

Volume 13 · Issue 10 | October 2021



mdpi.com/journal/diversity ISSN 1424-2818

# Diversity, Volume 13, Issue 10 (October 2021) - 51 articles

Open Access Article



# Overlooked Species Diversity and Distribution in the Antarctic Mite Genus Stereotydeus

by 🚇 Claudia Brunetti, 🚇 Henk Siepel, 🚇 Peter Convey, 🚇 Pietro Paolo Fanciulli, 👰 Francesco Nardi and Antonio Carapelli

Diversity 2021, 13(10), 506; https://doi.org/10.3390/d13100506 - 19 Oct 2021

Viewed by 154

Abstract In the harsh Antarctic terrestrial ecosystems, invertebrates are currently confined to sparse and restricted ice free areas, where they have survived on multi-million-year timescales in refugia. The limited dispersal abilities of these invertebrate species, their specific habitat requirements, and the presence of geographical [...] Read more.

(This article belongs to the Special Issue 2021 Feature Papers by Diversity's Editorial Board Members)

▶ Show Figures

Open Access Article



# The Trade-Offs and Synergistic Relationships between Grassland Ecosystem Functions in the Yellow River Basin

by O Jie Yang, Baopeng Xie and Degang Zhang

Diversity 2021, 13(10), 505; https://doi.org/10.3390/d13100505 (registering DOI) - 19 Oct 2021

Viewed by 144

Abstract Grassland is the most important land use/cover type in the Yellow River basin. Studying its ecosystem services and the trade-off and synergistic relationships between its various functions is of great significance to high-quality development and the protection of the ecological environment in the [...] Read more.

▶ Show Figures

Open Access Article



## Is a High Abundance of Spring Diatoms in the Photic Zone of Lake Baikal in July 2019 Due to an Upwelling Event?

by 🚇 Mikhail Grachev, 🚇 Yurij Bukin, 🚇 Vadim Blinov, 🚇 Oleg Khlystov, 🚇 Alena Firsova, 📵 Maria Bashenkhaeva, 🜔 Oxana Kamshilo, 🜔 Lubov Titova, 🜔 Elvira Bairamova, 🜔 Yekaterina Bedoshvili, 问 Maria Sakirko and Yulia Zakharova

Diversity 2021, 13(10), 504; https://doi.org/10.3390/d13100504 - 19 Oct 2021

Viewed by 154

Abstract A high abundance of planktonic microalgae is typically thought to be related to their 'bloom', that is, to active population growth. Diatom blooms in the photic zone of Lake Baikal generally occur during hydrological spring (April-June); when the summer arrives and the surface [...] Read more.

(This article belongs to the Special Issue Diatom Diversity in the Lakes)

Show Figures

Open Access Article



### The Hidden Wood-Decaying Fungal Diversity: Rhizochaete from East Asia

by Chang-Lin Zhao

Diversity 2021, 13(10), 503; https://doi.org/10.3390/d13100503 - 17 Oct 2021

Viewed by 180

Abstract Wood-decaying fungi play crucial roles as decomposers in forest ecosystems. In this study, two new corticioid fungi, Rhizochaete fissurata and R. grandinosa spp. nov., are proposed based on a combination of morphological features and molecular evidence. Rhizochaete fissurata is characterized by resupinate basidiomata [...] Read more.

(This article belongs to the Special Issue The Hidden Fungal Diversity in Asia)

▶ Show Figures





Article

# The Hidden Wood-Decaying Fungal Diversity: Rhizochaete from East Asia

Zi-Rui Gu 1,2 and Chang-Lin Zhao 1,2,3,4,\*

- Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, China; fungiziruig@126.com
- College of Biodiversity Conservation, Southwest Forestry University, Kunming 650224, China
- School of Life Sciences, Tsinghua University, Beijing 100084, China
- <sup>4</sup> Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming 650201, China
- \* Correspondence: fungi@swfu.edu.cn

Abstract: Wood-decaying fungi play crucial roles as decomposers in forest ecosystems. In this study, two new corticioid fungi, *Rhizochaete fissurata* and *R. grandinosa* spp. nov., are proposed based on a combination of morphological features and molecular evidence. *Rhizochaete fissurata* is characterized by resupinate basidiomata with a cracking hymenial surface, a monomitic hyphal system with simple-septa generative hyphae, presence of subfusiform to conical cystidia encrusted at the apex or coarse on the upper half, and ellipsoid basidiospores. *Rhizochaete grandinosa* differs in its resupinate basidiomata with a smooth hymenial surface, presence of two types of cystidia, and ellipsoid basidiospores. Sequences of ITS and nLSU rRNA markers of the studied samples were employed, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony, and Bayesian inference methods on two datasets (ITS+nLSU and ITS). Both dataset analyses showed that two new species clustered into the genus *Rhizochaete*, in which, based on the ITS+nLSU dataset, *R. fissurata* was sister to *R. belizensis*, and *R. grandinosa* grouped with *R. radicata*; the phylogram inferred from ITS sequences inside *Rhizochaete* indicated that *R. fissurata* formed a monophyletic lineage with a lower support; *R. grandinosa* grouped closely with *R. radicata*. In addition, an identification key to all *Rhizochaete* species worldwide is provided.

