



Article

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

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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.



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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;

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 rot-decay [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 parentheses structure of some corticioid fungi, Bianchinotti et al. [12] reported that three *Rhizochaete* species had perforate septal dolipore caps or parentheses. 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

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].

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

Species Name	Specimen No.	GenBank Accession No.		References	Country
		ITS	nLSU		
<i>Byssomerulius corium</i>	FP 102382	KP135007	KP135230	[11]	USA, Wisconsin
<i>Hapalopilus eupatorii</i>	Dammrich 10744	KX752620	KX752620	[6]	Germany
<i>H. nidulans</i>	JV 0206/2	KX752623	KX752623	[6]	Sweden
<i>H. percoctus</i>	Miettinen 2008	KX752597	KX752597	[6]	Botswana
<i>Phanerochaete affinis</i>	KHL 11839	EU118652	EU118652	[20]	Sweden
<i>P. ericina</i>	HHB 2288	KP135167	KP135247	[11]	USA, North Carolina
<i>P. laevis</i>	HHB 15519	KP135149	KP135249	[11]	USA, Alabama
<i>P. rhodella</i>	FD-18	KP135187	KP135258	[11]	USA, Massachusetts
<i>P. velutina</i>	LE 298547	KP994360	KP994385	[21]	Russia
<i>Phaeophlebiopsis caribbeana</i>	HHB-6990	KP135415	KP135243	[11]	USA, Florida
<i>P. peniophoroides</i>	FP 150577	KP135417	KP135273	[11]	USA, Hawaii
<i>Phlebiopsis crassa</i>	KKN 86	KP135394	KP135215	[11]	USA, Arizona
<i>P. crassa</i>	MAFF 420737	AB809163	AB809163	[22]	Japan
<i>P. flavidoalba</i>	KHL 13055	EU118662	EU118662	[20]	Costa Rica
<i>P. gigantea</i>	FBCC 315	LN611131	LN611131	[23]	Sweden
<i>Rhizochaete americana</i>	FP-102188	KP135409	KP135277	[11]	USA, Illinois
<i>R. americana</i>	HHB 2004	AY219391	AY219391	[9]	USA, Georgia
<i>R. belizensis</i>	FP 150712	KP135408	KP135280	[11]	Belize
<i>R. borneensis</i>	WEI 16-426	MZ637070	MZ637270	Unpublished	China
<i>R. brunnea</i>	MR 11455	AY219389	AY219389	[9]	Argentina
<i>R. filamentosa</i>	FP 105240	KP135411	AY219393	[8]	USA, Indiana
<i>R. filamentosa</i>	HHB 3169	KP135410	KP135278	[11]	USA, Maryland
<i>R. fissurata</i>	CLZhao 2200	MZ713640	MZ713844	Present study	China
<i>R. fissurata</i>	CLZhao 7965	MZ713641	MZ713845	Present study	China
<i>R. fissurata</i>	CLZhao 10407	MZ713642	MZ713846	Present study	China

Table 1. Cont.

Species Name	Specimen No.	GenBank Accession No.		References	Country
		ITS	nLSU		
<i>R. fissurata</i>	CLZhao 10418	MZ713643	MZ713847	Present study	China
<i>R. flava</i>	PR 1141	KY273030	KY273033	[8]	Puerto Rico
<i>R. flava</i>	PR 3148	KY273029	-	[8]	Puerto Rico
<i>R. fouquieriae</i>	KKN 121	AY219390	GU187608	[8]	USA, Arizona
<i>R. fouquieriae</i>	KKN-121-sp	KY948786	KY948858	[24]	United States
<i>R. grandinosa</i>	CLZhao 3117	MZ713644	MZ713848	Present study	China
<i>R. radicata</i>	FD 123	KP135407	KP135279	[11]	USA, Massachusetts
<i>R. radicata</i>	FD 338	KP135406	-	[11]	USA, Massachusetts
<i>R. radicata</i>	HHB 1909	AY219392	AY219392	[9]	USA, North Carolina
<i>R. rubescens</i>	Wu 0910-45	LC387335	MF110294	[25]	China
<i>R. sulphurina</i>	DLL 2014-176	KY273032	-	[8]	USA, Idaho
<i>R. sulphurina</i>	HHB 5604	KY273031	GU187610	[8]	USA, Montana
<i>R. sulphurosa</i>	KHL 16087	KT003523	-	[10]	Brazil
<i>R. sulphurosa</i>	URM 87190	KT003522	KT003519	[10]	Brazil

