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***Lactarius vividus* sp. nov. (Russulaceae, Russulales), a widely distributed edible mushroom in central and southern China**

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Abstract

Lactarius sect. *Deliciosi* includes a group of edible mushrooms with a worldwide geographical distribution. Although several species are considered choice edible mushrooms, taxonomic knowledge on this group in Asia is still fragmentary. A new species in *L.* sect. *Deliciosi* with orange latex, *L. vividus*, is described as an important wild edible mushroom that is widely distributed in central and southern China. Morphological analyses demonstrate that *L. vividus* encompasses high infraspecific morphological variation, which makes it difficult to clearly delimit its taxonomic boundary. Compared with other species having orange latex in *L.* sect. *Deliciosi*, *L. vividus* can be recognized by the vividly colored, subdistant to distant lamellae and heavy basidiospore ornamentation. It has been found to grow with four species of *Pinus* subg. *Pinus* including *P. latteri*, *P. massoniana*, *P. taiwanensis* and *P. thunbergii*. Phylogenetic analyses of the ITS region and glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*) support species delimitation and suggest ITS and *gpd* are good DNA markers to identify this new species.

Key words: ectomycorrhizal fungi, milkcap, pines, taxonomy

Introduction

Lactarius sect. *Deliciosi* (Fr.: Fr.) Redeuilh *et al.* is a group of edible fungi that are collected and traded commercially worldwide (Nuytinck 2005). *Lactarius* sect. *Deliciosi* can be readily identified by the orange, red, or bluish basidiocarps with orange, red or bluish latex but species boundaries are extremely difficult to delimit due to high morphological similarity and close affinity between species (Nuytinck *et al.* 2007). In Asia and North America, species that were previously identified with European names often turn out to be different species that are endemic to those continents (Nuytinck *et al.* 2006a, 2006b, 2007; Nuytinck & Ammirati 2014).

In the descriptions of Asian representatives of *L.* sect. *Deliciosi*, Nuytinck *et al.* (2006b) reported a distinct species (“*Lactarius* sp. 2”) from central-southern China (Hunan Province). In the worldwide phylogeny of *L.* sect. *Deliciosi* based on ITS region and the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene, sequences of this species formed a very early diverging branch (Nuytinck *et al.* 2007). Additional conspecific sequences were subsequently submitted to GenBank and published by Chinese researchers (Guo *et al.* 2011b; Chen *et al.* 2013; Xue *et al.* 2013). Well-documented collections by the first author during several field trips in China over the past five years make detailed descriptions of the morphology, host specificity and geographical distribution possible.

Material and methods

Thirteen specimens of *L. vividus* sp. nov. collected from 10 provinces in central and southern China (Fig. 1) were used for morphological observations and phylogenetic analyses. When the specimens were collected, special attention was

paid to the presence and identity of coniferous trees, since species in *L.* section *Deliciosi* are known to form almost exclusive symbioses with conifers. Morphological methods, DNA extraction and PCR protocols followed Wang *et al.* (2012). The ITS region and part of the *gpd* gene were amplified for all 13 specimens. For the amplification of *gpd* genes, primers CTK-132F and CTK-108_{rev} were used (Kreuzinger *et al.* 1996). Besides the three ITS sequences and one *gpd* sequence published by Nuytinck *et al.* (2007), five additional published ITS sequences of *L. vividus* were retrieved from GenBank. These sequences added one extra province (Guangxi) to the distribution area.



FIGURE 1. Localities of specimens of *Lactarius vividus* collected in China.

To test the relationship between *L. vividus* and other species of *L.* sect. *Deliciosi* in a worldwide spectrum, a preliminary phylogenetic analysis was made based on a dataset including all species of *L.* sect. *Deliciosi* with published sequences (tree not shown). Due to the close affinities among the species within the section, the preliminary analysis did not solve the relationships between *L. vividus* and the other species with high support, but suggested that *L. laeticolor* (S. Imai) Imazeki ex Hongo, *L. salmoneus* Peck, *L. salmonicolor* R. Heim & Leclair, *L. sanguifluus* (Paulet) Fr., *L. semisanguifluus* R. Heim & Leclair, and *L. thynos* A.H. Sm. were relatively closer to *L. vividus* than the other species. Consequently, a smaller sampling was made to include 22 ITS and 18 *gpd* sequences of these six species and *L. akahatsu* Tanaka, *L. deliciosus* (L. : Fr.) Gray, *L. hatsudake* Tanaka, *L. horakii* Nuytinck & Verbeken, *L. subindigo* Verbeken & E. Horak as well as an unnamed species ("*Lactarius* sp. 1" in Nuytinck *et al.* (2007). The additional six species were chosen to represent the known species of *L.* sect. *Deliciosi* from Asia (except for *L. thakalorum* Bills & Cotter, whose ITS sequence needs to be verified). *Lactarius rufus*, a species of *L.* subg. *Russularia* (Fr.) Kauffman was used as outgroup. Specimens used for phylogenetic analyses are listed in Table 1.

