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Article

- [Phylogenetic position of Argentinian and Chilean endemic species of *Senecio* ser. *Culcitium* \(Asteraceae\) with an evolutionary analysis of morphological characters](#) [PDF \(12MB\)](#) 
177–209
- LUCIANA SALOMÓN, SILVANA M. SEDE, SUSANA E. FREIRE
- [*Podoscypha yunnanensis* sp. nov. \(Polyporales, Basidiomycota\) evidenced by morphological characters and phylogenetic analyses](#) [PDF \(3MB\)](#) 
210–218
- YA-XING WU, SHAN SHEN, CHANG-LIN ZHAO
- [Marine red algae \(Rhodophyta\) of economic use in the algal drifts from the Yucatan Peninsula, Mexico](#) [PDF \(128MB\)](#) 
219–240
- MARÍA LUISA NÚÑEZ RESENDIZ, KURT M. DRECKMANN, ABEL SENTÍES, MICHAEL J. WYNNE, HILDA LEÓN TEJERA
- [Taxonomic Studies on Zingiberaceae of Myanmar I: A new species of *Curcuma* \(Subgenus *Ecomatae*\) from Myanmar](#) [PDF \(854KB\)](#) 
241–248
- NOBUYUKI TANAKA, MU MU AUNG
- [A reinvestigation on the taxonomic identity of *Rhynchospora panduranganii* \(Cyperaceae\)](#) [PDF \(1MB\)](#) 
249–254
- S. DEEPU, WILLIAM WAYT THOMAS
- [*Ripartitella brunnea*, a new species from subtropical China](#) [PDF \(2MB\)](#) 
255–261
- MING ZHANG, TAI-HUI LI, TIE-ZHENG WEI, XI-SHEN LIANG, ZHU-XIANG LIU
-

Correspondence

- [Validation of the name *Oenothera italica* \(Onagraceae\)](#) [PDF \(3MB\)](#) 
262–264
- MONIKA WOŹNIAK-CHODACKA
- [Lectotypification of the name *Linaria tonziqii* Lona \(Plantaginaceae\)](#) [PDF \(229KB\)](#) 
265–266
- SIMONE ORSENIGO, GABRIELE GALASSO
- [*Peucedanum shanianum*, a new replaced name for *P. rubricaula* R.H. Shan & M.L. Sheh \(Apiaceae\)](#) [PDF \(275KB\)](#) 
267–268
- FENGLIN CHEN, YUNFEI DENG



Podoscypha yunnanensis sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analyses

YA-XING WU¹, SHAN SHEN¹ & CHANG-LIN ZHAO^{1,2*}

¹ College of Biodiversity Conservation and Utilisation, Southwest Forestry University, Kunming 650224, P.R. China

² Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education,

Southwest Forestry University, Kunming 650224, P.R. China

* Corresponding author's e-mail: fungichanglinz@163.com

Abstract

A new wood-inhabiting fungal species, *Podoscypha yunnanensis* sp. nov., is proposed based on morphological and molecular characters. The species is characterized by annual, gregarious basidiocarps with spatulate to flabelliform pilei, a dimitic hyphal system with clamped generative hyphae, caulocystidia cylindrical with an apex, and ellipsoid to subglobose basidiospores measuring $3\text{--}3.5(-4) \times 2.5\text{--}3(-3.5)$ μm . The internal transcribed spacer (ITS) and the large subunit (LSU) regions of the nuclear ribosomal RNA gene sequences of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analyses based on molecular data of ITS sequences showed that *P. yunnanensis* belonged to the genus *Podoscypha* and was closely related to *P. fulvonitens* and *P. mellissii*. Phylogeny based on ITS+nLSU sequences demonstrated that the new species formed a monophyletic lineage with a high support (100% BS, 100% BP, 1.00 BPP).