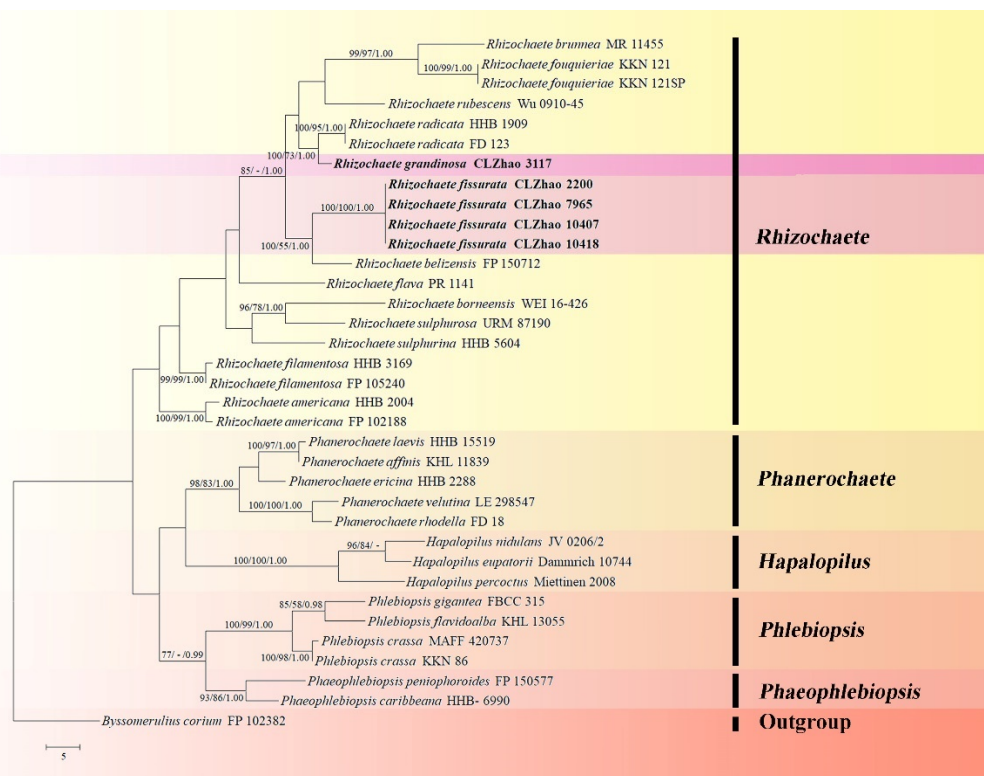


Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of the two new species and related species within the family Phanerochaetaceae based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50%, and Bayesian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

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.

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 (BP) > 70%, or Bayesian posterior probabilities (BPP) > 0.95.

