### ORIGINAL ARTICLE



# DGfM

# *Phylloporia minutipora and P. radiata* spp. nov. (Hymenochaetales, Basidiomycota) from China and a key to worldwide species of *Phylloporia*

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Abstract *Phylloporia* is a monophyletic genus within the Hymenochaetaceae as recovered by nuclear large subunit ribosomal DNA (nLSU) sequences. According to the summarization of 38 species accepted in this genus, Phylloporia is characterized by an absence of setae and the presence of abundant thick-walled, colored and tiny basidiospores, although its other morphological characters are highly diverse. Nine herbarium specimens from China, fitting the morphological concept of Phylloporia, were morphologically and phylogenetically studied in detail. The phylogeny inferred from nLSU sequences shows that the nine specimens formed three terminal lineages within the Phylloporia clade. Two lineages being composed of four specimens from Hainan and three from Guizhou were newly described as Phylloporia minutipora and P. radiata, respectively. In Phylloporia, P. minutipora is distinct by a combination of annual, sessile and imbricate basidiocarps, distinctly concentrically sulcate pileal surface with obtuse margin, angular pores of 12–15 per mm, duplex context separated by a black zone, a dimitic hyphal system, and broadly ellipsoid basidiospores of  $2.5-3 \times 2-2.5 \mu m$ , while P. radiata is distinct by a combination of annual, sessile and imbricate basidiocarps, faintly sulcate and radially striate pileal surface, sharp pileal margin, angular pores of 8-10 per mm, duplex context separated by a black zone, a monomitic hyphal system, and broadly ellipsoid basidiospores of 2.5- $3.5 \times 2-2.5$  µm. The third lineage, comprising two specimens from Hainan, was morphologically determined as Phylloporia pulla. This species was recently combined to Phylloporia

Li-Wei Zhou liwei\_zhou1982@163.com based on only morphological characters, and the current study for the first time generated its molecular sequences for phylogenetic reference. A key to all 40 species of *Phylloporia* is provided.

**Keywords** Hymenochaetaceae · Phylogenetic analysis · Polypore · Taxonomy

# Introduction

*Phylloporia* Murrill was introduced as a monotypic genus, with *P. parasitica* Murrill growing on a living leaf as generic type (Murrill 1904). Since Wagner and Ryvarden (2002) redefined the concept of *Phylloporia* with descriptions of species, a key and a preliminary phylogenetic analysis, many species have been newly introduced for *Phylloporia* with the aid of molecular phylogeny (Valenzuela et al. 2011; Zhou and Dai 2012; Decock et al. 2013, 2015; Gafforov et al. 2014; Yombiyeni et al. 2015; Zhou 2015b, c). A total of 38 species were accepted in *Phylloporia* before the current study, 15 of which were originally described from China (Cui et al. 2010; Zhou and Dai 2012; Zhou 2013, 2015b, c; Liu et al. 2015).

*Phylloporia* is a monophyletic genus within the Hymenochaetaceae as recovered by nuclear large subunit ribosomal DNA (nLSU) sequences (Decock et al. 2015; Zhou 2015b). According to the summarization of current accepted species, the diagnostic characters for *Phylloporia* within the Hymenochaetaceae are a lack of setae and the presence of abundant thick-walled, colored and tiny basidiospores. However, its other morphological characters are highly diverse. For instance, the habit could be annual or perennial, the basidiocarps stipitate or sessile, the context homogeneous or duplex, the duplex context separated by a black zone or not, and the hyphal system could be monomitic or dimitic. Most

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species of *Phylloporia* appear to colonize on specific living host genera as potential forest pathogens, while a few species grow on dead wood.

Although the diversity and phylogeny of Hymenochaetaceae have been extensively explored in China, resulting in newly introducing several genera and many species (Dai 2010; Zhou and Xue 2012; Zhou and Qin 2013; Wu et al. 2015; Zhou 2015a; Zhou et al. 2016a, b), there are still many unidentified Chinese hymenochaetoid specimens at the herbaria of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP) and the Institute of Microbiology, Beijing Forestry University (BJFC). When reexamining this kind of specimen, several specimens appeared to fit well with the concept of *Phylloporia*. After morphological examination and phylogenetic analysis, these specimens were identified to represent *Phylloporia pulla* (Mont. & Berk.) Decock & Yombiyeni, and two undescribed species that are newly introduced in the current study. In addition, an identification key to worldwide species of *Phylloporia* is provided.

### Materials and methods

### Morphological examination

The microscopic procedure followed Dai (2010). Specimen sections, stained in cotton blue (CB), Melzer's reagent (IKI) and 5 % potassium hydroxide (KOH), were examined using a Nikon Eclipse 80i microscope at magnification up to 1000× under phase contrast illumination. All measurements were taken from sections under CB. When presenting basidiospore size variations, 5 % of the measurements were excluded from each end of the range and are given in parentheses. Special color terms follow Petersen (1996). Microscopic structures were drawn with the aid of a drawing tube. In the text, L stands for mean basidiospore length (arithmetic average of all basidiospores), Q for the variation of L/W ratio between the specimens studied, and n for number of basidiospores measured from a given number of specimens.

