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Lactarius section *Deliciosi* (Russulales, Basidiomycota) and its ectomycorrhiza: a morphological and molecular approach

Lactarius sectie *Deliciosi* (Russulales, Basidiomycota) en hun ectomycorrhiza: een morfologische en moleculaire benadering

Jorinde Nuytinck

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Promotor: Prof. Dr. Annemieke Verbeken

Promotor:
Prof. Dr. Annemieke Verbeken (Universiteit Gent)

Overige leden leescommissie:
Dr. Ursula Eberhardt (Swedish University of Agricultural Sciences, Uppsala)
Dr. Machiel E. Noordeloos (Nationaal Herbarium Nederland, Leiden)

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Prof. Dr. Paul Goetghebeur (Universiteit Gent)
Dr. Olivier De Clerck (Universiteit Gent)
Dr. Peter Weekers (Universiteit Gent)

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Contents

Appendix

Abbreviations and acronyms

Note: herbarium abbreviations are in accordance with HOLMGREN *et al.* (1990).

!	(collection) seen and examined by the author(s)
: Fr.	name sanctioned in Fries' Systema Mycologicum (1821), see note[*]
→	in author citation: refers to the author of a replaced illegitimate name that is the basionym of the newly created name (proposed by KUYPER & VAN VUURE, 1985)
µm	micrometer (1 µm = 10^{-6} m)
AFLP	"Amplified Fragment Length Polymorphism"
a.o.	among others
AK	Alaska
a.s.l.	above sea level
AZ	Arizona
bp	base pairs
CA	California
CI	Consistency Index
CLSM	Confocal Laser Scanning Microscopy
CO	Colorado
CTAB	3D cetyltrimethylammonium bromide
dH_2O	distilled H_2O
diagn. Lat.	Latin diagnosis
diam.	diameter
DNA	deoxyribonucleic acid
ECM	ectomycorrhiza
EDTA	ethylenediaminetetraacetic acid
EF1-α	elongation factor 1-α
e.g.	exempli gratia (= for example)
FAA	5% formaldehyde, 45% ethanol, 5% acetic acid
FL	Florida
ft.	foot (1 ft. = 0.3048 m)
gpd	gene coding for glyceraldehyde-3-phosphate dehydrogenase
h	hour(s)
ICBN	International Code of Botanical Nomenclature (GREUTER *et al.*, 2000)
i.e.	id est (= in other words)
IFBL	Instituut voor de Floristiek van België en Luxemburg
IGS	Intergenic Spacer
ITS	Internal Transcribed Spacer(s) of the nuclear ribosomal DNA
L	Likelihood
leg.	legit (= collected by)
LRT	Likelihood Ratio Tests
max.	maximum
min.	minute(s)
ML	Maximum Likelihood
MP	Maximum Parsimony
MS	Mississippi
NC	North Carolina
NCBI	National Center for Biotechnology Information
nom. illeg.	nomen illegitimum (= illegitimate name)
nom. inval.	nomen invalidum (= invalidly published name)
nom. nud.	nomen nudum (= name without description)
nom. prov.	nomen provisorium (= provisional name)
nrDNA	nuclear ribosomal DNA

NY	New York
OR	Oregon
PCR	Polymerase Chain Reaction
pers. comm.	personal communication
p.p.	pro parte (= partly)
Q	length:width ratio (of the spores)
rDNA	ribosomal DNA
RC	Rescaled Consistency index
RI	Retention Index
RPB1	largest subunit of RNA polymerase II
rpm	rounds per minute
s	seconds
SEM	Scanning Electron Microscopy
SD	Standard Deviation
SDS	sodium dodecylsulphate
sp. nov.	species nova (= new species)
syn.	synonym
syn. nov.	synonymum novum (= new synonym)
TE	Tris and EDTA (10mM Tris-HCl, pH 8 and 0.1 mM EDTA)
TBR	Tree Bissection and Reconnection
Tris.HCl	Trishydroxymethyl aminomethane HCl
UPGMA	Unweighted Pair Group Method using Arithmic averages
UT	Utah
VA	Virginia
viz.	videlicet (= namely, that is)
WA	Washington
WY	Wyoming
WV	West Virginia

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[*] International Code of Botanical Nomenclature (Greuter *et al.*, 2000):

Art. 13.1. (d) Valid publication of names for fungi (including slime moulds and lichen-forming fungi) is treated as beginning at the following dates: 1 May 1753 (Linnaeus, *Species plantarum*, ed. 1). Names in the Uredinales, Ustilaginales, and Gasteromycetes (s. l.) adopted by Persoon (*Synopsis methodica fungorum*, 31 December 1801) and names of other fungi (excluding slime moulds) adopted by Fries (*Systema mycologicum*, vol. 1 (1 January 1821) to 3, with additional *Index* (1832), and *Elenchus fungorum*, vol. 1-2), are sanctioned (see Art. 15). For nomenclatural purposes names given to lichens shall be considered as applying to their fungal component.

Art. 15.1. Names sanctioned under Art. 13.1(d) are treated as if conserved against earlier homonyms and competing synonyms. Such names, once sanctioned, remain sanctioned even if elsewhere in the sanctioning works the sanctioning author does not recognize them.

Chapter 1

General introduction and outline of this thesis

Fungi form a very important but understudied group of eukaryotes. The species-richness is very high, while the taxonomical study is still in its developmental stage. Fungi function as decomposers, pathogens and partners in diverse symbioses (lichens, mycorrhizae). Fungal morphology encompasses a wide spectrum of forms, including mushrooms, bracket fungi, jelly fungi, puffballs and many others. Despite their morphological variability, fungi are anatomically simple and they have a notoriously poor fossil record. Consequently, the evolutionary relationships of fungi are still rather poorly understood.

Even macrofungi remain an understudied group. Numerous new species are still waiting to be discovered, even in well-studied areas as Europe. Moreover, there is no international, taxonomical consensus for many groups of macrofungi, and the nomenclature is often unstable and remains confusing. This becomes problematic for fungi useful to a wider public or to other branches in science (ecology, biochemistry, biotechnology) and leads to situations where information on a certain species is spread under different or wrong names.

The main focus of this study is on a section of the species-rich genus *Lactarius* Pers., namely *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.). Members of this section are believed to form a well-defined, monophyletic group of closely related species. Worldwide, 74 names have been published in this section, but most of these taxa are doubtful or badly known. *Lactarius* sect. *Deliciosi* is traditionally defined by a number of eye-catching, macroscopical features, such as the presence of strikingly coloured latex (from carrot orange over vinaceous red to indigo blue) that discolours the context greenish upon bruising. The guaiane sesquiterpenes that cause these colours have been studied in some well-known European representatives in order to discriminate between them (SCHMITT, 1974). Practically all species are edible and tasty; many species are being sold freshly, dried or canned. Members of the section form ectomycorrhiza mainly with conifers, and these associations are often highly species-specific. Many recent studies focussed on artificial inoculation of *Pinus* spp. with e.g. *L. deliciosus* (L.: Fr.) Gray or *L. sanguifluus* (Paulet) Fr. (GONZÁLES-OCHOA *et al.*, 2003; GUERIN-LAGUETTE *et al.*, 2003; PARLADE *et al.*, 2004). The known, natural distribution of *Lactarius* sect. *Deliciosi* is largely limited to the northern hemisphere.

Based on morphology there is no reason to question the monophyly of the section but this has never been examined thoroughly or with the aid of molecular data. It is beyond doubt however that within the section taxonomical and nomenclatural confusion prevails. Moreover, it seems that many wrong species-identifications circulate in non-taxonomical literature (ecological, chemistry, forestry), undermining the value of important data. We used detailed morphological data combined with the study of ectomycorrhiza and molecular data to deal with these problems.

Our original goal was to examine which species belonging to *Lactarius* sect. *Deliciosi* occur in Europe and how these species can be identified. An approach combining macro- and microscopical characters with molecular data seemed most suitable for this. Following questions were addressed: i) what is the value of characters such as the colour and discolouration of the latex and context for the identification of the European species? ii) are informative microscopical features available to aid in the identification? iii) how extensive is the morphological variation within the species and is this related to geographical data (e.g. northern versus southern Europe)? iv) are the eastern European species conspecific with the western European species and if not, where is the boundary between both mycotas?

Gradually, it became clear that non-European taxa had to be included in this study to solve the nomenclatural problems we encountered. This was mainly necessary to investigate the supposed conspecificity between Europe, Asia and North and Central America. Additional questions that were addressed are: i) how many taxa and species have been described on a worldwide scale and how many of them can we confirm by a combined morphological and molecular approach? ii) can we confirm the occurrence of species like *L. deliciosus*, *L. deterrimus* Gröger and *L. salmonicolor* R. Heim & Leclair in Asia and America? iii) how are the species in this section related, are e.g. the colour of the latex and its discolouration on the context phylogenetically informative characters as generally supposed?

Most chapters in this thesis are adapted from papers and can be read independently. As a consequence some overlap exists between the introduction and materials & methods sections. There is no separate materials & methods chapter; used techniques are described in sufficient detail within every corresponding chapter. In papers that are not published yet, information on GenBank and TreeBASE numbers is still lacking. This information will become accessible as soon as these papers are published.

Chapter 2 represents an introduction to *Lactarius* sect. *Deliciosi*, mainly based on literature data. A historical overview is given of the taxonomically important publications. Furthermore the current knowledge on the ecology, geographical distribution and biochemistry of the latex is this section is summarised. The often-encountered parasites on the basidiocarps and the use of the species for human consumption and artificial mycorrhization are discussed. We also list and explain the most important macro- and micromorphological features that were used in this study and discuss the peculiarities encountered in *Lactarius* sect. *Deliciosi*.

Chapter 3 deals with one of the first taxonomical problems we encountered in Europe, namely the distinction between *L. sanguifluus* and the much rarer *L. vinosus* (Quélet→) Bataille. The latter is regarded as a variety of *L. sanguifluus* by several authors although some striking morphological differences can be found. These are often obscured however by the frequently encountered, intermediate forms of both species. A molecular approach was applied to solve this question. Accompanying complicated nomenclatural problems were treated with care. In its original form this paper also includes a detailed morphological comparison of *L. sanguifluus* and *L. vinosus* but this part is included in chapter 6 of this thesis.

A more complete phylogenetic treatment of all the European species described in *Lactarius* sect. *Deliciosi* can be found in **chapter 4**. DNA sequences from two nuclear regions (ITS and a part of the *gpd* gene) were used here. Emphasis was put on the intra- and interspecific variability encountered in this section. For this purpose many specimens per species were included in the analysis. The distinction between another couple of closely related species, *L. deterrimus* and *L. fennoscandicus* Verbeken & Vesterh., was investigated here with the aid of the AFLP technique. Morphological characters were included in the phylogenetic analysis in a total evidence approach.

As data were also available on American and Asian taxa in *Lactarius* sect. *Deliciosi*, **chapter 5** is an attempt to reconstruct the phylogeny of this section on a worldwide scale. Results are based again on ITS and *gpd* gene sequences and the analysis includes all species known so far in the section. Basal phylogenetic relationships are not well resolved but most morphologically defined species are confirmed. Conclusions are drawn on the conspecificity between the continents and the phylogenetic value of some morphological features is discussed.

The phylogenetic analyses contributed to the final acceptance of 31 species in *Lactarius* sect. *Deliciosi* that are illustrated and described in detail in **chapters 6 to 8**. For each species all known nomenclatural and taxonomical synonyms are listed, a description of the macroscopy and microscopy is given and ecological and geographical data are provided. For every taxon a discussion is included in which a comparison is made with closely related and very similar taxa and nomenclatural and taxonomical questions are addressed. The descriptions of the European species are obviously more detailed than those of the American and Asian taxa and an identification key is included for the European species.

Chapter 9 is the result of the study of the ectomycorrhizae of some European species in *Lactarius* sect. *Deliciosi*. We wanted to examine here whether the morphological identification of the root tips is possible. This could be very helpful in more applied approaches, dealing with the ecology of the ectomycorrhizal symbiosis.

The two following chapters deal with the European species in *Lactarius* sect. *Piperites* (Fr. ex J. Kickx f.) Burl. and with a hypogeous, truffle-like *Lactarius*. Both taxa belong to the same subgenus as *Lactarius* sect. *Deliciosi*, viz. *Lactarius* subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman. They resulted from interesting collections made by the co-authors of these papers. Both chapters were included in this dissertation because they deal with species belonging to the same subgenus as *Lactarius* sect *Deliciosi* and similar techniques and approaches are explored and combined.

Chapter 10 deals with the European representatives of *Lactarius* sect. *Piperites*, a section comprising two well-known, common, birch-associated milk caps, which are widely distributed Europe: *L. torminosus* (Schaeff.: Fr.) Pers. and *L. pubescens* Fr. Special attention is paid to the ectomycorrhiza formed by a Mediterranean representative of this section, *L. tesquorum* Malençon. This research is part of a broader study, conducted at the University of L'Aquila, Italy and aiming at extending our knowledge on the distribution, phylogeny and ectomycorrhizal biology of *Lactarius* species in selected, predominantely Mediterranean ecosystems.

Within a wider taxonomic context, the genera *Lactarius* and *Russula* are placed in the order Russulales together with several genera of secotioid and gasteroid genera and some former Aphyllophorales (e.g. *Auriscalpium*, *Bondarzewia*, *Hericium*, *Peniophora*; LARSSON & LARSSON, 2003). The family Russulaceae has been defined as limited to the agaricoid genera *Lactarius* and *Russula*. Several recent, DNA-based studies have suggested however that secotioid, gasteroid and pleurotoid species have arisen several times independently within *Lactarius* and *Russula* (CALONGE & MARTÍN, 2000; HENKEL *et al.*, 2000; MARTÍN *et al.*, 1999; MILLER *et al.*, 2001). Also in *Lactarius* sect. *Deliciosi*, the hypogeous *L. rubriviridis* Desjardin, Saylor & Thiers was discovered (DESJARDIN, 2003). Most recent molecular approaches to elucidate the relationships in the Russulaceae/Russulales have not resulted yet in a well-resolved phylogeny (EBERHARDT, 2002; MILLER *et al.*, 2001). A hypothesis is that the genus *Lactarius* is derived from within *Russula*. This would make *Russula* a paraphyletic genus while *Lactarius* stays monophyletic when including the gasteroid and hypogeous *Zelleromyces* and *Arcangeliella*. Both *Zelleromyces* and *Arcangeliella* have moreover been shown to be polyphyletic (MILLER *et al.*, 2001).

We describe and combine the hypogeous *Zelleromyces stephensii* and *Arcangeliella borziana* in the genus *Lactarius* in **chapter 11**. The placement of these species was verified in a molecular phylogenetic approach using ITS sequences.

Chapter 2

Introduction

1. *Lactarius* sect. *Deliciosi*: a short historical-taxonomical overview

1.1. What’s in a name?

Through history, mycologists have been using different names for this popular and easily recognisable group of milk caps. Most current authors incorrectly use the name *Lactarius* sect. *Dapetes* (Fr. ex J. Kickx f.) Burl. The name *Dapetes* is derived from the Latin ‘daps’, meaning ‘meal, religious feast’. FRIES (1821) however, mentioned *Agaricus* sect. *Deliciosi* before *Agaricus* “Tribus” *Dapetes* (nom. inval.; FRIES, 1838) and before *Lactarius* sect. *Dapetes* (BURLINGHAM, 1908). FRIES (1821) more specifically writes ‘GALORRHEI LIMACINI l. *deliciosi*’, meaning *Lactarius* sect. *Limacini* or *Deliciosi* (all milk caps were grouped in *Agaricus* tribus *Galorrheus* and ‘l.’ stands for the Latin ‘vel’ and means ‘or’; MELOT, 1996). The name *Lactarius* sect. *Limacini* became illegitimate with REDEUILH *et al.* (2001): the first choice between two alternative names to be effectively published establishes the priority of the chosen name (art. 11.5 ICBN, GREUTER *et al.*, 2000). The correct name to be used is thus ***Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn**.
The American authors HESLER & SMITH (1979) used *Lactarius* subgenus *Lactarius* because they incorrectly considered *L. deliciosus* (L.: Fr.) Gray to be the type species for the genus *Lactarius* (EARLE (1909) indicated *L. piperatus* (L.: Fr.) Pers. as the type species for *Lactarius*). This makes *Lactarius* subgenus *Lactarius* sensu HESLER & SMITH (1979) a misapplication for *Lactarius* sect. *Deliciosi*.

Chronological overview of the names used for *Lactarius* section *Deliciosi*:

- FRIES (1821) establishes the name *Agaricus* sect. *Deliciosi* (and sect. *Limacini*).
- FRIES (1838) now mentions *Agaricus* “Tribus” *Dapetes*
- KICKX (1867) recognises *Lactarius* [unranked] *Dapetes*
- BURLINGHAM (1908) uses *Lactarius* sect. *Dapetes* but also *Lactarius* “group” *Deliciosi* (as “*Deliciosae*”[*])
- HESLER & SMITH (1960) use *Lactarius* sect. *Lactarius*.
- HESLER & SMITH (1979) raise sect. *Lactarius* to the rank of subgenus.
- REDEUILH *et al.* (2001) finally rectify the name *Lactarius* sect. *Deliciosi*.

1.2. Position within the genus Lactarius

Lactarius sect. *Deliciosi* was recognised as a natural group early on by many mycologists but its position within the genus changed numerous times (BURLINGHAM, 1908; FRIES, 1821 & 1838; LANGE, 1940; RICKEN, 1915; etc.). For an overview of the history of the infrageneric classification of

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• Both PERSOON (1797) and BURLINGHAM (1908) use the name *Lactaria*. The spelling of *Lactarius* is now conserved (KORF, 1988).

LACTARIUS Pers. 1797, Tent. Disp. Meth. Fung.: 63 (ut “*Lactaria*”), orth. cons. (Taxon 37: 456, 1988)
lectotype (Earle, *Bull. New York Bot. Gard.* **5**: 409, 1909): *L. piperatus* (L.: Fr.) Pers. (≡ *Agaricus piperatus* L.: Fr.)
original diagnosis : Pileus carnosus depressus, lamellae (nonnullae subramosae) lactescentes.

Lactarius, see VERBEKEN (1996). The most recent classification schemes of the genus include *Lactarius* sect. *Deliciosi* in *Lactarius* subgenus *Piperites* (BASSO, 1999a; HEILMANN-CLAUSEN *et al.*, 1998). This position is confirmed by molecular analyses based on ITS (and LSU) sequences (EBERHARDT, 2000) and is also supported in this study.
Following is the currently used definition of *Lactarius* sect. *Deliciosi* (adapted from HESLER & SMITH, 1979 and HEILMANN-CLAUSEN *et al.*, 1998):
Latex yellow, orange, red, vinaceous brown, purplish or blue coloured when first exuded; injured tissues staining green quickly to slowly (sometimes overnight) in most taxa; latex itself mostly not staining green; pileus and stipe may or may not be viscid or slimy when fresh; pileus often zonate.

1.3. Representatives of *Lactarius* sect. *Deliciosi*

Lactarius deliciosus is the oldest and most widely used name in *Lactarius* sect. *Deliciosi*. LINNAEUS (1753) described a species named *Agaricus deliciosus* in his Species Plantarum, PERSOON (1797) named it *Lactaria lateritia*, but FRIES (1821) used the name *A. deliciosus* again. GRAY (1821) was the first to combine this species epithet in *Lactarius*. From their very short descriptions, it is difficult to find out what exactly LINNAEUS (1753) and FRIES (1821) meant by *A. deliciosus*. This has caused some nomenclatural instability (e.g. SCHAEFER, 1970), and only the designation of a neotype for *L. deliciosus* can definitively settle this discussion. Until the 1950's and even up to now, the name *L. deliciosus* has been used for different species with orange latex. HEIM & LECLAIR (1950) and ROMAGNESI (1958) are among the first to give unmistakable descriptions of *L. deliciosus*. Their attentiveness and observational capacities enabled them to describe several new European species that are morphologically very similar to *L. deliciosus*.

Before these key-publications, great taxonomical and nomenclatural obscurity prevailed in Europe. In 1811, PAULET published the name *Hypophyllum sanguifluum* (but the common name Rougillon was already published in the first edition of his work in 1873); he was thus the first to describe a species with red latex. FRIES (1838) combined the species epithet *sanguifluus* in *Lactarius*. *Agaricus* or *Lactarius deliciosus* var. *lamelliporus* was used for *L. deliciosus* basidiocarps infected with *Hypomyces lateritius* (see below). BARLA (1855, 1859) draws and describes *A. deliciosus* var. *violaceus*, a species with a violet-red colour in all parts of the fruiting body. For a detailed discussion on the nomenclatural problems concerning this taxon see chapters 3 and 6. European authors introduced many new names in the late 19th-early 20th century; none of them are currently being used:

- *L. deliciosus* var. *violascens* Panizzi, 1861
- *L. rubrifluus* Gillet, 1879
- *L. deliciosus* var. *aeruginosus* Bommer & Rousseau, 1879
- *L. haemorrheus* Lowe, 1888
- *L. violaceocaeruleus* Voglino, 1894
- *L. sanguifluus* var. *yvreus* C. Martin, 1894
- *L. deliciosus* var. *tenuis* Naveau, 1923.
-

After World War II, two authors from Eastern Europe and Russia focussed on the variability they observed in *L. deliciosus*. SMOTLACHA (1947) invalidly describes three varieties of *L. deliciosus*: var. *piceus*, var. *pinicola* and var. *abietinus*, all from former Czechoslovakia. VASSILKOV (1948) describes *L. deliciosus* var. *piceae* and var. *pini* from the former USSR. For all of these names Latin diagnoses are lacking, even though the ICBN imposes this since January 1st 1935.

A turning point was the publication by HEIM & LECLAIR (1950) of *L. semisanguifluus* and *L. salmoneus* (later renamed *L. salmonicolor*; HEIM, 1953), followed by the excellent descriptions of *L. quieticolor* and *L. hemicyaneus* (ROMAGNESI, 1958) and *L. deterrimus* (GRÖGER, 1968). For the first time, mycologists realised the importance of the colour change of the context for species identification. Despite some confusion in the first years after their description (e.g. NEUHOFF, 1956; GRÖGER, 1968), most of these European species are now widely accepted and "easily" recognised.

This period of elucidation was unfortunately followed by a series of unclear and often too short descriptions (e.g. *Lactarius quieticolor* f. *semisanguinascens* Bon, 1975; *Lactarius deliciosus* var. *atrovirens* J. Blum, 1976; *Lactarius deliciosus* var. *lateritius* J. Blum ex J. Blum, 1976).

Even very recently, several new taxa have been described from Europe:

- *L. pinastri* Romagnesi, 1980
- *L. sanguineovirescens* Fillion, 1989
- *L. fennoscandicus* Verbeken & Vesterholt, 1998
- *L. cyanopus* Basso, 1998.

Outside of Europe, the striking, bright blue-coloured *L. indigo* is the first species being described (as *Agaricus indigo* by VON SCHWEINITZ, 1822). PECK (1872, 1878, 1898) contributed to the knowledge of *Lactarius* sect. *Deliciosi* in America by describing *L. chelidonium*, *L. subpurpureus* and *L. salmoneus*. COKER (1918), BEARDSLEE & BURLINGHAM (1940) and MURRILL (1945) further added new species to the section. HESLER & SMITH (1960, 1979) published an important revision of the section and later a monograph on the genus *Lactarius* in North America. They described several new species and varieties in *Lactarius* sect. *Deliciosi*, including numerous varieties of *L. deliciosus*. *Lactarius miniatosporus* and the hypogeous *L. rubriviridis* are the most recently discovered species on the American continent (MONTOYA & BANDALA, 2004; DESJARDIN, 2003).

On the Asian continent, *L. akahatsu* and *L. hatsudake* were early on described from Japan by TANAKA (1890). Also from Japan, *L. laeticolor* was described by IMAI (1935). Several species, varieties and forms have since then been described from this underexplored continent; most of them are badly known or doubtful. Following taxa have been described most recently:

- *L. thakalorum* Bills & Cotter, 1989
- *L. deliciosus* var. *indicus* Atri, Saini & D.K. Mann, 1991
- *L. subindigo* Verbeken & E. Horak, 2000
- *L. sanguifluus* var. *asiaticus* Dörfelt, Kiet & A. Berg, 2004.

In spite of these recent efforts, Asia and also North and Central America remain underexplored continents.

The total number of names published in *Lactarius* sect. *Deliciosi* adds up to 74: 41 from Europe, 20 from America and 13 from Asia. Appendix 1 lists all of these names, together with a reference to the protologue, eventual nomenclatural synonyms, etymology, original description or diagnosis, type and type studies (where present). Extensive morphological descriptions of all species, accompanied by a taxonomical discussion, can be found in chapters 6 to 8.

2. Ecology and biogeographical distribution

Members of *Lactarius* sect. *Deliciosi* form ectomycorrhiza with hosts belonging to the Pinaceae (often *Pinus* but also *Abies*, *Picea*, *Larix*, *Tsuga* and *Pseudotsuga*) or Fagaceae (*Quercus*, *Castanopsis*). Their distribution is accordingly largely limited to the northern hemisphere. *Lactarius subindigo* however was described from Papua New Guinea and is associated with *Castanopsis* (VERBEKEN & HORAK, 2000). A second species is described from the southern hemisphere (Java) in this study.

L. deliciosus and several related species travelled with seedlings for planting to places remote from their natural area; they are a.o. reported from South-Africa, Australia and New Zealand. *Lactarius thyinos*, a North American species, was recorded from South Africa (G. Lincoff, pers. comm.). A south Australian collection from a *Pinus radiata* plantation (an American pine species) was included in this study and grouped with the European and Asian specimens of *L. deliciosus* in our molecular analyses. *Lactarius deliciosus* is reported to be one of the few northern hemisphere mushroom species that has made the “accidental” journey to the southern hemisphere and it is one of the few wild mushrooms collected commercially in Australia (WANG *et al.*, 2001). Recently *L. deliciosus* has been imported in New Zealand to serve as secondary crop in *Pinus radiata* plantations (WANG *et al.*, 2001).

European representatives of *Lactarius* sect. *Deliciosi* show a well-documented and remarkable host-specificity (but see remark under *L. deterrimus* in chapter 4). The species seem to be strongly linked to a certain host genus: *L. salmonicolor* is associated with *Abies*, *L. deterrimus* and *L. fennoscandicus* with *Picea*, *L. porninsis* with *Larix* and all other European species are associated with *Pinus* spp. This specificity is not well examined in other continents. For the American *L. indigo,* a relatively well-known species, two hosts are consistently mentioned: *Pinus* and *Quercus*. The distribution area of *L. indigo* ranges from southeast Canada to Colombia. This striking species seems to show a tendency to be associated with *Pinus* spp. in the northern range of its area but with *Quercus* when moving to the south (B. Buyck, pers. comm.). *Quercus* itself also shows a more southern distribution than *Pinus.* The distribution maps in Fig. 1 show that the southern limit of *Pinus* is situated in central Central America, while the range of *Quercus* reaches down to Colombia. *Lactarius indigo* is reported from Colombia, where it grows with *Quercus humboldtii* (R. Halling, pers. comm.).
In vitro synthesis experiments by MOLINA & TRAPPE (1982a) revealed that a North American (Pacific northwest) isolate of "*L. deliciosus"* (see chapter 5) was capable of forming ectomycorrhiza with *Larix*, *Picea*, *Pinus, Pseudotsuga* and *Tsuga* but the same isolate could not form ectomycorrhiza with 11 species of *Eucalyptus* (MALAJCZUK *et al.*, 1982). HUTCHISON & PICHÉ (1995) found that under in vitro synthesis conditions, an isolate of *L. subpurpureus* could only develop ectomycorrhiza on its host *Tsuga canadensis*, and not with *Abies*, *Picea*, *Pinus*, *Larix*, *Betula* or *Alnus*. MOLINA & TRAPPE (1982b) pointed out however that under in vitro conditions, the ericaceous plants *Arbutus menziesii* and *Arctostaphylos uva-ursi* lacked mycorrhizal specificity and could form arbutoid mycorrhizae with a broad range of ECM fungi, including *L. deliciosus*.
The mechanisms that are responsible for host recognition and the precise development of the ectomycorrhizal interaction are not well understood yet. Hyphal systems of *Lactarius* spp. are undoubtedly attracted to root exudates of appropriate hosts (HORAN & CHILVERS, 1990) and SIRRENBERG *et al.* (1995) found that callus tissue of *Picea abies* stimulated the doubling of vegetative growth of *L. deterrimus*. Lectins, which are found on the surface of fungal hyphae, probably play an important role in host recognition and the specificity observed in species of *Lactarius* sect. *Deliciosi*. They are capable of binding to specific saccharides found on the cell walls of the host tree roots. Lectins from *L. deliciosus*, *L. deterrimus* and *L. salmonicolor* have a different molecular structure and bind only to specific oligosaccharides found on the root cells of their appropriate hosts (*Pinus*, *Picea* and *Abies* respectively; GIOLLANT *et al.*, 1993; GUILLOT *et al.*, 1991 & 1994).

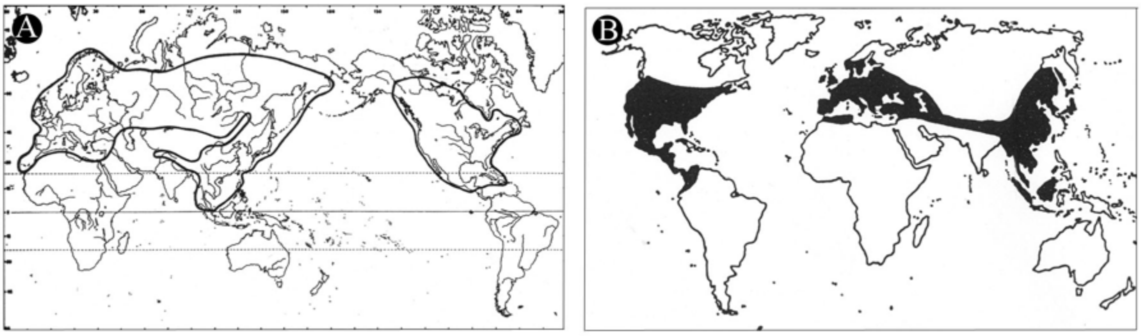

Fig. 1: A. Map showing the distribution of the genus *Pinus* (after MIROV, 1967 in FARJON, 1984); **B.** Distribution of the genus *Quercus* (after SOEPADMO, 1976 in JENKINS, 1993).

L. deliciosus is one of the few species occurring in both Europe and Asia (chapter 5). This name is misapplied in North America, no species overlap was found between North America and Eurasia (chapter 5). How exactly the current biogeographic distribution of the species in this section has been achieved remains unclear. Most clades that were revealed in our worldwide phylogenetic analyses contain American, European and/or Asian species and thus several ancestors must have been present when migration between these continents was still possible. Very recent migration between America and Eurasia seems unlikely as there is almost no species overlap between the continents (see chapter 5). Certainly, the changes in the distribution and occurrence of the host trees, strongly influenced by the climate changes during the latest glacial and interglacial periods, determined the current distribution of *Lactarius* sect. *Deliciosi*. Host-switches must have occurred several times and might have been followed by periods of rapid speciation (see chapter 4 and 5).

Most European species seem to occur throughout the continent. North African collections were also included in this study; they were examined within the framework of the revision of the Flore du Maroc (BERTAULT, 1978). *Lactarius fennoscandicus* is an example of a species with a relatively restricted distribution with records from Sweden and Finland and possibly also from the Alps. *Lactarius vinosus* on the other hand is a species with an explicit southern range. Several species in *Lactarius* sect. *Deliciosi* show the tendency to fruit abundantly. This was for example observed in *L. deterrimus* and *L. deliciosus*, two of the most common European representatives. Most European species seem to prefer a somewhat calcareous soil but *L. quieticolor* can be found on acid, often sandy, sometimes wet soils. *Lactarius sanguifluus* and *L. vinosus* are clearly thermophilous species with a mainly Mediterranean distribution, although *L. sanguifluus* is also recorded more northwards on calcareous, southern exposed slopes. Basidiocarps are most often found in grassy places or along trails but are also encountered in young tree plantations (especially *L. deterrimus*) or older forests.

Distributional and ecological data for the American species in *Lactarius* sect. *Deliciosi* are again much more limited than for the European species. Very striking is the separation between eastern North America, extending from the Great Plains to the Atlantic Ocean, and the western United States, bounded in the east by the Great Plains. No species overlap is found between these two areas, except maybe for "*L. deliciosus*", a very insufficiently known complex of varieties reported from mainly western but also eastern North America. The extensive grasslands of the Great Plains may actually act as a barrier to the migration of the species (HESLER & SMITH, 1979).

3. Morphology: macro- and microscopical descriptive characters

The morphological part of this thesis (chapters 6 to 8) gives a detailed macro- and microscopic description of every species known in *Lactarius* sect. *Deliciosi* and accepted in this thesis. The macroscopic descriptions of most European species are based on personal observations on many fresh collections from a wide geographic range and are very complete and elaborate. The descriptions of the American and Asian taxa are in many cases adapted from literature data and short field descriptions accompanying herbarium collections. They are made as complete as possible but only limited field work has been carried out here. Colour photographs are included where available. The microscopic characters are described in detail and illustrated extensively.

In this introduction only characters and character states of use in *Lactarius* sect. *Deliciosi* are discussed. A more complete treatment of the morphological characters in *Lactarius* can be found in VERBEKEN (1996). A total of about 450 fresh and/or herbarium collections were studied morphologically in the study presented here. They represent 38 taxa and were collected in 28 countries. Fig. 2 shows a map of the world with the distribution of the sampling sites.

3.1. Macromorphology

Pileus

Several aspects of the pileus are very useful to identify species in *Lactarius* sect. *Deliciosi*. The colour can be bright to dull orange, pink to vinaceous red, brownish, greyish, sometimes with a lilac sheen or blue. Some species show a very constant pileus colour, others are highly variable (e.g. *L. quieticolor*). Many species show a green discolouration of the pileus when bruised or in age. This discolouration ranges from (nearly) absent (e.g. *L. salmonicolor*, *L. thyinos*, *L. laeticolor*) to very strong (e.g. *L. semisanguifluus*). We frequently observed that, for many species, the primordial stages are entirely deep bluish green coloured.

In many species a white layer covers the pileus, making it look very pale. When examining the zoned pileus surface in more detail it becomes apparent that the zones are caused by the specific arrangement of large to mostly small scrobicules or pits (brightly coloured, rounded depressions) against the pale background colour. This surface-aspect is related to the microscopic structure of the pileipellis. We avoid using the term pruinose (= with a frost-like or flour-like surface covering) in our descriptions because the aspect we refer to is stronger than that.

Other common features are a smooth (even) and glabrous (hairless) pileus that is sticky to viscid when humid but soon becomes dry. The shape of the pileus evolves from convex in young basidiocarps to slightly or strongly infundibuliform (funnel-shaped). A slightly striate margin is reported from several species (e.g. *L. subpurpureus*, *L. deterrimus*). The diameter of the pileus ranges from about 4 cm to almost 20 cm in full-grown specimens, making them medium sized to large milk caps.

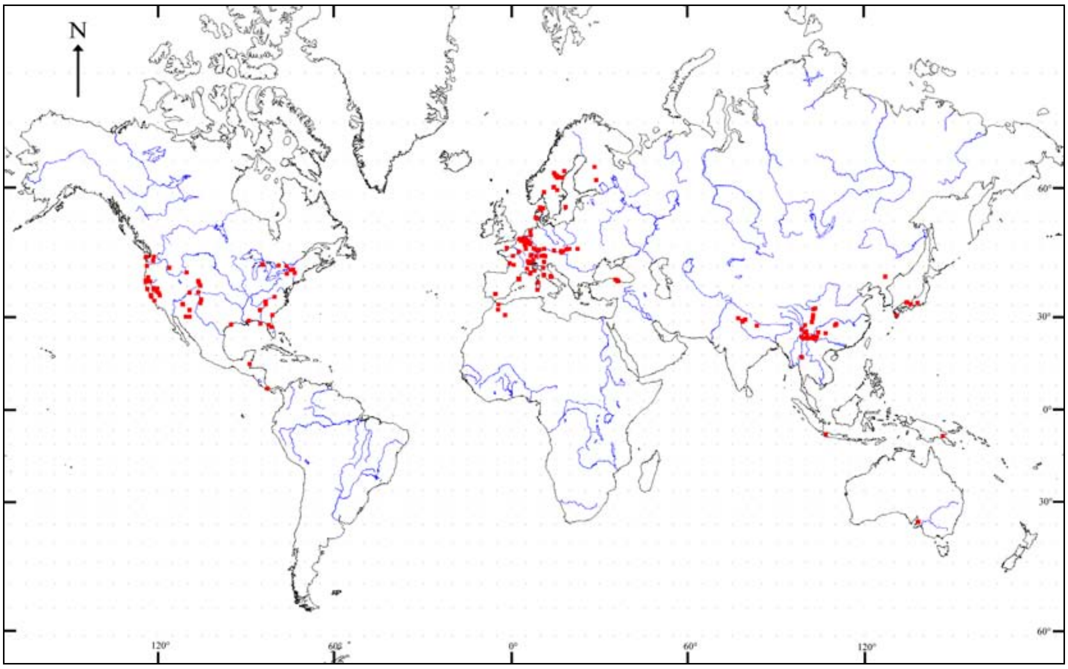

Fig. 2: Map of the world showing the distribution of the sampling sites of the specimens used in this study.

Stipe

Species in *Lactarius* sect. *Deliciosi* have a centrally attached, more or less cylindrical stipe. Only *L. indigo* is reported to sometimes have an eccentrical stipe (Hesler & Smith, 1979). The stipe is similarly coloured to the pileus or paler because of the very well developed white surface layer. The very apex of the stipe shows a completely white zone in many species. The presence or absence of brightly coloured scrobicules is an important character for identification (Fig. 3 A, B). All species have a smooth, glabrous and dry to viscid stipe. Some species typically have a short and robust stipe (e.g. *L. deliciosus*), while others have a more slender stipe (e.g. *L. deterrimus*). For *L. paradoxus* and *L. pseudodeliciosus* the base of the stipe is reported to be tapering and ending into a short root-like extension (Beardslee & Burlingham, 1940; Hesler & Smith, 1979).

Lamellae

According to Marxmüller & Romagnesi (1991) the actual colour of the lamellae or gills is pale yellowish. The observed colour is caused by the presence of numerous lactifers in the trama, giving the lamellae their nice and bright colour (pure orange, wine red, indigo blue (Fig. 3 C) …). The greenish discolouration, typical for members of this section, can be present very strongly in the lamellae. The edge is entire and concolorous or slightly paler. The spacing and thickness of the subdecurrent lamellae is fairly constant in the section. *Lactarius subpurpureus* however has somewhat more widely spaced lamellae while several unidentified Asian collections are characterised by strikingly dense lamellae (chapter 7 and 8). Anastomosing and branching of the lamellae is very common near the attachment of the stipe, it is not taxonomically important in *Lactarius* sect. *Deliciosi*.

Context

The consistency of the context or flesh is quite firm to very firm, but the stipe soon becomes hollow when the basidiocarps age. The colour varies from whitish, becoming buff in age, to yellow in the stipe cavity (in *L. subpurpureus*). Just underneath the pileipellis, the context may be (deep) blue-green coloured; this was observed in several specimens with a dark pileus colour (frequently observed in *L. quieticolor* and *L. fennoscandicus*). Absence or presence of this colour in these species does not have a taxonomic importance.

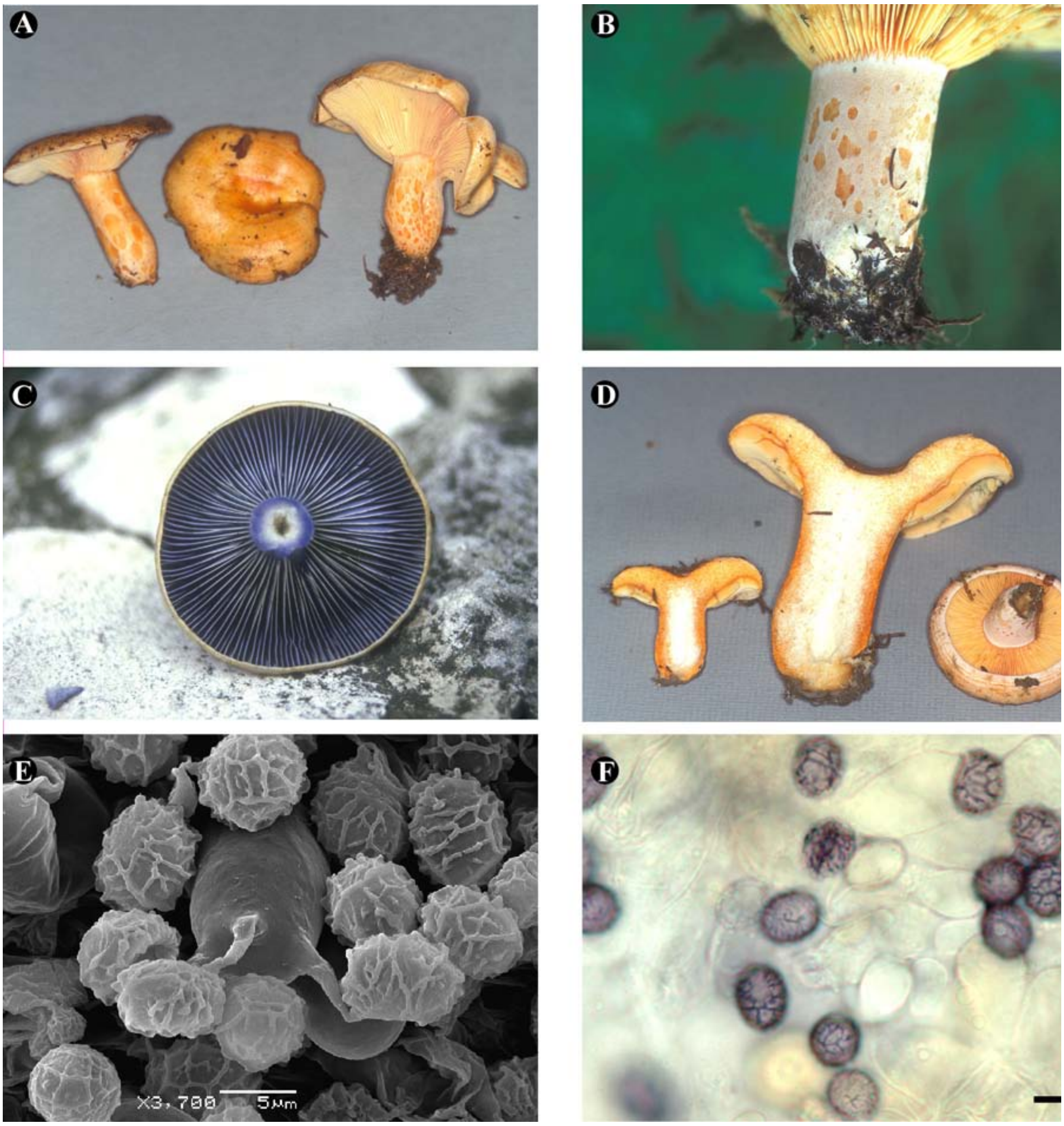

Fig. 3: A. *L. salmonicolor* basidiocarps showing abundant, small to large, bright orange scrobicules on the stipe; **B.** Stipe of *L. deliciosus* with a distinct white surface layer, a white zone at the apex and bright orange scrobicules (photo R. Walleyn); **C.** *L. indigo* with blue coloured lamellae and latex (photo L. Ryvarden); **D.** Basidiocarps of *L. deliciosus* showing the orange colour of the latex on the context when cut; **E.** SEM picture of a basidium with spores in *L. hatsudake*; **F.** *L. deliciosus* spores as observed in Melzer's reagens under the light microscope (scale bar = 10 µm).

Very important for the identification of the species in *Lactarius* sect. *Deliciosi* are the colour and the colour changes of the latex on the context. The context underneath the pilei- and stipitipellis and above the lamellae contains abundant lactifers. The latex colour varies from bright, carrot orange (in most species, Fig. 3 D) to dingy yellow, vinaceous red and bright but deep blue. The latex in the lactifers and the enzymes that act on the guaiane sesquiterpenes present in the latex are separated in the intact basidiocarps. Upon bruising or cutting of the basidiocarps a species-specific enzymatic reaction takes place, often resulting in the green discolouration of the context after several hours. In most species the orange latex changes more or less slowly (30 min. to 1 hour) to red. In *L. semisanguifluus* this reaction takes place in 5 to 10 min., an infallible feature for identification. In species with red latex, the context stays deep red or discolours brownish and finally greenish. *Lactarius indigo*, one of the most remarkable agarics, stains almost entirely deep blue when cut. Other species show different colours when cut: *L. cyanopus* stains blue except in the base of the stipe, there the latex is orange; *L. chelidonium* var. *chelidonioides* stains azure blue in the upper half of the pileus but paler to dingy yellowish near the lamellae. The colour change of the latex on the context is a rather reliable character, useful for identification of most species, provided that it is observed for a long enough period (about 1 hour, see Fig. 4). Earlier descriptions of European species (HEIM & LECLAIR, 1950; ROMAGNESI, 1958) mention unchanging orange milk for species like *L. deliciosus* and *L. quieticolor*, while we now know that the orange colour slowly changes to red in the majority of the basidiocarps of these species. This led to numerous misidentifications and confusion with *L. semisanguifluus* (BON, 1975; NEUHOFF, 1956). As with many macroscopical characters in *Lactarius* sect. *Deliciosi*, some variability can be observed in the colour change of the latex. ROMAGNESI (1958) for example mentions the case of *L. salmonicolor*, where he observed a rather rapid change from orange to red in warm and dry weather, while in cold and humid conditions the latex stayed orange for hours.

The smell and taste of the context and latex are similar in all species in the section and are not very useful for identification. Most species smell fruity, agreeable and a little sweetish. *Lactarius chelidonium* var. *chelidonioides* is reported to smell subnauseous, similar to *Morchella esculenta* (HESLER & SMITH, 1960). *Lactarius porninsis* smells like mandarins. The taste is mild but often slightly bitter and slowly becoming a little acrid when chewing a larger amount for some time.

Latex

The latex or milk is only scantily present in species in *Lactarius* sect. *Deliciosi*. This makes observations on the latex, separated from the context, difficult. The taste of the latex is mild in all species where this was tested. The colour changes of the isolated latex are different from the changes on the context, as this is an enzymatic reaction. Isolated latex dries brownish in most species but dirty greenish in *L. indigo*.

Spore deposit

There is almost no variation present in *Lactarius* sect. *Deliciosi* as far as the spore deposit is concerned; the colour is pale yellow-orange (4A3). Only *L. miniatosporus* is reported to have a yellowish brown deposit (MONTOYA & BANDALA, 2004) while *L. indigo* var. *diminutivus* has a white deposit (but this observation was done on a thin deposit; HESLER & SMITH, 1979).

Macrochemical reactions

Macrochemical reactions can be of taxonomic value in *Lactarius*. Certain reactions (with $FeSO_4$, gaiac or KOH for example) have proved to be very helpful. In this study KOH, $FeSO_4$, gaiac, phenol, HCl, NH_4OH, H_2SO_4 and NH_3 were tested on some specimens. The data on macrochemical characters are nevertheless very incomplete for *Lactarius* sect. *Deliciosi*. Most reagents show no reaction on any of the specimens tested ($FeSO_4$, phenol, HCl, NH_4OH and NH_3). Gaiac was tested on specimens of many species and showed a slow change to blue in most cases. H_2SO_4 turned black when applied on the context of *L. salmoneus* (MONTOYA & BANDALA, 1996). *Lactarius cyanopus* and *L. indigo* are the only species that show some reaction to KOH (changing to brown-grey in *L. cyanopus* (BASSO, 1998) and changing to yellowish or pale watery orange on the pileipellis, stipitipellis and context in *L. indigo* and dingy orange on the hymenium (Petersen in HESLER & SMITH, 1979). Whether this reaction to KOH is very constant and reliable remains to be tested. For the moment, it can be stated that these macrochemical tests are probably not useful for the identification of species in *Lactarius* sect. *Deliciosi*.

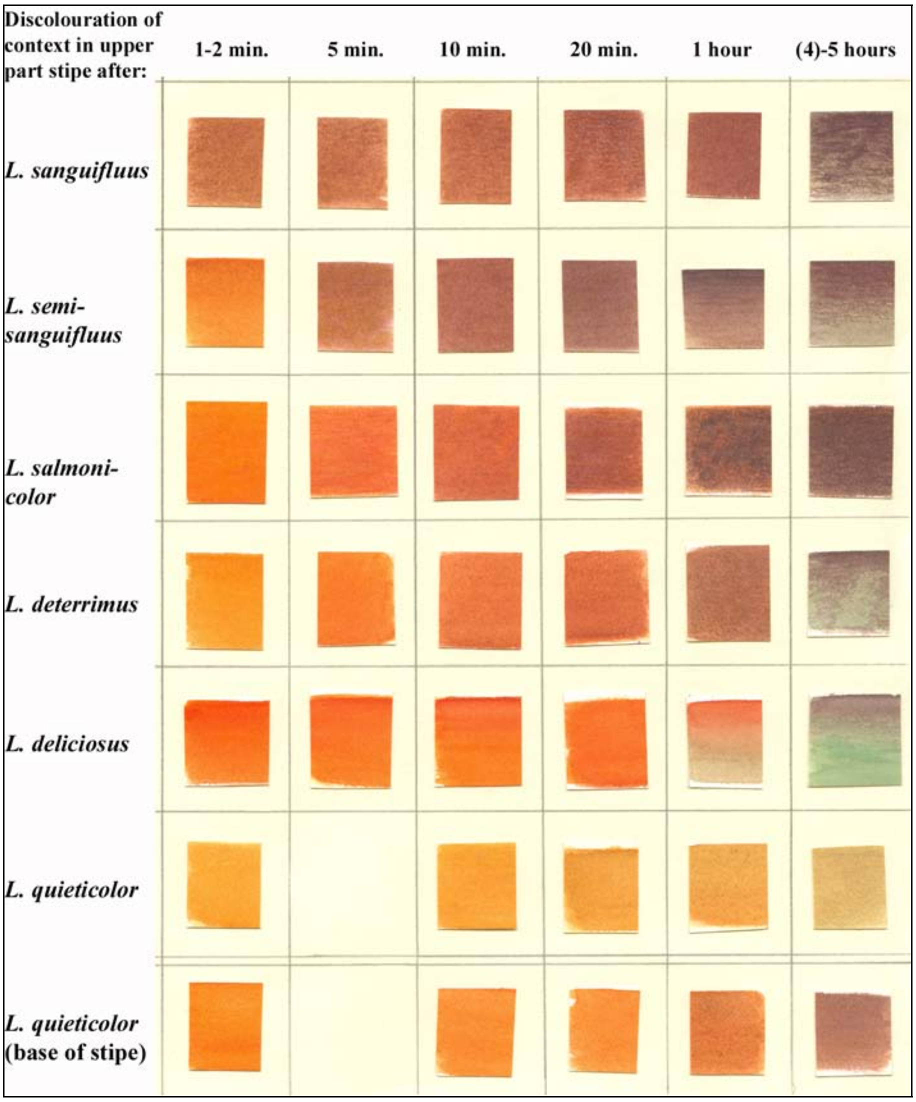

Fig. 4: Discolouration of the latex on the context after 1 min. to 5 hours as observed in several European species (watercolour Omer Van de Kerckhove).

Hypogeous habitus

L. rubriviridis was the first hypogeous or truffle-like species ever described in the genus *Lactarius* (DESJARDIN, 2003). Before, hypogeous species were placed in the genera *Zelleromyces* or *Arcangeliella*. The macromorphology of hypogeous species obviously is very different from most milk caps and the inclusion of this species seriously changes our concept and definition of this section (see chapter 12). For a detailed description of *L. rubriviridis*, see chapter 7.

3.2. Micromorphology

Spores

When observing *Lactarius* spores in Melzer's reagens (CLÉMENÇON, 1972), it is possible to distinguish the amyloid ornamentation of the spore wall, the hilar appendix (often referred to as apiculus) and the plage. The hilar appendix is a short, cylindrical to tapering projection at the base of a spore that abaxially and subterminally carries the hilum, the place where the spore is attached to the sterigma of a basidium (Fig. 3 E). The punctum lacrymans is the adaxial place where the Buller's drop is emitted during the active release of the spore. The plage is the adaxial zone above the hilar appendix with very aberrant or absent ornamentation. VERBEKEN (1996) distinguished four main types of amyloidity of the plage (non-amyloid, centrally amyloid, distally amyloid and totally amyloid). For the microscopical descriptions in chapter 6 to 8, we paid attention to the amyloidity of the plage, the peculiarities of the ornamentation and the dimensions and shape of the spores.

Most species in *Lactarius* sect. *Deliciosi* have a plage that is distally slightly amyloid. Small, irregular amyloid spots occurring scattered over the plage have been encountered occasionally. HEILMANN-CLAUSEN *et al*. (1998) report a centrally amyloid plage in *L. deliciosus*, but this was not confirmed by our observations.

The pattern of the amyloid spore ornamentation is one of the few useful microscopical features for distinguishing species in this section (Fig. 3 F). Nevertheless, all species have a rather similar spore ornamentation. The maximal height of the ornamentation ranges from 0.3 to 1 µm. Spores from most species show an ornamentation composed of thin to rather thick ridges and scattered isolated warts, forming an incomplete reticulum. Species with an almost complete reticulum include *L. cyanopus*, *L. rubriviridis* and *L. barrowsii*. The latter two species have an exceptional spore ornamentation, forming very small meshes. The thickness of the ornamentation can also be helpful for determination; *L. quieticolor* for example is a European species with heavily ornamented spores that distinguish it from the sometimes macroscopically very similar *L. deliciosus*.

Length and width of the spores were measured in side view, excluding the ornamentation. To determine the shape of the spores we used the length:width ratio (quotient- or Q-value). The following critical values and classification of the spore shapes were used (according to BAS, 1969):

- subglobose	Q: 1.05–1.15
- broadly ellipsoid	Q: 1.15–1.30
- ellipsoid	Q: 1.30–1.60

Following the reasoning of HEINEMANN & RAMMELOO (1985), twenty spores were measured per collection for as many collections as was possible or feasible per species. Spore dimensions and shape are useful characters for identification of the species in *Lactarius* sect. *Deliciosi*. The smallest spores were found in *L. subindigo* (6.6–7.5 × 5.3–5.9 µm on average); the largest in *L. subpurpureus* (10.0–10.6 × 7.1–7.3 µm on average) and *L. thyinos* (10.1–10.7 × 7.4–8.2 µm on average).

Scanning Electron Microscopy (SEM) pictures were made for all treated species and added to the descriptions. The additional magnification and resolution of SEM result in a very detailed image of the spore surface and ornamentation. Line drawings of the spores, as observed in Melzer's reagens and with the use of light microscopy, are however essential for identification.

Basidia

The taxonomic importance of the characters concerning basidia is considered to be very low within *Lactarius*, and within the Agaricales in general (VERBEKEN, 1996). In this study, basidia are described in terms of general shape, dimensions, content and sterigmata characters. Fig. 5 A shows a basidium in the hymenium of *L. rubriviridis*. The described basidia were observed halfway up the lamellae; basidia close to the margin are significantly smaller. The shape of the basidia in *Lactarius* sect. *Deliciosi* ranges from almost cylindrical to subclavate. Drawings are provided for every taxon studied to complement this rather unsatisfying terminology for the shape. The length of the basidia was measured excluding the sterigmata and generally was (35) 40 to 60 µm or exceptionally slightly longer. The width was measured at the broadest place and ranged from 8 to 14 µm. It is generally accepted that there is a relationship between the volume of the spores and the volume of the basidia (VERBEKEN, 1996). Basidia often contain relatively large oil-droplets and/or smaller guttules; a granular content may also often be observed. Observations of the content of the basidia on older herbarium material are unreliable however. All species in *Lactarius* sect. *Deliciosi* have 4-spored basidia, but in certain species 2-spored basidia were observed in small quantities. In these species (*L. semisanguifluus*, *L. subpurpureus*, *L. thyinos*) exceptionally large spores were encountered. Basidia were sometimes found on the lamella edge but this does not seem to be constant within a species or even within a basidiocarp in *Lactarius* sect. *Deliciosi*.

Cystidia

Cystidia can be defined as sterile elements, frequently of distinct shape, occurring at any surface of a basidioma, particularly the hymenium from which they frequently project (KIRK *et al.*, 2001). Cystidia have been classified and named according to their origin, position, form and contents. In the present study, following terminology is used:

- **Pseudocystidia** are the extremities of laticiferous hyphae in the hymenophoral trama and are thus directly in contact with such a differentiated hypha; there is no basal septum. Pseudocystidia ascend in the hymenium between basidioles and basidia and are only rarely emergent in *Lactarius* sect. *Deliciosi*. They are strongly tortuous near the subhymenium, but almost cylindrical near their apex. Pseudocystidia are (1.5) 2 to 6 µm wide in *Lactarius* sect. *Deliciosi*; the broadest pseudocystidia were observed in *L. vinosus* (up to 8 µm). Pseudocystidia are mostly abundantly present in the hymenium and have an ochre-yellow refractive content. Nevertheless, they are often hard to observe as they are embedded in the hymenium and their apex is sometimes hyaline. Fig. 5 B shows a pseudocystidium on the lamella edge in *L. deterrimus*.
- **Macrocystidia** are (sub)fusiform with a tapering, capitate to moniliform apex. They have a needle-like or granular content that is sometimes very similar to the content of the lactifers. Pleuromacrocystidia occur on the face of the lamellae while cheilomacrocystidia can be found on the edge. In Fig. 5 C a transverse section of a lamella is shown with abundant cheilomacrocystidia. Most species in *Lactarius* sect. *Deliciosi* have macrocystidia with a strongly moniliform apex. Hyaline macrocystidia are sometimes observed but occur intermixed with macrocystidia with a clear content. Thick-walled cystidia do not occur in this section. The presence and abundance of pleuro- and cheilomacrocystidia are often useful characters to identify species in *Lactarius* sect. *Deliciosi*. In several species however, quite some variability was found in the abundance of macrocystidia (e.g. *Lactarius deterrimus* and *L. fennoscandicus*, see chapter 6). These characters should thus be used with care. Macrocystidia are generally considered to be scarce in *Lactarius* sect. *Deliciosi* (BASSO, 1999a; HEILMANN-CLAUSEN *et al.*, 1998; ROMAGNESI, 1958) but have been overlooked in some species and are sometimes very prominent (e.g. the very emergent and striking cystidia in *L. salmonicolor* and to a lesser extent in *L. cyanopus*). Macrocystidia are often abundant close to the lamella edge but become scarce further away. The distinction between cheilo- and pleuromacrocystidia is for that reason not always clear.
- **Cheiloleptocystidia** are the thin-walled, mostly hyaline, clavate to irregularly shaped cells with a rounded apex that can be found on the lamella edge in all species in *Lactarius* sect. *Deliciosi*. In our terminology we consider cheiloleptocystidia as well as cheilomacrocystidia as marginal cells. In one species we observed cheiloleptocystidia with a remarkable dark content (see chapter 7). The term paracystidia is sometimes used for this type of cystidia (CLÉMENCON, 1997; HEILMANN-CLAUSEN *et al.*, 1998). Their taxonomic value seems to be very limited.

Lactifers

A clear difference between two types of hyphae with a distinct content, as described by VERBEKEN (1996), was not observed in *Lactarius* sect. *Deliciosi*. The lactifers in this section are 4–12 (15) µm broad and have a distinct, dense, ochre coloured content as observed in KOH or Congo red (Fig. 5 C, D). They are often very abundant and tortuous in the trama of the lamellae. The chemical composition of the latex is discussed below.

Hymenophoral trama

Almost no variation was found in the structure of the hymenophoral trama in *Lactarius* sect. *Deliciosi*. Sphaerocytes were never observed. When squashed, the trama appears to be entirely filamentous. As the hyphae are arranged very densely and irregularly, many cut hyphae and short fragments are observed in a transverse section (appearing like subglobose to irregularly shaped small cells), together with many refractive lactiferous hyphae. The subhymenium consists of a tangled mass of narrow filaments giving in sections the appearance of a cellular tissue because the hyphae are cut transversally. Lactifers are mostly absent in the trama close to the lamella edge, causing the edges to be slightly paler in colour. When lactifers do occur close to the lamella edge, cheilopseudocystidia can occur (Fig. 5 B).

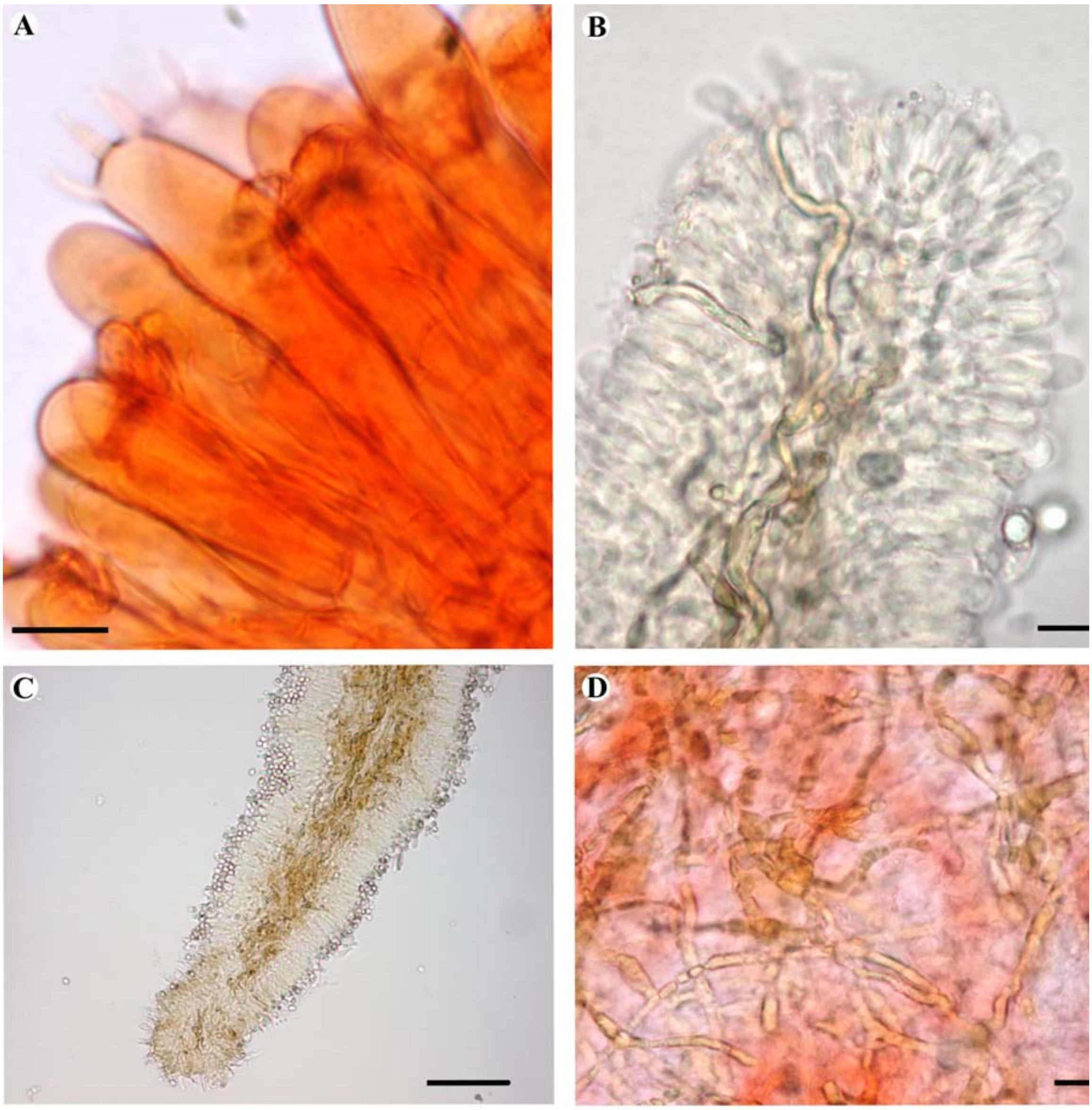

Fig. 5: **A.** Basidium of *L. rubriviridis* as seen in Congo red (scale bar = 10 µm); **B.** Lamella edge of *L. deterrimus* in KOH with an ochre coloured cheilopseudocystidium (scale bar = 10 µm); **C.** Transverse section of a lamella of *L. deterrimus* with abundant lactifers in the trama and very abundant cheilomacrocystidia (scale bar = 100 µm); **D.** Ochre coloured lactifers as observed in Congo red in *L. rubriviridis* (scale bar = 10 µm).

Pilei- & stipitipellis

Lactarius sect. *Deliciosi* is very homogeneous as to the structure of the pilei- and stipitipellis. In nearly all species the pileipellis is an ixocutis. The slime-layer is often thin and indistinct. Lactifers and pigmented hyphae were often observed to occur in the pileipellis. Only *L. salmoneus* has a somewhat deviating type of pileipellis with many hyphae arranged perpendicular to the surface and united into bundles. It is not appropriate however, to call this structure a true trichoderm. The stipitipellis is a cutis in most species but in some cases a clear ixocutis was observed with a very obvious slime-layer. The stipitipellis is generally thinner than the pileipellis. The typical scrobiculate aspect of many species seems to be caused to by a locally thinner cuticle structure. In between scrobicules a clearly thicker layer of parallel hyphae was observed with more intercellular space. BASSO (1999a) explains the scrobicules by a local absence of cystidia-like cells on the pileus or stipe.

In conclusion, species in *Lactarius* sect. *Deliciosi* form a macro- and micromorphologically very homogeneous group. Typical basidiocarps in fresh condition and in their growing place can mostly be identified without problem but ageing and weathering can seriously complicate identification. Identification based on microscopical characters alone is almost impossible. Only few species with strikingly different spores can be recognised but even then comparison with reference collections is recommended.

4. Parasitism

Parasitism on basidiocarps of *Lactarius* sect. *Deliciosi* is a very commonly observed phenomenon. Heavy infestation by dipterous larvae makes the basidiocarps often unsuitable as human food. Members of *Lactarius* sect. *Deliciosi* are especially prone to these infections. This might correlate with the limited antifeedant properties of the mild-tasting latex. HACKMAN & MEINANDER (1979) indeed observed that acrid-tasting species (e.g. *L. torminosus* (Schaeff.: Fr.) Pers. and *L. pyrogalus* (Bull.: Fr.) Fr.) and species with very abundant latex (e.g. *L. repraesentaneus* Britzelm. and *L. volemus* (Fr.: Fr.) Fr.) are comparatively rarely infected. The most important pest of the *L. deliciosus* group is *Mycetophila blanda*, a species rarely found in other fungi. *Exechia pseudocincta* also occurs regularly on the '*L. deliciosus* species complex' while it is not found in the other fungi examined (HACKMAN & MEINANDER, 1979). A total of 16 dipterous parasite species were reared from the 42 basidiocarps examined (HACKMAN & MEINANDER, 1979); other literature sources revealed another 15 species of Diptera parasitising *L. deliciosus* and relatives (DELY-DRASKOVITS, 1972a&b & 1974; DELY-DRASKOVITS & MIHÁLYI, 1972; HENNIG, 1964; LUNDBECK, 1922; PLASSMANN, 1969 in HACKMAN & MEINANDER, 1979). HACKMAN & MEINANDER (1979) furthermore mention that more than 80% of the examined basidiocarps were infested with larvae. *Lactarius deliciosus* and *L. deterrimus* were not treated separately by these authors because no significant differences were found in their dipterous pests.

Species in *Lactarius* sect. *Deliciosi* are also parasitised by other fungi. *Hypomyces* (Fr.) Tul. is a genus belonging to the Hypocreales (Ascomycota) and species of this genus are specialized in growing on basidiocarps of other fungi (they occasionally also grow on wood, bark or litter). The hosts are mostly homobasidiomycetes including Agaricales, Boletales, Russulales and aphyllophoralean basidiomycetes from several orders. Currently 51 teleomorph species are accepted in the genus *Hypomyces*. In addition, about 25 species are known that reproduce only asexually, but are nested within *Hypomyces* (POLDMAA *et al.*, 2004).

A number of species that grow on representatives of the Russulales and some other agaricoid basidiomycetes are obligate parasites, only found growing on their host, causing systemic infections and resulting in the mummification of host basidiocarps. Many of these species are restricted to basidiocarps of a certain (group of closely related) species.

All the species of *Hypomyces* are characterized by the development of light or brightly coloured perithecia in a ± concolorous subiculum (Fig. 6B). The subiculum is composed of loosely intertwined or compacted hyphae, appearing in the form of a thin, cottony mat to a firm, almost pseudoparenchymatous, stroma-like tissue in a wide array of colours. Perithecia are solitary to gregarious, immersed except for the papillae to almost superficial on the subiculum or sometimes seated directly on the host tissue. Perithecia are generally pyriform, smooth, 0.1 to 0.4 mm diam. and nearly hyaline or in various colours.

The most common of the *Hypomyces* species infecting Russulaceae is *H. lateritius* (Fr.: Fr.) Tul. & C. Tul. *H. lateritius* grows on species in *Lactarius* sect. *Deliciosi*, causing hardening of the basidiocarps. The popular name 'stone milk cap' originates from this phenomenon. The hardening prolongs the lifespan of the basidiocarp and provides the parasitic fungus with sufficient time to develop its own fruitbodies. A *H. lateritius* infection is externally recognizable by a complete suppression of gill development and a white layer of parasitic hyphae on the hymenium (Fig. 6A), while internally the flesh of the basidiocarp is entirely infected and hard in all parts. It was demonstrated that infection extends beyond the fruiting body to include the ectomycorrhizae of the host species (PILLUKAT & WANNER, 1996). Infected ectomycorrhizae of *L. salmonicolor* develop cone-shaped ends that increase the diameter of the side-branches of the ramification system. The parasitic hyphae grow between the host hyphae inside the mantle and form conidia on the surface of the ectomycorrhiza (PILLUKAT & WANNER, 1996).

We have found *H. lateritius* growing on *L. deterrimus*, *L. vinosus*, *L. salmonicolor* and *L. deliciosus*. It is also reported infecting *L. thyinos*, *L. sanguifluus*, *L. chelidonium* and *L. porninsis* (MOINGEON, 2004; POLDMAA *et al.*, 2004) and apparently occurs wherever these *Lactarius* species occur. According to ROGERSON & SAMUELS (1994) *H. lateritius* is not restricted to species of *Lactarius* sect. *Deliciosi*. They also report *H. lateritius* from *L. uvidus*, *L. camphoratus*, *L. vellereus* and *L. volemus*, among others. *H. lithuanicus* is a very similar species growing on basidiocarps of *L. torminosus* and *L. pubescens*. The most famous *Hypomyces* species is without doubt the American *H. lactifluorum* that parasitises *Lactarius* and *Russula* species, turning them into lobster mushrooms. The infected basidiocarps that show a red "crust" on the outside (the *H. lactifluorum* subiculum) and are white inside, are considered a delicacy.

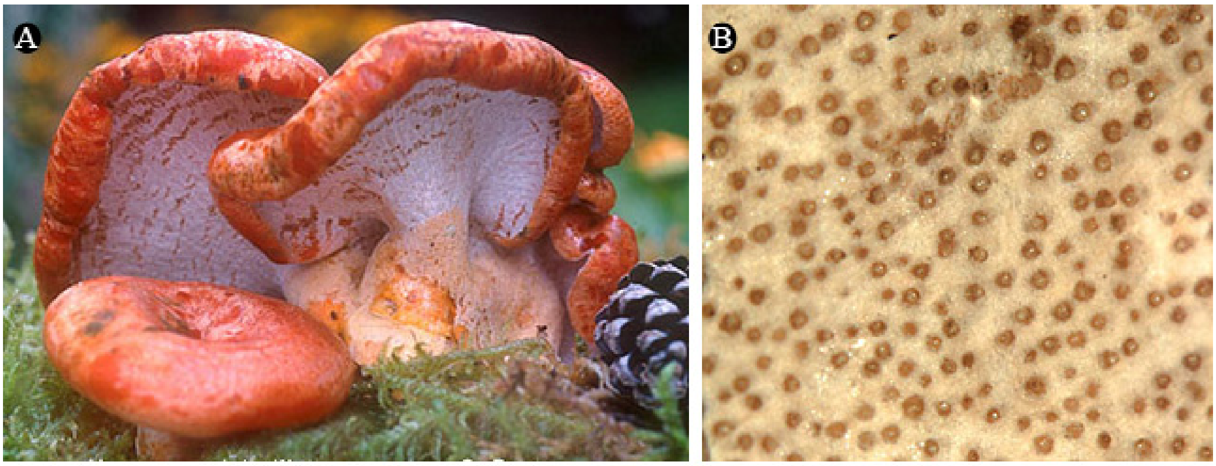

Fig. 6: A. Basidiocarps of a member of *Lactarius* sect. *Deliciosi* infected with *Hypomyces lateritius* (Photo C. Burgos); **B.** Young perithecia of *H. lateritius* immersed in the subiculum (POLDMAA *et al.*, 2004).

5. Biochemistry of the latex

The chemistry of the sesquiterpenes and esters in the latex of *Lactarius* species has been studied within the framework of the chemical defence system that protects pungent or bitter Russulaceae species against parasites and predators. Acrid-tasting *Lactarius* species, such as *L. necator*, *L. piperatus* and *L. rufus*, possess natural defensive compounds in their latex with antibiotic and antifeedant activities and are likely to be toxic (STERNER & ANKE, 1995). An antifeedant is defined as a substance that deters or inhibits feeding by an insect but does not necessarily kill it. Nevertheless these mushrooms are consumed by people, but only in restricted regions (e.g. in parts of Finland and Russia). In the basidiocarps of these species, within seconds after a physical injury, an apparently inactive precursor (a fatty acid ester of a marasmane sesquiterpene) is converted enzymatically into a range of pungent sesquiterpenes with an unsaturated dialdehyde functionality, possessing potent antimicrobial and cytotoxic activities. The injury brings the precursor, which is present as an emulsion in the latex within the lactifers, in contact with the enzyme systems that are kept apart in the intact basidiocarps.

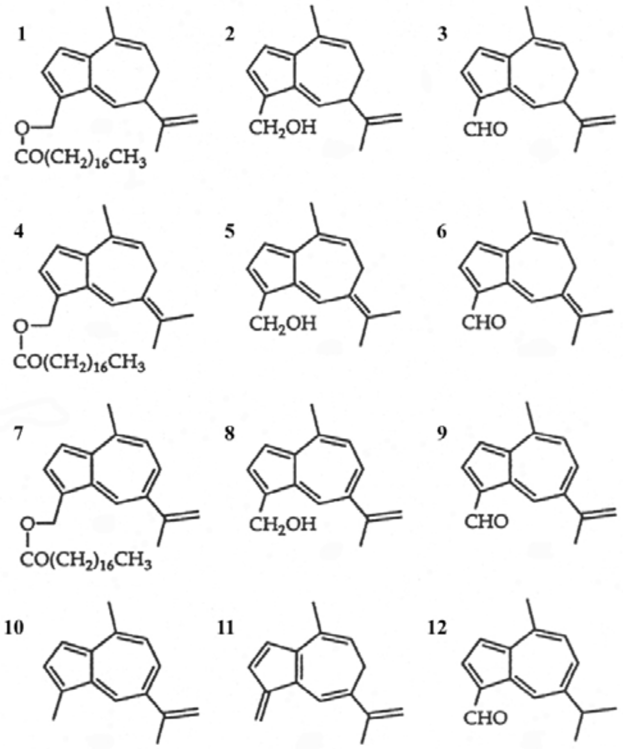

Fig. 7: Compounds with a guaiane sesquiterpenoid backbone isolated from members of *Lactarius* sect. *Deliciosi*: **1.** orange ester present in intact basidiocarps of *L. deliciosus* and *L. deterrimus*; **2.** alcohol derived from compound 1 after injury; **3.** delicial; **4.** red coloured compound found in *L. sanguifluus*; **5.** sangol; **6.** aldehyde isolated from *L. sanguifluus*; **7.** blue ester isolated only from *L. indigo*; **8.** deterrol; **9.** lactaroviolin; **10.** lactarazulene; **11.** lactarofulvene isolated from Californian specimens of "*L. deliciosus*"; **12.** aldehyde isolated from Indian specimens of "*L. deterrimus*". The green colour of the injured mushroom tissue emerges from a mixture of the orange-yellow compounds 1, 2 and 3 and the violet-blue compounds 8 and 9.

Species belonging to *Lactarius* sect. *Deliciosi* are among the most desirable by many consumers of wild mushrooms in large parts of the world. They contain precursors with a very different chemical structure that are converted in a similar way in response to injury, but to products with less striking biological activities and with still unknown function. Accordingly they lack the resistance to parasites, typical for the pungent species (STERNER & ANKE, 1995). The bright colours of the latex are caused by the presence and formation of azulene and hydroazulene sesquiterpenoids with a guaiane skeleton. In the intact basidiocarps, fatty acid (mainly stearic acid) esters of the sequiterpenes are present, which are converted to sesquiterpenoid alcohols and aldehydes upon bruising. Important differences with acrid tasting species are that the amounts of sesquiterpenoids are much smaller, that the enzymatic conversions are slower, and that the chemical functionalities present in the guaiane sesquiterpenes make them considerably less biologically active. STERNER & ANKE (1995) give a comprehensive overview of the esters and derived sesquiterpenes found so far in *Lactarius* sect. *Deliciosi* (Fig. 7).
Before, SCHMITT (1974) had published an elaborate study on the chemistry of the pigments in the latex of the species in *Lactarius* sect. *Deliciosi*. Special attention was paid to the taxonomical implications. Five common European species were included in this study: *L. deliciosus*, *L. deterrimus*, *L. salmonicolor*, *L. semisanguifluus* and *L. sanguifluus*. Results indicated that the orange, yellow and blue coloured components are the same in all species. The typical green discolouration of the basidiocarps is caused by a mixture of yellow-orange and blue coloured sesquiterpenoid compounds. Red coloured pigments like lactaroviolin were found in all five species but the initially red latex of *L. sanguifluus* contains additional red pigments like sangol. The different latex colours in the species are thus caused by different concentrations of mainly the same substances. This might partly explain why red and blue latex were derived many times independently during evolution (see chapter 5). Another observation was that after bruising and waiting for a certain colour change to have occurred,

concentrations of the colouring agents are much lower than in intact basidiocarps; indicating the instability of these pigments (SCHMITT, 1974).

6. Uses

6.1. Culinary aspects of *Lactarius* sect. *Deliciosi*

As the name of the section and its most popular species give away, many species in *Lactarius* sect. *Deliciosi* are edible and delicious. In fact, all species are probably edible but some are very inferior in taste (e.g. *L. salmonicolor*, HEIM & LECLAIR, 1950). Much depends however on the way of preparation; in several regions a mix of any *Lactarius* sect. *Deliciosi* species is collected or sold (e.g. in Slovakia and Barcelona, own observations; in Mexico, KONG LUZ, 1995 and in China, WANG *et al.*, 2004). The culinary properties of *Lactarius* sect. *Deliciosi* are especially appreciated in southern Europe (Catalunya, but also in the rest of Spain and southern France and on a smaller scale in Greece and Italy), eastern Europe and Russia, Turkey, Mexico, Guatemala, the Himalaya, China and Japan. People from Catalunya seem to prefer the taste of *L. sanguifluus* and even more the taste of *L. vinosus*. *Lactarius vinosus* is said to be most tasteful when greenish stains have developed on the pileus by the first frosts. In Catalunya, these species are commercially the second most important, after the *Tuber* spp. *Lactarius deliciosus* must have been popular since a long time in Mediterranean region. A fresco from Herculaneum, a city that was buried during the same series of eruptions that destroyed Pompeii in the year 79, accurately depicts several basidiocarps of *L. deliciosus* together with pheasants (AINSWORTH, 1976).

One of the reasons contributing to the popularity of these mushrooms is the ease by which they can be recognised. The presence of orange, red or blue coloured latex makes confusion with poisonous species impossible. Another advantage is that they often fruit in large quantities.

Many local, popular names circulate, mostly without making a distinction between the very similar species, e.g. 'pebrasus' in Ibiza; 'rovellón' or 'níscalo' in Catalunya and 'rovello', 'rovello albá' or 'rovelló de pí negre' in Valencia. In Mexico, *L. indigo* is sold at markets (also in Mexico City), and is known as 'añil', 'azul', 'hongo/corneta azul', 'zuin', 'zuine' or 'quexque' (MONTOYA *et al.*, 1996). Species with orange latex are called 'enchilado' (KONG LUZ, 1995).

L. deliciosus and relatives are being sold freshly or canned and are eaten roasted, prepared in a dish or in a cold salad. Numerous recipes can be found (e.g. PHILLIPS, 1983; GUERIN-LAGUETTE, 1998).

Eating *L. deliciosus* (and other species with orange latex?) may cause the urine to be blood-red, while this is not the case for red-milked species such as *L. sanguifluus* (HEIM & LECLAIR, 1950; MARCHAND, 1971; STERNER & ANKE, 1995). This is by no means harmful.

In a Russian study the protein content of *L. deliciosus* was determined (STANKYAVICHENE & URBONAS, 1988). Nineteen amino acids, including all essential ones, were found and these mushrooms were recommended as valuable food. The lipid content of *L. deliciosus* was shown to be only 1.02 % (ONDRUŠEK & PROŠTENIK, 1978). *Lactarius deliciosus* and relatives have also been used in several studies focussing on the concentrations of trace elements such as Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn and in edible mushrooms (FALANDYSZ *et al.*, 2002; ISILOGLU *et al.*, 2001). *Lactarius sanguifluus* e.g. was shown to accumulate easily several of these metals (a.o. Cd, Mn, Pb and Zn), especially when growing close to a road (ISILOGLU *et al.*, 2001).

6.2. Artificial mycorrhization

One of the most interesting applications of the species in *Lactarius* sect. *Deliciosi* is their use in artificial mycorrhization. The controlled mycorrhizal inoculation of seedlings in nurseries usually promotes the establishment of forest plantations, mainly by improving initial seedling growth (GROVE & LE TACON, 1993). Species like *L. deliciosus*, *L. sanguifluus* and *L. vinosus* present an additional income for forestry farms through the potential production of edible basidiocarps. In order to develop the use of an ectomycorrhizal species in forestry, a prerequisite step is to set up a reliable method of controlled mycorrhization of trees. A first step in this process is the efficient inoculation of seedlings. Methods for inoculating *Pinus sylvestris, P. halepensis* and *P. pinaster* with strains of *L. deliciosus* and *L. sanguifluus* have been developed and improved recently (GONZÁLEZ-OCHOA *et al.*, 2003; GUERIN-LAGUETTE *et al.*, 2000; PARLADE *et al.*, 2004). This has even lead to the fruiting of *L. deliciosus* under controlled, soil-less conditions (GUERIN-LAGUETTE *et al.*, 2000). YAMADA *et al.* (2001a&b) also managed to artificially inoculate *Pinus densiflora* with *L. akahatsu* and to obtain the development of *L. akahatsu* basidiocarps in open-pot soil under growth-chamber conditions. However, despite the fact that a high degree of mycorrhization on roots may contribute both to the field performance of trees after outplanting and to the persistence of the associated mycobiont, the ability of the introduced fungal isolate to develop in soil and to compete with indigenous mycorrhizal fungi is essential for determining the actual success of inoculation practices (GROVE & LE TACON, 1993; GUERIN-LAGUETTE *et al.*, 2003). GUERIN-LAGUETTE *et al.* (2003) use a forest soil bioessay approach to analyse the development on roots and the symbiotic efficiency of a rDNA internal transcribed spacer-selected isolate of *L. deliciosus*.

Researchers of the New Zealand Institute for Crop & Food Research imported European cultures of *L. deliciosus* to New Zealand in 1998 and carried out a series of inoculation experiments with seedlings of *Pinus radiata*, *Pinus densiflora* and *Picea abies*. Techniques were developed for producing sufficient *Pinus radiata* seedlings infected with *L. deliciosus* to establish two experimental plantations in 2000 on the North and South Island. After nine months, *L. deliciosus* infections had extended onto the new host root and contamination from competing fungi was minimal (WANG *et al.*, 2001). Further plantations were carried out in 2002 with the aim of producing basidiocarps of *L. deliciosus* as a secondary crop in *Pinus radiata* plantations.

Several companies, mainly in the Mediterranean region, have developed inoculum or inoculated seedlings on a commercial scale.

6.3. Other uses

The European species in *Lactarius* sect. *Deliciosi* have been the subject of study in pharmaceutical research and chemistry, in their search for new and useful natural products. Antimicrobial screening of 5 species occurring in Turkey for example revealed antagonistic activities against several important Gram (+) and Gram (-) pathogens but not against yeasts (DULGER *et al.*, 2002). AYER & TRIFONOV (1994) examined the useful metabolites produced by *L. deliciosus* in liquid culture.

Chapter 3

Lactarius sanguifluus versus *Lactarius vinosus*

Adapted from:
NUYTINCK J. & VERBEKEN A. (2003) – *Lactarius sanguifluus* versus *Lactarius vinosus* – molecular and morphological analyses. *Mycological Progress* **2**: 227-234.

Abstract

Lactarius vinosus, a relatively rare Mediterranean representative of *Lactarius* sect. *Deliciosi*, is often considered as a variety of *L. sanguifluus*. Morphological reasons (especially macroscopical and spore characters) and molecular arguments (based on ITS sequences) are given here to treat it as a separate species. The relationship of *L. vinosus* with the closely related *L. sanguifluus* and *L. semisanguifluus* is discussed.

1. Introduction

The results presented here are part of a more extensive study of *Lactarius* section *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.). The species in this section are easily recognised by their orange or red to blue coloured latex, caused by the presence of guaiane sesquiterpenes (BERGENDORFF & STERNER, 1988; SCHMITT, 1974; STERNER & ANKE, 1995). Members of *Lactarius* sect. *Deliciosi* are highly appreciated for their culinary qualities, especially *L. deliciosus* (L.: Fr.) Gray, which has orange coloured milk, and *L. sanguifluus* (Paulet) Fr. with vinaceous-red coloured latex. *Lactarius sanguifluus* is since long a well-established species in Europe, described in detail by KONRAD & MAUBLANC (1952), KÜHNER & ROMAGNESI (1953) and NEUHOFF (1956). This is not the case for another species with wine-red latex, *L. vinosus* (Quélet→) Bataille (see discussion for explanation of author citation). As LALLI *et al.* (2002) pointed out, there are two quite opposite opinions on the concept of *L. vinosus*. MARXMÜLLER & ROMAGNESI (1991) use this name for dull orange coloured, pale basidiocarps while BLUM (1976), COURTECUISSE & DUHEM (1994), MOSER & JÜLICH (1996) and BASSO (1999a) use it for collections with mostly vinaceous red tinges. Moreover, *L. vinosus* is often regarded as a variety of *L. sanguifluus* (BASSO, 1999a; BLUM, 1976; MARXMÜLLER & ROMAGNESI, 1991).

This paper clarifies the status of the taxa *L. sanguifluus* and *L. vinosus* and the relationships between them and their closest allies by using ITS sequences. For an extensive morphological description and comparison of both taxa, we refer to chapter 6.

2. Materials & Methods

2.1. Examined material

This study is based on herbarium material deposited in GENT (see Table 1 for the collections used in the phylogenetic analyses). Most collections have recently been made by the first author of this paper and have been described and compared extensively in fresh condition.

2.2. DNA preparation

Two methods were used for DNA extraction.

1) Dried material (about 50 mg) was first ground in liquid N_2. One ml extraction buffer (0.1 M Tris-HCl pH=8, 0.5 M NaCl, 0.05 M EDTA and 0.01 M β-mercapto-ethanol) and 50 µl 10% SDS were added and the mixture was incubated for 30 min. at 65°C. This extraction was centrifuged for 10 min. at 14000 rpm (Eppendorf centifuge, Hamburg, Germany). An equal volume of isopropanol was added

to the supernatant and after mixing the solution, it was centrifuged again as above. The pellet was washed with 70% ethanol, air dried and dissolved in 400 µl dH_2O. Then, 400 µl CTAB buffer (2% w/v CTAB, 0.2 M Tris-HCl pH=7.5, 2 M NaCl and 0.05 M EDTA) was added and after incubation for 15 min. at 65°C, the mixture was extracted twice with chloroform/iso-amylalcohol (24:1). Two volumes of 96% ethanol were added and the mixture was centrifuged again as above to pellet the DNA. The pellet was washed with 70% ethanol and dissolved in 100 µl TE.

2) Fresh material (about 200 mg) was put in a microcentifuge tube with 2x CTAB buffer (2% w/v CTAB, 100 mM Tris, 1.4 M NaCl and 20 mM EDTA) and stored at 4°C. The material was ground at room temperature in the buffer and incubated for 1 h at 65°C. Proteins were removed by two chloroform/iso-amylalcohol extractions. DNA was precipitated by adding 500 µl cold isopropanol and centrifuging for 10 min. at 5000 rpm. Pellets were washed with 70% ethanol and dissolved in 200 µl TE.

Quality and concentration of the DNA preparations was checked on 0.8% agarose gel.

Table 1: Samples used in phylogenetic analyses; including voucher number, geographic origin and GenBank accession numbers.

Species	Voucher (GENT)	Locality	GenBank accession number
outgroup			
L. subsericatus	-	-	AF140254
L. srobiculatus	-	-	AF140262
Lactarius* sect. *Deliciosi			
L. deliciosus	J. Nuytinck 2001-046	Slovakia, Moravsk˘y Svatý jàn village	AY332557
"L. semisanguifluus"	-	-	AF096988
L. sanguifluus	-	-	AF096981
L. sanguifluus	-	-	AF249289
L. sanguifluus	-	-	AF249290
L. sanguifluus	A. Verbeken 99-246	France, Manoir de Syrignac	AY332544
L. sanguifluus	J. Nuytinck 2000-006	Belgium, Ave et Auffe (1)	AY332548
L. sanguifluus	J. Nuytinck 2000-008	Belgium, Tellin (2)	AY332547
L. sanguifluus	J. Nuytinck 2001-050	Slovakia, near Ploveckli Podhra dic	AY332546
L. sanguifluus	J. Nuytinck 2001-096	Italy, Poggio Roio	AY332545
L. semisanguifluus	-	-	AF140268
L. semisanguifluus	-	-	AF249292
L. semisanguifluus	A. Verbeken 96-1032	Belgium, close to Rochefort	AY332553
L. semisanguifluus	C. Lange 96-081	Sweden, Tjaucklehagen	AY332555
L. semisanguifluus	J. Nuytinck 2001-049	Slovakia, Bratislava	AY332554
L. semisanguifluus	J. Nuytinck 2001-093	Italy, Chiarino	AY332556
L. vinosus	A. Peksen & G. Hatat 230	Turkey, Samsun	AY332549
L. vinosus	J. Nuytinck 2001-104a	Spain, St Hilary Sacalm (1)	AY332551
L. vinosus	J. Nuytinck 2001-104c	Spain, St Hilary Sacalm (2)	AY332552
L. vinosus	J. Nuytinck 2001-110	Spain, Ermita Sart Ponç (3)	AY332550

2.3. ITS amplification

The basidiomycete specific primers ITS1-F and ITS4-B (GARDES & BRUNS, 1993) were used to amplify the ITS region (part of the 18S rDNA, the ITS1 region, the 5.8S region, ITS2 region and part of the 28S region). The following touchdown profile was used: 95°C during 2 min, then 10 cycles of denaturation at 95°C (during 15 sec), primer annealing at 63°C (during 20 sec) with the temperature decreasing with 1°C at each cycle, polymerisation during 1 min. at 72°C; followed by 35 cycles with the same profile for denaturation and polymerisation but with a constant annealing temperature of 53°C; polymerisation was completed by an incubation of 2 min. at 72°C.

2.4. ITS sequencing

The obtained PCR products were purified using ExoSAP (USB, USA). DNA sequencing reactions were performed with the ABI PRISM® BigDye^{TM} Terminators v3.0 Cycle Sequencing Kit using primers ITS1-F, ITS2, ITS3 and ITS4-B (GARDES & BRUNS, 1993; WHITE *et al.*, 1990) on an ABI PRISM® 377 DNA Sequencer. The Phred and Phrap Software (EWING & GREEN, 1998; EWING *et al.*, 1998) was used to process the raw data.

