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Hyphoderma moniliforme and *H. nemorale* (Basidiomycota) newly recorded from China

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Abstract

Hyphoderma moniliforme and *H. nemorale*, saprobically growing on wood, are recorded as new for mycobiota of China. Both species were collected in mountains at the altitudes of 1850–3000 m, from Yunnan Province (southwestern China). *Hyphoderma moniliforme* is also a new record for Eurasia, and previously known only from South Africa. The collections of *H. nemorale* in this study represent the most eastern and the most southern localities for this species known for Eurasia. Both species bear moniliform cystidia. Bayesian inference of phylogeny based on ITS and partial 28S nuclear ribosomal DNA sequences indicated that *H. moniliforme* is united in one clade with *H. litschaueri* from North America. 28S-based phylogram demonstrated that Chinese *H. nemorale* belong to the same clade with the holotype of this species collected from Europe. Morphology descriptions and illustrations for these two species are provided.

Key words – corticioid fungi – Meruliaceae – Polyporales – taxonomy – wood-decay fungi

Introduction

Hyphoderma Wallr. (Meruliaceae, Polyporales) is one of the most species-rich and taxonomically complicated genera among corticioid fungi. The main generic features of this genus are effused basidiomata, broad subicular hyphae with relatively small clamp-connections (hyphae of 'hyphodermoid' morphology), typically large, subclavate basidia with a slight constriction at middle, and inamyloid basidiospores, which in most species are median-large, cylindrical and thin-walled. According to phylogenetic evidence, Larsson (2007) treated most species of *Hyphoderma* s. l. as the genera *Hyphoderma* s. str. and *Peniophorella* P. Karst.

According to the list of Dai (2011), 14 species, now belonging to *Hyphoderma* s. str., were recorded in mycobiota of the whole China. The present paper adds two new species of *Hyphoderma* s. str. based on the material collected from Yunnan Province of China.

Materials & Methods

Collections and morphology study

Herbarium specimens were collected in Yunnan Province by S.H. Wu in 1995 and 2002, and deposited in TNM; duplicates were deposited in MSK (herbarium acronyms follow *Index Herbariorum*, http://sweetgum.nybg.org/ih). Isolates are kept in culture collection at TNM.

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Descriptions of macromorphology were based on dry basidiomata. Microscopic measurements and drawings were done from the material mounted in 3% KOH water solution. Crystalline incrustations on hyphae, cystidia, and basidia, and spore wall amyloid or dextrinoid reaction were studied in Melzer's reagent. Sporal wall cyanophily was tested in cotton blue-lactophenol solution.

DNA extraction, amplification, and sequencing

In addition to morphological data, nuclear ribosomal DNA sequences were obtained to clarify taxonomic placement of the specimens. The materials for DNA isolation were the mycelia grown in pure culture (*Wu 0211-42*, *Wu 0211-46*, *Wu 9508-14*) or fragments of the basidioma (TNM F3931). DNA was extracted with Plant Genomic DNA Extraction Miniprep Kit (Viogene, Taiwan), according to manufacturer's protocol. Primer pair ITS1/ITS4 was used for amplification of internal transcribed spacer region, including ITS1, 5.8S and ITS2. DNA fragment at the beginning of ribosomal large subunit gene (28S), was amplified with primer pair LR0R/LR5. Amplifications were run on Mastercycler Gradient 5331 thermal cycler (Eppendorf, Germany). Amplification products were purified with PCR-M Clean Up kit (Viogene) and sequenced with ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit on ABI 3730 DNA sequencer (Applied Biosystems, USA). The original sequences were deposited in NCBI GenBank (Table 1).

Table 1 Data on species, specimens, and their sequences used in phylogenetic analysis.

