWZ 20180417-23 | MT319499 | – | China | Wang et al. (2021b) |
| | *Xylodon daweishanensis* | CLZhao 18357 | OP730715 | – | China | Guan et al. (2023) |
| | *Xylodon detriticus* | Zíbarová 30.10.17 | MH320793 | – | Russia | Wang et al. (2021b) |
| | *Xylodon dissiliens* | Ryvarden 44817 | OK273856 | – | Finland | Viner et al. (2021) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Schizoporaceae | *Xylodon echinatus* | OM 18237 | OQ645464 | – | Germany | Yurchenko et al. (2024a) |
| | *Xylodon filicinus* | MSK-F 12869 | MH880199 | – | Germany | Wang et al. (2021b) |
| | *Xylodon fissilis* | CLZhao 18740 | OR096211 | – | China | Dong et al. (2024) |
| | *Xylodon fissuratus* | CLZhao 9407 | OP730714 | – | China | Guan et al. (2023) |
| | *Xylodon flaviporus* | FR-0249797 | MH880201 | – | Germany | Wang et al. (2021b) |
| | *Xylodon flocculosus* | CLZhao 18342 | MW980776 | – | China | Unpublished |
| | *Xylodon follis* | FR-0249814 | MH880204 | – | Germany | Wang et al. (2021b) |
| | *Xylodon gloeocystidiifer* | BLS M-5232 | OQ645467 | – | Germany | Yurchenko et al. (2024a) |
| | *Xylodon gossypinus* | CLZhao 8375 | MZ663804 | – | China | Luo et al. (2021a) |
| | *Xylodon grandineus* | CLZhao 6425 | OM338090 | – | China | Luo et al. (2022) |
| | *Xylodon hastifer* | K(M) 172400 | NR166558 | – | USA | Riebesehl and Langer (2017) |
| | *Xylodon heterocystidiatus* | Wei 17-314 | MT731753 | – | China | Wu et al. (2021) |
| | *Xylodon hjortstamii* | Gorjon 3187 | ON188816 | – | Finland | Direct Submission |
| | *Xylodon hydnoides* | CLZhao 17991 | OR096203 | – | China | Dong et al. (2024) |
| | *Xylodon hyphodontinus* | KAS-GEL9222 | MH880205 | – | Germany | Riebesehl et al. (2019) |
| | *Xylodon jacobaeus* | MA-Fungi 91340 | MH430073 | – | Spain | Wang et al. (2021b) |
| | *Xylodon kunmingensis* | TUB-FO 42565 | MH880198 | – | Germany | Wang et al. (2021b) |
| | *Xylodon laceratus* | CLZhao 9892 | OL619258 | – | China | Qu et al. (2022) |
| | *Xylodon lagenicystidiatus* | LWZ 20180515-14 | MT319633 | – | China | Wang et al. (2021b) |
| | *Xylodon lagenicystidiatus* | LWZ 20180513-16 | MT319634 | – | China | Wang et al. (2021b) |
| | *Xylodon lanatus* | CFMR FP-101864-A | OQ645474 | – | Germany | Yurchenko et al. (2024a) |
| | *Xylodon laxiusculus* | Ryvarden 44877 | OK273827 | – | Finland | Viner et al. (2021) |
| | *Xylodon lenis* | Wu 890714-3 | KY081802 | – | Germany | Yurchenko et al. (2024a) |
| | *Xylodon luteodontioides* | CLZhao 3207 | MH114740 | – | China | Yuan and Zhao (2024) |
| | *Xylodon luteodontioides* | CLZhao 18494 | PP505422 | – | China | Yuan and Zhao (2024) |
| | *Xylodon macrosporus* | CLZhao 10226 | MZ663809 | – | China | Luo et al. (2021a) |
| | *Xylodon magallanesii* | MA: Fungi:90397 | MT158729 | – | Spain | Fernandez-Lopez et al. (2020) |
| | *Xylodon mantiqueirensis* | MV 529 | OQ645478 | – | Germany | Yurchenko et al. (2024a) |
| | *Xylodon mollissimus* | LWZ 20160318-3 | KY007517 | – | China | Kan et al. (2017b) |
| | *Xylodon montanus* | CLZhao 8179 | OL619260 | – | China | Qu et al. (2022) |
| | *Xylodon muchuanensis* | LWZ 20200819-3a | OQ540903 | – | China | Liu et al. (2024) |
| | ***Xylodon musicola*** | **CLZhao 35567*** | **PQ523358** | – | **China** | **Present study** |
| | *Xylodon neotropicus* | MV 580 | OQ645479 | – | Germany | Yurchenko et al. (2024a) |
| | *Xylodon nesporii* | LWZ 20180921-35 | MT319655 | – | China | Wang et al. (2021b) |
| | *Xylodon nesporii* | LWZ 20190814-17a | ON063679 | ON063879 | China | Wang et al. (2023) |
| | *Xylodon niemelaei* | CLZhao 3746 | MK269038 | – | China | Unpublished |
| | *Xylodon nongravis* | GC 1412-22 | KX857801 | – | China | Chen et al. (2017) |
| | *Xylodon nothofagi* | ICMP 13842 | AF145583 | – | Sweden | Wang et al. (2021b) |
| | *Xylodon olivaceobubalinus* | CLZhao 25174 | OR167772 | – | China | Dong et al. (2024) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Schizoporaceae | *Xylodon ovisporus* | LWZ 20170815-31 | MT319666 | – | China | Wang et al. (2021b) |
| | *Xylodon ovisporus* | LWZ 20190817-6b | ON063680 | ON063880 | China | Wang et al. (2023) |
| | *Xylodon papillosus* | CBS 114.71 | MH860026 | – | Germany | Vu et al. (2018) |
| | *Xylodon paradoxus* | Dai 14983 | MT319519 | – | China | Wang et al. (2021b) |
| | *Xylodon patagonicus* | ICMP 13832 | AF145581 | – | Sweden | Wang et al. (2021b) |
| | *Xylodon pingbianensis* | CLZhao 19029 | OR096208 | – | China | Dong et al. (2024) |
| | *Xylodon poroides* | CLZhao 17845 | PP505420 | – | China | Yuan and Zhao (2024) |
| | *Xylodon pruinosus* | Spirin 2877 | MH332700 | – | Russia | Wang et al. (2021b) |
| | *Xylodon pruniaceus* | Ryvarden 11251 | OK273828 | – | Finland | Viner et al. (2021) |
| | *Xylodon pseudolanatus* | FP-150922 | MH880220 | – | Germany | Wang et al. (2021b) |
| | *Xylodon pseudotropicus* | Dai 10768 | KF917543 | – | China | Wang et al. (2021b) |
| | *Xylodon puerensis* | CLZhao 8142 | OP730720 | – | China | Guan et al. (2023) |
| | *Xylodon punctus* | CLZhao 17691 | OM338092 | – | China | Luo et al. (2022) |
| | *Xylodon punctus* | CLZhao 17908 | OM338093 | – | China | Luo et al. (2022) |
| | *Xylodon punctus* | CLZhao 17916 | OM338094 | – | China | Luo et al. (2022) |
| | *Xylodon quercinus* | Spirin 12030 | OK273841 | – | Finland | Viner et al. (2021) |
| | *Xylodon raduloides* | FCUG 2433 | AF145570 | – | Sweden | Wang et al. (2021b) |
| | *Xylodon ramicida* | Spirin 7664 | NR138013 | – | Russia | Direct Submission |
| | *Xylodon reticulatus* | Wu 1109-178 | KX857805 | – | China | Wang et al. (2021b) |
| | *Xylodon reticulatus* | GC 1512-1 | KX857808 | – | China | Wang et al. (2021b) |
| | *Xylodon rimosissimus* | LWZ 20180904-28 | ON063682 | ON063882 | China | Wang et al. (2023) |
| | *Xylodon rhizomorphus* | Dai 12367 | NR154067 | – | China | Zhao et al. (2014) |
| | *Xylodon rhododendricola* | LWZ 20180513-9 | MT319621 | – | China | Wang et al. (2021b) |
| | *Xylodon serpentiformis* | LWZ 20190816-12a | ON063683 | ON063883 | China | Wang et al. (2023) |
| | *Xylodon sinensis* | CLZhao 9197 | MZ663810 | – | China | Luo et al. (2021a) |
| | *Xylodon sinensis* | CLZhao 11120 | MZ663811 | – | China | Luo et al. (2021a) |
| | *Xylodon spathulatus* | LWZ 20180804-10 | MT319646 | – | China | Wang et al. (2021b) |
| | *Xylodon subclavatus* | FO 42167 | MH880232 | – | China | Wang et al. (2021b) |
| | *Xylodon subflaviporus* | TNM F29958 | NR184880 | – | USA | Chen et al. (2017) |
| | *Xylodon submucronatus* | Renvall 1602 | OK273830 | – | Finland | Viner et al. (2021) |
| | *Xylodon subserpentiformis* | LWZ 20180512-16 | MT319486 | – | China | Wang et al. (2021b) |
| | *Xylodon subtilissimus* | Spirin 12228 | ON188818 | – | Finland | Direct Submission |
| | *Xylodon subtropicus* | LWZ 20180510-24 | MT319541 | – | China | Wang et al. (2021b) |
| | *Xylodon taiwanianus* | CBS 125875 | MH864080 | – | New Zealand | Vu et al. (2018) |
| | *Xylodon tropicus* | CLZhao 3351 | OL619261 | – | China | Qu et al. (2022) |
| | *Xylodon ussuriensis* | KUN 1989 | NR166241 | – | Russia | Direct Submission |
| | *Xylodon verecundus* | KHL 12261 | DQ873642 | – | USA | Wang et al. (2021b) |
| | *Xylodon victoriensis* | LWZ 20180510-29 | MT319487 | – | China | Wang et al. (2021b) |
| | *Xylodon wenshanensis* | CLZhao 15729 | OM338097 | – | China | Luo et al. (2022) |
| | *Xylodon wumengshanensis* | CLZhao 32517 | PP645439 | – | China | Yuan and Zhao (2024) |
| | *Xylodon xinpingensis* | CLZhao 9174 | MW394657 | – | China | Ma and Zhao (2021) |
| | *Xylodon yarraensis* | LWZ 20180510-5 | MT319639 | – | China | Wang et al. (2021b) |
| | *Xylodon yunnanensis* | LWZ 20180922-47 | MT319660 | – | China | Wang et al. (2021b) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Sideraceae | *Sidera lenis* | Miettinen 11036 | FN907914 | FN907914 | Finland | Miettinen and Larsson (2011) |
| | *Sidera minutipora* | Cui 16720 | MN621349 | MN621348 | Australia | Du et al. (2020b) |
| | *Sidera srilankensis* | Dai 19654 | MN621344 | MN621346 | Sri Lanka | Du et al. (2020b) |
| | *Sidera tenuis* | Dai 18697 | MK331865 | MK331867 | Singapore | Liu et al. (2022) |
| | *Sidera tibetica* | Dai 21057 | MW198484 | MW192009 | Belarus | Liu et al. (2022) |
| -/Skvortzoviaceae | *Skvortzovia dabieshanensis* | LWZ 20210918-15b | ON063694 | ON063894 | China | Wang et al. (2023) |
| | *Skvortzovia pinicola* | LWZ 20210623-18b | ON063695 | ON063895 | China | Wang et al. (2023) |
| | *Skvortzovia qilianensis* | LWZ 20180904-20 | ON063693 | ON063893 | China | Wang et al. (2023) |
| | *Skvortzovia yunnanensis* | CLZhao 16084 | MW472754 | MW473473 | China | Dong et al. (2021) |
| -/Tubulicrinaceae | *Tubulicrinis accedens* | ACD0414 | OL756001 | – | USA | Unpublished |
| | ***Tubulicrinis albobadius*** | **CLZhao 26202*** | **PQ523361** | **PQ523364** | **China** | **Present study** |
| | ***Tubulicrinis albobadius*** | **CLZhao 26330** | **PQ523362** | **PQ523365** | **China** | **Present study** |
| | *Tubulicrinis australis* | MA Fungi:88838 | KX017591 | – | Spain | Unpublished |
| | *Tubulicrinis australis* | MA Fungi:88839 | KX017593 | – | Chile | Unpublished |
| | *Tubulicrinis borealis* | DK14_93 | OL436811 | – | USA | Unpublished |
| | *Tubulicrinis calothrix* | LWZ 20210919-1b | ON063704 | – | China | Wang et al. (2023) |
| | *Tubulicrinis chaetophorus* | Spirin 12616 (H) | ON188814 | – | Slovenia | Direct Submission |
| | *Tubulicrinis chaetophorus* | UC2023055 | KP814255 | – | USA | Rosenthal et al. (2017) |
| | *Tubulicrinis chaetophorus* | UC2023059 | KP814233 | – | USA | Rosenthal et al. (2017) |
| | *Tubulicrinis glebulosus* | LWZ 20180903-13 | ON063705 | ON063905 | China | Wang et al. (2023) |
| | *Tubulicrinis glebulosus* | DK16_14 | OL436905 | – | USA | Unpublished |
| | *Tubulicrinis glebulosus* | UC2023229 | KP814463 | – | Canada | Rosenthal et al. (2017) |
| | *Tubulicrinis globisporus* | KHL 12133 (GB) | DQ873655 | – | Sweden | Larsson et al. (2006) |
| | *Tubulicrinis gracillimus* | PDD 95851 | HQ533047 | – | New Zealand | Unpublished |
| | *Tubulicrinis hirtellus* | KHL 11717 (GB) | DQ873657 | – | Sweden | Larsson et al. (2006) |
| | *Tubulicrinis inornatus* | KHL 11763 (GB) | DQ873659 | – | Sweden | Larsson et al. (2006) |
| | *Tubulicrinis inornatus* | OTU782 | MT596347 | – | Japan | Unpublished |
| | *Tubulicrinis martinicensis* | GG-MAR12-206 | NR_163282 | – | France | Unpublished |
| | *Tubulicrinis pini* | CLZhao 6881 | OR096210 | – | China | Dong et al. (2024) |
| | *Tubulicrinis subulatus* | UC2023072 | KP814430 | – | USA | Rosenthal et al. (2017) |
| | *Tubulicrinis subulatus* | LWZ 20190914-7 | ON063706 | ON063906 | China | Wang et al. (2023) |
| | *Tubulicrinis xantha* | CLZhao 2868 | MT153874 | – | China | He et al. (2020) |
| | *Tubulicrinis xantha* | CLZhao 2869 | MT153875 | – | China | He et al. (2020) |
| | *Tubulicrinis yunnanensis* | CLZhao 3418 | MT153879 | – | China | He et al. (2020) |
| | *Tubulicrinis yunnanensis* | CLZhao 9717 | MT153880 | – | China | He et al. (2020) |
| -/Umbellaceae | *Umbellus sinensis* | LWZ 20190615-27 | OR242616 | OR236212 | China | Wang and Zhou (2024) |
| | *Umbellus sinensis* | LWZ 20190615-39 | OR242617 | OR236213 | China | Wang and Zhou (2024) |
| -/Incertae sedis | *Alloclavaria purpurea* | M. Korhonen 10305 | MF319044 | MF318895 | Finland | Unpublished |
| | *Atheloderma mirabile* | TAA 169235 | DQ873592 | DQ873592 | Estonia | Larsson et al. (2006) |

| Order/Family | Species Name | Sample No. | GenBank Accession No. | | | References |
|---|---|---|---|---|---|---|
| | | | ITS | nLSU | | |
| -/Incertae sedis | *Blasiphalia pseudogrisella* | P. Joijer 4118 | MF319047 | MF318898 | Finland | Unpublished |
| | *Bryopistillaria sagittiformis* | IO.14.164 | MT232349 | MT232303 | Sweden | Olariaga et al. (2020) |
| | *Cantharellopsis prescotii* | H6059300 | MF319051 | MF318903 | Finland | Unpublished |
| | *Contumyces vesuvianus* | 203608 | – | MF318913 | Italy | Unpublished |
| | *Ginnsia viticola* | Wu 0010-29 | MN123802 | GQ470670 | China | Wu et al. (2021) |
| | *Globulicium hiemale* | Hjm 19007 | DQ873595 | DQ873595 | Sweden | Larsson et al. (2006) |
| | *Gyroflexus brevibasidiata* | IO.14.230 | MT232351 | MT232305 | Sweden | Olariaga et al. (2020) |
| | *Hastodontia halonata* | HHB-17058 | MK575207 | MK598738 | Mexico | Yurchenko et al. (2020) |
| | *Hastodontia hastata* | KHL 14646 | MH638232 | MH638232 | Norway | Larsson (2007) |
| | *Lawrynomyces capitatus* | KHL 8464 | DQ677491 | DQ677491 | Sweden | Larsson (2007) |
| | *Loreleia marchantiae* | Lutzoni 930826-1 | U66432 | U66432 | USA | Lutzoni F (1997) |
| | *Lyoathelia laxa* | Spirin 8810a | MT305998 | MT305998 | USA | Sulistyo et al. (2021) |
| | *Muscinupta laevis* | V. Haikonen 19745 | MF319066 | MF318921 | Finland | Unpublished |
| | *Sphaerobasidium minutum* | KHL 11714 | DQ873652 | DQ873653 | Finland | Larsson et al. (2006) |
| | *Tsugacorticium kenaicum* | CFMR HHB17347 | – | JN368221 | USA | Nakasone (2012) |
| Polyporales/ Fomitopsidaceae | *Fomitopsis pinicola* | AFTOL 770 | AY854083 | AY684164 | USA | Lutzoni et al. (2004) |
| -/Grifolaceae | *Grifola frondosa* | AFTOL 701 | AY854084 | AY629318 | USA | Lutzoni et al. (2004) |
| -/Thelephoraceae | *Thelephora ganbajun* | ZRL20151295 | LT716082 | KY418908 | China | Zhao et al. (2017) |

## Results

### Sequence similarity search

The ITS+nLSU dataset (Fig. 1) comprised 80 specimens representing 77 species of the phylogeny of the order Hymenochaetales. The dataset had an aligned length of 2,548 characters, of which 1,018 characters are constant, 476 are variable and parsimony uninformative, and 1,054 are parsimony informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 10768, CI = 0.2612, HI = 0.7388, RI = 0.4087, and RC = 0.1068). The best model for the ITS+nLSU dataset, estimated and applied in the Bayesian analysis, was GTR+I+G. Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.044070 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 645.

The ITS dataset (Fig. 2) comprised 78 specimens representing 69 species of the one new species and related species in the genus *Hymenochaete*. The dataset had an aligned length of 470 characters, of which 209 characters are constant, 35 are variable and parsimony uninformative, and 226 are parsimony informative. Maximum parsimony analysis yielded 68 equally parsimonious trees (TL = 1574, CI = 0.3018, HI = 0.6982, RI = 0.6876, and RC = 0.2075). The best model for the ITS dataset, estimated and applied in the Bayesian analysis, was GTR+I+G. Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies =

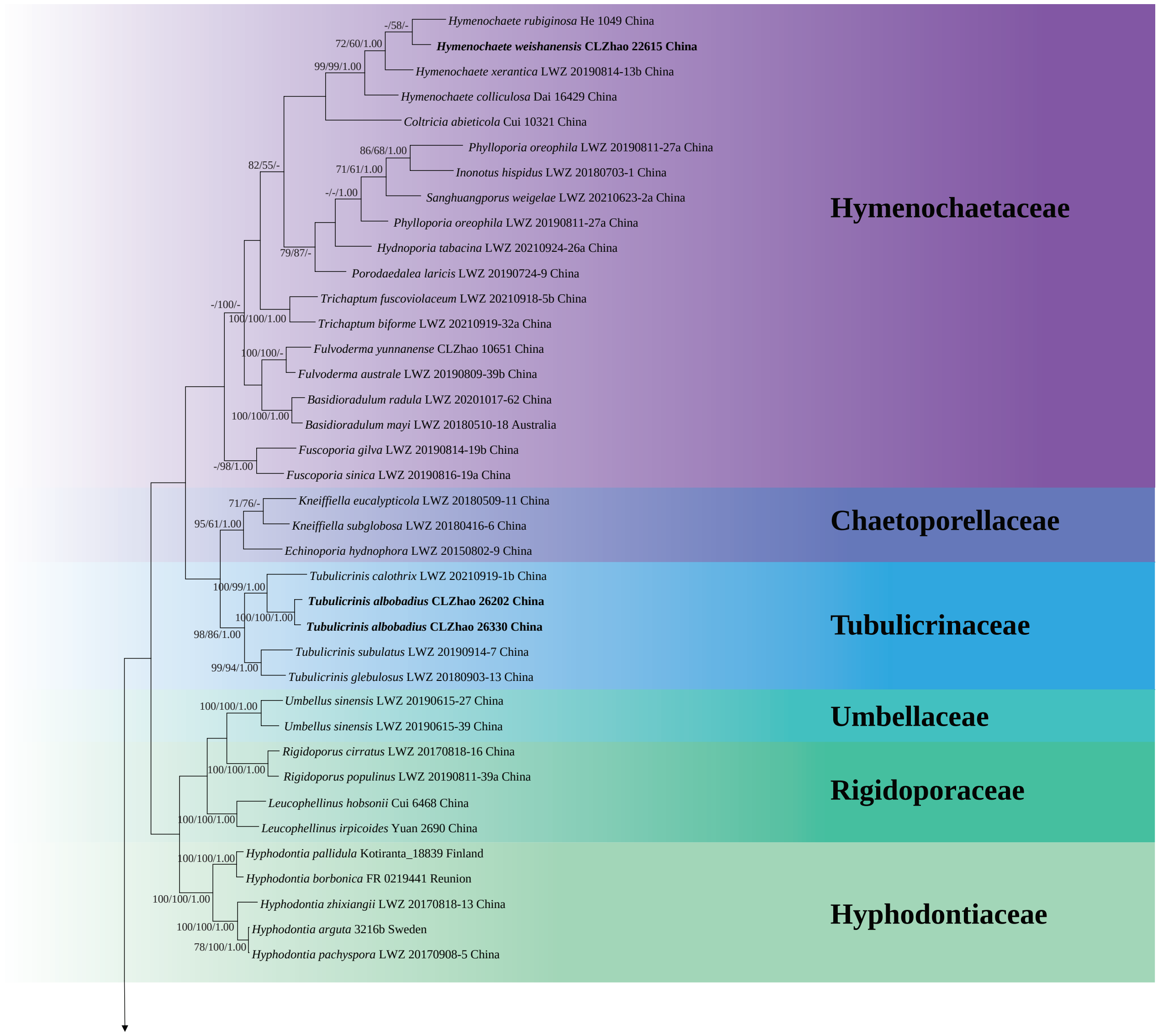

**Figure 1.** Maximum parsimony strict consensus tree illustrating the phylogeny of the order Hymenochaetales based on ITS+nLSU sequences. Branches are labelled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

0.012875 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 269.5.

The ITS dataset (Fig. 3) comprised 81 specimens representing 56 species of two new species and related taxa in the genus *Lyomyces*. The dataset had an aligned length of 470 characters, of which 209 characters are constant, 35 are variable and parsimony uninformative, and 226 are parsimony informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (TL = 1574, CI = 0.3018, HI = 0.6982, RI = 0.6876, and RC = 0.2075). The best model for the ITS dataset, estimated and applied in the Bayesian analysis, was GTR+I+G. Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.012875 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 269.5.

The ITS dataset (Fig. 4) comprised 28 specimens representing 17 species of new species and related taxa in the genus *Tubulicrinis*. The dataset had an

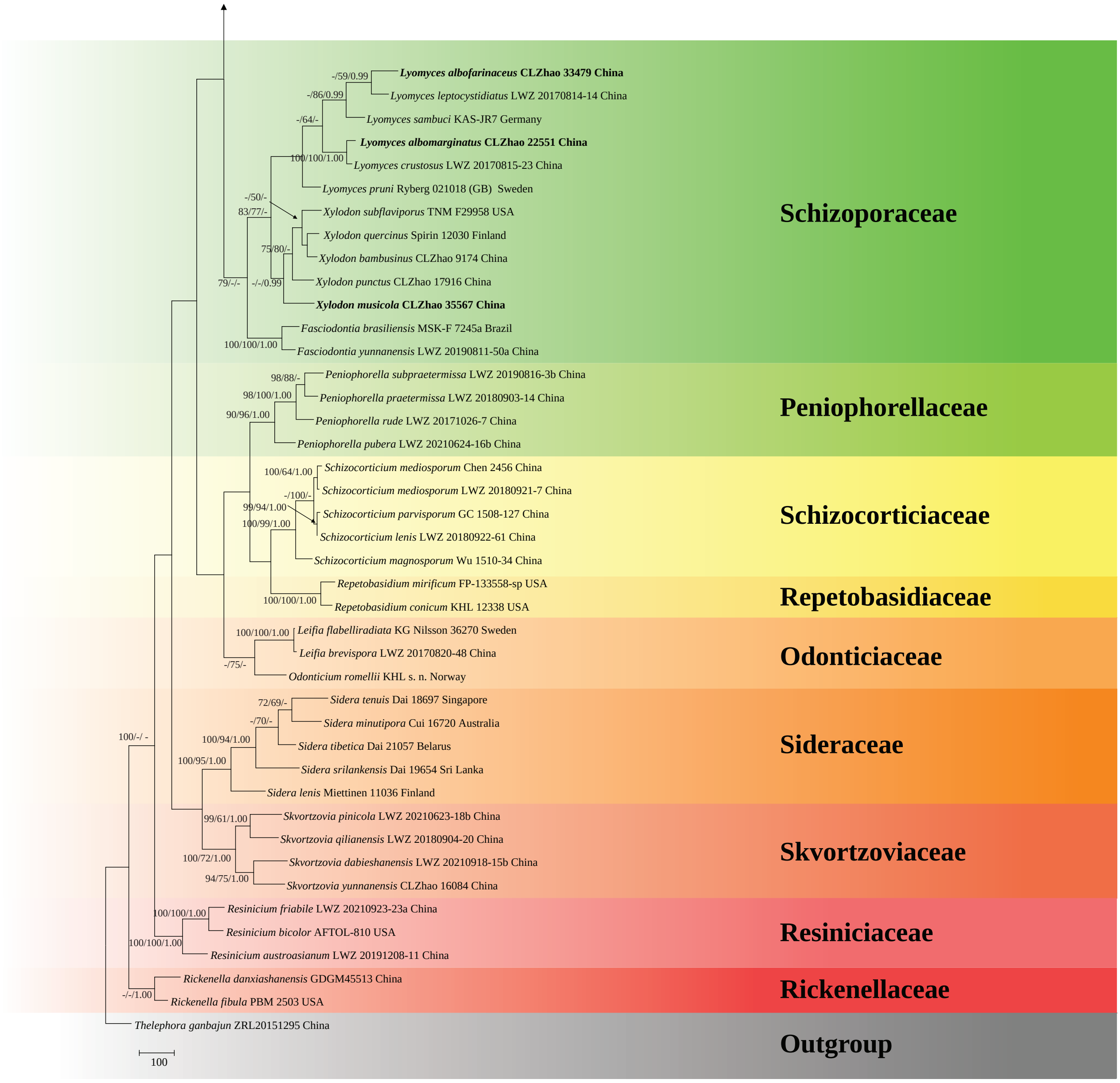

**Figure 1.** Continued.

aligned length of 772 characters, of which 284 characters are constant, 152 are variable and parsimony uninformative, and 336 are parsimony informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 1409, CI = 0.5777, HI = 0.4223, RI = 0.6248, and RC = 0.3610). The best model for the ITS dataset, estimated and applied in the Bayesian analysis, was GTR+I+G. Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.006683 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 633.

The ITS dataset (Fig. 5) comprised 104 specimens representing 97 species of the new species and related taxa in the genus *Xylodon*. The dataset had an aligned length of 673 characters, of which 233 characters are constant, 81 are variable and parsimony uninformative, and 359 are parsimony informative. Maximum parsimony analysis yielded 5,000 equally parsimonious trees

**Figure 2.** Maximum parsimony strict consensus tree illustrating the phylogeny of the one new species and related species in the genus *Hymenochaete* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

**Figure 2.** Continued.

(TL = 3842, CI = 0.2140, HI = 0.7860, RI = 0.4337, and RC = 0.0928). The best model for the ITS dataset, estimated and applied in the Bayesian analysis, was GTR+I+G. Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.022556 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs is double of the average ESS (avg ESS) = 1143.5.

**Figure 3.** Maximum parsimony strict consensus tree illustrating the phylogeny of the new species and related species in the genus *Lyomyces* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

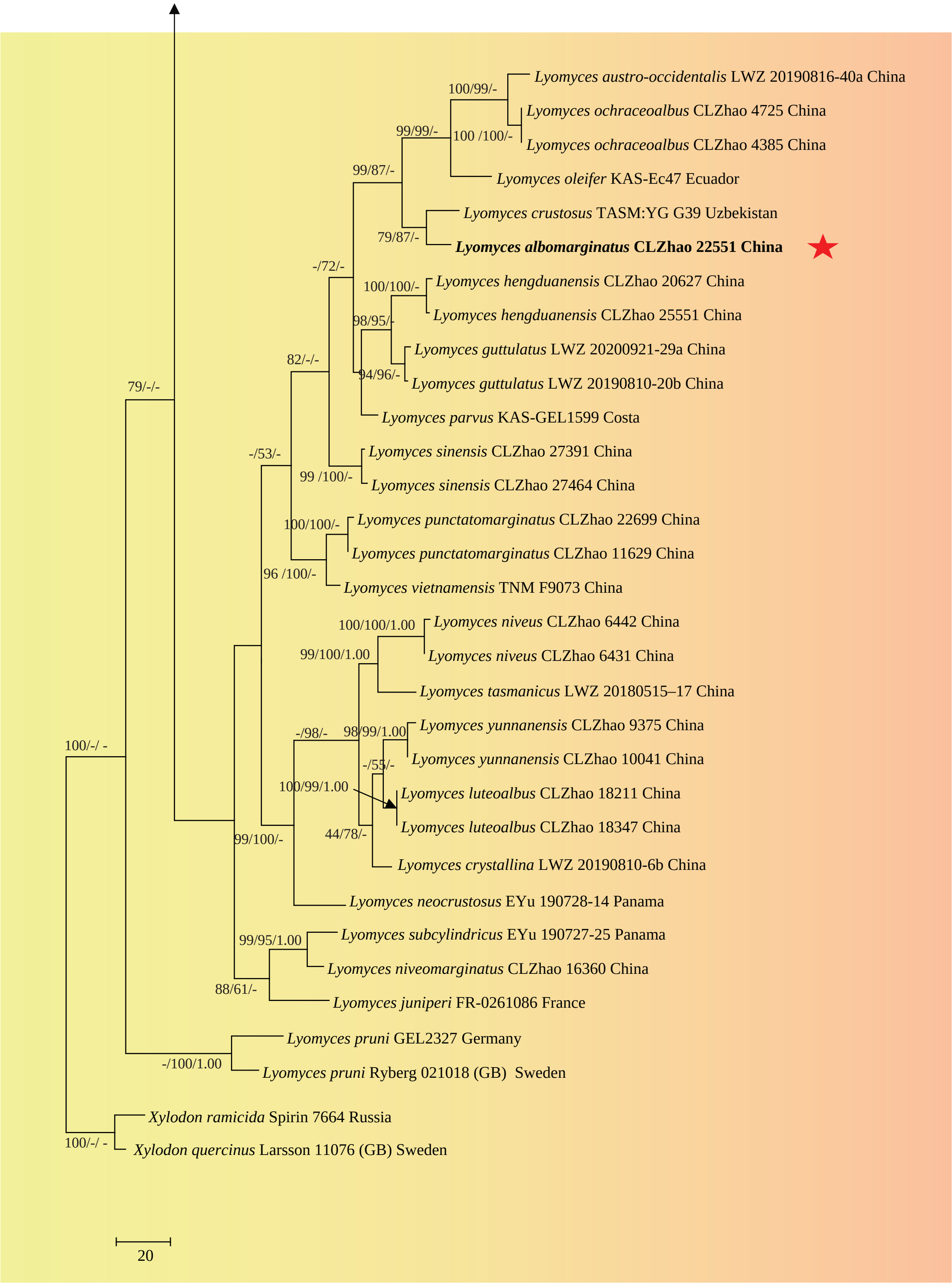

**Figure 3.** Continued.

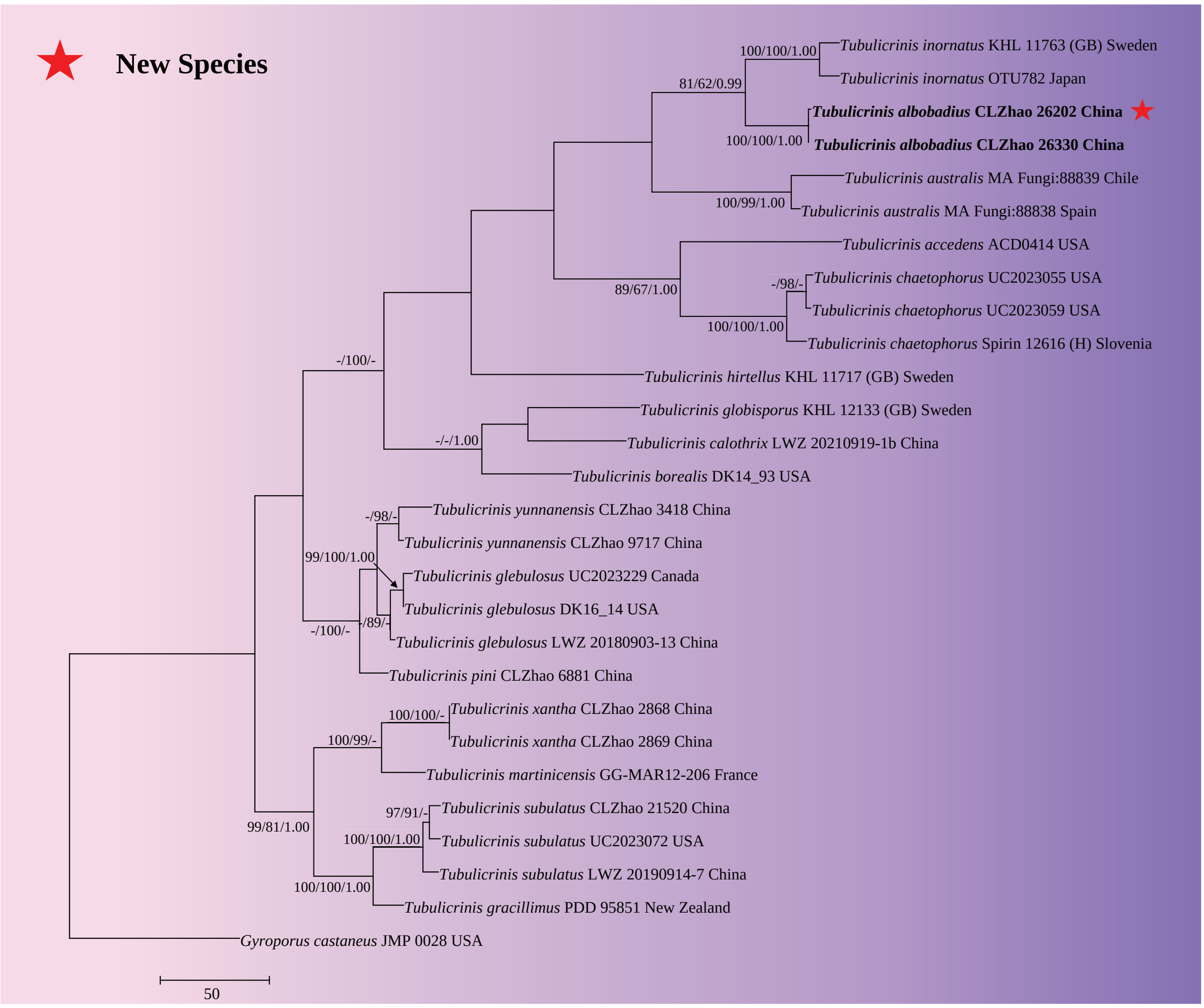

**Figure 4.** Maximum parsimony strict consensus tree illustrating the phylogeny of the new species and related species in the genus *Tubulicrinis* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

The phylogram based on the combined ITS+nLSU sequences (Fig. 1) analysis showed that five new species *Hymenochaete weishanensis*, *Lyomyces albofarinaceus*, *Lyomyces albomarginatus*, *Tubulicrinis albobadius* and *Xylodon musicola* were assigned to the genera *Hymenochaete*, *Lyomyces*, *Tubulicrinis* and *Xylodon* within the order Hymenochaetales, individually. The phylogenetic tree based on ITS sequences (Fig. 2), revealed that *H. weishanensis* was retrieved as a sister to *H. luteobadia*. The taxon based on the ITS sequences (Fig. 3) revealed that *L. albofarinaceus* was grouped with *L. albopulverulentus* and *L. qujingensis*. *L. albomarginatus* was sister to *L. crustosus*. The topology based on the ITS sequences (Fig. 4), revealed that *T. albobadius* was grouped with *T. australis* and *T. inornatus*. The phylogenetic tree, based on ITS sequences (Fig. 5), revealed that *X. musicola* grouped with three taxa: *X. gloeocystidiifer*, *X. hydnoides*, and *X. neotropicus*.

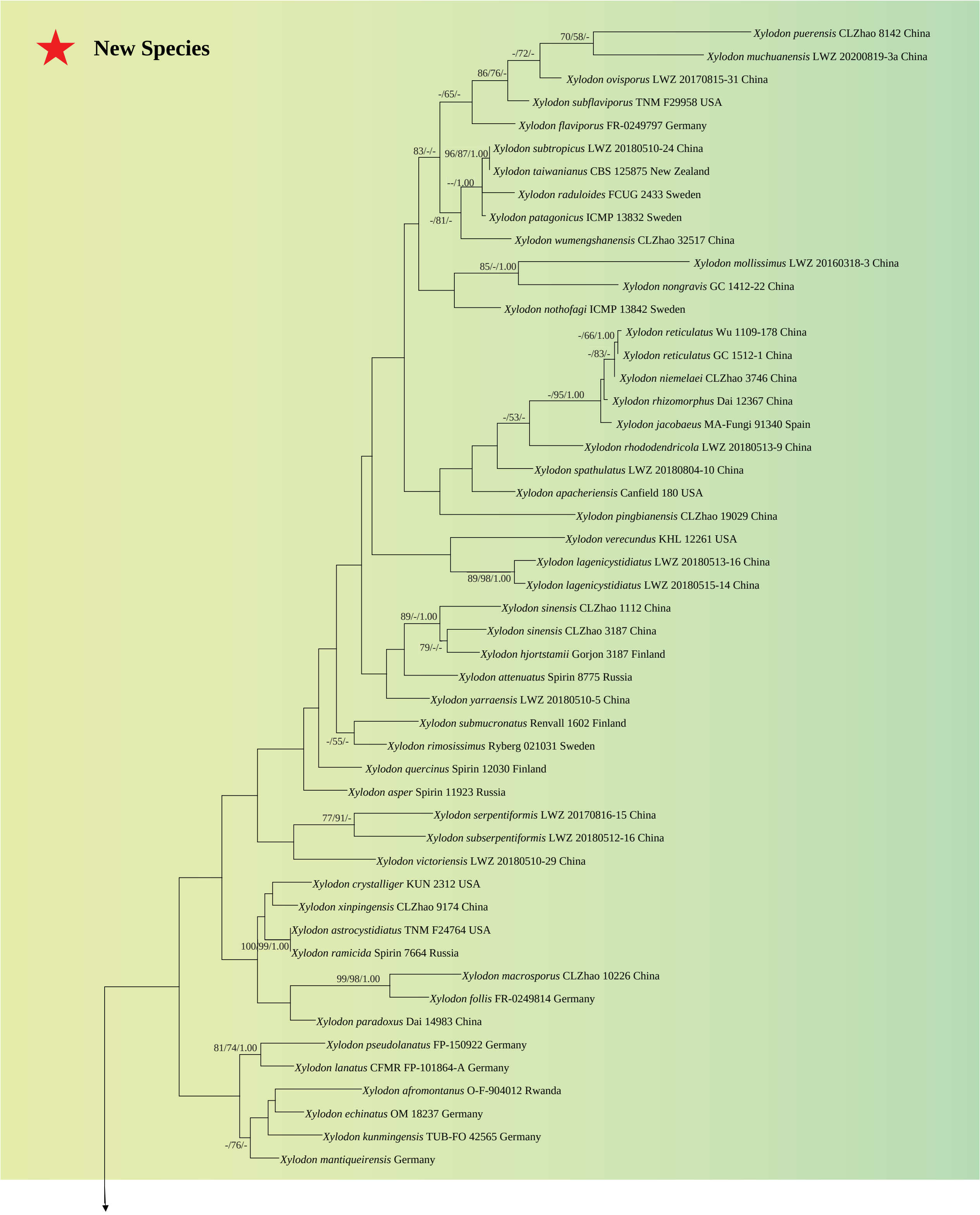

**Figure 5.** Maximum parsimony strict consensus tree illustrating the phylogeny of the new species and related species in the genus *Xylodon* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

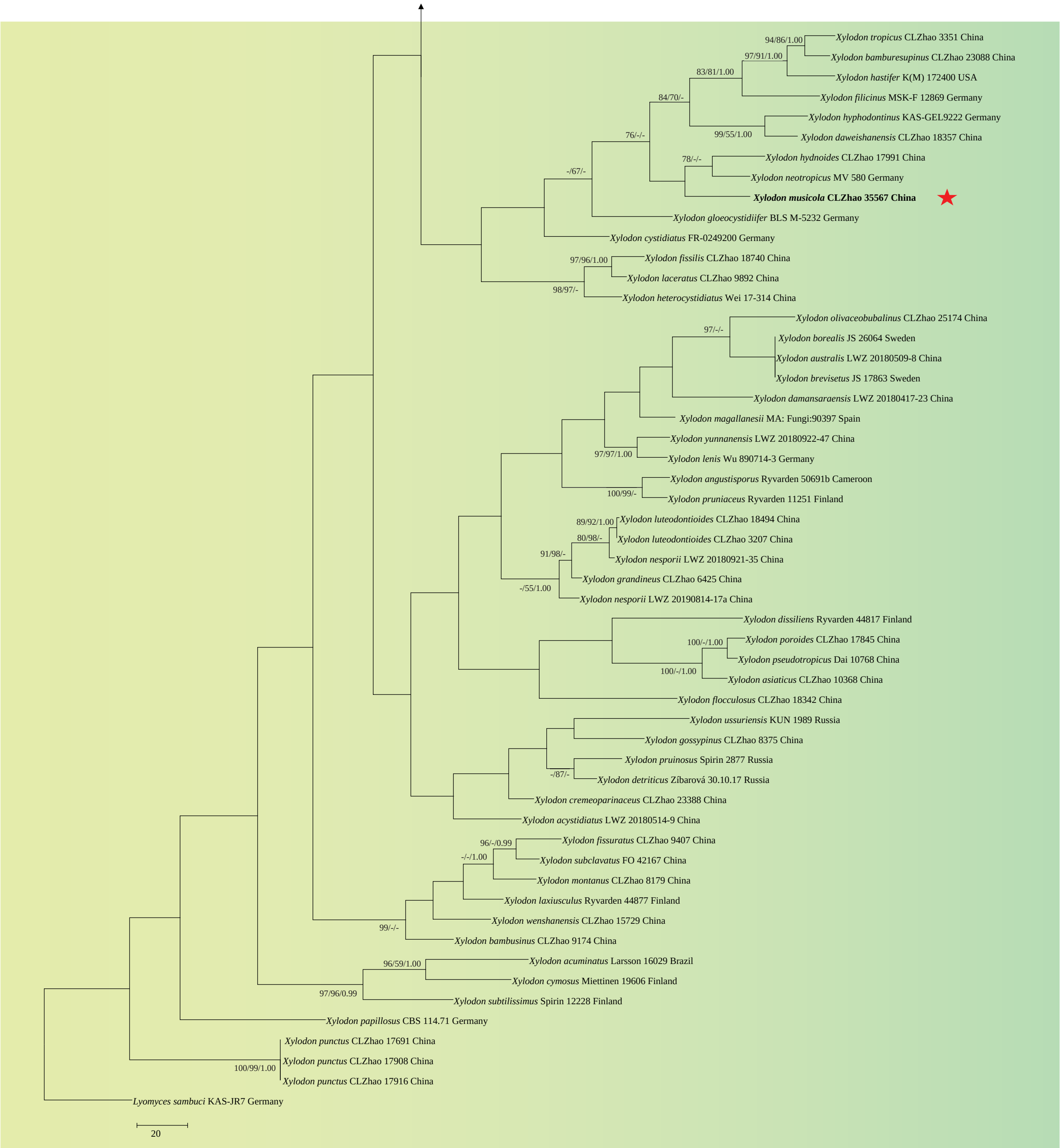

**Figure 5.** Continued.

## Taxonomy

***Hymenochaete weishanensis*** **Y.F. Dai & C.L. Zhao, sp. nov.**
MycoBank No: 856316
Figs 6, 7

**Holotype.** China • Yunnan Province, Dali, Weishan County, Leqiu Town, Zhongyao Village, GPS coordinates 25°02'N, 100°16'E, evel. 1910 m a.s.l., on a fallen branch of angiosperm, leg. C.L. Zhao, 19 July 2022, CLZhao 22615 (SWFC).

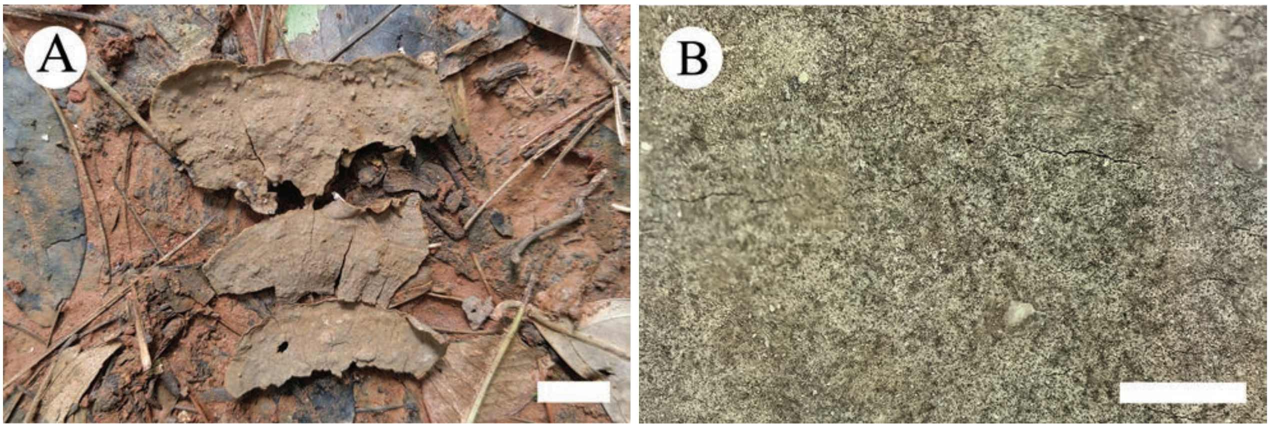

**Figure 6.** Basidiomata of *Hymenochaete weishanensis* in general and detailed views (CLZhao 22615, holotype). Scale bars: 1cm (**A**); 1mm (**B**).

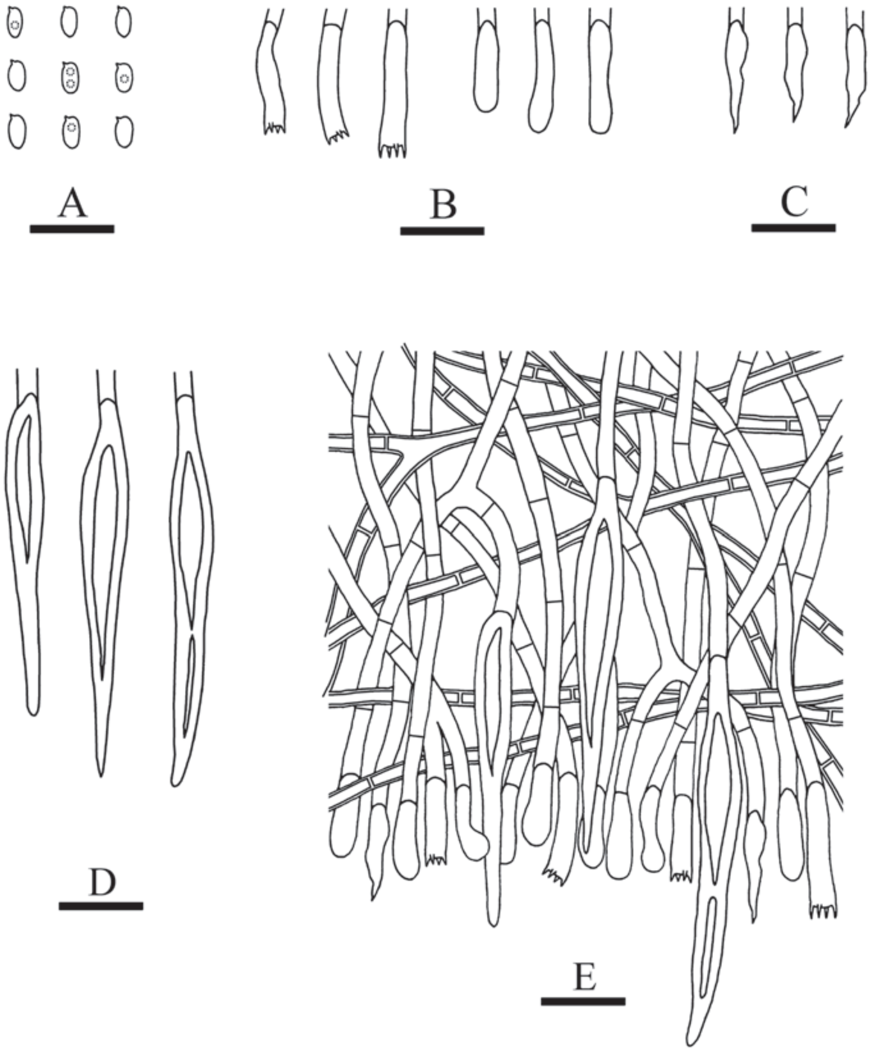

**Figure 7.** Microscopic structures of *Hymenochaete weishanensis* (holotype, CLZhao 22615). **A** Basidiospores **B** basidia and basidioles **C** tapering cystidia **D** setae **E** part of the vertical section of hymenium. Scale bars: 10 μm (**A–E**).

**Etymology.** *weishanensis* (Lat.), refers to the locality (Weishan) of the holotype.

**Basidiomata.** Annual, effused-reflexed, thin, coriaceous, without odor or taste when fresh, up to 5 cm long, 2.5 cm wide, and 150 μm thick. Pileal surface dark brown upon drying. Hymenial surface tuberculate, lightly brown when fresh, turning to gray brown upon drying. Sterile margin narrow, slightly gray-brown, up to 1 mm wide.

**Hyphal system.** Monomitic, generative hyphae with simple-septa, colorless, thin to slightly thick-walled, frequently branched, interwoven, 2.0–2.8 μm in diameter, IKI–, CB–; tissues unchanged in KOH.

**Hymenium.** Cystidia tapering, thin-walled, smooth, 9.5–20.5 × 2.0–3.5 μm; cystidioles absent. Hymenial setae abundant, subulate, reddish brown, thick-walled, smooth, 33.0–61.5 × 5.0–8.5 μm, projecting above the hymenium. Basidia subclavate, colorless, thin-walled, simple-septum, with four sterigmata, 6.5–24.0 × 2.5–4.0 μm; basidioles in shape similar to basidia, but slightly smaller.

**Basidiospores.** Elipsoid to narrow ellipsoid, colorless, thin-walled, smooth, with one or two guttate, IKI–, CB–, 4.0–5.0(–5.5) × 2.0–3.0 μm, L = 4.76 μm, W = 2.52 μm, Q = 1.86–1.92 (n = 60/2).

**Additional specimens examined (Paratype).** China • Yunnan Province, Dali, Weishan County, Leqiu Town, Zhongyao Village, on a fallen angiosperm branch, 19 July 2022, CLZhao 40297 (SWFC).

### ***Lyomyces albofarinaceus*** **Y.F. Dai & C.L. Zhao, sp. nov.**

MycoBank No: 856317
Figs 8, 9

**Holotype.** China • Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 28°05'N, 104°20'E, evel. 1600 m a.s.l., on a fallen branch of angiosperm, leg. C.L. Zhao, 20 September 2023, CLZhao 33479 (SWFC).

**Etymology.** *albofarinaceus* (Lat.), refers to the white and pruinose hymenophore surface.

**Basidiomata.** Annual, resupinate, adnate, without odor or taste when fresh, up to 8 cm long, 3.5 cm wide, and 150 μm thick. Hymenial surface pruinose, smooth, white when fresh, to white to cream upon drying. Sterile margin narrow, white, up to 1 mm wide.

**Hyphal system.** Monomitic, generative hyphae with clamp connections, colorless, thin to slightly thick-walled, frequently branched, interwoven, 2.0–3.0 μm in diameter, IKI–, CB–; tissues unchanged in KOH, subhymenial hyphae densely covered by crystals.

**Hymenium.** Cystidia of two types: (1) tapering, thin-walled, smooth to be covered by crystals, 11.5–44.0 × 4.5–7.5 μm; (2) capitate, thin-walled, smooth to be covered by crystals, slightly constricted at the neck, with a globose tip, 23.5–40.0 × 3.5–5.5 μm; cystidioles absent. Basidia clavate, colorless, thin-walled, with four sterigmata, 15.0–27.0 × 5.0–9.5 μm; basidioles in shape similar to basidia, but slightly smaller.

**Basidiospores.** Broadly ellipsoid, colorless, thin-walled, smooth, with one guttate, IKI–, CB–, (5.5–)6.0–7.0(–7.5) × (4.5–)5.0–6.0(–6.5) μm, L = 6.46 μm, W = 5.62 μm, Q = 1.07–1.15 (n = 60/2).

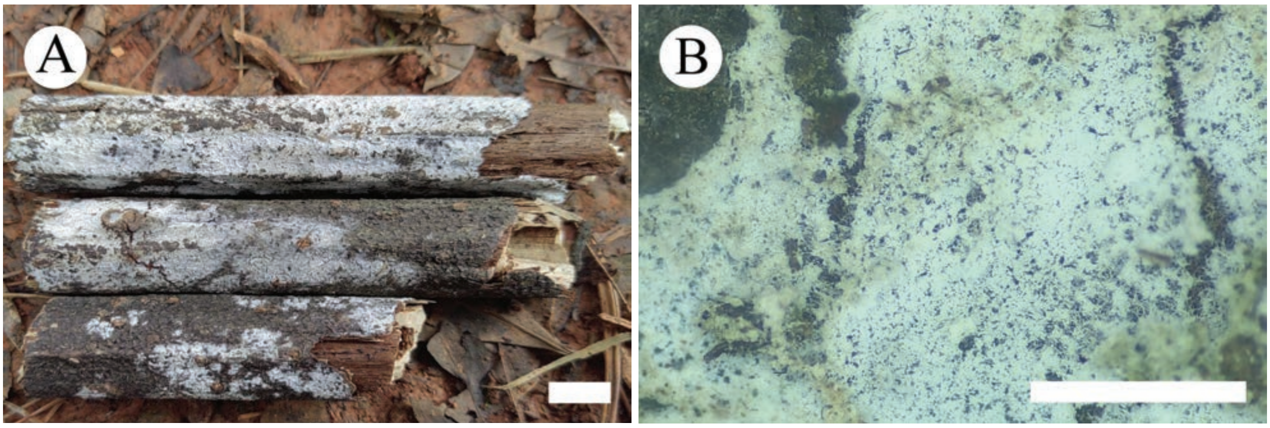

Figure 8. Basidiomata of *Lyomyces albofarinaceus* in general and detailed views (CLZhao 33479, holotype). Scale bars: 1cm (**A**); 1mm (**B**).

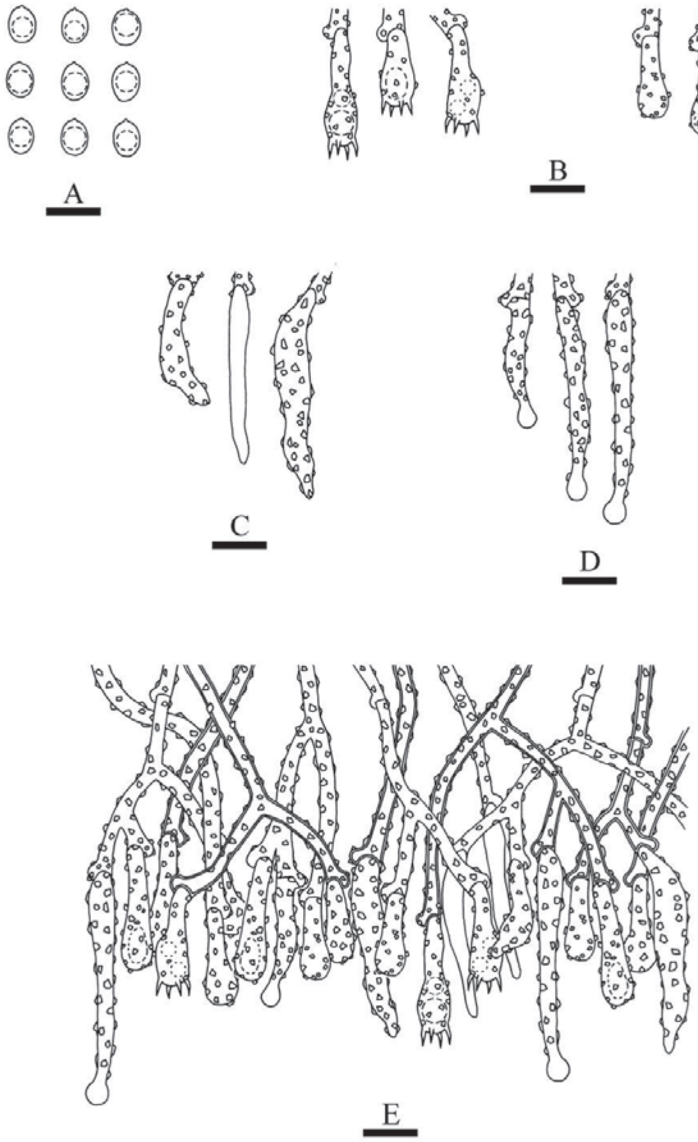

Figure 9. Microscopic structures of *Lyomyces albofarinaceus* (holotype, CLZhao 33479). **A** Basidiospores **B** basidia and basidioles **C** tapering cystidia **D** Capitate cystidia **E** part of the vertical section of hymenium. Scale bars: 10 μm (**A–E**).

**Additional specimens examined (Paratype).** China • Yunnan Province, Qujing, Qilin District, Cuishan Forestry Park, on a fallen angiosperm branch, 5 November 2022, CLZhao 26661 (SWFC).

***Lyomyces albomarginatus* Y.F. Dai & C.L. Zhao, sp. nov.**
MycoBank No: 856737
Figs 10, 11

**Holotype.** China • Yunnan Province, Dali, Weishan County, QinghuaTown, Green Peacock Reserve, Jiangzui Village, GPS coordinates 25°01'N, 100°11'E, evel. 1500 m a.s.l., on a fallen branch of angiosperm, leg. C.L. Zhao, 18 July 2022, CLZhao 22551 (SWFC).

**Etymology.** *albomarginatus* (Lat.), refers to the white margin of the basidiomata.

**Basidiomata.** Annual, resupinate, adnate, without odor or taste when fresh, up to 8 cm long, 3 cm wide, and 150 μm thick. Hymenial surface cracked, slightly buff when fresh, turning to buff to slightly yellowish upon drying. Sterile margin slightly buff, up to 3 mm wide.

**Hyphal system.** Monomitic, generative hyphae with clamp connections, colorless, thin to slightly thick-walled, rarely branched, interwoven, 2.0–3.5 μm in diameter, IKI–, CB–; tissues unchanged in KOH.

**Hymenium.** Cystidia numerous, tapering, thin-walled, smooth, 22.5–30.0 × 2.0–4.0 μm; cystidioles absent. Basidia cylindrical, colorless, thin-walled, with four sterigmata, 15.0–19.0 × 3.5–4.3 μm; basidioles in shape similar to basidia, but slightly smaller.

**Basidiospores.** Elliposoid, colorless, thin-walled, smooth, with one guttate, IKI–, CB–, (3.5–)4.0–5.5(–6.0) × (2.5–)2.7–3.5(–3.7) μm, L = 4.89 μm, W = 3.13 μm, Q = 1.56 (n = 30/1).

***Tubulicrinis albobadius* Y.F. Dai & C.L. Zhao, sp. nov.**
MycoBank No: 856318
Figs 12, 13

**Holotype.** China • Yunnan Province, Qujing, Qilin District, Cuishan Forest Park, GPS coordinates 25°32'N, 103°42'E, evel. 2250 m a.s.l., on a fallen branch of angiosperm, leg. C.L. Zhao, 5 Novermber 2022, CLZhao 26202 (SWFC).

**Etymology.** *albobadius* (Lat.), refers to the white basidiomata.

**Basidiomata.** Annual, resupinate, thin, adnate, without odor or taste when fresh, up to 8 cm long, 1.5 cm wide, and 150 μm thick. Hymenial surface arachnoid, white when fresh and became white to gray when drying. Sterile margin narrow, white, up to 1 mm.

**Hyphal system.** Monomitic, generative hyphae with clamp connections, colorless, thick-walled, branched, interwoven, 2.0–4.0 μm in diameter, IKI–, CB–; tissues unchanged in KOH.

**Hymenium.** Cystidia and cystidioles absent. Lyocystidia projecting, thick-walled, with a globose tip, some of the globose tips are thin-walled, 38.0–71.0 × 8.3–10.0 μm. Basidia barred, colorless, thin-walled, with four sterigmata, 9.5–14.0 × 4.0–5.0 μm; basidioles in shape similar to basidia, but slightly smaller.

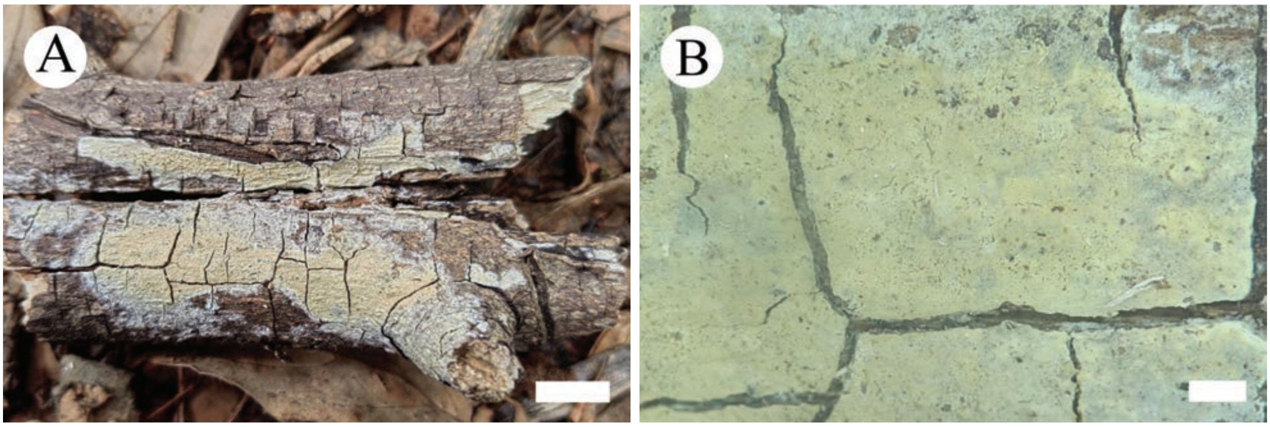

**Figure 10.** Basidiomata of *Lyomyces albomarginatus* in general and detailed views (CLZhao 22551, holotype). Scale bars: 1cm (**A**); 1mm (**B**).

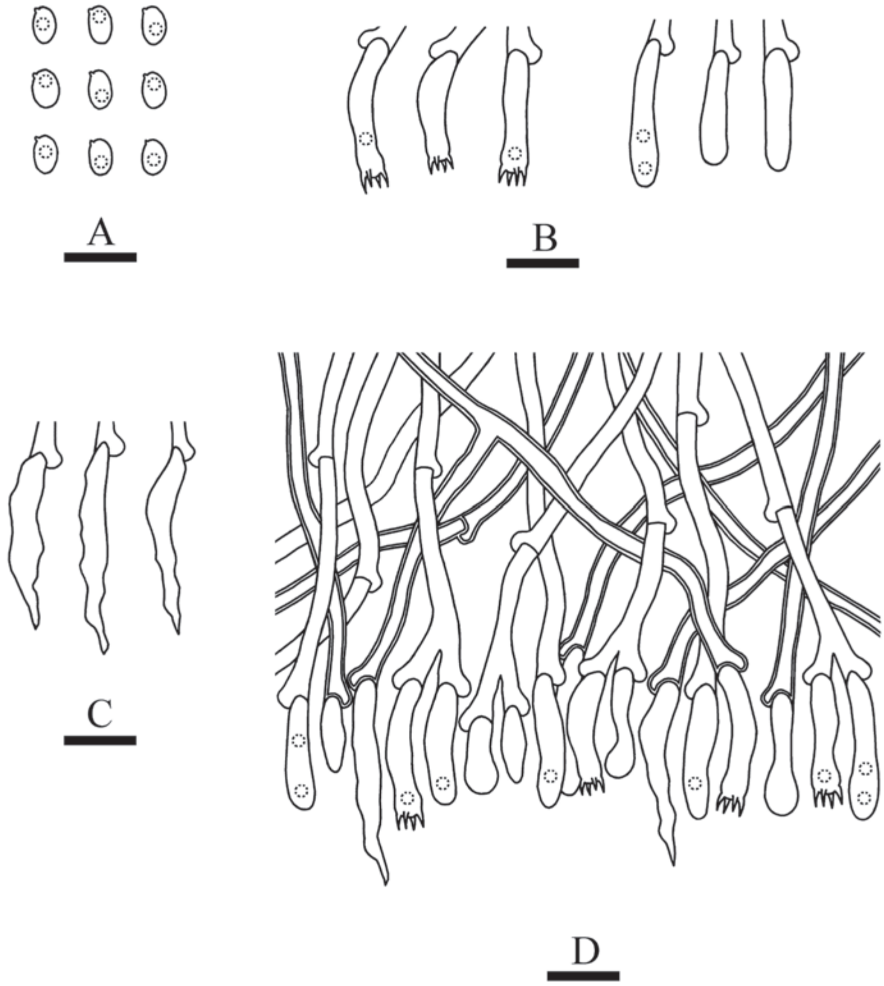

**Figure 11.** Microscopic structures of *Lyomyces albomarginatus* (holotype, CLZhao 22551). **A** Basidiospores **B** basidia and basidioles **C** tapering cystidia **D** part of the vertical section of hymenium. Scale bars: 10 μm (**A–D**).

**Basidiospores.** Cylindrical to allantoid, colorless, thin-walled, smooth, with one or two guttate, IKI–, CB–, (3.5–)4.0–6.0(–6.5) × 1.5–2.2(–2.5) μm, L = 5.09 μm, W = 1.89 μm, Q = 2.55–2.77 (n = 60/2).

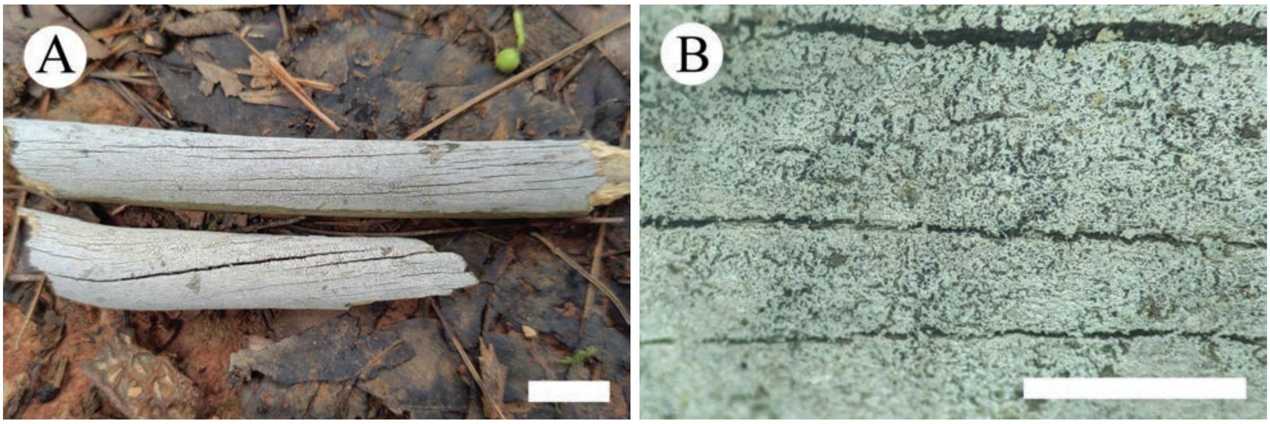

**Figure 12.** Basidiomata of *Tubulicrinis albobadius* in general and detailed views (CLZhao 26202, holotype). Scale bars: 1cm (**A**); 1mm (**B**).

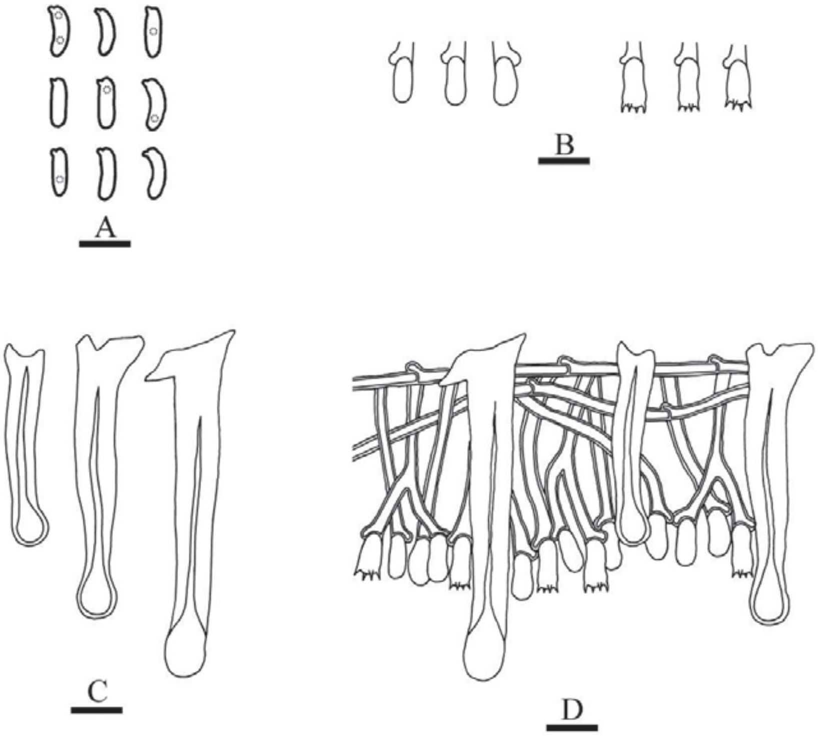

**Figure 13.** Microscopic structures of *Tubulicrinis albobadius* (holotype, CLZhao 26202). **A** Basidiospores **B** basidia and basidioles **C** lyocystidia **D** part of the vertical section of hymenium. Scale bars: 5 µm (**A**); 10 µm (**B–D**).

**Additional specimens examined (Paratype).** China • Qujing, Qilin District, Cuishan Forest Park, on a fallen angiosperm branch, 5 November 2022, CLZhao 26330 (SWFC).

***Xylodon musicola*** **Y.F. Dai & C.L. Zhao, sp. nov.**
MycoBank No: 856319
Figs 14, 15

**Holotype.** China • Yunnan Province, Zhaotong, Yongshan County, Mugan Town, Wumengshan Nature Reserve, GPS coordinates 28°05'N, 103°58'E, evel.

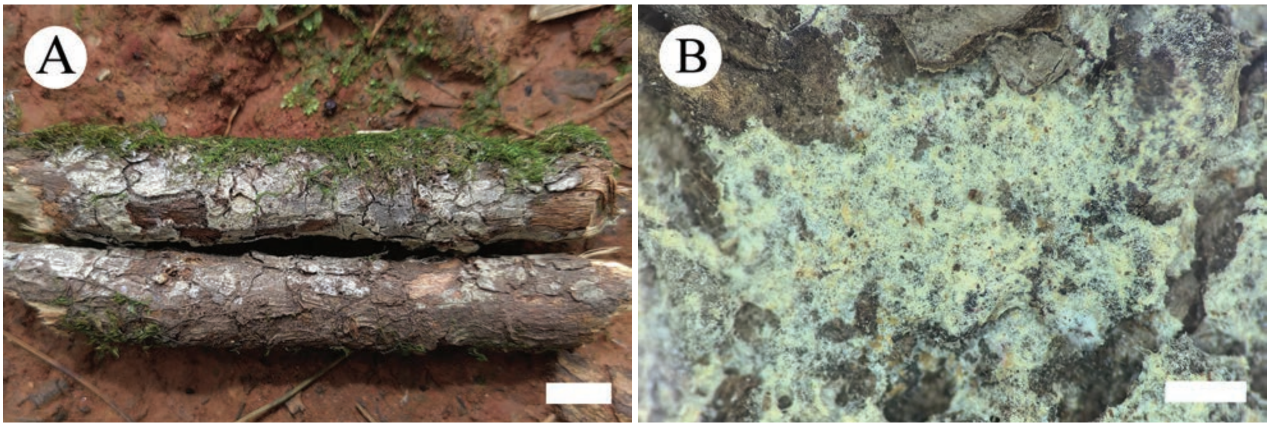

**Figure 14.** Basidiomata of *Xylodon musicola* in general and detailed views (CLZhao 35567, holotype). Scale bars: 1cm (**A**); 1mm (**B**).

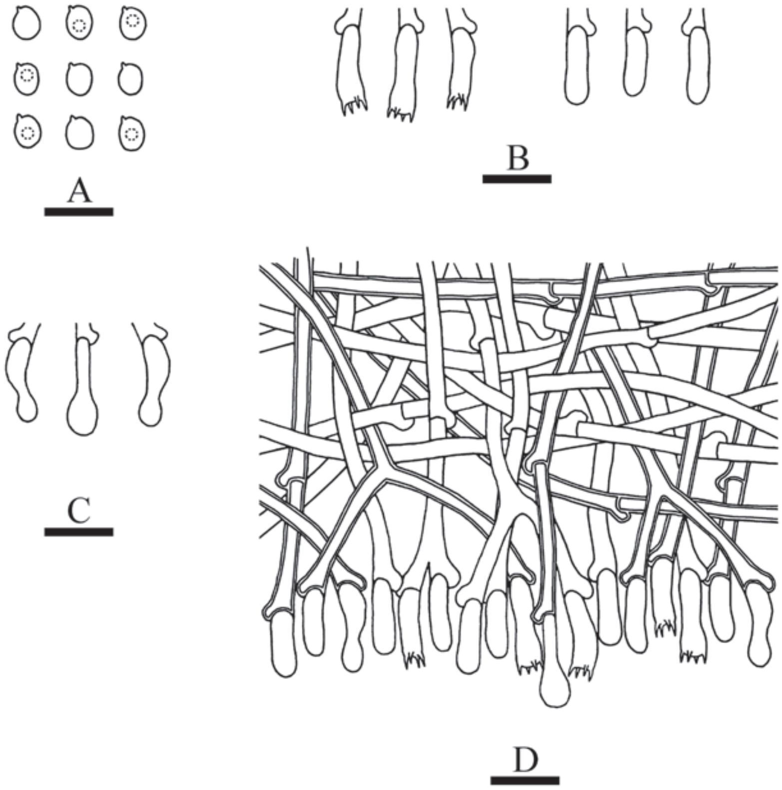

**Figure 15.** Microscopic structures of *Xylodon musicola* (holotype, CLZhao 35567). **A** Basidiospores **B** basidia and basidioles **C** capitate cystidia **D** part of the vertical section of hymenium. Scale bars: 10 μm (**A–D**).

2200 m a.s.l., on a fallen angiosperm branch, leg. C.L. Zhao, 7 November 2023, CLZhao 35567 (SWFC).

**Etymology.** *musicola* (Lat.), refers to the growth on the mosses, which is located Bryophyta.

**Basidiomata.** Annual, resupinate, adnate, very difficult to separate from substrate, without odor or taste when fresh, up to 7 cm long, 2 cm wide, and 150 µm thick. Hymenial surface arachnoid, white when fresh and becoming white to cream when drying. Sterile margin narrow, slightly cream, up to 1 mm wide. The basidiomata grow on the surface of muscus.

**Hyphal system.** Monomitic, generative hyphae with clamp connections, colorless, thin to slightly thick-walled, rarely branched, interwoven, 2.5–4.0 µm in diam, IKI–, CB–; tissues unchanged in KOH.

**Hymenium.** Cystidia capitate, thin-walled, smooth, slightly constricted at the neck, with a globose tip, 12.5–20.0 × 3.5–5.0 µm; cystidioles absent. Basidia clavate, colorless, thin-walled, with four sterigmata, 11.0–15.5 × 3.5–5.0 µm; basidioles in shape similar to basidia, but slightly smaller.

**Basidiospores.** Broadly ellipsoid to globe, colorless, thin-walled, smooth, with one guttate, IKI–, CB–, 4.0–5.5(–6.0) × 3.5–5.0(–5.5) µm, L = 4.77 µm, W = 4.35 µm, Q = 1.07–1.13 (n = 60/2).

**Additional specimens examined (Paratype).** China • Yunnan Province, Zhaotong, Yongshan County, Mugan Town, Wumengshan Nature Reserve, on a fallen angiosperm branch, 7 November 2023, CLZhao 40298 (SWFC).

## Discussion

The order Hymenochaetales comprises many representative corticioid fungal taxa, including hydnoid, corticioid, and polyporoid fungi possessing basidiomata with diverse hymenophoral and cystidial morphology (Riebesehl et al. 2019; Wu et al. 2022; Guan et al. 2023; Zhang et al. 2024). In the present study, five new species *Hymenochaete weishanensis*, *Lyomyces albofarinaceus*, *L. albomarginatus*, *Tubulicrinis albobadius*, and *Xylodon musicola* are described based on the phylogenetic analyses and morphological characteristics.

Based on ITS topology (Fig. 2), *Hymenochaete weishanensis* grouped closely with two species *H. luteobadia* and *H. anomala*. However, *H. anomala* can be delimited from *H. weishanensis* by its smooth hymenial surface and narrower basidiospores (3.5–4.5 × 1.8–2.3 µm; Parmasto 2001). *Hymenochaete Luteobadia* differs from *H. weishanensis* due to its sulcate and zonate hymenial surface and wider basidia (15.0–20.0 × 4.0–5.0 µm; Parmasto 2001). Based on ITS topology (Fig. 3), the taxon *Lyomyces albofarinaceus* grouped closely with *L. albopulverulentus* and *L. qujingensis*. The taxon *L. albomarginatus* was sister to *L. crustosus*. However, *L. qujingensis* differs from *L. albofarinaceus* due to its longer capitate cystidia (40.0–60.0 × 4.0–7.5 µm) and by possessing clavate cystidia (16.5–18.0 × 7.5–8.5 µm; Dong et al. 2024). *L. albopulverulentus* can be delimited from *L. albofarinaceus* by its longer basidiospores (8.0–10.5 × 5.5–7.0 µm; Guan et al. 2023). *Lyomyces crustosus* can be delimited from *L. albomarginatus* due to its odontoid hymenial surface and longer basidia (20–30 × 4.0–5.0 µm; Maekawa 1994). Based on ITS topology (Fig. 4), the taxon *Tubulicrinis albobadius* grouped closely with *T. australis* and *T. inornatus*. However, *T. inornatus* differs from *T. albobadius* by its reticulate to porulose hymenial surface and wider basidiospores (4.0–5.0 × 2.5–3.5 µm; Maekawa 2021). Based on ITS topology (Fig. 5), the taxon *Xylodon musicola* grouped closely with *X. gloeocystidiifer*, *X. hydnoides* and *X. neotropicus*. However, morphologically, *X. gloeocystidiifer* differs from *X. musicola* by its odontioid hymenial surface and smaller basidiospores (3.5–4.0 × 2.8–3.5 µm; Yurchenko et al. 2024b), *X. hydnoides* differs from *X. musicola* by its hydnoid hymenial surface and by possessing clavate cystidia (29.5–38.5 × 3.5–4.5 µm; Dong et al. 2024). *Xylodon neotropicus* can be delimited from *X.*

*musicola* due to its odontioid to short hydnoid hymenial surface and shorter basidiospores (3.5–4.0 × 3.3–3.7 µm; Yurchenko et al. 2024b).

Morphologically, *Hymenochaete weishanensis* resembles *H. colliculosa* (Sacc.) Parmasto, *H. biformisetosa* Jiao Yang & S.H. He and *H. sharmae* Hembrom, K. Das & A. Parihar by ellipsoid basidiospores. However, *H. colliculosa* differs from *H. weishanensis* due to its dimitic hyphal system and wider basidia (20.0–24.0 × 5.0–6.0 µm; He et al. 2017). The species *H. biformisetosa* differs from *H. weishanensis* by its smooth hymenial surface and wider basidiospores (4.3–6.0 × 3.0–4.2 µm; Yang and He 2014). The taxon *H. sharmae* differs from *H. weishanensis* by its smooth hymenial surface and wider basidia (12.0–16.0 × 4.0–6.0 µm; Wang et al. 2019)

Morphologically, *Lyomyces albofarinaceus* resembles *L. incanus* J.H. Dong & C.L. Zhao, *Lyomyces lancangjiangensis* Q. Li & C.L. Zhao, and *L. yunnanensis* C.L. Zhao by ellipsoid basidiospores. However, *L. lancangjiangensis* differs from *L. albofarinaceus* due to its membranaceous hymenial surface and narrower basidia (13.0–23.0 × 3.0–4.5 µm; Li et al. 2024b). The taxon *L. incanus* differs from *L. albofarinaceus* by its furfuraceous hymenial surface and narrower basidiospores (5.0–6.5 × 4.0–5.0 µm; Dong et al. 2024). The species *L. yunnanensis* differs from *L. albofarinaceus* by its grandinioid hymenial surface and narrower basidiospores (5.0–7.0 × 3.0–4.5 µm; Guan et al. 2023).

Morphologically, *Lyomyces albomarginatus* resembles *L. lincangensis* J.H. Dong & C.L. Zhao, *L. luteoalbus* J.H. Dong & C.L. Zhao and *L. sinensis* J.H. Dong & C.L. Zhao by ellipsoid basidiospores. However, *L. lincangensis* differs from *L. albomarginatus* due to its coriaceous hymenial surface and wider basidiospores (4.5–6.5 × 3.5–5.0 µm; Dong et al. 2024). *L. luteoalbus* differs from *L. albomarginatus* due to its membranaceous hymenial surface and shorter tapering cystidia (12.0–17.0 × 2.0–3.5 µm; Dong et al. 2024). *L. sinensis* differs from *L. albomarginatus* due to its coriaceous hymenial surface and wider basidiospores (4.5–6.0 × 3.5–4.5 µm; Dong et al. 2024).

Morphologically, *Tubulicrinis albobadius* resembles *T. hirtellus* (Bourd. & Galz.) John Erikss, *T. orientalis* Parmasto and *T. xantha* C.L. Zhao by cylindrical basidiospores. However, *T. hirtellus* differs from *T. albobadius* due to its porulose hymenial surface and longer basidiospores (7.0–8.5 × 2.0–2.5 µm; Hjortstam et al. 1988). The species *T. orientalis* differs from *T. albobadius* due to its hispidulous hymenial surface and narrower basidia (11.0–16.0 × 3.5–4.0 µm; Maekawa and Nordén 2022). The species *T. xantha* differs from *T. albobadius* by its furfuraceous hymenial surface and longer and narrower lyocystidia (78.0–192.5 × 5.8–7.5 µm; He et al. 2020).

Morphologically, *Xylodon musicola* resembles *X. cremeoparinaceus* Q. Yuan & C.L. Zhao, *X. luteodontioides* Q. Yuan & C.L. Zhao and *X. wumengshanensis* Q. Yuan & C.L. Zhao by ellipsoid basidiospores. However, *X. cremeoparinaceus* differs from *X. musicola* due to its farinaceous hymenial surface and narrower basidiospores (3.5–4.5 × 2.5–3.5 µm; Yuan and Zhao 2024). The species *X. luteodontioides* differs from *X. musicola* due to narrower basidiospores (3.5–4.5 × 2.5–3.5 µm) and by possessing schizopapillate cystidia (29.5–37.0 × 2.5–3.5 µm; Yuan and Zhao 2024). The species *X. wumengshanensis* differs from *X. musicola* due to its bigger basidia (22.5–33.0 × 5.0–5.5 µm) and by possessing fusoid cystidia (14.5–22.0 × 5.5–6.5 µm; Yuan and Zhao 2024).

