

Three new species of *Lactarius* sect. *Deliciosi* from subalpine-alpine regions of central and southwestern China

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Abstract – Three new species of *Lactarius* sect. *Deliciosi* are described from montane central and southwestern China. *Lactarius abieticola* is associated with *Abies* trees. The pure orange basidiomata with only faint greenish discoloration resemble *L. laeticolor*, *L. salmonicolor* and *L. thynos*, but the smaller size and whitish tomentum at the pileus margin make it different. *Lactarius hengduanensis* is similar to and closely related with *L. deterrimus* due to the orange latex and association with *Picea* trees. The contrast between the vivid orange stipe and paler lamellae and the spores with more reticulate ornamentation can be used to separate it from *L. deterrimus*. *Lactarius pseudohatsudake* has vinaceous red latex, similar to Asian *L. hatsudake*. The symbiotic relationship with *Picea* trees and crowded lamellae are differences from *L. hatsudake*. Comparison and phylogenetic analysis on the ITS sequences supported the three species to be distinct. The three new species are representative ectomycorrhizal fungi in the subalpine-alpine coniferous forests and putative choice edible mushrooms for the local people.

culinary mushrooms / Eastern Himalayas / ectomycorrhizal fungi / firs / spruces

INTRODUCTION

Eastern Himalayas and Hengduan Mts. and Central China are two of the three eminent diversity centers of higher fungi in China (Yang 2005). The alpine-subalpine parts in these two regions are of special biogeographical interest. The cold climate and forests that are dominated by spruces, firs, larches and birches are highly similar to those in circumboreal regions, but different geological history and host tree species are believed to bear a unique mycobiota.

Species of *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh *et al.* are among the most remarkable mushrooms in the subalpine-alpine coniferous forests in central and southwestern China. The vividly colored basidiomata make them easy to be found, but the highly similar morphology makes them difficult to be identified. Nuytinck *et al.* (2006) and Wang (2008) cited some specimens collected from *Picea* and *Abies* forests in subalpine Hengduan Mts. under *L. deliciosus* (L.: Fr.) Gray, a pine-symbiotic species in Europe. These specimens, however, were not included in any molecular analyses and their identities are questionable. During the monographic work on Chinese milk caps, new specimens of this section were collected and

sequenced. Molecular analyses revealed at least three new species, which are important representatives of ectomycorrhizal fungi in the coniferous forests but they passed under some common names, e.g. *L. deliciosus*, *L. deterrimus* Gröger, *L. hatsudake* Tanaka and *L. laeticolor* (S. Imai) Imazeki ex Hongo. Two of the three species have been encountered in the local markets, sold under the same local names as *L. deliciosus* and *L. hatsudake* (“Gushoujun” and “Tonglūjun”). To give decent names for these new species, formal publication is needed.

MATERIAL AND METHODS

Morphological methods, DNA extraction and PCR protocols followed Wang *et al.* (2012). Eighteen specimens of *L. abieticola*, 17 of *L. hengduanensis*, and 18 of *L. pseudohatsudake*, most collected from the subalpine-alpine coniferous forests and a few from local markets in five provinces, Hubei, Shanxi, Sichuan, Tibet, and Yunnan were sequenced (except for an relatively old specimen KUN-HKAS 39213) and studied with morphological approach. The generated ITS sequences were submitted to BLASTn to find sequences with high similarity (> 98%, query cover > 90%). An ITS dataset with all own 52 sequences and those retrieved from GenBank was used for a primary phylogenetic analysis. After that, duplicate sequences generated by this study, if not representing a different locality or host tree species, were removed. In addition, 37 sequences of *L. aestivus* Nuytinck & Ammirati, *L. akahatsu* Tanaka, *L. deliciosus*, *L. deterrimus*, *L. fennoscandicus* Verbeken & Vesterh., *L. hatsudake*, *L. laeticolor*, *L. salmoneus* Peck, *L. subindigo* Verbeken & E. Horak, *L. thynos* A.H. Sm. and *L. vividus* X.H. Wang *et al.* were included. These species are either sympatric species in China or representing the genetic divergence of the whole section, as demonstrated by former studies (Nuytinck *et al.* 2007; Nuytinck & Ammirati 2015; Wang *et al.* 2015). Sequences generated by this study are deposited in GenBank as accessions KY174900-KY174951 and specimen vouchers in herbarium KUN (Table 1).

ITS Alignment was made using the online version of the multiple sequence alignment program MAFFT v7 (Kato and Toh 2008), applying the L-INS-I strategy, and were manually adjusted in BioEdit v.7.1.3.0 (Hall 1999). Neighbor-Joining (NJ) phylogenetic analysis, implemented in MEGA5 (Tamura *et al.* 2011), was employed to construct the phylogenetic relationships, using the following settings: bootstrap method (with 1000 replicates) as test of phylogeny, p-distance as substitution model, transitions and transversions both included for substitutions, uniform rates among sites, homogenous pattern among lineages and pairwise deletion for gaps treatment. *Lactarius rufus*, a species of *L.* subg. *Russularia* (Fr.) Kauffman was used to root the tree. Bootstrap consensus tree was viewed and exported as PDF in FigTree v1.3.1.

Table 1. Taxa and samples of *Lactarius* used for molecular analyses in this study. Sequences produced in the present study are in bold

