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Two new species of *Steccherinum* (Polyporales, Basidiomycota) from southern China based on morphology and DNA sequence data

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Short Communication

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Full paper

Two new species of *Steccherinum* (Polyporales, Basidiomycota) from southern China based on morphology and DNA sequence data

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ABSTRACT

Two new wood-inhabiting fungal species, *Steccherinum hirsutum* and *S. yunnanense* spp. nov., are proposed based on a combination of morphological features and molecular evidence. Sequences of internal transcribed spacer and large subunit region of nuclear ribosomal RNA gene of the studied samples were generated, and phylogenetic analyses were performed using maximum likelihood, maximum parsimony and bayesian inference methods. *Steccherinum hirsutum* is characterized by an annual growth habit, stipitate basidiocarps with scarlet to red, odontoid hymenial surface, a dimittic hyphal system with clamped generative hyphae negative in Melzer's reaction, and acyanophilous basidiospores measuring $2.5\text{--}3.5 \times 1.5\text{--}2.5 \mu\text{m}$. *Steccherinum yunnanense* is distinguished by resupinate basidiomata with odontoid hymenial surface, a dimittic hyphal system with clamped generative hyphae, strongly encrusted cystidia and ellipsoid, hyaline, thin-walled, smooth basidiospores ($3.5\text{--}4.5 \times 2\text{--}3.5 \mu\text{m}$). The phylogenetic analyses confirmed that two new species nest in *Steccherinum*, in the residual polyporoid clade.

Keywords: Molecular phylogeny, *Steccherinaceae*, taxonomy, wood-inhabiting fungi, Yunnan Province

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1. Introduction

Steccherinum Gray (*Steccherinaceae*, *Polyporales*), typified with *S. ochraceum* (Pers. ex J.F. Gmel.) Gray, was originally described by Gray (1821), and it is characterized by resupinate to effused-reflexed or pileate basidiomes with a membranaceous consistency and odontoid to hydroid hymenophore. Microscopically, it presents a monomittic or dimittic hyphal system with clamped or simple-septate generative hyphae, some characteristically encrusted at the apices, numerous pseudocystidia, subclavate to clavate basidia and basidiospores that are colourless, thin-walled, smooth, ellipsoid to subcylindrical, acyanophilous and negative to Melzer's reagent (Gray, 1821; Bernicchia & Gorjón, 2010).

So far, about 75 species have been accepted in the genus worldwide (Fries, 1821; Banker, 1906, 1912; Cunningham, 1958; Snell & Dick, 1958; Ryvarden, 1978; Lindsey & Gilbertson, 1977, 1979; Burdsall & Nakasone, 1981; Melo, 1995; Legon & Roberts, 2002; Yuan & Dai, 2005; Spirin, Zmitrovitch, & Malysheva, 2007; Hjortstam & Ryvarden, 2008; Bernicchia & Gorjón, 2010; Miettinen, Larsson, Sjökvist, & Larsson, 2012; Yuan & Wu, 2012; Miettinen & Ryvarden, 2016; Westphalen, Rajchenberg, Tomšovský, & Gugliotta, 2018; Westphalen, Motato-Vásquez, Tomšovský, & Gugliotta, 2021).

2018; Westphalen, Motato-Vásquez, Tomšovský, & Gugliotta, 2021).

Recently, some molecular studies of *Steccherinum* have been carried out (Miettinen et al., 2012; Justo et al., 2017; Westphalen et al., 2018; Westphalen et al., 2021). Miettinen et al. (2012) revealed unaccounted diversity and morphological plasticity in a group of dimittic polypores (*Polyporales*, *Basidiomycota*), in which the phylogeny of the poroid and hydroid genera *Antrodiella* Ryvarden & I. Johans., *Junghuhnia* Corda and *Steccherinum* (*Polyporales*, *Basidiomycota*) was studied. The genus *Steccherinum* was shown to contain both hydroid and poroid species, and *Junghuhnia crustacea* (Jungh.) Ryvarden (generic type) nests in a different clade, apart from other poroid *Steccherinum*. Justo et al. (2017) revised family-level classification of the *Polyporales* (*Basidiomycota*), including eighteen families, and showed that *Steccherinum* belongs to *Steccherinaceae* Parmasto, grouping as a sister clade to *Cerrenaceae* and *Panaceae*. Westphalen et al. (2018) studied neotropical *Junghuhnia* s.lat. based on morphological and multigene analyses, introducing a new species, *Steccherinum neonitidum* Westphalen & Tomšovský and three new combinations, *S. meridionale* (Rajchenb.) Westphalen, Tomšovský & Rajchenberg, *S. polycystidiferum* (Rick) Westphalen, Tomšovský & Rajchenb. and *S. undigerum* (Berk. & M.A. Curtis) Westphalen & Tomšovský. Westphalen et al. (2021) presented morphological and phylogenetic analyses on hydroid specimens of *Steccherinaceae* in which the species studied nested in four genera:

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Cabalodontia Piatek, *Etheiron* Banker, *Metuloidea* G. Cunn., and *Steckerinum* and the authors described three new neotropical species, including *S. larssonii* Westphalen & Motato-Vásq. Recently, phylogenetic analyses on *Steckerinum* taxa from China were carried out, in which based on ITS+nLSU sequences and morphological studies, several new *Steckerinum* species were described: *S. puerense* Y.X. Wu, J.H. Dong & C.L. Zhao, *S. rubigimaculatum* Y.X. Wu, J.H. Dong & C.L. Zhao, *S. tenuissimum* C.L. Zhao & Y.X. Wu and *S. xanthum* C.L. Zhao & Y.X. Wu (Wu, Dong, & Zhao, 2021a; Wu, Wu, & Zhao, 2021b).

During our investigations on the diversity of wood-rotting fungi in southern China, two undescribed hydroid species similar to *Steckerinum* spp. were found. To confirm their placement in *Steckerinum*, morphological examination and phylogenetic analyses based on the internal transcribed spacer (ITS) and the large subunit nuclear ribosomal RNA (nLSU) genes, were carried out.