2.5. Data analysis

Sequences were deposited in GenBank (NCBI, http://www.ncbi.nlm.nih.gov/, under accession numbers AY332544 to AY332557). Sequences were aligned in ClustalW (THOMPSON *et al.*, 1994) and the alignment was manually corrected where necessary. Phylogenetic analyses were executed using PAUP*4b10 (SWOFFORD, 2002). A maximum parsimony analysis was performed with all sites treated as unordered and unweighted and with gaps treated as missing data. A total of 1000 random-addition sequence replicates were used, the branch-swapping algorithm was TBR, the MulTrees option was in effect and the steepest descent option was not in effect. Support for the internodes was estimated by 1000 bootstrap replicates with a heuristic search with 100 random-addition sequences for each replicate and the TBR branch-swapping algorithm. For maximum likelihood analysis, the substitution model of DNA evolution with corresponding parameters that fitted the data best was determined using likelihood ratio tests (LRT's) as implemented in Modeltest 3.06 (POSADA & CRANDALL, 1998). Heuristic search settings were random taxon addition with 10 replicates, TBR branch swapping, MulTrees option in effect and steepest descent option not in effect. A bootstrap analysis with 100 replicates was used to assess the reliability of individual branches in the phylogenetic tree. Alternative phylogenetic hypotheses were tested using the Shimodaira-Hasegawa test (SHIMODAIRA & HASEGAWA, 1999; GOLDMAN *et al.,* 2000), using the RELL option and 1000 bootstrap replicates (default settings in PAUP*).

3. Results

3.1. Sequence analysis and alignment

We sequenced the ITS region of 14 European and Turkish collections belonging to 4 taxa. Additionally, 8 sequences from GenBank were used for our phylogenetic analysis, bringing the total number of taxa in the analysis to 6. Four of these taxa belong to *Lactarius* sect. *Deliciosi*: *L. deliciosus*, *L. sanguifluus*, *L. semisanguifluus* R. Heim & Leclair and *L. vinosus*. The 2 species used as outgroup were *L. subsericatus* (Kühner & Romagn.) ex Bon and *L. scrobiculatus* (Scop.: Fr.) Fr.

The total length of ITS1, 5.8S and ITS2 regions ranged from 594 to 598 bp for the ingroup taxa and from 594 to 609 bp when the outgroup taxa are included. When looking only at the ingroup, the number of variable sites in the ITS1 region (219-231 bp) was 43. The ITS2 region (209-220 bp) had 35 variable sites within the ingroup. This means 16.7% of the sites in the alignment of ITS1 and ITS2 was variable within the ingroup species.

The highest value of interspecific sequence diversity between the species in this analysis is 13.5%. The interspecific sequence diversity between the ingroup species ranges from 1.4% to 7.5%. The intraspecific sequence divergence in the ingroup taxa ranges from 0.1% to 1.4%. These values are similar to the ones found by EBERHARDT (2000). Within *Lactarius* species an ITS sequence diversity of less than 1% is most frequent but larger differences exist. Despite of the overlap of intra- and interspecific sequence diversity, conspecific isolates cluster in the phylogenetic analysis (EBERHARDT, 2000 and this study).

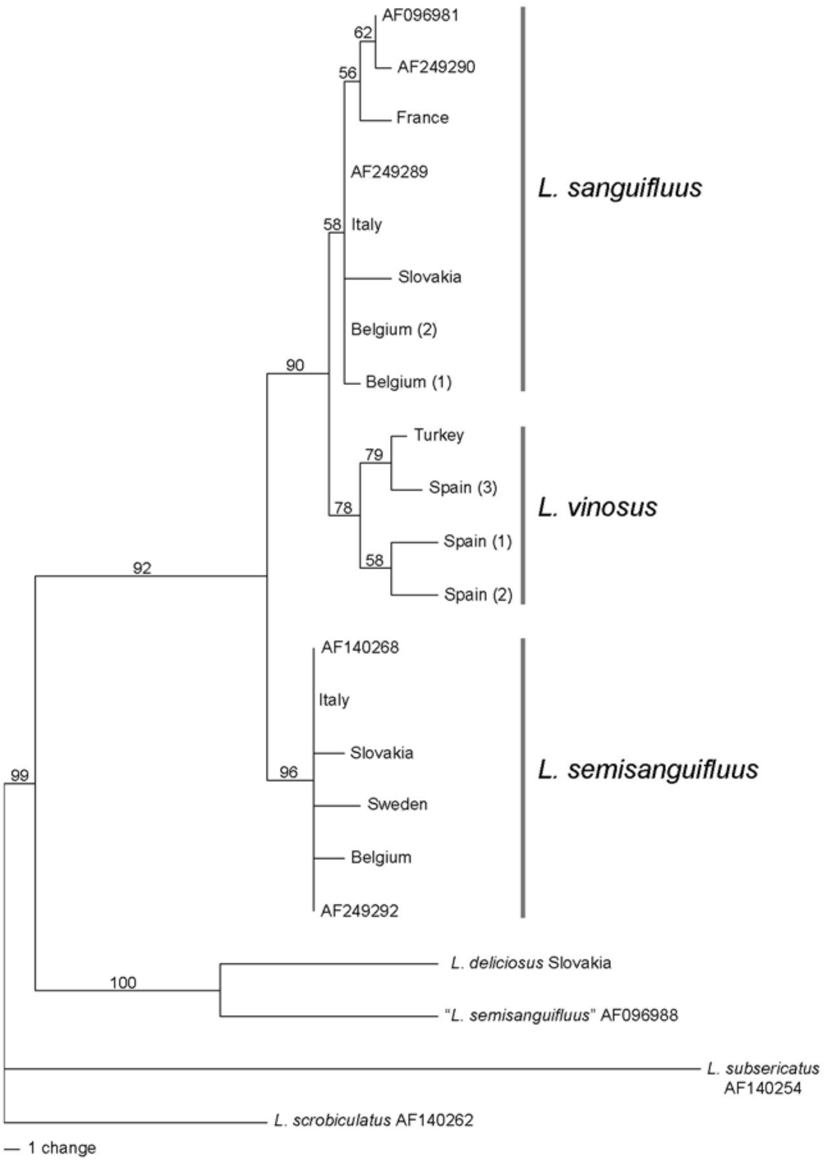

Fig. 1: Phylogenetic relationships of *L. vinosus* based on 47 parsimony-informative characters from ITS sequences of the nuclear ribosomal DNA. Best (-ln L = 1731.564) of six most parsimonious trees. Used specimens are labelled according to their origin (see also table 1). Numbers above the internodes represent the bootstrap values.

3.2. Molecular phylogeny

The alignment of the entire sequenced region yielded a total of 704 characters, 584 of which were constant, 73 variable parsimony-uninformative and 47 parsimony-informative. Maximum parsimony analysis, with gaps treated as missing data, resulted in six most parsimonious trees of 150 steps, CI = 0.920, RI = 0.891, RC = 0.820. All six trees are on the same tree island, are retrieved in all 100 replicates and show *L. sanguifluus*, *L. semisanguifluus*, *L. vinosus* and *L. deliciosus* as separate clusters. They differ only by some rearrangements within the *L. semisanguifluus* clade. The topology of the strict consensus of the shortest trees is identical to the tree shown in Fig. 1. Only the *L. deliciosus* and *L. semisanguifluus* clades are highly supported (bootstrap support of 100% and 93% respectively); *L. vinosus* receives a bootstrap value of 78% in the maximum parsimony analysis and clusters always separately from *L. sanguifluus*.

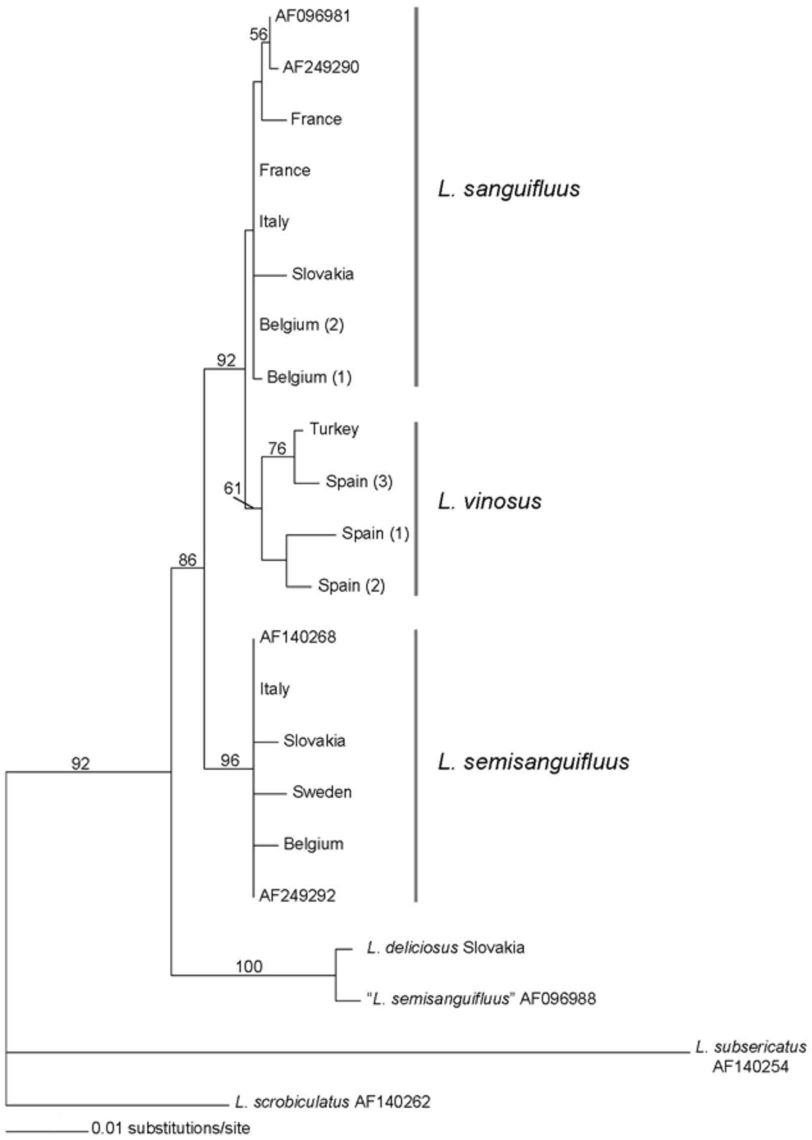

Fig. 2: ML tree based on ITS sequences, -ln likelihood = 1842.24786. Likelihood settings (from Modeltest): K81+G, R = (1, 11.184, 2.890, 2.890, 11.184) and gamma shape parameter 0.4230. Used specimens are labelled according to their origin (see also table 1). Numbers above the internodes represent the bootstrap values.

Based on the results of Modeltest LRT evaluations, maximum likelihood analysis was performed with the K81+G model (KIMURA, 1981; SWOFFORD *et al.*, 1996), with values: Base = equal, R = (1, 11.184, 2.890, 2.890, 11.184), Pinvar = 0 and gamma shape parameter = 0.4230 . The analysis resulted in a single tree (-ln likelihood = 1842.24786) with a similar topology as the consensus tree resulting from the maximum parsimony analysis (Fig. 2). The six most parsimonious trees and the maximum likelihood tree differ only in the arrangement of the accessions within the *L. semisanguifluus* clade. The bootstrap value of the *L. vinosus* clade is lower (61%) in the maximum likelihood tree than in the maximum parsimony analysis.

The Shimodaira-Hasegawa test was used to compare the maximum likelihood tree shown in Fig. 2 with a tree in which *L. sanguifluus* and *L. vinosus* are collapsed into one branch. The test showed that the topologies of these trees yielded significantly different (P<0.05) likelihood scores.

In both analyses the GenBank accession "*L. semisanguifluus*" AF096988 falls outside the *L. semisanguifluus* clade, preliminary analysis has shown that it is conspecific with *L. deliciosus* (see chapter 4).

4. Discussion

The phylogenetic analyses were performed with 22 specimens representing six taxa. These include two well-known species in *Lactarius* section *Deliciosi*, *L. deliciosus* and *L. semisanguifluus*, characterised by latex which is bright orange when it is first secreted but turns more or less quickly to wine-red. *Lactarius sanguifluus* and *L. vinosus* are the only two European taxa with latex that is vinaceous red from the start. The outgroup taxa, *L. scrobiculatus* and *L. subsericatus* represent other *Lactarius* sections: *Lactarius* sect. *Zonarii* (Quél.) Bon (same subgenus as sect. *Deliciosi*, viz. *Piperites* (Fr. ex J. Kickx f.) Kauffman) and *Lactarius* sect. *Russularia* Fr. ex Burl. (in *L.* subgenus *Russularia* (Fr. ex Burl.) Kauffman) respectively. *Lactarius sanguifluus* and *L. vinosus* form two separate clades in the maximum parsimony and maximum likelihood analysis but are not supported by (high) bootstrap values. The Shimodaira-Hasegawa test also convinced us to reject the hypothesis that *L. sanguifluus* and *L. vinosus* are conspecific. Together with the available morphological arguments (see below), we propose to treat *L. sanguifluus* and *L. vinosus* as separate species. The allozyme-analysis of LALLI *et al.* (2002) confirms our results. Sistergroup to these two species is *L. semisanguifluus* with milk that turns from orange to red in just a few minutes. This contradicts the traditional division of *Lactarius* sect *Deliciosi* in two subsections *Deliciosini* Bon & Basso and *Sanguifluini* Bon & Basso (BASSO, 1999a; BON, 1980) in which the latter species is included in the *Deliciosini*.

Morphologically there are some striking differences between *L. sanguifluus* and *L. vinosus*. Macroscopically the colour of the cap, stipe and lamellae is more (pale) vinaceous red in *L. vinosus*, which is almost completely lacking orange tinges. This is in accordance with the oldest descriptions of *L. vinosus* (BARLA, 1859; QUÉLET, 1881) and contradicts the opinion of MARXMÜLLER & ROMAGNESI (1991). Possibly their concept of *L. vinosus* refers to a pale form of *L. sanguifluus*, but we had no specimens available to check this. The stipe is more distinctly tapering downwards and the latex colours the context of the cap more intensely and completely in *L. vinosus*. Microscopically the reticulum on the spores of *L. vinosus* is less complete and less dense than in *L. sanguifluus*. In *L. sanguifluus* the ridges are more regular and evenly thick, while in *L. vinosus* they are locally thinner (see chapter 6).

Some confusion exists about the correct epitheton and authority of *L. vinosus* (see chapter 6 for a more elaborate discussion). The oldest known reference to *L. vinosus* is from BARLA (1855): a drawing of half a basidiocarp with the name *Agaricus deliciosus* var. *violaceus*. This publication is effective and valid. The description was given in a later work of BARLA (1859) without reference to the earlier drawing. QUÉLET (1881) explicitly refers to the work by Barla but changes the epitheton from *violaceus* to *vinosus* ("*Lactarius sanguifluus* var. *vinosus*"). Quélets name is thus an illegitimate synonym. BATAILLE (1908) is the first author to use the name *vinosus* on species level. Although its basionym is illegitimate, this combination has to be considered as a legitimate nom. nov. The correct author citation is thus *L. vinosus* Bataille, or *L. vinosus* (Quél.→) Bataille, as proposed by KUYPER & VAN VUURE (1985) to refer to a replaced illegitimate name that typifies the newly created name.

No type has been indicated for *L. vinosus,* but the lectotype becomes automatically the drawing by Barla from 1855 (see also chapter 6).

LALLI *et al.* (2002) studied the taxa with vinaceous-red latex by an electrophorethical analysis of 11 enzyme loci. The results show that their 5 morphological forms (based on colour variations of the pileus), separated into two clear groups. These correspond with *L. sanguifluus* and *L. vinosus* sensu COURTECUISSE & DUHEM (1994) and MOSER & JÜLICH (1996). These results are confirmed by our analysis.

Acknowledgements

The authors wish to thank Maria Teresa Basso, Aysun Peksen, Gürsel Hatat and Andreas Gminder for kindly providing us with collections. The Department of Plant Systems Biology (VIB, UGent) is thanked for help with sequencing. This research was financed by the Institute for the promotion of Innovation by Science and Technology in Flanders, Belgium.

Chapter 4

Species delimitation and phylogenetic relationships in *Lactarius* sect. *Deliciosi* in Europe

Adapted from:
NUYTINCK J. & VERBEKEN A. (2005) – Species delimitation and phylogenetic relationships in *Lactarius* sect. *Deliciosi* in Europe. *Mycological Research*: accepted.

Abstract

The phylogeny of *Lactarius* sect. *Deliciosi* was investigated employing both molecular and morphological characters. We used the nrDNA ITS sequences and an 800 bp fragment of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*). Results confirm that *Lactarius* sect. *Deliciosi* forms a monophyletic group within subgenus *Piperites*. The section contains nine accepted species in Europe, eight of which are traditionally being placed in sect. *Deliciosi. Lactarius porninsis*, a species with white latex that is usually classified in sect. *Zonarii*, also falls within in sect. *Deliciosi*. Some recently described species are not confirmed here (*L. sanguineovirescens*) or their status remains doubtful because of a lack of collections (*L. cyanopus*). AFLP results show a clear distinction between the closely related *L. deterrimus* and *L. fennoscandicus*.

1. Introduction

European representatives of *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn can easily be recognised from other *Lactarius* species by their bright orange to red coloured latex and general appearance. The characteristic green discolouration of the basidiocarps is caused by a mixture of yellow-orange and blue coloured sesquiterpenoid compounds, formed by enzymatic conversion of orange and red coloured fatty acid esters of sesquiterpenes, present in the latex (STERNER & ANKE, 1995). The basidiocarps often have a distinctly zonate pileus and scrobiculate stipe. The colour change of the latex and the host tree species are some of the key characters for identification of the species. For good morphological species descriptions we refer to HEILMANN-CLAUSEN *et al.* (1998) and BASSO (1999a).

All European members of *Lactarius* sect. *Deliciosi* form ectomycorrhiza with coniferous trees (*Pinus*, *Picea* and *Abies*) and exhibit a remarkable host specificity. Fungal lectins are possibly involved in the recognition between the symbionts (GUILLOT *et al.*, 1994). Several studies and experiments concerning artificial inoculation of pine trees with *L. deliciosus* and *L. sanguifluus* have been undertaken (GONZÁLES-OCHOA *et al.*, 2003; GUERIN-LAGUETTE *et al.*, 2003; PARLADE *et al.*, 2004).

Lactarius sect. *Dapetes* (Fr. ex J. Kickx f.) Burl. is a synonym of *Lactarius* sect. *Deliciosi*, a name recently rectified by REDEUILH *et al.* (2001).

Although for Europe only, 41 species, variety and form names have been published in this section between 1753 and now, only seven species are commonly and currently recognised by most authors (BASSO, 1999a; BON, 1980; COURTECUISSE & DUHEM, 1994; HEILMANN-CLAUSEN *et al.*, 1998). These are *L. deliciosus* (L.: Fr.) Gray, *L. sanguifluus* (Paulet) Fr., *L. salmonicolor* R. Heim & Leclair, *L. semisanguifluus* R. Heim & Leclair, *L. quieticolor* Romagn., *L. deterrimus* Gröger and *L. vinosus* (Quélet→) Bataille. Some of these species are well known for their excellent edibility and are being sold on markets, mainly in southern and eastern Europe. We are preparing a detailed account of the taxonomy and nomenclature of the section. Until 1950, only two species were recognised in this striking group: *Lactarius deliciosus* with orange milk and *L. sanguifluus* with red milk. With the publications by HEIM & LECLAIR (1950), HEIM (1953), ROMAGNESI (1958) and GRÖGER (1968) a better understanding of the section was created, resulting in more species. Several species were described very recently and some of them are still very poorly known (BASSO, 1998; FILLION, 1989; ROMAGNESI, 1980; VERBEKEN & VESTERHOLT, 1998). *L. cyanopus* Basso and *L. sanguineovirescens* Fillion are known from a few localities only. Due to the relatively recent description of most of the species and the variability of some of the important macroscopical characters (e.g. the colour and zonation of the pileus and the colour and colour change of the latex on the context), misidentifications are common and not all authors share the same species concepts.

In this study we used a combination of the ITS region, an 800 bp fragment of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and morphology data to analyse species delimitations and phylogenetic structure within *Lactarius* sect. *Deliciosi*. We studied the molecular and morphological inter- and intraspecific variability, the intragenomic variability of the ITS region, paid attention to the evolution pattern of the ectomycorrhizal host species and the biogeographic patterns within species.

2. Materials & Methods

2.1. Fungal collections

For the morphological part of this work, 230 fresh and herbarium specimens collected in Europe were studied (for their distribution see Fig. 1). All commonly accepted *Lactarius* sect. *Deliciosi* species were included plus all of the more recently described and poorly known taxa. Herbarium material was deposited in or obtained from GENT, BR, LIP, PC, MPU and the personal herbaria of P.A. Moreau and M. Contu.

2.2. Sequence data

Per species several specimens were used for sequencing in order to cover the range of morphological and geographical variation and hence test for morphological species concepts. For 54 collections we sequenced the nrDNA ITS region and for 31 collections a part of the *gpd* gene (Table 1). Table 2 lists the GenBank sequences that were used in this study.

Three DNA extraction methods were used: the CTAB-based methods as described in NUYTINCK & VERBEKEN (2003) and a protocol using benzyl chloride adapted from ZHU *et al.* (1993). For the latter method, 500 µl extraction buffer (100 mM Tris-HCl, pH 9.0, 40 mM EDTA), 100 µl 10% SDS and 300 µl benzyl chloride were added to the ground sample. After a 30 min. incubation at 50 °C, 300 µl 3 M NaOAc, pH 5.0 was added and the tube was kept on ice for 15 min. The supernatant was then collected after centrifugation at 6,000 g and DNA was precipitated with isopropanol and washed with 70% ethanol.

Primers ITS1-F and ITS4-B (GARDES & BRUNS, 1993) were used to amplify the ITS region. A touchdown PCR profile was used as in NUYTINCK & VERBEKEN (2003). The *gpd* gene was amplified using primers CTK-132 and CTK-108$_{rev}$ and the PCR program described for the *Lactarius*-specific primers in KREUZINGER *et al.* (1996). The obtained PCR products were purified using ExoSAP (USB). DNA sequencing reactions were performed with the ABI PRISM® BigDye^{TM} Terminators v3.0 Cycle Sequencing Kit using the same primers on an ABI PRISM® 377 DNA Sequencer. The Phred and Phrap Software (EWING *et al.*, 1998; EWING & GREEN, 1998) was used to process the raw data. Sequences were deposited in GenBank (accession numbers see Table 1).

For 26 specimens the ITS PCR products were cloned and two clones per collection were sequenced. We used the pGEM$^{®}$-T vector (Promega) for this purpose and followed the instructions provided by the manufacturer.

Table 1: Collections used for molecular analyses. GenBank (ITS, *gpd*) accession numbers will be completed. Herbarium number, origin and host tree are indicated. All collections are deposited in the GENT herbarium, collections by P.A. Moreau are kept in his personal herbarium. Collections marked with an asterisk were used in the AFLP analysis.

Species	**Voucher collection**	**GenBank accession numbers**	**Country of origin and locality**	**Trees at collection locality**
L. cyanopus	M.T. Basso 98102204		Italy, Civezza, Type locality	*Pinus*
L. deliciosus	J. Vesterholt 96-312		Denmark, Estrupvej	*Pinus*
L. deliciosus	J. Vesterholt 96-457		Italy, cala violina NW of Grosseto (1)	*Pinus*
L. deliciosus	A. Peksen & G. Hatat 229		Turkey, Samsun	*Pinus*
L. deliciosus	A. Verbeken 2000-104		Italy, Tempio Pausania (2)	*Pinus halepensis*
*L. deliciosus**	J. Nuytinck 2001-005		Sweden, Borgsjö	*Pinus sylvestris*
*L. deliciosus**	J. Nuytinck 2001-046	AY332557	Slovakia, Moravskˇy Svatý jàn village	*Pinus sylvestris*
L. deterrimus	A. Verbeken 96-1004		Denmark, Varming plantage (1)	*Picea*
L. deterrimus	J. Vesterholt 96-332		Denmark, Rubjerg Knude Plantage (2)	*Picea* in *Abies* dominated forest
L. deterrimus	J. Vesterholt 96-333		Denmark, Rubjerg Knude Plantage (3)	*Picea* in *Abies* dominated forest
L. deterrimus	J. Heilmann-Clausen 97-176		Sweden, Bräcke, Giman (1)	*Picea*
L. deterrimus	A. Verbeken 97-561		Sweden, West of Ånge (2)	*Picea*
L. deterrimus	J Vesterholt 97-217		Sweden, Jämtland, Brunflo (3)	Mixed forest
L. deterrimus	J. Nuytinck 2000-002		Belgium, Ruisseau d'Alise	*Picea*
L. deterrimus	J. Nuytinck 2000-005		Netherlands, Adamskamp	*Picea*
L. deterrimus	J. Nuytinck 2000-010		Belgium, Kessel-Lo (2)	*Picea*
*L. deterrimus**	J. Nuytinck 2001-008		Sweden, Borgsjö (4)	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-014	-	Sweden, Brunflo	*Pinus strobus*!
*L. deterrimus**	J. Nuytinck 2001-053		Slovakia, Dubina forest , Gaderskà dolina valley (1)	*Picea abies*
L. deterrimus	J. Nuytinck 2001-073		Slovakia, Malužiná village, Michalovo valley (2)	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-076		Slovakia, Dubové village (3)	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-077	-	Slovakia, Dubové village	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-078	-	Slovakia, Dubové village	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-079a	-	Slovakia, Dubové village	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-079b	-	Slovakia, Dubové village	*Picea abies*
*L. deterrimus**	J. Nuytinck 2001-099		Italy, Castel del Monte	*Picea abies*
L. fennoscandicus	J. Vesterholt 95-330		Sweden, Reva, at Indalsälven (1)	*Picea*
L. fennoscandicus	A. Verbeken 97-530 TYPE		Sweden, Liljanfors (2)	*Picea*
L. fennoscandicus	J. Heilmann-Clausen 97-119		Sweden, Liljanfors (3)	*Picea*

Species	**Voucher collection**	**GenBank accession numbers**	**Country of origin and locality**	**Trees at collection locality**
L. fennoscandicus	J. Heilmann-Clausen 97-183		Sweden, Bräcke, Giman (4)	*Pinus* and *Picea*
L. fennoscandicus	P.A. Moreau 05-09-1999		France, St Germain de Jaux	Mixed *Picea-Abies* wood
L. fennoscandicus	R. Walleyn 1577BIS		Sweden, Ange, along Geteraen river (5)	*Picea*
L. fennoscandicus	J. Nuytinck 2001-018		Sweden, Medelpad (6)	*Picea abies*
*L. fennoscandicus**	J. Nuytinck 2001-027	-	Sweden, Bergasen, close to Borgsjö	*Pinus, Picea, Betula* and *Salix*
*L. fennoscandicus**	J. Nuytinck 2001-028	-	Sweden, Bergasen, close to Borgsjö	*Pinus, Picea, Betula* and *Salix*
*L. fennoscandicus**	J. Nuytinck 2001-030		Sweden, close to Ange (7)	*Picea abies*
*L. fennoscandicus**	J. Nuytinck 2001-031		Sweden, close to Granboda (8)	*Picea abies, Pinus sylvestris* and *Betula*
L. fennoscandicus	J. Nuytinck 2001-039		Finland, Enonkoski	*Picea abies*
L. porninsis	J. Nuytinck 2001-082		Slovakia, Demanovska dolina valley, Demanova village	*Picea abies* and *Larix decidua*
L. porninsis	J. Nuytinck 2002-019		Italy, Trento	*Larix decidua*
L. quieticolor	R. Walleyn 1503		Belgium, Wingene, St. Pietersveld (1)	*Pinus*
L. quieticolor	J. Nuytinck 2000-009		Belgium, Wingene, Gulke putten (2)	*Pinus*
L. quieticolor	J. Nuytinck 2000-011		Belgium, Aalter, Drongengoed (3)	*Pinus*
L. quieticolor	J. Nuytinck 2001-004		Sweden, close to Mora	*Pinus sylvestris*
*L. quieticolor**	J. Nuytinck 2001-084	-	Slovakia, close to Bratislava	*Pinus*
L. salmonicolor	A. Verbeken 98-056		France, Forêt de Dorans, Bois de Chevrey (1)	*Abies*
L. salmonicolor	J. Nuytinck 2001-087		Belgium, close to Rochefort	*Abies alba*
L. salmonicolor	J. Nuytinck 2001-123		France, close to Aix les Bains (2)	*Abies alba*
L. salmonicolor	J. Nuytinck 2002-027, leg. M. Contu		Italy, Sassari, Madonna Della Neve	*Abies cephalonica*
L. sanguifluus	A. Verbeken 99-246	AY332544	France, Manoir de Syrignac	*Pinus sylvestris*
L. sanguifluus	J. Nuytinck 2000-006	AY332548	Belgium, Ave et Auffe (1)	*Pinus nigra*
L. sanguifluus	J. Nuytinck 2000-008	AY332547	Belgium, Tellin (2)	*Pinus*
L. sanguifluus	J. Nuytinck 2001-050	AY332546	Slovakia, near Ploveckli Podhra dic	*Pinus nigra*
L. pinastri/sanguineo-virescens?	P.A. Moreau 30-09-2001		France, Sevrier	
L. sanguineovirescens?	P.A. Moreau 13-10-2001		France, Minzier	*Pinus*?
L. semisanguifluus	C. Lange 96-081	AY332555	Sweden, Tjaucklehagen	*Pinus*
L. semisanguifluus	A. Verbeken 96-1032	AY332553	Belgium, close to Rochefort	*Pinus*
L. semisanguifluus	J. Nuytinck 2001-049	AY332554	Slovakia, Bratislava	*Pinus sylvestris*
L. semisanguifluus	J. Nuytinck 2001-093	AY332556	Italy, Chiarino	*Pinus nigra*

Species	**Voucher collection**	**GenBank accession numbers**	**Country of origin and locality**	**Trees at collection locality**
L. vinosus	A. Peksen & G. Hatat 230	AY332549	Turkey, Samsun	*Pinus*
L. vinosus	J. Nuytinck 2001-104a	AY332551	Spain, St Hilary Sacalm (1)	*Pinus*
L. vinosus	J. Nuytinck 2001-104c	AY332552	Spain, St Hilary Sacalm (2)	*Pinus*
L. vinosus	J. Nuytinck 2001-110	AY332550	Spain, Ermita Sart Ponç (3)	*Pinus*
L. tesquorum	J. Nuytinck 2001-121		Spain, Ca'l Coix, Maçanet de la Selva	*Cistus* sp.
L. rufus	J. Nuytinck 2002-008		Norway, Frognerseteren, close to Oslo	*Picea abies*

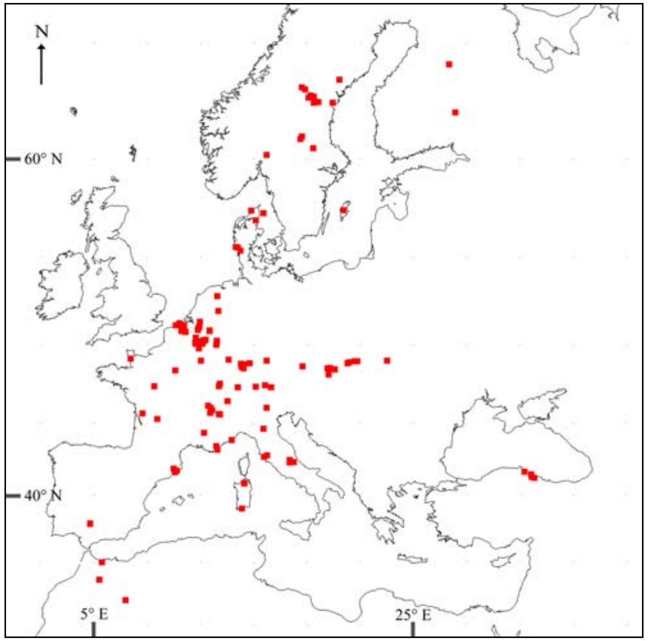

Fig. 1: Map of Europe with the distribution of the studied collections.

2.3. Phylogenetic analyses

We aligned the sequences using ClustalX 1.83 (THOMPSON *et al.*, 1997) and manually corrected and refined the alignments[1]. The alignments are available through TreeBASE (ID numbers will be added). The initial ITS data matrix contained 135 accessions, representing all common and widely recognised European species in *Lactarius* sect. *Deliciosi*, plus some of the more recently described and badly known taxa. It included our ITS sequences (Table 1), 30 extra *Deliciosi* sequences available from GenBank and another 21 ITS sequences from *Lactarius* species, belonging to subgenera *Piperites* (Fr. ex J. Kickx f.) Kauffman*, Russularia* (Fr. ex Burl.) Kauffman and *Plinthogali* (Burl.) Hesler & A.H. Sm. (Table 2). Sequence characteristics were calculated using this alignment (including only ITS1, 5.8S nrDNA and ITS2).

Table 2: GenBank sequences used in this study.

Species	GenBank accession numbers
L. deliciosus	AF092838, AF093456 (bad quality), AF096984, AF096985, AF096988 (as *L. semisanguifluus*) , AF101783, AF230892, AF249283, AF249284, U80999
L. deterrimus	AF096990 (as *L. deliciosus*), AF140266, AF140267, AF249285, AF249286
L. quieticolor	AF140269
L. salmonicolor	AF140258, AF140264, AF140265, AF249287, AF249288
L. sanguifluus	AF096980, AF096981, AF249289, AF249290, AF249291, AY296130
L. semisanguifluus	AF140268, AF249292
L. vinosus (as *L. rubrozonatus*)	AY292987
L. acerrimus	AJ278139
L. blennius	AY331015
L. chrysorrheus	AF096983
L. controversus	AJ272244
L. fluens	AY331014
L. fuliginosus	AY606947
L. fulvissimus	AF204679
L. hepaticus	AF096989
L. intermedius	AF140256
L. lignyotus	AY606949
L. mitissimus	AF157412
L. pterosporus	AY331013
L. quietus	AJ272247
L. repraesentaneus	AY331011
L. scrobiculatus	AF140262
L. serifluus s.l.	AY332558
L. subdulcis	AY331016
L. subsericatus	AF140254
L. tabidus (as *L. theiogalus*)	AF349716
L. tesquorum	AF096986
L. trivialis	AJ534935
L. uvidus	AJ534936

A first phylogenetic analysis was performed using ITS sequences from 87 collections (23 sequences from the initial alignment were identical to another sequence; they were removed from further analyses, together with the GenBank sequences). The alignment included the entire sequenced region: the 3' part of the 18S nrDNA, ITS1, 5.8S nrDNA, ITS2 and the 5' part of the 28S nrDNA. Three

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[1] The inclusion of secondary structure information of the ITS region to improve the alignment of this region seems infeasible (for the moment) within the Russulaceae when taking into account the very limited knowledge we have on this subject. It seems unlikely that the ITS processing and thus ITS folding follows the same pattern in the Russulaceae as in yeast, the only fungus of which the secondary structure of the ITS has ever been determined experimentally (U. Eberhardt, pers. comm.). Several attempts to use secondary structure information to improve the ITS alignments of species in *Lactarius* sect. *Deliciosi* have failed (U. Eberhardt, pers. comm.).

Plinthogali species were designated as the outgroup: *L. pterosporus* Romagn., *L. lignyotus* Fr. and *L. fuliginosus* (Fr.: Fr.) Fr.

ITS and partial *gpd* sequences were combined for 32 taxa. We used *L. rufus* (Scop.: Fr.) Fr. and *L. tesquorum* Malençon as outgroup. For the same subset a morphological data matrix, with 14 macro- and microscopical characters was designed. Table 4 summarizes the characters; Table 5 represents the morphology data matrix.

Phylogenetic analyses were performed with PAUP* 4b10 (SWOFFORD, 2002) and MrBayes 3.0b4 (HUELSENBECK & RONQUIST, 2001). Modeltest 3.06 (POSADA & CRANDALL, 1998) was used to determine the substitution model of DNA evolution with corresponding parameters that fitted the data best.

For the large ITS dataset (87 accessions) a Maximum Parsimony (MP) analysis was performed using 100 heuristic searches employing TBR branch swapping and random sequence addition with a limit of 100 trees saved per replicate. Other settings were: gaps treated as missing, all characters of type unordered and equally weighted, multistate characters interpreted as uncertainty, starting trees obtained via stepwise addition, one tree held at each step during stepwise addition, steepest descent option not in effect, branches collapsed (creating polytomies) if minimum branch length is zero and MulTrees option in effect. The SaveReps option was on (saving optimal trees from each replicate, even if they are not optimal overall) but only the shortest trees were used for further analyses. Bootstrap supports were evaluated using 1000 bootstrap replicates with 10 heuristic searches per replicate, random sequence addition and TBR branch swapping.

We performed a MP and a Bayesian analysis on the more restricted dataset (32 accessions) including ITS and partial *gpd* sequences, both in- and excluding morphological characters. Compatibility of the data sets was tested with the partition homogeneity test (FARRIS *et al.*, 1995); we used PAUP* to perform 1000 replicate searches. A MP analysis was carried out using 1000 heuristic searches employing TBR branch swapping and random sequence addition. MaxTrees was set to auto-increase, other settings were identical to the larger ITS data matrix. Support for the internodes of the most parsimonious trees was estimated by 1000 bootstrap replicates with 10 heuristic searches per replicate, random sequence addition and TBR branch swapping. For the Bayesian analysis three partitions were used for the three data sets (ITS, *gpd* and morphology). Parameters of the likelihood model were set to correspond with the results of the hierarchical likelihood ratio tests for the ITS and *gpd* partitions. Three independent analyses of two million generations were run, keeping one tree every 100 generations. The burn-in value was set to 20%. The remaining trees were used to calculate a 50% majority rule consensus tree.

The Shimodaira-Hasegawa test (SHIMODAIRA & HASEGAWA, 1999; GOLDMAN *et al.,* 2000) was used to test alternative topologies. The test was performed as implemented in PAUP*, using the RELL option and 1000 bootstrap replicates (default settings in PAUP*).

Table 3: Absolute and ML (HKY85+I+G) distance with their averages (av.) calculated for different data subsets. Values are based on comparison of the ITS1, 5.8S and ITS2 region. Between brackets in the first column is the number of sequences compared.

	abs. dist. (bp)	**HKY85+I+G**
within *L. deliciosus* (15)	0-11 (av. 4.1)	0.00-1.78% (av. 0.65%)
within *L. quieticolor* (5)	0-2 (av. 0.8)	0.00-0.32% (av. 0.13%)
within the *L. fennoscandicus*-clade (11)	0-10 (av. 3.7)	0.00-1.66% (av. 0.59%)
within the *L. deterrimus*-clade (18)	0-13 (av. 3.7)	0.00-2.13% (av. 0.59%)
within *L. sanguifluus* (10)	0-7 (av. 4.1)	0.00-1.13% (av. 0.65%)
within *L. vinosus* (5)	3-10 (av. 7.2)	0.47-1.62% (av. 1.16%)
within *L. semisanguifluus* (7)	0-8 (av. 3.1)	0.00-1.30% (av. 0.50%)
within *L. porninsis* (2)	1	0.15%
within *L. salmonicolor* (9)	0-5 (av. 1.3)	0.00-0.79% (av. 0.20%)
between *L. deterrimus* and *L. fennoscandicus*	3-18 (av. 8.5)	0.47-3.09% (av. 1.37%)
between *L. sanguifluus* and *L. cyanopus*	3-7 (av. 4.7)	0.47-1.13% (av. 0.75%)
between *L. sanguifluus* and *L. vinosus*	6-12 (av. 8.8)	0.96-1.98% (av. 1.43%)
interspecific in ingroup	3-39 (av. 20.1)	0.47-7.12% (av. 3.46%)
interspecific between *Deliciosi* and non-*Deliciosi*	18-94 (av. 50.5)	4.35-24.23% (av. 10.53%)

2.4. AFLP

The AFLP analysis basically followed the protocol described by Vos *et al.* (1995). 50-200 ng of pure DNA was digested by *Sac*I and *Mse*I during one hour at 37 °C. *Sac*I and *Mse*I adapters were ligated to the fragments and the ligation mixture was diluted seven times and stored at –20 °C. For pre-amplification one selective base was added to each primer. Pre-amplifications were diluted 10 times and stored at –20 °C. The *Sac*I primer was radioactively labelled with [γ-^{33}P]ATP before the selective PCR amplification. Primer combinations were *Sac*I+CA / *Mse*I+ATA, +AAG, +AT and *Sac*I+CC / *Mse*I+ATA, +AT. The samples were combined with an equal volume of formamide dye, denatured and run on a 4.5% denaturing polyacrylamide gel for 2.5 hours at 100 Watts. After drying the gels were exposed overnight to a phospho-imager. The scans were scored as dominant systems with bands as present (1) or absent (0). All monomorphic and polymorphic bands were scored. We used PAUP* 4b10 (Swofford, 2002) and NTSYS-pc 2.10L (Rohlf, 2000) to analyse the AFLP data.

Table 4: Overview of the morpholgical characters used in the phylogenetic analysis of *Lactarius* sect. *Deliciosi.*

1. **Pileus colour:**
   - 0. pale to bright orange
   - 1. red to vinaceous red
   - 2. bluish
   - 3. pale to dark brown
   - 4. yellowish
2. **Pileus zonations:**
   - 0. not zonate
   - 1. indistinctly zonate
   - 2. clearly zonate
3. **Stipe colour:**
   - 0. pale orange
   - 1. bright orange
   - 2. (pale) red to vinaceous red
   - 3. bluish
   - 4. whitish to buff
   - 5. pale to dark brown
4. **Scrobicules on stipe:**
   - 0. absent
   - 1. present
5. **Latex colour immediately after cutting the basidiocarp:**
   - 0. orange
   - 1. vinaceous red
   - 2. white
   - 3. bluish
6. **Latex colour 10 min. after cutting the basidiocarp**
   - 0. orange
   - 1. vinaceous red
   - 2. white
   - 3. bluish
7. **Latex colour 20-30 min. after cutting the basidiocarp:**
   - 0. orange
   - 1. vinaceous red
   - 2. white
   - 3. bluish
8. **Lamella colour:**
   - 0. orange
   - 1. reddish
   - 2. bluish
   - 3. white/pinkish/buff
9. **Host tree:**
   - 0. *Pinus*
   - 1. *Picea*
   - 2. *Abies*
   - 3. *Larix*
   - 4. Deciduous trees
10. **Soil acidity:**
    - 0. neutral to calcareous
    - 1. acidic
11. **Spore ornamentation: ridges:**
    - 0. rather heavy
    - 1. rather thin
12. **Spore ornamentation: reticulum:**
    - 0. (very) incomplete
    - 1. (nearly) complete
13. **Abundance pleuromacrocystidia:**
    - 0. (very) scarce
    - 1. abundant locally or near the lamella edge
    - 2. abundant to very abundant
14. **Abundance cheilomacrocystidia:**
    - 0. absent
    - 1. (very) scarce
    - 2. (very) abundant

3. Results

3.1. Characteristics of the ITS sequences and alignment

The total length of the alignment of ITS1, 5.8S nrDNA and ITS2 sequences was 722 bp. This region varied in length from 631 bp in *L. quieticolor* to 658 bp in *L. porninsis* Rolland*. Lactarius quieticolor* had a 19 bp deletion in the ITS1 region. The total length of the alignment used for the phylogenetic analyses (including the 3' part of the 18S and the 5' part of the 28S nrDNA) was 967 bp. Table 3 summarizes intra- and interspecific variability of the ITS region. The raw dissimilarity (in bp) was measured, together with a ML estimate of the distance. We used Modeltest to find HKY85+I+G (Hasegawa *et al.*, 1985) as the model that fitted the data best. The highest divergence within one species or terminal clade was 2.13 % (13 bp) for *L. deterrimus*. The distance between the very closely related *L. sanguifluus* and *L. vinosus* ranged from 0.96 to 1.98 % (1.42 % on average) and in *L. deterrimus*–*L. fennoscandicus* Verbeken & Vesterh. from 0.47 to 3.09 % (1.37% on average). The average interspecific distance within *Lactarius* sect. *Deliciosi* was 3.46 % with a maximum of 7.12 %. Only 8 pairs of cloned ITS sequences from 26 collections were identical, the average distance being 3.8 bp. *Lactarius deterrimus* J. Nuytinck 2000-002 showed the maximum intragenomic variability with 12 differences on a total of 887 bp. *Lactarius deliciosus*, *L. deterrimus* and *L. fennoscandicus* seem to show a much higher intragenomic variability than the other species.

Table 5: Datamatrix of 14 macro- and microscopical characters (see Table 4) for *Lactarius* sect. *Deliciosi*.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
L. cyanopus M.T. Basso 98102204	0+2	2	3	1	0+3	1+3	1+3	0+2	0	0	1	1	2	2
L. deliciosus J. Vesterholt 96-457	0	2	0	1	0	0	0	0	0	0	0	1	0	2
L. deliciosus A. Peksen & G. Hatat 229	0	2	0	1	0	0	0	0	0	0	0	1	0	2
L. deliciosus A. Verbeken 2000-104	0	2	0	1	0	0	0	0	0	0	0	1	0+1	1
L. deliciosus J. Nuytinck 2001-005	0	2	0	1	0	0	0	0	0	0	0	1	0	2
L. deliciosus J. Nuytinck 2001-046	0	2	0	1	0	0	0	0	0	0	0	1	0	2
L. deterrimus J. Nuytinck 2000-010	0	0	1	0	0	0	1	0	1	0	1	0	0	1
L. deterrimus J. Nuytinck 2001-053	0	1	1	0	0	0	1	0	1	0	1	0	0	0
L. deterrimus J. Nuytinck 2001-099	0	1	1	0	0	0	1	0	1	0	1	0	0	1
L. fennoscandicus? P.A. Moreau 05-09-1999	0+3	2	0	0	0	0	1	0	1	0	1	0	1	1
L. fennoscandicus A. Verbeken 97-530 TYPE	0+3	2	0	0	0	0	1	0	1	0	1	0	0	1
L. fennoscandicus J. Nuytinck 2001-018	0+3	2	0	0	0	0	1	0	1	0	1	0	0	1
L. fennoscandicus J. Nuytinck 2001-039	0+3	2	0	0	0	0	1	0	1	0	1	0	1	1
L. porninsis J. Nuytinck 2001-082	0	2	0	0	2	2	2	0	3	0	1	0	2	2
L. porninsis J. Nuytinck 2002-019	0	2	0	0	2	2	2	0	3	0	1	0	2	1
L. quieticolor J. Nuytinck 2000-011	0+3	2	0+1	0	0	0	0	0	0	1	0	1	2	1
L. quieticolor J. Nuytinck 2001-004b	0	2	1	0	0	0	1	0	0	1	0	1	0	1
L. salmonicolor J. Nuytinck 2001-087	0	0+1+2	1	1	0	0	1	0	2	0	1	0	2	2
L. salmonicolor J. Nuytinck 2001-123	0	1	1	1	0	0	1	0	2	0	1	0	2	2
L. salmonicolor J. Nuytinck 2002-027	0	1	1	1	0	0	1	0	2	0	1	0	2	2
L. sanguifluus J. Nuytinck 2000-008	0	1	2	1	1	1	1	0+1	0	0	0	0	1	1
L. sanguifluus J. Nuytinck 2001-050	0	1	2	1	1	1	1	0	0	0	0	0	2	2
L. sanguineovirescens? P.A. Moreau 13-10-2001	0+2	2	0	1	0	?	?	0	0	?	0	0	1	2
L. pinastri/sanguineovirescens? P.A. Moreau 30-09-2001	0	2	0	1	0	1	1	0	0	?	0	0	1	2
L. semisanguifluus A. Verbeken 96-1032	0	1	1	0+1	0	1	1	0	0	0	0	0	2	2
L. semisanguifluus C. Lange 96-081	0	2	0+1	0+1	0	1	1	0	0	0	1	0	2	2
L. semisanguifluus J. Nuytinck 2001-049	0	1	1	0+1	0	1	1	0	0	0	1	0	0	1
L. semisanguifluus J. Nuytinck 2001-093	0	1	1+2	0+1	0	1	1	0	0	0	1	0	0	2
L. vinosus A. Peksen & G. Hatat 230	1	2	2	1	1	1	1	1	0	0	1	0	2	2
L. vinosus J. Nuytinck 2001-104a	1	2	2	1	1	1	1	1	0	0	1	0	2	2
L. tesquorum	4	0	4	0	2	2	2	3	4	0	1	0	0	2
L. rufus	3	0	4+5	0	2	2	2	3	0+1+4	1	1	1	2	1

3.2. ITS phylogeny

The alignment included 87 sequences (representing 32 taxa) and 967 characters, of which 191 were parsimony-informative. The three species belonging to *Lactarius* subgenus *Plinthogali* were assigned to the outgroup[2]. One of the MP trees with the smallest –ln Likelihood (L) is depicted in Fig. 2. Branches that collapse in the strict consensus tree of all 7900 MP trees are indicated with an asterisk. The tree shows that *Lactarius* sect. *Deliciosi* forms a monophyletic group (89 % bootstrap support) within *Lactarius* subgenus *Piperites*. Nine species belong to *Lactarius* sect. *Deliciosi*: eight species that are traditionally being placed in this section and *L. porninsis*, usually classified in *Lactarius* sect. *Zonarii* (Quél.) Bon (nom. inval.).

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[2] Excluding the *Plinthogali* species from the analysis and assigning the *Russularia* species (as indicated in Fig.2) to the outgroup does not improve the resolution of the phylogeny when comparing the MP strict consensus or bootstrap consensus trees. The bootstrap values of the most basal clades (subgenus *Piperites* including *L. serifluus, L. chrysorrheus* and *L. quietus,* and subgenus *Piperites* alone) do improve but none of the support values within *Lactarius* sect. *Deliciosi* change.

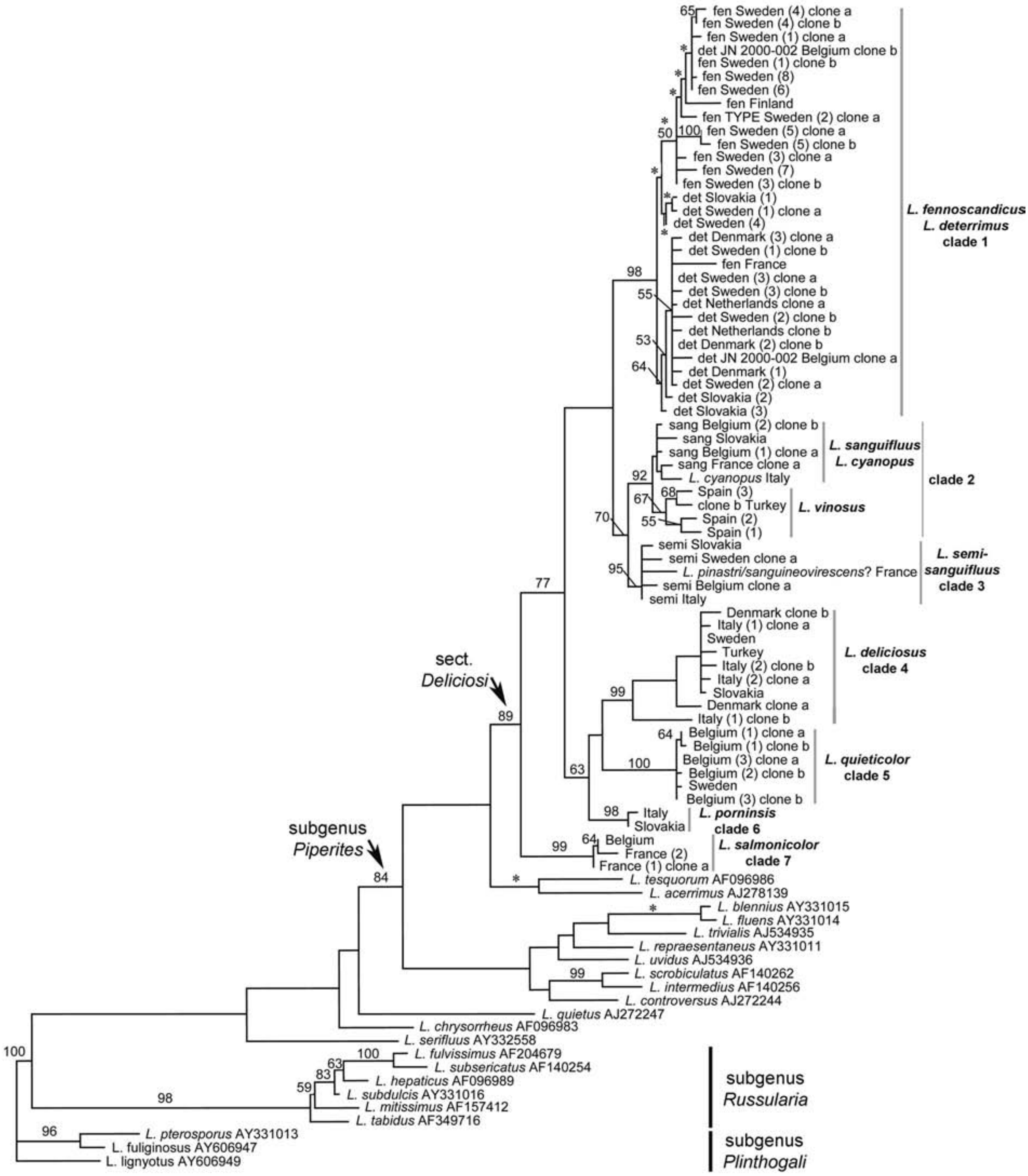

Fig. 2: One of four best (–ln L = 8088.44102) trees, out of 7900 most parsimonious trees based on ITS data. Collections are labelled according to their origin (see Table 1). Abbreviations: fen = *L. fennoscandicus*, det = *L. deterrimus,* sang = *L. sanguifluus* and semi = *L. semisanguifluus.* Clades 1 to 7 are mentioned in the text. Bootstrap supports > 50% are indicated above the branches. Branches marked with an asterisk collapse in the strict consensus tree. Tree length = 671 steps, CI = 0.6051, RC = 0.5015, RI = 0.8288.

Most of the species receive a high bootstrap support, except two groups of closely related taxa *L. deterrimus*-*L. fennoscandicus* and *L. sanguifluus*-*L. vinosus.* The seven clades indicated in Fig. 2 correspond to the five well-supported species and the two latter species-groups. *Lactarius salmonicolor* (clade 7), associated with *Abies*, is basal to the rest of *Lactarius* sect. *Deliciosi*. *L. porninsis* (clade 6) is associated with *Larix;* this analysis is not conclusive about its sister group relationships but it is firmly positioned within *Lactarius* sect. *Deliciosi* (see also below). *L. deliciosus* (clade 4) and *L. quieticolor* (clade 5) are both growing under *Pinus*. Colour variants of *L. quieticolor* are common. This analysis includes both pale orange and dark brown coloured specimens; the latter showing a clear blue zone underneath the pileipellis. No differentiation can be seen between these colour variants of *L. quieticolor*. *L. semisanguifluus* (clade 3) and *L. sanguifluus* plus *L. vinosus* (clade 2) are also associated with *Pinus*. Clade 2 and 3 group with a bootstrap support of 70% *L. deterrimus* and *L. fennoscandicus* (clade 1), form ectomycorrhiza with *Picea* and are not separating well in this analysis; several collections, morphologically identified as *L. deterrimus* cluster with *L. fennoscandicus*. *L. fennoscandicus* P.A. Moreau 5-9-1999 (fen France in Fig. 2), collected in the French Alps, is part of the *L. deterrimus clade*. The relationships between these clades remain largely unresolved. The *L. sanguineovirescens* and *L. cyanopus* specimens included do not form separate branches and come out within *L. semisanguifluus* and *L. sanguifluus* respectively.

Most cloned sequences come out in pairs; but the ITS clones of *L. deterrimus* J. Heilmann-Clausen 97-176 (det Sweden (1) clone a and b in Fig. 2) are in 2 different *L. deterrimus* clades, and *L. deterrimus* J. Nuytinck 2000-002 has one clone in *L. deterrimus* and one in *L. fennoscandicus*. No evident geographical pattern can be discerned within the species.

3.3. Combining ITS and *gpd* sequences and morphology

Partition homogeneity tests suggest no topological incongruence between ITS and *gpd* data sets ($P = 0.578$) and between ITS and morphological data ($P = 0.011$) but significant heterogeneity among *gpd* and morphological data ($P = 0.003$). Phylogenetic analyses (MP and Bayesian inference) were performed combining only molecular data and combining molecular data with morphology. Combining incongruent data sets may still be advantageous, since the overall accuracy of the combined data may be increased by a larger number of characters applied to parts of the tree unaffected by the mismatch (WIENS, 1998). When employing the total evidence approach there is the concern that molecular data will overwhelm morphological data if the two are combined, but it is the pattern of character covariation, not the number of characters alone, that generates the phylogenetic signal (EERNISSE & KLUGE, 1993). Situations where a smaller morphological data set, combined with much larger molecular data sets, nevertheless determined the results have been observed by several authors (CHAVARRIA & CARPENTER, 1994 & references therein).

The ITS partition contributed 936 sites to the alignment, the partial *gpd* gene 965 sites and the eventual morphology data 14 characters. Of the total of 1915 characters, 1459 were constant and 201 were parsimony-informative. Of these potentially parsimony-informative characters, ITS contributed 68 and *gpd* 119; all 14 morphological characters are informative. Using Modeltest the K80+G model (KIMURA, 1980) was found for the ITS partition and the HKY85+G model (HASEGAWA *et al.*, 1985) for the *gpd* partition. Trees obtained by MP and Bayesian analyses of molecular data alone and combined molecular and morphology data did not differ significantly from each other. MP analysis of the combined molecular data sets yielded 1400 shortest trees (all on one island, retrieved in 990 out of 1000 repeats, length = 661 steps, CI = 0.8245, RC = 0.6680, RI = 0.8101) (Fig. 3). When combining molecular with morphological data the MP analysis generated only 54 shortest trees (all on one island, retrieved in 998 out of 1000 repeats, length = 725 steps, CI = 0.7917, RC = 0.6286, RI = 0.7940). The obtained Bayesian topology is shown in Fig. 4.

The ITS phylogeny is largely confirmed by the analysis of the combined data sets, but a better resolution and higher bootstrap support values become evident (Fig. 3 and 4). *Lactarius sanguifluus* and *L. vinosus* receive support values of 97% and 100% respectively in Fig. 3; *L. deterrimus* and *L. fennoscandicus* form separate clades with moderate to low support (68% and 75% respectively in Fig. 3). *Lactarius fennoscandicus* P.A. Moreau 5-9-1999 from France still falls in the *L. deterrimus* clade, even when morphology is taken into account in the analysis (Fig. 4). There is some support for a close relationship between *L. deterrimus*-*L. fennoscandicus* and *L. porninsis* (posterior probability value of 74%) but the basal relationships within the section remain largely unresolved. The topological constraint excluding *L. porninsis* from *Lactarius* sect. *Deliciosi* yields a significantly less likely tree and can be rejected based on the available data ($P < 0.05$ using the Shimodaira-Hasegawa test). Two specimens identified as *L. sanguineovirescens* fall in the *L. semisanguifluus* clade. Both on morphological (after examination of the type material) and molecular grounds we believe *L. sanguineovirescens* should be considered a synonym of *L. semisanguifluus*. From *L. cyanopus* we only managed to sequence the ITS region, the species was until very recently known from the type locality only and all material is in rather bad condition. *Lactarius cyanopus* comes out as a sister species of *L. sanguifluus* and *L. vinosus* (Fig. 3 and 4) or within *L. sanguifluus* (in some of the MP trees, results not shown). More collections from other localities are needed here to draw further conclusions. Recent collections from Spain might elucidate the situation.

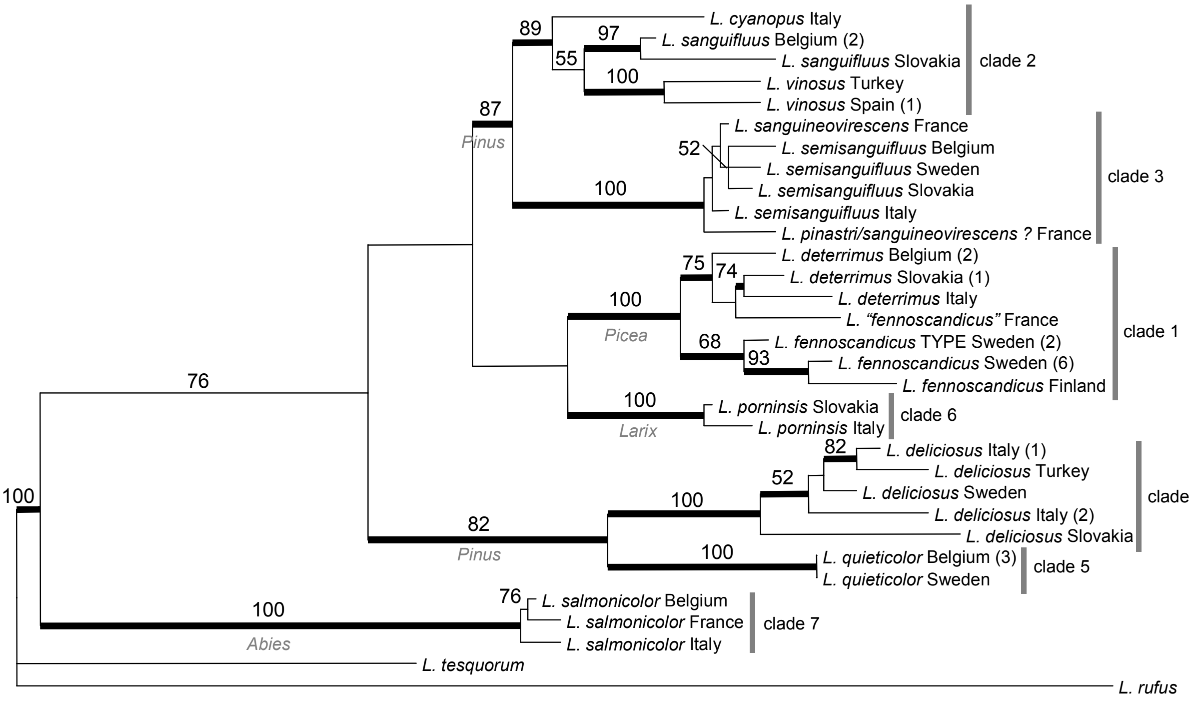

Fig. 3: One of 1400 most parsimonious trees resulting from a MP analysis of the combined molecular (ITS and *gpd*) data sets. See Table 1 for detailed collection data. Bootstrap support values > 50% are indicated above the branches. Thickened branches receive a posterior probability of ≥ 95% in the Bayesian analysis.

3.4. AFLP

The AFLP fingerprinting technique was used to test whether the closely related *L. deterrimus* and *L. fennoscandicus* could be distinguished. Based on morphology (the pileus colour and zonation and the abundance of cheilocystidia), a clear separation between both taxa would be expected, but molecular data (ITS and *gpd* sequences) do not confirm this. AFLP is a sensitive technique, capable of assessing variation both within and between fungal species (BOCK *et al.*, 2002; MAJER *et al.*, 1996). Because of the multitude of markers generated by AFLP, the technique is expected to be more suitable than the ITS sequence analysis to distinguish between closely related species and even reveal geographical patterns. Furthermore AFLP is known to be highly reproducible, an important advantage on RAPD.

A data matrix was generated for nine *L. deterrimus* specimens (both from distant and nearby localities), four *L. fennoscandicus* specimens (from Sweden), two *L. deliciosus* specimens and one *L. quieticolor* specimen (Table 1). Unfortunately, the quality of the DNA obtained from dried specimens was too poor to be useful in AFLP analysis so we were highly dependent on freshly collected material. Using five primer combinations, a total of 424 markers were scored for these 16 samples. Only 28 of them were monomorphic for all four taxa; *L. deterrimus* and *L. fennoscandicus* shared 93 markers. Genetic similarities between all pairs of accessions were calculated using the Nei & Li coefficient (NEI & LI, 1979). The similarity matrix was analysed by UPGMA cluster analysis (Unweighted Pair Group Method using Arithmic averages; SOKAL & MICHENER, 1985, Fig. 5). Reliability of the branches in the dendrogram was tested by bootstrap analysis with 1000 replicates. Additionally a PCO (principle coordinate) analysis was performed by using the DCENTER and EIGEN procedures in NTSYS-pc.

The AFLP analysis consistently distinguishes between *L. deterrimus* and *L. fennoscandicus*. Both in the UPGMA dendrogram (Fig. 5) and the PCO plot (not shown) they are separated; their branches are supported by a 100 % bootstrap value in the dendrogram. *Lactarius deterrimus* J. Nuytinck 2001-053, a collection that seemed more closely related to *L. fennoscandicus* in the ITS phylogeny, now falls within *L. deterrimus*. Very low levels of intraspecific diversity within *L. deterrimus* and *L. fennoscandicus* were observed (0.028 and 0.025 respectively). Interspecific diversity between *L. deterrimus* and *L. fennoscandicus* was 0.077 and low when compared with the distance to *L. deliciosus* and *L. quieticolor* (0.179 on average). A more detailed study with greater numbers of isolates from different locations is required to reveal an eventual geographic pattern.

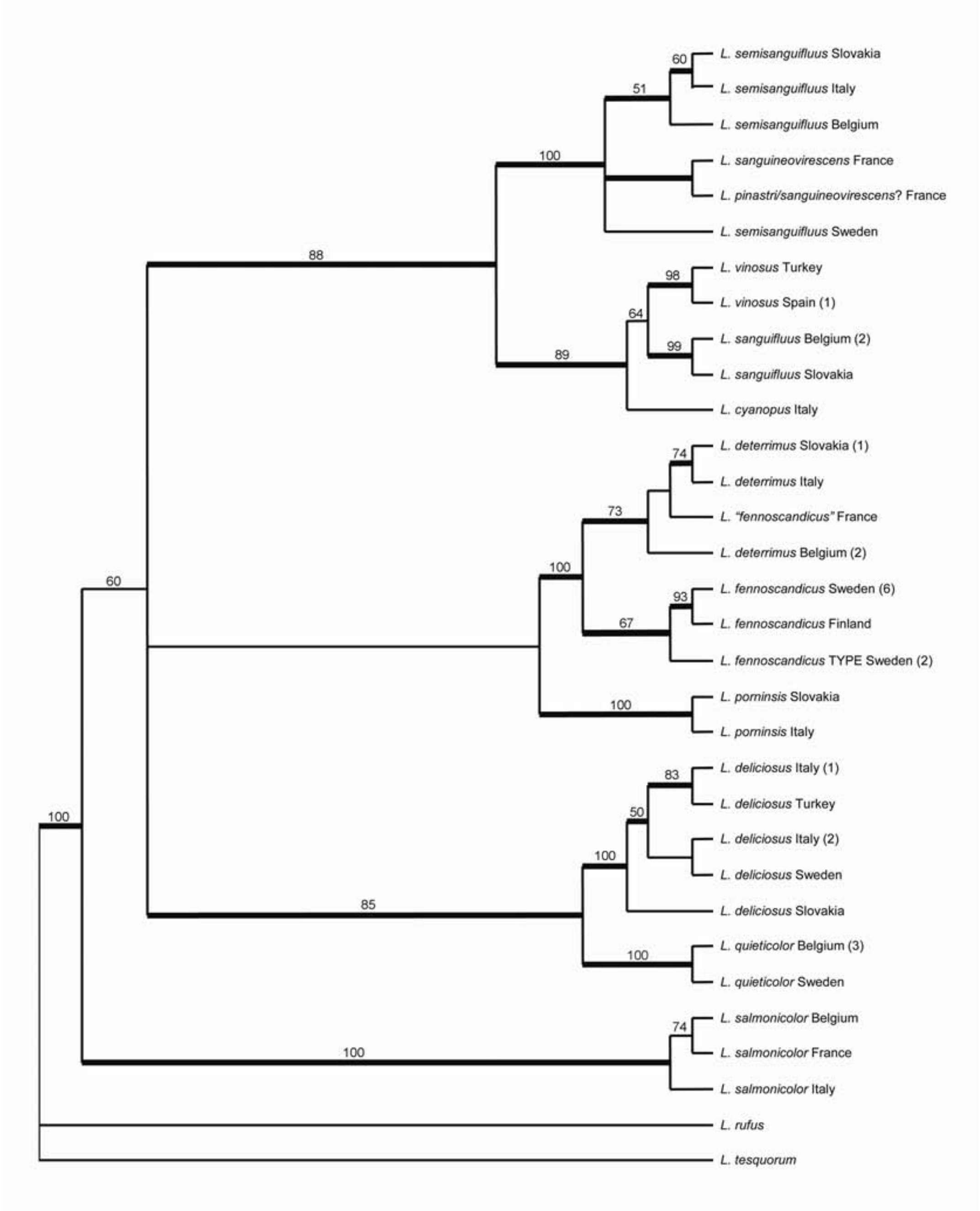

Fig. 4: 50% majority rule consensus tree based on the outcome of a Bayesian analysis of ITS, *gpd* and morphology data. Thickened branches receive posterior probabilities of ≥ 95%. MP bootstrap values > 50% are indicated above the branches.

4. Discussion

4.1. Sequence variation in the ITS region

The sequence variability we found within and between European species in *Lactarius* sect. *Deliciosi* is largely in accordance with the values EBERHARDT (2000) and EBERHARDT *et al.* (2000) obtained for *Lactarius*. However, the intra- and interspecific variability ranges strongly overlap and the resolution of the phylogenetic analyses could be affected by this lack of delimitation in variability. In spite of this overlap, most species separate reasonably well in the ITS phylogeny. All, except the very closely related *L. sanguifluus*-*L. vinosus* and *L. deterrimus*-*L. fennoscandicus*, receive high bootstrap values. ITS sequences may not contain enough information to admit reliable identification between these species pairs. Although per species a wide sample of specimens from distant European localities was included in our analyses, no geographical pattern could be discerned. This is in correspondence with the findings of EBERHARDT *et al.* (1999) and EBERHARDT (2000) for *Russula* and *Lactarius* and SHINOHARA *et al.* (1999) for *Cenococcum*. We found differences of up to 12 bp between cloned ITS sequences in *L. deterrimus. Lactarius fennoscandicus* and *L. deliciosus* also showed a relatively high intragenomic variability. Polymorphisms in the ITS region in the same individual have been reported several times in fungi (AANEN, 1999; O'DONNELL & CIGELNIK, 1997) and show the potential danger of using single ribosomal sequences for reconstructing species phylogenies. Multigene families, such as the ribosomal DNA, are subject to concerted evolution. The processes of gene conversion and unequal crossing over tend to homogenise the copies within a genome and within species, while differences accumulate between species. The mechanism is continuous during speciation and accordingly it should be deficient or unfinished in very recent species (ODORICO & MILLER, 1997).

Both the low interspecific and the high intragenomic sequence divergence suggest recent speciation in groups of *Lactarius* sect. *Deliciosi*, namely in *L. deterrimus*-*L. fennoscandicus* and *L. sanguifluus*-*L. vinosus*.

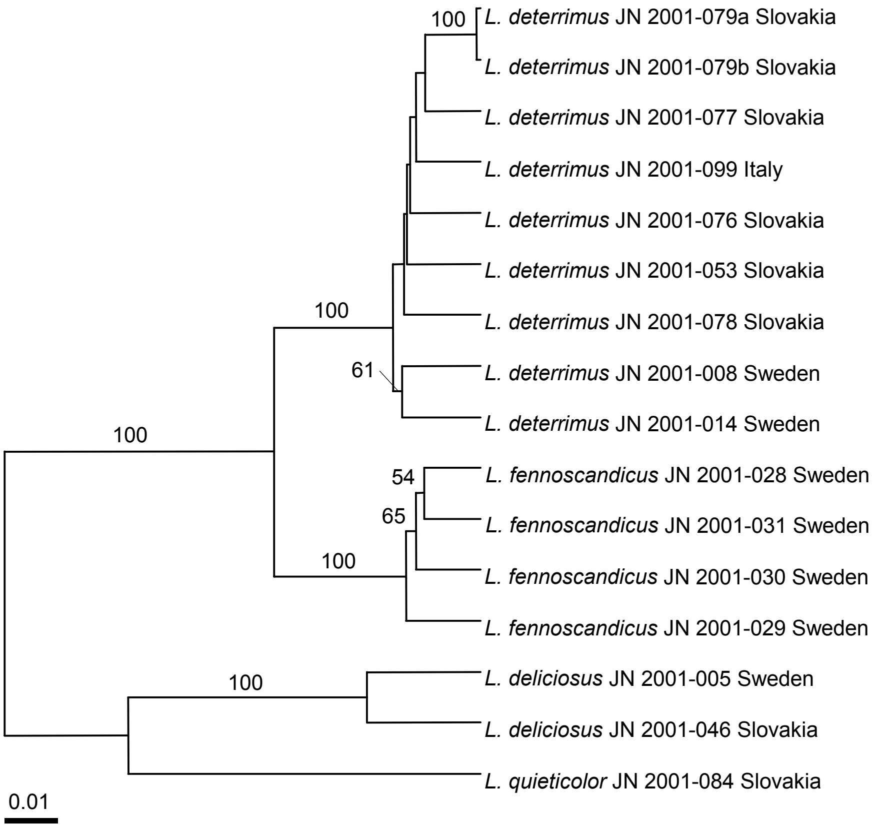

Fig. 5: UPGMA cluster analysis of AFLP data generated by five primer combinations (424 markers). Initials of the collector are used in the herbarium numbers. Bootstrap values > 50% (1000 replicates) are indicated above the branches.

4.2. AFLP and the closely related *L. deterrimus* and *L. fennoscandicus*

AFLP is a powerful DNA marker technique. It is based on the detection of DNA restriction fragments by PCR amplification. The obtained fingerprints are a rich source for restriction fragment polymorphisms, called AFLP markers. The frequency with which AFLP markers are found is dependent on the sequence polymorphisms between the tested DNA samples (VOS & KUIPER, 1997). The technique differs importantly from other random fingerprinting techniques by its robustness and reproducibility (VOS *et al.*, 1995). MAJER *et al.* (1996) were the first to test the AFLP technique on fungi. They used it to detect inter- and intraspecific genetic variation between the fungal phytopathogens *Cladosporium fulvum* and *Pyrenopeziza brassicae*. They showed that the technique is very efficient at detecting polymorphisms, even in species where very little variation could previously be found by RFLP analysis. AFLP fingerprinting is a relatively cheap and effective way to investigate several aspects of fungal molecular biology and genetic diversity (MAJER *et al.*, 1996). Several isolates of plant pathogens (BOCK *et al.*, 2002; CILLIERS *et al.*, 2000; GONZÁLEZ *et al.*, 1998) have been characterised by this technique. For a limited number of species and isolates from the Ustilaginales a comparison was made between ITS sequences and AFLP fingerprints as phylogenetic markers (BAKKEREN *et al.*, 2000). Here, the results obtained by both methods were in agreement, but AFLP fingerprints allowed to separate isolates from different regions or from closely related species while this was hard or impossible using ITS sequences.

L. deterrimus is distributed widely in Europe while *L. fennoscandicus* is known from Sweden and Finland and has been reported from the Alps (FLORIANI, 1999). *Lactarius deterrimus* has an orange pileus that is often totally azonate or only zonate near the margin. *Lactarius fennoscandicus* has a greyish lilac to brownish shade all over the distinctly zonate pileus. Microscopically they differ only in the abundance of cheilomacrocystidia, but this character has to be treated with care because exceptions occur. Both species are unique in the section by their association with *Picea*. AFLP differentiates clearly between both species while ITS sequences do not. The genetic distance between both species is significantly smaller than between the other *Deliciosi* species. Situations where a host switch is followed by an episode of rapid speciation have been reported for *Leccinum* (DEN BAKKER *et al.*, 2004). This might be the case also in this group, after the host switch from *Pinus* to *Picea*.

4.3. Phylogeny and systematics of *Lactarius* sect. *Deliciosi* in Europe

Five *Deliciosi* species can be clearly defined based on a phylogenetic analysis of their ITS sequences and receive high bootstrap values. In addition, *L. deterrimus*, *L. fennoscandicus*, *L. sanguifluus* and *L. vinosus* are well supported when taking the total evidence and AFLP analyses into consideration. This result agrees with the species delimitations previously described on the basis of morphology. *Lactarius* sect. *Deliciosi* forms a monophyletic group within subgenus *Piperites* and surprisingly contains *L. porninsis*, a white-milked species. Because of the lack of striking orange latex and the spectacular colour changes, *L. porninsis* has never been placed in *Lactarius* sect. *Deliciosi*, but several other characters (the pileus colour and aspect, the association with a coniferous tree) are shared with traditional members of *Lactarius* sect. *Deliciosi* while they are unknown in *Lactarius* sect. *Zonarii*.

It seems reasonable on both molecular (AFLP and total evidence analyses) and morphological grounds to distinguish *L. fennoscandicus* as a separate species from *L. deterrimus*. The "*L. fennoscandicus*" collection from the French Alps is more closely related to *L. deterrimus*, despite of its dark and strongly zonate cap, typical features for *L. fennoscandicus*. More problematic is the Belgian collection *L. deterrimus* J. Nuytinck 2000-002 with one ITS type in the *L. fennoscandicus* clade and the other in the *L. deterrimus* clade. This could be an indication that both species are not entirely reproductively isolated and that hybridisation events occur. Further research, including more specimens in the AFLP analysis and sequencing more nuclear and mitochondrial markers, is needed to elucidate the evolutionary history of both taxa and confirm eventual hybridisation events. Another process that could cause a conflicting signal between gene phylogeny and organismal phylogeny is incomplete lineage sorting. This hypothesis is worth mentioning in the light of a possible rapid speciation event (see above and see also chapter 5). Ancestral ITS variation present in the population prior to speciation of e.g. *L. deterrimus* and *L. fennoscandicus* coupled with the differential survival of those alleles could have resulted in an incongruent pattern between the ITS phylogeny we observe and the true organismal phylogeny.

As suggested in NUYTINCK & VERBEKEN (2003), *L. sanguifluus* and *L. vinosus* should be treated as distinct species, but discoloured forms of both species can complicate their identification. LALLI *et al.* (2002) share the same view, but have a different opinion on the nomenclatural history of these taxa. They propose the new name *L. rubrozonatus* Lalli & Pacioni for what we call *L. vinosus* and consider *L. vinosus* in its original sense as a form of *L. sanguifluus*.

The exact position and status of *L. cyanopus* remain unclear: this taxon is part of the *L. sanguifluus* clade or a sister species to *L. sanguifluus* and *L. vinosus.* In addition, clear morphological differences, such as the colour of the basidiocarps and latex, the ornamentation of the spores and the abundance of the cysitidia, separate *L. cyanopus* from the other European *Deliciosi* (BASSO, 1999b). More collections from other localities are needed for this taxon, which is known from three localities only.

Both morphological and molecular evidence is convincing enough to synonymise *L. sanguineovirescens* with *L. semisanguifluus*. The *L. sanguineovirescens* collection reported from Sardinia (MELIS *et al.*, 1999) is microscopically identical to *L. quieticolor*.

Identification of *L. quieticolor* collections is often problematic due to the highly variable pileus colour. HEILMANN-CLAUSEN *et al.* (1998) synonymise both *L. hemicyaneus* Romagn. and *L. pinastri* Romagn. with *L. quieticolor* because no microscopical differences were found and because the macromorphological differences between these species fall within the variability of these characters. Our molecular analyses comprise the wide colour variability in *L. quieticolor*, but do not show any differentiation. This confirms the opinion of HEILMANN-CLAUSEN *et al*. (1998).

Recently, the subdivision of *Lactarius* section *Deliciosi* into subsection *Deliciosini* Bon & Basso and subsection *Sanguifluini* Bon & Basso was proposed (BASSO, 1999a; BON, 1980). *Lactarius* subsection *Deliciosini* contains species with orange latex, at least when first exuding, and *Lactarius* subsection *Sanguifluini* contains species with red or blue coloured latex when first exuding, at least locally. This division is not supported here: e.g. *L. semisanguifluus*, classified in *Lactarius* subsection *Deliciosini* is closest related to *L. sanguifluus* and *L. vinosus* belonging to *Lactarius* subsection *Sanguifluini*.

Based on site by site comparison of the ITS region, EBERHARDT *et al.* (2000) supposed a smaller sequence divergence between *L. deterrimus*, *L. salmonicolor* and *L. semisanguifluus*, as an adaptation to different hosts, than between the *Pinus* associated species, which adapted to different environmental conditions. This study, based on more samples, demonstrates that this is certainly not true for *L. salmonicolor*, basal to the *Deliciosi*, associated with *Abies* and clearly more divergent. Host associations are plotted on the tree in Fig. 3 and show that the *Pinus* associated species are not more distantly related than the species associated with other hosts.

Acknowledgements

We would like to thank following people for supplying collections: Maria Teresa Basso, Marcel Contu, Gürsel Hatat, Jacob Heilmann-Clausen, Christian Lange, Pierre-Arthur Moreau, Aysun Peksen, Jan Vesterholt & Ruben Walleyn and the curators of the herbaria BR, LIP, PC & MPU. The Department of Plant Systems Biology (VIB, UGent) is indebted for help with sequencing. Two anonymous reviewers are thanked for their constructive remarks. The research of the first author is financed by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT), Belgium.

Chapter 5

Worldwide phylogeny of *Lactarius* sect. *Deliciosi* inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences

Adapted from:
NUYTINCK J., VERBEKEN A. & MILLER S.L. (2005) – Worldwide phylogeny of *Lactarius* sect. *Deliciosi* inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Will soon be submitted.

Abstract

A phylogenetic analysis of *Lactarius* sect. *Deliciosi* was performed based on collections of all known species. Several samples of each species were included, originating from a wide geographic range. The two DNA regions we used (ITS and a part of the gene encoding glyceraldehyde-3-phosphate dehydrogenase), showed an incongruent phylogenetic signal. Much attention was paid to carefully observed macro- and micromorphological characters to draw taxonomic conclusions. We currently accept 38 taxa (31 species and 7 varieties) in *Lactarius* sect. *Deliciosi* on a worldwide scale; four species are new to science. More sampling is needed to resolve the status of the North American varieties. Our knowledge of the Asian species in this section remains fragmentary. The monophyly of the section and its position within *Lactarius* subgenus *Piperites*, as proposed in recent morphology-based classification schemes, is confirmed. The intrasectional relationships however, do not coincide with the colour of the latex (as previously supposed). Intercontinental conspecificity is very low in general. The name *L. deliciosus* is wrongfully applied in North and Central America and only two species seem to occur in both Asia and Europe.

1. Introduction

Although many species in *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn are widely known and popular edible mushrooms in different parts of the world, taxonomical and nomenclatural problems prevail. Different opinions on the taxonomic status of a number of taxa exist among different authors. The morphological differences between species are often subtle and many characters are highly variable. This complicates identification and results in the spread of information under wrong names.

Several macroscopic character states make *Lactarius* sect. *Deliciosi* an easy group to recognise, but differ only slightly between species. The colour of the latex ranges from carrot orange and dingy yellow over vinaceous red and brown to indigo blue. This colour changes more or less rapidly once the latex is exuded on the context by an enzymatic activity and in most cases ends up green; the time needed for this change is species-specific. Most species however start off with orange latex, slowly (30 min.–1h) changing to red and finally to green. Recent research has proven that *L. porninsis* Rolland, a species with unchanging, white latex, surprisingly falls within *Lactarius* sect. *Deliciosi* (NUYTINCK & VERBEKEN, 2005b). Other macroscopical features that members of the section have in common concern the general aspect of the pileus and stipe: colour, zonation, size, presence of scrobicules, etc. Weather and growing-conditions unfortunately have a significant influence on these characters, thus hampering identifications.

Microscopical characters of (more limited) use are the size and ornamentation of the spores and the size and abundance of pleuro- and cheilomacrocystidia. The spore size and ornamentation are clearly divergent in only a few species and very similar in most other species. These spore characters are useful for identification when reference specimens are available. The size and especially the abundance of macrocystidia seem quite variable, making this character less reliable.

Representatives of *Lactarius* sect. *Deliciosi* form ectomycorrhiza with Pinaceae (*Pinus, Picea, Larix, Abies, Tsuga* and *Pseudotsuga*) or Fagaceae (*Quercus* and *Castanopsis*), and are consequently largely limited to the northern hemisphere in their distribution. Some species (e.g. *L. thyinos* (G. Lincoff, pers. comm.) and *L. deliciosus*) do occur in exotic pine plantations in Australia, New Zealand, South Africa. Ectomycorrhizal relationships are thought to be highly species specific (i.e. a species in *Lactarius* sect. *Deliciosi* is strictly associated with a tree genus), although this is only well documented for Europe. *Lactarius indigo* (Schwein.) Fr. is an example of an exception as it is consistently reported to be associated with both *Pinus* and *Quercus*.

So far, 74 names have been published in *Lactarius* sect. *Deliciosi*: 41 from Europe, 20 from America and 13 from Asia. Previous molecular and morphological research has lead to the acceptance of ten species in *Lactarius* sect. *Deliciosi* in Europe (NUYTINCK & VERBEKEN, 2005a&b). The situation in North and Central America and in Asia is less well studied and remains unclear. The publications by HESLER & SMITH (1960, 1979) provided an important step forward in the knowledge of *Lactarius* sect. *Deliciosi*, and the genus *Lactarius* in general, in North America. However, several European names are encountered in their work as well as numerous varieties, indicating uncertainty on the status of several taxa and illustrating their variability. Central America and Asia remain largely underexplored and mycologists often use European or North American names here because of superficial resemblance, without evaluating eventual intercontinental conspecificity.

For many groups of macrofungi, morphological as well as molecular studies have been limited to a certain continent and the degree of conspecificity over the continents has only rarely been investigated. A recent DNA study on a worldwide sample of matsutake (*Tricholoma matsutake* and allies) for example, resulted in a first comprehensive definition of natural groupings in this species complex (CHAPELA & GARBELOTTO, 2004). SHINOHARA *et al.* (1999) compared *Cenococcum geophilum* isolates from North America and Europe and concluded that they form a single taxonomic entity and possibly a single species. A molecular phylogenetic study of 51 isolates of *Grifola frondosa* (maitake) on the contrary, revealed a species partition separating eastern North American and Asian isolates (SHEN *et al.*, 2002).

About 400 *Lactarius* species are currently known worldwide (VERBEKEN, 2001). Systematic research in tropical Africa, Australia and South America revealed until now only endemic species (except for some introduced species in plantations; VERBEKEN, 2001). In contrast, many European *Lactarius* epithets circulate in North America and North American and European names are often being applied in Asia, although few comparative studies, focussing on real conspecificity, have been carried out. KYTÖVUORI (1984) reported that all American records of *L. scrobiculatus* (Scop.: Fr.) Fr. he examined are erroneous. Other authors report the same species from northern Europe and Greenland or Alaska after morphological comparison of the material (GULDEN *et al.*, 1988; KNUDSEN & BORGEN, 1994). Molecular studies have never been used to confirm any of these observations. *Lactarius deliciosus* (L.: Fr.) Gray, *L. salmonicolor* R. Heim & Leclair and *L. deterrimus* Gröger are European names commonly used for American and Asian taxa with orange latex. This is the first study critically comparing these and other European *Lactarius* taxa with material from outside of Europe both by morphological and molecular methods.

Evolutionary relationships between the species in *Lactarius* sect. *Deliciosi* are unclear. Morphological hypotheses on these relationships invariably group those species with similarly coloured latex. Several European authors for example have divided the section into subsections (and stirps) using the colour of the latex as a main character (BASSO, 1999; BON, 1980; SCHAEFER, 1970). In order to understand the relationships between these species and to elucidate morphological and ecological characters supporting these relationships, we included all known species in the section in our phylogenetic analyses.

In this study we addressed the following questions: 1) does *Lactarius* sect. *Deliciosi* form a monophyletic clade within *Lactarius* subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman, as proposed by most current authors; 2) which morphologically defined species are confirmed by our molecular analyses; 3) are morphologically similar taxa that occur on different continents conspecific; 4) what are the relationships between species and 5) is the colour of the latex useful for a further division of the section? We used nrDNA ITS sequences and an 800-bp fragment of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and combined the results with morphological data to answer these questions. The mutations of the ITS sequences are presumed to be neutrally selected and variable enough for the depth of resolution required to resolve species complexes (CHAPELA & GARBELOTTO, 2004). Because of the relatively high level of variability in the *gpd* DNA sequence, this region should be appropriate for phylogenetic comparison with the ITS region at about the same taxonomic levels (BERBEE *et al.*, 1999). This DNA region has been used successfully for various groups of ascomycetes (BERBEE *et al.*, 1999; CÂMARA *et al.*, 2002; YUN *et al.*, 1999) and for the basidiomycete genus *Leccinum* (DEN BAKKER *et al.*, 2004).

2. Materials & Methods

2.1. Examined specimens

The present study focuses on the molecular part of a broader taxonomic examination of *Lactarius* sect. *Deliciosi*, in which a detailed morphological approach of the basidiocarps and ectomycorrhizal root tips, is combined with a molecular analysis. More than 400 fresh and dried specimens from a broad geographic range, representing 38 taxa (31 species and 7 varieties) and collected in 28 countries have been examined morphologically. Type specimens have been included where possible. Detailed morphological descriptions can be found in NUYTINCK & VERBEKEN (2005a & in preparation) and NUYTINCK *et al.* (in preparation). Herbarium material was deposited in or obtained from BPI, BR, FLAS, GENT, H, HKAS, LIP, MICH, MPU, NY, NYS, PC, RMS, SFSU, TMI, XAL, ZT and the personal herbaria of P.A. Moreau and M. Contu. 89 specimens belonging to 30 species in *Lactarius* sect. *Deliciosi* are being used in the phylogenetic analyses presented here (Table 1).

2.2. DNA extraction, amplification and sequencing

Tissue from freshly collected basidiocarps and herbarium specimens ground in liquid nitrogen was placed in 2× CTAB buffer and ground with a plastic pestle. Genomic DNA was extracted using CTAB-based methods as previously described (HIBBETT & VILGALYS, 1993; NUYTINCK & VERBEKEN, 2003 & 2005b) and then purified using GENECLEAN III (Qbiogene, Carlsbad, CA, USA). Aqueous genomic preparations were frozen at - 20˚C until use.

PCR amplification of the ITS region was performed using tailed primer pair M13-ITS5/M13-ITS4 (WHITE *et al.*, 1990) or primers ITS1-F and ITS4-B (GARDES & BRUNS, 1993). A touchdown PCR profile was used as in NUYTINCK & VERBEKEN (2003). The *gpd* gene was amplified using primers CTK-107, CTK-132 and CTK-108$_{rev}$ and the PCR program described for the *Lactarius*-specific primers in KREUZINGER *et al.* (1996). The obtained PCR products were purified using ExoSAP (USB, USA) or Wizard PCR Preps (Promega Corporation, Madison, WI, USA). DNA sequencing reactions were performed with the ABI PRISM® BigDye^{TM} Terminators v3.0 Cycle Sequencing Kit using the same primers on an ABI PRISM® 377 DNA Sequencer or using primers M13-Forward (-29) and M13-Reverse labeled with IRD-700 and IRD-800, respectively, in preparation for simultaneous bidirectional sequencing (LI-COR Biotechnology Division, Lincoln, NE, USA). Amplified PCR products were then sequenced with the SequiTherm EXCEL II DNA Sequencing Kit (Epicentre Technologies, Madison, WI, USA) and analyzed on a LI-COR Gene ReadIR 4200-2 automated sequencer. The Phred and Phrap Software (EWING *et al.*, 1998; EWING & GREEN, 1998) or Base ImagIR (vers. 4.0, LI-COR) was used to process the raw data.

Sequences were deposited in GenBank (accession numbers see Table 1).

2.3. Phylogenetic analyses

We aligned the sequences using ClustalX 1.83 (THOMPSON *et al.*, 1997) and manually corrected and refined the alignments. Both ITS and gpd sequences were easily aligned across all taxa studied. The alignments are available through TreeBASE (ID numbers will be added).

In order to verify non-random structuring of the data, a left-skewness (g_1) test was performed (HILLIS & HUELSENBECK, 1992) using 10 000 randomly generated trees under the parsimony criterion. Likelihood mapping (STRIMMER & HAESELER, 1997) was applied to further visualize and compare the phylogenetic content of the ITS and *gpd* alignments. Tree-Puzzle 5.2 (SCHMIDT *et al.*, 2002) was used for this purpose. Likelihood values are computed for the three possible tree topologies of every quartet in a dataset and represented as a point inside an equilateral triangle. This triangle is partitioned into different regions corresponding to the strength of the phylogenetic signal. Points plotted toward the

Table 1: Collections used in the phylogenetic analyses, GenBank accession numbers will be added.