Species name	Isolate / Specimen voucher	Contry of origin	GenBank accession no. for nrDNA	
			ITS1-5.8S-ITS2	28S
Hyphoderma cremeoalbum	FCUG 2270 / NH 11538 (GB)	Turkey	–	DQ677492
Hyphoderma definitum	FCUG 2426 / NH 12266	Russia (Krasnodar krai)	AJ534293	–
Hyphoderma granuliferum	/ KHL 12561 (O)	Costa Rica	JN710545	–
Hyphoderma incrustatum	FCUG 2029 / KHL 6685	Sweden	–	AY586668
Hyphoderma litschaueri	/ CFMR:DLL2011-050	USA	KJ140573	–
Hyphoderma litschaueri	FCUG 786 / NH 7603 (GB)	Canada	–	DQ677496
Hyphoderma macaronesicum	E09/57-9 / TFC:Mic.15981	Canary Islands	HE577027	–
Hyphoderma macaronesicum	E06/61-10 / MA:Fungi:16099Tell	Azores	HE577028	–
Hyphoderma medioburiense	FCUG 2113 / NH 10950 (GB)	Spain	–	DQ677497
Hyphoderma moniliforme*	**Wu 0211-42 / TNM F14735**	China	**KC928282**	**KC928283**
Hyphoderma moniliforme	**Wu 0211-46 / TNM F14739**	China	**KC928284**	**KC928285**
Hyphoderma nemorale	**Wu 9508-14 / TNM F3910**	China	**KC928280**	**KC928281**
Hyphoderma nemorale	**/ TNM F3931**	China	**KJ885183**	**KJ885184**
Hyphoderma nemorale	FCUG 2324 / EM 2793	Switzerland	–	AY586669
Hyphoderma nudicephalum	/ TMIC 30479	Japan	AJ534267	–
Hyphoderma nudicephalum	Wu 9508-225 /	China	AJ534268	–
Hyphoderma obtusum	/ JS 17804	Norway	–	AY586670
Hyphoderma occidentale	/ KHL 8469G (GB)	Sweden	–	AY586674
Hyphoderma occidentale	/ KHL 8477 (GB)	Sweden	–	DQ677499
Hyphoderma prosopidis	E09/58-9 / ARIZ:H.H. Burdsall 8479	USA	HE577029	–
Hyphoderma roseocremeum	FCUG 1945 / NH 10545	Denmark	–	AY586672
Hyphoderma setigerum	/ GEL 4001	Germany	–	AJ406511
Hyphoderma setigerum	FCUG 1264 / NH 8544 (GB)	Sweden	–	FN907905
Hyphoderma setigerum	CFMR:HHB2578 /	USA	GQ409523	–
Hyphoderma setigerum	CFMR:FP150263 /	Jamaica	GQ409528	–
Hyphoderma setigerum	/ CFMR:DLL2011-267	USA	KJ140750	–
Hyphoderma subsetigerum	Wu 9508-155 /	China	AJ534275	–
*Mutatoderma heterocystidium***	FCUG 780/2 / NH 7574 (GB)	Canada	–	DQ677495
Mutatoderma mutatum	FCUG 2403 / NH 12026 (GB)	Russia	–	DQ677498
Phanerochaete sordida	/ KHL 12054 (GB)	Norway	EU118653	EU118653

*Data in bold belong to the sequences obtained in this study; **under the name *Hyphoderma heterocystidiatum* in GenBank

Sequence alignment and reconstruction of phylogeny

The datasets were composed of both the sequences obtained in this study and extracted from GenBank (Tab. 1). The sequences, most similar to specimens of *Hyphoderma* from Yunnan, traced by BLAST and belonged to *Hyphoderma* and *Mutatoderma* (Parmasto) C.E. Gómez, were selected for ITS and 28S datasets. *Phanerochaete sordida*, from a sister clade to Meruliaceae (Larsson 2007), was assigned as the outgroup in both datasets. Sequences were aligned in MAFFT v. 7 at the web server (http://mafft.cbrc.jp/alignment/server), using E-INS-i strategy for ITS and G-INS-i strategy for 28S (Katoh et al. 2009). Before alignment, sequences were arranged in such the way that presumably more related taxa were dispersed among less related. Too long 3' and 5' protruding ends in sequences were cut before the final alignment. Aligned datasets were edited manually in MEGA v. 3.1 software (Kumar et al. 2004). Ready datasets and the resulting phylograms were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S16138). The best-fit models of nucleotide evolution were estimated by MrModeltest v. 2.3 (https://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html), using Akaike Information Criterion.