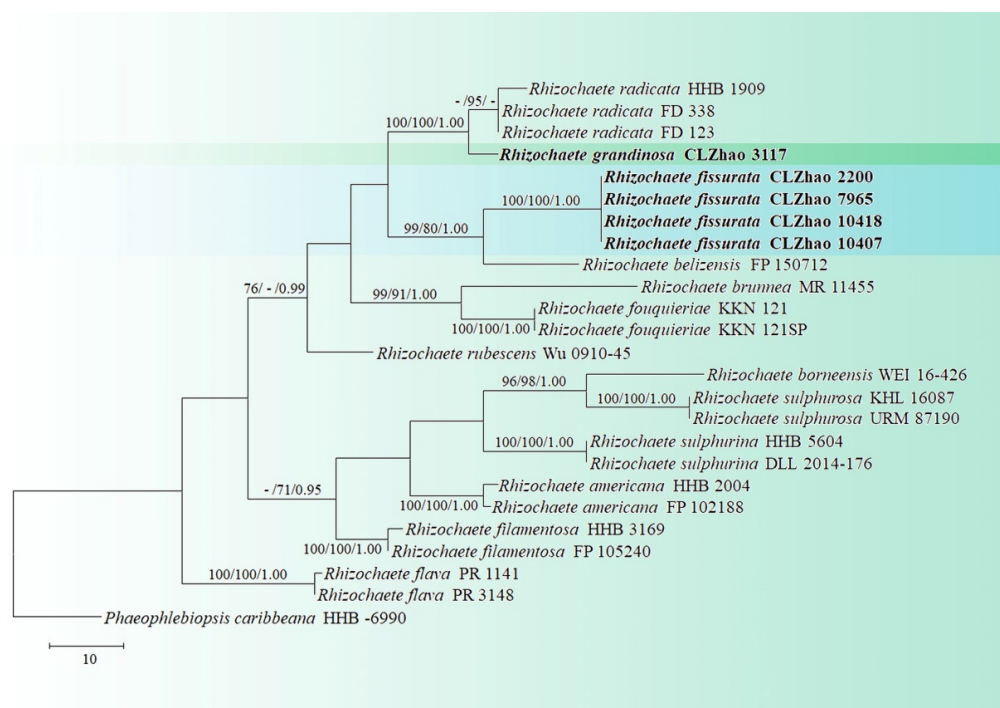


Figure 2. Maximum parsimony strict consensus tree illustrating the phylogeny of the two new species and related species in *Rhizochaete* based on ITS sequences. Branches are labeled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50%, and Bayesian posterior probabilities > 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 characters 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.