Keywords: Meruliaceae, phylogenetic analysis, taxonomy, wood-rotting fungi

Introduction

Podoscypha Pat. (1900: 70) (Meruliaceae, Polyporales), was typified by *P. nitidula* (Berk.) Pat. (1903: 21), which is a cosmopolitan genus characterized by a combination of flabelliform to infundibuliform, more or less stipitate basidiocarps, hymenophore smooth to more or less rugose, a dimitic hyphal structure with clamped generative hyphae, cystidia hyaline, thin- to thick-walled, basidia clavate and basidiospores hyaline, thin-walled, smooth, ellipsoid to cylindrical, acyanophilous and negative in Melzer's reagent (Patouillard 1900, Bernicchia & Gorjón 2010). So far about 48 species have been accepted in the genus worldwide (Patouillard 1900, Boidin 1959, 1960, Reid 1965, Dhingra 1987, Ryvarden 1997, 2015, Drechsler-Santos *et al.* 2007, Bernicchia & Gorjón 2010).

Recently, molecular studies involving *Podoscypha* have been carried out (Larsson 2007, Sjökvist *et al.* 2012, Binder *et al.* 2013, Justo *et al.* 2017). Larsson (2007) introduced a new division for part of the Polyporales, effectively renaming the phlebioid and residual polyporoid clades and suggested that *P. multizonata* (Berk. & Broome) Pat. (1928: 6) nested in the family Meruliaceae Rea. Sjökvist *et al.* (2012) explored DNA-phylogeny-based representatives of the genera *Cotylidia* P. Karst. (1881: 22), *Cymatoderma* Jungh. (1840: 290), *Muscinipta* Redhead, Lücking & Lawrey (2009: 1167), *Podoscypha* and *Stereopsis* D.A. Reid (1965: 290), which can be reconciled in the basidiomycetes and eleven species nested in the family Meruliaceae and grouped with *Abortiporus biennis* (Bull.) Singer (1944: 68) and *Cymatoderma*. Binder *et al.* (2013), using a molecular study based on multi-gene datasets, demonstrated that four species of *P. multizonata*, *P. parvula* (Lloyd) D.A. Reid (1965: 220), *P. petalodes* (Berk.) Boidin (1959: 230) and *P. venustula* (Speg.) D.A. Reid (1965: 260) belonged to the residual polyporoid clades and appeared to group with *A. biennis*. By using the multi-gene datasets, Justo *et al.* (2017) revised the classification of the Polyporales (Basidiomycota) at the family level, included eighteen families and showed that *P. parvula* belongs to Podoscyphaceae from the residual polyporoid clade, but it showed low support in the phylogenetical tree.

The family Meruliaceae P. Karst. is a cosmopolitan group and has a rich diversity, growing in boreal, temperate, subtropical, and tropical vegetation biomes (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Bernicchia &

Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Dai *et al.* 2015). During investigations on wood-inhabiting fungi in southern China, an additional taxon of *Podoscypha* was found which could not be assigned to any described species. In this study, the authors expand samplings from previous studies to examine the taxonomy and phylogeny of this new species within the *Podoscypha*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Materials and methods

Morphological studies:—The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens and observed under a light microscope following Dai (2010a). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer’s reagent, IKI– = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

DNA extraction, amplification, sequencing and phylogenetic analyses:—The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd, USA) was used to obtain PCR products from dried specimens, according to the manufacturer’s instructions with some modifications. ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al.* 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C 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 directly sequenced at the Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited in GenBank (Table 1).

