



## ***Phanerochaete porostereoides*, a new species in the core clade with brown generative hyphae from China**

Liu SL<sup>1</sup> and He SH<sup>1\*</sup>

<sup>1</sup> Institute of Microbiology, Beijing Forestry University, Beijing 100083, China

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### **Abstract**

A new species, *Phanerochaete porostereoides*, is described and illustrated from northwestern China based on the morphological and molecular evidence. It is characterized by a effused brown basidiocarp, a monomitic hyphal system, yellowish brown generative hyphae without clamp connections, numerous hyphal ends in hymenium and subhymenium, and small ellipsoid basidiospores  $4.7\text{--}5.3 \times 2.5\text{--}3.1 \mu\text{m}$ . Morphologically, *P. porostereoides* resembles *Porostereum*, but phylogenetic analyses inferred from the combined sequences of ITS and nLSU show that it is nested within the *Phanerochaete* s.s. clade, and not closely related to *Porostereum spadiceum*, type of the genus.

**Key words** – *Porostereum* – taxonomy – wood-inhabiting fungi

### **Introduction**

*Phanerochaete* P. Karst., typified by *Thelephora velutina* DC., is a widespread genus, and characterized by the membranaceous basidiocarps, a monomitic hyphal system, simple-septate generative hyphae (single or multiple clamps may present in subiculum), clavate basidia and smooth thin-walled inamyloid basidiospores (Eriksson et al. 1978, Burdsall 1985, Bernicchia & Gorjón 2010, Wu et al. 2010). Recent molecular research (de Koker et al. 2003, Wu et al. 2010, Floudas & Hibbett 2015) show that *Phanerochaete* s.l. is polyphyletic and distributed across several lineages in the phlebioid and other clades of Polyporales. Most species of *Phanerochaete* have hyaline generative hyphae, but distinctly brown subicular hyphae present in several species, such as *P. singularis* (G. Cunn.) Burds. and *P. stereoides* Sheng H. Wu.

Two specimens with effused brown basidiocarps were collected by the senior author from temperate forest in Shaanxi Province, northwestern China, and at first they were identified as a member of *Porostereum* Pilát because of the yellowish brown generative hyphae and hyphal ends in hymenium and subhymenium. However, subsequent further morphological examination and phylogenetic analyses based on combined sequences ITS and nLSU showed that it should be placed in *Phanerochaete* and was new to science. It is described and illustrated as *Phanerochaete porostereoides* below.

### **Materials & Methods**

#### **Morphological studies**

Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC). Samples for microscopic examination were mounted in cotton blue or 2% potassium hydroxide

(KOH). The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) measured from given number of specimens (b), IKI- = without reaction in Melzer's reagent, CB- = acyanophilous, Color codes and names are from Kornerup & Wanscher (1978).

### DNA extraction and sequencing

A CTAB rapid plant genomic DNA extraction Kit (Aidlab, Beijing, China) was used to extract total genomic DNA from dried specimens and perform the polymerase chain reaction (PCR), according to the manufacturer's instructions. The ITS and nLSU ribosomal RNA gene regions were amplified with the primer pairs ITS4 and ITS5 (White et al. 1990), and LR0R and LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>), respectively. The PCR procedure for ITS was: initial denaturation at 95 °C for 4 min, followed by 34 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min, and for nLSU was: initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, and the sequences were deposited in GenBank (Table 1).

### Phylogenetic analyses

The molecular phylogeny was inferred from a combined dataset of ITS and nLSU sequences from 34 ingroup species and an outgroup species: *Phlebia radiata* Fr. (Wu et al. 2010, Floudas & Hibbett 2015). Most sequences were retrieved from open datasets and originated from Floudas & Hibbett 2015. The sequences were aligned using the ClustalX v.1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit v.7.0.5.3 (Hall 1999). Sequence alignment was deposited at TreeBase (<http://treebase.org/treebase-web/home.html>, submission ID 19563).

