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Lactarius subgenus *Russularia* (Russulaceae) in South-East Asia: 3. new diversity in Thailand and Vietnam

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Abstract

Lactarius subgenus *Russularia* is a dominant group of milkcaps in Southeast Asia. This paper reveals the large diversity of the subgenus, with eight new species and one known species being described from montane evergreen and coniferous forests. All new species are supported by both morphological and molecular data, the latter using Maximum likelihood and Bayesian analysis based on the ITS region. Complete macro- and micro-morphological descriptions and illustrations are given. A key to the new taxa is provided. *Lactarius chichuensis* is reported for the first time from Thailand.

Keywords: Russulales, identification, ectomycorrhizal fungi, biodiversity

Introduction

Tropical Southeast Asia is recognized as one of the world's biodiversity hotspots and contains a high concentration of endemic species (Myers *et al*. 2000). The high number of endemic plants includes several families of ectomycorrhizal trees (e.g. Dipterocarpaceae, Fagaceae, Betulaceae, and Pinaceae). However, biodiversity is strongly declining in Southeast Asia due to deforestation for urbanization, logging, and agricultural expansion by local people and agricultural companies, who clear vast areas for crop cultivation (Sodhi *et al*. 2010). Since ectomycorrhizal (ECM) fungi are obligate symbionts of ECM trees and shrubs, an inevitable consequence of forest logging is the loss of ECM species, including those belonging to the genus *Lactarius* Pers. Issues regarding forest logging have therefore become of great concern in many countries (Mortimer *et al*. 2012).

Mycorrhizal trees have been used for reforestation programs in many regions such as tropical Africa, South America and Southeast Asia. Thus many attempts used mycorrhizal symbionts to improve the reforestation performance of transplanted mycorrhizal trees (Bâ *et al*. 2009, Ergiles *et al*. 2009, Sanon *et al*. 2010, Aggangan *et al*. 2012). Since ECM fungi facilitate water and nutrient uptake for their host plants, they are considered as microorganisms that can promote plant growth in forests. There is little information regarding the use of *Lactarius* species in tree seedling production. *Lactarius deliciosus* (L.: Fr.) Gray appears to be the most used *Lactarius* species applied to improve seedling establishment of *Pinus* trees (Guerin-Laguette *et al*. 2003, Parladé *et al*. 2004, Diaz *et al*. 2009). Our research aims at exploring the biodiversity of *Lactarius* subgenus *Russularia* (Fr.) Kauffman in Southeast Asia, as in local ecosystems it is one of the dominant groups in terms of numbers of basidiocarps covering the forest floor.

Lactarius subgenus *Russularia* is one of the three major subgenera of *Lactarius*. Species traditionally placed in this subgenus are recognized by basidiomata which are typically dry and vary in color from orange to warm brown (Heilmann-Clausen *et al*. 1998). As mentioned in Wisitrassameewong *et al*. (2014b), it is remarkable that most taxa in this group have unchanging latex and that the group as a whole is characterized by few color changes in the latex as compared to the other subgenera of *Lactarius* and *Lactifluus* (which together form the large group of milkcaps). Some species are described as having white to pale yellow latex e.g. *L. quietus* (Fr.: Fr.) Fr., and *L. decipiens* Quél.

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Lactarius tabidus Fr. has white latex which slowly turns straw yellow, while *L. duplicatus* A.H. Sm. has white latex that becomes sulphur yellow (Heilmann-Clausen *et al*. 1998). In the Northern hemisphere, this group is well-studied in Europe (Heilmann-Clausen *et al*. 1998, Basso 1999), North America (Hesler & Smith 1979) and Japan (Hongo 1957a, b, 1971).

Many mycological excursions have focused on milkcap diversity (both *Lactarius* and *Lactifluus*) in Southeast Asia in the last decade (Le *et al*. 2007a, 2007b, 2007c, Stubbe *et al*., 2007, 2008, Van de Putte *et al*. 2010, Verbeken *et al*. 2014, Wisitrassameewong *et al*. 2014a, 2014b). Compared to the exploration rate between *L.* subg. *Russularia* and other groups of milkcaps, the exploration rate in *Russularia*-group is quite low. So far about 21 known species belonging to *L.* subg. *Russularia* have been reported, including also eleven species from Papua New Guinea and Indonesia for which we are still missing molecular data. We need a comprehensive contribution to show the diversity of *L.* subg. *Russularia* for this subcontinent. This paper contributes to the knowledge of the biodiversity of the subgenus in Thailand and Vietnam. Only one significant contribution to milkcap diversity in Vietnam is given by Morozova *et al*. (2013) but that was on two new species of *Lactifluus*, therefore this will be the first report of *Lactarius* subgenus *Russularia* for the country. We collected specimens mainly from montane evergreen forests dominated by Fagaceae and coniferous forests dominated by *Pinus kesiya* Royle ex Gordon from Northern Thailand and Southern Vietnam. The aim was to use both morphological and molecular tools to reveal the diversity of this ectomycorrhizal group in order to stress their importance when it comes to conservation of the whole ecosystem.

Materials and methods

Taxon sampling

Fresh basidiomata were gathered in montane rainforests and planted coniferous forests in Northern Thailand and the regions around Dalat in Vietnam by the first author. Some collections were made by Huyen Thanh Le and Eske De Crop. The studied materials are deposited in the herbarium Universitatis Gandavensis (GENT), Belgium; Mae Fah Luang University herbarium (MFLU) and Chiang Mai University (CMU), Thailand; and/or San Francisco State University (SFSU), USA.

Morphological study

Macromorphological characters were observed on fresh material. Specimens were described and photographed in fresh condition during daylight hours. Color coding is according to Kornerup and Wanscher (1978). Macro-morphological features of the basidiocarp were documented in term of size, shape and features of pileus, lamellae and stipe. Latex features were tested by recording color when the latex was immediately exposed to the air, color change after exposing to the air for a certain time, color change when a drop of latex tested with 10% KOH and color change when a drop of latex touched on white tissue paper and a white cotton handkerchief. For morphological terminology, we refer to Vellinga (1988) and to Verbeken (1996) and Heilmann-Clausen *et al*. (1998) particularly for pileipellis structures. Microscopic features were studied from dried material, mainly in Congo Red in L4 (Clémençon 1973). Basidiospores were observed in Melzer's reagent. Basidia were measured excluding sterigmata length. The spore measurement included at least 20 spores from each collection, and excludes the ornamentation. Basidiospore measurements are represented as {(MIN) [AVa-2×SD]−*AVa–AVb*−–[AVb+2×SD] (MAX)} length × {(MIN) [AVa-2×SD]–*AVa–AVb*−[AV+2×SD] (MAX)} width, in which MIN = the minimum value, MAX = the maximum value, AVa = lowest mean value for the measured collection, AVb = highest mean value for the measured collection and SD = standard deviation. Q corresponds to spore "length/width ratio" and is given as (MINQa) Qa–Qb (MAXQb), where Qa and Qb are the lowest and the highest mean ratio for a measured specimen, respectively. All line drawings were made by Komsit Wisitrassameewong (KW).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted from fresh material stored in 2×CTAB buffer using the protocol described by Nuytinck & Verbeken (2003) with the modifications described in Van de Putte *et al*. (2010). The internal transcribed spacer of the nuclear ribosomal DNA (ITS) was amplified and sequenced using the ITS1-F and ITS4 primers (White *et al*. 1990, Gardes & Bruns 1993). Sequencing was conducted with an ABI 3730XL or ABI 3700 by MACROGEN (Amsterdam,

the Netherlands). Obtained sequences were assembled and edited with the software Sequencer™ v5.0 (Gene Code corporation, Ann Arbor, Michigan, U.S.A.).

Nucleotide alignment and phylogenetic reconstruction

This study comprises the DNA sequence data of most European and Southeast Asian representatives of *L.* subg. *Russularia*, most of which were generated from GENT. Additional sequences of European species were obtained from GenBank and UNITE. We included all European representatives except *L. duplicatus* A.H. Sm. The sequences of North American taxa, *L. rubidus* (Hesler & A.H. Sm.) Methven, *L. subserifluus* Longyear and *L. strigosipes* Montoya & Bandala were retrieved from GenBank. Representatives belonging to the other subgenera of *Lactarius* were included in the analysis. Three species of *L.* subgenus *Plinthogalus* (Berk.) Hesler & A.H. Sm., *L. pterosporus* Romagn., *L. fuliginosus* Romagn. and *L. friabilis* H.T. Le & Stubbe, were used as the outgroup in the phylogeny. Table 1 shows an overview of all sequences used in the phylogenetic analysis. Nucleotide sequence alignment was made using MAFFT v7 (Katoh & Standley 2013) and later manually edited in MEGA6 (Tamura *et al*. 2013). The program Gblocks v0.91b (Castresana 2000) was used to eliminate poorly aligned positions in the alignment, with settings allowing gaps within selected blocks, smaller blocks (minimum 5 bp) and bigger segments with contiguous non-conserved positions (maximum 10 bp). The Alignment Transformation Environment (ALTER) was used to convert sequence alignment formats (Glez-Peña *et al*. 2010). RAxML v7.0.3 (Stamatakis 2006) was used to infer the maximum likelihood (ML) topology, applying the Rapid Bootstrapping algorithm for 1000 replicates using the GTRGAMMA model. To determine the model of character evolution of each gene partition, we used MrModeltest v2.3 (Nylander 2004) and the suggested parameters were applied for Bayesian inference (BI) analysis. Four separate runs in parallel with 10 million Markov chain Monte Carlo (MCMC) generations were executed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Sample frequency was set at 100. To recognize that the number of generations was sufficient, we looked for a stationary likelihood graph and the effective sample size (ESS) value (should exceed 200) from independent runs using the graphing function in Tracer v1.6 (Drummond & Rambaut 2007). The proper burn-in value for the dataset was observed using this program. All phylograms were displayed using FigTree v1.3.1 (Rambaut 2009).

TABLE 1: List of specimens and GenBank accession number of sequences used in the phylogenetic analyses.

Species	Voucher collection	Origin	ITS accession no.	
Lactarius subg. Russularia				***Lactarius subg. Russularia***
L. rubrobrunneus sp. nov.	AV12-044 (GENT, MFLU) (Type)	Thailand	KF432985	*L. rubrobrunneus sp. nov.*
L. rubrobrunneus sp. nov.	LTH334 (GENT, CMU, SFSU)	Thailand	KR025598	*L. rubrobrunneus sp. nov.*
L. rubrobrunneus sp. nov.	LTH149 (GENT, CMU, SFSU)	Thailand	KR025599	*L. rubrobrunneus sp. nov.*
L. fuscomaculatus sp. nov.	KW111 (GENT, MFLU)	Thailand	KF433021	*L. fuscomaculatus sp. nov.*
L. fuscomaculatus sp. nov.	KW112 (GENT, MFLU)	Thailand	KR025602	*L. fuscomaculatus sp. nov.*
L. fuscomaculatus sp. nov.	KW128 (GENT, MFLU)	Thailand	KR025601	*L. fuscomaculatus sp. nov.*
L. fuscomaculatus sp. nov.	KW370 (GENT, MFLU)	Thailand	KR025600	*L. fuscomaculatus sp. nov.*
L. fuscomaculatus sp. nov.	KW373 (GENT, MFLU) (Type)	Thailand	KR025603	*L. fuscomaculatus sp. nov.*
L. austrorostratus sp. nov.	KW108 (GENT, MFLU) (Type)	Thailand	KF433012	*L. austrorostratus sp. nov.*
L. austrorostratus sp. nov.	KW109 (GENT, MFLU)	Thailand	KF433013	*L. austrorostratus sp. nov.*
L. austrorostratus sp. nov.	KW110 (GENT, MFLU)	Thailand	KF433014	*L. austrorostratus sp. nov.*
L. rubrocorrugatus sp. nov.	KW453 (GENT, MFLU)	Thailand	KR025592	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	KW042 (GENT, MFLU)	Vietnam	KF433010	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	KW043 (GENT, MFLU)	Vietnam	KR025589	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	KW045 (GENT, MFLU)	Vietnam	KR025591	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	KW123 (GENT, MFLU)	Thailand	KR025588	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	KW294 (GENT, MFLU)	Thailand	KF433011	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	KW384 (GENT, MFLU) (Type)	Thailand	KR025590	*L. rubrocorrugatus sp. nov.*
L. rubrocorrugatus sp. nov.	EDC14-505 (GENT, MFLU)	Thailand	KR025587	*L. rubrocorrugatus sp. nov.*
L. aquosus sp. nov.	LTH262 (GENT, CMU, SFSU)	Thailand	KR025622	*L. aquosus sp. nov.*
L. aquosus sp. nov.	KW231 (GENT, MFLU) (Type)	Thailand	KF432984	*L. aquosus sp. nov.*
L. tangerinus sp. nov.	KW091 (GENT, MFLU)	Thailand	KR025626	*L. tangerinus sp. nov.*
L. tangerinus sp. nov.	LTH203 (GENT, CMU, SFSU) (Type)	Thailand	KR025627	*L. tangerinus sp. nov.*
L. tangerinus sp. nov.	EDC14-475 (GENT)	Thailand	KR025625	*L. tangerinus sp. nov.*

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TABLE 1. (Continued)