2. Materials and methods

2.1. Morphological studies

The studied specimens are deposited at the herbarium of Southwest Forestry University (SWFC), Yunnan Province, P.R. China. Macromorphological descriptions are based on field notes. Petersen (1996) was followed for the colour terms. Micromorphological data were obtained from the dried specimens and observed under a light microscope Eclipse E 80i (Nikon, Tokyo) following Dai (2012). The following abbreviations were used for the micro characteristics description: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, IKI = Melzer's reagent, IKI- = both non-amyloid 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.

2.2. Molecular procedures and phylogenetic analyses

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions. ITS region was amplified with primer pairs ITS5 and ITS4 (White, Bruns, Lee, & Taylor, 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (https://sites.duke.edu/vilgalys-lab/rdna_primers_for_fungi/). 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 sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 (<https://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 28971). Sequences of *Climacocystis borealis* (Fr.) Kotl. & Pouzar obtained from GenBank were used as outgroups to root trees in the ITS analysis (Fig. 1), and *Byssomerulius corium* (Pers.) Parmasto and *Irpex lacteus* (Fr.) Fr. were used as an outgroup in the ITS+nLSU (Fig. 2).

Table 1. List of species, specimens, and GenBank accession numbers of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Antella americana</i>	HHB 4100	KP135316	KP135196	Floudas & Hibbett (2015)
<i>A. americana</i>	KHL 11949	JN710509	JN710509	Miettinen et al. (2012)
<i>A. chinensis</i>	Dai 8874	JK110843	KC485544	Yuan (2013a)
<i>A. chinensis</i>	Dai 9019	JK110844	KC485542	Yuan (2013a)
<i>Antrodia faginea</i>	KHL 11977	JN710514	JN710514	Miettinen et al. (2012)
<i>A. foliaceodentata</i>	X 1238	JN710515	JN710515	Miettinen et al. (2012)
<i>A. ichnusana</i>	X 131	JN710516	JN710516	Miettinen et al. (2012)
<i>A. pallescens</i>	X 1080	JN710518	JN710518	Miettinen et al. (2012)
<i>A. romellii</i>	X 154	JN710520	JN710520	Miettinen et al. (2012)
<i>A. semisupina</i>	X 242	JN710521	JN710521	Miettinen et al. (2012)
<i>A. stipitata</i>	FD-136	KP135314	KP135197	Floudas & Hibbett (2015)
<i>A. stipitata</i>	Yuan 5640	KC485525	KC485544	Yuan (2013b)
<i>Atraporcella neotropica</i>	Leif Ryvarden 44447	HQ659271	HQ659271	Miettinen & Rajchenberg (2012)
<i>A. yunnanensis</i>	CLZhao 604	MF962482	MF962485	Wu et al. (2017)
<i>A. yunnanensis</i>	CLZhao 605	MF962483	MF962486	Wu et al. (2017)
<i>Butyrea japonica</i>	Nun'ez 1065	JN710556	JN710556	Miettinen et al. (2012)
<i>B. luteoalba</i>	FP 105786	KP135226	KP135226	Floudas & Hibbett (2015)
<i>B. luteoalba</i>	KH Larsson 13238b	JN710558	JN710558	Miettinen et al. (2012)
<i>Byssomerulius corium</i>	FP 102382	KP135007	KP135230	Floudas & Hibbett (2015)
<i>Climacocystis borealis</i>	Dai 3703	KJ566626	KJ566636	Song et al. (2014)
<i>Etheiron fimbriatum</i>	HR 98811	MT849300	—	Westphalen et al. (2021)
<i>E. purpureum</i>	MCW 642/18	MT849301	MT849301	Westphalen et al. (2021)
<i>Flaviporus brownii</i>	MCW 362/12	KY175008	KY175008	Westphalen et al. (2018)
<i>F. brownii</i>	X 462	JN710538	JN710538	Miettinen et al. (2012)
<i>F. liebmannii</i>	X 666	JN710540	—	Miettinen et al. (2012)
<i>F. liebmannii</i>	X 249	JN710539	JN710539	Miettinen et al. (2012)
<i>F. liebmannii</i>	Yuan 1766	KC502914	—	Yuan (2013b)
<i>F. liebmannii</i>	TFRI 676	EU232262	—	Miettinen et al. (2012)
<i>F. subundatus</i>	MCW 367/12	KY175004	KY175004	Westphalen et al. (2018)
<i>F. subundatus</i>	MCW 457/13	KY175005	KY175005	Westphalen et al. (2018)
<i>Frantisia fissiliformis</i>	CBS 435.72	MH860521	MH872232	Vu et al. (2019)
<i>F. menschulensis</i>	BRNM 710170	FJ496728	FJ496728	Tomšovský et al. (2010)
<i>F. menschulensis</i>	AH 1577	JN710544	JN710544	Miettinen et al. (2012)
<i>F. ussuri</i>	Dai 8249	KC485526	—	Yuan (2013b)
<i>F. ussuri</i>	Wei 3081	KC485527	KC485545	Yuan (2013b)
<i>Irpex lacteus</i>	DO 421/1951208	JK109852	JK109852	Binder et al. (2013)
<i>Jianghuhnia austrosinensis</i>	Dai 17540	MN871755	MN877768	Du et al. (2020)
<i>J. austrosinensis</i>	Dai 17679	MN871756	MN877769	Du et al. (2020)
<i>J. crustacea</i>	X 262	JN710553	JN710553	Miettinen et al. (2012)
<i>J. nandinae</i>	Dai 21107	MN833677	MN833679	Du et al. (2020)
<i>J. nandinae</i>	Dai 21108	MN833678	MN833680	Du et al. (2020)
<i>J. pseudocrustacea</i>	Yuan 6160	MF139551	—	Yuan et al. (2019)
<i>J. pseudocrustacea</i>	Zhou 283	MF139552	—	Yuan et al. (2019)
<i>Loweomyces fractipes</i>	MT 13/2012	KX378866	KX378866	Westphalen et al. (2016)
<i>L. fractipes</i>	X 1149	JN710570	JN710570	Miettinen et al. (2012)
<i>L. spissius</i>	MCW 488/14	KX378869	KX378869	Westphalen et al. (2016)