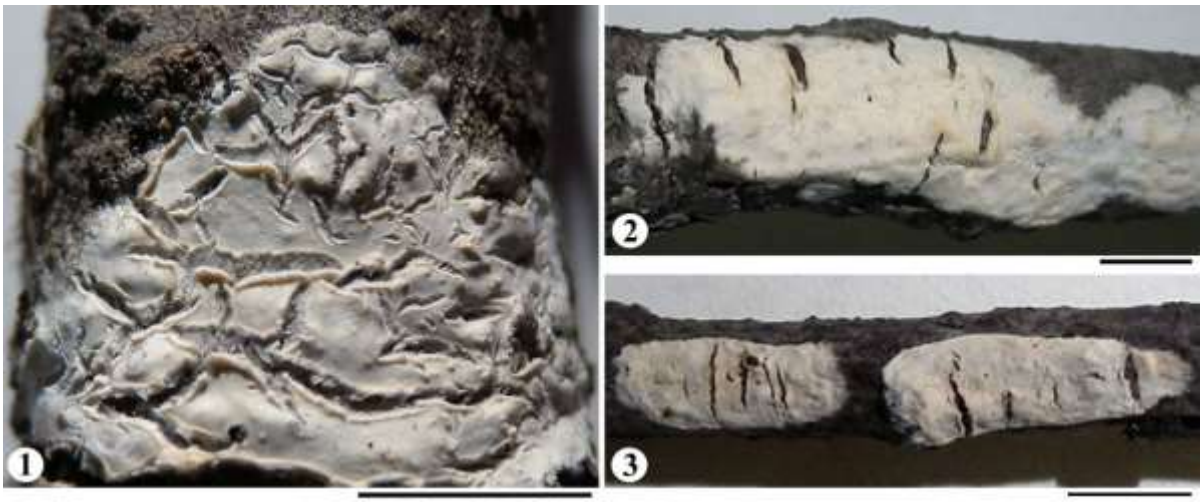
Bayesian analysis of phylogeny was performed in MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003). Both ITS and 28S datasets were individually analyzed using two independent runs, each with four MC^3 chains running for 500000 generations, with tree and parameter sampling every 500 generations. Burn-in discarded 25% of samples. The ITS datamatrix was analysed with different parameter set for the two partitions, 5.8S and ITS1+ITS2, according to the best-fit models.