TABLE 1. A list of species, specimens and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Abortiporus biennis</i>	FD 319	KP135300	KP135195	Binder <i>et al.</i> 2013
<i>Podoscypha bolleana</i>	32034	JQ675334	—	Binder <i>et al.</i> 2013
<i>P. bolleana</i>	32032	JQ675332	—	Binder <i>et al.</i> 2013
<i>P. bolleana</i>	CBS 333.66	JN649354	JN649354	Sjökvisst <i>et al.</i> 2012
<i>P. brasiliensis</i>	17586	JQ675312	—	Binder <i>et al.</i> 2013
<i>P. brasiliensis</i>	LR 37812	JN649355	JN649355	Sjökvisst <i>et al.</i> 2012
<i>P. bubalina</i>	17500	JQ675311	—	Binder <i>et al.</i> 2013
<i>P. cristata</i>	8667	JQ675320	—	Binder <i>et al.</i> 2013
<i>P. disseminata</i>	DMC 232	JQ675326	—	Binder <i>et al.</i> 2013
<i>P. elegans</i>	CBS 426.51	JN649356	MH868453	Sjökvisst <i>et al.</i> 2012
<i>P. fulvonitens</i>	17483	JQ675315	—	Binder <i>et al.</i> 2013
<i>P. fulvonitens</i>	18332	JQ675316	—	Binder <i>et al.</i> 2013
<i>P. fulvonitens</i>	C 1	JQ675322	—	Binder <i>et al.</i> 2013
<i>P. gillesii</i>	32036	JQ675335	—	Binder <i>et al.</i> 2013
<i>P. involuta</i>	CBS 654.84	MH861804	MH873497	Sjökvisst <i>et al.</i> 2012
<i>P. involuta</i>	CBS 113.74	JN649358	JN649358	Sjökvisst <i>et al.</i> 2012
<i>P. involuta</i>	Larsson	JN649357	—	Sjökvisst <i>et al.</i> 2012
<i>P. mellissii</i>	LR 41658	JN649359	—	Sjökvisst <i>et al.</i> 2012
<i>P. moelleri</i>	17588	JQ675313	—	Binder <i>et al.</i> 2013

.....continued on the next page

TABLE 1. (Continued)

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>P. multizonata</i>	Jahn 751012	JN649360	—	Sjökvisst <i>et al.</i> 2012
<i>P. multizonata</i>	CBS 662.84	MH861808	—	Sjökvisst <i>et al.</i> 2012
<i>P. multizonata</i>	CBS 663.84	MH861809	MH873501	Sjökvisst <i>et al.</i> 2012
<i>P. multizonata</i>	CBS 661.84	MH861807	MH873500	Sjökvisst <i>et al.</i> 2012
<i>P. multizonata</i>	CBS 660.84	MH861806	MH873499	Sjökvisst <i>et al.</i> 2012
<i>P. multizonata</i>	3005	JN710581	JN710581	Miettinen <i>et al.</i> 2012
<i>P. parvula</i>	32055	JQ675338	—	Binder <i>et al.</i> 2013
<i>P. parvula</i>	DMC 226	JQ675328	—	Binder <i>et al.</i> 2013
<i>P. parvula</i>	CBS 331.66	JN649361	—	Sjökvisst <i>et al.</i> 2012
<i>P. petalodes</i>	CBS 332.66	JN649363	MH870450	Sjökvisst <i>et al.</i> 2012
<i>P. petalodes</i>	CBS 659.84	JN649362	MH873498	Sjökvisst <i>et al.</i> 2012
<i>P. ravenelii</i>	CBS 664.84	JN649364	—	Sjökvisst <i>et al.</i> 2012
<i>P. venustula</i>	LR 40821	JX109851	—	Sjökvisst <i>et al.</i> 2012
<i>P. venustula</i>	CBS 656.84	JN649367	—	Sjökvisst <i>et al.</i> 2012
<i>P. vespillonea</i>	CBS 111.74	JN649368	MH872572	Sjökvisst <i>et al.</i> 2012
<i>P. vespillonea</i>	CBS 348.66	MH858820	MH870457	Sjökvisst <i>et al.</i> 2012
<i>P. vespillonea</i>	DMC 220	JQ675331	—	Binder <i>et al.</i> 2013
<i>P. yunnanensis</i>	CLZhao 3963	MK298400	MK298404	Present study
<i>P. yunnanensis</i>	CLZhao 4035	MK298403	MK298407	Present study
<i>P. yunnanensis</i>	CLZhao 3973	MK298401	MK298405	Present study
<i>P. yunnanensis</i>	CLZhao 3979	MK298402	MK298406	Present study

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Kato & Toh 2008; <http://mafft.cbrc.jp/alignment/server/>) using the “G-INS-I” strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 23475). Sequences of *Abortiporus biennis* obtained from GenBank were used as outgroups to root trees following Binder *et al.* (2013) in the ITS and ITS+nLSU analyses (Figs. 1 & 2).

