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

Dong-Qiong Wang and Chang-Lin Zhao

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 × 1.5–2.7 μm. 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 × 2.3–4.8 μm.

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 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...
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, nrlTS, 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 | Specimen No. | GenBank Accession No. |
|--------------|--------------|----------------------|
|              |              | ITS                  | nLSU                  |
| Bjerkandera adusta | FP-101236    | KPI34982             |                      | [21]        |
| B. adusta    | HHB-12826    | KPI34983             | KPI135198             | [21]        |
| B. fumosa    | Dai 12674B   | MW507112             | MW520213              | [28]        |
Table 1. Cont.

| Species Name          | Specimen No. | GenBank Accession No. | References |
|-----------------------|--------------|-----------------------|------------|
|                       |              | ITS                   | nLSU       |            |
| **B. fumosa**         | Dai 21087    | MW507110              |            | [28]       |
| **Byssomeriesus corium** | FP-102382   | KP135007              | KP135230   | [21]       |
| **B. corium**         | FP-107055    | KP135008              |            | [21]       |
| **Ceraceomyces serpens** | HHB-15692-Sp | KP135031              | KP135200   | [21]       |
| **C. serpens**        | L-11105      | KP135032              |            | [21]       |
| **Ceriporia purpurea** | KKN-223-Sp   | KP135044              | KP135203   | [21]       |
| **C. purpurea**       | HHB-3964     | KP135042              |            | [21]       |
| **C. reticulata**     | RLG-11354    | KP135041              | KP135204   | [21]       |
| **C. reticulata**     | L-7837       | KP135040              |            | [21]       |
| **Efibula gracilis**  | FD-455       | KP135027              | MZ637116   |            |
| **E. tropica**        | Wu 0809-8    | MZ636968              | MZ637130   |            |
|                       | WEI 18-149   | MZ636967              | MZ637129   |            |
| **Gloeoporus dichrous** | FP-151129    | KP135058              | KP135213   | [21]       |
| **G. pannocinctus**   | L-15726-Sp   | KP135060              | KP135214   | [21]       |
| **Hyphodermodella poroides** | Dai 12045   | KX008367              | KX011852   | [29]       |
| **H. poroides**       | Dai 10848    | KX008368              | KX011853   | [29]       |
| **H. rosae**          | FP-150552    | KP134978              | KP135223   | [21]       |
| **H. rosea**          | MA-Fungi     | FN600389              | JN939588   | [30]       |
| **Irpex lacteus**     | FD-9         | KP135026              | KP135224   | [21]       |
| **I. lacteus**        | FD-93        | KP135025              |            | [21]       |
| **Meruliposis**       | HHB-10729    | KP135051              | KP135229   | [21]       |
| **M. albostramineus** | HHB-7105-sp  | KP135081              | KP135240   | [21]       |
| **M. albostramineus** | L-9778       | KP135052              |            | [21]       |
| **M. taxicola**       | CBS 45548    | MH856432              | MH867978   | [31]       |
| **M. taxicola**       | Kuljok 00/75 (GB) | EU118648            |            | [32]       |
| **Phaeophlebiopsis caribbeana** | HHB-6990    | KP135415              | KP135243   | [21]       |
| **P. caribbeana**     | FD-442 (TYPE) | KP135416             |            | [21]       |
| **P. ignieri**        | FD-425       | KP135418              |            | [21]       |
| **P. peipholihovides** | 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   | GQ470656              |            | [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. bradusa**        | 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. burdsalli**      | He 2066      | MT235690              | MT248177   | [19]       |
| **P. burdsalli**      | CFMR: RP9JR  | KU668973              |            |            |
| **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  | MT235660              | MT248140   | [19]       |
| **P. carnosa**        | He 5172      | MT235660              | MT248140   | [19]       |
| **P. carnosa**        | HHB-9195     | KP135129              | KP135242   | [21]       |
| **P. chrysosporium**  | HHB-6251     | KP135094              | KP135246   | [21]       |
