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# The genus *Hebeloma* in the Rocky Mountain Alpine Zone

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## Abstract

Numerous taxa of *Hebeloma* have been reported in association with *Salix*, *Dryas*, and *Betula* in arctic-alpine habitats. However, species are notoriously difficult to delineate because morphological features overlap, and previously there was little reliable molecular data available. Recent progress in ITS-sequencing within the genus, coupled with an extensive database of parametrically described collections, now allows comparisons between species and their distributions. Here we report 16 species of *Hebeloma* from the Rocky Mountain alpine zone from some of the lowest latitudes (latitude 36°–45°N) and highest elevations (3000–4000 m) for arctic-alpine fungi in the northern hemisphere. Twelve of these species have been reported from arctic-alpine habitats in Europe and Greenland and are now molecularly confirmed from the Middle and Southern Rockies, greatly expanding their distribution. These are: *Hebeloma alpinum*, *H. aurantioumbrinum*, *H. dunense*, *H. hiemale*, *H. marginatulum*, *H. mesophaeum*, *H. nigellum*, *H. oreophilum*, *H. subconcolor*, *H. spetsbergense*, *H. vaccinum*, and *H. velutipes. Hebeloma hygrophilum* is known from subalpine habitats in Europe, but was never recorded in arctic-alpine ecology. Three species recorded from the Rockies, but as yet not reported from Europe, are *H. alpinicola*, *H. avellaneum*, and *H. excedens*. The last two have never previously been reported from an arctic-alpine habitat. For all three of these species, the holotypes have been studied morphologically and molecularly, and have been incorporated into the analysis.

## Keywords

A.H. Smith, Arctic-alpine, ectomycorrhizal, fungal biodiversity, *Hymenogastraceae*, ITS, systematics

## Introduction

The alpine is defined as the life zone above treeline on high mountain tops and this biome constitutes 3% of the earth's land (Körner 1999). In northern latitudes, it is characterized by low, open vegetation and a climate dominated by cold temperatures (Chapin and Körner 1995). Diurnal temperature fluctuations and periodic strong winds during the short growing season affect both plant development and basidiome production. Ectomycorrhizal fungi are critical to the survival of alpine woody plants such as *Salix*, *Dryas*, *Betula*, and non-woody plants such as *Persicaria* (*Bistorta*) and *Kobresia* in the alpine zone (Cripps and Eddington 2005). The most diverse ectomycorrhizal fungal genera in the Northern Hemisphere alpine are *Cortinarius*, *Inocybe*, *Hebeloma*, *Laccaria*, *Entoloma*, *Lactarius* and *Russula* (Gardes and Dahlberg 1996; Cripps and Horak 2008).

The Rocky Mountain alpine exists as islands on high mountain tops and plateaus separated by vast forests and grasslands. The middle and southern Rockies span some of the lowest latitudes (36°–45° N) and highest elevations (3000–4000 m) known for northern hemisphere alpine. Yet, species of *Inocybe* and *Lactarius* from the Rocky Mountain alpine zone have been found to be conspecific with those occurring in arctic and alpine habitats in the Alps, Pyrenees, Norway, Sweden, Finland, Svalbard, and Greenland through molecular matching of ITS (internally transcribed spacer) sequences (Cripps et al. 2010; Larsson et al. 2014; Barge et al. 2016; Barge and Cripps 2016).

The genus *Hebeloma* is common in arctic and alpine habitats, but species are poorly known. It is phylogenetically placed in the *Hymenogastraceae* Vittad. (Matheny et al. 2006) and is characterized by smooth to roughened brown spores that lack a visible germ pore, distinct cheilocystidia, an absence (usually) of pleurocystidia, and an ixocutis resulting in a smooth viscid pileus which is often two-colored (usually darker in the center). Distinctive odors, typically of radish or raw potato described as raphanoid are often present (Vesterholt 2005). However, not all species exhibit all features and character states overlap. Although most experienced mycologists will normally be able to identify a mushroom as a *Hebeloma* with relative ease, taxa are notoriously difficult to delineate at the species level because of variable morphological features and, until recently, a lack of reliable reference literature and a lack of confirmed DNA reference sequences of type materials. While the recent monograph by Beker et al. (2016) provides a great deal of reference material, this was centered on the *Hebeloma* of Europe; overlap between the European and American continents is currently being studied.

Numerous taxa of *Hebeloma* have been reported in association with *Persicaria*, *Betula*, *Salix*, and *Dryas* from arctic-alpine habitats including those in the Alps (Favre 1955; Bon 1986; Bruchet 1974; Debaud et al. 1981; Kühner and Lamoure 1986; Senn-Irlet 1990; Senn-Irlet 1993; Jamoni 2008; Graf 1994; Brunner et al. 2017), Iceland (Eyjolfsdottir 2009), Scandinavia (Vesterholt 2005, 2008; Knudsen and Vesterholt 2008), Svalbard (Hutinen 1987; Ohenoja 1971; Gulden and Torkelsen 1996; Beker et al. 2018), Pyrenees (Corriol 2008), and the Carpathians (Eberhardt et al. 2015b). In North America, there are reports from Greenland (Lange 1957; Borgen

2006; Borgen et al. 2006), Canada (Ohenoja and Ohenoja 1993, 2010), Alaska (Miller 1998), and the Rocky Mountains (Miller and Evenson 2001; Cripps and Horak 2008; Beker et al. 2010). A table comparing the occurrence of species in various arctic and alpine locations was presented in Beker et al. (2018); this table indicates 10 species from the Rocky Mountains. Beker and co-workers (2016) list 25 species occurring in arctic or alpine habitats, 14 of which appear (almost) restricted to these habitats; others also occur in a variety of habitats from subalpine or boreal with coniferous and hardwood trees right down to sand dunes where they grow with dwarf *Salix*. The veiled species of *Hebeloma* in Western North America have been treated in a monograph by Smith et al. (1983), but few (if any) of their collections are from above treeline, although many are from high elevations in the Rocky Mountains. While recent work on the genus *Hebeloma* in Europe now provides a basis for comparison of morphological and molecular data for a significant number of species and make possible comparisons of distribution patterns (Vesterholt 2005; Beker et al. 2016), much more work is needed before we will have a complete picture of the different species that occur on the different continents and their distribution across those continents. Here we delineate 16 species of veiled and unveiled *Hebeloma* primarily with *Salix* from the Rocky Mountain alpine zone. Thirteen of these taxa were described in detail in Beker et al. (2016) but three species described here were not included in that discussion of European *Hebeloma*. These three species (*H. alpinicola* A.H. Sm., Evenson & Mitchel, *H. avellaneum* Kauffman, and *H. excedens* (Peck) Sacc.), whose holotypes have been studied morphologically and molecularly, are described within this paper and their relationship with other *Hebeloma* species is explored.

As demonstrated in Beker et al. (2016), morphological differences do exist between species and although separation between species does need careful work, in almost all cases a morphological analysis may be used for determination of species and in some cases morphology is even better suited for species delimitation than the data of the five loci applied. Here we have carried out a morphological analysis to determine species and have found no conflict between our morphological placement and that provided by our molecular analysis based on ITS data. Tree and network building methods have been applied to demonstrate the taxonomic placement of the Rocky Mountains collections in relation to type specimens and confirmed collections of species treated by Beker et al. (2016). For the three species not treated in Beker et al. (2016) we include type sequences from American types. We do not provide lists of synonyms in the species descriptions, because we have not yet re-evaluated all species described outside Europe and any list that we could give would be provisional. Where we deem it necessary, synonyms are mentioned in species discussions. Species names and their synonyms from Europe have been treated to great detail by Beker et al. (2016).

A great majority of the encountered species was shown to be paraphyletic and part of species complexes by Beker et al. (2016) and previous works (Eberhardt et al. 2015a, 2016; Grilli et al. 2016). In the course of the studies for this work we found that the same is true for two species (*H. alpinicola* and *H. excedens*) not treated by Beker et al. (2016). We have chosen to illustrate the problems of species recognition

and delimitation based on ITS data by showing networks for taxa treated by Eberhardt et al. (2015a, 2016) and Grilli et al. (2016), i.e. members of the *H*. sects. *Denudata* and *Velutipes*; and in addition to trees for members of *H.* sect. *Hebeloma*. The ITS region of members of these species complexes often differs only by a small number of base pairs between species, and comparable differences occur within species. Additionally, species often do not form monophyla within these complexes.

Median-Joining Networks have been recommended for inferring intraspecific phylogenies (i.e. Bandelt et al. 1999). Pruned quasi-median networks (Ayling and Brown 2008) are a tool to visualize DNA sequence variation when evolution has not necessarily been treelike. No assumptions are made as to which evolutionary mechanisms (i.e. hybridization, recombination, etc.) have been responsible for the observed variation. In the networks, observed sequence variants are shown as circles and the size of each circle represents the number of times the respective sequence variant has been observed. Two circles connected by an unsegmented line differ in 1 bp. So-called quasi-medians, a kind of placeholder for unobserved sequence variants, are placed between observed sequence variants that each differ from the quasi-median by 1 bp. The number of segments to a line represents the number of base pair changes between two sequence variants or a sequence variant and a quasi-median. A pruning mechanism is applied to reduce the complexity of the networks while depicting at least one shortest path between all pairs of sequence variants (Ayling and Brown 2008).

Ideally, we would have been able to present networks of haplotypes. What we here refer to as 'ITS variants' are sequencing results of dikaryotic material; in many cases, the sequences do not seem to correspond to a single haplotype. Although the ITS exists in multiple copies in the genome, it has been shown to behave like a dikaryotic locus in *Hebeloma* (Aanen et al. 2001) and other fungi (i.e. Schnabel et al. 2005; Hughes et al. 2013). Even good quality reads of ITS and other nuclear loci of many *Hebeloma* species contain one or several ambiguous positions and/or indications of indels, which we consider as evidence of variation between haplotypes of the same locus. Here, the level of variation was such that attempts to phase all ITS data into haplotypes (Flot et al. 2006; Flot 2010) were aborted and each collection is represented by a single ITS variant, i.e. the consensus sequence of both 'haplotypes'.

## Methods

### Study sites

Our primary study sites are in the Middle-Northern and Southern Floristic zones of the Rocky Mountains that extend from Montana to Colorado (Fig. 1); the phytogeography is described in Cripps and Horak (2008) and further site details are in Barge et al. (2016) and Osmundson et al. (2005). Primary collecting sites include the Beartooth Plateau (latitude 45° N, elevation 3000–3500 m) in Montana and Wyoming, and the Front Range, Sawatch Range, and San Juan Mountains in Colorado (latitude 36°–

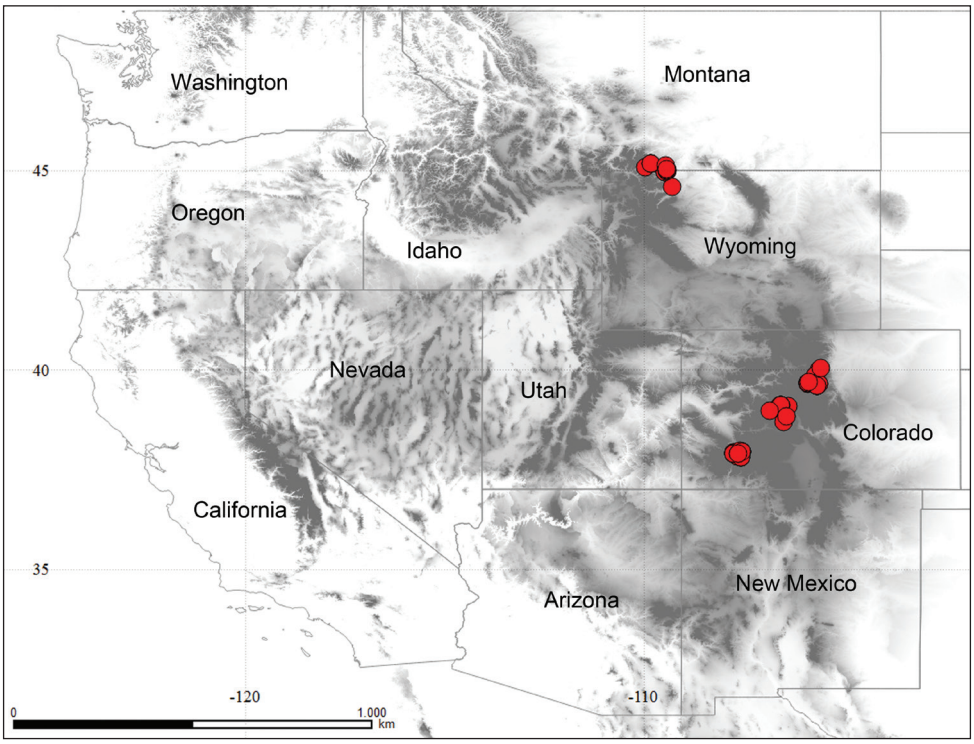


**Figure 1.** Distribution of Rocky Mountain alpine collections of *Hebeloma*. The map was generated with QGIS version 2.2.0 using WGS84 (EPDG: 4326; QGIS Development Team 2018). Shapefiles were provided by the Database of Global Administrative Areas (GADM, https://gadm.org/), accessed April 2018.

38° N, elevation 3600–4000 m). Ectomycorrhizal vascular plants include *Salix reticulata*, *S. arctica*, *S. rotundifolia*, *S. cascadensis*, *S. planifolia*, *S. glauca*, *Betula glandulosa* (= *B. nana*), *Dryas octopetala*, *Persicaria vivipara*, and *Kobresia mysuroides* (Cripps and Eddington 2005). While our study was focused on areas of tundra above the tree line, occasionally small *Picea* shrubs also occurred and it was not possible to unambiguously specify the mycorrhizal partner.

## Collections and morphological descriptions

Basidiomes were collected from late July through August, which constitutes the field season, from 1980 to 2017. Most collections were described in fresh condition, photographed, and dried on a dehydrator overnight. Dehydrated material was deposited in the MONT herbarium (Montana State University), ETH (Zurich, Switzerland), DBG (Denver Botanic Gardens), and/or the HJB private herbarium. Microscopic examination of dried material was done in 5% KOH to measure spores, cystidia, basidia, and other important features and in Melzer's solution to assess dextrinoid reactions follow-

ing Beker et al. (2016) and Vesterholt (2005). Within the species descriptions below we conform to spore descriptions based on spore ornamentation measures (O1–O4), spore dextrinoidity measures (D0–D3) and perispore loosening measure (P0–P3), as described in Beker et al. (2016). Similarly, cheilocystidia measurements include length, maximum width near the apex, minimum width in the median part of the cystidium and maximum width in the basal part of the cystidium. No distinction is made in the spore measurements for spores from two- and four-spored basidia. Measurements for the two types of spores are given separately in the Suppl. material 1. Exsiccate were also described. Unless otherwise mentioned, the species descriptions given are based on the collections from the Rocky Mountains cited here.

## Molecular analyses

ITS sequence data from the 115 *Hebeloma* collections from the Rocky Mountains (which is referred to as the RM dataset), 221 reference sequences including some type sequences from Europe (referred to as the FE (Fungi Europaei) dataset, see Beker et al. 2016) and 10 type collections of species described from the US, pertinent to the RM collections, were generated using a variety of protocols (Eberhardt 2012; Eberhardt et al. 2016). Newly generated sequences were submitted to GenBank (acc. no. MK280985–MK281025, MK286558–MK286561, and MK305906–MK305939).

The DNA of old material was extracted using the Gentra Puregene kit (Qiagen, Hilden, Germany), modifying the procedure that is described in the manual (version 2014) for yeasts, generally replacing any pipetting of DNA-containing fluids by pouring (see Eberhardt et al. 2016). A small amount of basidiome material was crushed in a TissueLyser II (Qiagen), suspended in 300 µl suspension solution plus 1.5 µl lytic enzyme for 30 min at 37 °C. The samples were centrifuged for 5 min at 8000 rpm and the supernatant poured out. Lysis was done in 300 µl of Cell Lysis Solution, the samples mixed by vortexing and incubated overnight at 37 °C, followed by 1 h at 65 °C. Samples were cooled to room temperature and 100 µl Protein Precipitation Solution added. Prior to centrifugation (maximum speed, 5 min), the samples were placed in the freezer for 10–15 min. Each sample was then poured into a prepared tube with 300 µl absolute isopropanol and 1 µl of glycogen (Life Technologies, Darmstadt; diluted 1:1 with ultrapure water). After mixing by repeatedly inverting for 1 min, the DNA was precipitated overnight to several days in the fridge. The pellets were washed in 300 µl 70% ethanol, air-dried for 30 min and re-desolved in 50 µl DNA Hydration Solution. The purified DNA was re-desolved by heating the samples for one hour at 65 °C and keeping them overnight at room temperature. DNA extracts were diluted for PCR as required. ITS1 and ITS2 were amplified separately in 35–40 cycles of PCR (30 s denaturation at 95 °C, 45 s annealing at 55 °C, and 60 s elongation at 72 °C) with 1.25 U/25 µl MyTaq Red (Bioline, Luckenwalde, Germany), using the primer pairs ITS1F/ITS2 and 58SF/ITS4 (White et al. 1990; Gardes and Bruns 1993; Tedersoo et al. 2013 [who erroneously

ascribed the primer 58SF (3' - ATG CAT CGA TGA AGA ACG C -5' to Martin and Rygiewicz 2005]). Sequencing was carried out at LGC (Berlin, Germany).

Taxonomic assignment to section and species cluster was done via BLAST searches against the collections analyzed in depth by Beker et al. (2016), the FE dataset, in Geneious R10 (version 10.2.3, Biolmatters, Auckland, NZ). To illustrate the taxonomic placement of the RM collections, eight alignments were assembled using Mafft online with the G-INS-I option (Katoh et al. 2017), breaking up the large number of sequences into manageable datasets based on BLAST results. Alignments include RM and FE representatives of the target species, i.e. species occurring in the Rockies, relevant types for non-European species, and (where applicable) FE sequences of taxa that cannot be unambiguously distinguished from the target taxa, i.e. neither target species nor sister species forming monophyla in the ITS analyses of Beker et al. (2016) for arctic-alpine species. For better readability, non-arctic-alpine sister species clearly distinct from the target species were excluded from the final analyses. Species excluded from the analyses were *H. crustuliniforme* (Bull.) Quél. and *H. salicicola* Beker, Vesterh. & U. Eberh. for the *H. alpinum* complex; *H. psammophilum* Bon and *H. subtortum* P. Karst for the *H. mesophaeum* complex; as well as *H. monticola* Vesterh. and *H. fuscatum* for the *H. nigellum* complex. Also, for better readability, the number of European representatives of the included species was restricted to 10 (if available) or, for species present in the RM dataset in more than 10 collections, matching (if possible) the number of collections of the RM dataset. An exception was made for *H. velutipes*, for which 20 sequences were included because of the known high intraspecific diversity of this species. For each included species, the selection of included representatives from Beker et al. (2016) was random, but only considering sequences with high quality reads. For illustrating the placement of *H. avellaneum*, not included in Beker et al. (2016), a small alignment was assembled representing all species accepted by Beker et al. (2016) in *H.* sect. *Naviculospora*. For tree analyses, outgroup sequences were added; selection of outgroup taxa followed Beker et al. (2016). Details are given in Table 1 for the sequences of Rockies collections, in Table 2 for other American collections, the majority types, and in Suppl. material 1 for FE data (Supplementary Data). Alignments were viewed and reformatted using AliView version 1.24 (Larsson 2014) and have been submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S23704). In summary, seven networks were calculated, one each for *H. alpinum* (J. Favre) Bruchet, *H. aurantioumbrinum* Beker, Vesterh. & U. Eberh., *H. hiemale* Bres. and *H. vaccinum* Romagn. *Hebeloma subconcolor* Bruchet and *H. velutipes* Bruchet are treated together, as are *H. excedens*, *H. marginatulum* (J. Favre) Bruchet, *H. mesophaeum* (Pers.) Quèl., and *H. alpinicola* as well as *H. hygrophilum* Poumarat & Corriol, *H. nigellum* Bruchet, *H. spetsbergense* Beker & U. Eberh., and *H. oreophilum* Beker & U. Eberh.

Maximum Likelihood analyses were calculated in RaxML (version 8.2.10, Stamatakis 2014) as implemented on Cipres (Miller et al. 2010), with the GTRGAMMA option, five searches for the best ML tree, using the MRE option to limit the number of fast bootstrap replicates. Trees were visualized using FigTree version 1.4.2 (Rambaut 2014).

**Table 1.** Taxon, voucher (Herbarium), locality information, elevation, and GenBank accession numbers for DNA sequences from Rockies collections described here. HJB refers to the herbarium of H.J. Beker; other herbarium acronyms follow Thiers http://sweetgum.nybg.org/ih/(continuously updated). The database numbers refer to the project database of H.J. Beker (Beker et al. 2016).

| Database no. | Herbarium | Voucher | Location | State | Elev. (m) | GenBank acc. no. ITS |
|---|---|---|---|---|---|---|
| ***Hebeloma alpinum*** | | | | | | |
| HJB15331 | MONT; HJB | CLC2855 | Lulu Pass, near Cooke City | USA: MT | 3000 | MK281073 |
| ***Hebeloma aurantioumbrinum*** | | | | | | |
| HJB12445 | HJB | HJB12445 | Beartooth Plateau, Wyoming Creek | USA: WY | 3176 | KM390714, KM390715 |
| HJB12446 | HJB | HJB12446 | Beartooth Plateau, Wyoming Creek | USA: WY | 3176 | KM390716, KM390717 |
| HJB12447 | HJB | HJB12447 | Beartooth Plateau, Wyoming Creek | USA: WY | 3176 | MK281061 |
| HJB12448 | HJB | HJB12448 | Beartooth Plateau, Wyoming Creek | USA: WY | 3177 | KM390718, KM390719 |
| HJB12450 | HJB | HJB12450 | Beartooth Plateau, Wyoming Creek | USA: WY | 3177 | MK281062 |
| HJB12451 | HJB | HJB12451 | Beartooth Plateau, Wyoming Creek | USA: WY | 3177 | KM390720, KM390721 |
| HJB12452 | HJB | HJB12452 | Beartooth Plateau, Wyoming Creek | USA: WY | 3177 | MK281059 |
| HJB12453 | HJB | HJB12453 | Beartooth Plateau, Wyoming Creek | USA: WY | 3177 | MK281063 |
| HJB12454 | HJB | HJB12454 | Beartooth Plateau, Wyoming Creek | USA: WY | 3177 | MK281060 |
| HJB12456 | HJB | HJB12456 | Beartooth Plateau, Wyoming Creek | USA: WY | 3176 | KM390722 |
| HJB12583 | ZT; HJB | ZT12730 | Beartooth Mts., Hellroaring Plateau | USA: MT | 3400 | MK281119 |
| HJB12584 | ZT; HJB | ZT12731 | Beartooth Mts., Hellroaring Plateau | USA: MT | 3400 | MK281118 |
| HJB15300 | MONT; HJB | CLC1565 | Beartooth Plateau, Highline Trail | USA: MT | 3100 | MK281076 |
| HJB15316 | MONT; HJB | CLC1822 | San Juan Range, Stony Pass | USA: CO | 3840 | MK281074 |
| HJB15332 | MONT; HJB | CLC3093 | Beartooth Plateau, Frozen Lake | USA: WY | 3200 | MK281075 |
| ***Hebeloma avellaneum*** | | | | | | |
| HJB15496 | DBG | DBG-F-020434 | Front Range, Loveland Pass Lake | USA: CO | 3620 | MK281025 |
| HJB15525 | DBG | DBG-F-019533 | Front Range, Niwott Ridge | USA: CO | 3200 | MK281026 |
| ***Hebeloma dunense*** | | | | | | |
| HJB12578 | ZT; HJB | ZT9001 | San Juan Range, Cinnamon Pass W | USA: CO | 3700 | MK281120 |
| HJB15290 | MONT; HJB | CLC1411 | San Juan Range, Cinnamon Pass | USA: CO | 3700 | MK281079 |
| HJB15293 | MONT; HJB | CLC1434 | San Juan Range, Cinnamon Pass | USA: CO | 3700 | MK281080 |
| HJB15315 | MONT; HJB | CLC1821 | San Juan Range, Stony Pass | USA: CO | 3840 | MK281077 |
| HJB15321 | MONT; HJB | CLC1845 | San Juan Range, Mineral Basin | USA: CO | 3835 | MK281078 |
| ***Hebeloma excedens*** | | | | | | |
| HJB12573 | ZT; HJB | ZT7475 | Sawatch Range, Independence Pass | USA: CO | 3760 | MK281122 |
| HJB12575 | ZT; HJB | ZT8074 | Front Range, Loveland Pass | USA: CO | 3750 | MK281124 |
| HJB12577 | ZT; HJB | ZT8136 | Sawatch Range, Independence Pass | USA: CO | 3680 | MK281123 |
| HJB12582 | ZT; HJB | ZT9830 | Sawatch Range, Independence Pass | USA: CO | 3700 | MK281121 |
| HJB15308 | MONT; HJB | CLC1685 | San Juan Range, U.S. Basin | USA: CO | 3658 | MK281081 |
| HJB15312 | MONT; HJB | CLC1732 | Sawatch Range, Independence Pass | USA: CO | 3760 | MK281082 |
| ***Hebeloma hiemale*** | | | | | | |
| HJB12457 | HJB | HJB12457 | Beartooth Plateau, Quad Creek | USA: MT | 3004 | GQ869529 |
| HJB12571 | ZT; HJB | ZT6417 | Beartooth Plateau, Highline Trail | USA: WY | 3200 | GQ869530 |
| HJB12574 | ZT; HJB | ZT8072 | Front Range, Loveland Pass | USA: CO | 3750 | MK281083 |
| HJB12581 | ZT; HJB | ZT9828 | Sawatch Range, Independence Pass | USA: CO | 3750 | MK281084 |
| HJB15301 | MONT; HJB | CLC1574 | Beartooth Plateau, Quad Creek | USA: MT | 3020 | MK281037 |
| HJB15306 | MONT; HJB | CLC1668 | San Juan Range, Mineral Basin, | USA: CO | 3835 | MK281027 |
| HJB15333 | MONT; HJB | CLC3094 | Beartooth Plateau, Frozen Lake | USA: WY | 3200 | MK281028 |
| HJB15493 | DBG | DBG-F-019162 | Front Range, Loveland Pass | USA: CO | 3655 | MK281029 |
| HJB15495 | DBG | DBG-F-021418 | Front Range, Loveland Pass | USA: CO | 3620 | MK281030 |

| Database no. | Herbarium | Voucher | Location | State | Elev. (m) | GenBank acc. no. ITS |
|---|---|---|---|---|---|---|
| HJB15497 | DBG | DBG-F-020440 | Front Range, Loveland Pass | USA: CO | 3597 | MK281031 |
| HJB15498 | DBG | DBG-F-020437 | Front Range, Loveland Pass | USA: CO | 3655 | MK281032 |
| HJB15499 | DBG | DBG-F-019241 | Front Range, Loveland Pass | USA: CO | 3749 | MK281033 |
| HJB15500 | DBG | DBG-F-020551 | Front Range, Mt. Goliath | USA: CO | 3658 | MK281038 |
| HJB15501 | DBG | DBG-F-021194 | Front Range, Loveland Pass | USA: CO | 3620 | MK281036 |
| HJB15502 | DBG | DBG-F-020431 | Front Range, Loveland Pass | USA: CO | 3597 | MK281034 |
| HJB15503 | DBG | DBG-F-020433 | Front Range, Loveland Pass | USA: CO | 3571 | MK281035 |
| HJB15518 | DBG | DBG-F-019597 | Front Range, Loveland Pass | USA: CO | 3620 | MK281067 |
| HJB15519 | DBG | DBG-F-016104 | Front Range, W Caribou townsite | USA: CO | 3200 | MK281068 |
| HJB15520 | DBG | DBG-F-020550 | Front Range, Mt. Goliath | USA: CO | 3810 | MK281069 |
| HJB17303 | MONT; HJB | CLC3574 | Beartooth Plateau, site 1 | USA: MT | 3000 | GQ869526 |
| HJB17304 | MONT; HJB | CLC3575 | Beartooth Plateau, site 1 | USA: MT | 3000 | GQ869528 |
| HJB17307 | MONT; HJB | CLC3533 | Beartooth Plateau, site 1 | USA: MT | 3000 | MK281085 |
| ***Hebeloma hygrophilum*** | | | | | | |
| HJB15296 | MONT; HJB | CLC1462 | Sawatch Range, Independence Pass | USA: CO | 3760 | MK281086 |
| HJB15297 | MONT; HJB | CLC1476 | Sawatch Range, Independence Pass | USA: CO | 3660 | MK281088 |
| HJB15329 | MONT; HJB | CLC1948 | Beartooth Plateau, Frozen Lake | USA: MT | 3200 | MK281087 |
| HJB15531 | DBG | DBG-F-021349 | Front Range, Loveland Pass | USA: CO | 3658 | MK281039 |
| ***Hebeloma marginatulum*** | | | | | | |
| HJB12458 | HJB | HJB12458 | Beartooth Plateau, Quad Creek | USA: MT | 2996 | MK281064 |
| HJB12579 | ZT; HJB | ZT9002 | San Juan Range, Cinnamon Pass | USA: CO | 3800 | MK281126 |
| HJB12580 | ZT; HJB | ZT9813 | San Juan Range, Black Bear Pass | USA: CO | 3900 | MK281125 |
| HJB15291 | MONT; HJB | CLC1413 | San Juan Range, Cinnamon Pass, | USA: CO | 3700 | MK281089 |
| HJB15294 | MONT; HJB | CLC1448 | San Juan Range, Black Bear Basin | USA: CO | 3830 | MK281090 |
| HJB15295 | MONT; HJB | CLC1449 | San Juan Range, Black Bear Basin | USA: CO | 3830 | MK281091 |
| HJB15298 | MONT; HJB | CLC1478 | Sawatch Range. Independence Pass | USA: CO | 3760 | MK281100 |
| HJB15299 | MONT; HJB | CLC1545 | Beartooth Plateau, Quad Creek | USA: MT | 3020 | MK281092 |
| HJB15305 | MONT; HJB | CLC1667 | San Juan Range, Mineral Basin | USA: CO | 3835 | MK281093 |
| HJB15310 | MONT; HJB | CLC1718 | San Juan Range, Black Bear Basin | USA: CO | 3760 | MK281103 |
| HJB15314 | MONT; HJB | CLC1811 | San Juan Range, Cinnamon Pass | USA: CO | 3700 | MK281094 |
| HJB15317 | MONT; HJB | CLC1824 | San Juan Range, Stony Pass | USA: CO | 3840 | MK281095 |
| HJB15318 | MONT; HJB | CLC1826 | San Juan Range, Stony Pass | USA: CO | 3840 | MK281101 |
| HJB15319 | MONT; HJB | CLC1836 | San Juan Range, Imogene Pass | USA: CO | 3850 | MK281102 |
| HJB15320 | MONT; HJB | CLC1840 | San Juan Range, Imogene Pass | USA: CO | 3850 | MK281096 |
| HJB15322 | MONT; HJB | CLC1860 | San Juan Range, Mineral Basin | USA: CO | 3835 | MK281097 |
| HJB15323 | MONT; HJB | CLC1861 | Mineral Basin, San Juan Range | USA: CO | 3835 | MK281104 |
| HJB15324 | MONT; HJB | CLC1874 | San Juan Range, Emma Lake | USA: CO | 3688 | MK281098 |
| HJB15326 | MONT; HJB | CLC1880 | San Juan Range, Emma Lake | USA: CO | 3688 | MK281099 |
| HJB15487 | DBG | DBG-F-027694 | Front Range, Loveland Pass | USA: CO | 3911 | MK281048 |
| HJB15488 | DBG | DBG-F-027695 | Front Range, Summit Lake Park | USA: CO | 3911 | MK281040 |
| HJB15491 | DBG | DBG-F-027682 | Front Range, Summit Lake Park | USA: CO | 3911 | MK281041 |
| HJB15505 | DBG | DBG-F-020708 | Front Range, Loveland Pass | USA: CO | 3655 | MK281042 |
| HJB15506 | DBG | DBG-F-020841 | Sawatch Range, Independence Pass | USA: CO | 3687 | MK281046 |
| HJB15507 | DBG | DBG-F-020856 | Sawatch Range, Independence Pass | USA: CO | 3687 | MK281047 |
| HJB15512 | DBG | DBG-F-021405 | Front Range, Loveland Pass | USA: CO | 3620 | MK281043 |
| HJB15533 | DBG | DBG-F-021388 | Front Range, Loveland Pass | USA: CO | 3655 | MK281044 |
| HJB15534 | DBG | DBG-F-020843 | Sawatch Range, Independence Pass | USA: CO | 3687 | MK281045 |
| HJB17308 | MONT; HJB | CLC3545 | Beartooth Plateau, Solufluction Terr | USA: WY | 3400 | MK281070 |
| ***Hebeloma mesophaeum*** | | | | | | |
| HJB12576 | ZT; HJB | ZT8082 | Front Range, Loveland Pass | USA: CO | 3750 | MK281127 |
| HJB15289 | MONT; HJB | CLC1245 | Sawatch Range, Independence Pass | USA: CO | 3760 | MK281105 |

| Database no. | Herbarium | Voucher | Location | State | Elev. (m) | GenBank acc. no. ITS |
|---|---|---|---|---|---|---|
| ***Hebeloma nigellum*** | | | | | | |
| HJB12572 | ZT; HJB | ZT6425 | Beartooth Plateau, Pass N | USA: WY | 3350 | MK281128 |
| HJB15292 | MONT; HJB | CLC1420 | San Juan Range, Engineer Pass | USA: CO | 3900 | MK281106 |
| HJB15309 | MONT; HJB | CLC1707 | San Juan Range, Cinnamon Pass | USA: CO | 3700 | MK281107 |
| HJB15313 | MONT; HJB | CLC1778 | Beartooth Plateau, Frozen Lake | USA: WY | 3200 | MK281108 |
| HJB17305 | MONT; HJB | CLC3614b | Beartooth Plateau, Billings Fen | USA: WY | 3400 | MK281071 |
| ***Hebeloma nigromaculatum*** | | | | | | |
| HJB12439 | HJB | HJB12439 | Beartooth Plateau, Quad Creek | USA: MT | 2988 | MK281065 |
| HJB15302 | MONT; HJB | CLC1577 | Beartooth Plateau, Quad Creek | USA: MT | 3020 | MK281109 |
| HJB15529 | DBG | DBG-F-020565 | Front Range, Little Echo Lake | USA: CO | 3505 | MK281050 |
| HJB15530 | DBG | DBG-F-020582 | Front Range, Little Echo Lake | USA: CO | 3505 | MK281049 |
| ***Hebeloma oreophilum*** | | | | | | |
| HJB12449 | HJB | HJB12449 | Beartooth Plateau, Wyoming Creek | USA: WY | 3176 | MK281066 |
| HJB12585 | ZT; HJB | ZT12733 | Beartooth Mts., Hellroaring Plateau | USA: MT | 3400 | MK281129 |
| HJB15288 | MONT; HJB | CLC1102 | Beartooth Plateau, Quad Creek | USA: MT | 3020 | MK281110 |
| HJB15328 | MONT; HJB | CLC1937 | Beartooth Plateau, Highline Trail | USA: MT | 3100 | MK281111 |
| HJB15489 | DBG | DBG-F-027674 | Front Range, Summit Lake Park | USA: CO | 3911 | MK281054 |
| HJB15504 | DBG | DBG-F-022788 | Front Range, Summit Lake Park | USA: CO | 3912 | MK281051 |
| HJB15508 | DBG | DBG-F-020053 | Elk Mountain Range, Pearl Pass | USA: CO | 3658 | MK281052 |
| HJB15521 | DBG | DBG-F-020558 | Front Range, Mount Goliath | USA: CO | 3658 | MK281053 |
| HJB17306 | MONT; HJB | CLC3607 | Beartooth Plateau, Billings Fen | USA: WY | 3048 | MK281072 |
| ***Hebeloma spetsbergense*** | | | | | | |
| HJB15325 | MONT; HJB | CLC1879 | San Juan Range, Horseshoe Basin | USA: CO | 3688 | MK281112 |
| HJB15490 | DBG | DBG-F-027678 | Front Range, Summit Lake Park | USA: CO | 3911 | MK281055 |
| ***Hebeloma subconcolor*** | | | | | | |
| HJB15510 | DBG | DBG-F-022785 | Front Range, Summit Lake Park | USA: CO | 3912 | MK281056 |
| HJB15511 | DBG | DBG-F-022786 | Front Range, Summit Lake Park | USA: CO | 3912 | MK281057 |
| ***Hebeloma vaccinum*** | | | | | | |
| HJB15327 | MONT; HJB | CLC1881 | San Juan Range, Horseshoe Basin | USA: CO | 3688 | MK281113 |
| ***Hebeloma velutipes*** | | | | | | |
| HJB12570 | ZT; HJB | ZT6100 | Beartooth Plateau, N of E Summit | USA: MT | 3320 | MK281130 |
| HJB15303 | MONT; HJB | CLC1646 | Sawatch Range, Cottonwood Pass | USA: CO | 3694 | MK281116 |
| HJB15304 | MONT; HJB | CLC1651 | Sawatch Range, Cumberland Pass | USA: CO | 3668 | MK281117 |
| HJB15311 | MONT; HJB | CLC1725 | Sawatch Range, Cottonwood Pass | USA: CO | 3694 | MK281115 |
| HJB15330 | MONT; HJB | CLC1980 | Beartooth Plateau, Quad Creek | USA: MT | 3020 | MK281114 |
| HJB15524 | DBG | DBG-F-005617 | Front Range, Herman Gulch | USA: CO | 3170 | MK281058 |

Pruned quasi-median network analyses were carried out in SplitsTree (version 4.14.6, Huson and Bryant 2006) using the default settings apart from activating the 'scale nodes by taxa' and 'subdivide edges' options. Nodes representing different classes of sequences (differentiated by species and origin, RM versus FE) were replaced in Adobe Illustrator CS6 by pie charts of corresponding diameters, showing the relative numbers of sequences for each class.

Distances between sequences were calculated in PAUP* (Swofford 2003), as the total number of differences of standard data, disabling the default 'equate' scheme for sequence data. By doing this, ambiguity reads like i.e. 'y' are not equated with the corresponding bases, here 'c' and 't'. Missing data were recoded as '?'; gaps were treated as standard characters. In addition, differences in PAUP* 'standard DNA/RNA absolute'

**Table 2.** Other North American collections considered. HJB refers to the herbarium of H.J. Beker; other herbarium acronyms follow Thiers http://sweetgum.nybg.org/ih/(continuously updated). The database numbers refer to the project database of H.J. Beker (Beker et al. 2016).

| Database no. | Herbarium | Voucher | Location | State | Elev. (m) | GenBank acc. no. ITS |
|---|---|---|---|---|---|---|
| ***Hebeloma alpinicola*** | | | | | | |
| HJB1000311 | MICH | MICH 5549† | Heavens Gate Ridge, Seven Devils Mountains | USA: Idaho | 2560 | MK280987 |
| HJB1000338 | DBG | DBG-F-002473‡ | Park County, Pike National Forest, Sacramento, west of Fairplay, north side of old house | USA: Colorado | 3600 | MK286559 |
| HJB1000416 | MICH | MICH 10760§ | Hancock, Bar Harbor, Mt Desert Island | USA: Maine | 25 | MK286558 |
| HJB1000435 | MICH | MICH 10778\| | Clackamas, Rhododendron | USA: Oregon | 495 | MK280989 |
| HJB1000500 | DBG | DBG-F-004877¶ | Gilpin County, Roosevelt National Forest, Perigo, north slope | USA: Colorado | 2865 | MK286560 |
| HJB1000147 | MICH | MICH 10730# | Chelsea, Lyndon Town Hall Park, Washtenaw Co. | USA: Michigan | 300 | MK280985 |
| HJB1000501 | DBG | DBG-F-007947†† | Conejos County, San Juan National Forest, Green Lake area south of Platero | USA: Colorado | 3353 | MK286561 |
| ***Hebeloma avellaneum*** | | | | | | |
| HJB14320 | FNL‡‡; HJB | HJB14320 | Pinware River | Canada: Labrador | 15 | MK281019 |
| HJB1000322 | MICH§§ | MICH 10722 | Grays Harbor, Lake Quinault, Olympic National Park | USA: Washington | 75 | MK280988 |
| ***Hebeloma excedens*** | | | | | | |
| HJB1000268 | NYS | NYS-F-001123\|\| | Saratoga, Saratoga | USA: New York | 100 | MK280986 |
| ***Hebeloma incarnatulum*** | | | | | | |
| HJB1000136 | MICH | MICH 10752¶¶ | Mud Lake Bog west of Whitmore Lake, Washtenaw | USA: Michigan | 275 | KT218477 |

†This is the holotype of *Hebeloma alpinicola*, 5 Jul 1958, A.H. Smith (58632).
‡This is the holotype of *Hebeloma chapmaniae*, 10 Sep 1969, S. Chapman.
§This is the holotype of *Hebeloma littenii*, 29 Oct 1980, W. Litten.
|This is the holotype of *Hebeloma nigromaculatum*, 1 Oct 1944, A.H. Smith (19314).
¶This is the holotype of *Hebeloma perigoense*, 13 Aug 1974, S. Chapman, S. Mitchel, A.H. Smith.
#This is the holotype of *Hebeloma smithii*, 10 Nov 1977, A.H. Smith (88295).
††This is the holotype of *Hebeloma subargillaceum*, 23 Aug 1978, V. Evenson.
‡‡Foray Newfoundland and Labrador herbarium http://www.nlmushrooms.ca/index.html
§§This is the holotype of *Hebeloma avellaneum*, 8 Nov 1925, C.H. Kauffman.
||This is the holotype of *Hebeloma excedens*, Oct 1870, C.H. Peck.
¶¶This is the holotype of *Hebeloma incarnatulum*, 14 Oct 1961, A.H. Smith (64680).

differences with default settings (equating scheme in place; gaps treated as missing data) are given in square brackets. For those who wish to convert absolute to relative distances, alignment length was between 698–722 bp.

## Results and general discussion

Species recognition is often not easy in *Hebeloma*, and although species can normally be identified by morphology alone, species are delimited by a combination of morphology, multi-locus molecular data and ecology. In some sections (*H*. sects. *Denudata* and *Velutipes*) the efforts of Aanen and co-workers (i.e. Aanen and Kuyper 1999, 2004, Aanen and Kuyper 2004) also gave some evidence with regard to the limits of biological species. As described earlier (Eberhardt et al. 2015a, 2015b, 2016, Beker et al. 2016, Grilli et al. 2016), species definitions based on several lines of evidence may share ITS or other loci' haplotypes, presumably as a result of incomplete linage sorting, hybridization or other population processes. The molecular distance between some species is so small that we assume that not all groups we recognize as species had sufficient time to reach monophyly in all loci. Thus, we do not necessarily expect species to form monophyla in ITS trees. In spite of this, and this is visualized by the networks, certain haplotypes or combination of haploypes (as in dikarya, here referred to as "variants") is normally characteristic for a single species and occurs only rarely in sister species. Therefore, in spite of its lack of resolution in phylogentic trees, BLAST searches against an ITS database of well identified collections very often retrieve the correct species name in relation to other lines of evidence. We are not aware of a single locus that can differentiate between all species of *Hebeloma*. In particular in *H*. sect. *Hebeloma*, the search for a locus that is more powerful in recognizing species than the loci used by Beker et al. (2016), namely ITS, *RPB2*, *Tef1a*, and variable regions of the mitochondrial SSU, is still ongoing. We are at the beginning of our research into the *Hebeloma* funga of America and all of our conclusions rest heavily on our insights into *Hebeloma* of Europe and there on the available material. For some species, for example *H. velutipes*, we have hundreds of collections to choose from, while for other species, like *H. pubescens* we have only a few specimens. As our research goes on and more data becomes available, we will revisit and if necessary rectify the conclusions drawn here.

Sixteen species of *Hebeloma* were identified morphologically among the collections from the Rocky Mountains alpine zone. The molecular analysis carried out supported the morphological analysis. A key is given below. In all, 115 collections and 10 relevant types from North America were sequenced successfully for the ITS region (Tables 1, 2).

Figure 2 shows the taxonomic positions of the treated species (complexes) mapped on the ITS tree of Beker et al. (2016). Of the 16 species collected in the Rockies, three were not treated by Beker et al. (2016), namely *H. alpinicola*, *H. avellaneum* and *H. excedens*. These species were named based on type studies. Figure 3 shows that *H. avellaneum* is a member of *H*. sect. *Naviculospora* and forms a monophylum. The only other species encountered in the Rocky Mountains that is clearly distinct in the ITS region is *H. hiemale* (Beker et al. 2016; Eberhardt et al. 2016; Fig. 4B). For all other species, several taxa were included in a single network (Figs 4A, 4C, 4D, 5, 6). The networks show that there are usually only a small number of unambiguous base pair differences between members of the same species, irrespective of their origin, even though some

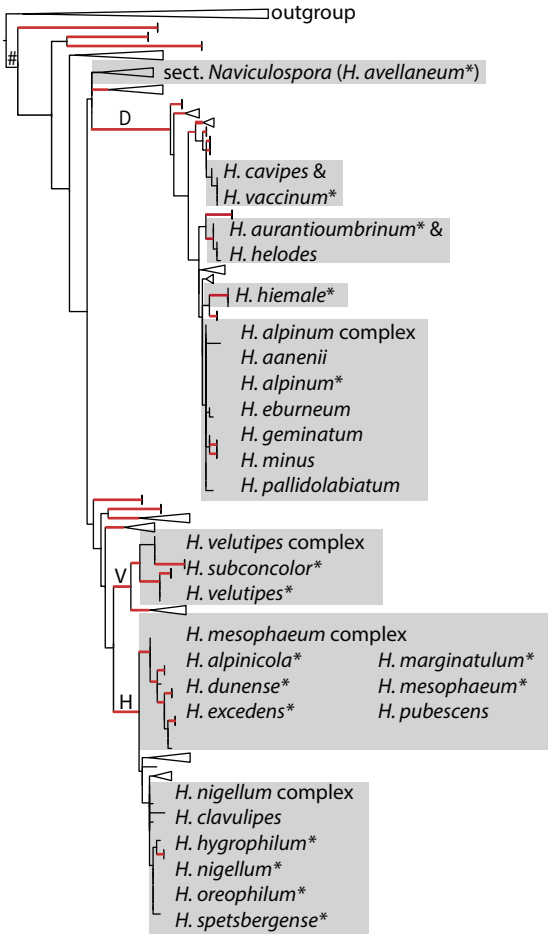


**Figure 2.** ITS overview tree of the genus *Hebeloma* in Europe from Beker et al. (2016) fig. 12A modified. Grey boxes indicate species clusters represented in separate tree or network figures. Red lines indicate branches with ML bootstrap support of ≥ 80%. # = genus *Hebeloma*; D = *H.* sect. *Denudata*; H = *H.* sect. *Hebeloma*; V = *H.* sect. *Velutipes*; * = species recorded from the Rocky Mountains. For further details see Beker et al. (2016) and the running text.

parts of some networks (*H. aurantioumbrinum*, *H. marginatulum*) are exclusively of RM origin. While ITS trees were published for *H.* sects. *Denudata* and *Velutipes* (Eberhardt et al. 2015a, 2016; Grilli et al. 2016), this is not the case for *H.* sect. *Hebeloma*. Therefore ITS ML trees, rooted with *H. grandisporum* Beker, U. Eberh. & A. Ronikier, are shown in Figure 6. Details, including base pair (bp) differences between species, are discussed in the Taxonomy section.

Beker et al. (2016) showed that in a number of *Hebeloma* species clusters or complexes, morphology is better suited for species distinction and delimitation than molecular data. The majority of the species encountered in the Rocky Mountains belong

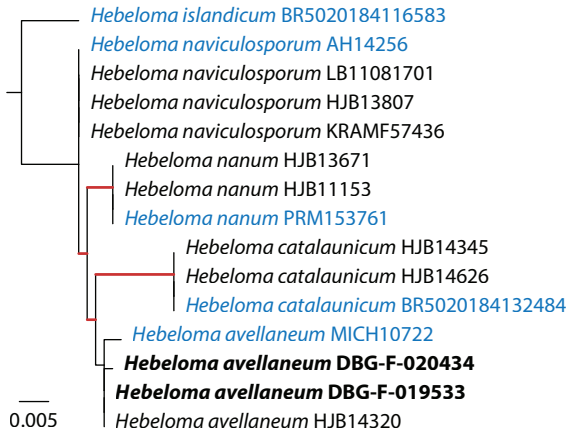


**Figure 3.** ML result of *Hebeloma* sect. *Naviculospora* rooted in accordance with the results of Beker et al. (2016) with *H. islandicum* (internal outgroup). Branches supported by ≥ 80% bootstrap (1000 replicates) are indicated in red. Collections from the Rocky Mountains are indicated in bold, type sequences are indicated in blue.

to these species complexes. Thus, it is not surprising that the ITS analyses are only clear for two species, namely *H. avellaneum* and *H. hiemale*. For the other species, there is at least one other species with very similar ITS sequences. In some cases such as for *H. aurantioumbrinum* and *H. vaccinum*, the only sister taxa that cannot be distinguished by ITS sequence differ in habitat (Beker et al. 2016). Also, in the larger complexes, not all of the considered species are associated with the same hosts or habitats as the target species. *Hebeloma clavulipes* Romagn., *H. eburneum* Malençon, *H. incarnatulum* A.H. Sm., and *H. leucosarx* P.D. Orton are not expected to occur in the habitats sampled in the Rocky Mountains; *H. aanenii* Beker, Vesterh. & U. Eberh. and *H. geminatum* Beker, Vesterh. & U. Eberh. hardly ever grow in such habitats (Beker et al. 2018).

In the Taxonomy part, minute levels of sequence variation are discussed. We do that against the background of multilocus analyses presented by Beker et al. (2016) and other works, indicating in which cases the ITS is wanting for species differentiation. Thus, even though ITS differences between species may be slight or not constant, and even considering that morphological distinction in some cases relies on minute differences, the combination of morphology, ecology, and ITS data provides a reliable set of information for species assignment.

Based on previous studies, delimitation of most species is now well understood (Eberhardt et al. 2015a, 2016; Beker et al. 2016; Grilli et al. 2016), and consequently we did not consider it necessary to include all species discussed as morphologically similar in the same molecular analysis. Our aim has been to show what information, even in the case when it is sparse, is contained in ITS data.