TABLE 1. Taxa and collections used for molecular phylogenetic analyses in this study. Sequences produced in the present study are in bold. ITS = internal transcribed spacer regions 1 and 2 and 5.8S rRNA, *gpd* = glyceraldehyde-3-phosphate dehydrogenase gene.

Species	Voucher (herbarium)	Geographical Origin	GenBank Accession	
			ITS	<i>gpd</i>
<i>Lactarius akahatsu</i>	X.H. Wang 2558 (KUN-HKAS)	Jilin, China	KT163421	KT163404
<i>L. akahatsu</i>	L.P. Tang 1235 (KUN-HKAS)	Yunnan, China	KT163422	KT163405
<i>L. akahatsu</i>	22601 (TMI)	Torreri, Japan	EF685045	EF685103
<i>L. akahatsu</i>	A. Verbeken 2004-141 (GENT)	Thailand	EF685097	EF685143
<i>L. deliciosus</i>	A. Verbeken 2000-104 (GENT)	Italy	DQ922489	DQ890392
<i>L. deliciosus</i>	X.H. Wang 2714 (KUN-HKAS)	Inner Mongolia, China	KT163423	KT163406
<i>L. hatsudake</i>	M. Härkönen Kiina62 (H, GENT)	Hunnan, China	EF685076	EF685125
<i>L. hatsudake</i>	X.H. Wang 3188 (KUN-HKAS)	Yunnan, China	KT163424	KT163407
<i>L. hatsudake</i>	24414 (TMI)	Kagoshima, Japan	EF685062	EF685115
<i>L. horakii</i>	E. Horak 8336 (ZT, GENT)	Java, Indonesia	EF685069	EF685121
<i>L. horakii</i>	E. Horak 9983 (ZT, GENT)	Java, Indonesia	EF685070	EF685122
<i>L. laeticolor</i>	23149 (TMI)	Tottori, Japan	EF23149	EF685126
<i>L. laeticolor</i>	OKM21714 (RSM)	Tottori, Japan	EF685091	NA
<i>L. rufus</i>	J. Nuytinck 2002-008 (GENT)	Norway	EF685089	DQ890419
<i>L. salmonicolor</i>	J. Nuytinck 2001-087 (GENT)	Belgium	DQ922548	DQ890406
<i>L. salmonicolor</i>	J. Nuytinck 2001-123 (GENT)	France	DQ922549	DQ890407
<i>L. salmoneus</i>	BK 11-08-2004-14 (RMS)	Mississippi, USA	EF685090	EF685136
<i>L. sanguifluus</i>	J. Nuytinck 2000-008 (GENT)	Belgium	DQ922539	DQ890409
<i>L. sanguifluus</i>	J. Nuytinck 2001-050 (GENT)	Slovakia	AY332546	DQ890410
<i>L. semisanguifluus</i>	A. Verbeken 96-1032 (GENT)	Belgium	AY332553	DQ890413
<i>L. semisanguifluus</i>	J. Nuytinck 2001-093 (GENT)	Italy	AY332556	DQ890389
<i>L. subindigo</i>	M. Härkönen Kiina 114 (H, GENT)	Hunnan, China	EF685073	NA
<i>L. subindigo</i>	K259-99 (RMS)	India	EF685068	EF685120
<i>L. thyinos</i>	SLM 9648 (RMS)	New York, USA	EF685102	EF685149
<i>L. vividus</i>	AH3 (CSUFTD)	Anhui, China	JX852626	NA
<i>L. vividus</i>	AH4 (CSUFTD)	Anhui, China	JX852627	NA
<i>L. vividus</i>	AH5 (CSUFTD)	Anhui, China	JX852628	NA
<i>L. vividus</i>	X.H. Wang 3033 (KUN-HKAS)	Anhui, China	KT163425	KT163408
<i>L. vividus</i>	X.H. Wang 2428 (KUN-HKAS)	Chongqing, China	KT163426	KT163409
<i>L. vividus</i>	D. Wang214 (KUN-HKAS)	Guangdong, China	KT163427	KT163410
<i>L. vividus</i>	GX2 (CSUFTD)	Guangxi, China	JX852629	NA
<i>L. vividus</i>	H.J. Li 140924-29 (KUN-HKAS)	Guizhou, China	KT163428	KT163411
<i>L. vividus</i>	H.J. Li 140928-19 (KUN-HKAS)	Guizhou, China	KT163429	KT163412
<i>L. vividus</i>	X.H. Wang 2404 (KUN-HKAS)	Guizhou, China	KT163430	KT163413
<i>L. vividus</i>	X.H. Wang 2417 (KUN-HKAS)	Guizhou, China	KT163431	KT163414
<i>L. vividus</i>	N.K. Zeng 552 (KUN-HKAS)	Hainan, China	KT163432	KT163415

...Continued on next page

TABLE 1. (Continued)