<i>L. lentosus</i>	MCW 366/12	KX378870	KX378870	Miettinen et al. (2012)
<i>L. wynneae</i>	X 1215	JN710604	JN710604	Miettinen et al. (2012)
<i>Metuloidea fragrans</i>	LE 295277	KC585281	—	Westphalen et al. (2019)
<i>M. murashkinsky</i>	X 449	JN710588	JN710588	Miettinen et al. (2012)
<i>M. reniformis</i>	MCW 523/17	MT849302	MT849302	Westphalen et al. (2021)
<i>M. reniformis</i>	MCW 542/17	MT849303	MT849303	Westphalen et al. (2021)
<i>M. rhinocephala</i>	X 460	JN710562	JN710562	Miettinen et al. (2012)
<i>Mycorrhaphium adustum</i>	KHL 12255	JN710573	JN710573	Miettinen et al. (2012)
<i>M. hispidum</i>	MCW 363/12	MH475306	MH475306	Westphalen et al. (2019)
<i>M. hispidum</i>	MCW 429/13	MH475307	MH475307	Westphalen et al. (2019)
<i>M. subadustum</i>	Dai 10173	KC485537	KC485545	Yuan (2013b)
<i>M. subadustum</i>	Yuan 12976	MW491378	MW488040	Cao et al. (2021)
<i>Steckerinum aridum</i>	Barsd 110510	JN710583	JN710583	Miettinen et al. (2012)
<i>S. autumnale</i>	VS 2957	JN710549	JN710549	Miettinen et al. (2012)
<i>S. bourdatii</i>	HHB 9743	KY948818	—	Justo et al. (2017)
<i>S. bourdatii</i>	Saarenoksa 10195	—	JN710584	Miettinen et al. (2012)
<i>S. ciliatatum</i>	Ryvarden 47033	JN710585	JN710585	Miettinen et al. (2012)
<i>S. collabens</i>	KHL 11848	JN710552	JN710552	Miettinen et al. (2012)
<i>S. confragosum</i>	CBS 746.81	MH861473	—	Vu et al. (2019)
<i>S. fimbriatellum</i>	OM 2091	JN710555	JN710555	Miettinen et al. (2012)
<i>S. formosanum</i>	TFRI 652	EU232184	EU232268	Westphalen et al. (2019)
<i>S. hirsutum</i>	CLZhao 4222	MW290040	MW290054	Present study
<i>S. hirsutum</i>	CLZhao 4523	MW290041	MW290055	Present study
<i>S. larssonii</i>	MCW 593/17	MT849306	MT849306	Westphalen et al. (2021)
<i>S. larssonii</i>	MCW 594/17	MT849307	MT849307	Westphalen et al. (2021)
<i>S. laeticolor</i>	FP-102480	KY948823	KY948868	Justo et al. (2017)
<i>S. lacernum</i>	TN 8246	JN710557	JN710557	Miettinen et al. (2012)
<i>S. lisshaueri</i>	X 1236	JN710587	JN710587	Miettinen et al. (2012)
<i>S. meridionalis</i>	MR 10466	KY174994	KY174994	Westphalen et al. (2018)
<i>S. meridionalis</i>	MR 11086	KY174993	KY174993	Westphalen et al. (2018)
<i>S. meridionalis</i>	MR 284	KY174992	KY174992	Westphalen et al. (2018)
<i>S. neomitidum</i>	MCW 371/12	KY174990	KY174990	Westphalen et al. (2018)
<i>S. neomitidum</i>	RP 79	KY174991	KY174991	Westphalen et al. (2018)
<i>S. nitidum</i>	KHL 11903	JN710560	JN710560	Miettinen et al. (2012)
<i>S. nitidum</i>	MT 33/12	KY174989	KY174989	Westphalen et al. (2018)
<i>S. nitidum</i>	FP 105195	KP135323	KP135227	Floudas & Hibbett (2015)
<i>S. ochraceum</i>	KHL 11902	JN710590	JN710590	Miettinen et al. (2012)
<i>S. oreophilum</i>	HHB-13202	KY948824	—	Justo et al. (2017)
<i>S. oreophilum</i>	X 214	JN710548	JN710548	Miettinen et al. (2012)
<i>S. polycystidiferum</i>	RP 140	KY174996	KY174996	Westphalen et al. (2018)
<i>S. polycystidiferum</i>	MCW 419/12	KY174995	KY174995	Westphalen et al. (2018)
<i>S. pseudozillingianum</i>	MK 1004	JN710561	JN710561	Miettinen et al. (2012)
<i>S. puerense</i>	CLZhao 3122	MW682341	—	Wu et al. (2021a)
<i>S. puerense</i>	CLZhao 3644	MW682342	MW682338	Wu et al. (2021a)
<i>S. robustius</i>	GB 1195	JN710591	—	Miettinen et al. (2012)
<i>S. rubigimaculatum</i>	CLZhao 4069	MW682343	MW682339	Wu et al. (2021a)
<i>S. rubigimaculatum</i>	CLZhao 10638	MW682344	—	Wu et al. (2021a)
<i>S. stramineum</i>	KHL 13849	JN710597	JN710597	Miettinen et al. (2012)
<i>S. subcollabens</i>	Dai 19344	MN871758	MN877771	Du et al. (2020)
<i>S. subcollabens</i>	Dai 19345	MN871759	MN877772	Du et al. (2020)
<i>S. tenue</i>	FP 102082	KY948817	—	Justo et al. (2017)
<i>S. tenue</i>	KHL 12316	JN710598	JN710598	Miettinen et al. (2012)
<i>S. tenuispinum</i>	OM 8065	JN710599	JN710599	Miettinen et al. (2012)
<i>S. tenuispinum</i>	LE231603	KM411452	KM411452	Westphalen et al. (2018)
<i>S. tenuispinum</i>	VS 2116	JN710600	JN710600	Miettinen et al. (2012)
<i>S. undigerum</i>	MCW 426/13	KY174986	KY174986	Westphalen et al. (2018)
<i>S. undigerum</i>	MCW 472/13	KY174987	KY174987	Westphalen et al. (2018)
<i>S. undigerum</i>	MCW 496/14	KY174988	KY174988	Westphalen et al. (2018)
<i>S. xanthum</i>	CLZhao 5024	MW204587	MW204576	Wu et al. (2021b)
<i>S. xanthum</i>	CLZhao 5044	MW204590	MW204579	Wu et al. (2021b)
<i>S. yunnanense</i>	CLZhao 1445	MW290042	MW290056	Present study
<i>S. yunnanense</i>	CLZhao 2822	MW290043	MW290057	Present study
<i>Trullitella confiericola</i>	Yuan 12655	MT269760	MT259326	Cao et al. (2021)
<i>Trullitella confiericola</i>	Yuan 12657	MT269761	MT259327	Cao et al. (2021)
<i>T. dentipora</i>	AS 2288	KY970064	KY952634	Westphalen et al. (2019)
<i>T. dentipora</i>	WX 95	KY969748	KY969732	Westphalen et al. (2019)
<i>T. duracina</i>	MCW 410/13	MH475309	MH475309	Westphalen et al. (2019)
<i>T. duracina</i>	RP 96	MH475310	MH475310	Westphalen et al. (2019)
<i>T. polyporoides</i>	JV 1008/68	KY446068	KY446068	Kout et al. (2017)

