Fungal Melanin and the Mammalian Immune System

Volume 7 - Issue 4 [April 2021]
Genome Sequence Analysis of the Oleaginous Yeast, *Rhodotorula diobovata*, and Comparison of the Carotenogenic and Oleaginous Pathway Genes and Gene Products with Other Oleaginous Yeasts

by Irene Fakankun, Brian Fristensky and David B. Levin

*J. Fungi* 2021, 7(4), 320; [https://doi.org/10.3390/jof7040320 - 20 Apr 2021]

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Abstract *Rhodotorula diobovata* is an oleaginous and carotenogenic yeast, useful for diverse biotechnological applications. To understand the molecular basis of its potential applications, the genome was sequenced using the Illumina MiSeq and Ion Torrent platforms, assembled by AbySS, and annotated using the JGI annotation [...] Read more.

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Aequorin as a Useful Calcium-Sensing Reporter in *Candida albicans*

by Dominique Sanglard

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Abstract In *Candida albicans*, calcium ions (Ca$^{2+}$) regulate the activity of several signaling pathways, especially the calcineurin signaling pathway. Ca$^{2+}$ homeostasis is also important for cell polarization, hyphal extension, and plays a role in contact sensing. It is therefore important [...] Read more.

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*Trichoderma* and the Plant Heritable Priming Responses

by María E. Morán-Diez, Ángel Emilio Martínez de Alba, M. Belén Rubio, Rosa Hermosa and Enrique Monte

*J. Fungi* 2021, 7(4), 318; [https://doi.org/10.3390/jof7040318 - 19 Apr 2021]

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Abstract There is no doubt that *Trichoderma* is an inhabitant of the rhizosphere that plays an important role in how plants interact with the environment. Beyond the production of cell wall degrading enzymes and metabolites, *Trichoderma* spp. can protect plants by inducing faster and [...] Read more.

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Investigating Host Preference of Root Endophytes of Three European Tree Species, with a Focus on Members of the Phialocephala fortinii—Acephala applanata Species Complex (PAC)

by Sophie Stroheker, Vivanne Dubach, Irina Vögltli and Thomas N. Sieber

J. Fungi 2021, 7(4), 317; https://doi.org/10.3390/jof7040317 - 19 Apr 2021

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Abstract Host preference of root endophytes of the three European tree species of Norway spruce (Picea abies), common ash (Fraxinus excelsior), and sycamore maple (Acer pseudoplatanus) were investigated in two forest stands near Zurich, Switzerland. The focus was [...] Read more.

(This article belongs to the Special Issue Fungal Endophytes in Agriculture and Ecosystems)

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Emerging Fungi and Diagnosis of Fungal Infections: Current Knowledge and New Developments

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Abstract

I would like to thank all the authors contributing to this Special Issue [...] Full article

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Trichoderma Biological Control to Protect Sensitive Maize Hybrids against Late Wilt Disease in the Field

by Ofir Degani and Shlomit Dor


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Abstract Late wilt, a disease severely affecting maize fields throughout Israel, is characterized by the relatively rapid wilting of maize plants from the tasseling stage to maturity. The disease is caused by the fungus Magnaportheopsis maydis, a soil and seed-borne pathogen. The pathogen [...] Read more.

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Abstract Rhizosphere-resident fungi that are helpful to plants are generally termed as ‘plant growth promoting fungi’ (PGPF). These fungi are one of the chief sources of the biotic inducers known to give their host plants numerous advantages, and they play a vital role in [...] Read more.

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Performance of LDBio Aspergillus WB and ICT Antibody Detection in Chronic Pulmonary Aspergillosis

by Anna Rozaliyani, Fandra Setiantingrum, Sresta Azahra, Asriyani Abdullah, Ayu Eka Fatril, Harmi Rosianawati, Erlina Burhan, Diah Handayani, Arief Riadi Arifin, Jamal Zaini, Mulyati Tugiran, Robiatul Adawiyah, Ridhawati Syam, Heri Wibowo, Retno Wahyuningsih, Chris Kosmidis and David W Denning

J. Fungi 2021, 7(4), 311; https://doi.org/10.3390/jof7040311 - 18 Apr 2021

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Abstract The detection of Aspergillus antibody has a key role in the diagnosis of chronic pulmonary aspergillosis. Western blot (WB) and immunochromatography (ICT) lateral flow detection of Aspergillus antibody can be used as confirmatory and screening assays but their comparative performance in TB patients [...] Read more.

(This article belongs to the Special Issue Chronic Pulmonary Aspergillosis)

Exploring the Species Diversity of Edible Mushrooms in Yunnan, Southwestern China, by DNA Barcoding

by Ying Zhang, Mei Mo, Liu Yang, Fei Mi, Yang Cao, Chunli Liu, Xiaozhao Tang, Pengfei Wang and Jianping Xu

J. Fungi 2021, 7(4), 310; https://doi.org/10.3390/jof7040310 - 17 Apr 2021

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Abstract Yunnan Province, China, is famous for its abundant wild edible mushroom diversity and a rich source of the world's wild mushroom trade markets. However, much remains unknown about the diversity of edible mushrooms, including the number of wild edible mushroom species and their [...] Read more.