This discovery of five new species *viz*. *Hymenochaete weishanensis*, *Lyomyces albofarinaceus*, *L. albomarginatus*, *Tubulicrinis albobadius*, and *Xylodon musicola* enrich our knowledge of fungal diversity in the order Hymenochaetales. We anticipate that more undescribed taxa will be discovered throughout China after extensive collection combined with morphological and molecular analyses.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization, CZ; methodology, CZ, HY and YD; software, CZ, YD and QY; validation, CZ and YD; formal analysis, CZ and YD; investigation, RL, DL, CZ and YD; resources CZ, RL and HY; writing – original draft preparation, CZ, YD, QY, XY and RL; writing – review and editing, CZ, HY, and YD; visualization, CZ and YD; supervision, CZ and HY; project administration, CZ; funding acquisition, CZ and HY. All authors have read and agreed to the published version of the manuscript.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# Morphological characteristics and phylogenetic analyses revealed five new species (Basidiomycota) from Southwestern China

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## Abstract

In the present study, five new wood-inhabiting fungal species, *Conferticium albocremeum* (Stereaceae, Russulales), *Dendrocorticiopsis luteoalba* (Punctulariaceae, Corticiales), *Eichleriella biluoxueshanensis* (Auriculariaceae, Auriculariales), *Gloeohypochnicium yunnanense* (Incertae sedis, Russulales), and *Punctularia nigrodontea* (Punctulariaceae, Corticiales), collected from southern China, are proposed based on a combination of morphological features and phylogenetic evidence. *Conferticium albocremeum* is characterized by membranaceous and smooth basidiomata with white to cream surface and ellipsoid and verrucose basidiospores (9–11 × 5–7 µm); *Dendrocorticiopsis luteoalba* is characterized white to buff, membranaceous basidiomata and ellipsoid, thin-walled and smooth basidiospores (7–8 × 4.5–5.5 µm); *Eichleriella biluoxueshanensis* is characterized by coriaceous and grandinioid basidiomata with buff to cinnamon-buff hymenophore and allantoid, thin-walled basidiospores (13.5–17.5 × 7–9 µm); *Gloeohypochnicium yunnanense* is characterized by buff to cream, coriaceous and smooth basidiomata and globose, thick-walled and warted basidiospores (10–12 × 10–11.5 µm), and *Punctularia nigrodontea* is characterized by resupinate to effused-reflexed basidiomata and ellipsoid, thin-walled and smooth basidiospores (8.5–10 × 5–6 µm). Sequences of the internal transcribed spacers (ITS) and the large subunit (nLSU) of the nuclear ribosomal DNA (rDNA) markers of the studied samples were generated. Phylogenetic analyses performed based on the ITS+nLSU rDNA gene regions with the maximum likelihood, maximum parsimony, and Bayesian inference methods revealed that five new species belong to the genera *Conferticium*, *Dendrocorticiopsis*, *Eichleriella*, *Gloeohypochnicium*, and *Punctularia*. Descriptions, illustrations, phylogenetic analysis results, and a comparison with closely related taxa of the five new species are provided.

**Key words:** Biodiversity, molecular systematics, new taxa, taxonomy, wood-inhabiting fungi, Yunnan Province

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## Introduction

The kingdom of fungi is one of the most diverse groups of living organisms on earth; its members occur across a broad range of ecosystems, including extreme environments, with an estimated number of species in the range of 2–3 million (Bhunjun et al. 2022, 2024; Wijayawardene et al. 2022, 2024; Dong et al. 2024a; Hyde et al. 2024a, b). Based on molecular phylogenetic evidence, numerous new taxa have been discovered in the past ten years (Wang and Cai 2023; Yuan et al. 2023; Zhao et al. 2023; Deng et al. 2024b; Dong et al. 2024b; He et al. 2024; Qin et al. 2024).

The genus *Conferticium* Hallenb. (Stereaceae, Russulales) was erected in 1980 and typified by *C. insidiosum* (Bourdot & Galzin) Hallenb., which is characterized by the resupinate basidiomes with membranaceous to ceraceous, smooth to tuberculate hymenophore, a monomitic simple-septate hyphae, and the numerous cylindrical, sinuous gloeocystidia (Bernicchia and Gorjón 2010). Based on the MycoBank database (http://www.mycobank.org, accessed on 27 January 2025) and the Index Fungorum (http://www.indexfungorum.org, accessed on 27 January 2025), six specific and infraspecific names were registered in *Conferticium*, and it is a small genus only including five widely recognized species up to now.

The genus *Dendrocorticiopsis* Sheng H. Wu et al. (Punctulariaceae, Corticiales) was introduced by Sheng H. Wu, with the description of one species, *D. orientalis* Sheng H. Wu et al. (Wei et al. 2022). It is conventionally defined by having strictly resupinate basidiomata, an ivory hymenophore, a monomitic hyphal system with clamped hyphae, encrusted cystidia, dendrohyphidia, and ellipsoid to ovoid basidiospores (Wei et al. 2022). Based on the MycoBank database (http://www.mycobank.org, accessed on 27 January 2025) and the Index Fungorum (http://www.indexfungorum.org, accessed on 27 January 2025), *Dendrocorticiopsis* is a monotypic genus.

The genus *Eichleriella* Bres. was introduced in 1903, typified by *E. incarnata* Bres., and it is a species-rich genus that belongs to Auriculariaceae (Auriculariales). The genus is characterized by annual or short-living perennial, leathery to ceraceous basidiomata with smooth, pale-colored hymenophore (in some species covered by spines), a monomitic to dimitic hyphal system with clamped genitive hyphae, cystidia often present, longitudinally septate basidia with 2- or 4-celled, and colorless, cylindrical to narrowly cylindrical basidiospores (Malysheva and Spirin 2017; Li et al. 2023; Deng et al. 2024a). Based on the MycoBank database (http://www.mycobank.org, accessed on 27 January 2025) and the Index Fungorum (http://www.indexfungorum.org, accessed on 27 January 2025), the genus *Eichleriella* has 32 specific and registered names, with 22 species accepted worldwide (Malysheva and Spirin 2017, Liu et al. 2019, Li et al. 2023; Deng et al. 2024a).

The genus *Gloeohypochnicium* (Parmasto) Hjortstam (Russulales), typified by *G. analogum* (Bourdot & Galzin) Hjortstam (Bernicchia and Gorjón 2010), is characterized by the resupinate basidiomes with smooth to tuberculate hymenophore, a monomitic hyphal system with clamps on generative hyphae, the numerous cylindrical, sinuous gloeocystidia, and globose to ellipsoid, thick-walled, warted basidiospores (Bernicchia and Gorjón 2010). Based on the MycoBank database (http://www.mycobank.org, accessed on 27 January 2025) and the Index Fungorum (http://www.indexfungorum.org, accessed on 27 January 2025), *Gloeohypochnicium* has registered two specific and infraspecific names, and it is a small genus only including two widely recognized species so far (Bernicchia and Gorjón 2010; He et al. 2024).

*Punctularia* Patouillard (Punctulariaceae, Corticiales) was typified with *P. tuberculosa* (Pat.) Pat. & Lagerh. (current name *P. atropurpurascens* (Berk. & Broome) Petch, which is characterized by resupinate to effused-reflexed basidiomata, gelatinous when fresh, rigid upon drying, tuberculate or radial ridges hymenophore, a monomitic hyphal system with clamped generative hyphae, yellowish to brown dendrohyphidia, and thin-walled, smooth, ellipsoid, acyanophilous basidiospores (Bernicchia and Gorjón 2010). Based on the MycoBank database (http://www.mycobank.org, accessed on 27 January 2025) and the Index Fungorum (http://www.indexfungorum.org, accessed on 27 January 2025), *Punctularia* has registered six specific and infraspecific names, and three species have been recognized worldwide up to now (Bernicchia and Gorjón 2010; Guan et al. 2021; He et al. 2024).

Molecular phylogenetic approaches have revolutionized the fungal taxonomy of Basidiomycota in the last decades, and these advances have greatly enhanced our knowledge of species diversity in Basidiomycota (Lücking et al. 2021; He et al. 2022; Wang et al. 2023; Dong et al. 2024a). The family Auriculariaceae is the largest and best-supported clade in the order Auriculariales and consists of a large group of wood-decaying fungi with varied basidiomes (Dong et al. 2024b; He et al. 2024). Corticioid and stereoid taxa are numerous in Auriculariaceae and are typically classified into three main genera: *Eichleriella* Bres., *Exidiopsis* (Bref.) A. Møller, and *Heterochaete* Pat. (Malysheva and Spirin 2017; Li et al. 2023; Deng et al. 2024a). DNA sequence-based classification and identification of the genus *Eichleriella* (Auriculariaceae) have reported that six species have been described from China recently (Li et al. 2023; Deng et al. 2024a). Corticiales K.H. Larss. is a small order of corticioid fungi with four families *viz*. Corticiaceae Herter, Dendrominiaceae Ghobad-Nejhad, Punctulariaceae Donk, and Vuilleminiaceae Maire ex Lotsy (Wei et al. 2022; He et al. 2024). Most of the previous studies of Punctulariaceae focused on European species (Bernicchia and Gorjón 2010; Gorjón and Bernicchia 2017). In China, the research on this family mainly focuses on the genera *Dendrocorticiopsis* and *Punctularia*; some new taxa have been proposed based on a combination of morphological features and molecular data (Guan et al. 2021; Wei et al. 2022; He et al. 2024). Members of the Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David exhibit diverse basidiome structures, which can range from agaricoid to discoid, clavarioid, polyporoid, corticoid, and even gasteroid, and the multigene phylogenetic analyses clarify the evolutionary relationships of some small genera in the latest study (Yuan et al. 2021; He et al. 2024). According to recent research in molecular systematics, the genus *Conferticium* (Stereaceae, Russulales) has reported one new species, *C. fissuratum* Xin Yang & C.L. Zhao from Yunnan Province (Bernicchia and Gorjón 2010; Shen et al. 2024). The taxonomic status of the genus *Gloeohypochnicium* is unclear, and it was only placed in the order Russulales, and it is a mystery genus; no new taxa in the genus have been described for nearly a decade (He et al. 2022, 2024). However, the phylogeny of the genus *Gloeohypochnicium* is ambiguous due to a lack of molecular evidence and morphological data.

In this paper, we presented the morphological characteristics and multigene molecular analyses with ITS and nLSU DNA markers to support the taxonomy and phylogenetic position of five new species.

## Materials and methods

### Sample collection and herbarium specimen preparation

Fresh basidiomata growing on angiosperm branches were collected from Dehong, Diqing, Tengchong, and Xishuangbanna of Yunnan Province, P.R. China. The samples were photographed *in situ*, and fresh macroscopic details were recorded. Photographs were recorded using a Jianeng 80D camera (Tokyo, Japan). Specimens were dried in an electric food dehydrator at 45 °C (Hu et al. 2022), then the specimens were sealed in an envelope and zip-lock plastic bags and labeled (Zhao et al. 2023). The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

### Morphology

The macromorphological descriptions were based on field notes and photos captured in the field and lab. The color terminology follows Petersen (1996). The micromorphological data were obtained from the dried specimens observed under a Nikon Eclipse E100 light microscope following Zhao and Wu (2017). The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB– = acyanophilous, IKI+ = amyloid, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and n = a/b (number of spores (a) measured from given number (b) of specimens).

### DNA extraction, PCR, and sequencing

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from the dried specimens according to the manufacturer's instructions. The nuclear ribosomal internal transcribed spacer (ITS) region was amplified with the primer pair ITS5/ITS4 (White et al. 1990). The nuclear large subunit (nLSU) region with the primer pair LR0R/LR7 (Vilgalys and Hester 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, P.R. China). All newly generated sequences were deposited in GenBank (Table 1).

### Molecular phylogeny

The sequences were aligned in MAFFT version 7 using the G-INS-i strategy (Katoh et al. 2019). The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). (1) *Hyphoderma cremeoalbum* (Höhn. & Litsch.) Jülich was assigned as an outgroup to root trees in the ITS+nLSU analysis (Fig. 1) (Dong et al. 2024a); (2) *Vararia fissurata* Y.L. Deng & C.L. Zhao were

**Table 1.** Names, voucher numbers, references, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses. [* indicates type materials; – indicates sequence unavailability].

| Taxa | Voucher no. | Locality | GenBank accession no. | | References |
|---|---|---|---|---|---|
| | | | ITS | nLSU | |
| *Acanthobasidium bambusicola* | He2357 | China | KU559343 | KU574833 | Tian et al. 2018 |
| *Acanthobasidium phragmitis* | CBS 233.86 | France | – | AY039305 | Wu et al. 2001 |
| *Acanthophysium bisporum* | T614 | USA | – | AY039327 | Maekawa et al. 2023 |
| *Acanthophysium lividocaeruleum* | FP-100292 | USA | – | AY039319 | Maekawa et al. 2023 |
| *Adustochaete punctata* | CLZhao 29675 | China | PP852052 | PP849035 | Dong et al. 2024b |
| *Aleurobotrys botryosus* | He2712 | China | KX306877 | KY450788 | Tian et al. 2018 |
| *Aleurodiscus bambusinus* | He4261 | China | KY706207 | KY706219 | Tian et al. 2018 |
| *Alloexidiopsis australiensis* | LWZ 20180514-18 | China | OM801934 | OM801919 | Liu et al. 2022 |
| *Alloexidiopsis calcea* | LWZ 20180904-14 | China | OM801935 | OM801920 | Liu et al. 2022 |
| *Alloexidiopsis grandinea* | CLZhao 33798 | China | PP852058 | – | Dong et al. 2024b |
| *Alloexidiopsis xantha* | CLZhao 25093 | China | PP852060 | PP849040 | Dong et al. 2024b |
| *Alloexidiopsis yunnanensis* | CLZhao 8106 | China | MT215569 | MT215565 | Guan et al. 2020 |
| *Amphistereum leveilleanum* | FP-106715 | USA | KX262119 | KX262168 | Malysheva and Spirin 2017 |
| *Amphistereum schrenkii* | HHB 8476 | USA | KX262130 | KX262178 | Malysheva and Spirin 2017 |
| *Amylostereum chailletii* | NH 8031 | – | AF506406 | AF506406 | Larsson and Larsson 2003 |
| *Amylostereum laevigatum* | NH 12863 | – | AF506407 | AF506407 | Larsson and Larsson 2003 |
| *Aporpium caryae* | Miettinen 14774 | Finland | JX044145 | – | Miettinen et al. 2012 |
| *Aporpium caryae* | WD 2207 | Japan | AB871751 | AB871730 | Sotome et al. 2014 |
| *Artomyces niveus* | CLZhao 19094 | China | OR094479 | OR461459 | Dong et al. 2024a |
| *Artomyces yunnanensis* | CLZhao 7118 | China | OR094476 | OR461461 | Dong et al. 2024a |
| *Auricularia auricula-judae* | JT 04 | UK | KT152099 | KT152115 | Tohtirjap et al. 2023 |
| *Auricularia cornea* | Dai 13621 | China | MZ618936 | MZ669905 | Tohtirjap et al. 2023 |
| *Auricularia mesenterica* | FO 25132 | Germany | AF291271 | AF291292 | Weiß and Oberwinkler 2001 |
| *Auricularia polytricha* | TUFC 12920 | Japan | AB871752 | AB871733 | Sotome et al. 2014 |
| *Auricularia tibetica* | Dai 13336 | China | MZ618943 | MZ669915 | Tohtirjap et al. 2023 |
| *Australovuilleminia coccinea* | BCP5551 | New Zealand | HM046875 | HM046930 | Ghobad-Nejhad et al. 2010 |
| *Basidiodesertica hydei* | SQUCC 15289 | Oman | MW077150 | MW077159 | Wei et al. 2022 |
| *Bondarzewia occidentalis* | AFTOL-ID 452 | Canada | DQ200923 | DQ234539 | Zhou et al. 2021 |
| *Bondarzewia podocarpi* | Dai 9261 | China | KJ583207 | KJ583221 | Zhou et al. 2021 |
| *Conferticium albocremeum* | CLZhao 35693* | China | PQ197729 | PQ783842 | Present study |
| *Conferticium albocremeum* | CLZhao 36343 | China | PQ783832 | PQ783843 | Present study |
| *Conferticium albocremeum* | CLZhao 37176 | China | PQ783833 | PQ783844 | Present study |
| *Conferticium albocremeum* | CLZhao 39238 | China | PQ783834 | – | Present study |
| *Conferticium fissuratum* | CLZhao 34654 | China | PQ201856 | – | Shen et al. 2024 |
| *Conferticium fissuratum* | CLZhao 34662 | China | PQ201857 | – | Shen et al. 2024 |
| *Conferticium heimii* | CBS321.66 | African | AF506381 | AF506381 | Tian et al. 2018 |
| *Conferticium ochraceum* | CLZhao 21515 | China | ON211619 | – | Present study |

| Taxa | Voucher no. | Locality | GenBank accession no. | | References |
|---|---|---|---|---|---|
| | | | ITS | nLSU | |
| *Conferticium ochraceum* | G07_P24A | Switzerland | KT943933 | – | Stroheker et al. 2018 |
| *Conferticium ravum* | CBS:125849 | Estonia | MH863805 | MH875269 | Vu et al. 2019 |
| *Conferticium ravum* | NH13291 | USA | AF506382 | AF506382 | Larsson and Larsson 2003 |
| *Corticium roseum* | MG252 | China | MW805872 | MW805836 | Ghobad-Nejhad et al. 2021 |
| *Corticium thailandicum* | MG242 | Tailand | MW805868 | MW805831 | Ghobad-Nejhad et al. 2021 |
| *Cytidia salicina* | MG49 | Finland | GU590881 | HM046921 | Wei et al. 2022 |
| *Dendrocorticiopsis luteoalba* | CLZhao 30380* | China | PQ783828 | PQ783838 | Present study |
| *Dendrocorticiopsis orientalis* | He 4195 | China | MW580926 | MW580921 | Wei et al. 2022 |
| *Dendrocorticiopsis orientalis* | WEI 20-166 | China | MW580922 | MW580924 | Wei et al. 2022 |
| *Dendrocorticium polygonioides* | CBS:106.56 | France | MH857525 | MH869062 | Vu et al. 2019 |
| *Dendrocorticium roseocarneum* | KUC20121109-32 | Korea | KJ668559 | KJ668413 | Ghobad-Nejhad and Duhem 2014 |
| *Dendrominia dryina* | MG159 | France | JX892936 | JX892937 | Ghobad-Nejhad and Duhem 2014 |
| *Dendrominia ericae* | MG162 | France | JX892938 | JX892939 | Ghobad-Nejhad and Duhem 2014 |
| *Dentipellicula austroafricana* | Dai 12580 | Africa | KJ855274 | KJ855275 | Zhou et al. 2021 |
| *Dentipellicula taiwaniana* | Dai 10867 | China | JQ349115 | JQ349101 | Zhou et al. 2021 |
| *Dentipellopsis dacrydicola* | Dai 12004 | – | JQ349104 | JQ349089 | Zhou and Dai 2013 |
| *Dentipellopsis dacrydicola* | Dai 12010 | – | – | JQ349090 | Zhou and Dai 2013 |
| *Disporotrichum dimorphosporum* | CBS:419.70 | Netherland | MH859776 | MH871538 | Vu et al. 2019 |
| *Disporotrichum dimorphosporum* | CBS:433.85 | USA | MH861895 | MH873584 | Vu et al. 2019 |
| *Eichleriella alliciens* | He4055b | Thailand | MH178244 | – | Li et al. 2023 |
| *Eichleriella alliciens* | HHB 7194 | USA | KX262120 | KX262169 | Malysheva and Spirin 2017 |
| *Eichleriella alpina* | He 20120916-1 | China | MH178245 | MH178268 | Li et al. 2023 |
| *Eichleriella bactriana* | TAAM 104431 | Uzbekistan | KX262138 | KX262186 | Malysheva and Spirin 2017 |
| *Eichleriella bambusicola* | Dai 6391 | China | MH178246 | – | Li et al. 2023 |
| *Eichleriella biluoxueshanensis* | CLZhao 34516* | China | PQ783827 | PQ783837 | Present study |
| *Eichleriella biluoxueshanensis* | CLZhao 34793 | China | PQ787846 | – | Present study |
| *Eichleriella crocata* | He2969 | China | MH178248 | MH178271 | Li et al. 2023 |
| *Eichleriella crocata* | TAAM 101077 | Russia | KX262100 | KX262147 | Malysheva and Spirin 2017 |
| *Eichleriella delicata* | CLZhao 25143 | China | PQ757163 | – | Present study |
| *Eichleriella delicata* | He3469 | China | MH178250 | MH178273 | Li et al. 2023 |
| *Eichleriella desertorum* | LR 49350 | Namibia | KX262142 | KX262190 | Malysheva and Spirin 2017 |
| *Eichleriella discolor* | He4584 | China | MH178252 | MH178275 | Li et al. 2023 |
| *Eichleriella discolor* | He4763 | China | MH178253 | MH178276 | Li et al. 2023 |
| *Eichleriella flavida* | LR 49412 | UK | KX262137 | KX262185 | Malysheva and Spirin 2017 |
| *Eichleriella leucophaea* | LE 303261 | Russia | KX262111 | KX262161 | Malysheva and Spirin 2017 |

| Taxa | Voucher no. | Locality | GenBank accession no. | | References |
|---|---|---|---|---|---|
| | | | ITS | nLSU | |
| *Eichleriella macrospora* | He2189 | USA | MH178251 | MH178274 | Li et al. 2023 |
| *Eichleriella ochracea* | SP467242 | Brazil | MK391514 | – | Alvarenga et al. 2019 |
| *Eichleriella shearii* | LR 23258 | – | KX262139 | – | Malysheva and Spirin 2017 |
| *Eichleriella shearii* | USJ 54609 | Costa Rica | AF291284 | AF291335 | Weiß et al. 2001 |
| *Eichleriella sicca* | OM 17349 | USA | KX262143 | KX262191 | Malysheva and Spirin 2017 |
| *Eichleriella sinensis* | CLZhao 29368 | China | PQ757164 | – | Present study |
| *Eichleriella sinensis* | CLZhao 31647 | China | PQ757165 | PQ757166 | Present study |
| *Eichleriella sinensis* | He4196 | China | MH178254 | MH178277 | Li et al. 2023 |
| *Eichleriella tenuicula* | CLZhao 35797 | China | PQ197731 | – | Present study |
| *Eichleriella tenuicula* | He3483 | China | MH178256 | MH178279 | Li et al. 2023 |
| *Eichleriella xinpingensis* | CLZhao 836 | China | MK560879 | MK560883 | Liu et al. 2019 |
| *Eichleriella xinpingensis* | CLZhao 842 | China | MK560880 | MK560884 | Liu et al. 2019 |
| *Eichleriella yunnanensis* | CLZhao 31317 | China | PP889850 | PP897009 | Deng et al. 2024a |
| *Eichleriella yunnanensis* | CLZhao 31350 | China | PP889852 | PP897010 | Deng et al. 2024a |
| *Elmerina cladophora* | Miettinen 14314 | Indonesia | MG757509 | MG757509 | Malysheva et al. 2018 |
| *Elmerina sclerodontia* | Miettinen 16431 | Malaysia | MG757512 | MG757512 | Malysheva et al. 2018 |
| *Erythricium hypnophilum* | MG169 | France | MW805858 | MW805823 | Ghobad-Nejhad et al. 2021 |
| *Erythricium laetum* | MG72 | – | GU590875 | GU590878 | Ghobad-Nejhad et al. 2021 |
| *Exidia candida* | VS 3921 | Russia | KY801867 | KY801892 | Spirin et al. 2018 |
| *Exidia glandulosa* | MW 355 | Germany | AF291273 | AF291319 | Weiß et al. 2001 |
| *Exidia pithya* | MW 313 | Germany | AF291275 | AF291321 | Weiß et al. 2001 |
| *Exidia reflexa* | Dai 20833 | China | MN850386 | MN850362 | Ye et al. 2020 |
| *Exidia subglandulosa* | Wu 270 | China | MN850381 | MN850357 | Ye et al. 2020 |
| *Exidiopsis effusa* | OM 19136 | Finland | KX262145 | KX262193 | Malysheva and Spirin 2017 |
| *Gloeocystidiellum bisporum* | KHL11135 | Norway | AY048877 | AY048877 | Larsson and Larsson 2003 |
| *Gloeocystidiellum clavuligerum* | FCUG2731 | Russia | AF310083 | AF310083 | Larsson and Larsson 2003 |
| *Gloeodontia eriobotryae* | Dai 12080 | – | JQ349116 | JQ349103 | Zhou and Dai 2013 |
| *Gloeodontia pyramidata* | LR15502 | – | AF506446 | AF506446 | Larsson and Larsson 2003 |
| *Gloeohypochnicium analogum* | NZFS:4549 | New Zealand | MH409974 | – | Hood et al. 2018 |
| *Gloeohypochnicium analogum* | PDD:91626 | New Zealand | GQ411521 | – | Fukami et al. 2010 |
| *Gloeohypochnicium yunnanense* | CLZhao 30018 | China | PQ783830 | PQ783840 | Present study |
| *Gloeohypochnicium yunnanense* | CLZhao 30049* | China | PQ783831 | PQ783841 | Present study |
| *Gloeophyllum abietinum* | H 22988 | Switzerland | JX524619 | KC782733 | He et al. 2014 |
| *Hericium abietis* | NH 6990 | Canada | AF506456 | AF506456 | Zhou et al. 2021 |
| *Hericium coralloides* | NH 282 | Sweden | AF506459 | AF506459 | Zhou et al. 2021 |
| *Heterobasidion annosum* | Dai 20962 | China | ON417163 | ON417213 | Liu et al. 2022 |
| *Heteroradulum australiense* | LWZ 20180512-25 | Australia | MZ325255 | MZ310425 | Li et al. 2022 |
| *Heteroradulum degluben* | LE 38182 | Sweden | KX262112 | KX262162 | Malysheva and Spirin 2017 |

| Taxa | Voucher no. | Locality | GenBank accession no. | | References |
|---|---|---|---|---|---|
| | | | ITS | nLSU | |
| *Heteroradulum kmetii* | He 4915 | China | MH178262 | MH178286 | Li et al. 2023 |
| *Heteroradulum labyrinthinum* | Yuan 1600 | China | KM379139 | KM379140 | Yuan et al. 2018 |
| *Heteroradulum mussooriense* | Dai 17193 | China | MH178265 | MH178289 | Li et al. 2023 |
| *Hyphoderma cremeoalbum* | CLZhao 17007 | China | OM985716 | OM985753 | Dong et al. 2024a |
| *Lactarius crocatus* | KVP08034 | Thailand | HQ318243 | HQ318151 | Wu et al. 2020 |
| *Lactarius deceptivus* | AFTOL-ID 682 | USA | AY854089 | AY631899 | Wu et al. 2020 |
| *Laetisaria fuciformis* | CBS:182.49 | Netherlands | MH856485 | MH868023 | Vu et al. 2019 |
| *Laetisaria roseipellis* | CBS:299.82 | – | EU622846 | EU622844 | Vu et al. 2019 |
| *Lawreymyces palicei* | Palice 2509 | Ecuador | AY542864 | AY542864 | Lücking and Moncada 2017 |
| *Lawreymyces palicei* | Palice 4369 | Ecuador | AY542865 | AY542865 | Lücking and Moncada 2017 |
| *Lentinellus sublineolatus* | TENN 059307 | Austria | NR119505 | – | Dong et al. 2024a |
| *Lentinellus vulpinus* | 7267 | Sweden | AY513230 | – | Kneal and Smith 2015 |
| *Marchandiomyces aurantioroseus* | FCUG 1166 | Sweden | KP864659 | HM046929 | Ghobad-Nejhad et al. 2021 |
| *Marchandiomyces corallinus* | JL128-98 | – | AY583327 | AY583331 | DePriest et al. 2005 |
| *Megalocystidium diffissum* | V.Spirin4244 | Sweden | MT477147 | MT477147 | Spirin et al. 2021 |
| *Megalocystidium leucoxanthum* | HK9808 | Sweden | AF506420 | AF506420 | Spirin et al. 2021 |
| *Mycobernardia incrustans* | CBS:172.36 | Canada | MH855759 | MH867272 | Vu et al. 2019 |
| *Mycobernardia incrustans* | Duhem 3613 | France | MW805860 | MW805825 | Ghobad-Nejhad et al. 2021 |
| *Neoaleurodiscus fujii* | He2921 | China | KU559357 | KU574845 | Dai et al. 2017 |
| *Neoaleurodiscus fujii* | Wu0807-41 | Japan | – | FJ799924 | Dai et al. 2017 |
| *Peniophora halimi* | CBS:864.84 | France | MH861845 | MH873533 | Vu et al. 2019 |
| *Peniophora incarnata* | CBS:398.50 | France | MH856680 | MH868197 | Vu et al. 2019 |
| *Protodaedalea foliacea* | Yuan 5691 | China | JQ764666 | JQ764644 | Zhou and Dai 2013 |
| *Protodaedalea hispida* | WD 548 | Japan | AB871768 | AB871749 | Sotome et al. 2014 |
| *Punctularia atropurpurascens* | UC 2022981 | USA | KP814559 | – | Knijn & Ferretti 2018 |
| *Punctularia atropurpurascens* | WEI 17-662 | China | MW570883 | MW570888 | Wei et al. 2022 |
| *Punctularia bambusicola* | CLZhao 4133 | China | MW559982 | MW559984 | Guan et al. 2021 |
| *Punctularia bambusicola* | CLZhao 9098 | China | MW559983 | MW559985 | Guan et al. 2021 |
| *Punctularia nigrodontea* | CLZhao 30592* | China | PQ783829 | PQ783839 | Present study |
| *Punctularia strigosozonata* | AFTOL-ID 1248 | – | DQ398958 | AF518642 | Wei et al. 2022 |
| *Punctularia strigosozonata* | CBS:34534 | – | MH855559 | MH867064 | Vu et al. 2019 |
| *Punctulariopsis efibulata* | Burdsall 8824 | USA | KR494276 | KR494277 | Wei et al. 2022 |
| *Punctulariopsis obducens* | MG70 | Ethiopia | HM046918 | HM046933 | Ghobad-Nejhad et al. 2010 |
| *Punctulariopsis subglobispora* | FCUG 2535 | Argentina | HM046917 | HM046932 | Guan et al. 2021 |
| *Russula blennia* | 569/BB08.066 | Switzerland | MH545687 | KU237556 | Wu et al. 2020 |
| *Russula pseudociliata* | 545/BB08.061 | Switzerland | MH545688 | KU237537 | Wu et al. 2020 |
| *Sclerotrema griseobrunneum* | TN 2722 | Canada | KX262144 | KX262192 | Malysheva and Spirin 2017 |
| *Sclerotrema griseobrunneum* | VS 7674 | Russia | KX262140 | KX262188 | Malysheva and Spirin 2017 |
| *Scytinostroma acystidiatum* | Dai 24608 | China | OQ689127 | OQ629351 | Zhang et al. 2023 |

| Taxa | Voucher no. | Locality | GenBank accession no. | | References |
|---|---|---|---|---|---|
| | | | ITS | nLSU | |
| *Scytinostroma bambusinum* | JXH 643 | China | OR510627 | PP660873 | Ji et al. 2024 |
| *Sistotrema brinkmannii* | 236 | Netherlands | JX535169 | JX535170 | Alvarenga and Gibertoni 2021 |
| *Stereodiscus pseudotrivialis* | SPG6799 | Argentina | OR506747 | OR506751 | Gorjón and Greslebin 2024 |
| *Stereodiscus pseudotrivialis* | SPG6874 | Argentina | OR506744 | OR506746 | Gorjón and Greslebin 2024 |
| *Stereum hirsutum* | CBS:108532 | Russia | MH862810 | MH874407 | Vu et al. 2019 |
| *Stereum sanguinolentum* | CBS:529.50 | Canada | MH856746 | MH868268 | Vu et al. 2019 |
| *Terrestriporia alba* | Dai 18546 | Malaysia | MT068562 | MT068558 | Wu et al. 2020 |
| *Terrestriporia alba* | Dai 18547 | Malaysia | MT068563 | MT068559 | Wu et al. 2020 |
| *Tremellochaete atlantica* | URM90199 | Brazil | MG594381 | MG594383 | Alvarenga et al. 2019 |
| *Tremellochaete cilliata* | SP467241 | Brazil | MK391523 | MK391529 | Alvarenga et al. 2019 |
| *Tremellochaete japonica* | LE 303446 | Russia | KX262110 | KX262160 | Malysheva and Spirin 2017 |
| *Vararia fissurata* | CLZhao 8171 | China | OQ025219 | OR539503 | Deng et al. 2024b |
| *Vararia tropica* | CBS:704.81 | France | MH861447 | MH873189 | Vu et al. 2019 |
| *Vuilleminia comedens* | AFTOL-ID 1247 | – | DQ398959 | AF518666 | Wei et al. 2022 |
| *Vuilleminia coryli* | MG136 | Turkmenistan | JN387996 | JN388005 | Ghobad-Nejhad and Ginns 2012 |
| *Vuilleminia cystidiata* | KUC20131022-26 | Korea | KJ668433 | KJ668285 | Wei et al. 2022 |
| *Vuilleminia erastii* | MG97 | Canada | JN387998 | JN388007 | Ghobad-Nejhad and Ginns 2012 |
| *Vuilleminia macrospora* | MG167 | France | JX892940 | JX892941 | Ghobad-Nejhad and Duhem 2014 |
| *Vuilleminia nilsii* | MG171 | France | JX892947 | JX892948 | Ghobad-Nejhad and Duhem 2014 |
| *Vuilleminia pseudocystidiata* | MG69 | France | HM046888 | HM046928 | Ghobad-Nejhad et al. 2010 |
| *Waitea circinata* | CBS:472.82 | USA | MH861518 | MH873265 | Vu et al. 2019 |
| *Waitea guianensis* | GUY13-110 | Guiana | MW449090 | MW449101 | Wei et al. 2022 |
| *Xylobolus frustulatus* | He2231 | USA | KU881905 | KU574825 | Tian et al. 2018 |
| *Xylobolus subpileatus* | FP-106735 | USA | – | AY039309 | Tian et al. 2018 |

assigned as an outgroup to root trees following the ITS+nLSU analysis (Fig. 2) (Deng et al. 2024b); (3) *Gloeophyllum abietinum* (Bull.) P. Karst. was assigned as an outgroup to root trees following the ITS+nLSU analysis (Fig. 3) (He et al. 2014); (4) *Sistotrema brinkmannii* (Bres.) J. Erikss. was assigned as an outgroup to root trees following the ITS+nLSU analysis (Fig. 4) (Alvarenga and Gibertoni 2021); (5) *Adustochaete punctata* J.H. Dong & C.L. Zhao were assigned as an outgroup to root trees following the ITS+nLSU analysis (Fig. 5) (Dong et al. 2024b).

Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses were applied to the combined datasets following a previous study (Wu et al. 2022; Dong et al. 2024a), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree generated. The multiple sequence alignment was also analyzed using maximum likelihood (ML) in RAxML-HPC2 (Miller et al. 2012). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each dataset for Bayesian inference (BI), which was performed using MrBayes 3.2.7a with a general time reversible model of DNA substitution and a gamma distribution rate variation across sites (Ronquist et al. 2012). Four Markov chains were run twice from a random starting tree for 0.4 million generations of the datasets (Fig. 1), 0.4 million generations of the datasets (Fig. 2), 1.6 million generations of the datasets (Fig. 3), 0.8 million generations of the datasets (Fig. 4), and for 0.6 million generations of the datasets (Fig. 5), and the tree was sampled every 1000 generations. The first one-fourth of all generations were discarded as burn-in. The majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received a maximum likelihood bootstrap value (BS) ≥70%, maximum parsimony bootstrap value (BT) ≥50%, or Bayesian posterior probabilities (BPP) ≥0.95.

## Results

### The phylogeny of *Conferticium*

The datasets based on ITS+nLSU (Fig. 1) comprise sequences from 44 fungal specimens representing 39 species. The datasets had an aligned length of 2,270 characters, of which 1,183 characters are constant, 293 are variable and parsimony-uninformative, and 794 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 4447, CI = 0.4160, H = 0.5840, RI = 0.5275, RC = 0.2194). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.008678 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 144.5. The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that the new species *Conferticium albocremeum* grouped into the genus *Conferticium* and clustered into the family Stereaceae (Russulales), in which it was retrieved as a sister to *C. heimii* (Boidin) Sheng H. Wu.

The datasets based on ITS+nLSU (Fig. 2) comprise sequences from 31 fungal specimens representing 23 species. The datasets had an aligned length of 2,136 characters, of which 1,456 characters are constant, 298 are variable and parsimony-uninformative, and 382 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 1491, CI = 0.6237, HI = 0.3763, RI = 0.5681, RC = 0.3544). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.013157 (BI), and the effective sample size (ESS) across

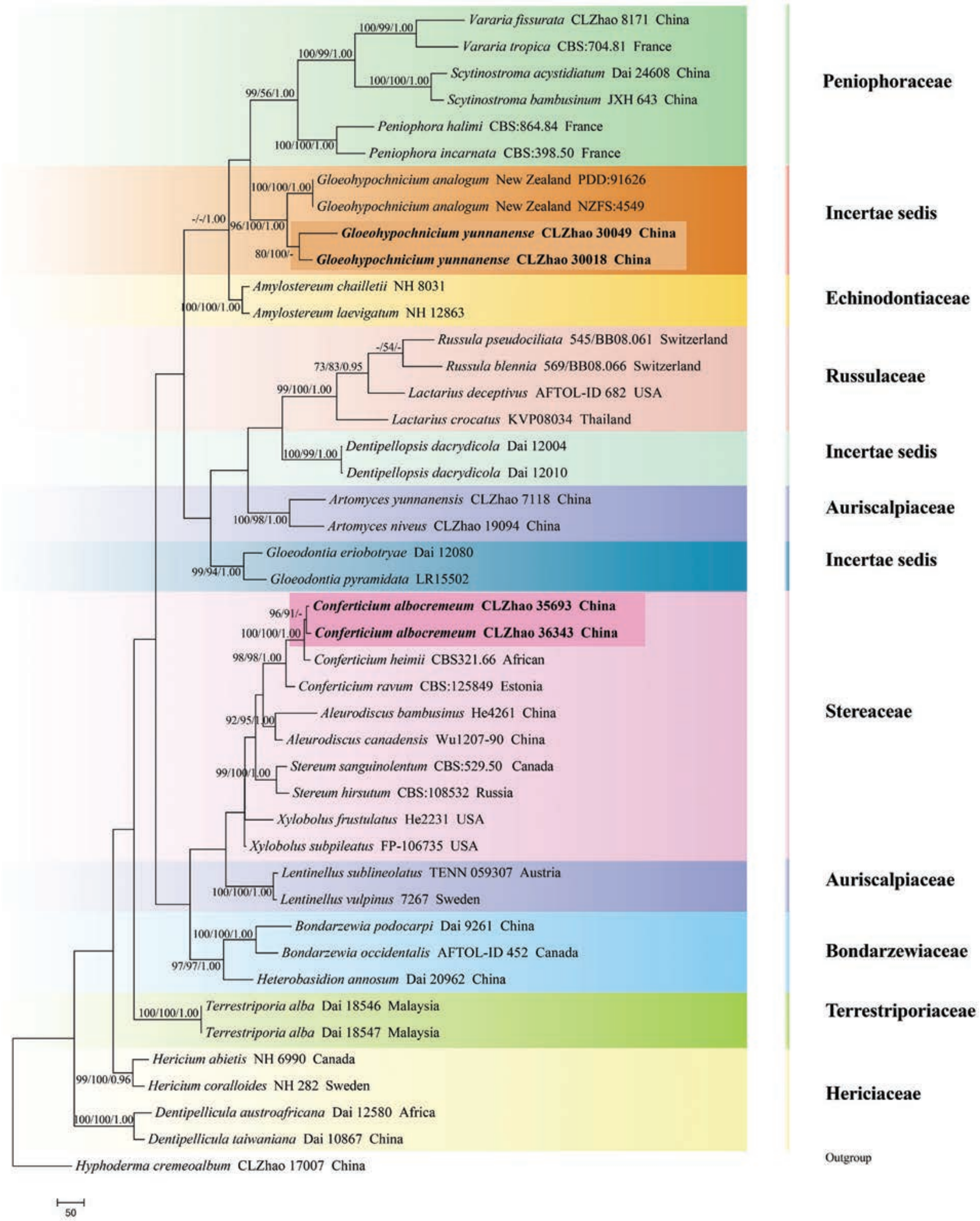

**Figure 1.** Maximum parsimony strict consensus tree illustrating the phylogeny of *Conferticium* and *Gloeohypochnicium* and related genera in the order Russulales, based on ITS+nLSU sequences; branches are labeled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

the two runs is double the average ESS (avg ESS) = 265.5. The phylogenetic tree (Fig. 2) inferred from ITS+nLSU sequences revealed that *Conferticium albocremeum* grouped into the genus *Conferticium* and clustered into the family Stereaceae, in which it was closely related to *C. heimii*.

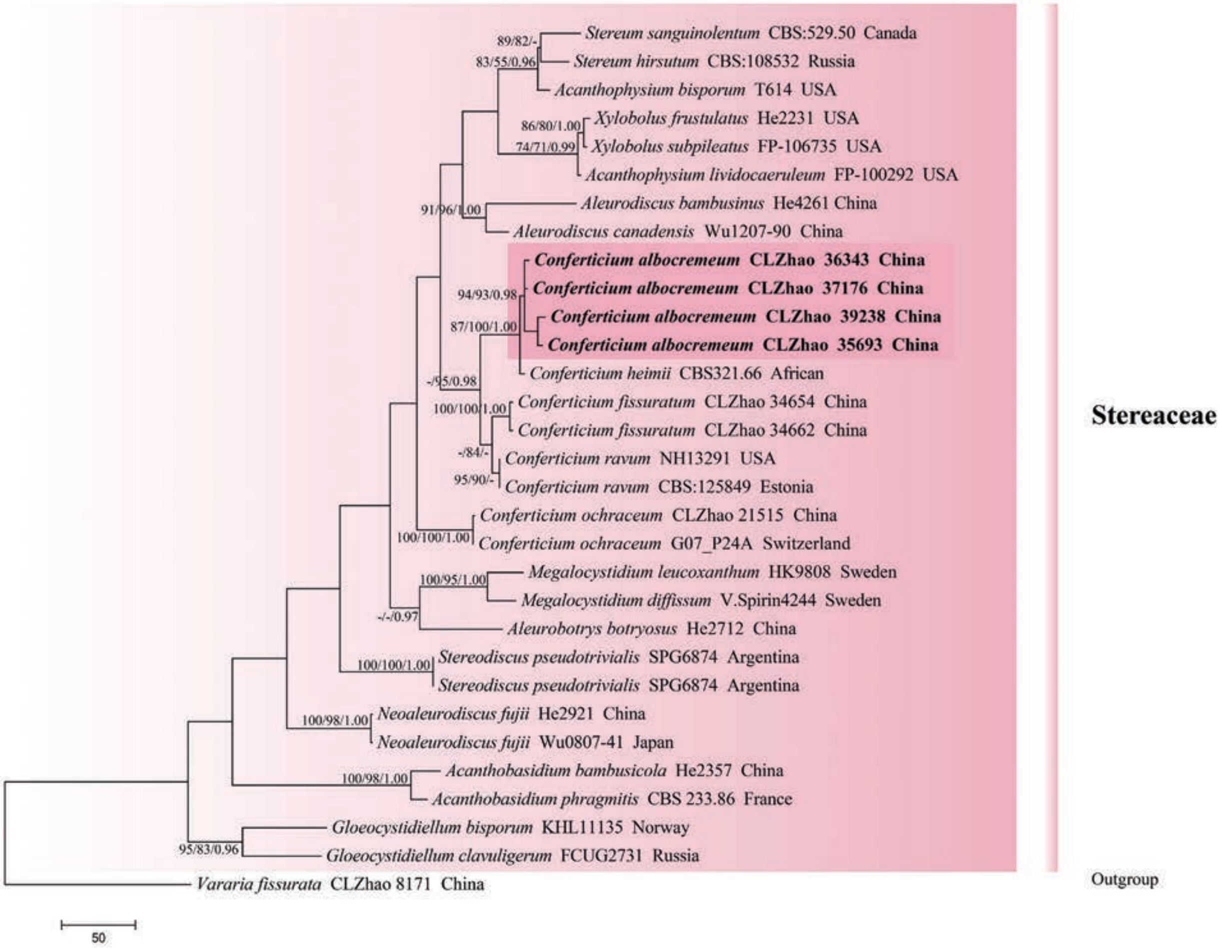

Figure 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Conferticium* and related genus in the family Stereaceae, based on ITS+nLSU sequences; branches are labeled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

## The phylogeny of *Dendrocorticiopsis*

The datasets based on ITS+nLSU (Fig. 3) comprise sequences from 44 fungal specimens representing 37 species. The datasets had an aligned length of 2,181 characters, of which 1,313 characters are constant, 232 are variable and parsimony-uninformative, and 736 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 3423, CI = 0.4368, HI = 0.5632, RI = 0.5695, RC = 0.2488). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.013154 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 658. The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that the new species *Dendrocorticiopsis luteoalba* grouped into the genus *Dendrocorticiopsis* and clustered into the family Punctulariaceae (Corticiales), in which it was retrieved as a sister to *D. orientalis* Sheng H. Wu et al.

## The phylogeny of *Eichleriella*

The datasets based on ITS+nLSU (Fig. 4) comprise sequences from 45 fungal specimens representing 43 species. The datasets had an aligned length of

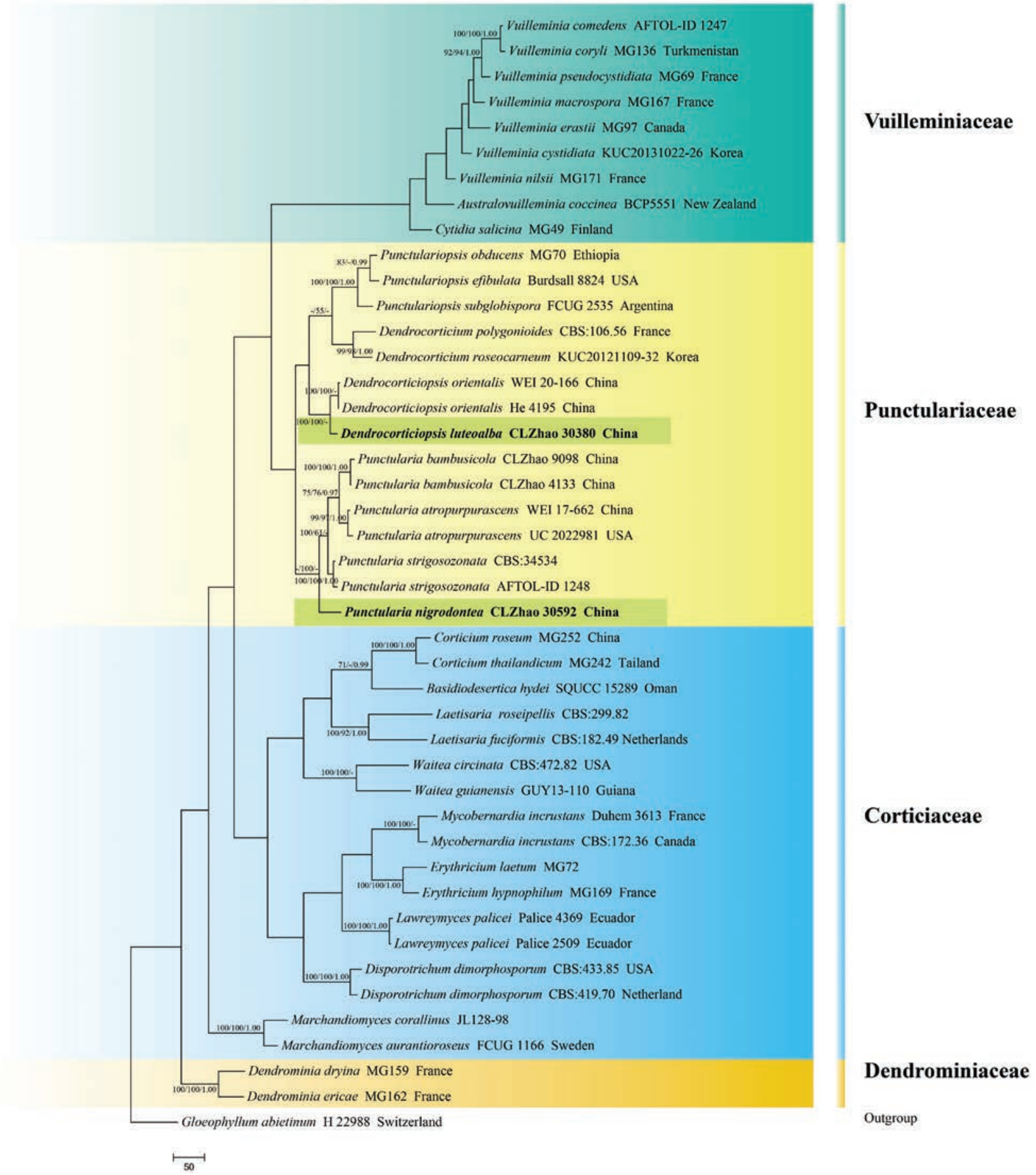

**Figure 3.** Maximum parsimony strict consensus tree illustrating the phylogeny of *Dendrocorticiopsis* and *Punctularia* and related genera in the order Corticiales, based on ITS+nLSU sequences; branches are labeled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

2,104 characters, of which 1,516 characters are constant, 254 are variable and parsimony-uninformative, and 334 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 1783 CI = 0.4773, HI = 0.5227, RI = 0.5633, RC = 0.2688). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.0024569 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 361.5. The phylogenetic tree (Fig. 4)

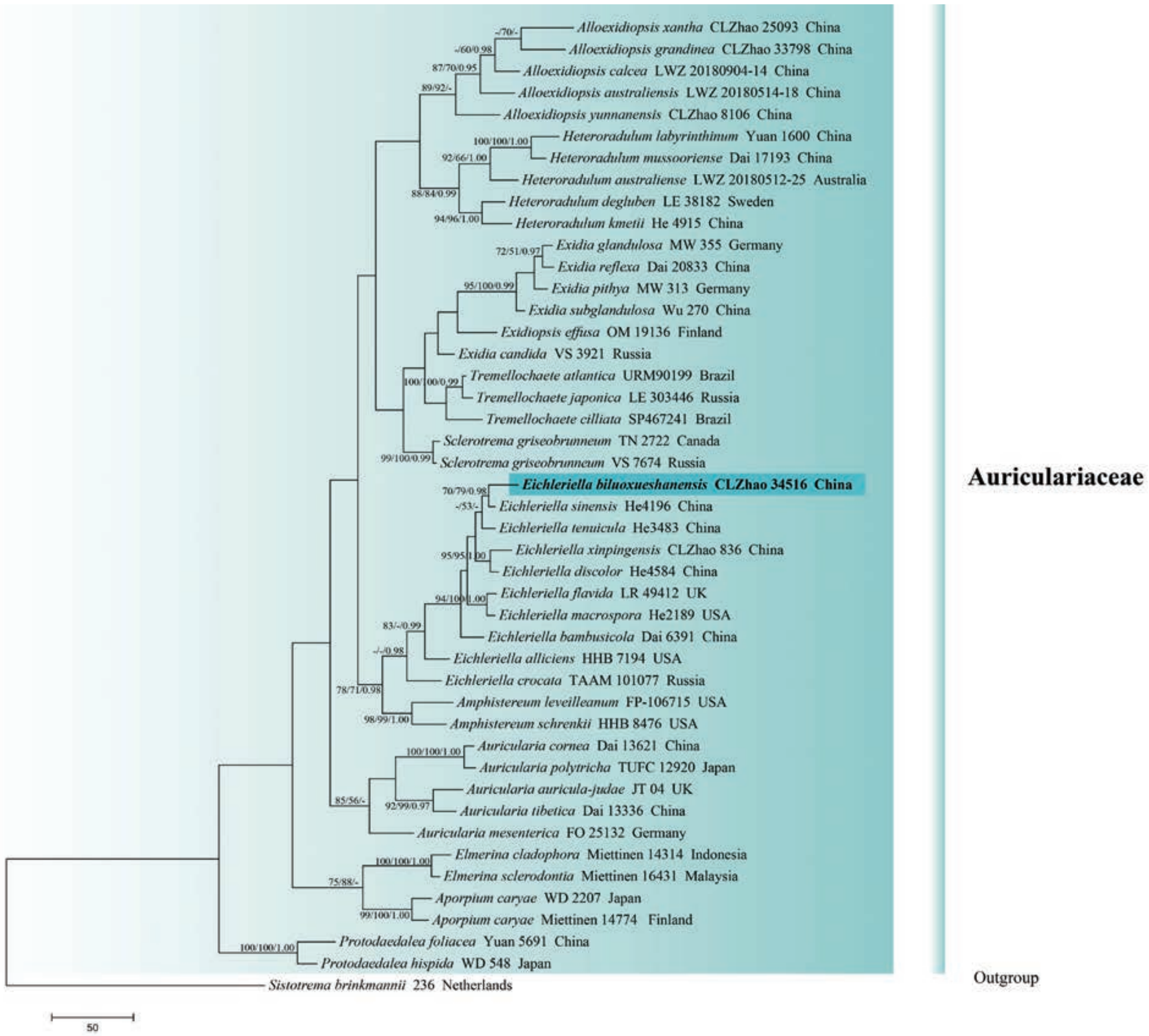

**Figure 4.** Maximum parsimony strict consensus tree illustrating the phylogeny of *Eichleriella* and related genera in the family Auriculariaceae, based on ITS+nLSU sequences; branches are labeled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

inferred from ITS+nLSU sequences revealed that *Eichleriella biluoxueshanensis* grouped into the genus *Eichleriella* and clustered into the family Auriculariaceae, in which it was retrieved as a sister to *E. sinensis* (Teng) S.H. He & Nakasone.

The datasets based on ITS+nLSU (Fig. 5) comprise sequences from 31 fungal specimens representing 24 species. The datasets had an aligned length of 1,905 characters, of which 1,687 characters are constant, 101 are variable and parsimony-uninformative, and 117 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 402, CI = 0.6542, HI = 0.3458, RI = 0.7495, RC = 0.4094). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.009368 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 264.5. The phylogenetic tree (Fig. 5) inferred from ITS+nLSU sequences revealed that *Eichleriella biluoxueshanensis* grouped into the genus *Eichleriella*, in which it was grouped with the clade comprising *E. sinensis* (Teng) S.H. He & Nakasone.

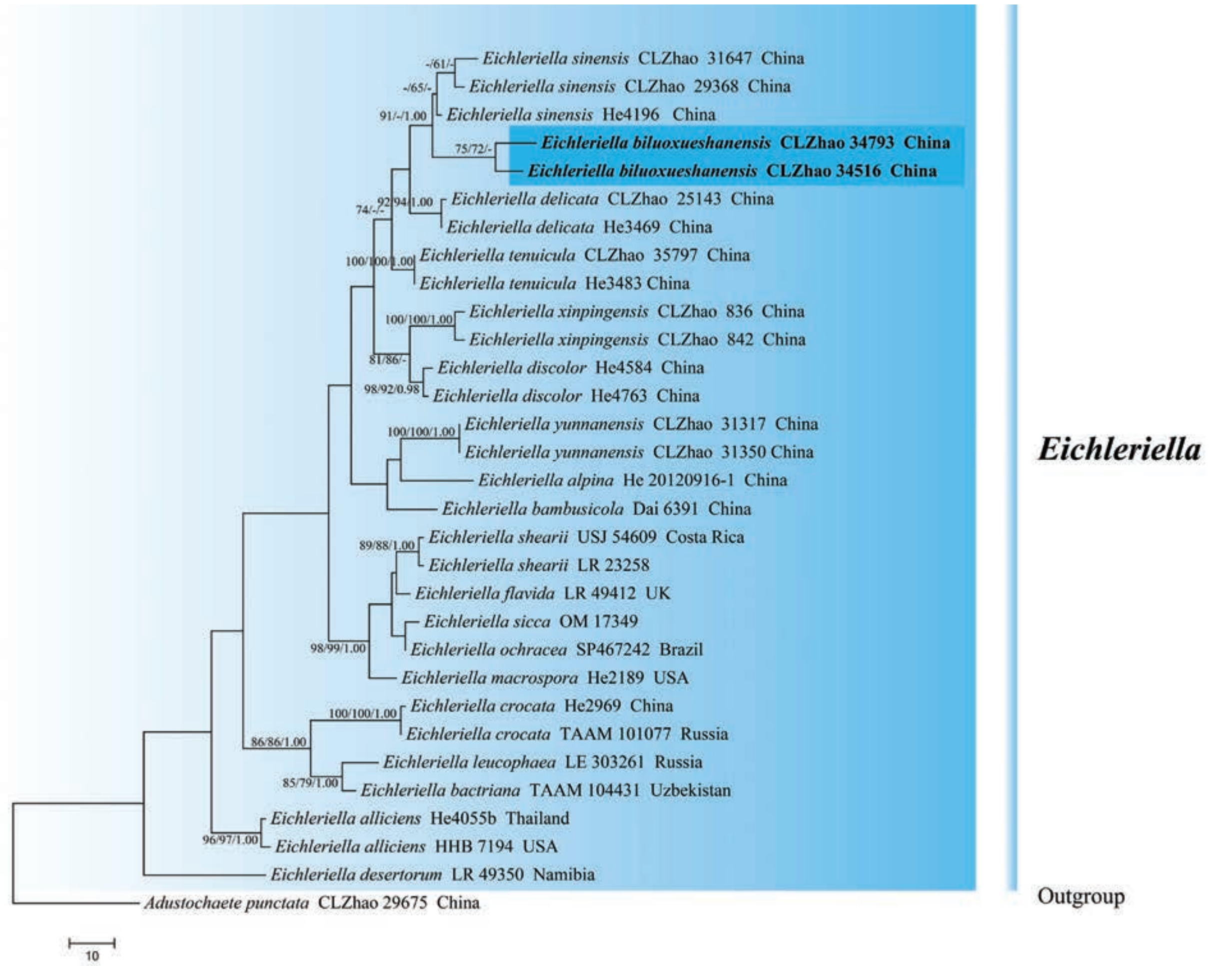

**Figure 5.** Maximum parsimony strict consensus tree illustrating the phylogeny of *Eichleriella* and related species in the genus *Eichleriella*, based on ITS+nLSU sequences; branches are labeled with maximum likelihood bootstrap value ≥ 70%, parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

### The phylogeny of *Gloeohypochnicium*

The datasets based on ITS+nLSU (Fig. 1) comprise sequences from 44 fungal specimens representing 39 species. The datasets had an aligned length of 2,270 characters, of which 1,183 characters are constant, 293 are variable and parsimony-uninformative, and 794 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 4447, CI = 0.4160, HI = 0.5840, RI = 0.5275, RC = 0.2194). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.008678 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 144.5. The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that *Gloeohypochnicium yunnanense* grouped into the genus *Gloeohypochnicium* and clustered into the order Russulales, in which it was closely related to *G. analogum* (Bourdot & Galzin) Hjortstam.

### The phylogeny of *Punctularia*

The datasets based on ITS+nLSU (Fig. 3) comprise sequences from 44 fungal specimens representing 37 species. The datasets had an aligned length of

2,181 characters, of which 1,313 characters are constant, 232 are variable and parsimony-uninformative, and 736 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 3423, CI = 0.4368, HI = 0.5632, RI = 0.5695, RC = 0.2488). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.013154 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 658. The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that *Punctularia nigrodontea* grouped into the genus *Punctularia* and clustered into the family Punctulariaceae (Corticiales), in which it was grouped with the clade comprising *P. atropurpurascens* (Berk. & Broome) Petch, *P. bambusicola* C.L. Zhao and *P. strigosozonata* (Schwein.) P.H.B. Talbot.

## Taxonomy

***Conferticium albocremeum*** **L. Wang & C.L. Zhao, sp. nov.**
MycoBank No: 856958
Figs 6–8

**Typification.** China. Yunnan Province • Xishuangbanna, Wild Elephant Valley, GPS coordinates: 22°10'N, 100°51'E, altitude: 900 m asl., on the fallen angiosperm branch, leg. C.L. Zhao, 25 January 2024 CLZhao 35693 (SWFC!).

**Etymology.** *Albocremeum* (Lat.) refers to the new species having white to cream hymenophore.

**Basidiomata.** Annual, resupinate, closely adnate, membranaceous, without odor or taste when fresh, up to 10 cm long, 2 cm wide, and 700 μm thick. Hymenophore smooth, white (60) to cream (4A2/3) when fresh, cream (4A2/3) upon drying. Sterile margin narrow, cream (4A2/3), up to 1 mm.

**Hyphal system.** Monomitic; generative hyphae with simple-septa, colorless, thin-walled, smooth, rarely branched, interwoven, IKI+, CB–, 2–3 μm in diameter; tissues unchanged in KOH.

**Hymenium.** Gloeocystidia subclavate, flexuous, colorless, mostly constricted in the middle, thin-walled, smooth, 33–47.5 × 5–8 μm. Basidia subcylindrical to subclavate, slightly flexuous, with a basal simple septum and four sterigmata, 22–36 × 4.5–7 μm; basidioles numerous, in shape similar to basidia.

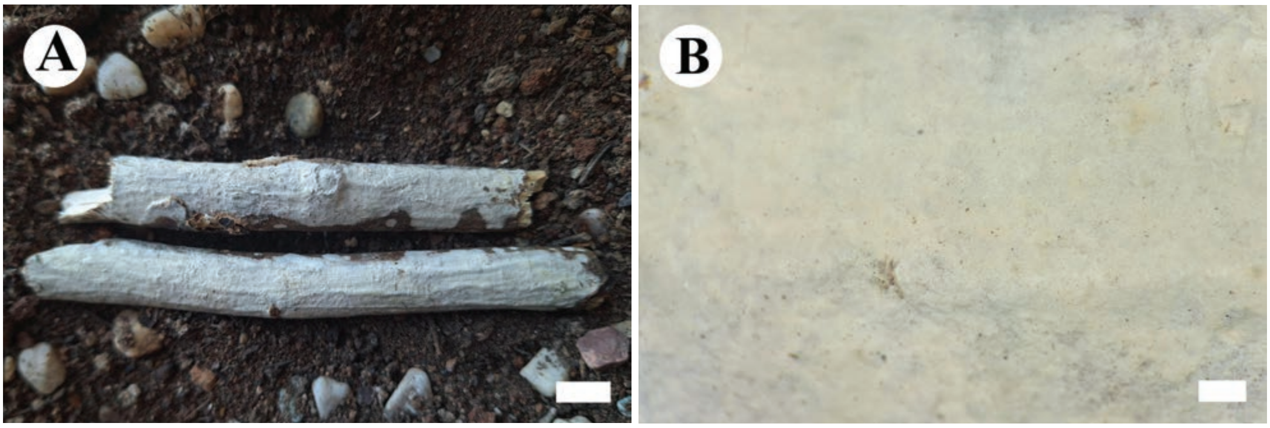

**Figure 6.** Basidiomata of *Conferticium albocremeum* (holotype CLZhao 35693). Scale bars: 1 cm (**A**); 1 mm (**B**).

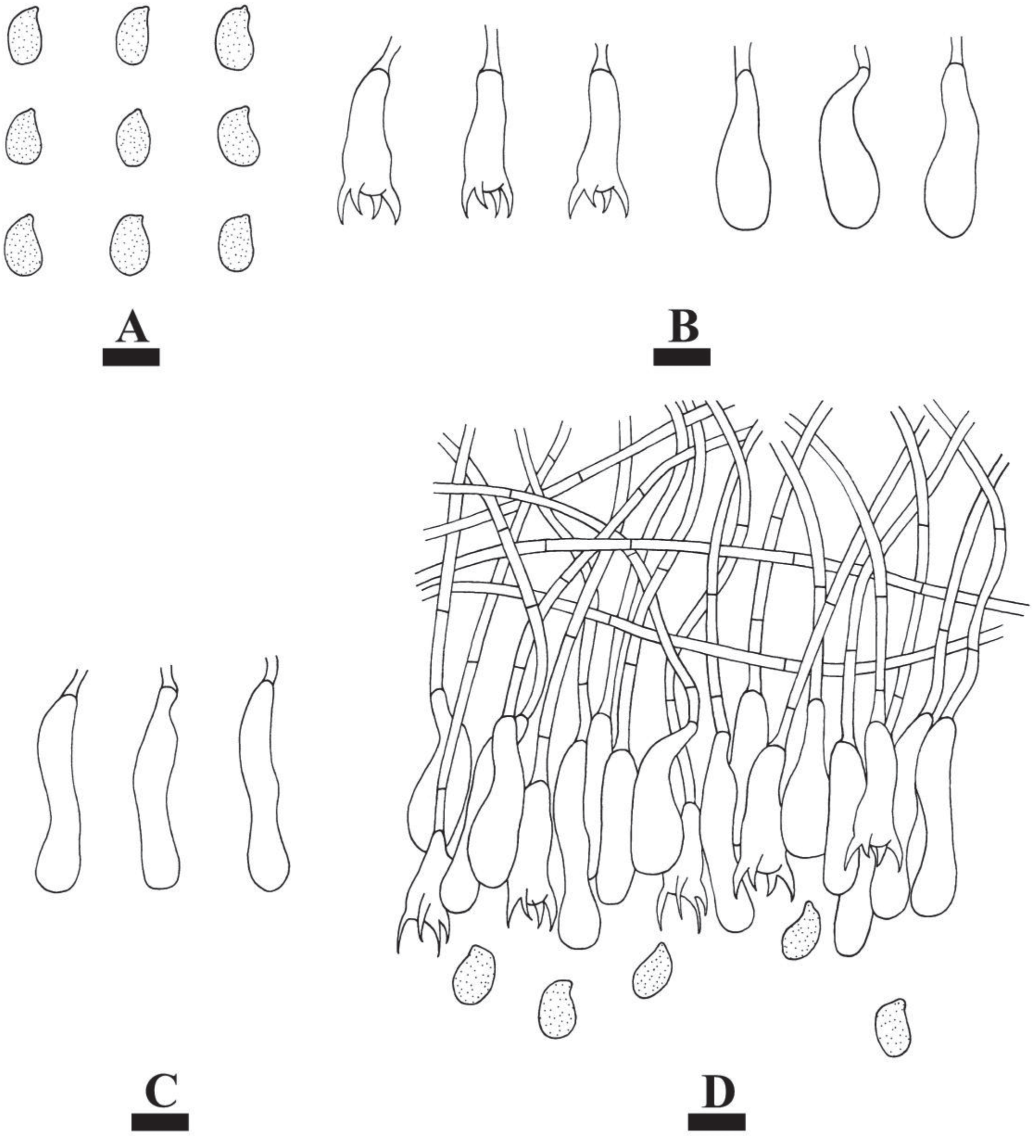

**Figure 7.** Microscopic structures of *Conferticium albocremeum* (holotype CLZhao 35693): basidiospores (**A**); basidia and basidioles (**B**); gloeocystidia (**C**); a section of the hymenium (**D**). Scale bars: 10 μm (**A–D**).

**Spores.** Basidiospores ellipsoid with a distinct apiculus, colorless, thin-walled, finely verrucose but appearing smooth by light microscope, IKI+, CB–, 9–11 × 5–7 μm, L = 9.82 μm, W = 5.84 μm, Q = 1.36–1.68 (n = 90/3).

**Additional specimens examined (paratypes).** China. Yunnan Province • Dehong, Mang City, Mengga Town, Tongbiguan Provincial Nature Reserve, GPS coordinates: 24°46'N, 97°34'E, altitude: 1300 m asl., on the fallen angiosperm branch, leg. C.L. Zhao, 29 June 2024, CLZhao 36343 • Dehong, Yingjiang County, Tongbiguan Provincial Nature Reserve, GPS coordinates: 25°50'N, 97°36'E, altitude: 1000 m asl., on the fallen angiosperm branch, leg. C.L. Zhao, 2 July 2024, CLZhao 37176 • Tengchong, Tuantian Town, Gaoligongshan National Nature Reserve, GPS coordinates: 25°27'N, 98°46'E, altitude: 2500 m asl., on the fallen angiosperm branch, leg. C.L. Zhao, 7 July 2024, CLZhao 39283 (SWFC!).

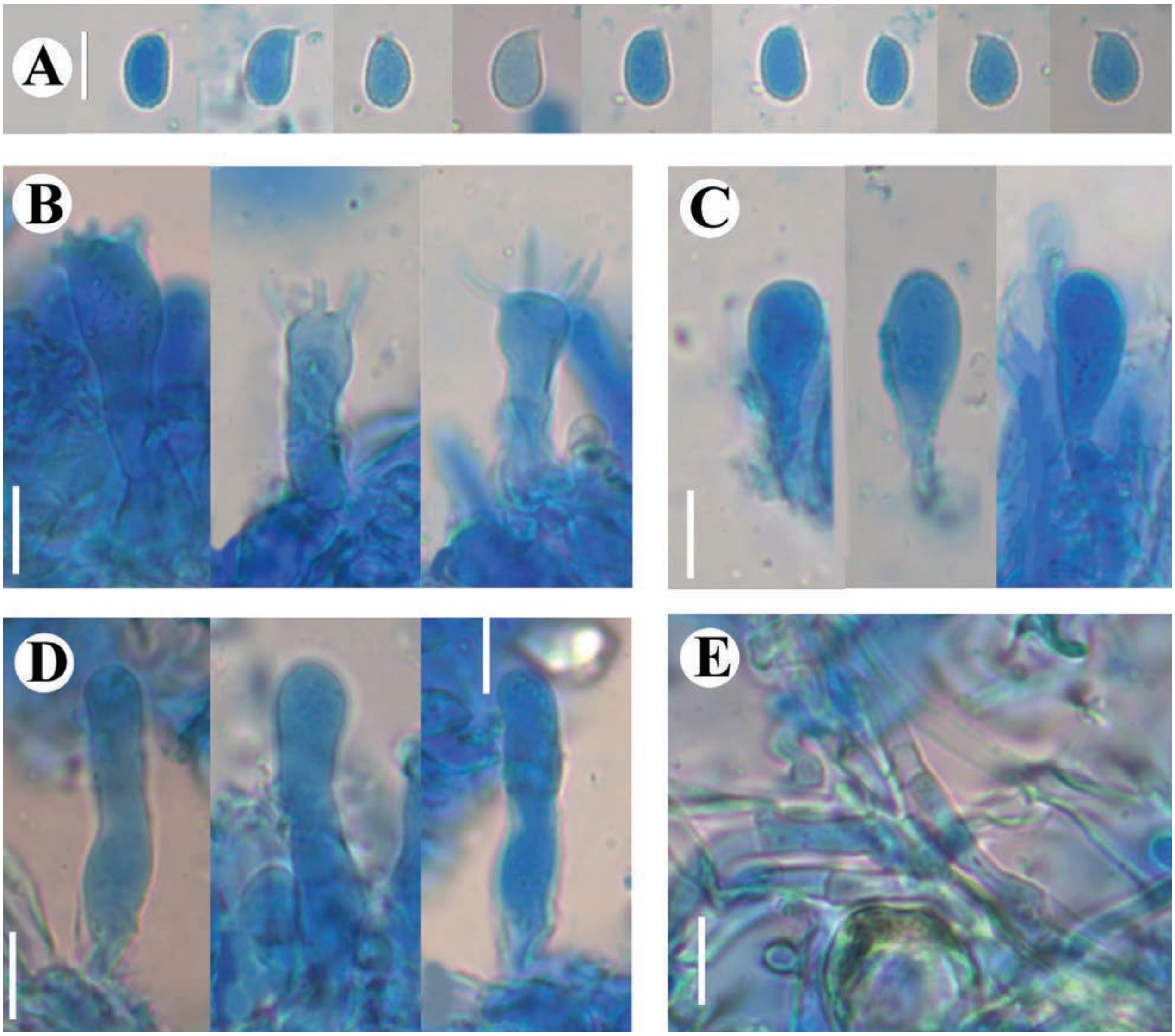

**Figure 8.** Sections of hymenium of *Conferticium albocremeum* (holotype CLZhao 35693): basidiospores (**A**); basidia (**B**); basidioles (**C**); gloeocystidia (**D**); a section of the generative hyphae (**E**). Scale bars: 10 µm (**A–E**); 10 × 100.

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### ***Dendrocorticiopsis luteoalba*** **L. Wang & C.L. Zhao, sp. nov.**

MycoBank No: 856959
Figs 9–11

**Typification.** China. Yunnan Province • Dehong, Yingjiang County, Tongbiguan Provincial Nature Reserve, GPS coordinates: 25°50'N, 97°36'E, altitude: 1000 m asl., on the fallen angiosperm branch, leg. C.L. Zhao, 19 July 2023, CLZhao 30380 (SWFC!).

**Etymology.** *Luteoalba* (Lat.) refers to the new species having a white to buff hymenophore.

**Basidiomata.** Annual, resupinate, closely adnate, membranaceous, without odor or taste when fresh, up to 6 cm long, 2 cm wide, and 300 µm thick. Hymenophore smooth, white (60) when fresh, white (60) to buff (4A4) upon drying. Sterile margin narrow, white (60) to buff (4A4), up to 1 mm.

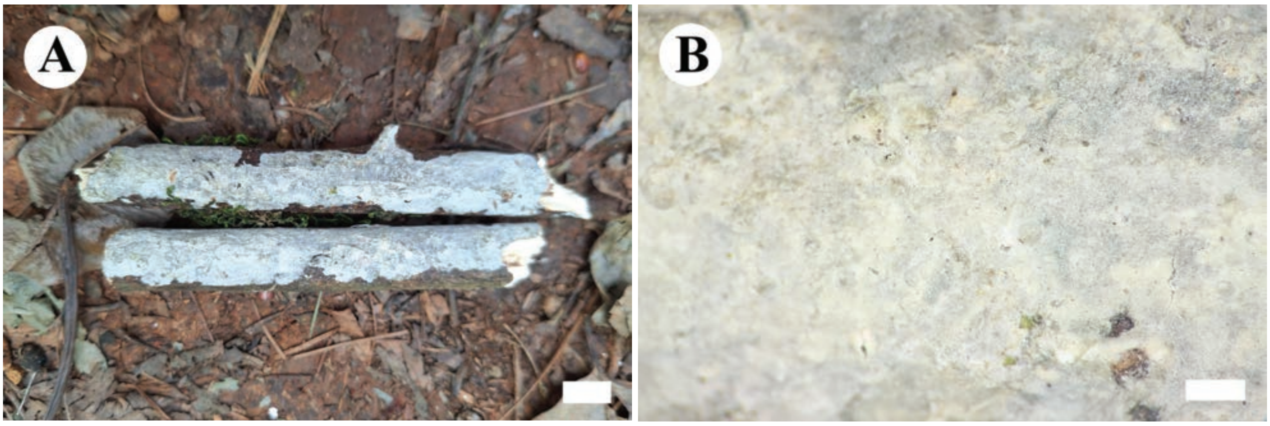

**Figure 9.** Basidiomata of *Dendrocorticiopsis luteoalba* (holotype CLZhao 30380). Scale bars: 1 cm (**A**); 1 mm (**B**).

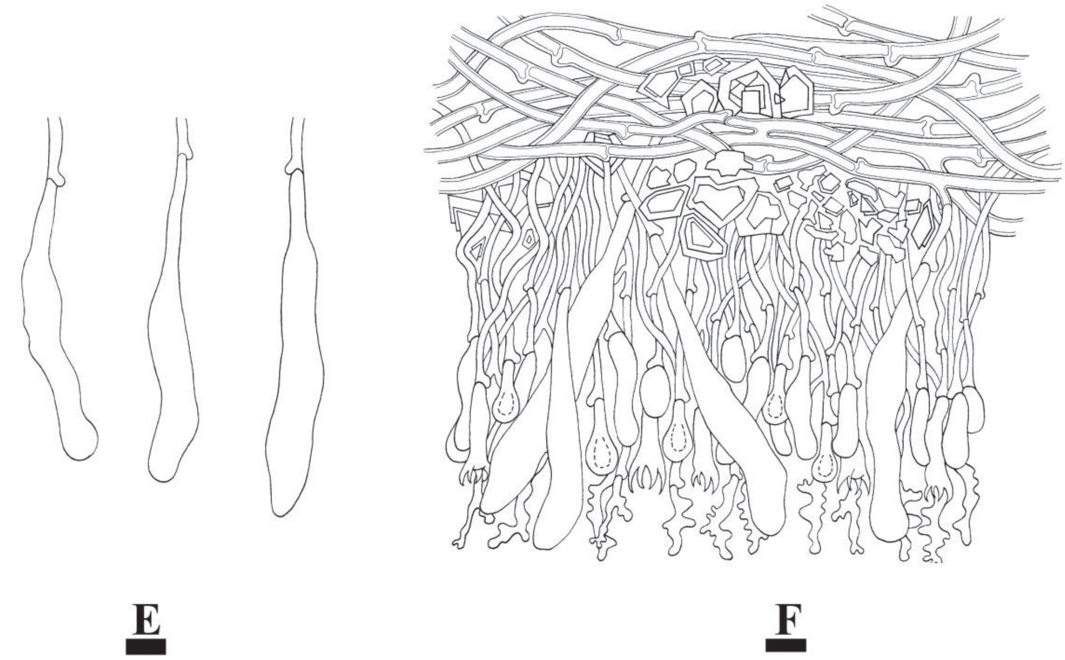

**Figure 10.** Microscopic structures of *Dendrocorticiopsis luteoalba* (holotype CLZhao 30380): basidiospores (**A**); basidia and basidioles (**B**); dendrohyphidia (**C**); cystidia (**D**); gloeocystidia (**E**); a section of the hymenium (**F**). Scale bars: 10 μm (**A–F**).

**Hyphal system.** Monomitic; generative hyphae with clamp connections, colorless, thin- to slightly thick-walled, smooth, branched, interwoven, usually with crystal masses, IKI–, CB–, 2.5–4 μm in diameter; tissues unchanged in KOH.

**Hymenium.** Cystidia are of two types: (1) capitate, apically with resinous materials, gradually dissolving in KOH, colorless, thin-walled, smooth, 8.5–14 ×

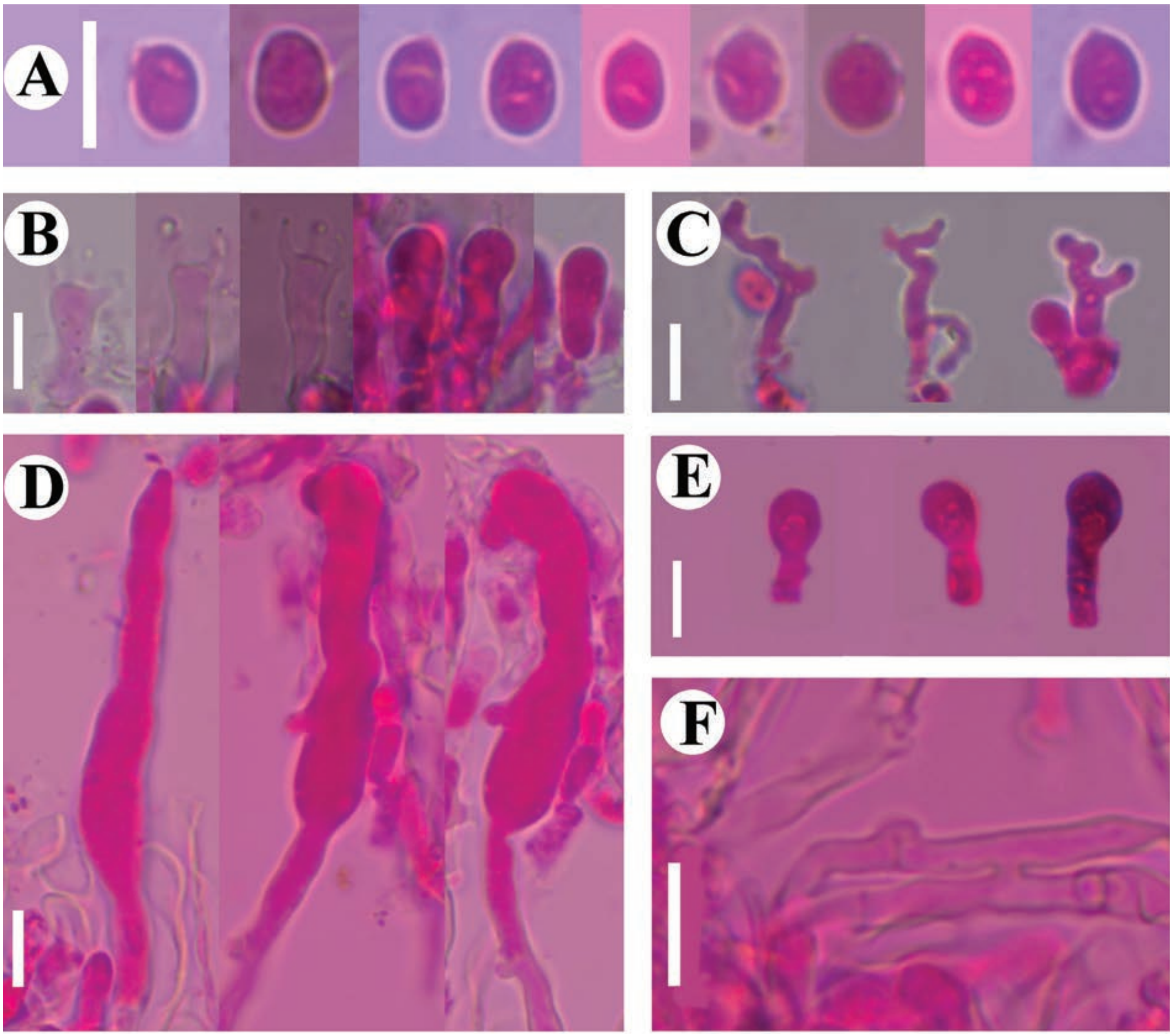

**Figure 11.** Sections of hymenium of *Dendrocorticiopsis luteoalba* (holotype CLZhao 30380): basidiospores (**A**); basidia and basidioles (**B**); dendrohyphidia (**C**); gloeocystidia (**D**); cystidia (**E**); a section of generative hyphae (**F**). Scale bars: 10 μm (**A–F**); 10 × 100.

6.5–8.5 μm; (2) gloeocystidia, clavate to subulate, slightly flexuous, colorless, thin-walled, smooth, 68.5–90 × 8–10 μm. Dendrohyphidia numerous, thick-walled toward base, colorless, 16–19 × 2.5–3.5 μm. Basidia subclavate to clavate, flexuous, with a basal clamp connection and four sterigmata, 15.5–28.5 × 4–5.5 μm; basidioles numerous, in shape similar to basidia.

**Spores.** Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, (6.5–)7–8 × (4–)4.5–5.5 μm, L = 7.29 μm, W = 4.97μm, Q = 1.47 (n = 30/1).

### ***Eichleriella biluoxueshanensis*** **L. Wang & C.L. Zhao, sp. nov.**

MycoBank No: 856960
Figs 12–14

**Typification.** CHINA. Yunnan Province • Diqing, Weixi County, Weideng, Songpo, GPS coordinates: 27°05'N, 99°13'E, altitude: 1400 m asl., on the fallen angiosperm branch, leg. C.L., 13 November 2023, CLZhao 34516 (SWFC!).

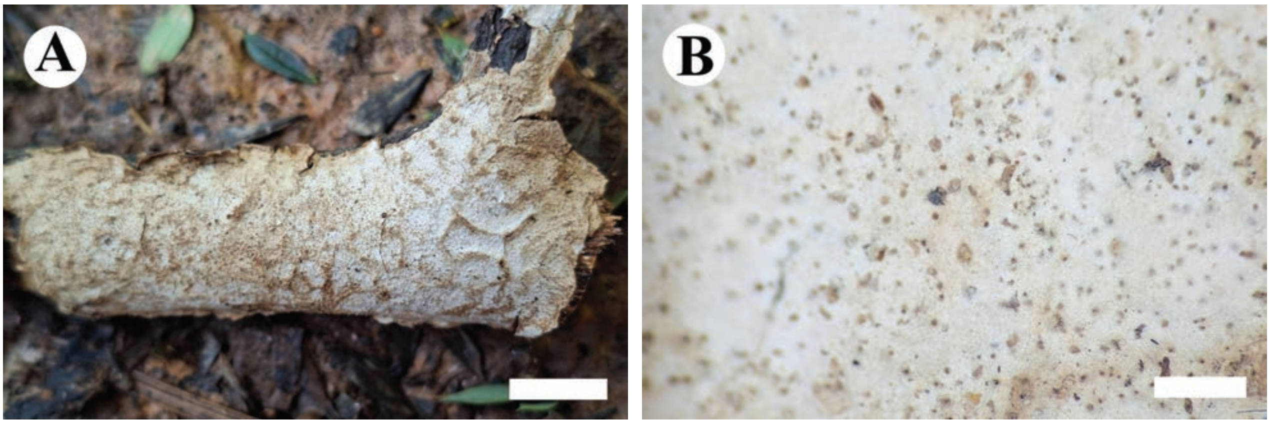

Figure 12. Basidiomata of *Eichleriella biluoxueshanensis* (holotype CLZhao 34516). Scale bars: 1 cm (**A**); 1 mm (**B**).