We have made an effort to combine sequence analyses based on different subsets of data and displaying different levels of complexity in the visualization. We have considered several different methods for analyzing ITS sequence data: ML trees, pruned quasi-median networks, and base pair difference counts between aligned se-

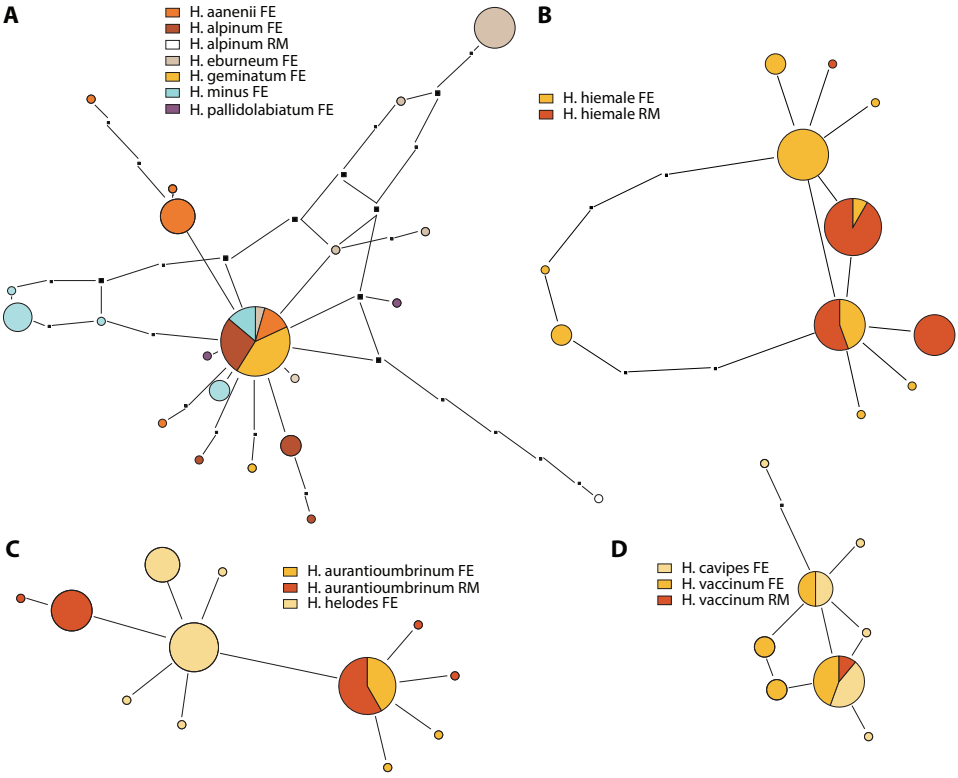


**Figure 4.** Pruned quasi-median networks of species and species clusters of *Hebeloma* sect. *Denudata*. **A** *H. alpinum* complex **B** *H. hiemale* **C** *H. aurantioumbrinum* and *H. helodes* **D** *H. cavipes* and *H. vaccinum*. In networks, the size of the circles corresponds to the number of sequences they represent. Circles shared by two or more taxa are divided according to the number of representatives for each species. FE and RM refer to the origin of the collections, Europe or Rocky Mountains, respectively.

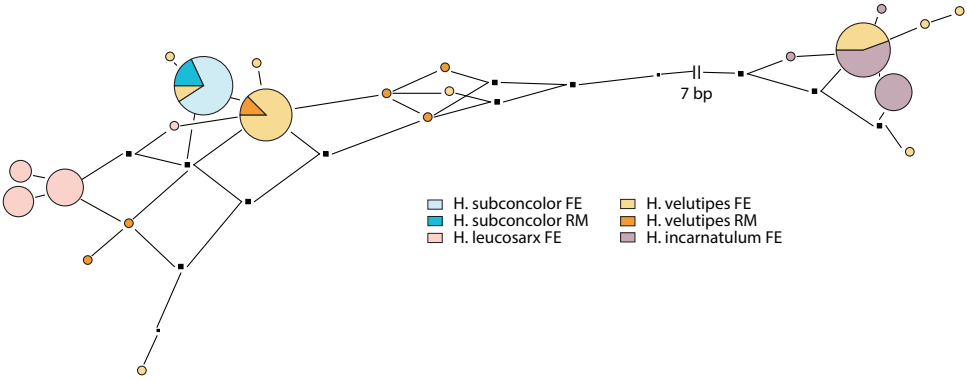


**Figure 5.** Pruned quasi-median networks of the *Hebeloma velutipes* complex. Circles shared by two or more taxa are divided according to the number of representatives for each species. FE and RM refer to the origin of the collections, Europe or Rocky Mountains, respectively.

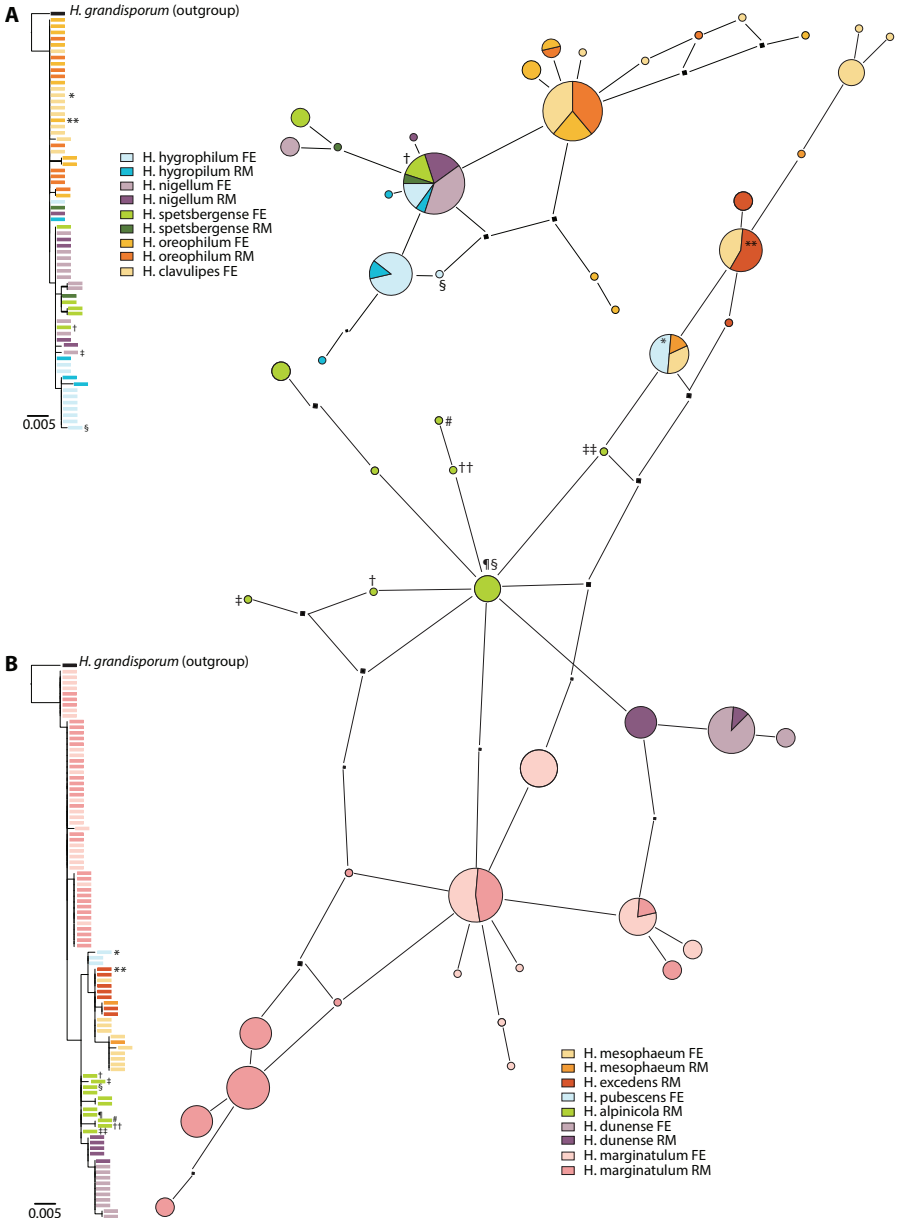


**Figure 6.** ML results and pruned quasi-median networks of species complexes of *Hebeloma* sect. *Hebeloma.* **A** *H. nigellum* complex **B** *H. mesophaeum* complex. In ML trees, branches supported by ≥ 80% bootstrap (1000 replicates) are double width. In networks, the size of the circles corresponds to the number of sequences they represent. Circles shared by two or more taxa are divided according to the number of representatives for each species. FE and RM refer to the origin of the collections, Europe or Rocky Mountains, respectively. Placement of type sequences is indicated as follows: **A** * = *H. clavulipes.* ** = *H. oreophilum*, † = *H. spetsbergense*, ‡ = *H. nigellum* (not included in the network analysis), § = *H. hygrophilum*; **B** * = *H. pubescens*, ** = *H. excedens*, † = *H. subargillaceum*, ‡ = *H. nigromaculatum*, § = *H. littenii*, ¶ = *H. alpinicola*, # = *H. perigoense*, †† = *H. chapmaniae* and ‡‡ = *H. smithii*.

quences. Sometimes, the relationship between sequences and species may appear differently between trees, networks and difference counts. In the ML analyses, gaps are treated as missing data and ambiguous reads are equated. The networks are based on clean base pair exchanges and gaps; polymorphic positions with two states, i.e. positions with ambiguous codes are treated as missing data. Owing to the complexity of networks displaying this kind of information in full, such networks are, as far as we are aware, used for data verification rather than for data analysis (Bandelt and Dürr 2007; Brandstätter et al. 2007). For the direct sequence difference counts, all kinds of differences were counted equally, thus giving the maximum number of differences plus giving absolute DNA differences in square brackets, which do not count gaps and polymorphic positions as different. Whereas ML trees pruned quasi-median networks and absolute DNA differences are prone to omitting observed intragenomic and thus intraspecific variation, total distance counts are overestimates. In spite of that, we have decided to present these values here, because they could influence species identificaton.

## Key to *Hebeloma* species of the Rocky Mountain Alpine Zone

1 Cortina absent; pileus mostly uniform in color, lamellae often with droplets; stipe base usually not dark; cheilocystidia mostly clavate or capitate (swollen near the apex, sometimes also in the lower half); spores mostly amygdaliform ..... **2**

2 Pileus small, 10–20(–25) mm, stipe 2–4 mm wide; and with 20–40 full length lamellae ..... **3**

3 Spores on ave. at least 12 µm long, distinctly finely verrucose, dextrinoid; pileus brown, reddish brown; stipe cream; with *Salix* ..... **1. *H. vaccinum***

3* Spores on ave. <12 µm long, not or weakly ornamented, slightly dextrinoid; pileus a different color ..... **4**

4 Pileus uniformly pinkish buff, orange brown; margin crenate with white rim; stipe whitish; cheilocystidia significantly constricted below the apex, ave. median width at most 5 µm; with *S. planifolia* or *S. arctica* ..... **2. *H. aurantioumbrinum***

4* Pileus brown, grayish brown, pruinose; stipe buff; cheilocystidia tapering more gently towards base, ave. median width at least 5 µm; with *Salix* ..... **3. *H. subconcolor***

2* Pileus larger, 20–60 mm; stipe wider 5–15 mm; and with 40–100 full length lamellae ..... **5**

5 Spores distinctly verrucose, not or weakly dextrinoid, on ave. 10–12.5 × 5–7 µm; cheilocystidia swollen at apex and also in the lower half; pileus cream, pinkish buff, isabella; stipe clavate, floccose; mostly with *S. reticulata* in the Rockies ..... **4. *H. hiemale***

5* Spores only slightly rough, weakly to strongly dextrinoid ..... **6**

6 Pileus rich brown, orange brown, cinnamon brown, margin rolled under; lamellae pale, stipe whitish; odor fruity; spores on ave. 8.5–10 ×

5–5.5 µm, narrow, distinctly dextrinoid; in lower alpine with conifers (poss. *Salix*) ..... **5. *H. avellaneum***

6* Pileus paler; spores somewhat larger; with *Dryas* or dwarf *Salix* ..... **7**

7 Pileus pale buff, pinkish buff; stipe stout, white, half floccose, often long, often with bulbous base; often with *Dryas* in the Rockies alpine; spores moderately to strongly dextrinoid ... **6. *H. velutipes***

7* Pileus cream to pale brown, robust; stipe mostly equal, shorter; with *Dryas* or *Salix*; spores at most weakly dextrinoid ..... **7. *H. alpinum***

1* Cortina present; pileus often two-colored, with darker center and paler margin; lamellae not or minimally weeping; stipe often black or dark at base; cheilocystidia lageniform to ventricose (swollen in lower half); spores elliptical or amygdaliform ..... **8**

8 Spores elliptical; rather smooth, not dextrinoid; slightly larger types with wider stipes (typically 4–8 mm); with *Salix* spp ..... **9**

9 Pileus with darker coloration, brown, reddish brown ..... **10**

10 Pileus dark brown, hoary; lamellae deeply emarginated; margin turned in and coated with veil remnants; spores on ave. at least 10 × 6 µm .... ..... **8. *H. marginatulum***

10* Pileus robust, reddish brown with grayish cast; stipe stout, base often encased in sand, cespitose; spores on ave. <10 µm long and <6 µm wide ..... ..... **9. *H. alpinicola***

9* Pileus with paler coloration, pinkish buff, light brown, yellowish brown, can be dark in center ..... **11**

11 Spores on average at least 10 × 6 µm, slightly ornamented; pileus pinkish buff, brown, hoary, more unicolor; lamellae subdecurrent or sinuate; yellow contents in some cystidia; with dwarf willows or *S. planifolia* ..... **10. *H. dunense***

11* Spores on ave. <10 µm long, almost smooth; with *Salix glauca* in alpine Rockies ..... **12**

12 Pileus ocher, darker in center, two-toned .... **11. *H. mesophaeum***

12* Pileus pale brown, pinkish brown, more uniform; margin can exceed lamellae ..... **12. *H. excedens***

8* Spores amygdaliform, finely verrucose, dextrinoid; smaller types with thinner stipes, 1–4(–8) mm in diam.; mostly with *S. planifolia* ..... **13**

13 Pileus 20–40 mm, brown, lamellae >40, stipe 3–8 mm wide; in moss or not; spores on ave. 11–14 × 6.8–7.2 µm ..... **13. *H. oreophilum***

13* Pileus 8–25 mm, pale brown with blackish brown center; lamellae <40; stipe thin, 1–4 mm wide; typically in moss ..... **14**

14* Spores on ave. 11.4 × 6.8 µm wide; epicutis >100 µm thick ..... ..... **14. *H. hygrophilum***

14* Spores on ave. 11.9 × 7.2 µm; epicutis less than 100 µm thick ..... ..... **15. *H. nigellum***

14** Spores on ave. at least 7.5 µm wide; on av 12.5 × 7.6 µm..... ..... **16. *H. spetsbergense***

## Taxonomy

### Descriptions of Rocky Mountain Collections

Descriptions of Rocky Mountain *Hebeloma* species 1–16 are presented in the order shown in the key for convenient access.

### *Hebeloma* sections *Denudata* (Fr.) Sacc., *Velutipes* Vesterh., and *Naviculospora* Beker & U. Eberh. – species without a cortina.

**1. *Hebeloma vaccinum* Romagn., Bull. Trimest. Soc. Mycol. Fr. 81: 333 (1965)**
Figures 4D, 7, 23(1)

**Etymology.** From *vaccinus*, meaning dun color (i.e. dull grayish brown).

**Description.** Cortina not observed. Pileus 10–11 mm in diameter, convex, buff to brownish with a hoary coating, rather unicolor, smooth, shiny, tacky; margin turned down, a bit crenulate, faintly striate; edges white. Lamellae adnexed, L = 38 plus lamellulae, buff to milk coffee. Stipe 10 × 3 mm, equal, cream, finely floccose at apex and fibrillose for length, delicate. Context cream. Odor not apparent, but previously noted as raphanoid. Exsiccate: very tiny, brown, not shiny, lamellae not blackening.

Basidiospores yellowish brown, amygdaliform, limoniform, with a snout and small apiculus, distinctly verrucose (O3), with loosening perispore observed in a few spores (P1, P2), dextrinoid (D3), 10–14 × 6–8 µm, on average 12.2 × 7.1 µm, Q = 1.71; some larger spores present –18 × –9 µm. Basidia 27–35 × 7–9 µm, four-spored, possibly a few two-spored because of larger spores present. Cheilocystidia clavate-lageniform, some slightly more swollen at apex, 35–70 × 6–8 µm at apex, 3–5 µm in middle, and 6–10 µm at base, occasionally septate, no thickening observed. Pleurocystidia absent. Epicutis thickness 40–125 µm, with some encrusted hyphae.

**Rocky Mountain Ecology** results are based on a single collection of two small basidiomes found in the Colorado alpine with *Salix arctica*.

**Rocky Mountain specimen examined.** U.S.A. COLORADO: San Juan County, San Juan Mountains, Mineral Basin, with *Salix arctica*, 3320 m, 31 July 2002, CLC1881 (MONT), C. Cripps.

**Discussion.** Beker and co-workers (Beker et al. 2016; Eberhardt et al. 2016; including ML ITS analyses) showed that *H. vaccinum* can be recognized by its ITS region from all species apart from *H. cavipes* Huijsman, which differs in morphology and ecology. The RM *H. vaccinum* collection fits in with the diversity found within the species (Fig. 4D) it differs in 0–5 [0] bp from other included members of the species. The intraspecific variation of the included FE members of *H. vaccinum* is 0–8 [0] bp.

This species is usually described as larger (13–40 mm) than the Rocky Mountain specimens described here. Microscopically, the species has spores that are strongly dextrinoid (D3) with a frequently loosening perispore. The spores and cheilocystidia characteristics (swollen at the apex and at the base but constricted in the middle part) put

**Figure 7.** *Hebeloma vaccinum* HJB11135 from Swiss alpine zone.

it in *H.* sect. *Denudata*, subsect. *Clepsydroida. Hebeloma vaccinum* is known to occur in low elevation dunes and woodlands with *Salix*; it is widespread in Northern Europe. Other arctic-alpine collections are from the European Alps, the Carpathians in Slovakia, and Greenland, always with *Salix* species (Beker et al. 2016; Eberhardt et al. 2015b). It could be recognized in the Rocky Mountains by its association with dwarf *Salix*, small size, lack of a veil, and distinct spores and cystidia; compare with *H. aurantioumbrinum*.

### 2. *Hebeloma aurantioumbrinum* Beker, Vesterh. & U. Eberh., Persoonia 35: 116 (2015)

Figures 4C, 8, 23(2)

**Etymology.** From *aurantius*, orange and *umbrinus*, umber.

**Description.** Cortina absent. Pileus small, 10–20 mm in diameter, convex, slightly conic-convex, appearing smooth, greasy, not hygrophanous, cream, then buff, pinkish buff, orange brown, can be lighter towards margin but not clearly two-toned, somewhat hoary; margin weakly involute, possibly crenate with a white rim. Lamellae deeply indented, deeply sinuate-arcuate, rather distant, L = 25–40 plus lamellulae, cream, then buff, pinkish buff, milk coffee; edges fimbriate, white but graying, drops visible. Stipe 15–28 × 2–3 mm, equal, bit curved, dingy whitish cream but darkening at base to watery brown (in CLC3093), floccose/pruinose for top third and smooth-fibrous below. Context dingy whitish. Odor faint or raphanoid. Exsiccate: pileus buff, lamellae brown; stipe very thin, whitish.

Basidiospores yellowish brown, slightly amygdaliform, with almost obtuse ends, with tiny apiculus, with slight ornamentation (O2), no loosening perispore (P0, P1), slightly dextrinoid (D1, D2), 10–13(–14) × 6–7.5 µm, on average 11.5 × 6.7 µm, Q = 1.72. Basidia 30–35 × 8–10 µm, clavate, two- and four-spored. Cheilocystidia long

**Figure 8.** *Hebeloma aurantioumbrinum*, CLC3093 and CLC1822.

with swollen apex, clavate-stiptate, occasionally clavate-lageniform, 40–70 × 6–9 µm at apex, 3–5.5 µm in middle, and 3–6.5 µm in base. Pleurocystidia absent. Epicutis thickness 70–100 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** In the alpine with willows *Salix glauca*, *Salix planifolia*, and *S. arctica*, reported from Colorado, Montana and Wyoming.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: San Juan/Hinsdale County, San Juan Mountains, Stony Pass, with *Salix arctica*, 28 July 2002, CLC1822 (MONT), C. Cripps. WYOMING: Park County, Beartooth Plateau. Frozen Lakes with *S. planifolia*, 14 Aug 2014, CLC3093 (MONT), C. Cripps; WY/MT stateline with *S. planifolia*, 14 July 2001, CLC1565 (MONT), C. Cripps. Wyoming Creek 6 Aug 2008 with *S. arctica* and *S. glauca*, HJB12445, C. Cripps & H.J. Beker; HJB12446, C. Cripps; HJB12447, C. Cripps; HJB12448, H.J. Beker; HJB12450, HJB12452, HJB12453, H.J. Beker; HJB12451 with *S. planifolia*, H. Knudsen; HJB12454, E. Horak. Upper Wyoming Creek, with *Salix arctica*, 8 Aug 2008, HJB12456, J. Antibus. Hell-Roaring Plateau, with *Salix* sp., 14 Aug 2007, ZT12730 (ETH), ZT12731 (ETH), E. Horak.

**Discussion.** Beker and co-workers (Beker et al. 2016; Eberhardt et al. 2015a) showed that *H. aurantioumbrinum* cannot be distinguished from the non-arctic-alpine *H. helodes* J. Favre based on ITS sequencing, but it can be separated from all other members of *H.* sect. *Denudata*. An ITS tree is given in Eberhardt et al. (2015a). The RM dataset includes more collections of *H. aurantioumbrinum* (15) than the FE dataset (7). Therefore, it is not surprising that the molecular diversity of the RM sequences is higher than that of the FE dataset (Fig. 4C). There are 0–6 [0] bp differences among the FE sequences of *H. aurantioumbrinum*, 0–9 [0–3] bp differences among the sequences of RM *H. aurantioumbrinum* and 2–11 [0–3] bp differences between *H. aurantioumbrinum* and *H. helodes*. Morphologically, *H. aurantioumbrinum* and *H. helodes* are quite different and can be easily separated, for example *H. helodes* always has a distinct thickening of the cheilocystidium wall at the apex, a feature that is absent

in *H. aurantioumbrinum*. Further, they occur in very different habitats; *H. helodes* has never, to our knowledge, been confirmed in arctic-alpine habitats.

*Hebeloma aurantioumbrinum* may have been confused with *H. pusillum* J.E. Lange, although *H. pusillum* has much more slender basidiomes that are distinctly two-toned. *Hebeloma aurantioumbrinum* is squatter and rarely two-toned. Additionally, we are not aware of any confirmed records of *H. pusillum* in arctic-alpine habitats. Both these species, without any veil (beyond the primordial stage) and with clavate-stiptate cheilocystidia, belong to the *Crustuliniformia* subsection of section *Denudata*. This subsection contains many small species that are arctic-alpine specialists that occur with *Salix*, and these species have only recently been split out and described (Eberhardt et al. 2015a). Collections of *H. aurantioumbrinum* have been confirmed from a number of arctic and alpine habitats, including Greenland, Iceland, Scandinavia, and Svalbard (Beker et al. 2016). In the Rockies, this species can be recognized by its alpine habitat, association with willows (primarily *S. planifolia*), small size, lack of veil, and pinkish buff to orange brown uniformly colored pileus often with a white, crenate margin.

### 3. *Hebeloma subconcolor* Bruchet, Bull. Mens. Soc. Linn. Lyon 39 (6, suppl.): 127 (1970)

Figures 5, 9, 23(3)

**Etymology.** *concolor* for the similar coloration of pileus and stipe, which is not a consistent feature.

**Description.** Cortina absent. Pileus 15–20 mm, convex, with or without a low broad umbo, becoming plane, smooth, moist, light to medium brown, pruinose with a grayish tint or sheen, lighter towards margin but not distinctly two-toned; margin turned down or not, entire. Lamellae adnexed, subdistant, well-separated, medium broad to broad, L = 25–32 plus lamellulae, dull brown, light brown; edges lighter. No beaded drops reported. Stipe 15–30 × 3–4 mm, equal, apex somewhat lighter tan and pruinose, below totally covered with longitudinal white fibers over a brownish ground base. Context buff. Odor astringent. Exsiccate: pileus medium brown, not two-toned, with grayish tint, dull; lamellae broad, warm cinnamon; stipe long, dull brown, narrow.

Basidiospores yellowish brown, amygdaliform, with a small apiculus, weakly ornamented (O2), loosening perispore observed in a few spores (P0, P1), distinctly dextrinoid (D2, D3), 10.5–12.5 × 6.5–7.5 µm, on average 11.6 × 7.1 µm, Q = 1.65. Basidia 25–34 × 8–10 µm, four-spored. Cheilocystidia gently clavate, some slightly swollen at apex and base, 40–60 × 6–11 µm at apex, 4.5–7 µm in middle, and 4–7 – (8) µm at base. Pleurocystidia absent. Epicutis thickness 60–75 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** Two collections reported under willow at alpine elevations of 4000 m in Colorado; noted as cespitose to gregarious.

**Figure 9.** *Hebeloma subconcolor*, DBG-F-022785 and DBG-F-022786.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Clear Creek County, Summit Lake Park, under *Salix*, some in moss, at 4000 m, 22 Aug 2012, DBG-F-022785; DBG-F-022786, L. Gillman.

**Discussion.** The sequences of the two collections for *H. subconcolor* from the Rocky Mountains are identical. The RM sequence differs by 1–4 [0] bp from the *H. subconcolor* collections described in Beker et al. (2016) and Grilli et al. (2016), where the ITS ML results were also shown. The closest *H. velutipes* sequence included in the dataset used in Fig. 5 differs in 3 [0] bp. *Hebeloma velutipes* is the only species of *Hebeloma* that cannot be distinguished from *H. subconcolor* by ITS sequence (Beker et al. 2016; Grilli et al. 2016; Fig. 5). However, morphologically these two species are very different and can be easily separated.

This small species has a grayish cast not found in other taxa in sections *Denudata* and *Velutipes* that we report from the Rocky Mountains; also, the lamellae are well separated and few in number. It should be compared to the other non-veiled, small species such as *H. aurantioumbrinum* and *H. vaccinum*. *Hebeloma velutipes* has a different coloration and is larger with many more full length lamellae. *Hebeloma subconcolor* is known from arctic and alpine locations in the European Alps, Greenland, Iceland and Scandinavia (Beker et al. 2016, 2018).

### 4. *Hebeloma hiemale* Bres., Fung. Trident. 2: 52 (1898)

Figures 4B, 10, 23(4)

**Etymology.** From *hiemalis*, winter or wintry, presumably to denote the production of basidiomes in colder seasons or habitats

**Description.** Cortina absent. Pileus 15–35 mm in diameter, slightly conic-convex or domed-convex, smooth, greasy, pinkish buff, yellowish buff, to pale cream at the margin, with uniform coloration, somewhat hoary, with or without a white rim a few

**Figure 10.** *Hebeloma hiemale*, CLC3094 and CLC3574.

mm wide at margin; margin turned down or rolled in, then wavy. Lamellae narrowly attached, emarginate, somewhat crowded, L = 48–60 plus lamellulae, white to pale milk coffee, pale brown, wood brown; edges white floccose, with drops of liquid. Stipe 20–45 × 5–12 mm, equal, slightly clavate towards the base, whitish cream, totally pruinose (big floccules) for most of length and smoother below. Context white to watery cream, firm. Odor raphanoid, faint. Exsiccate: pileus yellowish brown, not distinctly two-toned; lamellae brown with white edges; stipe white and slimmer than for *H. alpinum*.

Basidiospores yellowish brown, some coloring slightly brown in Melzer's, fat-bellied amygdaliform, limoniform, with short snout, apiculate, distinctly ornamented (O2), a few with slightly loosening perispore (P0,P1), rarely guttulate, with thickish wall, slightly dextrinoid (D1, rarely D2), 10–12 × 6–7 µm, on average, 11.1 × 6.8 µm, Q = 1.64. Basidia 25–35 × 7–9, most four-spored, maybe a few two-spored, occasionally with long sterigmata (–5 µm). Cheilocystidia long, gently clavate, clavate-lageniform, some with septa, 35–75 µm long, at apex 6–9 µm, in middle 4–6 µm, at base 4.5–9 µm, thickening sometimes observed in the middle. Pleurocystidia absent. Epicutis thickness 60–200 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** In the alpine zone with dwarf willows, *Dryas* and *Betula*, confirmed from Colorado, Montana, and Wyoming.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Summit County, Loveland Pass, 3750 m, with *Salix* in scrubland, 7 Aug 1999, ZT8072 (ETH), E. Horak; 15 Aug 1997, 3655 m, with *Salix*, DBG-F-019162, B. Rognerud; 21 Aug 2003, with *Salix* sp., DBG-F-021418, H. Miller; 20 Aug 1999, 3597 m, with *Betula*, DBG-F-020440, O.K. Miller; 22 Aug 1999, 3655 m, with *Salix* sp., DBG-F-020437, O.K. Miller; 16 Aug 1997, 3749 m, with *Salix* sp., DBG-F-019241, S. Trudell; 19 Aug 1999, 3620 m, DBG-F-021194, V.S. Evenson; 20 Aug 1999, 3620 m, with *Salix* sp., DBG-F-20431, V.S. Evenson; 20 Aug 1999, 3571 m, with *Betula nana*, DBG-F-020433, V.S. Evenson; 24 Aug 1999, 3620 m, with *Salix* sp., DBG-F-019597, N. Smith Weber; Clear Creek County, Mount Goliath, 3658 m, with *Salix*, 1 Sept 1999, DBG-F-020551, V.S. Evenson; 1 Sept 1999, 3810 m, DBG-F-020550, V.S. Evenson; Boulder County, West of Caribou townsite, 10 July 1988, DBG-F-016104, V.S. Evenson. Sawatch Range, Independence Pass, 13 Aug 2001, 3759 m, with *Dryas octopetala* and *S.*

*reticulata*, ZT9828, E. Horak. San Juan County, San Juan Mountains, Mineral Basin, 3835 m, with *Salix arctica*, 7 Aug 2001, CLC1668 (MONT), C. Cripps. MONTANA: Carbon County, Beartooth Plateau (at the stateline with WY), 3100 m near *S. reticulata*, 19 July 2001, CLC1574 (MONT), C. Cripps; site 2 at the stateline MT/WY, with *S. reticulata* 14 Aug 2014, CLC3094 (MONT), C. Cripps; Quad Creek, 3004 m, with *S. reticulata* and *Persicaria vivipara*, 8 Aug 2008, HJB12457, M. Nauta; site 1 in *Dryas*, 11 Aug 2017, CLC3533 (MONT), C. Cripps; with *S. planifolia* and *S. glauca*, 17 Aug, 2017, CLC3574 (MONT), C. Cripps; with *Salix planifolia*, 17 Aug 2017, CLC3575 (MONT), C. Cripps. WYOMING: Park County, Highline Trail, 3200 m, with *Dryas octopetala* and *S. reticulata*, 8 Aug 2008, ZT6417 (ETH), E. Horak.

**Discussion.** An ITS tree including *H. hiemale* is given by Eberhardt et al. (2016); the respective network is shown in Figure 4B. The RM dataset includes ITS sequences from 22 collections. These were matched by the same number of sequences from the FE dataset. *Hebeloma hiemale* ITS sequences were shown to form a well-supported monophylum in ML results presented in earlier studies (Beker et al. 2016; Eberhardt et al. 2016). Beker et al. (2010) showed that it is a species with a relatively high number of different ITS variants. The disparity between variants is mostly caused by gaps and SNPs (single-nucleotide polymorphisms). The number of differences between any pair of sequences of the presented *H. hiemale* data set is 0–9 [0–2] bp, within the RM sequences 0–8 [0] bp.

This species is widespread across Europe occurring from the subalpine to the alpine, in lowland dunes, shrublands, gardens, and parks; it occurs with a wide array of deciduous and coniferous trees and this includes a number of willow species, including dwarf *Salix*. Confirmed arctic-alpine reports include those from Canada, Greenland, Iceland, Scandinavia, and Svalbard with *Salix herbacea* and *S. polaris* as well as *Dryas* and *Persicaria* (Beker et al. 2016). Here it is confirmed with *S. reticulata*. *Hebeloma hiemale* has rarely been reported from North America in either subalpine or alpine habitats (Beker et al. 2010), but many collections previously labeled *H. alpinum* are now confirmed as *H. hiemale.*

This species looks like a small version of *Hebeloma crustuliniforme* but usually has more color in the pileus, particularly at the center. It has cheilocystidia that are generally swollen in the lower half, giving an hourglass appearance. The spores are verrucose, more warty than those of *H. alpinum*, but less so than the spores of *H. vaccinum*. There was some ambiguity around the delineation of *H. hiemale*, which was ultimately resolved with selection of an epitype (Beker et al. 2010; Eberhardt et al. 2015a).

### 5. *Hebeloma avellaneum* Kauffman, Papers of the Michigan Academy of Sciences 17: 171 (1933)

Figures 3, 11, 23(5)

**Etymology.** For the color of hazelnuts, such as *Corylus avellana*.

**Description.** Cortina absent. Pileus 20–40 mm across, hemispherical, convex, can be domed, glabrous-viscid, rich Sayal brown, ochraceous to orange brown, cin-

namon brown, with frosty canescence; margin turned down, or rolled in, remaining light colored, downy. Lamellae adnate to subdecurrent, narrow, L = 90 plus lamellulae, pale avellaneous, pale cinnamon, not dark at maturity; edges floccose, beaded. Stipe 25–35 mm × 8–10 mm, equal to clavate, sturdy, white to cream, pruinose at apex, scurfy scales below. Context thick over pileus area, whitish, watery, not changing, or browning a bit in stipe but not from base up. Odor fruity or herbal tones. Exsiccate: medium-sized, cespitose in one group, hemispherical with margin inrolled, evenly colored, ochraceous, smooth to aereolate; stipe white, sturdy.

Basidiospores yellowish brown, amygdaliform, with a small apiculus, weakly ornamented (O1, O2), loosening perispore observed in a few spores (P0, P1), distinctly dextrinoid (D3), 8–11 × 5–6 µm, on average 9.5 × 5.4 µm, Q = 1.76. Basidia 25–34 × 6.5–8.5 µm, two- and four-spored. Cheilocystidia variable, many cylindrical, but also gently clavate, capitate and capitate-stipitate as well as clavate-lageniform, 30–80 × 4–13(–15) µm at apex, 3.5–6.5 µm in middle, and 4–8(–9) µm at base. Pleurocystidia absent. Epicutis thickness 80–130 µm, no encrusted hyphae recorded.

**Rocky Mountain ecology.** Cespitose, or clustered, in low alpine krummholz with conifers and willows. Both collections we have studied are from Colorado.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Summit County, Loveland Pass Lake, 4000 m, under willows, 20 Aug 1999, DBG-F-020434, no conifers mentioned but present in the general area, O.K. Miller Jr; Boulder County, above Mountain Research Station, 3200 m, with small willows (*Salix planifolia*) and one spruce within 2 m, 1 Aug 1998, DBG-F-019533, V.S. Evenson.

**Other American specimens examined.** U.S.A. WASHINGTON: Grays Harbor County, Lake Quinault, Olympic National Park, at 75 m, on mossy edge of forest clearing, 8 Nov 1925, MICH 10722, C.H. Kauffman (**holotype**). CANADA. NEWFOUNDLAND AND LABRADOR: Pinware River at 15 m, under conifers, 7 Sep 2005, HJB14320, leg. J. May.

**Discussion.** Based on ITS data, *H. avellaneum* is monophyletic, but unsupported by bootstrap values (Fig. 3). In terms of phylogeny, its closest relative is *H. catalaunicum* Beker, U. Eberh., Grilli & Vila, a Mediterranean species. It is also close to *H. naviculosporum* Heykoop, G. Moreno & Esteve-Rav. and *H. nanum* Velen. All three species appear to associate with *Pinaceae* (Beker et al. 2016). The identification of *H. avellaneum* is supported by type studies. The fourth collection used in Fig. 3 is from Canada (Newfoundland) and has been presented by Voitk et al. (2016) as "*Hebeloma* sp. sect. *Naviculospora*".

Based on our studies of this taxon and of the habitats where it has been collected, we strongly suspect that this species is typically associated with conifers in temperate to subalpine or subarctic habitats. The holotype was collected in a temperate rainforest within the Olympic Peninsula in western Washington state. The often pruinose pileus with distinctive orange tones is indicative of *H*. sect. *Naviculospora*. These specimens were found in the low alpine where conifers are possible, and indeed *Picea* was noted for one collection, but only willows for the other. In the low alpine of the Rocky

**Figure 11.** *Hebeloma avellaneum*, DBG-F-019533 and UMICH 10722 (holotype).

Mountains, the species might be confused with *H. alpinum*, *H. velutipes*, or *H. hiemale* because of its robust habit and lack of veil, however there are more orange color tones of the pileus; the spores are smaller and more dextrinoid than one would expect for *H. alpinum* and *H. hiemale*.

**6. *Hebeloma velutipes* Bruchet, Bull. Mens. Soc. Linn. Lyon 39 (6, suppl.): 127 (1970)**
Figures 5, 12, 23(6)

**Etymology.** *velutinus*, for the velvety appearance of the stipe surface.

**Description.** Cortina absent. Pileus 20–60 mm in diameter, convex, convex-domed, tacky to kidskin, smooth, not spotting, not hygrophanous, nearly unicolor, very pale buff, pale salmon buff, with hoary coating (pruinose); margin incurved but not involute, entire. Lamellae narrowly attached, sinuate or marginate, narrow to broad, slightly crowded, L = 50–75 plus lamellulae, white at first, then milk coffee color; edges white-floccose; beaded drops observed on some. Stipe (25–)30–60 × 7–15 mm, robust, equal and either narrowing or swollen at base up to 20 mm wide, slightly curved or not, pruinose or floccose in top half, longitudinally fibrous in lower half or more smooth. Context whitish, thick in pileus, firm in stipe, stuffed/hollow. Odor raphanoid. Exsiccate: largest of all species recorded; uniform pale buff pileus, lamellae, and stipe.

Basidiospore print deep Sayal brown. Basidiospores yellowish brown, amygdaliform, with a slight snout, apiculate, not guttulate, a bit rough (O1, O2), moderately dextrinoid (D2, D3), no obvious loosening perispore (P0), 10–12 × 6–7 µm, on average 10.4 × 6.6 µm, a few large spores (–18 × –7) present, Q = 1.57. Basidia 26–32 × 7.5–9 µm, clavate, four-spored. Cheilocystidia gently clavate, thin-walled, occasionally bifurcate at apex, 55–80 µm × 7–12 µm at apex, 5–8 µm in middle, 4–7 µm at base. Pleurocystida absent. Epicutis thickness 80–200 µm, with some encrusted hyphae.

**Rocky Mountain alpine ecology.** In alpine situations, mostly reported with *Dryas octopetala* and also with *Salix* in Montana and Colorado.

**Figure 12.** *Hebeloma velutipes*, CLC1651 and ZT8072.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Gunnison County, Sawatch Range: Cumberland Pass, 3660 m, near *Salix glauca* but *Dryas* in vicinity, 4 Aug 2001, CLC1651 (MONT), C. Cripps; Cottonwood Pass, 3700, in pure *Dryas octopetala*, 4 Aug 2001, CLC1646 (MONT), 12 Aug 2001, CLC1725 (MONT), both C. Cripps. Summit County, Herman Gulch Trailhead, 3200 m, with *Salix* spp., 26 Aug 1983, DBG-F-005617, V.S. Evenson. MONTANA: Carbon County, Beartooth Plateau, site 1, 3000 m, in pure *D. octopetala*, 27 July 2004, CLC1980 (MONT), C. Cripps; N of East Summit, with *Dryas* and *Salix reticulata*, 30 July 1997, ZT6100 (ETH), E. Horak.

**Discussion.** Grilli and co-workers (2016) showed that in ITS ML analyses *H. velutipes* falls into three unsupported clusters, i.e. one with *H. incarnatulum*, one with *H. leucosarx*, and one with *H. subconcolor*. The latter is discussed above; the former two species do not occur in the kind of habitats sampled in the Rocky Mountains (Beker et al. 2016; Grilli et al. 2016). *Hebeloma velutipes* cannot be distinguished from these three species based on ITS, but it is distinct from all other species treated in Beker et al. (2016). The reason for the intraspecific variation observed in *H. velutipes* has already been shown by Aanen et al. (2001), namely that *H. velutipes* possesses ITS alleles that differ greatly. In the Rocky Mountains, representatives of two of the clusters were found, the *H. leucosarx* cluster and the *H. subconcolor* cluster, and the collections from Montana fall into the first of these clusters while those from Colorado fall in the latter cluster. Accordingly, the number of differences are between 2–23 [0–5] bp; seven pairs with 2–6 [0–1] bp differences and seven pairs with 20–23 [2–5] bp differences. Looking at all included collections, the overall figure hardly changes (1–23 bp), although the collections randomly selected from the FE dataset include representatives of all three clusters (Fig. 5). To date we have not observed any morphological or ecological differences between members of the different clusters. The geographical differentiation of the RM representatives of *H. velutipes* is possibly a sampling artifact.

This species displays the characteristic features of *H.* sect. *Velutipes*, i.e. the absence of a veil, presence of a velutinate stipe, and rather strongly dextrinoid spores (reaction can take a while), as well as the gently clavate cheilocystidia. It is known to be common and widely distributed in Europe at lower elevations primarily with deciduous trees but also with coniferous hosts. There are a number of arctic and alpine records, particularly from Svalbard with *Dryas octopetala* and *Salix polaris* (Beker et al. 2016), and it has been previously reported from the North American alpine zone (Beker et al. 2010). This species produces relatively large basidiomes for the genus in the alpine; but because of its pale coloration and lack of a veil, young specimens may have been incorrectly identified as *H. alpinum* or *H. hiemale*, which are typically smaller. Phylogenetically *H. velutipes* is not close to these two species but, as mentioned, is related to *H. subconcolor*, which is smaller with fewer lamellae, grayer coloration and is also reported from the Rocky Mountain alpine zone. Interestingly, almost all Rocky Mountain specimens of *H. velutipes* were found with *Dryas*, which might help with field recognition, in addition to its robust stature, and stout white stipe.

**7. *Hebeloma alpinum* (J. Favre) Bruchet, Bull. Mens. Soc. Linn. Lyon 39 (6 suppl.): 68 (1970)**
Figures 4A, 13, 23(7)

**Etymology.** *alpinum* from the alpine.

**Description.** Cortina absent. Pileus 20–35 mm in diameter, convex to broadly domed, buff to pale brown, rarely brown, slightly paler at margin but not two-toned, smooth, cracking when dry; margin turned down or in. Lamellae attached, emarginate, somewhat broad, pale milk coffee, L = 40–70 plus lamellulae; edges white fimbriate, beaded. Stipe 15–30 × 4–10 mm, rather short, equal, sometimes slightly restricted in middle, clavate, white, firm. Context buff. Odor slightly raphanoid. Exsiccate: pileus brown, slightly caramel color; lamellae dark rusty brown; stipe short, cream color.

Basidiospores yellowish brown, amygdaliform with a snout, more symmetrical in side view, apiculate, sometimes guttulate, weakly ornamented (O1, O2), no loosening perispore noted (P0), very slightly dextrinoid (D0, D1), 10–12 × 6–7 µm, on average 11.2 × 6.6 µm, a few large spores present –18 × –8 µm, Q = 1.69. Basidia 32–40 × 8.5–10.5, mainly four-spored, some possibly two-spored. Cheilocystidia mostly clavate-stiptate, 55–75 µm long, apex width 6.5–10.5 µm, median width 4–5.5 µm, base width 3.5–4.5 µm. Pleurocystidia absent. Epicutis thickness 60–160 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** Information is based on one collection from Montana, with mixed dwarf and shrub *Salix* species.

**Rocky Mountain specimens examined.** U.S.A. MONTANA: Park County, Lulu Pass, 3000 m in *Salix arctica* and *S. glauca*, 11 Aug 2012, CLC2855 (MONT), C. Cripps.

**Discussion.** The only confirmed report we have for this species from the Rocky Mountains relies on a single collection of a few specimens found near Cooke City,

**Figure 13.** *Hebeloma alpinum*, CLC2855 and HJB11123 (Switzerland).

Montana at an elevation of 3000 m with dwarf and shrub *Salix* species. In the network Fig. 4A, this single RM representative of *H. alpinum* appears rather distant from its European counterparts, which are clustered at one of the centers of the network, i.e. the biggest circle, of the *H. alpinum* complex. An ITS tree including the *H. alpinum* complex is given in Eberhardt et al. (2015a). Although this collection appears molecularly quite far removed from its conspecifics, 6–10 [1–2] bp, the total distance is largely due to a 5 bp indel repeating a sequence motif generally present in members of the *H. alpinum* complex. Thus, the molecular results do not argue against this being *H. alpinum*. This species is quite variable molecularly as well as morphologically (see the discussion of the alpinum-complex in Beker et al. 2016). The spores of this collection are on the lower end of the range for this taxon, as given in Beker et al. 2016, but still comfortably within the range.

*Hebeloma alpinum* has been reported previously in North America from the Rocky Mountain alpine zone (Cripps and Horak 2008) and Alaska (Miller 1998), however, most sightings were not molecularly confirmed. There are three records from the Canadian Arctic collected in 1971 and 1974 (Ohenoja and Ohenoja 2010), which have been confirmed molecularly (Beker et al. 2018). Ten collections at the Denver Botanic Garden, originally labeled *H. alpinum*, are now molecularly confirmed as *H. hiemale* (see comments for this species).

Favre originally described this species from the Swiss Alps as *Hebeloma crustuliniforme* var. *alpinum* Favre (Favre 1955) and Bruchet (1970) elevated it to species level. *Hebeloma alpinum* appears confined to arctic-alpine habitats and has been reported from such regions of the European Alps, Carpathians, Pyrenees, Greenland, Iceland, Scandinavia, Svalbard, and Switzerland, primarily with *Salix reticulata*, *S. polaris*, *S. retusa*, and *Dryas octopetala* as well as *Persicaria* (Beker et al. 2016). The species is in *H.* sect. *Denudata*, subsect. *Crustuliniformia* because of the lack of a veil, the clavate-stipitate shape of the cheilocystidia and molecular data (Eberhardt et al. 2015a). As a relatively robust alpine species, it should be compared to *H. hiemale* and *H. velutipes*; the latter has a robust floccose white stipe.

## Hebeloma section *Hebeloma*

We will address this next section in two parts, again following the outline of the key: first those that have ellipsoid indextrinoid spores (*H. alpinicola*, *H. dunense*, *H. excedens*, *H. marginatulum*, and *H. mesophaeum*), also referred to as the *H. mesophaeum* complex and secondly those with amagdaliform spores that are rather strongly dextrinoid (*H. hygrophilum*, *H. nigellum*, *H. oreophilum*, and *H. spetsbergense*), also referred to as the *H. nigellum* complex.

## *Hebeloma* section *Hebeloma*, Part one: cortina present, spores ellipsoid, not dextrinoid

**8. *Hebeloma marginatulum* (J. Favre) Bruchet, Bull. Mens. Soc. Linn. Lyon 39 (6, suppl.): 43 (1970)**
Figures 6B, 14, 23(8)

**Etymology.** From *marginatus*, with a margin or border, emphasizing a thin line of tissue near the margin.

**Description.** Cortina present, remnants distinctly present in some. Pileus 15–40(–50) mm in diameter, slightly conic-convex, domed convex, irregular, sometimes with a flat center that can even be dished, smooth or rough due to velipellus, shiny, strongly canescent, underneath dark brown, dark chestnut, to dark caramel color, mostly uniform but two-toned in some and then lighter at margin (more hoary, dingy whitish, or ochraceous in one), with a fine white border around the pileus perimeter a few mm in from margin, not hygrophanous; margin turned down or in, rather persistently so, and then covered with copious veil, often irregular, wavy, fragile. In one collection, the cuticle is rather thick and rubbery. Lamellae deeply emarginate and squared off, some pulling away, somewhat broad, L = 30–40 plus lamellulae, cream, then pinkish buff, darkening to medium coffee brown; edges fimbriate. Stipe 20–40(–45) mm × 2–6(–10) mm, equal, undulating or not, pale buff (some with possible yellow tint), and dark (up to black) at base, pruinose at apex, longitudinally fibrous lower, with a few longitudinal fibrils. Context dingy whitish, some with yellowish tones and dark at base. Odor raphanoid or sourish, sometimes faint. Exsiccate: pileus pale brown to dark brown, some obviously canescent; lamellae medium brown; stipe buff or ocher, darker at base.

Basidiospores yellowish gray, pale in Melzers, elliptical with rounded end, inequilateral in side view, no big apiculus, not guttulate, smooth to slightly punctate or rough (O1, O2), indextrinoid (D0, D1), perispore not loosening (P0), 9–12(–13) × 5.5–7(–8) µm, on average 10.1 × 6.4 µm, Q = 1.59. Basidia 25–35 × 8–9 µm, clavate, two and four-spored. Cheilocystidia lageniform, ventricose, often with very long equal neck, and somewhat gradually swollen base, occasionally clavate at apex, sometimes cylindrical, 35–80 µm long × 4–7 µm at apex, 4–6 in middle, and 7–12 (13) at base, no thickening noticed. Pleurocystidia absent. Epicutis thickness 40–100 µm, with some encrusted hyphae.

**Figure 14.** *Hebeloma marginatulum* DBG-F-020841 and CLC3545.

**Rocky Mountain ecology.** In the Rocky Mountain alpine zone, with various willows, including dwarf willows *Salix arctica* and *S. reticulata*, and *shrub willow S. planifolia.* Known from both Colorado and Montana.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Front Range, Loveland Pass, 12 Aug 2013, in *Dryas*, DBG-F-027694, C. Cripps; 12 Aug 2013, with *Salix* sp., DBG-F-027695, C. Cripps; 25 Aug 2000, with *Salix* sp., DBG-F-020708, V.S. Evenson; 21 Aug 2003, with *Salix* sp., DBG-F-021388, V.S. Evenson; 20 Aug 2013, DBG-F-027682, L. Gillman; 21 Aug 2003, with *Salix* sp., DBG-F-021405, O.K. Miller, Jr; San Juan County, Cinnamon Pass, 3700 m, with *Salix arctica*, 29 July 2000, CLC 1413 (MONT), C. Cripps, 3700 m, with *Salix arctica*, 27 July 2002, CLC1811 (MONT), C. Cripps; 29 July 2000, with *S. reticulata* and *Salix* sp., ZT9002 (ETH), E. Horak; Black Bear Basin, 2 Aug 2000, 3830 m, with *S. planifolia*, CLC1448 (MONT), C. Cripps; 8 Aug 2000, with *S. arctica*, CLC1449 (MONT), C. Cripps; 11 Aug 2001, with *S. reticulata*, ZT9813 (ETH), E. Horak; 3760 m, with *Salix arctica*, 11 Aug 2001, CLC1718 (MONT), C. Cripps; Emma Lake/Horseshoe Basin, 3688 m, with *S. arctica*, 31 July 2002, CLC1874 (MONT), C. Cripps; 31 July 2002, with *S. arctica*, CLC1880 (MONT), C. Cripps; Imogene Pass, 29 July 2002, 3850 m, with *S. arctica*, CLC1836 (MONT), C. Cripps; Mineral Basin, 3850 m, with *S. arctica*, 29 July 2002, with *S. arctica*, CLC1840 (MONT), C. Cripps; without obvious host, although *Salix* in the vicinity, 30 July 2002, CLC1860 (MONT), C. Cripps; with *S. arctica* and *S. planifolia*, 30 July 2002, CLC1861 (MONT), C. Cripps; 3835 m, with *S. arctica*, 7 Aug 2001, CLC1667 (MONT), C. Cripps; Stony Pass, 3840 m, with *S. arctica*, 28 July 2002, CLC1824 (MONT), C. Cripps; 3840 m, with *S. arctica*, 28 July 2002, CLC1826 (MONT), C. Cripps. Sawatch Range, Independence, 3 Aug 2000, with *Salix* sp., DBG-F-020841, DBG-F-020856, V.S. Evenson; 3 Aug 2000 with *Salix* sp., DBG-F-020843, V.S. Evenson; 3760 m, with *S. planifolia*, 7 Aug 2000, CLC1478 (MONT), C. Cripps. MONTANA: Carbon County, Beartooth Plateau, site 1, 9 Sept 2000, with *S. planifolia*, CLC1545 (MONT), C. Cripps; Quad Creek, 8 Aug 2008, with *S. planifolia*, HJB12458, A. and M. Ronikier; 11 Aug 2017; with *Salix reticulata* and *S. planifolia*, 11 Aug 2017, CLC3545 (MONT), C. Cripps.