Keywords: China; corticioid fungi; diversity; Phanerochaetaceae; molecular systematics; taxonomy



Citation: Gu, Z.-R.; Zhao, C.-L. The Hidden Wood-Decaying Fungal Diversity: *Rhizochaete* from East Asia. *Diversity* **2021**, *13*, 503. https://doi.org/10.3390/d13100503

Academic Editor: Tine Grebenc

Received: 17 September 2021 Accepted: 11 October 2021 Published: 17 October 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

#### 1. Introduction

Fungi make up an under-described, poorly documented clade of eukaryotes, in which they have immense ecological and economic impacts; many fungi are microscopic or have cryptic life cycles, which makes detection difficult [1]. Based on the ratio of vascular plants and fungi in different regions, Hawksworth [2] conservatively estimated that there were 1,500,000 fungal species worldwide, with about 69,000 species known at that time, and later, Blackwell [3] indicated that fungal species numbers were estimated to be as high as 5,100,000, with 97,861 known species. However, Hawksworth [4] proposed that the number of existing fungal species should be between 1,500,000 and 3,000,000, which is currently accepted by many mycologists [5,6]. Wood-decaying fungi are eukaryotic microorganisms that play fundamental ecological roles as decomposers of plants in the fungal tree of life [7], which drive carbon cycling in forest soils, mediate mineral nutrition of plants, and alleviate carbon limitations of other soil organisms [5].

Rhizochaete Gresl., Nakasone & Rajchenb. is a small, distinctive genus of wood-decaying fungi that produces hyphal cords and has a world-wide distribution. It was typified by *R. brunnea* Gresl., Nakasone & Rajchenb., and the genus is characterized by resupinate to effused, loosely adnate basidiomata of pellicular to membranous, fragile consistency, with smooth to tuberculate hymenophore covering a yellow, orange, brown, olivaceous, or violaceous hymenial surface, usually turning red to violet in KOH solution;

Diversity **2021**, 13, 503

fimbriate to fibrillose margin, often with hyphal cords; monomitic hyphal system with simple septae or clamp connections on generative hyphae; usually present cystidia; clavate to subcylindrical basidia, 4-sterigmate; cylindrical to ellipsoid basidiospores, which are thin to slightly thick-walled, smooth, acyanophilous, not reacting to Melzer's reagent; occurring on wood and bark of angiosperms and gymnosperms, associated with a white rotdecay [8]. Currently, about 14 species have been accepted in *Rhizochaete* worldwide [8–10]. Index Fungorum (http://www.indexfungorum.org; accessed on 25 August 2021) and MycoBank (https://www.mycobank.org; accessed on 25 August 2021) register 14 specific and infraspecific names in *Rhizochaete*.

Rhizochaete was distinguished from Phanerochaete P. Karst. by morphological and molecular characters [9], in which six species were separated from Phanerochaete and transferred to Rhizochaete. Phylogenetic studies indicated Rhizochaete in the Phanerochaete clade [11] and the Phanerochaetaceae Jülich [6]. Based on studying the parenthesome structure of some corticioid fungi, Bianchinotti et al. [12] reported that three Rhizochaete species had perforate septal dolipore caps or parenthesomes. Phylogenetic reconstruction of corticioid fungi using ITS and nLSU regions revealed that three species should be transferred to the genus Rhizochaete—R. sulphurosa (Bres.) Chikowski, K.H. Larss. & Gibertoni, R. sulphurina (P. Karst.) K.H. Larss., and R. violascens (Fr.) K.H. Larss.—and three new combinations were made (Include reference). Floudas and Hibbett [11] revealed that Rhizochaete was monophyletic in multigene phylogenetic analyses of the Phanerochaete clade and was represented by four species. Chikowski et al. [10] resolved Rhizochaete as monophyletic in the phylogenetic analyses of ITS sequence data, which included six Rhizochaete species. On the basis of the combined ITS and nLSU analyses by Miettinen et al. [6], seven Rhizochaete species were included in a nine-way polytomy in the Phlebiopsis clade, in which Rhizochaete was resolved as a distinct subclade within the Phlebiopsis clade. Morphological studies and molecular sequence data from two nuclear ribosomal DNA regions (ITS and LSU) supported the recognition of Rhizochaete, in which R. belizensis was closely related to R. radicata, and three new combinations were proposed. An in-depth study of the phylogeny and taxonomy of the corticioid genus Phlebiopsis (Phanerochaetaceae) was conducted, in which Rhizochaete clustered as a sister clade to Phaeophlebiopsis and Hapalopilus, and ten species of Rhizochaete grouped together [13].

In this study, two undescribed species of wood-decaying fungi from forest ecosystems were collected in Yunnan Province, China. We present morphological and molecular phylogenetic evidence that supports the recognition of two new species in *Rhizochaete* based on the internal transcribed spacer ITS and nLSU sequences.

#### 2. Materials and Methods

#### 2.1. Sample Collection and Herbarium Specimen Preparation

Fresh fruiting bodies of the fungi growing on angiosperm stumps and trunks were collected from Dali, Puer, Wenshan, and Yuxi of Yunnan Province, China. The samples were photographed in situ, and their fresh macroscopic details were recorded. Photographs recording the bioluminescence in complete darkness were taken with a Jianeng 80D camera. All photos were focus stacked and merged using Helicon Focus software. Macroscopic details were recorded in situ. Samples were transported to a field station where the fruit bodies were dried on an electronic food dryer at 35 °C. The dried specimens were deposited in the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

#### 2.2. Morphology

Macromorphological descriptions are based on field notes and photos captured in the field and lab. Color terminology follow Petersen [14]. Micromorphological data were obtained from the dried specimens observed under a light microscope following Dai [15]. The following abbreviations were used: KOH = 5% potassium hydroxide water solution, CB = Cotton Blue, CB- = acyanophilous, IKI = Melzer's reagent, IKI- = both inamyloid

Diversity **2021**, 13, 503 3 of 12

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, and n = a/b (number of spores (a) measured from given number (b) of specimens).