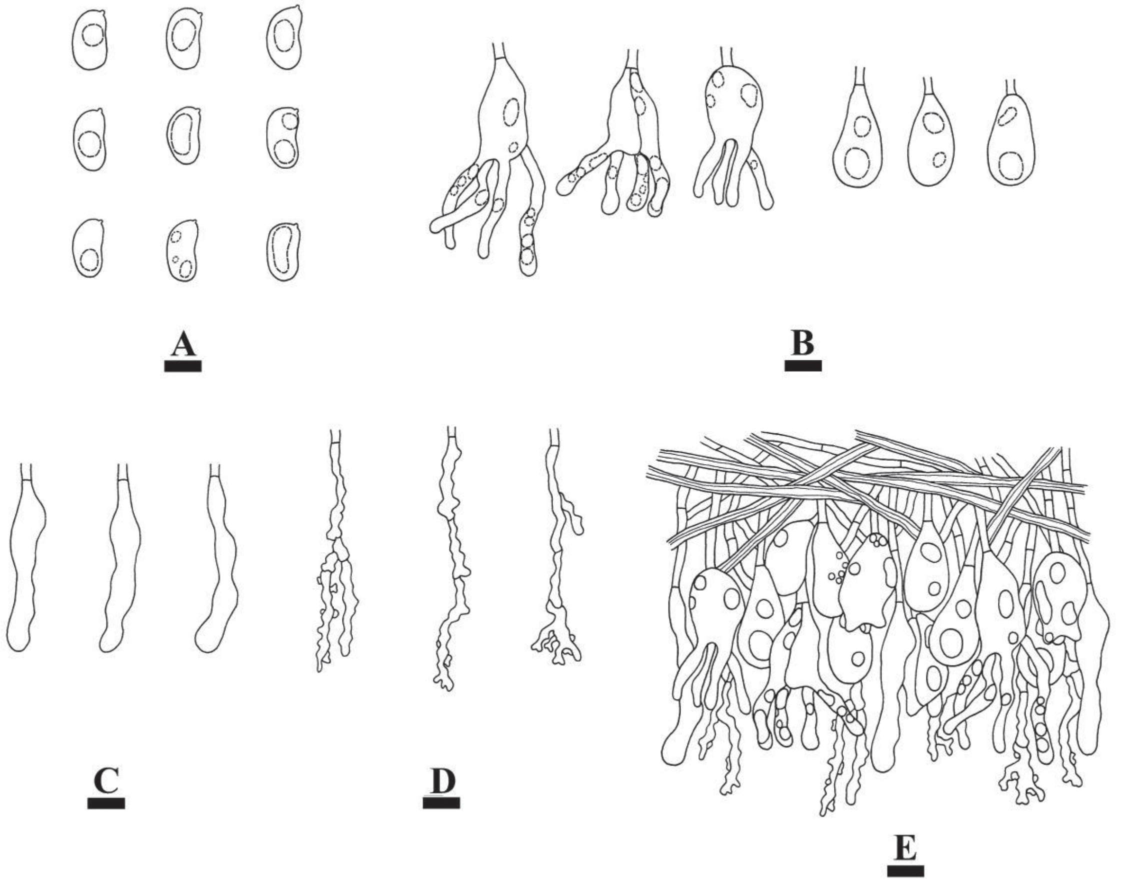

**Figure 13.** Microscopic structures of *Eichleriella biluoxueshanensis* (holotype CLZhao 34516): basidiospores (**A**); basidia and basidioles (**B**); cystidia (**C**); hyphidia (**D**); a section of the hymenium (**E**). Scale bars: 10 µm (**A–E**).

**Etymology.** *Biluoxueshanensis* (Lat.) refers to the locality (Biluoxueshan) of the holotype specimen.

**Basidiomata.** Annual, resupinate, closely adnate, coriaceous, without odor or taste when fresh, up to 8 cm long, 2 cm wide, and 700 µm thick. Hymenophore grandinioid, cream (4A2/3) to buff (4A4) when fresh, buff (4A4) to cinnamon-buff (4/5B4) upon drying. Sterile margin narrow, white (60) to cream (4A2/3), up to 1 mm.

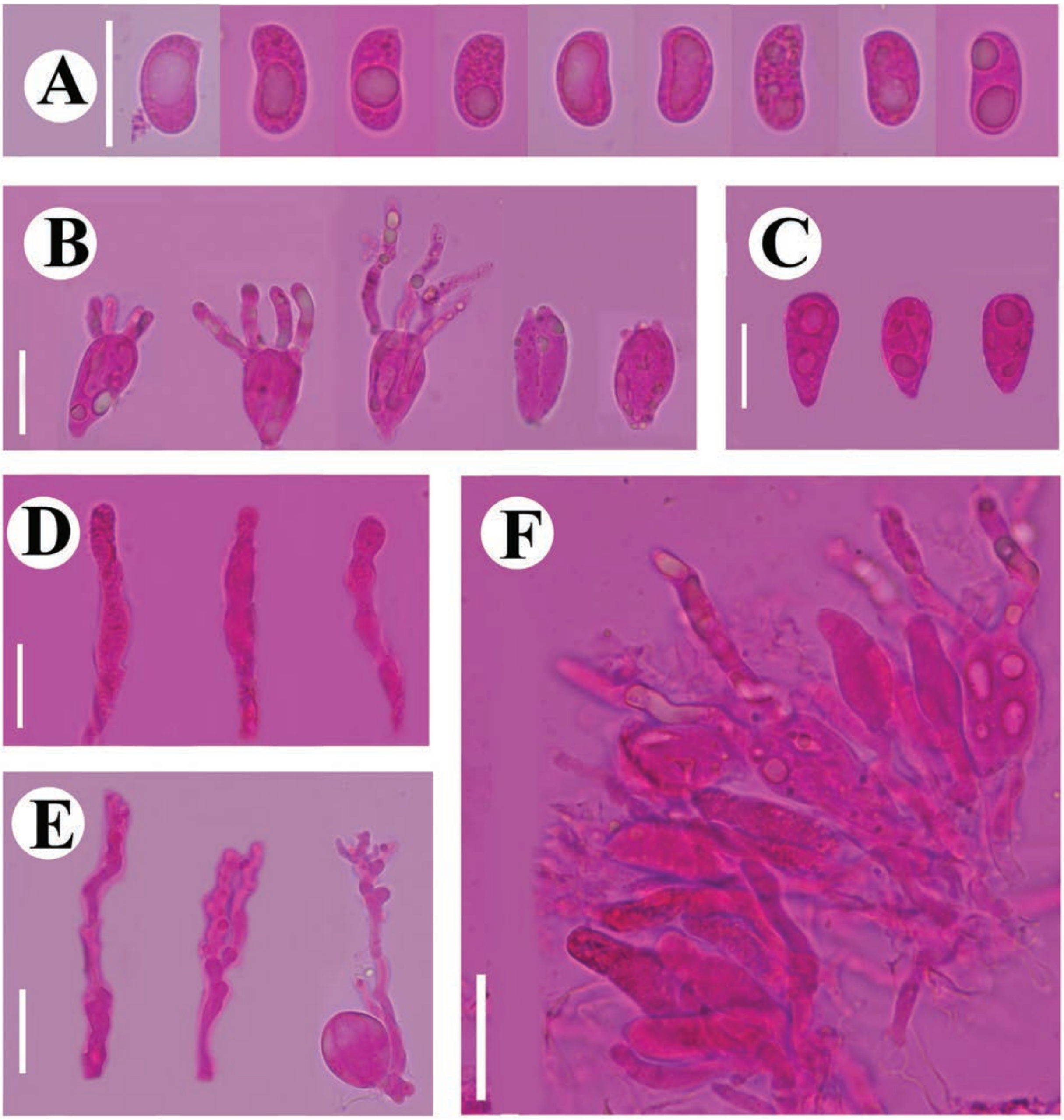

**Figure 14.** Sections of hymenium of *Eichleriella biluoxueshanensis* (holotype CLZhao 34516): basidiospores (**A**); basidia (**B**); basidioles (**C**); cystidia (**D**); hyphidia (**E**); a section of the hymenium (**F**). Scale bars: 10 µm (**A–F**); 10 × 100.

**Hyphal system.** Dimitic; generative hyphae simple-septate, colorless, thin-walled, smooth, rarely branched, interwoven, 3–4 µm in diameter; skeletal hyphae distinctly thick-walled, smooth, unbranched, interwoven, IKI–, CB–, 3–4 µm in diameter; tissues unchanged in KOH.

**Hymenium.** Cystidia subclavate, flexuous, colorless, thin-walled, smooth, 40–45.5 × 6–9.5 µm. Basidia narrowly ovoid to ellipsoid, longitudinally septate, four-celled, 21–29 × 11.5–15 µm; basidioles numerous, in shape similar to basidia but smaller. Hyphidia arising from generative hyphae, nodulose, branched, colorless, thin-walled, 58–72.5 × 2.5–4 µm in diameter.

**Spores.** Basidiospores allantoid, colorless, thin-walled, smooth, usually with one or more oil drops, IKI–, CB–, (12–)13.5–17.5(–18) × (6.5–)7–9(–9.5) µm, L = 15.57 µm, W = 7.99 µm, Q = 1.95–2.06 (n = 60/2).

**Additional specimen examined (paratype).** CHINA. Yunnan Province • Diqing, Weixi County, Zhonglu, Lagaluo, GPS coordinates: 27°9'N, 99°8'E, altitude: 1710 m asl., on the fallen angiosperm branch, leg. C.L. Zhao, 10 October 2023, CLZhao 34793 (SWFC!).

### ***Gloeohypochnicium yunnanense*** **L. Wang & C.L. Zhao, sp. nov.**

MycoBank No: 856961
Figs 15–17

**Typification.** CHINA. Yunnan Province • Dehong, Yingjiang County, Tongbiguan Provincial Nature Reserve, GPS coordinates: 25°50'N, 97°36'E, altitude: 1000 m asl., on the dead bamboo, leg. C.L. Zhao, 18 July 2023, CLZhao 30049 (SWFC!).

**Etymology.** *Yunnanense* (Lat.) refers to the locality "Yunnan Province" of the holotype specimen.

**Basidiomata.** Annual, resupinate, closely adnate, coriaceous, without odor or taste when fresh, up to 5 cm long, 3 cm wide, and 600 µm thick. Hymenophore smooth, slightly buff (4A4) when fresh, buff (4A4) to cream (4A2/3) upon drying. Sterile margin narrow, white (60) to buff (4A4), up to 1 mm.

**Hyphal system.** Monomitic; generative hyphae with clamp connections, colorless, thick-walled, smooth, branched, interwoven, 1.5–4 µm in diameter; IKI–, CB–, tissues unchanged in KOH.

**Hymenium.** Cystidia subcylindrical to subulate, flexuous, thin-walled, encrusted with whitish to yellowish crystals, 75–115.5 × 11.5–15 µm. Basidia subcylindrical to clavate, slightly flexuous, a basal clamp connection and four sterigmata, 55.5–70 × 9.5–11 µm; basidioles numerous, in shape similar to basidia but smaller.

**Spores.** Basidiospores globose, colorless, thick-walled, warted, IKI–, CB–, (9.5–)10–12 × (9.5–)10–11.5 µm, L = 10.91 µm, W = 10.45 µm, Q = 1.04–1.05 (n = 60/2).

**Additional specimen examined (paratype).** CHINA. Yunnan Province • Dehong, Yingjiang County, Tongbiguan Provincial Nature Reserve, GPS coordinates: 27°52'N, 97°38'E, altitude: 1000 m asl., on dead bamboo, leg. C.L. Zhao, 18 July 2023, CLZhao 30018 (SWFC!).

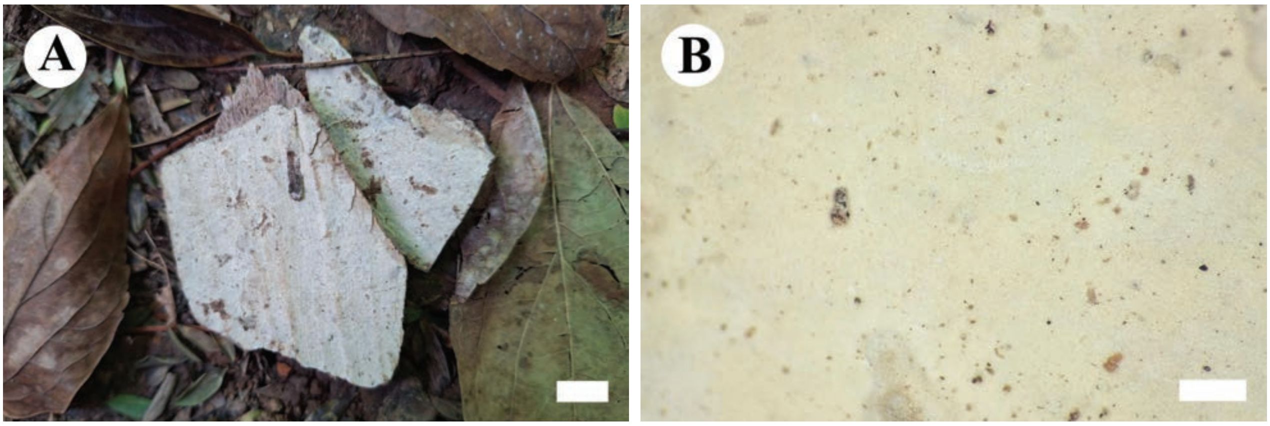

**Figure 15.** Basidiomata of *Gloeohypochnicium yunnanense* (holotype CLZhao 30049). Scale bars: 1 cm (**A**); 1 mm (**B**).

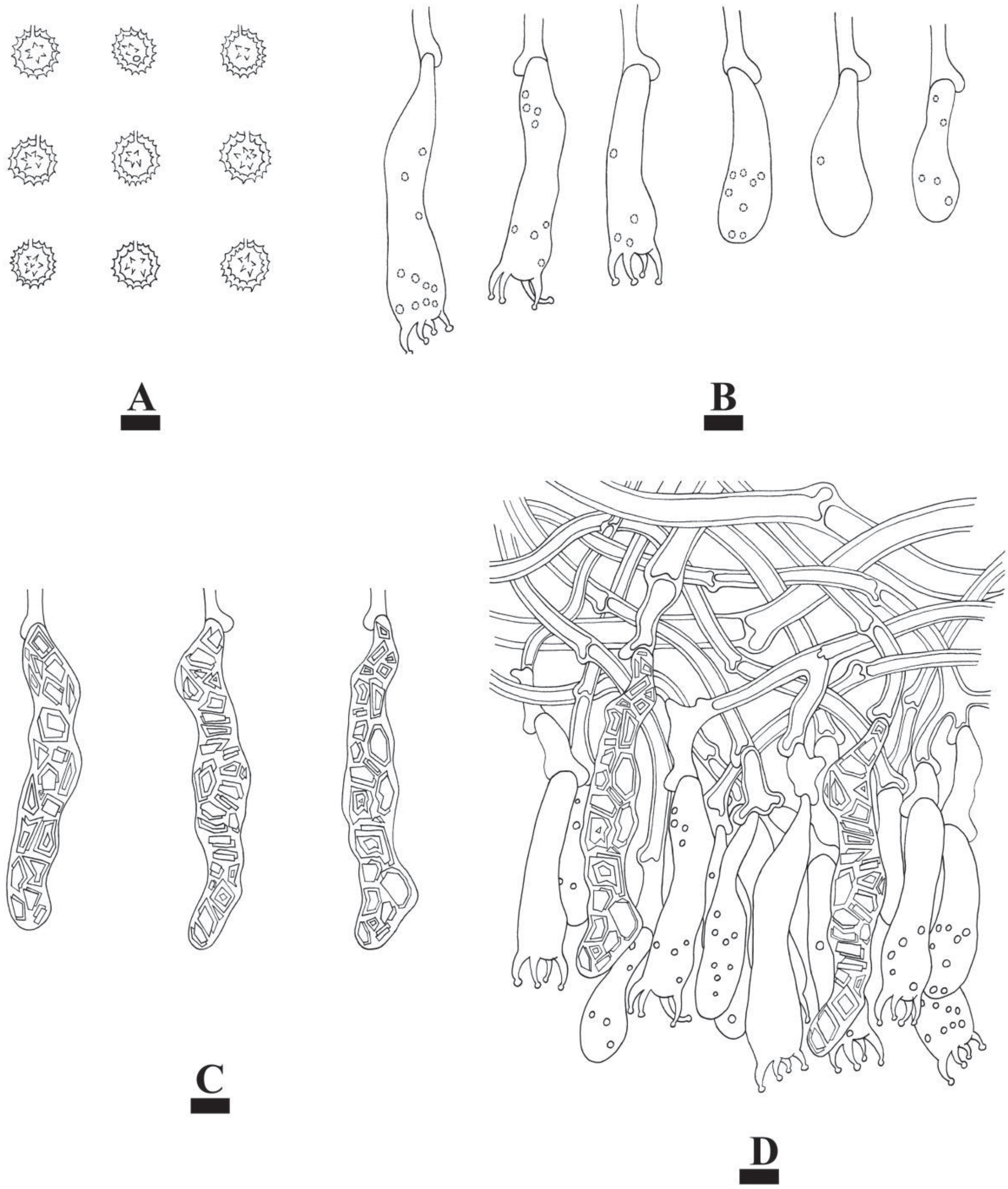

**Figure 16.** Microscopic structures of *Gloeohypochnicium yunnanense* (holotype CLZhao 30049): basidiospores (**A**); basidia and basidioles (**B**); cystidia (**C**); a section of the hymenium (**D**). Scale bars: 10 µm (**A–D**).

***Punctularia nigrodontea*** **L. Wang & C.L. Zhao, sp. nov.**
MycoBank No: 856962
Figs 18–20

**Typification.** CHINA. Yunnan Province • Dehong, Yingjiang County, Tongbiguan Provincial Nature Reserve, GPS coordinates: 25°50'N, 97°36'E, altitude: 1000 m asl., on the angiosperm trunk, leg. C.L. Zhao, 20 July 2023, CLZhao 30592 (SWFC!).

**Etymology.** *Nigrodontea* (Lat.) refers to the new species having black basidiomata.

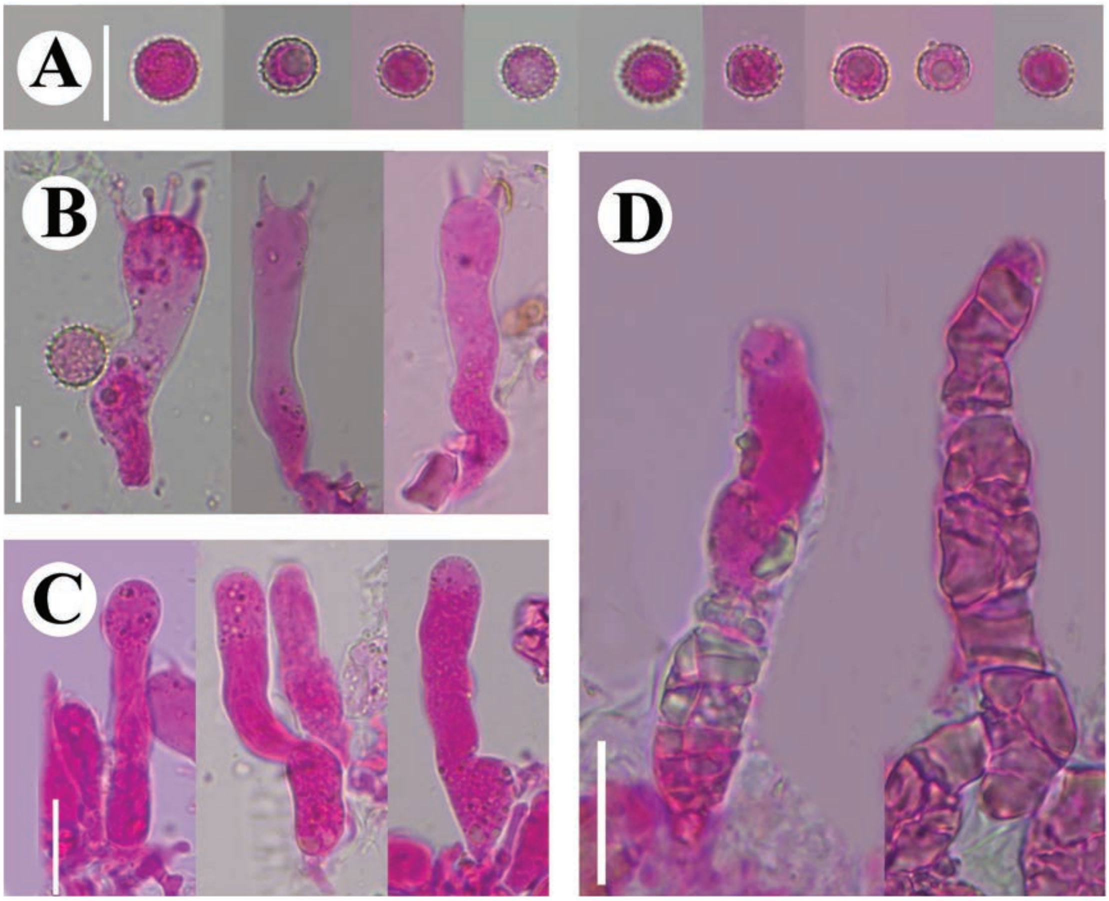

Figure 17. Sections of hymenium of *Gloeohypochnicium yunnanense* (holotype CLZhao 30049): basidiospores (**A**); basidia (**B**); basidioles (**C**); gloeocystidia (**D**). Scale bars: 20 µm (**A–D**); 10 × 100.

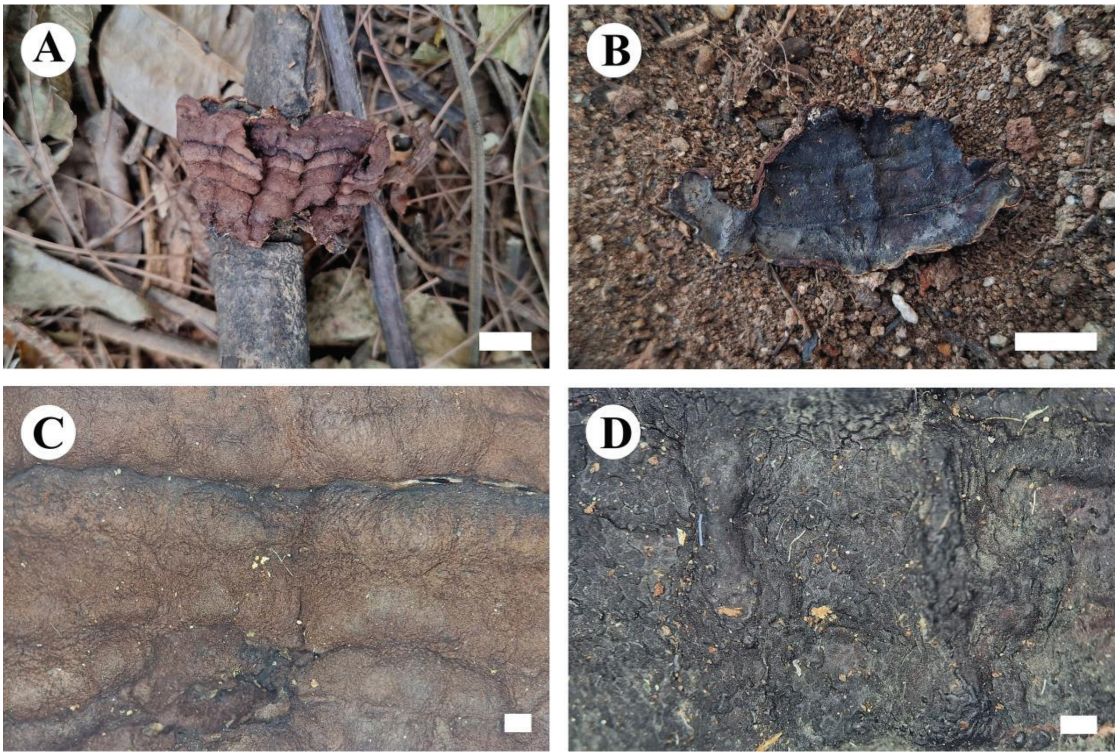

Figure 18. Basidiomata of *Punctularia nigrodontea* (holotype CLZhao 30592). Scale bars: 1 cm (**A, B**); 1 mm (**C, D**).

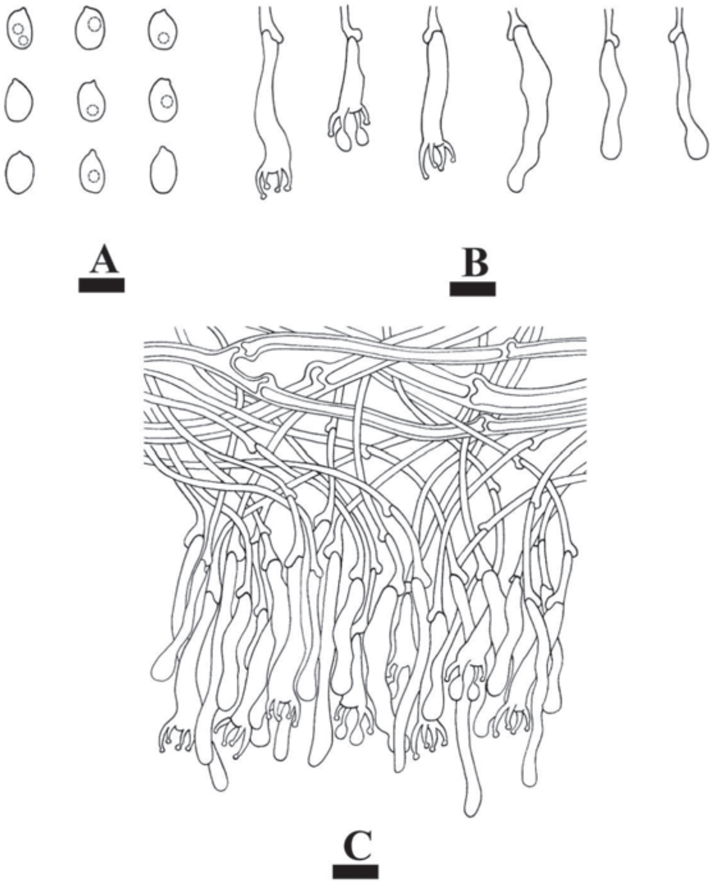

**Figure 19.** Microscopic structures of *Punctularia nigrodontea* (holotype CLZhao 30592): basidiospores (**A**); basidia and basidioles (**B**); a section of the hymenium (**C**). Scale bars: 10 µm (**A–C**).

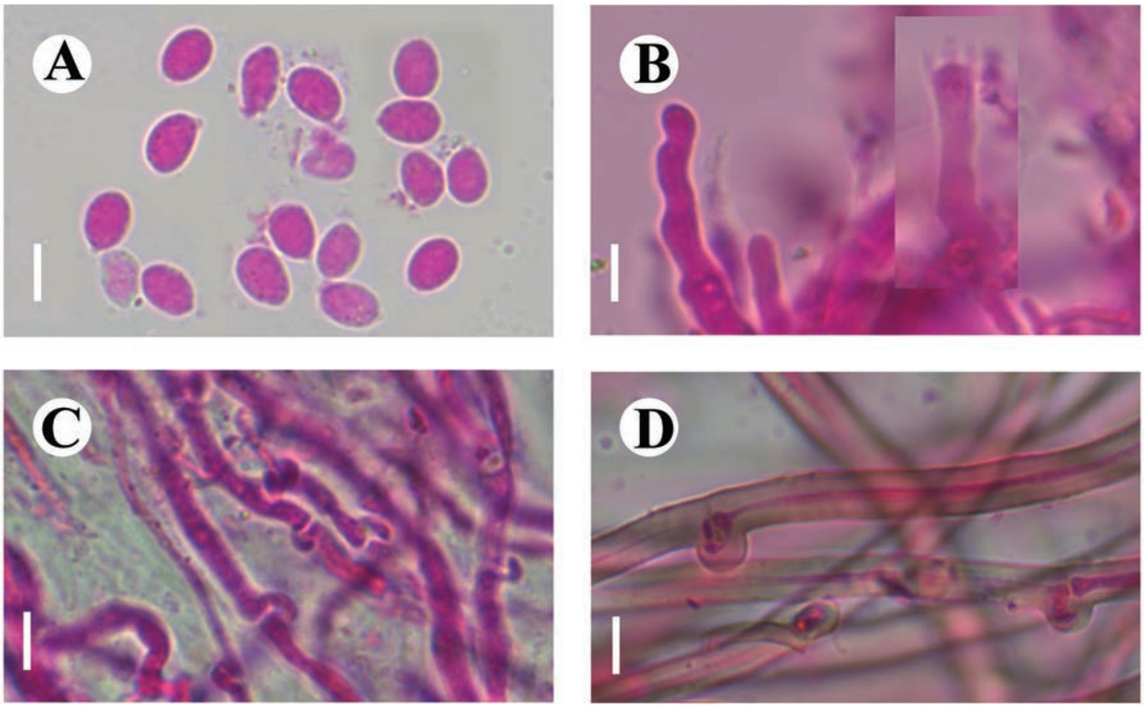

**Figure 20.** Sections of hymenium of *Punctularia nigrodontea* (holotype CLZhao 30592): basidiospores (**A**); basidia and basidioles (**B**); a section of thin-walled generative hyphae (**C**); a section of thick-walled generative hyphae (**D**). Scale bars: 10 µm (**A–D**); 10 × 100.

**Basidiomata.** Annual, resupinate to effused-reflexed, adnate but easily separable, gelatinous, without odor or taste when fresh, up to 7 cm long, 3 cm wide, and 600 μm thick. Pileal surface smooth, rigid, fuscous (5/6F5) when fresh, fuscous (5/6F5) to black (51) upon drying; pileal back cushion-shaped grandinioid, rigid, black (51) when fresh, black (51) upon drying. Sterile margin narrow, black (51), up to 1 mm.

**Hyphal system.** Monomitic; generative hyphae clamp connections, colorless, thin to thick-walled, smooth, rarely branched, interwoven, 3–7 μm in diameter; IKI–, CB–, tissues unchanged in KOH.

**Hymenium.** Cystidia absent. Basidia clavate, flexuous, with a basal clamp connection and four sterigmata, 16–25 × 3–4.5 μm; basidioles numerous, in shape similar to basidia but smaller.

**Spores.** Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, 8.5–10(–10.5) × (4.5–)5–6(–6.5) μm, L = 9.24 μm, W = 5.47 μm, Q = 1.69 (n = 30/1).

## Discussion

For fungal groups that are difficult to identify based on their morphological features, it is believed (and in most cases also proved) that the application of DNA sequences is able to delimit/recognize species much more easily and unequivocally (Wu et al. 2020; Bhunjun et al. 2024; He et al. 2022, 2024; Hyde et al. 2024a, b; Zhao et al. 2024; Zhou et al. 2024). Over time, understanding different aspects of fungi (i.e., taxonomy, diversity, species number) has improved rapidly by incorporating molecular and bioinformatics tools with traditional approaches (Cui et al. 2019; Wang et al. 2021; Hyde et al. 2023; Zhou et al. 2023; Dong et al. 2024a; Wang et al. 2024a; Wijayawardene et al. 2024).

Phylogenetically, the phylogenetic tree (Figs 1, 2) inferred from ITS+nLSU sequences revealed that the new species *Conferticium albocremeum* was nested into the family Stereaceae within the order Russulales, in which it was retrieved as a sister to *C. heimii*. However, *C. heimii* is distinguished from *C. albocremeum* by having a grayish orange hymenial surface and smaller basidiospores (5.3–6.2 × 3.5–4.2 μm; Wu 1996). Morphologically, *C. fissuratum* and *C. ravum* are similar to *C. albocremeum* by having finely verrucose, ellipsoid, and thin-walled basidiospores (Bernicchia and Gorjón 2010; Shen et al. 2024). However, *C. fissuratum* differs in its tuberculate, rough, white to cream, and cracking hymenial surface and longer subcylindrical cystidia (37–54.5 × 4–8 μm; Shen et al. 2024), and *C. ravum* differs in its smooth, brownish orange to grayish orange hymenial surface and bigger gloeocystidia (30–60 × 6–15 μm; Bernicchia and Gorjón 2010).

Phylogenetically, the multiple genes with ITS+nLSU analysis (Fig. 3) showed that the new species *Dendrocorticiopsis luteoalba* was nested into the family Punctulariaceae within the order Corticiales, and it is closely related with *D. orientalis*. Morphologically, *D. luteoalba* is similar to *D. orientalis* by having clavate apically with resinous cystidia and clavate to subclavate basidia (Wei et al. 2022). However, *D. orientalis* is delimited from *D. luteoalba* by its finely cracked, grayish ivory hymenial surface and smaller, ellipsoid to ovoid basidiospores (5–7 × 3.2–5.2 μm; Wei et al. 2022).

The phylogenetic tree (Figs 4, 5) inferred from ITS+nLSU sequences revealed that *Eichleriella biluoxueshanensis* grouped into the genus *Eichleriella* and clustered into the family Auriculariaceae, in which it was grouped with the clade comprising *E. sinensis*. However, *E. sinensis* is distinguished from *E. biluoxueshanensis* by having narrower basidiospores (10.5–16 × 5.5–7 µm; Li et al. 2023). Morphologically, *E. xinpingensis* C.L. Zhao and *E. yunnanensis* Y.L. Deng & C.L. Zhao are similar to *E. biluoxueshanensis* by all having subcylindrical to allantoid, thin-walled, and smooth basidiospores (Liu et al. 2019; Deng et al. 2024a). However, *E. xinpingensis* differs in its soft, leathery to ceraceous, flesh-pink to clay-pink, and covered by blunt-pointed spines hymenial surface and shorter basidia (15–28 × 5–9 µm; Liu et al. 2019), and *E. yunnanensis* differs in its cream to flesh-pink hymenial surface and smaller basidiospores (7.5–11.5 × 3.5–5 µm; Deng et al. 2024a).

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that *Gloeohypochnicium yunnanense* grouped into the genus *Gloeohypochnicium* and clustered into the order Russulales, in which it was closely related to *G. analogum*. Morphologically, *G. yunnanense* is similar to *G. analogum* in that it has subglobose, thick-walled, and warted basidiospores (Bernicchia and Gorjón 2010). However, *G. analogum* is delimited from *G. yunnanense* by its coriaceous, cream to ochraceous hymenial surface with a fibrillose margin and smaller gloeocystidia (40–60 × 6–10 µm; Bernicchia and Gorjón 2010).

Based on the ITS+nLSU sequence data (Fig. 3), *Punctularia nigrodontea* was nested into the family Punctulariaceae within the order Corticiales and grouped with the clade comprising *P. atropurpurascens*, *P. bambusicola*, and *P. strigosozonata*. Morphologically, *P. nigrodontea* is similar to *P. atropurpurascens*, *P. bambusicola*, and *P. strigosozonata* by having smooth, thin-walled, and ellipsoid basidiospores (Bernicchia and Gorjón 2010; Guan et al. 2021). However, *P. atropurpurascens* is delimited from *P. nigrodontea* by having the effuse-reflexed and reddish-brown to dark purplish-brown or bluish hymenial surface and larger basidia (40–65 × 5–6 µm; Guan et al. 2021). *Punctularia bambusicola* differs in its resupinate, tuberculate with rose tints, pink to purple hymenial surface, and smaller basidiospores (6.5–8.5 × 3.5–5 µm; Guan et al. 2021). *Punctularia strigosozonata* differs in its resupinate to effuse-reflexed basidiomata with a brown, velutinous margin and longer basidia (40–60 × 4–5 µm; Bernicchia and Gorjón 2010).

Fungi are an ancient, diverse, and heterogeneous group of organisms; they can be found in a wide range of habitats, and play key roles in ecosystems as decomposers, mutualists, and pathogens (Dai et al. 2015, 2021; Cui et al. 2023; Wei 2021; Bhunjun et al. 2022). The Yunnan Province is rich in woody plant species, providing excellent substrates for wood-inhabiting fungi (Dong et al. 2023, 2024a; Wang and Cai 2023; Deng et al. 2024b; Wang et al. 2024b; Zhu et al. 2024). Our study is helpful to further understand the species diversity of wood-inhabiting fungal groups in Yunnan and explore their evolutionary relationships.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Conceptualization, CZ, AY, and LW; methodology, CZ and LW; software, CZ, YJ, and LW; validation, CZ, SG, and HY; formal analysis, CZ, YJ, and LW; investigation, CZ and LW; resources, CZ; writing – original draft preparation, CZ, AY, LW, and JY; writing – review and editing, CZ and LW; visualization, CZ and LW; supervision, CZ; project administration, CZ; funding acquisition, CZ. All authors have read and agreed to the published version of the manuscript.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# Three new species of *Peroneutypa* (Diatrypaceae, Xylariales) and a first record of *Eutypa camelliae* in China with updated description

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## Abstract

Diatrypaceae is a diverse family with a worldwide distribution, occurring on a wide range of hosts in terrestrial and marine environments, some of which are important plant pathogens. During a survey of ascomycete diversity in Guizhou Province, China, three new taxa within *Peroneutypa* are proposed based on morphological comparisons and phylogenetic analyses of combined ITS and *tub2* sequences data. The newly proposed species are *Peroneutypa guizhouensis*, *P. wanfenglinensis* and *P. zhujiashanesis.* In addition, *Eutypa camelliae* was recorded for the first time from China, with an updated description. Detailed morphological descriptions, illustrations, comparative analyses, and a tabular comparison of the new species with related and similar taxa are provided.

**Key words:** 3 new species, diatrypaceous fungi, fungal systematics, Karst environment

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## Introduction

Diatrypaceae is a diverse and ecologically important family of higher ascomycetes within the order Xylariales, inhabiting a wide variety of hosts in both terrestrial and marine environments worldwide (Chlebicki 1986; Glawe and Jacobs 1987; Carmarán and Romero 1992; Carmarán et al. 2006; Trouillas and Gubler 2010; de Almeida et al. 2016; Li et al. 2023). The members of Diatrypaceae are distributed worldwide, can be found on a wide range of plant species, from economically important crops to forest trees with different life modes, functioning as saprobes, pathogens, and endophytes (Vasilyeva and Ma 2014; Dayarathne et al. 2016; Mayorquin et al. 2016; Senwanna et al. 2017; Hyde et al. 2020; Konta et al. 2020). Members of the Diatrypaceae are characterized by black or dark brown, immersed or erumpent, eustromatic or pseudostromatic stromata, 8-spored or polysporous asci with a very long pedicel and J-/J+ apical apparatus, hyaline to light brown allantoid ascospores, and a libertella-like asexual morph (Senanayake et al. 2015; Wijayawardene et al. 2017).

In Wijayawardene et al. (2022) 22 genera within Diatrypaceae have been documented. However, recent taxonomic advances have led to the addition of three more genera, *Alloeutypa, Pseudoeutypa*, and *Stromatolinea*, bringing the total to 25 recognized genera (Ma et al. 2023; Habib et al. 2024; Zhang et al. 2024). Collectively, these genera encompass approximately 1000 species. Most of the species belong to *Cryptosphaeria*, *Diatrype*, *Diatrypella*, *Eutypa*, and *Eutypella* (Wijayawardene et al. 2022) and are polyphyletic. The polyphyletic nature of these genera arises primarily from the phenotypic plasticity and anatomical similarities observed within Diatrypaceae, where stromatal characteristics are highly variable and often unreliable for clear species delineation (Zhu et al. 2021; Li et al. 2023; Habib et al. 2024). Previous studies have highlighted the challenges in distinguishing members of Diatrypaceae based on morphology alone, with many taxa still lacking molecular data. This absence of molecular data complicates the classification of Diatrypaceae (de Almeida et al. 2016; Shang et al. 2018; Du et al. 2022). Similar difficulties are evident in *Peroneutypa*. Currently, 38 species of *Peroneutypa* are listed in Species Fungorum (https://www.speciesfungorum.org), but molecular data are available for only 20 of these species.

*Peroneutypa* was established by Berlese (1902), but no type species was designated at the time. Later, Rappaz (1987) proposed *P. bellula* as the type species and considered the genus as a synonym of *Eutypella*. Carmarán et al. (2006) reinstated *Peroneutypa* as a distinct genus based on its ascal type and phylogenetic analyses. *Peroneutypa* is distinguished by its urn-shaped asci, which have a truncate apex and are wider in the middle, where ascospores tend to cluster. In contrast, *Eutypella* features spindle-shaped asci with ascospores that cluster and swell in the upper portion (Carmarán et al. 2006). *Peroneutypa* species are characterized by valsoid stroma, ascomata with long prominent necks, sessile to long-stalked asci, with truncate apices and allantoid hyaline or yellowish ascospores (Carmarán et al. 2006; Vasilyeva and Rogers 2010; Shang et al. 2017). Species of the genus are known as saprobes or pathogens and are widely distributed in terrestrial and marine habitats (Shang et al. 2017; Dayarathne et al. 2020; Du et al. 2022)

In a study focused on the diversity of ascomycetes in Guizhou, China, we identified several Diatrypaceae specimens that did not match any known species. To clarify their taxonomic status, we performed phylogenetic analyses using the internal transcribed spacer (ITS) and β-tubulin (*tub2*) gene regions. These analyses led to the discovery of three new species belonging to *Peroneutypa*. We present a brief diagnosis, descriptions, images, and phylogenetic placement of these new species.

## Materials and methods

### Collection and isolation

Ascomycetous fungi associated with decayed branches and twigs of various plants were collected during surveys conducted in Guizhou Province, China. All related habitat information, including details about elevation, climatic conditions, and geographical features, was recorded. The photos of the collected materials were taken using a Canon G15 camera (Canon Corporation, Tokyo, Japan). Materials were placed in paper bags and were taken to the lab for examination. To preserve the freshness of the specimens, they were dried at room temperature. Fungal isolates were obtained through single spore isolation, following the method described by Senanayake et al. (2020). Spores were observed under a Stereo Zoom microscope and transferred to potato dextrose agar (PDA; 39 g/L in distilled water, Difco potato dextrose). Cultures were incubated at 25–30 °C for 1–4 weeks with regular observations. Cultural characteristics, including mycelial color, shape, texture, and growth rate, were documented under normal light conditions.

Herbarium specimens were deposited in the Cryptogams Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences (KUN-HKAS), and the Guizhou Provincial Key Laboratory of Agricultural Biotechnology (GZAAS).

## Morphological study

Macroscopic characteristics were observed under an Olympus SZ61 stereomicroscope and photographed with a Canon 700D digital camera fitted to a light microscope (Nikon Ni). The morphological characteristics of specimens were examined, and photomicrographs were taken as described in Senanayake et al. (2020). Materials were mounted in water for anatomical examination, and Melzer's reagent was used where necessary. More than 30 ascospores and 30 asci were measured using the Tarosoft ® image framework (v. 0.9.0.7). Images were arranged using Adobe Photoshop CS6 (Adobe Systems, USA).

## DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from mycelium sourced from colonies cultured on PDA after 1–2 weeks at 25 °C, using the BIOMIGA Fungal gDNA Isolation Kit (BIOMIGA, Hangzhou City, Zhejiang Province, China). The DNA samples were stored at –20 °C. Internal transcribed spacers (ITS), and β-tubulin (*tub2*), were amplified by PCR with primers ITS1/ITS4 (White et al. 1990; Gardes and Bruns 1993), and Bt2a / Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997), respectively. The components of a 25 μL volume PCR mixture was: 9.5 μL of double distilled water, 12.5 μL of PCR Master Mix, 1 μL of each primer and 1 μL of template DNA. The PCR amplification was conducted as reported by Samarakoon et al. (2022). Qualified PCR products were checked through 1.5% agarose gel electrophoresis stained with GoldenView, and sent to Qingke Biotech Chongqing, China, for sequencing.

## Phylogenetic analyses

The newly generated forward and reverse sequences from this study were assembled in the BioEdit v. 7.0.5 (Hall 1999) then were subjected to BLASTn search against the GenBank nucleotide database at the National Center for Biotechnology Information (NCBI) to identify closely related sequences. Sequence data of related taxa were obtained from previous publications (Long et al. 2021; Zhu et al. 2021; Li et al. 2023) and downloaded from the GenBank database (Table 1). The sequences were aligned using MAFFT v.7 online web server (Katoh et al. 2019) under default settings. Alignment was adjusted manually using BioEdit

**Table 1.** Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers.

| Taxa | Strain number | GenBank Accession number | | Reference |
|---|---|---|---|---|
| | | ITS | β-tubulin | |
| *Allocryptovalsa castaneae* | CFCC52428 | MW632945 | MW656393 | Zhu et al. (2021) |
| *Allocryptovalsa castaneicola* | CFCC52432 | MW632947 | MW656395 | Zhu et al. (2021) |
| *Allocryptovalsa cryptovalsoidea* | HVFIG02$^{T}$ | HQ692573 | NA | Trouillas et al. (2011) |
| *Allocryptovalsa elaeidis* | MFLUCC150707 | MN308410 | MN340296 | Konta et al. (2020) |
| *Allocryptovalsa polyspora* | MFLU 17-1218 | NR153588 | MG334556 | Senwanna et al. (2017) |
| *Allocryptovalsa rabenhorstii* | WA08CB | HQ692619 | HQ692523 | Trouillas et al. (2011) |
| *Allocryptovalsa rabenhorstii* | GMB0416 | OP935171 | OP938733 | Li et al. (2023) |
| *Allocryptovalsa sichuanensis* | HKAS107017 | MW240633 | MW775592 | Samarakoon et al. (2022) |
| *Allocryptovalsa xishuangbanica* | KUMCC21-0830 | ON041128 | ON081498 | Maharachchikumbura et al. (2022) |
| *Allodiatrype albelloscutata* | IFRD9100 | OK257020 | NA | Li et al. (2022) |
| *Allodiatrype arengae* | MFLUCC 15-0713 | MN308411 | MN340297 | Konta et al. (2020) |
| *Allodiatrype elaeidicola* | MFLUCC15-0737a | MN308415 | MN340299 | Konta et al. (2020) |
| *Allodiatrype elaeidis* | MFLUCC150708a | MN308412 | MN340298 | Konta et al. (2020) |
| *Allodiatrype eleiodoxae* | MFLU23-0357 | OR571761 | OR591484 | Unpublished |
| *Allodiatrype dalbergiae* | MFLU23-0349 | OR571759 | OR771026 | Unpublished |
| *Allodiatrype dalbergiae* | MFLU23-0350 | OR571760 | OR591487 | Unpublished |
| *Allodiatrype taiyangheensis* | IFRDCC2800 | OK257021 | OK345036 | Li et al. (2022) |
| *Allodiatrype thailandica* | MFLUCC153662 | KU315392 | NA | Li et al. (2016) |
| *Allodiatrype trigemina* | FCATAS842 | MW031919 | MW371289 | Peng et al. (2021) |
| *Alloeutypa flavovirens* | E48C | AJ302457 | DQ006959 | Rolshausen et al. (2006) |
| *Alloeutypa milinensis* | FCATAS4309 | OP538689 | OP557595 | Ma et al. (2023) |
| *Anthostoma decipiens* | JL567 | JN975370 | JN975407 | Luque et al. (2012) |
| *Cryptosphaeria ligniota* | CBS273.87 | KT425233 | KT425168 | Acero et al. (2004) |
| *Cryptosphaeria multicontinentalis* | HBPF8 | KT425178 | NA | Trouillas et al. (2015) |
| *Cryptosphaeria pullmanensis* | ATCC52655 | KT425235 | KT425170 | Trouillas et al. (2015) |
| *Cryptosphaeria pullmanensis* | HBPF24 | KT425202 | KT425137 | Trouillas et al. (2015) |
| *Cryptosphaeria subcutanea* | CBS240.87 | KT425232 | KT425167 | Trouillas et al. (2015) |
| *Cryptovalsa ampelina* | A001 | GQ293901 | GQ293972 | Trouillas and Gubler (2010) |
| *Cryptovalsa ampelina* | DRO101 | GQ293902 | GQ293982 | Trouillas and Gubler (2010) |
| *Diatrypasimilis australiensis* | ATCC MYA-3540 | NR111369 | NA | Schoch et al. (2014) |
| *Diatrype bullata* | UCDDCh 400 | DQ006946 | DQ007002 | Rolshausen et al. (2006) |
| *Diatrype camelliae-japonicae* | GMB0427 | OP935172 | OP938734 | Li et al. (2023) |
| *Diatrype disciformis* | GNA14 | KR605644 | KY352434 | Senanayake et al. (2015) |
| *Diatrype lancangensis* | GMB0045 | MW797113 | MW814885 | Long et al. (2021) |
| *Diatrype macowaniana* | Isolate D15C | AJ302431 | NA | Acero et al. (2004) |
| *Diatrype quercicola* | CFCC-52418 | MW632938 | MW656386 | Zhu et al. (2021) |
| *Diatrype rubi* | GMB0429 | OP935182 | OP938740 | Li et al. (2023) |
| *Diatrypella atlantica* | HUEFS 194228 | KM396615 | KR363998 | de Almeida et al. (2016) |
| *Diatrypella banksiae* | CPC29118 | KY173402 | NA | Crous et al. (2013) |
| *Diatrypella betulicola* | CFCC52411 | MW632935 | MW656383 | Zhu et al. (2021) |
| *Diatrypella delonicis* | MFLUCC15-1014 | MH812994 | MH812994 | Hyde et al. (2019) |
| *Diatrypella elaeidis* | MFLUCC15-0279 | MN308417 | MN340300 | Konta et al. (2020) |
| *Diatrypella fatsiae-japonica* | GMB0422 | OP935184 | OP938744 | Li et al. (2023) |
| *Diatrypella frostii* | UFMGCB 1917 | HQ377280 | NA | Vieira et al. (2012) |
| *Diatrypella heveae* | MFLUCC17-0368 | MF959501 | MG334557 | Senwanna et al. (2017) |
| *Diatrypella hubeiensis* | CFCC52413 | MW632937 | NA | Zhu et al. (2021) |
| *Diatrypella iranensis T* | KDQ18 | KM245033 | KY352429 | Mehrabi et al. (2015) |
| *Diatrypella longiasca T* | KUMCC 20-0021 | MW039349 | MW239658 | Dissanayake et al. (2021) |
| *Diatrypella macrospora T* | KDQ15 | KR605648 | KY352430 | Mehrabi et al. (2016) |
| *Diatrypella major* | ANM1947 | KU320613 | NA | de Almeida et al. (2016) |
| *Diatrypella pulvinata* | H048 | FR715523 | FR715495 | de Almeida et al. (2016) |
| *Diatrypella tectonae* | MFLUCC120172b | KY283085 | KY421043 | Shang et al. (2017) |
| *Diatrypella vulgaris* | HVFRA02 | HQ692591 | HQ692503 | Trouillas et al. (2011) |
| *Diatrypella yunnanensis* | VT01 | MN653008 | MN887112 | Zhu et al. (2021) |
| *Eutypa armeniacae* | ATCC28120 | DQ006948 | DQ006975 | Rolshausen et al. (2006) |

| Taxa | Strain number | GenBank Accession number | | Reference |
|---|---|---|---|---|
| | | ITS | β-tubulin | |
| *Eutypa astroidea* | CBS292.87 | DQ006966 | DQ006966 | Rolshausen et al. (2006) |
| *Eutypa camelliae* | HKAS107022 | MW240634 | MW775593 | Samarakoon et al. (2022) |
| *Eutypa camelliae* | GZAAS24-0013 | PP528182 | PQ301430 | This study |
| *Eutypa camelliae* | HKAS-107022 | NR175674 | MW775593 | This study |
| *Eutypa cerasi* | GMB0048 | MW797104 | MW814893 | Long et al. (2021) |
| *Eutypa consobrina* | F091 | AJ302447 | KY111596 | Acero et al. (2004) |
| *Eutypa crustata* | CBS210.87 | AJ302448 | DQ006968 | Rolshausen et al. (2006) |
| *Eutypa laevata* | CBS291.87 | AJ302449 | NA | Acero et al. (2004) |
| *Eutypa lata* | EP18 | HQ692611 | HQ692611 | Trouillas et al. (2011) |
| *Eutypa lejoplaca* | CBS248.87 | DQ006922 | DQ006974 | Rolshausen et al. (2006) |
| *Eutypa maura* | CBS219.87 | DQ006926 | DQ006967 | Rolshausen et al. (2006) |
| *Eutypa microasca* | BAFC51550 | KF964566 | KF964572 | Grassi et al. (2014) |
| *Eutypa petrakii var. hedarae* | BENT014 | OP038000 | OP079836 | Unpublished |
| *Eutypa sparsa* | 38023b | AY684220 | AY684201 | Trouillas and Gubler (2004) |
| *Eutypa tetragona* | CBS284.87 | DQ006923 | DQ006960 | Rolshausen et al. (2006) |
| *Eutypella cerviculata* | EL59C | AJ302468 | NA | Acero et al. (2004) |
| *Eutypella motuoensis* | FCATAS4082 | OP538693 | OP557599 | Ma et al. (2023) |
| *Eutypella persica* | IRAN 2540C | KX828144 | KY352451 | Mehrabi et al. (2019) |
| *Eutypella quercina* | IRAN2543C | KX828139 | KY352449 | Mehrabi et al. (2019) |
| *Eutypella semicircularis* | MP4669 | JQ517314 | NA | Mehrabi et al. (2016) |
| *Eutypella virescens* | CBS205.36 | MH855778 | MH867286 | Vu et al. (2019) |
| *Halocryptovalsa salicorniae* | MFLUCC 15-0185 | MH304410 | MH370274 | Dayarathne et al. (2020) |
| *Halodiatrype avicenniae* | MFLUCC 150953 | KX573916 | KX573931 | Dayarathne et al. (2016) |
| *Halodiatrype salinicola* | MFLUCC 15-1277 | KX573915 | KX573932 | Dayarathne et al. (2016) |
| *Kretzschmaria deusta* | CBS 826.72 | KU683767 | KU684190 | U'Ren et al. (2016) |
| *Monosporascus cannonballus* | CMM3646 | JX971617 | NA | Unpublished |
| *Monosporascus cannonballus* | ATCC26931 | FJ430598 | NA | Unpublished |
| *Neoeutypella baoshanensis* | HMAS255436 | MH822887 | MH822888 | Phookamsak et al. (2019) |
| *Paraeutypella citricolca* | MFLU23-0352 | OR563996 | NA | Unpublished |
| *Paraeutypella guizhouensis* | KUMCC 20-0017 | MW036142 | MW239661 | Dissanayake et al. (2021) |
| *Paraeutypella pseudoguizhouensis* | GMB0420 | OP935186 | OP938748 | Li et al. (2023) |
| *Pedumispora rhizophorae* | BCC44877 | KJ888853 | NA | Klaysuban et al. (2014) |
| *Peroneutypa aquilariae* | KUNCC-2210817 | NR185767 | OP572195 | (Du et al. 2022). |
| *Peroneutypa anomianthe* | KUNCC-2315540 | PP584741 | PQ046048 | Dissanayake et al. (2024) |
| *Peroneutypa anomianthe* | MFLU-210242 | OK393705 | NA | de Silva et al. (2022) |
| *Peroneutypa alsophila* | CBS250.87 | AJ302467 | NA | Acero et al. (2004) |
| *Peroneutypa comosa* | BAFC393 | KF964568 | NA | Grassi et al. (2014) |
| *Peroneutypa curvispora* | HUEFS136877 | KM396641 | KM396641 | de Almeida et al. (2016) |
| *Peroneutypa diminutiasca* | MFLUCC17-2144 | MG873479 | NA | Shang et al. (2018) |
| *Peroneutypa diminutispora* | HUEFS192196 | KM396647 | NA | de Almeida et al. (2016) |
| ***Peroneutypa guizhouensis*** | **GZAAS24-0087** | **PQ878089** | **PQ876910** | **This study** |
| ***Peroneutypa guizhouensis*** | **GZAAS24-0088** | **PQ878090** | **PQ876911** | **This study** |
| *Peroneutypa hainanensis* | GMB0424 | OP935179 | OP938746 | Li et al. (2023) |
| *Peroneutypa hainanensis* | GMB0425 | OP935180 | OP938747 | Li et al. (2023) |
| *Peroneutypa hongheensis* | KUNCC-23-16753 | PP584742 | PP951427 | Dissanayake et al. (2024) |
| *Peroneutypa indica* | NFCCI 4393 | MN061368 | MN431498 | Dayarathne et al. (2020) |
| *Peroneutypa kochiana* | F092 | AJ302462 | NA | Carmarán et al. 2006 |
| *Peroneutypa kunmingensis* | HKAS 113189 | MZ475070 | MZ475070 | Phukhamsakda et al. (2022) |
| *Peroneutypa leucaenae T* | MFLU 18-0816 | MW240631 | MW775591 | Samarakoon et al. (2022) |
| *Peroneutypa longiasca* | MFLUCC170371 | MF959502 | MG334558 | Senwanna et al. (2017) |
| *Peroneutypa mackenziei* | MFLUCC16-0072 | KY283083 | KY706363 | Shang et al. (2017) |
| *Peroneutypa mangrovei* | PUFD526 | MG844286 | MH094409 | Phookamsak et al. (2019) |
| *Peroneutypa nayariophyti* | MFLU 23-0077 | OQ981955 | OR019690 | Unpublished |
| *Peroneutypa polysporae* | NFCCI4392 | MN061367 | MN431497 | Dayarathne et al. (2020) |
| *Peroneutypa qianensis* | GMB0431 | OP935177 | NA | Li et al. (2023) |
| *Peroneutypa qianensis* | GMB0432 | OP935178 | NA | Li et al. (2023) |
| *Peroneutypa rubiformis* | MFLU 17-1185 | MG873477 | MH316763 | Shang et al. (2018) |

| Taxa | Strain number | GenBank Accession number | | Reference |
|---|---|---|---|---|
| | | ITS | β-tubulin | |
| *Peroneutypa scoparia* | MFLUCC 17-2143 | MG873477 | NA | Shang et al. (2018) |
| ***Peroneutypa wanfenglinensis*** | **GZAAS24-0021** | **PP852356** | **PQ301419** | **This Study** |
| ***Peroneutypa wanfenglinensis*** | **GZAAS24-0022** | **PP852353** | **PQ301420** | **This Study** |
| ***Peroneutypa zhujiashanesis*** | **GZAAS24-0023** | **PP852363** | **PQ301421** | **This Study** |
| ***Peroneutypa zhujiashanesis*** | **GZAAS24-0024** | **PP852362** | **PQ301422** | **This Study** |
| *Pseudodiatrype hainanensis* | GMB0054 | MW797111 | MW814883 | Long et al. (2021) |
| *Quaternaria quaternata* | CBS278.87 | AJ302469 | NA | Acero et al. (2004) |
| *Quaternaria quaternata* | GNF13 | KR605645 | NA | Mehrabi et al. (2016) |
| *Stromatolinea grisea* | GMB4508 | PQ113921 | PQ115209 | Habib et al. (2024) |
| *Stromatolinea guizhouensis* | GMB4523 | PQ113922 | PQ115210 | Habib et al. (2024) |
| *Stromatolinea guizhouensis* | GMB4515 | PQ113923 | PQ115211 | Habib et al. (2024) |
| *Stromatolinea hydei* | GMB4509 | PQ113924 | PQ115212 | Habib et al. (2024) |
| *Stromatolinea linearis* | MFLUCC 15-0198 | KU940149 | MW775587 | Habib et al. (2024) |
| *Stromatolinea xishuiensis* | GMB4522 | PQ113928 | PQ115216 | Habib et al. (2024) |
| *Vasilyeva cinnamomic* | GMB0418 | OP935174 | OP938737 | Li et al. (2023) |
| *Xylaria hypoxylon* | CBS 122620 | AM993141 | KX271279 | Peršoh et al. (2009) |

v.7.0.5.3 (Hall 1999) where necessary. The combined sequence data was used to perform maximum likelihood (ML) and Bayesian inference analysis (BI). The ML analysis was implemented in RAxML v.8.2.12 using the GTR substitution model with 1,000 bootstrap replicates (Stamatakis 2014). Bayesian inference analysis was conducted in MrBayes v. 3.2.2 (Ronquist et al. 2012) online, with Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.2.2 (Ronquist et al. 2012) used to calculate posterior probabilities (PP). Six simultaneous Markov chains were run for 1,000,000 generations, and trees were sampled every 1,000th generation. The convergence of the MCMC procedure was assessed from the effective sample size scores (all > 100) using MrBayes. The first 25% of the trees were discarded as burn-ins. The remainder was used to calculate the posterior probabilities (PPs) for individual branches. The phylogenetic tree was visualized in FIGTREE v.1.4.3 (Rambaut 2012). All analyses were run on the CIPRES Science Gateway v 3.3 web portal (Miller et al. 2010).

## Results

### Phylogenetic analyses

The combined ITS and *tub2* dataset consisted of 125 ingroup strains and two outgroups: *Kretzschmaria deusta* (CBS 826.72) and *Xylaria hypoxylon* (CBS 122620). After the exclusion of ambiguously aligned regions and long gaps, the final combined data matrix contained 1,350 characters. The final ML optimization likelihood value of the best RAxML tree was −34243.792546. The tree topology derived from Maximum Likelihood (ML) analysis closely resembled that of Bayesian Inference (BI) analysis. The best-scoring RAxML tree is shown in Fig. 1.

The phylogenetic tree based on BI and ML approaches confirmed the position of our new species nested within the phylogenetic branch of the genus *Peroneutypa* (Fig. 1). According to the phylogenetic structure of the tree, *Peroneutypa* formed a large clade. However, the presence of *Eutypa microasca* (BAFC 51550) in *Peroneutypa* clade renders its status polyphyletic.

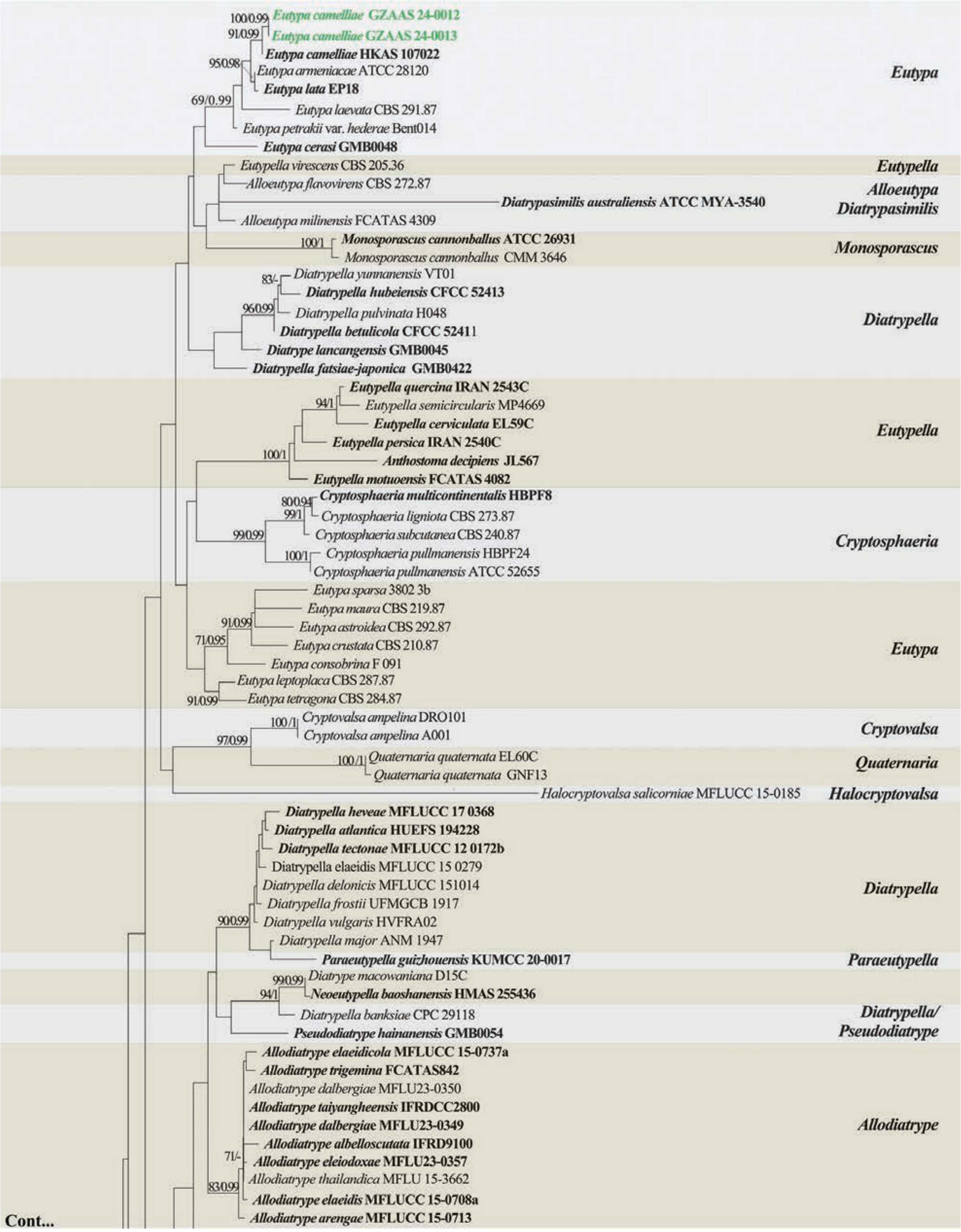

**Figure 1.** Phylogram generated from maximum likelihood analysis (RAxML) based on combined ITS and *tub2* sequences data. Bootstrap support values for maximum likelihood (ML) greater than 70% and Bayesian posterior probabilities (BPP) greater than 0.90 are displayed at the respective branches (ML/BPP). The newly described species are marked bold in red, and the new record is marked bold in green. Ex-type/type strains are indicated in black bold.

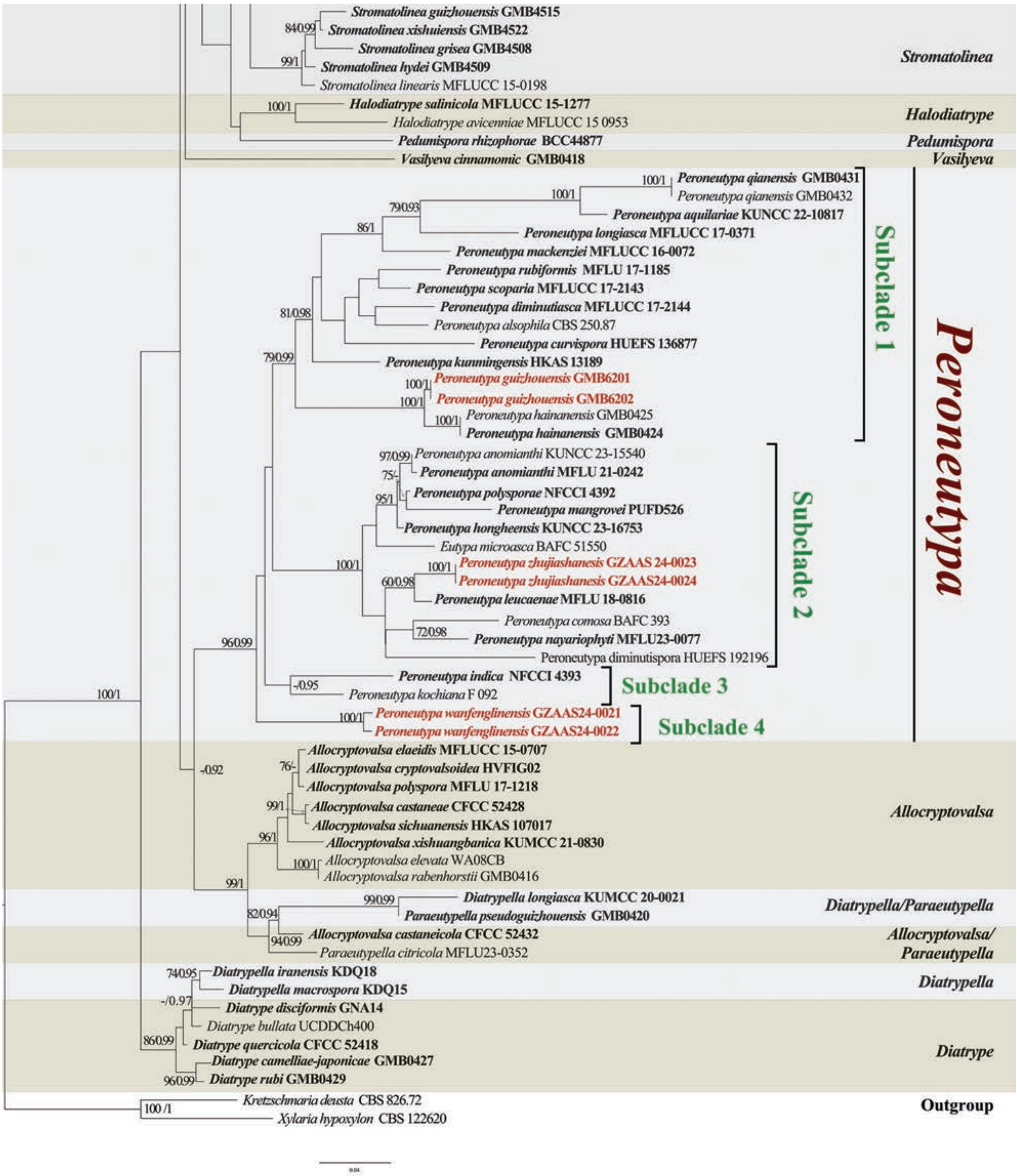

**Figure 1.** Continued

This suggests that the taxonomic status of *Eutypa microasca* should be revisited for clarification. In the phylogram, the *Peroneutypa* clade is represented with 4 subclades. Subclade 1 includes 12 species, with the new species *P. guizhouensis* forming a sister relationship with *P. hainanensis*, supported strongly (ML/BI = 100/1). Subclade 2 contains 10 species, including the new species *P. zhujiashanesis*, which appears as a sister to *P. leucaenae* with moderate support (ML/BI = 60/0.99). Subclade 3 consists of two species, *P. indica*

(NFCCI 4393) and *Peroneutypa kochiana* (F092). Subclade 4 represents solely the new species *P. wanfenglinensis*, forming a distinct, well-supported clade (ML/BI = 96/0.99) at the basal position of the *Peroneutypa* clade. The newly generated sequences of *Eutypa camelliae* clustered with the type strain *E. camelliae* (HKAS107022). The sister branch to this clade includes *E. lata* (EP18) and *E. americana* (ATCC28120).

## Taxonomy

### ***Eutypa camelliae* Samarakoon, M.C & Hyde, K.D, Fungal Diversity 112 (1), 1–88 (2022)**

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Fig. 2

**Description.** Saprobic on a dead branch of an unknown tree. Sexual morph: Stromata 3.1–8.3 mm diam., immersed in the bark, carbonaceous, effuse, confluent into irregularly elongated shape with diffuse margins, dark grayish to dull black surface, rarely with white dots on the surface, 20–50 loculate. Ascomata 390–620 µm in height, 190–340 µm in diameter ($\bar{x}$ = 530 × 260 µm, n = 10), perithecia, coated with a white powdery substance in between, vary from globose to akin to an inverted flask, Ostiole slightly raised, conspicuous, 114–128 µm wide. Peridium 13.5–25 µm thick, two-layered, outer layer dark brown of textural angular cell, inner layer hyaline of elongated cell. Paraphyses septate, 3.5–7.2 µm ($\bar{x}$ = 5.6 µm, n = 20) wide, constricted at the septa, longer than asci. Asci 50–120 × 4–6.6 µm ($\bar{x}$ = 77.5 × 5.4 µm, n = 30), 8–spored clavate, with a rounded to truncate apex, J- apical rings. Ascospore 4–5.8 × 1.1–1.8 µm ($\bar{x}$ = 4.93 × 1.35 µm, n = 30), overlap, allantoid, slightly curved, subhyaline, smooth, aseptate, often with a guttulae at both ends. Asexual morph: undetermined.

**Culture characteristics.** Colonies on PDA reach 60 mm in diameter after seven days at 28 °C. They are cottony, moderately dense, fluffy aerial mycelium, white from above and pale yellowish from below. Mycelium is composed of branched, septate, smooth-walled, hyaline hyphae.

**Specimens examined.** China • Guizhou Province, Kaili City, Leigongshan State Reserve (108°11'47"E, 26°22'43"N), altitude 1664 m, on a dead branch of an unknown tree, 23 August 2023, Xin Y Mao & Y.Q. Kang, LGS19 (GZAAS24-0012, KUN-HKAS133146, strain number GZCC 24-0187). GenBank accession numbers (ITS: PP528179; *tub2*: PQ301429). Guizhou Province, Libo County, MaoLan National Nature Reserve (108°4'9"E, 25°17'8"N), altitude 694 m, on dead branches of an unknown tree, 22 March 2022, Xin Y Mao & Y.Q. Kang, LBML10 (GZAAS24-0013, KUN-HKAS133147; strain number GZCC 24-0188). GenBank accession numbers (ITS: PP528182; *tub2*: PQ301430).

**Notes.** The sequence of our collection GZAAS24-0012 clustered with *Eutypa camelliae* in the phylogenetic tree, and ITS sequence BLAST searches also confirmed a 100% match with *E. camelliae* (HKAS 107022). The holotype description of this species was based on immature stromata, no asci or ascospores were observed in the material and the isolates were obtained from internal tissue of the stromata (Samarakoon et al. 2022). Our study represents the first report of *Eutypa camelliae* from China and provides the complete anatomical details, including mature stromata with asci and ascospores.

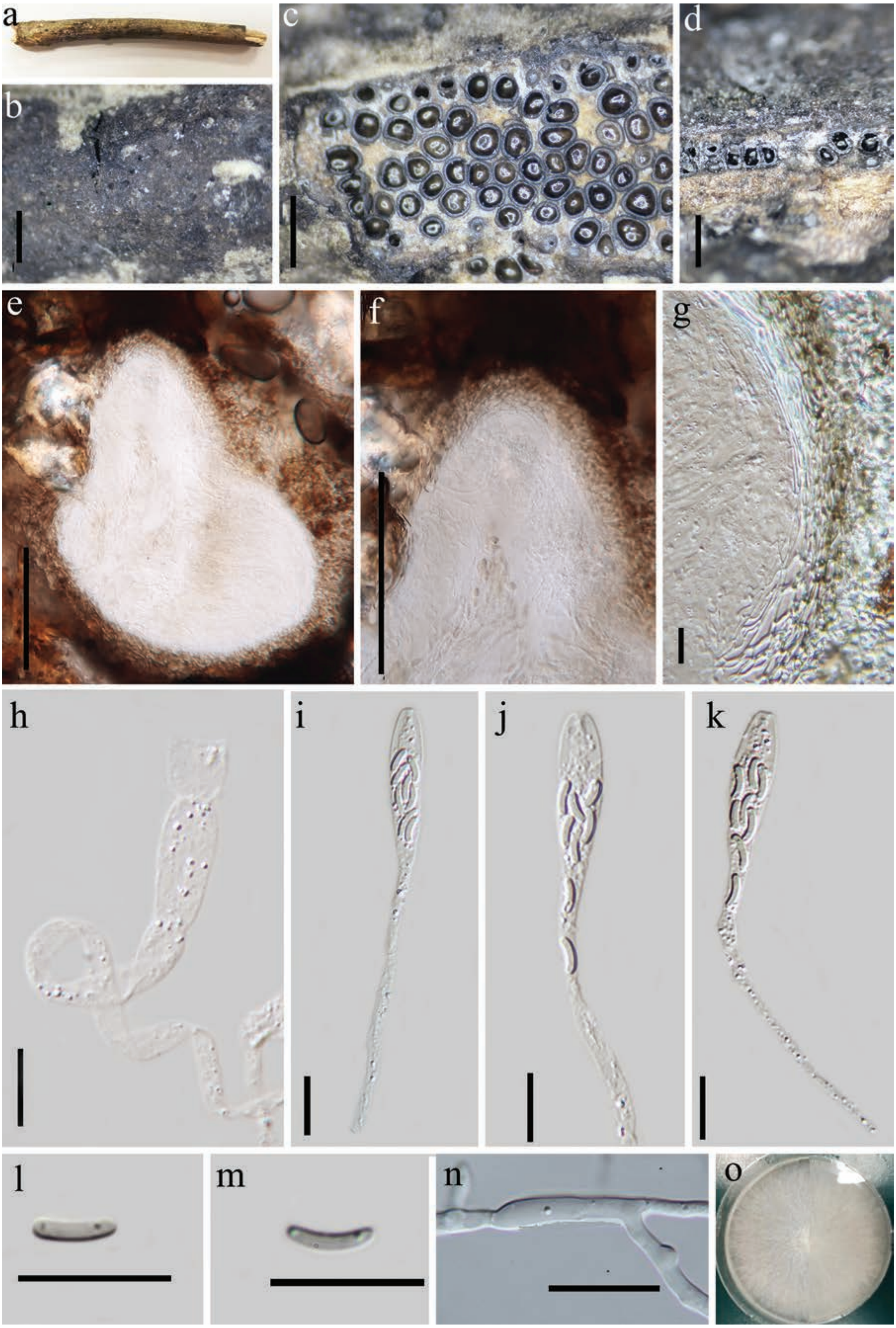

**Figure 2.** *Eutypa camelliae* **a, b** stromata on dead branch **c** cross section of a stroma showing perithecia **d, e** vertical sections of ascomata **f** ostiole **g** peridium **h** paraphyses **i–k** asci **l, m** ascospores **n** germinating ascospore **p** culture on PDA. Scale bars: 1 mm (**a–d**); 100 μm (**e, f**); 10 μm (**i–o**).

### ***Peroneutypa guizhouensis*** **X.Y. Mao, K. Habib & Y.Q. Kang, sp. nov.**

Index Fungorum: IF903236

Fig. 3

**Etymology.** The epithet refers to the name of the province from where the samples were collected.

**Type.** China • Guizhou Province, Guiyang City, Panlongshan Forest Park. (106°49'18"E, 26°44'58"N), altitude 1242.1 m, on branch of an unidentified plant, 8 June 2024. Xin Y Mao & Y.Q. Kang, PLS29 (Holotype GZAAS24-0087; ex-type cultures GZCC 24-0296; Isotype KUN-HKAS 145344). GenBank accession numbers (ITS: PQ878089; *tub2*: PQ876910).

**Description.** Saprobic on dead branches of an unidentified plant. Sexual morph: Stromata 0.5–1.5 mm in diameter, immersed in the host surface, ostiolar canals protruding through the bark, poorly developed, solitary, rarely gregarious, 1–4 locules, usually two, arranged irregularly, dark brown to black, glabrous, circular to irregular in shape, Ascomata (excluding neck) perithecia 400–720 µm high, 400–600 µm diam. ($\bar{x}$ = 650 × 400 µm, n = 20), immersed in a stroma, black, globose to sub-globose, each has an individual ostiole with a long neck. Ostiolar canals: erumpent, smooth, 300–570 ($\bar{x}$ = 435 µm) in length, cylindrical, smooth, curved at the apex. Peridium 48–56 µm ($\bar{x}$ = 52.4 µm) thick, composed of two layers, outer layer dark brown to black, cells thick-walled, texture angularis, inner layers hyaline, cells flattened. Paraphyses 3–5.8 µm ($\bar{x}$ = 4.9 µm, n = 20) wide, wider at the base, long, septate, smooth-walled. Asci 16–33 × 3.6–6.8 µm ($\bar{x}$ = 24.1 × 5.0 µm, n = 30), unitunicate, 8-spored, clavate, apically truncates, with a J- apical ring. Ascospore 2.2–4.7 × 1.1–1.8 µm ($\bar{x}$ = 3.3 × 1.4 µm, n = 30), overlapping, allantoid, subhyaline, smooth, aseptate, strongly curved, with 1–2 small guttules. Asexual morph: undetermined.

**Culture characteristics.** Colonies growing fast on PDA, reach 55 mm in 1 week at 28 °C, effuse, thin towards the edge, from above at first white, becoming dirty white at the edge after 2 weeks, from below brownish at the center, the rest white.

**Additional specimens examined.** China • Guizhou Province, Zunyi county, Dashahe Natural Reserve (107°34'19"E, 29°7'32"N) altitude: 1900 m, on branches of an unidentified plant, 26 April 2024; Xin Y Mao & Y.Q. Kang, XHP01 (Paratype GZAAS24-0088, Isotype KUN-HKAS 145343, ex-paratype cultures GZCC 24-0297).). GenBank accession numbers (ITS: PQ878090; *tub2*: PQ876911).

**Notes.** *Peroneutypa guizhouensis* is morphologically and phylogenetically like *P. hainanensis*, mainly due to its strongly curved ascospores. However, *P. guizhouensis* can be distinguished by its longer ostiolar necks (300–570 µm vs. 105–420 µm), smaller asci (16–33 µm in length, $\bar{x}$ = 24.1 × 5.0 µm vs. 28.5–40 µm, $\bar{x}$ = 33.5 × 5.5 µm), and significantly smaller ascospores (2.2–4.7 × 1.1–1.8 µm vs. 5.0–7.3 × 1–2 µm) (Li et al. 2023).

In addition to *Peroneutypa hainanensis*, *P. guizhouensis* shares similarities with *P. diminutiasca*, *P. curvispora*, and *P. qianensis* due to its strongly curved ascospores.

Compared to *P. diminutiasca*, *P. guizhouensis* has significantly longer ostiolar necks (300–570 µm vs. 105–280 µm), a thicker peridium (48–56 µm vs. 15–32 µm), and smaller ascospores (2.2–4.7 × 1.1–1.8 µm vs. 3.1–5.9 × 1.3–2.2 µm) (Shang et al. 2018). *Peroneutypa curvispora* differs from *P. guizhouensis* in having much longer ostiolar necks (400–800 µm vs. 300–570 µm), small-

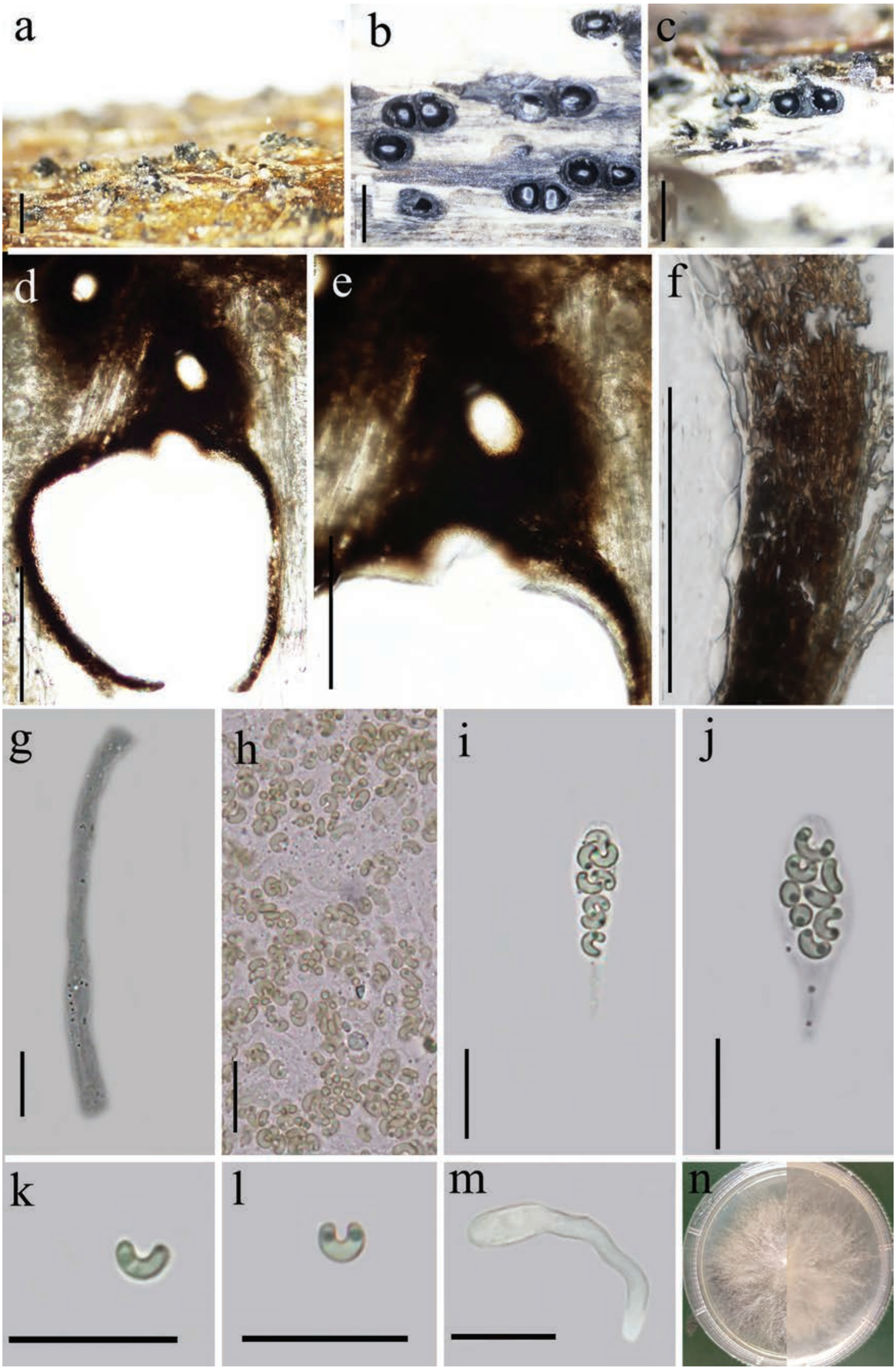

**Figure 3.** *Peroneutypa guizhouensis* (Holotype GZAAS24-0087) **a–c** stromata surface view **d** vertical section of ascomata **e** ostiole **f** peridium **g** paraphyses **h** numerous ascospores **i-j** asci **k, l** ascospores **m** germinating ascospore **n** culture on PDA. Scale bars: 1 mm (**a–d**); 100 μm (**e–g**); 10 μm (**h–o**).

er asci (9–16.5 × 4–6 μm vs. 16–33 μm), and the absence of paraphyses (vs. present) (de Almeida et al. 2016).