Maximum likelihood (ML), Maximum parsimony (MP) and Bayesian inference (BI) analyses were conducted for the dataset. RAxML v.7.2.6 (Stamatakis 2006) was used for ML analysis. All parameters in the ML analysis used the default setting, and statistical support values were obtained using nonparametric bootstrapping with 1000 replicates (Hillis & Bull 1993). MP analyses were performed using PAUP\* 4.0b10 (Swofford 2002). Gaps in the alignments 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 a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each tree generated. For Bayesian inference (BI), best models of evolution were estimated by using MrModeltest 2.2 (Nylander 2004), and posterior probabilities (BPP) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), using the estimated model of evolution. Four simultaneous Markov chains were run for 1,000,000 generations, and trees were sampled every 100th generation. A majority rule consensus tree of all remaining trees was calculated.

## Results

### Phylogenetic analyses

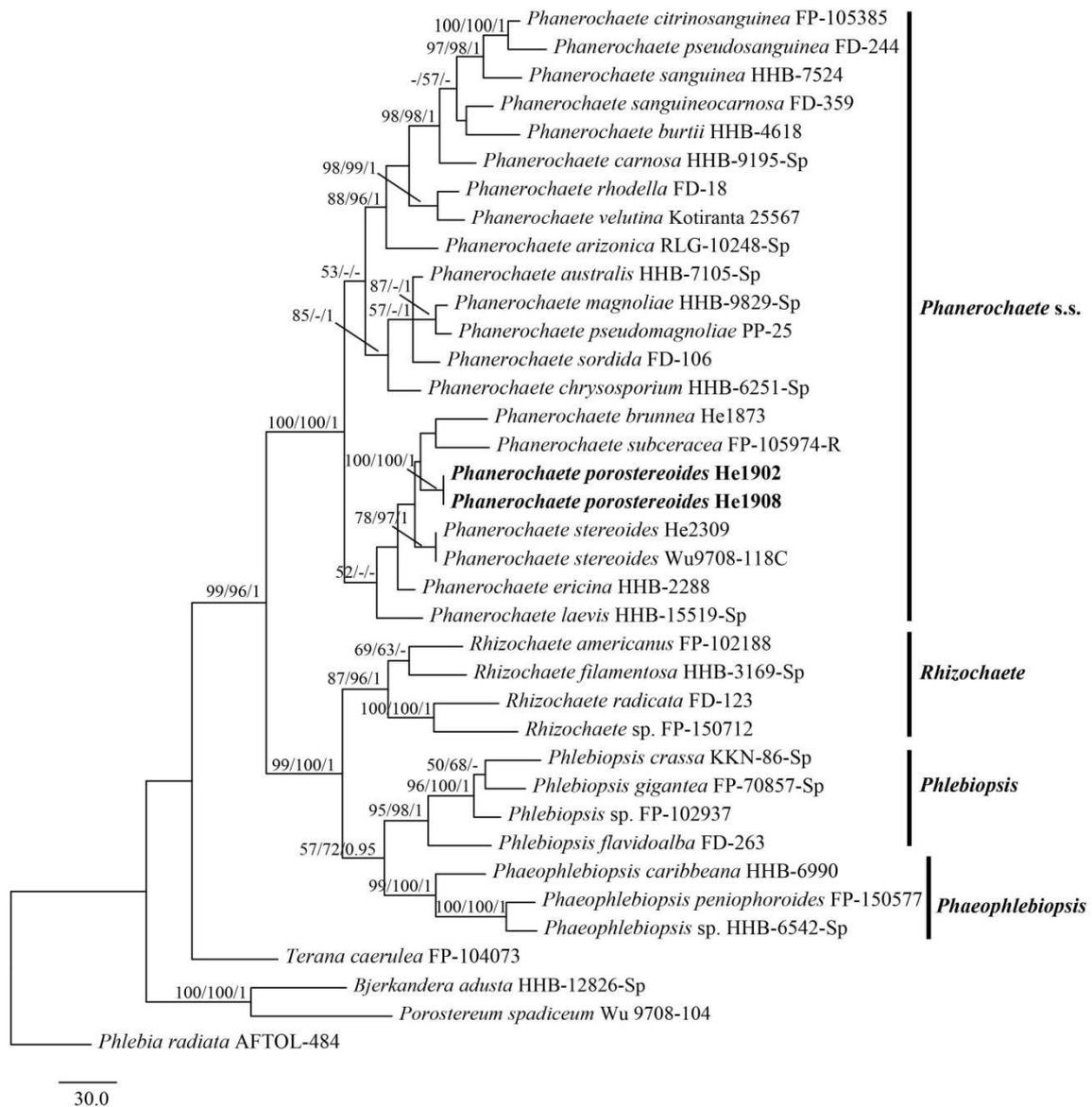
The combined dataset had an aligned length of 2003 characters, of which 345 are parsimony-informative. MP analysis yielded four equally parsimonious trees (TL = 1661, CI = 0.499, RI = 0.632, RC = 0.315, HI = 0.501). The best model estimated and applied in the Bayesian analysis was a GTR+I+G model. BI and ML analyses resulted in a topology similar to that with MP analysis. Only the ML tree is provided in Fig. 1 with the BT values ( $\geq 50\%$ ) and BPPs ( $\geq 0.95$ ) are shown at the nodes. In the tree, species of *Phanerochaete*, *Rhizochaete* Gresl., Nakasone & Rajchenb., *Phaeophlebiopsis* D. Floudas & Hibbett and *Phlebiopsis* Jülich formed four independent