Results

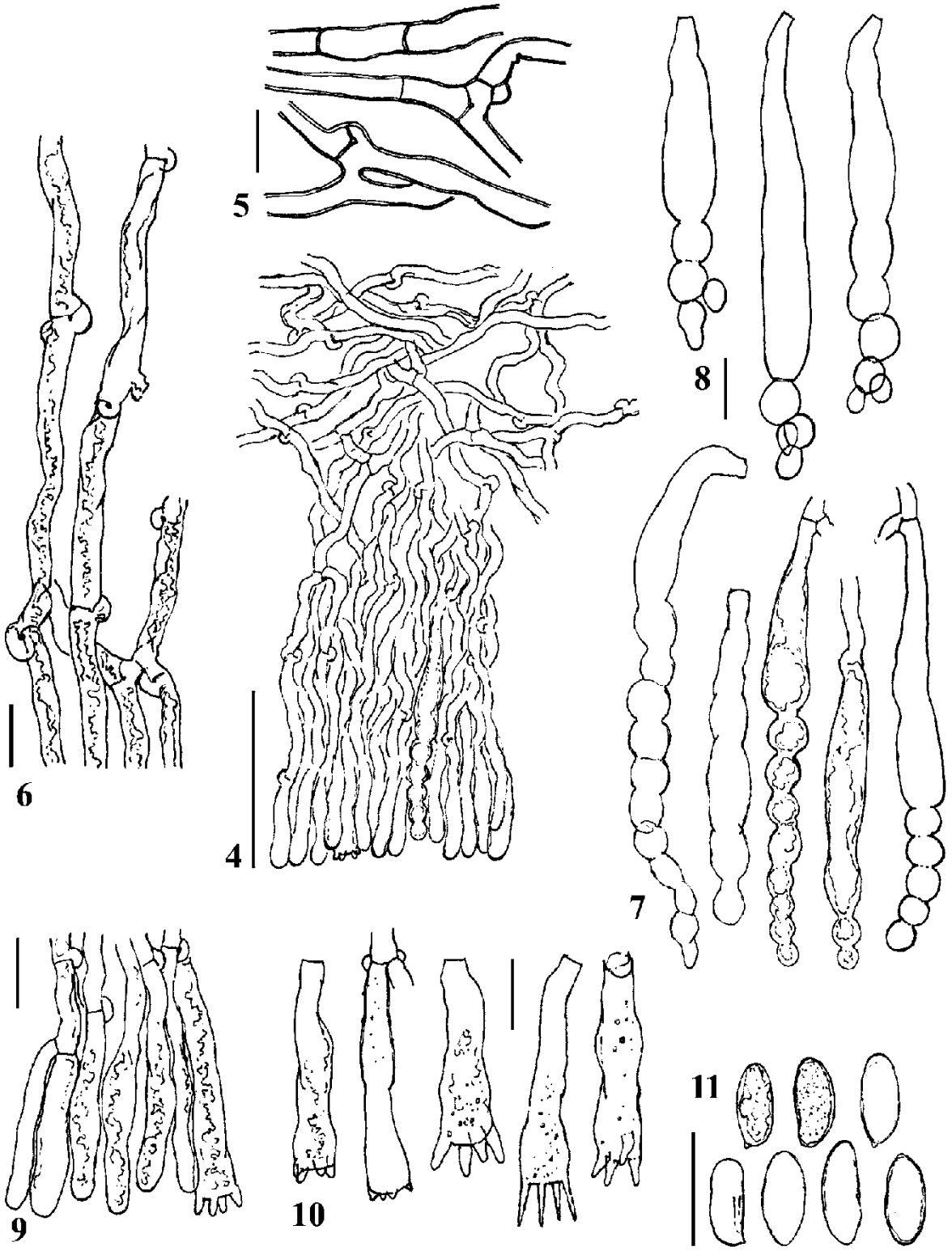
Species descriptions

Hyphoderma moniliforme (P.H.B. Talbot) Manjón, G. Moreno & Hjortstam, Mycotaxon 33: 261 (1988). Figs 1–11

Basidiomata effused, with age broadly cracking. Hymenophore smooth, milk white or creamish. Hyphae clamped at all primary septa, colorless. Subicular hyphae loosely arranged, sparsely branched, disordered (except thin layer of horizontal hyphae next to the substratum), (2.5–)3–5.5 μm diam, naked, with thin to thickened walls. Subhymenium thickening, compact, of thin-walled, naked or slightly encrusted hyphae 3.5–4 μm diam. Cystidia enclosed, 50–80 × 6–8 μm, moniliform in general or apically moniliform, thin-walled, colorless. Basidia clavate-subcylindrical, slightly median-constricted, 20–38 × 6–7.5(–8) μm, colorless, thin-walled, with four large sterigmata. Basidiospores oblong to cylindrical, (7.5–)8–9(–9.5) × (3.2–)3.5–4 μm, thin-walled, colorless, inamyloid, indextrinoid, with small or idistinct apiculus.



Figs 1–3 – Macromorphology of *Hyphoderma moniliforme*. 1 TNM F14732. 2 TNM F14735. 3 TNM F14739. Bars = 1 cm.



Figs 4–11 – Micromorphology of *Hyphoderma moniliforme* (TNM F14732). 4 Vertical section through basidioma. 5, 6 Subicular hyphae. 7 Unbranched cystidia. 8 Branched cystidia. 9 Portion of hymenium. 10 Basidia. 11 Basidiospores. Bars: for 4 = 50 μm, for 5–11 = 10 μm.

Material examined – China, Yunnan Province, Kunming, Chinlunghsia, alt. 1850 m, 7 Nov 2002, S.H. Wu, *Wu 0211-32* (TNM F14732, dup. in MSK); Chuhsiung, Tzuhsishan, alt. 2250 m, 8 Nov 2002, S.H. Wu, *Wu 0211-42* (TNM F14735), alt. 2400 m, 9 Nov 2002, S.H. Wu, *Wu 0211-46* (TNM F14739). All collections are from dead corticated branches of unidentified angiosperms.

