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Porodaedalea chinensis (*Hymenochaetaceae*, Basidiomycota) — a new polypore from China

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Abstract

Porodaedalea chinensis is described and illustrated as a new species occurring on *Pinus yunnanensis* from southwestern China based on morphological and molecular characters. Phylogenetic analyses of the combined internal transcribed spacer (ITS) of ribosomal RNA gene and translation elongation factor 1-alpha (*tef1-α*) sequences show that the new species forms a distinct lineage separating it from other *Porodaedalea* species. *P. chinensis* is characterized by perennial, pileate basidiocarps, relatively small pores (2–3 per mm), a dimitic hyphal system with generative hyphae bearing simple septa which are frequent in trama and skeletal hyphae dominant in context and trama, broadly ellipsoid, hyaline, thin-to slightly thick-walled, smooth, moderately cyanophilous basidiospores measured as 4–6 × 3–4.8 µm, and having a distribution in southwestern China.

Key words – Hymenochaetales – phylogeny – taxonomy – wood-inhabiting fungi

Introduction

Porodaedalea Murrill, typified by *Porodaedalea pini* (Brot.) Murrill, is characterized by perennial, effused-reflexed to pileate basidiocarps, a dimitic hyphal structure with simple septa on generative hyphae, ventricose to subulate hymenial setae originating from subhymenim, and subglobose, broadly ellipsoid to ovoid, slightly to fairly thick-walled, moderately cyanophilous basidiospores (Murrill 1905, Dai 2010, Tomšovský et al. 2010). All species of the genus grow on gymnosperm wood and cause a white pocket rot.

A basic outline for *Porodaedalea* was established by Fiasson & Niemelä (1984) based on morphological and biochemical features. Wagner & Fischer (2002) confirmed *Porodaedalea* as an independent genus through the nuclear large subunit (nLSU) ribosomal RNA-based phylogeny. In Europe, *Porodaedalea pini*, *P. chrysoloma* (Fr.) Fiasson & Niemelä and *P. laricis* (Jacz. ex Pilát) Niemelä are well known and have a wide distribution (Niemelä et al. 2005, Tomšovský et al. 2010, Ryvarden & Melo 2014). Another species, *P. niemelaei* M. Fischer growing on *Larix* from Finland was described by Fischer (2000), but the name was considered as a synonym of *P. laricis* by Niemelä et al. (2005).

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Based on morphology, *Phellinus chrysoloma* (Fr.) Donk, *P. pini* (Brot.) Bondartsev & Singer and *P. piceina* (Peck) Pat. were recognized in North America (Niemelä 1985, Gilbertson & Ryvarden 1987), then *Porodaedalea cancriformans* (M.J. Larsen, Lombard & Aho) T. Wagner & M. Fischer was described in northwestern North America by Wagner & Fischer (2002).

In addition, based on the mating tests, Fischer (1994) reported seven unique taxa of *Porodaedalea* in North America: *P. piceina* (Peck) Niemelä (group 1) and six taxa as *Porodaedalea* group 2, 3, 4, 5, 6 and 7. Larsen (2000) described *Porodaedalea* group 3 as *P. gilbertsonii* M.J. Larsen. Based on inter-sterility tests and PCR-RFLP, Fischer (1996) indicated that inter-sterility group 4 was identical with group 6 and 7, and inter-sterility group 5 was identical with a northern Asian taxon. Brazee & Lindner (2013) performed a multilocus phylogenetic analysis, which confirmed that a group labeled the 'Holarctic group' from *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* is distributed in North America, and that *P. pini* s.s. and *P. chrysoloma* s.s. do not occur in North America. Similar conclusions were published by Fischer (1994, 1996), Larsen & Melo (1996), Larsen & Stenlid (1999) and Larsen (2000). Recently only *P. piceina* and *P. cancriformans* were listed in North American polypores (Zhou et al. 2016).

Five species of *Porodaedalea*, *P. chrysoloma*, *P. himalayensis* (Y.C. Dai) Y.C. Dai, *P. laricis*, *P. pini* and *P. yamanoi* (Imazeki) Y.C. Dai, were recorded in East Asia (Dai 2010). Nevertheless, these species are poorly supported due to the lack of molecular data.