(This article belongs to the Special Issue Fungal Biodiversity and Ecology)

Characterizing the Assemblage of Wood-Decay Fungi in the Forests of Northwest Arkansas

by Nawaf Alshammari, Fuad Ameen, Muneera D. F. AlKahtani and Steven Stephenson

J. Fungi 2021, 7(4), 309; https://doi.org/10.3390/jof7040309 - 16 Apr 2021

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Abstract The study reported herein represents an effort to characterize the wood-decay fungi associated with three study areas representative of the forest ecosystems found in northwest Arkansas. In addition to specimens collected in the field, small pieces of coarse woody debris (usually dead branches) [...] Read more.

(This article belongs to the Section Environmental and Ecological Interactions of Fungi)
Taxonomy and Phylogeny of the Wood-Inhabiting Fungal Genus *Hyphoderma* with Descriptions of Three New Species from East Asia
by Qian-Xin Guan and Chang-Lin Zhao
*J. Fungi* 2021, 7(4), 308; https://doi.org/10.3390/jof7040308 - 16 Apr 2021
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Abstract Three new wood-inhabiting fungi, *Hyphoderma crystallinum*, *H. membranaceum*, and *H. microporoides* spp. nov., are proposed based on a combination of morphological features and molecular evidence. *Hyphoderma crystallinum* is characterized by the resupinate basidiomata with smooth hymenial surface scattering scattered nubby crystals, a [...] Read more.
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Systemic Resistance in Chilli Pepper against Anthracnose (Caused by *Colletotrichum truncatum*) Induced by *Trichoderma harzianum, Trichoderma asperellum* and *Paenibacillus dendritiformis*
by Mukesh Yadav, Manish Kumar Dubey and Ram Sanmukh Upadhyay
*J. Fungi* 2021, 7(4), 307; https://doi.org/10.3390/jof7040307 - 16 Apr 2021
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Abstract In the present study, *Paenibacillus dendritiformis, Trichoderma harzianum, and Trichoderma asperellum* were appraised as potential biocontrol agents that induce resistance in chilli (*Capsicum annuum*) against the devastating pathogen *Colletotrichum truncatum*, which causes anthracnose. Bright-field and scanning electron micrographs showed the [...] Read more.
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Favorable Effects of Voriconazole Trough Concentrations Exceeding 1 µg/mL on Treatment Success and All-Cause Mortality: A Systematic Review and Meta-Analysis
by Yuki Hanai, Yukihiro Hamada, Toshimi Kimura, Kazuaki Matsumoto, Yoshiko Takahashi, Satoshi Fujii, Kenji Nishizawa, Yoshitsugu Miyazaki and Yoshiro Takesue
*J. Fungi* 2021, 7(4), 306; https://doi.org/10.3390/jof7040306 - 16 Apr 2021
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Abstract This systematic review and meta-analysis examined the optimal trough concentration of voriconazole for adult patients with invasive fungal infections. We used stepwise cutoffs of 0.5–2.0 µg/mL for efficacy and 3.0–6.0 µg/mL for safety. Studies were included if they reported the rates of all-cause [...] Read more.
(This article belongs to the Special Issue Invasive Fungal Infections 2021)
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**Abstract:** Three new wood-inhabiting fungi, *Hyphoderma crystallinum*, *H. membranaceum*, and *H. microporoides* spp. nov., are proposed based on a combination of morphological features and molecular evidence. *Hyphoderma crystallinum* is characterized by the resupinate basidiomata with smooth hymenial surface scattering scattered nubby crystals, a monomitic hyphal system with clamped generative hyphae, and numerous encrusted cystidia present. *Hyphoderma membranaceum* is characterized by the resupinate basidiomata with tuberculate hymenial surface, presence of the moniliform cystidia, and ellipsoid to cylindrical basidiospores. *Hyphoderma microporoides* is characterized by the resupinate, cottony basidiomata distributing the scattered pinholes visible using hand lens on the hymenial surface, presence of halocystidia, and cylindrical to allantoid basidiospores. Sequences of ITS+nLSU rRNA gene regions of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony, and Bayesian inference methods. These phylogenetic analyses showed that three new species clustered into *Hyphoderma*, in which *H. crystallinum* was sister to *H. variolosum*, *H. membranaceum* was retrieved as a sister species of *H. sinense*, and *H. microporoides* was closely grouped with *H. nemorale*. In addition to new species, map to show global distribution of *Hyphoderma* species treated in the phylogenetic tree and an identification key to Chinese *Hyphoderma* are provided.