**Table 1** Taxa and GenBank accession numbers for ITS and nLSU sequences used in the phylogenetic analyses.

Species	Sample no.	GenBank accession no.	
		ITS	nLSU
<i>Bjerkandera adusta</i>	HHB-12826-Sp	KP134983	KP135198
<i>Phaeophlebiopsis caribbeana</i>	HHB-6990	KP135415	KP135243
<i>Phaeophlebiopsis peniophoroides</i>	FP-150577	KP135417	KP135273
<i>Phaeophlebiopsis</i> sp.	HHB-6542-Sp	KP135413	KP135413
<i>Phanerochaete arizonica</i>	RLG-10248-Sp	KP135170	KP135239
<i>Phanerochaete australis</i>	HHB-7105-Sp	KP135081	KP135240
<i>Phanerochaete brunnea</i>	He1873	KX212220 <sup>a</sup>	KX212224 <sup>a</sup>
<i>Phanerochaete burtii</i>	HHB-4618	KP135117	KP135241
<i>Phanerochaete carnosa</i>	HHB-9195-Sp	KP135129	KP135242
<i>Phanerochaete chrysosporium</i>	HHB-6251-Sp	KP135094	KP135246
<i>Phanerochaete citrinosa</i>	FP-105385	KP135100	KP135234
<i>Phanerochaete ericina</i>	HHB-2288	KP135167	KP135247
<i>Phanerochaete laevis</i>	HHB-15519-Sp	KP135149	KP135249
<i>Phanerochaete magnoliae</i>	HHB-9829-Sp	KP135089	KP135237
<i>Phanerochaete porostereoides</i>	He1902	KX212217 <sup>a</sup>	KX212221 <sup>a</sup>
<i>Phanerochaete porostereoides</i>	He1908	KX212218 <sup>a</sup>	KX212222 <sup>a</sup>
<i>Phanerochaete pseudomagnoliae</i>	PP-25	KP135091	KP135250
<i>Phanerochaete pseudosanguinea</i>	FD-244	KP135098	KP135251
<i>Phanerochaete rhodella</i>	FD-18	KP135187	KP135258
<i>Phanerochaete sanguinea</i>	HHB-7524	KP135101	KP135244
<i>Phanerochaete sanguineocarnosa</i>	FD-359	KP135122	KP135245
<i>Phanerochaete sordida</i>	FD-106	KP135070	KP135253
<i>Phanerochaete stereoides</i>	He2309	KX212219 <sup>a</sup>	KX212223 <sup>a</sup>
<i>Phanerochaete stereoides</i>	Wu9708-118	–	GQ470661
<i>Phanerochaete subceracea</i>	FP-105974-R	KP135162	KP135255
<i>Phanerochaete velutina</i>	Kotiranta 25567	KP994354	KP994387
<i>Phlebia radiata</i>	AFTOL-484	AY854087	AF287885
<i>Phlebiopsis crassa</i>	KKN-86-Sp	KP135394	KP135215
<i>Phlebiopsis flavidoalba</i>	FD-263	KP135402	KP135271
<i>Phlebiopsis gigantea</i>	FP-70857-Sp	KP135390	KP135272
<i>Phlebiopsis</i> sp.	FP-102937	KP135391	KP135270
<i>Porostereum spadiceum</i>	Wu 9708-104	–	DQ679918
<i>Rhizochaete americana</i>	FP-102188	KP135409	KP135277
<i>Rhizochaete filamentosa</i>	HHB-3169-Sp	KP135410	KP135278
<i>Rhizochaete radicata</i>	FD-123	KP135407	KP135279
<i>Rhizochaete</i> sp.	FP-150712	KP135408	KP135280
<i>Terana caerulea</i>	FP-104073	KP134980	KP135276