Maximum parsimony analyses were applied to the ITS and ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Wu *et al.* (2017), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 5 million generations (Fig. 1), for 3 million generations (Fig. 2) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BT) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (BT) and 0.95 (BPP) were considered as significantly supported, respectively.

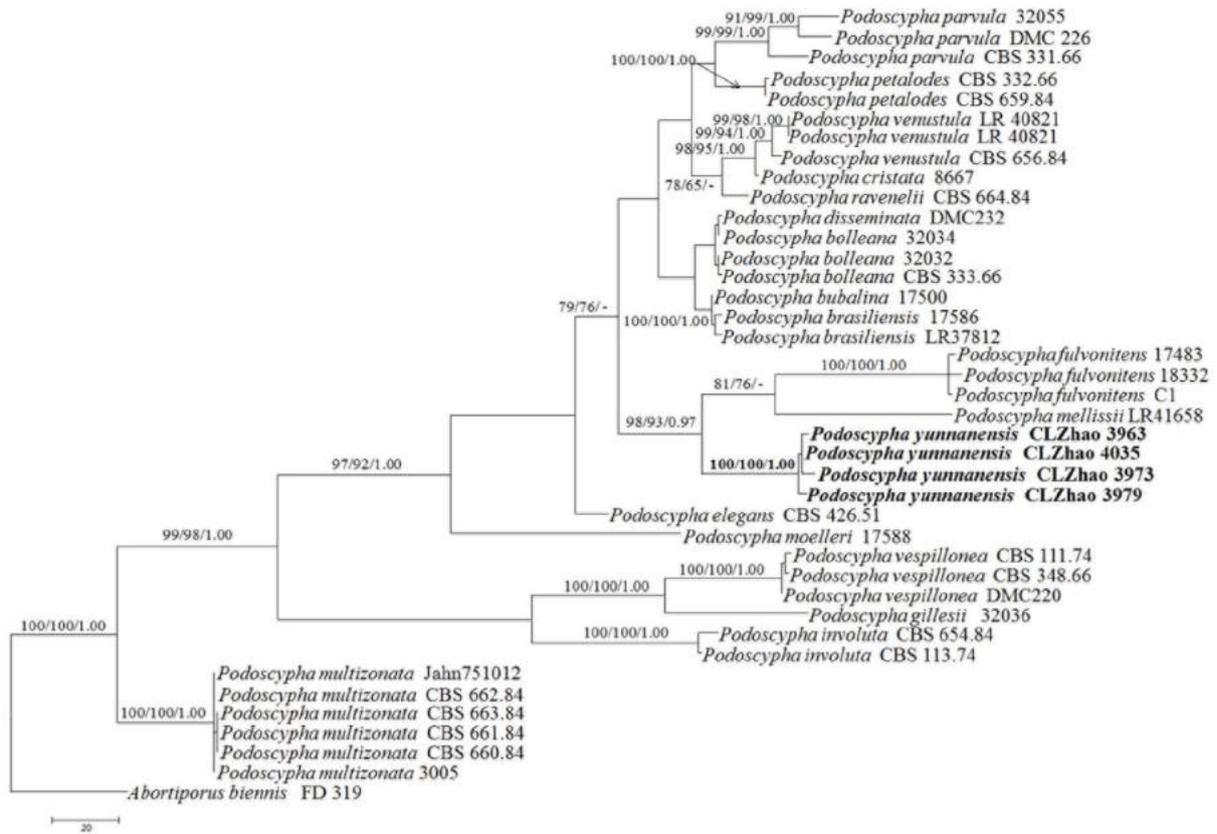


FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Podoscypha yunnanensis* and related species based on ITS sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