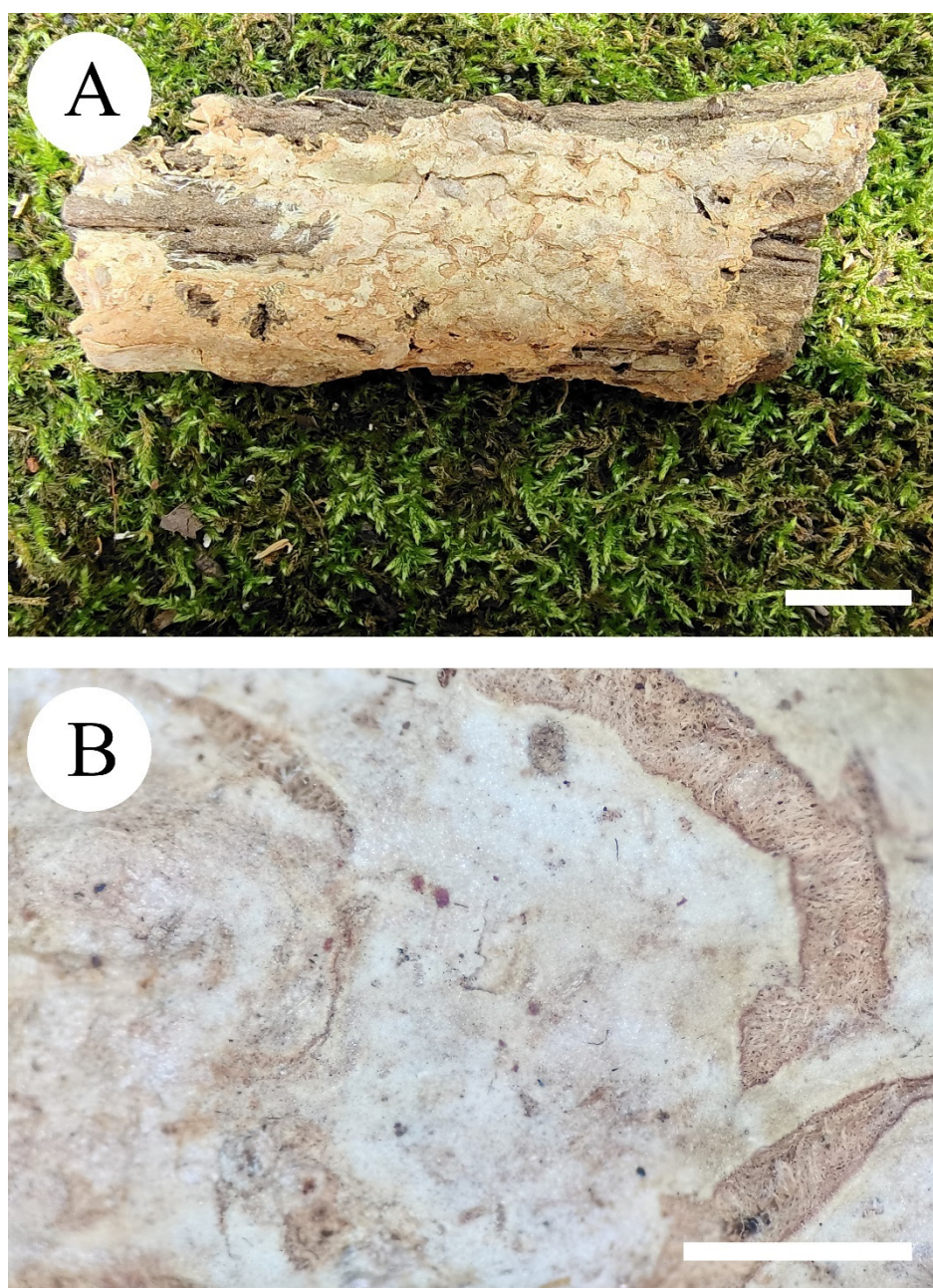


Figure 3. Basidiomata of *Rhizochaete fissurata* (holotype). Bars: (A) = 1 cm and (B) = 1 mm.

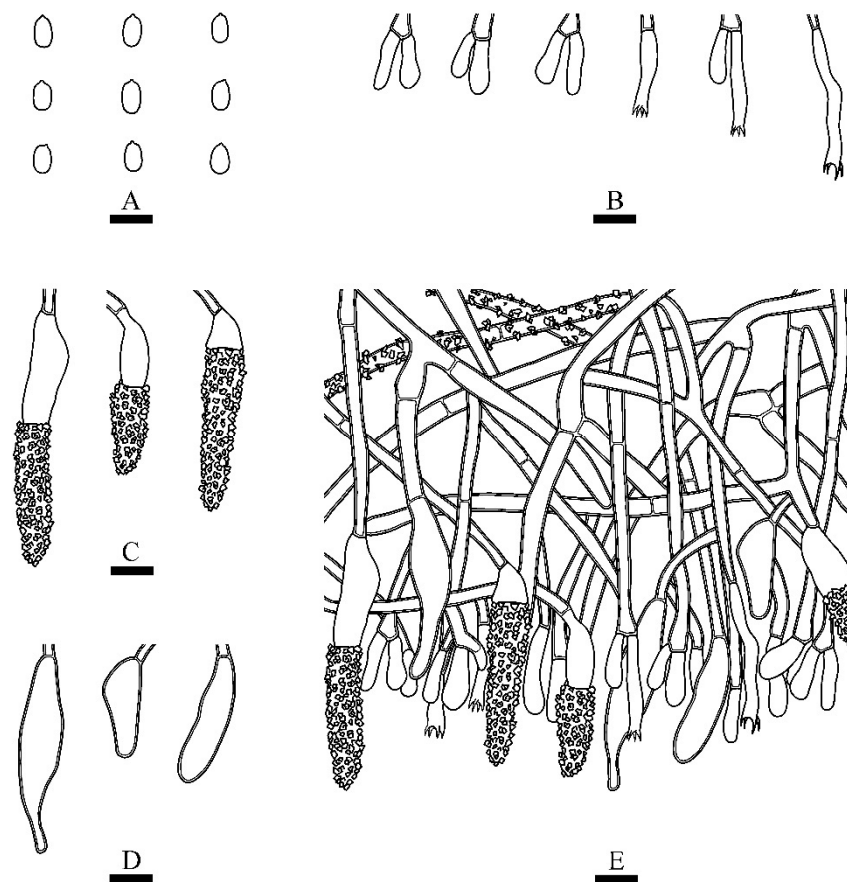


Figure 4. Microscopic structures of *Rhizochaete fissurata* (holotype): (A) basidiospores; (B) basidia and basidioles; (C,D) cystidia; (E) a section of hymenium. Bars: (A) = 5 μm , (B–E) = 10 μm .

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 μm 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 μm 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 μm thick, encrusted at the apex or with coarse upper half, 18–60.5 \times 6–11 μm ; basidia cylindrical, slightly constricted in the middle to somewhat sinuous, with four sterigmata and simple septa, 11–33 \times 3–5 μm .

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

Additional specimens examined—China. Yunnan Province, Yuxi, Xiping 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,

100°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.

