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Source: Cryptogamie, Mycologie, 35(4):313-322.
Published By: Association des Amis des Cryptogames
DOI: http://dx.doi.org/10.7872/crym.v35.iss4.2014.313
URL: http://www.bioone.org/doi/full/10.7872/crym.v35.iss4.2014.313

Cryptogamie, Mycologie, 2014, 35 (4): 313-322

Phylloporia yuchengii sp. nov. (Hymenochaetales, Basidiomycota) from Western Tien Shan Mountains of Uzbekistan based on phylogeny and morphology

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Abstract – *Phylloporia yuchengii* is newly described and illustrated from alpine ecosystem, Western Tien Shan Mountains in the Tashkent Province of Uzbekistan. This species is distinguished from other *Phylloporia* species in a combination of hard corky consistency of basidiocarps with thick base (up to 3.5 cm) and azonate pileal surface, pores as 6–8 per mm, a monomitic hyphal system with regularly arranged, interwoven and subparallel generative hyphae, respectively, in context, tomentum and trama, and ellipsoid to oblong-ellipsoid and cyanophilous basidiospores (3.2-4 × 2.3-3 µm). In nLSU-based phylogeny, *P. yuchengii* nested within the *Phylloporia* clade and formed a distinct lineage with strong supports. The morphological differences between *P. yuchengii* and other related *Phylloporia* species in morphology and geography are discussed.

Central Asia / Hymenochaetaceae / Polypore / Taxonomy

INTRODUCTION

Phylloporia Murrill (Hymenochaetaceae, Basidiomycota) was established in 1904 with *P. parasitica* Murrill as type, a species growing on living leaves (Murrill, 1904). Morphologically, *Phylloporia* is characterized by annual to perennial and sessile to stipitate basidiocarps with duplex to

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doi/10.7872/crym.v35.iss4.2014.313

homogenous context, a mono- to dimitic hyphal system, and tiny, colored and thick-walled basidiospores (Zhou & Dai, 2012). *Phylloporia* species are known to be parasitic and saprotrophic on the living trees, dead trunks, stems and root of trees. This genus comprises for the time being 27 species (Wagner & Ryvarden, 2002; Ipulet & Ryvarden, 2005; Cui *et al.*, 2010; Valenzuela *et al.*, 2011; Zhou & Dai, 2012; Campos-Santana *et al.*, 2015; Decock *et al.*, 2013; Zhou, 2013, 2015).

The species diversity of *Phylloporia* has been explored worldwide, especially in China (Cui *et al.*, 2010; Zhou & Dai, 2012; Zhou, 2013, 2015); nevertheless, this genus is still poorly known in Central Asia, where only *Phylloporia ephedrae* (Woron.) Parmasto and *P. ampelina (*Bondartsev & Singer) Bondartseva are known previously (Parmasto, 1985).

During a survey of wood-inhabiting fungi in forests of Western Tien Shan Mountains in Uzbekistan, Central Asia, two *Phylloporia* specimens were found on dead trunks and basal stems of an unknown angiosperm in areas of alpine ecosystem along the river Oqtosh and Xojikent valley in the Ugam ridge. After morphological examination and phylogenetic analysis, an unknown species was identified from these two specimens. It is described and illustrated as *Phylloporia yuchengii* in the present paper.

MATERIALS AND METHODS

Morphological examination – The studied specimens are deposited at TASM and IFP (herbarium acronyms follow Index herbariorum). The microscopic procedure follows Zhou (2014). Cotton Blue (CB), Melzer's reagent (IKI) and 5% potassium hydroxide (KOH) were used to stain the specimens. Sections were studied using a Nikon Eclipse 80i microscope at magnifications up to ×1000. All values were measured in CB. When presenting the basidiospore size variation, the upper and lower 5% of measurements are excluded from the range and the extreme values are presented in parentheses. The following abbreviations are used: L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens, and n = number of basidiospores measured/ number of specimens measured. Line drawings were made with the aid of a light tube.

