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Two New Edible *Lyophyllum* Species from Tibetan Areas, China

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Abstract: Two new species, *Lyophyllum yiqunyang* and *L. heimogu*, that belong to the section *Differmia* of the genus *Lyophyllum*, are described based on collections from Tibetan areas, China. The two species are delicious edible low-temperature mushrooms and are widely collected and eaten by local people. *Lyophyllum yiqunyang* sp. nov. is saprotrophic and has medium-sized basidiomata, olive-grey pileus, cheilocystidia, absent pleurocystidia, globose to subglobose basidiospores (6.12–6.31 × 6.02–6.23 µm) and clamp connections at the pileus context, hymenophoral trama and stipe. *Lyophyllum heimogu* sp. nov. is saprotrophic and has a dark grey to olive pileus, medium-sized basidiomata and globose to subglobose basidiospores (5.31–5.63 × 5.22–5.41 µm). In the phylogenetic analyses, our two new species formed distinct clades that are well supported by posterior probabilities and bootstrap proportions. Detailed descriptions, colour photos, illustrations and a phylogenetic tree to show the positions of the two new species are presented.

Keywords: ITS; Lyophyllaceae; morphology; novel taxa; phylogeny; taxonomy

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1. Introduction

The genus *Lyophyllum* P. Karst is classified under the family Lyophyllaceae, order Agaricales, http://www.indexfungorum.org/Names/Names.asp accessed on 10 June 2023, and is mainly characterised by basidiomata colour that is unchanging or changes to dark when injured; scattered, gregarious or solitary growth; a smooth pileus surface; a solid cylindrical or upwards-tapering stipe; basidiospores with variable shape (globose, ellipsoid or broadly fusiform); and saprophytic and symbiotic habits [1,2].

There is a complex without blackening when injured and with caespitose-growing basidiomata in the section *Differmia* [3]. *Lyophyllum* sect. *Differmia* includes 14 species worldwide, most of these species from Europe (*L. brunneum* Dähncke, Contu & Vizzini; *L. calabrum* Lavorato & Contu; *L. cistophilum* Vila & Llimona; *L. decastes* (Fr.) Singer; *L. lanzonii* Candusso; *L. pergamenum* [Sacc. & P. Syd.] Horniček; *L. pseudoloricatum* Dähncke, Contu & Vizzini; *L. subglobisporum* Consiglio & Contu; *L. soniae* Picillo & Contu) and a few from North/South America (*L. multiforme* [Peck] H.E. Bigelow and *L. tucumanense* Singer) and Asia (*L. shimeji* [Kawam.] Hongo and two novel species, *L. yiqunyang* and *L. heimogu*) [4–11]. *Lyophyllum* consists of 60 species worldwide [12].

Dai 1979 [13] identified the first *Lyophyllum* species in China, *L. cinerascens* (Bull.) Konrad & Maubl., which is now *L. decastes*. Since then, 20 additional *Lyophyllum* species have been recorded in China [14]. These species were identified based on phenotypic similarities to European *Lyophyllum* and lacked detailed descriptions and molecular data.

Many *Lyophyllum* species names have been combined and changed with the advancement of molecular biology methods. To date, 16 *Lyophyllum* species have been reported in China, *viz*. *L. atrofuscum* S.W. Wei, Q. Wang & Yu Li (Tibet); *L. decastes* (Yunnan, Sichuan, Qinghai, Liaoning and Fujian, etc.); *L. deqinense* Y.H. Ma, W.M. Chen & Y.C. Zhao (Yunnan);

L. immundum (Berk.) Kühner (not clear); *L. infumatum* (Bres.) Kühner (not clear); *L. loricatum* (Fr.) Kühner (Tibet); *L. macrosporum* Singer (not clear); *L. ochraceum* (R. Haller Aar.) Schwöbel & Reutter (Sichuan); *L. pallidofumosum* (Yunnan); *L. rhombisporum* Shu H. Li & Y.C. Zhao (Yunnan); *L. semitale* (Fr.) Kühner (Yunnan, Tibet, Qinghai, Heilongjiang, Shanxi); *L. subalpinarum* S.W. Wei, Q. Wang & Yu Li (Tibet); *L. subdecastes* S.W. Wei, Q. Wang & Yu Li (Gansu); *L. trigonosporum* (Bres.) Kühner (Yunnan, Tibet); *L. transforme* (Lapl.) Singer (Yunnan, Tibet, Qinghai, Liaoning, Heilongjiang); and *L. shimeji* (Yunnan) [14–18].

Most species of *Lyophyllum* have been described as well-known edible mushrooms, such as *L. shimeji*, *L. decastes* and *L. fumosum*. Currently, the classification of this genus and the cultivation of *L. shimeji* have been studied extensively in Italy, Japan, Sweden and Switzerland [19].

During a survey of *Lyophyllum* in China, two saprotrophic new species, *viz*. *L. yiqunyang* and *L. heimogu*, belonging to sect. *Difformia* were collected and identified based on morphological features and molecular data.

2. Materials and Methods

2.1. Site Description

Jiuzhaigou and Bomi are important and well-preserved nature sites on the edge of the Tibetan Plateau. The Tibetan area is made up of high-altitude hydrological and tectonic activity. The rock strata are mostly carbonate rocks such as dolomite and tufa, as well as some sandstone and shales. *Lyophyllum yiqunyang* and *L. heimogu* were found growing on soil in mixed coniferous and broad-leaved forests dominated by *Abies* spp., *Picea* spp. and *Salix cupularis*, with an elevation of 2000–3000 m a.s.l., a temperate climate and annual rainfall of 550 mm.

