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Phylogeny and taxonomy of three new *Ctenomyces* (Arthrodermataceae, Onygenales) species from China

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Abstract

Twelve *Ctenomyces* (Arthrodermataceae, Onygenales) strains were obtained and identified during a survey of keratinophilic fungi in soils from China. We used molecular identification combined with morphological evidence to delimit species, circumscribing five species in the genus. Three new species are herein described: *C. albus* **sp. nov.**, *C. obovatus* **sp. nov.** and *C. peltricolor* **sp. nov.** We also described, illustrated and compared the novel species with related species in the morphology.

Keywords

3 new species, Filamentous fungi, *Ctenomyces*, Morphology, Multigene

Introduction

The genus *Ctenomyces* belongs in the family Arthrodermataceae in the order Onygenales (Wijayawardene et al. 2018) with *C. serratus* as the type species (Eidam 1880, Wijayawardene et al. 2017). *Trichophyton lacticolor* and *Microsporum mentagrophytes* were transferred to *Ctenomyces*, being renamed *C. lacticolor* and *C. mentagrophytes* (Langeron and Milochevitch 1930). Subsequently, ten species (*Trichophyton denticulatum*, *T. equinum*, *T. eriotrephon*, *T. farinulentum*, *T. felineum*, *T. griseum*, *T. persicolor*, *T. gypseum* var. *radioplicatum*, *T. viannai* and *Epidermophyton gypseum*) were transferred to the genus (Nannizzi 1934). Thereafter, two new species, *C. bossae* and *C. trichophyticus*, were also described (Milochevitch 1935, Szathmáry 1960). Later studies showed that *C. bossae*

was misnamed; *C. trichophyticus* was invalid; *C. felineus* was a synonym of *C. serratus*; *C. persicolor* was transferred to *Nannizzia*, and named as *N. persicolor*; *C. mentagrophytes*, *C. equinus* and *C. eriotrephon* were transferred to *Trichophyton* and named as *T. mentagrophytes*, *T. equinum* and *T. eriotrephon*, respectively; *C. lacticolor* and *C. denticulatus* were transferred to *T. mentagrophytes*; the remaining five species *C. farinulentus*, *C. griseus*, *C. radioplicatus*, *C. viannai* and *C. gypseus* were transferred back to *Trichophyton* and *Epidermophyton*, which were eventually regarded as invalid or unclear (Orr and Huehn 1963, de Hoog et al. 2000, de Hoog et al. 2017). Therefore, *C. serratus* was ultimately regarded as the only valid species within the genus (Orr and Huehn 1963).

The main diagnostic criteria of *Ctenomyces* (*sensu* Oorschot 1980) are that conidia are verrucose, thick-walled, lightly pigmented, commonly with ampulliform swellings and mostly longer than 8 μm. Oorschot (1980) regarded *Ctenomyces* as a sexual morph of *Myceliophthora*. Furthermore, he transferred *Chrysosporium asperatum* to *Myceliophthora* as *M. vellerea* in a taxonomic revision of *Chrysosporium* and allied genera. *Myceliophthora vellerea* was regarded as a synonym of the asexual morph of *Ctenomyces serratus* (Guarro et al. 1985, Chabasse 1988). Phylogenetic analyses, based on ITS rDNA, demonstrated *Myceliophthora vellerea* to be a synonym of *Ctenomyces serratus* (van den Brink et al. 2012). De Hoog et al. (2017) expanded the breadth and understanding of dermatophytes in Arthrodermatacea based on multi-locus molecular data and *Ctenomyces serratus* is used as the only previously validated species of the genus *Ctenomyces*.

Investigation of keratinophilic fungi has been given more attention in some countries (Anbu et al. 2004, Zarrin and Haghgoo 2011, Shadzi et al. 2002). Many researchers have shown that keratinophilic fungi distribution is closely related to human and animal activity (Sharma and Sharma 2010). Therefore, we conducted a survey of keratinophilic fungi in places with high human activity in Guizhou, Shanxi and Gansu provinces in China and isolated 12 strains. By combining the ITS sequence and a multi-gene phylogeny and the morphological characteristics, we identify and describe three new species and one new record of *Ctenomyces* from China.

Materials and methods

Isolates

Twelve *Ctenomyces* strains were obtained from soil samples collected in Guizhou, Shanxi and Gansu province of China using a baiting technique (Vanbreuseghem 1952). Sterile chicken feather and human hair were combined with the soil samples and the samples were placed in sterile Petri dishes, which were moistened with sterile distilled water. The baited soil sample Petri dishes were incubated at 25 °C for 1 month and remoistened as necessary. When fungal growth was observed, those feathers with fungal growth were mixed with 9 ml of sterile water in an Erlenmeyer flask and 1 ml of suspensions were evenly spread on plates containing Sabouraud's dextrose agar (SDA) with chloramphenicol and cycloheximide medium. The plates were incubated

at 25 °C. The pure culture were then transferred to potato dextrose agar (PDA) plates for purification, the isolates were inoculated to test-tube slants and stored at 4 °C.

All holotypes and isotypes were deposited in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). Type strains and ex-type living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC) and the Institute of Fungus Resources, Guizhou University (GZUIFR). Taxonomic information of the new taxa was deposited in MycoBank (www.MycoBank.Org).

Morphology

Isolates were transferred to potato dextrose plates, incubated at 25 °C for 14 days and subjected to macroscopic examination. Fungal microscopic features were examined with a Nikon Ti-U microscope (Nikon, Japan) and photographed. Diagnostic features were then illustrated on the basis of these observations. Finally, the fungi were morphologically identified according to colony characteristics, conidiogenous structures and conidia (*sensu* Oorschot 1980).

DNA extraction, PCR amplification, sequencing

Total genomic DNA was extracted from fresh sporulating cultures after 14 days at 25 °C using a Fungal DNA Mini Kit (Omega Biotech, Doraville, GA, USA) according to the manufacturer's protocol and then stored at -20 °C. Three regions were amplified and sequenced, including the internal transcribed spacer (ITS) region using primers ITS1 and ITS4 (White et al. 1990); partial fragments of the RNA polymerase II largest subunit 2 (RPB2) gene region using primers 5F-Eur and 7cR-Eur (van den Brink et al. 2012); partial fragments of the translation elongation factor 1-alpha (EF1A) gene region using primers EF1-983F and EF1-2218R (van den Brink et al. 2012). The PCR mixture was prepared using a commercial kit (TSINGKE Biological Technology, Kunming, China) and contained 5 μl 10 × reaction buffer, 0.4 μl dNTPs (25μM), 0.2 μl T6 DNA polymerase (5 U/μl), 1 μl of each primer and 2 μl DNA template in a final volume of 25 μl. Reaction mixtures were pre-heated at 98 °C for 2 min and PCR was performed as follows: 30 cycles of 10 s at 98 °C, 10 s at 55 °C and 10 s 72 °C, with a final extension at 72 °C for 5 min and cooling at 4 °C. The PCR conditions were the same for all three markers. The resulting PCR products were sequenced by TSINGKE Biological Technology (Kunming, China) using the corresponding primers.

Phylogenetic analysis

Sequence data from the nine genera of Arthrodermataceae and *Myceliophthora lutea* sequences were used in the phylogenetic analysis. Details of newly generated and refer-

ence sequences retrieved from GenBank are listed in Table 1. Multiple sequence alignments for ITS, EF1A and RPB2 were achieved with MAFFT v.7.037b (Katoh and Standley 2013) and manually edited in the MEGA 6.06 (Tamura et al. 2013).

A total of 50 ITS sequences of 23 species and including *Myceliophthora lutea* (CBS 145.77 and MUCL 10070) as the outgroup taxon were used in the analysis. The data were analysed phylogenetically using Bayesian Markov chain Monte Carlo (MCMC) and maximum likelihood (ML). For the Bayesian analysis, two simultaneous Bayesian Inference (BI) Markov chain Monte Carlo runs were also executed for 10,000,000 generations, saving trees every 500 generations. Modeltest v3.7 suggested the GTR+I+G as the best-fit evolutionary model for dataset (Posada and Crandall 1988). After the BI analysis, each run was examined using the programme Tracer v1.5 (Drummond and Rambaut 2007) to determine whether the burn-in period was sufficient and to confirm that both runs had converged. ML analyses were performed using RAXML (Stamatakis and Alachiotis 2010) with the graphical user interface (GUI) (Silvestro and Michalak 2012) implementation and the GTRGAMMA model. The BI and ML analysis trees are available in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23736) and a consensus tree is presented in Figure 1.

A concatenated dataset (ITS+EF1A+RPB2) of five *Ctenomyces* species and *Myceliophthora lutea* (CBS 145.77 and MUCL 10070) was assembled using SequenceMatrix v. 1.7.8 (Vaidya 2011). Concordance between genes was assessed with the 'hompart' command of PAUP4.0b10 (Swofford 2002). Maximum likelihood (ML) phylogenetic analyses of the datasets were performed using RAxML (Stamatakis and Alachiotis 2010) with the graphical user interface (GUI) (Silvestro and Michalak 2012) implementation and the General Time Reversible (GTR) model. Bootstrap analysis with 1,000 replicates was used to estimate nodal support. Two simultaneous Bayesian Inference Markov chain Monte Carlo runs were also executed for 10,000,000 generations, saving trees every 500 generations. Modeltest v3.7 suggested the GTR+I+G as the best-fit evolutionary model for the dataset (Posada and Crandall 1988). After the BI analyses, each run was examined using the programme Tracer v1.5 (Drummond and Rambaut 2007) to determine whether the burn-in period was sufficient and to confirm that both runs had converged. The BI and ML analyses trees are available in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23736) and a consensus tree is presented in Figure 2.

Results

Phylogeny analysis

The ITS sequence alignment comprised 50 strains of 23 species (Table 1). The final dataset comprised 609 characters after alignment, which included nine genera of Arthrodermataceeae: *Arthroderma*, *Ctenomyces*, *Epidermophyton*, *Guarromyces*, *Lophophyton*, *Microsporum*, *Nannizzia*, *Paraphyton* and *Trichophyton* and *Myceliophthora lutea* (CBS 145.77 and MUCL 10070). No significant differences in topology were observed between the BI and ML phylogenies. The phylogenies show that each genus

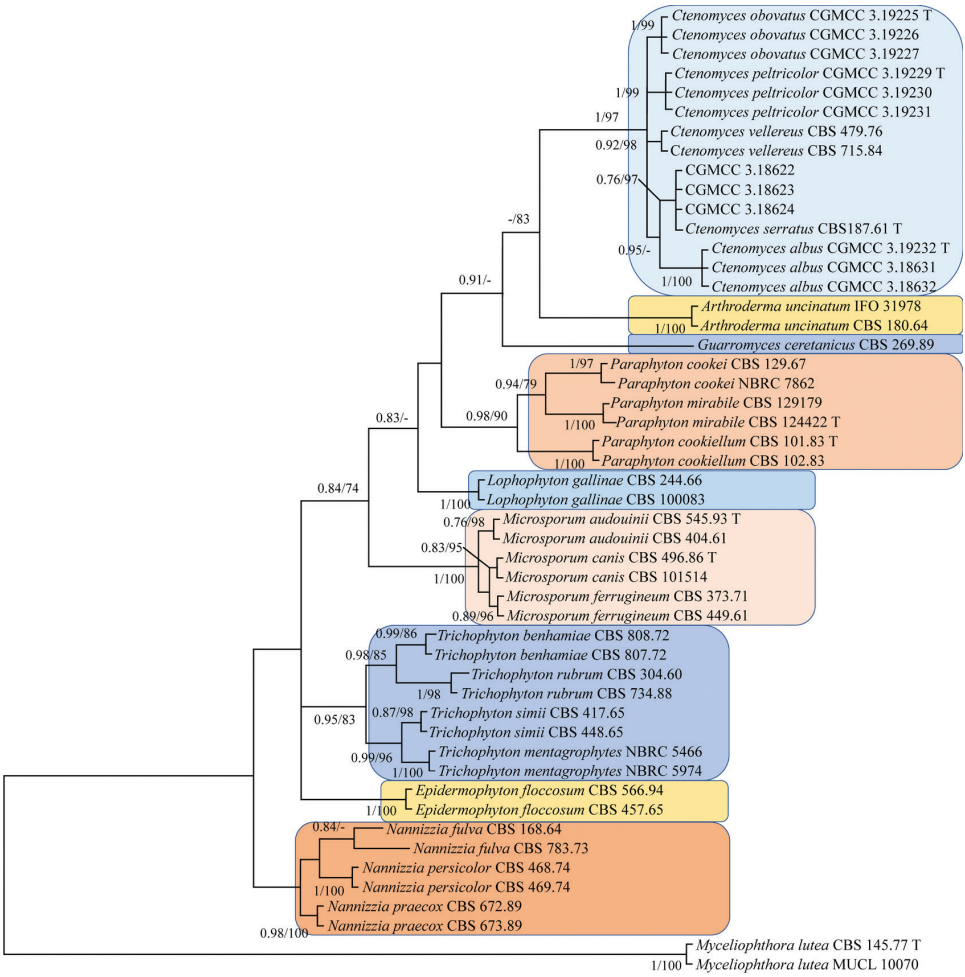

Figure 1. Phylogenetic tree of Arthrodermataceae based on the ITS dataset and *Myceliophthora lutea* (CBS 145.77 and MUCL 10070) as the outgroup taxon. Numbers at nodes are Bayesian posterior probabilities (left, BPP ≥0.75) and maximum likelihood bootstrap values (right, BS ≥70%).

to cluster into the expected subclades (Figure 1). The *Ctenomyces* strains all cluster in a single clade with good nodal support (BPP 1, BS 97%) and were divided into four subclades, including the *C. vellereus* and the type species, *C. serratus*. All species of *Ctenomyces* clustered in separate well-supported subclades comprising *C. serratus* (BPP 0.76, BS 97%), *C. vellereus* (BPP 0.92, BS 98%), *C. albus* (BPP 1, BS 100%), *C. obovatus* (BPP 1, BS 99%) and *C. peltricolor* (BPP 1, BS 99%).

The combined ITS+EF1A+RPB2 sequence alignment comprised 17 taxa of six species within *Ctenomyces* and *Myceliophthora* (Table 1). The total length of sequences were 1,719 (ITS: 453 bp, EF1A: 728 bp, RPB2: 538 bp) characters and *M. lutea* (CBS 145.77 and MUCL 10070) was the designated outgroup taxon. The BI and ML analysis yielded congruent tree topology (Figure 2). The phylogenies show that each genus

Table 1. Strains included in the phylogenetic analysis.

Taxa	Strain	GenBank accession		
		ITS	EF1A	RPB2
Arthroderma uncinatum	IFO 31978	JN134092	KM678197	
	CBS 180.64	MH858408	KM678070	
Ctenomyces albus	**CGMCC 3.19232 T = GZUIFR-QL17.10**	**MH793455**	**MH801900**	**MH801914**
	CGMCC 3.18631 = GZUIFR-QL17.11	**MH793456**	**MH801901**	**MH801915**
	CGMCC 3.18632 = GZUIFR-QL17.12	**MH793457**	**MH801902**	**MH801916**
C. obovatus	**CGMCC 3.19225 T = GZUIFR-L15**020	**MH793449**	**MH801894**	**MH801908**
	CGMCC 3.19226 = GZUIFR-L15021	**MH793450**	**MH801895**	**MH801909**
	CGMCC 3.19227 = GZUIFR-L15022	**MH793451**	**MH801896**	**MH801910**
C. peltricolor	**CGMCC 3.19229 T = GZUIFR-C03010**	**MH793458**	**MH801903**	**MH801917**
	CGMCC 3.19230 = GZUIFR-C03011	**MH793459**	**MH801904**	**MH801918**
	CGMCC 3.19231 = GZUIFR-C03012	**MH793460**	**MH801905**	**MH801919**
C. serratus	CBS 187.61 **T**	AJ877222		
	CGMCC 3.18622 = ZUIFR-S37.1	**MH793452**	**MH801897**	**MH801911**
	CGMCC 3.18623 = GZUIFR-S37.2	**MH793453**	**MH801898**	**MH801912**
	CGMCC 3.18624 = GZUIFR-S37.3	**MH793454**	**MH801899**	**MH801913**
C. vellereus	CBS 479.76	HQ871797	HQ871749	HQ871840
	CBS 715.84	HQ871795	HQ871747	HQ871841
Epidermophyton floccosum	CBS 566.94	MH862489		
	CBS 457.65	MH858667		
Guarromyces ceretanicus	CBS 269.89	MF926403		
Lophophyton gallinae	CBS 244.66	MH858789		
	CBS 100083	MF926355		
Microsporum audouinii	CBS 545.93 **T**	KT155940		
	CBS 404.61	MF926387		
M. canis	CBS 496.86 **T**	KT155928		
	CBS 101514	KT155672		
M. ferrugineum	CBS 449.61	KT155902		
	CBS 373.71	KT155886		
Nannizzia fulva	CBS 168.64	MH378229		
	CBS 783.73	MH378230		
N. persicolor	CBS 468.74	AJ000615		
	CBS 469.74	AJ000614		
N. praecox	CBS 672.89	MH378245		
	CBS 673.89	MH378246	KM678113	
Paraphyton cookei	CBS 129.67	MH858923	KM678064	
	NBRC 7862	JN134140	KM678208	
P. cookiellum	CBS 101.83 **T**	KT155670		
	CBS 102.83	KT155674		

Taxa	Strain	GenBank accession		
		ITS	EF1A	RPB2
P. mirabile	CBS 129179	MF926385		
	CBS 124422 **T**	MF926384		
Trichophyton benhamiae	CBS 808.72	MH860614	KM678050	
	CBS 807.72	MH860613	KM678118	
T. mentagrophytes	NBRC 5466	JN134100	KM678200	
	NBRC 5974	JN134103	KM678206	
T. rubrum	CBS 304.60	AJ270807	KM678081	
	CBS 734.88	AJ270800	KM678115	
T. simii	CBS 417.65	MH858646	KM678090	
	CBS 448.65	MH858665	KM678099	
Myceliophthora lutea	CBS 145.77 **T**	HQ871775	HQ871722	HQ871816
	MUCL 10070	LK932701	LK932710	LK932724

T= type strains, strain and sequences generated in this study are shown in **bold.**

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection; NBRC: NITE Biological Resource Centre, Japan; IFO: Institute for Fermentation, Osaka, Yodogawa-ku, Osaka, Japan; GZUIFR: Guizhou University, Institute of Fungus Resources; MUCL: Belgian Co-ordinated Collections of Micro-organisms, Belgium.

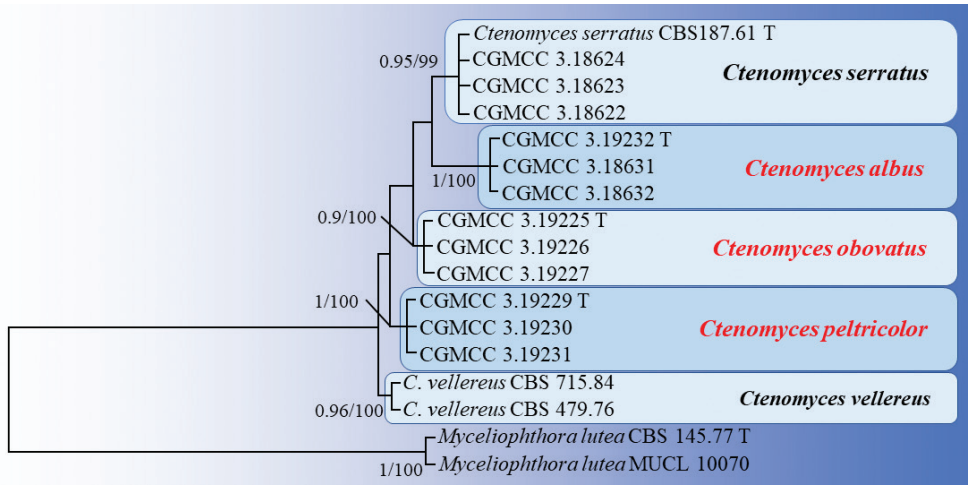

Figure 2. Phylogenetic tree of *Ctenomyces* based on the ITS+EF1A+RPB2 dataset and *Myceliophthora lutea* (CBS 145.77 and MUCL 10070) as the outgroup taxon. Numbers at nodes are Bayesian posterior probabilities (left, BPP ≥0.9) and maximum likelihood bootstrap values (right, ≥95%).

was sorted into expected subclades. Particularly, *C. vellereus* CBS 479.76 and CBS 715.84 strains cluster together with strong support values (BPP 0.96, BS 100%) and were separate from *C. serratus* (CBS 187.71, CGMCC 3.18622, CGMCC 3.18623 and CGMCC 3.18624) clustering together with strong support (BPP 0.95, BS 99%). In addition, our proposed three new species: *C. albus*, *C. obovatus* and *C. peltricolor*, had high support as a single subclade 1/100%, 0.9/100% and 1/100%, respectively.

Taxonomy

Ctenomyces albus **Y.F. Han, Z.Q. Liang & Z.Y. Zhang, sp. nov.**
MycoBank: MB827872
Figure 3

Holotype. CHINA, Guizhou Province, on soil, Sept. 2016, Z.Y. Zhang (HMAS 255389, holotype, ex-type culture CGMCC 3.19232).

Paratypes. CHINA, Guizhou Province, on soil, Sept. 2016, Z.Y. Zhang, dried cultures HMAS 255442 and HMAS 255443, isolates CGMCC 3.18631 (GZUIFR-QL17.11) and CGMCC 3.18632 (GZUIFR-QL17.12).

Etymology. Referring to the white colony.

Description. Aerial hyphae hyaline, smooth, septate, branched, 1.1–2.4 μm wide; racquet hyphae absent. Terminal and lateral conidia borne on hyphae, short protrusions, side branches or an ampulliform swelling. Conidia solitary or in series of up to 2–3 conidia connected by short and slim hypha, ellipsoid, smooth- or rough-walled, verrucose, 12.8–18.6 × 10.8–14.7 μm ($\bar{x}$ = 15.4 × 12.5 μm, n=15). Intercalary conidia present, subglobose or ellipsoidal, smooth- or rough-walled, 13.1–16.9 × 11.2–14.4 μm (x^{-} = 14.5 × 12.6 μm, n=15).

Culture characteristics. Colonies on PDA growing in the dark reaching 32 mm diam. in 14 d at 25 °C, white, short fluffy to powdery, appearing some annulations, rounded, margin regular and defined. Reverse yellowish.

Notes. *Ctenomyces albus* is distinct from other species as it is the only species with intercalary conidia in the genus. In addition, our ITS and polygenic phylogeny showed that three isolates of *C. albus* were in a clade sister to *C. serratus* (Figures 1, 2) and clearly separate from other species. Following Jeewon and Hyde's (2016) guidelines on new species delimitation, there were 37 bp (base pair) differences amongst 508 nucleotides ITS sequences between the isolate CGMCC 3.19232 and *C. serratus* CBS 187.61 (only ITS sequence data are available, EF1A and RPB2 are lacking), which also supports them as distinct different species. Therefore, we introduce *C. albus* sp. nov. in this study.

Ctenomyces obovatus **Y.F. Han, Z.Q. Liang & Z.Y. Zhang, sp. nov.**
MycoBank: MB827869
Figure 4

Holotype. CHINA, Shanxi Province, on soil, Nov. 2017, Z.Y. Zhang (HMAS 255446, holotype, ex-type culture CGMCC 3.19225).

Paratypes. CHINA, Shanxi Province, on soil, Nov. 2017, Z.Y. Zhang, dried cultures HMAS 255447 and HMAS 255448, isolates CGMCC 3.19226 (GZUIFR-L15021) and CGMCC 3.19227 (GZUIFR-L15023).

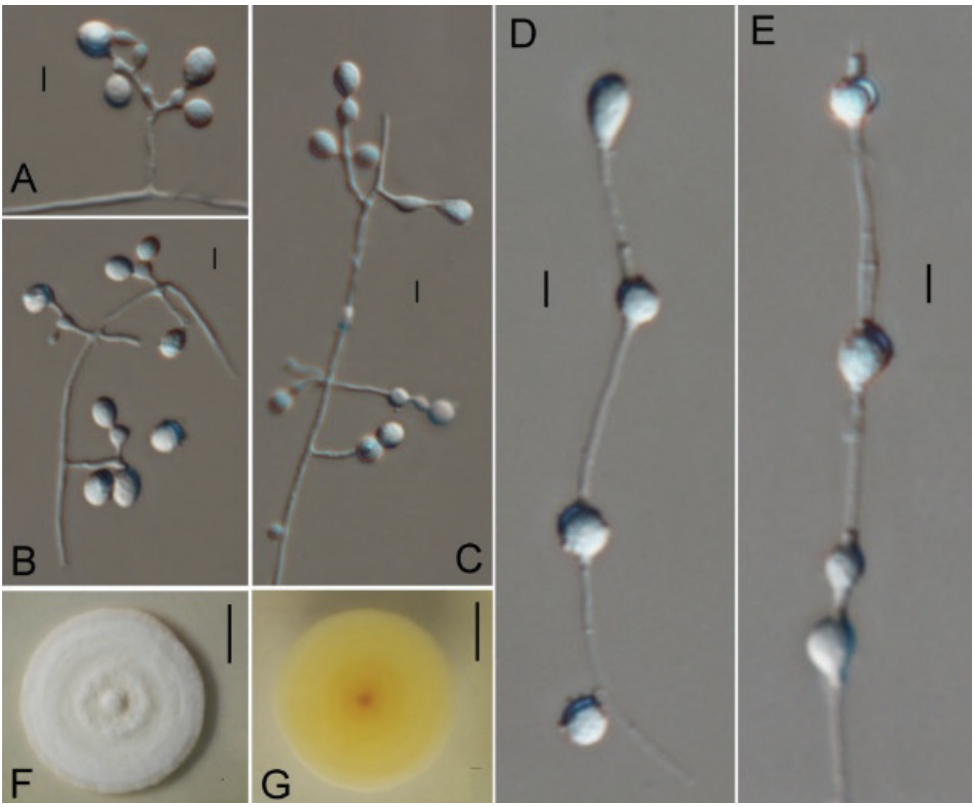

Figure 3. *Ctenomyces albus* (from ex-holotype strain CGMCC 3.19232). **A–C** Conidiogenous structures and conidia **D, E** Intercalary conidia **F, G** Colony on PDA at day 14. Scale bars: 10 μm (**A–E**); 10 mm (**F, G**).

Etymology. Referring to the obovoid conidia.

Description. Aerial hyphae hyaline, smooth, septate, abundant branched, 1.2–2.4 μm wide; racquet hyphae absent. Terminal and lateral conidia borne on hyphae, short protrusions, side branches or an ampulliform swelling. Conidia solitary or in series of up to 2 conidia, ellipsoidal, obovoid, smooth- or rough-walled, verrucose, spinate, 10.3–17.3 × 9.7–10.5 μm ($\bar{x}$ = 14.5 × 10 μm, n=15). Intercalary conidia absent.

Culture characteristics. Colonies on PDA growing in the dark reaching 14–15 mm diam. in 14 d at 25 °C, yellowish, white in the margin; fluffy; rounded, margin regular. Reverse brown.

Notes. *Ctenomyces obovatus* resembles *C. vellereus* in conidia size and conidiogenous cells. However, *C. obovatus* is the only species that produces obovoid conidia in this genus. Furthermore, our ITS and multigene phylogeny shows that three isolates of *C. obovatus* formed a single clade separate from other species (Figure 1), which indicates that *C. obovatus* is a new species.

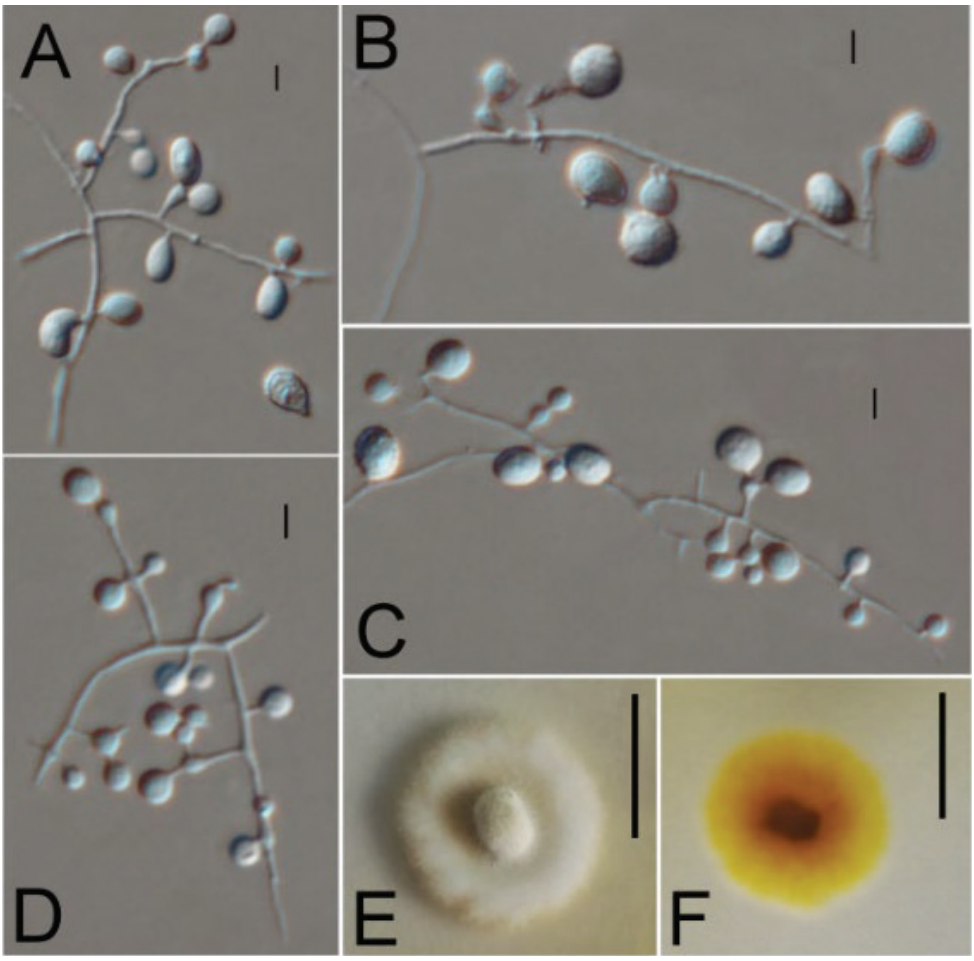

Figure 4. *Ctenomyces obovatus* (from ex-holotype strain CGMCC 3.19225). **A–D** Conidiogenous structures and conidia **E, F** Colony on PDA at day 14. Scale bars: 10 μm (**A–D**); 10 mm (**E, F**).

Ctenomyces peltricolor **Y.F. Han, Z.Q. Liang & Z.Y. Zhang, sp. nov.**
MycoBank: MB827873
Figure 5

Holotype. CHINA, Gansu Province, on soil, Nov. 2017, Z.Y. Zhang (HMAS 255387, holotype, ex-type culture CGMCC 3.19229).

Paratypes. CHINA, Gansu Province, on soil, Nov. 2017, Z.Y. Zhang, dried cultures HMAS 255439 and HMAS 255440, isolates CGMCC 3.19230 (GZUIFR-C03011) and CGMCC 3.19231 (GZUIFR-C03012).

Etymology. Referring to the pewter colony.

Description. Aerial hyphae hyaline, smooth, septate, branched, 1.2–3.3 μm wide; racquet hyphae absent. Terminal and lateral conidia borne on hyphae, short protru-

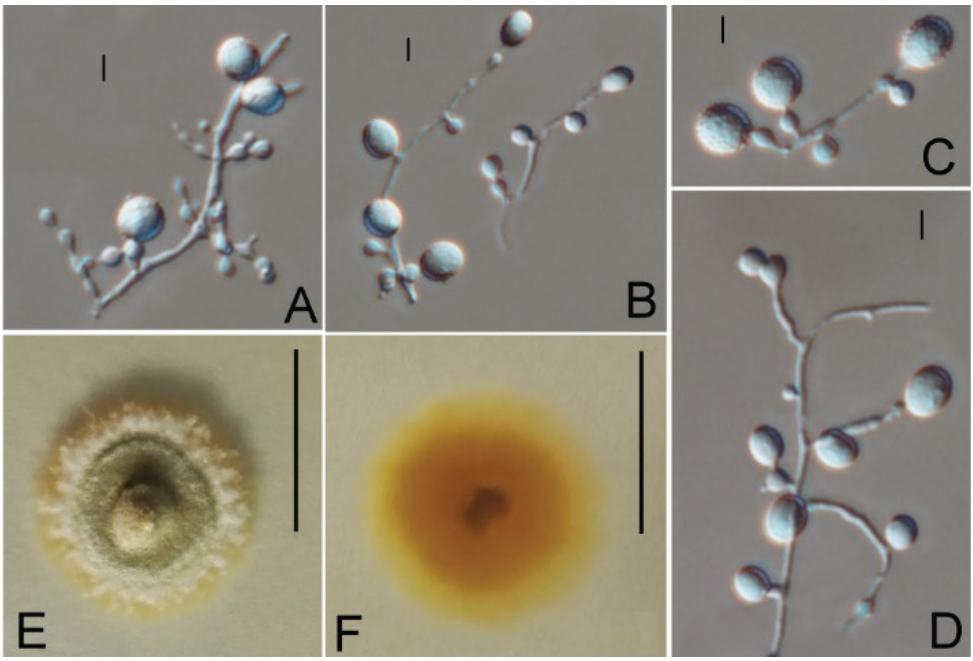

Figure 5. *Ctenomyces peltricolor* (from ex-holotype strain CGMCC 3.19229) **A–D** Conidiogenous structures and conidia **E, F** Colony on PDA at day 14. Scale bars: 10 μm (**A–D**); 10 mm (**E, F**).

sions, side branches or an ampulliform swelling. Conidia solitary, usually only 1 borne on ampulliform swellings; subglobose or globose; smooth- or rough-walled, verrucose, spinate, 8.3–20.2 μm ($\bar{x}$ = 15.5 μm, n=15). Intercalary conidia absent.

Culture characteristics. Colonies on PDA growing in the dark reaching 12 mm diam. in 14 d at 25 °C, pewter at the centre, white in the margin; powdery to floccose at the centre, fluffy in the margin; appearing a circle of annulation; rounded, margin regular. Reverse brown at the centre, yellowish in the margin.

Notes. *Ctenomyces peltricolor* is distinct from other species in its single conidia borne on ampulliform swellings and colony colour. Phylogenetically, the ITS-based phylogenetic analysis (Figure 1) showed that three isolates of *C. peltricolor* cluster in a single clade (BPP 1, BS 100%), consistent with the results of multigene phylogenetic analysis (BPP 1, BS 100%) (Figure 2). Therefore, based on both morphological and phylogenetic evidence, *C. peltricolor* was identified as a new species of *Ctenomyces*.

***Ctenomyces serratus* Eidam, in Beitrag zur Kenntnis der Gymnoasceen, Beiträge zur Biologie der Pflanzen 3: 267–305 (1880)**
MycoBank: MB827871
Figure 6

Description. Aerial hyphae hyaline, smooth, septate, branched, 0.9–3.3 μm wide; racquet hyphae absent. Terminal and lateral conidia borne on hyphae, short protrusions,

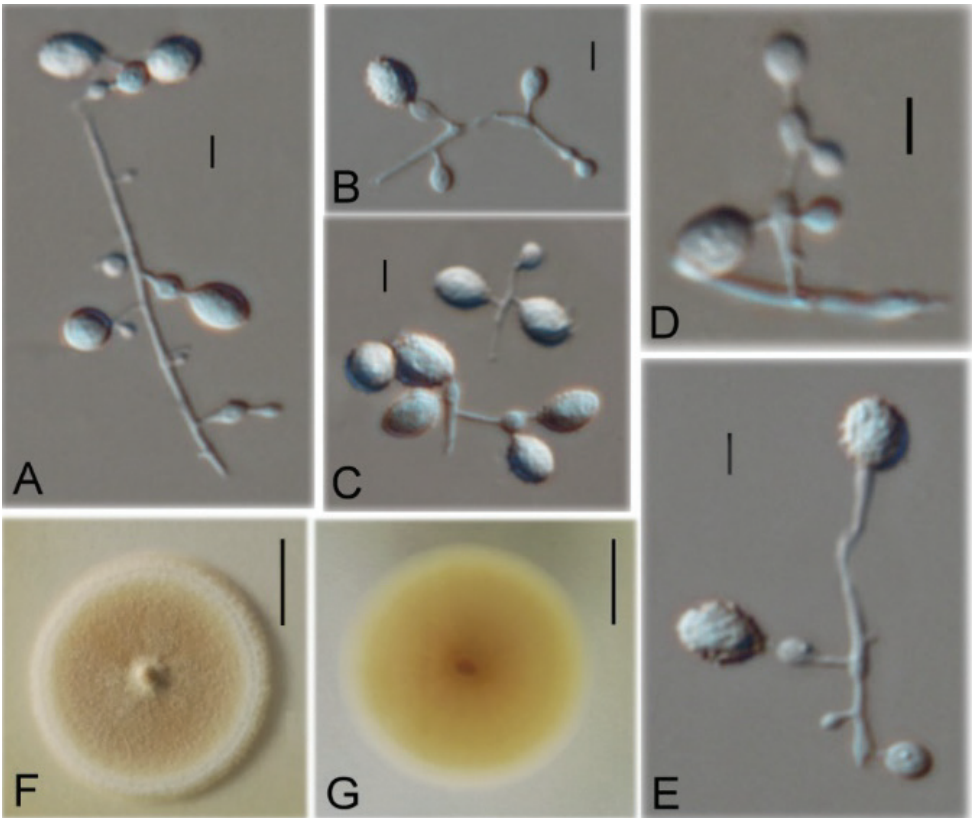

Figure 6. *Ctenomyces serratus* (from strain CGMCC 3.18622) **A–E** Conidiogenous structures and conidia **F, G** Colony on PDA at day 14. Scale bars: 10 μm (**A–E**); 10 mm (**F, G**).

side branches or an ampulliform swelling; ampulliform swelling solitary or 2 in series. Conidia solitary or in series of up to 2–3 conidia connected by short and slim hypha, mostly ellipsoidal, sometimes subglobose; smooth- or rough-walled, verrucose, spinate, 11.5–21.9 × 8–15.2 μm ($\bar{x}$ = 18.5 × 13.2 μm, n=15). Intercalary conidia absent.

Culture characteristics. Colonies on PDA growing in the dark reaching 30 mm diam. in 14 d at 25 °C, brown, white in the margin, floccose at the centre, short fluffy in other part, appearing obvious annulation; rounded, margin regular and defined. Reverse yellowish.

Specimens examined. CHINA, Guizhou Province, on soil, Sept. 2016, Z.Y. Zhang, dried cultures HMAS 255390, HMAS 255444 and HMAS 255445, isolates CGMCC 3.18622 (GZUIFR-S37.1), CGMCC 3.18623 (GZUIFR-S37.2) and CGMCC 3.18624 (GZUIFR-S37.3).

Known distribution. Currently known from Australia, England, India, Argentina, Germany (*sensu* Oorschot 1980) and China (this study).

Notes. The Australian collection of *C. serratus* (CBS 187.61) lacks racquet hyphae, conidia occur occasionally on short protrusions and usually 1–2 are borne on ampulliform swellings, solitary or in series of up to 3 conidia separated by short, nar-

row hyphal segments, initially subhyaline, thin- and smooth-walled, soon becoming reddish-brown, verrucose and thick-walled, ellipsoid, 5–23 × 3.5–12 μm, mature conidia usually 12–23 × 10.5–12 μm, with narrow basal scars (approx. 1 μm) (*sensu* Oorschot 1980). The characteristic features data from the China collections matched rather well with the original description of *C. serratus* reported from Australia. Phylogenetically, our isolates CGMCC 3.18622, CGMCC 3.18623 and CGMCC 3.18624 shared a close relationship with *C. serratus* (Figures 1, 2). Therefore, the isolates CGMCC 3.18622, CGMCC 3.18623 and CGMCC 3.18624 were identified as *C. serratus* which was new to China.

Discussion

Members of the family Arthrodermataceae (Onygenales) were common in nature, mostly found as saprobes in soil on keratin-rich substrates or associated with vertebrate causing dermatophytosis and other infections. The widely accepted morphology-based taxonomy of dermatophytes in the genera *Trichophyton*, *Microsporum* and *Epidermophyton* was established by Emmons (Emmons 1934). There are three common ecological groups, anthropophilic, zoophilic or geophilic (de Hoog et al. 2017). *Trichophyton* and *Epidermophyton* usually belonged to anthropophiles or zoophilic taxa, *Microsporum* were considered to zoophilic, *Arthroderma* was geophilic. Some species cannot be clearly attributed to one of these groups due to insufficient data. Geophilic dermatophytes have their reservoir in the soil around burrows of specific terrestrial mammals, feeding on keratinous debris. Hence, the difference between geophilic and zoophilic dermatophytes is not always well-resolved. The genus of *Ctenomyces* is usually saprobic or closely related to keratin-rich substrates (Wijayawardene et al. 2017, Deshmukh et al. 2018). In addition, these strains of our study were isolated by using hair and feathers as baiting material. Therefore, we infer the genus *Ctenomyces* to be geophilic and/or zoophilic.

Phylogenetic studies based on the ITS (Graser et al. 2008), partial LSU, the ribosomal 60S protein, partial β-tubulin and translation elongation factor 3 for Arthrodermataceae have shown that the genus *Trichophyton* was polyphyletic and resulted in establishing nine genera, i.e. *Arthroderma*, *Ctenomyces*, *Epidermophyton*, *Guarromyces*, *Lophophyton*, *Microsporum*, *Nannizzia*, *Paraphyton* and *Trichophyton* and it suggested that ITS was the optimal barcoding marker (de Hoog et al. 2017). Therefore, we selected the ITS sequences for phylogenetic analysis of Arthrodermataceae in this study and our results are consistent with the previous studies (de Hoog et al. 2017). *Ctenomyces vellereus* (strains CBS 479.76 and CBS 715.84) had ITS, EF1A and RPB2 sequence data. Hence, we selected the ITS, EF1A and RPB2 genes for phylogenetic analysis of *Ctenomyces*. The results show that the ITS-based and multigene-based phylogenetic analyses have similar results. Although our study revealed that *Ctenomyces* was closely related to *Arthroderma* in Arthrodermataceae, Onygenales, the species of *Ctenomyces* nearly all produce ampulliform swellings, a feature absent in *Arthroderma*.

Guarro et al. (1985), Chabasse (1988) and van den Brink et al. (2012) proposed that *C. vellereus* was a synonym of *C. serratus*, however, these two species have several different characters, the conidia of *C. serratus* are ellipsoid, 12–23 × 10.5–12 μm, while those of *C. vellereus* are subglobose, pyriform or ellipsoid, 4–13 × 3–9 μm (*sensu* Oorschot 1980). Van den Brink et al. (2012) conducted phylogenetic analysis of ITS sequences including only one sequence of *C. serratus* and three sequences of *C. vellereus*. This study used more ITS sequences in *Ctenomyces* for phylogenetic analysis and multigenic phylogeny analysis showed that they were two different species. In our phylogenetic tree, *C. albus*, *C. obovatus*, *C. peltricolor*, *C. serratus* and *C. vellereus* were grouped in five clear clades with good supported value and they are distinct from each other. Thus, based on the present molecular phylogeny, derived from nuclear and ribosomal DNA sequence data, together with morphological evidence, three distinct new *Ctenomyces* species, *C. albus*, *C. obovatus*, *C. peltricolor* and one new record, *C. serratus*, were proposed.