**Keywords:** corticioid fungi; *Hyphoderma*; hyphodermataceae; molecular systematics; Yunnan Province

**1. Introduction**

Fungi are an ecologically important branch of the tree of life based on its distinct and diverse characteristics, in which these organisms play a vital role in ecosystems as diverse as soil, forest, rocks, and ocean, but their roles are primarily enacted behind the scenes, literally as hidden layers within their substrate [1]. On the basis of the nature of their intertwined partners in numerous symbiotic interactions, they have mostly marched via stepwise codiversification with the plants [2]. Fungi have evolved numerous strategies to degrade hard-to-digest substrates for outcompeting with other microbes, while combating competitors using an arsenal of bioactive metabolites, such as the familiar antibiotics, ethanol, and organic acids [3]. Taxonomy plays a central role in understanding the diversity of life, discovering into systems of names that capture the relationships between species, and translating the products of biological exploration [4]. Despite the early embrace of the molecular systematics by mycologists, both the discovery and classification of fungi are still in great flux, particularly among the more basal branches of the tree, in which the true diversity is only now coming to light from genomic analyses and environmental DNA surveys [1]. The researches revealed that perhaps less than 5% of the estimated two to four million species have been formally described, therefore, the hidden and microscopic nature of many fungi also means that their diversity is undersampled [5,6].
The genus *Hyphoderma* Wallr. is one of the most important fungal groups because of its key role in the carbon cycle and being the most efficient wood decomposers in the forest ecosystem [7]. This genus is typified by *H. setigerum* (Fr.) Donk [8]. *Hyphoderma* 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 [9,10]. Currently, about 100 species have been accepted in *Hyphoderma* worldwide [8,11–15]. Index Fungorum (http://www.indexfungorum.org; accessed on 16 April 2021) and MycoBank (https://www.mycobank.org; accessed on 16 April 2021) register 192 specific and infraspecific names in *Hyphoderma*.

Molecular systematics covering *Hyphoderma* revealed the classification of corticioid fungi and showed that *H. obtusum* J. Erikss. and *H. setigerum* clustered into Meruliaceae Rea and then grouped with *Hypochnicium polonense* (Bres.) Á. Strid, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences [16]. Telleria et al. [17] discussed the relationships between *Hyphoderma* and *Peniophorella* P. Karst., in which some species from *Hyphoderma* and *Peniophorella* are grouped and they proposed a new species, *H. macaronesicum* Telleria et al. The research on studying *Hyphoderma setigerum* complex showed that *H. pinicola* Yurch. and Sheng H. Wu represented a fifth species in this complex, which revealed that this complex was a white-rot wood-decaying corticioid fungal species and occurred worldwide from tropical to temperate regions [18]. A revised family-level classification of the Polyporales revealed that four *Hyphoderma* species nested into the residual polyporoid clade belonging to *Hyphodermataceae*, and then, they were grouped with three related genera *Meripilus* P. Karst., *Physisporinus* P. Karst., and *Rigidoporus* Murrill [19]. Chinese *Hyphoderma* species were compared with closely related taxa, and two new species were proposed, *H. fissuratum* C.L. Zhao and X. Ma and *H. mopanshanense* C.L. Zhao [15].

In this study, three undescribed species of corticioid fungi were collected from Yunnan Province, China. Morphological characteristics and molecular phylogenetic analyses of combined ITS+nLSU rRNA sequences supported the recognition of three new species within *Hyphoderma*.

2. Materials and Methods

2.1. Morphology

The studied specimens are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. Macromorphological descriptions are based on field notes and photos captured in the field and laboratory. Color terminology follows Petersen [20]. Micromorphological data were obtained from the dried specimens, which were observed under a light microscope following Dai [21]. The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer’s reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for 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 Phylogeny

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from dried specimens, according to the manufacturer’s instructions [22]. ITS region was amplified with primer pair ITS5 and ITS4 [23]. Nuclear nLSU region was amplified with primer pair LR0R and LR7 (http://lutzonilab.org/nuclear-ribosomal-dna/; accessed on 16 April 2021). 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 for 1 min, and 72 °C for 1.5 min; and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, China. All newly generated sequences were deposited in NCBI GenBank (Table 1).