Extraction of DNA, amplification and sequencing – For extraction of DNA from *Phylloporia* dried basidiocarps, small pieces were placed in 2 ml centrifuge tubes with a screw cap and homogenized using FastPrep FP120 machine (Savant Instrument Inc. Holbrook, NY, USA). Then, 0.8 ml of CTAB buffer (3% cetyltrimetylammonium bromide, 2 mM ethylenediamine tetraacetic acid, 150 mM Tris–HCl, 2.6 M NaCl, pH 8) was added to each tube, and these were incubated at 65°C for 1 h. Following incubation, an equal volume of chloroform was added to the tubes, samples were vortexed and centrifuged for 7 min at 13 000 rpm. The supernatant was transferred to new 1.5 ml centrifuge tubes; DNA was precipitated by adding an equal volume of 2-propanol and pelleted by centrifugation for 20 min at 13 000 rpm. The resulting DNA pellets were washed in 200 µl 70% ethanol, dried, dissolved in 30 µl of sterile deionized water and stored at – 20°C.

The amplification by PCR of nLSU was performed using primers LR0R and LR7 (Vilgalys & Hester, 1990). Each PCR contained 200 μM deoxyribonucleotide triphosphates, 0.2 mM of each primer, 0.03 U/μl Thermo Green Taq polymerase with reaction buffer Green, and 2.75 μM final concentration of $MgCl_2$. The thermal cycling was carried out using an Applied Biosystems GeneAmp PCR System 2700 thermal cycler (Foster City, CA, USA). An initial denaturation step at 95°C for 5 min was followed by 35 amplification cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. The thermal cycling was ended by a final extension step at 72°C for 7 min. PCR products were size-separated on 1% agarose gels stained with ethidium bromide, and visualized under UV light. The PCR products were purified with Qiagen DNA extraction PCR M kit (Qiagen, Hilden, Germany). Sequencing was performed by Macrogen Inc., Seoul, Korea, utilizing ABI 3730 XL automated sequencers (Applied Biosystems) using primers LR0R and LR5 (Vilgalys & Hester, 1990). Raw sequence data were analysed using the SeqMan Pro version 10.0 software from DNASTAR package (DNASTAR, Madison, WI, USA). The assembled sequences are deposited at GenBank (http://www.ncbi.nlm.nih.gov/genbank/; Table 1).

Phylogenetic *analysis* – The newly generated sequences were added to the nLSU dataset of previous studies with *Inonotus hispidus* (Bull.) P. Karst. as outgroup (Zhou, 2015). The new dataset was aligned using MAFFT 7 (Katoh & Standley, 2013) with Q-INS-i option (Katoh & Toh, 2008). The alignment is deposited at TreeBASE (http://www.treebase.org; accession number S16221). Maximum likelihood (ML) and maximum parsimony (MP) analyses were used to conduct phylogenetic analysis. ML tree was constructed using raxmlGUI 1.2 (Stamatakis, 2006; Silvestro & Michalak, 2012) under GTR + I + G model that is selected as the best-fit evolutionary model by jModelTest 2.1.4 according to corrected Akaike information criterion (Darriba *et al.*, 2012; Guindon & Gascuel, 2003) and auto FC option (Pattengale *et al.*, 2010) in bootstrap (BS) replicates. MP analysis was performed using PAUP* 4.0b10 (Swofford, 2002) with heuristic searches and 1000 BS replicates. All characters were equally weighted and gaps were set as missing data. Other conditions were as follows: starting tree obtained via stepwise addition, tree-bisection-reconnection branch swapping, steepest descent option not in effect, and “multrees” option in effect.

RESULTS

Molecular phylogeny – The dataset referred to in the current phylogeny has 58 nLSU sequences and resulted in an alignment with 924 characters, of which 571 are constant, 98 parsimony-uninformative and 255 parsimony-informative. The BS search for ML analysis stopped after 300 replicates. The MP analysis generated six equally most-parsimonious trees of 1276 steps (CI = 0.350, RI = 0.548). The ML and MP trees had nearly congruent topologies, and thus only ML tree was presented along with BS values from MP analysis (Fig. 1).