Recently, *Porodaedalea cedrina* Pilát ex Tomsovsky & Kout occurring on *Cedrus atlantica* and *C. libani* was described from North Africa and Western Asia by phylogenetic analyses of the ITS rDNA region (internal transcribed spacer ribosomal DNA) and *tef1-α* (translation elongation factor 1-α) genes (Tomšovský & Kout 2013).

During investigations on poroid wood-inhabiting fungi on gymnosperms in China, one undescribed species belonging to *Porodaedalea* occurring on the living tree of *Pinus yunnanensis* was found in Yunnan Province, southwestern China. To confirm the affinity of these collections, phylogenetic analyses were carried out based on ITS and *tef1-α* sequences.

Materials & Methods

Morphological studies

The studied specimens are deposited at the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC), National Museum Prague of Czech Republic (PRM), and the private herbarium of J. Vlasák (JV), and microscopic procedures followed He & Li (2011) and Zhao et al. (2015). Microscopic features were described from dried materials prepared with cotton blue (CB), 5% potassium hydroxide (KOH), and Melzer's reagent (IKI). Sections were studied at magnifications up to 1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. At least 30 basidiospores and hymenial setae were measured each specimen, in presenting spores size data, 5% of the measurements were excluded from each end of the range, and shown in parentheses. The following abbreviations were used: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios, and n = number of spores measured from given number of specimens. Drawings were made with a drawing tube. Color terms followed Petersen (1996).

Molecular study

DNA was extracted from dried fruiting bodies. The DNA loci including the ITS and *tef1-α* were selected for the analysis, primer pair ITS5/ITS4 and 1487F/2218R were used, respectively (White et al. 1990, Rehner et al. 2005). The PCR reaction of ITS was performed as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles at 94°C for 40 seconds, 54°C for 45 seconds and 72°C for 1 minute, and a final extension of 72°C for 10 minutes. For the amplification of *tef1-α*, the PCR protocol was using a following touchdown regime: initiated with a 2 minutes denaturation at 94°C. The annealing temperature in the first amplification cycle was 60°C, which

was subsequently incrementally reduced by 1°C per cycle over the next 9 cycles. An additional 35 amplification cycles were then performed, each consisting of 45 seconds denaturation at 94°C, a 90 seconds annealing step at 53°C, and a 2 minutes extension at 72°C, concluding with a 10 minutes incubation at 72°C. All newly generated sequences were deposited in GenBank database (Table 1).

Table 1 A list of species, vouchers and GenBank accession number of sequences used in this study.

Species	Sample no.	Locality	Substrate	GenBank accession no.	
				ITS	*tef1-α*
Porodaedalea cedrina	JK 0908/04-3	Morocco, Middle Atlas	*Cedrus atlantica*	JQ772468	JQ772474
P. cedrina	MCF 03/1167	Turkey, Fethiye-Babadağ Mt.	*Cedrus libani*	JQ772467	JQ772473
P. chrysoloma	BRNM 712790	Sweden, Uppsala	*Picea abies*	FJ775543	FJ775576
P. chrysoloma	BRNM 712788	Czech, Moravian Karst	*Picea abies*	FJ775547	FJ775577
P. chrysoloma	Dai 12674 (BJFC)	Finland, Helsinki	*Picea abies*	KY000004[a]	KY000009[a]
P. chrysoloma	JV 1408/40-J	Czech, Hrensko	*Picea abies*	KY000006[a]	KY000011[a]
P. chinensis	Cui 10252 (BJFC)	China, Yunnan Province	*Pinus yunnanensis*	KX673606[a]	KX852283[a]
P. chinensis	Dai 16864 (BJFC)	China, Yunnan Province	*Pinus yunnanensis*	KX852282[a]	KX852284[a]
P. himalayensis	Cui 9620 (BJFC)	China, Tibet	*Picea likiangensis*	KX673605[a]	KX852286[a]
P. himalayensis	Cui 9320 (BJFC)	China, Tibet	*Picea likiangensis*	JQ772471	JQ772477
P. himalayensis	Cui 9618 (BJFC)	China, Tibet	*Picea likiangensis*	KX673604[a]	KX852285[a]
P. laricis	Dai 15862 (BJFC)	China, Xinjiang	*Larix* sp.	KY000005[a]	KY000010[a]
P. laricis	TFC 1981-38	Russia, Primorsk Territory	*Picea* sp.	FJ775559	FJ775585
P. laricis	PRM 892094	France, Pelvoux	*Larix decidua*	FJ775567	FJ775587
P. pini	BRNM 737548	Turkey, Isparta Province	*Pinus* sp.	JQ772470	JQ772476
P. pini	BRNM 712792	Croatia, Isle of Korčula	*Pinus halepensis*	FJ775554	FJ775599
P. pini	MT 11/13	Sweden	*Pinus sylvestris*	KY000008[a]	KY000012[a]
P. yamanoi	Dai 14795 (BJFC)	China, Jilin Province	*Picea jezoensis*	KX673607[a]	KX852287[a]
P. yamanoi	TFC 1971-24	Russia, Sakhalin Island	*Picea jezoensis*	FJ775551	FJ775592
P. yamanoi	Dai 8202 (BJFC)	China, Jilin Province	*Picea jezoensis*	JQ772469	JQ772475
P. sp. 1	Miettinen 10543	China, Jilin Province	*Pinus koraiensis*	KM011978[a]	KY024313[a]
P. sp. 1	Spirin 3918	Russia, Khabarovsk Reg	*Larix gmelinii*	KM011979[a]	KY024314[a]
P. sp. 2	Spirin 5568-1	Russia, Khabarovsk Reg	*Pinus pumila*	KM011968[a]	KY024315[a]
P. sp. 2	Spirin 5567	Russia, Khabarovsk Reg	*Pinus pumila*	KM011967[a]	KY024316[a]
P. sp. 3	Korhonen 1136	India, Himachal Pradesh	*Cedrus deodara*	KM011966[a]	–
Onnia leporina	BRNM 712782	Czech, Jihlava	*Picea abies*	FJ775542	FJ775573