Species	**Origin**	**Collection (Herbarium)**	**GenBank ITS/*gpd***
L. akahatsu	Japan, Tottori	22601 (TMI)	
L. akahatsu	Thailand	A. Verbeken 2004-141 (GENT)	
L. barrowsii	USA, Washington	N. Gray 91878 (RMS)	
L. barrowsii	USA, Arizona	States J. AEF 1271 (MICH)	
L. chelidonium var. *chelidonium*	USA, New York	S.L. Miller 9649 (RMS)	
L. deliciosus	Italy, Sardegna (1)	A. Verbeken 2000-104 (GENT)	
L. deliciosus	Sweden, Medelpad	J. Nuytinck 2001-005 (GENT)	
L. deliciosus	Slovakia	J. Nuytinck 2001-046 (GENT)	AY332557/
L. deliciosus	Italy, Toscana (2)	J. Vesterholt 96-457 (GENT)	
L. deliciosus	Turkey, Samsun	A. Peksen & G. Hatat 229 (GENT)	
L. deliciosus	Australia	U. Eberhardt 41	
L. deliciosus originally identified as *L. akahatsu*	China, Yunnan	39045 (HKAS)	
L. deliciosus var. *areolatus*	USA, Colorado	J. Walker 381 (RMS)	
L. deliciosus var. *areolatus*	USA, Utah	B. Kropp (RMS)	
L. deliciosus var. *deterrimus*	USA, California	S.L. Miller 214-03 (RMS)	
L. deliciosus var. *deterrimus*	USA, Wyoming (1)	J.R. Herr 650 (RMS)	
L. deliciosus var. *deterrimus*	USA, Wyoming (2)	J. Nuytinck 2003-017 (GENT)	
L. deliciosus var. *deterrimus*	USA, Wyoming (3)	J. Nuytinck 2003-041a (GENT)	
L. deliciosus var. *deterrimus*	USA, Wyoming (4)	J. Nuytinck 2003-041b (GENT)	
L. deliciosus var. *olivaceosordidus*	USA, Oregon	P.B. Matheny 2054 (RMS)	
L. deliciosus var. *olivaceosordidus*	USA, Washington	J. Ammirati 10762 (RMS)	
L. sp. originally identified as *L. chelidonium*	USA, Virginia	G. Bills 943 (VTMH 1057)	
L. sp. originally identified as *L. paradoxus*	USA, North Carolina	S.L. Miller 9666 (RMS)	
L. sp. originally identified as *L. rubrilacteus*	USA, Oregon	S.L. Miller 9878 (RMS)	
L. sp. originally identified as *L. rubrilacteus*	USA, Alaska	O.K. Miller 18143 (RMS)	
L. deterrimus	Switzerland	O.K. Miller 21256 (RMS)	
L. deterrimus	Slovakia	J. Nuytinck 2001-053 (GENT)	
L. deterrimus	Italy, Abruzzo	J. Nuytinck 2001-099 (GENT)	
L. deterrimus			U30876
L. deterrimus originally identified as *L. fennoscandicus*	France, Ain	P.A. Moreau 5-9-1999 (pers. herb.)	
L. fennoscandicus	Sweden (1)	A. Verbeken 97-530 (GENT) **TYPE**	
L. fennoscandicus	Sweden, Jämtland (2)	J. Vesterholt 95-330 (GENT)	
L. fennoscandicus	Sweden, Medelpad (3)	J. Nuytinck 2001-018 (GENT)	
L. fennoscandicus	Finland (1)	O.K. Miller 17447 (RMS)	
L. fennoscandicus	Finland (2)	J. Nuytinck 2001-039 (GENT)	
L. hatsudake	Thailand	A. Verbeken 2004-159 (GENT)	
L. hatsudake	China, Yunnan (1)	38541 (HKAS)	

Species	**Origin**	**Collection (Herbarium)**	**GenBank ITS/*gpd***
L. hatsudake	China, Yunnan (2)	39047 (HKAS)	
L. hatsudake	Japan, Kagoshima	24414 (TMI)	
L. hatsudake	China, Hunan (3)	M. Härkönen KIINA 62 (H, GENT)	
L. indigo var. *diminutivus*	USA, Virginia	M.C. Aime 811 (RMS)	
L. indigo var. *indigo*	USA, West Virginia	S.L. Miller 9579 (RMS)	
L. indigo var. *indigo*	Belize	B. Kropp 29-10-2000-15 (RMS)	
L. laeticolor	Japan, Tottori	23149 (TMI)	
L. laeticolor originally identified as *L. salmonicolor*	Korea	O.K. Miller 21714 (RMS)	
L. miniatosporus	Mexico	3830 (XAL) **TYPE**	
L. paradoxus	USA, Florida	D. Mitchell 1-02-2002 (RMS)	
L. porninsis	Slovakia	J. Nuytinck 2001-082 (GENT)	
L. porninsis	Italy, Trentino	J. Nuytinck 2002-019 (GENT)	
L. pseudodeliciosus var. *pseudodeliciosus*	USA, Florida	D. Mitchell 02-2002 (RMS)	
L. quieticolor	Belgium	J. Nuytinck 2000-011 (GENT)	
L. quieticolor	Sweden, Dalarna (1)	J. Nuytinck 2001-004b (GENT)	
L. quieticolor	Sweden (2)	S.L. Miller 192-03 (RMS)	
L. rubrilacteus	USA, Arizona	J. States AEF 1055 (MICH)	
L. rubrilacteus	USA, California (1)	S.L. Miller 11-04 (RMS)	
L. rubrilacteus	USA, California (2)	S.L. Miller 19-04 (RMS)	
L. rubriviridis	USA, California	D.E. Desjardin 7312 (SFSU) **TYPE**	
L. salmoneus	USA, Mississippi	B. Kropp 11-08-2000-14 (RMS)	
L. salmonicolor	Belgium	J. Nuytinck 2001-087 (GENT)	
L. salmonicolor	France, Savoie	J. Nuytinck 2001-123 (GENT)	
L. salmonicolor	Italy, Sardegna	J. Nuytinck 2002-027 (GENT)	
L. sanguifluus	Belgium	J. Nuytinck 2000-008 (GENT)	AY332547/
L. sanguifluus	Slovakia	J. Nuytinck 2001-050 (GENT)	AY332546/
L. semisanguifluus	Belgium	A. Verbeken 96-1032 (GENT)	AY332553/
L. semisanguifluus	Sweden, Gotland (1)	C. Lange 96-081 (GENT)	AY332555/
L. semisanguifluus	Slovakia	J. Nuytinck 2001-049 (GENT)	AY332554/
L. semisanguifluus	Italy, Abruzzo	J. Nuytinck 2001-093 (GENT)	AY332556/
L. semisanguifluus	Sweden	S.L. Miller 197-03 (RMS)	
L. semisanguifluus originally as *L. pinastri/sanguineovirescens*	France (1)	P.A. Moreau 30-9-2001 (pers. herb.)	
L. semisanguifluus originally identified as *L. sanguineovirescens*	France (2)	P.A. Moreau 13-10-2001 (pers. herb.)	
L. sp.1	China, Hunan	M. Härkönen KIINA 112 (H, GENT)	
L. sp.2	China, Hunan (1)	M. Härkönen KIINA 35 (H, GENT)	
L. sp.2	China, Hunan (2)	M. Härkönen KIINA 36 (H, GENT)	
L. sp.2	China, Hunan (3)	M. Härkönen KIINA 113 (H, GENT)	
L. sp.3	USA, California (1)	S.L. Miller 213-03 (RMS)	

Species	**Origin**	**Collection (Herbarium)**	**GenBank ITS/*gpd***
L. sp.3	USA, California (2)	S.L. Miller 216-03 (RMS)	
L. sp.4	Java (1)	8336 (ZT)	
L. sp.4	Java (2)	9983 (ZT)	
L. sp. originally identified as *L. deliciosus* var. *olivaceosordidus*	USA, Washington	MTS 3445 (RMS)	
L. subindigo	China, Hunan	M. Härkönen KIINA 114 (H, GENT)	
L. subindigo originally identified as *L. indigo*	India, Garhwal Himalaya	Kamal 259-99 (RMS)	
L. subpurpureus	USA, Virginia (1)	S.L. Miller 9572 (RMS)	
L. subpurpureus	USA, Virginia (2)	J.R. Herr 574 (RMS)	
L. thakalorum	Nepal	VC1313 (BPI) **TYPE**	
L. thyinos	USA, New York	S.L. Miller 9648 (RMS)	
L. vinosus	Spain, Catalunya (1)	J. Nuytinck 2001-104a (GENT)	AY332551/
L. vinosus	Turkey, Samsun	A. Peksen & G. Hatat 230 (GENT)	AY332549/
L. vinosus	Spain, Catalunya (2)	J. Nuytinck 2001-110 (GENT)	AY332550/
L. acerrimus			AJ278139
L. blennius			AY331015
L. chrysorrheus			AF096983
L. controversus			AJ272246
L. croceus	USA, Virginia	S.L. Miller 8-17-1997 (RMS)	
L. fluens			AY331014
L. fulvissimus			AF204679
L. hepaticus			AF096989
L. intermedius			AF140256
L. mitissimus			AF157412
L. olympianus	USA, Wyoming	J. Nuytinck 2003-032 (GENT)	
L. quietus			AF096982
L. repraesentaneus			AY331011
L. rufus	Norway	J. Nuytinck 2002-008 (GENT)	
L. scrobiculatus			AF140263
L. serifluus s.l.	Belgium	A. Verbeken 99-242 (GENT)	AY332558
L. tabidus			AF349716
L. tesquorum	Spain, Catalunya	J. Nuytinck 2001-121 (GENT)	AF096986/
L. trivialis			AJ534935
L. uvidus			AJ534936
L. subsericatus			AF140254
L. pterosporus			AY331013
L. fuliginosus	Germany, Bayern	fo 46889 (TUB)	AY606947
L. lignyotus	Germany, Bayern	lw 098 (TUB)	AY606949

centre represent a situation whereby each of the possible topologies is more or less equally likely. Regions close to the apices of the triangle represent well-resolved phylogenies, i.e. highly structured data, while the region between the apices corresponds to areas where it is difficult to distinguish between two of the three trees. The final distribution of points reveals the degree of hierarchical structure or tree-likeness present in the dataset (SMITH *et al.*, 2004).

Maximum parsimony (MP) analyses were performed with PAUP* 4b10 (SWOFFORD, 2002) using 100 or 1000 heuristic searches, employing TBR branch swapping and random sequence addition with a limit of 1000 trees saved per replicate. Other settings were: gaps treated as missing, all characters of type unordered and equally weighted, multistate characters interpreted as uncertainty, starting trees obtained via stepwise addition, one tree held at each step during stepwise addition, steepest descent option not in effect, branches collapsed (creating polytomies) if minimum branch length is zero and MulTrees option in effect. Bootstrap supports were evaluated using 1000 bootstrap replicates with 10 heuristic searches per replicate, random sequence addition and TBR branch swapping.

Maximum likelihood (ML) analyses were performed using PAUP*. The model of sequence evolution was optimized using likelihood ratio tests as implemented in Modeltest version 3.06 (POSADA & CRANDALL, 1998). Gaps were treated as missing data and phylogenies were obtained using the heuristic search option and TBR branch swapping. One MP tree was used as a starting tree. Bootstrap support for branches was calculated with 10 000 replicates of the fast bootstrap option in PAUP*.

MrBayes 3.0b4 (HUELSENBECK & RONQUIST, 2001) was used to perform Bayesian analyses. Parameters of the likelihood model were set to correspond with the results of the hierarchical likelihood ratio tests. Three independent analyses of 2×10^6 generations were run, starting with a random tree and keeping one tree every 100 generations. The burn-in value was set to 20%. The remaining trees were used to calculate a 50% majority rule consensus tree and to determine the posterior probabilities for the individual branches.

Compatibility of the data sets was tested with the partition homogeneity test (FARRIS *et al.* 1995); we used PAUP* to perform 1000 replicate searches.

3. Results

DNA sequences from two regions were used in our phylogenetic analyses: ITS sequences (the 3’ part of the 18S nrDNA, ITS1, the 5.8S nrDNA, ITS2 and the 5’ part of the 28S nrDNA) and a ± 800-bp fragment of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*). A total of 88 ITS sequences were produced by the authors and 21 were obtained from GenBank (Table 1). All 78 *gpd* sequences, with the exception of *L. deterrimus* GenBank U30876, were generated for this study (Table 1). All species included in the phylogenetic analyses were studied in detail morphologically. Microscopical features of the collections included here were compared with type specimens for most species to confirm identifications. The analyses include 30 species, of which 26 were previously described and 4 are undescibed. For detailed morphological descriptions and publication of the new species, see NUYTINCK & VERBEKEN (2005a & in preparation) and NUYTINCK *et al.* (in preparation). For as many species as possible we included two or more specimens in our analyses.

3.1. Data quality and hierarchical structure

The skewness value g_1 equalled –0.62 for the ITS data set and -0.31 for the *gpd* data set. This indicates that significant ($p > 0.01$), non-random structure is present, reflecting phylogenetic signal (HILLIS & HUELSENBECK, 1992).

The *gpd* alignment contains slightly less tree-like information than the ITS alignment (Fig. 1). 84.3 % of the quartets from the likelihood mapping analysis map into regions of the triangle representing well resolved phylogenies for the *gpd* data, while this is 87.1 % for the ITS data.

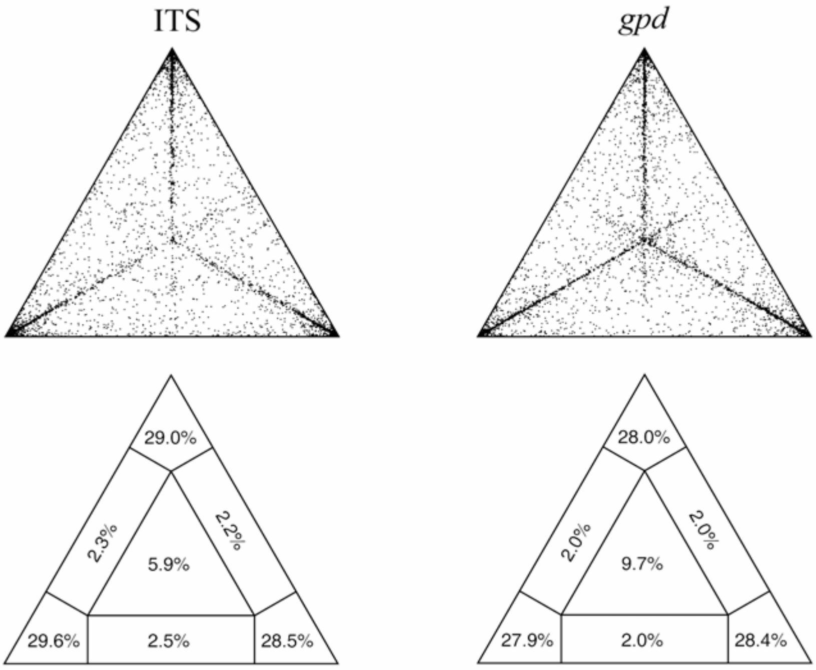

Fig. 1: Likelihood maps for the two DNA regions used in this study (ITS and *gpd*).

3.2. ITS phylogeny

The alignment of the 109 ITS sequences resulted in a 930-bp data set, of which 397 bp were variable and 247 bp were parsimony informative. Four species shared a 19-bp deletion in ITS1 (*L. quieticolor* Romagn., *L. hatsudake* Tanaka, *L.* sp.1 and *L.* sp.4, an undescribed species from Hunan, China). A total of 24 species, not belonging to *Lactarius* sect. *Deliciosi* were included in the analyses to test the monophyly of the section and its position within *Lactarius*. *Lactarius pterosporus* Romagn., *L. lignyotus* Fr. and *L. fuliginosus* (Fr.: Fr.) Fr. (representatives of *Lactarius* subgenus *Plinthogali* (Burl.) Hesler & A.H. Sm.) were assigned to the outgroup.

The TrN+I+G model (TAMURA & NEI, 1993) was chosen as the best fitting model using Modeltest. Variable sites were assumed to follow a gamma distribution (shape = 0.6712), nucleotide frequencies were set to A 0.2471, C 0.2463, G 0.2345 and T 0.2721 and substitution rates to 3.4613 (AG), 5.6180 (CT) and 1 for all transversions. The proportion of invariable sites was set to 0.2337.

The obtained ML phylogeny (-ln Likelihood = 7050.37) is depicted in Fig. 2. The overall topology of the ML tree corresponds with the strict consensus tree of the MP analysis and the 50% majority rule consensus tree resulting from the Bayesian analysis. The Bayesian analysis shows that *Lactarius* sect. *Deliciosi* (including *L. porninsis*) forms a monophyletic group (supported by a posterior probability of 100%) within *Lactarius* subgenus *Piperites*. The MP analysis (100 replicates, saving maximum 1000 trees per replicate) produced 57 000 shortest trees divided over 57 islands with a length of 1008 steps (CI = 0.5188, RC = 0.3904, RI = 0.7524).

Basal phylogenetic relationships within the section are not well resolved, irrespective of the inference technique used. Several smaller species groups do receive a strong support (posterior probability > 95%, bootstrap value > 70%): *L. salmonicolor* and *L. thyinos* A.H. Sm. form a well supported clade (A), as well as *L. quieticolor*, *L. hatsudake*, *L.* sp.1 and *L.* sp.4 (clade C). Furthermore, the angiocarpic species *L. rubriviridis* Desjardin, Saylor & Thiers groups with *L. barrowsii* Hesler & A.H. Sm. (clade D), *L. porninsis* with *L. rubrilacteus* Hesler & A.H. Sm. (clade E), *L. vinosus* (Quélet→) Bataille with *L. sanguifluus* (Paulet) Fr. (clade B) and *L. miniatosporus* Montoya & Band.-Muñoz with *L. paradoxus* Beardslee & Burl. (clade F); all of these clades receive a high support. The Bayesian inference tree also shows a strong support for the clade containing *L. porninsis*, *L. rubrilacteus*, *L. paradoxus*, *L. miniatosporus*, *L. deliciosus* and the varieties of *L. deliciosus* described from North America.

Lactarius salmonicolor, *L. thyinos* and *L. laeticolor* (S. Imai) Imazeki ex Hongo show a basal position, as well as *L.* sp.2, an undescribed species from Hunan, China.

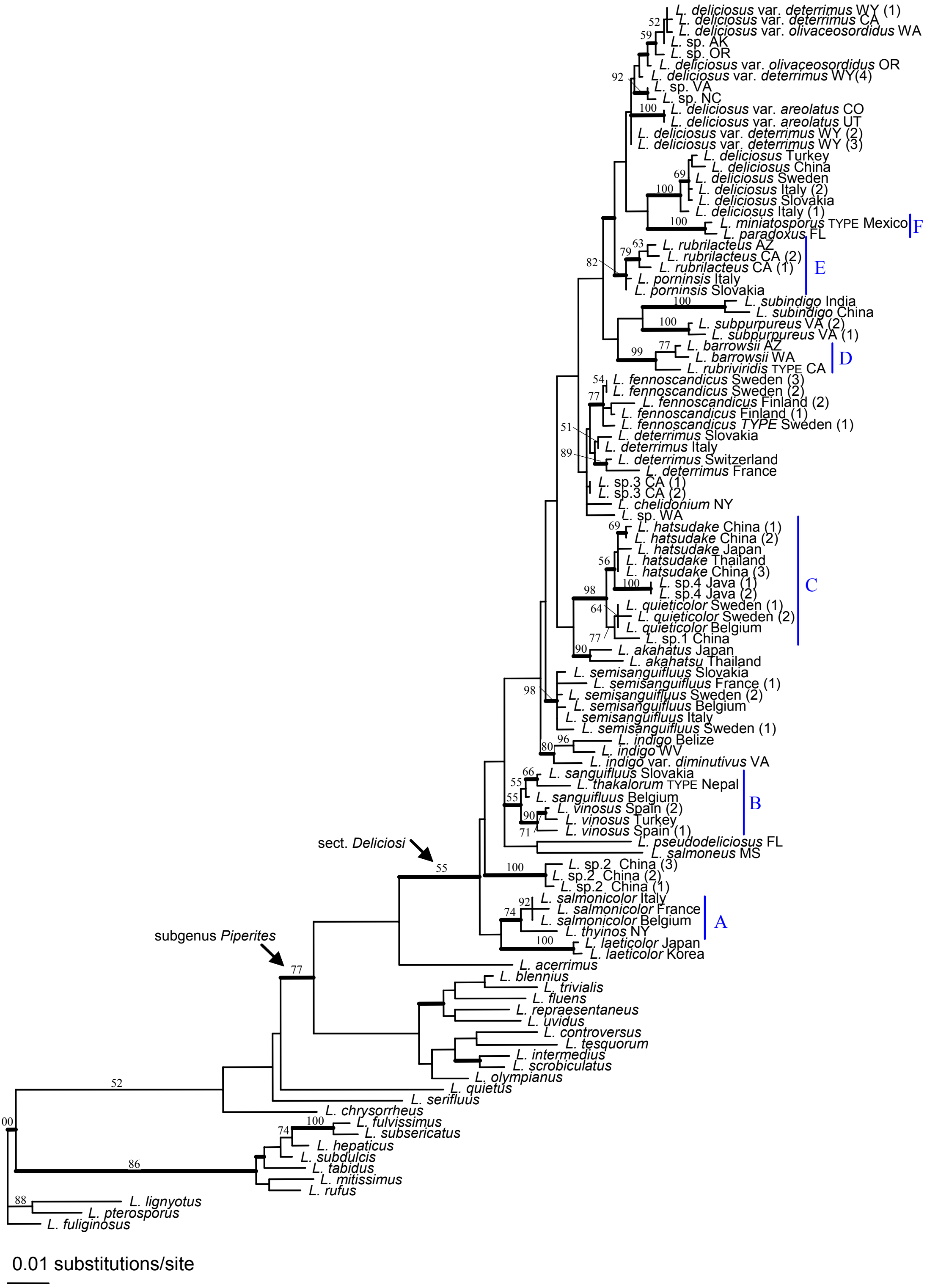

Fig. 2: ML phylogeny of *Lactarius* sect. *Deliciosi* based on ITS sequences. Bootstrap percentages > 50 %, calculated via maximum parsimony, are indicated above the branches. Thickened branches receive a posterior probability > 95% in the Bayesian analysis. Clades indicated with blue are mentioned in the text (under 3.2.).

The ITS data seem to support the morphologically identified entities and almost all species receive a high bootstrap support. *Lactarius thakalorum* Bills & Cotter (sequence obtained from the Nepalese type specimen) invariably is placed together with *L. sanguifluus* and is the only species not confirmed here. Most identification problems have emerged in the clade that unites the varieties of *L. deliciosus* described from North America.

3.3. *gpd* phylogeny

Alignment of the *gpd* sequences resulted in a 835-bp data set, of which 337 bp were variable and 203 bp where parsimony informative. Four species not belonging to *Lactarius* sect. *Deliciosi* were included: *L. tesquorum* Malençon, *L. olympianus* Hesler & A.H. Sm., *L. rufus* (Scop.: Fr.) Fr. and *L. croceus* Burl.

The TrN+I+G model (TAMURA & NEI, 1993) was again chosen as the best fitting model. Variable sites were assumed to follow a gamma distribution (shape = 0.8657), nucleotide frequencies were set to A 0.2391, C 0.2773, G 0.2357 and T 0.2479 and substitution rates to 4.2907 (AG), 5.4938 (CT) and 1 for all transversions. The proportion of invariable sites was set to 0.2953.

Fig. 3 shows one of the > 100 000 shortest trees obtained by the MP analysis (100 replicates, saving max. 1000 trees per replicate). The obtained MP, Bayesian and ML topologies show the same overall toplogy. *Lactarius* sect. *Deliciosi* received a bootstrap support of 75% in the MP analysis and a 100% posterior probability in the Bayesian analysis. The *gpd* data support most of the morphologically recognised species. Exceptions are the collections identified as *L. deliciosus* or a variety of that species from North America, that fall into two clades, and the collection identified as *L. indigo* var. *diminutivus* Hesler & A.H. Sm. that does not group with *L. indigo*. *Lactarius* sp.1 and *L.* sp.4 are not separated from *L. hatsudake*. Again, the basal relationships in *Lactarius* sect. *Deliciosi* did not receive significant support. *Lactarius barrowsii* and *L. rubriviridis*; *L. laeticolor*, *L. thyinos* and *L. salmonicolor*; and *L. porninsis* and *L. rubrilacteus* do group with high support. The Australian collection included was growing under *Pinus radiata*, an American pine species, but groups with the European and Asian collections of *L. deliciosus*.

3.4. Compatibility of the ITS and *gpd* datasets

As clearly shown by the partition homogeneity test, the phylogenetic signal in the ITS and *gpd* data sets are incongruent ($P = 0.001$). However, whether to combine the data despite a significant incongruence remains debatable (HIBBETT & DONOGHUE, 2001). Both phylogenies are not well resolved in the basal nodes of *Lactarius* sect. *Deliciosi* but show resolution for the morphologically recognised taxa and seem to support mostly the same groups of species. The general topology differs strongly however: in the trees based on the *gpd* gene *L. subindigo* Verbeken & E. Horak, *L.* sp.2 and *L. salmoneus* Peck show a basal position in the section, while the ITS trees show *L. salmonicolor* and its Asian and American counterparts at the base. The MP strict consensus trees from the individual data sets were compared and examined for conflicts involving nodes with bootstrap values > 70% (MASON-GAMER & KELLOGG, 1996). Most striking is the different placement of specimens *L. deliciosus* J. Nuytinck 2001-046, *L. deliciosus* var. *areolatus* A.H. Sm. JW 381, *L. indigo* var. *diminutivus* M.C. Aime 811, *L.* sp.1 K112 and *L. hatsudake* HKAS 38541. Moreover, the ITS data provide high support for the species *L. akahatsu* Tanaka, *L.* sp.4 and *L. fennoscandicus* Verbeken & Vesterh., while the *gpd* data do not. Vice versa the *gpd* data support strongly *L. porninsis*, *L. sanguifluus* and *L.* sp.3 (an undescribed species from California) while the ITS data do not.

Disregarding the incongruence test combining both data sets in a total evidence approach, results in improved resolution and higher bootstrap support for several nodes in the obtained trees. The MP strict consensus tree is shown in Fig. 4. This phylogeny reflects the *gpd* topology in the basal nodes but shows much of the species and species groupings from the ITS topology. ML and Bayesian topologies did not show a different general topology than this MP tree. *Lactarius subindigo*, *L.* sp.2 and *L. salmoneus* are basal to the rest of *Lactarius* sect. *Deliciosi*. Well supported clades (bootstrap value > 70%) are: (A) a clade formed by *L. barrowsii*, *L. rubriviridis* and *L. subpurpureus* Peck; (B) a clade comprising *L. laeticolor*, *L. thyinos* and *L. salmonicolor*; and (C) a clade uniting *L. deliciosus*, *L. hatsudake*, *L. quieticolor, L.* sp.1 and *L.* sp.4. *Lactarius hatsudake* becomes paraphyletic when excluding the latter three taxa. Furthermore, the data support strongly the grouping of *L. sanguifluus* and *L. vinosus*, of *L. deterrimus* and *L. fennoscandicus* and of *L. paradoxus* and *L. miniatosporus*. The specimens identified as *L. deliciosus* or one of its varieties collected in North America are not maintained as a monophyletic group.

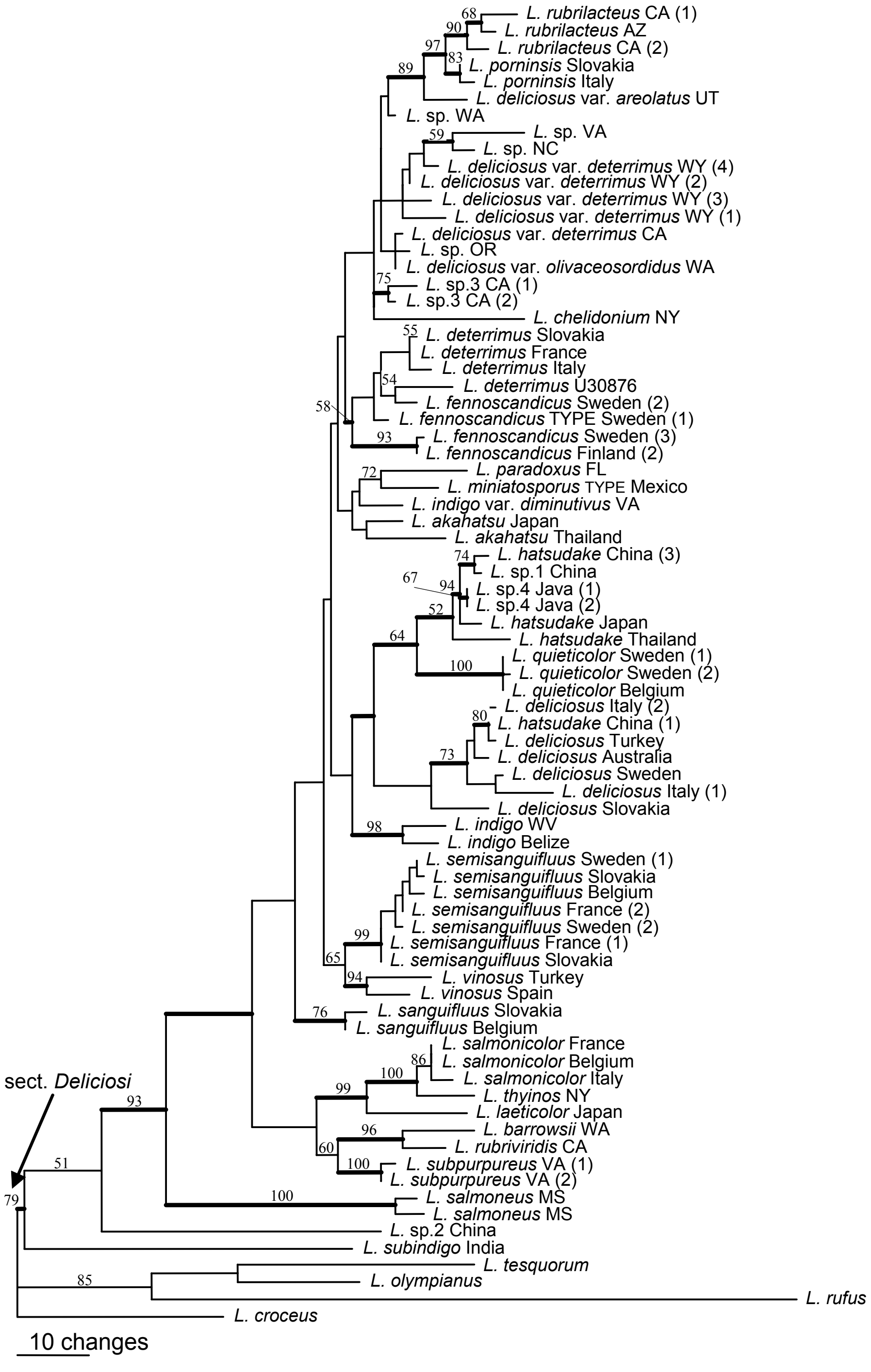

Fig. 3: Best (-ln Likelihood) tree of > 100.000 shortest trees obtained by the MP analysis of the *gpd* dataset (length = 748 steps, CI = 0.5802, RC = 0.4021, RI = 0.6931). Thickened branches receive a posterior probability of > 95% in the Bayesian analyses. Values above the branches represent bootstrap values > 50% (MP analysis).

4. Discussion

4.1. Monophyly of *Lactarius* sect. *Deliciosi* and its position in the genus

The position of *Lactarius* sect. *Deliciosi* in *Lactarius* subgenus *Piperites*, as proposed in most recent classification schemes (BASSO, 1999; HEILMANN-CLAUSEN *et al.*, 1998), is confirmed in our analyses while the classification of HESLER & SMITH (1979), placing these milk caps in a separate subgenus (*Lactarius* subgenus *Lactarius*), can be rejected. The phylogeny of *Lactarius* subgenus *Piperites* is not well understood yet. Results based on ITS and LSU sequences indicate a close relationship between *Lactarius* sect. *Piperites*, *Zonarii* and *Deliciosi* (EBERHARDT, 2000). The delimitation of sections within *Lactarius* subgenus *Piperites* is based on morphological characters (BASSO, 1999; HEILMANN-CLAUSEN *et al.*, 1998) and has not been confirmed yet by molecular studies (EBERHARDT, 2000; NUYTINCK *et al.*, 2004).

That *Lactarius* sect *Deliciosi* forms a monophyletic group is not surprising, as many morphological characters are unique for this section and very similar in the sometimes barely discernible species. However, *L. porninsis*, a species with white latex, has to be included in this section and thus the definition of *Lactarius* sect *Deliciosi* will have to be broadened. We cannot exclude the possibility that more white-milked species have to be included but have no indications as to a particular species. More sampling needs to be done to solve this question.

4.2. Species in *Lactarius* sect. *Deliciosi*

The total of 89 samples included in this phylogenetic study represents 30 species in *Lactarius* sect. *Deliciosi*. This means all known species are included, except for *L. cyanopus* Basso, which was included and discussed in detail in previous studies, together with the other European species (NUYTINCK & VERBEKEN, 2005a&b). The current state of knowledge of the American and Asian species is discussed here. The inclusion of several collections per species for most species, allows us to draw conclusions on the delimitation of these species, on their intra- and interspecific variability and on the frequency of misidentifications.

Twenty names have been published from Central and North America. HESLER & SMITH (1979) accept ten species in their important monograph. Since then only *L. miniatosporus* and the hypogeous *L. rubriviridis* have been described from the American continent (MONTOYA & BANDALA, 2004; DESJARDIN, 2003). It is important to note that many varieties of North American species have been described. HESLER & SMITH (1979) mention eight varieties belonging to five species: *L. chelidonium* var. *chelidonioides* (A.H. Sm.) Hesler & A.H. Sm., *L. deliciosus* var. *areolatus*, var. *deterrimus* (Gröger) Hesler & A.H. Sm., var. *olivaceosordidus* Hesler & A.H. Sm. and var. *piceus* Smotl. (nom. inval.), *L. indigo* var. *diminutivus*, *L. pseudodeliciosus* var. *paradoxiformis* (Murrill) Hesler & A.H. Sm. and *L. salmoneus* var. *curtisii* (Coker) Hesler & A.H. Sm. Some of these varieties are not very well known and recent collections are scarce (e.g. *L. pseudodeliciosus* var. *paradoxiformis*). As a consequence, we were not able to include all of them in our analyses. *Lactarius indigo* var. *diminutivus*, several North American varieties of *"L. deliciosus"* and *L. salmoneus* var. *curtisii* are included. We strongly doubt however, that the distinction between *L. salmoneus* var. *salmoneus* and var. *curtisii* can be maintained. HESLER & SMITH (1979) already expressed their doubts on the value of the only reported difference: presence or absence of a greenish discolouration. Observations in well-known European species show that the greenish discolouration is a variable character, sometimes absent, sometimes strikingly present, and this convinced us to label the concerned specimens as *L. salmoneus*.

Ten American species are indisputably confirmed in our analyses (*L. barrowsii*, *L. indigo*, *L. miniatosporus*, *L. paradoxus*, *L. pseudodeliciosus* Beardslee & Burl., *L. rubrilacteus*, *L. rubriviridis*, *L. salmoneus*, *L. subpurpureus* and *L. thyinos*). We are not sure about *L. chelidonium* Peck as we only have one representative in our analyses (S.L. Miller 9649). This specimen was compared with two

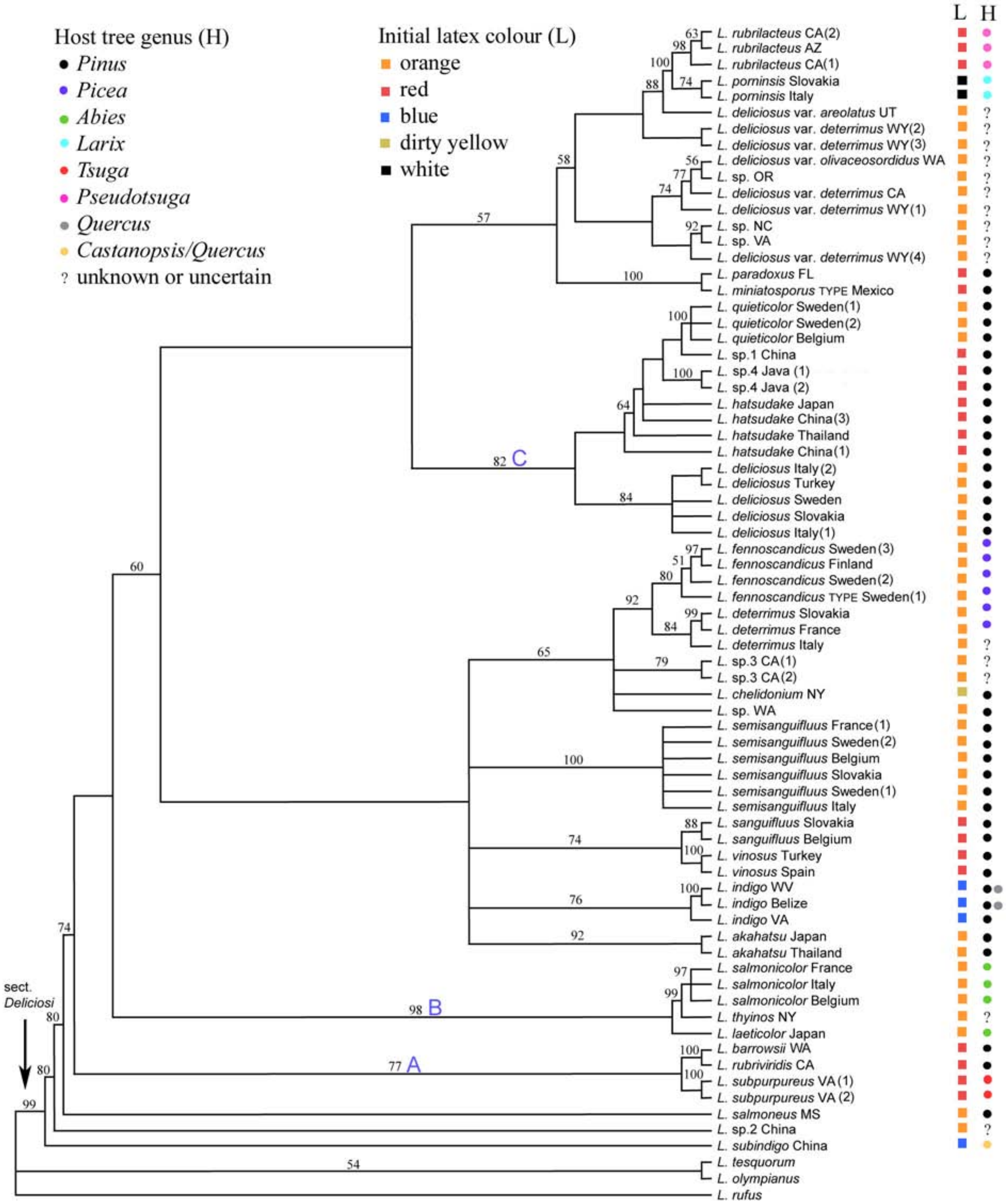

Fig. 4: Strict consensus of the most parsimonious trees obtained by combining ITS and *gpd* data. The MP analysis (1000 replicates, saving max. 1000 trees per replicate) resulted in 2255 most parsimonious trees of length 1278 divided over 60 islands (CI = 0.5978, RC = 0.4175, RI = 0.6984). Bootstrap values >50 % are indicated above the branches. Initial latex colour and host tree genus are indicated for each taxon. Clades A-C are mentioned in the text (under 3.4.).

specimens of *L. chelidonium* identified by Peck (collections Peck, 21 Aug., Bolton landing (NYS) and Sept., Bethlehem (NYS); the type is lost). The spores are similar but differences were found in the abundance of cheilo- and pleuromacrocystidia, which is maybe not a stable feature (NUYTINCK & VERBEKEN, 2005b). More, well-documented specimens are needed here. One new species was found (*L.* sp. 3, collections S.L. Miller 213-03 and 216-03), described in NUYTINCK *et al.* (in preparation). *Lactarius indigo* var. *diminutivus* is forming a well-supported group with *L. indigo* var. *indigo* in the ITS and the combined ITS-*gpd* analyses but not in the analysis of the *gpd* data alone, indicating a potentially strong difference between both taxa. More collections need to be examined to decide on the status of these varieties.

None of the North American specimens identified as *L. deliciosus* are conspecific with the European *L. deliciosus*. Detailed observations on the morphology (colour and general (surface) aspect of the pileus and stipe, and colour (change) of the latex) and microscopy are needed to elucidate the delimitations of taxa in the American "*L. deliciosus*" clade. *Lactarius deliciosus* var. *areolatus* is characterised by its distinctly larger spores and the lack of pleuromacrocystidia and is reported to be the most common variety of "*L. deliciosus*" western North America (HESLER & SMITH, 1979; METHVEN, 1997). Other varieties are mainly distinguished by the presence and abundance of pleuromacrocystidia, the colour of the pileus and the staining reaction of the context, three characters that show quite some intraspecific variability in this section.

From Asia, only the recently described *L. deliciosus* var. *indicus* Atri, Saini & D.K. Mann and *L. sanguifluus* var. *asiaticus* Dörfelt, Kiet & A. Berg are not included in the analyses. These taxa are unfortunately accompanied by very incomplete descriptions, making it difficult to draw any conclusions on their status. *Lactarius akahatsu*, *L. laeticolor* and *L. subindigo* form well supported clades. *Lactarius hatsudake* on the contrary, seems to be a heterogeneous group. Based on morphological evidence we distinguish three species: *L. hatsudake*, *L.* sp.1 and *L.* sp.4.

It is obvious that our currently used species concept in Asia is inevitably wider than in a well-studied area as Europe. The lack of detailed macroscopical descriptions from Asia, forcing us to rely on the less informative microscopy, contributes to this difference. The specimen collected in Yunnan, China, and identified as *L. deliciosus* indeed falls in the European *L. deliciosus* clade. *Lactarius thakalorum*, described from Nepal, is possibly conspecific with the European *L. sanguifluus* but more material is needed to confirm this. This study revealed three new species in Asia (labelled here as *L.* sp.1, *L.* sp.2 and *L.* sp.4). The number of Asian species in *Lactarius* sect. *Deliciosi* now adds up to nine. More species certainly remain to be discovered, as our knowledge from this underexplored continent is very poor and fragmentary.

Wrongly identified collections were very frequently encountered during our research. This was most striking for the Asian material but also in the American "*L. deliciosus"* complex.

In conclusion, we accept 38 taxa (31 species and 7 varieties) in *Lactarius* sect. *Deliciosi* on a worldwide scale but admit that the status of the 7 varieties needs further study. Moreover, a few collections could not be reconciled with any of these taxa; one of those collections is included here as *L.* sp. MTS 3445 (originally identified as *L. deliciosus* var. *olivaceosordidus*). As macroscopical descriptions are entirely lacking for these collections, we did not draw any further conclusions, keeping in mind the importance of macroscopical characters in this section. Microscopical descriptions of these collections can be found in NUYTINCK & VERBEKEN (in preparation) and NUYTINCK *et al.* (in preparation). The fact that the majority of the species, described with the aid of morphological data alone, is confirmed by our molecular approach is quite striking when taking into account the strong macro- and microscopical similarity of many taxa.

4.3. Intercontinental conspecificity

Intercontinental conspecificity in this section seems much lower than assumed so far. No overlap could be shown in this study between America and Eurasia. Further research is needed, including more samples from boreal North America and Asia, to exclude the existence of circum boreal species. Only *L. deliciosus* and *L. sanguifluus* seem to occur in both Asia and Europe. This misconception has for a large part originated from insufficient attention to morphological characters. *Lactarius deliciosus* and its varieties recognised in North America differ strongly macroscopically from the Eurasian *L. deliciosus* (personal observations on fresh collections). A new name for the American *L. deliciosus* is not proposed yet, full understanding of the status of the varieties is needed first and that requires more and better macro- and microscopical observations from a wide geographic range.

L. indigo has often been reported from Asia (HONGO & YOKOYAMA, 1978; IMAZEKI *et al.*, 1988; WU & MUELLER, 1997) but all collections examined by us are *L. subindigo*. The two species show distinct morphological differences in spore size and ornamentation (VERBEKEN & HORAK, 2000). There are records of *L. salmonicolor* from North and Central America but we had no material available yet to check conspecificity. Describing Asian taxa under European or American names (recent examples are *L. deliciosus* var. *indicus* and *L. sanguifluus* var. *asiaticus*) is unacceptable without a thorough comparison.

4.4. Relationships between the species and evolutionary trends

Due to the low resolution and support we obtained for the basal relationships and the differences resulting from the analysis of the ITS and *gpd* regions, it is impossible to propose a further division of *Lactarius* sect. *Deliciosi* in subsections. Several morphology-based classifications group those species with similarly coloured latex (BASSO, 1999; BON, 1980; SCHAEFER, 1970). The initial colour of the latex is plotted on the tree in Fig. 4 and seems to be of limited value to determine relationships in *Lactarius* sect. *Deliciosi*. Apparently, this colour, caused by the presence of azulene and hydroazulene sesquiterpenoids with a guaiane skeleton (SCHMITT, 1974; STERNER & ANKE, 1995), changed frequently during evolution of the section. The two species with indigo blue latex never clustered in any of our analyses; red coloured latex must have evolved at least five times independently and *L. porninsis* has lost the striking pigmentation of the latex. Nevertheless, some well-supported clades in our phylogenetic analyses are also supported by morphological evidence. *Lactarius salmonicolor*, *L. thyinos* and *L. laeticolor* share the lack of a green discolouration, the rather large spores with a thin ornamentation and the striking macrocystidia. Furthermore *L. barrowsii* and *L. rubriviridis* have very similar, densely ornamented spores and share the red coloured latex and the large size of the spores with *L. subpurpureus*. Spore characters (in this case the heavy ornamentation) also support the *L. hatsudake*, *L. quieticolor, L.* sp.1 and *L.* sp.4 clade.

When considering the geographic origin of the samples it is striking that many clades are composed of species from distant areas. This suggests that several ancestors must have existed when migration between the continents was still possible. Very recent migration between North America and Eurasia seems improbable, given the fact that until now no single conspecific taxon was found. The ancestors must have been very similar in morphology to the extant species, since some species in this section (e.g. the ones with orange latex that are found in nearly every clade of the tree) are strikingly similar and often hard to distinguish. The phylogenetic trees showed generally short branch lengths within *Lactarius* sect. *Deliciosi*, indicating a rather low divergence between the taxa. However, several, mainly North American species, are placed on longer branches (e.g. *L. salmoneus* and *L. pseudo-deliciosus*).

Host trees were plotted on the ITS and *gpd* tree in Fig. 4. The majority of species form ectomycorrhiza with *Pinus* but other coniferous hosts are *Picea*, *Abies*, *Larix*, *Pseudotsuga* and *Tsuga. Lactarius indigo* and *L. subindigo* are reported to be (also) associated with Fagaceae (*Quercus* and *Castanopsis* respectively). Host associations remain unclear for several species (e.g. *L. thyinos* and *L. salmoneus*) and deserve more attention. The well-documented host specificity of the European species has yet to be confirmed for North American and Asian species. The mostly mixed woods in North America can strongly complicate the host designation. Some species are presumably associated with more than one host (e.g. *L. indigo* is reported with *Pinus* and *Quercus*). The host switch from Pinaceae to Fagaceae or the other way around must have occurred at least twice. Careful comparative host-specificity and host-preference studies are necessary to verify these suggestions and draw more conclusions.

L. rubriviridis is a hypogeous, sequestrate species with forcibly discharged spores (DESJARDIN, 2003). It was the first hypogeous species described in the genus *Lactarius*. Based on morphological arguments, unambiguous designation to *Zelleromyces* or *Arcangeliella* turned out to be impossible. Moreover, it has been demonstrated that the latter two genera are polyphyletic (MILLER *et al.*, 2001; PETER *et al.*, 2001) and since then other hypogeous species have been assigned to the genus *Lactarius* as well (EBERHARDT & VERBEKEN, 2004; NUYTINCK *et al.*, 2003). DESJARDIN (2003) states that the red latex, green stains, forcibly discharged basidiospores and pine association of *L. rubriviridis* suggest that the species is relatively recently derived from an epigeous agaricoid ancestor, allied with

L. rubrilacteus. *Lactarius rubriviridis* is in our analyses very closely related to another species with red coloured latex, namely *L. barrowsii*. But as indicated above, red latex originated several times in the section. The species with red latex from North and Central America seem to fall into three distinct clades: (1) a clade formed by *L. rubriviridis*, *L. barrowsii* and *L. subpurpureus*; (2) a clade comprising *L. paradoxus* and *L. miniatosporus* and (3) a clade uniting *L. rubrilacteus* with *L. porninsis* and several North American collections identified as "*L. deliciosus*".

4.5. Future perspectives and open questions

The phylogenetic signal in the ITS and *gpd* data sets is incongruent. As revealed by likelihood mapping both data sets contain about the same amount of phylogenetic information, the skewness value however, is much lower for the *gpd* dataset. The ITS phylogeny agrees better with our morphological observations (e.g. separating *L. deterrimus* and *L. fennoscandicus*, grouping all specimens identified as *L. hatsudake* and as "*L. deliciosus*" in North America). A possible explanation for the different signal in both data sets is that we are dealing with paralogous copies of either gene. Paralogous copies have been reported for both genes in plants (FIGGE *et al.*, 1999; ÁLVAREZ & WENDEL, 2003). But both analytical factors (limited data availability, specific assumptions in the modelling of sequence evolution,…) and biological factors (the action of natural selection or genetic drift) may cause the history of the genes to obscure the history of the taxa (ROKAS *et al.*, 2003). Differences between data sets can also result from inclusion of reticulate taxa (MASON-GAMER & KELLOGG, 1996), but more research is needed to understand the evolutionary history and eventual hybridisation events between taxa like *L. deterrimus* and *L. fennoscandicus* (see also chapter 4). Sequencing more genes might be a solution for obtaining a robust phylogenetic hypothesis for *Lactarius* sect. *Deliciosi* (ROKAS *et al.*, 2003).

The low resolution of the phylogenies we obtained, especially in the basal clades, indicates that the used genes do not contain enough congruent information to solve these relationships. On the other hand, both DNA regions were successfully applied previously at the same or even a lower taxonomic level (BERBEE *et al.*, 1999; CHAPELA & GARBELOTTO, 2004; HIBBETT *et al.*, 1998; SHEN *et al.*, 2002). A hypothesis is that rapid speciation events caused this low resolution. This would also make incomplete lineage sorting a possible explanation for the incongruent phylogenetic signals we observe in *Lactarius* sect. *Deliciosi* (see also chapter 4).

The remaining taxonomic problems, such as the delimitation of taxa in the American "*L. deliciosus*" complex and the proposal of new names for these taxa but also for the undescribed species (named *L.* sp. 1 to 3 here) awaits additional sampling accompanied by detailed morphological descriptions.

Acknowledgements

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Chapter 6

Morphology and taxonomy of the European species in *Lactarius* sect. *Deliciosi*

Adapted from:
NUYTINCK J. & VERBEKEN A. (2005) – Morphology and taxonomy of the European species in *Lactarius* sect. *Deliciosi* (Russulales). *Mycotaxon* **92:** in print.

Abstract

Elaborate macro- and micromorphological descriptions are presented for the representatives of *Lactarius* sect. *Deliciosi,* together with taxonomical and nomenclatural remarks, partly based on previous molecular phylogenetic analyses (NUYTINCK & VERBEKEN, 2003 & 2005). Ten accepted European species are treated and an identification key is provided. Lectotypes are designated for *L. sanguifluus*, *L. semisanguifluus* and *L. vinosus*.

1. Introduction

This paper deals with the European species in *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.) and is part of an extensive study of this group of striking milk caps. Most representatives of *Lactarius* sect. *Deliciosi* are popular species, which are highly appreciated for their culinary qualities, mainly in southern and eastern Europe. All but one species in this section are easily recognisable by their orange or red to blue coloured latex, caused by the presence of guaiane sesquiterpenes (SCHMITT, 1974; BERGENDORFF & STERNER, 1988). Because of the variability of the colours and colour changes, not all current authors share the same species concepts, and 41 names are available for this limited number of European species. It is also this high variability of some important macroscopical characters used for identification (e.g. the colour and zonation of the cap and the discolouration of the latex) that leads to common misidentifications. Fresh collections in good condition are needed, preferably together with ecological data (host tree species, soil acidity), to achieve a reliable field determination.

A detailed molecular approach to the species delimitations and phylogenetic relationships for this section is presented in NUYTINCK & VERBEKEN (2003 & 2005). All commonly recognized European species (BASSO, 1999a; Bon, 1980; COURTECUISSE & DUHEM, 1994; HEILMANN-CLAUSEN *et al.*, 1998) were confirmed by analyses based on morphological and molecular characters. An unexpected result was the inclusion of *L. porninsis* Rolland, traditionally classified in *Lactarius* sect. *Zonarii* (Quél.) Bon (nom. inval.). In addition, we concluded that *L. deterrimus* Gröger and *L. fennoscandicus* Verbeken & Vesterh. on the one hand, and *L. sanguifluus* (Paulet) Fr. and *L. vinosus* (Quél.→) Bataille on the other hand, are very closely related and recently speciated taxa. In some cases, and this is true for nearly all species in this section, intermediate forms may strongly complicate correct identification.

This review starts with a key to the species and includes a macro- and microscopical and ecological description for every species, together with a discussion on the taxonomy and nomenclature. A list of insufficiently known and doubtful names is given at the end.

2. Materials & Methods

This study is based on herbarium material deposited in BR, GENT, LIP, MPU, PC and the personal herbaria of P.A. Moreau and M. Contu.

Macroscopical characters are based on fresh material. Colour codes are from KORNERUP & WANSCHER (1962). Microscopic measurements and drawings were made under oil immersion at 1000× with a Zeiss Axioscop 2 microscope and drawing tube. All observations and measurements (except for the spores) were made in Congo red in L_4 (7.2 g KOH, 160 ml glycerine, 840 ml dH_2O, 7.6 g NaCl and 5 ml Invadin (Ciba-Geigi), CLÉMENÇON, 1972). Where necessary a short pre-treatment in 10% KOH was used to rehydrate the tissue. Basidia lengths exclude sterigmata lengths. We use the term cheiloleptocystidia for the thin-walled, mostly clavate to irregularly shaped cystidia, without specific content and with a rounded apex on the lamella edge. Observations and measurements of basidiospores where made in Melzer's reagent. Spores were measured in side view, excluding ornamentation and the measurements are given as (MIN) [Ava-2×SDa] – *Ava* – *Avb* – [Avb+2×SDb] (MAX) in which Ava = lowest mean value for the measured collections, Avb = greatest mean value and SDa/b = standard deviation of the lowest and greatest mean value respectively. MIN is the lowest value measured, MAX the highest value; MIN and MAX are only given when they exceed [Ava-2×SDa] or [Avb+2×SDb] respectively. Q stands for 'quotient length/width' and is given as MINQ – *Qa* – *Qb* – MAXQ in which Qa and Qb stand for the lowest and the highest mean quotient for the measured specimens respectively. MINQ/MAXQ stands for the minimum/maximum value over the quotients of all available measured spores. 20 spores were measured per collection.

Scanning electron photographs were taken with a JEOL JSM-5600 LV microscope. Small pieces of lamellae were taken from dried specimens and soaked overnight in strongly diluted ammonia. The material was then treated with 70% ethanol (2 × 15 min.) and dimethoxymethane (2 × 30 min.), before being submitted to the process of critical point drying. This was done with a BAL-TEC CDP 030 dryer. The samples were then coated with gold in a JEOL JFC-1200 Fine Coater for 60 sec. at 8 Pa and 30 mA, until a 15 nm thick layer covered the spores.

3. Key to the European species of *Lactarius* sect. *Deliciosi*

Keep in mind that very small basidiocarps and buttons are often entirely deep blue-green, regardless of the species (phenomenon observed in L. salmonicolor, L. deliciosus, L. quieticolor and L. deterrimus).

1. - Latex white; species growing with *Larix*. .... **5. *L. porninsis***
   - Latex orange, red or blue; species growing with other coniferous trees. 2

2. - Pileus entirely salmon orange, sometimes with a paler margin, without green tinges; green tinges seldomly present in very young or very old basidiocarps; species associated with *Abies*; pleuromacrocystidia very abundant and large (up to 96 µm long). ....**7. *L. salmonicolor***
   - Pileus mostly not purely orange but with green, blue, brownish, greyish or red tinges; discolouring green when bruised or when older; species associated with *Pinus* or *Picea*; pleuromacrocystidia max. 70 µm long, often quite rare. 3

3. - Latex blue in the major part of the fruiting body but orange in the lower half of the stipe; stipe with clear blue tinges; species restricted to the mediterranean region; associated with *Pinus halepensis*. . ...**1. *L. cyanopus***
   - Latex orange or red, rarely with a bluish or greenish zone right underneath the pileipellis; stipe mainly orange, reddish or with green tinges; not restricted to the mediterranean region; associated with *Pinus* or *Picea*. 4

4. - Latex red when exuded; lamellae pinkish to vinaceous red; associated with *Pinus*. 5
   - Latex orange when exuded; lamellae orange; associated with *Pinus* or *Picea*. 6

5. - Pileus and stipe with orange tinges; pileus generally azonate; pileus slowly and relatively slightly discolouring greenish; stipe cylindrical or rarely tapering downwards; lamellae pinkish. ....**8. *L. sanguifluus***

- Pileus and stipe with pale to deep vinaceous red tinges; pileus generally clearly zonate; pileus strongly discolouring greenish after picking the basidiocarps; stipe mostly short and strongly tapering downwards; lamellae pale violet to vinaceous. ....**10. *L. vinosus***

6. - Orange latex becoming quickly vinaceous red (in 5 to 8 min.); often with a lot of green in pileus, especially in older specimens. ....**9. *L. semisanguifluus***
- Latex remaining orange for more than 10–15 min.; some green may be present in the pileus, especially in bruised spots, but basidiocarp never entirely greenish. 7

7. - Stipe without (obvious) scrobicules; species associated with *Picea*; spore ornamentation consisting of isolated warts and short ridges, never reticulate. 8
- Stipe mostly with scrobicules; species associated with *Pinus*; spores ornamented with an incomplete reticulum. 9

8. - Pileus typically azonate or with a few zones near the margin, with mainly bright orange colours and green tinges; stipe unicolourous bright orange without scrobicules but often with green hues; cheilomacrocystidia mostly quite abundant; species common everywhere in Europe. .... **3. *L. deterrimus***
- Pileus very clearly zonate and brown-orange with sometimes lilac-grey tinges; stipe pallid to dull orange-buff, rarely with unclear scrobicules; cheilomacrocystidia mostly rare; a subboreal, boreal and subalpine species. . ... **4. *L. fennoscandicus***

9. - Latex orange, remaining orange or changing slowly to red in more than 30 min.; pileus orange, mostly with a whitish aspect, mostly clearly zonate; stipe clearly scrobiculate; species occurring on neutral to calcareous soil; spores not very heavily ornamented. . .. **2. *L. deliciosus***
- Latex orange, changing to red in about 20 min.; pileus colour highly variable, from orange to brownish and sometimes bluish, but mostly dull; stipe almost without or with inconspicuous scrobicules; species growing on acidic soil; spores ornamented with very thick ridges. .... **6. *L. quieticolor***

4. Descriptions and taxonomical conclusions

4.1. ***Lactarius cyanopus* Basso**, Bull. Trimestriel Soc. Mycol. France 114: 67 (1998)

Figs. 1, 2

Holotype: 8630 (LUG), Italy: Liguria, Imperia, Civezza.

DESCRIPTION (macroscopy after BASSO, 1999b): *Pileus* 3–8 (12) cm diam., plano-convex with a slightly inrolled margin when young, flattened to funnel-shaped when full-grown, margin only slightly bent downwards or sometimes almost straight, regularly shaped, especially when young, to wavy, lobed or gibbous, margin thin and smooth; surface smooth, sticky and even slightly viscid when humid; colour pale ivory blue, with orange and blue shades or even almost entirely pale blue when young, more rarely with more or less regular, darker zones, sometimes with scarce, greenish to ochre-blue or clear blue-green spots, in age becoming creamy blue, creamy green, pale creamy ochre and washed-out to whitish. *Lamellae* subdecurrent to decurrent, medium dense, thin in young and thicker in older specimens, sometimes forking near the stipe, very fragile; colour pale cream to pale creamy orange but always with blue-green tones, becoming creamy green, sometimes even pale green with almost no cream shade, becoming bluish and then bluish green when bruised; edge entire, concolorous. *Stipe* 1.7–5 × 0.9–2.1 cm, short in relation to pileus diameter, subcylindric or slightly broader to more narrow at the base, full and firm when young but soon hollow; surface slightly irregular because of the presence of dimples, small humps or more rarely small scrobicules that are hardly any darker than the rest of the surface; colour white to whitish blue and pruinose in the zone of contact with the lamellae, whitish blue, blue to intense greenish blue with age in the upper part, paler, creamy blue centrally and white with ochre spots at the base. *Context* thin but rather firm when young, then becoming a little elastic, whitish in the pileus and underneath the pileipellis, white with a bluish tone in the central part of the stipe, sometimes with faint ochre or violet tones, blue to bluish above the lamellae and underneath the stipitipellis halfway down the stipe or sometimes right down to the base, becoming progressively dark blue-green, orange, becoming red and finally greenish in the lower half of the stipe or only at the base; smell indistinct, fruity, resembling the smell of much of the other species in the section, taste pleasant, mild, absolutely not acrid nor bitter, even after chewing for a long time. *Latex* very scarce, orange in the base of the stipe, changing to red but blue or bluish in the rest of the stipe and in the cap; taste mild. *Spore deposit* unknown.

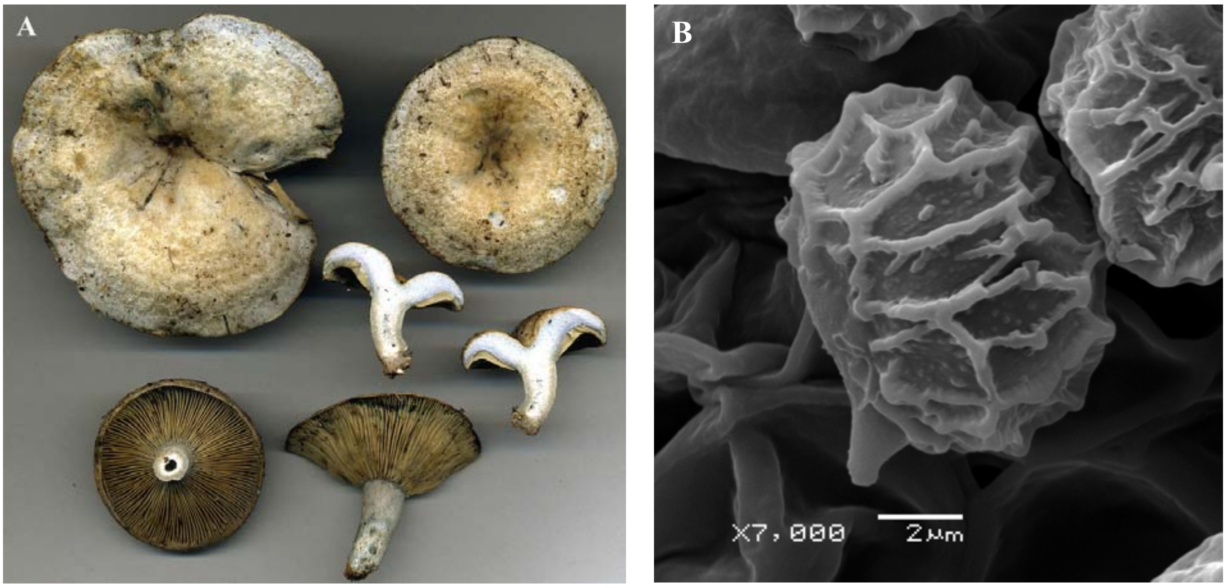

Fig. 1: **A.** basidiocarps of *L. cyanopus* collected in Spain (photo M.A. Pérez-De-Gregorio); **B.** SEM image of a spore of *L. cyanopus* M.T. Basso 98103001.

KOH slowly turning brown-grey, *gaiac* no reaction.

Spores 6.9–*8.3–8.8*–9.7 (9.9) × 5.8–*6.8–7.1*–7.8 µm, subglobose to ellipsoid but generally broadly ellipsoid (Q = 1.12–*1.22–1.24*–1.34); ornamentation rather low (< 0.5 µm), of rather thin and slender ridges, often angular, forming mostly a complete network, isolated warts rare to absent; plage non-amyloid. *Basidia* 49–67 × 9–12 µm, subclavate, 4-spored, content sometimes granular or with oil droplets; sterigmata 4.5–7.5 µm long. *Pleuromacrocystidia* abundant, 38.5–70 × 5.5–8.5 µm, emergent, subcylindric to subfusiform with an obtuse to mostly moniliform apex, sometimes with a branching apex, often containing small, needle-shaped crystals, thin-walled. *Pseudocystidia* relatively rare, not or rarely emergent, cylindrical to tortuous, slender (2–5.5 µm broad), with a rounded apex, content similar to the lactifers but hyaline in the apex. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia 11–28 × 3–9.5 µm, short and rounded to longer and cylindrical, hyaline, thin-walled; cheilomacrocystidia 29–42 × 6–8 µm, subfusiform, often with a moniliform apex, content needle-like, thin-walled. *Subhymenium* composed of globose to more angular cells, more or less arranged in rows. *Hymenophoral trama* irregularly filamentous; with rather abundant but slender lactifers. *Pileipellis* an ixocutis, up to 200 µm thick, slime-layer thin, of thin-walled, frequently branching and strongly interwoven hyphae, 2–5 µm diam., some are gelatinised or shrivelled. *Stipitipellis* a cutis to ixocutis, 50–120 µm thick, slime-layer thin where present, of thin-walled, interwoven hyphae, 2–3.5 µm diam., few hyphae gelatinised, some hyphae shrivelled, lactifers numerous and close to the surface, 3–10 µm diam. *Clamp-connections* absent.

HABITAT: Associated with *Pinus halepensis*; in mediterranean carigue vegetation with *Cistus albidus*, *Spartium junceum* and *Thymus vulgaris* (type locality, BASSO, 1999b); calcareous soil (PÉREZ-DE-GREGORIO & CARBÓ, 2004).

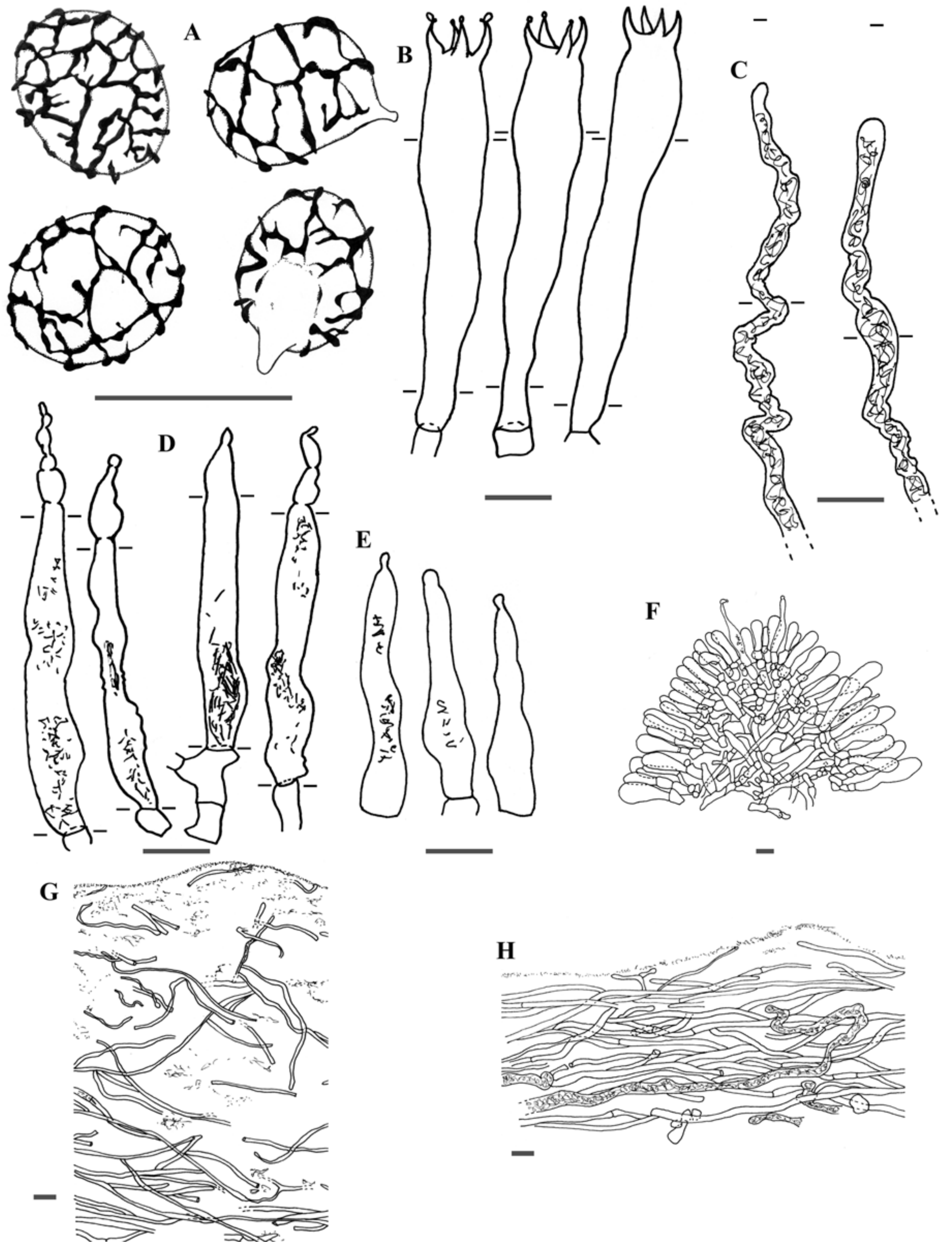

Fig. 2: *Lactarius cyanopus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** lamella edge; **G.** section through the pileipellis; **H.** section through the stipitipellis. From M.T. Basso 98102204 and M.T. Basso 98103001. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISTRIBUTION: Reported from Spain and Italy.

COLLECTIONS EXAMINED: Italy, Civezza (Imperia), road to Monte Faudo (type locality), 320 m a.s.l., *Cistus albidus* with *Pinus halepensis,* 22.10.1998, M.T. Basso 98102204 (GENT) – ibid., 30.10.1998, M.T. Basso 98103001 (GENT) – Spain, Girona, Baix Empordà, Vall-llobrega, 100 m a.s.l., under *Pinus halepensis*, on calcareous soil, 18.11.2002, M.A. Pérez-De-Gregorio 181102 (GENT).

DISCUSSION: *Lactarius cyanopus* was described in 1998 and is reported from three localities: Civezza (Imperia, Italy: type locality; BASSO, 1998), Vall-llobrega (close to Girona, Spain) and Mallorca (PÉREZ-DE-GREGORIO & CARBÓ, 2004). A forest fire destroyed the type locality in 2000 (Basso, pers. comm.), making it impossible for us to study fresh specimens. The macroscopical description given here is a summary of the description by BASSO (1999b). In this original description, *Cistus* is stated erroneously as the host species for *L. cyanopus*. *Pinus halepensis* grows nearby and is almost certainly the host tree (Basso, pers. comm.).
The extensive blue colours in the cap and stipe in *L. cyanopus* are unique in Europe. Young specimens of *L. quieticolor* can also be strikingly blue to bluish green in the pileus (HEILMANN-CLAUSEN *et al.*, 1998). Microscopically however, *L. cyanopus* is very different from *L. quieticolor*: the spore ornamentation in particular is much heavier in *L. quieticolor*.
The spore ornamentation in *L. cyanopus* is peculiar in that it often forms a complete network, which is rare in the other European *Deliciosi*. This very complete network was not observed in the Spanish collection. *Lactarius sanguifluus* has smaller and more rounded spores with thicker ridges forming an incomplete network. The profile of the spores of *L. semisanguifluus* is similar to the *L. cyanopus* spores but the ornamentation is more incomplete; isolated warts are more abundant.
According to our molecular analyses, *L. cyanopus* belongs to the same clade as *L. sanguifluus* and *L. vinosus* or even falls within *L. sanguifluus* and would thus be conspecific (NUYTINCK & VERBEKEN, 2005). However, we do not synonymise both names here. The very poor state of the *L. cyanopus* specimens made it hard to extract good quality DNA and several attempts were needed to amplify the ITS region. From a morphological point of view it seems very unlikely that both are conspecific. Most importantly because of differences in the spore ornamentation and spore shape, but also because *L. cyanopus* has orange coloured latex in the lower half of the stipe. *Lactarius sanguifluus* has vinaceous red latex and only very exceptionally some orange latex in the very base of the stipe or where the mycelium is attached has been observed (never halfway up the stipe; ROMAGNESI, 1958). Unfortunately the original description of *L. cyanopus* does not state clearly how quickly this orange latex in the base of the stipe turns reddish (maybe because of the bad state of the specimens?).

4.2. ***Lactarius deliciosus* (L.: Fr.) Gray**, Nat. Arr. Br. Pl. 1: 624 (1821)

Figs. 3, 4

Basionym: ≡ ***Agaricus deliciosus*** L., Species Pl.: 1172 (1753)
Synonyms: ≡ ***Lactifluus deliciosus*** (L.: Fr.) Kuntze, Revisio Generum Plantarum, Pars II: 856 (1891)
= ***Lactaria lateritia*** Pers., Tent. Disp. Meth. Fung.: 64 (1797)
= ***L. deliciosus*** var. ***lamelliporus*** (Barla) Sacc., Sylloge Fungorum 5: 438 (1887)
≡ ***Agaricus deliciosus*** var. ***lamelliporus*** Barla, Champ. Prov. Nice: 35 (1859)
= ***L. deliciosus*** var. ***pini*** Vassilkov, [Edible and poisonous fungi of central parts Europ.distr. U.S.S.R.]: 60 (1948), nom. inval., nom. nud.
= ***L. pinicola*** Smotl. ex. Z. Schaef., Schweiz. Z. Pilzk. 48: 141 (1970)
≡ ***L. deliciosus*** var. ***pinicola*** Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 219 (1947), nom. inval., nom. nud.
= ***L. deliciosus*** f. ***rubescens*** J.A. Schmitt, Z. Pilzk. 39: 238 (1974)
= ***L. deliciosus*** var. ***lateritius*** J. Blum ex J. Blum, Lactaires: 216 (1976)
Excluded: ***L. deliciosus*** sensu LANGE (1940) and many earlier authors from central and eastern Europe (= *L. deterrimus*)

Type: not selected, described from southern Sweden.

DESCRIPTION: *Pileus* 4–12 cm diam., sometimes larger, convex with an inrolled margin and a slightly depressed centre at first, later with a decurved to expanded margin and with a depressed centre to infundibuliform, sometimes ± irregularly shaped; surface viscid but soon dry, ± smooth; colour pale orange (5A2/4) to salmon orange (6A4/5), typically with a whitish aspect (a white bloom), with deeper orange (6B7/8) spots of irregular shape, sometimes forming a few broad, concentric zones, towards margin often with narrow zones, discolouring reddish and then greenish where bruised, but not very extensively. *Lamellae* slightly decurrent to decurrent, rather narrow to medium broad, rather crowded, often forking near the stipe; colour salmon to yellowish orange (5A6/7 to 6A/B7), discolouring red and then green where bruised; edge slightly paler, entire. *Stipe* 2.5–5 × 1–3 cm, typically relatively short and chunky, almost cylindrical or slightly tapering downwards; surface dry, colour orange with a well developed whitish surface layer (6A4/5), almost white at the very top, with a few to numerous, small to large, deeper orange scrobicules (6A/B8) (scrobicules only very rarely absent), greenish where bruised. *Context* firm, with an irregular cavity in the stipe, white to pale cream in the central parts, bright orange just above the lamellae, underneath the pileipellis and near the stipe surface (5/6A8), orange parts remaining orange (orange often slowly fading) or very slowly turning brownish orange to orange-red (7B/C7/8, after 30 min. to 1 h), greenish after several hours; smell fruity; taste mild to very slightly acrid at first and then slightly bitter. *Latex* scarce, bright orange (5/6A8), often very slowly changing to red on the context; taste mild. *Spore deposit* pale yellow-orange (4A3).

$FeSO_4$ no reaction, *KOH* no reaction, *phenol* no reaction, *gaiac* very slowly turning blue on context, *HCl* no reaction.

Spores 6.5–*8.1–9.7*–10.6 × 5.4–*6.2–7.0*–7.7 µm, broadly ellipsoid to ellipsoid, rarely subglobose (Q = 1.10–*1.25–1.39*–1.50); ornamentation up to 0.5 µm high, of rather thick and broad ridges, forming a coarse, incomplete to almost complete reticulum, with some short ridges and isolated warts present; ridges rarely less amyloid at the top, giving them a split appearance; plage mostly slightly amyloid distally, often with some small amyloid spots centrally. *Basidia* 43–65 × 9.5–12 µm, ± cylindrical to subclavate, 4-spored, sometimes with oil-droplets; sterigmata 4–6 µm long. *Pleuromacrocystidia* (very) scarce but more abundant near the lamella edge, 34–55 × 6.5–9 µm, slightly emergent, subfusiform, with a narrowing, obtuse or moniliform apex, often with a needle-like content, thin-walled. *Pseudocystidia* rather abundant, 2–6 µm diam., mostly not emergent, often branching and irregularly shaped, content similar to the lactifers but often hyaline in the apex. *Lamella edge* sterile (or rarely with a few basidia) with (very) abundant cheilomacrocystidia; cheiloleptocystidia 8–25 × 3–10 µm, subclavate to irregularly shaped, hyaline, thin-walled; cheilomacrocystidia 30–40 × 5–7 µm, subfusiform to fusiform, with a moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* appearing like short, irregularly shaped cells, often in rows. *Hymenophoral trama* irregularly filamentous; abundant lactifers present with an ochre content. *Pileipellis* an ixocutis, up to 200 µm thick, with a very thin slime-layer, composed of thin-walled, frequently branching hyphae, 2–7 µm diam.; few hyphae gelatinised or very narrow (1–2 µm diam.), most hyphae with slightly refringent walls and larger diam., some with short knot-like branches. *Stipitipellis* a cutis, up to 150 µm thick, of thin-walled hyphae, 2–4 µm diam.; emergent hyphae frequent, giving sometimes the impression of a trichoderm; emergent hyphae absent where scrobicules occur. *Clamp-connections* absent.

HABITAT: On neutral to calcareous soils, forming ectomycorrhizae with *Pinus* species (UHL, 1988).

DISTRIBUTION: Widespread in Europe, also found in Turkey and Morocco, but certainly not the most common species in central and eastern Europe. Reported from all over the world, with indigenous and introduced *Pinus* spp., identifications often doubtfull (NUYTINCK *et al.*, in preparation)

COLLECTIONS EXAMINED: Austria, Nordtirol, Vomperberg, 900 m NN, 23.10.1993, leg. & det. Plenk, KR 164/93 (GENT) – Belgium, Westerloo, 1.11.1937, M. Herregods 188 (GENT) – West-Vlaanderen, De Haan, close to *Pinus*, in sand dunes, 1.11.1984, I. Cauwels 84-36 (GENT) – Oostvoordeduinen, Under *Pinus* at graveyard, 22.10.1993, A. Verbeken 93-133 (GENT) – Nismes, J5.41.14, *Pinus sylvestris*, border limestone grassland, 30.09.1995, R. Walleyn 0378 (GENT) – Zedelgem, Vloethemveld, C1.47.22, in grassy, nutrient-poor roadside (close to *Pinus sylvestris*), 20.10.2002, R. Walleyn 2784 (GENT) – Close to Ave-et-Auffe, 244 m a.s.l., N50°07.060' E005°08.025', under *Pinus*, 26.10.2003, J. Nuytinck 2003-047 (GENT) – Denmark, NEJ: Høstemark Skov S of Mou, UTM NJ7511, TBU 11, under *Pinus*, 1.10.1995, J. Vesterholt 95-

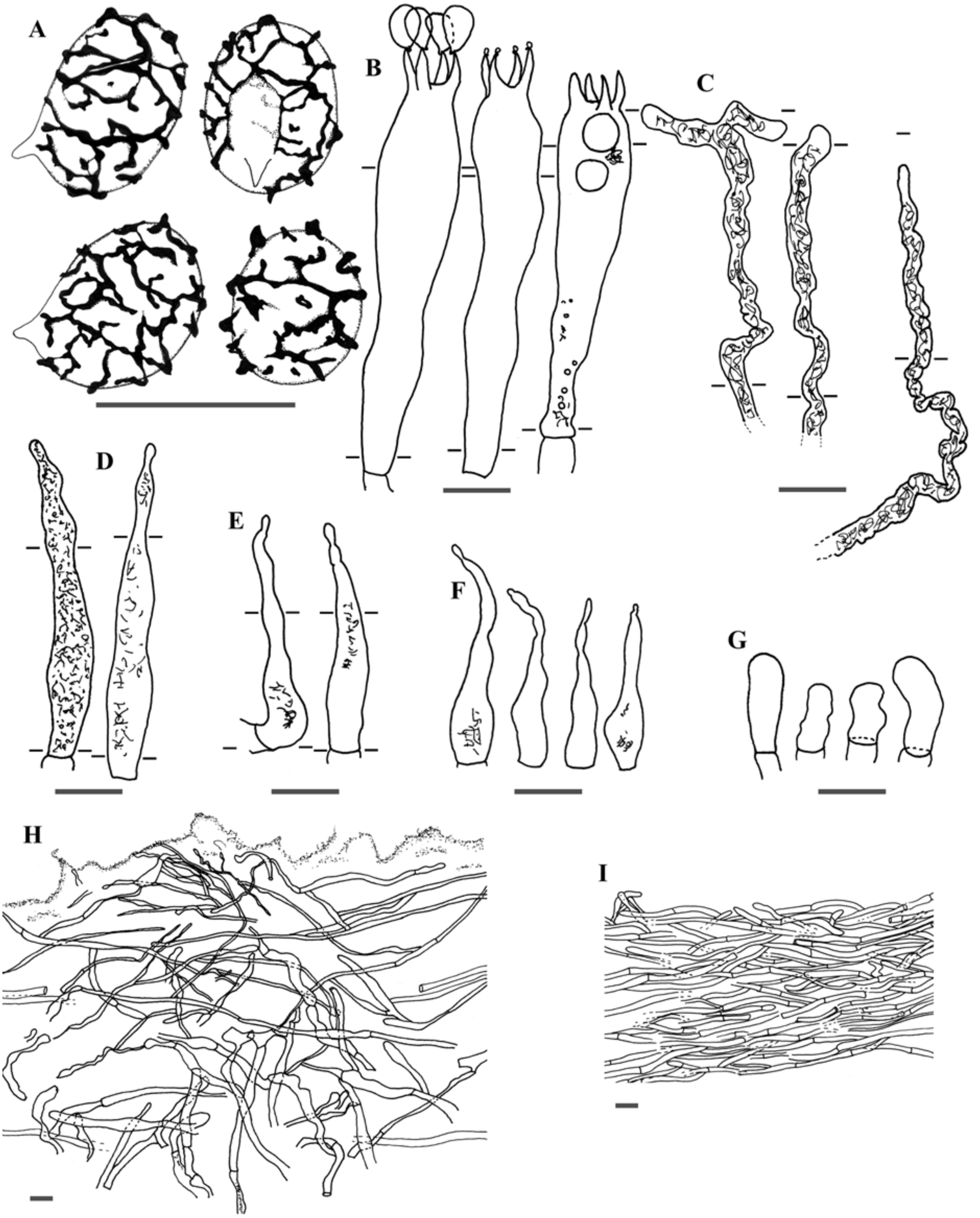

Fig. 3: *Lactarius deliciosus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** macrocystidia close to the lamella edge; **F.** cheilomacrocystidia; **G.** cheiloleptocystidia; **H.** section through the pileipellis; **I.** section through the stipitipellis. From A. Peksen & G. Hatat 229, J. Vesterholt 96-312 and J. Nuytinck 2001-006, 2001-036, 2001-101, 2001-117, 2002-042, 2003-057. Scale bars = 10 µm, small bars indicate the height of the hymenium.

482 (GENT) – NEJ: Læsø, Estrupvej, SW of Vesterø Havn, UTM PJ1549 TBU 03, at roadside under *Pinus*, 16.10.1996, Vesterholt 96-312 (GENT) – Finland, road from Riikinranta to Sotkamo, spruce and pine forest, 6.09.2001, J. Nuytinck 2001-036 (GENT) – France, Dept. Tours, under *Pinus*, 28.10.1991, R. Walleyn (GENT) – Forêt Domaniale de Fontainebau, Hippodrome La Solle, under *Pinus* on calcareous, dry, sandy soil, in grassy vegetation, 4.10.2001, leg. P-J. Keizer, J.

Nuytinck 2001-129 (GENT) – Germany, Baden-Württemberg, Deufringen, Mesobromethum auf Muschelkalk, under *Pinus*, 27.10.1988, H. Haas 4000430 (GENT) – Baden-Württemberg, Stuttgart-Weilimdorf, Neuer Friedhof, 340m, 7120/4.34, 23.09.1989, Bollmann A., KR 566/91 (GENT), as *Lactarius deliciosus* f. *rubescens* – Baden-Württemberg, Schafhausen, MTB 7219/3.23, Wacholderheide auf Muschelkalk, under *Pinus silvestris* and *Juniperus communis*, 28.10.2001, A. Gminder (GENT) – Italy, Toscana, Cala Violina NW of Grosseto, under *Pinus*, 7.11.1996, leg. M. Christensen, J. Vesterholt 96-457 (GENT) – Sardegna, Tempio Pausania, road SS133, at about 2 km from centre, plateau (old vineyard?), soil rather calcareous, under *Pinus halepensis*, 1.11.2000, A. Verbeken 2000-104 (GENT) – Slovakia, Zàhorská ´mžina lowland, 4 km SW of Moravsky Svatý jàn village, on the river side of the Morava (W Slovakia, <1 km from Austrian border), 120-130 m a.s.l., N48°33.480´ E016°56.978´, *Pinus* forest on sand, under *Pinus sylvestris*, 2.10.2001, J. Nuytinck 2001-045 (GENT) – ibid., 2.10.2001, J. Nuytinck 2001-046 (GENT) – Zàhorská ´mžina lowland, 3 km NWW of Lakšàrska Nová Ves village, 200m a.s.l., *Pinus sylvestris* plantation, 3.10.2001, J. Nuytinck 2001-052 (GENT) – Nízke Tatry mountains; 2,5 km N to Malužiná village, Michalovo valley, 707m a.s.l., N49°00.094´ E019°45.470´, In pasture on slope under *Pinus sylvestris*, calcareous soil, 6.10.2001, J. Nuytinck 2001-071 (GENT) – Spain, Surroundings of St Hilary Sacalm, 3.11.2001, bought on a local market, J. Nuytinck 2001-101 (GENT) – ibid., 3.11.2001, bought on a local market, J. Nuytinck 2001-102 (GENT) – Between Coll Sacren and east-side of A7, Montenegre mountain, 6.11.2001, J. Nuytinck 2001-108 (GENT) – Close to Lloretta del Mar, 134 m a.s.l., N41°45.032´ E002.48.527´, mediterranean vegetation with *Pinus* sp. and *Quercus ilex*, 8.11.2001, J. Nuytinck 2001-117 (GENT) – St Hilary Sacalm, 8.11.2001, J. Nuytinck 2001-118 (GENT) – Close to Fuenteheridos, region of Aracena, 690 m a.s.l., N37°54.195' W006°40.846', under *Pinus pinaster*, 4.11.2003, J. Nuytinck 2003-049 (GENT) – El Talenque, 587 m a.s.l., N37°55.749' W006°40.452', under *Pinus*, J. Nuytinck 2003-055 (GENT) – Between Linares de la Sierra and Alajar, 685m, N37°52.789' W006°38.792', under *Pinus pinea*, 7.11.2003, J. Nuytinck 2003-057 (GENT) – Sweden, Borgsjö, youth hostel, ±120 m a.s.l., N62°32.481´ E015°54.049´, grassy place, 12 m from closest *Pinus sylvestris* tree, 25.08.2001, J. Nuytinck 2001-005 (GENT) – ibid., 25.08.2001, J. Nuytinck 2001-006 (GENT) – ibid., 25.08.2001, J. Nuytinck 2001-007a (GENT) – ibid., 25.08.2001, J. Nuytinck 2001-007b (GENT) – ibid, 25.08.2001, J. Nuytinck 2001-007c (GENT) – City park in Ange, ±240 m a.s.l., N62°31.127´ E015°39.574´, lawn on small hill with *Pinus sylvestris* and *Betula*, 28.08.2001, J. Nuytinck 2001-016 (GENT) – Graveyard Bräcke, under *Pinus sylvestris*, 29.08.2001, leg. D. Laber, J. Nuytinck 2001-021 (GENT) – Turkey, Samsun region, under *Pinus*, 30.08.1997, A. Peksen & G. Hatat 104 (GENT) – Samsun region, under *Pinus*, 26.11.1997, A. Peksen & G. Hatat 229 (GENT) – Kocadağ, 682 m a.s.l., N41°19.279´ E036°07.631´, mainly *Pinus* forest on slope of hill, 22.10.2002, J. Nuytinck 2002-041 (GENT) – Kocadağ, 702 m a.s.l., N41°19.279´ E036°07.545´, mainly *Pinus* on slope, 22.10.2002, J. Nuytinck 2002-042 (GENT).

Fig. 4: A. *L. deliciosus* J. Nuytinck 2001-006 collected in Sweden (photo R. Walleyn); **B.** *L. deliciosus* J. Nuytinck 2003-055 collected in Spain.

DISCUSSION: Several features are characteristic for *L. deliciosus*: the orange cap with the whitish zonate aspect, the scrobiculate, pale orange stipe, the bright orange latex staying orange on the context for more than 30 min. and often longer, the often large dimensions of the cap combined with a short and chunky stipe and the association with *Pinus*. The green discolouration is never very strong. Not all specimens have the combination of all of these features! Different weather and growing conditions make the appearance of the pileus very variable. The spores are ornamented with rather thick ridges compared to most other species, but this difference is subtile, and best observed by comparison with reference specimens.

In his detailed study of specific guaiane sesquiterpenes colouring the latex in *Lactarius* sect. *Deliciosi*, SCHMITT (1974) describes *L. deliciosus* f. *rubescens*. This form has reddening latex (after about one hour) while in the forma typica the latex is supposed to stay orange. Most authors describe *L. deliciosus* with unchanging, orange milk, fading after some time and eventually becoming greenish (BON, 1980; NEUHOFF, 1956; ROMAGNESI, 1958). However, we observed a very slow colour change

from orange to red in most *L. deliciosus* specimens. BASSO (1999a) confirms this observation. Therefore, we do not consider *L. deliciosus* f. *rubescens* to have any taxonomical significance.
Some uncertainty exists about what LINNAEUS (1753) and FRIES (1821) actually meant by *L. deliciosus*. Both *L. deliciosus* and *L. deterrimus* are common in the Swedish forests, where *Pinus* and *Picea* often occur mixed. According to SCHAEFER (1970), *L. deliciosus* sensu Linnaeus and Fries is what we currently call *L. deterrimus* and he renamed the *Pinus* associated species *L. pinicola* Smotl. ex. Z. Schaef. One of his main arguments was that Fries' expression "in pinetis" means growing under coniferous trees in general and he was strengthened in his opinion after studying table 6 in FRIES (1861): the plate shows without doubt the *Picea* associated species. It seems very likely to us however, that *L. deliciosus* and *L. deterrimus* have not been considered to be separate species for a long time. According to BASSO (1999a), FRIES (1821 and later) refers to several other plates in his work that do represent the typical *L. deliciosus* (e.g. BARLA 1859; KROMBHOLZ, 1831–1846; SCHAEFFER, 1762–1774). The plate of *L. deliciosus* that was painted in 1851 under the supervision of Fries also depicts *L. deliciosus* (plate number S0691, ÄG 06, by E. Pettersson, STRID, 1994). There is a general consensus now to use the name *L. deliciosus* for the *Pinus* associated species. Designation of a neotype from the region where it was originally described will definitively settle this discussion.
BLUM (1976) described *L. deliciosus* var. *lateritius*, with a brownish orange cap and clear greyish brown zonations, strongly resembling some *L. zonarius* or *L. torminosus* collections. This name is currently not widely used. No collection is available from PC with collection number 62.2 (number indicated by Blum as the type collection). Two of Blum's collections (PC 1644 & PC 1653), labeled as *L. deliciosus* var. *lateritius,* were examined instead. Microscopically they are identical to *L. deliciosus* and the synonymy of *L. deliciosus* var. *lateritius* as also proposed by BASSO (1999a) is accepted here.