<i>Species</i>	<i>Voucher (herbarium)</i>	<i>Location</i>	<i>ITS GenBank Accession</i>
<i>L. aestivus</i>	J. F. Ammirati 13112 (WTU)	Washington, USA	KJ769667
<i>L. aestivus</i>	J. Nuytinck 2008-032 (GENT)	Washington, USA	KJ769670
<i>L. aurantiosordidus</i>	S.L. Miller 213-03 (RMS)	California, USA	EF685094
<i>L. aurantiosordidus</i>	S.L. Miller 216-03 (RMS)	California, USA	EF685096
<i>L. abieticola</i>	X.H. Wang 2948 (KUN)	Hubei, China	KY174936
<i>L. abieticola</i>	X.H. Wang 2755 (KUN)	Shanxi, China	KY174937
<i>L. abieticola</i>	X.H. Wang 2918 (KUN)	Shanxi, China	KY174939
<i>L. abieticola</i>	X.H. Wang 2853 (KUN)	Sichuan, China	KY174938
<i>L. abieticola</i>	X.H. Wang 3607 (KUN)	Sichuan, China	KY174935
<i>L. abieticola</i>	X.H. Wang 2778 (KUN)	Sichuan, China	KY174943
<i>L. abieticola</i>	X.H. Wang 2777 (KUN)	Sichuan, China	KY174941
<i>L. abieticola</i>	Z.W. Ge 1224 (KUN)	Sichuan, China	KY174947
<i>L. abieticola</i>	Q. Cai 1135 (KUN)	Tibet, China	KY174946
<i>L. abieticola</i>	Y.J.Hao 1215 (KUN)	Tibet, China	KY174942
<i>L. abieticola</i>	S.H.Li 2417 (KUN)	Yunnan, China	KY174948
<i>L. abieticola</i>	R. Wang bm24 (KUN)	Yunnan, China	KY174950
<i>L. abieticola</i>	R. Wang bm25 (KUN)	Yunnan, China	KY174951
<i>L. abieticola</i>	X.H. Wang 2241 (KUN)	Yunnan, China	KY174940
<i>L. abieticola</i>	X.H. Wang 3202 (KUN)	Yunnan, China	KY174944
<i>L. abieticola</i>	X.H.Wang 3213 (KUN)	Yunnan, China	KY174949
<i>L. abieticola</i>	J.P. Zhang 56 (KUN)	Yunnan, China	KY174945
<i>L. akahatsu</i>	X.H. Wang 2558 (KUN)	Jilin, China	KT163421
<i>L. akahatsu</i>	L.P. Tang 1235 (KUN)	Yunnan, China	KT163422
<i>L. akahatsu</i>	22601 (TMI)	Torrori, Japan	EF685045
<i>L. akahatsu</i>	A. Verbeken 2004-141 (GENT)	Thailand	EF685097
<i>L. deliciosus</i>	X.H. Wang 2714 (KUN)	Inner Mongolia, China	KT163423
<i>L. deliciosus</i>	A. Verbeken 2000-104 (GENT)	Italy	DQ922489
<i>L. deliciosus</i>	J. Nuytinck 2001-005 (GENT)	Sweden	DQ922485
<i>L. deterrimus</i>	JN2001-053 (GENT)	Slovakia	DQ922515
<i>L. deterrimus</i>	J. Nuytinck 2001-099 (GENT)	Italy	DQ922519
<i>L. deterrimus</i>	J. Vesterholt 96-333 (GENT)	Denmark	DQ922520
<i>L. deterrimus</i>	J. Nuytinck 2001-076 (GENT)	Slovakia	DQ922536
<i>L. fenoscandicus</i>	A. Verbeken 97-530 (GENT)	Sweden	DQ922510
<i>L. fenoscandicus</i>	J. Nuytinck 2001-018 (GENT)	Sweden	DQ922510
<i>L. fenoscandicus</i>	A. Verbeken 95-330 (GENT)	Sweden	DQ922500
<i>L. hatsudake</i>	M. Härkönen Kiima62 (H, GENT)	Hu'nan, China	EF685076
<i>L. hatsudake</i>	X.H. Wang 3188 (KUN)	Yunnan, China	KT163424
<i>L. hatsudake</i>	24414 (TMI)	Kagoshima, Japan	EF685062
<i>L. hengduanensis</i>	R. Wang dc39 (KUN)	Sichuan, China	KY174925
<i>L. hengduanensis</i>	R. Wang dc40 (KUN)	Sichuan, China	KY174926
<i>L. hengduanensis</i>	R. Wang lt54 (KUN)	Sichuan, China	KY174924
<i>L. hengduanensis</i>	K. Hosaka CH-15-001 (KUN)	Yunnan, China	KY174932
<i>L. hengduanensis</i>	S.H. Li 2362 (KUN)	Yunnan, China	KY174920
<i>L. hengduanensis</i>	J. Qin 37 (KUN)	Yunnan, China	KY174921

Table 1. Taxa and samples of *Lactarius* used for molecular analyses in this study. Sequences produced in the present study are in bold (*continued*)

<i>Species</i>	<i>Voucher (herbarium)</i>	<i>Location</i>	<i>ITS GenBank Accession</i>
<i>L. hengduanensis</i>	R. Wang rcb-84-1 (KUN)	Yunnan, China	KY174931
<i>L. hengduanensis</i>	R. Wang rcb-84-2 (KUN)	Yunnan, China	KY174930
<i>L. hengduanensis</i>	X.H. Wang 2242 (KUN)	Yunnan, China	KY174922
<i>L. hengduanensis</i>	X.H. Wang 2815 (KUN)	Yunnan, China	KY174923
<i>L. hengduanensis</i>	X.H. Wang 2816 (KUN)	Yunnan, China	KY174918
<i>L. hengduanensis</i>	X.H. Wang 2822 (KUN)	Yunnan, China	KY174919
<i>L. hengduanensis</i>	X.H. Wang 2267 (KUN)	Yunnan, China	KY174928
<i>L. hengduanensis</i>	X.H. Wang 2276 (KUN)	Yunnan, China	KY174929
<i>L. hengduanensis</i>	X.H. Wang 3191 (KUN)	Yunnan, China	KY174927
<i>L. hengduanensis</i>	X.H. Wang 3559 (KUN)	Yunnan, China	KY174933
<i>L. hengduanensis</i>	X.H. Wang 3560 (KUN)	Yunnan, China	KY174934
<i>L. laeticolor</i>	23149 (TMI)	Tottori, Japan	EF685077
<i>L. laeticolor</i>	OKM21714 (RSM)	Korea	EF685091
<i>L. pseudohatsudake</i>	Y.J. Hao 12 (KUN)	Yunnan, China	KY174903
<i>L. pseudohatsudake</i>	K. Hosaka CH-15-107 (KUN)	Yunnan, China	KY174909
<i>L. pseudohatsudake</i>	J. Qin 10 (KUN)	Yunnan, China	KY174900
<i>L. pseudohatsudake</i>	J. Qin 23 (KUN)	Yunnan, China	KY174916
<i>L. pseudohatsudake</i>	J. Qin 33 (KUN)	Yunnan, China	KY174911
<i>L. pseudohatsudake</i>	R. Wang rcb-88 (KUN)	Yunnan, China	KY174913
<i>L. pseudohatsudake</i>	X.H. Wang 2213 (KUN)	Yunnan, China	KY174912
<i>L. pseudohatsudake</i>	X.H. Wang 2245 (KUN)	Yunnan, China	KY174915
<i>L. pseudohatsudake</i>	X.H. Wang 2809 (KUN)	Yunnan, China	KY174904
<i>L. pseudohatsudake</i>	X.H. Wang 2831 (KUN)	Yunnan, China	KY174906
<i>L. pseudohatsudake</i>	X.H. Wang 3200 (KUN)	Yunnan, China	KY174914
<i>L. pseudohatsudake</i>	X.H. Wang 3214 (KUN)	Yunnan, China	KY174907
<i>L. pseudohatsudake</i>	J.P. Zhang 36 (KUN)	Yunnan, China	KY174908
<i>L. pseudohatsudake</i>	Q. Zhao 665 (KUN)	Yunnan, China	KY174902
<i>L. pseudohatsudake</i>	Q. Zhao 688 (KUN)	Yunnan, China	KY174910
<i>L. pseudohatsudake</i>	Q. Zhao 705 (KUN)	Yunnan, China	KY174917
<i>L. pseudohatsudake</i>	Q. Zhao 855 (KUN)	Yunnan, China	KY174905
<i>L. pseudohatsudake</i>	X.T. Zhu 14 (KUN)	Yunnan, China	KY174901
<i>L. rufus</i>	J. Nuytinck 2002-008 (GENT)	Norway	EF685089
<i>L. salmonicolor</i>	J. Nuytinck 2001-087 (GENT)	Belgium	DQ922548
<i>L. salmonicolor</i>	J. Nuytinck 2001-123 (GENT)	France	DQ922549
<i>L. salmonicolor</i>	J. Nuytinck 2002-027 (GENT)	Italy	DQ922552
<i>L. salmoneus</i>	BK 11-08-2004-14 (RMS)	Mississippi, USA	EF685090
<i>L. sanguifluus</i>	J. Nuytinck 2000-008 (GENT)	Belgium	DQ922539
<i>L. sanguifluus</i>	J. Nuytinck 2001-050 (GENT)	Slovakia	AY332546
<i>L. subindigo</i>	M. Härkönen Kiina 114 (H, GENT)	Hu'nan, China	EF685073
<i>L. subindigo</i>	K 259-99 (RMS)	Garhwal Himalaya, India	EF685068
<i>L. thynos</i>	Voitk 09-09-06 (priv. herb.)	New foundland, Canada	KJ769680
<i>L. thynos</i>	SLM 9648 (RMS)	New York, USA	EF685102
<i>L. vividus</i>	X.H. Wang 3039 (KUN)	Hubei, China	KT163433
<i>L. vividus</i>	M. Härkönen Kiina 35 (H, GENT)	Hu'nan, China	EF685074
<i>L. vividus</i>	M. Härkönen Kiina 113 (H, GENT)	Hu'nan, China	EF685072
<i>L. vividus</i>	X.H. Wang 2356 (KUN)	Hu'nan, China	KT163434