[a]Sequences newly generated in this study.

Phylogenetic analyses

Sequences were aligned in BioEdit with ClustalX and edited manually as necessary (Hall 1999). The resulting alignment was used in phylogenetic analyses with Maximum parsimony (MP) and Bayesian inference (BI). The sequences of *Onnia leporina* (Fr.) H. Jahn were chosen as the outgroup following Tomšovský & Kout (2013).

For MP analysis, the tests were implemented in PAUP* 4.0b10 using 1000 random sequence additions during the heuristic search, and all characters of the sequences were equally weighted and gaps were treated as missing data. Max-trees were set to 5000 branches of zero length were collapsed, and all parsimonious trees were saved. Branch supports was assessed using a bootstrap (BT) analysis by performing 1000 replicates datasets with random sequences addition for each bootstrap replicate (Felsenstein 1985). Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were calculated for each tree generated.

The best-fit evolution model was determined by MrMODELTEST2.3 (Nylander 2004, Posada & Crandall 1998) for BI. Four Markov chains were run for two independent runs from random starting trees with 4,000,000 generations, keeping one tree every 1,000 generation. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

Results

Molecular phylogeny

The combined dataset had an aligned length of 1843 characters, of which 1496 were constant, 261 were variable and parsimony-uninformative, and 86 were parsimony-informative. MP analysis yielded four equally parsimonious trees (TL = 413, CI = 0.889, HI = 0.111, RI = 0.846, RC = 0.751). The Bayesian analysis was performed using the GTR+I+G model for the ITS and *tef1-α* dataset. BI result is similar topology with MP, and only the MP tree is shown with both BT and BPPs values at the nodes (Fig. 1). *Porodaedalea chinensis* formed a distinct lineage and clustered within *Porodaedalea* clade with a strong support (BT = 99%, BPP = 0.99). The new species is closely related *P. yamanoi*, *P. cedrina* and *P. pini* with a low support (BT = 78%, BPP = 0.97). In addition, *Porodaedalea* sp. 1 (BT = 98%, BPP = 1.00) form Jilin Province of China and the Russia Far East, *Porodaedalea* sp. 2 (BT = 90%, BPP = 1.00) from Khabarovsk of Russia and *Porodaedalea* sp. 3 (BT = 62%, BPP = 0.96) from India also formed three distinct lineages, and they most probably are undescribed species.