Species	Voucher collection	Origin	ITS accession no.	
L. inconspicuus sp. nov.	KW339 (GENT, MFLU) (Type)	Thailand	KR025584	*L. inconspicuus sp. nov.*
L. inconspicuus sp. nov.	KW100 (GENT, MFLU)	Thailand	KF433001	*L. inconspicuus sp. nov.*
L. inconspicuus sp. nov.	KW003 (GENT, MFLU)	Thailand	KR025583	*L. inconspicuus sp. nov.*
L. inconspicuus sp. nov.	LTH256 (GENT, CMU, SFSU)	Thailand	KF433003	*L. inconspicuus sp. nov.*
L. inconspicuus sp. nov.	LTH098 (GENT, CMU, SFSU)	Thailand	KF433004	*L. inconspicuus sp. nov.*
L. kesiyae sp. nov.	KW034 (GENT, MFLU)	Vietnam	KF432995	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	AV12-022 (GENT, MFLU)	Thailand	KR025618	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW427 (GENT, MFLU)	Thailand	KR025614	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW346 (GENT, MFLU)	Thailand	KF432993	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW033 (GENT, MFLU)	Vietnam	KF432994	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW219 (GENT, MFLU)	Thailand	KR025616	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW210 (GENT, MFLU)	Thailand	KR025617	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW036 (GENT, MFLU)	Vietnam	KR025620	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW224 (GENT, MFLU)	Thailand	KR025615	*L. kesiyae sp. nov.*
L. kesiyae sp. nov.	KW032 (GENT, MFLU) (Type)	Vietnam	KR025619	*L. kesiyae sp. nov.*
L. sublaccarioides	KW300 (GENT, MFLU) (Type)	Thailand	KF432996	*L. sublaccarioides*
L. sublaccarioides	KW323 (GENT, MFLU)	Thailand	KF432997	*L. sublaccarioides*
L. sublaccarioides	KW332 (GENT, MFLU)	Thailand	KF432998	*L. sublaccarioides*
L. camphoratus	AV10-40 (GENT)	Norway	KF432971	*L. camphoratus*
L. camphoratus	JV2006-20 (GENT)	Belgium	KR025610	*L. camphoratus*
L. subumbonatus	JKLAC110902 (GENT)	Germany	KR025596	*L. subumbonatus*
L. subumbonatus	RC-KVP10-002 (GENT)	Belgium	KF432981	*L. subumbonatus*
L. subumbonatus	EDC11-237 (GENT)	Belgium	KR025595	*L. subumbonatus*
L. serifluus	JV2006-028 (GENT)	Belgium	KR025597	*L. serifluus*
L. crenulatulus	KW125 (GENT, MFLU)	Thailand	KR025605	*L. crenulatulus*
L. crenulatulus	KW368 (GENT, MFLU)	Thailand	KJ458979	*L. crenulatulus*
L. crenulatulus	KW383 (GENT, MFLU) (Type)	Thailand	KR025604	*L. crenulatulus*
L. pasohensis	KW355 (GENT, MFLU)	Thailand	KF432988	*L. pasohensis*
L. pasohensis	DS06-231 (GENT, KEP)	Malaysia	KF432987	*L. pasohensis*
L. pasohensis	DS06-245 (GENT, KEP) (Type)	Malaysia	KF432986	*L. pasohensis*
L. perparvus	KW320 (GENT, MFLU) (Type)	Thailand	KJ458981	*L. perparvus*
L. perparvus	KW337 (GENT, MFLU)	Thailand	KJ458982	*L. perparvus*
L. glabrigracilis	KW093 (GENT, MFLU) (Type)	Thailand	KR025606	*L. glabrigracilis*
L. glabrigracilis	KW335 (GENT, MFLU)	Thailand	KJ458985	*L. glabrigracilis*
L. glabrigraclis	KW321 (GENT, MFLU)	Thailand	KR025607	*L. glabrigraclis*
L. rubidus	M.Kuo 01131106 (NY)	USA	KC691205	*L. rubidus*
L. laccarioides	KW336 (GENT, MFLU) (Type)	Thailand	KF432991	*L. laccarioides*
L. laccarioides	KW360 (GENT, MFLU)	Thailand	KF432992	*L. laccarioides*
L. atlanticus	LAC11121201 (GENT)	Spain	KF432976	*L. atlanticus*
L. atlanticus	JKLAC13122801 (GENT)	Portugal	KR025611	*L. atlanticus*
L. atlanticus	AV13-047 (GENT)	Italy	KR025612	*L. atlanticus*
L. subserifluus	JMP0046	USA	EU819486	*L. subserifluus*
L. strigosipes		Mexico	JN003629	*L. strigosipes*
L. gracilis	KW096 (GENT, MFLU)	Thailand	KR025609	*L. gracilis*
L. gracilis	KW354 (GENT, MFLU)	Thailand	KR025608	*L. gracilis*
L. gracilis	KW334 (GENT, MFLU)	Thailand	KF433017	*L. gracilis*
L. hirtipes	XHW1243 (HKAS)	China	KF433007	*L. hirtipes*
L. chichuensis	XHW1236 (HKAS)	China	KF475766	*L. chichuensis*
L. chichuensis	KW271 (GENT, MFLU)	Thailand	KR025593	*L. chichuensis*
L. chichuensis	KW012 (GENT, MFLU)	Thailand	KF433008	*L. chichuensis*
L. chichuensis	KW359 (GENT, MFLU)	Thailand	KF433009	*L. chichuensis*
L. chichuensis	KW421 (GENT, MFLU)	Thailand	KR025594	*L. chichuensis*
L. rostratus	691(MUVE)	Italy	JF908276	*L. rostratus*
L. falcatus	KVP08-038 (GENT) (Type)	Thailand	KF133262	*L. falcatus*
L. quietus	JN2012-040 (GENT)	Germany	KR025623	*L. quietus*
L. quietus	KW131 (GENT)	Belgium	KF432972	*L. quietus*
L. quietus	KW133 (GENT)	Belgium	KR025624	*L. quietus*

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TABLE 1. (Continued)

Species	Voucher collection	Origin	ITS accession no.	
L. omphaliformis	PAM08083009	France	HQ714719	*L. omphaliformis*
L. lacunarum	JKLAC11092901 (GENT)	Germany	KF432982	*L. lacunarum*
L. lacunarum	EDC11-231 (GENT)	Belgium	KR025570	*L. lacunarum*
L. lacunarum	JKLAC13122201 (GENT)	Portugal	KR025569	*L. lacunarum*
L. tabidus	KW130 (GENT)	Belgium	KR025582	*L. tabidus*
L. tabidus	IMN98142	France	KR025581	*L. tabidus*
L. aurantiacus	JN11-089 (GENT)	Greece	KR025580	*L. aurantiacus*
L. aurantiacus	JN2001-60 (GENT)	Slovakia	KF432974	*L. aurantiacus*
L. lanceolatus	IA-F20	Norway	UDB002454	*L. lanceolatus*
L. brunneohepaticus	PAM08090315	France	HQ714726	*L. brunneohepaticus*
L. obscuratus	ED2008-15 (GENT)	USA	KR025579	*L. obscuratus*
L. obscuratus	LVL02-006 (GENT)	Belgium	KF432978	*L. obscuratus*
L. cyathuliformis	UE04.09.2004-2 (UPS)	Sweden	KF133266	*L. cyathuliformis*
L. hepaticus	JN02-049 (GENT)	Belgium	KF432980	*L. hepaticus*
L. hepaticus	JV2006-025(GENT)	Belgium	KR025574	*L. hepaticus*
L. hepaticus	JV2006-021 (GENT)	Belgium	KR025573	*L. hepaticus*
L. hispanicus	MA-Fungi 53339	Spain	AJ555567	*L. hispanicus*
L. fulvissimus	JN2012-025 (GENT)	Germany	KR025576	*L. fulvissimus*
L. fulvissimus	JV2006-006 (GENT)	Belgium	KR025577	*L. fulvissimus*
L. borziana		Switzerland	AF373599	*L. borziana*
L. rubrocinctus	EDC12-210 (GENT)	Germany	KF432977	*L. rubrocinctus*
L. rubrocinctus	JKLAC10082201 (GENT)	Germany	KR025575	*L. rubrocinctus*
L. sphagneti	JKLAC11091502 (GENT)	Germany	KF432975	*L. sphagneti*
L. badiosanguineus	AV04-235 (GENT)	France	KF432983	*L. badiosanguineus*
L. badiosanguineus	AV10-44 (GENT)	Norway	KR025578	*L. badiosanguineus*
L. decipiens	VDKO882 (GENT)	Belgium	KR025586	*L. decipiens*
L. decipiens	AV2000-137 (GENT)	Italy	KF432973	*L. decipiens*
L. decipiens	AV13-044 (GENT)	Italy	KR025585	*L. decipiens*
L. subdulcis	ED2008-27 (GENT)	Belgium	KR025572	*L. subdulcis*
L.subdulcis	JN2012-020 (GENT)	Germany	KR025571	*L.subdulcis*
L. subg. Lactarius				***L. subg. Lactarius***
L. austrozanarius	FH12-007 (GENT, MFLU)	Thailand	KF432965	*L. austrozanarius*
L. purpureus	FH12-008 (GENT, MFLU)	Thailand	KF432966	*L. purpureus*
L. scrobiculatus	JN01-058 (GENT)	Slovakia	KF432968	*L. scrobiculatus*
L. hatsudake	JN2011-065 (GENT)	Vietnam	KF432967	*L. hatsudake*
L. tornimosus	JN11-086 (GENT)	Greece	KR025613	*L. tornimosus*
L. subg. Plinthogalus				***L. subg. Plinthogalus***
L. fuliginosus	MTB97-24 (GENT)	Sweden	JQ446111	*L. fuliginosus*
L. pterosporus	DS09-614 (GENT)	Italy	KR025628	*L. pterosporus*
L. friabilis	FH12-103 (GENT, MFLU)	Thailand	KF432961	*L. friabilis*

Results

Phylogeny

The ITS multiple sequence alignment consists of 119 sequences and 1099 bases (including gaps). Gblocks retained 76% of the original sequence alignment; the excluded regions comprising about 257 bases, which mostly are at the beginning and the end of the multiple sequence alignment. The excluded regions of this study are largely due to the length variability of sequences available in GenBank. Twenty three, ten and three known *Russularia* species from Europe, Asia and North America respectively are included in the study. Figure 1 shows the ML topology based on the ITS sequence alignment. The bootstrap values (BS) and posterior probabilities (PP) are indicated in the phylogram when BS and PP exceed 50 and 0.85, respectively. All new species are indicated in boldface. Taxa in blue are obtained from public databases. Based on ITS sequence data, taxa from *L.* subg. *Russularia* form a monophyletic group apart from *L.* subg. *Lactarius*, but with low bootstrap support (48%). Except for Indonesian and Papuan representatives from Verbeken *et al.* (2001) and Verbeken & Horak (2000), we gathered all known Southeast Asian representatives

here and the molecular evidence shows that all newly proposed ones are new species. They are well-delimited in subg. *Russularia* (Figure 1).

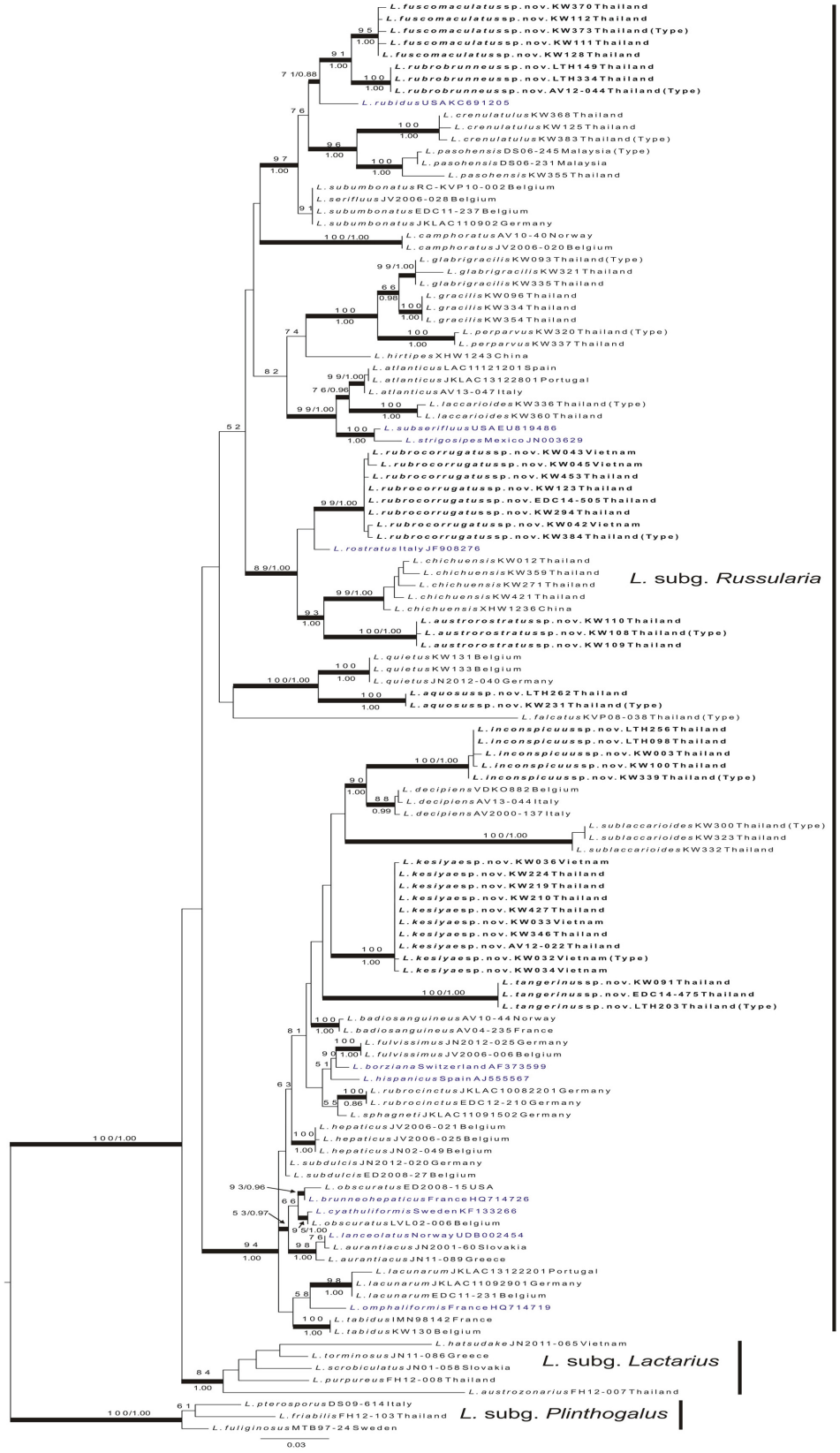


FIGURE 1. The obtained ML phylogeny based on ITS sequences. Names in boldface are new species. Names in blue are obtained from public databases. Bootstrap values and posterior probabilities are indicated if they exceed 50% and 0.85, respectively. The bar scale represents the expected number of nucleotide changes per site.

Taxonomy

Lactarius aquosus H.T. Le & K.D. Hyde, *sp. nov.* (Figure 2 and 11a)

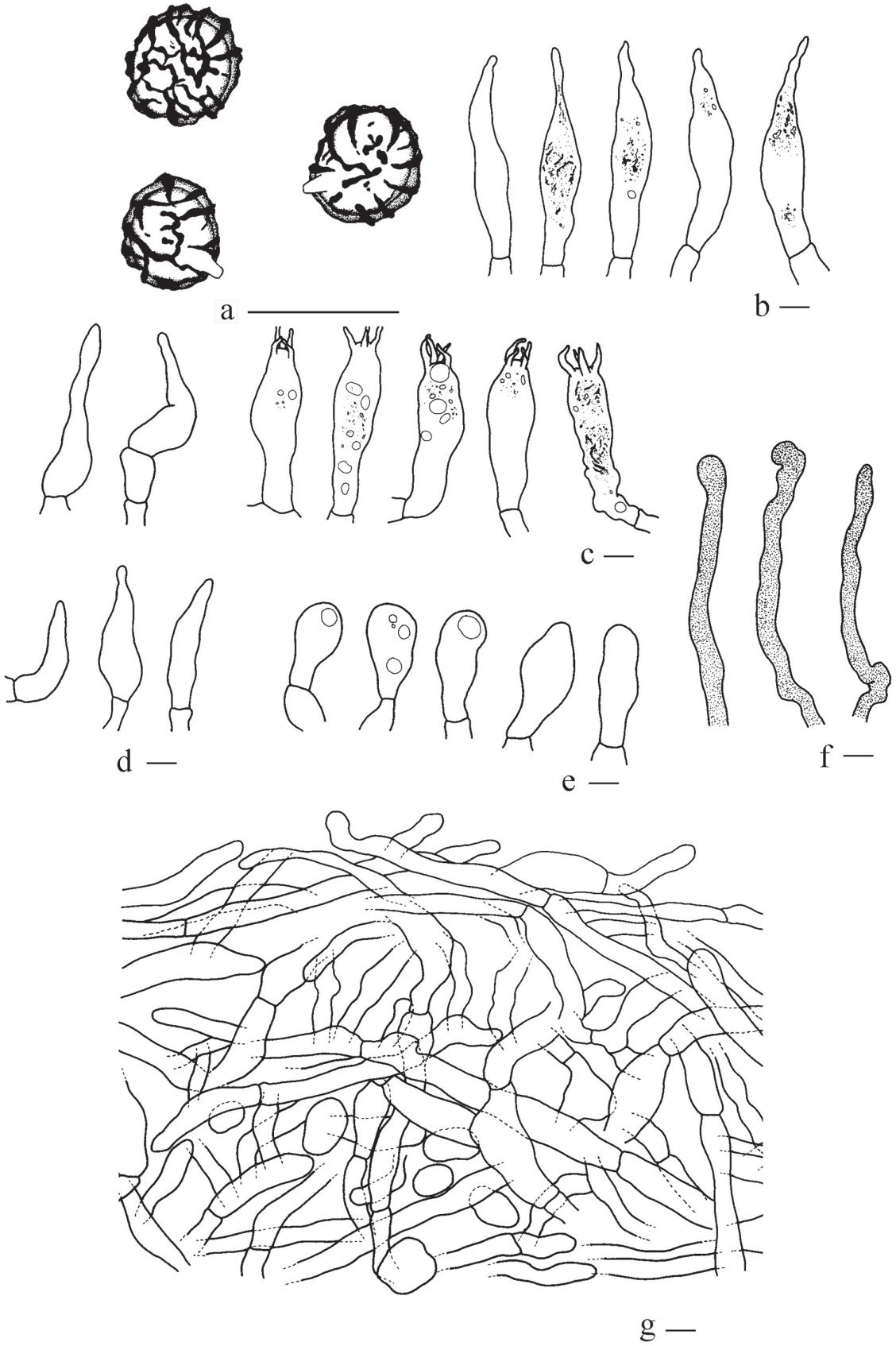


FIGURE 2. *L. aquosus*: a. basidiospore, b. pleuromacrocystidia, c. basidia, d. cheilocystidia, e. marginal cell, f. pseudocystidia, g. pileipellis (a–g: KW231, holotype) (scale bar = 10 μm).