### Molecular sequencing

The nLSU region was directly amplified from herbarium specimens using the Phire<sup>®</sup> Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's instructions, with primers LR0R and LR7 (Vilgalys and Hester 1990). The PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 48 °C for 5 s and 72 °C for 5 s, and a final extension at 72 °C for 10 min. The PCR products were sequenced using primers LR0R, LR7, LR3 and LR3R (Vilgalys and Hester 1990) at the Beijing Genomics Institute, China. All newly generated sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Fig. 1).

### **Phylogenetic analysis**

To explore the phylogenetic positions of the newly sequenced specimens, their nLSU sequences were incorporated into the data sets of previous phylogenetic studies (Decock et al. 2015; Zhou 2015b). Inonotus hispidus (Bull.) P. Karst. was selected as the outgroup (Zhou 2014). The related information to sequences included in the current nLSU data set is summarized in Table 1. The current nLSU data set was aligned using MAFFT 7.110 (Katoh and Standley 2013) with the Q-INS-i option (Katoh and Toh 2008) and the resulting alignment was deposited in TreeBASE (http://www.treebase.org; accession numbers S19032). The best-fit evolutionary model of the alignment was estimated using jModelTest 2.1.4 (Guindon and Gascuel 2003; Darriba et al. 2012), and was set for subsequent phylogenetic analysis. Phylogenetic analysis was performed under maximum likelihood (ML) and Bayesian inference (BI) algorithms. ML algorithm was inferred using raxmlGUI 1.2 (Stamatakis 2006; Silvestro and Michalak 2012). The bootstrap (BS) values were tested under the auto FC option (Pattengale et al. 2010). BI algorithm was conducted using MrBayes 3.2 (Ronquist and Huelsenbeck 2003). Two independent runs with a Metropolis-coupled Markov chain Monte Carlo method were employed. Each run with four chains for 10 million generations starts from random trees. Trees were sampled every 1000th generation. Of the sampling trees, the first 25 % were discarded as burn in, whereas all remaining trees were used to construct a 50 % majority consensus tree and for calculating Bayesian posterior probabilities (BPPs).

### Results

### Molecular phylogeny

The nLSU sequences were newly sequenced from nine specimens (Fig. 1). The current nLSU data set of 119 sequences resulted in an alignment with 951 characters. Its best-fit evolutionary model was estimated as GTR+I+G. The BS search for ML algorithm stropped after 250 replicates. All chains converged in BI algorithm after 10 million generations, where the effective sample sizes of all parameters were more than 1000 and the potential scale reduction factors were close to 1.000. ML and BI algorithms constructed congruent topologies in main lineages, and thus only the topology from the ML algorithm is presented along with statistical values from both algorithms at the nodes.

The current phylogeny inferred from nLSU data set (Fig. 1) shows that the newly sequenced specimens fell into the strongly

Fig. 1 Phylogenetic positions of *Phylloporia minutipora*, *P. pulla* and *P. radiata* inferred from the nLSU data set. The topology is from the maximum likelihood algorithm with bootstrap values and Bayesian posterior probabilities, respectively, from maximum likelihood and Bayesian inference algorithms, if simultaneously above 50 % and 0.8, at the nodes. The newly sequenced specimens are in boldface



supported *Phylloporia* clade (96 %/1) as three new terminal lineages. The lineage comprising specimens Dai 9627 and Cui 5251 (100 %/1) is determined as *P. pulla*, while the other two, being composed of specimens Dai 9257, LWZ 20150531-13, LWZ 20150531-14 and LWZ 20150531-15 (100 %/1), and LWZ 20141122-5, LWZ 20141122-6 and LWZ 20141122-19 (99 %/1), are described as two new species.

### Taxonomy

*Phylloporia minutipora* L.W. Zhou, **sp. nov.** (Figs. 2 and 3). MycoBank no.: MB 816182

*Holotype*: China, Hainan, Jianfengling National Nature Reserve, on the base of a living angiosperm tree, 17 November 2007, Dai 9257 (IFP).

 Table 1
 Information about GenBank accession numbers for nLSU sequences, origin, hosts, and voucher numbers of the specimens used in the phylogenetic analysis