Key to the species of the genus *Ctenomyces*

1	Intercalary conidia absen	**2**
–	Intercalary conidia present, subglobose or ellipsoidal	***C. albus***
2	Mostly 1–2 conidia borne on ampulliform swellings	**3**
–	Usually only 1 conidia borne on ampulliform swellings	***C. peltricolor***
3	Conidia less than 20 μm long	**4**
–	Conidia more than 20 μm long	***C. serratus***
4	Conidia obovoid or ellipsoidal	***C. obovatus***
–	Conidia subglobose, pyriform or ellipsoid	***C. vellereus***

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RESEARCH ARTICLE

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Rostania revised: testing generic delimitations in Collemataceae (Peltigerales, Lecanoromycetes)

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Abstract

Here, we test the current generic delimitation of *Rostania* (Collemataceae, Peltigerales, Ascomycota) utilizing molecular phylogeny and morphological investigations. Using DNA sequence data from the mitochondrial SSU rDNA and two nuclear protein-coding genes (MCM7 and β-tubulin) and utilizing parsimony, maximum likelihood and Bayesian phylogenetic methods, *Rostania* is shown to be non-monophyletic in the current sense. A new generic delimitation of *Rostania* is thus proposed, in which the genus is monophyletic, and three species (*Rostania coccophylla*, *R. paramensis*, *R. quadrifida*) are excluded and transferred to other genera. *Rostania occultata* is further non-monophyletic, and a more detailed investigation of species delimitations in *Rostania* s. str. is needed. The new combinations *Leptogium paramense* and *Scytinium quadrifidum* are proposed.

Keywords

Classification, cyanolichens, nomenclature, systematics, taxonomy, thallus anatomy

Introduction

Collemataceae is a large group of predominantly foliose lichenized fungi commonly known as the "jelly lichens" due to their gelatinous habit. This is caused by a polysaccharide matrix around the *Nostoc* cyanobacterial photobionts that swells and becomes extremely gelatinous when wet. Until very recently, the generic classification of the

Collemataceae s. str. was very unnatural and based solely on one character, presence (*Leptogium*) or absence (*Collema*) of a cellular cortex (Degelius 1954, 1974; Jørgensen 2007). Already Degelius (1954) questioned the monophyly of *Collema* and *Leptogium*. This was also supported by molecular phylogenies (Wiklund and Wedin 2003; Miadlikowska and Lutzoni 2004; Miadlikowska et al. 2014), and somewhat surprisingly, gelatinous genera with one-septate spores that earlier were classified in Collemataceae, were shown to belong to the Pannariaceae (Wedin et al. 2009; Otálora et al. 2010; Ekman et al. 2014; Weerakoon et al. 2018) or Arctomiaceae (Otálora and Wedin 2013). Not until Otálora et al. (2013a, 2013b) investigated the family in detail was a modern classification of Collemataceae s. str. proposed. *Collema* and *Leptogium* were confirmed as highly non-monophyletic, and Otálora et al. (2013b) instead suggested accepting 10 more or less morphologically distinct monophyletic groups from their tree, as genera. In addition to *Collema* and *Leptogium* in restricted senses, six old generic names were resurrected (*Blennothallia* Trevis., *Enchylium* (Ach.) Gray, *Lathagrium* (Ach.) Gray, *Pseudoleptogium* Müll. Arg., *Rostania* Trevis., and *Scytinium* (Ach.) Gray), and two new genera were described (*Callome* Otálora & Wedin and *Paracollema* Otálora & Wedin).

Rostania, the focus of the present study, corresponds to the *Occultatum*-group of *Collema* (Degelius 1954, 1974). It is a comparatively small genus with eight currently accepted, mainly epiphytic species, characterised by very small to medium sized (ca 0.3–5 cm in diam.) subcrustaceous to subfoliose thalli with very small apothecia (ca 0.2–0.8(–1) mm in diam.) and cuboid to oblong muriform spores. All five species included in the *Occultatum* group by Degelius were treated in *Rostania* by Otálora et al. (2013b); *Rostania callibotrys* (Tuck.) Otálora, P.M. Jørg. & Wedin, *Rostania ceranisca* (Nyl.) Otálora, P.M. Jørg. & Wedin, *Rostania coccophylla* (Nyl.) Otálora, P.M. Jørg. & Wedin, *Rostania occultata* (Bagl.) Otálora, P.M. Jørg. & Wedin and *Rostania multipunctata* (Degel.) Otálora, P.M. Jørg. & Wedin. In addition, *Rostania laevispora* (Swinscow & Krog) Otálora, P.M. Jørg. & Wedin was included in the genus. Two further species were later added, *Rostania paramensis* (P.M. Jørg. & Palice) P.M. Jørg. & Palice (Jørgensen and Palice 2015) and *Rostania quadrifida* (D.F. Stone & McCune) McCune (McCune et al. 2014). Degelius (1954) divided *Collema occultatum* into two varieties: var. *populinum* which was characterised by a squamulose, somewhat lobate thallus, and which almost exclusively grew on the bark of *Populus*, and var. *occultatum* with a granulose thallus and which occurred on many deciduous trees, including *Populus*. Perlmutter and Rivas Plata (2018) combined var. *populinum* in *Rostania*, as *R. occultata* var. *populina* (Th. Fr.) Perlmutter & Rivas Plata.

Otálora et al. (2013a, 2013b) included only three species (*R. ceranisca, R. multipunctata* and *R. occultata*) in their phylogenies, and thus the taxonomical position of most species has not been tested by molecular methods. As there is a substantial variation in shape and size of the lobes, apothecia and ascospores, as well as the hyphal arrangement in the thallus among the *Rostania* species, and as several former Collemataceae taxa have been shown to belong outside the family, the delimitation of the whole genus needs investigation. Here, we will test the generic delimitation

of *Rostania* and investigate the relationships of any species falling outside *Rostania* s. str. Finally, we will note and comment on any indication of species non-monophyly, in this genus.

Material and methods

Taxon sampling and morphological studies

We sampled 52 specimens of Collemataceae for the molecular study, including six of the eight currently accepted *Rostania* species and representatives of all genera within the family Collemataceae, including type species. Sequences originating from the study of Otálora et al. (2013a) were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/) and all sequences used in this work are summarized in Table 1. Our own collections were deposited in UPS and S, and additional herbarium material from the herbaria PRA, GZU, UPS and S was also included (Table 1). Additional herbarium type material from the herbaria H and O was studied morphologically only (listed on the end of the manuscript). Herbarium acronyms follow Thiers (2018). Three species of *Rostania* not included in earlier studies were successfully added (*R. callibotrys*, *R. quadrifida* and *R. paramensis*). The sampling of *Rostania occultata* included specimens of both varieties. To enable testing of generic monophyly and family placement of taxa potentially to be excluded from *Rostania*, we added secondary outgroups including newly produced sequences of two species from the sister family Placynthiaceae (*Placynthium nigrum* and *P. rosulans*) and sequences available in GenBank of two from the more distantly related Pannariaceae (*Pannaria rubiginosa* and *Staurolemma omphalarioides*). Finally, *Peltigera aphthosa* was used as outgroup to root the tree.

We studied morphological and anatomical characters under the light microscope and dissecting microscope. We used hand-cut longitudinal sections of apothecia to observe internal and microscopic characteristics, in water. Microscopic examinations of the thalli were conducted on transversal cross-sections of lobes in water, or lactic blue.

Data generation

Two apothecia or (in the case of sterile samples) a thallus fragment, were selected for extraction. We extracted total DNA using the Plant DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturers' instructions. We amplified ca 0.6 kb of the small subunit of the mitochondrial rDNA (mtSSU), ca 0.6 kb of the two protein-coding genes DNA replication licensing factor mini-chromosome maintenance complex component 7 (MCM7) and the β-tubulin gene (b-tub) using the same primer combinations and PCR settings as in previous studies (Otálora et al. 2013a; Košuthová et al. 2016). We assembled and edited DNA sequences using Geneious version R8 (http://www.geneious.com; Kearse et al. 2012).

Table 1. Sequences utilized in this study (newly produced sequences in bold, remaining sequences produced by Otálora et al. (2013a) and some of the outgroup sequences are taken from Wiklund and Wedin (2003), Buschbom and Mueller (2004), Otálora et al. (2010), Prieto et al. (2013)). In case of *Rostania* species, origin of both, state and provinces are given.

Taxon	Geographic origin, voucher	GenBank accession number		
		mtSSU	b-tub	MCM7
Blenothallia crispa1	Hungary: Thor 7021a (UPS–L48439)	JX992918	KC119040	JX992976
Blenothallia crispa 3	Spain: Westberg (S–F315217)	**MK445278**	**MK451934**	**MK451920**
Callome multipartita 1	Norway: Haugan 7015 (O–L117369)	GQ259019	–	–
Callome multipartita 2	Austria: Hafellner 74818 (GZU18–2009)	**MK445271**	**MK451935**	–
Collema leptaleum	Argentina: Wedin 8822 (S–F335749)	JX992928	KC119038	JX992986
Collema nigrescens	Spain: Aragón 80/04 (MA–16262)	EU982563	KC119016	JX992989
Collema subconveniens	New Zealand: Wedin 9225 (S–F335747)	JX992937	KC119019	JX992996
Enchylium bachmanianum	Sweden: Nordin 1521 (UPS–L133627)	JX992914	**MK451936**	JX992974
Enchylium polycarpon 3	Sweden: Odelvik 04700 (S–L316455)	JX992934	**MK451937**	JX992993
Enchylium tenax 1	Spain: Etayo 20214 (MA–L13396)	EU982556	KC128823	JX992998
Enchylium tenax 2	Spain: Sarrión 1509 (MA–L14789)	EU982579	KC128824	–
Lathagrium auriforme	Spain: Otálora 20904 (MA–L16249)	JX992913	KC119008	JX992973
Lathagrium cf. fuscovirens	Sweden: Wedin 9701 (S–F332476)	**MK445277**	**MK451938**	**MK451921**
Lathagrium fuscovirens	Sweden: Tibell 23588 (UPSL–145162)	JX992923	KC119013	JX992983
Leptogium azureum	Chile: Cornejo 26507 (MA–16273)	JX992939	KC119021	JX993002
Leptogium byssinum	Norway: Westberg (S–F264803)	KT240180	–	KT240183
Leptogium denticulatum	Argentina: Wedin 8690 (S–F332474)	JX992947	KC119025	JX993012
Leptogium terrenum	Portugal: van den Boom 41781 (hb. van den Boom)	KT240181	–	KT240184
Paracollema italicum 1	Croatia: Nordin 2708 (UPS–L076283)	JX992925	KC119015	JX992984
Paracollema italicum 3	Croatia: Nordin 2763 (UPS–L076284)	JX992926	–	JX992985
Pseudoleptogium diffractum 1	Sweden: Nygren 007 (UPS–L129612)	GQ259029	–	–
Pseudoleptogium diffractum 3	Sweden: Nordin 2529 (UPS–L153952)	JX992949	–	JX993015
Rostania callibotrys 1	Kenya: Moberg 4431a (UPS–L22044)	**MK445270**	**MK451939**	–
Rostania callibotrys 2	Costa Rica: Sipman 20495 (GZU–113_8P)	**MK445269**	**MK451940**	–
Rostania ceranisca 1	Norway, Troms: Nordin 5721 (UPS–L130978)	**MK445280**	**MK451941**	–
Rostania ceranisca 2	Sweden, Pite Lappmark: Westberg PL433 (UPS-L931677)	**MK445267**	**MK451942**	**MK451922**
Rostania ceranisca 3	Austria, Salzburg: MW_HOCH020 (S–F262465)	**MK445268**	**MK451943**	**MK451923**
Rostania multipunctata 1	Greece, Crete: Nordin 3160 (UPS–L027750)	JX992930	**MK451944**	JX992988
Rostania multipunctata 2	Greece, Korfu: Poelt 8852 (GZU–2–93)	**MK445273**	**MK451945**	–
Rostania occultata v. occultata 1	Sweden, Pite Lappmark: Westberg PL467 (UPS-L931673)	**MK445266**	**MK451946**	**MK451924**
Rostania occultata v. occultata 2	Sweden, Dalarna: Westberg (S–F304739)	**MK445259**	–	**MK451925**
Rostania occultata v. occultata 3	Sweden, Uppland: Westberg (UPS–L834451)	**MK445257**	–	**MK451926**
Rostania occultata v. populina 1	Sweden, Södermanland: Nordin 5407 (UPS–L120396)	JX992931	–	JX992991
Rostania occultata v. populina 2	Greece, Crete: Llop 56060303 (S–F233720)	JX992932	**MK451947**	JX992990
Rostania occultata v. populina 3	Sweden, Gästrikland: Odelvik 01269 (S–L42490)	**MK445260**	**MK451948**	**MK451927**
Rostania occultata v. populina 4	Sweden, Jämtland: Kosuthova 174 (S–F332481)	**MK445265**	**MK451949**	**MK451928**
Rostania paramensis	Ecuador, Carchi: Palice 2796 (PRA–00013999) (HOLOTYPE)	**MK445279**	–	–
Rostania quadrifida 1	USA, Oregon: McCune 2744 (UPS–L513233)	**MK445272**	**MK451950**	–
Rostania quadrifida 2	USA, Oregon: McCune 28536 (UPS–L513222) (ISOTYPE)	**MK445274**	**MK451951**	–
Scytinium biatorinum	Sweden: Jonsson 5500 (UPS–L186460)	JX992940	KC119022	JX993003
Scytinium imbricatum	Sweden: Hermansson 18777 (UPS–L706500)	**MK445264**	**MK451952**	**MK451929**
Scytinium intermedium	Sweden: Nordin 7385 (UPS–L587203)	**MK445263**	**MK451953**	**MK451930**
Scytinium magnussonii	Spain: Otálora 20104 (MA)	EU982565	KC119004	JX993022
Scytinium palmatum	Sweden: Nordin 5369 (UPS–L113313)	JX992959	KC119027	JX993025
Scytinium parvum	Sweden: Thor 4300 (UPS–L174011)	JX992933	KC119018	JX992992

Taxon	Geographic origin, voucher	GenBank accession number		
		mtSSU	b-tub	MCM7
Scytinium plicatile	Sweden: Nordin 5566 (UPS–L124847)	GQ259033	KC119030	JX993030
Scytinium pulvinatum	Russia: Pystina 17352 (UPS–L738570)	**MK445262**	**MK451954**	**MK451931**
Scytinium sp_Palice2273	Ecuador: Palice 2273 (PRA–00013997)	**MK445275**	**MK451955**	–
Scytinium sp_Palice2274a	Ecuador: Palice 2274a (PRA–00013998)	**MK445276**	–	–
Scytinium subtile	Sweden: Ågren 686 (UPS–L163890)	JX992869	KC119034	–
Scytinium tenuissimum	Spain: Aragón 1682/97 (MA)	JX992971	KC119036	–
Scytinium turgidum	Spain: Aragón 1671/98 (MA–12868)	EU982592	KC119037	JX993040
Outgroups:				
Placynthium rosulans	Sweden: Westberg URL222 (UPS–L854413)	**MK445258**	**MK451956**	**MK451932**
Placynthium nigrum	Sweden: Kosuthova 35 (S–F332479)	**MK445261**	–	**MK451933**
Pannaria rubiginosa	Portugal: Purvis et Smith 27/4/95 (BM)	AY340513	–	JX993042
Staurolemma omphalarioides	Spain: Aragón 83/04 (MA), mtSSU only Spain: Hafellner & Hafellner 41399 (UPS), MCM7 only	EU982560	–	JX993043
Peltigera apthosa	Sweden: Wedin 6164 (UPS)	AY340515	AY536792	JX000176

Sequence alignment and analysis

To identify and avoid contaminants among the new sequences, we used Megablast high similarity matches in Geneious version R8 (http://www.geneious.com; Kearse et al. 2012). Alignments were constructed using AliView 1.09 (Larsson 2014) with the "ClustalW/Multiple alignment" option and subsequent manual adjustments. All ambiguously aligned regions (sensu Lutzoni et al. 2000) were excluded from analysis.

The mitochondrial and the two protein-coding datasets were analysed separately before concatenation using parsimony jackknifing (JK) in WinClada (Nixon 1999–2002) with 100–200 replicates and otherwise default settings. As no significant (JK support above 70%) incongruence was detected, the alignments were concatenated. Final alignments have been deposited in TREEBASE (http://www.treebase.org) with accession number (http://purl.org/phylo/treebase/phylows/study/TB2:S23889). After concatenation, we inferred phylogenetic relationships using parsimony, maximum likelihood and Bayesian phylogenetic methods with indels treated as missing data. Partitions scheme and optimal model of nucleotide substitution for Bayesian analysis were selected using PartitionFinder2 (Guindon et al. 2010; Lanfear et al. 2012, 2016). PartitionFinder was set as follow: linked branch lengths, data blocks according to each codon position of each genetic region (mtSSU, MCM7, b-tub), the greedy search scheme, the Bayesian information criterion as selection metric and only models that are implemented in MrBayes. The selected substitution model schemes are provided in Table 2.

We performed parsimony JK in WinClada (Nixon 1999–2002) with 2000 replicates and otherwise default settings. For maximum likelihood and ML bootstrapping we used RAxML 8 (Stamatakis 2014) implementing a general time reversible (GTR) model of nucleotide substitution with gamma distributed rate heterogeneity GTR+G (GTRGAMMA)following recommendations in the user manual. We used 4 parti-

Table 2. Evolutionary models and partitions according to the Best scheme calculated in PartitionFinder. In RAxML only the GTR+G (GTRGAMMA) model was used for all partitions.

Subset name	Analyses type	Nr of sites	Codon position	Best model	Partition
mtSSU	MrBayes	735	–	HKY+I+G	1
MCM7	MrBayes	194	1	SYM+I+G	2
MCM7	MrBayes	194	2	SYM+I+G	2
MCM7	MrBayes	194	3	HKY+I+G	3
b-tub	MrBayes	210	1	SYM+I+G	2
b-tub	MrBayes	210	2	JC	4
b-tub	MrBayes	210	3	HKY+I+G	3
mtSSU	RAxML	735	–	–	1
MCM7	RAxML	194	1	–	2
MCM7	RAxML	194	2	–	3
MCM7	RAxML	194	3	–	4
b-tub	RAxML	210	1	–	2
b-tub	RAxML	210	2	–	3
b-tub	RAxML	210	3	–	4

tions determined by PartitionFinders (Table 2). 1000 bootstrap (BS) replicates were completed using the parametric (BS) algorithm of RAxML-HPC2 on the Cipres Web Portal (Miller et al. 2010). Bayesian phylogenetic analysis was inferred using MrBayes 3.2.5 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2011) with the evolutionary models following the partitioning scheme from PartitionFinder (Table 2). We estimated posterior probabilities (PP) by running one cold and two heated chains for 2 130 000 generations in parallel mode, saving trees every 100th generation. To test whether the Markov chain converged, we monitored the average standard deviation of split frequencies (ASDSF), which should fall below 0.01 when comparing two independent runs. We discarded the 25% of generations before the point where the ASDSF fell below 0.01 as burn-in. All remaining trees were summarized as a Bayesian 50% majority rule (MR) consensus tree with PP calculated for each clade.

Results and discussion

We produced 61 new sequences (Table 1) for the phylogenetic analyses (24 mtSSU, 15 MCM7, 22 b-tub) including 57 taxa and 1947 nucleotide positions (735 for mtSSU and 582 for MCM7 and 630 for b-tub) for the final matrix. The alignment contained 618 parsimony-informative characters (177 for mtSSU, 237 for MCM7 and 204 for b-tub). The most likely tree from the RAxML analysis is presented in Figure 1 with likelihood BS, Bayesian PP and parsimony JK support superimposed.

The analyses resulted in a topology (Fig. 1) very similar to the results of Otálora et al. (2013a, 2013b). Some of the backbone topology, however, has unfortunately no or low support. In Otálora et al. (2013b) *Callome* was the sister to *Rostania*, but in our

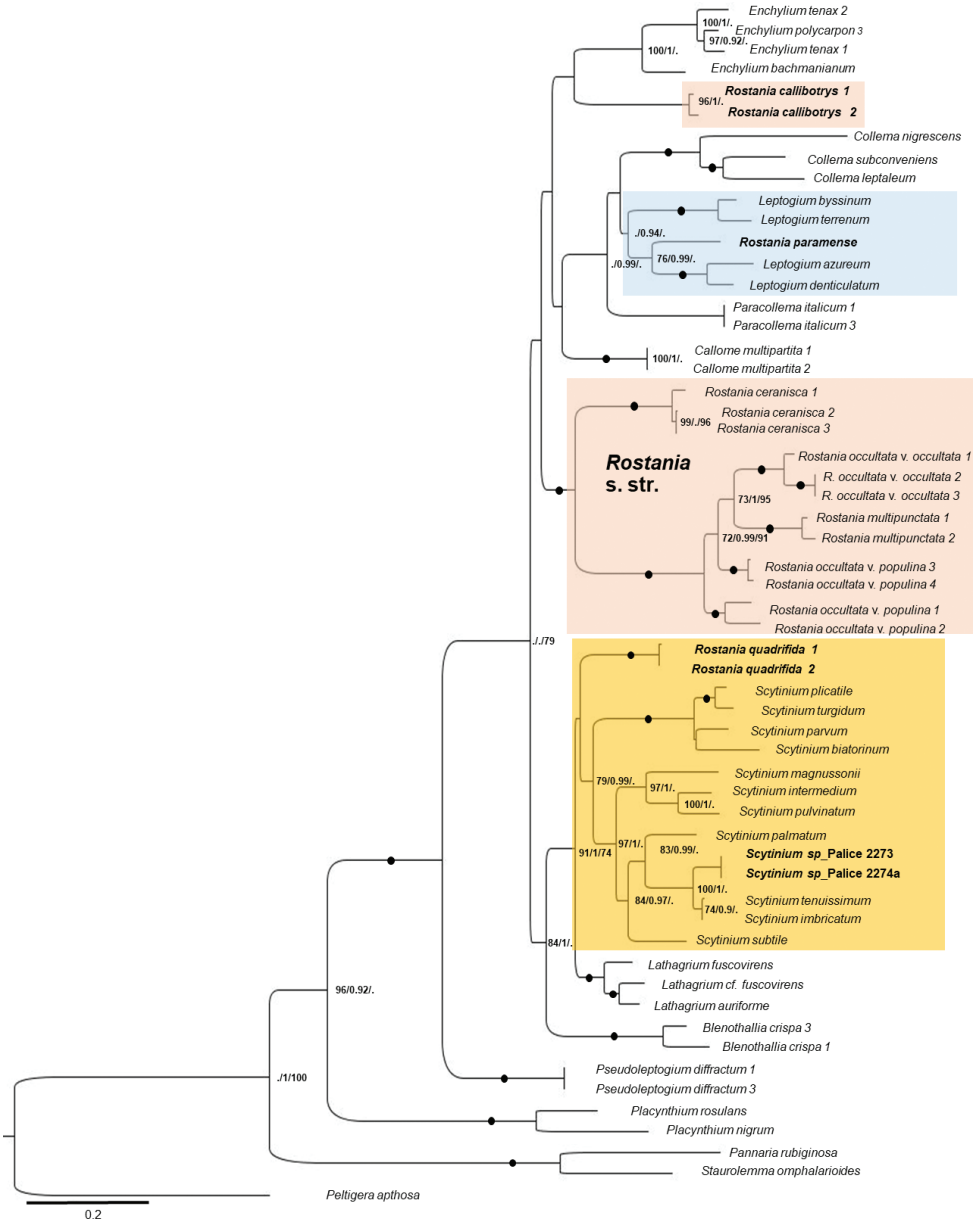

Figure 1. The most likely tree from the combined RAxML analysis based on 1947 aligned characters of mtSSU rDNA, MCM7 and b-tub from 57 specimens. Support values (Likelihood BS/Bayesian PP/parsimony JK) given when BS ≥ 70%, PP ≥ 0.90 and parsimony JK ≥ 70%. Branches receiving BS ≥ 75 %, PP ≥ 0.95 and JK ≥ 75% are indicated with a black dot. The different colour indicate different genera: blue = *Leptogium*, pink = *Rostania*, orange = *Scytinium*.

study this relationship is not formed. All *Rostania* species are nested within Collemataceae, but *Rostania* in the sense of Otálora et al. (2013b) is non-monophyletic. Three species form a core group, which we here treat as *Rostania* s. str. *Rostania* s. str. is well

supported and includes *Rostania occultata* (Fig. 2A), *R. ceranisca*, and *R. multipunctata*. We can conclude that *R. occultata* as currently circumscribed is non-monophyletic. *Rostania multipunctata* (Fig. 2B) shares the cuboid shape and size of the spores with *R. occultata* s. lat. (Fig. 3A), but the thallus differs in size (the lobes are generally larger, up to ca 2.5 cm long in *R. multipunctata*, while in *R. occultata* s. lat. they are up to ca 3 mm long). It has also accessory lobules developing from the wrinkles (Fig 2B), which do not occur in *R. occultata* s. lat. The delimitation of the two varieties of *R. occultata* is unclear, as is the separation from *R. multipunctata*. Our study is not designed to study species-delimitations and we will extend our investigation of this species complex in a larger study currently in preparation.

Rostania ceranisca, the only terricolous *Rostania*, is sister to the group consisting of *R. multipunctata* and *R. occultata* s. lat. In addition to its terricolous ecology, it is easily recognized by the erect accessory finger-like lobules (Fig. 2C), which grow from the edge of the main lobes. The spores in *R. ceranisca* differ in shape from the cuboid spores in *R. multipunctata* and *R. occultata* s. lat. (Fig. 3A) in being oblong (Fig. 3B). Although Degelius (1954) noted only four spores in the ascus, we have usually observed eight spores, even if four of them may be aborted or are at least not clearly visible when mature (Fig. 3B).

Rostania callibotrys does not group with *Rostania* s. str. (Fig. 1), but forms an unsupported group with *Enchylium*. *Rostania callibotrys* has a comparatively distinct thalline apothecium margin, similar to some species of *Enchylium*. However, this is a widespread feature in the family including some species of *Rostania* s. str. The thallus with characteristic accessory lobules in *R. multipunctata* (Fig. 2B) and *R. laevispora* (Fig. 2D) is very similar to *R. callibotrys* (Fig. 2E). *Rostania callibotrys* also has spores that are very similar to the typical cuboid to oblong *Rostania*-spores in *R. multipunctata* and *R. occultata* s. lat. (Fig. 3A, B), but the spores in *R. callibotrys* have fewer cells (Fig. 3C) than in these species. *Rostania laevispora* (Fig. 2D), a rarely collected species that we did not manage to get sequences from, is very similar and likely very closely related to *R. callibotrys* (Fig. 2E). As there is no support for excluding these species, and no distinct morphological evidence suggests any other relationship, we tentatively leave both *R. callibotrys* and *R. laevispora* in *Rostania*.

We did not manage to get molecular data from *R. coccophylla* (Fig. 4A), a tropical and rarely collected species where the available material was too old. Although *R. coccophylla* is similar to *R. callibotrys* and *R. multipunctata*, the apothecia in *R. coccophylla* are very different in that they are convex and stipitate when mature (compared to concave and initially immersed and later sessile, in *Rostania*) and considerably larger compared to other *Rostania* species. The apothecia of *R. coccophylla* are similar to several species in *Collema* sensu Otálora (2013b), where this species originally was placed. Although we preferably would want molecular data to test the correct placement of this species, we suggest that it is re-instated in *Collema*, where the name *Collema coccophyllum* Nyl. is available.

Rostania quadrifida and *R. paramensis* are not closely related to *Rostania* s. str. *Rostania quadrifida* was described by Stone and McCune (2010) as *Collema quadrifidum*, and was later included in *Rostania* based on spore shape and thallus morphology (McCune et al. 2014). It differs from *Rostania* s. str. by having spores with fewer septa

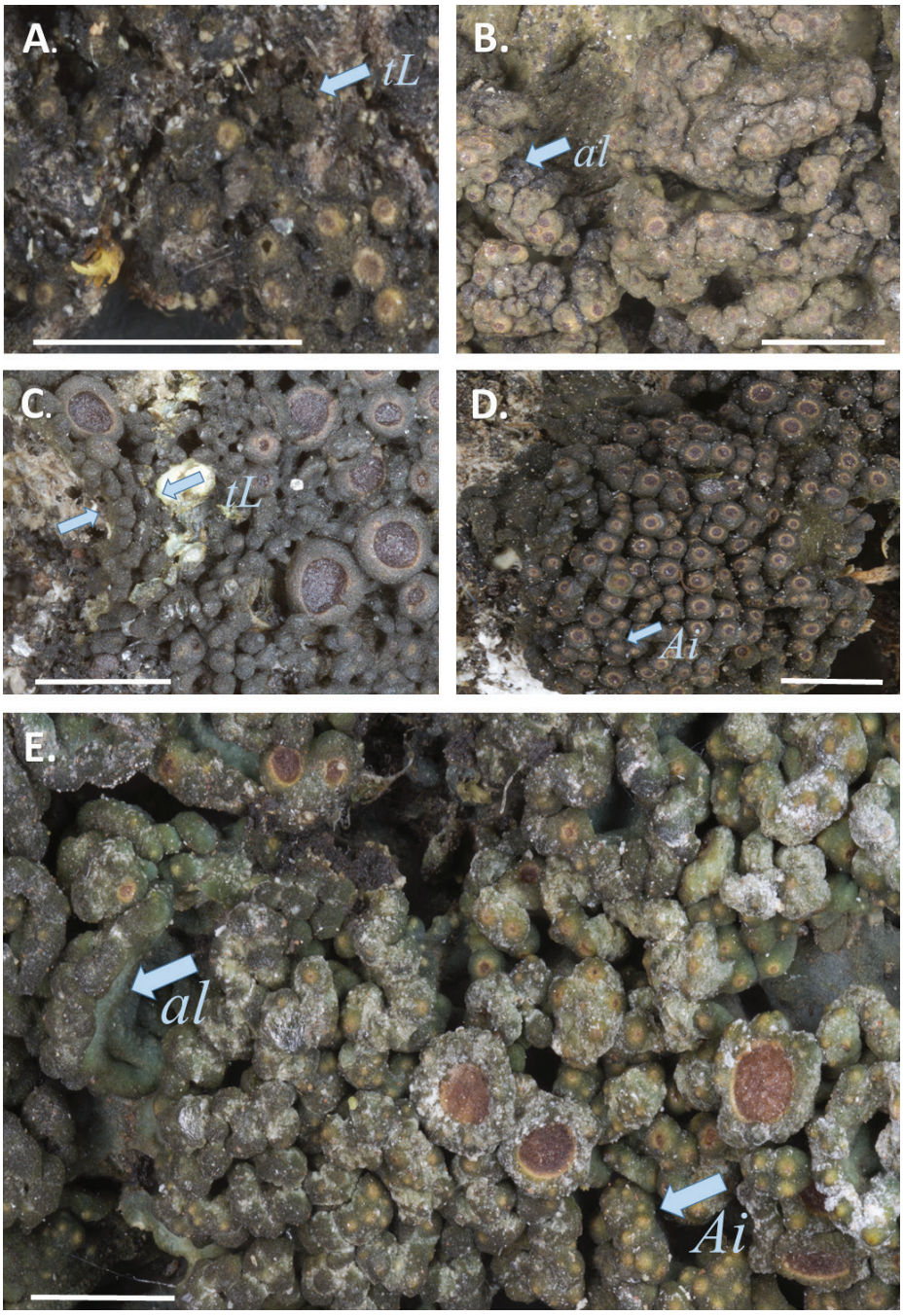

Figure 2. Thallus habitus: **A** *Rostania occultata* var. *populina* (Odelvik 1269, S), thallus lobes (arrow) **B** *Rostania multipunctata* (Poelt 8852, GZU), accessory lobules (arrow) **C** *Rostania ceranisca* (MW_HOCH020, S), accessory finger-like lobules (arrow) **D** *Rostania laevispora* (isotype of *Collema laevisporum* Swinscow & Krog, Tanzania, 1986, Swinscow & Krog T 3/6, O-00298), apothecium in initial stage (arrow) **E** *Rostania callibotrys* (Moberg 4431a, UPS), apothecium in initial stage (arrow). ***tL*** = thallus lobes, ***al*** = accessory lobules, ***Ai*** = apothecium in initial stage covering the top of the accessory lobules. Scale bar: 1 cm.

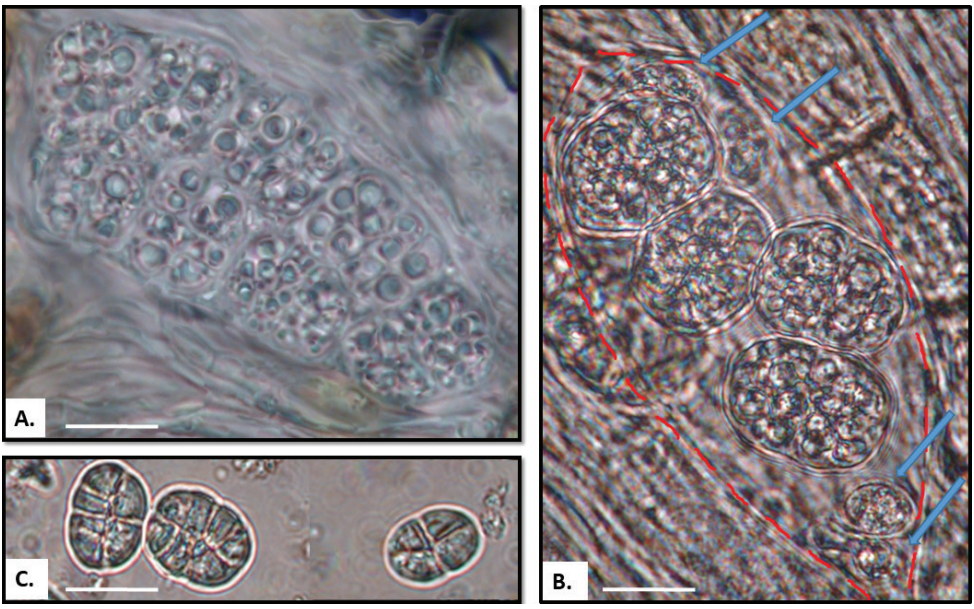

Figure 3. Ascospores: **A** *Rostania occultata* var. *populina* (Llop 56060303, S), cubic spores **B** *Rostania ceranisca* (Westberg L271_PL433, UPS), oblong spores; ascus (red line) with only four mature spores visible but remnants of four aborted spores can be seen (arrows) **C** *Rostania callibotrys* (Sipman 2049, GZU), oblong spores. Scale bar: 10 μm.

(Fig. 5A). Here it forms the sister group to *Scytinium* (Fig. 1), within a well-supported group consisting of *Blennothallia*, *Lathagrium* and *Scytinium*. *Rostania quadrifida* has a thallus composed by densely interwoven hyphae, and with a pseudocortex (Fig. 6A), features that do not occur in *Rostania* s. str., but in some species of *Scytinium* (similar to e.g. *Scytinium intermedium* and *S. magnussonii*; Jørgensen 1994). These similarities support including it in *Scytinium*, which we do below.

The generic position of *R. paramensis* has been complicated to assess. Jørgensen and Palice (2012) described it as *Collema paramense*, based on the holotype (Palice 2796) and another sample from a second locality in Ecuador (Palice 2273). As the thallus has a pseudocortex, Otálora et al. (2013b) transferred it to *Scytinium*. Jørgensen and Palice (2015) later studied another sample from the second locality (Palice 2274). They concluded that the spores in the holotype must have been unusually developed, and transferred it to *Rostania* based on the oblong spores (similar to *R. ceranisca*) found in Palice 2274. Our re-examination of these three specimens, including the holotype, shows that Palice 2273 and Palice 2274 contain two distinct Collemataceae species (Fig. 4C, D). One of these (Fig. 4D), present in small amounts only in both samples, is identical with holotype of *Collema paramense* and is characterised by a matt dark olive thallus with a pseudocortex (Fig. 6B), and hyaline, muriform, ellipsoid spores with acute ends (Fig.5B). This is very different from the spores in *Rostania,* but typical for species in *Leptogium* s. str. (Fig. 5C). We

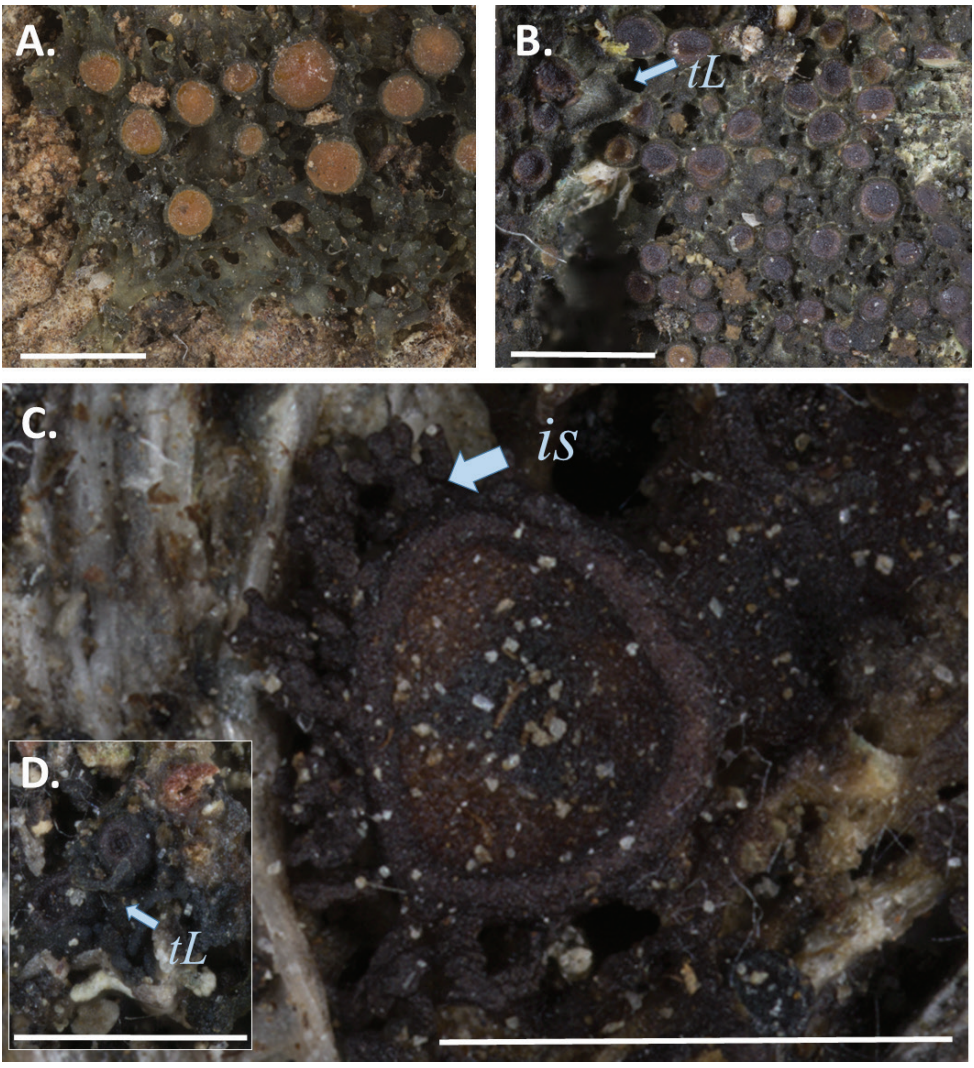

Figure 4. Thallus habitus: **A** *Rostania coccophylla* (isotype of *Collema coccophyllum* Nyl., India, 1858, Perrotet s.n., H-NYL 42355, H–9201376) **B** *Rostania paramensis* (Palice 2796, PRA-00013999; holotype of *Collema paramense* PM Jørg. & Palice) **C** *Scytinium* sp. Palice 2274a **D** *Rostania paramensis* Palice 2274b. ***tL*** = thalline lobes, ***is*** = isidia. Scale bar: 1 cm.

sequenced the holotype, and we can conclude that among the *Leptogium* species we have sampled, it forms a group with *Leptogium azureum* (the conserved type of *Leptogium*; Jørgensen et al. 2013) and *L. denticulatum* (Fig. 1). It has a thallus which is appressed to the substrate and composed by relatively small lobes (Fig. 4B) which is rare in other *Leptogium* s. str., and in section it has straight and unbranched hyphae which are perpendicular to the surface (Degelius 1954; Fig. 6B). This character is present in several groups in Collemataceae. It was observed by Degelius (1954) in

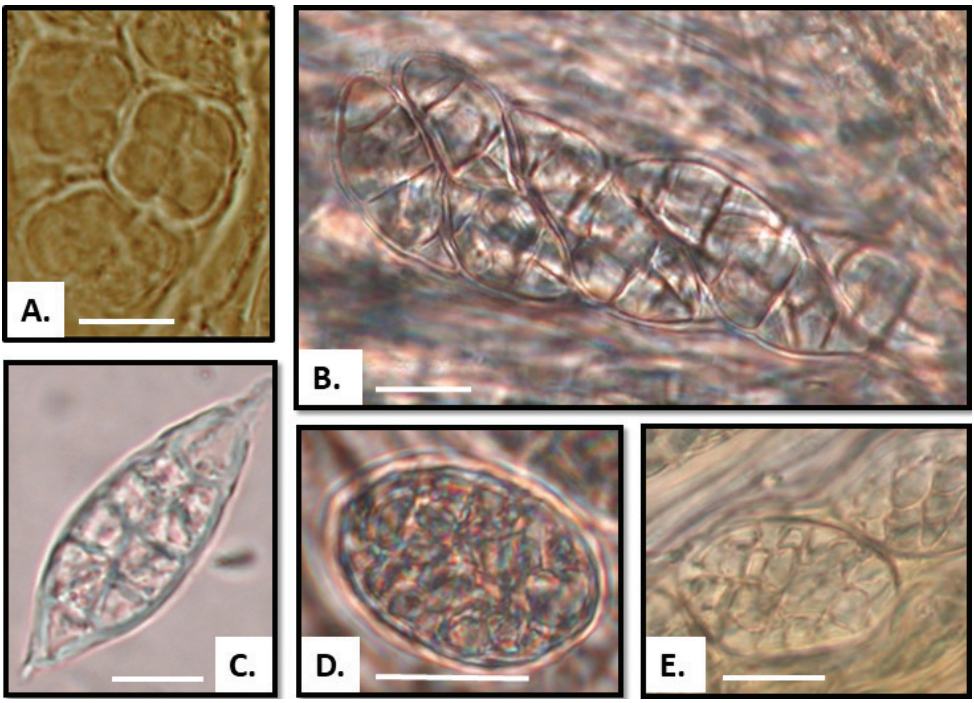

Figure 5. Ascospores: **A** *Rostania quadrifida* (McCune 2744, UPS), cubic spores with 2–5 cells **B** *Rostania paramensis* (Palice 2796, holotype of *Collema paramense*), ellipsoid spores with acute ends **C** *Leptogium azureum* (Tehler 3140, S), ellipsoid spores with acute ends **D** *Scytinium* sp. (Palice 2273), oblong spores, **E** *Scytinium* sp. (Palice 2274), oblong spores. Scale bar = 10 μm

some *Collema* species, and has also been noted in the newly described *Leptogium antarcticum* by Kitaura et al. (2018) who used the term "columnar hyphae" for the same hyphal arrangement. We have observed this hyphal arrangement in *Leptogium azureum* (Fig. 6C) and *L. denticulatum* too, but it is apparently not present in *Rostania.* The second species present in Palice 2273 and Palice 2274, apparently confused Jørgensen and Palice (2015) as their observation of oblong spores (Fig. 5D, E) refer to this species, which has a shiny brown thallus (Fig. 4C) and not a matt dark olive thallus as in "*Rostania*" *paramense* (Fig. 4B). The second species differs from *Rostania* by having a proper eucortex (Fig. 6D), and by producing isidia along the apothecium margin (Fig. 4C). The thallus is paraplectenchymateous throughout (Fig. 6D). This hyphal arrangement is present in several groups in Collemataceae, including *Rostania occultata* s. lat. (Fig. 6E). Already Degelius (1954) noted this hyphal arrangement in his *Occultatum*-group and Otálora et al. (2013b) observed the same in *Blennothallia*, *Pseudoleptogium* and in *Scytinium*. We sequenced also this species and we can confirm that both samples belong in *Scytinium*, but the species remains to be identified.