**Discussion.** *Hebeloma marginatulum* is distinct from other species of the *H. mesophaeum* complex, but not by much as to molecular distance (Fig. 6B). The species is paraphyletic in relation to the monophylum including the other taxa of the complex. With 0–19 [0–2] bp, the intraspecific variation is quite extensive in *H. marginatulum* in terms of total differences. Within each dataset, the ITS variation is also quite large, 0–14 [0–2] bp for the RM (29 sequences) and 0–17 [0–2] bp for the FE dataset (21 sequences). However, the total number of considered sequences is also larger than for other species.

This taxon was first described as *H. versipelle* var. *marginatulum* by Favre (1955) from the alpine region of the Swiss Alps and was later raised to species level by Bruchet (1970). It is now considered to be restricted to arctic and alpine habitats primarily with dwarf willows (Beker et al. 2016, 2018). Confirmed records show it to be present in these habitats in Canada, Greenland, Iceland, Scandinavia, Svalbard as well as the European Alps and the Carpathians and Rocky Mountains (Eberhardt et al. 2015b; Beker et al. 2016). Vesterholt (2005) described *H. polare* as a darker brown closely related species, but this has been synonymized with *H. marginatulum* (Beker et al. 2016). The Rocky Mountain specimens are also mostly uniformly dark brown with a canescent sheen.

Collections from the alpine that are very hoary and dark brown have been misinterpreted as *H. bruchetii* Bon (Miller and Evenson 2001) before molecular techniques; *H. bruchetii*, first described as an alpine species, has now been synonymized with *H. mesophaeum* and should have smaller spores. *Hebeloma marginatulum* is mentioned as a subalpine species (in Idaho) by Smith et al. (1983) who described two varieties (var. *fallax*, var. *proximum*) from the subalpine in Colorado. Smith's spore descriptions (dextrinoid with sharp ends) for his varieties may not fit this species, but the authors recognize that these varieties of *H. marginatulum*, and indeed other closely related species, need more study in North America.

This species is in *H.* sect. *Hebeloma* because of basidiomes with a cortina and the ventricose cheilocystidia together with the non-dextrinoid, or barely dextrinoid, spores that are primarily elliptical; within this group, it has an arctic-alpine habitat and relatively large spores (greater than 10 × 6 μm).

### 9. *Hebeloma alpinicola* A.H. Sm., V.S. Evenson & Mitchel, Veiled species of *Hebeloma* in the western United States (Ann Arbor): 48 (1983)

Figures 6B, 15, 23(9)

**Etymology.** *alpini-* and *cola*, meaning dweller, to emphasise its alpine habitat, although this taxon is not found exclusively in such habitats.

**Description.** Cortina present. Pileus robust, fleshy, 20–40 mm in diameter, irregular convex, somewhat domed or not, reddish brown center with grayish tones, outwards ocher and lighter towards margin (buff not white), not particularly two-toned, with hoary canescent coating that dries shiny; margin turned in at first, and then

turned down. Lamellae narrowly attached, slight emarginate, or with a tooth, or pulling away, somewhat broad, milk coffee, L = 36–44; edges white floccose. Stipe 30–40 × 5–10 mm, equal, straight or not, whitish and pruinose at apex, dingy ocher and longitudinally fibrillose and striate in lower part, base sometimes encased in sand or earth. Context dingy whitish, darker below, and flesh staining brown; stipe solid or slightly hollow. Odor raphanoid. Exsiccate: pileus and stipe medium ochraceous brown; lamellae dark brown; stipe base encased in soil in the large collection (CLC1577).

Basidiospores elliptical, or some slightly amygdaliform or ovoid, with rounded end, smooth to slightly rough (O0, O1), small apiculus, not guttulate, not dextrinoid (D0), perispore not loosening (P0), 8–11 × 5–6, on average 9.1 × 5.6 µm, Q = 1.63 Basidia clavate, four-spored, 30–35 × 7–8 µm. Pleurocystidia usually absent but occasionally present, sometimes rostrate. Cheilocystidia mostly cylindrical for the top two thirds and then swollen near the base (lageniform or ventricose), 30–70 µm long × 3–8 µm at apex, 3–7 µm in middle, and 6–11 µm at base, no yellow contents noted. Epicutis thickness up to 200 µm, with no encrusted hyphae recorded.

**Rocky Mountain ecology.** Collected from two different sites, one in Montana, the second in Colorado. The first site is a mixture of *Dryas*, *Salix planifolia* and *S. reticulata*, with some *Persicaria* present. The second site is a low alpine zone with dwarf willows. In both cases the growth habit was gregarious, sometimes in rings, sometimes cespitose, but not completely joined.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Gilpin County, Roosevelt National Forest, Little Echo Lake shoreline, near dwarf willows, 3500 m, 4 Sept 1999, DBG-F-020565, V.S. Evenson, M. Brown; 4 Sept 1999, DBG-F-020582, V.E. Evenson. MONTANA/WYOMING state line: Beartooth Plateau, 3020 m, with *Persicaria*, *Geum*, sedges, grasses, and quite distant *S. planifolia*, 19 July 2001, CLC1577 (MONT), C. Cripps; Quad Creek, 4 Aug 2008 with *Dryas octopetala* and *S. reticulata*, HJB12439, C. Cripps.

**Other specimens examined.** See Table 2.

**Discussion.** Figure 6B shows *H. alpinicola* as paraphyletic and closely related but not mixed with species from the *H. mesophaeum* complex other than *H. marginatulum*. The *H. alpinicola* representatives differ by 0–13 [0–2] bp from each other. Based on morphology and ITS results, the types of seven species, namely *H. alpinicola*, *H. chapmaniae* A.H. Sm., *H. littenii* A.H. Sm., *H. nigromaculatum* A.H. Sm., *H. perigoense* A.H. Sm., *H. smithii* = *H. angustifolium* A.H. Sm. et al. nom. illegit. (the name *Hebeloma angustifolium* (Britzelm.)Sacc. already existed) and *H. subargillaceum* A.H. Sm. are synonyms. The inclusion of the seven types increases the absolute intraspecific variation to 0–16 [0–4] bp. The distance from other species of the complex is 3–22 [0–7] bp within the sample. Although *H. alpinicola* has not yet been fully tested in multilocus analyses, we consider its distinctive morphology combined with the ITS evidence to be sufficient to assign the four RM collections to this species.

This taxon, with its small ellipsoid, indextrinoid spores and ventricose cheilocystidia is a member of *H.* sect. *Hebeloma*. Morphologically it is closely related to *H. excedens* and *H. mesophaeum.* It is generally more robust than these two species, espe-

**Figure 15.** *Hebeloma alpinicola*, DBG-F-020565 and CLC1577.

cially the stipe, and the pileus is not as two-toned. Colorado collections were described as having gray tones. While further work is needed to decide whether this really is a species distinct from the other two, the molecular evidence coupled with the morphological evidence suggest this to be the case. We have studied a number of collections, from a variety of habitats within North America that all appear to represent this taxon. *Hebeloma chapmaniae*, *H. littenii*, *H. nigromaculatum*, *H. perigoense*, and *H. subargillaceum* were all published by Smith et al. (1983) in the same publication that featured *H. alpinicola*; the replacement name *H. smithii* is later (Quadraccia 1987). Although there is some molecular variation between these seven collections, it is very small and we see insufficient evidence to separate these species. We have selected the name *Hebeloma alpinicola* on the grounds that although not all collections are strictly alpine, the majority are at least subalpine.

**10. *Hebeloma dunense* L. Corb. & R. Heim, Mém. Soc. Natn. Sci. Nat. Math. Cherbourg 40: 16 (1929)**
Figures 6B, 16, 23(10)

**Etymology.** Originally found in sand in dunes.

**Description.** Cortina present. Pileus 10–28 mm in diameter, convex, slightly conic-convex, with or without a slight umbo (one papillate), or almost applanate, some sunken in center, smooth, greasy, pale pinkish buff at first, becoming caramel color in center, outwards remaining pale, with a hoary coating, some flecks of white in outer part, mostly appearing pale unicolor; margin turned in or down, covered with white veil tissue or not. Lamellae emarginate to subdecurrent, or pulling away, variable, L = 25–48 plus lamellulae, a bit distant, cream buff to pinkish buff at first, then milk coffee; edges white fimbriate. Stipe 20–50 × 2–6 mm, equal or narrowing a bit at base, dingy whitish buff in top part, sometimes pruinose and base darkening to golden color then blackish brown (not always obvious unless cut open), with fibrils on lower part

**Figure 16.** *Hebeloma dunense*, CLC1821 and CLC1845.

and/or a few 'patches of tissue'. Context dingy white, watery buff, dark at base, sometimes splitting, often hollow when mature; tough in base. Odor faintly raphanoid or absent. Exsiccate: mostly pale; pileus buff or more ochraceous buff, center a bit caramel or not; lamellae pale light ocher; stipe buff, not obviously darker at base.

Basidiospores yellowish gray in Melzer's, mostly elliptical, a few slightly amygdaliform but typically without much snout, no big apiculus, not guttulate, look smooth but may be slightly rough in Melzer's (O1, O2), not or only very slightly dextrinoid (D0, D1), and no perispore loosening (P0), 9.5–11.5 × 5.5–7 µm, on average 10.3 × 6.2 µm, Q = 1.65. Basidia 20–30 × 8–9 µm, clavate, four-spored mostly. Pleurocystidia absent. Cheilocystidia cylindrical in the upper part and slightly swollen to more swollen at the base, 40–55 µm long × 4.5–6 µm at apex, 4–6 µm in middle, and 7–10.5 µm wide at base, with occasional thickening of the apical wall, some septate and clamped; many with dense yellow contents. Epicutis thickness 25–75 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** In the alpine zone of the San Juan Mountains, with dwarf willows *S. reticulata* and *S. arctica*, and shrub willow *S. planifolia*, some in moss or near streams.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: San Juan County, San Juan Mountains, Cinnamon Pass, 3700 m, with dwarf *Salix* near stream, 29 July 2000, CLC1411 (MONT), C. Cripps; with *Salix reticulata*, 8 Aug 2000, CLC1434 (MONT), C. Cripps; 29 July 2000 with *Salix reticulata*, ZT9001 (ETH), E. Horak; Stony Pass, 3840 m, with *S. arctica*, 28 July 2002, CLC1821 (MONT), C. Cripps; Mineral Basin, with *S. arctica* and *S. planifolia*, in moss, 3835 m, 30 July 2002, CLC1845 (MONT), C. Cripps.

**Discussion.** Based on ITS data, *Hebeloma dunense* is phylogenetically not clearly distinguishable, but neither is it molecularly identical to other members of the *H. mesophaeum* complex (Fig. 6B). The intraspecific variation is 0–10 [0–2] bp (17 sequences), within the RM dataset (5 sequences), 1–7 [0–1] bp. The exclusively RM circle in Fig. 6B is a result of the data selection; this corresponds to ITS variants that do occur in the FE dataset, but did not come up in the random selection of sequences for this species.

For the Rocky Mountain collections, so far, *H. dunense* has been found more often with dwarf willows *S. arctica*, *S. reticulata*, and shrub willow *S. planifolia* in contrast to *H. mesophaeum* and *H. excedens*, which were more often with *S. glauca*. Originally described from low-elevation dunes with *Salix*, this species has been more recently recognized in arctic and alpine habitats and from Canada, Greenland, Svalbard, the European Alps, and the Carpathians (Beker et al. 2016; Beker et al. 2018; Eberhardt et al. 2015b).

Rocky Mountain specimens of *H. dunense* are pale, often with narrow subdecurrent lamellae; the cortina can be scant or absent, some cheilocystidia have dense yellow contents, and the spores, which are ellipsoid and distinctly but not strongly ornamented, are slightly larger than those of *H. mesophaeum* and *H. excedens*.

**11. *Hebeloma mesophaeum* (Pers.) Quél., Mém. Soc. Émul. Montbéliard, sér. 2, 5: 128 (1872)**
Figures 6B, 17, 23(11)

**Etymology.** From Greek *meso*, in the middle, and *phaeus*, dark-colored. Persoon (1872) particularly mentioned the peculiar reddish brown pileus center "disco rufofusco peculiaris" which is characteristic of this taxon.

**Description.** Cortina present. Pileus 10–20 mm in diameter, convex with low indistinct umbo, or conic-convex, smooth, shiny, greasy, yellowish brown in center, outwards lightening to pale ocher, at margin buff, two-toned, non-translucent; margin entire, turned in when young, covered with veil or not. Lamellae attached, adnate, L = 38–40, pale buff, pinkish buff, then pinkish brown; edges fimbriate. Stipe: 30–45 × 3–5(–8 at base), very gradually larger at base, white, pruinose at apex, and fibrillose and darker below to ocher yellow and then blackish at very base. Context pale, dark in base of stipe. Odor raphanoid. Exsiccate: pileus pale brown, stipe with yellow sheen and darker at base.

Basidiospores yellow brown, elliptical, a few slightly ovoid, no big apiculus, not guttulate, looks almost smooth even under high magnification (O1), not or only very slightly dextrinoid (D0, D1), and no perispore loosening (P0), 8–10.5(–11) × 5–6.5 µm, on average 9.7 × 5.8 µm, Q = 1.66. Basidia 20–30 × 6–9 µm, clavate, four-spored mostly. Pleurocystidia absent. Cheilocystidia cylindrical in the upper part and slightly swollen to more swollen at the base, rarely fully cylindrical, 30–55 µm long × 4–7 µm at apex, 4–7 µm in middle, and 6–9.5(–10.5) µm wide at base, with occasional thickening of the apical wall, some septate. Epicutis thickness 60–350 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** Known so far only from the Colorado alpine with *Salix glauca.*

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Sawatch Range, Independence Pass, 3760 m, with *Salix glauca*, 8 Aug 1998, CLC1245 (MONT), C. Cripps; Front Range, Loveland Pass, 7 Aug 1999 with *Salix* sp., ZT8082 (ETH), E. Horak.

**Figure 17.** *Hebeloma mesophaeum*, CLC1245 and ZT8082.

**Discussion.** Only two collections from the RM dataset turned out to be *H. mesophaeum* that differ in their ITS region by 7 [2] bp (Fig. 6B). The sequence variation among all *H. mesophaeum* sequences (12) of the sample is 1–11 [0–4] bp. Beker et al. (2016) did not manage to delimit *H. mesophaeum* based on several loci. They suspected that there might be several species hidden within the sample assigned to *H. mesophaeum*. It appears likely that *H. excedens* and *H. alpinicola* are among these 'cryptic' taxa. We made sure that the 10 selected sequences from the FE dataset belong to *H. mesophaeum* in the strict sense. Among the *H. mesophaeum* representatives of the RM dataset, there is one collection that is reminiscent of *H. pubescens*. However, because of its ambiguous morphology we decided to keep it in *H. mesophaeum*. The respective collection (CLC1245) differs by 2–4 [1–2] bp from the available *H. pubescens* data (3 sequences).

Previously *Hebeloma bruchetii* Bon was one of the most commonly reported species from arctic and alpine areas, but it has now been synonymized with and folded into *H. mesophaeum* (Beker et al. 2016). *Hebeloma mesophaeum* has relatively small elliptical spores that are smooth to slightly rough and not dextrinoid. *Hebeloma mesophaeum* is a widespread species reported in almost all arctic and alpine habitats, as well as from subalpine, boreal, and lower elevation habitats with a wide variety of hosts (Beker et al. 2016). Also, many varieties have been described in North America (Smith et al. 1983) and in Europe (Vesterholt 2005). Some of the European taxa have been synonymized by the authors (Beker et al. 2016) and it remains to check the 12 North American varieties delineated by Smith et al. (1983).

### 12. *Hebeloma excedens* (Peck) Sacc., Syll. Fung. 5: 806 (1887)

Figures 6B, 18, 23(12)

**Etymology.** For the pileus cuticle which can exceed the lamellae.

**Description.** Cortina present. Pileus 10–25 mm in diameter, shallow convex, campanulate, then almost applanate, slight umbo or not, viscid or greasy, medium cocoa brown to orange caramel in center and pale brown on most of the pileus, with

**Figure 18.** *Hebeloma excedens*, CLC1685 and ZT9830.

or without white tissue at margin, or with whitish rim; margin originally described as extending beyond the lamellae. Pileus thin-fleshed. Lamellae sinuate, subdecurrent, narrow, becoming broader and eroded, very pale, cream with pinkish buff tint, L = 32–48 plus lamellulae. Stipe 30–50 × 2–4 mm, equal, slightly curved, pale cream, silky, pruinose above ring zone, more dingy brown below but still pale, with a golden brown fibrils in zones, blackening towards base. Context whitish in pileus and stipe apex and yellowish brown in lower stipe down to blackish at base; stipe tough, rubbery. Odor: raphanoid or none. Exsiccate: small, pale buff overall, base of stipe dark in some.

Basidiospores yellow brown, elliptical, a few slightly ovoid, no big apiculus, not guttulate, looks almost smooth to very slightly rough even under high magnification (O1), not or only very slightly dextrinoid (D0,D1), and no perispore loosening (P0), 7–11 × 5–6.5 µm, on average 9.1 × 5.8 µm, Q = 1.55. Basidia 20–30 × 6–9 µm, clavate, four-spored mostly. Pleurocystidia absent. Cheilocystidia cylindrical in the upper part and slightly swollen to more swollen at the base, rarely fully cylindrical, 30–60 µm long × 4–7 µm at apex, 4–6.5 µm in middle, and 6–10 µm wide at base, some septate. Epicutis thickness 65–200 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** In alpine with shrub willow *Salix glauca*, Colorado.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: San Juan County, San Juan Mountains. U.S. Basin, 3658 m, with *Salix glauca*, 8 Aug 2001, CLC1685 (MONT), C. Cripps. Sawatch Range, Independence Pass, 14 Aug 1999 with *Salix* sp., ZT7475 (ETH), E. Horak; 12 Aug 1999 with *Salix* sp., ZT8136 (ETH), E. Horak; 14 Aug 2001 with *Salix glauca* and *S. planifolia*, ZT9830 (ETH), E. Horak; 3760 m, with *Salix glauca*, 13 Aug 2001, CLC1732 (MONT), C. Cripps. Front Range, Loveland Pass, 7 Aug 1999 with *Salix* sp., ZT8074 (ETH), E. Horak.

**Other specimens examined.** NEW YORK: Saratoga at approx. 100 m, with *Pinus* sp. on sandy soil in woodland, Oct 1870, NYS-F-001123, C.H. Peck (**holotype**).

**Discussion.** *Hebeloma excedens* was not treated by Beker et al. (2016). The type of *H. excedens* fits in with the majority of the RM *H. excedens* collections, but the species cannot be clearly separated from *H. mesophaeum* (Fig. 6B). Looking at absolute differ-

ences, the intraspecific variation of the *H. excedens* sample (RM + type = 7 sequences) is 0–8 [0–1] bp, whereas the variation in the sample between *H. excedens* and *H. mesophaeum* is 2–11 [0–4] bp. In terms of absolute differences, the type of *H. excedens* is 5–8 [0–1] bp different from other collections referred to this species, but as Fig. 6B shows it is not strongly differentiated from other members of *H. excedens*, if ambiguous positions are treated as missing data as in networks or equated to their constituting bases as in the ML tree. In terms of absolute differences, the type of *H. excedens* is 5–11 [0–3] bp away from the *H. mesophaeum* sequences of the sample. Thus, within the limited support ITS data can give in this case, we do consider the species identification of the RM *H. excedens* collections as molecularly supported. Until the question of the distinctness and delimitation of this species can be clarified, we prefer to treat it as an independent taxon.

*Hebeloma pubescens* Beker & U. Eberh. is another species from the *H. mesophaeum* complex that might occur in the sampled habitats of the Rocky Mountains and is close to *H. excedens* in Fig. 6B. Based on a small sample (3 sequences available for *H. pubescens*; 7 sequences for *H. excedens*), the species vary 5–10 [1–3] bp in their ITS region.

*Hebeloma excedens* was first described by North American mycologist C.H. Peck; the species, with its lageniform to ventricose cheilocystidia and small elliptical, almost smooth, indextrinoid spores belongs to *H.* sect. *Hebeloma*. It is closely allied with *Hebeloma mesophaeum*, with which we believe it has often been confused. Separating these two taxa morphologically is rather difficult, but it does appear that the pileus of *H. excedens* may be more evenly colored, less yellow brown, less brown in the center, and it was originally described as having a cuticle that extended beyond the lamellae. The stipe surface appears to have fibrils arranged in zones, in contrast to that of *H. mesophaeum*. However, further work is required before we can have confidence that these characters are consistently different.

We have examined a number of collections from North America that are morphologically and molecularly consistent with this taxon. Based on these studies it would appear that *Hebeloma excedens* is widespread across North America and occurs in a wide variety of habitats.

## *Hebeloma* section *Hebeloma*, Part two: cortina present, spores amygdaliform, rather strongly dextrinoid

### 13. *Hebeloma oreophilum* Beker & U. Eberh., Mycologia 107: 1295 (2016) [2015]

Figures 6A, 19, 23(13)

**Etymology.** From *oreophilus*, mountain loving to emphasize its presence in alpine habitats.

**Description.** Cortina present. Pileus 15–30 mm in diameter, convex, hemispherical, not umbonate, smooth, dry or greasy, medium brown, bay brown, reddish brown, dark black brown, with white to cream rim of fibrillose veil remnants at margin, with hoary coating; margin even or weakly scalloped. Thick waxy pellicle mentioned in one collection. Lamellae emarginate, subdistant, L = 40–50 plus lamellulae, cream at first

**Figure 19.** *Hebeloma oreophilum*, DBG-F-027674 and ZT12733.

then milk coffee color, pinkish cinnamon; margin floccose, white. Stipe 15–60 × 3–8 mm, equal or slightly enlarged at base, a bit curved or undulating, whitich, tan, brown, in top part and darkening to blackish brown at base, pruinose in top half and fibrous below, with patches of fibrils. Context watery buff with yellow tint, and blackish brown in base, stipe hollow. Odor raphanoid. Exsiccate pale brown all over, not dark.

Basidiospores amygdaliform, with a small snout, apiculate, not guttulate, finely verrucose (O1, O2), distinctly dextrinoid (D2, D3), no perispore loosening observed (P0), 10–14 × 6–8 µm, on average 11.7 × 6.9 µm, Q = 1.68. Basidia clavate, 25–35 × 8–10 µm, mostly four-spored. Cheilocystidia lageniform, with subcapitate apex, long neck (sometimes wiggly), with gradually swollen base, sometimes septate, length 30–70 × 4–7 µm at apex, 3–6.5 µm in middle, and up to 13 µm at base, no thickening noticed. Pleurocystidia absent. Epicutis thickness 40–75 µm, with no encrusted hyphae recorded.

**Rocky Mountain ecology.** In low alpine with *Salix* species in Montana and Colorado.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Clear Creek County, Denver Mountain Park, Summit Lake, 3911 m, in *Salix arctica* and *S. glauca*, 20 Aug 2013, DBG-F-027674, L, Gillman; Summit Lake Park, 3912 m, with *Salix* sp., 22 Aug 2012, DBG-F-022788, L. Gillman; Arapaho National Forest, Nature Trail, Mount Goliath, 3658 m, in *Salix* sp, 1 Sept 1999, DBG-F-020558, V.S. Evenson; Pitkin County, White River National Forest, junction of Montezuma Basin and Pearl Pass, in *Salix* sp., 3658 m, 6 Aug 1999, DBG-F-020053, V.S. Evenson. MONTANA: Carbon County, Beartooth Plateau, Frozen Lakes, with dwarf *Salix*, 26 July 1997, 3200 m, CLC1102 (MONT), C. Cripps; site 2, 3100 m, 8 Aug 2002, CLC1937 (MONT), with *Salix planifolia*, C. Cripps; Billings Fen, in moss near *S. planifolia*, 3048 m, 23 Aug 2017, CLC 3607 (MONT). WYOMING: Beartooth Plateau, Wyoming Creek, with *Salix planifolia*, 3176 m, 6 Aug 2008, HJB12449, C. Cripps; Beartooth Plateau, Hell-roaring Plateau, near *Salix* sp., 14 Aug 2007, ZT12733 (ETH), E. Horak.

**Discussion.** *Hebeloma oreophilum* is a member of the *H. nigellum* complex that cannot always be distinguished from *H. nigellum* based on ITS data (Fig. 6A). In terms

of differences, the *H. oreophilum* sequences from the sample (9 RM, 10 FE) differ by 0–9 [0–3] bp; 0–8 [0–1] bp within the RM sample. Most similar to *H. oreophilum* is *H. clavulipes*, which in this sample differs by 1–11 [0–3] bp. The two species do not share the same habitats. The differences between species sharing the same habitats (*H. nigellum* and *H. spetsbergense*) are 3–10 [0–5] bp. Morphologically, the easiest way to separate *H. oreophilum* from *H. hygrophilum* and *H. nigellum* is by the number of full length lamellae, always at least 40 for *H. oreophilum* and less than 36 for the others. *Hebeloma clavulipes* is not known from arctic-alpine habitats and has spores with an average width at most 6.6 µm while the average spore width for *H. oreophilum* is on average at least 6.8 µm. *Hebeloma oreophilum* has a persisting cortina and the lageniform/ventricose cheilocystidia of *H*. sect. *Hebeloma*.

This species was first described from the western Carpathians (Slovakia) with *Salix reticulata*, *S. retusa*, or *Dryas octopetala* on calcareous soil (Eberhardt et al. 2015b). It has since been reported from Canada, Greenland, Scandinavia, Svalbard, and the Rocky Mountains (Beker et al. 2016; Beker et al. 2018).

### 14. *Hebeloma hygrophilum* Poumarat & Corriol, Fungi Europaei 14 (Lomazzo): 138 (2016)

Figures 6A, 20, 23(14)

**Etymology.** *hygrophilus*, because it is often found in moist, wet, boggy ground.

**Description.** Cortina present. Pileus 15–25 mm in diameter, convex to almost plane, smooth, greasy, center dark brown, reddish brown, lighter towards margin to buff; margin entire. Lamellae emarginate and strongly curved outwards, a bit distant, L = 24 plus lamellulae, pale buff becoming milk coffee color; edges lighter or darker. Stipe 25–35 × 1–2 mm, long and thin, undulating, dingy cream in top half, darkening to blackish at base, apex pruinose, below with longitudinal fibrils. Context dingy cream and brownish black in stipe base. Odor raphanoid. Exsiccate: small; pileus, two-toned, dark brown center, cream towards margin; stipe thin, whitish with a darker base.

Basidiospores slightly amygdaliform, a few with a snout, apiculate, not guttulate, finely verrucose (O2), distinctly dextrinoid (D2, D3), no perispore loosening observed (P0), 10–13 × 6–7.5 µm, on average 11.4 × 6.8 µm, Q = 1.67; a few spores larger –16 × –7 µm present. Basidia clavate, 25–30 × 7–9 µm, four-spored, possibly some two-spored because of larger spores present. Cheilocystidia lageniform, with subcapitate apex, long neck (sometimes wiggly), occasionally septate, with gradually swollen base, or almost cylindrical, length 35–70 × 4–6.5 µm or wider at apex, 4–6 µm in middle, and up to 7–13 µm at base, no thickening noticed. Pleurocystidia absent. Epicutis thickness 100–130 µm, with some encrusted hyphae.

**Rocky Mountain ecology.** Based on four collections from Colorado and Montana, in the alpine zone; all with *Salix*, and the presence of *Sphagnum* is mentioned for one.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: Pitkin/Lake County, Sawatch Range, Independence Pass, 6 Aug 2000, under *S. planifolia*, 3660 m, CLC1462

**Figure 20.** *Hebeloma hygrophilum*, DBG-F-021349 and CLC1462.

(MONT), C. Cripps; 7 Aug 2000, *Salix planifolia*, CLC1476 (MONT), 3660 m, C. Cripps. Summit County, near Summit Lake, with *Sphagnum* sp. and *Salix* sp., 3658 m, 10 Aug 2003, DBG-F-021349, V.S. Evenson. MONTANA: Beartooth Plateau, Frozen Lakes, at 3200 m, near *S. planifolia*, 29 Aug 2002, CLC1948 (MONT), C. Cripps.

**Discussion.** Figure 6A supports Beker et al. (2016) in that *H. hygrophilum* is paraphyletic in relation to the other members of the *H. mesophaeum* complex based on the ITS sequence, although some genotypes seem to be restricted to this species. The four *H. hygrophilum* representatives from the Rocky Mountains differ by 2–20 [0–2] bp in their ITS, whereas the intraspecific variation of *H. hygrophilum* within the sample is 1–22 [0–3] bp (14 sequences). Responsible for the high distance values is sample CLC1476 (HJB15297), which differs from all other conspecifics by 15–22 [0–1] bp and from all sequences of the ingroup by 14–22 [0–2] bp, while all other *H. hygrophilum* samples differ by only 1–9 [0–2] bp from each other. The morphologically closest taxon occurring in the Rocky Mountains is *H. nigellum* which differs by 3–10 [0–5] (14–21 [0–2]) bp. The values in round brackets are for CLC1476. An unusually high number of SNP positions in CLC1476 is responsible for the large total differences. However, sequences with numerous SNP positions occur occasionally in *Hebeloma* and are normally reproducable (Beker et al. 2016).

*Hebeloma hygrophilum* was first described from the Pyrenees in non-alpine habitats above 1250 m (Poumarat and Corriol 2009) and it is known in boreal habitats from northern Europe (Beker et al. 2016). Thus it is typically in subalpine or subarctic habitats. It appears to have been found mostly with *Salix* and usually in wet areas with moss, typically *Sphagnum*. Here we report it for the first time in the alpine habitat (with *S. planifolia*); at least one collection was found in *Sphagnum* moss. It is molecularly close to *H. clavulipes*, *H. nigellum* and *H. oreophilum* (see below). When found in the alpine, it could be confused with *H. nigellum*, which is morphologically very similar. However, the spore width of *H. nigellum* is reported typically with an average over 7 µm, while that for *H. hygrophilum* is reported with an average of less than 7 µm; to add confusion, both appear to have occasional very large spores likely from two-spored basidia.

**15. *Hebeloma nigellum* Bruchet, Bull. Mens. Soc. Linn. Lyon 39 (6 suppl.): 126 (1970)**
Figures 6A, 21, 23(15)

**Etymology.** From *nigellus*, meaning blackish for the dark pileus.

**Description.** Cortina present. Pileus 8–20 mm in diameter, broadly convex to hemispherical to almost plane with a small umbo, greasy, smooth or slightly fibrous, in center dark date brown, chocolate brown, or blackish brown, at margin paler even to cream, appearing two-toned, with hoary sheen, glazed-looking, not hygrophanous; margin inrolled at first, then even (not rimose). Lamellae emarginate, even with a tooth, normally spaced, L = 24–32 with lamellulae, whitish, then pale milk coffee, pale brown, paleness persisting; edges floccose. Stipe 15–50 × 1.5–4 mm, long and slim, equal, undulating a bit, pale dingy whitish in top half darkening to black brown at base, pruinose at apex, below silky-shiny, smooth to fibrillose. Context dingy whitish, darkening to brownish at base, rubbery in stipe. Odor raphanoid. Exsiccate: pileus small, two-toned, center dark brown, outwards cream; lamellae brown, red-brown; stipe long and very thin, cream, dark at base.

Basidiospores yellowish brown, amygdaliform, a few ellipsoid in certain view, no/slight snout, no big apiculus, slightly rough (O1, O2), perispore occasionally observed loosening very slightly (P0, P1), usually distinctly dextrinoid (D2, D3), not guttulate, 10–14.5 × 6–8 µm, on average 11.9 × 7.2 µm, Q = 1.6. Basidia 27–40 × 7.58–10.5 µm, sterigma 2–3 µm, clavate, mainly four-spored. Cheilocystidia lageniform, more or less swollen at the base, top half cylindrical, some apical thickening, some septate, 30–80 × 3.5–6.5 µm at apex, 3.5–6 µm in middle, 6.5–12.5 µm at base. Pleurocystidia absent. Epicutis thickness 40–75 µm, with no encrusted hyphae recorded.

**Rocky Mountain ecology.** Alpine mostly near *Salix planifolia* and in moss; reported from Colorado and Montana.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: San Juan County, San Juan Mountains, Engineer Pass, in *Salix planifolia*, 30 July 2000, CLC1420 (MONT), C. Cripps; Cinnamon Pass, in *Salix* spp., 10 Aug 2001, CLC1707 (MONT), C. Cripps. MONTANA: Beartooth Plateau, Frozen Lakes: at 3200 m in moss near *S. planifolia*, 21 Aug 2001, CLC1778 (MONT), C. Cripps; N Pass, with *S. planifolia*, 9 Aug 1998, ZT6425 (ETH), E. Horak; Billings Fen, in moss near *S. planifolia*, 23 Aug 2017, CLC3614b (MONT), C. Cripps.

**Discussion.** According to Beker et al. (2016), *H. nigellum* is paraphyletic in the ITS region, but monophyletic and bootstrap supported in multi-locus analyses. The corresponding network is in Figure 6A. *Hebeloma nigellum* is similar in its variabiltiy within the Rocky Mountains (1–7 [0–1] bp differences based on 5 sequences when compared with the random selection of 11 sequences from the FE dataset (0–8 [0–3] bp). As discussed above, *H. nigellum* is close to and not always distinguishable from *H. hygrophilum* by ITS sequence. Another arctic and alpine species is *H. spetsbergense* (discussed below) that cannot be distinguished from *H. nigellum* by ITS sequence either.

*Hebeloma nigellum* is a small, slim species with a dark-centered pileus and rather large, dextrinoid, amygdaliform spores. It is widespread across northern Europe,

**Figure 21.** *Hebeloma nigellum*, CLC3614b and CLC1420.

not only in arctic-alpine habitats, and is reported from alpine and arctic habitats in Canada, Greenland, Iceland, Svalbard and the European Alps (Beker et al. 2016, 2018). In molecular and morphological features it is close to *H. hygrophilum* (which normally associates with *Salix* in non-arctic-alpine habitats). *Hebeloma kuehneri* Bruchet, a commonly reported arctic-alpine species, was described in the same paper as *H. nigellum* with the main differentiation being that the former has more brownish coloration and the latter more blackish tones (Bruchet 1970); a distinction that could not be supported by other lines of evidence. The holotype of *H. kuehneri* was lost, however, and a new lectotype (selected from the paratypes) has been established (Beker et al. 2016; LY BR66-15); it is sequenced and is a molecular match to *H. nigellum*. We here follow Beker et al. (2016) in selecting the name *H. nigellum* over *H. kuehneri* for this species.

### 16. *Hebeloma spetsbergense* Beker & U. Eberh., Fungi Europaei 14 (Lomazzo): 180 (2016)

Figures 6A, 22

**Etymology.** Originally found in Svalbard.

**Description.** Cortina present. Pileus 10–25 mm in diameter, shallow convex, almost applanate with indistinct umbo or not, smooth, tacky to dry, brown in center, outwards paler brown or more cinnamon, with white edge, not hygrophanous; margin turned down in young specimens, entire. Lamellae attached, adnexed, medium close, L = 26–30, pale cream to milk coffee, to brown; edges indistinct fimbriate. Stipe long and thin, 20–40 × 2–3 mm, equal, cream at apex to dark brown at base, fibrils at apex, and below silky-smooth with longitundinal fibrils. Context cream and brown to black in lower part. Odor raphanoid. Exsiccata: pileus brown, darker brown in center; lamellae reddish brown; stipe thin, cream but darkening at base.

Basidiospores yellow brown, amygdaliform, without a large snout, apiculate, not guttulate, finely verrucose (O1, O2), distinctly and sometimes strongly dextri-

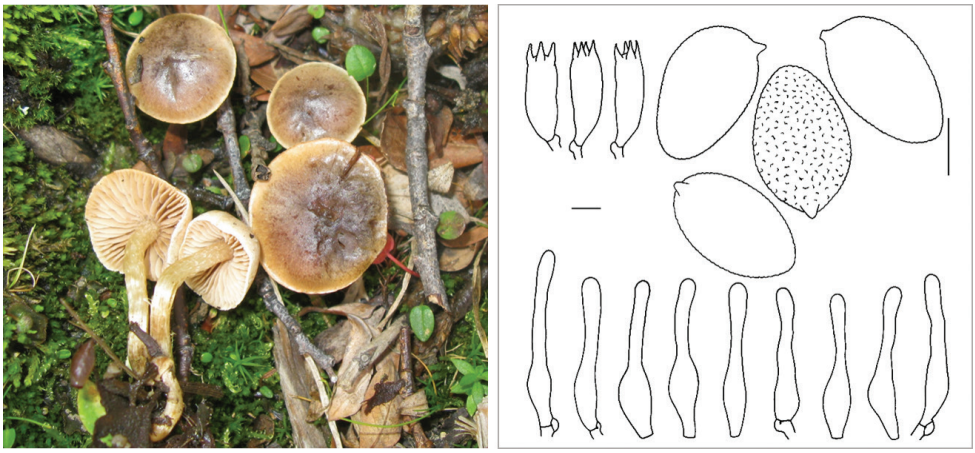

**Figure 22.** *Hebeloma spetsbergense*, DBG-F-027678 and BR5020184126599 (HJB 11982, from Svalbard). Scale bar for basidia and cheilocystidia 5 µm, for spores 10 µm. Drawing G. Walther, reproduced from Beker et al. (2016).

noid (D2, D3), no loosening perispore observed (P0), 11–14 × 7–8.5 µm, on average 12.5 × 7.6 µm, Q = 1.65. Basidia 28–35 × 8–10 µm, clavate, mostly four-spored. Cheilocystidia lageniform, with long cylindrical neck, 30–80 × 4–7 µm at apex, 4–5.5 µm in middle, and 7–10.5 µm at base. Pleurocystidia absent. Epicutis thickness 30–35 µm, with no encrusted hyphae recorded.

**Rocky Mountain ecology.** In alpine habitats in Colorado, in moss near *Salix* species.

**Rocky Mountain specimens examined.** U.S.A. COLORADO: San Juan County, San Juan Mountains, Mineral Basin, 31 July 2002, CLC1879 (MONT), C. Cripps. Clear Creek County, Denver Mountain Park, Summit Lake, 3911 m, in *Salix arctica* and *S. glauca*, 20 Aug 2013, DBG-F-027678, L. Gillman.

**Discussion.** According to Beker et al. (2018), *H. spetsbergense* cannot be distinguished from similar species by ITS. The two RM collections (Fig. 6A) differ by 4 [0] bp, the variation of *H. spetsbergense* within the sample (7 sequences) is 0–5 [0–2] bp. *Hebeloma nigellum* is the most similar species occurring in the same habitat and, within this sample, differs in its ITS by 1–8 [0–3] bp from *H. spetsbergense*. Morphologically *H. spetsbergense* is similar to *H. hygrophilum* and *H. nigellum*, but its spores appear to be larger. Previously this species was only known from Svalbard (Beker et al. 2016, 2018), and we report it here from North America for the first time. In Svalbard, it was found with *Salix Polaris* near sea level at a latitude of 78°N. In Colorado, it is reported at elevations of 3700–3800 m and latitudes from 36–38°N, and there is a distance between localities of 6500 km, greatly extending its disjunct range. It remains to be seen, if it also occurs in other arctic and alpine habitats.

With the persistent presence of a cortina and the lageniform or ventricose cheilocystidia, this taxon clearly belongs in *H.* sect. *Hebeloma*. The rather strongly dextrinoid amygdaloid spores, less than 14 µm long but more than 7.5 µm wide, distinguish this taxon from the other alpine-arctic species of this section.

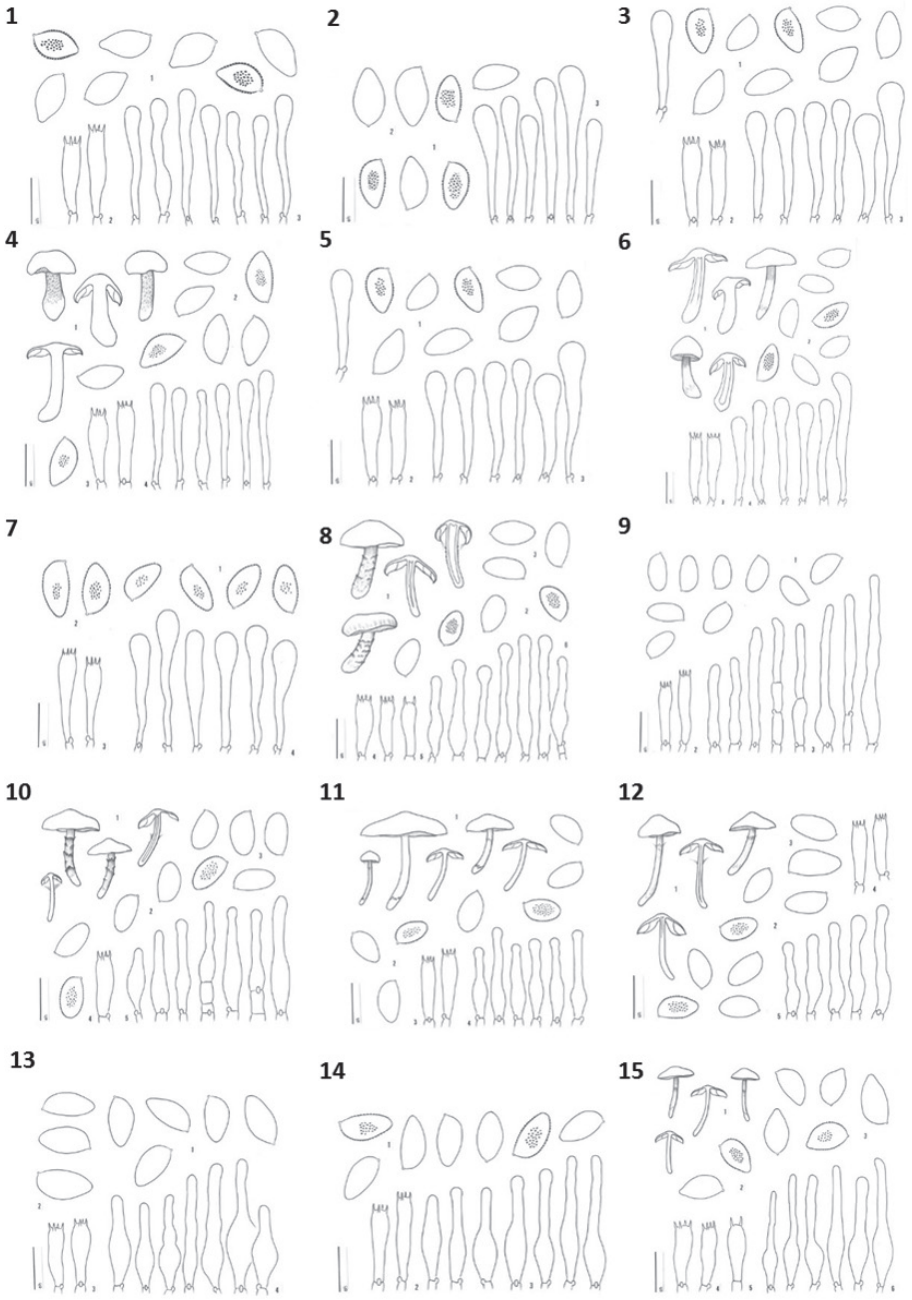


**Figure 23.** Micro-morphological features (basidiospores, basidia, cheilocystidia) of *Hebeloma* species found in the Rocky Mountain alpine zone. **1** *H. vaccinum* (holotype, Herb. PC) **2** *H. aurantioumbrinum* ZT12730 **3** *H. subconcolor* ZT 13776 **4** *H. hiemale* ZT9828 **5** *H. avellaneum* DBG-F-019533 **6** *H. velutipes* ZT6100 **7** *H. alpinum* CLC2875 **8** *H. marginatulum* ZT9002 **9** *H. alpinicola* ZT13763 **10** *H. dunense* ZT9001 **11** *H. mesophaeum* ZT8082 **12** *H. excedens* ZT7475 **13** *H. oreophilum* ZT12733 **14** *H. hygrophilum* CLC1462 **15** *H. nigellum* ZT6425 **16** *H. spetsbergense* micro in Fig. 22. Both two and four-spored basidiospores shown for **2, 5, 7, 8, 10, 12, 13, 15**. Scale bar: 10 µm. All drawings by E. Horak.

## Conclusions

The 16 species of *Hebeloma* we report from the Rocky Mountain alpine zone are from some of the lowest latitudes (latitude 36°–45° N) and highest elevations (3000–4000 m) for arctic-alpine fungi in the northern hemisphere. Twelve of these species have been reported from arctic-alpine habitats in Europe and Greenland, and are now molecularly confirmed from the middle and southern Rockies, greatly expanding their distributions. These are: *Hebeloma alpinum*, *H. aurantioumbrinum*, *H. dunense*, *H. hiemale*, *H. marginatulum*, *H. mesophaeum*, *H. nigellum*, *H. oreophilum*, *H. spetsbergense*, *H. subconcolor*, *H. vaccinum*, and *H. velutipes*. *Hebeloma hygrophilum* is known from subalpine habitats in Europe, but has never been recorded in arctic-alpine ecology. Interestingly, hosts can overlap or vary among continents and while Rocky Mountain collections are primarily with *S. arctica*, *S. reticulata*, *S. glauca*, *S. planifolia*, and *Dryas octopetala*, those from other continents were with these plants or additionally with *S. herbacea*, *S. polaris*, *S. retusa*, *Persicaria vivipara*, and *Helianthemum* sp. (Beker et al. 2016; Eberhardt et al. 2015b).

Three species, not known from Europe, have never previously been reported from a true arctic or alpine habitat; they are *H. alpinicola*, *H. avellaneum*, and *H. excedens*. All are species first reported as growing with *Pinaceae* in North America (Peck 1872; Kauffman and Smith 1933; Smith et al. 1983; Hesler unpublished manuscript). We note that the *H. avellaneum* collections described above are from the low alpine and conifers (and conifers are noted in some original descriptions); we do suspect that the ectomycorrhizal association is indeed with *Pinaceae*. The Rockies *H. excedens* collections were all reported with *Salix* in the alpine, yet the holotype was with pine in New York state. This species, like *H. dunense*, *H. mesophaeum*, and *H. nigellum*, appears not to be confined to alpine and arctic habitats. Similarly, *H. alpinicola* appears to be found with a variety of hosts in both alpine and subalpine habitats.

The Rocky Mountain alpine exists as islands on high mountain tops and plateaus far from the arctic and alpine areas of other mountain ranges. While the recent trend, due to molecular analysis, has been to discover differences between European and North American taxa given the same names, in the alpine the reverse appears to be true. Of the ectomycorrhizal genera, a majority of *Inocybe*, *Lactarius*, and *Cortinarius* species from the Rocky Mountain alpine zone have been found to be conspecific with those occurring in arctic and alpine habitats in the European Alps, Pyrenees, Scandinavia, Svalbard, and Greenland through molecular matching of ITS sequences (Cripps et al. 2010; Larsson et al. 2014; Barge et al. 2016; Barge and Cripps 2016). Only a few alpine species of Agaricales and Russulales are so far considered endemic to the Rocky Mountain alpine including *Laccaria pseudomontana* Osmundson, C.L. Cripps & G.M. Muell. (Osmundson et al. 2005) and *Lactarius pallidomarginatus* Barge & C.L. Cripps (Barge et al. 2016).

The distributions of various ectomycorrhizal plant hosts in the Rocky Mountains alpine have been shaped by glaciation, topography, parent rock, and climate. Glaciation during the quaternary allowed mixing at the glacial forefronts, interspersed with glacial retreat and withdrawal of cold-adapted plants to mountain tops, which include

dwarf *Salix* and *Dryas* (Birks 2008). Tertiary connections have also been suggested (Webber 2003). A view from the North Pole shows Arctic areas as more contiguous than generally considered, and corridors during interglacial periods stretched from the Rockies to the Arctic and Siberia allowing migration and genetic mixing.

Alpine areas, like the arctic, are known to be sensitive to climate change. Greening of these areas is primarily due to shrub encroachment (Tape et al. 2012), and this involves ectomycorrhizal host plants; consequently, ectomycorrhizal fungi communities are likely to change with the loss or gain of different hosts (Geml et al. 2015; Morgado et al. 2015).

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## Supplementary material 1

**GenBank accession numbers of reference sequences of *Hebeloma* spp. from Europe**

Authors: Cathy L. Cripps, Ursula Eberhardt, Nicole Schütz, Henry J. Beker, Vera S. Evenson, Egon Horak

Data type: GenBank accession numbers

Explanation note: GenBank accession numbers of reference sequences of *Hebeloma* spp. from Europe (FE dataset). These have been used to assemble species descriptions and distribution patterns of *Hebeloma* sp. in Europe by Beker et al. (2016), where additional information on these collections can be found. The database numbers refer to the project database of H.J. Beker (Beker et al. 2016).



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RESEARCH ARTICLE

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# New and noteworthy boletes from subtropical and tropical China

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## Abstract

The morphology, ecology, and phylogenetic relationships of specimens of the family Boletaceae from subtropical and tropical China were investigated. Four species, *Butyriboletus huangnianlaii*, *Lanmaoa macrocarpa*, *Neoboletus multipunctatus*, and *Sutorius subrufus*, are new to science. *Chalciporus radiatus* and *Caloboletus xiangtoushanensis* are redescribed. *Caloboletus guanyui* is proposed to replace *Boletus quercinus* Hongo, an illegitimate later homonym. The recently described *Tylopilus callainus* is synonymized with the Japanese *Boletus virescens*, and the new combination *T. virescens* (Har. Takah. & Taneyama) N.K. Zeng et al. is proposed. Moreover, *Neoboletus* is treated as an independent genus based on evidence from morphology and molecular phylogenetic data in the present study, and many previously described taxa of *Sutorius* are recombined into *Neoboletus*: *N. ferrugineus* (G. Wu et al.) N.K. Zeng et al., *N. flavidus* (G. Wu & Zhu L. Yang) N.K. Zeng et al., *N. hainanensis* (T.H. Li & M. Zang) N.K. Zeng et al., *N. obscureumbrinus* (Hongo) N.K. Zeng et al., *N. rubriporus* (G. Wu & Zhu L. Yang) N.K. Zeng et al., *N. sanguineoides* (G. Wu & Zhu L. Yang) N.K. Zeng et al. , *N. sanguineus* (G. Wu & Zhu L. Yang) N.K. Zeng et al., and *N. tomentulosus* (M. Zang et al.) N.K. Zeng et al.

