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Xylopsora canopeorum (Umbilicariaceae), a new lichen species from the canopy of *Sequoia sempervirens*

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Abstract

Xylopsora canopeorum Timdal, Reese Næsborg & Bendiksby is described as a new species occupying the crowns of large *Sequoia sempervirens* trees in California, USA. The new species is supported by morphology, anatomy, secondary chemistry and DNA sequence data. While similar in external appearance to *X. friesii*, it is distinguished by forming smaller, partly coralloid squamules, by the occurrence of soralia and, in some specimens, by the presence of thamnolic acid in addition to friesiic acid in the thallus. Molecular phylogenetic results are based on nuclear (ITS and LSU) as well as mitochondrial (SSU) ribosomal DNA sequence alignments. Phylogenetic hypotheses obtained using Bayesian Inference, Maximum Likelihood and Maximum Parsimony all support *X. canopeorum* as a distinct evolutionary lineage belonging to the *X. caradocensis*–*X. friesii* clade.

Keywords

California, epiphytic, *Hypocenomyce*, integrative taxonomy, morphology, multiple DNA sequence alignment, phylogeny, redwood forest, TLC

* Contributed equally to the study. Shared co-first authors.

Introduction

The squamulose lichen genus *Xylopsora* Bendiksby & Timdal consists of two species, *X. caradocensis* (Nyl.) Bendiksby & Timdal and *X. friesii* (Ach.) Bendiksby & Timdal. The two species were formerly placed in *Hypocenomyce* M. Choisy and referred to as the *H. friesii* group (Timdal 1984, 2001) until Bendiksby and Timdal (2013) showed that *Hypocenomyce* was highly polyphyletic. *Xylopsora* is the phylogenetic sister of the clade consisting of the two foliose genera *Lasallia* Mérat and *Umbilicaria* Hoffm. Those three genera make up the sister clade of the genus *Fulgidea* Bendiksby & Timdal, another *Hypocenomyce* segregate. The four genera together constitute the Umbilicariaceae (Bendiksby and Timdal 2013). *Fulgidea* consists of two species, *F. oligospora* (Timdal) Bendiksby & Timdal and *F. sierrae* (Timdal) Bendiksby & Timdal. *Fulgidea* and *Xylopsora* are morphologically, anatomically and ecologically very similar and differ mainly in secondary chemistry; alectorialic acid and thamnolic acid occur in the former, friesiic acid (= "friesii unknown") in the latter (Timdal 2001, 2002). All species of *Fulgidea* and *Xylopsora* grow on bark and wood and, with the exception of *X. caradocensis*, show preference for burnt stumps and trunks of conifers.

Coast redwood (*Sequoia sempervirens*) forests are an important component of California's ecosystems. Spanning more than six degrees of latitude along the Pacific coast (Van Pelt 2001) and containing individual trees that can live for more than 2000 years (Rocky Mountain Tree Ring Research 2017), these forests provide important habitats for many terrestrial species (Sawyer et al. 1999). However, biodiversity occupying the redwood forest canopies remains relatively under-explored because access into the tree crowns, which often grow to over 100 m in height, is challenging. The epiphytic lichen flora in old-growth redwood forests appears to be particularly species rich; an epiphyte survey of just nine large redwood trees yielded 137 lichen species including a new species of *Calicium* (Williams and Sillett 2007, Williams and Tibell 2008).

Recent epiphyte surveys in the crowns of additional large coast redwood trees in the southern part of the geographic range (Reese Næsborg 2017) revealed a previously undescribed species of *Xylopsora*. Here the authors have provided detailed morphologic, anatomic, chemical and molecular description of this new species, as well as characterising the habitat and substrates it occupies.

Establishing a multiple DNA sequence alignment (MSA) of non-coding loci, which often have unequal lengths due to indels, can be both time-consuming and highly subjective with regard to structural correctness. There has been great activity in recent years in the development of multiple sequence alignment tools (reviewed by Kamena and Notredame 2009). Moreover, there is no single recommendation as to what phylogenetic algorithm to use to transform the MSA into a reliable phylogenetic hypothesis. The current, moderately sized dataset has been used to test whether a less time-consuming and more objective approach, SATé-II (Liu et al. 2012), provides similar and meaningful results. Both manual and automatic approaches have been used to establish a concatenated MSA of three loci, of which one is non-coding and highly variable (nrITS). Different methods representing three classes of phylogenetic inference (Bayesian, likelihood and parsimony) have also been used.

Material and methods

The specimens

Five specimens of an unknown *Xylopsora* species were collected from *Sequoia sempervirens* trees in the southern part of the geographic range of coast redwood. The new species was documented on five trees in Big Basin Redwoods State Park, Santa Cruz County and on another five trees in Armstrong Redwoods State Natural Reserve, Sonoma County, California. The morphology, anatomy, chemistry and DNA sequences of these newly collected specimens have been studied and then compared to existing descriptions of *Xylopsora* and relatives (Bendiksby and Timdal 2013). A total of 50 accessions (Umbilicariales + Fuscideaceae) and their respective DNA sequences were reused from Bendiksby and Timdal (2013). Vouchers of the newly collected specimens are deposited at JEPS, NY and O.

Anatomy

Microscope sections were cut on a freezing microtome and mounted in water, 10 % KOH (K), 50 % HNO_3 (N), lactophenol cotton blue and a modified Lugol's solution in which water was replaced by 50 % lactic acid. Amyloid reactions were observed in the modified Lugol's solution after pretreatment in K. Ascospore measurements are given as X ± 1.5×SD, rounded to 0.5 µm, where X is the arithmetic mean and SD the standard deviation.

Secondary chemistry

Thin-layer chromatography (TLC) was performed in accordance with the methods of Culberson (1972), modified by Menlove (1974) and Culberson and Johnson (1982).

DNA extraction, PCR, and sequencing

DNA was extracted from the apothecia of four of the five newly collected specimens. The DNA extraction, PCR amplification (nrITS and mtSSU), PCR product purification, cycle sequencing and DNA sequence assembly and editing were performed as described by Bendiksby and Timdal (2013), including a subset of the oligonucleotide primers used (i.e. the forward primers ITS5 and mtSSU1 and the reverse primers ITS4 and mtSSU3R). The four DNA isolates were deposited in the DNA collection at O (Natural History Museum, University of Oslo).

DNA sequence analysis

The newly produced DNA sequences (mtSSU and nrITS) were aligned manually using BioEdit 7.2.3 (Hall 1999) into a trimmed version of the DNA sequence alignments used by Bendiksby and Timdal (2013). The resultant concatenated alignment comprised three genetic regions (nrLSU, mtSSU and nrITS) and a subset of 54 accessions representing the Elixiaceae, the Fuscideaceae, the Ophioparmaceae and the Umbilicariaceae. In addition to this alignment, hereafter referred to as "MSAmanual", the software SATé-II version 2.2.7 (Liu et al. 2012) was also used to establish an automated alignment, referred to as "MSAsate". Both alignments were analysed phylogenetically using Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) algorithms. The outgroup was defined as a clade consisting of two accessions representing the Fuscideaceae, which were also used for rooting. SeqState v.1.36 (Müller 2005) was used to convert alignments between different formats and FigTree 1.4.0 (Rambout 2006−2012) for visualising and editing output trees.

The BI analyses were performed as described in Bendiksby and Timdal (2013), but with only six million generations due to the smaller dataset. All trees saved prior to the point where the average standard deviation of split frequencies (ASDSF) fell below 0.01 were discarded as burn-in. For the sake of comparability of results, the evolutionary models GTR+G were used for both loci in the BI analysis (only a limited number of evolutionary models are available in SATé-II).

The software SATé-II simultaneously estimates multiple sequence alignments and ML phylogenetic trees. Prior to analyses, MSAmanual was divided into non-orphan (no empty sequences), single-locus datasets and were de-aligned (i.e. all gaps deleted). The MSAsate and its corresponding ML tree were estimated as a multilocus dataset in SATé-II using MAFFT (Katoh et al. 2005, Katoh and Toh 2008) as the aligner, MUSCLE (Edgar 2004a, b) as the merger and RAxML v.7.2.8 (Stamatakis 2006) as the tree estimator with the GTRCAT model. The limit of iterations was set to 50 and otherwise default settings were used. For comparison, MSAmanual was also analysed phylogenetically using SATé-II under the same settings.

For the MP analyses, NONA (Goloboff 1999) was used in combination with WinClada 1.0 (Nixon 1999), applying the heuristic search option with 2000 replicates and maxtrees set to 10000 and otherwise default settings. Parsimony jack-knifing (JK; Farris et al. 1996) with 2000 replicates was performed and otherwise default setting. Parsimony jackknifing was also performed on single-locus datasets for assessing potential gene-tree incongruence prior to estimating phylogenetic hypotheses based on all three loci.

Results

Four nrITS and three mtSSU sequences were generated (GenBank accession numbers MG309307–MG309313; Table 1). Preliminary parsimony jack-knife analyses of the individual three loci, regardless of the alignment approach, produced congruent gene-

Table 1. Specimens used in this study with voucher information, major lichen substances, and GenBank accession numbers.

Taxon, Specimen	Voucher Information	Major Lichen Substances	GenBank Accession Number		
			ITS	LSU	mtSSU
*Boreoplaca ultrafrigida**	(1) Russia, Sakha Rep., *Haugan & Timdal* YAK03/84 (O-L-138395; ITS). (2) YAK03/39 (O L-127, holotype; LSU, mtSSU)	lecanoric acid	HM161512	AY853360	AY853312
Elixia cretica 1	Australia, New South Wales, *Streimann & Curnow* 50968 p.p. (CANB 9304299 p.p.)	–	KF360371	KF360448	–
Elixia cretica 2	Mexico, Chihuahua, *Timdal* SON78/03 (O L-15969)	none	KF360372	KF360449	KF360419
Elixia cretica 3	Greece, *Spribille* 13340 (GZU, holotype)	–	–	–	GQ892058
Elixia flexella 1	Austria, *Halda, Palice & Steinova* 12407 (O L-157191)	–	KF360373	KF360450	KF360420
Elixia flexella 2	Turkey, *Palice* s.n. (hb. Palice)	–	–	AY853368	AY853320
Elixia flexella 3	*Palice* (ESS 21517)	–	–	AY300837	AY300887
Elixia sp. 1	U.S.A., Arizona, *Nash III* 11177 (ASU)	none	KF360374	KF360451	–
Elixia sp. 2	U.S.A., Arizona, *Nash III* 41750 (ASU)	none	KF360375	KF360452	–
Fulgidea oligospora 1	U.S.A., Arizona, *Nash III* 42735a (O L-767; holotype)	thamnolic acid	KF360395	KF360465	-
Fulgidea oligospora 2	U.S.A., Arizona, *Rui & Timdal* US215/01 (O L-59862)	alectorialic acid	KF360396	KF360466	KF360434
Fulgidea oligospora 3	U.S.A., Arizona, *Rui & Timdal* US272/01 (O L-59992)	alectorialic acid, thamnolic acid	KF360397	KF360467	KF360435
Fulgidea oligospora 4	Russia, Sakha Rep., *Haugan & Timdal* YAK04/05 (O L-18713)	alectorialic acid, thamnolic acid	KF360398	KF360468	-
Fulgidea sierrae 1	U.S.A., California, *Rui & Timdal* US249/01 (O L-59964)	alectorialic acid, thamnolic acid	KF360402	KF360471	KF360437
Fulgidea sierrae 2	U.S.A., California, *Timdal* SON125/01 (O L-60059; holotype)	alectorialic acid, thamnolic acid	KF360403	–	-
Fuscidea mollis	Sweden, *Ihlen* 1372 (UPS)	–	–	AY853369	AY853321
Hypocenomyce australis 1	Australia, Australian Capital Territory, *Elix* 19801 (O L-144372)	lecanoric acid	KF360380	–	–
Hypocenomyce australis 2	Australia, Victoria, *Krog* Au14/2 (O L-144373)	–	**	–	–
Hypocenomyce australis 3	Australia, Australian Capital Territory, *Weber & McVean* s.n. (O L-201, isotype)	lecanoric acid	KF360381	–	–
Hypocenomyce australis 4	Australia, Victoria, *Thor* 6047a (S)	–	KF360382	–	–
Hypocenomyce scalaris 1	Norway, *Timdal* 11022 (O L-158534)	–	KF360401	KF360470	KF360436
Hypocenomyce scalaris 2	U.S.A., North Carolina, *Amtoft* 2058 (DUKE 47763)	–	DQ782852	DQ782914	DQ912274

Taxon, Specimen	Voucher Information	Major Lichen Substances	GenBank Accession Number		
			ITS	LSU	mtSSU
Hypocenomyce scalaris 3	France, *Miadlikowska & Gueidan* 05/24/04-7 (DUKE 47529)	–	HQ650632	DQ986748	DQ986861
Hypocenomyce scalaris 4	Sweden, *Wedin* 7141 (UPS)	–	–	AY853373	AY853325
Hypocenomyce scalaris 5	Sweden, *Wedin* 7008 (UPS)	–	–	AY853374	AY853326
Hypocenomyce tinderryensis 1	Australia, Western Australia, *Elix* 38733 (CANB-790800)	–	KF360407	–	KF360440
Hypocenomyce tinderryensis 2	Australia, Australian Capital Territory, *Elix* 33386 (CANB-9801742.1)	–	KF360408	–	–
Hypocenomyce tinderryensis 3	Australia, Australian Capital Territory, *Elix* 33387 (CANB-676257)	–	KF360409	–	–
Hypocenomyce tinderryensis 4	Australia, New South Wales, *Streimann & Curnow* 50968 (CANB 9304299, holotype)	–	KF360410	–	–
Hypocenomyce tinderryensis 5	Australia, Australian Capital Territory, *Streimann & Curnow* 35001 (CANB 610213.1)	–	**	–	–
Lasallia pennsylvanica	U.S.A., *Culberson* 22287 (DUKE)	–	HM161513	AF356665	AY631278
Lasallia pustulata	Norway, *Hestmark* 3202 (DUKE 47908)	–	HM161456	DQ883690	DQ986889
*Maronea constans**	(1) Castello and Campagnolo 15972 (TBS; LSU). (2) China, Sipman 50094 (B; mtSSU)	–	–	AY640956	EF659771
Meridianelia maccarthyana	Australia, Tasmania, *Kantvilas* 752/03 (F)	–	–	–	FJ763185
Ophioparma handelii	China, Tibet, *Obermayer* 5135 (O L-168529)	–	KF360413	–	–
Ophioparma lapponica	Norway, *Timdal* 12353 (O L-170853)	divaricatic acid, usnic acid	KF360414	–	KF360443
Ophioparma ventosa 1	Norway, *Haugan* 7615 (O L-151477)	–	KF360415	KF360474	KF360444
Ophioparma ventosa 2	Norway, *Bjelland* 60 (BG)	–	AY011013	AY853380	AY853331
Umbilicaria africana	Peru, *Hestmark* 5081B (O)	–	HM161482	HM161545	HM161572
Umbilicaria aprina	Bolivia, *Hestmark* 5030B (O)	–	HM161483	HM161514	HM161573
Umbilicaria crustulosa	Norway, *Hestmark* 9017 (O)	–	HM161496	HM161590	HM161612
*Umbilicaria proboscidea**	(1) U.K., E:DNA:EDNA10-00739 (ITS). (2) *Lumbsch* 12165b (F; LSU, mtSSU)	–	FR799305	AY300870	AY300920
Umbilicaria spodochroa	Norway, *Hestmark* 3201 (DUKE 47907)	–	HM161481	DQ986773	DQ986815

Taxon, Specimen	Voucher Information	Major Lichen Substances	GenBank Accession Number		
			ITS	LSU	mtSSU
Xylopsora canopeorum	U.S.A., California, *Reese Næsborg* 1544 (NY)	friesiic acid	–	–	–
Xylopsora canopeorum 1	U.S.A., California, *Reese Næsborg* 1522 (JEPS, holotype)	friesiic acid, thamnolic acid	**MG309307**	–	**MG309311**
Xylopsora canopeorum 2	U.S.A., California, *Reese Næsborg* 1707 (O 1316)	friesiic acid	**MG309309**	–	–
Xylopsora canopeorum 3	U.S.A., California, *Reese Næsborg* 1597 (O 1315)	friesiic acid	**MG309308**	–	**MG309312**
Xylopsora canopeorum 4	U.S.A., California, *Reese Næsborg* 1775 (JEPS)	friesiic acid	**MG309310**	–	**MG309313**
Xylopsora caradocensis 1	Norway, *Timdal* 2410 (O L-32967)	friesiic acid	KF360383	–	–
Xylopsora caradocensis 2	Sweden, *Westling* s.n. (S L-53582)	–	KF360384	–	–
Xylopsora caradocensis 3	Sweden, *Odelvik* 599 (S L-29227)	–	KF360385	–	KF360425
Xylopsora friesii 1 - cf.	Norway, *Timdal* 11029 (O L-158541)	friesiic acid	KF360388	KF360459	KF360428
Xylopsora friesii 2	Norway, *Breili* L3615 (O L-167185)	friesiic acid	KF360389	KF360460	KF360429
Xylopsora friesii 3	Sweden, *Wedin* 7139 (UPS)	–	–	AY853372	AY853324
Xylopsora friesii 4	Norway, *Timdal* 1055 (O L-56480)	friesiic acid	KF360390	–	–

New sequences are indicated by accession numbers in bold.

* DNA sequences obtained from different vouchers; checked for gene-tree incongruence prior to concatenation.

** DNA sequences shorter that 200 bp and therefore not accepted for submission to GenBank. See Bendiksby and Timdal (2013) for sequences.

trees that were resolved to various extents (not shown). In subsequent analyses, the three loci were analysed in concert. The MSAmanual alignment (i.e. three loci, manually aligned) was 10 characters shorter than MSAsate (i.e. three loci, automatically aligned) and had 12 fewer parsimony informative characters (PIC; Table 2). Both alignments are provided as Suppl. material 1, 2 (MSAmanual.nex, MSAsate.nex). The MSAmanual dataset produced 1220 most parsimonious trees (MPTs) of length 1479, whereas MSAsate produced 10 MPTs of length 1485. Homoplasy measures (Farris 1989) differ negligibly between the two (RC: 46.6 vs 46).The likelihood scores from the RAxML analyses in SATé-II were very similar (Table 2). The ASDSF fell below 0.01 faster in the BI analysis of MSAsate (at generation 820) than in the BI analysis of MSAmanual (around generation 1300). All significantly supported clades were congruent amongst the BI, ML and MP analyses, regardless of the dataset analysed (MSAmanual vs MSAsate). Only results from analyses of the MSAsate dataset are shown (Fig. 1). The authors regarded clade support of at least 60% jack-knife (JK) and at least 0.9 posterior probability (PP) as significant.

Table 2. Tree statistics from various phylogenetic analyses (MP, ML, BI) of the MSAmanual and MSAsate alignments.

	Locus	Taxa	AL-length	PIC*	MPTs	MP Tree-length	CI	RI	RC	SATe ML score	BI ASDSF**	BI burn-in
MSAmanual	nrLSU	32	865	111								
	mtSSU	35	787	160								
	nrITS	45	505	164								
	concat	54	2157	435	1220	1479	60	79	46.6	-8926.214413	0.003623	21.7%
MSAsate	nrLSU	32	865	111								
	mtSSU	35	793	162								
	nrITS	45	509	174								
	concat	54	2167	447	10	1485	59	79	46	-9030.310897	0.005327	13.7%

* ingroup

** at termination (i.e. generation no 6 mill)

The four accessions of the tentatively new species group with significant support and showed themselves as sister to a clade consisting of three accessions of *Xylopsora friesii* (2, 3 and 4; Fig. 1). Four characters varied amongst the four accessions of the tentatively new species (three in the nrITS and one in mtSSU), none of which were parsimoniously informative within the group. *Xylopsora* cf *friesii* 1 differed from *X. friesii* 2, 3 and 4 in eight characters in the nrLSU, at least four in the mtSSU and at least 16 in the nrITS. The MP analyses supported *Elixia* as monophyletic (JK MSAmanual = 94 %; JK MSAsate = 87 %) with *Meridianelia* as sister (JK MSAmanual = 95 %; JK MSAsate = 93 %). In the ML and BI analyses, *Elixia* monophyly was not supported, as *Meridianelia* grouped with accessions of *E. flexella* and *Elixia* sp. and excluded *Elixia cretica.* This topology was not significantly supported by any analyses. The sister-relation between the Elixiaceae and the Ophioparmaceae was significantly supported only by Bayesian PP (Fig. 1).

Discussion

Forest canopies in general are relatively understudied because accessing the tree crowns requires technical expertise and equipment (Lowman et al. 2012). Therefore, the potential for encountering new species is relatively high compared to more easily accessible forest floor environments. The new species presented here, *Xylopsora canopeorum*, has so far only been registered from the crowns of large coast redwood trees, but other similar habitats, like the fibrous bark of other large members of Cupressaceae, should be explored for the species. The collected *Xylopsora canopeorum* specimens occurred on stable bark surfaces of old, large redwood trees together with several species previously classified in the genus *Hypocenomyce* (e.g. *Carbonicola anthracophila*, *Fulgidea oligospora*, *F. sierrae*, and *H. scalaris*), which was recently shown to be highly polyphyletic (Bendiksby and Timdal 2013). Together, these species covered a substantial portion of the trunk surface.

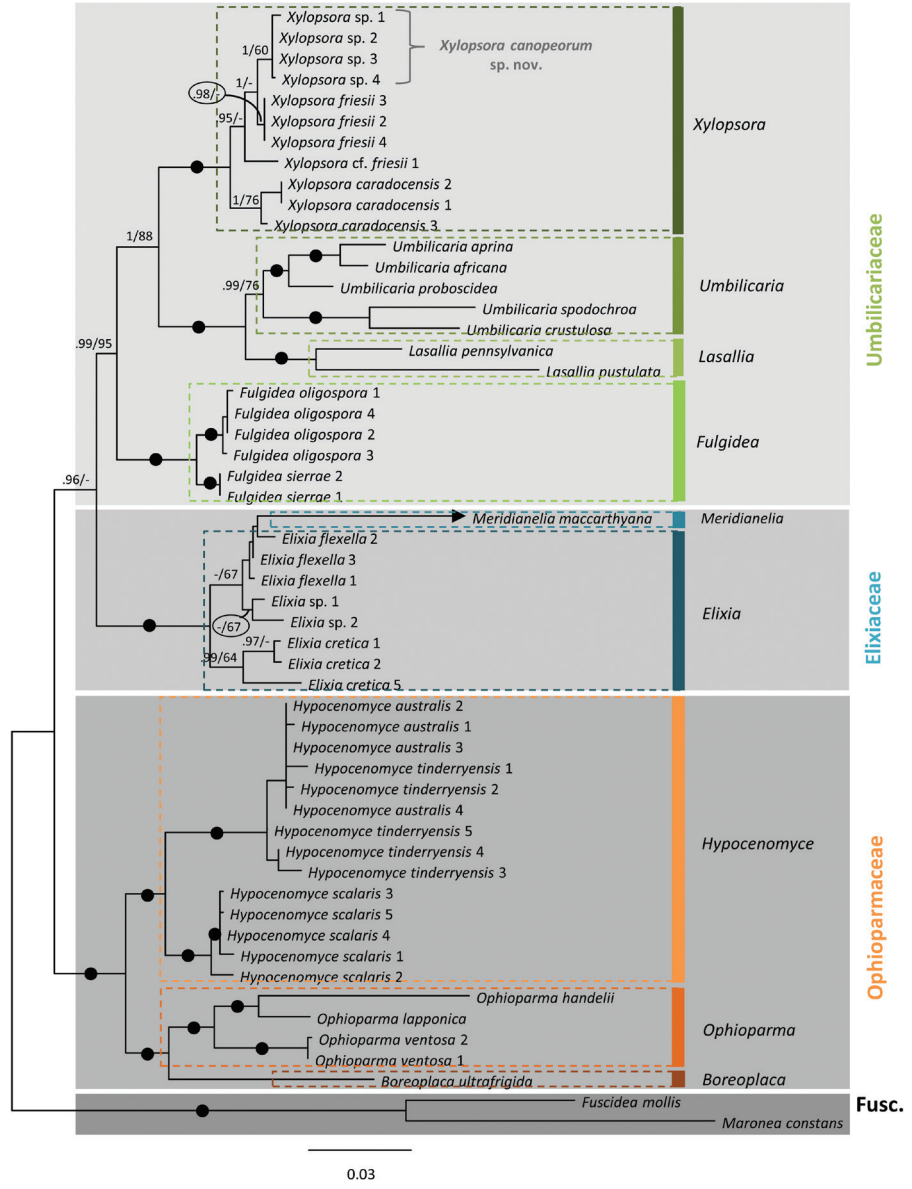

Figure 1. Hypothesis of the phylogenetic relationships and placement of the potentially undescribed species of *Xylopsora* based on DNA sequence data. The depicted topology is based on an automated alignment (MSAsate) of two nuclear (ITS and LSU) and one mitochondrial (SSU) ribosomal loci and is the "best tree" from a RAxML analysis using SATé-II. Clade support over certain values from Bayesian inference (posterior probability; PP) and parsimony jackknifing (JK) analyses are superimposed: PP >0.9 and JK>60% (PP/JK). Clades receiving maximum PP support (1.0) and at least 90% JK support are indicated with a black dot. Multiple accessions of the same taxon are numbered according to Table 1 (also corresponding to the numbering in Bendiksby & Timdal 2013). Family and genus circumscriptions are indicated. Abbreviation: Fusc. = Fuscideaceae. One accession in the tree (*Meridianelia maccarthyana*) appeared on a very long branch that is manually shortened (arrow tipped) to reduce the size of a broad figure.

Molecular analyses

Automatic alignment by SATé-II differed only slightly from the manually aligned dataset. Only areas with ambiguous alignment solutions varied between the manually aligned multi-locus alignment (MSAmanual) and the one aligned automatically (MSAsate). Moreover, the two alignments rendered highly similar topologies when analysed using the same algorithm. MSAsate contained slightly more parsimony phylogenetic information and produced fewer MPTs. Although the different algorithms produced variously resolved trees, the same significantly supported clades were present in all output trees. This suggests significant time-savings by using SATé-II and software of similar quality for both automated alignment and phylogenetic analyses.

