



## ***Coniophoropsis bambusicola* sp. nov. (Coniophoraceae, Basidiomycota) from southern Vietnam**

YA-NAN ZHAO<sup>1</sup>, SHI-LIANG LIU<sup>1</sup>, KAREN K. NAKASONE<sup>2</sup> & SHUANG-HUI HE<sup>1\*</sup>

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

<sup>2</sup>Center for Forest Mycology Research, Northern Research Station, U.S. Forest Service, Madison, WI 53726-2398, USA

\* Corresponding author's e-mail: [shuanghuihe@yahoo.com](mailto:shuanghuihe@yahoo.com)

### **Abstract**

*Coniophoropsis bambusicola* sp. nov. is described and illustrated from southern Vietnam based on morphological and molecular evidence. It is the second species in the genus, and differs from the type, *C. obscura*, by having much smaller basidiospores measuring  $5.5\text{--}7 \times 4\text{--}5 \mu\text{m}$  and growing on the culms of dead bamboo. The phylogenetic analyses based on a combined dataset of nuc rDNA ITS1-5.8S-ITS2 (ITS) and nuc 28S rDNA (28S) sequences of Boletales show that *Coniophoropsis bambusicola* is closely related to *Coniophora*.

**Keywords:** Bambusicolous fungi, Boletales, *Coniophora*, corticioid fungi, taxonomy

### **Introduction**

The monotypic genus *Coniophoropsis* Hjortstam & Ryvar den (1986: 540) was erected for *C. obscura* Hjortstam & Ryvar den (1986: 540) that has, so far, only been found in Argentina (Hjortstam & Ryvar den 1986). The genus is characterized by having brown, resupinate basidiocarps with a smooth hymenophore, a monomitic hyphal system, simple-septate generative hyphae, and thick-walled, verrucose and cyanophilous basidiospores (Hjortstam & Ryvar den 1986). Except for the ornamented basidiospores, *Coniophoropsis* is very similar to *Coniophora* DC. (1815: 34). Larsson (2007) treated *Coniophoropsis* as *Incertae sedis* in Boletales since the type species had not been (and to date still has not been) sequenced.

In the present paper, we describe and illustrate a new species of *Coniophoropsis* collected from southern Vietnam on bamboo. The phylogenetic position of the new species in the Boletales is inferred from a combined dataset of ITS and 28S sequences.

### **Materials and methods**

#### *Morphological studies*

The studied specimens are deposited at the herbarium of Beijing Forestry University (BJFC), Beijing, China. Freehand sections were made from the dried basidiocarps and mounted in 2% (w/v) potassium hydroxide (KOH), Melzer's reagent or Cotton Blue. Microscopic examination was carried out with a Nikon Eclipse 80i microscope at magnifications up to 1000 $\times$ . Drawings were made with the aid of a drawing tube. 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 number of specimens (b). Color codes and names follow Kornerup & Wanscher (1978).

#### *DNA extraction and sequencing*

A CTAB plant genome rapid extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing, China) was used for DNA extraction and PCR amplification from dried specimens. The primers ITS5 and ITS4 (White *et al.* 1990) were used to amplify the ITS region by using the following cycling protocol: 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 of 72°C for 10 min. The 28S gene region was amplified with primer pair LR0R and LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>), using the following procedure: 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 of 72°C for 10 min. DNA sequencing was performed using the same primers as for the PCR at the Beijing Genomics Institute, and the sequences were deposited in GenBank.

#### Phylogenetic analyses

The molecular phylogeny was inferred from the combined dataset of ITS and 28S sequences of Boletales (Table 1). *Athelia arachnoidea* (Berk.) Jülich (1972: 53), *A. epiphylla* Pers. (1818: 67) and *Jaapia argillacea* Bres. (1911: 428) were selected as the outgroup following Jarosch & Besl (2001), Binder & Hibbett (2006), Binder *et al.* (2010) and Baldoni *et al.* (2012). The sequences were aligned by using MAFFT v.6 (Kato & Toh 2008, <http://mafft.cbrc.jp/alignment/server/>) with a Q-INS-I algorithm.