MycoBank number: MB811741; *Facesoffungi number*: FoF00634

Diagnosis: a medium-sized species with a smooth cap surface which is zonate, brown to orange-brown coloration and a paler margin, transparent latex, completely transparent, incompletely to almost completely reticulate basidiospores, protruding pleuromacrocystidia and a cutis as pileipellis.

Etymology:—'aquosus' is referring to the transparent latex.

Typus:—THAILAND, Chiang Mai Province, Mae-on district, Mae Takraii National Park, Thepsaded Waterfall, alt. 1150 m , N18º55.32 E99º21.31, 01/06/2012, KW231 (holotype, GENT!, isotype, MFLU!).

Basidiocarps medium-sized. *Pileus* 21−37 mm diam., rather plane with depressed center to infundibuliform at maturity, typically darker colored in center and appearing more or less zonate near the margin; surface smooth, dry and glossy in moist condition, reddish brown (8E8) in center, reddish blond (5C4) to dark blond (5D4) to reddish brown (8D5), margin pale orange (5A3) to cream colored margin with short and inconspicuous striations, slightly crenulate. *Lamellae* subdecurrent to decurrent, 1−4.5 mm broad, crowded, with 2−4 series of lamellulae, cream to yellowish white (4A1−2) to light yellow (4A4), with light brown (7D6) discolorations in older specimens, yellowish brown (5D5) when bruised. *Stipe* 25−48 ×5−15 mm, cylindrical, hollow; surface smooth, rugose when old, often with short hairs and whitish pruinose at base, brownish orange (6C6) to light brown (6−7D6), darkening to brown (7E7) near base. *Context* 2−4.5 mm broad in pileus, pale yellow (3A3) to grayish yellow (3B4) to light grayish orange (6B−C4), unchanging when cut; smell strong, raphanoid; taste mild or slightly bitter and astringent. *Latex* watery, unchanging on exposure, moderately abundant, unchanging with 10 % KOH, unchanging on white tissue paper and on white cotton handkerchief. *Macrochemical reaction* no reaction on the context with 10 % KOH and with $FeSO_4$.

Basidiospores subglobose to broadly ellipsoid, 6.1−*6.9*−*7.2*−7.9(−8.0) × 5.4−*5.9*−*6.2*−6.9 µm; Q = 1.06−*1.15*−*1.17*−1.27 (n=60); ornamentation amyloid, composed of ridges up to 1 µm high, forming an incomplete to almost complete reticulum, with short ridges connected by finer lines which are blunt to subacute; isolated warts common; plage inamyloid. *Basidia* 48−52 × 12−16 µm, 4-spored, subclavate, sometimes bent near base, typically with needle-like to guttate contents. *Pleuromacrocystidia* 55−74 × 9−16 µm, abundant, subfusiform to subclavate with mucronate or moniliform apex, protruding up to 30 µm, with fine granules and needle-like contents. *Pleuropseudocystidia* 3−4 µm diam., not emergent, irregularly cylindrical, bent or curved near base; apex round or capitate. *Lamellar edge* heterogeneous, consisting of basidia, capitate to subclavate marginal cells 27−38 × 12−17 µm and cheilocystidia 34−58 × 8−14 µm, abundant, subfusiform, some irregularly curved, with mucronate to slightly moniliform apex, protruding up to 35 µm above the hymenium. *Lamellar trama* consisting of smaller and larger globose cells, septate hyphae and lactiferous hyphae. *Pileipellis* a cutis, 90−140 µm thick, consisting of parallel, repent and sometimes slightly erect hyphae.

Habitat: gregarious or scattered on slope, in montane tropical forest, with Fagaceae.

Collections examined: THAILAND, Chiang Mai Province, Mae-on district, Mae Takraii National Park, Thepsaded Waterfall, alt. 1150 m, N18º55.32 E99º21.31, 01/06/2012, KW231 (holotype, GENT!, isotype, MFLU!); Chiang Mai province, Mae Teang district, Bahn Pha Deng village, alt. 900 m., N19º17.12 E98º44.00, 01/06/2004, LTH102 (GENT!, CMU!, SFSU!);—ibid. 05/06/2005, LTH262 (GENT!, CMU!, SFSU!).

Comments: Lactarius aquosus is easily recognized in the field by its smooth pileus surface, zonate cap and transparent latex. Microscopically, this species has incompletely reticulate basidiospores and a cutis as a pileipellis.

Lactarius austrorostratus Wisitrassameewong & Verbeken, *sp. nov.* (Figure 3)

MycoBank number: MB811742; *Facesoffungi number*: FoF00635

Diagnosis: a medium sized, reddish brown species, with incompletely reticulate basidiospore ornamentation in a zebroid pattern, rostrate pleuromacrocystidia and a hyphoepithelium as a pileipellis.

Etymology:—'austrorostratus' refers to the occurrence in South East Asia and the rostrate cystidia.

Typus:—THAILAND, Chiang Mai province, Jomthong district, Bahn Luang sub-district, Doi Inthanon, nature trail at highest spot, N18º35.20 E98º29.03, alt. 2565 m, 06/07/2011, KW108 (holotype, GENT!, isotype, MFLU!).

Pileus: 10−30 mm diameter, at first convex to broadly convex with incurved margin, later expanding and with a more depressed center, or slightly infundibuliform, papillate in center; surface smooth when young, then becoming rugulose, particularly in center, dark brown (9F7) initially, turning dark brown (9F6−7) to reddish brown (9E8); margin inflexed, short striation, crenulate. *Lamellae* 1−3 mm broad, crowded, with 1−2 series of lamellulae, subdecurrent, cream to dark cream; edge minutely crenulate. *Stipe* 30−43 × 4 mm, cylindrical, central to slightly eccentric, fragile, fistulose; surface wrinkled, reddish brown (8D7) to dark brown (7F6) at base in youth, turning darker brown (8F8) when mature. *Context* 0.5−2 mm broad, fragile, pale reddish brown, unchanging when cut; odor like *L. quietus* or

Pentatomidae bugs, taste mild. *Latex* somewhat watery white to whey-like, sparse to moderately abundant, unchanging on exposure, unchanging with 10% KOH, unchanging on white tissue paper and on white cotton handkerchief, taste mild. *Macrochemical reaction* no reaction on the context with 10 % KOH and with $FeSO_4$.

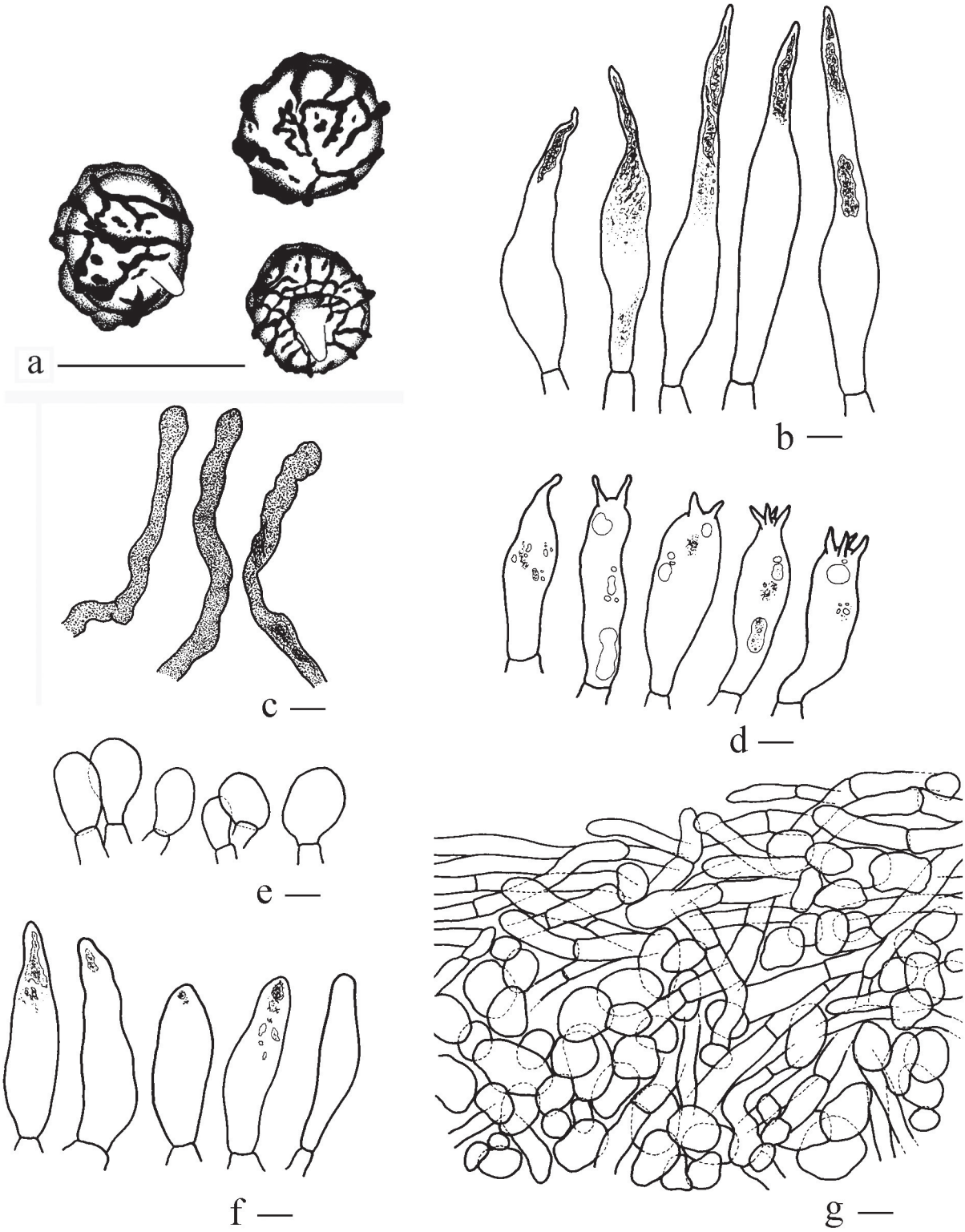


FIGURE 3. *L. austrorostratus*: a. basidiospore, b. pleuromacrocystidia, c. pseudocystidia, d. basidia, e. marginal cell, f. cheilocystidia, g. pileipellis (a and g: KW109, b–f: KW108, holotype) (scale bar = 10 µm).

Basidiospores subglobose to ellipsoid, (6.1–)6.4–*7.1*–7.8 × (5.6–)5.7–*6.2*–6.8(–6.9) µm; Q = 1.07–*1.14–1.15*–1.30,

(n = 40); ornamentation amyloid, up to 1 µm high, consisting of blunt irregular ridges, forming an incomplete reticulum, at times with a zebroid aspect; isolated warts abundant; plage distally amyloid. *Basidia* 34−52 × 10−17 µm, mostly 4-spored, sometimes 1-or 2-spored, irregularly subclavate, some irregularly cylindrical, with guttate contents and fine granules. *Pleuromacrocystidia* abundant, 54−107 × 9−18 µm, lanceolate to subfusiform, somewhat slender, protruding up to 40 µm, rostrate apex, often tortuous particularly near the apex, mostly with refractive contents containing fine granules. *Pleuropseudocystidia* abundant, 3−6 µm diam., cylindrical, but tortuous near the base, with fine granules, needle-like contents and sometimes with crystalline contents. *Lamellar edge* sterile, with marginal cells and cheilocystidia; marginal cells 13−26 × 8−18 µm, clavate to obovoid; cheilocystidia 32−58 × 10−16 µm, scattered to abundant, subfusiform with mucronate to rounded apex, with refractive contents containing fine granules, protruding up to 15 µm. *Lamellar trama* consisting of smaller and larger globose cells, septate hyphae and lactiferous hyphae. *Pileipellis* a hyphoepithelium, suprapellis a thin layer of repent hyphae; subpellis about 60−80 µm thick, consisting of large globose cells which are up to 35 µm diam.

Habitat: solitary or gregarious on ground in evergreen rainforest, under *Castanopsis*.

Collections examined: THAILAND, Chiang Mai province, Jomthong district, Bahn Luang sub-district, Doi Inthanon, nature trail at highest spot, N18º35.20 E98º29.03, alt. 2565 m, 06/07/2011, KW108 (holotype, GENT!, isotype, MFLU!);−ibid., 06/07/2011, KW109 (GENT!, MFLU!);−ibid., 06/07/2011, KW110 (GENT!, MFLU!).

Comments: This species is in some aspects similar to *L. rostratus,* e.g. in basidiocarp size, color and latex features. Both species have rostrate pleuromacrocystidia but the Asian species has larger pleurocystidia. Both species also share basidiospore ornamentation characteristics (an incomplete reticulum with a slightly zebroid pattern) and a hyphoepithelium pileipellis. *Lactarius austrorostratus* grows under *Castanopsis* whereas *L. rostratus* is associated with *Fagus* in moss cushions. Another closely related species, *L. rubrocorrugatus*, is a small reddish brown species with completely transparent latex. The other distinguishable character between *L. austrorostratus* and *L. rubrocorrugatus* is the pleuromacrocystidia. *L. austrorostratus* has conspicuous protruding cystidia (up to 40 µm) with acute apex whereas in *L. rubrocorrugatus* the cystidia are not protruding to slightly protruding up to 20 µm with a mucronate to moniliform apex.

Lactarius chichuensis W.F. Chiu, *Lloydia* 8(1): 38, 1945 (Figure 4 and 11j).

Basidiocarps small to medium-sized. *Pileus* 5−37 mm diam., broadly convex to convex initially with umbo, becoming infundibuliform with age; surface dry, smooth to wrinkled, color generally varying from brown (6E4−7E6) to reddish brown (8D8, 9D−E8), with paler shade near margin, brownish orange (6C4−6) to light brown (6D7−8), in some specimens brownish (7C7) to light brown (7D4−5) to grayish brown (7E3) to brown (7E4) with cream-colored margin, sometimes with a fine whitish powder covering the surface; margin not striate, involute initially and becoming incurved to straight in age. *Lamellae* subdecurrent to decurrent, crowded, 0.5−3 mm broad, grayish orange (5B4) to brownish orange (5C4), light brown (7D4) to brown (7E4) when older, with 2−3 series of lamellulae. *Stipe* 8−24 × 2−6.5 mm, cylindrical, central to eccentric; surface dry, smooth, light brown (7D6) to brown (6−7E6). *Context* 1−3 mm broad in pileus, hollow in stipe, pale orange (5A3) to grayish orange (5B3−B4); odor strong, like *L. quietus* or Pentatomidae bugs; taste mild. *Latex* abundant, watery white to white, unchanging on exposure, unchanging with 10 % KOH, pale yellow on white tissue paper and unchanging on white cotton handkerchief. *Macrochemical reaction* on context: olive brown (4D6) to light brown (5D6) with 10 % KOH, brown (6E7) with $FeSO_4$.