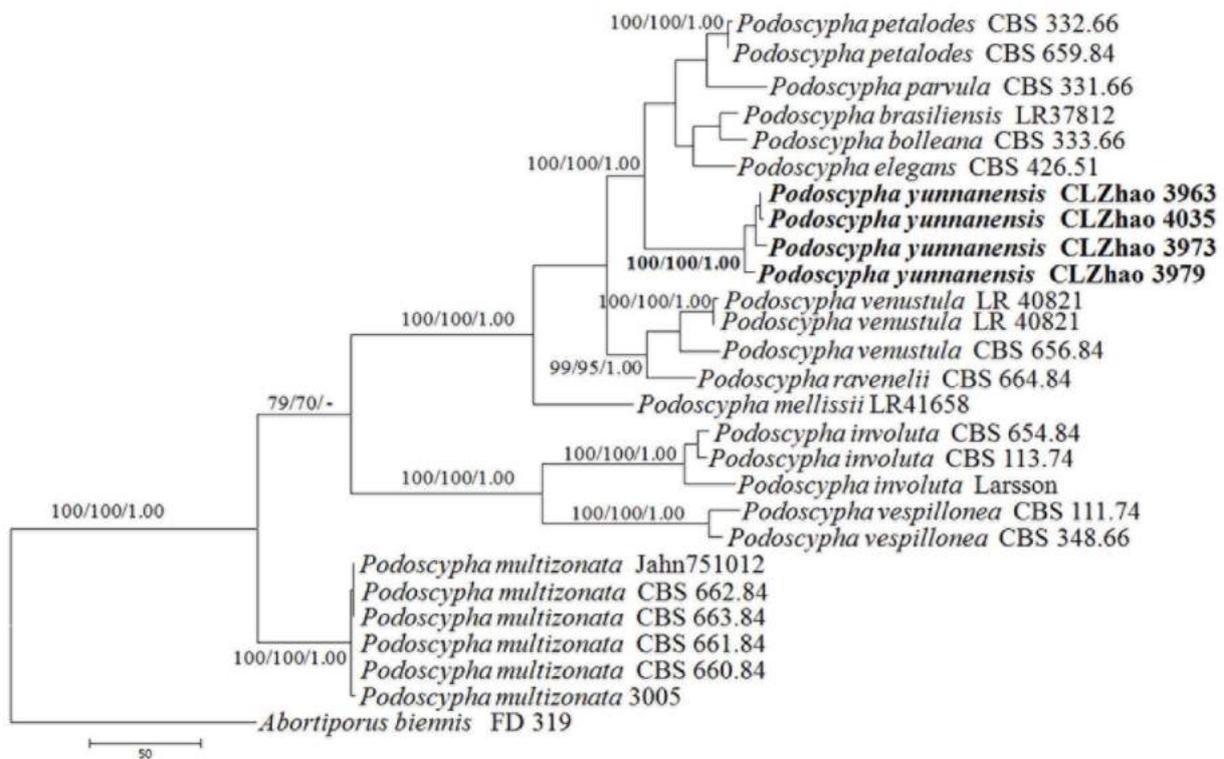


FIGURE 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Podoscypha yunnanensis* and related species based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

Results

Molecular phylogeny

The ITS dataset included sequences from 40 fungal specimens representing 19 species. The dataset had an aligned length of 756 characters, of which 347 characters are constant, 90 are variable and parsimony-uninformative, and 319 are parsimony-informative. Maximum parsimony analysis yielded 100 equally parsimonious trees (TL = 10277, CI = 0.660, HI = 0.339, RI = 0.828, RC = 0.547). The ITS+nLSU dataset (Fig. 2) included sequences from 28 fungal specimens representing 13 species. The dataset had an aligned length of 2221 characters, of which 1355 characters are constant, 494 are variable and parsimony-uninformative, and 372 are parsimony-informative. Maximum parsimony analysis yielded 100 equally parsimonious trees (TL = 1386, CI = 0.788, HI = 0.211, RI = 0.850, RC = 0.670). Best model for the ITS and ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.003157 (ITS) and 0.001352 (ITS+nLSU).