Species	Voucher collections	Hosts	Origin	Accession number
Coltricia cf. stuckertiana (Speg.) Rajchenb. & J.E. Wright	Robledo 218	Angiosperm	Argentina	KC136220
C. cf. stuckertiana	Robledo 219	Angiosperm	Argentina	KC136219
C. cf. stuckertiana	Robledo 281	Angiosperm	Argentina	KC136221
Fomitiporella cavicola (Kotl. & Pouzar) T. Wagner & M. Fisch.	N 153	Fagus sylvatica	UK	AY059052
F. umbrinella (Bres.) Murrill	CBS 303.66	Deciduous wood	Georgia, USA	AY059036
Fulvifomes fastuosus (Lév.) Bondartseva & S. Herrera	CBS 213.36	Gliricidia	Philippines, USA	AY059057
F. robiniae (Murrill) Murrill	CBS 211.36	Robinia pseudo-acacia	Maryland, USA	AF411825
P. afrospathulata Yombiyeni & Decock	MUCL 53983 (Paratype)	Angiosperm	Gabon	KJ743249
P. afrospathulata	MUCL 54511 (Isotype)	Angiosperm	Gabon	KJ743248
P. bibulosa (Lloyd) Ryvarden	Ahmad 27088	Peristropha bicalyculata	Pakistan	AF411824
P. chrysites (Berk.) Ryvarden	N.W. Legon	Dead root	Puerto Rico	AF411821
P. chrysites	MUCL 52763	-	Mexico	HM635665
P. Chrysiles	MUCL 52/64		Mexico	HM033000
P. Chrysties	MUCL 52862	Neopringie	Mexico	HM03300/
P. clausende L.W. Zhou	Vian 2528 (Paratima)	<i>Clausena</i>	Hainan, China	KJ/8//90
P. clausende P. cuatecci I. W. Zhou & V.C. Doi	Pai 11014 (Heletime)	Crista cours	Funnan, China	KJ/8//95
P. crataegi	Dai 11014 (Holotype)	Cratagus	Liaoning, China	JF/12922 JE712023
P. cylindrispora I. W. Zhou	Vuan 6144 (Holotype)	Angiosperm	Guangyi China	K 1787707
P cylindrispora	Yuan 6148 (Paratyne)	Angiosperm	Guangxi, China	K 1787798
P dependens VC Dai	Dai 13167 (Holotype)	Angiosperm	Yunnan China	KP698746
P enhedrae (Woron) Parmasto	ТАА 72-2	Enhedra	Turkmenistan	AF411826
P flabelliforma Decock & Yombiyeni	MUCL 55568 (Paratype)	Dichostemma	Gabon	KU198350
P. flabelliforma	MUCL 55569 (Isotype)	Dichostemma	Gabon	KU198349
P. flabelliforma	MUCL 55570 (Paratype)	Dichostemma	Gabon	KU198351
P. flacourtiae L.W. Zhou	Yuan 6204 (Paratype)	Angiosperm	Guangxi, China	KJ787799
P. flacourtiae	Yuan 6360 (Paratype)	Angiosperm	Guangxi, China	KJ787800
P. flacourtiae	Yuan 6362 (Paratype)	Angiosperm	Guangxi, China	KJ787801
P. flacourtiae	Zhou 140 (Holotype)	Flacourtia	Guangxi, China	KJ787802
P. fontanesiae L.W. Zhou & Y.C. Dai	Li 194 (Paratype)	Fontanesia	Henan, China	JF712924
P. fontanesiae	Li 199 (Holotype)	Fontanesia	Henan, China	JF712925
P. cf. fruticum (Berk. & M.A. Curtis) Ryvarden	ENCB TR&RV858	_	Mexico	HM635669
P. cf. fruticum	MUCL 52762	_	Mexico	HM635668
P. cf. fruticum	MUCL 52863	-	Mexico	HM635670
P. fulva Yombiyeni & Decock	MUCL 54472 (Isotype)	Angiosperm	Gabon	KJ743247
P. gabonensis Decock & Yombiyeni	MUCL 55571 (Paratype)	Dichostemma	Gabon	KU198352
P. gabonensis	MUCL 55572 (Isotype)	Dichostemma	Gabon	KU198353
P. gutta L.W. Zhou & Y.C. Dai	Dai 4103 (Paratype)	Angiosperm	Sichuan, China	JF712926
P. gutta	Dai 4197 (Holotype)	Abelia	Sichuan, China	JF712927
P. hainaniana Y.C. Dai & B.K. Cui	Dai 9460 (Holotype)	Angiosperm	Hainan, China	JF/12928
P. homocarnica L.w. Zhou	Yuan 5750 (Holotype)	Angiosperm	Guangxi, China	KJ/8/803
P. nomocarnica D inconstaides Vembiueni & Decestr	MUCL 54468 (Isotume)	Crotonomino manufana	Guangxi, Unina Gabon	KJ/8/804
P. inonotoidas	MUCL 54468 (Isotype)	Crotonogyne manniana Crotonogyne manniana	Gabon	KJ743230 K 1743251
P inonotoides	MUCL 54470 (Paratype)	Crotonogyne manniana Crotonogyne manniana	Gabon	K 1743251
P minutinora I W Zhou	Dai 9257 (Holotype)	Angiosperm	Hainan China	KU904464
P minutipora	LWZ 20150531-13 (Paratype)	Angiosperm	Hainan, China	KU904465
P minutipora	LWZ 20150531-14 (Paratype)	Angiosperm	Hainan, China	KU904466
P. minutipora	LWZ 20150531-15 (Paratype)	Angiosperm	Hainan, China	KU904467
P. minutispora Inulet & Ryvarden	Inulet 706 (Isotype)	Ground	Uganda	JF712929
P. minutispora	MUCL 52865	Ground	COD	HM635671
P. nandinae L.W. Zhou & Y.C. Dai	Dai 10588 (Holotype)	Nandina domestica	Jiangxi, China	JF712930
P. nandinae	Dai 10625 (Paratype)	Nandina domestica	Jiangxi, China	JF712931
P. nouraguensis Decock & Castillo	MUCL 53816 (Holotype)	Myrcia	French Guiana	KC136222
P. nouraguensis	MUCL 53817 (Paratype)	Myrcia	French Guiana	KC136223
P. nouraguensis	MUCL 53818 (Paratype)	Myrcia	French Guiana	KC136224
P. oblongospora Y.C. Dai & H.S. Yuan	Zhou 179 (Holotype)	Angiosperm	Guangxi, China	JF712932
P. oreophila L.W. Zhou & Y.C. Dai	Cui 2219 (Paratype)	Angiosperm	Gansu, China	JF712933
P. oreophila	Cui 9503 (Holotype)	Angiosperm	Tibet, China	JF712934
P. osmanthi L.W. Zhou	Yuan 5655 (Holotype)	Osmanthus	Guangxi, China	KF729938