2.2. Morphological Studies

Five specimens were photographed in situ and collected from Tibetan areas in China. After collecting, samples were wrapped in aluminium foil and placed in a collection box until they were examined. Macro-morphological features were recorded from fresh collections, and colour was determined following Kornerup and Wanscher [20]. Specimens were dried at 50 °C in a food drier, stored in sealed plastic bags and deposited at the Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming and Herbarium of Cryptogams at the Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Micromorphological features were observed under a Y-TV55 light microscope (Nikon, Tokyo, Japan) from the dried material that was studied. All tissues were revived in 5–10% KOH and mounted in Congo Red. Scanning electron microscopy (SEM) images were captured using Regulus 8100 (Hitachi, Tokyo, Japan). Twenty basidia and more than fifty basidiospores were measured and photographed using a Nikon Eclipse 80i microscope at magnifications up to ×1000. The notation [x/y/z] specified that measurements were made on 'x' basidiospores from 'y' basidiomata and 'z' collections. Basidiospore dimensions were given as '(a) b–n–c (d)'. Where 'a' and 'd' refer to the lower and upper extremes of all measurements, respectively, 'b–c' refers to the range of 95% of the measured basidiospores, 'n' refers to the average dimension, 'Q' is the length/width ratio of basidiospores and 'Q_m' is the average Q of all basidiospores.

2.3. DNA Extraction, PCR Amplification and Sequencing

Molecular analyses were performed at the Yunnan Academy of Agricultural Sciences, China. Genomic DNA was extracted from dry specimens using the Ezup Column Fungi Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China) following the manufacturer's protocol. Primer pairs (forward/reverse) used for PCR cycling of the ITS regions were ITS1/ITS4 [21]. PCR was carried out using a C1000 Thermal Cycler (Bio-Rad, Beijing, China) with a cycling program as follows. ITS: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 90 s and a final extension at 72 °C for 10 min [21].

PCR products were visualised via UV light after electrophoresis on 1% agarose gels stained with ethidium bromide. The PCR amplicons were sent to Sangon Biotech (Shanghai, China) for Sanger sequencing in both directions using the PCR primers.

2.4. Sequence Alignment and Phylogenetic Analyses

The sequences of new *Lyophyllum* identified in this study were submitted to the NCBI database. The Basic Local Alignment Search Tool for the GenBank database was used to check whether the newly generated sequences were amplified contaminant DNA and examine clusters with closely related sequences (see Table 1). DNA sequences were retrieved and assembled using SeqMan version 5.0. The sequence alignments were aligned using MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/, accessed on 10 June 2023) [22], and each gene was analysed using BioEdit v. 7 [23]. Maximum likelihood (ML) analysis was performed using RAxML-HPC2 v. 8.2.12 [24] as implemented in the CIPRES (https://www.phylo.org/portal2/login!input.action, accessed on 10 June 2023) portal [25] using the GTR+G+I model and 1000 rapid bootstrap (BS) replicates for all genes. A reciprocal 70% bootstrap support approach was used to check for conflicts between the tree topologies from individual genes. Since there was no significant incongruence in topology between the ML trees, the ITS was partitioned for phylogenetic analyses. For Bayesian inference (BI), the best substitution model for each partition was determined by MrModeltest 2.2 [26]. The results suggested ITS1: JC+I, 5.8S: GTR+G+I, ITS2: K80+I+G. Bayesian analysis was performed using MrBayes ver. 3.2.7a [27] in the CIPRES portal. Four parallel runs, each consisting of one cold and three heated chains, were performed for 10 million generations with sampling every 100 generations for the single gene trees and 50 million generations with sampling every 1000 generations. Parameter convergence > 200 was verified in Tracer v. 1.7 [28]. Phylogenetic clades were strongly supported if bootstrap support value (BS) was ≥70% and/or posterior probability (PP) was ≥0.95.

Table 1. Names, specimen numbers, origin, references and corresponding GenBank accession numbers of *Lyophyllum* sequences used in the phylogenetic analyses. Newly generated sequences are in bold black, and a "*" before taxa indicates holotypes.

Taxon Name	Specimen Number	Origin	ITS	Reference
Calocybe gambosa	HC78/64	Switzerland	AF357027	[7]
C. carnea	CBS552.50	Switzerland	AF357028	[7]
C. persicolor	HC80/99	Switzerland	AF357026	[7]
Hypsizygus marmoreus	V.1611	Germany	AJ494834	[11]
H. marmoreus	V.3133	China	FJ609271	[11]
H. marmoreus	HMW2	Malaysia	HM561971	[11]
H. marmoreus	HZND-1	China	JX046028	[11]
H. marmoreus	1-1	Korea	KF192813	[11]
H. tessulatus	AFTOL-ID 1898	USA	DQ917653	[11]
H. tessulatus	L2	China	FJ467372	[11]
H. ulmarius	CBS 286.77	Korea	AY265850	[29]
H. ulmarius	DUKE-JM/HW	USA	EF421105	[30]
L. ambustum	CBS452.87	Switzerland	AF357057	[7]
L. anthracophilum	HC79/132	Switzerland	AF357055	[7]
L. atratum	CBS709.87	Switzerland	AF357053	[7]
L. atrofuscum	HMJAU63461	China	OP605493	[16]
L. atrofuscum	HMJAU63456 *	China	OP605494	[16]
L. caerulescens	HC80-140	Switzerland	AF357052	[7]
L. caerulescens	V.15759	USA	JF908339	[11]
L. crassifolium	V.5077	Italy	JF908331	[11]
L. decastes	dd08054	China	FJ810160	[11]
L. decastes	Ld418	China	HM119485	[11]
L. deqinense	YAASM6949 *	China	OQ418117	[18]
L. deqinense	YAASM6948	China	OQ418116	[18]
L. deliberatum	V.15032	Slovenia	JF908338	[11]