4.3. ***Lactarius deterrimus* Gröger**, Westfäl. Pilzbriefe 7: 10 (1968)

Figs. 5, 6

Synonyms: ≡ ***L. deliciosus*** var. ***deterrimus*** (Gröger) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 94 (1979)
= ***L. deliciosus*** var. ***piceus*** Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 218 (1947), nom. inval., nom. nud.
= ***L. deliciosus*** var. ***piceae*** Vassilkov, [Edible and poisonous fungi of central parts Europ.distr. U.S.S.R.]: 60 (1948), nom. inval., nom. nud.
Misapplications: ***L. deliciosus*** sensu LANGE (1940) and many earlier authors from central and eastern Europe (see below)
L. semisanguifluus sensu NEUHOFF (1956)

Holotype: DR (requested information on type number not received from DR), described from Germany: Krahnberg, near Gotha.

DESCRIPTION: *Pileus* 4–11 cm diam., typically convex when young, becoming slightly depressed to broadly infundibuliform, with a straight to slightly decurved margin when mature, sometimes staying convex, even when full-grown or old, mostly regularly shaped, margin smooth when young, becoming substriate when old; surface smooth, viscid and greasy when young but soon dry, azonate or sometimes with some zones, especially near the margin; colour rather variable, often (light) salmon orange (6A4/5) with darker zones (6C7) especially near the margin and a brownish orange centre (6D6), sometimes brighter orange (6A7), when dry often very pale orange (6A3, 5A3/5) and azonate, where bruised or when old turning (pale) greyish green, sometimes extensively. *Lamellae* broadly adnate to slightly decurrent, rather narrow to medium broad and rather crowded, often forking or anastomosing near the stipe; colour (pale) orange (6A6 to 5B7 when old), quickly becoming red when bruised and then slowly turning green (after 1–2 h); edge entire and ± concolorous. *Stipe* 2–7.5 (10) × 0.8–2 (2.5) cm, mostly cylindrical, typically long and slender (in comparison with *L. deliciosus*); surface smooth and dry, scrobicules almost always completely absent (when present small and scarce);

colour bright, carrot orange (6A6/7 to 6B7/8), sometimes paler, turning green where bruised or after frost, typically with a white zone just underneath the lamellae. *Context* rather firm but thin in the pileus, especially near the margin, becoming hollow in stipe, white to buff, underneath pileipellis and stipitipellis and above the lamellae bright orange when cut (6A8), becoming red in (10) 15–20 min. (7/8B8), vinaceous red in 1 h (up to 10E6 but often paler) and green in 12 h; smell agreeable, fruity; taste mild but when chewing a large amount for a while becoming slightly acrid and bitter. *Latex* bright orange (6A8) at first, becoming red after 15–20 min. when drying on the context; taste mild. *Spore deposit* pale yellow-orange (4A3).

$FeSO_4$ no reaction, *KOH* no reaction, *phenol* no reaction, *gaiac* slowly turning blue on context.

Spores 7.6–*8.5*–*9.4*–10.4 × 5.9–*6.6*–*7.1*–8.0 µm, broadly ellipsoid to mostly ellipsoid (Q = 1.25–*1.34*–*1.38*–1.50); ornamentation up to 0.5 µm high, of mainly warts and short, rather broad ridges with some interconnecting finer lines, forming a very incomplete reticulum; plage very faintly amyloid distally. *Basidia* 45–60 × 9.5–12 µm, ± cylindrical to subclavate, 4-spored, with oil-droplets or a granular content; sterigmata 4.5–5.5 µm long. *Pleuromacrocystidia* scarce but locally abundant near the lamella edge, 45–65 × 5–8 µm, sometimes smaller near the lamella edge, emergent, subfusiform, with a narrowing or mostly moniliform apex, often with a fine, granular content, thin-walled. *Pseudocystidia* abundant, 4–6 µm broad, sometimes emergent but often shorter than the basidioles, cylindrical to rather tortuous, with an ochre-yellow content similar to the lactifers but almost invisible near the apex. *Lamella edge* mostly sterile with few to rather abundant cheilomacrocystidia; cheiloleptocystidia 15–25 × 5–10 µm, subclavate or irregular, hyaline or with a fine, granular content, thin-walled; cheilomacrocystidia 25–50 × 6–8 µm, subfusiform with a moniliform apex, hyaline or with a granular content, thin-walled. *Subhymenium* composed of rather small, globose cells arranged in short rows to irregular. *Hymenophoral trama* irregular; lactifers abundant and conspicuous with an ochre-yellow content. *Pileipellis* an ixocutis, up to 200 µm thick; slime-layer relatively thick, of interwoven hyphae with a diam. of 1–4 (7) µm and refringent walls; shrivelled and gelatinised hyphae abundant. *Stipitipellis* a cutis, up to 100 µm thick, of interwoven hyphae with a diam. of 3–5 µm, with very few shrivelled and gelatinised hyphae. *Clamp-connections* absent.

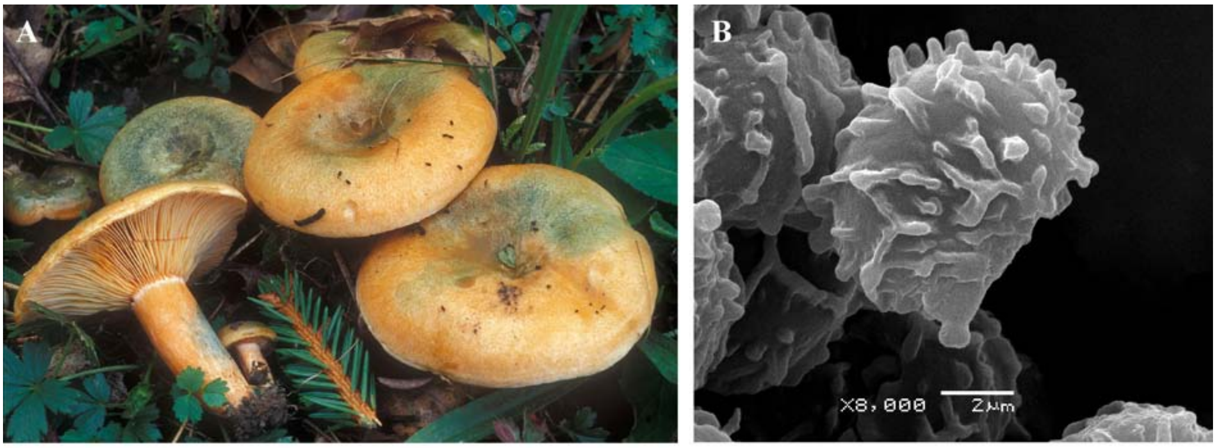

Fig. 5: A. *L. deterrimus* basidiocarps (photo R. Walleyn); **B.** SEM image of the spores of *L. deterrimus* J. Nuytinck 2001-076.

HABITAT: Forming ectomycorrhiza with *Picea* (AGERER, 1986b; MÜNZENBERGER *et al.*, 1986) and often very common where the host tree occurs, on calcareous to neutral soil, often in young *Picea* plantations.

DISTRIBUTION: Widespread in Europe, extending eastwards to Russia (VASSILKOV, 1948), very common in northern, central and eastern Europe. Certainly more common than *L. deliciosus* in central Europe (GRÖGER, 1968; SCHAEFER, 1970).

COLLECTIONS EXAMINED: Austria, kleines Walsertal, Moos-Alpe, 21.09.1998, V. Walther 4006560 (GENT) – Belgium, Ravels, 26.11.1961, Verheyen K. 996 (GENT) – Nismes, *Picea*-plantation, 30.09.1995, R. Walleyn 0379 (GENT) – Nukerke, Visputten, IFBL: E2.48.42, under *Picea*, 28.09.1998, leg. W. Termonia, R. Walleyn 1892 (GENT) – Ruisseau d'Alise, direction Moulin de Chestion, Forêt dominale, under *Picea* on acidic soil, 25.09.2000, J. Nuytinck 2000-002 (GENT) – Kessel-lo, entrance Wimmershof IFBL: E5.14.42, in lawn, underneath *Picea*, on lawn, 21.10.2000, J. Nuytinck 2000-010 (GENT) – Road between Bourdon and Marenne, N50°14.661' E005°24.422', 268 m a.s.l., in mixed forest under *Picea abies*, calcareous soil, at road side in grass, 22.09.2001, J. Nuytinck 2001-041 (GENT) – Barvaux, les Mignées, IFBL: H7.21.22, with young *Picea* trees on dry, calcareous soil, 30.09.2001, R. Walleyn 2183 (GENT) – Close to Roly, S of Philippeville, under *Picea abies*, 4.09.2002, J. Nuytinck 2002-013 (GENT) – Waulsort (Close to Dinant), in mixed forest under *Picea abies* on calcareous soil, 25.09.2002, J. Nuytinck 2002-018 (GENT) – Czech Republic, South Bohemia, Malonty, Hodonický potok, 7353a (SZ grid), ~630 m a.s.l., under young *Picea* on sandy, acid soil, 25.09.2003, R. Walleyn & A. Verbeken 3198 (GENT) – Denmark, W-Jylland, Varming plantage, W of Ribe, UTM: MG9427, *Picea*-plantation, 28.09.1996, leg. M. Christensen, AV 96-1004 (GENT) – NEJ: Rubjerg Knude Plantage, UTM: NJ4667, under *Picea* in *Abies* dominated forest on sandy, calcareous soil, 17.10.1996, J. Vesterholt 96-332 (GENT) – ibid., in *Abies* dominated forest on sandy, calcareous soil, no *Picea* seen nearby, but *Picea* occurs scattered in the plantation., 17.10.1996, J. Vesterholt 96-333 (GENT) – France, Beaulieu-en-Argonne, young *Picea* plantation, 31.10.1988, R. Walleyn 88/39bis (GENT) – Saint Germain_de Jaux (Ain), 1100m, mixed *Picea*-*Abies* wood, calcareous soil, 5.09.1999, leg. A. Bidaud, P.A. Moreau 5-9-99 (pers. herbarium P.A. Moreau), as *L. fennoscandicus* – Germany, Eifel, Wallersheim Wald, under *Picea*, 18.10.1984, I. Cauwels 84-10 (GENT) – Eifel, Duppach, *Picea* forest, 10.09.1986, B. Buyck 2434 (GENT) – Eifel, Kopp, *Picea* forest, 11.09.1986, B. Buyck 2440 (GENT) – Eifel, Kopp, *Picea abies*, 24.09.1993, A. Verbeken 93-65 (GENT) – Baden-Würtemberg, Welzheim, Wieslaufschlucht, 14.10.1997, V. Walther 4006348 (GENT) Duplum ex 4006348 KR – Baden-Württemberg, Tiefenbronn, MTB 7118/4.21, *Picea* reafforestation, slightly acidic soil (?), under *Picea abies*, *Betula*, *Abies* and *Pinus*, 28.10.2001, A. Gminder (GENT) – Italy, 13 km from Castel del Monte, N42°23.062' E013°45.365', 1433 m a.s.l., small mixed conifer forest on slope, under *Picea abies*, 1.11.2001, J. Nuytinck 2001-099 (GENT) – ibid., 1.11.2001, J. Nuytinck 2001-100 (GENT) – Trentino, N46°01.555' E011°13.035', 773 m a.s.l., in mixed forest, under *Picea abies*, calcareous soil, 2.10.2002, J. Nuytinck 2002-021 (GENT) – Maso Postel, Trentino, in mixed forest, under *Picea abies*, calcareous soil, 3.10.2002, leg. R. Flores, JN 2002-022 (GENT) – Maso Postel (Pergine Valsugama), Trentino, N46°02.254' E011°12.761', 824 m a.s.l., under *Picea abies*, calcareous soil, 4.10.2002, J. Nuytinck 2002-024 (GENT) – Netherlands, Adamskamp (close to Winterswijk), under *Picea*, 8.10.2000, J. Nuytinck 2000-005 (GENT) – Stokhem, Limburg, under *Picea* and *Pinus* on calcareous soil, 29.09.2001, leg. P-J Keizer, J. Nuytinck 2001-128 (GENT) – Norway, Akershus county, Nannestad municipality, Tromte, biological station, N60.16 E11.2, 200 m a.s.l., 15.09.1996, A. Verbeken 96-917 (GENT) – Slovakia, Velka Fatra hills, Dubina forest in Gaderska dolina valley, 5 km NE of Blatnica village, N48°57.030' E018°57.271', 542 m a.s.l., *Picea abies* forest on steep slope, with *Quercus* trees on top of hill, 5.10.2001, J. Nuytinck 2001-053 (GENT) – ibid., 5.10.2001, J. Nuytinck 2001-054 (GENT) – ibid., 5.10.2001, J. Nuytinck 2001-055 (GENT) – Turcianska kotlina valley, Diviacky haj forest, 3 km W from Turcianska Teplice town, N48°51.861' E018°50.223', 494 m a.s.l., Mixed forest with mainly *Picea abies*, 5.10.2001, J. Nuytinck 2001-056 (GENT) – ibid., 5.10.2001, J. Nuytinck 2001-057 (GENT) – Nizke Tatry mountains, 2.5 km N to Maluzina village, Michalovo valley, N49°00.156' E019°45.481', 674 m a.s.l., swampy place along river with *Picea abies* and some deciduous trees, 6.10.2001, J. Nuytinck 2001-072 (GENT) – ibid., 6.10.2001, J. Nuytinck 2001-073(1) (GENT) – Nizke Tatry mountains, 2 km S of Dubove village, 750 m a.s.l., open forest with *Pinus sylvestris* and *Picea abies*, 8.10.2001, J. Nuytinck 2001-075 (GENT) – Nizke Tatry mountains, 2.5 km S of Dubove village, N49°00.485' E019°31.038', 761 m a.s.l., open *Picea abies* forest, 8.10.2001, J. Nuytinck 2001-076 (GENT) – ibid., N49°00.423' E019°30.999', 765 m a.s.l., open *Picea abies* forest, 8.10.2001, J. Nuytinck 2001-077 (GENT) – ibid., N49°00.400' E019°30.986', 767 m a.s.l., open *Picea abies* forest, 8.10.2001, J. Nuytinck 2001-078 (GENT) – ibid., N49°00.393' E019°30.977', 769 m a.s.l., open *Picea abies* forest, 8.10.2001, J. Nuytinck 2001-079 (GENT) – Nizke Tatry mountains, Demanovska dolina valley, 7 km S of Demanova village, N49°00.019' E019°34.995', 772 m a.s.l., *Picea abies* forest along river on calcareous soil, 9.10.2001, J. Nuytinck 2001-080 (GENT) – ibid., 9.10.2001, J. Nuytinck 2001-081 (GENT) – Sweden, Jämtland, 5 km SE of Brunflo, in rich mixed forest, 4.09.1997, J. Vesterholt 97-217 (GENT) – Jämtland, Bräcke, Giman, under *Picea* on dry and rich soil, 5.09.1997, J. Heilmann-Clausen 97-176 (GENT) – West of Ange in *Picea*-forest, 7.09.1997, A. Verbeken 97-561 (GENT) – Borgsjö, youth hostel, N62°32.466' E015°54.060', ~240 m a.s.l., grassy place, under *Picea abies* on calcareous ground, 25.08.2001, J. Nuytinck 2001-008 (GENT) – Borgsjö, nearby E14, direction Ange, upside hill, N62°33.252' E015°52.312', ~180 m a.s.l., rich calcareous soil, under *Picea abies*, between grass and fallen needles, 26.08.2001, J. Nuytinck 2001-010 (GENT) – Borgsjö, nearby E14, direction Ange, nearby lake, N62°33.128' E015°52.277', ~150 m a.s.l., Close to swamp, under *Picea abies*, rich soil, 26.08.2001, J. Nuytinck 2001-011 (GENT) – Graveyard of Brunflo, N63°05.469' E014°49.141', ~430 m a.s.l., in lawn under *Pinus strobus*, 27.08.2001, J. Nuytinck 2001-014 (GENT) – Nearby Östersund, N63°10.265' E014°31.784', ~500 m a.s.l., under *Picea abies* on rich soil, 27.08.2001, J. Nuytinck 2001-015 (GENT) – Ange, opposite Shell station, N62°31.481' E015°40.085', ~150 m a.s.l., in grass under *Pinus cembra*, 28.08.2001, J. Nuytinck 2001-017 (GENT) – Nearby Borgsjö, ~5 km along small side road of E14, N62°32.230' E016°06.121', ~150 m a.s.l., Along river, in *Picea abies* and *Betula* forest, 28.08.2001, J. Nuytinck 2001-019 (GENT) – Ange, opposite Shell station, N62°31.481' E015°40.085', ~150 m a.s.l., in grass under *Pinus cembra*, 29.08.2001, J. Nuytinck 2001-022 (GENT) – Bergasen, mountain close to Borgsjö, ~N62°32.673' E015°54.748', ~230 m a.s.l., mixed forest with *Pinus*, *Picea*, *Betula* and *Salix*, rich, calcareous soil, 30.08.2001, J. Nuytinck 2001-026 (GENT) – ibid., ~180 m a.s.l., mixed forest with *Pinus*, *Picea*, *Betula* and *Salix*, rich, calcareous soil, nearby river, 30.08.2001, J. Nuytinck 2001-029 (GENT) – Switzerland, Adelboden, 1km NW of the village, 1800 m a.s.l., under 80 yr old *Picea abies*, on slope exposed to E, soil with slate and limestone, with loamy upper layer, 28.07.2001, leg. P.J. Keizer, J. Nuytinck 2001-038 (GENT).

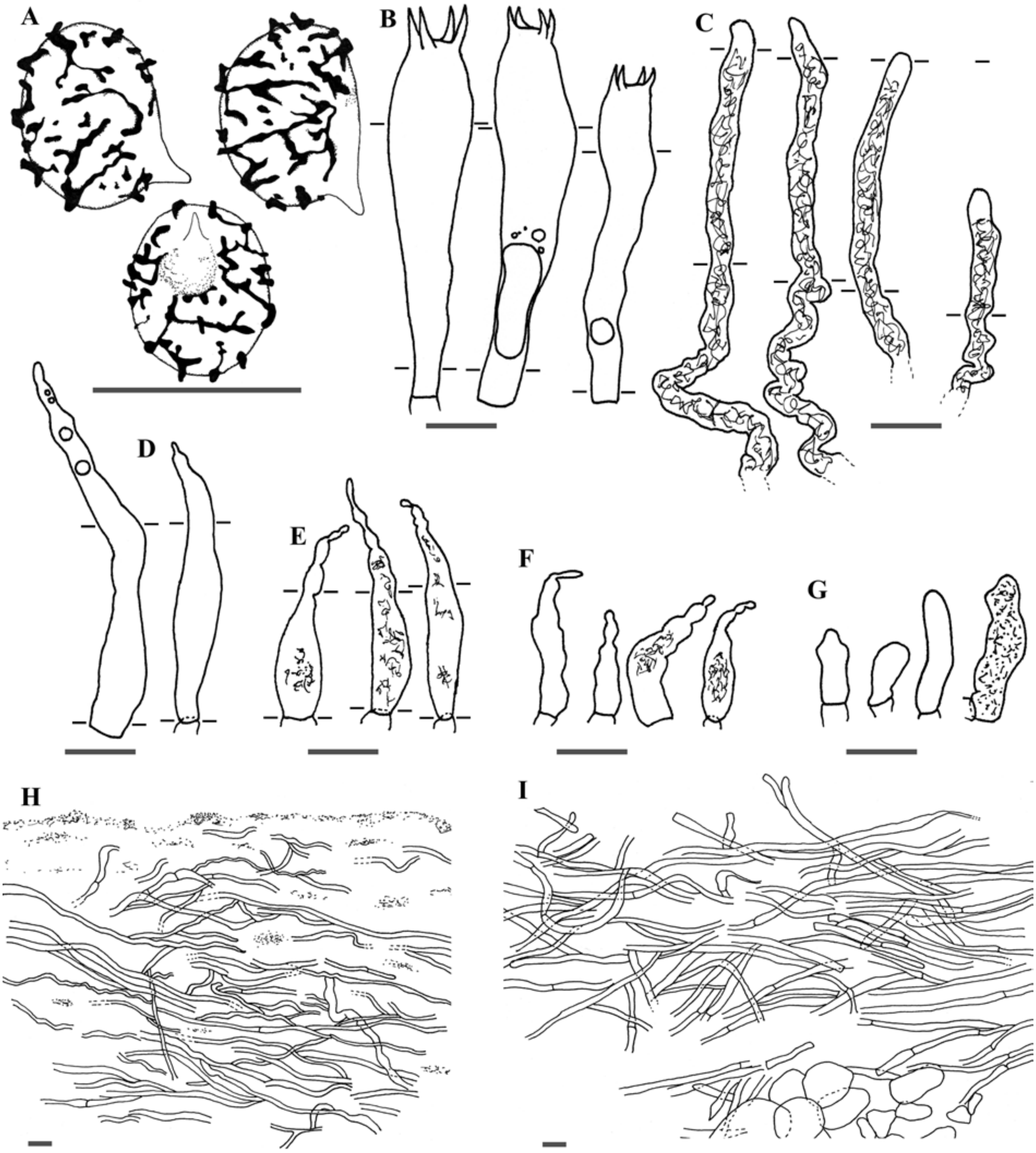

Fig. 6: *Lactarius deterrimus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** macrocystidia close to the lamella edge; **F.** cheilomacrocystidia; **G.** cheiloleptocystidia; **H.** section through the pileipellis; **I.** section through the stipitipellis. From J. Nuytinck 2000-010, 2001-022 and 2001-073. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: *Lactarius deterrimus* is associated with *Picea*, it has a characteristic bright orange stipe without scrobicules, with a white zone at the top. The pileus is orange with green and not to slightly zonate; the bright orange latex generally turns red in 15–20 min. The epitheton 'deterrimus' means 'the worst' and this name was chosen by GRÖGER (1968) because of the bitter taste. In some regions however this species (or actually a mix of any *Lactarius* sect. *Deliciosi* species) is very popular for consumption.

Although this common taxon was only given a species rank in 1968, it was recognised as a distinct variety from *L. deliciosus* before (VASSILKOV, 1948 as *L. deliciosus* var. *piceae*; SMOTLACHA, 1947 and ROMAGNESI, 1958 as *L. deliciosus var. piceus*, see also under *L. deliciosus*). *Lactarius deterrimus* is more common than *L. deliciosus* in large parts of central and eastern Europe (GRÖGER, 1968; SCHAEFER, 1970). This explains why *L. deterrimus* is most frequently depicted as *L. deliciosus* by eastern European authors from the first half of the 20th century (e.g. BRITZELMAYR, 1885; GRAMBERG, 1921; LANGE, 1940; MICHAEL, 1895–1905 & 1927; PILÁT, 1951; PILÁT & UŠÁK, 1951; SMOTLACHA, 1947).

NEUHOFF (1956) created confusion concerning the identities of *L. deterrimus* and *L. semisanguifluus* by describing *L. deterrimus* as *L. semisanguifluus*.

HESLER & SMITH (1979) consider the species as one of their numerous varieties of *L. deliciosus*. We are not convinced that this American taxon is conspecific with the European one, as molecular analyses show that so far not one American species of *Lactarius* sect. *Deliciosi* is conspecific with the European taxa (NUYTINCK *et al.*, in preparation).

Three *L. deterrimus* collections (J. Nuytinck 2001-014, J. Nuytinck 2001-017 and J. Nuytinck 2001-022) from Sweden, were growing underneath introduced *Pinus* trees (*Pinus strobus* L. from eastern North America and *Pinus cembra* L. from the Alps and Carpathians). A switch of host tree may have occurred, but it is also possible that *Picea* trees were nevertheless present in the close environment.

4.4. ***Lactarius fennoscandicus* Verbeken & Vesterh.**, Cryptog. Mycol. 19: 87 (1998)

Figs. 7, 8, 9

Misapplication: = ***L. deterrimus*** p.p. sensu M. Korhonen, Suomen rouskut (1984), photos p. 108 and 109 (Sipoo 2758 and Vantaa 4882)

Holotype: Verbeken 97-530 (GENT!), Sweden: Siljanfors.

DESCRIPTION: *Pileus* 3–8 cm diam., convex with a slightly depressed centre and a decurved margin, later becoming more depressed; surface smooth and sticky to slightly viscid, distinctly zonate with very narrow zones at the extreme margin and fewer, broader zones near the inside, with a generally dark appearance, background (pale) brownish orange (5/6C3/4) to greyish pink (7D3 to 8D2) with darker, brownish zones (7D6, 7E5 to 8D4), centrally reddish brown (6D7) with darker brown spots (6E4), locally green to bluish green. *Lamellae* slightly decurrent, medium crowded and rather narrow and thin, sometimes branching; colour (pale) orange (5B5/6 to 6B6), slowly turning green where bruised; edge entire and concolorous. *Stipe* 4–8 (11) × 1–2.5 cm, cylindrical or slightly broader near the base; surface dry, slightly pruinose, dull-coloured, pinkish buff to brownish orange (6B4, 6D7), often with a narrow white zone at the extreme apex, without scrobicules, turning green where bruised. *Context* rather to moderately firm, becoming hollow in the stipe, whitish in the central part, bluish grey (21B2) directly underneath the pileipellis, becoming green-grey (28B3) after 1–2 h, bright orange (6A8) above the lamellae and near the stipitipellis, orange parts becoming red in about 30 min. and green after several hours; smell indistinct or very slightly fruity; taste mild or becoming a little acrid and bitter. *Latex* rather scarce, orange (7C8), drying red and then green to greenish grey on the context. *Spore deposit* pale yellow-orange (4A3).

$FeSO_4$ no reaction, *KOH* no reaction, *phenol* no reaction.

Spores 6.8–*7.5*–*9.4*–10.2 × 5.6–*6.1*–*6.9*–7.9 µm, broadly ellipsoid to ellipsoid, rarely subglobose (Q = 1.10–*1.22*–*1.41*–1.50); ornamentation up to 0.5 µm high, of narrow ridges and often abundant rather thick isolated warts, forming an incomplete reticulum; plage very slightly amyloid distally or inamyloid. *Basidia* 40–60 × 9–11 µm, ± cylindrical to subclavate, 4-spored, with a granular content; sterigmata 4–5 µm long. *Pleuromacrocystidia* rather scarce but more abundant near the lamella edge, 35–55 × 4–8 µm, emergent, subfusiform, with a narrowing or moniliform apex, with a

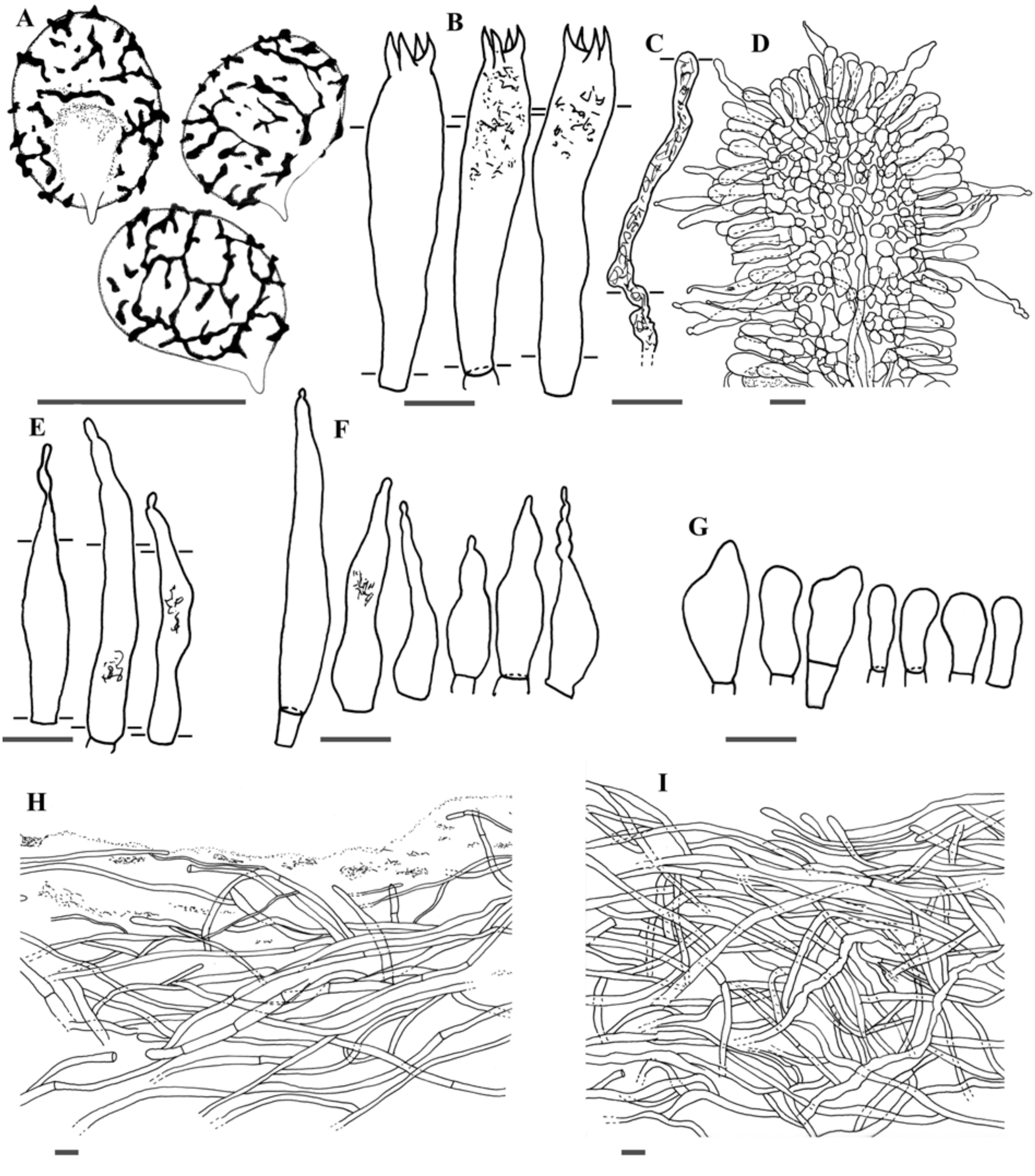

Fig. 7: *Lactarius fennoscandicus*: **A.** spores; **B.** basidia; **C.** pseudocystidium; **D.** lamella edge; **E.** pleuromacrocystidia close to the lamella edge; **F.** cheilomacrocystidia; **G.** cheiloleptocystidia; **H.** section through the pileipellis; **I.** section through the stipitipellis. From J. Nuytinck 2001-018, 2001-030, 2001-039 and R. Walleyn 1577BIS. Scale bars = 10 µm, small bars indicate the height of the hymenium.

needle-like or granular content, thin-walled. *Pseudocystidia* very abundant, 3–5 µm broad, not to slightly emergent, rarely clearly emergent, cylindrical but rather tortuous near the subhymenium, with an indistinct ochre-yellow content, almost invisible near the apex. *Lamella edge* sterile with mostly very few to rarely abundant cheilomacrocystidia; cheiloleptocystidia 8–20 × 3.5–9.5 µm, subclavate or

± cylindrical to irregular, hyaline, thin-walled; cheilomacrocystidia 15–35 × 4–7.5 µm, subfusiform to fusiform, with a narrowing to moniliform apex, thin-walled. *Subhymenium* composed of rather small, globose cells arranged in short rows to irregular. *Hymenophoral trama* irregular; lactifers abundant and conspicuous with an ochre-yellow content. *Pileipellis* an ixocutis, 60–300 µm thick; hyphae 1–5 µm broad, strongly shrivelled and gelatinised in the upper layer, more regularly shaped away from the surface. *Stipitipellis* a cutis, 70–150 µm thick; hyphae 3–5 µm broad, not shrivelled or gelatinised, only rarely branching; lactifers abundant and close to the surface. *Clamp-connections* absent.

HABITAT: Associated with *Picea* and found in late summer and early fall.

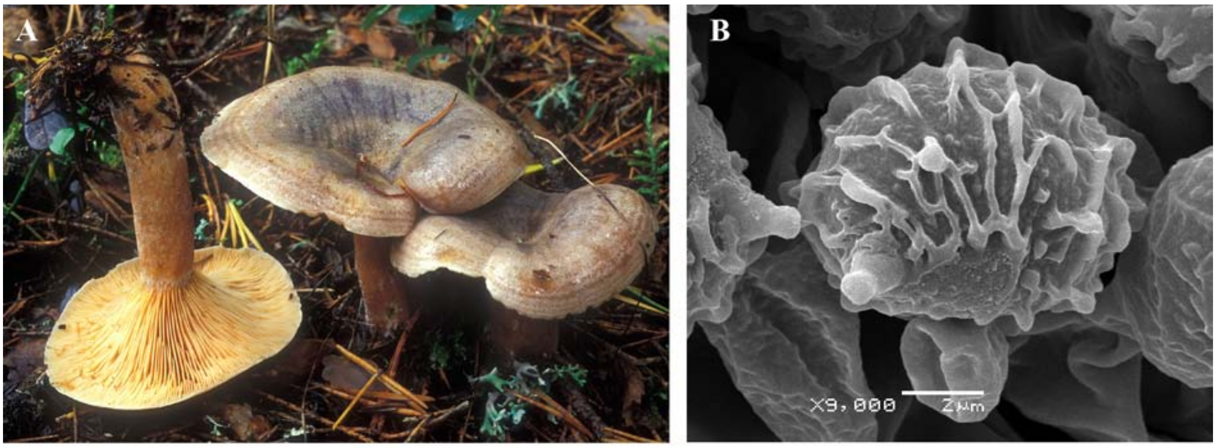

Fig. 8: A. *L. fennoscandicus* (photo R. Walleyn); **B.** SEM image of a spore of *L. fennoscandicus* J. Nuytinck 2001-030.

DISTRIBUTION: Described from boreal Sweden and Finland where it seems, at least locally, to be almost as common as *L. deterrimus*. More recently reported from higher altitudes in the Alps (FLORIANI, 1999).

COLLECTIONS EXAMINED: Finland, Enonkoski, ± 5 km S of town, under *Picea abies*, 1.09.2001, leg. A. Gminder, J. Nuytinck 2001-039 (GENT) – Sweden, Jämtland: Fors par., Reva, at Indalsälven, under *Picea*, 23.08.1995, leg. J.H. Petersen, J. Vesterholt etc., J. Vesterholt 95-330 (GENT) – Angermanland, skulenskogens Nationalpark, *Picea*, 28.08.1997, H. Huijser (GENT) – Liljansfors, in *Picea* forest, acidic soil, with *Sphagnum*, *Vaccinium* etc., 31.08.1997, leg. Christensen M., A. Verbeken 97-530 TYPUS (GENT) – Liljansfors, under *Picea* in mixed rich forest, 31.08.1997, leg. Christensen M., J. Heilmann-Clausen 97-119 (GENT) – Jämtland, Bräcke, Giman, in moist forest with mosses under *Pinus* and *Picea*, 5.09.1997, J. Heilmann-Clausen 97-183 (GENT) – W of Ange, 7.09.1997, A. Verbeken 97-569 (GENT) – Medelpad, Ange, along Geteraen river, *Picea*-dominated coniferous forest with *Betula* and *Populus tremula* on rich soil, 21.08.1999, leg. G. Redeuilh, R. Walleyn 1577BIS (GENT) – Nearby road between Hunge and Sidsjö, N62.73° E15.15°, ± 300 m a.s.l., under *Picea abies*, 27.08.2001, J. Nuytinck 2001-012 (GENT) – Prov. Medelpad, under *Picea abies*, on rich ground, 28.08.2001, J. Nuytinck 2001-018 (GENT) – Bergasen, mountain close to Borgsjö, N62°32.825' E015°54.733', ± 230 m a.s.l., mixed forest with *Pinus*, *Picea*, *Betula* and *Salix*; rich, calcareous soil, 30.08.2001, J. Nuytinck 2001-027 (GENT) – ibid., ± N62°32.825' E015°54.733', ± 230 m a.s.l., mixed forest with *Pinus*, *Picea*, *Betula* and *Salix*; rich, calcareous soil, 30.08.2001, J. Nuytinck 2001-028 (GENT) – Garden near road to Ange (E14), *Picea abies* forest behind garden, 31.08.2001, J. Nuytinck 2001-030 (GENT) – Side road of E14 to Granboda, N62°34.122' E015°44.433', ± 208 m a.s.l., along river in *Picea abies*, *Pinus sylvestris* and *Betula* forest, 31.08.2001, J. Nuytinck 2001-031 (GENT).

DISCUSSION: *Lactarius fennoscandicus* was described from Scandinavia as a close relative of *L. deterrimus* (VERBEKEN & VESTERHOLT, 1998). It also forms ectomycorrhiza with *Picea. Lactarius fennoscandicus* has a strongly zonate cap (containing lilac greyish hues mixed with brownish orange, and locally greenish) and bright orange milk, which is slowly reddening.

The spores are described as remarkably small: 7.5–8.0 x 6.0–6.5 µm in *L. fennoscandicus* versus 9.5 x 7.5 µm in *L. deterrimus* (VERBEKEN & VESTERHOLT, 1998). Another indicated microscopical difference between these species is that cheilomacrocystidia almost completely lack in *L. fennoscandicus* while they are moderately abundant in *L. deterrimus* (VERBEKEN & VESTERHOLT, 1998). These microscopic differences are not completely confirmed here. From our measurements the spore size does not seem to differ significantly (Fig. 9). There is a general tendency for the cheilomacrocystidia to be more abundant in *L. deterrimus* than in *L. fennoscandicus*, but exceptions occur. 10 to 20 % of the examined *L. fennoscandicus* collections have moderately to very abundant cheilomacrocystidia and 10 % of the *L. deterrimus* collections have very scarce cheilomacrocystidia.

The ITS region was sequenced for a large number of *L. deterrimus* and *L. fennoscandicus* accessions for this project (NUYTINCK & VERBEKEN, 2005). We see a division into three clades in the inferred phylogenetic trees, two clades with mainly *L. deterrimus* and one with mainly *L. fennoscandicus* accessions, including the type specimen. The smaller *L. deterrimus* clade clusters with *L. fennoscandicus* in the majority of the MP trees. Moreover, there are several problematic collections: e.g. the *L. fennoscandicus*-like collection from the French Alps falls in the *L. deterrimus* clade, conversely a typical *L. deterrimus* collection from Belgium (J. Nuytinck 2000-002) has one cloned ITS sequence in the large *L. deterrimus* clade while the other is placed in *L. fennocandicus*. However, based on more detailed and elaborate molecular analyses (AFLP and a total evidence approach including ITS and *gpd* sequences, and morphological characters, for more details see NUYTINCK & VERBEKEN, 2005), we conclude that both taxa should be treated as separate species for the moment.

As noted by VERBEKEN & VESTERHOLT (1998), an enormous variety can be observed in the appearance of the species of *Lactarius* sect. *Deliciosi* growing with *Picea* in Scandinavia. Apparently, transitional forms between the typical *L. deterrimus* and *L. fennoscandicus* are often encountered. A hypothesis is that *L. deterrimus* and *L. fennoscandicus* are very young, recently evolved species or that the speciation event is actually still going on. A switch of host (from *Pinus* to *Picea* in this case) could have triggered the speciation process, similarly to what happened in *Leccinum* (DEN BAKKER *et al.*, 2004).

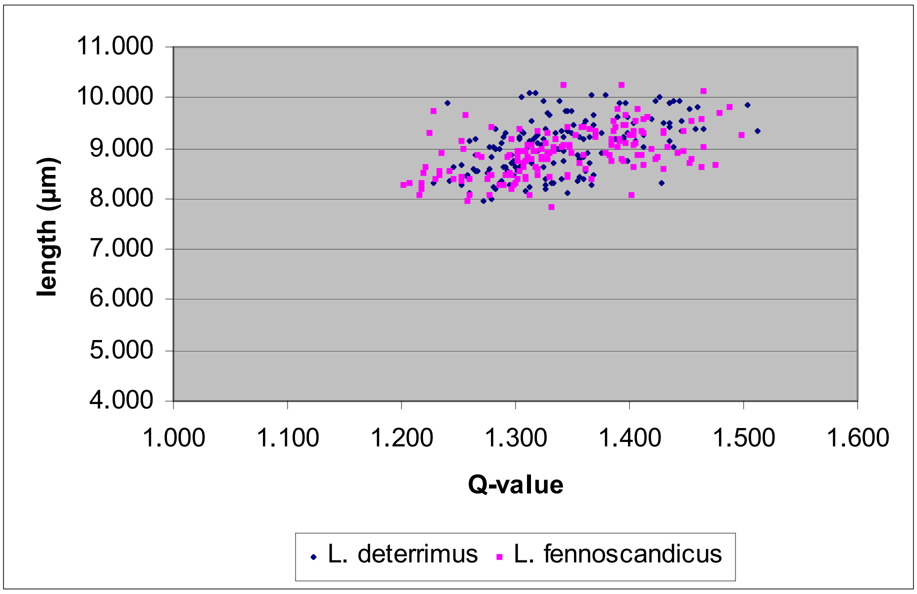

Fig. 9: Length values (in µm) and Q-values plotted against each other for 160 *L. deterrimus* and 160 *L. fennoscandicus* spores. There is a total overlap between both species.

4.5. *Lactarius porninsis* Rolland, Bull. Trimestriel Soc. Mycol. France 5: 168 (1889)

Figs. 10, 11

Note:	as ***L. porninae*** Sacc. in Sylloge Fungorum 9: 57 (1891) and CABI Index of Fungi, named after Mrs. Pornin.
Misapplications:	***L. tithymalinus*** sensu QUÉLET (1893); sensu BATAILLE (1908) ***L. aurantiacus*** sensu QUÉLET (1880); sensu BRESADOLA (1929)

Lectotype: Bull. Trimestriel Soc. Mycol. France 5, pl. XIVbis: 2!, depicted from Switzerland, Chamonix (designated in HEILMANN-CLAUSEN *et al.*, 1998).

DESCRIPTION: *Pileus* 3.5–12 cm diam., convex with a slightly depressed centre when young, becoming applanate with a depressed centre and a slightly decurved margin, regular but sometimes becoming a bit wavy when older; surface greasy to viscid, then dry, with concentric zones, especially near the margin; colour ochraceous orange, bright orange to brown orange (5A6, 6B6, 6B8, 6C8) often darkest in the centre, paler parts ochraceous salmon to pale cream (5A2–6A6). *Lamellae* slightly decurrent, thin, crowded, sometimes forking near the stipe; colour pale orange-buff (5A5); edge entire, concolorous. *Stipe* 2.5–8 × 0.7–2 cm, ± cylindrical but often slightly broader in the middle, sometimes bent; surface smooth; colour creamy buff, buff-yellow (4A4/5) to (light) ochraceous buff (5A4/5), often with a whitish zone at the top, sometimes with a few, indistinct scobicules. *Context* rather firm, hollow in the stipe; colour whitish, becoming buff with age in the stipe; taste mild at first, becoming bitter; smell fruity, like mandarins. *Latex* not very abundant, white, unchanging; taste mild. *Spore deposit* pale ochraceous buff (4A2).

Spores 6.3–*7.7*–*8.5*–9.6 × 5.2–*6.0*–*6.6*–7.3 µm, broadly ellipsoid to ellipsoid (Q = 1.15–*1.25*–*1.34*–1.45); ornamentation up to 0.5 µm high, of fine ridges, forming an incomplete reticulum with wide meshes, some isolated warts present; plage inamyloid or very slightly amyloid distally. *Basidia* 44–67 × 10.5–12.5 µm, subclavate, 4-spored, often with a fine, granular or needle-shaped content; sterigmata 4–6 µm long. *Pleuromacrocystidia* (very) abundant to dispersed, 41–60 × 6–10 µm, emergent, subfusiform, with a narrowing to moniliform or rarely a forked apex, with a needle-like or granular content, thin-walled. *Pseudocystidia* abundant, 2–5 µm broad, not emergent, cylindrical to tortuous. *Lamella edge* mostly sterile with abundant cheilomacrocystidia; cheiloleptocystidia 15–30 × 5–12 µm, cylindrical to clavate, hyaline, thin-walled; cheilomacrocystidia 20–40 × 5–8 µm, fusiform, with a narrowing apex, thin-walled. *Subhymenium* composed of almost isodiametric, rather small cells, arranged in short rows. *Hymenophoral trama* irregular; lactifers abundant and conspicuous with an opaque to transparent content. *Pileipellis* an ixocutis, 100–200 µm thick, of interwoven, rather broad hyphae, 5–7 µm diam., rarely shrivelled or gelatinised, sometimes branching; extracellular, fine, granular pigment present on some broad hyphae. *Stipitipellis* a cutis, up to 100 µm thick, of interwoven hyphae, 2.5–5.5 µm diam., not gelatinised nor shrivelled. *Clamp-connections* absent.

Fig. 10: A. *L. porninsis* (photo R. Walleyn); **B.** SEM image of a spore of *L. porninsis* J. Nuytinck 2002-025.

HABITAT: Ectomycorrhizal with *Larix* (TREU, 1990) and found from July to October.

DISTRIBUTION: Common in mountain-forests in central Europe; elsewhere rare and presumably introduced with its mycorrhizal partner, recorded from southern Europe to Denmark (HEILMANN-CLAUSEN *et al.*, 1998).

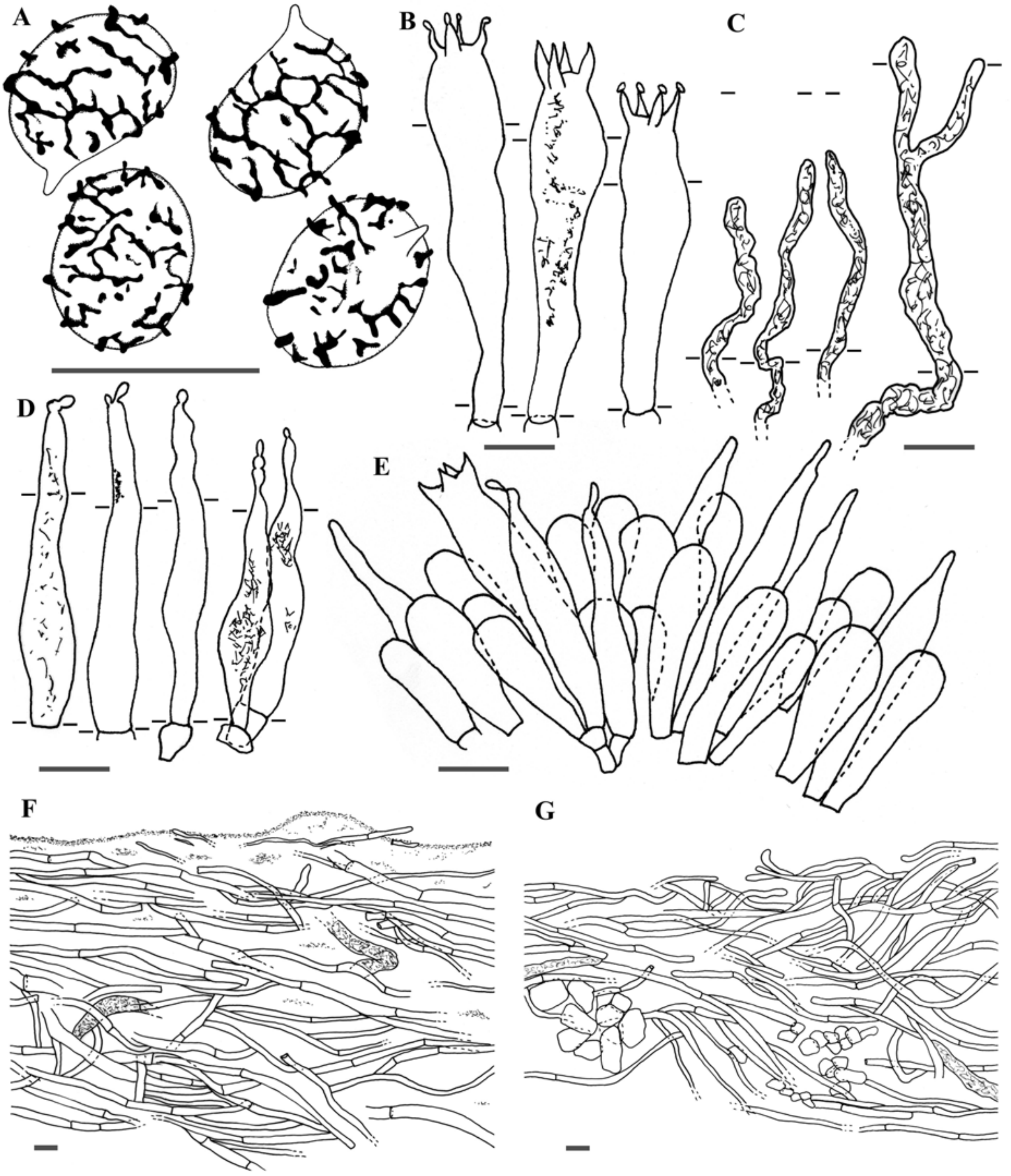

Fig. 11: *Lactarius porninsis*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocysitidia; **E.** part of the lamella edge; **F.** section through the pileipellis; **G.** section through the stipitipellis. From R. Walleyn 1233 and J. Nuytinck 2001-082, 2002-019, 2002-025. Scale bars = 10 µm, small bars indicate the height of the hymenium.

COLLECTIONS EXAMINED: France, Hautes-Alpes, Le Casset , ± 1500 m a.s.l., roadside, grassy place under *Larix*, 6.07.1996, A. De Haan 96-008 (GENT) – Savoie, les Arcs 1800, Bourg St.-Maurice, 16.08.1997, R. Walleyn 1233 (GENT), slides in situ – Savoie, les Arcs 1800, *Larix*-*Picea*, 17.08.1997, R. Walleyn 1256 (GENT) – Forêt d'Ecot, Doubs 25, *Larix*, *Picea* and some deciduous trees, 7.10.1998, leg. M. Pelerin, A. Verbeken 98-081 (GENT) – Italy, N46°01.555' E011°13.035', 773 m a.s.l., in mixed forest, under *Larix decidua*, calcareous soil, 2.10.2002, J. Nuytinck 2002-019 (GENT) – Maso Postel, Trentino, in mixed forest, under *Larix decidua*, calcareous soil, 3.10.2002, leg. R. Flores, J. Nuytinck 2002-023 (GENT) – Maso Postel (Pergine Valsugama), Trentino, N46°02.254' E011°12.761', 824 m a.s.l., under *Larix decidua*, calcareous soil, 4.10.2002, J. Nuytinck 2002-025 (GENT) – Slovakia, Nizke Tatry mountains, Demanovska dolina valley, 7 km S of

Demanova village, Demanovska dolina nature reserve, N49°00.027' E019°35.058', 809 m a.s.l., mixed forest with *Picea abies* and *Larix decidua* on steep slope, 9.10.2001, J. Nuytinck 2001-082 (GENT) – ibid., 9.10.2001, J. Nuytinck 2001-083 (GENT).

DISCUSSION: The combination of an orange and zonate cap, the smell of mandarins, mild-tasting, white latex and a host association with *Larix* makes *L. porninsis* an easily recognizable species. HEILMANN-CLAUSEN *et al.* (1998) and BASSO (1999a) place *L. porninsis* in section *Zonarii* (Quél.) Bon (nom. inval.)*,* subsection *Zonarii* (Quél.) Basso, together with *L. acerrimus* Britzelm.*, L. evosmus* Kühner & Romagn.*, L. illyricus* Piltaver*, L. zonarius* (Bull.) Fr.*, L. zonarioides* Kühner & Romagn. and *L. controversus* Pers.: Fr. All of these species are characterised by a more or less viscid, zonate cap and white, usually unchanging latex. It is surprising to find *L. porninsis* included in the *Deliciosi* clade in the ITS and *gpd*-based phylogenetic trees (NUYTINCK & VERBEKEN, 2005). EBERHARDT (2000) found the same relationships based on LSU and ITS nrDNA sequences. The position of *L. porninsis* in *Lactarius* section *Deliciosi* can be supported though by some chemical, macroscopical and ecological characters. A study of the secondary latex metabolites in *L. porninsis* showed it is very different from the other *Zonarii* (Marco Cleruzio, pers. comm.). The pileus of *L. porninsis* has an orange and zonate aspect that is remarkably similar to that of species in *Lactarius* sect. *Deliciosi* (BLUM, 1976) and the species is outstanding in *Lactarius* subsect. *Zonarii* by its association with a coniferous tree (while this is characteristic for all representatives of *Lactarius* sect. *Deliciosi*). NUYTINCK *et al.* (in preparation) show several examples of how a specific latex colour evolved two or more times independently in the section. A loss of pigmentation could explain the position of *L. porninsis.*

4.6. ***Lactarius quieticolor* Romagn.**, Rev. Mycol. (Paris) 23: 280 (1958)

Figs. 12, 13

Synonyms:
- ≡ ***L. deliciosus*** var. ***quieticolor*** (Romagn.) J. Blum, Lactaires: 203 (1976), nom. inval.
- ≡ ***L. deliciosus*** var. ***quieticolor*** (Romagn.) Krieglst., Beitr. kenntn. Pilze Mitteleuropas 7: 68 (1991), nom. inval.
- = ***L. hemicyaneus*** Romagn., Rev. Mycol. (Paris) 23: 280 (1958)
  - ≡ ***L. deliciosus*** var. ***hemicyaneus*** (Romagn.) Krieglst., Beitr. Kenntn. Pilze Mitteleuropas 7: 61 (1991), nom. inval.
  - ≡ ***L. quieticolor*** var. ***hemicyaneus*** (Romagn.) Basso, Fungi Europaei 7. *Lactarius* Pers.: 275 (1999)
  - = ***L. deliciosus*** var. ***indigoides*** Kavina, nom. inval., unpublished herbariumname (PR), cited in Schaefer Z., Schweiz. Z. Pilzk. 48 (9): 139 (1970)
- = ***L. quieticolor*** f. ***semisanguinascens*** Bon, Rev. Mycol. (Paris) 38: 202 (1975); as 'f. ***sanguinascens***' in Bon (1980)
- = ***L. pinastri*** Romagn., Bull. Trimestriel Soc. Mycol. France 96: 308 (1980)

Misapplication: ***L. sanguineovirescens*** sensu MELIS *et al.* (1999)

Holotype: Romagnesi 13-10-1957 (PC!), France: Seine-et-Oise, forêt de Rambouillet.

DESCRIPTION: *Pileus* 4–10.5 cm diam., convex in young specimens, soon a bit depressed, becoming infundibuliform with age, margin at first incurved, then straight, mostly regular; surface smooth, sticky and wet in young specimens but soon becoming dry, strongly zoned when older, especially near the margin, but sometimes only vaguely zoned; colour very variable from one basidiome to another, mostly greyish orange with some brownish tinges (5B/C5), sometimes even deep brown (5F5/6) near the centre, sometimes with dirty orange spots, more rarely brighter orange and similar to *L. deliciosus*, often with irregular green spots; young basidiocarps (up to 5 cm diam.) sometimes completely deep bluish green (26F4/6). *Lamellae* slightly decurrent to decurrent with a small tooth, moderately to rather distant, medium broad, sometimes forked; colour orange-buff (5A4/5 to 5B5), staining wine red and later green; edge entire, a bit paler. *Stipe* 4–9 × 1.2–2.5 cm, cylindrical, often bent, sometimes tapering downwards; surface smooth, rather dry, sometimes scrobiculate and then with regular rounded spots; colour bright to paler orange when young (6/7A6 to 5A5), becoming dirty, pale orange (5B2/3) in older specimens, mostly with a whitish zone right under the lamellae and often with greenish stains when old. *Context* firm, thick, becoming more brittle and hollow in the stipe, whitish cream-coloured, staining orange (6A7/8) when cut, especially near the pellis, in the stipe especially bright orange near the base, remaining whitish in the most central part, in some specimens with a bright blue zone (24/25DE4) underneath the pileipellis; orange colours turning wine red in 20 min. to maximum 1 h; smell not remarkable, agreeable; taste at first mild and agreeable, then becoming bitter and/or slightly acrid. *Latex* not abundant, bright orange (6A7/8), turning wine red and drying greenish on the context; taste mild. *Spore deposit* pale yellow-orange (4A3).

KOH no reaction, *$FeSO_4$* no reaction, *gaiac* turning blue after 1 min. on context.

Spores 7.9–*8.6–9.8*–10.4 × 6.1–*6.7–7.5*–8.3 µm, broadly ellipsoid to ellipsoid (Q = 1.15–*1.26–1.36*–1.44); ornamentation up to 0.8 µm high, of remarkably thick and broad ridges, forming a coarse, almost complete reticulum, some isolated warts present; plage amyloid distally. *Basidia* 48–60 × 8–11 µm, ± cylindrical to subclavate, 4-spored, often containing oil-droplets; sterigmata 2.5–5.5 µm long. *Pleuromacrocystidia* very scarce to rather abundant, 34–60 × 4.5–8 µm, (slightly) emergent, subfusiform, with an acute to (long) moniliform apex, often with a needle-shaped content, thin-walled. *Pseudocystidia* abundant but often hardly visible, 2.5 to 6 µm broad, mostly not emergent, cylindrical to tortuous (especially the lower part). *Lamella edge* sterile with scarce cheilomacrocystidia; cheiloleptocystidia 13–25 × 3–8 µm, cylindrical, subclavate to clavate, hyaline or with a fine, granular content, thin-walled; cheilomacrocystidia 16–33 × 3.5–7 µm, subfusiform to fusiform, with a narrowing to moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* composed of globose or slightly elongated, small cells. *Hymenophoral trama* very irregular; with abundant lactifers. *Pileipellis* an ixocutis, 50–200 (300) µm thick, with a rather thin slime-layer, composed of thin-walled, branching hyphae, 1–5 µm diam.; gelatinised and very narrow hyphae abundant, most of them with slightly refringent walls. *Stipitipellis* a cutis, ± 100 µm thick, composed of thin-walled, branching, interwoven, regularly shaped hyphae, 3–6 µm diam., no gelatinised or shrivelled hyphae. *Clamp-connections* absent.

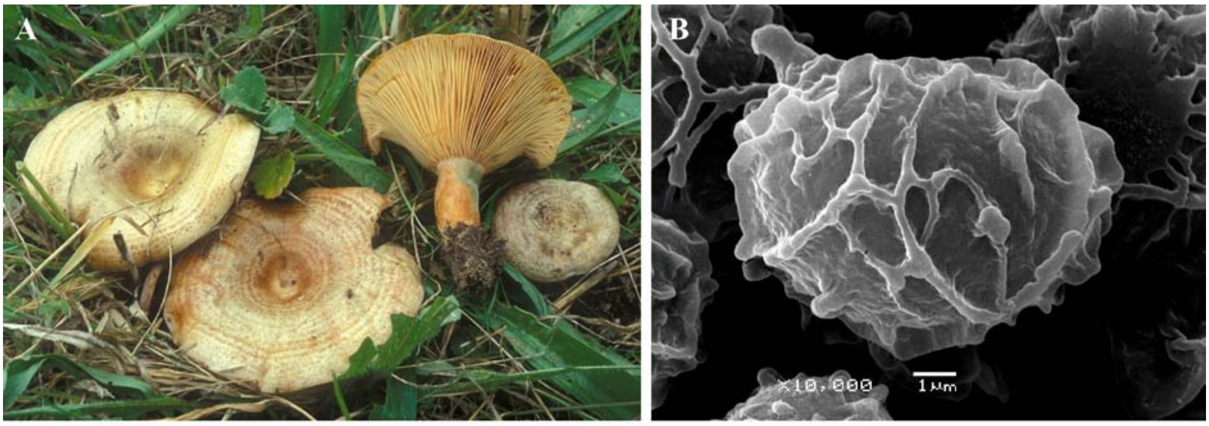

Fig. 12: A. *L. quieticolor* (photo R. Walleyn); **B.** SEM image of a spore of *L. quieticolor* J. Nuytinck 2002-032.

HABITAT: Associated with *Pinus*, found from August to October on acid, often sandy, sometimes wet soils.

DISTRIBUTION: Locally fairly common, distributed throughout the area where its mycorrhizal host is present.

COLLECTIONS EXAMINED: Austria, Nordtirol, Vomperberg, 900 m a.s.l., 23.10.1993, Plenk 166/93 (GENT), Duplum ex KR 166/93, as *Lactarius deliciosus* var. *hemicyaneus* – Belgium, Wingene, Gulke putten, IFBL: D2.13.24, in lawn, under *Pinus sylvestris*, on acidic soil, 13.10.1987, B. Buyck 2677 (GENT) – Herselt, Langendonken, IFBL: D5.26.43, *Pinus sylvestris* on nutrient-poor soil, 21.10.1995, R. Walleyn 0425 (GENT) – ibid., 21.10.1995, R. Walleyn 0426 (GENT) – ibid., *Pinus*, acidic soil, humid, 13.10.1996, R. Walleyn 595 (GENT) – Wingene, Gulke putten, IFBL: D2.13.24, grassland, under *Pinus*, 4.10.1998, R. Walleyn 1503 (GENT) – ibid., 4.10.1998, R. Walleyn 1504 (GENT) – ibid., 20.10.2000, J. Nuytinck 2000-009 (GENT) – Ursel, Drongengoed, under *Pinus*, 30.10.2000, J. Nuytinck 2000-011 (GENT) – ibid., in mixed *Pinus* and *Picea* forest, on acidic soil, 28.09.2001, leg. O. Van de Kerckhove, J. Nuytinck 2001-042 (GENT) – Wingene, Gulke Putten, Under *Pinus sylvestis*, on acidic soil, 9.10.2002, leg. A. Verbeken, J. Nuytinck 2002-032 (GENT) – Wachtebeke-Moerbeke, Heidebos, under *Pinus*, in grassy place, 11.11.2002, J. Nuytinck 2002-048 (GENT) – Patersmote Marke, IFBL: E2.32.13, *Pinus*, 10.11.2003, leg. C. Hanssens, R. Walleyn 3324 (GENT) – Czech Republic, South Bohemia, Malonty, Hodonický potok, 7353a (CZ grid), ~ 630 m a.s.l., under young *Pinus* on sandy acidic soil, 25.09.2003, R. Walleyn & A. Verbeken 3193 (GENT) – Denmark, WJ: Marbaek Plantage N of Esbjerg, UTM: MG5758, under *Pinus*, 2.10.1994, J. Vesterholt 94-766 (GENT) – Marbaek, West Coast, 1.10.1996, A. Verbeken 96-1024c (GENT) – France, Lande de Lessay, forêt de PIROU (Manche), in *Pinus pinaster* forest on acidic soil, 30.08.1971, H. Romagnesi 71-214 (PC), *Lactarius pinastri* TYPE – Forêt Domaniale de Fontainebau, south of Gave de Thomery, under *Pinus sylvestris*, mixed with some *Carpinus* and *Betula*, on sandy, acid soil, almost no herbs, 5.10.2001, leg. P.J. Keizer, J. Nuytinck 2001-130 (GENT) – Germany, Rheinland-Pfalz, Ludwigswinkel, NSG Rösselsweiher, 23.09.1979, A. Bollmann 563/91 (GENT), Duplum ex KR 563/91, det. H. Schwöbel as *Lactarius deliciosus* var. *hemicyaneus* – Italy, Mt. Cresia, commune di Sinnae-CA, 620 m a.s.l., *Pinus radiata*, 18.10.1997, Contu M., Mua A. & Melis M., LSV 181097, as *Lactarius sanguineovirescens* – Netherlands, Drenthe, 3.10.2001, leg. S. Van der Linde, J. Nuytinck 2001-127 (GENT) – Slovakia, Bratislava, under *Pinus sylvestris*, 8.10.2001, J. Nuytinck 2001-084 (GENT) – Sweden, road to Mora, Rather grassy place under *Pinus sylvestris*; poor, acidic soil, 24.08.2001, J. Nuytinck 2001-004a (GENT) – ibid., 24.08.2001, J. Nuytinck 2001-004b (GENT).

DISCUSSION: *Lactarius quieticolor* has a highly variable pileus colour: from greyish and brownish orange to brick, often with green tones. The latex typically changes from orange to wine red in about 20 min. This species prefers acid, often sandy soils and grows with *Pinus*. Microscopically, the species is (easily) recognisable because of the coarsely ornamented spores. The abundance of macrocystida is less reliable because highly variable.

Confusion with *L. deliciosus* may arise when pale orange forms of *L. quieticolor* are found. In these cases, examining the spore ornamentation is needed for exact identification.

BON (1975) described *L. quieticolor* f. *semisanguinascens*, a form with orange latex changing to vinaceous red in 15 min. Although *L. quieticolor* was originally described having unchanging orange latex (ROMAGNESI, 1958), personal observations show that most *L. quieticolor* basidiocarps have latex changing colour rather quickly (see also REIL, 1992), some have slowly changing latex (after about 1 hour), but they mostly end up with wine-red latex.

L. quieticolor shows a remarkable variability in pileus colour. This can even be observed within one and the same mycelium. For this reason, we are not in favor of subdividing *L. quieticolor* in varieties and forms.

VERBEKEN & VESTERHOLT (1998) synonymize *L. hemicyaneus* with *L. quieticolor*. Both taxa have identical microscopical features and the only macroscopical difference, the bright blue zone underneath the pileipellis in *L. hemicyaneus*, is shown to be very variable within one collection of basidiocarps, occurring more often in young specimens. This is also reported by KRIEGLSTEINER (1991a) who observed *L. hemicyaneus* basidiocarps of the same mycelium for several successive years and saw the blue zone becoming weaker as the mycelium aged, and finally disappearing. We included specimens with a very clear blue to bluish green zone in our phylogenetic analyses. They do not form a separate clade in our trees (NUYTINCK & VERBEKEN, 2005). It is commonly observed that specimens with a very dark coloured pileipellis have a clear bluish green zone underneath the pileipellis.

The type specimen of *L. pinastri* was carefully checked, no microscopical differences were found with *L. quieticolor*. The spores are ornamented with heavy ridges, forming an almost complete, dense reticulum with warts up to 0.8–1 µm high. Macrocystidia are scarce to very scarce, on the edge as well as the face of the lamellae. It is synonymised with *L. quieticolor* (see also HEILMANN-CLAUSEN *et al.*, 1998).

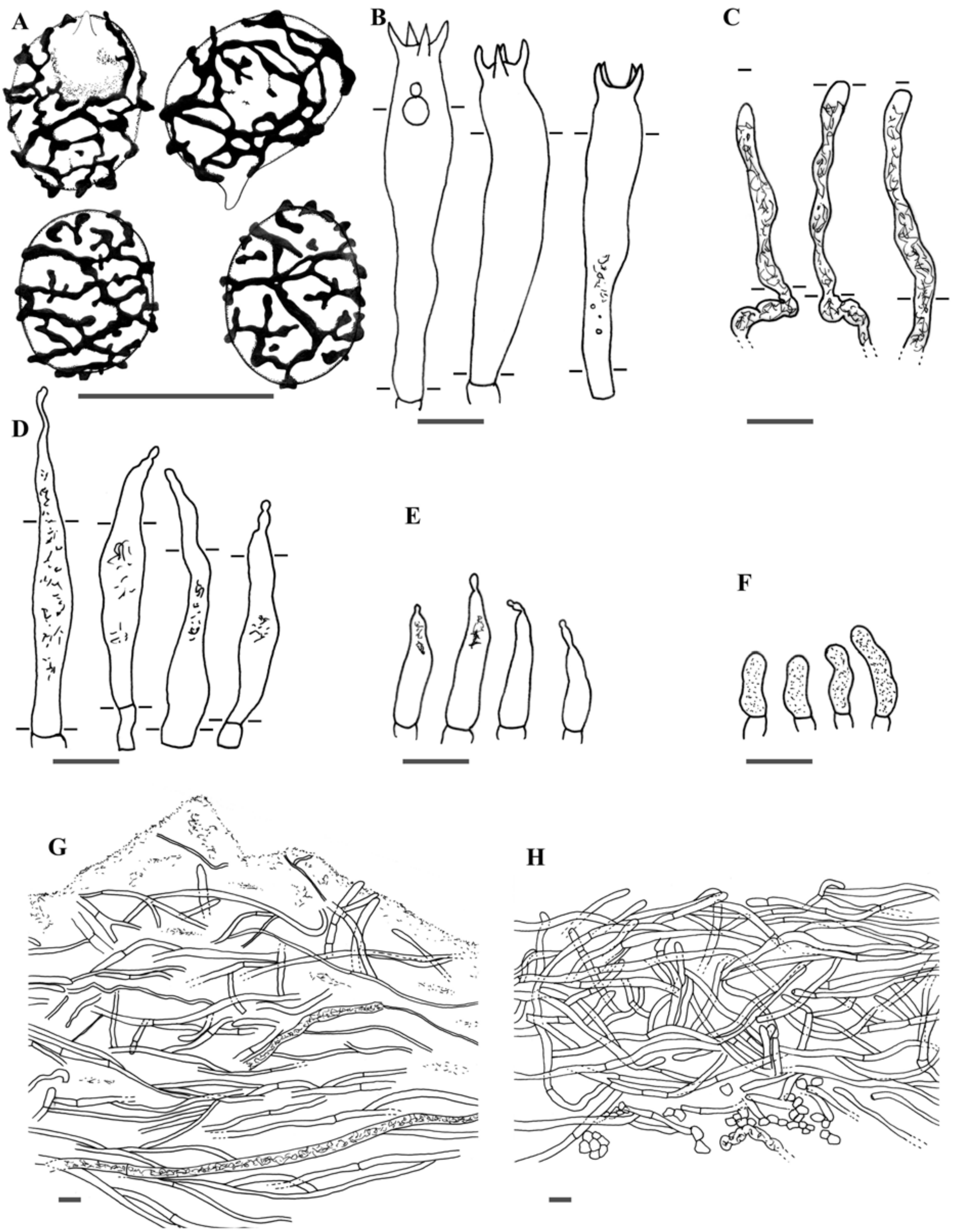

Fig. 13: *Lactarius quieticolor*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From J. Nuytinck 2000-009, 2000-011 and 2001-004b. Scale bars = 10 µm, small bars indicate the height of the hymenium.

4.7. ***Lactarius salmonicolor* R. Heim & Leclair**, Rev. Mycol. (Paris) 18: 221 (1953)

Figs. 14, 15

Replaced synonym: ≡ ***L. salmoneus*** R. Heim & Leclair, non PECK (1898), Rev. Mycol. (Paris) 15: 79 (1950)

Synonyms: ≡ ***L. subsalmoneus*** Pouzar, Česká Mykol. 8: 44 (1954)
≡ ***L. deliciosus*** var. ***salmoneus*** (R. Heim & Leclair) Kühner & Romagn., Flore Analytique des Champignons Supérieurs: 474 (1953)
= ***L. salmonicolor*** f. ***brigantiacus*** L. Remy, Bull. Trimestriel Soc. Mycol. France 80: 487 (1965), nom. inval., nom. nud.
= ***L. deliciosus*** var. ***abietinus*** Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 219 (1947), nom. inval., nom. nud.
?= ***L. flammeolus*** (Pollini: Fr.) Fr., Epicr. Syst. Mycol.: 341 (1838)
    ≡ ***Agaricus flammeolus*** Pollini, Giorn. Fis. 9: 34 (1816)
    ≡ ***Lactifluus flammeolus*** (Pollini: Fr.) Kuntze, Revisio Generum Plantarum, Pars II: 856 (1891)

Lectotype: France, Bellême, no information available on the number of the type collection (PC) (designated in HEILMANN-CLAUSEN *et al.*, 1998).

DESCRIPTION: *Pileus* 4.5–19 cm diam., when young convex with a slightly depressed centre, becoming infundibuliform when full-grown with a straight to inrolled margin, sometimes with an irregularly shaped to lobed margin; surface smooth and viscid; colour salmon orange (6A5), sometimes paler (light) ochraceous buff (5A4/5), typically clearly and finely zonate near the margin with the darker zones ochraceous orange-coloured (5A6/7 to 6A6/7), greenish tones almost always completely absent, only very small and very old specimens with some green discolouration. *Lamellae* slightly decurrent, with an (indistinct) decurrent tooth in older specimens, moderately spaced, often anastomosing near the insertion of the stipe; colour salmon orange or slightly paler (5A6/5), turning red when bruised and sometimes very faintly green after some hours; edge entire and concolorous. *Stipe* 2–7 × 0.8–2.5 cm, ± cylindrical, rather long and slender to rarely more short and robust, becoming hollow; surface dry to viscid, smooth; colour salmon orange or slightly more bright orange (6A5/6), sometimes with an unclear white bloom (5A3), deeper orange (6A7) scrobicules very conspicuous in most specimens and often large; stipe sometimes with a white zone at the top, without green tinges. *Context* firm, whitish, underneath the stipitipellis and in the entire pileus bright orange when cut (6A8 to 7A8), then (slowly) turning red: scarlet (8A/B8) after 20 min., vinaceous red (9D7) after 30 min., vinaceous brown after 1h; smell slightly fruity; taste mild to slightly acrid and then bitter after 10 sec. *Latex* scarce to rather abundant, bright orange (7A8), drying vinaceous red in 20–30 min. on the context; taste mild. *Spore deposit* pale yellow-orange (4A3).

Spores 8.5–*10.0–10.6*–11.8 × 6.7–*7.3–8.2*–9.0 µm, ellipsoid, rarely broadly ellipsoid (Q = 1.16–*1.27–1.35*–1.54); ornamentation up to 0.5 µm high, of rather narrow ridges with abundant short ridges and isolated warts, forming an incomplete reticulum; plage clearly visible and rather big but only slightly amyloid distally. *Basidia* 40–64 × 10–14 µm, almost cylindrical to subclavate, 4-spored, often containing oil droplets; sterigmata 3–7 µm long. *Pleuromacrocystidia* 40–96 × 6–12 µm, abundant over the entire lamella face, conspicuous and very emergent, ± cylindrical to fusiform, often with a moniliform apex, often with a granular to needle-like content, thin-walled. *Pseudocystidia* quite abundant, slender, 2–4 µm broad, mostly not to slightly (but rarely clearly) emergent, cylindrical to irregularly tortuous, content similar to the lactifers. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia 15–30 × 5–8 µm, ± cylindrical to irregular, thin-walled; cheilomacrocystidia 24–45 × 4–7 µm, fusiform, with a moniliform apex, most often with a needle-shaped content, thin-walled. *Subhymenium* irregular, composed of globose or slightly elongated, small cells. *Hymenophoral trama* irregularly filamentous, with very abundant lactifers. *Pileipellis* an ixocutis, up to 350 µm thick, with a thin and indistinct slime-layer, of frequently branching and interwoven hyphae, 2–6 µm diam., only few shrivelled hyphae. *Stipitipellis* an ixocutis, up to 200 µm thick, with a distinct (10–20 µm thick) slime-layer, composed of frequently branching and interwoven hyphae, 2–5 µm diam., few shrivelled hyphae. *Clamp-connections* absent.

HABITAT: Forming ectomycorrhiza with *Abies* (PILLUKAT, 1996a&b; EBERHARDT *et al.*, 2000), on calcareous soils, found from September to November.

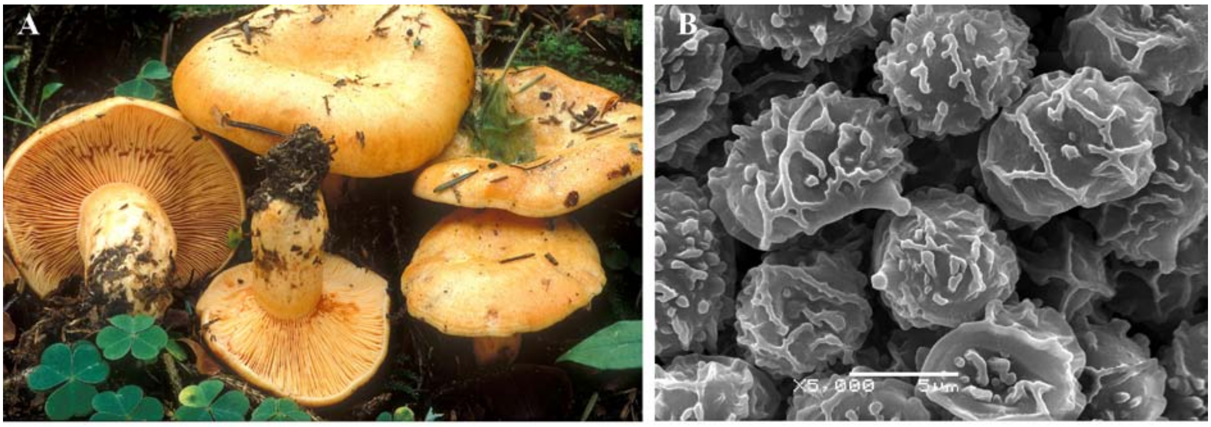

Fig. 14: A. *L. salmonicolor* (photo J. Vesterholt); **B.** SEM image of the spores of *L. salmonicolor* J. Nuytinck 2001-090.

DISTRIBUTION: Distributed widely in Europe where the host tree occurs, not known from Scandinavia.

COLLECTIONS EXAMINED: Belgium, Rochefort, Les Fèches, under *Abies*, young plantation, 5.10.1996, A. Verbeken & R. Walleyn 96-1033 (GENT) – ibid., 6.10.1996, R. Walleyn 0570 (GENT) – ibid., N50°10.209' E005°10.398', 189 m a.s.l., in *Abies alba* plantation, 27.10.2001, J. Nuytinck 2001-085 (GENT) – ibid., 27.10.2001, J. Nuytinck 2001-086 (GENT) – ibid., 27.10.2001, J. Nuytinck 2001-087 (GENT) – ibid., 10.10.2002, J. Nuytinck 2002-031 (GENT) – ibid., 30.10.2002, J. Nuytinck 2002-046 (GENT) – France, Villaroger (73), "Le Pré Derrière", 1300 m a.s.l., in a meadow borded by *Picea*, 25.08.1994, P.A. Moreau 94082501 (GENT) – Forêt de Dorans, Bois de Chevrey, between Dorans and Durbuy, *Abies* forest on rich, calcareous clayey soil, 4.10.1998, A. Verbeken 98-056 (GENT) – Close to Aix les Bains, *Abies alba* plantation on slope, 11.11.2001, J. Nuytinck 2001-123 (GENT) – ibid., 11.11.2001, J. Nuytinck 2001-126 (GENT) – Italy, Sardegna, Prov. Sassari, Monte Limbara, Loc. Madonna Della Neve, close to *Abies cephalonica*, 12.06.2000, leg. M. Contu, J. Nuytinck 2002-027 (ex. Herbar. Mycol. M. Contu) – ibid., 17.06.2000, leg. M. Contu, J. Nuytinck 2002-028 (ex. Herbarium Mycologicum M. Contu) – ibid., 22.06.2000, leg. M. Contu, J. Nuytinck 2002-029 (ex. Herbar. Mycol. M. Contu) – Chiarino (Passo delle Capannelle), ~N42°30.376' E013°24.679', 985 m a.s.l., planted, mixed forest on mountain slope, 29.10.2001, J. Nuytinck 2001-090 (GENT) – ibid., 29.10.2001, J. Nuytinck 2001-091 (GENT) – Sardegna, Prov. Sassari, Monte Limbara, Loc. Madonna Della Neve, close to *Abies cephalonica*, 24.06.2002, leg. M. Contu, J. Nuytinck 2002-030 (ex. Herbarium Mycologicum M. Contu) – Slovakia, Nizke Tatry mountains; 2,5 km N to Maluzina village, Michalovo valley, N48°59.898' E019°45.448', 725 m a.s.l., under *Abies alba* in mixed forest on steep slope, 6.10.2001, J. Nuytinck 2001-067 1 (GENT) – ibid., 6.10.2001, J. Nuytinck 2001-067 2 (GENT) – Nizke Tatry mountains; 2,5 km N to Maluzina village, Michalovo valley, N48°59.898' E019°45.448', 725 m a.s.l., under *Abies alba* in mixed forest on steep slope, 6.10.2001, J. Nuytinck 2001-067 3+4 (GENT) – ibid., 6.10.2001, J. Nuytinck 2001-067 rest (GENT) – Eastern Carpathians, Poloniny N.P., Stuzica virgin forest, terrestrial under *Abies alba*, 5.10.2003, R. Walleyn 3257 (GENT).

DISCUSSION: *Lactarius salmonicolor* is a medium sized to very large species with a salmon orange coloured cap, typically completely lacking green tinges, often with a strikingly scrobiculate stem and orange milk turning red in ± 20–30 min. It is outstanding in the section because of its association with *Abies*. The wrong indication of *Picea* as host tree in the original description caused some initial confusion on the identification of *L. salmonicolor* (e.g. BERTAULT, 1978).
Microscopically the abundant and emergent pleuromacrocystidia are striking. They were mentioned in the original description (HEIM & LECLAIR, 1950) and we observed them in all studied specimens. Nevertheless, caution is necessary when using macrocystidia for identification in *Lactarius* sect. *Deliciosi*. In HEILMANN-CLAUSEN *et al.* (1998) the abundant pleuromacrocystidia are not mentioned. The large spore size is another striking microscopical character. The high Q-value as indicated in the original description (HEIM & LECLAIR, 1950), refers only to a small portion of the spores (NEUHOFF, 1956; HEILMANN-CLAUSEN *et al.*, 1998).

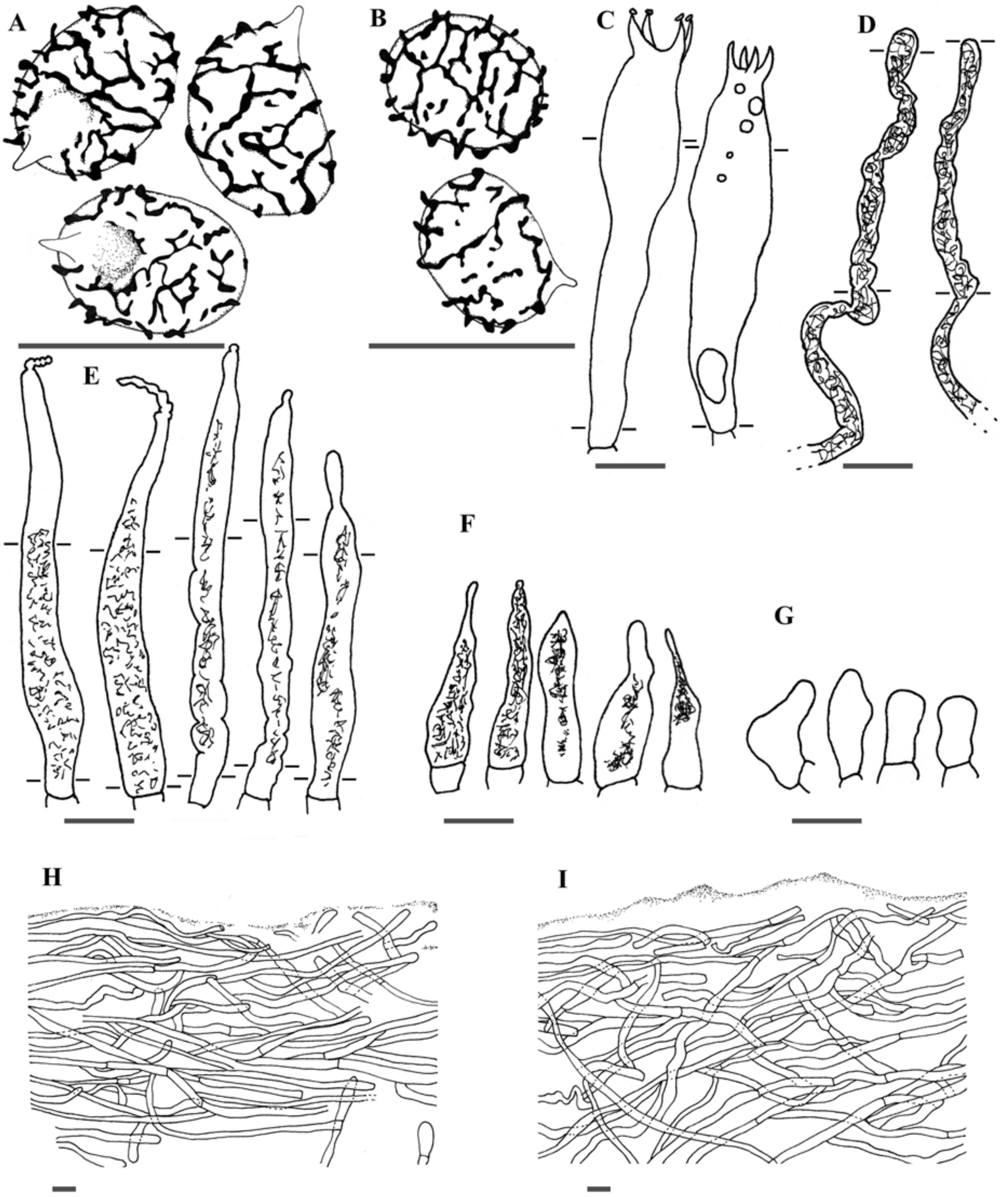

Fig. 15: *Lactarius salmonicolor*: **A.** spores; **B.** significantly smaller spores from J. Nuytinck 2002-027; **C.** basidia; **D.** pseudocystidia; **E.** pleuromacrocystidia; **F.** cheilomacrocystidia; **G.** cheiloleptocystidia; **H.** section through the pileipellis; **I.** section through the stipitipellis. From A. Verbeken 98-056, R. Walleyn 3257 and J. Nuytinck 2001-067, 2001-085, 2001-123, 2002-027, 2002-030. Scale bars = 10 µm, small indicate the height of the hymenium.

L. salmonicolor was first described as *L. salmoneus* (HEIM & LECLAIR, 1950) and subsequently renamed (HEIM, 1953) because the name *L. salmoneus* had already been used by PECK (1898) for a North-American species. Little time later POUZAR (1954) proposed the name *L. subsalmoneus* to replace *L. salmoneus*. The name *L. subsalmoneus*, although superfluous, has since then popped up several times. SCHAEFER (1970) even interpreted *L. subsalmoneus* and *L. salmonicolor* as two different species.

Agaricus flammeolus, described in 1816 by POLLINI and combined in *Lactarius* by FRIES (1838) is possibly a synonym of *L. salmonicolor* (fide NEUHOFF, 1956), but several key characters are lacking in the original description.
M. Contu collected deviating *L. salmonicolor* basidiocarps under introduced *Abies cephalonica* trees in Sardinia. The spores are remarkably smaller than in the typical *L. salmonicolor* collections (Fig. 15B) and the pleuromacrocystidia even more abundant, emergent and striking. Molecular analyses however, do not differentiate these collections from the other *L. salmonicolor* accessions (NUYTINCK & VERBEKEN, 2005).
L. laeticolor (S. Imai) Imazeki ex Hongo, described from Japan, is strikingly similar to *L. salmonicolor*. Based on this morphological resemblance, *L. laeticolor* has been proposed as a synonym of *L. salmonicolor* (HEILMANN-CLAUSEN *et al.*, 1998; EBERHARDT *et al.*, 2000). Our molecular data however, reject this hypothesis; both species are closely related but do form separate clades (NUYTINCK *et al.*, in preparation).
Similarly, several Mexican collections remind very strongly of *L. salmonicolor*. A molecular and detailed morphological study is needed here to come to reliable conclusions.

4.8. ***Lactarius sanguifluus* (Paulet) Fr.**, Epicr. Syst. mycol.: 341 (1838)

Figs. 16, 17

Basionym: ≡ ***Hypophyllum sanguifluum*** Paulet, Traité des champignons: 2 (9ed): pl. 81, fig. 3-5 (1811)
Synonym: ≡ ***Lactifluus sanguifluus*** (Paulet) Kuntze, Revisio Generum Plantarum, Pars II: 857 (1891)
= ***L. sanguifluus*** f. ***roseus*** Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003)

Lectotype: Traité des champignons: 2 (9th ed): plate 81, fig. 3–5! (PAULET, 1811), depicted from France (**designated here**). LALLI *et al.* (2003) invalidly neotypified *L. sanguifluus* (AQ (92/15) (AQUI), Italy, L'Aquila, S. Guiuliano). Because the drawing by PAULET (1811) does not reflect the typical *L. sanguifluus* morphology, we designate collection AQ (92/15) (AQUI), as the epitype for *L. sanguifluus* (**designated here**).

DESCRIPTION: *Pileus* 4–9 cm diam., convex with a slightly depressed centre, later becoming more depressed but margin remaining slightly incurved, mostly regular; surface smooth, viscid but soon dry, azonate to faintly zoned; colour orange-buff (5A2–4), pinkish buff (6A3) with sometimes ochraceous orange (6B/C7) in an irregular pattern or with ochraceous orange pits, sometimes with greyish green tinges, green to greyish green where bruised. *Lamellae* slightly decurrent, sometimes with a decurrent tooth, medium broad, rather crowded, often forked near the stipe; colour pale vinaceous (7A/B3/4) or with a pale pinkish buff (5B3) tint; edge entire and somewhat paler. *Stipe* 2–4.5 × 1–2.5 cm, mostly cylindrical; surface smooth, dry; colour deep red just where the gills are attached, with a whitish zone mostly present below the lamellae attachment, major part orange-buff (7B3) to orange-red (9A2), pale because of a white surface layer; scrobicules variable in size and abundance, deeper brownish orange (6D7) or more reddish. *Context* rather firm in the pileus, becoming hollow in the stipe, pale pinkish buff but vinaceous to deep wine red (9/10D6) just above the lamellae and under the stipe and pileus surface, drying brownish; smell faint, fruity; taste mild, shortly afterwards becoming very slightly bitter and slightly acrid. *Latex* rather scarce, vinaceous red (9/10D6); taste mild. *Spore deposit* pale yellow-orange (4A3).

KOH no reaction, *phenol* no reaction, *NH_4OH* no reaction.

Spores 7.3–*8.1–8.8*–9.7 × 5.7–*6.4–7.1*–7.8 µm, mostly subglobose to broadly ellipsoid, rarely ellipsoid (Q = 1.10–*1.24–1.29*–1.36); ornamentation up to 0.5 (0.7) µm high, of rather broad ridges, forming an incomplete reticulum, isolated warts and short ridges numerous; plage distally very slightly amyloid. *Basidia* 50–60 (70) × 9–11.5 µm, subclavate, 4-spored, containing fine granules or oil-droplets; sterigmata 4–6 µm long. *Pleuromacrocystidia* rather scarce to abundant, 45–65 × 6–9 µm, emergent, subfusiform to fusiform, tapering or moniliform at the apex, with a granular to needle-like content, thin-walled. *Pseudocystidia* abundant, 3–4 µm thick, not to slightly emergent, almost cylindrical to branching and tortuous, sometimes with a mucronate apex. *Lamella edge* sterile with rather scarce to abundant cheilomacrocystidia; cheiloleptocystidia 22–30 × 5–8 µm, subclavate to more irregular, with a fine, granular content, thin-walled; cheilomacrocystidia 35–45 × 6–8 µm, emergent, fusiform, with a moniliform to sometimes branching apex, with a granular to needle-shaped content, thin-walled. *Subhymenium* irregular, appearing like small, subglobose cell in rows under the hymenium elements. *Hymenophoral trama* irregular; with abundant lactifers. *Pileipellis* an ixocutis, up to 200 µm thick, with a very thin slime-layer; hyphae 2–6 µm broad, often branching, strongly interwoven, only few gelatinised or shrivelled, mostly with almost refringent walls; lactiferous hyphae locally present. *Stipitipellis* a cutis, up to 150 µm thick, of regularly shaped, strongly interwoven hyphae, 2–5 µm diam., without shrivelled or gelatinized hyphae; thin lactifers present relatively close to the surface. *Clamp-connections* absent.

HABITAT: Associated with *Pinus*, on calcareous soils, rather thermophilous, found from September to November (December in most southern Europe).