<sup>a</sup> Sequences newly generated in this study

clades with strong supports (Fig. 1). The two new collections of *Phanerochaete porostereoides* formed a highly supported lineage within the *Phanerochaete* clade.



**Fig. 1** – Phylogeny of *Phanerochaete porostereoides* and taxa in *Phanerochaete* s.l. and related genera generated by maximum parsimonious analysis based on combined ITS and nLSU sequences. Branches are labeled with maximum likelihood bootstrap value higher than 50%, parsimony bootstrap value higher than 50%, and Bayesian posterior probabilities more than 0.95.

***Phanerochaete porostereoides* S.L. Liu & S.H. He, sp. nov.**

Figs 2–3

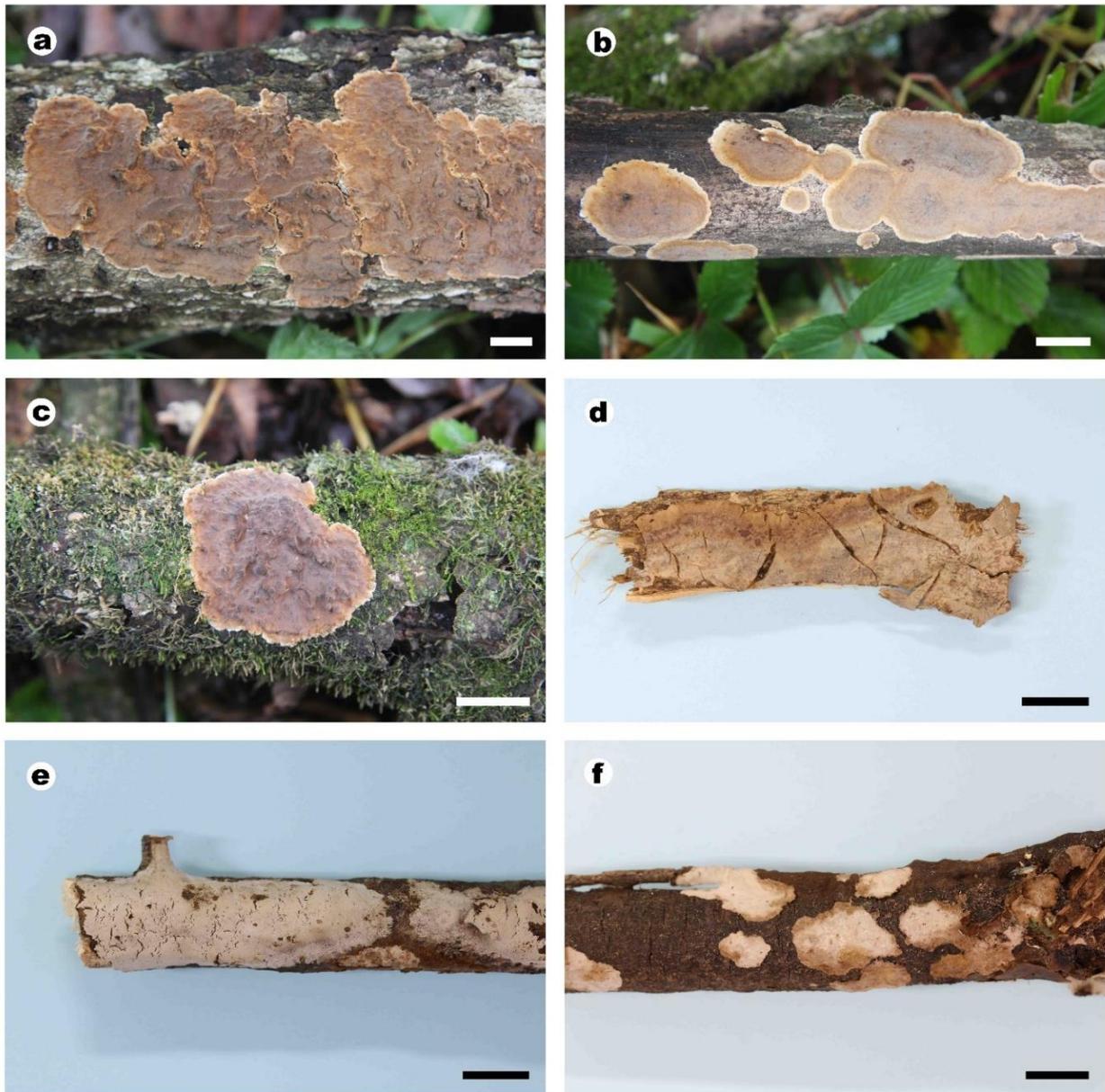
Mycobank 817074

*Facesoffungi* number: FoF 02625

Type – China, Shannxi Province, Foping County, Foping Nature Reserve, on dead angiospermous tree, 11 September 2013, He 1908 (BJFC016375, holotype).

Etymology – “*porostereoides*” (Lat.) refers to the morphological resemblance to *Porostereum*.

Fruiting body – Basidiocarps annual, adnate, effused to slightly effused-reflexed, coriaceous, leathery to soft corky, first as small round patches, later confluent up to 15  $\mu$ m long, up to 200  $\mu$ m thick. Hymenial surface smooth or tuberculate with scattered tubercles, light brown [6D(5–8)], brown [6E(5–8)] to dark brown [6F(5–8)], unchanged in KOH, not cracked or slightly cracked with age; margin thinning out, white (6A1), orange [6A(6–7)] to brownish orange [6C(4–8)] when juvenile, becoming concolorous with hymenial surface when mature, usually slightly lifted and curved inward, up to 2 mm wide.