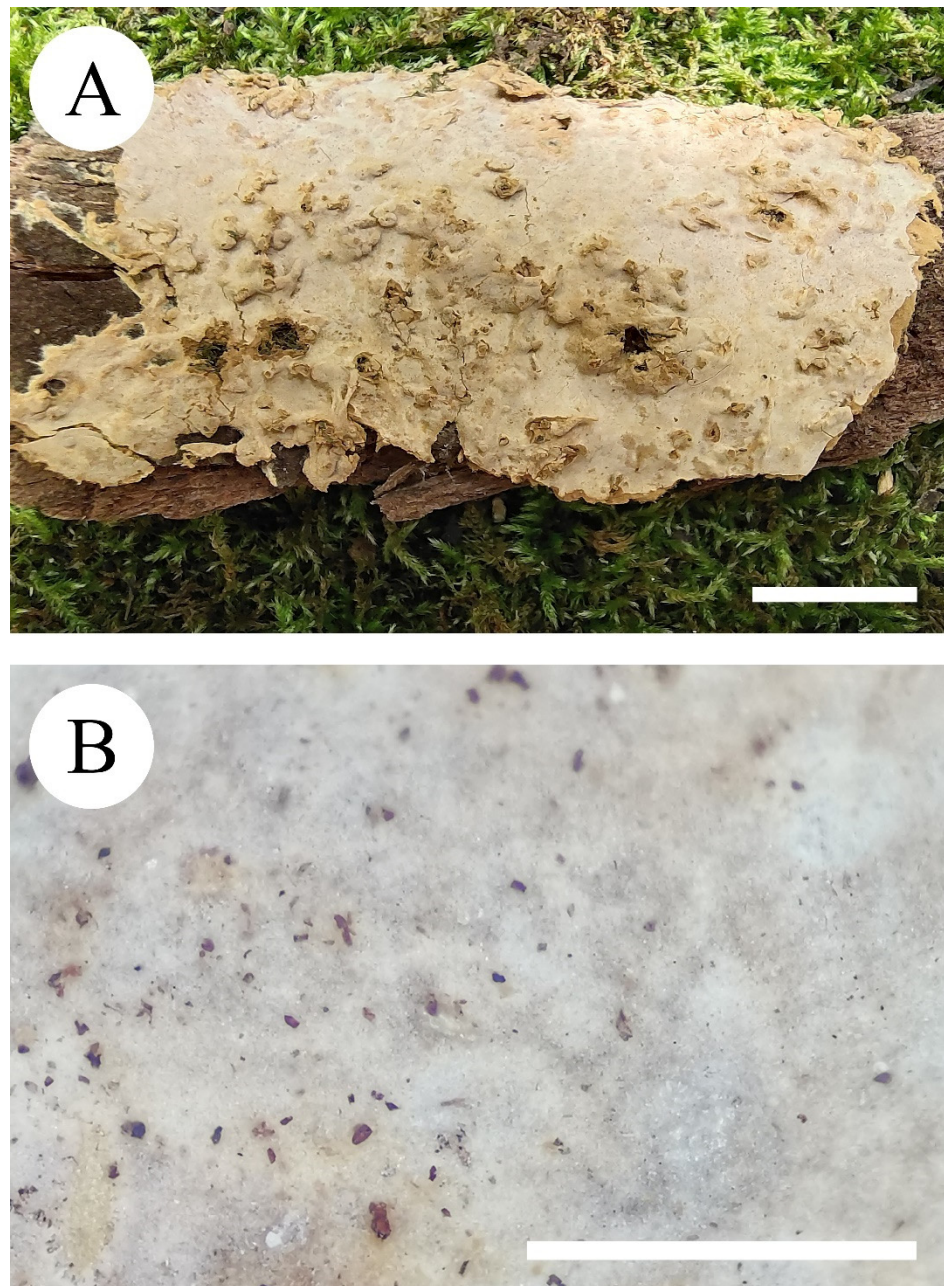


Figure 5. Basidiomata of *Rhizochaete grandinosa* (holotype). Bars: (A) = 1 cm and (B) = 1 mm.

