



Tales of the unexpected: angiocarpous representatives of the *Russulaceae* in tropical South East Asia

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Key words

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gasteroid fungi
hypogeous fungi
Lactarius
Martellia
morphology
phylogeny
Zelleromyces

Abstract Six new sequestrate *Lactarius* species are described from tropical forests in South East Asia. Extensive macro- and microscopical descriptions and illustrations of the main anatomical features are provided. Similarities with other sequestrate Russulales and their phylogenetic relationships are discussed. The placement of the species within *Lactarius* and its subgenera is confirmed by a molecular phylogeny based on ITS, LSU and *rpb2* markers. A species key of the new taxa, including five other known angiocarpous species from South East Asia reported to exude milk, is given. The diversity of angiocarpous fungi in tropical areas is considered underestimated and driving evolutionary forces towards gasteromycetization are probably more diverse than generally assumed. The discovery of a large diversity of angiocarpous milkcaps on a rather local tropical scale was unexpected, and especially the fact that in Sri Lanka more angiocarpous than agaricoid *Lactarius* species are known now.

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INTRODUCTION

Sequestrate and angiocarpous basidiomata have developed in several groups of Agaricomycetes. Various plausible selective pressures have been proposed to explain this transformation from agaricoid to gasteroid basidiomata (Miller et al. 2001). It is often assumed that changing environmental conditions led to enclosed basidiome morphology and eventually to the hypogeous gasteroid fruiting bodies, offering protection against frost and moisture loss from the hymenium and thus preventing desiccation (Thiers 1984a, Bruns et al. 1989, Bougher & Lebel 2001, Wilson et al. 2011). Arid or seasonally dry climates thus exert a selection pressure towards a sequestrate fruiting body, especially in ectomycorrhizal fungi which provide the plants with extra water through their mycelium and help them to survive the xeric conditions (Trappe & Claridge 2005, Smith et al. 2006). The observation that gasteroid and hypogeous gasteroid russuloid taxa are rare or absent in the humid tropics seems to support this idea (Buyck 1995).

Gasteroid *Russulales* are indeed particularly well-represented and well-studied in Australia and New Zealand (Bougher 1997, Bougher & Lebel 2001, Lebel 2001, 2002, 2003a, b, Lebel & Castellano 2002) and North America (Zeller & Dodge 1919, 1936, Singer & Smith 1960, Smith 1963, Thiers 1984b, Miller & Lebel 1999, Desjardin 2003, Smith et al. 2006). Tropical records seem rare and occasional. Only eight species that are currently accepted in the *Russulales* have been described from tropical Asia. Corner & Hawker (1953) described one *Arcangeliella* species and two *Elasmomyces* species from Malaysia and Heim (1959) described *Elasmomyces densus* from Thailand. In China, Zhang & Yu (1990) described two angiocarp *Rus-*

sulales species (*Gymnomyces lactifer* B.C. Zhang & Y.N. Yu and *Martellia ramispina* B.C. Zhang & Y.N. Yu) and Tao et al. (1993) described *Martellia nanjingensis* B. Liu & K. Tao and *Zelleromyces sinensis* B. Liu, K. Tao & Ming C. Chang.

Tropical Africa seems even poorer in sequestrate *Russulales* with only *Lactarius dolichocaulis* (Pegler) Verbeken & U. Eberh., *L. angiocarpus* Verbeken & U. Eberh. and *Cystangium capitis-orae* (Dring) T. Lebel (Dring & Pegler 1978, Eberhardt & Verbeken 2004, Verbeken & Walley 2010).

It is now generally accepted and in many cases molecularly confirmed that *Gymnomyces*, *Martellia*, *Cystangium* and *Elasmomyces* are synonyms of *Russula* and that *Zelleromyces* and *Arcangeliella* are included in *Lactarius* (Miller et al. 2001, Eberhardt & Verbeken 2004, Nuytinck et al. 2004).

This study reports on collections of gasteroid representatives of *Russulales*, encountered in tropical forests in the area around Shinharaja Forest Reserve, Sri Lanka, and around Chiang Mai, Northern Thailand. None of the collected specimens fits with previously described taxa, therefore six new species in the genus *Lactarius* are described here. Molecular data were used to ascertain their phylogenetic position and full descriptions and illustrations are given.

MATERIALS AND METHODS

The study is based on collections made by Kobeke van de Putte, Annemieke Verbeken and Dirk Stubbe. The studied material is deposited in the Herbarium Universitatis Gandavensis (GENT). An overview of the studied specimens, including information on the collection locality and ecology is given after each species description section.

Morphological study

Descriptions of macromorphological features are based on fresh material. Colours were described in daylight conditions following the colour guide by Kornerup & Wanscher (1978) and Petersen (1996, indicated as FK in descriptions). Latex coloration was recorded as it was exuded from the mushroom,

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Table 1 Specimens and GenBank accession numbers of DNA sequences used in the molecular analyses.

Species	Voucher collection	Origin	ITS accession no.	LSU accession no.	rpb2 accession no.
<i>Amylostereum laevigatum</i>	olrim409/CBS623.84		AY781246	AF287843	AY218469
<i>Arcangeliiella borziana</i>		Switzerland	AF286204		
		Switzerland	AF373599		
		Italy	JF908775		
<i>A. camphorata</i>		USA	EU644700		
		USA	EU644701		
		USA	EU644702		
		USA	EU834192		
		USA	EU846241		
<i>A. crassa</i>		USA	AY558740		
<i>A. sp.</i>		Thailand	FJ454900		
		Australia, Tasmania	JF960610		
		USA	JX415331		
<i>Auriscalpium vulgare</i>	AFTOL1897/DAOM128994		DQ911613	DQ911614	AY218472
<i>Echinodontium tinctorium</i>	AFTOL455		AY854088	AF393056	AY218482
<i>Lactarius acris</i>	EU014 (UPS)	Germany	DQ421988	DQ421988	DQ421922
<i>L. akahatsu</i>	AV2004-141 (GENT)	Thailand	KF133269	KF133301	KF133333
<i>L. albocarneus</i>	AV98-080 (GENT)	France	KF241545		
<i>L. alboscrobiculatus</i>	LTH175 (CMU, SFSU, GENT)	Thailand	EF141538		
<i>L. angiocarpus</i>	DA00-448 (GENT)	Zambia	AY606942	AY606970	DQ421921
<i>L. atroviridis</i>	AV05-306 (GENT)	USA	KF133270	KF133302	KF133334
<i>L. auriolla</i>	RW1601 (GENT)	Sweden	KF133257	KF133288	KF133321
<i>L. azonites</i>	AV00-124 (GENT)	Belgium	KF241540		
<i>L. baliophaeus</i>	AV05-155 (GENT)	Malawi	GU258277	GU265576	GU258312
<i>L. camphoratus</i>	UE04.09.2004 (UPS)	Sweden	DQ422009	DQ422009	DQ421933
<i>L. chichuensis</i>	Wang1236 (HKAS)	China	KF241541		
<i>L. chromospermus</i>	AV99-174 (GENT)	Zimbabwe	KF133260	KF133292	KF133324
<i>L. chrysorrheus</i>	UE04.10.2002-8 (UPS)	Italy	KF133261	KF133293	KF133325
<i>L. citriolens</i>	UE20.09.2004-03 (UPS)	Sweden	DQ422003	DQ422003	DQ421931
<i>L. controversus</i>	AV00-117 (GENT)	Italy	KF241544		
<i>L. crassiusculus</i>	LTH369 (GENT)	Thailand	EF560684	KF133303	KF133335
<i>L. cyanescens</i>	DS06-058 (GENT)	Malaysia	GU258278	CU265581	GU258317
<i>L. cyathuliformis</i>	UE04.09.2004-2 (UPS)	Sweden	KF133266	KF133298	KF133330
<i>L. deliciosus</i>	JN2001-046 (GENT)	Slovakia	KF133272	KF133305	KF133337
<i>L. echinellus</i> sp. nov.	AV07-169 (GENT)	Sri Lanka	KF133287	KF133320	KF133352
	AV07-175 (GENT)	Sri Lanka	KF133286	KF133319	KF133351
<i>L. echinus</i> sp. nov.	AV07-168 (GENT)	Sri Lanka	KF133273	KF133306	KF133338
<i>L. falcatus</i> sp. nov.	KVP08-038 (GENT)	Thailand	KF133274	KF133307	KF133339
<i>L. flexuosus</i>	UE06.09.2002-1 (UPS)	Sweden	DQ421992	DQ421992	DQ421925
<i>L. formosus</i>	LTH382 (CMU, SFSU, GENT)	Thailand	EF141549		
<i>L. fuliginosus</i>	MTB97-24 (GENT)	Sweden	JQ446111	JQ446180	JQ446240
<i>L. helvus</i>	UE08.09.2004-1 (UPS)	Sweden	KF133263	KF133295	KF133327
<i>L. hispidulus</i>	AB152 (GENT)	Guinea	KF133258	KF133289	KF133322
<i>L. kabansus</i>	AV99-205 (GENT)	Zimbabwe	KF133259	KF133291	KF133323
<i>L. lignyotus</i>	UE06.09.2003-5 (UPS)	Sweden	DQ421993	DQ421993	DQ421926
<i>L. lilacinus</i>	RW3774 (GENT)	Belgium	KF133275	KF133308	KF133340
<i>L. luridus</i>	OB11-011 (GENT)	Belgium	KF241547		
<i>L. mairei</i>	AV00-118 (GENT)	Italy	AY336950		
<i>L. mammosus</i>	UE09.09.2004-5 (UPS)	Sweden	KF133265	KF133297	KF133329
<i>L. montoyae</i>	KD1065 (BSHC)	India	EF560673	GU265641	GU258380
<i>L. necator</i>	AV04-231 (GENT)	France	KF133276	KF133309	KF133341
<i>L. peckii</i>	JN2004-020 (GENT)	USA	KF133277	KF133310	KF133342
<i>L. pomiolens</i> sp. nov.	AV07-159 (GENT)	Sri Lanka	KF133282	KF133315	KF133347
<i>L. pubescens</i>	AV96-931 (GENT)	Norway	AY336958		
	UE15.09.2002-2 (UPS)	Sweden	DQ421996	DQ421996	DQ421929
<i>L. quieticolor</i>	UE10.09.2004-1 (UPS)	Sweden	DQ422002	DQ422002	DQ42930
<i>L. quietus</i>	UE16.09.2004 (UPS)	Sweden	KF133264	KF133296	KF133328
<i>L. romagnesii</i>	UE29.09.2002-6 (UPS)	France	DQ421989	DQ421989	DQ421923
<i>L. rubriviridis</i>	DED7312 (SFSU)	USA	EF685088		
<i>L. rufus</i>	JN2002-008 (GENT)	Norway	KF241543		
<i>L. saturnisporus</i> sp. nov.	AV07-170 (GENT)	Sri Lanka	KF133283	KF133316	KF133348
	DS07-488 (GENT)	Sri Lanka	KF133284	KF133317	KF133349
	DS07-490 (GENT)	Sri Lanka	KF133285	KF133318	KF133350
<i>L. shoreae</i> sp. nov.	AV07-164 (GENT)	Sri Lanka	KF133278	KF133311	KF133343
<i>L. sphagneti</i>	PL2805 (pers. herb. P. Leonard)	UK	KF133268	KF133300	KF133332
<i>L. spinosulus</i>	AT2003068 (UPS)	Sweden	KF133262	KF133294	KF133326
<i>L. stephensii</i>		UK	EU784439		
	RW2930 (GENT)	Belgium	AY331012		
<i>L. subdulcis</i>	JV2006-024 (GENT)	Belgium	KF133279	KF133312	KF133344
<i>L. subplinthogalus</i>	AV04-219 (GENT)	USA	KF241539		
<i>L. subsericatus</i>	UE11.10.2004-8 (UPS)	Sweden	DQ422011	DQ422011	DQ421934
<i>L. tenellus</i>	DKA3598 (BR)	Benin	KF133280	KF133313	KF133345
<i>L. thyinos</i>	A.Voitk23-08-2004 (GENT)	Canada	KF133271	KF133304	KF133336
<i>L. torminosus</i>	LVL2002-013 (GENT)	Belgium	AY336959		
	RW3183 (GENT)	Czech Republic	KF133281	KF133314	KF133346
<i>L. trivialis</i>	UE27.08.2002-17a (UPS)	Sweden	DQ421991	DQ421991	DQ421924
<i>L. uvidus</i>	KVP10-027 (GENT)	Russia	KF241546		
<i>L. vietus</i>	UE11.19.2004-1 (UPS)	Sweden	KF133267	KF133299	KF133331

