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Two new corticioid species, *Hyphoderma sinense* and *H. floccosum* (Hyphodermataceae, Polyporales), from southern China

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Abstract: Two new corticioid fungal species, *Hyphoderma sinense* and *H. floccosum* from Yunnan Province, southwestern China, are proposed based on a combination of morphological features and molecular evidence. *Hyphoderma sinense* is characterized by the resupinate basidiomata with smooth hymenial surface, presence of encrusted and moniliform cystidia, and cylindrical to allantoid basidiospores (8–11.5×3–5μm). *Hyphoderma floccosum* is characterized by the farinaceous hymenial surface, tubular cystidia and leptocystidia, and ellipsoid to allantoid basidiospores. Sequences of ITS+nLSU nrRNA gene regions of the studied samples were generated and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analysis based on molecular data of ITS+nLSU sequences showed that two new species clustered into the genus *Hyphoderma*, in which *H. sinense* grouped with *H. translens*, and *H. floccosum* grouped with a clade comprising *H. pinicola, H. setigerum* and *H. subsetigerum*. The new taxa are described, illustrated and compared with morphologically similar species. An identification key to 23 accepted species in China is provided.

Key words: Basidiomycota, molecular phylogeny, new taxa, taxonomy, wood-rotting fungi


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中国南方两个革菌新种：中国丝皮革菌和棉絮丝皮革菌（丝皮革菌科，多孔菌目）

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摘要：依据形态学和分子系统学研究结果，描述了产自中国云南省的丝皮革菌属 2 个新种，即中国丝皮革菌 *Hyphoderma sinense* 和棉絮丝皮革菌 *H. flocosum*。中国丝皮革菌以光滑子实层体表面、被结晶和念珠状的囊状体以及椭圆形至近圆形的担孢子（8–11.5×3–5μm）为主要识别特征，棉絮丝皮革菌具有棉絮状至粉状子实层体表面、囊状和分隔状囊状体及椭圆形至近圆形的担孢子。对新种的 ITS 和 nLSU 片段进行了测序和分析，采用最大似然法和贝叶斯推断法对研究样本的 ITS+nLSU nrRNA 基因序列进行系统发育分析，结果显示两个新种归属于丝皮革菌属，其中，中国丝皮革菌与变形丝皮革菌同为一类；棉絮丝皮革菌与松生丝皮革菌、丝皮革菌和拟丝皮革菌聚为一类。本研究提供了新种的详细描述、线粒图、生态照片及与相似种的区别，同时编写了我国丝皮革菌属 23 个种的检索表。

关键词：担子菌门、分子系统学、新单元、分析学、多孔菌目

INTRODUCTION

*Hyphoderma* Wallr. was typified by *H. setigerum* (Fr.) Donk (Donk 1957), which is characterized by the resupinate to effuse-reflexed basidiomata with ceraceous consistency, and smooth to tuberculate or hydnoid hymenophore and a monomitic hyphal structure (rarely dimitic) with clamp connections on generative hyphae, presence of cystidia or not, basidia suburniform to subcylindrical and cylindrical, ellipsoid to subglobose, smooth, thin-walled basidiospores (Wallroth 1833; Bernicchia & Gorjón 2010). Currently, about one hundred species have been accepted in *Hyphoderma* worldwide (Wallroth 1833; Donk 1957; Reid 1965; Berthet & Boidin 1966; Jüllich 1974; Eriksson & Ryvarden 1975; Lindsey & Gilbertson 1977; Burdsall & Nakasone 1983; Vries 1987; Hjortstam et al. 1988; Wu 1990; Boidin & Gilles 1991, 1994, 2004; Bernicchia 1993; Wu 1997a, 1997b; Larsson 1998; Gilbertson 2001; Dai et al. 2004; Gilbertson & Hemmes 2004; Hjortstam & Ryvarden 2005, 2007; Nakasone 2008; Wu et al. 2010; Singh et al. 2010; Priyanka & Dhangra 2012; Telleria et al. 2012; Yurchenko & Wu 2014a, 2014b, 2015; Kaur et al. 2015; Baltazar et al. 2015; Martín et al. 2018; Ma et al. 2021).

