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Abstract Cercospora leaf spot (CLS), caused by the fungal pathogen Cercospora beticola, is the most important foliar pathogen of sugar beet worldwide. Extensive reliance on fungicides to manage CLS has resulted in the evolution of fungicide resistance in C. beticola worldwide, including populations [...] Read more.

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Article Morphological and Phylogenetic Evidence for Recognition of Two New Species of *Phanerochaete* from East Asia

Dong-Qiong Wang ^{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; fungiwangdongqiong@163.com
- ² College of Biodiversity Conservation, Southwest Forestry University, Kunming 650224, China
- ³ Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
- ⁴ School of Life Sciences, Tsinghua University, Beijing 100084, China
- Correspondence: fungi@swfu.edu.cn or fungichanglinz@163.com

Abstract: Two new corticioid fungal species, *Phanerochaete pruinosa* and *P. rhizomorpha* spp. nov. are proposed based on a combination of morphological features and molecular evidence. *Phanerochaete pruinosa* is characterized by the resupinate basidiomata with the pruinose hymenial surface, a monomitic hyphal system with simple-septate generative hyphae and subcylindrical basidiospores measuring as $3.5-6.7 \times 1.5-2.7 \mu m$. *Phanerochaete rhizomorpha* is characterized by having a smooth hymenophore covered by orange hymenial surface, the presence of rhizomorphs, subulate cystidia, and narrower ellipsoid to ellipsoid basidiospores. Sequences of ITS+nLSU nrRNA gene regions of the studied specimens were generated and phylogenetic analyses were performed with maximum likelihood, maximum parsimony, and Bayesian inference methods. These phylogenetic analyses showed that two new species clustered into genus *Phanerochaete*, in which *P. pruinosa* was sister to *P. yunnanensis* with high supports (100% BS, 100% BT, 1.00 BPP); morphologically differing by a pale orange to greyish orange and densely cracked hymenial surface. Another species *P. rhizomorpha* was closely grouped with *P. citrinosanguinea* with lower supports; morphologically having yellow to reddish yellow hymenial surface, and smaller cystidia measuring as $31-48 \times 2.3-4.8 \mu m$.

Keywords: corticioid fungi; Phanerochaetaceae; molecular systematics; taxonomy; Yunnan Province

1. Introduction

Corticioid fungi is a large group of Basidiomycota with simpler basidiomata with the diverse morphological features when compared with polypores, but the phylogenetic diversity of this group is less intensively studied [1,2]. In the subtropical-tropical areas, many corticioid taxa have not been discovered and described worldwide. The genus *Phanerochaete* P. Karst. is a member of the corticioid fungi, which is typified by *P. alnea* (Fr.) P. Karst. [3], and the genus is characterized by the resupinate, membranaceous basidiomata with or without rhizomorphs, a monomitic hyphal system with primarily simple-septate generative hyphae, clavate basidia with four sterigmata, and smooth, thin-walled, inamyloid basidiospores [1,4,5]. Index Fungorum (http://www.indexfungorum.org; accessed on 9 December 2021) registers 187 specific and infraspecific names in *Phanerochaete*. The diversity and taxonomy of *Phanerochaete* s.l. in China have been studied for the last 30 years [6–19].

Molecular studies involving *Phanerochaete* based on the ribosomal DNA (rDNA) sequences, revealed the phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi, in which *P. chrysosporium* Burds. nested into phlebioid clade in Polyporales [20]. Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset and extensive ITS sampling indicated that *Phanerochaete* sensu lato was polyphyletic and distributed across nine lineages in the phlebioid clade, in which



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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/). six lineages were associated to described genera [21]. Miettinen et al. [22]. explored the DNA-phylogeny-based and morphology-based to reconcile the polypores and genus concepts in the family Phanerochaetaceae, which the macromorphology of fruiting bodies and hymenophore construction did not reflect monophyletic groups, and *Ceriporia inflata* B.S. Jia and B.K. Cui was combined into *Phanerochaete*. Amplifying nrLSU, nrITS, and rpb1 genes across the Polyporales revealed that eleven genera clustered into family Phanerochaetaceae, and two families Hapalopilaceae and Bjerkanderaceae were placed as synonyms of Phanerochaetaceae [23]. Recently, the research supported by morphological studies and the phylogenetic analyses, showed that many new taxa of *Phanerochaete* s.s. were found and displayed the taxonomic status for the new taxa within genus *Phanerochaete* [14,19].