Table 1 (cont.)

Species	Voucher collection	Origin	ITS accession no.	LSU accession no.	rpb2 accession no.
<i>L. vinaceorufescens</i>	JN2007-018 (GENT)	Canada	KF241542		
<i>Lactifluus deceptivus</i>	AV04-181 (GENT)	USA	DQ422020	DQ422020	DQ421935
<i>Lf. edulis</i>	AV99-041 (GENT)	Zimbabwe	DQ421977	DQ421977	DQ421916
<i>Lf. emergens</i>	AV99-005 (GENT)	Zimbabwe	AY606979	KF133290	DQ421919
<i>Lf. gerardii</i>	AV05-375 (GENT)	USA	GU258254	GU265616	GU258353
<i>Lf. longisporus</i>	AV99-197 (GENT) / BB 00.1519 (PC)	Zimbabwe/Madagascar	DQ421971 (AV)	DQ421971 (AV)	DQ421910 (PC)
<i>Lf. nodosicystidiosus</i>	BB97-072 (PC)	Madagascar	DQ421976	DQ421976	DQ421915
<i>Lf. phlebophyllus</i>	BB00-1388 (PC)	Madagascar	DQ421979	DQ421979	DQ421918
<i>Lf. piperatus</i>	UE09.08.2004-6 (UPS)	Sweden	DQ422035	DQ422035	DQ421937
<i>Lf. vellereus</i>	UE20.09.2004-22 (UPS)	Sweden	DQ422034	DQ422034	DQ421936
<i>Lf. velutissimus</i>	AV99-185 (GENT)	Zimbabwe	DQ421973	DQ421973	DQ421912
<i>Lf. volemus</i>	UE09.08.2004-5 (UPS)	Sweden	DQ422008	DQ422008	DQ421932
<i>Multifurca furcata</i>	RH7804 (NY)	Costa Rica	DQ421994	DQ421994	DQ421927
<i>M. ochricompacta</i>	BB02.107 (PC)	USA	DQ421984	DQ421984	DQ421940
<i>M. zonaria</i>	DED7442 (PC)	Thailand	DQ421990	DQ421990	DQ421942
<i>Russula aeruginea</i>	AT2003017 (UPS)	Sweden	DQ421999	DQ421999	DQ421946
<i>R. albonigra</i>	AT2002064 (UPS)	Sweden	DQ422029	DQ422029	DQ421966
<i>R. camarophylla</i>	PAM01081108 (PC)	France	DQ421982	DQ421982	DQ421938
<i>R. earlei</i>	WCRW00-412 (PC)	USA	DQ422025	DQ422025	DQ421963
<i>R. emetica</i>	UE05.10.2003-11 (UPS)	Sweden	DQ421997	DQ421997	DQ421943
<i>R. firmula</i>	AT2004142 (UPS)	Sweden	DQ422017	DQ422017	DQ421958
<i>R. gracillima</i>	UE23.08.2004-14 (PC)	Sweden	DQ422004	DQ422004	DQ421949
<i>R. heterophylla</i>	UE20.08.2004-2 (UPS)	Sweden	DQ422006	DQ422006	DQ421951
<i>R. illota</i>	UE26.07.2002-3 (UPS)	Sweden	DQ422024	DQ422024	DQ421967
<i>R. lepida</i>	HJB9990 (UPS)	Belgium	DQ422013	DQ422013	DQ421954
<i>R. nigricans</i>	UE20.09.2004-07 (PC)	Sweden	DQ422010	DQ422010	DQ421952
<i>R. ochrospora</i>	GD20.07.2004 (UPS)	Italy	DQ422012	DQ422012	DQ421953
<i>R. parazurea</i>	BW06.09.2002-16 / MF01.10.2003 (UPS)	Sweden	DQ422007 (MF)	DQ422007 (MF)	DQ421945 (BW)
<i>R. pectinatoides</i>	AT2001049 (UPS)	Sweden	DQ422026	DQ422026	DQ421964
<i>R. persicina</i>	UE21.09.2003-01 (UPS)	Sweden	DQ422019	DQ422019	DQ421960
<i>R. risigallina</i>	UE03.07.2003-08 (UPS)	Sweden	DQ422022	DQ422022	DQ421961
<i>R. vesca</i>	AT2002091 (UPS)	Sweden	DQ422018	DQ422018	DQ421959
<i>R. virescens</i>	HJB9989 (UPS)	Belgium	DQ422014	DQ422014	DQ421955
<i>Stereum hirsutum</i>	AFTOL492		AY854063	AF393078	AY218520
<i>Zelleromyces gardneri</i>		USA	DQ453696		
		USA	JN022500		
<i>Z. giennensis</i>		Spain	AF230900		
<i>Z. hispanicus</i>		Spain	AF231911		
		Spain	AF231912		
		Spain	AF231913		
		Spain	AJ555566		
		Spain	AJ555567		
<i>Z. sp.</i>		Australia, Tasmania	JF960852		
		Australia, Tasmania	JF960853		
		Australia, Tasmania	JF960854		

but also from a drop placed on a glass slide held over white paper, and from a drop placed directly on white paper. Pictures of the basidiocarps will be published on the *Russulales* News website (<http://www2.muse.it/russulales-news/>).

Micromorphological characters were registered from the dried specimens. Spores were observed in Melzer's reagent for measurements and drawings; other structures in 2–5 % KOH or Congo-red. For each collection the length and width of at least 20 spores were measured in side view in Melzer's reagent, excluding the ornamentation. Measurements are given as $(MIN_a) [AV_a - 2 \times SD] - AV_a - AV_b - [AV_b + 2 \times SD] (MAX_b)$ in which AV_a = lowest mean value for the measured collections, AV_b = greatest mean value and SD = standard deviation calculated for the measurements of one collection. Q stands for 'quotient length/width' and is given as $(MINQ) - Q_a - Q_b - (MAXQ)$ in which Q_a and Q_b stand for the lowest and the highest, respectively mean quotient for the measured specimens. In case only one collection was available spore measurements are given as $(MIN_a) [AV_a - 2 \times SD] - AV_a - [AV_a + 2 \times SD] (MAX_a)$. Micromorphological features were illustrated with the aid of a drawing tube attached to an Olympus CX-41 research compound microscope. For the details of description and terminology of micromorphological features see Verbeken (1998) and Verbeken & Walley (2010).