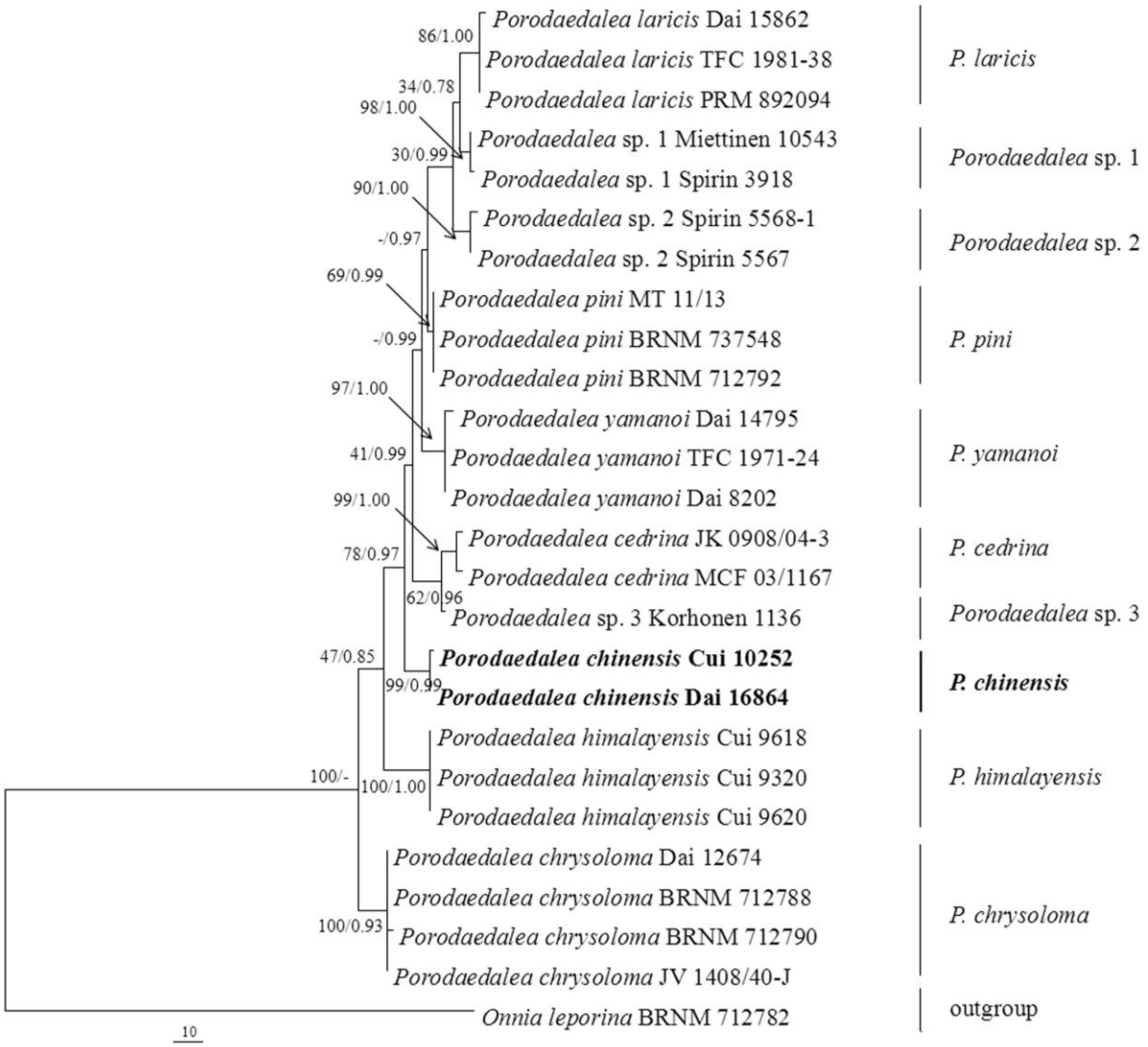

Figure 1 – Phylogenetic position of *Porodaedalea chinensis* inferred from the combined two genes (ITS and *tef1-α*).

Taxonomy

Porodaedalea chinensis S.J. Dai & F. Wu, **sp. nov.** Figs 2–3

Index Fungorum number: IF 553333; Facesoffungi number: FoF 02918

Etymology – *chinensis* = refers to the locality in China.