### Table 1 (continued)

Species	Voucher collections	Hosts	Origin	Accession number
P. pectinata (Klotzsch) Ryvarden	R. Coveny 113	Rhodania rubescens	Australia	AF411823
P. pulla (Mont. & Berk.) Decock & Yombiyeni	Cui 5251	Angiosperm	Hainan, China	KU904468
P. pulla	Dai 9627	Angiosperm	Hainan, China	KU904469
P. radiata L.W. Zhou	LWZ 20141122-5 (Paratype)	Liana	Guizhou, China	KU904470
P. radiata	LWZ 20141122-6 (Holotype)	Liana	Guizhou, China	KU904471
P. radiata	LWZ 20141122-19 (Paratype)	Liana	Guizhou, China	KU904472
P. resupinata Douanla-Meli & Ryvarden	Douanla-Meli 476 (Isotype)	Entandrophragma cylindricum	Cameroon	JF712935
P. ribis (Schumach.) Ryvarden	MF 82-828	Ribes uva-crispa	Germany	AF311040
P. rzedowskyi R. Valenz. & Decock	ENCB RV8750 (Holotype)	Hybanthus mexicanus	Mexico	HM635672
P. rzedowskyi	MUCL 52859 (Paratype)	Hybanthus mexicanus	Mexico	HM635673
P. rzedowskyi	MUCL 52860 (Paratype)	Hybanthus mexicanus	Mexico	HM635674
P. rzedowskyi	MUCL 52861 (Paratype)	Hybanthus mexicanus	Mexico	HM635675
P. spathulata (Hook.) Ryvarden	Chay 456	Apocynaceae	Mexico	AF411822
P. sp.	ICN/ISA 007	_	Brazil	KJ743265
P. sp.	ICN/ISA 117	_	Brazil	KJ743271
P. sp.	ICN/ISA 333	_	Brazil	KJ743272
<i>P</i> . sp.	ICN/ISA 352	_	Brazil	KJ743267
P. sp.	ICN/ISA 553	_	Brazil	KJ743266
P. sp.	ICN/ISA 555	_	Brazil	KJ743274
P. sp.	ICN/ISA 610	_	Brazil	KJ743273
P. sp.	ICN/ISA G70	_	Brazil	KJ743275
P. sp.	MUCL 43733	_	Cuba	KJ743278
P. sp.	MUCL 45062	Angiosperm	Cuba	KJ743284
P. sp.	MUCL 52684	Angiosperm	Ecuador	KJ743276
P. sp.	MUCL 52864	Angiosperm	Ecuador	HM635676
P. sp.	MUCL 53433	Angiosperm	Mexico	KC136231
P. sp.	MUCL 54288	_	Brazil	KJ743268
<i>P</i> . sp.	MUCL 54295	_	Brazil	KJ743269
P. sp.	MUCL/CU-05-249	Angiosperm	Cuba	KJ743282
P. sp.	MUCL/FG-10-321	Angiosperm	French Guiana	KJ743277
P. sp.	MUCL/FG-11-462	Angiosperm	French Guiana	KC136228
P. sp.	MUCL/FG-11-506	Angiosperm	French Guiana	KC136227
P. sp.	MUCL/FG-11-506	Angiosperm	French Guiana	KJ743258
P. sp.	MUCL/FG-12-522	Angiosperm	French Guiana	KJ743259
P. sp.	MUCL/FG-12-523	Angiosperm	French Guiana	KJ743260
P. sp.	MUCL/GA-12-812	Angiosperm	Gabon	KJ743281
P. sp.	MUCL/GA-12-813	Angiosperm	Gabon	KJ743253
<i>P</i> . sp.	MUCL/GA-12-814	Angiosperm	Gabon	KJ743256
P. sp.	MUCL/GA-12-815	Angiosperm	Gabon	KJ743257
P. sp.	MUCL/GA-12-816	Angiosperm	Gabon	KJ743255
P. sp.	MUCL/GA-12-846	Angiosperm	Gabon	KJ743254
P. sp.	MUCL/FG-13-670	Angiosperm	French Guiana	KJ743262
P. sp.	MUCL/FG-13-754	Angiosperm	French Guiana	KJ743261
P. sp.	MUCL/FG-13-721	Angiosperm	French Guiana	KJ743263
P. sp.	MUCL/FG-13-722	Angiosperm	French Guiana	KJ743264
P. sp.	MUCL/FG-13-726	Angiosperm	French Guiana	KJ743279
P. sp.	MUCL/FG-13-749	Angiosperm	French Guiana	KJ743280
P. sp.	MUCL/YOM 5	Liana	Gabon	KJ743283
P. sp.	Robledo 351	Angiosperm	Argentina	KC136226
P. sp.	Robledo 1220	Angiosperm	Argentina	KC136225
P. terrestris L.W. Zhou	Yuan 5738 (Holotype)	Ground	Guangxi, China	KC778784
P. tiliae L.W. Zhou	Yuan 5491 (Holotype)	Tilia	Hunan, China	KJ787805
P. ulloai R. Valenz., T. Raymundo, Cifuentes & Decock	MUCL 52866 (Paratype)	Liana	Mexico	HM635677
P. ulloai	MUCL 52867 (Holotype)	Liana	Mexico	HM635678
P. ulloai	MUCL 52870 (Paratype)	Liana	Mexico	HM635679
P. weberiana (Bres. & Henn. ex Sacc.) Ryvarden	Dai 9242	Angiosperm	Hainan, China	JF712936
P. yuchengii Yu.Sh. Gafforov, Tomšovský, E. Langer & L.W. Zhou	YG 033 (Holotype)	Angiosperm	Uzbekistan	KM264324
P. yuchengii Outgroup	YG 051 (Paratype)	Angiosperm	Uzbekistan	KM264325
Inonotus hispidus (Bull.) P. Karst.	MF 92-829	Fraxinus excelsior	Germany	AF311014