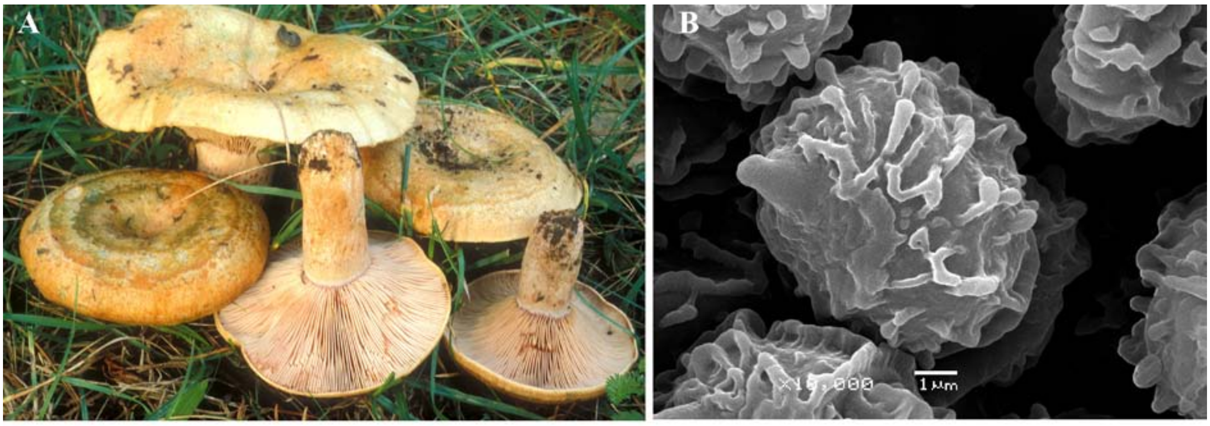

Fig. 16: A. *L. sanguifluus* (photo J. Vesterholt); **B.** SEM image of the spores of *L. sanguifluus* J. Nuytinck 2001-051.

DISTRIBUTION: Widespread in mainly southern Europe.

COLLECTIONS EXAMINED: Belgium, Nismes, coniferous forest, 15.10.1984, I. Cauwels 84-43 (GENT) – Nismes, Fondri des Chiens, N50°04'26.63" E04°33'31.53", *Pinus sylvestris* on calcareous soil, 19.11.1995, A. Verbeken and R. Walleyn (GENT) – Ave-et-Auffe, N50°06'75.7" E005°10'39.0", 171 m a.s.l., *Pinus nigra* plantation on calcareous, S-SE exposed slope, 19.10.2000, J. Nuytinck 2000-006 (GENT) – Tellin, in garden under *Pinus*, 19.10.2000, J. Nuytinck 2000-008 (GENT) – Barvaux, les Mignées, N50°19'46.35" E005°29'12.29", in ruderal grassland on limestone under *Pinus*, 6.10.2001, R. Walleyn 2193 (GENT) – France, Forêt de la Brosse, between Danjoutin and Andelnans, along road near forest with *Pinus*, 5.10.1998, A. Verbeken 98-063 (GENT) – Ramtuelle, Gassin, 16.11.1999, D. Laber 4007287 (STU) – Causse de flames, Coulaures, under *Pinus sylvestris* on calcareous soil, 24.10.1999, A. Verbeken 99-245 (GENT) – Manoir de Syrignac (Salignac), under *Pinus sylvestris* on calcareous soil, 24.10.1999, leg. H. Van Nieuwenhove, A. Verbeken 99-246 (GENT) – Germany, Baden-Württemberg, Schaffhausen, MTB 7219/3.23, Wacholderheide on calcareous soil under *Pinus silvestris* and *Juniperus communis*, 28.10.2001, A. Gminder (STU) – Italy, Toscana, Cipressata di S. Agnese, on slope in forest dominated by *Cupressus sempervirens* with scattered *Quercus ilex* and *Pinus*, 5.11.1996, J. Vesterholt 96-423 (GENT) – Poggio Roio, N42°20'64.9" E013°22'44.4", 865 m a.s.l., mixed forest on small mountain (*Pinus*, *Abies*, *Picea* and deciduous trees), 31.10.2001, J. Nuytinck 2001-095 (GENT) – ibid., 31.10.2001, J. Nuytinck 2001-096 (GENT) – ibid., 31.10.2001, J. Nuytinck 2001-097 (GENT) – ibid., 31.10.2001, J. Nuytinck 2001-098 (GENT) – Morocco, Tamrabta, Under *Pinus maritimus*, 10.11.1953, G. Malençon 2567A&B (MPU) – Marabout Sidi-Bou-Hassan RIF, Under *Pinus halepensis*, 30.11.1960, G. Malençon & R. Bertault 4091 (MPU) – Slovakia, Mali Karpaty hills near Ploveckli Podhra dic, N48°29'00.2" E017°16'05.2", 320-360 m a.s.l., *Pinus nigra* plantation on S exposed slope, 3.10.2001, J. Nuytinck 2001-051 (GENT) – ibid., 3.10.2001, J. Nuytinck 2001-050 (GENT) – Spain, Surroundings of St Hilary Sacalm, 5.11.2001, J. Nuytinck 2001-103 (GENT) – Sweden, Gotland, Endre par., Ölbäck, in open calcareous grassland under *Pinus*, 13.10.1996, leg. C. Lange, E. Bohus and P. Johansson, C. Lange 96-080 (GENT).

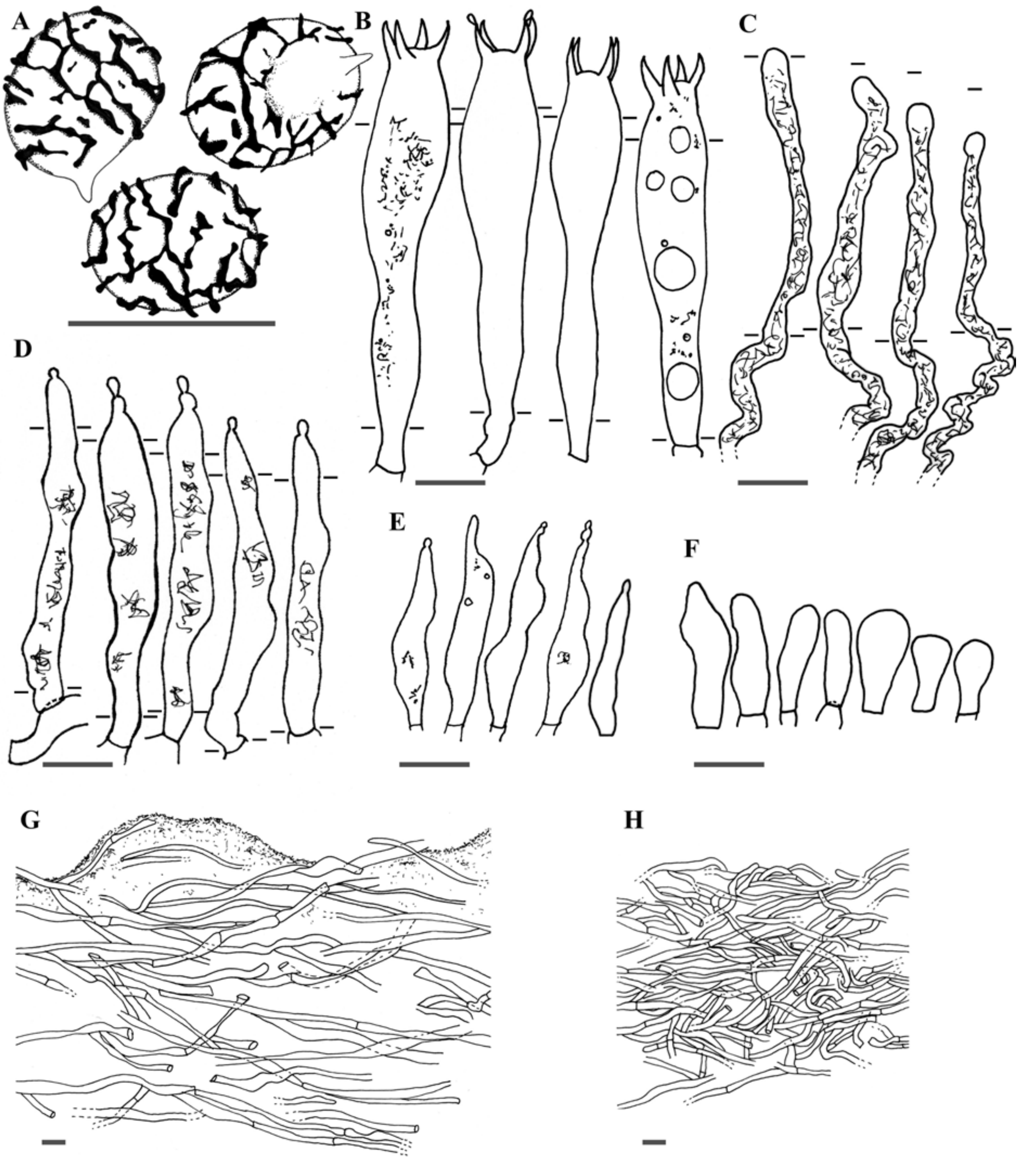

Fig. 17: *Lactarius sanguifluus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From J. Nuytinck 2000-008, 2001-095 and 2001-097. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: *Lactarius sanguifluus* is a medium-sized to rather large species, associated with *Pinus* and exuding vinaceous red latex when cut. ROMAGNESI (1958) remarks the accidental occurrence of spots of orange coloured milk at the base of the stipe. The pileus and stipe have a generally dull ochraceous orange appearance; the lamella colour is very characteristic pale vinaceous, clearly more reddish than in *L. deliciosus* but much paler than in the entirely vinaceous coloured *L. vinosus*. Microscopically, the rather small and mostly subglobose, quite heavily ornamented spores are characteristic. *Lactarius sanguifluus* and *L. vinosus* are considered to be some of the most delicious edible species in the Mediterranean area.

L. sanguifluus and *L. vinosus* form two separate clades in the molecular phylogenetic analyses (NUYTINCK & VERBEKEN, 2003, 2005). Maximum parsimony analysis of the ITS nrDNA shows that the two clades are not supported by very high bootstrap values, but a total evidence approach reveals much higher support values. We consider it justified to treat *L. sanguifluus* and *L. vinosus* as separate species as several ecological and macro- and microscopical features distinguish them (see under *L. vinosus*). Sister group to these two species is *L. semisanguifluus* with latex that turns from orange to red in just a few minutes.

LALLI *et al.* (2003) state that, due to the existence of multiple forms and intermediates and due to the lack of clearly differentiating features, it can be very hard to distinguish species in the "*L. sanguifluus*-complex" (see discussion on nomenclature under *L. vinosus*). The appearance of the cap may indeed change quite drastically with age and even more with exposure to direct sunlight and drought. For this reason, LALLI *et al.* (2003) described two forms: *L. sanguifluus* f. *roseus* with a greyish-whitish discoloured cap and a general pinkish appearance and *L. sanguifluus* f. *vinosus* with a less clearly zonate cap without much green and a more lilac-pink sheen in the lamellae. The nominate form is stated to have ochre-orange lamellae with a salmon sheen (LALLI *et al.*, 2003). We do not agree with this delimitation of forms as they are part of a continuous morphological variation.

4.9. ***Lactarius semisanguifluus*** **R. Heim & Leclair**, Rev. Mycol. (Paris) 15: 79 (1950)

Figs. 18, 19

Synonym: = ***L. sanguineovirescens*** Fillion, Bull. Trimestriel Féd. Mycol. Dauphiné-Savoie 29 (113): 21 (1989)

Excluded: ***L. semisanguifluus*** sensu NEUHOFF (1956) (= *L. deterrimus*)

Lectotype: Heim 1949-610 (PC!), France: Bellême (**designated here**) (this specimen was studied by HEIM & LECLAIR (1950) but was not designated as type specimen[*]).

DESCRIPTION: *Pileus* 4–7 cm diam., convex with a slightly depressed centre when young, becoming broadly infundibuliform with an inrolled to flattened margin and a slightly depressed centre; surface smooth, sticky but soon becoming dry, (almost) not zonate to slightly zonate (close to the margin) or clearly zonate; colour sometimes bright orange but often dull orange (6B6) or brownish (6C7, 6D7) to greyish orange (6D3), exceptionally greyish with pink and buff shades, discolouring red where bruised, very often with a lot of green. *Lamellae* slightly decurrent, medium broad, medium crowded, rarely forked or anastomosing; colour pale salmon pink to buff to brighter orange (5A4/7 to 6A6), turning red in 5 min. when bruised, then becoming green (in 30 min. or more); edge entire and slightly paler. *Stipe* 2–6.5 × 0.9–2 cm, ± cylindrical or more irregular; surface smooth, dry; colour pale to brighter orange (5A6 to 6A5), often with a white bloom, turning red and then green where bruised or when old, with (a few) darker orange scrobicules (sometimes absent). *Context* quite firm and thick, also in the pileus margin, becoming hollow in the stipe, whitish but turning bright orange (6/7A8) when cut, changing to red (8C7) in 5–10 min. and becoming deep vinaceous red (8E6 to 10D/E7/8) in 20 min., becoming green in a few hours, zone just underneath pileipellis sometimes greenish from the beginning; smell faintly fruity; taste ± mild or very slightly acrid at first and then becoming bitter. *Latex* scarce, bright orange (6/7A8) but quickly (5–10 min.) drying vinaceous red on the context; taste mild. *Spore deposit* pale yellow-orange (4A3).

KOH no reaction, *NH_4OH* no reaction.

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[*] ICBN art. *37.1.* Publication on or after 1 January 1958 of the name of a new taxon of the rank of genus or below is valid only when the type of the name is indicated

Spores 7.7–*8.6–9.0*–9.7 × 6.1–*6.7–7.0*–7.7 µm, broadly ellipsoid to ellipsoid (Q = 1.16–*1.28–1.32*–1.42), exceptionally large spores rarely present; ornamentation up to 0.5 µm high, of fine ridges and many isolated warts and short ridges, forming a very incomplete reticulum, rarely with slightly thicker ridges; plage slightly amyloid distally. *Basidia* 40–60 × 8.5–11 µm, ± cylindrical to subclavate, mostly 4-spored, rarely 1- or 2-spored; sterigmata 3.5–6.5 µm long. *Pleuromacrocystidia* moderately to quite abundant, 41–64 × 6.5–12 µm, emergent, often with a moniliform apex, with a needle-like or granular content, thin-walled. *Pseudocystidia* abundant, 2.5–5 µm broad, not emergent, cylindrical and tortuous, sometimes branching, with a yellowish content similar to the lactifers. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia 10–25 × 4–8 µm, cylindrical to subclavate, hyaline or with a fine granular content, thin-walled; cheilomacrocystidia 20–50 × 5–9 µm, subfusiform or irregular with a moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* irregular, composed of ± isodiametric or elongated cells, arranged in rows under hymenium elements. *Hymenophoral trama* irregular with abundant lactifers. *Pileipellis* an ixocutis, up to 200 µm thick, composed of interwoven, branching hyphae, 2–7 µm thick; gelatinised and shrivelled hyphae not abundant. *Stipitipellis* a cutis, up to 100 µm thick, composed of interwoven hyphae of 2–6 µm diam. *Clamp-connections* absent.

HABITAT: Associated with *Pinus*, linked to rich, calcareous soils, found from September to November.

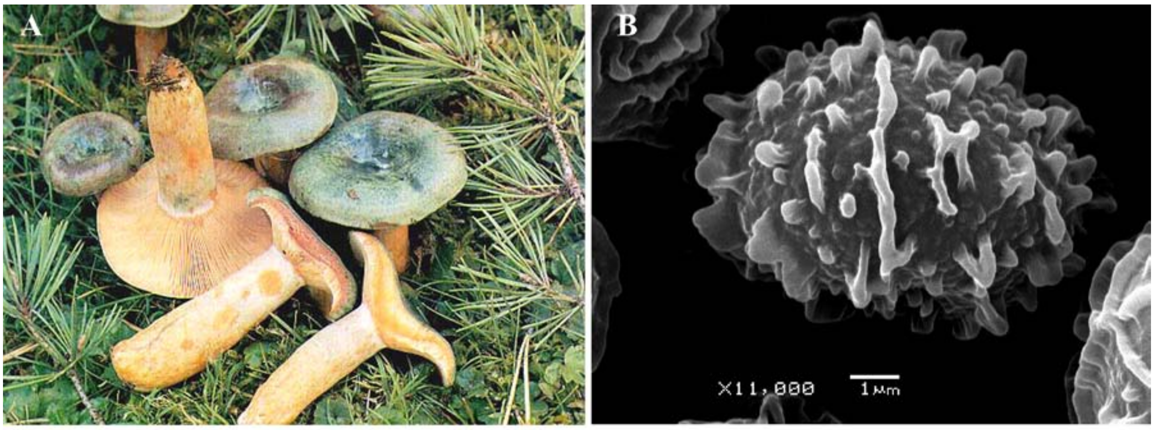

Fig. 18: A. *L. semisanguifluus*; **B.** SEM image of a spore of *L. semisanguifluus* J. Nuytinck 2001-075.

DISTRIBUTION: Distributed throughout Europe, but mainly in the southern part, eastwards known up to Turkey.

COLLECTIONS EXAMINED: Belgium, Bagrimont, 27.08.1948, M. Herregods 1019 (GENT) Herregods, as *L. sanguifluus* – Heure-en-Famenne, Bois d'Heure, IFBL: D6.36.24, grassy place, *Pinus sylvestris*, 19.09.1994, R. Walleyn & A. Verbeken 94-737 (GENT) – Nismes, IFBL: J5.41.14, *Pinus sylvestris*, border calcareous grassland, 30.09.1995, R. Walleyn 0377 (GENT) – Matagne-La Grande, Les Mires, IFBL: J5.23.14, *Pinus sylvestris*, border calcareous grassland, 25.09.1996, R. Walleyn 0515 (GENT) – Parking place 5 km before Rochefort on road from Ciergnon to Rochefort, *Pinus*, grassy, calcareous soil, 5.10.1996, A. Verbeken 96-1032 (GENT) – Close to Rochefort, S-SE exposed slope, calcareous soil, under *Pinus nigra*, 19.10.2000, J. Nuytinck 2000-007 (GENT) – Resteigne, N50°05.355' E005°10.990', 240 m a.s.l., under *Pinus* on calcareous soil, on forest path, 22.09.2001, J. Nuytinck 2001-040 (GENT) – Close to Rochefort, N50°10.209' E005°10.398', 189 m a.s.l., under *Pinus sylvestris*, in lawn, 30.10.2002, J. Nuytinck 2002-045 (GENT) – Denmark, NEJ: Hostemark Skov S of Mou, UTM: NJ7511, under *Pinus*, 1.10.1994, L. Vesterholt & S. Hansen, J. Vesterholt 94-749 (GENT) – France, 1.10.1949, Heim 1949-610 (PC), Lectotype – Close to Bellegarde, Under *Pinus*, on acidic soil, leg. Bon etc., det Bon & Fillion, Bon 88127 (LIP), Isotype of *L. sanguineovirescens* – Sevrier (74), 30.09.2001, P.A. Moreau 30-09-2001 (pers. herbarium P.A. Moreau), as *Lactarius pinastri/sanguineovirescens*? – Minzier, 13.10.2001, P.A. Moreau 13-10-2001 (pers. herbarium P.A. Moreau), as *Lactarius sanguineovirescens* – Germany, Baden-Würtemberg, Bebenhausen, Goldersbachtal, MTB 7420/1.213, *Picea* and *Pinus*, 4.10.2001, A. Gminder (GENT) – Italy, Chiarino (Passo delle Capannelle), N42°30.376' E013°24.679', 981 m a.s.l., small *Pinus nigra* stand on slope with deciduous trees, 29.10.2001, J. Nuytinck 2001-092 (GENT) – ibid., 29.10.2001, J. Nuytinck 2001-093 (GENT) – Morocco, Lot. Mediouna, under *Pinus pinea*, 03.01.1959, G. Malençon 5905 (MPU) – Massif de Tamrabta, under *Pinus pinaster*, 21.11.1961, G. Malençon 5530 (MPU) – Slovakia, Botanical Institute, Bratislava, N48°10.256' E017°04.266', 260 m a.s.l., in lawn under *Pinus sylvestris*, 2.10.2001, J. Nuytinck 2001-044 (GENT) – Zàhorská mžina lowland, 4 km SW of Moravskˇy Svatý jàn village, on the river side of the Morava, N48°33.480' E016°56.978', 120-130 m a.s.l., *Pinus* forest on sand, under *Pinus sylvestris*, 2.10.2001, J. Nuytinck 2001-047 (GENT) –

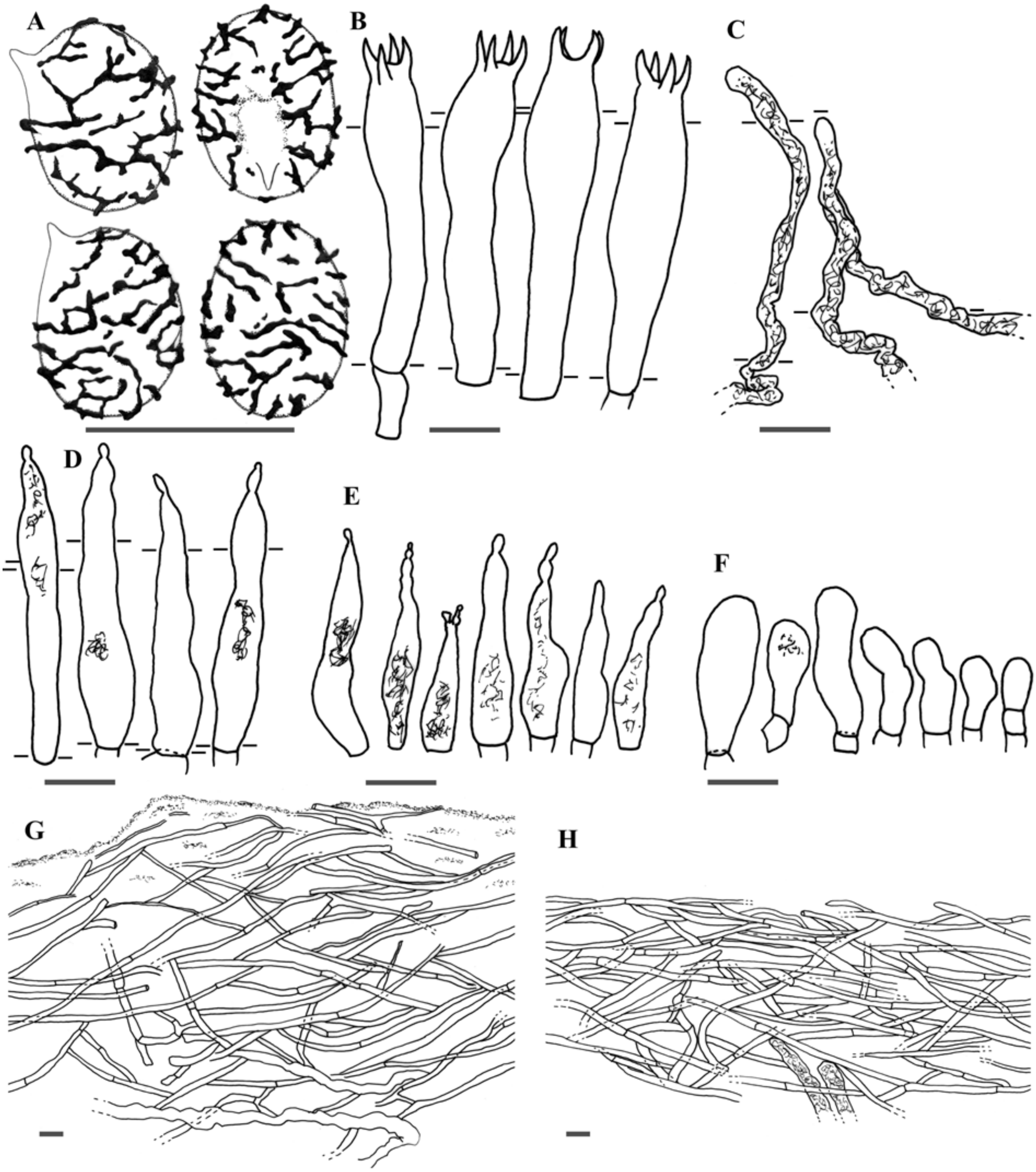

Fig. 19: *Lactarius semisanguifluus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From A. Peksen & G. Hatat 220, A. Verbeken 96-1032, R. Walleyn and J. Nuytinck 2001-049, 2001-092. Scale bars = 10 µm, small bars indicate the height of the hymenium.

ibid., 2.10.2001, J. Nuytinck 2001-048 (GENT) – Botanical Institute, Bratislava, N48°10.256' E017°04.266', 260 m a.s.l., in lawn under *Pinus sylvestris*, 3.10.2001, J. Nuytinck 2001-049 (GENT) – Sweden, Gotland, Viklau par., Tjaucklehagen 2km S viklau kyrka, in open grazed *Pinus* woodland, 13.10.1996, C. Lange, E. Bohus & P. Johansson, C. Lange 96-081 (GENT) – Turkey, Black sea region, under *Pinus*, 20.11.1997, A. Peksen & G. Hatat 220 (GENT) – Samsun district, Ondokuzmayis forestry service area, N41°30.106' E036°03.025', 27 m a.s.l., in mixed forest, under *Pinus nigra*, on calcareous soil, 20.10.2002, J. Nuytinck 2002-038 (GENT) – ibid., N41°29.996' E036°02.911', 51 m a.s.l., in mixed forest, under *Pinus nigra*, on calcareous soil, 20.10.2002, J. Nuytinck 2002-039, 2002-040 (GENT) – Kocadag, N41°19.215' E036°07.660', 647 m a.s.l., mainly *Pinus* on slope, 22.10.2002, J. Nuytinck 2002-043 (GENT) – Malgälû-Alaçan, N41°40.806' E035°25.031', 12 m a.s.l., young *Pinus brutia* plantation, 25.10.2002, J. Nuytinck 2002-044 (GENT).

DISCUSSION: *Lactarius semisanguifluus* is a small to medium-sized species, associated with *Pinus*, with bright orange latex turning wine red in 5 min. The pileus typically stains greenish, even in young basidiocarps. The spores in most studied collections are ornamented with a very incomplete reticulum, composed of isolated warts and some connective, fine ridges. Some variation in this pattern was observed however; some collections have spores with a more complete network (e.g. J. Nuytinck 2001-049) or with slightly thicker ridges (e.g. A. Verbeken 96-1032).

NEUHOFF (1956), who described *L. deterrimus* as *L. semisanguifluus*, initially created confusion concerning the identities of both species. The mistake was adopted by several later authors (e.g. FAVRE, 1960). REIL (1992) points out that the pileus never becomes deeply infundibuliform in *L. semisanguifluus* and that the context in the pileus margin is more thick and firm than in *L. deterrimus*.

L. sanguineovirescens was described in 1989 from the Savoie in south-east France (FILLION, 1989). It is characterised by its greyish pileus with pink and buff shades, contrasting bright orange lamellae, orange latex turning red in 5 minutes, a greenish blue zone underneath the pileipellis and a distinctly acrid taste after some chewing; it grows with *Pinus* on neutral to slightly acidic soil. In ROMAGNESI's opinion (1990) *L. sanguineovirescens* might be conspecific with *L. pinastri*. After sequencing collections identified as *L. sanguineovirescens* from near the type locality and after microscopical examination of the type material, we are convinced that *L. sanguineovirescens* is a synonym of *L. semisanguifluus* (NUYTINCK & VERBEKEN, 2005).

So far, "*L. sanguineovirescens"* has been reported from one other locality only (i.e. Sardinia, MELIS *et al.*, 1999). Examination of this material revealed that there are no microscopic differences with *L. quieticolor*. The picture in MELIS *et al.* (1999) shows very dry basidiocarps (the pileipellis has become "metallic" and the lamellae show a strong discolouration to greenish blue).

4.10. ***Lactarius vinosus*** **(Quél.→) Bataille**, Fl. Monogr. Astérosporales: 28 (1908)

Figs. 20, 21

Synonyms: ≡ ***Agaricus deliciosus*** var. ***violaceus*** Barla, Tableau comparatif des champignons comestibles et vénéneux de Nice tab. 4, fig. 24 (1855)
≡ ***L. sanguifluus*** var. ***vinosus*** Quél., Compt. Rend. Assoc. Franç. Avancem. Sci. 9: 668 (1881 ("1880"))
≡ ***L. sanguifluus*** var. ***violaceus*** (Barla) Basso, Fungi Europaei 7. *Lactarius* Pers.: 308 (1999)
≡ ***L. sanguifluus*** f. ***vinosus*** (Quél.→Bataille) Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003)
= ***L. rubrozonatus*** Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003)
= ***L. rubrozonatus*** f. ***carneus*** Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121–132 (2003)
?= ***L. deliciosus*** var. ***violascens*** Panizzi, Comment. Soc. Crittog. Ital. 1: 174 (1862)
?= ***L. sanguifluus*** var. ***yvreus*** C. Martin, Bull. Trav. Soc. Bot. Genève 7: 184 (1894)
?= ***L. haemorrheus*** Lowe, Grevillea 16: 121 (1888)

Lectotype: Tableau comparatif des champignons comestibles et vénéneux de Nice: tav. 4, fig. 24! (BARLA, 1855), depicted from France, around Nice (**designated here**).

DESCRIPTION: *Pileus* 4–8 cm diam., at first convex with a decurved margin and a slightly depressed centre, later with a decurved to expanded margin and a depressed centre, sometimes slightly lobed-irregular, margin mostly regular; surface smooth, slightly viscid, becoming dry, typically distinctly zoned but sometimes only faintly zoned, sometimes scrobiculate; colour vinaceous red (7D6) with paler, more orange and/or darker zones, green where bruised, paler, discoloured forms rather common. *Lamellae* subdecurrent, medium broad, rather crowded, sometimes forked and often anastomosing near the stipe; colour pale violet (7A3) to vinaceous, strongly discolouring greenish where bruised; edge entire and slightly paler than the gills. *Stipe* 1.5–4.5 × 0.7–1.8 cm, clearly tapering downwards in most basidiomes; surface smooth, dry; colour brownish-red (10C4) but mostly strongly pruinose and then pinkish-violet, green where bruised, usually with numerous wine red (10E6) scrobicules of variable size. *Context* firm in the cap but hollow in the stipe, whitish buff in the central part of the stipe cavity, dark vinaceous red (11E/F5) in the entire pileus and near the stipe surface, turning greenish after several hours; smell faintly fruity; taste mild, shortly afterwards becoming very slightly bitter and slightly acrid. *Latex* rather scarce, deep wine-red (11E/F5), drying brownish (8E8); taste mild. *Spore deposit* unknown.

KOH no reaction, *phenol* no reaction.

Spores 7.2–*7.8–8.6*–9.3 × 5.6–*6.1–6.7*–7.4 µm, subglobose to ellipsoid (Q = 1.07–*1.23–1.36*–1.46); ornamentation up to 0.5 µm high, of rather narrow ridges, forming an incomplete reticulum, scattered isolated warts and short ridges present; plage slightly amyloid in the distal part. *Basidia* 45–60 × 9–11 µm, ± cylindrical to subclavate, 4-spored, often with abundant, oil-like droplets; sterigmata 4–6 µm long. *Pleuromacrocystidia* rather abundant, 35–55 × 6–9 µm, subfusiform to fusiform, tapering or moniliform at the apex, thin-walled. *Pseudocystidia* very abundant, 4–8 µm broad, not emergent to very slightly emergent, cylindrical to tortuous, with a refractive content. *Lamella edge* sterile with (quite) abundant cheilomacrocystidia; cheiloleptocystidia 7.5–25 × 4–8 µm, cylindrical to subclavate, hyaline or with a fine, granular content, thin-walled; cheilomacrocystidia 35–45 × 6–8 µm, fusiform, thin-walled. *Subhymenium* irregular, appearing like small, subglobose cell in rows under the hymenium elements. *Hymenophoral trama* irregularly filamentous; with abundant lactifers. *Pileipellis* an ixocutis, up to 200 µm thick, of frequently branching and interwoven hyphae, 2–6 µm diam., only few gelatinised or shrivelled, lactiferous hyphae present. *Stipitipellis* a cutis or ixocutis, 50–100 µm thick, of regularly shaped, strongly interwoven hyphae, 2–6 µm broad, no shrivelled nor gelatinized hyphae, sometimes with very abundant lactifers. *Clamp-connections* absent.

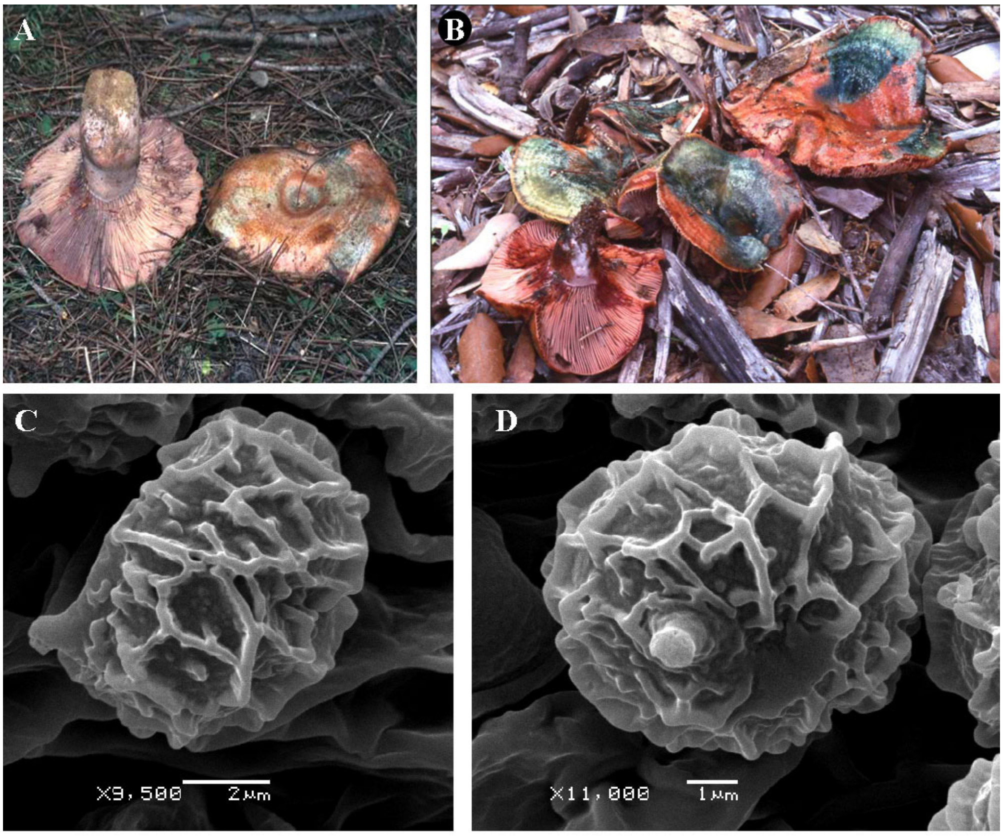

Fig. 20: A. and **B.** *L. vinosus* (B: photo P.A. Moreau); **C.** and **D.** SEM images of spores of *L. vinosus* J. Nuytinck 2001-122.

HABITAT: Associated with *Pinus*, on calcareous soils but presumably less strictly than *L. sanguifluus*, mainly found in late autumn.

DISTRIBUTION: Mediterranean (distribution more southern than *L. sanguifluus*), also known from Turkey.

COLLECTIONS EXAMINED: Italy, Toscana, Marina de Grosseto, Pineta sul Mare, 4.11.1996, leg. D. Maggiori, M.T. Basso 961104 – Spain, Surroundings of St Hilary Sacalm, 6.11.2001, leg. G. Gulden, J. Nuytinck 2001-104a+b+c (GENT) – Ermita Sart Ponç (Tordera), N41°42'56.6" E002°40'25.6", 3 m a.s.l., 7.11.2001, J. Nuytinck 2001-116 (GENT) – St Hilary Sacalm, 7.11.2001, J. Nuytinck 2001-122 (GENT) – Close to Fuenteheridos, region of Aracena, N37°54.195' W006°40.846', 690 m a.s.l., Under *Pinus pinaster*, 4.11.2003, J. Nuytinck 2003-050 – Between Linares de la Sierra and Alajar, N37°52.789' W006°38.792', 685 m a.s.l., Under *Pinus pinea*, 6.11.2003, leg. L. Llorens, J. Nuytinck 2003-058 – Turkey, Samsun, under *Pinus*, 26.11.1997, A. Peksen and G. Hatat 230 (GENT) – Samsun district, Ondokuzmayis forestry service area, N49°02'25.4" E011°12'76.1", 29 m a.s.l., in mixed forest, under *Pinus nigra* on calcareous soil, 20.10.2002, J. Nuytinck 2002-037 (GENT).

DISCUSSION: *Lactarius vinosus* has a striking violet-red to deep vinaceous red overall tinge. The cap is zonate, the generally tapering stipe is strongly pruinose and mostly scrobiculate. There is a strong tendency to discolouring greenish after bruising or picking of the basidiocarps. The latex is vinaceous red from the beginning.

L. vinosus and *L. sanguifluus* are very close relatives (NUYTINCK & VERBEKEN, 2005) but there are some striking differences between the typical representatives of both. Macroscopically the colour of the cap, stipe and lamellae is more vinaceous red in *L. vinosus*, which is almost completely lacking orange tinges. This is in accordance with the oldest descriptions of *L. vinosus* (BARLA, 1859; QUÉLET, 1881) and contradicts the opinion of MARXMÜLLER & ROMAGNESI (1991). Possibly their concept of *L. vinosus* refers to a pale form of *L. sanguifluus*, but we had none of their collections available to check this. The stipe is more distinctly tapering downwards in *L. vinosus* and the latex colours the context of the cap more intensely and completely. Microscopically, the reticulum on the spores of *L. vinosus* is less complete and less dense than in *L. sanguifluus*. In *L. sanguifluus* the ridges are more regular and evenly thick, while in *L. vinosus* they are locally thinner. Discoloured forms of both species, complicating the identification, are often observed.

Some confusion exists about the correct epitheton and authority of *L. vinosus*. The oldest known reference to *L. vinosus* is from BARLA (1855): a drawing of half a fruiting body with the name *Agaricus deliciosus* var. *violaceus*. This publication is effective and valid (art. 29, 42.3 & 44 ICBN, GREUTER *et al*., 2000). A description was given in a later work of BARLA (1859) without reference to the earlier drawing. Although it is hard to be sure on the basis of one drawing and a short description, we interprete Barla's *Agaricus deliciosus* var. *violaceus* as the same as *L. vinosus* (in the sense of most current authors). The description (BARLA, 1859) contains several indications that Barla indeed had *L. vinosus* in mind. The colour of the basidiocarp is described as violet-red, even from the beginning, with the lamellae having the same tint or even more pronounced, more bright. The stipe is described as short, tapering downwards and pruinose, whitish. It is described as a species fruiting slightly later in the year, when the first frosts are nearing. These are all typical features of *L. vinosus*. QUÉLET (1881) explicitly refers to the work by Barla but changes the epitheton from *violaceus* to *vinosus* (*Lactarius sanguifluus* var. *vinosus*). Quélets name is thus an illegitimate nomenclatural synonym (art. 52 ICBN, GREUTER *et al*., 2000). BATAILLE (1908) is the first author to use the epitheton *vinosus* on species level (although the rank Bataille used is disputable). Even though its basionym is illegitimate, this combination has to be considered as a legitimate nom. nov. (art. 58 ICBN, GREUTER *et al*., 2000) and thus the name *L. vinosus* has to be used for this taxon on species level (art. 11.2 ICBN, GREUTER *et al*., 2000). The correct author citation, taking into consideration art. 46.3 ICBN (GREUTER *et al*., 2000) is thus *L. vinosus* Bataille, or *L. vinosus* (Quél. →) Bataille, as proposed by KUYPER & VAN VUURE (1985) to refer to a replaced illegitimate name that typifies the newly created name. LALLI *et al.* (2003) interpret *Agaricus deliciosus* var. *violaceus* Barla as identical to *L. sanguifluus,* and argue that if Barla had known *L. sanguifluus* he would have made his var. *violaceus* subordinate to the latter. They do not find a discriminating character between *Agaricus deliciosus* var. *violaceus* and *L. sanguifluus* in the later description (BARLA, 1859). In the description of QUÉLET (1881, 1888), who explicitly refers to BARLA (1855) and produces only a simple interpretation of his drawing, they interpret *L. sanguifluus* var. *vinosus* as a discoloured form of *L. sanguifluus*. Strengthened by MARXMÜLLER & ROMAGNESI's (1991) concept of *L. sanguifluus* var. *vinosus*, they propose the new name *L. rubrozonatus* for what we call *L. vinosus* in this work. Furthermore they describe *L. rubrozonatus* f. *carneus* for the paler, discoloured forms.

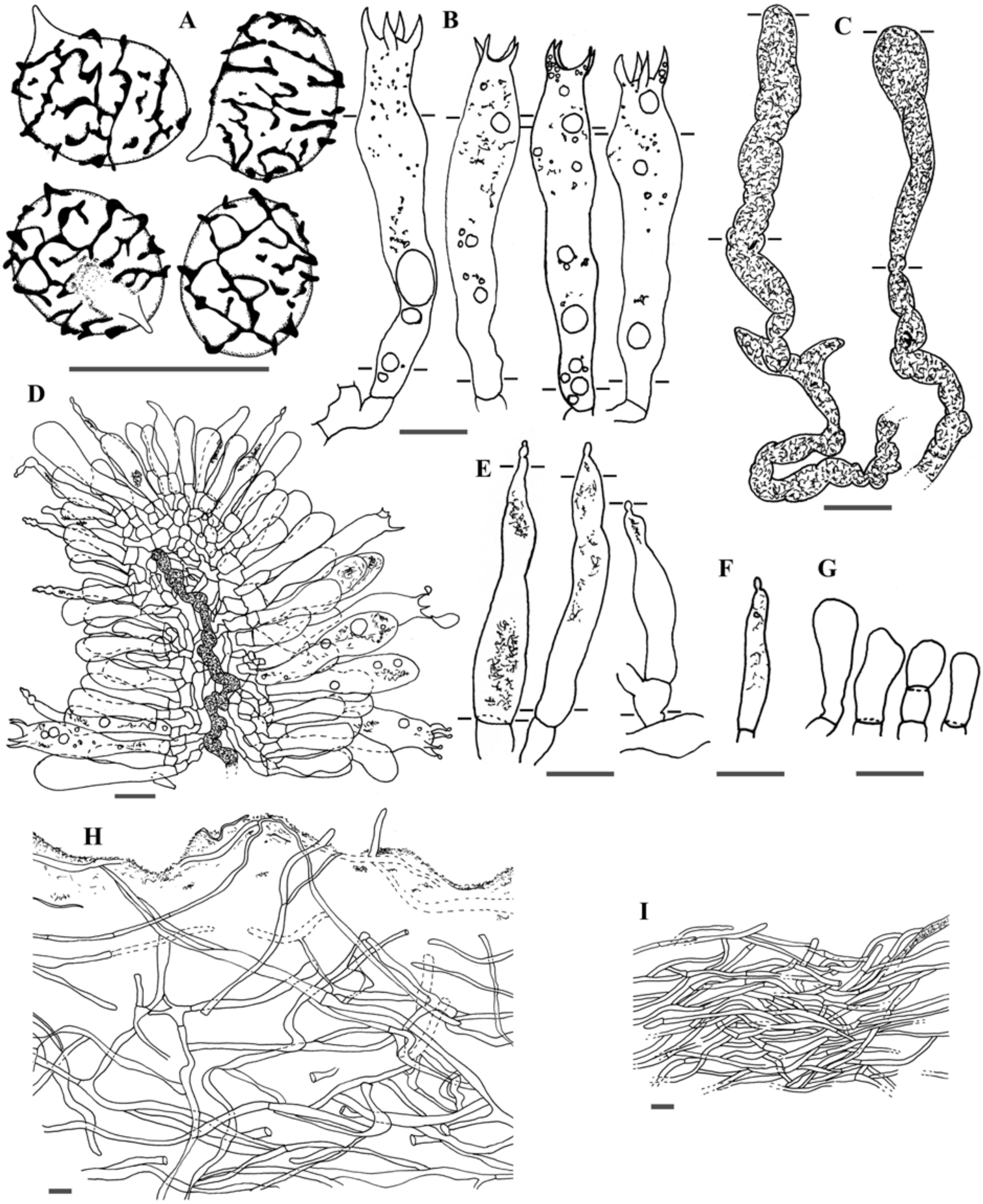

Fig. 21: *Lactarius vinosus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** lamella edge; **E.** pleuromacrocystidia; **F.** cheilomacrocystidium; **G.** cheiloleptocystidia; **H.** section through the pileipellis; **I.** section through the stipitipellis. From M.T. Basso 961104 and J. Nuytinck 2001-104, 2001-122, 2003-058. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Several old publications describe taxa that correspond also well to *L. vinosus*, *L. sanguifluus* or a discoloured form of them; no type specimens were indicated and no certainty exists about their identity:
PANIZZI (1862) describes *Lactarius deliciosus* var. *violascens* from the region of Baiardo (Imperia, Italy). The description corresponds best to *L. vinosus*: the cap is zonate, the stipe is '*subconicus*', whitish-violet and pitted, the lamellae are '*livido-violascentes*'. This taxon could maybe also correspond to a form of *L. sanguifluus*: the cap colour is described as pale orange.
L. sanguifluus var. *yvreus* C. Martin seems to be very similar in every aspect to a discoloured form of *L. vinosus*. MARTIN (1894) thinks var. *yvreus* is different from *L. sanguifluus* because it often has a hollow stipe which is amethyst coloured and not reddish-orange and because the lamellae have an amethyst sheen and are never cream to pinkish-orange. He also mentions this taxon is very close to Barla's var. *vinosus*.
From the short description of *L. haemorrheus* (Lowe in COOKE, 1888), collected in Madeira, we can only decide that this taxon must also correspond to a form of *L. sanguifluus* or *L. vinosus*.

5. Insufficiently known and doubtful taxa from Europe

All names that are listed here are considered doubtful, insufficiently known or could not with certainty be synonymised with one of the well-known taxa.

aeruginosus. *Lactarius deliciosus* var. *aeruginosus* E. Bommer & M. Rousseau, Bull. Soc. Roy. Bot. Belgique 18: 94 (1879), nom. inval., nom. nud.
BOMMER & ROUSSEAU (1879) mention '*sous les sapins entre Boisfort et Auderghem. Août-septembre*' for this variety, without further description. No type was designated.

atrovirens. *Lactarius deliciosus* var. *atrovirens* J. Blum, Lactaires: 217 (1976)
This variety is described as very small and deep green in the pileus, stipe and lamellae from the beginning. The type is missing from PC. Given the fact that many *Deliciosi* species start as deep blue-green buttons, it is hard to draw any further conclusions. This name should be treated as a nomen dubium.

flammeolus. *Lactarius flammeolus* (Pollini: Fr.) Fr., Epicr. Syst. Mycol.: 341 (1838)
≡ *Agaricus flammeolus* Pollini, Giorn. Fis. 9: 34 (1816)
≡ *Lactifluus flammeolus* (Pollini: Fr.) Kuntze, Revisio Generum Plantarum, Pars II: 856 (1891)
See under *L. salmonicolor.*

haemorrheus . *Lactarius haemorrheus* Lowe, Grevillea 16: 121 (1888)
See under *L. vinosus.*

rubrifluus. *Lactarius rubrifluus* Gillet, Bull. Soc. Linn. Normandie II 4: 255 (1870)
Gillet describes *L. rubrifluus* very elaborately, especially when considered the early time of publication, but no type material was selected. Unfortunately we cannot decide from his description with which currently used name *L. rubrifluus* coincides. The description bears much resemblance with *L. deterrimus* and *L. semisanguifluus* but two key characters are lacking to decide upon the identity: the host species ('sous sapins' was used for any coniferous tree) and the rate at which the colour of the latex changes. This taxon was subject for debate several times. SCHAEFER (1970) interpreted the description as *L. pinicola* (= *L. deliciosus* sensu most current authors). ROMAGNESI (1958), GRÖGER (1968) and BASSO (1999a) share the same opinion and stay inconclusive about eventual synonymy with *L. deterrimus* or *L. semisanguifluus*. We consider *L. rubrifluus* a nomen dubium for the sake of nomenclatural stability.

violaceocaeruleus. *Lactarius violaceocaeruleus* Voglino, Boll. Soc. Bot. Ital. 1894: 122 (1894)

This very remarkable find by VOGLINO (1894) under *Castanea* (!?) is described as having a violet-blue cap, abundant blue milk and blue coloured lamellae. According to SINGER (1942) it is identical to *L. indigo*. As SCHAEFER (1970) already indicates, this is probably not true as the stipe in *L. violaceocaeruleus* is pale ochre-yellow and the cap is not zonate and violet-blue coloured and thus different from *L. indigo*. The description by Voglino also reminds of the very recently described *L. cyanopus*. BASSO (1999a) writes that *L. cyanopus* differs from *L. violaceocaeruleus* by the habitat, the colour of the cap and lamellae, and the colour of the latex that is only partially blue in *L. cyanopus*.

violascens. *Lactarius deliciosus* var. *violascens* Panizzi, Comment. Soc. Crittog. Ital. 1: 174 (1862)
See under *L. vinosus.*

yvreus. *Lactarius sanguifluus* var. *yvreus* C. Martin, Bull. Trav. Soc. Bot. Genève 7: 184 (1894)
See under *L. vinosus.*

6. Excluded taxa

L. deliciosus **var.** ***tenuis*** **Naveau,** Natuurw. Tijdschr. 5: 70 (1923)
Holotype: BR-130746,87 (BR !), Belgium : Peerdsbos (Herentals, near Antwerpen)
Spores ellipsoid, ~ 7.5 × 6 µm (Q = 1.30); ornamentation clearly deformed with rounded, dropplet-like warts, breaking off easily, warts 1 µm high and higher, connected by fine, low and often unclear ridges.
COLLECTIONS EXAMINED: Belgium, Peerdsbos (Herentals, near Antwerp), Muscinetum under *Fagus*, 26.09.1920, R. Naveau, BR-130746,87 (BR), holotype.
DISCUSSION: Although the type material is deformed (probably by age) we exclude the possibility that this taxon belongs to *Lactarius* sect. *Deliciosi*. The spores are too small for *L. deliciosus*. This taxon could belong to *Lactarius* sect. *Zonarii* or sect. *Piperites*.

Acknowledgements

The curators of the herbaria BR, LIP, MPU & PC and P.A. Moreau & M. Contu are thanked for providing herbarium material. We would also like to thank M.T. Basso, A. Gminder, J. Heilmann-Clausen, P.J. Keizer, C. Lange, M.A. Pérez-De-Gregorio, J. Vesterholt, R. Walleyn and all other collectors listed in the examined specimens lists. Ruben Walleyn is thanked for the help with the nomenclature and Chiel Noordeloos is thanked for the review. The research of the first author is financed by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT), Belgium.

Chapter 7

A taxonomical treatment of the North and Central American species in *Lactarius* sect. *Deliciosi*

Adapted from:
NUYTINCK J., MILLER S. & VERBEKEN A. (2005) – A taxonomical treatment of the North and Central American species in *Lactarius* sect. *Deliciosi* (Russulales). Will soon be submitted.

Abstract

This paper deals with the 13 American species in *Lactarius* sect. *Deliciosi* that were confirmed or revealed in a molecular phylogenetic study of this section on a worldwide scale (NUYTINCK *et al.*, in preparation). Elaborate macro- and microscopical descriptions are given and illustrated for every species. Type specimens for nearly all taxa were examined in this study. None of the taxa treated here seems to occur in Asia or Europe. The name *L. deliciosus* is misapplied in North America but more research is needed to solve the taxonomy of and relationships in this complex of varieties.

1. Introduction

In North and Central America, *L. indigo* (Schwein.) Fr. is the most striking representative of *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl., syn. subgenus *Lactarius* sensu HESLER & SMITH, 1979). It differs from the other species in the section by its deep blue coloured latex. Most representatives have orange latex that more or less slowly turns reddish or have vinaceous red to brown latex from the beginning. The basidiocarps are similarly coloured but often paler due to the presence of a whitish layer covering the surface of the pileus and stipe. Other characteristic features are the mostly viscid pileus, and in some species also the viscid stipe, the often zonate pileus, the frequent presence of scrobicules and the green stains that develop upon bruising or with age.

L. indigo was also the first species that was described from North America in this section (as *Agaricus indigo* by VON SCHWEINITZ, 1822). PECK (1872, 1878, 1898) contributed to the knowledge of *Lactarius* sect. *Deliciosi* in America by describing *L. chelidonium* Peck, *L. subpurpureus* Peck and *L. salmoneus* Peck. COKER (1918), BEARDSLEE & BURLINGHAM (1940) and MURRILL (1945) furthermore described *L. curtisii* Coker, *L. paradoxus* Beardslee & Burl., *L. pseudodeliciosus* Beardslee & Burl. and *L. paradoxiformis* Murrill. HESLER & SMITH (1960) published an important revision in which they divide *Lactarius* sect *Lactarius* (= sect. *Deliciosi*) in three subsections. The pileipellis structure was considered very important. *Lactarius* subsection *Versicolores* Hesler & A.H. Sm. contains species with a dry pileus: *L. salmoneus*, *L. curtisii* and *L. subaustralis* Hesler (the latter species was later transferred to *Lactarius* subgenus *Plinthogali*). *Lactarius* subsection *Lactarii* contains species with a viscid pileus and thus includes most species; a separate subsection *Caerulei* Hesler & A.H. Sm. was created for *L. indigo*. Most European species known at that time were treated in HESLER & SMITH (1960); some of them were also recognised to occur in North America (*L. salmonicolor* R. Heim & Leclair, *L. sanguifluus* (Paulet) Fr., *L. deliciosus* (L.: Fr.) Gray var. *deliciosus* and *L. quieticolor* Romagn.). In their later monograph of the genus *Lactarius* in North America, HESLER & SMITH (1979) raise the section to the level of subgenus and give up their subdivision of *Lactarius* subgenus *Lactarius* (= sect. *Deliciosi*). Furthermore, many European taxa are no longer included; the European names still present are *L. salmonicolor*, *L. deliciosus* var. *deliciosus*, var. *deterrimus* (Gröger) Hesler & A.H. Sm. and var. *piceus* Smotl. However, in an appendix listing extralimital species that have been reported form North America and are expected to be eventually verified, they mention many European taxa. Four new species and varieties were described by HESLER & SMITH (1979) (*L. barrowsii* Hesler & A.H. Sm., *L. indigo* var. *diminutivus* Hesler & A.H. Sm., *L.*

rubrilacteus Hesler & A.H. Sm. and *L. deliciosus* var. *olivaceosordidus* Hesler & A.H. Sm.) and four recombinations were made. The many recognised varieties indicate uncertainty about the status of several taxa, but HESLER & SMITH (1979) explicitly state they wanted to stress the diversity in the genus. Since then, only two new species have been discovered: *L. miniatosporus* Montoya & Band.-Muñoz and the hypogeous *L. rubriviridis* Desjardin, Saylor & Thiers from Central and North America respectively (MONTOYA & BANDALA, 2004; DESJARDIN, 2003).

A molecular approach, using two DNA regions and including 33 North and Central American collections, confirmed 13 species in *Lactarius* sect. *Deliciosi* for the area (NUYTINCK *et al.*, in preparation). Many of the described varieties however were not included. This paper lists all the species in *Lactarius* sect. *Deliciosi*, we provisionally recognise in North and Central America. For every species a macroscopical description is given, often based on literature data, and a detailed microscopical description is elaborately illustrated. A short discussion summarizes the most important diagnostic features per species and the conclusions of our molecular phylogenetic approach (NUYTINCK *et al.*, in preparation). For most species, the type specimen was examined. Most problematic remains the status of the many varieties and the delimitation of taxa in the "*L. deliciosus*" complex. Moreover, the molecular data prove that the name *L. deliciosus* is misapplied in North and Central America (NUYTINCK *et al.*, in preparation).

2. Materials & Methods

This study is based on herbarium material deposited in or obtained from FLAS, GENT, MICH, NY, NYS, PC, RMS, SFSU, XAL and ZT.

Macroscopical characters are based on fresh material or are compiled from literature. Colour codes are from KORNERUP & WANSCHER (1962). Microscopic measurements and drawings were made under oil immersion at 1000× with a Zeiss Axioscop 2 microscope and drawing tube. All observations and measurements (except for the spores) were made in Congo red in L_4 (7.2 g KOH, 160 ml glycerine, 840 ml dH_2O, 7.6 g NaCl and 5 ml Invadin (Ciba-Geigi), CLÉMENÇON, 1972). Where necessary a short pre-treatment in 10% KOH was used to rehydrate the tissue. Basidia lengths exclude sterigmata lengths. We use the term cheiloleptocystidia for the thin-walled, mostly clavate to irregularly shaped cystidia, without specific content and with a rounded apex on the lamella edge. Observations and measurements of basidiospores where made in Melzer's reagent. Spores were measured in side view, excluding ornamentation and the measurements are given as (MIN) [Ava-2×SDa] – *Ava* – *Avb* – [Avb+2×SDb] (MAX) in which Ava = lowest mean value for the measured collections, Avb = greatest mean value and SDa/b = standard deviation of the lowest and greatest mean value respectively. MIN is the lowest value measured, MAX the highest value; MIN and MAX are only given when they exceed [Ava-2×SDa] or [Avb+2×SDb] respectively. Q stands for 'quotient length/width' and is given as MINQ – *Qa* – *Qb* – MAXQ in which Qa and Qb stand for the lowest and the highest mean quotient for the measured specimens respectively. MINQ/MAXQ stands for the minimum/maximum value over the quotients of all available measured spores. 20 spores were measured for every collection mentioned in the examined collections section. For species that are microscopically described using only one specimen, the measurements are given as (MIN) [Av-2×SD] – *Av* – [Av+2×SD] (MAX) in which Av is the mean value of the 20 spores measured from that collection and as MINQ – *Q* – MAXQ in which Q stands for the mean quotient of the measured spores.

Scanning electron photographs were taken with a JEOL JSM-5600 LV microscope. Small pieces of lamellae were taken from dried specimens and soaked overnight in strongly diluted ammonia. The material was then treated with 70% ethanol (2 × 15 min.) and dimethoxymethane (2 × 30 min.), before being submitted to the process of critical point drying. This was done with a BAL-TEC CDP 030 dryer. The samples were then coated with gold in a JEOL JFC-1200 Fine Coater for 60 sec. at 8 Pa and 30 mA, until a 15 nm thick layer covered the spores.

3. Descriptions and taxonomical conclusions

3.1. *Lactarius barrowsii* Hesler & A.H. Sm., N. Amer. Species Lactarius: 74 (1979)

Figs. 1, 2

Type: Barrows 35 of 1969 (MICH!), USA: New Mexico, near Santa Fe

DESCRIPTION (macroscopical description after HESLER & SMITH, 1979): *Pileus* 3–10 cm diam., convex to depressed with an incurved margin; surface glabrous, viscid but soon dry, azonate or only near the margin faintly zoned; colour whitish to light pinkish cinnamon at first, becoming mottled creamy to ochraceous orange or finally ± pinkish buff, often flushed green or olive or more olive-grey. *Lamellae* becoming decurrent, close, narrow; colour ochraceous to pinkish orange, becoming flushed green, green where injured. *Stipe* 2–4 × 1–2.5 cm, equal or nearly so, soon hollow; surface dry, not scrobiculate, at first with a white bloom, yellowish beneath this but soon stained green. *Context* firm, thick; colour whitish to pale pinkish cinnamon (especially around worm-holes), becoming flushed greenish, staining green quickly where cut; taste mild to peppery. *Latex* scanty, dark red (port-wine red). *Spore deposit* yellowish.

Spores 8.5–*9.3*–*9.4*–10.1 (10.4) × 6.3–*7.0*–*7.1*–7.6 (7.8) µm, broadly ellipsoid to ellipsoid (Q = 1.22–*1.31*–*1.34*–1.48); ornamentation up to 0.5 µm high, of medium thick ridges with some thinner ridges and isolated warts, forming a nearly complete reticulum with small meshes; plage distally weakly amyloid. *Basidia* 42–60 × 7–11 µm, subclavate, 4-spored, with a needle-shaped content. *Pleuromacrocystidia* scarce and inconspicuous, 39–50 × 4.7–8 µm, slightly emergent, subfusiform with a moniliform or narrowing apex, often with an ochre, smooth content or with small needle-shaped crystals, thin-walled. *Pseudocystidia* rather abundant, 2-6 µm broad, sometimes slightly emergent, tortuous, content oleiferic and ochre-brownish in KOH. *Lamella edge* mostly fertile and with abundant cheilomacrocystidia and basidioles; cheilomacrocystidia 34–50 × 5–7 µm, emergent, subfusiform with a moniliform to obtuse or capitate apex, with a needle-like to granular content, thin-walled. *Subhymenium* composed of small, ± rounded cells. *Hymenophoral trama* irregularly filamentous; lactifers abundant. *Pileipellis* an ixocutis, 100–200 µm thick, slime layer thin and inconspicuous, composed of regularly shaped, densely interwoven hyphae, 2–7 µm diam., shrivelled hyphae scarce. *Stipitipellis* a very dense cutis, up to 100 µm thick, of regularly shaped hyphae, 2–4 µm diam. *Clamp-connections* absent.

HABITAT: Scattered under *Pinus ponderosa* and *Pinus cembroides* in montane habitat (type locality, HESLER & SMITH, 1979).

DISTRIBUTION: Reported from New Mexico, Washington and Arizona.

COLLECTIONS EXAMINED: USA, New Mexico, Santa Fe Co., Near Santa Fe, Under Pinon pine, 01.09.1969, C.A. Barrows 35 (MICH), holotype – Washington, Spokane, Whitworth College Campus near MacKay Hall, 18.09.1978, leg. N. Gray (RMS) – Arizona, Cochise Co., east side Onion Saddle, Turkey Creek Road, Coal outcrop, 1620 m a.s.l., 03.09.1993, J. States AEF 987 (MICH) – Arizona, Cochise Co., Pinery Canyon Road, *Pinus*, 04.09.1993, J. States AEF 1039 (MICH) – Arizona, Santa Cruz Co., Santa Rita Mountains, 03.09.1994, J. States AEF 1271 (MICH) – Arizona, Coconino Co., Walnut Canyon National Monument, *Pinus edulis*, N35°10' W111°30.26', 10.09.1994, J. States AEF 1151 (MICH).

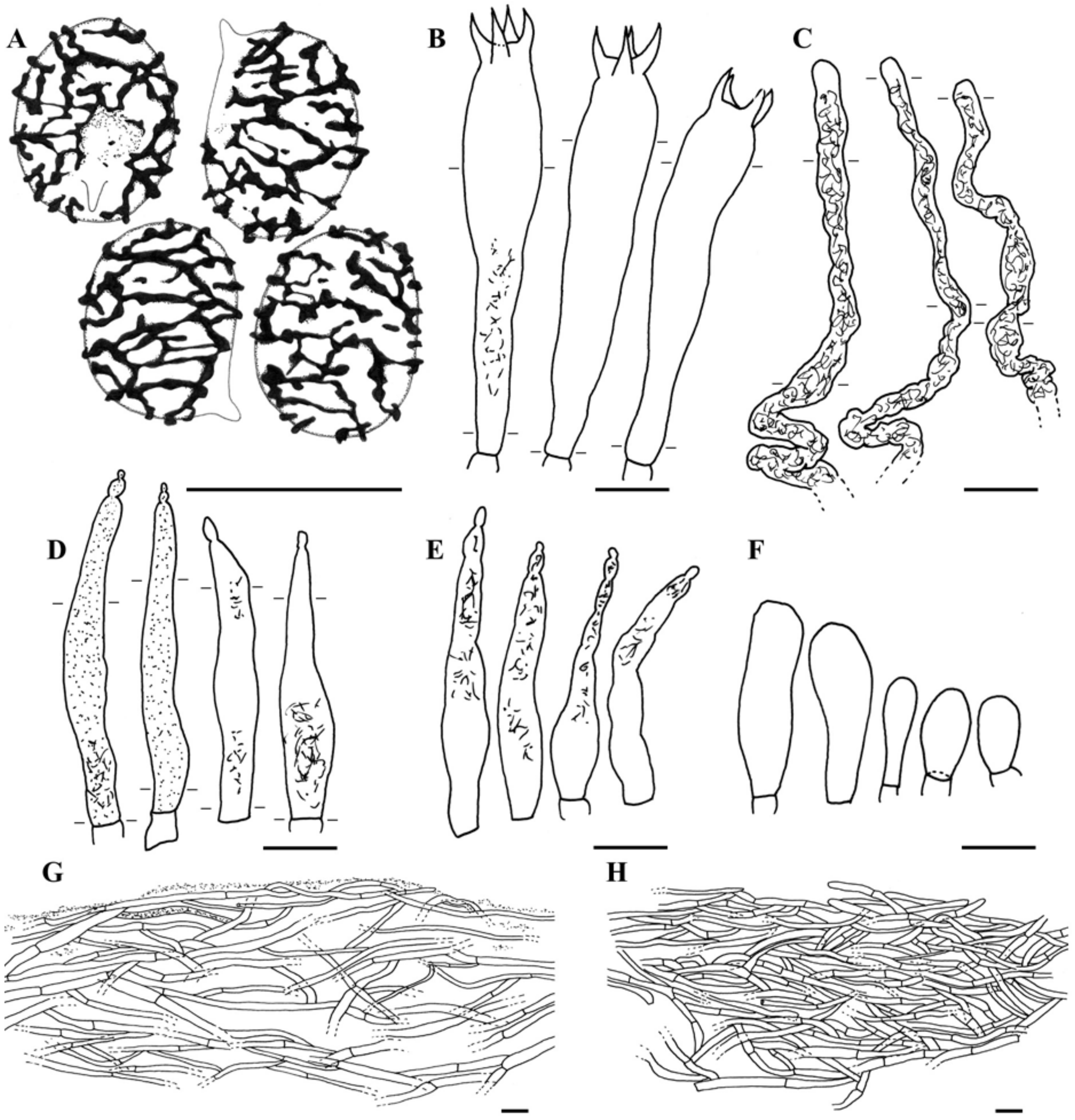

Fig. 1: *Lactarius barrowsii*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** basidioles and marginal cells frm the lamella edge; **G.** section through the pileipellis; **H.** section through the stipitipellis. From C.A. Barrows 35 (type), J. States AEF 987, J. States AEF 1039, J. States AEF 1271 and J. States AEF 1151. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: HESLER & SMITH (1979) remark that this species is amply distinct in the colour of the pileus from the other western American species with red latex, *L. rubrilacteus*. The latter species has an orange coloured and clearly zoned pileus. Both species also differ in spore size and ornamentation. *Lactarius barrowsii* has larger spores that are ornamented with a denser and more complete reticulum. Another striking feature of *L.barrowsii* is the frequently fertile lamella edge and as a consequence the occurrence of basidioles on the edge. *Lactarius barrowsii* is so far only know from New Mexico, Washington and Arizona and seems to be associated with *Pinus* spp. in mountainous habitat. We included two *L. barrowsii* specimens (originating from Arizona and Washington) in our phylogenetic analyses. They form a well-supported clade and group with a high confidence value with *L. rubriviridis*, a hypogeous species described from California and collected in the same type of habitat as *L. barrowsii. Lactarius barrowsii* does not seem to be very closely related to *L. rubrilacteus*.

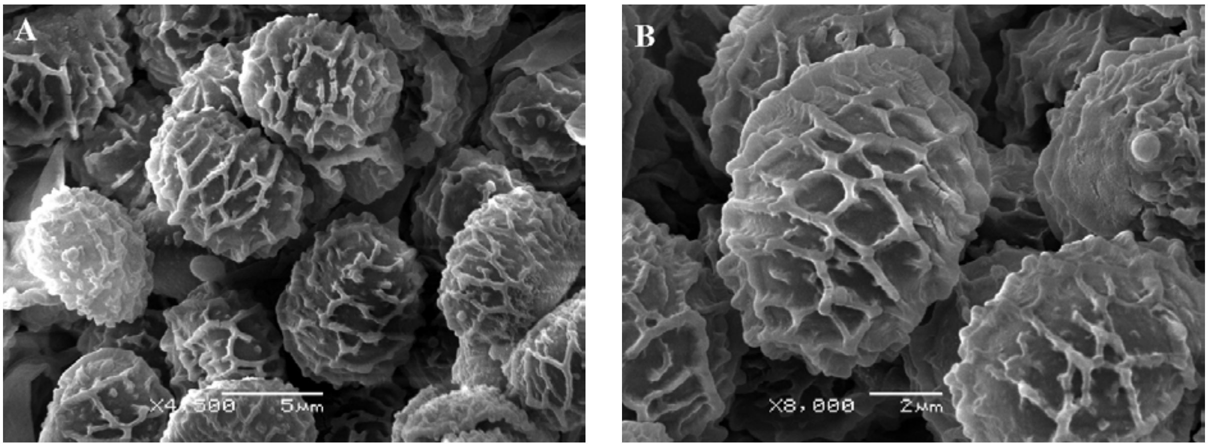

Fig. 2: A., B. SEM images of the spores of *L. barrowsii* J. States AEF 1151.

3.2.1. *Lactarius chelidonium* Peck, Rep. (Annual) New York State Mus. Nat. Hist. 24: 74 (1872) var. *chelidonium*

Figs. 3, 4

Holotype: missing from NYS, collected by Peck in New York, Saratoga.

DESCRIPTION (macroscopy after PECK, 1872): *Pileus* fleshy, firm, centrally depressed; surface smooth, slightly viscid, of a greyish green colour with blue and yellow tinges and a few narrow zones on the margin. *Lamellae* close, narrow, forked and wavy at the base, sometimes anastomosing; colour greyish yellow. *Stipe* short, subequal, hollow; surface smooth; coloured like the pileus. *Context* when bruised at first stained yellowish, then changing to blue and finally to green. *Latex* sparse, of a yellowish colour resembling the juice of Celandine (*Chelidonium majus*) or the liquid secreted from the mouth of grasshoppers; taste mild. *Spore deposit* yellowish.

Spores (8.0) 8.1–*8.6*–9.2 (9.3) × 5.9–*6.4*–7.0 µm, broadly ellipsoid to ellipsoid (Q = 1.24–*1.35*–1.48); ornamentation up to 0.5 µm high, of medium thick ridges and quite abundant isolated warts, forming an incomplete reticulum; plage distally amyloid or with scattered, small amyloid spots. *Basidia* 40–55 × 8–10 µm, subclavate, 4-spored, sometimes with a needle-shaped content; sterigmata 4–6 µm long. *Pleuromacrocystidia* scarce, 45–65 × 4.5–7 µm, emergent, subfusiform with a narrowed to strongly constricted (moniliform) apex, sometimes with a needle-shaped content, thin-walled. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia 12–25 × 3–6 µm, subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia 30–40 × 4.5–7 µm, emergent, subfusiform with a tapering, capitate or moniliform apex, with a needle-like content, thin-walled. *Subhymenium* of irregularly shaped cells, often arranged in short rows. *Hymenophoral trama* with abundant lactifers of 4–9 µm diam. *Pileipellis* an ixocutis, 100-200 µm thick. *Clamp-connections* absent.

HABITAT: Sandy soil, under pine trees (type locality; PECK, 1872).

DISTRIBUTION: HESLER & SMITH (1979) cite collections from Alabama, Michigan, Tennessee and Wisconsin. The type locality is in New York.

COLLECTIONS EXAMINED: USA, New York, Bolton Landing, August 1921, C.H. Peck (NYS) – New York, Bethlehem, September 1921, C.H. Peck (NYS) – New York, St. Lawrence Co., near Star Lake, pine woods, 26.09.1997, S.L. Miller 9649 (RMS).

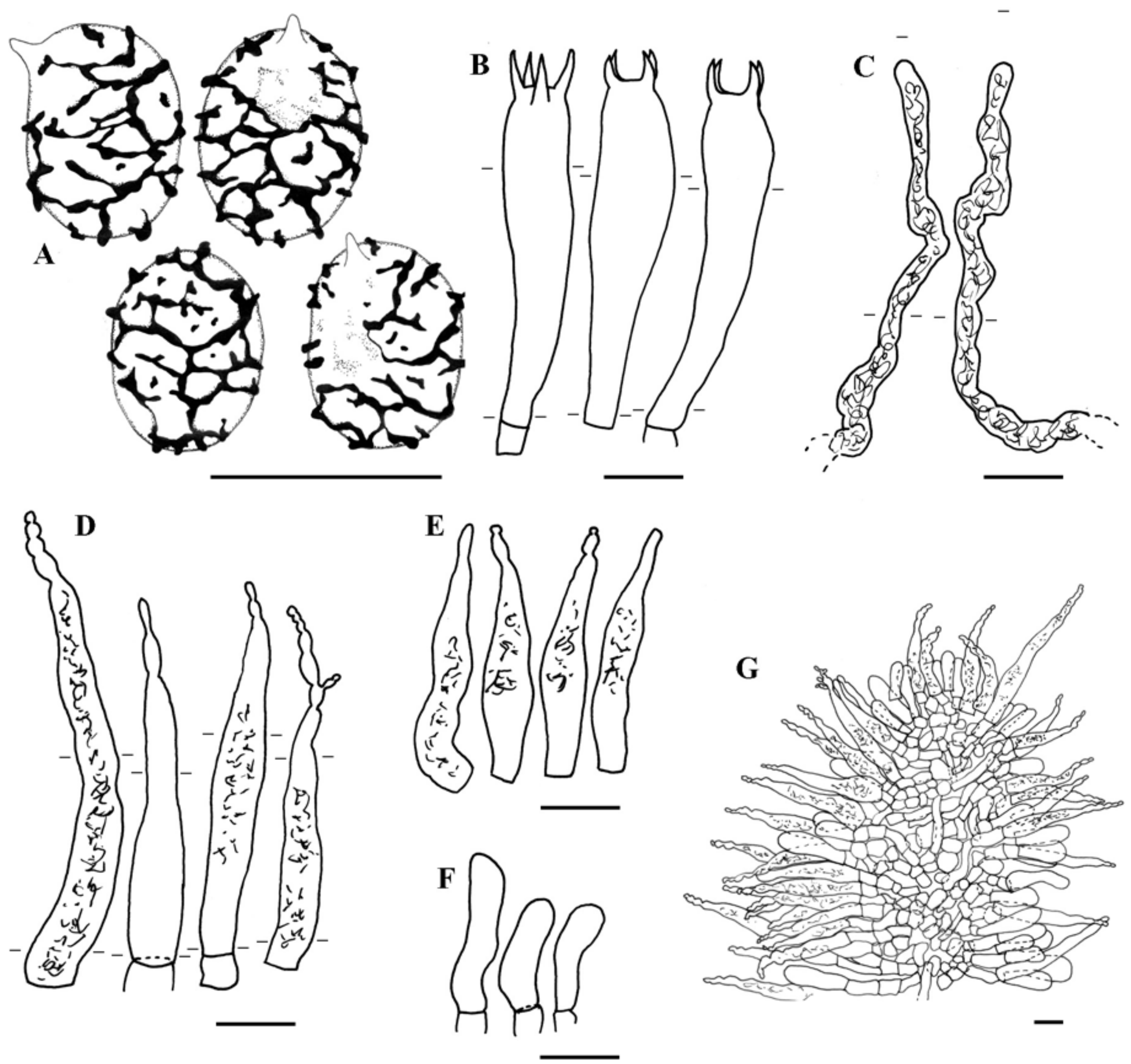

Fig. 3: *Lactarius chelidonium* var. *chelidonium*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** lamella edge. From Bethlehem, September 1921, C.H. Peck and S.L. Miller 9649. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: HESLER & SMITH (1979) state that *L. chelidonium* has been generally misidentified since Peck's time, which has led to much confusion with "*L. deliciosus*". The yellowish colour of the latex and the greyish and bluish tinges in the cap should make a clear distinction between both species possible. The blue colour of the pileus context is independent of the very commonly observed change to green in this section. This has been wrongly interpreted since the time of Peck (HESLER & SMITH, 1979). As the type of *L. chelidonium* is missing, we examined two collections identified by Peck. The collection from Bolton landing however is in a very bad state.
Collection S.L. Miller 9649 (RMS) from New York was identified as *L. chelidonium* in the field and is included in our molecular analyses. Because the microscopical features are slightly deviating from the ones observed in Peck's material, a short description is given here separately.
Spores 7.8–*8.4*–8.9 × 5.8–*6.4*–6.9 (7.0) µm, broadly ellipsoid to ellipsoid (Q = 1.25–*1.32*–1.37); ornamentation up to 0.5 µm high, of medium thick ridges and some isolated warts, forming an incomplete reticulum; plage distally amyloid or with scattered amyloid spots. *Basidia* 37–55 × 8–12 µm, subclavate, mostly 4-spored, sometimes 2-spored, mostly hyaline; sterigmata 4–7 (8) µm long. *Pleuromacrocystidia* quite abundant, 35–70 × 5.3–7 µm, emergent, subfusiform with a narrowing to

moniliform apex, with a granular or needle-shaped content, thin-walled. *Pseudocystidia* relatively scarce, 2.5–5.5 µm broad, subcylindric to tortuous, with a yellowish content. *Lamella edge* sterile with very abundant cheilomacrocystidia; cheiloleptocystidia 12–30 × 4–6 µm, mostly subclavate, hyaline, thin-walled; cheilomacrocystidia 27–58 × 4–8 µm, subfusiform with a narrowing apex, with a granular to needle-shaped content, thin-walled.
This collection differs from the collections made by Peck by the abundant cheilo- and pleuromacrocystidia. Macrocystidia are only abundant in a zone of 100 µm, close to the lamella edge in Peck's collections. A picture of this collection is shown in Fig. 4 B, but doesn't allow a sure identification. More collections are needed to find out whether these differences fall into the intraspecific variation of *L. chelidonium*.

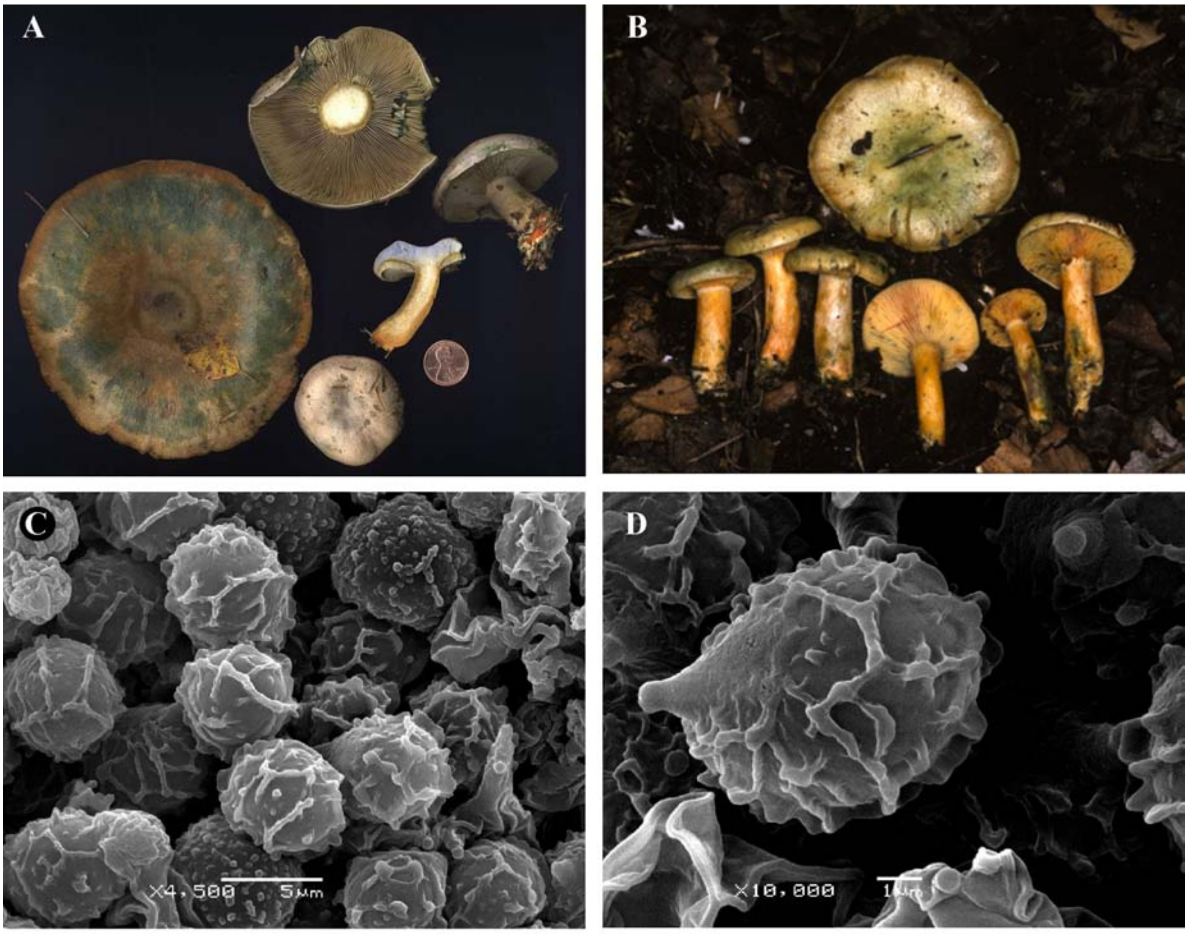

Fig. 4: **A.** *L. chelidonium* var. c*helidonioides* (photo M. Kuo); **B.** *L. chelidonium* S.L. Miller 9649; **C.** and **D.** SEM images of the spores of *L. chelidonium* S.L. Miller 9649.

3.2.2. ***Lactarius chelidonium*** **var.** ***chelidonioides*** **(A.H. Sm.) Hesler & A.H. Sm.**, N. Amer. Species Lactarius: 84 (1979)

Figs. 4, 5

Synonym: ≡ ***L. chelidonioides*** A.H. Sm., Brittonia 12: 127 (1960)

Holotype: Smith 62026 (MICH!), USA: Michigan, Dexter

DESCRIPTION (macroscopy after HESLER & SMITH, 1979 and KUO, 2003): *Pileus* 3–8 cm diam., applanate to convex or shallowly depressed, becoming shallowly infundibuliform; surface smooth or finely roughened, glabrous, viscid but soon dry, azonate to slightly zonate, often mottled with watery spots; colour dirty bluish when young, passing through stages of yellowish brown and dull orange, readily bruising dark green, in age often green overall. *Lamellae* decurrent, narrow, fairly crowded; colour dull dingy yellowish to tawny olive (yellow-brown) but soon discolouring greenish, in old basidiocarps olive buff overall and stained darker green locally. *Stipe* 3–6 (8) × 1–2.5 cm, equal or enlarged below; surface fairly smooth, glabrous, dry, without scrobicules; colour similar to the cap but paler and more dingy yellowish orange at the base. *Context* becoming hollow in the stipe, pallid, staining azure blue in the upper half of the pileus, with a paler to dingy yellowish line above the lamellae, dingy pale yellow-brown in the cortex of the stipe, in age azure blue throughout except for the line above the lamellae and dirty yellowish orange in the stipe; smell subnauseous, like that of *Morchella esculenta*; taste tardily and slightly peppery. *Latex* very scarse (likely to be observed only in very young primordia), dirty yellowish to yellowish brown. *Spore deposit* pale buff.

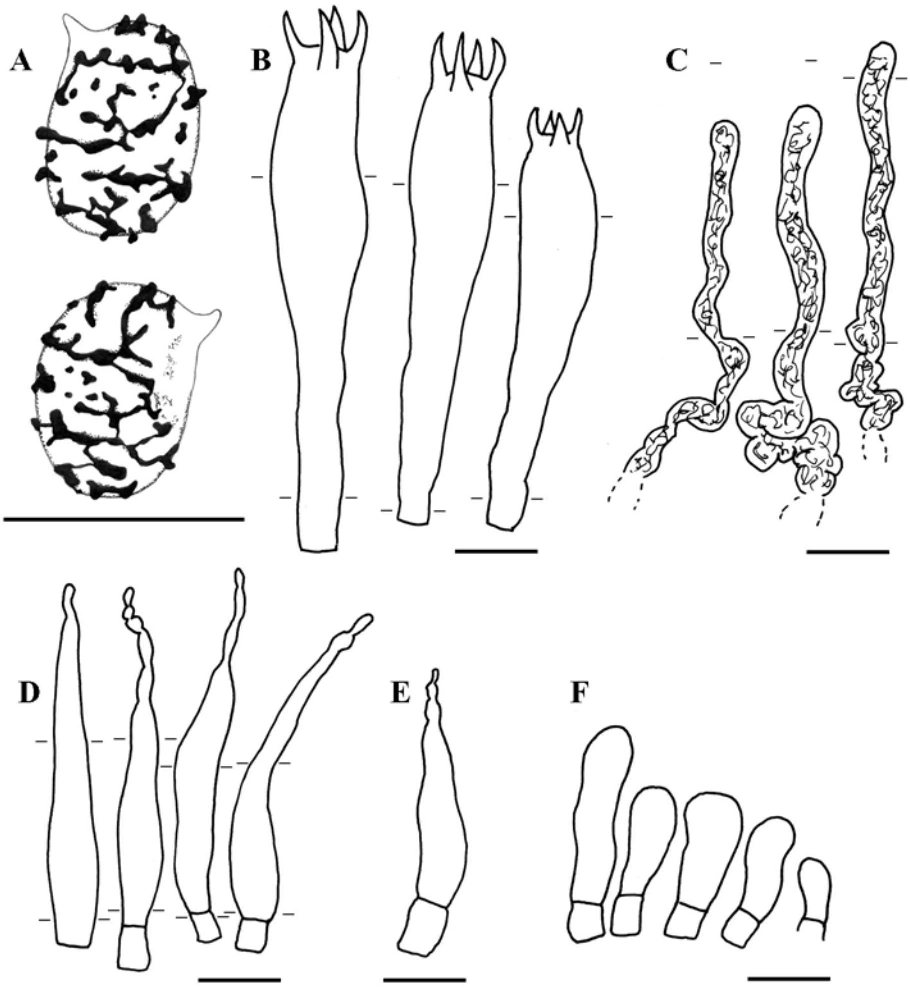

Fig. 5: *Lactarius chelidonium* var. *chelidonioides*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** macrocystidium close to the lamella edge; **F.** cheiloleptocystidia. From A.H. Smith 62026 (type). Scale bars = 10 µm, small bars indicate the height of the hymenium.

Spores 8.1–*8.7*–9.2 × 5.9–*6.6*–7.0 µm, broadly ellipsoid to ellipsoid (Q = 1.27–*1.35*–1.42); ornamentation up to 1 µm high, of large isolated warts and some rather short and medium thick ridges, not organized into a reticulum or only a very incomplete one; plage distally slightly amyloid. *Basidia* 40–65 × 8–11 µm, subclavate, 4-spored, mostly hyaline; sterigmata 3–7 µm long. *Pleuromacrocystidia* absent but macrocystidia abundantly present very close to the edge (see under lamella edge). *Pseudocystidia* rather scarce, 2.5–5 µm broad, cylindrical to tortuous, with an ochre-yellow content. *Lamella edge* sterile with abundant macrocystidia 100–150 µm away from the edge, no real cheilomacrocystidia; cheiloleptocystidia 7–22 × 3.5–7.5 µm, mostly subclavate, hyaline, thin-walled; macrocystidia close to the edge 40–60 × 4.5–10 µm, subfusiform, with a moniliform apex, mostly hyaline, thin-walled. *Subhymenium* or irregularly shaped cells, arranged in short rows. *Hymenophoral trama* of interwoven, mainly irregularly arranged hyphae and abundant lactifers. *Pileipellis* an ixocutis of narrow, gelatinous, hyaline and appressed hyphae. *Stipitipellis* a cutis. *Clamp-connections* absent.

HABITAT: Under conifers, especially *Pinus strobus* and *Pinus resinosa*, late summer and fall.

DISTRIBUTION: Uncertain but probably limited to eastern North America; recorded in Michigan, Massachusetts, Vermont, Illinois and Quebec (KUO, 2003).

COLLECTION EXAMINED: USA, Michigan, Washtenaw Co., Stinchfield Woods, NW of Dexter, Under *Pinus strobus*, 12.10.1972, A.H. Smith 62026 (MICH), holotype.

DISCUSSION: Originally, HESLER & SMITH (1960) described *L. chelidonioides* as a separate species, differing from *L. chelidonium* by its muddy yellow to brownish latex, its *Morchella*-like smell and the blue context of pileus. The colour of the context was observed at all stages from buttons 1 cm in diameter to the largest caps, and it became more intense and widespread up to maturity. In old basidiocarps the colour had faded out to greyish pallid (HESLER & SMITH, 1979). Because of the lack of well-defined differences, HESLER & SMITH (1979) re-evaluated the status of *L. chelidonium* and treated it as a variety of *L. chelidonium*.

Microscopically, the most striking difference between Peck's collections of *L. chelidonium* var. *chelidonium* and the holotype of *L. chelidonium* var. *chelidonioides* is the ornamentation of the spores. The ornamentation is clearly higher in var. *chelidonioides* and forms a less complete network. In many spores the warts and short ridges are not organised in a reticulate pattern at all.

This variety seems to be collected more frequently than the type variety (e.g. KUO, 2003); the latter differs from var. *chelidonioides* by its mild taste, its indistinct smell and the latex that is bright yellow at first (becoming a pale tobacco to dirty brown; HESLER & SMITH, 1979). More detailed research is needed on both varieties of *L. chelidonium* to decide on their taxonomic status.

3.3.1. ***Lactarius indigo* (Schwein.) Fr.**, Epicr. Syst. Mycol.: 341 (1838) **var. *indigo***

Figs. 6, 7

Synonyms:
≡ ***Agaricus indigo*** Schwein., Schr. Nat. Ges. Leipzig 1: 7 (1822)
≡ ***Lactifluus indigo*** (Schwein.) Kuntze, Revisio Generum Plantarum, Pars II: 857 (1891)
= ***L. canadensis*** Winder, Mushrooms Canada, addenda [24] (1871)

Excluded: ***L. indigo*** sensu IMAZEKI *et al.* (1988), NAGASAWA (1998) and probably several other authors (= *L. subindigo*) (see discussion under *L. subindigo*, Chapter 8).

Type: not typified, described from North Carolina.

DESCRIPTION: *Pileus* 5–15 cm diam., convex-depressed becoming deeply infundibuliform, with an inrolled margin at first; surface smooth (but actually with a lot of very small scroicules), slightly viscid, nearly azonate to clearly zonate, often with fine but clear zones near the margin; colour bright blue when fresh and moist, fading to greyish to almost white (21A2) with a silvery sheen; zones staying deeper blue (22A-C4/5). *Lamellae* slightly decurrent, with a small decurrent tooth, rather close, medium thick, sometimes forkedg near the stipe; colour bright indigo blue (21B5/6) or paler, sometimes appearing yellowish from the mature spores, staining green where bruised; edge entire, slightly paler. *Stipe* 2–8 × 1–2.5 cm, central to more rarely eccentric, cylindrical, equal or tapering downwards; surface smooth, viscid but soon dry, mostly scrobiculate; colour indigo blue but often much paler (23A2/3) and with a whitish top layer; scrobicules deep blue (22B/C4/5). *Context* firm, hollow in the stipe, whitish buff to yellowish in the stipe, when cut immediately indigo blue in almost the entire pileus surface and the margins of the stipe, becoming deep bluish green after 1 h (26E5); smell agreeable, quite strongly fruity; taste mild to very slightly acrid after some chewing. *Latex* not very abundant, bright to dark blue (19/20C/D8 to 23D/E7/8), slowly turning green on the context; taste mild. *Spore deposit* cream colour (3A3/4).