In 2018–2019, we collected the material supposedly belonging to the two undescribed species of corticioid fungi from Yunnan Province, China. We present the morphological and molecular phylogenetic evidence that support the recognition of two new species within the *Phanerochaete* s.s., based on the internal transcribed spacer (ITS) and regions nLSU sequences.

2. Materials and Methods

2.1. Morphology

The fruiting bodies were observed growing on the ground of broad-leaved treemixed forest. The fruiting bodies were dried in an electric food dehydrator at 40 °C, then sealed and stored in an envelope bag. They were then transported to Kunming where microscopic morphology and phylogeny to be studied at the mycology laboratory of Southwest Forestry University, Kunming, Yunnan Province, China. The for-study specimens were deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. Macromorphological descriptions were based on field notes and photos captured in the field and lab. Color terminology follow Petersen [24]. Micromorphological data were obtained from the dried specimens, and observed under a light microscope following Dai [25]. 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, n = a/b (number of spores (a) measured from given number of specimens (b)).

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 followed previous study [26]. ITS region was amplified with primer pair ITS5 and ITS4 [27]. nLSU region was amplified with primer pair LR0R and LR7 (http://lutzonilab.org/nuclear-ribosomal-dna/; accessed on 28 September 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.

Species Name	Saasin on Na	GenBank A	D (
	Specimen No	ITS	nLSU	References
Bjerkandera adusta	FP-101236	KP134982		[21]
B. adusta	HHB-12826	KP134983	KP135198	[21]
B. fumosa	Dai 12674B	MW507112	MW520213	[28]

		GenBank A	- /	
Species Name	Specimen No. –	ITS	nLSU	References
B fumosa	Dai 21087	MW507110		[28]
Bussomerulius corium	FP-102382	KP135007	KP135230	[20]
B. corium	FP-107055	KP135008	14 100-00	[21]
Ceraceomuces servens	HHB-15692-Sp	KP135031	KP135200	[21]
C. sernens	L-11105	KP135032	14 100200	[21]
Ceriporia nurnurea	KKN-223-Sp	KP135044	KP135203	[21]
C. nurnurea	HHB-3964	KP135042	14 100-00	[21]
C. reticulata	RLG-11354	KP135041	KP135204	[21]
C. reticulata	L-7837	KP135040	14 100-01	[21]
Efibula oracilis	FD-455	KP135027	MZ637116	[21]
E. gracilis	FP-102052	KP135028	112007110	[21]
E. tropica	W11 0809-8	MZ636968	MZ637130	unpublished
E. tropica	WEI 18-149	MZ636967	MZ637129	unpublished
Gloeoporus dichrous	FP-151129	KP135058	KP135213	[21]
G. pannocinctus	L-15726-Sp	KP135060	KP135214	[21]
Hyphodermella poroides	Dai 12045	KX008367	KX011852	[29]
H. poroides	Dai 10848	KX008368	KX011853	[29]
H. rosae	FP-150552	KP134978	KP135223	[21]
H rosae	MA-Fungi	FN600389	IN939588	[30]
Irnex lacteus	FD-9	KP135026	KP135224	[21]
I Jacteus	FD-93	KP135025	14 100221	[21]
Merulionsis	12 70	1010020		
albostramineus	HHB-10729	KP135051	KP135229	[21]
M. albostramineus	L-9778	KP135052		[21]
M taxicola	CBS 45548	MH856432	MH867978	[31]
M taxicola	Kuliok 00/75 (GB)	EU118648		[32]
Phaeonhlehionsis	144.000,70 (02)	20110010		[0-]
caribbeana	HHB-6990	KP135415	KP135243	[21]
P. caribbeana	FD-442 (TYPE)	KP135416		[21]
P. jonerij	FD-425	KP135418		[21]
P. peiophoroides	FP-150577	KP135417	KP135273	[21]
Phanerochaete, albida	FD-31	KP135308	KP135210	[19]
P. alnea	OM 8110	KP135171		[21]
P. alnea	KHL 12054	EU118653	EU118653	[32]
P. argillacea	Wu 9712-18	20110000	GO470656	[13]
P. arizonica	RLG-10248-sp	KP135170	KP135239	[21]
P. australis	He 6013	MT235656	MT248136	[19]
P. australis	HHB-7105-sp	KP135081	KP135240	[21]
P. australosanguinea	20098 Tell		MH233928	[33]
P. australosanguinea	20102 Tell		MH233929	[33]
P. bambucicola	He 3606	MT235657	MT248137	[19]
P. bambucicola	Wu 0707-2	MF399404	MF399395	[15]
P. brunnea	He 4192	MT235658	MT248138	[19]
P. brunnea	He 1873	KX212220	KX212224	[17]
P. burdsallii	He 2066	MT235690	MT248177	[19]
P. burdsallii	CFMR: RF9JR	KU668973		unpublished
P. burtii	HHB-4618-sp	KP135117	KP135241	[21]
P. burtii	FD-171	KP135116		[21]
P. calotricha	Vanhanen-382	KP135107		[21]
P. canobrunnea	He 5726	MT235659	MT248139	[19]
P. canobrunnea	CHWC 1506-66	LC412095	LC412104	[14]
P. canolutea	Wu 9211-105		GO470641	[13]
P. carnosa	He 5172	MT235660	MT248140	[19]
P. carnosa	HHB-9195	KP135129	KP135242	[21]
P. chrysosvorium	HHB-6251	KP135094	KP135246	[21]
P. chrysosporium	He 5778	MT235661	MT248141	[19]