*Etymology: minutipora* (Lat.): referring to the extremely small pores.

Basidiocarps annual, sessile, imbricate, without odor or taste, woody. Pilei dimidiate to flabelliform, fused together,



Fig. 2 Basidiocarps of Phylloporia minutipora (Dai 9257). Scale bar: 2 cm

applanate, single pileus projecting up to 10 cm, 7 cm wide and 0.5 cm thick at base. *Pileal surface* yellowish brown to dark brown, velutinate, distinctly concentrically sulcate with narrow to wide zones; *margin* honey-yellow, obtuse. *Pore surface* honey-yellow, more or less shining; *sterile margin* 



**Fig. 3** Microscopic structures of *Phylloporia minutipora* (drawn from the holotype). **a**. Basidiospores. **b**. Basidia and basidioles. **c**. Hyphae from trama. **d**. Hyphae from lower context. Scale bars:  $a = 5 \mu m$ ,  $b - d = 10 \mu m$ 

distinct, curry-yellow, up to 2 mm wide; *pores* angular, 12–15 per mm; *dissepiments* thick, entire. *Context* up to 3 mm thick, duplex, with a black zone, *lower context* cinnamon-buff, woody, up to 1.5 mm thick, *upper tomentum* dark brown, soft, up to 1.5 mm thick. *Tubes* honey-yellow, woody, up to 2 mm long.

Hyphal system dimitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH. Context: in the lower context, generative hyphae vellowish, slightly thick-walled, rarely branched, frequently septate, 2.5-4 µm in diam; skeletal hyphae yellow, thick-walled with a wide lumen, unbranched, aseptate, interwoven, 3–5 µm in diam; in the upper tomentum, generative hyphae yellow, slightly thick-walled, unbranched, frequently septate, 2.5-4 µm in diam; skeletal hyphae brown, thick-walled with a wide lumen, unbranched, aseptate, loosely interwoven, 3.5-5.5 µm in diam; in the black zone, hyphae dark brown, distinctly thickwalled with a narrow lumen, strongly agglutinate, interwoven. *Tubes*: generative hyphae hyaline to yellowish, thin- to slightly thick-walled, occasionally branched, frequently septate, 2-3 µm in diam; skeletal hyphae dominant, yellow, thick-walled with a wide lumen, unbranched, aseptate, interwoven, 3-5 µm in diam. Cystidia and cystidioles absent. Basidia barrelshaped, hyaline, thin-walled, with four sterigmata and a simple septum at the base,  $5-7 \times 3-4$  µm; *basidioles* clavate, slightly smaller than basidia. Basidiospores broadly ellipsoid, pale yellowish, slightly thick-walled, smooth, IKI-, CB-, 2.5- $3 \times (1.5)^{2-2.5} \mu m$ , L=2.74  $\mu m$ , W=2.14  $\mu m$ , Q=1.26-1.29 (n = 120/4).