Maximum parsimony (MP) and Bayesian inference (BI) analyses were performed using PAUP\* v.4.0b10 (Swofford 2002) and MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003), respectively. In MP analysis, gaps were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm. All characters were given equal weight. Branch support for all parsimony analyses was estimated by performing 1000 bootstrap replicates (Felsenstein 1985), with a heuristic search of 10 random-addition replicates for each bootstrap replicate. For BI, best models of evolution were estimated using MrModeltest 2.2 (Nylander 2004), and posterior probabilities were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). Four Markov chains were run from a random starting tree for 3 million generations, and sampled every 100 generations. The first quarter trees representing the burn-in phase of the analysis were discarded.

**TABEL 1.** Species and sequences used in the phylogenetic analyses. Newly generated sequences are set in bold.

Taxa	Voucher	Location	ITS	LSU
Boletales				
<i>Aureoboletus thibetanus</i> (Pat.) Hongo & Nagas.	HKAS 41151	China	DQ200917	AY700189
<i>Boletellus projectellus</i> (Murrill) Singer	AFTOL 713	—	AY789082	AY684158
<i>Boletus edulis</i> Bull.	R380/88	—	—	AF462353
<i>B. pallidus</i> Frost	179/97	USA	DQ534564	AF457409
<i>Bondarcevomyces taxi</i> (Bondartsev) Parmasto	Dai 2524	China	DQ534575	DQ534672
<i>Calostoma cinnabarinum</i> Desv.	AW 136	—	AY854064	AY645054
<i>Chamonixia caespitosa</i> Rolland	92/83	Germany	DQ534565	AF336245
<i>Chroogomphus vinicolor</i> (Peck) O.K. Mill.	TDB 1010	—	DQ533974	AF071529
<i>Coniophora arida</i> (Fr.) P. Karst.	FP 104367	USA	GU187510	GU187573
<b><i>C. arida</i></b>	<b>He 4658</b>	<b>China</b>	<b>MG763875</b>	<b>MH476322</b>
<b><i>C. hanoiensis</i> Pat.</b>	<b>He 5197</b>	<b>Vietnam</b>	<b>MG763873</b>	—
<b><i>C. hanoiensis</i></b>	<b>He 5202</b>	<b>Vietnam</b>	<b>MG763874</b>	<b>MH476323</b>
<i>C. marmorata</i> Desm.	MUCL 31667	Belgium	GU187515	GU187571
<i>C. olivacea</i> (Fr.) P. Karst.	FP 104386	USA	GU187516	GU187572
<b><i>C. olivacea</i></b>	<b>He 2082</b>	<b>USA</b>	<b>MG763871</b>	—
<b><i>C. olivacea</i></b>	<b>He 2990</b>	<b>China</b>	<b>MG763872</b>	—
<i>C. prasinoides</i> (Bourdot & Galzin) Bourdot & Galzin	FP 105969	USA	GU187519	GU187576
<i>C. puteana</i> (Schumach.) P. Karst.	MUCL 1000	Germany	GU187521	GU187578
<b><i>C. puteana</i></b>	<b>He 2909</b>	<b>China</b>	<b>MG763876</b>	<b>MH476324</b>
<b><i>Coniophoropsis bambusicola</i></b>	<b>He 5208</b>	<b>Vietnam</b>	<b>MG763877</b>	<b>MH476325</b>
<b><i>C. bambusicola</i></b>	<b>He 5210</b>	<b>Vietnam</b>	<b>MG763878</b>	<b>MH476326</b>
<i>Gomphidius roseus</i> (Fr.) Fr.	MB 95-038	Germany	DQ534570	DQ534669

.....continued on the next page

TABLE 1. (Continued)

