Lepiota amplicystidiata, a new species from Tibet

J. F. Liang*

Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou 510520, People's Republic of China

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The new species *Lepiota amplicystidiata,* sp. nov., is described and illustrated from Tibet. It is characterized by its pileus with radially sulcate striate margin and spores fusiform in frontal view and penguin-shaped with straight or almost straight abaxial side in side view distinctly narrowed towards the apex, widely clavate cheilocystidia, and a trichodermal pileus covering of elongate, subcylinderical elements.

Keywords: Agaricales, Agaricaceae, lepiotaceous fungi, taxonomy, phylogeny.

Lepiota (Pers.) S. F. Gray is an important genus in Agaricaceae. There are nearly 70 species reported from China (Teng 1936, Chiu 1948, Li *et al*. 1993, Bi *et al*. 1986, Yang 1994, Yuan & Sun 1995, Tolgor & Li 2004, Wang & Yang 2005, Liang 2007). Teng (1936) and Chiu (1948) reported some species from China, but did not mention the specimen accession numbers. A re-examination of cited and thus traceable specimens showed that a number of them, including some originally described from China, were misidentified (Liang *et al*. 2010, Liang *et al*. 2011; Liang & Yang 2011). Based on the author's estimate, there are more than 100 species of *Lepiota* in China. Some specimens collected from Tibet were reported as *L. clypeolaria* (Bull.) P. Kumm. and *L. ventriosospora* D. A. Reid by Mao (1995). Re-examination of these collections revealed that they are only one taxon, which is different from the known species. Thus, we describe and illustrate it as a new species.

Materials and methods

Specimens were borrowed from the Herbarium Mycologicum Academiae Sinicae (HMAS). Terminology for descriptive terms follows Vellinga & Noordeloos (2001) and colour designations, Kornerup & Wanscher (1981). Herbarium abbreviations follow Thiers (2012).

Morphology

For microscopic observations, sections of specimens were cut by hand and mounted in 5 % KOH, Congo red, and Melzer's reagent. Basidiospores

* e-mail: jfliang2000@163.com

were mounted in cresyl blue to test for a metachromatic reaction (Singer 1986). Sizes of basidia, basidiospores, cheilocystidia, and elements of the pileus covering were measured. At least 20 elements of each fungal structure were measured. The abbreviation [n/m/p] indicates that measurements were made on n basidiospores in m basidiomata from p collections. Dimensions of basidiospores are given using a notation of the form (a) b–c (d). The range b–c contains a minimum of 90 % of the measured values. Extreme values are given in parentheses. The following abbreviations are used: Q refers to the length/breadth ratio of basidiospores; Q_{av} refers to the average Q of all basidiospores ± standard deviation.

Molecular identification

DNA was extracted from herbarium material using a CTAB protocol (Zhou & Liang 2011). The internal transcribed spacer (ITS) of the nuclear ribosomal repeat was amplified by PCR with the fungal specific primers ITS1 and ITS4 (White *et al*. 1990). Sequencing of both strands was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services CO., Ltd, Shanghai). DNA sequences were edited and aligned using SeqMan (DNASTAR Package) and ClustalX (Thompson *et al*. 1997). The matrix was manually checked and modified. Ambiguous positions were excluded. Thirty nine taxa were included in the phylogenetic analyses. Among them 21 were downloaded from Genbank. Their accession numbers and their detailed information about each specimen are shown in Tab. 1. *Leucoagaricus leucothites* (Vittadini) Wasser and *La. nympharum* (Kalchbr.) Bon were selected as outgroup.

The matrix was analyzed with Bayesian inference using the parallel version of MrBayes 3.1.2 with default priors (Ronquist & Huelsenbeck 2003, Altekar *et al*. 2004) and maximum likelihood with the RAxML BlackBox online server (Stamatakis *et al*. 2008). Bayesian analysis was implemented on a Linux cluster under a general-time-reversible (GTR) model (nst = 6) and rate heterogeneity parameters (gamma-distributed substitution rate and a proportion of invariable sites) following the AIC criterion in Modeltest 3.06 (Posada & Crandall 1998).

Results and discussion

Phylogenetic study

We analyzed a dataset of ITS sequences with 755 nucleotide sites for 39 taxa. The dataset contained all hitherto available sequences of sect. *Lepiota* except two sequences (*L. pongduadensis* HQ718461 and *L. eurysperma* HQ718462) and of most species of other sections in *Lepiota*. Two recently described species from Thailand, *L. pongduadensis* and *L. eurysperma* (Sysouphanthong *et al*. 2012) are different from each other according to molecular sequences and morphological characters. However, the two downloaded

Table 1. Samples used in the present study and their GenBank sequence accession numbers (ITS).