The phylogenetic analysis shows that the two Uzbekistan specimens formed a strongly supported terminal lineage within the *Phylloporia* clade defined by previous studies (Decock *et al.*, 2013; Zhou, 2015). The affinity of this lineage with other known *Phylloporia* species was not resolved.

Table 1. Information of nLSU sequences used in the phylogenetic analysis

Species	*Voucher specimens/cultures*	*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	AY059057
F. robiniae (Murrill) Murrill	CBS 211.36	*Robinia pseudo-acacia*	Maryland, USA	AF411825
Phylloporia 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. chrysites	MUCL 52764	–	Mexico	HM635666
P. chrysites	MUCL 52862	*Neopringle*	Mexico	HM635667
P. crataegi L.W. Zhou & Y.C. Dai	Dai 11014 (Holotype)	*Crataegus*	Liaoning, China	JF712922
P. crataegi	Dai 11016 (Paratype)	*Crataegus*	Liaoning, China	JF712923
P. ephedrae (Woron.) Parmasto	TAA 72-2	*Ephedra*	Turkmenistan	AF411826
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. *frutica* (Berk. & M.A. Curtis) Ryvarden	ENCB TR&RV858	–	Mexico	HM635669
P. cf. *frutica*	MUCL 52762	–	Mexico	HM635668
P. cf. *frutica*	MUCL 52863	–	Mexico	HM635670
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	JF712928
P. minutispora Ipulet & Ryvarden	Ipulet 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/FG-11-400 (Holotype)	*Myrcia*	French Guiana	KC136222
P. nouraguensis	MUCL/FG-11-404 (Paratype)	*Myrcia*	French Guiana	KC136223

Table 1. Information of nLSU sequences used in the phylogenetic analysis *(continued)*

Species	*Voucher specimens/cultures*	*Hosts*	*Origin*	*Accession number*
P. nouraguensis	MUCL/FG-11-409 (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
P. pectinata (Klotzsch) Ryvarden	R. Coveny 113	*Rhodania rubescens*	Australia	AF411823
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. rzedowskii R. Valenz. & Decock	ENCB RV8750 (Holotype)	*Hybanthus mexicanus*	Mexico	HM635672
P. rzedowskii	MUCL 52859	*Hybanthus mexicanus*	Mexico	HM635673
P. rzedowskii	MUCL 52860	*Hybanthus mexicanus*	Mexico	HM635674
P. rzedowskii	MUCL 52861	*Hybanthus mexicanus*	Mexico	HM635675
P. spathulata (Hook.) Ryvarden	Chay 456	Apocynaceae	Mexico	AF411822
P. sp. 1	MUCL 53433	Angiosperm	Mexico	KC136231
P. sp. 2	MUCL/FG-11-462	Angiosperm	French Guiana	KC136228
P. sp. 3	MUCL/FG-11-506	Angiosperm	French Guiana	KC136227
P. sp. 4	MUCL/GA-06-166	Angiosperm	Gabon	KC136229
P. sp. 5	MUCL/Yom-47	Angiosperm	Gabon	KC136230
P. sp. 6	Robledo 351	Angiosperm	Argentina	KC136226
P. sp. 7	Robledo 1220	Angiosperm	Argentina	KC136225
P. terrestris L.W. Zhou	Yuan 5738 (Holotype)	Ground	Guangxi, China	KC778784
P. ulloai R. Valenz., T. Raymundo, Cifuentes & Decock	MUCL 52866	Lianas	Mexico	HM635677
P. ulloai	MUCL 52867 (Holotype)	Lianas	Mexico	HM635678
P. ulloai	MUCL 52870	Lianas	Mexico	HM635679
P. weberiana (Bres. & Henn. ex Sacc.) Ryvarden	Dai 9242	Angiosperm	Hainan, China	JF712936
P. yuchengia Yu.Sh. Gafforov *et al.*	YG 033 (Holotype)	Angiosperm	Uzbekistan	KM264324
P. yuchengia	YG 051 (Paratype)	Angiosperm	Uzbekistan	KM264325
Outgroup				
Inonotus hispidus (Bull.) P. Karst.	MF 92-829	*Fraxinus excelsior*	Germany	AF311014