RESULTS

Phylogenetic Analysis

In BLASTn, for *L. abieticola*, sequences with the highest scores are from *L. salmonicolor*; for *L. hengduanensis* the hits are from *L. aurantiosordidus* Nuytinck & S.L. Miller and *L. sanguifluus* (Paulet) Fr. For *L. pseudohatsudake*, all the 11 hits with the highest similarity 98% are from unidentified samples. By comparing with available identified sequences, seven of them (from Alaska, USA) were identified to *L. aurantiosordidus*. The rest four (from Italy) were left un-identified and not included in the following analysis. After removing duplicate sequences, 74 sequences of 18 species were used for a final phylogenetic analysis.

The aligned ITS dataset includes 699 characters: 259 of ITS1 (complete), 155 of 5.8S, and 285 of ITS2 (complete). Among the 699 bp aligned nucleotides, 540 are constant, 43 are variable but parsimony-uninformative and 116 are parsimony-informative. *Lactarius abieticola* has an ITS region of 649 bp, *L. hengduanensis* 642 bp and *L. pseudohatsudake* 640 bp. No length variation (INDELs) exists among the individuals of each species. The ITS matrix is available at TreeBASE under accession S20218.

NJ analysis produced a bootstrap consensus tree with all the backbones not highly supported (Fig. 1). All high support values (70%) are on the branches leading to species. Samples of *L. abieticola* were grouped into a clade with NJ bootstrap proportion (NJ-BP) 75%, including two major branches supported by NJ-BP 85% and 76%, respectively. *Lactarius abieticola*, *L. laeticolor*, *L. salmonicolor*, *L. thynos*, and *L. vividus*, all having vivid orange or salmon-colored basidiomata, formed a clade but without support. Among them *L. vividus* is associated with trees of *Pinus* subg. *Pinus* (Wang *et al.* 2015), *L. thynos* grows in woods of *Thuja* (Hesler & Smith 1979; Nuytinck *et al.* 2006), and the other three with firs. Samples of *L. hengduanensis* formed a highly supported clade (84%), which is sister to the clade formed by *L. aurantiosordidus*, *L. deterrimus* and *L. fennoscandicus* with NJ-BP 76%. *Lactarius pseudohatsudake* is supported by NJ-BP 98%, but the grouping with its sister clade *L. hengduanensis*-*L. aurantiosordidus*-*L. deterrimus*-*L. fennoscandicus* is without support. All these species are *Picea*-associated, except that the symbiotic relationship between *L. aurantiosordidus* and *P. sitchensis* is not fully confirmed yet (Nuytinck *et al.* 2006, 2007).

Taxonomy

Lactarius abieticola X.H. Wang, sp. nov.

Fig. 2a-c, Fig. 3

Mycobank: MB819141

Holotypus: CHINA. Sichuan Prov., Puge Co., Luoji Mt., N27°34'51.04" E107°22'42.98", elev. 3610 m, under *A. georgei*, 11 Sept. 2010, X.H. Wang 2777 (KUN-HKAS 61973)

Etymology: Named after the symbiotic relationship with *Abies* trees.

Basidiomata small to medium-sized, fleshy, fragile. **Pileus** 25-50 mm in diam., margin involute when young, soon plano-applanate with depressed center, faintly to clearly zonate; margin acute, often with short strigose tomentum, transparently sulcate when fully mature, apricot (5B6), orange (6B6), salmon-orange (6A4-6A6), Persian orange (6A7), carrot red (6B7), rarely with brownish orange