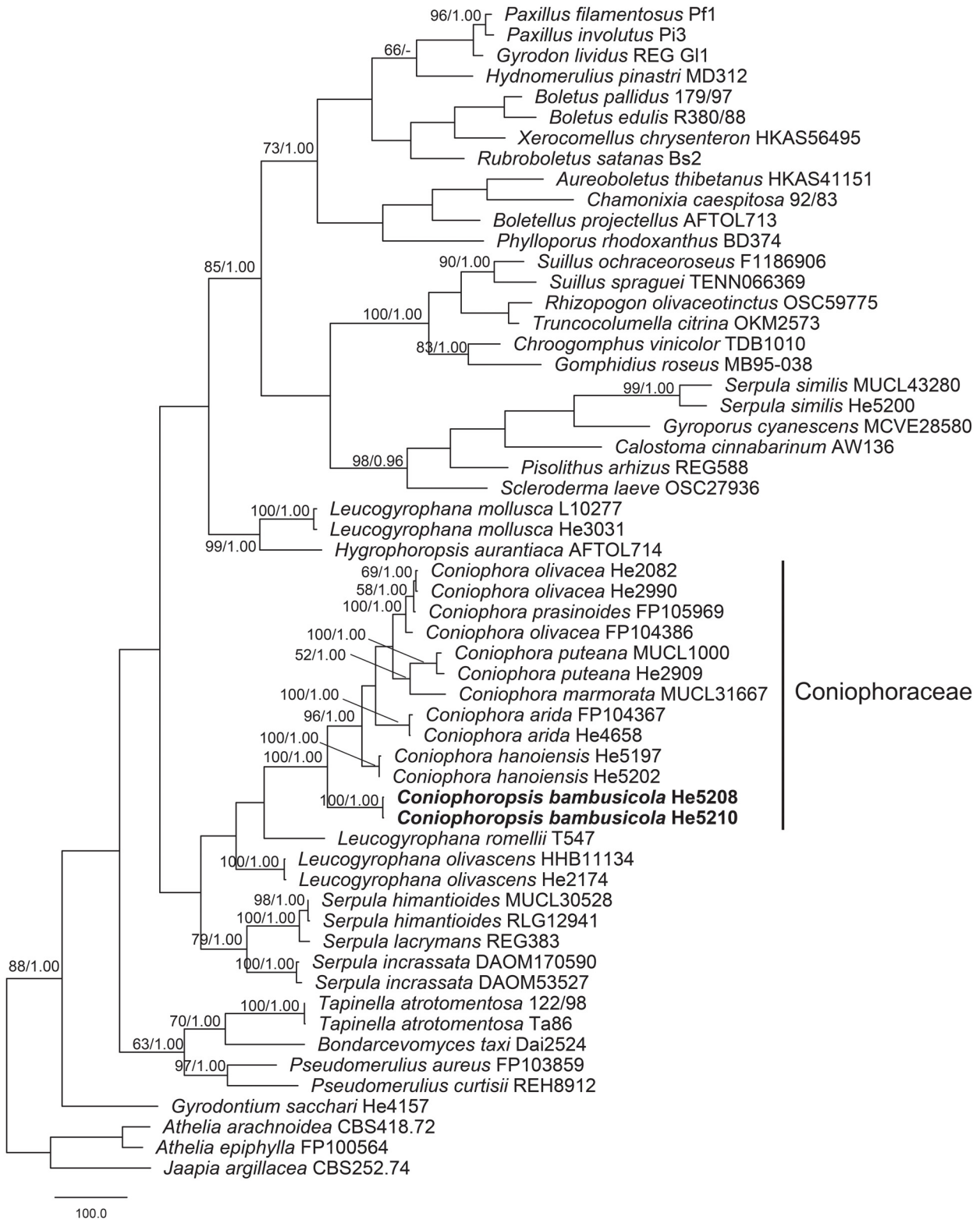
Taxa	Voucher	Location	ITS	LSU
<i>Gyrodon lividus</i>	REG G11	Germany	DQ534568	AF098378
<b><i>Gyrodontium sacchari</i> (Spreng.) Hjortstam</b>	<b>He 4157</b>	<b>Thailand</b>	<b>MG763879</b>	<b>MH476327</b>
<i>Gyroporus cyanescens</i> (Bull.) Quéf.	MCVE 28580	Italy	KT363684	KT363685
<i>Hydnomerulius pinastris</i> (Fr.) Jarosch & Besl	MD 312	USA	GU187523	GU187580
<i>Hygrophoropsis aurantiaca</i> (Wulfen) Maire	AFTOL 714	USA	AY854067	AY684156
<i>Leucogyrophana mollusca</i> (Fr.) Pouzar	L 10277	USA	GU187525	GU187584
<b><i>L. mollusca</i></b>	<b>He 3031</b>	<b>China</b>	<b>MG763881</b>	<b>MH476328</b>
<i>L. olivascens</i> (Berk. & M.A. Curtis) Ginns & Weresub	HHB 11134	USA	GU187532	GU187587
<b><i>L. olivascens</i></b>	<b>He 2174</b>	<b>USA</b>	<b>MG763882</b>	<b>MH476329</b>
<i>L. romellii</i> Ginns	T 547	Canada	GU187529	GU187586
<i>Paxillus filamentosus</i> (Scop.) Fr.	Pf 1	—	—	AF167680
<i>P. involutus</i> (Batsch) Fr.	Pi 3	—	—	AF167682
<i>Phylloporus rhodoxanthus</i> (Schwein.) Bres.	BD 374	USA	JN021070	HQ161851
<i>Porphyrellus porphyrosporus</i> (Fr. & Hök) E.-J. Gilbert	MB 97-023	Germany	DQ534563	DQ534643
<i>Pseudomerulius aureus</i> (Fr.) Jülich	FP 103859	USA	GU187534	GU187590
<i>P. curtisii</i> (Berk.) Redhead & Ginns	REH 8912	Australia	GU187533	GU187589