Name	Voucher	Location	GenBank accession No.
Lepiota amplicystidiata	HMAS 53632	CHINA, Tibet, Palong Co., Zhangbu Riverside	JN203141[1]
L. andegavensis	Roux 2121	France, Coudre, congres de Thonon	AY176461[4]
L. apatelia	Huijser-26IX1990	The Netherlands, Limburg Prov., Brunssum, mine Hendrik	AY176462[4]
L. aff. aspera	HKAS 7645	China, Jilin Prov., Antu Co., White Rriver	EU681782[7]
L. attenuata	HKAS 42320	China, Taiwan Prov., Nantou Co., Jiujiufeng	EU681777[7]
L. boudieri	HKAS 5803	China, Jilin Prov., Fusong Co., Lushui River	EU416280[6]
L. brunneoincarnata	HMAS 63488	China, Yinxia Autonomous Region, Yinchuan City	EU416302[6]
L. castanea	HKAS48817	China, Yunnan Prov., Lijiang City, Mt. Xiang	EU416282[6]
L. castaneidisca	UCB-ecv2411	USA, California, San Mateo Co., Skyline Blv	AF391063[3]
L. clypeolaria	HKAS 5217	China, Jilin Prov., Fusong Co., Lushui River	EU416284[6]
L. cortinarius	HKAS 46095	China, Tibet, Leiwuqi Co.	EU416306[6]
L. cristata	HKAS49258	China, Sichuan Prov., Xinlon Co., Shadui Town	EU081937[5]
L. cristatoides	Huijser-21IX1998	The Netherlands, Limburg Prov.,Cadier en Keer, Riesenberg	AY176363[4]
L. cystophoroides	L-ecv2142	France, Dépt. I'Hérault, Cêdraie d'Escandorgue	AF391031[2]
L. echinacea	Huijser-21IX1998	The Netherlands, Noord-Brabant Prov., Best, Steenfabriek	AY176469[4]
L. echinella	Huijser-4X1998	Belgium, Luxembourg Prov., Bois de Resteigne	AY176366[4]
L. elaiophylla	L-ecv1006	The Netherlands, Noor-Holland Prov., Uithoorn, de Kwakel	AF391024[2]
L. erminea	L-ecv2290	The Netherlands, Zeeland Prov., Schouwen-Duivenland	AY176470[4]
L. felina	HKAS 5801	China, Jilin Prov., Fusong Co., Lushui River	EU416286[6]
L. grangei	Huijser-4X1998	Belgium, Luxembourg Prov., Bois de Resteigne	AY176471[4]
L. hymenoderma	L-ecv2017	The Netherlands, Limburg Prov., Elsloo-Geulle, Bunderbos	AF391083[2]
L. hystrix	Huijser-25X1998	France, BeaulieuenArgonne, Triancourt	AY176377[4]
L. ignicolor	Huijser-17X1999	The Netherlands, Limburg Prov., Savelsbos, Trichterberg	AY176472[4]

Name	Voucher	Location	GenBank accession No.
L. ignivolvata	L-ecv2127	France, Dépt. Aude, Saissac, exhibition	AY176473[4]
L. kuehneriana	HKAS 45723	China, Tibet, Leiwuqi Co.	GU199360[7]
L. luteophylla	MICH-Smith25VIII1972	USA, Michigan, Washtenaw Co., Sharon Hollow	AY176475[4]
L. magnispora	HKAS 8247	China, Jilin Prov., Antu Co.,White river	EU416288[6]
L. metulispora	HMAS 61859	China, Hong Kong, The Chinese University	EU681778[7]
L. oreadiformis	HMAS 63449	China, Beijing City,Yangfang	GU199361[7]
L. pilodes	Huijser-19IX1998	The Netherlands, Limburg Prov., Elsloo-Geulle, Bunderbos	AY176476[4]
L. pseudolilacea	HKAS 8288	China, Jilin Prov., Antu Co.,White river	EU416304[6]
L. pyrochroa	L-ecv2006	The Netherlands, Limburg Prov., Elsloo-Geulle, Bunderbos	AY176477[4]
L. revelata	HKAS 50115	China,Yunnan Prov., Mengla Co., Lüshilin Park	GU199359[7]
L. rufipes	Huijser-9X1991	The Netherlands, Limburg Prov., Bemelen	AF391066[4]
L. spheniscispora	UC-ecv2438	USA, California, Marin Co., Mt. Tamalpais	AF391003[2]
L. subgracilis	HKAS 5802	China, Jilin Prov., Fusong Co., Lushui River	EU416290[6]
L. subincarnata	L-ecv2234	The Netherlands, Limburg Prov., Elsloo-Geulle, Bunderbos	AY176491[4]
L. thiersii	UC-ecv2602	USA, California, San Mateo Co., San Francisco Watershed	AY176492[4]
L. thrombophora	HKAS 48451	China, Hainan Prov., Ledong Co., Jianfengling	EU681779[7]
Leucoagaricus leucothites	HMAS 88854	China, Beijin City, Haidian, Zhongguanchun	EU416308[6]
La. nympharum	HMAS 99343	China, Tibet, Nyingchi Co., Zarao	EU416310[6]