| **P. chrysosporium**  | He 5778      | MT235661              | MT248141   | [19]       |
| Species Name       | Specimen No. | GenBank Accession No. | References |
|--------------------|--------------|-----------------------|------------|
| *P. cinerea*       | He 6003      | MT248172              | [19]       |
| *P. citrinosanguinea* | He 4298     | MT248178              | [19]       |
| *P. citrinosanguinea* | FP-105385-sp | KP135100              | [21]       |
| *P. concrescens*   | He 4657      | MT248142              | [19]       |
| *P. concrescens*   | Spirin 7322  | KP994380              | [34]       |
| *P. cumulodontata* | He 2995      | MT248144              | [19]       |
| *P. cumulodontata* | LE 298935    | KP994359              | [34]       |
| *P. cystidiata*    | He 4224      | MT248145              | [19]       |
| *P. cystidiata*    | Wu 1708-326  | LC412097 LC412100     | [14]       |
| *P. deflectens*    | FCUG 2777    | GQ470644              | [13]       |
| *P. ericina*       | He 4285      | MT248146              | [19]       |
| *P. ericina*       | HHB-2288     | KP135167 KP135247     | [21]       |
| *P. exilis*        | HHB-6988     | KP135001 KP135236     | [21]       |
| *P. cumulodontata* | LE 298935    | KP994359              | [34]       |
| *P. cystidiata*    | He 4224      | MT248145              | [19]       |
| *P. cystidiata*    | Wu 1708-326  | LC412097 LC412100     | [14]       |
| *P. deflectens*    | FCUG 2777    | GQ470644              | [13]       |
| *P. ericina*       | He 4285      | MT248146              | [19]       |
| *P. ericina*       | HHB-2288     | KP135167 KP135247     | [21]       |
| *P. exilis*        | HHB-6988     | KP135001 KP135236     | [21]       |
| *P. hymenochaetoides* | He 5988    | MT248173              | [19]       |
| *P. incarnata*     | He 20120728-1 | MT248149 | [19]       |
| *P. incarnata*     | WEI 16-075   | MT248149              | [19]       |
| *P. inflata*       | Dai 10376    | JX623929 JX644062     | [35]       |
| *P. inflata*       | Cui 7712     | JX623930 JX644063     | [35]       |
| *P. krikiophora*   | HHB-5796     | KP135164 KP135268     | [21]       |
| *P. laevis*        | He 20120917-8 | MT248150 | [19]       |
| *P. laevis*        | HHB-15519    | KP135149 KP135249     | [21]       |
| *P. leptocystidiata* | He 5853    | MT248168              | [19]       |
| *P. leptocystidiata* | Dai 10468   | MT248167              | [19]       |
| *P. livescens*     | He 5010      | MT248151              | [19]       |
| *P. livescens*     | FD-106       | KP135253              | [21]       |
| *P. magnoliae*     | He 3321      | MT248152              | [19]       |
| *P. magnoliae*     | HBB-9829-sp  | KP135089 KP135237     | [21]       |
| *P. metuloidea*    | He 2565      | MT248163              | [19]       |
| *P. metuloidea*    | He 2766      | MT248164              | [19]       |
| *P. minor*         | He 3988      | MT248170              | [19]       |
| *P. minor*         | He 3977      | MT248169              | [19]       |
| *P. parmastoii*    | He 4570      | MT248153              | [19]       |
| *P. parmastoii*    | Wu 880313-6  | GQ470654              | [13]       |
| *P. porostereoides* | He 1902    | KX212217 KX212221     | [17]       |
| *P. pruinosa*      | CLZhao 7712  | MZ435346 MZ435350     | Present study |
| *P. pruinosa*      | CLZhao 7713  | MZ435347 MZ435351     | Present study |
| *P. pseudosanguinea* | PP-25      | KP135250              | [21]       |
| *P. queletii*      | HHH-11463   | 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. rhedella*      | FD-18        | KP135258              | [21]       |
| *P. robusta*       | Wu 1109-69   | MZ435244              | [21]       |
| *P. sanguinea*     | HHB-7524     | KP135101 KP135244     | [21]       |
| *P. sanguineocarnosa* | FD-359    | KP135122 KP135245     | [21]       |
| *P. sinensis*      | He 4660      | MT248175              | [19]       |
| *P. sinensis*      | GC 1809-56   | MT248176              | [19]       |
| *P. sordida*       | He 5400      | MT248157              | [19]       |
| *P. sordida*       | FD-241       | KP135252              | [21]       |
| *P. stereoidea*    | He 5824      | MT248158              | [19]       |
| *P. subceracea*    | FP-105974-R  | KP135255              | [21]       |
| *P. subrosea*      | He 2421      | MT248174              | [19]       |
| *P. taiwaniana*    | He 5269      | MT248161              | [19]       |
### Table 1. Cont.

