

Full Length Research Paper

A new subspecies of *Lentinellus* and its phylogenetic relationship based on ITS sequence

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***Lentinellus castoreus* is a complex group. A new taxon under this species was found in northeast of China in this study. The new one was distinguished from the other sub-taxon by its morphological characteristics of denty tomentum, subdistant lamellae and inamyloid hyphae. The assignment of the new taxon was supported by phylogenetic analyses based on the internal transcribed spacer (ITS) sequences. The results of molecular analyses demonstrated that the subspecies was clustered in a clade which included the sequences of Chinese, Russian and Japanese. It is named as *Lentinellus castoreus* subsp. *orientalis* Yu Liu and Tolgor Bau after the authors examined specimens with the morphological and molecular taxonomic methods.**

Key words: New subspecies, morphology, taxonomy, phylogenetic tree.

INTRODUCTION

The genus *Lentinellus* was erected by P. Karsten in 1879, and belongs to the family Auriscalpiaceae, in Russulales, Agaricomycetes, and Basidiomycota (Kirk et al., 2008). This genus is characterized by possessing mostly pleurotoid basidiomata with serrate lamellae, nodulose and amyloid basidiospores. *Lentinellus* which comprises about 45 species under 65 names were known all over the world. And among them, 10 species were reported in China (Tai, 1979, Teng, 1963, Wang and Ayinuer, 2004, Petersen and Hughes, 2004).

Lentinellus castoreus was named in 1934, and appears to be distributed over South America, all of temperate North America, much of Europe and Russian Caucasus, Australia and New Zealand (Petersen and Hughes, 2004). More recently, it was also found in southwest of China (Liu and Bau, 2011a). *L. castoreus* has outstanding characteristics of crepidotoid basidiomata, ochraceous buff pileus with heavy tomentum on the whole surface, crowded lamellae and heavy amyloid hyphae in the trama

tissue. So far, *L. castoreus* is a complex group with 3 subspecies and 3 variety species under the species rank (Kirk et al., 2008).

A new taxon of *Lentinellus* was found in Jilin, Heilongjiang and Inner Mongolia provinces of China by the authors of this study. It belongs to the species of *L. castoreus* by confirming with the morphological and molecular taxonomic methods. But some of its morphological characters were distinct from all the groups of *L. castoreus*. To establish its taxonomic position, it is named as *Lentinellus castoreus* subsp. *orientalis* Yu Liu and Tolgor Bau after the authors doing morphological and molecular taxonomic studies.

MATERIALS AND METHODS

The specimens identified in this research are collected from some places of Jilin province, Heilongjiang province and Inner Mongolia. Morphological characteristics of these specimens were described

Table 1. The Genbank numbers of ITS sequences in the phylogenetic tree.

Sequence source	GenBank Number	Specie	Location	Specimen Number
Download sequences	AY513133	<i>Auriscalpium vulgare</i>	Sweden	-
	AY299367	<i>L. castoreus</i>	North Carolina of USA	-
	AY513180	<i>L. castoreus</i>	Tennessee of USA	-
	AY513177	<i>L. castoreus</i>	Florida of USA	-
	AY513174	<i>L. castoreus</i>	California of USA	-
	AY513189	<i>L. castoreus</i>	Australia	-
	AY513195	<i>L. castoreus</i>	Russia	-
	AY513175	<i>L. castoreus</i>	Russia	-
	AY513182	<i>L. castoreus</i>	Costa Rica	-
	AY513188	<i>L. castoreus</i>	Japan	-
	AY513190	<i>L. castoreus</i>	New Zealand	-
	AY513176	<i>L. castoreus</i>	Mexico	-
	AY513187	<i>L. castoreus</i>	Costa rica	-
	AY513185	<i>L. castoreus</i>	Argentina	-
	Testing sequences	FJ869340	<i>L. sinensis</i>	Jilin of China
FJ869341		<i>L. brunnescens</i>	Jilin of China	HMJAU 20186
GQ219734		<i>L. castoreus</i> subsp. <i>orientalis</i>	Inner mongolia of China	HMJAU 6664
GQ219727		<i>L. castoreus</i> subsp. <i>orientalis</i>	Jilin of China	HMJAU 6359
FJ869344		<i>L. ursinus</i>	Inner mongolia of China	HMJAU 6371
GQ142012		<i>L. flabelliformis</i>	Inner mongolia of China	HMJAU 6384

HMJAU, Herbarium of Mycology of Jilin Agricultural University, Changchun, China.

and illustrated with macro- and micro-morphological methods. For microscopic studies, the basidiomata were examined using a 5%KOH solution as the developing solvent and 1% Congo red solution as the staining agent. At least, 20 basidiospores, basidia and cystidia per specimen were measured. The specimens identified in this research were all deposited on the Herbarium Mycologicum Academiae Sinicae (HMAS) and Herbarium of Mycology of Jilin Agricultural University (HMJAU) in China. The authors prefer using the terms "dimitic" and "trimitic" in describing the types of hyphal construction of tramal tissue, and among them, "dimitic trama" are bearing generative hyphae and gloeoplerous hyphae; "trimitic trama" are bearing generative hyphae, gloeoplerous hyphae and skeletal hyphae.

