
***Albatrellus piceiphilus* sp. nov. on the basis of morphological and molecular characters**

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Albatrellus piceiphilus is described as new from Gansu Province, China based on morphological characters and sequence data from the ITS region of nuclear ribosomal DNA. The new species is characterized by its pale yellow to yellowish-brown basidiocarps, simple septate generative hyphae, slightly thick-walled and distinctly amyloid basidiospores, and its habit on the ground in a *Picea crassifolia* forest. Parsimony analyses were applied to the ITS dataset. Our results suggest that *Albatrellus* is not monophyletic, and two clades of *Albatrellus* species are recognized. One clade including *A. piceiphilus*, *A. citrinus* and *A. ovinus* is strongly supported, which probably represents the core of *Albatrellus* in the russuloid clade. *Albatrellus syringae* is supported as a sister group to some polypores in the polyporoid clade, distantly away from the other *Albatrellus* species in the tree.

Key words: *Albatrellus*, ITS, phylogeny, polypore, taxonomy.

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Introduction

Albatrellus species are fairly common in northern temperate forests, and they usually produce medium to large fleshy fruiting bodies of various colours. Traditionally, species of *Albatrellus* has been classified as polypores due to their poroid hymenophore on the lower surface of basidiocarps. Most species are terrestrial having mycorrhizal connections with trees, while some may be wood-decaying taxa (Pouzar, 1972; Canfield, 1981; Ryvarden and Gilbertson, 1993). Basidiospores of *Albatrellus* species are ellipsoid to subglobose, smooth, and either amyloid or negative in Melzer's reagent. Recent phylogenetic studies using molecular markers showed that *Albatrellus* consists of two separate groups with affinities to the polyporoid clade and the russuloid clade (Gardes and Bruns, 1996; Hibbett *et al.*, 1997; Hibbett and Thorn, 2001; Larsson and Larsson, 2003; Greslebin *et al.*, 2004). While most of the species belong to the russuloid clade, the

closest relatives of *Albatrellus syringae* (Parmasto) Pouzar are members of *Antrodiella* Ryvarden & I. Johans., *Junghuhnia* Corda, *Steccherinum* Gray and *Ceriporiopsis* Domański, of the polyporoid clade (Binder *et al.*, 2005).

During a survey of polypores in Northwest China, two stipitate poroid specimens of Aphyllophorales were collected on the ground in a *Picea crassifolia* forest. Their macroscopic and microscopic features fit well with *Albatrellus* but do not match with the descriptions of any existing species in the genus. Members of both the polyporoid and the russuloid clades are highly diverse in their fruiting body morphology and microscopic characters, such as characters of spores and hyphae (Binder *et al.*, 2005). To confirm the affinity of the taxon and to infer the relationships within the genus *Albatrellus*, phylogenetic analyses were carried out based on the internal transcribed spacer sequences of ribosomal DNA (ITS) sequences data, in

addition to comparative studies of morphological characters. ITS sequences have been shown particularly valuable for the classification of fungi at the species level (e.g., Yao *et al.*, 1999; Liu *et al.*, 2001; Ryman *et al.*, 2003). With accumulating ITS data of *Albatrellus* species in the GenBank, a phylogenetic reclassification of the genus becomes possible. The results of both the morphological and DNA studies supported the recognition of the newly found species and its placement in *Albatrellus*, and the taxon is therefore described as new.

Materials and methods

Morphological studies

The studied specimens are deposited at the Herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Collections were examined in the microscope, extensive measurements in particular of spore dimensions were made, and pictures were drawn from slide preparations stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. IKI = Melzer's reagent, KOH = 5% potassium hydroxide, and CB = Cotton Blue. CB+ = cyanophilous and CB- = acyanophilous, and IKI- = both inamyloid and indextrinoid. In presenting the variation in the size of the spores, 5% of the measurements were excluded from each end of the range, and are given in parentheses. In the text the following abbreviations are used: L = mean spore length (arithmetical mean of all spores), W = mean spore width (arithmetical mean of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = number of spores measured from given number of specimens. The width of a basidium was measured at the thickest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. Sections were studied at magnification up to $\times 1000$ by using a Nikon Eclipse E600 microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Colour descriptions follow Petersen (1996) and Anonymous (1969).