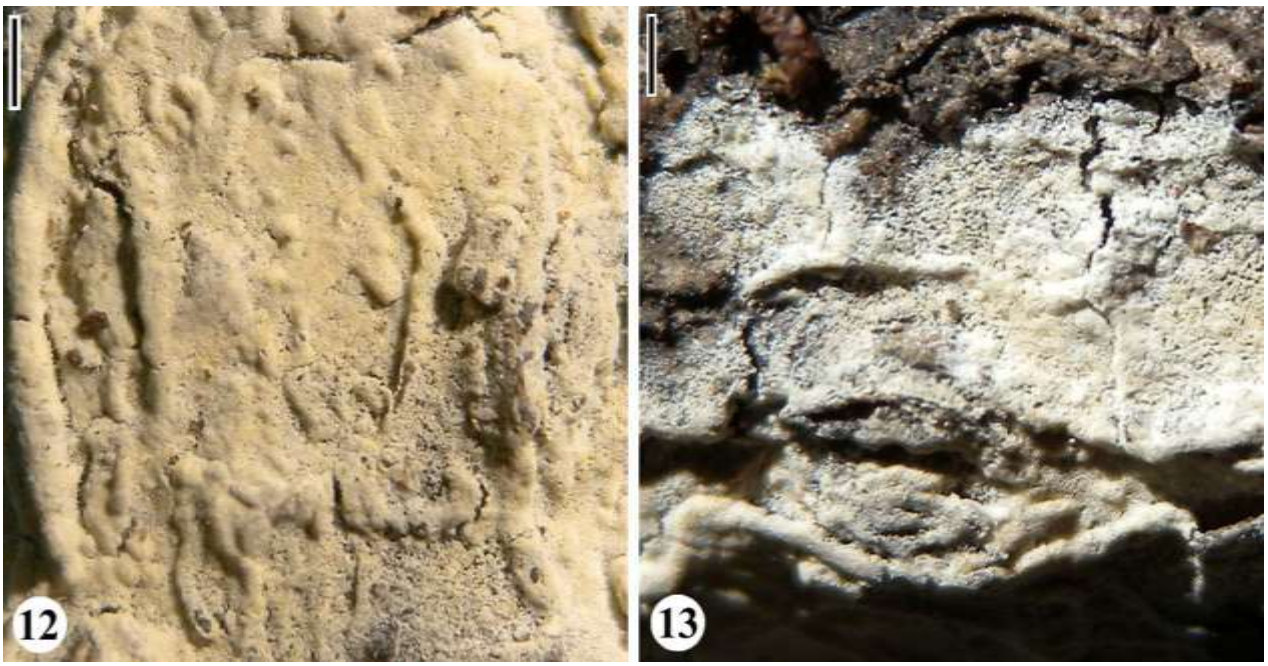
Hyphoderma nemorale K.H. Larss., Nordic J. Bot. 18(1): 123 (1998). Figs 12–22

Basidiomata effused, soft-membranaceous or subpellicular, 90–180 μm thick. Hymenial surface colliculose, cream, when young minutely porulose, then almost continuous and slightly cracking. Margin farinaceous, paler colored, up to 2.5 mm broad. Hyphal system monomitic, hyphae clamped at all primary septa, moderately branched, 2.5–4 μm wide, colorless, thin- or almost thin-walled, naked to richly encrusted. Subiculum of loosely arranged, disordered or subvertical hyphae, richly interspersed by middle-sized crystalline material. Subhymenium not clearly differentiated. Cystidia scattered, subcylindrical to somewhat moniliform and capitate, slightly projecting, 35–70 × 7–8 μm, aseptate of with several (up to 5) adventitious septa, colorless, thin-walled, naked or somewhat encrusted basally; cystidioles numerous, suburniform to subcylindrical, often slightly capitate, a little protruding, 20–35 × 4–8 μm, colorless, thin-walled, more or less encrusted, with capitate apex 3.5–4.5(–5.5) μm broad. Basidioles slightly to richly encrusted mostly in lower and middle part. Basidia clamped at base, clavate or subclavate, slightly median constricted, 20–30 × 6.5–9.5 μm, thin-walled, more or less encrusted in lower and middle part, unicellular or with 1–3 adventitious septa, with 2–4 conical sterigmata 4–5 × 1.3–1.5 μm. Spores cylindrical, adaxially slightly convex, flat, or somewhat depressed, (8.5–)9.5–14(–15) × (3.5–)4–5(–6) μm, colorless or almost so, thin-walled, smooth; contents more or less granular or large-guttulate; wall negative in Melzer's reagent, slightly cyanophilous; apiculus very short or unclear.