As expected, the overall tree-topology (Fig. 1) largely corresponded to previous findings (Bendiksby and Timdal 2013: fig. 2A), the only exception being that *Elixia* monophyly was not supported by the BI and ML analyses and supported only by MP (JK MSAmanual = 94 %; JK MSAsate = 87 %). The grouping of *Meridianelia* with accessions of *E. flexella* and *Elixia* sp., however, was not significantly supported by any analyses (Fig. 1) and was not considered of taxonomical significance. More importantly, monophyly of the four newly included accessions was significantly supported regardless of alignments or analyses algorithm. Likewise, this clade's sister relation to *X. friesii* was significantly supported. The low and non-informative genetic variation between the four newly included accessions strongly suggests they belong to a single species. The specimen *Xylopsora* cf *friesii* 1, on the other hand, differed significantly from *X. friesii* 2, 3 and 4. It is hypothesised that *X.* cf. *friesii* 1 represents a species distinct from *X. friesii*, but more material will need to be studied prior to drawing additional taxonomic conclusions.

Taxonomy

Xylopsora canopeorum **Timdal, Reese Næsborg & Bendiksby, sp. nov.**
Mycobank: MB823500
Fig. 2

Diagnosis. The species differs from *X. caradocensis* and *X. friesii* mainly in forming more minute, coralloid and sometimes, sorediate squamules and sometimes (the holotype) in containing thamnolic acid in addition to friesiic acid; it also differs from the former in having shorter, non-septate ascospores.

Type. USA, California, Santa Cruz Co., 75 m E of North Escape Road, 125 m S of the third gate on North Escape Road in Big Basin Redwoods State Park, 37°10'46"N, 122°12'58"W, 341 m alt., on bark of main trunk more than 100 cm diameter, from the upper trunk of old *Sequoia sempervirens* in old-growth redwood forest, fall (autumn) 2015, R. Reese Næsborg 1522 (JEPS, holotype [TLC: friesiic acid (major), thamnolic acid (submajor); GenBank: MG309307 (ITS), MG309311 (mtSSU)]).

Figure 2. *Xylopsora canopeorum,* holotype. Scale bar: 1 mm.

Description. Thallus crustose to squamulose; individual squamules up to 0.5 mm diam. but often soon breaking up into a coralloid crust, adnate when young, later ascending and more or less geotropically imbricate; soralia occurring patchily, labriform, bluish;

upper surface greyish-green to medium brown, dull; margin crenulate or incised, concolorous with upper surface. Upper cortex up to 15 µm thick but mostly poorly defined. Apothecia common, up to 0.6 mm diam., plane, black, epruinose, egyrose; margin remaining prominent, entire or flexuose; proper exciple composed of closely conglutinated hyphae, olivaceous brown in inner part, brownish black in the rim, not containing crystals, K–, N–; hymenium ca. 50 µm high, pale olivaceous brown; hypothecium pale olivaceous brown; epihymenium dark reddish brown, not containing crystals, K–, N–; paraphyses ca. 2 µm thick, simple, without swelling or pigment cap in apical cell; ascus clavate, ca. 30 µm tall, with a thin, evenly amyloid tholus and covered by an amyloid cap, with orange pigment in the cytoplasm when young. Ascospores ellipsoid, simple, hyaline, with orange pigment in the cytoplasm when young, 4–7 × 2.5–4.5 µm (n = 20, from holotype). Pycnidia not seen.

Chemistry. Friesiic acid (major) and thamnolic acid (absent to submajor). Thallus PD– or PD+ yellow, K– or K+ yellow, C–, UV+ bluish white.

Distribution. Specimens were collected from central coastal California in Big Basin Redwoods State Park (37.1°N, 11 km from the Pacific Ocean) and Armstrong Redwoods State Natural Reserve (38.3°N, 18 km from the Pacific Ocean).

Ecology. *Xylopsora canopeorum* was observed on coarse, fibrous bark and occasionally on charred bark between 5 and 75 m above ground level along the trunks of large coast redwood trees in old-growth redwood forests. The species commonly co-occurred with *Carbonicola anthracophila*, *Fulgidea oligospora*, *F. sierrae*, *Hertelidea botryosa* and *Hypocenomyce scalaris*, which together covered substantial portions of the trunk surface. *Xylopsora canopeorum* appeared to have an affinity for old and stable bark surfaces on the main trunks of large redwood trees.

Etymology. The specific epithet "*canopeorum*" refers to the habitat in which the species was encountered ¾ in the canopy of old-growth redwood forests.

Remarks. The species differs from *X. caradocensis* and *X. friesii* morphologically by forming more minute squamules (less than 0.5 mm diam.) which soon break up into a coralloid crust and sometimes into soralia. In *X. caradocensis* and *X. friesii*, the squamules are up to 1.0 (–1.5) mm diam. and always esorediate. In the former, the squamules are bullate or irregularly ascending; in the latter more or less plane, adnate or somewhat ascending (Timdal 1984). In *X. caradocensis*, the ascospores are longer (6.5–14 × 2–4 µm) than those of *X. canopeorum* and often 1- or 3-septate; in *X. friesii,* the ascospores hardly differ (4.5–7.5 × 2.5–3.5 µm) from those of *X. canopeorum*. *Xylopsora caradocensis* and *X. friesii* contain friesiic acid only (Timdal 1984, as "friesii unknown").

In the current Californian lichen checklist (Tucker 2014), *Lecidea xanthococcoides* Zahlbr. is the only species unknown to the authors that could be assumed to be an earlier name for *X. canopeorum*. That species was described from conifer trunks at 1700 m alt. in the San Bernardino Mountains, i.e. in an area and habitat where *X. canopeorum* possibly can occur. The holotype (H.E. Hasse 705) was not found in W upon enquiry. Details in the original description (Zahlbruckner 1900) indicate that it is a different species, however – Apothecia becoming convex and immarginate, hymenium 160–180 µm high and ascospores 12–15 × 5.5–6 µm.

Additional specimens examined. USA. California. *Santa Cruz Co.*: label data as for holotype, R. Reese Næsborg 1544 (NY); 800 m WNW of North Escape Road up

Rodgers Creek in Big Basin Redwoods State Park, 37°11'44"N, 122°13'34"W, 403 m alt., on bark of branch less than 50 cm diameter in the lower crown of an old *Sequoia sempervirens* tree in an old-growth redwood forest, spring 2015, R. Reese Næsborg 1597 (O L-1315); 400 m E of North Escape Road along Sequoia Trail in Big Basin Redwoods State Park, 37°11'13"N, 122°12'54"W, 422 m alt., on bark of trunk more than 100 cm diameter in the upper trunk of an old *Sequoia sempervirens* tree in an old-growth redwood forest, Fall 2015, R. Reese Næsborg 1707 (O L-1316). *Sonoma Co.*: 50 m SW of Colonel Armstrong Tree parking area in Armstrong State Natural Reserve, 38°32'13"N, 123°00'29"W, 49 m alt., on bark from the upper trunk of an old *Sequoia sempervirens* tree in an old-growth redwood forest, fall 2015, R. Reese Næsborg 1775 (JEPS).

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Supplementary material 1

MSAmanual.nex

Authors: Mika Bendiksby, Rikke Reese Næsborg, Einar Timdal

Data type: Sequence alignment

Explanation note: A manually aligned concatenated multiple sequence alignment comprising three genetic regions (nrLSU, mtSSU and nrITS) and a subset of 54 accessions representing the Elixiaceae, the Fuscideaceae, the Ophioparmaceae, and the Umbilicariaceae.

Link: https://doi.org/10.3897/mycokeys.30.22271.suppl1

Supplementary material 2

MSAsate.nex

Authors: Mika Bendiksby, Rikke Reese Næsborg, Einar Timdal

Data type: Sequence alignment

Explanation note: An automatically aligned (using the software SATé-II version 2.2.7) concatenated multiple sequence alignment comprising three genetic regions (nrLSU, mtSSU and nrITS) and a subset of 54 accessions representing the Elixiaceae, the Fuscideaceae, the Ophioparmaceae, and the Umbilicariaceae.

Link: https://doi.org/10.3897/mycokeys.30.22271.suppl2

MycoKeys 30: 17–30 (2018)
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RESEARCH ARTICLE

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Phylloporia lonicerae (Hymenochaetales, Basidiomycota), a new species on *Lonicera japonica* from Japan and an identification key to worldwide species of *Phylloporia*

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Abstract

Phylloporia, in the Hymenochaetaceae, is a polypore genus with a worldwide distribution. The new taxon *Phylloporia lonicerae* is introduced, which is the first *Phylloporia* species to originate from Japan. This species grows exclusively on living *Lonicera japonica* and is distinguished by annual, sessile basidiocarps that occur in clusters, pileal surface of narrow, concentrically sulcate zones, 6–8 pores per mm, duplex context separated by a black zone, dimitic hyphal system and broadly ellipsoid basidiospores, 3.2–4 × 2.3–3.1 µm. Phylogenetically, *P. lonicerae* is nested within the *Phylloporia* clade as a distinct terminal lineage with full statistical supports and sister to the clade of *P. minutispora*, *P.* cf. *pulla* and *P. terrestris* with weak supports. Besides *Phylloporia bibulosa*, *P. chrysites* and *P. spathulata*, *P. lonicerae* is the fourth species of *Phylloporia* recorded from Japan. An identification key to all accepted 48 species of *Phylloporia* is provided.

Keywords

Hymenochaetaceae, key, *Lonicera japonica*, polypore, taxonomy

* These two authors contributed equally to this work.

Introduction

Phylloporia Murrill, in the Hymenochaetaceae Donk, was introduced for an unusual polypore species, *P. parasitica* Murrill growing on the underside of living leaves in Columbia (Murrill 1904). For nearly 70 years, *Phylloporia* was forgotten until Ryvarden (1972) transferred five taxa into the genus. Renewed interest in *Phylloporia* was stimulated by Wagner and Ryvarden's (2002) phylogenetic and morphological study in which they accepted 12 species. Since then, a number of new species have been described from Africa (Ipulet and Ryvarden 2005, Decock et al. 2015, Yombiyeni et al. 2015, Yombiyeni and Decock 2017), the Americas (Valenzuela et al. 2011, Decock et al. 2013, Ferreira-Lopes et al. 2016) and Asia, especially China (Gafforov et al. 2014, Cui et al. 2010, Zhou and Dai 2012, Zhou 2013, 2015a, 2015b, 2016, Liu et al. 2015, Chen et al. 2017, Ren and Wu 2017).

Phylloporia began as a monophyletic genus based on phylogenic studies of the large subunit of the nuclear ribosomal gene (nLSU) (Wagner and Ryvarden 2002) but is now paraphyletic with the inclusion of *Coltricia* cf. *stuckertiana* (Speg.) Rajchenb. & J.E. Wright in the *Phylloporia* clade (Valenzuela et al. 2011, Decock et al. 2013). The genus is morphologically quite diverse and includes species with annual or perennial basidiocarps with resupinate, sessile or stipitate habits, homogenous or duplex context, monomitic or dimitic hyphal system and cylindrical to subglobose basidiospores (Wagner and Ryvarden 2002, Cui et al. 2010, Zhou 2015a). Substrate preferences of *Phylloporia* species are equally diverse. Some species are saprobes that colonise woody debris (Ipulet and Ryvarden 2005, Zhou 2015b, Ferreira-Lopes et al. 2016) and others are parasites usually of specific plant hosts (Zhou 2015a, Ren and Wu 2017, Yombiyeni and Decock 2017).

There are three species of *Phylloporia* reported from Japan – *P. bibulosa* (Lloyd) Ryvarden, *P. chrysites* (Berk.) Ryvarden and *P. spathulata* (Hook.) Ryvarden (Núñez and Ryvarden 2000). In this paper, a new species, *Phylloporia lonicerae*, is described from Nara, Japan, growing on living vines of *Lonicera japonica*. Morphological and molecular data support the recognition of this new species. In addition, an updated key to the known species of *Phylloporia* is presented.

Materials and methods

Morphological examination

The studied specimens were deposited at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP) in China. The macroscopic characters were observed from dried specimens with the aid of a stereomicroscope. Specimen sections were mounted in Cotton Blue (CB), Melzer's reagent (IKI) and 5 % potassium hydroxide (KOH) for observation using a Nikon Eclipse 80i microscope at magnification up to 1000×. Special colour terms follow Petersen (1996). All measurements were taken

from sections mounted in CB. When presenting the size variation of basidiospores, 5% of measurements from each end of the range were put in parentheses. Line drawings of microscopic characters were made with the aid of a drawing tube. The abbreviations used in the description are as follows: L = mean basidiospore length (arithmetic average of all measured basidiospores), W = mean basidiospore width (arithmetic average of all measured basidiospores), Q = variation in the L/W ratios between the specimens studied and n = number of basidiospores measured from a given number of specimens.

Molecular sequencing

The PCR products were directly amplified from the extracts of the basidiocarps with the Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's protocol. The PCR protocol was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles of denaturation at 98 °C for 5 s, annealing at 48 °C for 5 s and extension at 72 °C for 5 s and a final extension of 72 °C for 10 min. The primers LR0R and LR7 (Vilgalys and Hester 1990) were used for PCR amplification and subsequent sequencing at the Beijing Genomics Institute, China. The newly generated sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank; Fig. 1).

Phylogenetic analysis

To explore the phylogenetic relationship of *P. lonicerae*, six nLSU sequences were incorporated into previous nLSU datasets of *Phylloporia* (Zhou 2016, Chen et al. 2017, Ren and Wu 2017, Yombiyeni and Decock 2017). Several species of *Fomitiporella* Murrill and *Fulvifomes* Murrill were included in the dataset and *Inonotus hispidus* (Bull.) P. Karst. was selected as the outgroup taxon.

The nLSU dataset was aligned with MAFFT 7.110 (Katoh and Standley 2013) with the G-INI-I option (Katoh et al. 2005). The best-fit evolutionary model for the resulting alignment that was deposited in TreeBASE (http://www.treebase.org; accession number S21971), was estimated as GTR + I + G using jModelTest 2.1.4 (Darriba et al. 2012). Following this model, maximum likelihood (ML) and Bayesian Inference (BI) algorithms were used to infer the phylogeny of the alignment. The ML analysis was conducted using raxmlGUI 1.2 (Silvestro and Michalak 2012, Stamatakis 2006) under the auto FC option for bootstrap (BS) replicates (Pattengale et al. 2010). MrBayes 3.2 (Ronquist et al. 2012) was carried out for BI analysis, which employed two independent runs, each including four chains of 10 million generations and starting from random trees. Trees were sampled every 1000th generation. Of the sampled trees, the first 25 % was deleted and the remaining trees were used to construct a 50 % majority consensus tree and calculate Bayesian posterior probabilities (BPPs). Chain convergence was determined using Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/).

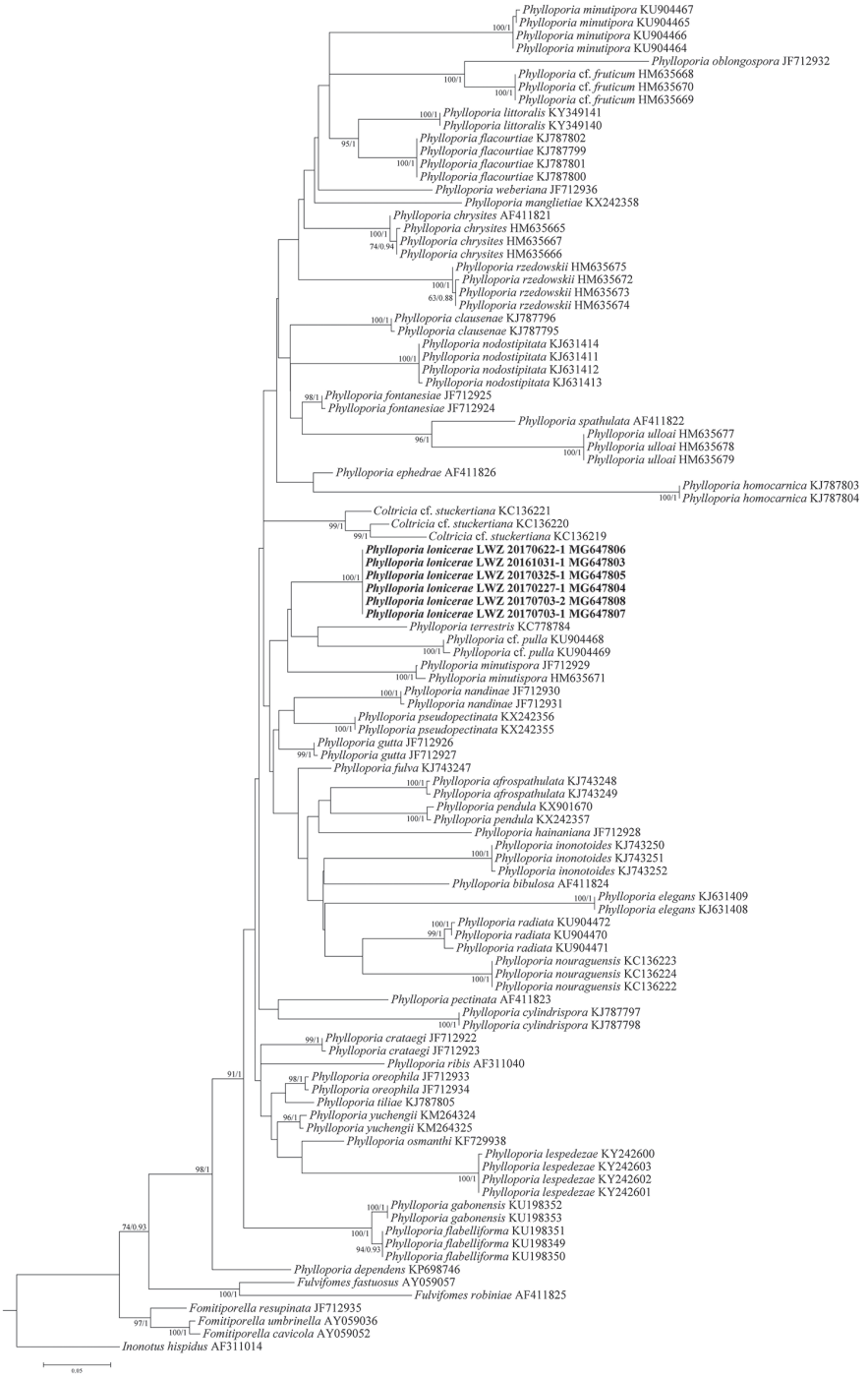

Figure 1. Phylogenetic position of *Phylloporia lonicerae* inferred from the nLSU dataset. The topology is inferred by maximum likelihood algorithm, while bootstrap values above 50 % and Bayesian posterior probabilities above 0.8 are given at the nodes. Newly sequenced specimens are in boldface.

Results

Six nLSU sequences of *P. lonicerae* were generated and included in a dataset of 105 sequences and 942 characters. ML analysis was ended after 250 BS replicates. BI analysis converged all chains as indicated by the effective sample sizes of all parameters above 2000 and the potential scale reduction factors close to 1.000. As the ML and BI analyses generated congruent topologies in main lineages, the ML tree is presented in Figure 1. Values of BS above 50 % and BPPs above 0.8 are given at the nodes. The phylogenic tree (Fig. 1) shows that the strongly supported *Phylloporia* clade (98 % in ML, 1 in BI) consists of 44 terminal lineages and the six *P. lonicerae* samples formed a new lineage with full statistical supports (100 % in ML, 1 in BI). The *Phylloporia lonicerae* lineage is sister to the clade that includes *P. minutispora* Ipulet & Ryvarden, *P.* cf. *pulla* (Mont. & Berk.) Decock & Yombiy and *P. terrestris* L.W. Zhou with weak supports.

Taxonomy

***Phylloporia lonicerae* W.M. Qin, Xue W. Wang, T. Sawahata & L.W. Zhou, sp. nov.**
MycoBank: MB823715
Figs 2, 3

Holotype. JAPAN. Nara, Research Forest of Faculty of Agriculture, Kindai University, 3 Jul 2017, on living vine of *Lonicera japonica*, LWZ 20170703-2 (IFP 019172).

Etymology. *Lonicerae* (Lat.): referring to *Lonicera*, the host tree genus.

Description. Basidiocarps annual, sessile, imbricate, rarely solitary, without odour or taste, woody. Pilei semi-circular, flabelliform or fused together, applanate, single pileus projecting up to 1.5 cm long, 3 cm wide and 0.5 cm thick at base. Pileal surface greyish-brown to yellowish-brown, velutinate, concentrically sulcate with narrow zones; margin pale yellow or concolorous, sharp. Pore surface honey-yellow, slightly glancing; sterile margin distinct, curry-yellow, up to 0.5 mm wide; pores circular to angular, 6–8 per mm; dissepiments thin, entire. Context up to 2 mm thick, duplex, with a black zone, lower context olivaceous buff, hard corky, up to 1 mm thick, upper tomentum cinnamon-buff, soft, up to 1 mm thick. Tubes honey-yellow, corky, up to 3 mm long.

Hyphal system dimitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH. Context: in the lower context, generative hyphae hyaline to pale yellowish, slightly thick- to thick-walled with a wide lumen, frequently branched and septate, 2–4 μm in diam; skeletal hyphae golden yellow, thick-walled with a narrow lumen, unbranched, aseptate, interwoven, 2–4.5 μm in diam; in the upper tomentum, generative hyphae infrequent, pale yellowish, slightly thick- to thick-walled with a wide lumen, rarely branched, frequently septate, 2–4 μm in diam; skeletal hyphae golden yellow, thick-walled with a narrow to wide lumen, unbranched, aseptate, loosely interwoven, 2.5–5 μm in diam; in the black zone, hyphae dark brown, thick-walled with a narrow lumen, strongly agglutinated, interwoven. Tubes: generative hyphae hyaline to pale yellowish, thin- to

Figure 2. Basidiocarps of *Phylloporia lonicerae* in situ. **a** LWZ 20170703-2 (holotype) **b** LWZ 20170622-1 (paratype). Scale bars: 2 cm.

slightly thick-walled, occasionally branched, frequently septate, 1.8–4 μm in diam; skeletal hyphae golden yellow, thick-walled with a narrow lumen, unbranched, aseptate, interwoven, 2–4 μm in diam. Setae absent. Cystidia and cystidioles absent. Basidia clavate, with four sterigmata up to 3 μm long and a simple septum at the base, 7–11 × 4–6 μm; basidioles in shape similar to basidia, but slightly smaller. Basidiospores broadly ellipsoid, pale yellowish, thick-walled, smooth, indextrinoid, inamyloid, acyanophilous, (3–)3.2–4 × (2.1–)2.3–3.1(–3.3) μm, L = 3.61 μm, W = 2.77 μm, Q = 1.28–1.33 (n = 90/3).

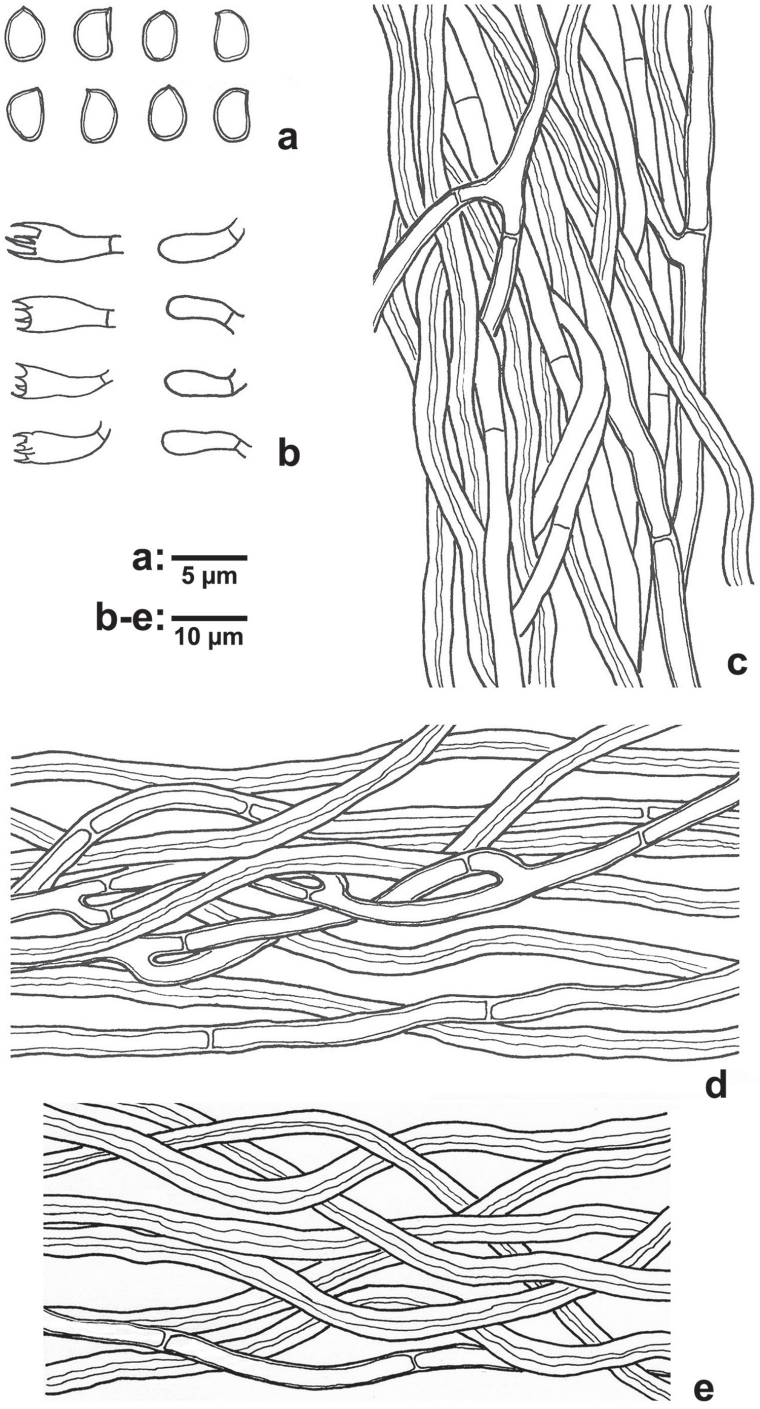

Figure 3. Microscopic structures of *Phylloporia lonicerae* (drawn from the holotype, LWZ 20170703-2). **a** Basidiospores **b** Basidia and basidioles **c** Hyphae from trama **d** Hyphae from lower context **e** hyphae from upper tomentum. Scale bars: **a** = 5 µm, **b–e** = 10 µm.