#### 2.3. Molecular Phylogeny

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from the dried specimens using the manufacturer's instructions (as done in [16]). The nuclear ribosomal ITS region was amplified with primers ITS5 and ITS4 [17]. The nuclear ribosomal LSU gene was amplified with primers LR0R and LR7 [18,19]. 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 sequenced at Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, China. All newly generated sequences were deposited in NCBI GenBank (Table 1).

Sequences were aligned in MAFFT 7 (https://mafft.cbrc.jp/alignment/server/, accessed on 5 October 2021) using G-INS-i strategy for ITS+nLSU and ITS datasets, and they were manually adjusted in BioEdit [26]. The datasets were deposited in TreeBASE WEB (submission ID 28787). *Byssomerulius corium* (Pers.) Parmasto was selected as an outgroup for the phylogenetic analysis of ITS+nLSU (Figure 1), referred to following [8], and *Phaeophlebiopsis caribbeana* Floudas & Hibbett was selected as an outgroup taxon in ITS phylogenetic analysis following a previous study [11].

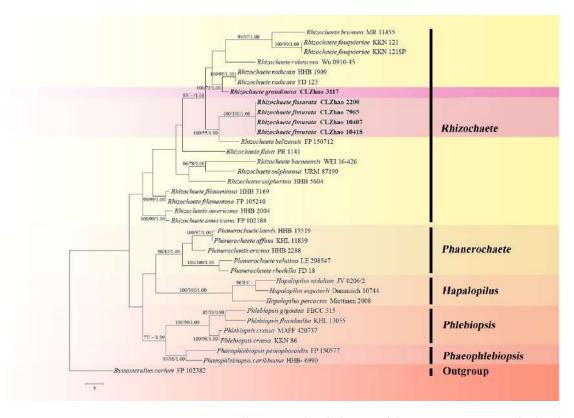


Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of the two new species and related species within the family Phanerochaetaceae based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50%, and Bayesian posterior probabilities > 0.95, respectively.

Diversity 2021, 13, 503 4 of 12

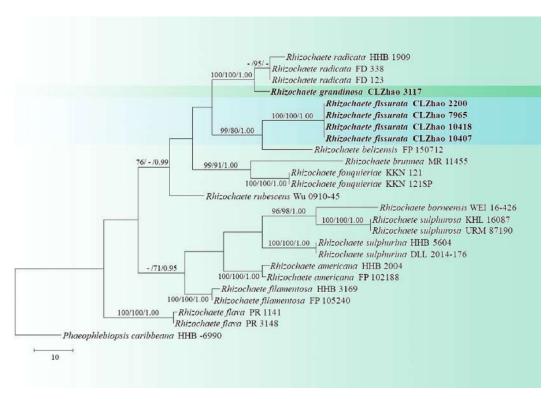
Table 1. List of species, specimens, and GenBank accession numbers of sequences used in this study.

Species Name	Specimen No. —	GenBank Accession No.			
		ITS	nLSU	References	Country
Byssomerulius corium	FP 102382	KP135007	KP135230	[11]	USA, Wisconsin
Hapalopilus eupatorii	Dammrich 10744	KX752620	KX752620	[6]	Germany
H. nidulans	JV 0206/2	KX752623	KX752623	[6]	Sweden
H. percoctus	Miettinen 2008	KX752597	KX752597	[6]	Botswana
Phanerochaete affinis	KHL 11839	EU118652	EU118652	[20]	Sweden
P. ericina	HHB 2288	KP135167	KP135247	[11]	USA, North Carolina
P. laevis	HHB 15519	KP135149	KP135249	[11]	USA, Alabama
P. rhodella	FD-18	KP135187	KP135258	[11]	USA, Massachusetts
P. velutina	LE 298547	KP994360	KP994385	[21]	Russia
Phaeophlebiopsis caribbeana	HHB-6990	KP135415	KP135243	[11]	USA, Florida
P. peniophoroides	FP 150577	KP135417	KP135273	[11]	USA, Hawaii
Phlebiopsis crassa	KKN 86	KP135394	KP135215	[11]	USA, Arizona
P. crassa	MAFF 420737	AB809163	AB809163	[22]	Japan
P. flavidoalba	KHL 13055	EU118662	EU118662	[20]	Costa Rica
P. gigantea	FBCC 315	LN611131	LN611131	[23]	Sweden
Rhizochaete americana	FP-102188	KP135409	KP135277	[11]	USA, Illinois
R. americana	HHB 2004	AY219391	AY219391	[9]	USA, Georgia
R. belizensis	FP 150712	KP135408	KP135280	[11]	Belize
R. borneensis	WEI 16-426	MZ637070	MZ637270	Unpublished	China
R. brunnea	MR 11455	AY219389	AY219389	[9]	Argentina
R. filamentosa	FP 105240	KP135411	AY219393	[8]	USA, Indiana
R. filamentosa	HHB 3169	KP135410	KP135278	[11]	USA, Maryland
R. fissurata	CLZhao 2200	MZ713640	MZ713844	Present study	China
R. fissurata	CLZhao 7965	MZ713641	MZ713845	Present study	China
R. fissurata	CLZhao 10407	MZ713642	MZ713846	Present study	China
R. fissurata	CLZhao 10418	MZ713643	MZ713847	Present study	China
Ř. flava	PR 1141	KY273030	KY273033	[8]	Puerto Rico
R. flava	PR 3148	KY273029	-	[8]	Puerto Rico
R. fouquieriae	KKN 121	AY219390	GU187608	[8]	USA, Arizona
R. fouquieriae	KKN-121-sp	KY948786	KY948858	[24]	United States
R. grandinosa	CLZhao 3117	MZ713644	MZ713848	Present study	China
R. radicata	FD 123	KP135407	KP135279	[11]	USA, Massachusetts
R. radicata	FD 338	KP135406	-	[11]	USA, Massachusetts
R. radicata	HHB 1909	AY219392	AY219392	[9]	USA, North Carolina
R. rubescens	Wu 0910-45	LC387335	MF110294	[25]	China
R. sulphurina	DLL 2014-176	KY273032	=	[8]	USA, Idaho
R. sulphurina	HHB 5604	KY273031	GU187610	[8]	USA, Montana
R. sulphurosa	KHL 16087	KT003523		[10]	Brazil
R. sulphurosa	URM 87190	KT003522	KT003519	[10]	Brazil