Table 1. *Cont.*

Taxon Name	Specimen Number	Origin	ITS	Reference
L. favrei	BSI94cp2	Switzerland	AF357035	[7]
L. favrei	V.6334	Italy	JF908333	[11]
L. fumosum	SJ02/006	Sweden	HM572539	[11]
L. fumosum	LAS00/144	Sweden	HM572541	[11]
L. fumosum	V.16077	Italy	JF908340	[11]
L. fumosum	LfumNlf24	Japan	JN983977	[11]
L. fumosum	L2010512371	China	JX966310	[11]
L. fumosum	YAASM6215	China	ON681708	[17]
L. fumosum	YAASM6340	China	ON681709	[17]
L. gangraenosum	V.12332	Italy	JF908335	[11]
L. heimogu	**L3026 ***	**China**	**KY434100**	**This study**
L. heimogu	**L3033**	**China**	**KY434101**	**This study**
L. heimogu	**L3035**	**China**	**KY434102**	**This study**
L. infumatum	V.10152	Italy	JF908334	[11]
L. leucophaeatum	Hae251.97	Switzerland	AF357032	[7]
L. littoralis	CA20091210	Italy	JX280410	[11]
L. loricatum	V.13175	USA	JF908336	[11]
L. loricatum	CA20090202.03	Italy	JX280406	[11]
L. loricatum	01.12.09	Italy	JX280407	[11]
L. moncalvoanum	PDD 96328 *	New Zealand	NR_137615	[2]
L. moncalvoanum	PDD 102581	New Zealand	KJ461912	[2]
L. ochraceum	BSI94.cp1	Switzerland	AF357033	[7]
L. ochraceum	V.537	Italy	JF908329	[11]
L. rhombisporum	L1762*	China	JX966307	[11]
L. rhombisporum	L2082	China	JX966308	[11]
L. semitale	HC85/13	Switzerland	AF357049	[7]
L. semitale	EL187-09	Sweden	HM572552	[8]
L. shimeji	Olsen821006	Sweden	HM572530	[8]
L. shimeji	NZ4Q88	New Zealand	JN983985	[11]
L. shimeji	L2010512377	China	JX966311	[11]
L. sp.	PBM 2688	USA	DQ182502	[11]
L. sp.	SB102	China	FJ687273	[11]
L. sp.	Aase811014	Sweden	HM572550	[8]
L. sp.	TO-2011	Italy	JF908337	[11]
L. sp.	JN001	China	FJ687270	[11]
L. sp.	O73586	Netherlands	GU234137	[11]
L. sp.	Cultivar Jpn	Sweden	HM572551	[8]
L. sp.	SL-2013	China	JX966308	[11]
L. sykosporum	IFO30978	Switzerland	AF357050	[7]
L. subalpinarum	HMJAU63447 *	China	OP605490	[16]
L. subalpinarum	HMJAU63453	China	OP605491	[16]
L. subdecastes	HMJAU63470	China	OP605488	[16]
L. subdecastes	HMJAU63467 *	China	OP605489	[16]
L. turcicum	KATO-2971 *	Turkey	KJ158159	[11]
L. yiqunyang	**L4206**	**China**	**KY434104**	**This study**
L. yiqunyang	**L2989 ***	**China**	**KY434103**	**This study**
Tephrocybe boudier	BSI96/84	USA	DQ825427	[11]

3. Result

3.1. Phylogenetic Analysis

Five newly generated and 64 retrieved sequences from GenBank were used as the ingroup. Three sequences of *Calocybe gambosa*, *C. carnea* and *C. persicolor* retrieved from GenBank were used as the outgroup [30]. The ITS was 575 characters in length, of which 284 characters were constant, 291 were variable but parsimony-uninformative and 231 were parsimony-informative. Estimated base frequencies were as follows: A = 0.231384, C = 0.218313, G = 0.226647, T = 0.323655, substitution rates AC = 1.548998, AG = 4.813675,

AT = 2.464671, CG = 0.797610, CT = 7.018015, GT = 1.000000; gamma distribution shape parameter α = 0.394616.

ML and BI analyses generated nearly identical tree topologies with minimal variation in statistical support values. Thus, only the ML tree is displayed (Figure 1). Phylogenetic data and thorough morphological analysis (see below) showed that the two newly described taxa in this study are significantly distinguished from other known *Lyophyllum* species.