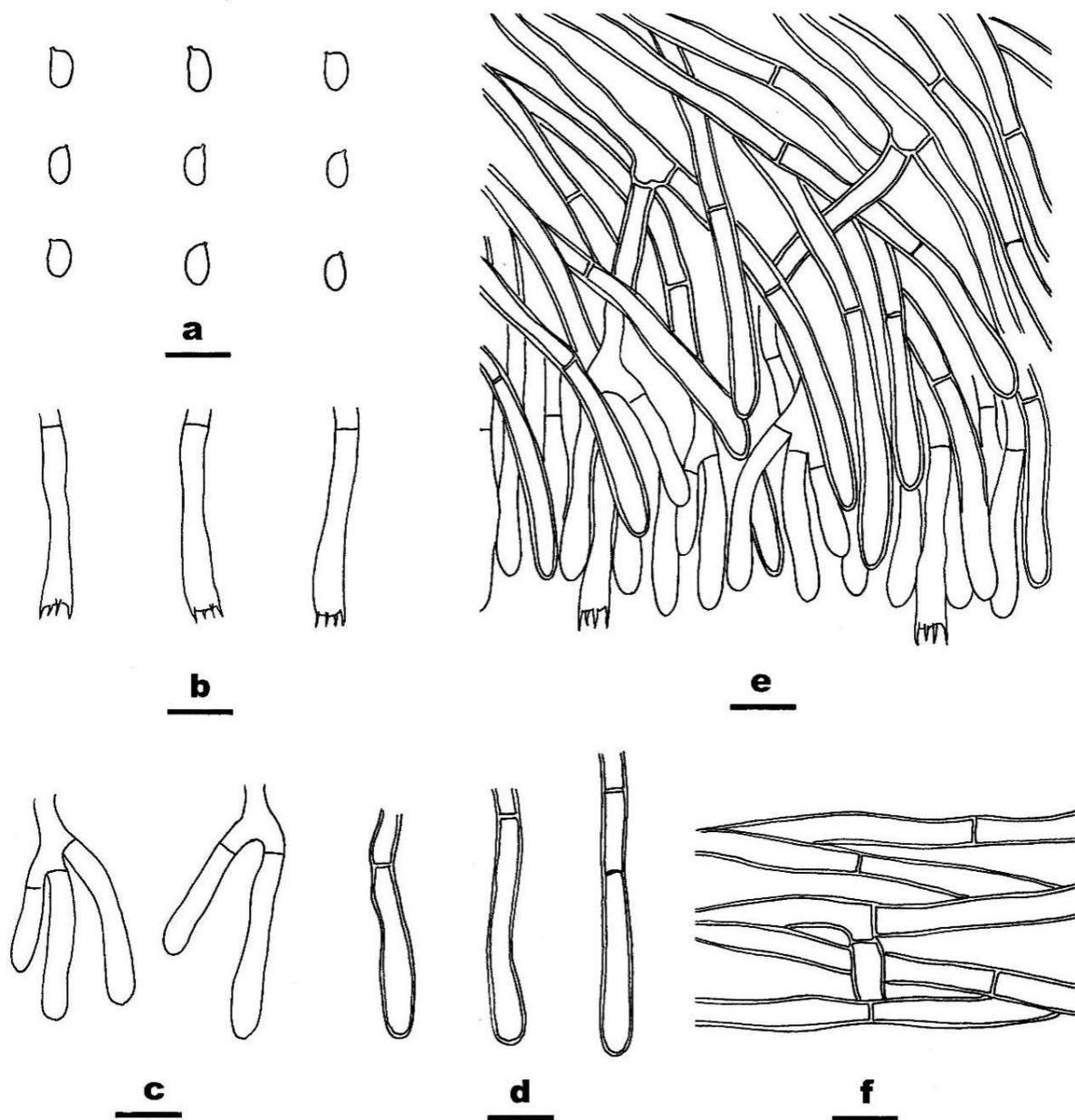


**Fig. 2** – Basidiocarps of *Phanerochaete* species with brown subicular hyphae. a–c. *P. porostereoides* (a. holotype He 1908, b–c paratype He 1902). d. *P. monomitica* (F8333). e. *P. brunnea* (He 1873). f. *P. stereoides* (He 2309). Bars = 10  $\mu$ m.

**Hyphal structure** – Hyphal system monomitic, all hyphae without clamps, not darkening in KOH. Subiculum well developed, subicular hyphae yellowish brown, thick-walled, moderately branched at right angles, more or less parallel to substrate, 3–7 (10)  $\mu$ m in diam. Subhymenium indistinct, hyphae in this layer similar to subicular hyphae.

**Hymenium** – Hyphal ends scattered to numerous, thick-walled, yellowish brown, originated from subiculum, with blunt tips, not projecting outside of hymenium. Cystidia and cystidioles absent. Basidia scattered to rare, clavate, hyaline, thin-walled, with a basal simple septum and four sterigmata, 23–35  $\times$  4–5.3  $\mu$ m; basidioles numerous, similar basidia but slightly smaller.

**Spores** – Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (4.5–)4.7–5.3(–5.5)  $\times$  (2.3–)2.5–3.1(–3.3)  $\mu$ m, L = 4.98  $\mu$ m, W = 2.85  $\mu$ m, Q = 1.76 (n = 60/2).



**Fig. 3** – Microscopic structures of *Phanerochaete porostereoides* (holotype, He 1908). a. Basidiospores. b. Basidia. c. Basidioles. d. hyphal ends from hymenium. e. Section through part of basidiocarp. f. Generative hyphae from subiculum. Bars = 10  $\mu$ m.