Maximum parsimony analysis was applied to the combined ITS+nLSU and ITS datasets. The approach followed the previous study by Zhao and Wu [16], and the tree construction procedure was performed in PAUP\* version 4.0a169 (http://phylosolutions.com/paup-test/, accessed on 5 October 2021). 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 [27]. 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. The combined dataset was also analyzed using maximum likelihood (ML) in RAxML-HPC2 through the Cipres Science Gateway [28]. Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

Diversity **2021**, 13, 503 5 of 12

MrModeltest 2.3 [29] was used to determine the best-fit evolution model for each dataset (ITS+nLSU and ITS) for Bayesian inference (BI). BI was calculated with MrBayes version 3.2.7a [30]. Four Markov chains were run for 2 runs from random starting trees for 250 thousand generations for ITS+nLSU (Figure 1) and 200 thousand generations for ITS (Figure 2). 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 2.** Maximum parsimony strict consensus tree illustrating the phylogeny of the two new species and related species in *Rhizochaete* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50%, and Bayesian posterior probabilities > 0.95, respectively.

#### 3. Results

#### 3.1. Molecular Phylogeny

The ITS+nLSU dataset (Figure 1) included sequences from 35 fungal specimens representing 27 taxa. The dataset had an aligned length of 738 characters, of which 388 characters are constant, 99 are variable and parsimony-uninformative, and 251 are parsimony-informative. Maximum parsimony analysis yielded 61 equally parsimonious trees (TL = 412, CI = 0.4733, HI = 0.5267, RI = 0.6146, and RC = 0.2909). The best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis with an average standard deviation of split frequencies = 0.009664 (BI). The phylogram inferred from ITS+nLSU sequences within family Phanerochaetaceae highlighted two undescribed species nested in genus *Rhizochaete*, in which *R. fissurata* was sister to *R. belizensis* Nakasone, K. Draeger & B. Ortiz with a medium supported lineage (99% BS, 79% BP and 1.00 BPP); *R. grandinosa* grouped with *R. radicata* (Henn.) Gresl., Nakasone & Rajchenb. (100% BS, 99% BP and 1.00 BPP).

The ITS-alone dataset (Figure 2) included sequences from 25 fungal specimens representing 13 taxa. The dataset had an aligned length of 728 characters, of which 459 charac-

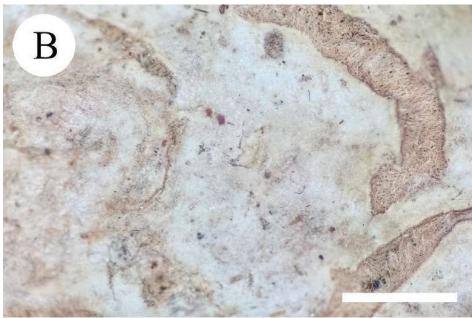
Diversity 2021, 13, 503 6 of 12

ters are constant, 62 are variable and parsimony-uninformative, and 207 are parsimony-informative. Maximum parsimony analysis yielded 18 equally parsimonious trees (TL = 334, CI = 0.5749, HI = 0.4251, RI = 0.7380, and RC = 0.4242). The best model for the ITS dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis with an average standard deviation of split frequencies = 0.008927 (BI). The phylogram inferred from ITS sequences within genus *Rhizochaete* revealed that *R. fissurata* formed a monophyletic lineage with a lower support; *R. grandinosa* grouped closely with *R. radicata*.

#### 3.2. Taxonomy

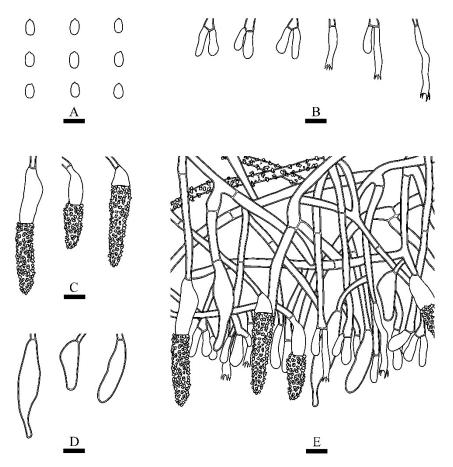
Rhizochaete fissurata C.L. Zhao sp. nov. Figures 3 and 4.





**Figure 3.** Basidiomata of *Rhizochaete fissurata* (holotype). Bars: (A) = 1 cm and (B) = 1 mm.

Diversity 2021, 13, 503 7 of 12



**Figure 4.** Microscopic structures of *Rhizochaete fissurata* (holotype): (**A**) basidiospores; (**B**) basidia and basidioles; (**C**,**D**) cystidia; (**E**) a section of hymenium. Bars: (**A**) =  $5 \mu m$ , (**B**–**E**) =  $10 \mu m$ .

MycoBank no.: 841215

Holotype—China. Yunnan Province, Dali, Nanjian County, Lingbaoshan National Forestry Park, GPS co-ordinates 24°44′N, 100°29′E, altitude 2300 m asl., on an angiosperm trunk, leg. C.L. Zhao, 10 January 2019, CLZhao 10,407 (SWFC).