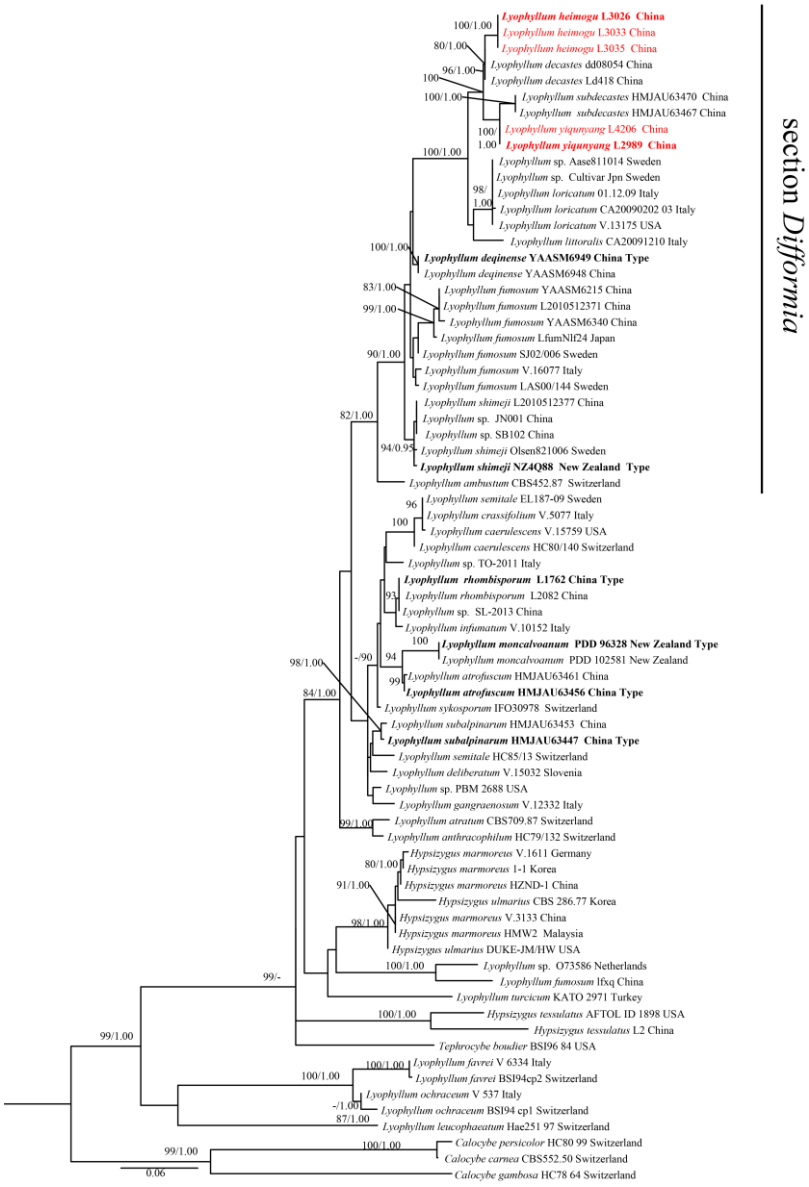

Figure 1. Phylogram generated from maximum likelihood (RAxML) analysis based on combined sequence data of ITS1−5.8S−ITS2 alignment of *Lyophyllum*. *Calocybe persicolor*, *C. carnea* and *C. gambosa* were used as the outgroup. ML bootstrap support values/Bayesian posterior probability greater than 70%/0.95 are indicated. Species names in red represent new species.

In our phylogeny, our species *L. yiqunyang* (L4206 and L2989) and *L. heimogu* (L3026, L3033 and L3035) formed independent branches. Comparing the ITS sequences, there were 12/575 (2.09%), 18/575 (3.13%), 16/575 (2.78%) and 6/575 (1.04%) differences between *L. yiqunyang* (L2989, holotype) and *L. heimogu* (L3026, holotype), *L. loricatum* (V.13175), *L. littoralis* (CA20091210) and *L. subdecastes* (HMJAU 63467 holotype), respectively, as well as 15/575 (2.78%), 15/575 (2.78%), 13/575 (2.26%) and 9/575 (1.57%) ITS sequence differences

between *L. heimogu* (L3026, holotype) and *L. loricatum* (V.13175), *L. littoralis* (CA20091210), *L. subdecastes* (HMJAU63467 holotype) and *L. decastes* (Ld418), respectively.

3.2. Taxonomy

Lyophyllum yiqunyang Shu H. Li sp. nov. Figure 2, Figure 3 and Figure 6.

Figure 2. Fresh basidiomata of *Lyophyllum yiqunyang* ((**a**) YAAS L2989; (**b**) YAAS L4206). Scale bars: (**a**,**b**) = 1 cm.

MycoBank: 849794.

Holotype: China, Sichuan Province, Jiuzhaigou County, 103°54′37″ E, 33°10′34″ N, elev. 3251 m, in a forest dominated *Abies* spp., *Picea* spp. and *Salix cupularis*, Shu−Hong Li, 10 August 2014. (YAAS L2989, holotype!).

Etymology: "yiqunyang" refers to the common name used in mushroom markets in China.

Basidiomata mid-sized (Figure 2), Pileus 3.0–6.0 cm diameter, fleshy, fragile, variable in shape according to growth conditions, hemispherical when young, becoming convex with age; surface smooth, dry, olive-grey (2E-F), unchanging, without umbo, deflexed aspects of margin; pileus context thick, 0.1–0.3 cm wide, white (1A1), watery soaked in wet weather. Lamellae moderately close together, ventricose to broadly ventricose, adnate to narrowly adnate, broad, white (1A1) to pale grey (1B1), non-discolouring when bruised; edge even or entire. Stipe 3.0–7.0 × 0.5–1.5 cm, cylindrical to clavate, white or light grey, pruinose at the base, sometimes tapered towards at the base, often twisted, white (1A1) to pale grey (1B1), smooth, sometimes squamous on the surface, unchanging in colour when injured. Odour and taste not distinctive.