Additional specimens (paratypes) studied. (All on living vine of *Lonicera japonica*)—JAPAN. Nara, Research Forest of Faculty of Agriculture, Kindai University, 31 Oct 2016, LWZ 20161031-1 (IFP 019173); 27 Feb 2017, LWZ 20170227-1 (IFP 019174); 25 Mar 2017, LWZ 20170325-1 (IFP 019175); 22 Jun 2017, LWZ 20170622-1 (IFP 019176); 3 Jul 2017, LWZ 20170703-1 (IFP 019177).

Discussion

Phylloporia lonicerae is morphologically distinct from other species in *Phylloporia* by its annual, sessile basidiocarps that occur in clusters, pileal surface of narrow, concentrically sulcate zones, 6–8 pores per mm, duplex context separated by a black zone, dimitic hyphal system and broadly ellipsoid basidiospores, 3.2–4 × 2.3–3.1 μm. In the field, it is readily identified by fruiting on living vines, >1.5 cm diameter, of *Lonicera japonica*. *Phylloporia lonicerae* is most similar to *P. pseudopectinata* Yuan Y. Chen & B.K. Cui and *P. minutipora* L.W. Zhou by sharing annual, sessile basidiocarps in clusters and a dimitic hyphal system, but easily distinguished from *P. pseudopectinata* by larger pores (8–9 per mm) and subglobose basidiospores (Chen et al. 2017) and from *P. minutipora* by larger pores and basidiospores and the specific host (Zhou 2016). An updated key, based on Zhou (2016), to all accepted 48 species of *Phylloporia* is provided below.

Lonicera japonica is a well-known and important medicinal plant (Li 1578). Therefore, the potential medicinal applications of fungi growing on this plant are intriguing. Li et al. (2010) studied the medicinal metabolites from basidiocarps of *Phylloporia ribis* (Schumach.) Ryvarden that were collected on *Lonicera japonica* in China. Recent phylogenetic evidence, however, indicates that Chinese specimens of *P. ribis* collected on hosts other than *Ribes* are distinct from a *P. ribis* specimen collected on *Ribes* in Germany (Zhou and Dai 2012). As *P. ribis* was originally described from Denmark (Larsen and Cobb-Poulle 1990), *P. ribis* specimens used by Li et al. (2010) in their study are likely *P. lonicerae* or another undescribed species.

Some species of *Phylloporia* are parasitic and appear to be restricted by host and geographic distribution of its host. For example, *Phylloporia crataegi* L.W. Zhou & Y.C. Dai, which occurs exclusively on living *Crataegus* and *P. fontanesiae* L.W. Zhou & Y.C. Dai, which colonises living *Fontanesia*, are widely distributed in China (Zhou and Dai 2012, unpublished data). Similarly, in central African rainforests, *P. flabelliformis* Decock & Yombiy is found on living trunks of *Dichostemma* and *Anthostema* whereas *P. gabonensis* Decock & Yombiy occurs only on *Dichostemma* (Decock et al. 2015). In contrast, *Lonicera japonica* has a worldwide distribution and so far is host to a single species of *Phylloporia*. It will be interesting to determine if *P. lonicerae* is found elsewhere on *Lonicera japonica* or if different species of *Phylloporia* are found on living *Lonicera japonica* in other geographic regions.

Since 2010, 21 new species of *Phylloporia* have been described from China (Cui et al. 2010, Zhou and Dai 2012, Zhou 2013, 2015a, 2015b, 2016, Liu et al. 2015, Chen

et al. 2017, Ren and Wu 2017). Yet in Japan, only four *Phylloporia* species, including *P. lonicerae*, are known. It is hoped that this paper will draw attention to this genus in Japan and lead to the discovery of additional species.

Key to worldwide species of *Phylloporia*

1 Basidiocarps resupinate ..... ***P. parasitica***
– Basidiocarps sessile or stipitate **2**
2 Basidiocarps stipitate and terrestrial (woody debris) **3**
– Basidiocarps sessile and on aerial wood **9**
3 Context homogeneous ..... ***P. minutispora***
– Context duplex **4**
4 Basidiospores > 4 µm long, > 3 µm wide ..... ***P. verae-crucis*** **(Berk. ex Sacc.) Ryvarden**
– Basidiospores < 4 µm long, < 3 µm wide **5**
5 Cystidia present **6**
– Cystidia absent 7
6 Hyphae in tomentum short and anticlinal ..... ***P. elegans*** **Ferreira-Lopes, Robledo & Drechsler-Santos**
– Hyphae in tomentum loosely interwoven ..... ***P. nodostipitata*** **Ferreira-Lopes & Drechsler-Santos**
7 Pores < 10 per mm ..... ***P. spathulata***
– Pores > 10 per mm **8**
8 Basidiospores < 3.3 µm long, < 2.3 µm wide ..... ***P. terrestris***
– Basidiospores > 3.3 µm long, > 2.3 µm wide ..... ***P. afrospathulata*** **Yombiy & Decock**
9 Hyphal system dimitic **10**
– Hyphal system monomitic **18**
10 Basidiocarps perennial **11**
– Basidiocarps annual **12**
11 Pores 6–8 per mm ..... ***P. manglietiae*** **Yuan Y. Chen & B.K. Cui**
– Pores 8–11 per mm ..... ***P. pectinata*** **(Klotzsch) Ryvarden**
12 Basidiocarps solitary ..... ***P. nouraguensis*** **Decock & G. Castillo**
– Basidiocarps in cluster **13**
13 Pileal surface lighter (greyish-orange to pale cinnamon) ..... ***P. fulva*** **Yombiy & Decock**
– Pileal surface darker (yellowish-brown to dark brown) **14**
14 Pileus attached by a small vertex and pendant **15**
– Pileus widely attached to the substratum **16**
15 Pores 7–9 per mm; basidiospores > 3.5 µm long ..... ***P. pendula*** **Yuan Y. Chen & B.K. Cui**
– Pores 11–12 per mm; basidiospores < 3.5 µm long ..... ***P. pulla***

16 Pores 12–15 per mm; basidiospores < 3 µm long, < 2.5 µm wide..... ***P. minutipora***
– Pores 6–9 per mm; basidiospores > 3 µm long, > 2.5 µm wide..... **17**
17 Pores 6–9 per mm; basidiospores broadly ellipsoid (Q = 1.28–1.33)..... ***P. lonicerae***
– Pores 8–9 per mm; basidiospores subglobose (Q = 1.21–1.23)..... ***P. pseudopectinata*** **Yuan Y. Chen & B.K. Cui**
18 Pores 2–4 per mm..... **19**
– Pores 4–12 per mm..... **22**
19 Basidiospores broadly ellipsoid to subglobose..... ***P. fruticum*** **(Berk. & M.A. Curtis) Ryvarden**
– Basidiospores oblong-ellipsoid, subcylindrical to cylindrical..... **20**
20 Context duplex..... ***P. rzedowskii*** **R. Valenz. & Decock**
– Context homogeneous..... **21**
21 Context < 1 mm thick; on living branch..... ***P. oblongospora*** **Y.C. Dai & H.S. Yuan**
– Context 2–4 mm thick; on living trunk.. ***P. inonotoides*** **Yombiy & Decock**
22 Basidiocarps annual to perennial, dense and hard consistency..... **23**
– Basidiocarps annual, soft corky at least at tomentum layer..... **29**
23 Pores 10–12 per mm; on living *Tilia*..... ***P. tiliae*** **L.W. Zhou**
– Pores 6–9 per mm; on other angiosperms..... **24**
24 Pileal surface zonate and sulcate..... **25**
– Pileal surface azonate..... ***P. yuchengii*** **Gafforov, Tomšovský, Langer & L.W. Zhou**
25 Pores 6–7 per mm..... **26**
– Pores 7–9 per mm..... **27**
26 Basidiospores ellipsoid; mostly on *Ribes*..... ***P. ribis***
– Basidiospores subglobose; mostly on *Ephedra*, *Cotoneaster* or *Jasminum*..... ***P. ephedrae*** **(Woron.) Parmasto**
27 Basidiospores > 2.7 µm wide..... ***P. dependens*** **Y.C. Dai**
– Basidiospores < 2.7 µm wide..... **28**
28 Basidiospores ellipsoid to oblong-ellipsoid with a guttule; on *Abelia*..... ***P. gutta*** **L.W. Zhou & Y.C. Dai**
– Basidiospores broadly ellipsoid without a guttule; on living *Crataegus*..... ***P. crataegi***
29 Basidiospores broadly ellipsoid to subglobose..... **30**
– Basidiospores ellipsoid, oblong-ellipsoid to cylindrical..... **40**
30 Pores 5–6 per mm..... **31**
– Pores 6–11 per mm..... **35**
31 Context duplex, separated by a black zone..... **32**
– Context not separated by a black zone..... **33**

32 Pileal surface azonate, lower context 1–4 μm thick ..... ***P. ampelina* (Bondartsev & Singer) Bondartseva**

– Pileal surface zonate and sulcate, lower context 1 μm thick ..... ***P. weberiana* (Bres. & Henn. ex Sacc.) Ryvarden**

33 Basidiocarps solitary covered by a thick tomentum layer, pileal surface not radially faintly wrinkled ..... ***P. littoralis* Decock & Yombiy**

– Basidiocarps in cluster without a distinct tomentum layer, pileal surface radially faintly wrinkled **34**

34 Pileus < 1.5 mm thick, margin regular ..... ***P. flabelliformis***

– Pileus > 1.5 mm thick, margin irregular ..... ***P. gabonensis***

35 Basidiocarps > 8 cm wide, > 15 mm thick; contextual hyphae > 5 μm in diam ..... ***P. ulloai* R. Valenz., Raymundo, Cifuentes & Decock**

– Basidiocarps < 8 cm wide, < 15 mm thick; contextual hyphae < 5 μm in diam **36**

36 Contextual hyphae regularly arranged **37**

– Contextual hyphae interwoven **38**

37 Pileus distinctly sulcate, not radially striate, margin obtuse, basal context separated by two black zones; hyphae in tomentum > 4 μm in diam; on living angiosperm trunk ..... ***P. clausenae* L.W. Zhou**

– Pileus faintly sulcate, radially striate, margin sharp, context duplex thoroughly; hyphae in tomentum < 4 μm in diam; on living liana ..... ***P. radiata* L.W. Zhou**

38 Contextual hyphae slightly thick-walled with a wide lumen, frequently septate, large rhomboid crystals absent **39**

– Contextual hyphae thick-walled with a narrow lumen, occasionally septate, large rhomboid crystals present in trama and context ..... ***P. chrysites***

39 Pores 10–12 per mm; basidiospores < 3 μm long; on living *Fontanesia* ..... ***P. fontanesiae***

– Pores 7–9 per mm; basidiospores > 3 μm long; on other angiosperms ..... ***P. oreophila* L.W. Zhou & Y.C. Dai**

40 Basidiospores mostly > 3 μm wide **41**

– Basidiospores mostly < 3 μm wide **42**

41 Pores 4–6 per mm ..... ***P. hainaniana* Y.C. Dai & B.K. Cui**

– Pores 8–10 per mm ..... ***P. capucina* (Mont.) Ryvarden**

42 Basidiocarp solitary **43**

– Basidiocarp imbricate **46**

43 Context homogeneous ..... ***P. homocarnica* L.W. Zhou**

– Context duplex **44**

44 Context not separated by a black zone; on living *Flacourtia* ..... ***P. flacourtiae* L.W. Zhou**

– Context separated by a black zone; on other angiosperms **45**

45 Pileal surface azonate, pores 6–8 per mm; basidiospores cylindrical.....
.....***P. cylindrispora*** **L.W. Zhou**
– Pileal surface zonate and sulcate, pores 8–9 per mm; basidiospores ellipsoid.....
.....***P. lespedezae*** **G.J. Ren & F. Wu**
46 Basidiospores mostly < 2.5 µm wide **47**
– Basidiospores mostly > 2.5 µm wide ..... ***P. bibulosa***
47 Pores 5–6 per mm, context duplex, not separated by a black zone; basidiospores > 3.5 µm long, contextual hyphae interwoven; on living *Nandina*....
.....***P. nandinae*** **L.W. Zhou & Y.C. Dai**
– Pores 7–9 per mm, context duplex, separated by a black zone; basidiospores < 3.5 µm long, contextual hyphae regularly arranged; on living *Osmanthus*...
.....***P. osmanthi*** **L.W. Zhou**

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COMMENTARY

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New light on names and naming of dark taxa

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Abstract

A growing proportion of fungal species and lineages are known only from sequence data and cannot be linked to any physical specimen or resolved taxonomic name. Such fungi are often referred to as "dark taxa" or "dark matter fungi". As they lack a taxonomic identity in the form of a name, they are regularly ignored in many important contexts, for example in legalisation and species counts. It is therefore very urgent to find a system to also deal with these fungi. Here, issues relating to the taxonomy and nomenclature of dark taxa are discussed and a number of questions that the mycological community needs to consider before deciding on what system/s to implement are highlighted.

Keywords

Taxonomy, nomenclature, mycology, biodiversity

Introduction

> *The first step in wisdom is to know the things themselves; this notion consists in having a true idea of the objects; objects are distinguished and known by classifying them methodically and giving them appropriate names. Therefore, classification and name-giving will be the foundation of our science.*
>
> Linnaeus (1735)

Public DNA sequence databases abound with fungal entries that defy all attempts at taxonomic identification. These poorly understood lineages are known from more or less all imaginable substrates and environments, including soil, wood and water but also spacecraft, tumuli and residential areas (e.g. Nilsson et al. 2016). They have been referred to as "dark taxa" or "dark matter fungi" (Page 2016; Grossart et al. 2016) and most of them likely represent undescribed taxa (Tedersoo and Smith 2017), while a limited proportion presumably represents described, but never before sequenced, taxa (cf. Nagy et al. 2011). The number of dark taxa is growing rapidly in the wake of the increasing use of sequence-based approaches to characterisation of biodiversity. Today, few researchers would contest that dark taxa merit both scientific and societal interest, and there is a growing need to classify and communicate these taxa and to record and accumulate data on them. Biodiversity data collections and legislation, in sharp contrast, are largely centred on names of species and higher groups. Being nameless, the dark taxa are not easily incorporated into many of these contexts and are consequently often omitted. They are, for example, usually left out of species counts of areas and therefore from decisions on nature conservation. This unsatisfactory situation where much of our resources and infrastructure cannot properly accommodate dark taxa has spurred a debate in the life sciences on how they should be handled (Samyn and De Clerck 2012; Patterson 2014).

The mycological community has been aware of the problem of dark taxa for a long time (Nilsson et al. 2005; Porter et al. 2008; Ryberg et al. 2008; Hibbett et al. 2009) and several solutions have been proposed. The UNITE database for molecular identification of fungi, for instance, has presented a system where ITS sequence clusters are presented as species hypotheses tagged with unique digital object identifiers (DOIs) to facilitate unambiguous communication (Kõljalg et al. 2016), while Hawksworth et al. (2016) suggested that it should be possible to publish valid names under the International Code of Nomenclature for algae, fungi and plants (ICN) using a DNA sequence, instead of a physical specimen, as type. However, there is no consensus on how the dark taxa should be handled and mycology clearly faces a number of extremely difficult questions in the very near future. These questions will dictate how we refer to, name and to some extent identify, fungi – in short, how mycology is done. The pivotal nature of the pending decisions suggests that all perspectives and points of view must be brought to the surface and vetted thoroughly. Yet it is felt, when following the debate, that this has not been the case so far. In this opinion piece, the authors wish to clarify several overlooked and perhaps obscured aspects of dark taxa and their naming. While a stand will not be taken regarding whether DNA sequences should suffice as species types, it is hoped that several matters that should be resolved will be identified before such a decision can be made. This commentary's contribution is meant for mycologists at large, because dark taxa concern mycology at large. Through longstanding work with difficult-to-identify fungal lineages from soil and other environmental samples, the authors can attest to the frequency and the widespread nature of dark taxa across the fungal tree of life. Disregarding these un-named taxa for the

simple reason that they lack a formal name would be a severe, costly mistake – and one that has already impeded mycology for far too long. This is an issue whose resolution can no longer be postponed.

Nomenclature and taxonomy

In the context of dark taxa, the distinction between taxonomy (delimiting and characterising taxa) and nomenclature (naming and, to some extent, diagnosing taxa) must be stressed. While these are connected and commonly discussed together, blurring their distinction, they are fundamentally different (de Queiroz 2006). The ICN only governs taxon names and the process of naming taxa; it is by design essentially silent on the processes of characterising and delimiting taxa. A name can therefore be the correct name for a taxon according to the code, even if the taxon delimitation/characterisation with which it was published does not suit the taxon. In analogy, a taxon description can be suitable for a taxon even if the name with which it is published is not the correct name or even a validly published name.

According to the ICN, only a validly published name can be considered for the correct name of a taxon (the name that should be used for the taxon). Two of the requirements for a name to be validly published are that it is published with a diagnosis or description and that a type is designated. The diagnosis can be based on molecular characters (Tripp and Lendemer 2014; Sheikh et al. 2017), while the type of a species name needs to be a physical specimen or exceptionally, an illustration of a specimen. Names of taxa of higher rank have a taxon at the immediate lower rank as their type and thus also refer back to a type specimen in the end. The type indicates what taxon a name refers to, and all validly published names whose type is included in the taxon should be considered for that taxon. Determining which name goes where is sometimes made more complicated by the fact that types may be difficult to get hold of or may even be missing altogether for some names. In other cases, the type may not manifest all characters needed to clarify to which taxon it belongs. When the type is missing or lacking important characters, new or additional types can be designated (neo- and epitypes, respectively; Ariyawansa et al. 2014). The basic principle to establish the correct name for a taxon is that the first validly published name should be used, although there are exceptions to this rule. Given that all validly published names must be considered for a taxon, an inflation in names that are difficult to interpret or apply may hamper taxonomy more than an inflation in species descriptions without valid names.

Since the description of taxa is only controlled by the regular scientific principles for publishing, taxa may be described and diagnosed based solely on molecular characters. Such taxa can also be given a name (De Beer et al. 2016), although such names cannot be considered validly published unless there is a specimen to serve as type. This is what the Hawksworth et al. (2016) proposal seeks to amend. The issue with dark taxa is thus not that they cannot be described, but that they cannot be given formal names for communication.

What are names for?

Names are used for communicating objects and concepts. Unless an object or concept is very straightforward indeed, the lack of a name is a major obstacle in its communication and may – implicitly or not – be taken to mean that its communication is not necessary to begin with. This is a general societal issue, but it certainly pertains to mycology as well. Many newly described taxa have, in fact, been represented in DNA sequence databases for 10 years or more before they piqued somebody's interest or were possible to typify according to the ICN (see examples in Nilsson et al. 2016). Upon closer inspection, several of these taxa were found to be both ubiquitous and of significant taxonomic and ecological interest (Rosling et al. 2011; James and Seifert 2017). There is, thus, data to suggest both that the lack of names for dark taxa have retarded progress on their study and that there is ample reason to study and communicate dark taxa in the first place.

Communication of taxa includes aspects such as incorporation into biodiversity datasets, sequence repositories and legislation but also regular scientific and societal communication. What type of name to use will depend on the identity of the communicating parties. For computers, accession numbers such as DOIs will suffice for communication. DOIs are, however, less suitable for human communication (e.g. http://dx.doi.org/10.15156/BIO/SH004915.07FU versus *Vishniacozyma victoriae*). For scientists, latinised binomials may work well, while society at large may prefer vernacular names.

For efficient communication, it is important to consider that one taxon should have only one name and that any name should refer to only one taxon. One of the major purposes of the ICN is to ensure and uphold these relations, while vernacular names are not governed by and, indeed, often violate such rules. The use of parallel naming systems does little to facilitate unambiguous naming in biological systematics. For instance, one and the same name can be the correct or valid name for different taxa under the ICN and the International Code for Zoological nomenclature at the same time, e.g. *Erica* in Ericaceae (Viridiplantae) and Arachnida (Metazoa). For ambiregnal taxa, different names may be the correct/valid name under different nomenclature codes. For instance, a dinoflagellate genus was named *Phalacroma* under the ICN, a name that subsequently was found to be already occupied under the zoological code. Thus, the name *Prodinophysis* was introduced for the same genus (Taylor et al. 1987). In the case where different names are used for the same taxon, databases can link the different uses of names across the systems.

Delimitation of taxa

Descriptions of taxa may be based on different sets of characters, for example sexual or asexual reproductive structures, physiological parameters or DNA bases. It may therefore be difficult to tell whether a taxon, described based on one type of character, is the same as a taxon described from another character type or set of characters. This is the basis behind both the former dual system of naming for "Eumycota" vis-à-vis "Deuteromycota" and the situation which is now faced with dark taxa. In the case of

dark taxa, it is not immediately clear how to correlate a species delimited from environmental sequence data to, say, a range of physiological parameters quantified in the lab or a handful of morphological traits gleaned from microscopy studies of soil samples. Obtaining such additional data and mapping them to individual species will not be straightforward from heterogeneous, mixed-species substrates such as soil and water, but emerging single-cell techniques (e.g. Castelle et al. 2015) offer promise in this regard. As overlapping character sets gradually become available, improved understanding of the underlying taxon will follow piece by piece and the correct name can eventually be assigned according to the nomenclature code. In the context of "Eumycota" and "Deuteromycota", molecular data are often used to link the teleomorph and anamorph stages of species, thus resolving the issue (e.g. Piątek et al. 2017). This is also the reason why sequence data from type specimens are very valuable to sort out nomenclature and DNA barcoding issues and to bring dark taxa under the realm of taxonomy by providing them with a name (Robbertse et al. 2017; Torres-Cruz et al. 2017).

As taxon delimitation and naming are two different things, another complication in what a name refers to is that different taxonomists may advocate different circumscriptions of taxa while the name itself is determined by the ICN. In these cases, the same name may be the correct name for different taxa, with little overlap in the underlying organisms. Furthermore, a name can be correct for some taxon or a synonym of another name depending on the specific taxonomy. If it is required that taxa be monophyletic, then changes in the taxonomy should be expected due to changes in the understanding of evolutionary relationships. Even if this stabilises with time as better estimates of evolutionary relationships are obtained, there may still be conflicts as to what clades are considered as taxa and at what taxonomic level. For example, Hibbett et al. (2007) treat Monoblepharidomycetes as a class in Chytridiomycota while Powell and Letcher (2014) classify it in the monotypic phylum Monoblepharidomycota. Similarly, the small genus *Entorrhiza* is variously recognised as a basidiomycete lineage or as a separate phylum depending on what resource is turned to (Bauer et al. 2015).

The species level is often viewed as a separate evolutionary lineage of special standing (Mayden 1997; but see, e.g. Baum 2009), but there will still be disagreement on how species are delimited. Any species delimitation will always be a hypothesis and different lines of evidence may disagree as to which hypothesis is best supported. Although molecular data provide significant explanatory power in systematics and taxonomy, their use is not devoid of complications. Clustering of sequences into operational taxonomic units, for instance, depends on the choice of clustering algorithm and the parameter settings used (e.g. selection of sequence similarity cut-off) as well as the choice of genetic marker and the individual sequences to be clustered. Thus, equating a sequence-derived operational taxonomic unit with a species is problematic (Schoch et al. 2012; Ryberg 2015). A sequence-derived operational taxonomic unit may, nevertheless, be a species hypothesis.

Without a reference to which taxonomy is employed, what is referred to by a name is more or less ambiguous. The UNITE species hypothesis system provides an unambiguous way to refer to sets of sequences at approximately the species level and additions and removals of sequences to those species hypotheses can be traced back in time

(e.g. https://unite.ut.ee/bl_forw_sh.php?sh_name=SH181628.07FU). However, this approach is limited to sequences included in the underlying dataset, the given set of hypotheses and to taxa represented by ITS sequence data in the first place. Changes between taxonomies are a part of the progress in understanding nature and only scientific advances, together with a dialogue amongst scientists to arrive at a consensus, can resolve this problem. However, without some sort of names for communication such progress seems difficult.

Outlook

The number of dark taxa increases with more or less every new metabarcoding study, but the pace at which these taxa are formalised is many orders of magnitude slower (James and Seifert 2017). This hints at an untenable situation and it is becoming increasingly clear that a system to handle dark taxa in the context of taxonomy, nomenclature and biodiversity at large is needed. The authors plea for the adoption of dark taxa into regular mycology and argue for an expedient establishment of a system or approach to handle dark taxa in mycology and elsewhere. When constructing such a system, many urgent questions present themselves. What are being communicated: sequence clusters, taxa at an undefined level or taxa as recognised by the ICN? Who are communicating: is it computers, scientists, the society at large or any combination of these? If a system with computer-facilitated communication on sequence clusters is wanted, UNITE already fills this role. If a system with a flexible set of taxonomic hypotheses that consider more than just ITS sequence distances is also wanted, something more is needed. For such a system, the impact on biodiversity research should also be considered. Will it encourage and/or deter research and, if so, what kind of research? Should it, unlike the traditional nomenclature codes, encourage best practices in taxonomy? If so, will it achieve these best practices or will it engender poor practices and increased confusion? There is clearly a risk that allowing sequences as types will inflate the number of (rogue?) names and serve to hamper taxonomy in the end (cf. Seifert 2018). As sequences are fundamentally different from a physical specimen, consideration should also be given as to how well sequences will serve as types. It is true that they are perfectly well amenable to digitalisation and that they are easy to share and compare. At the same time, they contain little additional information if further taxonomic resolution is needed and may increase the need for epitypes. Finally, it should be asked if the aim is a separate, DNA-based system for the dark taxa or an integrated system including other characters and taxa too. If the aim is comparable units and not the creation of disjunct taxonomic systems, one system is certainly recommended.

These questions are urgent, because dark taxa permeate mycology and the fungal tree of life, and *ad hoc* names are being used to communicate them without any system to ensure stability of those names (De Beer et al. 2016; Tedersoo and Smith 2017). The answers to the above questions are not immediately clear and they may furthermore differ depending on personal perspectives. A heated debate is therefore expected, perhaps without any consensus at the end. Whatever system is implemented, there needs to be an active discussion in the mycological community as to what confidence should

be required for the named taxa and whether a specific system should be implemented to safeguard these quality measures (cf. Tedersoo et al. 2015).