## Keywords

Molecular phylogeny, morphology, new taxa, taxonomy

## Introduction

Boletaceae Chevall. (Boletales) is a large, cosmopolitan family with abundant species. Many of them are interesting and important for their mycorrhizal relationships with trees, edibility, medicinal value, and toxicity (Wang et al. 2004; Roman et al. 2005; Wu et al. 2013; Chen et al. 2016). In China, species of Boletaceae have received much attention by mycologists, and many taxa have been discovered across the country (Chiu 1948; Zang 2013; Zeng et al. 2013, 2016, 2017; Liang et al. 2016, 2017; Wu et al. 2016a). However, the diversity of species still remains poorly known in subtropical and tropical China, a biodiversity hotspot. During field trips in the past several years, many collections of boletes have been made in subtropical and tropical China. Evidence from morphology, molecular phylogenetic analyses, and ecological data indicate that these collections belong to *Butyriboletus* D. Arora & J.L. Frank, *Caloboletus* Vizzini, *Chalciporus* Bataille, *Lanmaoa* G. Wu & Zhu L. Yang, *Neoboletus* Gelardi et al., *Sutorius* Halling et al., and *Tylopilus* P. Karst. Thus, they are described/redescribed in an effort to (i) further demonstrate the species diversity in subtropical and tropical China, (ii) resolve some taxonomic quandaries in Boletaceae.

## Materials and methods

### Abbreviations of generic names used in the study

The abbreviations of *Boletus*, *Butyriboletus*, *Caloboletus*, *Chalciporus*, *Crocinoboletus*, *Lanmaoa*, *Neoboletus*, *Sutorius*, *Tylopilus* mentioned in this work are *B.*, *But.*, *C.*, *Ch.*, *Cr.*, *L.*, *N.*, *S.* and *T.*, respectively.

### Collection sites and sampling

Specimens were collected from subtropical and tropical China including Hainan and Fujian Provinces. Specimens examined are deposited in the Fungal Herbarium of Hainan Medical University (FHMU), Haikou City, Hainan Province, China, the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), and the Mycological Herbarium of Pharmacy College, Kunming Medical University (MHKMU).

### Morphological studies

The macroscopic descriptions are based on detailed notes and photographs taken from fresh basidiomata. Color codes are from Kornerup and Wanscher (1981). Sections of the pileipellis were cut radial-perpendicularly and halfway between the center and

margin of the pileus. Sections of the stipitipellis were taken from the middle part along the longitudinal axis of the stipe. Five percent KOH was used as a mounting medium for microscopic studies. All microscopic structures were drawn by freehand from rehydrated material. The number of measured basidiospores is given as *n*/*m*/*p*, where *n* represent the total number of basidiospores measured from *m* basidiomata of *p* collections. Dimensions of basidiospores are given as (*a*)*b* – *c*(*d*), where the range *b* – *c* represents a minimum of 90% of the measured values (5$^{th}$ to 95$^{th}$ percentile), and extreme values (*a* and *d*), whenever present (*a* < 5$^{th}$ percentile, *d* > 95$^{th}$ percentile), are in parentheses. *Q* refers to the length/width ratio of basidiospores; $Q_m$ refers to the average *Q* of basidiospores and is given with a sample standard deviation.

## DNA extraction, primers, PCR and sequencing

Total genomic DNA was obtained with Plant Genomic DNA Kit (TIANGEN Company, China) from materials dried with silica gel according to the manufacturer's instructions. The primers used for amplifying the nuclear ribosomal large subunit RNA (28S) were LROR/LR5 (Vilgalys and Hester 1990; James et al. 2006), ITS5/ITS4 (White et al. 1990) for the nuclear rDNA region encompassing the internal transcribed spacers 1 and 2, along with the 5.8S rDNA (ITS), the translation elongation factor 1-α gene (*tef1*) with 983F/1567R (Rehner and Buckley 2005) and the RNA polymerase II second largest subunit gene (*rpb2*) with RPB2-B-F1/RPB2-B-R (Wu et al. 2014). PCR products were checked in 1% (w/v) agarose gels, and positive reactions with a bright single band were purified and directly sequenced using an ABI 3730xl DNA Analyzer (Guangzhou Branch of BGI, China) with the same primers used for PCR amplifications. Assembled sequences were deposited in GenBank (Table 1).

## Dataset assembly

For the concatenated multilocus dataset of *Butyriboletus*, 14 sequences (four of 28S, four of ITS, four of *tef1*, and two of *rpb2*) from four collections were newly generated (Table 1) and then combined with selected sequences from previous studies (Table 1). *Rugiboletus extremiorientalis* (Lj.N. Vassiljeva) G. Wu & Zhu L. Yang was chosen as outgroup on the basis of the phylogeny in Wu et al. (2016a). For the concatenated multilocus dataset of *Caloboletus*, *Neoboletus*, and *Sutorius*, 68 sequences (21 of 28S, 16 of ITS, 20 of *tef1*, 11 of *rpb2*) from 23 collections were newly generated and deposited in GenBank (Table 1) and then combined with selected sequences from previous studies (Table 1). *Crocinoboletus laetissimus* (Hongo) N.K. Zeng et al. and *Cr. rufoaureus* (Massee) N.K. Zeng et al. were chosen as outgroup based on the phylogeny in Wu et al. (2016a). For the concatenated multilocus dataset of *Lanmaoa*, eight sequences (three of 28S, two of ITS, and three of *tef1*) from

**Table 1.** Taxa, vouchers, locations, and GenBank accession numbers of DNA sequences used in this study.

| Taxon | Voucher | Locality | 28S | ITS | *tef1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|
| *Baorangia pseudocalopus* | HKAS63607 | Yunnan, SW China | KF112355 | – | KF112167 | – | Wu et al. 2014 |
| *Baorangia pseudocalopus* | HKAS75081 | Yunnan, SW China | KF112356 | – | KF112168 | – | Wu et al. 2014 |
| *Butyriboletus abieticola* | Arora11087 | California, USA | KC184413 | KC184412 | – | – | Arora and Krank 2014 |
| *Butyriboletus appendiculatus* | Bap1 | Germany | AF456837 | KJ419923 | JQ327025 | – | Binder and Bresinsky 2002 |
| *Butyriboletus appendiculatus* | BR50200893390-25 | Meise, Belgium | KT002609 | KT002598 | KT002633 | – | Zhao et al. 2015 |
| *Butyriboletus appendiculatus* | BR50200892955-50 | Zoniënwoud, Belgium | KJ605677 | KJ605668 | KJ619472 | KP055030 | Zhao et al. 2014a |
| *Butyriboletus appendiculatus* | MB000286 | Germany | KT002610 | KT002599 | KT002634 | – | Zhao et al. 2015 |
| *Butyriboletus autumniregius* | Arora11108 | California, USA | KC184424 | KC184423 | – | – | Arora and Krank 2014 |
| *Butyriboletus brunneus* | NY00013631 | Connecticut, USA | KT002611 | KT002600 | KT002635 | – | Zhao et al. 2015 |
| *Butyriboletus fechtneri* | AT2003097 | – | KF030270 | KC584784 | – | – | Nuhn et al. 2013 |
| *Butyriboletus frostii* | JLF2548 | New Hampshire, USA | – | KC812303 | – | – | Arora and Krank 2014 |
| *Butyriboletus frostii* | NY815462 | Costa Rica | JQ924342 | – | KF112164 | KF112675 | Wu et al. 2014 |
| *Butyriboletus hainanensis* | N.K. Zeng 1197 (FHMU 2410) | Hainan, southern China | KU961651 | KU961653 | – | KU961658 | Liang et al. 2016 |
| *Butyriboletus hainanensis* | N.K. Zeng 2418 (FHMU 2437) | Hainan, southern China | KU961652 | KU961654 | KU961656 | KX453856 | Liang et al. 2016 |
| *Butyriboletus huangnianlaii* | N.K. Zeng 3245 (FHMU 2206) | Fujian, SE China | **MH879688** | **MH885350** | **MH879717** | **MH879740** | this study |
| *Butyriboletus huangnianlaii* | N.K. Zeng 3246 (FHMU 2207) | Fujian, SE China | **MH879689** | **MH885351** | **MH879718** | **MH879741** | this study |
| *Butyriboletus peckii* | 3959 | Tennessee, USA | JQ326999 | – | JQ327026 | – | Halling et al. 2012 |
| *Butyriboletus persolidus* | Arora11110 | California, USA | – | KC184444 | – | – | Arora and Krank 2014 |
| *Butyriboletus primiregius* | DBB00606 | Dunsmuir, California, USA | KC184451 | – | – | – | Arora and Krank 2014 |
| *Butyriboletus pseudoregius* | BR50201618465-02 | Eprave, Belgium | KT002613 | KT002602 | KT002637 | – | Zhao et al. 2015 |
| *Butyriboletus pseudoregius* | BR50201533559-51 | Meise, Belgium | KT002614 | KT002603 | KT002638 | – | Zhao et al. 2015 |
| *Butyriboletus pseudospeciosus* | HKAS59467 | Yunnan, SW China | KF112331 | – | KF112176 | KF112672 | Wu et al. 2014 |
| *Butyriboletus pseudospeciosus* | HKAS63513 | Yunnan, SW China | KT990541 | – | KT990743 | KT990380 | Wu et al. 2016a |
| *Butyriboletus pseudospeciosus* | HKAS63596 | Yunnan, SW China | KT990542 | – | KT990744 | KT990381 | Wu et al. 2016a |
| *Butyriboletus pseudospeciosus* | N.K. Zeng 2127 (FHMU 1391) | Yunnan, SW China | **MH879687** | **MH885349** | **MH879716** | – | this study |
| *Butyriboletus pseudoregius* | MG383a | Lazio, Italy | – | KC184458 | – | – | Arora and Krank 2014 |
| *Butyriboletus pulchriceps* | DS4514 | Arizona, USA | KF030261 | – | KF030409 | – | Nuhn et al. 2013 |
| *Butyriboletus pulchriceps* | R. Chapman 0945 | Arizona, USA | KT002615 | KT002604 | KT002639 | – | Zhao et al. 2015 |
| *Butyriboletus quercriegius* | Arora11100 | California, USA | – | KC184461 | – | – | Arora and Krank 2014 |
| *Butyriboletus regius* | MB000287 | Germany | KT002616 | KT002605 | KT002640 | – | Zhao et al. 2015 |
| *Butyriboletus regius* | MG408a | Lazio, Italy | KC584790 | KC584789 | – | – | Arora and Krank 2014 |
| *Butyriboletus regius* | PRM:923465 | Czech Rep. | KJ419931 | KJ419920 | – | – | Šutara et al. 2014 |
| *Butyriboletus roseoflavus* | Arora11054 | Yunnan, SW China | KC184435 | KC184434 | – | – | Arora and Krank 2014 |
| *Butyriboletus roseoflavus* | HKAS63593 | Yunnan, SW China | KJ184559 | KJ909517 | KJ184571 | – | Zhao et al. 2015 |
| *Butyriboletus roseoflavus* | HKAS54099 | Yunnan, SW China | KF739665 | KJ909519 | KF739779 | – | Zhao et al. 2015 |
| *Butyriboletus roseoflavus* | N.K. Zeng 2123 (FHMU 1387) | Yunnan, SW China | **MH879686** | **MH885348** | **MH879715** | – | this study |
| *Butyriboletus roseopurpureus* | E.E. Both3765 | New York, USA | KT002617 | KT002606 | KT002641 | – | Zhao et al. 2015 |
| *Butyriboletus roseopurpureus* | JLF2566 | West Virginia, USA | KC184467 | KC184466 | – | – | Arora and Krank 2014 |
| *Butyriboletus roseopurpureus* | MB06-059 | New York, USA | KF030262 | KC184464 | KF030410 | – | Nuhn et al. 2013 |
| *Butyriboletus sanicibus* | Arora99211 | Yunnan, SW China | KC184470 | KC184469 | – | – | Arora and Krank 2014 |
| *Butyriboletus* sp. | MHHNU7456 | China | KT990539 | – | KT990741 | KT990378 | Wu et al. 2016a |
| *Butyriboletus* sp. | HKAS52525 | Yunnan, SW China | KF112337 | – | KF112163 | KF112671 | Wu et al. 2014 |
| *Butyriboletus* sp. | HKAS57774 | Yunnan, SW China | KF112330 | – | KF112155 | KF112670 | Wu et al. 2014 |

| Taxon | Voucher | Locality | 28S | ITS | *tef1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|
| *Butyriboletus* sp. | HKAS59814 | Hunan, central China | KF112336 | – | KF112199 | KF112699 | Wu et al. 2014 |
| *Butyriboletus* sp. | HKAS63528 | Sichuan, SW China | KF112332 | – | KF112156 | KF112673 | Wu et al. 2014 |
| *Butyriboletus subappendiculatus* | MB000260 | Germany | KT002618 | KT002607 | KT002642 | – | Zhao et al. 2015 |
| *Butyriboletus subsplendidus* | HKAS52661 | Yunnan, SW China | KF112339 | – | KF112169 | KF112676 | Wu et al. 2014 |
| *Butyriboletus yicibus* | Arora9727 | Yunnan, SW China | KC184475 | KC184474 | – | – | Arora and Krank 2014 |
| *Butyriboletus yicibus* | HKAS57503 | Yunnan, SW China | KT002620 | KT002608 | KT002644 | – | Zhao et al. 2015 |
| *Butyriboletus yicibus* | HKAS68010 | Yunnan, SW China | KT002619 | KJ909521 | KT002643 | – | Zhao et al. 2015 |
| *Caloboletus calopus* | Bc1 | Bavaria, Germany | AF456833 | DQ679806 | JQ327019 | – | Zhao et al. 2014a |
| *Caloboletus calopus* | BR5020159063805 | Montenau, Belgium | KJ184554 | KJ605655 | KJ184566 | – | Zhao et al. 2014a |
| *Caloboletus calopus* | 112606 | California, USA | KF030279 | – | – | – | Nuhn et al. 2013 |
| *Caloboletus firmus* | MB06-060 | New York, USA | KF030368 | – | KF030408 | – | Nuhn et al. 2013 |
| *Caloboletus firmus* | NY00796115 | Cayo, Belize | KJ605678 | KJ605656 | KJ619464 | – | Zhao et al. 2014a |
| *Caloboletus guanyui* | N.K. Zeng 3058 (FHMU 2019) | Hainan, southern China | **MH879708** | **MH885365** | **MH879734** | **MH879751** | this study |
| *Caloboletus guanyui* | N.K. Zeng 3079 (FHMU 2040) | Hainan, southern China | **MH879709** | **MH885366** | **MH879736** | **MH879752** | this study |
| *Caloboletus guanyui* | N.K. Zeng 3257 (FHMU 2218) | Fujian, SE China | **MH879705** | – | **MH879732** | **MH879748** | this study |
| *Caloboletus guanyui* | N.K. Zeng 3261 (FHMU 2222) | Fujian, SE China | **MH879706** | – | **MH879733** | **MH879749** | this study |
| *Caloboletus guanyui* | N.K. Zeng 3263 (FHMU 2224) | Fujian, SE China | **MH879707** | **MH885364** | **MH879735** | **MH879750** | this study |
| *Caloboletus guanyui* | N.K. Zeng 3344 (FHMU 2809) | Hainan, southern China | – | – | **MK061357** | – | this study |
| *Caloboletus inedulis* | MB06-044 | New York, USA | JQ327013 | – | JQ327020 | – | Halling et al. 2012 |
| *Caloboletus inedulis* | HKAS80478 | Florida, USA | KJ605671 | KJ605657 | KJ619465 | – | Zhao et al. 2014a |
| *Caloboletus panniformis* | HKAS56164 | Yunnan, SW China | KJ605674 | KJ605667 | KJ619466 | – | Zhao et al. 2014a |
| *Caloboletus panniformis* | HKAS57410 | Yunnan, SW China | KJ184555 | KJ605659 | KJ184567 | – | Zhao et al. 2014a |
| *Caloboletus panniformis* | HKAS77530 | Yunnan, SW China | KJ605670 | KJ605661 | KJ619470 | – | Zhao et al. 2014a |
| *Caloboletus polygonius* | K(M)60247 | Greece | KU317763 | KU317753 | – | – | GenBank |
| *Caloboletus radicans* | HKAS80856 | France | KJ184557 | KJ605662 | KJ184569 | – | Zhao et al. 2014a |
| *Caloboletus* sp. | HKAS53353 | China | KF112410 | – | KF112188 | KF112668 | Wu et al. 2014 |
| *Caloboletus taienus* | GDGM44081 | Guangdong, southern China | KY800414 | KY800420 | – | – | Zhang et al. 2017 |
| *Caloboletus xiangtoushanensis* | GDGM44725 | Guangdong, southern China | KY800416 | KY800422 | – | – | Zhang et al. 2017 |
| *Caloboletus xiangtoushanensis* | GDGM44833 | Guangdong, southern China | KY800415 | KY800421 | KY800418 | – | Zhang et al. 2017 |
| *Caloboletus xiangtoushanensis* | GDGM45160 | Guangdong, southern China | KY800417 | KY800423 | KY800419 | – | Zhang et al. 2017 |
| *Caloboletus xiangtoushanensis* | N.K. Zeng 1330 (FHMU 883) | Fujian, SE China | **MH879702** | – | – | – | this study |
| *Caloboletus xiangtoushanensis* | N.K. Zeng 1331 (FHMU 884) | Fujian, SE China | **MH879703** | **MH885362** | – | – | this study |
| *Caloboletus xiangtoushanensis* | N.K. Zeng 1354 (FHMU 906) | Fujian, SE China | **MH879704** | **MH885363** | – | – | this study |
| *Caloboletus yunnanensis* | HKAS69214 | Yunnan, SW China | KJ184556 | KJ605663 | KJ184568 | – | Zhao et al. 2014a |
| *Caloboletus yunnanensis* | HKAS58694 | Yunnan, SW China | KJ605672 | KJ605664 | KJ619470 | – | Zhao et al. 2014a |
| *Chalciporus radiatus* | N.K. Zeng 1379 (FHMU 930) | Fujian, SE China | **MH879710** | **MH885367** | **MH879738** | – | this study |
| *Chalciporus radiatus* | N.K. Zeng 1414 (FHMU 959) | Fujian, SE China | **MH879711** | – | **MH879739** | – | this study |
| *Chalciporus radiatus* | N.K. Zeng 1808 (FHMU 2494) | Hainan, southern China | – | – | **MH879737** | – | this study |
| *Costatisporus cyanescens* | Henkel9067 | Guyana | LC053662 | LC054831 | – | – | Smith et al. 2015 |
| *Crocinoboletus laetissimus* | HKAS50232 | Yunnan, SW China | KT990567 | – | KT990762 | – | Wu et al. 2016a |

| Taxon | Voucher | Locality | 28S | ITS | *tef1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|
| *Crocinoboletus rufoaureus* | HKAS53424 | Hunan, central China | KF112435 | – | KF112206 | KF112710 | Wu et al. 2014 |
| *Cyanoboletus brunneoruber* | HKAS63504 | Yunnan, SW China | KF112368 | – | KF112194 | – | Wu et al. 2014 |
| *Cyanoboletus brunneoruber* | HKAS80579-1 | Yunnan, SW China | KT990568 | – | KT990763 | – | Wu et al. 2016a |
| *Cyanoboletus brunneoruber* | HKAS80579-2 | Yunnan, SW China | KT990569 | – | KT990764 | – | Wu et al. 2016a |
| *Cyanoboletus hymenoglutinosus* | DC14-010 | India | KT860060 | KT907355 | – | – | Li et al. 2016 |
| *Cyanoboletus instabilis* | HKAS59554 | Yunnan, SW China | KF112412 | – | KF112186 | – | Wu et al. 2014 |
| *Cyanoboletus instabilis* | FHMU1839 | Yunnan, SW China | MG030466 | MG030473 | MG030478 | – | Chai et al. 2018 |
| *Cyanoboletus pulverulentus* | 9606 | USA | KF030313 | – | KF030418 | – | Nuhn et al. 2013 |
| *Cyanoboletus pulverulentus* | RW109 | Belgium | – | – | KT824046 | – | Raspe et al. 2016 |
| *Cyanoboletus pulverulentus* | MG126a | Italy | KT157062 | KT157053 | – | – | Gelardi et al. 2015 |
| *Cyanoboletus pulverulentus* | MG456a | Azores Islands, Portugal | KT157063 | KT157054 | – | – | Gelardi et al. 2015 |
| *Cyanoboletus pulverulentus* | MG628a | Italy | KT157064 | KT157055 | KT157073 | – | Gelardi et al. 2015 |
| *Cyanoboletus sinopulverulentus* | HKAS59609 | Yunnan, SW China | KF112366 | – | KF112193 | – | Wu et al. 2014 |
| *Cyanoboletus* sp. | HKAS76850 | Hainan, southern China | KF112343 | – | KF112187 | – | Wu et al. 2014 |
| *Cyanoboletus* sp. | HKAS52639 | Yunnan, SW China | KF112367 | – | KF112195 | – | Wu et al. 2014 |
| *Cyanoboletus* sp. | HKAS52601 | Yunnan, SW China | KF112469 | – | – | – | Wu et al. 2014 |
| *Cyanoboletus* sp. | HKAS50292 | Yunnan, SW China | KF112470 | – | – | – | Wu et al. 2014 |
| *Cyanoboletus* sp. | HKAS59418 | China | KT990570 | – | KT990765 | – | Wu et al. 2016a |
| *Cyanoboletus* sp. | HKAS90208-1 | China | KT990571 | – | KT990766 | – | Wu et al. 2016a |
| *Cyanoboletus* sp. | HKAS90208-2 | China | – | – | KT990767 | – | Wu et al. 2016a |
| *Cyanoboletus* sp. | PRM944518 | USA | MF373585 | – | – | – | Braeuer et al. 2018 |
| *Exsudoporus frostii* | SAT1221511 | Tennessee, USA | KP055021 | – | KP055018 | KP055027 | Zhao et al. 2014b |
| *Exsudoporus frostii* | TENN067311 | Tennessee, USA | KT002612 | KT002601 | KT002636 | – | Zhao et al. 2015 |
| *Lanmaoa angustispora* | HKAS74765 | Yunnan, SW China | KF112322 | – | KF112159 | – | Wu et al. 2014 |
| *Lanmaoa angustispora* | HKAS74752 | Yunnan, SW China | KM605139 | – | KM605154 | – | Wu et al. 2016b |
| *Lanmaoa angustispora* | HKAS74759 | Yunnan, SW China | KM605140 | – | KM605155 | – | Wu et al. 2016b |
| *Lanmaoa asiatica* | HKAS54094 | Yunnan, SW China | KF112353 | – | KF112161 | – | Wu et al. 2014 |
| *Lanmaoa asiatica* | HKAS63516 | Yunnan, SW China | KT990584 | – | KT990780 | – | Wu et al. 2016a |
| *Lanmaoa asiatica* | HKAS63603 | Yunnan, SW China | KM605142 | – | KM605153 | – | Wu et al. 2016b |
| *Lanmaoa asiatica* | FHMU1389 | Yunnan, SW China | MG030470 | MG030477 | MG030481 | – | Chai et al. 2018 |
| *Lanmaoa asiatica* | FHMU1775 | Yunnan, SW China | MG030469 | – | MG030480 | – | Chai et al. 2018 |
| *Lanmaoa flavorubra* | NY775777 | Costa Rica | JQ924339 | – | KF112160 | – | Wu et al. 2014 |
| *Lanmaoa macrocarpa* | N.K. Zeng 3021 (FHMU 1982) | Hainan, southern China | **MH879684** | – | **MH879713** | – | this study |
| *Lanmaoa macrocarpa* | N.K. Zeng 3251 (FHMU 2212) | Fujian, SE China | **MH879685** | **MH885347** | **MH879714** | – | this study |
| *Lanmaoa pseudosensibilis* | DS615-07 | USA | KF030257 | – | KF030407 | – | Nuhn et al. 2013 |
| *Lanmaoa rubriceps* | FHMU 1756 | Hainan, southern China | MG030465 | MG030472 | – | – | Chai et al. 2018 |
| *Lanmaoa rubriceps* | FHMU 1757 | Hainan, southern China | MG030467 | MG030474 | – | – | Chai et al. 2018 |
| *Lanmaoa rubriceps* | FHMU 1763 | Hainan, southern China | MG030468 | MG030475 | MG030479 | – | Chai et al. 2018 |
| *Lanmaoa rubriceps* | FHMU 2801 | Hainan, southern China | MG030471 | MG030476 | – | – | Chai et al. 2018 |
| *Lanmaoa rubriceps* | N.K. Zeng 3006 (FHMU 1967) | Hainan, southern China | **MH879683** | **MH885346** | **MH879712** | – | this study |
| *Lanmaoa* sp. | HKAS52518 | Yunnan, SW China | KF112354 | – | KF112162 | – | Wu et al. 2014 |
| *Neoboletus brunneissimus* | HKAS52660 | Yunnan, SW China | KF112314 | – | KF112143 | KF112650 | Wu et al. 2014 |
| *Neoboletus ferrugineus* | HKAS77617 | Guangdong, southern China | KT990595 | – | KT990788 | KT990430 | Wu et al. 2016a |

| Taxon | Voucher | Locality | 28S | ITS | *tef1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|
| *Neoboletus ferrugineus* | HKAS77718 | Guangdong, southern China | KT990596 | – | KT990789 | KT990431 | Wu et al. 2016a |
| *Neoboletus flavidus* | HKAS58724 | Yunnan, SW China | KU974140 | – | KU974137 | KU974145 | Wu et al. 2016a |
| *Neoboletus flavidus* | HKAS59443 | Yunnan, SW China | KU974139 | – | KU974136 | KU974144 | Wu et al. 2016a |
| *Neoboletus hainanensis* | HKAS59469 | Yunnan, SW China | KF112359 | – | KF112175 | KF112669 | Wu et al. 2016a |
| *Neoboletus hainanensis* | HKAS90209 | Hainan, southern China | KT990615 | – | KT990809 | KT990450 | Wu et al. 2016a |
| *Neoboletus hainanensis* | HKAS63515 | Yunnan, SW China | KT990614 | – | KT990808 | KT990449 | Wu et al. 2016a |
| *Neoboletus hainanensis* | HKAS74880 | Yunnan, SW China | KT990597 | – | KT990790 | KT990432 | Wu et al. 2016a |
| *Neoboletus hainanensis* | N.K. Zeng 2128 (FHMU 1392) | Yunnan, SW China | **MH879690** | – | **MH879719** | – | this study |
| *Neoboletus luridiformis* | AT2001087 | Berkshire, England | JQ326995 | – | JQ327023 | – | Halling et al. 2012 |
| *Neoboletus magnificus* | HKAS54096 | Yunnan, SW China | KF112324 | – | KF112149 | KF112654 | Wu et al. 2014 |
| *Neoboletus magnificus* | HKAS74939 | Yunnan, SW China | KF112320 | – | KF112148 | KF112653 | Wu et al. 2014 |
| *Neoboletus multipunctatus* | HKAS76851 | Hainan, southern China | KF112321 | – | KF112144 | KF112651 | Wu et al. 2014 |
| *Neoboletus multipunctatus* | N.K. Zeng 2498 (FHMU 1620) | Hainan, southern China | **MH879693** | **MH885354** | **MH879722** | – | this study |
| *Neoboletus multipunctatus* | N.K. Zeng3324 (FHMU 2808) | Hainan, southern China | **MK061360** | **MK061359** | **MK061358** | – | this study |
| *Neoboletus obscureumbrinus* | HKAS63498 | Yunnan, SW China | KT990598 | – | KT990791 | KT990433 | Wu et al. 2016a |
| *Neoboletus obscureumbrinus* | HKAS89027 | Yunnan, SW China | KT990600 | – | KT990794 | KT990436 | Wu et al. 2016a |
| *Neoboletus obscureumbrinus* | N.K. Zeng 3091 (FHMU 2052) | Hainan, southern China | **MH879694** | **MH885355** | **MH879723** | **MH879742** | this study |
| *Neoboletus obscureumbrinus* | N.K. Zeng 3094 (FHMU 2055) | Hainan, southern China | **MH879695** | **MH885356** | **MH879724** | **MH879743** | this study |
| *Neoboletus obscureumbrinus* | N.K. Zeng 3098 (FHMU 2059) | Hainan, southern China | **MH879696** | **MH885357** | **MH879725** | **MH879744** | this study |
| *Neoboletus rubriporus* | HKAS83026 | Yunnan, SW China | KT990601 | – | KT990795 | KT990437 | Wu et al. 2016a |
| *Neoboletus rubriporus* | HKAS89174 | Yunnan, SW China | KT990602 | – | KT990796 | KT990438 | Wu et al. 2016a |
| *Neoboletus rubriporus* | HKAS89181 | Yunnan, SW China | KT990603 | – | KT990797 | – | Wu et al. 2016a |
| *Neoboletus rubriporus* | HKAS90210 | Yunnan, SW China | KT990604 | – | KT990798 | KT990439 | Wu et al. 2016a |
| *Neoboletus rubriporus* | MHKMU-L.P. Tang 1958 | Yunnan, SW China | – | **MH885358** | **MH879726** | – | this study |
| *Neoboletus sanguineoides* | HKAS55440 | Yunnan, SW China | KF112315 | – | KF112145 | KF112652 | Wu et al. 2014 |
| *Neoboletus sanguineoides* | HKAS57766 | Yunnan, SW China | KT990605 | – | KT990799 | KT990440 | Wu et al. 2016a |
| *Neoboletus sanguineoides* | HKAS63530 | Sichuan, SW China | KT990607 | – | KT990801 | – | Wu et al. 2016a |
| *Neoboletus sanguineoides* | HKAS80823 | Yunnan, SW China | KT990605 | – | KT990799 | KT990440 | Wu et al. 2016a |
| *Neoboletus sanguineus* | HKAS80849 | Yunnan, SW China | KT990609 | – | KT990803 | KT990443 | Wu et al. 2016a |
| *Neoboletus sanguineus* | HKAS90211 | Xizang, SW China | KT990610 | – | KT990804 | KT990444 | Wu et al. 2016a |
| *Neoboletus sanguineus* | HKAS68587 | Yunnan, SW China | KF112329 | – | KF112150 | KF112657 | Wu et al. 2014 |
| *Neoboletus* sp. | CMU58-ST-0237 | – | KX017292 | KX017301 | – | – | GenBank |
| *Neoboletus* sp. | HKAS76851 | Hainan, southern China | KF112321 | – | KF112144 | KF112651 | Wu et al. 2014 |
| *Neoboletus* sp. | HKAS50351 | Yunnan, SW China | KF112318 | – | – | KF112658 | Wu et al. 2014 |
| *Neoboletus* sp. | HKAS76660 | Henan, Central China | KF112328 | – | KF112180 | KF112731 | Wu et al. 2014 |
| *Neoboletus thibetanus* | HKAS57093 | Xizang, China | KF112326 | – | – | KF112655 | Wu et al. 2014 |
| *Neoboletus tomentulosus* | HKAS53369 | Fujian, SE China | KF112323 | – | KF112154 | KF112659 | Wu et al. 2014 |
| *Neoboletus tomentulosus* | HKAS77656 | Guangdong, southern China | KT990611 | – | KT990806 | KT990446 | Wu et al. 2016a |
| *Neoboletus tomentulosus* | N.K. Zeng 1285 (FHMU 841) | Fujian, SE China | **MH879691** | **MH885352** | **MH879720** | – | this study |
| *Neoboletus tomentulosus* | N.K. Zeng 1286 (FHMU 842) | Fujian, SE China | **MH879692** | **MH885353** | **MH879721** | – | this study |
| *Neoboletus venenatus* | HKAS57489 | Yunnan, SW China | KF112325 | – | KF112158 | KF112665 | Wu et al. 2014 |
| *Neoboletus venenatus* | HKAS63535 | Sichuan, SW China | KT990613 | – | KT990807 | KT990448 | Wu et al. 2016a |
| *Rugiboletus brunneiporus* | HKAS68586 | Xizang, SW China | KF112402 | – | KF112197 | – | Wu et al. 2014 |

| Taxon | Voucher | Locality | 28S | ITS | *tef1* | *rpb2* | References |
|---|---|---|---|---|---|---|---|
| *Rugiboletus brunneiporus* | HKAS83009 | Xizang, SW China | KM605133 | – | KM605146 | – | Wu et al. 2016b |
| *Rugiboletus extremiorientalis* | HKAS76663 | Henan, Central China | KM605135 | – | KM605147 | KM605170 | Wu et al. 2016b |
| *Rugiboletus extremiorientalis* | HKAS74754 | China | KT990639 | – | KT990832 | KT990469 | Wu et al. 2016a |
| *Rubroboletus latisporus* | HKAS63517 | Yunnan, SW China | KP055022 | – | KP055019 | KP055028 | Zhao et al. 2014b |
| *Rubroboletus latisporus* | HKAS80358 | Chongqing, SW China | KP055023 | – | KP055020 | KP055029 | Zhao et al. 2014b |
| *Rubroboletus sinicus* | HKAS68620 | Yunnan, SW China | KF112319 | – | KF112146 | KF112661 | Zhao et al. 2014b |
| *Sutorius* aff. *eximius* | HKAS56291 | Yunnan, SW China | KF112400 | – | KF112208 | KF112803 | Wu et al. 2014 |
| *Sutorius* aff. *eximius* | MHKMU-S.D. Yang 010 | Yunnan, SW China | **MH879697** | **MH885359** | **MH879727** | – | this study |
| *Sutorius australiensis* | REH9280 | Australia | JQ327031 | – | JQ327031 | – | Arora and Krank 2014 |
| *Sutorius australiensis* | REH9441 | Australia | JQ327006 | – | JQ327032 | MG212652 | Halling et al. 2012 |
| *Sutorius eximius* | REH9400 | USA | JQ327004 | – | JQ327029 | – | Arora and Krank 2014 |
| *Sutorius eximius* | HKAS52672 | Yunnan, SW China | KF112399 | – | KF112207 | KF112802 | Wu et al. 2014 |
| *Sutorius eximius* | HKAS50420 | Yunnan, SW China | KT990549 | – | KT990750 | KT990387 | Wu et al. 2016a |
| *Sutorius eximius* | HKAS59657 | China | KT990707 | – | KT990887 | KT990505 | Wu et al. 2016a |
| *Sutorius eximius* | 8594 | Costa Rica | JQ327008 | – | JQ327027 | – | Halling et al. 2012 |
| *Sutorius eximius* | 995 | Costa Rica | JQ327010 | – | JQ327030 | – | Halling et al. 2012 |
| *Sutorius eximius* | 986 | Costa Rica | JQ327009 | – | JQ327028 | – | Halling et al. 2012 |
| *Sutorius eximius* | 8069 | Indonesia | JQ327003 | – | – | – | Halling et al. 2012 |
| *Sutorius* sp. | N.K. Zeng 3297 (FHMU 2258) | Fujian, SE China | **MH879701** | – | **MH879731** | – | this study |
| *Sutorius* sp. | ECV3603 | Thailand | JQ327000 | – | JQ327033 | – | Halling et al. 2012 |
| *Sutorius* sp. | 01-528 | Zambia | JQ327002 | – | – | – | Halling et al. 2012 |
| *Sutorius subrufus* | N.K. Zeng 3043 (FHMU 2004) | Hainan, southern China | **MH879698** | **MH885360** | **MH879728** | **MH879745** | this study |
| *Sutorius subrufus* | N.K. Zeng 3045 (FHMU 2006) | Hainan, southern China | **MH879699** | **MH885361** | **MH879729** | **MH879746** | this study |
| *Sutorius subrufus* | N.K. Zeng 3140 (FHMU 2101) | Hainan, southern China | **MH879700** | – | **MH879730** | **MH879747** | this study |

three collections were newly generated and deposited in GenBank (Table 1), and then combined with selected sequences from previous studies (Table 1). *Rugiboletus brunneiporus* G. Wu & Zhu L. Yang was chosen as outgroup on the basis of the phylogeny in Wu et al. (2016a). To test for phylogenetic conflict among the different genes in three combined datasets (*Butyriboletus*, *Caloboletus* + *Neoboletus* + *Sutorius*, *Lanmaoa*), the partition homogeneity (PH) or incongruence length difference (ILD) test was performed with 1000 randomized replicates, using heuristic searches with simple addition of sequences in PAUP* 4.0b10 (Swofford 2002). The results of the partition homogeneity test showed that the phylogenetic signals present in the different gene fragments were not in conflict. Then the sequences of different genes in three combined datasets (*Butyriboletus*, *Caloboletus* + *Neoboletus* + *Sutorius*, *Lanmaoa*) were aligned with MAFFT v. 6.8 using algorithm E-INS-i (Katoh et al. 2005) and manually optimized on BioEdit v. 7.0.9 (Hall 1999). The sequences of the different genes were concatenated in three combined datasets (*Butyriboletus*, *Caloboletus* + *Neoboletus* + *Sutorius*, *Lanmaoa*) using Phyutility v. 2.2 for further analyses (Smith and Dunn 2008).

### Phylogenetic analyses

The three combined datasets (*Butyriboletus*, *Caloboletus* + *Neoboletus* + *Sutorius*, *Lanmaoa*) were all analyzed by using maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood tree generation and bootstrap analyses were performed with the program RAxML 7.2.6 (Stamatakis 2006) running 1000 replicates combined with an ML search. Bayesian analysis with MrBayes 3.1 (Huelsenbeck and Ronquist 2005) implementing the Markov Chain Monte Carlo (MCMC) technique and parameters predetermined with MrModeltest 2.3 (Nylander 2004) was performed. The model of evolution used in the Bayesian analysis was determined with MrModeltest 2.3 (Nylander 2004). For the combined dataset of *Butyriboletus*, the best-fit likelihood models of 28S, ITS1+ITS2, 5.8S, *tef1* and *rpb2* were GTR+I+G, HKY+I+G, K80, SYM+I+G and K80+I+G, respectively; for the combined dataset of *Caloboletus*, *Neoboletus*, and *Sutorius*, the best-fit likelihood models of 28S, ITS1+ITS2, 5.8S, *tef1* and *rpb2* were GTR+I+G, HKY+I+G, K80, SYM+I+G and SYM+I+G, respectively; for the combined dataset of *Lanmaoa*, the best-fit likelihood models of 28S, ITS1+ITS2, 5.8S and *tef1* were GTR+I+G, GTR+I, K80 and SYM+G, respectively. Bayesian analysis was run with one cold and three heated chains and sampled every 100 generations; trees sampled from the first 25% of the generations were discarded as burn-in; the average standard deviation of split frequencies was restricted to be below 0.01, and Bayesian posterior probabilities (PP) were then calculated for a majority consensus tree of the retained Bayesian trees.

## Results

### Molecular data

The four-locus dataset (28S + ITS + *tef1* + *rpb2*) of *Butyriboletus* consisted of 52 taxa and 3116 nucleotide sites (Fig. 1). The aligned dataset was submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23508). The molecular phylogenetic analyses showed that the collections numbered as FHMU 2206 and FHMU 2207 respectively grouped together with a high statistical support (BS = 100, PP = 1), forming an independent lineage within *Butyriboletus* (Fig. 1).

The four-locus dataset (28S + ITS + *tef1* + *rpb2*) with *Caloboletus*, *Neoboletus*, and *Sutorius* consisted of 93 taxa and 3228 nucleotide sites (Fig. 2). The aligned dataset was submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23509). The molecular phylogenetic analyses indicated each of the previously described genera, viz. *Neoboletus*, *Sutorius*, *Costatisporus* T.W. Henkel & M.E. Sm., and *Caloboletus*, forms an independent clade with a high statistical support respectively (Fig. 2). In the genus *Neoboletus*, one collection numbered as FHMU 1392 and one previously described *S. hainanensis* (T.H. Li & M. Zang) G. Wu and Zhu L. Yang grouped together with a strong statistical support (BS = 100, PP = 1), forming an independent lineage; two collections numbered as FHMU 841 and FHMU 842 respectively and one previously described


**Figure 1.** Phylogenetic placement of *Butyriboletus huangnianlaii* inferred from a multilocus (28S, ITS, *tef1*, *rpb2*) dataset using RAxML. BS ≥ 50% and PP ≥ 0.95 are indicated above or below the branches as RAxML BS/PP.

*S. tomentulosus* (M. Zang et al.) G. Wu & Zhu L. Yang grouped together with a high statistical support (BS = 100, PP = 1), forming an independent lineage; one collection tentatively named *Sutorius* sp. (HKAS 76851) in a previous study (Wu et al. 2016a) and one specimen numbered as FHMU 1620 grouped together with a high statistical support (BS = 100, PP = 1), forming an independent lineage; three specimens numbered as FHMU 2052, FHMU 2055, FHMU 2059 respectively and one previously described *S. obscureumbrinus* (Hongo) G. Wu & Zhu L. Yang grouped together with a high statistical support (BS = 100, PP = 1), forming an independent lineage (Fig. 2). In the genus *Sutorius*, the specimens numbered as FHMU 2004, FHMU 2006 and FHMU 2101 respectively grouped together with a high statistical support (BS = 100, PP = 1), forming an independent lineage (Fig. 2). In the genus *Caloboletus*, the materials numbered as FHMU 883, FHMU 884, FHMU 906 respectively and the holotype of *C. xiangtoushanensis* Ming Zhang et al. grouped together with a high statistical support (BS = 100, PP = 1),

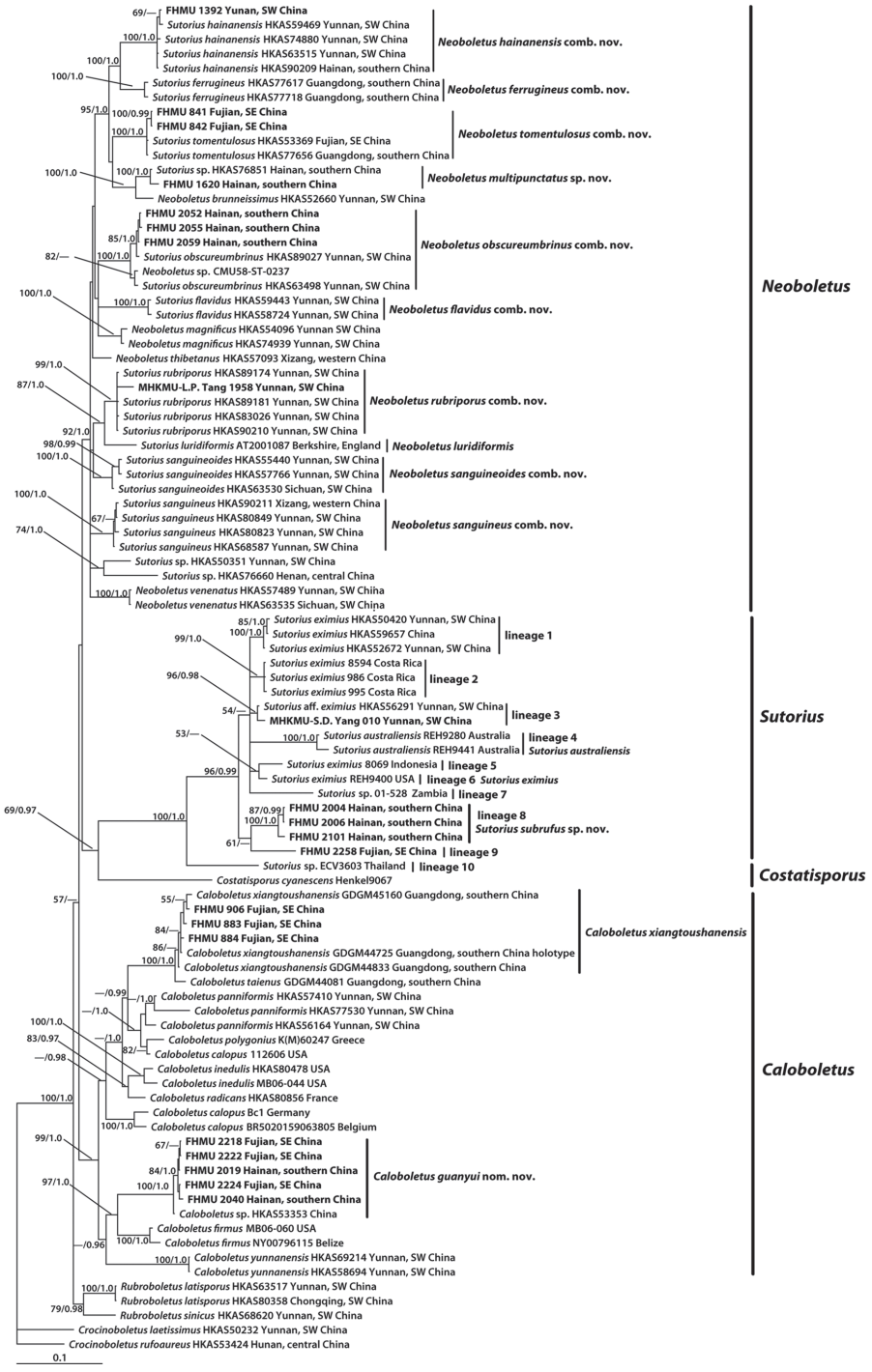


**Figure 2.** Phylogenetic placement of *Neoboletus multipunctatus*, *Sutorius subrufus* and *Caloboletus guanyui* inferred from a multilocus (28S, ITS, *tef1*, *rpb2*) dataset using RAxML. BS ≥ 50% and PP ≥ 0.95 are indicated above or below the branches as RAxML BS/PP.

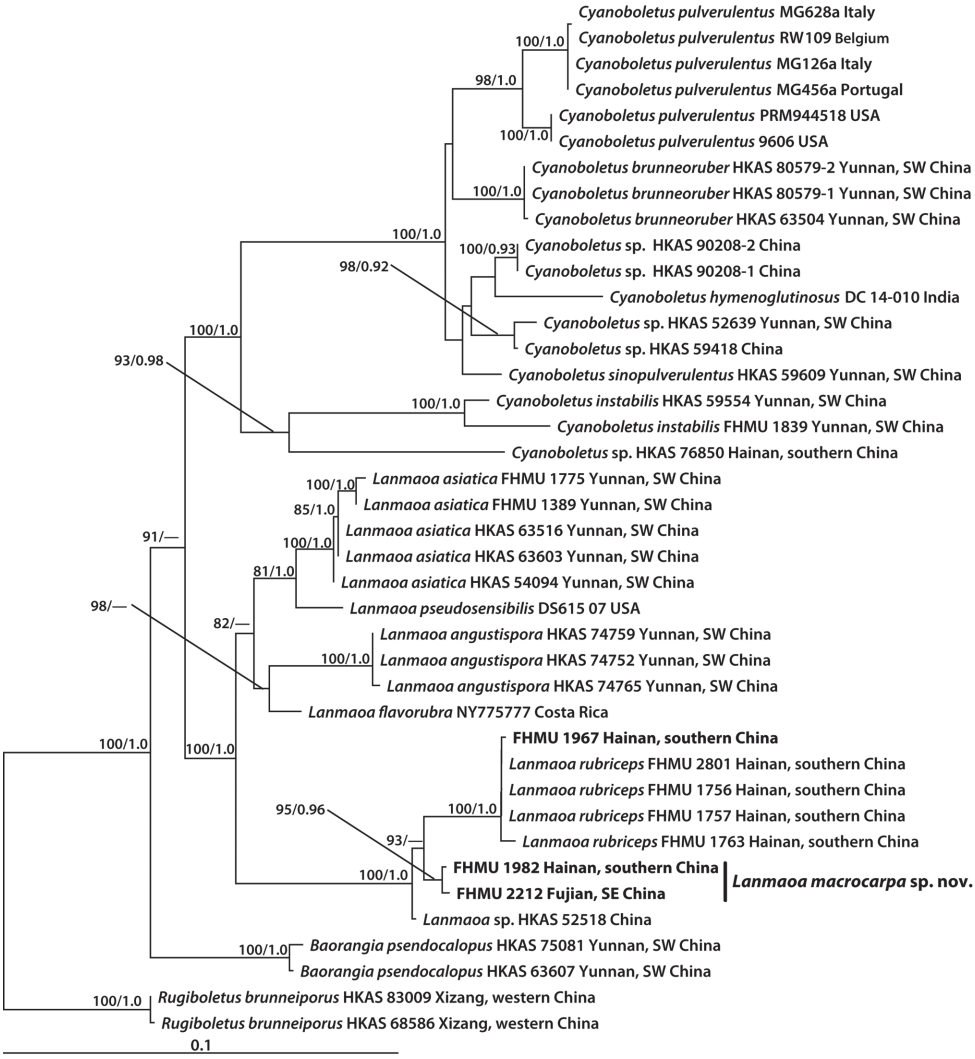


**Figure 3.** Phylogenetic placement of *Lanmaoa macrocarpa* inferred from a multilocus (28S, ITS, *tef1*) dataset using RAxML. BS ≥ 50% and PP ≥ 0.95 are indicated above or below the branches as RAxML BS/PP.

forming an independent lineage; the collections numbered as FHMU 2019, FHMU 2040, FHMU 2218, FHMU 2222 and FHMU 2224 respectively grouped together with a strong statistical support (BS = 100, PP = 1), forming an independent lineage (Fig. 2).

The three-locus dataset (28S + ITS + *tef1*) of *Lanmaoa* consisted of 40 taxa and 2007 nucleotide sites (Fig. 3). The aligned dataset was submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23510). The molecular phylogenetic analyses showed that the collections numbered as FHMU 1982 and FHMU 2212 respectively grouped together with a high statistical support (BS = 100, PP = 1), forming an independent lineage within *Lanmaoa* (Fig. 3).

## Taxonomy

### *Butyriboletus* D.Arora & J.L. Frank

*Butyriboletus*, typified by *But. appendiculatus* (Schaeff.) D. Arora & J.L. Frank, was erected to accommodate the "butter boletes", which are mainly characterized by yellow hymenophore and context staining blue when injured and stipe surface usually covered with reticulations (Arora and Frank 2014; Zhao et al. 2015). Until now, six species, including *But. hainanensis* N.K. Zeng et al., *But. pseudospeciosus* Kuan Zhao & Zhu L.Yang, *But. roseoflavus* (Hai B. Li & Hai L.Wei) D.Arora & J.L. Frank, *But. sanicibus* D. Arora & J.L. Frank, *But. subsplendidus* (W.F. Chiu) Kuan Zhao et al., and *But. yicibus* D. Arora & J.L. Frank have been described from China (Arora and Frank 2014; Liang et al. 2016; Wu et al. 2016a). Herein, we describe another novel species.

**1. *Butyriboletus huangnianlaii* N.K. Zeng, H. Chai & Zhi Q. Liang, sp. nov.**
MycoBank: MB828521
Figures 4a, b, 7

**Typification.** CHINA. Fujian Province: Sanming City, Geshikao National Forest Park, elev. 420 m, 16 August 2017, *N.K. Zeng 3246* (FHMU 2207, holotype). GenBank accession numbers: 28S = MH879689, ITS = MH885351, *tef1* = MH879718, *rpb2* = MH879741.

**Etymology.** Latin, "*huangnianlaii*" is named after Chinese mycologist Nian-Lai Huang, in honor of his contribution to mycology.