Fruiting body – Basidiocarps perennial, pileate, solitary to clusters, without odour or taste, hard corky when fresh, heavy and woody hard when dry. Pilei projecting up to 4.8 cm, 10 cm wide and 1.1 cm thick at base. Pileal surface greyish brown to black, concentrically sulcate with narrow zones, irregularly cracked and become encrusted with age; margin obtuse. Pore surface cinnamon to yellowish brown when fresh, becoming honey-yellow to rust-brown and slightly glancing when dry. Pores circular to angular, 2–3 per mm; dissepiments thick, entire. Context cinnamon, woody hard, up to 0.5 mm thick. Tubes yellowish brown, woody hard, up to 0.6 cm long.

Figure 2 – Basidiocarps of *Porodaedalea chinensis* (**paratype**, Dai 16864). Scale bar = 1 cm.

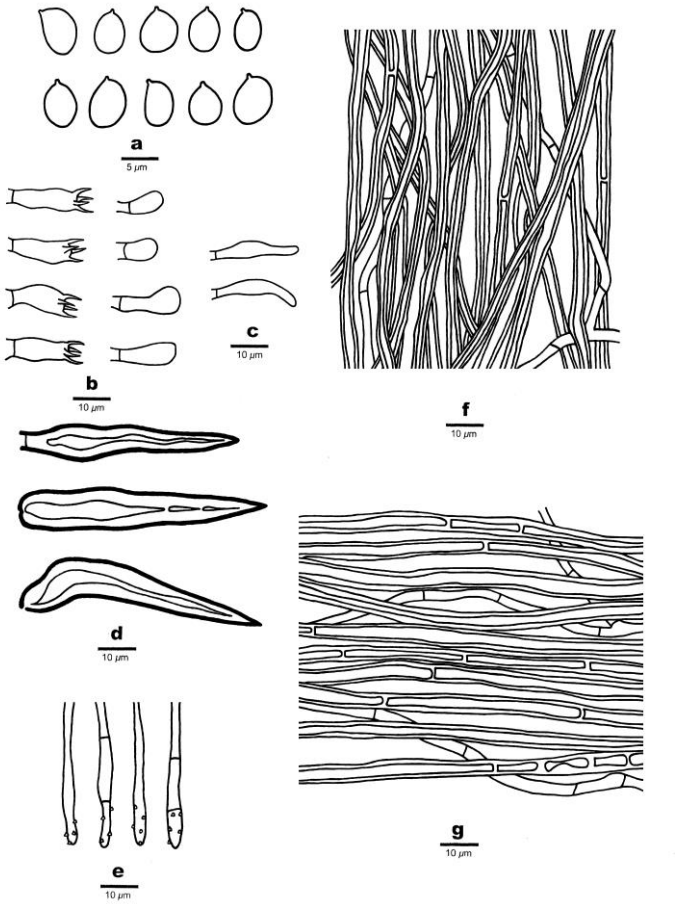

Figure 3 – Microscopic structures of *Porodaedalea chinensis* (**holotype**). **a** Basidiospores. **b** Basidia and basidioles. **c** Cystidioles. **d** Setae. **e** Hyphae at dissepimental edge. **f** Hyphae from trama. **g** Hyphae from context.

Hyphal structure – Hyphal system dimitic; generative hyphae simple septate; tissue darkening but unchanged in KOH.

Context – Generative hyphae hyaline, thin-walled, occasionally branched, frequently simple septate, 1.5–3 μm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a wide lumen, rarely branched, occasionally septate, straight, regularly arranged, 3–5 μm in diam.

Tubes – Generative hyphae frequent, hyaline, thin-walled, occasionally branched, frequently septate, some bearing crystals at dissepimental edges, 1–2 μm in diam; skeletal hyphae yellowish brown, thick-walled with a narrow to wide lumen, unbranched, occasionally septate, straight, parallel along the tubes, 2–5 μm in diam. Setae frequent, mostly originating from subhymenim, sometimes embedded in trama, but not seen at dissepimental edges, subulate, dark brown, thick-walled, (33–)35–69(–74) × 5–14(–15) μm; cystidioles infrequent, clavate, hyaline, thin-walled; basidia clavate, bearing four sterigmata and a simple septum at the base, 11–20 × 4–7 μm; basidioles dominating in hymenium, pear-shaped to capitate, mostly shorter than basidia.