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

<table>
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<th>Species Name</th>
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</tr>
<tr>
<td><em>H. geogenium</em></td>
<td>NH 10910</td>
<td>DQ677509 DQ677509</td>
<td>[16]</td>
</tr>
<tr>
<td><em>H. geogenium</em></td>
<td>MA-Fungi 48308</td>
<td>FNS52534 JN939576</td>
<td>[30]</td>
</tr>
<tr>
<td><em>H. micheli</em></td>
<td>MA-Fungi 79155</td>
<td>SR119742 NG060635</td>
<td>[30]</td>
</tr>
<tr>
<td><em>H. punctulatum</em></td>
<td>FP101698sp</td>
<td>KY948827 KY948860</td>
<td>[19]</td>
</tr>
<tr>
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<td>KY948803 KY948861</td>
<td>[19]</td>
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<td>FNS52531 JN939577</td>
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<tr>
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<td>Dai 15917</td>
<td>KY131870 KY131926</td>
<td>[31]</td>
</tr>
<tr>
<td><em>P. subcrocatus</em></td>
<td>Dai 12800</td>
<td>KY131869 KY131925</td>
<td>[31]</td>
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<tr>
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<td>Cui 9588</td>
<td>KY131873 KY131929</td>
<td>[31]</td>
</tr>
<tr>
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<td>Cui 9518</td>
<td>KY131872 KY131928</td>
<td>[31]</td>
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<td><em>Rigidoporus eminens</em></td>
<td>Dai 17200</td>
<td>MT279690 MT279911</td>
<td>[31]</td>
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<td><em>R. undatus</em></td>
<td>Miettinen-13591</td>
<td>KY948731 KY948870</td>
<td>[19]</td>
</tr>
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</table>

New sequences are shown in bold.

Sequences were aligned in MAFFT 7 (https://mafft.cbrc.jp/alignment/server/; accessed on 10 April 2021) using the “G-INS-i” strategy for ITS+nLSU and manually adjusted in BioEdit [32]. The dataset was aligned first and then, ITS and nLSU sequences were combined with Mesquite. Alignment datasets were deposited in TreeBASE (submission ID 27983). Climacocystis borealis (Fr.) Kotl. and Diplomitoporus crustulinus (Bres.) Domanski were selected as an outgroup for phylogenetic analysis of ITS+nLSU phylogenetic tree (Figure 1) following a previous study [19].

Maximum parsimony analysis was applied to the combined (ITS+nLSU) dataset. Its approaches followed previous study [22], and the tree construction procedure was performed in PAUP* version 4.0b10 [33]. 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 bootstrap (BT) analysis with 1000 replicates [34]. 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; accessed on 8 April 2021) [35]. Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 [36] 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 [37]. Four Markov chains were run for 2 runs from random starting trees for 6 million generations for ITS+nLSU (Figure 1). The first one-fourth of all generations was discarded as burn-in. The majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap value (BS) > 70%, maximum parsimony bootstrap value (BT) > 70%, or Bayesian posterior probabilities (BPP) > 0.95.
Figure 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of three new species and related species in *Hyphoderma* within Polyporales based on internal transcribed spacer + nuclear ribosomal RNA gene (ITS+nLSU) sequences. Branches are labeled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50% and Bayesian posterior probabilities > 0.95. The new species are in bold. Clade names follow previous study [19].
3. Results

3.1. Molecular Phylogeny

The ITS+nLSU dataset (Figure 1) included sequences from 78 fungal specimens representing 44 taxa. The dataset had an aligned length of 2086 characters, of which 1245 characters are constant, 127 are variable and parsimony-uninformative, and 714 are parsimony-informative. Maximum parsimony analysis yielded 5000 equally parsimonious trees (TL = 3441, CI = 0.3787, HI = 0.6213, RI = 0.7178, RC = 0.2718). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G (iset nst = 6, rates = invgamma; prset statefreqpr = 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 = 0.007698 (BI).

The phylogram inferred from ITS+nLSU sequences (Figure 1) demonstrated that three new species are clustered into genus *Hyphoderma*, in which *H. crystallinum* was sister to *H. variolosum* Boidin, Lanq. and Gilles, *H. membranaceum* was retrieved as a sister species of *H. sinense* C.L. Zhao and Q.X. Guan, and *H. microporoides* was closely grouped with *H. nemorale* K.H. Larss. (100% BS, 100% BP, and 1.00 BPP).

3.2. Taxonomy

*Hyphoderma crystallinum* C.L. Zhao and Q.X. Guan, sp. nov. Figures 2 and 3.

**Figure 2.** *Hyphoderma crystallinum* (holotype) (**A**): basidiomata on the substrate (**B**); scattered nubby crystals. Bars: **A** = 2 cm and **B** = 1 mm.
Figure 3. Microscopic structures of *Hyphoderma crystallinum* (holotype) (A): basidiospores (B), basidia and basidioles (C), tubular cystidia (D), and encrusted cystidia (E). A section of hymenium. Bars: A–E = 10 µm.

MycoBank no.: MB 839276.

**Holotype**—China, Yunnan Province, Puer, Jingdong County, the Forest of Pineapple, E 100°48′, N 24°21′, 2113 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 4 January 2019, C.L. Zhao 9338 (SWFC).