**Description.** *Basidiomata* medium-sized to large. *Pileus* 5–11 cm in diameter, convex to applanate; surface dry, finely tomentose, pale brown (5D1–4D2), brown to reddish brown (5C2–6C2); context 0.6–2.2 cm thick in the center of the pileus, yellowish to yellow, changing blue quickly when injured. *Hymenophore* poroid, adnate or slightly depressed around apex of stipe; pores angular, about 0.5 mm in diameter, yellowish white (30A2) to yellowish brown (4A4), changing blue quickly when injured; tubes 0.4–0.8 cm in length. *Stipe* 4.5–8 × 1.3–2.5 cm, central, subcylindric, solid; surface dry, yellowish (30A2) when young, then brownish red (8D5), reticulate nearly to base; reticulum yellowish (1A2) when young, then brownish red (8D5); context yellowish to yellow, changing blue quickly when injured; basal mycelium white (1A1). *Odor* indistinct.

*Basidia* 20–31 × 6–9 μm, clavate, thin-walled, colorless to yellowish in KOH; four-spored, sterigmata 3–4 μm in length. *Basidiospores* [40/2/2] (7–)7.5–10.5(–11) × 3–4 μm, Q=(2.00–)2.14–2.86(–3.14), $Q_m$=2.51 ± 0.27, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid; composed of colorless to yellowish in KOH, 3–10 μm wide, thin- to slightly thick-walled (to 0.5 μm) hyphae. *Cheilocystidia* 32–53 × 7–12 μm, fusiform or subfusiform, thin-walled, yellowish in KOH, no


**Figure 4.** Basidiomata of boletes. **a, b** *Butyriboletus huangnianlaii* (FHMU 2207, holotype) **c–f** *Caloboletus guanyui* (**c–d** from FHMU 399; **e** from FHMU 2224; f from FHMU 2222) **g–j** *Caloboletus xiangtoushanensis* (**g** from FHMU 883 **h, j** from FHMU 906 **i** from FHMU 884) **k, l** *Chalciporus radiatus* (FHMU 930). Photos by N.K. Zeng.

encrustations. *Pleurocystidia* 40–60 × 8–13 μm, fusiform or subfusiform, thin-walled, yellowish in KOH, no encrustations. *Pileipellis* a trichoderm about 110 μm thick, composed of slightly interwoven, nearly colorless in KOH, 4–6 μm wide, thin-walled hyphae; terminal cells 30–50× 4–8 μm, clavate or subclavate, with obtuse apex. *Pileal trama* made up of hyphae 8–12 μm in diameter, thin-walled, colorless in KOH. *Stipitipellis* hymeniform about 120–140 μm thick, composed of thin- to slightly thick-walled (to 0.5 μm) emergent hyphae, colorless to yellowish in KOH, with clavate, subclavate, fusiform or subfusiform terminal cells (15–45 × 4–9 μm) , and occasionally with scattered clavate, 4-spored basidia. *Stipe trama* composed of longitudinally arranged, paral-

lel hyphae 3.5–7 μm wide, cylindrical, thin- to slightly thick-walled (up to 0.5 μm), colorless to yellowish in KOH, parallel hyphae. *Clamp connections* absent in all tissues.

**Habitat.** Scattered on the ground in forests dominated by *Castanopsis kawakamii* Hay.

**Distribution.** Southeastern China.

**Additional specimens examined.** CHINA. Fujian Province: Sanming City, Geshikao National Forest Park, elev. 420 m, 16 August 2017, *N.K. Zeng 3245* (FHMU 2206).

**Note.** *Butyriboletus huangnianlaii* is characterized by a medium-sized to large basidioma, pileal surface densely covered with pale brown to reddish brown squamules, smaller basidiospores, and its association with fagaceous trees. It is both morphologically similar and phylogenetically related to *But. pseudospeciosus* and *But. roseoflavus* (Fig. 1). However, *But. pseudospeciosus*, originally described from Yunnan Province of southwestern China, has a tomentose pileus without a reddish tinge, surface of pileus and stipe promptly staining blue when bruised, narrower cystidia and longer basidiospores measuring 9–11 × 3.5–4 μm (Wu et al. 2016a); *But. roseoflavus*, originally described from Zhejiang Province of southeastern China, has a pinkish to purplish red or rose-red pileus with tomentose surface, longer basidiospores measuring 9–12 × 3–4 μm, and its association with *Pinus* spp. (Arora and Frank 2014; Li et al. 2014; Wu et al. 2016a).

## *Caloboletus* Vizzini

*Caloboletus*, typified by *C. calopus* (Pers.) Vizzini, is mainly characterized by yellow tubes, yellow or more rarely orange to red pores changing to blue when injured, bitter taste of the context due to the presence of calopin and cyclocalopin (Hellwig et al. 2002; Vizzini 2014; Zhao et al. 2014a; Wu et al. 2016a; Zhang et al. 2017). Until now, four species, including *C. panniformis* (Taneyama & Har. Takah.) Vizzini, *C. taienus* (W.F. Chiu) Ming Zhang and T.H. Li, *C. xiangtoushanensis* Ming Zhang et al., and *C. yunnanensis* Kuan Zhao & Zhu L. Yang, have been found in China (Zhao et al. 2014a; Wu et al. 2016a; Zhang et al. 2017). We describe two *Caloboletus* species here.

### 2. *Caloboletus guanyui* N.K. Zeng, H. Chai & S. Jiang, nom. nov.

MycoBank: MB828522

Figures 4c–f, 8

*Boletus quercinus* Hongo, Memoirs of Shiga University 17: 92, 1967 (nom. illeg., later homonym)

non *Boletus quercinus* Schrad., Spicilegium Florae Germanicae 1: 157, 1794

non *Boletus quercinus* (Pilát) Hlaváček, Mykologický Sborník 67(3): 87, 1990 (nom. illeg., later homonym)

**Etymology.** Latin, "*guanyui*" is named for Guan Yu, a historic Chinese hero, said to have a reddish face, and thus sharing the same color of pores of the species when young.

**Description.** *Basidiomata* medium-sized to large. *Pileus* 5–10 cm in diameter, convex to applanate; surface dry, finely tomentose, dirty white to pale brown; context 0.5–1.8 cm thick in the center of the pileus, white, changing bluish quickly when injured, then back to white. *Hymenophore* poroid, depressed around apex of stipe; pores subround, 0.3–0.5 mm in diameter, reddish to reddish brown when young, then yellow or yellowish brown, changing bluish black when injured; tubes about 0.5–1 cm in length, yellowish, changing bluish quickly when injured. *Stipe* 5.5–9 × 0.7–1.5 cm, central, subcylindric, solid, usually flexuous; surface dry, densely covered with pale brown, brown to reddish brown, minute squamules; context white, sometimes tinged with pale red, unchanging in color when injured; basal mycelium white. *Odor* indistinct.

*Basidia* 21–30 × 6–8 μm, clavate, thin-walled, colorless to yellowish in KOH; four-spored, sterigmata 3–4 μm in length. *Basidiospores* [220/12/5] (8.5–)9–11(–12) × 3.5–4.5 μm, Q=(2.00–)2.22–2.67(–2.86), $Q_m$=2.43 ± 0.17, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid; composed of yellowish in KOH, 4–10 μm wide, thin-walled hyphae. *Cheilocystidia* 25–40 × 7–10 μm, fusiform or subfusiform, thin-walled, colorless to yellowish in KOH, no encrustations. *Pleurocystidia* 35–45 × 6–11 μm, fusiform or subfusiform, thin-walled, colorless to yellowish in KOH, no encrustations. *Pileipellis* a trichoderm about 100–200 μm thick, composed of slightly interwoven, nearly colorless in KOH, 5–8 μm wide, thin-walled hyphae; terminal cells 28–35 × 5–10 μm, clavate or subclavate, with obtuse apex. *Pileal trama* made up of hyphae 4–8 μm in diameter, slightly thick-walled (to 0.5 μm), colorless to yellowish in KOH. *Stipitipellis* hymeniform about 80–100 μm thick, composed of thin-walled emergent hyphae, yellowish in KOH, with clavate, subclavate, fusiform or subfusiform terminal cells (27–43 × 6–11 μm), and occasionally with scattered clavate, 4-spored basidia. *Stipe trama* composed of longitudinally arranged, parallel hyphae 3–6 μm wide, cylindrical, thin-walled, colorless to yellowish in KOH. *Clamp connections* absent in all tissues.

**Habitat.** Gregarious on the ground in forests dominated by *Castanopsis kawakamii* Hay. or *Lithocarpus* spp.

**Distribution.** Southeastern and southern China; Japan (Hongo 1967).

**Specimens examined.** CHINA. Hainan Province: Ledong County, Yinggeling National Nature Reserve, elev. 650 m, 4 June 2017, *N.K. Zeng 3058* (FHMU 2019); same location, 5 June 2017, *N.K. Zeng 3079* (FHMU 2040). Fujian Province: Zhangping County, Tiantai National Forest Park, elev. 350 m, 28 August 2009, *N.K. Zeng 635* (FHMU 399); Sanming City, Geshikao National Forest Park, elev. 420 m, 16 August 2017, *N.K. Zeng 3257* (FHMU 2218); same location and date, *N.K. Zeng 3261* (FHMU 2222); Yongan City, Tianbaoyan National Nature Reserve, elev. 600 m, 17 August 2017, *N.K. Zeng 3263* (FHMU 2224).

**Note.** *Caloboletus guanyui* was originally described as *B. quercinus* from Japan (Hongo 1967). Nomenclaturally, the epithet *quercinus* of this species is an illegitimate

name, because Schrader (1794) described a species using the same epithet before Hongo (1967). Therefore, the new epithet *guanyui* is proposed here for this species. Moreover, morphological and molecular evidence indicates the taxon is a member of the genus *Caloboletus* (Fig. 2), and is characterized by a dirty-white to pale-brown pileus, pores reddish to reddish brown when young, then yellow or yellowish brown, changing bluish black when injured, and a stipe densely covered with pale-brown, brown to reddish-brown squamules. Morphologically, *C. taienus* and *C. xiangtoushanensis* also have reddish pores (Bessette et al. 2016; Zhang et al. 2017), however, a dirty-white to pale-brown pileus easily distinguishes *C. guanyui* from the two taxa. Phylogenetically *C. guanyui* is closely related to *C. firmus* (Frost) Vizzini (Fig. 2), however, *C. firmus* has a stipe covered with whitish or reddish reticula, and it is restricted to North and Central America (Bessette et al. 2016).

### 3. *Caloboletus xiangtoushanensis* Ming Zhang, T.H. Li & X.J. Zhong, Phytotaxa 309: 119, 2017

Figures 4g–j, 9

**Description.** *Basidiomata* medium-sized to large. *Pileus* 5.5–11 cm in diameter, convex to plane; surface dry, tomentose, yellowish brown, pale brown to brown; context 1–1.5 cm thick in the center of the pileus, yellowish, changing blue quickly when injured. *Hymenophore* poroid, adnate to depressed around apex of stipe; pores subround to angular, 0.5–1 mm in diameter, yellow, sometimes brownish red, changing blue quickly when injured; tubes 0.5–1.4 cm in length, yellowish, changing blue quickly when injured. *Stipe* 5–9 × 0.9–1.6 cm, central, subcylindric, solid, usually flexuous; surface dry, upper part covered with reddish brown, minute squamules, middle and lower part covered with brown minute squamules; context yellowish, changing blue quickly when injured; basal mycelium white. *Odor* indistinct.

*Basidia* 25–35 × 5–10 μm, clavate, thin-walled, colorless to yellowish in KOH; four-spored, sterigmata 3–4 μm in length. *Basidiospores* [140/8/3] (9.5–)10–11.5(–13) × 3.5–4.5 μm, Q=(2.11–)2.44–3.00(–3.29), $Q_m$=2.76 ± 0.21, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid; composed of colorless to yellowish in KOH, 4–10 μm wide, thin-walled hyphae. *Cheilocystidia* 25–45 × 7–10 μm, fusiform or subfusiform, thin-walled, colorless in KOH, no encrustations. *Pleurocystidia* 30–50 × 7–12 μm, fusiform or subfusiform, thin-walled, colorless in KOH, no encrustations. *Pileipellis* a trichoderm about 70–100 μm thick, composed of slightly interwoven, colorless or yellowish in KOH, 4–7 μm wide, thin-walled hyphae; terminal cells 35–55 × 4–7 μm, clavate or subclavate, with obtuse apex. *Pileal trama* made up of hyphae 3.5–7 μm in diameter, thin-walled, colorless to yellowish in KOH. *Stipitipellis* hymeniform about 60–80 μm thick, composed of thin- to slightly thick-walled (to 0.5 μm) emergent hyphae, colorless to yellowish in KOH, with clavate, subclavate,

fusiform or subfusiform terminal cells (15–46 × 5–8 μm), and occasionally with scattered clavate, four-spored basidia. *Stipe trama* composed of longitudinally arranged, parallel hyphae 3.5–8 μm wide, cylindrical, thin- to slightly thick-walled (to 0.5 μm), yellowish in KOH. *Clamp connections* absent in all tissues.

**Habitat.** Solitary or gregarious on the ground in forests dominated by fagaceous trees.

**Distribution.** Southeastern and southern China.

**Specimens examined.** CHINA. Fujian Province: Zhangping County, Xinqiao Town, Chengkou Village, elev. 350 m, 30 July 2013, *N.K. Zeng 1330* (FHMU 883); same location and date, *N.K. Zeng 1331* (FHMU 884); same location, 1 August 2013, *N.K. Zeng 1354* (FHMU 906).

**Notes.** Our recent collections and the holotype of *C. xiangtoushanensis*, a species originally described from Guangdong Province of southern China (Zhang et al. 2017), phylogenetically group together with a strong statistical support (Fig. 2), which indicates that these specimens should be recognized as *C. xiangtoushanensis*. It is new to Fujian Province. Morphologically, several features of our collections also match well with the protologue of *C. xiangtoushanensis* (Zhang et al. 2017), but reticulations on the stipe were not observed in our specimens. Moreover, pores of our specimens are sometimes brownish red. In appearance, *C. xiangtoushanensis* is highly similar to Japanese *B. bannaensis* Har. Takah., which needs further confirmation for generic placement (Takahashi 2007). However, *B. bannaensis* has rufescent and faintly cyanescent context, small basidiospores measuring 6.5–9 × 3.5–4 μm, and narrower cystidia (Takahashi 2007). The molecular analyses also indicates that *C. xiangtoushanensis* is closely related to *C. taienus* (W.F. Chiu) Ming Zhang and T.H. Li (Fig. 2), a species originally described from Yunnan Province (Chiu 1948); their morphological differences have been elucidated in a previous study (Zhang et al. 2017).

### *Chalciporus* Bataille

*Chalciporus*, typified by *Ch. piperatus* (Bull.) Bataille, is an early branching lineage in the Boletaceae (Nuhn et al. 2013; Wu et al. 2014, 2016b) and is characterized by a pinkish-red to reddish-brown hymenophore. Several taxa, including *Ch. citrinoaurantius* Ming Zhang & T.H. Li, *Ch. hainanensis* Ming Zhang & T.H. Li, *Ch. radiatus* Ming Zhang & T.H. Li, and *Ch. rubinelloides* G.Wu & Zhu L. Yang, were recently described from China (Zhang et al. 2015, 2017; Wu et al. 2016b). Here, *Ch. radiatus* is redescribed based on new collections from subtropical and tropical China.

#### 4. *Chalciporus radiatus* Ming Zhang & T.H. Li, Mycoscience 57: 21, 2016

Figures 4k, l, 10

**Description.** *Basidiomata* small. *Pileus* 2.5–5 cm in diameter, subhemispherical to convex when young, then applanate; surface dry, pale yellowish brown, densely cov-

ered with pale yellowish-brown, yellowish-brown, brown to reddish-brown squamules; margin decurved; context 0.6–1 cm thick in the center of the pileus, yellowish, unchanging in color when injured. *Hymenophore* poroid, slightly decurrent; pores radially strongly elongated, yellow to pale yellowish brown, reddish with age, unchanging in color when injured; tubes 0.2–0.4 cm in length, yellowish, unchanging in color when injured. *Stipe* 2.5–4.5 × 0.5–1 cm, central, subcylindric, solid; surface dry, yellow, covered with yellowish brown, brown to reddish-brown squamules; context yellowish, unchanging in color when injured; annulus absent; basal mycelium yellow. *Odor* indistinct.

*Basidia* 23–34 × 7–10 μm, clavate, thin-walled, four-spored; sterigmata 5–6 μm in length. *Basidiospores* [101/5/4] 6–7(–8) × 3–4 μm, Q = (1.63–)1.71–2.14(–2.33), $Q_m$ = 1.91 ± 0.15, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid. *Cheilocystidia* 57–75 × 8–10 μm, abundant, subfusiform or fusiform, thin-walled, with pale yellowish-brown to yellowish-brown contents, without encrustations. *Pleurocystidia* 60–76 × 7–9 μm, abundant, fusiform or subfusiform, thin-walled, with pale yellowish-brown to yellowish-brown contents, without encrustations. *Pileipellis* a trichoderm 200–230 μm thick, composed of rather vertically arranged, sometimes slightly interwoven, pale yellowish-brown to yellowish-brown in KOH, thin-walled hyphae 4–10 μm in diameter; terminal cells 25–50 × 6–9 μm, narrowly clavate or subcylindrical, with obtuse apex. *Pileal trama* composed of thin- to slightly thick-walled (up to 0.5μm) hyphae 2–8 μm in diameter. *Stipitipellis* hymeniform composed of thin- walled hyphae with clavate, subclavate, subfusiform or fusiform terminal cells (13–80 × 5–9 μm). *Stipe trama* composed of cylindrical, thin- to slightly thick-walled (to 0.5 μm) parallel hyphae 5–11 μm in diameter. *Clamp connections* absent in all tissues.

**Habitat.** Solitary, scattered or gregarious on the ground in forests of *Pinus massoniana* Lamb. or *P. latteri* Mason.

**Distribution.** Central (Zhang et al. 2015), southeastern, and southern China.

**Specimens examined.** CHINA. Fujian Province: Zhangping County, Xinqiao Town, Chengkou Village, elev. 370 m, 4 August 2013, *N.K. Zeng 1379* (FHMU 930); same location, 17 August 2013, *N.K. Zeng 1414* (FHMU 959); same location, 16 August 2014, *N.K. Zeng 1633* (FHMU 2493). Hainan Province: Dongfang County, Exian Mountain, elev. 633 m, 5 October 2014, *N.K. Zeng 1808* (FHMU 2494).

**Notes.** Our molecular phylogenetic analyses indicate that the new collections and the holotype of *Ch. radiatus*, a species first described from Hunan Province of central China, group together with a strong statistical support based on a two-locus dataset (28S + *tef1*) (data not shown). This indicates that our specimens should be recognized as *Ch. radiatus* (Zhang et al. 2015). This species is new to Fujian and Hainan Province. Zhang et al. (2015) reported *Ch. radiatus* from under *Cunninghamia lanceolata* (Lamb.) Hook, *Cyclobalanopsis* spp. and *Castanopsis* spp. We found the species associated with *Pinus* spp.

### *Lanmaoa* G.Wu & Zhu L.Yang

*Lanmaoa*, typified by *L. asiatica* G. Wu & Zhu L. Yang, was erected recently. However, *Lanmaoa* and its closely related genus *Cyanoboletus* share overlapping morphological features and the most important diagnostic feature of *Lanmaoa* defined by Wu et al. (2016a) is not constant (Chai et al. 2018). Here, we treat *Lanmaoa* as an independent genus until the true taxonomic relationship between *Lanmaoa* and *Cyanoboletus* can be studied.

### 5. *Lanmaoa macrocarpa* N.K. Zeng, H. Chai & S. Jiang, sp. nov.

MycoBank: MB828523
Figures 5a–c, 11

**Typification.** CHINA. Hainan Province: Qiongzhong County, Yinggeling National Nature Reserve, elev. 750 m, 28 May 2017, *N.K. Zeng 3021* (FHMU 1982, holotype). GenBank accession numbers: 28S = MH879684, *tef1* = MH879713.

**Etymology.** Latin, "*macrocarpa*", meaning the new species has a large pileus.

**Description.** *Basidiomata* large. *Pileus* 10–13 cm in diameter, subhemispherical when young, then convex to applanate; surface dry, finely tomentose, brownish red (8B6–9B6); context about 2.5 cm thick in the center of the pileus, yellowish, changing blue quickly when injured. *Hymenophore* poroid, depressed around apex of stipe; pores subround to angular, 1–2 mm in diameter, yellow (3A5), changing blue quickly, then turning brown slowly when injured; tubes about 1.5 cm in length. *Stipe* 8–11 × 1.5–2 cm, central, subcylindric, solid; surface dry, brownish red (9C6), sometimes reticulate at apex; context yellow, changing blue quickly when injured; basal mycelium yellowish (2A4). *Odor* indistinct.

*Basidia* 18–28 × 6–10 μm, clavate, thin-walled, colorless to yellowish in KOH; four-spored, sterigmata 3–4 μm in length. *Basidiospores* [40/2/2] (9–)10–12(–13) × 4.5–5 μm, Q=(2.00–)2.10–2.60(–2.67), $Q_m$=2.39 ± 0.16, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid; composed of colorless to yellowish in KOH, 4.5–9 μm wide, thin- to slightly thick-walled (to 0.5 μm) hyphae. *Cheilocystidia* 25–42 × 7–10 μm, ventricose, fusiform or subfusiform, thin-walled, yellowish in KOH, no encrustations. *Pleurocystidia* 25–45 × 7–11 μm, fusiform or subfusiform, thin-walled, yellowish in KOH, no encrustations. *Pileipellis* a trichoderm 120–160 μm thick, composed of rather vertically arranged, nearly colorless in KOH, 4.5–6 μm wide, thin-walled hyphae; terminal cells 21–32 × 4–6 μm long, clavate or subclavate, with obtuse apex. *Pileal trama* made up of hyphae 3–10 μm in diameter, thin-walled, nearly colorless in KOH. *Stipitipellis* hymeniform about 100 μm thick, composed of thin- to slightly thick-walled (to 0.5 μm) emergent hyphae, colorless in KOH, with clavate, subclavate, fusiform, or subfusiform terminal cells (22–43 × 3–9 μm), and oc-


**Figure 5.** Basidiomata of boletes. **a–c** *Lanmaoa macrocarpa* (a from FHMU 2212; **b–c** from FHMU 1982, holotype) **d–f** *Neoboletus hainanensis* (HKAS 90209) **g–l** *Neoboletus multipunctatus* (**g, i–j, l** from FHMU 2808 **h, k** from FHMU 1620, holotype). Photos by N.K. Zeng.

casionally with scattered clavate, 4-spored basidia. *Stipe trama* composed of longitudinally arranged, parallel hyphae 3–8 μm wide, cylindrical, thin- to slightly thick-walled (to 0.5 μm), yellowish in KOH. *Clamp connections* absent in all tissues.

**Habitat.** Solitary on the ground in forests dominated by *Castanopsis kawakamii* Hay. or *C. fissa* (Champ. ex Benth.) Rehd. et Wils.

**Distribution.** Southeastern and southern China.

**Additional specimens examined.** CHINA. Fujian Province: Sanming City, Geshikao National Forest Park, elev. 400 m, 16 August 2017, *N.K. Zeng* 3251 (FHMU 2212).

**Note.** *Lanmaoa macrocarpa* is characterized by its large basidioma, brownish red pileus and stipe, thickness of hymenophore 3/5 times that of pileal context, and its

association with *Castanopsis* spp. It is both morphologically similar and phylogenetically related to Chinese *L. rubriceps* N.K. Zeng & Hui Chai (Chai et al. 2018) and one collection tentatively named "*Lanmaoa* sp. HKAS 52518" (Fig. 3). However, *L. rubriceps* has a red to crimson, orange-red pileus, pores stuffed when young, sometimes tinged with reddish when old, and smaller basidiospores measuring 8–11 × 4–5 µm (Chai et al. 2018); careful examinations showed that *Lanmaoa* sp. HKAS 52518 has a smaller basidioma, a reddish to red or blackish-red pileus, and surface of stipe turning blue when injured.

### *Neoboletus* Gelardi, Simonini & Vizzini

*Neoboletus*, typified by *N. luridiformis* (Rostk.) Gelardi et al., is characterized by stipitate-pileate or sequestrate; when basidiomata stipitate-pileate, pores brown, dark brown to reddish brown when young, becoming yellow when old (Fig. 6c, d, f), tubes always yellow (Figs 5f, l, 6e, h), hymenophore and context staining blue, and stipe usually covered with punctuations (Vizzini 2014; Wu et al. 2016a). The monophyly of *Neoboletus* has been assessed, and many species of the genus were described (Wu et al. 2014, 2016b). Astonishingly, the same authors recombined *Neoboletus* species in the genus *Sutorius* after a short time (Wu et al. 2016a). As a matter of fact, the stipe ornamentation pattern, spore print color, and colors of pores and tubes are fully different between the two genera (Halling et al. 2012; Vizzini 2014; Gelardi 2017). Furthermore, with more sequences added, our molecular data infers that *Neoboletus* forms an independent clade with strong support, and the genus *Sutorius* is sister to *Costatisporus* T.W. Henkel & M.E. Sm. (Smith et al. 2015) (Fig. 2). Thus, we recognize *Neoboletus* as an independent genus.

#### 6. *Neoboletus hainanensis* (T.H. Li & M. Zang) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.

MycoBank: MB828527
Figure 5d–f

*Boletus hainanensis* T.H. Li & M. Zang, Mycotaxon 80: 482, 2001
*Sutorius hainanensis* (T.H. Li & M. Zang) G. Wu & Zhu L. Yang, Fungal Diversity 81: 135, 2016

**Habitat.** Solitary on the ground in forests dominated by fagaceous trees including *Lithocarpus* spp.

**Distribution.** Southern and southwestern China.

**Note.** *Boletus hainanensis* T.H. Li & M. Zang was first described from Hainan Province of southern China (Zang et al. 2001). It was later also reported from Yunnan Province of southwestern China (Wu et al. 2016a) and was transferred to the genus

*Sutorius*. It is called the "Black bolete" in Yunnan Province, and largely traded in local mushroom markets (Wang et al. 2004).

**Specimens examined.** CHINA. Hainan Province: Changjiang County, Bawangling National Nature Reserve, elev. 650 m, 20 August 2009, *N.K. Zeng 523* (HKAS 90209). Yunnan Province: Kunming City, bought from market, 11 July 2015, *N.K. Zeng 2128* (FHMU 1392).

### 7. *Neoboletus multipunctatus* N.K. Zeng, H. Chai & S. Jiang, sp. nov.

MycoBank: MB828528
Figures 5g–l, 12

**Typification.** CHINA. Hainan Province: Qiongzhong County, Yinggeling National Nature Reserve, elev. 800 m, 3 August 2015, *N.K. Zeng* 2498 (FHMU 1620, holotype). GenBank accession numbers: 28S = MH879693, ITS = MH885354, *tef1* = MH879722.

**Etymology.** Latin, "*multipunctatus*", referring to the many punctuations on the stipe.

**Description.** *Basidiomata* medium-sized. *Pileus* 5.7–7 cm in diameter, convex to applanate; surface dry, finely tomentose, brown (4D7), dark brown (5C7) to blackish brown (5D5); context 1–1.5 cm thick in the center of the pileus, yellowish (1A5), changing blue quickly when injured. *Hymenophore* poroid, depressed around apex of stipe; pores subround, 0.3–0.4 mm in diameter, brown (7B5) to reddish brown (6C8), changing bluish black quickly when injured; tubes 0.5–0.7 cm in length, yellowish (1A5), changing blue quickly when injured. *Stipe* 7–7.4 × 1–1.3 cm, central, subcylindric, solid, usually flexuous; surface dry, covered with reddish-brown (7B5) squamules; context yellow (1A3), changing blue (21B3) quickly when injured; basal mycelium yellow (1A3). *Odor* indistinct.

*Basidia* 27–37 × 6–10 μm, clavate, thin-walled, colorless to yellowish in KOH; four-spored, sterigmata 5–6 μm in length. *Basidiospores* [80/4/3] 8.5–11(–12) × 4–5 μm, Q=(1.80–)1.90–2.50(–2.75), $Q_m$=2.22 ± 0.22, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid; composed of colorless to yellowish in KOH, 4–8 μm wide, thin-walled hyphae. *Cheilocystidia* 27–34 × 5–7 μm, fusiform or subfusiform, thin-walled, fawn to tawny in KOH, no encrustations. *Pleurocystidia* 38–61 × 6–8 μm, fusiform or subfusiform, thin-walled, colorless to tawny in KOH, no encrustations. *Pileipellis* a trichoderm about 120 μm thick, composed of vertically arranged, nearly colorless to yellowish in KOH, 3–5 μm wide, thin-walled hyphae; terminal cells 21–70 × 3–5 μm, clavate or subclavate, with obtuse apex. *Pileal trama* made up of hyphae 3–8 μm in diameter, thin-walled, colorless to yellowish in KOH. *Stipitipellis* hymeniform about 100 μm thick, composed of thin-walled emergent hyphae, colorless to yellowish in KOH, with clavate, subclavate, fusiform or subfusiform

terminal cells (25–44 × 3–9 μm), and occasionally with scattered clavate, 4-spored basidia. *Stipe trama* composed of longitudinally arranged, parallel hyphae 4–9 μm wide, cylindrical, thin to slightly thick-walled (to 0.5 μm), colorless in KOH. *Clamp connections* absent in all tissues.

**Habitat.** Solitary on the ground in forests dominated by fagaceous trees including *Lithocarpus* spp.

**Distribution.** Southern China.

**Additional specimens examined.** CHINA. Hainan Province: Changjiang County, Bawangling National Nature Reserve, elev. 600 m, 22 August 2009, *N.K. Zeng 559* (HKAS 76851); Ledong County, Yinggeling National Nature Reserve, elev. 620 m, 6 May 2018, *N.K. Zeng 3324* (FHMU 2808).

**Note.** *Neoboletus multipunctatus* is characterized by a brown, dark brown to blackish brown pileus, brown to reddish-brown pores changing bluish black when injured, stipe surface densely covered with brown to reddish-brown punctuations, smaller basidiospores, and its association with fagaceous trees. It is both morphologically similar and phylogenetically related to *N. brunneissimus* (W.F. Chiu) Gelardi et al. (Fig. 2), a species originally described from Yunnan Province of southwestern China. However, *N. brunneissimus* has larger basidiospores measuring 10–14 × 4.5–5 μm, and it occurs in temperature regions in addition to subtropical belts (Wu et al. 2016a). *Neoboletus multipunctatus* is also similar to *N. hainanensis* and *N. sinensis* (T.H. Li & M. Zang) Gelardi et al. morphologically. However, both pileal and stipe surface of *N. hainanensis* stain blue when injured, with white basal mycelium on the stipe, relatively larger basidiospores measuring 9.5–13.5 × 4–5 μm, and a trichodermium to ixotrichodermium pileipellis (Zang et al. 2001; Wu et al. 2016a). *Neoboletus sinensis*, a species also described from Hainan Province, has a cherry red stipe with reticulations, larger basidiospores measuring 13–19 × 5–6.5 μm, and wider cystidia (Zang et al. 2001; Vizzini 2014).

### 8. *Neoboletus obscureumbrinus* (Hongo) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.

MycoBank: MB828529
Figure 6a–e

*Boletus obscureumbrinus* Hongo, Mem. Fac. Lib. Arts. Educ. Shiga Univ. Nat. Sci., 18: 4, 1968
*Sutorius obscureumbrinus* (Hongo) G. Wu & Zhu L. Yang, Fungal Diversity 81: 138, 2016

**Habitat.** Solitary or gregarious on the ground in forests dominated by fagaceous trees including *Lithocarpus* spp.

**Distribution.** Southern and southwestern China; Japan (Hongo 1968).

**Note.** *Boletus obscureumbrinus* Hongo was originally described from Japan (Hongo 1968) and later reported from Guangdong Province of southern China and Yunnan Province of southwestern China (Wu et al. 2016a). It was transferred to the genus

*Sutorius* by Wu et al. (2016a); in the present study, we place the species in *Neoboletus* according to the evidence referred to above (Fig. 2). It is new to Hainan Province. The fruit body of this species is eaten by the Li people who live in the region (our own investigations).

**Specimens examined.** CHINA. Hainan Province: Ledong County, Yinggeling National Nature Reserve, elev. 620 m, 5 June 2017, *N.K. Zeng 3091*, *3094, 3098* (FHMU 2052, 2055, 2059); same location, 6 May 2018, *N.K. Zeng 3310, 3353* (FHMU 2271, 2814).

**9. *Neoboletus tomentulosus* (M. Zang, W.P. Liu & M.R. Hu) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.**
MycoBank: MB828530
Figure 6f–h

*Boletus tomentulosus* M. Zang, W.P. Liu & M.R. Hu, Acta Botanica Yunnanica 13: 150, 1991
*Sutorius tomentulosus* (M. Zang, W.P. Liu & M.R. Hu) G. Wu & Zhu L. Yang, Fungal Diversity 81: 142, 2016

**Habitat.** Solitary or gregarious on the ground in forests dominated by *Castanopsis kawakamii* Hay.

**Distribution.** Southeastern China.

**Note.** *Boletus tomentulosus* M. Zang et al. was first described from Fujian Province of southeastern China (Zang et al. 1991) and later reported from Guangdong Province of southern China (Wu et al. 2016a). Although the description of the protologue was brief (Zang et al. 1991), it has been well studied by Wu et al. (2016a). Our new collections were encountered near the type locality and augments our understanding of the species and the genus *Neoboletus*.

**Specimens examined.** CHINA. Fujian Province: Zhangping County, Xinqiao Town, Chengkou Village, elev. 350 m, 27 July 2013, *N.K. Zeng 1285, 1286* (FHMU 841, 842).

## *Sutorius* Halling, Nuhn & N.A. Fechner

*Sutorius*, typified by *S. eximius* (Peck) Halling et al., is mainly characterized by pores and tissues that are tinged with reddish at all growth stages, tissues not stained blue, a reddish-brown spore print, and transversely scissurate scales on stipe surface (Smith and Thiers 1971; Halling et al. 2012). Until now, only two taxa, *S. australiensis* (Bougher & Thiers) Halling and N.A. Fechner, and *S. eximius* (Peck) Halling et al., were described, excluding those in Wu et al (2016a). Herein, we describe another species new to science.


**Figure 6.** Basidiomata of boletes. **a–e** *Neoboletus obscureumbrinus* (**a, e** from FHMU 2271 **b, d** from FHMU 2055 **c** from FHMU 2814 ) **f–h** *Neoboletus tomentulosus* (**h–i** from FHMU 842, **j** from FHMU 841) **i–k** *Sutorius subrufus* (FHMU 2004, holotype) **l** *Tylopilus virescens* (FHMU 1004). Photos by N.K. Zeng.

### 10. *Sutorius subrufus* N.K. Zeng, H. Chai & S. Jiang, sp. nov.

MycoBank: MB828531
Figures 6i–k, 13

**Typification.** CHINA. Hainan Province: Qiongzhong County, Yinggeling National Nature Reserve, elev. 850 m, 29 May 2017, *N.K. Zeng 3043* (FHMU 2004, holotype).

GenBank accession numbers: 28S = MH879698, ITS = MH885360, *tef1* = MH879728, *rpb2* = MH879745.

**Etymology.** Latin, "*subrufus*" refers to the stipe surface and context of the species turning reddish when injured.

**Description.** *Basidiomata* medium to large. *Pileus* 5–10 cm in diameter, subhemispherical to convex when young, then applanate; surface dry, finely tomentose, brown to pale reddish brown (10C2–11C3); context about 1.6 cm thick in the center of the pileus, white (6A1), changing reddish (9C3) when injured. *Hymenophore* poroid, adnate or slightly depressed around apex of stipe; pores angular, about 0.3 mm in diameter, pale brown (8C3), brown (7E2) to pale reddish brown (10C2), mostly unchanging in color when injured, but sometimes changing reddish; tubes about 1 cm in length, pale brown (8D3), unchanging in color when injured, but sometimes changing reddish. *Stipe* 6–10 × 1–2.2 cm, central, subcylindric, solid; surface dry, gray-white, but brownish yellow at base, covered with pale reddish-brown (7B2) to blackish-brown squamules, usually changing reddish when injured; context white (1D1–2), changing reddish (9C3) when injured; annulus absent; basal mycelium white (1A1). *Odor* indistinct.

*Basidia* 18–30 × 6–9 μm, clavate, thin-walled, colorless to yellowish in KOH; four-spored, sterigmata 2–3 μm in length. *Basidiospores* [200/24/3] (8–)9–12(–13.5) × 3.5–4.5 μm, Q=(2.25–)2.50–3.00(–3.29), $Q_m$=2.79 ± 0.21, subfusoid and inequilateral in side view with a weak or distinct suprahilar depression, elliptic-fusiform to subfusiform in ventral view, slightly thick-walled (to 0.5 μm), olive-brown to yellowish brown in KOH, smooth. *Hymenophoral trama* boletoid; composed of colorless to yellowish in KOH, 5–10 μm wide, thin- to slightly thick-walled (up to 0.5 μm) hyphae. *Cheilocystidia* 28–45 × 7–10 μm, ventricose, fusiform or subfusiform, thin-walled, colorless to yellowish in KOH, no encrustations. *Pleurocystidia* 35–50 × 7–10 μm, fusiform or subfusiform, thin-walled, colorless to yellowish in KOH, no encrustations. *Pileipellis* a trichoderm about 100–150 μm thick, composed of rather vertically arranged, yellowish in KOH, 3.5–6 μm wide, thin-walled hyphae; terminal cells 30–43 × 3.5–6 μm, clavate or subclavate, with obtuse apex. *Pileal trama* made up of hyphae 4.5–10 μm in diameter, thin-walled, nearly colorless in KOH. *Stipitipellis* hymeniform about 60–80 μm thick, composed of thin-walled emergent hyphae, colorless in KOH, with clavate, subclavate terminal cells (22–28 × 4–9 μm), and occasionally with scattered clavate, four-spored basidia. *Stipe trama* composed of longitudinally arranged, parallel hyphae 4–8 μm wide, cylindrical, thin- to slightly thick-walled (to 0.5 μm), fawn to tawny in KOH, parallel hyphae. *Clamp connections* absent in all tissues.

**Habitat.** Scattered, gregarious or caespitose on the ground in forests dominated by fagaceous trees, including *Lithocarpus* spp.

**Distribution.** Southern China.

**Additional specimens examined.** CHINA. Hainan Province: Qiongzhong County, Yinggeling National Nature Reserve, elev. 860 m, 29 May 2017, *N.K. Zeng 3045* (FHMU 2006); Ledong County, Yinggeling National Nature Reserve, elev. 650 m, 27 July 2017, *N.K. Zeng* 3140 (FHMU 2101).

**Note.** *Sutorius subrufus* is characterized by a brown to pale reddish-brown pileus, stipe surface and context turning reddish when injured, relatively smaller basidi-

ospores, and it is restricted in tropical China. It is both morphologically similar and phylogenetically related to *S. eximius* (Peck) Halling et al. and *S. australiensis* (Bougher & Thiers) Halling and N.A. Fechner. However, stipe surface and context of *S. eximius* does not change when injured. Moreover, *S. eximius* has larger basidiospores, and a distribution in North and Central America (Singer 1947; Smith and Thiers 1971; Halling et al. 2012); *S. australiensis* has relatively larger basidiospores, a distribution in Australia, and is associated with Myrtaceae and Casuarinaceae (Halling et al. 2012).

### *Tylopilus* P. Karst.

*Tylopilus*, typified by *T. felleus* (Bull.) P. Karst., is characterized by the pallid, pinkish, vinaceous and pinkish-brown hymenophore, white to pallid context without color change, but some species becoming rufescent or sea-green when injured, and the bitter taste of the context (Baroni and Both 1998; Henkel 1999; Fulgenzi et al. 2007; Osmundson and Halling 2010; Wu et al. 2016a; Magnago et al. 2017; Liang et al. 2018). In China, although lots of species of the genus have been previously discovered (Li et al. 2002; Fu et al. 2006; Gelardi et al. 2015; Wu et al. 2016a; Liang et al. 2018), still there are a large number of undescribed taxa in this region.

#### 11. *Tylopilus virescens* (Har. Takah. & Taneyama) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.

MycoBank: MB828532
Figure 6l

*Boletus virescens* Har. Takah. & Taneyama, The fungal flora in southwestern Japan, agarics and boletes 1: 45, 2016
*Tylopilus callainus* N.K. Zeng, Zhi Q. Liang & M.S. Su, Phytotaxa 343 (3): 271, 2018

**Habitat.** Solitary or gregarious on the ground in forests dominated by fagaceous trees including *Lithocarpus* spp. or *Castanopsis kawakamii* Hay.

**Distribution.** Southeastern and southern China; Japan (Terashima et al. 2016).

**Note.** *Tylopilus callainus* N.K. Zeng et al. was described from the south of China (Liang et al. 2018). This taxon was previously thought to be different from *B. virescens* Har. Takah. & Taneyama, a species described from Japan (Terashima et al. 2016). After a careful re-evaluation of specimens, we now know that the two taxa are conspecific, and *T. callainus* is synonymized with *B. virescens*. Clarifying the taxonomic relationship between the two taxa also indicated that the *B. virescens* is a member of *Tylopilus*, and thus the new combination is proposed. Illustrations and a full description have been provided by Liang et al. (2018).

**Specimens examined.** CHINA. Fujian Province: Zhangping County, Xinqiao Town, Chengkou Village, elev. 350 m, 22 August 2013, *N.K. Zeng 1360, 1459* (FHMU

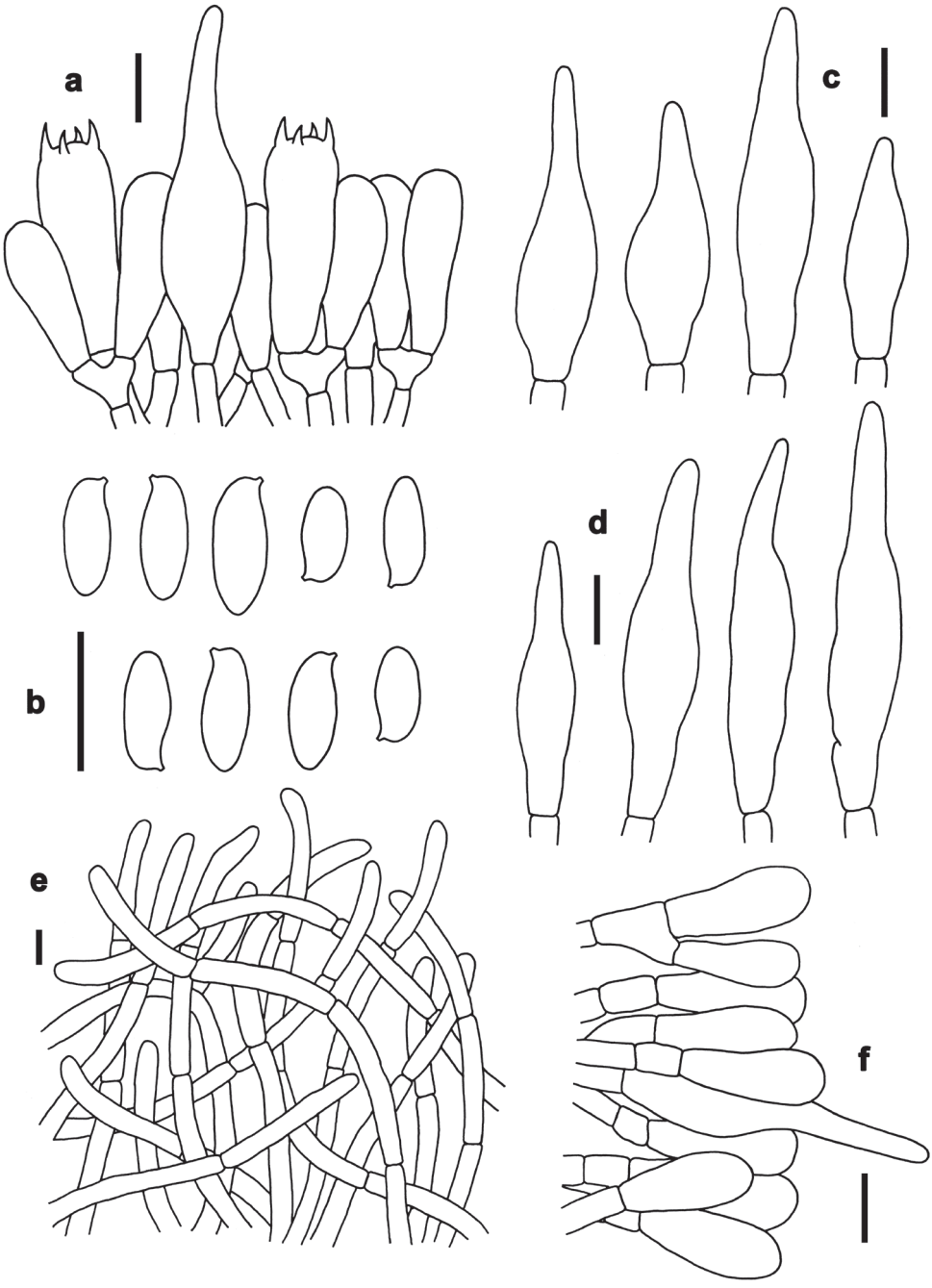


**Figure 7.** Microscopic features of *Butyriboletus huangnianlaii* (FHMU 2207, holotype). **a** Basidia and pleurocystidium **b** Basidiospores **c** Cheilocystidia **d** Pleurocystidia **e** Pileipellis **f** Stipitipellis. Scale bars: 10 μm.

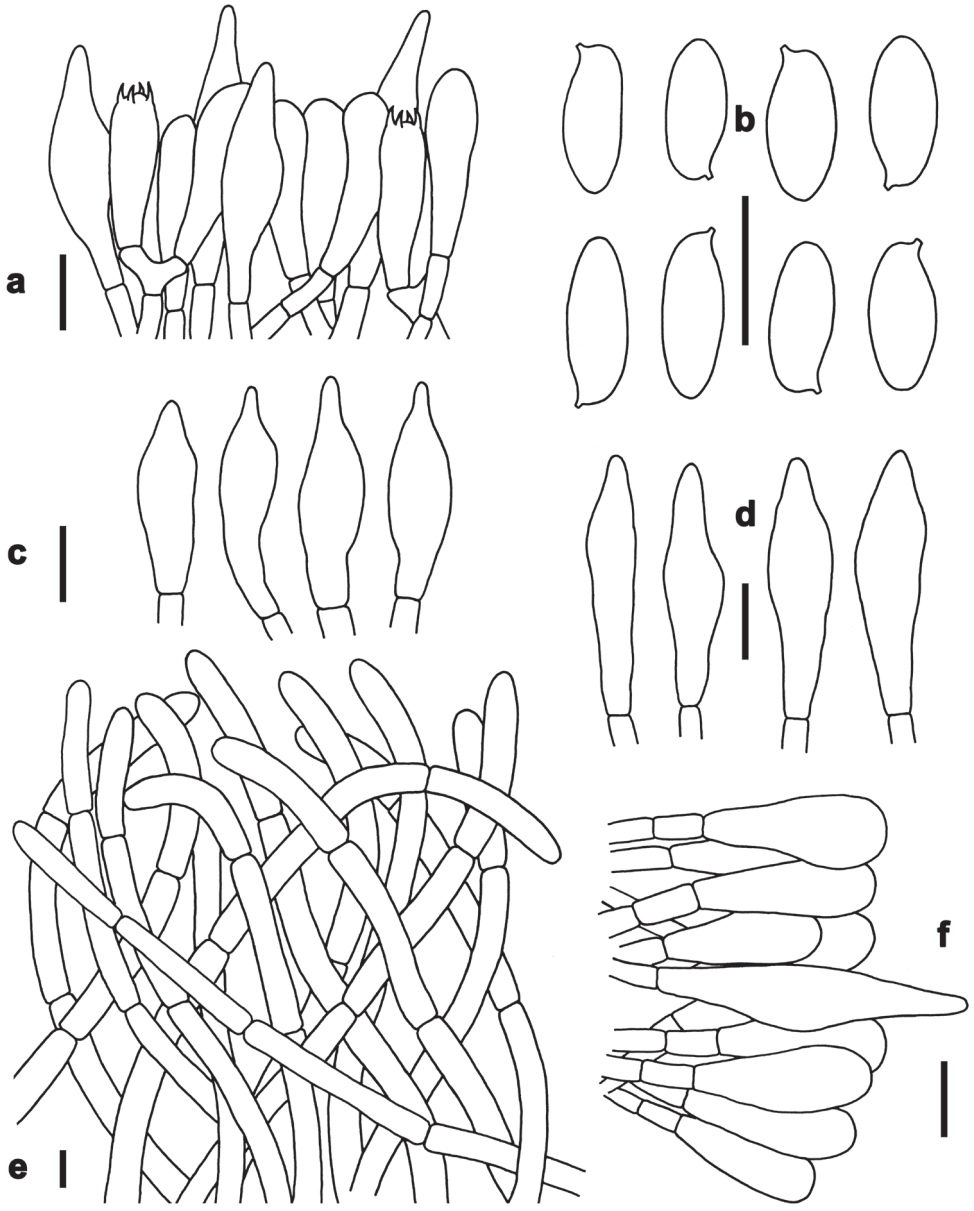


**Figure 8.** Microscopic features of *Caloboletus guanyui* (FHMU 2040). **a** Basidia and pleurocystidia **b** Basidiospores **c** Cheilocystidia **d** Pleurocystidia **e** Pileipellis **f** Stipitipellis. Scale bars: 10 μm.