Basidiospores [120/2/2] 6.1–6.3 × 6.0–6.2 μm (Figure 3 and Figure 6), Q = 1.1–1.3, Q_m = 1.18 ± 0.35, av. 6.21 ± 0.12 × 6.15 ± 0.24 μm, globose, subglobose to broadly ellipsoid, hyaline, smooth. Basidia 31.0–42.0 × 7.8–9.0 μm (N = 20), av. 38.1 ± 3.4 × 8.3 ± 1.28 μm, four–spored, sometime with basal clamp connections, clavate, siderophilous granulation. Subhymenium made up of moderately thin-walled hyphae, 5–15 μm across. Hymenophoral trama regular, consisting of thin and hyaline hyphae, some with clamp connection. Marginal cells absent. Pileipellis an interwoven trichodermium to a subcutis composed of almost hyaline interwoven filamentous hyphae, terminal cells 20–83 × 3–8 μm, almost cylindrical to subcylindrical, occasional hyphal tips flexuous and sometimes inflate, some with clamp connections. Stipitipellis a cutis of elongate hyphae. Clamp connection present at some septa. Thromboplerous hyphae present on the hymenophoral trama, 5–8 μm wide.

Ecology and distribution: saprotrophic (growing on fallen wood or soil); when growing on the soil, the soil's upper layer has a relatively thick humus layer; gregarious in forests of *Abies* spp., *Picea* spp. or *Salix cupularis*. Summer to autumn (August to September). Known only from Sichuan Province, China.

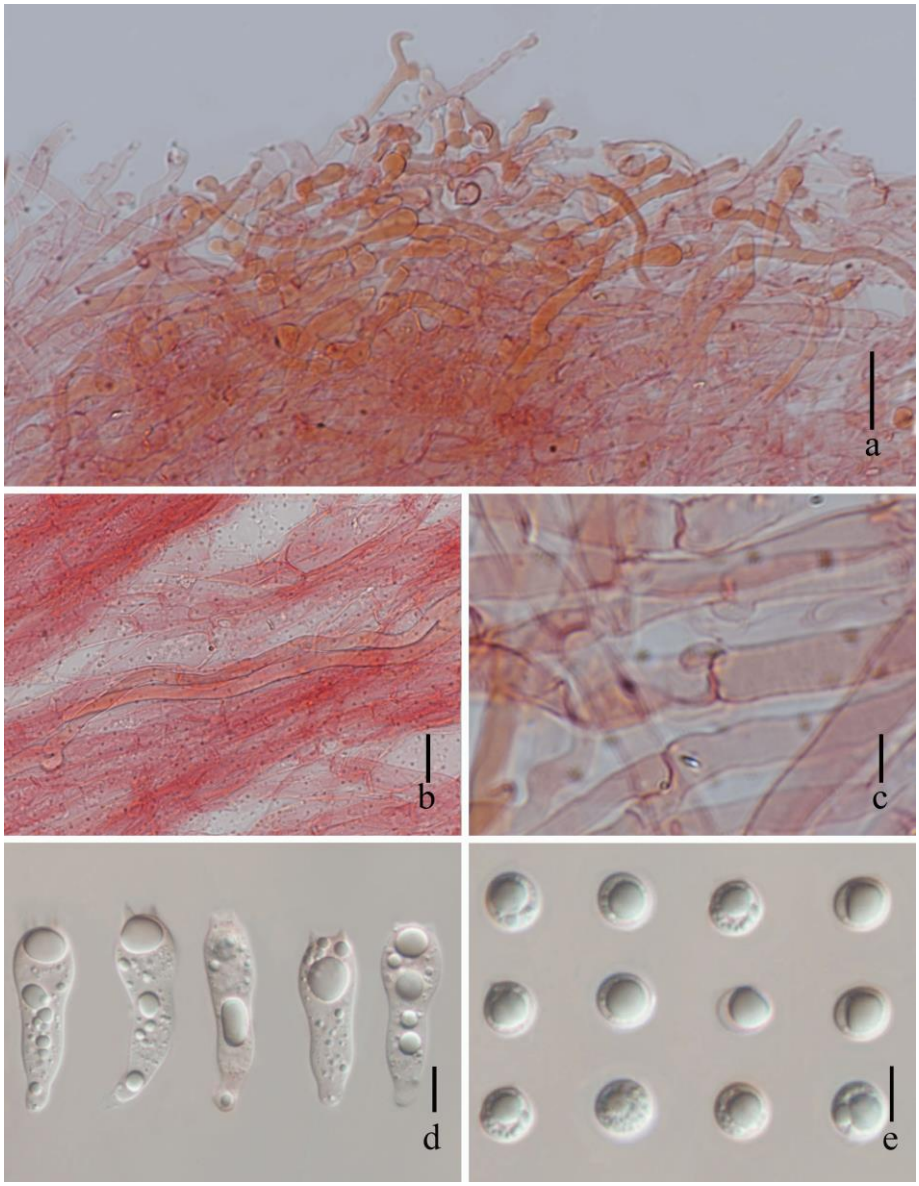

Figure 3. *Lyophyllum yiqunyang* (YAAS L2989). (**a**) Pileipellis; (**b**) Thromboplerous hyphae; (**c**) Clamp connection; (**d**) Basidia; (**e**) Basidiospores. Scale bars: (**a**) = 20 μm, (**b**–**e**) = 5 μm.

Additional specimens examined: China, Sichuan province, Jiuzhaigou County, elev. 3200 m, latitude 103°54′37″ E and longitude 33°10′34″ N, in a forest dominated by *Abies* spp. and *Salix cupularis*, Shu-Hong Li, 8 August 2015, (L4206).

