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A new edible mushroom resource, *Pleurotus abieticola*, in southwestern China

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Abstract: Species of the genus *Pleurotus* are very important edible mushrooms and many of them can be cultivated in commercial scale. Although *P. abieticola* was originally described from Russian Far East, and then reported from northeastern China and northwestern Russia, its distribution range is still largely unknown. Our morphological and molecular phylogenetic evidence indicated that this species is also distributed in subalpine mountains of southwestern China. This paper documented the taxon based on morphological and ecological features, and DNA sequences generated from materials collected from Sichuan Province and the Tibet Autonomous Region.

Key words: Basidiomycetes, new distribution, edible mushroom, taxonomy

冷杉侧耳——中国西南一种新的食用菌资源

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摘 要：侧耳属 *Pleurotus* 真菌具有重要经济价值，该属不少种类可以商业化人工栽培。冷杉侧耳 *P. abieticola* 原初报道于俄罗斯远东地区，后来在我国东北和俄罗斯西北也有记载，但因为文献中记载的标本有限，我国研究人员对该种并不十分了解。在开展侧耳属的研究中，作者发现该种在我国西南亚高山地区也有分布。基于采自四川和西藏的标本，利用形态、生态特征及 DNA 序列证据，作者对该种进行了描述，以期为该种的资源开发利用提供科学依据。

关键词：担子菌，新分布，食用菌，分类

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INTRODUCTION

Many species of the genus *Pleurotus* (Fr.) P. Kumm. are very important edible mushrooms (Dai *et al.* 2010). Several of them are cultivated in large commercial scales (Sánchez 2010). Due to their importance, very rich studies on the genus were carried out in the past (Corner 1981; Hilber 1982; Singer 1986; Vilgalys *et al.* 1993; Vilgalys & Sun 1994; Segedin *et al*. 1995; Petersen & Krisai-Greilhuber 1996; Li & Yao 2004, 2005; Huang *et al.* 2010; Li *et al.* 2014).

During the study of edible mushroom resources in *Pleurotus*, we encountered an interesting species, *P. abieticola* R.H. Petersen & K.W. Hughes, which was originally described from Far East Russia, and then reported from northeastern China with a limited number of collections (Petersen & Hughes 1997; Albertó *et al.* 2002; Li *et al.* 2014). Our morphological and molecular phylogenetic evidence indicated that this species is also distributed in subalpine regions in southwestern China. This paper documented the taxon based on materials collected from Sichuan and Tibet.

1 MATERIALS AND METHODS

1.1 Specimens and morphological descriptions

Basidiomata were photographed and collected with field-notes. Specimens were dried and then kept in the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Morphological descriptions of the basidiomata are based on field notes. Method for microscopic observation and explanations of basidiospore data followed Yang & Feng (2013).

1.2 DNA extraction, PCR and sequencing

Total DNA was isolated from silica-gel dried materials using the CTAB method (Doyle & Doyle 1987). The internal transcribed spacer (ITS) region was amplified with primer pair ITS1/ITS4 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR program was as follows: pre-denaturation at 94°C for 4min; then followed by 35 cycles of denaturation at 94°C for 40s, annealing at 50°C for 50s, elongation at 72°C for 90s; afterwards, a final elongation at 72°C for 8min was included. PCR products were depurated with the Gel Extraction & PCR Purification Combo Kit (Spin-column, Bioteke, Beijing, China), and then sequenced on an ABI-3730-XL sequence analyzer (Applied Biosystems, Foster City, CA, USA) using the same primer combinations used for the PCR. Forward and reverse sequences were assembled and edited with SeqMan (DNA STAR package; DNAStar Inc., Madison, WI, USA). Sequences used in this study and additional sequences obtained by us were listed in Table 1.