Additional specimens examined – *Phanerochaete porostereoides*: China, Shaanxi Province, Foping County, Foping Nature Reserve, on dead angiospermous tree, 11 September 2013, He 1902 (BJFC016369, paratype). *Phanerochaete monomitica*: Argentina, Corrientes, Ituzaingó, Isla Apipé Grande, Monte Grande, interior de selva, 26 September 1988, leg. O. Popoff et al. 602 (F8332, TNM); Capital, Richuelo, en selva degradada, 28 September 1990, leg. O. Popoff et al. 729 (F8333, TNM). Paraguay, Itapúa, Alto paran, Reserva Biol. Itabó, 6 km N de la Administración, interior de la selva, 9 October 1990, leg. O. Popoff & Caballero 800 (F8331, TNM). *Phanerochaete brunnea*: China, Zhejiang Province, Qingyuan County, Baishanzu Nature Reserve, on fallen angiospermous branch, 15 August 2013, He 1873 (BJFC 16340). *Phanerochaete stereoides*: China, Hunan Province, Zhangjiajie County, Zhangjiajie Forest Park, on fallen angiospermous branch, 8 July 2015, He 2309.

## Discussion

Five species of *Phanerochaete*, *P. brunnea* Sheng H. Wu, *P. fuscomarginata* (Burt) Gilb., *P. monomitica* (G. Cunn.) Sheng H. Wu & Popoff, *P. singularis* and *P. stereoides*, with brown subicular hyphae were accepted by Wu (1995). However, Hjortstam and Ryvarden (2002) established a new genus, *Australicum* Hjortstam & Ryvarden, to accommodate *Corticium singulare* G. Cunn., and referred *P. fuscomarginata* and *P. monomitica* to *Porostereum*. In our phylogenetic tree, *P. brunnea*, *P. stereoides* and *P. porostereoides* described above nested within the *Phanerochaete* s.s. clade (Fig. 1). Thus, we would like to keep them all in *Phanerochaete*.

*Phanerochaete porostereoides* is similar to *P. monomitica*, both of which resemble *Porostereum* and have brown hymenial surface, simple septate and brown generative hyphae, and hyphal ends in hymenium and subhymenium. However, *P. monomitica* differs from *P. porostereoides* by its larger basidiospores ( $6\text{--}7 \times 3.5\text{--}4.5 \mu\text{m}$ , Hjortstam & Ryvarden 1990;  $6\text{--}7.3 \times 3.8\text{--}4.6 \mu\text{m}$ , measured by the authors from F8333 in TNM, Fig. 2d) and distribution in the South Hemisphere (New Zealand, Argentina and Paraguay, Wu 1995). *Phanerochaete porostereoides* is also reminiscent of *Porostereum perplexum* (D.A. Reid) Hjortstam & Ryvarden, which however is reported only from the type locality in Australia and has distinctly larger basidiospores ( $6.5\text{--}10 \times 3\text{--}3.5 \mu\text{m}$ , Hjortstam & Ryvarden 1990).

*Phanerochaete brunnea* Sheng H. Wu reported from Taiwan was considered as a synonym of *Australicum singulare* (G. Cunn.) Hjortstam & Ryvarden (Hjortstam & Ryvarden 2002). However, according to Wu (1995), *P. brunnea* has smaller basidiospores than *A. singulare*. A specimen (He 1873, Fig. 2e) collected from southeastern China has basidiospores measured as  $4\text{--}5 \times 2\text{--}3 \mu\text{m}$ , which is in the range of spore size of *P. brunnea*. We identified it as *P. brunnea*, and this is the first report of *P. brunnea* from mainland China. At present, we would like to accept *P. brunnea* as an independent species before more evidence show that it is conspecific with *P. singularis*. *P. brunnea* can be easily distinguished from *P. porostereoides* by its basidiocarp texture, color of hymenial surface and the anatomical structure (Wu 1990, 1995).