Seifert (2018) asks what is the point of a name when there is no additional information attached to it. At the same time, he gives a species the name "the brain fungus" (not valid according to the ICN) to be able to talk about it and makes a plea for more information on it. However, without a stable and precise name as an identifier, it will be difficult to accumulate precise knowledge about exactly this species. A name is not an end to our understanding of a taxon, but a means and a beginning. If mycology is to be the study of all fungi and not just the perhaps < 10 % which can be readily observed, then dark taxa should be welcomed into the light.

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RESEARCH ARTICLE

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The genus *Parasola* in Pakistan with the description of two new species

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Abstract

Parasola is a genus of small, veil-less coprinoid mushrooms in the family Psathyrellaceae (Agaricales). The genus is not well documented in Asia, specifically in Pakistan. In this study we describe two new species *Parasola glabra* and *P. pseudolactea* from Pakistan, based on morphological and molecular data. Phylogeny based on three DNA regions: nuc rDNA region encompassing the internal transcribed spacers 1 and 2 along with the 5.8S rDNA (ITS), nuc 28S rDNA D1-D2 domains (28S) and translation elongation factor 1*a* gene (*TEF1a*) show that the new taxa are clustered in a clade formed by the members of section *Parasola* of genus *Parasola*. *Parasola glabra* with grayish pileus, slightly depressed pileal disc, lamellae separated from the stipe by pseudocollarium, basidiospores 14.5–16.5 × 9.5–11.5 × 8.0–10.5 µm, in front view broadly ovoid to oblong, some with rhomboidal outline, in side view ellipsoid, with eccentric germ-pore of 1.5 µm diameter. *Parasola pseudolactea* with yellowish brown to dull brown pileus, disc indistinctly umbonate, lamellae free, pseudocollarium absent, basidiospores 13.5–14.5 × 10.5–12.0 × 9.5–10.5 µm, in face view rounded triangular to heart shaped, rarely ovoid to subglobose, in side view ellipsoid to oblong, with eccentric germ-pore of 1.5 µm diam. In addition to these new species, *P. auricoma* and *P. lilatincta* were also studied. Morphological descriptions for the new species and comparison with known *Parasola* species are provided. Our observations highlight the diversity of *Parasola* in northern Pakistan and further document the need for additional systematic focus on the region's fungi.

Keywords

Basidiomycota, diversity, *Parasola*, phylogeny, taxonomy

Introduction

Parasola Redhead, Vilgalys & Hopple is a genus of small, veil-less coprinoid mushrooms belonging to family Psathyrellaceae Vilgalys, Moncalvo & Redhead (Redhead et al. 2001, Nagy et al. 2009, Schafer 2010). These fungi are saprotrophs of decayed organic matter in bare soil, grassland, on woody debris including wood chips and on herbivore dung (Schafer 2014). The genus *Parasola* typified by *Parasola plicatilis* (Curtis) Redhead, Vilgalys & Hopple (Redhead et al. 2001), currently comprises 18 established species, distributed world-wide. The genus is well documented in Europe (Orton and Watling 1979, Uljé and Bas 1988, Uljé and Bender 1997, Schafer 2014, Szarkándi et al. in press). Some species are reported from North America, Africa, Lesser Antilles (Pegler 1966, 1983, Dennis 1970), and Asia (Ahmad 1980, Pegler 1986, Hongo 1987, Hussain et al. 2016, 2017) and Australia (Grgurinovic 1997).

Species of *Parasola* are divided into section *Auricomi* (Singer) D.J. Schaf. and section *Parasola* Redhead, Vilgalys & Hopple (previous references to *Parasola* section *Glabri* (Lange) D.J. Schaf. – see Schafer (2010) – should be replaced by *Parasola* section *Parasola* to conform with the International Code of Nomenclature for Algae, Fungi and Plants (Schafer, D.J., personal communication). The sections are distinguished on the basis of presence or absence of hair-like, golden- to dark brown, thick walled sclerocystidia in the pileipellis (Schafer 2010). In mature fruitbodies during basidiospore discharge, the gill cystidia of *Parasola* lose turgor and collapse, a characteristic feature of the genus (Nagy et al. 2009).

Basidiospore shape and size are the main descriptive features for species identification in *Parasola* (Nagy et al. 2009, 2010, Schafer 2014, Hussain et al. 2017).

Previously, five species of this genus (*Parasola auricoma* (Pat.) Redhead, Vilgalys & Hopple, *P. lilatincta* (Bender & Uljé) Redhead, Vilgalys & Hopple, *P. malakandensis* S. Hussain, N. Afshan & H. Ahmad, *P. plicatilis* and *P. setulosa* (Berk. & Broome) Redhead, Vilgalys & Hopple) have been reported from Pakistan (Ahmad 1980, Hussain et al. 2016, 2017). In this study, we describe two new species *P. glabra* and *P. pseudolactea*, based on morphological characters and phylogenetic analyses of nuc rDNA region encompassing the internal transcribed spacers 1 and 2, along with the 5.8S rDNA (ITS), nuc 28S rDNA D1-D2 domains (28S) and translation elongation factor 1α gene (*TEF1α*). In addition to these new species we also studied *P. auricoma* and *P. lilatincta.*

Materials and methods

Sampling and morphological characterization

Specimens were collected from Malakand, Shangla and Swat districts of Khyber Pakhtunkhwa, Pakistan in summer seasons, 2013–2017. Basidiomata were photographed, tagged and field notes were made. Munsell (1975) was used for determination of color. The specimens were air-dried and kept in zip-lock bags. Specimens examined in this study

are deposited in the Herbaria of Hazara University Mansehra, Pakistan (HUP), University of the Punjab, Lahore, Pakistan (LAH) and University of Swat, Pakistan (SWAT).

For anatomical studies slides were prepared in 5% aqueous KOH (w/v). Microscopic features such as size and shape of basidiospores, basidia, cheilocystidia, pleurocystidia and pileipellis were studied under a light microscope (MX4300H, Meiji Techo Co., Ltd., Japan) with at least 20 structures measured in each instance. Cheilocystidia and pleurocystidia were observed and measured by cutting the gill edge from the rest of gill to avoid confusion between the two types of cystidia. In the case of basidiospores, 50 spores were measured in face view and/or side view through 1000× magnification with a calibrated optical micrometer and measurements were rounded to the nearest 0.5 µm. Basidiospores measurements are presented as follows: length range × breadth range × width range. Q values were calculated as: Q_1 = length divided by breadth; Q_2 = length divided by width (Nagy et al. 2010).

DNA extraction, PCR and sequencing

We extracted genomic DNA using the DNeasy Plant Mini Kit (Qiagen, Redwood City, California, USA.). We amplified nuc rDNA internal transcribed spacer (ITS) and 28S loci and translation elongation factor 1α gene (*TEF1a*) using the primer combinations ITS1F/ITS4; LR0R/LR5 and EF1-983F/EF1-1567R, respectively (White et al. 1990, Gardes and Bruns 1993, Rehner and Buckley 2005). For PCR amplification, we followed Hussain et al. (2017). PCR products were purified using the QIAquick PCR Purification kit (Qiagen). Sequencing was performed with the same PCR primers using the Big Dye Sequencing Kit v.3.1 on an ABI-3730-XL DNA Analyzer (Applied Biosystems, Foster City, California, USA). Sequences produced for this study have been deposited in GenBank (Table 1).

Alignments and phylogenetic inference

ITS, 28S and *TEF1a* sequences were aligned using BIOEDIT v 7.2.5 (Hall 1999) and CLUSTAL X 2.1 (Larkin et al. 2007). The ITS, 28S and *TEF1a* alignments were concatenated into a supermatrix. *Psathyrella candolleana* (Fr.) Maire was selected as outgroup. Alignments are submitted to TreeBase (Treebase ID 21639). Phylogenetic inference was conducted using Bayesian and Maximum Likelihood (ML) methods. For Bayesian inference, we used BEAST 1.6.2 (Drummond and Rambaut 2007) with a Markov chain Monte Carlo (MCMC) coalescent approach. A Yule tree prior (Gernhard 2008) was used in all simulations, and the starting tree was randomly generated. Four independent runs were undertaken. Chain length was 10 million generations, with a sampling frequency of 1000. TRACER 1.6 (Rambaut et al. 2014) was used to check the effective sample size (ESS), and burn in values were adjusted to achieve an overall ESS (Effective Sample Size) of ≥ 200. Maximum clade credibility tree (20% burn-in) was generated

Table 1. Voucher numbers, geographic origins and GenBank Accession numbers for the specimens included, in boldface are sequences produced in this study.

Species	Geographic origin	Voucher number	GenBank Accessions		
			ITS	28S	*TEF1α*
Parasola auricoma	**Pakistan**	**LAH-SHP-P6**	KX212106	**KY461729**	**MG587083**
	Pakistan	**LAH-SHP-P7**	**KY461721**	**KY461730**	**MG587084**
	Pakistan	**LAH-SHP-P11**	**KY621802**	**KY461728**	
	Hungary	NL0268	FM163186	FM160723	
	Hungary	NL0087	FM163185	FM160724	FM897236
P. conopilus	Hungary	NL0465	FM160686	FM163223	
	Hungary	NL0286	FM160685	FM163224	
	Hungary	NL0285	FM160684	FM163225	KJ732832
P. glabra	**Pakistan**	**LAH-SHP-5** (Holotype)	**KY461717**	**KY621806**	**KY461735**
	Pakistan	**HUP-SHP-23**	**KY461718**	**KY621805**	
P. hercules	Netherlands	Uljé 1269 (L)	FM163190	FM160719	
	Netherlands	L146 holotype	HQ847027	HQ847112	
P. kuehneri	Netherlands	Uljé 904 (L)	FM163191	FM160718	
P. lactea	Hungary	NL0466	FM163192	FM160717	FM897241
	Sweden	NL0095	FM163188	FM160721	
	Germany	NL0283	FM163194	FM160715	FM897239
	Sweden	NL0288	FM163193	FM160716	
	Hungary	NL6601	FM163187	FM160722	
	USA	MICH232885	KM403384		
	Latvia	KuP6.2.2.1	KP698198		
P. pseudolactea	**Pakistan**	**HUP-SU-412** (Holotype)	**KY461719**	**KY621799**	**KY461733**
	Pakistan	**HUP-SU-413**	**KY461720**	**KY621800**	**KY461734**
P. lilatincta	**Pakistan**	**LAH-SHP-8**	**KY461722**	**KY461725**	**KY461731**
	Pakistan	**LAH-SHP-31**	**KY461723**	**KY461726**	**KY461732**
	Pakistan	**LAH-SHP-12**	**KY461724**	**KY461727**	
	Hungary	NL0683	FM163203	FM160706	FM897231
	Hungary	NL0660	FM163195	FM160714	FM897230
	Hungary	NL0472	FM163199	FM160709	
	Hungary	NL0667	FM163198	JQ045886	
	Pakistan	SH4	KP886462		
	Pakistan	SHP2	KP886463		
	Pakistan	SHP9	KP886464		
P. aff. *lilatincta*	Hungary	NL0086	FM163204	FM160705	
	Sweden	NL0096	FM163205	FM160704	
P. megasperma	Denmark	C 19683	FM163206	FM160703	
	Sweden	NL1924	FM163208	FM160701	FM897232
P. malakandensis	Pakistan	LAH-SHP-17	KU599827	KU599830	KU599832
	Pakistan	HUP 17501	KP738713	KU599829	KU599831
P. misera	Hungary	NL0677	FM160698	FM163211	FM897240
	Hungary	NL0280	FM160699	FM163210	
	Hungary	NL0490	FM163209	FM160700	
P. plicatilis	Sweden	NL0477	FM163212	FM160697	FM897235
	Hungary	NL0295	FM163216	FM160693	FM897242
P. plicatilis	Sweden	NL0097	FM163215	FM160694	
	Hungary	NL0075	FM163214	FM160695	
	Hungary	NL0284	FM163189	FM160720	
P. schroeteri	Netherlands	LBrier:1051999	FM163219	FM160690	
P. setulosa	Hungary	L32	HQ847030	HQ847115	
Parasola sp.	Norway	NL3167	JN943136	JQ045865	
Parasola sp.	Norway	NL3621	JN943134	JQ045875	

Species	Geographic origin	Voucher number	GenBank Accessions		
			ITS	28S	*TEF1α*
Parasola sp.	Hungary	NL4175	HQ847025	HQ847110	
Parasola sp.	Hungary	NL0287	FM163218	FM160691	
Parasola sp.	Hungary	NL2952	HQ847028		
Psathyrella candolleana	Hungary	NL2937	FN396114	FN396165	FN396220

using TREEANNOTATOR 1.6.2 (Drummond and Rambaut 2007). Maximum Likelihood analyses were run in RAXML-VI-HPC (Stamatakis 2006). Rapid bootstrap analysis/search for best-scoring ML tree (-f a) was configured. For the bootstrapping phase, the GTRCAT model was selected. One thousand rapid bootstrap replicates were run. Nodes were considered strongly supported when maximum likelihood bootstrap (MLB) were ≥ 70% and Bayesian posterior probability (BPP) were ≥ 0.95.

Results

Phylogenetic analyses

Sequence length varied from 631 bp (SHP-8) to 644 bp (SHP-11) for our 10 new ITS (ITS1-5.8S-ITS2) sequences and 1042 bp (SHP-12) to 1144 bp (SHP-8) for 10 28S sequences. The 7 *TEF1a* sequences generated for this study varied from 402 bp (SHP-5) to 502 bp (SU-412). The ITS dataset contained 52 taxa and 631 characters long after being trimmed (Trimming was done manually in BIOEDIT v 7.2.5). The combined ITS-28S dataset represented 47 taxa and 1892 characters long after being trimmed. Similarly, the combined ITS-28S-*TEF1a* dataset comprised 20 species and with 2890 nucleotides, after being trimmed.

The results of phylogenetic analyses of ITS, ITS-28S and combined ITS-28S-*TEF1a* datasets are summarized in Figures 1, 2 and 3, respectively. Each tree represents ML phylogeny produced by RAXML analysis. Maximum likelihood bootstrap (MLB) percentages > 70% are given above or below the branch node, followed by Bayesian posterior probabilities (BPP) > 0.95. The novel sequences in this study are represented in boldface (Figures 1, 2 and 3), their Genbank accessions are provided in Table 1.

Using Bayesian and ML methods, *P. auricoma*, *P. conopilus*, *P. setulosa* and *P. malakandensis* were recovered as basal groups with strong support, collectively forming section *Auricomi*, whereas species of section *Parasola* fall in a single clade represented as gray highlighted, called 'the crown *Parasola*' clade (Nagy et al. 2009). Statistical support for the specimens that represent *P. pseudolactea* was strong in ITS dataset (MLB 98% and BPP 1), and excellent in combined ITS-28S and ITS-28S-*TEF1a* datasets, respectively (MLB 100% and BPP 1). Similarly, statistical support for *P. glabra* in both ITS and combined ITS-28S datasets was maximal (MLB 100% and BPP 1). In combined ITS-28S-*TEF1a* dataset *P. glabra* was represented by a single specimen and poorly recovered (Figure 3).

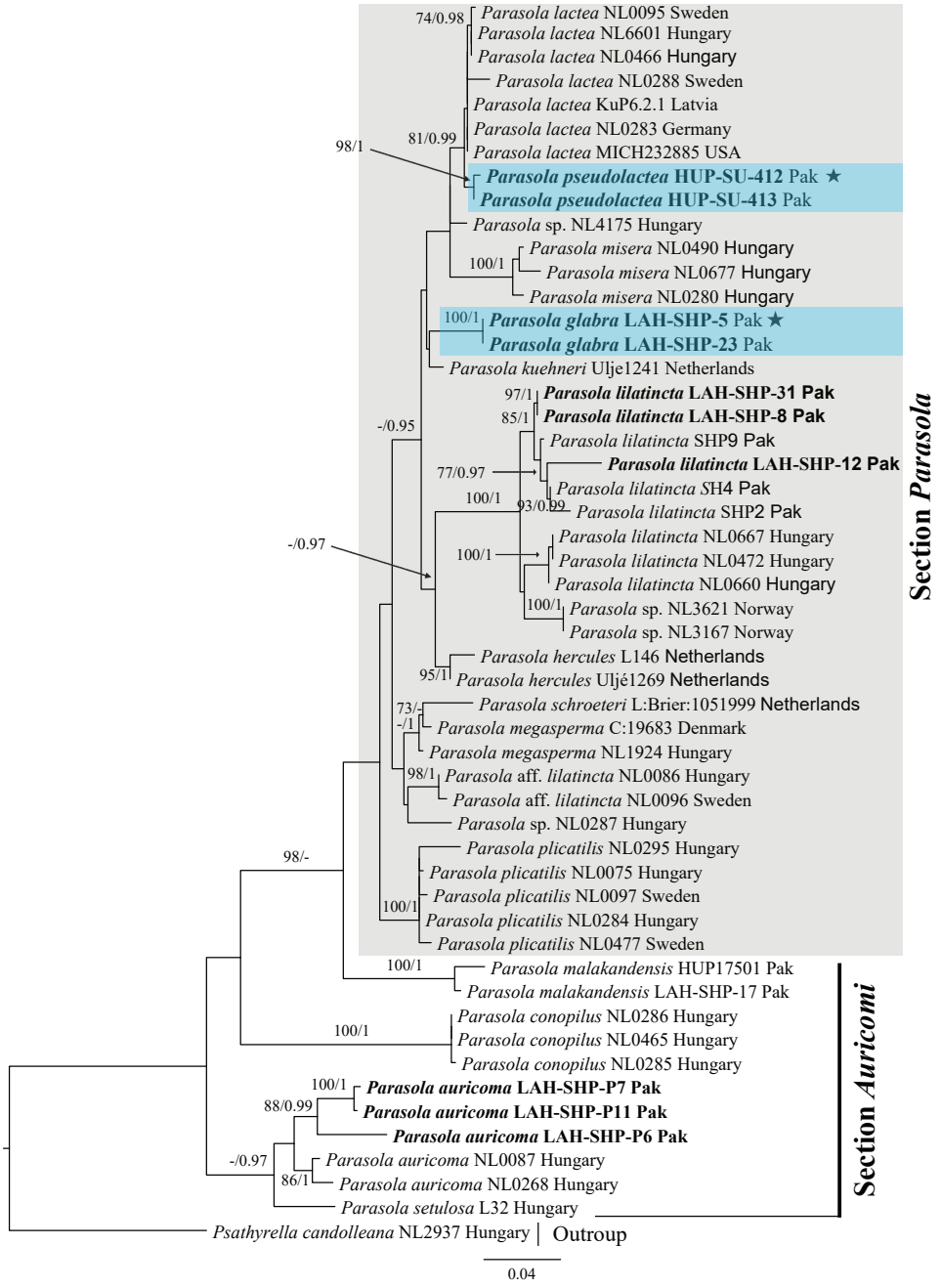

Figure 1. Phylogeny of *Parasola* species based on 52 ITS sequences. Our sequences are indicated in boldface. Other sequences are from Nagy et al. (2009). Numbers above or below branches indicate maximum likelihood bootstrap percentages followed by Bayesian posterior probabilities. Species in section *Parasola* are gray highlighted where the new species are shown as light-blue highlighted, while the HOLOTYPE collection for *P. glabra* (LAH-SHP-5) and *P. pseudolactea* (HUP-SU-412) are represented by stars (*).

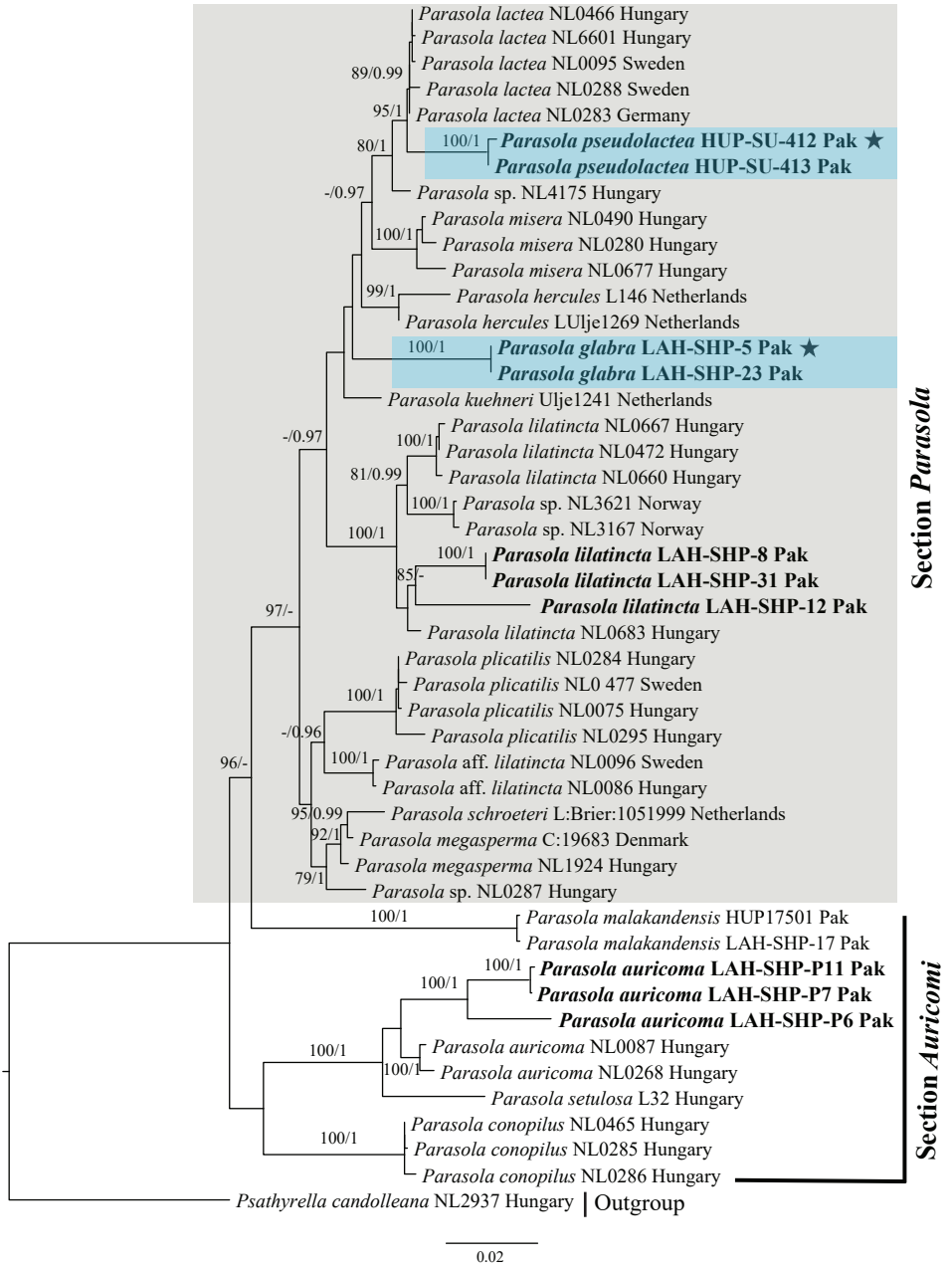

Figure 2. Phylogeny of *Parasola* species based on 47 sequences of combined ITS-28S dataset. Our sequences are indicated in boldface. Other sequences are from Nagy et al. (2009). Numbers above or below branches indicate maximum likelihood bootstrap percentages followed by Bayesian posterior probabilities. Species in section *Parasola* are gray highlighted where the new species are shown as light-blue, while the HOLOTYPE collection for *P. glabra* (LAH-SHP-5) and *P. pseudolactea* (HUP-SU-412) are represented by stars (*).

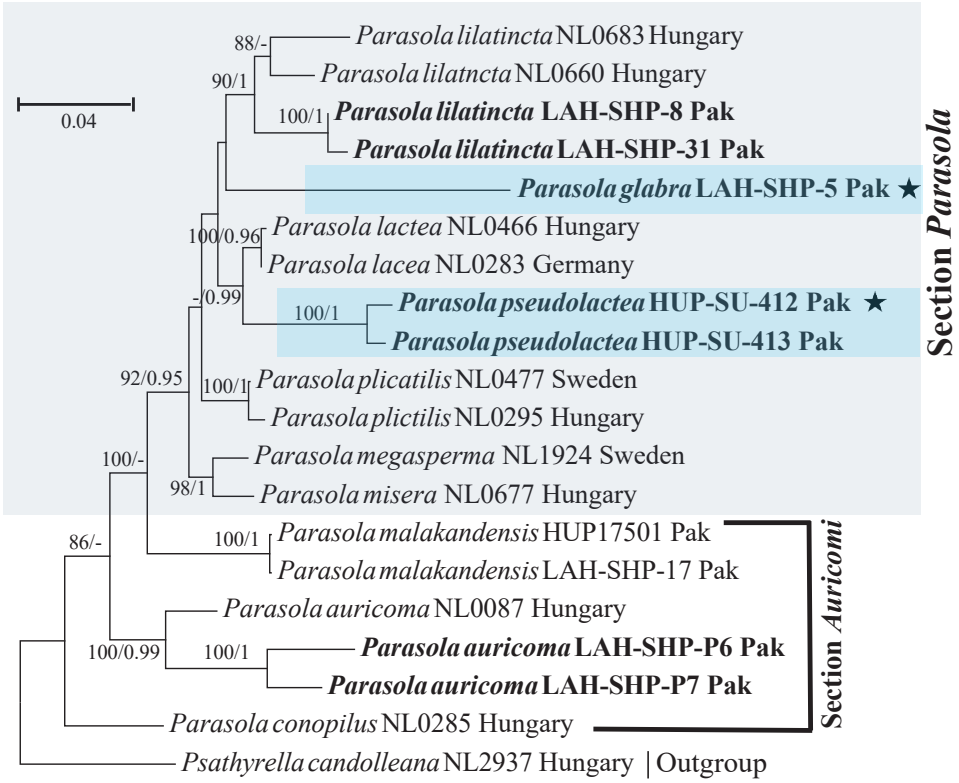

Figure 3. Phylogeny of *Parasola* species based on 20 sequences of combined ITS-28S-*TEF1a* dataset. Our sequences are indicated in boldface. Other sequences are from Nagy et al. (2009, 2011). Numbers above or below branches indicate maximum likelihood bootstrap percentages followed by Bayesian posterior probabilities. Species in section *Parasola* are light-brown highlighted where the new species are shown as light-blue, while the HOLOTYPE collection for *P. glabra* (LAH-SHP-5) and *P. pseudolactea* (HUP-SU-412) are represented by stars (*).