Etymology—*fissurata* (Lat.): referring to the cracking hymenial surface.

Fruiting body—Basidiomata annual, resupinate, loosely adnate, soft, membranous to pellicular, without odor and taste when fresh, up to 10 cm long, 3 cm wide, 500–900  $\mu m$  thick, violet in KOH. Hymenial surface smooth, obviously cracking, buff to olivaceous buff when fresh, olivaceous buff to pale brown on drying. Margin sterile, thinning out, narrow, cream. Hyphal cords fimbriate.

Hyphal system—Monomitic, generative hyphae having simple septa, colorless, thin-walled, frequently branched, interwoven, 2–7  $\mu m$  in diameter; IKI–, CB–; tissues unchanged in KOH; subhymenial hyphae sometimes densely covered with crystals.

Hymenium—Cystidia numerous, subfusiform to conical, slightly thick-walled, <1  $\mu m$  thick, encrusted at the apex or with coarse upper half, 18–60.5  $\times$  6–11  $\mu m$ ; basidia cylindrical, slightly constricted in the middle to somewhat sinuous, with four sterigmata and simple septa, 11–33  $\times$  3–5  $\mu m$ .

Spores—Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, 3–4.5  $\times$  (2–)2.5–3  $\mu$ m, L = 3.68  $\mu$ m, W = 2.74  $\mu$ m, Q = 1.23–1.51 (n = 120/4).

Additional specimens examined—China. Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, GPS co-ordinates 23°46′ N, 101°16′ E, altitude 2214 m asl., on an angiosperm trunk, leg. C.L. Zhao, 18 August 2017, CLZhao 2200 (SWFC); altitude 2322 m asl., on an angiosperm trunk, leg. C.L. Zhao, 19 August 2018, CLZhao 7965 (SWFC); Dali, Nanjian County, Lingbaoshan National Forestry Park, GPS co-ordinates 24°44′ N,

Diversity 2021, 13, 503 8 of 12

 $100^{\circ}29'$  E, altitude 2300 m asl., on an angiosperm stump, leg. C.L. Zhao, 10 January 2019, CLZhao 10,418 (SWFC).

Habitat and ecology—Climate of the sample collection site is monsoon humid, the forest type is evergreen broad-leaved forest, and samples were collected on an angiosperm trunk.

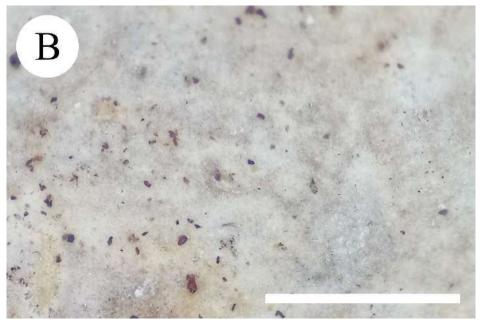
*Rhizochaete grandinosa* C.L. Zhao & Z.R. Gu, sp. nov. Figures 5 and 6.

MycoBank no.: 841216

Holotype—China. Yunnan Province, Puer, Laiyanghe National Forestry Park, GPS co-ordinates 22°36′ N, 101°1′ E, altitude 1500 m asl., on an angiosperm trunk, leg. C.L. Zhao, 30 September 2017, CLZhao 3117 (SWFC).

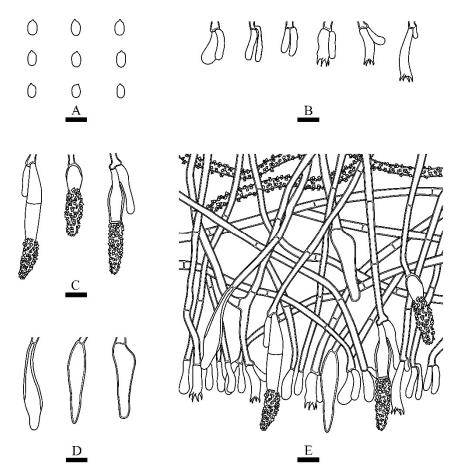
Etymology—grandinosa (Lat.): referring to the grand nose or protrusion of the basidiomata.





**Figure 5.** Basidiomata of *Rhizochaete grandinosa* (holotype). Bars: (A) = 1 cm and (B) = 1 mm.

Diversity 2021, 13, 503 9 of 12



**Figure 6.** Microscopic structures of *Rhizochaete grandinosa* (holotype): (**A**) basidiospores; (**B**) basidia and basidioles; (**C**,**D**) cystidia; (**E**) a section of hymenium. Bars: (**A**) =  $5 \mu m$ , (**B**–**E**) =  $10 \mu m$ .

Fruiting body—Basidiomata annual, resupinate, loosely adnate, soft, membranous when fresh, cottony upon drying, up to 9 cm long, 4.5 cm wide, 300–500  $\mu$ m thick, violet in KOH. Hymenial surface smooth, with grand nose or protrusion, curry-yellow when fresh, curry-yellow to cinnamon-buff upon drying. Margin sterile, slightly brown, 1 mm wide. Hyphal cords fimbriate.

Hyphal system—Monomitic, generative hyphae with simple septa, colorless, thick-walled, rarely branched, interwoven, 3–6 μm in diameter, IKI–, CB–; tissues unchanged in KOH.