Basidiospores globose to broadly ellipsoid, 6.1−*6.7*−*7.3*−8.1(−8.3) × 5.5−*6.0*−*6.4*−7.2(−7.3) µm; Q = 1.02−*1.10*−*1.16*−1.28; ornamentation amyloid; ridges up to 1 µm high forming a zebroid ornamentation composed of parallel irregular, short and long ridges, never reticulate; plage inamyloid. *Basidia* 50−57 × 13−18 µm, 4-spored, subclavate, with fine granules. *Pleuromacrocystidia* rare to abundant, 45−71 × 10−15 µm, protruding up to 30 µm, subfusiform, bent to straight with mucronate to moniliform apex, typically with refractive contents at apex. *Pseudocystidia* 3−6 µm diam., slightly protruding, cylindrical, tortuous to straight, with fine granulate content. *Lamellar edge* heterogeneous, consisting of basidia, marginal cells and cheilocystidia; marginal cells 12−28 × 7−15 µm, subcylindrical to clavate; cheilocystidia abundant 31−45 × 10−19 µm, protruding up to 15 µm, subfusiform to subclavate, rarely clavate, with refractive contents at apex. *Lamellar trama* consisting of globose cells, septate hyphae and lactiferous hyphae. *Pileipellis* a hyphoepithelium, with a dense upper layer of repent hyphae and an underlying layer of globose cells, suprapellis composed of cylindrical hyphae about 20−40 µm thick; subpellis consisting of globose cells about up to 20 µm diam.

Habitat: gregarious on ground in montane mixed forest with Fagaceae and *Pinus kesiya*.

Distribution: reported from China (Chiu 1945, Wang & Liu 2002), Thailand.

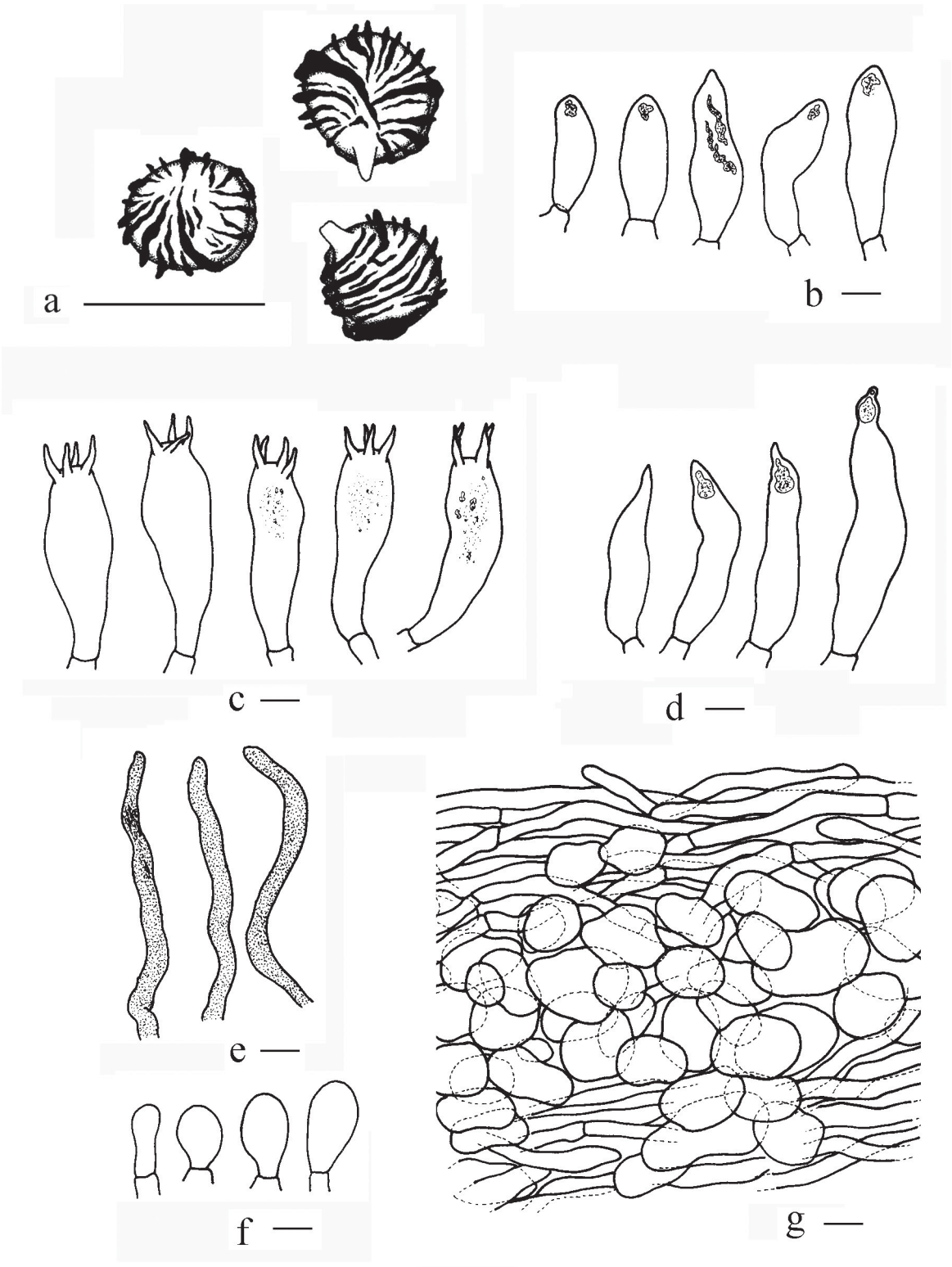


FIGURE 4. *L. chichuensis*: a. basidiospore, b. cheilocystidia, c. basidia, d. pleuromacrocystidia, e. pseudocystidia, f. marginal cells, g. pileipellis (a: KW271, b–g: KW421) (scale bar = 10 μm).

Collections examined: THAILAND, Chiang Rai province, Mae Fah Luang district, Doi Mae Salong Nok sub-district, Doi Mae Salong, alt. 1269 m., N20º16.90 E99º62.30, 13/07/2012, KW352 (GENT!, MFLU!);−ibid., 27/07/2012, KW372 (GENT!, MFLU!);−ibid., 08/08/2012, KW388 (GENT!, MFLU!);−ibid., 16/09/2012, KW403 (GENT!, MFLU!);−ibid., 28/08/2013, KW465 (GENT!, MFLU!);−ibid., 10/09/2013, KW467 (GENT!, MFLU!); Chiang Mai province, Mae Teang district, Pa Pae sub-district, Bahn Pha Deng village, mushroom research center, N19º17.12 E98º44.00, alt. 900 m, 13/05/2011, KW012 (GENT!, MFLU!); Chiang Mai province, Mae Teang district,

Pa Pae sub-district, Bahn Pha Deng village,Pathummikaram Temple, forest trail, alt. 1050 m, 07/06/2012, KW271 (GENT!, MFLU!);−ibid., 07/06/2012, KW272 (GENT!, MFLU!).

Comments: Lactarius chichuensis is a distinctive species in the subgenus because of its zebroid basidiospore ornamentation. The species has small, reddish brown basidiocarps and a strong *L. quietus*-like or Pentatomidae bug odor. The species was discovered by Chiu (1945). The holotype is in poor condition and only the spore ornamentation was studied by Chiu. Later the species was described in more detail and distinguished from a look-alike species, *L. hirtipes* J.Z. Ying by Wang & Liu (2002) on account of its different basidiospore ornamentation. Morphological characters of our specimens are consistent with the documentation of Wang & Liu (2002). In our collections we sometimes observed the pileus to be whitish pruinose. Wang & Liu (2002) stated that the species is found in forests with Fagaceae, whereas we found the species in mixed forest.

Lactarius fuscomaculatus Wisitrassameewong & Verbeken, *sp. nov.* (Figure 5 and 11b)

MycoBank number: MB811743; *Facesoffungi number*: FoF00636

Diagnosis: a medium sized species with a brown to orange-brown pileus and remarkably dark brown spots or tinges, white latex, a strong odor of Pentatomidae bugs, incompletely reticulate basidiospore ornamentation, pleuromacrocystidia rare and pileipellis a hyphoepithelium.

Etymology:—'fuscomaculatus' refers to the dark, brownish spots on the pileus.

Typus:—THAILAND, Chiang Rai province, Muang district, Thasai sub-district, forest near Doi Pui Reverse Signal Station, Doi Pui, N19º49.26 E99º52.19, alt. 655 m, 31/07/2012, KW373 (holotype, GENT!, isotype, MFLU!). *Basidiocarps* medium sized. *Pileus* 25−62 mm diam., broadly convex initially, turning slightly infundibuliform to deeply infundibuliform in age, with a more or less distinct papilla; surface dry, more rugose in the center in age, brown (7D8 to 7E7) to dark brown (8F7) in the center, towards the margin, paler, brownish orange (5C5) to brown (6D7) to pale yellow (3A3), typically becoming uneven in color in age, with dark brown (8F7) discolorations, varying from spotted to brushed over the whole surface except the margin; margin indistinctly striate, slightly crenulate. *Lamellae* subdecurrent to decurrent, 1−3 mm broad, crowded, with 3−4 series of lamellulae, pale yellow (4A3) to cream-colored, discoloring light brown (6D6) to brown (7E6), spotted in age. *Stipe* 34−76 x 4−7 mm, cylindrical, central, rarely slightly eccentric; surface dry, smooth, whitish pruinose, particularly in young specimens, dull yellow (3B3) to olive brown (4D6) at the apex, yellowish brown (5E4) to dark brown (7F5) towards the base, sometimes brownish orange (7C7), with 1−2 mm long hairs at the base. *Context* 0.5−4 mm broad in the pileus, partially hollow to completely hollow in stipe, pale yellow (3A3); odor strong, reminiscent of *L. quietus* or Pentatomidae bugs; taste mild. *Latex* watery white to white, abundant, unchanging on exposure, unchanging with 10% KOH, unchanging on white tissue paper and on white cotton handkerchief; taste slightly astringent and becoming faintly peppery. *Macrochemical reaction* no reaction on the context with 10% KOH, or with $FeSO_4$.

Basidiospores globose to broadly ellipsoid, 6.0−*6.6*−*7.2*−7.7(−7.8) × 5.5−*6.1*−*6.6*.−7.0(−7.2) µm, Q = 1.02−*1.08*−*1.10*−1.21 (n=100); ornamentation amyloid, composed of irregular ridges up to 1 µm high, forming an incomplete reticulum, warts and ridges connected by fine lines; isolated warts present; plage inamyloid to distally amyloid. *Basidia* 42−53 × 13−17 µm, 4-spored, some 2-spored, subclavate to clavate; with guttate contents. *Pleuromacrocystidia* rare, protruding up to 10 µm, 55−68 × 13−17 µm, fusiform, with a mucronate, occasionally ramified apex. *Pleuropseudocystidia* scarce to abundant, 3−6 µm diam., not emergent to slightly emergent, cylindrical to tortuous with an obtuse apex, often broadened at the apex. *Lamellar edge* heterogeneous, composed of basidia, abundant cylindrical to subclavate, thin-walled marginal cells 13−26 × 5−13 µm; cheilocystidia absent to rare, 34−38 ×11−14 µm, not emergent, fusiform with a mucronate apex. *Lamellar trama* consisting of lactifers and sphaerocysts. *Pileipellis* a hyphoepithelium, with a thin upper layer of repent or oblique hyphae; suprapellis composed of cylindrical hyphae, 10−20 µm thick; subpellis a layer of large globose cells, up to 30 µm diam., mixed with cylindrical to inflated hyphae.

Habitat: gregarious to scattered on the ground in montane tropical forests with Fagaceae.

Collections examined: THAILAND, Chiang Rai province, Muang district, Thasai sub-district, forest near Doi Pui Reverse Signal Station, Doi Pui, N19º49.26 E99º52.19, alt. 655 m, 31/07/2012, KW373 (holotype, GENT!, isotype, MFLU!);−ibid., 25/07/2011, KW111 (GENT!, MFLU!);−ibid., 25/07/2011, KW112 (GENT!, MFLU!);−ibid., 01/09/2011, KW126 (GENT!, MFLU!);−ibid., 01/09/2011, KW128 (GENT!, MFLU!);−ibid., 01/09/2011, KW129 (GENT!, MFLU!);−ibid., 25/07/2012, KW370 (GENT!, MFLU!); Chiang Rai province, Mae Fah Luang district, Doi Mae Salong Nok sub-district, Doi Mae Salong, alt. 1269 m, N20°16.90 E99°62.30, 30/05/2012, KW221 (GENT!, MFLU!);−ibid., 30/05/2012, KW223 (GENT!, MFLU!); Chiang Rai province, Mae Fah Luang district, Doi Mae Salong Nok sub-district, Doi Mae Salong, N20º17.23 E99º61.69, alt. 1193 m, 22/07/2012, KW365 (GENT!, MFLU!);−ibid.,

28/08/2013, KW466 (GENT!, MFLU!); Chiang Mai province, Mae-On district, Huaikaew sub-district, Bahn Pok M.1, N18º53.08 E99º21.45, alt. 1040 m, 02/06/2012, KW236 (GENT!, MFLU!); Chiang Mai province, Mae-On district, Huaikaew sub-district, Bahn Mae Kampong, N18º51.43 E99º22.09, alt. 1450 m, 03/06/2012, KW249 (GENT!, MFLU!).