Compared to *P. qianensis*, *P. guizhouensis* differs in having longer ostiolar necks (300–570 μm vs. 105–420 μm), larger asci (16–33 × 3.6–6.8 vs. 16.5–20.5 × 4–6 μm), and smaller ascospores (2.2–4.7 × 1.1–1.8 μm vs. 4.5–6.3 × 1.5–0.3 μm) and presence of paraphyses (vs. lack) (Li et al. 2023).

These morphological differences (Table 2), combined with phylogenetic evidence, highlight the distinctiveness of *P. guizhouensis* and confirm its status as a new species.

### ***Peroneutypa wanfenglinensis*** **X.Y. Mao, K. Habib & Y.Q. Kang, sp. nov.**

Index Fungorum: IF902676
Fig. 4

**Etymology.** The epithet refers to the name of the location (Wan Feng Lin State Reserve), where the type specimen was collected.

**Type.** China • Guizhou Province, Xingyi City, Wan Feng Lin State Reserve (104°55'28"E, 24°59'26"N), altitude 896.8 m, on dead branches of *Betula platyphylla*, 28 May 2022, Xin Y Mao & Y.Q. Kang, WFL02 (Holotype GZAAS24-0021; Isotype KUN-HKAS133157, ex-type cultures GZCC 24-0196). GenBank accession numbers (ITS: PP852356; *tub2*: PQ301419).

**Description.** Saprobic on decaying branches of *Betula platyphylla*. Sexual morph: Stromata 1.90–2.5 mm in diameter, interior, solitary to gregarious, with 1–4 perithecia, immersed, erumpent by a long ostiolar canal, dark brown to black, surface glabrous, shape circular to irregular, arranged irregularly. Ascomata (excluding necks) 330–620 μm high, 280–520 μm diam. ($\bar{x}$ = 540 × 320 μm, n = 10), immersed in a stroma, black, monostichous to distichous, circular to oval, each has an individual ostiole with a short neck. Ostiolar canals erumpent, smooth, 120–140 μm ($\bar{x}$ = 135 μm, n = 10) long, arch-shaped, sulcate, and curved at the apex. Peridium 20–45 μm ($\bar{x}$ = 31.85 μm) thick, composed of two layers, outer layer brown to dark, cells thick-walled, texture angularis, inner layers hyaline, cells flattened, texture angularis. Paraphyses septate, slightly swollen at the septa, 3.5–6 μm ($\bar{x}$ = 5.4 μm, n = 20) wide. Asci 20–30.5 × 3–5 μm ($\bar{x}$ = 25 × 4 μm, n = 30), unitunicate, 8-spored, clavate, with apically rounded to truncate ends, with a J- apical ring. Ascospore 3–4.2 × 1–2 μm ($\bar{x}$ = 3.6 × 1.4 μm, n = 30), overlapping, allantoid, subhyaline, smooth, aseptate, with 1–2 oil droplets. Asexual morph: undetermined.

**Culture characteristics.** Colonies growing fast on PDA, reach 60 mm in 1 week at 28 °C, effuse, velvety to hairy, nearly circular, dense towards the edge, fluffy aerial mycelium, appear white from above and pale from below. Mycelium is composed of branched, septate, smooth-walled, hyaline hyphae.

**Additional specimens examined.** China • Guizhou Province, Zunyi City, Chishui Zhuhai National Forest Park (105°99'14"E, 28°47'19"N), altitude 838 m, on branches of an unidentified plant, 21 July 2023. Xin Y Mao, CSZH01 (Paratype GZAAS24-0022; KUN-HKAS133156; ex-paratype cultures, GZCC 24-0197). GenBank accession numbers (ITS: PP852353; *tub2*: PQ301420).

**Notes.** BLAST results reveal that *Peroneutypa wanfenglinensis* is closely related to *P. kochiana*. However, *P. wanfenglinensis* differs morphologically from

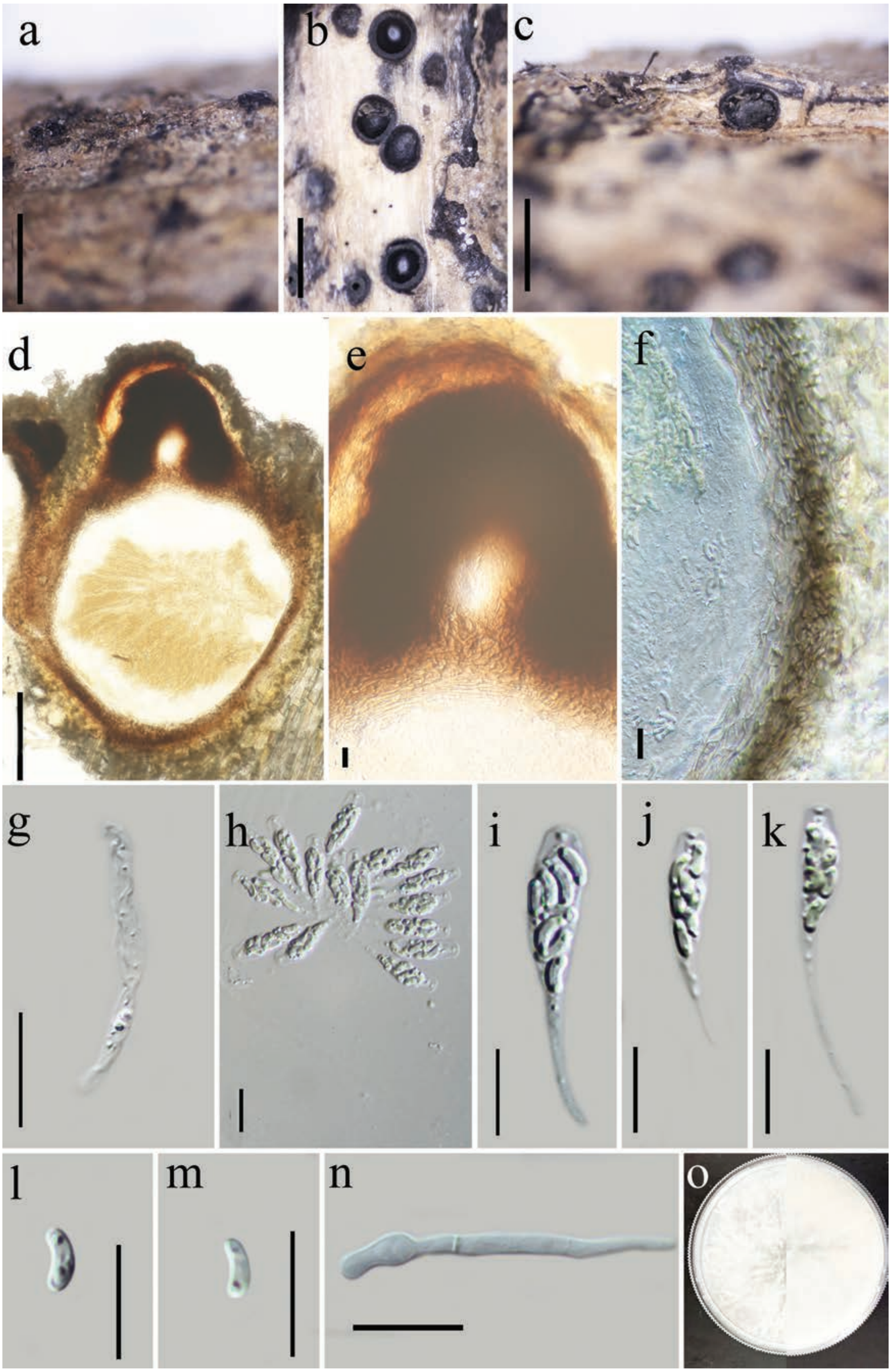

**Figure 4.** *Peroneutypa wanfenglinensis* (Holotype GZAAS24-0021) **a** surface view of stromata **b** cross section of a stroma showing perithecia **c, d** vertical sections of ascomata **e** ostioles **f** peridium **g** paraphyses **h–k** asci **l, m** ascospores **n** germinating ascospore **o** culture on PDA. Scale bars: 1 mm (**a–c**); 10 μm (**d, e**); 100 μm (**f–o**).

Table 2. Comparison of new taxa with closely related species.

| Species | Stromata (mm wide) | Ascomata (µm) | Ostiolar canal (µm) | Peridium (µm) | Paraphyses (µm) | Asci (µm) | Ascospores (µm) | Country | Host | References |
|---|---|---|---|---|---|---|---|---|---|---|
| ***P. guizhouensis*** | 0.5–1.5 | 400–720 × 400–600 | 300–570 long | 48–56 | 3–5.5 | 16–33 × 3.6–6.8, J– apical ring, short pedicellate | 2.2–4.7 × 1.1–1.8, strongly curved | China | Unknown tree branch | This Paper |
| ***P. wanfenglinensis*** | 1.9–2.5 | 330–620 × 280–520 | 120–140 long | 20–45 | 3.5–6 | 20–30.5 × 3–5, J– apical ring, long pedicellate | 3–4.2 × 1–2, slightly curved | China | *Betula platyphylla* | This Paper |
| ***P. zhujiashanesis*** | 1–2.8 | 550–910 × 400–570 µm | 50–145 long | 24–42 | 3.5–6 | 23–31 × 3.5–7, J– apical ring, long pedicellate | 3.5–5 × 1–1.5, slightly curved | China | Unknown tree branch | This Paper |
| ***P. indica*** | N/A | 375 × 202 | 100–350 long, | 15–35 | 1–2 | 42 × 3.5, short pedicellate, J– apical ring | 5.5 × 1.3, slightly curved | India | *Suaeda monoica* | Dayarathne et al. 2020 |
| ***P. curvispora*** | 0.6–3 | 300–700 | 400–800 long | N/A | Absent | 9–16.5 × 4–6, long pedicellate | 3–5 × 1–2, strongly curved | Brazil | Unidentified plant | de Almeida et al. (2016) |
| ***P. diminutiasca*** | 1.2–1.4 | 75–220 × 99–340 | 193 × 48 | 15–32 | 4–7 | 12–33 × 2.8–5, J– apical ring, long pedicellate, | 4.2 × 1.7 µm, slight to moderately curved | China, Thailand | Unidentified wood | Shang et al. 2018; Du et al. 2022 |
| ***P. hainanensis*** | 0.4–0.7 | 350–600 × 130–300 | 105–420 × 80–120 | 45–65 | N/A | 28.5–40 × 3.5–6.5, J– apical ring | 5.0–7.3 × 1–2, strongly curved | China | Unidentified plant | Li et al. 2023 |
| ***P. kochiana*** | N/A | 150 | Neck not prominent | N/A | N/A | 18–28 long, J+ apical ring | 4.5–6 × 1.5–2 slightly curved | Russia, Spain | *Atriplex halimus* | Acero et al. 2004; Carmarán et al. 2006 |
| ***P. leucaenae*** | N/A | 655 × 525 | 275–350 long | 22–43 | 3.2–7 wide, septate | 33 × 4.2, J+ apical ring, long pedicellate | 2.9–3.7 × 0.9–1.3 slightly curved | Thailand | *Leucaena leucocephala* | Samarakoon et al. 2022 |
| ***P. qianensis*** | 1.5–2 | 320–540 × 175–290 | 105–420 × 80–120 | 45–65 | N/A | 16.5–20.5 × 4–6, J– apical ring | 4.5–6.3 × 1.5–0.3, slightly curved | China | Unidentified plant | Li et al. 2023 |

*P. kochiana* in having smaller ascospores (3–4.2 × 1.0–1.9 µm vs. 4.5–6 × 1.5–2 µm), larger ascomata (330–620 µm high, 280–520 µm diam. vs. 150 µm diam.), and asci with a J- (non-amyloid) apical ring, compared to the J+ (amyloid) apical ring in *P. kochiana* (Carmarán et al. 2006). Sequence analysis also indicates a notable difference between *P. wanfenglinensis* and *P. kochiana*, showing a relatively low ITS similarity of 91%.

In terms of ascomata size and apical ring, *Peroneutypa wanfenglinensis* is more like *P. indica*. However, their ascus and ascospore dimensions can differentiate the two species. *Peroneutypa indica* has longer asci (35–47 µm vs. 20–30.5 µm) and ascospores (4–8 µm vs. 3–4.2 µm) compared to *P. wanfenglinensis* (Dayarathne et al. 2020).

In terms of ascomata and ascospore dimensions, *Peroneutypa wanfenglinensis* is comparable to *P. leucaenae*. However, *P. leucaenae* can be distinguished by its significantly longer ostiolar neck (275–350 µm vs. 120–140 µm) and larger asci (average 33 × 4.2 μm vs. average 25 × 4 μm). Additionally, *P. leucaenae* is characterized by a J+ (amyloid) apical ring, contrasting with the J− (non-amyloid) apical ring observed in *P. wanfenglinensis* (Du et al. 2022).

These distinct morphological features (Table 2), together with their distinct phylogenetic position, support the recognition of *P. wanfenglinensis* as a new species.

### ***Peroneutypa zhujiashanesis*** **X.Y. Mao & Y.Q. Kang, sp. nov.**

Index Fungorum: IF902672
Fig. 5

**Etymology.** The epithet refers to the name of the location where the type specimen was collected, Zhujiashan National Forest Park.

**Type.** China • Guizhou Province, Douyun City, Weng‘an County, Zhujiashan National Forest Park (107°38'35"E, 26°58'35"N), altitude 848 m, on branches of an unidentified plant, 14 February 2022. Xin Y Mao & Y.Q. Kang, ZJS14 (Holotype GZAAS24-0023; KUN-HKAS133155; ex-type GZCC 24-0198). GenBank accession numbers (ITS: PP852363; *tub2*: PQ301421).

**Description.** Saprobic on decaying branches of an unknown tree. Sexual morph: Stromata 1–2.8 mm in diameter, immersed in the host surface, with necks conspicuously protruding through the bark, erumpent through an ostiolar canal, solitary to gregarious,1–3 locules, mostly solitary, arranged irregularly, dark brown to black, glabrous, circular to irregular in shape, arranged irregularly, delimited by a black zone in host tissues. Ascomata (excluding neck) immersed in a stroma, dark brown to black, perithecia 550–910 μm high, 400–570 μm diam. ($\bar{x}$ = 790 × 520 μm, n = 10), black, single to aggregated, globose to sub-globose, each has an individual ostiole with a long neck. Ostiolar canals erumpent, smooth, 50–145 μm ($\bar{x}$ = 134.69 μm) in length, cylindrical, and curved at the apex. Peridium 24–42 μm ($\bar{x}$ = 32.73 μm) thick, composed of two layers, outer layer dark brown to black, cells thick-walled, texture angularis, inner layers hyaline, cells flattened, texture angularis. Paraphyses 3.5–6 μm ($\bar{x}$ = 5.4 μm, n = 20) wide, wider at the base, long, septate, smooth-walled, constricted at septa. Asci 23–31 × 3.5–7 μm ($\bar{x}$ = 27.5 × 5.5 μm, n = 30), unitunicate, 8-spored, clavate, apically truncates, with a J- apical ring. Ascospore 3.5–5 × 1–1.5 μm ($\bar{x}$ = 4.2 × 1.3 μm, n = 30), overlapping, allantoid, subhyaline, smooth, aseptate, with 1–2 small guttules. Asexual morph: undetermined.

**Culture characteristics.** Colonies grow fast on PDA, reach 60 mm in 1 week at 28 °C, effuse towards the edge, from above at first white, becoming dirty white at the edge after 2 weeks, from below black at center, the rest white.

**Additional specimens examined.** China • Guizhou Province, Anlong county, Xianheping National Forest Park (105°36'26"E, 24°58'39"N) altitude: 1298 m, on branches of an unidentified plant, 30 May 2022; Xin Y Mao & Y.Q. Kang, XHP01 (Paratype GZAAS24-0024; KUN-HKAS-133158; ex-paratype GZCC 24-0199). GenBank accession numbers (ITS: PP852362; *tub2*: PQ301422).

**Notes.** Phylogenetically, *Peroneutypa zhujiashanesis* is closely related to *P. leucaenae*. Morphologically, it also shares similarities with *P. leucaenae* in terms of ascomata size and the shape and size of paraphyses. However, *P. zhujiashanesis* can be distinguished from *P. leucaenae* by its smaller asci

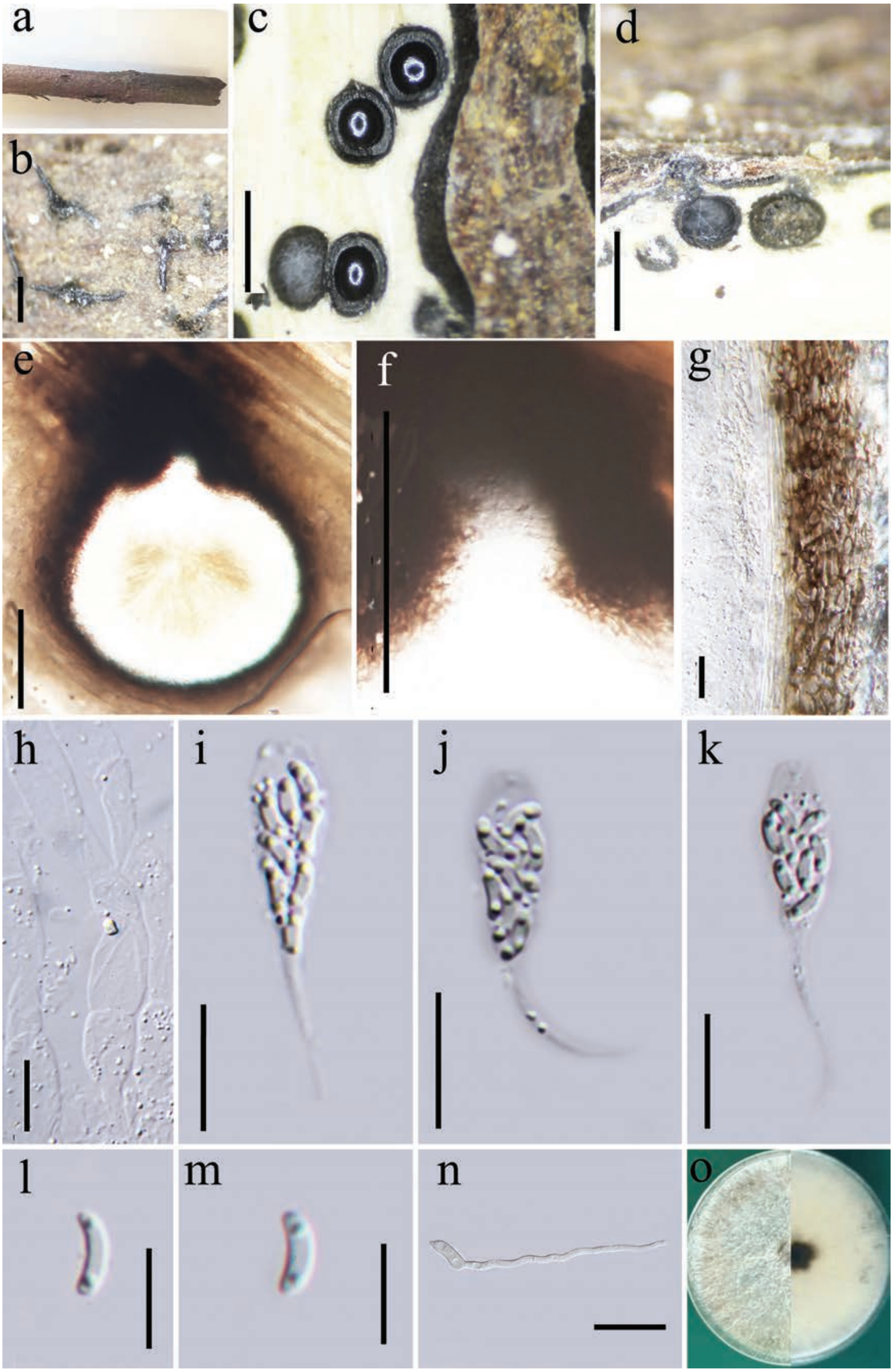

Figure 5. *Peroneutypa zhujiashanesis* (Holotype GZAAS24-0023) **a, b** stromata on dead branch **c** transverse section of ascomata **d, e** vertical section of ascomata **f** ostioles **g** peridium **h** paraphyses **i–k** asci **l, m** ascospores **n** germinating ascospore **o** culture on PDA. Scale bars: 1 mm (**a–d**); 100 µm (**e–f**); 5 µm (**i–o**).

(23–31 × 3.5–7 µm vs. 30–37 × 3.8–4.5 µm) and longer ascospores (3.5–5 µm vs. 2.9–3.7 µm) (Samarakoon et al. 2022). Additionally, *P. zhujiashanesis* has smaller ostiolar necks (50–145 µm) and a J– apical ring, whereas *P. leucaenae* has longer ostiolar necks (275–350 µm) and an amyloid apical ring.

Morphologically, *Peroneutypa zhujiashanesis* is also like *P. diminutiasca* in ascospore size and presence of a J– subapical ring. However, *P. diminutiasca* differs by having smaller ascomata (147–218 µm in diameter), with longer ostiolar neck (average 193 µm vs 134.6 µm), and possessing 1–10 locules per ascomata (vs 1–3 loculate, mostly single) (Du et al. 2022).

Based on these morphological differences (Table 2) and phylogenetic evidence, we introduce our collection as a new species, *Peroneutypa zhujiashanesis*.

## Discussion

The taxonomy of the Diatrypaceae has long been challenging, with unstable generic boundaries that lack strong morphological or phylogenetic support. Current classifications often fail to reflect the evolutionary relationships among these fungi accurately. Our phylogenetic analyses, based on ITS and β-tubulin sequences, corroborate previous findings (Shang et al. 2017; Dissanayake et al. 2021; Zhu et al. 2021; Long et al. 2021; Li et al. 2023) and reveal that several genera, such as *Cryptosphaeria*, *Diatrype*, *Diatrypella*, and *Eutypa*, are not monophyletic and contain multiple problematic clades.

The phylogeny of the family reveals many clades that may represent distinct genera, suggesting that the current classification is too simplistic. A comprehensive revision involving extensive sampling and a combination of taxonomic methods (integrating morphology, molecular data, chemical profiles and genomic information) is essential to resolve these complex relationships and achieve a more natural classification for the Diatrypaceae. *Peroneutypa* exemplifies the unresolved taxonomic and phylogenetic issues that are common within Diatrypaceae.

Phylogenetically, *Peroneutypa* species form a well-supported clade (Fig. 1). However, the inclusion of *Eutypella microasca* within this clade suggests its polyphyletic nature. In our investigation, we found that molecular data did not correlate well with morphological characteristics. Species that are phylogenetically close differed significantly in morphology. For instance, *P. polysporae*, characterized by multispored asci, clustered with *P. mangrovei*, which possesses eight-spored asci. Similarly, the newly described species *Peroneutypa guizhouensis* clustered with *P. hainanensis*, both of which possess strongly curved ascospores. However, other strongly curved ascospore-bearing species, such as *P. curvispora*, *P. diminutiasca*, *P. obesa*, and *P. qianensis*, are phylogenetically distant. Another example is *Peroneutypa zhujiashanesis*, which has a J- apical ring, clustering with *P. leucaenae*, a species that possesses a J+ apical ring. Only six species in the genus are known to have a J+ apical ring (*P. alsophila*, *P. comosa*, *P. exigua*, *P. iranica*, *P. kochiana*, and *P. leucaenae*), and they are not closely clustered phylogenetically.

These analyses suggest that the genus *Peroneutypa* exhibits a complex evolutionary history, where phylogenetic relationships are not always reflected in morphological traits. The discordance between molecular and morphological data underscores the need for a comprehensive integrative approach. The use of additional genetic regions, such as LSU, SSU, *rpb2*, and *tef-1α*, should be explored to achieve more accurate phylogenetic analyses. Coupled with detailed morphological studies, these efforts will enable the precise delineation of species boundaries and provide deeper insights into the evolutionary relationships within this genus. The inclusion of newly described species in future studies, such as those introduced in this research, will continue to refine the phylogeny and enhance understanding of the genus's diversity and evolutionary patterns, and contribute to a more robust and accurate classification system.

*Peroneutypa* species are mostly reported from plant species belonging to the families Thymelaeaceae, Rubiaceae, Moraceae, Fabaceae, and Euphorbiaceae, among others (Du et al. 2022) and primarily associated with woody angiosperms, particularly trees and shrubs, and are rarely found on herbaceous plants. To date, there are no reports of *Peroneutypa* species occurring on gymnosperms. Among angiosperms, *Peroneutypa* species are mainly recorded on dicots, with the exception of *P. scoparia* and *P. bellula*, which have been documented on monocots.

Within this genus, species can be distinguished based on several morphological traits, including the size of the ascomata, ostiolar canal length, asci, and ascospores. Additional characteristics, such as the shape of the asci and ascospores, the reaction of the ascus apex in Melzer's reagent, and the presence or absence of paraphyses, are also used in differentiating species (Shang et al. 2017; Du et al. 2022). *Peroneutypa polymorpha* and *P. rubiformis* are unique within the genus for having larger asci, measuring more than 40 μm in length. Most other species in the genus have smaller asci. Similarly, ascospore size is generally consistent across the genus, ranging from 3–6 × 1–2 μm, except *P. polysporae*, which has notably larger ascospores measuring up to 9 × 1.8 μm.

Ostiolar canal length is another key characteristic for species delimitation. Species such as *P. coffea*, *P. comosa*, *P. curvispora*, *P. cylindrica*, *P. cyphelioides*, *P. exigua*, *P. komonoensis*, *P. macroceras*, *P. philippinarum*, and *P. variabilis* all have longer ostiolar canals (> 500 μm), distinguishing them from other species in the genus that have shorter canals (< 400 μm). Curved ascospores have been observed in *P. curvispora*, *P. diminutiasca*, *P. hainanensis*, *P. obesa* and *P. qianensis*, another prominent differentiating feature within the species.

Overall, the morphological variability in *Peroneutypa* requires using a combination of these traits for accurate species identification.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Yingqian Kang conceived and designed the experiments and performed the experiment. Xinying Mao analyzed the data and wrote the manuscript. Hongde Yang dealt with some of the sequences. Kamran Habib and Rizwana Zulfiqar reviewed and polished the language and approved the final version of the manuscript. All authors contributed extensively to the study presented in the manuscript.

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## Data availability

The datasets generated during and/or analyzed during the current study are available in the MycoBank repository (included in the manuscript) and GenBank (included in Table 1). Also, the datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Research Article

# Three new species of *Gerronema* (Agaricales, Basidiomycota) from southern China

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## Abstract

Three new species of *Gerronema* are discovered from southern China. *Gerronema angustum* is characterized by its small basidiomata, greenish-green pileus, slender stipe, narrow, and close lamellae. *Gerronema pubescence* is characterized by its pubescent pileus when young, yellowish white to pale yellow lamellae that are lighter towards the margin, narrowly cylindrical to lageniform pleurocystidia, and absent cheilocystidia. *Gerronema rhizomorpha* is characterized by its yellowish white to brown pileus, well-developed rhizomorphs at the stipe base, absent cheilocystidia and pleurocystidia, and not growing on rotten wood. Molecular phylogenetic analyses of nrITS + nrLSU support the species delimitation. In this study, detailed descriptions, photos of the basidiomata, line drawings, and discussions with related species are comprehensively provided. A key to the known *Gerronema* species in China is also provided.

**Key words:** New taxa, phylogenetic analysis, Porotheleaceae, taxonomy

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## Introduction

*Gerronema* Singer (Porotheleaceae, Agaricales, Agaricomycetes, Basidiomycota), typified by *G. melanomphax* Singer, was first proposed to accommodate the three "rebellious" species from Argentina, which were characterized by their somewhat thick-walled hyphae and consequently more elastic-toughish consistency, strongly irregular hymenophoral trama, strongly intracellular pigmentation, and lignicolous habitat (Singer 1951). In this case, species of *Gerronema* only differ from species in *Omphalina* Quél. by the absence of fuscous, intraparietal, or incrusting pigments (Singer 1964, 1986; Bigelow 1970, 1982, 1985). Lange (1981) suggested that the characters defining various segregates of *Omphalina* are hardly of sufficient significance on the generic level, and *Gerronema* was regarded as a subgenus of *Omphalina*. Later, the characteristics of *Gerronema* were redefined as basidiomata omphalinoid to clitocyboid, pileus convex to infundibuliform or umbilicate, lamellae decurrent and subdistant, stipe central, basidiospores thin-walled, smooth, inamyloid, tramal tissue sarcodimitic, occasionally with cystidia and clamp connections, and lignicolous habitat (Redhead 1986; Norvell et al. 1994). And *Chrysomphalina strombodes* (Berk. & Mont.) Clémençon and *Clitocybe xanthophylla* Bres. were transferred into *Gerronema* (Norvell et al. 1994). Up to now, this definition is widely accepted by some scholars (Bañares et al. 2006; Antonín et al. 2008; Latha et al. 2018; Liu et al. 2019; Jayawardena et al. 2022; Na et al. 2022, 2024).

In previous studies, *Gerronema* was considered to be heterogenous by some scholars (Clémençon 1982; Moser 1983; Kuyper 1986; Singer 1986; Norvell et al. 1994). However, some researchers regarded *Gerronema* as a monophyletic group as restricted by Norvell et al. (1994) and included into the hydropoid clade together with *Clitocybula* (Singer) Singer ex Métrod, *Hydropus* Kühner ex Singer, *Megacollybia* Kotl. & Pouzar, and *Porotheleum* Fr. (Norvell et al. 1994; Moncalvo et al. 2002; Antonín et al. 2008, 2019; Yang et al. 2012). Molecular phylogenetic analyses provided new perspectives, and *Gerronema* was proved to be a polyphyletic group (Lutzoni 1997; Moncalvo et al. 2002; Redhead et al. 2002; Latha et al. 2018). The genus was resolved into several clades and closely related to the genera *Megacollybia* and *Trogia* Fr. in the family Porotheleaceae (Vizzini et al. 2019; Na et al. 2022, 2024). Up to now, the phylogenetic systematic position of *Gerronema* remains unclear due to the insufficient number of specimens and the limitation of phylogenetic research progress.

*Gerronema* is a small genus; only 75 species names have been recorded in Index Fungorum (http://www.indexfungorum.org, 1 November 2024). Most species of *Gerronema* are distributed in subtropical to tropical regions (Singer 1970; Norvell et al. 1994). China is one of the countries with the highest biodiversity and rich species, but only twelve *Gerronema* species have been reported, namely, *G. albidum* (Fr.) Singer, *G. baisanzuens* Q. Na, H. Zeng & Y.P. Ge, *G. brunneosquamulosum* Q. Na & Y.P. Ge, *G. chrysocarpum* P.G. Liu, *G. confusum* L. Fan & T.Y. Zhao, *G. indigoticum* T. Bau & L.N. Liu, *G. lapidescens* (Horan.) Ming Zhang & W.X. Zhang, *G. kuruvense* K.P.D. Latha & Manim., *G. microcarpum* Q. Na, H. Zeng & Y.P. Ge, *G. nemorale* Har. Takah., *G. strombodes* (Berk. & Mont.) Singer and *G. zhujian* Q. Na, H. Zeng & Y.P. Ge, seven of them are originally described from China (Liu 1995; Liu et al. 2019; Dai et al. 2010; Li et al. 2021; Wu et al. 2021; Na et al. 2022, 2024; Zhao and Fan 2022; Zhang et al. 2024). During our field investigation in southern China, three new species of *Gerronema* were found; they were formally described and introduced in morphological characters with molecular data in the present study.

## Materials and methods

### Sample collection and morphological study

Macroscopic morphological characteristics were derived from observation records and color images of fresh specimens collected in the field. Color descriptions were obtained according to Kornerup and Wanscher (1978). Samples were dried using an electric dryer at 50 °C and then deposited in the Fungarium of the Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China (GDGM). The specific operations of the microscopic morphological characteristics are as follows: Sample each tissue section with tweezers or blades, place it in the slide floating carrier (1 drop of 5% KOH), stain with 1% Congo red solution, and observe the microstructure and measure it with a light microscope (Nikon Ni-U, Nikon Corporation, Japan). Twenty mature spores and 10 basidia were selected for measurement and represented by (a)b–c(d), where a or d indicates the extreme values, and b–c contains 90% of the measurements. L, W, and Q refer to length, width, and L/W ratio, respectively; $L_m$, $W_m$, and $Q_m$ refer to the mean length, width, and Q value of all basidiospore samples ± the standard deviation, respectively.

### DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Genomic DNA samples were extracted from dried specimens using the HiPure Fungal DNA Kit (Magen Biotechnology Co., Ltd., Guangzhou, China) and kept in a -20 °C refrigerator. The internal transcribed spacer (nrITS) and the large subunit nuclear ribosomal DNA gene (nrLSU) were respectively amplified with primer pairs ITS1/ITS4 and LROR/LR7 (White et al. 1990; Hopple and Vilgalys 1999). PCR reactions were performed in a total volume of 25 μL containing 1 μL template DNA, 9.5 μL distilled water, 1 μL of each primer, and 12.5 μL 2 × PCR mix (DreamTaq$^{tm}$ Green PCR Master Mix, Fermentas) (Zhang et al. 2022). The PCR procedure amplification was as follows: pre-denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C (for nrITS)/50 °C (for nrLSU) for 40 s, extension at 72 °C for 50 s, and final extension at 72 °C for 10 min. After the amplification products were tested by agarose gel electrophoresis, PCR products were sent to Beijing BGI Co., Ltd. (Guangzhou, China) for sequencing. Then the sequences of the bidirectional sequencing were checked and assembled by the Geneious Pro trial 4.8.4 (Biomatters Limited Company). The newly obtained sequences were submitted to GenBank.

### Phylogenetic analyses

The nrITS and nrLSU datasets were concatenated, including newly generated sequences, some valuable *Gerronema* sequences, and related genera (*Clitocybula*, *Hydropus*, *Leucoinocybe* Singer ex Antonín, Borovička, Holec & Kolařík, *Marasmiellomycena* De la Peña-Lastra, Mateos, Kolařík, Ševčíková & Antonín, *Megacollybia*, *Porotheleum*, *Pseudohydropus* Vizzini & Consiglio, *Pulverulina* Matheny & K.W. Hughes, and *Trogia*) sequences in GenBank were selected for phylogenetic analyses based on previous studies (Na et al. 2022, 2024) and listed in Table 1. *Mycena purpureofusca* (Peck) Sacc. was selected as the outgroup according to a previous study (Na et al. 2022). Using the auto strategy with MAFFT v7.505 (Katoh and Standley 2013) and then manually edited in MEGA v11.0.10 (Koichiro et al. 2021). The best model of nucleotide evolution for the dataset (nrITS + nrLSU) was identified using PartitionFinder 2 (Lanfear et al. 2016). Bayesian Inference (BI) and Maximum Likelihood (ML) bootstrap analyses were performed using the best-fit substitution models identified in PhyloSuite v1.2.3 (Zhang et al. 2020). The BI analysis was carried out in MrBayes 3.2.6 (Ronquist et al. 2012) under the best-fit substitution model, in which the initial 25% of sampled data were discarded as burn-in. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the edge-linked partition model for 5000 ultrafast (Minh et al. 2013) bootstraps.

**Table 1.** Information for the sequences used in the phylogenetic analyses. Newly generated sequences are in bold.

| Taxon | Voucher | Locality | GenBank accession No. | | Reference |
|---|---|---|---|---|---|
| | | | nrITS | nrLSU | |
| *Clitocybula abundans* | STU:SMNS-B-FU-2017/00898 | Germany | MF627833 | – | Direct Submission |
| *C. familia* | PRM 921866 | Czech Republic | JF730327 | JF730320 | Antonín et al. (2011) |
| *C. fuscostriata* | FFAAS1030 | China | OR238882 | OR238894 | Na et al. (2024) |
| *Gerronema albidum* | H:6050710 | USA | – | MF318923 | Direct Submission |
| *G. albidum* | H:6059277 | USA | – | MF318924 | Direct Submission |
| ***G. angustum*** | **GDGM 88662** | **China** | **PQ452698** | **PQ350413** | **This study** |
| ***G. angustum*** | **GDGM 88663** | **China** | **PQ452699** | **–** | **This study** |
| *G. atrovirens* | BKF10264 | Thailand | MZ452088 | MZ452671 | Jayawardena et al. (2022) |
| *G. atrovirens* | BKF10265 | Thailand | MZ452668 | MZ452672 | Jayawardena et al. (2022) |
| *G. baishanzuense* | FFAAS0359 | China | OL985962 | OL985984 | Na et al. (2022) |
| *G. baishanzuense* | FFAAS0360 | China | OL985963 | – | Na et al. (2022) |
| *G. baishanzuense* | FFAAS0361 | China | OL985964 | – | Na et al. (2022) |
| *G. baishanzuense* | FFAAS0362 | China | OL985965 | OL985986 | Na et al. (2022) |
| *G. baishanzuense* | FFAAS0363 | China | OL985966 | OL985987 | Na et al. (2022) |
| *G. baishanzuense* | FFAAS0366 | China | OL985967 | OL985988 | Na et al. (2022) |
| *G. brunneosquamulosum* | FFAAS1032 | China | OR238884 | OR238896 | Na et al. (2024) |
| *G. brunneosquamulosum* | FFAAS1033 | China | OR238885 | OR238897 | Na et al. (2024) |
| *G. citrinum* | G7458 | French | MN994795 | – | Jaouen et al. (2019) |
| *G. citrinum* | G7785 | French | MN994822 | – | Jaouen et al. (2019) |
| *G. citrinum* | PC0713130 | French | MN994747 | – | Jaouen et al. (2019) |
| *G. citrinum* | PC0714037 | French | MN994655 | – | Jaouen et al. (2019) |
| *G. confusum* | BJTC FM1592 | China | OK161262 | – | Zhao and Fan (2022) |
| *G. confusum* | BJTC FM1624 | China | OK161271 | – | Zhao and Fan (2022) |
| *G. indigoticum* | HMJAU47636 | China | MK693727 | MK693732 | Liu et al. (2019) |
| *G. indigoticum* | HMJAU47942 | China | MK693728 | MK693733 | Liu et al. (2019) |
| *G. indigoticum* | HMJAU47943 | China | MK693729 | MK693734 | Liu et al. (2019) |
| *G. keralense* | BKF10263 | Thailand | MZ452107 | MZ452144 | Direct Submission |
| *G. keralense* | CAL 1666 | India | MH156555 | MH153979 | Latha et al. (2018) |
| *G. kuruvense* | BKF10266 | Thailand | MZ452090 | MZ452669 | Direct Submission |
| *G. kuruvense* | CAL 1665 | India | MH156554 | MH153978 | Latha et al. (2018) |
| *G. kuruvense* | DCY3362(HGASMF01-15010) | China | MZ951144 | – | Direct Submission |
| *G. kuruvense* | FCATAS9085 | China | PP622159 | – | Direct Submission |
| *G. kuruvense* | KUC20220701_03 | Korea | OR600252 | – | Cho et al. (2024) |
| *G. lapidescens* | GDGM 85271-1 | China | OR736197 | – | Zhang et al. (2024) |
| *G. lapidescens* | GDGM 85271-2 | China | OR736198 | – | Zhang et al. (2024) |
| *G. lapidescens* | GDGM 86705 | China | OR736202 | – | Zhang et al. (2024) |
| *G. microcarpum* | FFAAS0371 | China | OL985968 | OL985990 | Na et al. (2022) |
| *G. microcarpum* | FFAAS0372 | China | OL985969 | OL985991 | Na et al. (2022) |
| *G. microcarpum* | FFAAS0373 | China | OL985970 | OL985992 | Na et al. (2022) |
| *G. microcarpum* | FFAAS0374 | China | OL985971 | – | Na et al. (2022) |

| Taxon | Voucher | Locality | GenBank accession No. | | Reference |
|---|---|---|---|---|---|
| | | | nrITS | nrLSU | |
| *G. microcarpum* | FFAAS0375 | China | OL985972 | OL985993 | Na et al. (2022) |
| *G. nemorale* | HMJAU59063 | China | OK560883 | – | Direct Submission |
| *G. nemorale* | HMJAU59064 | China | OK560871 | – | Direct Submission |
| *G. nemorale* | FA236 | Pakistan | MN744687 | – | Aqdus and Khalid (2021) |
| *G. nemorale* | FA239 | Pakistan | MN744688 | – | Aqdus and Khalid (2021) |
| *G. nemorale* | FA249 | Pakistan | MN744686 | – | Aqdus and Khalid (2021) |
| *G. nemorale* | FFAAS0389 | China | OL985981 | OL986002 | Na et al.(2022) |
| *G. nemorale* | FFAAS0392 | China | OL985982 | OL986003 | Na et al.(2022) |
| *G. nemorale* | FFAAS0410 | China | OL985983 | OL986004 | Na et al.(2022) |
| ***G. pubescence*** | **GDGM 93936** | **China** | **PQ452700** | **PQ350414** | **This study** |
| ***G. pubescence*** | **GDGM 94001** | **China** | **PQ452701** | **PQ350415** | **This study** |
| ***G. rhizomorpha*** | **GDGM 87835** | **China** | **PQ452702** | **–** | **This study** |
| ***G. rhizomorpha*** | **GDGM 92067** | **China** | **PQ452703** | **PQ350416** | **This study** |
| *Gerronema* sp. | HMJAU59018 | China | OK491123 | – | Direct Submission |
| *G. strombodes* | FLAS-F-60957 | USA | MH016911 | – | Direct Submission |
| *G. strombodes* | FLAS-F-71339 | USA | OR438652 | – | Direct Submission |
| *G. strombodes* | TENN:F-60009 | USA | KY271083 | – | Direct Submission |
| *G. strombodes* | TFB12519/TENN60718 | USA | EU623640 | – | Hughes et al. (2007) |
| *G. strombodes* | TFB12783/TENN61350 | USA | EU623641 | – | Hughes et al. (2007) |
| *G. strombodes* | DJL05NC72 | USA | EU623639 | – | Hughes et al. (2007) |
| *G. subclavatum* | FLAS-F-60986 | USA | MH016932 | – | Direct Submission |
| *G. subclavatum* | FLAS-F-61518 | USA | MH211945 | – | Direct Submission |
| *G. subclavatum* | FLAS-F-71359 | USA | OR242635 | – | Direct Submission |
| *G. subclavatum* | iNaturalist # 8545787 | India | MN906021 | – | Direct Submission |
| *G. subclavatum* | Mushroom Observer # 243440 | USA | MK607510 | – | Direct Submission |
| *G. subclavatum* | S.D. Russell MycoMap # 6854 | India | MN906138 | – | Direct Submission |
| *G. subclavatum* | Smith-2018 iNaturalist # 17333993 | USA | MK573888 | – | Direct Submission |
| *G. viridilucens* | DED 7822 | Brazil | – | OR449361 | Direct Submission |
| *G. waikanaense* | PDD:87667 | New Zealand | JQ694117 | – | Direct Submission |
| *G. xanthophyllum* | PRM 924657 | Czech Republic | LT854023 | LT854023 | Antonín et al. (2019) |
| *G. xanthophyllum* | SYKOf3970 | Russia | OR915457 | – | Direct Submission |
| *G. zhujian* | FFAAS0370 | China | OL985974 | OL985995 | Na et al. (2022) |
| *G. zhujian* | FFAAS0376 | China | OL985975 | OL985996 | Na et al. (2022) |
| *Hydropus fuliginarius* | S.D. Russell ONT iNaturalist # 130794969 | USA | OP643427 | – | Direct Submission |
| *H. marginellus* | OSC 112834 | USA | EU669314 | EU852808 | Direct Submission |
| *H. rugosodiscus* | MGW1257 | USA | KY777386 | – | Direct Submission |
| *Leucoinocybe danxiashanensis* | GDGM 80184 | China | MZ667478 | MZ667482 | Direct Submission |
| *L. lishuiensis* | FFAAS0115 | China | MW424491 | MW424495 | Na et al. (2021) |
| *L. subglobispora* | FFAAS1034 | China | OR238886 | OR238898 | Na et al. (2024) |

| Taxon | Voucher | Locality | GenBank accession No. | | Reference |
|---|---|---|---|---|---|
| | | | nrITS | nrLSU | |
| *Oudemansiella* aff. | platyphylla 360-630 | Japan | AB509870 | – | Direct Submission |
| *Marasmiellomycena pseudoomphaliiformis* | BRNM:552721 | USA | OR913562 | OR913566 | Senanayake et al. (2023) |
| *M. tomentosa* | FFAAS1036 | China | OR238888 | OR238900 | Na et al. (2024) |
| *Megacollybia clitocyboidea* | TENN62231 | USA | EU623664 | – | Hughes et al. (2007) |
| *M. marginata* | HR 91607 | Czech Republic | LT854051 | – | Antonín et al. (2019) |
| *M. platyphylla* | BRNM 737654 | Czech Republic | LT854048 | LT854036 | Antonín et al. (2019) |
| *Mycena purpureofusca* | HMJAU43554 | China | MG654740 | MK629356 | Na and Bau (2018) |
| *M. purpureofusca* | HMJAU43624 | China | MG654741 | MK629357 | Na and Bau (2018) |
| *Porotheleum fimbriatum* | CLZhao 1120 | China | MH114870 | – | Direct Submission |
| *P. fimbriatum* | Dai 12276 | China | KX081137 | KX161656 | Direct Submission |
| *Pseudohydropus floccipes* | BRNM 816173 | Czech Republic | OM422758 | OM423634 | Direct Submission |
| *P. floccipes* | BRNM 825631 | Spain | OM422760 | OM423636 | Consiglio et al. (2022) |
| *P. globosporus* | BAP 661 | USA | MH414566 | MH385340 | Cooper et al. (2019) |
| *Pulverulina flavoalba* | FFAAS1039 | China | OR238891 | OR238903 | Na et al. (2024) |
| *P. flavoalba* | FFAAS1040 | China | OR238892 | OR238904 | Na et al. (2024) |
| *P. ulmicola* | TFB13871 | USA | MT237476 | MT237446 | Matheny et al. (2020) |
| *Trogia benghalensis* | CUH AM031 | India | KU647630 | – | Dutta et al. (2017) |
| *T. infundibuliformis* | KUN_HKAS56709 | China | JQ031776 | JQ031781 | Yang et al. (2012) |
| *T. venenata* | KUN_HKAS54710 | China | JQ031772 | JQ031778 | Yang et al. (2012) |

Phylogenetic trees were visualized using FigTree v.1.4.4. The maximum likelihood bootstrap over 50% (MLB ≥ 50%) and the Bayesian posterior probability over 0.90 (BPP ≥ 0.90) were shown.

## Results

### Molecular phylogenetic results

The final concatenated dataset consisted of 95 nrITS and 52 nrLSU sequences from 46 taxa of 11 genera of Physalacriaceae, Porotheleaceae, and Mycenaceae, which comprised 1927 nucleotide sites (942 for nrITS, 985 for nrLSU), of which 752 were parsimony-informative, 277 were singleton sites, and 898 were constant sites. For the ML analyses, the best-fit substitution models selected for nrITS and nrLSU region partitions in the concatenated dataset were HKY+I+G and GTR+I+G, respectively. For the BI analysis, the best-fit substitution model selected for each of the two DNA regions was GTR+I+G (2 parallel runs, 2000000 generations), and the average standard deviation of split frequencies was stably dropped under 0.01. The phylogenetic trees generated from BI and ML analyses show almost similar topologies and few variations in statistical support, so only the ML tree is displayed (Fig. 1).

In the phylogenetic tree (Fig. 1), *Clitocybula*, *Gerronema*, *Hydropus*, *Leucoinocybe*, *Marasmiellomycena*, *Megacollybia*, *Porotheleum*, *Pseudohydropus*, *Pulverulina*,

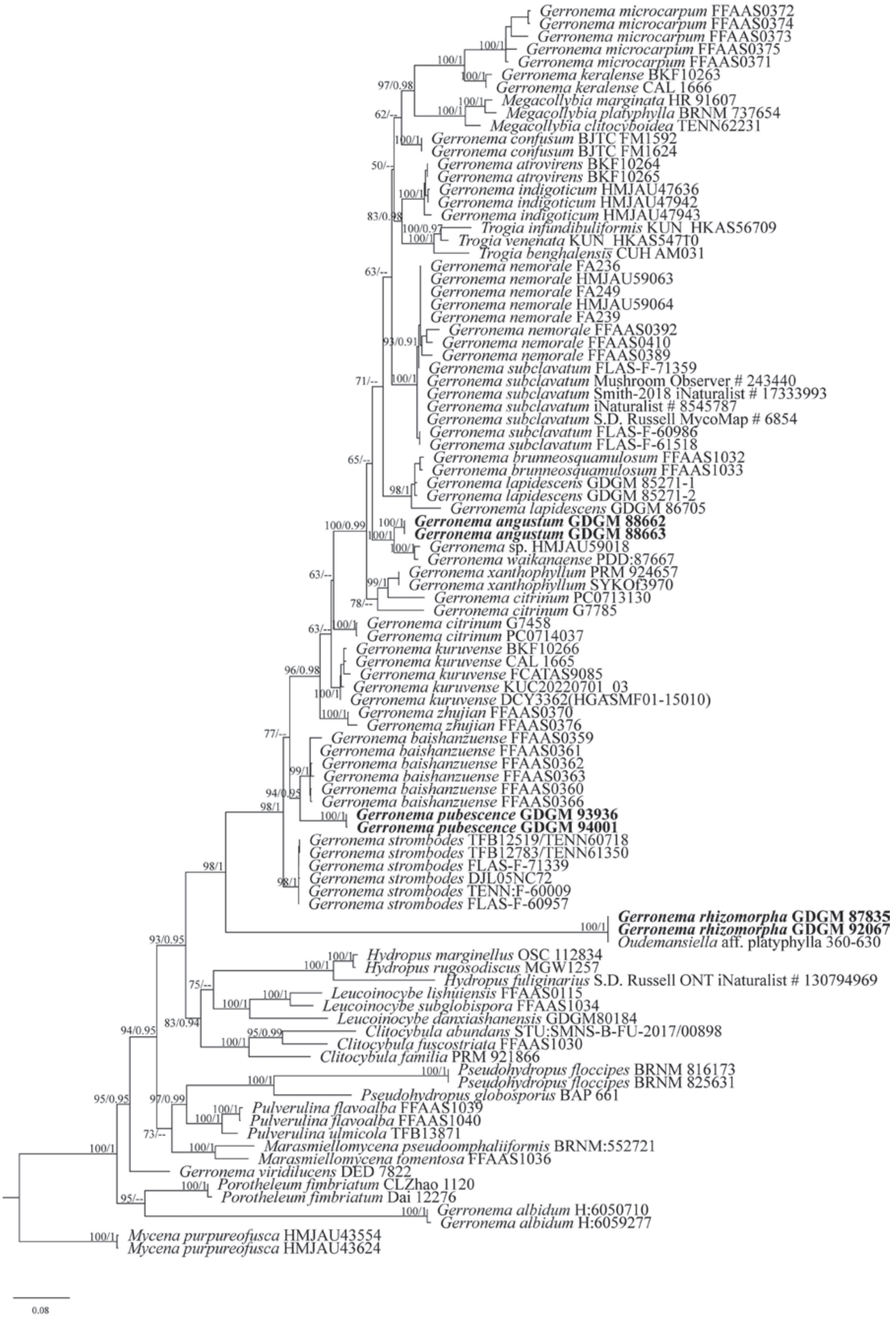

**Figure 1.** Phylogenetic consensus tree of *Gerronema* species inferred from the maximum likelihood and Bayesian inference based on a concatenated nrITS and nrLSU (MLB ≥ 50%, BPP ≥ 0.90 are shown). The tree is rooted with *Mycena purpureofusca*. New taxa are shown in bold.

and *Trogia* were nested in the core clade of Porotheleace with significant support (MLB = 100%, BPP = 1.00). The proposed new species formed three independent lineages within the genus *Gerronema* (MLB = 98%, BPP = 1.00), named *G. angustum*, *G. pubescence*, and *G. rhizomorpha*. In addition, *G. angustum* is sister to *G. waikanaense* (G. Stev.) J.A. Cooper and an unnamed specimen (HMJAU59018) (MLB = 100%, BPP = 1.00). *Gerronema pubescence* closely related to *G. baishanzuense* with a well-supported (MLB = 94%, BPP = 0.95). *Gerronema rhizomorpha* is placed at the base of the *Gerronoma* clade.

## Taxonomy

### ***Gerronema angustum*** **Ming Zhang & W.X. Zhang, sp. nov.**

Fungal Names: FN 572081
Figs 2, 3

**Diagnosis.** Distinguished from other *Gerronema* species by the combination characters, including caespitose habit, greenish green pileus, narrow and close lamellae, slender stipe, baidiospores measuring (4)4.5–5.5 × 2.5–3.5 µm, mainly clavate to narrowly utriform cheilocystidia.

**Holotype.** China • Guangdong Province: Shaoguan City, Nanling National Forest Park; 24°53'54"N, 113°2'24"E; 210 m asl.; 7 July 2022; Bin Song, Guo-Rui Zhong, and De-Chun Xie (GDGM 88662).

**Etymology.** *angustum* (Latin), referring to the narrow lamellae of this species.

**Description.** Basidiomata small-sized. Pileus 10–18 mm broad, infundibuliform, umbilicate to deeply umbilicate at center, greyish brown to brown (6E3–4) when young, greyish green (30B3–4, 30C2–3) when old, greyish green (30E5–6) at center, surface moist, glabrous, margin inflexed, radially striped with greenish grey to dull green (30B4–5, 30D4–5) lines. Lamellae decurrent, close, narrow, arcuate, even, white (30A1) to greenish grey (30B2), with 1–3 lamellulae. Stipe 45–60 × 2–5 mm, slender, centric, cylindrical, hollow, fragile, grey to greyish green (30C1–3), covered with white (30A1) fibrils. Odor and taste not recorded.

Basidiospores (4)4.5–5.5 × 2.5–3.5 µm, $L_m$ = 4.88 ± 0.51 µm, $W_m$ = 2.96 ± 0.32 µm, Q = (1.33)1.43–1.83, $Q_m$ = 1.66 ± 0.18, ellipsoid to oblong, smooth, thin-walled, hyaline, guttulate, inamyloid. Basidia 18–26 × 5.5–7 µm, clavate, thin-walled, hyaline, 4-spored, with sterigmata 2.3–4.4 µm long. Cheilocystidia 26–45 × 6–9.5 µm, clavate, fusiform to narrowly utriform, thin-walled, hyaline. Pleurocystidia not seen. Lamellar trama regular to subregular, hyphae 3–22 µm wide, cylindrical, thin-walled, hyaline. Pileipellis a cutis, hyphae 1.5–24.5 µm wide, smooth, hyaline; pileocystidia 22.5–65 × 8–15.5 µm, oblong to utriform, thin-walled, greyish brown to light brown pigmented in KOH. Pileus trama subregular, sarcodimitic. Stipitipellis a cutis, hyphae 3.5–25 µm wide, sometimes upturned hyphae, smooth, thin-walled, hyaline; caulocystidia 56–72 × 10.5–20.5 µm, narrowly cylindrical to oblong, thin-walled, hyaline. Stipe trama regular, sarcodimitic. Clamp connections present in all tissues.

**Habit and distribution.** Caespitose on the rotten wood in broad-leaved forests. Currently only known from the type locality in China.

**Additional specimen examined.** China • Guangdong Province: Shaoguan City, Nanling National Forest Park; 24°55'39"N, 113°3'20"E; 225 m asl.; 7 July 2022; Bin Song, Guo-Rui Zhong, and De-Chun Xie (GDGM 88663).

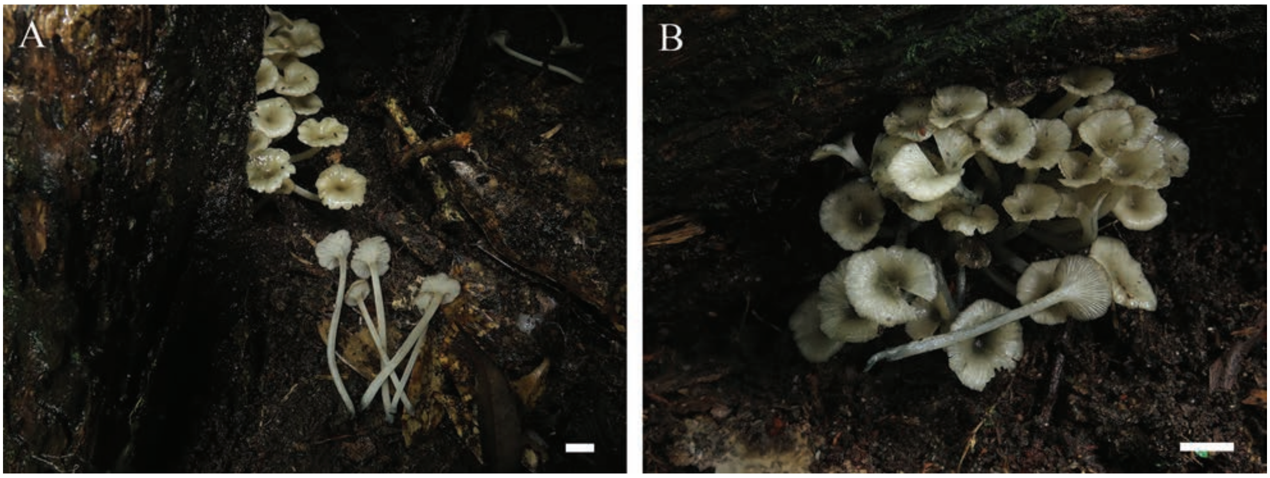

**Figure 2.** Basidiomata of *Gerronema angustum* **A** collection GDGM 88662, holotype **B** collection GDGM 88663. Photographed by Bin Song. Scale bars: 10 mm.

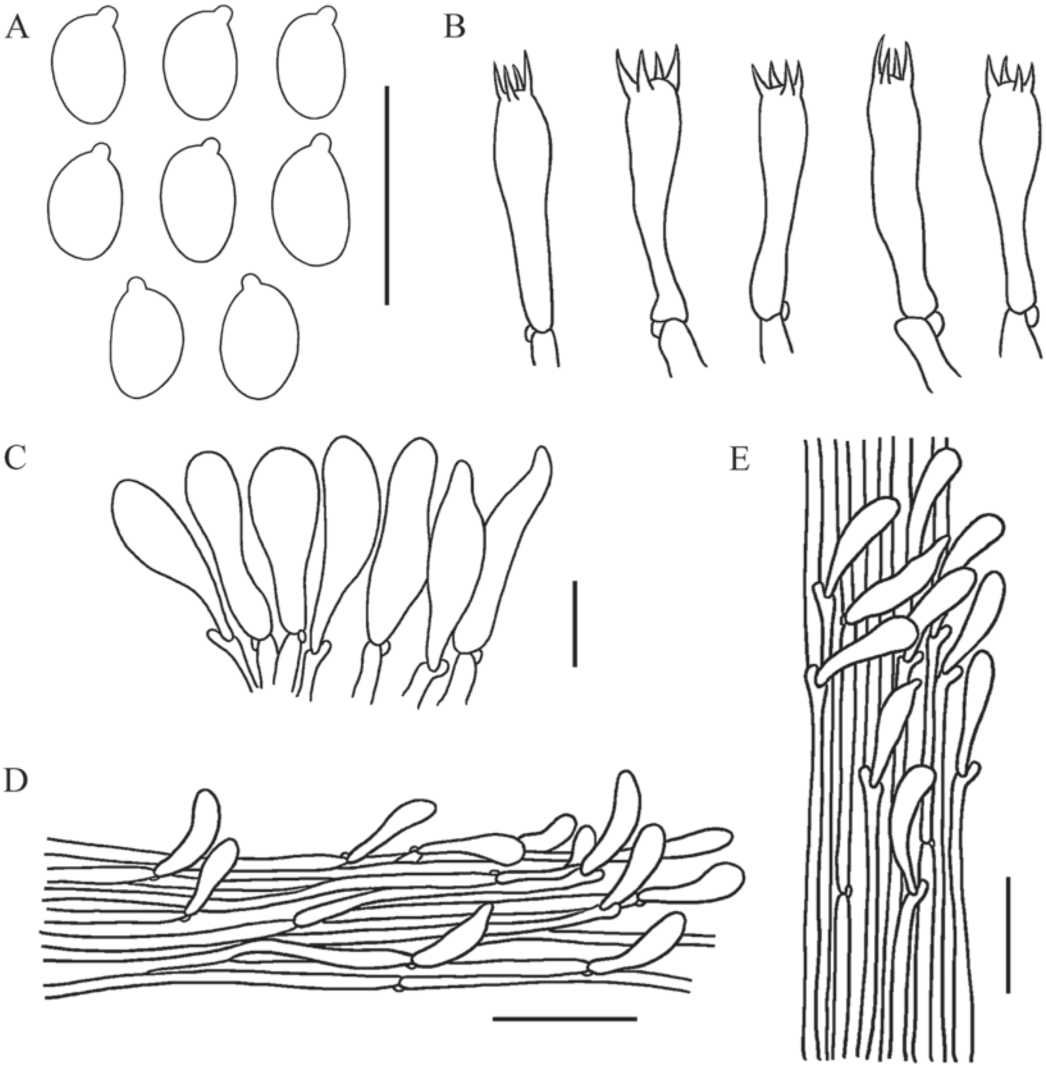

**Figure 3.** Microscopic features of *Gerronema angustum* (GDGM 88662, holotype) **A** Basidiospores **B** Basidia **C** Cheilocystidia **D** Pileipellis **E** Stipitipellis. Scale bars: 10 µm (**A–C**); 50 µm (**D–E**).

***Gerronema pubescence*** **Ming Zhang & W.X. Zhang, sp. nov.**
Fungal Names: FN 572082
Figs 4, 5

**Diagnosis.** Distinguished from other *Gerronema* species by the combination characters of the pastel grey pileus covered with pubescence when young, yellowish white to pale yellow lamellae are paler towards the margin, the absence of cheilocystidia, and the narrow cylindrical to utriform pleurocystidia.

**Holotype.** China • Guangdong Province: Huizhou City, Xiangtou Mountain Nature Reserve; 23°26'N, 114º37'E; 335 m asl.; 19 September 2023; Hao Huang and Wei-Xin Zhang (GDGM 94001).

**Etymology.** *pubescence* (Latin), referring to the species pileus usually covered with pubescence when young.

**Description.** Pileus 12–70 mm broad, hemispherical to plano-convex, depressed at center, grey (1E1), covered with pubescence when young, white to yellowish white (1A1–2) with age, grey to greyish brown (5E2–3, 6C1–2) at center, shallowly sulcate, surface dry, glabrous or pubescent, distinctly radially striped with grey to brownish orange (4C1–2, 6C2–3) lines, margin inflexed to reflexed. Lamellae subdecurrent, subdistant, ventricose, even, yellowish white to pale yellow (4A2–3), white (4C1) towards margin, with 1–5 lamellulae. Stipe

**Figure 4.** Basidiomata of *Gerronema pubescence* **A, B** collection GDGM 94001, holotype **C, D** collection GDGM 93936. **A, B** Photographed by Hao Huang **C, D** photographed by Ming Zhang. Scale bars: 30 mm (**A, B**); 10 mm (**C, D**).

15–40 × 2–7 mm, central, cylindrical, hollow, white (1A1) to grey (1C1), covered with white granulose or fibrils. Odor and taste not recorded.

Basidiospores (6)6.5–8 × (3.5)4–4.5 µm, $L_m$ = 7.13 ± 0.57 µm, $W_m$ = 4.08 ± 0.29 µm, Q = (1.5)1.56–2, Qm = 1.75 ± 0.18, ellipsoid to oblong, smooth, thin-walled, hyaline, guttulate, inamyloid. Basidia 24.5–39 × 5–7.5 µm, clavate, thin-walled, hyaline, 2- or 4-spored, with sterigmata 2.5–5 µm long. Cheilocystidia absent. Pleurocystidia 40–104 × 8.5–12.5 µm, narrowly cylindrical to lageniform, thin-walled, hyaline. Lamellar trama regular to subregular, hyphae 3.5–26.5 µm wide, thin-walled, hyaline. Pileipellis a cutis, hyphae 3–25 µm wide, smooth, hyaline; pileocystidia 39–100 × 10–21 µm, oblong to narrowly clavate, apex sometimes rostrate, thin-walled, greyish brown pigmented in KOH. Pileus trama regular to subregular, sarcodimitic. Stipitipellis a cutis, hyphae 2.5–38.5 µm wide, smooth, thin-walled, hyaline; caulocystidia 38.5–84.5 × 8.5–19 µm, narrowly cylindrical to clavate, thin-walled, hyaline. Stipe trama regular, sarcodimitic. Clamp connections present in all tissues.

**Habit and distribution.** Scattered or caespitose on the rotten wood in broad-leaved forests. Currently only known from the type locality in China.

**Additional specimen examined.** China • Guangdong Province: Zhaoqing City, Dinghu Mountain Nature Reserve; 23°10'43"N, 112°33'10"E; 150 m asl.; 9 April 2024; Ming Zhang, Guo-Rui Zhong, and Wen-Xiao Xia (GDGM 93936).

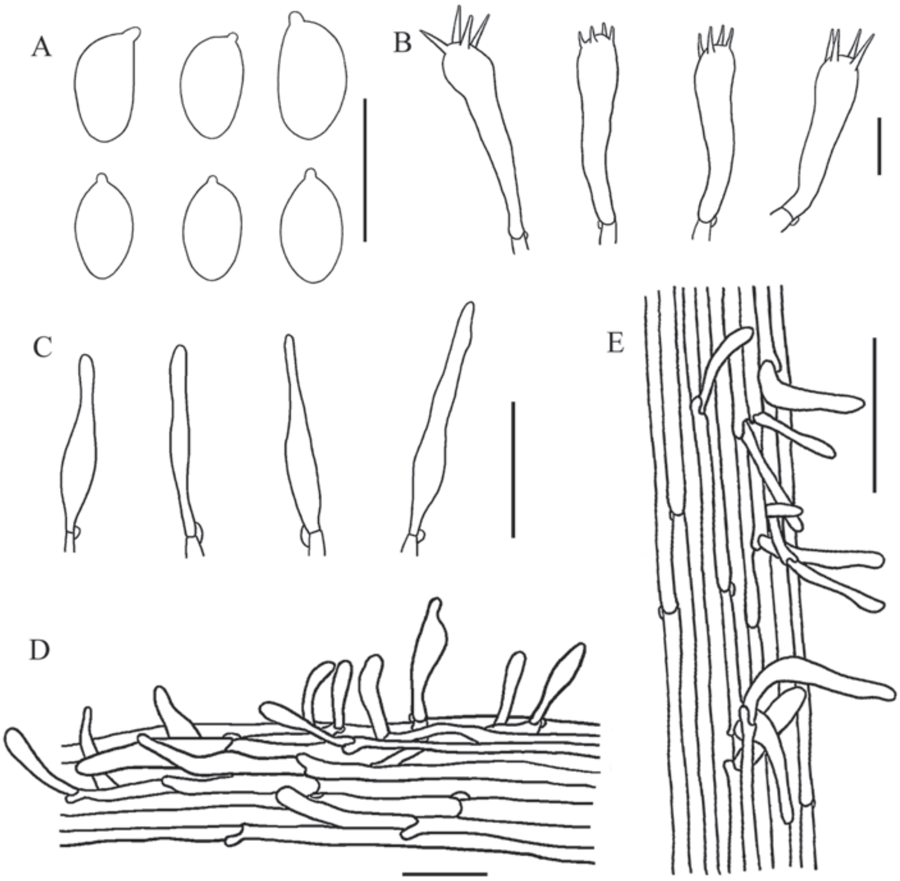

**Figure 5.** Microscopic features of *Gerronema pubescence* (GDGM 94001, holotype) **A** Basidiospores **B** Basidia **C** Pleurocystidia **D** Pileipellis **E** Stipitipellis. Scale bars: 10 mm (**A, B**); 50 mm (**C–E**).

### ***Gerronema rhizomorpha*** **Ming Zhang & W.X. Zhang, sp. nov.**

Fungal Names: FN 572083

Figs 6, 7

**Diagnosis.** Distinguished from other *Gerronema* species by the combination characters of medium-sized basidiomata, yellowish white to brown pileus, well-developed rhizomorphs at stipe base, the absence of cheilocystidia, and pleurocystidia.

**Holotype.** CHINA • Guangdong Province: Fengkai County, Zhaoqing City, Heishiding provincial natural reserve; 23°26'30"N, 111°53'28"E; 340 m asl.; 25 May 2023; Ming Zhang and Guo-Rui Zhong (GDGM 92067).

**Etymology.** *rhizomorpha* (Latin), referring to the species, usually has long and well-developed rhizomorphs at stipe base.

**Description.** Basidiomata medium-sized. Pileus 35–95 mm broad, hemispherical to plano-convex, depressed at center, brown (5E5–6) when young, yellowish white (2A2–3) to brown (5E4–5) at maturity, usually brown (5E4–5) to dark brown (6F4–5) at center, surface dry, distinctly radially striped with brown (5E6–7, 6E4–5) lines, margin inflexed. Lamellae subdecurrent, subdistant, ventricose, even, white to yellowish white (1A1–2), with 1–3 lamellulae.

**Figure 6.** Basidiomata of *Gerronema rhizomorpha* **A, B** collection GDGM 92067, holotype **C, D** collection GDGM 87835. Photographed by Ming Zhang. Scale bars: 30 mm (**A, B**); 50 mm (**C**); 30 mm (**D**).

Stipe 40–110 × 4–10 mm, central to eccentric, cylindrical, hollow, white to yellowish white (1A1–2), sometimes yellowish grey to greyish yellow (2C2–3) at the base, covered with yellowish grey to greyish yellow (2C2–4) fibrils, base with developed and white (1A1) rhizomorphs. Odor and taste not recorded.

Basidiospores 7–8.5(9.5) × 5–6(6.5) µm, $L_m$ = 7.80 ± 0.71 µm, $W_m$ = 5.53 ± 0.42 µm, Q = (1.23)1.27–1.58(1.6), $Q_m$ = 1.41 ± 0.13, broadly ellipsoid to ellipsoid, smooth, thin-walled, hyaline, guttulate, inamyloid. Basidia 25.5–32 × 7.5–11.5 µm, clavate, thin-walled, hyaline, 4-spored, with sterigmata 2.8–6 µm long. Hymenial cystidia absent. Lamellar trama regular to subregular, hyphae 3–22 µm wide, thin-walled, hyaline. Pileipellis a cutis, hyphae 3–25 µm wide, thin-walled, light yellow in KOH; pileocystidia 25.5–63.5 × 6–14 µm, narrowly utriform to cylindrical, thin-walled, greyish brown to light brown pigmented in KOH. Pileus trama subregular, sarcodimitic. Stipitipellis a cutis, hyphae 1.5–11 µm wide, smooth, thin-walled, hyaline; caulocy stidia 12.5–33 × 3.5–8 µm, narrowly cylindrical to oblong, thin-walled, hyaline. Stipe trama regular, sarcodimitic. Clamp connections present in all tissues.

**Habit and distribution.** Solitary or scattered, growing on the damp soil under broad-leaved forests. Currently known from China and Japan.

**Additional specimen examined.** China • Guangdong Province: Shaoguan City, Nanling National Forest Park; 24°56'48"N, 113°3'19"E; 490 m asl.; 9 June 2022; Ming Zhang, Guo-Rui Zhong, and Shi-Zheng Wang (GDGM 87835).

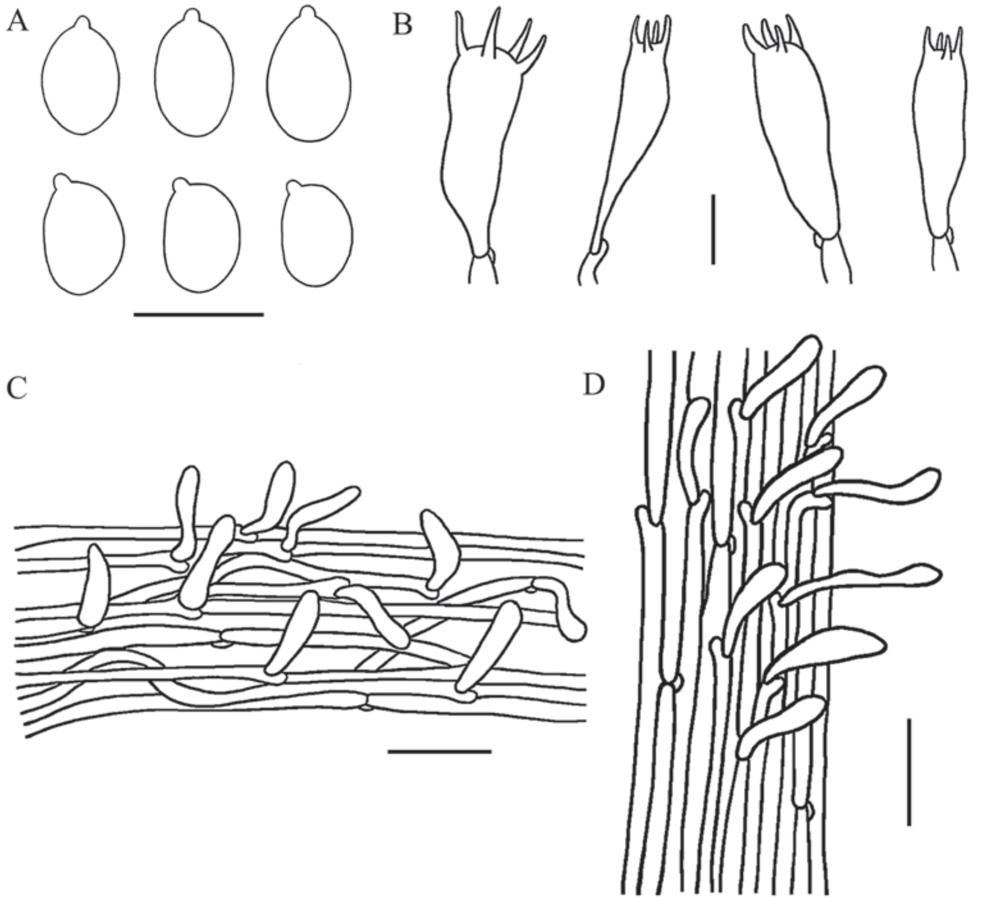

Figure 7. Microscopic features of *Gerronema rhizomorpha* (GDGM 92067, holotype) **A** Basidiospores **B** Basidia **C** Pileipellis **D** Stipitipellis. Scale bars: 10 mm (**A, B**); 50 mm (**C, D**).

## Discussion

Morphologically, *Gerronema angustum* can be easily distinguished from other species in *Gerronema* by its caespitose habit, slender basidiomata, greenish-green pileus, narrow and close lamellae, and ellipsoid to oblong basidiospores measuring (4)4.5–5.5 × 2.5–3.5 µm. *Gerronema angustum* is similar to *G. albidum*, which has been recorded in China (Singer 1962). But the distinctly white basidiomata and the absence of cheilocystidia can be used to distinguish *G. albidum* from *G. angustum*.

*Gerronema pubescence* is characterized by its hemispherical to plano-convex pileus covered with pubescence when young, yellowish white to pale yellow lamellae paler towards the pileus margin, absent cheilocystidia, and narrowly cylindrical to utriform pleurocystidia. *Gerronema pubescence* is similar to *G. keralense* K.P.D. Latha & Manim. and *G. zhujian*. However, *G. keralense*, originally described from India, can be distinguished by its small yellowish-brown pileus (4–17 mm broad), greyish-yellow stipe gradually greyish-brown towards the base, flexuose or irregular cheilocystidia, and absent pleurocystidia (Latha et al. 2018). *Gerronema zhujian* can be distinguished by the greyish-white pileus with a brown tinge at the center, slightly brown and narrow stipe (19–25 × 1.0–1.5 mm), subfusiform cheilocystidia, and the absence of pleurocystidia (Na et al. 2022).

*Gerronema rhizomorpha* is mainly characterized by its yellowish white to brown pileus, well-developed rhizomorphs at stipe base, and absent cheilocystidia and pleurocystidia. *Gerronema rhizomorpha* is similar to *G. confusum* in some extent as sharing relatively large basidiomata and brown pileus. But *G. confusum* from the north of China can be distinguished by its lignicolous habitat, greyish-brown stipe surface covered with dark brown granulose, absent rhizomorphs, 1–2-spored basidia, and abundant subcylindrical to cylindrical cheilocystidia (Zhao and Fan 2022). *Gerronema rhizomorpha* is also similar to *G. atrialbum* (Murrill) Borovička & Kolařík, with the stipe base often with rhizomorphs, white lamellae, and the absence of cheilocystidia and pleurocystidia. However, *G. atrialbum*, which is originally described from the USA, can be distinguished by its greyish brown to grey pileus and brown to pale stipe, relatively longer basidia (36–50 × 6.5–9.5 µm), which mainly has 2-spored. In addition, *G. atrialbum* mainly grows on humus or rotting hardwood (Murrill 1913; Antonín et al. 2019).

Phylogenetically (Fig. 1), our new species formed three distinct lineages according to the ML and BI phylogenetic analyses of the concatenated dataset and can be easily distinguished from other species with known sequences. *Gerronema angustum* formed a distinct lineage in *Gerronema* and is sister to *G. waikanaense* with high statistical support (MLB = 100%, BPP = 1.00). However, *G. waikanaense*, reported from New Zealand, differs by its dark leaden grey basidiomata, minutely fibrillose stipe surface, distant lamellae, and absent cheilocystidia (Cooper 2014).

*Gerronema pubescence* is phylogenetically related to *G. baishanzuense* (MLB = 94%, BPP = 0.95). But *G. baishanzuense* can be distinguished by its relatively small pileus (3–25.5 mm broad), relatively short stipe (4.5–26 mm) densely covered with pruinose when young, clavate or subfusiform cheilocystidia usually swollen at apex, and absent pleurocystidia (Na et al. 2022).

*Gerronema rhizomorpha* was gathered together with an ITS sequence (AB509870) named *Oudemansiella* sp. (platyphylla 360–630) from Japan

(MLB = 100%, BPP = 1.00) and shows that they represent the same phylogenetic species. It is noteworthy that *G. rhizomorpha* with well-developed rhizomorphs at stipe base and not growing on rotten wood is rare in *Gerronema*, which is morphologically more similar to species of the genus *Megacollybia*, but it does not belong to *Megacollybia* in phylogenetic analyses. Additionally, *G. rhizomorpha* has significant variability in the ITS1 region, has a low similarity rate with *Gerronema*, and is placed at the base of the *Gerronema* clade, possibly representing a separate evolutionary lineage. As the *Gerronema* genus is not a monophyletic group, *G. rhizomorpha* was temporarily classified as a member of *Gerronema* in the present study.

Due to *Gerronema* being widely distributed and many species being misidentified, only ten of the 20 species reported in Asia are from China. Recent investigations have found a high species diversity of *Gerronema* in southern China, and there are still many other species waiting to be reported, which can enrich the species diversity of the genus. In addition, the intraspecific and intergeneric phylogenetic relationships of *Gerronema* still remain highly controversial. Upon defining the diversity of species explicitly, the systematic phylogenetic framework of the genus *Gerronema* needs to be further refined based on more samples and sequence fragments to solve the classification problem of the *Gerronema*.

## Key to species of *Gerronema* in China

| | | |
|---|---|---|
| 1 | Baisidiomata grow on soil, stipe base with well-developed rhizomorphs | **2** |
| – | Baisidiomata grow on decaying woods, stipe base without developed rhizomorphs | **3** |
| 2 | Sclerotia can be found at the base | ***G. lapidescens*** |
| – | Sclerotia not be found or recorded | ***G. rhizomorpha*** |
| 3 | Basidiomata blue | ***G. indigoticum*** |
| – | Basidiomata without blue tinge | **4** |
| 4 | Basidiomata greyish green | ***G. angustum*** |
| – | Basidiomata without greyish green tinge | **5** |
| 5 | Basidiomata white | ***G. albidum*** |
| – | Basidiomata without white tinge | **6** |
| 6 | Pleurocystidia present | **7** |
| – | Pleurocystidia absent | **8** |
| 7 | Pileus viscid | ***G. chrysocarpum*** |
| – | Pileus dry, usually covered with pubescence when young. | ***G. pubescence*** |
| 8 | Pileus densely covered with deep brown fur or scales | **9** |
| – | Pileus without fur or scales | **10** |
| 9 | Stipe fuscous, basidiospores 9.0–12.9 × 4.9–7.2 µm | ***G. brunneosquamulosum*** |
| – | Stipe white, basidiospores 6.3–8.5 × 3.2–4.8 µm | ***G. zhujian*** |
| 10 | Basidiomata small-sized, Pileus usually ≤ 11 mm in diam. | **11** |
| – | Basidiomata small to medium-sized, Pileus ≥ 11 mm in diam | **12** |
| 11 | Basidiospores 6.1–7.5 × 3.5–4.3 µm, cheilocystidia present | ***G. microcarpum*** |
| – | Basidiospores 8–11 × 4–6 µm, cheilocystidia absent | ***G. kuruvense*** |
| 12 | Basidia 1–2-spored | ***G. confusum*** |
| – | Basidia 4-spored | **13** |

13 Pileus up to 25 mm in diam, stipe 4.5–26.0 × 0.5–2.0 mm .......................................................................................................... ***G. baishanzuense***

– Pileus less than 20 mm in diam, stipe 19.0–36.0 × 1.0–2.5 mm ............................................................................................................. ***G. nemorale***

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

All authors have contributed to the manuscript and approved the version submitted for publication.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# *Sticta flakusiorum* and *S. kukwae*—two additional new species from the Neotropics (Peltigerales, Peltigeraceae)

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## Abstract

Two additional species of *Sticta* are described as new to science based on material from Bolivia and Peru and supported by phylogenetic analysis of the fungal ITS barcoding marker. The two new species represent lineages within clade I on the global *Sticta* phylogeny. *Sticta flakusiorum* Ossowska, B. Moncada & Lücking is a species in the *S. humboldtii* morphodeme and is characterized by lobes partly to entirely covered with white hairs, also covering the margins of submarginal and laminal apothecia, and the scabrid basal membrane of cyphellae, which is white to yellow, or partly brown, and when yellow K+ purple. The taxon was discovered at a single locality in Bolivia, but it is closely related to a potentially new *Sticta* species from Peru, which is here left undescribed. The other new species, *S. kukwae* Ossowska, Magain & Sérus., belongs to the *S. weigelii* morphodeme. It has lobes with sinuous margins and dark, palmate to corymbose phyllidia. It was collected at several locations in Peru and a single locality in Bolivia.