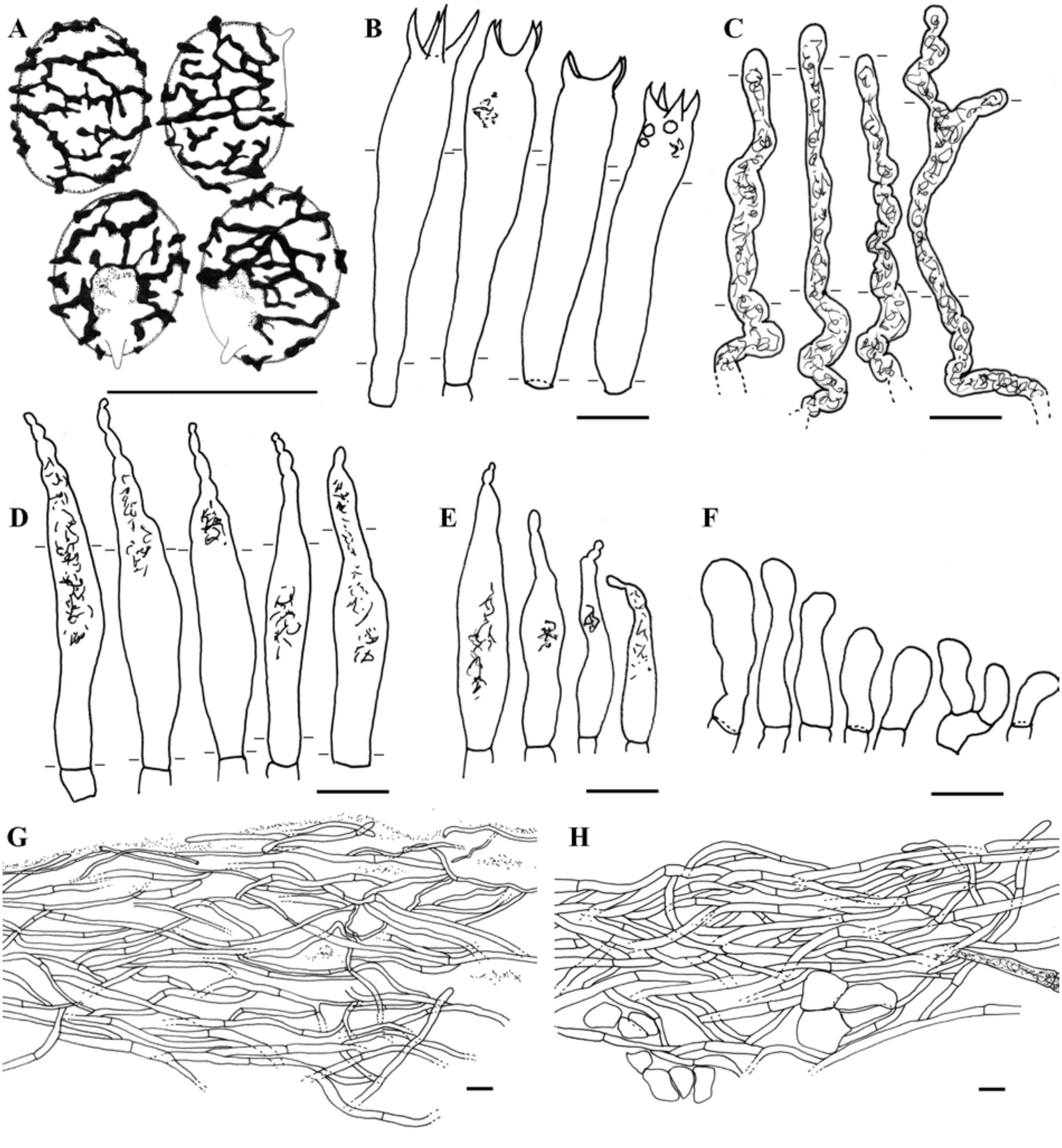

Fig. 6: *Lactarius indigo* var. *indigo*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystdia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From B. Buyck 01.158, B. Kropp 29-Oct.-00-15, R.E. Halling 8143, R.F. Cain 24421 and S.L. Miller 9579. Scale bars = 10 µm, small bars indicate the height of the hymenium.

$FeSO_4$ no reaction, *KOH* yellowish to pale watery orange on the pileus cuticle, stipe context and stipe cuticle, dirty orange on the hymenium, *gaiac* no reaction.

Spores (6.9) 7.0–*7.7*–*8.1*–8.9 (9.2) × 5.3–*5.9*–*6.2*–6.8 µm, subglobose to ellipsoid (Q = 1.19–*1.28*–*1.34*–1.45); ornamentation up to 0.5 µm high, of medium thick ridges and low warts, forming an incomplete reticulum; plage distally slightly amyloid. *Basidia* 37–53 × 7.3–10.5 µm, cylindrical to subclavate, 4-spored, sometimes containing oil-droplets; sterigmata 3–7 µm long. *Pleuromacrocystidia* very abundant near the edge, becoming scarcer further away, 30–62 × 4–8 µm, emergent, subfusiform with a moniliform apex, with a granular content, thin-walled. *Pseudocystidia* very abundant, 3–6 µm broad, tortuous, mostly not emergent, with a dark ochre content in KOH. *Lamella edge* sterile with scarce to very abundant cheilomacrocystidia; cheiloleptocystidia 8–25 × 3–6 µm, mostly subclavate, hyaline, thin-walled; cheilomacrocystidia 18–37 × 3–7.3 µm (up to 50 µm in some collections), subfusiform with a moniliform apex, emergent, with a granular content, thin-walled. *Subhymenium* composed of compactly arranged, short cells. *Hymenophoral trama* composed of irregularly interwoven hyphae; lactifers numerous. *Pileipellis* an ixocutis, 70–200 µm thick, of strongly interwoven hyphae, 1–5 µm diam.; shrivelled and gelatinised hyphae scarce; near the surface slender and strongly tortuous hyphae sometimes present. *Stipitipellis* a cutis, 50– 60 µm thick, of regularly shaped hyphae, (1) 3–6 µm diam., lactifers present close to the surface. *Clamp-connections* absent.

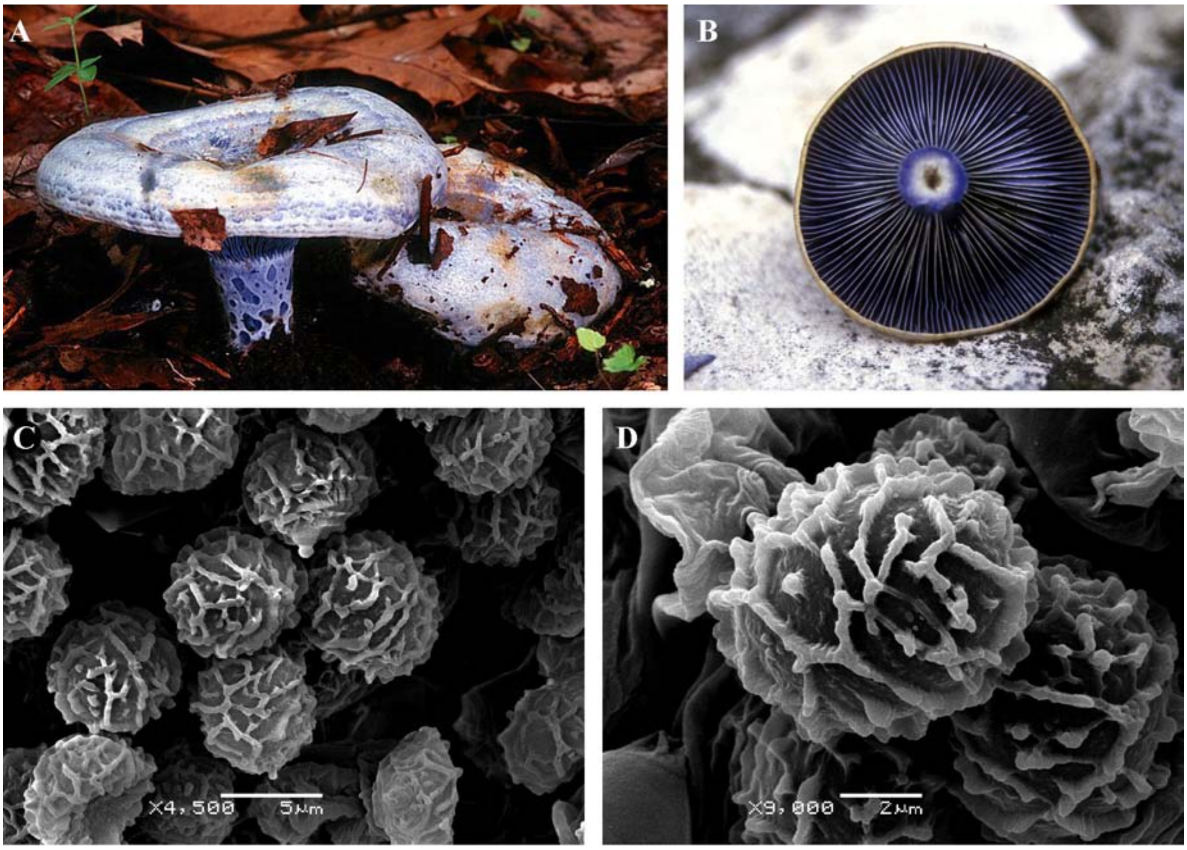

Fig. 7: **A.** *L. indigo* (photo P. Kaminski); **B.** blue coloured lamellae of *L. indigo* (photo L. Ryvarden); **C.** and **D.** SEM images of the spores of *L. indigo* R.E. Halling 8143.

HABITAT: Scattered to gregarious on soil, in both *Pinus* and *Quercus* woods.

DISTRIBUTION: Not particularly common but fairly widespread in its distribution, found throughout eastern North America, southwards to southern Colombia (R. Halling, pers. comm.).

COLLECTIONS EXAMINED: Belize, Mtn. Pire Ridge, Cayo district, *Pinus caribaea*, 29.10.2000, B. Kropp 29-Oct.-00-15 (RMS) – Cayo district, 03.08.2003, B. Kropp 3-Aug.-03-1 (RMS) – Costa Rica, B. Buyck 01.158 (PC) – Coto Brus, Las Mellizas, Zona Protectora Las Tablas, Finca La Cafrosa, Camino a Portones por El Tajo, *Quercus seemannii* & *Quercus* spp., scattered, on soil, 1475 m a.s.l., N8°55.034' W82°46.000', 07.06.2001, R.E. Halling 8143 (NY) – USA, R.F. Cain 24421 (ZT) – unknown, brought, West Virginia Mycological Association Foray in Green Briar State Forest, 09.09.1997, S.L. Miller 9579 (RMS) – Tennessee, Cocke Co., Greenbrier biological station (Tudor road), under Red Oak sp. and *Pinus* sp., 488 m a.s.l., N35°44.038' W083°25.45', 12.07.2004, J. Nuytinck 2004-002 (GENT).

DISCUSSION: Without a doubt, this is one of the most striking agarics seen anywhere. It is an unmistakable species, but HESLER & SMITH (1979) warn for confusion of dried out specimens with *L. paradoxus*. This species shows quite some variability in colour (from young to old specimens) and in the placement of the stipe that can be eccentric (HESLER & SMITH, 1979). The macroscopical description given here is compiled from our own observations, HESLER & SMITH (1979) and KONG LUZ (1995).

At the very base of the stipe a whitish orange mycelium can be observed, that scarcely exudes a reddish orange latex (7A7/8 to 8C8); pale reddish orange coloured rhizomorphs can also be present at the base of the stipe (KONG LUZ, 1995; P. Leacock, pers. comm.).

Found throughout eastern North America, *L. indigo* is distributed south to southern Colombia where it is associated with *Quercus humboldtii*. Tree partners in Costa Rica include *Q. seemannii, Q. copeyensis, Q. oocarpa, Q. oleoides*, *Q. corrugata* and *Q. costaricensis* (R. Halling, pers. comm.). *Lactarius indigo* tends to be associated with *Pinus* in the northern part of its distribution area but with *Quercus* when moving southwards (B. Buyck, pers. comm.). Records of *L. indigo* associated with *Pinus* are nonetheless known from Mexico (KONG LUZ, 1995).

There is a form (or perhaps a distinct taxon), found near Palo Verde in the northern Talamancas, that has narrow crowded lamellae, an azonate pileus and smaller stature (R. Halling, pers. comm.). We had no material available for our phylogenetic analysis.

Microscopically the small spores are characteristic. This character state is shared with the very similar but not very closely related *L. subindigo* Verbeken & Horak, known from SE Asia to India (VERBEKEN & HORAK, 2000; NUYTINCK & VERBEKEN, in preparation).

3.3.2. ***Lactarius indigo* var. *diminutivus* Hesler & A.H. Sm.**, N. Amer. Species Lactarius: 69 (1979)

Fig. 8

Holotype: Hillhouse 249 (MICH!), USA: Texas, Brazoria County

DESCRIPTION: *Pileus* 3–5 cm broad at maturity, plano-depressed becoming broadly infundibuliform, rarely remaining convex, margin inrolled at first; surface glabrous, viscid to slimy, when dry appearing unpolished (almost velvety); colour dark blue when young and fresh, grey-blue with a silvery sheen when dry, in age grey-green to olive green overall; margin often finely striate when moist, subzonate (zones visible at maturity only). *Lamellae* decurrent to longly decurrent, fairly broad, concolorous with the pileus, quickly staining dark indigo where cut or bruised and then slowly turning to grey-green or olive, green in age. *Stipe* 1.5–2.5 × 0.5–0.8 cm, solid, soon becoming hollow, tapering downwards, concolorous with the pileus. *Context* white but quickly changing to dark blue by the abundant latex, changing to green in ± 0.5 h. *Latex* deep indigo blue, unchanging. *Spore deposit* white in a thin deposit.

Spores 7.0–*7.5*–*7.6*–8.2 × 5.5–*5.9*-*6.0*–6.4 µm, broadly ellipsoid (Q = 1.18–*1.26*–*1.29*–1.34); ornamentation up to 0.5 µm high, of (rather) thick ridges, rarely intermixed with finer ridges, with some small isolated warts, forming an almost complete reticulum; plage distally very slightly amyloid. *Basidia* 40–55 × 7–10 µm, subclavate to almost cylindrical, 4-spored, sometimes containing oil-droplets; sterigmata 3–6 µm long. *Pleuromacrocystidia* apparently absent. *Pseudocystidia* rather abundant, 2.5–5.5 µm broad, tortuous to cylindrical, sometimes branching, oleiferic, ochre-brown in KOH. *Lamella edge* often fertile, with scarce cheilomacrocystidia; cheiloleptocystidia 9–30 × 2.7–7 µm, subclavate, sometimes containing oil-droplets, thin-walled; cheilomacrocystidia 28–45 × 5.5–7 µm, emergent, subfusiform with a capitate to moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* composed of subglobose cells, often arranged in rows. *Hymenophoral trama* irregular, with (short) hyphae and abundant, conspicuous lactifers. *Pileipellis* an ixocutis, 150–250 µm thick, slime layer thin, few shrivelled or gelatinised hyphae; lactifers rather abundant and often situated close to the surface. *Stipitipellis* a thin ixocutis of hyaline narrow hyphae but no slime zone extending beyond the hyphal zone (surface lubricous to subviscid in wet weather). *Clamp-connections* absent.

HABITAT: Type locality: on bottom land along sides of a muddy ditch under grasses and weeds, loblolly pine (*Pinus taeda*) nearby; gregarious in tufts of 2–3 or scattered; December and January (generally abundant but never found in any other than low muddy spots, Hillhouse in HESLER & SMITH, 1979).

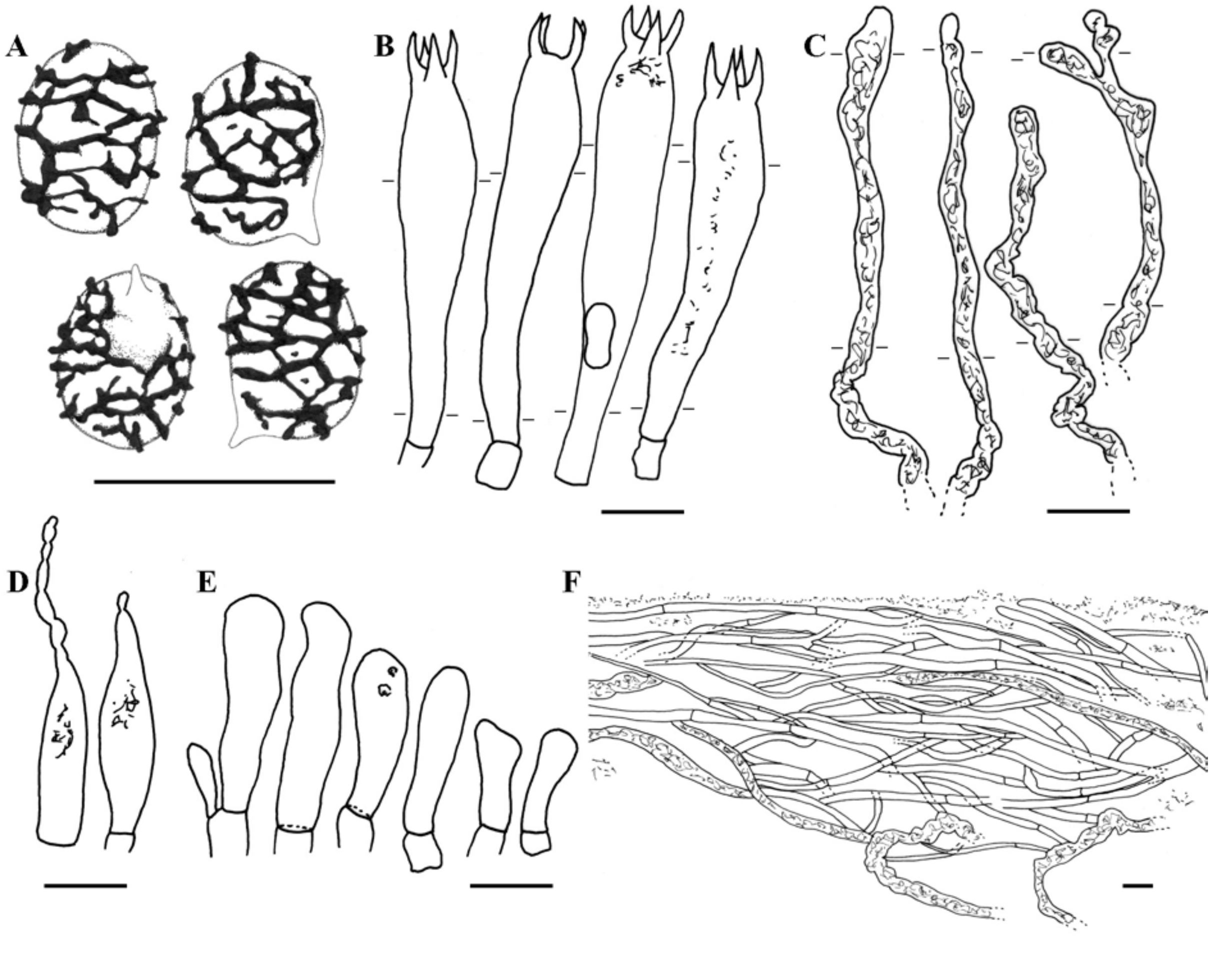

Fig. 8: *Lactarius indigo* var. *diminutivus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** cheilomacrocystidia; **E.** cheiloleptocystidia; **F.** section through the pileipellis. From E. Hillhouse 249 (type) and M.C.Aime 811. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISTRIBUTION: Described from Brazoria County, Texas, possibly also in Virginia.

COLLECTIONS EXAMINED: USA, Texas, Brazoria Co., Liverpool, along ditch bottom and sides of old pipeline crossing, 26.12.1970, E. Hillhouse 249 (MICH), holotype – Virginia, Montgomery Co., Pandapas Pond Rec. Area, Jefferson National Forest, gregarious in one spot in bottom of moist creek bed, mixed woods, 26.08.1999, M.C.Aime 811 (RMS).

DISCUSSION: *Lactarius indigo* var. *diminutivus* differs from var. *indigo* by its small size, the pronounced change to green, the locally thicker spore ornamentation and the scarcer macrocystidia. A fertile lamella edge has only been observed in *L. indigo* var. *diminutivus*. More collections need to be thoroughly described macro- and microscopically to decide on the status of this taxon. We sequenced only one collection, collected in Virginia. It groups with the *L. indigo* var. *indigo* collections in the ITS trees but not in the *gpd* trees, indicating a possible genetic difference.

3.4. ***Lactarius miniatosporus*** **Montoya & Band.-Muñoz**, Mycotaxon 89: 48 (2004)

Fig. 9

Holotype: Montoya 3830 (XAL!), Mexico: Veracruz, Mpio. Xico.

DESCRIPTION (macroscopy after MONTOYA & BANDALA, 2004): *Pileus* 4–11 cm diam., convex to plano-convex, centrally depressed; margin incurved, tomentose when young, expanding and becoming glabrous and striate in age; surface viscid, azonate or with diffuse and vanishing zones; colour pinkish with pale reddish tinges to pale pinkish vinaceous (7A2–B3, 8A/B2), with dark pinkish vinaceous stains (8C/D5), fading to straw colour or yellowish white (4A2–5A2/3–B2–B4); margin remaining vinaceous pink or vinaceous red (10B–D8); centre yellowish white with pale pinkish tinges, irregularly staining blue-green (25D3–25E8). *Lamellae* adnate to subdecurrent, close to moderately subdistant, forked near the stipe; colour pinkish grey (7B3/4, 8B2/3) or vinaceous red when observed in mass; colour ± pale pinkish vinaceous (10C5) as seen singly, staining blue-green (25D3–25E8) when handled; cut areas staining vinaceous red by the latex. *Stipe* 1.5–7 × (0.8) 1–2 cm, cylindrical; surface smooth, dry, very rarely superficially spotted; colour dull pink (8A2–B3) to vinaceous pink (7B3–8A2–C4/5, 9B4–10B3) or at times vinaceous red (10C/D5), whitish and subtomentose at the apex, staining blue-green (25D3–E8). *Context* whitish, staining pink (5A2, 7/8A2) when exposed, especially towards the margin of both pileus and stipe, staining vinaceous red by the latex and staining blue-green (25D3–D4–E8) in all parts; smell mild or of chlorine; taste mild to faintly astringent. *Latex* vinaceous red (8C/B7, 9E7/8). *Spore deposit* pale yellowish brown.

Spores 7.0–*7.5*–8.1 (8.2) × 5.5–*5.8*–6.2 (6.3) µm, broadly ellipsoid to ellipsoid (Q = 1.21–*1.30*–1.41); ornamentation up to 0.5 µm high, composed of medium thick ridges and some isolated warts, forming a rather complete reticulum; plage distally slightly amyloid. *Basidia* 36–70 × 8–10 µm, subclavate, mostly 4-spored, often containing oil-droplets or with a granular content; sterigmata 4–5.5 µm long. *Pleuromacrocystidia* absent. *Pseudocystidia* abundant, 2–5.5 µm diam., often slightly emergent, tortuous and often branching, with an ochre-yellow content. *Lamella edge* sterile with rather scarce to moderately abundant cheilomacrocystidia; cheiloleptocystidia 8-30 × 2.6-8 µm, subclavate to irregularly shaped, hyaline, thin-walled; cheilomacrocystidia 28–46 × 5.3–9 µm, subfusiform with a moniliform apex, emergent, with a needle-shaped to granular content, thin-walled. *Subhymenium* composed of irregular to subglobose cells, sometimes arranged in short rows. *Hymenophoral trama* irregularly filamentous; with very abundant lactifers. *Pileipellis* an ixocutis, up to 250 µm thick, composed of strongly interwoven hyphae, 1–5 µm diam.; shrivelled and gelatinised hyphae abundant near the surface. *Stipitipellis* a cutis, up to 100 µm thick, composed of strongly interwoven, regularly shaped hyphae, 2–5 µm diam.; no shrivelled hyphae. *Clamp-connections* absent.

HABITAT: Associated with *Pinus* (type locality: in pure or mixed stands of *Pinus nubicola*, *P. pseudostrobus* and *P. patula*).

DISTRIBUTION: Known from the central region of the Gulf area of Mexico only.

COLLECTIONS EXAMINED: Mexico, Veracruz, Mpio. Xico, E Cofre Perote, Ejido Ingenio El Rosario, El Revolcadero, 2850 m a.s.l., under *Pinus patula*, 3.07.2002, Montoya 3830 (XAL), holotype.

DISCUSSION: *Lactarius miniatosporus* is a medium sized to quite large species, characterised by its pinkish pileus, soon fading to yellowish white, its red latex and its small spores.
MONTOYA & BANDALA (2004) suppose a close relationship with *L. subpurpureus*, *L. thakalorum* and *L. sanguifluus/L. vinosus*, four species with vinaceous red latex and reddish tones in the basidiocarps. *Lactarius miniatosporus* differs from these species by its smaller spores and the absence of pleuromacrocystidia. Characteristic for *L. miniatosporus* is the tendency of the pileus colour to become duller in age (the colour becomes straw colour to yellowish white, the vinaceous pigmentation is only observed at the margin). Our molecular analyses reveal a well-supported, close relationship of *L. miniatosporus* with *L. paradoxus*. Branch lengths are very short for these taxa in the ITS trees but clearly longer in the *gpd* trees. Only one specimen was included for both *L. miniatosporus* and *L. paradoxus* and thus results need to be interpreted with care.

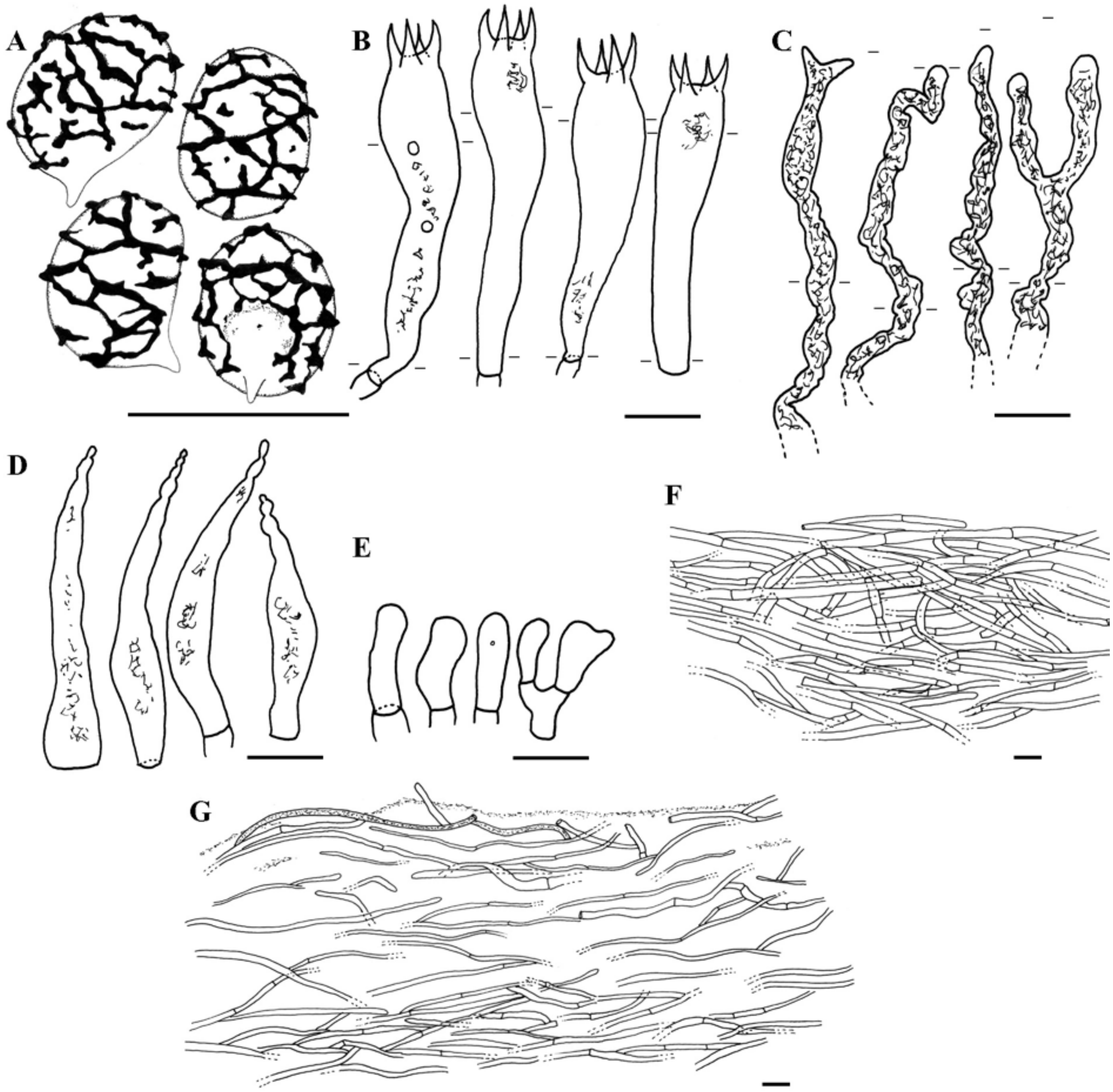

Fig. 9: *Lactarius miniatosporus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** cheilomacrocystidia; **E.** cheiloleptocystidia; **F.** section through the stipitipellis; **G.** section through the pileipellis. From Montoya 3830 (type). Scale bars = 10 µm, small bars indicate the height of the hymenium.

3.5. ***Lactarius paradoxus*** **Beardslee & Burl.**, Mycologia 32: 584 (1940)

Figs. 10, 11

Lectotype: Burlingham, 16-11-1939 (NY!), USA: Florida, Fort Christmas Land Development Co., hammock at "Old Faithful", near Fort Christmas (designated in HESLER & SMITH, 1979).

DESCRIPTION: *Pileus* 4.3–8 cm diam., broadly convex, becoming centrally depressed, in age finally infundibuliform; margin inrolled at first, thin and even, translucently striate; surface glabrous, viscid when wet, with concentric canescent zones, becoming more conspicuous when dry; colour greyish indigo to deep blue (20D/E3), paler between the zones, at first with an overall silvery shade, fading to pale blue in age, with yellowish straw stains (3/4A3), staining green when bruised; margin initially vinaceous (11B3/4), becoming reddish vinaceous to pinkish vinaceous (9D5/6) when mature. *Lamellae* adnate to decurrent, close, narrow, becoming broad, some forked near the stipe; colour pinkish vinaceous (8B3), often with salmon to ochraceous (6A2-B3) or vinaceous salmon (7/8A3) tinges, green (25E5/6) where bruised. *Stipe* 2–3.5 × 1–2 cm, subcylindric to attenuate near the base, sometimes tapering downwards to a root-like extension; surface dry, superficially scrobiculate, rugose to fibrose; colour pinkish vinaceous (9C4-D5) to vinaceous (10B/C4-12B4), often with bluish shades, with a dingy vinaceous apex at the line of gill attachment and pruinose below this, staining green (25E7) in age and where bruised; base with whitish rhizomorphs. *Context* thick and firm, becoming hollow in the stipe; colour pallid with a pink to green tinge, vinaceous and staining blue near the pileipellis, dark vinaceous near the stipitipellis, slowly becoming greenish where bruised; smell none; taste mild or slowly peppery to slightly acrid and only slightly bitter. *Latex* scarce, dark vinaceous brown to blood red (9E7/8), in age staining green on the context. *Spore deposit* yellow-ochre (5B5/6).

KOH no reaction.

Spores 7.8–*8.5–8.6*–9.3 × 5.8–*6.2–6.3*–6.7 (6.9) µm, broadly ellipsoid to ellipsoid (Q = 1.23–*1.36–1.38*–1.46); ornamentation up to 0.5 µm high, of rather thick ridges, forming an almost complete reticulum, isolated warts scarce; plage distally slightly amyloid. *Basidia* 47–54 × 8-11 µm, subclavate to cylindrical, 4-spored, often containing oil-droplets or needle-shaped crystals; sterigmata 3–6 µm long. *Pleuromacrocystidia* rather scarce to abundant, 37–76 × 5.3–8 µm, emergent, subfusiform, with a narrowing to strongly moniliform apex, sometimes with a granular, ochre-coloured content, thin-walled. *Pseudocystidia* abundant, 2–4 µm broad, slender, mostly not emergent, cylindrical to tortuous, with an oleiferic, ochre content. *Lamella edge* sterile with rather scarce to abundant cheilomacrocystidia; cheiloleptocystidia 10–23 × 4–8 µm, subclavate or more irregular, hyaline, thin-walled; cheilomacrocystidia 25–50 × 3.3–8 µm, emergent, subfusiform with a moniliform apex, often with a granular content, thin-walled. *Subhymenium* composed of small, globose to irregular cells, often in short rows. *Hymenophoral trama* composed of strongly interwoven hyphae; with abundant lactifers. *Pileipellis* an ixocutis, up to 200 µm thick, of strongly interwoven hyphae, 1–4 µm diam.; shrivelled hyphae abundant near the surface. *Stipitipellis* a cutis, up to 100 µm thick, of regular, strongly interwoven hyphae, 2-5 µm thick; shrivelled hyphae absent. *Clamp-connections* absent.

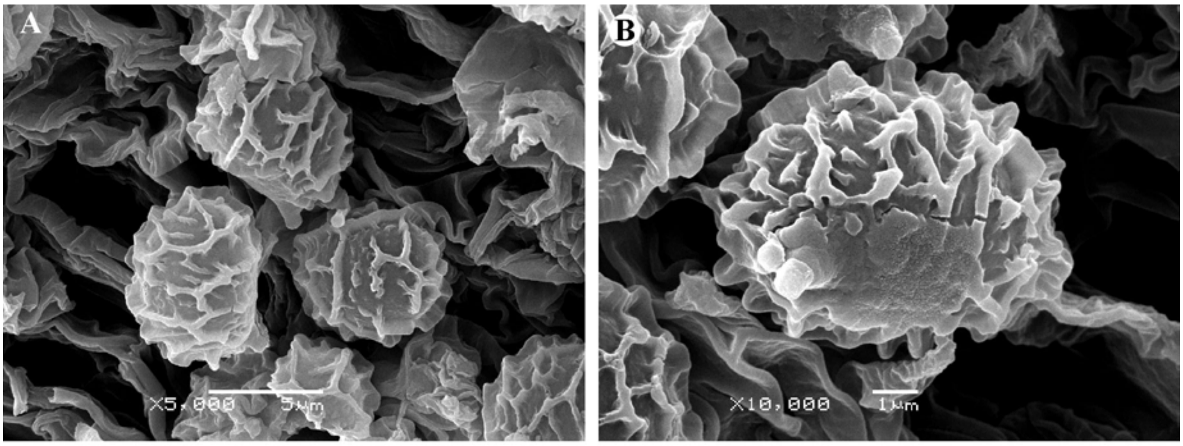

Fig. 10: A. and **B.** SEM images of spores of *L. paradoxus* G.S. Burlingham 184233 (lectotype).

HABITAT: Scattered on sandy soil or in lawns; reported under *Quercus virginiana*, *Pinus banksiana*, *P. pseudostrobus*, *P. carribaea* and other *Pinus* spp.; found in the late summer and fall (August to September in the north, October to February in the south).

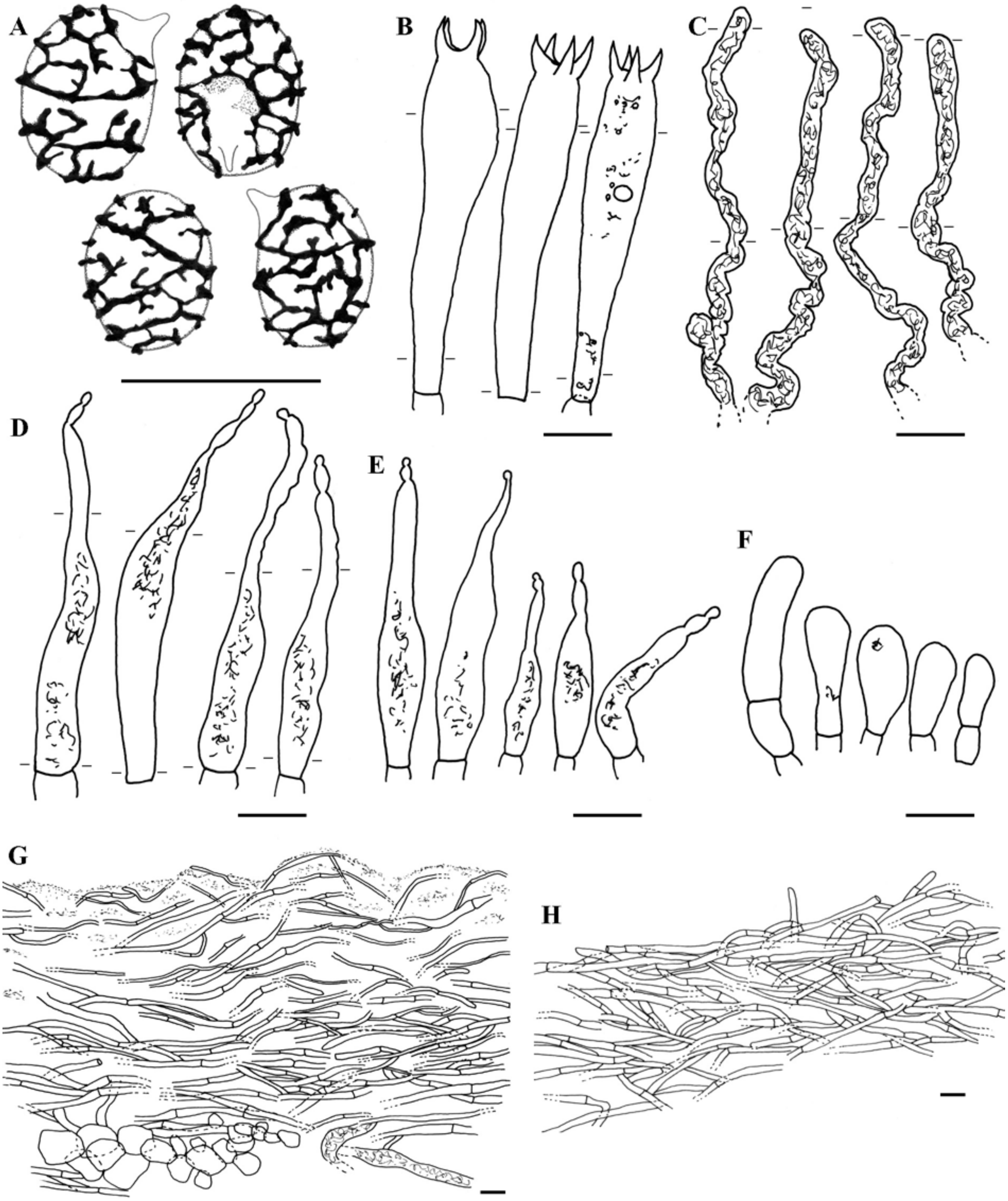

Fig. 11: *Lactarius paradoxus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From G.S. Burlingham 184233 (lectotype), G.S. Burlingham 184263 and D. Mitchell 01.02.2002. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISTRIBUTION: Known from the eastern USA (Florida, Michigan, Tennessee, Alabama, Mississippi, Texas and New York), southeast Canada (Ontario), Mexico and Cuba (MONTOYA *et al*., 1998).

COLLECTIONS EXAMINED: USA, Florida, Old Faithful, under cabbage palmetto and live oaks in grass, 16.11.1939, G.S. Burlingham 184233 (NY), lectotype – Florida, 20.11.1939, G.S. Burlingham 184263 (NY), labelled as co-type, possible syntype – Florida, Gulf County, Port St. Joe, Port St. Joe Historical cemetery, 01.02.2002, D. Mitchell (RMS).

DISCUSSION: *Lactarius paradoxus* is a medium sized species, characterised by the bluish and often greenish tinges in the pileus and the vinaceous red to brown latex. Remarkable is the short root-like projection of the stipe that is often present. The macroscopical description given here was compiled from the original description, from HESLER & SMITH (1979) and from MONTOYA *et al.* (1998). Quite some variability in microscopical characters was found in this species. HESLER & SMITH (1979) report several collections with differently ornamented spores. A remarkable variability in the abundance of macrocystidia was observed by us (even between the two collections made by Burlingham); MONTOYA *et al.* (1998) report the pleuromacrocystidia to be absent.

3.6.1. ***Lactarius pseudodeliciosus* Beardslee & Burl.**, Mycologia 32: 582 (1940) **var. *pseudodeliciosus***

Figs. 12, 13

Lectotype: Beardslee & Burlingham 22-12-1937 (NY!), USA: Florida, Orange Co., Rock Springs, Kelly Park (designated in HESLER & SMITH, 1979).

DESCRIPTION (macroscopy after BEARDSLEE & BURLINGHAM, 1940): *Pileus* 6–8.5 cm diam., broadly convex, deeply umbilicate, expanding to infundibuliform, margin thin agglutinated-fibrous when young (under a lens); surface very viscid when wet, azonate to faintly zonate; colour nearly white at first, becoming yellowish with age, centrally pale pinkish buff to ochraceous-buff. *Lamellae* adnate to adnate-decurrent, intervenose; colour honey yellow as seen singly (except at the base, which is orange), with orange tones as seen in position. *Stipe* 1.2–2.5 × 1–2 cm, short, solid, extending to a root-like point on one side, somewhat scrobiculate; colour isabelline (dirty whitish), white-tomentose on the lower half. *Context* turning drab-green where bruised; smell none. *Latex* scanty, neutral orange; taste slowly peppery. *Spore deposit* ochraceous.

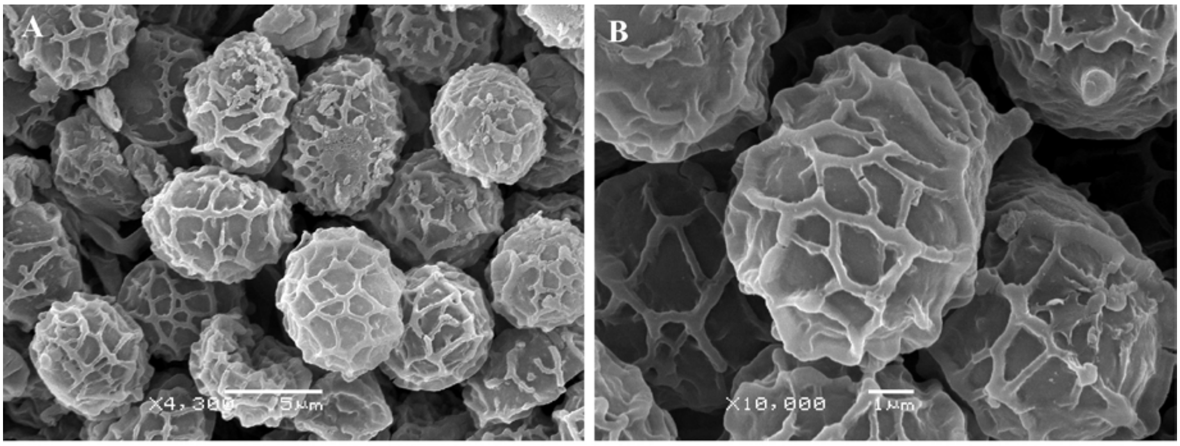

Fig. 12: A. and **B.** SEM images of the spores of *L. pseudodeliciosus* var. *pseudodeliciosus* Beardslee & Burlingham 22.12.1937 (lectotype).

Spores 7.9–*8.4*–8.9 × 5.8–*6.3*–6.7 µm, broadly ellipsoid to ellipsoid (Q = 1.27–*1.34*–1.43); ornamentation up to 0.5 µm high, composed of mainly medium thick ridges with some thinner ridges and isolated warts, forming an incomplete reticulum; plage distally slightly amyloid. *Basidia* 35–45 × 6–9 µm, subclavate, 4-spored, with oil-droplets or a granular content; sterigmata 4–6 µm long. *Pleuromacrocystidia* not very abundant and often inconspicuous, 45–65 × 5–8 µm, subfusiform with a moniliform apex, sometimes branching, with a needle-shaped content, thin-walled. *Pseudocystidia* abundant, cylindrical to tortuous, usually not emergent, with a yellow-ochre content. *Lamella edge* sterile with abundant cheilomacrocystidia on the edge or close to the edge; cheiloleptocystidia 13–25 × 4–7 µm, mostly subclavate, some irregular, hyaline, thin-walled; cheilomacrocystidia 26–47 × 3.5–8 µm, emergent, subfusiform with a moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* composed of small, irregular cells. *Hymenophoral trama* with rather abundant, pale ochre coloured lactifers. *Pileipellis* an ixocutis, 150–300 µm thick, with abundant shrivelled and some gelatinised hyphae, 1–4 µm diam. *Stipitipellis* a cutis, of rather narrow hyphae. *Clamp-connections* absent.

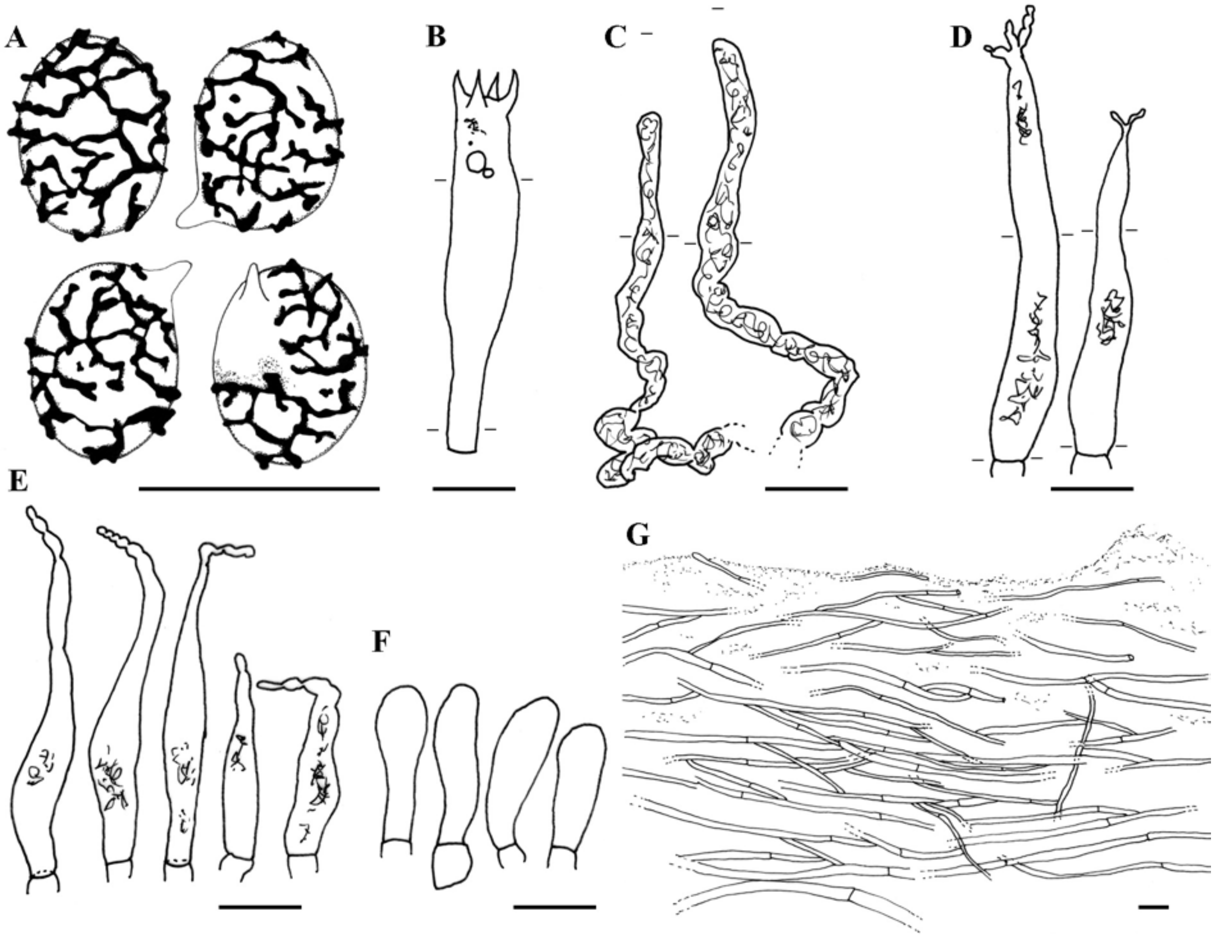

Fig. 13: *Lactarius pseudodeliciosus* var. *pseudodeliciosus*: **A.** spores; **B.** basidium; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis. From Beardslee & Burlingham 22.12.1937 (lectotype). Scale bars = 10 µm, small bars indicate the height of the hymenium.

HABITAT: Under *Quercus* and *Pinus*, November to February.

DISTRIBUTION: Only known from Florida.

COLLECTIONS EXAMINED: USA, Florida, Orange County, Rock Springs, Wood road, laurel wood, oak & pine, sand, 22.12.1937, Beardslee & Burlingham (NY), lectotype.

DISCUSSION: This rather badly known and apparently uncommon species seems to be characterised by the general pale colours of the pileus and stipe, the orange latex and the development of greenish stains upon bruising. BEARDSLEE & BURLINGHAM (1940) mention that *L. pseudodeliciosus* differs from "*L. deliciosus*" by the agglutinated fibrous edge as seen with a lens, the nearly white and usually azonate pileus and the lack of odour. Microscopical differences we observed are the smaller spores that are ornamented with thinner ridges.
The specimen included in the phylogenetic analyses (from which only the ITS sequence was obtained) shows that *L. pseudodeliciosus* clusters with *L. salmoneus*. Both species are on very long branches, indicating a high divergence.

3.6.2. ***Lactarius pseudodeliciosus* var. *paradoxiformis* (Murrill) Hesler & A.H. Sm.**, N. Amer. Species Lactarius: 82 (1979)

Fig. 14

Basionym: ≡ ***L. paradoxiformis*** Murrill, Lloydia 7: 304 (1945)

Holotype: Murrill, F19250 (FLAS!), USA: Florida, Marion Co., South of Orange Lake, Cherry Hill

DESCRIPTION (macroscopy after MURRILL, 1945): *Pileus* 6–8 cm diam., convex to applanate with a depressed centre; margin deflexed, even or striate over the lamellae, entire to undulate, paler; surface viscid, smooth, glabrous, cream-coloured, becoming in part or totally bluish green. *Lamellae* short-decurrent, arcuate, crowded or sub-crowded, rather narrow; colour ochraceous, becoming bluish green with age in places; edge entire. *Stipe* 2–5 × 1–1.5 cm, equal; surface smooth, glabrous; colour ochraceous, bluish green where bruised. *Context* pallid above, ferruginous below, unchanging, slightly fragrant, slightly but distinctly acrid. *Latex* not copious enough to form drops but stains orange to rusty. *Spore deposit* ochroleucous.

Spores 7.7–*8.4*–9.1 (9.2) × 5.7–*6.2*–6.7 µm, broadly ellipsoid to ellipsoid (Q = 1.25–*1.35*–1.43); ornamentation up to 0.5 µm high, of medium thick to thin ridges and isolated warts, forming an incomplete reticulum; plage distally slightly amyloid. *Basidia* 35–52 × 8–11 µm, subclavate, 4-spored, often with oil-droplets or a granular content. *Pleuromacrocystidia* not very abundant, inconspicuous, 36–50 × 5.5–7 µm, subfusiform with a moniliform apex, with a granular content, thin-walled. *Pseudocystidia* scattered, 2–5 µm diam., not emergent, tortuous. *Lamella edge* sterile with rather abundant cheilomacrocystidia; cheiloleptocystidia 10–20 × 4–8.6 µm, subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia 18–30 × 3–5 µm, emergent, subfusiform with a moniliform apex, often with a needle-shaped content, thin-walled. *Pileipellis* a thick ixocutis. *Clamp-connections* absent.

HABITAT: On the ground under hardwoods, especially red oak, fall to early winter (type locality).

DISTRIBUTION: Only known from Florida

COLLECTIONS EXAMINED: USA, Florida, Marion County, Cherry Hill, south of Orange Lake, Under red oak and hawthorn, 07.12.1941, Murrill F19250 (FLAS), holotype.

DISCUSSION: HESLER & SMITH (1960) treated this taxon as a synonym of *L. pseudodeliciosus* but changed their mind in their later monograph of *Lactarius*. Their decision was based on the following differences in the original descriptions: *L. pseudodeliciosus* var. *paradoxiformis* has a striate pileus margin, a slightly fragrant smell and a distinctly acrid taste, while var. *pseudodeliciosus* has a thinly agglutinated fibrous edge, no distinct smell and a slowly, merely peppery taste. Another indicated difference is the lack of pleuromacrocystidia in *L. pseudodeliciosus* var. *paradoxiformis*, but we did observe (rather scarce and inconspicuous) pleuromacrocystidia in the type collection. Certainly more collections need to be examined to draw any further conclusion about this taxon. We should remark here that the type collection is in a very bad state.

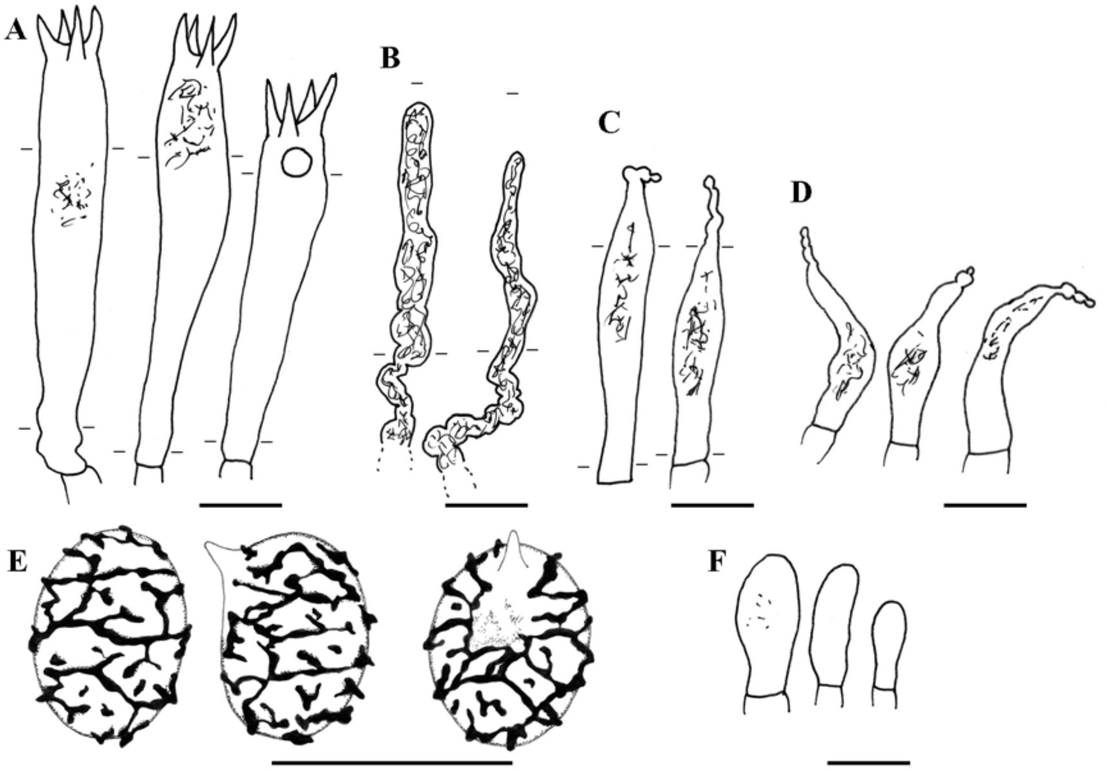

Fig. 14: *Lactarius pseudodeliciosus* var. *paradoxiformis*: **A.** basidia; **B.** pseudocystidia; **C.** pleuromacrocystidia; **D.** cheilomacrocystidia; **E.** spores; **F.** cheiloleptocystidia. From Murrill F19250 (type). Scale bars = 10 µm, small bars indicate the height of the hymenium.

3.7. ***Lactarius rubrilacteus* Hesler & A.H. Sm.**, N. Amer. Species Lactarius: 76 (1979)

Figs. 15, 16

Misapplication: *L. sanguifluus* sensu HESLER & SMITH (1960).

Holotype: W.B. Gruber 1200 (MICH!), USA: Oregon, Grants Pass.

DESCRIPTION (macroscopy after HESLER & SMITH, 1979 and METHVEN, 1997): *Pileus* 4–15 cm diam., convex to applanate, depressed in the centre, becoming broadly infundibuliform with age; margin inrolled when young; surface glabrous, (sub)viscid, zonate, with light orange zones (5A2-4), alternating with brownish orange zones (6C5-7), developing irregular, dull green stains (26D3/4), generally duller and paler in age. *Lamellae* adnate to subdecurrent, close to crowded, narrow to moderately broad, forked near the stipe; colour light orange (6A2-4) to greyish orange (6B2-4), staining reddish brown (8D4-6) and then greyish green (25C6-8) when bruised, in age stained greenish; edge entire. *Stipe* 2.5–8 × 1–3 cm, equal to tapering downwards; surface glabrous to pruinose, dry, sometimes scrobiculate; colour light orange (6A3/4) to brownish orange (7C3/4) or greyish red (8C2/3); with a light orange (8C2/3) tomentum at the base. *Context* firm, brittle, thick, becoming hollow in the stipe; colour dingy yellowish white, staining reddish brown (8D4-6) when cut, staining greenish in age; smell becoming faintly subaromatic; taste mild. *Latex* scanty, in young specimens brownish red (8D4-6), often paler and dingy orange-red in mature specimens; taste not distinctive. *Spore deposit* pale yellow (4A2/3).

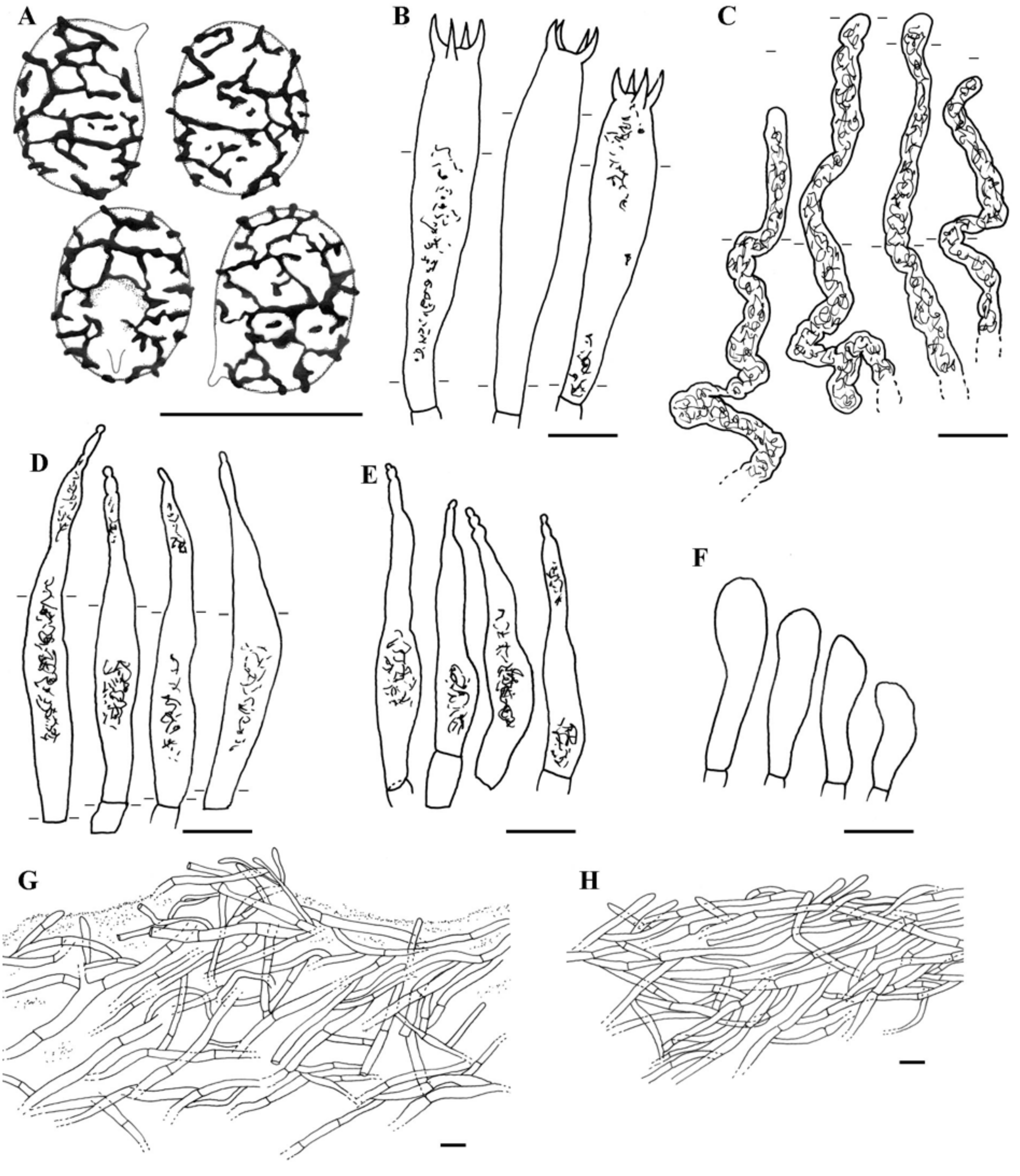

Fig. 15: *Lactarius rubrilacteus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From W.B. Gruber 1200 (type), S.L. Miller 19-04, J. States AEF 1055 and A.S. Methven 485. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Spores 8.1–*8.6–8.9*–9.4 × 5.9–*6.3–6.5*–7.5 µm, broadly ellipsoid to ellipsoid (Q = 1.22–*1.32–1.37*–1.45); ornamentation up to 0.5 µm high, of thin to medium thick ridges and very abundant warts, forming a (very) incomplete reticulum; plage distinct but almost inamyloid. *Basidia* 43.5–56 × 8–10.5 µm, subcylindrical to subclavate, 4-spored, hyaline or containing small guttules, sometimes content granular; sterigmata 4–5 µm long. *Pleuromacrocystidia* rather abundant, especially close to the edge, 48–75 × 5.4–7 µm, emergent, subfusiform, with a moniliform apex, content granular or needle-shaped, thin-walled. *Pseudocystidia* rather abundant, 3.5–5.5 µm broad, mostly not emergent, cylindrical to tortuous, with a refractive, ochre content. *Lamella edge* sterile with very abundant cheilomacrocystidia; cheiloleptocystidia 14–30 × 3.4–6.7 µm, mostly subclavate, hyaline, thin-walled; cheilomacrocystidia 35–55 × 5–6 µm, emergent, subfusiform, with a moniliform apex, with a needle-shaped, refractive content, thin-walled. *Subhymenium* composed of irregular, small cells. *Hymenophoral trama* irregularly filamntous; with abundant, ochre coloured lactifers. *Pileipellis* a thick ixocutis, up to 350 µm thick, with an unclear slime-layer, hyphae (1) 3–8 µm diam.; shrivelled hyphae scarce, many hyphae orientated anticline to the surface. *Stipitipellis* a cutis, up to 80 µm thick, of strongly interwoven, regularly shaped hyphae, 2–5 µm diam. *Clamp-connections* absent.

HABITAT: Scattered to gregarious, often abundant, under Douglas fir (*Pseudotsuga menziesii*) and *Pinus* spp.

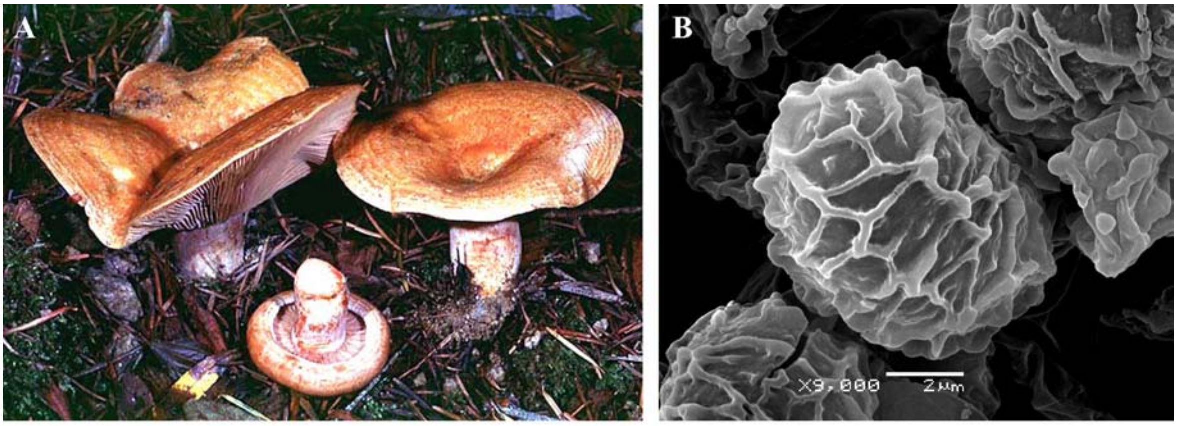

Fig. 16: A. *L. rubrilacteus* (photo M. Woods); **B.** SEM image of the spores of *L. rubrilacteus* J. States AEF 1055.

DISTRIBUTION: Occurring throughout the Pacific Northwest, south into California and in New Mexico, fruiting during the summer in the southern Rocky Mountains and in the fall in the Pacific Northwest (HESLER & SMITH, 1979).

COLLECTIONS EXAMINED: USA, Oregon, Josephine Co., Grants Pass, 17.11.1947, W.B. Gruber 1200 (MICH), holotype – California, Butte Co., Paradise, 2000 ft. a.s.l., 26.11.1968, D. Ripley 1626 (SFSU) – Arizona, Road between Big Lake & Alpine, Apache Natl. Forest, solitary in humus in mixed woods, 28.07.1972, H.D. Thiers 29827 (SFSU) – Washington, Cispus Work Center near Randle, scattered in humus under Douglas fir, 20.10.1972, H.D. Thiers 30197 (SFSU) – California, Yuba Co., Bullard's Bar Reservoir, solitary to scattered in duff in mixed forest, 19.11.1981, H.D. Thiers, A.S. Methven 485 (SFSU) – California, Medocino Co., Jackson State Forest, "*Suillus* Park", gregarious in duff in mixed forest especially near *Pseudotsuga menziesii*, 6.11.1982, A.S. Methven 1912 (SFSU) – California, Santa Cruz Co., Hwy 9 near San Lorenzo Park, solitary in duff in hardwood-coniferous forest, 16.12.1982, H.D. Thiers, A.S. Methven 2360 (SFSU) – Washington, Kittatas Co., Stampede Pass Road, off I-90, East of Snoqualmie Pass, gregarious on moss and duff, under conifers, 9.10.1984, A.S. Methven 3444 (SFSU) – California, Sierra Co., Wild Plum campground, near Sierra City, solitary in soil under Douglas fir, 4.10.1989, H.D. Thiers 52786 (SFSU) – New Mexico, Hwy 64 between Taos and Angel Fire, in soil under mixed conifer hardwoods, 24.08.1991, E. Thiers & H.D. Thiers 53639 (SFSU) – Arizona, Coconino Co., Bismark Lake Trail, mixed conifer-*Populus*, 26.08.1992, J. States, AEF 947 (MICH) – California, Marin Co., Mt. Tamalpais watershed, Rock Springs area, Benstein Trail, collected under pure *Arctostaphylos glandulosa*, 22.12.1992, D.E. Desjardin 5595 (SFSU) – Arizona, Cochise Co., Onion Saddle, *Quercus* and *Pinus edulis*, 4.09.1993, J. States, AEF 1055 (MICH) – Arizona, Cochise Co., Onion Pass, *Pinus*, *Juniperus*, *Quercus* and *Pseudotsuga menziesii*, 4.09.1993, J. States, AEF 1060 (MICH) – California, 14.01.2004, S.L. Miller 11-04 (RMS) – California, 14.01.2004, S.L. Miller 19-04 (RMS).

DISCUSSION: *Lactarius rubrilacteus* is characterised by a zonate, brownish orange pileus and reddish brown latex (METHVEN, 1997). Confusion with similar species, occurring in the same area, seems unlikely. It is reported under both *Pseudotsuga menziesii* and *Pinus* spp.; collection D.E. Desjardin 5595 (SFSU) was found in a pure *Arctostaphylos glandulosa* stand.

For years HESLER & SMITH (1960, 1979) used the name *L. sanguifluus* for this species. After comparison with the plates of *L. sanguifluus* by PAULET (1811, plate 81, fig. 3–5) and NEUHOFF (1956, plate 6, fig. 24), they decided that the species depicted on these plates could not be reconciled with the "*L. sanguifluus*" from the western United States (HESLER & SMITH, 1979). Our phylogenetic analyses indeed confirm this and even place *L. sanguifluus* and *L. rubrilacteus* in very different clades. Strangely enough, according to the phylogenetic analyses of both ITS and *gpd* genes, *L. rubrilacteus* is very closely related to *L. porninsis*, a European species with white latex, associated with *Larix*.

3.8. *Lactarius rubriviridis* Desjardin, Saylor & Thiers, Mycologia 95: 148 (2003)

Figs. 17, 18

Holotype: DED 7312 (SFSU!), USA: California, Sierra Co., Cold Creek Campground.

DESCRIPTION (macroscopy after DESJARDIN, 2003): *Basidiomes* hypogeous, 2.5–6 cm long × 2–4.5 cm broad × 1.5–3 cm thick, irregularly globose to ovoid or ellipsoid, sometimes lobed. *Peridium* absent, exterior surface alveolate to ridged and pitted from exposed locules and hymenophoral tramal tissue; ridges reddish brown to dark brown (8/9E/F5–7), staining deep greenish grey to bluish green or dark green (25–27F5–7) where bruised. *Gleba* loculate; *locules* irregular in shape, often elongate, 1–5 mm × 0.5–1.5 mm, filled with yellowish white to orange-white (4/5A2) or cream-coloured (4A3) basidiospores at maturity; *hymenophoral tramal tissu*e firm, waxy, white to yellowish white (4A2), but becoming reddish brown (9D7/8) when cut from exuded latex; *columella* rudimentary to well-developed, composed of radiating dendritic veins arising from a central main vein, 1–4 mm diam., initially white, but staining greenish white (27A2/3) to greyish green (26C6/7) over time when cut. *Latex* scant, deep red (9B/C7/8), discolouring tramal tissue to dark brownish red (11/12E7/8). *Odour* not distinctive or sweet. *Taste* mild. *Spores* forcibly discharged, leaving an orange-white (5A2) to yellowish white (4A2) or cream (4A3) deposit.

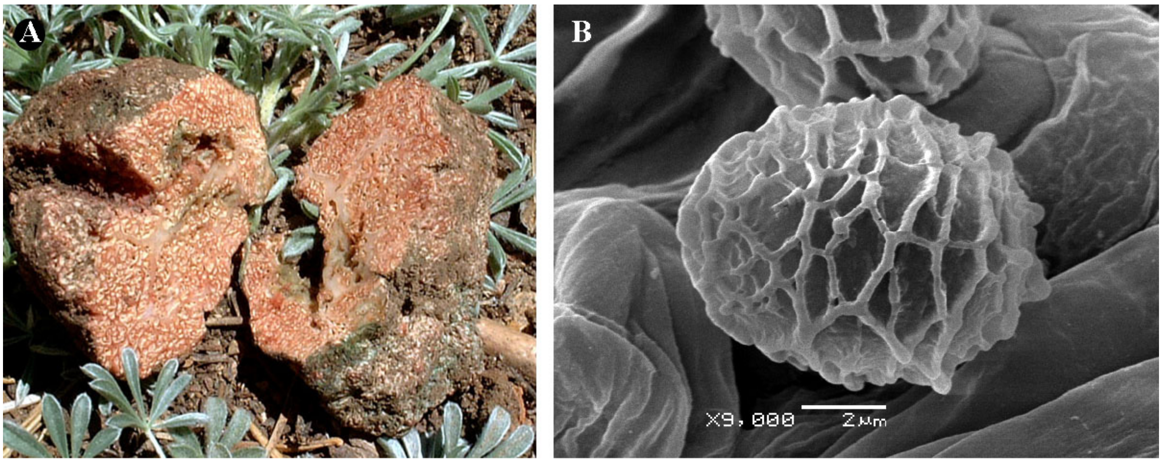

Fig. 17: **A.** *L. rubriviridis* (photo D. Desjardin); **B.** SEM image of the spores of *L. rubriviridis* D.E. Desjardin 7312 (type).

Spores 8.0–*8.9–9.2*–10.8 × 6.0–*6.7–6.9*–7.9 µm, broadly ellipsoid to ellipsoid (Q = 1.26–*1.33–1.34*–1.43), inequilateral in profile with an eccentric hilar appendix; ornamentation up to 0.5 µm high, of dense, rather tick ridges, forming a complete reticulum with very small meshes, isolated warts rare; plage rather large but only weakly amyloid. *Basidia* 40–60 × 10-12.7 µm, subclavate, 4-spored, sometimes with a granular content; sterigmata 3.3–6 µm long. M*acrocystidia* rather scarce, 34–67 × 4.3–8 µm, often hardly visible, rarely emergent, subfusiform with a moniliform apex, with a granular to needle-shaped content, thin-walled. *Pseudocystidia* rather abundant, 2–4 µm broad, slender and short, mostly not emergent, content oleiferic. *Subhymenium* of short-celled hyphae, not very well differentiated. *Hymenophoral trama* composed of densely compact, agglutinated hyphae, 3–10 µm diam., cylindrical to subglobose; lactifers abundant, contorted to strangulate, 3–5 (10) µm diam., refractive, yellowish orange in KOH. *Columella* tissues similar to the hymenophoral trama. *Sphaerocytes* absent in all tissues. *Clamp-connections* absent.

HABITAT: Buried under needle duff of conifers (*Abies* spp., *Pinus* spp.), June (type locality).

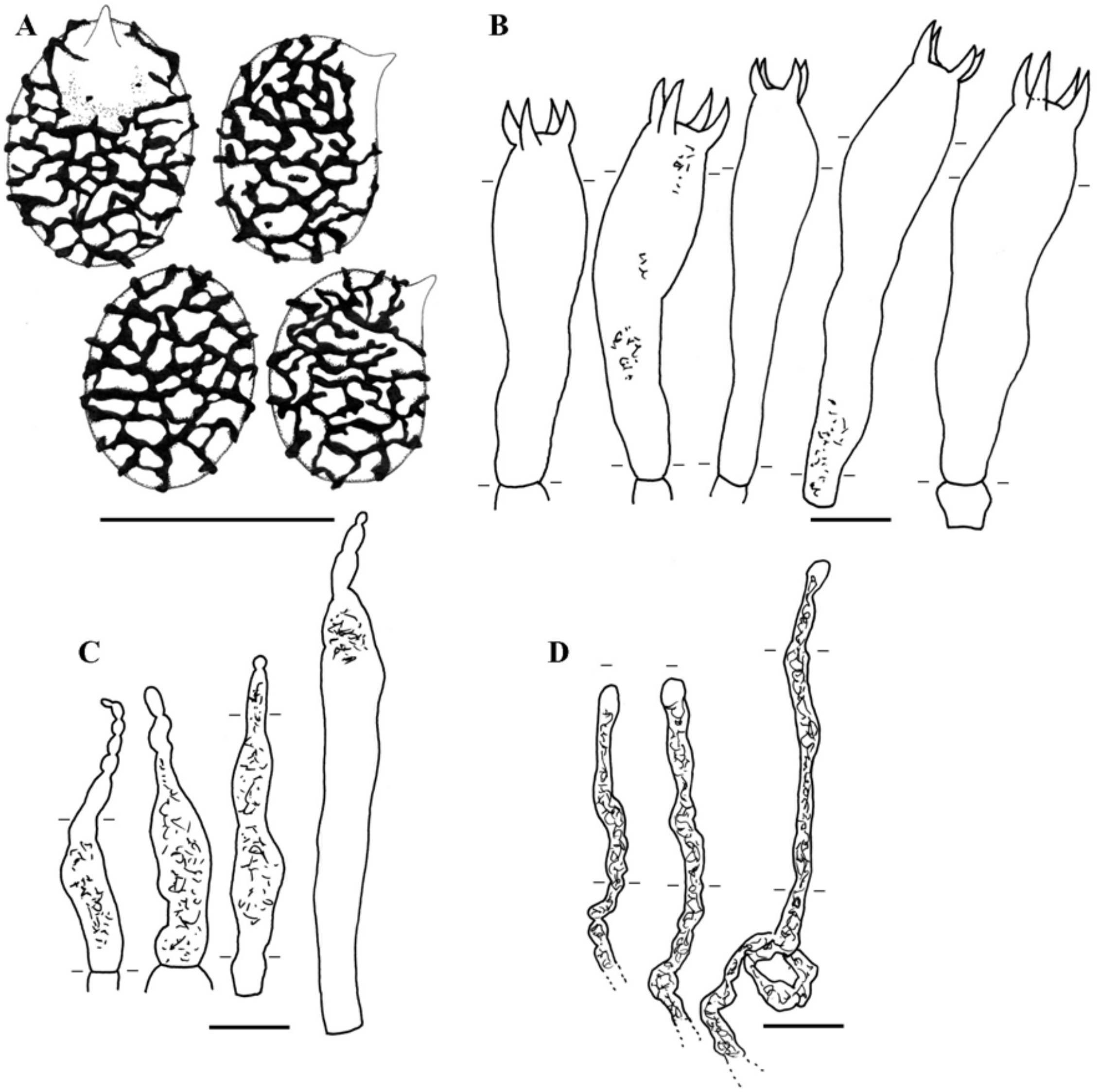

Fig. 18: *Lactarius rubriviridis*: **A.** spores; **B.** basidia; **C.** macrocystidia; **D.** pseudocystidia. From D.E. Desjardin 7312 (type). Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISTRIBUTION: Only known from the Sierra Nevada, California.

COLLECTIONS EXAMINED: <u>USA</u>, California, Sierra Co., Highway 89, Cold Creek Campground, solitary, hypogeous under *Abies* and *Pinus* in montane conifer forest, 14.06.2001, D.E. Desjardin 7312 (SFSU), holotype.

DISCUSSION: Diagnostic features of *L. rubriviridis* are the gasteroid, hypogeous basidiome that lacks a peridium, the red latex, the green stains developing on the tissues, the well-developed dendritic columella and the reticulate ballistospores. The size and ornamentation of the spores are very similar in *L. rubriviridis* and its closest relative *L. barrowsii*. This is an extra indication that *L. rubriviridis* was recently derived from its epigeous, agaricoid ancestor *L. barrowsii*, that can be found in the same type of habitat.

DESJARDIN (2003) mentions that macrocystidia are absent in *L. rubriviridis.* Macrocystidia were probably confused here with pseudocystidia that are described as cylindrical with a tapered apex, projecting slightly beyond the basidia and hyaline or with orange globular contents.

3.9.1. *Lactarius salmoneus* Peck, Bull. Torrey Bot. Club 25: 369 (1898) **var. *salmoneus***

Fig. 19

Holotype: Earle & Baker (NYS!), USA: Alabama, Vaughn's Mills

DESCRIPTION (macroscopy after PECK, 1898): *Pileus* 2.5–3.8 cm broad, rather thin, convex, becoming nearly applanate or slightly depressed in the centre; surface dry, subvelvety, sometimes irregular; colour white, becoming reddish where bruised. *Lamellae* adnate or decurrent, narrow, close; colour bright salmon colour. *Stipe* about 2.5 × 3–6 cm, short, solid, central or occasionally eccentric; surface velvety; colour white, salmon within. *Context* taste mild, slightly aromatic. *Latex* bright salmon coloured.

Spores 6.9–*7.6–7.9*–8.4 (8.5) × 5.1–*5.1–5.6*–6.1 µm, mostly ellipsoid, more rarely broadly ellipsoid (Q = 1.30–*1.34–1.41*–1.47); ornamentation 0.2–0.4 µm high, of medium thick ridges and some finer lines, forming an incomplete reticulum, isolated warts rather scarce; plage indistinct. *Basidia* 33–45 × 5.5–8 µm, mostly subcylindrical, 4-spored, often containing oil-droplets; sterigmata 3.5–6 µm long. *Pleuromacrocystidia* (very) scarce but locally abundant close to the edge, 35–60 × 5.5–8 µm, emergent, subfusiform with a moniliform apex, with a refractive, needle-shaped content, thin-walled. *Pseudocystidia* scarce, 2.5–4 µm broad, often emergent, mostly cylindrical, often branching, inconspicuous, with a pale yellow content. *Lamella edge* sometimes fertile, with scarce cheilomacrocystidia; cheiloleptocystidia 11–25 × 3.5–7 µm, subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia 17–24 × 4–5.5 µm, subfusiform with a moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* composed of small, isodiametric cells. *Hymenophoral trama* composed of irregular, enlarged cells and abundant, yellow to ochre coloured lactifers. *Pileipellis* a cutis inclining towards a trichoderm locally, up to 200 µm thick, of regularly shaped, strongly interwoven and frequently branched hyphae, 2–4 µm diam., no shrivelled hyphae present; erect hyphae present at the surface, forming tufts. *Stipitipellis* a cutis, up to 70 µm thick, of regularly shaped, strongly interwoven hyphae, 2–4 µm diam. *Clamp-connections* absent.

HABITAT: In wet swampy places, usually on inundated ground, collected in August (Earle & Baker, type locality in Alabama); probably associated with *Pinus*.

DISTRIBUTION: Reported from Alabama, North Carolina and Tennessee, USA (HESLER & SMITH, 1979).

COLLECTIONS EXAMINED: USA, Alabama, Vaughn's Mills, in wet swampy places, usually on naked ground that has been overflowed, August, Earle & Baker (NYS), holotype.

DISCUSSION: PECK (1898) describes *L. salmoneus* as “a small but very distinct species easily recognized by the salmon colour of the milk and the change in the colour of the bruised flesh (turning reddish)". The spores are comparatively small. The white epicuticular covering of the pileus is the outstanding feature of both *L. salmoneus* var. *salmoneus* and var. *curtisii* (HESLER & SMITH, 1979). The inrolled margin is cottony but not as much as in *L. deceptivus* Peck. In both *L. salmoneus* var. *salmoneus* and var. *curtisii* anticline to ascending hyphae, single or in tufts are observed, but never forming a trichoderm. These elements become matted down in age and finally the layer is eroded. The layer is not distinct anatomically from the cuticular region of the pileus, but according to HESLER & SMITH (1979) the entire region features very intricately interwoven much-branched relatively uninflated hyphae forming a layer quite unusual for the genus.

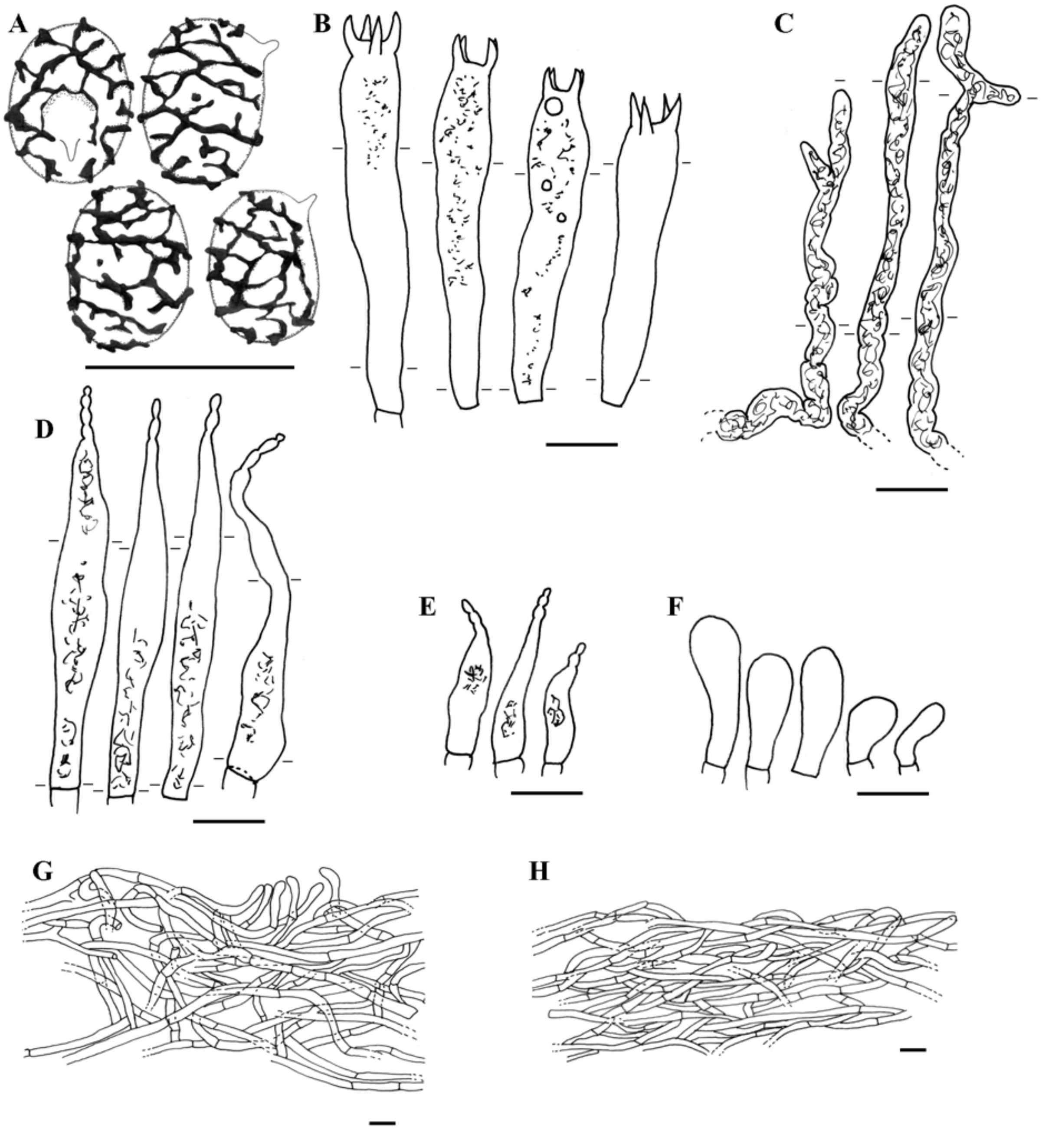

Fig. 19: *Lactarius salmoneus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From Earle & Baker (type), B. Kropp 29-Oct.-00-3 and B. Kropp 11-Aug.-00-14. Scale bars = 10 µm, small bars indicate the height of the hymenium.

3.9.2. *Lactarius salmoneus* var. *curtisii* (Coker) Hesler & A.H. Sm., N. Amer. Species Lactarius: 72 (1979)

Figs. 19, 20

Synonym: ≡ ***L. curtisii*** Coker, J. Elisha Mitchell Sci. Soc. 34: 41 (1918)

Holotype: Coker 1845 (NCU), USA: North Carolina, hill southwest of Sparrow's Mill

DESCRIPTION (macroscopy after MONTOYA *et al.*, 1996 and HESLER & SMITH, 1979): *Pileus* 2–6 (9) cm diam., convex becoming broadly depressed, with an arched margin or the margin nearly applanate and depressed in the centre; margin inrolled and cottony; surface dry and felty (as if covered with a layer of cotton), not at all zonate; colour evenly white or with a cream orange shade (5A3) at first, in age staining bluish green where bruised. *Lamellae* subdecurrent, many forked at the base, narrow, close; colour cadmium orange to ochraceous salmon, readily staining green where injured, sometimes faded to whitish; edge whitish. *Stipe* 1–4 × 0.9–1.5 cm, equal or enlarged above; covered by a white, fine felt-like coating (similar to that of pileus) at first; colour cadmium orange. *Context* solid, whitish, in age pallid orange, staining cadmium orange when cut, discolouring green; smell and taste not distinctive. *Latex* scanty, cadmium orange when first exposed, unchanging but finally greenish (4C3-4B3) on the context; taste faintly peppery in young basidiocarps, mild at maturity. *Spore deposit* yellowish.

$FeSO_4$ pale blue on the context, *phenol* no reaction, *NH_4OH* no reaction, *H_2SO_4* black on the context (MONTOYA & BANDALA, 1996).

Spores 6.9–*7.6–7.8*–8.7 × 4.7–*5.1–5.3*–6.4 µm, mostly ellipsoid, more rarely broadly ellipsoid, shape quite variable (Q = 1.21–*1.34–1.42*–1.54); ornamentation less than 0.5 µm high, of mainly rather broad, irregular ridges and some finer lines, forming an incomplete reticulum, isolated warts rather scarce; plage inconspicuous and small, distally slightly amyloid. *Basidia* 33–55 × 5.5–8 µm, slender, mostly (sub)cylindrical, 4-spored, with a granular content and often containing oil-droplets; sterigmata 3.5–6 µm long. *Pleuromacrocystidia* (very) scarce but locally abundant close to the edge, 50–60 × 5.5–7.5 µm, emergent, subfusiform with a narrowing to moniliform apex, with a refractive, needle-shaped content, thin-walled. *Pseudocystidia* scarce, 2.5–4.5 µm broad, often emergent, mostly cylindrical, often branching, inconspicuous, with a pale yellow content. *Lamella edge* sometimes fertile, with scarce cheilomacrocystidia; cheiloleptocystidia 10–22 × 3.5–7 µm, subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia 17–25 × 4–5.5 µm, subfusiform with a moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* composed of small, isodiametric cells. *Hymenophoral trama* composed of irregularly arranged hyphae and abundant, yellow to ochre coloured lactifers. *Pileipellis* a cutis inclining towards a trichoderm locally, 120–150 µm thick, of regularly shaped, strongly interwoven and frequently branched hyphae, 2–4 µm diam., no shrivelled hyphae present; upright hyphae present at the surface, united into fascicles. *Stipitipellis* a cutis, 50–70 µm thick, of regularly shaped, strongly interwoven hyphae, 2–4 µm diam. *Clamp-connections* absent.

HABITAT: Moist areas of lower altitude, under *Pinus* spp.

DISTRIBUTION: Distributed throughout the pine belt of the southeast USA, also reported from Mexico (CIFUENTES *et al.*, 1989; MONTOYA *et al.*, 1996) and Belize.

COLLECTIONS EXAMINED: Belize, Cayo district, Mtn. Pire Ridge, under *Pinus caribaea*, 29.10.2000, B. Kropp 29-Oct.-00-3 (RMS), notes: latex orange, greenish stain, cap whitish – USA, Mississippi, Harrison Co., Harrison Exp. forest, under *Pinus*, 11.08.2000, B. Kropp 11-Aug.-00-14 (RMS), notes: latex orange, slowly staining greenish, det.: Cibula B.

DISCUSSION: HESLER & SMITH (1979), focussing on the diversity in the genus *Lactarius*, lowered *L. curtisii* to the rank of variety under *L. salmoneus* but admit there are few arguments to keep two different taxa. The only differences they report between both varieties are the lack of green discolouration and slightly larger spores in *L. salmoneus* var. *salmoneus*. Our request to see the holotype of *L. salmoneus* var. *curtisii* was never answered by NCU. The examined specimens from Belize and Mississippi showed a greenish staining reaction and were thus identified as *L. salmoneus* var. *curtisii*. It is very doubtful, that the distinction between both varieties can be maintained. The greenish discolouration in *Lactarius* sect. *Deliciosi* is often delayed, sometimes very faint and in some species sometimes erratic. HESLER & SMITH (1979) expect that green staining will be found in the type variety of *L. salmoneus* when it becomes better known. Microscopical features are identical for both varieties. We were not able to study fresh collections from either variety of *L. salmoneus* and thus do not draw any further conclusions. As the microscopical features are identical drawings were united into one plate (Fig. 19).

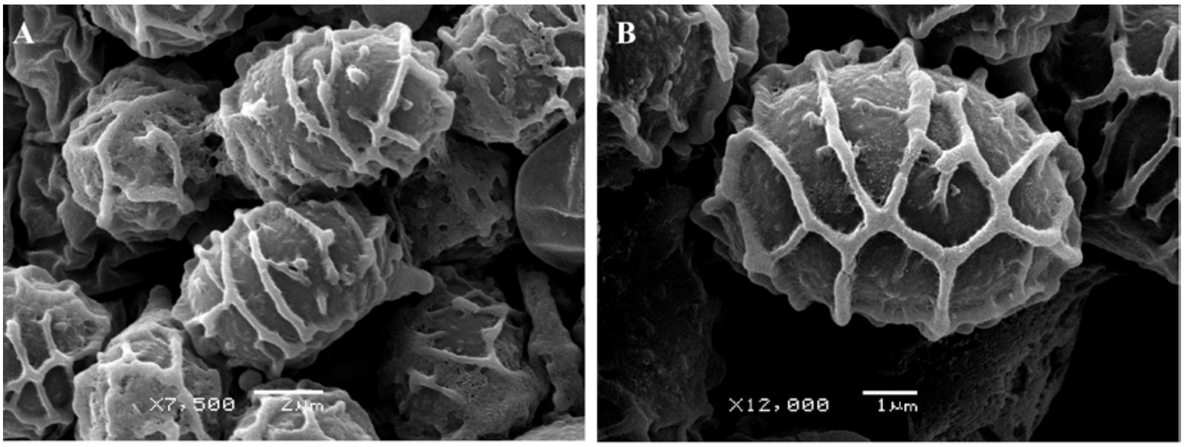

Fig. 20: **A.** and **B.** SEM images of the spores of *L. salmoneus* var. *curtisii* B. Kropp 11-Aug.-00-14.