1.3 Phylogenetic analyses

ITS sequences of genus *Pleurotus* were retrieved from GenBank and were combined with the ITS sequences generated in this study to form a dataset. *Pleurotus purpureo-olivaceus* were chosen as outgroups (Moncalvo *et al.* 2002). The dataset was then aligned using MAFFT v7.130b (Katoh & Standley 2013) and manually optimized on Bioedit v7.0.9 (Hall 1999).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were applied using RaxML (Stamatakis 2008) and MrBayes (Ronquist &

Table 1 Specimens used in molecular phylogenetic studies and their GenBank accession numbers

Species	Collections	GenBank accession #				
		ITS	*tef1-α*	*rpb1*	*rpb2*	LSU
Pleurotus abieticola	HKAS45720	KP771696	KP867895	KP867886	KP867879	KP867907
Pleurotus abieticola	TENN52359	AY450348	-	-	-	-
Pleurotus abieticola	HKAS45507	KP771697	KP867896	KP867887	KP867880	KP867908
Pleurotus abieticola	HKAS46100	KP771695	KP867897	KP867888	KP867881	KP867909
Pleurotus abieticola	TENN58284	AF345656	-	-	-	-
Pleurotus albidus	Duke327	AF345658	-	-	-	-
Pleurotus albidus	BAFC 50.261	AF345659	-	-	-	-
Pleurotus australis	CBS100127	EU424276	-	-	-	-
Pleurotus australis	PDD87/021XP	AY315764	-	-	-	-
Pleurotus citrinopileatus	HKAS85956	KP867919	KP867898	-	-	KP867910
Pleurotus citrinopileatus	HMAS63344	AY696301	-	-	-	-
Pleurotus citrinopileatus	TFM-M-E793	AB115043				
Pleurotus cornucopiae	TENN55191	AY450341				
Pleurotus cornucopiae	H-14	JQ837484				
Pleurotus cystidiosus subsp. *abalonus*	CBS80391	AY315806	-	-	-	--
Pleurotus cystidiosus subsp. *abalonus*	VT2476	AY315802	-	-	-	-
Pleurotus cystidiosus	IFO30607	AY315778	-	-	-	-
Pleurotus cystidiosus	AG55	FJ608592	-	-	-	-
Pleurotus eryngii	HIK135	HM998833	-	-	-	-
Pleurotus eryngii	HIK154	HM998841	-	-	-	-
Pleurotus eryngii	HIK139	HM998837	-	-	-	-
"*Pleurotus* cf. *eryngii*"	C24	FJ514570	-	-	-	-
Pleurotus eryngii var. *tuoliensis*	HIK152	HM998839	-	-	-	-
Pleurotus eryngii var. *tuoliensis*	HIK138	HM998836	-	-	-	-
Pleurotus eryngii var. *tuoliensis*	CCMSSC01433	KP867912	-	-	KP867873	KP867900
Pleurotus fossulatus	HIK127	HM998828	-	-	-	-
Pleurotus fossulatus	ATCC 52666	AY265833	-	-	-	-
Pleurotus levis	TENN58298	AF345662				
Pleurotus nebrodensis	HIK125	HM998826	-	-	-	-
Pleurotus nebrodensis	UPA6	HM998816	-	-	-	-
Pleurotus nebrodensis	HIK137	HM998835	-	-	-	-
Pleurotus ostreatus	HKAS84903	KP867913	KP867889	-	KP867874	KP867901

Table 1 continued

Table 1 continued

Pleurotus ostreatus	HKAS53480	KP867914	KP867890	-	KP867875	KP867902
Pleurotus ostreatus	CCMSSC06141	KP867915	KP867891	-	-	KP867903
Pleurotus ostreatus	TENN 53662	AY854077	-	-	-	-
Pleurotus populinus	TENN56749	AY450346	-	-	-	-
Pleurotus populinus	ATCC 90083	AY368667	-	-	-	-
Pleurotus pulmonarius	HMAS76672	AY696299				
Pleurotus pulmonarius	HKAS76382	KP867916	KP867892	KP867883	KP867876	KP867904
Pleurotus pulmonarius	ECS-0158	GU722283	-	-	-	-
Pleurotus purpureo- olivaceus	ICMP17077	GQ411512				
Pleurotus purpureo- olivaceus	PDD91632	GQ411523				
Pleurotus tuber-regium	DMC172	EU908193				
Pleurotus tuber-regium	RV95/947.1	AF109966				

Note: KP771695-KP771697, KP867873-KP867920 are sequences generated in this study.