The molecular taxonomic study is based on sequence analysis of internal transcribed spacer (ITS). The methods of DNA extraction and polymerase chain reaction (PCR) amplification were according to Petersen and Hughes (2004), and products of PCR amplification were sequenced by Sangon Co., Shanghai, China. 20 sequences of *Lentinellus* were selected to construct phylogenetic tree. Among them, 6 sequences are tested by ourselves in this study, and 14 sequences of the closest species were downloaded from GenBank and for construction of the consensus tree with *Auriscalpium vulgare* Gray as the outgroup. Their GenBank Accession numbers are listed in Table 1. Phylogenetic relationships were analyzed using the neighbor-joining method (Saitou and Nei, 1987) with the software Mega 4.0.

RESULTS

Taxonomy

Lentinellus castoreus subsp. *orientalis* Yu Liu and T. Bau,

subsp. nov (Figure 1).

MycoBank MB 804815

Ut Lentinellus castoreus, sed: *Lamellae latus, subdistantibus. Pileus tomentulosus. Skeletal hyphae non amyloideae. In Northeast Asia.*

Holotype: Lushuihe, Fusong County, Jilin Province, China, 4 July 2008, Tolgor Bau, HMJAU 6664.

Etymology: from its main distributed place.

Basidiomata is up to 1.5-6.5 × 1-4.5 cm, gregarious to subimbricate, crepidotoid to conchate. Pileus is yellow brown or cinnamonaceous when young, brown to reddish brown when mature, often with felty tomentum, inward appearing carpet- or leather-like by adherent together, sometimes up to 1 mm thick; outward pileus to margin, tomentum reduced to short spikes and dark brown dots representing spike bases. Flesh is thick, soft, sand-colored to pale watery brown when fresh. Lamellae convergent at attachment, subdistant, up to 5 mm deep, thick, pale ochraceous to tawny, avellaneous when dry, dull brown overall and often with whitish powder; margin serrate. Stipe, none while spore prints are white.

Basidiospores is 4-5(-5.2) × 3-3.8 (-4) μm, 4-5 × 3-3.5 μm on average, ellipsoid to broadly ellipsoid, thin-walled, with scattered wart on the surface, amyloid. Basidia is 17-22 × 5-6 μm, subclavate, more or less cylindrical or cigar-shaped; sterigmata 4, 4-5 μm long. Pleurocystidia is

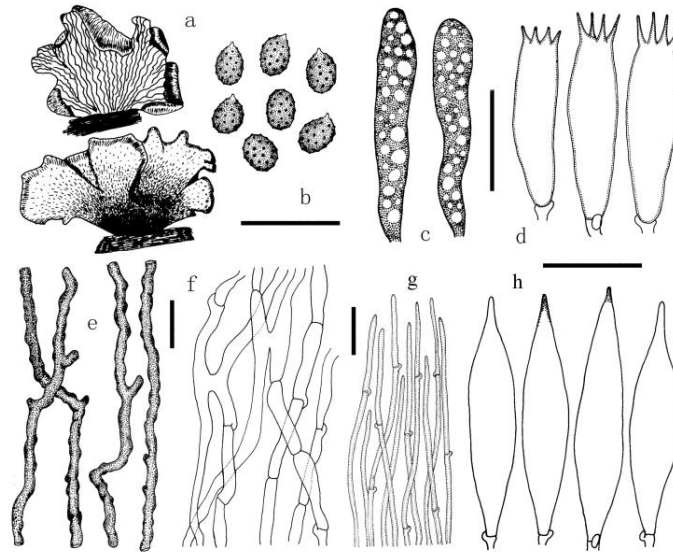


Figure 1. *Lentinellus castorceus* subsp. *orientalis* Yu Liu and Tolgor Bau (HMJAU 6664). Bar = 10 μ m. a, Basidiomata; b, basidiospores; c, gloeocystidia; d, basidia; e, skeletal hyphae; f, generative hyphae; g, suprapellis hyphae; h, pleurocystidia.