3.10. ***Lactarius subpurpureus* Peck**, Rep. (Annual) New York State Mus. Nat. Hist. 29: 43 (1878)

Figs. 21, 22

Synonym: ≡ ***Lactifluus subpurpureus*** (Peck) Kuntze, Revisio Generum Plantarum, Pars II: 857 (1891)

Holotype: Peck (NYS!), USA: New York, Sandlake

DESCRIPTION: *Pileus* 4–10 cm diam., convex when young, becoming applanate when mature with a slightly depressed centre, never deeply infundibuliform; margins bent downwards to flattened, vaguely striate; surface smooth, viscid, zonate to scrobiculate; zones fading in age; colour pinkish to vinaceous buff (9B2/3), paler flesh-coloured to pink in the centre (10A2), but deep pink to brownish near the margin (10D4), hygrophanous, becoming very pale vinaceous pink (9A2) in the centre, margin staying deeper pink (9C4/5), green discolouration (28A2) never very extensive. *Lamellae* adnate to subdecurrent, sometimes with a small decurrent tooth, moderately broad and subdistant, rarely anastomosing; colour vinaceous buff to dirty pinkish or pale vinaceous red (9B2/3 to 10A/B4), turning slightly green when bruised; edge entire, ± concolorous. *Stipe* 3–8 × 0.7–1.5 cm, almost cylindrical or broader at the base, relatively long and slender; surface smooth, dry, tomentose at the base with whitish orange coloured subiculum sometimes present, with scarce to rather abundant, darker scrobicules; colour pinkish (10C3 to 11C2/3), covered with a whitish layer, almost white near the apex and the base, greenish discolouration mainly near the base. *Context* not very firm and rather thin, turning hollow in the stipe, whitish buff but yellowish to brown in the stipe cavity, staining vinaceous red (10E/F7) underneath the pilei- and stipitipellis and above the lamellae, ± unchanging, green where affected by parasites; smell sweetish and fruity, agreeable; taste mild becoming very slightly acrid. *Latex* scarce, vinaceous red (10D5), very dark brownish red (11F8) after 1 h on the context; taste mild. *Spore deposit* pale yellow-orange (4A3).

KOH no reaction, *gaiac* slowly turning blue on context.

Spores 9.3–*10.0*–*10.6*–11.2 (12) × 6.8–*7.1*–*7.3*–7.7 µm, ellipsoid (Q = 1.30–*1.38*–*1.45*–1.52); ornamentation up to 0.5 µm high, of rather thick ridges mixed with finer interconnecting lines, some isolated warts present, forming an incomplete reticulum; plage distally slightly amyloid. *Basidia* 46–60 × 9–11 µm, subclavate, 4-spored, rarely 2-spored, containing oil-droplets; sterigmata 4–6 µm long. *Pleuromacrocystidia* abundant, 37–78 × 4.5–8 µm, emergent and very conspicuous, subfusiform with a tapering to moniliform, sometimes branching apex, with a needle-shaped content, thin-walled. *Pseudocystidia* abundant, 1.6–4 µm broad, rather slender, not to slightly emergent, mainly tortuous, sometimes branching, oleiferic, content deep ochre coloured. *Lamella edge* sterile with rather abundant to rather scarce cheilomacrocystidia; cheiloleptocystidia 12.5–18 × 4–6 µm, subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia 25–33 × 3.4–6 µm, emergent, subfusiform, with a moniliform apex, with a needle-shaped to granular content, thin-walled. *Subhymenium* composed of densely interwoven hyphae, appearing like subglobose cells, mostly arranged in short rows. *Hymenophoral trama* of short hyphae, irregularly arranged; lactifers very abundant and conspicuous. *Pileipellis* an ixocutis, 70–150 µm thick, of strongly interwoven hyphae, 1–5 µm diam., shrivelled and gelatinised hyphae abundant. *Stipitipellis* a cutis to ixocutis, thin, up to 50 (70) µm thick, of strongly interwoven, regularly shaped hyphae, 2-4 µm diam. *Clamp-connections* absent.

HABITAT: On soil in coniferous and mixed woods, associated with hemlock (*Tsuga canadensis*), found in the summer and early fall (HESLER & SMITH, 1979).

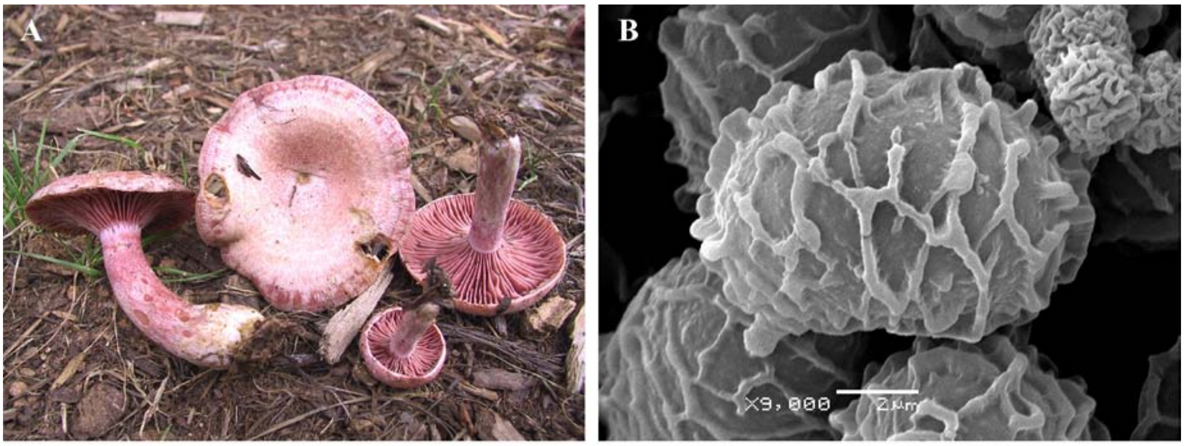

Fig. 21: **A.** *L. subpurpureus* basidiocarps, collection J. Nuytinck 2004-014; **B.** SEM image of the spores of *L. subpurpureus* J. Nuytinck 2004-014.

DISTRIBUTION: Eastern half of the USA and southern Canada, south to the Gulf Coast region (HESLER & SMITH, 1979).

COLLECTIONS EXAMINED: USA, New York, Sandlake (& Brewerton), mossy ground in swamps, October, C.H. Peck (NYS), holotype – Virginia, Cherokee flats near interiors VA, in moist *Rhododendron* flat with hemlock and 2-needle pine, 21.08.1997, S.L. Miller 9572 (RMS) – Virginia, Poverty Creek Trail, Jeff. Nat. Forest, under hemlock, 09.03.1998, J.R. Herr 050 (RMS) – Virginia, Montgomery Co., Poverty Hollow, Jeff. Nat. Forest, Road 708, along trail through mixed hardwoods/conifers, 07.10.1999, J.R. Herr 491 (RMS) – Virginia, Giles Co., Cherokee Flats, Jeff. Nat. Forest, along trail through mixed hardwood/conifer forest, 09.10.1999, J.R. Herr 348 (RMS) – Virginia, Montgomery Co., Poverty Hollow, Pandapas Pond Rec. Area, Jeff. Nat. Forest, off Service Road 708, under mixed conifers/hardwoods, 07.08.2000, J.R. Herr 574 (RMS) – Tennessee, Cocke Co., Great Smoky Mountains National Park, Maddron Bald trail, between Gabed mountain trail & the Allbright grove, near Cosby, 777 m a.s.l., N35°45.352' W083°16.321', under *Tsuga canadensis*, 12.06.2004, J. Nuytinck 2004-003 (GENT) – Tennessee, Cocke Co., Great Smoky Mountains National Park, Greenbrier Ranger Station, second growth forest with *Pinus* spp. *Quercus* spp., *Tsuga canadensis* etc., 13.06.2004, J. Nuytinck 2004-006 (GENT) – North Carolina, Transylvania Co., Pisgah forest, close to Asheville, rich and mixed forest, under *Tsuga canadensis,* along creek, 17.06.2004, J. Nuytinck 2004-014 (GENT).

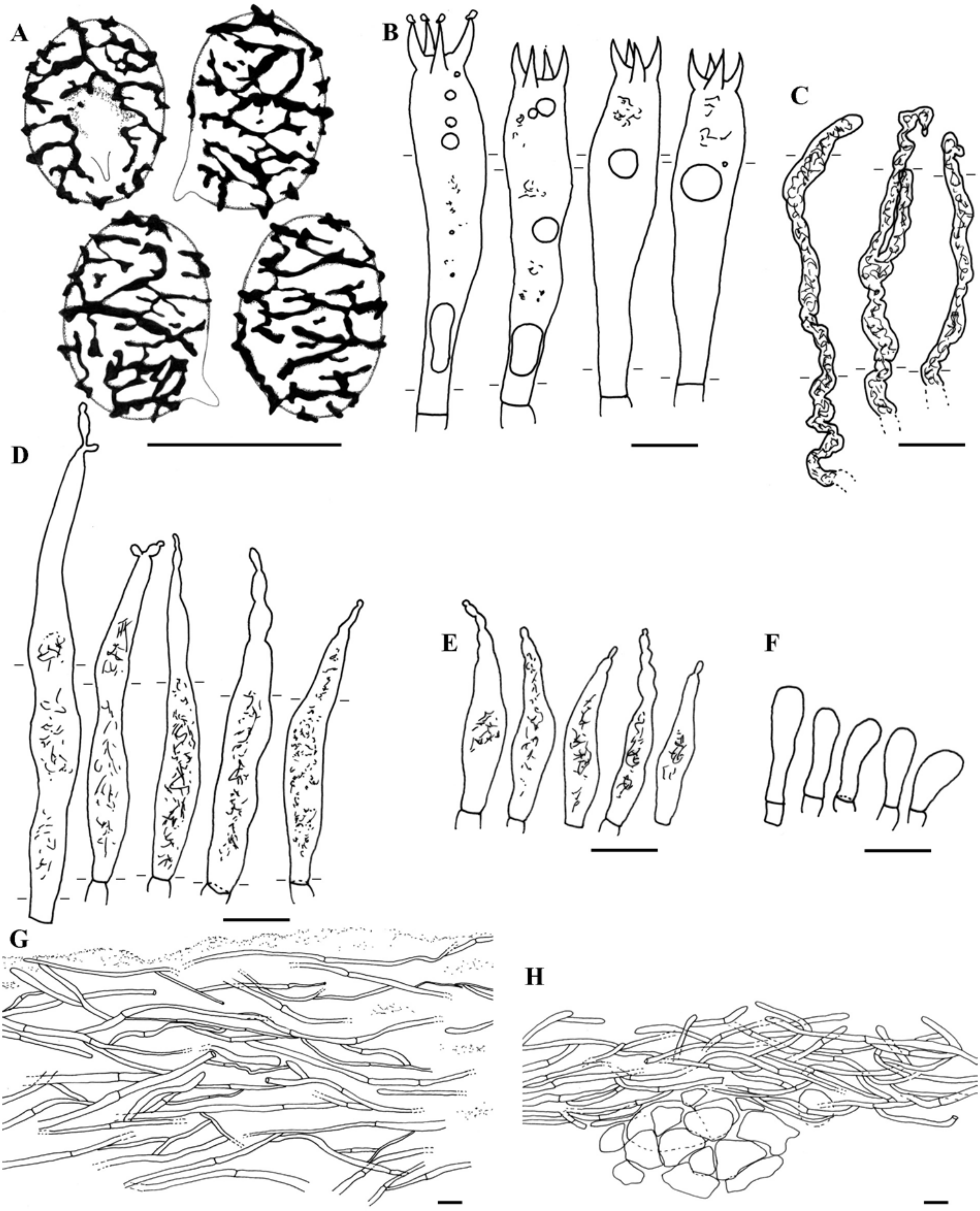

Fig. 22: *Lactarius subpurpureus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From C.H. Peck (type), J.R. Herr 348, J.R. Herr 574 and J. Nuytinck 2004-014. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: The dirty pinkish colour of the basidiocarps, the vinaceous red latex and the subdistant lamellae make *L. subpurpureus* an easy species to recognise. Microscopically, the large, ellipsoid spores and conspicuous pleuromacrocystidia are characteristic. *Lactarius paradoxus* is an eastern species with similarly coloured latex but is readily distinguished from *L. subpurpureus* by the colour of the cap and the spacing of the lamellae. *Lactarius miniatosporus* is superficially similar to *L.*

subpurpureus but has much smaller spores and lacks pleuromacrocystidia (Montoya & Bandala, 2004). *Lactarius subpurpureus* is closely related to two western species with red latex: *L. barrowsii* and *L. rubriviridis*.

3.11. ***Lactarius thyinos*** **A.H. Sm.**, Brittonia 12: 135 (1960)

Figs. 23, 24

Holotype: Smith 22150 (MICH!), USA: Michigan, Cheboygan County, Reese's Bog.

Description (macroscopy after Hesler & Smith, 1979 and Kuo, 2004): *Pileus* 3–9 cm diam., convex at first, becoming applanate, with a shallow central depression to broadly infundibuliform; margin bent downwards to straight; surface viscid when fresh to thinly slimy, glabrous, zonate, zones carrot orange to salmon orange, alternating with pallid yellow zones, in age weathering to greyish. *Lamellae* broadly adnate to decurrent, close at first, subdistant when mature; colour at first bright ochraceous salmon, gradually paler orange, when bruised staining (dark) vinaceous brown, not staining green at any stage. *Stipe* 4–8 × 0.8–2.0 (3.5) cm, more or less equal, or tapering somewhat to base; surface smooth, when young and fresh covered with a thin layer of slime but soon dry, often with a whitish sheen especially above; colour bright ochraceous salmon or paler, slowly staining dull vinaceous red to vinaceous brown where cut. *Context* thin, becoming hollow and fragile in the stipe, staining orange-buff when cut, in the base of the stipe staining slowly to vinaceous red; smell faintly fragrant; taste mild. *Latex* bright orange, slowly staining tissues (especially base of stipe) vinaceous red, no stains to green evident anywhere. *Spore deposit* pale yellow.

$FeS0_4$ no reaction, *KOH* no reaction, NH_4OH no reaction.

Fig. 23: **A.** *L. thyinos* basidiocarps; **B.** *L. thyinos* basidiocarps, lower-right specimen is parasitised by *Hypomyces* (photo T. Chiu); **C.** and **D.** SEM images of spores of *L. thyinos* S.L. Miller 9648.

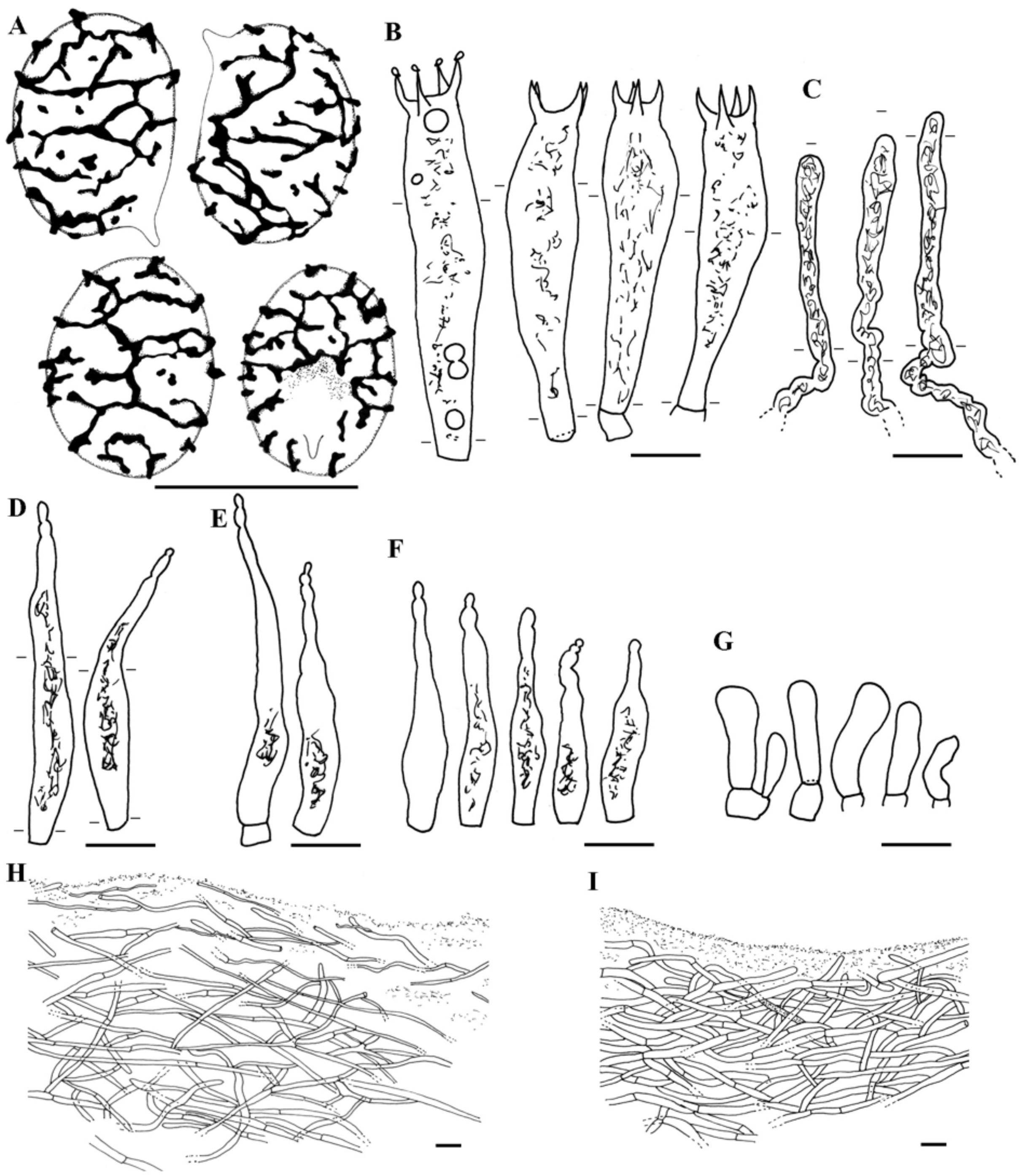

Fig. 24: *Lactarius thyinos:* **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** macrocystidia close to the lamella edge; **F.** cheilomacrocystidia; **G.** cheiloleptocystidia; **H.** section through the pileipellis; **I.** section through the stipitipellis. From A.H. Smith 22150 (type) and S.L. Miller 9648. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Spores 9.2–*10.1–10.7*–11.7 × 6.7–*7.4–8.2*–8.7 µm, broadly ellipsoid to ellipsoid (Q = 1.24–*1.31–1.36*–1.45); ornamentation up to 0.5 µm high, of thin ridges and isolated warts, forming an incomplete reticulum with rather wide meshes; plage distinct but almost inamyloid to distally slightly amyloid. *Basidia* 40–55 (60) × 8–11 µm, subclavate, 4-spored or rarely 2-spored, often with a granular content and containing oil-droplets; sterigmata 4–6 µm long. *Pleuromacrocystidia* abundant near the lamella edge, 40–80 × 4.5–8 µm, strongly emergent, subfusiform with a (long) moniliform apex, with a refractive, granular to needle-shaped content, thin-walled. *Pseudocystidia* relatively scarce, 3.3–5 µm broad, tortuous to cylindrical, inconspicuous, not emergent, pale yellow coloured (observation in

KOH), hyaline in the apex. *Lamella edge* sterile with rather scarce cheilomacrocystidia; cheiloleptocystidia 8–20 × 2.7–6 µm, subclavate to more irregular, hyaline, thin-walled; cheilomacrocystidia 25–55 × 4–8 µm, strongly emergent, subfusiform with a moniliform apex. *Subhymenium* composed of almost isodiametric cells, sometimes arranged in rows. *Hymenophoral trama* with very abundant, pale yellow to ochre coloured lactifers. *Pileipellis* an ixocutis, 100–200 µm thick, composed of strongly interwoven and mainly shrivelled hyphae, 1–4 µm diam. *Stipitipellis* an ixocutis, up to 70 µm thick, with a distinct slime-layer; composed of regularly shaped hyphae, 2–4 µm diam. *Clamp-connections* absent.

HABITAT: Scattered to gregarious in woods of *Thuja* and in cold *Thuja* bogs and swamps, summer and fall, common, host tree unknown.

DISTRIBUTION: Northeastern USA (Vermont, New York and Michigan) and Canada (Nova Scotia, Ontario and Quebec).

COLLECTIONS EXAMINED: USA, Michigan, Cheboygan, Carp Creek, Reese's Bog, 20.08.1946, A.H. Smith 22150 (MICH), holotype – New York, St. Lawrence Co., Star Lake, 26.09.1997, S.L. Miller 9648 (RMS).

DISCUSSION: *Lactarius thyinos* is a medium-sized species with a viscid (when fresh), zonate pileus and a carrot to salmon orange coloured pileus and stipe. When bruised the basidiocarps show vinaceous brown stains but never turn green. The spores are large and ornamented with a rather fine, incomplete reticulum. *Lactarius thyinos* has all of these characters in common with its European and Asian sister species *L. salmonicolor* and *L. laeticolor*. It is unclear with which tree *L. thyinos* forms ectomycorrhiza (*Thuja* spp. are reported not to be ectomycorrhizal).

3.12. The "*L. deliciosus*" complex in North America

Solving the taxonomic problems in this complex of varieties described from or recognised in North America was beyond the scope of our study, which included a broad, worldwide approach on one hand and a more detailed approach in Europe on the other, where we had better collecting facilities. More observations on fresh collections are indispensable to solve this North American complex. The four varieties that HESLER & SMITH (1979) report from eastern and western North America are *L. deliciosus* var. *olivaceosordidus*, var. *areolatus*, var. *piceus* and var. *deterrimus*.

First and most importantly, we proved with our phylogenetic analyses that these taxa form a separate clade and are not conspecific with *L. deliciosus* nor with *L. deterrimus* occurring in Europe (and Asia). BESSETTE (1997) already suggested the name *L. deliciosus* is misapplied in North America. Second, we want to remark that in our opinion *L. deliciosus* var. *piceus* (an invalid name) and *L. deterrimus* are taxonomic synonyms and should not be used alongside. But finally, we did not obtain any further resolution or obvious separation of taxa within this complex in our molecular analysis. Only one collection identified as *L. deliciosus* var. *olivaceosordidus* is convincingly different from the other collections in our phylogenetic analyses. It is described under the insufficiently known taxa and unidentified collections here under.

Main morphological characters that are used to separate the varieties are the presence and abundance of pleuromacrocystidia, the colour of the pileus and the staining reaction of the context (remaining orange or slowly staining red). These characters have to be used with care because they are known to show quite some variability in *Lactarius* sect. *Deliciosi*.

HESLER & SMITH (1979) mention the problem of obtaining apparently mixed collections. Following is an example of this. Specimens J. Nuytinck 2003-041a and b were collected at the same site and time in Wyoming and showed no differences at first sight. Taking a closer look at the discolouration of the latex learned that J. Nuytinck 2003-041a had unchanging to very slowly (> 1 h) changing orange latex while J. Nuytinck 2003-041b possessed orange latex becoming red in 10 to 15 min. ITS nor *gpd* sequences from the separated collection were identical but they did both fall in the "*L. deliciosus"* clade together with all other collections of "*L. deliciosus*" from North America.

One taxon, *L. deliciosus* var. *areolatus*, is characterised by clearly larger spores. Sequenced specimens of this variety do group together in the ITS tree but fall within the larger "*L. deliciosus"* clade. The *gpd* phylogeny shows a closer relationship to *L. porninsis*, *L. rubrilacteus* and an unidentified collection from Washington.

For the moment however, we decided not to draw any further taxonomical conclusions and consequently not to propose any new names. We describe *L. deliciosus* var. *areolatus* and var. *olivaceosordidus* in the following part; for both taxa the type specimen was studied. The macroscopical descriptions were adapted from HESLER & SMITH (1960 & 1979) and METHVEN (1997) and we refer to these publications for more details on the other varieties. METHVEN (1997) distinguishes four varieties in California: *L. deliciosus* var. *olivaceosordidus*, var. *areolatus*, var. *piceus* and var. *deliciosus*. Table 1 gives a comparison of the distinguishing characters METHVEN (1997) describes.

Table 1: Summary of the most important features distinguishing the varieties of "*L. deliciosus*" treated in METHVEN (1997).

***L. deliciosus* var.**	**Colour pileus**	**Colour latex**	**Pleuromacro-cystidia**	**Habitat-host tree**
areolatus	brownish to greyish orange or plain orange	orange, staining context reddish	absent	montane coniferous-deciduous forests
deliciosus	obscurely zonate, brownish to greyish orange	orange, unchanging	absent	*Picea sitchensis*?
olivaceosordidus	greyish green with orange tinges	orange, unchanging	present	*Picea sitchensis*?
piceus	light to greyish orange	orange, staining context reddish	present	*Picea* spp.

3.12.1. *Lactarius deliciosus* var. *areolatus* A.H. Sm., Brittonia 12: 135 (1960)

Fig. 25

Holotype: Smith 46914 (MICH!), USA: Idaho, Payette Lakes

DESCRIPTION (after HESLER & SMITH, 1960 & 1979 and METHVEN, 1997): *Pileus* 5–15 cm diam., convex becoming convex-depressed to ± plano-depressed or broadly infundibuliform in age; surface glabrous and shining, thinly slimy to merely viscid and soon becoming dry, sometimes becoming areolate-scaly, zonate to azonate; colour variable, orange (6A6/7), brownish orange (5C4-6) or greyish orange (6B4-6), soon staining greyish green (26C3/4) to dull green (26D3/4), often sordid in age as the green staining becomes apparent. *Lamellae* adnate to subdecurrent, narrow to moderately broad, close to subdistant, forked near the stipe; colour light orange (5A3/4), slowly staining reddish brown (8D4-6), then dull green (26D3-4), finally entirely green in age; edge concolorous. *Stipe* 2–5 (10) × 1–3 (4) cm, ± equal; surface glabrous, dry, not to slightly scrobiculate; colour light orange (6A3/4) and dull, pruinose at first. *Context* firm, thick, brittle, soon hollow in the stipe; colour white to pale yellow (4A2/3), staining pale orange-buff, slowly changing to greyish red (8C3/4) or reddish brown (8D4-6) on exposure; smell not distinctive; taste mild becoming bitterish to ± acrid. *Latex* (very) scarce, orange (6A6/7), slowly staining purplish red to vinaceous red on the context. *Spore deposit* pale yellow (4A2/3).

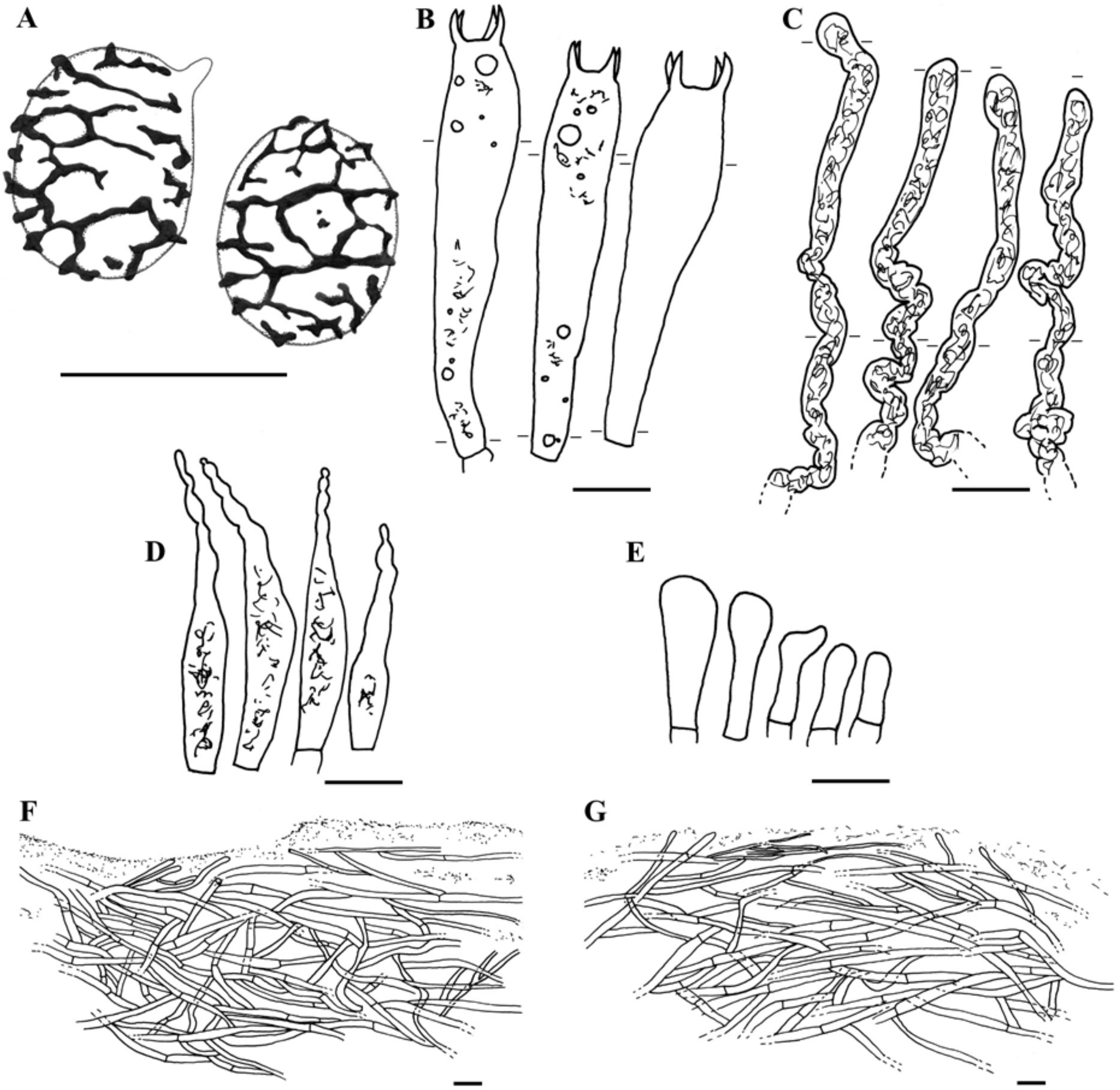

Fig. 25: *Lactarius deliciosus* var. *areolatus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** cheilomacrocystidia; **E.** cheiloleptocystidia; **F.** section through the pileipellis; **G.** section through the stipitipellis. From A.H. Smith 46914 (type) and A.S. Methven 360. Scale bars = 10 µm, small bars indicate the height of the hymenium.

$FeSO_4$ no reaction, *KOH* no reaction, *phenol* no reaction, NH_3 no reaction (Wells & Kempton in Hesler & Smith, 1979).

Spores 8.5–*9.2–9.8*–10.5 (11) × 6.0–*6.5–7.1*–7.6 µm, broadly ellipsoid to ellipsoid (Q = 1.31–*1.36–1.40*–1.46); ornamentation up to 0.5 µm high, of medium thick ridges and some isolated warts, forming an incomplete reticulum with rather wide meshes; plage distinct and distally slightly amyloid. *Basidia* 45–60 × 8–12 µm, subclavate, 4-spored, often containing oil-droplets; sterigmata 3.5–4.5 µm long. *Pleuromacrocystidia* absent or very scarce. *Pseudocystidia* abundant, 3.5–5.5 µm diam., tortuous, sometimes slightly emergent, sometimes branching, with an ochre content. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia 8.5–20 × 3.2–7.3 µm, subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia 30–45 × 5.3–6.7 µm, subfusiform with a moniliform apex, emergent, with and ochre, refractive, granular to needle-shaped content, thin-walled.

Subhymenium composed of mainly small, irregular cells. *Hymenophoral trama* with abundant, wide lactifers, with an ochre-brown content in KOH. *Pileipellis* an ixocutis, 200–300 µm thick, of strongly interwoven, rather dense, regularly shaped hyphae, 2–4 µm diam.; few gelatinised and shrivelled hyphae present near the surface. *Stipitipellis* a cutis to ixocutis, up to 100 µm thick, of mostly regularly shaped hyphae, 3–4 µm diam.; shrivelled and gelatinised hyphae (1 µm diam.) present near the surface. *Clamp-connections* absent.

HABITAT: Scattered to gregarious under mixed conifers (HESLER & SMITH, 1979).

DISTRIBUTION: Western North America; reported from Alaska, California, Colorado, Idaho, New Mexico and Wyoming.

COLLECTIONS EXAMINED: USA, Idaho, Valley Co., Payette Lake, Payette National Forest, 27.08.1954, A.H. Smith 46914 (MICH), holotype – California, Sierra Co., Lincoln Creek Campground, Highway 49, scattered in duff in mixed forest under *Abies* and *Pinus*, 25.09.1982, A.S. Methven 1619 (SFSU) – California, Yuba Co., Schoolhouse Campground, Bullard's Bar Reservoir, gregarious in soil in mixed wood, 30.10.1982, H.D. Thiers 45335 (SFSU) – California, Mendocino Co., Jackson State Forest, solitary to scattered in duff in mixed forest, 07.11.1982, A.S. Methven 360 (SFSU) – California, Medocino Co., Jackson State Forest, "*Suillus* Park": densely gregarious in duff in mixed forest, 12.12.1982, A.S. Methven 2301 (SFSU).

DISCUSSION: *Lactarius deliciosus* var. *areolatus* seems to be the most common variant of the "*L. deliciosus*" group in western North America (HESLER & SMITH, 1979; METHVEN, 1997). The areolate condition of the cap, considered as a characteristic feature in the original description, is the result of dry weather conditions and is not present in normally developing basidiocarps (HESLER & SMITH, 1979). HESLER & SMITH (1979) give an overview of the variability they encountered in the colour of the pileus, the taste and the discolouration of the latex in different collections from different regions.

3.12.2. *Lactarius deliciosus* var. *olivaceosordidus* Hesler & A.H. Sm., N. Amer. Species Lactarius: 95 (1979)

Fig. 26

Holotype: Smith 83831 (MICH!), USA: Oregon, Pacific City

DESCRIPTION (macroscopy after HESLER & SMITH, 1979 and METHVEN, 1997): *Pileus* 4-8 cm diam., plano-convex, soon becoming centrally depressed and in age broadly infundibuliform; margin incurved, faintly translucently striate; surface glabrous, thinly viscid, soon dry, obscurely zonate or scrobiculate; colour greyish green (27D4-6) on an orange (5A6/7), golden yellow (5B6/7) or brownish yellow (5C6/7) ground colour. *Lamellae* (sub)decurrent, close to subdistant, forked near the stipe; colour light orange (5A3-5), becoming dingy yellow, staining deep green where cut (25E7/8). *Stipe* 3–5 (7) × 0.5-1.0 (1.2) cm, slightly enlarged downwards; surface glabrous, moist to dry, not scrobiculate, orange (5A6/7), staining deep green (25E7/8). *Context* thin, yellowish centrally, olive underneath the pileipellis, staining orange when cut, unchanging; smell not distinctive; taste mild to very slowly slightly acrid. *Latex* scarce, orange (6A6/7), unchanging on the context; taste mild. *Spore deposit* pale yellow (4A2/3).

Spores 8.4–*9.0–9.4*–9.9 × (6.3) 6.4–*6.8–7.1*–7.4 µm, broadly ellipsoid to ellipsoid (Q = 1.23–*1.30–1.36*–1.44); ornamentation up to 0.5 µm high, of thin and medium thick ridges and some isolated warts, forming an incomplete reticulum; plage almost inamyloid. *Basidia* 36–50 × 7–9 µm, subclavate, 4-spored, mostly hyaline; sterigmata 3–5.5 µm long. *Pleuromacrocystidia* abundant near the lamella edge, 45–70 × 4.5–7 µm, subfusiform but very slender, with a moniliform apex, containing needle-shaped crystals, thin-walled. *Pseudocystidia* rather scarce to abundant, 3–5.5 µm broad, cylindrical to tortuous, not emergent, with a deep ochre coloured content in KOH. *Lamella edge* sterile with very abundant cheilomacrocystidia; cheiloleptocystidia 12–21 × 3.3–6 µm, subclavate, hyaline, thin-walled; cheilomacrocystidia 25–47 × 4.5–7.3 µm, subfusiform and slender, with a moniliform or capitate apex, containing needle-shaped crystals, thin-walled. *Subhymenium* of irregularly arranged, small cells. *Hymenophoral trama* containing short hyphae and ochre coloured lactifers. *Pileipellis* an ixocutis, up to 300 µm thick, composed of mostly rather thick hyphae, 2–6 µm thick; shrivelled hyphae scarce; lactifers present close to the surface. *Stipitipellis* an ixocutis to cutis, up to 100 µm thick. *Clamp-connections* absent.

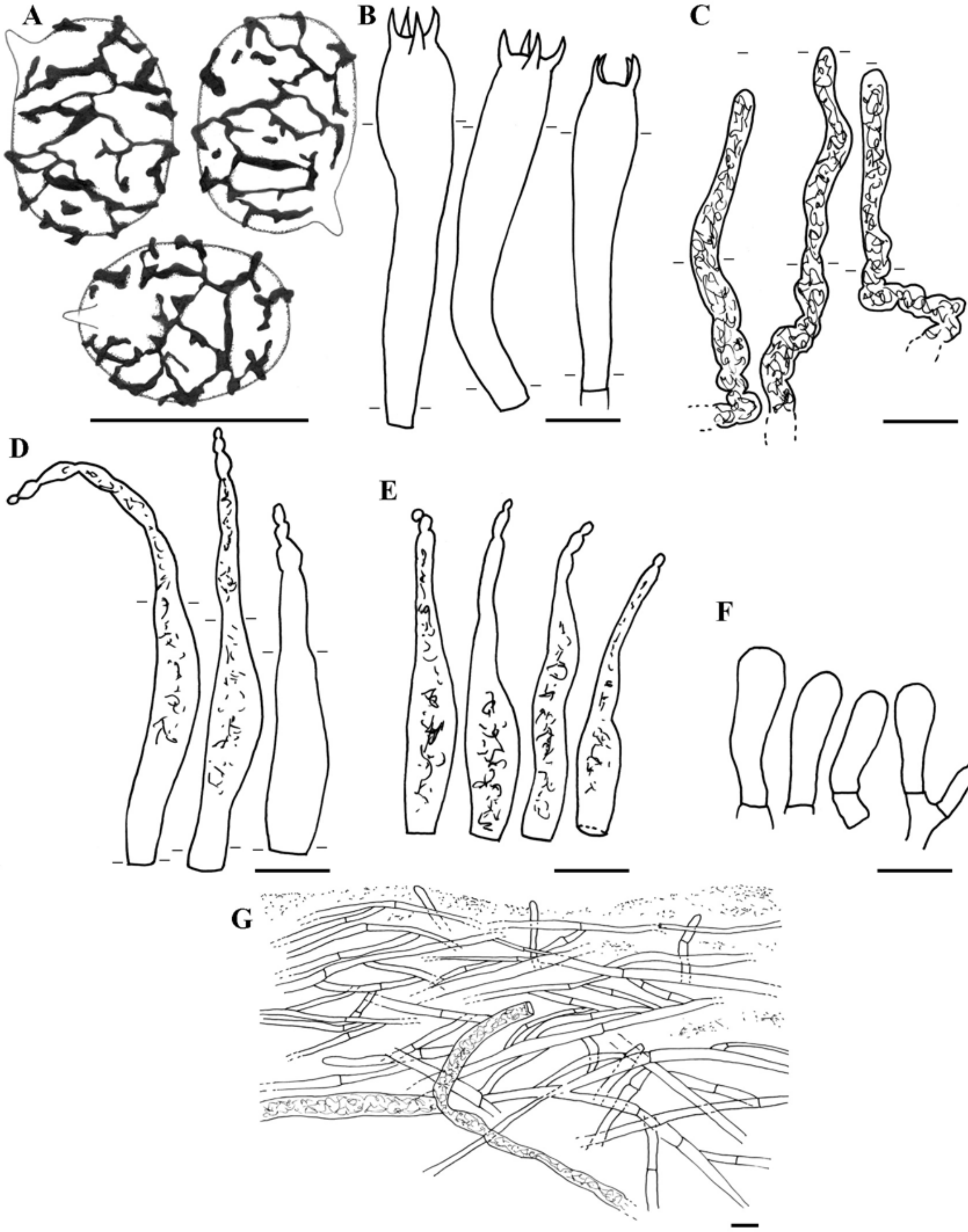

Fig. 26: *Lactarius deliciosus* var. *olivaceosordidus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis. From A.H. Smith 83831 (type). Scale bars = 10 µm, small bars indicate the height of the hymenium.

HABITAT: Scattered to gregarious under *Picea sitchensis*.

DISTRIBUTION: Western USA, reported to be uncommon in California (METHVEN, 1997).

COLLECTIONS EXAMINED: USA, Oregon, Tillamook Co., Pacific City, under spruce, 15.11.1972, A.H. Smith 83831 (MICH), holotype – California, Del Norte Co., Wilson Creek Rd near Klamath, scattered in duff under *Picea sitchensis*, 23.10.1982, D.E. Desjardin 1622 (SFSU) – California, Del Norte Co., Wakefield's Farm, Northcrest Dr, Crescent City, scattered in duff under *Picea sitchensis*, 23.10.1982, A.S. Methven 1840 (SFSU) – California, Humbolt Co., Big Lagoon, Patrick's Pt State Park, gregarious in duff under *Picea sitchensis*, 24.10.1982, A.S. Methven 1895 (SFSU) – Washington, Grays Harbor Co., Quinault Research Natural Area, 10-1-d-11, in humus, litter & mosses, *Tsuga* and *Picea*, 02.10.1992, JA 10762 (RMS) – Washington, Grays Harbor Co., Griffiths-Priday Ocean State Park, under Sitka spruce in sandy soil, 12.11.2000, P.B. Matheny 2054 (RMS).

DISCUSSION: The green to olive stains are a prominent feature of even young material in this variety. The dried basidiocarps are uniformly dingy orange-brown, washed green. Only a slight tendency to become red on cut surfaces was noted (HESLER & SMITH, 1979).

3.13. *Lactarius* sp. 3

Figs. 27, 28

DESCRIPTION: *Pileus* 2–5.5 cm diam., broadly convex at first, then plane to slightly wavy or slightly upturned, shallowly infundibuliform with a small central depression with age; margin slightly incurved to downturned when young, becoming plane, mostly entire but occasionally with faint or moderately well developed transverse wrinkles or lobes, not striate; surface smooth, glabrous, thinly viscid and shiny when moist, drying quickly, often faintly zonated; colour pale dirty orange (6B3–5) when young, zonations slightly paler (5B3), soon flushed or mottled with olive to greyish green (28/29D4–6), often darker green (to 28/29E6/7) centrally but green tinges mostly lacking at the margin. *Lamellae* adnate to slightly decurrent, crowded when young, becoming close; colour salmon to pinkish orange (6A3) when young, dull greyish orange (6B3) in age; edge entire. *Stipe* 2.5–5.5 × 0.6–1.2 cm, mostly equal, occasionally tapered below, sometimes irregularly shaped; surface faintly tomentose or felted when young, remaining so at the base but felted aspect soon disappearing where handled, basal tomentum whitish to buff, scrobicules rare or absent, concolorous with the pileus, frequently paler at the apex. *Context* firm and solid when young, becoming hollow in the stipe; colour pale greyish orange (6A2) when young but greenish immediately beneath the pileipellis, discolouring orange near the lamellae and in the stipe when cut; smell indistinctive; taste mild to very slowly faintly acrid. *Latex* scarce, dark orange when fresh, slowly turning to green on the context in an hour or more. *Spore deposit* unknown.

Fig. 27: **A.** basidiocarps of *Lactarius* sp. 3 collection S.L. Miller 213-03; **B.** basidiocarps of *Lactarius* sp. 3 collection S.L. Miller 216-03.

Spores 8.7–*9.3*–9.9 × (6.5) 6.6–*7.0*–7.5 µm, broadly ellipsoid to ellipsoid (Q = 1.24–*1.32*–1.39); ornamentation up to 0.5 µm high, of medium thick ridges and isolated warts, forming an incomplete reticulum; plage mostly not amyloid. *Basidia* 40–55 × 8–11 µm, subclavate to almost cylindrical, with a striking, dark coloured, granular content and abundant, pigmented oil-droplets. *Basidioles* with a similar content to the basidia, only very few hyaline basidioles present. *Pleuromacrocystidia* very scarce, 40–45 × 6–8 µm, slightly emergent, subfusiform with a narrowing apex, hyaline, thin-walled. *Pseudocystidia* very abundant, 2.5–5.5 µm broad, sometimes slightly emergent, tortuous to cylindrical, sometimes branching, with a striking, deep ochre-brown colour. *Lamella edge* sterile without cheilomacrocystidia; marginal cells 20–35 × 7–10 µm, subclavate, with a dark coloured granular content and oil-droplets. *Pileipellis* a thin ixocutis, up to 100 µm thick, composed of strongly interwoven hyphae, 1.5–5 µm broad, shrivelled hyphae scattered, pigmented hyphae and lactifers present close to the surface. *Clamp-connections* absent.

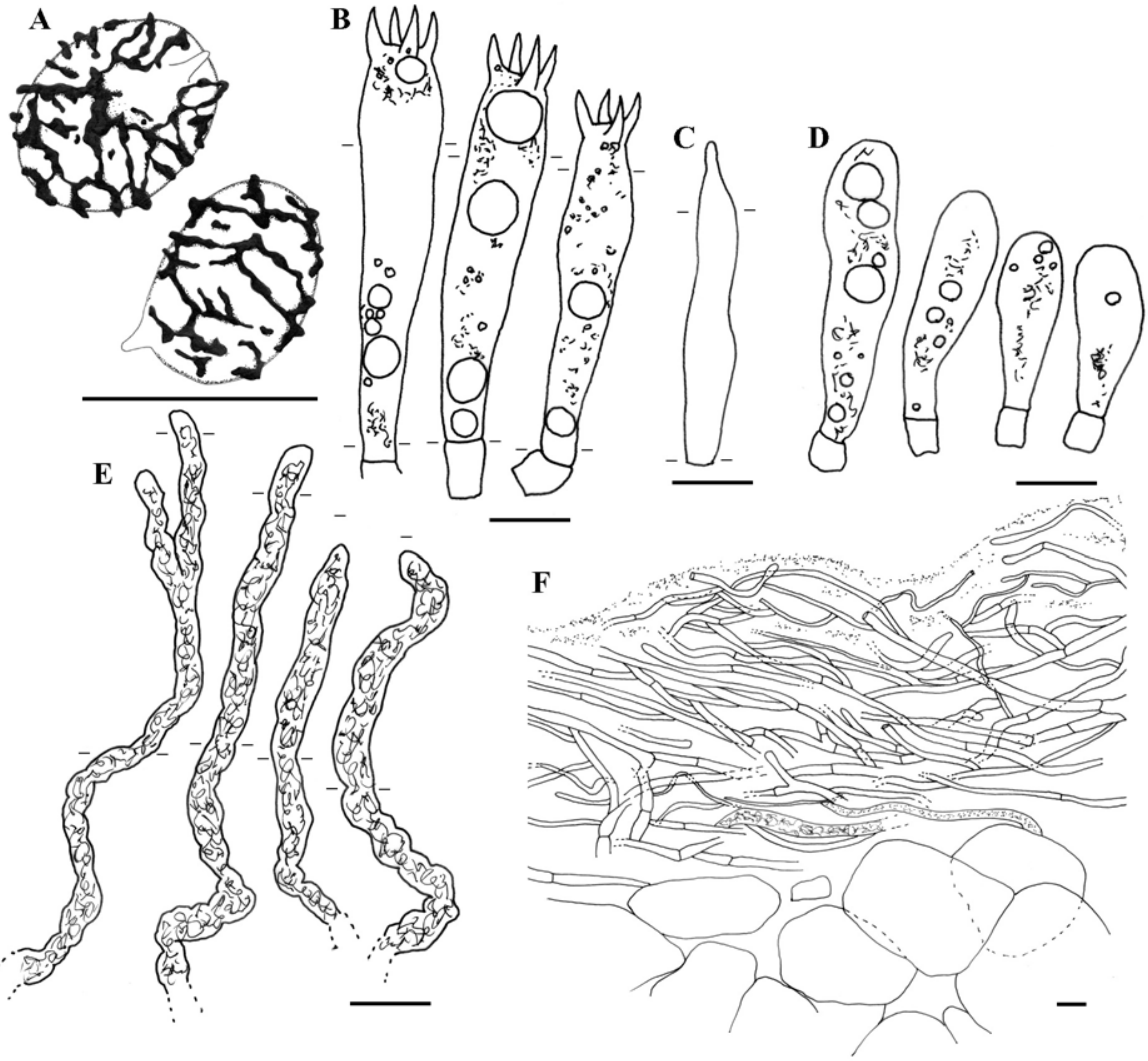

Fig. 28: *Lactarius* sp. 3: **A.** spores; **B.** basidia; **C.** pleuromacrocystidium; **D.** marginal cells; **E.** pseudocystidia; **F.** section through the pileipellis. From S.L. Miller 213-03 and S.L. Miller 216-03. Scale bars = 10 µm, small bars indicate the height of the hymenium.

COLLECTIONS EXAMINED: USA, California, Davison Road, near Humbolt, N41° 21.287' W124° 04.127', scattered or in small groups under Redwood, Sitka spruce with Hemlock and Red alder nearby, S.L. Miller 213-03 (RMS) – California, Davison Road, near Humbolt, N41° 21.287' W124° 04.127', scattered or in small groups under Redwood, Sitka spruce with Hemlock and Red alder nearby, S.L. Miller 216-03 (RMS).

DISCUSSION: In our molecular analyses these collections (labelled as *L.* sp.3) do not group with any of the known species. Although there is a resemblance with *L. deliciosus* var. *olivaceosordidus*, these specimens do not group with the collections of "*L. deliciosus*" from North America. This species is characterised by its small size and its dirty orange tinges and extensive dirty greenish discolouration. The dried specimens are conspicuously dark in colour. Microscopically, the strongly and deeply pigmented content of nearly all hymenium elements is striking. Strongly pigmented hyphae were also noted in the pileipellis. The collections lack cheilomacrocystidia and pleuromacrocystidia are scarce. We will describe this taxon as a new species.

4. Insufficiently known taxa and unidentified collections from North and Central America

salmonicolor. *Lactarius salmonicolor* R. Heim & Leclair, Rev. Mycol. (Paris) 18: 221 (1953)
HEIM (1953) reports *L. salmonicolor* from the col de Toluca, Mexico under *Abies religiosa*. The herbarium material that he deposited in PC unfortunately seems to be lost. KONG LUZ (1995) also gives a detailed macro- and microscopical description of collections of *L salmonicolor* from Mexico that resembles the European *L. salmonicolor* very well. Only the spore size he reports (8.8–9.35 × 6.43–7.28 µm on average) is smaller than what we measured from European material (10.0–10.6 × 7.3–8.2 µm on average). Furthermore KONG LUZ (1995) admits a more detailed comparison with *L. thyinos* is necessary. We were unable to study collections identified as *L. salmonicolor* from Mexico or elsewhere in North America and thus do not draw any further conclusions.

***Lactarius* sp.** Collection MTS 3445 (RMS) originally identified as *L. deliciosus* var. *olivaceosordidus* Fig. 29
MICROSCOPICAL DESCRIPTION: *Spores* (8.5) 8.6–*9.1*–9.6 × 6.3–*6.8*–7.4 µm, broadly ellipsoid to ellipsoid (Q = 1.27–*1.33*–1.40); ornamentation up to 0.5 µm high, of medium thick ridges and isolated warts, forming an incomplete reticulum; plage distally slightly amyloid. *Basidia* 40–50 × 9–11 µm, subclavate, 4-spored, mostly hyaline, sometimes with a granular content; sterigmata 3.5–5.5 µm long. *Pleuromacrocystidia* scarce, 45–55 × 5–7 µm, emergent, subfusiform with a tapering apex, sometimes slightly constricted at the apex but not capitate and never moniliform, hyaline and inconspicuous, thin-walled. *Pseudocystidia* abundant, 2–5 µm broad, not emergent, tortuous to cylindrical, with and ochre-yellow content. *Lamella edge* sterile with rather scarce cheilomacrocystidia; cheiloleptocystidia 7–25 × 3.5–8 µm, subclavate or more irregular, hyaline, thin-walled; cheilomacrocystidia small, 20–25 × 4–7 µm, only slightly emergent, subfusiform with a capitate to rarely moniliform apex, often apex merely tapering, content granular or needle-shaped, thin-walled. *Subhymenium* composed of small, ± isodiametric cells. *Hymenophoral trama* with abundant lactifers except near the edge. *Pileipellis* an ixocutis, up to 250 µm thick, composed of strongly interwoven hyphae, 1–5 µm broad, shrivelled hyphae only present near the surface, some extracellularly pigmented hyphae and lactifers present close to the surface. *Clamp-connections* absent.

COLLECTION EXAMINED: USA, Washington, Grays Harbor Co., Quinault Research Natural Area, plot 10-1-c-11 (0), solitary on moss covered conifer twig & surrounding conifer debris, common, 07.10.1992, G. Walker, J. Ammirati & M. Seidl, MTS 3445 (RMS).

DISCUSSION: This specimen was included in our phylogenetic analyses and was originally identified as *L. deliciosus* var. *olivaceosordidus*. In both ITS and *gpd* phylogenies, this collection does not fall in the "*L. deliciosus*" clade from North America. It was collected in Washington at the same site as collection JA 10762 (RMS) that also was identified as *L. deliciosus* var. *olivaceosordidus*. Collection JA 10762 however, groups with the other specimens identified as "*L. deliciosus*" or one of its varieties collected in North America in the molecular analyses. This means that *L. deliciosus* var. *olivaceosordidus* as currently identified in North America is polyphyletic. Based on microscopical data alone, it is impossible to draw any further conclusions. The microscopical features of this collection are very similar to those of the type of *L. deliciosus* var. *olivaceosordidus*. The spores are practically identical in size and ornamentation. The only differences noted concern the pleuro- and cheilomacrocystidia. Collection MTS 3445 (RMS) has rather exceptional pleuromacrocystidia with a narrowing or sometimes a slightly constricted apex that never is capitate or moniliform as in the type material of *L. deliciosus* var. *olivaceosordidus*. Furthermore the cheilomacrocystidia are scarce in MTS 3445 while they are very abundant in the type of *L. deliciosus* var. *olivaceosordidus*.

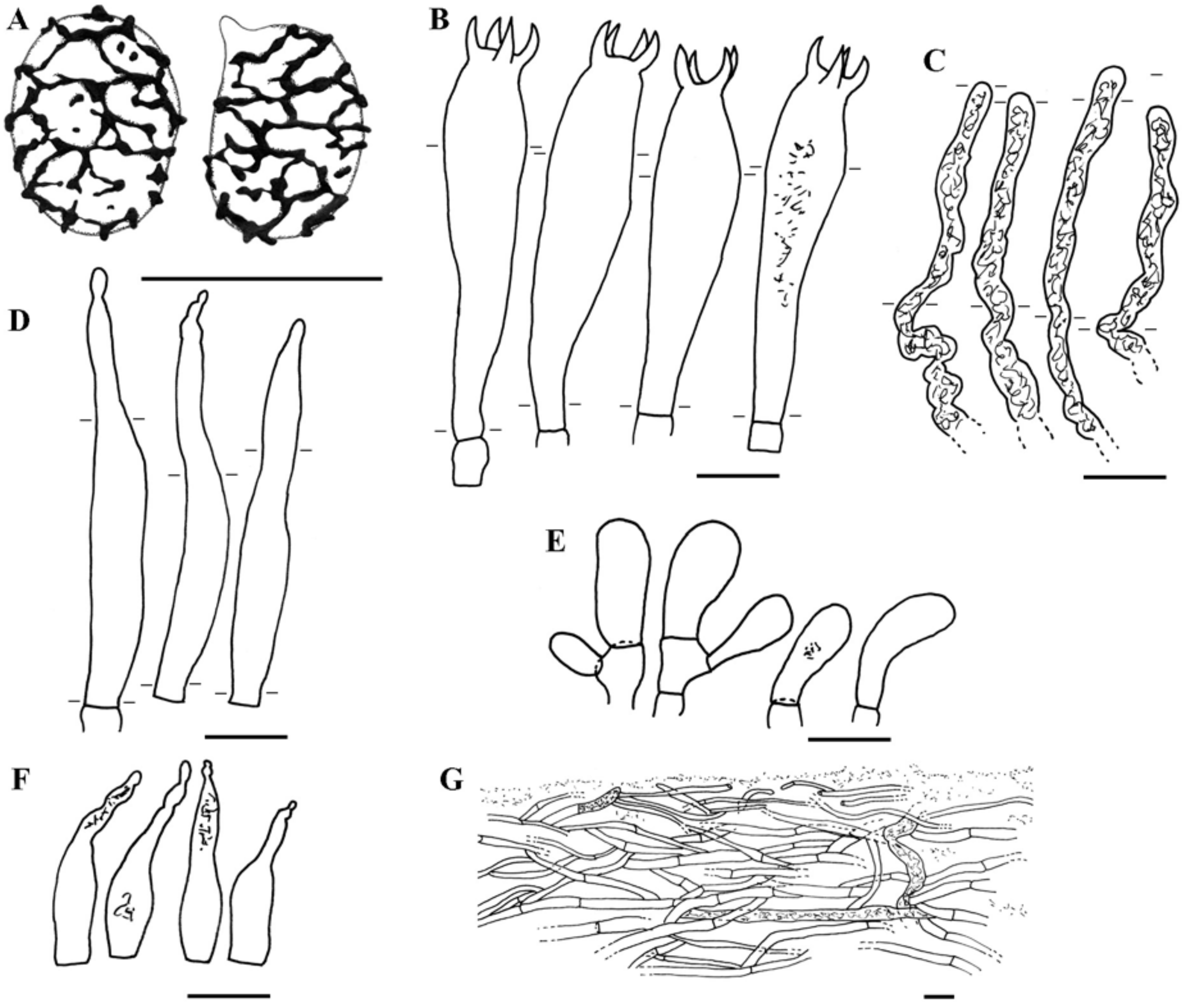

Fig. 29: *Lactarius* sp.: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheiloleptocystidia; **F.** cheilomacrocystidia; **G.** section through the pileipellis. From MTS 3445. Scale bars = 10 µm, small bars indicate the height of the hymenium.

In conclusion and as HORTON (2002), who revealed three different ITS-RFLP types in "*L. deliciosus*" from a locality in Oregon, already indicated, especially the "*L. deliciosus*"-complex needs more taxonomic attention in North America.

Acknowledgements

We thank the curators of the herbaria FLAS, MICH, NY, NYS, PC, RMS, SFSU, XAL and ZT and the collectors mentioned in the examined specimens sections. Steven L. Miller is thanked for the opportunity to visit his lab, do field work in the Rocky Mountains and the invitation to the MSA meeting in Asheville, 2004. This research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT), Belgium.

Chapter 8

Descriptions and taxonomy of the Asian representatives of *Lactarius* sect. *Deliciosi*

Adapted from:
NUYTINCK J. & VERBEKEN A. (2005) – Descriptions and taxonomy of the Asian representatives of *Lactarius* sect. *Deliciosi* (Russulales). *Fungal Diversity*: submitted.

Abstract

Nine Asian species belonging to *Lactarius* sect. *Deliciosi* are described and illustrated in detail in this paper. All of them were confirmed in a previous molecular phylogenetic study (NUYTINCK *et al.*, in preparation). Only *L. thakalorum* is possibly conspecific with the European *L. sanguifluus*. *Lactarius deliciosus* seems to be one of the rare species in this section that occurs in both Asia and Europe. *Lactarius* sp. 4 will be described as a new species. Two other new species were discovered but as detailed macroscopical notes are lacking we decided not to describe them as new species yet. Several unidentified and insufficiently known taxa are briefly discussed.

1. Introduction

In Asia, the knowledge of the genus *Lactarius* Pers. is very poor and fragmentary. Several publications focussed on the milk caps associated with mainly Fagales (*Castanopsis*, *Lithocarpus*, *Nothofagus*) from the tropical, mostly montane rainforests of Southeast Asia (VERBEKEN & HORAK, 1999 & 2000; VERBEKEN *et al.*, 2002). In Japan, the study of *Lactarius* is relatively well advanced. Sixty species are recorded for this country; most of them receive European or North American names. A general, critical overview of the reported species and the proportion of endemic species does not yet exist for the Asian continent. One of the major problems to be overcome is the lack of modern and illustrated descriptions and revisions from this continent (VERBEKEN, 2001).

Thirteen names have been published so far from Asia in *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.): seven species, three varieties, one form and two invalid names. We recognise five of those seven species here; we add *L. deliciosus* (L.: Fr.) Gray to the list and give descriptions of three new species. This brings the total number of species to nine. Only one of the three new species is effectively published as detailed macroscopical descriptions lack for the other two species. More taxa undoubtedly will be discovered as more fieldwork will be done in this underexplored area. Several unidentified collections and insufficiently known taxa are listed at the end of this paper. Furthermore, the species concept we (provisionally) apply here is wider than our species concept in a well-studied region as Europe. This is for example reflected in the large genetical and morphological variability we observed in *L. hatsudake* Tanaka.

All nine Asian species described here were included in a molecular analysis of this section on a worldwide scale, based on ITS and *gpd* sequences (NUYTINCK *et al.*, in preparation). Although this analysis indicates a possible conspecificity of *L. thakalorum* Bills & Cotter with *L. sanguifluus* (Paulet) Fr., *L. thakalorum* is provisionally maintained as a separate species here. In the discussion under *L. thakalorum* we give an overview of the morphological differences with *L. sanguifluus*. More evidence, in the form of more, well documented collections, is needed to come to a confident conclusion on the eventual conspecificity. The description of *L. deliciosus* that we provide here is entirely based on specimens collected in China (Guizhou, Yunnan and Sichuan). A description based on European collections can be found in NUYTINCK & VERBEKEN (2005). These descriptions were kept separately in order to maintain information on the regional differences in the morphology of this species.

2. Materials & Methods

This study is based on herbarium material obtained from BPI, H, HKAS, RMS, TMI and ZT or deposited in GENT. Macroscopical characters are based on observations on fresh collections or are compiled from literature and field notes attached to herbarium collections. Colour codes are according to KORNERUP & WANSCHER (1962). Microscopic measurements and drawings were made under oil immersion at 1000× with a Zeiss Axioscop 2 microscope and drawing tube. All observations and measurements (except for the spores) were made in Congo red in L_4 (7.2 g KOH, 160 ml glycerine, 840 ml dH_2O, 7.6 g NaCl and 5 ml Invadin (Ciba-Geigi), CLÉMENÇON, 1972). Where necessary a short pre-treatment in 10% KOH was used to rehydrate the tissue. Basidia lengths exclude sterigmata lengths. We use the term cheiloleptocystidia for the thin-walled, mostly clavate to irregularly shaped cystidia, without specific content and with a rounded apex on the lamella edge. Observations and measurements of basidiospores where made in Melzer's reagent. Spores were measured in side view, excluding ornamentation and 20 spores were measured for every collection mentioned under the examined collections section. Measurements are given as (MIN) [Ava-2×SDa] – *Ava* – *Avb* – [Avb+2×SDb] (MAX) in which Ava = lowest mean value for the measured collections, Avb = greatest mean value and SDa/b = standard deviation of the lowest and greatest mean value respectively. MIN is the lowest value measured, MAX the highest value; MIN and MAX are only given when they exceed [Ava-2×SDa] or [Avb+2×SDb] respectively. Q stands for 'quotient length/width' and is given as MINQ – *Qa* – *Qb* – MAXQ in which Qa and Qb stand for the lowest and the highest mean quotient for the measured specimens respectively. MINQ/MAXQ stands for the minimum/maximum value over the quotients of all available measured spores. For species that were microscopically described using only one specimen, the measurements are given as (MIN) [Av-2×SD] – *Av* – [Av+2×SD] (MAX) in which Av is the mean value of the 20 spores measured from that collection and as MINQ – *Q* – MAXQ in which Q stands for the mean quotient of the measured spores.

Scanning electron photographs were taken with a JEOL JSM-5600 LV microscope. Small pieces of lamellae were taken from dried specimens and soaked overnight in strongly diluted ammonia. The material was then treated with 70% ethanol (2 × 15 min.) and dimethoxymethane (2 × 30 min.), before being submitted to the process of critical point drying. This was done with a BAL-TEC CDP 030 dryer. The samples were then coated with gold in a JEOL JFC-1200 Fine Coater for 60 sec. at 8 Pa and 30 mA, until a 15 nm thick layer covered the spores.

3. Descriptions and taxonomical conclusions

3.1. *Lactarius akahatsu* Tanaka, Bot. Mag. (Tokyo) 4: 394 (1890)

Figs. 1, 2, 3

Synonyms:	≡ ***L. hatsudake*** var. ***akahatsu*** (Tanaka) Kawam., Bot. Mag. (Tokyo) 28: 525 (1914)
	= ***L. deliciosus*** f. ***virescens*** S. Imai, Bot. Mag. (Tokyo) 49: 607 (1935)
Misapplication:	***L. deliciosus*** sensu Yasuda (1913); sensu Kobayshi (1939)

Type: not selected, described from Japan.

INTRODUCTION: We did not observe fresh *L. akahatsu* collections from Japan and thus had to adapt the macroscopical description from the original description and other literature data (TANAKA, 1890; HONGO, 1977). Microscopical features were observed on herbarium material from Japan (one collection only). Molecular analysis revealed a very close relationship between this Japanese specimen and some recent collections from Thailand. Several macro- and microscopical differences, such as the behaviour of the latex and the occurrence of macrocystidia, persuaded us to keep the descriptions separately. Obviously, more collections from other localities are needed to arrive at a reliable and detailed description of *L. akahatsu*.

DESCRIPTIONS:

Macroscopical description compiled from TANAKA (1890) & HONGO (1977):

Pileus 2–10 cm diam., at first convex with an incurved margin, then flattened to depressed; margin smooth, becoming slightly undulated, rather thin and acute; surface smooth, glabrous, viscid when moist, zoned; colour orange to brownish orange (6A/B6), then pale, margin pale orange, staining green with age. *Lamellae* decurrent, rather thin, crowded; colour orange (5A6-6A6), staining green where bruised. *Stipe* 3–6 × 1.2–2 cm, subequal, sometimes curved, more or less rugulose, becoming hollow, concolorous with the pileus to pruinose. *Latex* scarce, orange, slowly becoming vinaceous red on the context; taste mild or slightly acrid.

Microscopical observations on *L. akahatsu* TMI 22601 (TMI), collected in Japan:

Spores 8.0–*8.5*–9.1 (9.3) × 5.9–*6.4*–7.0 µm, broadly ellipsoid to ellipsoid (Q = 1.22–*1.33*–1.44); ornamentation up to 0.7 µm high, of rather broad ridges and some isolated warts and short ridges, forming an almost complete reticulum; plage distally slightly amyloid, often with scattered, light amyloid spots. *Basidia* 45–55 × 9–11 µm, subclavate to almost cylindrical, 4-spored, content sometimes slightly granular or with oil-droplets; sterigmata 4.5–6.5 µm long. *Pleuromacrocystidia* rather abundant but hardly visible, 30–50 × 6–8.5 µm, not to slightly emergent, fusiform with an obtuse to (more rarely) capitate apex, with a needle-shaped content, thin-walled. *Pseudocystidia* rather abundant, (2) 3–5 µm broad, not to slightly emergent, cylindrical but tortuous near the base, content oleiferic. *Lamella edge* sterile with quite abundant cheilomacrocystidia; cheiloleptocystidia 11–25 × 3–5 µm, subclavate to irregular, hyaline or with a pale brownish to ochre, granular content (observation in 10% KOH); cheilomacrocystidia 19–25 × 4–6 µm, subfusiform but often irregular, with a moniliform apex, hyaline or with a granular content, thin-walled. *Pileipellis* an ixocutis, 90–120 µm thick, with only very few shrivelled hyphae. *Clamp-connections* absent.

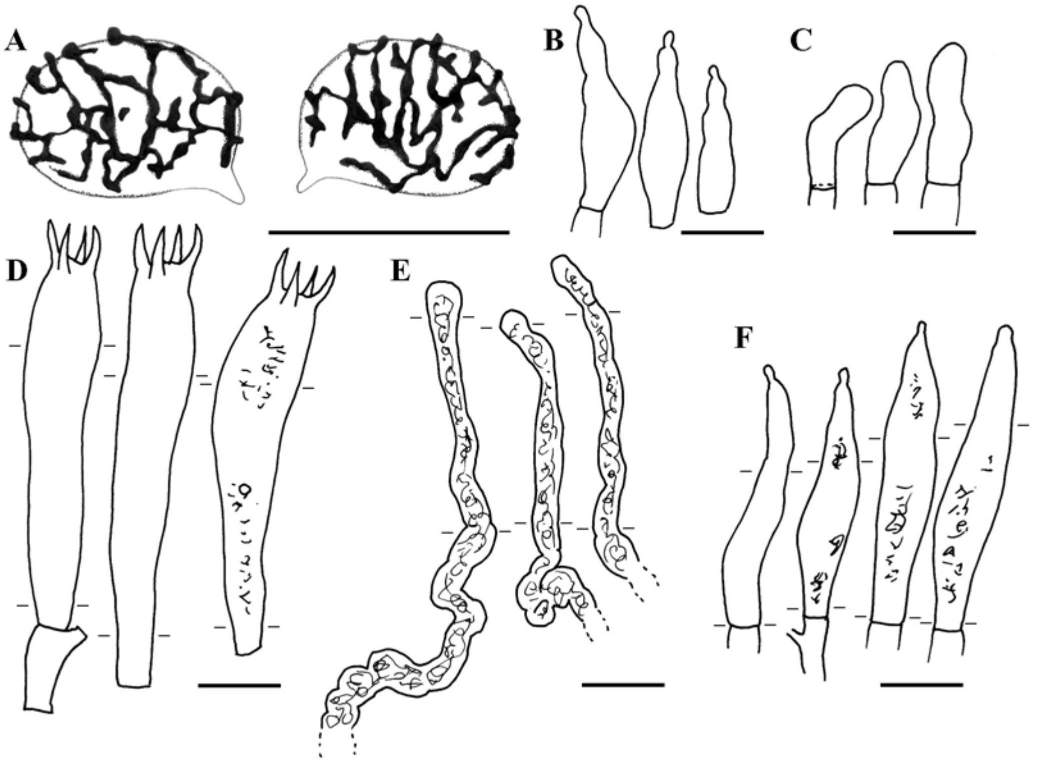

Fig. 1: *Lactarius akahatsu*: **A.** spores; **B.** cheilomacrocystidia; **C.** cheiloleptocystidia; **D.** basidia; **E.** pseudocystidia; **F.** pleuromacrocystidia. From TMI 22601. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Macroscopical observations on A. Verbeken 2004-015, A. Verbeken 2004-036 and A. Verbeken 2004-076 (Thailand):

Pileus 6–12 cm diam., plano-convex to widely infundibuliform, irregular; margin straight to very irregular wavy, (translucently) striate (up to 12 mm); surface smooth, sticky, greasy to slightly viscid, weakly zonate in some specimens, with distinct zones near the margin in other specimens, zones consisting of watery spots; colour partly pale orange (4A4/5 near the centre), deeper orange in other places (5A/B6 to 6A7/8), more whitish orange when dry, with emerald green shades when older. *Lamellae* decurrent with a small tooth, distant, abundant; colour orange (5A5) but staining greenish when bruised, dirty brownish orange when older; edge entire, paler. *Stipe* 3–4.6 × 1.1–2 cm, irregularly cylindrical, tapering downwards, central to slightly eccentric; surface smooth, slightly viscid; colour orange-buff (5A4–6). *Context* rather firm, ± 6 mm thick in the pileus, hollow in the stipe, pale cream to whitish but soon pinkish buff to pale orange when cut, especially underneath the pellis; colour unchanging; smell agreeable, sweetish or flowery; taste agreeable, mild, like nuts. *Latex* scarce, bright orange (6A8).

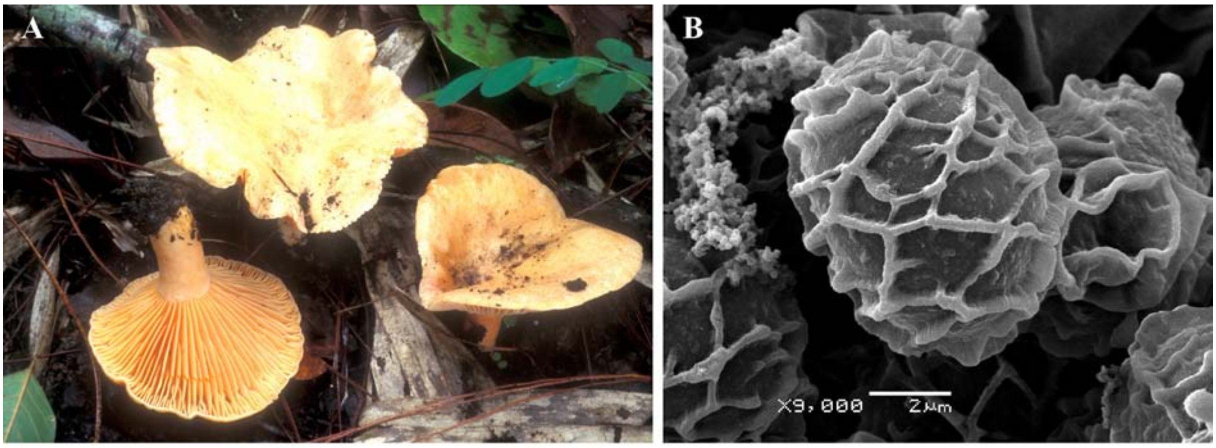

Fig. 2: **A.** *L. akahatsu* A. Verbeken 2004-076 (photo R. Walleyn); **B.** SEM image of the spores of *L. akahatsu* A. Verbeken 2004-141.

Microscopical observations on A. Verbeken 2004-141 (Thailand):

Spores 7.5–*7.9*–8.4 × 5.5–*6.0*–6.5 (6.8) µm, broadly ellipsoid to ellipsoid (Q = 1.22–*1.33*–1.44); ornamentation up to 0.5 µm high, of rather broad ridges and isolated warts, forming a rather complete reticulum; plage distally slightly amyloid, ± centrally additional amyloid spots often present. *Basidia* 35–50 × 9–10 µm, subclavate, 4-spored, often containing oil-droplets; sterigmata 3.5 –5 µm long. *Pleuromacrocystidia* very scarce, 50–55 × 6–8 µm, emergent, fusiform with a moniliform apex, content needle-shaped, thin-walled. *Pseudocystidia* abundant, 4–5.5 µm broad, not to slightly emergent, often tortuous, content oleiferic. *Lamella edge* sterile without cheilomacrocystidia; cheiloleptocystidia 9.5–22 × 4–8 µm, subclavate to irregular, hyaline or with an ochre granular content (observation in 10% KOH), thin-walled. *Pileipellis* an ixocutis, 50–250 µm thick; composed of mostly strongly shrivelled and gelatinised hyphae in the upper layer, hyphae 1–3 µm diam. *Stipitipellis* a cutis, 70–100 µm thick, composed of thin-walled, strongly interwoven hyphae, 2–4 µm diam., no shrivelled hyphae. *Clamp-connections* absent.

HABITAT: On the ground in lowland *Pinus* forests, widely distributed along with *Pinus* spp., such as *P. densiflora*, *P. thunbergii*, *P. liuchuensis* etc., found in the autumn (to the winter on the Bonin Islands) (NAGASAWA, 1998); the Thailand collections were found under *Pinus kesiya*.

DISTRIBUTION: Reported from Japan and introduced on the Bonin Islands (HONGO, 1960 & 1977). A very closely related, probably conspecific taxon is found in Thailand

COLLECTIONS EXAMINED: Japan, Tottori, Tottori-shi, Uemachi (Bairian), under planted *Pinus thunbergii* in garden, 27.09.1997, leg. E. Nagasawa, TMI 22601 (TMI) – Thailand, Chiang Mai Prov., Mae Teng distr., Toung Yoa village, forest trail, 1300 m a.s.l., N19°08.07' E98°38.90', secondary forest with *Pinus kesiya*, *Castanopsis* etc., 20.06.2004, leg. R. Walleyn & A. Verbeken 2004-015 (GENT) – ibid., road side, 1350 m a.s.l., N19°08.07' E98°38.90', under *Pinus kesiya*, 21.06.2004, leg R. Walleyn & A. Verbeken 2004-036 (GENT) – Chiang Mai Prov., Mae Teng distr., Highway 1095 at km "22", 750 m a.s.l., N19°07.57' E98°45.65', xeric broad-leaf forest (*Dipterocarpus* spp. + teak) with *Pinus kesiya*, under *Pinus*, 23.06.2004, leg. R. Walleyn & A. Verbeken 2004-076 (GENT) – Chiang Mai Prov., Huai Nam Dang National Park, nature trail, 1530 m a.s.l., N19°18.29' E98°35.88', forest with *Pinus kesiya*, bamboo & scattered *Castanopsis*, 28.06.2004, leg. R. Walleyn & A. Verbeken 2004-141 (GENT).

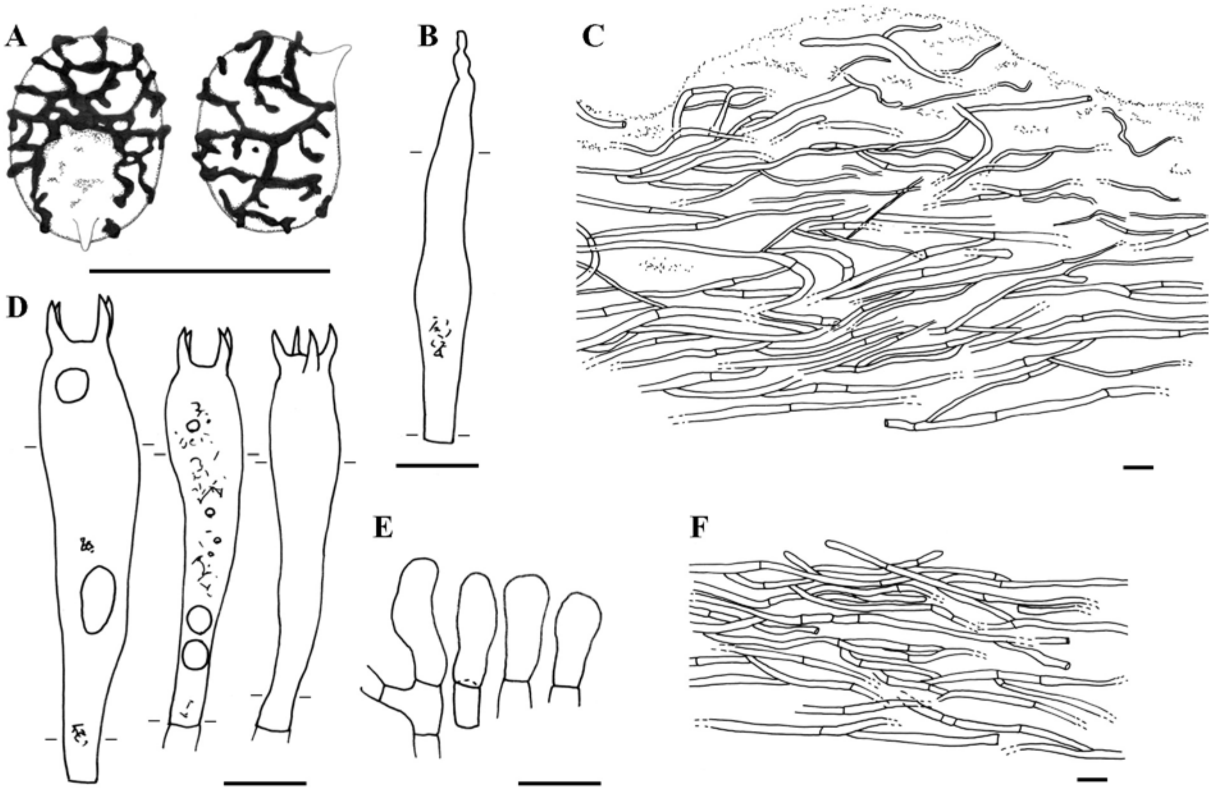

Fig. 3: *Lactarius akahatsu*: **A.** spores; **B.** pleuromacrocystidium; **C.** section through the pileipellis; **D.** basidia; **E.** cheiloleptocystidia; **F.** section through the stipitipellis. From A. Verbeken 2004-141. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: *Lactarius akahatsu* seems to be a common species in Japan, characterised by its generally orange appearance and orange latex that slowly becomes red on the context. It is unmistakably different from *L. hatsudake*, a species with red latex from the beginning. TANAKA (1890) moreover, remarks that the lamellae in *L. akahatsu* are more narrow than those of *L. hatsudake*. The descriptions by TANAKA (1890) and HONGO (1977) include short notes on the microscopy. TANAKA (1890) reports much larger spores (9–11 × 6–8 µm). The microscopical description by HONGO (1977), that mentions abundant cheilomacrocystidia but pleuromacrocystidia difficult to demonstrate, corresponds best with our observations on collection 22601 (TMI). The pleuromacrocystidia we observed on collection TMI 22601 are remarkable in that their shape is slightly different from the pleuromacrocystidia in most other members of *Lactarius* sect. *Deliciosi*. The apex is obtuse to (more rarely) capitate here, while a moniliform apex is observed in most species. Certainly, more observations on more collections are needed to confirm these findings.

HONGO (1977) thinks *L. akahatsu* is very closely related to or even conspecific with *L. semisanguifluus*. He concludes this mainly because of the colour change of the latex. SINGER (1986) suggests the same. IMAI (1935) suggests *L. akahatsu* and *L. deliciosus* are conspecific. He distinguishes a form growing in *Pinus* and *Picea* woods (*L. deliciosus* f. *virescens*), and a form from *Abies* woods that doesn't discolour greenish (*L. deliciosus* f. *laeticolorus*). Our molecular approach reveals that *L. akahatsu* is a species well separated from all other species in *Lactarius* sect. *Deliciosi*. The exact position in the section remains unclear.

The Japanese specimens differ from the ones collected in Thailand by the behaviour of the latex, which slowly becomes vinaceous red or stays orange respectively. Microscopically pleuro- and cheilomacrocystidia are more abundant in the Japanese collection.

3.2. *Lactarius deliciosus* (L.: Fr.) Gray, Nat. Arr. Br. Pl. 1: 624 (1821)

Figs. 4, 5

Basionym: ≡ ***Agaricus deliciosus*** L., Species Pl.: 1172 (1753)
Synonyms: = ***Lactaria lateritia*** Pers., Tent. Disp. Meth. Fung.: 64 (1797)
= ***L. deliciosus*** var. ***lamelliporus*** (Barla) Sacc., Sylloge Fungorum 5: 438 (1887)
 ≡ ***Agaricus deliciosus*** var. ***lamelliporus*** Barla, Champ. Prov. Nice: 35 (1859)
= ***L. deliciosus*** var. ***pini*** Vassilkov, [Edible and poisonous fungi of central parts Europ.distr. U.S.S.R.]: 60 (1948), nom. inval., nom. nud.
= ***L. pinicola*** Smotl. ex. Z. Schaef., Schweiz. Z. Pilzk. 48: 141 (1970)
 ≡ ***L. deliciosus*** var. ***pinicola*** Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 219 (1947), nom. inval., nom. nud.
= ***L. deliciosus*** f. ***rubescens*** J.A. Schmitt, Z. Pilzk. 39: 238 (1974)
= ***L. deliciosus*** var. ***lateritius*** J. Blum ex J. Blum, Lactaires: 216 (1976)

Type: not selected, described from southern Sweden.

DESCRIPTION: *Pileus* 3–9 cm diam., sometimes larger, convex with an inrolled margin and a slightly depressed centre when young, becoming infundibuliform; surface smooth, viscid; colour pale orange, yellowish to greyish orange, often with darker spots or concentric zones, especially when young, becoming bluish green when bruised. *Lamellae* (slightly) decurrent, rather crowded, often forked near the stipe; colour pale orange to yellowish but often more intense than the pileus colour, when bruised discolouring greenish to bluish. *Stipe* 2–4 × 0.6–2 cm, cylindrical or tapering near the base; surface smooth, scrobicules present in most basidiocarps; colour pale orange to yellowish, whitish near the lamellae, discolouring greenish when bruised. *Context* hollow in the stipe, white in the central parts, very pale orange near the pilei- and stipitipellis, orange above the lamellae, unchanging to rarely slowly becoming red (uncertain observation), discolouring bluish green. *Latex* scarce, orange to reddish; taste sometimes slightly acrid.

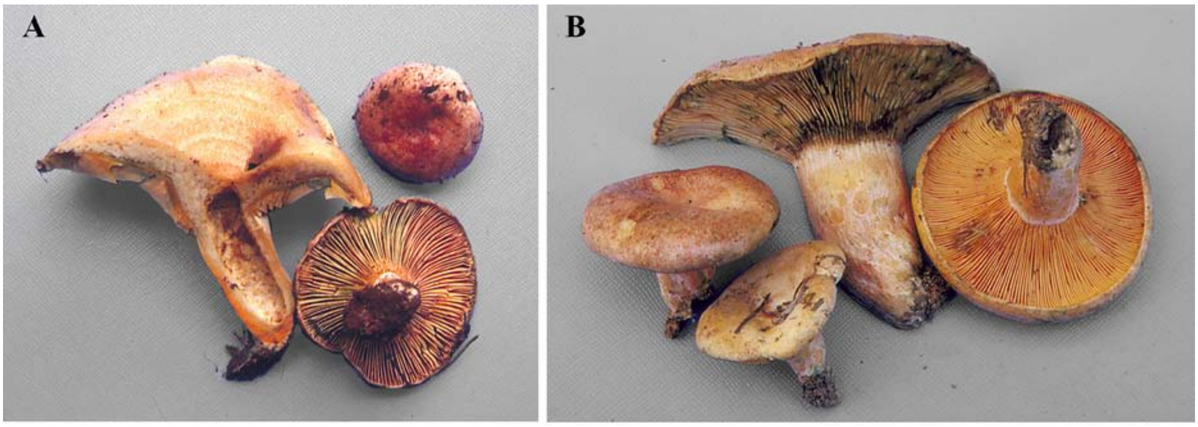

Fig. 4: A. and **B.** *L. deliciosus* collected in Yunnan, showing the macroscopical variability (photo's X.H. Wang).

Spores 7.2–*8.0*–*8.8*–9.3 × 5.7–*6.4*–*6.6*–7.3 µm, subglobose to ellipsoid, mainly broadly ellipsoid (Q = 1.13–*1.24*–*1.33*–1.43); ornamentation up to 0.7 µm high, of rather thick ridges and quite some isolated warts and short ridges, forming an incomplete or seldom almost complete reticulum; plage inconspicuous and with scattered amyloid spots to slightly amyloid distally. *Basidia* 40–60 × 8–11 µm, subclavate, 4-spored, often containing oil-droplets or with a granular content. *Pleuromacrocystidia* rather abundant, 40–62 × 5.5–10.5 µm, emergent, subfusiform, with a moniliform or capitate apex, with a granular to needle-shaped content, thin-walled. *Pseudocystidia* not very abundant, 2–4 µm broad, rarely emergent, rather slender, tortuous in the lower part, content oleiferic and ochre-brown coloured. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia 6–20 × 2.5–6 µm, subclavate to almost cylindrical but often bent or irregular, hyaline, thin-walled; cheilomacrocystidia 24–35 × 4–6 µm, subfusiform, with a moniliform apex, often with a granular content, thin-walled. *Subhymenium* composed of globose to more irregularly shaped, small cells. *Hymenophoral trama* irregularly filamentous; with remarkably abundant and broad lactifers. *Pileipellis* an ixocutis, 100–350 µm thick, of strongly interwoven hyphae, 1–6 µm diam., shrivelled and gelatinised hyphae abundant near the surface. *Stipitipellis* an ixocutis, up to 100 µm thick, of regularly shaped and strongly interwoven hyphae, 2-4 µm diam., only very few shrivelled hyphae. *Clamp-connections* absent.

HABITAT: Growing under *Pinus yunnanensis* and other *Pinus* species.

DISTRIBUTION: The examined material was collected in three Chinese provinces: Guizhou, Yunnan and Sichuan.

COLLECTIONS EXAMINED: China, Guizhou Prov.: Weining county, Heishitou, 15.09.1993, M. Zang 3610, HKAS 29133 (HKAS) – Yunnan Prov.: Lijiang county, Yulong Mountain, under *Pinus densata*, 29.07.1995, M. Zang,12, HKAS 30003 (HKAS) – Sichuan Prov.: Xiaojin county, Rilong, Shuangqiaogou, 3400 m a.s.l., 23.08.1996, 2503, HKAS 30958 (HKAS) – Sichuan Prov.: Luding county, Hailuogou, 2600 m a.s.l., 28.08.1996, 2513, HKAS 30970 (HKAS) – Sichuan Prov.: Luding county, Hailuogou, 3000 m a.s.l., 02.09.1996, M. Zang 2584, HKAS 31054 (HKAS) – Yunnan Prov.: Qujing City, 04.10.1997, X.H. Wang 171, HKAS 31722 (HKAS) – Sichuan Prov.: Xiaojin county, Rilong, 3200 m a.s.l., *Picea, Betula, Populus,* 19.07.1998, M.S. Yuan 3020, HKAS 33954 (HKAS) – Yunnan Prov.: near the city of Baoshan, 1800 m a.s.l., *Pinus yunnanensis,* 30.09.1998, Z.L. Yang 2574, HKAS 32125 (HKAS) – Sichuan Prov.: Hongyuan county, Shuajingsi, 3500 m a.s.l., *Picea, Betula*, 22.08.1998, M.S. Yuan 3471, HKAS 33824 (HKAS) – Yunnan Prov.: Lijiang county town, 2000 m a.s.l., 11.09.1999, P.Q. Sun. 4537, HKAS 34741 (HKAS) – Yunnan Prov.: Chuxiong, Zixishan, *Pinus yunnanensis,* 11.07.2001, F.Q. Yu 437, HKAS 39044 (HKAS) –Yunnan Prov.: Lijiang county, Laojunshan, 2800 m a.s.l., *Pinus yunnanensis,* 29.07.2001, Z.L. Yang 3109, HKAS 38286 (HKAS) – Yunnan Prov.: Kunming, Heilongtan Park, 1890 m a.s.l., *Pinus yunnanensis,* 11.10.2001, F.Q. Yu 769, HKAS 39045 (HKAS).

DISCUSSION: Molecular evidence shows that the included Asian collection of *L. deliciosus* is conspecific with the European ones. Small microscopical differences we observed are the slightly smaller spores, the more abundant pleuromacrocystidia and the ixocutis of the stipe in the Asian specimens. The abundance of pleuromacrocystidia is however a variable feature within many species in *Lactarius* sect. *Deliciosi*. HESLER & SMITH (1979) mention that the occurrence of a cutis or ixocutis as stipe cuticle can also be variable within a species.

Apparently, quite some variability is observed in the macromorphology of *L. deliciosus*. W.H. Wang (pers. comm.) describes the variability as follows: some collections consist of smaller basidiocarps with dirty, ochraceous to pale orange colours, often with green hues; they are rather slender and never have very incurved margins; they grow with *Pinus* (Fig. 4 A). Other collections are more robust and have brighter orange colours, especially in the lamellae; the margins are often inrolled; they also grow under *Pinus* (Fig. 4 B). Both types are very commonly sold on the markets in Yunnan, often mixed. A third, less common type from higher elevations has pure but rather pale orange colours and grows in *Abies*-*Picea* forests; the former two forms are clearly duller in colour. All these collections do not differ microscopically; they were not yet included in any molecular analysis.