Huelsenbeck 2003), respectively. For phylogenetic analysis, GTR+G was chosen as the best fit model for the dataset by using Mrmodeltest 2.3 (Nylander 2004). As GTR is the only model available in RAxML, we thus used GTRGAMMA with the default setting in ML analysis. Statistic supports were calculated using nonparametric bootstrapping with 1 000 replicates. For BI analysis, GTR+G model was used with the default setting. We set the generations to 2 million and used the stoprul command with the value of stopval set to 0.01. Trees were sampled every 100 generation. Statistic supports were obtained by the using sumt command implemented in MrBayes by discarding the first 25% of generations as burn-ins.

2 RESULTS

2.1 Molecular phylogeny

Our target species, *P. abieticola*, was related to the *P. ostreatus* - *P. pulmonarius* species complex, including *P. nebrodensis* (Inzenga) Quél., *P. eryngii* (DC.) Quél., *P. fossulatus* Cooke, *P. eryngii* var. *tuoliensis* C.J. Mou, *P. ostreatus* (Jacq.) P. Kumm., *P. populinus* O. Hilber & O.K. Mill., *P. albidus* (Berk.) Pegler, and *P. pulmonarius* (Fr.) Quél. with high statistical supports (Bootstrap values 100%, and Bayesian posterior probabilities 1) (Fig. 1). Three sequences generated from different collections of *P. abieticola* collected from southwestern China were clustered to the sequences generated from the same species collected from Russia (Bootstrap values 88%, and Bayesian posterior probabilities <0.90).

2.2 Taxonomy

Pleurotus abieticola R.H. Petersen & K.W. Hughes, Mycologia 89: 175, 1997. Figs. 2–3

Basidiomata small to medium-sized. Pileus flabelliform, 3–10cm from attachment to margin, 3–8cm in width; surface greyish, grey to brownish grey, becoming paler when mature, glabrous, smooth, finely innately streaked toward margin, appearing hygrophanous or slightly viscid when