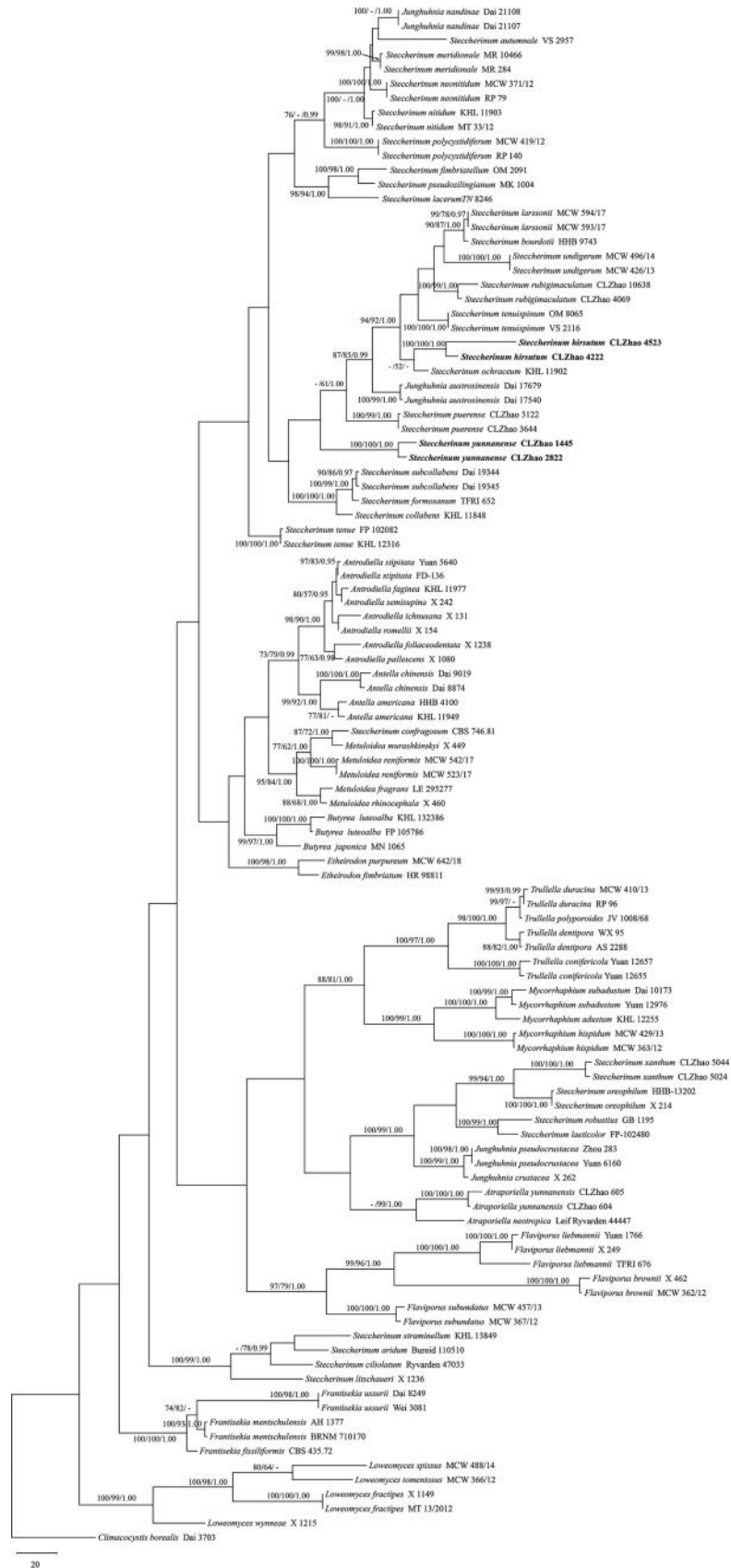


Fig. 1. – Maximum parsimony strict consensus tree illustrating the phylogeny of two new species and related species based on ITS+nLSU sequences in the family Steccheriaceae. Branches are labeled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap values higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