uncommon, 25-28 \times 3-5 μ m, narrowly fusiform to fusiform, lanceolate apically, hyaline, thin-walled, clamped. Gloeocystidia is 18-22 \times 5-6 μ m, clavate to subcapitate, yellow-refringent; contents uni- or biguttulate. The lamellar trama is interwoven, trimitic: generative hyphae abundant, 3-8 μ m diameter, hyaline, thin-walled, clamped; skeletal hyphae common, 3.5-5 μ m diameter, thick-walled to cell lumen occluded, bent or interwoven, strongly sinuate, with more knobby prominence, inamyloid to weakly amyloid; gloeoplerous hyphae abundant, 4-5 μ m diameter, thin-walled, contents often with discontinuous resinous drops. Pileipellis generative hyphae coherent, involved in mucus; hyphae 3.5-7 μ m diameter, watery brown in mass, thin-walled, sometimes ornamented with minute granules or banded, clamped. Suprapellis generative hyphae clamped, thick-walled, pallid yellow-brown singly, deep yellow-brown in mass, gathered into conical, coherent spines as tomentum on the surface of pileus. The pileus trama is tightly interwoven, trimitic: generative hyphae 3.5-7 μ m diameter, smooth on the surface, thin- to thick-walled, clamped; skeletal hyphae frequent, same as the lamellar trama; gloeoplerous hyphae rare, 3.5-7.5 μ m diameter; contents often with discontinuous resinous drops. The habitat is on rotten wood of broad-leaved tree with distributions in China and probably in Japan, Russia.

Specimens examined include Heilongjiang (China): Longwangmiao, Mishan City, 01-Sep-2003, Tolgor Bau (HMJAU3032); Rudai forest farm, Raohe City, 07-August-2004, Tolgor Bau (HMJAU2508). Jilin(China): Qinggouzi, Dunhua City, 13-August-1999, Tolgor Bau (HMJAU1865); Zuojiia Town, Jilin City, 16-sep-2000, Tolgor Bau (HMJAU1495); Jingyuetan, Changchun City, 29-July-

2004, Jianrui Wang (HMJAU3480); Jingyuetan, Changchun City, 10-June-2005, Jianrui Wang (HMJAU6368, HMJAU6369); Lushuihe Town, Fusong County, 30-June-2005, Tolgor Bau (HMJAU4052); Lushuihe Town, Fusong County, 4-July-2008, Tolgor Bau (HMJAU6664)(Typus!); Dashitou Town, Dunhua City, 11-August-1986, Kai Tao and Y. M. Li (HMAS85956); Erdaobaihe Town, Antu County, 10-Jul-2007, Yuguang Fan (HMJAU6359); Inner Mongolia (China): Yiershi town, Aershan City, 8-Sep-2002, Tolgor Bau (HMJAU20062); Daqinggou, Tongliao City, 1997, Tolgor BAU(HMJAU1512, HMJAU1708); Feitianling, Aershan City, 30-August-2006, Yu Wan (HMJAU20199).

DISCUSSION

The main characteristics of the new taxon in this study which were collected in northeast of China by the authors, are often with astipitate and brownish basidiocap, felty tomentum on the whole cap and appearing carpet- or leather-like by adherent together. These characteristics are all typical morphological characters of the genus *Lentinellus*. Appearance characteristics of this new taxon are similar to *Lentinellus ursinus* (Fr.) Kühner at first glance, especially its tomentose cap surface. But the other characteristics of this subspecies with distant and thick lamellae, inamyloid skeletal hyphae are very different from other groups under the species of *L. castoreus* and *L. ursinus*. Further more, those subtaxon are all distributed on America and Europe, such as *L. castoreus* f. *galeiformis* R.H. Petersen and *L. castoreus* f.