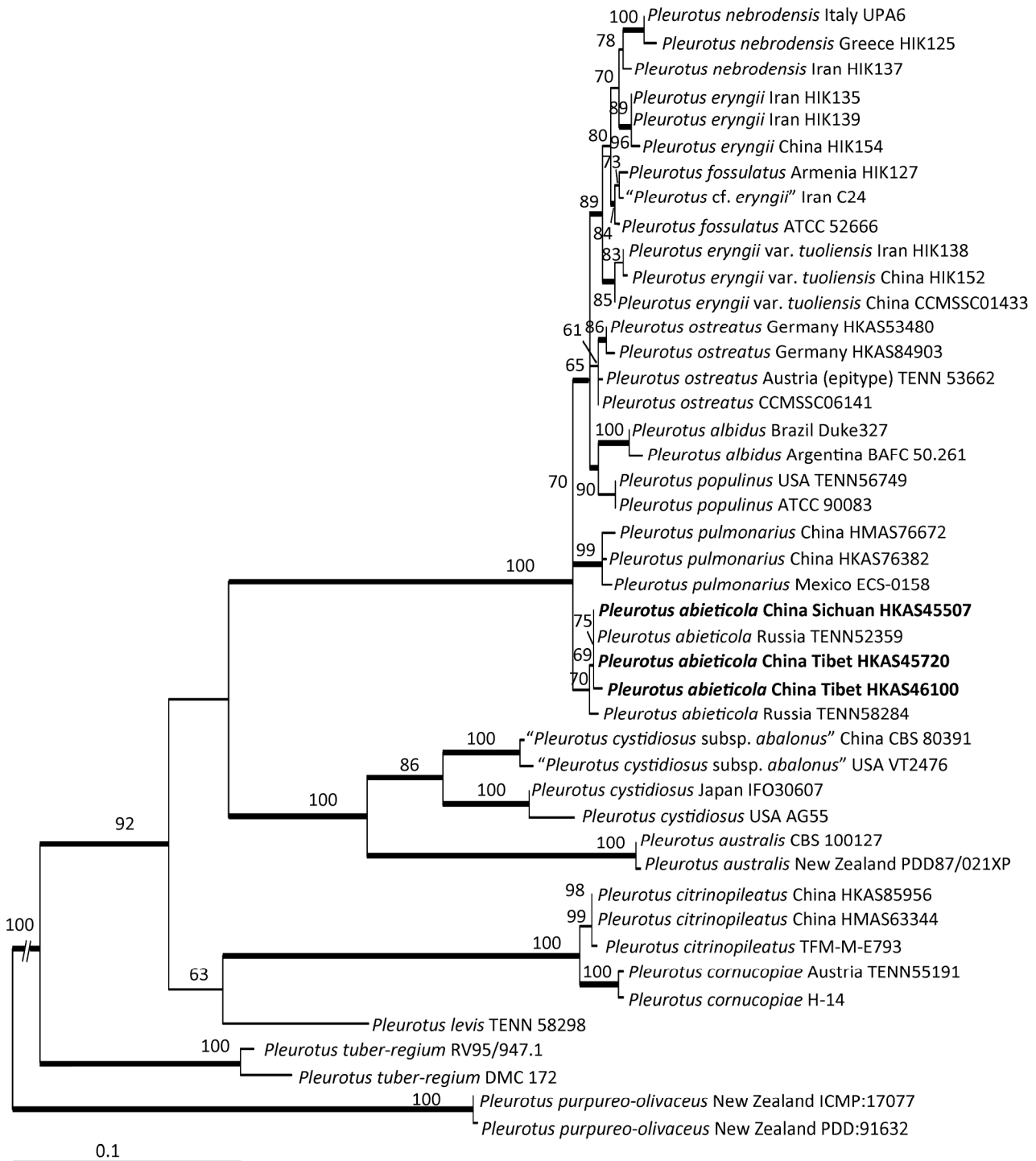

Fig. 1 Phylogenetic analysis of *Pleurotus* species inferred from Maximum Likelihood (ML) analysis of ITS sequences. Bootstrap values (ML, >50%) are shown above or beneath individual branches, Bayesian posterior probabilities (BI, ≥0.95) are indicated with thick branches. Sequences of the target species obtained in this study are in bold face.

Fig. 2 Basidiomata of *Pleurotus abieticola*. A: HKAS 45720; B: HKAS 46100.

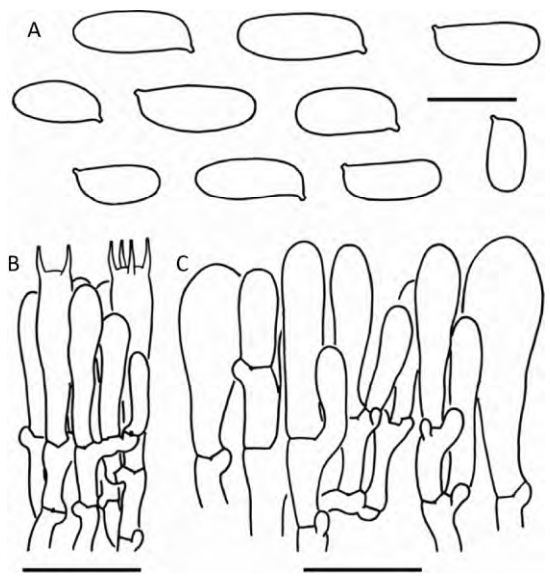

Fig. 3 Microscopic characters of *Pleurotus abieticola* (HKAS 45720). A: Basidiospores; B: Basidia; C: Cheilocystidia. Bars: A= 10μm, B and C= 20μm.

wet; margin inrolled when young, becoming straight by maturity; context white, unchanging, relatively thin (up to 8mm in thickness near attachment). Lamellae strongly decurrent, dense to subdistant, white; lamellar edge entire, concolorous with lamella surface; lamellulae 2–3 tiers. Stipe lateral to strongly eccentric, 0.5–2×0.5–1.5cm, subcylindric, whitish to white, longitudinally striate as extensions of lamellae but forming a reticulum on surface of stipe. Odour none; taste mild. Spore print white to cream-colored.