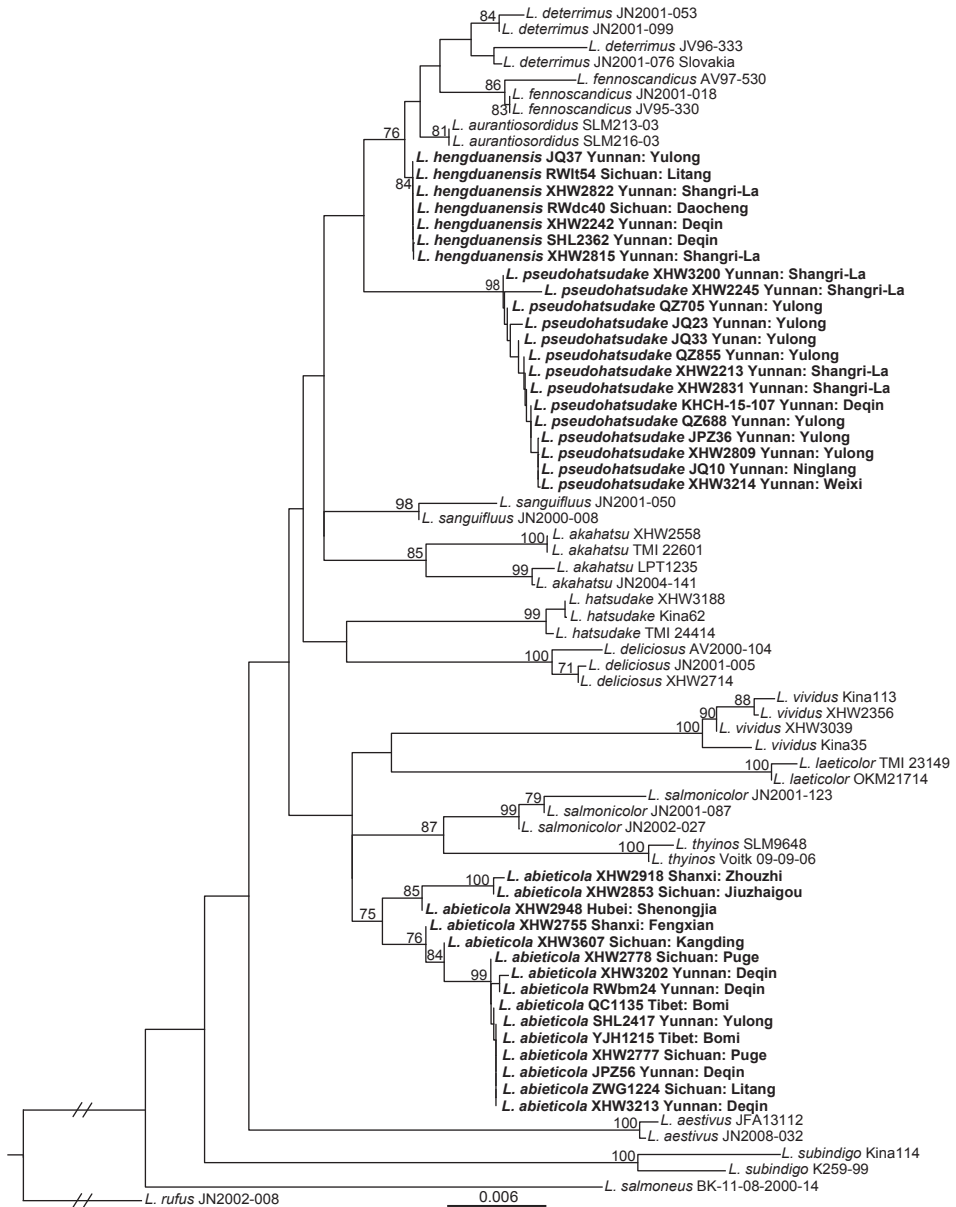


Fig. 1. Bootstrap consensus tree generated by Neighbor-Joining phylogenetic analysis, rooted with *Lactarius rufus*. The bootstrap proportions higher than 70% are indicated above or by the nodes. Sample numbers correspond to the collector or herbarium numbers in Table 1. Samples of the three new species are in bold.



Fig. 2. Basidiomata: **a.** *Lactarius abieticola* (KUN-HKAS 61973, holotype), **b.** *L. abieticola* (KUN-HKAS 61951, showing strigose pileus margin), **c.** *L. abieticola* (KUN-HKAS 61951, showing scrobicules on the stipe), **d.** *L. hengduanensis* (KUN-HKAS 62018, showing contrast between the vividly orange stipe and paler lamellae), **e.** *L. hengduanensis* (KUN-HKAS 62011, holotype), **f.** *L. deterrimus* (KUN-HKAS 73425, showing the less orange stipe), **g.** *L. pseudohatsudake* (KUN-HKAS 62005, immature basidiomata); **h.** *L. pseudohatsudake* (KUN-HKAS 60404, holotype), **i.** *L. hatsudake* (KUN-HKAS 75818, showing distant lamellae).

tinge (6C8), strongly hygrophanous; surface greasy to strongly sticky. **Context** 1-3 mm thick in the pileus, pale orange or nearly white, not discoloring when bruised, with pale greenish tinge when old. **Lamellae** 2-4 mm wide, subcrowded to crowded, short decurrent, often forking or anastomosing near stipe, melon (5A6), light orange

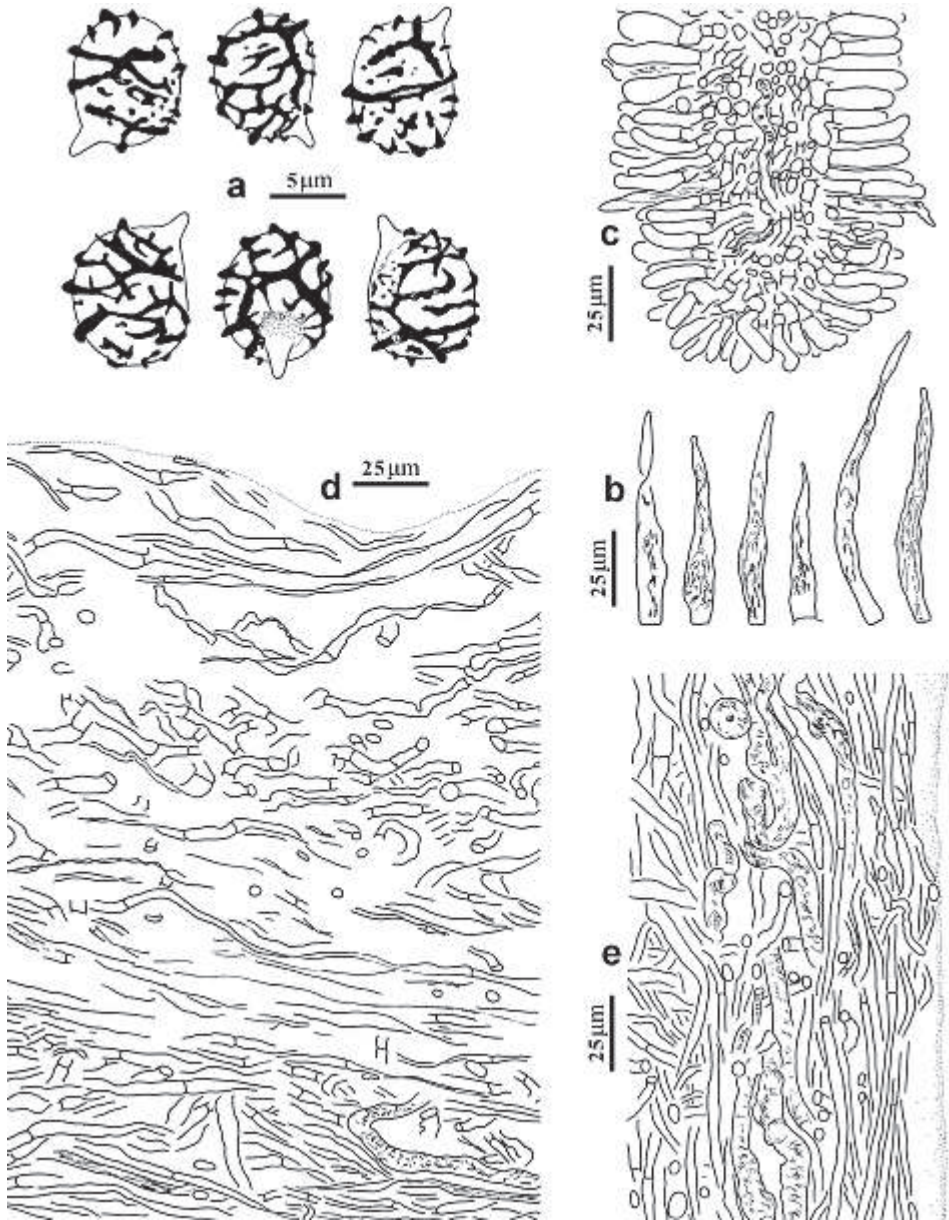


Fig. 3. *Lactarius abieticola* (KUN-HKAS 61973, holotype): a. basidiospores, b. pleuromacrocytidia, c. lamella edge, d. pileipellis, e. stiptipellis.

to melon (5A5-5A6), orange (5A7), orange to Persian orange (5A7-6A7, 6A6), unchanging or discoloring faint green when old or bruised. **Stipe** 20-50 × 5-10 (15) mm, central to eccentric, equal or slightly tapering upwards, orange (6A6), base concolorous or paler, not discoloring or only having very faint greenish discoloration, subhygrophanous, sometimes with small scrobicules, subsolid to hollow; surface greasy to sticky; base shortly strigose with cream-colored to pale orange or pale greenish hairs. **Latex** orange red, carrot-colored, unchanging.