Taxonomy

Parasola glabra **Hussain, Afshan, Ahmad & Khalid, sp. nov.**
MycoBank: MB819601
Figures 4, 5

Diagnosis. The diagnostic features of *Parasola glabra* are grayish pileus, deeply plicate towards margin; disc slightly depressed, strong reddish orange; lamellae free, separated from the stipe by pseudocollarium; basidiospores 14.5–16.5 × 9.5–11.5 × 8.0–10.5 µm, in front view broadly ovoid to oblong, some with rhomboidal outline, in side view ellipsoid, with eccentric germ-pore of 1.5 µm diam.

Type. PAKISTAN. Khyber Pakhtunkhwa Province, Malakand, Qaldara, scattered under herbaceous plants, 480 m alt., 15 August 2014, S. Hussain SHP5 (holotype:

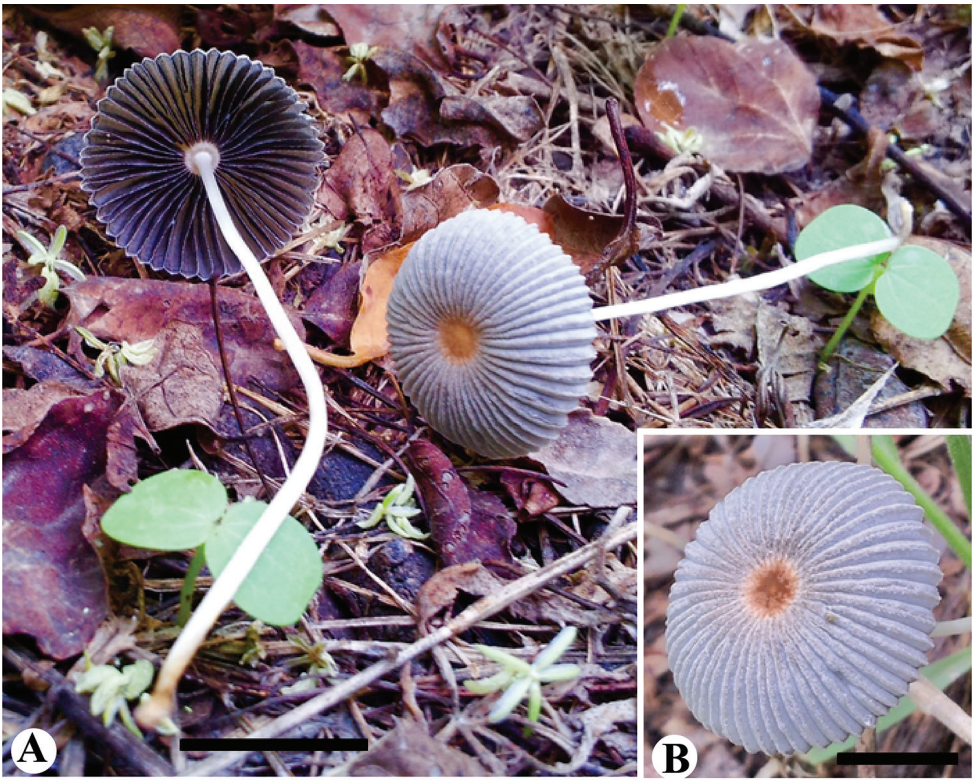

Figure 4. Basidiomata of *Parasola glabra* sp. nov. **A, B** Collection SHP-5 (HOLOTYPE LAH-SHP-5). Scale bars: 20 mm.

LAH SH-P5; GenBank accessions: ITS = KY461717; 28S = KY621806; *TEF1a* = KY461735).

Description. Pileus 20–30 mm diam, initially subglobose, later convex to hemispheric; at first smooth, without veil, the center glabrous at maturity, becoming deeply plicate towards the margin; light gray (2.5R 6/2) to moderate gray (7.5R 6/2); disc slightly depressed, strong reddish orange (7.5R 5/12). Lamellae free, fairly crowded, separated from the stipe by pseudocollarium, 0–2 lamellulae, regular, initially whitish, then dark brown becoming black at maturity, finally losing turgor and collapsing. Stipe 30–60 × 2–3 mm, central, equal, smooth, slightly sub-bulbous at the base, hollow, white, fragile, without annulus.

Basidiospores (13)14.5–16.5(18) × (7.5)9.5–11.5(15) × (9)8.0–10.5(11.5) µm, on average 15.8 × 10.9 × 10.1 µm, Q_1 = 1.3–1.5, Q_2 = 1.4–1.6, avQ = 1.4; in face view broadly ovoid to oblong, some with rhomboidal outline, in side view ellipsoid, germ-pore eccentric and upto 1.5 µm diam; wall upto 1.5 µm thick, dark brown to blackish in KOH. Basidia 28–41 × 10–13 µm, clavate to cylindrical, 4-spored, hyaline in KOH. Cheilocystidia 50–63 × 17–23 µm, oblong, ellipsoid, narrowly to broadly utriform, hyaline. Pleurocystidia 60–75 × 22–38 µm, clavate to broadly lageniform,

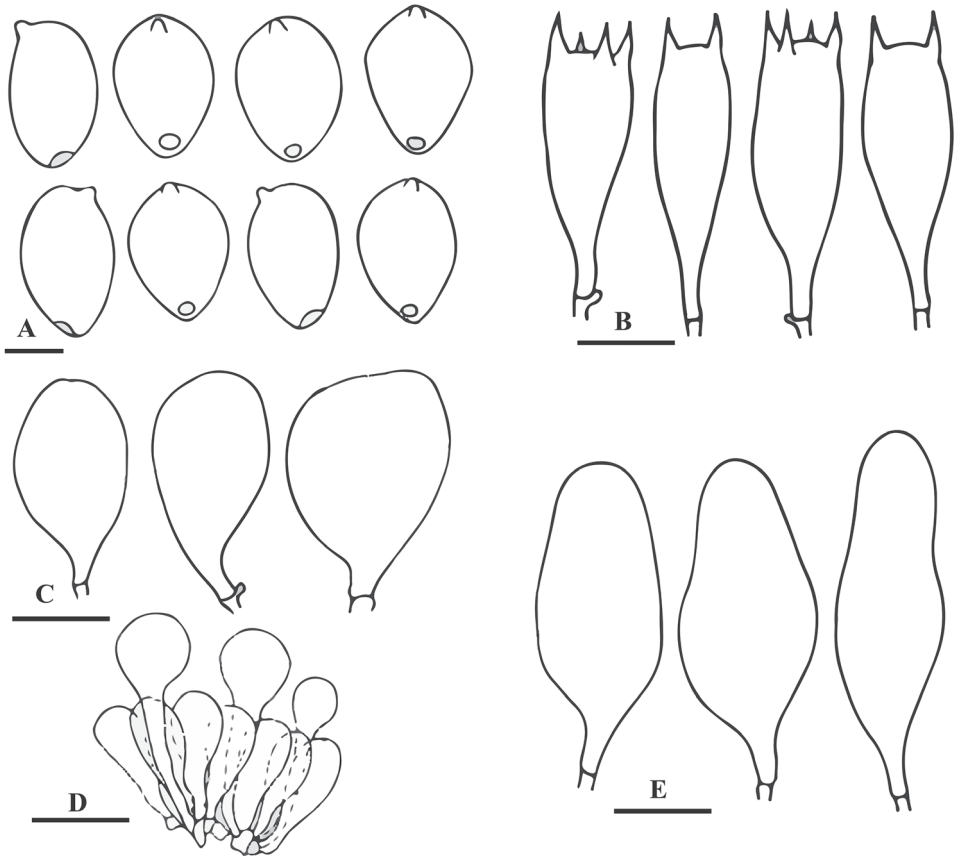

Figure 5. Anatomical features of *Parasola glabra* sp. nov. (LAH-SHP-5). **A** Basidiospres **B** Basidia **C** Pleurocystidia **D** Pileipellis **E** Cheilocystidia. Scale bars: 12 μm (**A**), 20 μm (**B–E**).

hyaline. Pileipellis hymeniform, consisting of clavate cells 47–60 × 13–16 μm, bright yellow at the base in KOH. Clamp connections present mostly in the pileipellis and at the base of basidia. Sclerocystidia absent.

Habitat and distribution. Saprotrophic, scattered under herbaceous plants on grass land. So far only known from the lowland of northern Pakistan. This species is, however, common in lowland northwest Pakistan.

Etymology. Specific epithet '*glabra*' refers to the glabrous cap found in species of section *Parasola* of the genus *Parasola*, where this species belongs.

Additional specimen examined. PAKISTAN, Khyber Pakhtunkhwa Province, Malakand, Qaldara, 480 m alt., 28 May 2015, S. Hussain SHP23 (HUP SHP-23).

Comments. The distinguishing features of the new species *P. glabra* are: basidiospores broadly ovoid to oblong, some with rhomboidal outline in face view, ellipsoid in side view, on range 14.5–16.5 × 9.5–11.5 × 8.0–10.5 μm, pileus light gray to moderate gray but reddish orange at the disk, without sclerocystidia. Lacking sclerocystidia, *P. glabra* belongs

Table 2. Characteristics distinguishing *Parasola glabra* and *P. pseudolactea* from the remaining species in section *Parasola*.

Taxa	Pileus diam; and pileus color	Stipe size	Basidiospores size, length/breadth (Q_1), length/width (Q_2) ratios	Basidiopores shape and germ-pore position	References
P. glabra	20–30 mm diam, light-gray to moderate-gray	30–60 × 2–3 mm	15.8 × 10.9 × 10.1 µm; Q_1 = 1.3–1.5, Q_2 = 1.4–1.6, avQ = 1.4	In face view broadly ovoid to oblong, some with rhomboidal outline, in side view ellipsoid; germ-pore eccentric, upto 1.5 µm diam.	Observed during this study.
P. hercules	15–20 mm diam, orange-brown to red-brown	75 × 1.5 mm	15.83 × 15.42 × 10.63 µm; Q_1 = 1–1.15, Q_2 = 1.4–1.5	In face view rounded triangular to quadrangular, rarely subglobose to ovoid, in side view ellipsoid to amygdaliform; germ-pore eccentric, upto 2.7µm diam.	Nagy et al. 2010, Schafer 2014.
P. kuehneri	35 mm diam, dark light grayish-brown	100 × 3 mm	9.36 × 7.85 × 5.9 µm; Q_1 = 1.1–1.2, Q_2 = 1.4–1.6	In face view ovoid to rounded triangular, rhomboid to mitriform, in side view amygdaliform; germ- pore eccentric, 1.5 µm diam.	Nagy et al. 2010, Schafer 2014.
P. lactea	15–23 mm diam, yellow-brown to dull red-brown	140 × 3 mm	10.73 × 8.81 × 6.73 µm; Q_1 = 1.02–1.25, Q_2 = 1.66–2.10	In face view mostly broadly ovoid to subglobose, rarely angular to rounded triangular, in side view broadly ellipsoid to ellipsoid; germ-pore eccentric, upto 1.8 µm diam.	Nagy et al. 2010, Schafer 2014.
P. pseudolactea	15–25 mm diam, initially yellow-brown to dull-brown, moderate gray at maturity	30–50 × 1 mm	14.0 × 11.3 × 9.7 µm; Q_1 = 1.3–1.5, Q_2 = 1.4–1.5, avQ = 1.4	In face view mostly rounded triangular to heart shape, rarely ovoid to subglobose, in side view ellipsoid to oblong, germ-pore eccentric, upto 1.5 µm diam.	Observed during this study
P. lilatincta	30–50 mm diam, dark red-dish brown, not plicate	70–100 ×2–4 mm	14.4 × 10.8 × 9.2 µm; Q_1 = 1.3–1.4, Q_2 = 1.3–1.5	In face view rounded triangular to quadrangular, in side view ellipsoid to amygdaliform; germ-pore eccentric, upto 2.5 µm diam.	Uljé and Bender 1997, Nagy et al. 2010, Schafer 2014, Hussain et al. 2016.
P. megasperma	35 mm diam, chestnut-brown to red-brown or ochre-tawny	50–100 × 1.5–3 mm	16.5 × 10.66 × 8.5 µm; Q_1 = 1.40–1.78, Q_2 = 1.83–1.95	In face view ellipsoid to broadly ellipsoid, rarely ovoid, in side view ellipsoid to subamygdaliform; germ-pore slightly eccentric, upto 2.3 µm diam.	Nagy et al. 2010, Schafer 2014.
P. misera	2–5 × 1–3 mm, tawny-orange to cinnamon-brown	50 × 0.5 mm	7.0–10.6 × 6.5–10.0 × 5.9–6.6 µm	In face view heart-shape to rounded triangular, irregularly globose, in side view ellipsoid; sometimes broader than long; germ-pore eccentric.	Schafer 2014.
P. plicatilis	35 mm diam, yellow-brown to dull pinkish-brown	30–70 × 0.5–3 mm	12.41 × 8.21 × 7.14 µm; Q_1 = 1.34–1.67, Q_2 = 1.61–1.86	In face view mostly leminiform-subhexagonal, rarely ovoid, in side view ellipsoid to subamygdaliform; germ-pore ec-centric, 2.3 µm diam.	Nagy et al. 2010, Schafer 2014.
P. schroeteri	20–30 mm diam, yellow-brown to grayish red-brown	40–60 × 1 mm	14.44 × 11.83 × 9.72 µm, Q_1 = 1.16–1.27, Q_2 = 1.46–1.68	In the face view rounded triangular to subglobose, in side view ovoid to amygdaliform; germ-pore eccentric, upto 2.5 µm diam.	Uljé and Bender 1997, Nagy et al. 2010, Schafer 2014.

in section *Parasola*. On basidiospore dimensions, it could be thought close to *P. plicatilis* and *P. megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple but these are distinguishable on the basis of spores shape, length and breadth together and on the color of the cap disk. Using maximum likelihood phylogeny, these two species are clearly distinct from *P. glabra* and, based on ITS and 28S loci, the more closely related species are: *P. hercules* (Uljé & Bas) Redhead, Vilgalys & Hopple; *P. kuehneri* (Uljé & Bas) Redhead, Vilgalys & Hopple; *P. lilatincta* and *P. schroeteri* (P. Karst.) Redhead, Vilgalys & Hopple. The new species can be distinguished from these species on account of basidiospore morphology: among these species, *P. hercules* has the largest spore breadth (11.3–16.9 µm), followed by *P. schroeteri* (9–13 µm), *P. glabra* (9.5–11.5 µm), *P. lilatincta* (9–11.2 µm) and smallest spore breadth (5.5–8.4 µm) in *P. kuehneri*. On the basis of basidiospore length/breadth ratio (Q_1), the new taxon *P. glabra* (Q_1 = 1.3–1.5), can be easily distinguished from these species: in *P. hercules* (Q_1 = 1.04–1.28), *P. schroeteri* (Q_1 = 1.16–1.27), *P. lilatincta* (Q1 = 1.14–1.33) and *P. kuehneri* (Q1 = 1.12–1.28), respectively (Nagy et al. 2010, Schafer 2014). Comparison of morphological characters of *P. glabra* with regards to these and other species of section *Parasola* genus *Parasola* are set out further in Table 2.

Parasola pseudolactea Sadiqullah, Hussain & Khalid, sp. nov.

MycoBank: MB819600
Figures 6, 7

Diagnosis. Pileus yellowish brown to dull brown, deeply plicate towards margin; disc subumbilicate, deep orange yellow; lamellae free, pseudocollarium absent; basidiospores 13.5–14.5 × 10.5–12.0 × 9.5–10.5 µm, in face view rounded triangular to heart shape, rarely ovoid to subglobose, in side view ellipsoid to oblong, with eccentric germ-pore of 1.5 µm diam; sclerocystidia absent.

Type. PAKISTAN, Khyber Pakhtunkhwa Province, Shangla, solitary to scattered under *Quercus incana*, 1480 m alt., 9 July 2014, Sadiq Ullah SU412 (holotype: HUP SU-412; GenBank accessions: ITS = KY461719; 28S = KY621799; *TEF1a* = KY461733).

Description. Pileus 15–25 mm diam, initially obtusely conical, later becoming applanate and deeply plicate towards margin; yellowish brown to dull brown (10YR 6/4) when young, moderate gray (7.5R 6/2) on maturity; disk subumbilicate, deep orange-yellow (7.5YR 6/12). Lamellae free, 0–2 lamellulae, distant, pseudocollarium absent, initially dark gray, becoming blackish at maturity and finally losing turgor and collapsing. Stipe 30–50 × 1 mm, equal, smooth, grayish-brown, translucent, hollow, without annulus.

Basidiospores (12.0)13.5–15.0(16.0) × (9.5)10.5–12.0(13.0) × (7.5)9.5–10.5(12.0) µm, on average 14.0 × 11.3 × 9.7 µm, Q_1 = 1.3–1.5, Q_2 = 1.4–1.5, avQ = 1.4; in face view mostly rounded triangular to heart shaped, rarely ovoid to subglobose, in side view ellipsoid to oblong, with eccentric germ pore of 1–1.5 µm diam, dark to blackish in KOH. Basidia 24–31 × 8–12 µm, clavate to cylindrical, 4-spored. Cheilocystidia 55–70 × 22–29 µm, clavate, broadly clavate to broadly cylindrical. Pleurocys-

Figure 6. Basidiomata of *Parasola pseudolactea* sp. nov. *P. auricoma* and *P. lilatincta*. **A** *Parasola pseudolactea* sp. nov. collection SU-412 (HOLOTYPE HUP SU-412) **B** *Parasola lilatincta* collection SHP-8 (HUP-SHP-8) **C** *Parasola auricoma* collection SHP7 (LAH-SHP-7). Scale bars: 20 mm.

tidia 44–67 × 19–23 µm, utriform to lageniform. Pileipellis hymeniform, consisting of clavate cells, 33–38 × 17–22 µm. Clamp connections present. Sclerocystidia absent.

Habitat and distribution. Solitary to scattered on humus rich loamy soil, under *Quercus incana*. So far only known from northwest Pakistan.

Etymology. The prefix "*pseudo*" means similar and "*lactea*" refers to the epithet of the species (*Parasola lactea*) that this species closely resembles. This species is known so far from low to moderate altitude mountains of northwest Pakistan.

Additional specimens examined. PAKISTAN, Khyber Pakhtunkhwa Province, Shangla, 1480 m alt., 9 July 2014, Sadiq Ullah SU413 (HUP SU-413).

Comments. The new species belongs to *Parasola* section *Parasola* due to the absence of sclerocystida in the pileipellis. This species resembles *Parasola lactea* and is close to that species in the molecular phylograms. However, its spores are substantially larger, closer to *P. schroeteri* or *P. hercules* in size. The spores of *P. pseudolactea* are mostly rounded triangular, rarely ovoid to subglobose in face view and larger (14.0 × 11.3 × 9.7 µm), whereas those of *P. lactea* are mostly broadly ovoid to subglobose, rarely rounded triangular in face view, and comparatively smaller (10.73 × 8.81 × 6.73 µm). Other species similar to the new taxon are *P. megasperma* and *P. plicatilis*. Both these species share pileus color with *P. pseudolactea*. Lamellae of *P. megasperma* and *P. plicatilis* are separated from the stipe by a pseudocollarium, whereas in *P. pseudolactea*, a pseudocollarium is generally absent. Basidiospores are more ellipsoid rarely ovoid in face view and on average 16.5 ×

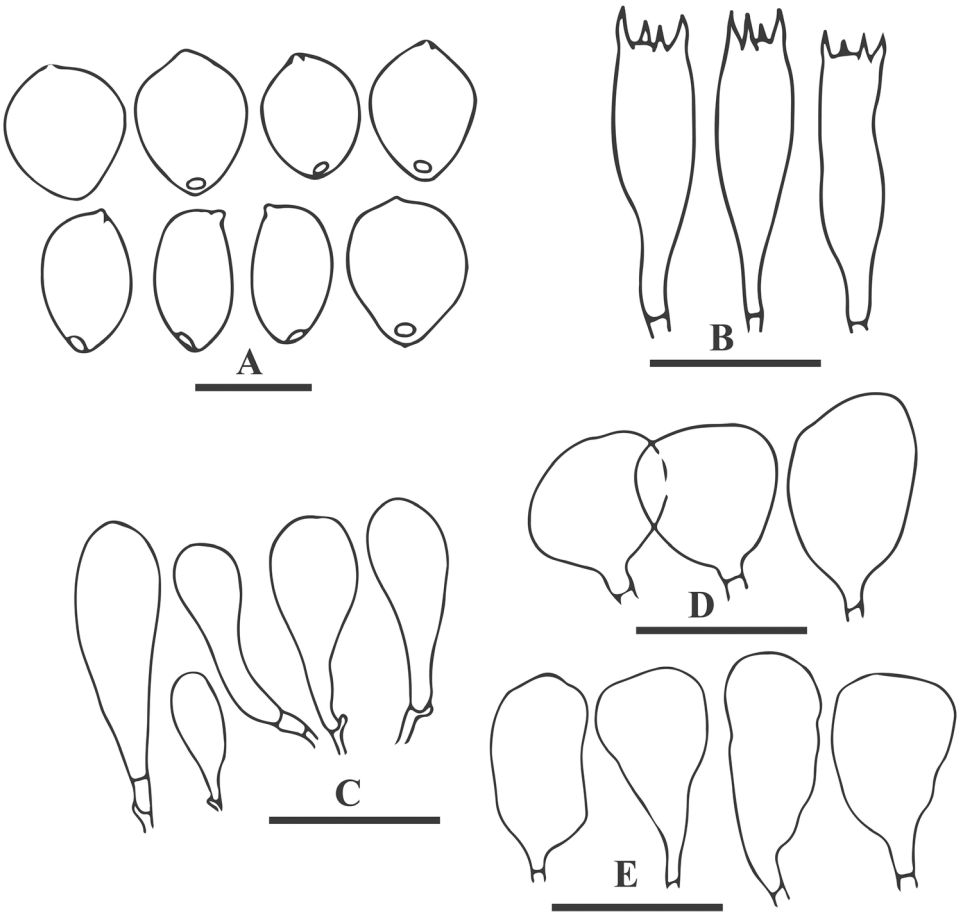

Figure 7. Anatomical features of *Parasola pseudolactea* sp. nov (HUP-SU-412). **A** Basidiospores **B** Basidia **C** Pileipellis **D** Pleurocystidia **E** Cheilocystidia. Scale bars: 12 µm (**A**), 20 µm (**B–E**).

10.66 × 8.5 µm in *P. megasperma*. Basidiospore shape is quite variable in *P. plicatilis*, in face view mostly limoniform-subhexagonal, rarely ovoid, in side view broadly ellipsoid, on average 12.41 × 8.21 × 7.14 µm (Nagy et al. 2010). Comparison of morpho-anatomical features of *P. pseudolactea* with regards to other species of the genus *Parasola* are set out in Table 2, where the new species can be differentiated by careful comparison of the morphology of its basidiospores.

***Parasola auricoma* (Pat.) Redhead, Vilgalys & Hopple, Taxon 50: 235. 2001.**
Figures 6, 8

Synonymy. *Coprinus auricomus* Pat., Tab. analyt. Fung. 5: 200, 1886.

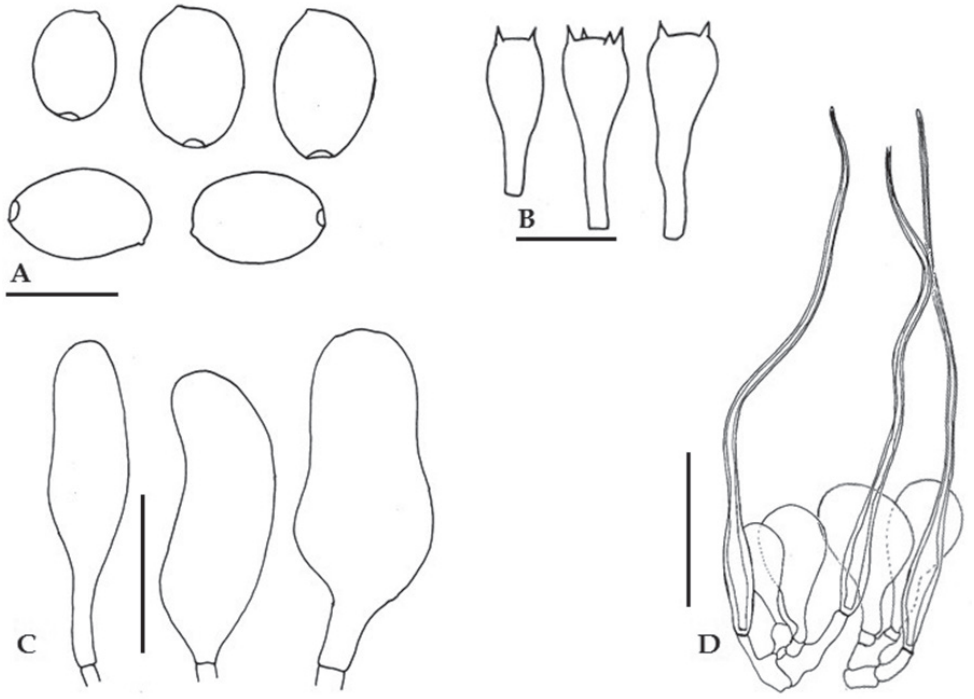

Figure 8. Anatomical features of *Parasola auricoma* (LAH-SHP-7). **A** Basidiospores **B** Basidia **C** Cheilocystidia **D** Pileipellis. Scale bars: **A** = 10 μm, **B–D** = 20 μm.

Description. Pileus 15–30 mm diam, convex to broadly convex, deeply plicate towards the margin, light grayish-brown (2.5YR 5/2) to grayish reddish-brown (2.5YR 3/2); disc indistinctly umbonate to umbilicate, dark reddish orange (7.5R 4/8) to grayish reddish orange (2.5YR 5/6). Lamellae free and remote, pseudocollarium absent, closed, initially concolorous with pileus, later on dark black, finally losing turgor and collapsing. Stipe 40–65 × 2–5 mm, equal, smooth, central, hollow, without annulus.

Basidiospores (10.5)12.5–13.5(15.0) × (8.0)8.5–9.5(10.0) × (7.0)8.0–9.0(10.0) μm, on average 12.9 × 9.0 × 8.5 μm, Q_1 = 1.5–1.6, Q_2 = 1.3–1.4, avQ = 1.5; in face view subcylindrical to ellipsoid or ovoid, in side view ellipsoidal to elliptical; with central germ-pore, 2–2.5 μm diam, wall 1.5 μm thick, strong reddish-brown to blackish in KOH. Basidia 30–38 × 7–11 μm, clavate to subcylindrical, 2- or 4-spored. Cheliocystidia 33–45 × 12–25 μm, subclavate to subglobose, abundant. Pleurocystidia 30–40 × 11–15 μm, cylindrical to clavate, pale brown at the base, rare. Sclerocystidia 90–170 × 4–7 μm, dark brown, with acute apex and bulbous base, wall 1.5–2 μm thick. Clamp connection present.