Table 1. Cont.

Species Name

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GenBank A	ccession No.	D (
'S	nLSU	References	
	MT248172	[19]	
35691	MT248178	[19]	
E100	KD125224	[01]	

Table 1. Cont.

Specimen No.

Species Name	Specimen No.	ITS	nLSU	Keferences
P. cinerea	He 6003		MT248172	[19]
P. citrinosanguinea	He 4298	MT235691	MT248178	[19]
P. citrinosanguinea	FP-105385-sp	KP135100	KP135234	[21]
P. concrescens	He 4657	MT235662	MT248142	[19]
P. concrescens	Spirin 7322	KP994380	KP994382	[34]
P. cumulodentata	He 2995	MT235664	MT248144	[19]
P. cumulodentata	LE 298935	KP994359	KP994386	[34]
P. cystidiata	He 4224	MT235665	MT248145	[19]
P. cystidiata	Wu 1708-326	LC412097	LC412100	[14]
P. deflectens	FCUG 2777		GQ470644	[13]
P. ericina	He 4285	MT235666	MT248146	[19]
P. ericina	HHB-2288	KP135167	KP135247	[21]
P. exilis	HHB-6988	KP135001	KP135236	[21]
P. fusca	Wu 1409-163	LC412099	LC412106	[14]
P. hymenochaetoides	He 5988		MT248173	[19]
P. incarnata	He 20120728-1	MT235669	MT248149	[19]
P. incarnata	WEI 16-075	MF399406	MF399397	[15]
P. inflata	Dai 10376	JX623929	JX644062	[35]
P. inflata	Cui 7712	JX623930	JX644063	[35]
P. krikophora	HHB-5796	KP135164	KP135268	[21]
P. laevis	He 20120917-8	MT235670	MT248150	[19]
P. laevis	HHB-15519	KP135149	KP135249	[21]
P. leptocystidiata	He 5853	MT235685	MT248168	[19]
P. leptocystidiata	Dai 10468	MT235684	MT248167	[19]
P. livescens	He 5010	MT235671	MT248151	[19]
P. livescens	FD-106	KP135070	KP135253	[21]
P. magnoliae	He 3321	M1235672	M1248152	[19]
P. magnoliae	HHB-9829-sp	KP135089	KP135237	[21]
P. metuloidea	He 2565	MTOOLCOO	M1248163	[19]
P. metulolueu D. minor	He 2766	NT225686	MT248104	[19]
P. minor	Не 3966	IVI 1 233000	MT248160	[19]
D narmastoi	He 4570	MT235673	MT248153	[19]
P narmastoi	W11 880313-6	111233073	GO470654	[17]
P norostereoides	He 1902	KX212217	KX212221	[13]
P nruinosa	CLZhao 7712	MZ435346	MZ435350	Present study
P nruinosa	CLZhao 7713	MZ435347	MZ435351	Present study
P. nseudomaonoliae	PP-25	KP135091	KP135250	[21]
P. pseudosanguinea	FD-244	KP135098	KP135251	[21]
P. queletii	HHB-11463	KP134994	KP135235	[21]
P. queletii	FP-102166	KP134995		[21]
P. rhizomorpha	CLZhao 10470	MZ435348	MZ435352	Present study
P. rhizomorpha	CLZhao 10477	MZ435349	MZ435353	Present study
P. rhodella	FD-18	KP135187	KP135258	[21]
P. robusta	Wu 1109-69	MF399409	MF399400	[15]
P. robusta	Ghobad 2288	KP127068	KP127069	[16]
P. sanguinea	HHB-7524	KP135101	KP135244	[21]
P. sanguineocarnosa	FD-359	KP135122	KP135245	[21]
P. sinensis	He 4660	MT235688	MT248175	[19]
P. sinensis	GC 1809-56	MT235689	MT248176	[19]
P. sordida	He 5400	MT235676	MT248157	[19]
P. sordida	FD-241	KP135136	KP135252	[21]
P. stereoides	He 5824	MT235677	MT248158	[19]
P. subceracea	FP-105974-R	KP135162	KP135255	[21]
P. subrosea	He 2421	MT235687	MT248174	[19]
P. taiwaniana	He 5269	MT235680	MT248161	[19]