DNA extraction, PCR amplification and sequencing

Nine gasteroid *Lactarius* collections were used for the molecular analyses. DNA was extracted from dried and fresh fruiting bodies using the methods described by Nuytinck & Verbeken (2003) with slight modifications (van de Putte et al. 2010). Three loci were amplified and sequenced: 1) the internal transcribed spacer region of the nuclear ribosomal DNA (ITS), using primers ITS1-F and ITS4 (White et al. 1990, Gardes & Bruns 1993); 2) a part of the nuclear ribosomal large subunit region (LSU), using primers LR0R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994); and 3) the region between domains 6 and 7 of the nuclear gene encoding the second largest subunit of RNA polymerase II (*rpb2*), using primers bRPB2-6F and fRPB2-7cR (Liu et al. 1999, Matheny 2005).

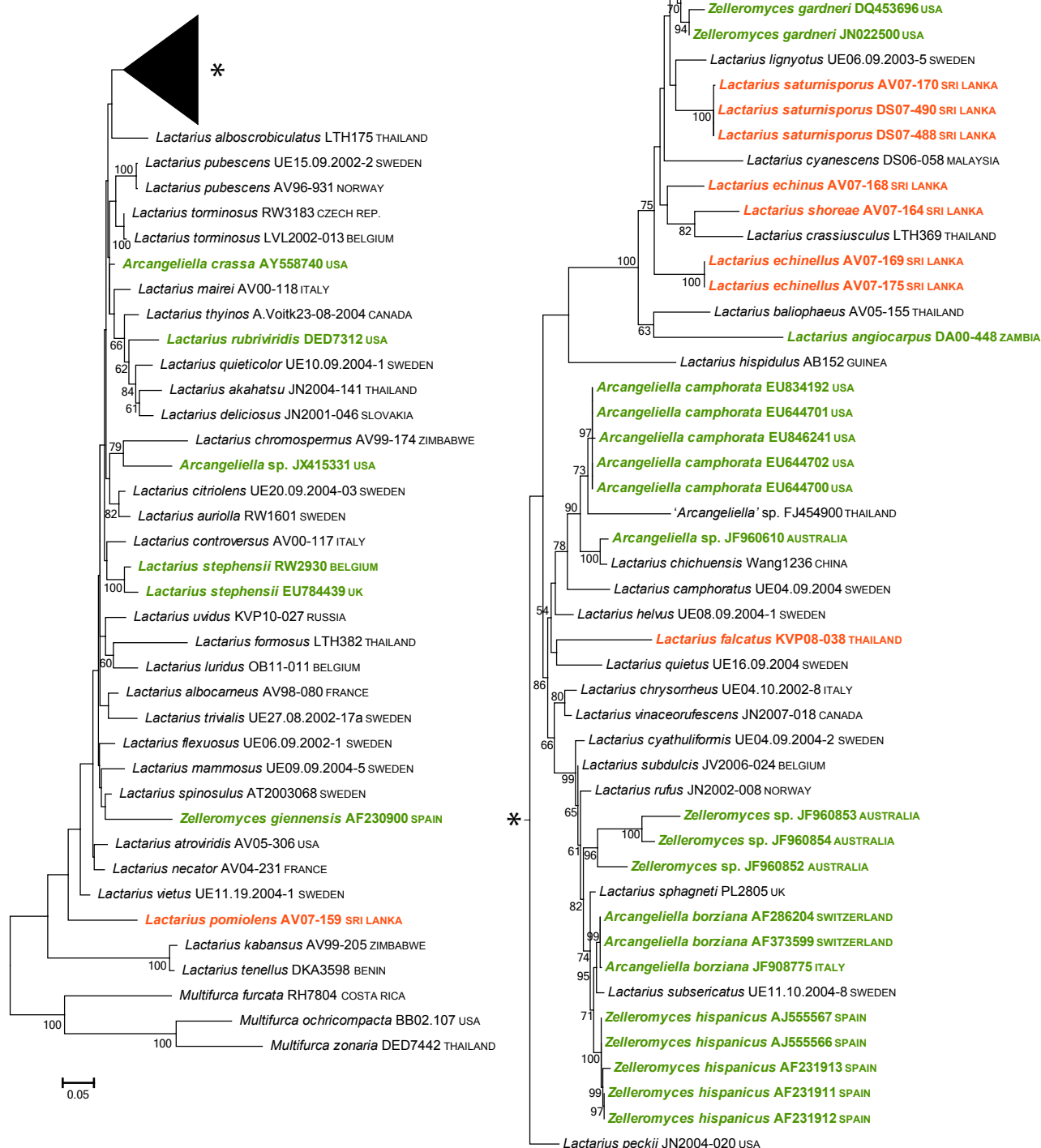
Protocols for PCR amplification and sequencing follow Le et al. (2007), sequencing was also conducted with an ABI 3730XL or ABI 3700 by MACROGEN (Amsterdam, The Netherlands). Sequences were assembled and edited with the software Sequencher™ v4.9 (GeneCodes Corporation, Ann Arbor, Michigan, USA). Other sequences were gained following the DNA extraction, PCR and sequencing protocols described by Eberhardt (2002) or Taylor et al. (2006).

Alignment and phylogenetic analyses

Table 1 shows an overview of all specimens and sequences used in the phylogenetic analyses, including GenBank accession numbers. Two alignments were constructed. The first alignment consists of ITS sequences only; it includes all sequestrate *Lactarius*, *Arcangeliella* and *Zelleromyces* sequences available from GenBank, the newly discovered sequestrate collections from Sri Lanka and Thailand, and a broad selection of agaricoid *Lactarius* taxa; three *Multifurca* species were used as the outgroup. This alignment is used to confirm that our new taxa are well supported and differ from the sequestrate milk cap

species that have been known and sequenced before. The second alignment consists of ITS, LSU and *rpb2* sequences, including only specimens for which all three loci are available. The sampling covers a broader selection of *Russulaceae*: the four genera of *Russulaceae* (*Lactarius*, *Lactifluus*, *Multifurca* and *Russula*, see Buyck et al. 2008) are represented. Outgroup *Russulales* species are *Auriscalpium vulgare* Gray, *Stereum hirsutum* (Willd.) Pers., *Amylostereum laevigatum* (Fr.) Boidin and *Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh. This second alignment is used to study the phylogenetic placement of the newly described species within *Lactarius* and its

Fig. 1 ML tree (RAXML) based on ITS sequences. Bootstrap values are indicated if they exceed 50 %. Names in orange are the new angiocarpous species described in this paper, names in green are angiocarpous *Lactarius* species for which ITS sequences are available on GenBank. *Arcangeliella* sp. FJ454900 was obtained by sequencing plant roots; the fruiting body was not observed, and thus it is unclear whether this sequence is actually from an angiocarpous species. The scale bar represents the number of nucleotide changes per site.



subgenera. Alignments were constructed with the online version of MAFFT v6 (Kato & Toh 2008), applying the E-INS-I strategy, a very slow method recommended for less than 200 sequences with multiple conserved domains and long gaps. The alignments were manually refined in BioEdit v7.0.9.0 (Hall 1999) and made available in TreeBASE (www.treebase.org, study ID: S14274). For the second alignment, ambiguously aligned positions (mainly within ITS1 and 2) were detected using Gblocks v0.91b (Castresana 2000), specifying less stringent conditions than default in order to keep gapped sites. Apart from the positions identified by Gblocks, the intron region of *rpb2* was also deleted from the analyses to avoid the inclusion of ambiguous alignment. Sequence data were partitioned as follows: 1) ITS was partitioned into the ribosomal genes 18S (partial) and 5.8S and the spacer regions ITS1 and ITS2; 2) LSU; and 3) *rpb2* was partitioned into codon positions 1, 2 and 3.

Maximum Likelihood (ML) analyses were performed in RAxML v7.0.3 (Stamatakis 2006), combining a ML search with the Rapid Bootstrapping algorithm for 1 000 replicates. The model GTRGAMMA was estimated for each partition separately. The analyses were first run for the individual loci. Incongruence between loci was checked by comparing clades with a bootstrap support of 70 % or higher.

Bayesian Inference (BI) analyses were carried out in MrBayes v3.2.0 (Ronquist & Huelsenbeck 2003). The general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR+I+G) was used. Rates and model parameters were unlinked between all partitions. Two analyses were run: 1) an analysis on a desktop computer with 2 runs and 1 chain per run was executed for 20 million generations (Ronquist et al. 2009); and 2) 4 independent, parallel runs of 1 cold and 3 heated chains were run for 20 million generations on a High Performance Computer (HPC) of the Ghent University. Sample frequency was set at 100. The log probability of the data given the parameter values and effective sample size statistics (ESS) of the runs were examined with Tracer v1.5 (Drummond & Rambaut 2007). To check convergence, 1) the standard deviation of split frequencies across the 2 runs on the desktop computer was assessed; and 2) topologies and posterior probabilities from the 4 runs on the HPC were compared. An appropriate burn-in value was determined visually using Tracer.