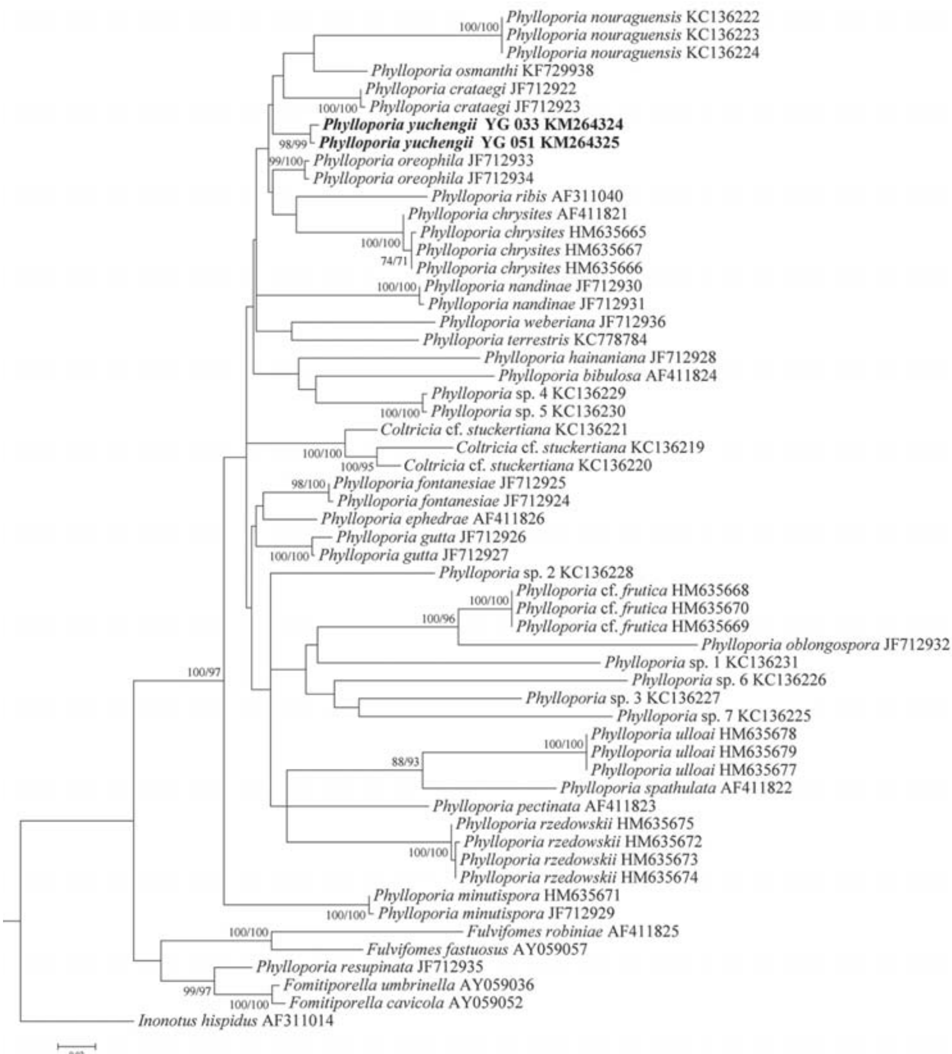

Fig. 1. Phylogenetic position of *Phylloporia yuchengii* (in boldface) inferred from nLSU sequences. The topology is from maximum likelihood analysis, while bootstrap values from maximum likelihood (before slash) and maximum parsimony (after slash) analyses are indicated when both above 50%.

Taxonomy

Phylloporia yuchengii Yu. Sh. Gafforov, Tomšovský, E. Langer & L.W. Zhou, **sp. nov.** **Figs 2-4**

MycoBank no.: MB 809827

Diagnosis: Differs from other *Phylloporia* species in a combination of hard corky consistency of basidiocarps (up to 3.5 cm thick at base) and azonate pileal surface, 6-8 per mm of pores, a monomitic hyphal system with regularly

Fig. 2. A basidiocarp of *Phylloporia yuchengii* (holotype).

Fig. 3. Imbricate basidiocarps of *Phylloporia yuchengii* (holotype).