Additional specimens studied (paratypes): China, Hainan, Wuzhishan National Nature Reserve, on living angiosperm trunk, 31 May 2015, LWZ 20150531-13 (IFP), LWZ 20150531-14 (IFP), LWZ 20150531-15 (IFP).

*Phylloporia radiata* L.W. Zhou, **sp. nov.** (Figs. 4 and 5). MycoBank no.: MB 816183

*Holotype*: China, Guizhou, Fanjingshan National Nature Reserve, on living liana, 22 November 2014, LWZ 20141122-6 (IFP).

*Etymology: radiata* (Lat.): referring to the radially striate pileal surface.

*Basidiocarps* annual, sessile, attached by a small vertex, imbricate, rarely solitary, without odor or taste, corky. *Pilei* 

dimidiate, flabelliform or spathulate, sometimes fused together, applanate, single pileus projecting up to 2.5 cm, 3 cm wide and 0.5 cm thick at base. *Pileal surface* honey-yellow, velutinate, faintly concentrically sulcate with wide zones, radially striate; *margin* honeyyellow, sharp. *Pore surface* reddish brown, more or less shining; *sterile margin* distinct, curry-yellow to cinnamon-buff, up to 1 mm wide; *pores* angular, 8–10 per mm; *dissepiments* thin, entire. *Context* up to 4 mm thick, duplex, with a black zone, *lower context* honey-yellow, corky, up to 2 mm thick, *upper tomentum* concolorous with the lower context, soft, up to 2 mm thick. *Tubes* cinnamon-buff, corky, up to 1 mm long.



Fig. 4 Basidiocarps of *Phylloporia radiata* (LWZ 20141122-5). a. Pileal surface. b. Pore surface. Scale bars: a-b=1 cm



**Fig. 5** Microscopic structures of *Phylloporia radiata* (drawn from the holotype). **a**. Basidiospores. **b**. Basidia and basidioles. **c**. Hyphae from trama. **d**. Hyphae from lower context. Scale bars:  $a = 5 \mu m$ ,  $b-d = 10 \mu m$ 

Hyphal system monomitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH. Context: hyphae in the lower context yellow, thickwalled with a wide lumen, unbranched, frequently septate, regularly arranged, 3-5 µm in diam; hyphae in the upper tomentum yellowish, thick-walled with a wide lumen, unbranched, frequently septate, loosely interwoven, 2.5-4 µm in diam; hyphae in the black zone dark brown, distinctly thick-walled with a narrow lumen, strongly agglutinate, interwoven. Tubes: hyphae yellow, thick-walled with a wide lumen, rarely branched, frequently septate, subparallel along the tubes, 2-4 µm in diam. Cystidia and cystidioles absent. Basidia clavate, hyaline, thin-walled, with four sterigmata and a simple septum at the base,  $10-15 \times 4-7 \mu m$ ; basidioles in shape similar to basidia, but slightly smaller than basidia. Basidiospores broadly ellipsoid, pale yellowish, slightly thick-walled, smooth, IKI-, CB-,  $2.5-3.5 \times 2-$ 2.5(-3)  $\mu$ m, L=3.02  $\mu$ m, W=2.42  $\mu$ m, Q=1.24-1.26 (n = 90/3).

Additional specimens studied (paratypes): China, Guizhou, Fanjingshan National Nature Reserve, on living liana, 22 November 2014, LWZ 20141122-5 (IFP), LWZ 20141122-19 (IFP).

Other specimens studied: Phylloporia pulla. China, Hainan, Wuzhishan National Nature Reserve, on living angiosperm trunk, 22 May 2008, Dai 9627 (IFP); Jianfengling National Nature Reserve, on living angiosperm trunk, 19 November 2007, Cui 5251 (BJFC).

### Discussion

*Phylloporia minutipora* and *P. radiata* lack setae and bear thick-walled, colored and tiny basidiospores, which correspond to the morphological concept of *Phylloporia*. Moreover, the phylogeny inferred from nLSU sequences (Fig. 1) also confirmed these two species to be members of *Phylloporia*.

*Phylloporia minutipora* is characterized in the genus by a combination of annual, sessile and imbricate basidiocarps, distinctly concentrically sulcate pileal surface with obtuse margin, angular pores, duplex context separated by a black zone, a dimitic hyphal system, and broadly ellipsoid basidiospores. Its most astonishing characters are extremely small pores of 12–15 per mm and basidiospores of  $2.5-3 \times 2-2.5 \mu m$  even in *Phylloporia* that is a genus known for tiny basidiospores.

The imbricate pilei and a dimitic hyphal system of *Phylloporia minutipora* bring *P. fulva* Yombiyeni & Decock, *P. pectinata* (Klotzsch) Ryvarden and *P. pulla* to mind. However, comparing with *P. minutipora*, besides larger pores and basidiospores, *P. fulva* and *P. pulla* also differ in their pendant pilei being attached to the substrata by a small vertex (Yombiyeni et al. 2015), and *P. pectinata* has a perennial habit (Wagner and Ryvarden 2002).