The phylogeny (Fig. 1) inferred from ITS sequences demonstrated that the new species was closely related to *Podoscypha fulvonitens* (Berk.) D.A. Reid (1965: 176) and *P. mellissii* (Berk. ex Sacc.) Bres (1915: 300).

Phylogeny (Fig. 2) inferred from the combined ITS+nLSU sequences demonstrated that the new species formed a monophyletic entity with a high 100% BS, 100% BP and 1.00 BPP.

Taxonomy

Podoscypha yunnanensis C.L. Zhao, *sp. nov.* Figs. 3, 4

Mycobank no.: MB 829067

Type: CHINA. Yunnan Province, Puer, Jingdong county, Ailaoshan National Nature Reserve, on the angiosperm trunk, 4 October 2017, *CLZhao 4035* (holotype, SWFC!)

Etymology:—*Yunnanensis* (Lat.): referring to the locality (Yunnan Province) of the type specimen.

Description:—*Basidiocarps* annual, gregarious, without odor or taste and corky when fresh, becoming hard corky upon drying. *Pilei* spatulate to flabelliform, more or less stipitate, projecting up to 2 cm wide, 1 cm thick at centre. Pileal surface slightly tomentose, zonate, buff to ochraceous when fresh and ochraceous to pale brown upon drying. *Hymenophore surface* smooth, cream to buff when fresh, turn to buff to pale brown upon drying. *Hyphal structure* dimitic; generative hyphae with clamps, thin- to thick-walled, unbranched, 2.5–3.5 µm in diam.; skeletal hyphae colorless, thick-walled with a wide to narrow lumen, unbranched, 3–4.5 µm in diam.; IKI–, CB–, tissues unchanged in KOH.

Hymenium:—Cystidia (caulocystidia) cylindrical with an apex or not, present in subiculum, thin- to thick-walled, 30–55 × 3–5 µm, cystidioles absent; *basidia* narrowly clavate to subcylindrical, with 4(2) sterigmata and a basal clamp, 25–32 × 2.5–4 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. *Basidiospores* ellipsoid to subglobose, hyaline, thin-walled, smooth, IKI–, CB–, 3–3.5(–4) × 2.5–3(–3.5) µm, L = 3.34 µm, W = 2.71 µm, Q = 1.2–1.31 (n = 120/4).

Additional specimens examined:—CHINA. Yunnan Province, Puer, Jingdong county, Ailaoshan National Nature Reserve, on the angiosperm trunk, 4 October 2017, *CLZhao 3963, 3973, 3979*, (SWFC!).

Discussion

In the present study, a new species, *Podoscypha yunnanensis*, is described based on phylogenetic analyses and morphological characters.

In the molecular analysis (Fig. 1), *Podoscypha yunnanensis* grouped with *P. fulvonitens* and *P. mellissii* inferred from the ITS analysis. However, morphologically *P. fulvonitens* differs from *P. yunnanensis* by its basidiocarps with a cuticle and narrower basidiospores (1.7–2.2 µm in width, Reid 1965). *Podoscypha mellissii* can be distinguished by its basidiocarps with long stipes and presence of hymenial metuloids (Drechsler-Santos *et al.* 2007).



FIGURE 3. A basidiomata of *Podoscypha yunnanensis* (holotype). Scale bars: a–b–2 cm. Photos by: Chang-Lin Zhao