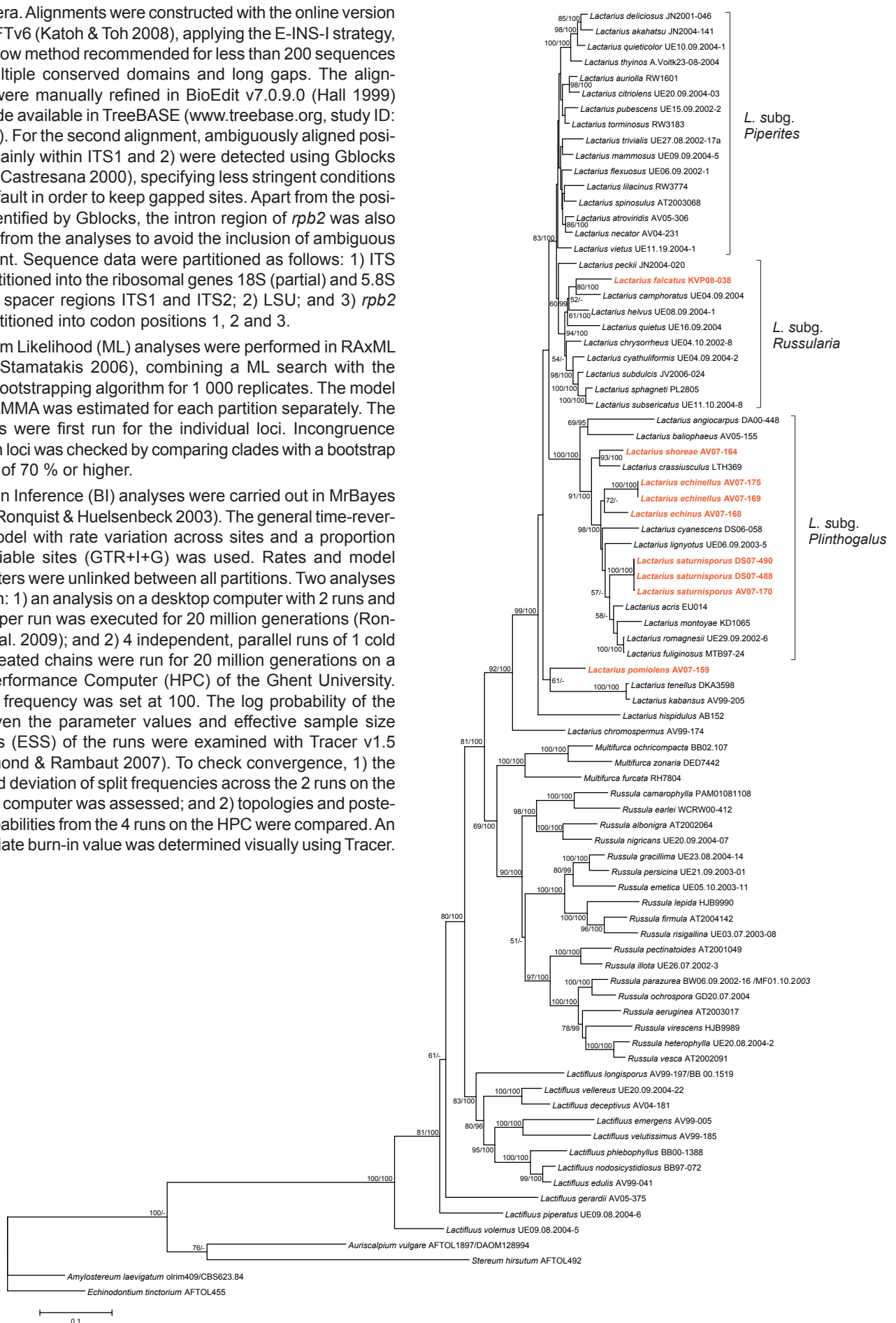


Fig. 2 ML tree (RAxML) based on ITS, LSU and *rpb2* sequences. Bootstrap values and Posterior Probabilities (resulting from Bayesian analysis using the HPC) are indicated if they exceed 50 % or 95 %, respectively (BS/PP). Names in orange are the new angiocarpous species described in this paper. The scale bar represents the number of nucleotide changes per site.

RESULTS

Phylogeny

Fig. 1 shows the obtained ML topology based on the alignment including only ITS sequences; bootstrap (BS) values are indicated on the branches. The six new sequestrate *Lactarius* species from South East Asia are indicated in orange and are clearly distinct from the previously known and sequenced sequestrate milk cap species (indicated in green).

The analysis based on all three sampled loci (ITS, LSU and *rbp2*) reveals the position of our South East Asian collections within the genus *Lactarius*. Since there was no conflict among the single loci trees in clades with a bootstrap support of 70 % or higher, a combined analysis was performed. Fig. 2 shows the obtained ML topology with BS values and Bayesian posterior probabilities (PP). ML and both Bayesian phylogenies differ only in the placement of some terminal, non-gasteroid taxa. All 3 analyses show 3 well-delimited genera in the *Russulaceae* (*Lactarius* BS 92 % - PP 100 %, *Multifurca* BS 100 % - PP 100 % and *Russula* BS 90 % - PP 100 %) but fail to support the monophyly of the genus *Lactifluus*. Instead, *Lactifluus* consistently comes out as paraphyletic and basal to the other *Russulaceae* genera. *Lactifluus volemus*, *Lactifluus piperatus* and *Lactifluus gerardii* are not included in a monophyletic 'core'-group of *Lactifluus* (BS 83 % - PP 100 %) represented by *Lactifluus* subg. *Lactifluus* p.p. (excluding section *Lactifluus*), subg. *Edules* and subg. *Lactariopsis*.

All gasteroid milkcaps included in this study belong to the genus *Lactarius*. *Lactarius falcatus* sp. nov. is member of *L.* subg. *Russularia*, while *L. saturnisporus* sp. nov., *L. echinus* sp. nov., *L. echinellus* sp. nov. and *L. shoreae* sp. nov. belong to *L.* subg. *Plinthogalus*. The affinities of *L. pomiolens* sp. nov. are less clear. It appears as one of the long, basal branches of the genus *Lactarius* for which no subgeneric subdivisions are available.

Taxonomy

All newly proposed species (*L. pomiolens*, *L. echinus*, *L. echinellus*, *L. saturnisporus*, *L. shoreae* and *L. falcatus*) produce milky exudates or latex. The additional known angiocarpous species from South East Asia reported to exude milk are also included in the species key. These are: *Arcangeliella lactifera* (B.C. Zhang & Y.N. Yu) J.M. Vidal, *A. densa* (R. Heim) Singer & A.H. Sm., *Zelleromyces ramispinus* (B.C. Zhang & Y.N. Yu) Trappe, T. Lebel & Castellano, *Z. sinensis* B. Liu, K. Tao & Ming C. Chang and *Martellia nanjingensis* (B. Liu & K. Tao) J.M. Vidal. Although we also consider these species to be members of the genus *Lactarius*, new combinations are not proposed here because we did not study the type specimens, or obtained molecular data.

It is striking that all tropical species have a very high spore ornamentation, either consisting of wings, or of isolated high spines, while all known Australian species have a much lower ornamentation that is usually subreticulate or formed of irregular warts, to sometimes even extremely low resulting in almost smooth spores, as in *Zelleromyces glabrellus* (Zeller & C.W. Dodge) Singer & A.H. Sm. (Zeller & Dodge 1936). The only angiocarpous milkcap species that have spores with a winged aspect are *Zelleromyces striatus* (G. Cunn.) G.W. Beaton, Pegler & T.W.K. Young and *Zelleromyces malaiensis* (Comer & Hawker) A.H. Sm., but the ridges are not exceeding 0.5 µm height (Pegler & Young 1979, Grgurinovic 1997). The latter species is also reported from India and Malaysia, but only with *Eucalyptus* (Trappe et al. 2002, Desjardin 2003).

KEY TO THE SPECIES

1. Spores winged, reticulate, usually with high ridges 2
1. Spores echinulate, with isolated warts or spines 5
2. Spores lowly ornamented, with small ridges that are 0.5–1.5 µm high *Z. sinensis*
2. Spores distinctly winged, with ornamentation that is clearly exceeding 2 µm high 3
3. Spores > 10 µm *L. pomiolens*
3. Spores < 10 µm 4
4. Spore ornamentation with ridges up to 3–4 µm high, with distinct transverse striations; peridiopellis a strongly interwoven trichopalisade, embedded in a narrow and incrustated slime-layer *L. saturnisporus*
4. Spore ornamentation with ridges up to 2.5–3.5 µm high, lacking striations; peridiopellis a strongly interwoven palisade to trichopalisade, without obvious slime layer *L. shoreae*
5. Basidia 4-spored, 2-spored basidia sometimes present 6
5. Basidia exclusively 2-spored 8
6. Spores ornamented with irregular warts to short spines, never more than 1 µm long *A. densa*
6. Spores ornamented with spines up to 2.5 µm long 7
7. Basidia only 4-spored; spines straight and slender, not branched *L. echinellus*
7. Basidia 2- and 4-spored; spines often branched on top *Z. ramispinus*
8. Spores on average 12 × 11.5 µm, ornamented with slender and straight spines up to 4 µm long *L. echinus*
8. Spores on average 10 × 9.3 µm or smaller 9
9. Spores ornamented with spines up to 4 µm long *A. lactifera*
9. Spores ornamented with spines that are at most 2 µm long 10
10. Spores ornamented with irregular and curved spines up to 2 µm long *L. falcatus*
10. Spores ornamented with conical to blunt spines up to 1.5 µm long *M. nanjingensis*

1. *Lactarius pomiolens* Verbeke & Stubbe, sp. nov. — MycoBank MB804182; Fig. 3

Holotype. SRI LANKA, near Sinharaja Forest, trail along river, on sandy wet soil in rainforest with *Shorea trapezifolia*, *Shorea disticta* and *Dipterocarpus hispidus*, 13 Dec. 2007, Verbeke 07-159 (GENT).

Etymology. With the smell of apples.

Basidiocarp 25–45 mm diam, subglobose, rather regular. *Peridium* very slightly tomentose, felty, ochraceous to leather-brown (FK13–14), buff to ochraceous, irregularly coloured, with patches. *Stipe* absent. *Columella* absent. *Gleba* strongly labyrinthoid, with small loculi, with some, but very few gelatinous veins among them, greyish yellow (4B4), a bit more flesh-coloured, staining dark brown where eaten by insect larvae, firm in youngest ones, more compressible, rubbery in older specimens. *Latex* rather abundant, white, staining immediately sulphur yellow to greenish yellow (1A5–6) on white paper, slowly changing yellow on the context then apparently disappearing, when isolated turning golden yellow in a 10 % aqueous potassium hydroxide solution, not forming a whitish layer on the gleba when drying. *Taste* bitter, astringent, not just dry. *Smell* very sweet, fruity, like apples, *Russula fellea*-like.