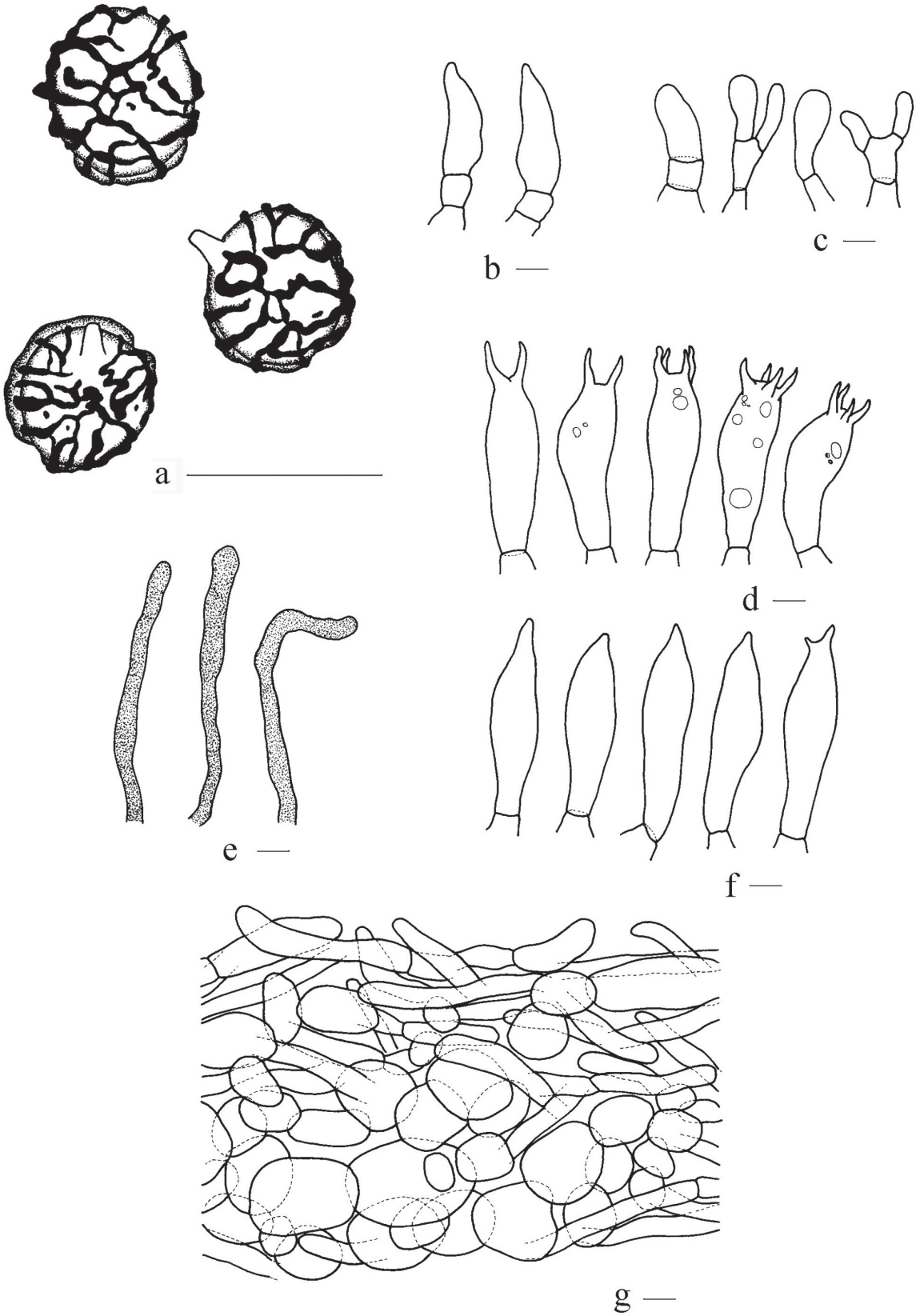


FIGURE 5. *L. fuscomaculatus*: a. basidiospore, b. cheilocystidia, c. marginal cell, d. basidia, e. pseudocystidia, f. pleuromacrocystidia, g. pileipellis (a and c−f: KW365, b and g: KW373, holotype) (scale bar = 10 μm).

Comments: Lactarius fuscomaculatus is widely distributed in tropical forests with *Castanopsis* and *Quercus* in Northern Thailand. The species can be confused with a closely related species, *L. rubrobrunneus* due to similarities in basidiocarps size, color, latex features and habitat. Both species are very similar in the immature stage, however, according to our experience, brown spots on the pileus are often found in fully mature fruiting bodies of *L. fuscomaculatus*, while *L. rubrobrunneus* is more unicolored. Under the microscope, both species have basidiospores with an incomplete reticulum and not many cystidia. A slight difference was observed in their pileipellis structures; *L. rubrobrunneus* has in part a very thin layer of repent hyphae which is like a transition between an epithelium and a hyphoepithelium, whereas *L. fuscomaculatus* has a more complete layer of repent hyphae covering the pileus. *Lactarius fuscomaculatus* may also be confused with *L. tangerinus* in the field. *Lactarius tangerinus* has smaller basidiomata, is typically paler in color without dark brown spots and possesses transparent latex. For more details on the difference between these two species, see under *L. tangerinus*.

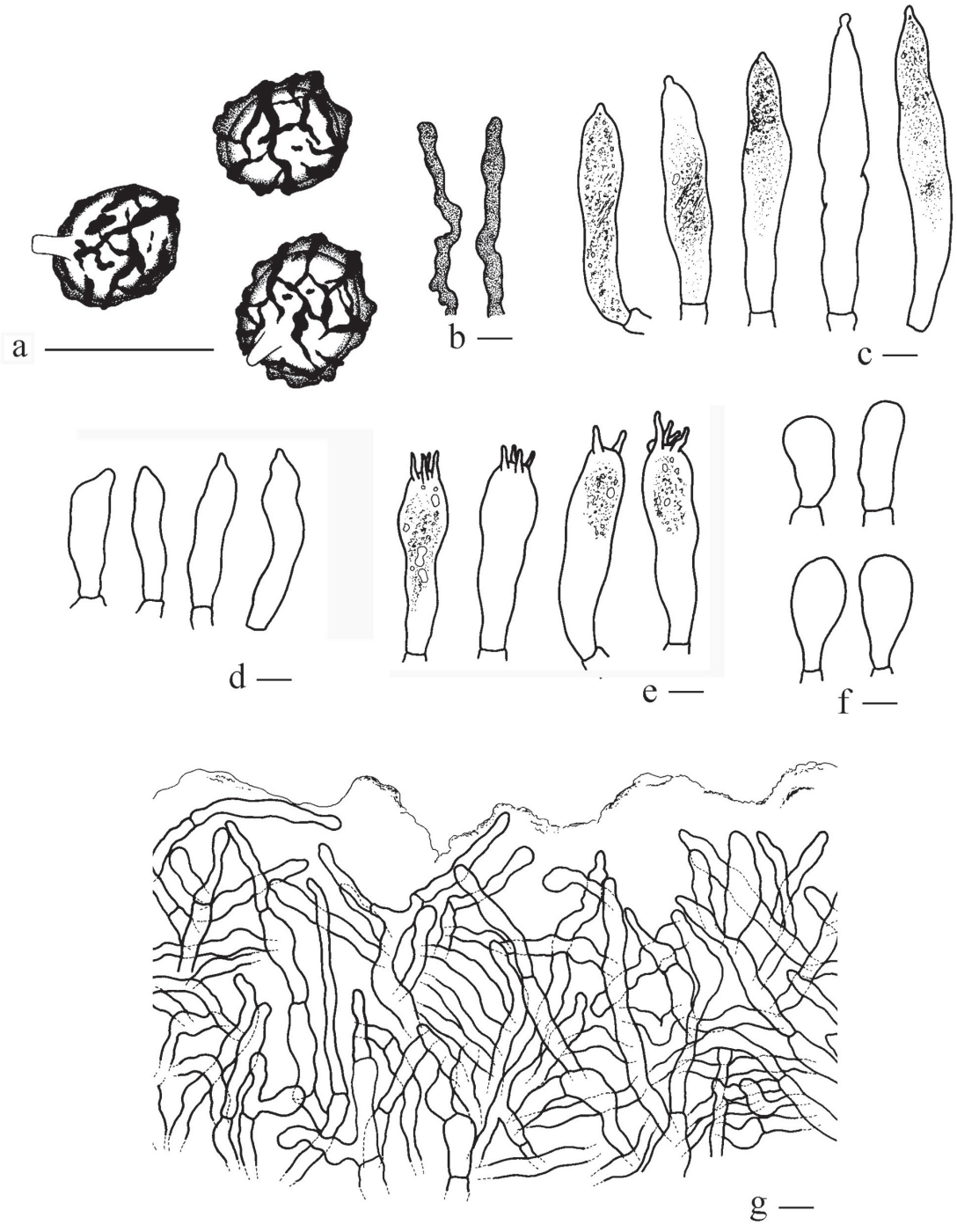


FIGURE 6. *L. inconspicuus*: a. basidiospore, b. pseudocystidia, c. pleuromacrocystidia, d. cheilocystidia, e. basidia, f. marginal cell, g. pileipellis (a–f: LTH256, g: KW339, hylotype) (scale bar = 10 μm).

Lactarius inconspicuus H.T. Le & F. Hampe, *sp. nov.* (Figure 6 and 11d)

MycoBank number: MB811744; *Facesoffungi number*: FoF00637

Diagnosis: a small to medium sized species recognized by the brownish orange cap with paler margin, white latex turning pale yellow on exposure and on white tissue paper and white handkerchief, with incomplete reticulate basidiospore ornamentation, large and protruding pleuromacrocystidia and an ixotrichodermal pileipellis.

Etymology:—'inconspicuus' refers to the inconspicuous macromorphological characters of the species.

Typus:—THAILAND, Chiang Mai province, Doi Suthep-Pui national park, Sangasabhasri Lane to Huai Kok Ma Village, N18º48.62 E98º54.60, alt. 1145 m, 05/07/2012, KW339 (holotype, GENT!, isotype, MFLU!).

Basidiocarps small to medium sized. *Pileus* 10−56 mm diam., convex-umbonate in immature specimens, becoming plano-convex with depressed disc; surface minutely rugulose, dry to slightly sticky, glossy when moist, brown (7E6) to dark brown (7F8) in center or at least on umbo, brown (6E−F6) to light brown (6D7) to brownish orange (6C−D6), paler and cream-colored at margin; margin estriate when immature, becoming striate with age, crenulate. *Lamellae* subdecurrent to decurrent, 1−3 mm broad, crowded, with 3−4 series of lamellulae, yellowish white (2A2), pale yellow (4A3) to light yellow (4A4), turning brownish orange (6B−C7, 7C−D6) to reddish brown (8E5) when bruised. *Stipe* 20−75 × 2−8 mm, cylindrical to tapering upwards; surface dry to moist, smooth, brownish yellow (6B−C6) to brownish orange (6C8−7C8) to brown (7E−F8), fistulose, whitish pruinose at base. *Context* 1−3 mm thick in pileus, pale cream to orange-white (5A2) to brownish orange (5C−D6); odor faint, like *L. quietus* or Pentatomidae bugs; taste mild. *Latex* abundant, watery white, slowly turning pale yellow on exposure, unchanging with 10 % KOH, pale yellow on white tissue paper, pale yellow on white handkerchief; taste mild. *Macrochemical reaction* on the context: light brown (5D4−D5) with 10 % KOH, grayish yellow (4B3) with $FeSO_4$.

Basidiospores typically subglobose to broadly ellipsoid, rarely globose, 6.1−*6.9*−*7.4*−8.0(−8.1) × 5.5−*5.8*−*6.3*−6.9(−7.0) µm; Q = 1.03−*1.13*−*1.16*−1.28 (n = 100); ornamentation amyloid, up to 1.3 µm high, consisting of blunt to subacute thick ridges forming an incomplete to almost complete reticulum; isolated warts common; plage inamyloid, sometimes slightly distally amyloid. *Basidia* 30−62 × 8−18 µm, mostly 4-spored, sometimes 2-spored, subcylindrical to subclavate, with fine granules and guttate contents. *Pleuromacrocystidia* abundant, 30−114 × 8−20 µm, protruding up to 30 µm, subcylindrical to subfusiform, with mucronate to moniliform apex, with needle-like contents and fine granules. *Pleuropseudocystidia* not protruding, 3−5 µm diam., tortuous to straight, cylindrical, with fine granules. *Lamellar edge* heterogeneous, consisting of basidia, cylindrical to subclavate marginal cells 15−36 × 8−17 µm and abundant cheilocystidia 30−52 × 8−18 µm, not protruding to slightly protruding up to 10 µm, subfusiform to fusiform, with mucronate to moniliform apex. *Lamellar trama* consisting of globose cells, septate hyphae and lactiferous hyphae. *Pileipellis* an ixotrichoderm, 100−120 µm thick, consisting of erect cylindrical hyphae, or sometimes, repent cylindrical hyphae.

Habitat: solitary to gregarious on soil in montane tropical forests, under *Castanopsis armata*.

Collections examined: THAILAND, Chiang Mai province, Doi Suthep-Pui national park, Sangasabhasri Lane to Huai Kok Ma Village, N18º48.62 E98º54.60, alt. 1145 m, 02/06/2005, LTH256 (GENT!, CMU!, SFSU!);−ibid., 30/05/2004, LTH098 (GENT!, CMU!, SFSU!);−ibid., 24/06/2005, LTH306 (GENT!, CMU!, SFSU!);−ibid., 24/06/2005, LTH307 (GENT!, CMU!, SFSU!);−ibid., 21/04/2011, KW003 (GENT!, MFLU!);−ibid., 05/07/2012, KW339 (holotype, GENT!, isotype, MFLU!); Chiang Mai province, Mae Taeng district, Bahn Mae Sae, 50 km marker on highway 1095, N19º14.59 E98º39.45, alt. 962 m, 03/06/2011, KW016 (GENT!, MFLU!).

Comments: This species has the general features of the subgenus but lacks any striking distinguishing characters. In the field this species is similar to *L. tangerinus*. A microscopic examination revealed that these species can be distinguished by: (1) larger pleuromacrocystidia in *L. inconspicuus*; (2) presence of a narrow mucus layer in the pileipellis in *L. inconspicuous*; and (3) higher basidiospore ornamentation in *L. tangerinus*.

Lactarius kesiyae Verbeken & K.D. Hyde *sp. nov.* (Figure 7, 11g and 11h)

MycoBank number: MB811745; *Facesoffungi number*: FoF00638

Diagnosis: a medium sized species with smooth cap and glossy surface, brownish gray to brownish orange cap with grayish green tints, latex watery white turning yellow on a white handkerchief, incompletely reticulate basidiospores, protruding pleuromacrocystidia, an ixotrichoderm and association with *P. kesiya*.

Etymology:—'kesiyae' refers to the ectomycorrhizal tree, *P. kesiya*.

Typus:—VIETNAM, Lam Dong province, Lac Duong district, Xa Lat, Lang Biang National park, alt. 1545 m, N12º01.57 E108º25.58, 12/06/2011, KW032 (holotype, GENT!, isotype, MFLU!).

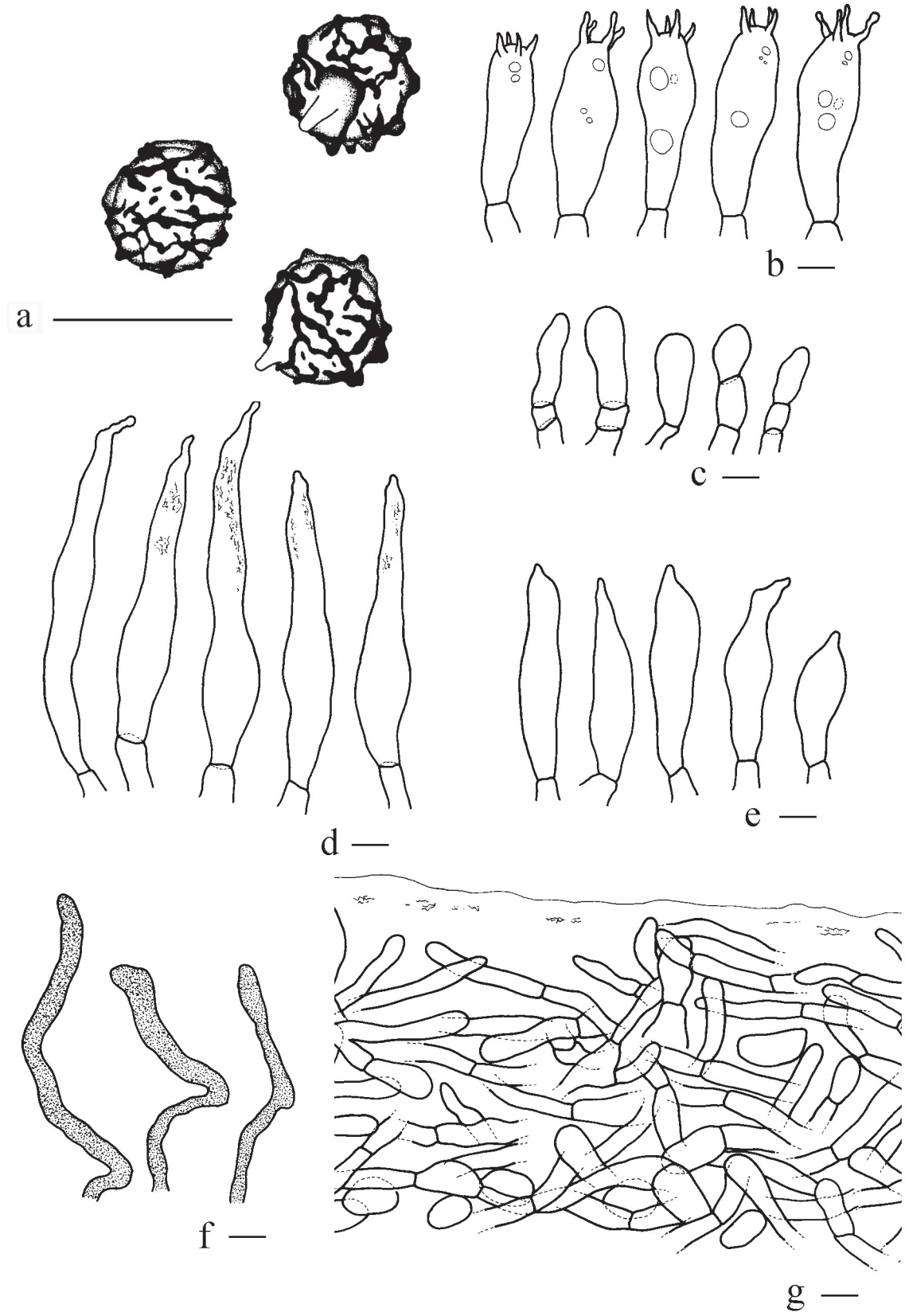


FIGURE 7. *L. kesiyae*: a. basidiospore, b. basidia, c. marginal cell, d. pleuromacrocystidia, e. cheilocystidia, f. pseudocystidia, g. pileipellis (a–g: KW353) (scale bar = 10 μm).