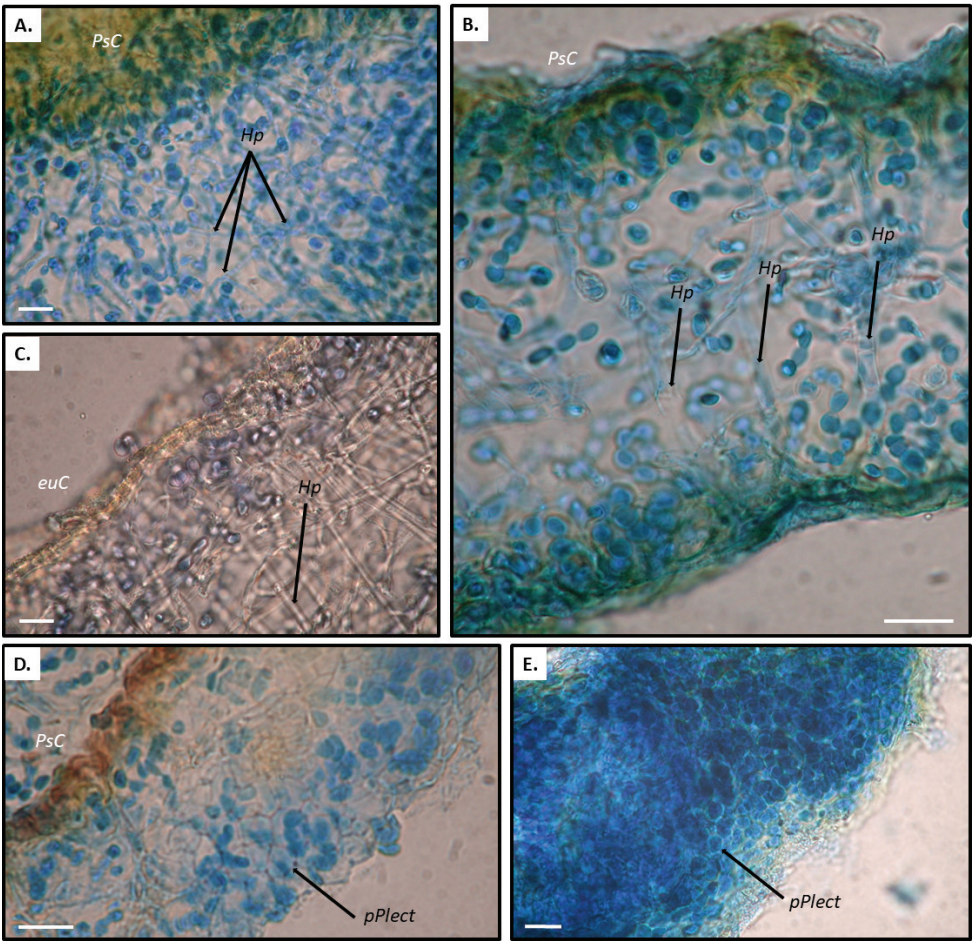

Figure 6. Thalli, transversal cross-sections: **A** Thallus with pseudocortex and densely interwoven hyphae (*Rostania quadrifida*, McCune 2744, UPS) **B** Thallus with pseudocortex and straight and unbranched hyphae which are perpendicular to the surface (*Rostania paramensis*, Palice 2796, holotype of *Collema paramense*) **C** Thallus with eucortex and straight and unbranched hyphae which are perpendicular to the surface (*Leptogium azureum*, Tehler 3140, S) **D** Thallus with eucortex and paraplectenchymateous throughout (*Scytinium* sp. Palice 2273) **E** Thallus paraplectenchymateous throughout (*Rostania occultata* var. *populina*, Llop 56060303, S) **A–E** in lactic blue **C** in water. euC = eucortex, PsC = pseudocortex, Hp = hyphae, pPlect = paraplechtenchyma. Scale bar = 10 µm

Conclusions

Here we have tested the current generic concept of *Rostania* and conclude that at least three of the species should be excluded and that the position of *R. callibotrys* and *R. laevispora* in *Rostania* is uncertain. *Rostania* is characterized by crustose to subfoliose thallus with initially immersed apothecia (Fig. 2D, E), which only later become sessile. The disc is concave when young and plane when older, but never convex. The spores are muri-

form with at least 5 cells, cuboid to oblong, but never fusiform to ellipsoid (Fig. 3A–C). Most species are comparatively small, and all lack cortex, rhizines and isidia.

Rostania includes six taxa: *R. callibotrys*, *R. ceranisca*, *R. laevispora*, *R. multipunctata*, *R. occultata* var. *occultata*, and *R. occultata* var. *populina*. *Rostania occultata* s. lat. is non-monophyletic and this species complex will be investigated in the near future.

New combinations

Leptogium paramense **(P.M.Jørg. & Palice) A.Košuth. & Wedin, comb. nov.**
MB829590

Basionym. *Collema paramense* P.M. Jørg. & Palice, Biblioth. Lichenol. 108: 136 (2012)

Type. ECUADOR. Carchi: volcan Chiles, wet paramo, Palice 2796 (PRA-00013999!–holotype, BG, QCA–isotypes).

Scytinium quadrifidum **(D.F.Stone & McCune) A.Košuth. & Wedin, comb. nov.**
MB829591

Basionym. *Collema quadrifidum* D.F. Stone & McCune, *N. Amer. Fung.* 5(2): 2 (2010)

Type. U. S. A. OREGON, Douglas County: Bushnell-Irwin Rocks ACEC, McCune 28536 (OSC–holotype, US, UPS–L513222!–isotypes).

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Tuber pulchrosporum sp. nov., a black truffle of the Aestivum clade (Tuberaceae, Pezizales) from the Balkan peninsula

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Abstract

Knowledge on the diversity of hypogeous sequestrate ascomycetes is still limited in the Balkan Peninsula. A new species of truffle, *Tuber pulchrosporum*, is described from Greece and Bulgaria. Specimens were collected from habitats dominated by various oak species (i.e. *Quercus ilex*, *Q. coccifera*, *Q. robur*) and other angiosperms. They are morphologically characterised by subglobose, ovoid to irregularly lobed, yellowish-brown to dark brown ascomata, usually with a shallow basal cavity and surface with fissures and small, dense, almost flat, trihedral to polyhedral warts. Ascospores are ellipsoid to subfusiform, uniquely ornamented, crested to incompletely reticulate and are produced in (1–)2–8-spored asci. Hair-like, hyaline to light yellow hyphae protrude from the peridium surface. According to the outcome of ITS rDNA sequence analysis, this species forms a distinct well-supported group in the Aestivum clade, with *T. panniferum* being the closest phylogenetic taxon.

Keywords

Ascomycota; Tuberaceae; truffle; ectomycorrhizal fungi; taxonomy; phylogeny; fungal diversity

Introduction

The genus *Tuber* F.H. Wigg. (Ascomycota, Pezizales, Tuberaceae) is globally famous and historically appreciated for the production of hypogeous ascomata, known as 'truffles'; several of them are highly prized due to their unique aroma and culinary value. Moreover, the genus is known for the symbiotic ectomycorrhizal associations that its members form with several gymnosperm and angiosperm forest-tree species as well as with orchids (Riousset et al. 2001; Selosse et al. 2004; Mello et al. 2006; Trappe et al. 2009). Furthermore, truffles are also important for serving as a primary or supplementary source of nutrition for soil micro-fauna and several mammals (Hanson et al. 2003; Trappe and Claridge 2010; Schickmann et al. 2012).

A continuous interest in the study of this particular group has resulted in several recent reports on new *Tuber* species from various parts of the world (e.g. Crous et al. 2017; Fan et al. 2015; Guevara-Guerrero et al. 2018; Piña Páez et al. 2018). It is estimated that their number ranges between 180 and 220 (Zambonelli et al. 2016) nested in 11 major phylogenetic clades (Bonito et al. 2013). In particular, the Aestivum clade is composed of species associated with a large spectrum of host plants and are reported to occur in the Old World, i.e. Europe, North Africa and/or Asia (Jeandroz et al. 2008; Bonito et al. 2013; Payen et al. 2014). Indicative examples are *T. aestivum* Vittad. (the type species of the genus), *T. panniferum* Tul. & Tul., *T. malenconii* Donadini, Riousset, G. Riousset & G. Chev. and *T. mesentericum* Vittad., as well as *T. sinoaestivum* Zhang & Liu recently described from China (Zambonelli et al. loc. sit.; Zhang and Chen 2012). The morphologically diverse and economically important species *T. magnatum* Picco also forms part of this clade (Bonito et al. 2010a; 2013).

Although *Tuber* diversity is well documented in Europe (Bonito at al. 2010a, Ceruti et al. 2003, Jeandroz et al. 2008), the south-eastern part of the continent and especially the Balkan Peninsula was until recently poorly investigated. Indicative of this fact is that, by the end of the last century, only three *Tuber* species had been recorded in Greece (Zervakis et al. 1999). However, during the last two decades, an ever increasing interest in the collection of truffles led to a remarkable increase in the number of pertinent records (e.g. Diamandis and Perlerou 2008; Konstantinidis 2009; Agnello and Kaounas 2011; Alvarado et al. 2012a,b; Gyosheva et al. 2012); thus, to date, 15 *Tuber* spp. are reported from Greece. Similarly, only two *Tuber* spp. had been recorded in Bulgaria by the end of the last century; however, this number is fast-growing during the last few years and 14 species are currently known to exist (Dimitrova and Gyosheva 2008; Gyosheva et al. 2012; Lacheva 2012; Nedelin et al. 2016; Assyov and Slavova 2018). Regarding adjacent countries, 12 truffle species were reported to occur in Serbia, including one recently described (Marjanović et al. 2010; Milenković et al. 2015), while six *Tuber* spp. were recorded in Montenegro, five in FYROM and four in Albania (Pacioni 1984; Marjanović et al. 2010).

In the frame of this work, several truffle specimens originating from north and central continental Greece and from Bulgaria were studied with respect to their morphology and phylogenetic relationships to other *Tuber* taxa and a new species is hereby proposed.

Methods

Sampling and Morphological characterisation

Specimens used for this study were collected during 2008–2017 from north and central Greece (Regions of Epirus, Thessaly, Eastern Macedonia and Thrace, Western Greece and Attica), as well as from Bulgaria (Regions of Eastern Stara Planina and Black Sea coast). Specimens are deposited in the fungaria of the Laboratory of General and Agricultural Microbiology (Agricultural University of Athens, ACAM), of the Institute of Biodiversity and Ecosystem Research (SOMF) and the authors' personal collections. Macroscopic characters such as size, peridium surface texture, colour and odour were observed in fresh ascomata. Colour coding and terminology is derived from the "Flora of British Fungi – Colour Identification Chart" (Royal Botanic Garden Edinburgh 1969).

Microscopic characters were examined by hand-cut sections on fresh and dried material, using a Zeiss Axioimager A2 microscope under bright field and Differential Interference Contrast (DIC) and an AmScope T360B. Microphotographs were taken with the aid of a mounted digital camera (Axiocam). Microscopic observations were performed in water, 3% (w/v) potassium hydroxide (KOH) and Melzer's reagent. To assess the ascospore size, a minimum of 30 mature ascospores from each type of asci (2 to 8-spored) were measured and dimensions are provided as (minimum) average ± standard deviation (maximum); quotient (Q), i.e. length divided by the width, was calculated for each ascospore and the median value (Qm) is given. For scanning electron microscopy (SEM), ascospores were scraped from the hymenial surface and mounted on aluminium foil, which was then fixed on a microscope holder and sputter-coated with gold. Observations were performed in JEOL JSM-5510.

DNA sequencing and Phylogenetic analyses

Total genomic DNA was extracted from herbarium specimens using the Nucleospin Plant II DNA kit (Macherey and Nagel, Germany) following the manufacturer's protocol with minor modifications. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) was amplified using the primer combination ITS1/ITS4 (White et al. 1990). Polymerase chain reactions (PCR) were performed in 50 µl containing 50 ng DNA template, 0.25 µM of each primer, 0.2 mM of each dNTP, 1× HiFi Buffer (Takara BIO INC., Japan) and 1 U HiFi Taq DNA polymerase (Takara BIO INC., Japan). Conditions for PCR amplification were as follows: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 sec, 50 °C for 30 sec and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were purified using Invitrogen PureLink kit (Thermo Fisher Scientific, Korea) and were submitted for sequencing to CeMIA SA (Larissa, Greece). DNA sequences were then visualised, manually edited and assembled using UGENE (Okonechnikov et al. 2012). Validated sequences, generated in this study, were deposited in GenBank under the accession numbers MK113975 to

Table 1. Details of ITS sequences deriving from *Tuber pulchrosporum* sp. nov. and from reference material used for the construction of the phylogenetic tree. Clades names are placed in the order they appear in Fig. 5.

Species/ Clade	Collection code	GenBank Accession No.	Origin	Reference
Excavatum Clade				
Tuber fulgens	M2435	HM485358	Italy	Bonito et al. 2010a
	HMT37	HM151976*	Austria	Urban et al. 2010
Tuber excavatum	SA1TE	KJ524533*	Poland	Hilszczanska et al. 2014
	JST62014	KX354295	Germany	Schiebold et al. 2017
Gennadii Clade				
Tuber lacunosum	AH39255	JN392212	Spain	Alvarado et al. 2012a
	AH38932	JN392213	Spain	Alvarado et al. 2012a
Tuber gennadii	B M1904	HM485361	Italy	Bonito et al. 2010a
	AH39251	JN392211	Spain	Alvarado et al. 2012a
	AH31113	JN392203	Spain	Alvarado et al. 2012a
	AH38957	JN392204	Spain	Alvarado et al. 2012a
Regianum Clade				
Tuber bernardinii	2172	KY420104	Italy	Merenyi et al. 2017
	NA	KY420105	Italy	Merenyi et al. 2017
Tuber magentipunctatum	MO793	KY420089	Italy	Merenyi et al. 2017
	ZB4293	JQ288909**	Hungary	Merenyi et al. 2017
Tuber regianum	ZB3081	KY420098	Slovakia	Merenyi et al. 2017
	erd-2590	KY420102	Spain	Merenyi et al. 2017
Macrosporum Clade				
Tuber macrosporum	Macro1	AF106885*	Italy	Rubini et al. 1998
	HMSFI_TUBMAC/141207A	FM205634*	Slovenia	Grebenc et al. 2008
Aestivum Clade				
Tuber magnatum	JT19460	HM485374	Italy	Bonito et al. 2010a
	GB12	JQ925645	Italy	Bonito et al. 2013
Tuber malenconii	MA:Fungi:28384/ 02MLC	FM205597*	Spain	Grebenc et al. 2008
	17110	JF908743	Italy	Osmundson et al. 2013
Tuber sinoaestivum	L4213	KY081688*		Wang and Wang 2016
	JP-Zhang-140	JN896355	China	Zhang et al. 2012
Tuber aestivum	TaeW016I-E134	AJ888090	Italy	Weden 2005
	S19	HQ706002	Slovakia	Gryndler et al. 2011
Tuber uncinatum	MA: Fungi: 24605	FM205618*	Spain	Grebenc et al. 2008
	228	AJ492199	Italy	Mello et al. 2002
Tuber mesentericum	CW105	HM485375	Sweden	Bonito et al. 2010a
	UASWS1612	KY197989*	Switzerland	Cochard et al. 2016
Tuber panniferum	–	AF132507		Roux et al. 1999
	JT12835	HM485380	Spain	Bonito et al. 2010a
Tuber pulchrosporum	1945 F8517	MK113981	Bulgaria	This work
sp. nov.	1961 F0388	MK113982	Bulgaria	This work
	VN091 (holotype)	MK113975	Greece	This work
	GK3801	MK113979	Greece	This work
	LT1183	MK113976	Greece	This work
	GK9408	MK113977	Greece	This work
	VK4482	MK113980	Greece	This work
	GK6538	MK113978	Greece	This work
Multimaculatum Clade				
Tuber multimaculatum	OSC 62169	HM485377	Spain	Bonito et al. 2010a
Rufum Clade				
Tuber rufum	1785	EF362475	Italy	Iotti et al. 2007
	S90	JF926123	Germany	Stobbe et al. 2012

Species/ Clade	Collection code	GenBank Accession No.	Origin	Reference
Melanosporum Clade				
Tuber pseudoexcavatum	T14_HKAS44325b	GU979039	China	Chen et al. 2011
	Tpse-yn05	DQ329374	China	Wang et al. 2006
Tuber regimontanum	ITCV 909	EU375838	Mexico	Guevara et al. 2008
Tuber indicum	Ascocarpe I1	AF300822	China	Mabru et al. 2001
	HKAS 39501	AY514305	China	Zhang et al. 2005
Tuber melanosporum	SB2-6	MF693845	France	Schneider-Maunoury et al. 2018
	P_Qr	KP972070	Canada	Berch and Bonito 2016
Tumericum Clade				
Tuber turmericum	BJTC FAN475	KT758839	China	Fan et al. 2015
	BJTC FAN473	KT758837	China	Fan et al. 2015
Gibbosum Clade				
Tuber oregonense	DUKE GB284	FJ809874	USA	Bonito et al. 2010b
Tuber gibbosum	OSC 40964	FJ809863	USA	Bonito et al. 2010b
Maculatum Clade				
Tuber maculatum	A15	AM406673	Italy	El Karkouri et al. 2007
	Db-A	MH040280*		Sikora 2018
Latisporum Clade				
Tuber latisporum	HKAS 44315	DQ898183	China	Chen and Liu 2007
Tuber pseudosphaerosporum	BJTC Fan250	KF744063	China	Fan and Yue 2013
Puberulum Clade				
Tuber cistophilum	AH 39275	JN392231	Spain	Alvarado et al. 2012a
Tuber borchii	Tar042	KT165326	Italy	Belfiori et al. 2016
Tuber sphaerospermum	AH38930	JN392244	Morocco	Alvarado et al. 2012a
	AH39190	JN392246	Spain	Alvarado et al. 2012a
Outgroup				
Choiromyces alveolatus	22830	AF501258		Ferdman et al. 2005
	p612i	EU697268*		Gordon 2008

* unpublished sequence.
** this sequence appears as "T. regianum" in GenBank (unpublished; Merenyi et al. 2011).

MK113982 (Table 1). Moreover, the percent sequence identity was estimated by using ClustalOmega (Sievers and Higgins 2018) through the EMBL-EBI portal.

A total of 62 *Tuber* ITS rDNA sequences were used for phylogenetic analysis by including eight sequences of *T. pulchrosporum* sp. nov. and 54 sequences from GenBank (nine of them representing type specimens) which correspond to 31 *Tuber* taxa mainly of European distribution (Table 1). *Choiromyces alveolatus* (Harkn.) Trappe (AF501258, EU697268) was used as the outgroup. Sequence alignment was performed through the online version of the multiple sequence alignment programme MAFFT v7 (Katoh and Standley 2013) by applying the Q-INS-I strategy and alignments were inspected and manually adjusted at misaligned sites by using MEGAX (Kumar et al. 2018). The pertinent matrix was deposited in TreeBASE under the accession number 23587.

Phylogenetic relationships of taxa were inferred by using maximum likelihood (ML) and Bayesian Inference (BI) through the CIPRES portal (www.phylo.org; Miller et al. 2010). ML analysis of the ITS dataset was conducted by RAxML v8.2 (Stama-

takis 2014) with 1,000 bootstrap replicates and search for the best-scoring ML tree. BI analysis was performed by MrBayes v3.2.1 (Ronquist et al. 2012) and the General Time Reversible + Gamma (GTR+G) model was selected as the best model under the Akaike Information Criterion (AIC) implemented in MrModeltest v2.3 (Nylander 2004). To estimate posterior probabilities, 20,000,000 Markov chain Monte Carlo (MCMC) simulation generations were run in two parallel independent runs of four chains, one cold and three heated, with trees sampled every 1,000 generations and the first 25% of trees were omitted as burn-in. A 50% majority rule consensus tree was built and visualised with iTOL (Letunic and Bork 2016). Clades with bootstrap support (BS) ≥ 70% and Bayesian posterior probability (PP) ≥ 95% were considered as significantly supported.

Results

Taxonomy

Tuber pulchrosporum **Konstantinidis, Tsampazis, Slavova, Nakkas, Polemis, Fryssouli & Zervakis, sp. nov.**
MycoBank: MB 828883
GenBank: MK113975
Fig. 1a

Type. GREECE. Ioannina Prefecture: Ioannina city, 39°36'39"N, 20°50'05"E, 500 m alt., in soil under a pure stand of *Quercus coccifera* L., 27 Apr 2016, coll. V. Nakkas, VN091, holotype: ACAM 2016-007 (ACAM!); isotype: SOMF 29980 (SOMF!).

Diagnosis. Ascomata 0.6–7(–10) cm in diam., subglobose, ovoid to irregularly lobed, usually with shallow basal cavity, surface with fissures and small, dense, almost flat trihedral to polyhedral warts, yellowish-brown to dark brown. Ascospores 25.0–37.0 × 18.2–25.6 µm in (1–)2–8-spored asci, ellipsoid to subfusiform on average, Qm=1.4, crested to incompletely reticulate. Hair-like, hyaline to light yellow-brown hyphae protruding from peridium surface.

T. panniferum, the closest phylogenetically-related species, produces smaller ascospores (23–26 × 18–20 µm), broadly ellipsoid to subglobose on average, with isolated warts; moreover, the peridium surface is woolly-felted due to the presence of dense rusty brown hair-like hyphae.

Etymology. "*pulchrosporum*" refers to the uniquely distinct/impressive ornamentation of the ascospores.

Description. ***Ascomata*** 0.6–7(–10) cm in diameter, tuberous, subglobose, ovoid to irregularly lobed, usually depressed with a shallow - occasionally prominent - basal cavity (excavated), covered up with whitish to yellowish rhizomorphs, fragile, initially greyish to yellowish-brown [fawn (29), sienna (11), fulvous (12)], darkening in maturity to brown [snuff brown (17), umber (18), bay (19), to date brown (24)] or with

Figure 1. *T. pulchrosporum* sp. nov.: **a** ascomata in situ (holotype) **b** ascomata in situ (paratype) **c** detail of peridium surface (paratype) **d** section of peridium (paratype).

some shades of purple tinges [purplish date (22), purplish chestnut (21) to brown vinaceous (25)], sometimes with darker black [fuscous black (38)] spots, surface rarely almost smooth, usually rough, with fissures and small, dense, almost flat trihedral to polyhedral warts. *Gleba* with one of more cavities, initially pinkish-grey [vinaceous buff (31), clay pink (30)], then greyish-brown [milky coffee (28)], yellowish-brown [fulvous (12)], brown [snuff brown (17), umber (18), bay (19)], to purplish-brown in maturity [purplish date (22) to purplish chestnut (21)], with bay (19) to rusty tawny (14) coloured areas close to the cavity, marbled with relatively few and thick white veins, that sometimes are reddening (Fig. 1). *Odour* pleasant truffle-like.

Peridium 120–370 µm thick, consisting of two layers; the outer layer 50–160 µm thick, pseudoparenchymatous, composed of yellowish-brown and subglobose inwards to subangular dark brown cells outwards; 4.0–16.3 × 2.5–13.2 µm, thick-walled (1.5–2.5 µm); the inner layer 70–210 µm, composed of pale yellow or hyaline and thick-walled, interwoven hyphae, 2–10 µm in diameter, forming an intricate texture, becoming agglutinated when dried. Surface with abundant isolated, hyaline to golden-yellow (in water or KOH), thick-walled hair-like hyphae (walls 1.0–1.5 µm), 30–140 µm long (occasionally exceeding 300 µm in Bulgarian specimens) and 2.5–4.5 µm broad at base, 1–2 septate (Figs 1, 2).

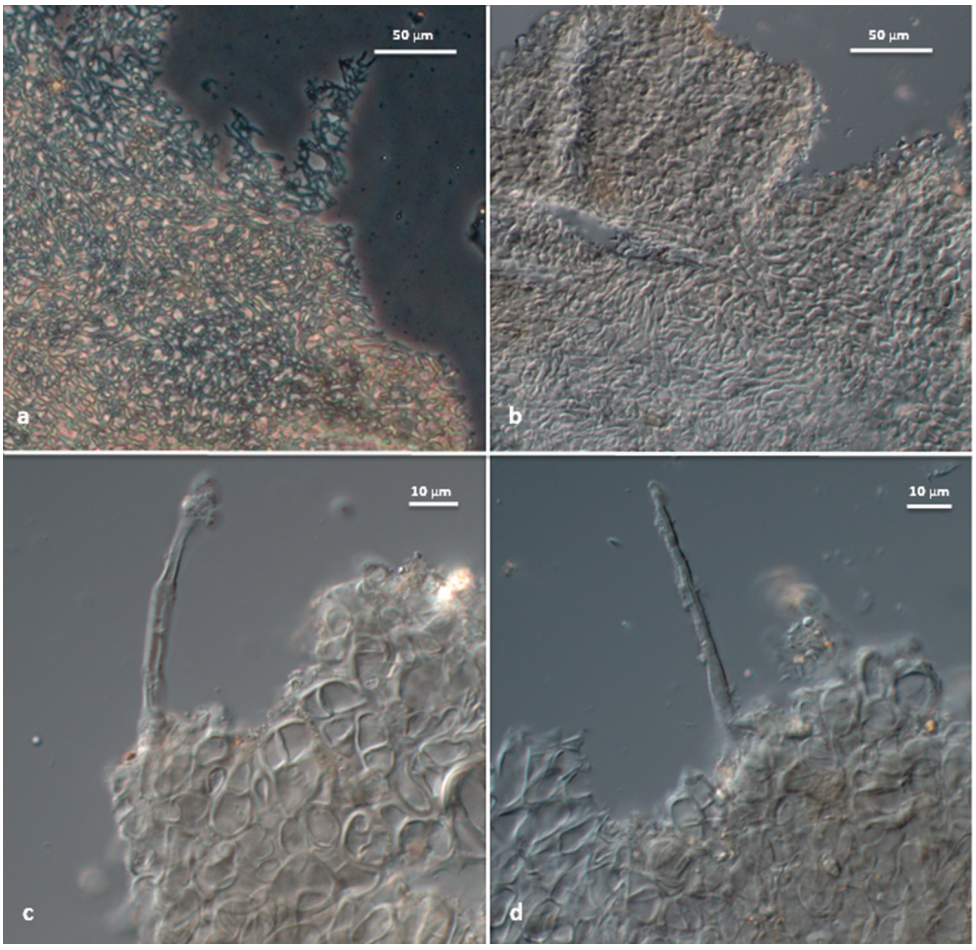

Figure 2. *T. pulchrosporum* sp. nov.: **a, b** peridium structure **c, d** hair-like hyphae on peridium surface.

Ascospores hyaline when young then yellowish, yellow-brown to brown, at most ellipsoid to subfusiform, some broadly ellipsoid, subglobose to globose, rarely almost limoniform in initial stages, thin-walled and smooth when young, becoming thick-walled at maturity, walls 2–3.5(–4) µm thick, usually crested to incompletely reticulate, measured (excluding the ornamentation) in the rare 1-spored asci (28–) 46.7±7.4 (–57) × (20–) 29.4±4.6 (–34) µm, in 2-spored asci (27–) 39.5±5.8 (–53) × (21–) 27.3±4.2 (–41) µm, in 3-spored asci (24–) 34.5±5.3 (–49) × (19–) 24.5±2.6 (–31) µm, in 4-spored (21–) 30.9±4.9 (–39) × (18–) 22.2±2.7 (–30) µm, in 5-spored asci (22–) 30.3±3.7 (–44) × (16–) 21.2±2.2 (–28) µm, in 6-spored asci (22–) 28.9±4.6 (–37) × (17–) 20.6±2.0 (–28) µm, in 7-spored asci (21–) 27.8±3.3 (–35) × (13–) 19.9±2.7 (–27) µm and in 8-spored asci (20–) 25.4±2.6 (–31) × (14–) 18.4±3.1 (–26) µm (Fig. 3); Q=1.0–2.2, Qm=1.43±0.19; ornamentation with (0–)1–2(–4) thick veins across the long axis with few to several transverse outgrowths, rarely al-

Figure 3. *T. pulchrosporum* sp. nov.: asci and ascospores.

most completely reticulate in maturity and then with (0–)2–10(–15) meshes in the longitudinal direction; circumferentially with 22–42 conical warts, with pointed or blunt, straight or curved apices, rarely forked, 1.5–6(–8) µm tall (Fig. 4); not reacting with Melzer's reagent. *Asci* (64–) 78–96 (–121) × (50–) 65–84 (–98) µm (excluding stalk), globose, subglobose, ellipsoid, rarely subangular, with a short stalk, 6.5–9(–15) × 6.5–7.5(–10.5) µm, (1–)2–8-spored (Fig. 3).

Distribution and ecology. Hypogeous, in soil, appearing solitary or in small groups from March to June, under *Quercus* sp., *Q. coccifera* or *Q. ilex* L. or under *Carpinus* sp. or in mixed stands of *Quercus* sp. and *Pinus nigra* J.F. Arnold or of *Q. ilex* and *Pinus halepensis* Miller or of *Quercus robur* L., *Corylus* sp., *Carpinus* sp. and *Acer* sp. It seems to be rather common in continental (northern and central) Greece, while it also occurs in the regions of Eastern Stara Planina and the Black Sea coast of Bulgaria.

Additional collections examined (paratypes). GREECE. Xanthi Prefecture: Toxotes, in soil under a mixed stand dominated by *Q. coccifera*, 20 June 2008, GK3186b (ACAM 2010-127), coll. P. Panagiotidis. Aitoloakarnania Prefecture: Xiromero, in soil under pure forest of *Quercus* sp., 10 May 2009, GK3801 (ACAM 2010-129), coll. Ch. Chrysopoulos and K. Giatra (GenBank: MK113979); Xiromero, in soil under pure for-

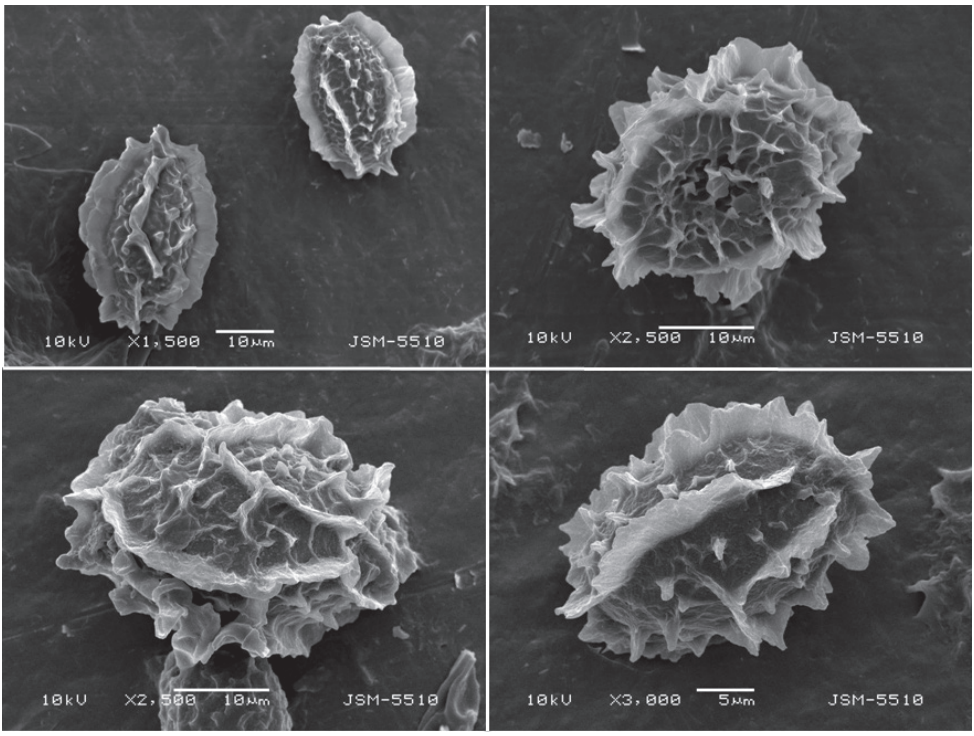

Figure 4. *T. pulchrosporum* sp. nov.: SEM of ascospores.

est of *Quercus* sp., 10 May 2009, GK3799 (ACAM 2010-128), coll. Ch. Chrysopoulos and K. Giatra. Trikala Prefecture: Koziakas Mt., in soil under mixed forest of *Quercus* sp. and *P. nigra*, 2 April 2013, GK6538 (ACAM 2013-073), coll. K. Papadimitriou (GenBank: MK113978); Koziakas Mt., in soil under mixed forest of *Quercus* sp. and *P. nigra*, 2 April 2013, GK6537 (ACAM 2013-074), coll. K. Papadimitriou. Ioannina Prefecture: Metsovo, in soil under pure stand of *Q. coccifera*, 18 April 2016, GK9408 (ACAM 2016-001), coll. A. Bideris (GenBank: MK113977); Metsovo, in soil under pure stand of *Q. coccifera*, 19 April 2016, GK9409 (ACAM 2016-002), coll. A. Bideris; Metsovo, in soil under pure stand of *Q. coccifera*, 19 April 2016, GK9410 (ACAM 2016-003), coll. A. Bideris; Demati, in soil under pure stand of *Q. coccifera*, 22 March 2017, GK10231 (ACAM 2017-033), coll. A. Bideris. Attica Prefecture: Katsimidi, in soil under mixed forest of *Q. ilex* and *P. halepensis*, 22 March 2016, VK4482 (ACAM 2016-004), coll. V. Kaounas (GenBank: MK113980); Katsimidi, in soil under mixed forest of *Q. ilex* and *P. halepensis*, 12 April 2016, VK4506 (ACAM 2016-005), coll. V. Kaounas (GenBank: MK113980). Ioannina Prefecture: Neochoropoulo, in soil under a mixed stand of *Q. coccifera* and *Q. ilex*, 27 April 2016, LT1183 (ACAM 2016-006), coll. V. Nakkas (GenBank: MK113976). BULGARIA. Varna, Dolishte village, in soil under pure stand of *Carpinus* sp., 07 June 2017, MSL 1945 F8517 (SOMF 29978; ACAM 2017-034), coll. R. Radev (GenBank: MK113981). Sliven, in soil under a mixed stand of *Quercus robur*,

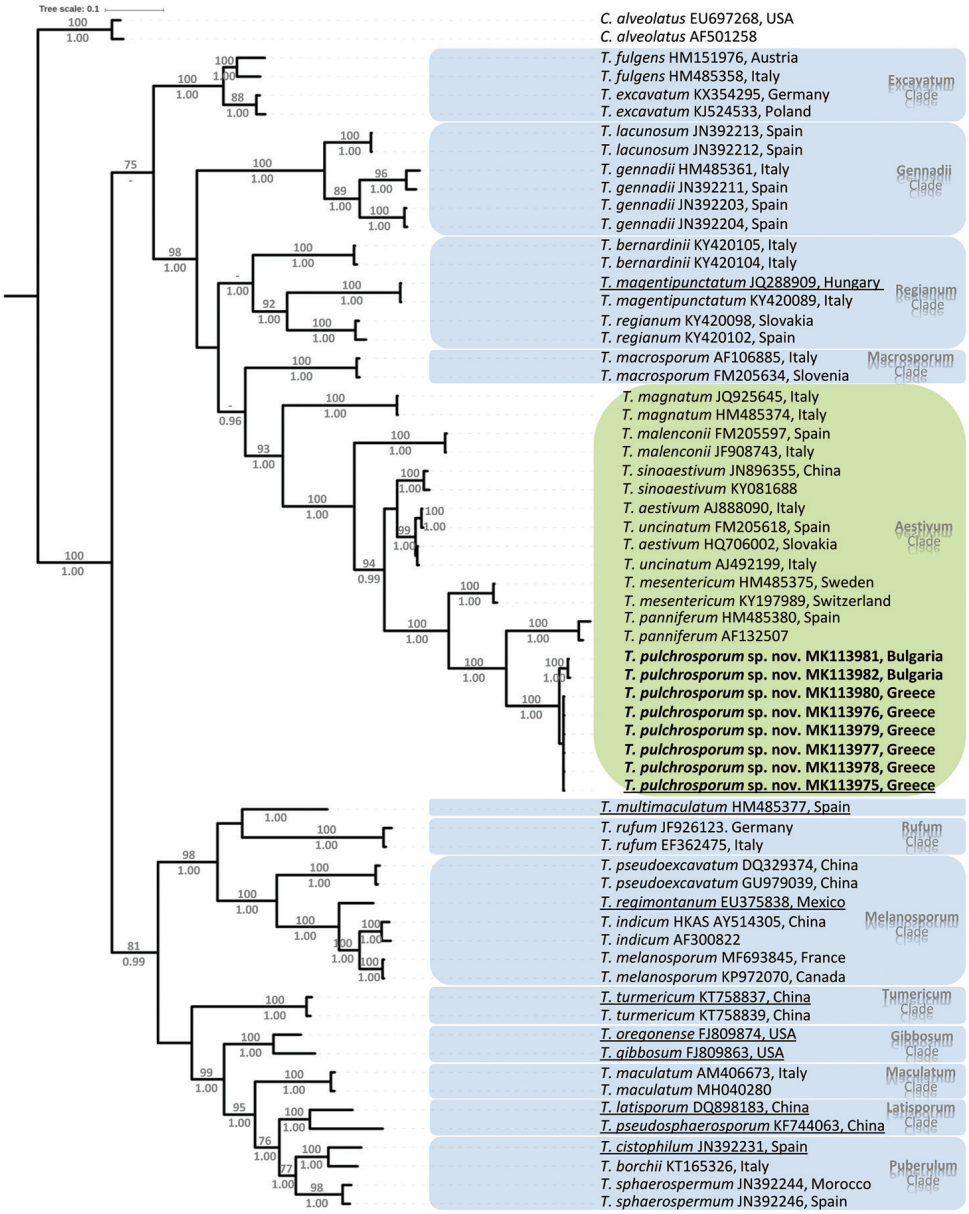

Figure 5. Phylogenetic tree inferred from Bayesian analysis including 62 ITS sequences assigned to 31 *Tuber* taxa, including members of major clades of the genus. Sequences are labelled with Latin binomials, GenBank accession numbers and geographic origin. *T. pulchrosporum* sp. nov. is indicated in boldface. Reference sequences deriving from type material are underlined. *Choiromyces alveolatus* (Tuberaceae) was used as the outgroup. Bootstrap (BS) values from Maximum Likelihood (ML) analysis (≥ 70%) and Posterior Probabilities (PPs) from Bayesian Inference (≥ 0.95) are shown at the nodes of branches.

Corylus sp., *Carpinus* sp. and *Acer* sp., 09 August 2017, MSL 1961 F0388 (SOMF 29979; ACAM 2017-035), coll. K. Pilasheva & P. Neikov (GenBank: MK113982).

Phylogenetic aspects. The resultant ITS sequence data comprises of 64 sequences which were aligned at 780 sites, 738 of which represent the ITS1-5.8S-ITS2 region, i.e. between the end of the SSU motif (CATTA) and the beginning of LSU motif (TAGGG) (Bonito et al. 2010a). ML and BI analyses yielded similar tree topologies and only the tree inferred from the Bayesian analysis is presented (Fig. 5). The morphologically variable genus *Tuber* is monophyletic (BS: 100%, PP: 1.00) and several lineages are revealed; for the purposes of this study, the following highly supported clades were included: Aestivum, Excavatum, Gennadii, Gibbosum, Latisporum, Maculatum, Macrosporum, Melanosporum, Puberulum, Regianum, Rufum, Tumericum (=Japonicum).

According to the phylogenetic analysis performed, *T. pulchrosporum* belongs to the Aestivum clade. All eight sequences of this new taxon form a distinct highly supported subclade (BS: 100%, PP: 1.00). Greek specimens possessed almost identical ITS sequences (99.8 – 100%) and so did Bulgarian samples, whereas the comparison between collections from the two countries resulted in sequence identity values of 98.13 ± 0.08%. In total, intraspecific sequence identity values for *T. pulchrosporum* exceeded 98% (i.e. 98.05 – 100%). The new species is sister to *T. panniferum* (BS: 100%, PP: 1.00); the respective sequences demonstrated low sequence identity (73.21 – 75.08%) further evidencing their distinct taxonomic status.

Discussion

The molecular analysis evidenced that the eight sequences representing *T. pulchrosporum* are grouped within the Aestivum clade by forming a distinct terminal group supported with high BS and PP values. The closest phylogenetic relative of *T. pulchrosporum* is *T. panniferum* Tul. & C. Tul., i.e. a Mediterranean species with analogous ecological preferences (Jeandroz et al. 2008). *T. panniferum* also exhibits a rather similar macromorphology characterised by a brownish pubescent peridium, absence of pyramidal warts and ascomata often bearing a cavity, although the tomentum is much more prominent, exhibiting thus a felted appearance. However, the microscopic features of the two species are clearly different. In *T. panniferum*, the ornamentation consists of isolated spines never exceeding 3 μm in height, while the peridial surface is covered by rusty brown hyphae which form a dense cottony mass (Montecchi and Sarasini 2000; Riousset et al. 2001; Moreno-Arroyo et al. 2005).

By morphology alone, *T. pulchrosporum* is easily distinguishable within the Aestivum clade since no other species produces ascospores bearing such a uniquely crested ornamentation. The more distant *T. aestivum* (Wulfen) Spreng. (including *T. uncinatum* Chatin) and *T. sinoaestivum* J.P. Zhang & P.G. Liu could be distinguished macroscopically thanks to their blackish peridial surface with prominent pyramidal warts and ascospores bearing a complete reticulum. Ascospores of *T. mesentericum* Vittad. show some affinity in their outline to those of *T. pulchrosporum* but they clearly possess a much more reticulate network; moreover, the peridial surface is black with pyramidal warts as in *T. aestivum*.

Although phylogenetically more distant, some other species with asci containing 1–8 ascospores may superficially resemble *T. pulchrosporum*. Hence, *T. regianum* Montecchi & Lazzari, the recently described *T. magentipunctatum* Z. Merényi, I. Nagy, Stielow & Bratek and *T. bernardinii* Gori, all belonging to the Regianum clade (Zambonelli et al. 2016; Crous et al. 2017), possess a reddish-brown to brown peridial surface with dense and rather flat warts as in the case of *T. pulchrosporum*. However, they all produce ascospores with pointed spines which are connected to form a complete reticulum. Ascomata of *T. malenconii* Donadini, Riousset, G. Riousset & G. Chev and *T. pseudoexcavatum* Y. Wang, G. Moreno, Riousset, Manjón & G. Riousset also show a macroscopic resemblance to *T. pulchrosporum*, with their rough indistinctly warty peridial surface (black for the former and brown for the latter), often with a similar basal cavity as well. However, ascospores of both *T. malenconii* and *T. pseudoexcavatum* have short spines, basally/broadly connected, exhibiting a more or less regular reticulum (Donadini et al. 1979; Manjón et al. 2009). Therefore, the unique type of ornamentation of *T. pulchrosporum* ascospores clearly distinguishes it from all species with similar macroscopic appearance.

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Two new entomopathogenic species of *Ophiocordyceps* in Thailand

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Abstract

Ophiocordyceps is entomopathogenic and the largest studied genus in the family *Ophiocordycipitaceae*. Many species in this genus have been reported from Thailand. The first new species introduced in this paper, *Ophiocordyceps globiceps*, differs from other species based on its smaller perithecia, shorter asci and secondary ascospores and additionally, in parasitising fly species. Phylogenetic analyses of combined LSU, SSU, ITS, TEF1α and RPB1 sequence data indicate that *O. globiceps* forms a distinct lineage within the genus *Ophiocordyceps* as a new species. The second new species, *Ophiocordyceps sporangifera*, is distinguished from closely related species by infecting larvae of insects (Coleoptera, Elateridae) and by producing white to brown sporangia, longer secondary synnemata and shorter primary and secondary phialides. We introduce *O. sporangifera* based on its significant morphological differences from other similar species, even though phylogenetic distinction is not well-supported.