Spores globose to subglobose, 10.5–12.0–13.3 × 10.2–11.5–12.8 µm, n = 20, Q = 1.01–1.04–1.13; ornamentation amyloid, very highly winged; ridges up to 3–4 µm high, seldomly branched, rather broad and not completely amyloid but

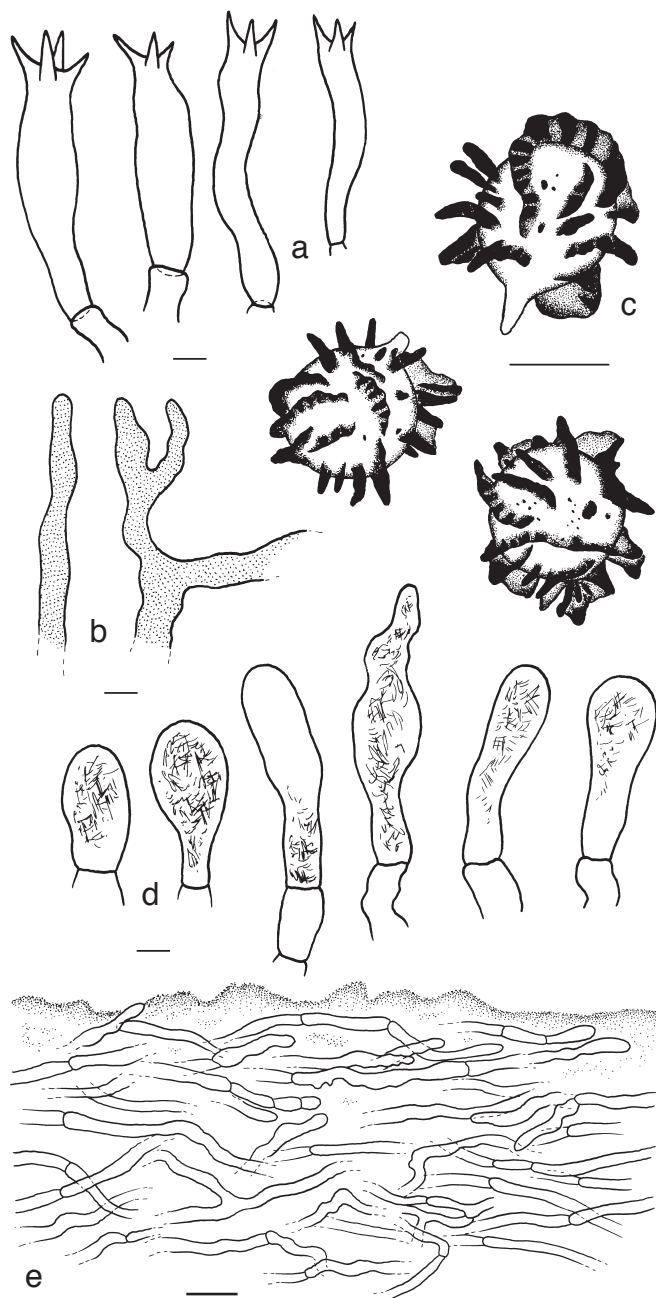


Fig. 3 *Lactarius pomiolens* (holotypus). a. Basidia; b. pseudocystidia; c. basidiospores; d. cystidia; e. peridiopellis. — Scale bar = 10 µm.

with strongly amyloid transversal bands; surface with amyloid spots between the ridges; plage not distinct, not amyloid. *Basidia* 40–55 × 5–13 µm, slender and cylindrical to subclavate, 4-spored, thin-walled, hyaline; sterigmata up to 8 µm long. *Pseudocystidia* present, irregular, sometimes branching, not emergent, 5–7 µm diam. *Cystidia* extremely abundant, mostly cylindrical, subclavate or clavate and regularly rounded on top, sometimes fusiform, 25–60 × 10–16 µm, with very dense needle-like and yellowish brown contents, with walls slightly refringent to very slightly thickened. *Peridiopellis* an ixocutis, composed of intricate, mostly pericline hyphae, 3–5 µm diam, sometimes with small bulges.

Habitat — Rainforest with *Shorea* sp. and *Dipterocarpus* sp.

Specimen examined. SRI LANKA, Kudawa, near Sinharaja Forest, trail along river, on sandy wet soil in rainforest with *Shorea trapezifolia*, *Shorea disticta* and *Dipterocarpus hispidus*, 13 Dec. 2007, A. Verbeken GENT AV07-159, holotype.

Notes — The species is outstanding because of its very large (average 12 × 11.5 µm) spores and wings. The distinct

smell of apples seems a striking character, but more records are needed to evaluate the stability of this feature.

2. *Lactarius saturnisporus* Verbeken & Stubbe, sp. nov. — MycoBank MB804180; Fig. 4

Holotype. SRI LANKA, Kudawa, near Sinharaja Forest Reserve, primary rainforest with *Shorea* spp., 14 Dec. 2007, Verbeken 07-170 (GENT).

Etymology. Referring to the spores that are so spectacularly winged that they are reminiscent of the planet Saturn and its ring system

Basidiocarp 15–25 mm diam, 10–15 mm high, subglobose to flattened or irregular, sometimes with minute papilla, slightly rooting. **Peridium** surface, minutely velutinous, chamois-leather-like, locally smooth or wrinkled, ochraceous cream coloured, sometimes with pinkish and purplish tinges. **Stipe** absent. **Columella** absent. **Gleba** rather soft and compressible, with labyrinthuloid and rounded loculi, 1–3 per mm, dull cream coloured to pale or greyish orange (6AB3) sometimes with pinkish and purplish tinges near the margin, faintly staining yellow, ultimately becoming pinkish but drying pale fawn, indistinct reaction with ferrous sulphate. **Latex** scarce to abundant, whitish hyaline, unchanging or slightly yellowing on the gleba, staining white paper yellow, becoming yellow in a 10 % aqueous potassium hydroxide solution. **Taste** bitter to astringent, disagreeable but also somewhat acrid. **Smell** not remarkable or somewhat like citrus fruit.

Spores globose to subglobose, 8.0–8.9–9.7 × 7.6–8.3–9.0 µm, n = 20, Q = 1.01–1.07–1.12; ornamentation amyloid, very highly winged; ridges up to 3–4 µm high with distinct transversely

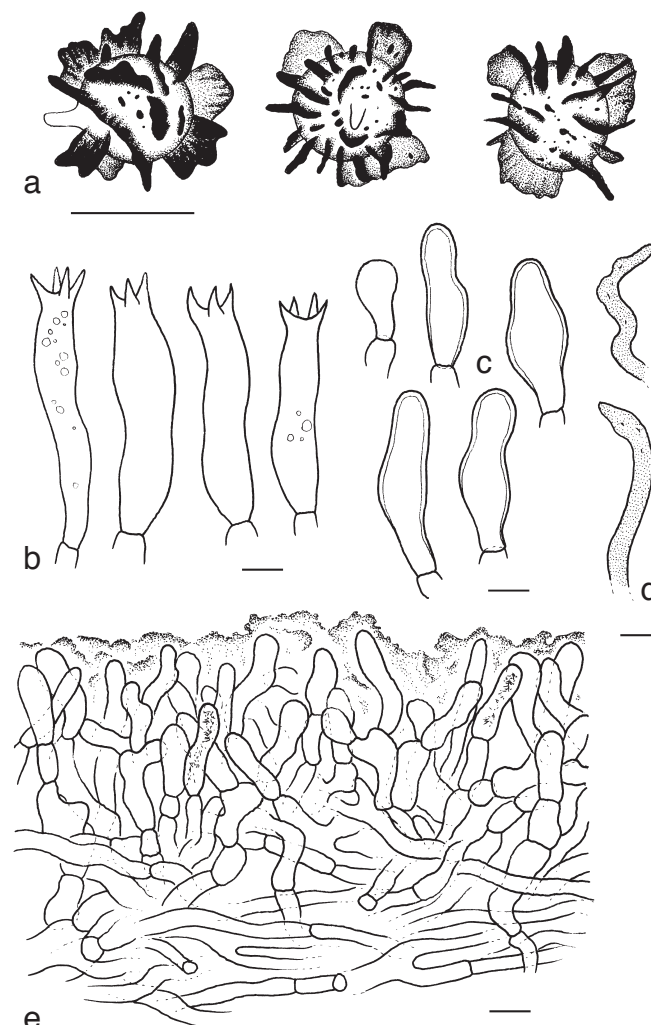


Fig. 4 *Lactarius saturnisporus* (holotypus). a. Basidiospores; b. basidia; c. cystidia; d. pseudocystidia; e. peridiopellis. — Scale bar = 10 µm.

striped and bifurcating pattern, mostly unbranching, sometimes branched but never forming a reticulum, edges sharp and mostly crenate; surface roughly amyloid and verrucose in between the ridges; plage not distinct, not amyloid. *Basidia* 4-spored, subcylindrical, $45\text{--}60 \times 10\text{--}12\ \mu\text{m}$, thin-walled, hyaline or with some oil-drops; sterigmata up to $8\ \mu\text{m}$ long. *Cystidia* present in the hymenial cavities, rather abundant, variable in shape, some clavate, some slightly utriform, $20\text{--}45 \times 10\text{--}15\ \mu\text{m}$, with slightly thickened wall, hyaline. *Pseudocystidia* less abundant, cylindrical to somewhat tortuous, $4\text{--}6\ \mu\text{m}$ diam. *Peridiopellis* a strongly interwoven trichopalisade, embedded in a narrow and incrustated slime-layer, some small globose elements present but rare, terminal elements usually on top of intricate and short hyphae; terminal elements cylindric to subclavate, $20\text{--}25(45) \times 4\text{--}7\ \mu\text{m}$, some with a prominent needle-like content, thin-walled.