**Etymology**—*crystallinum* (Lat.): referring to the numerous and larger crystals on the hymenial surface.

**Fruiting body**—Basidiomata annual, resupinate, adnate, without odor and taste when fresh, membranaceous when fresh, becoming hard membranaceous upon drying, and up to 15 cm long, 3 cm wide, and 30–100 µm thick. Hymenial surface white to pale gray when fresh, pale gray to slightly cream upon drying, with scattered nubby crystals. Margin sterile indistinct and white.

**Hyphal system**—Monomitic, generative hyphae with clamp connections, colorless, thin-walled, frequently branched, interwoven, 2–3.5 µm in diameter, IKI-, CB-; tissues unchanged in KOH.

**Hymenium**—Cystidia of two types: (1) tubular cystidia, colorless, thin-walled, 32–51 µm × 5–10 µm and (2) encrusted cystidia, numerous, colorless, encrusted by crystals, 14–46 µm × 4–11 µm. Basidia clavate to subcylindrical, slightly constricted in the middle to somewhat sinuous, with 4 sterigmata and a basal clamp connection, 21.5–31 µm × 6–8.5 µm.
Spores—Basidiospores allantoid, colorless, thin-walled, smooth, with oil drops inside, IKI-, CB-, (10.5–)11–14.5(–15) µm × 4–5.5(–6) µm, L = 12.99 µm, W = 4.81 µm, Q = 2.47–2.98 (n = 90/3).

Additional specimens examined—China, Yunnan Province, Puer, Jingdong County, the Forest of Pineapple, E 100°48', N 24°21', 2113 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 4 January 2019, C.L. Zhao 9374 (SWFC); Dali, Nanjian County, Lingbaoshan National Forestry Park, E 100°30', N 24°46', 1963 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 9 January 2019, C.L. Zhao 10224 (SWFC); Wenshan, Funing County, Guying village, E 105°35', N 23°36', 976 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 20 January 2019, C.L. Zhao 11723 (SWFC); Wenshan, Xichou County, Jiguanshan Forestry Park, E 103°46', N 23°33', 1670 m asl., on fallen angiosperm trunk, leg. C.L. Zhao, 22 July 2019, C.L. Zhao 15841 (SWFC); Honghe, Pingbian County, Daweishan National Nature Reserve, E 103°35', N 22°53', 1990 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 3 August 2019, C.L. Zhao 18459 (SWFC).

*Hyphoderma membranaceum* C.L. Zhao and Q.X. Guan sp. nov. Figures 4 and 5.

Figure 4. Basidiomata (A) of *Hyphoderma membranaceum* (B) (holotype). Bars: A = 2 cm and B = 1 mm.
Hymenium—Cystidia moniliform, thin-walled, 28–60 µm × 6.5–10.5 µm; basidia clavate to subcylindrical, slightly constricted in the middle to somewhat sinuous, with 4 sterigmata and a basal clamp connection, 21.5–31 µm × 5–7.5 µm.

Spores—Basidiospores ellipsoid to cylindrical, colorless, thin-walled, smooth, with irregular vacuole inside, IKI-, CB-, (10.5–)11–13.5(–14) µm × 4.5–5.5(–6) µm, L = 12.52 µm, W = 5.18 µm, Q = 2.42 (n = 60/2).

Additional specimens examined—China, Yunnan Province, Puer, Zhenyuan County, Heping Town, Liangzizhai, E 101°25′, N 23°56′, 2246 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 15 Jan 2018, C.L. Zhao 5844 (SWFC).

Hyphoderma microporoides C.L. Zhao and Q.X. Guan sp. nov. Figures 6 and 7.

MycoBank no.: MB 839277.

Holotype—China, Yunnan Province, Chuxiong, Zixishan Forestry Park, E 101°24′, N 25°01′, 2356 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 1 July 2018, C.L. Zhao 6971 (SWFC).

Etymology—membranaceum (Lat.): referring to the membranous hymenophore.

Fruiting body—Basidiomata annual, resupinate, adnate, membranous, without odor and taste when fresh, and up to 15 cm long, 2 cm wide, and 30–100 µm thick. Hymenial surface tuberculate, white to pale gray when fresh, pale gray to cream on drying, with cracking. Margin sterile, narrow, and gray.

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

Hymenium—Cystidia moniliform, thin-walled, 28–60 µm × 6.5–10.5 µm; basidia clavate to subcylindrical, slightly constricted in the middle to somewhat sinuous, with 4 sterigmata and a basal clamp connection, 21.5–31 µm × 5–7.5 µm.

Spores—Basidiospores ellipsoid to cylindrical, colorless, thin-walled, smooth, with irregular vacuole inside, IKI-, CB-, (10.5–)11–13.5(–14) µm × 4.5–5.5(–6) µm, L = 12.52 µm, W = 5.18 µm, Q = 2.42 (n = 60/2).