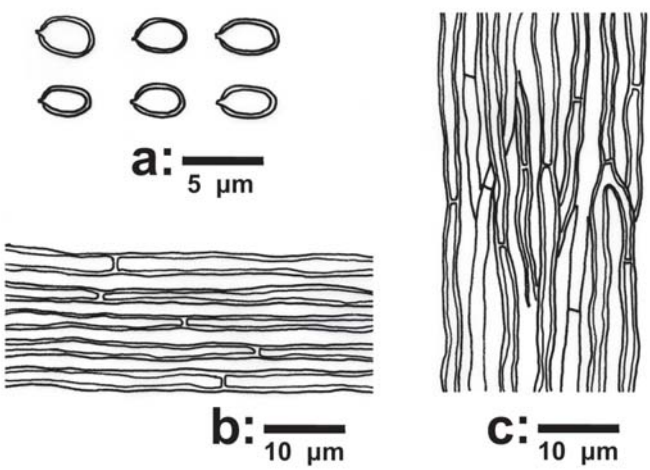

Fig. 4. Microscopic structures of *Phylloporia yuchengii* (drawn from holotype). a: Basidiospores; b: Hyphae in context; c: Hyphae in trama.

arranged, interwoven and subparallel generative hyphae, respectively, in context, tomentum and trama, and ellipsoid to oblong-ellipsoid and cyanophilous basidiospores (3.2-4 × 2.3-3 μm).

Holotypus: **UZBEKISTAN**, Tashkent Province, Oqtosh River, Ugam-Chatkal State National Natural Park, Ugam ridge of the Western Tien Shan Mountains, on dead angiosperm trunk and stem, 1 Jun 2011, YG 033 (TASM; Isotype at IFP).

Etymology. yuchengii (Lat.): in honour of Chinese mycologist, Prof. Dr. Yu-Cheng Dai.

Basidiocarps perennial, pileate, nodulose, broadly attached to the substrate, imbricate rarely solitary, without odor or taste when fresh, hard corky. **Pilei** projecting up to 5 cm, 7 cm wide and 3.5 cm thick at base. **Pileal surface** golden brown to umber brown when mature, finely velutinous when juvenile to glabrous, azonate. **Margin** sharp. **Pore surface** golden brown with greyish or brownish tints with age, dull; sterile margin irregular, 1.5-3 mm thick, paler than pore surface. **Pores** circular to angular, 6-8 per mm; **dissepiments** thin, entire. **Context** golden brown or yellowish brown, darkening with age, up to 3 cm thick, duplex, with a black line, separating lower hard corky context, up to 2.5 cm thick, and upper tomentum, softer than context, up to 5 mm thick. **Tubes** concolorous with the context or slightly paler, up to 1 mm long.

Hyphal system monomitic; generative hyphae simple septate; tissue becoming red in KOH but otherwise unchanged. **Context** hyphae brown, thick-walled with a wide lumen, unbranched, occasionally simple septate, straight, regularly arranged, 1.5-3 µm in diam; hyphae in tomentum yellowish brown, thick-walled with a wide lumen, unbranched, occasionally simple septate, loosely interwoven, 2-6 µm in diam; hyphae in the black zone dark brown, distinctly thick-walled with a narrow lumen, strongly agglutinate, interwoven. **Tubes** hyphae hyaline to yellowish, thin- to slightly thick-walled, unbranched, frequently septate, more or less straight, some of them at dissepiment edges and in hymenium encrusted with small crystals, 1.5-3 µm in diam; yellowish brown, thick-walled with a wide lumen, rarely branched, occasionally septate, straight, 2-3 µm in diam; both types subparallel along the tubes. **Setae** absent; **cystidia** and **cystidioles** absent. **Hymenium** collapsed in investigated specimens, basidia and basidioles not seen. **Basidiospores** ellipsoid to oblong-ellipsoid, yellowish, thick-walled, smooth, neither amyloid nor dextrinoid, cyanophilous, (3-)3.2-4 × (2-)2.3-3(-3.2) µm, L = 3.56 µm, W = 2.7 µm, Q = 1.30-1.34 (n = 60/2).