Habitat — Primary tropical forest with *Shorea* spp.

Specimens examined. SRI LANKA, Kudawa, near Shinharaja Forest Reserve, primary rainforest with *Shorea* spp., 14 Dec. 2007, A. Verbeken GENT AV07-170, holotype; Kudawa, near Sinharaja Forest Reserve, alongside Pitakele river with mostly *S. trapezifolia* and some *Dipterocarpus hispidus* stands, half buried in the soil near *Shorea* spp., 13 Dec. 2007, D. Stubbe GENT DS07-488, DS07-490.

Notes — The species is easily recognized among most other known angiocarpous *Lactarius* species because of the extremely high wings in the spore ornamentation. With a height of $3\text{--}4\ \mu\text{m}$ on relatively small spores, they are so far known, the highest winged *Lactarius* spores. They do share this character with *L. pomiolens*, which has a similar high ornamentation up to $4\ \mu\text{m}$, but remarkably larger spores (see further).

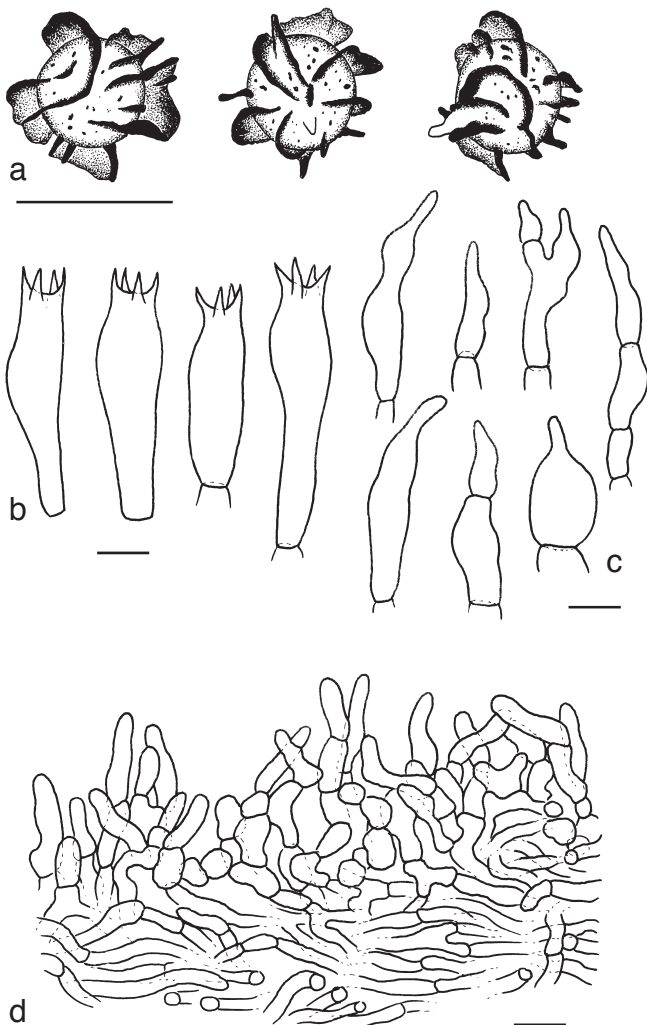


Fig. 5 *Lactarius shoreae* (holotypus). a. Basidiospores; b. basidia; c. cystidia; d. peridiopellis. — Scale bar = $10\ \mu\text{m}$.

3. *Lactarius shoreae* Stubbe & Verbeken, sp. nov. — MycoBank MB804181; Fig. 5

Holotype. SRI LANKA, near Shinharaja Forest, primary rainforest with *Shorea* spp., 13 Dec. 2007, Verbeken 07-164 (GENT).

Etymology. Referring to the association with the ectomycorrhizal host *Shorea* spp.

Basidiocarp 15 mm diam, 10 mm high, irregular. *Peridium* irregularly shaped, with bulges and folds; pale yellow (2A3), in some places darker; surface smooth, showing the loculi by transparency. *Stipe* absent. *Columella* absent. *Gleba* with rounded and labyrinthoid, small loculi, buff, pale yellow (4A3). *Latex* white, rather abundant, but soon after cutting becoming hyaline and disappearing. *Taste* mild, very dry. *Smell* not very remarkable, a bit sweetish and rubber-like.

Spores globose to subglobose, $7.9\text{--}9.1\text{--}10.3 \times 7.6\text{--}8.7\text{--}9.9\ \mu\text{m}$, $n = 20$, $Q = 1.01\text{--}1.04\text{--}1.10$; ornamentation amyloid, very highly winged; ridges up to $2.5\text{--}3.5\ \mu\text{m}$ high, sharp, mostly unbranching, sometimes branched without forming a reticulum; surface roughly amyloid and verrucose in between the ridges; plage not distinct, not amyloid. *Basidia* 4-spored, subcylindrical to subclavate, $40\text{--}60 \times 12\text{--}15\ \mu\text{m}$, thin-walled, hyaline, sometimes with oil-drops; sterigmata up to $7\ \mu\text{m}$ long. *Cystidia* present in the hymenial cavities where they occur dispersed between the basidia but also locally clustered, hyaline, thin-walled (occasionally slightly thick-walled parts are observed), very variable in shape, some fusiform or very narrow, others irregular and somewhat knotty. *Pseudocystidia* rare, cylindrical, $4\text{--}6\ \mu\text{m}$ diam. *Peridiopellis* a strongly interwoven palisade to trichopalisade, with very small globose cells present; terminal elements partly anticline, but sometimes adpressed and intricate, cylindrical, $10\text{--}20 \times 4\text{--}6\ \mu\text{m}$, with thin or slightly thickened walls.

Habitat — Primary forest with *Shorea* spp.

Specimen examined. SRI LANKA, Kudawa, near Shinharaja Forest Reserve, primary rainforest with *Shorea* spp., 13 Dec. 2007, A. Verbeken GENT AV07-164, holotype.

Notes — Like the previous species, *L. saturnisporus*, this species has rather small ($< 10\ \mu\text{m}$) but highly winged spores. It differs with *L. saturnisporus*, however, by the lower wings ($2.5\text{--}3.5\ \mu\text{m}$) without striations, and the lack of a slime layer in the peridiopellis.

4. *Lactarius echinellus* Verbeken & Stubbe, sp. nov. — MycoBank MB804184; Fig. 6

Holotype. SRI LANKA, near Sinharaja Forest, 13 Dec. 2007, Verbeken 07-157 (GENT).

Etymology. Latin for small sea urchin or small hedgehog, referring to the spores that are small and echinate.

Basidiocarp globose to irregularly subglobose and even knotty, somewhat flattened, $15\text{--}35\ \text{mm}$ diam, often with short rhizomorphs. *Peridium* smooth but mostly with several pleats, sometimes with venose wrinkles, forming a thin layer ($< 1\ \text{mm}$ thick) around the gleba, somewhat translucent revealing loculoid structure underneath; surface glabrous with chamois-leather-like patches, dry, often pruinose and whitish in pleats and dents, predominantly buff to pale ochraceous, reddish blond to brownish orange (5C4–5), slightly more ochraceous (FK13–14), pale yellow to pale orange locally (4A3–5A3) with some small whitish cracks. *Stipe* absent. *Columella* absent. *Gleba* with very small, round or labyrinthoid, irregular loculi ($\pm 3\ \text{per mm}$), firm, hardly compressible, pale yellow to greyish yellow (4A3–4B4), dark cream coloured, mostly with a pinkish tinge after exposure, pinkish buff to pale orange (5A3) in older specimens. *Latex* white, abundant, thick and sticky, unchanging

or staining the gleba slightly pinkish, drying soon and leaving a whitish layer on the gleba, not hyaline at all, unchanging in a 10 % aqueous potassium hydroxide solution. *Smell* distinct but variable sweetish rancid or reminiscent of *L. azonites*, *Geranium robertianum*, motor oil, boiled rice. *Taste* mild, immediately very dry, then mild. Both gleba and peridium unchanging with ferrous sulphate.

Spores globose to subglobose, $6.8\text{--}7.4\text{--}7.8\text{--}8.6(8.8) \times (5.8)6.0\text{--}6.4\text{--}6.6\text{--}7.1\text{ }\mu\text{m}$, $n = 40$, $Q = 1.10\text{--}1.16\text{--}1.19\text{--}1.29$; apiculus $2\text{--}4\text{ }\mu\text{m}$ long; ornamentation echinate, composed of long, isolated spines up to $2.5\text{ }\mu\text{m}$, rather slender and straight, sometimes slightly curved, rounded on top, not acute. *Basidia* 4-spored, some subclavate, some very long and narrowly cylindrical, but mostly irregularly shaped, $25\text{--}40(55) \times 8\text{--}12\text{ }\mu\text{m}$, sterigmata up to $5\text{ }\mu\text{m}$ long. *Cystidia* absent. *Pseudocystidia* present, irregular, tortuous to moniliform, $2\text{--}4\text{ }\mu\text{m}$ diam. *Lactifers* very abundant in the gleba. *Peridiopellis* a loose ixotrichoderm; terminal elements irregularly shaped and branched, with intricate finger-like bulges, $10\text{--}20 \times 2\text{--}10\text{ }\mu\text{m}$, some locally with thickened wall.