Basidiospores [70/3/3] (8–) 8.5–13 (–14)×4–5 (–5.5)μm, Q= (1.7–) 1.82–2.75 (–2.89) (**Q**= 2.26 ± 0.28), elongate to nearly cylindrical, colorless and hyaline, thin-walled, smooth, non-amyloid, non-dextrinoid. Basidia 27–40×5.5–8.5μm, narrowly clavate, hyaline, thin-walled, 4-spored, sometimes 2-spored; sterigmata 3–5μm long. Cheilocystidia abundant, broadly clavate, clavate, narrowly clavate to nearly cylindrical, 15–40×5–14μm, colorless and hyaline, thin-walled. Pleurocystidia absent. Lamellar trama monomitic, composed of ± irregularly arranged thin- to thick-walled (up to 2μm thick), colorless and hyaline, filamentous hyphae 3–10μm wide. Pileipellis a 40–60μm thick cutis composed of repent, radially arranged, yellowish to brownish filamentous hyphae 2–5μm wide. Trama of pileus monomitc, composed of radially to irregularly arranged thin- to slightly

thick-walled (up to 1μm thick), colorless and hyaline, filamentous hyphae 3–15μm wide. Trama of stipe monomitc, composed of vertically to irregularly arranged thin- to thick-walled (up to 2μm thick), colorless and hyaline, filamentous hyphae 3–10μm wide. Stipitipellis composed of vertically arranged, branching and sometimes anastomosing hyphae 3–5μm wide. Clamp connections abundant in all tissues.

Habitat and known distribution: growing on rotten wood of *Picea* in subalpine forests dominated by *Picea*; in summer at elev. 3 600–4 100m in southwestern China. Also known from northeastern China and Russia.

Specimens examined: CHINA, Sichuan Province, Xiangcheng County, Reda, alt. 3 600m, 14 July 2004, Z.L. Yang 4122 (HKAS 45507). Tibet Autonomous Region, Leiwuqi County, Mengda, alt. 4 100m, 9 August 2004, Z.L. Yang 4341 (HKAS 45720); Leiwuqi County, Haola, alt. 3 900m, 10 August 2004, Z.W. Ge 320 (HKAS 46100).

3 DISCUSSION

Although the genus *Pleurotus* harbors many economically important species due to their well known usage as vegetable or food (Dai *et al*. 2010), species of *Pleurotus* are morphologically not easily separated from each other. Both morphological and molecular phylogenetic data should be employed in the characterization of the species of the genus. In addition, cultural characters and mating tests can also provide useful evidence for species delimitation (Petersen & Hughes 1993, 1997).

Morphologically, *Pleurotus abieticola* is very similar to the other species of the *P. ostreatus* - *P. pulmonarius* complex, including *P. pulmonarius*, *P. ostreatus*, *P. populinus*, *P. albidus*, *P. nebrodensis*, *P. eryngii*, *P. fossulatus*, and *P. eryngii* var. *tuoliensis*, which produce monomitic basidiomata. However, it differs from the other taxa by its occurrence mainly on coniferous rotten wood and the common presence of cheilocystidia (Petersen & Hughes 1997; Albertó *et al.* 2002; our observations).

Phylogenetically, *Pleurotus abieticola*, was related to the *P. ostreatus* - *P. pulmonarius* species complex (Fig. 1). *Pleurotus abieticola* was basal to all the other species mentioned above, which is consistent with the results of Albertó *et al.* (2002).

Geographically, *P. abieticola* was originally described from far-eastern Russia (Sichote Alin Biosphere Preserve), and then reported from northeastern China (Songjianghe and Baihe in Jilin Prov.) and northwestern Russia (north of St. Petersburg) based on all the five collections available then (Petersen & Hughes 1997; Albertó *et al.* 2002; Li *et al.* 2014). Our collections made in southwestern China largely extend the known distribution range of the species.

Although the epithet "*abieticola*" may indicate that the species has a preference for substrates of *Abies*, *P. abieticola* can also grow on rotten wood of *Picea* (Albertó *et al.* 2002; our observations in the field). In addition, according to Albertó *et al.* (2002), this taxon was on *Alnus* or *Salix* in northwestern Russia.

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