Molecular studies regarding to *Hyphoderma* were employed, and based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences, Larsson (2007) revealed the classification of corticioid fungi, in which *H. obtusum* J. Eriksson and *H. setigerum* clustered into family Meruliaceae Rea and grouped with *Hyphochlamium polonense* (Bres.) Å. Strid. Telleria et al. (2012) discussed the relationships

Recently, we collected two undescribed taxa from Yunnan Province, P.R. China, that could not be assigned to any known species. Morphological and molecular phylogenetic evidence supported the recognition of the two new species within *Hyphoderma*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

1 MATERIALS AND METHODS

1.1 Morphology

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological descriptions are based on field notes. Colour terms follow Petersen (1996). Micromorphological data were obtained from the dried specimens, and observations under light microscope follow Dai (2010). The following abbreviations were used: KOH=5% potassium hydroxide, CB=Cotton Blue, CB=acianophilous, IKI=Melzer's reagent, IKI= both inamyloid and indexinoid, L=mean spore length (arithmetic average for all spores measured), W=mean spore width (arithmetic average for all spores measured), 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.

1.2 Molecular phylogeny

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 that a small piece of dried fungal specimen (about 30mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5ml centrifuge
tube, suspended in 0.4mL of lysis buffer, and incubated at 85°C in a water bath for 60min. After that, 0.4mL phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13000g/min for 5min, 0.3mL of supernatant was transferred to a new tube and mixed with 0.45mL of binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13000g/min for 0.5min. Then, 0.5mL of inhibitor removal fluid was added in AC for a centrifugation at 12000g/min for 0.5min. After washing twice with 0.5mL of washing buffer, the AC was transferred to a clean centrifuge tube and 100mL elution buffer was added to the middle of adsorbed film to elute the genome DNA. ITS region was amplified with primer pair ITS5 and ITS4 (White et al. 1990). Nuclear LSU region was amplified with primer pair 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 3min, followed by 35 cycles at 94°C for 40s, 58°C for 45s and 72°C for 1min, and a final extension of 72°C for 10min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1min, followed by 35 cycles at 94°C for 30s, 48°C 1min and 72°C for 1.5min, and a final extension of 72°C for 10min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, P.R. China. All newly generated sequences were deposited at GenBank (Table 1).

Sequences were aligned in MAFFT 7 (https://mafft.cbrc.jp/alignment/server/) using the "G-INS-i" strategy for ITS+nLSU, and manually adjusted in BioEdit (Hall 1999). The dataset align firstly and then combine ITS and nLSU sequences with Mesquite. Alignment datasets were deposited in TreeBase [submission ID 27484]. Climacocystis borealis (Fr. Kotl. & Pouzar) and Diplomitoporus crustulinus (Bres.) Domarăski were selected as an outgroup for phylogenetic analysis of ITS+nLSU phylogenetic tree (Fig. 1) followed previous study (Justo et al. 2017).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Cui et al. (2019), 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 1000 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. Datamatrix was also analyzed using maximum likelihood (ML) approach with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org, Miller et al. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.
Table 1 List of species, specimens and GenBank accession numbers of sequences used in this study

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<td>Dai 15917</td>
<td>KY131870</td>
<td>KY131926</td>
<td>Wu et al. (2017)</td>
</tr>
<tr>
<td>Physosporus submaculatus</td>
<td>Dai 12003</td>
<td>KY131880</td>
<td>KY131925</td>
<td>Wu et al. (2017)</td>
</tr>
<tr>
<td>Physosporus tibeticus</td>
<td>Cui 9588</td>
<td>KY131873</td>
<td>KY131929</td>
<td>Wu et al. (2017)</td>
</tr>
<tr>
<td>Physosporus tibeticus</td>
<td>Cui 9518</td>
<td>KY131872</td>
<td>KY131928</td>
<td>Wu et al. (2017)</td>
</tr>
<tr>
<td>Rigidosporus eminens</td>
<td>Dai 17200</td>
<td>MT279690</td>
<td>MT279691</td>
<td>Wu et al. (2017)</td>
</tr>
<tr>
<td>Rigidosporus undatus</td>
<td>Miettinen-13301</td>
<td>KY348731</td>
<td>KY348780</td>
<td>Justo et al. (2017)</td>
</tr>
</tbody>
</table>