Species	Voucher (herbarium)	Geographical Origin	GenBank Accession	
			ITS	<i>gpd</i>
<i>L. vividus</i>	X.H. Wang 3039 (KUN-HKAS)	Hubei, China	KT163433	KT163416
<i>L. vividus</i>	M. Härkönen Kiina 35 (H, GENT)	Hunnan, China	EF685074	NA
<i>L. vividus</i>	M. Härkönen Kiina 36 (H, GENT)	Hunnan, China	EF685075	NA
<i>L. vividus</i>	M. Härkönen Kiina 113 (H, GENT)	Hunnan, China	EF685072	EF685124
<i>L. vividus</i>	HH1 (CSUFTD)	Hunnan, China	HQ323351	NA
<i>L. vividus</i>	X.H. Wang 2356 (KUN-HKAS)	Hunnan, China	KT163434	KT163417
<i>L. vividus</i>	X.H. Wang 3048 (KUN-HKAS)	Jiangxi, China	KT163435	KT163418
<i>L. vividus</i>	X.H. Wang 2987 (KUN-HKAS)	Shangdong, China	KT163436	KT163419
<i>L. vividus</i>	X.H. Wang 2769 (KUN-HKAS)	Sichuan, China	KT163437	KT163420
<i>Lactarius</i> sp	M. Härkönen Kiina 112 (H, GENT)	Hunnan, China	EF695071	EF685123

Alignments were made using the online version of the multiple sequence alignment program MAFFT v7 (Katoh and Toh 2008), applying the L-INS-I strategy and manually adjusted in BioEdit. To test the incongruence between the ITS and *gpd* datasets, the ML-BP (Maximum Likelihood Bootstrap Proportions) for the same set of taxa were compared. A conflict was assumed to be significant when two different relationships (one monophyletic and the other non-monophyletic) for the same set of taxa were both supported with ML-BP $\geq 70\%$.

Phylogenetic analyses were conducted using ML in RAxML v7.2x (Stamatakis 2006) and BI (Bayesian Inference) in MrBayes v3.2.1 (Ronquist *et al.* 2012). ML analyses were executed applying the Rapid Bootstrapping algorithm with 1000 replicates, followed by a ML tree search. In the BI analysis, the GTR+I+G model was used and all parameter values, except branch lengths and tree topologies, were set unlinked. The BI analyses were conducted using two runs with four chains each for 10000000 generations sampling every 100th tree. Runs were terminated when the average standard deviation of split frequencies went below 0.01 and effective sampling sizes were > 200 . A 50% majority rule consensus tree was built after discarding trees from a 25% burnin. Trees generated by the two analyses were viewed and exported in FigTree v1.3.1.

Results

The aligned ITS dataset includes 46 samples and 691 characters: 251 of ITS1 (complete), 155 of 5.8S, and 285 of ITS2 (complete). Among the 691 bp aligned nucleotides, 544 are constant, 55 are variable but parsimony-uninformative and 92 are parsimony-informative. All the collections of *L. vividus* have an ITS region of 650 bp long and no length variation (INDELs) exists. Within *L. vividus*, the ITS region has five variable sites, two of which are parsimony-informative. The aligned *gpd* dataset includes 37 samples and 765 characters, among which 538 are constant, 112 are variable but parsimony-uninformative and 115 are parsimony-informative. Within *L. vividus*, the *gpd* gene (754 bp long) has seven variable sites, five of which are parsimony-informative. The two matrices are available at TreeBASE under accession Nos. Submission ID 17823 (ITS) and Submission ID 17825 (*gpd*).

The ITS and *gpd* datasets produced different typologies regarding the relationships between the taxa of the ingroup, but no significant conflict was found when comparing the ML-BP of monophyletic and non-monophyletic relationships for the same set of taxa. To show the uncertain phylogenetic position of *L. vividus* in the two datasets, and the infraspecific variation within *L. vividus*, the ITS and *gpd* datasets were analyzed separately and the trees produced by the ML analyses of both datasets are shown (Fig. 2).

ML and BI analyses of the ITS dataset produced similar topologies which differ in that *L. salmoneus* is the most early diverging branch in the ML analysis whereas in the BI analyses *L. subindigo* is the most early diverging. The 21 samples of *L. vividus* formed a highly supported clade on a long branch with a ML-BP value of 99% and a BI-Posterior Probability (BI-PP) support of 1.00. The relationship between *L. vividus* and the other species was not resolved. ML and BI analyses of the *gpd* dataset produced identical topologies with comparable support values. The 14 samples of

L. vividus formed a highly supported clade with a long branch and a ML-BP value of 100% and BI-PP of 1.00. This clade included two subclades among which one received significant support of ML-BP = 89%. All species except for *L. subindigo* and *L. vividus* formed a highly supported clade with a ML-BP value of 94% and a BI-PP of 1.00. The monophyly of *L. sect. Deliciosi* was not statistically supported either in the analysis of the ITS dataset or that of the *gpd* dataset.