*Phylloporia radiata* is characterized in the genus by a combination of annual, sessile and imbricate basidiocarps, faintly sulcate and radially striate pileal surface, sharp pileal margin, angular pores of 8-10 per mm, duplex context separated by a black zone, a monomitic hyphal system, and broadly ellipsoid basidiospores of  $2.5-3.5 \times 2-2.5$  µm.

Phylloporia clausenae L.W. Zhou resembles P. radiata by its annual and sessile basidiocarps, duplex context, a monomitic hyphal system, and broadly ellipsoid basidiospores of  $3-3.5 \times 2-3 \ \mu m$  (Zhou 2015b). However, P. clausenae differs mainly in its distinctly sulcate pileal surface, obtuse pileal margin, basal context separated by two black zones, and wider hyphae in the tomentum (4–6  $\mu$ m in diam; Zhou 2015b). Moreover, P. clausenae grows on living angiosperm trunk (Zhou 2015b), whereas P. radiata is only known on living liana. Phylloporia ulloai R. Valenz. et al. is another species of Phylloporia that was found exclusively on living liana, and it shares with P. radiata annual and sessile basidiocarps, wsharp pileal margin, duplex context separated by a black zone, a monomitic hyphal system and broadly ellipsoid basidiospores (Valenzuela et al. 2011). However, P. ulloai, originating from Mexico, is distinct from P. radiata by its much larger basidiocarps (> 4 cm long, > 8 cm wide, and > 1.5 cm thick), larger pores (6–8 per mm), wider hyphae in context (> 5  $\mu$ m in diam) and slightly larger basidiospores  $(3.2-3.6 \times 2.5-3.2 \text{ }\mu\text{m}; \text{ Valenzuela et al. 2011})$ . The current nLSU-based phylogeny does not recover any reliable relationship at the specific level as in previous studies (Yombiyeni et al. 2015; Zhou 2015b). Therefore, it is impossible to tell whether P. radiata and P. ulloai have a common ancestor restricted to growth on living liana, or if they evolved the habit of living liana separately.

Polyporus pullus Mont. & Berk. was described from Java, Indonesia in 1844 (Montagne and Berkeley 1844). Recently, the holotype of this species has been morphologically reexamined, and its taxonomic position was set in Phylloporia (Yombiyeni et al. 2015). It is very difficult, if possible, to obtain any molecular sequence from the holotype that was collected more than 150 years ago. Therefore, according to morphological comparison, the specimens Cui 5251 and Dai 9627 from Hainan, tropical China were tentatively identified as P. pulla. Both Hainan and Java locate in tropical Asia, and more importantly, the two Chinese specimens share identical morphology in main taxonomic characters with the holotype. The only difference is that the concentric sulcus in pileal surface of the Chinese specimens is distinct and that of the holotype is faint, which might be caused by the more aged Chinese specimens. The nLSU sequences from the two Chinese specimens could represent P. pulla in future phylogenetic analyses of Phylloporia.

An identification key to 30 species of Phylloporia was recently provided by Zhou (2015b), which is essential for identifying specimens of this genus. However, during the time that paper was under review, eight more species, viz. Phylloporia afrospathulata Yombiyeni & Decock, P. dependens Y.C. Dai, P. flabelliforma Decock & Yombiyeni, P. fulva, P. gabonensis Decock & Yombiyeni, P. inonotoides Yombiyeni & Decock, P. pulla and P. yuchengii Yu.Sh. Gafforov et al., were added to Phylloporia (Gafforov et al. 2014; Decock et al. 2015; Liu et al. 2015; Yombiyeni et al. 2015). With the addition of *P. minutipora* and *P. radiata*, newly described in the current study, a total of 40 species are accepted in Phylloporia. An updated key to Phylloporia is provided below.

- 1. Basidiocarps resupinate
   *P. parasitica* 

   1. Basidiocarps sessile or stipitate
   2
- 2. Basidiocarps stipitate and terrestrial (on buried wood or roots) – \_\_\_\_\_3
- 2. Basidiocarps sessile and on aerial wood—7
- 3. Pores > 10 per mm
   4

   3. Pores < 10 per mm</td>
   5
- *terrestris* L.W. Zhou
- afrospathulata
- 5. Basidiospores mostly < 3 µm long—*P. minutispora* Ipulet & Ryvarden
- 5. Basidiospores > 3 µm long -6
- (Berk. ex Sacc.) Ryvarden

- Ryvarden 7. Hyphal system dimitic -8 7. Hyphal system monomitic \_\_\_\_\_12 9. Basidiocarps solitary, pores < 9 per mm-P. nouraguensis Decock & G. Castillo 9. Basidiocarps in cluster, pores > 9 per mm—10 10. Pileal surface lighter (grayish orange to pale cinnamon), pores < 11 per mm \_\_\_\_\_\_P. fulva 10. Pileal surface darker (vellowish brown to dark brown), pores 11–15 per mm \_\_\_\_\_11
- 11. Pileus attached by a small vertex and pendant, pores < 12 per mm; basidiospores mostly > 2.5 µm wide—P. pulla
- 11. Pileus widely attached to the substratum, pores>12 per
- 12. Pores 2–4 per mm
   13