This study[1],Vellinga 2001 b[2],Vellinga 2001 c[3],Vellinga 2003[4], Liang *et al.* 2009[5], Liang et al. 2010[6], Liang *et al.* 2011[7]

sequences HQ718461 and HQ718462) are identical. As one of them is possibly incorrect, they were excluded from the present phylogenetic analyses.

Bayesian and RAxML phylogenetic analyses (Fig. 1) show that the new species is nested in a single group (clade 2) with 100 % bootstrap support and 1.0 posterior probability. Most species in clade 2 share a trichodermal

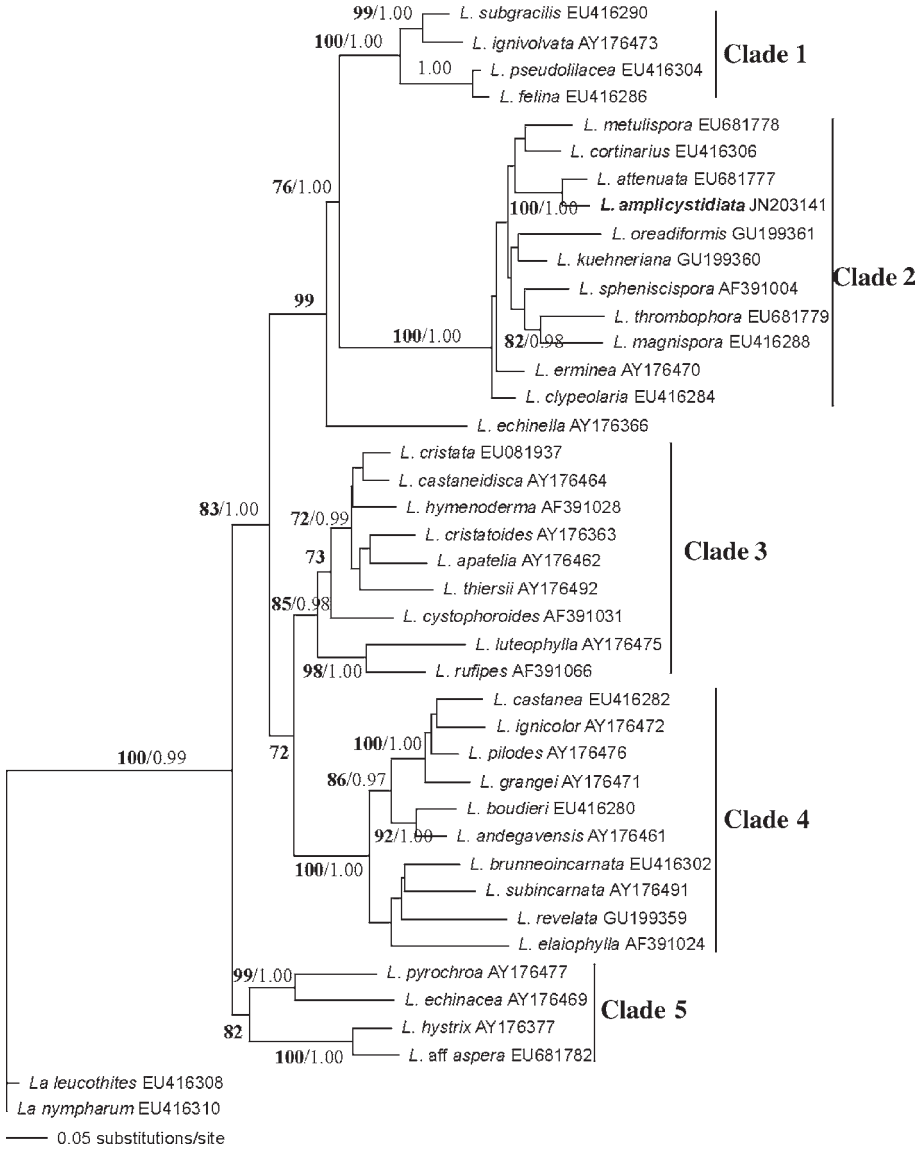

Fig. 1. One of 100 RAxML likelihood trees (-In L 9197.94777) based on the ITS dataset. Support values in bold type are RAxML likelihood bootstrap (≥70%). Values in normal type are Bayesian posterior probabilities (≥0.95).

pileus covering and fusiform-amygdaliform spores (*L. clypeolaria* and *L. erminea* (Fr.) Gillet) or penguin-shaped spores (*L. magnispora* Murrill and *L. cortinarius* J. E. Lange). According to the definition of Singer (1986) and Vellinga (2003), species in clade 2 belong to *Lepiota* sect. *Lepiota*.