Molecular techniques

DNA was extracted from dried herbarium materials following the methods of Grades and Bruns (1993), Yao *et al.* (1999) and Liu *et al.* (2001), with some modifications. About 10-30 mg dried specimen material was ground in liquid nitrogen, and then was mixed well with 600 μ l pre-warmed 2.5% CTAB extraction buff, containing 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), and 1.4 mol/L NaCl, followed by a water bath at 65°C for 50 to 80 minutes. 600 μ l chloroform-isoamyl alcohol (24:1 in volume) was added to precipitate unwanted cell components and proteins. 30 μ l 3 M NaAc was added to the aqueous phase followed by 200 μ l cold (-20°C) isopropanol to precipitate the DNA. The precipitated DNA was collected by centrifuge, washed with 500 μ l ice-cold 70% ethanol, and dried, then dissolved in 50 μ l sterile distilled water and stored at -20°C.

PCR amplifications follow the methods of Yao *et al.* (1999) and Liu *et al.* (2001). Primers for the PCR amplification were ITS1 and ITS4 described by White *et al.* (1990). 50 μ l reaction mixtures for the PCR were: genomic DNA in optimum concentration, 0.5 μ M of each primer, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.08% Nonidet P40, 1.5 mM magnesium chloride (MgCl₂), 200 μ M each of dATP, dCTP, dGTP and dTTP (Promega Co., USA), and 2 units Taq DNA polymerase (Sangon Ltd., Canada). Mineral oil (about 40 μ l) was overlaid on the reaction mixture. The PCR was performed in a MiniCyclerTM (MJ Research Inc., USA) for 35 cycles, including 94°C for 35 s, 50°C for 45 s, and 72°C for 45 s. PCR products used for sequencing were cleaned using the QIAquick PCR purification columns (Qiagen Ltd., UK). The DNA sequences were determined through direct PCR sequencing by GeneCore Biotechnologies (Shanghai, China). Sequences were edited with Sequencher version 3.1 (geneCodes Corporation, Ann Arbor, Michigan) and deposited in GenBank (Table 1).

Phylogenetic analyses

Taxa used in the phylogenetic analyses are listed in Table 1. One data set was prepared based on ITS sequences of 25 taxa, among which 18 taxa represent nine *Albatrellus*

Table 1. Species and sequences database accession numbers used in this study.

Species name	Collection no.	Locality	GenBank no.
<i>Albatrellus caeruleoporus</i> (Peck) Pouzar	K.A. Harrison 8825		AY963565
<i>Albatrellus citrinus</i> Ryman	Muskos 850928	Sweden	AY198190
<i>Albatrellus citrinus</i> Ryman	Ryman 9077	Sweden	AY198192
<i>Albatrellus citrinus</i> Ryman	Strid 16319A	Switzerland	AY198194
<i>Albatrellus ellisii</i> (Berk.) Pouzar			AY621803
<i>Albatrellus flettii</i> Morse ex Pouzar			AY061738
<i>Albatrellus ovinus</i> (Schaeff.:Fr.) Kotl. & Pouzar	Fransson 2	Sweden	AY198198
<i>Albatrellus ovinus</i> (Schaeff.:Fr.) Kotl. & Pouzar	Fransson 3	Sweden	AY198201
<i>Albatrellus ovinus</i> (Schaeff.:Fr.) Kotl. & Pouzar	Ryman 9132	Sweden	AY198202
<i>Albatrellus ovinus</i> (Schaeff.:Fr.) Kotl. & Pouzar	Danell 11/8 00	Sweden	AY198203
<i>Albatrellus piceiphilus</i> B.K. Cui & Y.C. Dai	Cui 2220	China	DQ789396
<i>Albatrellus piceiphilus</i> B.K. Cui & Y.C. Dai	Cui 2221	China	DQ789397
<i>Albatrellus similis</i> Pouzar			AY963566
<i>Albatrellus subrubescens</i> (Murrill) Pouzar	Ryman 6085	Sweden	AY198208
<i>Albatrellus syringae</i> (Parmasto) Pouzar	Cui 2183	China	DQ789394
<i>Albatrellus syringae</i> (Parmasto) Pouzar	Cui 2177	China	DQ789395
<i>Albatrellus syringae</i> (Parmasto) Pouzar	Ryman 6388	Sweden	AY198209
<i>Albatrellus syringae</i> (Parmasto) Pouzar	K.A. Harrison 6224		AY621804
<i>Antrodiella americana</i> Ryvardeen & Gilb.	Haikonen 14727	Finland	AF126877
<i>Antrodiella pallasii</i> Renvall <i>et al.</i>	T. Renvall 89a	Finland	AF126896
<i>Antrodiella romellii</i> (Donk) Niemelä	Renvall 3501		AF126902
<i>Auricularia</i> sp.			DQ200918
<i>Hericium alpestre</i> Pers.			AY534580
<i>Junghuhnia collabens</i> (Fr.) Ryvardeen			AF533965
<i>Leucophleps spinispora</i> Fogel	F3082		AY621749