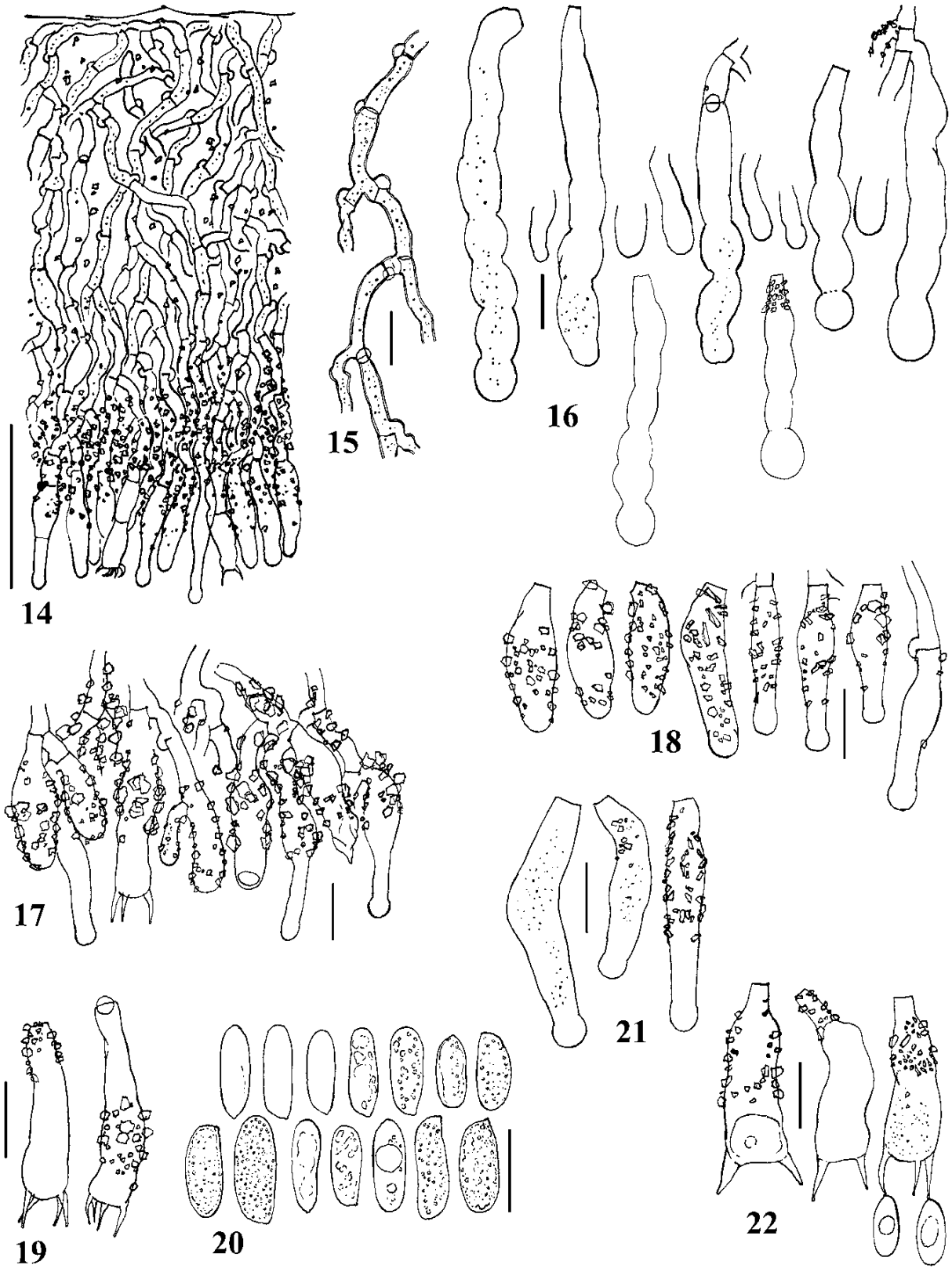
Material examined – China, Yunnan Province, Lijiang, Santaowan, alt. 3000 m, 1 Aug 1995, S.H. Wu & J.Y. Tseng, *Wu 9508-14* (TNM F3910; dup. in MSK), *Wu 9508-43* (TNM F3931). Both specimens were collected from dead twigs and branches of unidentified angiosperms.