Additional specimens examined—China, Yunnan Province, Puer, Zhenyuan County, Heping Town, Liangzizhai, E 101°25′, N 23°56′, 2246 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 15 Jan 2018, C.L. Zhao 5844 (SWFC).

Hyphoderma microporoides C.L. Zhao and Q.X. Guan sp. nov. Figures 6 and 7.
Figure 6. *Hyphoderma microporoides* (holotype) (A): basidiomata on the substrate (B) and scattered pinholes. Bars: A = 2 cm, B = 1 mm.

MycoBank no.: MB 839277.

**Holotype**—China, Yunnan Province, Chuxiong, Zixishan Forestry Park, E 101°24’, N 25°01’, 2313 m asl., on fallen angiosperm trunk, leg. C.L. Zhao, 30 June 2018, C.L. Zhao 6857 (SWFC).

**Etymology**—*microporoides* (Lat.): referring to the scattered pinholes on the hymenophore that are visible under hand lens.

**Fruiting body**—Basidiomata annual, resupinate, adnate, without odor and taste when fresh, cottony when fresh, fragile upon drying, and up to 22 cm long, 2.5 cm wide, and 50–100 µm thick. Hymenial surface smooth with scattered pinholes visible under hand lens, cream to pale buff when fresh, and slightly buff upon drying. Margin sterile, indistinct, and white to cream.

**Hyphal system**—Monomitic, generative hyphae with clamp connections, colorless, thin-walled, frequently branched, interwoven, 3–5 µm in diameter, IKI-, CB-; tissues unchanged in KOH.

**Hymenium**—Halocystidia capitate, thin-walled, smooth, 18–51 µm × 4.5–7 µm; basidia clavate, constricted, somewhat sinuous, with 4 sterigmata and a basal clamp connection, 18.5–29.5 µm × 5–7 µm.
Spores—Basidiospores cylindrical to allantoid, colorless, thin-walled, smooth, with oil drops inside, IKI-, CB-, 8.5–10(–10.5) µm × 2.5–3.5(–4) µm, L = 9.29 µm, W = 3.24 µm, Q = 2.87 (n = 30/1).

Additional specimens examined—China, Yunnan Province, Puer, Jingdong County, Taizhong Town, Ailaoshan Ecological Station, E 100°56′, N 24°29′, 1938 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 25 August 2018, C.L. Zhao 8695 (SWFC).

Figure 7. Microscopic structures of *Hyphoderma microporoides* (holotype) (A): basidiospores (B), basidia and basidioles (C), and cystidia (D). A section of hymenium. Bars: A = 5 µm, B–D = 10 µm.

4. Discussion

In the present study, three new species, *Hyphoderma crystallinum*, *H. membranaceum*, and *H. microporoides* are described based on phylogenetic analyses and morphological characteristics.

Phylogenetically, the family-level classification of the Polyporales (Basidiomycota) amplified nLSU, nITS, and rpb1 genes across the Polyporales, was employed, in which four species *Hyphoderma macaronesicum*, *H. medioburiense* (Burt) Donk, *H. mutatum* (Peck) Donk, and *H. setigerum* nested into family Hyphodermataceae within the residual polyporoid clade [19]. In the present study, three new taxa clustered into *Hyphoderma*, in which *Hyphoderma crystallinum* was sister to *H. variolosum*, *H. microporoides* grouped closely with *H. nemorale*, and *H. membranaceum* grouped with *H. sinense* and *H. transiens* (Bres.) Parmasto (Figure 1). However, morphologically, *H. variolosum* differs from *H. crystallinum* by its narrower tubular cystidia (40–50 µm × 4–6 µm) [38]; *H. nemorale* is separated from *H. microporoides* by having the colliculose hymenial surface, wider moniliform cystidia (35–70 µm × 7–8 µm) and basidiospores (9.5–14 µm × 4–5 µm) [27]; *H. sinense* differs from *H. membranaceum* by having the encrusted cystidia and smaller basidiospores (8–11.5 µm × 3–5 µm) [25], and another species *H. transiens* differs in its odontoid hymenial surface and narrower basidiospores (9–13 µm × 3–4.5 µm) [39].
Morphologically, *Hyphoderma ayresii* (Berk. ex Cooke) Boidin and Gilles, *H. cremeum* Sheng H. Wu and *H. rimulosum* Sheng H. Wu are similar to *H. crystallinum* by having encrusted cystidia. However, *H. ayresii* differs in its larger encrusted cystidia (70–130 μm × 13–20 μm) and wider basidiospores (9.5–12.5 μm × 6–8 μm) [38]; *H. cremeum* differs from *H. crystallinum* by having both larger encrusted cystidia (40–90 μm × 10–15 μm) and basidia (35–50 μm × 6.5–8 μm) [40]; *H. rimulosum* is separated from *H. crystallinum* by smaller basidiospores (6–7 μm × 3.9–4.1 μm) [41]. *Hyphoderma incrustatum* K.H. Larss., *H. medioburiense*, *H. multicystidium* (Hjortstam and Ryvarden) Hjortstam and Telléria and *H. rosocremum* (Bres.) Donk are similar to *H. crystallinum* by having tubular cystidia. However, *H. incrustatum* differs from *H. crystallinum* by the porulose hymenial surface and the larger tubular cystidia (50–80 μm × 6–10 μm) [42]; *H. medioburiense* is separated from *H. crystallinum* by the porulose hymenial surface and the larger tubular cystidia (60–100 μm × 7–10 μm) [8]; *H. multicystidium* differs in its larger tubular cystidia (60–80 μm × 5–7 μm), larger basidia (35–50 μm × 5–7 μm) and smaller basidiospores (8–10 μm × 4.5–5 μm) [43]; *H. rosocremum* differs from *H. crystallinum* by having larger tubular cystidia (80–100 μm × 6–9 μm) and smaller basidiospores (8–12 μm × 3–4 μm) [8].