Specimens examined. Pakistan, Khyber Pakhtunkhwa Province, Malakand, Kharkai, alt. 460 m, scattered in grassland under herbaceous plants, 10 August 2014, S. Hussain SHP6 (LAH-SHP-6), 10 August 2014, S. Hussain SHP7 (LAH-SHP-7),

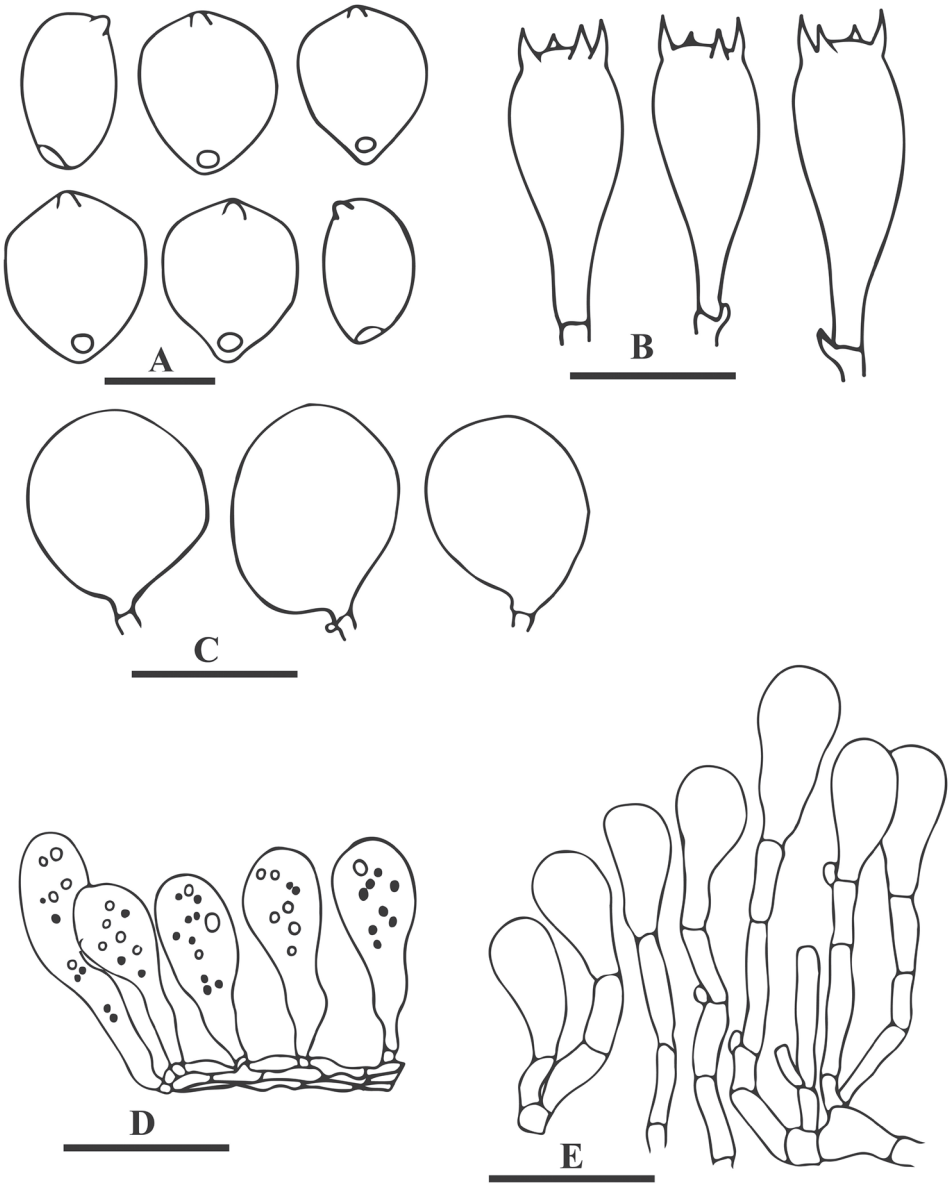

Figure 9. Anatomical features of *Parasola lilatincta* (LAH-SHP-8). **A** Basidiospores **B**Basidia **C** Pleurocystidia **D** Cheilocystidia **E** Pileipellis. Scale bars: **A** = 10 μm, **B–E** = 20 μm.

Malakand, Qaldara 10 August 2014, S. Hussain SHP11 (LAH-SHP-11); Khyber Pukhtunkhwa Province, Swat, Kanju Township, alt. 1023 m, 27 July 2017, S. Hussain SHP34 (SWAT SHP-34).

***Parasola lilatincta* (Bender & Uljé) Redhead, Vilgalys & Hopple, Taxon 50: 236. 2001.**
Figures 6, 9

Synonymy. *Coprinus lilatinctus* Bender & Uljé, Persoonia 16: 373, 1997.

Description. Pileus 20–30 mm diam, hemispheric to pulvinate, smooth, deeply plicate towards margin, yellow brown (2.5R 9/2–5R 9/2) to grayish red brown (2.5R 7/2–5R 7/2); disc slightly depressed, brilliant orange (2.5YR 8/12 – 5YR 8/12) to strong orange (2.5YR 6/12–5YR 6/12). Lamellae free, separated from the stipe by pseudocollarium, distant, lamellae edge blackish while faces initially concolorous with the pileus but later on black and finally losing turgor and collapsing. Stipe 40–60 × 1 mm, equal, smooth, white, fragile, without annulus with slightly sub-bulbous base.

Basidiospores (12)13–14.5(15.5) × (11.5)12–12.5(13.5) × (6.0)8.5–11(13.5) µm, on average 14.5 × 12.5 × 9.9 µm, Q_1 = 1.1–1.2, Q_2 = 1.2–1.5, avQ = 1.3; in the face view rounded triangular to subglobose, in side view ovoid to amygdaliform, with eccentric germ-pore of 2–2.5 µm diam; wall upto 2 µm thick, dark brown in KOH. Basidia 17–22 × 6–9 µm, 4-spored, cylindrical to clavate, hyaline in KOH. Cheilocystidia 25–29 × 23–26 µm, rounded to globose, rare. Pleurocystidia 34–40 × 11–14 µm, cylindrical to subclavate. Pileipellis of clavate cells, 33–37 × 9–12 µm, with rounded apex, bright yellow at the base. Clamp connections present in most of the tissues. Sclerocystidia absent.

Specimens examined. PAKISTAN, Khyber Pakhtunkhwa Province, Malakand, Qaldara, alt. 430 m, scattered under herbaceous plants, 11 August 2014, S. Hussain SHP-8, SHP-31, SHP-12 (LAH SHP-8; LAH SHP-31; LAH SHP-12); Khyber Pakhtunkhwa Province, Swat, Kanju Township, alt. 1023 m, on road trails, 27 July 2017, S. Hussain SHP35 (SWAT SHP-35).

Discussion

The incorporation of molecular phylogenetics has significantly benefited the systematic and taxonomic studies of coprinoid mushrooms. These mushrooms are deliquescent or, at least, have morphological characters like gill cystidia, coloration and surface features that are quickly changed during basidioma maturation. So morphology based taxonomy of coprinoid mushrooms is always a difficult task for mushroom biologists. In the present study two new species of mushroom genus *Parasola* are described from Pakistan, based on morphological and molecular data.

On account of absence of sclerocystidia in the pileipellis, both the new species *P. glabra* and *P. pseudolactea* belong to section *Parasola* of genus *Parasola*. *Parasola glabra* with light gray to moderate gray pileus was collected in Malakand region of Pakistan. This region is rich in diversity of *Parasola* species (Hussain et al. 2016, 2017). The new species *P. glabra* with broadly ovoid to oblong, some with rhomboidal basdiospore is closely related to *P. hercules*. Morphological features of *P. glabra* are discussed with other species of section *Parasola* genus *Parasola*, set out in Table 2. Phylogenetic infer-

ence of *P. glabra* based on ITS and combined ITS-28S datasets was strongly supported (MLB 100% and BPP 1). While in combined ITS-28S-*TEF1a* dataset, *P. glabra* was represented by single specimen and was poorly recovered.

Similarly, the second new species *P. pseudolactea* in this study was collected in Shangla district, Khyber Pakhtunkhwa province of Pakistan. This species with yellow brown to dull brown pileus, basidiospores mostly rounded triangular to heart shape, was found in a *Quercus* forest. The species most closely related to *P. pseudolactea* on the basis of basidiospore morphology is *P. lactea*. Basidiospores are mostly rounded triangular to heart shape, rarely ovoid to subglobose in face view in *P. pseudolactea*; while spores are ovoid to subglobose, rarely rounded triangular in face view in *P. lactea*. A poorly described species *P. subprona* (Cleland) J.A. Simpson & Grgur. with elliptical basidiospores (15 × 8 µm) can be differentiated from both the new species on account of central germ-pore (Grgurinovic 1997). Phylogenetic analyses recovered *P. pseudolactea* well supported in ITS, combined ITS-28S and combined ITS-28S-*TEF1a* datasets (Figures 1, 2 and 3), respectively. Along with these new species, collections of *P. auricoma* and *P. lilatincta* from Pakistan were also documented in this study. The phylogenetic separation of *P. auricoma* collected in Pakistan from European collections (albeit into adjacent clades) suggests that the taxon from Pakistan may be a distinct, previously undescribed species. However, morphological features do not yet provide a basis for distinguishing separate species.

Conclusion

It is concluded form this study that low altitude mountains of northern Khyber Pakhtunkhwa Province of Pakistan are rich in the diversity of *Parasola* and other coprinoid mushrooms.

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RESEARCH ARTICLE

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Tuber aztecorum sp. nov., a truffle species from Mexico belonging to the Maculatum clade (Tuberaceae, Pezizales)

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Abstract

A new species of truffle, *T. aztecorum*, is described from central Mexico. *Tuber aztecorum* can be distinguished from other related *Tuber* species synoptically by a combination of morphological features including ascospore size, pellis cells with irregular thickness, cystidia, ascoma colour and associated host (*Abies religiosa* an endemic *Abies* species from central Mexico); sequence variation on the ITS rDNA also distinguishes *T. aztecorum* from related species. A phylogenetic analysis of the ITS rDNA demonstrates that *T. aztecorum* belongs to the Maculatum clade and is unique from other similar small, white-cream coloured *Tuber* species distributed in north-eastern Mexico such as *T. castilloi* and *T. guevarai*.

Keywords

Taxonomy, systematics, phylogeny, hypogeous fungi, cryptic species

Introduction

Tuber is one of the most important edible truffle genera in the world due to its economic importance and ecological role in forest ecosystems. *Tuber* spp. are known as 'true truffles' and their fruiting bodies are edible and highly valued. Ecologically, *Tuber* spp. form symbiotic ectomycorrhizal associations with gymnosperm and angiosperm trees and also orchids (Riousset et al. 2001, Wurzburger et al. 2001, Bidartondo et al. 2004, Walker et al. 2005, Mello et al. 2006, Trappe et al. 2009, Martin and Bonito 2012, Morcillo et al. 2015). In addition, *Tuber* spp. are consumed for nutrition by many kinds of invertebrates and vertebrates including primates (McGraw et al. 2002, Hanson et al. 2003, Hochberg et al. 2003, Maser et al. 2008, Beaune et al. 2013). A few *Tuber* species are now cultivated worldwide, including *Tuber melanosporum*, *T. aestivum* and *T. borchii* (Martin and Bonito 2012).

The Puberulum and Maculatum clades within the genus *Tuber* are two of the most species diverse and geographically widely dispersed of the eleven recognised clades. More recently, the related Latisporum clade was described from Asia, where the species are endemic (Fan et al. 2016). *Tuber* species in these three clades are often pale in colour and typically small in size (Trappe et al. 2009, Bonito et al. 2010a, 2013, Guevara et al. 2013b, Payen et al. 2014). Recent molecular analyses of *Tuber* spp. from northern and central Mexico and USA have shown that *Tuber* species are genetically unique compared to their European and Asian counterparts (Bonito et al. 2009, 2010a, Lancellotti et al. 2016). Many *Tuber* species belonging to these clades have been formally named recently. For example, five species within the *T. separans* complex of the Puberulum clade were described from Mexico. *Tuber bonitoi*, a large truffle (approx. 5 cm) found recently in Mexico, is morphologically similar to *T. borchii*. It was found associated with *Pinus hartwegi* and *Abies religiosa*. *Tuber brunneum*, a smaller, brownish truffle from central Mexico, was associated with *Quercus magnolifolia* as was *T. pseudoseparans* and *T. tequilanum*. *Tuber guzmanii* and *T. separans* are also found in Mexico and belong to the Puberulum clade (Guevara et al. 2015). Other *Tuber* species belonging to the Maculatum clade are known from north-eastern and central Mexico including *Tuber castilloi*, *T. gardneri*, *T. guevarai*, *T. maculatum*, *T. mexiusanum* and *T. miquihuanense* (Cázares et al. 1992, Guevara et al. 2008, 2013a,b, 2015). In addition, new findings on asexual anamorphic states have been discovered for some North American *Tuber* species, however the role of these structures is still unknown (Urban et al. 2004, Ouanphanivanh et al. 2008, Healy et al. 2013).

Studies on *Tuber* species from Mexico are still scarce. In this work, a morphological and molecular analysis was performed on recent *Tuber* collections. The authors report on a new taxon, which is described here as *T. aztecorum*. Phylogenetically, *T. aztecorum* is within the Maculatum clade, a group of small to medium sized, white truffles. It is associated with *Abies religiosa,* an endemic *Abies* species from central Mexico. *Tuber aztecorum* can be differentiated from related taxa by its morphology, ecology, biogeography and nuclear ITS ribosomal DNA. This research contributes to the knowledge of *Tuber* biodiversity and ecology in North America.

Materials and methods

Sampling and morphological characterisation

Tuber fruiting bodies were collected from central México and preserved following recommendations of Harkness (1899) and Castellano et al. (1989). Duplicate splits of sample collections are deposited in the herbaria José Castillo Tovar (ITCV), Oregon State University (OSC), Michigan State University (MSU) and Florida University (FLAS). Previously accessioned herbarium specimens of *Tuber*, including type collections from OSC and ITCV, were also examined during this study.

Morphological data were obtained by the methods of Castellano et al. (1989), Gilkey (1916, 1939) and Pegler et al. (1993). Examined characters included ascoma (fruiting body) size, surface texture and colour, peridial structure; spore length and width (excluding ornamentation), length/width ratio (Q), shape, wall thickness, number of reticular meshes, height of the meshes, colour and ascus size, shape, wall thickness and number of spores/ascus. Hand-cut sections were mounted in 5% KOH and Melzer's reagent for light microscopy. Spore measurements of *Tuber* spp. in KOH compared to those in water showed no KOH effect (J. Trappe, unpublished data). Microscopic structures were measured and photographed under a light microscope and stereo microscope.

DNA sequencing and phylogenetic analyses

Molecular protocols follow those of Guevara et al. (2008). DNA was extracted from truffle fruiting bodies with the chloroform extraction technique using CTAB 2X DNA extraction buffer. The ITS region was amplified with the primer pair ITS1f-ITS4 (Gardes and Bruns 1993, White et al. 1990). PCR products were cleaned enzymatically with antarctic phosphatase and endonuclease digestion (New England Biolabs, Ipswich MA). Sanger sequencing was performed by Big Dye chemistry v3.1 (Applied Biosystems, Foster City, CA) with the forward primer ITS1f and reverse primers ITS4. DNA sequences were determined on an ABI 3700 capillary sequencer (Applied Biosystems, Foster City CA). DNA sequences were viewed and manually edited in Sequencher 4.0 (Gene Codes, Ann Arbor, MI). Sequences were aligned with MUSCLE (Edgar 2004). Alignments were manually checked and ambiguous regions were excluded in Mesquite 2.5 (Maddison and Maddison 2009).

Phylogenetic analyses were conducted with maximum likelihood (ML) in PAUP* (Swofford 2002). The best fit nucleotide substitution model (GTR+G+I) was based on the Akaike information criterion and was implemented in PAUP* 4d106 (Swofford 2002). ML bootstrap support based on 1000 replicates was assessed with RAxML (Pattengale et al. 2009, Stamatakis et al. 2008, Stamatakis 2006) and executed on the CIPRES Science Gateway (Miller et al. 2010). Phylogenetic trees were rooted with species belonging to the Latisporum clade. Sequences produced in this study are deposited in GenBank under accession numbers KY271791 and KY271790, Table 1.

Table 1. List of *Tuber* species, GenBank accession numbers and reference for the ITS sequences used in the phylogenetic analysis. The sequences of the new taxon are in bold.

Taxon	GenBank	Reference
Tuber alboumbilicum Y. Wang & Shu H. Li	KJ742702	Li et al. 2014
T. anniae W. Colgan & Trappe	NR119860	Bonito et al. 2010a
T. aff. *asa* Tul. & C. Tul.	HM485341	Bonito et al. 2010a
T. aztecorum Guevara, Bonito & Smith	**KY271790, KY271791**	This paper
T. beyerlei Trappe, Bonito & G. Guevara	NR119866	Bonito et al. 2010a
T. bomiense K. M. Su & W. P. Xiong	KC517481	NCBI
T. bonitoi G. Guevara & Trappe	JT32421, KC152256,	Guevara et al. 2015
T. borchii Vittad.	HM485342	Bonito et al. 2010a
T. brunneum G. Guevara, Bonito & Trappe	JT33830, JT33837	Guevara et al. 2015
T. californicum Harkn.	HM485346	Bonito et al. 2010a
T. castilloi G. Guevara, Bonito & Trappe	NR119865	Guevara et al. 2013b
T. cistophilum P. Alvarado, G. Moreno, Manjón, Gelpi & J. Muñoz	JN392231	Alvarado et al. 2012
T. dryophilum Tul. & Tul.	HM485354	Bonito et al. 2010a
T. foetidum Vittad.	JQ288907	NCBI
T. guevarai Bonito & Trappe	JF419305	Guevara et al. 2013b
T. huizeanum L. Fan & C. L. Hou	JQ910651	Fan et al. 2012c
T. latisporum Juan Chen & P.G. Liu	NR119620	Chen and Liu 2007
T. lauryi Trappe, Bonito & G. Guevara	NR119862	Bonito et al. 2010a
T. lijiangense L. Fan & J.Z. Cao	GQ217541	Chen and Liu 2007
T. linsdalei Gilkey	HM485370	Bonito et al. 2010a
T. liyuanum L. Fan & J.Z. Cao	NR111717	Fan and Cao 2012a
T. maculatum Vitadd.	KJ524540	Hilszczanska et al. 2014
T. mexiusanum G. Guevara, Bonito & Cázares	NR119867	Guevara et al. 2013b
T. microsphaerosporum L. Fan & Y. Li	KF805726	Fan and Yue 2013
T. microverrucosum L. Fan & C.L. Hou	JN870099	Fan et al. 2012c
T. miquihuanense G. Guevara, Bonito & Cázares	NR119868	Guevara et al. 2013b
T. panzhihuanense X.J. Deng & Y. Wang	JQ978644	Deng et al. 2013
T. pseudoseparans G. Guevara, Bonito & Trappe	JT33778, JT33774 (KT897480)	Guevara et al. 2015
T. pseudogamagnatum L. Fan	NR111718	Fan and Cao 2012a
T. pseudosphaerosporum L. Fan	KF744063	Fan and Yue 2013
T. rapaeodorum Tul. & C. Tul.	DQ011849	NCBI
T. separans Gilkey	HM485385	Bonito et al. 2010a
T. shearii Harkn.	HM485389	Bonito et al. 2010a
T. sinosphaerosporum L. Fan, J.Z. Cao & Yu Li	JX092086	Fan and Yue 2013
T. sphaerosporum Gilkey	HM485390	Fan and Yue 2013
T. tequilanum G. Guevara, Bonito & Trappe	JT33755, JT33790 (KT897482)	Guevara et al. 2015
T. vesicoperidium L. Fan	JQ690071	Fan et al. 2012b
T. walkeri Healy, Bonito & G. Guevara	JF419265	Guevara et al. 2013b
T. zhongdianense X.Y. He, Hai M. Li & Y. Wang	DQ898187	Chen and Liu 2007
Tuber sp.	AB553464	Kinoshita et al. 2011
Tuber sp. 14	GQ221447	NCBI
Tuber sp. 36	JF419253, JF419256	Guevara et al. 2013b
Tuber sp. 47	HM485416	Bonito et al. 2010a
EcM Salix humboldtiana Willd.	KF742730	Berch and Bonito (2016)
EcMCU046	KJ595014	NCBI

Results

Molecular analyses

A total of 51 taxa including holotypes were analysed (Table 1). As previous studies have shown, the Maculatum clade was distinct from the Puberulum and Latisporum clades in ML and Bayesian Inference analyses (Fig. 1). The designation of *Tuber aztecorum* as a new species is supported by ITS rDNA analysis, morphological characters and ecology.

Taxonomy

Tuber aztecorum **Guevara, Bonito & Smith, sp. nov.**
MycoBank: MB819367
GenBank: KY271791
Figs 1, 2A–I

Type. MEXICO. *State of Mexico,* Toluca-Temascaltepec road, La Puerta, Parque Nacional Nevado de Toluca, 29 July 2010, Guevara 993 (ITCV [José Castillo Tovar herbarium] – holotype, MSU and FLAS – isotypes), GB KY271791.

Diagnosis. *Tuber aztecorum* is a sister species to *T. castilloi*, but *T. castilloi* differs by having larger spores, 27–63 × 20–40 µm, is without an irregular thickness to the cell wall on peridial hyphae and is associated mainly with *Quercus* spp. Also resembles *T. guevarai* but *T. guevarai* has narrow spores that are 18–55 × 16–42 µm and cream-yellow fruiting bodies and rDNA variation.

Etymology. "*aztecorum"* in reference to the ancient Aztec civilisation of Mexico.

Description. ***Ascomata*** 5–23 × 4–16 × 3–11 mm, subglobose, irregular, lobate or globose, light to orange brown or reddish-brown changing to dark brown when handled, finely verrucose or granulose, with 5–8 verrucae in 1 mm, solid, brittle, surface dry, base sessile. Peridium in cross-section undetachable <.5 mm wide, with one or several basal white to cream furrows or depressions that merge into veins. 5% KOH negative. Gleba marbled, white to greyish, white veins, some veins ending in the peridium. Odour fungoid to raw potato-like, taste not recorded.

Peridium 110–350 µm thick. Outer layer (epicutis) a pseudoparenchyma 62–250 µm thick, of hyphae 5–30 µm diam., versiform, angular or isodiametric, in some areas hyphae arranged perpendicular to the epicutis, hyaline to reddish-brown in mass in KOH, thick-walled (2 µm), without intracellular content. Surface hairs versiform, single hair-like hyphae or cystidia 53–97 µm long × 4–5 µm at the base, tapered to the tip, some with septa, scattered or in clusters, brittle, thin-walled, hyaline in KOH. Other hyphae present are claviform, erect, cylindrical or sinuate with an irregular thickness to the cell wall that resembles knobs or "spines". Some globose or constricted hyphae emerging from isodiametric hyphae, 3–10 µm wide. Inner layer (subcutis) 50–225 µm thick, of prostrate and interwoven, hyphae gradually intermixing into gleba, hyaline in KOH and trypan blue, hyphae

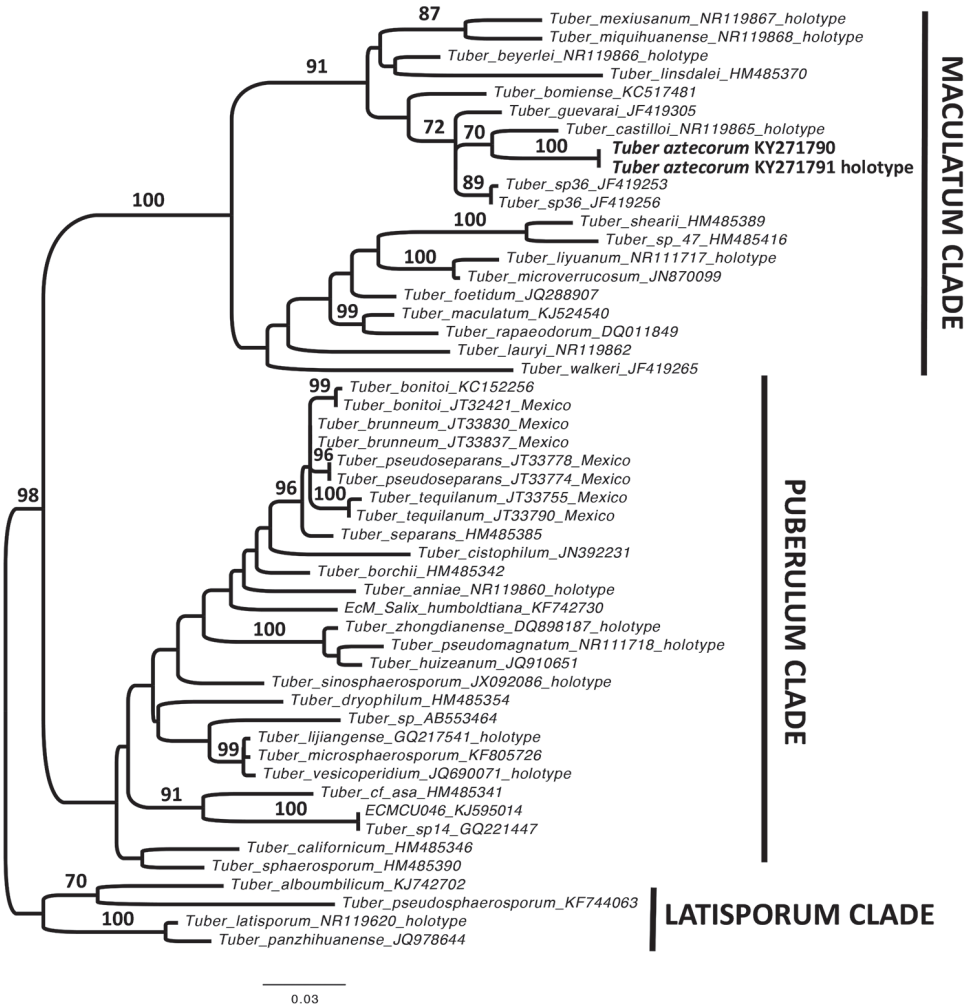

Figure 1. Phylogenetic tree inferred under the maximum-likelihood (ML) criterion from the ITS rDNA alignment corresponding to the *Tuber* dataset. The tree was rooted using midpoint rooting. Numbers on the branches represent support values from 1,000 ML bootstrap replicates. The branches are scaled in terms of the expected number of substitutions per site. The phylogeny is rooted with species belonging to the Latisporum clade. Accession numbers in the sequence labels indicate sequences from Genbank.