**Key words:** Bolivia, diversity, integrative taxonomy, ITS rDNA, Lobarioideae, Peltigeraceae, Peru

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## Introduction

Lobarioid lichens, long treated in their own family, Lobariaceae (Moncada et al. 2013a), are now recognized as a subfamily, Lobarioideae, within Peltigeraceae, along with subfamilies Nephromatoideae and Peltigeroideae (Kraichak et al. 2018; Lücking 2019; Lumbsch and Leavitt 2019; Widhelm et al. 2019, 2021). Traditionally, the presence or absence of regular pores in the lower cortex, called cyphellae, was the main feature that differentiated the genus *Sticta* (Schreb.) Ach. from other genera in the family (Galloway and Elix 2013; Moncada et al. 2013a); however, apart from *Sticta*, a second lineage, nested within the *Lobaria* clade and separated in the genus *Dendriscosticta* B. Moncada & Lücking, also features cyphellae (Moncada et al. 2013a; Simon et al. 2022). In addition to *Dendriscosticta*, another nine genera have recently been segregated from the collective genera *Lobaria* (Schreb.) Hoffm., *Pseudocyphellaria* Vain., and *Sticta*, including, e.g., *Yarrumia* D.J. Galloway (Galloway 2015) and *Emmanuelia* Ant. Simon, Lücking & Goffinet (Simon et al. 2020). Currently, close to 500 species have been accepted in the subfamily Lobarioideae (Kirk et al. 2008; Lücking et al. 2017), almost half of them within the genus *Sticta* (Moncada et al. 2014a, 2021a; Lücking et al. 2017; Ossowska et al. 2022a). The species of this genus are common in humid, cool to warm environments with high rainfall or humidity and are most diverse in tropical areas (Moncada 2012; Moncada et al. 2014b, 2020). This is illustrated by the example of Colombia, where more than 150 *Sticta* species have been identified following extensive field and laboratory work (Moncada 2012; Moncada and Lücking 2012; Moncada et al. 2013b, 2014a, b, 2015, 2021b). In comparison, the knowledge on the genus *Sticta* in neighboring countries is limited. For Ecuador, 46 names have been listed (Consortium of Lichen Herbaria 2023; Yánez-Ayabaca et al. 2023), but at least eight of these are doubtful records, including some known New Zealand endemics. Twenty-two species are included in the revised checklist for Brazil (Aptroot 2002; Dal Forno et al. 2018; Torres et al. 2021); however, Torres et al. (2021) suggest that the Cerrado forest ecoregion may host a significant number of novel *Sticta* species, indicating that the true diversity of species in Brazil may be higher. The checklist of lichens for Peru includes only ten *Sticta* species (Ramos 2014), but the majority of the records are historical and have not been critically checked. Meanwhile, twenty-eight *Sticta* taxa are known from Bolivia, mostly based on recent works (Moncada and Lücking 2012; Ossowska 2021; Ossowska et al. 2022a, b, 2024a, b; Crous et al. 2023).

This paper presents two additional new *Sticta* species, *S. flakusiorum* and *S. kukwae*, both supported by molecular data, from Peru and/or Bolivia. *Sticta flakusiorum* has been found so far at a single site in Bolivia, whereas *S. kukwae* has been collected from several localities in Peru and Bolivia. Detailed morphological and anatomical descriptions of both species are also given, together with a discussion on similar taxa.

## Materials and methods

### Taxon sampling

Fresh material for this study was collected during fieldwork in Bolivia in 2010–2017 and Peru in 2012. The collected material is deposited in the LPB, UGDA, and DUKE herbaria. All material was examined under a dissecting and a compound microscope (Nikon SMZ800N and ZEISS Axioskop). Character assessment was based on the morphological and anatomical traits for *Sticta* described by Moncada (2012) and Moncada et al. (2014a). Spot reactions were done with K (potassium hydroxide solution), C (sodium hypochlorite solution), Pd (paraphenylenediamine), and KC (K followed by C) on close spots of exposed medulla of the same thallus fragments; secondary compounds were analyzed using the thin-layer chromatography method (TLC) in solvents A and C (Orange et al. 2001).

Species that were informally distinguished by Moncada (2012) and Moncada et al. (2020) but have not yet been formally described are marked with quotes (e.g., '*S. arachnosylvatica*').

### DNA extraction, PCR amplification, and sequencing

Genomic DNA from the Bolivian samples was isolated, and the nuITS rDNA marker was amplified following the protocol described in Ossowska et al. (2022a). Sequencing was performed in a Macrogen sequencing system (http://www.macrogen.com). In the case of samples from Peru, DNA was extracted following the protocol of Cubero et al. (1999). PCR conditions and primers were the same as in Ossowska et al. (2022a).

### Alignment and sequence analyses

The newly generated sequences were compared with available data from the genus *Sticta* (Suppl. material 1), using our previous alignment (Ossowska et al. 2022a) based on a recent master alignment (Moncada et al. 2020). The new sequences were added to the existing alignment using MAFFT 7.164 with the "—add" option (Katoh and Frith 2012; Katoh and Standley 2013), followed by manual checking in BIOEDIT 7.0.9 (Hall et al. 2011). Phylogenetic analysis was performed using maximum likelihood in RAxML 8.2.0 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010), with non-parametric bootstrapping using 400 pseudoreplicates (based on an automated saturation criterion) under the universal GTR GAMMA model. Trees were visualized in FigTree 1.4.2 (Drummond and Rambaut 2007) and edited using Coral Draw 2019.

## Results

We generated seven new nuITS rDNA sequences that form two distinct lineages in the *Sticta* tree (Fig. 1), suggesting the presence of three new species, two of which are closely related sister species. The new sequences align close to other *Sticta* species, such as *S. sylvatica* (Huds.) Ach. and *S. aymara* Ossowska et al., within clade I (*fuliginosa* clade) sensu Widhelm et al. (2018).

The first of the new species, *S. kukwae*, is represented by one specimen from Bolivia and four from Peru. All specimens have a thallus with strongly sinuous margins and very dark phyllidia. One specimen (LG3227) from Peru had small and sparse apothecia, absent in the other specimens. However, other characteristics were consistent with the rest of the specimens in this clade (Fig. 1). Within clade I, the new species is closely related to *S. umbilicariiformis* Hochst. ex Flot. (Fig. 1).

The second new lineage is formed by two sister species. The lineage with a specimen from Bolivia is named in this paper *S. flakusiorum* and is closely related to the specimens from Peru, which potentially also represent a new species. At this point, we have named it *Sticta* sp. 36. It is a phyllidiate species (*S. flakusiorum* lacks vegetative diaspores), and morphologically it is similar to *S. phyllidiokunthii* B. Moncada & Lücking, with numerous, aggregated, palmate phyllidia that are marginal and laminal in *S*. sp. 36 and marginal in *S. phyllidiokunthii* (Moncada et al. 2013b). However, the material of *S*. sp. 36 is too sparse for a formal description at this point. Both species, *S. flakusiorum* and *S*. sp. 36, are nested in the clade of *S. viviana* Alej. Suárez & Lücking and are closely related to *S. phyllidiofuliginosa* B. Moncada, A. Suárez & Lücking.

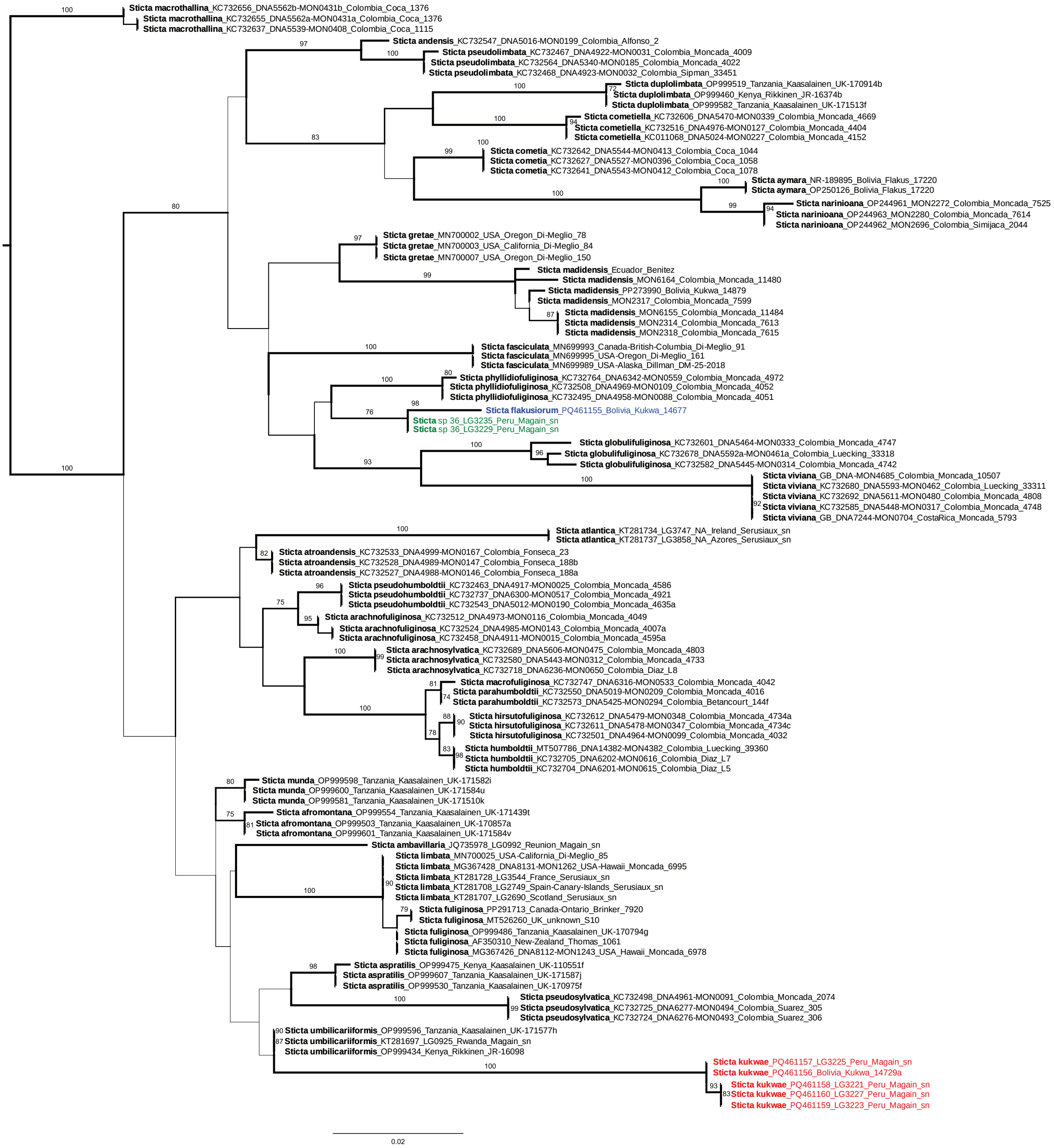

**Figure 1.** Best-scoring maximum likelihood tree of the *Sticta* target clade containing the new species *S. flakusiorum* from Bolivia (blue), *S. kukwae* from Bolivia and Peru (red), and *S.* sp. 36 from Peru (green), based on the fungal ITS barcoding marker. Branches associated with high bootstrap support values ( ≥ 70) are thickened and values are indicated near the branches.

Detailed descriptions of morphological and anatomical characteristics of *S. flakusiorum* and *S. kukwae*, together with figures and comparisons with similar and related species, are given below.

## Discussion

As a genus, *Sticta* is relatively easy to recognize in the field due to the foliose, large thallus with tomentum and cyphellae on the lower surface and the often characteristic fishy odor caused by the presence of methylamine products (Galloway

1994, 1997; Moncada 2012). However, species within this genus are much more difficult to distinguish, due to the lack of a clear concept of within-species variation, including the type of photobiont, the presence and type of vegetative propagules, their shape, distribution, and size, as well as the width and length of the lobes or their shape (Moncada 2012). Molecular data have helped to address this issue and to refine the set of potentially diagnostic characters, and Moncada et al. (2014a) identified a total of over 150 morphological and anatomical features that should be taken into account (see also Ossowska et al. 2024a).

Even so, accurate species recognition may be hindered by intraspecific variability, such as the formation of photosymbiodemes, apotheciate vs. non-apotheciate species pairs, and discrete morphodemes (Moncada et al. 2020, 2021b; Ossowska et al. 2022a, b, 2024a; Di Meglio and Goward 2023). For instance, the two vegetatively reproducing *S. fuliginosa* (With.) Ach. (with isidia) and *S. limbata* (Sm.) Ach. (with soredia) cannot be distinguished using the ITS barcoding marker (Moncada et al. 2014a; Magain and Sérusiaux 2015). Another case is found in the large foliose *S. filix* (Sw.) Nyl. vs. the delicate *S. lacera* (Hook. f. & Taylor) Müll. Arg., both New Zealand endemics (Lücking et al. 2022), or in *S. antoniana* B. Moncada & Lücking and *S. tomentosa* (Sw.) Ach. from Hawaii (Moncada et al. 2020, 2021a), as well as *S. arenosella* Di Meglio & Goward and *S. torii* Ant. Simon & Goward (Simon et al. 2018a; Di Meglio and Goward 2023; Ossowska et al. 2024a, b).The *Sticta fuliginosa* clade also contains apotheciate specimens devoid of isidia or soredia, suggesting the existence of individuals within the same species with different modes of reproduction. This was also observed in our new species, *S. kukwae*, with apothecia in one specimen from Peru, arranged in a clade together with non-apotheciate specimens. Similar cases were recently reported for *S. scabrosa* B. Moncada, Merc.-Díaz & Bungartz subsp. *scabrosa* and *S. cellulosa* Kaasalainen, originally described as sterile (Moncada et al. 2021b; Kaasalainen et al. 2023), but later found fertile in material from Bolivia (Ossowska et al. 2022b, 2024b). Some species, e.g., the widely distributed *S. andina*, show even greater variation in reproduction modes: this species was previously divided into three tentative taxa that differed in the type of propagation (Moncada et al. 2021a, b; Ossowska et al. 2022a; Kaasalainen et al. 2023), but later they were merged into one species due to genetic similarities (Moncada et al. 2021b).

Traditional taxonomy in *Sticta* was largely based on morphodemes, i.e., particular gross morphologies that were recognized as species, e.g., narrow-lobed, cyanobacterial individuals with marginal isidia as *S. weigelii* (Ach.) Vain., broad-lobed, cyanobacterial specimens with laminal isidia as *S. fuliginosa*, or green-algal, apotheciate individuals as *S. canariensis* (Bory) Bory ex Delise, *S. damicornis* (Sw.) Ach., or *S. dichotoma* Bory ex Delise. Molecular data have shown that these morphodemes consist of many, often only distantly related species (Moncada et al. 2013b, 2014a, 2015; Lücking et al. 2021; Ossowska et al. 2024a). The two additional new species of *Sticta* introduced in this paper are also part of morphodemes: *S. kukwae* is morphologically similar to *S. weigelii*, both having a brown, irregular to orbicular thallus with dark, marginal vegetative propagules, whereas *S. flakusiorum*, due to its hairy upper surface, represents the *S. humboldtii* morphodeme.

In recent years, research on *Sticta* has intensified, resulting in the addition of many new species in various parts of the world (e.g., Lendemer and Goffinet 2015; Magain and Sérusiaux 2015; Simon et al. 2018a, b; Dal Forno et al. 2018; Torres et al. 2021; Ossowska et al. 2022a, 2024a; Kaasalainen et al. 2023;

Di Meglio and Goward 2023; Yánez-Ayabaca et al. 2023). Many regions in the Neotropics, e.g., Colombia, Bolivia (Ossowska et al. 2024a) and Puerto Rico (Mercado-Díaz et al. 2020), but also Africa (Simon et al. 2018b; Kaasalainen et al. 2023) and Oceania, e.g., Hawaii (Moncada et al. 2020, 2021a), are being explored, but many other regions remain poorly studied in terms of the genus *Sticta*. For instance, the checklist of lichens from Peru includes only ten *Sticta* species (Ramos 2014), and these have not yet been critically restudied. Among the names in the Peruvian checklist are *S. fuliginosa*, *S. laciniata* Ach., *S. sylvatica*, and *S. weigelii*, in which many new species have recently been distinguished (Moncada et al. 2021b; Di Meglio and Goward 2023; Ossowska et al. 2024a; this study). The new species presented here, *S. kukwae*, and the as-yet-undescribed *S*. sp. 36, are the first specimens from Peru supported by molecular data.

## Taxonomy

### ***Sticta flakusiorum*** **Ossowska, B. Moncada & Lücking, sp. nov.**

MycoBank No: 856228
Fig. 2

**Diagnosis.** Differing from *S. humboldtii* in the absence of true cilia, the presence of submarginal apothecia with entire to crenate margins, completely to partly covered by white hairs, spongy to fasciculate primary tomentum, and scabrid basal membrane of cyphellae, white to yellow (then K+ purple), or partly brown.

**Type.** Bolivia. • Dept. La Paz; Prov. Bautista Saavedra, Área Natural de Manejo Integrado Nacional APOLOBAMBA, between La Curva and Charazani, 15°08'09"S, 69°02'03"W, 3780 m, open area with shrubs, Ceja de Monte Superior (Altimontano), on shrub, 15 Nov. 2014, M. Kukwa 14677 (holotype UGDA L-65223, isotype LPB).

**Description.** Stipe absent. Thallus orbicular, up to 5 cm diam., moderately branched, with 3–5 branches per 5 cm radius, branching pleurotomous to polytomous; lobes suborbicular to flabellate, interspaced to adjacent, involute, with their apices rounded, revolute, and undulate and their margins sinuous, slightly thickened; lobe internodes 2–20 mm long, 3–15 mm broad; thallus coriaceous. Upper surface pitted to rugose, yellowish brown to chocolate brown, darker near the apices in the herbarium, shiny; lobes entirely hirsute or rarely with some parts lacking tomentum, covered by white hairs, without papillae and maculae; true cilia absent, but lower tomentum partly projecting beyond the margins and resembling cilia, fasciculated to agglutinated, white to pale brown, up to 0.5 mm. Apothecia submarginal and laminal, subaggregated, sessile to shortly stipitate, with pronounced invagination on the lower side, up to 2.0 mm diam.; disc brown to chestnut-brown; margin entire to crenate, completely to partly covered by white hairs, up to 1 mm long, simple to agglutinated, margin brown to dark brown. Vegetative propagules absent. Lower surface ribbed, brown; primary tomentum dense and usually thick to sparse to the margin, spongy to fasciculate, soft, white to brown; secondary tomentum present, arachnoid. Rhizines absent. Cyphellae 1–20 per cm$^2$ towards the thallus center and 41–60 per cm$^2$ towards the margin, scattered, elongate to irregular, urceolate with wide pore to cupuliform, erumpent to sessile, remaining below the level of the primary tomentum, with the margin raised and involute to erect, cream to brown colored, with tomentum up to the pore; pore up to 1.5 mm diam.; basal membrane scabrid, white to yellow, or partly brown, when yellow K+ purple and C+ red-orange, KC–, P–. Medulla compact, white to yellow, or partly brown, when yellow K+ purple and C+ red-orange, KC–, P–. No substances detected by TLC.

Upper cortex paraplectenchymatous, up to 35 μm thick, uniform, up of 5 layers of cells, their walls up to 1.5 μm thick and their lumina rounded to isodiametric, up to 5–15 × 5–10 μm diam. Photobiont layer up to 150 μm thick, its cells up to 10 μm diam. Medulla up to 120 μm thick, its hyphae up to 5.0 μm broad. Lower cortex paraplectenchymatous, up to 50 μm thick, with up to 7 cell layers; cells up to 10 μm diam. Upper primary tomentum up to 100 μm long, simple or in fascicles formed of up to 7 hyphae, hyphae simple. Upper secondary tomentum not seen on upper surface. Lower primary tomentum up to 200 μm long, composed of fascicles formed of 10–15 hyphae, hyphae mostly simple, apically free, and flexuous. Lower secondary tomentum 30 μm long, of single, simple to branched hairs, moniliform. Cyphellae cavity up to 220 μm deep; cells of basal membrane without or rarely with up to 2 papillae. Apothecia biatorine, up to 500 μm high, with indistinct stipe, about 20 μm high; excipulum up to 400 μm broad, with projecting hairs, up to 1 μm long. Hymenium up to 300 μm high; epihymenium up to 5 μm high, orange-brown, pigment present in the gel and in the walls upper cells of paraphyses, with very gelatinous upper layer. Asci 4–8-spored, ascospores fusiform, 1–3-septate, 25–35 × 6–8 μm.

**Habitat and distribution.** *Sticta flakusiorum* is an epiphytic species found in an open area with shrubs at an altitude of 3780 m in the Department La Paz, Bolivia.

**Etymology.** The species is named in honor of two lichenologists, Adam Flakus and Pamela Rodriguez-Flakus, for their contributions to the taxonomy of lichens and lichenicolous fungi of Bolivia.

**Notes.** The new species, *S. flakusiorum*, forms part of the *S. humboldtii* morphodeme, which also includes *S. pseudohumboldtii* B. Moncada & Lücking and *S. parahumboldtii* B. Moncada & Lücking (Moncada et al. 2013b). However, unlike in the other species, the upper surface of *S. flakusiorum* is rather hirsute, while in *S. humboldtii* and the other species, the hairs are very dense and resemble the primary tomentum present on the lower surface (Moncada 2012). In addition, *S. parahumboldtii* has marginal soredia and lacks apothecia (Moncada et al. 2013b). Furthermore, all species differ in the color of the lower surface and tomentum. In the new species, the lower surface is brown, and the primary tomentum is white to cream. Other species have a cream-colored lower surface, and the primary tomentum is cream in *S. parahumboldtii*, cream-white in *S. pseudohumboldtii*, and cream to grey-brown in *S. humboldtii* (Moncada 2012; Moncada et al. 2013b). All species belong to clade I on the *Sticta* phylogeny (see Fig. 1), but the new species is more closely related to *S. viviana*. *Sticta humboldtii* and *S. parahumboldtii* are related to *'S. arachnosylvatica'*, while *S. pseudohumboldtii* is close to *S. arachnofuliginosa* B. Moncada & Lücking (Widhelm et al. 2018). Among the species of this morphodeme, *S. humboldtii* has been reported more frequently than other species (Moncada 2012; Moncada et al. 2013b), including records from Peru (Ramos 2014). However, only the Colombian records are supported by molecular data (Moncada et al. 2013b, 2014a), and therefore its presence in Peru needs to be verified. *Sticta pseudohumboldtii* and *S. parahumboldtii* are known so far only from Colombia (Moncada 2012; Moncada et al. 2013b, 2014a, b).

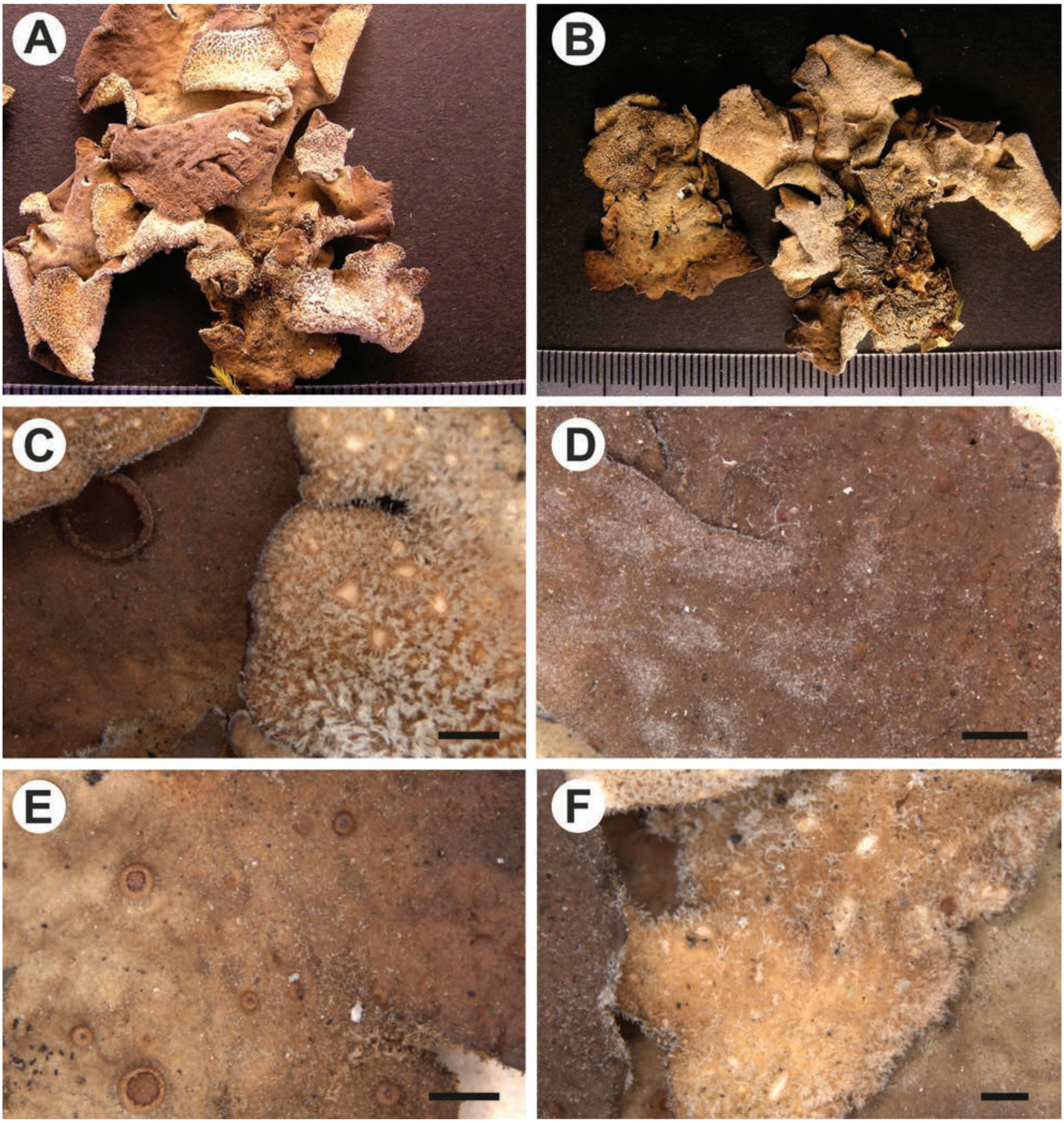

**Figure 2.** Morphology of *Sticta flakusiorum* (holotype) **A** upper surface **B** lower surface **C, D** hirsute upper surface with apothecium and lower surface with tomentum and cyphellae **E** apothecia with entire to crenate margins, covered by white hairs **F** primary tomentum spongy to fasciculate and cyphellae with scabrid basal membrane. Scale bars: 1 mm (**A–F**).

In the phylogenetic tree, *S. flakusiorum* forms a lineage sister to a clade of a potentially new species, referred to as *Sticta* sp. 36 (see above). This taxon is distinguished by its thallus with smooth upper surface, sparse and laminal apothecia, and abundant, marginal phyllidia. Furthermore, the primary tomentum is greyish gold, whereas in *S. flakusiorum* it is white to brown. The specimens of *S.* sp. 36 are fragmentary; thus, we have decided not to describe it at this moment. *Sticta* sp. 36 was found in Peru in Puno (Lampa, Santa Lucia).

The hirsute upper surface is also characteristic of '*S. arachnosylvatica*', *S. minutula* B. Moncada, A. Suárez & Lücking and *S. hirta* (Nyl.) Trevis (Moncada

2012; Moncada et al. 2014a, 2020), but these taxa differ from *S. flakusiorum* in the structure of the lobes, the presence of vegetative propagules, as well as the color of the lower surface and the structure of primary tomentum. In particular, the lobe margins in all these species are entire to crenate, whereas in *S. flakusiorum* they are sinusoidal; in addition, '*S. arachnosylvatica*' and *S. minutula* have isidia. The lower surface of '*S. arachnosylvatica*' is cream-white with primary tomentum dense to the margin (Moncada 2012), and in *S. minutula* the lower surface is cream-white with primary tomentum scarce over the whole area. Additionally, the latter taxon is distinguished by its sparse cyphellae (Moncada 2012). *Sticta hirta* has a creamy lower surface with irregular tomentum, sparse towards the margins, and it is fasciculate to spongy (Moncada 2012). All three species, '*S. arachnosylvatica*,' *S. minutula*, and *S. hirta*, have been molecularly confirmed only from Colombia (Moncada 2012; Moncada et al. 2014a, 2020) but have not been reported from Bolivia and Peru.

### ***Sticta kukwae*** **Ossowska, Magain & Sérusiaux, sp. nov.**

MycoBank No: 856229
Fig. 3

**Diagnosis.** Differing from *S. weigelii* in lobes with sinuous margins, in the presence of marginal phyllidia, and the scarce, submarginal apothecia, as well as the primary tomentum being light brown to brown, dense, and sparse towards the margins.

**Type.** Bolivia. • Dept. La Paz; Prov. Franz Tamayo, Área Natural de Manejo Integrado Nacional APOLOBAMBA, between la Cumbre and Pelechuco, close to Aguas Blancas, 14°49'12"S, 69°07'05"W, elev. 4070 m, open high Andean vegetation, Altoandino, saxicolous, 15 Nov. 2014, M. Kukwa 14729a (holotype UGDA L-65224, isotype LPB).

**Description.** Stipe absent. Thallus suborbicular to irregular, up to 10 cm diam., moderately branched, with 3–5 branches per 5 cm radius, branching anisotomous to polytomous; lobes ligulate to flabellate, undulate, with their apices rounded, revolute, and their margins sinuous, not thickened; lobe internodes 5–9 mm long, 4–9 mm broad; thallus coriaceous. Upper surface smooth to shallowly pitted, yellowish brown to brown when dry, shiny; surface glabrous, few lobes with papillae but without maculae; true cilia absent. Only two apothecia found, submarginal, with slightly pronounced invagination on lower side, up to 1.5 mm diam.; disc brown; margin smooth, brown to dark brown. Phyllidia present, marginal and laminal, simple, branched, palmate to corymbose, vertical to obliquely arranged, globular at first, then spatulate to squamiform, usually darker than the thallus. Lower surface uneven, light brown; primary tomentum dense and thick to the margin, sometimes absent at the very edge, fasciculate to spongy, soft, white to brown, sometimes brown with brighter apices; secondary tomentum present, arachnoid. Rhizines present, only on few lobes, whitish to brown, simple to branched, densely distributed. Cyphellae 1–20 per cm$^2$ towards the thallus center and 1–20 per cm$^2$ towards the margin, dispersed, rounded to irregular, urceolate with wide pore, erumpent to sessile, remaining below the level of the primary tomentum, with the margin elevated and involute, white to beige colored, with tomentum; pore up to 0.5 mm diam.; basal membrane scabrid, white, K+ yellowish, C–, KC–, P–. Medulla compact, white, K–, C–, KC–, P–. No substances detected by TLC.

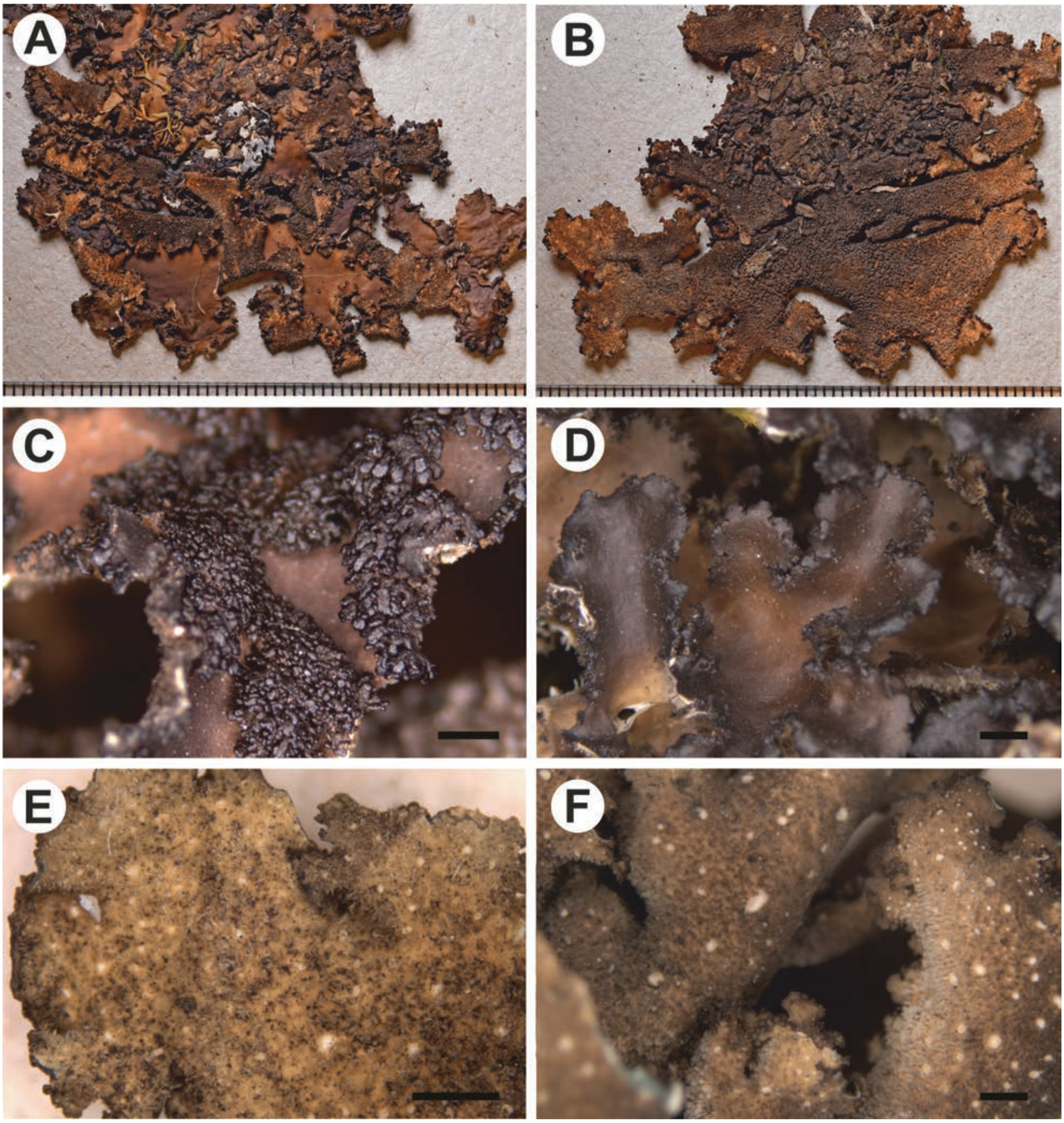

**Figure 3.** Morphology of *Sticta kukwae* (**A, B, E** holotype **C** LG3223, **D** LG3221, **F** LG3227) **A** upper surface **B** lower surface **C, D** lobes with sinuous margins and marginal phyllidia **E** lower tomentum with cyphellae and rhizines. Scale bars: 1 mm (**A–F**).

Upper cortex paraplectenchymatous, up to 65 µm thick, uniform, consisting of up to 7 cell layers with cells 5–10 µm diam., their walls up to 1.5 µm thick. Photobiont layer up to 130 µm thick, its cells up to 15 µm diam. Medulla up to 120 µm thick, its hyphae 4 µm broad, without crystals. Lower cortex paraplectenchymatous, up to 60 thick, with 7 cell layers; cells up to 10 µm diam., their walls up to 2.5 µm thick. Lower primary tomentum up to 400 µm long, with cells resembling secondary tomentum and probably representing thalloconidia, simple or in fascicles formed of up to 20 hyphae, hyphae simple. Lower secondary tomentum 70 mm long, simple to branched, moniliform. Cyphellae cavity up to 150 µm deep; cells of basal membrane without or with single papillae.

Apothecia lecanorine (with algal layer below cortex), up to 250 µm high, without distinct stipe; excipulum 150 µm broad, without projecting hairs. Hymenium up to 75 µm high; epihymenium 5 µm high, orange-brown with gelatinous upper layer. Asci immature. Ascospores not observed.

**Habitat and distribution.** *Sticta kukwae* is known from Bolivia and Peru. In Bolivia, it was found saxicolous and was collected at a single locality in the Área Natural de Manejo Integrado Nacional Apolobamba in the Department La Paz, at an altitude of 4070 m. In Peru, it was also saxicolous and found in four localities in Puno, in a vegetation type of Roquedal, Matorral de Puna, at an altitude of 3850 m.

**Etymology.** Named in honor of the lichenologist Martin Kukwa for his contribution to the taxonomy of lichens and lichenicolous fungi in Bolivia.

**Additional material examined.** Peru. • Puno - Carabaya, Ollachea - Macusani (20 km of Macusani), in a vegetation type of Roquedal, Matorral de Puna, on rocks on the ground/close to the ground, 23 May 2012, N. Magain (LG3225, LG3227, LG3221 & LG3223).

**Notes.** *Sticta kukwae* is another species in the *S. weigelii* morphodeme, along with the recently described *S. andina* B. Moncada, Lücking & Sérus., *S. scabrosa*, and *S. waikamoi* Moncada & Lücking. It differs from these species in the type of vegetative propagules and the presence of lobes with strongly sinuous margins, which have not been observed in the other species. *Sticta weigelii* s.str. and *S. waikamoi* produce isidia, and *S. andina* has isidia and phyllidia. *Sticta scabrosa*, as *S. kukwae*, produces phyllidia, but in this taxon, they are the same color as the thallus, whereas in the new species, they are blackish-brown. Both species can produce sparse apothecia, but in *S. kukwae* their margins are crenate and dark brown, whereas in *S. scabrosa* they are entire to very rarely shallowly crenate and in the same color as the thallus (Moncada et al. 2021b; Ossowska et al. 2022b). *Sticta andina* may also have apothecia, but they are abundant and with verrucose to crenate margins (Moncada et al. 2021a, b; Ossowska et al. 2022b). Another difference is found in the color of the lower surface, as *S. andina* has dark lower surface, in *S. scabrosa* it is yellow-brown, while in *S. weigelii* the color ranges from beige to dark brown, and in *S. waikamoi* it is dark brown (Moncada et al. 2020, 2021a, b; Ossowska et al. 2022b). The newly described species has a light brown lower surface. *Sticta andina* and *S. scabrosa* have a wide distribution (Moncada et al. 2021a, b; Kaasalainen et al. 2023). In contrast, *S. weigelii* was previously assumed to be widespread (Galloway 1994, 1997, 2006). However, recent research has shown that its distribution is probably limited to the Neotropics (Moncada et al. 2021b; Mercado-Díaz et al. 2023). All three taxa are also known from Bolivia (Ossowska 2021; Ossowska et al. 2022b). *Sticta waikamoi* is known from the Hawaiian islands (Moncada et al. 2020, 2021a). Only *S. weigelii* has been reported from Peru (Ramos 2014), but without molecular evidence.

In the phylogenetic tree (Fig. 1), the new species is closely related to *S. umbilicariiformis*. However, it has many marginal pustules, which can sometimes make it appear sorediate; thalli is often quite large, and lobes are thick with wavy to foveolate margins. Additionally, the lower surface is cream-colored to brown and thickly tomentose. *Sticta umbilicariiformis* has been documented in East Africa, with a high probability of its occurrence in other regions as well (Magain and Sérusiaux 2015; Kaasalainen et al. 2023).

The presence of lobes with sinuous margins is also a characteristic feature in the recently distinguished *S. monlueckiorum* Ossowska, Flakus & Rodr.-Flakus from Bolivia. In *S. monlueckiorum*, the thallus is larger (up to 10 cm) and moderately branched, while the apothecia are laminal with hirsute margins and without vegetative propagules (Crous et al. 2023), whereas *S. flakusiorum* has a hirsute upper surface with abundant, submarginal apothecia and without vegetative propagules. All three taxa differ also in the color of the lower surface and the density of the cyphellae. In *S. monlueckiorum*, the lower surface is beige to yellowish, and the cyphellae have a density of 41–60 per cm$^2$ towards the center and more than 100 towards the margins (Crous et al. 2023). In *S. flakusiorum*, the lower surface is brown, and the cyphellae are 1–20 per cm$^2$ towards the center and 41–60 per cm$^2$ towards the margins, and in *S. kukwae*, 1–20 per cm$^2$ towards the thallus center and margins.

The hyphae of primary tomentum of *Sticta kukwae* produce peculiar structures that resemble budding conidia forming chains. Similar structures were found in the isidiate *S. atlantica* Magain & Sérus., *S. fuliginoides* Magain & Sérus., and *S. fuliginosa* by Magain and Sérusiaux (2015), who stated in the case of *S. fuliginosa* they can act as conidia. These cells in the mentioned species are very similar to cells of secondary tomentum in several *Sticta* species, and possibly both can play a role of conidia. Such spores thus can be named thalloconidia, which are on the other hand known mainly in several species of the genus *Umbilicaria* Hoffm. (Hestmark 1990, 1991, 1992), but also in some crustose lichens (e.g., *Miriquidica nephaea* (Sommerf.) P.F. Cannon, *Protoparmelia leproloma* (R. Sant.) Rambold & Poelt, *Protoparmeliopsis peltata* (DC.) Arup, Zhao Xin & Lumbsch, *Rhizoplaca melanophthalma* (DC.) Leuckert & Poelt, *Sporastatia karakorina* (Obermayer & Poelt) Davydov & Yakovch.) (Poelt and Obermayer 1990). However, the ultrastructural study of their development must be performed prior to the final change in the conception of their role.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Emilia Anna Ossowska: conceptualization, descriptions of new species, determination of species, molecular laboratory work and analyses, chromatographic analyses, manuscript writing, and editing; Bibiana Moncada: descriptions of new species, phylogenetic analyses, manuscript editing; Robert Lücking: phylogenetic analyses, manuscript writing, and editing; Emmanuel Sérusiaux: molecular laboratory work and analyses, manuscript editing; Nicolas Magain: material collecting, molecular laboratory work and analyses, manuscript editing.

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## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Specimens of *Sticta* used in molecular analysis with locality, voucher information, GenBank accession numbers and list of references

Authors: Emilia Anna Ossowska, Bibiana Moncada, Robert Lücking, Emmanuel Sérusiaux, Nicolas Magain

Data type: xlsx

Explanation note: Sequences generated for this study are in bold.

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Research Article

# *Lijiangomyces laojunensis* gen. et sp. nov. (Mytilinidiaceae), and *Sclerococcum stictae* (Dactylosporaceae), a new lichenicolous species from Yunnan, China

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## Abstract

*Lijiangomyces laojunensis* **gen. et sp. nov.** and *Sclerococcum stictae* **sp. nov.** are reported from China and identified through DNA sequence analyses (LSU, ITS, and *tef*1-α) and morphological characteristics. Phylogenetic analysis showed that *L. laojunensis* forms a distinct lineage within Mytilinidiaceae, closely related to the *Mytilinidion* subclade, leading to the establishment of a new genus within this family. This saprotrophic species grows on the bark of *Abies fabri*, often surrounded by the thallus of *Pertusaria* species. *Lijiangomyces laojunensis* is characterized by broadly open, black hysterothecia, clavate asci, and uniseriate, hyaline muriform ascospores. *Sclerococcum stictae*, a new lichenicolous species, forms a sister clade relationship to a lichenicolous fungus, *S. ricasoliae*. It was found on the thallus of *Sticta*, and is characterized by black apothecia, elongate, cylindrical asci, and brown, elliptical, and 1-septate ascospores. Descriptions, illustrations, and phylogenetic analysis results of the new taxa are provided.

**Key words:** 2 new taxa, lichen, morphology, phylogeny, saprobe, taxonomy

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## Introduction

Mytilinidiaceae belongs to Mytilinidiales, Dothideomycetes (Hyde et al. 2024), and was introduced by Kirschstein (1924) to accommodate four genera that were originally classified under Hysteriaceae. Boehm et al. (2009a) introduced Mytilinidiales based on multigene phylogenetic analysis to accommodate Mytilinidiaceae. Pem et al. (2024) included eight genera

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within Mytilinidiaceae: *Actidium*, *Lophium*, *Mytilinidion*, *Ostreola*, *Peyronelia*, *Pseudocamaropycnis*, *Quasiconcha* and *Zoggium*. Hyde et al. (2024) added three more genera: *Bullatosporium*, *Camaroglobulus* and *Halokirschsteiniothelia*, increasing the total to eleven genera within this family. Members of Mytilinidiaceae are distinguished by their globose or obovoid pseudothecia which are typically oyster- or hatchet-shaped with a longitudinal keel and crested apex. They feature bitunicate asci containing eight ascospores that are arranged uniseriately, biseriately, or in aggregated clusters. The ascospores are hyaline to brown, exhibiting diverse morphologies such as scolecospores, didymospores, phragmospores, or dictyospores, and with bipolar symmetry (Boehm et al. 2009a).

*Mytilinidion* was established to accommodate the type species *M. aggregatum*. This genus is characterized by globoid to obovoid, erect, conchate, or dolabrate ascomata, and a thin-walled and peridium, bitunicate, 8-spored asci. The ascospores are hyaline to brown and transversely 3–5(–7)-septate (Boehm et al. 2009b; Jayasiri et al. 2018). *Ostreola* was introduced to accommodate two species, *O. consociate* and *O. sessilis*, characterized by conchiform to hatchet-shaped hysterothecia with a longitudinal slit that opens narrowly. The asci are cylindrical, containing eight uniseriate, brown and muriform ascospores (Darker 1963). *Ostreola* shares similarities with *Mytilinidion* and *Lophium* in the ascomatal morphology but is distinguished by the presence of muriform ascospores. Lumbsch and Huhndorf (2007) placed *Ostreola* within Mytilinidiaceae. The members of *Mytilinidion* are primarily saprobic and plant pathogens, while *Ostreola* species are primarily saprobic.

*Sclerococcum* belongs to Dactylosporaceae, Sclerococcales, Eurotiomycetes (Hyde et al. 2024), and was described by Fries (1819, 1825) to accommodate the parasitic hyphomycetous fungus *S. sphaerale*, which was previously classified as *Spiloma sphaerale*. Phylogenetic analysis by Diederich et al. (2013) revealed that *S. sphaerale* clusters with two species of *Dactylospora*. In a subsequent study incorporating expanded molecular data, Diederich et al. (2018) proposed adopting the family name Dactylosporaceae, with Sclerococcaceae as its synonym. They further recommended retaining *Sclerococcum* as the valid genus name and synonymizing *Dactylospora* under it, as *Sclerococcum* has nomenclatural priority over *Dactylospora*. Consequently, 46 *Dactylospora* species were transferred to *Sclerococcum* (Diederich et al. 2018). Dactylosporaceae comprises six genera: *Cylindroconidiis*, *Fusichalara*, *Gamsomyces*, *Pseudosclerococcum*, *Rhopalophora* and *Sclerococcum* (Hyde et al. 2024).

During a survey of microfungi in Yunnan Province (Thiyagaraja et al. 2024), two unidentified fungal specimens were collected. The first specimen was found on the bark of *Abies fabri*, and was often surrounded by the thallus of *Pertusaria* species while the second was observed growing on the thallus of a foliose lichen belonging to *Sticta*. Phylogenetic analyses and morphological comparison reveal that the first specimen represents a novel genus closely related to *Mytilinidion* within Mytilinidiaceae. The second specimen is proposed as a new lichenicolous species within the genus *Sclerococcum* based on morpho-molecular analyses.

## Materials and methods

### Sample collection and morphological examination

The specimens were collected from Yunnan Province, China and the important collection information was noted (Rathnayaka et al. 2024). Macro-morphological characteristics were observed using a stereomicroscope (Olympus ZX-16) and photographed with a fitted digital camera (Olympus SC180). The ascomata were sliced, temporarily mounted, and observed under a compound microscope (Nikon Y-TV55), with images captured using a fitted digital camera (Nikon DS-Ri2). Distilled water was used as a mounting slide solution and 10% potassium hydroxide (K) and Lugol's iodine solution (I) were used to stain and examine the hymenium. Photographic plates were assembled using Adobe Photoshop CC 2019 software (Adobe Systems, USA). Measurements were conducted using Image Framework software (Tarosoft, Version 0.9.7). The length, width, and length/width ratio (l/w) of asci and ascospores are provided (where n ≥ 10) as: (min–) [X−SD]–[X+SD] (–max), where "min" and "max" represent the extreme observed values, X is the arithmetic mean, and SD is the standard deviation. The number of measurements (n) is indicated. Unless otherwise specified, measurements were taken from water mounts, and procedures followed those specified by Senanayake et al. (2020). The holotype specimens are deposited in the Lichen Herbarium of Kunming Institute of Botany (KUN-L), Chinese Academy of Science, Yunnan, China.

### DNA extraction, PCR amplification, and sequencing

Apothecia were carefully removed with a razor blade under a dissecting microscope, and the lichen thallus was thoroughly cleaned. The sample was then transferred to a 200 µL centrifuge tube. Total genomic DNA was extracted using a Forensic DNA Kit (Omega Bio-Tek, Norcross, Georgia), following the manufacturer's instructions. The primer pairs ITS1f/ITS4, LR0R/LR5, 983F/2218R and mrSSU1/mrSSU3R were used respectively, to amplify the internal transcribed spacer region of rDNA (ITS), the 28S large-subunit of rDNA (LSU), the translation elongation factor 1-alpha (*tef*1-α), and the mitochondrial small subunit ribosomal RNA gene (mtSSU) (Vilgalys and Hester 1990; White et al. 1990; Gardes and Bruns 1993; Zoller et al. 1999; Rehner and Buckley 2005).

The polymerase chain reaction (PCR) was performed using a Mastercycler (Bio-RAD T-100) in a 25-µL reaction volume consisting of 12.5 µL of 2 × Mix (Solarbio, dNTPs Mix), 9.5 µL of double-distilled water (ddH$_2$O), 1.0 µL of each primer (10 mM), and 1.0 µL of the DNA template. The PCR conditions were as follows: an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 53 °C for ITS, LSU, and mtSSU or at 58 °C for *tef*1-α, for 90 seconds, and elongation at 72 °C for 1 minute. A final extension was performed at 72 °C for 10 minutes, and the reaction was then held at 4 °C indefinitely. PCR products were sequenced by Shanghai Sangon Biotech (Chengdu, Sichuan Province, China).

## Sequence alignment and phylogenetic analyses

The quality of chromatogram sequences was verified using BioEdit Sequence Alignment software (Version 7.0.9.0). Forward and reverse sequences were assembled with ContigExpress software (New York, USA). The newly generated sequences were subjected to BLASTn searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and deposited in the GenBank database. BLAST analysis of three genes (LSU, ITS, and *tef*1-α) from specimen KUN-L 88703 indicated that this species might belong to Gloniales, Hysteriales, or Mytilinidiales. To confirm its phylogenetic placement, all available sequence data from these orders were downloaded from GenBank (Table 1). Additionally, representative species from six adjacent orders, *viz.* Botryosphaeriales, Capnodiales, Dothideales, Jahnulales, Patellariales, and Pleosporales were included based on references from Boehm et al. (2009b). Two species of *Orbilia* (Orbiliomycetes, Orbiliales, Orbiliaceae) were selected as the outgroup taxa following Hongsanan et al. (2020) for phylogenetic analysis.

The BLAST analysis of three genes (LSU, ITS, and mtSSU) from the specimen KUN-L 88687 indicated that this species belongs to Sclerococcales. Sequence data for all available taxa within this order were retrieved from GenBank and are listed in Table 2. Two species of *Caliciopsis* (Coryneliaceae, Coryneliales, Coryneliomycetidae, Eurotiomycetes) were included as the outgroup following Olariaga et al. (2019).

Sequence alignment, concatenation, model selection, and format conversion were performed using the OFPT program (Zeng et al. 2023). Each gene region dataset was aligned using the ‘auto’ strategy in MAFFT (Katoh and Standley 2013) and trimmed with the ‘gappyout’ command in TrimAl (Capella-Gutierrez et al. 2009). The best-fit nucleotide substitution models for each dataset were selected using the Bayesian Information Criterion (BIC) from twenty-two common DNA substitution models with rate heterogeneity, as implemented in ModelFinder (Kalyaanamoorthy et al. 2017). The datasets were then concatenated with partition data for subsequent phylogenetic analyses.

Maximum likelihood (ML) analysis was conducted on the IQ-TREE web server applying the ultrafast bootstrap approximation with 1,000 replicates (Hoang et al. 2018), and the SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010; Nguyen et al. 2015). The consensus tree was summarized using the extended majority rule. For further verification, an additional ML analysis was performed with RAxML-HPC2 on ACCESS (v8.2.12), using the GTRGAMMA model with a rapid bootstrap analysis of 1000 replicates (Miller et al. 2010; Stamatakis 2014).

Bayesian inference was carried out using two parallel Metropolis-coupled Markov Chain Monte Carlo (MCMC) runs, each consisting of one ‘cold’ chain and three heated chains, in MrBayes (Ronquist et al. 2012). Trees were sampled every 1,000 generations, and the run was terminated when the average standard deviation of split frequencies fell below 0.01. The final tree was summarized after discarding the first 25% of samples as burn-in and visualized in FigTree v1.4.4 (Rambaut 2016). The newly identified taxon was registered in Index Fungorum and Faces of Fungi database (Jayasiri et al. 2015).

**Table 1.** Sequences used in phylogenetic analysis of Mytilinidiales and adjacent orders, with specimens or strains information and GenBank accession numbers. Newly obtained sequences are in bold font. "NA" indicates the sequence is unavailable.

| Species name | Voucher/strains | GenBank accession numbers | | |
|---|---|---|---|---|
| | | ITS | LSU | *tef*1-α |
| *Aigialus grandis* | BCC 20000 | NA | GU479775 | NA |
| *A. mangrovis* | BCC 33563 | NA | GU479776 | GU479840 |
| *A. parvus* | PUFD45 | MK028710 | MK026761 | MN520611 |
| *A. rhizophorae* | BCC 33572 | NA | GU479780 | GU479844 |
| *Aliquandostipite crystallinus* | AF007 | NA | EF175652 | NA |
| *A. khaoyaiensis* | MFLUCC 21-0106 | MT864350 | MT860428 | MT873577 |
| *Ascagilis guttulaspora* | MFLUCC 17-0244 | NA | NG_064432 | NA |
| *A. submersa* | MFLUCC 18-1143 | NR_171970 | MN888485 | NA |
| *A. thailandensis* | MFLUCC 18-1149 | NR_171969 | MN913693 | NA |
| *Botryosphaeria dothidea* | CBS 115476 | NA | DQ678051 | DQ767637 |
| *Brachiosphaera tropicalis* | SS 2523 | FJ887923 | JN819284 | JN819298 |
| *Bullatosporium taxicola* | a21-004; CBS 151403 | PP516536 | PP516533 | PP514386 |
| *B. taxicola* | a21-005; CBS 151402 | PP516535 | PP516534 | PP514385 |
| *Capnodium aciculiforme* | CBS 892.73 | NA | GU301847 | GU349045 |
| *C. alfenasii* | CBS 146151 | MN749233 | MN749165 | MN829346 |
| *C. coartatum* | CPC 17779 | MN749236 | MN749167 | MN829348 |
| *Cenococcum geophilum* | CG5 | KC967409 | NA | NA |
| *C. geophilum* | CG54 | KC967410 | NA | NA |
| *Chaetocapnodium insulare* | CBS 146159 | NR_168830 | NG_068681 | MN829359 |
| *C. philippinense* | MFLUCC 12-0110 | NR_168831 | KP744503 | MN829362 |
| *C. placitae* | CBS 124758 | MH863403 | MH874920 | MN829363 |
| *Conidiocarpus asiaticus* | MFLUCC10-0062 | NA | JN832612 | NA |
| *C. caucasicus* | GUMH937 | NA | KC833050 | NA |
| *C. fici-septicae* | MFLUCC 19-0072 | MW063143 | MW063206 | NA |
| *C. siamensis* | SICAUCC 23-0010 | OR405901 | OR405912 | OR671432 |
| *Delitschia chaetomioides* | DSE871 | MW209042 | MW209067 | MW238837 |
| *D. winteri* | AFTOL-ID 1599 | NA | DQ678077 | DQ677922 |
| | CBS 225.62 | | | |
| *Dothidea insculpta* | CBS 189.58 | NA | DQ247802 | DQ471081 |
| *D. sambuci* | DAOM 231303 | NA | AY544681 | DQ497606 |
| *Ericboehmia centramura* | chuni 70 | KM272258 | KM272256 | KM277819 |
| | MFLUCC 12-0808 | | | |
| *E. curtisii* | CBS 198.34 | NA | MH866967 | FJ161093 |
| *E. doimaeensis* | MFLUCC 16-0329 | MH535872 | MH535894 | NA |
| *Fusculina eucalypti* | CBS 120083 | DQ923531 | DQ923531 | NA |
| *Gloniopsis arciformis* | GKM L166A | NA | GU323211 | NA |
| *G. calami* | MFLUCC 15-0739 | NR_164398 | NG_059715 | KX671965 |
| *G. leucaenae* | MFLU 21-0201 | OL782134 | OL782050 | OL875100 |
| *G. percutanea* | FMR 8713 | AM286786 | LS997561 | LS997569 |
| *Glonium circumserpens* | CBS 123342 | NA | FJ161208 | NA |
| *G. circumserpens* | CBS 123343 | NA | FJ161200 | NA |
| *G. stellatum* | ANM 32; A. Miller 32, F | NA | GQ221887 | GQ221926 |
| *G. stellatum* | CBS 207.34 | MZ570257 | FJ161179 | FJ161095 |
| *Glyphium elatum* | EB 0365; BPI 892671 | KM220945 | KM220939 | KM220933 |
| *Gordonomyces mucovaginatus* | CBS 127273 | NR_157428 | NG_057941 | NA |
| *Graphyllium caracolinense* | HUEFS 42838 | NA | NG_060651 | NA |
| *Guignardia gaultheriae* | CBS 447.70 | MH859790 | DQ678089 | NA |

| **Species name** | **Voucher/strains** | **GenBank accession numbers** | | |
|---|---|---|---|---|
| | | **ITS** | **LSU** | ***tef*1-α** |
| *Halokirschsteiniothelia maritima* | 3124D | KM272366 | NA | NA |
| *H. maritima* | CBS 221.60 | NA | AY849943 | GU349001 |
| *H. maritima* | NWHC 45703-222 | MK782369 | NA | NA |
| *Hysterium angustatum* | KUMCC 21-0213 | OK482567 | OK482568 | NA |
| *H. pulicare* | EB 0238; CBS 123377 | NA | FJ161201 | FJ161109 |
| *H. rhizophorae* | MFLUCC 15-0950 | NR_189349 | NG_241879 | MF615401 |
| *Hysterobrevium baoshanense* | MFLUCC 16-2162 | MZ467049 | KX772765 | KX772769 |
| *H. constrictum* | KUN-HKAS102101 | MN429070 | MN429073 | MN442088 |
| *H. rosae* | CBS 149699 | OQ990113 | OQ990064 | OQ989245 |
| *Hysterodifractum partisporum* | HUEFS 42865 | NA | NG_060652 | NA |
| *Hysterographium didymosporum* | MFLUCC 10-0101 | NA | NG_064526 | NA |
| *H. fraxini* | CBS 109.43 | NA | FJ161171 | FJ161088 |
| *H. minus* | JCM 2758 | NA | NG_059814 | NA |
| *Hysteropatella elliptica* | AFTOL-ID 1790 | NA | DQ767657 | DQ767640 |
| | CBS 935.97 | | | |
| *H. prostii* | G.M. 2016-02-20.2 | MT341324 | MT341324 | NA |
| *Jahnula appendiculata* | BCC11400 | JN819280 | FJ743446 | JN819299 |
| *J. dianchia* | KUMCC 17-0039 | KY928456 | KY928457 | NA |
| *J. rostrata* | MFLU 20-0435 | MT627720 | MT627657 | NA |
| *Lophium arboricola* | CBS 758.71 | NA | MH872091 | NA |
| *L. arboricola* | FMR 3868 | KU705825 | KU705842 | NA |
| *L. arboricola* | P99; KRAM F-59986 | OR754902 | OR754924 | NA |
| *L. mytilinum* | CBS 114111 | EF596819 | EF596819 | NA |
| *L. mytilinum* | CBS 269.34 | OM337540 | MH867013 | NA |
| *L. zalerioides* | MFLUCC 14-0417 | MF621583 | MF621587 | NA |
| *Massaria inquinas* | WU 30527 | HQ599402 | HQ599402 | HQ599342 |
| *M. vomitoria* | WU 30606 | HQ599437 | HQ599437 | HQ599375 |
| *Mytilinidion acicola* | EB 0349; BPI 879794 | NA | GU323209 | NA |
| *M. acicola* | EB 0379; BPI 879793 | NA | GU397346 | NA |
| *M. andinense* | EB 0330; CBS 123562 | NA | FJ161199 | FJ161107 |
| *M. australe* | CBS 301.34 | NR_160067 | MH867035 | NA |
| *M. californicum* | EB 0385; BPI 879795 | NA | GU323208 | NA |
| *M. didymospora* | MFLUCC 16-0619 | NA | MH535902 | NA |
| *M. mytilinellum* | CBS 303.34 | NA | MH867037 | FJ161100 |
| *M. mytilinellum* | EB 0386; BPI 879796 CBS 303.34 | NA | GU397347 | NA |
| *M. resinicola* | CBS 304.34 | MH855535 | MH867038 | FJ161101 |
| *M. rhenanum* | CBS 135.34 | NA | NA | FJ161092 |
| *M. rhenanum* | EB 0341; CBS 135.45 | NA | GU323207 | NA |
| *M. scolecosporum* | CBS 305.34 | MH855536 | MH867039 | FJ161102 |
| *M. thujarum* | EB 0268; BPI 879797 | NA | GU323206 | NA |
| *M. tortile* | CBS 306.34 | MH855537 | MH867040 | NA |
| *M. tortile* | EB 0377; BPI 879798 | NA | GU323205 | NA |
| *Neocamarosporium goegapense* | CBS 138008 | KJ869163 | KJ869220 | NA |
| *N. phragmitis* | MFLUCC 17-0756 | MG844345 | NG_070431 | MG844351 |
| *Neomassaria fabacearum* | MFLUCC 16-1875 | NA | KX524145 | KX524149 |
| *N. formosana* | NTUCC 17-007 | NA | MH714756 | MH714762 |
| *Oedohysterium insidens* | ANM 1443 | NA | GQ221882 | NA |
| | A. Miller 1443, F | | | |
| *O. insidens* | CBS 238.34 | NA | FJ161182 | FJ161097 |
| *O. sinense* | EB 0339; BPI 879800 | NA | GU397348 | GU397339 |

| Species name | Voucher/strains | GenBank accession numbers | | |
|---|---|---|---|---|
| | | ITS | LSU | *tef*1-α |
| *Orbilia auricolor* | AFTOL-ID 906 | DQ491512 | DQ470953 | DQ471072 |
| | CBS 547.63 | | | |
| *O. vinosa* | AFTOL-ID 905 | DQ491511 | DQ470952 | DQ471071 |
| | CBS 917.72 | | | |
| *Ostreichnion sassafras* | CBS 322.34 | MH855548 | FJ161188 | NA |
| ***Lijiangomyces laojunensis*** | **KUN-L 88703** | **PQ049177** | **PQ047633** | **PQ267963** |
| *Patellaria apiculatae* | MCD 096; MFLU 19-1236 | MN047094 | MN017860 | NA |
| *P. atrata* | CBS 958.97 | NA | GU301855 | GU349038 |
| *P. chromolaenae* | MFLUCC 17-1482 | MT214381 | MT214475 | MT235796 |
| *Pseudocamaropycnis pini* | CBS 115589 | KU728518 | KU728557 | |
| *Pseudocenococcum floridanum* | Culture BA4b001 | NA | LC095431 | LC095383 |
| | NBRC 111599 | | | |
| | FLAS-F-59166 | | | |
| *Psiloglonium araucanum* | CBS 112412 | NA | FJ161172 | FJ161089 |
| *P. colihuae* | MFLU 11-0214 | KP744466 | KP744511 | NA |
| *P. macrosporum* | MFLU 18-2218 | OR225075 | OP612525 | OR140436 |
| *Purpurepithecium murisporum* | MFLUCC 16-0611 | NA | NG_059797 | KY887666 |
| *P. murisporum* | MFLUCC 17-0319 | NA | KY799174 | KY799177 |
| *Quasiconcha reticulata* | EB QR; RLG 14189 | NA | GU397349 | NA |
| *Quasiconcha* sp. | ZY 22.011 | OR680490 | OR680557 | OR865892 |
| | CGMCC 3.25498 | | | |
| *Quasiconcha* sp. | ZY 22.012 | OR680491 | OR680558 | OR865893 |
| | CGMCC 3.25498 | | | |
| *Quasiconcha* sp. | ZY 22.013 | OR680492 | OR680559 | OR865894 |
| | CGMCC 3.25498 | | | |
| *Rhytidhysteron bannaense* | KUMCC 21-0483 | OP526399 | OP526409 | OP572200 |
| *R. bruguierae* | SDBR-CMU 473 | OQ943970 | OQ940376 | OQ973477 |
| *R. camporesii* | KUNCC 22-12388 | OR807853 | OR801302 | OR832866 |
| *Yuccamyces citri* | CBS 143161 | MG386043 | MG386096 | NA |
| *Y. pilosus* | CBS 579.92 | MG386044 | MG386097 | NA |

**Table 2.** Sequences used in phylogenetic analysis of Dactylosporaceae with specimens or strains' information and GenBank accession numbers. Newly obtained sequences are in bold font. "NA" indicates the sequence is unavailable.

| Species name | Voucher/strains | GenBank accession numbers | | |
|---|---|---|---|---|
| | | LSU | ITS | mtSSU |
| *Umbilicaria* sp. | INB_io4503Q | KM242300 | KM242300 | NA |
| *Umbilicaria* sp. | INB_io4513J | KM242356 | KM242356 | NA |
| *Umbilicaria* sp. | INB_io4513L | KM242358 | KM242358 | NA |
| *Caliciopsis orientalis* | CBS 138.64 | NG_058741 | NA | FJ190654 |
| *C. pinea* | AFTOL-ID 1869 | DQ678097 | NA | FJ190653 |
| | CBS 139.64 | | | |
| *Cylindroconidiis aquaticus* | MFLUCC 11-0294 | MH236579 | MH236576 | NA |
| *Fusichalara minuta* | CBS 709.88 | KX537758 | KX537754 | KX537762 |
| *Gamsomyces aquaticum* | MFLUCC 18-1015 | MN335230 | MN335228 | NA |
| *G. chiangmaiensis* | MFLUCC 18-0982 | MN335229 | MN335227 | NA |
| *G. longisporus* | CBS 118.86 | MT020877 | MT020865 | NA |
| *G. longisporus* | CBS 240.89 | MT020878 | MT020866 | NA |
| *G. stilboideus* | CBS 146494 | MT020879 | MT020867 | NA |
| *Pseudosclerococcum golindoi* | ARAN-Fungi 6619 | NG_073673 | NR_171236 | MK759897 |

| Species name | Voucher/strains | GenBank accession numbers | | |
|---|---|---|---|---|
| | | LSU | ITS | mtSSU |
| *Rhopalophora clavispora* | CBS 281.75 | KX537756 | KX537752 | KX537760 |
| *R. clavispora* | CBS 129.74 | KX537755 | KX537751 | KX537759 |
| *R. clavispora* | CBS 637.73 | KX537757 | KX537753 | KX537761 |
| *Sclerococcum ahtii* | RP 23 | KY661659 | KY661630 | KY661686 |
| | F. Hognabba 1325a (H) | | | |
| *S. ahtii* | RP182 | NA | KY661622 | NA |
| | CHI17-37a (H) | | | |
| *S. chiangraiensis* | MFLU 16-0570 | NG_066422 | NR_163755 | NA |
| *S. deminutum* | RP235 | NA | KY661629 | NA |
| | J. Pykala 39390 (H) | | | |
| *S. fusiformis* | MFLU 16-0593 | NG_066423 | NR_163756 | NA |
| *S. fusiformis* | MFLU 18-0678 | NA | MH718442 | NA |
| *S. glaucomarioides* | RP275; Zhurbenko 13107 (LE 261065) | KY661660 | KY661632 | KY661683 |
| *S. glaucomarioides* | KUN-L 88756 | NA | OQ991232 | OR035764 |
| *S. haliotrephum* | AFTOL-ID 758 | FJ176855 | NA | KJ766382 |
| | ATCC MYA-3590 | | | |
| *S. haliotrephum* | J.K.5129B | FJ713617 | NA | NA |
| | AFTOL-ID 798 | | | |
| *S. lobariellum* | Diederich 18109 | MH698499 | NA | MH698503 |
| *S. lobariellum* | Diederich 17708 | MH698498 | NA | MH698502 |
| *S. lobariellum* | ARAN-Fungi 10091 | MK759891 | NA | MK759898 |
| *S. mangrovei* | AFTOL-ID 2108 | FJ176890 | NA | KJ766383 |
| *S. martynii* | D. Haelew. F_1567b | MZ221620 | MZ221612 | NA |
| | PUL F27737 | | | |
| *S. martynii* | D. Haelew. F_1570b | MZ221623 | MZ221616 | NA |
| | PUL F27739 | | | |
| *S. martynii* | D. Haelew. F_1577a | MZ221619 | MZ221610 | NA |
| | PUL F27741 | | | |
| *S. parasiticum* | ARAN-Fungi 2724 | MK759892 | NA | MK759899 |
| *S. parasiticum* | RP422 | KY661666 | KY661646 | KY661690 |
| | LE 260868 | | | |
| *S. parasiticum* | F-283586 | MK759894 | NA | MK759901 |
| *S. parasiticum* | F-283587 | MK759895 | NA | MK759902 |
| *S. parasiticum* | ARAN-Fungi A3044025 | MK759893 | NA | MK759900 |
| *S. pseudobactrodesmium* | CGMCC 3.25577T | OR514703 | OR514694 | OR588037 |
| *S. pseudobactrodesmium* | GZCC 23-0056 | OR514704 | OR514695 | OR588038 |
| *S. pseudobactrodesmium* | GZCC 23-0057 | OR514705 | OR514696 | OR588039 |
| *S. pseudobactrodesmium* | GZCC 23-0549 | OR514702 | OR514693 | OR588036 |
| *S. ricasoliae* | A.F. 29132 | MT153992 | MT153963 | MT153924 |
| *S. ricasoliae* | A.F. 25967 | MT153991 | MT153962 | MT153923 |
| *S. ricasoliae* | A.F. Fla6b | MT153993 | MT153964 | MT153925 |
| *S. ricasoliae* | A.F. 25611 | MT153990 | MT153961 | MT153922 |
| *S. simplex* | MFLU 21-0117 | MZ655912 | MZ664325 | MZ676669 |
| *Sclerococcum* sp. | A1153 | MF071425 | NA | MF085485 |
| *Sclerococcum* sp. | A1016 | KT263077 | NA | KT263115 |
| *Sclerococcum* sp. | RP391 | KY661664 | NA | KY661689 |
| *S. sphaerale* | Diederich 17283 | JX081673 | NA | JX081678 |
| *S. sphaerale* | Diederich 17279 | JX081672 | NA | JX081677 |
| *S. sphaerale* | Ertz 17425 (BR) | JX081674 | NA | JX081676 |

| Species name | Voucher/strains | GenBank accession numbers | | |
|---|---|---|---|---|
| | | LSU | ITS | mtSSU |
| ***S. stictae*** | **KUN-L 88687** | **PQ407923** | **PQ408029** | **PQ415057** |
| ***S. stictae*** | **KUN-L 88687-1** | NA | **PQ408030** | NA |
| *S. tardum* | ICMP 24355 | NA | NR_176187 | NA |
| *S. tardum* | PDD 91756 | NA | OL709435 | NA |
| *S. tardum* | PDD 105454 | NA | MK432753 | NA |
| *S. stygium* | ARAN-Fungi 00823 | NA | MK759886 | MK759904 |
| *S. stygium* | ARAN-Fungi 3395 | MK759896 | NA | MK759903 |
| *S. stygium* | BHI-F312 (FH) | NA | MF161218 | NA |
| *S. vrijmoediae* | NTOU 4002 | KC692153 | NR_138396 | NA |

**Abbreviations: ARAN-Fungi:** The ARAN-Fungi Fungarium**; BCC:** BIOTEC Culture Collection, Thailand; **BPI:** United States USDA ARS National Fungus Collections, Beltsville, MD; **CBS:** CBS Fungal Biodiversity Center, Utrecht, The Netherlands; **CGMCC:** China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; **CPC:** Collection of P.W. Crous; **DAOM:** Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; **FH:** FH Fungarium of Helsinki University Museum, Finland; **FLAS**: University of Florida Herbarium, located at the Florida Museum of Natural History in Gainesville, Florida, USA; **FMR:** Fungal Biodiversity Centre of the University of Valencia, Spain; **GZCC:** Guizhou Culture Collection, China; **GUMH:** Guangxi University Microbial Herbarium, China; **GZU:** Herbarium of the Institute of Botany, University of Graz, Austria; **HUEFS:** Herbário da Universidade Estadual de Feira de Santana, Brazil; **ICMP:** International Collection of Microorganisms from Plants, University of Auckland, New Zealand; **JAC:** University of Johannesburg Herbarium, USA; **JCM:** Japan Collection of Microorganisms; **KRAM:** Władysław Szafer Institute of Botany, Polish Academy of Sciences Herbarium; **KUMCC:** Kunming Institute of Botany Culture Collection; **KUN-HKAS:** Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China; **KUN-L:** Lichen Herbarium of Kunming Institute of Botany, Chinese Academy of Science, Yunnan, China; **MFLU:** the herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC:** Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC:** NITE Biological Resource Center, Japan; **NTOU:** National Taiwan Ocean University; **NWHC:** National Wildlife Health Center of U.S. Geological Survey; **PDD:** Plant Disease Database at the Auckland Museum, New Zealand; **PUFD:** Purdue University Forestry Department; **PUL:** Purdue University Herbarium; **RLG:** The Robert L. Gilbertson Mycological Herbarium at the University of Arizona; **SDBR-CMU:** Sustainable Development of Biodiversity Resources, Chiang Mai University, Thailand; **SICAUC:**Sichuan Agricultural University Culture Collection;**WU:** Herbarium of the University of Vienna.

## Results

In the analysis of Mytilinidiales, the final dataset comprised 116 taxa (Table 1) with 2246 aligned characters, including gaps (ITS 1–494 bp, LSU 495–1346 bp, and *tef*1-α 1347–2246 bp). The best-fit models for each gene, determined using the Bayesian information criterion (BIC), were as follows: ITS: SYM+I+G4, LSU: TN+F+R4, and *tef*1-α: TN+F+I+G4. For the combined dataset (LSU, ITS, and *tef*1-α), the parameters of the GTRGAMMA model were as follows: estimated base frequencies: A = 0.24, C = 0.25, G = 0.28, T = 0.23; substitution rate: AC = 1.12, AG = 3.35, AT = 1.77, CG = 0.88, CT = 8.54, GT = 1.00; gamma distribution shape parameter (α) = 0.325118; and tree-length = 6.868758. The best-scoring RAxML tree was constructed with a final maximum likelihood (ML) optimization likelihood value of -34,803.39. Bayesian posterior probabilities (BYPP) were calculated using MCMC analysis, achieving a final average standard deviation of split frequencies of 0.009998. The final tree topologies of ML and BYPP analyses were consistent. The best-scoring RAxML tree, based on combined LSU, ITS, and *tef*1-α sequence datasets, is presented in Fig. 1.

The resulting phylogram distinguishes eight order-level clades, most of which are well-supported, except for the Patellariales clade. Seven genera form a strongly supported Mytilinidiales clade, with *Mytilinidion* appearing polyphyletic. The newly identified species clusters within the Mytilinidiales clade are closely related to a *Mytilinidion* subclade containing eight species. The remaining *Mytilinidion* species form a separate clade alongside *Lophium*. *Halokirschsteiniothelia* and *Quasiconcha* form a sister clade to the *Ostreola*-*Mytilinidion* grouping.

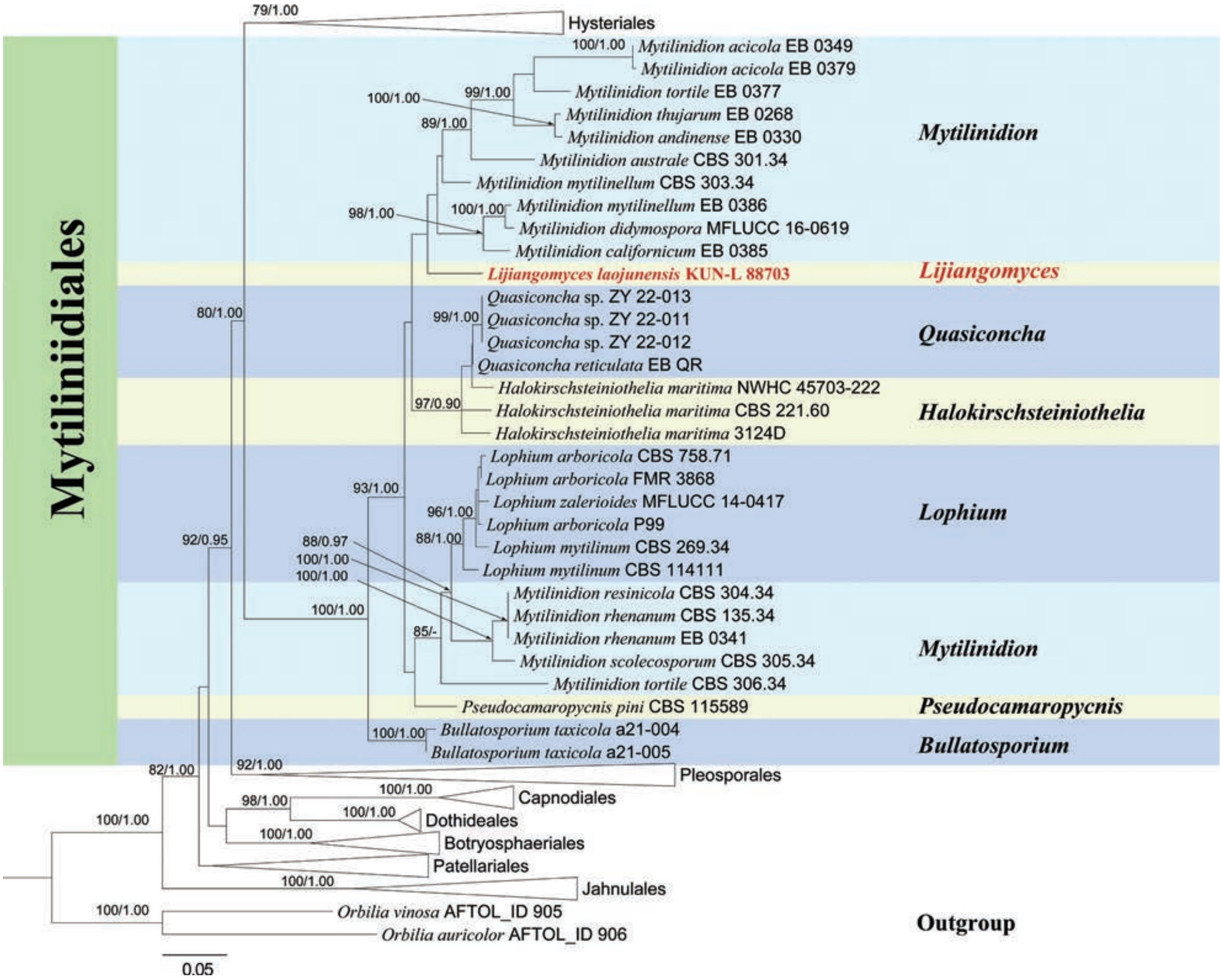

**Figure 1.** RAxML analysis of Mytilinidiales based on the combined LSU, ITS, and *tef*1-α sequence data. Bootstrap support values for maximum likelihood (ML ≥ 70%), and the Bayesian Posterior Probabilities (PP ≥ 0.90) are shown near the nodes as ML/PP. *Orbilia auricolor* (AFTOL-ID 906) and *O. vinosa* (AFTOL-ID 905) were used as outgroups. The newly generated sequence is in red bold font.

Additionally, two *Bullatosporium* strains form a distinct clade closely related to other genera within the Mytilinidiales order.

In the analysis of Dactylosporaceae, the final dataset comprised 62 taxa (Table 2) with 2041 aligned characters, including gaps (ITS 1–455 bp, LSU 456–1310 bp, and mtSSU 1311–2041 bp). The best-fit nucleotide substitution models, selected based on the Bayesian information criterion (BIC), were as follows: ITS: TIM2e+I+G4, LSU: TNe+I+G4, mtSSU: TVM+F+I+G4. The parameters for the GTRGAMMA model of the combined LSU, ITS, and mtSSU were as follows: estimated base frequencies: A = 0.29, C = 0.19, G = 0.25, T = 0.26, and substitution rate AC = 1.01, AG = 2.68, AT = 1.67, CG = 0.85, CT = 5.43, and GT = 1.00. Gamma distribution shape parameter α = 0.256278, and the tree length = 2.405501. The best-scoring RAxML tree was constructed with a final ML optimization likelihood value of - 14773.67. The final tree topologies of ML and BYPP analyses were consistent. The best-scoring RAxML tree, based on combined LSU, ITS, and mtSSU sequence datasets, is presented in Fig. 2. The newly collected specimen clusters within the *Sclerococcum* clade and is closely related to *S. ricasoliae*.

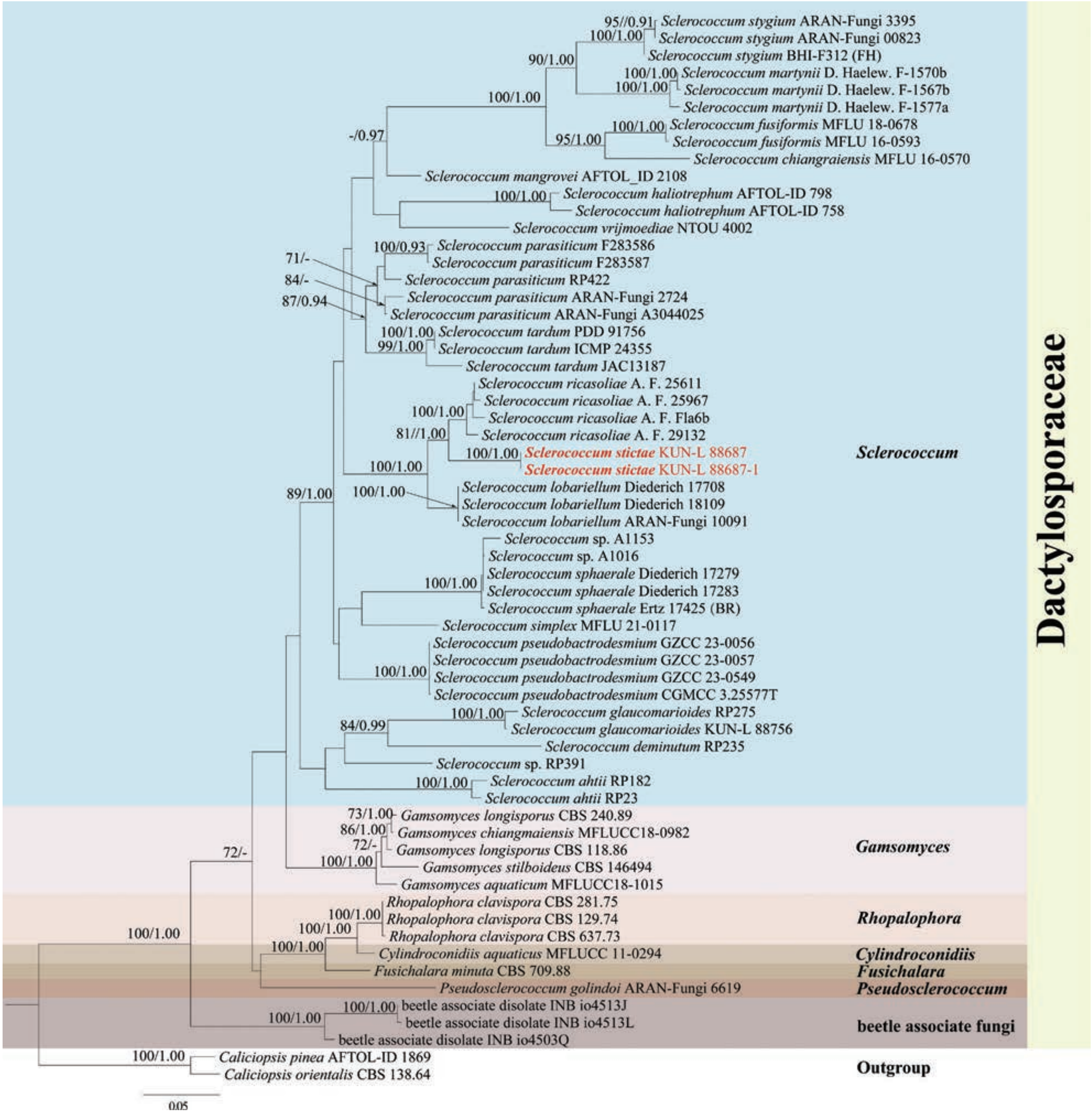

**Figure 2.** RAxML analysis of Dactylosporaceae based on the combined LSU, ITS and mtSSU sequence data. Bootstrap support values for maximum likelihood (ML ≥ 70%), and the Bayesian Posterior Probabilities (PP ≥ 0.90) are shown near the nodes as ML/PP. *Caliciopsis orientalis* (CBS 138.64) and *Caliciopsis pinea* (AFTOL-ID 1869) were used as outgroups. The newly generated sequence is in red bold font.