Notes: *Lyophyllum yiqunyang* is quite similar to *L. deqinense* in having an olive-greyish to greyish-orange pileus and subglobose to globose basidiospores, the *L. deqinense* basidia are shorter (24.1–33.8 × 7.3–9.9 μm). The pileipellis of *L. deqinense* is a cutis composed of parallel elements [18].

Lyophyllum yiqunyang is also similar to *L. subalpinarum* in having a greyish-yellow to olive-greyish pileus. *Lyophyllum subalpinarum* stipe is hollow, whitish-greyish to dark in the middle. The lamellae of *L. subalpinarum* change to black when touched or injured and present rounded-cylindrical basidiospores (6.9–8.7 × 4.3–5.1 μm) [16].

Lyophyllum yiqunyang is related to *L. subdecastes*; however, *L. subdecastes* has a yellowish-brown, brown, greyish-red pileus, relatively small basidiospores (3.9–5.0 × 3.7–5.0 μm) and larger basidia (36.7–50.6 × 8.4–10.9 μm) [16].

Lyophyllum heimogu S. H. Li sp. nov. Figures 4–6.

Figure 4. Fresh basidiomata of *Lyophyllum heimogu* ((**a**) YAAS L3026, holotype; (**b**) YAAS L3035; (**c**) YAAS L3533; (**d**) YAAS L3033). Scale bars: (**a**–**d**) = 1 cm.

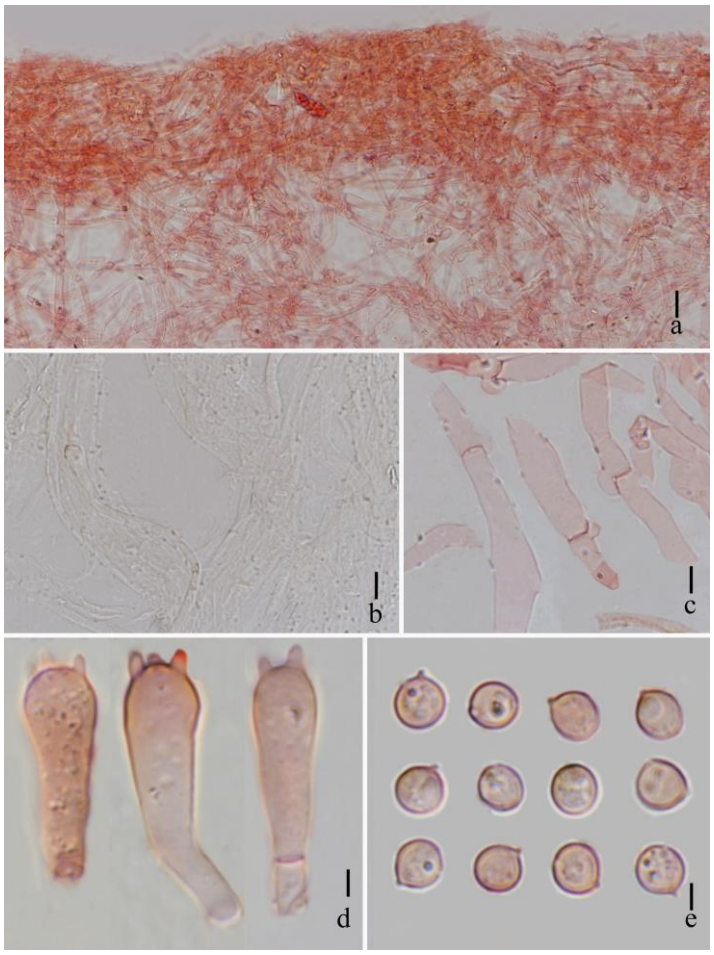

Figure 5. *Lyophyllum heimogu* (YAAS L3026, holotype) (**a**) Pileipellis; (**b**) Thromboplerous hyphae (**c**) Clamp connection; (**d**) Basidia; (**e**) Basidiospores. Scale bars: (**a**) = 20 μm, (**b**–**e**) = 5 μm.