2–5 µm wide. Some young specimens show noticeable prostrate cylindrical, claviform or vermiform hyphae along the subcutis that are thick-walled. Veins formed by hyaline, thin-walled, interwoven hyphae.

Ascospores subglobose, globose to broadly ellipsoid, 23–58 × 18–48 µm without ornamentation, alveoli 2–7 µm tall, 7–10 alveolar meshes along the spore length, 5–6 across, polygonal (4–6 sides), cell wall 2–3 µm thick. 1-spored asci have spores that are 42–58 × 27–48 µm, 2-spored asci have spores that are 25–52 × 23–40 µm, 3-spored asci have spores that are 27–40 × 20–30 µm, 4-spored asci have spores that

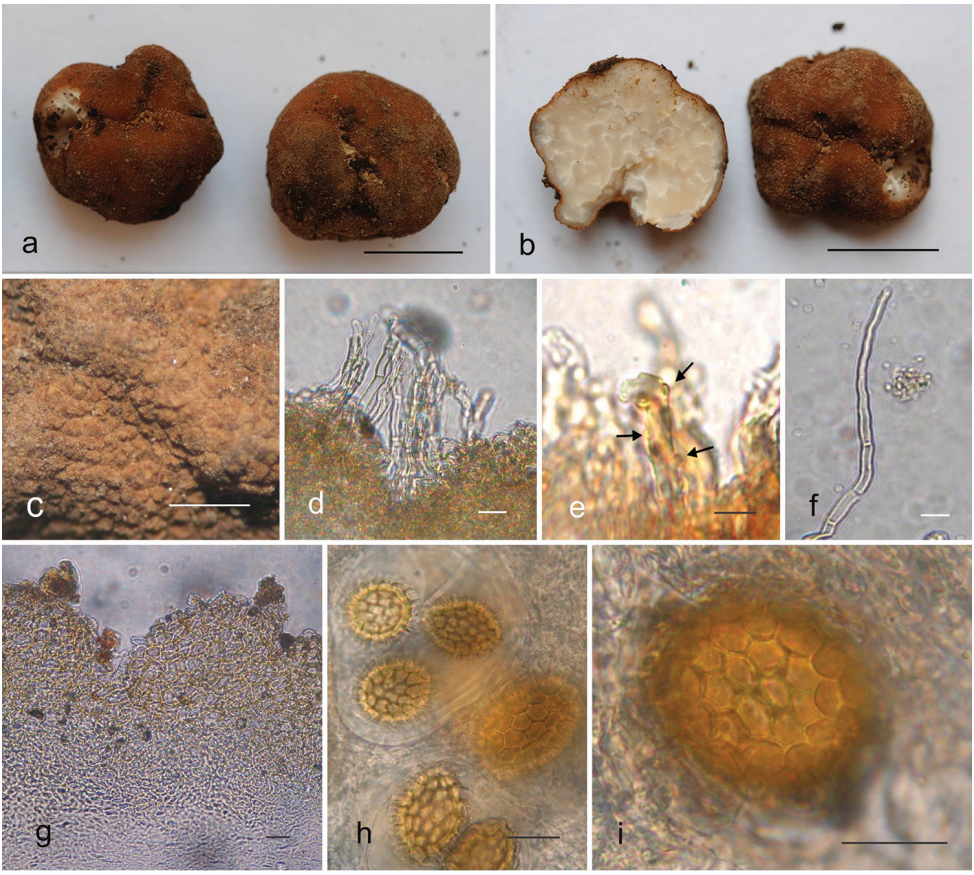

Figure 2. a–i *Tuber aztecorum* (holotype ITCV 993). a; Two ascomata showing the peridial surface (bar = 1 cm) **b** Ascoma in cross-section showing peridial surface and glebal surface (bar = 1 cm) **c** Peridial surface magnified showing the verrucose surface (bar = 1 mm) **d** Clusters of erect hyphae emanating from the peridial surface (bar =10 µm) **e** A single surface hair-like hypha (bar = 10 µm) **f** Cystidium (bar = 10 µm) **g** Cross section of peridium showing pseudoparenchyma-like epicutis (bar = 20 µm) **h** Ascospores within asci in surface view showing the alveoli (bar = 20 µm) **i** Ascospore within asci in surface view showing the alveoli magnified (bar = 20 µm).

are 23–38 × 18–28 µm, 5-spored asci have spores that are 25–32 × 18–25 µm, yellowish to light brown in KOH and Melzer´s reagent. Asci globose, subglobose to broadly ellipsoid, without pedicel, 62–95 × 57–77 mm, hyaline in KOH, yellowish to brownish in Melzer´s reagent, thin-walled (immature asci thick-walled, up to 7.5 µm thick).

Distribution and Ecology. MEXICO, state of Mexico La Puerta, National Park Nevado de Toluca. Hypogeous, gregarious in volcanic rock soil in an *Abies religiosa* forest at 3065 m. N 19°11.662', W099°48.537'. 29 July 2010.

Additional collections examined. Mexico, state of Mexico, La Puerta, National Park Nevado de Toluca, Guevara 1109 (paratype ITCV1109, GB KY271790), Guevara 1110 (paratype ITCV 1110), 29 July 2010.

Discussion

Molecular data confirm that *T. aztecorum* belongs to the Maculatum clade, which is distinct from the Puberulum and Latisporum clades. *Tuber aztecorum* is morphologically and ecologically distinct from other known *Tuber* species (Fig. 1). *Tuber aztecorum* is a sister species to *T. castilloi*, however, *T. castilloi* differs by having larger spores, 27–63 × 20–40 µm, without irregular thickness to the cell wall and is associated mainly with *Quercus* spp. (Guevara et al. 2013a, b). Also *T. aztecorum* resembles *T. guevarai* but *T. guevarai* has narrower spores that are 18–55 × 16–42 µm (Guevara et al. 2013b). Although *T. aztecorum* belongs to the Maculatum clade, it is also morphologically similar to other *Tuber* species outside this group. It was preliminarily identified as *T. gibbosum* in the Gibbosum clade due to its association with *Abies religiosa* and the presence on the peridium of hyphae with irregular swellings in *T. aztecorum* (Guevara et al. 2013a, Bonito et al. 2010b). However, close morphological analysis and further molecular analysis revealed that it was not closely related to the Gibbosum clade. *Tuber aztecorum* is also similar to other species that belong to the Puberulum clade. *Tuber foetidum* is similar in its dark brown to reddish-brown peridium. However, *T. foetidum* has peridial cells 20–30 µm wide, lacks hairs, ascospores that are 25–44 × 21–32 µm and it grows under *Larix*, *Quercus* and *Fagus* (Jeandroz et al. 2008, Pegler et al. 1993). It is similar to *T. puberulum*, *T. rapaeodorum* and *T. borchii* from Europe. These three *Tuber* species have a dense and conspicuous fine epicutis. *Tuber puberulum* is frequently found in association with both *Fagus* and *Larix*. *Tuber rapaeodorum* has a paler ascoma surface with thinner cystidia, ellipsoid spores and is associated with *Quercus*, *Larix*, *Taxus*, *Pinus*, and *Fagus*. *Tuber borchii* has a pale, whitish or yellowish ascoma surface with abundant peridial hairs, 80% of its spores are ellipsoid and it is usually associated with *Fagus* or *Larix* (Pegler et al. 1993, Montecchi and Sarasini 2000, Halász et al. 2005, Wang et al. 2007, Jeandroz et al. 2008). *Tuber latisporum* differs morphologically from *T. aztecorum* by its conspicuously pubescent peridium, spores that are 24–49 (-51) × 20–40 (-44) µm and its association with *Pinus armandii* in China (Chen and Liu 2007).

In conclusion, morphological and sequence analysis of ITS rDNA can distinguish *T. aztecorum* from previously described species with strong bootstrap support and confidence (Halász et al. 2005, Wang et al. 2007, Jeandroz et al. 2008, Bonito et al. 2010a, b). The number of formally described *Tuber* species continues to grow (Table 1).

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RESEARCH ARTICLE

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Three new species of *Fomitiporella* (Hymenochaetales, Basidiomycota) based on the evidence from morphology and DNA sequence data

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Abstract

Fomitiporella austroasiana, *F. mangrovei* and *F. vietnamensis* are described and illustrated as new species based on morphological characters and molecular evidence. They have annual to perennial, mostly resupinate basidiomata with grayish fresh pores, an indistinct subiculum, lack any kind of setae, have brownish, thick-walled basidiospores, and cause a white rot. The distinctive morphological characters of the new species and their related species are discussed. Phylogenies based on the nuclear ribosomal large subunit (28S) and the nuclear ribosomal ITS region show that these three new species form three distinct lineages in the *Fomitiporella* clade. A key to known species of *Fomitiporella* is given.

Keywords

Hymenochaetaceae, Polypore, Taxonomy, Phylogenetic analysis

Introduction

Fomitiporella Murrill was described by Murrill (1907) with *F. umbrinella* as type. The genus is characterized by perennial, resupinate and adnate basidiomata, a thin subiculum, stratified tubes, and brown, subglobose basidiospores (Murrill 1907). *Fomitiporella*

has been considered a synonym of *Phellinus* (Ryvarden and Johansen 1980, Larsen and Cobb-Poulle 1990, Ryvarden 1991, Ryvarden and Gilbertson 1994, Dai 1999, Núñez and Ryvarden 2000). A previous phylogenetic study based on 28S DNA sequence data confirmed *Fomitiporella* as an independent genus within Hymenochaetaceae, with *Phellinus caryophyllii* (Racib.) G. Cunn. and *P. cavicola* Kotl. & Pouzar transferred into *Fomitiporella* (Wagner and Fischer 2002). During the past five years, many new species were revealed based on morphological characters and molecular data (Zhou 2014, Ji et al. 2017). Recently, Ji et al. (2017) broadened the concept of *Fomitiporella* to accommodate species with resupinate to effused reflexed and annual basidiomata.

As a continuation of the revision of *Fomitipo*rella Murrill, phylogenetic inferences based on 28S and ITS DNA sequences revealed three new species. The taxonomic affinity and the evolutionary relationships among the new species and relates species are outlined.

Materials and methods

Morphological studies

Specimens studied are deposited in the herbarium of Beijing Forestry University (BJFC) and will be forwarded to the National Museum Prague of Czech Republic (PRM). The sections were prepared in 5% potassium hydroxide (KOH), Melzer's reagent (IKI) and Cotton Blue (CB). The following abbreviations were used: KOH = 5% potassium hydroxide, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, CB = Cotton Blue, CB+ = cyanophilous, CB(+) = cyanophilic after 12 hours stained with Cotton Blue, CB– = acyanophilous, L = mean spore length (arithmetic average of the spores), W = mean spore width (arithmetic average of the spores), Q = variation in the ratios of L/W between specimens studied and n = number of spores measured from new specimens. The microscopic procedure follows He and Li (2013) and the special color terms follow Petersen (1996). Sections were studied at magnifications up to 1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and illustrations were made from slide preparations stained with Cotton Blue. Spores were measured from sections cut from the tubes.

Molecular study and phylogenetic analysis

A CTAB-based rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens. The primer pair ITS4 and ITS5 was used for amplification of the ITS region (White et al. 1990), while the primer pair LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) was used for providing the D1–D4 regions of the 28S (https://unite.ut.ee/primers.php). The PCR procedure for ITS amplification was as follows: initial denaturation at 95°C for

3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for 28S was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at the Beijing Genomics Institute, China, with the same primers.

Reference ITS and 28S sequences from various species of *Fomitiporella*, available from GenBank (Benson et al. 2017), were compiled and complemented with sequences generated for this study. Additionally, we also used sequences from Ji et al. (2017) (Table 1). *Phellinus laevigatus* (P. Karst.) Bourdot & Galzin and *P. populicola* Niemelä were selected as the outgroup representatives both in the ITS dataset and 28S dataset (Wagner and Fischer 2002). The sequences were aligned using ClustalX 1.83 (Chenna et al. 2003) and alignments were curated manually in BioEdit 7.0.5.3 (Hall 1999). Prior to phylogenetic analyses, ambiguous regions at the start and the end were deleted. The sequence alignment was deposited at TreeBase (submission ID 22036; www.treebase.org). Phylogenetic analyses were carried out as described previously (Ji et al. 2017).

Maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses were performed for the two datasets. The three phylogenetic analysis algorithms generated nearly identical topologies for each dataset, thus only the topology from the MP analysis is presented along with statistical values from the ML, MP and BI algorithms (Bootstrap support < 50 % and Bayesian posterior probabilities < 0.9 are not shown) at the nodes. MP analyses were performed using PAUP* 4.0b10 (Swofford 2002) with gaps in the alignments treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm with all characters given equal weight. Branch supports (BS) for all parsimony analyses were estimated by performing 1,000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. Sequences were also analyzed using MLwith RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org). BI was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist and Huelsenbeck 2003). The ITS region was divided into three partitions, ITS1, 5.8S and ITS2, for the Bayesian analysis. MrModeltest2.3 (Posada and Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each dataset. Trees were visualized in TreeView 1.6.6 (Page 1996).

Results

Fifty-six 28S rDNA sequences, including eight sequences generated in this study (GenBank accession numbers MG657320–MG657327) and forty-six ITS rDNA sequences, including six sequences generated in this study (GenBank accession numbers MG657328–MG657333) were used to infer the phylogenetic trees. Sequence information is provided in Table 1. The 28S dataset had an aligned length of 898 characters, of

Table 1. Information on the sequences used in this study. Type specimens are shown in bold.

Species	Location	Sample no.	GenBank accession no.	
			ITS	28S
Fomitiporella americana	USA	JV 0312/26.6J	KX181291	–
F. americana	USA	JV 0212/8J	KX181292	–
F. americana	USA	JV 0904/149J	KX181293	KX181329
F. austroasiana	**China**	**Dai 16244**	**MG657328**	**MG657320**
F. austroasiana	China	Dai 16168	MG657329	MG657321
F. austroasiana	Singapore	Dai 17868	–	MG657322
F. austroasiana	Singapore	Dai 17871	–	MG657323
F. austroasiana	Singapore	Dai 17879	MG657330	MG657324
F. caryophyllii	India	CBS 448.76	AY558611	AY059021
F. cavicola	UK	N 153	–	AY059052
F. caviphila	China	LWZ 20130812-1	–	KF729937
F. chinensis	China	Cui 11097	KX181310	KX181342
F. chinensis	China	Cui 11091	–	KX181340
F. chinensis	China	LWZ 20130713-7	KJ787817	KJ787808
F. chinensis	China	LWZ 20130916-3	KJ787818	KJ787809
F. chinensis	China	Cui 11095	–	KX181341
F. chinensis	China	Cui 8725	–	KX181343
F. inermis	USA	JV 0509/57K	KX181305	KX181346
F. inermis	USA	JV 1109/19A	KX181304	–
F. inermis	USA	JV 1009/56	KX181306	KX181347
F. mangrovei	**USA**	**JV 1008/60**	**KX181313**	**KX181334**
F. mangrovei	France	JV 1612/25-J	MG657331	MG657325
F. micropora	USA	JV 1312/E2J	KX181294	KX181333
F. micropora	USA	JV 1407/46	KX181295	KX181332
F. micropora	USA	JV 0409/6J	KX181296	KX181331
F. micropora	USA	JV 1207/6.1J	KX181297	KX181330
F. resupinata	Cameroon	Douanla-Meli 476	KJ787822	JF712935
F. sinica	China	Cui 10139	KX181298	–
F. sinica	China	Dai 10461	KX181300	–
F. sinica	China	LWZ 20130809-8	KJ787820	KJ787811
F. sinica	China	LWZ 20140625-2	KX181301	KX181320
F. sinica	China	LWZ 20140624-5	KX181302	KX181321
F. sinica	China	Dai 12450	–	KX181326
F. sinica	China	Dai 13944	–	KX181324
F. sp. 1	China	Cui 6557	KX181303	–
F. sp. 2	China	Cui 11352	KX181315	KX181338
F. sp. 3	China	LWZ 20140721-2	KX181316	KX181337
F. sp. 4	Thailand	LWZ 20140729-22	KX181317	KX181339
F. sp. 5	Chile	Fv.Ch-7	–	DQ459301
F. sp. 6	Ethiopia	AM 12	JF895466	JQ910908
F. sp. 7	Ethiopia	AM 15	JF895467	JQ910909
F. sp. 8	Ethiopia	AM 18	JF895468	JQ910910
F. sp. 9	Ethiopia	AM 04	KX181318	KX181335
F. subinermis	China	Dai 15114	KX181308	KX181344
F. subinermis	China	Dai 15131	KX181307	KX181345

Species	Location	Sample no.	GenBank accession no.	
			ITS	28S
F. tenuissima	China	Dai 12365	KC456244	KC999901
F. tenuissima	China	Dai 12245	KC456242	KC999902
F. tenuissima	China	Dai 12255	KC456243	KC999903
F. tenuissima	China	Cui 10960	KX181319	–
F. umbrinella	USA	0509/114	KX181314	KX181336
F. umbrinella	USA	CBS 303.66	–	AY059036
F. vietnamensis	**Vietnam**	**Dai 18377**	**MG657332**	**MG657326**
F. vietnamensis	Vietnam	Dai 18382	MG657333	MG657327
Fulvifomes fastuosus	Thailand	LWZ 20140801-1	KR905675	KR905669
F. robiniae	USA	CBS 211.36	AY558646	AF411825
Inonotus hispidus	Germany	MF 92-829	–	AF311014
I. hispidus	–	CBS 386.61	AY558602	AY558664
I. obliquus	Germany	TW 705	–	AF311017
I. quercustris	Argentina	0193	AY072026	AY059050
I. andersonii	USA	CBS 312.35	–	AY059041
Phylloporia bibulosa	Pakistan	Ahmad 27088	–	AF411824
P. chrysites	Puerto Rico	N.W. Legon	–	AF411821
P. ephedrae	Turkmenistan	TAA 72-2	–	AF411826
P. pectinata	UK	R. Coveny 113	–	AF411823
P. ribis	Germany	MF 82-828	–	AF311040
P. spathulata	Mexico	Chay 456	–	AF411822
Phellinus laevigatus	Finland	TN 3260	–	AF311034
P. laevigatus	–	83-912	AY340051	–
P. populicola	Germany	MF 84-61	–	AF311038
P. populicola	Sweden	BRNM 714885	GQ383706	–

which 628 characters are constant, 84 are variable and parsimony-uninformative, and 186 (21%) are parsimony-informative. The best-fit model for the 28S dataset estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The ITS dataset had an aligned length of 854 characters, of which 350 are constant, 114 variable and parsimony-uninformative, and 390 (46 %) parsimony-informative. The best-fit models for the three partitions estimated and applied in the Bayesian analysis are as follows: HKY+I+G for ITS1, K80+I+G for 5.8S and HKY+G for ITS2. The Bayesian and ML analyses produced similar topologies compared to the MP analysis, with an average standard deviation of split frequencies = 0.006943 (BI) (28S). Bayesian analysis and ML analysis resulted in a similar topology as the MP analysis, with an average standard deviation of split frequencies = 0.009677 (BI) (ITS).

The current phylogenies (Figs 1, 2) confirmed that *Fomitiporella austroasiana*, *F. mangrovei* and *F. vietnamensis* formed three strongly supported clades (all received strong branch support in the ML, BI and MP analyses). These taxa have typical morphology of the current concept of *Fomitiporella* (Ji et al. 2017). However, each clade has its unique characters distinct from other *Fomitiporella* species. We therefore describe them as new species.

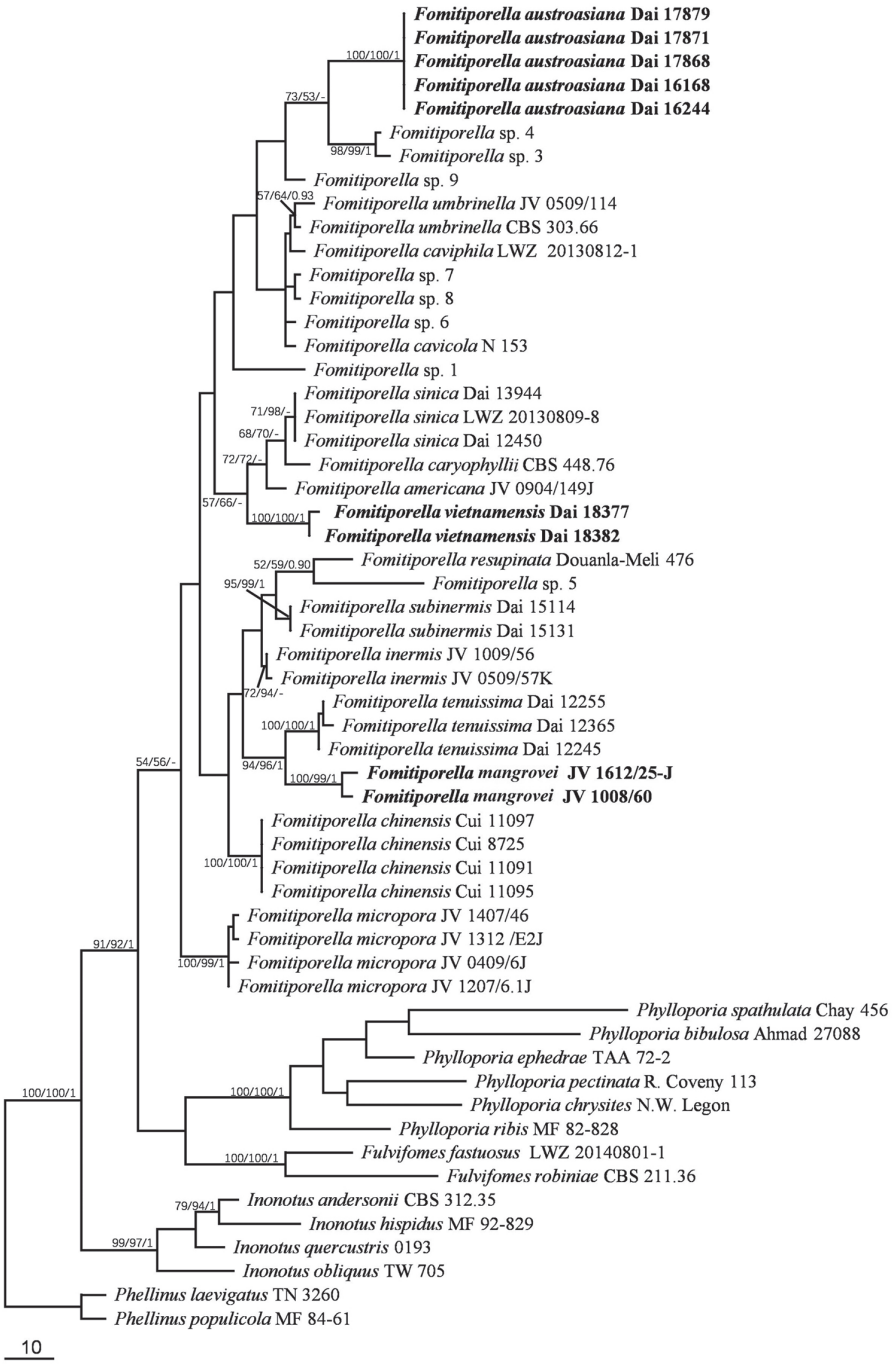

Figure 1. Phylogeny of *Fomitiporella* inferred from the 28S dataset. The topology is that of the MP analysis, and statistical values (ML/MP/BI) are indicated for each node that simultaneously received BS from ML and MP not below 50 %, and BPP from BI not below 0.9. *Phellinus laevigatus* and *P. populicola* are used to root the tree. Branch lengths reflect the number of steps as indicated by the scale.