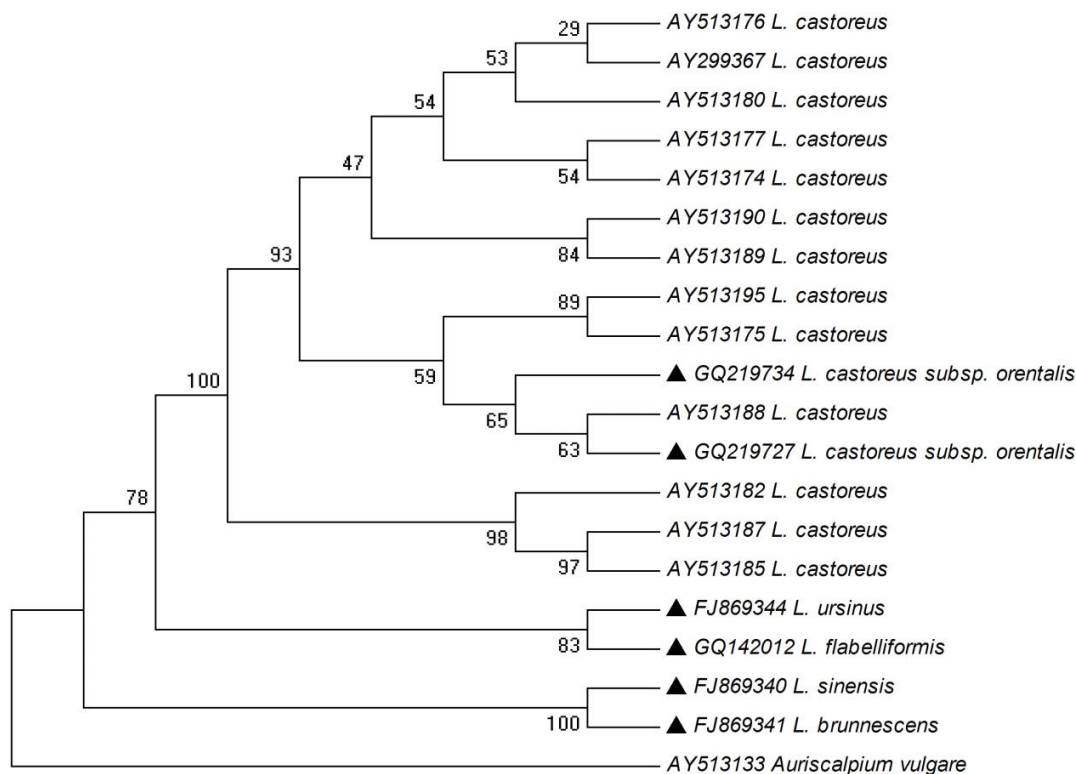


Figure 2. The phylogenetic tree of *Lentinellus* base on internal transcribed spacer (ITS) sequences by neighbor-joining (N-J) method. ("▲", testing sequences in this research).

ochropallidus P.-A. Moreau ex R.H. (Petersen and Hughes, 2004). These two species are all with closed lamellae and distributed on America and Europe. Though *L. castoreus* f. *pseudoporosus* (Petersen and Hughes, 2004) often with a sub-distant lamellae is as same as the new taxon, its pseudostipitate and distribution on Mexico and Central America are different from our new one.

Phylogenetic tree was constructed (Figure 2) in order to analyze the relationships among the groups of *L. castoreus* which distribute all over the world and some relatively closed groups, also with some species which were collected in northeast of China by the authors. The results show that, all the groups of *L. castoreus* analyzed in this study formed a clade, with a comparatively high support (100%). Among them, a single clade was formed by the groups originated from Russia, Japan and China, five of these sequences are more closed than any other groups which originated from other location such as Sweden, USA, Australia, Mexica, Argentina, New Zealand, Costa Rica and so on, with a comparatively high support (93%).

In the clade of Russia, Japan and our new taxon in the phylogenetic tree, the specimen of the sequence AY513188 originated from Japan. Petersen and Hughes (2004) recorded that the basidiomata of this specific specimen from Japan were effuse-reflexed, with widely spaced, coarsely digitate-serrate lamellae when was described a special example of *L. castoreus*, but its DNA-

ITS molecular phylogenetic reconstruction places the specimen clearly in the *L. castoreus* clade. Fortunately, its morphological characteristics were identical to our new taxon. Then, we downloaded this sequence (AY513188) and added it to the phylogenetic tree in this study. The result shows that it is the closest to the new taxon.

The new taxon belonged to *L. castoreus* by analysis of morphological and molecular taxonomy. Some characters of the new taxon are obviously different from other groups of *L. castoreus* such as morphological discussion, and it is more closely related to Japanese and Russian groups of *L. castoreus* according to molecular taxonomic messages. For this reason, we finally concluded it as a new geographic subspecies of *L. castoreus*, and named it as *L. castoreus* subsp. *orientalis* Yu Liu and Tolgor Bau.

L. vulpinus (Sowerby) Kühner and Maire was previously recorded from China including some brief descriptions of morphological characteristics (Tai, 1979; Teng, 1963). But these specimens were all found to be *L. castoreus* subsp. *orientalis* after the authors examined the voucher specimens in the recently research.

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