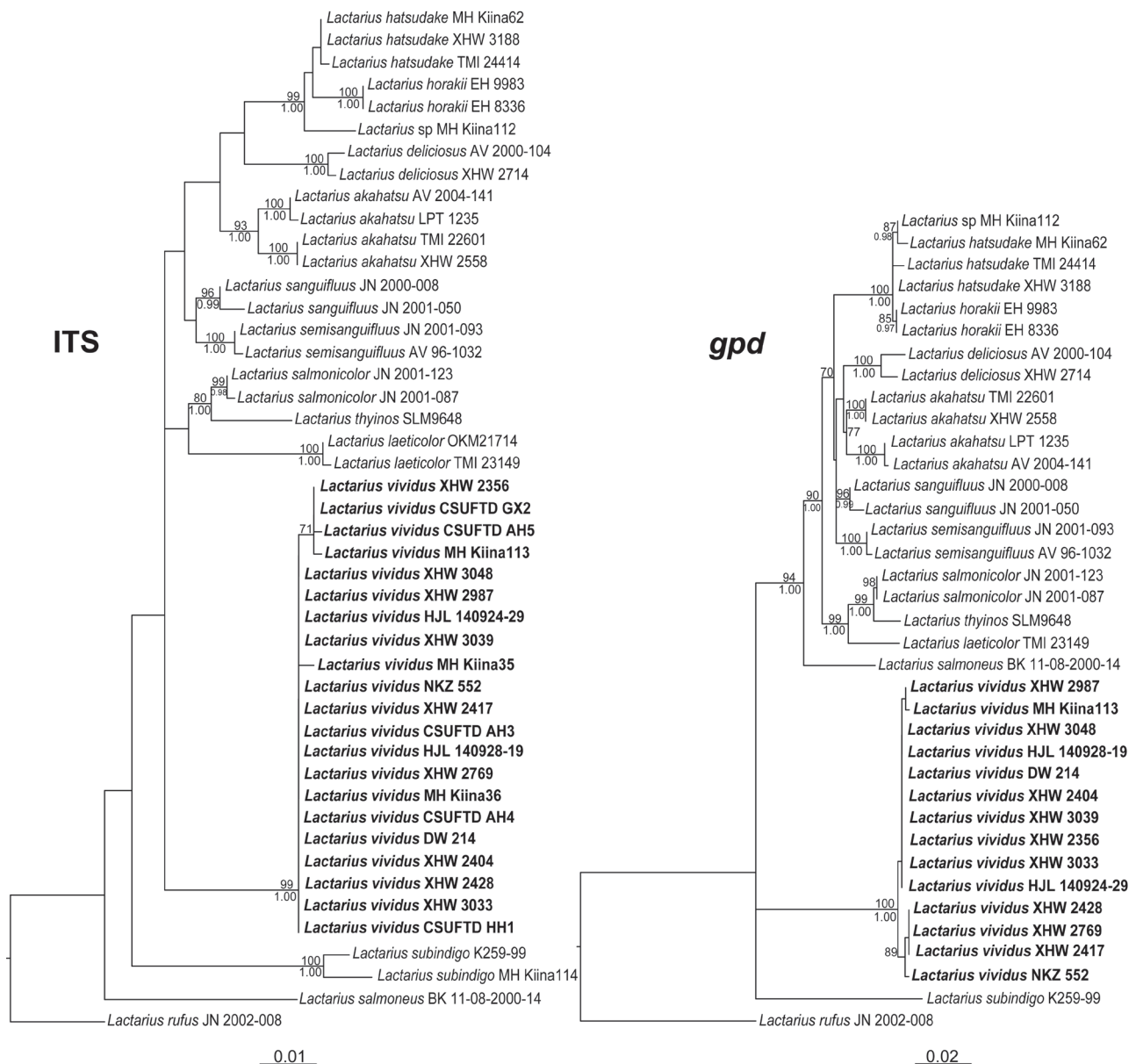


FIGURE 2. Maximum Likelihood (ML) phylogenetic trees of *Lactarius vividus* and its relatives based on the ITS region and the *gpd* gene, rooted with *L. rufus*. Bootstrap proportions higher than 70% in the ML analysis and Posterior probabilities higher than 95% in the Bayesian Inference analysis are indicated above and below the branches respectively. Initials of the sample numbers correspond to the collectors or herbaria in Table 1. Samples of *L. vividus* are in bold.

Taxonomy

Lactarius vividus X.H. Wang, Nuytinck & Verbeken, *sp. nov.* Fig. 3a–f, Fig. 4

Mycobank: MB 812841

Diagnosis:—*Lactarius vividus* differs from the other orange-colored species of *Lactarius* sect. *Deliciosi* by the subdistant to distant lamellae and heavy basidiospore ornamentation.

Holotypus:—CHINA. Hubei Province, Yingshan, Taohuachong Forest Park, N30°58'29.37" E116°02'00.68", elev. 960 m, under *Pinus taiwanensis*, 30 Aug. 2011, X.H. Wang 3039 (KUN-HKAS 735821).