2812, 1001); same location, 23 August 2013, *N.K. Zeng 1460* (FHMU 2813); same location, 24 August 2013, *N.K. Zeng 1464* (FHMU 1004). Hainan Province: Baisha County, Yinggeling National Nature Reserve, elev. 550 m, 1 August 2015, *N.K. Zeng 2436* (FHMU 1562); same location, 26 May 2017, *N.K. Zeng 2982* (FHMU 1943);

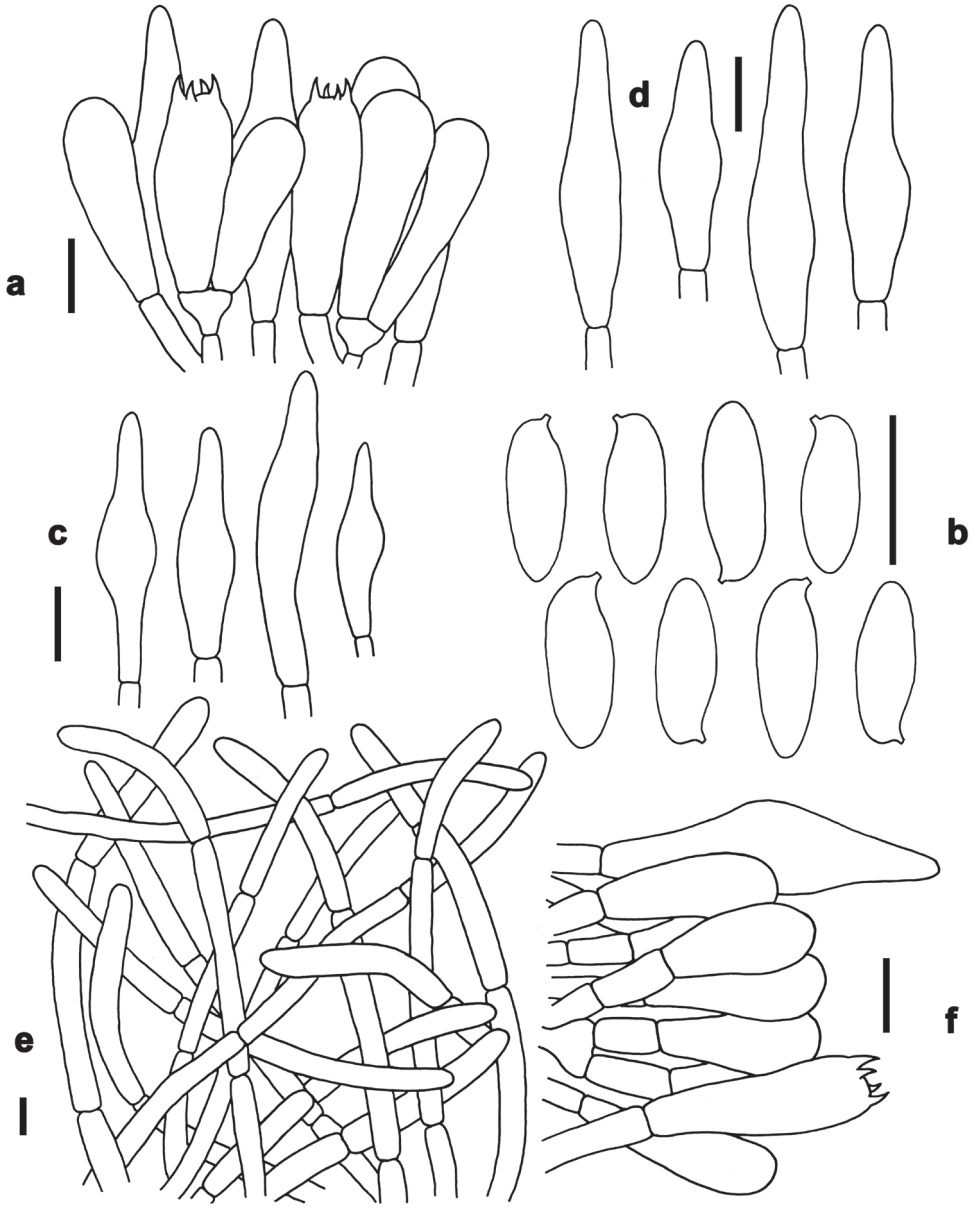


**Figure 9.** Microscopic features of *Caloboletus xiangtoushanensis* (FHMU 883). **a** Basidia and pleurocystidia **b** Basidiospores **c** Cheilocystidia **d** Pleurocystidia **e** Pileipellis **f** Stipitipellis. Scale bars: 10 μm.

same location, 27 May 2017, *N.K. Zeng 3001* (FHMU 1962); Ledong County, Jianfengling National Nature Reserve, elev. 850 m, 27 June 2018, *N.K. Zeng 3426, 3431* (FHMU 2810, 2811).

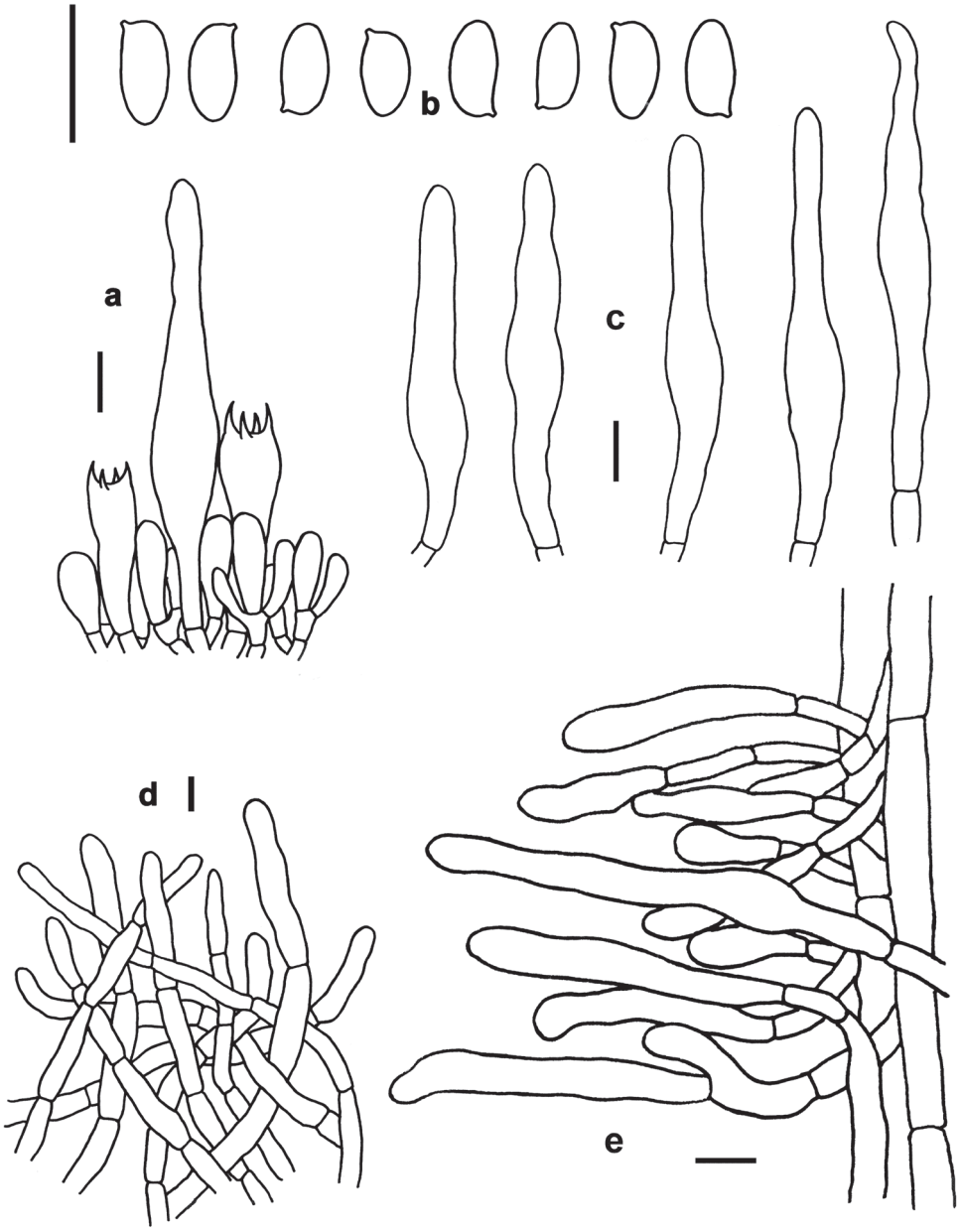


**Figure 10.** Microscopic features of *Chalciporus radiatus* (FHMU 930). **a** Basidia and pleurocystidium **b** Basidiospores **c** Cheilocystidia **d** Pileipellis **e** Stipitipellis. Scale bars: 10 μm.

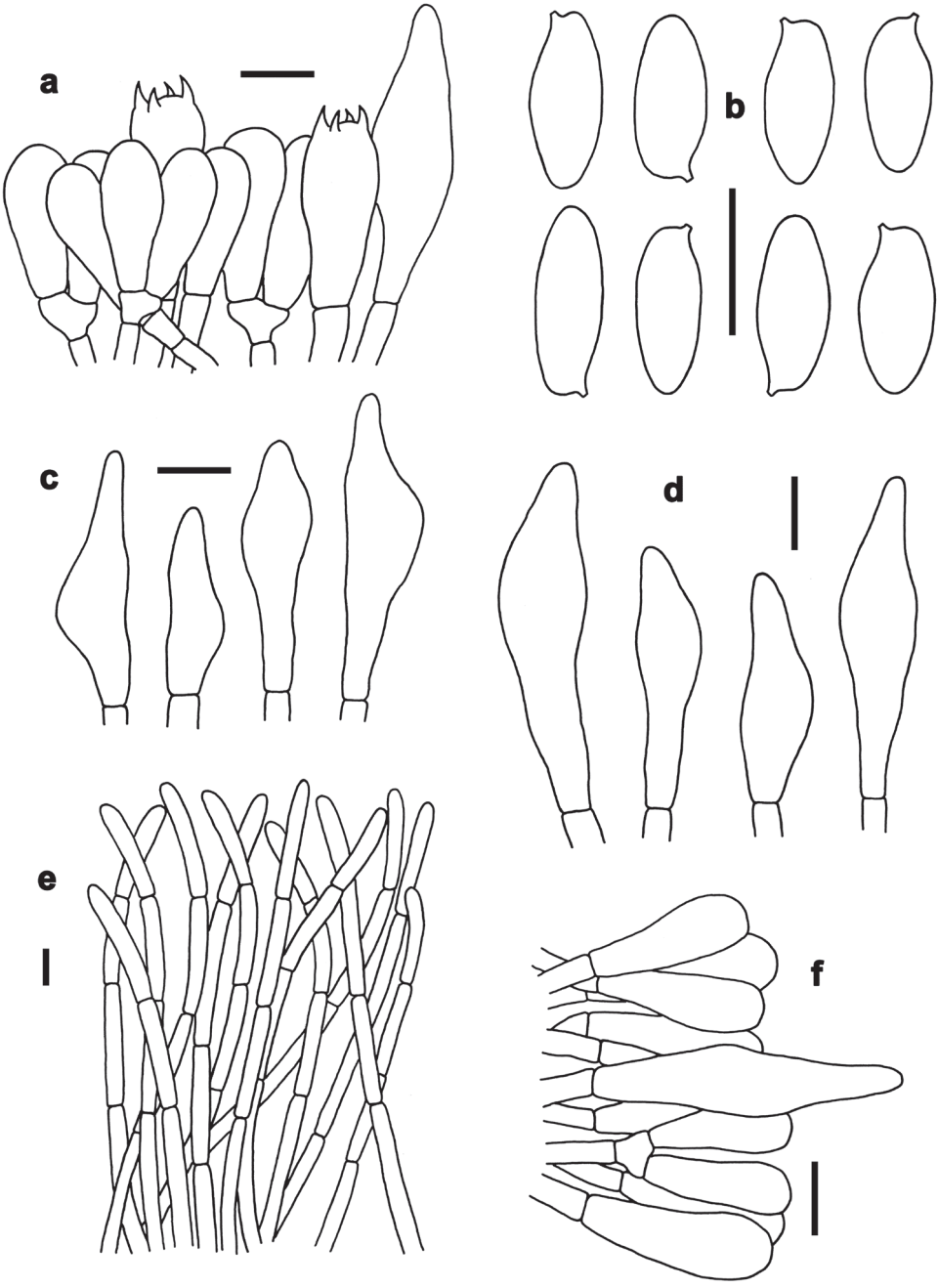


**Figure 11.** Microscopic features of *Lanmaoa macrocarpa* (**a–e** from FHMU 1982, holotype **f** from FHMU 2212). **a** Basidia and pleurocystidium **b** Basidiospores **c** Cheilocystidia **d** Pleurocystidia **e** Pileipellis **f** Stipitipellis. Scale bars: 10 μm.

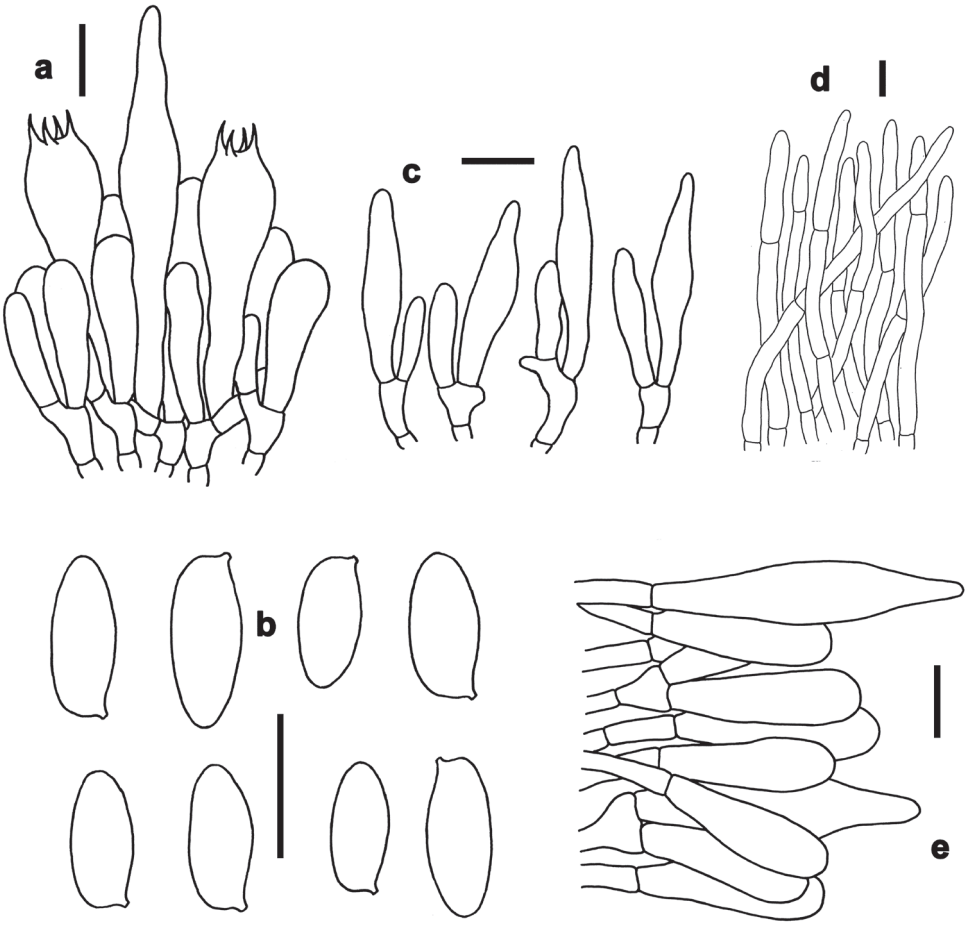


**Figure 12.** Microscopic features of *Neoboletus multipunctatus* (FHMU 1620, holotype). **a** Basidia and pleurocystidium **b** Basidiospores **c** Cheilocystidia **d** Pileipellis **e** Stipitipellis. Scale bars: 10 μm.

## New combinations

According to the analytical results presented here, the following new combinations are proposed:

***Neoboletus ferrugineus*** **(G. Wu, F. Li & Zhu L. Yang) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.**
MycoBank: MB828533

*Sutorius ferrugineus* G. Wu, Fang Li & Zhu L. Yang, Fungal Diversity 81: 134, 2016

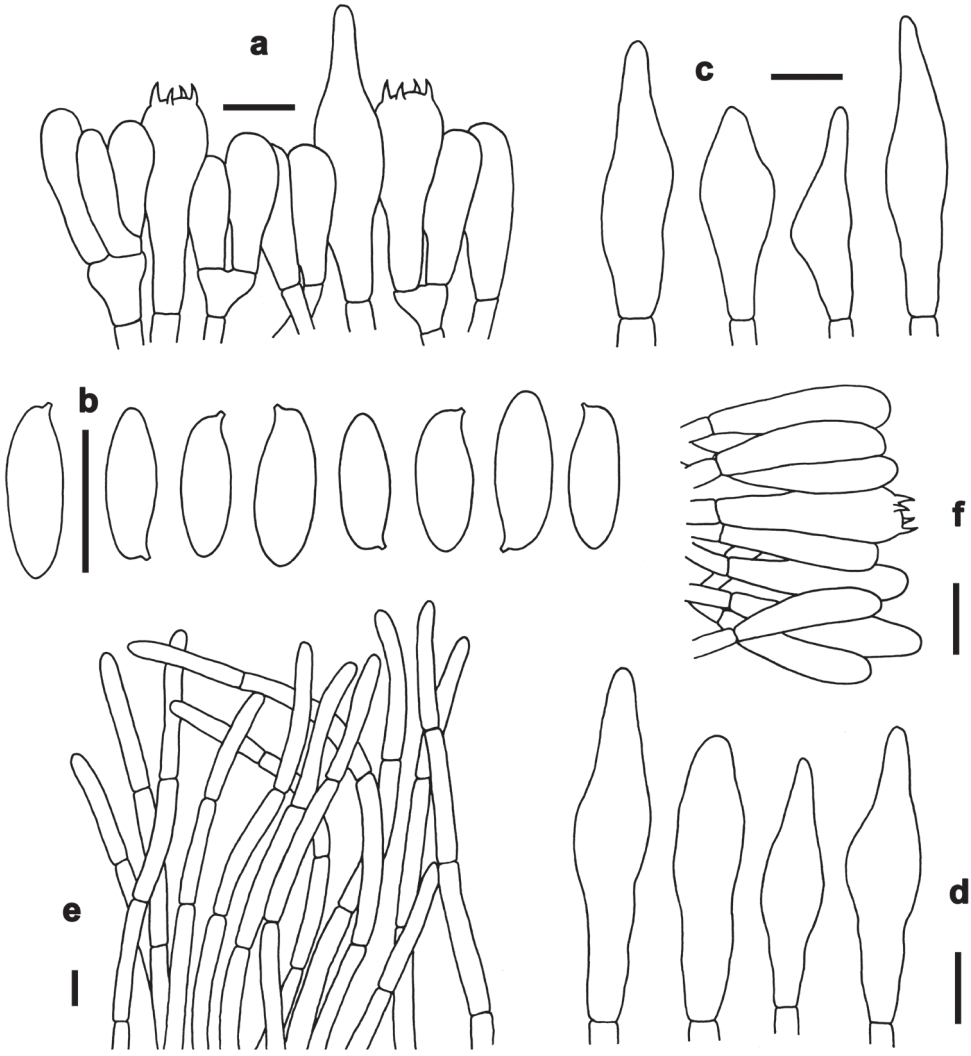


**Figure 13.** Microscopic features of *Sutorius subrufus* (FHMU 2004, holotype). **a** Basidia and pleurocystidium **b** Basidiospores **c** Cheilocystidia **d** Pleurocystidia **e** Pileipellis **f** Stipitipellis. Scale bars: 10 μm.

***Neoboletus flavidus*** **(G. Wu & Zhu L. Yang) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.**
MycoBank: MB828534

*Sutorius flavidus* G. Wu & Zhu L. Yang, Fungal Diversity 81: 135, 2016

***Neoboletus rubriporus*** **(G. Wu & Zhu L. Yang) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.**
MycoBank: MB828535

*Sutorius rubriporus* G. Wu & Zhu L. Yang, Fungal Diversity 81: 139, 2016

***Neoboletus sanguineoides*** **(G. Wu & Zhu L. Yang) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.**
MycoBank: MB828536

*Sutorius sanguineoides* G. Wu & Zhu L. Yang, Fungal Diversity 81: 140, 2016

***Neoboletus sanguineus*** **(G. Wu & Zhu L. Yang) N.K. Zeng, H. Chai & Zhi Q. Liang, comb. nov.**
MycoBank: MB828537

*Sutorius sanguineus* G. Wu & Zhu L. Yang, Fungal Diversity 81: 141, 2016

## Discussion

Molecular phylogenetic analyses have been used widely to define the genera of boletes, and as a result, many genera were erected or merged (Zeng et al. 2012, 2014b; Nuhn et al. 2013; Wu et al. 2014, 2016a, b). Recently, the genus *Neoboletus* was synonymized with *Sutorius* solely based on the evidence of molecular data (Wu et al. 2016a). Our molecular phylogenetic analyses based on a four-locus dataset (28S + ITS + *tef1* + *rpb2*) with sequences from taxa of *Neoboletus*, *Sutorius*, *Costatisporus*, and *Caloboletus* (Fig. 2) indicate those species that morphologically match the concept of genus *Neoboletus* do not belong in *Sutorius*; instead, they form an independent clade with strong support (Fig. 2). At the same time, the morphological features including the stipe ornamentation pattern, spore print color, and color change of tissues are different between the two genera and has been noted in previous studies (Halling et al. 2012; Gelardi 2017). It is noteworthy that the color of tubes of *Neoboletus* is always yellow (Figs 5f, l, 6e, h), and in this genus the pores usually become yellow when old (Fig. 6d, f), whereas the color of tubes and pores of *Sutorius* are always tinged with reddish at different growth stages (Fig. 6i–k).

The present study further shows that the most important diagnostic feature of the genus *Lanmaoa*, viz. "short hymenophoral tubes (thickness of hymenophore 1/3–1/5 times that of pileal context at the position halfway to the pileus center) and a slow color change when injured" defined by Wu et al. (2016b) is not constant (Chai et al. 2018), for the thickness of hymenophore is about 3/5 times that of pileal context in our newly described *L. macrocarpa*. Additionally, context and hymenophore of our new species turn quickly and strongly when injured (Fig. 5c).

According to current molecular data, 10 lineages (lineages 1–10) of *Sutorius* were found (Fig. 2). Lineages 4 and 6 were identified as *S. australiensis* and *S. eximius* respectively in a previous study (Halling et al. 2012). Lineages 1, 2, 3, 5, 7 and 9 may have not diverged enough (Fig. 2) and are treated here as a series of closely related taxa or disjunct populations of previously described entities; these will be assessed in the future with more DNA sequences and more collections. As to lineages 8 and 10, they should be treated as independent taxa due to their high degree divergence. Moreover, morphological and ecological features (described above) of specimens (FHMU 2004, FHMU 2006, FHMU 2101) in lineage 8 from Hainan Province are also different from the described taxa of *Sutorius*, and thus, the new taxon *S. subrufus* was proposed. Lineage 10 was not described due to the paucity of the materials (Halling et al. 2012).

Subtropical and tropical China is believed to be a biodiversity hotspot. Mycologists have paid much attention to boletes of the region in the past decade, and many taxa have been discovered (Bi et al. 1997; Zeng and Yang 2011; Zeng et al. 2012, 2013, 2014a, b, 2015a, b, 2016, 2017, 2018; Zang 2013; Liang et al.2016, 2017, 2018; Chai et al. 2018; Xue et al. 2018). Among of them, many have been found to be as North American or European species (Bi et al. 1997; Zang 2013), and recent studies have shown that species shared between subtropical/tropical China and North America/Europe are rare but that there are many common species between Japan and subtropical/tropical China (Zeng et al. 2013, 2016, 2017). Our study now reveals that the geographic distributions of the Japanese *C. guanyui*, *N. obscureumbrinus*, and *T. virescens* extend into subtropical or tropical China.

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# More pieces to a huge puzzle: Two new *Escovopsis* species from fungus gardens of attine ants

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## Abstract

*Escovopsis* (Ascomycota: Hypocreales, Hypocreaceae) is the only known parasite of the mutualistic fungi cultivated by fungus-growing ants (Formicidae: Myrmicinae: Attini: Attina, the "attines"). Despite its ecological role, the taxonomy and systematics of *Escovopsis* have been poorly addressed. Here, based on morphological and phylogenetic analyses with three molecular markers (internal transcribed spacer, large subunit ribosomal RNA and the translation elongation factor 1-alpha), we describe *Escovopsis clavatus* and *E. multiformis* as new species isolated from fungus gardens of *Apterostigma* ant species. Our analysis shows that *E. clavatus* and *E. multiformis* belong to the most derived *Escovopsis* clade, whose main character is the presence of conidiophores with vesicles. Nevertheless, the most outstanding feature of both new species is the presence of a swollen region in the central hypha of the conidiophore named swollen cell, which is absent in all previously described *Escovopsis* species. The less derived *Escovopsis* clades lack vesicles and their phylogenetic position within the Hypocreaceae still remains unclear. Considering the high genetic diversity in *Escovopsis*, the description of these new species adds barely two pieces to a huge taxonomic puzzle; however, this discovery is an important piece for building the systematics of this group of fungi.

## Keywords

Hypocreales, Taxonomy, Phylogeny, Parasitic fungi, Symbiosis

## Introduction

Microorganisms play important roles in the stability of social insect colonies (Hughes et al. 2008, Joop and Vilcinskas 2016, Vanderpool et al. 2018). The environment of these insects has a high potential to harbour unique fungal species (Attili-Angelis et al. 2014, Harrington et al. 2014, Menezes et al. 2015, Montoya et al. 2016). The evolutionary success of the fungus garden of the fungus-farming ants (Formicidae: Myrmicinae: Attini: Attina, the "attines") depends on complex symbiotic interactions amongst bacteria, fungi and the ants (Currie et al. 2003, Gerardo et al. 2006a, Kost et al. 2007). The association between attine ants and their mutualistic fungi (Basidiomycota: Agaricales) is the core of the attine colonies; however, *Escovopsis* (Ascomycota: Hypocreales: Hypocreaceae) can exploit this association. Although no specialised parasitic structures were found, studies showed that this parasite is able to kill the fungal cultivar as well as the ants and their mutualistic bacteria by chemical compounds (Currie 2001, Varanda-Haifig et al. 2017, Dhodary et al. 2018, Heine et al. 2018, Custodio and Rodrigues 2019). Despite the ecological relevance of *Escovopsis* as parasites of attine ant colonies, the taxonomy of this genus has been neglected.

Attine ants are classified in two sister clades: the Palaeoattina and Neoattina (Branstetter et al. 2017). Leafcutter ants (*Atta* and *Acromyrmex*) are considered the most derived attines within the Neoattina. Their behaviour is characterised by collecting fresh leaves and flowers to feed several cultivars from two clades of fungi in the Agaricaceae (Mueller et al. 2017, 2018). On the other hand, non-leafcutter ants also occur in both the Neoattina and Palaeoattina clades. Distinct from *Atta* and *Acromyrmex*, non-leafcutter ants collect seeds, insect frass and dry leaves to nourish a wide range of fungal cultivars in the Agaricaceae and Pterulaceae (Villesen et al. 2004, Schultz and Brady 2008).

The attine ant-fungus cultivar-*Escovopsis* symbiosis has been widely studied in leafcutter ants (Mueller and Gerardo 2002, Currie et al. 2003, Gerardo et al. 2004, 2006a,b). In addition to their contributions on the biology of *Escovopsis*, these studies also revealed considerable diversity of the parasite. Considering the variety of mutualistic fungi that non-leafcutter ants may cultivate, as well as the different substrates used for that purpose, a high diversity of *Escovopsis* species is unsurprising. This is especially true for *Apterostigma* (Gerardo et al. 2006b), a genus of non-leafcutter attine with species that cultivate different cultivars including *Leucoagaricus gongylophorus*, the domesticated fungus cultivated by many higher attine ant species, mostly leafcutter ants (Sosa-Calvo and Schultz 2010, Schultz et al. 2015, Ješovnik et al. 2016, Sosa-Calvo et al. 2017, Mueller et al. 2017, 2018).

While *Escovopsis* species exploiting gardens of *Atta*, *Acromyrmex*, *Trachymyrmex* and *Mycetophylax* were formally described, the morphological characters of the species associated with *Apterostigma* are unknown. A previous study associated clades of the parasite with the colour pattern of *Escovopsis* colonies (brown, yellow, white and pink; Gerardo et al. 2006b). However, no taxonomic studies were undertaken to formally describe these clades. Here, we describe *Escovopsis clavatus* and *E. multiformis* as new species isolated from the fungus garden of *Apterostigma*. The distinctive feature of

these lineages is the presence of swollen cells at the base of the conidiophore branches. This phenotype differentiates these two new species from previously described *Escovopsis*. Considering that previous studies showed a high genetic diversity within *Escovopsis*, the description of these species adds two pieces to the enormous taxonomic puzzle which is *Escovopsis*.

## Material and methods

### Sampling sites and *Escovopsis* isolation

Five *Escovopsis* isolates were obtained from fungus gardens of five different colonies of *Apterostigma* spp. (Suppl. material 1: Table S1). The isolates LESF 847, LESF 853, LESF 854 and LESF 855 were obtained from colonies found in the Atlantic Rain Forest in Florianópolis, State of Santa Catarina, Brazil (October 2015). The isolate LESF 1136 was obtained from a colony found in the Amazon Forest in Cotriguaçu, State of Mato Grosso, Brazil (October 2017). The nests were found inside or under rotten logs. Fungus gardens, along with tending workers and brood, were collected in UV-sterilised plastic containers using sterilised spoon and forceps. Samples were taken to the Laboratory of Fungal Ecology and Systematics (LESF) at the UNESP – São Paulo State University, Rio Claro, Brazil.

For fungal isolation, seven garden fragments (0.5–1 $mm^3$) were inoculated on plates (three plates per colony) containing potato dextrose agar (PDA, Neogen Culture Media, Neogen) supplemented with chloramphenicol (150 μg $mL^{-1}$, Sigma) and incubated at 25 °C in darkness. Plates were monitored daily for fungal growth and, when *Escovopsis* mycelia sprouted, they were transferred to new PDA plates. All isolates were prepared as axenic (monosporic) cultures and stored under sterile distilled water kept at 8 °C (Castellani 1963) and at –80 °C (as conidia suspensions in 10% glycerol).

### Morphological analysis

The morphological characters of the five isolates (LESF 847, LESF 853, LESF 854, LESF 855 and LESF 1136) were examined. Due to the lack of standardisation of culture conditions for *Escovopsis*, the macroscopic characters of the colonies, i.e. radial growth, mycelium colour, morphology and presence of soluble pigments, were evaluated on eight different media: PDA, malt agar 2% [MA2%: 20 g $L^{-1}$ of malt extract (Neogen Culture Media) and 15 g $L^{-1}$ of agar (Neogen Culture Media)], cornmeal agar (CMD, Neogen Culture Media), synthetic nutrient agar [SNA: 1 g $L^{-1}$ of $KH_2PO_4$ (Labsynth), 1 g $L^{-1}$ of $KNO_3$ (Labsynth), 0.5 g $L^{-1}$ of $MgSO_4(7H_2O)$ (Labsynth), 0.5 g $L^{-1}$ of KCl (Labsynth), 0.2 g $L^{-1}$ of Glucose (Labsynth), 0.2 g $L^{-1}$ of Sucrose (Labsynth) and 15 g $L^{-1}$ of Agar (Neogen Culture Media)], oatmeal agar (OA), potato carrot agar (PCA, HiMedia), malt extract agar 2% [MEA: 30 g $L^{-1}$ of malt extract (Neogen Cul-

ture Media), 5 g $L^{-1}$ of bacteriological peptone (Neogen Culture Media), 20 g $L^{-1}$ of glucose (Labsynth) and 15 g $L^{-1}$of Agar (Neogen Culture Media)] and Czapek yeast extract agar [CYA; 30 g $L^{-1}$ of Sucrose (Labsynth), 5 g $L^{-1}$ of Yeast extract (Neogen Culture Media), 1 g $L^{-1}$ of $KH_2PO_4$ (Labsynth), 0.3 g $L^{-1}$ of $NaNO_3$ (Synth), 0.05 g $L^{-1}$ of KCl (Labsynth), 0.05 g $L^{-1}$ of $MgSO_4(7H_2O)$ (Labsynth), 0.001 g $L^{-1}$ of $FeSO_4$ (Labsynth), 0.001 g $L^{-1}$ of $ZnSO_4$ (Labsynth), 0.0005 g $L^{-1}$ of $CuSO_4$ (Labsynth), 15 g $L^{-1}$ of Agar (Neogen Culture Media)] at five temperatures (10 °C, 20 °C, 25 °C, 30 °C and 35 °C). These temperatures correspond to the conditions used in previous studies that described *Escovopsis* species (Seifert et al. 1995, Augustin et al. 2013, Masiulionis et al. 2015, Meirelles et al. 2015a). For this purpose, 200 µl of conidia were spread on plates with water-agar (WA) and incubated for seven days at 25 °C in darkness. Then, mycelium fragments of 0.5 cm diameter were cut from the WA plates and inoculated in the centre of the plates (90 × 15 mm) containing the eight culture media. All the strains examined showed better development in the dark and with unsealed Petri dishes to allow air passage; therefore, incubation was carried out in the darkness and without sealing the plates, for 14 days. Three replicate plates were inoculated for each media and for each incubation temperature.

To examine the microscopic characters, i.e. the morphology, size, branching patterns, vesicles and swollen cells of the conidiophores, as well as phialides and conidia, slide cultures on PDA and MEA were performed. Briefly, we placed a 5 $mm^2$ fragment of culture medium on a microscopic slide and then we inoculated the fungus at the centre of the fragment. Then, the inoculated medium was covered with a coverslip and incubated at 25 °C for 4–7 days in the dark. After that, the coverslips, where the fungus grew, were removed and placed in new slides with a drop of lactophenol. Finally, the slides were examined under a light microscope (DM750, Leica, Germany). Fungal microscopic structures were photographed and measured (with 30 measurements per structure) in LAS EZ v.4.0 (Leica Application Suite).

Microscopic structures were also examined under scanning electron microscopy (SEM). Fungal samples (five days old cultures on PDA) were fixed in osmium tetroxide vapour for 72 h. Then, samples were dehydrated using a series of acetone concentrations (50, 75, 90, 95 and 100%) and dried to critical point using liquid $CO_2$ (Balzers CPD030). The dried material was sputtered with gold (Balzers SCD050) and examined under the scanning electron microscope (TM3000, Hitachi).

## DNA extraction, PCR and sequencing

DNA extraction of the five strains was performed, following the steps published in Meirelles et al. (2015a). Three molecular markers were amplified: the internal transcribed spacer (ITS) region (White et al. 1990, Schoch et al. 2012); translation elongation factor 1-alpha (*tef*1) (Taerum et al. 2007); and the large subunit ribosomal RNA (LSU) (White et al. 1990, Haugland and Heckman 1998, Currie et al. 2003) (Suppl. material 1: Table S2).

PCR and sequence reaction conditions followed the steps published in Meirelles et al. (2015b) for the ITS region, Meirelles et al. (2015a) for *tef*1 and Augustin et al. (2013) for LSU. The final amplicons were cleaned up with Wizard SV Gel and PCR Clean-up System kit (Promega), following the manufacturer's protocol. Sequences (forward and reverse) were generated in ABI3500 (Life Technologies). The LSU of 29 strains previously used in Meirelles et al. (2015b) was also amplified and sequenced for this study (Suppl. material 1: Table S1). The sequences were assembled in contigs in BioEdit v. 7.1.3 (Hall 1999) and deposited in GenBank (Suppl. material 1: Table S1 for accession numbers).

## Phylogenetic analyses

To infer the phylogenetic position of the new species in the *Escovopsis* clade, sequences from previous studies were retrieved from the GenBank and aligned with our new sequences in a dataset for each marker (Chaverri et al. 2003, Spatafora et al. 2007, Jaklitsch et al. 2011, Põldmaa 2011, Meirelles et al. 2015b). This data included sequences from the seven *Escovopsis* ex-type strains, from *Escovopsioides nivea* and some species from *Hypomyces* and *Trichoderma*, as the phylogenetic closest relatives of *Escovopsis*. First, the three datasets [46 sequences of ITS (619 bp), LSU (594 bp) and *tef*1 (758 bp)] were aligned separately in MAFFT v.7 (Katoh and Standley 2013). The end parts of each alignment were removed manually by considering a point where the sequences presented greater homogeneity (all alignments are deposited in Treebase: http://purl.org/phylo/treebase/phylows/study/TB2:S23689). Then, a phylogenetic tree was inferred using each dataset separately. The nucleotide substitution model was selected by independent runs in jModelTest 2 (Darriba et al. 2012) using the Akaike Information Criterion with a 95% confidence interval. Second, the three datasets were concatenated using Winclada v.1.00.08 (Nixon 2002). The final file comprised 46 sequences totalling 1971 bp. All phylogenetic trees were reconstructed using maximum likelihood (ML) in RAxML v.8 (Stamatakis 2014) with 1000 independent trees and 1000 bootstrap replicates (MLB) and Bayesian Inference (BI) in MrBayes v.3.2.2. (Ronquist et al. 2012). The ML phylogenetic trees were reconstructed using the GTR + G substitution model and the BI phylogenetic trees were performed with the GTR + I + G substitution model. In the case of BI, two separate runs were carried out, each consisting of three hot chains and one cold chain and a Markov Chain Monte Carlo (MCMC) sampling for two million generations to obtain Bayesian posterior probability (PP) values for the clades. Convergence occurred when the standard deviation of split frequencies fell below 0.01 and the first 25% of the generations of MCMC sampling were discarded as burn-in. The final phylogenetic trees were edited in FigTree v.1.4 and in Adobe Illustrator CC v.17.1. *Lecanicillium antillanum* CBS 350.85 was used as the outgroup in all trees, because it belongs to a family phylogenetically close to Hypocreaceae (Spatafora et al. 2007).

## Results

### Taxonomy

***Escovopsis clavatus*** **Q.V. Montoya, M.J.S. Martiarena, D.A. Polezel, S. Kakazu & A. Rodrigues, sp. nov.**
MycoBank: MB828328
Figs 1–3

**Etymology.** "*clavatus*" in reference to the predominantly clavate shape of vesicles.

**Typification.** BRAZIL. Santa Catarina, Florianópolis, (27°44'39.6"S, 48°31'10.14"W), elev. 46 m, fungus garden, 08, 2015. *A. Rodrigues*. Holotype: CBS H-23845 (dried culture on PDA). Ex-type strain LESF 853 (= CBS 145326).

**Sequences.** ITS (MH715096), *tef*1 (MH724270) and LSU (MH715110).

**Description.** *Colonies* grow only at 20 and 25 °C (Fig. 1). At both temperatures, growth starts on the third day on CMD, CYA, MA2%, MEA, OA, PCA, PDA; and on the sixth day on SNA. Colonies have floccose aerial mycelia with a pale-brown colour after seven days. Faster growth was observed on MA2% and heavy sporulation was identified on MA2%, PDA and OA. At 20 °C, colonies reached 0.5–0.7 cm, 1.5–2.5 cm and 0.5–1 cm on CMD, CYA and SNA, respectively. At this temperature, colonies reached the edge of the plate after 10 days on MA2% and PCA; after 12 days on OA and MEA; and after 14 days on PDA and CYA. At 25 °C, colonies reach 2 cm, 3–3.2 cm and 2 cm on CMD, CYA and SNA, respectively, after 14 days. At this temperature, colonies reached the plate edge after seven days on OA and PCA; and after 10 days on MA2%, MEA and PDA. Concentric rings were observed only on PCA at 20 °C (Fig. 1). No pustule-like structures were observed.

*Conidiophores* arising from aerial hypha alternated or opposite (Fig. 2A), with the main axis of 50–780 µm in length, some without branching and often with 1–2 levels of branching (Figs 2A, E, 3A, E). Branches arise from the main axis of the conidiophore in an alternated or opposite pattern, with a septum near to the central axis and before the vesicle, usually with 1–2 branches at each branching point (16–138 µm long) or 2–4 branches arising from swollen cells (28–35 µm long), mostly forming angles less than 90° and less frequently right angles, usually straight and sometimes slightly curved up or down. Each branch terminates in a vesicle, with 1–8 fertile heads per conidiophore. Swollen cells are present in 15% of the total of conidiophores examined (Figs 2C, D, 3E) and can measure 10–18 µm long × 7–9 µm wide. *Vesicles* with only a septum at the base, in various shapes: globose (8%), subglobose (24%), broadly ellipsoidal / clavate (33%), ellipsoidal (27%), cylindrical (8%) (Figs 2E–G and 3F–G); and reaching 9–27 µm long × 7–20 µm wide. *Phialides* lageniform formed on vesicles (Fig. 3H), with 5–8 µm in total length, elongated base (0.5–1.5 µm × 0.5–1 µm), followed by a swollen section (1.5–2.5 µm × 1–3 µm) and a thin neck (1.5–4 µm × 0.5 µm). *Conidia* with 1.5 µm–2.5 µm long × 0.5 µm–1.5 µm wide, in various shapes: broadly ellipsoidal (5%), ellipsoidal (43.3%), cylindrical (51.7%); brown, with smooth and slightly thickened walls and in chains (Figs 2H, 3I).

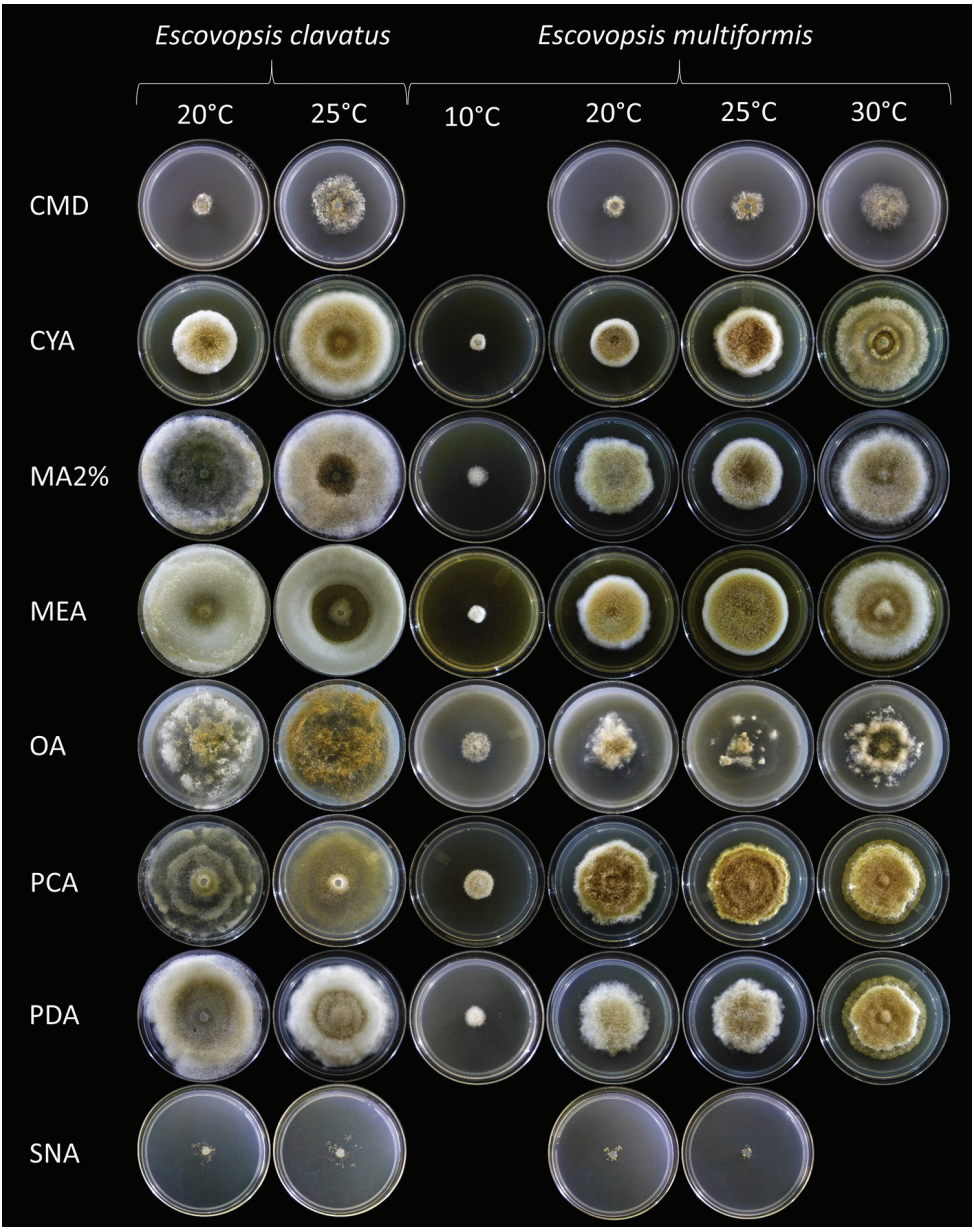


**Figure 1.** Colony macroscopic characters of *Escovopsis clavatus* and *Escovopsis multiformis* on CMD, CYA, MA2%, MEA, OA, PCA, PDA and SNA media after 14 days at 10, 20, 25 and 30 °C.

**Habitat.** Isolated from fungus gardens of *Apterostigma* sp.

**Additional specimens examined.** BRAZIL. Santa Catarina, Florianópolis, (27°44'38.94"S, 48°31'9.3"W), elev. 32 m, fungus garden, 08, 2015. *A. Rodrigues*. LESF 854 (ITS – MH715097, *tef*1 – MH724271 and LSU – MH715111). Santa Catarina, Florianópolis, (27°44'39.49"S, 48°31'9.72"W), elev. 38 m, fungus garden, 08, 2015. *A. Rodrigues*. LESF 855 (ITS – MH71509, *tef*1 – MH724272 and LSU – MH715112).

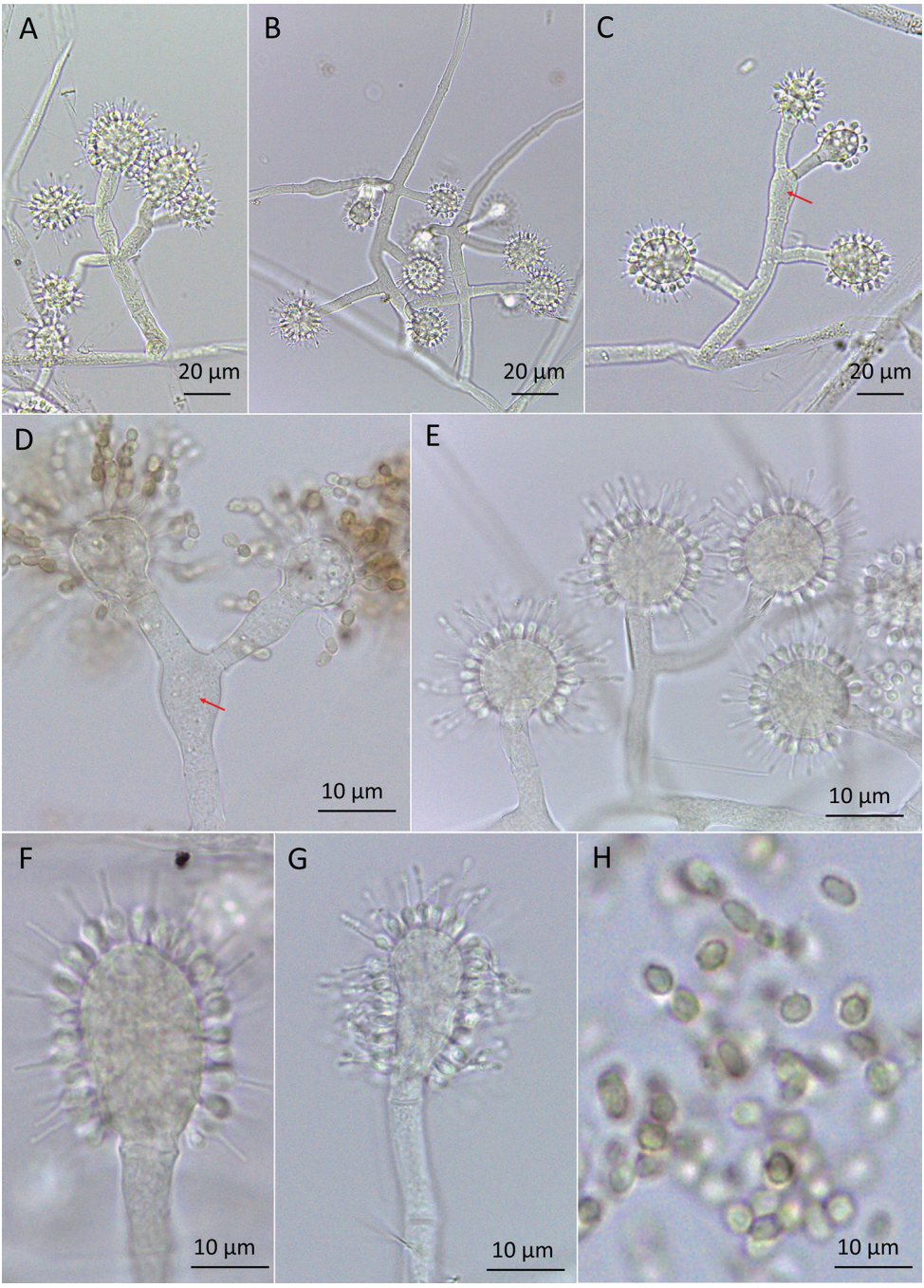


**Figure 2.** *Escovopsis clavatus*. **A, B** Conidiophores without "swollen cells" **C, D** Conidiophores with "swollen cells" (red arrows) **E–G** Vesicles in various shapes with phialides pattern **G** Conidia.

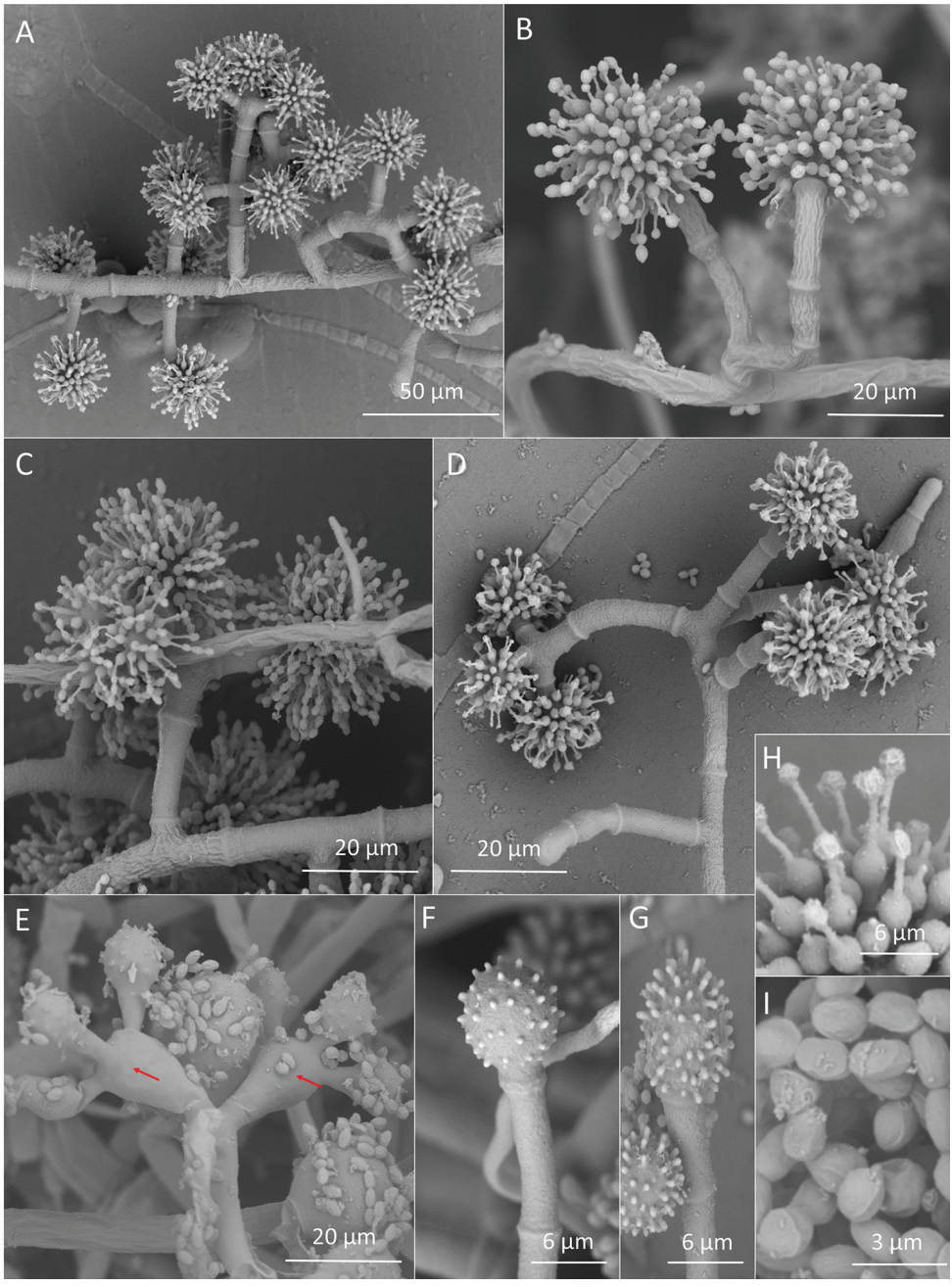


**Figure 3.** *Escovopsis clavatus*. SEM images **A–D** Conidiophores without "swollen cells" **E** Conidiophore with "swollen cells" (red arrows) **F, G** Vesicles **H** Phialides **G** Conidia.