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). BI was calculated with MrBayes 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 3 million generations for ITS+LSU (Fig. 1). 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 value (BS)>75%, maximum parsimony bootstrap value (BT)>75%, or Bayesian posterior probabilities (BPP)>0.95.
Fig. 1. Phylogenetic tree illustrating the phylogeny of two new species in Polychaeta.

Based on ITS2 and 18S rDNA sequences. Branches are labeled with maximum likelihood bootstrap values >70% and Bayesian posterior probabilities >0.95, respectively.
2 RESULTS

2.1 Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 68 fungal samples representing 41 taxa. The dataset had an aligned length of 2,080 characters in the dataset, of which 1,244 characters are constant, 135 are variable and parsimony-uninformative, and 701 are parsimony-informative. Maximum parsimony analysis yielded 107 equally parsimonious trees (TIC=3,153, CI=0.4050, HI=0.5950, RI=0.7034, RC=0.2849). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G [iset nst=6, rates=invgamma; preset statefreq=dirichlet (1,1,1,1)]. Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies=0.009632 (BI).

The phylogeny (Fig. 1) inferred from ITS+nLSU sequences in Polyporales, demonstrated that two new Hyphoderma species nested in the family Hyphodermaeaceae, in which H. floccosum grouped with a clade comprising H. pinicola, H. setigerum and H. subsetigerum Sheng. H. Wu. Another new species H. sinense clustered with to H. transiens (Bres.) Parmasto.

2.2 Taxonomy

**Hyphoderma sinense** C.L. Zhao & Q.X. Guan, sp. nov. Figs. 2, 3

Mycobank MB838137

Holotype. China. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, 22°53'45"N; 103°35'37"E, alt. 1,969 m, on fallen angiosperm branch, 1 Aug 2019, CL Zhao 17811 (SWFC).

**Fig. 2.** Basidiomata of **Hyphoderma sinense** (holotype). Bars: A=2cm; B=1mm.

**Fig. 3.** Microscopic structures of **Hyphoderma sinense** (holotype). A: Basidiospores; B: Basidia and basidioles; C: Cystidia; D: A section of hymenium. Bars: A–D=10µm.
Etymology. *sinense* (Lat.): referring to the locality (China) of the specimens.

Fruiting body. Basidiomata annual, resupinate, adnate, ceraceous when fresh, turn to membranaceous upon drying, without odor or taste when fresh, up to 14 cm long, 2 cm wide, 50–150 μm thick. Hymenial surface smooth, white to cream when fresh, cream on drying. Sterile margin narrow, white to cream, 0.3 cm wide.

Hyphal structure. Hyphal system monomitic, generative hyphae with clamp connections, colorless, thick-walled, frequently branched, interwoven, 2.5–4.5 μm in diameter, IKI–, CB–; tissues unchanged in KOH.

Hymenium. Cystidia of two types: (1) encrusted cystidia, rare, colorless, encrusted by crystals, 18.5–38 × 6–11 μm; (2) moniliform cystidia common, colorless, 30–60.5 × 6–10 μm. Basidia clavate to subcylindrical, median slightly constricted to somewhat sinuous, with 4 sterigmata and a basal clamp connection, with oil drops, 20.5–34 × 6–9.5 μm.

Sporation. Basidiospores cylindrical to slightly allantoid, colorless, thin-walled, smooth, with oily contents, IKI–, CB–, (7.5–)8–11.5 (–12) × 3–5 (–5.5) μm, L=9.69 μm, W=4.35 μm, Q=2.08–2.31 (n=120/4).