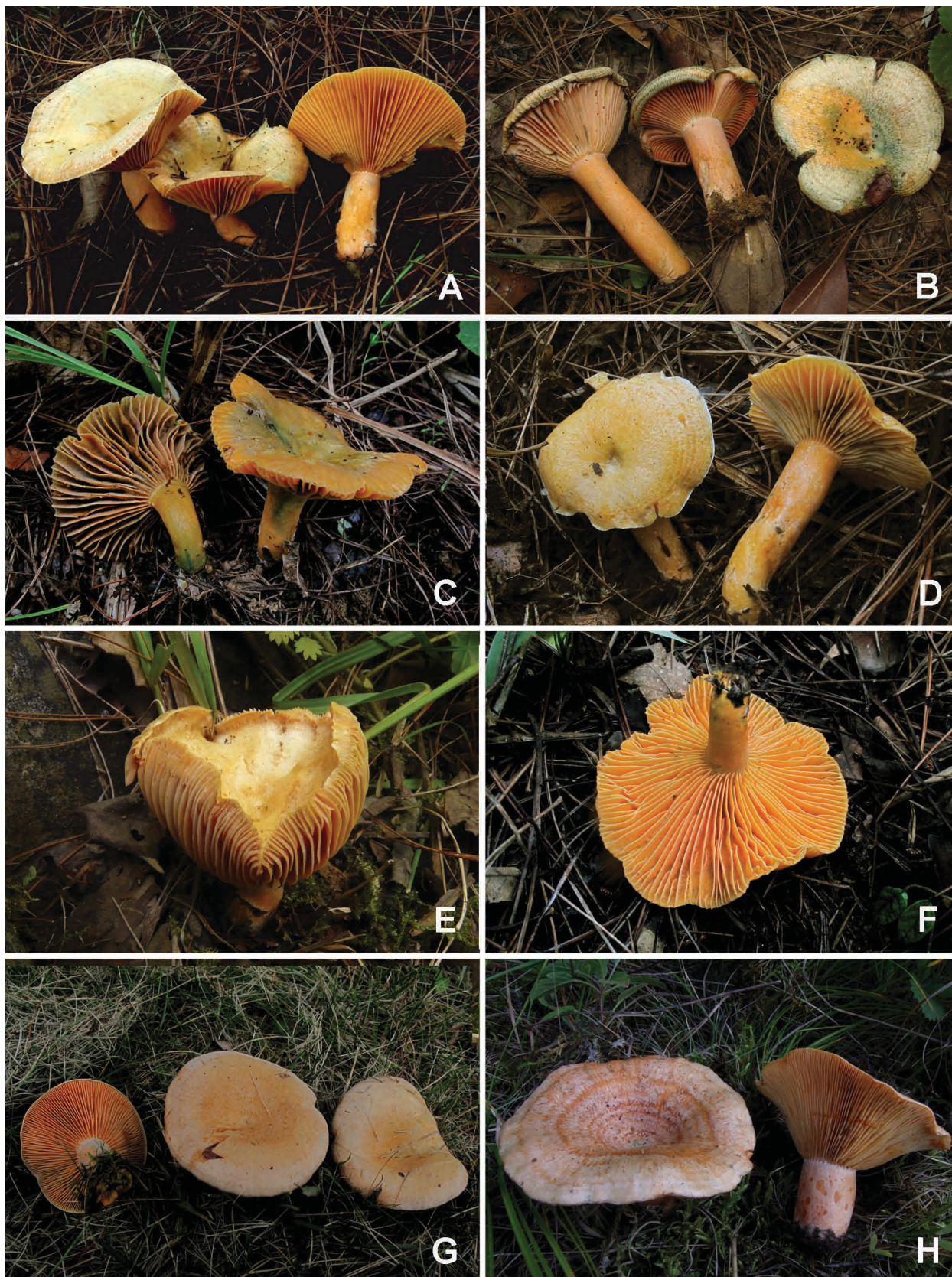


FIGURE 3. Basidiocarps of *Lactarius vividus*, *L. akahatsu* and *L. deliciosus*, showing the subdistant to distant lamellae in *L. vividus* in contrast with the crowded lamellae in the other two species. A: *L. vividus* (KUN-HKAS 73591, holotype); B: *L. vividus*, in dry habitat (KUN-HKAS 61742); C: *L. vividus*, old basidiocarps (KUN-HKAS 61725); D: *L. vividus*, undicoloring basidiocarps (KUN-HKAS 61965); E: *L. vividus*, undicoloring basidiocarp (KUN-HKAS 61325); F: *L. vividus*, with rich-colored subdistant lamellae and evenly colored stipe (KUN-HKAS 73576); G: *L. akahatsu*, with crowded lamellae (KUN-HKAS 61764); H: *L. deliciosus*, with crowded lamellae (KUN-HKAS 61910).

Etymology:—Named after the vividly colored lamellae.