Keywords

2 new taxa, Hypocreales, morphology, phylogenetic, taxonomy

Introduction

The genus *Ophiocordyceps* was introduced by Petch (1931) to accommodate species which have different features of asci and ascospores from *Cordyceps* (Petch 1931). *Ophiocordyceps* was treated as a subgenus of *Cordyceps* by Kobayasi (1941, 1982) and Mains (1958). Sung et al. (2007a) established the new family *Ophiocordycipitaceae* in Hypocreales (Sordariomycetes) and revised *Ophiocordyceps* as the type genus based on phylogenetic analyses. This is followed in the Outline of Ascomycetes (Wijayawardene et al. 2018). The main characters of the sexual morph species of *Ophiocordyceps* are fibrous, hard, pliant-to-wiry, dark stromata with superficial to immersed perithecia (Sung et al. 2007a, Ban et al. 2015). The asexual morphs in the majority of species have hirsutella-like and hymenostilbe-like features (Kepler et al. 2013, Maharachchikumbura et al. 2015, 2016). The hosts of species in *Ophiocordyceps* are larval lepidopterans and coleopterans, adult hymenopterans, hemipterans, dipterans, orthopterans or dragonflies (Odonata) and, in few cases, spiders (Kobayasi 1941, Mains 1958, Sung et al. 2007a, Ban et al. 2015). Hitherto, *Ophiocordyceps* included 233 species (Index Fungorum, June 2018) with a worldwide diversity (Sung et al. 2007a, Ban et al. 2015, Spatafora et al. 2015, Shrestha et al. 2017).

Thailand is located in the tropical areas with a rich biodiversity (Luangsa-ard et al. 2008, Aung et al. 2008, Luangsa-ard et al. 2010, Hyde et al. 2017, Hyde et al. 2018). A variety of entomopathogenic species (more than 400 species) (Index Fungorum, June 2018, Luangsa-ard et al. 2008, Luangsa-ard et al. 2010) were reported from Thailand after the first species recorded by Petch in 1932. In this study, we introduce two new species of *Ophiocordyceps*, which were found on larvae of insects (Lepidoptera, Cossidae) and adult Diptera. The descriptions of these two new species and phylogenetic evidence for the new taxa are provided. Morphological differences between two new species and their related species are also discussed.

Methods

Collection, isolation, and morphology study

Specimens were collected in The Mushroom Research Centre, Chiang Mai, Thailand, from soil and grass litter and taken to the laboratory. Fruiting bodies were examined using free hand sections under a stereomicroscope. Water-mounted slides were prepared for a microscope study and photographed under a compound microscope. Strains were isolated from single spores by using the protocol in Chomnunti et al. (2014). Cultures were incubated at 25 °C for 4–10 weeks on potato extract agar (PDA) in light-promoted sporulation.

DNA extraction, PCR amplification and determination of DNA sequences

DNA was extracted from both dried specimens and cultures by using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA), according to the manufacturers proto-

cols. Universal known primers were used in PCR amplification; ITS4/ITS5 for internal transcribed spacer gene region (ITS), NS1/NS4 for partial small subunit ribosomal RNA gene region (SSU), LROR/LR5 for partial large subunit rDNA gene region (LSU) (Vilgalys and Hester 1990, White et al. 1990), 983F/2218R for partial translation elongation factor 1-alpha gene region (TEF1α) (Sung et al. 2007b) and CRPB1A/RPB1Cr for partial RNA polymerase II largest subunit gene region (RPB1) (Castlebury et al. 2004). PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China. Specimen was performed by using TaKaRa PMD18-T vector system (TaKaRa Biotechnology, Dalian, China), while PCR products could not be sequenced directly.

Phylogenetic analyses

Sequence data were obtained from GenBank based on previous studies as listed in Table 1. MAFFT v.7 was used to align combined datasets of ITS, SSU, LSU, TEF1α and RPB1 regions (Katoh and Standley 2013, http://mafft.cbrc.jp/alignment/server/). BioEdit (Hall 2011) was used to check alignment manually. Gaps were treated as missing data. *Tolypocladium inflatum* W. Gams and *T. ophioglossoides* (J.F. Gmel.) C.A. Quandt et al. (Kepler et al. 2012, Schoch et al. 2012) were selected as outgroup taxa.

Maximum likelihood trees (ML) were estimated by using the software RAxML 7.2.8 Black Box (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES Science Gateway platform (Miller et al. 2010). MrModeltest v.2.3 (Nylander 2004) was used to determine the best-fit model of evolution for Bayesian analyses. MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) was used to evaluate posterior probabilities (PP) (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 10,000,000 generations, trees were sampled every 100th generation and 100,001 trees were obtained. The first 25% of trees (25,000) were discarded, as they represented the burn-in phase of the analyses, while the remaining trees (75,001) were used for calculation of posterior probabilities in the majority rule consensus tree (critical values for the topological convergence diagnostic is 0.01). Trees were figured in FigTree v1.4.0 programme (Rambaut 2012). Bayesian Posterior Probabilities (BYPP) equal to or great than 0.90 were given below each node (Fig. 1).

Results

Molecular phylogeny

Eighty-seven taxa (including the four with new sequence data) were included in the combined ITS, SSU, LSU, RPB1 and TEF1α dataset (Table 1), which comprises 3894 characters with gaps; 1011 characters for SSU, 824 for LSU, 561 for ITS, 880 for TEF1α and 618 for RPB1. Tree topology of the RAxML analysis was similar to the Bayesian analysis. The best scoring RAxML tree with a final likelihood value of

Figure 1. Phylogram of *Ophiocordyceps globiceps* and *O. sporangifera* generated from maximum likelihood (RAxML) analysis of ITS, SSU, LSU, RPB1 and TEF1α sequence data. *Tolypocladium inflatum* and *T. ophioglossoides* were used as outgroup taxon. Maximum likelihood bootstrap values greater than 75% and Bayesian posterior probabilities over 0.90 were indicated above the nodes. The new species are indicated in red.

-46932.268101 is presented (Fig. 1). The matrix had 2081 distinct alignment patterns, with 35.22% of undetermined characters or gaps. Parameters for the GTR model of the concatenated dataset were as follows: Estimated base frequencies; A = 0.240006, C = 0.270755, G = 0.276725, T = 0.212514; substitution rates AC = 1.073676, AG = 3.611556, AT = 1.170890, CG = 1.176549, CT = 6.339087, GT = 1.000; gamma distribution shape parameter α = 0.265589.

Table 1. Sources of isolates and GenBank accession numbers used in the paper.

Species	Insecta	Voucher	SSU	ITS	LSU	TEF1α	RPB1	References
H. dipterigena	Diptera	NHJ12170.02		GU723771		GU797126		Luangsa-ard et al. 2011
O. acicularis	Coleoptera (larva)	OSC 110988	EF468951		EF468804	EF468745	EF468853	Sung et al. 2007a
O. agriotidis	Coleoptera (larva)	ARSEF 5692	DQ522540	JN049819	DQ518754	DQ522322	DQ522368	Ban et al. 2015
O. amazonica	Orthoptera (Acrididae imago)	Ophama2026	KJ917562		KJ917571	KM411989	KP212902	Sanjuan et al. 2015
O. annulata	Coleoptera	CEM 303	KJ878915		KJ878881	KJ878962	KJ878995	Quandt et al. 2014
O. aphodii	Coleoptera	ARSEF 5498	DQ522541		DQ518755	DQ522323		Spatafora et al. 2007
O. appendiculata	Coleoptera (larva)	NBRC 106960	JN941728	JN943326	JN941413	AB968577	JN992462	Ban et al. 2015
O. arborescens	Cossida (larva)	NBRC 105891		AB968398	AB968414	AB968572		Ban et al. 2015
O. australis	Hymenoptera (ant)	Ophaus992	KC610785		KC610766	KC610731	KF658663	Ban et al. 2015
O. barnesii	Coleoptera (larva)	BCC28560	EU408776				EU408773	Luangsa-ard et al. 2010
O. brunneinigra	Hemiptera (Cicadellidae)	TBRC 8093			MF614654	MF614638	MF614668	Luangsa-Ard et al. 2018
O. brunneiperitheciata	Lepidoptera (larva)	TBRC 8100		MF614658		MF614643		Luangsa-Ard et al. 2018
O. brunneipunctata	Coleoptera (Elateridae larva)	OSC 128576	DQ522542		DQ518756	DQ522324	DQ522369	Spatafora et al. 2007
O. buquetii	Hymenoptera (Formicidae)	HMAS 199613	KJ878939		KJ878904	KJ878984	KJ879019	Quandt et al. 2014
O. citrina	Hemiptera	TNS F18537			KJ878903	KJ878983		Quandt et al. 2014
O. clavata	Coleoptera (larva)	NBRC 106962	JN941726	JN943328	JN941415	AB968587	JN992460	Schoch et al. 2012
O. coccidiicola	Insect	NBRC 100682	AB968404		AB968419	AB968583		Ban et al. 2015
O. coccidiicola	Insect	HMAS199612	KJ878917	AB027377	KJ878884	KJ878965	KJ878998	Quandt et al. 2014
O. coenomyia	Coenomyia (larva)	NBRC 108993	AB968384	AB968396	AB968412	AB968570		Ban et al. 2015
O. communis	Coleoptera	NHJ 12581	EF468973		EF468831	EF468775		Quandt et al. 2014
O. cossidarum	Lepidoptera (larva)	MFLU 17-0752	MF398186		MF398187	MF928403	MF928404	Hyde et al. 2017
O. crinalis	Lepidopteran (larva)	HIMGD17327		EU149926				Zhang et al. 2007
O. curculionum	Coleoptera (adult Curculionidae)	OSC 151910	KJ878918		KJ878885		KJ878999	Quandt et al. 2014
O. cylindrospora	Hymenoptera (adult wasp)	MFLU: 17-1961	MG553651	MG553635	MG553652			Hyde et al. 2018
O. dipterigena	Diptera (adult fly)	MY621		GU723764		GU797126		Luangsa-ard et al. 2011
O. dipterigena	Diptera (adult fly)	MRCIF71		EU573346				Freire 2015
O. dipterigena	Diptera (adult fly)	OSC 151912	KJ878920		KJ878887	KJ878967	KJ879001	Quandt et al. 2014
O. elongata	Lepidoptera (larva)	OSC 110989			EF468808	EF468748	EF468856	Sung et al. 2007a
O. emeiensis	Lepidoptera (larva)	G96031		AJ309347				Liu et al. 2002
O. entomorrhiza	Lepidoptera	KEW 53484	EF468954	JN049850	EF468809	EF468749	EF468857	Quandt et al. 2014
O. evansii	Hymenoptera (Pachycondylaharpax)	Ophsp 858	KC610796		KC610770	KC610736	KP212916	Sanjuan et al. 2015
O. forquignonii	Diptera (adult fly)	OSC 151908	KJ878922		KJ878889		KJ879003	Quandt et al. 2014

Species	Insecta	Voucher	SSU	ITS	LSU	TEF1α	RPB1	References
O. formicarum	*Camponotus* (Ant)	BCMU CF 01		AB222678				Freire 2015
O. formicarum	*Camponotus* (Ant)	BCMU CF 02		AB222679				Freire 2015
O. formosana	Coleoptera (larva)	MFLU: 15-3888						Li et al. 2016
O. fulgoromorphila	Hemiptera (Fulgoridae adult)	Ophara717	KC610794		KC610760	KC610729	KF658676	Sanjuan et al. 2015
O. geometridicola	Lepidoptera (Geometridae)	TBRC 8095			MF614648	MF614632	MF614663	Luangsa-Ard et al. 2018
O. globiceps	Diptera (adult fly)	MFLUCC 18-0495	MH725811	MH725815	MH725829	MH727387		This study
O. globiceps	Diptera (adult fly)	MFLU 18-0661	MH725812	NH725816	MH725830	MH727388		This study
O. gracilis	Lepidoptera (larva)	EFCC 8572	EF468956	JN049851	EF468811	EF468751	EF468859	Kepler et al. 2012
O. hemisphaerica	Diptera (adult fly)	FLOR 59525	KX197233					Hyde et al. 2016
O. heteropoda	Hemiptera (cicada nymph)	OSC 106404	AY489690		AY489722	AY489617	AY489651	Castlebury et al. 2004
O. irangiensis	Hymenoptera (adult ant)	OSC 128579	EF469123		EF469076	EF469060	EF469089	Sung et al. 2007a
O. issidarum	Hemiptera (adult)	MFLU:17-0751		MF398185	MF398188			Hyde et al. 2017
O. karstii	*Hepialus* (larva)	MFLU:15-3884	KU854952			KU854945	KU854943	Li et al. 2016
O. konnoana	Coleoptera (larva)	EFCC 7315	EF468959			EF468753	EF468861	Sung et al. 2007a
O. lanpingensis	*Hepialus* (larva)	YHOS0707	KC417459		KC417461	KC417463	KC417465	Chen et al. 2013
O. lloydii	Hymenoptera (Camponotus)	OSC 151913	KJ878924		KJ878891	KJ878970	KJ879004	Quandt et al. 2014
O. longissima	Hemiptera (cicada nymph)	NBRC 108989	AB968394	AB968407	AB968421	AB968585		Sanjuan et al. 2015
O. macroacicularis	lepidopterans (larvae)	NBRC 105888	AB968389	AB968401	AB968417	AB968575		Ban et al. 2015
O. melolonthae	Coleoptera (Scarabeidae larva)	OSC 110993	DQ522548		DQ518762	DQ522331	DQ522376	Spatafora et al. 2007
O. multiperitheciata	Lepidoptera (larva)	BCC 69008			MF614657	MF614641		Luangsa-Ard et al. 2018
O. myrmecophila	Hymenoptera (adult ant)	MFLU 16-2912	MF351730	MF351726	MF372585	MF372759		Xiao et al. 2017
O. myrmicarum	*Formicidae* (adult ant)	ARSEF11864	KJ680150			JX566973	KJ680151	Simmons et al. 2015
O. neovolkiana	Coleoptera	OSC 151903	KJ878930		KJ878896	KJ878976	KJ879010	Quandt et al. 2014
O. nigra	Hemiptera	TNS 16252	KJ878941		KJ878906	KJ878986		Quandt et al. 2014
O. nigrella	Lepidoptera (larva)	EFCC 9247	EF468963	JN049853	EF468818	EF468758	EF468866	Sung et al. 2007a
O. nutans	Hemiptera (Pentatomidae adult)	OSC 110994	DQ522549		DQ518763	DQ522333	DQ522378	Spatafora et al. 2007
O. odonatae	Odonata (Dragonfly)	TNS F18563	D86055	AB104725				Ito and Hirano 1997
O. pauciovoperithe-ciata	Lepidoptera (larva)	TBRC 8106			MF614652	MF614633		Luangsa-Ard et al. 2018
O. pseudoacicularis	Lepidoptera (larva)	TBRC 8102			MF614646	MF614630	MF614661	Luangsa-Ard et al. 2018
O. pulvinata	Hymenoptera (adult ant)	TNS-F 30044	GU904208			GU904209	GU904210	Quandt et al. 2014
O. purpureostromata	Coleoptera	TNS F18430	KJ878931		KJ878897	KJ878977	KJ879011	Quandt et al. 2014
O. pseudolloydii	Formicidae (adult ant)	MFLU 15-1425		MF351725		MF372758	MF372761	Xiao et al. 2017
O. ramosissimum	Lepidoptera (larva)	GZUHHN8	KJ028012	KJ028007		KJ028014	KJ028017	Wen et al. 2014
O. ravenelii	Coleoptera (larva)	OSC 110995	DQ522550		DQ518764	DQ522334	DQ522379	Spatafora et al. 2007

Species	Insecta	Voucher	SSU	ITS	LSU	TEF1α	RPB1	References
O. rhizoidea	Isoptera (adult termite)	NHJ 12529	EF468969		EF468824	EF468765	EF468872	Sung et al. 2007a
O. robertsii	Lepidoptera (Hepialidae larva)	KEW 27083			EF468826	EF468766		Sung et al. 2007a
O. rubiginosiperithe-ciata	Coleoptera (larva)	NBRC 106966	JN941704	JN943344	JN941437	AB968582	JN992438	Ban et al. 2015
O. sinensis	Lepidopteran pupa	EFCC7287	EF468971	JN049854		F468767	EF468874	Sung et al. 2007a
O. sobolifera	Hemiptera (cicada nymph)	NBRC 106967	AB968395	AB968409	AB968422	AB968590		Ban et al. 2015
O. sp		FMF147		KX197238				Freire 2015
O. sp		OSC 110997	EF468976			EF468774	EF468879	Quandt et al. 2014
O. spataforae	Hemiptera (Fulgoridae)	NHJ 12525	EF469125		EF469078	EF469063	EF469092	Sung et al. 2007a
O. sphecocephala	Hymenoptera (adult wasp)	NBRC 101753	JN941695	JN943350	JN941446	AB968592	JN992429	Ban et al. 2015
O. sporangifera	Lepidoptera (Cossidae)	MFLUCC 18-0492	MH725814	MH725818	MH725832	MH727390	MH727392	This study
O. sporangifera	Lepidoptera (Cossidae)	MFLU 18-0658	MH725813	MH725817	MH725831	MH727389	MH727391	This study
O. stylophora	Coleoptera (Elateridae larva)	OSC 111000	DQ522552	JN049828	DQ518766	DQ522337	DQ522382	Spatafora et al. 2007
O. superficialis	Insect	MICH 36253	EF468983				EF468883	Sung et al. 2007a
O. thanathonensis	Hymenotera (adult ant)	MFU 16-29010	MF882926	MF850375	MF850375	MF872614	MF872616	Xiao et al. 2017
O. tricentri	Hemiptera (Cercopidae)	NBRC 106968	AB968393	AB968410	AB968423	AB968593		Ban et al. 2015
O. unilateralis	Hymenoptera (Camponotus)	OSC 128574	DQ522554		DQ518768	DQ522339	DQ522385	Spatafora et al. 2007
O. variabilis	Diptera (larva)	OSC 111003	EF468985		EF468839	EF468779	EF468885	Sung et al. 2007a
O. xuefengensis	Lepidoptera (Hepialidae larva)	GZUH2012HN19	KC631788	KC631803		KC631794	KC631799	Wen et al. 2013
O. yakusimensis	Hemiptera (cicada nymph)	HMAS 199604	KJ878938		KJ878902		KJ879018	Quandt et al. 2014
T. inflatum	Coleoptera (larva)	OSC 71235	EF469124	JN049844	EF469077	EF469061	EF469090	Kepler et al. 2012
T. ophioglossoides	Fungi (*Elaphomyces* sp.)	NBRC 106332	JN941732	JN943322	JN941409		JN992466	Schoch et al. 2012

Taxonomy

Ophiocordyceps globiceps **Y.P. Xiao, T.C. Wen & K.D. Hyde, sp. nov.**
Index Fungorum number: IF555323
Faces of fungi number: FoF 04864
Fig. 2

Etymology. The specific epithet refers to the feature of the secondary hemispherical to globoid fertile head.

Sexual morph: *Stromata* 4–8 mm long × 0.5–1 mm diam., one or several from the host, stipitate, capitate, unbranched, cinnamon to yellow. *Stipe* 3.5–7.5 mm long, 0.2–0.5 mm diam., yellow, cylindrical, with a fertile apex. *Fertile head* 1–1.5 mm long, 1–1.2 mm diam., cinnamon to yellow, single, hemispherical to globoid. *Perithecia* 538–663 × 182–247 μm ($\bar{x}$= 600 × 214 μm, n = 60), immersed, ovoid to elongated pyriform, thick-walled, vertical with the ostioles opening on the upper surface of the head. *Peridium* 17–22 μm ($\bar{x}$ = 20 μm, n = 90) wide, hyaline, of *textura porrecta* to *textura prismatica* to *textura angularis*. *Asci* 373–454 × 5.7–8.2 μm ($\bar{x}$ = 413 × 7 μm, n = 90), 8-spored, hyaline, filiform, with a thick apex. *Apical cap* 4.4–6.4 × 4.9–5.7 μm ($\bar{x}$ = 5.4 × 5.3 μm, n = 60), thick, with a small channel in the centre. *Ascospores* 240–303 × 1.8–2.3 μm ($\bar{x}$ = 272 × 2.1 μm, n = 60), filiform, hyaline, multiseptate. *Secondary ascospores* 4–5.4 × 1.2–1.9 μm ($\bar{x}$ = 4.7 × 1.6 μm, n = 90) cylindrical to fusoid, 1-celled, straight, hyaline, smooth-walled. **Asexual morph:** Undetermined.

Culture characteristics. growing on PDA, reaching 5 cm diam., after 6 weeks at 25 °C, superficial cottony, whitened, loose, reverse yellow. After 10 weeks at 25 °C, reaching 6 cm diam., no conidiogenous structures observed.

Material examined. THAILAND, Ranong, Tambon Khao Niwet, parasitise on fly (Muscidae, Diptera) 7 mm long, 3 mm wide, brown to dark brown, without hyphae on the surface, collected on the grass stem, 19 July 2015, YuanPin Xiao, (MFLU 18–0661, **holotype**, ex-type living culture, MFLUCC 18–0495); Chiang Mai, Thailand, on adult fly (Diptera), 6.5 mm long, 2.7 mm wide, brown to dark brown, without hyphae on the surface, collected on the grass, 19 July 2017, YuanPin Xiao, (MFLU 18–0662, **paratypes**, living culture MFLUCC 18–0496).

Notes. In the phylogenetic tree, *Ophiocordyceps globiceps* is closely related to *O. dipterigena* (Berk. & Broome) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafor. (Thailand) and *O. hemisphaerica* Mafalda-Freire, Reck & Drechsler-Santos (Brazil), which infect flies (Luangsa-ard et al. 2008, Hyde et al. 2016). *Ophiocordyceps globiceps* also groups with *Ophiocordyceps* sp. (FMF147) (106bp ITS differ), which was introduced by ITS sequence data and without any other detail (Freire 2015). *Ophiocordyceps globiceps* has 60 bp that differ from *O. dipterigena* (MY621, Thailand) in the ITS region, 19 bp in TEF1α. It has 87 bp that differ from *Hymenostilbe dipterigena* Petch (NHJ12170, Thailand, asexual morph of *O. dipterigena*) in the ITS region and 20 bp in TEF1α. *Ophiocordyceps globiceps* also has 94 bp (ITS) that differ from *O. dipterigena* (MRCIF71, Thailand), which only has ITS and without any details. *Ophiocordyceps globiceps* has 104 bp that differ from *O. hemisphaerica* (FLOR 59525)

Figure 2. *Ophiocordyceps globiceps* (holotype MFLU 18–0661). **a** Habitat **b** Ascostroma emerging from infected fly **c** Host **d** Fertile head of ascostroma **e** Vertical section of the stroma **f** Section of ascomata **g** Peridium **h, i** Asci **k** Apical cap of asci **l, q** Part of ascospore **m, n** Secondary ascospores **o** Upper side of the culture **p** Reverse side of the culture. Scale bars: 1000 μm (**b–d**), 500 μm (**e, f**), 100 μm (**h, i**), 20 μm (**g**), 10 μm (**k, l**), 5 μm (**m, n, q**), 5 cm (**o, p**).

Table 2. Synopsis of *Ophiocordyceps* species discussed in the paper.

Species	Location	Host	Stromata (mm)	Stipe (mm)	Fertile part (mm)	Perithecia (μm)	Asci (μm)	Ascospores (μm)	Part-spores (μm)	Reference
C. sakishimensis	Japan	Diptera	6–7 long, cylindrical, white			500 × 250–260, superficial, ovoid			4–6 × 1, cylindrical	Kobayasi and Shimizu 1983
O. dipterigena (First record)	Sri Lanka		5–10 × 1, pale	Cylindrical	Globose				10 × 1.5	Berkeley and Broome 1873, Freire 2015
O. dipterigena	Japan	Diptera	5–8 long, 1–2 wide, 0.5–1 wide, orange-cinnamon or cinnamon-brown	0.2–0.5 thick, orange-cinnamon to light yellow		Narrowly ovoid or conoid, 700–900 × 240–400, wall 15–25 thick	480–600 long	Filiform, multiseptate	6–12 × 1–1.5, cylindric or fusoid fragments	Kobayasi 1941
O. dipterigena	Thailand	Diptera	4–10 long, pale cream-yellow to orange-brown		1–1.5 high, 1.5–2.5 diam., terminal, disc-like to subglobose	800–1000 × 200–300, narrowly ovoid to obclavate	450–600 × 4–6, cylindrical	Filiform, breaking up into 64 part-spore	6–12 × 1–1.5, cylindrical to fusiform	Luangsa-ard et al. 2008
O. discoideicapitata	Japan	Diptera	2.5–3.5 × 0.7–1.2, two		3–4, discoid, laterally conical	620–700 × 200–250, pyriform	5–6 diam., filiform		6–9 × 1, cylindrical, truncated	Kobayasi and Shimizu 1982
O. forquignonii		Diptera		3-6 long, subfiliform, with a cylindrical apex	Cylindrical	Ellipsoid			Oval, 8	Saccardo 1891
O. globiceps	Thailand	Diptera	4–8 long × 0.5–1 diam., unbranched, cinnamon to yellow, one or several from host	3.5–7.5 long, 0.2–0.5 diam., cinnamon to yellow, cylindrical, with a fertile apex	1–1.5 long, 1–1.2 diam., yellow, hemispherical to globoid	538–663 × 182–247, ovoid to elongated pyriform	373–454 × 5.7–8	240–303 × 1.8–2.3, filiform, hyaline, multiseptate	4–5.4 × 1.2–1.9, cylindrical to fusoid	This study
O. hemisphaerica	Brazil	Diptera (Muscidae)	12–20 × 0.8–1, unbranched, brown to greyish-brown	11–19 long, 0.8–1 wide, cylindrical, with a fertile apex	1–1.2 long, 2–4 diam., hemispherical	780–860 × 220–290, Obpyriform, slightly curved	500–640 × 5–6	Filiform, more than 52 septa	7–10 × 1–1.5, cylindrical to unusually fusoid	Hyde et al. 2016
O. lacrimoidis	Brazil	Diptera	4–5 × 1, two, simple	3–4 long, 1 wide, cylindrical, epidermal layer brown, medullar region white to cream	1.2 long, 1.8–2.2 diam., discoid, pale to dark yellowish	650–700 × 200–250, immersed, obpyriform, slightly curved	350–450 × 5, narrow cylindrical	Filiform, as long as asci, hyaline, more than 56 septa	8–14 × 2, cylindrical, hyaline	Hyde et al. 2016
O. muscicola = *C. muscicola*	Brazil	Diptera	9–13 × 0.5–1, two to six, rarely branched		2–4 × 1–1.2, discoid	850–920 × 230–300, pyriform	550–700 × 5, filiform	650–700 × 2, 64 part-spores	11–14 × 2, terminal cylindrical, intermediates fusoids 8–10 × 1–2	Möller 1901, Freire 2015

in the ITS region and has 21 bp in nr*SSU*, 97 bp in nr*LSU*, 74 bp in TEF1α that differ from *O. dipterigena* (OSC 151913).

We compared the new species with other *Ophiocordyceps* species which infect flies (Diptera) or are morphologically similar to *O. globiceps* (Table 2). *Ophiocordyceps globiceps* differs from three records of *O. dipterigena* found in Sri Lanka, Japan and Thailand by producing single smaller stroma, smaller and shorter perithecia, shorter asci and smaller ascospores (Table 2). *Cordyceps sakishimensis* Kobayasi & Shimizu, *Ophiocordyceps discoideicapitata* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, *Ophiocordyceps forquignonii* (Quél.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, *Ophiocordyceps hemisphaerica* Mafalda-Freire, Reck & Drechsler-Santos, *Ophiocordyceps lacrimoidis* Mafalda-Freire, Reck & Drechsler-Santos and *Cordyceps muscicola* Möller (= *Ophiocordyceps muscicola*) have been reported as fly infected taxa (Saccardo 1891, Möller 1901, Kobayasi and Shimizu 1982, Freire 2015, Hyde et al. 2016), but their morphology is different from *O. globiceps* (see Table 2). *Cordyceps sakishimensis* is distinct from *O. globiceps* in having white, longer, cylindrical stromata and larger superficial perithecia. *Ophiocordyceps discoideicapitata* differs from *O. globiceps* by producing smaller stromata, pyriform, larger perithecia and longer part-spores (Table 2) (Kobayasi and Shimizu 1982). *Ophiocordyceps forquignonii* is distinct from *O. globiceps* in having a cylindrical fertile apex and oval secondary ascospores (Table 2) (Saccardo 1891). Molecular data indicate that the new species has 26 bp in nrSSU and 89 bp in nrLSU that are different from *O. forquignonii*. *Ophiocordyceps hemisphaerica* is different from *O. globiceps* in having longer stomata, larger obpyriform perithecia, longer asci and longer fusoid part-spores (Hyde et al. 2016). *Ophiocordyceps lacrimoidis* (Diptera infected species) was not considered in our phylogenetic sampling as the DNA (ITS) sequence did not align well with other species, but its DNA sequence differed by 154 bp in the ITS region from the sequence of *O. globiceps*. However, *Ophiocordyceps lacrimoidis* is morphologically different from our new species in producing longer stipe, obpyriform, slightly curved perithecia, longer asci and longer part spores. *Cordyceps muscicola* was revised as *Ophiocordyceps muscicola* by Freire (2015), while it is different from *O. globiceps* in having longer stromata, larger pyriform perithecia, longer asci and longer part-spores (Möller 1901, Freire 2015). We would like to introduce *Ophiocordyceps globiceps* as a new species based on the phylogenetic and morphological analyses.

Ophiocordyceps sporangifera **Y.P. Xiao, T.C. Wen & K.D. Hyde, sp. nov.**
Index Fungorum number: IF555324
Faces of fungi number: FoF 04865
Figs 3, 4

Etymology. The specific epithet refers to the feature of the sporangium-bearing.

Sexual morph: Unknown. **Asexual morph:** *Primary synnema* 9–18 cm high 1–2 mm diam., arising from the head region of the larva, branching into 2–5, cylindrical, brown to deep brown, with small white fertile head on the top, not smooth.

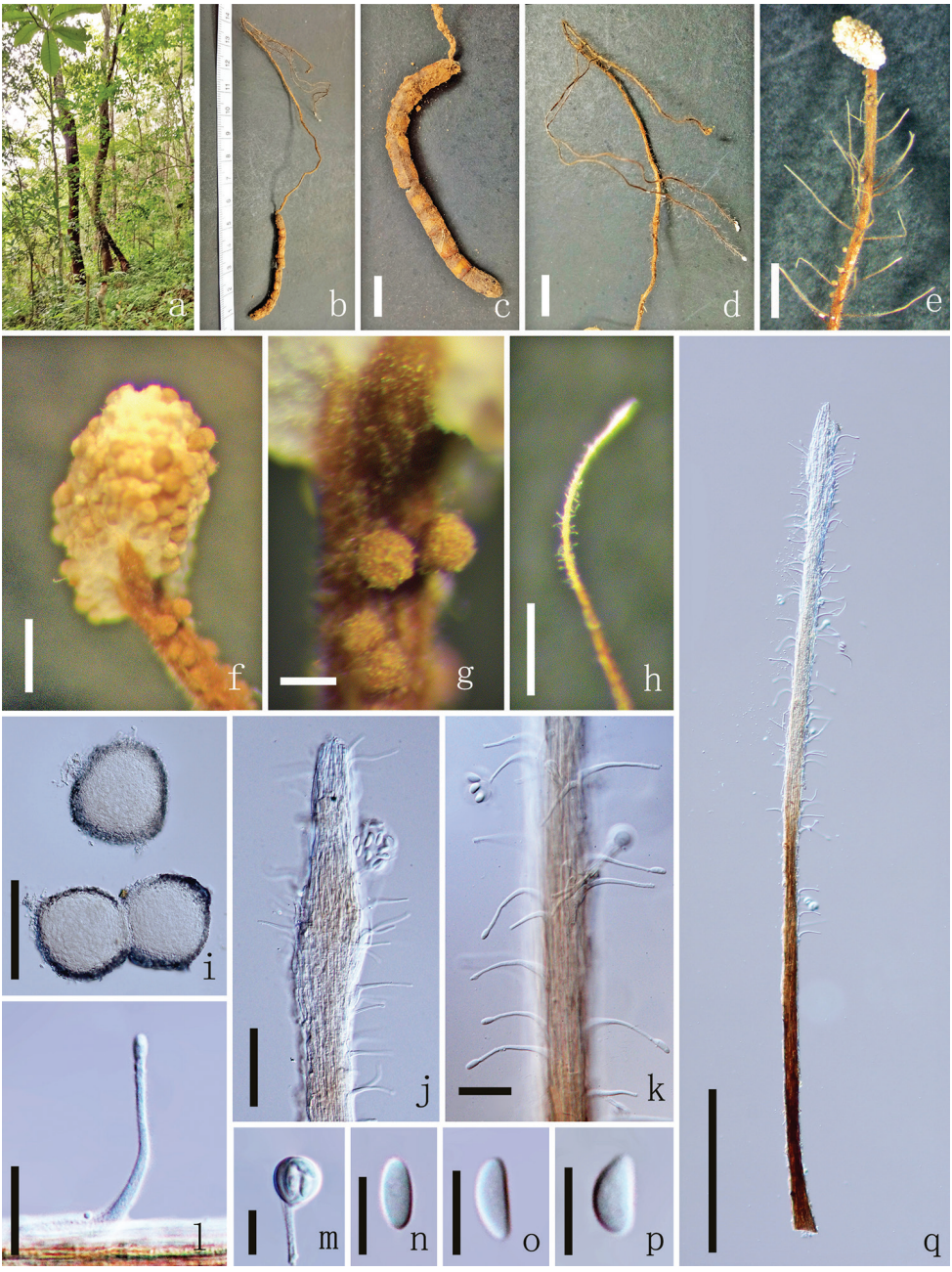

Figure 3. *Ophiocordyceps sporangifera* (holotype MFLU 18–0658). **a** Habitat **b** Synnemata on host surface **c** Host **d, e** Synnemata **f** Fertile head of primary synnema **g** Sporangium **h** Secondary synnema **i** Sporangium **j, k, q** Part of secondary synnema **l** Phialides **m** Conidia bound by deliquescing mucilaginous material **n–p** Conidia. Scale bars: 1 cm (**c, d**), 1000 μm (**e**), 200 μm (**f, h, q**), 100 μm (**g, i**), 50 μm (**j**), 20 μm (**k, l**), 10 μm (**m–p**).

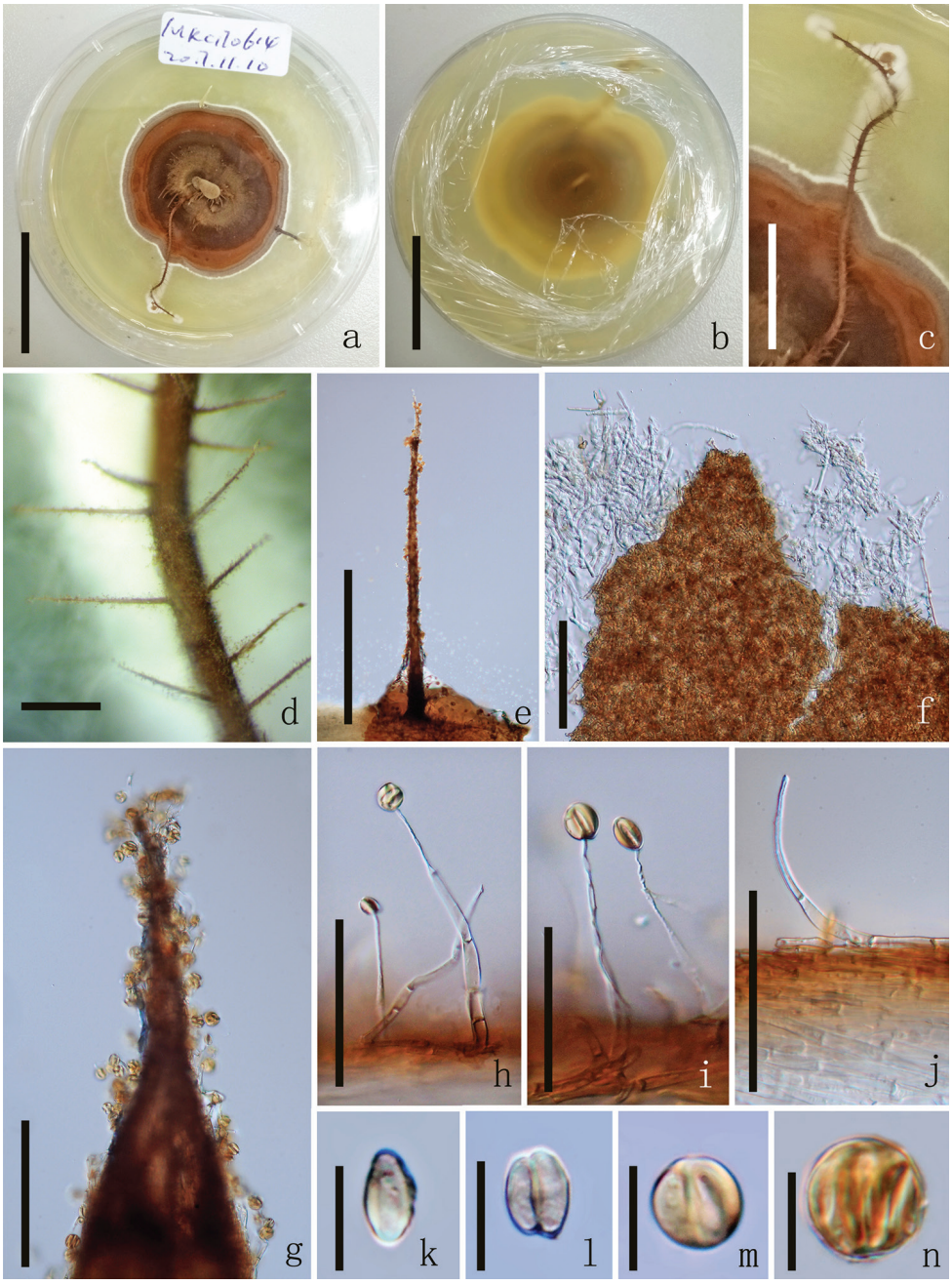

Figure 4. *Ophiocordyceps sporangifera* (culture) MFLUCC 18–0492. **a** Upper side of the culture **b** Reverse side of the culture **c, d** Synnemata growing on PDA medium **e, g** Synnemata **f** Mycelium **h–j** Phialides **k** Conidia **l–n** Conidia form mucilaginous spheres. Scale bars: 1 cm (**a, b**), 5000 µm (**c**), 1000 µm (**d**), 500 µm (**e**), 100 µm (**f, g**), 50 µm (**h–j**), 10 µm (**k–n**).

Fertile head 500–2000 µm long, 400–1000 µm diam., globose to subglobose, capitulum, white to brown, arising from the apical end of primary synnema, mess of sporangium on the surface. *Sporangium* 78–121 µm diam. ($\bar{x}$ = 100 µm, n = 60), spherical, arising from the apical end of primary synnema, white colour when immature, becoming brown to dark brown after maturity, consisting of thick-walled cells. *Secondary synnemata* 1092–1937 × 21–34 µm, ($\bar{x}$= 1515 × 27 µm, n = 60), laterally from the primary synnema, brown to white, cylindrical, not smooth. *Hyphae* 1.8–2.8 µm wide ($\bar{x}$ = 2.3 µm, n = 60), irregularly multi-septate, brown, cylindrical, smooth or rough, sometimes particularly expand. *Phialides* 25–40 × 1.3–2.5 µm ($\bar{x}$ = 33 × 1.9 µm, n = 60), hirsutella-like, hyaline, solitary, unbranched, narrow slender, smooth. *Conidia* 6.7–9.8 × 2.5–3.8 µm ($\bar{x}$ = 8.3 × 3.2 µm, n = 60), 1 cell, hyaline, subglobose to reniform, bound in mucilaginous spheres. *Mucilaginous spheres* 10.5–12.9 × 6.4–8.7 µm ($\bar{x}$ = 11.7 × 7.5 µm, n = 60), composed of 1–12 conidia, hyaline, at phialide apex.

Culture Characteristics. growing on PDA, reaching 2 cm diam., after 4 weeks at 25 °C, with circular, dense mycelium on the surface. After 6 weeks, the colour of the colony gradually deepened from white to dark brown from the periphery to the centre, with complex fold as 4 circle rings, reverse white to yellow in colour, with ring. Synnemata was produced after 8 weeks. Most of the characters are the same as the fresh collection except phialides and mucilaginous spheres. *Phialides* 56–86 µm long ($\bar{x}$ = 71 µm, n = 60), 3–5 µm wide at base ($\bar{x}$ = 4 µm, n = 60), 1.4–2.2 µm at top ($\bar{x}$ = 1.8 µm, n = 60), hirsutella-like, hyaline, solitary, unbranched, narrow slender, smooth, 1–4 septa, not observed on host. *Mucilaginous spheres* 10.5–15.9 × 8.2–14.7 µm ($\bar{x}$ = 12.7 × 11.5 µm, n = 60), 1–4 conidia, hyaline to brown. Observation stopped after 10 weeks.

Material examined. THAILAND, Chiang Mai, The Mushroom Research Centre, on dead larva of Elateridae, Coleoptera, 6.5 cm long 0.38 cm diam., brown to dark brown, with thallus inside (larva), 18 July 2015, YuanPin Xiao, (MFLU 18–0658, **holotype**); THAILAND, Chiang Mai, The Mushroom Research Centre, on dead larva of Elateridae, Coleoptera, 5.8 cm long 0.4 cm diam., brown to dark brown, with thallus inside (larva), 22 August 2015, YuanPin Xiao, (MFLU 18–0659, **paratypes,** ex-type living culture, MFLUCC 18–0492); THAILAND, Chiang Mai, Samoeng on larva insect of Elateridae, Coleoptera, 5.5 cm long 0.32 cm diam., brown to dark brown, with thallus inside (larva), 18 June 2017, YuanPin Xiao, (MFLU 18–0660, **paratypes,** living culture, MFLUCC 18–0493, MFLUCC 18–0494).

Notes. *Ophiocordyceps sporangifera* is closely related to *O. myrmicarum* D.R. Simmons & Groden in our phylogenetic tree (Fig. 1). The morphology of *O. sporangifera* is different from *O. myrmicarum* in having longer primary and secondary synnemata, a white to brown sporangium, shorter phialides and it infects insect larvae (Lepidoptera, Cossidae), while *O. myrmicarum* was found on an ant (*Myrmica rubra*) (Simmons et al. 2015). The phylogenetic analysis does not have good support, but *O. sporangifera* is distinct from *O. myrmicarum*. In the phylogenetic tree, the relationships of *O. sporangifera* and *O. myrmicarum* are obscure because they share one clade with short branch length (100% ML/ 1 BYPP), while the two strains of *O. sporangifera* clustered

Table 3. Synopsis of *Ophiocordyceps* species discussed in the paper.

Species	*Ophiocordyceps myrmicarum*	*Ophiocordyceps sporangifera*
Host	*Myrmica rubra* (Hymenoptera)	Elateridae, Coleoptera
Primary synnemata	Whitish-yellow aging to rufous brown	9–18 cm high 1–2 mm diam., brown to deep brown
Secondary synnemata (μm)	Hyaline aging to rufous brown, up to 350 long, narrow (25) at base, common on agar but not observed on host	Brown to white, not smooth 1092–1937 × 21–34, arising from the all parts of the primary synnemata, observed on both of the host and agar
Primary phialides (μm)	Subulate, hyaline or pigmented at base, 39.9–86.2 long, 3.6–5.4 wide at base	Slender, solitary, hyaline, unbranched, narrow, smooth, 25–40 × 1.3–2.5
Secondary phialides (μm)	Subulate, 27.2–47.0 long, 2.4–3.3 wide at base	Narrow slender, 56–86 long, 3–5 wide at base, 1.4–2.2 at top, 1–4 septa, common on culture but not observed on host
Sporangium (μm)	No observed	78–121 diam., spherical, white immature, brown after mature
Conidia (μm)	7.3–9.6 × 3.2–5.1 reniform to ovoid, bi-guttulate, aseptate	6.7–9.8 × 2.5–3.8, subglobose to reniform
Mucilaginous spheres (μm)	Composed of 1–4 conidia, hyaline to brown, at phialide apex	10.5–12.9 × 6.4–8.7, composed of 1–12 conidia, hyaline on host, 1–4 conidia on culture, hyaline to brown on culture
Reference	Simmons et al. 2015	This study

together with a low bootstrap support (88% ML/ 0.90 BYPP). The type strain of *O. sporangifera* has 0 bp in nr*SSU*, 3 bp in TEF1α and 5 bp in RPB1 that are different from *O. myrmicarum*. However, the morphological features of those two species are different, thus, they should be treated as two separate species (Table 3).