Species in clade 1 and clade 4 (both with 100 % bootstrap support and 1.0 posterior probability) also share a trichodermal pileus covering. Clade 1 includes species with fusiform and ellipsoid spores, while clade 4 comprises species with spurred and ellipsoid spores. Species in clade 3 (with 85 % bootstrap support and 0.98 posterior probability) are characterized by a hymenidermal pileus covering, while clade 5 (with 82 % bootstrap support) has species with a hymenidermal pileus covering and a pileus covering made up of rounded elements in chains.

Taxonomy

Lepiota amplicystidiata J. F. Liang, **sp. nov.** – Fig. 2.
MycoBank no.: MB 800111

Holotypus. – CHINA, Tibet, Milin Co., Daxiawan town, Zhongganglang, 23 Sep 1982, *leg.* X. L. Mao 635 (HMAS 53458).

Basidiomata small (Fig. 2 A). – Pileus 2–4 cm in diam., plano-convex to plano-concave with umbo; surface white, covered by yellowish brown, grayish brown, reddish brown to dark brown squamules (8D3-9E6), reddish brown to dark brown (9E6-9E4) at the disk; margin radially sulcate striate reaching intermediary area. – Lamellae free, whitish, moderately crowded with lamellulae of two lengths. – Stipe 4–8.2 × 0.3–1.0 cm, attenuate; hollow, whitish and glabrous at the apical part; surface whitish, covered by small reddish brown (8D3-9E4) squamules at base. – Annulus whitish, reddish brown (9E4) on the surface, evanescent. – Smell and taste not recorded.

Basidiospores (Fig. 2 B) [89/4/3] (13.0) 15.0–20.0 (21.5) × (4.0) 4.5–5.5 (6.0) µm [Q = (2.80) 3.00–4.00 (4.50), Q_{av} = 3.56 ± 0.32], penguin-shaped, i.e. with straight or almost straight abaxial side and ventricose adaxial side with a distinct suprahilar depression in side view, distinctly narrowed at the apex, fusiform in frontal view, hyaline, smooth, slightly thick-walled, weakly dextrinoid, congophilous, but turning colors slowly, not metachromatic in Cresyl Blue; germ pore absent. – Basidia 18–33 × 8–13 µm, narrowly clavate, 4-spored, rarely 2-spored. Lamella edge sterile. – Cheilocystidia (Fig. 2 C) 19–40 × 9–15.5 µm, widely clavate, rarely widely fusiform or clavate; walls smooth, thin, hyaline in KOH, congophilous. – Pleurocystidia absent. – Pileus covering (Fig. 2 D) a trichoderm of elongate, subcylindrical terminal elements 65–288 × 8–13 µm, sometimes attenuate, often with some short elements; pigment pale yellow brown, parietal and intracellular in upper part, sometimes in basal part also encrusted with a faint brown pigment. – Clamp connections abundant.

Etymology. – From Latin *amplicystidiata*, referring to the widely clavate cheilocystidia.