Basidiocarps medium-sized. *Pileus* 18–49 mm diam., at first convex to broadly convex, then becoming plano-convex with a more depressed disc, finally infundibuliform; surface smooth and slightly glossy, slightly sticky when

moist; color darkest in immature specimens, dark blond (5D4) when young, grayish yellow (3−4C4), brownish gray (5C2), reddish blond (5C3−4) to brownish orange (5C5) when mature, in some collections with grayish green (25B4−B6) tints, color paler in dry condition, pale orange (5A3) to pale grayish orange (5B3−4); margin, paler, yellowish white (2A2), estriate when immature, later becoming striate and slightly crenulate with age. *Lamellae* subdecurrent to decurrent, 1−3 mm broad, crowded, with 2 series of lamellulae, pale cream to cream, to grayish orange (6B5) when bruised. *Stipe* 30−56 × 3−8 mm, cylindrical, centrally attached; apex becoming concolorous with lamellae, darker when moist, brownish orange (5C5), pale orange when dry. *Context* 3−5 mm broad in pileus, whitish, hollow in stipe; smell fruity; taste mild, a bit sweet after a while, then faintly acrid. *Latex* watery white, moderately abundant, unchanging on exposure, unchanging with 10% KOH, unchanging on white tissue paper, soon turning yellow on a white cotton handkerchief; taste mild to slightly astringent. *Macrochemical reaction* no reaction on the context with 10% KOH, but becoming brownish (5E7) to light brown (6D6) with $FeSO_4$ after a while.

Basidiospores globose to broadly ellipsoid, 6.3−*6.9*−*7.1*−7.8(−7.9) × 5.7−*6.1*−*6.3*−6.8(−7.0) µm; Q = 1.02−*1.10*−*1.13*−1.19 (n=60); ornamentation amyloid, up to 1.2 µm high, composed of irregular to crenulate ridges, obtuse, forming an incomplete reticulum; isolated warts common; plage distally amyloid. *Basidia* 48−54 × 12−18 µm, 4-spored, clavate, with fine granules and guttate contents. *Pleuromacrocystidia* abundant, 54−100 × 11−16 µm, protruding up to 30 µm, irregular narrowly fusiform, slender, thin-walled, partially with granular and needle-like or guttate contents; apex mucronate, occasionally ramified. *Pleuropseudocystidia* abundant, 4−6 µm diam., not protruding, cylindrical to broadened at apex, tortuous. *Lamellar edge* heterogeneous, with basidia, cylindrical to subclavate, thin-walled marginal cells 14−28 × 6−10 µm,; cheilocystidia scarce to fairly abundant, 37−58 × 10−14 µm, slightly protruding up to 10 µm, narrowly fusiform, with mucronate apex, with granules. *Lamellar trama* consisting of abundant lactifers and sphaerocysts. *Pileipellis* an ixocutis to ixotrichoderm, covered by a thin glutinous layer; upper layer 150−200 µm thick, composed of repent to erect hyphae; subpellis composed of cylindrical and inflated hyphae with a few globose cells.

Habitat: gregarious or scattered on ground, in coniferous forest with *P. kesiya*.

Collections examimed: VIETNAM, Lam Dong province, Lac Duong district, Xa Lat, Lang Biang National park, alt. 1545 m, N12º01.57 E108º25.58 , 12/06/2011, KW032 (holotype, GENT!, isotype, MFLU!);−ibid., 12/06/2011, KW033 (GENT!, MFLU!); −ibid., 12/06/2011, KW034 (GENT!, MFLU!);−ibid., 12/06/2011, KW035 (GENT!, MFLU!);−ibid., 12/06/2011, KW036 (GENT!, MFLU!); THAILAND, Chiang Rai province, Mae Fah Luang district, Doi Mae Salong Nok sub-district, Doi Mae Salong, N20º08.67 E99º40.17, alt. 1015 m, 15/05/2012, KW207 (GENT!, MFLU!);−ibid., 19/05/2012, KW210 (GENT!, MFLU!),−ibid., 24/05/2012, KW219 (GENT!, MFLU!);−ibid., 30/05/2012, KW224 (GENT!, MFLU!);−ibid., 13/07/2012, KW353 (GENT!, MFLU!);−ibid., 13/07/2012, AV12-022, (GENT!, MFLU!); Mae Hong Son province, coniferous forest along highway 1095, near Huai Nam Dang national park, alt. 1322 m, N19º16.07 E98º37.86, 08/07/2012, KW346 (GENT!, MFLU!); Lampang province, Muangparn district, Chaesorn sub-district, forest along highway 1252, N18º55.43 E99º23.40, alt. 1420 m, 15/06/2013, KW427 (GENT!, MFLU!).

Comments: Lactarius kesiyae grows in coniferous forests dominated by *P. kesiya*. It can be recognized by its sticky and glossy appearance in moist conditions, and its pale brownish gray to pale brownish orange color with greenish or orange to even pinkish tints on the pileus. The latex is watery white and turns yellow on a white cotton handkerchief. Microscopically, the pileipellis is an ixotrichoderm and the pleuromacrocystidia, which are up to 100 µm long in length, protrude conspicuously from the hymenium.

Microscopically, the sticky pileus is reflected as an ixocutis and it is not a common character for this subgenus. A sticky pileus occurs in a few temperate species such as *L. decipiens*, *L. duplicatus*, and *L. badiosanguineus* Kühner & Romagn. and it seems to be less common in tropical Asia. There are two tropical Asian species recorded as having a thin slime layer in the pileipellis stucture, *L. austrotabidus* Verbeken & E. Horak, with an ixocutis or ixotrichoderm and *L. inconspicuus* with an ixotrichoderm. In the field *L. kesiyae* could be confused with representatives of *L.* subg. *Lactarius* (syn. *L.* subg. *Piperites* (Fr.) Kauffman) on account of this surface feature which is one of the dominant characters of that subgenus. However, the overall brownish orange colors of *L. kesiyae* suggest it is a member of subg. *Russularia*.

Lactarius rubrobrunneus H.T. Le & Nuytinck, *sp. nov.* (Figure 8 and 11c)

MycoBank number: MB811746; *Facesoffungi number*: FoF00639

Diagnosis: a medium sized species with reddish brown cap, incompletely reticulate basidiospores, pleuromacrocystidia rare, pileipellis an epithelium to hyphoepithelium.

Etymology:—'rubrobrunneus' refers to the reddish brown color of the cap.

Typus:—THAILAND, Chiang Mai province, Mae-On district, Huaikaew sub-district, Bahn Mae Kampong, N18º51.43 E99º22.09, alt. 1450 m, 15/07/2012, KW356 (AV12-044) (holotype, GENT!, isotype, MFLU!).

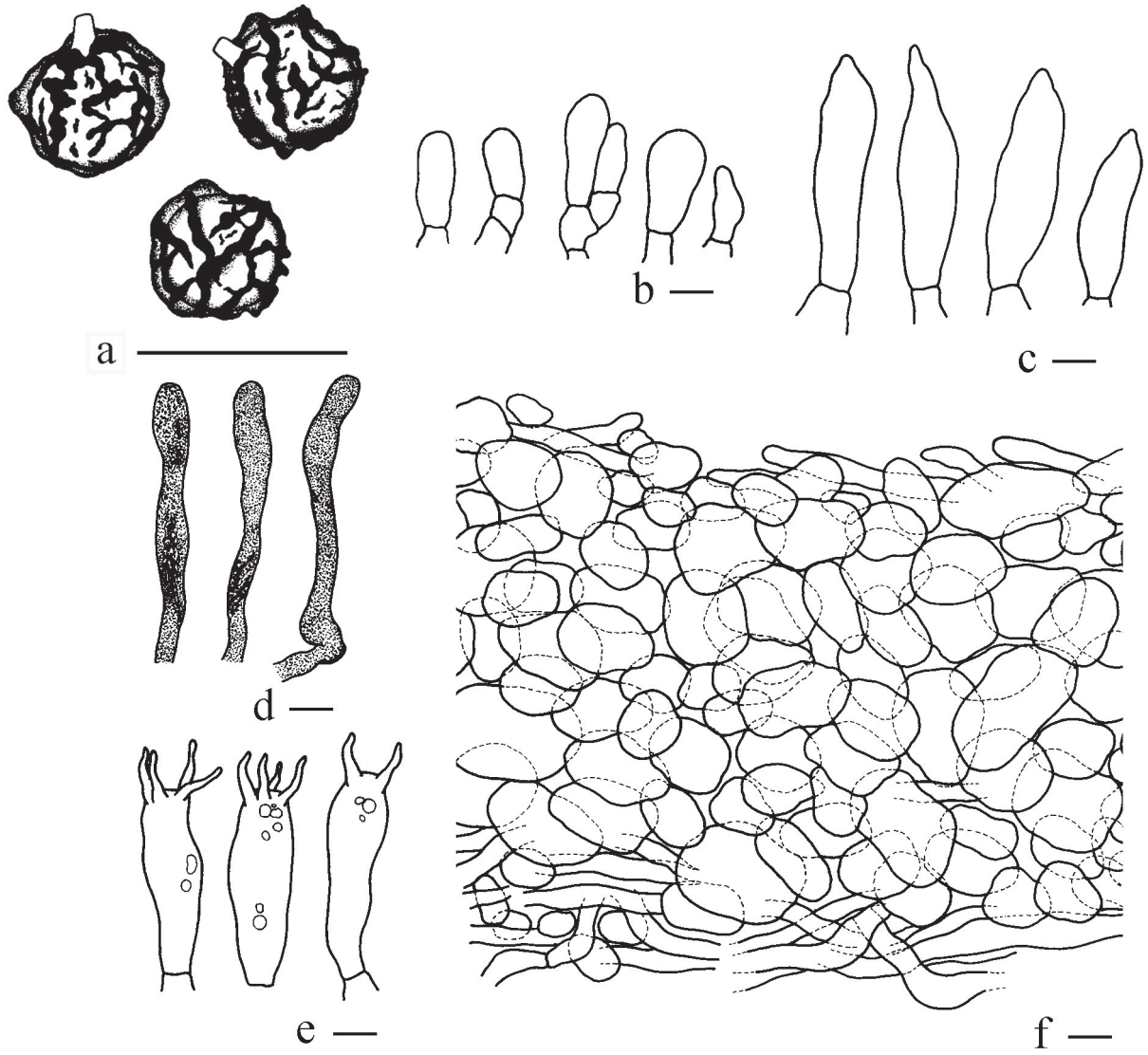


FIGURE 8. *L. rubrobrunneus*: a. basidiospore, b. marginal cell, c. pleuromacrocystidia, d. pseudocystidia, e. basidia, f. pileipellis (a−f: AV12-044, holotype) (scale bar = 10 µm).

Basidiocarps medium sized. *Pileus* 15−80 mm diam., convex to widely depressed or infundibuliform, papillate; surface dry, greasy, slightly rugulose, dark brown (8F5−6) in center, reddish orange (7A6−8) to light reddish brown to yellow-brown (6C6−7) to brown (6D7) at the margin. *Lamellae* 1−4 mm broad, decurrent, crowded, with 3−4 series of lamellulae, forked, cream to grayish orange (5B4−5), sometimes paler, discoloring with reddish brown spots in age. *Stipe* 50−105 × 2−8 mm, cylindrical to tapering upwards, central, dry, smooth, whitish to pale cream pruinose at apex, light brown (6D4) to brown (6E7) to dark brown (6F6), fistulose, hairy at base. *Context* 1−3 mm thick in pileus, cream to brownish cream (5B4−5), odor strong, reminiscent of *L. quietus* or Pentatomidae bugs; taste mild. *Latex* watery white to white, abundant, unchanging on exposure, unchanging on white tissue paper and on a white cotton handkerchief and with 10 % KOH, taste mild. *Macrochemical reaction* no reaction on context with 10% KOH, or with $FeSO_4$.

Basidiospores subglobose to broadly ellipsoid, 6.1−*6.6*−*6.9*−7.4(−7.5) × 5.6−*6.0*−*6.3*−6.9(−7.0) µm, Q = 1.05−*1.10*−*1.11*−1.23 (n = 40); ornamentation amyloid, an incomplete reticulum up to 1 µm high,, consisting of blunt and irregular ridges, connected by thinner ridges, isolated warts common; plage inamyloid to distally amyloid. *Basidia* 41−57 × 12−16 µm, 4-spored, some 2-spored, subclavate, with guttate contents. *Pleuromacrocystidia* rare, 38−50 ×

10−14 µm, not protruding, with guttate contents. *Pleuropseudocystidia* abundant, slightly protruding, 3−7 µm diam., with fine granules, irregular to tortuous, sometimes with broadened apex. *Lamellar edge* heterogeneous, consisting of basidia and marginal cells without cheilocystidia, marginal cells abundant, 15−36 × 5−12 µm, mostly cylindrical to subcylindrical to subclavate. *Lamellar trama* a combination of globose cells, septate hyphae and lactiferous hyphae. *Pileipellis* a transition between epithelium and hyphoepithelium, with or without a thin layer of about 5−10 µm thick, repent hyphae, subpellis layer consisting of small to large, globose cells, up to 35 µm diam., layer about 70−100 µm thick.

Habitat: gregarious in montane tropical forests with Fagaceae.

Collections examined: THAILAND, Chiang Mai province, Jomthong district, Bahn Luang sub-district, Doi Inthanon National Park, junction of highway 1009 and road to Mae Cham, N19º31.58 E98º29.64, alt. 1703 m, 25/06/2004, LTH149 (GENT!, CMU!, SFSU!); Mae Hong Son province, Huai Nam Dang National Park, nature trail, alt. 1538 m, N19º18.29 E98º35.88, 29/06/2005, LTH334 (GENT!, CMU!, SFSU!); Chiang Mai province, Mae-On district, Huaikaew sub-district, Bahn Mae Kampong, N18º51.43 E99º22.09, alt. 1450 m, 15/07/2012, KW356 (AV12-044) (holotype, GENT!, isotype, MFLU!).