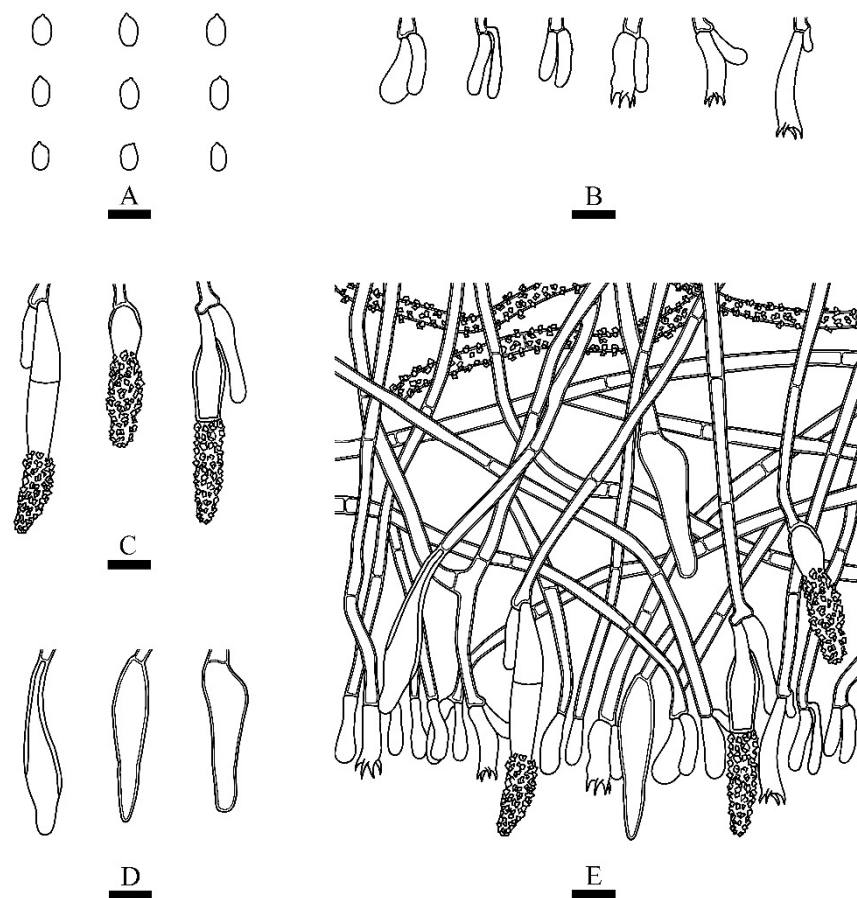


Figure 6. Microscopic structures of *Rhizochaete grandinosa* (holotype): (A) basidiospores; (B) basidia and basidioles; (C,D) cystidia; (E) a section of hymenium. Bars: (A) = 5 μ m, (B–E) = 10 μ m.

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 μ 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 μ m 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 μ m thick, upper half lightly to heavily encrusted with hyaline, insoluble crystals, 24–50 \times 4–9 μ m; basidia clavate to subcylindrical, constricted, somewhat sinuous, with four sterigmata and simple septa, 14.5–21 \times 4.3–5.2 μ m.

Spores—Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, 3–4(–4.5) \times 2.5–3(–3.3) μ m, L = 3.65 μ m, W = 2.79 μ 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

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 ($4\text{--}5 \times 2.5\text{--}3 \mu\text{m}$) [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 ($100\text{--}250 \times 8\text{--}15 \mu\text{m}$) [9]; *R. sulphurosa* is separated from *R. fissurata* by having the lemon yellow to mustard to buff hymenial surface and larger basidiospores ($4.5\text{--}5.5 \times 2\text{--}3 \mu\text{m}$) [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 ($4.5\text{--}5.5 \times 2\text{--}2.5 \mu\text{m}$) [32]; *R. percitrina* differs in its smooth to farinaceous hymenial surface with no change in KOH, and narrower cystidia ($22\text{--}35 \times 3.5\text{--}6 \mu\text{m}$) [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 ($22\text{--}36 \times 4\text{--}5 \mu\text{m}$) [31]; *R. rhizomorphosulphurea* differs from *R. grandinosa* by having the widely effused basidiomata, with sulfur yellow to light orange hymenial surface and larger basidiospores ($4\text{--}4.5 \times 2.8\text{--}3.6 \mu\text{m}$) [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 hyphae regularly clamped.....2
1. Generative hyphae primarily simple septa.....6
2. Cystidia absent.....*R. violascens*
2. Cystidia abundant.....3
3. Cystidia up to 250 μm long with thick walls, basidia > 40 μm in length.....*R. brunnea*
3. Cystidia up to 100 μm long with thin walls, basidia < 40 μm 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 μm in length.....*R. americana*
5. Basidiomes bright to dull yellow, cystidia > 60 μm in length.....*R. sulphurina*
6. Cystidia with thin or slightly thickened walls, < 1 μm thick.....7
6. Cystidia with distinctly thick walls, > 1 μm thick.....14
7. Hymenium turning violet or red in KOH.....8
7. Hymenium not reacting or changing to orange or brown in KOH.....12

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

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