*Hyphoderma litschaueri*, *H. moniliforme* (P.H.B. Talbot) Manjón, G. Moreno and Hjortstam, *H. para macaronesicum* Telléria et al., *H. prospidis* (Burds.) Telléria et al. and *H. sinense* are similar to *H. membranaceum* by having moniliform or apically moniliform cystidia. However, *H. litschaueri* differs from *H. membranaceum* by having larger moniliform cystidia (60–100 μm × 6–8 μm) and narrower basidiospores (9–12 μm × 3–4 μm) [44]; *H. moniliforme* differs from *H. membranaceum* by having smaller basidiospores (8–9 μm × 3.5–4 μm) [27]; *H. para macaronesicum* differs in its having both larger moniliform cystidia (70–124 μm × 8–13 μm) and basidia (40–48 μm × 6–9 μm), and wider basidiospores (12–15 μm × 5.5–7 μm) [14]; *H. prospidis* differs from *H. membranaceum* by the arachnoid to farinaceous hymenial surface and larger basidia (40–45 μm × 8–11 μm) [17]; and *H. sinense* differs in its having encrusted cystidia (18.5–38 μm × 6–11 μm) and smaller basidiospores (8–11.5 μm × 3–5 μm) [25].

*Hyphoderma clavatum* Sheng H. Wu, *H. etriuiae* Bernichia, *H. incrustatum*, *H. orphanellum* (Bourdot & Galzin) Donk, and *H. subclavatum* Sheng H. Wu are similar to *H. microporoides* by having capitulate cystidia. However, *H. clavatum* differs from *H. microporoides* by the tuber culate hymenial surface and larger basidiospores (10–13 μm × 4.2–5.2 μm) [41]; *H. etriuiae* differs from *H. microporoides* by the grandinioid hymenial surface and wider basidiospores (9–11 μm × 5.5–6.5 μm) [45]; *H. incrustatum* differs in having larger basidiospores (11–14 μm × 4–5 μm) [42]; *H. orphanellum* differs from *H. microporoides* by having larger capitulate cystidia (50–80 μm × 8–10 μm) and wider basidiospores (8–10 μm × 5–6 μm) [8]; *H. subclavatum* is separated from *H. microporoides* by having both larger basidia (40–55 μm × 7–8 μm) and basidiospores (10–12 μm × 4.2–5.3 μm) [41].

*Hyphoderma* species are an extensively studied group [10,46], mainly distributed in Europe (e.g., Austria, Russia, France, Germany, Poland, UK, The Netherlands, Portugal, Sweden, Italy, Denmark, Norway, Finland, Spain) (Figure 8) and mainly found on hardwood, although a few species grow on coniferous wood. Many species of *Hyphoderma* were found in Europe, but most of them have not been reported in northern China (Figure 8), in which we presumed that *Hyphoderma* are undersampled by mycologists. Several studies on new wood-decaying fungi of *Hyphoderma* from China have been reported [15,40,41,46], in which 26 *Hyphoderma* species were reported, *H. acystidiatum* Sheng H. Wu, *H. clavatum*, *H. cremeoealbum* (Höhn. and Litsch.) Jülich, *H. cremeum*, *H. definitum* (H.S. Jacks.) Donk, *H. densum* Sheng H. Wu, *H. fissuratum*, *H. floccosum* C.L. Zhao and Q.X. Guan, *H. litschaueri*, *H. crystallinum*, *H. medioburiense*, *H. micr cystidium* Sheng H. Wu, *H. microporoides*, *H. moniliforme*, *H. mopan Shanense*, *H. nemorale*, *H. obtusiforme* J. Erikss. and A. Strid, *H. pinicola*, *H. rimulosum*, *H. setigerum*, *H. sibiricum* (Parmasto) J. Erikss. and A. Strid, *H. sinense*, *H. sub clavatum*, *H. subsetigerum* Sheng H. Wu, *H. transiens*, and *H. membranaceum* [8,18,25,27,29,40,41,46]. Further studies should focus on the relationships between the host and *Hyphoderma* species, as well as trying to better understand the evolu-
tionary directions between plant and *Hyphoderma* species. The researches on the phylogeny of *Hyphoderma*, as well as many fungal studies on the molecular systematics [47–49], will be useful to push the further research on fundamental research and applied research of fungi. More species of *Hyphoderma* should be found in subtropical and tropical Asia as it was shown that wood-inhabiting fungi are rich in tropical China [50,51].