<i>Rhizopogon olivaceotinctus</i> A.H. Sm.	OSC 59775	USA	KT968553	KT968609
<i>Rubroboletus satanas</i> (Lenz) Kuan Zhao & Zhu L. Yang	Bs2	Germany	DQ534567	AF336242
<i>Scleroderma laeve</i> Lloyd	OSC 27936	—	EU718120	DQ683003
<i>Serpula himantioides</i> (Fr.) P. Karst.	MUCL 30528	Belgium	GU187545	GU187600
<i>S. himantioides</i>	RLG 12941	USA	GU187547	GU187602
<i>S. incrassata</i> (Berk. & M.A. Curtis) Donk	DAOM 170590	Canada	GU187541	GU187595
<i>S. incrassata</i>	DAOM 53527	Canada	HM135648	HM135698
<i>S. lacrymans</i>	REG 383	—	GU187542	GU187596
<i>S. similis</i> (Berk. & Broome) Ginns	MUCL 43280	Cameroon	GU187546	GU187601
<b><i>S. similis</i></b>	<b>He 5200</b>	<b>Vietnam</b>	<b>MG763880</b>	—
<i>Suillus ochraceoroseus</i> (Snell) Singer	F 1186906	USA	KU721258	KU721404
<i>S. spraguei</i> (Berk. & M.A. Curtis) Kuntze	TENN 066369	USA	KU721523	KU721502
<i>Tapinella atrotomentosa</i> (Batsch) Šutara	122/98	USA	GU187550	AY177260
<i>T. atrotomentosa</i>	Ta 86	USA	DQ534573	AF352048
<i>Truncocolumella citrina</i> Zeller	OKM 2573	—	—	AY612831
<i>Xerocomellus chrysenteron</i> (Bull.) Šutara	HKAS 56495	Germany	KC215210	KC215211
Outgroup				
<i>Athelia arachnoidea</i> (Berk.) Jülich	CBS 418.72	Netherlands	GU187504	GU187557
<i>A. epiphylla</i> Pers.	FP 100564	USA	GU187501	GU187558
<i>Jaapia argillacea</i> Bres.	CBS 252.74	Netherlands	GU187524	GU187581