Hymenium—Cystidia numerous, colorless, subfusiform to conical with an obtuse apex, simple septa at base, protruding or enclosed, sometimes with secondary septa, thin to slightly thick-walled, <1  $\mu m$  thick, upper half lightly to heavily encrusted with hyaline, insoluble crystals, 24–50  $\times$  4–9  $\mu m$ ; basidia clavate to subcylindrical, constricted, somewhat sinuous, with four sterigmata and simple septa, 14.5–21  $\times$  4.3–5.2  $\mu m$ .

Spores—Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, 3–4(–4.5)  $\times$  2.5–3(–3.3)  $\mu$ m, L = 3.65  $\mu$ m, W = 2.79  $\mu$ m, Q = 1.31 (n = 30/1).

Habitat and ecology—Climate of the sample collection site is a transition between tropical and subtropical climate, the forest type is tropical monsoon evergreen broad-leaved forest, and samples were collected on an angiosperm trunk.

#### 4. Discussion

In the present study, two new species, *Rhizochaete fissurata* and *R. grandinosa*, are described based on phylogenetic analyses and morphological characters.

A revised family-level classification of Polyporales (Basidiomycota) using nrLSU, nrITS, and rpb1 genes across Polyporales showed that the genus *Rhizochaete* nested into

Diversity **2021**, 13, 503

family Phanerochaetaceae, in which *Rhizochaete* was grouped with *Phlebiopsis*, *Phaeophlebiopsis*, and *Hapalopilus* [24]. In the present study, all species of the *Rhizochaete* group together and the genus cluster with a sister clade comprising *Phlebiopsis* and *Phaeophlebiopsis*.

Phylogenetically, the two new taxa were found to group into genus *Rhizochaete* based on the ITS+nLSU dataset, in which *R. fissurata* was sister to *R. belizensis*; *R. grandinosa* was grouped with *R. radicata* (Figure 1). Based on the ITS dataset, *R. fissurata* formed a monophyletic lineage; *R. grandinosa* grouped closely with *R. radicata* (Figure 2). However, morphologically, *R. belizensis* Nakasone, K. Draeger & B. Ortiz differs from *R. fissurata* by having the orange white to violaceous hymenial surface and generative hyphae with rare single clamps [8]; *R. radicata* differs from *R. grandinosa* by its yellowish buff to ochraceous hymenial surface, becoming reddish purple in KOH, and by larger basidiospores  $(4–5 \times 2.5–3 \ \mu m)$  [31].

Morphologically, *Rhizochaete fissurata* is similar to *R. brunnea* and *R. sulphurosa* in the hymenium turning violet in KOH. However, *R. brunnea* differs by its larger cystidia  $(100-250 \times 8-15 \,\mu\text{m})$  [9]; *R. sulphurosa* is separated from *R. fissurata* by having the lemon yellow to mustard to buff hymenial surface and larger basidiospores  $(4.5-5.5 \times 2-3 \,\mu\text{m})$  [10].

Rhizochaete fissurata is similar to R. filamentosa (Berk. & M.A. Curtis) Gresl., Nakasone & Rajchenb. and R. percitrina (P. Roberts & Hjortstam) Nakasone in having the simple-septa generative hyphae. However, R. filamentosa differs from R. fissurata by its orange-gray to brownish orange basidiomata and larger basidiospores (4.5–5.5  $\times$  2–2.5  $\mu$ m) [32]; R. percitrina differs in its smooth to farinaceous hymenial surface with no change in KOH, and narrower cystidia (22–35  $\times$  3.5–6  $\mu$ m) [8].

*Rhizochaete grandinosa* is similar to *R. americana* (Nakasone, C.R. Bergman & Burds.) Gresl., Nakasone & Rajchenb., *R. rhizomorphosulphurea* (B.K. Bakshi & Suj. Singh) Nakasone, and *R. rubescens* (Sheng H. Wu) Sheng H. Wu in having the thick-walled, encrusted cystidia. However, *R. americana* differs from *R. grandinosa* by its greyish brown to yellowish brown hymenial surface and larger basidia ( $22-36 \times 4-5 \mu m$ ) [31]; *R. rhizomorphosulphurea* differs from *R. grandinosa* by having the widely effused basidiomata, with sulfur yellow to light orange hymenial surface and larger basidiospores ( $4-4.5 \times 2.8-3.6 \mu m$ ) [8]; *R. rubescens* differs in its hymenial surface reddening in KOH and generative hyphae rarely clamped [33].

Rhizochaete species are worldwide distributed (e.g., America, Argentina, Belize, Borneo, Brazil, Burundi, Cameroon, Canada, China, Costa Rica, Cuba, Denmark, Finland, Germany, India, Jamaica, Japan, Mexico, New Zealand, Norway, Sweden, Switzerland, Uganda, Vietnam) and are mainly found on angiosperm bark and wood. Wood-decaying taxa were widely collected and studied from China [34–38], in which three Rhizochaete species—R. filamentosa, R. rubescens, and R. sulphurina—were reported [39]. Further studies should focus on the relationships between Rhizochaete species and their hosts as well as trying to better understand the evolutionary directions between hosts and Rhizochaete species.