species. ITS sequences of all the *Albatrellus* species included in this study were used as references to blast against GenBank, and sequences of *Leucophleps spinispora* Fogel, *Hericium alpestre* Pers., and four polypores including *Junghuhnia collabens* (Fr.) Ryvardeen and three species of *Antrodiella*, were downloaded from GenBank for high sequence similarity with *Albatrellus* species. DNA sequences were aligned with ClustalX using default setting (Thompson *et al.*, 1997) and further adjusted by eye in the data editor of PAUP* 4.0b (Swofford, 1999). ITS sequences were highly variable across sampled taxa, but sequences of two groups, one including *Albatrellus syringae* and four polypores, and the other comprising the other *Albatrellus* species, *Leucophleps* Harkn., and *Hericium* Pers., are alignable. Since our goal in this study was to investigate groups of closely related species within the genus *Albatrellus* under

current concept, gaps were introduced for an arbitrary alignment with these two classes and contributed partially to the long branch length observed in our tree. A proper outgroup for inferring relationships among these groups using ITS data is not possible. The data set in this study was arbitrarily rooted with *Hericium alpestre*. Data set was analyzed in PAUP* 4.0b (Swofford, 1999), with gaps treated as missing data.

Parsimony analyses were performed using equally weighting of characters and transformations. Heuristic searches were performed with one thousand replicate searches, each with one random taxon addition sequences. MAXTREES set to autoincrease, and TBR branch swapping. Robustness of individual branches was estimated by maximum parsimony bootstrap proportions (BP), using 500 replicates, each consisting of a single heuristic search with 50 random taxon