Species Name	Constant No.	GenBank Accession No.		
	Specimen No. –	ITS	nLSU	References
P. taiwaniana	Wu 0112-13	MF399412	MF399403	[15]
P. thailandica	2015_07	MF467737		[36]
P. velutina	He 3079	MT235681	MT248162	[19]
P. velutina	Kotiranta 25567	KP994354	KP994387	[34]
P. xerophila	HHB-8509-Sp	KP134996	KP135259	[21]
P. xerophila	KKN-172	KP134997		[21]
P. yunnanensis	He 2719	MT235683	MT248166	[19]
P. yunnanensis	He 2697		MT248165	[19]
Phlebiopsis flavidoalba	FD-263	KP135402	KP135271	[21]
P. flavidoalba	FD-374	KP135403		[21]
P. gigantea	FP-70857-sp	KP135390	KP135272	[21]
P. gigantea	FP-101815	KP135389		[21]
Pirex concentricus	OSC-41587	KP134984	KP135275	[21]
P. concentricus	Kropp160Bup6-R	KP134985		[21]
Rhizochaete filamentosa	HHB-3169	KP135410	KP135278	[21]
R. filamentosa	FP-105240	KP135411		[21]
R.radicata	FD-123	KP135407	KP135279	[21]
Terana caerulea	FP-104073	KP134980	KP135276	[21]
T. caerulea	T-616	KP134981		[21]
Trametopsis aborigena	Robledo 1238	KY655337		[37]
T. aborigena	Robledo 1236	KY655336		[37]
T. cervina	AJ-185	JN165020	JN164839	[21]
T. cervina	AJ-189	JN165021		[21]

Table 1. Cont.

New sequences are shown in bold.

Sequences were aligned in MAFFT 7 (https://mafft.cbrc.jp/alignment/server/; accessed on 28 September 2021) using G-INS-i strategy for ITS+nLSU combined dataset, and manually adjusted in BioEdit [38]. Aligned dataset was deposited in TreeBase (submission ID 28442). *Phlebiopsis gigantea* Fr. and *Rhizochaete radicata* (Henn.) Gresl., Nakasone and Rajchenb were selected as an outgroup for phylogenetic analyses of combined dataset following a previous study [19]. The taxon sampling strategy for the selection of sequences for phylogenetic trees was to choose (1) in a larger scale, focusing on the related genera in the families Phanerochaetaceae and Irpicaceae in Figure 1; (2) the related taxa based on BLAST search in GenBank within *Phanerochaete* s.l.; and (3) all species of *Phanerochaete* s.s.