Comments: Lactarius rubrobrunneus is recognized by its reddish brown to orange-brown cap with a darker brown shade in the center. *Lactarius rubrobrunneus* differs from *L. fuscomaculatus* by the lack of dark brown spots on cap. Compared to *L. fuscomaculatus*, all collections of *L. rubrobrunneus* have a thinner layer of terminal hyphae in the pileipellis, which is a transition between an epithelium and a hyphoepithelium. In addition, we have observed cheilocystidia in *L. fuscomaculatus* collections, while they are completely absent in *L. rubrobrunneus*.

Lactarius rubrocorrugatus Wisitrassameewong & Nuytinck, *sp. nov.* (Figure 9 and 11i)

MycoBank number: MB811747; *Facesoffungi number*: FoF00640

Diagnosis: A small to medium sized species, cap red to reddish brown with a rugulose surface, latex transparent, basidiospore ornamentation consisting of low ridges forming an incomplete reticulum, pleuromacrocystidia present and pileipellis a hyphoepithelium.

Etymology:—'rubrocorrugatus' refers to the red and wrinkled pileus.

Typus:—THAILAND, Chiang Rai province, Muang district, Thasai sub-district, forest at Doi Pui Reverse Signal Station, Doi Pui, alt. 740 m, N19º49.00 E99º52.03, 31/07/2012, KW384 (holotype, GENT!, isotype, MFLU!).

Basidiocarps small to medium sized. *Pileus* 7−44 mm diam., plane to infundibuliform, papillate initially, becoming depressed with or without a papilla; surface dry, smooth in immature specimens, later becoming wrinkled in mature specimens, hygrophanous, red (9B7−8) to reddish brown (9D7−8−E8), typically with dark brown (7F5) shade in center; margin not striate in young specimens, becoming slightly striate in age, incurved, crenulate. *Lamellae* decurrent, 1−2 mm broad, very crowded, sometimes forked, with 1−3 series of lamellulae, yellowish white to light yellow (4A4) to cream, turning brown (6E5−E6) when bruised; edge slightly crenulate to even. *Stipe* 11−42 × 2−8 mm, cylindrical, fistulose; surface dry, smooth to slightly wrinkled, brownish orange (7C7) to brown (7E7−E8), turning dark brown (7F8) when older, whitish pruinose at base. *Context* 1−3 mm broad in pileus, pale yellow (4A3) to cream, unchanging when cut; odor reminiscent of *L. quietus* or Pentatomidae bugs; taste mild, sometimes sweetish and bitter. *Latex* transparent, unchanging on exposure, unchanging on tissue paper and on a white cotton handkerchief. *Macrochemical reaction* on the context: unchanging or slowly turning pale yellow with 10 % KOH, grayish green (26E6) with $FeSO_4$.

Basidiospores globose to broadly ellipsoid, 5.8−*6.4*−*7.0*−7.9 × 5.2−*5.8*−*6.3*−6.9 µm; Q = 1.01−*1.09*−*1.13*−1.25 (n=120); ornamentation amyloid, composed of ridges up to 0.7 µm high, forming an incomplete reticulum; isolated warts common, sometimes clustered; plage inamyloid to distally amyloid. *Basidia* 45−73 × 9−18 µm, mostly 4-spored, sometimes 1-spored, subclavate to subcylindrical, with guttate contents. *Pleuromacrocystidia* 44−87 × 11−20 µm, abundant, not protruding to protruding up to 20 µm, subfusiform, with a mucronate to moniliform apex, with granules and guttate contents. *Pleuropseudocystidia* abundant, 3−6 µm diam., cylindrical, tortuous, with fine granules. *Lamellar edge* heterogeneous, with basidia, marginal cells and cheilocystidia; marginal cells 24−40 × 8−15 µm, subcylindrical, subclavate to clavate; cheilocystidia 25−51 × 7−13 µm, rare to abundant, subfusiform, bent or irregular, with a mucronate to moniliform apex, with granular contents. *Lamellar trama* consisting of globose cells, septate hyphae and lactiferous hyphae. *Pileipellis* a hyphoepithelium, with an upper layer of repent to oblique hyphae; suprapellis a thin layer of repent hyphae about 10−25 µm thick; subpellis 40−60 µm thick, consisting of globose cells up to 30 µm diam.

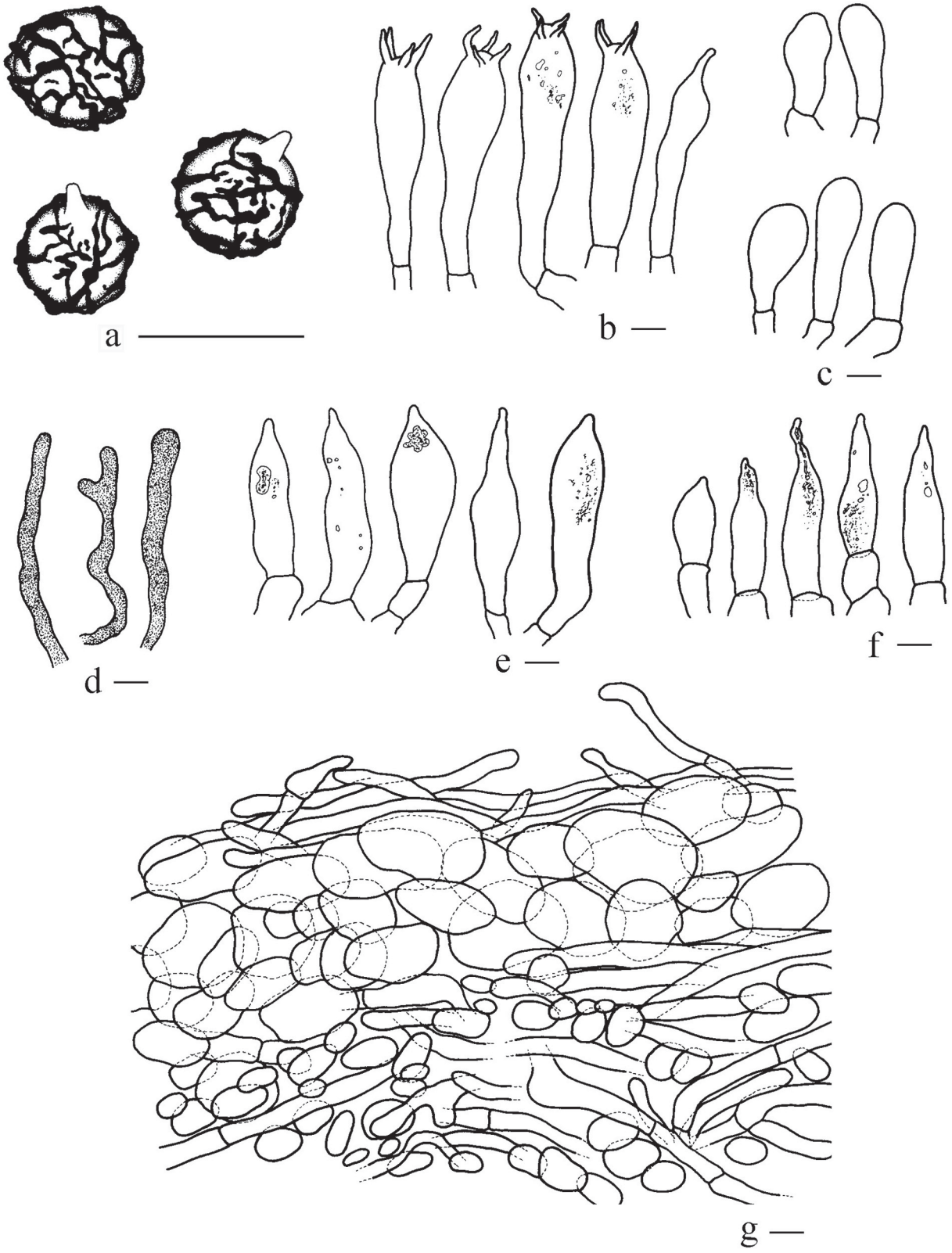


FIGURE 9. *L. rubrocorrugatus*: a. basidiospore, b. basidia, marginal cell, d. pseudocystidia, e. pleuromacrocystidia, f. cheilocystidia, g. pileipellis (a: KW384, holotype, b−g: KW294) (scale bar = 10 μm).

Habitat: gregarious on soil, in coniferous forest, dominated by *P. kesiya* and in tropical rain forests, dominated by *Shorea* sp., *Quercus* sp. and *Castanopsis* sp.

Collections examined: VIETNAM, Dalat province, Xa Xuan Truong, at km 7, left side of the main road, alt. 1499 m, N11º54.94 E108º32.01, 13/06/2012, KW042 (GENT!, MFLU!);—ibid. 13/06/2012, KW043 (GENT!,

MFLU!);−ibid. 13/06/2012, KW045 (GENT!, MFLU!); THAILAND, Chiang Mai province, Mae Taeng District, Pa Pae sub-district, Bahn Pha Deng village, Pathummikaram Temple, forest trail, alt. 1050 m, N19º06.28' E98º44.47, 9/06/2012, KW294 (GENT!, MFLU!); Chiang Rai province, Muang district, Thasai sub-district, forest at Doi Pui Reverse Signal Station, Doi Pui, alt. 740 m, N19º49.00 E99º52.03, 31/07/2012, KW381 (GENT!, MFLU!);−ibid. 31/07/2012, KW384 (holotype, GENT!, isotype, MFLU!);−ibid., 08/07/2013, KW453 (GENT!, MFLU!); Chiang Rai province, Chiang Khong district, forest near Bahn Nurnsomboon, alt. 450 m, N20º07.77 E100º26.40, 26/08/2011, KW123 (GENT!, MFLU!), Chiang Mai province, Mae Taeng District, Bahn Thapa, 22 marker on highway 1095, alt. 750 m, N19º07.41 E98º45.57, 31/07/2014, EDC14-505 (GENT!, MFLU!); Loei province, Phu Ruea district, junction from highway 203, alt. 1045 m, N17º28.09 E101º26.18, 27/06/2013, KW443 (GENT!, MFLU!).

Comments: Lactarius rubrocorrugatus can be easily distinguished from the other Southeast Asian species with transparent latex because of its small size, and a hygrophanous and rugulose cap. The other species with transparent latex described in the present work are *L. aquosus* and *L. tangerinus*. *Lactarius aquosus* differs from *L. rubrocorrugatus* by the zonate, smooth cap and a cutis for a pileipellis. *Lactarius tangerinus* has a reddish orange cap and a trichoderm pileipellis. *Lactarius rubrocorrugatus* is also similar to the European *L. rostratus* Heilmann-Clausen because of the characters mentioned above. The major difference can be found in the macrocystidia. *Lactarius rubrocorrugatus* has larger cystidia and the apex is not as acute as in *L. rostratus*. *Lactarius rubrocorrugatus* has been found in Vietnam and Thailand. The putative host range of the species is broad. The Vietnamese specimens were found in coniferous forest dominated by *P. kesiya* at higher altitude, around 1500 m above sea level, while the Thai specimens were found in evergreen forests dominated by members of the Fagaceae at lower altitude (alt. between 700–1000 m).

Lactarius tangerinus H.T. Le & De Crop, *sp. nov.* (Figure 10, 11e and 11f)

MycoBank number: MB811748; *Facesoffungi number*: FoF00641

Diagnosis: a small to medium sized species with reddish brown to reddish orange cap and a paler margin, watery latex, incompletely to almost completely reticulate basidiospores, pleuromacrocystidia present and pileipellis a trichoderm.

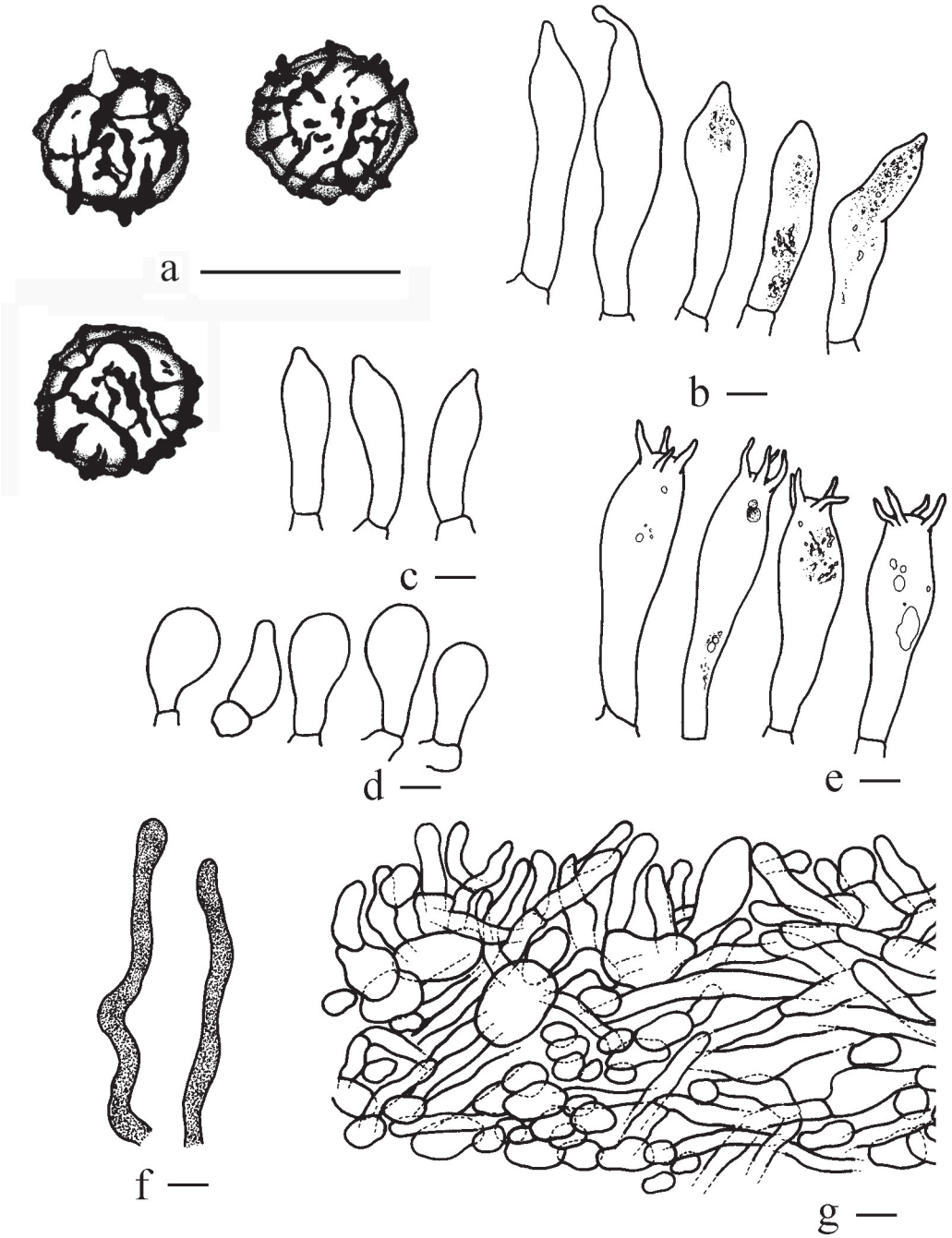
Etymology:—'tangerinus' refers to the reddish orange color on cap.

Typus:—THAILAND, Chiang Mai province, Mae Teang district, Pa Pae sub-district, Bahn Pha Deng village, mushroom research center, N19º17.12 E98º44.00, alt. 900 m, 28/07/2004, LTH 203 (holotype GENT!, isotype, CMU!, isotype, SFSU!).