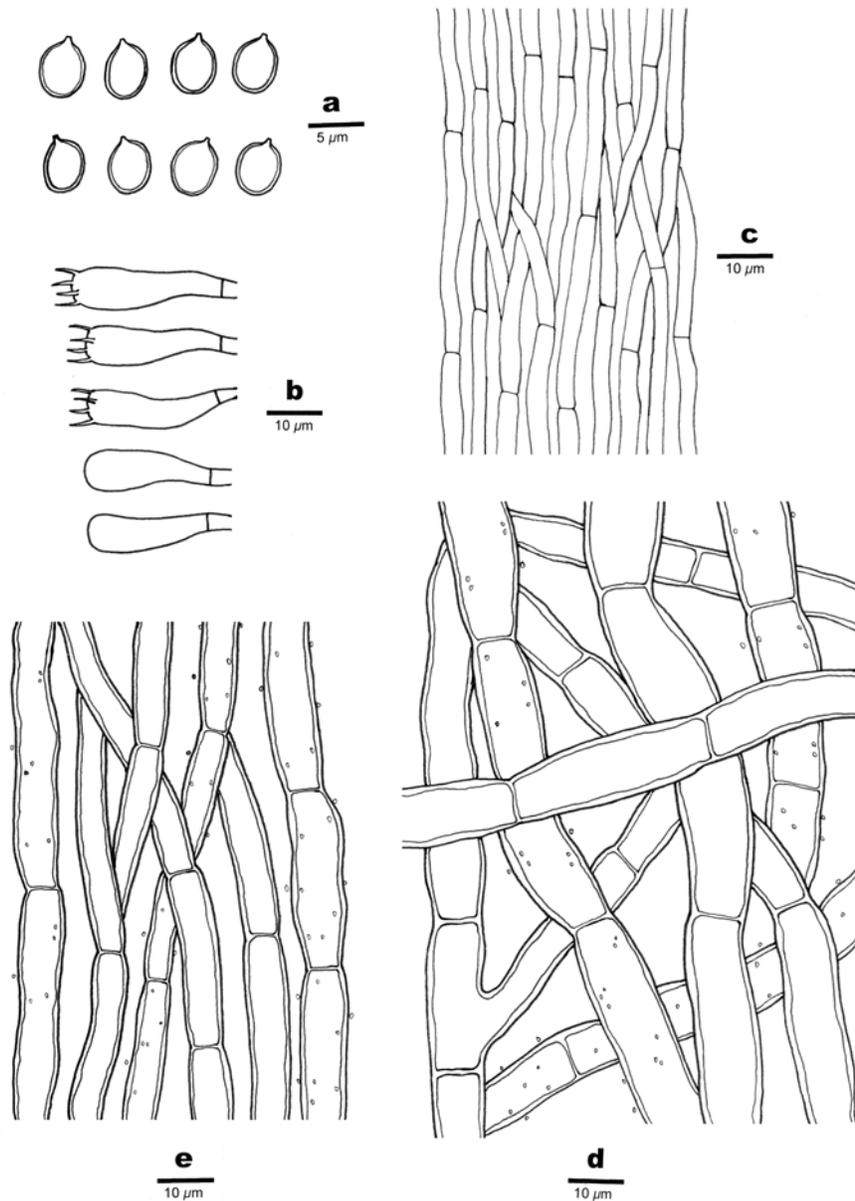


Fig. 1. Microscopic structures of *Albatrellus piceiphilus* B.K. Cui & Y.C. Dai (drawn from the holotype). **a.** Basidiospores. **b.** Basidia and basidioles. **c.** Hyphae from trama. **d.** Hyphae from context. **e.** Hyphae from stipe.

addition sequences, MAXTREES set to autoincrease, and TBR branch swapping.

Results

Albatrellus piceiphilus B.K. Cui & Y.C. Dai, **sp. nov.** (Fig. 1)

Mycobank: 510979.

Etymology: *Piceiphilus* (Lat.), growing on *Picea*.

Carpophorum annuum, stipitatum. *Facies* pororum pallide luteola vel luteola, pori 2-4 per mm. *Systema* hypharum monomiticum, *hyphae* generatoriae sine fibulis, *hyphae* contexti 5-10 μm in diam. *Sporae*

ellipsoideae vel perlate ellipsoideae, amyloideae, 4.4-5 × 3.5-4.1 μm.

Fruitbody - *Basidiocarps* annual, terrestrial, centrally or laterally stipitate, solitary, or several pilei fused to form more compound cluster, fleshy and watery, without odour or taste when fresh, becoming fragile with an unpleasant odour upon drying. *Pileus* more or less circular, sometimes depressed and infundibuliform, 7-12 cm in diam., and up to 6 mm thick at centre. *Pileal surface* yellowish to yellowish brown, smooth when fresh, becoming clay buff to pale brown, wrinkled