Pileus 3–10 cm in diam., involute when young, soon plano-applanate with slightly depressed center or even reflexed; surface faintly zonate, with only 1/2–2/5 of the margin with clear or faint concentric zones or azonate, hygrophanous, subgreasy, light yellow (4A5–5A5), orange (5A6), greyish orange (5B5), golden yellow (5B7), brownish yellow (5C7), ochraceous yellow mixed with grayish-green tinge in the center and orange (6B6) at the margin when old, orange mixed with bluish-green tinge when dry, locally with dull greenish patches; margin sulcate when fully mature in some basidiocarps, occasionally locally lobate. *Pileus context* 2–8 mm thick, pale orange (5A3, 5A4) or paler than the pileus, not discoloring when bruised, with pale greenish tinge when old. *Lamellae* 2–8 mm wide, thick, subcrowded initially, becoming subdistant to distant with age, adnate to shortly decurrent, light orange (5A4, 5A5), golden yellow (5B7), orange (6A6, 6A7, 6A6–6B6), unchanging or discoloring dull green (28E3) to green when old or bruised. *Stipe* 3–4.5 × 0.8–2 cm, equal or slightly tapering downwards; surface subhygrophanous, rarely scrobiculate, light orange (6A5, 5A5–6A5) to golden yellow (5B7), almost concolorous with the lamellae, locally paler, with a narrow whitish or paler zone at the apex, rarely with a concolorous apex, discoloring greenish where bruised; base with short, strigose, pale orange or pale greenish hairs. *Latex* scant, orange (5A6) to golden yellow (5B7), slowly discoloring dull blood-red in 2–10 minutes or when dry. *Spore print* pale ochraceous.