Molecular phylogeny

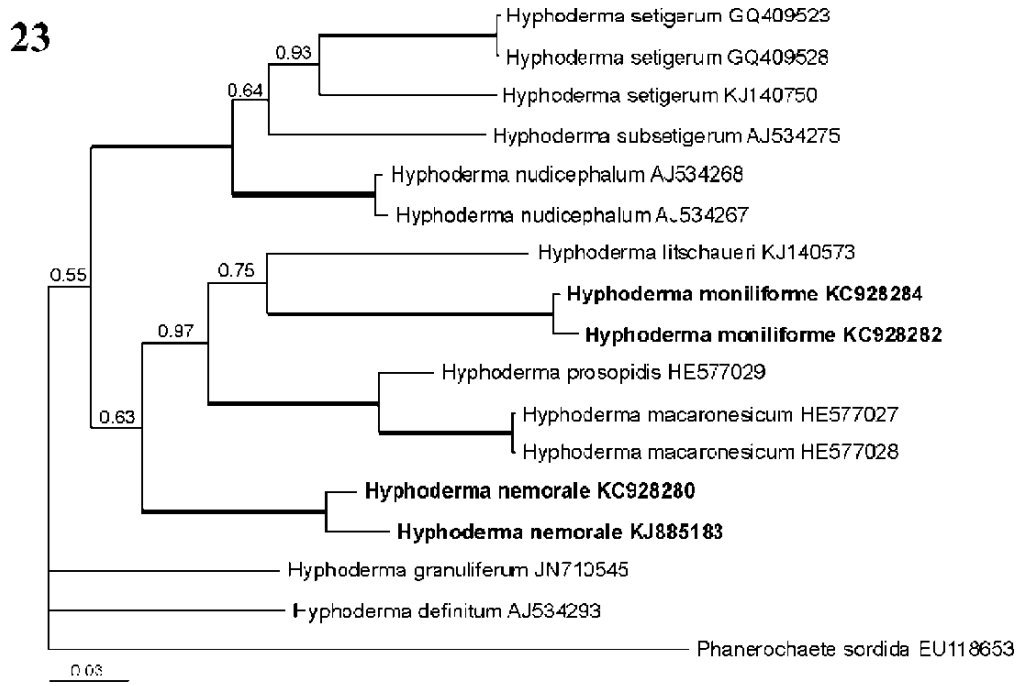
The aligned ITS dataset which was undergone by Bayesian analysis included 624 positions, 418 of which were constant. MrModeltest suggested GTR+G as the best-fit model of nucleotide evolution for ITS1+ITS2, and K80 for 5.8S. The aligned datamatrix of partial 28S sequences included 887 positions, 866 of which were constant. The best-fit model of nucleotide evolution suggested for it by MrModeltest was GTR+I+G.

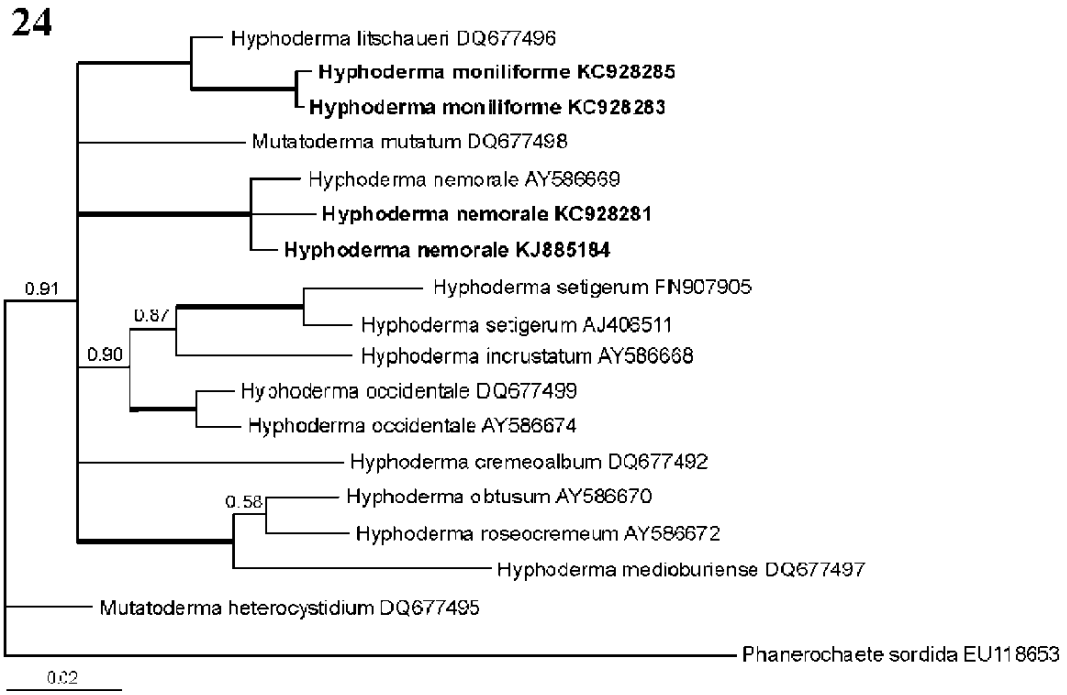


Figs 12–13 Macromorphology of *Hyphoderma nemorale* (TNM F3910). 12 Basidioma in central part. 13 Basidioma in marginal part. Bars = 1 mm.