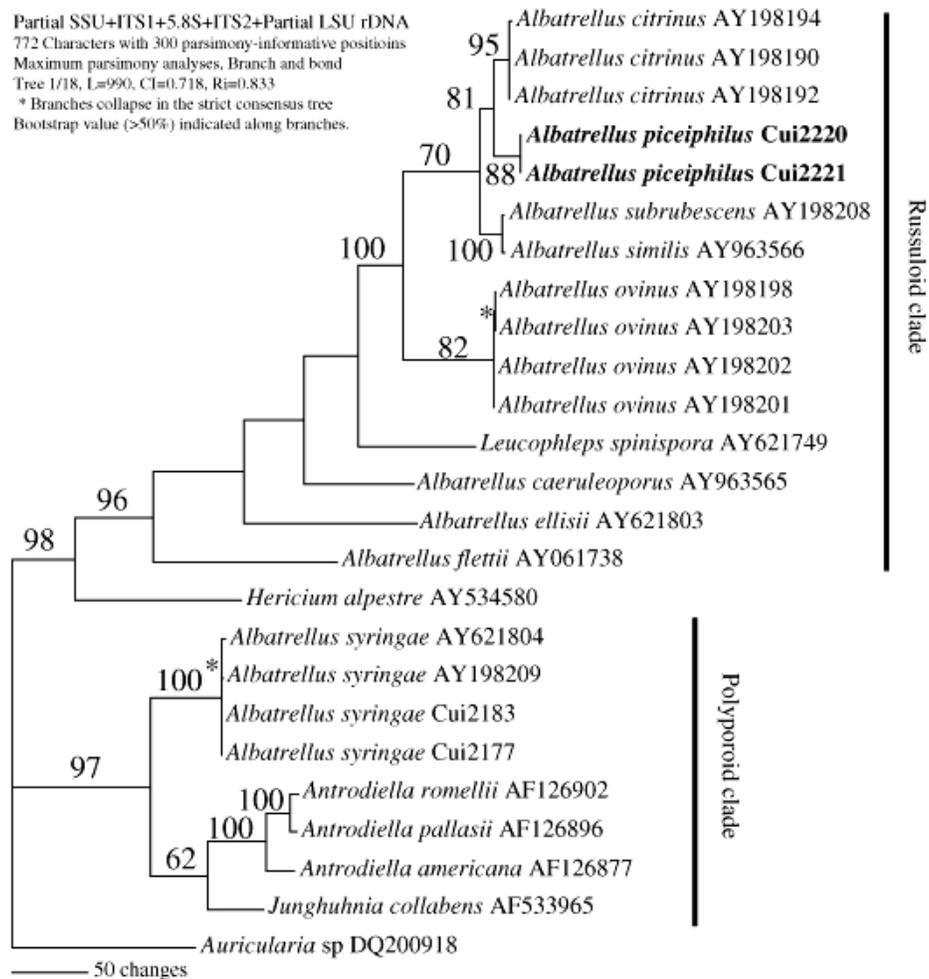


Fig. 2. Phylogenetic analyses of *Albatrellus piceiphilus* and the related species based on ITS region sequence data. Bootstrap less than 50% are not shown.

upon drying, azonate; margin sharp, sometimes lobed, yellowish when fresh, becoming pale brown upon drying. *Pore surface* pale yellow to yellow when fresh, becoming pale yellowish brown to olivaceous buff or cinnamon buff when dry; pores angular, 2-4 per mm, tube mouths thin, entire to slightly lacerate. *Context* yellowish, fleshy and watery when fresh, becoming yellowish to olivaceous buff, with a dark line next to the tubes, fragile to brittle upon drying, up to 3 mm thick. *Tubes* concolorous with pore surface, soft when fresh, become brittle when dry, up to 3 mm long. *Stipe* pale brownish vinaceous, watery and fleshy when fresh, becoming pale greyish

brown, wrinkled and brittle upon drying, up to 4 cm long and 1 cm in diam.

Hyphal structure — *Hyphal system* monomitic; generative hyphae simple septate, IKI-, CB-; tissue more or less darkening but otherwise unchanged in KOH.

Context — *Contextual hyphae* hyaline, thin- to slightly thick-walled, simple septate, occasionally branched, interwoven, 5-10 µm in diam., sometimes inflated, up to 23 µm in diam.; gloeoplerous hyphae absent; hyphae at stipe similar to contextual hyphae.

Tubes — *Tramal hyphae* hyaline, thin-walled, frequently simple septate, rarely branched, more or less straight and parallel

along the tubes, 2.6-5 µm in diam. Cystidia and cystidioles absent; basidia clavate, with four sterigmata and a basal simple septum, 15-25 × 5-7 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores — Basidiospores ellipsoid to broadly ellipsoid, hyaline, slightly thick-walled, smooth, distinctly amyloid, CB-, (4.2-) 4.4-5(-5.2) × (3.4-)3.5-4.1 (-4.4) µm, L = 4.78 µm, W = 3.85 µm, Q = 1.24-1.25 (n=60/2).

Habitat: On ground in *Picea crassifolia* forest.

Known distribution: Gansu Province of China.

Material examined: CHINA, Gansu Province, Yuzhong County, Xinglongshan Nature Reserve, on ground in *Picea crassifolia* forest, 26.VIII.2005 Cui 2221 (**holotype in IFP**, isotypes in H and HMAS); Gansu Province, Yuzhong County, Xinglongshan Nature Reserve, on ground in *Picea crassifolia* forest, 26.VIII.2005 Cui 2220 (paratype in IFP).

Relationships among the *Albatrellus* species were investigated using ITS region from 24 taxa. The data set had an aligned length of 772 base pair with 300 parsimony-informative positions.

Equally weighted parsimony analysis yielded 24 equally parsimonious trees of 900 steps with a consistency index CI = 0.718 (Fig. 2). Two strongly supported clades were recognized within targeted *Albatrellus* species: One consisted of *A. citrinus* Ryman, *A. piceiphilus*, *A. ovinus* (Schaeff.:Fr.) Kotl. & Pouzar, *A. subrubescens* (Murrill) Pouzar and *A. similis* Pouzar (BP = 100%), and within the clade, the new species *A. piceiphilus* shared a clade with *A. citrinus* (BP = 81%) with *A. ovinus* as weakly supported sister groups (BP = 70%). Relationships of *A. flettii* Morse ex Pouzar, *A. caeruleoporus* (Peck) Pouzar, and *A. ellisii* (Berk.) Pouzar to the clade and *Leucophleps* species were not resolved, though they were supported as clade with BP = 100%. Another clade (BP = 100%) consisted of four isolates of *A. syringae* with almost no difference in sequences, and this clade was strongly supported as the sister group to four investigated polypores (BP = 100%).