Basidiospores (7.0) 7.5-10.0 (10.5) × (5.5) 6.0-8.0 (8.5) μm [Q = (1.09) 1.14-1.34 (1.50), Q = 1.24 ± 0.06] (400/20/16), ellipsoid, rarely broadly ellipsoid; ornamentation up to 1.2 μm high, mostly 0.3-1.0 μm high, of short or long ridges and irregular warts partly connected, some fused into short chains, rarely forming closed meshes, some arranged in a more or less zebroid pattern; plage not amyloid or slightly distally amyloid. **Basidia** 45-60 × 9-15 μm, subclavate, 4-spored; sterigmata 5-7 μm long. **Pleuromacrocystidia** scarce to abundant, emergent, 40-80 (100) × 5-10 μm, projecting up to 30 μm beyond the basidia, subfusiform, sublanceolate, some with a moniliform apex, often with yellowish granular or crystal-like contents, rarely hyaline with sparse contents. **Pseudocystidia** uncommon to common, (2) 3-4 (5) μm wide, cylindrical, pale yellow to pale golden yellow, some forking. **Lamella edge** sterile. **Cheilomacrocystidia** absent to common, 25-40 × 5-6 (-10) μm, similar to pleuromacrocystidia in shape. **Hymenophoral trama** with sphaerocytes and abundant golden yellowish lactifers, lacking typical rosettes. **Pileipellis** an ixocutis, (50) 100-200 (300) μm thick, of loose hyphae; hyphae 2.5-5 μm diam., slightly to strongly gelatinized, often shrivelled, thin-walled, hyaline, rarely pale yellow. **Stipitipellis** often an ixocutis with a clear slime-layer, rarely a cutis, 20-70 (150) μm thick, of slightly to strongly gelatinized hyphae; hyphae shrivelled or not, 2-5 μm diam. **Trama of pileus and stipe** with abundant golden yellowish lactifers and rosettes. **Clamp-connections** absent.

Habitat: on ground, scattered or in group in *Abies* forests.

Other specimens examined: CHINA. **Hubei Prov.:** Fang Co.: Shennongjia scenic section, Xiaolongtan, N31°28'53" E110°18'14", elev. 2155 m, under *Abies fargesii*, 01 Aug. 2011, X.H. Wang 2948 (KUN-HKAS 73508). **Shanxi Prov.:** Feng Co.: Jianlingjiang Headstream scenic section, N34°10'03.21" E107°2'8.52", elev. 2500 m, under *A. chensiensis*, 7 Sept. 2010, X.H. Wang 2755 (KUN-HKAS 61951); Zhouzhi Co.: Houzhenzi, Taibai Mts., elev. 2300 m, under *A. chensiensis*, 27 Jul. 2011, X.H. Wang 2918 (KUN-HKAS 73479). **Sichuan Prov.:** Kangding Co.: Mugecuo National Forest Park, under *Abies* sp., 4 Aug. 2015, X.H. Wang 3607 (KUN-HKAS 89881); Jiuzhaigou Co.: Jiuzhaigou scenic section, elev. 2600 m, under *Abies ernestii*, 15 Jul. 2011, X.H. Wang 2853 (KUN-HKAS 73417); Litang Co.: narrow side road off Highway 318 from Yajiang to Litang, Gaowa, N30°10'6" E100°35'7", elev. 4300-4350 m, 8 Aug. 2006, Z.W. Ge 1224 (KUN-HKAS 50804); Puge Co.: Luoji Mt., N27°34'51.04" E107°22'42.98", elev. 3610 m, under *A. georgei*, 11 Sept. 2010, X.H. Wang 2778 (KUN-HKAS 61974). **Tibet Autonomous Region:** Bomi Co.: roadside from Bomi to Motuo, N29 47'26.1" E95 41'51.4", elev. 3653 m, under *A. delavayi* var. *motuoensis*, 1 Jul. 2014, Q. Cai 1135 (KUN-HKAS 83595); roadside from Bomi to Motuo 37 km, N29 47'52.46" E95°33'3.17", elev. 2718 m, 3 Jul. 2014, Y.J. Hao 1215 (KUN-HKAS 83006). **Yunnan Prov.:** Deqin Co.: Baima Snow Mts., under *A. georgei*, 19 Aug. 2014, R. Wang bm-24 (KUN-HKAS 86038), R. Wang bm-25 (KUN-HKAS 86039); *ibid*, 5 Sept. 2009, J.P. Zhang 56 (KUN-HKAS 59013); roadside from Deqin to Yakou of Baima Snow Mts., elev. 3500 m, under *A. georgei*, 06 Sept. 2008, X.H. Wang 2241 (KUN-HKAS 61373); *ibid*, 14 Oct. 2011, X.H. Wang 3213 (KUN-HKAS 72239); roadside from

Benzilan to Yakou of Baima Snow Mts., near Yakou, under *A. gerogei*, elev. 12 Oct. 2011, X.H. Wang 3202 (KUN-HKAS 72236); Yulong Co.: Laojun Mt. Nature Reserve, under *Abies* and *Picea* trees, 30 Aug. 2012, S.H. Li L2417 (KUN-HKAS 85395); *ibid.*, alt. 3400 m, 28 Jul. 2001, X.H. Wang 1283 (KUN-HKAS 39213).

Note: The specimens cited above are variable in microscopy. The pleuromacrocytidia, in general very emergent, can be abundant (KUN-HKAS 61974 and 72239), common (KUN-HKAS 61973, 73419, 73479, 73408, 83595, 86038, 89881), uncommon (KUN-HKAS 39213, 61951, 85395) or very rare (KUN-HKAS 50804, 59013, 72236, 86039). The lamella edge is sterile, mostly lacking cheilomacrocytidia, but in KUN-HKAS 61974 and 89881 cheilomacrocytidia are very common. The average measurements of basidiospores of 20 spores vary from 8.1×6.6 (KUN-HKAS 61974) to 9.8×7.5 μm (KUN-HKAS 73417), forming a continuous series.