## Results

### Phylogeny

The combined dataset contained 53 ITS and 53 28S sequences from 57 samples representing 42 species of Boletales and three outgroup taxa, of which 12 ITS and 8 28S sequences were newly generated and submitted to GenBank (ITS: MG763871–MG763882, 28S: MH476322–MH476329, Table 1). The dataset had an aligned length of 2381 characters, of which 1212 characters were constant, 286 variable characters were parsimony-uninformative, and 883

characters were parsimony informative. Fifty-six equally parsimonious trees were obtained in the MP analysis. The best-fit model for the dataset was GTR+I+G. The average standard deviation of split frequencies was 0.009680. The topologies of trees obtained from MP and BI were almost the same. Only the MP tree is shown in Fig. 1 with maximum parsimony bootstraps (PB)  $\geq 50\%$  and Bayesian posterior probabilities (PP)  $\geq 0.95$  labelled along the branches. In the tree, samples of *Coniophoropsis bambusicola* formed a lineage sister to *Coniophora* species with high support values (PB = 100%, PP = 1.00).



**FIGURE 1.** Phylogenetic tree inferred from maximum parsimony analysis based on the combined dataset of ITS and 28S sequences of Boletales species. Branches are labelled with maximum parsimony bootstraps  $\geq 50\%$  and Bayesian posterior probabilities  $\geq 0.95$ .



## Taxonomy

*Coniophoropsis bambusicola* S.H. He & Nakasone, *sp. nov.* (Figs. 2–4)

MycoBank: MB 824360

Diagnosis:—The species differs from *C. obscura* by having much smaller basidiospores measuring  $5.5\text{--}7 \times 4\text{--}5 \mu\text{m}$ .