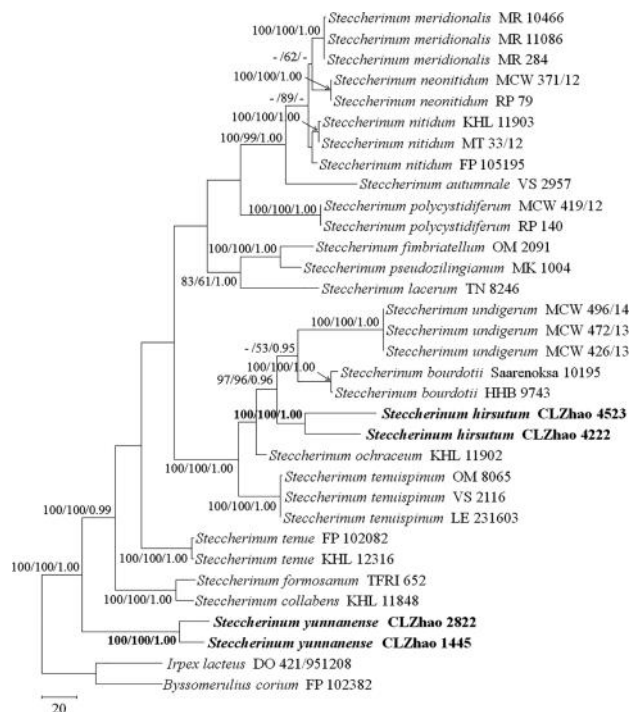


Fig. 2. – Maximum parsimony strict consensus tree illustrating the phylogeny of two new species and related species in *Steccherinum* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap values higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhao and Wu (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 generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller et al., 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Nylander, 2004) was used to determine the best-fit evolution model for data set for Bayesian inference (BI). BI was calculated with MrBayes v. 3.1.2 with a general time reversible (GTR+I+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 500,000 generations (Fig. 1), for 3,000,000 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 were considered as significantly supported if they received maximum likelihood bootstrap values (BS) >75%, maximum parsimony bootstrap values (BT) >75%, or Bayesian posterior probabilities (BPP) >0.95.

3. Results

3.1. Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 107 fungal specimens representing 71 taxa. The dataset had an aligned length of 2,183 characters, of which 1,514 characters were constant, 185 parsimony-uninformative and 484 parsimony-informative. MP analysis yielded 14 equally parsimonious trees (TL = 2,836, CI = 0.3586, HI = 0.6414, RI = 0.7103, RC = 0.2547). The best-fit model for ITS+nLSU alignment estimated and applied in the BI was GTR+I+G, Iset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies = 0.007604.

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences uncovered 30 species of *Steccherinum*, which demonstrated that *Steccherinum hirsutum* grouped with *S. ochraceum* with low support. *Steccherinum yunnanense* formed a monophyletic lineage with a strong support (100% BS, 100% BT, 1.00 BPP).

The ITS+nLSU dataset (Fig. 2) included sequences from 33 fungal specimens representing 19 taxa. The dataset had an aligned length of 2,069 characters, of which 1,652 characters were constant, 124 parsimony-uninformative and 293 parsimony-informative. MP analysis yielded 24 equally parsimonious trees (TL = 982, CI = 0.549, HI = 0.451, RI = 0.738, RC = 0.405). The best-fit model for ITS+nLSU alignment estimated and applied in the BI was GTR+I+G, Iset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies = 0.009785.

The phylogenetic tree (Fig. 2) inferred from ITS+nLSU sequences covered 17 species of *Steccherinum*, which demonstrated that the clade with 97% ML, 96% MP and 0.96 BPP, includes *S. undigerum*, *S. bourdotii* Saliba & A. David and *S. hirsutum*. *Steccherinum yunnanense* formed a monophyletic lineage with a strong support (100% BS, 100% BT, 1.00 BPP).

3.2. Taxonomy

Steccherinum hirsutum Y.X. Wu & C.L. Zhao, sp. nov. Figs. 3, 4. MycoBank no.: MB 838261.

Diagnosis: differs from other *Steccherinum* species by the stipitate basidiomata with scarlet to red, odontoid hymenial surface, a dimittic hyphal system with clamped generative hyphae and acyanophilous basidiospores measuring $2.5\text{--}3.5 \times 1.5\text{--}2.5 \mu\text{m}$.

Type: CHINA, Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, on the angiosperm trunk, 5 Oct 2017 (Holotype: CLZhao 4222) (SWFC).

Etymology: *hirsutum* (Lat.) referring to the hirsute pileal surface.

Basidiomata: Annual, laterally stipitate, subceraceous, without odor or taste when fresh, becoming leathery upon drying. Pilei flabelliform, projecting up to 1.5 cm wide, up to 1 cm long, 2 mm thick at centre. Pileal surface hirsute, zonate, smoke grey when fresh and grey to straw-yellow upon drying. Hymenophore hydroid, with conical aculei, 2–4 per mm, 2 mm long, scarlet to red when fresh, turn to fawn to pale brown upon drying. Sterile margin distinct, wavy, 0.5–1 mm wide, scarlet to red or slightly brown.

Hyphal structure: Hyphal system dimittic, generative hyphae with clamp connections, hyaline, thin-walled, branched, more or less interwoven, 2.5–3 μm diam; skeletal hyphae hyaline, thick-walled, 2.5–5 μm diam; all hyphae IKI–, CB+, hyphal cell-wall unchanged in KOH.