**Notes.** *Escovopsis clavatus* is phylogenetically closely related to *E. multiformis* and its most distinctive characters are its growth temperatures, the conidiophore branching and the swollen cells. It grows at 20 and 25 °C; nevertheless, *E. multiformis* grows at 10, 20, 25 and 30 °C. The conidiophore of *E. clavatus* is larger and more branched than the conidiophore of *E. multiformis*. In addition, the swollen cells of *E. clavatus* are less frequent and shorter than in *E. multiformis*. The character distinguishing *E. clavatus* from other species of *Escovopsis* is the swollen cell on the conidiophores and because it is phylogenetically placed in a distinct clade.

***Escovopsis multiformis*** **Q.V. Montoya, M.J.S. Martiarena, D.A. Polezel, S. Kakazu & A. Rodrigues, sp. nov.**
Mycobank: MB828329
Figs 1, 4, 5

**Etymology.** "*multiformis*" in relation to the different vesicle shapes found in the same isolate.

**Typification.** BRAZIL. Santa Catarina, Florianópolis, (27°28'11.28"S, 48°22'39.48"W), elev. 119 m, fungus garden, 08, 2015. *A. Rodrigues*. Holotype: CBS H-23846 (dried culture on PDA). Ex-type strain LESF 847 (= CBS 145327).

**Sequences.** ITS (MH715091), *tef*1 (MH724265) and LSU (MH715105).

**Description.** *Colonies* grow at 10, 20, 25 and 30 °C (Fig. 1). The best growth temperature was 30 °C. At this temperature, colonies reached 1.2–1.4 cm, 2.7–3 cm, 2.6–3 cm, 3.3–3.5 cm, 2.5–2.8 cm, 2.7–2.9 cm and 1.9–2.5 cm in radius on CMD, CYA, MA2%, MEA, OA, PCA and PDA, after 14 days, respectively. Colonies exhibit light-brown floccose mycelia (colony edge usually lighter or white). The colour shades and the character of the aerial mycelium vary on each culture medium (Fig. 1). Colonies present concentric rings with a hardened ring similar to a crust in the centre on CYA (Fig. 1) and the sporulation is more abundant on PCA and PDA. At 20 °C, on CMD, CYA, MA2%, MEA, OA, PCA, PDA and SNA, colonies attained 0.5–0.8 cm, 1.1–2.2 cm, 2–2.5 cm, 2.1–2.3 cm, 2–2.5 cm, 2.8 cm, 1.9–2.4 cm and 0–0.1 cm in radius, respectively. At 25 °C, colonies reached 1 cm, 2.1–2.3 cm, 2–2.4 cm, 2.5–2.6 cm, 2.2–2.7 cm, 2.8–3 cm, 1.8–2 cm and 0.1–0.2 cm in radius on CMD, CYA, MA2%, MEA, OA, PCA, PDA and SNA, respectively. Pustule-like structures were observed on OA and CMD at 20, 25 and 30 °C. At 10 °C, the colony growth was inconspicuous, reaching 0.2–0.3 cm, 0.2–0.4 cm, 0.3 cm, 0.6–0.8 cm, 0.8 cm and 0.3–0.5 cm in radius on CYA, MA2%, MEA, OA, PCA and PDA, respectively, after 14 days. At this temperature, growth started in these culture media after seven days and sporulation occurred only after the 12$^{th}$ day. No growth was observed at 35 °C.

*Conidiophores* arising from aerial hypha alternated or opposite (Fig. 3A), with the main axis of 41–293 µm in length, some without branching and most of them with one level of branching. Rarely, branches form two levels branching (Figs 4A–C, 5A, B). Branches arise from the main axis of the conidiophore alternated, with a septum near

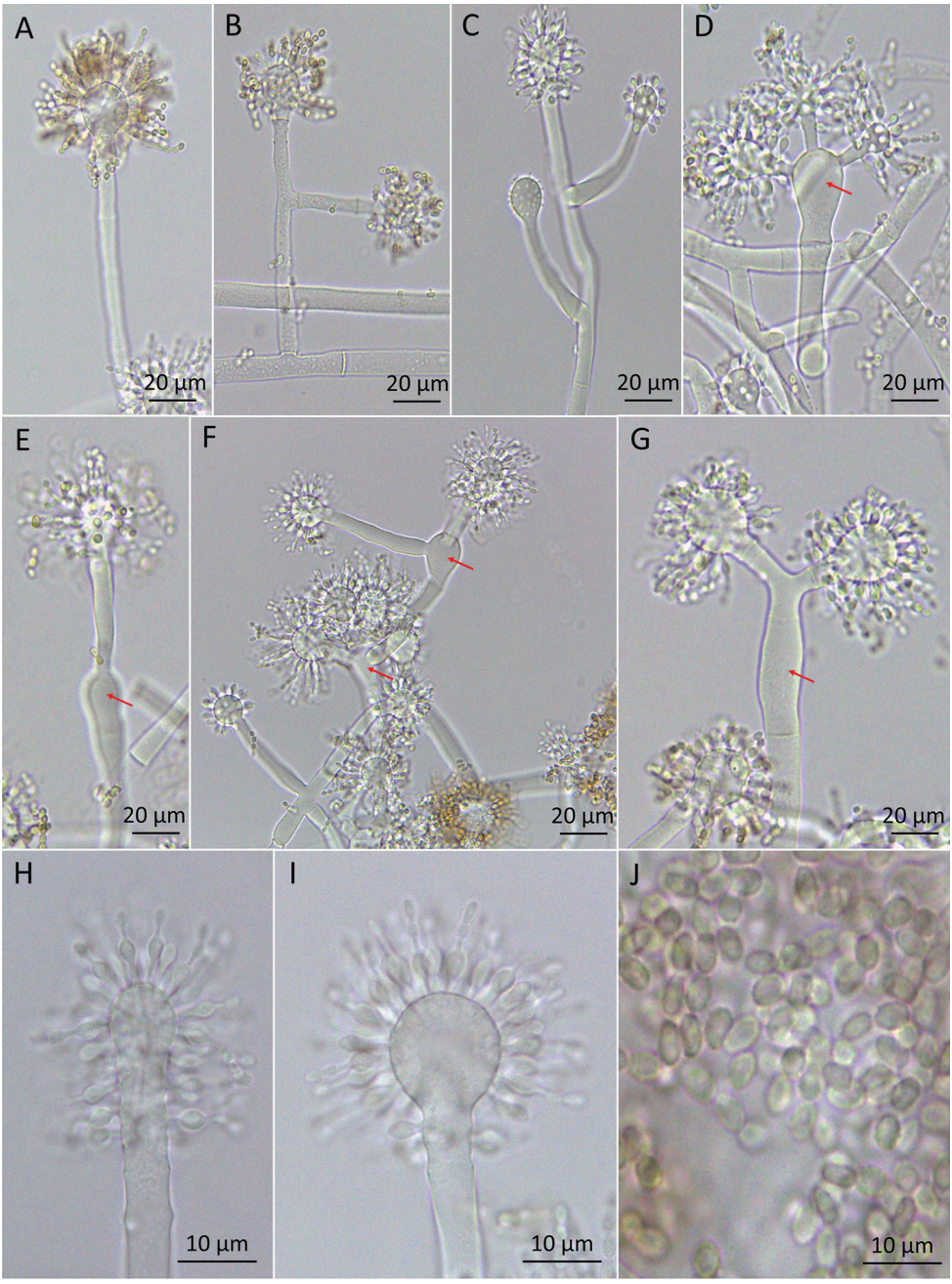


**Figure 4.** *Escovopsis multiformis*. **A–C** Conidiophores mono- and polycephalous without "swollen cells" **D–G** Conidiophores mono and polycephalous with "swollen cells" (red arrows) **H, I** Vesicles in various shapes **J** Conidia.

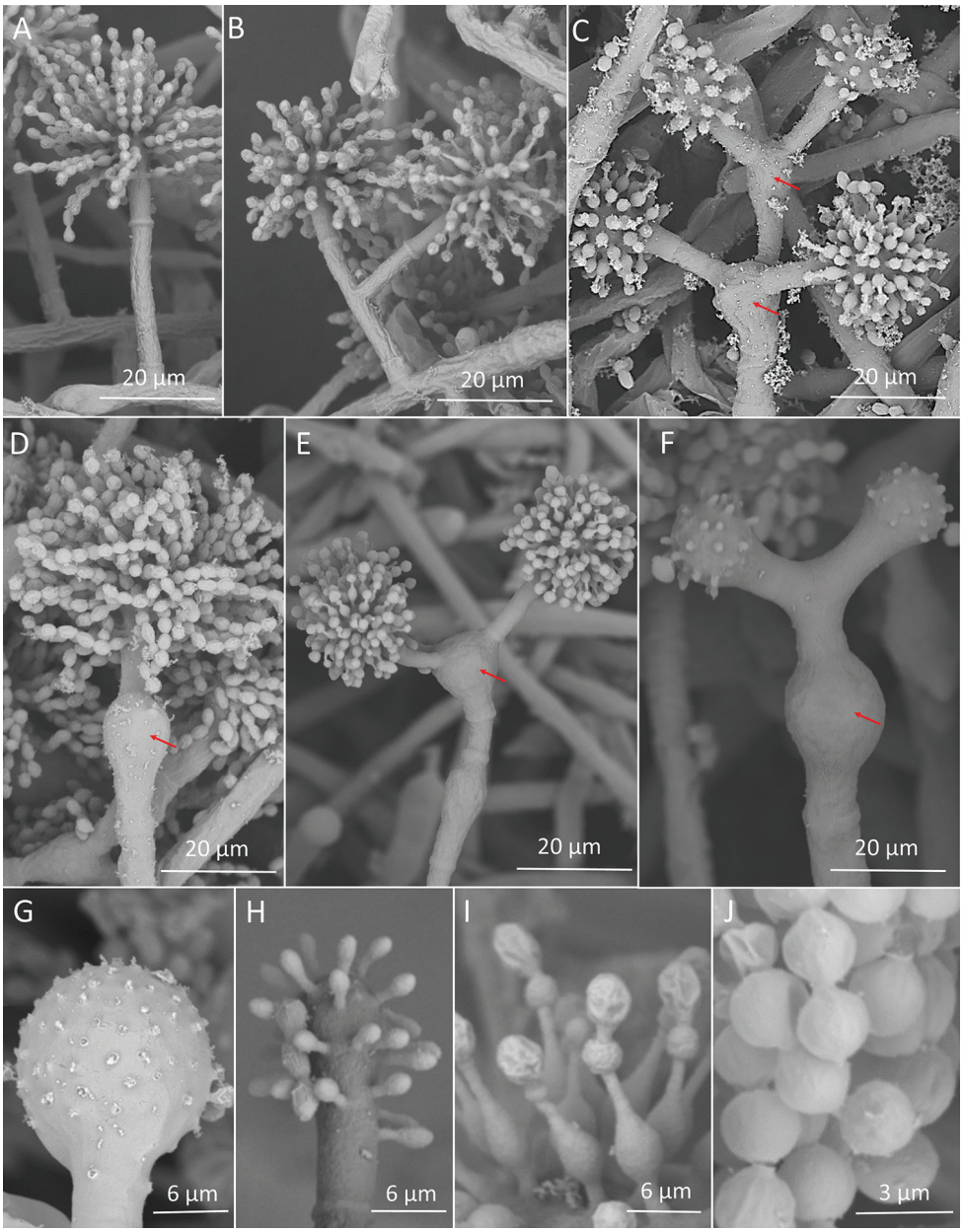


**Figure 5.** *Escovopsis multiformis*. SEM images **A, B** Conidiophores mono- and polycephalous without "swollen cells" **C–F** Conidiophores mono- and polycephalous with "swollen cells" (red arrows) **G, H** Vesicles **I** Phialides **J** Conidia.

the central axis and before the vesicle, usually with one branch at each branching point (32–84 µm long) or 2–4 branches arising from swollen cells (17–86 µm long), mostly forming right angles, usually slightly curved up. Each branch terminates in a vesicle, with 1–4 fertile heads per conidiophore. Swollen cells are present in 27% of the total of con-

idiophores examined (Figs 4D–G, 5C–F) and can measure 16–34 µm long × 9–20 µm wide. Sometimes, one swollen cells' branch gives rise to another swollen cell with more branches (Figs 2F, 3C). *Vesicles* with only a septum at the base, in various shapes: globose (22%), subglobose (37%), broadly ellipsoidal (26%), ellipsoidal (10%), cylindrical (5%) (Figs 4H, I, 5G, H); and reaching 12–27 µm × 9–17 µm wide. *Phialides* lageniform formed on vesicles (Fig. 5I), with 6–10 µm in total length, elongated base (1– 2.5 µm × 0.5–1µm), followed by a swollen section (2.5–4.5 µm × 2–3.5 µm) and a thin neck (1–4.5 µm × 0.5–1 µm). *Conidia* are 2.5–3.5 µm long × 1.5–2.5 µm wide, in various shapes: globose (2%), subglobose (3%), broadly ellipsoidal (33%), ellipsoidal (47%), cylindrical (15%); brown, with smooth and slightly thickened walls and in chains (Figs 4, 5J).

**Habitat.** Isolated from fungus garden of *Apterostigma* sp.

**Additional specimens examined.** BRAZIL. Mato Grosso, Cotriguaçu, (09°49'22.74"S, 58°15'32.04"W), elev. 252 m, fungus garden, 10, 2017. *Q. V. Montoya*. LESF 1136 (ITS – MH715092, *tef*1 – MH724266 and LSU – MH715106).

**Notes.** *Escovopsis multiformis* is closely related to *E. clavatus*. Different from *E. clavatus* that grow at 20 and 25 °C, *E. multiformis* grow at 10, 20, 25 and 30 °C. The optimum growth temperature of *E. multiformis* is 30 °C and that of *E. clavatus* is 25 °C. The conidiophores of *E. multiformis* are smaller and less branched than *E. clavatus* and the swollen cells are more frequent and larger than those found in *E. clavatus*. *E. multiformis* differs from other described species by the presence of conidiophores with a swollen cell, the presence of different vesicles shapes and because it is phylogenetically placed in a distinct clade.

## Morphological analyses

The isolates LESF 853 (*Escovopsis clavatus*, Figs 1–3) and LESF 847 (*Escovopsis multiformis*, Figs 1, 4, 5) differed from the seven previously described *Escovopsis* species, mainly in micro-morphological structures. All isolates had white colonies with a floccose appearance on all culture media, but *E. clavatus* had the most floccose colonies. After 5–7 days incubation, the centre of the colonies turned pale brown and, after 7 days, the entire colony gradually turned from white to light brown (not always from the middle to the edge in *E. multiformis*).

*Escovopsis multiformis* showed growth at wide ranges of temperature (from 10–30 °C); nonetheless, *E. clavatus* showed growth only at 20 and 25 °C (Fig. 1). None of the isolates grew at 35 °C. On all culture media, the best growth was obtained at 25 °C for *E. clavatus* and at 30 °C for *E. multiformis*. In all cases where growth was observed, it started between 24 to 36 hours and sporulation started on the third day.

All strains of both species have a unique type of conidiophore with a swollen cell, from which branches emerge (Figs 2C, D, 3E, 4D–F, 5C–F). These conidiophores were more frequent in *E. multiformis* than *E. clavatus* (27% and 15%, respectively). Mono or polycephalous conidiophores, without the swollen cells, that were described in the other *Escovopsis* species, were also present but with some differences in the size and branching pattern (Figs 2A, B, 3A–D, 4A–C, 5A, B). Conidiophores

with cruciform or opposed branches were rarely observed. On the other hand, the two new species had basipetal and smooth-walled conidia with slightly thickened walls, formed from phialides. No chlamydospores were observed in the aerial or submersed mycelia of any of the strains.

## Phylogenetic analyses

Separate phylogenetic analyses with the three molecular markers showed topological differences because of the incongruity placement of the formal described *Escovopsis* species and some strains that form new phylogenetic clades within the genus (Fig. 6). The phylogenetic placement of *E. multiformis* and *E. clavatus* also presented conflicts amongst the three molecular markers; however, the position of each strain that made up both new species was concordant through the three genealogies (PP= 1; MBL= 100%, Fig. 6).

The combined analysis also confirmed *E. multiformis* and *E. clavatus* as two new phylogenetic species in *Escovopsis* (PP= 1; MLB= 100%, Fig. 7) and showed the strain LESF 018 (a vesiculated *Escovopsis* species) as the closest relative of both. Nevertheless, the concatenated BI and ML trees also presented few differences between them with respect to the position of the *E. aspergilloides* and *E. lentecrescens*. The BI analysis placed *E. aspergil-*

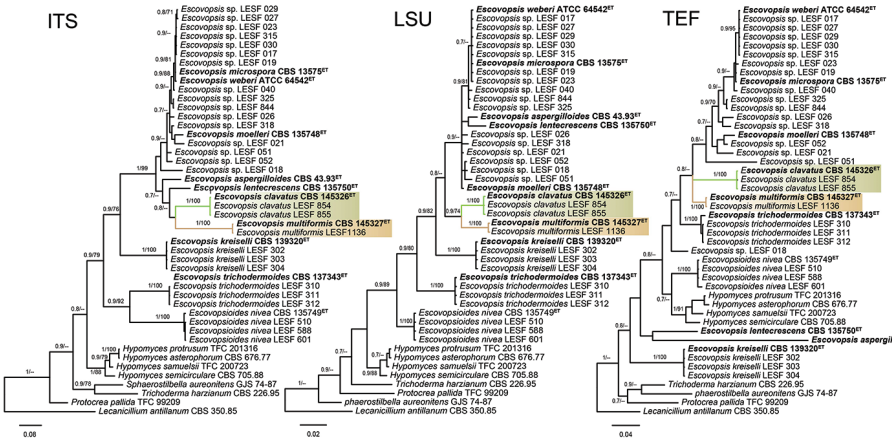


**Figure 6.** Phylogenetic position of *Escovopsis clavatus* and *Escovopsis multiformis* considering each molecular marker separately (ITS, LSU and *tef1*). The trees were reconstructed under Bayesian and Maximum Likelihood inferences. The numbers on branches indicate the posterior probabilities and the bootstrap support values, respectively. The seven *Escovopsis* ex-type strains are denoted in bold and the new species are highlighted in green (*E. clavatus*) and light brown (*E. multiformis*). The trees include a total of 46 *Escovopsis* sequences of each marker (ITS – 619 bp, LSU – 594 bp and *tef*1 – 758 bp) and *Escovopsioides*, *Hypomyces*, *Sphaerostilbella*, *Trichoderma* and *Protocrea* were included as the closest phylogenetic relatives of *Escovopsis*. *Lecanicillium antillanum* CBS 350.85 was used as the outgroup. ET: ex-type.

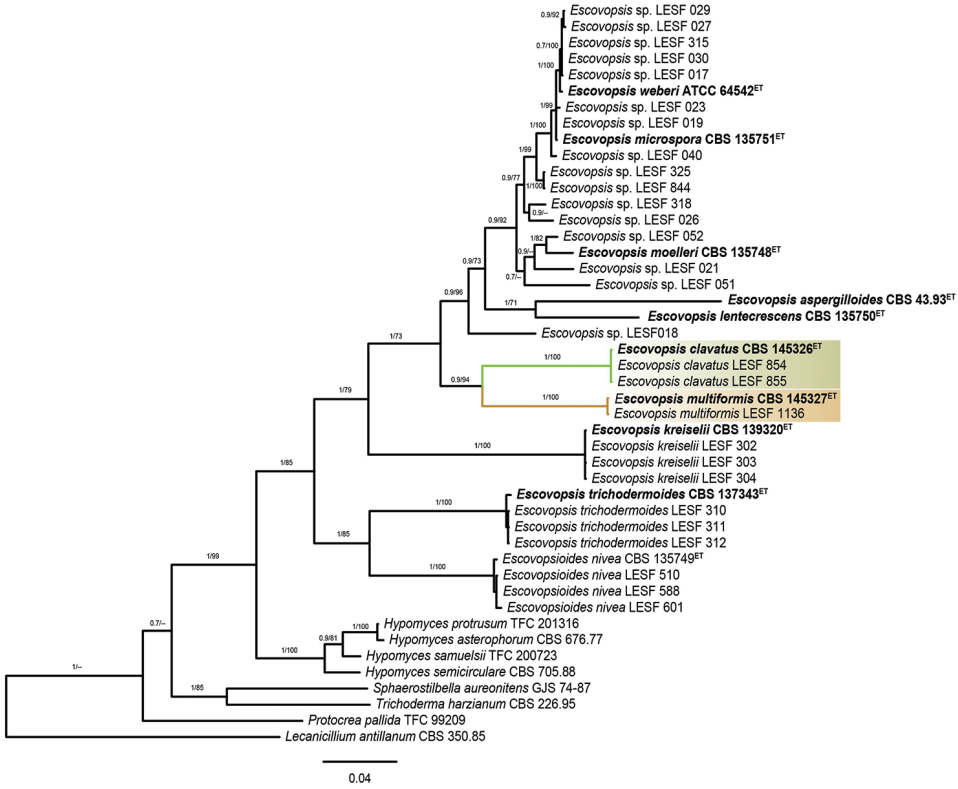


**Figure 7.** Phylogenetic position of *Escovopsis clavatus* and *Escovopsis multiformis*. The phylogenetic analysis is based on the concatenated sequences of ITS, LSU and *tef1*; and the tree was reconstructed using Bayesian and Maximum Likelihood inferences. Numbers on branches indicate the posterior probabilities and the bootstrap support values, respectively. All *Escovopsis* species previously described are denoted in bold and the new species are highlighted in green (*E. clavatus*) and light brown (*E. multiformis*). The tree includes a total of 40 *Escovopsis* sequences with 1971 bp (ITS – 619 bp, LSU – 594 bp and *tef*1 – 758 bp). The data also included sequences from *Escovopsioides*, *Hypomyces*, *Sphaerostilbella*, *Trichoderma* and *Protocrea* as the closest phylogenetic relatives of the parasite. *Lecanicillium antillanum* CBS 350.85 was used as the outgroup. ET: ex-type strains. Bar: 0.04 substitutions per nucleotide position.

*loides* and *E. lentecrescens* separate from *E. multiformis* and *E. clavatus* (Fig. 7); however, the ML analysis showed the former species as sister clades of *E. multiformis* and *E. clavatus*.

It is important to highlight that the concatenated analysis, as well as the trees inferred with ITS and LSU, showed the vesiculated *Escovopsis* (*E. aspergilloides*, *E. clavatus*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, *E. weberi*) as the most derived group, separated from the non-vesiculated *Escovopsis* (*E. kreiselii* and *E. trichodermoides*). In addition, both the combined and the analysis performed with ITS and *tef*1 showed some *Escovopsis* species (*E. aspergilloides*, *E. kreiselii*, *E. lentecrescens* and *E. trichodermoides*) often clustering with other Hypocreaceae genera or falling outside the *Escovopsis* clade, which reveals that *Escovopsis* is apparently paraphyletic (Figs 6, 7).

## Discussion

The attine ants have persisted for millions of years because of the biological relationships that these insects maintain with the beneficial microorganisms that inhabit their colonies. Several studies tried to understand how these biological relationships sculptured the evolutionary history of the attines (Mueller et al. 1998, Currie et al. 2003, Gerardo et al. 2006ab, Nygaard et al. 2016, Mueller et al. 2018). Nevertheless, the taxonomy of *Escovopsis*, the only known parasite in the attine's colony, has been poorly addressed. Considering that *Escovopsis* co-evolved with the attine ants' cultivar, improved knowledge about the taxonomy and systematics of this genus could shed light on the evolutionary success of these insects. Therefore, the discovery and description of new *Escovopsis* species is an important advance in understanding this system.

Subsequent to the formal description of *Escovopsis* (Muchovej and Della Lucia 1990), several studies showed a high genetic diversity of this genus in the colonies of both leafcutter and non-leafcutter attine ants (Gerardo et al. 2006a, Meirelles et al. 2015b). However, only seven species of the parasite have been described so far (Seifert et al. 1995, Augustin et al. 2013, Masiulionis et al. 2015, Meirelles et al. 2015a) and the morphological diversity and physiology of the parasite remain unknown. In addition, a lack of standardised conditions for describing the morphology of *Escovopsis* hinders researchers from identifying morphological characters that might help to distinguish *Escovopsis* species from one another and from the other related genera from the Hypocreaceae. Unfortunately, this fact made it difficult to describe new species of the parasite. Studies showed that the expressed phenotypic characters (phenotypic plasticity) of fungi are directly influenced by growth conditions (Slepecky and Starmer 2009, Sharma and Pandey 2010, Wrzosek et al. 2017, Kim et al. 2017). As the morphological plasticity of *Escovopsis* species is still poorly understood, the standardisation of cultivation conditions is imperative. The strains described as new species here were evaluated on eight different culture media (those used in the description of the seven previous species) and at five temperatures (to establish cardinal growth temperatures). Due to the lack of standard culture conditions, the comparison with each species previously described was only partial. Nonetheless, we are providing characters of these two new species in all the conditions previously used, to help future researchers to standardise the taxonomy of the genus.

Recent attempts to expand the morphological concept of *Escovopsis* generated inconsistencies in the taxonomy and systematics of this genus (Masiulionis et al. 2015, Meirelles et al. 2015a). The morphological characters that initially gave rise to the concept of *Escovopsis* (presence of terminal vesicles and phialidic conidiogenesis, see Muchovej and Della Lucia 1990) are distinctive to delineate *Escovopsis*, because no other genus in the Hypocreaceae family has such combined characters. However, some *Escovopsis* species described recently, namely *E. trichodermoides* and *E. kreiselii*, lack vesicles and each has a different kind of conidiogenesis (synchronous and sympodial, respectively). Besides, the results of the phylogenetic analysis performed in previous studies (Meirelles et al. 2015b, Masiulionis et al. 2015, Augustin et al. 2013), as well as the results from our analysis, reveal that *Escovopsis* is paraphyletic (Figs 6, 7). Therefore, future studies will have to reconsider if both species indeed belong to *Escovopsis*. For

this purpose, the taxonomic conditions need to be delimited and additional molecular markers will have to be included to help resolve those phylogenetic incongruities. Then the generic concept of *Escovopsis* species should be revisited.

Our study shows that the ex-type strains LESF 853 (*E. clavatus*) and LESF 847 (*E. multiformis*) form a monophyletic clade within most derived *Escovopsis* (vesiculated *Escovopsis*, PP = 1, BML = 100%). Most interesting, unlike the other *Escovopsis* species, the two new species present a unique type of conidiophore with a swollen cell, from which one to four branches arise. The newly described species also possess smooth conidia with slightly thickened walls. A recent study suggests the possibility that conidia ornamentation could be associated with the mechanism for horizontal transmission of *Escovopsis* between ant colonies and with the latency of the parasite conidia. This hypothesis was based on observations of some conidia adhering to the ant legs and in spore dormancy in vitro bioassays (Augustin et al. 2017). The same authors also argued that such character could be used as morphological markers for the taxonomy of the genus. However, because of scarce knowledge of the morphological features of the *Escovopsis* species, it is difficult to decipher which phenotypic character could be considered diagnostic for this genus. Therefore, future researchers need to carefully evaluate the phenotypic characters of each *Escovopsis* clade to determine which of characters are homologous versus those that are homoplasious to build taxonomic keys.

Considering the high genetic diversity of *Escovopsis* and the poor knowledge of its taxonomy, our study suggests that the fungus gardens of attine ants host a great diversity of *Escovopsis* that has yet to be discovered. Thus, the description of these new species are merely two small pieces of a complex puzzle. Nonetheless, our work should help future researchers to build the framework for the systematics of this parasitic fungus.

## Acknowledgements

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## Supplementary material 1

**Supplementary tables**

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Data type: phylogenetic data

Explanation note: Table S1. *Escovopsis* strains used in the phylogenetic analyses and their associated metadata. Table S2. Molecular markers, primers and PCR conditions used in this study.



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# *Neostagonosporella sichuanensis* gen. et sp. nov. (Phaeosphaeriaceae, Pleosporales) on *Phyllostachys heteroclada* (Poaceae) from Sichuan Province, China

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## Abstract

*Neostagonosporella sichuanensis* sp. nov. was found on *Phyllostachys heteroclada* collected from Sichuan Province in China and is introduced in a new genus *Neostagonosporella* gen. nov. in this paper. Evidence for the placement of the new taxon in the family Phaeosphaeriaceae is supported by morphology and phylogenetic analysis of a combined LSU, SSU, ITS and TEF 1-α DNA sequence dataset. Maximum-likelihood, maximum-parsimony and Bayesian inference phylogenetic analyses support *Neostagonosporella* as a distinct genus within this family. The new genus is compared with related genera of Phaeosphaeriaceae and full descriptions and illustrations are provided. *Neostagonosporella* is characterised by its unique suite of characters, such as multiloculate ascostromata and cylindrical to fusiform, transversely multiseptate, straight or curved ascospores, which are widest at the central cells. Conidiostromata are multiloculate, fusiform to long fusiform or rhomboid, with two types conidia; macroconidia vermiform or subcylindrical to cylindrical, transversely multiseptate, sometimes curved, almost equidistant between septa and microconidia oval, ellipsoidal or long ellipsoidal, aseptate, rounded at both ends. An updated phylogeny of the Phaeosphaeriaceae based on multigene analysis is provided.

## Keywords

2 new taxa, bambusicolous fungi, phylogeny, stem spot, taxonomy

## Introduction

The family Phaeosphaeriaceae is a large and important family of Pleosporales, initially introduced by Barr (1979) with *Phaeosphaeria oryzae* I. Miyake as the type species (Miyake 1909). The taxonomy of members within this family has often been confused with those of the Leptosphaeriaceae (Müller 1950, Holm et al. 1957, Munk 1957, Zhang et al. 2009, Phookamsak et al. 2014) and it is sometimes difficult to distinguish species. Criteria which have previously been used to differentiate species have been based mostly on the morphology of the peridial wall, asexual characteristics and host association (Eriksson 1967, 1981, Lucas and Webster 1967, Leuchtmann 1984, Shoemaker 1984, Barr 1987, Shoemaker and Babcock 1989, Shearer et al. 1990, Khashnobish and Shearer 1996, Câmara et al. 2002) and taxonomic schemes followed are those of Kirk et al. (2008), Zhang et al. (2009), Hyde et al. (2013), Phookamsak et al. (2014a) and Abd-Elsalam et al. (2016). However, this delimitation of taxa in Phaeosphaeriaceae and Leptosphaeriaceae, based solely on the above-mentioned features, is not feasible. Recent studies showed that it is very difficult to discriminate them only by such characters, because numerous new members have been introduced to these two families and these species are not significantly different in these features, but they can be differentiated by phylogenetic analysis (Zhang et al. 2012, Hyde et al. 2013, Ahmed et al. 2014, Ariyawansa et al. 2015a, 2018, Bakhshi et al. 2018). Hence there is a need to use the multigene sequence data analyses to infer relationships.

Barr (1979) originally introduced 15 genera in this family and subsequent researchers have revised this number (Barr 1992, Eriksson and Hawksworth 1993, Kirk et al. 2001, 2008, Lumbsch and Huhndorf 2007, 2010). The taxonomic placement of genera within this family has been changed in recent years based on phylogenetic analyses (Zhang et al. 2012, Hyde et al. 2013, Wijayawardene et al. 2014, Phookamsak et al. 2014a, 2017, Wanasinghe et al. 2018). Taxonomic revision of the genera in Phaeosphaeriaceae resulted in 28 genera based on morphology and phylogenetic evidence (Phookamsak et al. 2014a). Since 2014, many new genera have been introduced based on molecular data (Ariyawansa et al. 2015b, Ertz et al. 2015, Crous et al. 2015a, 2015b, 2017a, Jayasiri et al. 2015, Li et al. 2015, Phukhamsakda et al. 2015, Rossman et al. 2015, Tibpromma et al. 2015, 2017, Abd-Elsalam et al. 2016, Hernández-Restrepo et al. 2016, Hyde et al. 2016, 2017, Tennakoon et al. 2016, Wijayawardene et al. 2016, Ahmed et al. 2017, Huang et al. 2017, Karunarathna et al. 2017, Phookamsak et al. 2017, Bakhshi et al. 2018, Senanayake et al. 2018, Wanasinghe et al. 2018). The placement of some older genera has been reconfirmed with DNA sequence (Phookamsak et al. 2017, Senanayake et al. 2018). However, there are still a few genera lacking molecular data, such as *Bricookea*, *Dothideopsella*, *Eudarluca*, *Phaeostagonospora* and *Tiarospora*. At present, this family includes more than 800 species in 61 genera (25 genera are known only from asexual morphs) (Index Fungorum 2018, Wijayawardene et al. 2017, 2018). Many genera were introduced to accommodate a single or a few species in Phaeosphaeriaceae. Only 14 genera in the Phaeosphaeriaceae

contained 10–50 species, while *Ophiobolus* and *Phaeosphaeria* comprised more than 150 species. However, most species in *Ophiobolus* and *Phaeosphaeria* lack molecular data to confirm their phylogenetic affinities.

We are studying fungi on bamboo which is the main food for panda in Sichuan Province of China (Tang et al. 2007, Wang et al. 2017). The purpose of this paper is to introduce a new genus with one species in Phaeosphaeriaceae recovered from *Phyllostachys heteroclada* Oliv. Combined multigene (LSU, SSU, ITS and TEF 1-α) analyses confirm its phylogenetic position in Phaeosphaeriaceae. A comprehensive comparison with similar genera and detailed descriptions and illustrations are provided.

## Materials and methods

### Sampling and morphological study

The specimens were collected from Ya'an City of Sichuan Province in China, on living to near dead stems and branches of *Phyllostachys heteroclada*. The samples were kept in Ziplock plastic bags and brought to the laboratory. Fresh materials were examined by using stereo and compound microscopes. Vertical free-hand sections were made by using a razor blade and placed on a droplet of sterilised water on a glass slide (Gupta and Tuohy 2013). Lactate cotton blue reagent was used to observe the number of septa. Micromorphological characters were examined by using a Nikon ECLIPSE N*i* compound microscope fitted to a Cannon 600D digital camera. Fruiting tissues were observed by stereomicroscopy using NVT-GG (Shanghai Advanced Photoelectric Technology Co. Ltd, China) and photographed by VS-800C (Shenzhen Weishen Times Technology Co. Ltd, China). Measurements were taken using Tarosoft® Image Frame Work v.0.9.7.

### Isolation

Single ascospore and conidium isolation was carried out following the method described by Dai et al. (2017). Germinated ascospores and conidia were separately transferred to Potato Dextrose Agar media plates (PDA) and incubated at 25°C and the colonies were observed after 10 days and as outlined by Vijaykrishna et al. (2004) and Liu et al. (2010). Specimens are deposited in Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and Sichuan Agricultural University Herbarium (SICAU), Chengdu, China. Living cultures are deposited at the Culture Collection at Mae Fah Luang University (MFLUCC) and the Culture Collection at Sichuan Agricultural University (SICAUCC). Facesoffungi and Index Fungorum numbers were registered as in Jayasiri et al. (2015) and Index Fungorum (2018), respectively. New species are established following the recommendations of Jeewon and Hyde (2016).

## DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for seven days at 25°C and genomic DNA was extracted from fresh mycelia, following the protocols of Plant Genomic DNA Kit (Tiangen, China). If cultures were unavailable, fungal DNA was directly extracted from fruiting tissues according to Yang et al. (2017), Wanasinghe et al. (2018) and Zeng et al. (2018). The primers, LR0R and LR5 (Vilgalys and Hester 1990), NS1 and NS4, ITS5 and ITS4 (White et al. 1990) and EF1-983F and EF1-2218R (Rehner 2001) were used for the amplification of the 28S large subunit rDNA (LSU), 18S small subunit rDNA (SSU), internal transcribed spacers (5.8S, ITS) and translation elongation factor 1-α gene region (TEF 1-α), respectively. The amplification reactions were performed as stated by Phukhamsakda et al. (2015). Amplified PCR fragments were purified and sequenced at TsingKe Biological Technology Co., Ltd. (Chengdu, China). Newly generated sequences of LSU, SSU, ITS and TEF 1-α regions are deposited in GenBank.

## Molecular phylogenetic analysis

Sequence data, mainly from recent publications (Phookamsak et al. 2017, Wanasinghe et al. 2018), were downloaded for analyses (Table 1). Four Massarineae taxa *Cyclothyriella rubronotata* (CBS 121892), *C. rubronotata* (CBS 141486), *Didymosphaeria rubi-ulmifolii* (MFLUCC 14-0024) and *D. variabile* (CBS 120014) were chosen as outgroup taxa based on Tanaka et al. (2015) and Jaklitsch and Voglmayr (2016). DNA alignments were performed by using MAFFT v.7.407 online service (Katoh and Standley 2013) and ambiguous regions were excluded with BioEdit version 7.0.5.3 (Hall 1999). Multigene sequences were concatenated by Mesquite version 3.11 (build 766) (Maddison and Maddison 1997–2016). Multigene phylogenetic analyses of the combined LSU, SSU, ITS and TEF 1-α sequence data were obtained from maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses. The alignments were converted to NEXUS file (.nxs) by using ClustalX version 1.81 (Thompson et al. 1997) for MP and BI analyses. The symbols "ABCDEFGHIKLMNOPQRSTUVWXYZ" was deleted in PAUP v. 4.0b10 (Swofford 2002) for preparing data matrix of evaluated evolutionary model by MrModeltest v. 2.2 (Nylander 2004). The best nucleotide substitution model was determined by MrModeltest v. 2.2 (Nylander 2004) and the best-fit model for BI is GTR+I+G under the Akaike Information Criterion (AIC).

Maximum likelihood analysis was generated by using the CIPRES Science Gateway web server (Miller et al. 2010) and chosen RAxML-HPC BlackBox (8.2.10) (Stamatakis 2014). Maximum parsimony analysis was performed by PAUP v. 4.0b10 (Swofford 2002) with the heuristic search option with 1,000 random sequence additions and tree-bisection reconnection (TBR) as branch-swapping algorithm. All characters were unordered and of equal weight and gaps were regarded as missing data. Maxtrees were set up to 1,000, a zero of maximum branches length was collapsed and all multiple parsimonious trees were saved. Tree length [TL], consistency index [CI], retention index [RI], rela-

**Table 1.** Molecular data used in this study and GenBank accession numbers.

| Species | Strain/Voucher No. | GenBank Accession No. | | | | Refferences |
|---|---|---|---|---|---|---|
| | | LSU | SSU | ITS | TEF 1-α | |
| ***Acericola italica*** | **MFLUCC 13-0609** | MF167429 | MF167430 | MF167428 | - | Hyde et al. 2017 |
| *Allophaeosphaeria muriformia* | MFLUCC 13-0277 | KX910089 | KX950400 | KX926415 | - | Liu et al. 2015 |
| ***Allophaeosphaeria muriformia*** | **MFLUCC 13-0349** | KP765681 | KP765682 | KP765680 | - | Liu et al. 2015 |
| ***Amarenographium ammophilae*** | **MFLUCC 16-0296** | KU848197 | KU848198 | KU848196 | MG520894 | Wijayawardene et al. 2016, Phookamsak et al. 2017 |
| ***Amarenomyces dactylidis*** | **MFLUCC 14-0207** | KY775575 | - | KY775577 | - | Hyde et al. 2017 |
| *Ampelomyces quisqualis* | CBS 131.31 | JX681066 | - | AF035781 | - | Kiss and Nakasone 1998, Verkley et al. 2014 |
| *Ampelomyces quisqualis* | CBS 133.32 | JX681067 | - | - | - | Verkley et al. 2014 |
| ***Banksiophoma australiensis*** | **CBS 142163** | KY979794 | - | KY979739 | - | Crous et al. 2017 |
| ***Bhatiellae rosae*** | **MFLUCC 17-0664** | MG828989 | MG829101 | MG828873 | - | Wanasinghe et al. 2018 |
| *Boeremia exigua* | CBS 431.74 | EU754183 | EU754084 | FJ427001 | GU349080 | Aveskamp et al. 2009, de Gruyter et al. 2009, Schoch et al. 2009 |
| ***Camarosporioides phragmitis*** | **MFLUCC 13-0365** | KX572345 | KX572350 | KX572340 | KX572354 | Hyde et al. 2016 |
| ***Chaetosphaeronema achilleae*** | **MFLUCC 16-0476** | KX765266 | - | KX765265 | - | Hyde et al. 2016 |
| ***Chaetosphaeronema hispidulum*** | **CBS 216.75** | KF251652 | EU754045 | KF251148 | - | de Gruyter et al. 2009, Quaedvlieg et al. 2013 |
| *Cyclothyriella rubronotata* | CBS 121892 | KX650541 | - | KX650541 | KX650516 | Jaklitsch and Voglmayr 2016 |
| ***Cyclothyriella rubronotata*** | **CBS 141486** | KX650544 | KX650507 | KX650544 | KX650519 | Jaklitsch and Voglmayr 2016 |
| ***Dactylidina shoemakeri*** | **MFLUCC 14-0963** | MG829003 | MG829114 | MG828887 | MG829200 | Wanasinghe et al. 2018 |
| ***Dematiopleospora cirsii*** | **MFLUCC 13-0615** | KX274250 | - | KX274243 | KX284708 | Hyde et al. 2016 |
| ***Dematiopleospora fusiformis*** | **MFLU 15-2133** | KY239030 | KY239028 | KY239029 | - | Huang et al. 2018 |
| ***Dematiopleospora mariae*** | **MFLUCC 13-0612** | KJ749653 | KJ749652 | KJ749654 | KJ749655 | Wanasinghe et al. 2014 |
| *Didymocyrtis caloplacae* | CBS 129338 | JQ238643 | - | JQ238641 | - | Lawrey et al. 2012 |
| *Didymocyrtis ficuzzae* | CBS 128019 | JQ238616 | - | KP170647 | - | Lawrey et al. 2012, Trakunyingcharoen et al. 2014 |
| ***Didymocyrtis xanthomendozae*** | **CBS 129666** | JQ238634 | - | KP170651 | - | Lawrey et al. 2012, Trakunyingcharoen et al. 2014 |
| ***Didymosphaeria rubi-ulmifolii*** | **MFLUCC 14-0024** | KJ436585 | KJ436587 | - | - | Ariyawansa et al. 2014 |
| *Didymosphaeria variabile* | CBS 120014 | JX496139 | - | JX496026 | - | Verkley et al. 2014 |
| ***Dlhawksworthia alliariae*** | **MFLUCC 13-0070** | KX494877 | KX494878 | KX494876 | - | Hyde et al. 2016 |
| ***Dlhawksworthia clematidicola*** | **MFLUCC 14-0910** | MG829011 | MG829120 | MG828901 | MG829202 | Wanasinghe et al. 2018 |
| ***Dlhawksworthia lonicera*** | **MFLUCC 14-0955** | MG829012 | MG829121 | MG828902 | MG829203 | Wanasinghe et al. 2018 |
| *Dothidotthia aspera* | CPC 12933 | EU673276 | EU673228 | - | - | Phillips et al. 2008 |
| ***Dothidotthia symphoricarpi*** | **CPC 12929** | EU673273 | EU673224 | - | - | Phillips et al. 2008 |
| *Edenia gomezpompae* | AM04 | KM246015 | - | KM246160 | - | González et al. 2007 |
| ***Edenia gomezpompae*** | **CBS 124106** | FJ839654 | - | FJ839619 | - | Crous et al. 2009 |
| *Edenia* sp. | UTHSC: DI16-264 | LN907407 | - | LT796858 | LT797098 | Valenzuela-Lopez et al. 2017 |
| *Edenia* sp. | UTHSC: DI16-260 | LN907403 | - | LT796855 | LT797095 | Valenzuela-Lopez et al. 2017 |
| ***Embarria clematidis*** | **MFLUCC 14-0652** | KT306953 | KT306956 | KT306949 | - | Ariyawansa et al. 2015a |

| Species | Strain/Voucher No. | GenBank Accession No. | | | | Refferences |
|---|---|---|---|---|---|---|
| | | LSU | SSU | ITS | TEF 1-α | |
| *Embarria clematidis* | MFLUCC 14-0976 | MG828987 | MG829099 | MG828871 | MG829194 | Wanasinghe et al. 2018 |
| ***Equiseticola fusispora*** | **MFLUCC 14-0522** | KU987669 | KU987670 | KU987668 | MG520895 | Abd-Elsalam et al. 2016, Phookamsak et al. 2017 |
| *Foliophoma fallens* | CBS 161.78 | GU238074 | GU238215 | KY929147 | - | Aveskamp et al. 2010, Crous and Groenewald 2017 |
| *Foliophoma fallens* | CBS 284.70 | GU238078 | GU238218 | KY929148 | - | Aveskamp et al. 2010, Crous and Groenewald 2017 |
| ***Galiicola pseudophaeosphaeria*** | **MFLU 14-0524** | KT326693 | - | KT326692 | MG520896 | Phookamsak et al. 2017 |
| ***Italica achilleae*** | **MFLUCC 14-0959** | MG829013 | MG829122 | MG828903 | MG829204 | Wanasinghe et al. 2018 |
| ***Juncaceicola italica*** | **MFLUCC 13-0750** | KX500107 | KX500108 | KX500110 | MG520897 | Phookamsak et al. 2017 |
| ***Juncaceicola luzulae*** | **MFLUCC 13-0780** | KX449530 | KX449531 | KX449529 | MG520898 | Tennakoon et al. 2016, Phookamsak et al. 2017 |
| ***Leptospora galii*** | **KUMCC 15-0521** | KX599548 | KX599549 | KX599547 | MG520899 | Phookamsak et al. 2017 |
| *Leptospora rubella* | CPC 11006 | DQ195792 | DQ195803 | DQ195780 | - | Crous et al. 2006 |
| ***Leptospora thailandica*** | **MFLUCC 16-0385** | KX655549 | KX655554 | KX655559 | KX655564 | Hyde et al. 2016 |
| *Loratospora aestuarii* | JK 5535B | GU301838 | GU296168 | - | - | Schoch et al. 2009 |
| ***Melnikia anthoxanthii*** | **MFLUCC 14-1010** | KU848204 | KU848205 | - | - | Wijayawardene et al. 2016 |
| ***"Muriphaeosphaeria" ambrosiae*** | **MFLU 15-1971** | KX765264 | - | KX765267 | - | Hyde et al. 2016 |
| ***Muriphaeosphaeria galatellae*** | **MFLUCC 14-0614** | KT438329 | KT438331 | KT438333 | - | Phukhamsakda et al. 2015 |
| *Muriphaeosphaeria galatellae* | MFLUCC 15-0769 | KT438330 | KT438332 | - | - | Phukhamsakda et al. 2015 |
| ***Neocamarosporium lamiacearum*** | **MFLUCC 17-560** | MF434279 | MF434367 | MF434191 | MF434454 | Wanasinghe et al. 2017 |
| ***Neosetophoma clematidis*** | **MFLUCC 13-0734** | KP684153 | KP684154 | KP744450 | - | Liu et al. 2015 |
| *Neosetophoma rosae* | MFLUCC 17-0844 | MG829035 | MG829141 | MG828926 | MG829219 | Wanasinghe et al. 2018 |
| ***Neosetophoma rosae*** | **MFLU 15-1073** | MG829034 | MG829140 | MG828925 | MG829218 | Wanasinghe et al. 2018 |
| ***Neosphaerellopsis thailandica*** | **CPC 21659** | KP170721 | - | KP170652 | - | Trakunyingcharoen et al. 2014 |
| ***Neostagonospora arrhenatheri*** | **MFLUCC 15-0464** | KX910091 | KX950402 | KX926417 | MG520901 | Phookamsak et al. 2017, Thambugala et al. 2017 |
| ***Neostagonospora caricis*** | **CBS 135092** | KF251667 | - | KF251163 | - | Quaedvlieg et al. 2013 |
| ***Neostagonospora phragmitis*** | **MFLUCC 16-0493** | KX910090 | KX950401 | KX926416 | MG520902 | Phookamsak et al. 2017, Thambugala et al. 2017 |
| ***Neostagonosporella sichuanensis*** | **MFLUCC 18-1228** | MH368073 | MH368079 | MH368088 | MK313851 | This study |
| *Neostagonosporella sichuanensis* | MFLUCC 18-1231 | MH368074 | MH368080 | MH368089 | - | This study |
| *Neostagonosporella sichuanensis* | MFLU 18-1223 | MH394690 | MH394687 | MK296469 | MK313854 | This study |
| ***Neosulcatispora agaves*** | **CPC 26407** | KT950867 | - | KT950853 | - | Crous et al. 2015b |
| ***Nodulosphaeria guttulatum*** | **MFLUCC 15-0069** | KY496726 | KY501115 | KY496746 | KY514394 | Tibpromma et al. 2017 |
| ***Nodulosphaeria multiseptata*** | **MFLUCC 15-0078** | KY496728 | KY501116 | KY496748 | KY514396 | Tibpromma et al. 2017 |
| ***Nodulosphaeria scabiosae*** | **MFLUCC 14-1111** | KU708846 | KU708842 | KU708850 | KU708854 | Mapook et al. 2016 |
| ***Ophiobolopsis italica*** | **MFLUCC 17-1791** | MG520959 | MG520977 | MG520939 | MG520903 | Phookamsak et al. 2017 |
| ***Ophiobolus artemisiae*** | **MFLUCC 14-1156** | KT315509 | MG520979 | KT315508 | MG520905 | Phookamsak et al. 2017 |
| *Ophiobolus artemisiae* | MFLU 15-1966 | MG520960 | MG520978 | MG520940 | MG520904 | Phookamsak et al. 2017 |
| ***Ophiobolus disseminans*** | **MFLUCC 17-1787** | MG520961 | MG520980 | MG520941 | MG520906 | Phookamsak et al. 2017 |
| ***Ophiobolus italicus*** | **MFLUCC 14-0526** | KY496727 | - | KY496747 | KY514395 | Tibpromma et al. 2017 |