Maximum parsimony analysis was applied to the combined (ITS+nLSU) dataset. Its approaches followed Zhao and Wu [26], and the tree construction procedure was performed in PAUP* version 4.0b10 [39]. 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 [40]. 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 28 September 2021) [41]. Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 [42] was used to determine the best-fit evolution model for the dataset for Bayesian inference (BI). BI was calculated with MrBayes 3.1.7a [43]. Four Markov chains were run for 2 runs from random starting trees for 10 million generations for ITS+nLSU (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 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of two new species and related genera in Phanerochaetaceae and Irpicaceae based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values >70%, parsimony bootstrap values >70% and Bayesian posterior probabilities >0.95, respectively.



Figure 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of two new species and related species in *Phanerochaete* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values >70%, parsimony bootstrap values >70% and Bayesian posterior probabilities >0.95, respectively. The yellow backgrounds indicate new species.

3. Results

3.1. Molecular Phylogeny

The ITS+nLSU dataset (Figure 1) included sequences from 86 fungal specimens representing 50 species. The dataset had an aligned length of 2368 characters, of which 1170 characters are constant, 598 are variable and parsimony-uninformative, and 600 are parsimony-informative. Maximum parsimony analysis yielded one equally parsimonious tree (TL = 3476, CI = 0.3631, HI = 0.6369, RI = 0.7539, RC = 0.3512). Best model for the

ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G (lset 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 = 0.038487 (BI), and the effective sample size (ESS) across the two runs is the double of the average ESS (avg ESS) = 303.

The phylogeny (Figure 1) based on the combined ITS+nLSU sequences indicated that both species *Phanerochaete pruinose* and *P. rhizomorpha* clustered into *Phanerochaete* s.s and then *P. pruinose* grouped with *P. subceracea* (Burt) Burds.; *P. rhizomorpha* was sister to *P. citrinosanguinea* Floudas and Hibbett.

The ITS+nLSU dataset (Figure 2) included sequences from 83 fungal specimens representing 53 taxa. The dataset had an aligned length of 2017 characters, of which 1548 characters are constant, 164 are variable and parsimony-uninformative, and 395 are parsimony-informative. Maximum parsimony analysis yielded 35 equally parsimonious trees (TL = 1900, CI = 0.4095, HI = 0.5905, RI = 0.6456, RC = 0.2644). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G (lset 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 = 0.004260 (BI), and the effective sample size (ESS) across the two runs is the double of the average ESS (avg ESS) = 309.

The phylogram inferred from ITS+nLSU sequences (Figure 2) revealed that two new species were clustered into genus *Phanerochaete* s.s.; *P. pruinosa* sp. nova. was sister to *P. yunnanensis* Y.L. Xu and S.H. He with high supports (100% BS, 100% BT, 1.00 BPP), and then grouped with *P. robusta* Parmasto without supported data. Another species *P. rhizomorpha* sp. nova. was closely grouped with *P. citrinosanguinea* with lower supports, and then grouped with *P. pseudosanguinea* Floudas and Hibbett (–BS, 98% BP and 1.00 BPP) and *P. sanguinea* (Fr.) Pouzar (96% BS, 91% BP and 1.00 BPP).

3.2. Taxonomy

Phanerochaete pruinosa C.L. Zhao and D.Q. Wang, sp. nov. Figures 3 and 4. MycoBank no.: MB 841271.

Diagnosis: It differs from *P. yunnanensis* by its pruinose hymenophore with the white to slightly cream hymenial surface and lightly darkening in KOH.

Holotype—China, Yunnan Province, Chuxiong, Zixishan National Forestry Park, on the bark of fallen angiosperms, 101.4° E, 25.1° N, 1 July 2018, CLZhao 7113 (SWFC).

Etymology—*pruinosa* (Lat.): from Latin, referring to the white powder on hymenial surface of basidiomata.

Fruiting body—Basidiomata annual, resupinate, adnate, undetachable from substrate, membranaceous to coriaceous, without odor and taste when fresh, up to 15 cm long, 3 cm wide, 50–100 μ m thick. Hymenial surface smooth to have small verrucous process, pruinose, white when fresh, white to slightly cream on drying; lightly darkening in KOH. Margin sterile, narrow, white, attached.

Hyphal system—Hyphal system monomitic, generative hyphae simple-septa, colorless, thick-walled, unbranched, interwoven, 3–4.5 µm in diameter, subhymenial hyphae densely covered by larger crystals, basal hyphae regular; IKI–, CB–; tissues unchanged in KOH.