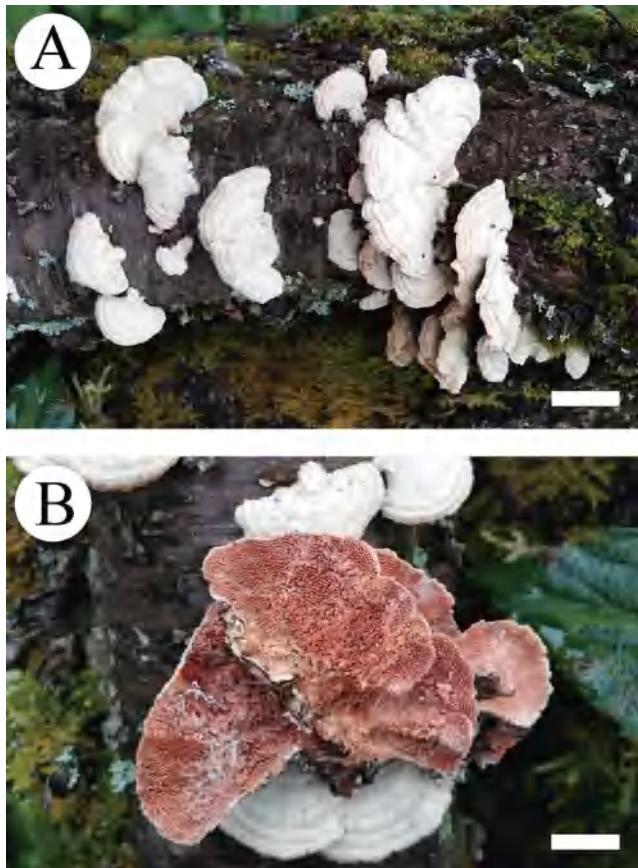


Fig. 3. – Basidiomata of *Steccherinum hirsutum* (holotype: CLZhao 4222). Bars: A 2 cm; B 1 cm.

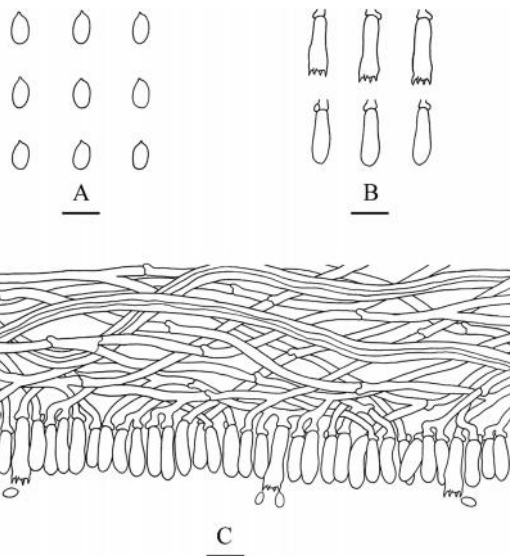


Fig. 4. – Microscopic structures of *Steccherinum hirsutum* (drawn from the holotype: CLZhao 4222). A: basidiospores, B: basidia and basidioles, C: a section of hymenium. Bars: A 5 μm ; B, C 10 μm .

Hymenium: Cystidia and cystidioles absent. Basidia clavate, with 4-sterigmata and basal clamp connections, $9\text{--}14.5 \times 2.5\text{--}4.5 \mu\text{m}$, basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores: Ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, $(2\text{--})2.5\text{--}3.5 \times 1.5\text{--}2.5 \mu\text{m}$, $L = 3.05 \mu\text{m}$, $W = 2.18 \mu\text{m}$, $Q =$

$1.38\text{--}1.4$ ($n = 60/2$).

Ecology and distribution: Lignicolous, causing a white rot. Found in China.

Additional specimen examined: CHINA, Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, on the angiosperm trunk, 6 Oct 2017, CLZhao 4523 (SWFC).

Steccherinum yunnanense Y.X. Wu & C.L. Zhao, sp. nov. Figs. 5, 6. MycoBank no.: MB 838262.

Diagnosis: differs from other *Steccherinum* species by the resupinate basidiomata with odontoid hymenial surface, a dimitic hyphal system with clamped generative hyphae, strongly encrusted cystidia and ellipsoid basidiospores ($3.5\text{--}4.5 \times 2.2\text{--}3.3 \mu\text{m}$).

Type: CHINA, Yunnan Province, Yuxi, Xiping County, Shimexia Forestry Park, on fallen branch of angiosperm, 21 Aug 2017 (Holotype: CLZhao 2822) (SWFC).

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

Basidiomata: Annual, resupinate, adnate, soft leathery, without odor or taste when fresh, becoming membranaceous upon drying, up to 10 cm long, up to 2 cm long, $50\text{--}100 \mu\text{m}$ thick. Hymenial surface odontoid, aculei $5\text{--}8$ per mm, up to 0.1 mm , white when fresh, turning to white to cream upon drying. Sterile margin white to cream, fimbriate, entire, $0.5\text{--}1 \text{ mm}$.

Hyphal system: Hyphal system dimitic, generative hyphae with clamp connections, hyaline, thin-walled, frequently branched, interwoven, $2\text{--}3.5 \mu\text{m}$ diam; skeletal hyphae hyaline, thick-walled, $2.5\text{--}3.5 \mu\text{m}$ diam; all hyphae IKI–, CB+, hyphal cell-wall unchanged in KOH.

Hymenium: Cystidia numerous, thin-walled, cylindrical, strongly encrusted in the surface and almost entirely, $14\text{--}31 \times 3.5\text{--}6 \mu\text{m}$. Basidia subclavate to barrel, with 4-sterigmata and basal clamp connections, $10.5\text{--}15 \times 5\text{--}6 \mu\text{m}$, basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores: Ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, $(3\text{--})3.5\text{--}4.5(-5) \times 2\text{--}3.5 \mu\text{m}$, $L = 3.99 \mu\text{m}$, $W = 2.83 \mu\text{m}$, $Q = 1.38\text{--}1.42$ ($n = 60/2$).

Ecology and distribution: Lignicolous, causing a white rot. Found in China.