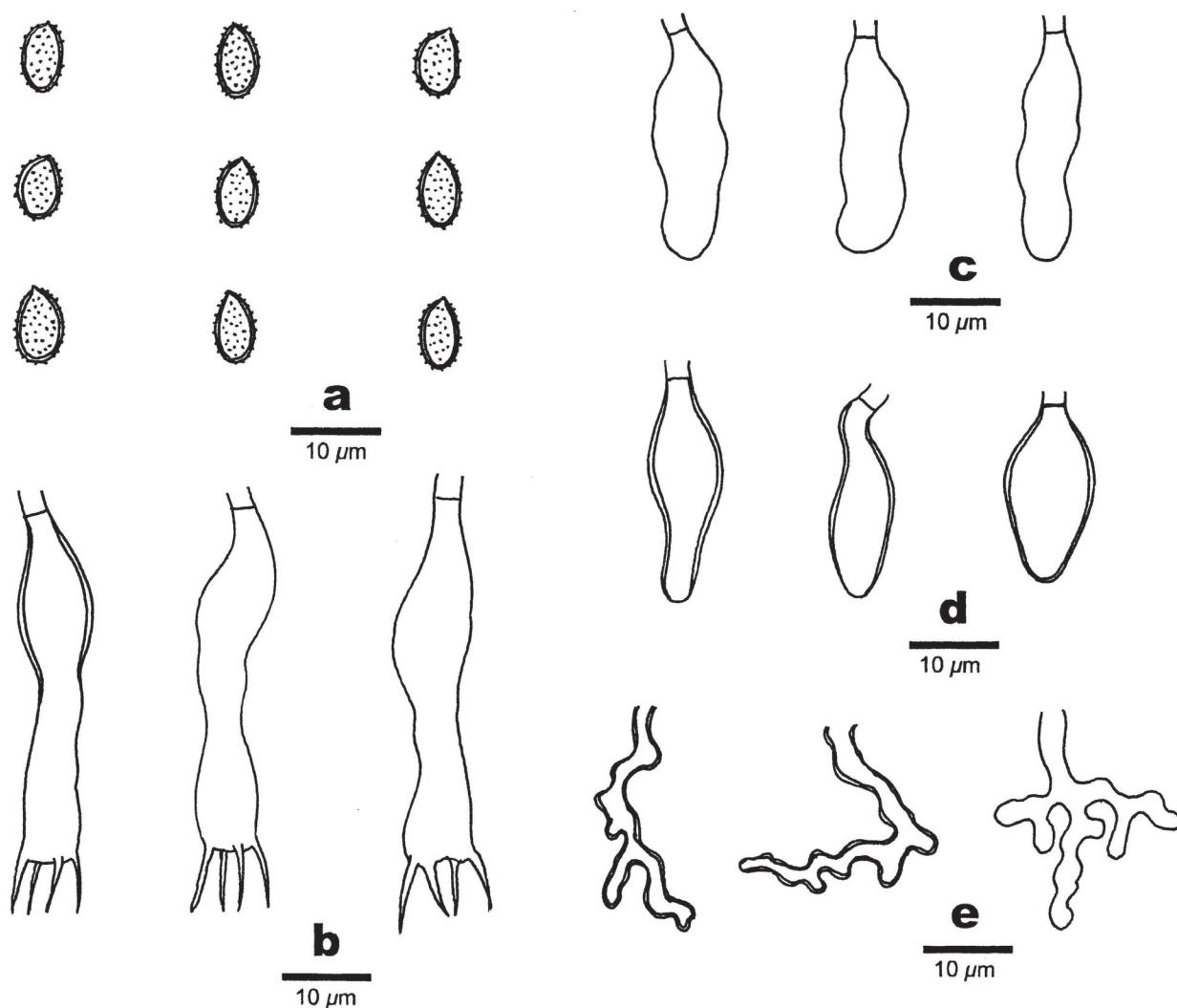
Type:—VIETNAM. Thac Mai Preservation Park, lowland rain forest Dipterocarp, on culm of dead bamboo, 14 October 2017, He 5208 (BJFC 024726, holotype).



**FIGURE 2.** Basidiocarps of *Coniophoropsis bambusicola* (He 5208, holotype). Bar: 1 cm. Photos by Shuang-Hui He.

Etymology:—“*bambusicola*” (Lat.) refers to growing on bamboo.

Description:—*Basidiocarps* annual, resupinate, effused, closely adnate, inseparable from substrate, coriaceous to crustose, first as irregular small patches, later confluent up to 20 cm long, 4 cm wide, 50–180  $\mu\text{m}$  thick. *Hymenophore surface* smooth, greyish brown [6(D–F)3], brown [6E(4–8)] to dark brown [6F(4–8)], not cracked; *margin* thinning, indeterminate, indistinct, concolorous or slightly darker than hymenophore surface. *Hyphal system* monomitic. *Subiculum* very thin to absent. *Subhymenium* thickening, yellowish brown; generative hyphae in this layer simple-septate, without verticillate clamps, hyaline to mostly yellowish brown, thin- to thick-walled, with a wide lumen, densely interwoven, agglutinated, frequently branched and septate, 2–4  $\mu\text{m}$  in diam. *Cystidia* abundant, ventricose, slightly thick-walled, 18–25  $\times$  8–10  $\mu\text{m}$ . *Dendrohyphidia* numerous, hyaline, thin- to slightly thick-walled, usually torulose. *Basidia* subcylindrical to suburniform with a distinct median constriction, tapering toward the base, thin-walled or slightly thick-walled at the base, hyaline, with four sterigmata and a basal simple septum, 32–40  $\times$  5–8  $\mu\text{m}$ ; *basidioles* similar to basidia but smaller. *Basidiospores* subfusiform to ellipsoid, with an apiculus, hyaline to pale yellow, thick-walled, verrucose, without color change in Melzer’s reagent, cyanophilous, 5.5–7 (–7.5)  $\times$  4–5 (–5.5)  $\mu\text{m}$ , L = 6.3  $\mu\text{m}$ , W = 4.7  $\mu\text{m}$ , Q = 1.3 (n = 30/1).