Hymenium—Hymenial cystidia and cystidoles absent; basidia clavate to subcylindrical, with four sterigmata and a simple-septum, 13–24 μ m × 3.5–4.5 μ m.

Spores—Basidiospores cylindrical, colorless, thin-walled, smooth, IKI–, CB–, (3.3–) 3.5–6.7(–7) μ m × 1.5–2.7(–2.9) μ m, L = 4.42 μ m, W = 1.94 μ m, Q = 2.21–2.35 (n = 60/2).

Additional specimen examined—China, Yunnan Province, Zixishan National Forestry Park, on fallen branch of angiosperm, 101.4° E, 25.1° N, 1 July 2018, C.L. Zhao 7112 (SWFC).

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



Figure 3. Basidiomata of *Phanerochaete pruinosa* (holotype) Bars: (A) = 2 cm and (B) = 1 mm.



Figure 4. Microscopic structures of *Phanerochaete pruinosa* (holotype): basidiospores (**A**), basidia and basidioles (**B**), A section of hymenium (**C**). Bars: (**A**) = 5 μ m, (**B**,**C**) = 10 μ m.



Phanerochaete rhizomorpha C.L. Zhao and D.Q. Wang sp. nov. Figures 5 and 6.

Figure 5. Basidiomata of *Phanerochaete rhizomorpha* (holotype) Bars: (A) = 2 cm and (B) = 1 mm.



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

MycoBank no.: MB 841272.

Diagnosis: It differs from *P. citrinosanguinea* by its orange hymenial surface and larger cystidia $48.5-71.5 \ \mu m \times 3-6.5 \ \mu m$)

Holotype—China, Yunnan Province, Dali, Nanjian Country, Lingbaoshan National Forestry Park, on the fallen branch of angiosperm, 24.7° N, 100.6° E, 10 January 2019, C.L. Zhao 10,477 (SWFC).

Etymology—*rhizomorpha* (Lat.): from Latin, referring to the rhizomorphic basidiomata of the specimens.

Fruiting body—Basidiomata annual, resupinate, adnate, easily detachable from substrate, membranaceous, up to 5 cm long, 3 cm wide, 200–300 μ m thick. Hymenial surface smooth, slightly orange when fresh, orange upon drying; lightly darkening in KOH. Margin sterile, buff to slightly orange, up to 1 mm wide, rhizomorphic.

Hyphal system—Hyphal system monomitic, generative hyphae simple-septa, colorless, thick-walled, frequently branched, interwoven, 3–6.5 μ m in diameter, basal hyphae regular, numerous crystals present among the abhymenium hyphae, IKI–, CB–; tissues unchanged in KOH.

Hymenium—Hymenium cystidia subulate or tapering, colorless, thick-walled, with 2–4 septa, 48.5–71.5 μ m × 3–6.5 μ m; basidia subcylindrical, with 4 sterigmata, 18.5–35.5 μ m × 3.5–5.5 μ m.

Spores—Basidiospores narrower ellipsoid to ellipsoid, colorless, thin-walled, smooth, with oil 1–2 drops inside, IKI–, CB–, 4.5–5.8(–6) μ m × 2.7–3.6(–3.8) μ m, L = 5.07 μ m, W = 3.19 μ m, Q = 1.58–1.60 (n = 62/2).

Additional specimen examined—China, Yunnan Province, Nanjian Country, Lingbaoshan National Forestry Park, on fallen branch of angiosperm, 24.7° N, 100.6° E, 10 January 2019, C.L. Zhao 10,470 (SWFC).

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

4. Discussion

In the present study, two new species, *Phanerochaete pruinosa* C.L. Zhao and D.Q. Wang and *P. rhizomorpha* C.L. Zhao and D.Q. Wang spp. nov., are described based on phylogenetic analyses and morphological characters. The nucleotide differences of phylogenetically similar species to *Phanerochaete pruinosa* and *P. rhizomorpha*.