#### Key to all species of *Rhizochaete* worldwide

1. Generative hypnae regularly clamped	
1. Generative hyphae primarily simple septa	6
2. Cystidia absent	R. violascens
2. Cystidia abundant	
3. Cystidia up to 250 $\mu$ m long with thick walls, basidia > 40 $\mu$ m in length	R. brunnea
3. Cystidia up to 100 $\mu$ m long with thin walls, basidia < 40 $\mu$ m in length	4
4. Basidiospores > 3 μm in width	R. fouquieriae
4. Basidiospores < 3 μm in width	5
5. Basidiomes olive brown to yellowish brown, cystidia $<$ 60 $\mu m$ in length	R. americana
5. Basidiomes bright to dull yellow, cystidia $> 60 \mu m$ in length	R. sulphurina
6. Cystidia with thin or slightly thickened walls, $< 1 \mu m$ thick	7
6. Cystidia with distinctly thick walls, $> 1 \mu m$ thick	14
7. Hymenium turning violet or red in KOH	8
7. Hymenium not reacting or changing to orange or brown in KOH	12

Diversity 2021, 13, 503 11 of 12

8. Hymenium turning red in KOH	9
8. Hymenium turning violet in KOH	10
9. Subiculum brown	R. filamentosa
9. Subiculum colorless	R. rubescens
10. Subiculum yellow	R. sulphurosa
10. Subiculum colorless	11
11. Hymenial surface smooth, cracking	R. fissurata
11. Hymenial surface grandinioid, not cracking	R. grandinosa
12. Basidiomes bright yellow, unchanged in KOH	R. percitrina
12. Basidiomes yellow to brownish orange, darkening in KOH	13
13. Basidia $> 30 \mu m$ in length	rhizomorphosulphurea
13. Basidia < 30 μm in length	R. flava
14. Cystidia < 50 μm in length	R. borneensis
14. Cystidia > 50 μm in length	15
15. Subiculum mustard yellow to brown, cystidia > 60 μm in length,	
basidiospores > 4 μm in length	R. radicata
15. Subiculum yellow, cystidia < 60 μm in length, basidiospores < 4 μm	L
in length	

**Author Contributions:** Conceptualization, C.-L.Z.; methodology, C.-L.Z. and Z.-R.G.; software, C.-L.Z. and Z.-R.G.; validation, C.-L.Z. and Z.-R.G.; formal analysis, C.-L.Z. and Z.-R.G.; investigation, C.-L.Z. and Z.-R.G.; resources, C.-L.Z.; writing—original draft preparation, C.-L.Z. and Z.-R.G.; writing—review and editing, C.-L.Z. and Z.-R.G.; visualization, C.-L.Z. and Z.-R.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:** The research was supported by the Yunnan Fundamental Research Project (Grant No. 202001AS070043) and the High-level Talents Program of Yunnan Province (YNQR-QNRC-2018-111).

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

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

**Data Availability Statement:** Publicly available datasets were analyzed in this study. These 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 28787; (accessed on: 17 September 2021).

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

#### References

- 1. Hibbett, D.; Abarenkov, A.; Kõljalg, U.; Öpik, M.; Chai, B.; Cole, J.; Crous, P.; Robert, V.; Helgason, T.; Herr, J.R.; et al. Sequence-based classification and identification of Fungi. *Mycologia* **2016**, *108*, 1049–1068. [CrossRef]
- 2. Hawksworth, D. The fungal dimension of biodiversity: Magnitude, significance, and conservation. *Mycol. Res.* **1991**, *95*, 641–655. [CrossRef]
- 3. Blackwell, M. The fungi: 1, 2, 3 ... 5.1 million species? Botany 2011, 98, 426–438. [CrossRef] [PubMed]
- 4. Hawksworth, D. Global species numbers of fungi: Are tropical studies and molecular approaches contributing to a more robust estimate? *Biodivers. Conserv.* **2012**, *21*, 2425–2433. [CrossRef]
- 5. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef] [PubMed]
- 6. Miettinen, O.; Spirin, V.; Vlasak, J.; Rivoire, B.; Stenroos, S.; Hibbett, D.S. Polypores and genus concepts in Phanerochaetaceae (Polyporales, Basidiomycota). *Mycokeys* **2016**, *17*, 1–46. [CrossRef]
- 7. James, T.Y.; Stajich, J.E.; Hittinger, C.T.; Rokas, A. Toward a fully resolved fungal tree of life. *Annu. Rev. Microbiol.* **2020**, 74, 291–313. [CrossRef]
- 8. Nakasone, K.K.; Draeger, K.R.; Ortiz-Santana, B. A contribution to the taxonomy of *Rhizochaete* (Polyporales, Basidiomycota). *Cryptogam. Mycol.* **2017**, *38*, 81–99. [CrossRef]
- 9. Greslebin, A.; Nakasone, K.K.; Rajchenberg, M. *Rhizochaete*, a new genus of phanerochaetoid fungi. *Mycologia* **2004**, *96*, 260–271. [CrossRef]
- 10. Chikowski, R.S.; Larsson, K.H.; Gibertoni, T.B. Three new combinations in *Rhizochaete* (Agaricomycetes, Fungi) and a new record to the Brazilian Amazonia. *Nova Hedwig.* **2016**, *102*, 185–196. [CrossRef]

Diversity 2021, 13, 503 12 of 12

11. Floudas, D.; Hibbett, D.S. Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset and extensive ITS sampling. *Fungal Biol.* **2015**, *119*, 679–719. [CrossRef]