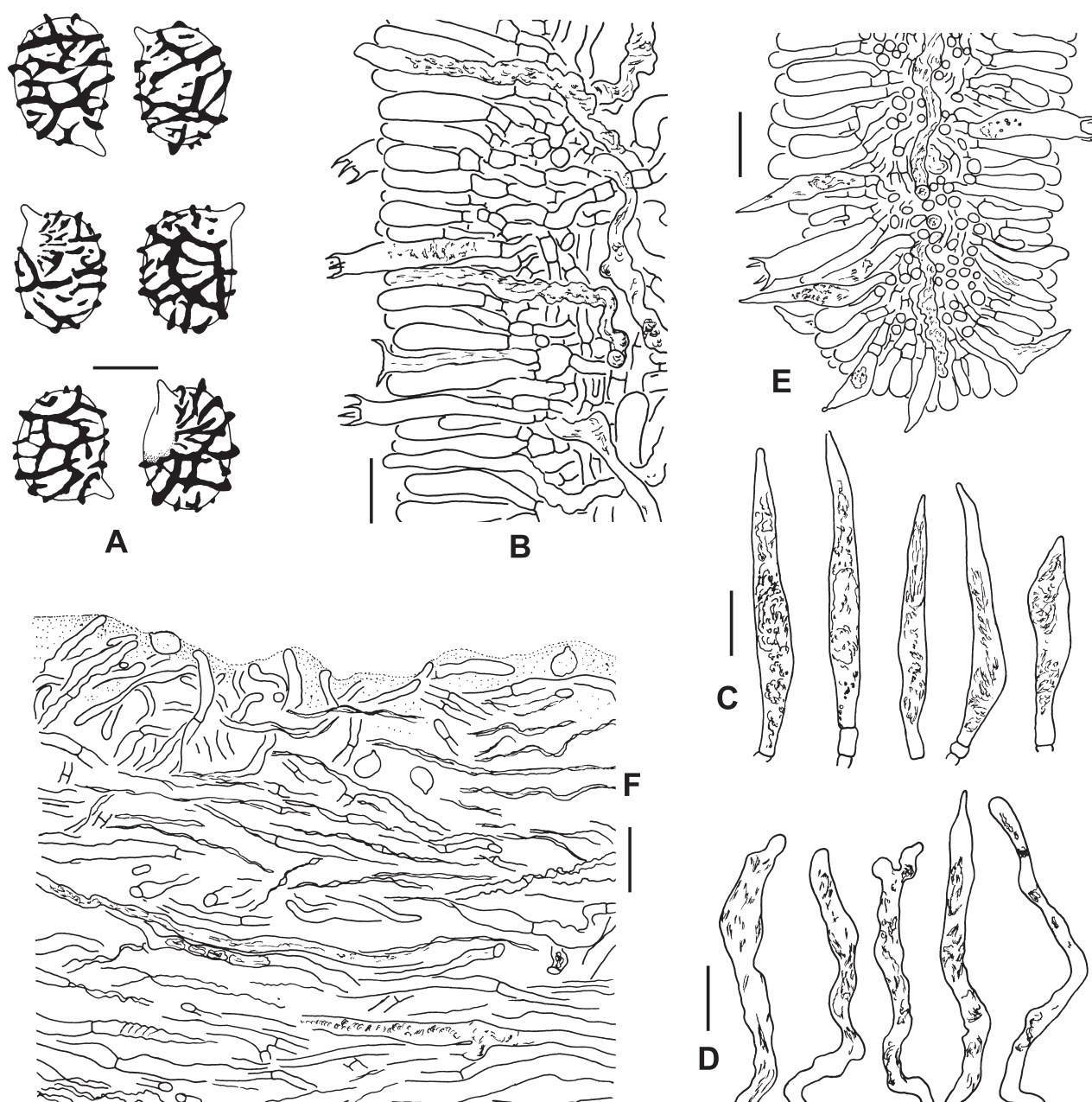


FIGURE 4. *Lactarius vividus* (all from holotype except for C from KUN-HKAS 73591). A: basidiospores, B: hymenium, C: pleuromacrocystidia, D: pleuropseudocystidia; E: lamellar edge, F: pileipellis. (A: scale bar = 5 µm, B–F: scale bars = 20 µm).

Basidiospores (5.5–) 7.0–10.0 (–11.0) × (5.0–) 5.5–7.0 (7.5) µm [$Q = (1.15–) 1.22–1.46 (–1.55)$, $Q = 1.34 \pm 0.07$] (320/16/11), ellipsoid; ornamentation up to 1.3 µm high, mostly 0.5–1.0 µm high, composed of ridges and irregular warts forming a broken to almost complete reticulum; plage not amyloid or slightly distally amyloid. *Basidia* 35–65 × 8–11 µm, subclavate to subcylindrical, 4-spored; sterigmata 5–8 µm long. *Pleuromacrocystidia* very rare to absent in some collections, rarely common, 35–100 × 7–10 µm, embedded in hymenium or projecting to 30 µm beyond hymenium, subfusiform, sublanceolate, sometimes with a moniliform apex, with a dense granular or massy content. *Pseudocystidia* abundant, (3–) 5–10 (–12) µm diam., emergent and broadened at apex, sometimes narrowing again at the very end, subcylindrical, rarely forking or branching, with a dense golden-brown to ochre-brown content. *Lamellar edge* sterile. *Cheilomacrocystidia* scarce to common, absent in some collections, 20–50 × 3–9 (–10) µm, subcylindrical, subfusiform or sublanceolate, sometimes with a moniliform apex, with a needle-like or granular content. *Hymenophoral trama* with sphaerocytes and abundant lactifers, lacking rosettes. *Pileipellis* an ixocutis, 40–150 µm thick, of compact thin-walled hyphae 2–6 µm diam., cylindrical with a round apex, strongly shrivelled and gelatinized, thin-walled, hyaline, parallel with the pileal surface, locally interwoven into an atypical ixolattice; slime layer very thin. *Stipitipellis* a cutis, rarely an ixocutis, very thin, of slightly gelatinized and shrivelled hyphae 2–5 µm diam. *Trama of pileus and stipe* with abundant golden-brown to ochre-brown lactifers and rosettes. *Clamp connections* absent.

Specimens Examined: CHINA. Anhui, Qianshan, Tianzhu Mt., near Foguang Temple, elev. 630 m, N30°43'17.73" E116°27'18.31", under *Pinus taiwanensis*, 29 August 2011, X.H. Wang 3033 (KUN-HKAS 73576); Chongqing, Nanchuan, Sanquan, Jinpo Mt., elev. 750 m, N29°07'19.20" E107°11'59.45", under *P. massoniana*, 29 July 2010, X.H. Wang 2428 (KUN-HKAS 61742); Guangdong, Lechang, Jiufeng, under *P. massoniana*, 04 October 2010, D. Wang 214 (KUN-HKAS 86667); Guizhou, Suiyang, Qinggangtang, elev. 768 m, N28°19'24.11" E107°09'06.69", under *P. massoniana*, 25 July 2010, X.H. Wang 2404 (KUN-HKAS 61725); Guizhou, Daozhen, Yangxi, elev. 1200 m, under *P. massoniana*, 28 July 2010, X.H. Wang 2417 (KUN-HKAS 61734); Guizhou, Meitan, Xinnan, Fenghuang village, elev. 1170m, N27°32'34.48" E107°21'03.85", under *P. massoniana*, 28 September 2014, H.J. Li 140928-19 (KUN-HKAS 88181); Guizhou, Wuchuan, Nigao, elev. 1070 m, under *P. massoniana*, 24 September 2014, H.J. Li 140924-29 (KUN-HKAS 88182); Hainan, Changjiang, Bawangling, under *P. latteri*, 22 August 2009, N.K. Zeng 552 (KUN-HKAS 60688); Hubei, Yingshan, Taohuachong Forest Park, elev. 960 m, N30°58'29.37" E116°02'00.68", 30 August 2011, X.H. Wang 3039 (holotype, KUN-HKAS 73582); Hunan, Zhangjiajie, Sanjiaguan, Yanshuijing, elev. 650 m, N29°11'44.52" E110°17'17.12", under *P. massoniana*, 15 June 2010, X.H. Wang 2356 (KUN-HKAS 61325); Jiangxi, Jiujiang, Lushan Botanical Garden, elev. 1110 m, N29°33'00.62" E115°58'43.34", under *P. taiwanensis*, 31 August 2011, X.H. Wang 3048 (KUN-HKAS 73591); Shandong, Qingdao, Lao Mt., Beijiushui, elev. 400 m, N36°12'18.72" E120°36'15.69", under *P. thunbergii*, 18 August 2011, X.H. Wang 2987 (KUN-HKAS 73543); Sichuan, Emei, Hongzhushan, elev. 550 m, N29°33'42.85" E103°26'37.05", under *P. massoniana*, 10 September 2010, X.H. Wang 2769 (KUN-HKAS 61965).