The pure orange basidiomata mostly lacking greenish discoloration, the viscid pileus and stipe surface and the exclusive symbiotic relationship with *Abies* trees of *L. abieticola* are strongly reminiscent of Japanese *L. laeticolor* and European *L. salmonicolor*. Wang (2008) misidentified one specimen (KUN-HKAS 39213) of *L. abieticola* as *L. laeticolor* and Nuytinck *et al.* (2006) took it as a “pure but rather pale orange” type of *L. deliciosus*. Compared with *L. laeticolor* and *L. salmonicolor* (Heilmann-Clausen *et al.* 1998; Wang & Liu 2010), *L. abieticola* has smaller basidiomata, not exceeding 5 cm in diam. Most specimens of *L. abieticola* have a pileus with short whitish strigose tomentum at the margin, which is clearly visible when young (Fig. 2b). This character is also found in North American *L. salmoneus* (Hesler & Smith 1979). Phylogenetic analysis of the ITS dataset supported *L. abieticola* to be a distinct species but the relationships with *L. laeticolor*, *L. salmonicolor* and *Pinus*-associated *L. vividus* (growing in subtropical-tropical China) are not well resolved. More loci are needed to investigate if the *Abies*-associated taxa form a monophyletic group or not.

Lactarius hengduanensis X.H. Wang, sp. nov.

Fig. 2d-e, Fig. 4

Mycobank: MB819142

Holotypus: CHINA. Yunnan, Shangri-La Co., Xiaozhongdian, roadside from Xiaozhongdian to Tianchi, elev. 3600 m, under *P. likiangensis*, 15 Sept. 2010, X.H. Wang 2815 (KUN-HKAS 62011)

Etymology: Named after the geographical origin of the species, i.e. Hengduan Mts.

Basidiomata medium-sized, fleshy, fragile. **Pileus** 50-70 mm in diam., margin involute when young, convex with a depressed center when mature, mostly faintly zonate, more clearly zonate towards margin, orange to Pompeian yellow (5C6), yellowish brown, vinaceous-red with brownish tinge, sometimes with bluish-green or bluish-lilac tinge at center, hygrophanous; surface greasy. **Context** 3-5 mm thick in the pileus, pale grayish red or pale orange, not discoloring when bruised. **Lamellae** 2-4 mm wide, crowded, short decurrent, light orange (5A4, 6A5), discoloring bluish green after bruised. **Stipe** 30-50 \times 12-15 mm, central to eccentric, equal or slightly tapering upwards, Persian orange (6B7), mandarin orange (6B8, or duller), grayish red (7B6), grayish orange to grayish red (6B6-7B6), discoloring bluish green when bruised, with a whitish zone underneath the lamellae attachment, with white to cream-orange strigose hairs at the base, solid to hollow; surface subgreasy to greasy. **Latex** deep orange to mandarin orange (6A8-6B8), discoloration not observed.

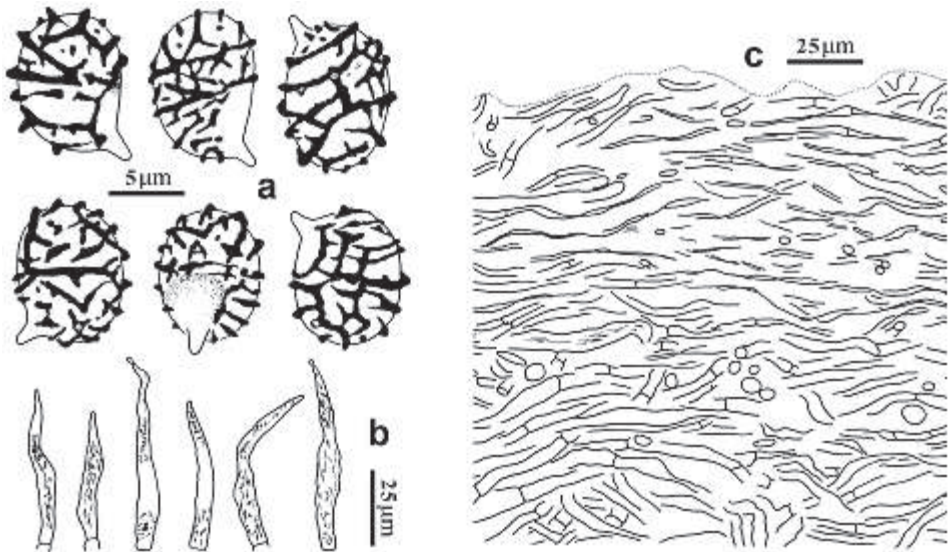


Fig. 4. *Lactarius hengduanensis* (KUN-HKAS 62011, holotype): a. basidiospores, b. pleuromacrocystidia, c. pileipellis.

Basidiospores (7.5) 8.0-9.5 (10.0) × (6.0) 6.5-8.0 (8.5) µm [Q = (1.14) 1.18-1.38 (1.43), Q = 1.26 ± 0.06] (160/8/7), ellipsoid, rarely broadly ellipsoid; ornamentation up to 1.0 µm high, mostly 0.3-0.7 µm high, of short or long ridges partly connected, sometimes forming an incomplete reticulum, with isolated warts, rarely arranged in a more or less zebraoid pattern; plage not amyloid or slightly distally amyloid. **Basidia** 50-60 × 9-13 µm, subclavate, 4-spored; sterigmata 5-7 µm long. **Pleuromacrocystidia** absent to common, often abundant near lamella edge, 30-70 × 5-8 µm, projecting up to 30 µm beyond the basidia, subfusiform, sublanceolate, some with a moniliform apex, with yellowish granular or crystal-like contents. **Pseudocystidia** common, (2) 3-4 (5) µm wide, cylindrical, pale yellow to pale golden yellow, some forking or multi-branching at apex. **Lamella edge** sterile. **Cheilomacrocystidia** absent. **Hymenophoral trama** with sphaerocytes and abundant golden yellowish-brown lactifers, lacking typical rosettes. **Pileipellis** an ixocutis, 70-170 µm thick, of compact hyphae; hyphae 2.5-6 µm diam., slightly to strongly gelatinized, often shrivelled, thin-walled, hyaline, rarely pale yellow. **Stipitipellis** a cutis, 20-60 µm thick, very rarely with an ixocutis 150-170 µm thick; hyphae strongly gelatinized, 2-5 µm diam. **Trama of pileus and stipe** with abundant golden yellowish brown lactifers and rosettes. **Clamp-connections** absent.

Habitat: on ground, gregarious or scattered in *Picea* forests.