Specimens examined. SRI LANKA, Kudawa, near Sinharaja Forest Reserve, patch dominated by *Dipterocarpaceae* (*Shorea congestiflora*, *S. trapezifolia*, *Dipterocarpus hispidus*, *D. zeylanicus*), near *S. congestiflora* half buried in the soil, 11 Dec. 2007, D. Stubbe GENT DS07-472, DS07-73, A. Verbeken GENT AV07-133; Kudawa, near Sinharaja Forest Reserve, alongside Pitakele river with mostly *S. trapezifolia* and some *D. hispidus* stands, half buried in the soil near *S. trapezifolia*, 13 Dec. 2007, D. Stubbe GENT DS07-489, DS07-492, A. Verbeken GENT AV07-157, holotypus; *ibid.*, 17 Dec. 2007, D. Stubbe GENT DS07-507; Kudawa, near Sinharaja Forest Reserve, primary rainforest, near *Shorea* spp., 14 Dec. 2007, D. Stubbe GENT DS07-498, DS07-499, DS07-500, DS07-169; *ibid.*, 15 Dec. 2007, A. Verbeken GENT AV07-175; *ibid.*, 16 Dec. 2007, D. Stubbe GENT DS07-505.

Notes — *Arcangeliella lactifera* (basionym: *Gymnomycetes lactifer* B.C. Zhang & Y.N. Yu) is a similar species described

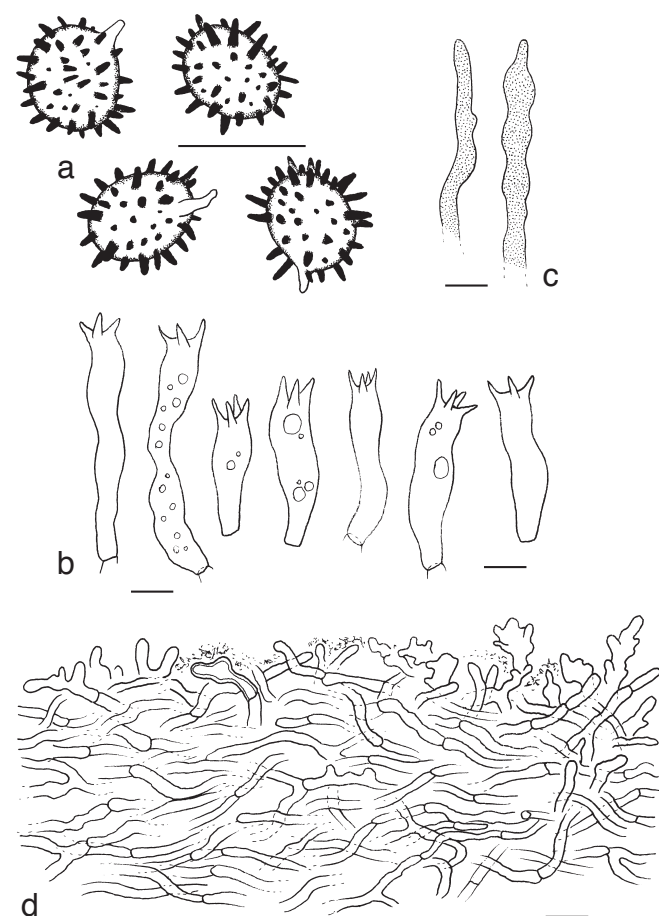


Fig. 6 *Lactarius echinellus* (holotypus). a. Basidiospores; b. basidia; c. pseudocystidia; d. peridiopellis. — Scale bar = $10\text{ }\mu\text{m}$.

from China. It is obviously similar to *L. echinus* and *L. echinellus* owing to the spores ornamented with isolated spines. It shares the 2-spored basidia with *L. echinus* but the spores are distinctly smaller: $8\text{--}10\text{ }\mu\text{m}$. Macroscopically the species is also characterized by globose, subglobose to flattened or irregular basidiomata without stipe or columella, a pale peridium and white milky latex. The peridiopellis, however, is described to be a layer of repent hyphae.

White milky latex is also present in the Chinese angiocarpous species *Zelleromyces ramispinus* (basionym: *Martellia ramispina* B.C. Zhang & Y.N. Yu), which differs by the striking spore ornamentation where $2\text{--}2.5\text{ }\mu\text{m}$ high spines have double or triple forked tips and the peridiopellis which is also a cutis.

Another gasteroid *Russulales* representative with spores bearing isolated spines is *Arcangeliella densa* (basionym: *Elasmo-mycetes densus* R. Heim), described from Thailand. The species has a better developed stipe than the ones proposed here, but we doubt whether this is a constant feature as intermediates between sequestrate species with a well-developed stipe and true angiocarpous species without stipe are possible. A more important difference is the peridiopellis which is an ixocutis resulting in a viscid peridium which is ochraceous and zonate. Heim (1959) suggests a connection with *Lactarius* species in *L.* section *Zonarii*. Judging from his drawings, the spines ornamenting the spores are also rather short compared to our Sri Lanka species.

5. *Lactarius echinus* Stubbe & Verbeken, sp. nov. — Myco-Bank MB804183; Fig. 7

Holotype. SRI LANKA, near Sinharaja Forest, primary rainforest with *Shorea* spp., 14 Dec. 2007, Verbeken 07-168 (GENT).

Etymology. Latin for sea urchin or hedgehog, referring to the spores that are large, round and distinctly echinate

Basidiocarp globose to subglobose, $10\text{--}15\text{ mm}$ diam. *Peridium* light orange to greyish orange (5AB4), smooth, slightly felty, in some places wrinkled, rugulose or strongly rugulose and deeply grooved, slightly pinkening after cutting. *Stipe* absent. *Columella* absent. *Gleba* greyish orange to brownish orange (5BC5), with very labyrinthuloid loculi. *Latex* white, very scarce to rather abundant. *Taste* mild. *Smell* distinctly of *Geranium robertianum*, but in other specimens not remarkable.

Spores globose to subglobose, $9.6\text{--}11.8\text{--}12.0\text{--}14.0(14.3) \times 9.4\text{--}11.2\text{--}11.4\text{--}13.4\text{ }\mu\text{m}$, $n = 40$, $Q = 1.01\text{--}1.04\text{--}1.07\text{--}1.15$; apiculus up to $5\text{ }\mu\text{m}$ long; ornamentation amyloid, echinate, composed of long, isolated spines; spines up to $4\text{ }\mu\text{m}$ long, rather slender and straight, sometimes slightly curved, rounded on top, not acute. *Basidia* 2-spored, some subclavate, but mostly irregularly shaped, $20\text{--}35 \times 8\text{--}14\text{ }\mu\text{m}$, sterigmata up to $5\text{ }\mu\text{m}$ long. *Cystidia* absent. *Pseudocystidia* present, tortuous to moniliform, sometimes branched, $2\text{--}4\text{ }\mu\text{m}$ diam. *Lactifers* very abundant in the gleba. *Peridiopellis* a palisade to trichopalisade, embedded in a thin and strongly incrustated slime-layer; terminal elements usually on a chain of subglobose, small elements or short hyphal parts; terminal elements clavate to irregularly subglobose, $10\text{--}17 \times 4\text{--}14\text{ }\mu\text{m}$, sometimes with slightly thickened wall.

Specimens examined. SRI LANKA, Kudawa, near Sinharaja Forest, primary rainforest with *Shorea* spp., 14 Dec. 2007, A. Verbeken GENT AV07-168, holotype; *ibid.*, 16 Dec. 2007, A. Verbeken GENT AV07-178.

Notes — Within the angiocarpous species with echinulate spore ornamentation, *L. echinus* is easily characterized by its very large spores (average $11.8 \times 11.2\text{ }\mu\text{m}$). Such spores are exceptionally large for the genus, but were also observed in *L. pomioleus*, though clearly different because highly winged. In *Lactarius*, the species with the largest spores are species

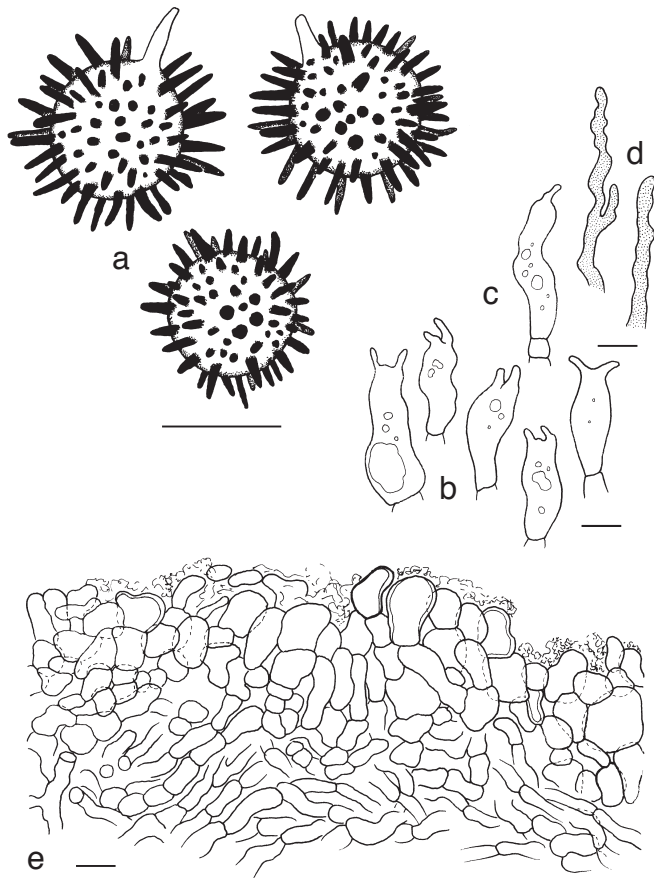


Fig. 7 *Lactarius echinus* (holotypus). a. Basidiospores; b. basidia; c. cystidium; d. pseudocystidia; e. peridiopellis. — Scale bar = 10 µm.

with 2-spored basidia (such as *L. acerrimus*). In contrast to *L. pomiolens*, which has strictly 4-spored basidia, this is the case here as well, but surprisingly the basidia themselves are very small. Besides giant spores in *L. echinus*, some smaller spores are also observed, probably produced by 4-spored instead of 2-spored basidia. Four-spored basidia could not be observed, however a single 1-spored basidium was recorded.