## Taxonomy

### *Lijiangomyces* Meng & Jayaward., gen. nov.

Index Fungorum: IF903174
Facesoffungi Number: FoF17069

**Etymology.** The genus name "*Lijiangomyces*" refers to "Lijiang", the city in Yunnan Province of China from where the holotype was collected.

**Description.** ***Sexual morph***: ***Ascomata*** hysterothecia, superficial, solitary, dispersed, sessile, obovoid to broadly shell-shaped or irregularly rounded, with a broadly open or slit-like disc. ***Margin*** black, vertically erect, fragile, with the disc surface appearing yellowish-brown. ***Peridium*** carbonaceous, black in the lateral and upper regions. ***Hymenium*** hyaline to slightly yellowish, with a densely packed hamathecium. ***Paraphyses*** filiform, hyaline, non-anastomosed, and non-septate. ***Hypothecium*** slightly yellowish. ***Asci*** bitunicate, 8-spored, elongated to clavate, with a rounded apex lacking ascal wall thickening, I-, K-. ***Ascospores*** uniseriate, arranged obliquely and parallelly, hyaline, thin-walled, smooth, fusiform to ellipsoidal, K-, I+ dark blue, aseptate at immature, becoming muriform at maturity with 4–7 transverse septa and 1–2 longitudinal septa. ***Asexual morph*:** Not observed.

**Type species.** *Lijiangomyces laojunensis* Meng & Jayaward.

**Notes.** The genus is distinguished by black, broadly shell-shaped to irregularly rounded hysterothecial ascomata, with a broadly open or occasionally closed disc, typically light brown to flesh-yellow. Asci are elongated to clavate, containing eight uniseriate, hyaline, and muriform ascospores. Phylogenetic analysis places this genus within the family Mytilinidiaceae (Mytilinidiales, Dothideomycetes) closely related to *Mytilinidion*. In the single-gene phylogenies, the new species is positioned outside the *Mytilinidia* sensu stricto clade in the ITS and LSU trees but clusters within it in the *tef*1-α tree. However, in the concatenated analysis combining all three genes, it is again placed outside the *Mytilinidia* sensu stricto clade. These results support its recognition as a distinct lineage within Mytilinidiaceae. Morphologically, this genus differs significantly from *Mytilinidion* in having obovoid to broadly shell-shaped or irregularly rounded, with broadly open ascomata (vs. globoid to obovoid, conchate, or dolabrate ascomata with narrow slit-like openings) and hyaline and muriform ascospores (vs. hyaline to brown and transverse septa). In addition, this genus shares similar morphology with *Ostreola* in having muriform ascospores but differs in the broadly shell-shaped or irregularly rounded ascomata (vs. conchiform to hatchet-shaped), a broad disc opening (vs. narrowly slit-like), and hyaline ascospores (vs. brown).

### *Lijiangomyces laojunensis* Meng & Jayaward., sp. nov.

Index Fungorum: IF902457
Facesoffungi Number: FoF16265
Fig. 3

**Etymology.** The species epithet "*laojunensis*" refers to the type locality "Laojun Mountain National Nature Reserve" in Yunnan Province of China.

**Holotype.** KUN-L 88703.

**Description.** ***Sexual morph*: *Ascomata*** hysterothecia, (0.8–)0.88–1.05(–1.1) × (0.4–)0.47–0.78(–0.8) mm ($\bar{x}$ = 0.97 × 0.63, n = 10), superficial, solitary, dispersed, sessile, non-stromatic, obovoid to broadly shell-shaped or irregularly rounded, with a broadly open or slit-like disc. ***Margin*** black, vertically erect, fragile, with a yellowish-brown, slightly depressed disc surface appearing below the rim of the lateral wall. ***Peridium*** 70–100 µm thick, carbonaceous, black laterally and apically, transitioning to grayish near the base. ***Hymenium*** 350–400 µm high, hyaline to slightly yellowish, densely packed with hamathecium. ***Paraphyses*** 1–2 µm wide, unbranched, hyaline, non-anastomosed, non-septate. ***Hypothecium*** 35–50 µm thick, slightly yellowish. ***Asci*** (120–)122.6–168.7(–190) × (13–)13.4–16.5(–18) µm ($\bar{x}$ = 145.7 × 14.9, n = 10), bitunicate, 8-spored, elongated to

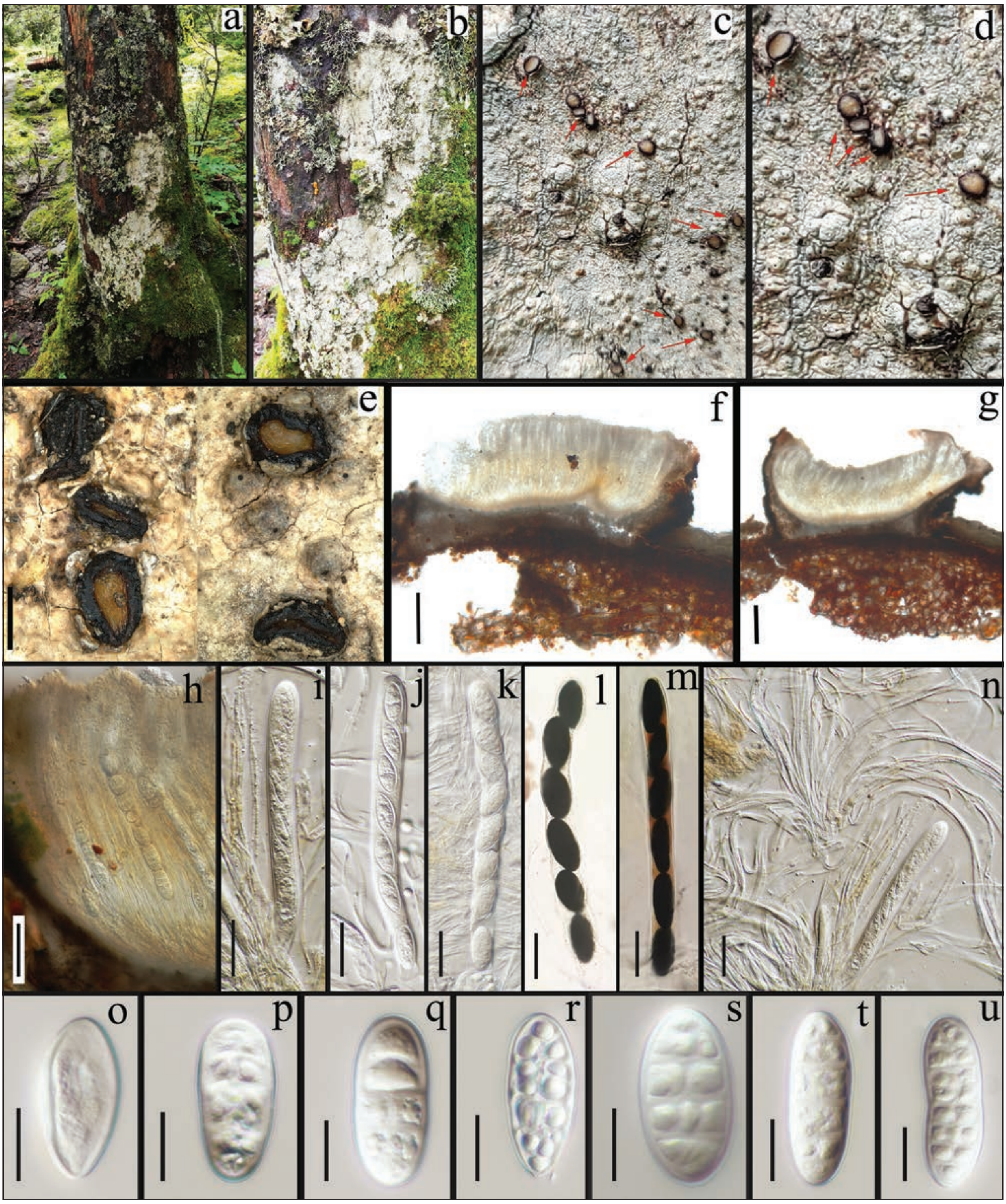

**Figure 3.** *Lijiangomyces laojunensis* sp. nov., (KUN-L 88703) **a, b** habitat **c–e** ascomata (arrows) **f–h** section of ascomata in water **i–k** asci in water **l, m** asci in IKI **n** paraphyses in water **o–u** ascospores in water. Scale bars: 500 µm (**e**); 200 µm (**f, g**); 50 µm (**h**); 25 µm (**i–n**); 10 µm (**o–u**).

clavate, rounded apex, without apical thickening of ascal wall, K-, I-. ***Ascospores*** (20–)20.9–28.8(–37.5) × (9–)10.1–15.4(–17.5) ($\bar{x}$ = 24.8 × 12.8, n = 30) µm, K-, I+ reddish brown, then turning to dark blue, uniseriate, arranged obliquely and parallelly, hyaline, thin-walled, smooth, fusiform to ellipsoidal, aseptate at immature, becoming muriform at maturity with 4–7 transverse septa and 1–2 longitudinal septa, sometimes slightly constricted at the median septum, rounded at the ends in aged ascospores. ***Asexual morph*:** Not observed.

**Material examined.** China • Yunnan Province, Lijiang City, Laojun Mountain National Nature Reserve, 26°39'N, 99°43'E, 3900 m elev., on the bark of *Abies fabri* (Pinaceae), 10 Apr 2022, Qing-feng Meng, ljs-52 (holotype KUN-L 88703).

**Notes.** *Lijiangomyces laojunensis* closely resembles *Ostreola consociata* (the type species of *Ostreola*) and *O. sessilis*, in having cylindrical asci and uniseriate muriform ascospores. However, the new species is distinguished by its ascomatal morphology, which is broadly shell-shaped or irregularly rounded with a widely opened disc, in contrast to *Ostreola* species which have conchiform to hatchet-shaped ascomata with a narrow slit-like opening. Furthermore, the ascospores of the new species are hyaline and larger in size (20.9–28.8 × 10.1–15.4 μm), in contrast, brown and smaller ascospores (14–22 × 6–8 μm) are the characteristic feature of *Ostreola* (Darker 1963).

Phylogenetic analysis places this species as a sister clade to *Mytilinidion*, however, it can be distinguished by its obovoid to broadly shell-shaped or irregularly rounded ascomata (vs. globoid to obovoid, erect, conchate, or dolabrate), and hyaline and muriform ascospores (vs. hyaline to dark brown and transversely septate) (Boehm et al. 2009b; Jayasiri et al. 2018).

### *Sclerococcum stictae* Meng, Diederich & Thiyagaraja, sp. nov.

Index Fungorum: IF903175
Facesoffungi Number: FoF17070
Fig. 4

**Etymology.** The species epithet "*stictae*" refers to "*Sticta*", the host lichen on which the holotype was found.

**Holotype.** KUN-L 88687.

**Description.** ***Sexual morph*: *Ascomata*** apothecioid, 200–300 μm in diam., rounded, cup-shaped, sessile, erumpent from the host thallus with a narrow base, 170–200 μm in diam., either dispersed or occurring in small groups, black, matte. ***Disc*** flat, black. ***Margin*** distinct, persistent, and concolorous with the disc. ***Exciple*** brown, paraplectenchymatous, laterally 40–70 μm wide. ***Epithecium*** (12–)14.3–21.0(–24) μm thick ($\bar{x}$ = 17.7, n = 30), with dark brownish granules. ***Hymenium*** (46–)65.2–94.8(–96) μm high ($\bar{x}$ = 80, n = 30), light brown, and distinctly gelatinized, K/I+ reddish with bluish in epihymenium. ***Paraphyses*** 2–3 μm wide, anastomosing, branched, septate, with swollen, pigmented apices. ***Hypothecium*** (86–)89.6–95.2(–97) μm thick ($\bar{x}$ = 92.4, n = 30), dark orange-brown, with irregularly shaped hypothecial cells. ***Asci*** (40–)44.8–67.9(–72) × (7–)7.8–11.9(–12) μm ($\bar{x}$ = 56.3 × 9.9, n = 10), bitunicate, narrowly clavate to cylindrical, ascus wall thickened at the apex, ocular chamber absent, 8-spored, K/I-, except for the K/I+ blue outer gelatinous coat, most intensely colored around the ascus apex. ***Ascospores*** (8–)9.2–11.1(–12) × (5–)5.1–6.2(–7) μm ($\bar{x}$ = 10.1 × 5.6, n = 30), l/w ratio = (1.5–)1.7–1.9(–2) ($\bar{x}$ = 1.8, n = 30), brown when mature, 1-septate, slightly constricted at the septum, verrucose, slightly asymmetric with a larger upper cell, ellipsoidal, sometimes soleiform. ***Asexual morph*:** Not observed.

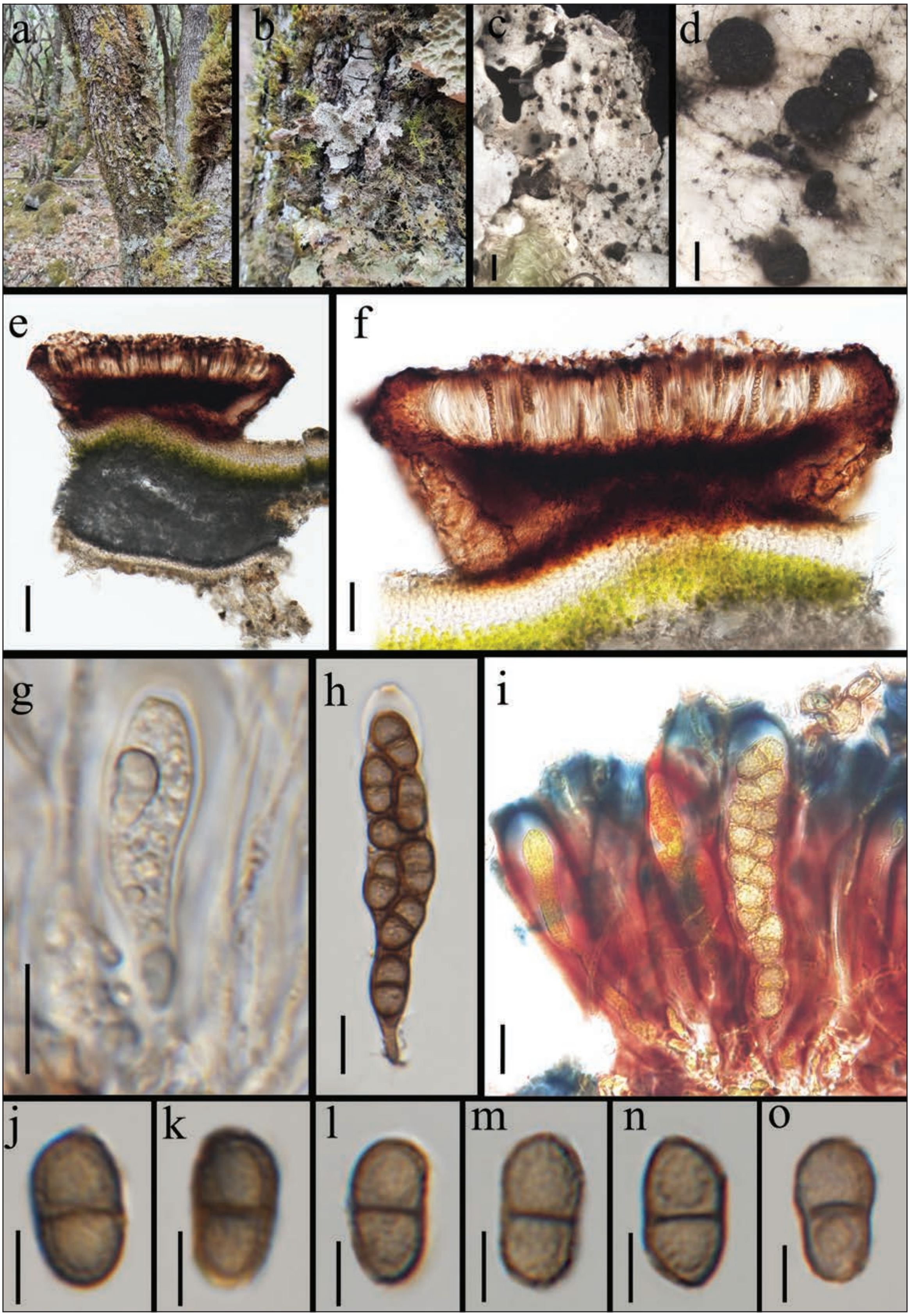

**Figure 4.** *Sclerococcum stictae* sp. nov., growing on the thallus of *Sticta* sp. (KUN-L 88687) **a, b** habitat and appearance of the host lichen **c, d** appearance of the apothecia **e, f** section of apothecium in water **g** immature ascus in water **h** mature ascus with ascospores in water **i** asci in I **j–o** ascospores in water. Scale bars: 1 mm (**c**); 200 μm (**d**); 100 μm (**e**); 50 μm (**f**); 10 μm (**g–i**); 5 μm (**j–o**).

**Material examined.** China • Yunnan Province, Diqing Autonomous Prefecture, Meili Mountain National Nature Reserve, 28°24'N, 98°48'E, 3300 m elev., on the thallus of *Sticta* sp., on the bark of *Rhododendron lapponicum* (Ericaceae), 21 Apr 2023, Qing-feng Meng, ml-68 (holotype KUN-L 88687).

**Notes.** *Sclerococcum stictae* clusters within a well-supported subclade along with *S. ricasoliae* and *S. lobariellum*. The basepair comparison with *S. ricasoliae* revealed 7.5% differences (34/455 bp) in ITS, 2.2% (19/855 bp) differences in LSU, and 1% (7/730 bp) differences in mtSSU sequences (Flakus et al. 2019). Compared to *S. lobariellum*, it exhibits 4.21% differences (36/855 bp) in LSU and 1.1% (8/730) in mtSSU. Morphologically, *S. stictae* resembles *S. ricasoliae* in its ascomatal and ascospore appearance but can be distinguished by its longer, narrower asci (c. 45–68 × 8–12 µm vs. 35–50 × 10–15 µm) and broader ascospores (c. 9–11 µm vs 4–6 µm), with a smaller length/width ratio (1.7–1.9 vs. 1.5–3.5) (Flakus et al. 2019). Phylogenetically, *S. stictae* is also related to *S. lobariellum*, but the basepair comparison revealed 4.3% (37/855 bp) differences in LSU and 1.1% (8/730 bp) differences in mtSSU sequences (Hafellner 1979). Hence based on recommendations outlined by Jeewon and Hyde (2016), the establishment of the new species is supported. Diederich et al. (2024) have shown that both *Sclerococcum lobariellum* and *S. ricasoliae* possess an asexual stage producing dark brown, dispersed, muriform conidia, often co-occurring with the sexual stage. In all other known lichenicolous *Sclerococcum* species with an asexual stage, conidia are produced within compact sporodochia, and this stage is never accompanied by a sexual stage. We anticipate, therefore, that an asexual stage with dispersed, muriform conidia also exists in the new *S. stictae* and should be searched for when more specimens become available.

Another species, *Sclerococcum dendriscostictae*, also found on *Sticta*, shares morphological traits with *S. stictae*. However, the new species can be distinguished by its longer asci (c. 45–68 × 8–12 µm vs. 33–44 × 9.5–13.5 µm) and verrucose ascospore ornamentation, in contrast to smooth-walled ascospores reported in *S. dendriscostictae* (Joshi 2021).

## Discussion

Hysteriaceous fungi are distinguished by their persistent, carbonaceous, navicular pseudothecia with a longitudinal slit opening. Historically, the mytilinidiaceous fungi, which possess fragile, shell-shaped pseudothecia that dehisce through a longitudinal cristate apex, were considered part of the hysteriaceous. However, Boehm et al. (2009a, 2009b) reclassified these fungi based on phylogenetic analyses, leading to the establishment of the order Mytilinidiales.

In our phylogenetic analysis, six genera within Mytilinidiales form a distinct yet complex clade. *Mytilinidion* is split into two subclades, indicating its polyphyletic nature. This is consistent with the previous study by Boehm et al. (2009a). One subclade includes four species of *Mytilinidion* alongside all *Lophium* species, forming a well-supported *Lophium*-*Mytilinidion* clade. The other subclade contains eight *Mytilinidion* species with our new collection, although this grouping is weakly supported. Morphologically, *Mytilinidion* species are defined by globoid to obovoid, conchate, or dolabrate ascomata with narrow slit-like openings, bitunicate, 8-spored asci, and hyaline to dark brown ascospores with 3–5(–7) transverse septa (Boehm et al. 2009b; Jayasiri et al.

2018). However, our new species displays distinct morphological features that diverge significantly from those of *Mytilinidion*, supporting its exclusion from this genus based on both phylogenetic and morphological evidence.

Hyde et al. (2024) accepted one family and eleven genera within Mytilinidiales, most of which lack muriform septation except *Ostreola* which was introduced by Darker (1963). Although molecular evidence for *Ostreola* remains unavailable, our new species shares features with it, such as cylindrical asci and uniseriate muriform ascospores but differs from *Ostreola* in having broadly opened hysterothecia, while *Ostreola* exhibits narrow, slit-like openings. Additionally, *Ostreola* typically has dull-brown muriform spores, whereas our species is distinguished by its hyaline muriform ascospore (Harkness and Cooke 1878; Darker 1963; Tilak and Kale 1968; Rao and Modak 1972; Barr 1987).

Phylogenetic analysis further positions our new species at a significant distance from the recently described genus *Bullatosporium* (Andreasen et al. 2024). Apart from the six genera included in our phylogenetic analysis, four additional genera within the family, *viz*. *Actidium*, *Camaroglobulus*, *Peyronelia*, and *Zoggium* lack molecular data but exhibit distinct morphological characteristics. *Actidium* is characterized by simple, rounded spores (Fries 1815). *Camaroglobulus* was introduced as the asexual morph of *Mytilinidion resinae*, though its taxonomic placement requires further molecular confirmation (Speer 1986). *Peyronelia* is defined by brown, fusiform conidia that form short chains of slender, septate, interconnected cells (Ciferri and Fragoso-Romualdo 1927) and *Zoggium* features broadly filiform or vermiform spores that are transversely septate and pale-colored (Vasilyeva 2001).

Given these morphological and phylogenetic distinctions, the establishment of a new genus to accommodate our newly identified species is both necessary and justified. This classification will provide a clearer framework for understanding diversity within the order Mytilinidiales.

The holotype of *Lijiangomyces laojunensis* exhibits another intriguing feature: the base of its apothecium is surrounded by the thallus of *Pertusaria* sp. This phenomenon has led us to mistakenly identify it as a lichenicolous species. Although lichenicolous behavior was not confirmed in this study, the close physical association suggests that the two fungi coexist without conflict. It is also possible that lichenicolous species may be identified as more specimens are collected and studied in the future.

Before 2018, *Dactylospora* and *Sclerococcum* were considered distinct genera, with *Sclerococcum* containing 21 species, 19 of which were lichenicolous. Diederich et al. (2018) synonymized *Dactylospora* with *Sclerococcum* and transferred 46 species from *Dactylospora* to *Sclerococcum*. Subsequently, Olariaga et al. (2019) transferred 14 non-lichenicolous species of *Dactylospora* to *Sclerococcum*. Johnston (2022) introduced six new saprophytic species of *Sclerococcum*. Since 2018, ten new lichenicolous species and three combinations have been added to the genus (Elix et al. 2019; Flakus et al. 2019; Fryday 2019; Navarro-Rosinés and Romero 2019; Spribille et al. 2020; Joshi 2021; Zhurbenko 2022; Paz-Bermúdez et al. 2023; Diederich et al. 2024; Zhurbenko and Diederich 2024). Diederich et al. (2024) have accepted a total of 85 species in *Sclerococcum*, with 64 lichenicolous species and the remainder being saprotrophs on liverworts, wood, and bark in both terrestrial and marine habitats (Dong et al. 2020; Thiyagaraja et al. 2022).

The first study of *Sclerococcum* in China was conducted by Thiyagaraja et al. (2022), who reported a new geographical record of *S. simplex*, collected from a corticolous *Pertusaria* thallus in Yunnan province. Ma et al. (2023) described the lignicolous asexual species, *S. pseudobactrodesmium* from Guizhou Province. Meng et al. (2024) reported a new geographical record of *S. glaucomarioides* found on *Ochrolechia akagiensis* from China. This study provides an additional new *Sclerococcum* species from China.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Qingfeng Meng and Ruvishika S. Jayawardena designed the experiments and structured the manuscript. Qingfeng Meng conducted the experiments, analyzed the data, and drafted the manuscript. Shaobin Fu provided funding. Paul Diederich, Vinodhini Thiyagaraja, Ruvishika S. Jayawardena, and Damien Ertz contributed to data analysis and manuscript revision. Xinyu Wang, Natsaran Saichana, Kevin D. Hyde, and Shaobin Fu revised the manuscript. All authors reviewed, edited, and approved the final version of the manuscript for publication.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# Unveiling four new taxa and *Nigrosynnema natarajanensis* comb. nov. in Stachybotryaceae (Hypocreales) from monocotyledon plants in Guangdong Province, China

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## Abstract

Members of Stachybotryaceae are distributed worldwide, with certain species playing a significant role as bio-degraders and some causing diseases in plants, humans, and animals. Other species within this family can be found in soil and have been reported as saprobes in various plants. In this study (2021–2022), fungal taxa resembling Stachybotryaceae, isolated from dead leaves of *Agave sisalana* and a dead stem of *Wurfbainia villosa* in Guangdong Province, China, are identified based on morphological characteristics and molecular data. Multi-locus phylogeny based on calmodulin (*cmdA*), internal transcribed spacer (ITS), the large subunit nuclear rDNA (LSU), RNA polymerase II second largest subunit (*rpb2*), the partial translation elongation factor 1-α (*tef1-α*), and β-tubulin (*tub2*) revealed that nine strains were grouped within Stachybotryaceae. *Nigrosynnema guangdongense* **gen. et sp. nov.**, typical of Stachybotryaceae but having unusual olivaceous brown to black synnemata that are narrower towards the apex and produce phialidic, aseptate, slimy conidia in black and glistening heads, is introduced. Additionally, *Brevistachys wurfbainiae* and *Sirastachys guangdongensis* are introduced as new species. *Stachybotrys microsporus* is a new host record for *Agave sisalana*. The present study provides comprehensive descriptions, illustrations, and molecular data analyses of the newly discovered taxa and newly recorded species as a taxonomic and phylogenetic contribution to Stachybotryaceae. Furthermore, a new combination, *Nigrosynnema natarajanensis*, is proposed for the previously described *Virgatospora natarajanensis*.

**Key words:** 4 new taxa, Asparagaceae, Hyphomycetes, saprobic fungi, Sordariomycetes, taxonomy, Zingiberaceae

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## Introduction

The family Stachybotryaceae (as Stachybotriaceae), belonging to Hypocreales, Sordariomycetes (Hyde et al. 2024), was established to accommodate the genera *Myrothecium*, *Peethambara*, and *Stachybotrys*, with *Stachybotrys* as the type (Crous et al. 2014). The members of this family are commonly isolated from soil and dead plant materials (Jie et al. 2012; Lombard et al. 2016; Hyde et al. 2017). Some species have been reported as pathogenic to plants and animals, with some posing a substantial risk to human health (Ben et al. 2015).

The polyphyletic nature of the genera *Myrothecium* and *Stachybotrys* was addressed by Lombard et al. (2016) through phylogenetic analyses using *cmdA*, ITS, LSU, *rpb2*, *tef1-α*, and *tub2*. As a result of their study, several species previously classified under *Myrothecium* and *Stachybotrys* were transferred into numerous other genera. Thirteen myrothecium-like genera were introduced, viz., *Albifimbria*, *Capitofimbria*, *Dimorphiseta*, *Gregatothecium*, *Inaequalispora*, *Myxospora*, *Neomyrothecium*, *Paramyrothecium*, *Parvothecium*, *Smaragdiniseta*, *Striaticonidium*, *Tangerinosporium*, and *Xenomyrothecium*. Eight stachybotrys-like genera were established, viz., *Achroiostachys*, *Brevistachys*, *Cymostachys*, *Globobotrys*, *Grandibotrys*, *Kastanostachys*, *Sirastachys*, and *Striatibotrys* (Lombard et al. 2016). In total, 33 genera were accommodated in the Stachybotryaceae, of which 21 were newly introduced and 20 were new combinations (Lombard et al. 2016). Additional novel genera were later introduced into this family by Hernandez-Restrepo et al. (2016), Gordillo and Decock (2017), Tibpromma et al. (2018), and Hyde et al. (2020). To date, 39 genera are accepted in this family (Hyde et al. 2020; Wijayawardene et al. 2022; Hyde et al. 2024). The family Stachybotryaceae is characterized by asexual morphs having mononematous, sporodochial, or synnematous conidiophores and phialidic conidiogenous cells that produce conidia in chains or in slimy masses (Crous et al. 2014; Wang et al. 2015; Lombard et al. 2016; Hyde et al. 2020). The sexual morph is described as having solitary ascomata, superficial or completely immersed in host tissue, bright to dark yellow, orange, or black that remain unchanged when treated with KOH, unitunicate asci rounded to nearly truncate at the apex with a refractive apical ring, and ellipsoidal to fusiform to broadly reniform ascospores (Subramanian and Bhat 1978; Crous et al. 2014; Lombard et al. 2016; Hyde et al. 2020).

The type genus *Stachybotrys* was introduced by Corda (1837) with *St. chartarum* as the type species. *Stachybotrys* shares a morphology similar to *Memnoniella* (introduced by Von Höhnel (1924), based on *Me. aterrima*) in having branched or unbranched, erect, thin-walled, smooth, or verrucous conidiophores (Bisby 1943; Wang et al. 2015). The conidia of *Memnoniella* are borne in dry chains, while those in *Stachybotrys* are produced in slimy masses. Smith (1962) considered both *Memnoniella* and *Stachybotrys* to be congeneric, arguing that the arrangement of conidia in dry chains (*Memnoniella*) or slimy masses (*Stachybotrys*) is insufficient for differentiating these two genera. In agreement with the argument provided by Smith (1962), Wang et al. (2015) synonymized *Memnoniella* under *Stachybotrys*. Phylogenetic analyses by Lombard et al. (2016), which included a broader sampling of taxa and more loci, clearly demonstrated that the isolate previously identified as *Memnoniella echinata* (CBS 216.32) (*Me. aterrima*) by Galloway (1933) formed a distinct and well-supported clade separate from the *Stachybotrys* str clade. Therefore, Lombard et al. (2016) resurrected *Memnoniella* and designated the type species of the genus, *Me*. *Echinata*, as the epitype, using Galloway's strain (Galloway 1933). Morphologically, *Memnoniella* can be distinguished from *Stachybotrys* by having mostly smooth, thick-walled, and unbranched conidiophores that give rise to linear dry chains of conidia (Lombard et al. 2016). The study by Lombard et al. (2016) was further supported by Lin et al. (2016), Doilom et al. (2017), Hyde et al. (2020), Mapook et al. (2020), Samarakoon et al. (2021), and Liu et al. (2024), all of which treated *Memnoniella* and *Stachybotrys* as distinct genera.

*Brevistachys*, introduced by Lombard et al. (2016) with *B*. *variabilis* as the type species, is characterized by conspicuously short conidiophores and conidiogenous cells that are borne either on conidiophores or directly from the vegetative hyphae, and obovoid to globose to ossiform to ellipsoidal conidia, aggregated in slimy masses. Five species are listed under *Brevistachys* in Index Fungorum (http://www.indexfungorum.org/names/names.asp; accessed on 12 October 2024). The asexual morph is only observed in *Brevistachys* species, which were isolated from *Musa* and *Zingiber* (Cooke 1883; Lombard et al. 2016). *Sirastachys*, introduced by Lombard et al. (2016), is typified by *Si*. *phaeospora*. The genus is characterized by cylindrical synnemata formed in culture, which consist of bundles of parallelly compacted, erect hyphae, with conidiophores arising laterally from synnemata. *Sirastachys* species were mainly isolated from leaves, with one species isolated from soil under *Thuja occidentalis* (Lombard et al. 2016; Crous et al. 2018; Tibpromma et al. 2018). Nine species are listed under *Sirastachys* Fungorum (http://www.indexfungorum.org/names/names.asp; accessed on 25 July 2024).

In this study, we introduce one new genus, three new species, and one new host record in Stachybotryaceae from dead stems of *Wurfbainia villosa* and a dead leaf of *Agave sisalana* in Guangdong Province, China, based on the morphological characteristics and multi-locus phylogenetic analyses of *cmdA*, ITS, LSU, *rpb2*, *tef1-α*, and *tub2*. The new taxa, *Brevistachys wurfbainiae* sp. nov., *Nigrosynnema guangdongense* gen. et. sp. nov., and *Sirastachys guangdongensis* sp. nov., are compared to morphologically and phylogenetically closely related taxa. A new host record, *Stachybotrys microsporus*, is presented with a detailed description and illustration supported by phylogenetic evidence. Additionally, a new combination, *Nigrosynnema natarajanensis* (= *Virgatospora natarajanensis*), is proposed based on the similarity in morphological characteristics aligning with the generic concept of *Nigrosynnema* and supported by phylogenetic evidence of the type species of *Virgatospora*, *V*. *echinofibrosa*.

## Materials and methods

### Sample collection, morphological studies, and isolation

Samples of dead leaves of *Agave sisalana* and dead stems of *Wurfbainia villosa* were collected in Guangdong Province, China, during the winter to spring seasons of 2021 and 2022, and the important collection information was noted (Rathnayaka et al. 2024). The morphological characteristics and microscopic examination of fungal structures were observed using the method described by Liao et al. (2023). Single spore isolation was performed following the methodology outlined in Senanayake et al. (2020). The living cultures were deposited in the Zhongkai University of Agriculture and Engineering Culture Collection (ZHKUCC), Guangdong, China. Specimens were deposited in the Mycological Herbarium of Zhongkai University of Agriculture and Engineering (MHZU), Guangzhou, China. The newly discovered species were registered in Faces of Fungi (FoF) (http://www.facesoffungi.org) (Jayasiri et al. 2015) and the Index Fungorum (IF) databases (http://www.indexfungorum.org/names/names.asp).

## DNA extraction, PCR amplification, and sequencing

The genomic DNA was extracted from the fungal mycelia cultivated in the dark at 25 °C on PDA for two weeks using the MagPure Plant DNA AS Kit, following the manufacturer's instructions (Guangzhou Magen Biotechnology Co., Ltd, Guangdong, China). Extracted DNA was preserved at -20 °C for further molecular studies. The calmodulin (*cmdA*), internal transcribed spacer (ITS), large subunit rDNA (LSU), RNA polymerase II second largest subunit (*rpb2*), the partial translation elongation factor 1-α (*tef1-α*), and β-tubulin (*tub2*) were amplified and sequenced using primer CAL-228F (Carbone and Kohn 1999) and CAL-2RD (Groenewald et al. 2013), ITS4 and ITS5 (White et al. 1990), LR5 and LR0R (Vilgalys and Hester 1990), rpb2-5f and rpb2-7cR (Liu et al. 1999), EF1-728F (Carbone and Kohn 1999), EF2 (O'Donnell et al. 1998), and BT2a and BT2b (Glass and Donaldson 1995), respectively.

The polymerase chain reaction (PCR) contained a total of 25 µl of mixture, including 9.5 µl of $ddH_2O$, 12.5 µl of 2 × Taq Master Mix (a mixture of dNTPs, optimized buffer, and Taq (Nanjing Vazyme Biotech Co., Ltd., Nanjing, China)), and 1 µl of each of the primer and DNA template. The PCR thermal cycling program for ITS and LSU amplification was conducted with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 30 sec; the annealing temperature was set to 52 °C for 30 sec; the extension step was performed at 72 °C for 1 min; and final elongation was carried out at 72 °C for 10 min. The annealing temperatures were altered to 53.5 °C for *cmdA* and *tef1-α* and 55 °C (45 sec) for *tub2*. PCR was performed for the *rpb2* in a thermal cycle as follows: an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, an annealing temperature of 52 °C for 2 min, and extension at 72 °C for 1.5 min, with a final elongation at 72 °C for 10 min. The PCR products were purified and sent for sequencing at Tianyi Huiyuan Gene Technology & Services Co. (Guangdong, China). All sequences obtained in this study have been deposited in GenBank (available online: http://www.ncbi.nlm.nih.gov).

## Phylogenetic analyses

The original sequence obtained from the sequencing company was cross-checked by verifying chromatograms using BioEdit v. 7.2.3 (Hall 1999), and subsequently, consensus sequences were generated using OFPT (Zeng et al. 2023) and SeqMan v. 7.0 (Lasergene, Madison, WI, USA). The consensus sequences of our fungal strains from each locus were subjected to a basic local alignment search tool (BLAST) search in GenBank. The reference sequences and outgroup taxa used for phylogenetic analyses were selected based on recent relevant literature (Lombard et al. 2016; Tibpromma et al. 2018; Mapook et al. 2020), obtained from GenBank. The phylogenetic analyses utilized 184 sequences (Table 1), with *Fusarium sambucinum* strains CBS 146.95 and CBS 136.24 used as the outgroup taxa.

**Table 1.** Names, culture collection numbers, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses. The new strains in this study are indicated in cells with light blue shading. “$^{T}$” is used to represent ex-type. “-” denotes unavailable information.

| Taxa | Culture collection numbers | GenBank accession numbers | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***cmdA*** | **ITS** | **LSU** | ***rpb2*** | ***tef1-α*** | ***tub2*** |
| *Achroiostachys humicola* | CBS 317.72 | KU845777 | KU845797 | KU845817 | KU845835 | KU845852 | KU845758 |
| *A. humicola* | CBS 868.73$^{T}$ | KU845779 | KU845799 | KU845819 | KU845837 | KU845854 | KU845760 |
| *A. levigata* | CBS 185.79$^{T}$ | KU845785 | KU845805 | KU845825 | KU845841 | KU845860 | KU845765 |
| *A. levigata* | CBS 363.58 | KU845786 | KU845806 | KU845826 | KU845842 | KU845861 | KU845766 |
| *Albosynnema elegans* | GB 3101$^{T}$ | - | - | AF193226 | - | - | - |
| *Albifimbria verrucaria* | CPC 30056 | KU845869 | KU845885 | KU845904 | KU845923 | KU845942 | KU845961 |
| *Al. verrucaria* | CBS 176.27 | KU845870 | KU845886 | KU845905 | KU845924 | KU845943 | KU845962 |
| *Al. viridis* | CBS 244.78 | - | KU845897 | KU845916 | KU845935 | KU845954 | KU845973 |
| *Al. viridis* | CBS 449.71$^{T}$ | KU845879 | KU845898 | KU845917 | KU845936 | KU845955 | KU845974 |
| *Alfaria caricicola* | CBS 113567$^{T}$ | KU845976 | KU845983 | KU845992 | KU846001 | KU846008 | KU846014 |
| *Alf. cyperiesculenti* | CPC 23153$^{T}$ | - | KJ869143 | KJ869200 | - | - | - |
| *Alf. thymi* | CBS 447.83$^{T}$ | KU845981 | KU845990 | KU845999 | - | KU846013 | KU846021 |
| *Alfariacladiella spartii* | CPC 24966 | - | NR_164243 | NG_070399 | - | - | - |
| *Brevistachys globosa* | CBS 141056$^{T}$ | KU846024 | KU846038 | KU846057 | - | KU846085 | KU846101 |
| *B. globosa* | CPC 15952 | KU846025 | KU846040 | KU846059 | - | KU846087 | KU846103 |
| *B. globosa* | CPC 16060 | - | KU846042 | KU846061 | - | KU846089 | KU846105 |
| *B. lateralis* | CBS 141058$^{T}$ | KU846027 | KU846043 | KU846062 | KU846074 | KU846090 | KU846106 |
| *B. ossiformis* | CBS 696.73$^{T}$ | - | KU846044 | KU846063 | - | - | KU846107 |
| *B. ossiformis* | CBS 112792 | KU846028 | KU846045 | KU846064 | KU846075 | KU846091 | KU846108 |
| *B. ossiformis* | CPC 16031 | KU846029 | KU846046 | KU846065 | - | KU846092 | KU846109 |
| *B. subsimplex* | ATCC 32888$^{T}$ | - | AF205439 | - | - | - | - |
| *B. variabilis* | CBS 141057 | KU846030 | KU846047 | KU846066 | KU846076 | KU846093 | KU846110 |
| *B. wurfbainiae* | ZHKUCC 23-1011$^{T}$ | PP746514 | PP645738 | PP683135 | PP746507 | PP746523 | PP746532 |
| *B. wurfbainiae* | ZHKUCC 23-1012 | PP746515 | PP645739 | PP683136 | PP746508 | PP746524 | PP746533 |
| *B. wurfbainiae* | ZHKUCC 23-1013 | PP746516 | PP645740 | PP683137 | PP746509 | PP746525 | PP746534 |
| *Capitofimbria compacta* | CBS 111739$^{T}$ | KU846261 | KU846287 | KU846317 | KU846349 | KU846378 | KU846404 |
| *C. compacta* | MUCL 50238 | - | KU878556 | KU878557 | KU878558 | - | KU878559 |
| *Cymostachys coffeicola* | CBS 252.76$^{T}$ | KU846035 | KU846052 | KU846071 | KU846081 | KU846097 | KU846113 |
| *Cy. coffeicola* | CPC 25009 | - | KU846053 | - | - | - | - |
| *Cy. fabispora* | CBS 136180$^{T}$ | KU846036 | KU846054 | KU846072 | KU846082 | KU846098 | KU846114 |
| *Cy. fabispora* | CPC 24352 | - | KU846055 | - | KU846083 | KU846099 | - |
| *Didymostilbe aurantispora* | CBS 616.85$^{T}$ | - | - | KU846344 | - | - | - |
| *D. matsushimae* | CBS 549.84 | - | - | KU846345 | - | - | - |
| *D. matsushimae* | CCFC 54984 | - | - | AY283545 | - | - | - |
| *Digitiseta dimorpha* | MUCL 54683$^{T}$ | - | KY389329 | KY389349 | KY389367 | KY769935 | KY366460 |
| *Di. multidigitata* | MUCL 41187$^{T}$ | - | KY389325 | KY389345 | KY389363 | KY769934 | KY366456 |
| *Di. parvidigitata* | MUCL 48180$^{T}$ | - | KY389326 | KY389346 | KY389364 | KY769931 | KY366457 |
| *Di. parvidigitata* | MUCL 48271 | - | KY389327 | KY389347 | KY389365 | KY769932 | KY366458 |
| *Di. parvidigitata* | MUCL 48260 | - | KY389328 | KY389348 | KY389366 | KY769933 | KY366459 |
| *Di. setiramosa* | CBS 534.88 | - | AY254156 | - | - | KY769930 | - |
| *Dimorphiseta terrestris* | CBS 127345$^{T}$ | KU846284 | KU846314 | KU846346 | KU846375 | KU846401 | KU846431 |
| *Fusarium sambucinum* | CBS 146.95 | - | - | KM231682 | KM232381 | - | - |
| *F. sambucinum* | CBS 136.24 | - | - | MH866281 | - | - | - |
| *Globobotrys sansevieriicola* | CBS 138872$^{T}$ | - | KR476717 | KR476752 | - | KR476793 | KR476794 |
| *Grandibotrys pseudotheobromae* | CBS 136170$^{T}$ | - | KU846135 | KU846161 | KU846188 | KU846215 | KU846241 |
| *G. pseudotheobromae* | CBS 136391 | - | KU846136 | KU846162 | KU846189 | KU846216 | KU846242 |
| *G. xylophila* | CBS 136179$^{T}$ | KU846115 | KU846137 | KU846163 | KU846190 | KU846217 | |
| *Gregatothecium humicola* | CBS 205.96$^{T}$ | KU846285 | KU846315 | KU846347 | KU846376 | KU846402 | KU846432 |
| *Hyalinostachys cylindrospora* | MFLUCC 17-2583 | - | NR_182450 | NG_148956 | - | - | - |
| *Inaequalispora prestonii* | CBS 175.73$^{T}$ | KU846286 | KU846316 | KU846348 | KU846377 | KU846403 | KU846433 |
| *Kastanostachys aterrima* | CBS 101310$^{T}$ | - | - | AF178565 | KU846191 | - | - |
| *Koorchaloma bambusae* | MFLU 19-2899 | - | MT185516 | MT183479 | MT432230 | - | - |

| Taxa | Culture collection numbers | GenBank accession numbers | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***cmdA*** | **ITS** | **LSU** | ***rpb2*** | ***tef1-α*** | ***tub2*** |
| *K. europaea* | PRM 953076 | - | LR963471 | - | - | - | - |
| *K. krabiense* | MFLUCC 16-0317$^{T}$ | - | MH388348 | MH376721 | MH412729 | - | - |
| *K. oryzae* | MFLUCC 21-0055$^{T}$ | - | MZ519544 | MZ519543 | MZ508427 | - | - |
| *K. spartinicola* | SAP 130 | - | AF422963 | - | - | - | - |
| *Koorchalomella salmonispora* | MD6018 | - | - | KX611345 | - | - | - |
| *Melanopsamma pomiformis* | CBS 325.90 | KU846031 | KU846048 | KU846067 | KU846077 | KU846094 | KU846111 |
| *M. pomiformis* | CBS 101322$^{T}$ | KU846032 | KU846049 | KU846068 | KU846078 | - | - |
| *M. pomiformis* | CBS 114119 | KU846033 | KU846050 | KU846069 | KU846079 | KU846095 | KU846112 |
| *M. xylophila* | CBS 100343$^{T}$ | KU846034 | KU846051 | KU846070 | KU846080 | KU846096 | - |
| *Memnoniella alishanensis* | MFLUCC 20-0168$^{T}$ | - | MW114372 | - | - | - | MW148278 |
| *Me. brunneoconidiophora* | CBS 109477 | - | KU846138 | KU846165 | KU846192 | KU846218 | KU846243 |
| *Me. brunneoconidiophora* | CBS 136191$^{T}$ | KU846116 | KU846139 | KU846166 | KU846193 | KU846219 | KU846244 |
| *Me. celtidis* | MFLUCC 20-0040$^{T}$ | - | MW114374 | - | - | - | MW148280 |
| *Me. celtidis* | NCYUCC 19-0326 | - | MW114375 | - | - | - | MW148281 |
| *Me. chromolaenae* | MFLUCC 17-1507 | - | NR_168873 | MT214465 | - | - | - |
| *Me. dichroa* | CBS 526.50 | KU846117 | KU846140 | KU846167 | KU846194 | KU846220 | - |
| *Me. dichroa* | CBS 123800 | KU846118 | KU846141 | KU846168 | KU846195 | KU846221 | - |
| *Me. echinata* | CBS 216.32$^{T}$ | KU846119 | KU846142 | KU846169 | KU846196 | KU846222 | KU846245 |
| *Me. echinata* | DAOMC 173162 | KU846125 | JN942886 | JN938868 | KU846202 | KU846228 | KU846250 |
| *Me. echinata* | DAOMC 235365 | KU846126 | KU846149 | KU846176 | KU846203 | KU846229 | KU846251 |
| *Me. ellipsoidea* | CBS 136199 | KU846127 | KU846150 | KU846177 | KU846204 | KU846230 | KU846252 |
| *Me. ellipsoidea* | CBS 136200 | KU846128 | KU846151 | KU846178 | KU846205 | KU846231 | KU846253 |
| *Me. ellipsoidea* | CBS 136201$^{T}$ | KU846129 | KU846152 | KU846179 | KU846206 | KU846232 | KU846254 |
| *Me. humicola* | CBS 463.74$^{T}$ | KU846130 | KU846154 | KU846181 | KU846208 | KU846234 | |
| *Me. longistipitata* | CBS 136197 | KU846131 | KU846155 | KU846182 | KU846209 | KU846235 | KU846256 |
| *Me. longistipitata* | ATCC 22699 | - | AF081471 | - | - | - | - |
| *Me. mori* | MFLUCC 18-1640$^{T}$ | - | MW114377 | - | - | - | MW148283 |
| *Me. nilagirica* | MFLUCC 15-0660 | - | KU760374 | - | KU760394 | - | - |
| *Me. oblongispora* | MFLUCC 17-2064 | - | MT310665 | - | MT394724 | - | - |
| *Me. oblongispora* | MFLUCC 15-1074 | KY124123 | KU760376 | - | KU760396 | | KY124127 |
| *Me. oenanthes* | ATCC 22844$^{T}$ | - | AF081473 | - | - | - | - |
| *Me. oenanthes* | CBS 388.73 | - | KU846156 | KU846183 | KU846210 | KU846236 | |
| *Me. Pseudodichroa* | BCRC FU31689$^{T}$ | - | ON692522 | - | LC714856 | LC714858 | LC714861 |
| *Me. Pseudodichroa* | BCRC FU31700 | - | ON692523 | - | LC714857 | LC714859 | LC714862 |
| *Me. pseudonilagirica* | CBS 136405$^{T}$ | KU846132 | KU846157 | KU846184 | KU846211 | KU846237 | KU846257 |
| *Me. putrefolia* | CBS 101177$^{T}$ | - | KU846158 | KU846185 | KU846212 | KU846238 | KU846258 |
| *Me. putrefolia* | CBS 136171 | KU846133 | KU846159 | KU846186 | KU846213 | KU846239 | KU846259 |
| *Me. sinensis* | YMF 1.05582$^{T}$ | - | MK773576 | - | MK773575 | MK772066 | MK773574 |
| *Memnoniella* sp. | MUCL 50191 | KU846134 | KU846160 | KU846187 | KU846214 | KU846240 | KU846260 |
| *Myrothecium inundatum* | CBS 196.74 | KU846434 | KU846451 | KU846473 | - | KU846513 | KU846532 |
| *My. inundatum* | CBS 275.48$^{T}$ | KU846435 | KU846452 | KU846474 | - | KU846514 | KU846533 |
| *My. inundatum* | CBS 120646 | KU846438 | KU846455 | KU846477 | - | KU846516 | KU846536 |
| *My. simplex* | CBS 582.93$^{T}$ | KU846439 | KU846456 | KU846478 | - | KU846517 | KU846537 |
| *Myxospora crassiseta* | CBS 731.83$^{T}$ | KU846442 | KU846459 | KU846481 | KU846497 | KU846520 | KU846540 |
| *Myx. crassiseta* | CBS 121141 | KU846443 | KU846460 | KU846482 | KU846498 | KU846521 | KU846541 |
| *Myx. masonii* | CBS 174.73$^{T}$ | KU846445 | KU846462 | KU846484 | KU846500 | KU846523 | KU846543 |
| *Neomyrothecium humicola* | CBS 310.96$^{T}$ | KU846448 | KU846467 | KU846488 | KU846505 | KU846527 | - |
| *Nigrosynnema guangdongense* | ZHKUCC 23-1014$^{T}$ | PP746517 | PP645741 | PP683138 | PP668100 | PP746526 | PP746535 |
| *N. guangdongense* | ZHKUCC 23-1015 | PP746518 | PP645742 | PP683139 | PP668101 | PP746527 | PP746536 |
| *Paramyrothecium foliicola* | CBS 113121$^{T}$ | KU846266 | KU846294 | KU846324 | - | KU846385 | KU846411 |
| *P. roridum* | CBS 212.95 | KU846269 | KU846299 | KU846329 | KU846360 | KU846389 | KU846416 |
| *P. roridum* | CBS 357.89$^{T}$ | KU846270 | KU846300 | KU846330 | KU846361 | KU846390 | KU846417 |
| *Parvothecium terrestre* | CBS 198.89$^{T}$ | KU846449 | KU846468 | KU846489 | KU846506 | KU846528 | KU846548 |
| *Parvothecium terrestre* | CBS 534.88 | KU846450 | KU846469 | KU846490 | KU846507 | KU846529 | KU846549 |
| *Peethambara sundara* | CBS 521.96 | - | KU846470 | KU846491 | KU846508 | KU846530 | KU846550 |
| *Pe. sundara* | CBS 646.77$^{T}$ | - | KU846471 | AF193245 | KU846509 | KU846531 | KU846551 |
| *Septomyrothecium maraitiense* | MUCL 47202$^{T}$ | - | - | KU846493 | KU846510 | - | - |

| Taxa | Culture collection numbers | GenBank accession numbers | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***cmdA*** | **ITS** | **LSU** | ***rpb2*** | ***tef1-α*** | ***tub2*** |
| *S. uniseptatum* | CBS 100966 | - | KU846472 | KU846494 | KU846511 | - | KU846552 |
| *S. uniseptatum* | MUCL 52944 | - | - | KU846495 | KU846512 | - | - |
| *Sirastachys castanedae* | CBS 164.97 | KU846553 | KU846658 | KU846771 | KU846885 | KU846990 | KU847094 |
| *Si. castanedae* | CBS 531.69 | KU846554 | KU846659 | KU846772 | KU846886 | KU846991 | KU847095 |
| *Si. castanedae* | CBS 136403$^{T}$ | KU846555 | KU846660 | KU846773 | KU846887 | KU846992 | KU847096 |
| *Si. castanedae* | CPC 20373 | KU846556 | KU846661 | KU846774 | KU846888 | KU846993 | KU847097 |
| *Si. cylindrospora* | CBS 136166$^{T}$ | KU846557 | KU846662 | KU846775 | KU846889 | - | KU847098 |
| *Si. cylindrospora* | CBS 13654 | KU846558 | KU846663 | KU846776 | KU846890 | KU846994 | KU847099 |
| *Si. cyperacearum* | CBS 143444 | - | MH107917 | MH107963 | - | - | - |
| *Si. guangdongensis* | ZHKUCC 23-1003$^{T}$ | PP746510 | PP645734 | PP683131 | PP754606 | PP746519 | PP746528 |
| *Si. guangdongensis* | ZHKUCC 23-1004 | PP746511 | PP645735 | PP683132 | PP754607 | PP746520 | PP746529 |
| *Si. longispora* | ATCC 32451$^{T}$ | - | AF081482 | | - | - | - |
| *Si. pandanicola* | CBS 136545$^{T}$ | - | KU846664 | KU846777 | - | - | KU847100 |
| *Si. phaeospora* | CBS 100155$^{T}$ | KU846560 | KU846666 | KU846779 | KU846891 | KU846995 | KU847102 |
| *Si. phangngaensis* | MFLUCC 15-0680 | - | NR_168202 | NG_068841 | MH412735 | - | - |
| *Si. phyllophila* | CBS 173.97 | KU846565 | KU846671 | KU846784 | KU846896 | KU846998 | KU847107 |
| *Si. phyllophila* | CBS 136169$^{T}$ | KU846566 | KU846672 | KU846785 | KU846897 | KU846999 | KU847108 |
| *Si. pseudolongispora* | CBS 417.93 | KU846567 | KU846673 | KU846786 | KU846898 | KU847000 | KU847109 |
| *Si. pseudolongispora* | CBS 100154$^{T}$ | KU846568 | KU846674 | KU846787 | KU846899 | - | KU847110 |
| *Sirastachys* sp. | CBS 308.56 | KU846569 | KU846675 | KU846788 | KU846900 | KU847001 | KU847111 |
| *Smaragdiniseta bisetosa* | CBS 459.82$^{T}$ | KU847206 | KU847229 | KU847255 | KU847281 | KU847303 | KU847319 |
| *Stachybotrys aloeticola* | CBS 137940$^{T}$ | KU846570 | KJ817888 | KJ817890 | KU846901 | - | KJ817886 |
| *St. aloeticola* | CBS 137941 | KU846571 | KJ817889 | KJ817891 | KU846902 | - | KJ817887 |
| *St. chartarum* | CBS 182.80$^{T}$ | KU846573 | KU846679 | KU846792 | KU846904 | KU847003 | KU847115 |
| *St. chartarum* | CBS 119371 | KU846594 | KU846700 | KU846813 | KU846925 | KU847024 | KU847135 |
| *St. chartarum* | CBS 485.48 | KU846577 | KU846683 | KU846796 | KU846908 | KU847007 | KU847119 |
| *St. chlorohalonata* | CBS 113.97 | KU846635 | KU846742 | KU846855 | KU846965 | KU847065 | KU847176 |
| *St. chlorohalonata* | CBS 127.94 | KU846636 | KU846743 | KU846856 | KU846966 | KU847066 | KU847177 |
| *St. chlorohalonata* | CBS 222.46 | KU846637 | KU846744 | KU846857 | KU846967 | KU847067 | KU847178 |
| *St. chlorohalonata* | CBS 250.89 | KU846617 | KU846723 | KU846836 | KU846948 | KU847047 | KU847158 |
| *St. chlorohalonata* | CBS 109283 | KU846622 | KU846728 | KU846841 | KU846953 | KU847052 | KU847163 |
| *St. chlorohalonata* | CBS 109285$^{T}$ | KU846623 | KU846729 | KU846842 | KU846954 | KU847053 | KU847164 |
| *St. chlorohalonata* | CBS 136158 | KU846626 | KU846732 | KU846845 | KU846956 | KU847056 | KU847167 |
| *St. dolichophialis* | DAOMC 227011 | KU846628 | KU846734 | KU846847 | KU846958 | - | KU847169 |
| *St. limonispora* | CBS 128809$^{T}$ | KU846629 | KU846735 | KU846848 | KU846959 | KU847058 | KU847170 |
| *St. limonispora* | CBS 136165 | KU846630 | KU846736 | KU846849 | KU846960 | KU847059 | KU847171 |
| *St. microsporus* | CBS 186.79 | KU846631 | KU846737 | KU846850 | DQ676580 | KU847060 | KU847172 |
| *St. microsporus* | ATCC 18852$^{T}$ | - | AF081475 | - | - | - | - |
| *St. microsporus* | MFLUCC 15-0830 | KY124124 | KU760377 | - | KU760397 | - | KY124128 |
| *St. microsporus* | MFLUCC 15-1076 | KY124125 | KU760378 | - | KU760398 | - | KY124129 |
| *St. microsporus* | MFLUCC 20-0190 | - | MW477992 | - | - | MW480237 | MW480235 |
| *St. microsporus* | ZHKUCC 23-1007 | PP746512 | PP645736 | PP683133 | PP668098 | PP746521 | PP746530 |
| *St. microsporus* | ZHKUCC 23-1008 | PP746513 | PP645737 | PP683134 | PP668099 | PP746522 | PP746531 |
| *St. musae* | MFLUCC 20-0188$^{T}$ | MW480232 | NR_173231 | - | MW480230 | - | MW480234 |
| *St. musae* | MFLUCC 20-0152 | MW480231 | MW477991 | - | MW480229 | - | MW480233 |
| *St. pallescens* | HGUP 0146$^{T}$ | | KC305345 | KC305345 | - | - | - |
| *St. phaeophialis* | KAS 525$^{T}$ | KU846632 | KU846738 | KU846851 | KU846962 | KU847061 | KU847173 |
| *St. reniformis* | CBS 976.95 | KU846633 | KU846739 | KU846852 | KU846963 | KU847062 | KU847174 |
| *St. reniformis* | CBS 136198 | - | KU846740 | KU846853 | - | KU847063 | - |
| *St. subcylindrospora* | HGUP 0201$^{T}$ | - | KC305354 | - | - | - | - |
| *St. subreniformis* | HGUP 1051$^{T}$ | - | KC305344 | - | - | - | - |
| *St. subsylvatica* | CBS 126205$^{T}$ | KU846634 | KU846741 | KU846854 | KU846964 | KU847064 | KU847175 |
| *Stachybotrys* sp. | CBS 525.50 | KU846645 | KU846752 | KU846865 | | KU847075 | KU847186 |
| *Striatibotrys eucylindrospora* | CBS 203.61$^{T}$ | KU846648 | KU846755 | KU846868 | KU846975 | KU847078 | KU847189 |
| *Str. eucylindrospora* | CBS 136399 | - | KU846757 | KU846870 | KU846977 | KU847080 | KU847191 |
| *Str. eucylindrospora* | CBS 136547 | KU846649 | KU846758 | KU846871 | KU846978 | KU847081 | KU847192 |
| *Str. neoeucylindrosporus* | UAMH 7211 | - | MW187767 | MW187732 | MW192603 | MW192605 | MW192606 |
| *Str. neoeucylindrosporus* | UAMH 7122 | - | MW187766 | MW187768 | MW192608 | MW192609 | MW192610 |
| *Striaticonidium brachysporum* | CBS 131.71 | KU847207 | KU847230 | KU847256 | KU847282 | KU847304 | KU847320 |

| Taxa | Culture collection numbers | GenBank accession numbers | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***cmdA*** | **ITS** | **LSU** | ***rpb2*** | ***tef1-α*** | ***tub2*** |
| *Stri. brachysporum* | CBS 513.71$^{T}$ | KU847209 | KU847232 | KU847258 | KU847284 | KU847305 | KU847322 |
| *Stri. brachysporum* | CBS 126552 | KU847210 | KU847233 | KU847259 | KU847285 | KU847306 | KU847323 |
| *Stri. cinctum* | CBS 373.50 | KU847214 | KU847237 | KU847263 | KU847289 | - | KU847327 |
| *Stri. cinctum* | CBS 932.69$^{T}$ | KU847216 | KU847239 | KU847265 | KU847290 | - | KU847329 |
| *Stri. Deklijnearum* | CBS 143232 | - | NR_156676 | NG_058527 | - | MG386158 | MG386171 |
| *Stri. humicola* | CBS 258.76$^{T}$ | - | KU847240 | KU847266 | | KU847311 | KU847330 |
| *Stri. humicola* | CBS 388.97 | KU847217 | KU847241 | KU847267 | KU847291 | KU847312 | KU847331 |
| *Stri. synnematum* | CBS 479.85$^{T}$ | KU847218 | KU847242 | KU847268 | KU847292 | - | KU847332 |
| *Tangerinosporium thalitricola* | CBS 317.61$^{T}$ | KU847219 | KU847243 | KU847269 | - | - | KU847333 |
| *Virgatospora echinofibrosa* | CBS 110115 | KU847220 | KU847244 | KU847270 | KU847293 | KU847313 | KU847334 |
| *V. echinofibrosa* | MUCL 39092 | - | KU847245 | KU847271 | KU847294 | - | KU847335 |
| *Xenomyrothecium tongaense* | CBS 598.80$^{T}$ | KU847221 | KU847246 | KU847272 | KU847295 | KU847314 | KU847336 |
| *Xepicula jollymannii* | CBS 276.48$^{T}$ | KU847223 | KU847248 | KU847274 | KU847297 | KU847316 | KU847338 |
| *X. jollymannii* | CBS 126168 | KU847224 | KU847250 | KU847276 | KU847298 | KU847317 | KU847340 |
| *X. leucotricha* | CBS 278.78 | KU847227 | KU847253 | KU847279 | KU847301 | - | KU847343 |
| *X. leucotricha* | CBS 483.78 | KU847228 | KU847254 | KU847280 | KU847302 | KU847318 | KU847344 |

Six loci, *cmdA*, ITS, LSU, *rpb2*, *tef1-α*, and *tub2*, were aligned using the MAFFT version v. 7 online program (https://mafft.cbrc.jp/alignment/server/; Katoh and Standley 2013). Subsequently, the dataset was trimmed with TrimAl v.1.3 using the Gappyout option (Capella-Gutiérrez et al. 2009). The alignments were transformed into NEXUS format using the ALignment Transformation EnviRonment online platform (http://www.sing-group.org/ALTER/).

The combined *cmdA*, ITS, LSU, *rpb2*, *tef1-α*, and *tub2* sequence data were performed using maximum likelihood (ML) and Bayesian inference (BI) analyses. The ML analysis was carried out in the CIPRES Science Gateway online platform (Miller et al. 2010) using RAxMLHPC v.8.2.12 on XSEDE (Stamatakis 2014) with GTR+G+I evolutionary substitution, with 1000 rapid bootstrap inferences followed by an extensive ML search. All free model parameters were estimated using the RAxML maximum likelihood method with 25 per-site rate categories. The likelihood of the final tree was evaluated and optimized under the GAMMA gamma distribution shape parameter. The Bayesian Inference (BI) analysis was conducted utilizing the Markov Chain Monte Carlo (MCMC) method and executed in MrBayes XSEDE (3.2.7a) (Huelsenbeck and Ronquist 2001). The simulation was conducted by running six concurrent Markov chains for 5,000,000 generations, with tree sampling occurring every 100th generation. The phylogenetic trees were visualized using FigTree v. 1.4.0 (Rambaut 2009) and formatted using PowerPoint 2010 (Microsoft Corporation, WA, United States). New species are established based on the recommendations of Jeewon and Hyde (2016).

## Results

### Phylogenetic analyses

The phylogenetic tree was constructed using the combined *cmdA*, ITS, LSU, *rpb2*, *tef1-α*, and *tub2* sequence data of 184 strains (including our new strains) through ML and BI analyses. The total length of the dataset, including gaps, was 5060 base pairs (*cmdA*: 1–930, ITS: 931–1657, LSU: 1658–2494, *rpb2*: 2495–3274, *tef1*-α: 3275–4646, *tub2*: 4647–5060). The topology of the ML analysis resembled that of the BI analysis. The highest-scoring RAxML tree, with a final ML optimization likelihood value of -87901.129587, is depicted in Fig. 1. The matrix consisted of 1060

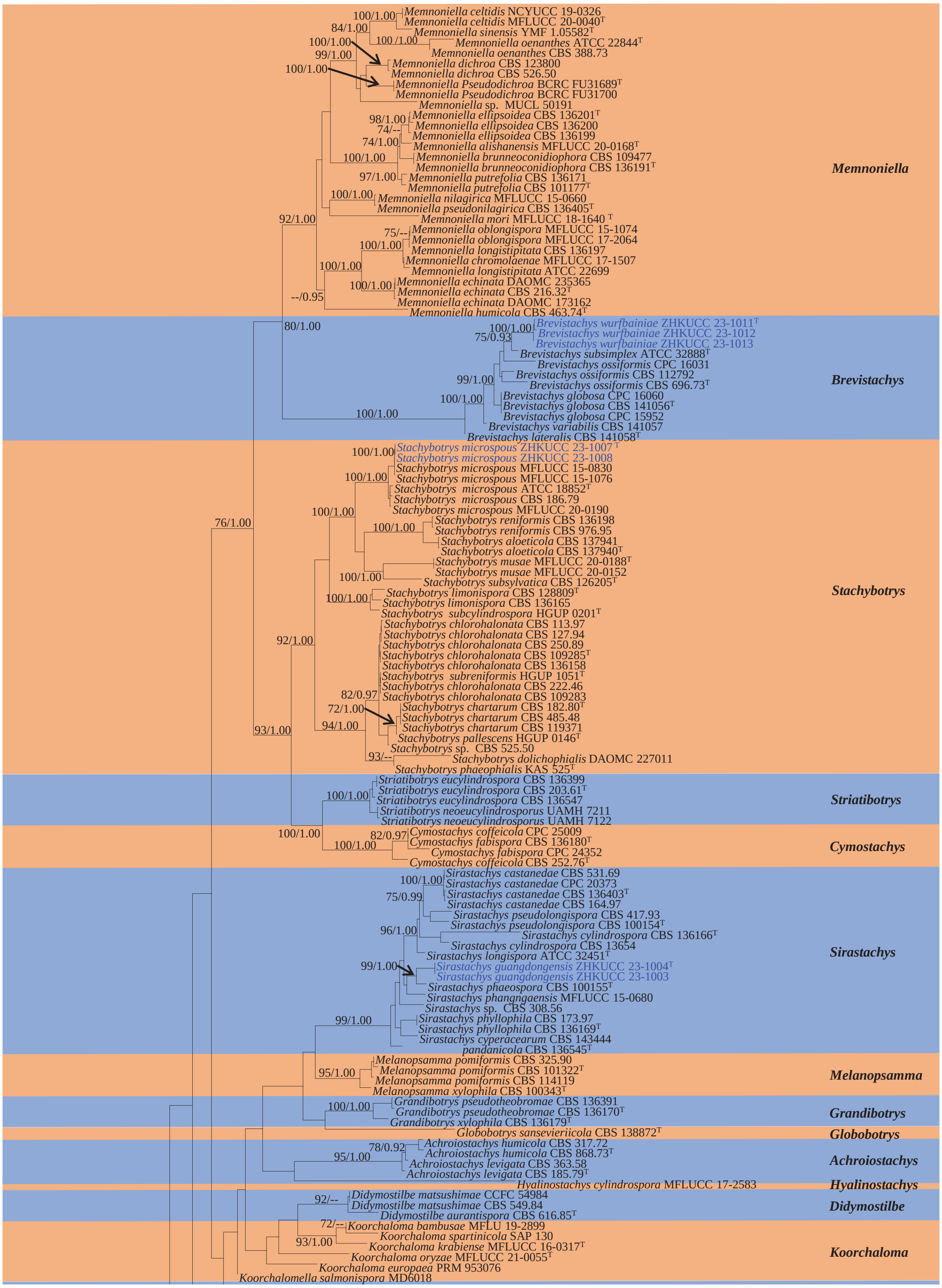

**Figure 1.** Phylogram generated from maximum likelihood analysis (RAxML) of strains in Stachybotryaceae based on the combined *cmdA*, ITS, LSU, *rpb2*, *tef1-α*, and *tub2* sequence data. Maximum likelihood bootstrap values ≥ 70% (ML) and Bayesian posterior probabilities ≥ 0.90 (ML/BYPP) are provided at the nodes. The tree is rooted with *Fusarium sambucinum* strains CBS 136.24 and CBS 146.95. The hyphen (-) represents support values < 70% ML and < 0.90 BYPP. The ex-type strains are denoted as "T", while the newly isolated strains are highlighted in blue.

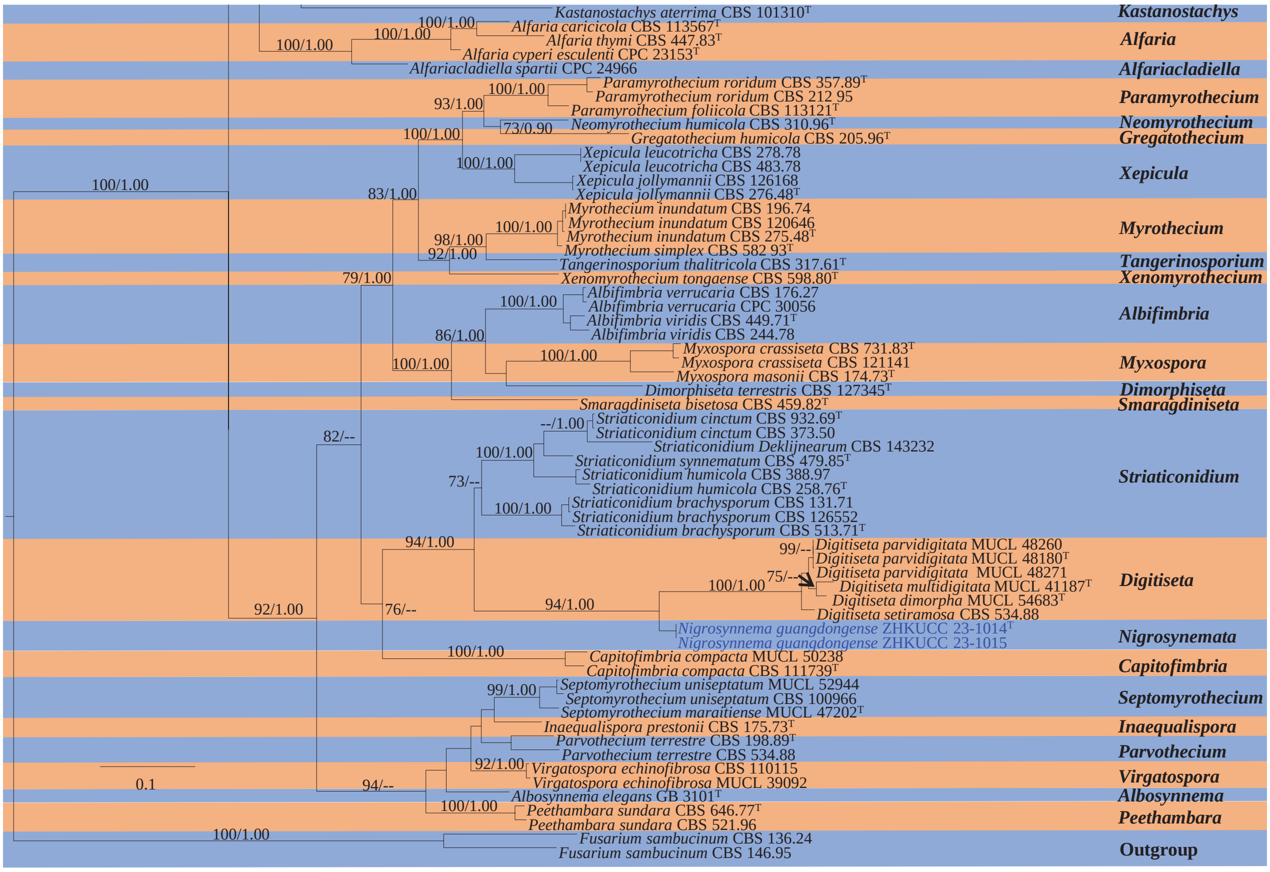

**Figure 1.** Continued.

distinct alignment patterns, with 44.52% undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.235675, C = 0.272976, G = 0.268686, T = 0.222663; substitution rates AC = 1.088667, AG = 3.185610, AT = 1.213744, CG = 0.825063, CT = 4.778952, GT = 1.000000; gamma distribution shape parameter α = 0.307577. In this study, the phylogenetic analyses showed that our strains belong to Stachybotryaceae. The tree topology in this study is almost similar to the previous studies of Lombard et al. (2016) and Hyde et al. (2020). However, Lombard et al. (2016) and Hyde et al. (2020) constructed the tree using LSU and *rpb2*. The inclusion of an increasing number of newly discovered genera and taxa, including *Digitiseta* gen. nov. and additions to *Inaequalispora* and *Parvothecium* as reported by Gordillo and Decock (2017), has resulted in slight alterations to the positions of some genera. Additionally, our phylogenetic tree showed that species of *Koorchaloma* are paraphyletic and grouped with *Didymostilbe* instead of *Koorchalomella* and *Alfariacladiella* as shown in Hyde et al. (2020). Two new strains (ZHKUCC 23-1007, ZHKUCC 23-1008) constituted a highly supported subclade with *Stachybotrys microsporus* (type strain, CBS 126205) with 100% ML and 1.00 BYPP. The novel strains ZHKUCC 23-1003 and ZHKUCC 23-1004 formed a sister subclade with *Sirastachys phaeospora* (type strain, CBS 100155) with 99% ML bootstrap support and 1.00 BYPP. Three strains, ZHKUCC 23-1011, ZHKUCC 23-1012, and ZHKUCC 23-1013, formed a sister subclade with *Brevistachys subsimplex* (type strain, ATCC 32888) with 75% ML and 0.93 BYPP. Two strains, ZHKUCC 23-1014 and ZHKUCC 23-1015, formed a distinct clade with *Digitiseta* species with 94% ML and 1.00 BYPP.

## Taxonomy

***Brevistachys wurfbainiae*** **C.F. Liao, K.D. Hyde & Doilom, sp. nov.**
Index Fungorum: IF902005
Facesoffungi Number: FoF15745
Fig. 2

**Etymology.** In reference to the host genus *Wurfbainia*, from which the holotype was isolated.

**Holotype.** MHZU 23-0254.

**Description.** ***Saprobic*** on dead stem of *Wurfbainia villosa*. ***Sexual morph*****:** undetermined. ***Asexual morph*****:** Colonies superficial on host substrate, effuse, hairy, gregarious, with numerous dark conidia on the substrate visible as black granular powder. ***Conidiophores*** 80–235 × 3–5.5 µm (av. 155 × 4.5 µm, n = 20), macronematous, mononematous, erect, simple, unbranched, straight or flexuous, subcylindrical, unevenly olivaceous brown, 1–3-septate, not constricted at the septa, smooth-walled to finely verruculose in the above half, thick-walled, with bulbous apices, bearing 5–8 conidiogenous cells at the tip, often intermixed with setiferous, flexuous, sterile filaments. ***Setae*** 230–390 × 3–6 µm (av. 305 × 4.5 µm, n = 20), arising from the basal stroma, adjacent to cells that give rise to fertile conidiophores, unbranched, straight, and subhyaline at base, mostly flexuous, olivaceous green, in above half, moderately thick-walled, smooth, septate, acute at apex. ***Conidiogenous cells*** 6–10 × 4–7 µm (av. 7.5 × 5.5 µm, n = 30), enteroblastic, monophialidic, discrete, determinate, terminal, elongate doliiform, pale to dark brown, smooth-walled, with a conspicuous collarette. ***Conidia*** 5–9 µm diam. (av. 7 µm, n = 30), acrogenous, solitary, dry, obovoid to subglobose, aseptate, hyaline, and smooth-walled when young, pale brown, mostly olivaceous to dark brown, verrucose to warty-surfaced at maturity.

**Culture characteristics.** Colonies on PDA reaching 2 cm in two weeks at 28 ± 2 °C, medium dense, raised, sparse, filamentous, floccose to fluffy, velvety, filiform at margin, cream to pale brown from above; brown to pale luteous from reverse.

**Material examined.** China • Guangdong Province, Yangchun City, Yongning Town (22.256185°N, 111.609037°E, 270 m), on dead stems of *Wurfbainia villosa* (Lour.) Škorničk. & A.D. Poulsen. (Zingiberaceae), 10 April 2022, C.F. Liao & Y.H. Yang, YAM16 (MHZU 23-0254, holotype) • ex-type, ZHKUCC 23-1011 • *ibid*., living culture ZHKUCC 23-1012, and ZHKUCC 23-1013.

**Notes.** *Brevistachys wurfbainiae* differs from other known species in the genus *Brevistachys* by having erect to flexuous, sterile, setiferous filaments intermixed with fertile conidiophores. *Brevistachys wurfbainiae* resembles *B*. *subsimplex* but differs from the latter in having slightly longer conidiophores with bulbous apices (80–235 × 3–5.5 µm vs. 80–200 (most frequently 100–140) × 3–5.5 µm) and shorter conidiogenous cells (6–10 × 4–7 µm vs. 8–13 × 4–6 µm). The conidiophores of *B*. *wurfbainiae* are 1–3-septate, while they are 2–6-septate in *B*. *subsimplex* (Deighton 1960). The phylogenetic analyses revealed that *B*. *wurfbainiae* (ZHKUCC 23-1011, ZHKUCC 23-1012, and ZHKUCC 23-1013) formed a separate branch from *B*. *subsimplex* (ex-type CBS 100155) with 75% ML bootstrap support and 0.93 BYPP (Fig. 1). Based on distinct morphology and phylogenetic support, we propose *B*. *wurfbainiae* as a new species.

**Figure 2.** *Brevistachys wurfbainiae* (MHZU 23-0254, holotype) **a, b** colonies on the surface substrate **c, d** conidiophores, setae or conidiophore-like (arrow), conidiogenous cells, and conidia **e, f** conidiogenous cells with conidia **g** conidia **h** germinated conidium **i, j** colonies on PDA (front and below). Scale bars: 100 μm (**b–d**); 10 μm (**e, f**); 5 μm (**g**); 20 μm (**h**).

***Nigrosynnema*** **C.F. Liao, K.D. Hyde & Doilom, gen. nov.**
Index Fungorum: IF902006
Facesoffungi Number: FoF15744

**Etymology.** The name refers to the characteristic black synnemata formed on natural substrate.

**Description.** Saprobic on dead plant material. ***Sexual morph*:** undetermined. ***Asexual morph*: *Conidiomata*** synnematous or sporodochial. ***Synnemata*** unbranched, subcylindrical, globose to subglobose head, robust at base, olivaceous brown to black, straight or curved in the upper portion, consisting of bundles of parallelly arranged, tightly compacted conidiophores. ***Sporodochia*** stromatic, superficial, scattered or gregarious, irregular, pulvinate, with white mycelium surrounding an olivaceous green mass of conidia. ***Conidiophores*** arising from basal stroma, macronematous, mononematous, septate, unbranched or branched, straight or flexuous, thin-walled, subcylindrical, olivaceous brown, verrucose, consisting of a stipe and a penicillately branched conidiogenous apparatus consisting of a whorl of primary branches, each terminating in number of conidiogenous cells. ***Conidiogenous cells*** enteroblastic, monophialidic, integrated, terminal, clavate to subcylindrical, hyaline to pale olivaceous brown, smooth, often verruculose at base, with a conspicuous collarette. ***Conidia*** solitary, fusiform to ellipsoidal, aseptate, initially hyaline, becoming olivaceous brown to dark brown, longitudinally striated at surface, with a distinct dark basal hilum.

**Type species.** *Nigrosynnema guangdongense* C.F. Liao, K.D. Hyde & Doilom

**Notes.** *Nigrosynnema* resembles *Striaticonidium* in having fusiform to ellipsoidal conidia with longitudinal striations. However, it can be distinguished from *Striaticonidium* by having synnematous conidiomata, the absence of setae on the sporodochia, as well as support from molecular data. The synnematous conidiomata of *Nigrosynnema* are subcylindrical, flexuous, narrower towards the apex of the stipe, and robust at the base. The sporodochia are devoid of setae. However, in *Striaticonidium*, they are cylindrical to pyriform, broadened towards the apex, and have sporodochia covered by setae (Lombard et al. 2016). The blastn search of NCBI GenBank revealed that two strains of *Nigrosynnema*, ZHKUCC 23-1014 and ZHKUCC 23-1015, have sequence similarities of 98.37%, 91.73%, 89.70%, 89.04%, and 82.03% to the type species of *Striaticonidium* (*Stri*. *cinctum* CBS 932.69, ex-type) in LSU, ITS, *tub2*, *rpb2*, and *cmdA* sequence data, respectively. However, *tef1-α* sequence data of *Stri*. *cinctum* CBS 932.69 (ex-type) is unavailable in the NCBI database.

*Nigrosynnema* resembles *Virgatospora* described by Finley (1967) in having synnematous conidiomata, phialidic conidiogenous cells, and striated conidia. However, olivaceous brown to black synnemata in *Nigrosynnema* are subcylindrical, robust at the base, and narrower towards the apex of the stipe. The conidia in *Nigrosynnema* are aseptate, fusiform to ellipsoidal, and different from the septate, slightly curved conidia with a protuberant hilum of the type species of *Virgatospora*, *V*. *echinofibrosa*. *Nigrosynnema* can be distinguished from its closely related genera, as shown in Table 2.

The phylogenetic analyses supported that our two strains (ZHKUCC 23-1014 and ZHKUCC 23-1015) formed a distinct clade from other morphologically closely related taxa and constituted a well-supported clade related to *Digitiseta* with 94% ML and 1.00 BYPP statistical support. The main distinguishing morphological characteristic between the two genera is the absence of hypha-like setoid structures in *Nigrosynnema*, whereas *Digitiseta*, introduced by Gordillo and Decock (2017), has short apical branches and digitated hypha-like setoids. Additionally, the conidial shape is fusiform to ellipsoidal in *Nigrosynnema*, while they are cylindrical in *Digitiseta*.

Based on morphological and molecular evidence, we introduce a novel asexual genus, *Nigrosynnema*, characterized by olivaceous to black synnematous or sporodochial conidiomata that produce phialidic, aseptate conidia in black,

**Table 2.** Morphological comparison of *Nigrosynnema* and its closely related genera.

| Genera | Synnematous conidiomata | | Sporodochial conidiomata | Conidiogenous cells | | Conidia | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shape | Color | Shape | Color | Shape | Color | Shape | Color | Number of septa | |
| *Nigrosynnema* | Subcylindrical, narrower towards the apex of the stipe with a robust base | Olivaceous brown to black | Irregular, with white mycelia surrounding an olivaceous green mass of conidia | Olivaceous brown to black | Enteroblastic, phialidic, monophialidic, subcylindrical | Mostly hyaline, sometimes pale olivaceous brown in the lower portion | Fusiform to ellipsoidal, longitudinally striated | Olivaceous brown to dark brown | Aseptate | This study |
| *Albosynnema* | Subcylindrical | Hyaline | - | - | Phialidic, cylindrical to subulate | Hyaline | Ellipsoidal to oblong | Blackish brown to black | 3-septate | Morris (1967) and Bills et al. (1994) |
| *Didymostilbe* | Cylindrical, slightly thinned downwards, apex in a globose-hemispherical cap | White-gray | - | - | - | - | Short oblong-fusoid | Hyaline | 0–1-septate | Saccardo and Saccardo (1906) |
| *Digitiseta* | - | - | Circular to ellipsoid | Hyaline | Penicillus biverticillate, phialides, cylindrical, finger-like, straight to slightly incurved inward the penicillus | Hyaline | Cylindrical to slightly asymmetrical | Hyaline to pale greenish | Aseptate | Gordillo and Decock (2017) |
| *Peethambara* | Cylindrical with a subglobose to oval head surmounted by conspicuous, slimy conidia | White | - | - | Phialide, mostly subcylindrical, sometimes wider in the middle than at either end | Hyaline | Ellipsoidal to limoniform, with mammiform basal and/or apical | Hyaline to subhyaline | 1-septate | Subramanian and Bhat (1978) |
| *Striaticonidium* | Cylindrical to pyriform, broadening towards the apex | Marginal hyphae of synnemata olivaceous green | Oval to elongate or irregular with a white to grey setose fringe surrounding an olivaceous green to dark green slimy mass of conidia | Olivaceous green to mouse grey | Phialidic, clavate to cylindrical to subcylindrical | Hyaline | Fusiform to ellipsoidal, longitudinally striated | Olivaceous green to brown | Aseptate | Lombard et al. (2016) |
| *Virgatospora* | Cylindrical throughout the greater part, somewhat expanded at the apex and base | White or yellow at the base, yellow-black or blackish black at the apex | - | - | - | - | Campanulate, cylindrical, or allantoid to fusiform | Pale, olive to fuscous | Mature 3-(sometimes 2-or 4)-septate | Finley (1967) |

slimy, glistening masses or heads. The conidia are fusiform to ellipsoidal, aseptate, longitudinally striated, and olivaceous brown to dark brown.