Discussion

We introduce two new entomopathogenic species of *Ophiocordyceps*, one from Coleoptera (Elateridae) and the other from flies (Diptera). Morphological and phylogenetic analyses have provided insights to resolve generic delimitation (Sung et al. 2007a, Jeewon and Hyde 2016). Most of the species of this genus are parasitic on insects (Sung et al. 2007a, Maharachchikumbura et al. 2015, Wijayawardene et al. 2017). The sexual morph species in this genus is characterised by fibrous, hard, pliant-to-wiry, dark-coloured stroma with superficial to immersed perithecia (Sung et al. 2007a, Ban et al. 2015, Maharachchikumbura et al. 2015), while the asexual morph species have mainly hymenostilbe-like and hirsutella-like features, branched or unbranched phialides with oval to fusiform conidia (Kepler et al. 2013, Maharachchikumbura et al. 2015, 2016).

Ophiocordyceps globiceps groups with *H. dipterigena*, *O. dipterigena*, *Ophiocordyceps* sp. and *O. hemisphaerica* in the phylogenetic tree with high bootstrap support, while four of these species are reported as fly (Diptera) parasitic fungi (Kobayasi 1941, Saccardo 1891, Luangsa-ard et al. 2011, Hyde et al. 2016). *Ophiocordyceps globiceps* dif-

fers from closely related species by producing capitate, stipitate ascostromata, vertical, narrowly ovoid to obclavate, occasionally irregular perithecia and cylindrical secondary ascospores. Both morphology and phylogenetic analyses clearly show *O. globiceps* as a new species within *Ophiocordyceps*.

Ophiocordyceps sporangifera is an asexual morph species and groups with *O. myrmicarum* in the phylogenetic tree (Fig. 1). *Ophiocordyceps sporangifera* can be distinguished from *O. myrmicarum* by infecting and parasitising larvae of insects (Lepidoptera, Cossidae), producing white to brown sporangium, longer primary and secondary synnemata and shorter primary and secondary phialides. The new species can be defined based on the distinctive morphological characters even through the phylogenies are not well-supported (Jeewon and Hyde 2016). In case of intricate differences between a gene tree and a species tree and, in addition, several morphs can be under the influence of many genes which are not really being reflected in the phylogeny (Jeewon and Hyde 2016). In our study, morphological characters strongly support *O. sporangifera* as a new species within *Ophiocordyceps*, even through phylogenetic analysis is not well-resolved. In this case, other loci which have more phylogenetic variation than the current loci may be able to differentiate these two species.

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Four new species of *Tremella* (Tremellales, Basidiomycota) based on morphology and DNA sequence data

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Abstract

In the present study, a total of 33 *Tremella* specimens in China were collected and examined using molecular phylogenetic analysis based on a combined sequence dataset of the nuc rDNA internal transcribed spacer (ITS) region and nuc 28S rDNA D1/D2 domain in conjunction with the morphological characters. Four new species, namely *Tremella basidiomaticola*, *T. cheejenii*, *T. erythrina*, and *T. salmonea*, are newly described based on their distinct phylogenetic relationships and the comparison of morphological characters with known *Tremella* species. Our results indicate a high species diversity of *Tremella* waiting to be discovered.

Keywords

Basidiomycota, morphology, phylogeny, taxonomy, *Tremella*

Introduction

Tremella Pers. has been traditionally considered to be the largest and most polyphyletic genus in Tremellaceae (Fell et al. 2000; Scorzetti et al. 2002; Sampaio et al. 2004; Boekhout et al. 2011; Millanes et al. 2011; Weiss et al. 2014; Liu et al. 2015a). The members of *Tremella* sensu lato are dimorphic fungi that contain both a haploid unicellular yeast stage and a dikaryotic filamentous stage. This genus is characterized by its mycoparasitic lifestyle and comprises species growing on the hymenium of Corticiales,

Polyporales, Rhytismatales, and Russulales, on the mycelium of Russulales such as *Peniophora* and *Stereum*, in the basidiomata of Dacrymycetales, Polyporales, Russulales, and Trechisporales, on the perithecia of Diaporthales, Pleosporales, and Xylariales, as well as on lichens (Bandoni 1961; Reid 1970; Brough 1974; Zugmaier et al. 1994; Bandoni 1995; Roberts 1995, 1999, 2001, 2007; Roberts and deMeijer 1997; Diederich 1996; Torkelsen 1997; Chen 1998; Hauerslev 1999; Van Ryckegem et al. 2002; Pippola and Kotiranta 2008; Zamora 2009).

Tremella sensu lato includes approximately 90 species, more than half of which are known to exclusively parasitize specific lichenized fungal hosts (Diederich and Marson 1988; Diederich and Christiansen 1994; Diederich 1996, 2003, 2007, Sérusiaux et al. 2003; Kirk et al. 2008; Zamora 2009, Zamora et al. 2011, 2016; Millanes et al. 2012, 2014, 2015, 2016; Diederich et al. 2014; Kout et al. 2015; Lindgren et al. 2015; Westberg et al. 2015; Spirin et al. 2017). This genus splits into eight monophyletic groups in combination with several isolated species in Tremellales. Four clades have been emended, namely *Tremella* sensu stricto, *Carcinomyces*, *Naematelia*, and *Phaeotremella,* and one proposed as new genus, namely *Pseudotremella*. The other three clades consist of lichenicolous species that were defined as *Tremella* clade I, II, and III (Millanes et al. 2011; Liu et al. 2015a, b). Their taxonomy remains be determined until more robust phylogeny is resolved and further morphological characters are found. The basidiomata colour and shape of species belonging to *Tremella* s. l. are generally variable between different clades. Non-lichenicolous species mainly exhibit jelly-like basidiomata with cerebriform, folise, lobe, or pulvinate macromorphology and white, yellow, orange, or brown colour. In addition, some species are intrahymenial parasites that occur within the hymenia of dacrymycetaceous or corticoid species. Their basidiomata are not macroscopically visible. Lichenicolous species usually produce inconspicuous gall deformations on the thallus of lichens, at least in early stages of growth, where as some species can induce the formation of large galls up to 15 mm in diameter (Diederich 1996, 2007). Some species can produce gelatinous basidiomata instead of gall formation (Diederich 1996; Lindgren et al. 2015; Millanes et al. 2015; Zamora et al. 2017). Moreover, some species grow intrahymenially without any external symptoms (Diederich 1996, 2007). Compared to the increasing knowledge of the diversity of lichenicolous species, few studies of non-lichenicolous *Tremella* species are published in recent years.

Tremella s. s. is now confined to Fuciformis and Mesenterica subclades containing more than 30 species. Basidiomata of some *Tremella* s. s. species have long been used as food or traditional medicine in China or other Asian countries. *Tremella fuciformis* and *T. aurantialba* have been cultivated in China for more than 30 years. The diversity and distribution of *Tremella* are poorly known in China, as comparatively few mycologists focus on these fungi (Peng 1982; Bandoni and Zang 1990). In the present study, four new species are described and characterised based on morphological characters and phylogenetic analyses of nuc rDNA ITS region and nuc 28S rDNA D1/D2 domain.

Materials and methods

Sampling and morphological examination

Specimens were collected from Guangdong, Guangxi, Heilongjiang, Jilin, Qinghai, Tibet, and Yunnan provinces in China. The specimens were air dried immediately after their collection. Macromorphological descriptions were based on field observations. Micromorphological examination followed the studies by Chen (1998) and Millanes et al. (2014). Microscopic structures, features, and measurements were observed using handmade sections stained with 1% Phloxin after pretreatment with 5% KOH and photographed with Zeiss AXIO Imager A2 coupled to an AxioCam MRc5 digital camera. Basidiospores and conidia measurements are present as follows: length range × width range, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios and *n* = number of spores measured. All specimens were preserved in the XZL culture collection (personal culture collection of Xin-zhan Liu housed in the Institute of Microbiology, Chinese Academy of Sciences). Type specimens were deposited in Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). The cultures were deposited in China General Microbiological Culture Collection Center (CGMCC) and the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.

DNA extraction, PCR amplification and sequencing

DNA was extracted directly from the specimens examined. A very small amount of dry tissue was soaked in sterile water for 30 min and dried with sterile filter papers. The tissue was taken into 2 ml eppendorf tube with quartz sand (1–2 mm), lyophilized using liquid nitrogen and immediately crushed with tissue grinder for 2 min using SCIENTZ-48 at 70 Hz (SCIENTZ, China). The sample was homogenized in 1 ml 5% CTAB preheated at 65 °C. The mixture was warmed up at 65 °C for 1 h and centrifuged by 15000 rpm for 15 min. The supernatant was purified with phenol:chloroform:isoamyl alcohol (25:24:1) for twice of which the second purification step without phenol. The supernatant was incubated for 30 min at 37 °C with 25 μl RNAase (20 mg/ml) and then purified again. The precipitation with 3 M sodium acetate and ethyl alcohol absolute was conducted. Finally, the DNA was washed twice with 70% (w/v) ethanol and then dissolved in 50 μl of pure water. The nuc rDNA ITS region and D1/D2 domain of nuc 28S rDNA were amplified using the protocols described previously (Liu et al. 2015a). PCR products were observed on 1% Agarose gel electrophoresis stained with ethidium bromide. Purification and sequencing of PCR products were carried out at TSINGKE Biological Technology, Beijing, China.

Phylogenetic analyses

Phylogenetic analyses were performed as described previously with modification (Millanes et al. 2011; Liu et al. 2015a, b). *Vishniacozyma carnescens* CBS 973^{T} was chosen as outgroup because the genera *Vishniacozyma* is the sister group of Tremellaceae (Liu et al. 2015a, b). Four partitions, i.e., ITS1, 5.8S, ITS2 and D1/D2 domain, were chosen as the appropriate scheme (Millanes et al. 2011; Zamora et al. 2017). Multiple sequences were aligned using MAFFT algorithm and the G-INS-I option (Standley 2013). Major insertions and ambiguous regions were identified and eliminated with Gblocks version 0.91b (Castresana 2000) using a relaxed selection (minimum number of sequences for a conserved position = 36, minimum number of sequences for a flank position = 60, maximum number of contiguous non-conserved positions = 10, minimum length of a block = 5 and allowed gap positions = 'with half'), following Talavera and Castresana (2007). PartitionFinder V2.1.1 (Lanfear et al. 2017) was used to determine the best-fit evolutionary model for each partition, with the following settings: the 'all' search algorithm, the corrected Akaike Information Criterion (AICc) for model selection and either the 'raxml' or 'mrbayes' set of models.

Dataset congruence was assessed manually by analyzing the datasets separately by maximum likelihood bootstrapping. Conflict among clades was considered significant if a significantly supported clade (bootstrap support ≥ 70%; Hillis and Bull 1993) for one marker was contradicted with significantly supported by another. Incongruence was found between topologies derived from ITS1, 5.8S, ITS2, and D1/D2 domain.

Maximum likelihood (ML) analyses of single gene were performed in RAxML-HPC V.8 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010). The GTR+G, GTR+G, GTR+I+G and GTR+I+G models were applied to each partition. The best-scoring tree was obtained using rapid bootstrap analysis by running 1000 replicates. Four single-gene trees estimated above were then used as input to infer the species tree with the coalescent-based approach implemented in the ASTRAL program v5.6.3 (Mirarab and Warnow 2015). The bootstrapping option of ASTRAL was used for 1000 replicates.

Bayesian analyses were conducted by Markov Chain Monte Carlo (MCMC) sampling for combined nucleotide sequences using MRBAYES 3.2.2 (Ronquist et al. 2012) on the CIPRES Science Gateway (Miller et al. 2010). Likelihood models were selected for each of the four gene partitions among the 24 models implemented in MrBayes. A HKY+I+G model was selected for the ITS1, a K80+G model was selected for the 5.8S, a SYM+I+G was selected for the ITS2 and a GTR+I+G model was selected for D1/D2 domain. Two independent runs were executed, each with four chains, three of which were incrementally heated. The analysis was conducted for 5 million generations with trees sampled every 5000 generations. The first 25% trees, which represent the burn-in phase of the analysis, were discarded after checking for stability on the log-likelihood curves and the split-frequencies of the runs in Tracer v.1.7 (Rambaut et al. 2018). The remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Branches that received bootstrap values (BP) for Maximum likelihood and Bayesian posterior probabilities (BPP) greater than or equal to 50% (BP) and 0.95 (BPP) were considered as significantly supported. The GenBank accession numbers for the sequences of the ITS region and D1/D2 domain used in this study are listed in Table 1.

Table 1. Sequences used in molecular phylogentic analysis. Entries in bold are newly generated for this study.

Species	Strain number	Voucher number	Country	ITS	D1D2
Tremella basidiomaticola	**CGMCC 2.5724^{T}**	–	**China, Fujian**	**MH712820**	**MH712784**
	CGMCC 2.5725	–	**China, Fujian**	**MH712821**	**MH712785**
	CBS 8225	–	**China, Fujian**	**MH712822**	**MH712786**
Tremella brasiliensis	CBS 6966^{R}	–	Costa Rica	AF444429	AF189864
	CBS 8231	–	Costa Rica	JN053465	JN043570
Tremella cerebriformis	–	LE 296436	Russia	KP986538	/
	–	LE 303455	Russia	KP986522	/
	–	VLA M-11693	Russia	KP986538	/
Tremella cerebriformis	–	**ZRL20170101**	**China, Heilongjiang**	**MH712823**	**MH712787**
	–	**ZRL20170269**	**China, Heilongjiang**	**MH712824**	**MH712788**
Tremella cheejenii	–	**GX20172598**	**China, Guangxi**	**MH712825**	**MH712789**
	–	**GX20172640**	**China, Guangxi**	**MH712826**	**MH712790**
Tremella dysenterica	–	LE 303447	Russia	KP986509	KP986542
	–	VLA M-18599	Russia	KP986531	/
Tremella erythrina	–	**GX20170141 (HMAS 255317)**	**China, Guangxi**	**MH712827**	**MH712791**
	–	**GX20170916001 (HMAS 279591)**	**China, Guangxi**	**MH712828**	**MH712792**
Tremella fibulifera	–	LE 303445	Russia	KP986518	KP986547
Tremella fibulifera	–	**GX20172028**	**China, Guangxi**	**MH712829**	**MH712793**
	–	**HMAS 52852**	**China, Tibet**	**MH712830**	**MH712794**
Tremella flava	CBS 8471^{R}	–	Taiwan	KY105681	KY105681
	–	CCJ 907	Taiwan	AF042221	AF042403
	–	CCJ 928	Taiwan	AF042223	AF042405
Tremella flava	–	**ZRL20180289**	**China, Yunnan**	**MH712834**	**MH712798**
	–	**ZRL20180156**	**China, Yunnan**	**MH712835**	**MH712799**
	–	**KM20170128**	**China, Yunnan**	**MH712836**	**MH712800**
	–	**YN135**	**China, Yunnan**	**MH712837**	**MH712801**
	–	**ZRL20180167**	**China, Yunnan**	**MH712838**	**MH712802**
	–	**ZRL20180164**	**China, Yunnan**	**MH712839**	**MH712803**
	–	**ZRL20180166**	**China, Yunnan**	**MH712840**	**MH712804**
	–	**ZRL20180348**	**China, Yunnan**	**MH712841**	**MH712805**
	–	**ZRL20180349**	**China, Yunnan**	**MH712842**	**MH712806**
	–	**23**	**China, Yunnan**	**MH712843**	**MH712807**
	–	**24**	**China, Yunnan**	**MH712844**	**MH712808**
	–	**YN177**	**China, Yunnan**	**MH712845**	**MH712809**
	–	**YN180**	**China, Yunnan**	**MH712846**	**MH712810**
Tremella fuciformis	CBS 6970^{R}		Taiwan	KY105683	AF075476
	–	CCJ 1072	Taiwan	AF042227	AF042409
	–	CCJ 1531	Taiwan	AF042254	AF042436
Tremella fuciformis	–	**GX20170212**	**China, Guangxi**	**MH712831**	**MH712795**
	–	**GX20172644**	**China, Guangxi**	**MH712832**	**MH712796**
	–	**HMAS 0274334**	**China, Tibet**	**MH712833**	**MH712797**
Tremella fuciformis	CBS 6971	–	USA	KY105682	KY109896
Tremella globispora	CBS 6972^{R}	–	Canada	AF444432	AF189869
	–	UBC 586	Canada	AF042425	AF042243
Tremella laurisilvae	–	Koschatzky s.n.	Portugal	JN053467	JN043572
Tremella lloydiae-candidae	–	VLA M-11702	Russia	KP986536	KP986559
	–	VLA M-11703	Russia	KP986537	KP986560
Tremella mesenterica	CBS 6973^{R}	–	Canada	AF444433	AF075518
	–	Ryman 9146	Sweden	JN053463	JN043568
	–	CCJ 1040	Taiwan	AF042408	AF042226
	–	FO 24610	German	AF042447	AF042265
Tremella mesenterica	–	**HMAS 270832**	**China, Guangdong**	**MH712847**	**MH712811**
	–	**HMAS 88438**	**China, Jilin**	**MH712848**	**MH712812**
	–	**HMAS 96841**	**China, Qinghai**	**MH712849**	**MH712813**
	–	**GX20170708**	**China, Guangxi**	**MH712850**	**MH712814**

Species	Strain number	Voucher number	Country	ITS	D1D2
Tremella resupinata	–	CCJ 1458	Taiwan	AF042421	AF042239
Tremella salmonea	–	**GX20172637**	**China, Guangxi**	**MH712851**	**MH712815**
Tremella samoensis	–	LE 262897	Russia	KP986511	/
	–	VLA M-18603	Russia	KP986532	KP986555
Tremella samoensis	–	**GX20172371**	**China, Guangxi**	**MH712852**	**MH712816**
	–	**GX20170536**	**China, Guangxi**	**MH712853**	**MH712817**
Tremella taiwanensis	–	CCJ 1151	Taiwan	AF042412	AF042230
	–	CCJ 1153	Taiwan	AF042413	AF042231
Tremella taiwanensis	–	**GX20170625**	**China, Guangxi**	**MH712854**	**MH712818**
	–	**GX20170629**	**China, Guangxi**	**MH712855**	**MH712819**
Tremella tropica	CBS 8483^{R}	–	Taiwan	KY105697	KY109908
	CBS 8486	–	Taiwan	KY105697	KY109909
	–	CCJ 1355	Taiwan	AF042433	AF042251
Tremella yokohamensis	JCM 16989^{T}	–	Japan	HM222926	HM222927
	–	VLA M-11700	Russia	KP986529	/
Outgroup	–	–	–	–	–
Cryptococcus depauperatus	CBS 7841^{T}	–	–	FJ534881	FJ534911

Results

Phylogenetic analyses

The combined dataset consisted of ITS1 region (44 bp), 5.8S region (156 bp), ITS2 region (168 bp), and D1/D2 domain (532 bp) (a total of 900 bp) for 57 specimens and 13 strains in genus *Tremella* with *Vishniacozyma carnescens* CBS 973^{T} as the outgroup. Two methods for phylogenetic tree construction resulted in a similar topology. Therefore, only the best scoring RAxML tree is shown with BP and BPP values simultaneously in Figure 1. All the *Tremella* specimens and strains in this study separated into 19 clades, representing 15 known and four new species. The four new species clustered into distinct clades supported with high bootstrap values.

Taxonomy

Tremella basidiomaticola **X.Z. Liu & F.Y. Bai, sp. nov.**
MycoBank: MB827184
Figure 2

Type. CHINA, Fujian Province, Ningde city, Gutian county, on the basidioma of *Tremella fuciformis*, July 2017, X.Z. Liu (holotype strain: CGMCC 2.5724^{T}, ex-holotype strain: CBS 15261^{T}).

Etymology. *Basidiomaticola* refers to the species isolated from the basidioma of *T. fuciformis*.

Description. Asexual morph: colonies yellowish, smooth, shiny, and slimy, with an entire margin. Pseudohyphae and hyphae are not formed on corn meal agar. Conidia hyaline, smooth, globose to subglobose, 3.0–6.0 × 2.5–5.0 μm, L = 4.8 ± 0.9 μm,

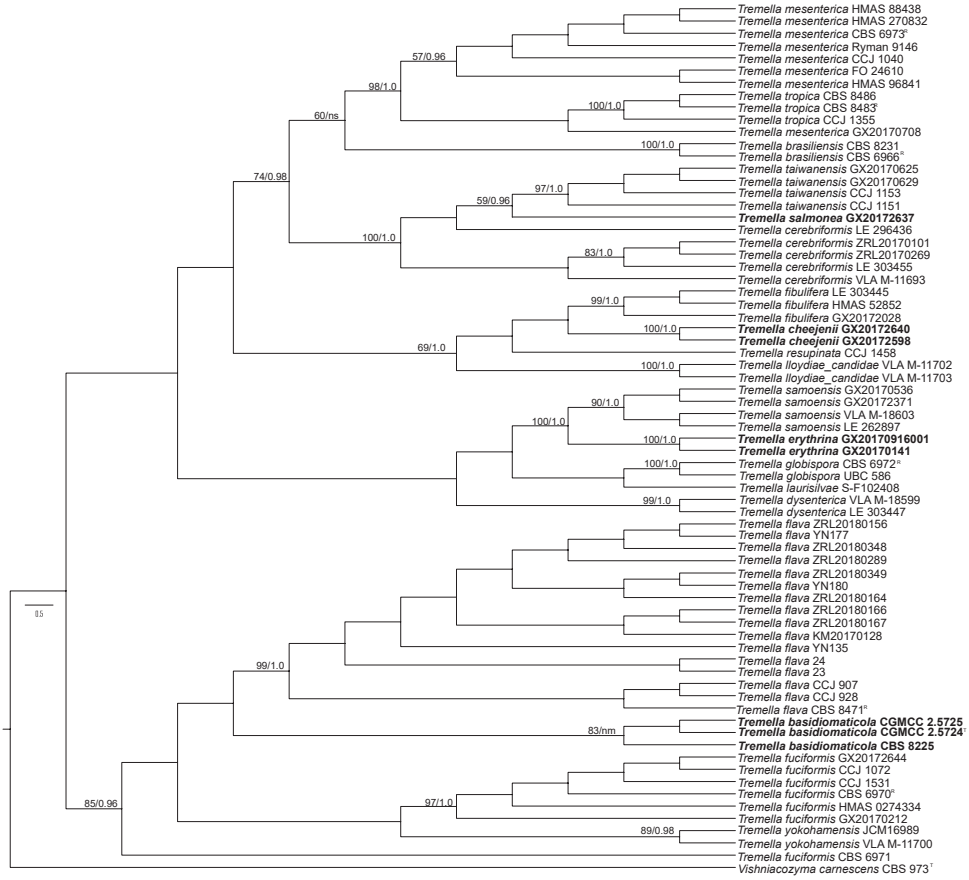

Figure 1. The maximum likelihood tree of the novel species and related taxa in *Tremella* sensu stricto based on the combined sequences of the nuc rDNA ITS region and nuc 28S rDNA D1/D2 domain. Bootstrap supports (BP) (> 50%) of maximum likelihood method and Bayesian posterior probability (BPP) values (> 0.9) are shown at each node. Note: ns, not supported (BP < 50% or PP < 0.9); nm, not monophyletic.

W = 3.9 ± 0.8 μm, Q = 1.0–1.7 (*n* = 30). Ballistoconidia, globose to subglobose on CMA agar, 5.0–7.0 × 3.5–6.0 μm, L = 6.0 ± 0.6 μm, W = 5.1 ± 0. 6 μm (*n* = 30). The comparison of physiological properties between this new species and its related taxa were listed in Suppl. material 1. **Sexual morph**: undetermined.

Additional isolate examined. CHINA, Fujian Province, on the basidioma of *Tremella fuciformis*, July 2017, X.Z. Liu, CGMCC 2.5725 = CBS 15262; Japan, isolated from Mori Ind. Co., Ltd, 1968, T. Suda, NBRC 8990 = CBS 8225.

Notes. Three strains representing *T. basidiomaticola* clustered in a well-supported clade that closely related to *T. yokohamensis*, *T. flava*, and *T. fuciformis*. *Tremella basidiomaticola* CGMCC 2.5724^{T} differed from *T. yokohamensis*, *T. flava*, and *T. fuciformis* by 97.4%, 94.4%–95.1%, and 97.8%–98.1% sequence identities in D1/D2 domain and 96.3%–96.6%, 94.4%–95.7%, and 96.6%–97.5% sequence identities in ITS

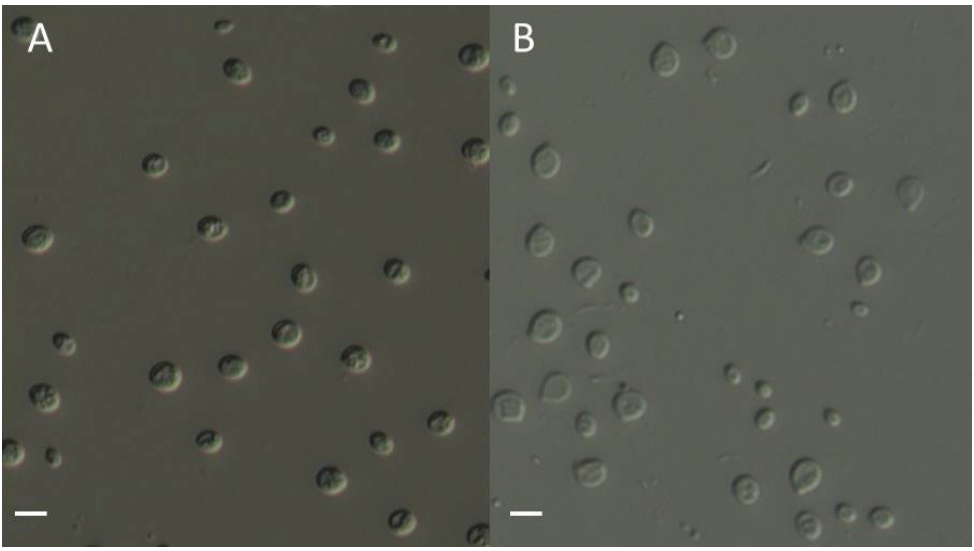

Figure 2. *Tremella basidiomaticola* CGMCC 2.5724^{T} **A** Vegetative cells grown in YM agar for 7 days at 17 °C **B B**allistoconidia produced on CMA agar for 7 days at 17 °C. Scale bars: 5 μm.

region. Physiologically, the ability to assimilate lactose, melibiose, raffinose, inulin, soluble starch, L-rhamnose, ethanol, glycerol, DL-lactic acid, and inositol were different between *T. basidiomaticola* and closely related taxa (Suppl. material 1: Table S1). Moreover, the novel species can grow in vitamin-free medium but not for its sister species.

***Tremella cheejenii* X.Z. Liu & F.Y. Bai, sp. nov.**
MycoBank: MB827187
Figures 3, 4

Type. CHINA. Guangxi Province, Hechi city, Luocheng county, Pingying village, Jiuwan Mountain National Nature Reserve, 108°48'E, 25°19'N, G.J. Li, H.S. Ma, Z.L. Lin & M.Z. Zhang, 7 August 2017, GX20172598 (HMAS 279589).

Etymology. *Cheejenii* was named in honor of Chee-Jen Chen for his contributions to systematics of tremellalean fungi.

Description. Basidiomata sessile, cerebriform, up to 1.0–3.0 cm in diameter, broadly attached to substratum, soft gelatinous, pale white when fresh and pale brown in dry condition. Hyphae smooth, thick-walled, slender, 2.0–4.5 μm in diameter, often anastomosing, clamp connections abundant, loop-like forming a large hollow. Haustoria rare, small, subglobose, ca 2.0 μm in diameter, with a single hypha. Hyphidia abundant, smooth, thin-walled, 2.5–4.0 μm in diameter, branched, hyphidia and basidia derived from the same hypha. Probasidial initials subglobose, ovoid or

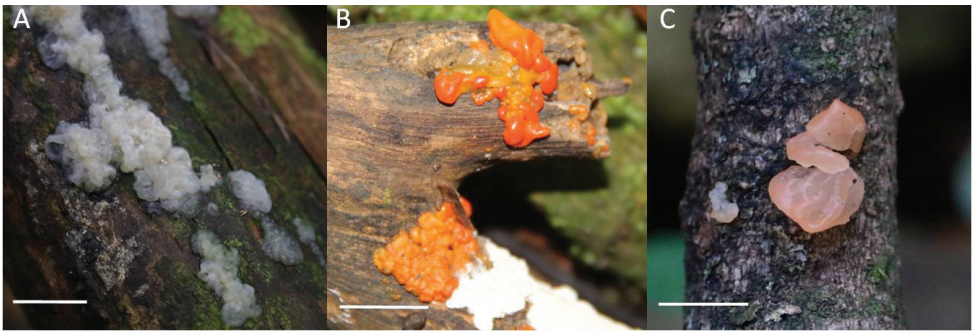

Figure 3. Macromorphology of *Tremella* basidiomata. **A** *T. cheejenii* **B** *T. erythrina* C *T. salmonea*. Scale bars: 1 cm.

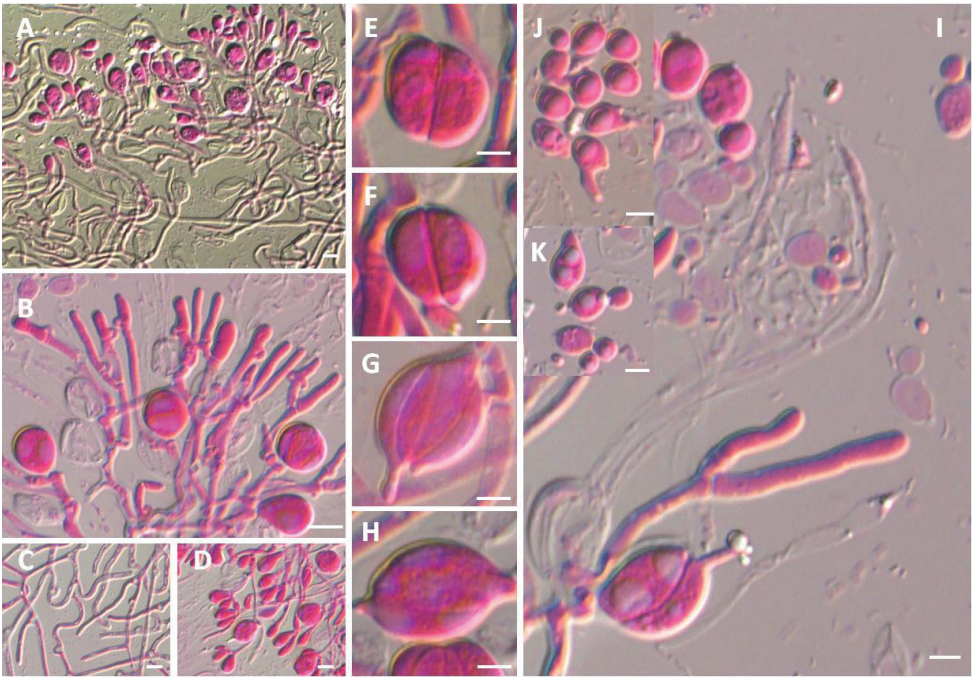

Figure 4. Microscopic structure of *Tremella cheejenii* (HMAS 279589). **A** Section through hymenium **B** Hyphidia from context **C** Hyphae from context **D** Probasidia **E–H** Mature basidia **I** Mature basidia and conidia produced from the sterigmata **J–K** Basidiospres and its germination with short sterigma. Scale bars: 10 μm (**A–D**), 5 μm (**E–J**).

pyriform. Mature basidia subglobose, broadly ellipsoid or ovoid, mostly two-celled, and occasionally four-celled, with apical protuberance, often longitudinally septate or occasionally oblique or cruciate-septate, thin-walled, 12.0–17.0 μm × 13.0–18.0 μm, stalked, 2.0–4.0 μm long, with sterigmata up to 70 μm, not swollen at apex. Ba-

sidiospores hyaline, smooth, thin-walled, subglobose to broadly ellipsoid, apiculate, 5.0–10.0 μm × 4.5–8.0 μm, L = 8.6 ± 1.1 μm, W = 6.6 ± 0.8 μm, Q = 1.1–1.8 (*n* = 40). Basidiospores forming secondary ballistoconidia by the formation of a sterigma. Conidia ellipsoid, smooth, hyaline, thin-walled, 2.2–4.0 μm × 1.8–3.0 μm, L = 3.1 ± 0.6 μm, W = 2.2 ± 0.3 μm, Q = 1.0–2.0 (*n* = 40), monokaryotic, budding from apex of sterigmata.

Habitat. On wood of deciduous tree, in forest dominated by Fagaceae, Lauraceae, Theaceae, Magnoliaceae, and Hamamelidaceae.

Additional specimens examined. CHINA. Guangxi Province, Hechi city, Luocheng county, Pingying village, Jiuwan Mountain National Nature Reserve, 108°48'E, 25°19'N, G.J. Li, H.S. Ma, Z.L. Lin & M.Z. Zhang, 7 August 2017, GX20172640 (HMAS 279590).

Notes. Two specimens form the sister group to *T. fibulifera*, *T. lloydiae-candidae*, and *T. resupinata* and represent a new species, *T. cheejenii*. The sequence identities between *T. cheejenii* and *T. fibulifera* are 95.7%–95.9% and 92.5%–93.2% in the D1/D2 domain and ITS region, respectively. Similarly, *T. cheejenii* and *T. lloydiae-candidae* showed 96.1%–96.2% and 92.1% sequence identities in the D1/D2 domain and ITS region, respectively. *Tremella cheejenii* and *T. resupinata* showed 90.4% and 89.9% sequence identities in the D1/D2 domain and ITS region, respectively. *Tremella cheejenii* is distinct from *T. fibulifera* in its bigger basidia (12.0–17.0 μm × 13.0–18.0 μm in *T. cheejenii* vs 14–16 μm × 10–13 μm in *T. fibulifera*). However, the basidia of *T. cheejenii* are smaller than that of *T. resupinata* (12.0–17.0 μm × 13.0–18.0 μm in *T. cheejenii* vs 27.0–40.0 μm × 22.0–31.0 μm in *T. resupinata*) (Chen 1998; Malysheva et al. 2015). Moreover, conidia are produced from the sterigmata in *T. cheejenii* compared to the absence of conidia in *T. fibulifera*, *T. lloydiae-candidae*, and *T. resupinata*.

***Tremella erythrina* X.Z. Liu & F.Y. Bai, sp. nov.**
MycoBank: MB827186
Figures 3, 5

Type. CHINA. Guangxi Province, Chongzuo city, Longzhou county, Qiang village, Nonggang National Nature Reserve, 106°54'E, 22°27'N, R.L. Zhao, M.Q. He, G.F. Mou, J.L. Qin, H.J. Wang & X.Y. Zhu, 30 July 2017, GX20170141 (HMAS 255317).

Etymology. *Erythrina* refers to the colour of the basidioma.

Description. Basidiomata sessile, cerebriform to foliose, with undulate broad lobes, lobes hollow, firm gelatinous, up to 1.3–1.8 cm in diameter, broadly attached to substrate, red and brownish orange when fresh and brownish orange when dry. Hyphae smooth, thin- or thick-walled, slender, hyaline, 1.0–3.0 μm, with clamp connections, branched with frequent anastomoses. Haustoria rare, small, subglobose, 1.5–2.0 μm in diameter, with single hyphae. Hyphidia present, smooth, thin-walled, 2.0–4.0 μm, branched. Probasidia mostly broadly ellipsoid. Mature basidia, globose to subglobose or broadly ellipsoid to ovoid, 12.0–18.0 μm × 13.0–19.0 μm, mostly four-celled, oc-

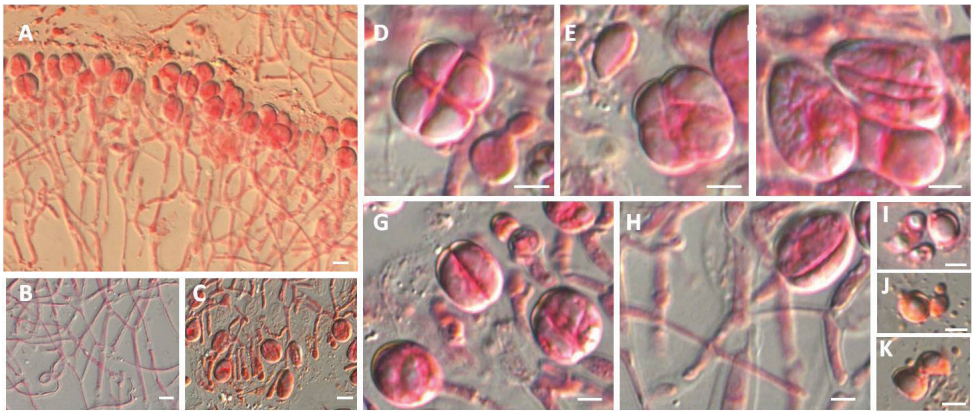

Figure 5. Microscopic structure of *Tremella erythrina* (HMAS 255317). **A** Section through hymenium **B** Hyphae from context **C** Hyphidia with basidia of different developmental stages **D–H** Mature basidia **I–K** Basidiospres. Scale bars: 10 μm (**A–C**), 5 μm (**D–K**).

casionally two-celled, without stalks, frenquently longitudianllly cruciate-septate. Basidiospores, smooth, thin-walled, ellipsoid to ovoid, apiculate, 7.0–10.0 μm × 5.0–7.0 μm, L = 8.2 ± 0.8 μm, W = 6.1 ± 0.6 μm, Q = 1.1–1.7 (*n* = 40).

Habitat. On decaying wood of deciduous tree, in forest dominated by Anacardiaceae, Palmae, Hypericaceae, and Sterculiaceae.

Additional specimens examined. CHINA. Guangxi Province, Chongzuo city, Longzhou county, Nonggang village, Nonggang National Nature Reserve, 106°56'E, 22°28'N, H.S. Ma, 16 September 2017, GX20170916001 (HMAS 279591).

Notes. Two specimens representing *T. erythrina* clustered in a well-supported clade and were closely related to *T. samoensis*. These two species showed 97.6%–97.8% and 93.7%–96.0% sequence identities in the D1/D2 domain and ITS region, respectively. Basidia in *T. erythrina* are larger than those of *T. samoensis* (12.0–18.0 μm × 13.0–19.0 μm in *T. erythrina* vs 12.0–18.0 μm × 8.0–12.0 μm in *T. samoensis*) (Chen 1998; Malysheva et al. 2015). Moreover, hyphidia are present and located in the hymenial structure and derived from the same generative hyphae with basidia in *T. erythrina*, whereas hyphidia are lacking in *T. samoensis* (Chen 1998; Malysheva et al. 2015).

***Tremella salmonea* X.Z. Liu & F.Y. Bai, sp. nov.**
MycoBank: MB827188
Figures 3, 6

Type. CHINA. Guangxi Province, Hechi city, Luocheng county, Jiuwan Mountain National Nature Reserve, 108°48'E, 25°19'N, G.J. Li, H.S. Ma, Z.L. Lin & M.Z. Zhang, 7 August 2017, GX20172637 (HMAS 279588).

Etymology. *Salmonea* refers to the colour of the basidioma.

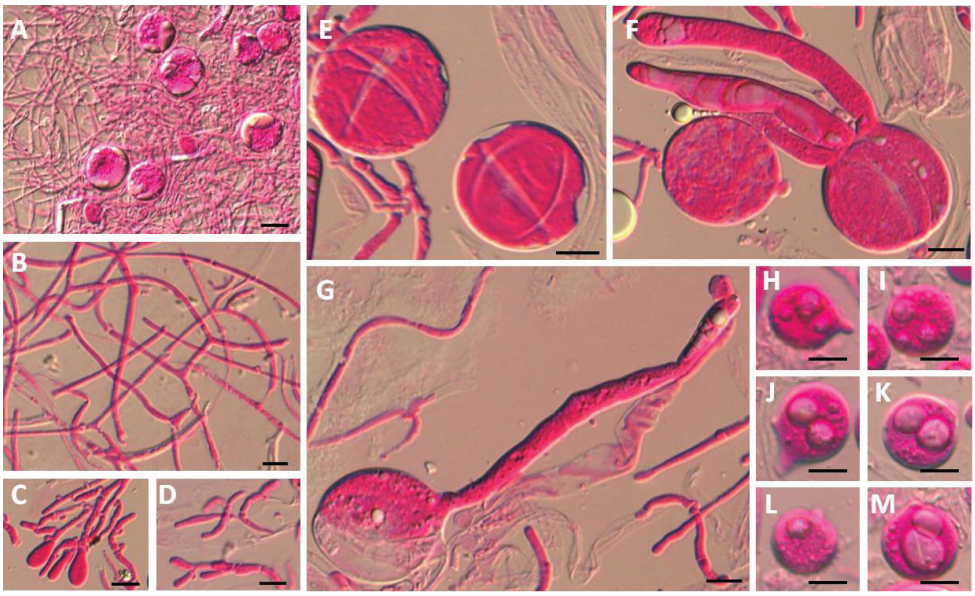

Figure 6. Microscopic structure of *Tremella salmonea* (HMAS 279588). **A** Section through hymenium **B** Hyphae from context **C** Swollen cells **D** Conidia in cluster **E–G** Mature basidia **H–M** Basidiospres. Scale bars: 10 μm (**A–M**).

Description. Basidiomata small, gyrose to cerebriform, 0.6–1.0 cm in diameter, firm gelatinous and thick, pale orange when fresh, yellow orange when dry, flat on the substrate. Hyphae smooth, thin-walled, slender, 2.0–3.5 μm in diameter, often with clamp connections. Haustoria rare, small, globose or subglobose, 2.0–4.0 μm in diameter, with single hyphae. Hyphidia rare, smooth, thin-walled, 2.0–4.0 μm, branched. Probasidial initials mostly subglobose to globose, sometimes broadly ellipsoid. Basidia, when mature, subglobose to globose, four-celled, occasionally two-celled, thin-walled, 31.0–38.0 μm × 29.0–37.0 μm, with longitudinally cruciate-septate, without stalk-like base; sterigmata up to 110.0 μm long, not swollen at apex. Basidiospores globose to subglobose, 16.0–22.0 μm × 15–20.0 μm, L = 18.3 ± 1.3 μm, W = 17.8 ± 1.4 μm, Q = 0.9–1.3 (*n* = 25), with a distinct apiculus. Conidia present, ellipsoid, fusiform to cylindrical, 8.0–17.0 μm × 2.0–5.0 μm, L = 10.7 ± 2.2 μm, W = 3.5 ± 0.5 μm, Q = 2–5.8 (*n* = 40), hyaline, clamped, arranged in cluster. Terminally and laterally swollen cells appearing abundant in the subhymenium, citriniform, pyriform or broadly ellipsoid, 9.0–20.0 μm × 5.6–13.0 μm, L = 14.2 ± 2.8 μm, W = 8.8 ± 1.8 μm, Q = 1.1–2.8 (*n* = 40).

Habitat. On wood of deciduous tree, in forest dominated by Rosaceae, Moraceae, Lauraceae, and Theaceae.