Additional specimens examined. China. Yunnan Province, Yuexi, Xingping County, Mopanshan National Forestry Park, 23°57′42″N; 101°57′41″E, alt. 2130 m, on fallen angiosperm branch, 19 Aug 2018, CLZhao 7963 (SWFC), CLZhao 7981 (SWFC); Cha Ma Ancient Road spot, 23°58′37″N; 101°31′53″E, alt. 2360 m, on fallen angiosperm branch, 21 Aug 2018, CLZhao 8298 (SWFC).

*Hyphoderma floccosum* C.L. Zhao & Q.X. Guan, sp. nov. Figs. 4, 5

*Mycobank: MB838138*

Holotype. China. Yunnan Province, Wenshan, Pingba Town, Wenshan National Nature Reserve, 23°19′32″N; 104°40′32″E, alt. 2480 m, on fallen angiosperm branch, 28 July 2019, CLZhao 17129 (SWFC).

Etymology. *floccosum* (Lat.): referring to the flocculence hymenial surface.

Fruiting body. Basidiomata annual, resupinate, ceraceous when fresh, turn to brittle upon drying, without odor or taste, up to 12 cm long, 2.5 cm wide, 50–200 μm thick. Hymenial surface farinaceous, white to cream when fresh, cream upon drying, with age initially discontinuous. Sterile margin narrow, white, 0.2 cm wide.

![Figure 4](image-url) Basidiomata of *Hyphoderma floccosum* (holotype). Bars: A=2 cm; B=1 mm.
Additional specimens examined. China, Yunnan Province, Wenshan, Pingla Town, Wenshan National Nature Reserve, 23°18′42″N; 104°42′37″E, alt. 2480 m, on fallen angiosperm branch, 26 July 2019, CLZhao 16492 (SWFC); 28 July 2019, CLZhao 17065 (SWFC), CLZhao 17079 (SWFC), CLZhao 17215 (SWFC), CLZhao 17296 (SWFC).

3 DISCUSSIONS

Phylogenetically, the family-level classification of the Polyporales employed nrLSU, nrITS, and rpb1 genes, in which four species, *Hyphoderma litschaueri*, *H. medioburiense* (Burt) Donk, *H. mutatum* (Peck) Donk and *H. setigerum*, clustered into family Hyphodermataceae within the residual polyporoid clade (Justo et al. 2017). In the present study, *H. sinense* was sister to *H. transiens and H. fioccosum* grouped with a clade comprising *H. pinicola*, *H. setigerum* and *H. substigerum*, based on ITS+nrLSU sequence data (Fig. 1). However, morphologically, *H. transiens* differs from *H. sinense* by its odontoid hymenial surface with grayish white to pale orange hymenophore and subcyllindrical to cylindrical cystidia (63–76×9.4–11.8μm; Parmasto 1968). *Hyphoderma pinicola* differs from *H. fioccosum* by its chalky white, minutely warty hymenial surface with porulose hymenophore, and larger basidiospores (13–16×4–4.5μm); in addition, it grows on the coniferous tree (Yurchenko & Wu 2014b). *H. setigerum* differs in its smooth to tuberculate hymenophore with white to yellowish to ochraceous hymenial surface and presence of
fimbriate margin, in addition, this taxon is distributed in Northern Europe (Bernicchia & Gorjón 2010); *H. subsetigerum* differs in its granulinoid hymenophore with white to ivory yellow hymenial surface and smaller basidiospores (6–8×2.8–3.2μm; Wu 1997a).