**_Nigrosynnema guangdongense_ C.F. Liao, K.D. Hyde & Doilom, sp. nov.**
Index Fungorum: IF902007
Facesoffungi Number: FoF15746
Figs 3, 4

**Etymology.** The epithet “guangdongense” refers to the locality, Guangdong Province, China, where the holotype was collected.

**Holotype.** MHZU 23-0255.

**Description.** ***Saprobic*** on dead stem of *Wurfbainia villosa*. ***Sexual morph*:** undetermined. Asexual morph: ***Synnemata*** on the natural substrate, 370–570 × 20–50 µm (av. 470 × 33 µm, n = 20), erect, unbranched, subcylindrical, with a robust base, narrowed towards fertile apex, olivaceous brown to black, straight or curved in the upper portion, consisting of parallelly arranged, tightly compacted conidiophores. ***Conidiophores*** 2–4 µm wide, subcylindrical, branched, olivaceous brown, slightly tapering towards the apex, verrucose. ***Conidiogenous cells*** 10.5–32.5 × 1.5–3 µm (av. 22 × 2.5 µm, n = 30), enteroblastic, monophialidic, discrete, terminal, subcylindrical, mostly hyaline, sometimes pale olivaceous brown in the lower portion, mostly smooth-walled in above half, often verruculose at below half, with a conspicuous collarette. ***Conidia*** 10–12.5 × 3–4.5 µm (av. 11.5 × 4 µm, n = 30), solitary, slimy, fusiform to ellipsoidal, aseptate, longitudinally striated, olivaceous brown to dark brown, guttulate, obtuse at both ends, with a distinct dark basal hilum.

**Culture characteristics.** Colonies on PDA reaching 4.5–6.5 cm in two weeks at 28 ± 2 °C, medium dense, flat or effuse, diffuse, rough, circular, filiform with curled, large circle in the middle becoming a wave and extends outward, cream from above; cream from the reverse. The spores produced on PDA after three weeks. ***Conidiomata*** 220–300 × 15–20 µm, sporodochial, superficial, scattered, irregular, with white mycelia surrounding an olivaceous green mass of conidia, with or without covering the slimy mass of conidia, without setae. ***Conidiophores*** arising from the basal stroma, consisting of a stipe and a penicillately branched conidiogenous apparatus; stipes unbranched or rarely branched, hyaline, septate, smooth, 10–30 × 2.5–3.5 µm (av. 18 × 3.0 µm, n = 20), conidiogenous apparatus consisting of a whorl of 2–5 primary branches, each terminating in 2–5 conidiogenous cells; primary branches, 1, 2-septate, smooth, unbranched, 8–20 × 2–6 µm, secondary branches, aseptate, smooth, unbranched, 6–20 × 2–5 µm. ***Conidiogenous cells*** 10–20 × 2–4 µm (av. 14 × 2.5 µm, n = 30), phialidic, terminal, with a conspicuous collarette, clavate to cylindrical, hyaline, smooth. ***Conidia*** 7–10 × 3–5 µm (av. 8.5 × 3.5 µm, n = 30), acrogenous, longitudinally striated, fusiform to ellipsoidal, aseptate, initially hyaline, becoming olivaceous green when mature.

**Material examined.** China • Guangdong Province, Yangchun City, Yongning Town (22.256185°N, 111.609037°E, 270 m), on dead stems of *Wurfbainia villosa* (Lour.) Škorničk. & A.D. Poulsen. (Zingiberaceae), 10 April 2022, C.F. Liao & Y.H. Yang, YAM19 (MHZU 23-0255, holotype) • ex-type, ZHKUCC 23-1014 • *ibid*., living culture ZHKUCC 23-1015.

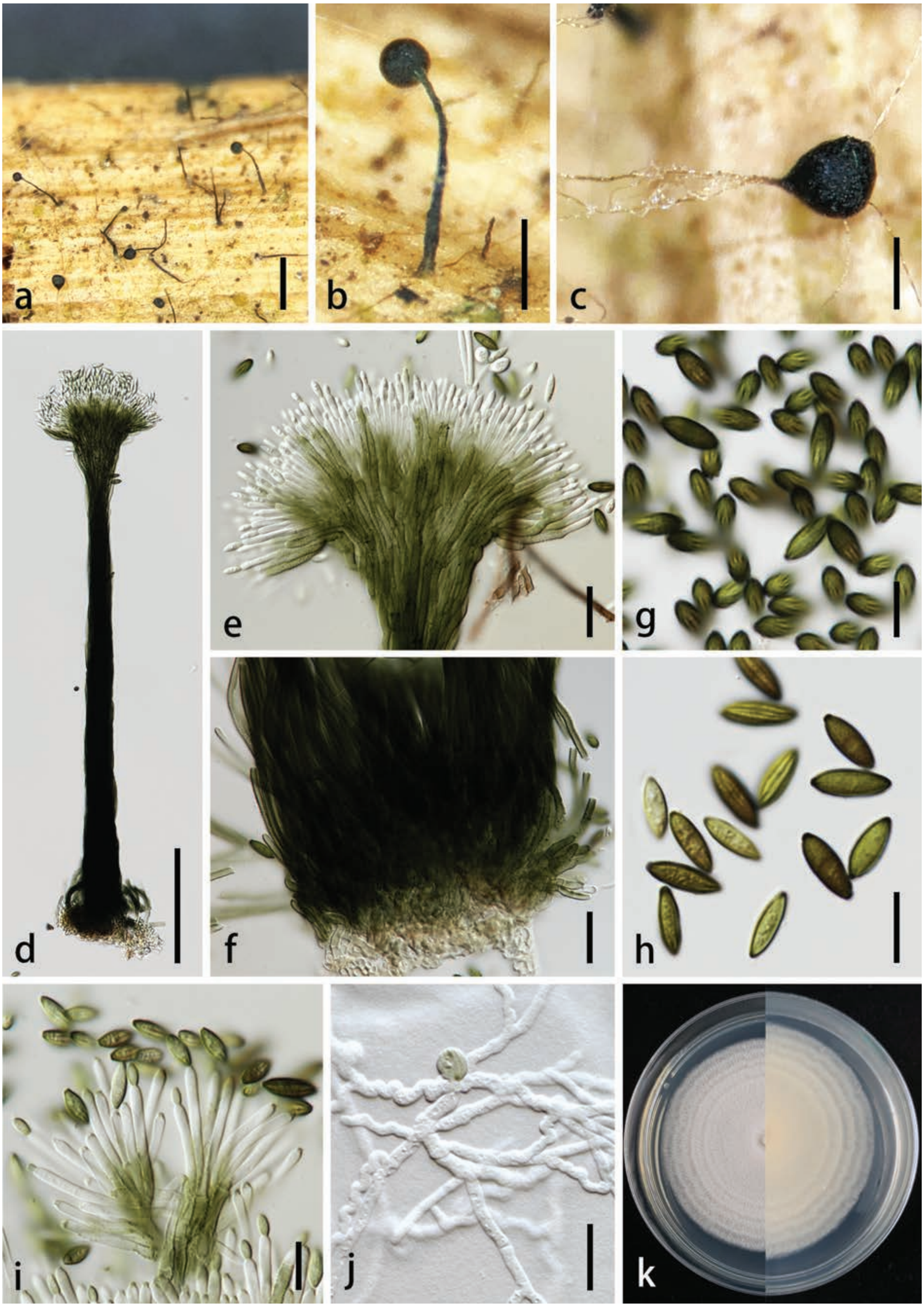

**Figure 3.** *Nigrosynnema guangdongense* (MHZU 23-0255, holotype) on natural substrate **a–c** synnemata on substrate **d** synnema **e** top of synnema **f** base of synnema **g, h** conidia **i** conidiogenous cells with conidia **j** germinated conidium **k** colonies on PDA (front and below). Scale bars: 500 µm (**a**); 200 µm (**b**); 100 µm (**c, d**); 20 µm (**e, f**); 10 µm (**g–j**).

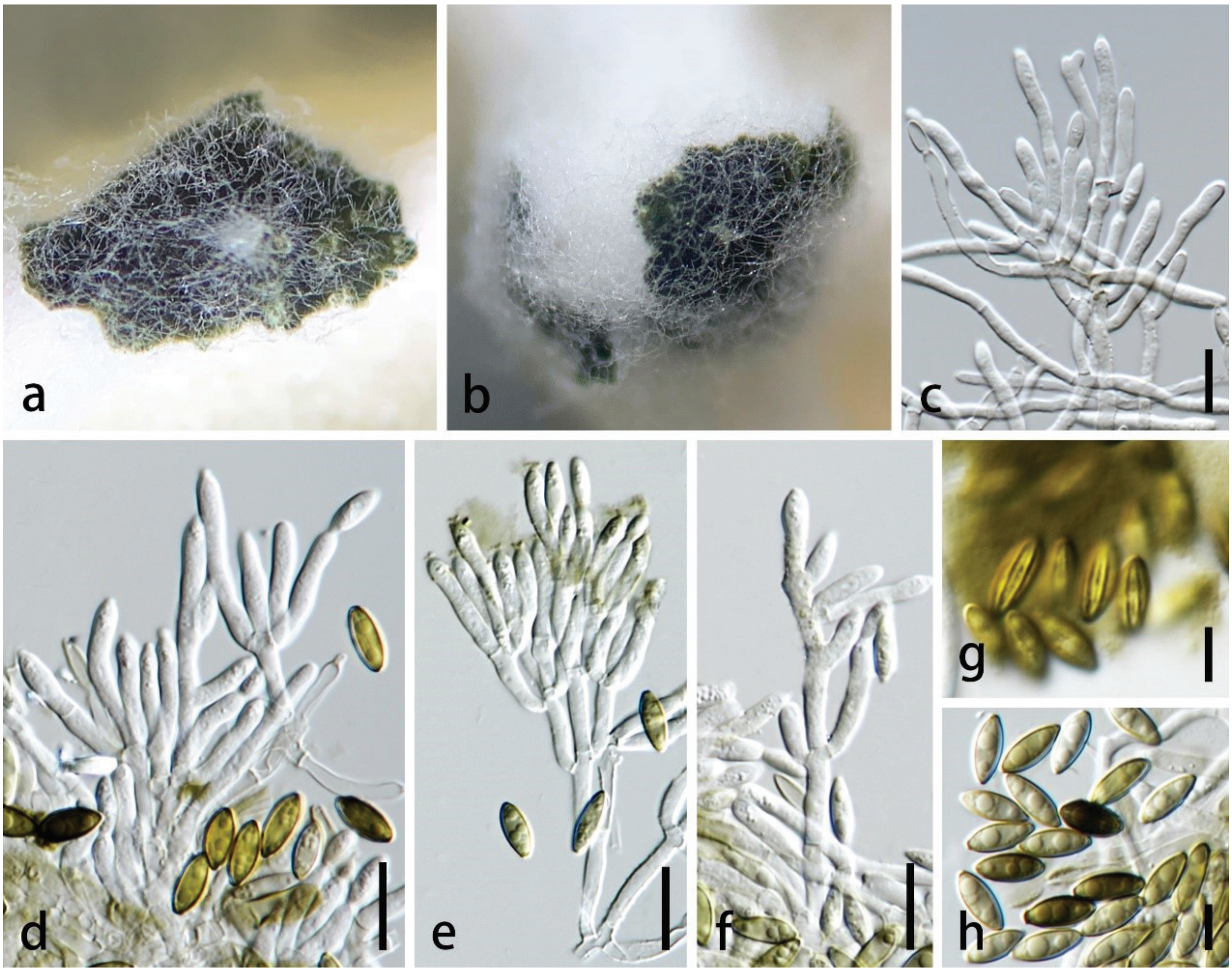

**Figure 4.** *Nigrosynnema guangdongense* (MHZU 23-0255) on PDA after three weeks **a, b** conidial masses on pda **c–f** conidiophores, conidiogenous cells with conidia **g, h** conidia. Scale bars: 10 µm (**c–f**); 5 µm (**g, h**).

**Notes.** *Nigrosynnema guangdongense* is established here as the type species. It is similar to *Virgatospora natarajanensis* described by D'Souza et al. (2002) in having synnematous conidiomata, with fusiform, aseptate, and striated conidia. However, *N. guangdongense* has verrucose, olivaceous, brown conidiophores, conidia with obtuse apices, and a distinct dark basal hilum, whereas *V. natarajanensis* has distinctly echinulate, subhyaline conidiophores that are narrower and smooth towards the apex, and conidia are rounded at both ends. Additionally, the conidiogenous cells of *V. natarajanensis* are occasionally found in the subterminal position, while they have not been observed in *N. guangdongense*. *Nigrosynnema guangdongense* has longer conidiogenous cells (10.5–32.5 × 1.5–3 µm) compared to *V. natarajanensis* (18–25 × 1.5–3.5 µm).

*Nigrosynnema guangdongense* and *Virgatospora echinofibrosa* (the type species of *Virgatospora*) (Finley 1967) are similar in having synnematous conidiomata and striate, phialidic conidia. However, *N. guangdongense* has shorter synnemata (370–570 × 20–50 µm) than *V. echinofibrosa* (up to 1500 µm). Synnemata of *N. guangdongense* are robust at the base, narrower towards the apex of the stipe, and olivaceous brown to black, whereas they are simple or branched, sometimes proliferated, cylindrical throughout the greater part, somewhat broader at the apex and base, white or yellow at the base, yellow-black or blackish black at the apex in *V. echinofibrosa*.

Additionally, *N. guangdongense* has smaller (10–12.5 × 3–4.5), fusiform, aseptate conidia compared to ellipsoidal to limoniform, 3-(sometimes 2- or 4)-septate conidia (39–50 × 9–15 µm) of *V. echinofibrosa*.

**_Nigrosynnema natarajanensis_ (D'Souza, S.K. Singh & Bhat) C.F. Liao, K.D. Hyde Doilom & Bhat, comb. nov.**
Index Fungorum: IF902010

**Basionym.** *Virgatospora natarajanensis* D'Souza, S.K. Singh & Bhat, Mycotaxon 82: 141 (2002).

**Holotype.** IMI 386680.

**Type information.** India • Middle Andaman Island, on dead leaves of *Calamus thwaitesii*, 15 December 2000, Rajiv Kumar, IMI386680 (holotype).

**Description.** See D'Souza et al. (2002) on page 141.

**Illustration.** See D'Souza et al. (2002) on page 140, Fig. 4a–d.

**Notes.** D'Souza et al. (2002) introduced *Virgatospora natarajanensis* based on the morphology and found it as a saprobe on dead leaves of *Calamus thwaitesii* from Middle Andaman Island, India. Although the DNA sequence data of *V. natarajanensis* is not available in NCBI, morphologically, it fits well within the generic concept of *Nigrosynnema* due to its synnematous conidiomata, phialidic conidiogenous cells, ellipsoidal to fusiform, aseptate conidia (amerosporous) with distinct longitudinal striations. The conidia in *V. echinofibrosa*, the type species of *Virgatospora*, are ellipsoidal to limoniform and 3-(sometimes 2- or 4)-septate (phragmosporous). Based on the morphological similarities between *V. natarajanensis* and *N. guangdongense* (the type species of *Nigrosynnema*), as well as support from molecular phylogenetic analyses of the type species of *Virgatospora*, we synonymize *V. natarajanensis* under *Nigrosynnema* as *N. natarajanensis*. However, it is desirable to obtain DNA sequence data from both the type specimen of *V. natarajanensis* and fresh collections to further support our proposal.

**_Sirastachys guangdongensis_ C.F. Liao, K.D. Hyde & Doilom, sp. nov.**
Index Fungorum: IF902009
Facesoffungi Number: FoF15747
Fig. 5

**Etymology.** The epithet "guangdongensis" refers to the locality, Guangdong Province, China, where the holotype was collected.

**Holotype.** MHZU 23-0250.

**Description.** ***Saprobic*** on dead stem of *Agave sisalana*. ***Sexual morph*:** undetermined. ***Asexual morph*:** Colonies superficial on host substrate, erect, gregarious, visible as numerous black conidial masses. ***Conidiophores*** 105–170 × 3.5–7 µm (av. 140 × 5.5 µm, n = 30), macronematous, mononematous, erect, simple, unbranched, straight or slightly flexuous, subcylindrical, hyaline, 1–5-septate, not constricted at septa, smooth-walled, or slightly verrucose, thick-walled, bearing 4–8 conidiogenous cells on the tip. ***Conidiogenous cells*** 6.5–12.5 × 4–5 µm (av. 10 × 4 µm, n = 30), enteroblastic, monophialidic, discrete, determinate, terminal, elongate doliiform to reniform, subhyaline to brown, smooth-walled, with

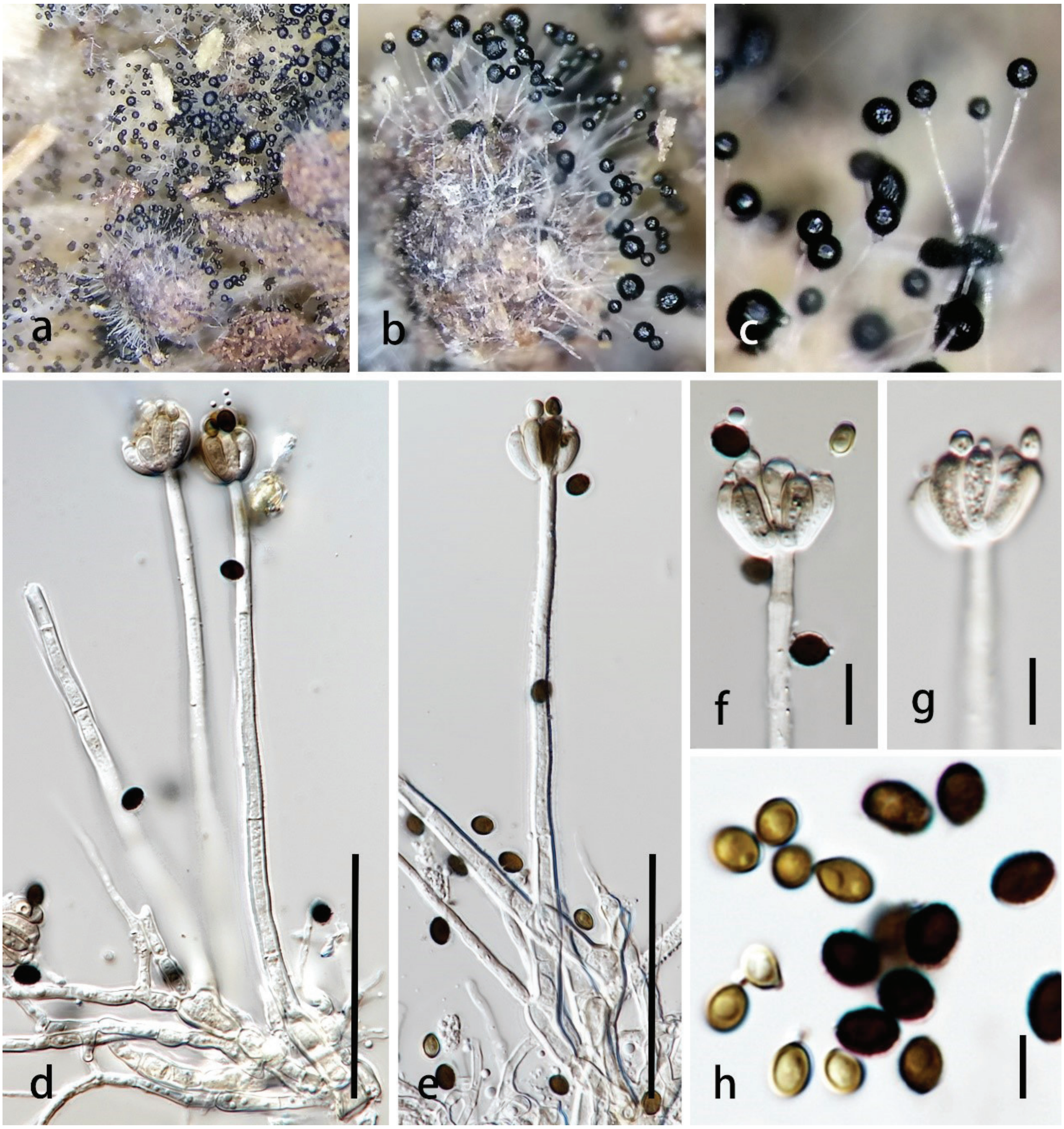

**Figure 5.** *Sirastachys guangdongensis* (MHZU 23-0250, holotype) **a–c** colonies on the surface substrate **d, e** conidiophores, conidiogenous cells with conidia **f, g** conidiogenous cells with conidia **h** conidia. Scale bars: 50 μm (**d, e**); 10 μm (**f, g**); 5 μm (**h**).

a conspicuous collarette. ***Conidia*** 5–6 × 4–5 μm (av. 5.5 × 4 μm, n = 30), acrogenous, aggregating in slimy masses, obovoid, with a prominent hilum, aseptate, brown, pale olivaceous brown, black, smooth-walled.

**Culture characteristics.** Colonies on PDA reaching 5.5–6.0 cm in two weeks at 28 ± 2 °C, medium dense, flat, circular, cream from above; pale luteous from the reverse, with no pigmentation.

**Material examined.** China • Guangdong Province, Guangzhou City, Zhongkai University of Agriculture and Engineering (23.10643°N, 113.28240°E, 20 m), on dead leaf of *Agave sisalana* Perr. ex Engelm. (Agavaceae), 17 November 2021, C.F. Liao & Y.H. Yang, JM02 (MHZU 23-0250, holotype) • ex-type, ZHKUCC 23-1003 • *ibid*., living culture ZHKUCC 23-1004.

**Notes.** *Sirastachys guangdongensis* resembles *Si*. *pandicola* and *Si*. *Phaeospora* that were described by Lombard et al. (2016). However, the former can be distinguished by the size of the conidiophores and conidia as well as other conidiophore characteristics. *Sirastachys guangdongensis* has longer conidiophores (105–170 µm) than those of *Si*. *pandicola* (55–75 µm) and *Si*. *phaeospora* (40–65 µm). *Sirastachys guangdongensis* has larger conidia (5–6 × 4–5 µm) than *Si*. *pandicola* (3–4 × 2–3 µm) and *Si*. *phaeospora* (4–5 × 2–3 µm). Conidiophores of *Si*. *guangdongensis* are 1–5-septate, while they are 1–3-septate in *Si*. *pandicola* and 1–2(–3)-septate in *Si*. *phaeospora*. Branched conidiophores are observed in *Si*. *phaeospora* (Lombard et al. 2016), while they are unbranched in *Si*. *guangdongensis*. The phylogenetic analyses supported *Si*. *guangdongensis* as a distinct species from other *Sirastachys* species and showed that *Si*. *guangdongensis* (ZHKUCC 23-1003 and ZHKUCC 23-1004) formed a distinct branch and sister to *Si*. *phaeospora* (ex-type CBS 100155) with 99% ML bootstrap support and 1.00 BYPP (Fig. 1). Based on distinct morphological and molecular evidence, we propose *Sirastachys guangdongensis* as a novel species.

### ***Stachybotrys microsporus*** **(B.L. Mathur & Sankhla) S.C. Jong & E.E. Davis [as 'microspora']**

Index Fungorum: IF627002
Facesoffungi Number: FoF09372
Fig. 6

**Description.** ***Saprobic*** on dead leaf of *Agave sisalana*. ***Sexual morph*****:** undetermined. ***Asexual morph*****:** Colonies superficial on host substrate, gregarious, visible as numerous black conidial masses. ***Conidiophores*** 35–70 × 2.5–5 µm (av. 48 × 4 µm, n = 30), macronematous, mononematous, irregularly or sympodially branched, straight or flexuous, subcylindrical, hyaline, becoming pale olivaceous brown in the above half, 1–3-septate, not constricted at the septa, smooth-walled, slightly rough-walled in the subterminal region, thick-walled, bearing 3–9 conidiogenous cells on the tip. ***Conidiogenous cells*** 6–10 × 4–6 µm (av. 8 × 5 µm, n = 30), enteroblastic, monophialidic, discrete, determinate, terminal, obovoid, sub-hyaline to pale olivaceous brown, smooth-walled. ***Conidia*** 5–7 µm diam. (av. 6 µm, n = 30), aggregating in slimy masses, globose, subglobose, aseptate, olivaceous brown to black, rough-walled, verrucose.

**Culture characteristics.** Colonies on PDA reaching 2.5–3.0 cm in two weeks at 28 ± 2 °C, medium dense, raised, flat, floccose to fluffy, velvety, irregular edge, gold brown at the center, pale brown, with conidiophores forming on the surface of the medium, carrying slimy olivaceous green from above; brown to pale luteous from the reverse. The conidia producing on PDA after three weeks: Conidiophores 30–80 × 3.5–5.5 µm (av. 46 × 4 µm, n = 30), most similar with the above description, 0–2-septate, unbranched or branched, bearing 2–10 conidiogenous cells at the tip. Conidiogenous cells 9–17 × 4.5–7.5 µm (av. 12 × 5.5 µm, n = 30), most similar to those on natural substrate. Conidia 6.5–10 × 3–6.5 µm (av. 8 × 5 µm, n = 30), often aggregated as large, slimy, glistening, blackheads,

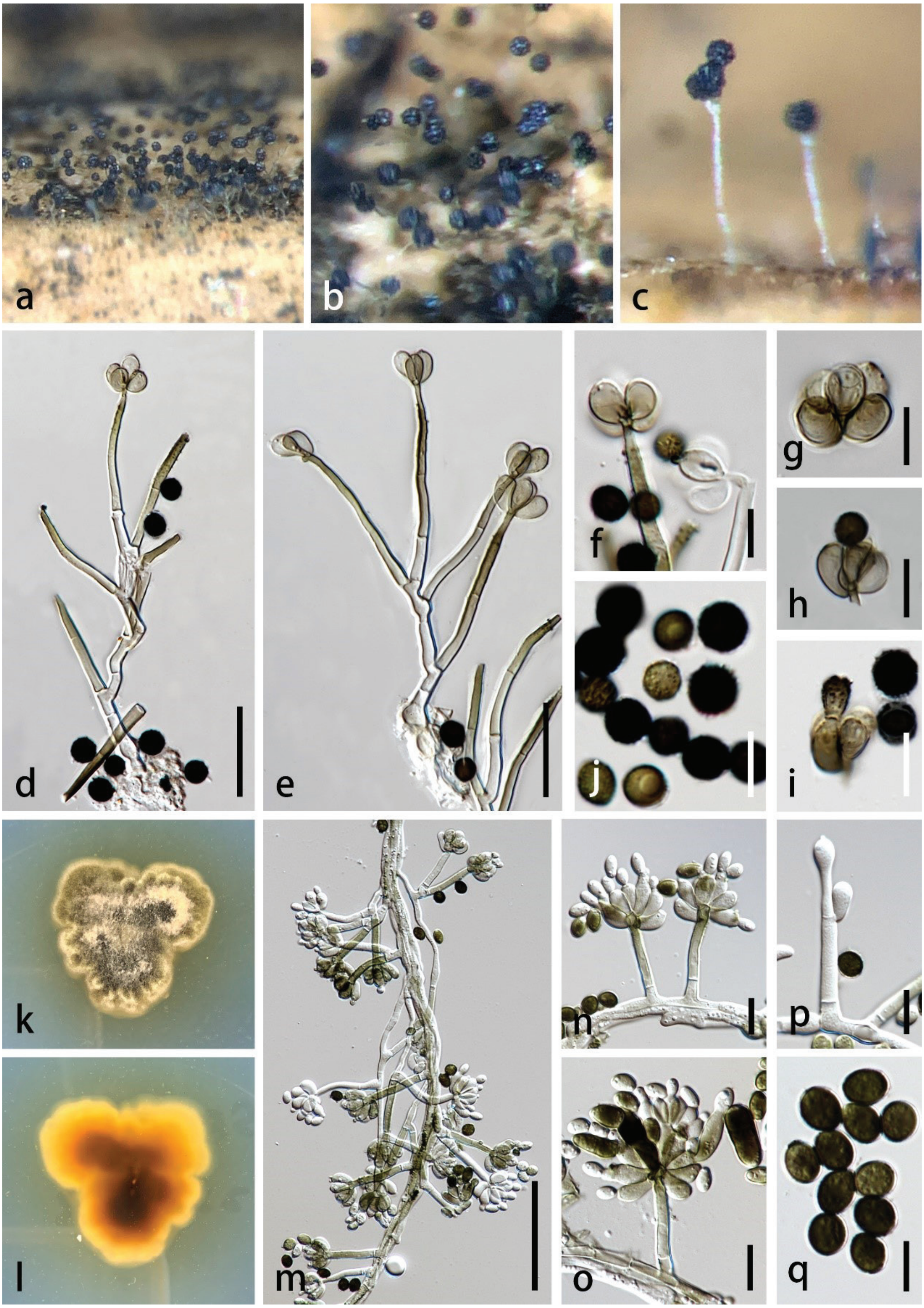

**Figure 6.** *Stachybotrys microsporus* (MHZU 23-0252, new host record) **a–c** colonies on the surface substrate **d, e** conidiophores **f–i** conidiogenous cells **j** conidia **k** colonies on pda (front) **l** colony on pda (below) **m** mycelium, conidiophores, and conidiogenous cells with conidia **n–p** conidiogenous cells with conidia **q** conidia. Scale bars: 20 µm (**d, e**); 50 µm (**f–j, m**); 10 µm (**n–q**).

initially hyaline to olivaceous green, oblong, obovoid to subglobose, becoming black-brown, globose, smooth to verruculose.

**Material examined.** China • Guangdong Province, Guangzhou City, Zhongkai University of Agriculture and Engineering (23.10643°N, 113.28240°E, 20 m), on dead leaf of *Agave sisalana* Perr. ex Engelm. (Agavaceae), 17 November 2021, C.F. Liao & Y.H. Yang, SR09A (MHZU 23-0252, new host record) • living culture, ZHKUCC 23-1007 • *ibid*., living culture ZHKUCC 23-1008.

**Known distribution.** • Canada, Cuba, India, Nigeria, and Pakistan (Ellis 1971; Jong and Davis 1976); • China (this study); Japan (Iwama et al. 2022); • New Guinea; Zaria (Jong and Davis 1976); • Thailand (Lin et al. 2016; Samarakoon et al. 2021); • Sudan (Lombard et al. 2016).

**Known hosts/substrates.** *Agave sisalana* (this study), *Arachishypogaea rhizosphere*, soil (Jong and Davis 1976), *Castanopsis cuspidata* var. sieboldii (Iwama et al. 2022), dead plants, paper, seeds, and textiles (Ellis 1971), decaying shrubs, wood (Lin et al. 2016), *Musa* sp. (Samarakoon et al. 2021), soil in *Mangifera* field (Lombard et al. 2016).

**Notes.** *Stachybotrys microsporus* (ZHKUCC 23-1007, ZHKUCC 23-1008) formed a subclade with the type and other strains of *St*. *microsporus* with 100% ML and 1.00 BYPP (Fig. 1). Our collection has similar morphs to *St*. *microsporus* described by Jong and Davis (1976), Lin et al. (2016), and Samarakoon et al. (2021) by having irregularly branched conidiophores with tapering apices, monophialidic, discrete conidiogenous cells, unicellular, globose, roughened, and black conidia. *Stachybotrys microsporus* has been reported from forest soil in New Guinea (Jong and Davis 1976), on decaying wood in Thailand (Jong and Davis 1976), and on dead leaf petiole of *Musa* sp. in Thailand (Samarakoon et al. 2021). We report *St*. *microsporus* here as a new host record on *Agave sisalana* in China.

## Discussion

Species of Stachybotryaceae have primarily been collected from soil and dead plant tissues. For example, *Albifimbria terrestris* was isolated from soil in mopane woodlands in Namibia and unidentified dead hardwood in the USA, while *Cymostachys fabispora* was obtained from decaying leaf material in Cuba and *Aloe ferox* in Tanzania (Lombard et al. 2016). In China, most taxa in this family have been reported from the soil, including *Stachybotrys pallescens*, *St*. *subcylindrospora*, *St*. *subreniformis*, and *St*. *subcylindrospora* (Jiang and Zhang 2009; Li and Jiang 2011; Jie et al. 2012). However, some species were also found in diseased plants and dead plant tissues. *Paramyrothecium roridum* (formerly known as *Myrothecium roridum*) has been identified as a pathogen causing leaf spot on *Abutilon megapotamicum* and *Zantedeschia aethiopica* plants from China (Li et al. 2014; Ben et al. 2015). *Myxospora aptrootii* was isolated from leaf litter in Hong Kong, China (Lombard et al. 2016). In this study, *Brevistachys wurfbainiae* and *Nigrosynnema guangdongense* were isolated from dead stems of *Wurfbainia villosa*, while *Sirastachys guangdongensis* and *Stachybotrys microsporus* were obtained from dead leaves of *Agave sisalana* in Guangdong, China. The present study contributes to the taxonomic and phylogenetic study of Stachybotryaceae by introducing a novel genus and two new species, along with the documentation of one newly recorded species from China.

The new genus, *Nigrosynnema*, is phylogenetically related to but distinct from *Digitiseta* and *Striaticonidium*. These taxa are classified into distinct genera based on differences in their asexual morphs, as detailed in the notes under *Nigrosynnema*. *Nigrosynnema* is also similar to *Peethambara* in having synnematous, erect conidiomata, unbranched or branched, septate, smooth conidiophores with phialidic conidiogenous cells (Subramanian and Bhat 1978). However, *Peethambara* has elongate or elongate-fusiform or broad-fusiform, hyaline, thick-walled, 1-septate conidia in green slimy masses, which are mostly widest in the middle, sometimes above or below the middle, and with smoothly rounded mamilla at the base (Subramanian and Bhat 1978; Lombard et al. 2016). Furthermore, *Nigrosynnema* can be distinguished from other asexual genera in Stachybotryaceae by its black, subcylindrical synnema that tapers towards the apex (Table 2) and the support from robust phylogeny. Additionally, our study found that *Nigrosynnema guangdongense*, the type species of this novel genus, produced synnematous conidiomata on the natural host substrates and sporodochial conidiomata on PDA. Synnemata observed on the natural substrate are erect, with a robust base, subcylindrical, narrower towards the apex of the stipe, and olivaceous brown to black in color. Synnemata appear slender, straight, or curved in the upper portion and consist of bundles of parallelly arranged, tightly compacted conidiophores. On the other hand, sporodochial conidiomata appeared on PDA, producing superficially scattered irregular structures on pulvinate conidiophores and surrounded by white mycelia and crowned by an olivaceous green mass of conidia with or without a slimy covering.

This study proposes *Sirastachys guangdongensis* as a novel species in the family Stachybotryaceae, represented by the strains ZHKUCC 23-1003 and ZHKUCC 23-1004. It is noted that Lombard et al. (2016) designated CBS 100155 as the ex-type of *Sirastachys phaeospora* and identified additional strains (CBS 136167, CBS 136185, CPC 16092, CPC 16093, and CBS 253.75) as *Si*. *phaeospora*. However, their phylogenetic analyses did not support the formation of a well-defined monophyletic lineage for these additional strains with the ex-type. Although the materials examined included CBS 136167, CBS 136185, and CPC 16092, only ex-type (CBS100155) was used to illustrate the morphological characteristics of *Si*. *phaeospora*. Therefore, it is recommended to confirm the taxonomic identification of these additional strains and determine whether they are congeneric with the ex-type of *Si*. *phaeospora*. In this study, we accepted only CBS 100155 as an authentic strain of *Si*. *phaeospora* for inclusion in our phylogenetic tree, in which our new collections (ZHKUCC 23-1003 and ZHKUCC 23-1004) formed a distinct branch sister to *Si*. *phaeospora* (ex-type CBS100155) with 99% ML bootstrap support and 1.00 BYPP (Fig. 1). The morphological characteristics observed in our collection differ from those described for *Si*. *phaeospora* based on characteristics of conidiophores, as well as the size of conidiophores and conidia, as described in the notes under *Si. guangdongensis*.

The taxonomic placement and phylogenetic relationship of many species, such as *Myrothecium atrocarneum*, *Stachybotrys asperulus*, *St*. *atrogriseus*, *St*. *atrus*, *St*. *clitoriae*, and *St*. *verrucosus* in Stachybotryaceae, remain unclear due to a lack of DNA sequence data of ex-type strains and fresh collections. Furthermore, many species in Stachybotryaceae are limited to ITS and LSU sequence data. There is a scarcity of reliable phylogenetic markers (e.g., *cmdA*, *rpb2*, *tef1*-α, and *tub2*) to identify the phylogenetic status within Stachybotryaceae accurately. Future taxonomic studies in this family should incorporate multi-locus genes, such as *cmdA*, ITS, LSU, *rpb2*, *tef1*-α, and *tub2*, along with morphological characteristics and other polyphasic approaches (e.g., physiology and secondary metabolites), while also considering hosts and their distribution to enhance our understanding in this issue (Maharachchikumbura et al. 2021). Additionally, the specimen of the type species should be revisited, and epitypification is needed to confirm their taxonomic placement.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: KWTC, WD, MD, CL. Data curation: CL. Formal analysis: MD, CL. Funding acquisition: MD. Investigation: DJB, CL, MD, KDH. Methodology: CL. Project administration: KDH. Resources: YY, MD, CL. Software: KDH, KWTC, CL. Supervision: KWTC, KT, MD. Validation: MD. Visualization: DJB, MD, CL. Writing - original draft: MD, CL. Writing - review and editing: FAO, WD, KWTC, KT, CL, MD, DJB, YY.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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Checklist

# Annotated checklist of arthropod-pathogenic species in the Entomophthoromycotina (Fungi, Zoopagomycota) in North America

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## Abstract

The subphylum Entomophthoromycotina (Phylum Zoopagomycota) includes many arthropod pathogens, some of which are renowned for their abilities to alter host behavior prior to death and cause epizootics that impact host populations. The last checklist of arthropod-pathogenic species in this group was published in 1963 and consisted of 39 species in a single genus. Since then, more species have been named, and their taxonomy has changed extensively. We have constructed an updated checklist for species of Entomophthoromycotina in North America; this checklist includes species in the continental United States, Canada, and Mexico. Data were compiled based on available published literature and metadata available from the ARSEF culture collection, adjusting names based on current taxonomy. In North America, the arthropod-pathogenic Entomophthoromycotina now include 80 species belonging to 14 genera, within two classes, plus one species in a form genus. This checklist provides a current framework for future studies of the biodiversity of this group of fungi.

**Key words:** Biodiversity, entomopathogenic fungi, Entomophthorales, mycodiversity, pathogen species list

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## Introduction

Most species in the fungal subphylum Entomophthoromycotina (Phylum Zoopagomycota; Spatafora et al. 2016) are pathogens of arthropods. Many species in this group naturally cause epizootics (Pell et al. 2001), highlighting their potential use for biological control of arthropod pests. Species in this group are also renowned for altering the behavior of infected arthropod hosts in fixed, predictable patterns (de Bekker et al. 2021), including 'summit behavior' just prior to host death (e.g., Elya et al. 2018) or producing psychoactive metabolites to stimulate continued flight by spore-ejecting infected cicada hosts (Boyce et al. 2019).

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Despite their promising roles in the control of insect pests and their fascinating biology, relatively few studies have documented the biodiversity, distribution, and ecology of entomophthoralean fungi in natural ecosystems. Most inventories of species of arthropod-pathogenic fungi in the Entomophthoromycotina originate from Europe. Twenty-six species have been listed for Spain (Niell and Santamaria 2001), 24 for Sweden (Gustafsson 1965), 12 for Norway (Klingen et al. 2002), 29 for Austria (Barta et al. 2005; Tkaczuk et al. 2011), and 35 for the United Kingdom (www.mapmate.co.uk/checklist). The much higher counts for Switzerland (95; Keller 2008; S. Keller pers. comm.) and Poland (94; Sosnowska et al. 2004; Mułenko et al. 2008; Dubiel 2016) are consistent with the suggestion that this group of fungi is most diverse in central Europe (Keller 2008). However, this statement might be biased by the prolific work of researchers specifically studying the subject in that geographical area. Outside of Europe, lists of species have also been reported for China (79; Zha et al. 2016), Israel (31; Ben-Ze'ev 1993), the Philippines (19; Villacarlos and Mejia 2004; Villacarlos 2008), Argentina (16; López Lastra et al. 2019), and Australia (10; Glare and Milner 1987), as well as in several different publications from Mexico (Remaudière and Latgé 1985; Sanchez-Peña 1990, 2000).

For North America, Roland Thaxter published a comprehensive monograph on the family Entomophthoraceae in the United States in 1888, in which he reported on 26 species (Thaxter 1888). Following this, Charles (1941) reported 36 species in this group for North America, and in 1963, Hutchison reported 39 species of entomophthoralean fungi that occurred in North, Central, and South America and provided information about hosts and geographic distributions of these species. In the 21st century, a protochecklist of all nonlichenized fungi in North America included 38 species of arthropod-pathogens in the Entomophthoromycotina (Bates and Miller 2018). The protochecklist entries were based on herbarium specimens from the US (plus its territories), Canada, and Mexico, and in many instances, the names of arthropod pathogens that were included are not the currently accepted names.

Although there has not been an up-to-date summary of arthropod pathogens in North American species after 1963, the discovery, taxonomic changes, and research since that time have been prolific. It is very clear that an updated checklist is sorely needed; for example, the most recent protochecklist for all North American fungi does not use current taxonomic genera and species designations for this group (Bates and Miller 2018), certainly because this information might not be easy to find. Therefore, our goal with this annotated checklist was to present arthropod hosts and distributions for arthropod-pathogenic species in the Entomophthoromycotina that occur in North America, using the currently accepted taxonomy. This will facilitate continued progress in this field and summarize disparate observations as a compendium.

## Materials and methods

### Study area

This checklist of arthropod-pathogenic North American species includes records from the continental United States, Canada, and Mexico.

## The data

Published records that were used to begin generating this checklist were found in Thaxter (1888), Charles (1941), Hutchison (1963), and Bałazy (1993). The catalogue of the USDA Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF 2020) was also used to search for North American records falling within the arthropod-pathogenic Entomophthoromycotina. Subsequently, search engines were used to explore records for additional North American species, using a recent worldwide list of species within the arthropod pathogens of the Entomophthoromycotina (Sacco and Hajek 2023). Personal communication records for R.A. Humber are from his notes regarding specimens in the Thaxter collection at the Farlow Herbarium, Harvard University. Throughout the checklist, only naturally occurring fungi associated with field-collected arthropod hosts were included. Samples from laboratory manipulations, often from studies of host specificity, have not been included.

Within the Entomophthoromycotina, extensive taxonomic reorganization has occurred in relatively recent years, and many generic names have changed. For example, in 1963, *Entomophthora* is given as the generic name for all 39 species in the Western Hemisphere (Hutchison 1963). However, for all except 4 of these species, the generic names have since changed. We used Humber (1989), Index Fungorum (http://www.indexfungorum.org), and MycoBank (Robert et al. 2005) to assist with all taxonomic decisions about fungi. We only include information from records with fungal identifications to the species level. For all arthropod hosts, only the currently accepted genus and species names are used.

For all records, hosts and distributions are provided with associated citations. For distributional information for North America, we generally referred to states or provinces but also provided citations for references where more specific details about collection site locations were found. For some records, only the name of the country was available. In at least one publication, the locations of species by country were not provided, and this record therefore could not be included (e.g., Remaudière et al. 1978). Two-letter, capitalized abbreviations of US states and Canadian provinces were used. For records from Mexico, we used ‘conventional’ abbreviations that are 2–4 letters long for states (https://en.wikipedia.org/wiki/Template:Mexico_State-Abbreviation_Codes). In all cases, the country name occurs alongside states or provinces. GPS coordinates for sites are not included, as for most entries, references are older, and GPS information was not available. Alternatively, descriptions of sites were too vague or broad for us to infer a GPS location. GPS information provided in more recent reports is available via the citations provided.

For information about hosts, only pathogens in the Entomophthoromycotina infecting arthropods were included (Sacco and Hajek 2023). For example, reports of vertebrate infections by *Conidiobolus coronatus* and reports of species only isolated from soil (e.g., many *Conidiobolus* spp.) were not included.

Many arthropod-pathogenic species in the Entomophthoromycotina have not been isolated into pure culture. Among those that have, numerous have been frozen and deposited in culture collections. The USDA-ARS entomopathogenic fungi collection (ARSEF) is the world’s largest repository of these isolates, and we have indicated for each species whether a culture is available in ARSEF. We have not included GenBank accession numbers for numerous reasons, including that DNA data for most of these fungi are lacking or difficult to link to specimens, as many of these are older records prior to the DNA era. We can only confidently link accessions correctly to a few known specimens, and these are scarce. For those fungal species that have been sequenced, accession numbers can be found in the references cited or in GenBank.

References are always provided, but we did not exhaustively include references; if several references report a host/fungus association from the same state/province, we only included one of such references. Summaries were cited if these existed.

## Results

Records for host species and distributions are reported below. For species with more extensive lists of hosts and distributions, detailed accounts are included in tabular form. For example, for some species reported from many states across much of North America, hosts and distribution are summarized below, but individual host species and specific collection locations are listed in tables. Species names below followed by an asterisk have arthropod pathogenic North American isolates in the ARSEF culture collection.

### Class Neozygitomycetes
### Order Neozygitales
### Family Neozygitaceae
### *Neozygites*

### [1] *Neozygites floridanus* (J. Weiser & Muma) Remaud. & S. Keller, 1980

In the US, *N. floridanus* has been reported infecting six species of mites in the family Tetranychidae (Arachnida, Trombidiformes) in the southeastern US states, as well as IA, KS, PA, and TX (US) (Lopes Ribeiro et al. 2009) (Table 1). This species is frequently referred to as *N. floridana*, which is taxonomically incorrect.

**Table 1.** Recorded arthropod hosts of *Neozygites floridanus* in the US, all in the family Tetranychidae (Arachnida, Trombidiformes).

| Host Species | Country | States | References |
|---|---|---|---|
| *Eotetranychus sexmaculatus* | US | FL | Lopes Ribeiro et al. 2009 |
| *Eutetranychus banksi* | US | FL, TX | Pickett and Gilstrap 1986;<br>Lopes Ribiero et al. 2009 |
| *Oligonychus pratensis* | US | KS | Dick and Buschman 1995 |
| *Panonychus citri* | US | FL | Lopes Ribiero et al. 2009 |
| *Tetranychus urticae* | US | AL, GA, IA, KS, MS, NC, NY, SC | Lopes Ribiero et al. 2009; ARSEF 2020 |
| *Bryobia* sp. | US | PA | C. Tkaczuk, unpubl. data |
| - | US | PA | C. Tkaczuk, unpubl. data |

### [2] *Neozygites fresenii* (Nowak.) Remaud. & S. Keller, 1980

On multiple aphid species (Hemiptera, Aphididae) from a broad distribution across the US, as well as ON (Canada) and Mexico (Fig. 1, Table 2).

**Figure 1.** Distribution of recorded occurrences of *Neozygites fresenii* in the US, Canada, and Mexico (see references in Table 2). Dark gray = locations by state in the US or province in Canada; light gray = a record from an unspecified area within Mexico.

**Table 2.** Recorded arthropod hosts of *Neozygites fresenii* in the US, Canada, and Mexico, all hosts Hemiptera.

| Host Family | Host Species | Country | States/Provinces | References |
|---|---|---|---|---|
| Aphididae | *Aphis glycines* | US | AR,NY | Nielsen and Hajek 2005; Galligan 2007 |
| | *Aphis gossypii* | US | AL, AR, GA, LA, MS, MO, NC, SC, TN, TX | Sanchez-Peña 1993; Steinkraus et al. 1996 |
| | *Aphis gossypii* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis pomi* | US | MA, ME, NC | Thaxter 1888 |
| | *Aphis solitaria* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis spiraecola* | US | FL | Charles 1941 |
| | *Capitophorus elaeagni* | Mex | - | Remaudière and Latgé 1985 |
| | *Myzus persicae* | US | FL | Charles 1941 |
| | *Periphyllus lyropictus* | US | NH | Charles 1941 |
| | *Schizaphis graminum* | US | ID | Feng et al. 1990 |
| | *Schizolachnus piniradiatae* | Can | ON | Soper and MacLeod 1963 |
| | - | US | PA | C. Tkaczuk, unpubl. data |
| Pseudococcidae | *Planococcus citri* | US | LA | Charles 1941 |

### [3] ***Neozygites fumosus*** (Speare) Remaud. & S. Keller, 1980

On the mealybug *Planococcus citri* (Hemiptera, Pseudococcidae) in FL and LA (US) (Speare 1922). This species is frequently referred to as *N. fumosa*, which is taxonomically incorrect.

### [4] ***Neozygites lageniformis*** (Thaxt.) Remaud. & S. Keller, 1980

Infecting ‘Aphides on *Betula populifolia*’ and ‘*Solidago*’ in MA, ME, and NC (US) (Thaxter 1888), and infecting *Macrosiphum euphorbiae* and *Aphis nasturtii* (Hemiptera, Aphididae) in ME (US) (Shands et al. 1972).

### [5] *Neozygites parvisporus* (D.M. MacLeod & K.P. Carl) Remaud. & S. Keller, 1980

On *Frankliniella* sp. (Thysanoptera, Thripidae) in BC (Mexico) (Remaudière and Latgé 1985). This species is frequently referred to as *N. parvispora*, which is taxonomically incorrect.

### [6] *Neozygites turbinatus* (R.G. Kenneth) Remaud. & S. Keller, 1980

On *Cinara curvipes* (Hemiptera, Aphididae, Lachninae) in Jal (Mexico) (Remaudière and Latgé 1985). This species is frequently referred to as *N. turbinata*, which is taxonomically incorrect.

## Class Entomophthoromycetes
## Order Entomophthorales
## Family Conidiobolaceae
## *Conidiobolus*

### [7] *Conidiobolus coronatus* (Constantin) A. Batko 1964*

On diverse insects, including termites (Isoptera, Kalotermitidae and Termitidae), aphids, and leafhoppers (Hemiptera), Diptera, Hymenoptera, and Thysanoptera. A truly polyphagous fungus, this species is also known to infect Collembola (Entognatha), Araneida, and Opiliones (Palpatores). It has been reported from across the US, Ver (Mexico), and AB (Canada) (Hutchison 1963; Matanmi et al. 1974; ARSEF 2020) (Table 3).

**Table 3.** Recorded arthropod hosts of *Conidiobolus coronatus* in the US, Canada, and Mexico.

| Host Class | Host Order /Suborder | Host Family | Host Species | Country | States/ Provinces | References |
|---|---|---|---|---|---|---|
| Arachnida | Araneida | - | - | Can | AB | ARSEF 2020 |
| | Opiliones/"Palpatores" | - | - | Can | AB | ARSEF 2020 |
| Entognatha | Collembola | - | - | US | NC | ARSEF 2020 |
| Insecta | Diptera | Anthomyiidae | *Delia platura* | US | WI | Matanmi et al. 1974 |
| | | | *Delia radicum* | US | WI | Matanmi et al. 1974 |
| | | Bibionidae | *Plecia nearctica* | US | FL | Kish et al. 1974 |
| | | Sciaridae | *Lycoriella ingenua* | Mex | Ver | ARSEF 2020 |
| | Hemiptera | Aphididae | *Aulacorthum solani* | US | ME | Harris 1948 |
| | | | *Macrosiphum euphorbiae* | US | ME | Harris 1948 |
| | | | *Metopolophium dirhodum* | US | ID | ARSEF 2020 |
| | | | *Myzus persicae* | US | ME | Harris 1948 |
| | | Cercopidae | *Aeneolamia albofasciata* | Mex | Oax | Remaudiére and Latgé 1985 |
| | | | *Aeneolamia contigua* | Mex | Tamps | Guzmán and Alcocer-Gómez 1972 |
| | | Cicadellidae | *Empoasca fabae* | US | NY | ARSEF 2020 |
| | Hymenoptera | Formicidae | - | Can | AB | ARSEF 2020 |
| | Isoptera | Kalotermitidae | - | US | CA, LA | Hutchison 1963 |
| | | Termitidae | - | US | CA, LA | Hutchison 1963 |
| | Orthoptera | Acrididae | - | Mex | - | Remaudiére and Latgé 1985 |
| | Thysanoptera | Thripidae | - | US | VT | ARSEF 2020 |
| | | | *Frankliniella occidentalis* | US | FL | ARSEF 2020 |

### Family Neoconidiobolaceae
### *Neoconidiobolus*

#### [8] *Neoconidiobolus thromboides* (Drechsler) B. Huang & Y. Nie, 2020*

Well known as an aphid (Hemiptera, Aphididae) pathogen: on numerous aphid hosts across the US and in AB, ON, and QC (Canada). It has also been reported from another hemipteran, the leafhopper *Empoasca fabae* in NY (US) (Hemiptera, Cicadellidae), a heleomyzid fly in ME (US) (Diptera, Heliomyzidae), and an acridid in MT (US) (Orthoptera, Acrididae) (ARSEF 2020; Castrillo and Harris-Shultz 2024). *Delia radicum* and *Delia platura* (Diptera, Anthomyiidae) were also reported infected in WI (US) (Matanmi et al. 1974) (Table 4).

**Table 4.** Recorded arthropod hosts of *Neoconidiobolus thromboides* in the US and Canada.

| Host Order | Host Family | Host Species | Country | States/ Provinces | References |
|---|---|---|---|---|---|
| Diptera | Anthomyiidae | *Delia platura* | US | WI | Matanmi et al. 1974 |
| | | *Delia radicum* | US | WI | Matanmi et al. 1974 |
| | Heliomyzidae | - | US | ME | ARSEF 2020 |
| Hemiptera | Aphididae | *Aphis glycines* | US | MN, NY | Nielsen and Hajek 2005 |
| | | *Diuraphis tritici* | US | MT | Feng et al. 1991 |
| | | *Macrosiphum euphorbiae* | US | ME | ARSEF 2020 |
| | | *Melanaphis sacchari* | US | GA | Castrillo and Shultz 2024 |
| | | *Metopolophium dirhodum* | US | ID | ARSEF 2020 |
| | | *Myzus persicae* | US | FL, ME, WI | ARSEF 2020 |
| | | *Schizaphis graminum* | Can | AB | ARSEF 2020 |
| | | *Sitobion avenae* | US | ID | ARSEF 2020 |
| | | *Therioaphis maculata* | US | CA | ARSEF 2020 |
| | | *Uroleucon* sp. | Can | QC | ARSEF 2020 |
| | Cicadellidae | *Empoasca fabae* | US | NY | ARSEF 2020 |
| Orthoptera | Acrididae | - | US | MT | ARSEF 2020 |

### Family Batkoaceae
### *Batkoa*

#### [9] *Batkoa apiculata* (Thaxt.) Humber, 1989*

Broad host range including four families of Lepidoptera (Erebidae, Noctuidae, Tortricidae, Geometridae), numerous aphid species (Hemiptera, Aphididae), leafhoppers (Hemiptera, Cicadellidae), a spittlebug (Hemiptera, Cercopidae), Scirtidae and Helotidae (Coleoptera), and nematocerans (Diptera). Collected across the northern US, as far south in the US as TN and NC but also in SLP (Mexico) (Thaxter 1888; Hutchison 1963; ARSEF 2020) (Table 5).

#### [10] *Batkoa major* (Thaxt.) Humber, 1989*

Initially, described from Ptilodactylidae (Coleoptera) in NC (US) by Thaxter (1888). Epizootics occurred in *Lycorma delicatula*, an invasive species in the Fulgoridae (Hemiptera) in PA (US) in 2018 (Clifton et al. 2019). Following field surveys of host range in PA and NY (US), confirmed infections were observed in 3 families of Coleoptera, 8 families of Diptera, 5 families of Hemiptera, 6 families of Lepidoptera, and 1 family of Psocomorpha (Gryganskyi et al. 2022a) (Table 6).

**Table 5.** Recorded arthropod hosts of *Batkoa apiculata* in the US and Mexico.

| Host Order | Host Family/Suborder | Host Species/Subfamily | Country | States | References |
|---|---|---|---|---|---|
| Coleoptera | Cantharidae | - | Mex | Oax | Remaudière and Latgé 1985 |
| | Scirtidae | - | US | TN | Hutchison 1963 |
| Diptera | Nematocera | - | US | ME, NC | Thaxter 1888 |
| | | - | Mex | Tamps | Remaudière and Latgé 1985 |
| Hemiptera | Aphididae | *Acyrthosiphon pisum* | US | NY | ARSEF 2020 |
| | Aphididae | *Macrosiphum euphorbiae* | US | ME | ARSEF 2020 |
| | Aphididae | *Myzus persicae* | US | ME | ARSEF 2020 |
| | Aphididae | *Rhopalosiphum maidis* | US | MT | ARSEF 2020 |
| | Aphididae | *Rhopalosiphum padi* | US | CO | ARSEF 2020 |
| | Cercopidae | *Prosapia simulans* | Mex | - | Remaudière and Latgé 1985 |
| | Cicadellidae | - | US | NH, NY | ARSEF 2020 |
| | Cicadellidae | *Typhlocyba* sp. | US | ME, NC | Thaxter 1888 |
| Lepidoptera | Erebidae | *Hyphantria cunea* | US | ME, NC | Thaxter 1888 |
| | Erebidae | listed as 'Deltoid sp.' | US | ME, NC | Thaxter 1888 |
| | Geometridae | *Petrophora* sp. | US | ME, NC | Thaxter 1888 |
| | Tortricidae | *Tortrix* sp. | US | ME, NC | Thaxter 1888 |

**Table 6.** Recorded arthropod hosts of *Batkoa major* in the US and Mexico.

| Host Order | Host Family | Host Species | Country | States | References |
|---|---|---|---|---|---|
| Coleoptera | Cantharidae | *Rhagonycha fraxini* | US | NY | Gryganskyi et al. 2022a |
| | | *Rhagonycha* sp. | US | NY | Gryganskyi et al. 2022a |
| | Elateridae | *Athous brightwelli* | US | NY | Gryganskyi et al. 2022a |
| | Ptilodactylidae | *Ptilodactyla serricollis* | US | NC | Thaxter 1888 |
| | Tenebrionidae | *Isomira sericea* | US | NY | Gryganskyi et al. 2022a |
| Diptera | Anthomyiidae | - | US | NY | Gryganskyi et al. 2022a |
| | Dolichopodidae | *Gymnopterus* sp. | US | NY | Gryganskyi et al. 2022a |
| | | *Medetera* sp. | US | PA | Gryganskyi et al. 2022a |
| | | *Thrypticus* sp. | US | NY | Gryganskyi et al. 2022a |
| | Drosophilidae | *Drosophila suzukii* | US | TN | ARSEF 2020 |
| | Heleomyzidae | *Tephrochlamys rufiventris* | US | NY | Gryganskyi et al. 2022a |
| | Lauxaniidae | *Homoneura inserta* | US | NY, PA | Gryganskyi et al. 2022a |
| | Milichiidae | *Madiza glabra* | US | PA | Gryganskyi et al. 2022a |
| | Psychodidae | - | US | NY | Gryganskyi et al. 2022a |
| | Rhagionidae | - | US | NY | Gryganskyi et al. 2022a |
| | | *Symphoromyia* sp. | US | NY | Gryganskyi et al. 2022a |
| | Sciaridae | - | US | NY, PA | Gryganskyi et al. 2022a |
| Hemiptera | Achilidae | - | US | NY | Gryganskyi et al. 2022a |
| | Aphididae | *Macrosiphum euphorbiae* | US | ME | ARSEF 2020 |
| | Cercopidae | *Prosapia simulans* | Mex | Chis, Tamps | Remaudiére and Latgé 1985 |
| | Cicadellidae | - | US | NY | Gryganskyi et al. 2022a |
| | | *Empoasca fabae* | US | NY | ARSEF 2020 |
| | Cixiidae | *Cixius* sp. | US | NY | Gryganskyi et al. 2022a |
| | Derbidae | *Apache degeeri* | US | NY | Gryganskyi et al. 2022a |
| | Fulgoridae | *Lycorma delicatula* | US | PA | Gryganskyi et al. 2022a |
| Lepidoptera | Blastobasidae | - | US | NY | Gryganskyi et al. 2022a |
| | Crambidae | *Eudonia* sp. | US | NY | Gryganskyi et al. 2022a |
| | Erebidae | *Lophocampa caryae* | US | NY | Gryganskyi et al. 2022a |
| | | *Lymantria dispar* | US | NY | Gryganskyi et al. 2022a |
| | Geometridae | *Lambdina fiscellaria* | US | NY | Gryganskyi et al. 2022a |
| | Oecophoridae | *Fabiola edithella* | US | NY | Gryganskyi et al. 2022a |
| | Tineidae | *Dryadaula* sp. | US | NY | Gryganskyi et al. 2022a |
| Psocoptera | Amphipsocidae | *Polypsocus corruptus* | US | NY | Gryganskyi et al. 2022a |
| | Psocomorpha | - | US | PA | Gryganskyi et al. 2022a |

### [11] *Batkoa obscura* (I.M. Hall & P.H. Dunn) Gryganskyi, 2022*

On numerous species of aphids in CA, NY, ID, MT, and ME (US) and QC (Canada) (Table 7).

**Table 7.** Recorded arthropod hosts of *Batkoa obscura* in the US, Canada, and Mexico, all in the family Aphididae (Hemiptera).

| Host Species | Country | States/ Provinces | References |
|---|---|---|---|
| *Acyrthosiphon pisum* | US | NY | ARSEF 2020 |
| *Aphis nasturtii* | US | ME | Shands et al. 1972 |
| *Aphis* sp. | Can | QC | ARSEF 2020 |
| *Aulacorthum solani* | US | ME | Shands et al. 1972 |
| *Capitophorus elaeagni* | Mex | - | Remaudiére and Latgé 1985 |
| *Capitophorus shepherdiae* | Mex | - | Remaudiére and Latgé 1985 |
| *Capitophorus xanthii* | Mex | - | Remaudiére and Latgé 1985 |
| *Hyperomyzus lactucae* | Mex | - | Remaudiére and Latgé 1985 |
| *Macrosiphum euphorbiae* | US | ME | Shands et al. 1962 |
| *Macrosiphum* spp. | Mex | - | Remaudiére and Latgé 1985 |
| *Metopolophium dirhodum* | US | MT | Feng et al. 1991 |
| *Microparsus* (*Picturaphis*) sp. | Mex | - | Remaudiére and Latgé 1985 |
| *Myzus persicae* | US | ME | Shands et al. 1962 |
| *Myzus persicae* | Mex | - | Remaudiére and Latgé 1985 |
| *Rhopalosiphum maidis* | US | MT | Feng et al. 1991 |
| *Rhopalosiphum maidis* | Mex | - | Remaudiére and Latgé 1985 |
| *Rhopalosiphum padi* | Can | QC | ARSEF 2020 |
| *Rhopalosiphum padi* | Mex | - | Remaudiére and Latgé 1985 |
| *Rhopalosiphum* sp. | Mex | - | Remaudiére and Latgé 1985 |
| *Therioaphis maculata* | US | CA | ARSEF 2020 |
| *Uroleucon ambrosiae* | Mex | - | Remaudiére and Latgé 1985 |
| *Uroleucon sonchi* | Mex | - | Remaudiére and Latgé 1985 |
| *Uroleucon* sp. | Can | QC | ARSEF 2020 |
| *Uroleucon* sp. | Mex | - | Remaudiére and Latgé 1985 |

### [12] *Batkoa papillata* (Thaxt.) Humber, 1989

On ‘several minute gnats’ (Diptera, Nematocera) from NH and NC (US). In North America, known only from these initial collections by Thaxter (1888).

## Family Entomophthoraceae

## Subfamily Entomophthoroideae

## *Arthrophaga*

### [13] *Arthrophaga myriapodina* K.T. Hodge & A.E. Hajek, 2017

Reported from three species of millipedes (Myriapodina, Polydesmidae): *Apheloria virginiensis corrugata*, *Nannaria* sp., and *Boraria infesta* in MA, MD, NC, NY, PA, and VA, Washington DC (US), and southern ON (Canada) (Hodge et al. 2017).

### *Entomophaga*

#### [14] *Entomophaga aulicae* (E. Reichardt) Humber, 1984 (*aulicae* species complex)*

*Entomophaga aulicae* is a complex of morphologically identical species infecting only Lepidoptera, within which only *E. maimaiga* has been named as a separate species (treated below). Aside from *E. maimaiga*, fungal populations belonging to the *E. aulicae* complex infect lepidopteran species in the Noctuidae, Erebidae, Geometridae, Tortricidae, Lasiocampidae, Notodontidae, Sphingidae, and Saturniidae families from around the US and Canada (FPMI 1990; Walsh 1996; Speare and Colley 1912; ARSEF 2020). An invasive species in the Hesperiidae (Lepidoptera) in QC (Canada) was also infected (McNeil and MacLeod 1982). Within the complex, genetic diversity has been detected, with two groups occurring in North America. Pathotype I includes hosts in the Geometridae, Tortricidae, Notodontidae, and Saturniidae from BC, ON, and NF (Canada) and ME, NY, and VT (US). Pathotype II includes species in the Noctuidae and Erebidae in GA, CA (US), and ON (Canada) (Hajek et al. 1996b; Walsh et al. 1990; Walsh 1996) (Table 8).

**Table 8.** Recorded arthropod hosts of *Entomophaga aulicae* in the US, Canada, and Mexico, with all hosts in the order Lepidoptera.

| Host Family | Host species | Country | States/ Provinces | References | Group |
|---|---|---|---|---|---|
| Erebidae | *Catocala* sp. | US | CT | Speare and Colley 1912 | - |
| | *Estigmene acrea* | US | CT | Speare and Colley 1912 | - |
| | *Estigmene acrea* | Mex | Son | Young and Sifuentes 1959 | - |
| | *Euchaetes egle* | US | - | Speare and Colley 1912 | - |
| | *Euproctis chrysorrhoea* | US | MA, ME | Kirkland 1906; Boyd et al. 2021 | - |
| | *Hypena scabra* | US | SC | Kalkar and Carner 2005 | - |
| | *Hyphantria cunea* | Can | ON | Walsh et al. 1990 | II |
| | *Orgyia antiqua nova* | Can | NF | Thaxter 1888 | - |
| | *Orgyia leucostigma* | Can | NS | van Frankenhuyzen et al. 2002 | - |
| | *Orgyia vetusta* | US | CA | Hajek et al. 1996b | II |
| | *Pyrrharctia isabella* | US | ME | Hitchings 1908 | - |
| | *Spilosoma virginica* | US | OH, TX | Webster 1894; Mitchell 1919 | - |
| Geometridae | *Epirrita autumnata* | Can | BC | FPMI 1990 | - |
| | *Lambdina fiscellaria* | Can | BC, NF | Walsh et al. 1990; Hajek et al. 1996b | I |
| | *Nepytia freemani* | Can | BC | FPMI 1990 | - |
| | *Rheumaptera hastata* | Can | ON | Walsh et al. 1990 | I |
| | *Sabulodes griseata* | Can | BC | ARSEF 2020 | - |
| Lasiocampidae | *Malacosoma americanum* | US | CT | Speare and Colley 1912 | - |
| | *Malacosoma disstria* | US | NY | ARSEF 2020 | - |
| Noctuidae | *Agrotis* sp. | US | CT | Thaxter 1891 | - |
| | *Amphipyra pyramidoides* | US | - | Speare and Colley 1912 | - |
| | *Helicoverpa zea* | US | GA | Hamm 1980 | - |
| | ?*Heliothis* sp. | US | GA | Walsh et al. 1990 | II |
| | *Heliothis virescens* | US | GA | Hamm 1980 | - |
| | *Lithophane* sp. | US | CT | Thaxter 1891 | - |
| | *Mamestra* sp. | US | CT | Thaxter 1891 | - |
| | *Mythimna* sp. | Can | ON | ARSEF 2020 | - |
| | *Spodoptera frugiperda* | US | GA | Hamm 1980 | - |
| Nolidae | *Nola cereella* | US | GA | Hamm 1980 | - |

| Host Family | Host species | Country | States/ Provinces | References | Group |
|---|---|---|---|---|---|
| Notodontidae | *Cecrita biundata* | Can | ON | ARSEF 2020 | |
| | *Cecrita guttivitta* | US | NY, VT | Hajek et al. 1991; 1996b | I |
| | *Ellida caniplaga* | Can | ON | ARSEF 2020 | - |
| Saturniidae | *Dryocampa rubicunda* | Can | ON | Walsh et al. 1990 | I |
| Sphingidae | - | US | ME | FPMI 1990 | - |
| | *Manduca quinquemaculata* | US | CT | Thaxter 1891 | - |
| | *Manduca sexta* | US | CT | Thaxter 1891 | - |
| | *Pachysphinx modesta* | US | ME | Farlow Herbarium unpubl. data | - |
| | *Eumorpha fasciata* | US | FL | ARSEF 2020 | - |
| Tortricidae | *Choristoneura fumiferana* | US | ME | Walsh et al. 1990 | |
| | *Choristoneura fumiferana* | Can | NF, ON | Walsh et al. 1990 | I |
| | *Choristoneura occidentalis* | Can | BC | Hajek et al. 1996b | I |

### [15] *Entomophaga batkoi* (Bałazy) S. Keller, 1888

On a species in the Phalangidae in ME (US) (R.A. Humber pers. comm.).

### [16] *Entomophaga calopteni* (Bessey) Humber, 1989 (*E. grylli* species complex)*

A member of the *Entomophaga grylli* species complex, also known as Pathotype 2. Infects species in the Acrididae, with most infections in the subfamily Melanoplinae, although lower levels of infection have been found in the subfamilies Oedopodinae and Gomphocerinae. Reported from AZ, IA, KS, MT, ND, SD, and WY (US), AB and SK (Canada), and NL and Coah (Mexico) (Thaxter 1888; Soper et al. 1983; Erlandson et al. 1988; Bidochka et al. 1995, 1996; Casique-Valdes et al. 2012, 2022; ARSEF 2020).

### [17] *Entomophaga grylli* Pathotype I (Fresen.) A. Batko, 1964 (*E. grylli* species complex)*

A member of the *Entomophaga grylli* species complex, also known as Pathotype 1 or *E. macleodii* (an unpublished name). Principally infects grasshopper species in the subfamily Oedopodinae (Acrididae), although also known to infect Gomphocerinae and occasionally Melanoplinae. Reported from AZ, ND, NY, MT, and OR (US), and AB, ON, and SK (Canada), and Coah (Mexico) (Bidochka et al. 1996; Casique-Valdes et al. 2012, 2022; Kistner and Belovsky 2013; ARSEF 2020).

### [18] *Entomophaga kansana* (J.A. Hutchison) A. Batko, 1964

Reported from Calliphoridae, Sarcophagidae, Muscidae, and Tachinidae (Diptera) near Lawrence, KS (US) (Hutchison 1962).

### [19] *Entomophaga maimaiga* Humber, Shimazu & R.S. Soper, 1988 (*aulicae* species complex)*

*Entomophaga maimaiga* is a North Asian species only infecting Lepidoptera, accidentally introduced to North America, probably from Japan, at some time after 1971, but before 1989 (Weseloh 1998; Nielsen et al. 2005). Principal hosts are larvae of *Lymantria dispar* (Lepidoptera, Erebidae). Surveys of naturally occurring host specificity documented low levels of infections in 3 of 7 lymantriines (Hajek et al. 2004). Rare infections also occurred in *Catocala ilia* (Erebidae) and *Malacosoma disstria* (Lasiocampidae) (Hajek et al. 1996a), *Agrochola bicolorago* (Noctuidae); an unidentified gelechiid (Lepidoptera) was also infected (Hajek et al. 2000). *E. maimaiga* was discovered in 1989 in seven northeastern US states (Hajek et al. 1990) and has since spread (naturally and with human assistance) into a total of 19 US states (Hajek et al. 2021). *E. maimaiga* has also been reported from ON and QC (Canada) (Nealis et al. 1999, S. Picq pers. comm.) (Fig. 2).

**Figure 2.** Distribution of recorded occurrences of *Entomophaga maimaiga* in the US and Canada, by state and province (Hajek et al. 2021; S. Picq pers. comm.).

### [20] *Entomophaga tabanivora* (J.F. Anderson & Magnar.) Humber, 1989

On *Tabanus nigrovittatus* and *Atylotus thoracicus* (Diptera, Tabanidae) in MA and NY (US) (Anderson and Magnarelli 1979; Mullens et al. 1983).

### [21] *Entomophaga tenthredinis* (Fresen.) A. Batko, 1964

On sawfly larvae (Hymenoptera, Tenthredinidae), including *Pristiphora erichsonii* in ON (Canada), larvae of an unidentified tenthredinid species in ME (US), and the introduced pine sawfly, *Diprion similis*, in WI (US) (Thaxter 1888; Klein and Coppel 1973; FPMI 1990).

## *Entomophthora*

### [22] *Entomophthora chromaphidis* O.F. Burger & Swain, 1918

An aphid pathogen (Hemiptera, Aphididae) on *Chromaphis juglandicola* in CA (Burger and Swain 1918), the cereal aphids *Metopolophium dirhodum* in ID, WA (US) and *Sitobion avenae* in ID (US) (Humber and Feng 1991), *Myzus per-*

*sicae* in ID (Kish et al. 1994), and *Aphis glycines* in NY (US) (Nielsen and Hajek 2005). Also reported on *Psocus* sp. (Psocodea, Psocidae) in CA (US) (Burger and Swain 1918). At least some of the entries under *E. planchoniana* (Table 9) could instead be *E. chromaphidis* (see Humber and Feng 1991; Barta and Cagáň 2006); further study is necessary.

### [23] *Entomophthora culicis* (A. Braun) Fresen., 1858

Reported by Thaxter (1888) on Diptera: "*Culex* [Culicidae], and numerous genera of minute flies or gnats" in MA, ME, and NH. On unidentified midges (Chironomidae) in NY (US) (Kramer 1981b) and unidentified black flies (Simuliidae) in AB (Canada) (Shemanchuk and Humber 1978).

### [24] *Entomophthora erupta* (Dustan) I.M. Hall, 1959

On Miridae (Hemiptera): *Neolygus communis*, *Adelphocoris lineolatus*, *Irbisia solani*, *Lygocoris pabulinus*, and *Plagiognathus* sp. in NS (Canada) (Dustan 1924), and *I. solani* in CA (US) (Hall 1959). Also reported from *A. lineolatus* from NY (US) (Wheeler 1972).

### [25] *Entomophthora muscae* (Cohn) Fresen., 1856*

*Entomophthora muscae* is a species complex of morphologically similar species infecting Diptera, including from 4 to 8 described species (Elya and De Fine Licht 2021). Three species within the complex occur in North America: *E. muscae sensu stricto* (s.s), *E. scatophagae* (treated separately), and *E. schizophorae* (treated separately). *Entomophthora muscae* s.s infects *Musca domestica* (Muscidae) in CA, NY, NC, and NE, and probably across most of the US and Canada, and in Coah (Mexico) (Sanchez-Peña unpubl. observations). Also on *Coenosia tigrina* (Muscidae) and *Delia radicum* (Anthomyiidae) in NC (US) (Gryganskyi et al. 2013), and six species of *Drosophila* (Drosophilidae) in CA (US): *Drosophila melanogaster*, *D. simulans*, *D. hydei*, *D. immigrans*, *D. pseudoobscura*, *D. repleta* (Elya et al. 2018). Further studies of hosts and distributions of the different members of this species complex in North America are necessary.

### [26] *Entomophthora planchoniana* Cornu, 1873

On diverse species of aphids (Aphididae) across the US and Mexico (Table 9). However, at least some of the entries under *E. planchoniana* in Table 9 could instead be *E. chromaphidis* (see Humber and Feng 1991; Barta and Cagáň 2006); further study is necessary.

**Table 9.** Recorded arthropod hosts of *Entomophthora planchoniana* in the US and Mexico, with all hosts in the family Aphididae (Hemiptera).

| Host Species | Country | States | References |
|---|---|---|---|
| *Acyrthosiphon malvae* | Mex | - | Remaudière and Latgé 1985 |
| *Acyrthosiphon pisum* | Mex | - | Remaudière and Latgé 1985 |
| *Aphis asclepiadis* | Mex | - | Remaudière and Latgé 1985 |
| *Aphis coreopsidis* | Mex | - | Remaudière and Latgé 1985 |

| Host Species | Country | States | References |
|---|---|---|---|
| *Aphis fabae* | Mex | - | Remaudière and Latgé 1985 |
| *Aphis gossypii* | Mex | - | Remaudière and Latgé 1985 |
| *Aphis lugentis* | Mex | - | Remaudière and Latgé 1985 |
| *Aphis nasturtii* | US | ME | Shands et al. 1972 |
| *Aphis solitaria* | Mex | - | Remaudière and Latgé 1985 |
| *Aphis spiraecola* | Mex | - | Remaudière and Latgé 1985 |
| *Aulacorthum solani* | US | ME | Shands et al. 1972 |
| *Brevicoryne brassicae* | Mex | - | Remaudière and Latgé 1985 |
| *Capitophorus elaeagni* | Mex | - | Remaudière and Latgé 1985 |
| *Capitophorus shepherdiae* | Mex | - | Remaudière and Latgé 1985 |
| *Capitophorus xanthii* | Mex | - | Remaudière and Latgé 1985 |
| *Chaetosiphon fragaefolii* | US | CA | Dara 2017 |
| *Cryptomyzus galeopsidis* | US | ME | Shands et al. 1962 |
| *Hayhurstia atriplicis* | Mex | - | Remaudière and Latgé 1985 |
| *Hyperomyzus* sp. | Mex | - | Remaudière and Latgé 1985 |
| *Latgerina orizabaensis* | Mex | - | Remaudière and Latgé 1985 |
| *Macrosiphum euphorbiae* | US | ME | Shands et al. 1962 |
| *Macrosiphum* spp. | Mex | - | Remaudière and Latgé 1985 |
| *Melanocallis caryaefoliae* | US | GA | Ekbom and Pickering 1990 |
| *Metopolophium dirhodium* | US | ID | Feng et al. 1991 |
| *Metopolophium dirhodium* | Mex | BC | Remaudière and Latgé 1985 |
| *Monellia caryella* | US | GA | Ekbom and Pickering 1990 |
| *Monelliopsis pecanis* | US | GA | Ekbom and Pickering 1990 |
| *Myzus ornatus* | Mex | - | Remaudière and Latgé 1985 |
| *Myzus persicae* | US | ME | Shands et al. 1962 |
| *Myzus persicae* | Mex | - | Remaudière and Latgé 1985 |
| *Obtusicauda* sp. | Mex | BC | Remaudière and Latgé 1985 |
| *Rhopalosiphum maidis* | US | ID | Feng et al. 1991 |
| *Rhopalosiphum maidis* | Mex | Coah | Sanchez-Peña 2000 |
| *Rhopalosiphum padi* | Mex | BC | Remaudière and Latgé 1985 |
| *Rhopalosiphum padi* | Mex | - | Remaudière and Latgé 1985 |
| *Rhopalosiphum* sp. | Mex | - | Remaudière and Latgé 1985 |
| *Sitobion avenae* | US | ME | Shands et al. 1962 |
| *Uroleucon ambrosiae* | Mex | - | Remaudière and Latgé 1985 |
| *Uroleucon sonchi* | Mex | - | Remaudière and Latgé 1985 |
| *Uroleucon* sp. | Mex | - | Remaudière and Latgé 1985 |
| *Utamphorophora crataegi* | Mex | - | Remaudière and Latgé 1985 |

### [27] *Entomophthora scatophagae* Giard, 1888

A member of the *E. muscae* species complex (Jensen et al. 2006). On *Scatophaga stercoraria* (Diptera, Anthomyiidae) in NY (Steinkraus and Kramer 1988).

### [28] *Entomophthora schizophorae* S. Keller & Wilding, 1988

A member of the *E. muscae* species complex, infecting only Diptera (Elya and De Fine Licht 2021). Isolates from NE and NY (US) infect *Pollenia rudis* (Diptera, Polleniidae), and both also infect *Musca domestica*, although at lower prevalence (Steinkraus et al. 1993a; Watson et al. 1993; Six and Mullens 1996). Also, infects *Hylemya* sp. (Anthomyiidae) in PA (US) (C. Tkaczuk unpubl. data).

## *Eryniopsis*

### [29] *Eryniopsis caroliniana* (Thaxter) Humber, 1984

On *Tipula* sp. (Diptera, Tipulidae) in NC (US) (Thaxter 1888).

### [30] *Eryniopsis lampyridarum* (Thaxter) Humber, 1984

On *Chauliognathus pensylvanicus* and *Chauliognathus marginatus* (Coleoptera, Cantharidae) in AR, MD, KS, NC, PA, SC, and VA (US) (Thaxter 1888; Carner 1980; Steinkraus et al. 2017) (Fig. 3). Also, on *Chauliognathus* sp. in Coah (Mexico) (RI Torres-Acosta & S. Sanchez-Peña, unpubl. observation: https://www.youtube.com/watch?v=NRBO7zc1J-o).

**Figure 3.** Distribution of recorded occurrences of *Eryniopsis lampyridarum* in the US and Mexico, by state (see references in the text).

## *Massospora*

### [31] *Massospora cicadina* Peck, 1878*

On species of the genus *Magicicada* (Hemiptera, Cicadidae) in the eastern US (Macias et al. 2020).

### [32] *Massospora diceroproctae* R.S. Soper, 1974

On *Diceroprocta delicata*, *Diceroprocta cinctifera*, *Diceroprocta vitripennis*, and *Diceroprocta biconica* (Hemiptera, Cicadidae) in TX (and possibly LA, FL) (US) (Macias et al. 2020).

### [33] *Massospora fidicinae* R.S. Soper, 1974

On *Fidicina* sp. (Hemiptera, Cicadidae) in Chis (Mexico) (Macias et al. 2020).

### [34] *Massospora levispora* R.S. Soper, 1963

On *Okanagana rimosa* and *Okanagana sperata* (Hemiptera, Cicadidae) in CA (US) and ON (Canada) and *Platypedia putnami* (Hemiptera, Cicadidae) in CA, NM, and UT (US) (Macias et al. 2020).

### [35] *Massospora spinosa* Cif., A.A. Machado & Vittal, 1956

On *Quesada gigas* (Hemiptera, Cicadidae) in NL (Mexico) (Macias et al. 2020).

### *Orthomyces*

### [36] *Orthomyces aleyrodis* Steinkr., Humber & Oliv., 1998

On *Trialeurodes abutiloneus* (Hemiptera, Aleyrodidae) in AL (US) (Steinkraus et al. 1998).

## Subfamily Erynioideae

### *Erynia*

### [37] *Erynia aquatica* (J.F. Anderson & Ringo ex J.F. Anderson & Anagnost.) Humber, 1981*

On larvae and pupae of Culicidae (Diptera) in the US: *Aedes canadensis* (CT, RI), *Culiseta morsitans* (CT), *Aedes stimulans* (NY), *Aedes fitchii* (NY), *Aedes cantator* (CT), *Aedes* sp. (NY) (Anderson and Ringo 1969; Molloy and Wraight 1982; Andreadis and Magnarelli 1983; Steinkraus and Kramer 1989; Christie 1996; ARSEF 2020).

### [38] *Erynia conica* (Nowak.) Remaud. & Hennebert, 1980*

On Chironomidae, Chaoboridae, Simuliidae, and Tipulidae (Diptera) in NC, NH, and NY (US) (Thaxter 1888; Cuebas-Incle 1992; ARSEF 2020). On Simuliidae (Diptera) in QC (Canada): *Simulium venustum* complex, *Simulium verecundum/ rostratum*, *Simulium vittatum* complex (Nadeau et al. 1994).

### [39] *Erynia curvispora* (Nowak.) Remaud. & Hennebert, 1980*

Reported from adult *Simulium decorum* (Diptera, Simuliidae) in NY (US) and QC (Canada) (Kramer 1983; Nadeau et al. 1994), *Aedes triseriatus* (Diptera, Culicidae) in ME (US), and unidentified *Simulium* in NY (US) and QC (Canada) and Trichoptera in QC (Canada) (ARSEF 2020).