Additional specimen examined: CHINA, Yunnan Province, Kunming, Xishan District, Haikou Forestry Park, on the fallen branch of *Alnus nepalensis* D.Don, 23 Apr 2017, CLZhao 1445 (SWFC).

4. Discussion

In the present study, two new species, *Steccherinum hirsutum* and *S. yunnanense* are described based on phylogenetic analyses and morphological characters.

Miettinen et al. (2012) employed the phylogeny of the poroid and hydroid genera *Antrodiella*, *Junghuhnia* and *Steccherinum* utilizing sequences of the gene regions ITS, nLSU, mtSSU, ATPase subunit 6 (*atp6*), RNA polymerase II second largest subunit (*rpb2*), and translation elongation factor 1-alpha (*tef1*), that revealed generic concepts need to be revised within *Steccherinaceae* and at least 16 transitions have taken place between poroid and hydroid hymenophore types in the family, and similar plasticity could be seen in microscopic characters. In the present study, *S. hirsutum* lacks the typical cystidia of the genus, but phylogenetically it is close to the generic type *S. ochraceum* and morphologically it presents other characteristics typical of the genus, as the hydroid or-

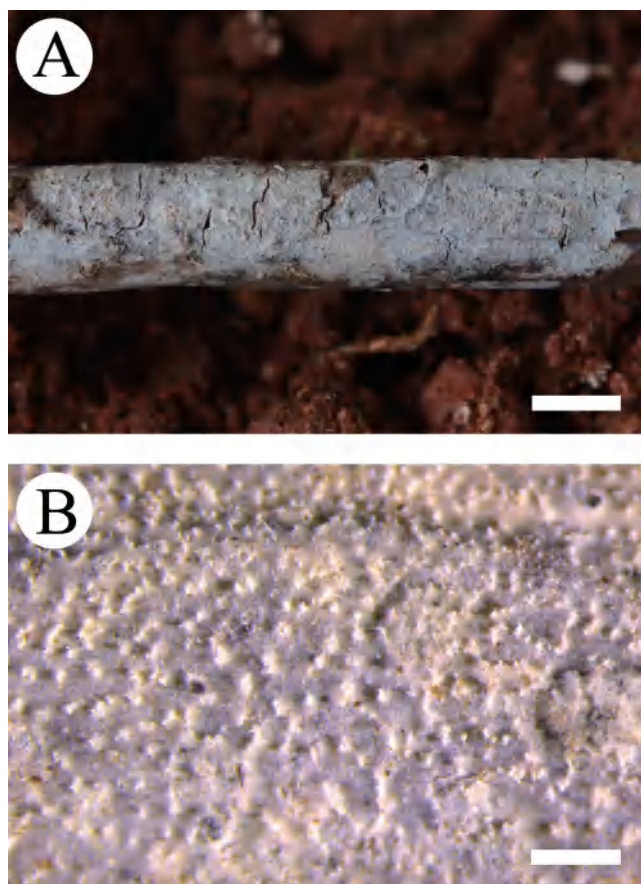


Fig. 5. – Basidiomata of *Steccherinum yunnanense* (holotype: CLZhao 2822). Bars: A 0.5 cm; B 0.5 mm.

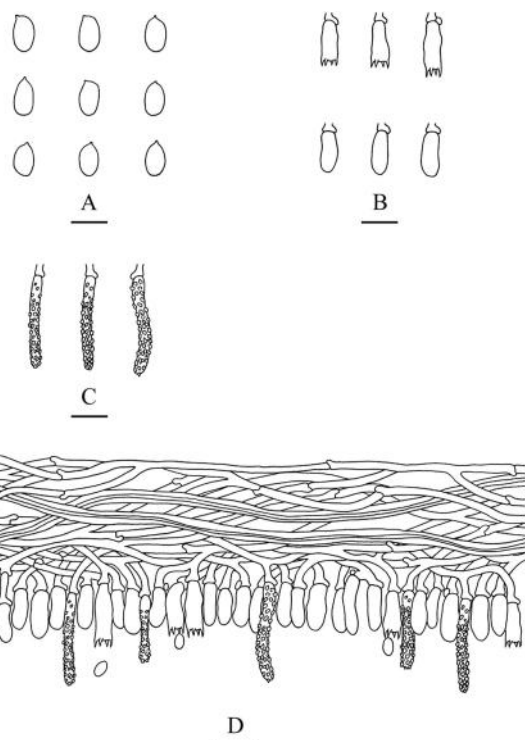


Fig. 6. – Microscopic structures of *Steccherinum yunnanense* (drawn from the holotype: CLZhao 2822). A: basidiospores, B: basidia and basidioles, C: pseudocystidia, D: a section of hymenium. Bars: A 5 µm; B–D 10 µm.

ange hymenophore, dimitic hyphal system and small basidiospores, therefore, we propose that it belongs to *Steccherinum* s.s. The other new taxon formed an isolated lineage within *Steccherinum* based on the molecular data obtained. In addition, it shares similar morphological characters with other species in the genus (odontoid basidiomes, encrusted cystidia, and a dimitic hyphal system).

Steccherinum hirsutum grouped closely with *S. ochraceum*, but morphologically *S. ochraceum* differs in having pale ochraceous to salmon hymenial surface and numerous cystidia (Bernicchia & Gorjón, 2010). *Steccherinum hirsutum* formed a sister clade to *S. bourdotii* and *S. undigerum* based on ITS+nLSU sequences (Fig. 2). However, morphologically, *S. bourdotii* differs from *S. hirsutum* by its cream to pale ochraceous hymenial surface, presence of cystidia and subglobose basidiospores (3–4.5 × 4.5–5.5 µm; Bernicchia & Gorjón, 2010). *Steccherinum undigerum* differs from *S. hirsutum* by its ochraceous basidiomata with poroid hymenophore and ellipsoid to subglobose to basidiospores (4–5 × 3.5–4 µm; Ryvarden, 1984).