**Morphologically, *Hyphoderma itschaueri*, *H. moniliforme* (P.H.B. Talbot) Manjón, G. Moreno & Hjortstam, *H. nemorale* K.H. Larss., *H. paramacaronesicum* Telleria, M. Dueñas, J. Fernández-López & M.P. Martín and *H. prosopidis* (Burd.) Telleria, M. Dueñas & M.P. Martín are similar to *H. sinense* on the basis of the character by having moniliform or apically moniliform cystidia. However, *Hyphoderma itschaueri* differs from *H. sinense* by having tuberculate hymenial surface and larger moniliform cystidia (60–100×6–8μm; Eriksson & Ryvarden 1975); *H. moniliforme* is separated from *H. sinense* by cracking hymenial surface and larger cystidia (50–80×6–8μm; Hjortstam et al. 1988); *H. nemorale* differs in its thin-walled generative hyphae and larger basidiospores (10.4–14.4×5.4–6.2μm; Larsson 1998); *H. paramacaronesicum* differs from *H. sinense* by having larger moniliform cystidia (70–124×8–13μm) and basidiospores (12–15×5.5–7μm; Martín et al. 2018); *H. prosopidis* differs in slightly tuberculate hymenial surface and larger basidiospores (9–12×5.5–8.5μm; Telleria et al. 2012).

**Hyphoderma medioburiense** (Burt) Donk, *H. occidentale* (D.P. Rogers) Boidin & Gilles and *H. roseocremeum* (Bres.) Donk are similar to *H. floccosum* in having leptocystidia cystidia. However, *H. medioburiense* differs from *H. floccosum* by having slightly tuberculate hymenial surface and larger basidiospores (11–15×4–5μm; Donk 1957); *H. occidentale* differs in its porulose hymenial surface and larger basidiospores (12–14×4.5–5μm; Boidin & Gilles 1994).

An identification key to 23 accepted species in China

1. Cystidia absent .......................................................... 2
2. Cystidia present .......................................................... 5
3. Hymenial surface granuloid ............................................. 3
4. Hymenial surface smooth ................................................. 3
5. Basidiospores >10.5μm in length ...................................... H. acystidiatum
6. Basidiospores <10.5μm in length ...................................... H. densum
7. Hymenophore cracked; basidiospores larger (8.5–10.3×3–4μm) ........................................ H. fissuratum
8. Hymenophore uncracked; basidiospores smaller (7–8.1×5–5.5μm) ...................................... H. sibiricum
9. Hymenophore smooth ................................................... 6
10. Hymenophore tuberculate, porulose, granuloid or oconuloid .................................. 13
11. Two types of cystidia present ......................................... 7
12. One type of cystidia present ........................................... 8
13. Moniliform cystidia absent ........................................... H. microcystidiun
14. Moniliform cystidia present ........................................... H. sinense
15. Hymenophore uncracked ............................................... H. definitum
16. Hymenophore cracked ................................................ H. 9
17. Cystidia moniliform ..................................................... H. 10
18. Cystidia cylindrical ..................................................... H. 11
19. Basidiospores larger (9–12×3–4μm) .................................. H. itshchauri
20. Basidiospores smaller (8–9×3.5–4μm) ................................ H. moniliforme
21. Basidiospores ellipsoid <10μm ....................................... H. rimulosum
22. Basidiospores cylindrical <10μm .................................... H. transiens
23. Basidiospores larger (12–14×0.5–5.5μm) ................................ H. cremeum
24. Basidiospores smaller (10–12×4.2–5.3μm) ................................ H. subclavatum
25. Hymenophore oconuloid or granuloid ................................ 14
26. Hymenophore tuberculate, porulose ................................ 15
27. Hymenophore oconuloid, basidiospores larger (9.5–10.6×3.4–4.2μm) ......................... H. 15
28. Hymenophore granuloid, basidiospores smaller (5–8×2.8–3.2μm) .......................... H. subsetigerum
29. Cystidia of two types .................................................. 16
30. Cystidia of one type .................................................... 17
31. Basidia 2–sterigmata, basidiospores larger (13–16×4–4.5μm) ................................ H. riicola
32. Basidia 4–sterigmata, basidiospores smaller (6–9.5×3–4.5μm) ................................ H. floccosum
33. Septate cystidia present ............................................... 18
34. Septate cystidia absent ............................................... 19
35. Hymenophore porulose to pilose, basidiospores smaller (15–28.5×3–4.5μm) ................. H. mopsanhanense
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