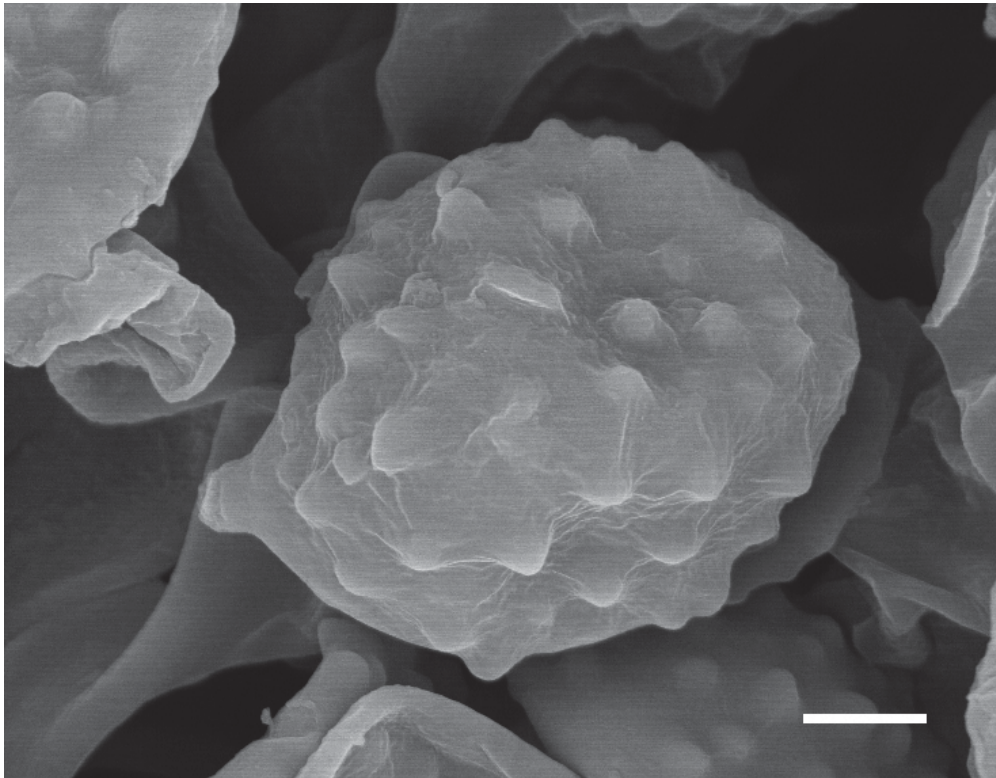


**FIGURE 3.** Microscopic structures of *Coniophoropsis bambusicola* (from the holotype). a. Basidiospores. b. Basidia. c. Basidioles. d. Gloeocystidia. e. Dendrohyphidia. Bars: 10 µm. Drawings by Shi-Liang Liu.

Additional specimen examined:—VIETNAM. Thac Mai Preservation Park, lowland rain forest Dipterocarp, on culm of dead bamboo, 14 October 2017, *He 5210* (BJFC 024726).