Other specimens examined: CHINA. **Sichuan:** Daocheng Co.: Sangdui town, Jiyi village, 21 Aug. 2014, R. Wang dc-39 (KUN-HKAS 86052), R. Wang dc-40 (KUN-HKAS 86053); Litang Co.: Haizi Mt., N29°19.221' E100° 5.012', elev. 4350 m, 22 Aug. 2014, R. Wang lt-54 (KUN-HKAS 86066). **Yunnan:** Chuxiong city town: wild mushroom market, 28 Aug. 2015, K. Hosaka CH-15-001 (KUN-HKAS 94954); Deqin Co.: Baima Snow Mts., roadside from Benzilan to Yakou, elev. 3450 m, under *P. likiangensis*, 6 Sept. 2008, X.H. Wang 2242 (KUN-HKAS 61374); Baima Snow Mts., elev. 3291 m, 27 Aug. 2012, S.H. Li L2362

(KUN-HKAS 85393); Kunming city town: Ciba wild mushroom market, 20 Aug. 2015, R. Wang rcb-84-1 (KUN-HKAS 90692), rcb-84-2 (HKAS 90690); Mushuihua wild mushroom market, 24 Jul. 2012, X.H. Wang 3559 (KUN-HKAS 76125), X.H. Wang 3560 (KUN-HKAS 76127); Yulong Co.: Daju town, Yulong Snow Mts., 12 Jul. 2010, J. Qin 37 (KUN-HKAS 67723); Shangri-La Co.: Bita lake, south routine, elev. 3700 m, under *P. likianensis*, 9 Sept. 2008, X.H. Wang 2267 (KUN-HKAS 60445); *ibid*, 10 Sept. 2008, X.H. Wang 2276 (KUN-HKAS 60445); Bita Lake, south routine, elev. 3600 m, *P. likianensis*, 16 Sept. 2010, X.H. Wang 2822 (KUN-HKAS 62018); Xiaozhongdian town, roadside from Xiaozhongdian to Tianchi, elev. 3600 m, under *P. likianensis*, 15 Sept. 2010, X.H. Wang 2816 (KUN-HKAS 62012); *ibid*, 10 Oct. 2011, X.H. Wang 3191 (KUN-HKAS 75962).

Note: For a long time, *L. hengduanensis* is confused with *L. deterrimus*, a sympatric species also growing with spruces and having orange basidiomata. Compared with *L. deterrimus*, *L. hengduanensis* shows a general tendency for the stipe to be more vividly colored, in contrast with the paler lamellae (compare Fig. 2d, e with f), and for the spores to be more reticulate (Fig. 4). ITS dataset supported the affinity with the *L. deterrimus*-*L. fennoscandicus* complex and North American *L. sordidoaurantiacus* (Fig. 1). In the ITS alignment, *L. hengduanensis* has five substitutions, a 11 bp deletion and a 3 bp insertion compared with *L. deterrimus*.

Lactarius pseudohatsudake X.H. Wang, sp. nov.

Fig. 2g-h, Fig. 5

Mycobank: MB819143

Holotypus: CHINA. Yunnan, Shangri-La Co., Xiaozhongdian, Jisha, elev. 3380 m, under *P. likiangensis*, 4 Sept. 2008, X.H. Wang 2213 (KUN-HKAS 60404)

Etymology: Named after the morphological similarity with *L. hatsudake*.

Basidiomata medium-sized to big, fleshy, fragile. **Pileus** 50-100 mm in diam., margin involute when young, convex with a depressed center when mature, faintly to clearly zonate; margin sometimes with small scrobicules, transparently sulcate when fully mature, grayish red, dull red, often mixed with bluish-green tinge or totally bluish green at center, strongly hygrophanous; surface greasy. **Context** 3-4 mm thick in the pileus, pale grayish red, not discoloring when bruised. **Lamellae** 2-4 mm wide, crowded, short decurrent, often forking or anastomosing near stipe, reddish brown (8D4), sometimes with bluish green tinge. **Stipe** 30-90 × 7-20 mm, central to eccentric, equal or slightly tapering upwards, cinnamon to light brown (6D6-7D5), with a whitish zone underneath the lamellae attachment, rarely with scrobicules, base white, solid; surface subgreasy to greasy. **Latex** in pileus brick red (7D7), in stipe brick red or with more ochraceous brown tinge, unchanging.

Basidiospores (7.0) 7.5-9.5 (11.0) × 6.0-7.5 (8.5) μm [Q = (1.14) 1.17-1.37 (1.43), Q = 1.27 ± 0.06] (180/9/8), ellipsoid, rarely broadly ellipsoid; ornamentation up to 1.0 μm high, mostly 0.3-0.7 μm high, of short or long ridges partly connected, some short ridges fused into longer chains, sometimes forming an incomplete reticulum, with isolated warts, some arranged in a more or less zebroid pattern; plage not amyloid or distally amyloid. **Basidia** 45-65 × 9-14 μm, subclavate, 4-spored; sterigmata 5-7 μm long. **Pleuromacrocyttidia** mostly absent or very scarce, rarely common, 40-70 × 6-10 μm, projecting up to 30 μm beyond the basidia, subfusiform, sublanceolate, some with a moniliform apex, with yellowish granular or crystal-like contents. **Pseudocystidia** uncommon to common, (2) 3-4 (5) μm wide, cylindrical, pale yellow to pale golden yellow, some forking. **Lamella edge** sterile. **Cheilomacrocyttidia** absent to very scarce, 20-25 × 5-6 μm, fusiform, with

crystal-like contents. *Hymenophoral trama* with sphaerocytes and abundant golden yellowish-brown lactifers, lacking typical rosettes. *Pileipellis* an ixocutis, 50-170 μm thick, of compact hyphae; hyphae 2.5-6 μm diam., slightly to strongly gelatinized, often shrivelled, thin-walled, hyaline, rarely pale yellow. *Stipitipellis* an ixocutis with a sparse slime-layer, rarely a cutis, 30-50 (70) μm thick, of strongly gelatinized hyphae; hyphae shrivelled or not, 2-5 μm diam. *Trama of pileus and stipe* with abundant golden yellowish lactifers and rosettes. *Clamp-connections* absent.

Habitat: on ground, gregarious or scattered in *Picea* forests.

Other specimens examined: CHINA. **Yunnan**: **Degin Co.**: Baima Snow Mts., K. Hosaka CH-15-107 (KUN-HKAS 94963); roadside from Benzilan to Yakou, elev. 3450 m, under *P. likiangensis*, 6 Sept. 2008, X.H. Wang 2245 (KUN-HKAS 60381); Kunming city town: Ciba wild mushroom market, 20 Aug. 2015, R. Wang