Discussion

Albatrellus piceiphilus is characterized by its simple septate generative hyphae,

slightly thick-walled and distinctly amyloid basidiospores, and by its occurrence on ground in *Picea crassifolia* forests. Its pileal surface is yellowish to yellowish brown when fresh, becoming clay buff to pale brown upon drying; pore surface pale yellow to yellow when fresh, becoming pale yellowish brown to olivaceous buff or cinnamon buff when dry. ITS phylogeny in this study strongly supports a close relationship among *A. citrinus*, *A. piceiphilus*, *A. ovinus*, *A. subrubescens* and *A. similis*, some of which have already been shown to belong to the russuloid clade (Larsson and Larsson, 2003; Binder *et al.*, 2005).

Phylogenetically, *Albatrellus piceiphilus* appeared to be closely related to *A. citrinus*, and both taxa having yellowish basidiocarps, simple septate generative hyphae, distinctly amyloid basidiospores and link with *Picea*. Basidiocarps of *A. citrinus* are white when fresh, and its context has no special odour when dry (Ryman *et al.*, 2003). On the contrary, *Albatrellus piceiphilus* has yellowish basidiocarps when fresh, and it has strongly unpleasant smell upon drying. In addition, basidiospores in *Albatrellus piceiphilus* are slightly wider than those in *A. citrinus* (4.4-5 × 3.4-4.1 µm, L = 4.78 µm, W = 3.85 µm, Q = 1.24-1.25 vs. 4-4.7 × 3-3.6 µm, L = 4.3 µm, W = 3.2 µm, Q = 1.3-1.4, Niemelä, 2005). *Albatrellus citrinus* was reported from China by Zheng *et al.* (2004), which has thin-walled basidiospores, while the spore size (4.5-5.5 × 3-4.5 µm) given by them is almost identical to *A. piceiphilus*.

Albatrellus piceiphilus is easily confused with *A. ovinus*, in particular because of the pale brown pileal colour, simple septate generative hyphae, and slightly thick-walled basidiospores (Gilbertson and Ryvarden, 1986; Ryvarden and Gilbertson, 1993). However, the latter species has gloeoplerous hyphae, and inamyloid and slightly smaller basidiospores (3.7-4.2 × 3.1-3.5 µm, Niemelä, 2005).

Albatrellus piceiphilus resembles *A. subrubescens* by having simple septate generative hyphae, and distinctly amyloid basidiospores (Gilbertson and Ryvarden, 1986; Ryvarden and Gilbertson, 1993),

however, *A. subrubescens* has gloeoplerous hyphae and smaller basidiospores ($3.8\text{-}4.6 \times 2.8\text{-}3.3 \mu\text{m}$, Niemelä, 2005).

Another Asian species, *Albatrellus dispansus* (Lloyd) Canf. & Gilb., has yellow basidiocarps; it was reported in Japan (Imazeki *et al.*, 1988), and we found it from central China, too. However, its pore surface is white and with a fragrant odour when fresh, and its basidiospores are negative in Melzer's reagent.

Macroscopically *Albatrellus piceiphilus* is similar to *A. syringae*, but the latter species has concentrically zonate pileal surface, clamped generative hyphae, and smaller, apically tapering basidiospores ($3.8\text{-}4.7 \times 2\text{-}3.5 \mu\text{m}$, $L = 4.24 \mu\text{m}$, $W = 3.15 \mu\text{m}$, Dai *et al.*, 2004; Niemelä, 1970). In addition, *Albatrellus syringae* was reported as a terrestrial species, mostly on lawn or in angiosperm forests, but it was recorded in spruce forest in Italy (Ryvarden and Gilbertson, 1993). We found this species on ground in *Salix* forest of northeast China, and on ground in spruce forest in northwest China. Also the ITS sequences of *Albatrellus syringae* are distinctly different from the other species of *Albatrellus*, our analysis of ITS regions placed *Albatrellus syringae* in the polyporoid clade, which congruent with previous studies (Bruns *et al.*, 1998; Binder and Hibbett, 2002; Ryman *et al.*, 2003).

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