Discussion

Observations on the macro- and microscopical characters of the specimens of *L. vividus* cited above demonstrate that this species is extremely variable in morphology. The pileus varies from clearly zonate to azonate; the stipe, although mostly lacking scrobicules, does have scattered scrobicules in some basidiocarps; greenish discoloration is mostly lacking in some collections but quite visible and intense in others; the basidiospores of different collections differ in size, shape and pattern of ornamentations; the abundance of pleuromacrocystidia is a continuous series from common (KUN-HKAS 60688, 73543, 73591) to scarce (KUN-HKAS 61742), to absent (KUN-HKAS 61325, 61725, 73582, 86667); this continuous series even exists between different basidiocarps of the same collection (KUN-HKAS 61965). The high morphological variability makes it hard to clearly delimit *L. vividus* from the other orange-colored species in *L. sect. Deliciosi*. Nevertheless the invariably vividly colored, relatively thick and subdistant to distant lamellae, combined with the heavy basidiospore ornamentation can be used to recognize this new species (Fig. 3). These characters, as well as the host specificity to *Pinus* subg. *Pinus*, can also be used to distinguish *L. vividus* from *L. salmonicolor* with which *L. vividus* has been misidentified in China (Chen *et al.* 2013).

The collections of *L. vividus* formed an early diverging clade in the phylogenetic trees of ITS and *gpd* of *L. sect. Deliciosi* (Nuytinck *et al.* 2007). The limited sampling in this study does not allow us to retrieve the phylogenetic position of *L. vividus* in *sect. Deliciosi* and the monophyly of *L. sect. Deliciosi* was not supported in the analyses of the two loci. The morphology of *L. vividus*, however, is so close to that of the species of *L. sect. Deliciosi* that we are

convinced that it is a member of that section. Although ITS and *gpd* do not contain enough phylogenetic information to reveal the exact evolutionary relationships between *L. vividus* and its lookalikes, they do support *L. vividus* as a distinct species. It can be seen that the branches leading to *L. vividus* in both ITS region and *gpd* gene are quite long, suggesting a significant genetic divergence from the other species. In contrast with the large interspecific distance, the genetic variation within the species is very low, although the samples are from a quite wide geographical origin, covering temperate, subtropical, and tropical zones (Fig. 1).

In central and southern China (e.g. Hunan and Yunnan Provinces), species of *L. sect. Deliciosi* are commonly collected, consumed, and commercialized, with various local names including “Songjun” (pine mushroom) and “Gushoujun” (mushroom fruiting when corn plants are ripe) (Wang 2000; Wang *et al.* 2003; Guo *et al.* 2011b; Li *et al.* 2007). Due to the high morphological similarity between species in sect. *Deliciosi*, debates on the species identifications prevailed for many years (Wang 2000). For example Wang *et al.* (2003) lumped all the species under the local name “Songjun” into *L. hatsudake*, whereas Guo *et al.* (2011b) discovered three additional species under this local name, i.e. *L. akahatsu*, *L. deliciosus*, and *Lactarius* sp. (*L. vividus*). According to the investigations of the first author (data unpublished) and ITS sequences published by other Chinese researchers (Guo *et al.* 2011a, 2011b; Li *et al.* 2012; Chen *et al.* 2013), the geographical distributions of *L. akahatsu*, *L. deliciosus* and *L. vividus* overlap in central and southern China. We believe that collections of *L. vividus* mostly passed under the names *L. deliciosus* and *L. akahatsu* in the Chinese literature. Although in theory *L. vividus* can be distinguished from *L. deliciosus* and *L. akahatsu* by the relatively thick, subdistant to distant lamellae, in practice it stays difficult to separate the orange-colored species of *L. sect. Deliciosi* solely based on morphology. For instance, the Thai samples of *L. akahatsu* with distant lamellae described by Nuytinck (2005) may complicate the morphological separation between *L. vividus* and *L. akahatsu*. In contrast with the morphological ambiguity, the low infraspecific diversity of ITS and *gpd* sequences in *L. vividus* and the clear genetic gap between *L. vividus* and its lookalikes allow easy delimitation of *L. vividus*. We conclude that the two genes are powerful markers to identify *L. vividus* correctly.

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