Notes. Only one specimen representing *T. salmonea* formed a distinct clade closely related to *T. taiwanensis* with 96.8%–98.3% sequence identities in D1/D2 domain and 95.4%–96.6% in ITS region, respectively. The affinity of *T. salmonea* to *T. taiwanensis*

lacked high support by the coalescent-based method (Fig. 1). *Tremella salmonea* differs from *T. taiwanensis* in its larger basidia (31.0–38.0 μm × 29.0–37.0 μm in *T. salmonea* vs 23.0–29.0 μm × 22.0–27.0 μm in *T. taiwanensis*) and basidiospores (16.0–22.0 μm × 15.0–20.0 μm in *T. salmonea* vs 14.0–18.0 μm ×14.0–20.0 μm in *T. taiwanensis*). In addition, hyphae-like conidiogenous cells and dikaryotic conidia were observed in *T. salmonea* compared to monokaryotic conidia produced from apex of sterigmata (Chen 1998). Swollen cells were located in the hymenium in *T. salmonea* whereas they were absent in *T. taiwanensis* (Chen 1998).

Discussion

Tremella s. s. is characterized by their tremella-like basidiomata. Many morphological characteristics have been used in taxonomic studies of *Tremella*, including the shape, colour, and size of basidiomata, basidia, and basidiospores, as well as other features such as length of the stalks and sterigmata, spore formation of the basidia, conidia, swollen cells, and hyphidia (Chen 1998). However, morphology-based taxonomy of *Tremella* species is very complicated. Almost 30 macromorphological and micromorphological characters need to be checked for identification at the species level (Chen 1998). Morphological taxonomy cannot provide enough evidence of phylogenetic relationship. Morphologically, *Tremella globispora* resembles species in the Indercorata group by its pyriform to capitates basidia and its spores that are broader than long (Chen 1998). Nevertheless, it is more related to species in the Fuciformis group, based on molecular data (Chen 1998; Fell et al. 2000; Scorzetti et al. 2002; Liu et al. 2015b). The application of molecular phylogenetics has significantly benefited the systematics and taxonomy of *Tremella*. In the present study, four new species of genus *Tremella* are described from China based on both morphological and molecular data.

The fruiting bodies of fungi harbour diverse microbial community including bacteria, yeasts and filamentous fungi (Buzzini et al. 2005; Barbieri et al. 2007; Pacioni et al. 2007). Microbial habitants could have roles in the development of the fruiting body, such as mycelium growth, nutrition supply, antifungal activity, and flavour formation (Sbrana et al. 2002; Barbieri et al. 2007; Antony-Babu et al. 2013; Seung-Yoon et al. 2018). There have been a new bacterial species found in the fruiting body of *T. fuciformis* which can cause infection (Wen et al. 2016). *Tremella basidiomaticola* was isolated from the fruit body of *T. fuciformis* and their relationship and contributions to the growth of fruiting body remain unknown.

Tremella salmonea is highly supported as belonging to the Mesenterica group. Microscopically, *T. salmonea* and *T. mesenterica* are similar in that both species share loose a hymenial structure with abundant hyphidia. However, these two species have different basidiomata colour: in *T. salmonea* basidiomata are salmon-orange, whereas in *T. mesenterica* they are yellowish. Other species in the *T. mesenterica* group with similar basidiomata colour include *T. roseolutescens* (basidia 20–27 μm × 18–27 μm) and *T.*

tropica (basidia 19–21 μm × 15–17 μm), but these are clearly different in the shape of their basidiomata and size of their basidia (Bandoni et al. 1996; Chen 1998; Roberts 2008).

The affiliation of *T. cheejenii* and *T. erythrina* to the Fuciformis or Mesenterica groups were not ascertained phylogenetically. *Tremella cheejenii* are closely related to T. fibulifer*a*, *T. lloydiae-candidae*, and *T. resupinata* in the phylogenetic analysis. Though they all have white basidiomata, there are clear differences in the shape and size of their basidiamata, length of their basidia and stalks, and length of their sterigmata (Bandoni and Oberwinkler 1983; Chen 1998; Malysheva et al. 2015). *Tremella hainanensis* also has whitish basidiomata, but it is distinguished from *T. cheejenii* by its filamentous lobes and ball-like basidiomata (Peng 1982). *Tremella erythrina* is closely related to *T. samoensis*, nevertheless, *T. erythrina* is distinguished by its salmon-orange cerebriform basidiomata that are larger than in *T. samoensis* (Chen 1998). Macroscopically, the most similar species to *T. erythrina* is *T. armeniaca*, *T. elastica*, *T. roseolutescens*, and *T. tawa*, all of which have orange basidiomata. *Tremella roseolutescens* (basidia 20–27 μm × 18–27 μm; basidiospores 11–15 μm × 9–11.5 μm) is diagnosed by its pulvinate basidiomata and larger basidia and basidiospores differing from *T. erythrina* (basidia 12–18 μm × 13–19 μm; basidiospores 7–10 μm × 5–7 μm) (Bandoni et al. 1996). Basidia in *T. erythrina* are slightly larger than those of *T. elastic* (10.0–15.0 μm × 6.0–9.0 μm) (Chen 1998). The presence of conidia and phialide-like conidiogenous cells in the hymenium of *T. armeniaca* has not been discovered in *T. erythrina* (Bandoni et al. 1996). *Tremella tawa* (basidia 20–30 μm × 13–18 μm) differs from *T. erythrina* in its clavate basidia and larger basidiomata and basidia (Bandoni and Buchanan 1990).

A total of 33 specimens of *Tremella* s. s. were collected from seven provinces (Guangdong, Guangxi, Heilongjiang, Jilin, Qinghai, Tibet, and Yunnan), which span a large portion of China and have different climates, humidity, and vegetation types. This implies the genus is really diverse beyond current knowledge. *Tremella* s. s. showed a significant deviation from the optimal range calculated for the genus rank using the phylogenetic rank boundary optimization (RPBO) analysis that indicates great genetic variation between different species in *Tremella* s. s. (Liu et al. 2015b). Two subclades, namely Mesenterica and Fuciformis, are included in this genus and can be featured by distinct ecological and morphological characters (Chen 1998, Liu et al. 2015b). They could probably be reclassified as two separate genera in the future. Further studies with additional fresh collections will clarify the systematic of this genus and enrich the knowledge of distribution, abundance, and ecology of *Tremella* species.

Key to the whitish species in *Tremella* s. s.

1 Basidia with sterigmata shorter than 35, hyphae grow from side of hyphae..... **2**
– Basidia with sterigmata longer than 35, hyphae grow from basidial clamp..... **3**
2 Basidiomata gyrose to cerebriform, 1–3 cm in diameter and basidia > 10 μm long ..... ***T. lloydiae-candidae***

– Basidiomata foliose, larger than 3 cm in diameter and basidia < 10 μm long **4**
3 Basidiomata filamentous lobes, conjunctive as a ball ..... ***T. hainanensis***
– Basidiomata resupinate or gyrose to cerebriform **5**
4 Basidia globose to subglobose ..... ***T. fuciformis***
– Basidia clavate with stalks ..... ***T. yokohamensis***
5 Basidiospores mostly broader than long ..... ***T. globispora***
– Basidiospores mostly longer than broad **6**
6 Basidiomata resupinate, < 1 cm in diameter ..... ***T. resupinata***
– Basidiomata gyrose to cerebriform, usually > 1 cm in diameter 7
7 Basidia size longer than 30 μm and basidiospores > 17 μm long ..... ***T. cerebriformis***
– Basidia size smaller than 20 μm and basidiospores ≤ 10 μm in long **8**
8 Basidia > 13 μm wide, with short stalk, sterigmata with inconspicuous apically swollen ..... ***T. cheejenii***
– Basidia < 13μm wide, without stalk, sterigmata with conspicuous apically swollen ..... ***T. fibulifera***

Key to the yellow, orange, or red species in *Tremella* s. s.

1 Basidiomata yellow **2**
– Basidiomata orange or red **11**
2 Basidia mostly > 25 μm long **3**
– Basidia mostly < 25 μm long **4**
3 Basidia < 22 μm wide and basidiospores 10–12 μm long ... ***T. philippinensis***
– Basidia > 26 μm wide and basidiospores > 13 μm long ..... ***T. brasiliensis***
4 Basidiomata pulvinate ..... ***T. subrubiginosa***
– Basidiomata gyrose to cerebriform or foliose **5**
5 Basidiomata gyrose to cerebriform **6**
– Basidiomata foliose **8**
6 Vesicles absent, haustoria rare, and conidia monokaryotic budding from apex of sterigmata ..... ***T. taiwanensis***
– Vesicles present, haustoria abundant, and conidia dikaryotic from hyphae-like conidiogenous cells 7
7 Basidiospores broadly ellipsoid or ovoid ..... ***T. mesenterica***
– Basidiospores globose to subglobose ..... ***T. mesenterella***
8 Basidia > 17 μm long and basidiospores > 7 μm wide ..... ***T. iduensis***
– Basidia < 17 μm long and basidiospores < 7 μm wide **9**
9 Basidiomata lobes not hollow ..... ***T. boninensis***
– Basidiomata lobes hollow **10**
10 Haustoria abundant and branched, probasidia mostly growing from side of the hymenial hyphae ..... ***T. flava***

–	Haustoria rare, probasidia proliferating directly from basidial clamps	***T. samoensis***
11	Basidiomata pulvinate	***T. roseolutescens***
–	Basidiomata gyrose to cerebriform or foliose	**12**
12	Basidiomata foliose and flat; basidia > 30 μm long	***T. salmonea***
–	Basidiomata gyrose to cerebriform; basidia < 30 μm long	**13**
13	Basidiomata reddish	**14**
–	Basidiomata orange	**15**
14	Basidia 17–21 μm long	***T. rubromaculata***
–	Basidia 11–15 μm long	***T. flammea***
15	Basidia predominantly clavate	***T. tawa***
–	Basidia globose to subglobose or ellipsoid to oval	**16**
16	Conidia present	**17**
–	Conidia absent	**18**
17	Conidiogenous cells globose or subglobose to ellipsoid, basidiospore > 12 μm long	***T. tropica***
–	Conidiogenous cells phialide-like, basidiospore 6–9 μm long	***T. armeniaca***
18	Hollow lobes	***T. erythrina***
–	Not having hollow lobes	***T. dysenterica***

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Supplementary material 1

Table S1. Physiological properties

Authors: Ying Zhao, Xin-zhan Liu, Feng-yan Bai

Data type: measurement

Link: https://doi.org/10.3897/mycokeys.47.29180.suppl1

Supplementary material 2

Sequence alignment

Authors: Ying Zhao, Xin-zhan Liu, Feng-yan Bai

Data type: phylogenetic data

Link: https://doi.org/10.3897/mycokeys.47.29180.suppl2

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RESEARCH ARTICLE

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Phylogenetic and morphological studies in *Xylodon* (Hymenochaetales, Basidiomycota) with the addition of four new species

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Abstract

Xylodon (Hymenochaetales, Basidiomycota) is the largest segregate genus of *Hyphodontia* s.l. Based on molecular and morphological data, 77 species are accepted in *Xylodon* to date. Phylogenetic analyses of ITS and 28S sequences, including 38 new ITS and 20 28S sequences of *Xylodon* species, revealed four species new to science. The new taxa *X. exilis*, *X. filicinus*, *X. follis* and *X. pseudolanatus* from Taiwan, Nepal, Réunion, Belize, and USA are described and illustrated. In addition, species concepts for *Odontia vesiculosa* from New Zealand and *Xylodon lanatus* from U.S.A. are revised and the new name *X. vesiculosus* is proposed. Phylogenetic analyses of the ITS region placed *X. spathulatus*, *X. bubalinus* and *X. chinensis* in a strongly supported clade and demonstrated that they are conspecific. *Palifer* and *Odontiopsis* are synonymised under *Xylodon* based on morphological and sequence data. The following new combinations are proposed: *X. erikssonii*, *X. gamundiae*, *X. hjortstamii*, *X. hyphodontinus*, *X. septocystidiatus* and *X. verecundus*. Line drawings of *X. cystidiatus*, *X. hyphodontinus*, *X. lanatus* and *X. vesiculosus*, as well as photographs of *X. raduloides* basidiomata, are provided. A key to *X. lanatus* and similar species is presented.

Keywords

Agaricomycetes, corticioid fungi, Schizoporaceae, *Schizopora*, *Odontia ambigua*, *Xylodon echinatus*

Introduction

The corticioid fungal genus *Xylodon* (Pers.) Gray, based on the generic type *X. quercinus* (Pers.) Gray, was described in 1801 by Persoon as *Sistotrema* sect. *Xylodon* and belongs in the Hymenochaetales (Basidiomycota). Species of *Xylodon* were usually placed in *Hyphodontia* J. Erikss. until Hjortstam and Ryvarden (2002, 2009) reorganised *Hyphodontia* s.l. into different genera based on morphological features.

The most recent generic description of *Xylodon* was published by Riebesehl and Langer (2017). With few exceptions, the hymenophore in *Xylodon* is odontioid or poroid with many different cystidia types and basidiospore shapes.

Xylodon spp. are primarily wood decomposers, causing a white-rot of angiosperms and gymnosperms (Eriksson and Ryvarden 1976, Yurchenko and Wu 2014). A few species have been collected on brown-rotten spruce stumps, palms or palm tree inflorescences, bamboo, ferns and on the herbaceous *Staehelina dubia* L. and *Fallopia sachalinensis* (F.Schmidt) Ronse Decr. (Burdsall and Nakasone 1981, Langer 1994, Nordén et al. 1999, Kotiranta and Saarenoksa 2000, Boidin and Gilles 2003, Hjortstam et al. 2005, Xiong et al. 2010, Jo et al. 2018). *Xylodon* has a worldwide distribution, with both cosmopolitan species and species restricted to a limited geographic area.

Palifer Stalpers & P.K.Buchanan (1991), based on *Peniophora verecunda* G.Cunn. from New Zealand, is another segregate genus of *Hyphodontia* s.l. recognised by Hjortstam and Ryvarden (2009). It is characterised by encrusted cystidia and remained monotypic until 2007 when three species were transferred to the genus (Hjortstam and Ryvarden 2007a). After a thorough morphological study of *Palifer* species and related taxa, Gorjón (2012) concluded that *Palifer* was probably a synonym of *Xylodon* but did not propose any new combinations. *Palifer* is represented by only one nuclear ribosomal internal transcribed spacer (ITS) sequence in the public record and phylogenetic analyses showed it to be embedded in *Xylodon* (Larsson et al. 2006). Riebesehl and Langer (2017), however, declined to synonymise *Palifer* with *Xylodon* based on one DNA sequence alone and chose to emphasise its morphological features.

Odontiopsis Hjortstam and Ryvarden (1980) is based on the type species *O. hyphodontina* Hjortstam & Ryvarden from Tanzania. It is characterised by an odontioid hymenium, encrusted hyphae projecting from the aculei, stout basidia and globose to subglobose basidiospores. Hjortstam and Ryvarden (1980) mentioned hyphal and basidial similarities with *Schizopora* and *Hyphodontia*.

In this study, we conducted an in-depth phylogenetic study of 36 *Xylodon* species represented by 96 strains or collections, including 58 new ITS and large subunit (28S) ribosomal DNA sequences. Phylogenetic analyses of the ITS and 28S sequence data uncovered four new taxa, *X. exilis*, *X. filicinus*, *X. follis* and *X. pseudolanatus*, that are described and illustrated. In addition, the species complex of *X. spathulatus* was identified and resulted in the synonymisation of two taxa. The genera *Palifer*

and *Odontiopsis* are re-evaluated and placed in synonymy with *Xylodon*, resulting in a number of new combinations. Morphological studies in *Xylodon lanatus* and *Odontia vesiculosa* were conducted and a key to morphologically similar species is provided. Line drawings of *X. cystidiatus*, *X. hyphodontinus* and *X. vesiculosus* are presented and *X. vesiculosus* is described.

Methods

Molecular study

Pieces of dried basidiomata served as material for DNA extractions with the E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-Tek, VWR, USA). Two nuclear ribosomal DNA markers were used in this study: the ITS region and the D1-D2 domains of 28S. The ITS region includes the internal transcribed spacers 1 and 2 as well as the intercalary 5.8S rRNA gene. For amplification of ITS, different combinations of the following primers were used: ITS1-F (Gardes and Bruns 1993), ITS1, ITS2, ITS3, ITS4, ITS5 (White et al. 1990) and ALR0 (Collopy et al. 2001). The last one was modified in one position (Riebesehl and Langer 2017). NL1, NL4 (O'Donnell 1993), LR0R (Bunyard et al. 1996) and LR5 (Vilgalys and Hester 1990) were used, also in different combinations, for the amplifications of the D1-D2 domains of 28S. PCR products were purified with innuPREP PCRpure Kit (Analytik Jena, Berlin, Germany) and the DNA sequencing was implemented by Eurofins Genomics (Ebersberg, Germany).

Newly generated sequences were edited with MEGA7 (Kumar et al. 2016). Their quality was checked following the five guidelines by Nilsson et al. (2012) and they were deposited in NCBI GenBank (Benson et al. 2018; Tab. 1). Other sequences used in this study were downloaded from the same database. *Phellinus gabonensis* Decock & Yombiyeni (Hymenochaetales) was chosen as the outgroup for rooting the phylograms. The two different alignments were calculated with MAFFT v.7 (Katoh and Standley 2013), using the L-INS-i algorithm for ITS and G-INS-i for 28S. Minimum Evolution (ME) and Bayesian inference (BI) trees were calculated for both datasets. The ME phylograms were computed with MEGA7, using the Tamura-Nei model (Tamura and Nei 1993) including 1000 bootstrap (BS) replications, partial deletion of gapped positions with 95% site coverage cut-off and other default settings. The BI phylograms were constructed with MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003), using DNA substitution models estimated by MrModeltest 2.4 (Nylander 2004) with 10 million generations and one tree saved for every 1000 generations; other parameters used default settings. A partitioned analysis was done for the ITS alignment with independent DNA substitution models and parameter values for ITS1, 5.8S and ITS2. MEGA7 and FigTree 1.4.2 (Rambaut 2012) were used for processing the phylograms.

Table 1. List of accepted species in *Xylodon* with some closely related species from other genera, including specimens used in the phylogenetic study. Newly generated sequences are shown in bold. *Xylodon* species without available ITS or 28S sequences are marked with 'not available' (n.a.); these have to date not been studied using ribosomal sequence data.

Species	Specimen voucher	GenBank accession number		Reference	Country
		ITS	28S		
Hastodontia hastata (Litsch.) Hjortstam & Ryvarden	KAS-GEL3124	DQ340311	–	unpublished	Sweden
	EL47/99 (GB)	–	DQ873620	Larsson et al. 2006	Sweden
***Hyphodontia borbonica* Riebesehl, Langer & Barniske**	**FR-0219441, Holotype**	KR349240	–	Riebesehl et al. 2015	**Réunion**
	–	–	**MH884915**	**This study**	–
H. pallidula (Bres.) J.Erikss.	KAS-GEL2097	DQ340317	DQ340372	unpublished	Germany
Kneiffiella barba-jovis (Bull.) P.Karst.	KHL 11730 (GB)	DQ873609	DQ873610	Larsson et al. 2006	Sweden
K. palmae Rick ex Hjortstam & Ryvarden	FR7	KP689185	–	Wang et al. 2016	China
	KAS-GEL3456	–	DQ340369	unpublished	Taiwan
Lagarobasidium calongei M.Dueñas, Tellería, Melo & M.P.Martín	MA-Fungi 73256	NR119737	n.a.	Dueñas et al. 2009	Azore Islands
Lyomyces crustosus (Pers.) P.Karst.	TASM YG-G39	MF382993	–	Gafforov et al. 2017	Uzbekistan
	KAS-GEL2325	–	DQ340354	unpublished	Germany
L. sambuci (Pers.) P.Karst.	KAS-JR7	KY800402	KY795966	Yurchenko et al. 2017	Germany
Phellinus gabonensis Decock & Yombiyeni	MUCL 52025	HM635715	HM635690	Yombiyeni et al. 2011	Gabon
Xylodon adhaerisporus (Langer) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. anmashanensis (Yurchenko, H.X.Xiong & Sheng H.Wu) Riebesehl, Yurchenko & Langer	–	n.a.	n.a.	–	–
X. apachericnsis (Gilb. & Canf.) Hjortstam & Ryvarden	Canfield 180, Holotype	KY081800	n.a.	Riebesehl and Langer 2017	USA, Arizona
X. archeri (Berk.) Kuntze	–	n.a.	n.a.	–	–
X. asperus (Fr.) Hjortstam & Ryvarden	UC2023169	KP814365	–	Rosenthal et al. 2017	USA, Montana
	KHL8530 (GB)	–	AY586675	Larsson et al. 2004	Sweden
X. astrocystidiatus (Yurchenko & Sheng H.Wu) Riebesehl, Yurchenko & Langer	Wu 9211-71	JN129972	JN129973	Yurchenko and Wu 2014	Taiwan
***X. australis* (Berk.) Hjortstam & Ryvarden**	**CIEFAP-11041 (CFMR)**	n.a.	**MH884895**	**This study**	**Argentina**
X. bisporus (Boidin & Gilles) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. borealis (Kotir. & Saaren.) Hjortstam & Ryvarden	UC2022850	KP814307	–	Rosenthal et al. 2017	USA, Connecticut
	JS26064	–	AY586677	Larsson et al. 2004	Norway
X. bresinskyi (Langer) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. brevisetus (P. Karst.) Hjortstam & Ryvarden	KHL 12386 (GB)	DQ873612	DQ873612	Larsson et al. 2006	Sweden
X. bubalinus (Min Wang, Yuan Y. Chen & B.K. Cui) C.C. Chen & Sheng	CLZhao 184	MG231628	n.a.	unpublished	China
	Cui 6834	KY290981	–	Wang and Chen 2017	China
	Cui 12887	KY290982	–	Wang and Chen 2017	China
	Cui 12888, Holotype	KY290983	–	Wang and Chen 2017	China
X. candidissimus (Berk. & M.A.Curtis) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. capitatus (G.Cunn.) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–

Species	Specimen voucher	GenBank accession number		Reference	Country
		ITS	28S		
X. chinensis (C.C.Chen & Sheng H.Wu) C.C.Chen & Sheng H.Wu	Wu 1307-42	KX857802	–	Chen et al. 2017	China
	Wu 1407-105, Holotype	KX857804	KX857811	Chen et al. 2017	China
X. crassihyphus (Douanla-Meli) Riebesehl & Langer	–	n.a.	n.a.	–	–
X. crassisporus (Gresl. & Rajchenb.) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. crustosoglobosus (Hallenb. & Hjortstam) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
***X. cystidiatus* (A.David & Rajchenb.) Riebesehl & Langer**	**FR-0249200**	**MH880195**	**MH884896**	**This study**	**Réunion**
X. detriticus (Bourdot) Tura, Zmitr., Wasser & Spirin	UC2023108	KP814412	n.a.	Rosenthal et al. 2017	USA, Michigan
X. echinatus (Yurchenko & Sheng H.Wu) Riebesehl, Yurchenko & Langer	–	n.a.	n.a.	–	–
X. erikssonii (M.Galán & J.E.Wright) Riebesehl & Langer	–	n.a.	n.a.	–	–
***X. exilis* Yurchenko, Riebesehl & Langer**	**MSK-F 7381**	**MH880196**	–	**This study**	**Taiwan**
	MSK-F 7431	–	**MH884897**	**This study**	**Taiwan**
	TUB-FO 42450	**MH880197**	–	**This study**	**Taiwan**
	TUB-FO 42565, Holotype	**MH880198**	**MH884898**	**This study**	**Taiwan**
***X. filicinus* Yurchenko & Riebesehl**	**MSK-F 12869, Holotype**	**MH880199**	**MH884899**	**This study**	**Taiwan**
	MSK-F 12870	**MH880200**	**MH884900**	**This study**	**Taiwan**
X. fimbriatus (Sheng H.Wu) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
***X. flaviporus* (Berk. & M.A.Curtis ex Cooke) Riebesehl & Langer**	FCUG 1053	AF145575	–	Paulus et al. 2000	Romania
	FR-0249797	**MH880201**	**MH884901**	**This study**	**Réunion**
	KAS-GEL3462	**MH880202**	–	**This study**	**Taiwan**
	KAS-GEL5047	**MH880203**	–	**This study**	**Réunion**
	KUC20130808-17	–	KJ668314	Jang et al. 2016	South Korea
***X. follis* Riebesehl, Yurchenko & Langer**	**FR-0249814, Holotype**	**MH880204**	**MH884902**	**This study**	**Réunion**
X. gamundiae (Gresl. & Rajchenb.) Riebesehl & Langer	–	n.a.	n.a.	–	–
X. gracilis (Hjortstam & Ryvarden) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. hallenbergii (Sheng H.Wu) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. hastifer (Hjortstam & Ryvarden) Hjortstam & Ryvarden	Ryvarden 19767, Holotype	KY081801	n.a.	Riebesehl and Langer 2017	Argentina
X. heterocystidiatus (H.X.Xiong, Y.C.Dai & Sheng H.Wu) Riebesehl, Yurchenko & Langer	Wu 9209-27	JX175045	–	Yurchenko and Wu 2014	Taiwan
	–	–	KX857821	Chen et al. 2017	–
X. hjortstamii (Gresl. & Rajchenb.) Riebesehl & Langer	–	n.a.	n.a.	–	–
***X. hyphodontinus* (Hjortstam & Ryvarden) Riebesehl, Yurchenko & G.Gruhn**	**KAS-GEL9222**	**MH880205**	**MH884903**	**This study**	**Kenya**
	LIP GG-GUY13-044	**MH880206**	**MH884904**	**This study**	**French Guyana**
	LIP GG-MAR12-238	**MH880207**	**MH884905**	**This study**	**Martinique**
	LIP GG-MAR15-127	**MH880208**	**MH884906**	**This study**	**Martinique**
X. knysnanus (Van der Byl) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. lanatus (Burds. & Nakasone) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. lenis Hjortstam & Ryvarden	Wu0808-32	–	KX857820	Chen et al. 2017	Taiwan
	Wu890714-3, Holotype	KY081802	–	Riebesehl and Langer 2017	Taiwan

Species	Specimen voucher	GenBank accession number		Reference	Country
		ITS	28S		
X. lutescens (Hjortstam & Ryvarden) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. mollissimus (L.W.Zhou) C.C.Chen & Sheng H.Wu	LWZ20160318-3	KY007517	n.a.	Kan et al. 2017	China
X. mussooriensis Samita, Sanyal & Dhingra	–	n.a.	n.a.	–	–
***X. nespori* (Bres.) Hjortstam & Ryvarden**	KAS-GEL3158	–	DQ340346	unpublished	Sweden
	KAS-JR14	**MH880210**	–	**This study**	**Germany**
	KUC20161012-50	MF774797	–	unpublished	South Korea
X. nesporina (Hallenb. & Hjortstam) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
***X. niemelaei* (Sheng H.Wu) Hjortstam & Ryvarden**	Dai 15358	KT989973	–	Chen et al. 2016	China
	FR-0219860	**MH880211**	–	**This study**	**Réunion**
	FR-0249174	**MH880212**	–	**This study**	**Réunion**
	FR-0249178	–	**MH884907**	**This study**	**Réunion**
	FR-0249225	**MH880213**	–	**This study**	**Réunion**
	FR-0249289	**MH880214**	–	**This study**	**Réunion**
	FR-0249744	**MH880215**	–	**This study**	**Réunion**
	FR-0249811	**MH880216**	–	**This study**	**Réunion**
	FR-0249846	**MH880217**	–	**This study**	**Réunion**
	GC 1508-146	KX857798	–	Chen et al. 2017	Taiwan
	KAS-GEL4904	**MH880218**	–	**This study**	**Réunion**
	KAS-GEL4998	EU583422	–	unpublished	Réunion
	Wu1010-62	–	KX857817	Chen et al. 2017	Taiwan
X. nongravis (Lloyd) C.C.Chen & Sheng H.Wu	GC1412-22	KX857801	KX857818	Chen et al. 2017	Taiwan
X. nothofagi (G.Cunn.) Hjortstam & Ryvarden	PDD:91630	GQ411524	–	Fukami et al. 2010	New Zealand
X. nudisetus (Warcup & P.H.B.Talbot) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. ovisporus (Corner) Riebesehl & Langer	ICMP 13830	AF145584	–	Paulus et al. 2000	New Zealand
	KAS-GEL3493	EU583421	–	unpublished	Taiwan
	KUC20130725-29	–	KJ668365	Jang et al. 2016	South Korea
X. papillosus (Fr.) Riebesehl, Yurchenko & Langer	–	n.a.	n.a.	–	–
***X. paradoxus* (Schrad.) Chevall.**	FCUG 2425	AF145571	–	Paulus et al. 2000	Russia
	KAS-GEL2511	–	AF518647	Hibbett and Binder 2002	Germany
	KAS-JR06	**MH880219**	–	**This study**	**Germany**
	KAS-JR28	–	**MH884908**	**This study**	**Austria**
X. pelliculae (H.Furuk.) Riebesehl, Yurchenko & Langer	–	n.a.	n.a.	–	–
X. poroideoefibulatus (Sheng H.Wu) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. pruniaceus (Hjortstam & Ryvarden) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
***X. pseudolanatus* Nakasone, Yurchenko & Riebesehl**	**FP-150922 (CFMR), Holotype**	**MH880220**	**MH884909**	**This study**	**Belize**
X. pseudotropicus (C.L.Zhao, B.K.Cui & Y.C.Dai) Riebesehl, Yurchenko & Langer	Dai 10768	KF917543	n.a.	Zhao et al. 2014	China
X. quercinus (Pers.) Gray	Otto Miettinen 15050,1 (H 6013352)	KT361632	–	Ariyawansa et al. 2015	Finland
	KHL11076 (GB)	KT361633	AY586678	Larsson et al. 2004	Sweden
***X. raduloides* (Pers.) Riebesehl & Langer**	ICMP 13833	AF145580	–	Paulus et al. 2000	Australia
	KAS-JR 02	**MH880221**	–	**This study**	**Germany**
	KAS-JR 03	**MH880222**	–	**This study**	**Germany**
	KAS-JR 09	**MH880223**	–	**This study**	**Germany**

Species	Specimen voucher	GenBank accession number		Reference	Country
		ITS	28S		
***X. raduloides* (Pers.) Riebesehl & Langer**	**KAS-JR 10**	**MH880224**	–	**This study**	**Germany**
	KAS-JR 26	**MH880225**	**MH884910**	**This study**	**Germany**
	LR 18813	**MH880226**	**MH884911**	**This study**	**Australia**
X. ramicida Spirin & Miettinen	Viacheslav Spirin 7664 (H), Holotype	KT361634	n.a.	Ariyawansa et al. 2015	Russia
X. reticulatus (C.C.Chen & Sheng H.Wu) C.C.Chen & Sheng H.Wu	GC1512-1	KX857808	KX857813	Chen et al. 2017	Taiwan
	KUC20160721B-26	MF774798	–	Kwon et al. 2018	South Korea
	Wu1109-178, Holotype	KX857805	–	Chen et al. 2017	Taiwan
X. rhizomorphus (C.L.Zhao, B.K.Cui & Y.C.Dai) Riebesehl, Yurchenko & Langer	Dai 12354	KF917544	n.a.	Zhao et al. 2014	China
	Dai 12367, Holotype	KF917545	–	Zhao et al. 2014	China
	Dai 12389	KF917546	–	Zhao et al. 2014	China
X. rickii (Hjortstam & Ryvarden) K.H. Larss.	–	n.a.	n.a.	–	–
X. rimosissimus (Peck) Hjortstam & Ryvarden	Ryberg 021031 (GB)	DQ873627	DQ873628	Larsson et al. 2006	Sweden
X. rudis (Hjortstam & Ryvarden) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. septocystidiatus (H.X.Xiong, Y.C.Dai & Sheng H.Wu) Riebesehl & Langer	–	n.a.	n.a.	–	–
***X. serpentiformis* (Langer) Hjortstam & Ryvarden**	**KAS-GEL3668**	**MH880227**	–	**This study**	**Taiwan**
	TUB-FO 40675	**MH880228**	–	**This study**	**Taiwan**
	TUB-FO 40985	–	**MH884912**	**This study**	**Taiwan**
	TUB-FO 42688	**MH880229**	**MH884913**	**This study**	**Taiwan**
X. sp. 1	Dai 15321	KT989969	n.a.	Chen et al. 2016	China
***X. spathulatus* (Schrad.) Kuntze**	KAS-GEL2690	KY081803	–	Riebesehl and Langer 2017	Germany
	KAS-MMS7224	**MH880230**	–	**This study**	**Czech Republic**
	KHL7085 (GB)	KY081804	–	Riebesehl and Langer 2017	Sweden
	MSK-F 12931	**MH880231**	**MH884914**	**This study**	**Russia**
***X. subclavatus* (Yurchenko, H.X.Xiong & Sheng H.Wu) Riebesehl, Yurchenko & Langer**	**TUB-FO 42167**	**MH880232**	n.a.	**This study**	**Taiwan**
***X. subflaviporus* C.C.Chen & Sheng H.Wu**	**KAS-GEL3466**	**MH880233**	–	**This study**	**Taiwan**
	Wu 0809-76	KX857803	KX857815	Chen et al. 2017	China
X. subglobosus Samita, Sanyal & Dhingra	–	n.a.	n.a.	–	–
X. submucronatus (Hjortstam & Renvall) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. subscopinellus (G.Cunn.) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. subtropicus (C.C.Chen & Sheng H.Wu) C.C.Chen & Sheng H.Wu	Wu 1508-2	KX857806	KX857812	Chen et al. 2017	China
	Wu 9806-105, Holotype	KX857807	KX857809	Chen et al. 2017	Vietnam
X. syringae (Langer) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. taiwanianus (Sheng H.Wu) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. tenellus Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. tenuicystidius (Hjortstam & Ryvarden) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. trametoides (Núñez) Riebesehl & Langer	–	n.a.	n.a.	–	–
X. tuberculatus (Kotir. & Saaren.) Hjortstam & Ryvarden	–	n.a.	n.a.	–	–
X. verecundus (G.Cunn.) Yurchenko & Riebesehl	KHL 12261 (GB)	DQ873642	n.a.	Larsson et al. 2006	USA
X. vesiculosus Yurchenko, Nakasone & Riebesehl	–	n.a.	n.a.	–	–

Morphological study

The studied specimens are deposited in herbaria CFMR, FR, KAS, LIP, MSK, and TUB (acronyms follow Index Herbariorum, http://sweetgum.nybg.org/science/ih). Morphological descriptions and figures employed dried basidiomata. Preparations in 3% potassium hydroxide (KOH) aqueous solution were used for microscopic measurements and most drawings. Crystalline deposits on hyphae were additionally examined in Melzer's reagent (Mz) and tap water. Amyloid and dextrinoid reactions of basidiospores were tested with Mz. Spore wall cyanophily was determined in Cotton Blue-Lactophenol solution (CBL). The following abbreviations are used to describe arithmetic averages for 30 basidiospores, randomly selected in squash preparations of one specimen: L – spore length, W – spore width, Q = length/width ratio.

Results

Phylogeny

The aligned ITS data matrix consisted of 92 taxa and 847 positions. The partial deletion of gapped positions resulted in 463 positions that were used in the ME phylogenetic analysis. The data matrix was partitioned as follows: ITS1 = positions 1–373, 5.8S rRNA gene = 374–541 and ITS2 = 542–847. The GTR + G model was used as DNA substitution model for ITS1 and ITS2 and SYM + I for 5.8S in the BI analysis. The aligned data matrix of the D1-D2 domains of 28S rRNA gene consisted of 47 taxa and 634 positions; 532 positions were used in the ME analysis. The GTR + I + G model was chosen as the DNA substitution model for the BI analysis. A high degree of agreement was observed between the ME and BI trees; therefore, the ME phylogram with BS and integrated posterior probability (PP) values from the BI phylogram are presented in Figures 1, 2. The sum of branch lengths in the resulting ME phylograms was 3017 for ITS (Fig. 1) and 1009 for 28S (Fig. 2). Multiple sequence alignments and trees are deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23512).

The ITS phylogram (Fig. 1) shows a number of clades with low BS support which is well-documented for *Xylodon* (see Chen et al. 2018, Riebesehl and Langer 2017). Figure 1 includes 83 sequences of *Xylodon* specimens or strains of which 38 were generated in this study. No significant distances were observed amongst sequences of *X. niemelaei*, *X. rhizomorphus*, and *X. reticulatus* nor amongst sequences of *X. spathulatus*, *X. chinensis*, and *X. bubalinus*. The strong BS (99 and 97) and PP (1) values of these two clades indicate that the taxa within each clade may be conspecific. Seven collections of *X. raduloides* form two distinct subclades (96 and 98 BS). Similarly, four collections of *X. flaviporus* formed two subclades (99 and 93 BS). The newly generated ITS sequences show that *X. cystidiatus*, *X. hyphodontinus*, *X. serpentiformis* and *X. subclavatus* form distinct lineages in *Xylodon*. The four new species introduced herein form

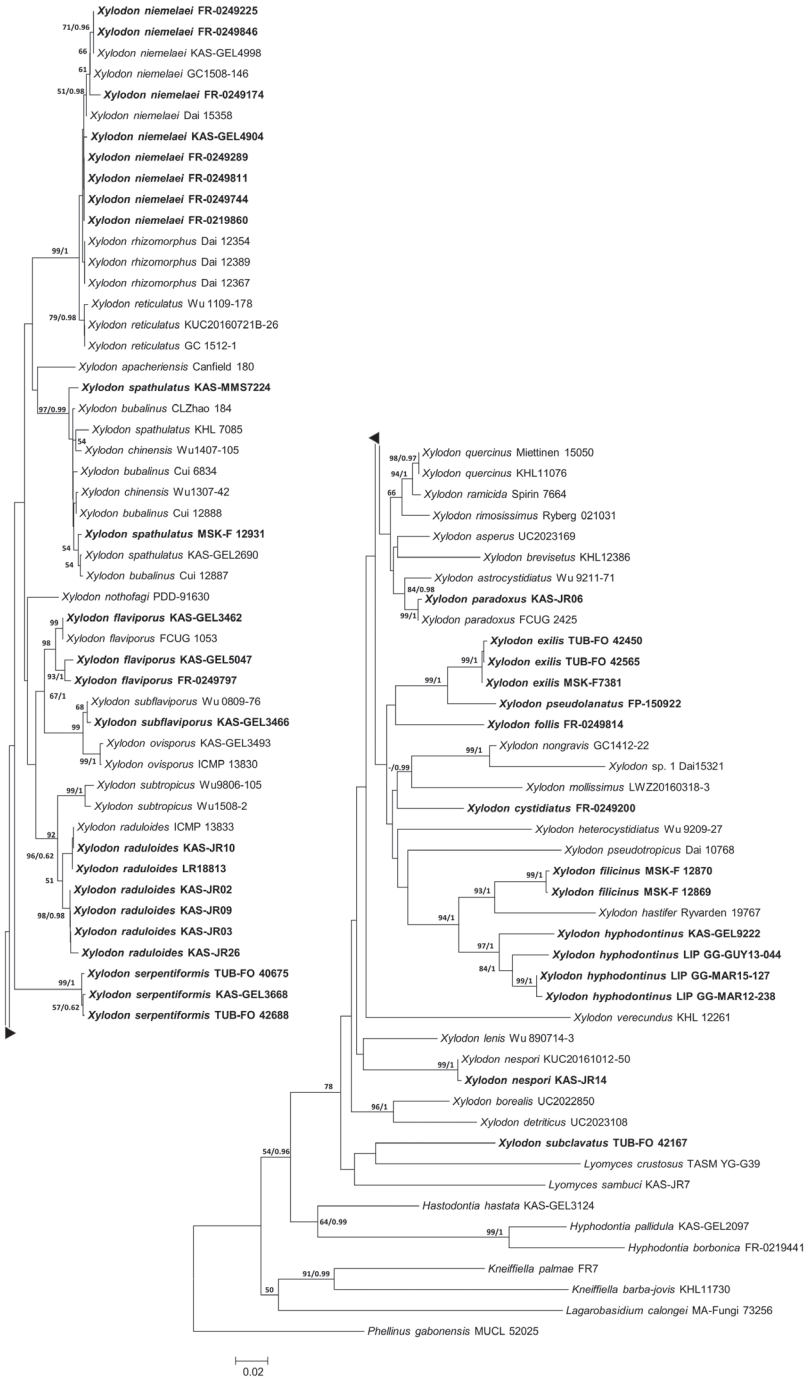

Figure 1. ITS-based Minimum Evolution phylogram for *Xylodon* and allied species. Bootstrap values >50 are shown next to the branches. The second number, if present, represents posterior probabilities received from BI analysis. Scale bar indicates estimated number of substitutions per site. Sequences generated in this study are shown in bold. Voucher numbers and species names are indicated in Table 1.

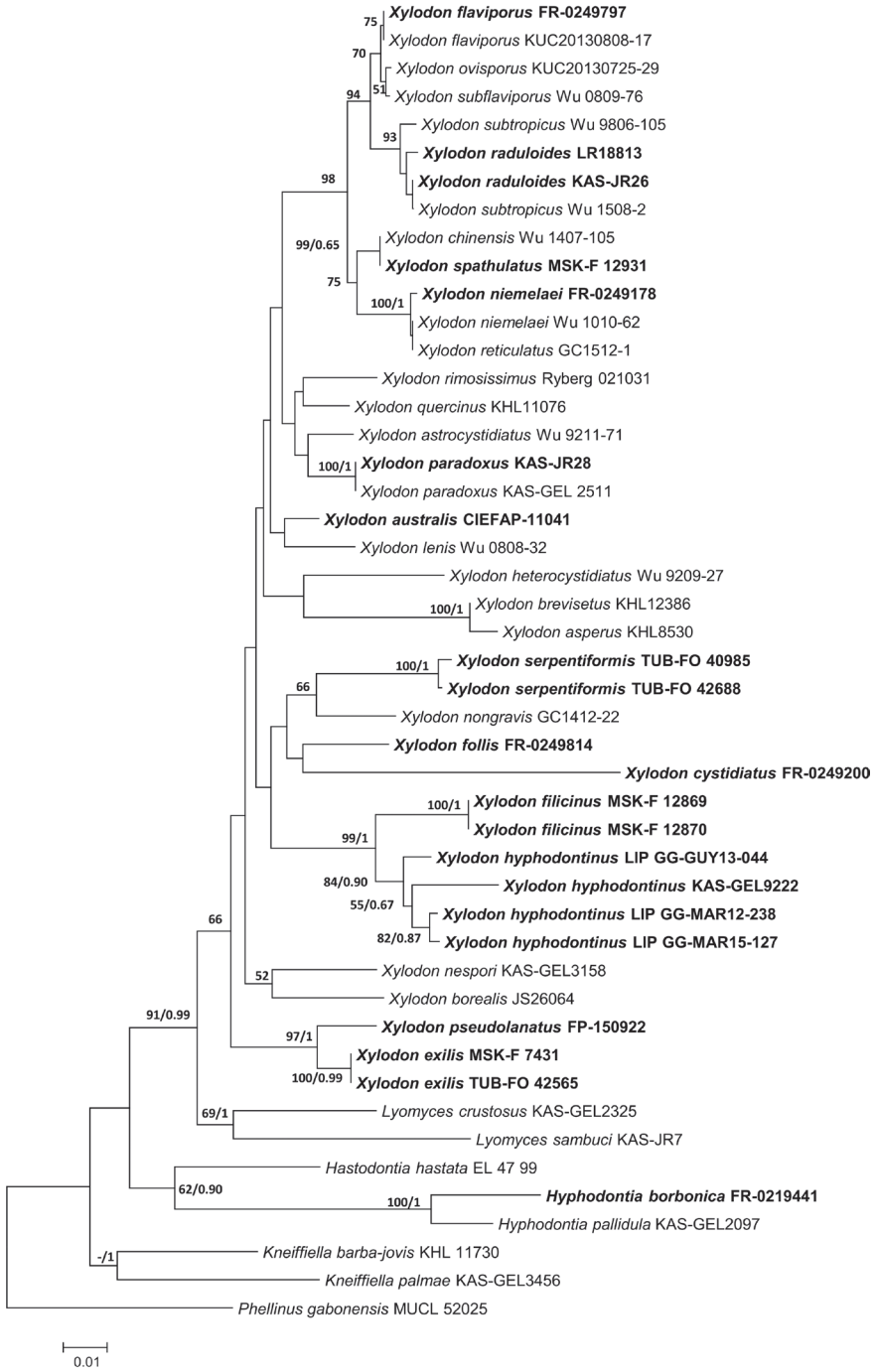

Figure 2. 28S-based Minimum Evolution phylogram for *Xylodon* and allied species. Bootstrap values >50 are shown next to the branches. The second number, if present, represents posterior probabilities received from BI analysis. Scale bar indicates estimated number of substitutions per site. Sequences generated in this study are shown in bold. Voucher numbers and species names are indicated in Table 1.

distinct lineages as well. *Xylodon pseudolanatus* and *X. exilis* are sister groups with 5.9% differences between their ITS sequences (*X. pseudolanatus*: FP-150922 and *X. exilis*: TUB-FO 42565). They cluster in a well-supported clade (99 BS), within a weakly supported lineage that includes *X. follis*. The closest relative of *X. filicinus* is *X. hastifer*; they form a clade (93 BS) that is sister to *X. hyphodontinus* s.l.