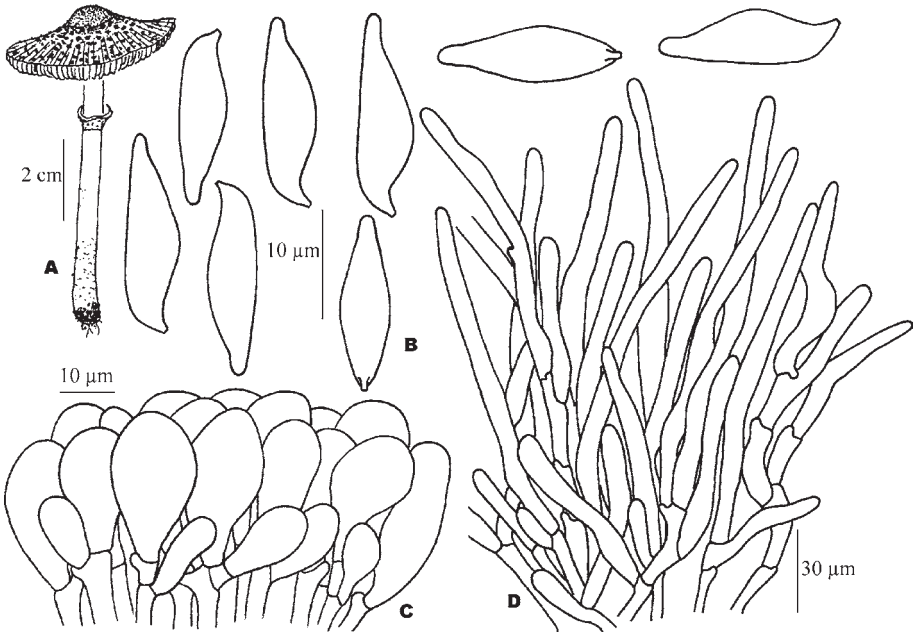

Fig. 2. ***Lepiota amplicystidiata***, holotype. **A.** Basidioma. **B.** Basidiospores. **C.** Cheilocystidia. **D.** Pileus covering.

Habitat and distribution. – Solitary, saprobic, on the ground in broadleaf forests of high altitudes in early autumn. Currently only known from Tibet, western China.

Additional specimens examined: –CHINA, Tibet, Milin Co., Dabiewan, Zhongzhalong, alt. 1950 m, 26 Sep 1982, *leg.* X. L. Mao 621 (HMAS 53363); Palong Co., Zhangbu Riverside, alt. 2000 m, 30 Sep 1982, *leg.* X. L. Mao 689 (HMAS 53632).

Notes. – *Lepiota amplicystidiata* is characterized by the pileus with radially sulcate striate margin, penguin-shaped spores distinctly narrowed towards the apex, widely clavate cheilocystidia and by the trichodermal pileus covering. Based on the trichodermal pileus covering and the penguin-shaped spores, it can be placed in sect. *Lepiota* (Singer 1986, Vellinga 2001 a).

The type was originally regarded as *L. clypeolaria*, and the other two specimens HMAS 53363 and HMAS 53632 were identified as *L. ventriosospora* by Mao (1995). However, *L. clypeolaria* differs from the new species by fusiform spores and narrowly clavate to clavate cheilocystidia. *Lepiota ventriosospora* is a synonym of *L. magnispora* (Vellinga 2000) which lacks a sulcate striate pileus, basidiospores narrowed at apex, and widely clavate cheilocystidia.

Phylogenetic analyses show that the new species, *Lepiota amplicystidiata* is different from the known species (Fig. 1) and is closely allied with *L. attenuata* J. F. Liang & Zhu L. Yang. Morphologically, *L. amplicystidiata* is also similar to *L. attenuata*. Both species have a pileus with radially sulcate

striate margin and penguin-shaped spores distinctly narrowed towards the apex. However, *L. attenuata* is distinguished from the new species by its inflated submoniliform or catenulate elements in the pileus covering and clavate or fusiform cheilocystidia (Liang *et al.* 2011).

Several other species also share penguin-shaped basidiospores and a trichodermal pileus covering, such as *L. cortinarius*, *L. spheniscispora* Vellinga, *L. microcarpa* Sysouphanthong, K. D. Hyde & Vellinga, *L. pongduadensis* Sysouphanthong, K. D. Hyde & Vellinga, *L. metulispora* (Berk. & Broome) Sacc. and *L. thrombophora* (Berk. & Broome) Sacc. However, *L. cortinarius* has sturdier basidiomata with a distinctly cortinate pileus margin and smaller spores while *L. spheniscispora* has narrowly clavate cheilocystidia and basidiospores not attenuated at apex (Candusso & Lanzoni 1990, Bon 1996, Vellinga 2001 a). Both *L. microcarpa* and *L. pongduadensis*, originally described from Thailand, have smaller basidiomata and basidiospores than the new species (Sysouphanthong *et al.* 2012). *Lepiota metulispora* differs from the new species by the lighter coloured squamules on the pileus, smaller basidiospores and narrowly clavate cheilocystidia while *L. thrombophora* is characterized by more slender basidiomata and smaller basidiospores (Pegler 1972, Liang *et al.* 2011)

The other species in the section, *L. erminea*, *L. eurysperma* Sysouphanthong, K. D. Hyde & Vellinga, *L. ignivolvata* Bousset & Joss., *L. oreadiformis* Velen. and *L. subgracilis* Kühner have fusiform spores without straight abaxial side and not penguin-shaped ones (Vellinga 2001 a, Sysouphanthong *et al.* 2012).

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