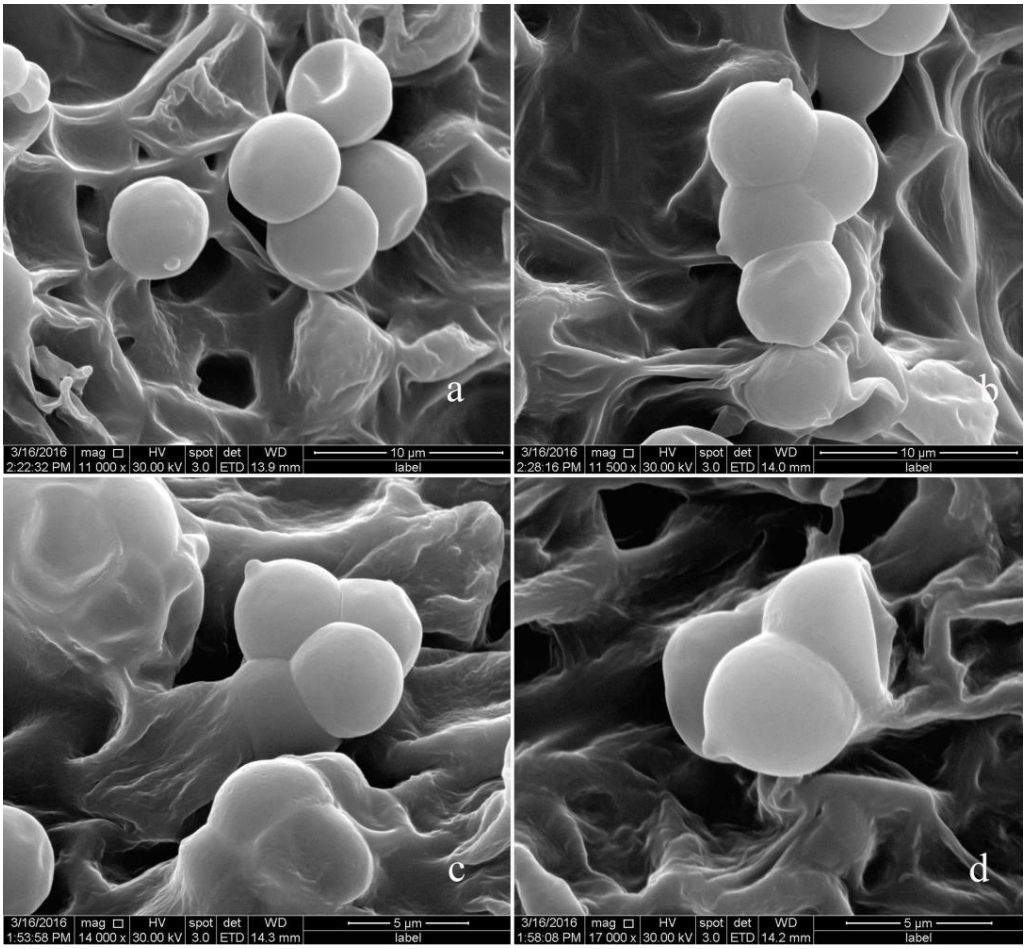

Figure 6. SEM photo of *Lyophyllum* Basidiospores. (**a**,**b**) *Lyophyllum heimogu* (YAAS L3026), (**c**,**d**) *Lyophyllum yiqunyang* (YAAS L2989).

MycoBank: 849795.

Holotype: China, Tibet Autonomous Region, Bomi County, elev. 2717 m, 95°43′06″ E, 29°51′41″ N, in a forest dominated by *Abies* spp. and *Picea* spp., Shu–Hong Li, 3 October 2013. (YAAS L3026, holotype!).

Etymology: "heimogu" refers to the common name used by local people.

Basidiomata mid-size (Figure 4), Pileus 3.0–6.5 cm wide, fleshy, fragile, hemispherical when young, becoming convex with age; surface smooth, dark grey (1F1) at the centre, becoming olive (1E-F3) towards the margin with an indistinctly striated margin, umbo papilla, dark (1F1), involute aspects of margin; pileus context thick, 0.1–0.2 cm wide, white (1A1). Lamellae moderately close together, subventricose to ventricose, free to adnexed, lamella edge even or entire, white when young, becoming yellowish-white with age. Context 0.3–0.8 cm thick, white to pale white, unchanging when injured. Stipe 2.5–7.0 × 0.5–2.0 cm, yellowish-brown (5E4-5), central, cylindrical to clavate, sometime bulbous at the base, solid, often twisted, smooth. Odour and taste not distinctive.

Basidiospores [120/2/2] 5.31–5.63 × 5.22–5.41 µm (Figures 5 and 6), Q = 1.1–1.2, Qm = 1.12 ± 0.03, av. 5.5 ± 0.24 × 5.3 ± 0.36 µm, globose to subglobose, hyaline, smooth. Basidia 28.5–32.0 × 7.1–8.3 µm (N = 20), av. 30.2 ± 3.51 × 7.5 ± 0.82 µm, four–spored, sometime with basal clamp connections, clavate, siderophilous granulation. Subhymenium made up of moderately thin-walled hyphae, 4–10 µm across. Hymenophoral trama regular, consisting of thin and hyaline hyphae, some with clamp connection. Marginal cells absent. Pileipellis an interwoven trichodermium to a subcutis composed of almost hyaline interwoven filamentous hyphae, terminal cells 25–100 × 2–5 µm, almost cylindrical to subcylindrical, occasional hyphal tips flexuous and sometimes inflate, some with clamp

connections. Stipitipellis a cutis of elongate hyphae. Clamp connection present at some septa. Thromboplerous hyphae present on the hymenophoral trama, 3–7 μm wide.

Ecology and distribution: saprotrophic (growing on fallen wood or soil); when growing on the soil, the soil has a relatively thick humus layer; clusters or gregarious in forests dominated by *Abies* or *Picea*. Summer to autumn (August to October). Known only from Tibet Autonomous Region, China.

Additional specimens examined: China, Tibet Autonomous Region, Bomi County, alt. 2700 m, in forest of *Abies* spp. and *Picea* spp., Shu–Hong Li, 95°43′15″ E, 29°51′52″ N, 18 September 2014 (YAAS L3035); ibid, Shu–Hong Li, 19 August 2014, YAAS L3533; ibid, Shu–Hong Li, 20 August 2014, YAAS L3033.

Notes: *Lyophyllum heimogu* is similar to *L. subdecastes*, having medium-sized basidiomata, brown to dark pileus and globose to subglobose basidiospores. However, *L. subdecastes* basidiospores are smaller (av. = 4.47 × 4.25 μm) and present fusoid-ventricose to broadly fusoid-ventricose pleurocystidia [16].