Morphologically, *S. yunnanense* resembles *Etheiroidon fimbriatum* (Pers.) Banker, *S. litschaueri* (Bourdot & Galzin) J. Erikss. and *S. robustius* (J. Erikss. & S. Lundell) J. Erikss. based on the cylindrical, encrusted cystidia and small basidiospores. *Etheiroidon fimbriatum* differs in its pale violaceous to pinkish hymenial surface and presence of fimbriate to rhizomorphic margin (Bernicchia & Gorjón, 2010). *Steccherinum litschaueri* differs from *S. yunnanense* by having the larger cystidia (60–80 × 6–8 µm) and larger basidiospores (4.5–5.5 × 2–2.2 µm; Bernicchia & Gorjón, 2010). *Steccherinum robustius* differs in its reddish orange to pale orange hymenial surface (Bernicchia & Gorjón, 2010).

Many species of *Steccherinum* were previously described from China, *S. subglobosum* H.S. Yuan & Y.C. Dai, *S. subulatum* H.S. Yuan & Y.C. Dai, *S. tenuissimum* and *S. xanthum*. However, morphologically, *S. subglobosum* differs in its effuse-reflexed to pileate basidiomata, velutinate to tomentose pilear surface and subglobose basidiospores (3.9–4.6 × 3.3–3.9 µm; Yuan & Dai, 2005). *Steccherinum subulatum* differs in resupinate to effuse-reflexed basidiomata with cream to buff hymenial surface, longer spines and unbranched skeletal hyphae (Yuan & Dai, 2005). *Steccherinum tenuissimum* differs in its aculei turning to cream to olivaceous buff upon drying, and basidiospores with oil drops (Wu et al., 2021b). *Steccherinum xanthum* differs in buff hymenial surface, longer cystidia (35.5–125 × 5–9 µm) and basidia (10–19.3 × 3–5.2 µm; Wu et al., 2021b).

Wood decaying fungi in *Polyporales* have been studied intensively in recent years (Bernicchia & Gorjón, 2010; Dai, 2011; Cui et al., 2019; Guan, Liu, Zhao, & Zhao, 2020; Wang, He, & Zhao, 2020; Westphalen et al., 2021; Wu et al., 2021a, 2021b), but hydroid species in this order are still not well investigated in China, yet. It is possible that new taxa will be found after further investigations and molecular analyses.

Key to species of *Steccherinum sensu lato* from China

1. Hyphal system monomitic in subiculum.....2
1. Hyphal system dimitic in subiculum.....6
2. Basidiospores <2 µm wide, cylindrical
.....*Mycorrhaphium adustum*
2. Basidiospores >2 µm wide, ellipsoid.....3
3. Skeletocystidia absent.....*Steccherinum fragile*
3. Skeletocystidia present.....4
4. Aculei >1mm long.....*S. aggregatum*
4. Aculei <1 mm long.....5

5. Aculei <0.3 mm long, basidiospores with oil drops
..... *S. xanthum*
5. Aculei >0.3 mm long, basidiospores without oil drops
..... *Cabalodontia queletii*
6. Skeletocystidia absent..... *S. hirsutum*7
6. Skeletocystidia present.....7
7. Skeletocystidia subulate, apex acute.....8
7. Skeletocystidia clavate, apex blunt.....10
8. Basidiospores >5 µm wide, aculei >1.5 mm long
..... *S. oreophilum*
8. Basidiospores <5 µm wide, aculei <1.5 mm long.....9
9. Basidiomata surface reddish to brick, basidiospores <2 µm wide
..... *S. laeticolor*
9. Basidiomata surface white to buff, basidiospores >2 µm wide
..... *S. subulatum*
10. Basidiomata resupinate.....11
10. Basidiomata effused-reflexed.....14
11. Basidiomata with broom-like rhizomorphs
..... *Etheirodon fimbriatum*
11. Basidiomata without broom-like rhizomorphs.....12
12. Basidiospores <2 µm wide..... *S. mukhni*
12. Basidiospores >2 µm wide.....13
13. Aculei <0.5 mm long, aculei <4 per mm..... *S. tenuissimum*
13. Aculei >0.5 mm long, aculei >4 per mm..... *S. ochraceum*
14. Sterile margin fimbriate.....15
14. Sterile margin not fimbriate.....16
15. Basidiospores <3.5 µm wide..... *S. yunnanense*
15. Basidiospores >3.5 µm wide..... *S. elongatum*
16. Basidiospores <4 µm long.....17
16. Basidiospores >4 µm long.....22
17. Aculei <2 mm long.....18
17. Aculei >2 mm long.....20
18. Basidiospores >3 µm wide, aculei >0.5 mm long
..... *S. subcollabens*
18. Basidiospores <3 µm wide, aculei <0.5 mm long.....19
19. Basidiospores subcylindrical to allantoid..... *S. puerense*
19. Basidiospores ellipsoid..... *S. cremicolor*
20. Aculei 3–4 mm long, pileus margin sharp
..... *Metuloidea murashkinskyi*
20. Aculei up to 2 mm long, pileus margin blunt.....21
21. Basidiospores >1.5 µm wide..... *S. rawakense*
21. Basidiospores <1.5 µm wide..... *S. confragosum*
22. Basidiospores subglobose.....23
22. Basidiospores ellipsoid.....24
23. Aculei <2 mm long, basidiospores with a normal guttule or not
..... *S. subglobosum*
23. Aculei >2 mm long, basidiospores with a distinct guttule
..... *S. hydneum*
24. Basidia <11 µm long..... *S. rubigimaculatum*
24. Basidia >11 µm long.....25
25. Basidiospores >3 µm wide..... *S. bourdotii*
25. Basidiospores <3 µm wide.....26
26. Aculei >0.5 mm long, pinkish buff to clay buff..... *S. robustius*
26. Aculei <0.5 mm long, cream to pale buff..... *S. ciliolatum*

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of the People's Republic of China.

Acknowledgments

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