The 28S phylogram (Fig. 2) includes 39 sequences of *Xylodon* of which 20 were generated here. Notable new 28S sequences include *X. australis*, *X. hyphodontinus*, *X. serpentiformis*, the four new species described herein, and furthermore *Hyphodontia borbonica*. As the 28S phylogram features several lineages with low BS support, the clades between the 28S and ITS trees are not identical throughout. Although clearly resolved with ITS sequences, the 28S gene analyses were not able to resolve the closely related *X. raduloides* and *X. subtropicus*. Some clades that were well supported with ITS sequences were also well supported in the 28S phylogram, for example, the *X. niemelaei* and *X. reticulatus* (100 BS) and the *X. chinensis* and *X. spathulatus* (99 BS) clades.

Morphology

***Xylodon exilis* Yurchenko, Riebesehl & Langer, sp. nov.**
MycoBank MB827462
Figs 3a, 4

Holotype. TAIWAN, Nantou county, south from Sun-Moon Lake, near Hua Lien, Lien-Hwa-Chi, 700 m a.s.l., on fallen angiosperm twig, leg. E. Langer, G. Langer, F. Oberwinkler, 10 Jul 1990 (TUB-FO 42565; isotypes in KAS and MSK).

Description. Basidiomata effused, 1–5 cm in extent, membranaceous, discontinuous at the periphery. Hymenial surface minutely odontioid, cream-coloured, between aculei 50–130 µm thick. Aculei peg-like, conical or subcylindrical, entire or slightly penicillate apically, 35–70 µm long, 15–50(–70) µm diam., 8–14/mm. Margin abrupt or somewhat thinning out. Hyphal system monomitic, hyphae colourless, with clamps at all primary septa. Subicular hyphae forming a loose tissue, rarely branched, 3–4(–4.5) µm wide, with slightly thick to thick walls (0.5–1.2 µm thick), with scattered adventitious septa, smooth. Subhymenial hyphae in a dense tissue, richly branched, 2–3 µm wide, thin-walled, smooth or slightly encrusted. Capitate cystidia enclosed, 18–22 × 5.5–8 µm, sometimes with an adventitious septum in stem, thin- to slightly thick-walled. Projecting hyphae in aculei flexuous, apically obtuse, 90–130 µm long, 3–4 µm wide, originating from thick-walled subicular hyphae, with simple and clamped septa, often constricted at septa, walls thickened at base then gradually thinning toward apex, moderately encrusted. Basidioles clavate or bowling pin-shaped, 10–20 × 4.5–5.5 µm. Basidia narrowly utriform, 20–25 × 4–5(–5.5) µm, thin-walled, smooth, with four sterigmata 2–4 × 0.3 µm. Spores narrowly ellipsoid, 5.5–6 × 2.5–3 µm, holotype L = 5.8 µm, W = 2.8 µm, Q = (1.6–)1.8–2.2, colourless, smooth, slightly thick-walled, negative in Mz, acyanophilous, with minute apiculus.

Figure 3. Basidiomata of *Xylodon* spp. **A** *X. exilis* (TUB-FO 42565, holotype) **B** *X. filicinus* (MSK-F 12369, holotype) **C** *X. follis* (FR-0249814, holotype) **D** *X. pseudolanatus* (FP-150922, holotype) **E** *X. pseudolanatus* (HHB-6925, paratype) **F** *X. vesiculosus* (PDD-18112, isotype) **G** *X. lanatus* (CFMR HHB-8925, holotype). Scale bars: 1 mm.

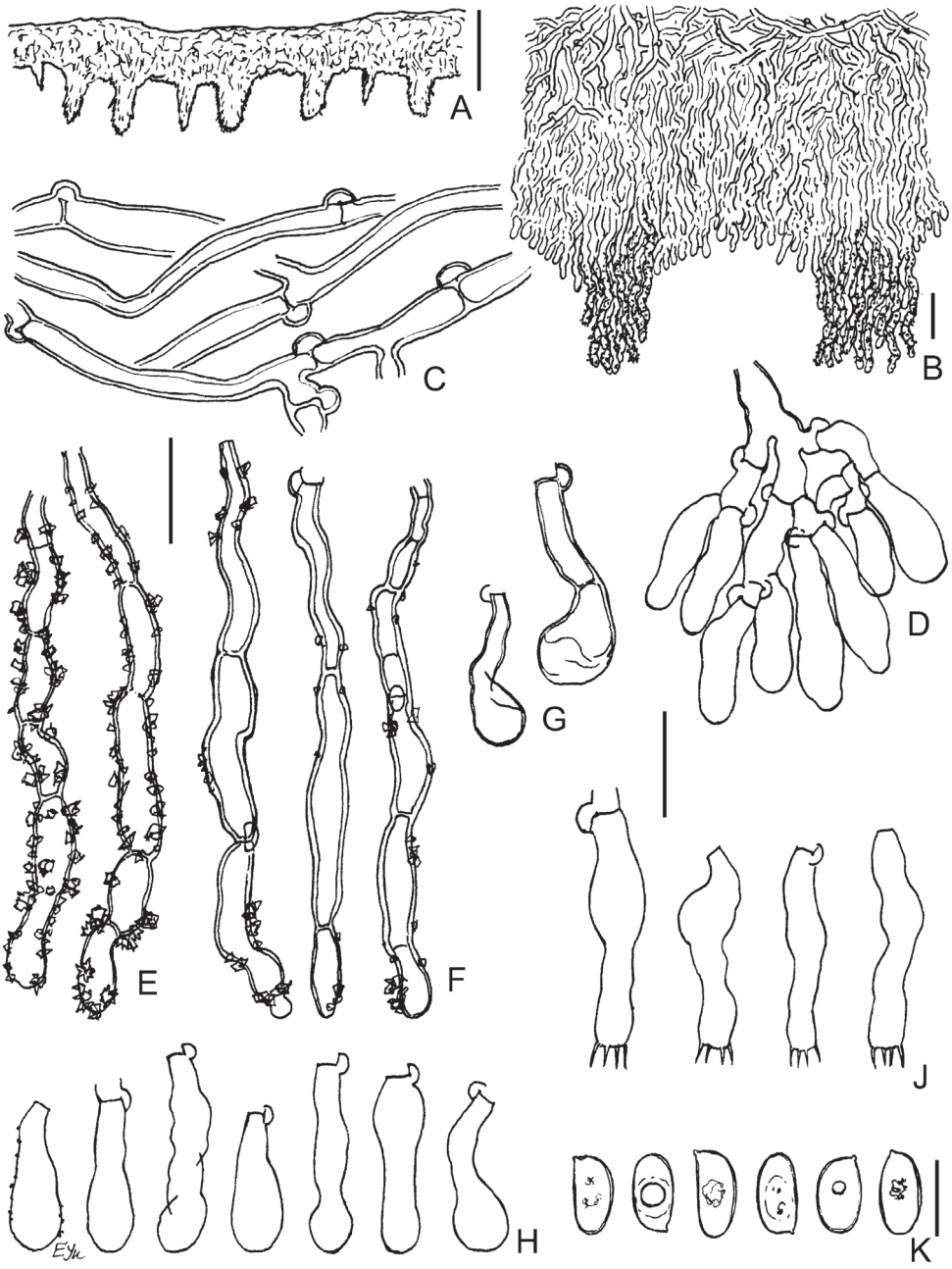

Figure 4. Micromorphology of *Xylodon exilis* (TUB-FO 42565, holotype): **A, B** vertical sections through basidioma **C** subicular hyphae **D** portion of hymenium and subhymenium **E** projecting aculeal hyphae in Mz **F** projecting aculeal hyphae in 3% KOH **G** capitate cystidia **H** basidioles **J** basidia **K** basidiospores. Scale bars: 100 μm (**A**); 20 μm (**B**); 10 μm (**C–J**); 5 μm (**K**).

Distribution and ecology. The species is known from Taiwan and Nepal. It grows on dead wood of angiosperms, with a preference for small branches and twigs.

Etymology. from Latin *exilis* – thin, fine, refers to the small and narrow aculei.

Additional specimens examined. TAIWAN, Nantou Co., west from Sun-Moon Lake, near Hua Lien, on dead wood, leg. E. Langer, G. Langer, F. Oberwinkler, 26 Mar 1989 (TUB-FO 40734; dupl. in KAS); south from Sun-Moon Lake, near Hua Lien, on fallen angiosperm twig, leg. E. Langer, G. Langer, F. Oberwinkler, 9 Jul 1990 (TUB-FO 42450; dupl. in KAS and MSK); Taichung Co., Shinshe, on fallen angiosperm twig, leg. E. Yurchenko, 2 Apr 2011 (MSK-F 12912); ibid., on fallen liana stem, leg. E. Yurchenko, 2 Apr 2011 (MSK-F 12913); ibid., on fallen angiosperm twig, leg. E. Yurchenko, 5 Jun 2011 (MSK-F 12914); Taipei Co., Wulai, Neidong Recreation Area, on fallen angiosperm twig, leg. E. Yurchenko, 23 Jun 2011 (MSK-F 7381; dupl. in KAS and LE); Miaoli Co., Sanyi, on fallen angiosperm branch, leg. E. Yurchenko, 3 Jul 2011 (MSK-F 7431); ibid., on fallen angiosperm branch, leg. E. Yurchenko, 19 Jul 2011 (MSK-F 7430). NEPAL: Gandaki Prov., Kuldi, Anapurna Trek, leg. L. Ryvarden, 7 Nov 1979 (O-LR 18918/B, dupl. in KAS).

Remarks. The species concept of *X. lanatus* is revised and restricted to specimens with a well-developed woolly subiculum. The distinctive characters of *X. exilis* are the minutely odontioid basidiomata with peg-like aculei composed of flexuous, encrusted, septate projecting hyphae that are constricted at the septa, embedded capitate cystidia and narrowly ellipsoid spores with slightly thickened walls. Earlier specimens of *X. exilis* from Taiwan (e.g. Langer 1994, p. 143 for illustration) and Nepal (Hjortstam and Ryvarden 1984) were originally identified as *X. lanatus*. Hyphae and spores of this species were also depicted by Yurchenko et al. (2013) under the name *X. lanatus*. These two species and other morphologically similar taxa are compared in the Discussion section below; a key is also presented.

Xylodon filicinus Yurchenko & Riebesehl, sp. nov.

MycoBank MB827463
Figs 3b, 5

Holotype. TAIWAN, Nantou Co., Xitou (Shitou) Forest Recreation Area, W slope of Phoenix Mt. Range, 1470 m a.s.l., 23°40'N, 120°48'E, old-growth sparse broadleaf forest, on dead detached rachis of *Cyathea* sp., leg. E. Yurchenko, 31 Jul 2011 (field No. 38; MSK-F 12869; isotype in KAS).

Description. Basidiomata effused, white, 2–4 cm in extent, farinaceous or pruinose, very loose or discontinuous, odontioid, 30–55 μm thick between aculei. Margin thinning out. Aculei conical or subcylindrical, 40–80 μm long, 15–45 μm diam., peg-like, of loose texture, 8–14/mm. Hyphal system monomitic, hyphae colourless, clamped at all septa. Subicular hyphae in a loose tissue, rarely branched, 2–3 μm diam., thin- or slightly thick-walled, loosely encrusted, under the subhymenium with inflations 5–6.5 μm wide. The largest crystals in subiculum 6–8 μm across, aggregated

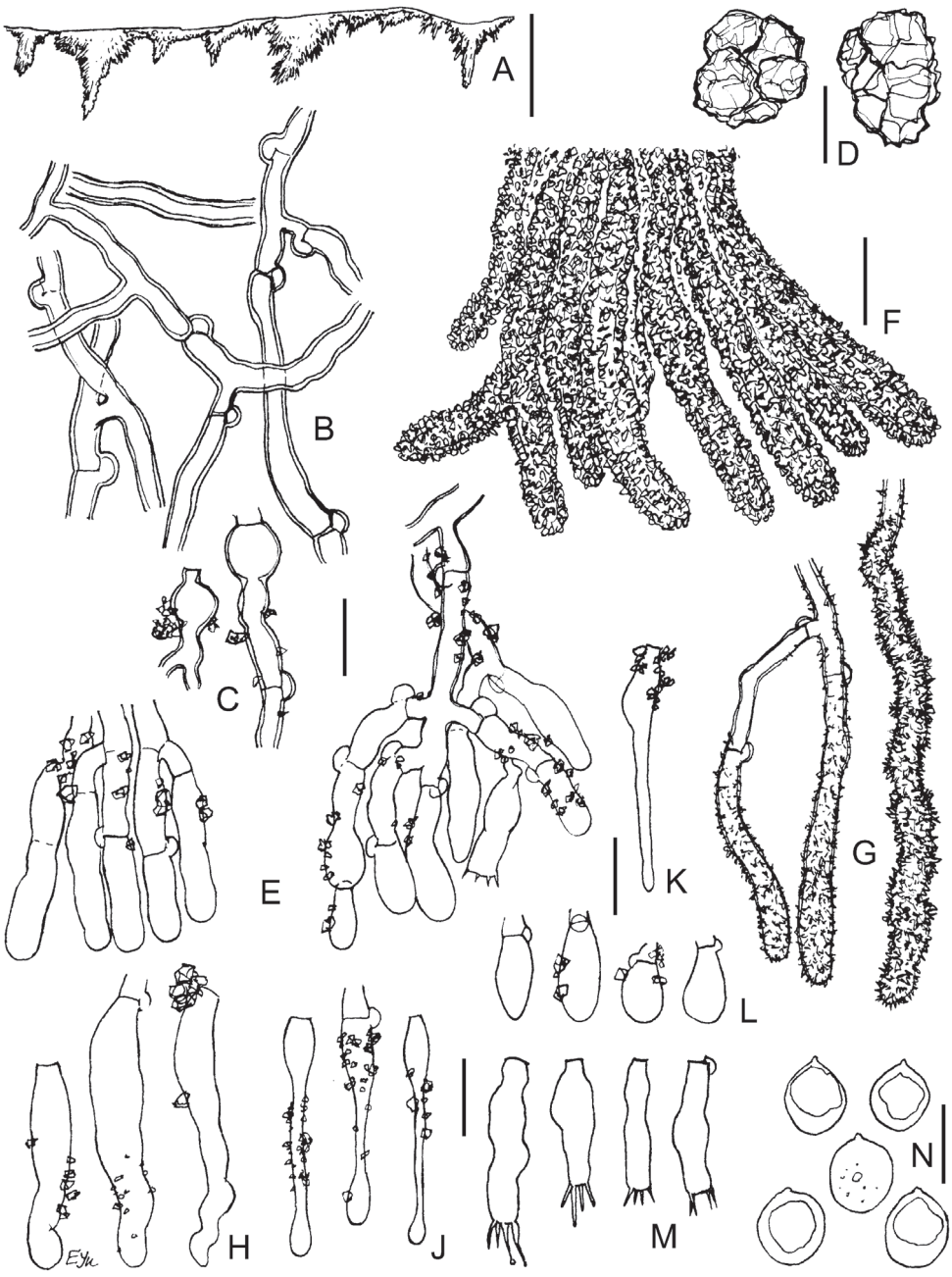

Figure 5. Micromorphology of *Xylodon filicinus* (MSK-F 12369, holotype): **A** vertical section through basidioma **B** subicular hyphae **C** inflations on hyphae in lower subhymenium **D** crystals from subiculum **E** portions of hymenium and subhymenium **F** bundle of encrusted projecting hyphae **G** separate projecting hyphae **H** subcylindrical cystidia **J** capitate cystidia **K** hyphoid cystidium **L** basidioles **M** basidia **N** basidiospores. Scale bars: 100 μm (**A**); 10 μm (**B–M**); 5 μm (**N**).

in clusters 15–18 µm diam. Subhymenial hyphae moderately branched, partly short-celled and slightly inflated, 2–3.5(–4) µm diam., lightly encrusted. Projecting hyphae in aculei richly encrusted, 20–45 × 5–7 µm in encrusted part, with clamped and simple septa, basally thick-walled, then becoming thin-walled, obtuse, sometimes subacute at apex. Cystidia in hymenium thin-walled, lightly encrusted, of three types: (1) subcylindrical, often slightly tapered to apex, numerous, 20–35 × 4.5–5.5 µm; (2) capitate, rare, 26–32 µm long, 3–5 µm wide at base, 2.5–3 µm wide at apex; (3) hyphoid to narrowly ventricose, about 30 × 4.5 µm. Basidioles ellipsoid, ovoid, clavate, 7.5–18 × 4.5–7.5 µm, more or less encrusted. Basidia utriform, (14–)16–20 × (3.5–)4.5–5.5 µm, thin-walled, smooth or sparsely encrusted, with four sterigmata 2–6.5 × 1–1.5 µm. Spores globose to subglobose, 4–5(–5.5) × (3.7–)4–4.5 µm, holotype L = 4.7 µm, W = 4.1 µm, Q = 1.1–1.2, thin-walled, often with one large oil-like globule, negative in Mz, weakly cyanophilous, with minute apiculus.

Distribution and ecology. From the lower mountainous belt in Taiwan, on dead fern rachises.

Etymology. from Latin *filix* − fern, refers to the occurrence on dead fern rachises.

Additional specimen examined. TAIWAN, the same locality and the same substrate as holotype, leg. E. Yurchenko, 31 Jul 2011 (field No. 18; MSK-F 12870; dup. in KAS).

Remarks. The distinctive features of this species are the pruinose, minutely odontioid basidiomata, fascicles of richly encrusted projecting hyphae in aculei and the three types of cystidia. *Xylodon filicinus* is morphologically similar to *X. hyphodontinus*, which differs in having projecting hyphae in the aculei that are straighter with more septa, a denser subhymenium composed of short-celled hyphae, short, ventricose cystidioles and spore walls that are slightly thickened at maturity (see Fig. 6).

Xylodon follis Riebesehl, Yurchenko & Langer, sp. nov.

MycoBank MB827464
Figs 3c, 7

Holotype. REUNION, Forêt Notre Dame de la Paix, Sentier botanique, 21°15.8'S, 55°36.1'E, 1720 m a.s.l., on angiosperm wood, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 12 Mar 2015 (FR-0249814; isotypes in KAS (as L1040) and MSK).

Description. Basidiomata effused, cream-coloured, about 1–5 cm in extent, soft-membranaceous, continuous, finely aculeate, between aculei 50–200 µm thick; aculei narrowly conical or nearly cylindrical, 80–170 × 20–40(–60) µm, 10–12/mm, fragile, slightly fimbriate at apices, sterile. Margin abrupt. Hyphal system monomitic; hyphae clamped and simple septate, colourless, (1–)2–3.5 µm diam. Subiculum little differentiated, composed of thin- to slightly thick-walled hyphae. Hyphae in aculeal trama mostly parallel, thin- to thick-walled (walls up to 1 µm thick), projecting through aculeal apices and loosely encrusted with crystals about 1–3 µm long in KOH. Subhymenium thickening; subhymenial hyphae moderately branched, thin-walled, smooth. Capitate cystidia numerous, projecting and immersed, in subhymenium, hymenium and aculei,

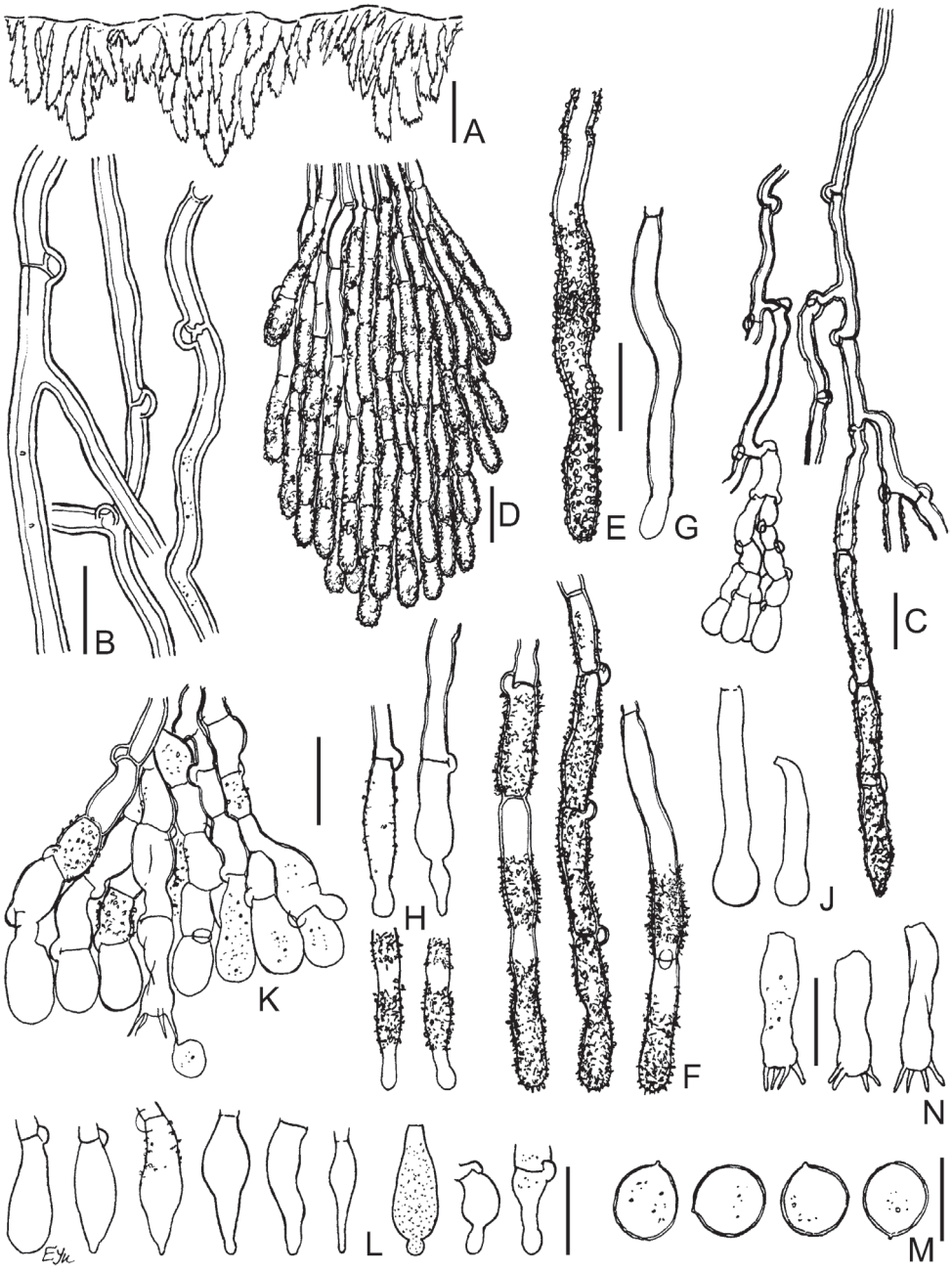

Figure 6. Micromorphology of *Xylodon hyphodontinus*. LIP GG-MAR 15-127: **A** vertical section through basidioma **B** subicular hyphae **C** excerpt of tramal hyphae to hymenium and projecting hyphae **D** bundle of encrusted aculeal hyphae **E** encrustation on projecting hypha in water **F** encrustation on projecting hyphae in 3% KOH **G** naked projecting hyphal end **H** variously shaped hyphal ends **J** capitate cystidia **K** portion of hymenium and subhymenium **L** basidioles and cystidioles **M** basidiospores. LIP GG-MAR 12-238: **N** basidia. Scale bars: 100 μm (**A**); 10 μm (**B–L, N**); 5 μm (**M**).

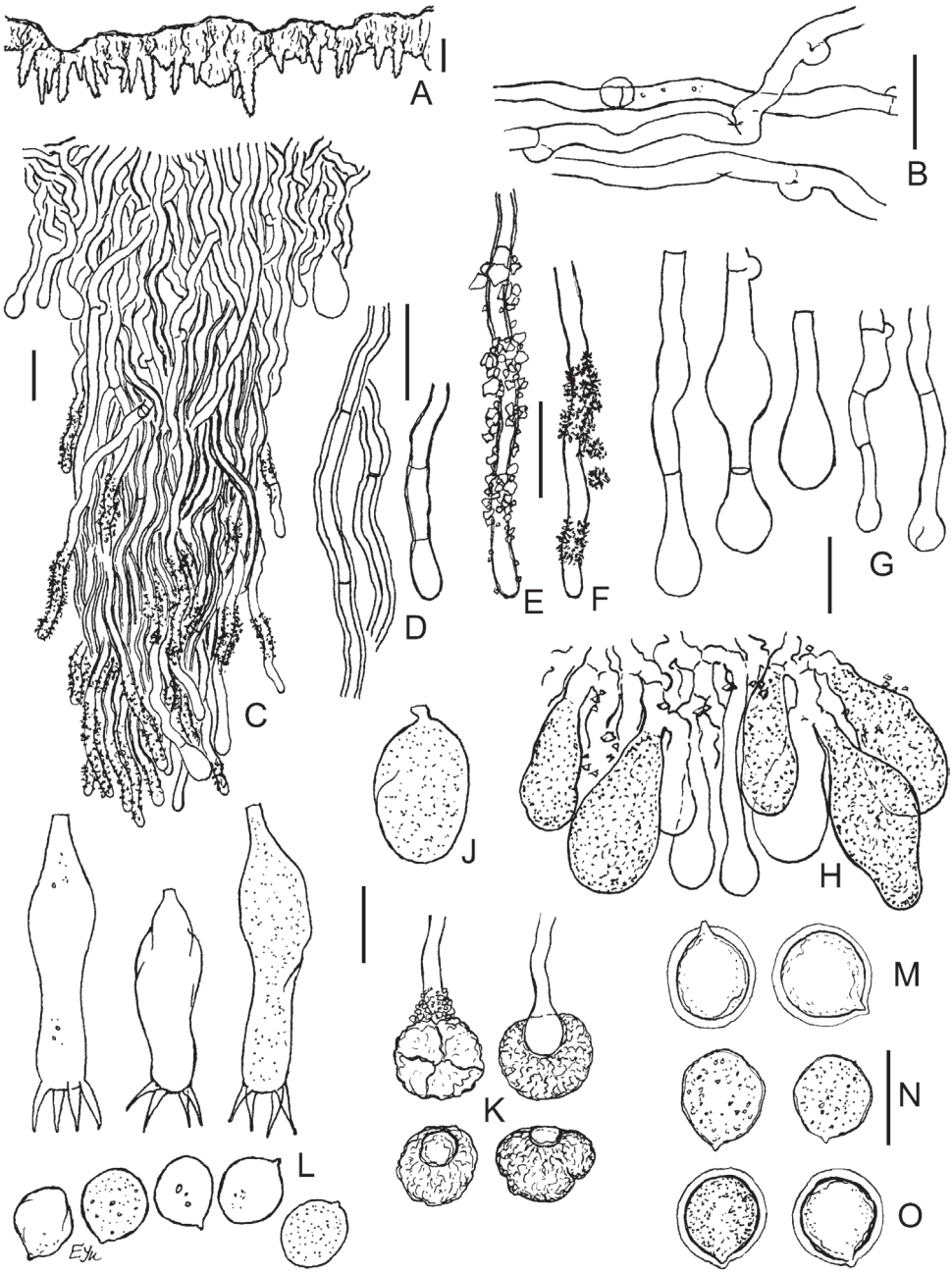

Figure 7. Micromorphology of *Xylodon follis* (FR-0249814, holotype): **A** vertical section through basidioma **B** subicular hyphae **C** vertical section through aculeus **D** lower and apical part of aculeal hyphae with adventitious septa **E** encrusted aculeal hyphae in water **F** partially dissolved crystals on aculeal hyphae in 3% KOH **G** capitate cystidia **H** portion of hymenium **J** vesicular basidiole **K** capitate encrusted cystidia and their detached resinous caps **L** basidia and basidiospores **M** basidiospores in 3% KOH **N** basidiospores in water **O** basidiospores in CBL. Scale bars: 100 μm (**A**); 10 μm (**B–N**).

17–30(–40) × 4.5–9 µm, with 1–2 adventitious septa in stalk, apical cap encased with resinous encrustation 6–12 µm wide, easily dissolving in KOH and Mz, unchanged in CBL. Hyphidial elements common in hymenium, 17–27 × 2.3–3.2 µm. Basidioles pyriform or ellipsoid, 17–28 × 8–12 µm, with granular contents, smooth or slightly encrusted. Basidia utriform or suburniform, thin-walled, smooth, 32–37 × 9–10 µm, with 4 sterigmata 4–6.5 × 1.3–2.3 µm. Spores globose to subglobose, colourless, with homogeneous or granular contents, smooth, inamyloid, indextrinoid, cyanophilous, thin-walled, (7.5–)8–9.5(–10) × 7–8.5 µm, holotype L = 8.6 µm, W = 7.6 µm, Q = 1.0–1.2, outer wall layer sometimes swelling in KOH and CBL, with rounded-triangular apiculus.

Distribution and ecology. The species is so far known from Réunion (Mascarene Archipelago) and inhabits dead wood.

Etymology. from Latin *follis* − bag or bubble, referring to shape of the spores, basidioles and capitate cystidia found in this species.

Additional specimen examined. REUNION, Forêt de Bébour, 1328 m asl., leg. E. Langer, G. Langer, E. Hennen, 20 Mar 1998 (KAS-GEL 4951; dupl. in MSK).

Remarks. This taxon differs from other *Xylodon* species by its unusually large basidia, large globose basidiospores with walls that swell in KOH and CBL and numerous simple septa as well as clamps on the hyphae. The swelling of spore walls was observed in some spores; spores were unaffected in water mounts. The hymenium has a granular appearance visible under 100× magnification because of the resinous cap developed on the capitate cystidia. The resinous caps are observed only in CBL and are easily detaching in squash preparations. Intermediate forms in morphology of hyphidia to capitate cystidia and of capitate cystidia to pyriform basidioles were frequently observed.

Xylodon pseudolanatus **Nakasone, Yurchenko & Riebesehl, sp. nov.**
MycoBank MB827465
Figs 3d, 8

Holotype. BELIZE: Cayo District, Mountain Pine Ridge, on corticated hardwood branch, leg. K.K. Nakasone, 24 Nov 2001 (CFMR FP-150922; isotypes in KAS and MSK; ex-type culture CFMR FP-150922-sp; ex-type ITS sequence MH880220; ex-type 28S sequence MH884909).

Description. Basidiomata effused, membranaceous, cream-coloured, 1–6 cm in extent, odontioid with conical aculei 50–120 µm long and 25–65 µm diam. at base, 8–14 aculei/mm. Subiculum between aculei very loose, minutely porulose, 100–150 µm thick. Margin pale cream-coloured, abrupt or diffuse, up to 2 µm wide. Hyphal system monomitic, hyphae clamped at all primary septa, colourless. Subicular hyphae little branched, mostly thick-walled, 2.5–4 µm diam., smooth or scarcely encrusted. Subhymenial hyphae richly branched, thin-walled, 2–3.5(–4.5) µm diam., smooth or slightly encrusted. Aculei consisting mostly of projecting hyphae. Projecting hyphae moderately flexuous, (3–)3.5–5 µm diam., slightly thick-walled, loosely encrusted, clamped at septa. Capitate cystidial elements found mostly in subhymenium and subiculum,

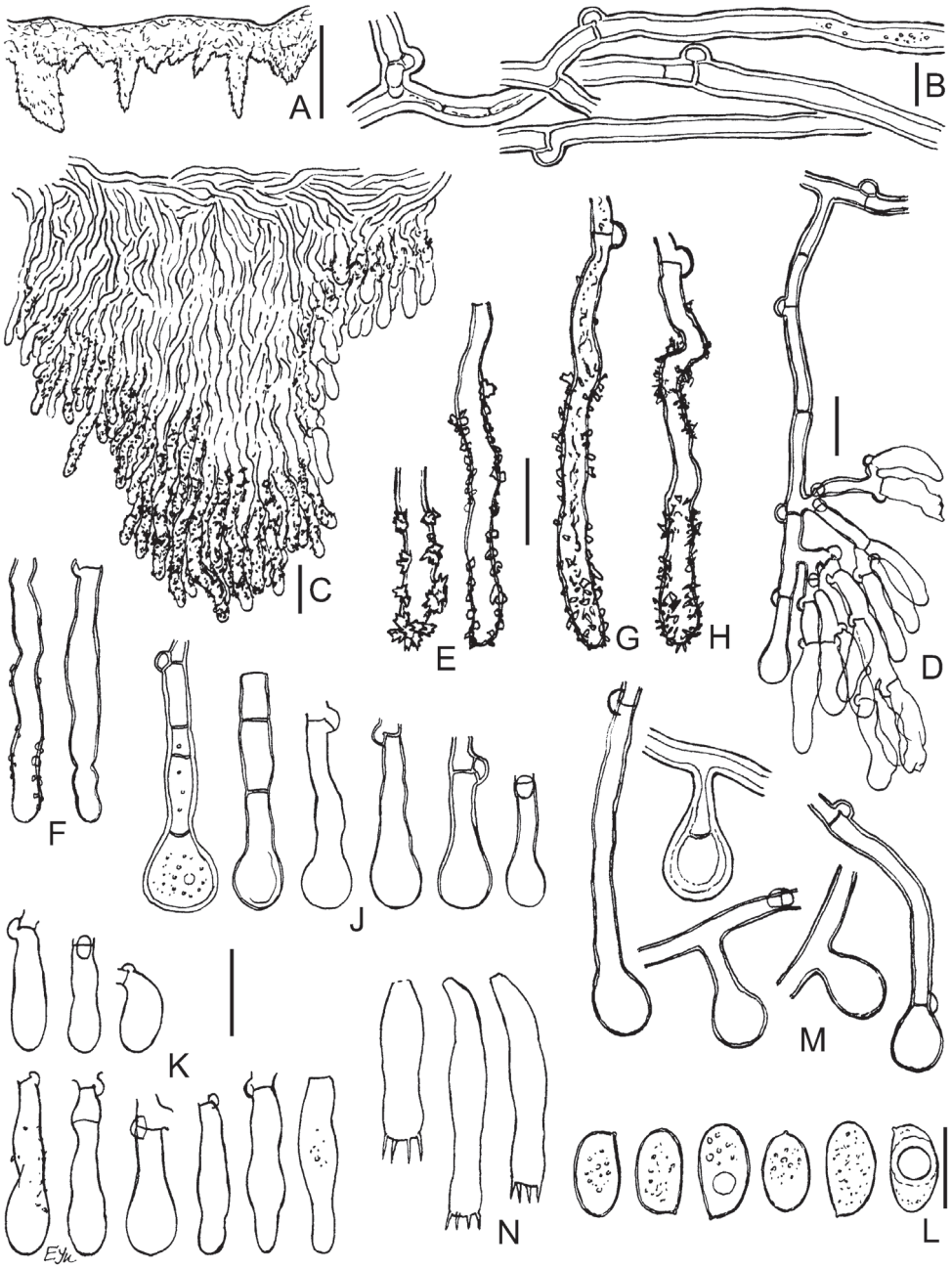

Figure 8. Micromorphology of *Xylodon pseudolanatus*. CFMR: FP-150922 (holotype): **A** vertical section through basidioma **B** subicular hyphae **C** vertical section through aculeus **D** detail of subicular hyphae and hymenium **E, F** projecting aculeal hyphae in 3% KOH **G** projecting aculeal hyphae in water **H** projecting aculeal hyphae in Mz **J** capitate cystidia in hymenium **K** basidioles **L** basidiospores. CFMR: HHB-6925: **M** capitate cystidia in subiculum **N** basidia. Scale bars: 100 μm (**A**); 10 μm (**C–K, M, N**); 5 μm (**B, L**).

scattered to frequent, terminal or lateral, smooth, thin- to thick-walled, aseptate or with adventitious septa, (8–)15–30 × (4.5–)5–6.5(–8.5) µm. Basidioles clavate to subcylindrical, sometimes slightly tapering to apex, 7–22 × 4–5 µm. Basidia cylindrical, sometimes slightly constricted, 16–30 × 4–4.3 µm, thin-walled, smooth, with four sterigmata about 2.5 × 0.2 µm. Spores narrowly ellipsoid to oblong, 5–6(–6.3) × (2.5–)3–3.5 µm, holotype L = 5.5 µm, W = 3.2 µm, Q = 1.7, thin- or slightly thick-walled, colourless, smooth, with minute apiculus, inamyloid, indextrinoid, weakly cyanophilous.

Distribution and ecology. South-eastern USA and Central America, on dead wood of angiosperms.

Etymology. From Greek *pseudo-* – false, refers to its similarity to *X. lanatus*.

Additional specimens examined. USA: Alabama, Escambia County, 3 miles east of Flomaton, on bark of *Taxodium distichum* (L.) Rich. (CFMR FP-103492), on bark of *Quercus* sp. (CFMR FP-103500), leg. A.S. Rhoades, 1 Nov 1952; Florida, Marion County, Okalawaha River, on dead inflorescence of *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult. f., leg. H.H. Burdsall, Jr., 3 Aug 1972 (CFMR HHB-6925; dupl. in KAS and MSK); Louisiana, Baton Rouge, on *Melia azedarach* L., leg. C.J. Humphrey & C.W. Edgerton, 29 Aug 1909 (CFMR FP-5519).

Remarks. The diagnostic features of this species are the minutely odontioid hymenophore, bundles of sparsely to moderately encrusted hyphae, projecting from aculeal apices, embedded capitate cystidia, cylindrical basidia and narrowly ellipsoid basidiospores. Some hymenial elements in this species are intermediate in morphology between basidioles, capitate cystidia and hyphal ends. *Xylodon pseudolanatus* can be distinguished from similar species in the key below (see Discussion).

Xylodon hyphodontinus **(Hjortstam & Ryvarden) Riebesehl, Yurchenko & G.Gruhn, comb. nov.**
MycoBank MB827758
Fig. 6

Odontiopsis hyphodontina Hjortstam & Ryvarden, Mycotaxon 12(1): 180 (1980) (Basionym). Typus of *O. hyphodontina*: TANZANIA, Morogoro Prov., Morogoro distr., Uluguri Mts., Morning Side Res. sta. ca. 5 km S of Morogoro, substrate unknown, leg. L. Ryvarden, 24–26 Feb 1973 (O L. Ryvarden 10949 – holotype).
= *Hydnum ambiguum* Berk. & Broome, Journal of the Linnean Society, Botany 14(73): 60 (1873). Typus of *H. ambiguum*: SRI LANKA, Central Province, on dead wood (Berkeley No. 974 – holotype).
= *Odontiopsis ambigua* (Berk. & Broome) Hjortstam, Mycotaxon 28(1): 35 (1987).
= *Pteridomyces sphaericosporus* Boidin, Lanq. & Gilles, Mycotaxon 16(2): 490 (1983).

Remarks. This new combination is based on the phylogenetic analyses of the ITS and 28S sequences as well as morphological study of specimens, including the holotype of *O. hyphodontina*. Originally, the collections from Martinique and French Guyana were

identified as *O. ambigua*, but the molecular data clearly show that these collections are embedded in *Xylodon* (Figs 1, 2). Although *H. ambiguum* is the oldest name for this taxon, it cannot be transferred to *Xylodon* because the name is preoccupied by *X. ambiguus* (Peck) Kuntze (= *Veluticeps ambigua* (Peck) Hjortstam & Telleria). *Odontiopsis ambigua*, *P. sphaericosporus* and *O. hyphodontina* were recognised as conspecific by Hjortstam (1987, 1991). *Odontiopsis hyphodontina* is the next oldest name and is chosen to represent this taxon. As *O. hyphodontina* is also the type of *Odontiopsis* Hjortstam & Ryvarden, *Odontiopsis* concomitantly becomes a synonym of *Xylodon*.

The newly generated ITS and 28S sequences of *X. hyphodontinus* hold comparable positions in a clade that includes three distinct lineages in both phylogenetic trees (Figs 1, 2). Specimens KAS-GEL9222 from Kenya and LIP GG-GUY13-044 from French Guyana each represent distinct lineages from the third lineage of LIP GG-MAR15-127 and LIP GG-MAR12-238 from Martinique. As species in *Hyphodontia* s.l. can be readily distinguished with ITS or 28S sequences, these three lineages should result in the recognition of three different species. However, we were not able to identify any definite morphological differences amongst the lineages in comparison with the holotype material from Tanzania. Cultures are not available for these specimens, thus intercompatibility tests are not possible. As a result, we decided to treat all three lineages as *X. hyphodontinus* at this time.

Xylodon vesiculosus **Yurchenko, Nakasone & Riebesehl, nom. nov.**
MycoBank MB827759
Figs 3e, 9

Replaced synonym. *Odontia vesiculosa* G. Cunn., Transactions and Proceedings of the Royal Society of New Zealand 86(1): 75 (1959) nom. inval.

Typus. NEW ZEALAND: Otago, Alton Valley, Tuatapere, leg. J.M. Dingley, Feb 1954 (PDD-18112 – holotype).

Cunningham (1959) described this new taxon as *Odontia vesiculosa* G. Cunn. Earlier, *Odontia vesiculosa* Burt was used for another species (Povah 1929). Consequently, *Odontia vesiculosa* G. Cunn. is an illegitimate name and a new name is required for this taxon (see Art. 6.11, 7.4 and 58.1 in Turland et al. 2018).

Below is a description based on the isotype of *X. vesiculosus* (CFMR).

Description. Basidiomata effused, odontioid, membranaceous, with a densely odontioid, ochraceous hymenial surface. Margin mostly abrupt, some parts thinning out. Hymenophoral aculei cylindrical to conical, acute apically, 130–350 µm long, 60–150 µm diam. at base, 4 per mm. Subiculum 100–150 µm thick, minutely cracking. Hyphal system monomitic; hyphae clamped at all primary septa. Subicular and tramal hyphae thick-walled (wall up to 1.5 µm), 2.5–4 µm wide, often with narrow lumen, smooth, colourless, looking faint yellowish in mass due to refractive walls. Subhymenium well developed; hyphae richly branched, thin- to slightly thick-walled, yellowish in mass. Aculei bearing skeletal-like, naked or poorly encrusted, immersed hyphal ends

and variously encrusted, thick-walled, projecting hyphae in bunches, 3.5–5 µm wide. Capitate elements common, as lateral branches on tramal or subhymenial hyphae, (25–)30–55 × 6.5–10.5 µm, thin- to thick-walled, aseptate or with 1–2 adventitious septa. Basidioles clavate, subcylindrical, utriform. Basidia utriform to subcylindrical and clavate, 15–22 × 4–5 µm, thin-walled, smooth, with four sterigmata ca. 2 × 0.5 µm. Spores ellipsoid to narrowly ellipsoid or short cylindric, 5.3–6.3(–7) × 3–4 µm, holotype L = 5.9, W = 3.4, Q = 1.8 (n = 22), with adaxial side flat to convex, smooth, thin-walled, colourless, with minute apiculus, inamyloid, indextrinoid, acyanophilous.