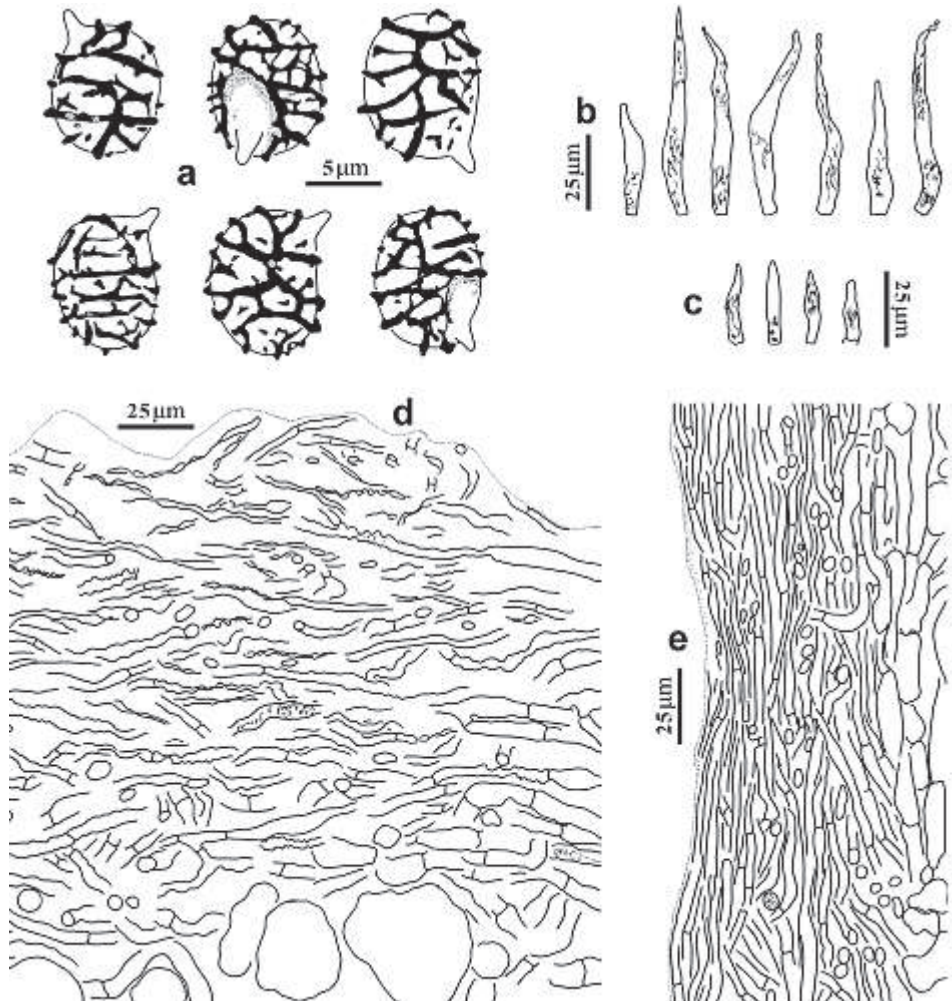


Fig. 5. *Lactarius pseudohatsudake* (holotype, KUN-HKAS 60404): a. basidiospores, b. pleuro-macrocyttidia; c. cheilomacrocyttidia; d. pileipellis, e. stipitipellis.

rcb-88 (KUN-HKAS 90693); Ninglang Co.: Lugu Lake, elev. 2740–3280 m, 10 Jul. 2010, J. Qin 10 (KUN-HKAS 63645), Y.J. Hao 12 (KUN-HKAS 67984), Q. Zhao 665 (KUN-HKAS 69386), X.T. Zhu 14 (KUN-HKAS 68190); Shangri-La Co.: Bitahai, south routine, parking spot, 27° 48'01" E99° 59'20", elev. 3700 m, under *P. likiangensis*, 16 Sept. 2010, X.H. Wang 2831 (KUN-HKAS 62027); Napahai, N27° 55'56" E99°36'37", elev. 3645 m, under *P. likiangensis*, 11 Oct. 2011, X.H. Wang 3200 (KUN-HKAS 75813); Weixi Co.: Pantiang town, roadside from Tacheng to Weixi, N27°20'48" E99°17'01", under *Picea* sp., elev. 2940 m, 15 Oct. 2011, X.H. Wang 3214 (KUN-HKAS 75821); Yulong Co.: Daju town, Yulong Snow Mts., elev. 3231 m, 12 Jul. 2010, J. Qin 33 (KUN-HKAS 67718), Q. Zhao 688 (KUN-HKAS 69409), Q. Zhao 705 (KUN-HKAS 69426); Ganhaizi, elev. 3200 m, under *P. likiangensis*, 14 Sept. 2010, X.H. Wang 2809 (KUN-HKAS 62005); Taian Town, Gaomeigu, elev. 3100 m, under *P. likiangensis*, 20 Jul. 2008, Q. Zhao 855 (KUN-HKAS 55056); Taian town, Lijiang Observatory, elev. 3230 m, under *P. likiangensis*, 03 Sept. 2009, J.P. Zhang 36 (KUN-HKAS 59017); Mingyin town, Yulong Snow Mts., under *P. likiangensis*, 11 Jul. 2010, J. Qin 23 (KUN-HKAS 63658).

Note: Most specimens examined above lack macrocystidia or have very scarce macrocystidia. Three collections, KUN-HKAS 60381, 60404 and 94963, however, have common pleuromacrocystidia. Cheilomacrocystidia are absent or very scarce in all collections examined. The stiptipellis often has an ixocutis, more or less similar to *L. abieticola*.

Together with *L. hatsudake*, *L. pseudohatsudake* is the second species in China having vinaceous-red latex and grayish red-dominant basidiomata [the distinct identity of another reddish species "*Lactarius* sp. 1" in Nuytinck *et al.* (2006) from *L. hatsudake* is not verified]. Compared with *L. hatsudake*, this new species has crowded lamellae (compare Fig. 2g, h with i). *Lactarius hatsudake* is associated with pines and in China it is distributed in subtropical regions or lower places < 2500 m (Li *et al.* 2011; Nuytinck *et al.* 2006; Wang *et al.* 2003; own observations), whereas *L. pseudohatsudake* invariably grows in subalpine spruce forests. Phylogenetically, *L. pseudohatsudake* seems more closely related with the *L. deterrimus*-*L. fennoscandicus* complex and *L. hengduanensis* other than *L. hatsudake* (Fig. 1). This relationship also excludes the possibility of being conspecific with Himalayan *L. thakalorum* Bills & Cotter, a species also having reddish latex but closely related with *L. sanguifluus* and growing with pines (Bills & Coker 1989; Nuytinck *et al.* 2007).

DISCUSSION AND CONCLUSION

Taxonomy of *Lactarius* sect. *Deliciosi* is notoriously difficult due to the high macro- and microscopical similarity among species. The three new species described in this study might easily be assigned to other species when molecular data are not used. For instance, *L. abieticola* could be easily identified as *L. laeticolor*, another Asian species. The wide intraspecific variation observed in all three new species for e.g. degree of abundance of macrocystidia, slimy degree of stipe or size and ornamentation of spores aggravates the difficulty of morphological species delimitation. Although there is a tendency for the lamellae of *L. pseudohatsudake* to be more crowded than those of *L. hatsudake*, the degree of space may be overlapping

among some individuals of the two species. The stipe of *L. hengduanensis* sometimes might be not so vivid orange and this will cause a dilemma in assigning such specimens to either *L. deterrimus* or *L. hengduanensis*.

Although NJ analysis of the ITS dataset supported the three newly described species to be distinct from the others, in the ITS alignment, these species only differ in several substitutions and 1-3 INDELs. Such high similarity seems to suggest very recent (or ongoing) speciation. These new species and their relatives would be good candidates for investigating speciation, populational genetics and co-evolution with host trees.

In our investigation, the three species described here are never found in pine forests in the lower montane regions (elev. < 3000 m). They are apparently endemic elements to the alpine-subalpine regions. Samples of *L. hengduanensis* and *L. pseudohatsudake* (often in large quantities) are commonly sold as culinary mushrooms (under the same local names of *L. deliciosus* and *L. hatsudake*) in some lower land markets (Kunming and Chuxiong, Yunnan). They are believed to be transported down from the alpine regions. The morphological and molecular data published in this study will help to identify these edible mushrooms and the names can be used as correct tags for the conservation of the natural resources.

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