Remarks:—*Coniophoropsis bambusicola* is characterized by having thin, brown resupinate basidiocarps with a smooth hymenophore, simple-septate generative hyphae, ventricose cystidia, hyaline dendrohyphida, ellipsoid, thick-walled and verrucose basidiospores, and growing on bamboo in the tropics. It is the second species in the genus. *Coniophoropsis obscura*, the generic type, can be easily distinguished from the *C. bambusicola* by having much larger basidiospores (15–18 × 7.5–9 µm), the absence of cystidia and a distribution in South America (Hjortstam & Ryvarden 1986). In the ITS-28S phylogenetic tree of Boletales, *Coniophoropsis bambusicola* and the *Coniophora* species formed a fully supported clade (Fig. 1). However, morphologically *Coniophoropsis* can be easily distinguished from *Coniophora* by having ornamented and non-dextrinoid basidiospores, and absence of verticillate or scattered clamps on the basal hyphae (Ginns 1982, Hjortstam & Ryvarden 1986).





**FIGURE 4.** Scanning electron microscope (SEM) of a basidiospore of *Coniophoropsis bambusicola* (from the holotype). Bar: 1  $\mu$ m. Photos by Shi-Liang Liu.

### Acknowledgements

This study was supported by the Fundamental Research Funds for the Central Universities (No. 2017PT09) and the National Natural Science Foundation of China (Nos. 31470144 & 31670013).

### References

- Baldoni, D.B., Coelho, G., Jacques, R.J.S., Silveira, R.M.B., Grebenc, T. & Antoniolli, Z.I. (2012) Brown rotting fungus closely related to *Pseudomerulius curtisii* (Boletales) recorded for the first time in South America. *Mycosphere* 3: 533–541.  
<https://doi.org/10.5943/mycosphere/3/5/1>
- Binder, M. & Hibbett, D.S. (2006) Molecular systematics and biological diversification of Boletales. *Mycologia* 98: 971–981.  
<https://doi.org/10.1080/15572536.2006.11832626>
- Binder, M., Larsson, K.H., Matheny, P.B. & Hibbett, D.S. (2010) Amylocorticiales ord. nov. and *Mycologia* 102: 865–880.  
<https://doi.org/10.3852/09-288>
- Jaapiales ord. nov.: early diverging clades of agaricomycetidae dominated by corticioid forms.
- Bresadola, G. (1911) Adnotanda mycologica. *Annales Mycologici* 9: 425–428.
- De Candolle, A.P. (1815) *Flore française* 6: 1–662.
- Felsenstein, J. (1985) Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* 39: 783–791.  
<https://doi.org/10.2307/2408678>
- Ginns, J. (1982) A monograph of the genus *Coniophora* (Aphyllphorales, Basidiomycetes). *Opera Botanica* 61: 1–61.
- Hjortstam, K. & Ryvarden, L. (1986) Some new and noteworthy fungi (Aphyllphorales, Basidiomycetes) from Iguazu, Argentina. *Mycotaxon* 25: 539–567.
- Jarosch, M. & Besl, H. (2001) *Leucogyrophana*, a polyphyletic genus of the order Boletales (Basidiomycetes). *Plant Biology* 3: 443–448.  
<https://doi.org/10.1055/s-2001-16455>

- Jülich, W. (1972) Monographie der Athelieae (Corticaceae, Basidiomycetes). *Willdenowia Beiheft* 7: 1–283.
- Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* 9: 286–298.  
<https://doi.org/10.1093/bib/bbn013>
- Kornerup, A. & Wanscher, J.H. (1978) *Methuen handbook of colour*. 3rd Ed. Eyre Methuen, London, pp. 1–252.
- Larsson, K.H. (2007) Rethinking the classification of corticioid fungi. *Mycological Research* 111: 1010–1063.  
<https://doi.org/10.1016/j.mycres.2007.08.001>
- Nylander, J.A.A. (2004) *MrModeltest 2.2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.
- Persoon, C.H. (1818) *Traité sur les Champignons Comestibles*. 276 pp.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.  
<https://doi.org/10.1093/bioinformatics/btg180>
- Swofford, D.L. (2002) *PAUP\*: Phylogenetic analysis using parsimony (\*and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- White, T.J., Bruns, T.D., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols, a guide to methods and applications*. Academic, San Diego, pp. 315–322.  
<https://doi.org/10.1016/B978-0-12-372180-8.50042-1>