### [40] *Erynia gracilis* (Thaxter) Remaud. & Hennebert, 1980

On “very minute gnats” (Diptera) in NC (US) (Thaxter 1888).

### [41] *Erynia ovispora* (Nowak.) Remaud. & Hennebert, 1980

On a ‘small gnat attached to bark’ in TN (Diptera) (US) (R.A. Humber pers. comm.).

### [42] *Erynia rhizospora* (Thaxter) Remaud. & Hennebert, 1980*

On Neuroptera and “several genera” of adult Phryganeidae (Trichoptera) in ME and NC (US) (Thaxter 1888). Also reported on Trichoptera in NY (US) (ARSEF 2020).

### [43] *Erynia sepulchralis* (Thaxter) Remaud. & Hennebert, 1980*

On unidentified adult crane flies (Diptera, Tipulidae) in western NC and eastern TN (US) (Thaxter 1888), and on *Tipula caloptera* in NY (US) (Kramer 1980).

### [44] *Erynia variabilis* (Thaxter) Remaud. & Hennebert, 1980

On 'minute gnats of various genera' in NC (Diptera) (US) (Thaxter 1888).

## *Pandora*

### [45] *Pandora americana* (Thaxter) S. Keller, 2007

On Diptera: *Musca domestica* (Muscidae), *Calliphora vomitoria*, and *Lucilia caesar* (Calliphoridae) and 'numerous other large flies.' Common in New England and less common in NC (US) (Thaxter 1888). Also reported in the US from *Calliphora* sp. (WI), *C. vomitoria* (AL), *Phormia regina* (TX) (Calliphoridae), and *Muscina stabulans* (TN) (Muscidae) (Charles 1941).

### [46] *Pandora blissi* (G. Lakon) D.M. MacLeod & Müller-Kögler, 1973; *nomen dubium*

On the chinch bug, *Blissus leucopterus* (Hemiptera, Blissidae) in IA, IL, KS, MN, and OH (Billings and Glenn 1911). This species was initially placed in the genus *Empusa* by Thaxter (Gillette 1888). The 1888 species description was minimal (Gillette 1888), and no types were designated (but 'co-types' are present at Harvard). The provisional genus *Pandora* is based on ongoing evaluation of museum specimens.

### [47] *Pandora blunckii* (G. Lakon ex G. Zimm.) Humber, 1989*

On *Plutella xylostella* (Lepidoptera, Plutellidae) in Gto. (Mexico) (ARSEF 2020) and on Diptera (possibly Sciaridae) in NY (US) (ARSEF 2020).

### [48] *Pandora bullata* (Thaxt. & D.M. MacLeod ex Humber) Humber, 1989

On Calliphoridae (Diptera): including *Phormia regina*, *Lucilia sericata*, *Protophormia terraenovae*, *Calliphora vomitoria*, and perhaps other *Calliphora* spp. and Sarcophagidae (Diptera): *Sarcophaga aldrichi* in ON (Canada) and NY, MI, and MA (US) (MacLeod et al. 1973; Kramer 1979; Nielsen et al. 2001).

### [49] *Pandora delphacis* (Hori) Humber, 1989*

On Hemiptera: *Spissistilus festinus* (Hemiptera, Membracidae) in AL, AR (US) (Miller and Harper 1987; ARSEF 2020), and *Empoasca fabae* (Hemiptera, Cicadellidae) and Miridae in NY (ARSEF 2020).

### [50] *Pandora dipterigena* (Thaxter) Humber, 1989*

On Diptera: "small Tipulidae; other small flies or gnats belonging especially to the Mycetophilidae" in MA, ME, NC, and NH (US) (Thaxter 1888), unknown dipterans in ME and NY (US) (ARSEF 2020), and nematocerans in Mich. (Mexico) (Remaudière and Latgé 1985).

### [51] *Pandora echinospora* (Thaxter) Humber, 1989

On *Minettia duplicata* (Diptera, Lauxaniidae) and 'rarely other smaller Diptera' in ME, NH, and NC (US) (Thaxter 1888).

### [52] *Pandora formicae* (Humber & Bałazy) Humber, 1989

On Formicinae (Hymenoptera, Formicidae) in ME (US) (R.A. Humber pers. comm.).

### [53] *Pandora gammae* J. Weiser ex. Humber, 1989

On noctuid larvae (Lepidoptera): *Chrysodeixis includens* in AL and GA (US) in GA (US) and Tamps (Mexico) and *Trichoplusia ni* in AL (US) and Coah (Mexico) (Harper and Carner 1973; Newman and Carner 1975; Gilreath et al. 1986; Sanchez-Peña 1990, 2000). Also on *Mocis* sp. in NL and Coah (Mexico) (S.R. Sanchez-Peña unpubl. data).

### [54] *Pandora gastropachae* (Racib.) Hajek & Gryganskyi, 2024*

In hardwood forests on *Malacosoma disstria* (Lepidoptera, Lasiocampidae), ranging from BC to QC (Canada) and ME to FL and AL (US) (Filotas et al. 2003).

### [55] *Pandora gloeospora* (Vuillemin) Humber, 1989*

On *Lycoriella mali* (Diptera, Sciaridae) in mushroom production facilities in MD, DE, and southeastern PA (US) (Miller and Keil 1990, AP Gryganskyi pers. comm.) and cadavers producing conidia found on oyster mushrooms (*Pleurotus* sp.) in FL (US) (MW Miller pers. comm.).

### [56] *Pandora heteropterae* (Bałazy) S. Keller, 2005*

On *Lygus lineolaris* (Hemiptera, Miridae) in AR (US) (Hannam and Steinkraus 2010).

### [57] *Pandora ithacensis* (Kramer) Hajek & Gryganskyi, 2024*

On Diptera: *Symphoromyia hirta* and *Rhagio mystaceus* (Rhagionidae) and *Empis obesa* (Empididae) in NY (US) (Kramer 1981a) and an unidentified rhagionid in PA (US) (C. Tkaczuk unpubl. data).

### [58] *Pandora montana* (Thaxter) Hajek & Gryganskyi, 2024

On Diptera on the alpine summit of Mt. Washington, NH (US), infecting "minute gnats, apparently *Chironomus* sp." (Thaxter 1888).

### [59] *Pandora muscivora* (J. Schröt.) S. Keller, 2005

On *Syrphus* sp. (Diptera, Syrphidae) in ME (US) (MyCoPortal 2024).

### [60] *Pandora neoaphidis* (Remaud. & Hennebert) Humber, 1989*

On diverse aphids (Hemiptera, Aphididae) across the US, Mexico, and QC (Canada). In addition, single records of *Lygus* sp. (Hemiptera, Miridae) in NY (US) (ARSEF 2020) and *Aeneolamia albofasciata* (Hemiptera, Cercopidae) in Mexico (Remaudière and Latgé 1985) (Table 10). These last records of this fungus on spittlebugs and mirids should be reconsidered due to the unambiguous nature of this fungus as a specialized aphid pathogen, and the brief and rather incomplete description in these records.

**Table 10.** Recorded arthropod hosts of *Pandora neoaphidis* in the US, Canada, and Mexico, all hosts in the order Hemiptera.

| Host Family | Host Species | Country | States/ Provinces | References |
|---|---|---|---|---|
| Aphididae | *Acyrthosiphon kondoi* | US | CA | Pickering and Gutierrez 1991 |
| | *Acyrthosiphon pisum* | US | CA, GA, ID, IL, MN, NY, PA, WA | Folsom 1909; Pickering et al. 1989; Pickering and Gutierrez 1991; ARSEF 2020; C. Tkaczuk unpubl. data |
| | *Acyrthosiphon pisum* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis asclepiadis* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis coreopsidis* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis fabae* | US | WA | ARSEF 2020 |
| | *Aphis fabae* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis glycines* | US | NY | ARSEF 2020; Nielsen and Hajek 2005 |
| | *Aphis gossypii* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis lugentis* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis solitaria* | Mex | - | Remaudière and Latgé 1985 |
| | *Aphis* sp. | Can | QC | ARSEF 2020 |
| | *Aphis* sp. | US | WA | ARSEF 2020 |
| | *Aphis spiraecola* | Mex | - | Remaudière and Latgé 1985 |
| | *Aulacorthum solani* | US | ME | Shands et al. 1972 |
| | *Brachycaudus helichrysi* | Mex | - | Remaudière and Latgé 1985 |
| | *Brachyunguis tetrapteralis* | Mex | - | Remaudière and Latgé 1985 |
| | *Brevicoryne brassicae* | Mex | - | Remaudière and Latgé 1985 |
| | *Capitophorus elaeagni* | Mex | - | Remaudière and Latgé 1985 |
| | *Capitophorus shepherdiae* | Mex | - | Remaudière and Latgé 1985 |
| | *Capitophorus xanthii* | Mex | - | Remaudière and Latgé 1985 |
| | *Cavariella hendersoni* | Mex | - | Remaudière and Latgé 1985 |
| | *Diuraphis noxia* | US | CO, ID | ARSEF 2020; Feng et al. 1990 |
| | *Hayhurstia atriplicis* | Mex | - | Remaudière and Latgé 1985 |
| | *Hyperomyzus lactucae* | Mex | - | Remaudière and Latgé 1985 |
| | *Hyperomyzus* sp. | Mex | - | Remaudière and Latgé 1985 |
| | *Illinoia* sp. | Mex | - | Remaudière and Latgé 1985 |
| | *Impatientinum americanum* | Mex | - | Remaudière and Latgé 1985 |
| | *Macrosiphum euphorbiae* | US | FL, ID, ME | ARSEF 2020; Feng et al. 1990 |
| | *Macrosiphum* spp. | Mex | - | Remaudière and Latgé 1985 |
| | *Melanaphis sacchari* | Mex | Coah | ARSEF 2020 |
| | *Metopolophium dirhodum* | US | ID, MT | ARSEF 2020; Feng et al. 1990, 1991 |
| | *Myzus ornatus* | Mex | - | Remaudière and Latgé 1985 |
| | *Myzus persicae* | US | AR, ID, ME, VA, WA | Harris 1948; Elkassabany et al. 1992; Kish et al. 1994; Dara and Semtner 2001 |
| | *Myzus persicae* | Mex | - | Remaudière and Latgé 1985 |

| Host Family | Host Species | Country | States/ Provinces | References |
|---|---|---|---|---|
| Aphididae | *Myzus persicae nicotianae* | US | KY, VA | ARSEF 2020; Dara and Semtner 2001 |
| | *Rhapalosiphum padi* | US | ID | Feng et al. 1990 |
| | *Rhodobium porosum* | Mex | - | Remaudière and Latgé 1985 |
| | *Rhopalosiphum maidis* | US | ID, MT | Feng et al. 1990, 1991 |
| | *Rhopalosiphum maidis* | Mex | - | Remaudière and Latgé 1985 |
| | *Rhopalosiphum padi* | Mex | - | Remaudière and Latgé 1985 |
| | *Rhopalosiphum* sp. | Mex | - | Remaudière and Latgé 1985 |
| | *Schizaphis graminum* | US | ID | ARSEF 2020; Feng et al. 1990 |
| | *Schizaphis graminum* | Mex | - | Remaudière and Latgé 1985 |
| | *Sibobion avenae* | US | ID | Feng et al. 1990 |
| | *Sitobion* sp. | Mex | - | Remaudière and Latgé 1985 |
| | *Uroleucon ambrosiae* | Mex | - | Remaudière and Latgé 1985 |
| | *Uroleucon sonchi* | Mex | - | Remaudière and Latgé 1985 |
| | *Uroleucon* sp. | Can | QC | FPMI 1990 |
| | *Uroleucon* sp. | Mex | - | Remaudière and Latgé 1985 |
| | *Utamphorophora crataegi* | Mex | - | Remaudière and Latgé 1985 |
| | *Wahlgreniella arbuti* | Mex | - | Remaudière and Latgé 1985 |
| Cercopidae | *Aenolamia albofasciata* | Mex | - | Remaudière and Latgé 1985 |
| Miridae | *Lygus* sp. | US | NY | ARSEF 2020 |

### [61] *Pandora nouryi* (Remaud. & Hennebert) Humber, 1989*

On aphids (Hemiptera: Aphididae) on potato in ME (US) (ARSEF 2020).

### [62] *Pandora pieris* (Z.Z. Li & Humber) Hajek & Gryganskyi, 2024*

On larvae of *Pieris rapae* (Lepidoptera, Pieridae) in NY (US) (Li and Humber 1984).

### [63] *Pandora sylvestris* Hajek & Gryganskyi, 2024

On larvae of *Lophocampa caryae* (Lepidoptera, Erebidae) from MI and VT (US) (Hajek et al. 2024a).

### [64] *Pandora virescens* (Thaxter) Hajek & Gryganskyi, 2024

On Noctuidae (Lepidoptera): *Mythimna unipuncta* in AR (US), *Dargida procinctus* in OR (US), and *Ochropleura fennica* in ON (Canada) (Steinkraus et al. 1993b).

### [65] *Pandora vomitoriae* (Rozsypal) Hajek & Gryganskyi, 2024

On adult Calliphoridae (Diptera): 'blue bottle flies' in Coah. (Mexico) (Sanchez-Peña 2000) and *Lucilia sericata* in NY (US) (B. Lovett unpubl. data).

## *Zoophthora*

### [66] *Zoophthora aphrophorae* (Rostr.) S. Keller, 2007

On the pine spittlebug, *Aphrophora parallela* (Hemiptera, Aphrophoridae), in PA (US) (Knull 1932).

### [67] *Zoophthora canadensis* (MacLeod, Tyrrell & Soper) Remaud. & Hennebert, 1980

On *Schizolachnus piniradiatae* (Hemiptera, Aphididae) in red pine plantations in ON (Canada) (MacLeod et al. 1979).

### [68] *Zoophthora forficulae* (Giard) A. Batko, 1964

On *Forficula auricularia* (Dermaptera, Forficulidae) in OR and WA (US) (Rockwood 1950; Hutchison 1963).

### [69] *Zoophthora geometralis* (Thaxt.) A. Batko, 1964

On adults of *Eupithecia* sp., *Petrophora* sp., and *Thera* sp. (Lepidoptera, Geometridae) in ME (US) (Thaxter 1888).

### [70] *Zoophthora ichneumonis* Bałazy, 1993

On an adult ichneumonid (Hymenoptera) in PA (US) (C. Tkaczuk, unpubl. data).

### [71] *Zoophthora independentia* A.E. Hajek, Humber & Gryganskyi, 2016

Resting spore stages occurred within adult *Tipula submaculata* (Diptera, Tipulidae) in NY (US) (Hajek et al. 2016).

### [72] *Zoophthora occidentalis* (Thaxter) A. Batko, 1964*

First reported on 'aphides on *Betula populifera*' in MA and ME (US) (Thaxter 1888). On aphids (Hemiptera, Aphididae), *Myzus persicae*, *Macrosiphum euphorbiae*, and *Aphis fabae* in ME (US); *Acyrthosiphon pisum* and *Aphis glycines* in NY (US); and *Sitobion avenae* in ID (US) (Feng et al. 1990; Nielsen and Hajek 2005; Barta and Cagáň 2006; ARSEF 2020).

### [73] *Zoophthora phalloides* A. Batko, 1966

Aphid pathogens (Hemiptera, Aphididae) on *Macrosiphum euphorbiae*, *Nearctaphis bakeri*, *Uroleucon* sp., and *Acyrthosiphon pisum* in QC (Canada), ME and NH (US) (Remaudiére et al. 1978). On *A. pisum* in Oax. (Mexico) and *Therioaphis maculata* in Mexico (ARSEF 2020) and NY (US) (Milner and Soper 1981).

### [74] *Zoophthora phytonomi* (Arthur) A. Batko, 1964*

Two genotypes infecting weevils in the genus *Hypera* (Coleoptera, Curculionidae) occur in North America (Hajek et al. 1995). The genotype principally infecting *Hypera postica* was first found in 1973 in ON (Canada) and subsequently in 21 eastern US states (Fig. 4). This genotype was also reported in *H. punctata* from NY (US). The second genotype infected *Hypera punctata* in ON (Canada) and NY and DE (US) (Hajek et al. 1995).

**Figure 4.** Distribution of recorded occurrences of *Zoophthora phytonomi* in the US and Canada (Hajek et al. 1995). Dark gray = states and provinces where this pathogen was reported from both *Hypera postica* and *Hypera phytonomi*; light gray = US states where this pathogen was only reported from *H. postica* (Hajek et al. 1995).

### [75] *Zoophthora porteri* (R.S. Soper) A.E. Hajek, Humber & Gryganskyi, 2016

Resting spore stages occurred within adult *Tipula colei* (Diptera, Tipulidae) in TN (US) (Hajek et al. 2016).

### [76] *Zoophthora radicans* (Bref.) A. Batko, 1964*

On hosts in diverse families across Hemiptera, Lepidoptera, Hymenoptera, and Diptera. Widespread distribution across the US, Canada, and Mexico (Table 11).

**Table 11.** Recorded arthropod hosts of *Zoophthora radicans* in the US, Canada, and Mexico.

| Host Order | Host Family | Host Species | Country | States/ Provinces | References |
|---|---|---|---|---|---|
| Diptera | Drosophilidae | - | US | FL | ARSEF 2020 |
| | Nematocera | - | Mex | Dgo | Remaudière and Latgé 1985 |
| | Tipulidae | - | US | ME | FPMI 1990 |
| Hemiptera | Aphididae | *Acyrthosiphon pisum* | US | GA | Pickering et al. 1989 |
| | | *Aphis nasturtii* | US | ME | Shands et al. 1972 |
| | | *Macrosiphum euphorbiae* | US | ME | Shands et al. 1962 |
| | | *Metopolophium dirhodum* | US | ID, MT | Feng et al. 1990 |
| | | *Myzus ornatus* | Mex | - | Remaudière and Latgé 1985 |
| | | *Sitobion avenae* | US | ID | Feng et al. 1990 |
| | | *Therioaphis maculata* | US | NY | ARSEF 2020 |
| | | *Therioaphis maculata* | Mex | CDMX | FPMI 1990 |
| | | *Therioaphis trifolii* | Mex | CDMX | Remaudière and Latgé 1985 |
| | Cicadellidae | *Empoasca fabae* | US | IL, MI, NY, WI | ARSEF 2020; McGuire et al. 1987 |
| | Pentatomidae | *Bagrada hilaris* | Mex | Coah | Torres-Acosta et al. 2016 |
| | Psyllidae | *Trioza* sp. | Can | QC | ARSEF 2020 |
| | | *Psylla trimaculata* | Can | QC | FPMI 1990 |
| | | *Cacopsylla mali* | Can | NS | Gilliatt 1925 |
| | Triozidae | *Bactericera cockerelli* | Mex | Coah | Torres-Acosta et al. 2016 |

| Host Order | Host Family | Host Species | Country | States/ Provinces | References |
|---|---|---|---|---|---|
| Hymenoptera | - | - | US | ME | ARSEF 2020 |
| | Diprionidae | *Neodiprion tsugae* | US | AK | ARSEF 2020 |
| Lepidoptera | Geometridae | *Lambdina fiscellaria* | Can | NF | Otvos et al. 1973 |
| | Hesperiidae | *Thymelicus lineola* | Can | QC | FPMI 1990 |
| | Noctuidae | *Autographa precationis* | US | IN | Yendol and Paschke 1987 |
| | | *Rachiplusia ou* | US | IN | Yendol and Paschke 1987 |
| | | *Trichoplusia ni* | US | IN | Yendol and Paschke 1987 |
| | | *Trichoplusia ni* | Mex | Coah | Sanchez-Peña 2000 |
| | Plutellidae | *Plutella xylostella* | Mex | Gto | ARSEF 2020 |
| | Tortricidae | *Acleris variana* | US | ME | ARSEF 2020 |
| | | *Acleris variana* | Can | NF | FPMI 1990 |
| | | *Archips argyrospila* | US | PA | Knull 1932 |
| | | *Choristoneura biennis* | Can | BC | FPMI 1990 |
| | | *Choristoneura fumiferana* | US | ME | Vandenberg and Soper 1975 |
| | | *Choristoneura fumiferana* | Can | BC, NS, ON | FPMI 1990 |
| | | *Rhopobota naevana* | US | MA | Sawyer 1933 |

### [77] *Zoophthora rhagonycharum* (Bałazy) S. Keller, 2007

Resting spore stages in adult *Rhagonycha vilis* and *Rhagonycha fraxini* (Coleoptera, Cantharidae) in NY (US) (Hajek et al. 2024b).

### *Strongwellsea*

### [78] *Strongwellsea castrans* A. Batko & Weiser, 1965

On *Delia platura* (Diptera, Anthomyiidae) in WI (US) (Strong et al. 1960) and *Delia radicum* in ON (Canada) (Nair and McEwen 1973).

### [79] *Strongwellsea magna* Humber, 1976

On *Fannia canicularis* (Diptera, Fanniidae) in CA (US) (Humber 1976).

## FORM GENUS

### *Tarichium*

### [80] *Tarichium megaspermum* Cohn, 1875

On two species of Noctuidae (Lepidoptera): *Euxoa messoria* and *Euxoa ochrogaster* in BC and ON (Canada) (Bucher and MacLeod 1974; Steinkraus et al. 1993b).

## Incomplete and questionable records

For some species we could not resolve the identification of the fungal species, especially older identifications based only on morphology or species for which confusion exists regarding the correct fungal species name to use. For example, the species *Entomophthora carpentieri*, named by Giard in 1888 from only resting spores collected in Europe, was identified by V.K. Charles from *Horistonus uhleri* (Coleoptera, Elateridae) collected in 1934 by J.N. Tenhet in SC (My-

CoPortal 2024). However, this fungal species is considered questionable by Keller (1991) and Bałazy (1993), and any potential synonymous species are not known in North America, and so it has not been included in this checklist.

One isolate of *Entomophaga conglomerata* is listed in the ARSEF culture collection (ARSEF 2020), but a recent publication demonstrated that this isolate instead belongs in the genus *Batkoa* (Gryganskyi et al. 2022b), and therefore this example was not included. Tipulids in NHand NC (US), collected by Thaxter, harbored a fungus named *Entomophthora thaxteri* (MacLeod and Müller-Kögler 1973), but this species was not adequately described. However, trying to find the correct genus and species for this species has been difficult. Over time, it has been suggested that this species could be *E. conglomerata*, *Entomophaga tipulae*, or *Entomophthora tipulae* (MacLeod and Müller-Kögler 1973; Bałazy 1993). In addition, because Thaxter found only resting spores in adults, perhaps this is *Zoophthora independentia* or *Z. porteri*. Therefore, this species (whatever it is) is not included in the checklist. In the case of *Pandora blissi*, this genus name is provided as *nomen dubium*; the native host of this species is no longer as abundant as in the past (Waldbauer 2005), and studies are currently underway to obtain specimens so the correct genus can be determined.

Sometimes, pathogen/host associations seem incorrect in initial reports. For example, in 1909, *Zoophthora radicans* was reported infecting the weevil *Hypera punctata* in IL (US) (Folsom 1909). However, now we know that *Zoophthora phytonomi* (not described until 1964) infects this weevil species in other regions (Fig. 4), and we have no examples of *Z. radicans* infecting Coleoptera (see Table 11). In this case, we have not included this location information under *Z. phytonomi* (Fig. 4) or under *Z. radicans* (Table 11).

Finally, as we do not include entries for which a species name has not been provided. Thus, we could not include an unidentified species infecting the economically important northern corn rootworm, *Diabrotica barberi* (Naranjo and Steinkraus 1988); in this case beetle cadavers only contained resting spores. We also could not include the only example from the Arctic. *Zoophthora* sp. was reported causing epizootics in outbreak populations of the noctuid *Eurois occulta* in West Greenland (Avery and Post 2013). The larval cadavers that had summited on the vegetation only contained resting spores, and the species could not be identified or described at that time.

## Discussion

In 1963, a checklist of entomophthoralean fungi from the Western Hemisphere listed 39 species, all ascribed to the same genus: *Entomophthora* (Hutchison 1963). Today, with increased collection data and the incorporation of taxonomic changes within this group, our survey of arthropod pathogens in the Entomophthoromycotina found in North America includes 80 species in 14 genera, within 2 classes, plus one species in a form genus. A recent checklist of all fungi in North America (Bates and Miller 2018) lists only 38 arthropod-pathogenic species belonging to the Entomophthoromycotina. This checklist most likely relied on the Hutchison (1963) checklist, as it was the most recent available checklist for this group (Hutchison, 1963), underscoring the necessity for an updated checklist of Entomophthoromycotina in North America.

In their checklist for all North American fungi, Bates and Miller (2018) discuss the issue that there are many instances where European names were initially assigned to North American taxa based on morphological similarities. However, North American strains could represent cryptic taxa native to this continent that should be described as separate species, particularly if supported by molecular findings. We assume that this type of work will be undertaken in the future. However, at present many species in this group have not been isolated *in vitro* and/or there are no herbarium samples that can be used for DNA extraction. Therefore, to move forward along these molecular lines, in many cases, new specimens must be collected for analyses.

In fact, many species of arthropod pathogenic Entomophthoromycotina found worldwide have not been isolated in culture, and sequences are not available for molecular identification. In nature, viable cells of these fungi are quite ephemeral, and it is therefore difficult to collect them for isolation. The exception to this would be cadavers bearing resting spores (azygospores or zygospores). However, resting spores are often not found or, if found, are dormant and difficult to either germinate or use for DNA extraction (but see Bidochka et al. 1995; Eilenberg and Jensen 2018; Hajek et al. 2018). The ARSEF collection has North American cultures or samples from 29 of the species included in this checklist (approximately 36%). We hope that this annotated checklist will make it more possible in the future for the culture and sequencing of additional species in North America.

Taxonomic changes in this group have been relatively frequent since the first publication on species of Entomophthoromycotina in the United States by Thaxter in 1888, referring to this group as the Entomophthoreae. These taxonomic changes pose challenges for understanding whether names for host/fungus associations in the older literature are accurate today. For example, *E. muscae* is now known to be a species complex (Elya and De Fine Licht 2021), with one species named *E. muscae* s.s delimited within the complex. Therefore, for older records when *E. muscae* is mentioned, it is unclear which of the species in the complex is being discussed and how to apply historical findings to the modern circumscription of the species *E. muscae* itself. Therefore, since this checklist treats the three species of this species complex known from North America separately, the older reports of *E. muscae* have usually not been included in order not to introduce errors. The *Entomophaga grylli* species complex raised similar problems. Today, two members of this complex occur in North America, and older literature did not differentiate between them, with the result that older reports for collection locations could not be included.

Likewise, confusion has occurred with *Pandora neoaphidis*, for which the correct nomenclature was only resolved in 1980 (Barta and Cagáň 2006). From 1888 (Thaxter) until 1980, in North America, *P. neoaphidis* was incorrectly known as *Empusa aphidis* and later *Entomophthora aphidis*. However, in 1980 *Entomophthora aphidis* was synonymized with *Zoophthora aphidis*, a species known only from relatively few host aphids in western and central Europe. We assume that records of *Empusa* or *Entomophthora aphidis* in North America before 1980 probably refer to *P. neoaphidis*, especially for collections from Maine, due to the common occurrence of *E. aphidis* and the presence of cultures of *P. neoaphidis* from Maine collected in 1972 and 1977 in the ARSEF collection (ARSEF 2020).

Our records of the distribution of fungal species are predominantly based on reports in the literature. The time of year, or even year of collection, is not always reported. Additionally, entomophthoralean fungi have been collected by only a handful of experts and remain vastly understudied across many locations in North America. Therefore, if a state or province is not listed, this is not definitive proof that a species is not present there, but rather this suggests that further surveys are necessary.

This annotated checklist provides data on these arthropod pathogens by connecting arthropod host species and fungal species at different locations within North America. Therefore, for each record, three specific pieces of information were needed: 1) host species, 2) fungal species, and 3) collection location. Unfortunately, some publications (although relatively few) did not provide separate data for these three metadata types and thus could not be included. Such problems arose with Remaudière et al. (1978), where all fungal pathogens and their aphid hosts were merged for one trip covering NH (US), and ON and QC (Canada). Similarly, we could not include data from Feng et al. (1990), in which data from collections of three species of aphid-pathogenic *Conidiobolus* species were merged so that locations and hosts of individual species were not listed (one of these species is now a *Batkoa*), or from Wraight et al. (1993), in which data for fungal infections of several cereal aphid species were merged.

## Conclusion

In conclusion, we provide an updated checklist of arthropod pathogenic fungi in the Entomophthoromycotina detected in North America, using the latest taxonomy and largely based on published literature. While this checklist includes many more species than the last checklist (Hutchison 1963), there are still many arthropod-pathogenic species known from this subphylum (see Sacco and Hajek 2023) that have not been found in North America. Additional sampling is needed to determine if these are truly absent from the continent or await discovery as well as whether new discoveries await. Additionally, given the paucity of molecular data (De Fine Licht et al. 2016), sequencing of additional species from verified specimens, together with improved taxonomies, is an area of future research that will enhance DNA-based identification of these fungi. This updated checklist provides a framework for future efforts sampling and documenting the biodiversity of this important, yet understudied, group of fungi.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: AEH. Data curation: AEH, KLS, CT, SSP. Formal analysis: AEH. Funding acquisition: KEB. Investigation: AEH, SSP, CT. Methodology: AEH. Project administration: AEH. Software: BL, KLS. Supervision: KEB, AEH. Validation: AEH. Visualization: KLS, BL. Writing - original draft: AEH. Writing - review and editing: CT, SSP, KLS, AEH, BL, KEB.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

# Four new species of *Cryptothecia* (Arthoniaceae, Ascomycota) and *Myriostigma* (Arthoniaceae, Ascomycota) from China, based on morphology and molecular phylogeny

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## Abstract

In this study, morphological and molecular phylogenetic approaches were used to investigate the species diversity of *Cryptothecia* and *Myriostigma* from southern China. We found four new species of *Cryptothecia* and *Myriostigma* (*C. disjecta*, *C. sorediatum*, *M. melanovillosa* and *M. visus-blotch*). *Cryptothecia disjecta* is distinguished by verrucose pseudisidia that are loosely scattered on the thallus, the upper parts of which are white or have darker dots. *Cryptothecia sorediatum* is distinguished by I– medulla and thalli having globose soralia. *Myriostigma melanovillosa* is distinguished by thalli with black or purple dots and hyaline to pale yellow ascospores (63–71 × 26–33 μm). *Myriostigma visus-blotch* is distinguished by hyaline ascospores and ascigerous areas, which have many irregular small patches that are scattered or clustered together radially elongated. In addition, a phylogenetic tree based on mtSSU, RPB2 and nuLSU illustrates the phylogenetic placement of the proposed four new taxa. Detailed descriptions of the morphological, ecological and chemical characteristics and illustrations of each species are provided. Two updated keys for all known Chinese *Cryptothecia* and *Myriostigma* species are also provided.

**Key words:** Diversity, identification key, Lichenized fungi, taxonomy

## Introduction

Arthoniaceae (Arthoniales, Arthoniomycetes, Ascomycota) is widely distributed in tropical and subtropical regions. Arthoniaceae is characterized by its crustose, sometimes poorly developed or immersed, effuse or determinate thallus; trentepohlioid or rarely chlorococcoid photobiont; not determinate, often elongated and/or branched ascomata with rudimentary walls but rarely thick- and dark-walled; reddish or brownish, I+ blue hymenium; thick walled, ± fissitunicate, usually with a large apical dome and often I+ blue asci; septate and often multi-septate, occasionally muriform ascospores; and small, inconspicuous, coelomycetous (pycnidial) or sporodochial anamorph (Cannon et al. 2020). According to previous studies, the topology of the Arthoniaceae phylogenetic tree is mainly divided into Arthonioid and Cryptothecioid clades (Frisch et al. 2014; Thiyagaraja et al. 2020). *Cryptothecia*, *Herpothallon* and *Myriostigma* belong to the Cryptothecioid clade, and their morphological characteristics are similar, so distinguishing them is difficult.

From 2020 to 2023, we collected a large number of lichen specimens from southern China while identifying a large number of species from these specimens. We found three new record species of *Cryptothecia*, eight new species and five new record species of *Herpothallon*, and three new species of *Myriostigma* (Chen et al. 2022; Liu et al. 2023a, 2023b; Xue et al. 2024; Zhang et al. 2024). Recently, we studied the remaining specimens, and we found that several of these specimens differed from the known species in their morphology and molecular phylogeny. Here, we describe two new species of *Cryptothecia* (*C. disjecta* and *C. sorediatum*) and two new species of *Myriostigma* (*M. melanovillosa* and *M. visus-blotch*).

## Materials and methods

### Morphology and anatomy

The study was based on specimens collected during fieldwork in Hainan and Yunnan Provinces, China, and preserved in the Lichen Section of Botanical Herbarium (SDNU, Shandong Normal University). Morphological features were studied with a dissecting microscope (COIC XTL7045B2), and photos were taken under a microscope (Olympus SZX16, Japan) with a DP72 camera system. The observations included the growth type, diameter and colour of the thallus; the shape and width of the prothallus; and the shape of the ascigerous areas. Anatomical features were observed and measured by a polarizing compound microscope (Olympus CX41, Japan), and images were taken under a microscope (Olympus BX61, Japan) with a DP72 camera system. The observations included the colour of the medulla and whether it contained crystals; the shape and size of the photobiont; the width of the hyphae; and the colour, shape and size of the asci, ascospores, pycnidia and conidia.

### Colour reaction and chemical analysis

Colour reactions were performed on the thallus surface and thin medulla sections with a saturated solution of aqueous sodium hypochlorite (C), a 10% aqueous solution of potassium hydroxide (K), a saturated solution of p-phenylenediamine in 95% ethyl alcohol (P), a 3% solution of Lugol's iodine (I) and long-wavelength UV light. Polarized light microscopy (pol) was used to detect the presence of calcium oxalate crystals. The secondary metabolites of the lichens were analyzed and identified via thin layer chromatography (TLC) with solvent C (Orange et al. 2010; Elix 2014).

### DNA extraction, PCR amplification and sequencing

We extracted genomic DNA from the collected specimens using the Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St. Louis, MO, USA) following the manufacturer's protocol, except that only 30 µL of extraction buffer and 30 µL of dilution buffer were used. For extraction, the clean growing portions of the thalli of the specimens were selected under a dissecting microscope (COIC XTL7045B2) with sterile blades and forceps.

We amplified three gene sequences: the mtSSU gene with the primer pairs mtSSU1 and mtSSU3R (Zoller et al. 1999), the RPB2 gene with RPB2-7cF and

RPB2-11aR (Liu et al. 1999), and the nuLSU gene with LIC24R and LR7 (Vilgalys and Hester 1990; Miadlikowska and Lutzoni 2000). We performed PCR amplification with a 50 µL volume containing 25 µL of 2 × Taq PCR MasterMix [Taq DNA Polymerase (0.1 unit/µL); 3 mM $MgCl_2$; 100 mM KCl; 0.5 mM dNTPs; and 20 mM Tris-HCl (pH 8.3)] (Tiangen, Beijing, China), 19 µL of dd $H_2O$, 2 µL of forward primer, 2 µL of reverse primer and 2 µL of DNA. The mtSSU gene was amplified via the following protocol: initial denaturation at 94 °C for 10 min; followed by 34 cycles at 95 °C for 45 s, 50 °C for 45 s, and 72 °C for 90 s; and a final extension at 72 °C for 10 min. The RPB2 gene was amplified via the following protocol: initial denaturation at 94 °C for 10 min; followed by 34 cycles at 94 °C for 45 s, 52 °C for 50 s, and 72 °C for 1 min; and a final extension at 72 °C for 5 min. The nuLSU gene was amplified via the following protocol: initial denaturation at 95 °C for 15 min; followed by 45 cycles at 95 °C for 45 s, 53 °C for 45 s, and 72 °C for 1 min; and a final extension at 72 °C for 7 min. PCR products were sequenced by BioSune Biological Technology (Shanghai, China) using the same primers.

### Sequence alignment and phylogenetic analysis

We compared the newly generated sequences with the available sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) to ensure the reliability of all the new sequences. Then, we selected new sequences with high similarity to the clothing species of the *Cryptothecia* and *Myriostigma* for further analysis. We assembled the new sequences via SeqMan v.7.0 (DNAstar packages). The sequences of other genera of Arthoniaceae used in this study were downloaded from GenBank (Table 1). We aligned the sequences via the online version of MAFFT v.7.0.26. The algorithm of MAFFT is chosen automatically (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depending on the data size). The sequences were edited via MEGA v.7.0. To construct the phylogenetic tree, the species *Chiodecton natalense* Nyl. was selected as the outgroup taxon (Woo et al. 2017).

The multigene phylogenetic trees were inferred via maximum likelihood (ML) and Bayesian inference (BI). The three gene sequences were combined via the Concatenate Sequence function in PhyloSuite v1.2.3 (Zhang et al. 2020). We used the CIPRES Science Gateway (http://www.phylo.org/portal2/) (Miller et al. 2010) and performed ML analyses via RaxML-HPC v. 8.2.12 (Stamatakis 2014) under the default parameters implemented in CIPRES. Support values were based on 1000 nonparametric bootstrap pseudoreplicates. Bootstrap support values for ML equal to or greater than 70 were given above or below the nodes in the phylogenetic tree (Fig. 1). We used PhyloSuite to infer BI phylogenies via MrBayes 3.2.6 (Ronquist et al. 2012) under a partition model, for which the initial 25% of the sampled data were discarded as burn-in. Four Markov chains were run for 2,000,000 generations for the dataset. Trees were sampled every 1000th generations. The stationarity of the analysis was determined by examining the standard deviation of the split frequencies (<0.01). Bayesian posterior probabilities equal to or greater than 0.95 were given above or below the nodes in the phylogenetic tree (Fig. 1). The phylogenetic trees generated were visualized via FigTree v1.4.2 (Rambaut 2012) and edited via Adobe Illustrator (AI). The new sequences were submitted to GenBank (Table 1).

**Table 1.** Specimens used for the phylogenetic analyses with the corresponding voucher information and GenBank accession numbers for the mtSSU, RPB2 and nuLSU sequences. Newly obtained sequences in this study are in bold, * represents type material.

| Species Name | Voucher Specimen | GenBank Accession Number | | |
|---|---|---|---|---|
| | | mtSSU | RPB2 | nuLSU |
| *Arthonia calcarea* | Thor 11/6a (UPS) | KJ850974 | KJ851105 | – |
| *Arthonia didyma* | Ertz 7587 (BR) | EU704047 | EU704010 | EU704083 |
| *Arthonia granitophila* | Frish 10/Se74 (UPS) | KJ850981 | KJ851107 | KJ851049 |
| *Arthonia graphidicola* | Frisch 10/Jp102 (UPS) | KJ850980 | – | KJ851034 |
| *Arthonia ilicina* | McCune 31067 | KJ850982 | – | – |
| *Arthonia radiata* | Frisch 10/Se29 (UPS) | KJ850968 | KJ851108 | – |
| *Arthonia subfuscicola* | Thor 11/1 (UPS) | KJ850971 | KJ851110 | – |
| *Arthothelium ruanum* | KoLRI 038018 | MF616609 | MF616619 | – |
| *Arthothelium ruanum* | KoLRI 038261 | MF616611 | MF616621 | – |
| *Arthothelium spectabile* | Frisch 12Jp179a (TNS) | KP870144 | KP870160 | – |
| *Chiodecton natalense* | Ertz 6576 (BR) | EU704051 | EU704014 | EU704085 |
| *Coniocarpon cinnabarinum* | Johnsen 111003 (UPS) | KJ850976 | KJ851103 | KJ851083 |
| *Coniocarpon fallax* | LD: L10075 | KJ850979 | KJ851101 | – |
| *Crypthonia palaeotropica* | Frisch 11/Ug457 (UPS) | KJ850961 | KJ851084 | – |
| *Cryptophaea phaeospora* | Van den Broeck 5809 (BR) | KX077541 | – | – |
| *Cryptothecia bartlettii* | Zhang et al. 20220297 (SDNU) | PP051262 | | PP583805 |
| *Cryptothecia bartlettii* | Zhang et al. 20220275 (SDNU) | PP051261 | | PP583804 |
| ***Cryptothecia disjecta**** | **Xue et al. 20230146 (SDNU)** | **PP587867** | – | – |
| ***Cryptothecia disjecta*** | **Xue et al. 20230145 (SDNU)** | **PP587868** | – | – |
| *Cryptothecia inexspectata* | Liu et al. 20230668 (SDNU) | PP051263 | PP109371 | |
| *Cryptothecia inexspectata* | Liu et al. 20230639 (SDNU) | PP051264 | PP109370 | |
| *Cryptothecia striata* | Liu et al. 20230938 (SDNU) | PP302048 | PP585251 | |
| *Cryptothecia striata* | Liu et al. 20233925 (SDNU) | PP302049 | PP585252 | PP585252 |
| ***Cryptothecia sorediatum**** | **Liu et al. 20230379 (SDNU)** | **PP587866** | – | – |
| ***Cryptothecia sorediatum*** | **Liu et al. 20230381 (SDNU)** | **PP587865** | – | – |
| *Cryptothecia subnidulans* | v.d.Boom 40613 (hd v.d. Boom) | KJ850952 | KJ851087 | – |
| *Cryptothecia subnidulans* | Joensson Guyana 6a (UPS) | KJ850953 | KJ851088 | – |
| *Glomerulophoron mauritiae** | Ertz 19164 (BR) | KP870153 | KP870167 | – |
| *Herpothallon inopinatum** | Rudolphi 12 (UPS) | KJ850964 | KJ851099 | – |
| *Herpothallon kigeziense** | Frisch 11/Ug26 (UPS) | KF707644 | KF707654 | – |
| *Herpothallon rubrocinctum* | Rudolphi 5 (UPS) | KF707643 | KF707655 | – |
| *Herpothallon* sp. | Frisch 11/Ug401 (UPS) | KF707645 | KF707653 | – |
| *Inoderma byssaceum* | Thor 25952 (UPS) | KJ850962 | KJ851089 | KJ851040 |
| *Inoderma nipponicum** | Frisch 12Jp227 (TNS) | KP870146 | KP870162 | – |
| *Leprantha cinereopruinosa* | Kukwa 17127 & Lubek (BR) | MG207692 | – | – |
| *Myriostigma candidum* | Ertz 9260 (BR) | EU704052 | EU704015 | HQ454520 |
| *Myriostigma candidum* | Frisch 11/Ug125 (UPS) | KJ850959 | KJ851096 | – |
| *Myriostigma flavescens** | Liu et al. 20230612 (SDNU) | PP051268 | PP130144 | |
| *Myriostigma flavescens* | Liu et al. 20230641 (SDNU) | PP051267 | | |
| *Myriostigma hainana** | Xue et al. 20230061 (SDNU) | PP051271 | PP101845 | |
| *Myriostigma hainana* | Xue et al. 20230050 (SDNU) | PP051272 | PP109365 | |
| *Myriostigma laxipunctata** | Liu et al. 20231052 (SDNU) | PP051265 | PP109368 | PP033943 |
| *Myriostigma laxipunctata* | Liu et al. 20231231 (SDNU) | PP051266 | PP109369 | PP033944 |
| ***Myriostigma melanovillosa**** | **Liu et al. 20230635 (SDNU)** | **PP587874** | **PP585246** | **PP583807** |
| ***Myriostigma melanovillosa*** | **Liu et al. 20230629 (SDNU)** | **PP587875** | **PP585247** | – |
| *Myriostigma miniatum** | Silva T2A29 (ISE—epitype) | KP843606 | – | – |
| ***Myriostigma visus-blotch**** | **Liu et al. 20231187 (SDNU)** | **PP587872** | **PP585249** | – |
| ***Myriostigma visus-blotch*** | **Liu et al. 20230837 (SDNU)** | **PP587873** | **PP585248** | – |
| *Pachnolepia pruinata* | Frisch 11/Se34 (UPS) | KJ850967 | KJ851098 | – |
| *Reichlingia leopoldii* | Ertz 13293 | JF830773 | HQ454722 | HQ454581 |
| *Reichlingia syncesioides** | Frisch 11/Ug14 (UPS) | KF707651 | KF707656 | KF707636 |
| *Snippocia nivea** | Ertz 17437 (BR) | MG207695 | – | – |
| *Stirtonia neotropica* | Cáceres & Aptroot 11112 (ISE) | KP843611 | – | – |

| Species Name | Voucher Specimen | GenBank Accession Number | | |
|---|---|---|---|---|
| | | mtSSU | RPB2 | nuLSU |
| *Sporodophoron gossypinum** | Frisch 12Jp186 (TNS) | KP870154 | KP870168 | – |
| *Sporodophoron primorskiense** | Ohmura 10509 (TNS) | KP870157 | KP870169 | – |
| *Synarthonia albopruinosa** | VDB 6086 (BR<BEL>) | MH251873 | – | – |
| *Synarthonia inconspicua* | VDB 7013B (BR<BEL>) | MH251881 | – | – |
| *Synarthonia ochracea* | VDB 6653 (BR<BEL>) | MH251884 | – | – |
| *Tylophoron galapagoense* | Bungartz 8749 (CDS) | JF830776 | – | JF295078 |
| *Tylophoron hibernicum* | Frisch 11/Ug220 (UPS) | KJ850966 | KJ851097 | KJ851065 |
| *Tylophoron moderatum* | Ertz 14504 (BR) | JF830780 | – | JF295085 |

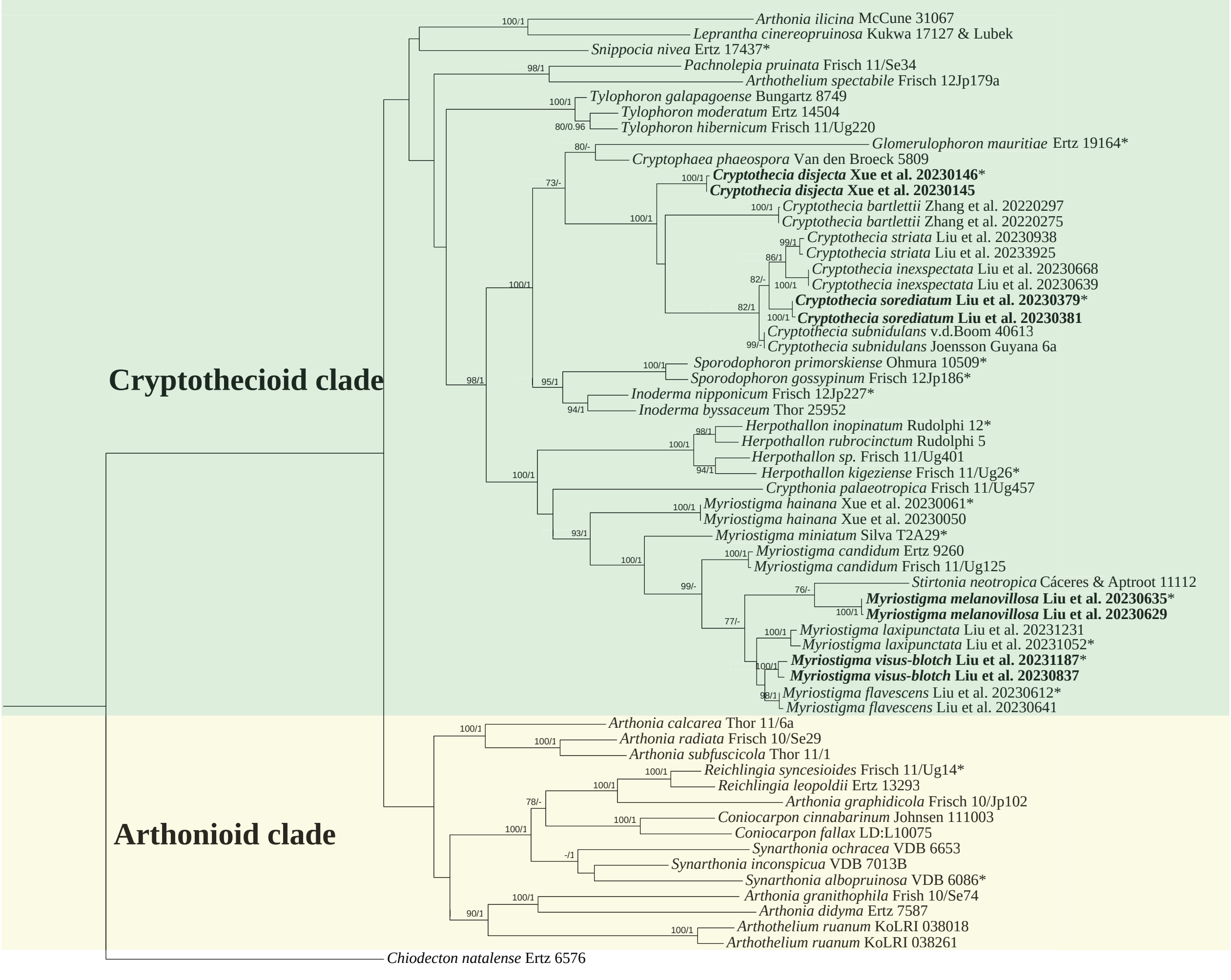

**Figure 1.** Phylogenetic tree constructed via maximum likelihood (ML) analysis of Arthoniaceae species on the basis of the concatenated mtSSU-RPB2-nuLSU dataset. Bootstrap support values ≥ 70 for ML and posterior probabilities ≥ 0.95 (second value) for Bayesian methods are indicated above or below the branches. Newly obtained sequences are marked in bold, * represents type material.

## Results

### Phylogenetic analyses

A total of 8 mtSSU sequences, 4 RPB2 sequences and 1 nuLSU sequence were newly generated from 8 specimens. We constructed ML and BI topologies on the basis of these mtSSU, RPB2 and nuLSU sequences and 108 additional sequences downloaded from NCBI (https://www.ncbi.nlm.nih.gov/) (Table 1). The phylogenetic trees obtained from the ML and BI analyses presented similar topologies; therefore, we present only the ML tree, with BS ≥ 70 for the ML analysis and PP ≥ 0.95 for the Bayesian analysis (Fig. 1).

The phylogenetic tree structure we obtained was similar to that described in previous studies (Frisch et al. 2014; Thiyagaraja et al. 2020). All the species positions' strongly supported the results of the ML and Bayesian analyses. Our phylogenetic tree revealed that *C. disjecta* and *C. sorediatum* clustered with *C. subnidulans* (the type species of *Cryptothecia*). However, on the basis of differences in phylogeny and morphology compared with those of *C. subnidulans*, which are described in detail below, they are classified as two new species. *M. melanovillosa* and *M. visus-blotch* clustered with *Myriostigma* and *Stirtonia neotropica* Aptroot. According to Aptroot (2009), *S. neotropica* has transversely septate ascospores; thus, on the basis of its morphological characteristics and phylogenetic analysis, we propose two new species in *Myriostigma*. Therefore, on the basis of morphological characteristics and phylogenetic analysis of the combined mtSSU, RPB2 and nuLSU sequence datasets, there is sufficient evidence to verify four previously unknown new species: *Cryptothecia disjecta* J.X. Xue & Lu L. Zhang, sp. nov.; *C. sorediatum* J.X. Xue & Lu L. Zhang, sp. nov.; *Myriostigma melanovillosa* J.X. Xue & Lu L. Zhang, sp. nov.; and *M. visus-blotch* J.X. Xue & Lu L. Zhang, sp. nov.

## Taxonomy

### *Cryptothecia disjecta* J.X. Xue & Lu L. Zhang, sp. nov.

MycoBank No: 855064
Fig. 2

**Diagnosis.** The new species is distinguished from other *Cryptothecia* species by its verrucose pseudisidia, which are loosely scattered on the thallus. The upper parts of the pseudisidia are white or have darker dots.

**Type.** China • Hainan Province, Baoting Li and Miao Autonomous County, Qixianling Hot Spring National Forest Park, 18°42'14.43"N, 109°41'47.92"E, alt. 325 m, on the bark of trees, 8 March 2023, J.X. Xue et al. 20230146 (SDNU, holotype).

**Description.** ***Thallus*** corticolous, up to 15 cm in diameter, ecorticate, cottony, dull, pale green, loosely attached to the substrate. ***Pseudisidia*** verrucose, loosely scattered on the thallus, upper parts white or with darker dots, and most pseudisidia have few projecting hyphae, 0.13–0.26 × 0.13–0.22 mm. ***Prothallus*** is usually distinct, thin, whitish byssoid, mainly composed of interwoven and radiating hyphae, 1.1–1.5 mm wide. ***Medulla*** white, with calcium oxalate crystals. ***Photobionts*** trentepohlioid, cells elliptical to oblong, single or aggregate into bundles, 10–23 × 7–18 μm. ***Hyphae*** 1–2.5 μm wide.

***Asci and pycnidia*** were not observed.

**Chemistry.** thallus C+ red, K+ pale yellow, P–, UV+ pale grey-white; medulla and paraphysoids I+ sky-blue. TLC: gyrophoric acid and lecanoric acid.

**Etymology.** The epithet refers to its pseudisidia, which are verrucose and loosely distributed across the thallus.

**Ecology and distribution.** This species is found only in China on the bark of trees in a humid tropical forest in Hainan Province.

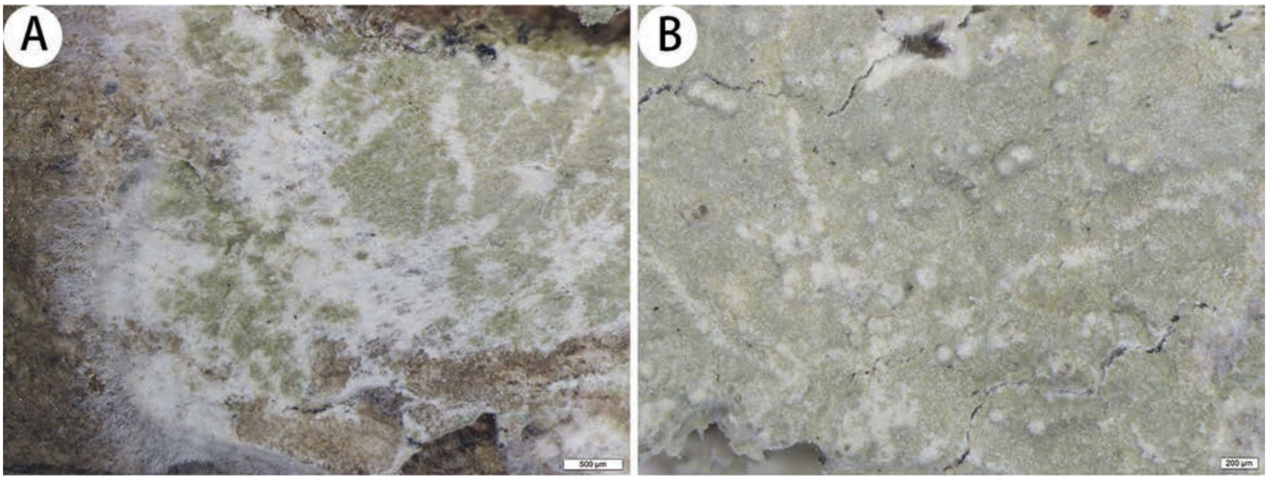

**Figure 2.** *Cryptothecia disjecta* (SDNU 20230144, type) **A** thallus and prothallus **B** pseudisidia. Scale bars: 500 μm (**A**); 200 μm (**B**).

**Notes.** Morphologically, *Cryptothecia disjecta* is similar to both *Herpothallon* and *Cryptothecia*. However, we describe *C. disjecta* on the basis of its morphological and chemical characteristics and assign it to *Cryptothecia* on the basis of sequencing (Aptroot et al. 2024). *Cryptothecia disjecta* is similar to *C. eungellae* G. Thor, as they both have whitish byssoid prothallus, I+ sky-blue medulla and C+ red thallus, but *C. eungellae* has 1-spored asci, muriform ascospores, and gyrophoric acid and norstictic acid as secondary metabolites (Thor 1997; Jagadeesh Ram and Sinha 2016).

Phylogenetically, *Cryptothecia disjecta* is clustered with *C. bartlettii* G. Thor. They both have cottony thallus, whitish byssoid prothallus and C+ red thallus, but *C. bartlettii* has delimited ascigerous areas (developing in the thallus centre and covered with globose isidia-like structures), 1-spored asci and muriform ascospores (Thor 1997).

**Additional specimens examined.** China • Hainan Province, Baoting Li and Miao Autonomous County, Qixianling Hot Spring National Forest Park, 18°42'14.43"N, 109°41'47.92"E, alt. 325 m, on the bark of trees, 8 March 2023, J.X. Xue et al. 20230144 (SDNU); • *ibid*., 20230145 (SDNU).

### *Cryptothecia sorediatum* J.X. Xue & Lu L. Zhang, sp. nov.

MycoBank No: 855065
Fig. 3

**Diagnosis.** The new species differs from other species of *Cryptothecia* in its soralia and I– medulla.

**Type.** China • Yunnan Province, Xishuangbanna Dai Nationality Autonomous Prefecture, Jinghong City, Jinuo Mountain, Jinuo Ethnic Township, 21°54'52.26"N, 101°11'33.04"E, alt. 630 m, on the bark of trees, 3 March 2023, L.L. Liu et al. 20230379 (SDNU, holotype).

**Description.** ***Thallus*** corticolous, up to 4 cm in diameter, ecorticate, cottony, dull, pale green, firmly attached to the substrate. ***Soralia*** globose, with many calcium oxalate crystals, 0.07–0.2 × 0.07–0.2 μm. ***Prothallus*** is usually distinct, thick, whitish byssoid, mainly composed of interwoven and radiating hyphae,

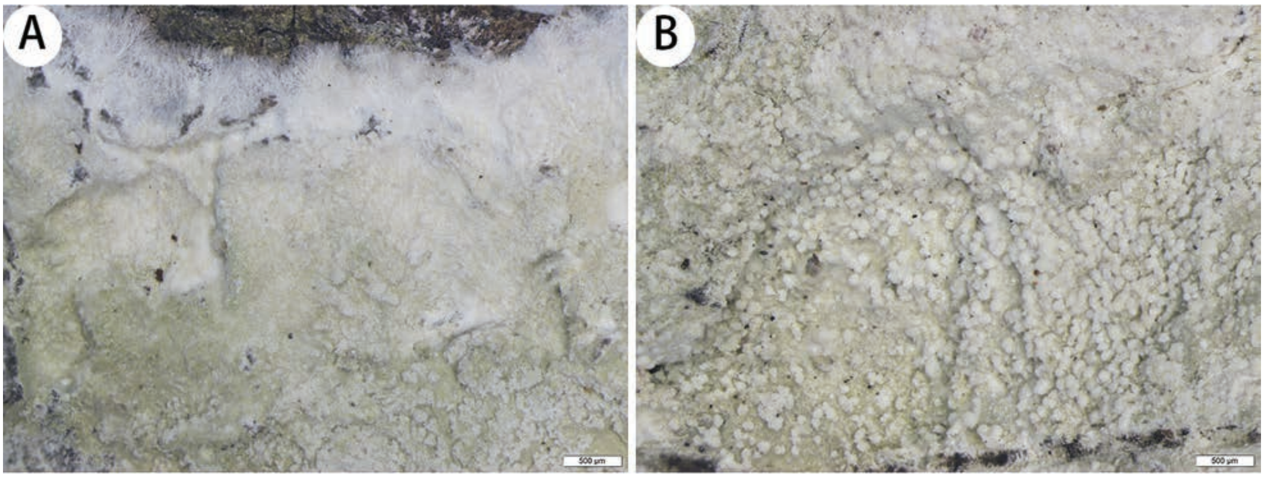

**Figure 3.** *Cryptothecia sorediatum* (SDNU 20230377, type) **A** thallus and prothallus **B** soralia. Scale bars: 500 μm (**A, B**).

0.9–1.7 mm wide. ***Medulla*** white, with calcium oxalate crystals. ***Photobionts*** trentepohlioid, cells rounded to elliptical, single or aggregate into bundles, 7–12 × 6–12 μm. ***Hyphae*** 1–2.5 μm wide.

***Asci and pycnidia*** were not observed.

**Chemistry.** thallus C+ red, K–, P–, UV+ pale grey-white; medulla and paraphysoids **I**–. TLC: gyrophoric acid and lecanoric acid.

**Etymology.** The epithet refers to the presence of soralia on its thallus.

**Ecology and distribution.** This species is found only in China on the bark of trees in a humid tropical forest in Yunnan Province.

**Notes.** Morphologically, *Cryptothecia sorediatum* is similar to *C. bartlettii* G. Thor, as they both have C+ red thallus and globose structures (*C. sorediatum* has globose soralia, and *C. bartlettii* has globose isidia-like structures) on their thallus, but *C. bartlettii* has **I**+ sky-blue medulla, 1-spored asci and muriform ascospores (Thor 1997).

Phylogenetically, *Cryptothecia sorediatum* is clustered with *C. subnidulans* Stirton, *C. inexspectata* G. Thor and *C. striata* G. Thor. They all have a cottony thallus and whitish byssoid prothallus, but *C. subnidulans* has a C– thallus, muriform ascospores (69–90 × 29–44 μm) and psoromic acid as a secondary metabolite (Thor 1997). *C. inexspectata* has whitish ascigerous areas, **I**+ sky-blue medulla and muriform ascospores (33–50 × 16–22 μm) (Thor 1997). *C. striata* has granular isidia-like structures on the thallus, **I**+ sky-blue medulla and muriform ascospores [(46–)55–70(–80) × (19–)23–29(–37) μm] (Thor 1991).

**Additional specimens examined.** China • Yunnan Province, Xishuangbanna Dai Nationality Autonomous Prefecture, Jinghong City, Jinuo Mountain, Jinuo Ethnic Township, 21°54'52.26"N, 101°11'33.04"E, alt. 630 m, on the bark of trees, 3 March 2023, L.L. Liu et al. 20230377 (SDNU); • *ibid*., 20230381 (SDNU).

### *Myriostigma melanovillosa* J.X. Xue & Lu L. Zhang, sp. nov.

MycoBank No: 855066
Fig. 4

**Diagnosis.** The new species differs from other species of *Myriostigma* in the presence of black or purple dots on the thalli and hyaline to pale yellow ascospores (63–71 × 26–33 μm).

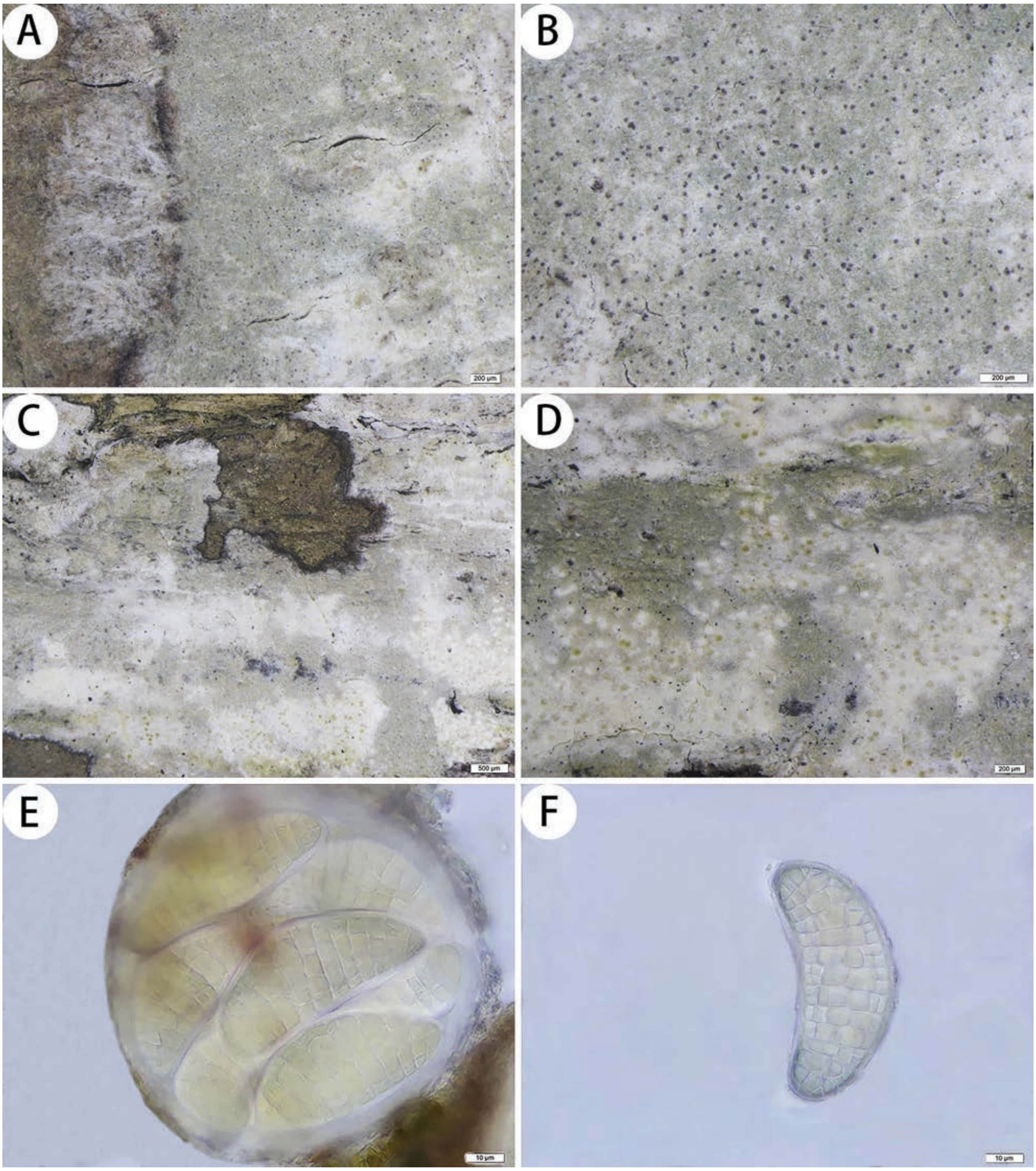

**Figure 4.** *Myriostigma melanovillosa* (SDNU 20230629, type for (**A, B**); SDNU 20230635, holotype for (**C, D, E, F**)) **A** prothallus **B** black or purple dots **C** thallus **D** ascigerous areas **E** asci **F** ascospores. Scale bars: 200 µm (**A, B, D**); 500 µm (**C**); 10 µm (**E, F**).

**Type.** CHINA • Yunnan Province, Xishuangbanna Dai Nationality Autonomous Prefecture, Jinghong City, Primitive Forest Park, 22°1'55.75"N, 100°52'37.47"E, alt. 689 m, on the bark of trees, 7 March 2023, L.L. Liu et al. 20230635 (SDNU, holotype).

**Description.** ***Thallus*** corticolous, up to 6 cm in diameter, ecorticate, cottony, dull, greenish grey to whitish grey, with black or purple dots, firmly attached to the substrate. ***Isidia*** not observed. ***Prothallus*** is usually distinct, thin, whitish byssoid, mainly composed of interwoven and radiating hyphae, 0.7–1.3 mm wide, forming a dark brown to black line while bordering different species. ***Medulla*** white, with calcium oxalate crystals. ***Photobionts*** trentepohlioid, cells rounded to elliptical, single or a few cells aggregated, 5–11 × 5–8 μm. ***Hyphae*** 1–2 μm wide.

***Ascigerous areas*** are distinct, generally delimited, erumpent, slightly raised above the thallus level, plaque, irregular in outline, white with dense brown dots indicating individual asci. ***Asci*** frequent, hyaline, pale yellow when mature, globose to subglobose, often covered by hyaline hyphae, 8-spored, 103–115 × 97–115 μm. ***Ascospores*** hyaline, pale yellow when mature, oblong, muriform, curved, often broader in the centre, 63–71 × 26–33 μm.

***Pycnidia*** were not observed.

**Chemistry.** thallus C+ red, K–, P–, UV+ pale grey-white; medulla and paraphysoids I+ sky-blue. TLC: gyrophoric acid, lecanoric acid and confluentic acid.

**Etymology.** The epithet refers to the presence of black or purple dots on the thallus.

**Ecology and distribution.** This species is found only in China on the bark of trees in a humid tropical forest in Yunnan Province.

**Notes.** Morphologically, *Myriostigma melanovillosa* is similar to *M. irregularis* Lücking, Aptroot, Kalb & Elix, as they both have irregular erumpent and with brown dots whitish ascigerous areas, but *M. irregularis* has narrower asci (40–70 μm wide) and psoromic acid, subpsoromic acid, 2'-*O*-demethylpsoromic acid and trace confluentic acid as secondary metabolites (Lücking et al. 2006).

Phylogenetically, *Myriostigma melanovillosa* is clustered with *Stirtonia neotropica* Aptroot. They both have C+ red thalli and 8-spored asci, but *S. neotropica* has linear shape ascigerous areas, ovoid asci and transversely septate ascospores (35–38 × 10–12 μm) (Aptroot 2009).

**Additional specimens examined.** China • Yunnan Province, Xishuangbanna Dai Nationality Autonomous Prefecture, Jinghong City, Primitive Forest Park, 22°1'55.75"N, 100°52'37.47"E, alt. 689 m, on the bark of trees, 7 March 2023, L.L. Liu et al. 20230629 (SDNU); • *ibid*., 20234628 (SDNU).

### ***Myriostigma visus-blotch*** J.X. Xue & Lu L. Zhang, sp. nov.

MycoBank No: 855067
Fig. 5

**Diagnosis.** The new species differs from other species of *Myriostigma* in ascigerous areas, which have many irregular small patches that are scattered or clustered together radially elongated; hyaline ascospores (31–)37–74 × (14–)17–29 μm.

**Type.** China • Yunnan Province, Xishuangbanna Dai Nationality Autonomous Prefecture, Jinghong City, Primitive Forest Park, 22°2'9.71"N, 100°53'5.81"E, alt. 716 m, on the bark of trees, 7 March 2023, L.L. Liu et al. 20230681 (SDNU, holotype); • *ibid*., Mengla County, Menglun Town, Xishuangbanna Tropical Botanical Garden, 21°55'12.06"N, 101°16'5.55"E, alt. 496 m, on the bark of trees, 5 March 2023, L.L. Liu et al. 20231187 (SDNU, paratype).

**Description.** ***Thallus*** corticolous or bambusicolous, up to 6.5 cm in diameter, ecorticate, cottony, dull, pale green to greenish grey, firmly attached to the substrate. ***Isidia*** not observed. ***Prothallus*** is usually distinct, thin, whitish byssoid, mainly composed of interwoven and radiating hyphae, 0.8–2.5 mm wide,

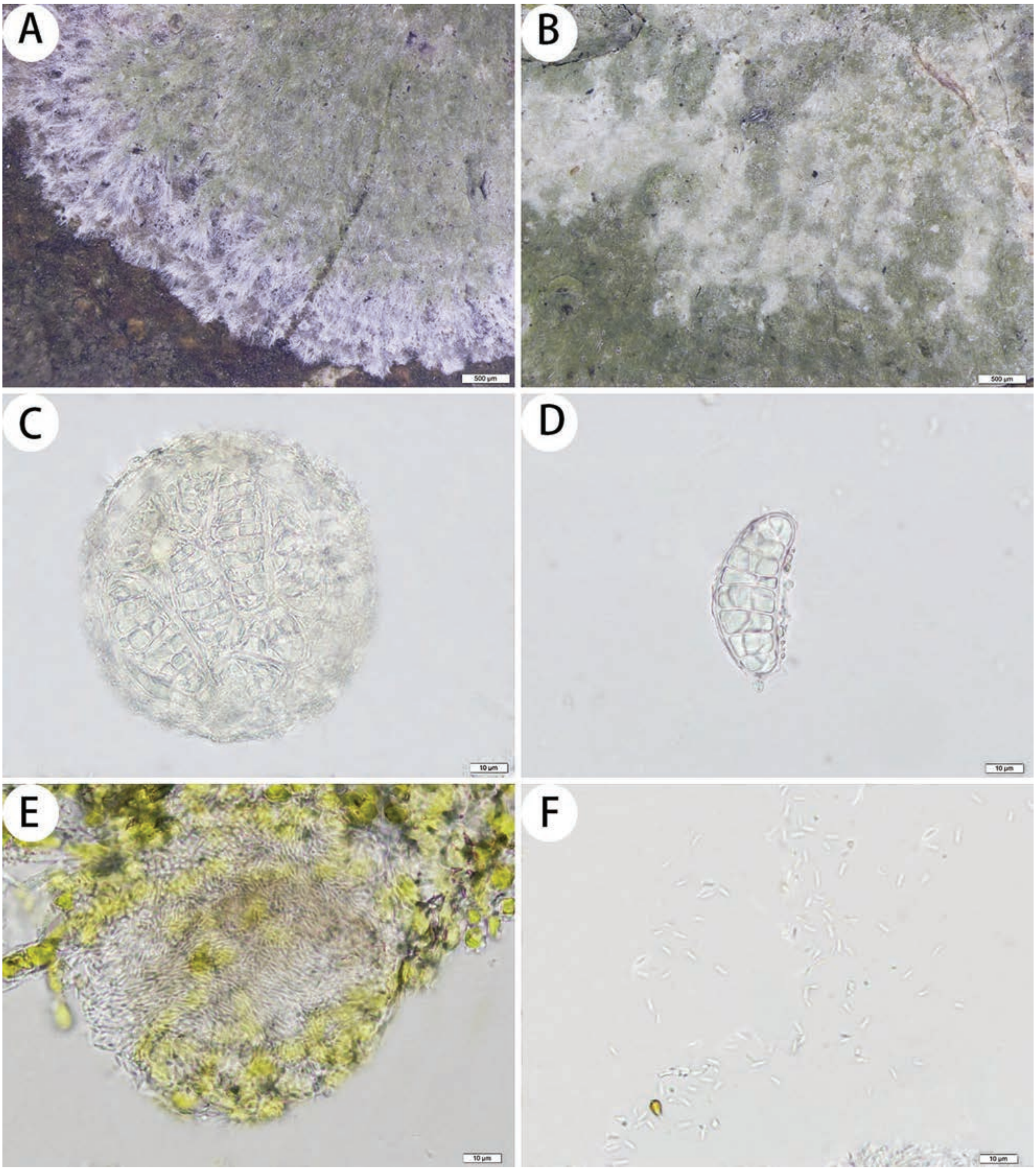

**Figure 5.** *Myriostigma visus-blotch* (SDNU 20230950, type for (**A, B**); SDNU 20230681, holotype for (**C, D**); SDNU 20230847, type for (**E, F**)) **A** thallus and prothallus **B** ascigerous areas **C** asci **D** ascospores **E** pycnidia **F** conidia. Scale bars: 500 μm (**A, B**); 10 μm (**C, D, E, F**).

forming a dark brown to black line while bordering different species. ***Medulla*** white, with calcium oxalate crystals. ***Photobionts*** trentepohlioid, cells elliptical to oblong, single or aggregate into bundles, 6–14 × 6–9 μm. ***Hyphae*** 1–2 μm wide.

***Ascigerous areas*** are distinct, generally delimited, slightly raised above the thallus level, developing in the thallus centre, colour lighter than the thallus, many irregular small patches that are scattered or clustered together radially elongated. ***Asci*** hyaline, globose to subglobose, often covered by hyaline hyphae, 8-spored, 82–91 × 81–90 µm. ***Ascospores*** hyaline, oblong, muriform, curved, often broader in the centre, (31–)37–74 × (14–)17–29 µm.

***Pycnidia*** hyaline, globose to subglobose, scattered and immersed across the thallus, 55–85 × 54–80 µm. ***Conidia*** hyaline, short bacilliform, 4–5 × 0.9–1 µm.

**Chemistry.** thallus C+ red, K+ pale yellow, P–, UV+ pale grey-white; medulla and paraphysoids I+ sky-blue. TLC: gyrophoric acid, lecanoric acid and confluentic acid.

**Etymology.** The epithet refers to its ascigerous areas having many irregular pale green small patches.

**Ecology and distribution.** This species is found only in China on bamboo and trees in humid tropical forests in Hainan and Yunnan Provinces.

**Notes.** *Myriostigma visus-blotch* is widely distributed in the Xishuangbanna Dai Nationality Autonomous Prefecture. The ascigerous areas of *M. visus-blotch* begin as a small bulge slightly higher than the thallus and gradually become multiple small patches or radially elongated in the centre of the thallus, and the colour of the ascigerous areas is pale green. In addition, we found that species with asci generally do not have pycnidia, and species with pycnidia generally do not have asci.

Phylogenetically, *Myriostigma visus-blotch* is clustered with *M. flavescens* J.X. Xue & Lu L. Zhang and *M. laxipunctata* J.X. Xue & Lu L. Zhang. They all have C+ red thalli, globose asci and muriform ascospores, but *M. flavescens* has a black linear shape prothallus, white ascigerous areas and yellow ascospores (58–76 × 19–28 µm). *M. laxipunctata* has complete ascigerous areas (not small patches or radially elongated), larger asci (95–124 × 93–119 µm), and loose brown dots indicating asci (Xue et al. 2024).

**Additional specimens examined.** China • Hainan Province, Baoting Li and Miao Autonomous County, Qixianling Hot Spring National Forest Park, 18°42'14.43"N, 109°41'47.92"E, alt. 325 m, on the bark of trees, 8 March 2023, J.X. Xue et al. 20230142 (SDNU); • *ibid*., 20230138 (SDNU); • *ibid*., 20230134 (SDNU); • Yunnan Province, Xishuangbanna Dai Nationality Autonomous Prefecture, Jinghong City, Mengla County, Menglun Town, Xishuangbanna Tropical Botanical Garden, 21°55'45.40"N, 101°15'29.17"E, alt. 518 m, on the bark of trees, 4 March 2023, L.L. Liu et al. 20230950 (SDNU); • *ibid*., 20230969 (SDNU); • *ibid*., 21°55'30.14"N, 101°15'42.72"E, alt. 527 m, on the bark of trees, 5 March 2023, L.L. Liu et al. 20233946 (SDNU); • *ibid*., 21°55'50.99"N, 101°15'33.29"E, alt. 512 m, on the bark of trees, 4 March 2023, L.L. Liu et al. 20230973 (SDNU); • *ibid*., 21°55'55.96"N, 101°14'35.00"E, alt. 560 m, on the bark of trees, 4 March 2023, L.L. Liu et al. 20230847 (SDNU); • *ibid*., on bamboo, 4 March 2023, L.L. Liu et al. 20230837 (SDNU); • *ibid*., Jinuo Mountain, Jinuo Ethnic Township, 21°54'52.26"N, 101°11'33.04"E, alt. 640 m, on the bark of trees, 3 March 2023, L.L. Liu et al. 20230502 (SDNU); • *ibid*., Wild Elephant Valley, 22°10'37.70"N, 100°51'24.54"E, alt. 749 m, on the bark of trees, 6 March 2023, L.L. Liu et al. 20230602 (SDNU); • *ibid*., Primitive Forest Park, 22°2'9.71"N, 100°53'5.81"E, alt. 746 m, on the bark of trees, 7 March 2023, L.L. Liu et al. 20230692 (SDNU).

## Discussion

*Cryptothecia* was established in 1877 (Stirton 1877), and *Myriostigma* was established in 1874 (Krempelhuber 1874). These genera are prevalent in the humid tropical and subtropical forests of southern China, boasting a remarkable species diversity. Nevertheless, their morphological distinctions are scarce, making molecular analyses indispensable for classification and species identification. This study focused on Cryptothecioid lichens in Yunnan and Hainan Provinces of China, employing phylogenetic analyses based on DNA sequence data, and identified four novel species: *C. disjecta*, *C. sorediatum*, *M. melanovillosa*, and *M. visus-blotch*.

Prior to this study, China had reported eight species of *Cryptothecia* (*C. aleurella*, *C. aleurocarpa*, *C. bartlettii*, *C. inexspectata*, *C. polymorpha*, *C. striata*, *C. subnidulans*, and *C. subtecta*) and four species of *Myriostigma* (*M. candidum*, *M. flavescens*, *M. hainana*, and *M. laxipunctata*), as documented by Aptroot and Sipman (2001), Aptroot and Sparrius (2003), Aptroot and Rodrigues (2005), Xue et al. (2024), and Zhang et al. (2024). The two new *Cryptothecia* species, *C. disjecta* and *C. sorediatum*, are readily distinguishable from all other Chinese *Cryptothecia* species by their sterile thalli featuring verrucose pseudisidia (*C. disjecta*) or globose soralia (*C. sorediatum*), along with gyrophoric acid and lecanoric acid as secondary metabolites. Among the other Chinese *Cryptothecia* species containing gyrophoric acid, namely *C. bartlettii*, *C. inexspectata*, and *C. striata*, all are fertile (Thor 1997; Xue et al. 2024; Zhang et al. 2024). *Myriostigma melanovillosa* is further distinguished from all other Chinese *Myriostigma* species by its thallus with black or purple dots, a whitish byssoid prothallus, and the presence of gyrophoric acid and confluentic acid. It also exhibits white plaque ascigerous areas with dense brown dots indicating individual asci, and hyaline to pale yellow ascospores. Although *M. flavescens* and *M. laxipunctata* also contain gyrophoric acid and confluentic acid, *M. flavescens* can be differentiated by its black line prothallus, smaller asci (95–100 × 83–95 μm), and yellow ascospores. Meanwhile, *M. laxipunctata* is distinguished by its pale greenish complete ascigerous area with loose brown dots (Xue et al. 2024). *M. visus-blotch* further distinguishes itself from all other Chinese *Myriostigma* species by its whitish byssoid prothallus, the presence of gyrophoric acid and confluentic acid, and pale greenish patchy (scattered or clustered together radially elongated) ascigerous areas. *Myriostigma hainana* also possesses a whitish byssoid prothallus and gyrophoric acid, but it can be differentiated by its indistinct ascigerous area, larger asci (120–138 × 120–135 μm), and the additional presence of methyl 2’-*O*-methylmicrophyllinate alongside gyrophoric acid (Xue et al. 2024).

In summary, the molecular phylogenetic and morphological results support the identification of the four new species in this study.

## Key to the *Cryptothecia* species occurring in China

1 Thallus sterile; without asci .......... **2**
– Thallus fertile; with asci .......... **3**
2 Thallus with verrucose pseudisidia; medulla I+ sky-blue .......... ***C. disjecta***
– Thallus with globose soralia; medulla I– .......... ***C. sorediatum***
3 Asci 1–2-spored .......... **4**
– Asci 8-spored .......... **7**
4 Thallus P+ yellow; with psoromic acid .......... ***C. subnidulans***
– Thallus P–; without psoromic acid .......... **5**

5 Thallus loosely attached to the substrate; ascigerous areas generally covered with globose isidia-like structures; ascospores (49–)68–100(–105) × (18–)23–36(–42) µm ........ ***C. bartlettii***
– Thallus firmly attached to the substrate; ascigerous areas whitish and usually radially elongated ........ **6**
6 Thallus generally with granula isidia-like structures; ascospores 54–80 × 21–42 µm ........ ***C. striata***
– Thallus generally without isidia-like structures; ascospores 33–50 × 16–22 µm ........ ***C. inexspectata***
7 Thallus without lichen substances ........ **8**
– Thallus with lichen substances ........ **9**
8 Ascospores narrow; 60–76 × 17–30 µm ........ ***C. aleurella***
– Ascospores broad; 65–108 × 42–50 µm ........ ***C. aleurocarpa***
9 Thallus P+ yellow; with psoromic acid; ascospores 50–70 × 30–37 µm ........ ***C. polymorpha***
– Thallus P–; without psoromic acid; ascospores 27–40 × 12–20 µm ........ ***C. subtecta***

## Key to the *Myriostigma* species occurring in China

1 Ascigerous areas indistinct; thallus with gyrophoric acid and methyl 2'-*O*-methylmicrophyllinate; ascospores 52–88 × 24–47 µm ... ***M. hainana***
– Ascigerous areas distinct ........ **2**
2 Thallus with 2'-*O*-methylanziaic and 2'-*O*-methylperlatolic acids; ascospores 40–65 × 12–25 µm ........ ***M. candidum***
– Thallus with gyrophoric and confluentic acids ........ **3**
3 Ascigerous areas without brown dots indicate individual asci; ascospores hyaline (31–)37–74 × (14–)17–29 µm; some specimens have pycnidia........ ***M. visus-blotch***
– Ascigerous areas with brown dots indicate individual asci ........ **4**
4 Thallus with black or purple dots; ascospores hyaline to pale yellow 63–71 × 26–33 µm ........ ***M. melanovillosa***
– Thallus without black or purple dots ........ **5**
5 Ascigerous areas with dense brown dots; ascospores yellow 58–76 × 19–28 µm ........ ***M. flavescens***
– Ascigerous areas with loosely brown dots; ascospores hyaline 57–78 × 24–33 µm ........ ***M. laxipunctata***

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

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## Author contributions

Junxia Xue performed the specimen collection, data analysis and draft writing; Zihao Yang and Ruotong Li performed the experimental work and draft modification; Lulu Zhang designed the project, supervised the research, revised the manuscript and provided funding.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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