In our phylogenetic analyses, the phylogenetic positions of *L. heimogu* and *L. decastes* within *Lyophyllum* were well supported (80/1.00) as monophyletic clades. However, *L. decastes* was found in Poland and has a broad pileus, ellipsoid basidiospores (5.0–7.0 μm) [4]; ITS sequence differences between *L. heimogu* (L3026, holotype) and *L. decastes* (Bengtsson 19910929, HM572545 from Sweden) were 12/616 (1.95%).

4. Discussion

From a morphological perspective, *L. yiqunyang* and *L. heimogu* are very similar to *L. decastes* and *L. shimeji* but differ in their trophic modes. While *L. yiqunyang* and *L. heimogu* are saprotrophic, *L. decastes* and *L. shimeji* are symbiotic. Molecularly, *L. yiqunyang* and *L. heimogu* are closely related to *L. decastes*. However, the original description of *L. decastes* from Sweden in 1818 [4] was shorter. When comparing the ITS sequences of *L. heimogu* (L3026, holotype) and *L. yiqunyang* (L2989, holotype) with *L. decastes* (Bengtsson 19910929, HM572545 from Sweden), the differences are 12/616 (1.95%) and 13/616 (2.11%), respectively. *Lyophyllum yiqunyang* and *L. heimogu* are considered the most delicious mushrooms that can be stored at low temperatures, making them potential candidates for commercialisation. Detailed comparisons of the diagnostic characteristics of *L. heimogu* and *L. yiqunyang* with similar species are mentioned in Table 2.

Species of sect. *Difformia* are characterised by their caespitose growth [31], which is seen in *L. decastes*, *L. fumosum* and *L. shimeji* belonging to sect. *Difformia*. In our phylogenetic analyses (Figure 1), these three species formed a strongly supported group (90/100) in the sect. *Difformia*. Therefore, we believe that sect. *Difformia* is monophyletic with *L. ambustum*, *L. decastes*, *L. fumosum*, *L. shimeji*, *L. loricatum*, *L. littoralis*, *L. subdecastes* and the two new species studied here, *L. yiqunyang* and *L. heimogu*.

Most species of *Lyophyllum* are delicious edible mushrooms, such as *L. decastes*, *L. shimeji* and *L. subdecastes*. Cases of poisoning associated with *Lyophyllum* species have not been reported. Wu et al. [32] have indicated the diversity of Chinese macrofungi, including seven edible *Lyophyllum* species that are distributed in China. Furthermore, some species of *Lyophyllum* have been used in traditional medicine, for example, *L. decastes*, which has been used to treat hypoglycaemia [33].

Lyophyllum species are mainly distributed in temperate to subtropical regions [11,31,34]. Most species are saprotrophic, with few being symbiotic [4,11,16,31]. An important characteristic for identifying *Lyophyllum* species is the shape of their basidiospores. In most species of *Lyophyllum*, basidiospores are globose to subglobose (e.g., *L. fumosum*, *L. heimogu*, *L. subdecastes*, *L. shimeiji*, *L. loricatum* and *L. yiqunyang*, among others) [3,11,16,31,33,35,36]. However, in rare cases, species may have irregular rhombus basidiospores (e.g., *L. subalpinarum* and *L. atrofuscum* among others) [16].

Table 2. Comparison of the diagnostic characteristics of *Lyophyllum heimogu* and *L. yiqunyang* with similar species.

Taxa	Pileus	Stipe	Spores	Basidia	Reference
L. decastes	Greyish-brown to yellowish-brown or brown, usually darker when young, 4–12 cm	Whitish-greyish, 5–10 cm	Broadly ellipsoid, 5–7 × 5–7 µm	-	[4,33,34]
L. fumosum	Dark to light grey, brown, 2–10 cm	Cream-coloured to brown, 2.5–10 cm	Globose to subglobose, 5.5–7 × 5–7 µm	40–45 × 8–10 µm	[11]
L. heimogu	**Dark grey to olive, 3.0–6.5 cm**	**Yellowish-brown, 2.5–7.0 × 0.5–2.0 cm**	**Globose to subglobose, 5.30–5.60 × 5.20–5.40 µm**	**28.5–32.0 × 7.1–8.3 µm**	**Present study**
L. subdecastes	Yellowish-brown, brown to greyish-red	Orange-white, reddish-grey to greyish-red 2.7–6.6 × 0.5–1.5 cm	Globose to subglobose, 3.9–5.0 × 3.7–5.0 µm	36.7–50.6 × 8.4–10.9 µm	[16]
L. shimeiji	Dark grey to grey, brown, 2–8 cm	White, 3–8 cm	Globose to subglobose, 4.0–6.0 × 4.0–6.0 µm	-	[11]
L. loricatum	Reddish-brown to chestnut brown 3–12 cm	Cream to pale brownish, grey-brown when old, 3.5–9 × 0.7–1.5 cm	Globose to subglobose, 5.0–6.0 × 4.5–5.3 µm	28.0–32.0 × 7.0–8.0 µm	[4]
L. littoralis	Grey to brownish-grey, 5–15 cm	Grey, 1.5–4 × 0.4–1.5 cm	Globose to subglobose, 4.5–5.5 × 4.5–5.5 µm	-	[36]
L. yiqunyang	**Olive-grey, 3.0–6.0 cm**	**White or light grey, 3.0–7.0 × 0.5–1.5 cm**	**Globose to subglobose, 6.1–6.3 × 6.0–6.2 µm**	**31.0–42.0 × 7.8–9.0 µm**	**Present study**

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