Figs 14–22 Micromorphology of *Hyphoderma nemorale*. 14–20 TNM F3910. 14 Vertical section through basidioma. 15 Subicular hyphae. 16 Submoniliform cystidia. 17 Portion of hymenium. 18 Basidioles (left) and subcapitate cystidioles (right). 19 Basidia. 20 Spores. 21, 22 TNM F3931. 21 Capitate cystidia. 22 Basidia. Bars: for 14 = 50 μm, for 15–22 = 10 μm.





Figs 23–**24** Consensus Bayesian phylograms for *Hyphoderma* species. 23 Based on ITS dataset. 24 Based on partial 28S dataset. Numbers above branches denote Bayesian posterior probability value (PP). Thick branches have PP = 0.99–1.00. Bars: number of substitutions per site.

Both phylograms generated using Bayesian approach (Figs 23, 24) confirmed that the considered specimens from Yunnan belong to the genus *Hyphoderma*. The branch pattern on both phylograms demonstrates that specimens of *H. moniliforme* TNM F14735 and TNM F14739 have little molecular divergence and belong to the same independent species. Phylogenetic distances between two *H. nemorale* specimens, TNM F3910 and TNM F3931, suggest that they are also conspecific, but molecular divergence between them is fairly high in comparison with other *Hyphoderma* species. On the phylogram based on partial 28S sequences these two specimens occurred to be joint together in a highly supported clade (Bayesian posterior probability value, PP = 1.00) with the holotype of *H. nemorale*.

Discussion

Characteristics of three studied specimens of *H. moniliforme* from Yunnan correspond with the description of *Corticium moniliforme* P.H.B. Talbot (Talbot 1958), except non-stratified context, shorter and somewhat narrower cystidia, and hyphal morphology. In Chinese specimens hyphae are fairly regular, while in *C. moniliforme* hyphae were described as tortuous and nodulose swollen. Our specimens differ from the similar species *H. litschaueri* (Burt) J. Erikss. & Å. Strid (Eriksson and Ryvarden 1975) by having non-porulose basidioma, longer and wider basidia, shorter and non-guttulate basidiospores. Moreover, in *H. litschaueri* the constrictions of cystidia are less pronounced. In *H. moniliforme* the constricted part of cystidia has a tendency to be as if composed of a chain of globose cells. The specimens of *H. moniliforme* were collected in central part of Yunnan Province of China. Previous record of this species was only in South Africa (Talbot 1958).

Bayesian analyses of phylogeny based on ITS and 28S sequences (Figs 23, 24) demonstrated that *H. moniliforme* belongs to *H. litschaueri*-clade. This clade gets high posterior probability support (PP=1.00) in 28S-based phylogram. Both species in the clade have constricted cystidia. In the same time, in terms of molecular phylogeny *H. moniliforme* is well distinguished from North American specimen of *H. litschaueri*.

The specimens of *H. nemorale* from Yunnan fairly fit the type description of this species (Larsson 1998), except bearing shorter submoniliform cystidia and presence of incrustations on hyphae and hymenial elements. No incrustations in hymenium were noted in a description of *H. nemorale* from Asia (Ghobad-Nejhad et al. 2008). Richly encrusted basidia and incrustations in subhymenium in *H. nemorale* were decribed and illustrated based on Italian material (Bernicchia and Gorjón 2010). However, the authors noted that the hiatus between Italian specimens of *H. nemorale* and *H. incrustatum* K.H. Larss. is not distinct. The phylogenetic data reveal that the material from China is a form of *H. nemorale* with encrusted hymenium and subhymenium.

Hyphoderma nemorale was collected in northwest part of Yunnan Province, from temperate forest montane belt. Larsson (1998) supposed that *H. nemorale* is not rare in boreal and nemoral zones of Eurasia. This species was reported from Britain, Norway, Sweden, Finland, Denmark, Germany, France, Switzerland, Spain, Italy, Ukraine, Russia (Arkhangel'sk, Yamal, and Karachayevo-Cherkessiya), Cyprus, Iran (Larsson 1998, Ghobad-Nejhad et al. 2009, Bernicchia & Gorjón 2010, Torrejón 2013). Ghobad-Nejhad et al. (2008) reported this species in NW Iran at the altitudes 1000–1800 m. The studied collections from Yunnan represent the most southern and the most eastern distribution locality known for this species.

Hyphoderma granuliferum P. Roberts (Roberts 2000) and *H. incrustatum* (Larsson 1998, Dämon 2000) also bear incrusted hymenial elements and more or less capitate cystidia. However, no close phylogenetic proximity among *H. nemorale*, *H. granuliferum* and *H. incrustatum* was demonstrated in the analyses (Figs 23, 24).

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