Additional specimen examined: UZBEKISTAN. Tashkent Province, Xojikent valley, Ugam-Chatkal State National Natural Park, Ugam ridge of the Western Tien Shan Mountains, on dead angiosperm trunk, 2 Nov 2011, YG 051 (TASM).

DISCUSSION

Phylloporia yuchengii is characterized by perennial mostly imbricate basidiocarps, hard corky and thick pilei (up to 3.5 cm thick at base), azonate pileal surface, sharp margin, circular to angular pores with 6-8 per mm, a monomitic hyphal system with regularly arranged, interwoven and subparallel generative hyphae, respectively, in context, tomentum and trama, and ellipsoid to oblong-ellipsoid and cyanophilous basidiospores (3.2-4 × 2.3-3 µm).

Phylloporia oreophila L.W. Zhou & Y.C. Dai shares with *P. yuchengii* imbricate basidiocarps, azonate pileal surface, sharp margin, similar pore size (7-9 per mm) and comparable basidiospores (3-3.7 × 2-3 µm). *Phylloporia oreophila* differs in its annual habit, soft corky consistency of tomentum, and interwoven arrangement of contextual hyphae (Zhou & Dai, 2012).

Phylloporia weberiana (Bres. & Henn. ex Sacc.) Ryvarden has nearly identical basidiospore size (3.4-4.1 × 2.2-3 μm) as *P. yuchengii*. However, its macromorphological characters, including annual and solitary basidiocarps, zonate pileal surface, obtuse pileal margin, and cottony tomentum (Dai, 2010), make it distinguished from *P. yuchengii*. In addition, in *P. weberiana* the (generative) hyphae are interwoven and regularly arranged, respectively, in trama and tomentum (Dai, 2010).

Phylloporia yuchengii also resembles *P. ribis* (Schumach.) Ryvarden, *P. ephedrae*, *P. crataegi* L.W. Zhou & Y.C. Dai and *P. gutta* L.W. Zhou & Y.C. Dai in having perennial, hard and imbricate basidiocarps, and similar pore sizes (6-7 per mm in *P. ribis* and *P. ephedrae*, Wagner & Ryvarden, 2002; 7-9 per mm in *P. crataegi* and *P. gutta*, Zhou & Dai, 2012). However, these four species differ from *P. yuchengii* mainly in their thin basidiocarps with zonate and sulcate pileal surfaces.

It is worth noting that *Phylloporia ephedrae* is reported from Central Asia (Parmasto, 1985). The type locality of *Phylloporia ampelina* is in Georgia (Wagner & Ryvarden, 2002) and its distribution also extends to Central Asia (Bondartseva, 1983). It resembles *P. yuchengii* in its thick basidiocarp (3 cm at base) with azonate pileal surface, but differs in its annual habit, soft and brittle tomentum, and larger pores (5-6 per mm) according to the original description (Wagner & Ryvarden, 2002).

Phylloporia yuchengii represents the third *Phylloporia* species known from Central Asia. The polypore diversity in Central Asia is still poorly known. The adjacent Caucasus region has a preliminary polypore checklist including four *Phylloporia* species (Ghobad-Nejhad, 2011). Polypores are also well known in neighbouring areas of China (Dai, 2012). A systematic survey of polypores in Central Asia is badly needed. Taken the special geographic position of this region, centre of Eurasia, into consideration, this kind of survey would extremely improve our knowledge on polypore diversity worldwide.

Acknowledgments. Prof. Yu-Cheng Dai (Beijing Forestry University) is thanked for improving this manuscript. This research was financed by the National Natural Science Foundation of China (Project No. 31200015) and the Youth Fund for Creative Research Groups, Institute of Applied Ecology, Chinese Academy of Sciences. YG is thankful to Erasmus Mundus Action 2 CASIA Postdoctoral Fellowship UZGT2-P3 and German Academic Exchange Service (DAAD) visiting Fellowship A/13/03723, for the financial support of a research stay at the Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Sweden and Department of Ecology, University Kassel, Germany. YG thanks to Prof. Jan Stenlid (Sweden) for the use of his laboratory facilities.

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