   12. Pores 4–12 per mm
   16
- 13. Basidiospores broadly ellipsoid to subglobose-P. fruticum (Berk. & M.A. Curtis) Ryvarden
- 13. Basidiospores oblong-ellipsoid, subcylindrical to cylindrical — -----14
- 14. Context duplex-P. rzedowskii R. Valenz. & Decock
- 14. Context homogeneous \_\_\_\_\_15
- 15. Context < 1 mm thick; on living branch—P. oblongospora Y.C. Dai & H.S. Yuan
- 15. Context 2-4 mm thick; on living trunk-P. inonotoides
- 16. Basidiocarps annual to perennial, dense and hard consis-\_\_\_\_\_17 tency -
- 16. Basidiocarps annual, soft corky at least at tomentum layer-\_\_\_\_\_23
- 17. Pores 10–12 per mm; on living Tilia—————P. tiliae L.W. Zhou
- \_\_\_\_\_19
- 18. Pileal surface zonate and sulcate
   19

   18. Pileal surface azonate
   *P. yuchengii*
- 19. Pores 7–9 per mm -21
- 20. Basidiospores ellipsoid; mostly on Ribes-P. ribis (Schumach.) Ryvarden
- 20. Basidiospores subglobose; mostly on Ephedra, Cotoneaster Parmasto
- 21. Basidiospores > 2.7 μm wide*P. dependens*21. Basidiospores < 2.7 μm wide</td>22
- 22. Basidiospores ellipsoid to oblong-ellipsoid with a guttule; on Abelia-P. gutta L.W. Zhou & Y.C. Dai
- 22. Basidiospores broadly ellipsoid without a guttule; on living Crataegus-----P. crataegi L.W. Zhou & Y.C. Dai

- 23. Basidiospores broadly ellipsoid to subglobose 24 23. Basidiospores ellipsoid, oblong-ellipsoid to cylindrical-32 24. Pores 5–6 per mm \_\_\_\_\_ -25 24. Pores 6–11 per mm -27 Bondartseva 25. Context homogeneous — -26 26. Pileus < 1.5 mm thick, margin regular—P. flabelliforma 26. Pileus > 1.5 mm thick, margin irregular—*P. gabonensis* 27. Basidiocarps > 8 cm wide, > 15 mm thick; contextual hyphae > 5  $\mu$ m in diam— ------P. ulloai 27. Basidiocarps < 8 cm wide, < 15 mm thick; contextual hyphae < 5 µm in diam \_\_\_\_\_ 28. Contextual hyphae regularly arranged -29 28. Contextual hyphae interwoven -30 29. Pileus distinctly sulcate, not radially striate, margin obtuse, basal context separated by two black zones; hyphae in tomemtum>4 µm in diam; on living angiosperm trunk — —————P. clausenae 29. Pileus faintly sulcate, radially striate, margin sharp, context duplex thoroughly; hyphae in tomemtum  $< 4 \mu m$  in 30. Contextual hyphae slightly thick-walled with a wide lumen, frequently septate, large rhomboid crystals absent --3130. Contextual hyphae thick-walled with a narrow lumen, occasionally septate, large rhomboid crystals present in trama and context-––––––––––P. chrysites (Berk.) Ryvarden 31. Pores 10–12 per mm; basidiospores  $< 3 \mu m \log$ ; on living Fontanesia ————P. fontanesiae L.W. Zhou & Y.C. Dai 31. Pores 7–9 per mm; basidiospores > 3  $\mu$ m long; on other angiosperms ———— P. oreophila L.W. Zhou & Y.C. Dai 32. Basidiospores mostly>3 µm wide—\_\_\_\_33 32. Basidiospores mostly < 3 µm wide-------34 33. Pores 4-6 per mm-P. hainaniana Y.C. Dai & B.K. Cui 33. Pores 8–10 per mm————P. capucina (Mont.) Ryvarden 34. Basidiocarp solitary — 
   34. Basidiocarp imbricate
   -35 -38 35. Context homogeneous———*P. homocarnica* L.W. Zhou 35. Context duplex — -36 36. Context not separated by a black zone; on living Flacourtia \_\_\_\_\_\_ P. flacourtiae L.W. Zhou 36. Context separated by a black zone; on other angiosperms --37 37. Pores circular; basidiospores mostly > 2.2  $\mu$ m wide-P. weberiana (Bres. & Henn. ex Sacc.) Ryvarden
- 38. Basidiospores mostly < 2.5 μm wide 39
- Basidiospores mostly>2.5 μm wide——*P. bibulosa* (Lloyd) Ryvarden
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- Context duplex, not separated by a black zone; basidiospores > 3.5 μm long, contextual hyphae interwoven; on living *Nandina*—*P. nandinae* L.W. Zhou & Y.C. Dai
- 39. Context duplex, separated by a black zone; basidiospores < 3.5 μm long, contextual hyphae regularly arranged; on living Osmanthus—P. osmanthi L.W. Zhou

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