Spores – Basidiospores broadly ellipsoid, hyaline, thin- to slightly thick-walled, smooth, without guttule, usually glued in tetrads, IKI–, moderately CB+, (3.9–)4–6 × 3–4.8 μm, L = 4.95 μm, W = 4.00 μm, Q = 1.19–1.27 (n = 60/2).

Known distribution – Yunnan Province, China.

Material examined – China, Yunnan Province, Chuxiong, Zixishan Forest Park, on living tree of *Pinus yunnanensis*, 16 April 2011, Cui 10252 (BJFC011148, **holotype**). China, Yunnan Province, Xishan Forest Park, on living tree of *Pinus yunnanensis*, 2 April 2016, Dai 16864 (BJFC011148, paratype); Chuxiong, Zixishan Forest Park, on living tree of *Pinus yunnanensis*, 16 April 2011, Cui 10254 (BJFC011149, paratype).

Discussion

Although *Porodaedalea* was established by Murrill in 1905, it was previously treated as the *Phellinus pini* complex (Gilbertson & Ryvarden 1987, Ryvarden & Melo 2014). The molecular analyses showed it was different from other groups in the *Phellinus* sensu lato and accepted as an independent genus (Wagner & Fischer 2002). Tomšovský & Kout (2013) employed the ITS and *tef1-α* to study *Porodaedalea* group that produced six strongly supported terminal clades. Our results confirmed that *P. chinensis* formed a distinct lineage from the analyses of ITS and *tef1-α* DNA sequences. *P. chinensis* was grouped with *P. pini*, *P. cedrina* and *P. yamanoi* in our phylogeny (Fig. 1). However, *P. pini* differs from *P. chinensis* in having wider hymenial setae (8–15 μm) and usually larger basidiospores (5.3–6.2 × 4–5.5 μm, Table 2). In addition, *P. pini* has slightly larger pores (1–3 per mm, Table 2). Compared with *P. chinensis*, *P. cedrina* also has bigger basidiospores (5–6 × 4.0–5.1 μm) and wider setae (6–18 μm, Tomšovský & Kout 2013). Moreover, it grows exclusively on *Cedrus* and has a distribution in Mediterranean area (Tomšovský & Kout 2013). *P. yamanoi* is different from *P. chinensis* in having interwoven tramal hyphae, and usually growing on *Picea jezoensis* (Dai 2010).

In fact, *Porodaedalea chinensis* is more similar to *P. laricis* in macromorphology, but *P. laricis* has shorter setae (30–58 × 6–12 μm) and slightly bigger basidiospores (4.9–5.5 × 3.5–4.2 μm, Table 2). The distribution of *P. chinensis* and *P. himalayensis* is overlap, but the latter species has smaller pores (5–7 per mm), shorter setae (27–39 μm), distinctly ovoid basidiospores, and grows mostly on *Picea* (Dai 2010).

Table 2 A comparison of morphological and ecological characters of *Porodaedalea* species.

Species	Distribution/ substrate	Pores (per mm)	Setae (μm)	Basidiospores (μm)
P. chrysoloma	Europe; *Picea*	2–3	30–55 × 6–11, L = 41.9, W = 8.1	4–5.3 × 3–4.5, L = 5, W = 4.1, Q = 1.24
P. chinensis	East Asia; *Pinus*	2–3	35–69 × 5–14, L = 52.9, W = 9.1	4–6 × 3–4.8, L = 4.9, W = 4, Q = 1.19–1.27
P. himalayensis	East Asia; *Picea*	5–6	24–43 × 6–10,	4.3–5.8 × 3.5–4.8,

P. laricis	East Asia; *Larix*	2–3	L = 33.4, W = 7.9 30–58 × 6–12, L = 44.5, W = 8.3	L = 4.8, W = 4, Q = 1.21 4.9–5.5 × 3.5–4.2, L = 5, W = 3.9, Q = 1.27
P. pini	Europe; *Pinus*	1–3	39–67 × 8–15, L = 50.8, W = 10.6	5.3–6.2 × 4–5.5, L = 5.9, W = 4.9, Q = 1.2
P. yamanoi	East Asia; *Picea*	3–4	36–65 × 7–13, L = 47.5, W = 10	4.2–5.8 × 3.8–4.5, L = 4.9, W = 4.1, Q = 1.19

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