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|---|---|---|---|---|---|---|
| | | LSU | SSU | ITS | TEF 1-α | |
| ***Ophiobolus rossicus*** | **MFLU 17-1639** | MG520964 | MG520983 | MG520944 | MG520909 | Phookamsak et al. 2017 |
| *Ophiobolus rudis* | CBS 650.86 | GU301812 | AF164356 | KY090650 | GU349012 | Liew et al. 2000, Schoch et al. 2009, Ahmed et al. 2016 |
| ***Ophiobolus senecionis*** | **MFLUCC 13-0575** | KT728366 | - | KT728365 | - | Tibpromma et al. 2015 |
| ***Ophiosimulans tanaceti*** | **MFLUCC 14-0525** | KU738891 | KU738892 | KU738890 | MG520910 | Tibpromma et al. 2016b, Phookamsak et al. 2017 |
| *Ophiosphaerella agrostidis* | MFLUCC 11-0152 | KM434281 | KM434290 | KM434271 | KM434299 | Phookamsak et al. 2014a |
| *Ophiosphaerella agrostidis* | MFLUCC 12-0007 | KM434282 | KM434291 | KM434272 | KM434300 | Phookamsak et al. 2014a |
| ***Ophiosphaerella aquatica*** | **MFLUCC 14-0033** | KX767089 | KX767090 | KX767088 | MG520911 | Ariyawansa et al. 2015a, Phookamsak et al. 2017 |
| ***Paraleptosphaeria rubi*** | **MFLUCC 14-0211** | KT454718 | KT454733 | KT454726 | - | Ariyawansa et al. 2015b |
| ***Paraophiobolus arundinis*** | **MFLUCC 17-1789** | MG520965 | MG520984 | MG520945 | MG520912 | Phookamsak et al. 2017 |
| ***Paraophiobolus plantaginis*** | **MFLUCC 17-0245** | KY815010 | KY815012 | KY797641 | MG520913 | Hyde et al. 2017, Phookamsak et al. 2017 |
| ***Paraphoma chrysanthemicola*** | **CBS 522.66** | GQ387582 | GQ387521 | KF251166 | - | de Gruyter et al. 2010, Quaedvlieg et al. 2013 |
| ***Paraphoma radicina*** | **CBS 111.79** | KF251676 | EU754092 | KF251172 | - | de Gruyter et al. 2009, Quaedvlieg et al. 2013 |
| ***Parastagonospora dactylidis*** | **MFLUCC 13-0375** | KU058722 | - | KU058712 | - | Li et al. 2015 |
| ***Parastagonospora italica*** | **MFLUCC 13-0377** | KU058724 | MG520985 | KU058714 | MG520915 | Li et al. 2015, Phookamsak et al. 2017 |
| ***Parastagonospora minima*** | **MFLUCC 13-0376** | KU058723 | MG520986 | KU058713 | MG520916 | Li et al. 2015, Phookamsak et al. 2017 |
| ***Parastagonospora uniseptata*** | **MFLUCC 13-0387** | KU058725 | MG520987 | KU058715 | MG520917 | Li et al. 2015, Phookamsak et al. 2017 |
| ***Parastagonosporella fallopiae*** | CBS 135981 | MH460545 | - | MH460543 | - | Bakhshi et al. 2018 |
| *Parastagonosporella fallopiae* | CCTU 1151.1 | MH460546 | - | MH460544 | - | Bakhshi et al. 2018 |
| ***Phaeopoacea festucae*** | **MFLUCC 17-0056** | KY824767 | KY824769 | KY824766 | - | Thambugala et al. 2017 |
| *Phaeopoacea phragmiticola* | CBS 459.84 | KF251691 | KY090700 | KF251188 | - | Quaedvlieg et al. 2013, Ahmed et al. 2016 |
| ***Phaeosphaeria acaciae*** | **MFLUCC 17-0320** | KY768868 | KY768870 | KY768869 | - | Hyde et al. 2017 |
| ***Phaeosphaeria chiangraina*** | **MFLUCC 13-0231** | KM434280 | KM434289 | KM434270 | KM434298 | Phookamsak et al. 2014a |
| *Phaeosphaeria musae* | MFLUCC 11-0151 | KM434278 | KM434288 | KM434268 | KM434297 | Phookamsak et al. 2014a |
| ***Phaeosphaeria oryzae*** | **CBS 110110** | KF251689 | GQ387530 | KF251186 | - | de Gruyter et al. 2010, Quaedvlieg et al. 2013 |
| ***Phaeosphaeria thysanolaenicola*** | **MFLUCC 10-0563** | KM434276 | KM434286 | KM434266 | KM434295 | Phookamsak et al. 2014a |
| ***Phaeosphaeriopsis dracaenicola*** | **MFLUCC 11-0157** | KM434283 | KM434292 | KM434273 | KM434301 | Phookamsak et al. 2014a |
| ***Phaeosphaeriopsis glaucopunctata*** | **MFLUCC 13-0265** | KJ522477 | KJ522481 | KJ522473 | MG520918 | Thambugala et al. 2014, Phookamsak et al. 2017 |
| ***Phaeosphaeriopsis triseptata*** | **MFLUCC 13-0271** | KJ522479 | KJ522484 | KJ522475 | MG520919 | Thambugala et al. 2014, Phookamsak et al. 2017 |
| *Phoma herbarum* | AFTOL-ID 1575 | DQ678066 | DQ678014 | - | DQ677909 | Schoch et al. 2006 |
| ***Stemphylium vesicarium*** | **CBS 191.86** | GU238160 | GU238232 | EF452449 | DQ471090 | Spatafora et al. 2006, Andrie et al. 2008, Aveskamp et al. 2010 |
| ***Stemphylium botryosum*** | **CBS 714.68** | KC584345 | KC584603 | EF452450 | DQ677888 | Schoch et al. 2006, Andrie et al. 2008, Woudenberg et al. 2013 |
| *Poaceicola arundinis* | MFLUCC 14-1060 | KX655548 | KX655553 | KX655558 | - | Hyde et al. 2016 |
| *Poaceicola arundinis* | MFLU 16-0158 | MG829057 | MG829162 | MG828947 | MG829229 | Wanasinghe et al. 2018 |
| ***Poaceicola forlicesenica*** | **MFLUCC 15-0470** | KX910095 | KX950406 | KX926422 | MG520922 | Phookamsak et al. 2017, Thambugala et al. 2017 |
| ***Poaceicola garethjonesii*** | **MFLUCC 15-0469** | KX954390 | KY205717 | KX926425 | MG520923 | Phookamsak et al. 2017, Thambugala et al. 2017 |
| ***Populocrescentia ammophilae*** | **MFLUCC 17-0665** | MG829059 | MG829164 | MG828949 | MG829231 | Wanasinghe et al. 2018 |

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|---|---|---|---|---|---|---|
| | | LSU | SSU | ITS | TEF 1-α | |
| ***Populocrescentia forlicesenensis*** | **MFLUCC 14-0651** | KT306952 | KT306955 | KT306948 | MG520925 | Ariyawansa et al. 2015a, Phookamsak et al. 2017 |
| ***Populocrescentia rosae*** | **TASM 6125** | MG829060 | MG829165 | - | MG829232 | Wanasinghe et al. 2018 |
| ***Pseudoophiobolus achilleae*** | **MFLU 17-0925** | MG520966 | - | MG520946 | - | Phookamsak et al. 2017 |
| ***Pseudoophiobolus galii*** | **MFLUCC 17-2257** | MG520967 | MG520989 | MG520947 | MG520926 | Phookamsak et al. 2017 |
| ***Pseudoophiobolus urticicola*** | **KUMCC 17-0168** | MG520975 | MG520996 | MG520955 | MG520933 | Phookamsak et al. 2017 |
| ***Pseudophaeosphaeria rubi*** | **MFLUCC 14-0259** | KX765299 | KX765300 | KX765298 | - | Hyde et al. 2016 |
| ***Pyrenochaeta nobilis*** | **CBS 407.76** | DQ678096 | - | EU930011 | DQ677936 | Ferrer et al. 2006, Schoch et al. 2006 |
| *Pyrenophora bromi* | DAOM 127414 | JN940074 | JN940954 | JN943666 | - | Schoch et al. 2012 |
| *Pyrenophora dactylidis* | DAOM 92161 | JN940087 | - | JN943667 | - | Schoch et al. 2012 |
| ***Sclerostagonospora lathyri*** | **MFLUCC 14-0958** | MG829066 | MG829170 | MG828955 | MG829235 | Wanasinghe et al. 2018 |
| *Sclerostagonospora* sp. | CBS 118152 | JX517292 | - | JX517283 | - | Crous et al. 2012. |
| ***Scolicosporium minkeviciusii*** | **MFLUCC 12-0089** | KF366382 | KF366383 | - | - | Wijayawardene et al. 2013 |
| ***Septoriella phragmitis*** | **CPC 24118** | KR873279 | - | KR873251 | - | Crous et al. 2015c |
| *Setomelanomma holmii* | CBS 110217 | GQ387633 | GQ387572 | KT389542 | GU349028 | Schoch et al. 2009, de Gruyter et al. 2010, Chen et al. 2015 |
| ***Setophoma chromolaena*** | **CBS 135105** | KF251747 | - | KF251244 | - | Quaedvlieg et al. 2013 |
| ***Setophoma sacchari*** | **CBS 333.39** | GQ387586 | GQ387525 | KF251245 | - | de Gruyter et al. 2010 |
| *Setophoma sacchari* | MFLUCC 12-0241 | KJ476147 | KJ476149 | KJ476145 | KJ461318 | Phookamsak et al. 2014b |
| *Setophoma sacchari* | MFLUCC 11-0154 | KJ476146 | KJ476148 | KJ476144 | KJ461319 | Phookamsak et al. 2014b |
| ***Setophoma vernoniae*** | **CPC 23123** | KJ869198 | - | KJ869141 | - | Crous et al. 2014 |
| *Staurosphaeria rhamnicola* | MFLUCC 17-0813 | MF434288 | MF434376 | MF434200 | MF434462 | Wanasinghe et al. 2017 |
| ***Staurosphaeria rhamnicola*** | **MFLUCC 17-0814** | MF434289 | MF434377 | MF434201 | MF434463 | Wanasinghe et al. 2017 |
| *Sulcispora pleurospora* | CBS 460.84 | - | - | AF439498 | - | Câmara et al. 2002 |
| ***Sulcispora supratumida*** | **MFLUCC 14-0995** | KP271444 | KP271445 | KP271443 | - | Senanayake et al. 2018 |
| ***Tintelnotia destructans*** | **CBS 127737** | KY090664 | KY090698 | KY090652 | - | Ahmed et al. 2016 |
| ***Tintelnotia opuntiae*** | **CBS 376.91** | GU238123 | GU238226 | KY090651 | - | Aveskamp et al. 2010, Ahmed et al. 2016 |
| ***Vagicola chlamydospora*** | **MFLUCC 15-0177** | KU163654 | KU163655 | KU163658 | - | Jayasiri et al. 2015 |
| ***Vrystaatia aloeicola*** | **CBS 135107** | KF251781 | - | KF251278 | - | Quaedvlieg et al. 2013 |
| ***Wojnowicia italica*** | **MFLUCC 13-0447** | KX430001 | KX430002 | KX342923 | KX430003 | Hyde et al. 2016 |
| ***Wojnowicia lonicerae*** | **MFLUCC 13-0737** | KP684151 | KP684152 | KP744471 | - | Liu et al. 2015 |
| ***Wojnowiciella dactylidis*** | **MFLUCC 13-0735** | KP684149 | KP684150 | KP744470 | - | Liu et al. 2015 |
| ***Wojnowiciella eucalypti*** | **CPC 25024** | KR476774 | - | KR476741 | - | Crous et al. 2015a |
| ***Wojnowiciella spartii*** | **MFLUCC 13-0402** | KU058729 | MG520998 | KU058719 | MG520937 | Li et al. 2015, Phookamsak et al. 2017 |
| *Xenoseptoria neosaccardoi* | CBS 120.43 | KF251783 | - | KF251280 | - | Quaedvlieg et al. 2013 |
| ***Xenoseptoria neosaccardoi*** | **CBS 128665** | KF251784 | - | KF251281 | - | Quaedvlieg et al. 2013 |
| *Yunnanensis phragmitis* | MFLUCC 17-0315 | MF684863 | MF684867 | MF684862 | MF683624 | Karunarathna et al. 2017 |
| ***Yunnanensis phragmitis*** | **MFLUCC 17-1361** | MF684865 | MF684864 | MF684869 | - | Karunarathna et al. 2017 |

tive consistency index [RC] and homoplasy index [HI] were determined under different optimality criteria. The robustness was assessed using bootstrap analysis with 1,000 replications (Hillis and Bull 1993). The Kishino-Hasegawa tests were made in order to determine whether trees were significantly different (Kishino and Hasegawa 1989).

Bayesian inference analysis was conducted with MrBayes v. 3.2.2 (Ronquist et al. 2012) and a Bayesian posterior probability (BYPP) was determined by Markov Chain Monte Carlo sampling (MCMC). The Bayesian parameters were set up to "Lset applyto= (all) nst=6 rates=invgamma; prset applyto= (all) statefreqpr=dirichlet (1,1,1,1)". Six simultaneous Markov chains were set up to 10,000,000 generations and trees were sampled every 100$^{th}$ generation. The programme was automatically terminated when the average standard deviation of split frequencies reached below 0.01 (Maharachchikumbura et al. 2015). The distribution of log-likelihood scores were examined to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence, using Tracer v.1.6 program (Rambaut et al. 2013). The first 10% of generated trees representing the burn-in phase were discarded and the remaining trees were used to calculate posterior probabilities of the majority rule consensus tree.

The tree was made in FigTree v. 1.4.3 (Rambaut 2016) and edited in Adobe Illustrator CS6 (Adobe Systems Inc., United States). The finalised alignment and tree were submitted in TreeBASE, submission ID: 23697 (http://www.treebase.org).

**Notes.** Ex-type strains are given in bold and the new species in this study is in red. "-" means that the sequence is missing or unavailable.

**Abbreviations. AFTOL**: Assembling the Fungal Tree of Life; **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **CCTU**: Culture Collection of Tabriz University, Tabriz, Iran; **CPC**: Culture Collection of P.W. Crous; **DAOM**: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; **JK**: J. Kohlmeyer; **KUMCC**: Kunming Institute of Botany Culture Collection, Chinese Academy of Sciences, Kunming, China; **MFLU**: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **TASM**: Tashkent Mycological Herbarium, Institute of Botany and Zoology, Uzbek Academy of Science, Uzbekistan; **UTHSC**: Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA.

## Results

### Phylogenetic analyses

In this phylogenetic analysis, we include all representative sequences of genera in Phaeosphaeriaceae and other representative genera and species in Pleosporineae and Massarineae. The final concatenated dataset containing 138 ingroup taxa within the suborder Pleosporineae, included 56 currently existing genera in Phaeosphaeriaceae, with 3559 characters including gaps (917 characters for LSU, 1046 for SSU, 681 for ITS and 915 for TEF 1-α). Single gene datasets of LSU, SSU, ITS and TEF 1-α were

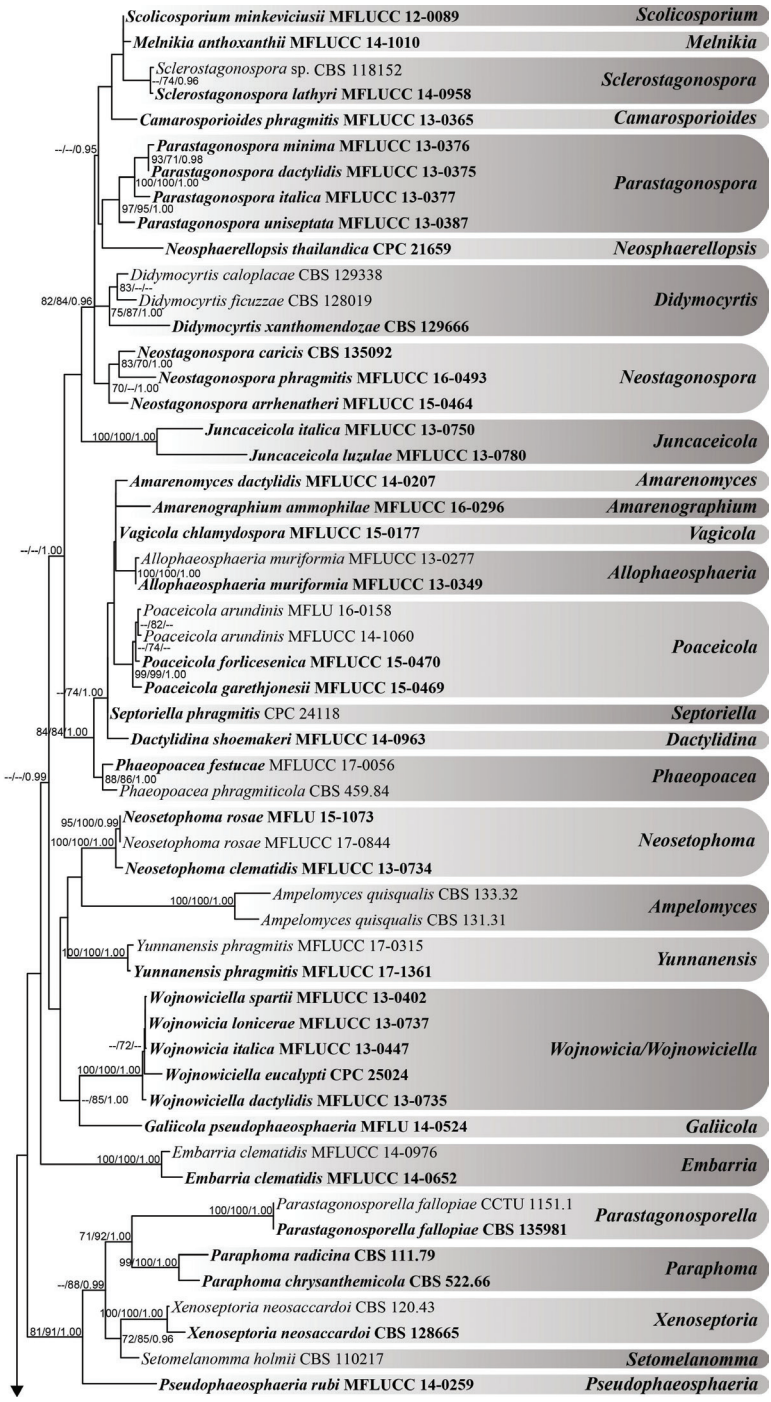


**Figure 1.** Phylogram generated from maximum likelihood analysis (RAxML) based on combined LSU, SSU, ITS and TEF 1-α sequenced data of taxa from the family Phaeosphaeriaceae and other representative species in Pleosporineae and Massarineae. The tree is rooted to *Cyclothyriella rubronotata* (CBS 121892), *C. rubronotata* (CBS 141486), *Didymosphaeria rubi-ulmifolii* (MFLUCC 14-0024) and *D. variabile* (CBS 120014).

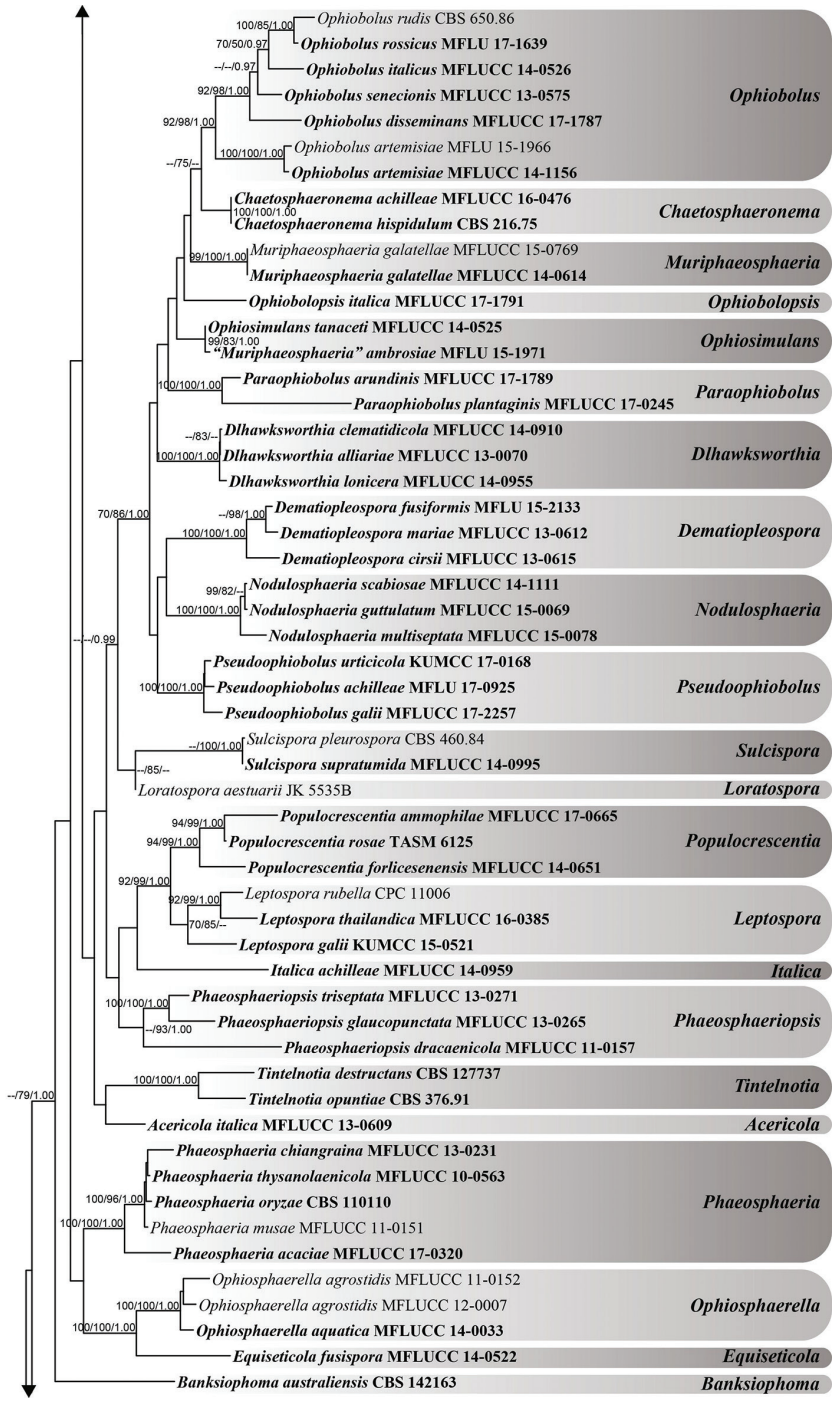


**Figure 1.** (Continued) Bootstrap support values of maximum parsimony and maximum likelihood (MPBP, left; MLBP, middle) equal to or greater than 70% and Bayesian posterior probabilities (BYPP, right) equal to or greater than 0.95 are provided. The type strains were highlighted in bold and the newly generated sequences are highlighted in red.

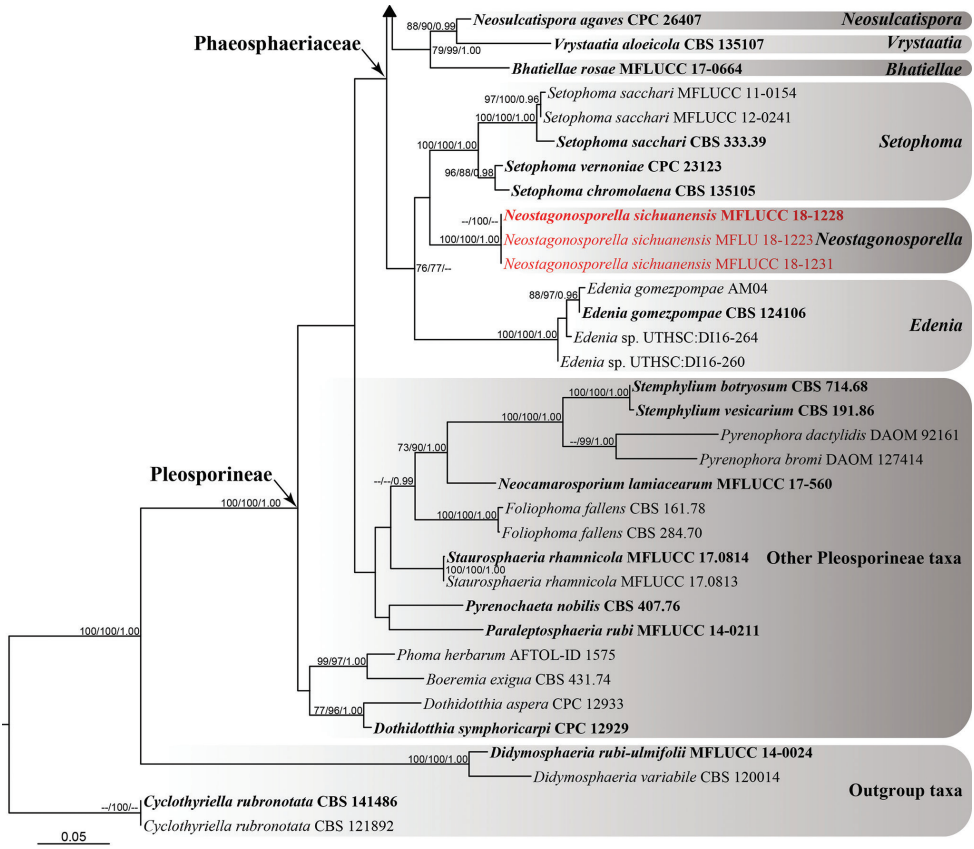


**Figure 1.** (Continued)

initially analysed and checked for topological congruence but these were not significantly different (data not shown). Support values of MP, ML and BI analyses (equal to or higher than 70% for MPBP and MLBP and 0.95 for BYPP) are shown in Fig. 1 which is the best scoring tree generated from ML. The phylogenetic trees generated from ML analyses were similar to previous phylogenies including Phaeosphaeriaceae (Phookamsak et al. 2014a, b, 2017, Jayasiri et al. 2015, Li et al. 2015, Liu et al. 2015, Phukhamsakda et al. 2015, Tibpromma et al. 2015, 2016, 2017, Hyde et al. 2016, Mapook et al. 2016, Ahmed et al. 2017, Huang et al. 2017, Karunarathna et al. 2017, Thambugala et al. 2017, Ariyawansa et al. 2018, Bakhshi et al. 2018, Senanayake et al. 2018, Wanasinghe et al. 2018).

The best scoring RAxML tree with the final optimisation had a likelihood value of -32702.569414. The matrix had 1387 distinct alignment patterns and 32.39% in this alignment is the gaps and completely undetermined characters. Estimated base frequencies were as follows: A=0.244424, C=0.233850, G=0.265929, T=0.255797, with substitution rates AC=1.171601, AG=2.805496, AT=2.145028, CG=0.771605, CT=6.035018 and GT=1.000000. The gamma distribution shape parameter $\alpha$=0.167161 and the Tree-Length=5.334112. The maximum parsimony dataset con-

sisted of 3559 characters, of which 2580 characters were constant, 217 were parsimony-uninformative and 762 were parsimony-informative. All characters were of type 'unord' with equal weight. The parsimony analysis resulted in a thousand equally most parsimonious trees with a length of 5829 steps (CI = 0.270, RI = 0.654, RC = 0.177, HI = 0.730). Bayesian posterior probabilities were determined by MCMC and the final average standard deviation of split frequencies was 0.009939.

*Neostagonosporella sichuanensis* clusters in the family Phaeosphaeriaceae with strong support (100% MLBP/100% MPBP/1.00 BYPP) and nucleotide sequences from all strains are the same and it confirms that our three collections are the same species. The multigene analyses show that *N. sichuanensis* is phylogenetically close to the genus *Setophoma* and *Edenia* and separated from the remaining genera of the family in a distinct clade with moderate bootstrap support.

## Taxonomy

***Neostagonosporella*** **C.L. Yang, X.L. Xu & K.D. Hyde, gen. nov.**
Index Fungorum number: IF555713
Facesoffungi number: FoF 05490

**Type species.** *Neostagonosporella sichuanensis* C.L. Yang, X.L. Xu & K.D. Hyde

**Etymology.** Name reflects the morphological similarity to the genus *Stagonospora*.

**Description.** *Parasitic* on living to nearly dead stems and branches of bamboo. ***Sexual morph:*** *Ascostromata* coriaceous, visible as raised to superficial on host, gregarious, multi-loculate, ellipsoidal, globose to subglobose or irregular in shape, dark brown to black, glabrous. *Locules* globose to subglobose, with a centrally located ostiole, lacking periphyses. *Peridium* multi-layered, of brown to dark brown, pseudoparenchymatous cells of *textura angularis*. *Hamathecium* comprising trabeculate, anastomosed pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded with an ocular chamber. *Ascospores* overlapping biseriate, hyaline, cylindrical to fusiform, septate, smooth-walled, surrounded by a distinct mucilaginous sheath. ***Asexual morph:*** Coelomycetous. *Conidiostromata* pycindial, coriaceous, superficial, dark brown to black, fusiform to long fusiform or rhomboid, multi-loculate, solitary, glabrous. *Pycnidia* globose to subglobose, ostiolate. *Pycnidial wall* comprising multi-layered, of dark brown to black, pseudoparenchymatous cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform to subcylindrical, smooth, hyaline, enteroblastic, phialidic, arising from inner layer of pycnidial wall. *Macroconidia* hyaline, subcylindrical to cylindrical, septate, nearly equidistant between septa, smooth-walled, sometimes surrounded by a mucilaginous sheath when immature. *Microconidia* hyaline, varied in shape, aseptate, smooth-walled, with small guttulate.

**Notes.** *Stagonospora* resembles *Neostagonosporella* in asexual status, but *Stagonospora* differs in having generally uni-loculate conidiomata, a thick-walled pycnidial wall, doliiform, holoblastic conidiogenous cells with several percurrent proliferations at the

apex and mostly smooth to verruculose conidia (Quaedvlieg et al. 2013, Hyde et al. 2016). Phylogenetic analyses based on a concatenated LSU, SSU, ITS and TEF 1-α sequence data (Fig. 1) show that *Neostagonosporella* is closely related to *Setophoma* and *Edenia* within Phaeosphaeriaceae. There are some significant differences in morphology between these genera and these are summarised in Table 2. Six species are currently accepted in *Setophoma* and two species in *Edenia* and both of them occur on different grasses but only our new collections are parasitic on bamboo. Comparison of DNA sequence data across four gene regions reveals base pair differences as shown in Table 3. Phylogenetic analyses also clearly differentiate these taxa (Fig. 1). It is the first time that species with massarineae-like morphology occurring on bamboo, were found in the Phaeosphaeriaceae. Based on molecular phylogeny, the new genus is introduced in Phaeosphaeriaceae to accommodate a massarineae-like taxon.

**Table 2.** Morphological comparison of *Neostagonosporella*, *Setophoma* and *Edenia*.

| Morphology | *Neostagonosporella* (Type: *N. sichuanensis*) | *Setophoma* (Type: *S. terrestris*) | *Edenia* (Type: *E. gomezpompae*) |
|---|---|---|---|
| Ascostromata | Multi-loculate, globose to subglobose or irregular | Uni-loculate, globose | |
| Locules | Globose to subglobose, with a central ostiole, lacking periphyses | Globose, with a central ostiole | |
| Pseudoparaphyses | Narrow, septate, trabeculae, longer than asci | Broad, septate, prominently branched, constricted at septa, sometimes anastomosing | |
| Asci | Cylindrical to cylindric-clavate, short-pedicellate | Cylindrical or subcylindrical, fasciculate, pedicellate | |
| Ascospores | Bi-seriate, hyaline, cylindrical to fusiform, smooth-walled, transversely multi-septate | Uni- to multi-seriate, light brown or red brown, fusiform, sometimes verruculose, 2–3-septate | |
| Conidiostromata | Multi-loculate | Uni-loculate | |
| Pycnidia | Globose to subglobose, smooth, ostiolate | Globose to subglobose, setose, with papillate ostiolate | |
| Conidia | Two types. Macroconidia subcylindrical to cylindrical, transversely multi-septate, hyaline. Microconidia oval, ellipsoidal or long ellipsoidal, aseptate, hyaline | One type. Ellipsoidal to subcylindrical to subfusoid, aseptate, hyaline | One type. Ellipsoidal or slightly narrowed at base, aseptate, subhyaline |
| Others | On PDA, grey white, reverse dark brown. Hyphae developing by different angle branched and without forming rope-like strands | On PDA, iron-grey-olivaceous, reverse same. Hyphae undescribed | On PDA, pinkish-white, reverse reddish-brown, velvety to floccose. Hyphae frequently developing by 90° angle branched and forming rope-like strands |
| References | This study | de Gruyter et al. 2010, Quaedvlieg et al. 2013, Phookamsak et al. 2014a, b, Crous et al. 2016, Thambugala et al. 2017 | González et al. 2007, Sun et al. 2013 |

**Table 3.** Comparison of DNA sequence data *Parastagonosporella* vs *Edenia* and *Setophoma*.

| Gene region | *Parastagonosporella* vs *Edenia* | *Parastagonosporella* vs *Setophoma* |
|---|---|---|
| LSU | 12/819 (1.47%) | 13/818 (1.6%) |
| SSU | NA* | 4/981 (0.4%) |
| TEF | 47/869 (5.41%) | 43/868 (5%) |
| ITS | 89/515 (17.28%) | 66/515 (12.8%) |

*SSU is not available for *Edenia*

***Neostagonosporella sichuanensis*** **C.L. Yang, X.L. Xu & K.D. Hyde, sp. nov.**
Index Fungorum number: IF555714
Facesoffungi number: FoF 05491
Figs 2–3

**Type.** CHINA, Sichuan Province, Ya'an City, Yucheng District, Kongping Township, Alt. 1133 m, 29°50.14'N 103°03'E, on living to nearly dead branches of *Phyllostachys heteroclada* Oliv. (Poaceae), 8 April 2016, C.L. Yang and X.L. Xu, YCL201604001 (MFLU 18-1212/SICAU 16-0001, **holotype**), ex-type living culture, MFLUCC 18-1228/SICAUCC 16-0001; Sichuan Province, Ya'an City, Yucheng District, Yanchang Township, Alt. 951 m, 29°43.57'N 103°04.74'E, on nearly dead stems of *Phyllostachys heteroclada* Oliv. (Poaceae), 9 April 2017, C.L. Yang and X.L. Xu, YCL201704001 (MFLU 18-1220/SICAU 17-0001, **paratype**), ex-type living culture, MFLUCC 18-1231/SICAUCC 17-0001; Sichuan Province, Ya'an City, Lushan County, Longmen Township, Alt. 949 m, 30°15.74'N 102°59.27'E, on nearly dead branches of *Phyllostachys heteroclada* Oliv. (Poaceae), 12 September 2017, C.L. Yang and X.L. Xu, YCL201709002 (MFLU 18-1223, **paratype**).

**Etymology.** in reference to Sichuan Province where the specimens were collected.

**Description.** *Associated* with stem spot disease on living to nearly dead stems and branches of *Phyllostachys heteroclada* (Poaceae). ***Sexual morph:*** *Ascostromata* (0.5–) 1–2 (–4.5) × 0.8–1.3 mm long ( $\bar{x}$ = 1.9 × 1 mm, n = 50), 230–340 µm high ($\bar{x}$ = 290 µm, n = 20), ellipsoidal, globose to subglobose or irregular in shape, immersed in host epidermis, becoming raised to superficial, coriaceous, solitary to gregarious, multi-loculate, erumpent through host tissue, with dark brown to black, glabrous, ostiole, usually generating subrhombic to rhombic pale yellow stripes at ascostromatal fringe. *Locules* 230–300 µm high ($\bar{x}$ = 264 µm, n = 20), 330–460 µm diam. ($\bar{x}$ = 393 µm, n = 20), clustered, gregarious, globose to subglobose, with a centrally located ostiole, lacking periphyses. *Peridium* 18–35 µm wide ($\bar{x}$ = 27 µm, n = 20), composed of several layers of small, brown to dark brown pseudoparenchymatous cells of *textura angularis*, with inner hyaline layer, slightly thin at base, thick at sides towards apex, upper part fused with host tissue. *Hamathecium* composed of 1–2 µm ($\bar{x}$ = 1.59 µm, n = 50) wide, filiform, septate, trabeculate, anastomosed pseudoparaphyses, embedded in a hyaline gelatinous matrix. *Asci* 90–125 × 12.5–14 µm ($\bar{x}$ = 108.1 × 13.3 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, 7.8–14 µm long ($\bar{x}$ = 11 µm, n=20), apically rounded with an ocular chamber. *Ascospores* 30–35 × 6–7 µm ($\bar{x}$ = 31.9 × 6.6 µm, n = 50), overlapping bi-seriate, hyaline, cylindrical to fusiform or subcylindric-clavate, with rounded to acute ends, narrower towards end cells, sometimes narrower at lower end cell, straight or slightly curved, 5–8 transversely septa, mostly 7-septate, slightly constricted at septa, nearly equidistant between septa, guttulate, smooth-walled, surrounded by a mucilaginous sheath, 5–9 µm thick ($\bar{x}$ = 6.9 µm, n = 30). ***Asexual morph:*** Coelomycetous. *Conidiostromata* 9–13 × 1–2 mm long ($\bar{x}$ = 11.2 × 1.6 mm, n = 10), 320–350 µm high ($\bar{x}$ = 332 µm, n=10), fusiform to long fusiform or rhomboid, coriaceous, superficial, dark brown to black, multi-loculate, solitary, scattered, glabrous. *Pycnidia* 180–240 µm high ($\bar{x}$ = 209 µm, n = 20),

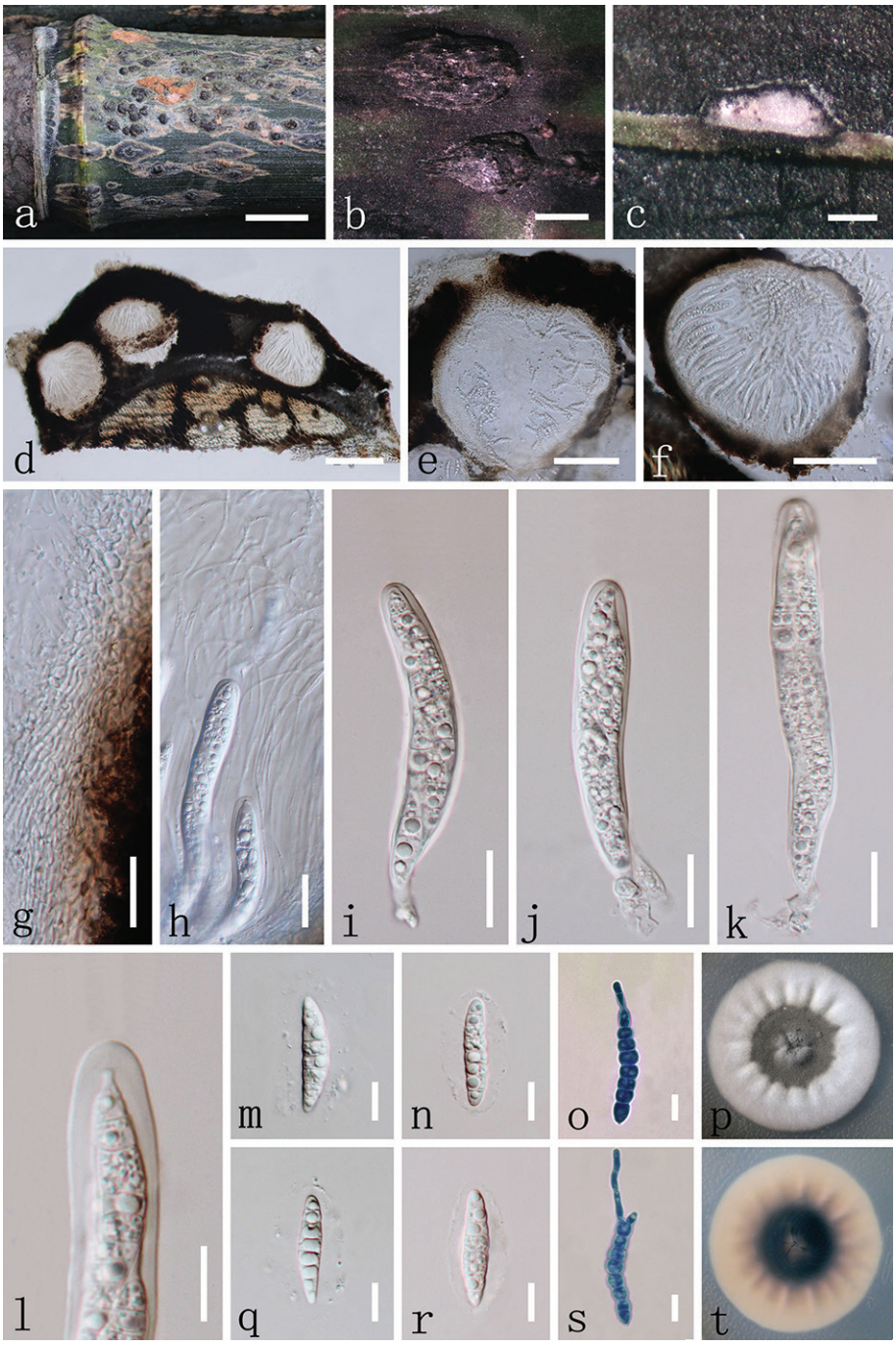


**Figure 2.** *Neostagonosporella sichuanensis* (MFLU 18-1212, holotype). **a** appearance of ascostromata on host **b** ascostroma **c, d** vertical section of ascostroma **e, f** close up of ascoma **g** peridium **h** trabeculate pseudoparaphyses and asci **i–k** asci **l** bitunicate asci, note ocular chamber **m, n, q, r** ascospores with mucilaginous sheath **o, s** germinated ascospores in lactate cotton blue reagent **p, t** colonies on PDA (p-from above, t-from below). Scale bars: 1 cm (**a**); 1 mm (**b**); 200 µm (**c, d**); 100 µm (**e, f**); 20 µm (**g–k**); 10 µm (**l–o, q–s**).

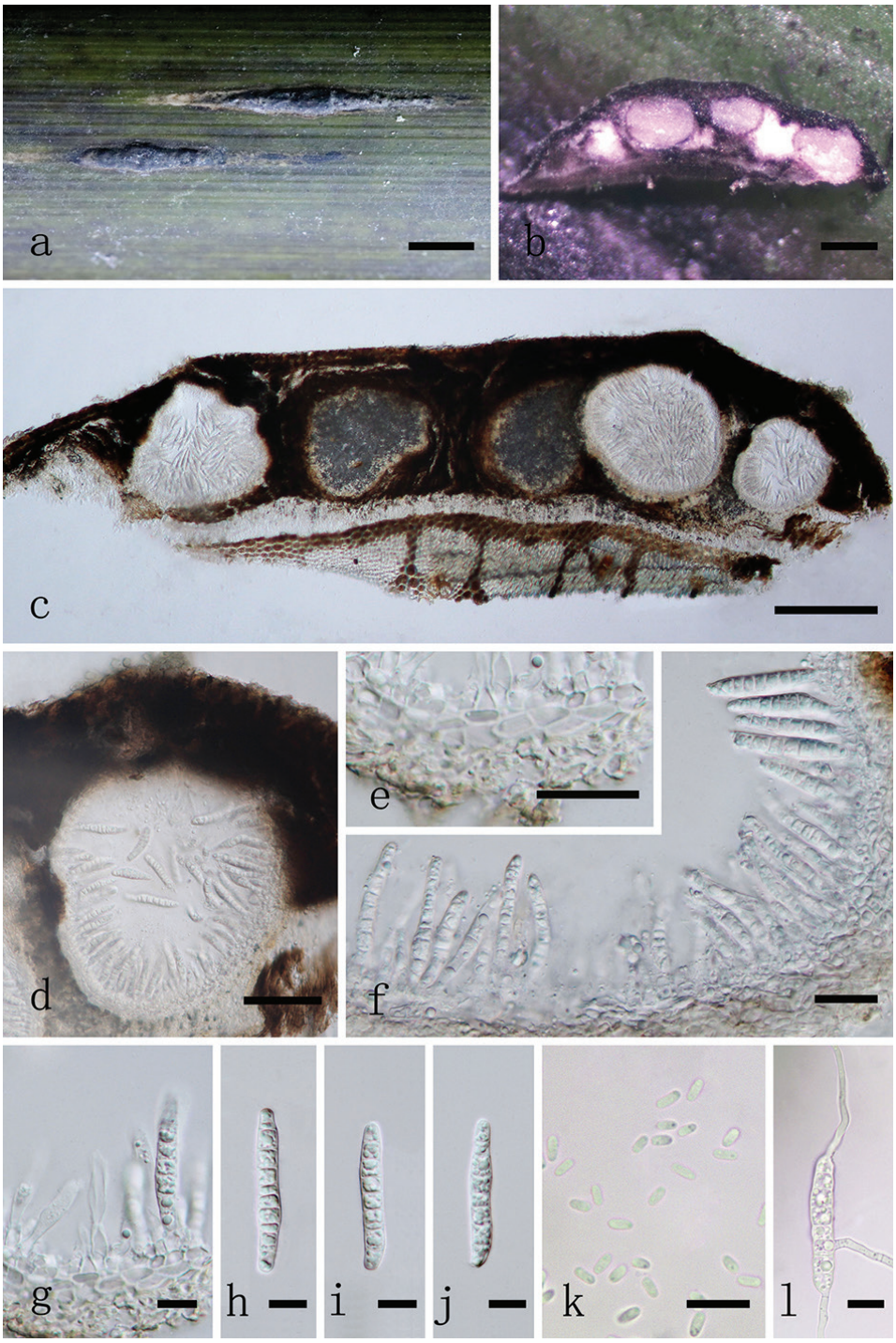


**Figure 3.** *Neostagonosporella sichuanensis* (MFLU 18-1220, paratype). **a** appearance of conidiomata on host **b, c** vertical section of conidioma **d** pycnidia **e** peridium **f, g** conidiogenous cells and developing conidia **h–l** conidia **m** germinated conidium. Scale bars: 1 cm (**a**); 200 μm (**b–d**); 20 μm (**e, f**); 10 μm (**g–m**).

170–240 µm diam. ($\bar{x}$ = 210 µm, n = 20), globose to subglobose, ostiolate. *Pycnidial wall* 12–18 (–23) µm wide ($\bar{x}$ = 15 µm, n = 20), comprising multi-layered, brown to dark brown pseudoparenchymatous cells, of *textura angularis*, paler towards inner layers, slightly thin at base, thick at sides towards apex, upper part fused with host tissue. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–5.5 (–7) × 3–4 µm ($\bar{x}$ = 4.17 × 3.29 µm, n = 20), ampulliform to subcylindrical, smooth, hyaline, enteroblastic, phialidic, formed from inner layer of pycnidial wall. *Macroconidia* (32.5–) 33.5–40 (–44) × (5–) 5.5–7 (–7.5) µm ($\bar{x}$ = 37.5 × 6.2 µm, n = 40), subcylindrical to cylindrical, narrowly rounded at both ends, sometimes curved, 7–13 transversely septa, nearly equidistant between septa, hyaline, smooth-walled, guttulate, sometimes surrounded by a mucilaginous sheath when immature. *Microconidia* (3–) 3.5–4 (–5) × (1–) 1.5–2 (–3) µm ($\bar{x}$ = 3.9 × 1.9 µm, n = 50), oval, ellipsoidal or elongate-ellipsoidal, aseptate, rounded at both ends, hyaline, smooth-walled, with small guttulate.

**Culture characteristics.** Ascospores germinating in sterilised water within 24 hours at 25°C, with germ tubes developed from each cell of ascospores, mostly from middle and end of spores. Colonies on PDA circular, with concentric circles, grey white in outer side, fawn in reverse side, grey in inner side, dark brown on back side. Conidial germination similar to ascospores. Conidiomata formed on PDA at 25°C after 75 days, pycnidial, solitary to gregarious, raised on agar, black dots, pyriform, globose to subglobose, or irregular, uniloculate, covered by white or grey hyphae. Conidia two types, macroconidia and microconidia and both longer than ones on host. Macroconidia (30–)40–48(–60.5) × (4–)5–6 µm ($\bar{x}$ = 43.8 × 5.2 µm, n = 50), hyaline, 4–7-septate, occasionally 3-septate, hyaline. Microconidia (3.5–)4–6(–12) × (1–)1.5–2(–3) µm ($\bar{x}$ = 5.3 × 1.9 µm, n = 50), aseptate, hyaline.

## Discussion

*Neostagonosporella* has a unique suite of characters that differentiate it from other genera in Phaeosphaeriaceae, such as multi-loculate ascostromata and trabeculate pseudoparaphyses. Trabeculate pseudoparaphyses have been shown to be uninformative at the higher taxonomic levels (Liew et al. 2000), but appear to be informative at the genus level. *Neostagonosporella* is the only genus of Phaeosphaeriaceae with this type of pseudoparaphyses. Phaeosphaeriaceous taxa have diverse morphological characteristics and the familial placement of some genera could not be resolved based on a concatenated phylogeny of three to four loci, because some genera contain only 1-2 described species (Crous et al. 2015a, 2015b, 2017a, Jayasiri et al. 2015, Phukhamsakda et al. 2015, Tibpromma et al. 2015, 2017, Abd-Elsalam et al. 2016, Hernández-Restrepo et al. 2016, Hyde et al. 2016, 2017, Wijayawardene et al. 2016, Ahmed et al. 2017, Karunarathna et al. 2017, Phookamsak et al. 2017, Bakhshi et al. 2018, Wanasinghe et al. 2018).

Species of Phaeosphaeriaceae have been found on various hosts and substrates, including plants, lichens, mushrooms, algae, human, soil and air (Saccardo 1883, Berlese and Voglino 1886, Phookamsak et al. 2014a, Ahmed et al. 2016, Karunarathna et al.

2017, Zhang et al. 2017, Joshi et al. 2018). However, most Phaeosphaeriaceous genera occur on plants of more than 65 host families, the majority of them being monocotyledons and herbaceous plants, such as Arecaceae, Asparagaceae, Compositae, Juncaceae, Leguminosae, Poaceae, Ranunculaceae, Restionaceae and Rosaceae etc. (Taylor and Hyde 2003, Quaedvlieg et al. 2013, Crous et al. 2015b, Hyde et al. 2016, Tibpromma et al. 2016a, Karunarathna et al. 2017, Phookamsak et al. 2017, Wanasinghe et al. 2018). Our new genus exists on Poaceae and at least 30 genera are reported within this family. Currently, 11 genera are observed only on Poaceae: *Amarenomyces*, *Bricookea*, *Camarosporioides*, *Dactylidina*, *Embarria*, *Melnikia*, *Neosphaerellopsis*, *Phaeopoacea*, *Sulcispora*, *Vagicola* and *Yunnanensis*, all of them being recently established except for *Amarenomyces*, *Bricookea* and *Sulcispora* (Eriksson 1981, Barr 1982, Shoemaker and Babcock 1989, Trakunyingcharoen et al. 2014, Ariyawansa et al. 2015b, Hyde et al. 2016, Wijayawardene et al. 2016, Karunarathna et al. 2017, Thambugala et al. 2017, Wanasinghe et al. 2018). Amongst them, all hosts are short herbaceous plants and there are no bamboo plants recorded so far, with the exception of a few species of *Ophiobolus* and *Phaeosphaeria* in the old literature (Penzig and Saccardo 1897, Miyake and Hara 1910). A large number of bamboo forests (more than 130 species) are distributed throughout Sichuan (Yi 1997) and, most likely, many Phaeosphaeriaceae species are waiting for exploration and discovery.

## Acknowledgements

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