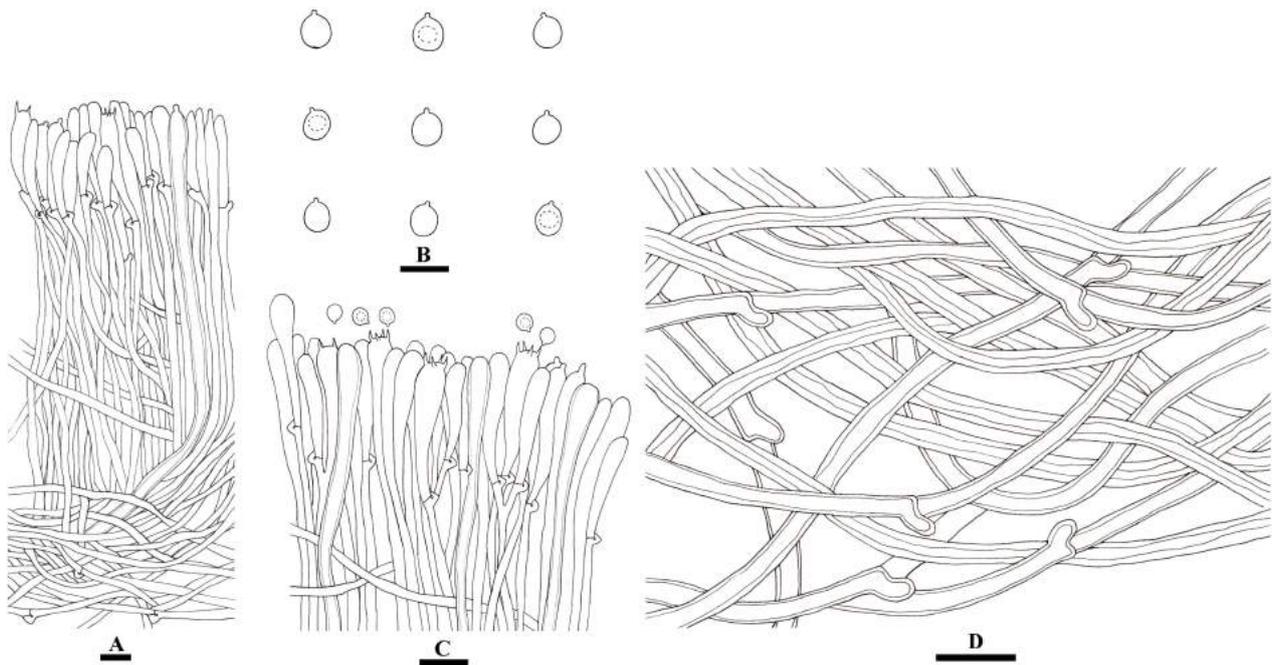


FIGURE 4. Microscopic structures of *Podoscypha yunnanensis* (drawn from the holotype). a. A section of basidiocarp. b. Basidiospores. c. A section of trama. d. Hyphae from context. Bars: a, c, d–10 µm; b–5 µm. Drawings by: Shan Shen

Two similar species in the genus *Podoscypha*: *P. bolleana* (Mont.) Boidin (1960: 30) and *P. moelleri* (Bres. & Henn.) D.A. Reid. (1965: 202) also have caulocystidia. *Podoscypha bolleana* differs from *P. yunnanensis* by having larger caulocystidia (40–125 × 7–15 µm, Boidin 1960, Drechsler-Santos *et al.* 2007). *Podoscypha moelleri* differs in having smaller basidiospores (2.2–3.2 × 2–2.2 µm, Reid 1965).

The diversity of *Podoscypha* species is low in China and only two species have been reported previously from this region: *Podoscypha brasiliensis* D.A. Reid (1965: 169) and *P. elegans* (G. Mey.) Pat. (1900: 71) (Wu 2003, Dai 2010b). However, morphologically *Podoscypha brasiliensis* differs from *P. yunnanensis* by larger basidiospores (5–7 × 3.5–4.5 µm, Reid 1965, Wu 2003, Drechsler-Santos *et al.* 2007). *Podoscypha elegans* can be distinguished by the presence of chlamydospores in the context and larger basidiospores (5–5.6 × 3.8–4.3 µm, Patouillard 1900). The diversity of *Podoscypha* in China is still not well known, especially in the subtropical and tropical regions and many recently described taxa of wood-rotting fungi were from these areas (Ren & Wu 2017, Yuan *et al.* 2017a, b). *Podoscypha yunnanensis*, is also from the subtropics. It is possible that new taxa will be found after further investigation.

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