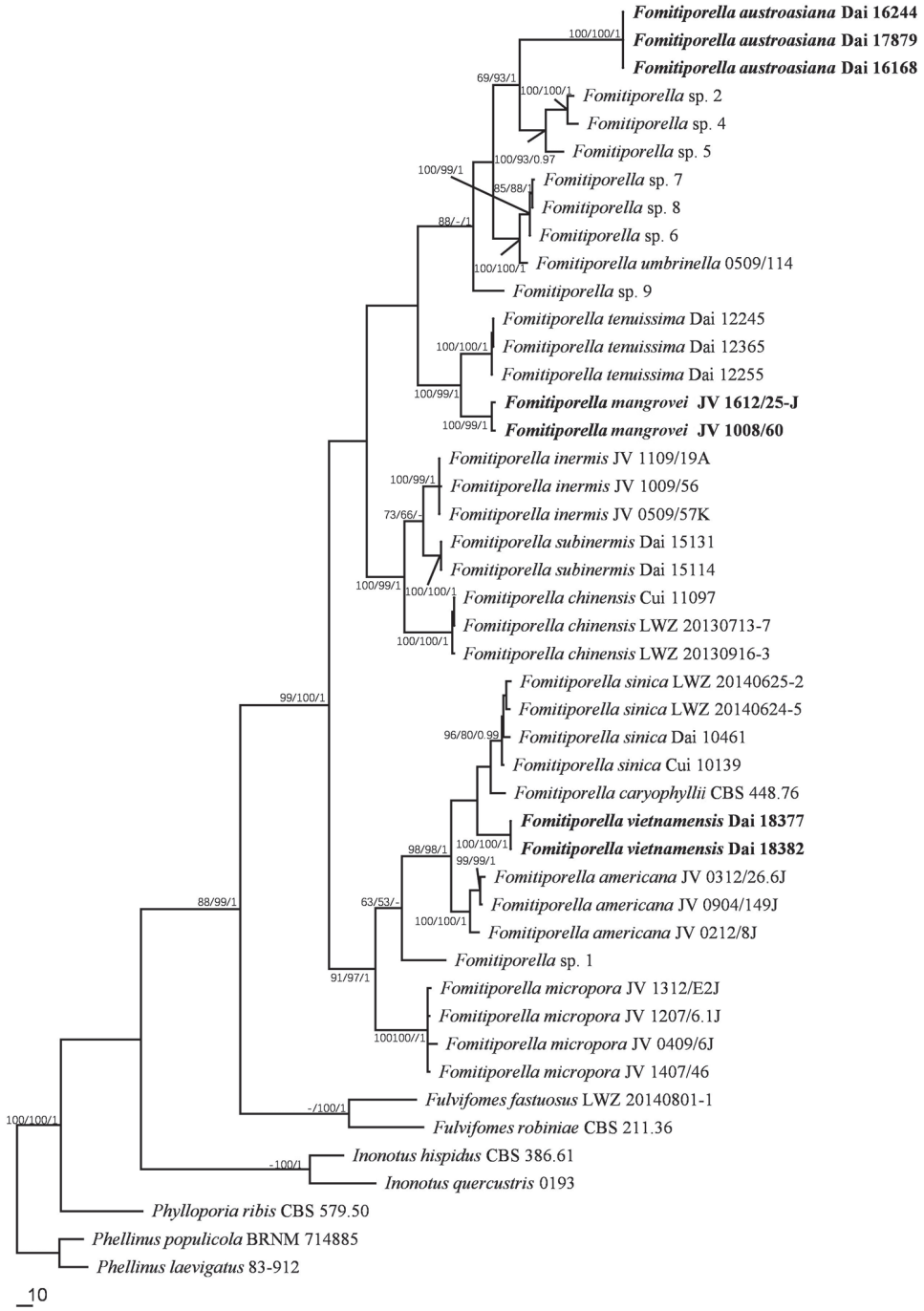

Figure 2. Phylogeny of *Fomitiporella* inferred from the ITS dataset. The topology is that of the MP analysis, and statistical values (ML/MP/BI) are indicated for each node that simultaneously received BS from ML and MP not below 50 %, and BPP from BI not below 0.9. *Phellinus laevigatus* and *P. populicola* are used to root the tree. Branch lengths reflect the number of steps as indicated by the scale.

Taxonomy

Fomitiporella austroasiana **Y.C. Dai, X.H. Ji & J. Vlasák, sp. nov.**
MycoBank: MB823738
Figs 3, 4

Holotype. CHINA. Hainan Province: Qiongzhong County, Limushan Forest Park, 15 Nov 2015, on fallen angiosperm trunk, *Dai 16244* (BJFC).

Etymology. *Austroasiana* (Lat.): referring to the distribution of the species in South Asia.

Basidiomata perennial, resupinate, hard corky and without odor or taste when fresh, woody hard when dry, up to 12 cm long, 5 cm wide and 12 mm thick at center.

Figure 3. A basidiocarp of *Fomitiporella austroasiana*. Scale bar: 1 cm.

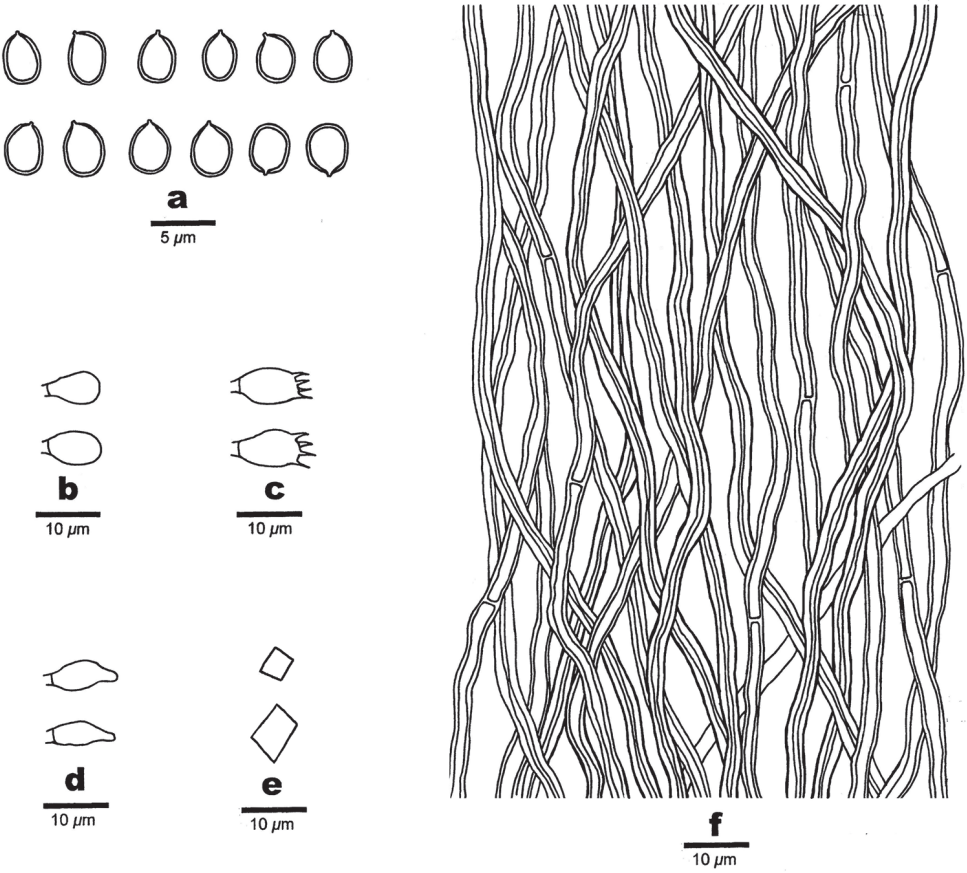

Figure 4. Microscopic structures of *Fomitiporella austroasiana*. **a** Basidiospores **b** Basidioles **c** Basidia **d** Cystidioles **e** Rhomboid crystals **f** Hyphae from trama.

Pore surface ash-gray to grayish brown when fresh, grayish brown to olivaceous, more or less shiny and uncracked on drying; margin yellowish-brown, less than 1 mm wide, thinning out; pores circular, 8–10 per mm; dissepiments thick, entire; tubes woody hard, concolorous with pores, each layer up to 2 mm deep, white mycelial strands present in old tubes. Subiculum very thin to almost lacking.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Tubes. Generative hyphae frequent, hyaline to pale yellow, thin- to slightly thick-walled, occasionally branched, frequently simple septate 1.5–2.5 μm in diam; skeletal hyphae pale brown to brown, thick-walled to almost solid, aseptate, 2–3 μm in diam; setae absent; cystidioles ventricose with elongated apical portion, 7–12 × 3–4 μm; basidia barrel-shaped, with four sterigmata and a simple basal septum, 8–11 × 5–6 μm; basidioles similar to basidia in shape, but slightly smaller; small or big rhomboid crystals abundant.

Spores. Basidiospores subglobose, yellowish-brown, thick-walled, IKI–, CB(+), (3.5–)3.8–4(–4.3) × 3–3.5 µm, L = 4 µm, W = 3.29 µm, Q = 1.2–1.21 (n = 60/2).

Additional specimens examined (paratypes). CHINA. Hainan Province: Wuzhishan, Wuzhishan Nature Reserve, 14 Nov 2015, on fallen angiosperm trunk, *Dai 16168* (BJFC). SINGAPORE. Bukit Timah Nature Reserve, 20 June 2017, *Dai 17868*; *Dai 17871*; *Dai 17879* (BJFC).

Fomitiporella mangrovei **Y.C. Dai, X.H. Ji & J. Vlasák, sp. nov.**
MycoBank: MB823743
Figs 5, 6

Holotype. USA. Florida: Collier-Seminole State Park, 28 Aug 2010, on *Conocarpus erectus*, *JV 1008/60* (BJFC).

Etymology. *Mangrovei* (Lat.): referring to the species growing in mangrove. Basidiomata annual, resupinate, inseparable, without odor or taste when fresh, woody hard on drying, up to 30 cm long, 7 cm wide and 5 mm thick at center. Pore surface ash-gray to bluish gray when fresh, becomes pale clay-buff to pale brown and uncracked when dry; pores angular, 3–5 per mm; dissepiments thin, more or less entire to slightly lacerate; tubes woody hard, dark brown, up to 5 mm long. Subiculum very thin to almost lacking.

Hyphal structure. Hyphal system monomitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH.

Tubes. Generative hyphae hyaline to pale yellowish, thin- to thick-walled with a wide lumen, occasionally branched, frequently simple septate, interwoven, 1.5–3 mm in diam; setae absent; cystidioles absent; basidia barrel-shaped, with four sterigmata and a simple basal septum, 12–15 × 4–6 µm; basidioles barrel-shaped to pyriform, slightly smaller than basidia in size.

Spores. Basidiospores broadly ellipsoid, yellowish-brown, thick-walled, smooth, IKI–, CB+, (5–)5.5–6(–6.3) × (4–)4.2–4.8(–5) µm, L = 5.82 µm, W = 4.47 µm, Q = 1.26–1.31 (n = 60/2).

Additional specimen examined (paratype). FRANCE. Guadeloupe: Grande-Terre, 25 Dec 2012, on *Conocarpus erectus*, *JV 1612/25-J* (BJFC).

Fomitiporella vietnamensis **Y.C. Dai, X.H. Ji & J. Vlasák, sp. nov.**
MycoBank: MB823744
Figs 7, 8

Holotype. VIETNAM. Lam Dong Province, Lac Duong District, Bidoup Nui Ba National Park, 15 Oct 2017, on angiosperm tree, *Dai 18377* (BJFC).

Etymology. *Vietnamensis* (Lat.): referring to the distribution of the species in Vietnam.

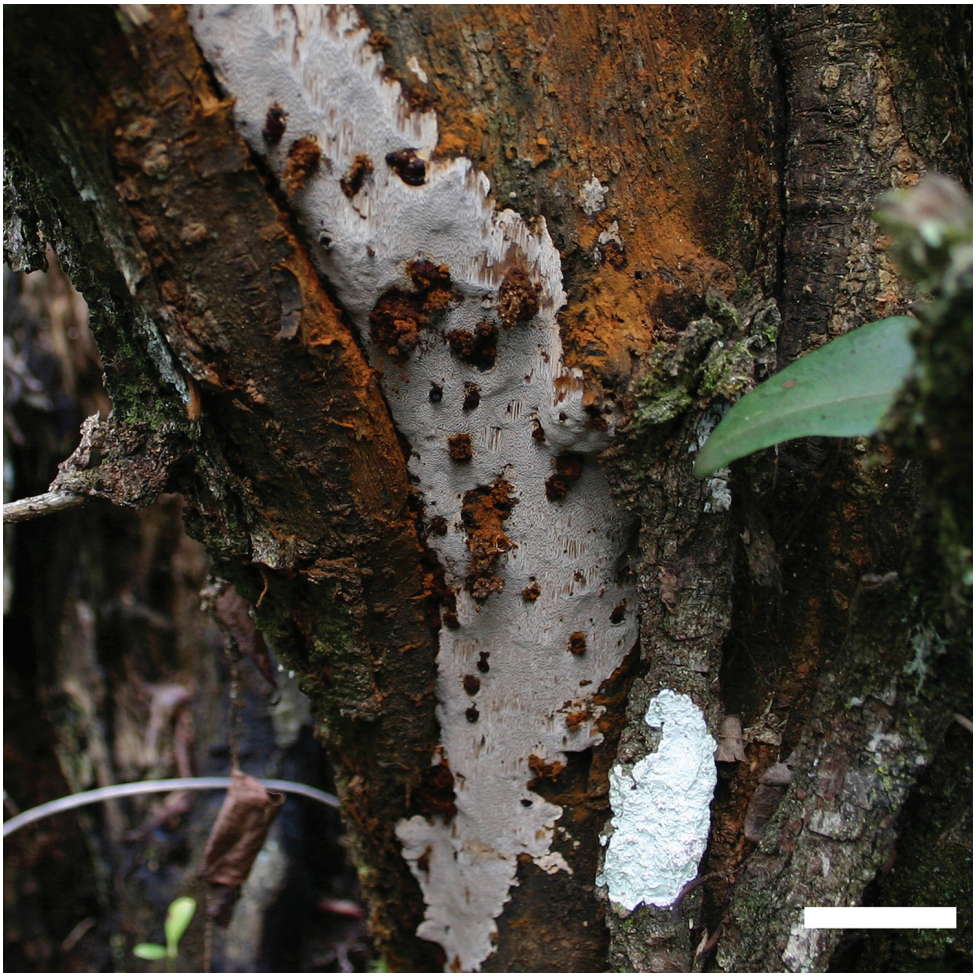

Figure 5. Basidiomata of *Fomitiporella mangrovei*. Scale bar: 5 cm.

Basidiomata perennial, effused-reflexed, imbricate, hard corky and without odor or taste when fresh, projecting up to 1 cm long, 4 cm wide and 5.5 mm thick. Pileal surface bearing curry-yellow and black zones when fresh, becoming deep olive when dry; pore surface bluish gray to ash-gray when fresh, becomes dark brick, shiny and uncracked on drying; margin yellowish-brown, less than 1 mm wide, thinning out; pores angular to circular, 4–7 per mm; dissepiments thin, slightly lacerate. Tubes rust-brown, paler contrasting with pores, up to 5 mm long. Subiculum dull brown, hard corky, up to 0.5 mm.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Subiculum. Generative hyphae rare, hyaline to pale yellowish, thick-walled, rarely branched and septate, 2–2.5 µm in diam; skeletal hyphae dominant, golden yellow,

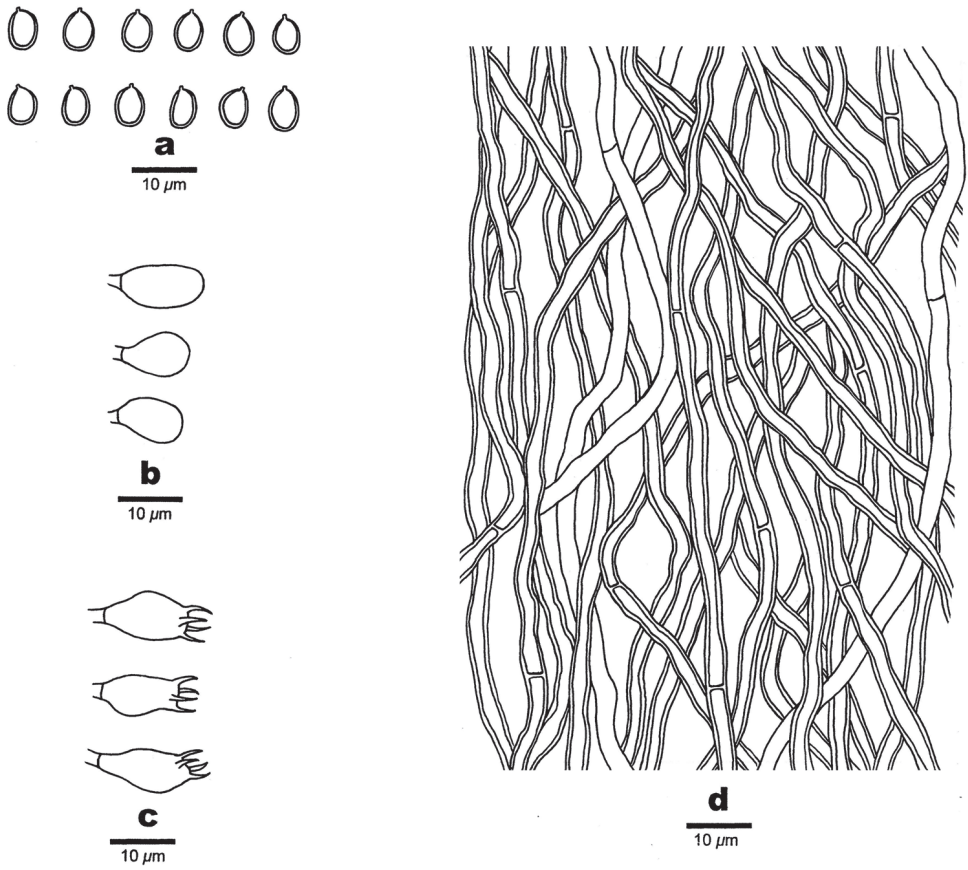

Figure 6. Microscopic structures of *Fomitiporella mangrovei*. **a** Basidiospores **b** Basidioles **c** Basidia **d** Hyphae from trama.

thick-walled with a wide lumen, unbranched, aseptate, more or less flexuous, interwoven, 2–3.5 μm in diam.

Tubes. Generative hyphae frequent, hyaline to pale yellowish, thin- to fairly thick-walled, occasionally branched, frequently septate, 2–2.7 μm in diam; skeletal hyphae dominant, golden yellow, thick-walled, unbranched, aseptate, straight, more or less parallel along the tubes, 2–3 μm in diam; setae absent; cystidioles ventricose with elongated apical portion, 7–14 × 3–5 μm; basidia barrel-shaped, with four sterigmata and a simple basal septum, 10–16 × 5–6 μm; basidioles similar to basidia in shape, but slightly smaller.

Spores. Basidiospores broadly ellipsoid, yellowish-brown, thick-walled, IKI–, CB+, 4–4.8(–5) × (3–)3.2–3.7(–4) μm, L = 4.41 μm, W = 3.52 μm, Q = 1.23–1.28 (n = 60/2).

Additional specimen examined (paratype). VIETNAM. Lam Dong Province, Lac Duong District, Bidoup Nui Ba National Park, 15 Oct 2017, on angiosperm tree, *Dai 18382* (BJFC).

Figure 7. Basidiomata of *Fomitiporella vietnamensis*. Scale bar: 1 cm.

Discussion

Fomitiporella austroasiana fits well in *Fomitiporella* (redefined in Ji et al. 2017). In the current phylogenies (Figs 1, 2), *F. austroasiana* forms a new, strongly supported clade. Macroscopically, *F. austroasiana* is similar to *F. micropora* Y.C. Dai, X.H. Ji & Vlasák in sharing perennial, resupinate basidiomata and small pores (8–10 per mm), a dimitic hyphal structure, and slightly cyanophilous basidiospores (3–4.5 × 2–3.5 µm), whereas *F. micropora* has ellipsoid basidiospores (Q=1.27–1.3, Ji et al. 2017). Moreover, the presence of the cystidioles in *F. austroasiana* makes it different from *F. micropora*.

Fomitiporella mangrovei was previously treated as an undescribed taxon (*Fomitiporella* sp.1) because only a single collection from Florida (USA) was available (Ji et al. 2017). Another specimen, collected from Guadeloupe, Lesser Antilles, was found to represent

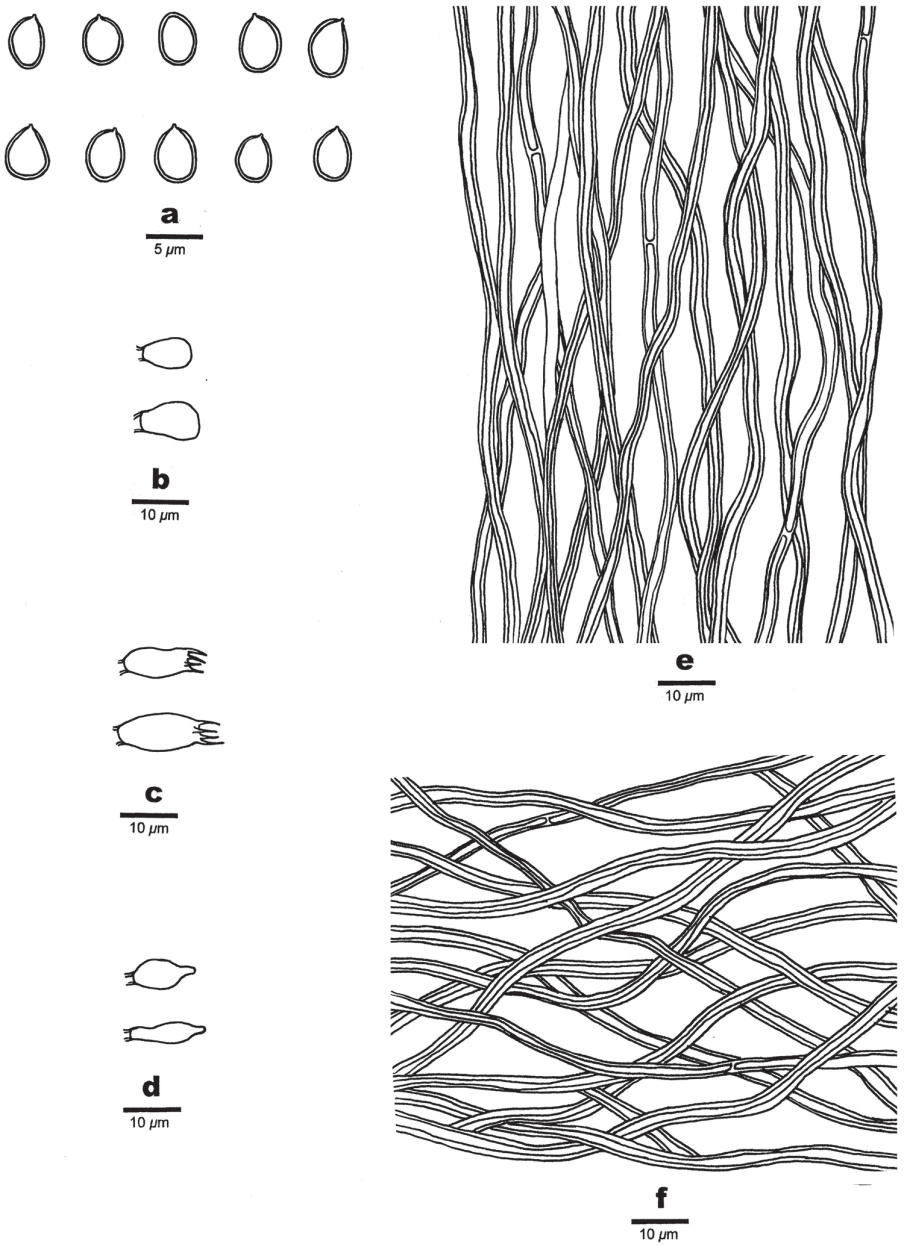

Figure 8. Microscopic structures of *Fomitiporella vietnamensis*. **a** Basidiospores **b** Basidioles **c** Basidia **d** Cystidioles **e** Hyphae from trama **f** Hyphae from subiculum.

the same taxon, allowing a better description. *Fomitiporella mangrovei* is characterized by annual, resupinate basidiomata with ash-gray to bluish gray pores when fresh, large pores (3–5 per mm), a monomitic hyphal structure, ellipsoid, yellowish and thick-walled basidiospores (5–6.3 × 4–5 μm), and growing on *Conocarpus erectus* (Combretaceae), in

mangrove ecosystem. Macroscopically it resembles *F. tenuissima* (H.Y. Yu, C.L. Zhao & Y.C. Dai) Y.C. Dai, X.H. Ji & J. Vlasák and the species are closely related (Figs 1, 2), but *F. tenuissima* differs in having smaller basidiospores (4–5 × 3–4 µm; Yu et al. 2013).

Fomitiporella vietnamensis is distinct by a combination of perennial, effused-reflexed and imbricate basidiomata, shiny and uncracked pore surface, a dimitic hyphal system, and broadly ellipsoid basidiospores, 4–5 × 3–4 µm. *Fomitiporella vietnamensis* is closely related to *F. caryophyllii* (Racib.) T. Wagner & M. Fisch in the current phylogenies (Figs 1, 2). Morphologically, both species share the perennial, effused-reflexed basidiomata and a dimitic hyphal system (Ryvarden and Johansen 1980). However, *F. caryophyllii* has smaller pores (7–9 per mm) and smaller basidiospores of 3–4 × 2.5–3 µm (Ryvarden and Johansen 1980). Another species close to *F. vietnamensis* is *F. americana* Y.C. Dai, X.H. Ji & J. Vlasák (Figs 1, 2), but *F. americana* has strictly resupinate basidiomata and lacks cystidioles (Ji et al. 2017).

The phylogenetic analyses based on 28S or the ITS dataset produced trees with near-identical topologies, and each of the three new species formed a distinct, well-supported clade.

An identification key to the accepted species of *Fomitiporella* is provided as follows:

Key to species of *Fomitiporella*

1 Basidiocarp pileate to effused-reflexed **2**
– Basidiocarp resupinate **4**
2 Pores 3–7 per mm; basidiospores > 4 µm long **3**
– Pores 7–9 per mm; basidiospores < 4 µm long ..... ***F. caryophyllii***
3 Basidiomata biennial; pores 3–4 per mm; basidiospores mostly > 4.5 µm long ..... ***F. chinensis***
– Basidiomata perennial; pores 4–7 per mm; basidiospores mostly < 4.5 µm long ..... ***F. vietnamensis***
4 Basidiomata annual; pore surface more or less grayish when fresh **5**
– Basidiomata perennial; pore surface brown when fresh **6**
5 Pore surface vinaceous gray when fresh; basidiospores < 5 µm long ..... ***F. tenuissima***
– Pore surface ash-gray to bluish gray when fresh; basidiospores > 5 µm long ..... ***F. mangrovei***
6 Cystidioles present **7**
– Cystidioles absent **9**
7 Pores 5–7 per mm; basidiospores mostly > 4.5 µm long **8**
– Pores 8–10 per mm; basidiospores < 4.5 µm long ..... ***F. austroasiana***
8 Basidiomata up to 3 mm thick at center; basidiospores broadly ellipsoid ..... ***F. inermis***
– Basidiomata up to 10 mm thick at center; basidiospores subglobose ..... ***F. subinermis***

9 Pores 5–6 per mm.....**10**
– Pores 6–10 per mm.....**11**
10 Basidiospores 4.7–5.5 µm long; growth mostly on *Fagus*.....***F. cavicola***
– Basidiospores 3.6–4.6 µm long; growth mostly on *Quercus*.....***F. americana***
11 Basidiospores ≤ 4 µm long.....***F. resupinata***
– Basidiospores ≥ 4 µm long.....**12**
12 Pores 6–8 per mm.....**13**
– Pores 8–10 per mm.....***F. micropora***
13 Basidiospores broadly ellipsoid to subglobose, CB(+).....**14**
– Basidiospores ellipsoid to broadly ellipsoid, CB– .....***F. umbrinella***
14 Basidiospores < 4.5 µm long in average.....***F. sinica***
– Basidiospores > 4.5 µm long in average.....***F. caviphila***

Acknowledgments

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