- 12. Bianchinotti, M.V.; Rajchenberg, M.; Greslebin, A.G. Parenthesome structure of some corticioid fungi. *Mycol. Res.* **2005**, *109*, 923–926. [CrossRef] [PubMed]
- 13. Zhao, Y.N.; He, S.H.; Nakasone, K.K.; Kumara, K.L.W.; Chen, C.C.; Liu, S.L.; Ma, H.X.; Huang, M.R. Global phylogeny and taxonomy of the wood-decaying fungal genus *Phlebiopsis* (Polyporales, Basidiomycota). *Front. Microbiol.* **2021**, *12*, 622460. [CrossRef] [PubMed]
- 14. Petersen, J.H. Farvekort. The Danish Mycological Society's Colour-Chart; Foreningen til Svampekundskabens Fremme: Greve, Danmark, 1996.
- 15. Dai, Y.C. Polypore diversity in China with an annotated checklist of Chinese polypores. *Mycoscience* **2012**, *53*, 49–80. [CrossRef]
- 16. Zhao, C.L.; Wu, Z.Q. *Ceriporiopsis kunmingensis* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. *Mycol. Prog.* **2017**, *16*, 93–100. [CrossRef]
- 17. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc. A Guide Methods Appl.* **1990**, *18*, 315–322. [CrossRef]
- 18. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Bacteriology* **1990**, 172, 4238–4246. [CrossRef] [PubMed]
- 19. Rehner, S.A.; Samuels, G.J. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol. Res.* **1994**, *98*, 625–634. [CrossRef]
- 20. Larsson, K.H. Re-thinking the classification of corticioid fungi. Mycol. Res. 2007, 111, 1040–1063. [CrossRef]
- 21. Volobuev, S.; Okun, M.; Ordynets, A.; Spirin, V. The *Phanerochaete sordida* group (Polyporales, Basidiomycota) in temperate Eurasia, with a note on *Phanerochaete pallida*. *Mycol. Prog.* **2015**, *14*, 80. [CrossRef]
- 22. Takano, M.; Abe, H.; Hayashi, N. Extracellular peroxidase activity at the hyphal tips of the white-rot fungus *Phanerochaete crassa* WD1694. *Wood Sci.* **2006**, *52*, 429–435. [CrossRef]
- 23. Kuuskeri, J.; Mäkelä, M.R.; Isotalo, J.; Oksanen, L.; Lundell, T. Lignocellulose-converting enzyme activity profiles correlate with molecular systematics and phylogeny grouping in the incoherent genus *Phlebia* (Polyporales, Basidiomycota). *BMC Microbiol*. **2015**, *15*, 217. [CrossRef] [PubMed]
- 24. Justo, A.; Miettinen, O.; Floudas, D.; Ortiz-Santana, B.; Sjokvist, E.; Lindner, D.; Nakasone, K.K.; Niemela, T.; Larsson, K.H.; Ryvarden, L.; et al. A revised family-level classification of the Polyporales (Basidiomycota). *Fungal Biol.* **2017**, 121, 798–824. [CrossRef] [PubMed]
- 25. Chen, C.C.; Wu, S.H.; Chen, C.Y. *Hydnophanerochaete* and *Odontoefibula*, two new genera of phanerochaetoid fungi (Polyporales, Basidiomycota) from East Asia. *MycoKeys* **2018**, *39*, 75–96. [CrossRef]
- Hall, T.A. Bioedit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 1999, 41, 95–98.
- 27. Felsenstein, J. Confidence intervals on phylogenetics: An approach using bootstrap. Evolution 1985, 39, 783–791. [CrossRef]
- 28. Miller, M.A.; Pfeiffer, W.; Schwartz, T. The CIPRES Science Gateway: Enabling High-Impact Science for Phylogenetics Researchers with Limited Resources. *Assoc. Comput. Mach.* **2012**, *39*, 1–8. [CrossRef]
- 29. Nylander, J.A.A. *MrModeltest v2. Program Distributed by the Author*; Evolutionary Biology Centre, Uppsala University: Uppsala, Sweden, 2004.
- 30. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Hohna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. Mrbayes 3.2, Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
- 31. Nakasone, K.K.; Bergman, C.R.; Burdsall Jr, H.H. *Phanerochaete filamentosa-Corticium radicatum* Species Complex in North America. *Sydowia* **1994**, 46, 44–62.
- 32. Burdsall Jr, H.H. Taxonomic and distributional notes on Corticiaceae (Homobasidiomycetes, Aphyllophorales) of the southern appalachians. *Algae Fungi* **1975**, 265–286.
- 33. Wu, S.H. Nine new species of *Phanerochaete* from Taiwan. Mycol. Res. 1998, 102, 1126–1132. [CrossRef]
- 34. Griffin, E.A.; Harrison, J.G.; McCormick, M.K.; Burghardt, K.T.; Parker, J.D. Tree diversity reduces fungal endophyte richness and diversity in a large-scale temperate forest experiment. *Diversity* **2019**, *11*, 234. [CrossRef]
- 35. Girometta, C.E.; Bernicchia, A.; Baiguera, R.M.; Bracco, F.; Buratti, S.; Cartabia, M.; Picco, A.M.; Savino, E. An italian research culture collection of wood decay fungi. *Diversity* **2020**, *12*, 58. [CrossRef]
- 36. Gargano, M.L.; Zervakis, G.I.; Isikhuemhen, O.S.; Venturella, G.; Calvo, R.; Giammanco, A.; Fasciana, T.; Ferraro, V. Ecology, phylogeny, and potential nutritional and medicinal value of a rare white "maitake" collected in a mediterranean Forest. *Diversity* **2020**, *12*, 230. [CrossRef]
- 37. Polemis, E.; Fryssouli, V.; Daskalopoulos, V.; Zervakis, G.I. Basidiomycetes associated with *Alnus glutinosa* habitats in Andros Island (Cyclades, Greece). *Diversity* **2020**, *12*, 232. [CrossRef]
- 38. Ogura-Tsujita, Y.; Tetsuka, K.; Tagane, S.; Kubota, M.; Anan, S.; Yamashita, Y.; Tone, K.; Yukawa, T. Differing life-history strategies of two mycoheterotrophic orchid species associated with leaf litter- and wood-decaying fungi. *Diversity* **2021**, *13*, 161. [CrossRef]
- 39. Dai, Y.C. A revised checklist of corticioid and hydnoid fungi in China for 2010. Mycoscience 2011, 52, 69–79. [CrossRef]