Remarks. This species was considered conspecific with *Xylodon lanatus* from North America (Burdsall and Nakasone 1981, Wu 1990, Gorjón and Greslebin 2012), but we observed significant morphological differences. For example, in *X. vesiculosus*, the basidiomata have a denser, tough-membranaceous texture compared to the soft woolly basidiomata of *X. lanatus*. In addition, the aculei in *X. vesiculosus* are larger and the basidia are thin-walled in contrast to the smaller aculei and basally thick-walled basidia found in *X. lanatus* (compare Figs 9, 10). See Discussion for a key to *X. lanatus*, *X. vesiculosus* and allied taxa.

Xylodon niemelaei **(Sheng H.Wu) Hjortstam & Ryvarden, Synopsis Fungorum 26: 28 (2009)**

≡ *Hyphodontia niemelaei* Sheng H. Wu, Acta Botanica Fennica 142:98 (1990).

Xylodon rhizomorphus **(C.L.Zhao, B.K.Cui & Y.C.Dai) Riebesehl, Yurchenko & Langer, Mycological Progress 16(6): 649 (2017).**

≡ *Hyphodontia rhizomorpha* C.L.Zhao, B.K.Cui & Y.C.Dai, Cryptogamie, Mycologie 35(1):92 (2014).

Xylodon reticulatus **(C.C.Chen & Sheng H.Wu) C.C.Chen & Sheng H.Wu, Mycoscience 59(5): 349 (2018).**

≡ *Hyphodontia reticulata* C.C.Chen & Sheng H.Wu, Mycological Progress 16(5): 558 (2017).

Remarks. Molecular and morphological analyses demonstrate that the three taxa listed above are very similar. The 11 samples of *X. niemelaei*, 3 of *X. rhizomorphus* and 3 of *X. reticulatus* formed a strongly supported clade (99 BS, 1 PP) in the ITS phylogram (Fig. 1). In addition, three samples representing two of the species are found in a strongly supported clade (100 BS, 1 PP) in the 28S tree (Fig. 2), differing in only one position in the associated alignment.

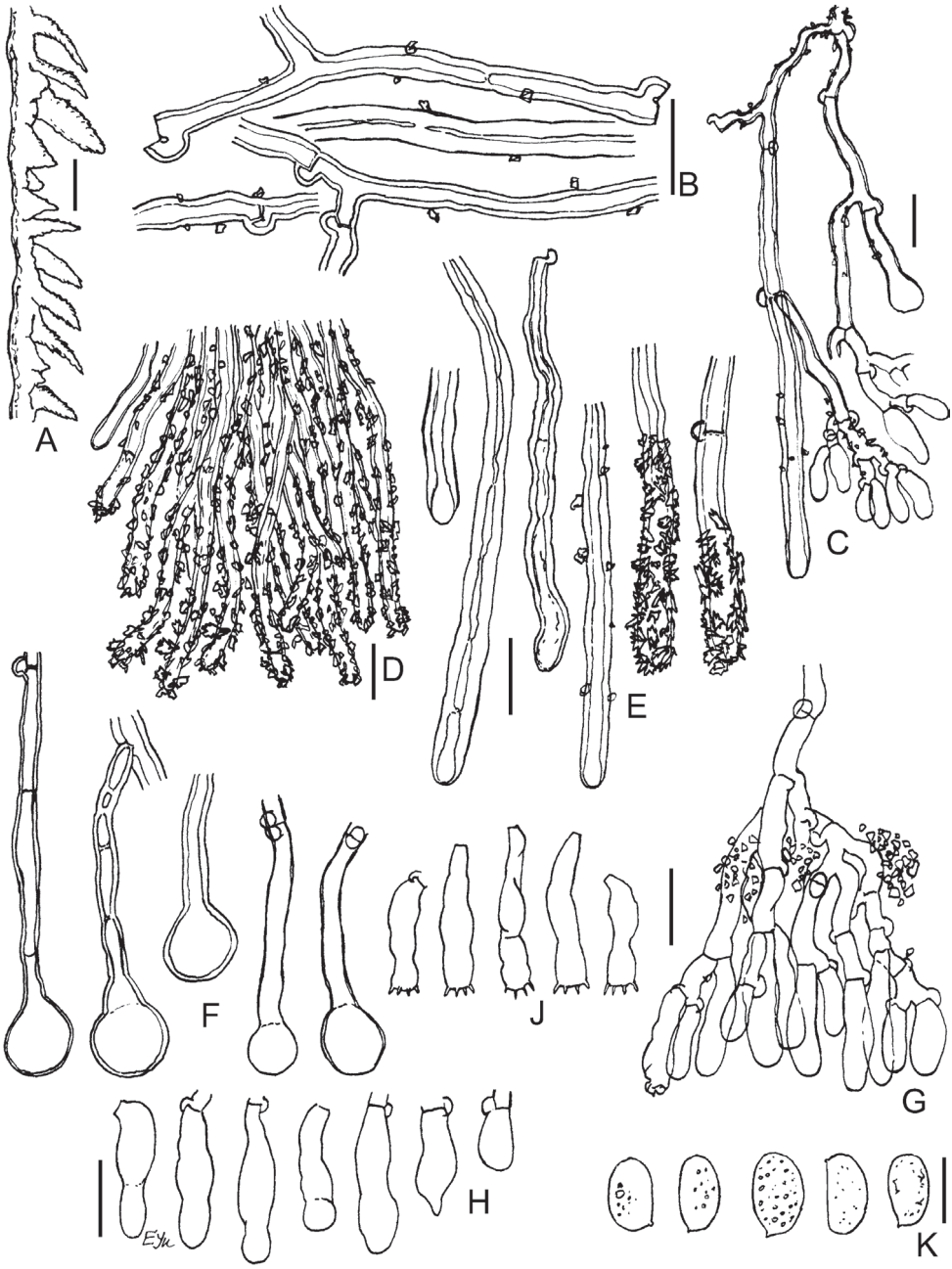

Figure 9. Micromorphology of *Xylodon vesiculosus* (PDD-18112, isotype): **A** vertical section through basidioma **B** subicular hyphae **C** excerpt of tramal hyphae to hymenium and skeletoid hyphae **D** bundle of projecting aculeal hyphae **E** smooth and variously encrusted aculeal hyphae **F** capitate cystidia **G** portion of hymenium and subhymenium **H** basidioles **J** basidia **K** basidiospores. Scale bars: 250 μm (**A**); 10 μm (**B–J**); 5 μm (**K**).

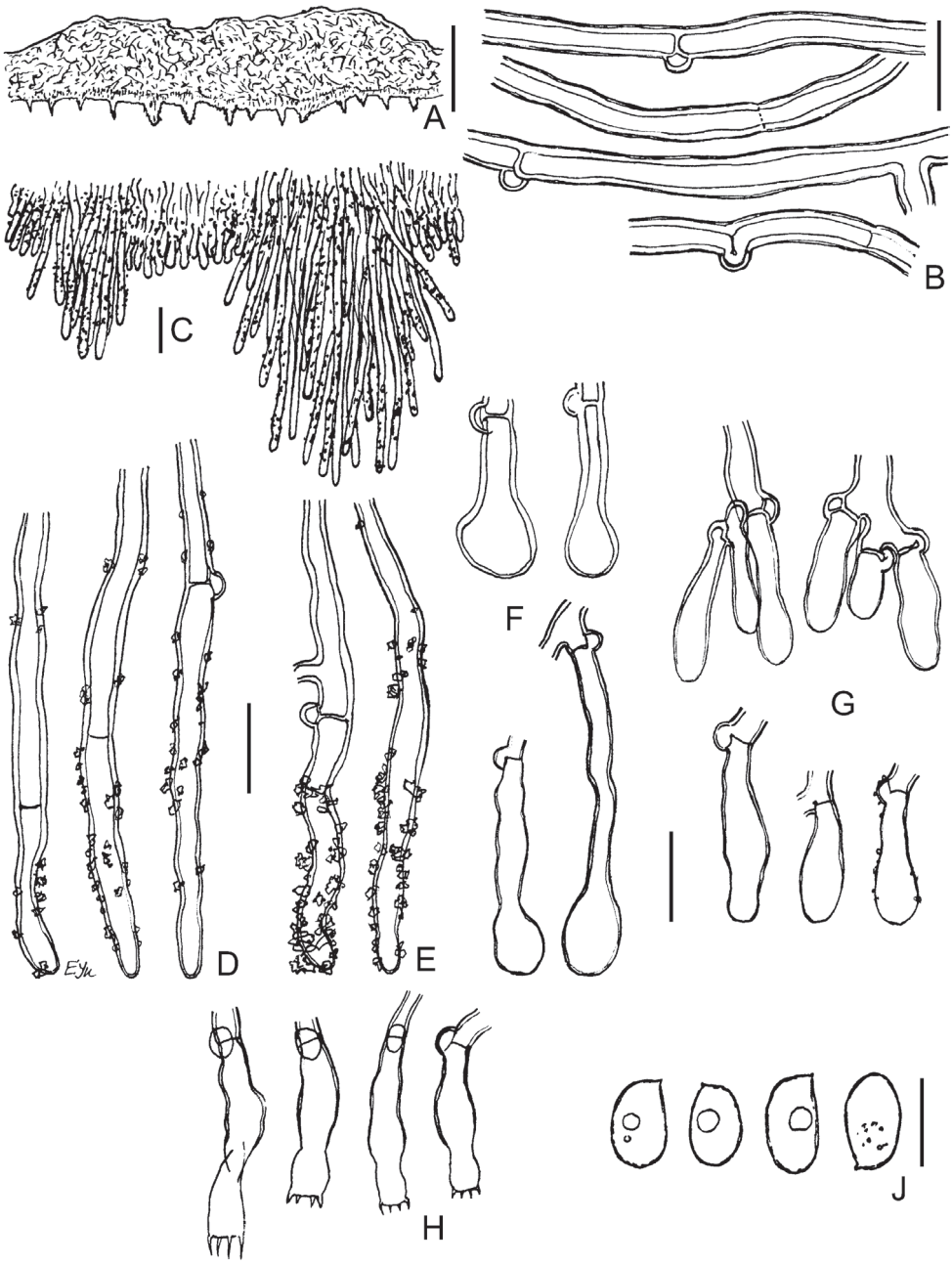

Figure 10. Micromorphology of *Xylodon lanatus* (CFMR: HHB-8925, holotype): **A** vertical section through basidiomata **B** subicular hyphae **C** vertical section through aculei and hymenium **D** projecting hyphae in 3% KOH **E** projecting hyphae in Mz **F** capitate cystidia **G** basidioles **H** basidia **J** basidiospores. Scale bars: 500 μm (**A**); 20 μm (**C**); 10 μm (**B, D–H**); 5 μm (**J**).

Xylodon niemelaei was described and illustrated in detail by Wu (1990) and Langer (1994). It is characterised by a poroid hymenophore, embedded and hymenial capitate cystidia, small, subulate or fusoid hymenial cystidia and encrusted hyphal ends mainly developed at the pore edges but sometimes also in other areas. At the morphological level, the bladder-like embedded cystidia and hyphal encrustations appear identical in *X. niemelaei* (Langer 1994), *X. rhizomorphus* (Zhao et al. 2014) and *X. reticulatus* (Chen et al. 2017). Spore size and spore quotient overlap in these three species. *Xylodon rhizomorphus* occurs in south-western China, whereas *X. reticulatus* occurs in Taiwan and Japan. *Xylodon niemelaei* is reported also from these three countries and furthermore from Réunion, Africa and South America (Langer 1994), but the last two reports require morphological and molecular confirmation.

Specimens examined. *Xylodon niemelaei* – REUNION: Forêt Mare-Longue, on dead stump of angiosperm wood, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 12 Mar 2015 (FR-0249846, dupl. as L1087 in KAS); on lumber, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 12 Mar 2015 (FR-0249174, dupl. as L1077 in KAS); on brown-rotten wood, leg. E. Langer, G. Langer, E. Hennen, 21 Mar 1998 (KAS-GEL 4998); Forêt Notre-Dame de la Paix, on dead wood of *Monimia rotundifolia* Thouars, leg. E. Langer, 11 Mar 2013 (FR-0219860, dupl. as L0002 in KAS); on dead angiosperm wood, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 10 Mar 2015 (FR-0249811, dupl. as L1031 in KAS); on white-rotten wood, leg. E. Langer, G. Langer, E. Hennen, 19 Mar 1998 (KAS-GEL 4904); Le Petit Tampon, on dead wood, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 9 Mar 2015 (FR-0249225, dupl. as L1007 in KAS); Piton Mont Vert, on dead wood, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 18 Mar 2015 (FR-0249178, dupl. as L1172 in KAS); Plaine des Fougères, on dead wood, leg. E. Langer, 12 Sep 2013 (FR-0249744, dupl. as L0698 in KAS); Sentier de Takamaka, on white-rotten wood, leg. J. Riebesehl, M. Schröder, M.M. Striegel, 26 Mar 2015 (FR-0249289, dupl. as L1269 in KAS).

Xylodon spathulatus (Schrad.) Kuntze, Revisio generum plantarum (Leipzig) 3(2):541 (1898)

≡ *Hydnum spathulatum* Schrad., Spicilegium Florae Germanicae: 178, t. 4:3 (1794).
= *Hyphodontia bubalina* Min Wang, Yuan Y.Chen & B.K.Cui, Phytotaxa 309(1):50 (2017).
≡ *Xylodon bubalinus* (Min Wang, Yuan Y.Chen & B.K.Cui) C.C.Chen & Sheng H.Wu, Mycoscience 59:349 (2018).
= *Hyphodontia chinensis* C.C.Chen & Sheng H.Wu, Mycological Progress 16(5): 554 (2017).
≡ *Xylodon chinensis* (C.C.Chen & Sheng H.Wu) C.C.Chen & Sheng H.Wu, Mycoscience 59: 349 (2018).

Remarks. Based on both molecular data and morphology, we place the taxa *X. bubalinus* and *X. chinensis* in synonymy under *X. spathulatus*. In our phylogenetic analysis of ITS sequence data, the recently described *X. bubalinus* (4 collections) and *X. chinensis* (2 collections) from China form a well-supported clade with *X. spathulatus* (4 collections)

from Europe (97 BS, 1 PP) that is sister to *X. apacheriensis* (Fig. 1). Within this clade are several subclades, with very low bootstrap support (<55), thus subspecies or varieties cannot be identified. The 28S rRNA gene analysis also supports conspecificity between *X. chinensis* and *X. spathulatus* (99 BS, 0.65 PP) (Fig. 2). *Xylodon spathulatus* has three main diagnostic features: prominent (1–2 mm tall) aculei of varied shape, numerous apically acute cystidia with 1–4 slight constrictions and capitate cystidia with a resinous cap. It is described and illustrated by Eriksson and Ryvarden (1976) and Langer (1994). Minor morphological variation amongst the three taxa was observed. For example, *X. chinensis* has ventricose cystidia, similar to those in *X. spathulatus*, but they are sometimes septate at the constrictions. Distinctly ventricose cystidia were not observed in *X. bubalinus*, which instead had hyphoid or subulate cystidioles (Wang and Chen 2017, Fig. 2f). Encrusted hyphal ends at apices of the aculei in *X. bubalinus* and *X. chinensis* are typical of those in *X. spathulatus*. Resinous caps enclosing capitate elements are often absent as in the case of *X. bubalinus*, *X. chinensis*, *X. spathulatus* KAS-GEL2690 (from Germany) and *X. spathulatus* MSK-F 12931 (from Russia). Spore shape and size are similar amongst the three taxa and the spore quotient 1.3–1.4(–1.5) overlaps (Eriksson and Ryvarden 1976, Wang and Chen 2017, Chen et al. 2017). A few spores in *X. chinensis* were up to 6 × 5 µm and may be due to better climatic conditions. The description of *X. spathulatus* is modified to include variable aculei from conical and subulate to distinctly spathuliform and the variable presence of cystidia with resinous caps, mucronate apices and a submoniliform type that are aseptate with more or less blunt apices. Thus *X. spathulatus* is a highly variable but distinctive species that is widely distributed from northern Europe (Eriksson and Ryvarden 1976) to southern China (Chen et al. 2017) and has a preference for old-growth forests (Dvořák et al. 2017). Reports of *X. spathulatus* from North and South America (Ginns and Lefebvre 1993, Hjortstam and Ryvarden 2007b) should be confirmed by molecular sequence data.

Specimens examined. *Xylodon spathulatus* – CZECH REPUBLIC: Zofinsky National Park, on dead deciduous wood, leg. M.M. Striegel, 16 Sep 2015 (KAS-MMS 7224); GERMANY: Baden-Wurttemberg, Bad Waldsee, on dead wood of *Picea abies* (L.) H.Karst., leg. E. Langer, G. Langer, 15 Oct 1992 (KAS-GEL 2690); SWEDEN: Gästrikland, Island Torrö, on dead wood of *Betula* sp., leg. K.H. Larsson, 29 Sep 1988 (GB KHL 7085, dupl. in KAS); RUSSIA: Udmurtia, near Izhevsk town, on *Sorbus aucuparia* L., leg. V.I. Kapitonov, 7 Aug 2012 (MSK-F 12931).

Xylodon cystidiatus (A.David & Rajchenb.) Riebesehl & Langer, Mycological Progress 16(6):645 (2017)

≡ *Schizopora cystidiata* A. David & Rajchenb., Mycotaxon 45:140 (1992).

Remarks. We undertook a thorough morphological analysis of the specimen FR-0249200 (Réunion, Plaine des Fougères, on fallen angiosperm twig, leg. E. Langer, 12 Sep 2013), because it provided the first sequences of *X. cystidiatus*. We are confident that FR-0249200 is *X. cystidiatus*, although we detected minor differences from the

Figure 11. Basidioma of *Xylodon cystidiatus* (FR-0249200). Scale bar: 1 cm.

descriptions in David and Rajchenberg (1992) and Langer (1994). Some of the differences we noticed include: (1) the encrusted cystidia in FR-0249200 are mostly thin-walled with finer crystals; (2) the spores in our specimen were slightly broader 5–6 × 3.5–4.3 µm (L = 5.4 µm, W = 3.9 µm, Q = 1.4) than in published records 5–6 × 3–4 µm. Photographs of the basidioma (Fig. 11) and drawings of the microscopic features (Fig. 12) of FR-0249200 are provided for future identifications.

Discussion

We recognise 77 species of *Xylodon* based on studies by Riebesehl and Langer (2017), Chen et al. (2017, 2018), Kan et al. (2017), Wang and Chen (2017) and results herein (Table 1). Our phylogenetic analyses included 122 ITS and 28S sequences representing 37 *Xylodon* species. The other 40 accepted species in *Xylodon* are based on morphological studies.

In the following discussion, we highlight some of the significant results.

Odontiopsis is a synonym of *Xylodon*

The monotypic *Odontiopsis* Hjortstam & Ryvarden was described in 1980 based on *O. hyphodontina* from Tanzania. Hjortstam (1987) transferred *Hydnum ambiguum* to *Odontiopsis* and placed *O. hyphodontina* in synonymy. Later, *Pteridomyces sphaericosporus* was placed in synonymy with *O. ambigua* by Hjortstam (1991). Analyses of ITS and 28S sequences placed specimens originally identified as *O. ambigua* in the *Xylodon* lineage. Due to nomenclature rules to choose the earliest possible epithet to represent a taxon (see Art. 11.4 in Turland et al. 2018), the name for this taxon is *Xylodon hyphodontinus* and *Odontiopsis* is reduced to a synonym of *Xylodon*.

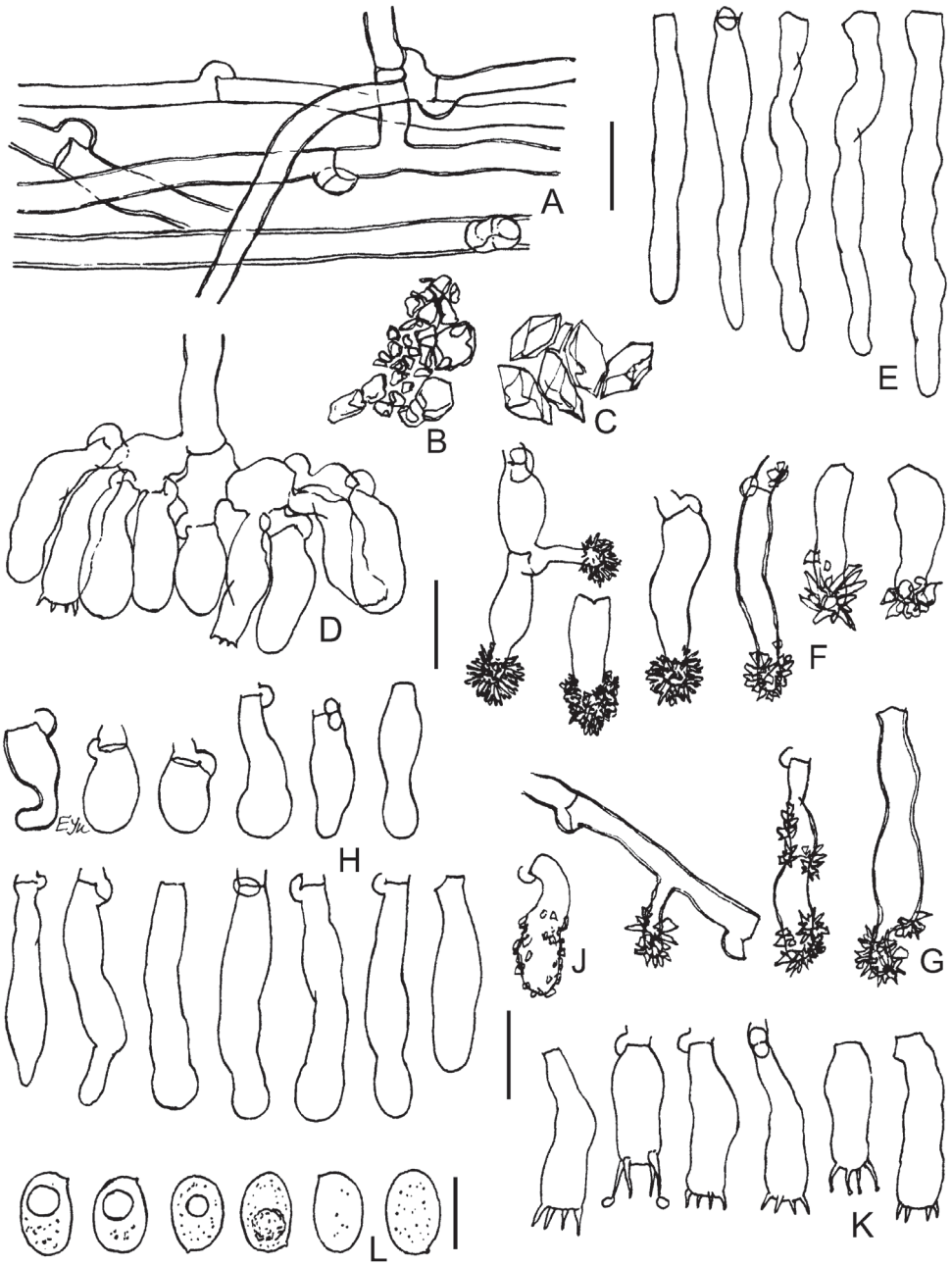

Figure 12. Micromorphology of *Xylodon cystidiatus* (FR-0249200): **A** subicular hyphae **B** crystals from dissepiment in 3% KOH **C** crystals from dissepiment in Mz **D** portion of hymenium and subhymenium **E** hyphal endings from dissepiment edges **F** encrusted cystidia in 3% KOH **G** encrusted cystidia in Mz **H** smooth basidioles and cystidioles **J** encrusted basidiole (in Mz) **K** basidia **L** basidiospores. Scale bars: 10 μm (**A–K**); 5 μm (**L**).

Palifer is a synonym of *Xylodon*

Species in *Palifer* have apically encrusted cystidia that are characteristic of the genus and distinctly different from the lagenocystidia of *Hyphodontia* s.s. (Hjortstam and Ryvarden 2009, Riebesehl and Langer 2017). *Palifer* is defined primarily by morphology because there is only a single ITS sequence available. Phylogenetic studies place *P. verecundus* amongst the *Xylodon* species (Larsson et al. 2006, Fig. 1). The recently described *X. mollissimus* has cystidia that are similar to those of *Palifer* species and ITS sequence analyses place it in a clade with *Xylodon* sp. 1 (Kan et al. 2017, Fig. 1). Although not closely related, *X. mollissimus* and *P. verecundus* are embedded within *Xylodon* and demonstrate that the distinctive cystidia developed in *Palifer* is not a phylogenetically significant character. Thus, we reduce *Palifer* to a synonym of *Xylodon* and propose the following transfers:

Xylodon erikssonii **(M.Galán & J.E.Wright) Riebesehl & Langer, comb. nov.**
MycoBank MB827760

Grandinia erikssonii M.Galán & J.E.Wright, in Galán, Lopez & Wright, Darwiniana 32(1–4):251 (1993) (Basionym).
≡ *Hyphodontia erikssonii* (M.Galán & J.E.Wright) Hjortstam & Ryvarden, Synopsis Fungorum 20: 63 (2005).
≡ *Palifer erikssonii* (M.Galán & J.E.Wright) Riebesehl, Yurchenko & Langer, in Riebesehl & Langer, Mycological Progress 16(6): 646 (2017).

Typus. ARGENTINA, Prov. Bonariae, Videla Dorna, on *Salix babylonica* L., May 1972, Deschamps et al. (BAFC 31920 – holotype).

Xylodon gamundiae **(Gresl. & Rajchenb.) Riebesehl & Langer, comb. nov.**
MycoBank MB827761

Hyphodontia gamundiae Gresl. & Rajchenb., Mycologia 92(6):1159 (2000) (Basionym).
≡ *Palifer gamundiae* (Gresl. & Rajchenb.) Hjortstam & Ryvarden, Synopsis Fungorum 22: 9 (2007).

Typus. ARGENTINA, Tierra del Fuego, Dpto. Ushuaia, Estancia El Valdéz, on *Nothofagus pumilio* (Poepp. & Endl.) Krasser, 4–5 Mar 1996, A. Greslebin (BAFC 50036 – holotype).

Xylodon hjortstamii **(Gresl. & Rajchenb.) Riebesehl & Langer, comb. nov.**
MycoBank MB827762

Hyphodontia hjortstamii Gresl. & Rajchenb., Mycologia 92(6):1160 (2000) (Basionym).

≡ *Palifer hjortstamii* (Gresl. & Rajchenb.) Hjortstam & Ryvarden, Synopsis Fungorum 22: 9 (2007).

Typus. ARGENTINA, Tierra del Fuego, Parque Nacional Tierra del Fuego, Río Pipo, on *Nothofagus* sp., 7 Nov 1998, A. Greslebin (BAFC 50037 – holotype).

***Xylodon septocystidiatus* (H.X.Xiong, Y.C.Dai & Sheng H.Wu) Riebesehl & Langer, comb. nov.**
MycoBank MB827764

Hyphodontia septocystidiata H.X.Xiong, Y.C. Dai & Sheng H.Wu, Mycologia 102(4):918 (2010) (Basionym).
≡ *Palifer septocystidiatus* (H.X.Xiong, Y.C.Dai & Sheng H.Wu) Riebesehl, Yurchenko & Langer, in Riebesehl & Langer, Mycological Progress 16(6): 649 (2017).

Typus. TAIWAN, Taipei, Kungliao, on rotten angiosperm branch, 25 Nov 1990, Y.F. Lin (TNM Lin 90202 – holotype).

***Xylodon verecundus* (G.Cunn.) Yurchenko & Riebesehl, comb. nov.**
MycoBank MB827765

Peniophora verecunda G.Cunn., Transactions and Proceedings of the Royal Society of New Zealand 83(2):262 (1955) (Basionym).
≡ *Palifer verecundus* (G.Cunn.) Stalpers & B.K.Buchanan, New Zealand Journal of Botany 29(3): 339 (1991).
≡ *Hyphodontia verecunda* (G.Cunn.) Hjortstam & Ryvarden, Mycotaxon 64: 237 (1997).

Typus. NEW ZEALAND, Auckland, Hauhangaroa Range, Taupo, on decayed decorticated wood of *Dacrydium cupessinum* Sol., Mar 1953, J.M. Dingley (PDD 12513 – holotype).

Notes

The three species *Palifer rickii* (Hjortstam & Ryvarden) Riebesehl, Yurchenko & Langer, *P. seychellensis* Dämmrich & Rödel and *P. wrightii* (Hjortstam & Ryvarden) Hjortstam & Ryvarden are today already accepted in other genera as *Xylodon rickii* (Hjortstam & Ryvarden) K.H. Larss. (Viner et al. 2018), *Sceptrulum inflatum* (Burt) K.H. Larss. (Larsson 2014) and *Hyphodontia wrightii* Hjortstam & Ryvarden (Gorjón 2012).

Xylodon lanatus and allied species

Xylodon lanatus was originally described by Burdsall and Nakasone (1981) based on collections from North America and New Zealand. A comparative morphological study of specimens, annotated as *X. lanatus* from Taiwan and North America, revealed that *X. lanatus* is a complex of morphologically similar species. The New Zealand specimen, *X. vesiculosus*, was discussed above and is considered to be a distinct species. The specimen *X. lanatus* (TUB-FO 40734) from Taiwan, depicted in Langer (1994), is *X. exilis*. The specimen of *X. lanatus* cited by Hjortstam and Ryvarden (1984) from Nepal is also *X. exilis*. In the protologue of *X. lanatus* (Burdsall and Nakasone 1981), the authors illustrated the paratype (HHB-6925 from Florida, U.S.A.) which is correctly identified as *X. pseudolanatus*. Hjortstam and Bononi (1987) reported *X. lanatus* from Brazil while noting the controversial taxonomic position of this species.

We accept *X. lanatus*, based on the type (CFMR HHB-8925; Figs 3f, 10) and paratype (CFMR HHB-4305), as a distinct species but with a restricted concept. We retain the same diagnostic features, noted in the protologue (basidiomata with well-developed woolly subiculum, terminal vesicular structures on subicular hyphae, poorly differentiated subhymenium, encrusted thick-walled hyphae in tooth apices and capitate cystidia) and add that walls of basidia and subhymenial hyphae directly under hymenial elements are slightly but distinctly thickened. The illustration of *Xylodon lanatus* from Taiwan, provided by Wu (1990), also shows basidia with walls thickened below, but hyphal pegs appear different from *X. lanatus* s.s. We have also determined that *X. echinatus* (Yurchenko et al. 2013) is the most morphologically similar species to *X. lanatus* s.s. A key to the taxa in the *X. lanatus* group is presented here.

1 Basidioma between aculei 0.3–0.5 mm thick, woolly; subhymenial hyphae somewhat thick-walled directly under hymenium **2**

– Basidioma between aculei 0.05–0.15 mm thick, membranaceous or subceraceous; subhymenial hyphae thin-walled **3**

2 Capitate cystidia present; basidia with slightly thickened walls in lower ½–2/3; spores 3–3.5 µm broad, Q = 1.8–2 ..... ***X. lanatus***

– Capitate cystidia absent; basidia thin-walled; spores 3.5–4(–5) µm broad, Q = 1.6–1.9 ..... ***X. echinatus***

3 Subicular hyphae strongly thick-walled (up to 1.5 µm thick), often with narrow lumen; hymenophoral aculei 0.13–0.35 mm long, 4 per mm ..... ***X. vesiculosus***

– Subicular hyphae moderately thick-walled (up to 1–1.2 µm thick), with wide lumen; hymenophoral aculei 0.03–0.12 mm long, 8–14 per mm **4**

4 Projecting hyphae in aculei strongly flexuous, thick-walled (up to 1–1.5 µm thick) in middle and lower part, provided with closely arranged simple and clamped septa, constricted at septa ..... ***X. exilis***

– Projecting hyphae in aculei slightly flexuous, slightly thick-walled, with remote septa, not constricted at septa ..... ***X. pseudolanatus***

Xylodon niemelaei, *X. reticulatus* and *X. rhizomorphus* are very closely related

Phylogenetic analyses of ITS sequences of 17 samples, including 8 new sequences of *X. niemelaei* and 28S sequences of three samples, demonstrate that the three taxa are very similar (Figs 1, 2). ITS sequences from holotypes of *X. reticulatus* and *X. rhizomorphus* were included in the analyses (Fig. 1). The ITS sequences were 98.2–99% similar amongst the taxa, differing at 6–11 sites. Minor morphological differences were noted amongst the taxa. We keep the taxa *X. niemelaei*, *X. reticulatus* and *X. rhizomorphus* as separate species following the results of phylogenetic studies of Chen et al. (2017) and Fernández-López et al. (2018a). The last work was published shortly before the completion of our study and therefore could not be considered further.

However, in our reconstruction, the phylogenetic distances between these taxa are very short, and comparable to those between the OTUs of *X. spathulatus*. Taking into account that these three taxa remain as monophyletic branches, we suppose that they can be subspecies or varieties of one species.

Xylodon bubalinus, *X. chinensis* and *X. spathulatus* are conspecific

Phylogenetic analyses of ITS sequences of 10 samples, including sequences from holotypes of *Xylodon bubalinus* and *X. chinensis* and 28S sequences of *X. spathulatus* and holotype of *X. chinensis* show that the three taxa are conspecific (Figs 1, 2). Amongst the taxa, ITS sequences were 98.7–99.8% similar, differing at up to 8 sites. The hymenophore is quite variable in this group and the presence of the different types of cystidia is also variable. The correct name for this group is *X. spathulatus* with *X. bubalinus* and *X. chinensis* reduced to synonyms.

The classification of *Xylodon australis* in *Xylodon* is confirmed

Xylodon australis is sequenced for the first time and shown in the 28S phylogenetic tree (Fig. 2) and its placement in *Xylodon* is confirmed. The sequenced specimen is from Argentina and was studied by Greslebin et al. (2000). They reported differences in spore morphology in specimens from Argentina, Australia and New Zealand. A molecular study may be able to resolve this species complex.

The paratype material of *Xylodon dimiticus* may be an independent species

Chen et al. (2017) showed that the holotype material of *Xylodon dimiticus* (Jia J.Chen & L.W.Zhou) Riebesehl & Langer Dai 11686 is conspecific with *X. nongravis*. In addition, they also proposed that the paratype material Dai 15321 may be an independent species as shown in the phylograms. We support this view and included Dai 15321 in our phylo-

grams as *Xylodon* sp. 1. The NCBI BLAST search of the ITS sequence of Dai 15321 shows an identity of 93% with the *X. dimiticus* holotype material Dai 11686 as well as with sequences of *X. nongravis*. Although this low similarity value indicates that Dai 15321 is a different species, a further study is needed to identify morphological differences.

Additions to the distribution and morphology of *Xylodon serpentiformis*

A BLAST search of the newly generated *Xylodon serpentiformis* ITS sequences revealed that they are 99% identical to a sequence from South Korea identified as *Hyphodontia* sp. (KUC20121019-31, Jang et al. 2016). *Xylodon serpentiformis* is known from Taiwan and the Canary Islands (Langer 1994). Based on the small distance between Taiwan and South Korea and the similarities of the sequences, the distribution of *X. serpentiformis* is expanded to include South Korea. Langer (1994) cited a specimen of *X. serpentiformis* from the Canary Islands, but this material needs molecular confirmation. A distinctive feature, described for this species, was the presence of flexuous, thick-walled tubular tramacystidia in the aculei (Langer 1994). After our morphological analysis of the holotype of *Hyphodontia serpentiformis* Langer (TUB-FO 40677) and three more specimens (TUB-FO 40675, TUB-FO 40985, TUB-FO 42688), we emend the diagnosis of *X. serpentiformis* as follows: aculei consisting mostly of flexuous, agglutinated projecting hyphae, hyphae slightly thickened or moderately thick-walled at base, then thinning toward apex and partly collapsing at maturity; spores broadly ellipsoid, ellipsoid, subovoid, sometimes narrowly ellipsoid.

Sequences of *Xylodon raduloides* form two subclades in phylogenetic trees

The two subclades of *Xylodon raduloides* in the ITS phylogeny (Fig. 1) appear also in the 28S phylogram (Fig. 2), although with the inclusion of the sister species *X. subtropicus*. Both subclades include specimens from Germany and Australia. Micromorphological distinctions between the clades were not observed, but we noted that, in one of the clades (KAS-JR 02, 03, 09, 26), the hymenophore is pale cream to cream whereas, in the second clade (KAS-JR10, LR 18813), it is yellow-brownish (Fig. 13). Nevertheless, the morphological as well as sequence-based differences are not sufficient to recognise two separate species.

Sequences of *Xylodon flaviporus* form two subclades in phylogenetic trees

In the ITS phylogram, two subclades are also present in the *Xylodon flaviporus* lineage (Fig. 1). Subclade 1 (FR-0249797, KAS-GEL 5047) comprises specimens from Réunion whereas subclade 2 (FCUG 1053, KAS-GEL 3462) comprises specimens from Romania and Taiwan. All other ITS sequences of *X. flaviporus* from the NCBI GenBank are from the northern hemisphere (Romania, South Korea, Taiwan, Turkey and USA) and clustered together in subclade 2 (data in Fig. 1 are reduced to specimens

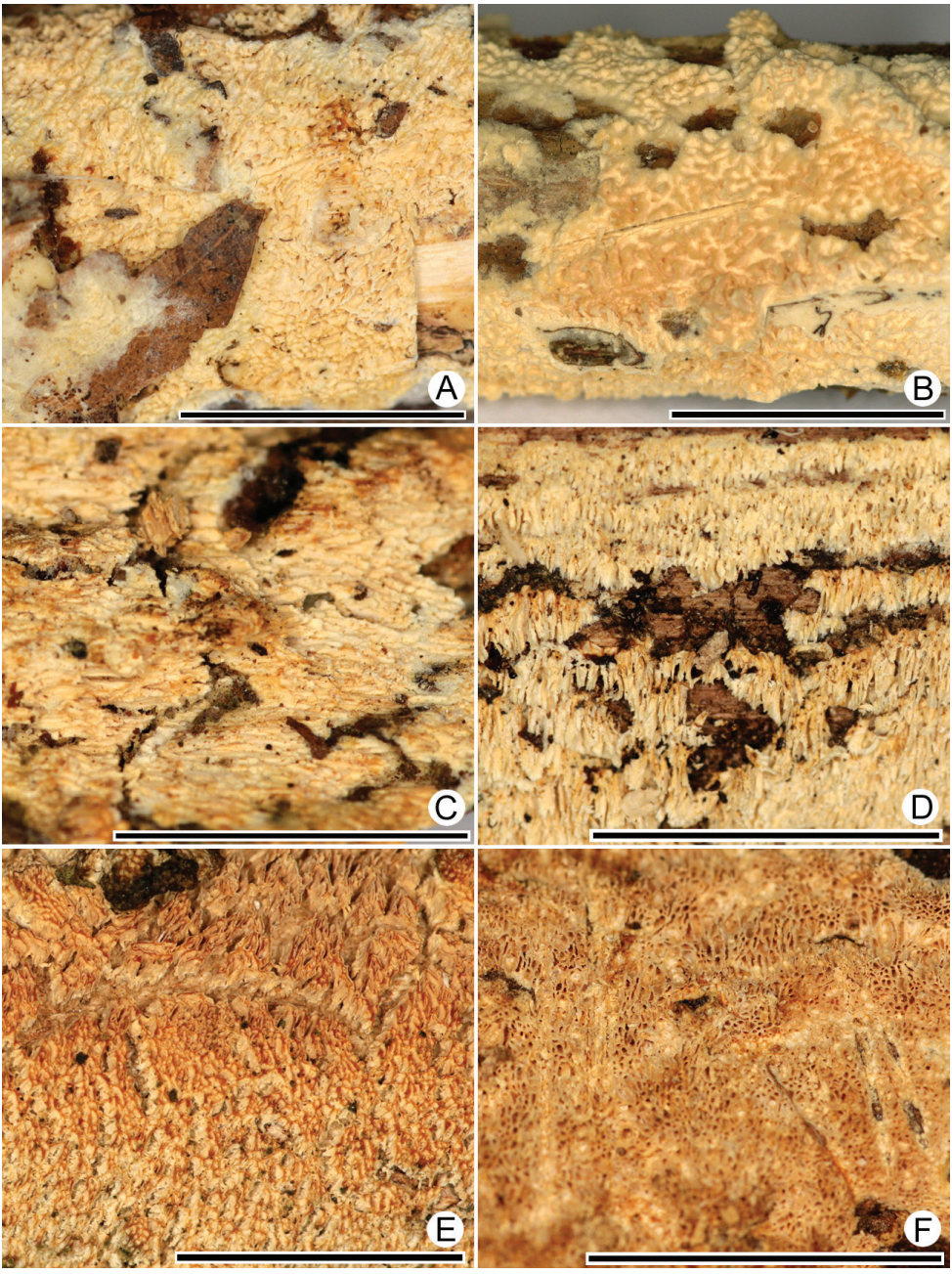

Figure 13. Hymenophores of *X. raduloides*. **A** KAS-JR 02, Germany **B** KAS-JR 03, Germany **C** KAS-JR 09, Germany **D** KAS-JR 26, Germany **E** KAS-JR 10, Germany **F** LR18813, Australia. Scale bars: 1 cm.

from Romania and Taiwan). We did not find micromorphological differences between specimens of the two subclades and the variation in the ITS sequences is too small to merit recognition of two different species.

Xylodon ramicida and *X. quercinus*

Xylodon ramicida and *X. quercinus* are morphologically similar (Ariyawansa et al. 2015), exhibiting slight differences in spore width and shape and different substrate preferences. Their ITS sequences are 98.8–99% similar, differing at just 6–7 positions (*X. quercinus*: Miettinen 15050 and *X. ramicida*: Spirin 7664). Taking into account the similarity values of other *Xylodon* species (*X. spathulatus* 98.7–99%, *X niemelaei* 98.2–99%) and small morphological differences between *X. ramicida* and *X. quercinus*, we believe that *X. ramicida* is a well-defined subspecies within *X. quercinus*. More sequences from both taxa are required, however, before the taxonomic status of *X. ramicida* can be clarified.

The taxonomic status of *Xylodon detriticus*

In this study, we accept *Hyphodontia detritica* (Bourdot) J. Erikss. in *Xylodon* as *X. detriticus*. This combination was introduced by Ţura et al. (2011), recognised as invalid in MycoBank (Art. 36.1a and b, Melbourne Code) and supported in the work by Rosenthal et al. (2017). The first sequenced specimen of this species was GB Nilsson 990902 (Larsson 2007). We have studied this specimen and it is identical to the concept of *Hypochnicium detriticum* (Bourdot) J. Erikss. & Ryvarden (Eriksson and Ryvarden 1976). The alignment between GB Nilsson 990902 and *X. detriticus* UC2023108 (Rosenthal et al. 2017) in ITS2 (ITS1 is unavailable for the previous specimen) showed nearly 100% similarity. We discovered that the ITS2 sequences between *Lagarobasidium detriticum* MA-Fungi 5758 (Dueñas et al. 2009) and GB Nilsson 990902 were 62% identical. Consequently, the taxonomic identity of *Lagarobasidium detriticum* MA-Fungi 5758 needs to be investigated. Viner et al. (2018) came to the same conclusion, but we could not integrate their further results, because this study was already finished when the work of Viner et al. was published.

Conclusion

The usefulness of ITS sequences alone in defining and identifying species in *Xylodon* is approaching its limits. Further studies in *Xylodon* will require sequences from additional genetic markers with more variation. Fernández-López et al. (2018b) published the first phylogenetic tree for *Xylodon* with rpb2 sequences, but it contains only sequences of six different species. Nevertheless, the topology is very similar to our ITS and 28S trees.

Morphological features for defining species in *Xylodon* is also limited. Species, such as *X. spathulatus* with its variability in aculei morphology and in cystidia occurrence and shape, present challenges for identification. In other cases such as *X. hyphodontinus*, ITS sequence differences are significant whereas morphological differences are elusive.

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