Basidiocarps small to medium sized. *Pileus* 8−36 mm diam., plane to slightly infundibuliform with a central depression; surface rugulose, typically darker in center, reddish brown (8E8−F8) to dark brown (7F8), reddish blond (6C6), to beige, paler towards the margin, orange-yellow (4A3−5), light yellow (4A5) at the margin; margin striate at maturity. *Lamellae* 1.5−4 mm broad, decurrent with tooth, crowded, with 3−4 series of lamellulae, yellowish white (4A1−2) to light yellow (4A4) to grayish orange (5B5). *Stipe* 10−36 × 2−6 mm, cylindrical to tapering downwards, central to eccentric; surface dry, smooth, rugose when old, with paler color at apex, grayish orange (6B4) to pale orange (5A3) at apex, brownish orange (6C4), light brown (6D4), light brown (7D7−8), whitish pruinose at the base solid to hollow. *Context* 1−2 mm broad in the pileus, pale pinkish cream (5A3) to gray cream (5A−B3); odor like *L. quietus* or Pentatomidae bugs; taste mild. *Latex* transparent, abundant, unchanging on exposure, unchanging with 10 % KOH; taste mild. *Macrochemical reaction* on context: becoming pale grayish green with 10 % KOH, light grayish blue with $FeSO_4$.

Basidiospores subglobose to broadly ellipsoid, (5.8−)6.1−*6.6*−*7.2*−7.9 × 5.4−*6.1*−*6.3*−7.0 (−7.2) µm; Q = 1.02−*1.09*−*1.13*−1.25 (n=40); ornamentation amyloid, up to 1.8 µm high, composed of blunt to subacute, thick irregular ridges, forming an incomplete to almost complete reticulum; short ridges interconnected by fine lines; plage inamyloid to slightly amyloid. *Basidia* 30−65 × 9−18 µm, 4-spored, mostly subclavate, rarely subcylindrical, with fine granules and guttate contents. *Pleuromacrocystidia* not abundant, 35−77 × 10−18 µm, subcylindrical to subclavate, with mucronate apex, protruding up to 10 µm. *Pleuropseudocystidia* 2−4 µm broad, not protruding, tortuous to straight, cylindrical, with fine granules. *Lamellar edge* heterogeneous, consisting of basidia, subcylindrical to subclavate to obovoid marginal cells 15−30 × 4−15 µm, and a few cheilocystidia 39−49 × 11−15 µm, not protruding, subclavate, with mucronate apex. *Lamellar trama* composed of cylindrical hyphae, lactiferous hyphae and sphaerocysts. *Pileipellis* a trichoderm, about 35−50 µm thick, consisting of erect cylindrical hyphae, subpellis mainly with small globose cells, around 10−20 µm diam., intermixed with hyphae.

Habitat: solitary or gregarious on soil among leaf litter, near *Lithocarpus thomsonii* and *L. elegon* stands.



FIGRUE 10. *L. tangerinus*: a. basidiospore, b. pleuromacrocystidia, c. cheilocystidia, d. marginal cell, e. basidia, f. pseudocystidia, g. pileipellis (a−g: LTH203, holotype) (scale bar = 10 μm).

Collections examined: THAILAND, Chiang Mai province, Mae Teang district, Pa Pae sub-district, Bahn Pha Deng village, mushroom research center, N19º17.12 E98º44.00, alt. 900 m, 28/07/2004, LTH 203 (holotype GENT!, isotype, CMU!, isotype, SFSU!);—ibid., 13/08/2004, LTH217 (GENT!, CMU!, SFSU!);−ibid., 01/07/2011, KW091 (GENT!, MFLU!);—ibid., 28/07/2014, EDC14-475 (GENT!, MFLU!).

Comments: Immature basidiocarps of this species might be mistaken for *L. fuscomaculatus* or *L. inconspicuus*. However, *L. fuscomaculatus* has watery white latex and develops dark brown spots on the cap in mature specimens. The latex also separates *L. tangerinus* from *L. inconspicuus*. The latter species has watery white latex that slowly turns yellowish white or pale yellow. For the microscopic differences between both species, see under *L. inconspicuus*.

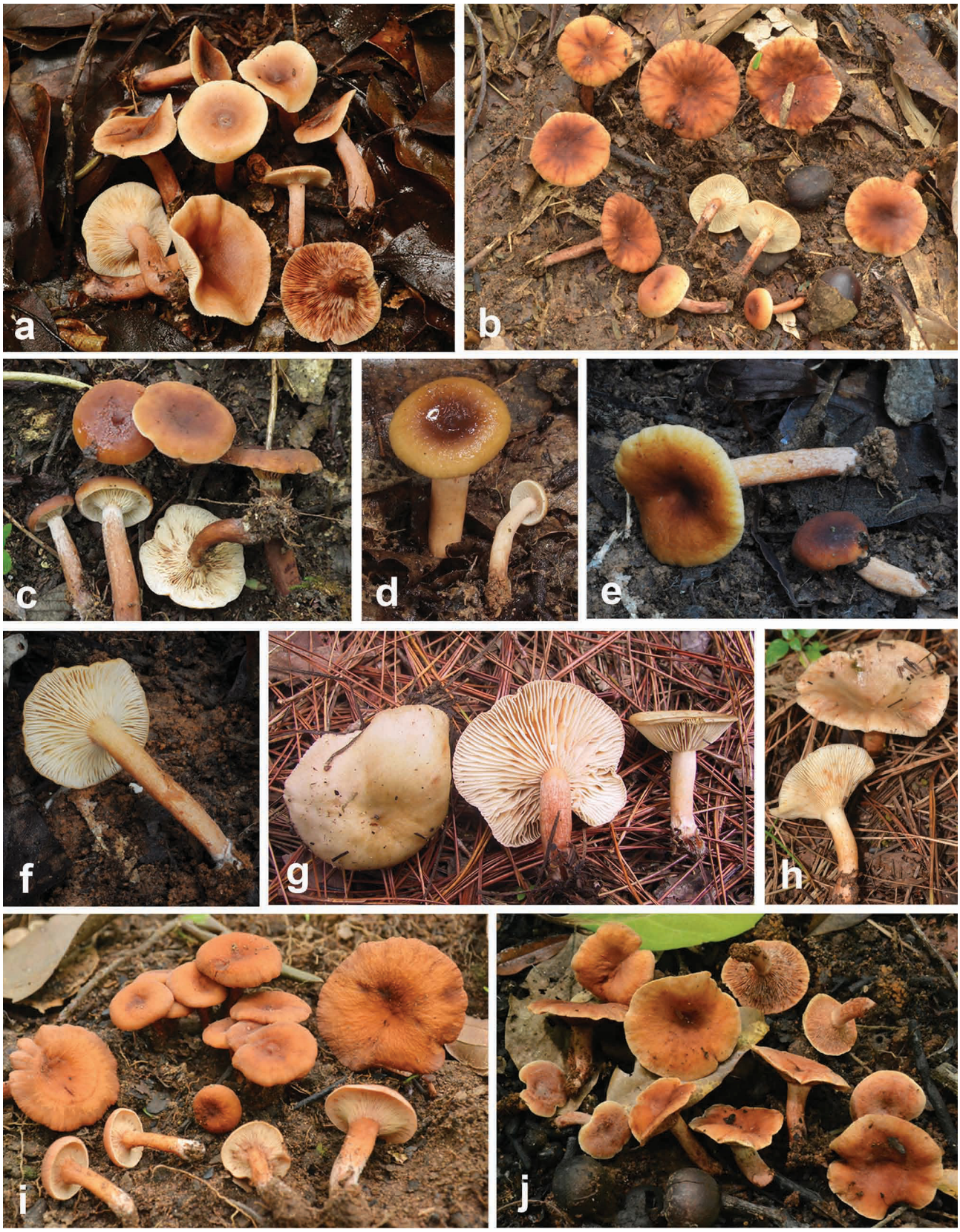


FIGURE 11. basidiocarps a. *L. aquosus* (KW231), b. *L. fuscomaculatus* (KW373), c. *L. rubrobrunneus* (AV12-044, photo by A. Verbeken), d. *L. inconspicuus* (KW339, photo by F. Hampe), e–f. *L. tangerinus* (EDC14-475, photo by E. De Crop), g–h. *L. kesiyae* (g: KW032, h: KW353), i. *L. rubrocorrugatus* (KW384), j. *L. chichuensis* (KW271).

Identification key to the Southeast Asian *Lactarius* species

1. Basidiocarps rather small sized, an average size around 20 mm ..... (2)
1. Basidiocarps medium sized ..... (5)
2. Pileus margin typically with triangle tufts ..... *Lactarius gracilis*
2. Pileus margin without tufts ..... (3)
3. Basidiospores complete reticulum; pleuromacrocystidia absent; stipe mostly with whitish pruinose at base ..... *Lactarius crenulatulus*
3. Basidiospores incomplete reticulum; pleuromacrocystidia present; stipe mostly with strigose at base ..... (4)
4. Pileus smaller dimension (5−7 mm diam.), with or without an inconspicuous papilla; pileipellis a cutis ..... *Lactarius perparvus*
4. Pileus larger dimension (5−40 mm diam.), with an acute papilla; pileipellis an epithelium ..... *Lactarius glabrigracilis*
5. Distant lamellae; pileus with sulcate striations ..... (6)
5. Crowded lamellae; pileus without sulcate striations ..... (9)
6. Pleuromacrocystidia present ..... (7)
6. Pleuromacrocystidia absent ..... (8)
7. Pleuromacrocystidia strikingly protruding from the hymenium; basidiospores complete reticulum ..... *Lactarius sublaccarioides*
7. Pleuromacrocystidia not protruding from the hymenium; basidiospores incomplete reticulum ..... *Lactarius stubbei*
8. Pileus surface non-velutinous; stipe long (26−71 mm in length) and often very hispid ..... *Lactarius laccarioides*
8. Pileus surface minutely velutinous; stipe shorter (9−22 mm in length) and hairs only at the base ..... *Lactarius pasohensis*
9. Latex transparent, completely watery and unchanging on exposure ..... (10)
9. Latex watery white to white ..... (13)
10. Pileus inconspicuously zoned, reddish brown to brown, paler towards margin; lamellae discoloring brown or with brown spots; pileipellis a cutis ..... ***Lactarius aquosus sp. nov.***
10. Pileus without zonation ..... (11)
11. Pileipellis a trichoderm ..... ***Lactarius tangerinus sp. nov.***
11. Pileipellis a hyphoepithelium ..... (12)
12. Pleuromacrocystidia with a rostrate apex, remarkably protruding up to 40 µm ..... ***Lactarius austrorostratus sp. nov.***
12. Pleuromacrocystidia different; basidiospores with low ornamentation (less than 1 µm high), an incomplete reticulum; pileus rugulose ..... ***Lactarius rubrocorrugatus sp. nov.***
13. Pileipellis covered with a thin slime layer ..... (14)
13. Pileipellis without a thin slime layer ..... (15)
14. Latex watery white, unchanging on exposure, turning yellow on a white handkerchief, associated with conifers (*Pinus*) ..... ***Lactarius kesiyae sp. nov.***
14. Latex watery white, slowly turning to pale yellow on exposure; growing with *Castanopsis* ..... ***Lactarius inconspicuus sp.nov.***
15. Basidiospore ornamentation zebroid, ridges up to 1 µm high ..... *Lactarius chichuensis*
15. Basidiospore ornamentation incomplete reticulum, ridges up to 1 µm high ..... (16)
16. Pileipellis a hyphoepithelium; pileus reddish brown to orange-brown with distinct dark brown discolorations and spots ..... ***Lactarius fuscomaculatus sp. nov.***
16. Pileipellis a transition between hyphoepithelium and epithelium; pileus more unicolorous, reddish orange to reddish brown ..... ***Lactarius rubrobrunneus sp. nov.***

Discussion

This contribution is the third in a series of publications reporting the diversity of *L.* subg. *Russularia* in Southeast Asia. According to the species concept stated in De Queiroz (2007), speciation occurs when a lineage acquires genotypic divergence and different recognizable characters. Thus, species delimitation is ideally based on the concordance of morphological characters and molecular evidence. Including the previous studies of Wisitrassameewong *et al.* (2014a, 2014b), we have described 15 new species of *L.* subg. *Russularia* from mycological expeditions in Thailand, Malaysia and Vietnam. Most of them grow in broadleaf evergreen forests with members of the Fagaceae, except for the conifer associated taxon, *L. kesiyae*. Relatively few distinct field characters could be defined and applied for all described species because of subtle macro-morphological differences among species on this subcontinent. *Lactarius fuscomaculatus* and *L. rubrobrunneus* seem to form one of the most complex groups. These two species can be easily confused in the field and are also closely related in our phylogenetic analysis. *Lactarius fuscomaculatus* mainly differs from *L. rubrobrunneus* by the dark brown spots on its pileus and the subtle difference in the terminal layer of the pileipellis. Although most Southeast Asian taxa are superficially similar in their basidiocarp color and latex features, which could lead to confusion in field identification, we consider none of the Southeast Asian species to be morphologically cryptic (as described in the part of identification keys). All 15 described species are well-delimited using ITS sequence data and the molecular evidence is consistent with their morphological differences. Distant gills, sulcate pileus striation and sticky pileus surface can be used as field characters in some species. We notice that representatives with a sticky

surface seem to be rare, at least in this region. Considering the low variety in latex features, this character is not particularly useful to differentiate among species. Basidiospore ornamentation, presence or absence of true cystidia and pileipellis structure appear to be more reliable for species delimitation. From our experience, many representatives possess basidiospores with an incomplete to almost complete reticulum while a complete reticulum, isolated warts and zebroid ornamentation sometimes occur. The loss of true cystidia arose during a speciation event in several taxa. The presence of a thin slime layer and presence or absence of terminal hyphal elements in the pileipellis are considered important characters.

Our molecular analysis involves specimens from Asia, North America and Europe. So far all Southeast Asian taxa are endemic to the subcontinent and no intercontinental conspecificity with temperate representatives has been found (Figure 1). All new species are well-supported in our molecular phylogram and we found concordance between field characters, microscopic characters and phylogenetic positions. The closest relative of *L. austrorostratus* is *L. chichuensis* and the newly proposed species is sister to the clade that includes *L. rostratus* and *L. rubrocorrugatus*. *Lactarius rubrocorrugatus*, *L. kesiyae* and *L. tangerinus* form distinct groups in the phylogram. *Lactarius aquosus* and the European *L. quietus* split from the same ancestor. Although both species are phylogenetically related and have an inconspicuous pileus zonation, other features are different. *Lactarius quietus* is easy to recognize due to its pinkish buff cap and strong Pentatomidae bug odor (Heilmann-Clausen *et al*., 1998). Microscopically, *L. aquosus* has a cutis and *L. quietus* has a trichopalisade as a pileipellis. *Lactarius inconspicuus* falls within a clade with *L. decipiens*. Both species have similar characters, such as yellowing milk, large and acute pleurocystidia and a thin glutinous layer with erect terminal hyphae in the pileipellis. *Lactarius decipiens* mainly differs from the Asian taxon by the pinkish fruiting body, the odor of *Pelargonium* and reticulate basidiospore ornamentation.

Our results and recent studies suggest that the diversity of *L.* subg. *Russularia* in Southeast Asia is higher than we previously thought. We emphasize that tropical rainforests in this continent comprise many ectomycorrhizal trees and a large diversity of *Lactarius* species. However, many regions in this continent are undersampled. DNA sequence data of several known Southeast Asian taxa are lacking. Further expeditions are necessary to explore undescribed indigenous species in undersampled areas and to obtain more sample collections and molecular data of known Asian species. Additional gene markers are necessary in order to better resolve the evolutionary relationship between species.

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