![Figure 8. Geographic distribution of *Hyphoderma* species treated in the phylogenetic tree.](image)

**Key to 26 accepted species of *Hyphoderma* in China**

1. Cystidia absent 2
2. Cystidia present 5
3. Hymenial surface grandinioid *H. acystidiatum*
4. Hymenial surface smooth 3
5. Basidiospores > 10.5 µm in length *H. densum*
6. Basidiospores < 10.5 µm in length 4
7. Hymenophore cracked; basidiospores > 8.5 µm in length *H. fissuratum*
8. Hymenophore uncracked; basidiospores < 8.5 µm in length *H. sibiricum*
9. Hymenophore smooth 6
10. Hymenophore tuberculate, porulose, grandinioid, or odontoid 14
11. Two types of cystidia present 7
12. One type of cystidia present 8
13. Moniliform cystidia absent *H. microcystidium*
14. Moniliform cystidia present *H. sinense*
15. Hymenophore uncracked 9
16. Hymenophore cracked 10
17. Basidiospores > 11 µm in length *H. definitum*
18. Basidiospores < 11 µm in length *H. microporoides*
19. Cystidia moniliform 11
20. Cystidia cylindrical 12
21. Basidiospores > 9 µm in length *H. litschaueri*
22. Basidiospores < 9 µm in length *H. moniliforme*
23. Basidiospores ellipsoid < 10 µm in length *H. rimulosum*
24. Basidiospores cylindrical > 10 µm in length 13
25. Basidiospores > 12 µm in length *H. cremeum*
26. Basidiospores < 12 μm in length *H. subclavatum*
27. Hymenophore odontoid or grandinioid 15
28. Hymenophore tuberculate, porulose 16
29. Hymenophore odontoid, basidiospores > 9 μm in length *H. transiens*
30. Hymenophore grandinioid, basidiospores < 9 μm in length *H. subsetigerum*
31. Cystidia of two types 17
32. Cystidia of one type 19
33. Septate cystidia absent
34. Septate cystidia present 20
35. Basidia 2-sterigmata, basidiospores > 13 μm in length *H. pinicola*
36. Basidia 4-sterigmata, basidiospores < 13 μm in length *H. floccosum*
37. Septate cystidia present 20
38. Septate cystidia absent 21
39. Hymenophore porulose to pilose, basidia < 5 μm in width *H. mopanshanense*
40. Hymenophore tuberculate, basidia > 5 μm in width *H. setigerum*
41. Hymenophore porulose *H. obtusiforme*
42. Hymenophore tuberculate, colliculose 22
43. Cystidia > 30 μm in length 23
44. Cystidia < 30 μm in length 25
45. Basidia > 30 μm in length 24
46. Basidia < 30 μm in length 25
47. Hymenophore cracking, cystidia < 10 μm in width *H. medioburiense*
48. Hymenophore not cracking, cystidia > 10 μm in width *H. clavatum*
49. Hymenophore colliculose *H. nemorale*
50. Hymenophore tuberculate *H. membranaceum*

**Author Contributions:** Conceptualization, C.-L.Z.; methodology, C.-L.Z. and Q.-X.G.; software, C.-L.Z. and Q.-X.G.; validation, C.-L.Z. and Q.-X.G.; formal analysis, C.-L.Z. and Q.-X.G.; investigation, C.-L.Z. and Q.-X.G.; resources, C.-L.Z.; writing—original draft preparation, C.-L.Z. and Q.-X.G.; writing—review and editing, C.-L.Z. and Q.-X.G.; visualization, C.-L.Z. and Q.-X.G.; supervision, C.-L.Z.; project administration, C.-L.Z.; funding acquisition, C.-L.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Yunnan Fundamental Research Project, grant number 202001AS070043.

**Institutional Review Board Statement:** Not applicable for studies involving humans or animals.

**Informed Consent Statement:** Not applicable for studies involving humans.

**Data Availability Statement:** Publicly available datasets were analyzed in this study. This data can be found here: [https://www.ncbi.nlm.nih.gov/; https://www.mycobank.org/page/Simple%20names%20search; http://purl.org/phylo/treebase, submission ID 27983; accessed on 16 April 2021].

**Conflicts of Interest:** The authors declare no conflict of interest.

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