6. *Lactarius falcatus* Verbeke & Van de Putte, sp. nov. — MycoBank MB804185; Fig. 8

Holotype. THAILAND, Chiang Mai Prov., Mae Tang District, Ban Mae sae village, 18 June 2008, *Van de Putte 08-038* (holo GENT; iso MFU).

Etymology. Latin for sickle-shaped, curved (like the wings of a falcon), referring to the shape of the spines on the spores.

Basidiocarp globose to subglobose, 17–22 mm diam. *Peridium* brown (6E6, but paler) in upper part, part buried in soil paler brown to buff (4A3, with brown tinge), smooth. *Stipe* absent. *Columella* absent. *Gleba* cream-coloured (3A3), discolouring pale greyish brown with light orange pinkish tinge (5A4). *Latex* moderately abundant, immediately bright pale yellow (1A4). *Taste* unknown. *Smell* unremarkable.

Spores globose to subglobose, (8.8)9.1–10.1–11.1 × 8.5–9.3–10.1 µm, $n = 40$, $Q = 1.01–1.07–1.13$; apiculus 3–4 µm long; ornamentation echinate, composed of long, isolated spines; spines up to 1.5(2) µm long, rather blunt and somewhat irregular, often curved, rounded on top, seldom acute. *Basidia* 2-spored, some subclavate, some with remarkable narrower part in the middle, mostly irregularly shaped, 35–45 × (3)7–10 µm, sterigmata up to 5 µm long. *Cystidia* absent. *Pseudocystidia* present, cylindrical, 2–4 µm diam. *Peridiopellis* a loose layer of intricate hyphae, arranged periclinally as well as anticlinally,

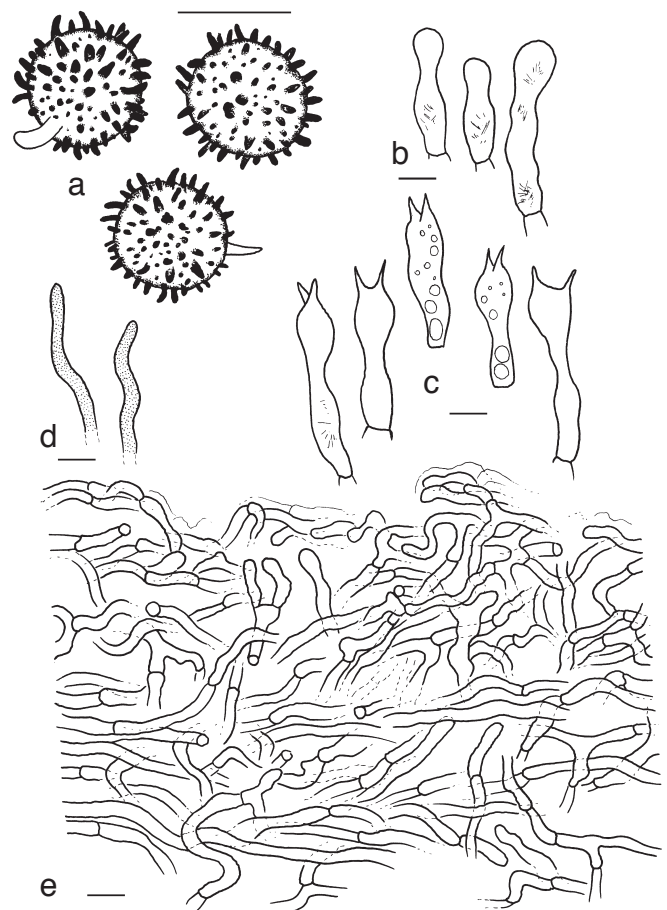


Fig. 8 *Lactarius falcatus* (holotypus). a. Basidiospores; b. cystidia; c. basidia; d. pseudocystidia; e. peridiopellis. — Scale bar = 10 µm.

no slime-layer present; terminal elements rather regular and cylindrical.

Specimens examined. THAILAND, Chiang Mai Prov., Mae Tang District, Ban Mae sae village, 18 June 2008, *K. Van de Putte* GENT KVP08-038, holotypus, MFU08-1214, isotype.

Notes — Within the angiocarpous species with echinulate spores, *L. falcatus* is easily recognized by the remarkably curved spines up to 2 µm long. Macroscopically it is characterized by the latex which is white in the beginning but soon turns bright pale yellow. *Martellia nanjingensis* differs by the lower spore ornamentation consisting of conical and blunt spines which are never curved as in *L. falcatus*. We assume it also differs in unchanging latex since a colour change to bright yellow is not mentioned in the description (Tao et al. 1993).

DISCUSSION

A striking diversity of sequestrate *Russulales* was encountered during these expeditions in tropical South East Asian forests. Six new species are described here and are phylogenetically placed in the genus *Lactarius*. We also found one angiocarpous *Russula* species which will be described in a separate paper (Hampe et al. In prep.). Worldwide, all known species of sequestrate milkcaps so far belong to the genus *Lactarius*; none are described in the genus *Lactifluus* (Verbeke & Nuytinck In press). The angiocarpous habit evolved several times in the genus and has been demonstrated in *L.* subg. *Russularia*, *L.* subg. *Piperites* and *L.* subg. *Plinthogalus*. The species described here largely confirm this: *L. echinellus*, *L. echinus*, *L. saturnisporus* and *L. shoreae* are included in *L.* subg. *Plinthogalus*; *L. falcatus* is a representative of *L.* subg. *Russularia*. *Lactarius pomiolens* has a rather isolated position and cannot be confined with cer-

tainy to one of the existing subgenera. There seems to be a close relation with the African *L. kabansus* and *L. tenellus*, for which recent phylogenetic studies show that a new infrageneric group has to be created (Stubbe 2012).

Studying *Arcangeliella*, Thiers (1984b) stated that there are two major evolutionary lines: *A. borziana* and *A. densa* (Thailand) have basidiospores ornamented with spines and rods, while the other line, represented by American and perhaps Australian species, has spores with either a broken or a complete reticulum. Both types of spore ornamentation are also encountered here, but it turns out that they do not represent true evolutionary lines and the spore ornamentation is not a phylogenetic informative feature at all. It is even striking that in *L.* subg. *Plinthogalus*, a subgenus characterized so far by reticulate to highly winged spores, two species with isolated spines are occurring.

Wilson et al. (2011) show that the gasteromycete lineages within the Agaricomycetes might now be diversifying at rates comparable to, or exceeding, those of their nongasteroid relatives. Their analyses suggest that the net diversification rate of gasteroid forms exceeds that of nongasteroid forms, and that gasteroid forms will eventually come to predominate over nongasteroid forms in the clades in which they have arisen. The low number of gasteroid forms in the Agaricomycetes as a whole may reflect the relatively recent origin of many gasteroid lineages. The even more recent origin of gasteromycetization in the order *Russulales* is suggested by several observations. Firstly, the anatomy of the basidiomes is relatively simple and in no way comparable to the complex and specialized tissues found in highly evolved gasteroid groups such as *Sclerodermatineae* or *Phallomycetidae*. Secondly, none of the gasteroid lineages in *Lactarius* has evolved into a clade containing a diversity of species. On the contrary, the gasteroid species appear as independent and isolated incidents within the phylogeny.

Hibbett et al. (1994) suggested that the genetic mechanisms resulting in the initial stages of gasteromycetization could be rather simple. It is generally assumed that dry climatic conditions are one of the driving forces that enhances the development of sequestrate fruiting bodies. However, gasteroid species occur also in Europe and North America in temperate climates, and the current findings demonstrate a strong presence in tropical rainforests as well. In Sri Lanka for instance, the number of known sequestrate *Lactarius* species now exceeds the number of known agaricoid species (Pegler 1986, Stubbe 2012). The exploration rate is rather low and the period of sampling may play an important role in the number of sequestrate fungi we encountered, but we assume that angiocarpous mushrooms are overlooked in these regions and that the phenology and ecology of these tropical rainforest angiocarpous species deserve further investigation. It seems likely to us that more sequestrate species are to be discovered in rainforest biotopes. Another impression we have from several expeditions is that production of angiocarpous basidiomes does not necessarily coincide with the seasonality of the majority of macromycetes. During our expedition in Sri Lanka, agaricoid mushrooms were not abundant, even scarce. The intensified search efforts lead us to find a greater number of small and inconspicuously growing species, among which many false truffles, such as the species presented in this paper. Perhaps the tropical angiocarpous species flourish during periods when unfavourable weather conditions cause too much stress for most agaricoid species. Another hypothesis is that angiocarpous fructifications are less susceptible to – or less dependent on – seasonal changes and are abundant year-round, only to be overlooked during the fructification season of the other mushrooms. The phenology and ecology of these tropical rainforest angiocarpous species deserve further investigation.

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