Phylogenetically, Xu et al. [19] revealed the taxonomy and phylogeny of Phanerochaete sensu stricto (Polyporales, Basidiomycota) with emphasis on Chinese collections, which showed that twenty-eight species of *Phanerochaete* s.s. from China are confirmed by morphology and DNA sequence data. In the present study (Figure 2), two new taxa clustered into Phanerochaete s.s., in which P. pruinosa was sister to P. yunnanensis, and then grouped with P. robusta. Another species P. rhizomorpha was closely grouped with P. citrinosanguinea with lower supports, and then grouped with *P. pseudosanguinea* and *P. sanguinea*. However, morphologically, P. yunnanensis is separated from P. pruinosa by having a pale orange to greyish orange and densely cracked hymenial surface [19]; P. robusta differs in its yellow basidiomata and two kinds of cystidia without encrustation, larger basidiospores (5.5–7 µm \times 2.4–2.9 µm) and a boreal distribution [34]. *Phanerochaete citrinosanguinea* differs from P. rhizomorpha by having yellow to reddish yellow hymenial surface, and smaller cystidia $(31-48 \ \mu m \times 2.3-4.8 \ \mu m)$ [21]; *P. pseudosanguinea* differs *P. rhizomorpha* in its light red or dark red hymenial surface, and narrower basidiospores (4–5.5 μ m \times 2–2.5 μ m) [21]; *P. sanguinea* is separated from *P. rhizomorpha* by having the thin-walled cystidia and the larger basidia $(25-45 \,\mu\text{m} \times 4-6 \,\mu\text{m})$ [1]; in addition, there is some coloration of wood as in *P. sanguinea*.

Morphologically, *Phanerochaete pruinosa* is similar to *P. concrescens* Spirin and Volobuev and *P. sordida* (P. Karst.) J. Erikss. and Ryvarden, based on presence of white or cream hymenial surface. However, *P. concrescens* differs from *P. pruinosa* by having the large basidia (27–39 μ m × 4–5 μ m) [34]; *P. sordida* is separated from *P. pruinosa* by presence of cystidia and wider basidiospores (5–7 μ m × 2.5–3.5 μ m) [1].

Phanerochaete rhizomorpha reminds four taxa of *Phanerochaete* based on the character by having the rhizomorph, *P. burdsallii* Y.L. Xu, Nakasone and S.H. He, *P. leptocystidiata* Y.L. Xu and S.H. He, *P. sinensis* Y.L. Xu, C.C. Chen and S.H. He and *P. subrosea* Y.L. Xu and S.H. He. However, *P. burdsallii* differs from *P. rhizomorpha* by having the cystidia encrusted with small crystals [19]; *P. leptocystidiata* differs in having a tuberculate hymenial surface and thin-walled cystidia encrusted at apex (24–30 μ m × 4–6 μ m) [19]; *P. sinensis* differs in its thin-walled cystidia and the shorter basidia (17–22 μ m × 4–5 μ m) [19]; *P. subrosea* is separated from *P. rhizomorpha* by having the thin-walled and smaller cystidia (33–55 μ m × 3–5 μ m) [19].

Phanerochaete rhizomorpha is similar to *P. aurantiobadia* Ghob.-Nejh., S.L. Liu, Langer and Y.C. Dai, *P. cumulodentata* (Nikol.) Parmasto and *P. hymenochaetoides* Y.L. Xu and S.H. He based on the character by the orange hymenial surface. However, *P. aurantiobadia* differs from *P. rhizomorpha* by having the larger basidiospores (5–8.3 μ m × 2–3 μ m) [16]; *P. cumulodentata* differs from *P. rhizomorpha* by a tuberculate hymenophore and shorter basidia (16.7–28.3 μ m × 3.7–5.2 μ m) [34]; *P. hymenochaetoides* differs from *P. rhizomorpha* by having both smaller cystidia (30–45 μ m × 3–4 μ m) and basidiospores (4–5.2 μ m × 2–2.8 μ m) [19].

In the ecology and biogeography, the taxa of *Phanerochaete* are a typical example of wood-rotting fungi, which are mainly distributed in Asia, Europe, and America, and the substrata are mostly hardwood [1,25], and this genus is an extensively studied group of Basidiomycota; nonetheless, the wood-rotting fungi diversity is still not well known in the subtropics and tropics [44–48]. The two new species, *Phanerochaete pruinosa* and *P. rhizomorpha* spp. nov., were found in subtropics, which enriches the diversity of wood-rotting fungi.

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