*Phanerochaete stereoides* (Fig. 2f) reported from China (Wu 1995, Xiong & Dai 2009) differs from *P. porostereoides* in pale grayish-brown hymenial surface, presence of cystidia, and larger basidiospores ( $6.5\text{--}8 \times 3.2\text{--}4 \mu\text{m}$ , Wu 1995). Meanwhile, *P. stereoides* and *P. porostereoides* formed two distinct lineages in the phylogenetic tree (Fig. 1). *Phanerochaete hyphocystidiata* Sheng H. Wu resembles *P. porostereoides* by having hyphal ends in hymenium and subhymenium and same size of basidiospores, but the former has hyaline subicular hyphae (Wu 1998).

Although the *Phanerochaete* s.s. clade got high support in the phylogenetic tree (100/100/1, Fig. 1), species of this clade sampled in this study show high morphological diversity. For example, hyphal cords, clamp connections and cystidia can be present or totally absent among the species. Meanwhile, it seems that the brown color of the subicular hyphae and hymenial surface is phylogenetically unimportant. Thus, in future, some other characters like chemical reactions should be used, and more species are needed to be sequences to resolve the generic positions of many species in *Phanerochaete* s.l.

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## References

Bernicchia A, Gorjón SP. 2010 – Fungi Europaei 12. Corticiaceae s.l. Edizioni Candusso, Alassio. pp. 489–508.

- Burdsall HH Jr. 1985 – A contribution to the taxonomy of the genus *Phanerochaete*. *Mycologia Memoirs* 10, 1–165.
- Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD. 2003 – Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* 31, 3497–3500.
- de Koker T, Nakasone KK, Haarhof J, Burdsall HH Jr., Janse BJH. 2003 – Phylogenetic relationships of the genus *Phanerochaete* inferred from the internal transcribed spacer region. *Mycological Research* 107, 1032–1040.
- Eriksson J, Hjortstam K, Ryvarde L. 1978 – The Corticiaceae of North Europe vol. 5: *Mycoaciella–Phanerochaete*. *Fungiflora*, Oslo, pp 987–1047.
- Felsenstein J. 1985 – Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* 39, 783–791.
- Floudas D, Hibbett DS. 2015 – Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset and extensive ITS sampling. *Fungal Biology* 119, 679–719.
- Hall TA. 1999 – Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95–98.
- Hillis DM, Bull JJ. 1993 – An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biodivers* 42, 182–192.
- Hjortstam K, Ryvarde L. 1990 – *Lopharia* and *Porostereum* (Corticiaceae). *Synopsis Fungorum* 4, 1–68.
- Hjortstam K, Ryvarde L. 2002 – *Australicum* (Basidiomycotina, Aphyllophorales), a new genus for *Corticium singulare* G. Cunn. *Synopsis Fungorum* 15, 18–21.
- Kornerup A, Wanscher JH. 1978 – *Methuen handbook of colour*. 3rd Ed. E. Methuen and Co., Ltd., London. pp 1–252.
- Nylander JAA. 2004 – MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Ronquist F, Huelsenbeck JP. 2003 – MRBAYES 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Stamatakis A. 2006 – RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Swofford DL. 2002 – PAUP\*: Phylogenetic analysis using parsimony (\*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.), *PCR Protocols: A guide to methods and applications*. Academic Press, San Diego, pp 315–322.
- Wu SH, Nilsson HR, Chen CT, Yu SY, Hallenberg N. 2010 – The white-rotting genus *Phanerochaete* is polyphyletic and distributed throughout the phlebioid clade of the Polyporales (Basidiomycota). *Fungal Diversity* 42, 107–118.
- Wu SH. 1990 – The Corticiaceae (Basidiomycetes) subfamilies Phlebioideae, Phanerochaetoideae and Hyphodermoideae in Taiwan. *Acta Botanica Fennica* 142, 1–123.
- Wu SH. 1995 – A study of the genus *Phanerochaete* (Aphyllophorales) with brown subicular hyphae. *Mycotaxon* 54, 163–172.
- Wu SH. 1998 – Nine new species of *Phanerochaete* from Taiwan. *Mycological Research* 102, 1126–1132.
- Xiong HX, Dai YC. 2009 – Notes on lignicolous corticioid fungi in China 3. *Phanerochaete* (Basidiomycota, Polyporales) in China. *Mycosystema* 28, 29–35.