| Species Name          | Specimen No. | GenBank Accession No. | 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**      | HHHB-8509-Sp | KP134996 KP135259     | [21]       |
| **P. xerophila**      | KKN-172      | KP134997               | [21]       |
| **P. yunnanensis**    | He 2719      | MT235683 MT248166     | [19]       |
| **P. gigantea**       | FP-70857-sp  | KP135390 KP135272     | [21]       |
| **P. flavidoalba**    | FD-263       | KP135402 KP135271     | [21]       |
| **P. flavidoalba**    | FD-374       | KP135403               | [21]       |
| **P. gigantea**       | FP-101815    | KP135389               | [21]       |
| **Pirex concentricus**| OSC-41587    | KP134984 KP135275     | [21]       |
| **R. filamentosa**    | HHHB-3169    | KP135410 KP135278     | [21]       |
| **R. radicata**       | FP-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]       |

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 analyses 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 Iripiceae 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 μm 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 μm × 3.5–4.5 μm.

**Spores**—Basidiospores cylindrical, colorless, thin-walled, smooth, IKI–, CB–, (3.3–) 3.5–6.7(–7) μm × 1.5–2.7(–2.9) μm, L = 4.42 μm, W = 1.94 μm, 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 µm, (B,C) = 10 µm.
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 µm, (B–D) = 10 µm.
MycoBank no.: MB 841272.

Diagnosis: It differs from *P. citrinosanguinea* by its orange hymenial surface and larger cystidia (48.5–71.5 μm × 3–6.5 μm).

**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 μm 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 μm 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 μm × 3–6.5 μm; basidia subcylindrical, with 4 sterigmata, 18.5–35.5 μm × 3.5–5.5 μm.

**Spores**—Basidiospores narrower ellipsoid to ellipsoid, colorless, thin-walled, smooth, with oil 1–2 drops inside, IKI–, CB–, 4.5–5.8 (–6) μm × 2.7–3.6 (–3.8) μm, L = 5.07 μm, W = 3.19 μm, 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.

**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 × 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 μm × 2.3–4.8 μm) [21]; *P. pseudosanguinea* differs from *P. rhizomorpha* in its light red or dark red hymenial surface, and narrower basidiospores (4–5.5 μm × 2–2.5 μm) [21]; *P. sanguinea* is separated from *P. rhizomorpha* by having the thin-walled cystidia and the larger basidia (25–45 μm × 4–6 μm) [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 μm × 4–5 μm) [34]. *P. sordida* is separated from *P. pruinosa* by presence of cystidia and wider basidiospores (5–7 μm × 2.5–3.5 μm) [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 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 μm × 4–6 μm) [19]; *P. sinensis* differs in its thin-walled cystidia and the shorter basidia (17–22 μm × 4–5 μm) [19]; *P. subrosea* is separated from *P. rhizomorpha* by having the thin-walled and smaller cystidia (33–55 μm × 3–5 μm) [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 μm × 2–3 μm) [16]; *P. cumulodentata* differs from *P. rhizomorpha* by a tuberculate hymenophore and shorter basidia (16.7–28.3 μm × 3.7–5.2 μm) [34]; *P. hymenochaetoides* differs from *P. rhizomorpha* by having both smaller cystidia (30–45 μm × 3–4 μm) and basidiospores (4–5.2 μm × 2–2.8 μm) [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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