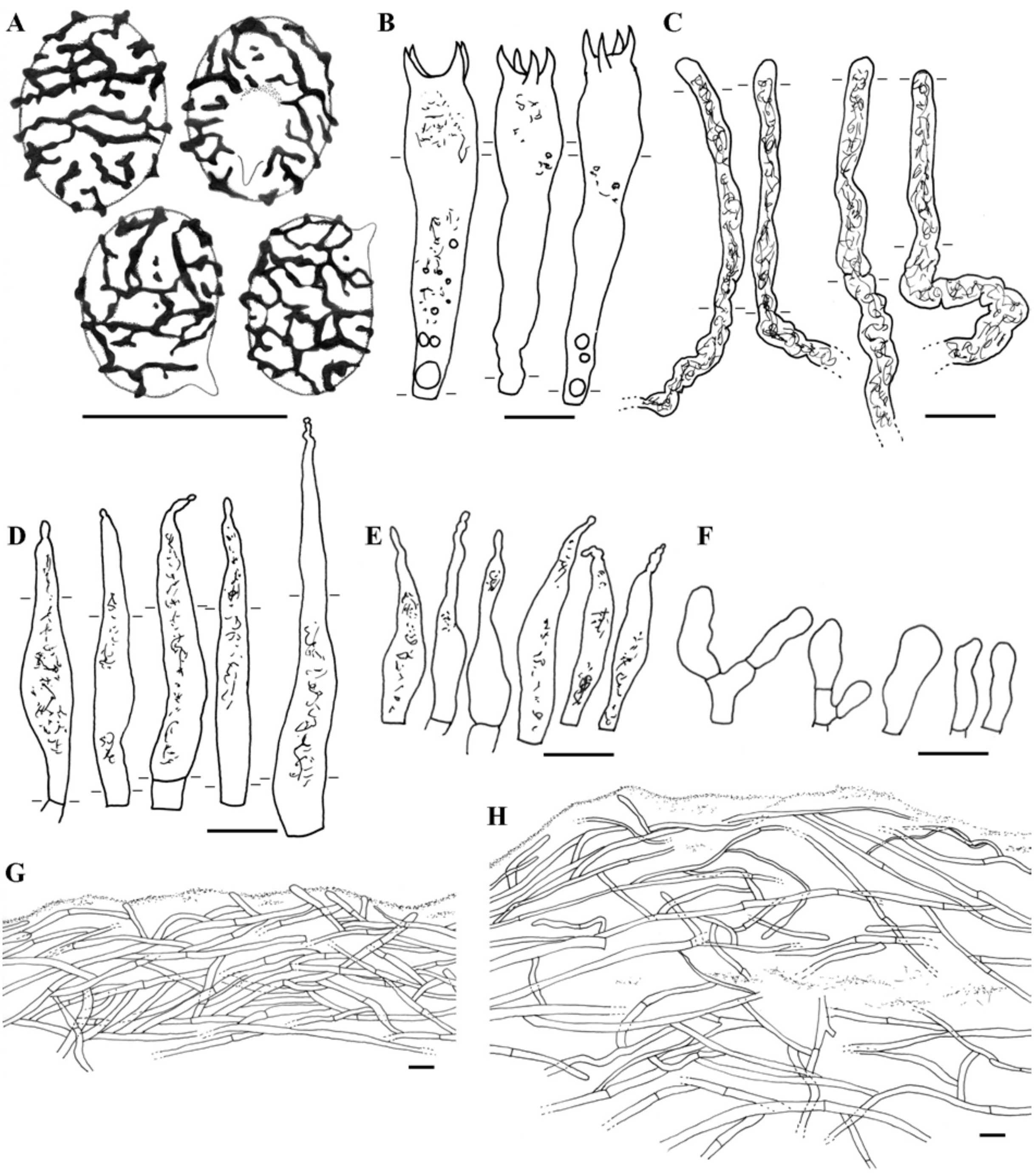

Fig. 5: *Lactarius deliciosus*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the stipitipellis; **H.** section through the pileipellis. From HKAS29133, HKAS31054, HKAS31722, HKAS32125, HKAS33954, HKAS38286 and HKAS39045. Scale bars = 10 µm, small bars indicate the height of the hymenium.

3.3. *Lactarius hatsudake* Tanaka, Bot. Mag. (Tokyo) 4: 393 (1890)

Figs. 6, 7, 8

Synonym: ?= ***L. lividatus*** Berk. & M.A. Curtis, Proc. Amer. Acad. Arts & Sciences 4: 119 (1860)

Type: not selected, described from Japan.

DESCRIPTION: *Pileus* 3–10 cm diam., at first convex with an incurved margin, sometimes with a papilla, then expanded and margin bent downwards to slightly incurved, finally widely infundibuliform; margin sometimes slightly translucently striate; surface smooth, greasy to slightly viscid when wet, zoned, especially near the margin; colour pale reddish buff to dirty brownish pink or with ochraceous tinges (7/8B3/4), sometimes with a darker centre (8C5), zones dull red, older or discoloured specimens very light orange or with a greenish ochraceous centre, discolouring bluish green in age (25F6), slightly hygrophanous. *Lamellae* decurrent, rather crowded to subdistant, often branched, rather broad; colour dull, pale reddish when young, vinaceous red when mature, becoming more ochraceous, mixed with greenish or bluish green in age; edge entire, paler. *Stipe* 1.5–4 × 0.6–2 cm, short, tapering downwards, becoming hollow; surface glabrous; colour dull reddish (9C/D5), often covered with a white layer, scrobicules absent, with a white zone at the apex. *Context* firm in the centre of the pileus to very thin at the margin, white to buff, staining vinaceous red (10/11C5) underneath the pileipellis or in the entire pileus surface and underneath the stipitipellis, later becoming greenish; smell indistinct to sweet, agreeable; taste mild. *Latex* scarce, vinaceous red (11D6/7), unchanging. *Spore deposit* unknown.

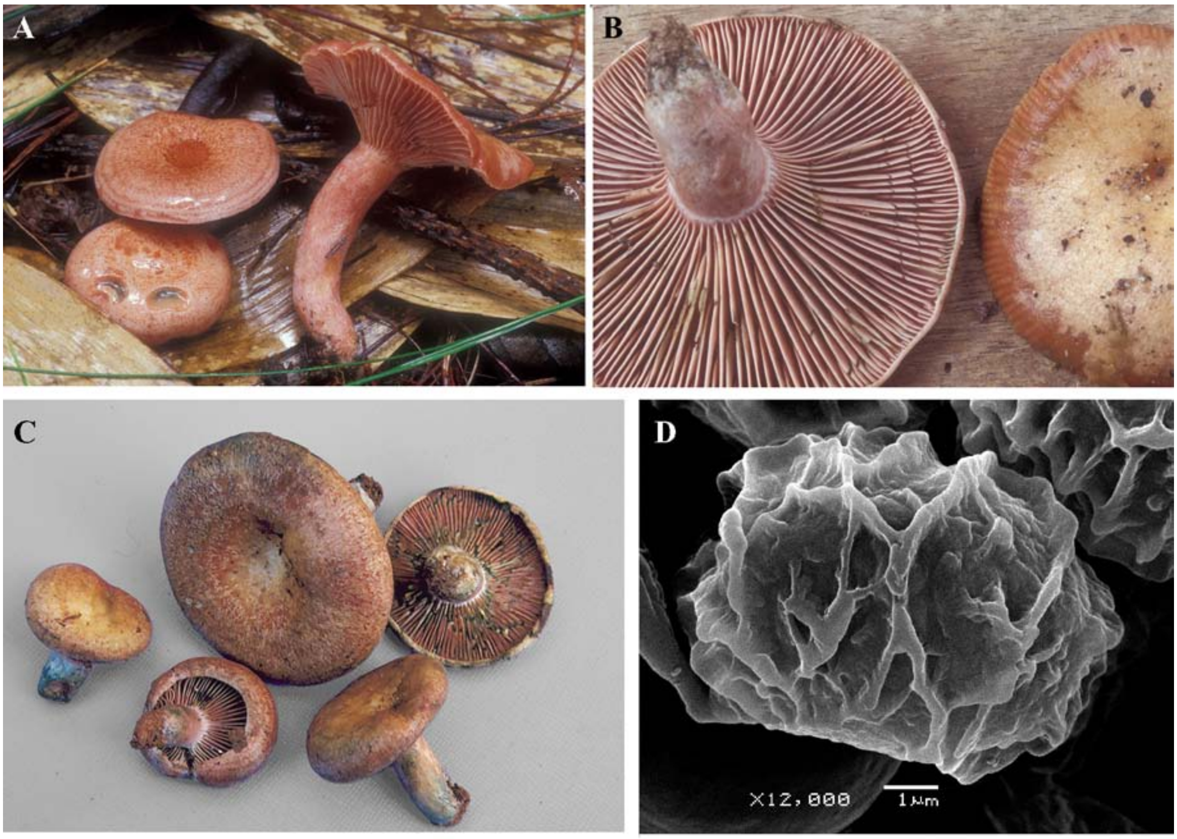

Fig. 6: A. and **B.** *L. hatsudake* collected in Thailand (photo's R. Walleyn); **C.** *L. hatsudake* collected in Yunnan, China (photo X.H. Wang); **D.** SEM image of a spore of *L. hatsudake* HKAS39045.

Spores 7.7–*8.3–9.0*–9.7 × 5.6–*6.2–6.6*–7.5 µm, broadly ellipsoid to ellipsoid (Q = 1.21–*1.30–1.39*–1.54); ornamentation up to 0.8 (1.0) µm high, composed of conspicuous, large, but rather faint amyloid spots connected with mainly thick ridges but also with scarcer thin ridges, forming an irregular, dense and almost complete network, some isolated warts present; plage often with an irregular, faintly amyloid pattern or distally amyloid. *Basidia* 40–52 × 8–14 µm, subclavate, 4-spored; sterigmata 4.5–8 µm long. *Pleuromacrocystidia* very scarce but more abundant close to the lamella edge, 42–48 × 7–9 µm, emergent, subfusiform, with a moniliform or acute apex, thin-walled. *Pseudocystidia* abundant, 2.5–5 µm diam., not to slightly emergent, cylindrical to tortuous, with an ochre-yellow content. *Lamella edge* sterile with scarce cheilomacrocystidia but macrocystidia mostly abundant close to the edge; cheiloleptocystidia mostly 8–16 × 3.5–6 µm but rarely very conspicuous and emergent, up to 35–40 µm long, subclavate to cylindrical or more irregular, hyaline, thin-walled; cheilomacrocystidia 24–35 × 4.5–6 µm, subfusiform with a moniliform apex, content needle-shaped or granular, thin-walled. *Subhymenium* composed of mainly small, ± isodiametric, irregularly arranged cells. *Hymenophoral trama* irregularly filamentous, with abundant lactifers. *Pileipellis* an ixocutis, 50–300 µm thick, of mainly shrivelled and gelatinised hyphae in the top layer, deformed and swollen hyphae also present; hyphae thin-walled and 1–6 µm broad. *Stipitipellis* a cutis, 30–70 µm thick, of thin-walled, strongly interwoven hyphae, 2–6 µm broad; no shrivelled or gelatinised hyphae. *Clamp-connections* absent.

HABITAT: Under *Pinus* spp., such as *P. thunbergii*, *P. densiflora*, *P. liuchuensis*, *P. yunnanensis*, *P. kesiya*; in Japan in lowland forests and gardens etc., abundant in (early) autumn; in Yunnan (Southwest China) throughout the mushroom season (May to September) (TANAKA, 1890; NAGASAWA, 1998; X.H. Wang, pers. comm.).

DISTRIBUTION: Recorded from China, Japan, the Bonin Islands, eastern Russia, Korea, Thailand and Taiwan.

COLLECTIONS EXAMINED: China, Yunnan Prov.: Lunan county, Stone Forest, 11.10.1995, P.G. Liu & Y. Doi, HKAS 29734 (HKAS) – Yunnan Prov.: Kunming, Ciba, 1900 m a.s.l., 30.08.1997, X.H. Wang 50, HKAS 31714 (HKAS) – Yunnan Prov.: Kunming, Longtoujie, 1900 m a.s.l., 03.09.1997, X.H. Wang 60, HKAS 31715 (HKAS) – Yunnan Prov.: Songming county, Baiyi, 2000 m a.s.l., *Pinus yunnanensis,* 22.07.1998, X.H.Wang 407, HKAS 32067(HKAS) – Yunnan Prov.: near the city of Baoshan, 1800 m a.s.l., under *Pinus yunnanensis*, 30.09.1998, Z.L. Yang 2577, HKAS 32124 (HKAS) – Yunnan Prov.: Songming county town, 28.08.1999, X.H. Wang 911, HKAS 39349 (HKAS) – Yunnan Prov.: Wuding county, Shizishan, *Pinus densata*, 05.07.2000, X.H. Wang 992, HKAS 39354 (HKAS) – Yunnan Prov.: Kunming Botanical Garden, 1900 m a.s.l., under *Pinus yunnanensis* & *Quercus*, 17.07.2000, X.H. Wang 1014, HKAS 39355 (HKAS) – Yunnan Prov.: Kunming, Laobailong, 2000 m a.s.l., *Pinus yunnanensis*, 24.07.2000, F.Q. Yu 12, HKAS 39034 (HKAS) – Yunnan Prov.: Kunming, Heilongtan Park, 1890 m a.s.l., *Pinus yunnanensis*, 06.09.2000, F.Q. Yu 395, HKAS 39031 (HKAS) – Yunnan Prov.: Kunming, Qiongzhusi, 2000 m a.s.l., *Pinus yunnanensis*, 14.09.2000, F.Q. Yu 413, HKAS 38689 (HKAS) – Yunnan Prov.: Nanjian county, Zhonghuashan, 1940 m a.s.l., *Pinus yunnanensis*, 13.08.2001, M. Zang 13859, HKAS 38541 (HKAS) – Yunnan Prov.: Kunming, Laobailong, 2000 m a.s.l., under *Pinus yunnanensis*, 14.08.2001, F.Q. Yu,512, HKAS 39047 (HKAS) – Yunnan Prov.: Chuxiong, Zixishan, 2200 m a.s.l., *Pinus yunnanenssis*, 23.08.2001, F.Q. Yu 541, HKAS 39032 (HKAS) – Yunnan Prov.: near Gongshan county town, 2000 m a.s.l., *Pinus yunnanensis*, 06.09.2001, F.Q. Yu 703, HKAS 39035 (HKAS) – Yunnan Prov.: Kunming Botanical Garden, 1900 m a.s.l., 06.09.2001, L.F. Zhang 88, HKAS 38454 (HKAS) – Yunnan Prov.: Gongshan county town, 07.09.2001, X.H. Wang 1391, HKAS 38746 (HKAS) – Japan,Tottori, Tottori-shi, Iwakura (Exp. For.), under *Pinus densiflora*, 17.10.1997, leg. E. Nagasawa, TMI 22646 (TMI) – Kagoshima, Aira-gun, Makizono-cho, Oonami-yama, 1060-1300 m a.s.l., under *Pinus densifolia*, in mixed coniferous and hardwood forest, 27.10.2000, leg. E. Nagasawa & T. Fujita, TMI 24414 (TMI) – Tottori, Tottori-shi, Hamasaka, Tottori Sand Dune, In *Pinus thunbergii* woods, 07.11.2000, leg. T. Hongo & E. Nagasawa, TMI 24398 (TMI) – Thailand, Chiang Mai Prov., Mae Teng distr., Toung Yoa village, forest trail, 1300 m a.s.l., N19°08.07' E98°38.90', secondary forest, under *Pinus kesiya*, on a steep wall of red soil along the trail, 20.06.2004, leg. R. Walleyn & A. Verbeken 2004-014 (GENT) – Chiang Mai Prov., Huai Nam Dang National Park, nature trail, 1530 m a.s.l., N19°18.29' E98°35.88', forest with *Pinus kesiya*, bamboo, scattered *Castanopsis*, 28.06.2004, leg. R. Walleyn & A. Verbeken 2004-134 (GENT) – Chiang Mai Prov., Huai Nam Dang National Park, nature trail, 1530 m a.s.l., N19°18.29' E98°35.88', forest *with Pinus kesiya*, bamboo, scattered *Castanopsis*, 28.06.2004, leg. R. Walleyn & A. Verbeken 2004-138 (GENT) – Chiang Mai Prov., Huai Nam Dang National Park, nature trail, 1530 m a.s.l., N19°18.29' E98°35.88', forest with *Pinus kesiya*, bamboo, scattered *Castanopsis*, 28.06.2004, leg. R. Walleyn & A. Verbeken 2004-139 (GENT) – Chiang Mai Prov., Mae Teng distr., Toung Yoa village, trail, 1300 m a.s.l., N19°08.07' E98°38.90', secondary forest with *Pinus kesiya*, *Castanopsis* etc., 30.06.2004, leg. R. Walleyn & A. Verbeken 2004-159 (GENT).

DISCUSSION: The macrodescription given here is compiled from the original description (TANAKA, 1890) and from field notes attached to collections X.H. Wang 992 (HKAS 39354), X.H. Wang 1014 (HKAS 39355), L.F. Zhang 88 (HKAS 38454) and A. Verbeken 2004-014, 2004-134, 2004-138, 2004-139 and 2004-159 (GENT). *Lactarius hatsudake* is one of the highly prized edible mushrooms in Japan and large parts of China and probably also in Korea and eastern Russia (NAGASAWA, 1998; WANG *et al.*, 2004).

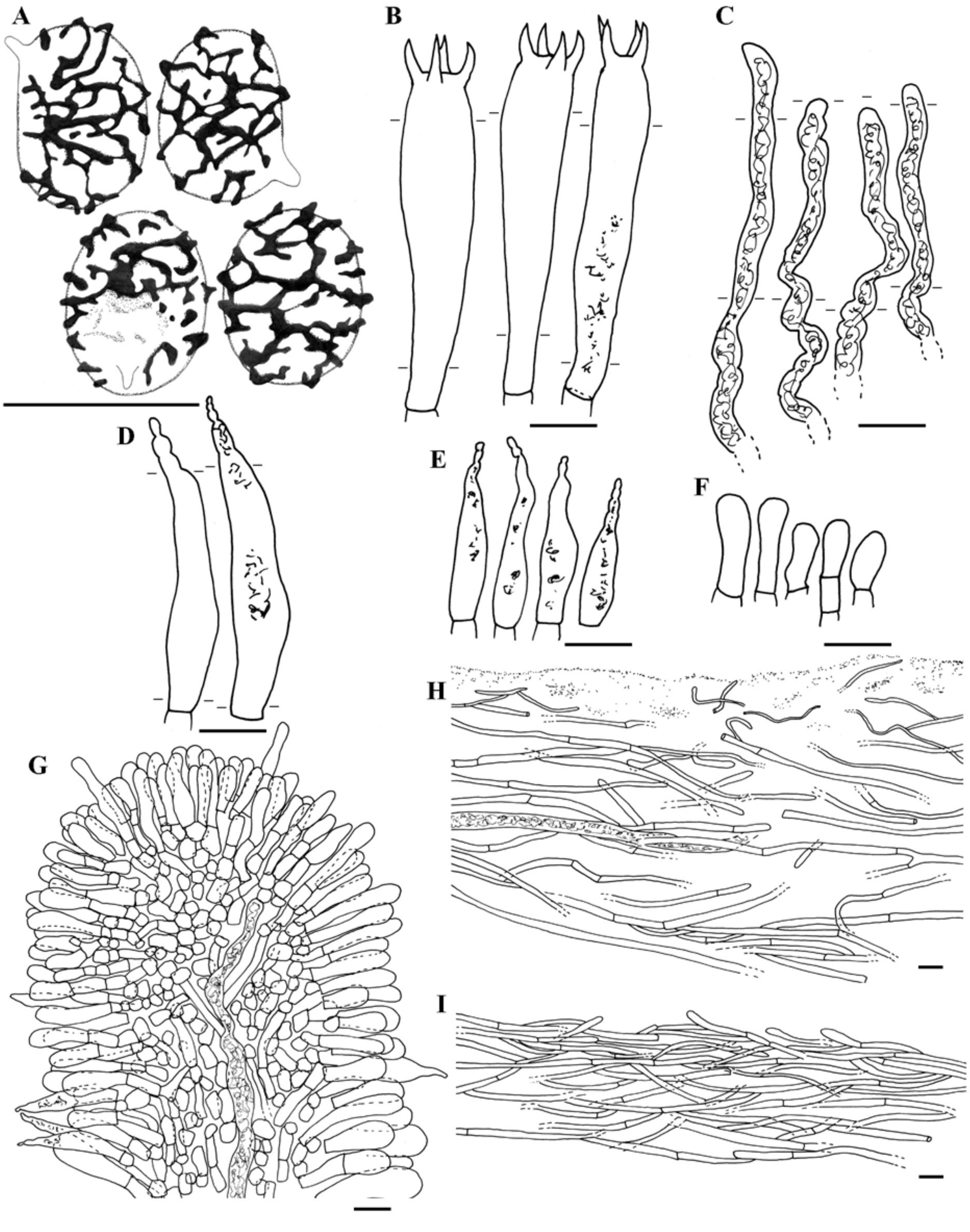

Fig. 7: *Lactarius hatsudake*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** lamella edge; **H.** section through the pileipellis; **I.** section through the stipitipellis. From HKAS31714, HKAS38746, HKAS39032, HKAS39047, HKAS39354, TMI22646 and TMI24414. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Lactarius hatsudake is characterised by its wine red latex and its association with *Pinus* spp. The size of the basidiocarps seems very variable; very small to rather large basidiocarps are often encountered together (X.H. Wang, pers. comm.). Microscopically the heavy spore ornamentation with the large but faintly amyloid spots is typical and a reliable feature for identification.

Although IMAI (1935, 1938, 1941) expected *L. hatsudake* to be conspecific to *L. sanguifluus*, both species do not seem to be very closely related. A difference with the indeed macroscopically rather similar *L. sanguifluus* is the absence of scrobicules on the stipe. A recently described variety of *L. sanguifluus* from Vietnam (*L. sanguifluus* var. *asiaticus*) might be conspecific to *L. hatsudake* or to *L.* sp. 4 but we did not have the opportunity to study the type specimen or any other material. The small size of the basidiocarps seems to point to a close resemblance to the latter.

Lactarius lividatus was originally described from the Amami-Oshima Islands, South of Japan. According to NEDA (1992), who examined the type collection of *L. lividatus,* it is identical with *L. hatsudake*. LALLI & PACIONI (1992) also studied the type and conclude that the type specimen is in very poor condition and that there is not enough evidence to arrive at a precise identification. According to them, the spores and cystidia are of the same type as those of *L. hygrophoroides* Berk. & M.A. Curtis, which belongs to a completely different group.

Molecular evidence shows that the genetic variability in *L. hatsudake* is high, which might indicate that our species concept is relatively wide here. Moreover, this molecular variability is also confirmed by morphological diversity (Fig. 6). The collections from Thailand e.g. showed a striking hygrophanous colour change of the cap that is not reported for the Chinese collections. It is possible that at least several varieties can be distinguished within *L. hatsudake* but more observations on fresh material are needed.

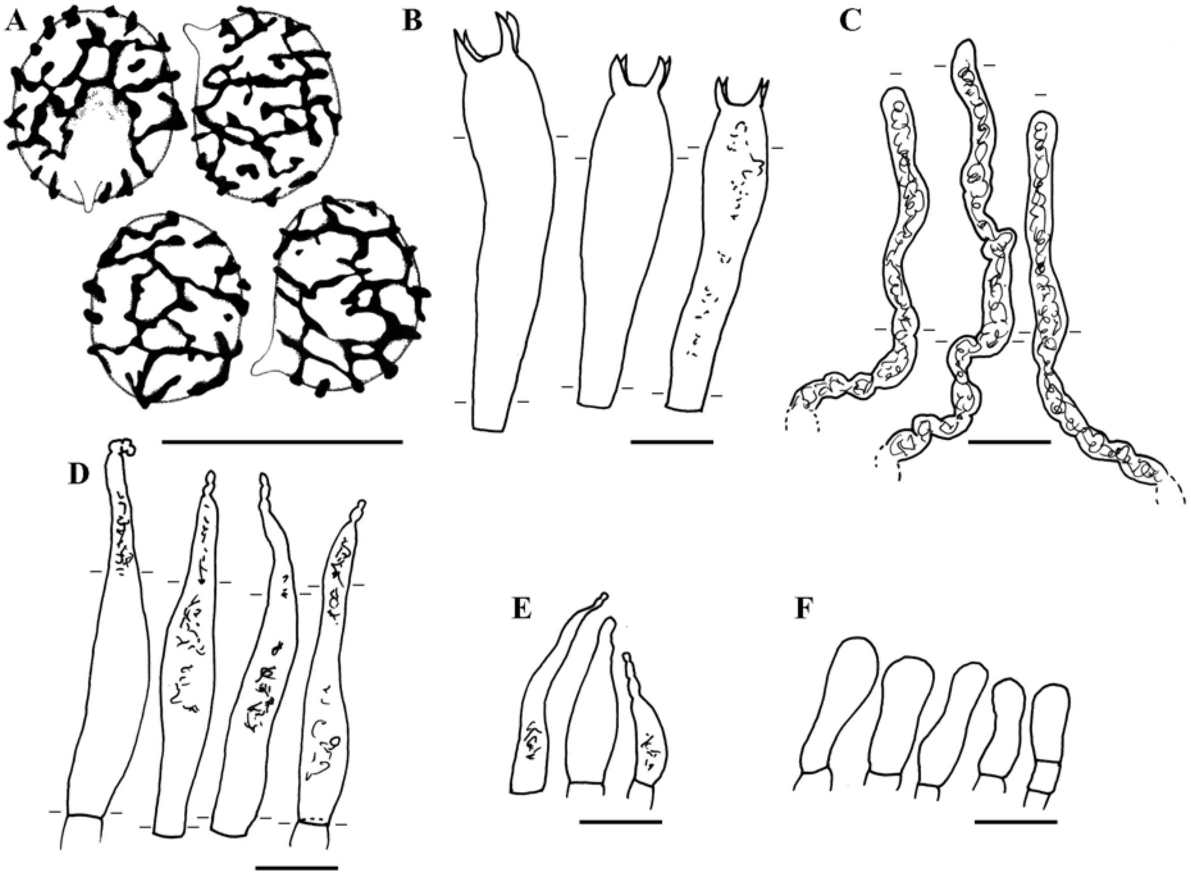

Fig. 8: *Lactarius hatsudake*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia. From M. Härkönen KIINA 62 and HKAS39378. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Several Chinese collections differ from the other *L. hatsudake* specimens by the broadly ellipsoid spores that have a slightly thinner ornamentation, the very scarce to absent cheilomacrocystidia and the more abundant pleuromacrocystidia, also further away from the edge. One of these collections (M. Härkönen, KIINA 62) was included in our molecular analyses (as *L. hatsudake* China 3) and seemed to be conspecific with or very closely related to *L. hatsudake.* The following is a description of the hymenial characters (Fig. 8):

Spores 7.5–*8.3*–9.0 × (5.7) 5.8–*6.5*–7.2 µm, mostly broadly ellipsoid (Q = 1.21–*1.27*–1.33); ornamentation up to 0.5 µm high, of medium thick ridges and some scarce isolated warts, forming a rather complete reticulum; plage distally slightly amyloid. *Basidia* 32–46 × 9.5–11 µm, rather short and broad, subclavate, 4-spored; sterigmata 4.5–6 µm long. *Pleuromacrocystidia* abundant to very abundant, 40–50 × 4.5–7 µm broad, emergent, subfusiform with a moniliform to obtuse apex, content granular and refractive, thin-walled. *Pseudocystidia* rather abundant, 2–4 µm broad, tortuous, with an ochre, refractive content. *Lamella edge* sterile with very scarce cheilomacrocystidia; cheiloleptocystidia 10–20 × 4.5–9.5 µm, subclavate, irregularly shaped to swollen, often with a granular content, thin-walled; cheilomacrocystidia 20–28 × 4–7 µm, subfusiform with a narrowing to moniliform apex, thin-walled.

COLLECTIONS EXAMINED: China, Hunan Prov.: Wulingyuan World Heritage Area, Zhangjiajie. Matianya, 1000 m a.s.l., N29°19' E110°27', forest with planted *Cunninghamia lanceolata*, *Pinus massoniana* and *Cryptomeria fortunei*, under young, cultivated *Pinus massoniana*., 16.09.1999, M. Härkönen KIINA 62 (H, GENT) – Yunnan Prov.: Tengchong county, Jietou, Datang, under *Pinus yunnanensis*, 04.08.2000, X.H. Wang 1098, HKAS 39378 (HKAS) – Yunnan Prov.: Wuding county, Shizishan, 2300 m a.s.l., *Pinus yunnanensis*, 17.08.2000, F.Q. Yu 141, HKAS 39033 (HKAS).

3.4. *Lactarius* sp. 4

Fig. 9

Latin description:

Pileus 45–65 mm diam., primo planoconvexus margine incurvo, tum depressus ad infundibiliformis, interdum papillatus in centro; pileipellis laevis, viscida, ex purpureo rubrobrunnea, juventute pallide rosea ad griseorosea, cum zonis concentricis fuscis, aetate provecta pallidior, azonata, flavovirens. Lamellae breve decurrentes, confertae, saepe anastomosae, pallide roseae, virescentes. Stipes 40–45 mm longus, 9–10 mm crassus, cylindricus, siccus, purpureogriseus ad pallide roseus, virescens, tomento albo virescenti. Contextus albidus, virescens, gustu mitis ad amaris. Latex vinosus.

Sporae late ellipsoideae ad ellipsoideae, 7.2–7.7–8.2 × (5.3) 5.4–5.8–6.2 µm, ornatae cristis usque ad 0.5 µm altis, dense subreticulatae, macula suprahilaris leviter distale amyloidea.. Basidia 35–45 x 7–9 µm, subclavata ad subcylindrata, tetraspora. Pleuromacrocystidia abundantia prope marginem lamellaris, 35–55 x 6–8 µm, emergentia, fusiformia. Pseudocystidia abundantia. Cellulae marginales 7.5–15 x 2.5–5 µm, subclavatae ad irregularis. Pileipellis ixocutis, 50–100 µm crassa. Ad terram in silva coniferarum (*Pinus merkusii*). Indonesia, Java, Bogor, Mt. Salak, Curug Nangka, 910 m, 02.01.2000, Horak 8336 (holotypus ZT, isotypus GENT).

Etymology: Named after the collector, Prof. Dr. E. Horak, for his important contribution to the study of a.o. *Lactarius* in the Southeast Asian region.

Synonym: ?= ***Lactarius sanguifluus*** var. ***asiaticus*** Dörfelt, Kiet & A. Berg, Feddes Repert. 115: 169 (2004)

Holotype: Horak 8336 (ZT!), 02.01.2000, Indonesia, Java, Bogor, North slope of Mt. Salak, Curug Nangka, 910 m a.s.l., under *Pinus merkusii* (native to Sumatra), planted in secondary broad-leaf montane rainforest, on soil; isoype GENT.

DESCRIPTION: *Pileus* up to 4.5–6.5 cm diam., plano-convex when young with an incurved margin, when expanding becoming depressed with straight margins to deeply infundibuliform, occasionally papillate in the centre; margin translucently-striate, entire to eroded; surface glabrous, sticky when moist, reddish-brown with a pale purplish tinge and with conspicuously darker ring-like zones when young, mature specimens light pink (6–8A2) to greyish pink (8C4), aged specimens changing to dirty pale beige and losing zoned aspect, staining lemon green (30A6/7) to lime green (27/28B8). *Lamellae* shortly decurrent, crowded, often anastomosing at the base; colour pale pink (8/9A/B2), greening upon bruising; edge slightly paler. *Stipe* 4–4.5 × 0.9–1 cm, cylindric, equal or slowly tapering towards the base, apex often costate otherwise smooth; surface dry, purple-grey (8C4) to pale pink (6–8A2), base and especially upon handling turning to pale or deep green; whitish mycelial tomentum or rhizomorphic strands present at base, this basal tomentum turning blue-green. *Context* hollow in the stipe; whitish to pale beige, lower half of the stipe blue-green, rind of stipe and above the lamellae blood red-brown, becoming pale to deep green after exposure; smell not distinctive to pleasant; taste mild to bitter. *Latex* vinaceous red (10/11D/E8).

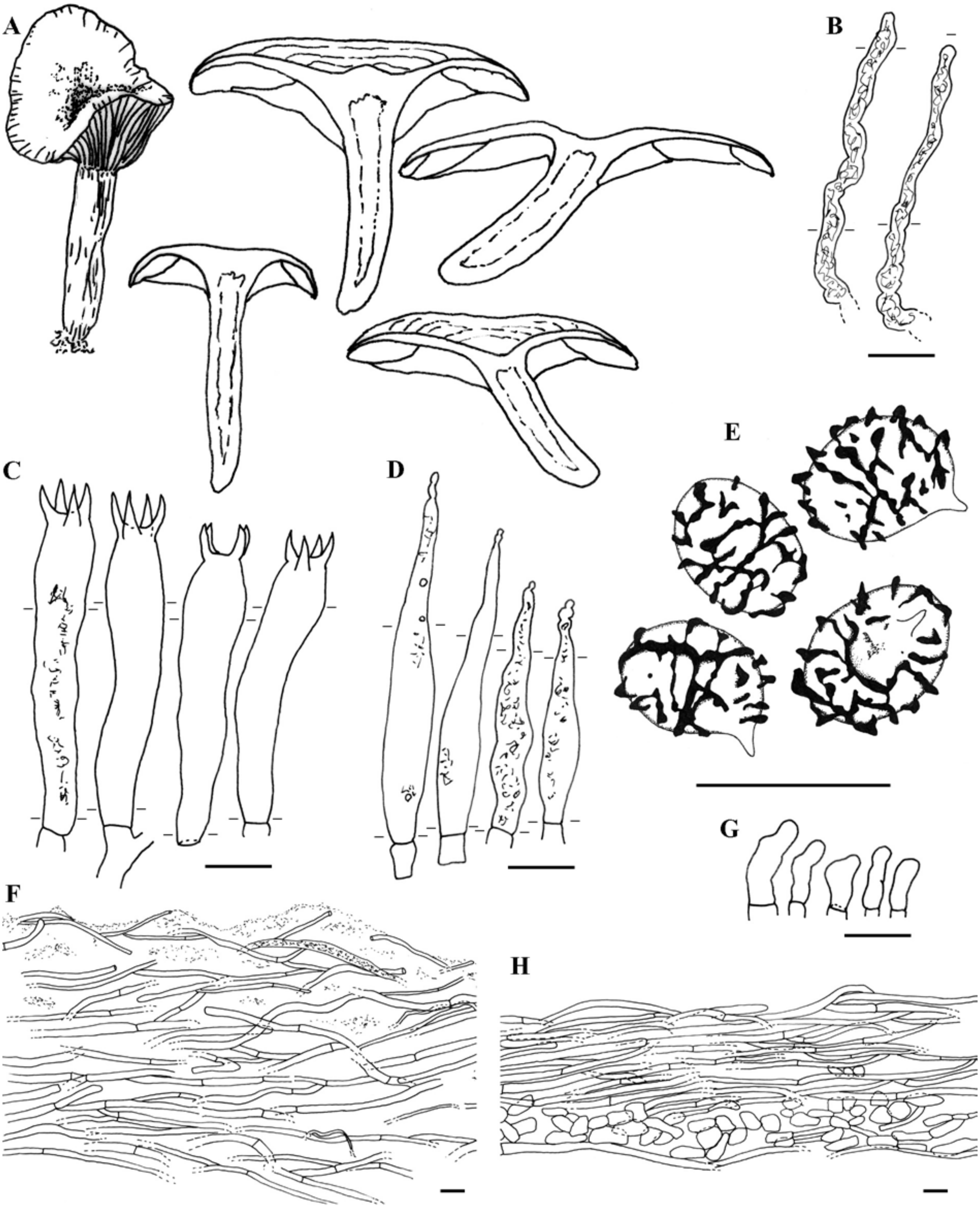

Fig. 9: *Lactarius* sp. 4: **A.** basidiocarps (life size); **B.** pseudocystidia; **C.** basidia; **D.** pleuromacrocystidia; **E.** spores; **F.** section through the pileipellis; **G.** cheiloleptocystidia; **H.** section through the stipitipellis. From E. Horak 8336 and E. Horak 9983. Scale bars = 10 µm, small bars indicate the height of the hymenium.

Spores 6.8–*7.3*–*7.7*–8.2 × (5.1) 5.2–*5.6*–*5.8*–6.2 µm, mainly broadly ellipsoid to sometimes ellipsoid (Q = 1.25–*1.32*–*1.34*–1.43); ornamentation up to 0.5 µm high, composed of medium broad ridges and some isolated warts forming a rather dense, incomplete to almost complete reticulum; plage very slightly amyloid distally. *Basidia* 35–45 × 7–9 µm, subclavate to subcylindric, hyaline or with a needle-like content, 4-spored; sterigmata up to 5.2 µm high. *Pleuromacrocystidia* abundant near the lamella edge, scarce elsewhere, 35–55 × 6–8 µm, emergent, fusiform, with an acute to moniliform apex, with needle- to cube-shaped crystals, thin-walled. *Pseudocystidia* abundant, 3–4 µm broad, cylindrical to tortuous, not to slightly emergent, with a refringent content. *Lamella edge* sterile without cheilomacrocystidia; cheiloleptocystidia 7.5–15 × 2.5–5 µm, subclavate to irregularly shaped, hyaline, thin-walled. *Subhymenium* irregular, appearing like angular cells, more or less arranged in rows. *Hymenophoral trama* irregularly filamentous, with abundant, ochre coloured lactifers. *Pileipellis* an ixocutis, 50–100 µm thick, composed of thin-walled hyphae ± parallel to the surface; with abundant shrivelled and gelatinous hyphae in the surface layer, hyphae 1–4 µm diam. *Stipitipellis* a cutis, 30–50 µm thick, composed of hyaline hyphae with somewhat refractive walls, arranged parallel to the surface; no shrivelled or gelatinised hyphae. *Clamp-connections* absent.

HABITAT & DISTRIBUTION: Only known from montane rainforest in Java, probably associated with *Pinus*.

COLLECTIONS EXAMINED: Indonesia, Java, Bogor, North slope of Mt. Salak, Curug Nangka, 910 m a.s.l., under *Pinus merkusii* (native to Sumatra), planted in secondary broad-leaf montane rainforest, on soil, 02.01.2000, E. Horak 8336 (ZT), holotype – Java, Bogor, North slope of Mt. Salak, Curug Nangka, 910 m a.s.l., under *Pinus merkusii* (native to Sumatra), planted in secondary broad-leaf montane rainforest, on soil, 01.11.2000, leg. A. Retnovati, AWW 30, E. Horak 9983 (ZT).

DISCUSSION: *Lactarius* sp. 4 is a small species with reddish brown colours in the pileus, a purple-grey stipe and red latex. It was collected in montane rainforest under *Pinus*. It has rather densely ornamented, small spores (7.3–7.7 × 5.6–5.8 µm on average), which are mostly broadly ellipsoid.

Our molecular analyses reveal a very close relationship to *L. hatsudake*. The most striking morphological differences with *L. hatsudake* are the general colour of the basidiocarps (*L. hatsudake* often has orange tinges) and the size of the basidiocarps. Microscopically the spores of *L.* sp. 4 are clearly smaller than those of *L. hatsudake* (8.3–9.0 × 6.2–6.6 µm on average in *L. hatsudake*) and *L.* sp. 4 lacks cheilomacrocystidia.

DÖRFELT *et al.* (2004) describe a seemingly very similar taxon from Vietnam: *L. sanguifluus* var. *asiaticus*. The size of the basidiocarps they describe ranges from 2 to 3 cm only. Many key-characters lack in their description to draw any further conclusions. Even if *L.* sp. 4 and *L. sanguifluus* var. *asiaticus* are shown to be the same taxon, the name *L. sanguifluus* var. *asiaticus* should be rejected, as there is no close relationship to the European *L. sanguifluus*.

3.5. ***Lactarius laeticolor* (S. Imai) Imazeki ex Hongo**, Acta Phytotax. Geobot 18: 139 (1959)

Figs. 10, 11

Basionym: ≡ ***L. deliciosus*** f. ***laeticolor*** S. Imai, Bot. Mag. (Tokyo) 49: 607 (1935), as ‘*laeticolorus*’

Synonyms: ≡ ***L. laeticolor*** (S. Imai) Imazeki, Genshoku-Kinoko: 153 (1959), as ‘*laeticolorus*’, nom. inval., no basionym indicated

= ***L. deliciosus*** var. ***japonicus*** Kawam. , The Japanese fungi: 55 (1929)

  ≡ ***L. japonicus*** (Kawam.) Lar.N. Vassiljeva, Agarics and Boletes (Agaricales) of the Primorsk Region: 302 (1973)

Type: not selected, described from Japan.

Fig. 10: A. *L. laeticolor* collected in Japan; **B.** SEM image of the spores of *L. laeticolor* TMI23149.

DESCRIPTION: *Spores* 8.0–*9.0*–10.0 × 6.4–*7.1*–7.8 µm, broadly ellipsoid to ellipsoid (Q = 1.16–*1.27*–1.39); ornamentation composed of medium thick ridges and some isolated warts, forming a rather complete network, ornamentation up to 0.5 µm high; plage distally amyloid. *Basidia* 42–50 × 8–12 µm, subclavate, 4-spored, often containing oil-droplets and fine granules; sterigmata 4–6 µm long. *Pleuromacrocystidia* not very abundant, 65–92 × 7.5–9 µm, subfusiform with a capitate or moniliform apex, very tall and conspicuous, strongly emergent, typically with crystal-shaped content in the base, thin-walled. *Pseudocystidia* rather abundant, 2.5–4 µm broad, cylindrical to tortuous, sometimes slightly emergent, mostly not, with an ochre-yellow content. *Lamella edge* sterile with quite abundant cheilomacrocysidia; cheiloleptocystidia 9.0–16 × 3.5–6 µm, subclavate or more irregular, hyaline or with a fine granular content, thin-walled; cheilomacrocystidia 32–45 × 5–7 µm, subfusiform with a moniliform apex, emergent, content needle-shaped, thin-walled. *Subhymenium* composed of small and rounded, irregularly arranged cells. *Hymenophoral trama* containing abundant lactifers. *Pileipellis* an ixocutis, 250–300 µm thick, of very widely spaced, shrivelled hyphae; hyphae 1–5 µm diam. *Stipitipellis* an ixocutis. *Clamp-connections* absent.

HABITAT: Associated with *Abies* (*A. firma*, *A. sachalinensis*, …), summer to autumn.

DISTRIBUTION: Japan (Hokkaido, Honshu, Kyushu & Bonin Islands), Korea.

COLLECTIONS EXAMINED: Japan, Tottori, Tottori-shi, Ochidani, in *Abies firma-Castanopsis cusoidata* woods, 06.11.1998, leg. E. Nagasawa *et al.*, TMI 23149 (TMI) – Korea, O.K. Miller 21714 (RMS), originally identified as *L. salmonicolor.*

DISCUSSION: No detailed macroscopical description is available for this species. *Lactarius laeticolor* is described as very distinctive in its bright orange colouration of the entire basidiocarp, its viscid pileus and stipe surface in wet conditions and the presence of concentric zones on the cap. The latex is described as reddish orange, not discolouring with time (NAGASAWA, 1998). The pictures in IMAZEKI *et al.* (1988) show distinct, large scrobicules on the stipe. The greenish discolouration is absent or very limited in *L. laeticolor*. All these features (except maybe the colour reaction of the latex) remind very strongly of *L. salmonicolor*, a European species also associated with *Abies,* and to a lesser extent of *L. thyinos*. Microscopically these three species are very similar by the presence of an ixocutis on the stipe and the presence of strongly emergent pleuromacrocystidia. In our molecular phylogeny these three species are clearly distinct but together form a rather well supported group, confirming their close relationship. Synonymising *L. laeticolor* with *L. salmonicolor* however (as done in HEILMANN-CLAUSEN *et al.*, 1998 & EBERHARDT *et al.*, 2000) is incorrect.

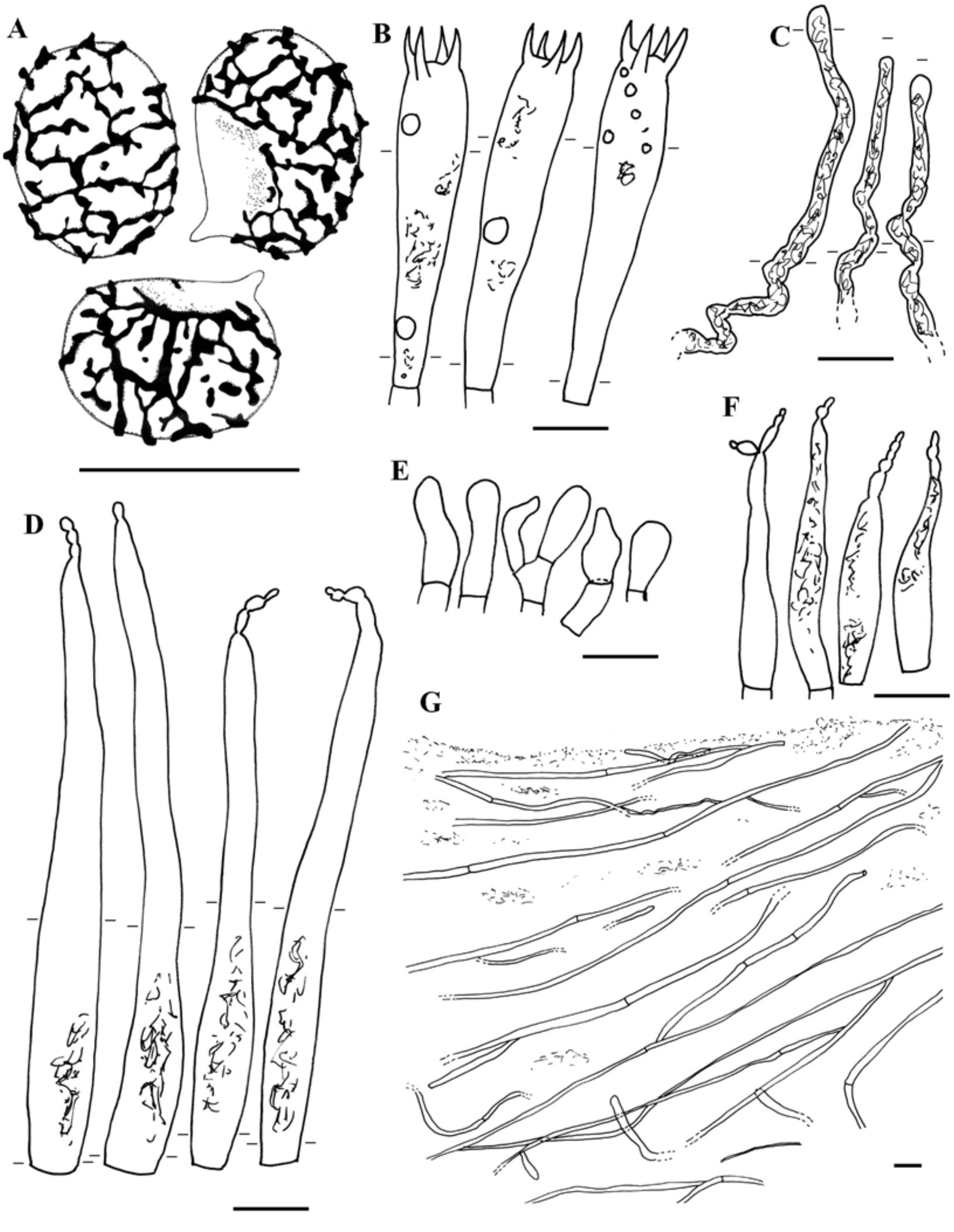

Fig. 11: *Lactarius laeticolor*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheiloleptocystidia; **F.** cheilomacrocystidia; **G.** section through the pileipellis. From TMI23149. Scale bars = 10 µm, small bars indicate the height of the hymenium.

3.6. ***Lactarius subindigo*** **Verbeken & E. Horak**, Australian Syst. Bot. 13: 651 (2000)

Figs. 12, 14

Misapplication: = ***L. indigo*** sensu IMAZEKI *et al.* (1988), NAGASAWA (1998) and probably several other authors (see under discussion).

Holotype: Horak 71-189 (CANB), Isotypes (GENT!, ZH), Papua New Guinea: Morobe District, Bulolo, Watut

DESCRIPTION (macroscopy adapted from VERBEKEN & HORAK, 2000): *Pileus* up to 7 cm diam., at first convex with a strongly incurved margin, centre sometimes with a conical papilla, becoming depressed to umbilicate in mature specimens, papilla absent; margin non-striate; surface viscid if moist, glabrous in dry condition, conspicuously and persistently zonate; colour conspicuously blue-green, centre turning pale ochre in age. *Lamellae* arcuate to decurrent, very dense, up to 4 mm wide, with numerous lamellulae; colour brilliant blue-green, turning dark green when bruised; edge entire, concolorous. *Stipe* 1–3 × 0.4–1 cm, cylindrical, equal or gradually attenuated towards the base; surface dry, glabrous, scrobiculate, concolorous with the pileus or paler, hollow, solitary (or rarely in pairs). *Context* brittle, immediately staining blue-green, in base turning to orange on exposure; smell fruity; taste mild. *Latex* milky, immediate blue on exposure. *Spore deposit* unknown.

KOH no reaction, *HCl* no reaction, NH_3 no reaction.

Spores 5.8–*6.6–7.5*–8.3 × (4.3) 4.5–*5.3–5.9*–6.5 µm, broadly ellipsoid, rarely subglobose or ellipsoid (Q = 1.10–*1.23–1.27*–1.39); ornamentation up to 0.5 µm high, of low but medium thick, strongly fragmented ridges, forming a very incomplete reticulum; plage not to distally slightly amyloid; apiculus often strikingly broad and large. *Basidia* 30–70 × (3) 6–9 µm, subclavate to mostly ± cylindric, 4-spored, containing oil-droplets or with a fine granular content; sterigmata 3–7 µm long. *Pleuromacrocystidia* very scarce, only present relatively close to lamella edge, 35–40 × 6–7 µm, subfusiform with a moniliform apex, containing needle-shaped crystals or with a granular content, slightly emergent, thin-walled. *Pseudocystidia* abundant, 3–7 µm broad, ± cylindric or tortuous, not to slightly emergent, with a deep ochre content. *Lamella edge* sterile, composed of mainly cheiloleptocystidia with very scarce cheilomacrocystidia; cheiloleptocystidia 8–25 × 3.5–8 µm, subclavate to more irregular, hyaline, thin-walled; cheilomacrocystidia 17–35 × 4–7 µm, subfusiform with a moniliform apex, slightly emergent, with a needle-shaped or granular content, thin-walled. *Subhymenium* composed of small, ± isodiametric cells, irregular. *Hymenophoral trama* irregular, composed of short hyphae and very abundant lactifers with a deep ochre-brown content. *Pileipellis* an ixocutis, 50–150 µm thick, with a thin slime-layer, composed of interwoven, hyaline hyphae, 1–6 µm diam., only few shrivelled hyphae present. *Stipitipellis* a cutis, 30–80 µm thick, composed of strongly interwoven, ± parallel hyphae, 2.5–5 µm diam.; lactifers with deep ochre content present close to surface. *Clamp-connections* absent.

HABITAT: Described from broad-leaf rainforest dominated by *Castanopsis acuminatissima* & *Lithocarpus* spp.; recorded under *Quercus leucotrichophora* & *Quercus semecarpifolia* in India and in both *Pinus* and broad-leaf forests with *Castanopsis* and *Quercus* in Japan (as *L. indigo*, NAGASAWA, 1998).

DISTRIBUTION: Described from Papua New Guinea, also found in India (Uttaranchal, north India), China (Hunan, China) and Japan (infrequent in the south of Tohoku district; as *L. indigo* in IMAZEKI *et al.*, 1988; NAGASAWA, 1998), summer to autumn.

COLLECTIONS EXAMINED: China, Hunan Prov.: Wulingyuan World Heritage Area, Zhangjiajie. Matianya, N29° E110°, 19.09.1999, M. Härkönen KIINA 114 (H, GENT), specimen bought from two local (commercial) mushroom pickers – India, Uttaranchal, Pauri Garhwal, Adwani, ~1900 m a.s.l., *Quercus leucotrichophora* & *Rhododendron arboreum*, 15.08.1999, RPB 547 (RMS) – Uttaranchal, Tehri Pauri, Majharatal forest, In deciduous forest, associated with *Quercus semecarpifolia*, 23.09.2000, K. Das 3030 (GENT) – Garhwal, Pauri, Near Hanuman Mandir (forest behind college campus), *Quercus* *leucotrichophora* & *Rhododendron arboreum*, 5.08.2002, KCS 475 (RMS) – Papua New Guinea, Morobe, Bulolo, road to Watut, 1000 m a.s.l., montane broad-leaf forest dominated by *Castanopsis acuminatissima-Lithocarpus* spp. on soil, 21.10.1971, E. Horak 71-189 (GENT), isotype.

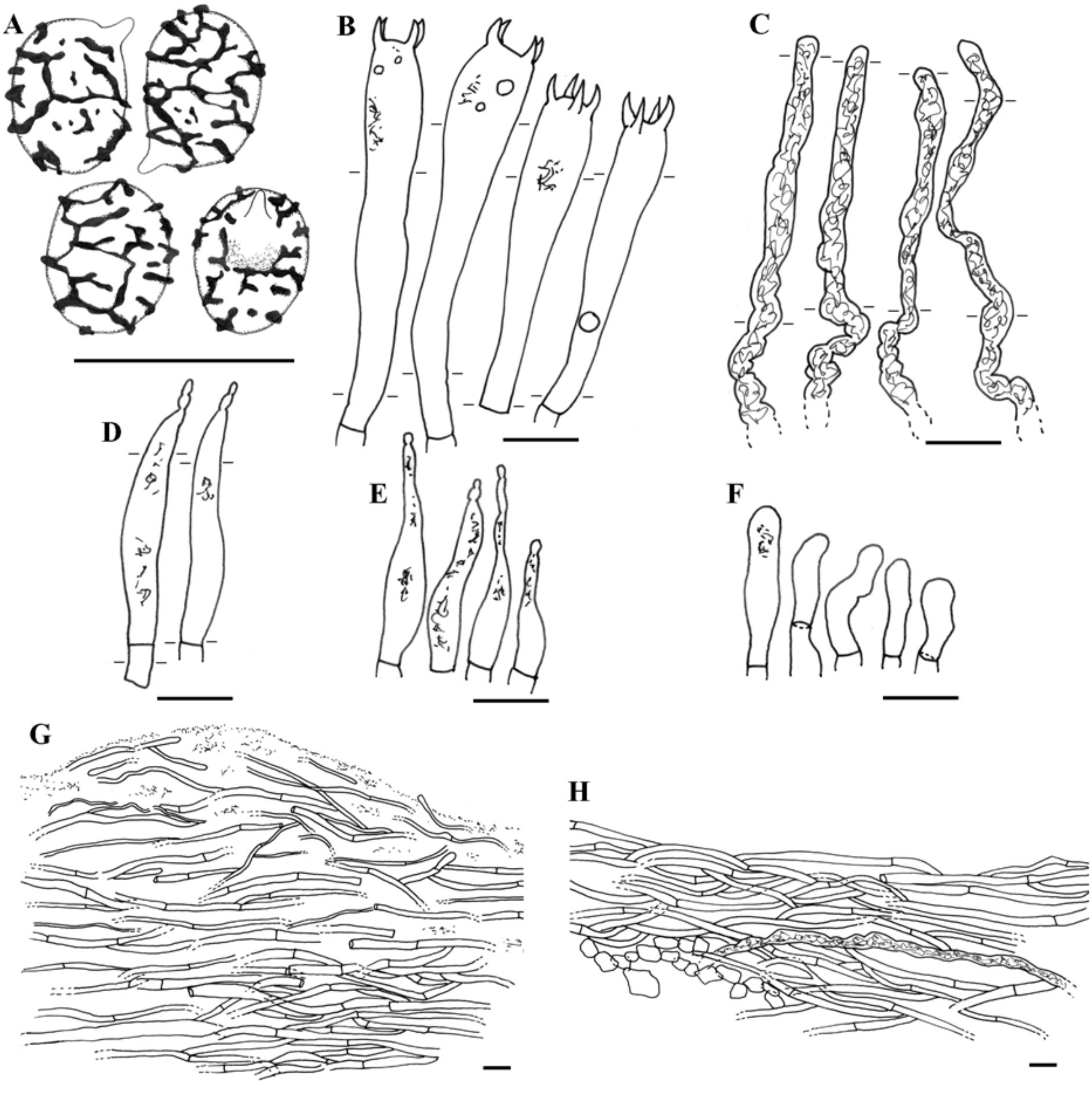

Fig 12: *Lactarius subindigo*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis; **H.** section through the stipitipellis. From M. Härkönen KIINA 114, RPB 547, K. Das 3030, KCS 475 and E. Horak 71-189 (isotype). Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: *Lactarius subindigo* has small basidiocarps, blue latex (except in the very base of the stipe) and blue tinges in the pileus and stipe. Microscopically it is characterised by its very small spores and very scarce pleuro- and cheilomacrocystidia. From the ecology notes attached to the examined collections, *L. subindigo* seems to be associated with both *Castanopsis* and *Quercus*.

We observed some variability in the spacing of the lamellae on the herbarium specimens we examined. The Chinese specimen (KIINA 114) for example clearly has more widely spaced lamellae (Fig. 14). The size and shape of the spores is also variable. Two Indian collections (KCS 475 and RPB

547) have very small spores (5.7–5.8 x 4.8 µm on average) that are subglobose to mostly broadly ellipsoid (Q = 1.16–1.22 on average).

The original description of *L. subindigo* states that pleuro- and cheilomacrocystidia are absent. The pleuromacrocystidia, erroneously illustrated in Fig.2, p. 653 (VERBEKEN & HORAK, 2000) show basidia-like cells, very unusual for pleuromacrocystidia in *Lactarius* sect. *Deliciosi*. Pleuro- and cheilomacrocystidia are present however in *L. subindigo* (also observed in the type collection), but are very scarce.

L. subindigo differs microscopically from *L. indigo* by the smaller spores that are ornamented with slightly wider and more rounded ridges. Both species are macroscopically very similar and many of the *L. subindigo* collections examined for this study were originally identified as *L. indigo*. Moreover, *L. indigo* has often been reported from Asia (BILLS & COTTER, 1989; HONGO & YOKOYAMA, 1978; IMAZEKI *et al.*, 1988; WU & MUELLER, 1997); these records should be critically re-checked. Surprisingly, the very close relationship of *L. indigo* and *L. subindigo*, as supposed by VERBEKEN & HORAK (2000), is not confirmed by molecular phylogenetic analyses. Both ITS and *gpd* data place *L. indigo* and *L. subindigo* in different clades in *Lactarius* sect. *Deliciosi*. This means blue coloured latex originated at least twice independently in this section.

3.7. ***Lactarius thakalorum*** **Bills & Cotter**, Mem. New York Bot. Garden 49: 193 (1989)

Fig. 13

DESCRIPTION (adapted from BILLS & COTTER, 1989): *Pileus* 1.8–7.2 cm diam., convex-depressed with an involute to incurved margin when young, soon shallowly to deeply depressed with an incurved margin, finally deeply depressed with an uplifted, lobed or undulating margin; margin glabrous, sometimes eroded in age; surface dry to moist, waxy, greasy or viscid, slightly rugulose, glabrous, concentrically zonate in young to medium-aged specimens, azonate in age; colour mottled pink to greyish red (4A/B3/4, 7/8B4, 8A2, 10C5), sometimes with pale reddish-purple hues, fading to pale yellow (4A2/3) with scattered reddish-purple hues, developing deep green to greyish green stains in age (25D-F6-8). *Lamellae* decurrent, close to subdistant, slightly intervenose, with many lamellulae, sometimes forked; colour greyish pink (10B2) to orange (6A6), becoming greyish orange (8B3) to pale orange (5/6B4) and finally pale yellow (4B4) to pale orange (6A2), with dull green stains (25D/E6, 24E7) where injured or in age. *Stipe* (1) 2–6 × 0.5–1 cm, equal or enlarged towards base; surface dry, with a white canescent bloom, concolorous with the pileus, developing green stains in age, with a white mycelium and deep orange mycelial strands attached to the base. *Context* brittle, becoming partially hollow in the stipe, 5–7 mm thick at junction of stipe and lamellae, white, becoming reddish-orange to reddish brown (7B6/7 to 9/10D7) from the latex, with a distinct reddish-brown line above the lamellae, sometimes with light orange hues at the base of the stipe; smell fragrant, fungoid; taste mild to slightly bitter or unpleasant. *Latex* reddish-brown (8-10E/F8), scant. *Spore deposit* pale yellow-orange (4A3/4).

$FeSO_4$ no reaction, *KOH* no reaction, *NH_4OH* no reaction.

Spores (8.2) 8.3–*9.1*–10 × 6.3–*6.9*–7.5 µm, broadly ellipsoid to ellipsoid (Q = 1.22–*1.32*–1.43); ornamentation composed of rather large and high warts (up to 1 µm high) interconnected with thick and thin ridges, some isolated smaller warts present, forming a nearly complete reticulum with small meshes; plage ornamented with fine and faintly amyloid lines and small warts or not amyloid. *Basidia* 40–55 × 10–12 µm, subclavate, 4-spored; sterigmata 5–6 µm high. *Pleuromacrocystidia* rather abundant near the lamella edge but becoming scarce elsewhere, 35–60 × 6–8 µm, subfusiform, with a moniliform apex, often containing needle-shaped crystals, thin-walled. *Pseudocystidia* 2–5 µm broad, narrowly cylindrical and often tortuous, with refractive ochre content, mostly not emergent. *Lamella edge* sterile; cheiloleptocystidia 10–20 × 3–5 µm, abundant, subclavate to irregular cylindric, hyaline, thin-walled; cheilomacrocystidia 25–40 × 4–7 µm, rather scarce on the edge but very abundant just beneath, emergent, subfusiform, with an acute to monilifom apex, often containing needle-shaped crystals. *Subhymenium* irregular, composed of angular to rounded, small cells. *Hymenophoral trama* irregular, containing abundant lactifers and interwoven hyphae. *Pileipellis* an ixocutis, 90–150 µm thick, with a thin slime-layer, composed of tightly interwoven, narrow, septate and rarely branching hyphae, 1–2 µm diam.; gelatinised and shrivelled hyphae abundant. *Stipitipellis* a cutis, 40–70 µm thick, consisting of interwoven, hyaline hyphae with somewhat refractive walls, 2–4 µm diam.; no shrivelled or gelatinised hyphae present. *Clamp-connections* absent.

HABITAT: Scattered to gregarious on the ground in forests and plantations of *Pinus wallichiana* or *P. roxburghii* or in second-growth angiosperm forests mixed with *P. wallichiana* or *P. roxburghii*, 1300–2600 m a.s.l., found from July to October.

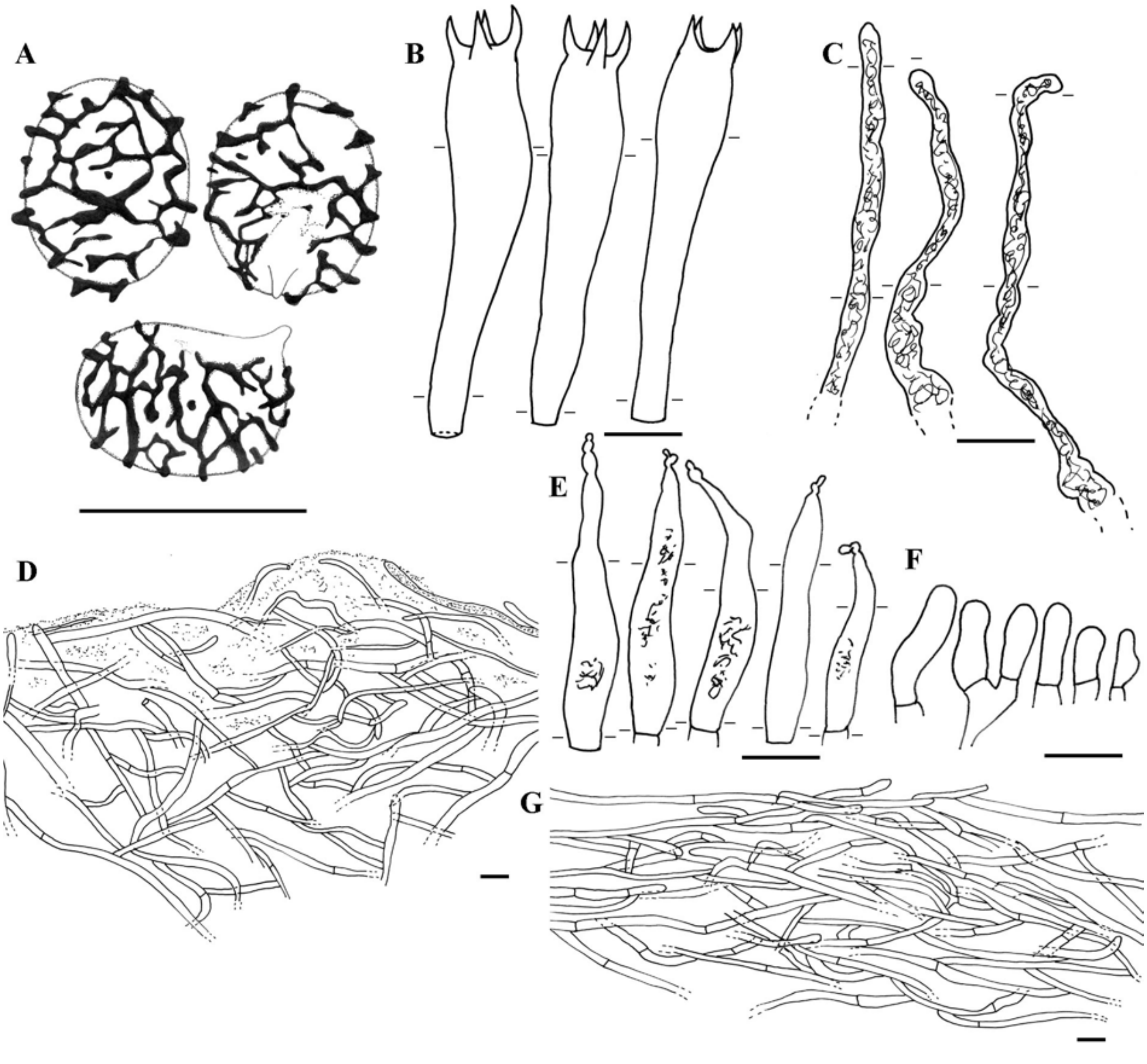

Fig. 13: *Lactarius thakalorum*: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** section through the pileipellis; **E.** macrocystidia close to the edge; **F.** cheiloleptocystidia; **G.** section through the stipitipellis. From VC 1313 (type). Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISTRIBUTION: Only reported from central Nepal.

COLLECTIONS EXAMINED: Nepal, Dhau-Lagiri Zone, Mustang Distr., upstream of Khobang, 03.09.1985, VC 1313 (BPI), holotype.

DISCUSSION: As explained in the introduction, molecular evidence points towards a possible conspecificity with *L. sanguifluus*. Both taxa seem to differ slightly in several macromorphological features however. *Lactarius thakalorum* is reported to have rather small basidiocarps with a dirty pink to greyish red pileus, fading to pale yellow, while *L. sanguifluus* also has orange tinges in the pileus and has medium-sized to large basidiocarps. Microscopically there are no striking differences.
L. thakalorum has recently been collected in Nepal (M. Christensen) and the study of this documented material might give clues on the eventual close relationship with *L. sanguifluus* and the morphological variability within *L. thakalorum*.

3.8. *Lactarius* sp. 1

Figs. 14, 15

DESCRIPTION: *Spores* 8.1–*8.7*–9.3 (9.4) × 6.0–*6.5*–7.1 µm, broadly ellipsoid to mostly ellipsoid (Q = 1.29–*1.34*–1.43); ornamentation up to 0.8 µm high, of medium thick to rather fine ridges and many, sometimes elongated warts, forming an irregular, incomplete network; plage very slightly to not amyloid. *Basidia* 40–55 × 7–10 µm, subclavate to almost cylindrical, mostly 4-spored, often with small oil-droplets; sterigmata 4–5 µm long. *Pleuromacrocystidia* absent. *Pseudocystidia* abundant, conspicuous, 2.5–4 µm broad, rarely slightly emergent, tortuous, with a yellowish ochre content. *Lamella edge* sterile, without cheilomacrocystidia; cheiloleptocystidia 7.5–25 × 2.5–5 µm, mostly cylindrical, sometimes irregular, often rather long and slender, hyaline, thin-walled. *Subhymenium* of small, irregularly arranged cells. *Hymenophoral trama* with very abundant, ochre-yellow coloured lactifers. *Pileipellis* an ixocutis, up to 200 µm thick, composed of strongly interwoven, irregularly arranged hyphae, 2-6 µm diam; few shrivelled or gelatinised hyphae. *Clamp-connections* absent.

Fig. 14: Collections from Hunan: left, M. Härkönen KIINA 112 (*L.* sp.1); middle, M. Härkönen KIINA 113 (*L.* sp.2); right, M. Härkönen KIINA 114 (*L. subindigo*).

HABITAT: No information available.

DISTRIBUTION: Hunan, China.

COLLECTIONS EXAMINED: <u>China</u>, Hunan Prov.: Wulingyuan World Heritage Area, Zhangjiajie, Yuanjiajie village, N29° E110°, 19.09.1999, M. Härkönen KIINA 112 (H, GENT), specimen bought from two local (commercial) mushroom pickers.

DISCUSSION: No detailed information is available on the macroscopy of this collection. From the picture (Fig. 14) it can be derived that this species looks similar to *L. hatsudake*. The specimen was bought from local commercial mushroom pickers.

This taxon (labelled *L.* sp.1) is consistently placed close to *L. quieticolor* in our molecular phylogenetic analyses and is clearly distinct from *L. hatsudake*. It also differs microscopically from the latter species in the differently ornamented spores and the absence of pleuro- and cheilomacrocystidia. Many deformed and also 3-spored basidia were observed in this specimen.

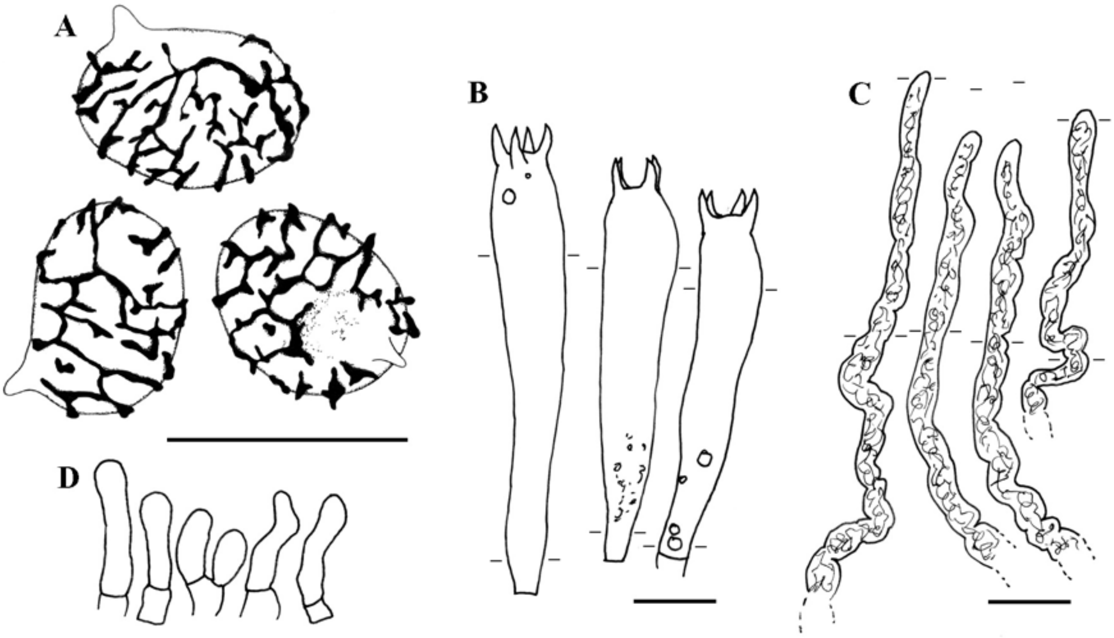

Fig. 15: *Lactarius* sp.1: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** cheiloleptocystidia. From M. Härkönen KIINA 112. Scale bars = 10 µm, small bars indicate the height of the hymenium.

3.9. *Lactarius* sp. 2

Figs. 14, 16, 17

DESCRIPTION: *Spores* 7.8–*8.4*–*9.1*–10.1 × 5.6–*6.1*–*6.6*–7.2 µm, broadly ellipsoid to mostly ellipsoid (Q = 1.26–*1.36*–*1.39*–1.52); ornamentation up to 0.7 µm high, composed of rather broad ridges, forming an almost complete, dense reticulum; plage not to distally slightly amyloid. *Basidia* 50–60 × 10–12 µm, subclavate to subcylindric, 4-spored, sometimes with a granular content; sterigmata 6–8 µm long. *Pleuromacrocystidia* very rare, 60–70 × 9–11 µm, subfusiform, with a moniliform apex, content somewhat granular, thin-walled. *Pseudocystidia* abundant, 6–10 µm diam., often emergent and broadened at the apex, irregularly cylindric, with ochre-brown intracellular pigmentation. *Lamella edge* sterile with scarce cheilomacrocystidia; cheiloleptocystidia 10–20 × 4–6 µm, subcylindric to subclavate, thin-walled; cheilomacrocystidia 26–30 × 6–9 µm, subfusiform, with an obtuse to moniliform apex, often with a granular content, thin-walled. *Subhymenium* composed of small, irregularly shaped and arranged cells. *Hymenophoral trama* with abundant lactifers. *Pileipellis* an ixocutis, 40–70 µm thick, of thin-walled, interwoven hyphae, 2–4 µm diam., and some lactifers. *Stipitipellis* an ixocutis, 30–50 µm thick, of thin-walled, interwoven and mostly gelatinised hyphae, 2–4 µm diam.; some shrivelled hyphae present near the surface; slime-layer very thin. *Clamp-connections* absent.

HABITAT: Collected in a forest with planted *Cunninghamia lanceolata*, *Pinus massoniana* and *Cryptomeria fortunei*. Growing under *Pinus massoniana.*

DISTRIBUTION: Specimens collected in Hunan, China.

COLLECTIONS EXAMINED: China, Hunan Prov.: Wulingyuan World Heritage Area, Zhangjiajie, Matianya, 1000 m a.s.l., N29°19' E110°27', forest with planted *Cunninghamia lanceolata*, *Pinus massoniana* and *Cryptomeria fortunei*, under *Pinus massoniana*., 16.09.1999, M. Härkönen KIINA 35 (H) – Hunan Prov.: Wulingyuan World Heritage Area, Zhangjiajie, Matianya, 1000 m a.s.l., N29°19' E110°27', forest with planted *Cunninghamia lanceolata*, *Pinus massoniana* and *Cryptomeria fortunei*., 16.09.1999, M. Härkönen KIINA 36 (H) – Hunan Prov.: Wulingyuan World Heritage Area, Zhangjiajie, Matianya, N29° E110°, 19.09.1999, M. Härkönen KIINA 113 (H), specimen bought from two local (commercial) mushroom pickers.

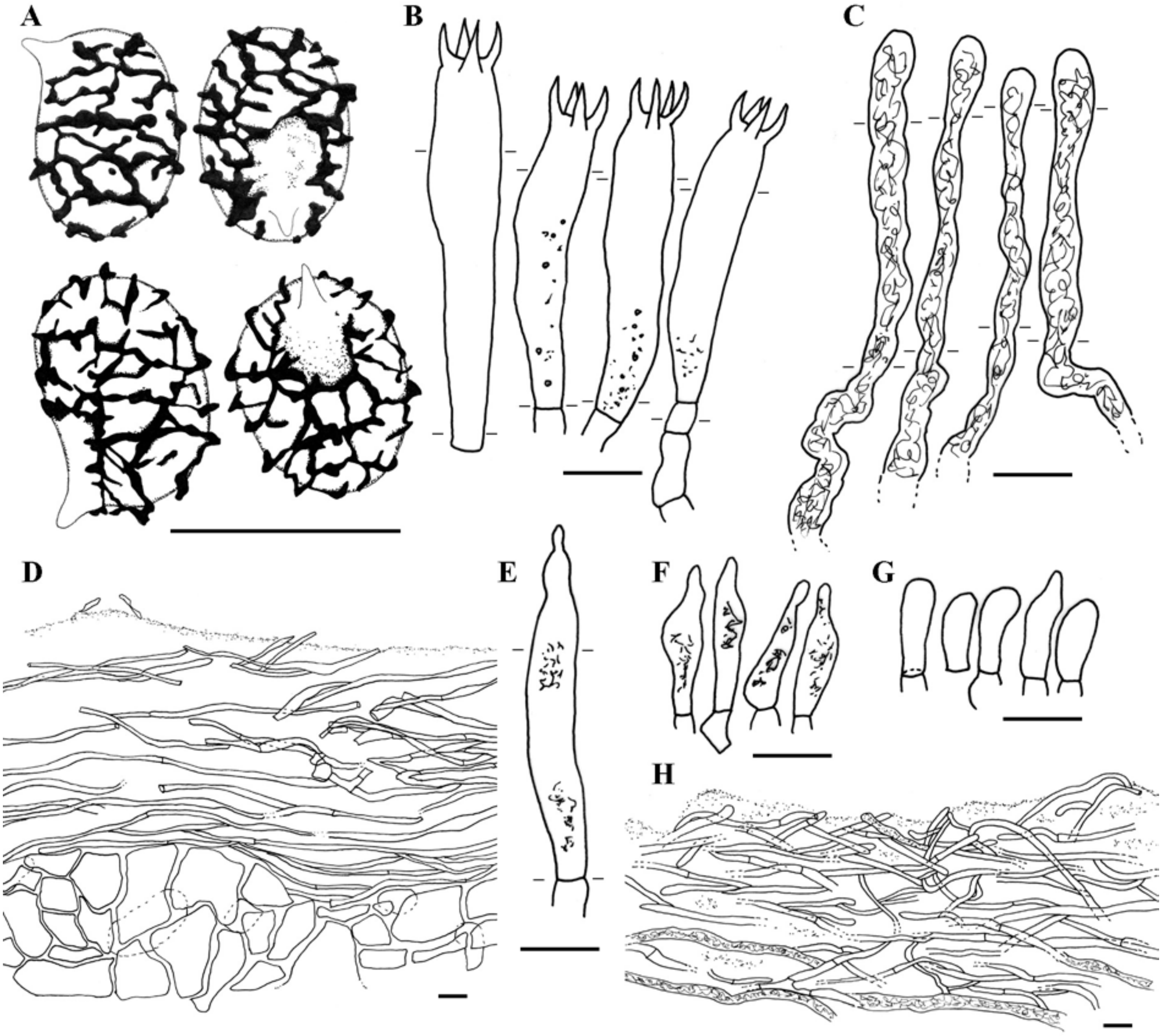

Fig. 16: *Lactarius* sp.2: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** section trough the pileipellis; **E.** macrocystidium; **F.** cheilomacrocystidia; **G.** cheiloleptocystidia; **H.** section through the stipitipellis. From M. Härkönen KIINA 35, 36 and 113. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: These specimens (labelled as *L.* sp.2) form a distinct, rather basal clade in our phylogenetic analysis and almost certainly represent an undescribed species. Since a macroscopical description is lacking, we do not describe this species as new. It differs microscopically from *L. salmonicolor* and *L. laeticolor* (two species that show a superficial macroscopical resemblance) in the absence of striking pleuromacrocystidia and the heavier spore ornamentation. Specimen KIINA 113 was bought from local mushroom pickers that gave it the local name ‘Tsong-jun’.

Fig. 17: **A.** *L.* sp.2 M. Härkönen KIINA 35; **B.** SEM image of a spore of M. Härkönen KIINA 35.

4. Unidentified collections from Asia

Apart from the variability described above under *L. deliciosus*, several clearly distinct taxa were collected that are however macroscopically closest to *L. deliciosus*. The microscopical features are described here. None of the examined specimens were included in our molecular analyses and more collections are needed to come to conclusions.

Lactarius **sp., collections HKAS 39043, HKAS 39196 and HKAS 30127 (HKAS)**
Fig. 18
DESCRIPTION: *Pileus* 6–9 cm diam., convex with inrolled margins and a slightly depressed centre when young, becoming infundibuliform; surface smooth, greasy when moist, obscurely zoned; colour dull orange with green tinges, becoming more carrot orange when mature, centre green to violaceous blue when young, greenish in mature specimens, whole surface extensively discolouring greenish when bruised. *Lamellae* decurrent, very dense and crowded, often forking; colour carrot orange, greenish when bruised. *Stipe* 5.5–9 × 1.2–1.6 cm, cylindrical or broader at the base; colour orange but white at the apex, no scrobicules present. *Context* quite firm, hollow in the stipe, whitish, staining orange near the pellis and above the lamellae, unchanging. *Latex* carrot orange, unchanging on the context.

Spores 7.9–*8.7–8.8*–9.5 (9.6) × 6.0–*6.7–6.8*–7.3 (7.5) µm, broadly ellipsoid to ellipsoid (Q = 1.22–*1.31*–1.39); ornamentation up to 0.5 µm high, of medium thick to rather thick ridges and isolated warts, forming a very incomplete reticulum; plage distally slightly amyloid. *Basidia* 42–50 × 9–10.5 µm, subclavate, 4-spored, often hyaline; sterigmata 4.5–6 µm long. *Pleuromacrocystidia* abundant, especially near the lamella edge, 40–60 × 5–6 µm, subfusiform with a moniliform or capitate apex, emergent, with a granular or needle-like content, thin-walled. *Pseudocystidia* rather abundant, 2–5 µm broad, mostly not emergent, cylindrical to tortuous, with a refractive content. *Lamella edge* sometimes fertile and with very abundant, emergent and striking cheilomacrocystidia; cheiloleptocystidia 11–22 × 3–10 µm, subclavate to more irregular, mostly hyaline, thin-walled; cheilomacrocystidia 32–50 × 4–7 µm, strongly emergent, subfusiform with mostly a moniliform apex, with a needle-shaped content, thin-walled. *Subhymenium* composed of sub-isodiametric cells, irregular. *Hymenophoral trama* composed of short hyphae and abundant, ochre-coloured lactifers. *Pileipellis* an ixocutis, 100–150 µm thick, of strongly interwoven hyphae; shrivelled and gelatinised hyphae present near the surface. *Clamp-connections* absent.

COLLECTIONS EXAMINED: China, Yunnan Prov.: Lijiang county, Yulong Mountain, Ganhaizi, 04.08.1995, M. Zang 12555, HKAS 30127 (HKAS), originally identified as *L. hatsudake* – Yunnan Prov.: Binchuan county, Jizushan, 2250 m a.s.l., *Pinus densata*, 16.07.2001, X.H. Wang 1264, HKAS 39196 (HKAS), originally identified as *L. deliciosus* – Yunnan Prov.: Binchuan county, Jizushan, 2300 m a.s.l., *Pinus de*nsata, 16.07.2001, F.Q. Yu 467, HKAS 39043 (HKAS), originally identified as *L. akahatsu.*

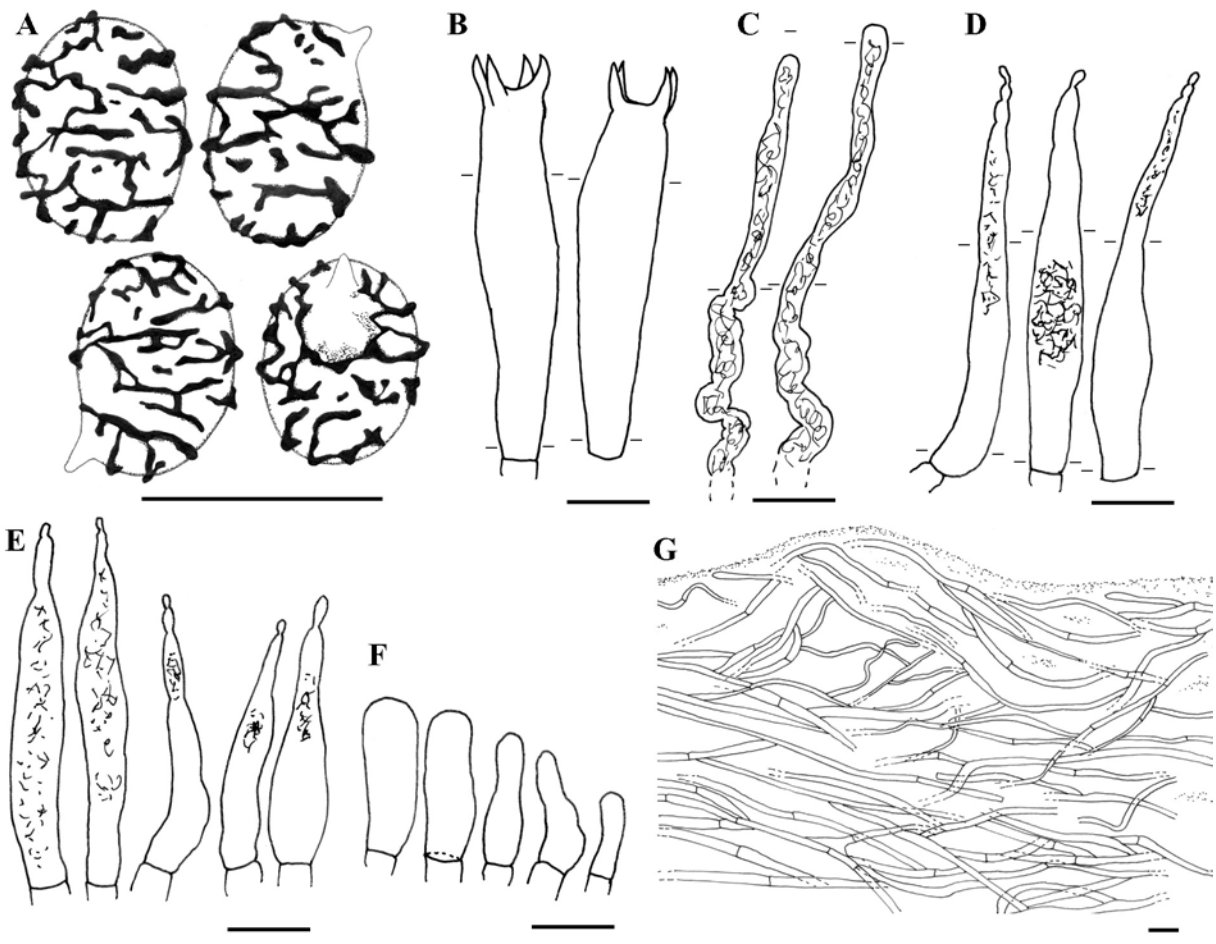

Fig. 18: *Lactarius* sp.: **A.** spores; **B.** basidia; **C.** pseudocystidia; **D.** pleuromacrocystidia; **E.** cheilomacrocystidia; **F.** cheiloleptocystidia; **G.** section through the pileipellis. From HKAS39043, HKAS39196 and HKAS30127. Scale bars = 10 µm, small bars indicate the height of the hymenium.

DISCUSSION: These specimens are strikingly larger and taller than the "typical *L. deliciosus"* sold on the markets in Yunnan. The colours are less bright and the greenish discolouration is much stronger. The very close lamellae seem to be characteristic. Microscopically the abundant, large, strongly emergent cheilomacrocystidia are striking. Very large (> 10 µm long) and strongly ellipsoid spores were occasionally observed but no 2-spored basidia were noticed. The examined collections all come from Yunnan.

***Lactarius* sp., collection HKAS 28382 (HKAS)**

Fig. 19

Spores 8.7–*9.5*–10.3 (10.5) × (6.6) 6.7–*7.3*–8.0 µm, broadly ellipsoid to ellipsoid (Q = 1.21–*1.29*–1.43); ornamentation up to 0.5 µm high, of medium broad ridges, forming an incomplete reticulum; plage slightly amyloid distally or in scattered spots. *Basidia* 40–50 × 8.5–10 µm, subclavate, 4-spored, mostly hyaline, many deformed basidia with very long sterigmata present. *Pleuromacrocystidia* rather scarce to locally abundant, 45–55 × 6–7.5 µm, subfusiform with a moniliform apex, emergent, often with a needle-shaped content, thin-walled. *Pseudocystidia* scarce, 2–5 µm broad. *Lamella edge* sterile with abundant cheilomacrocystidia; cheiloleptocystidia subclavate to irregular, hyaline, thin-walled; cheilomacrocystidia subfusiform with a moniliform to obtuse apex. *Pileipellis* an ixocutis, more than 150 µm high, of strongly interwoven hyphae, 1.5–3.5 µm diam.; shrivelled an gelatinised hyphae abundant near the surface. *Clamp-connections* absent.

COLLECTION EXAMINED: China, Yunnan Prov.: Lijiang county, Yunshanping, 3400 m a.s.l., *Picea* and *Pinus* forest, 08.10.1994, Tanaka & P.G. Liu 94-37, HKAS 28382 (HKAS), originally identified as *L. akahatsu.*

DISCUSSION: No description is available of the macroscopical features of this collection that was originally identified as *L. akahatsu*. The spore size is significantly larger than what we measured in *L. akahatsu* or *L. deliciosus*.

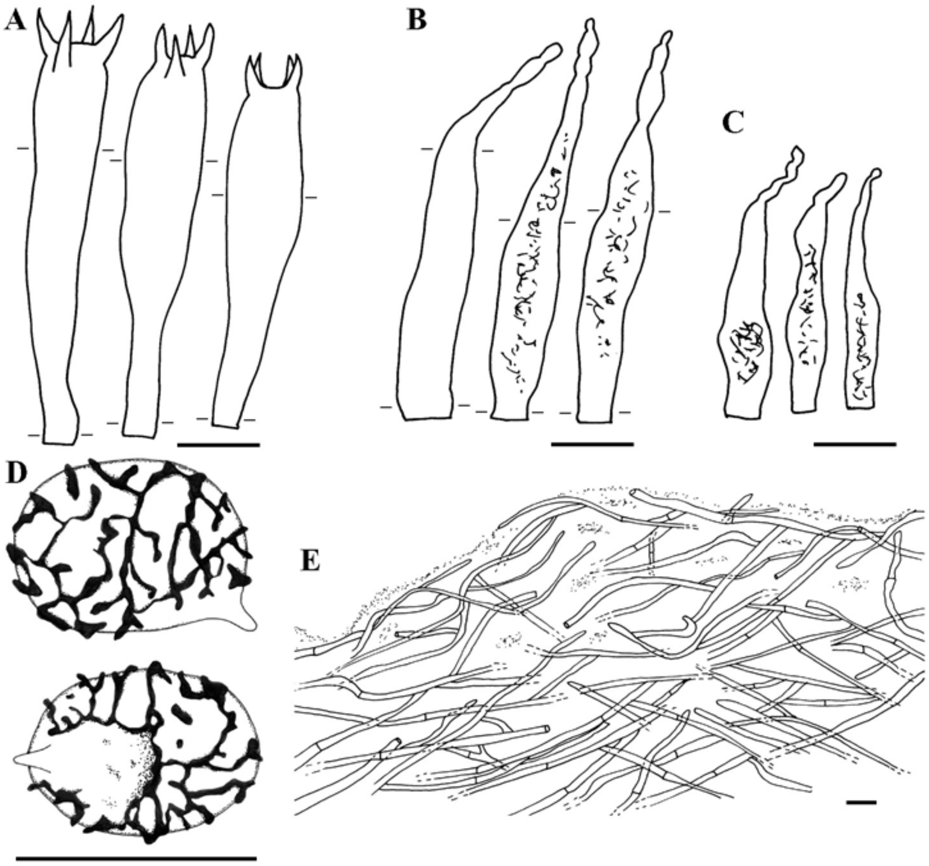

Fig. 19: *Lactarius* sp.: **A.** basidia; **B.** pleuromacrocystidia; **C.** cheilomacrocystidia; **D.** spores; **E.** section through the pileipellis. From HKAS 28382. Scale bars = 10 µm, small bars indicate the height of the hymenium.

5. Insufficiently known and doubtful taxa from Asia

- ***Lactarius ogasawarashimensis*** S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 55 (1940)

This species is described as having a pinkish to buff coloured pileus with dark zones, a light blue coloured stipe and olive-buff lamellae. The latex is described as scarce and blue coloured. The spores are 7.5–8.5 × 7–7.5 µm. *Lactarius ogasawarashimensis* is described as endemic to the Bonin Islands (from which the name was derived) and is found under *Pinus liuchuensis*. Nothing is known from this taxon except for its original description (NAGASAWA, 1998). There is no similar species described from Asia or elsewhere.

- ***Lactarius sanguineus*** Teng, unpubl., cited in Fungi of China: 414 (1996), nom. inval., nom. nud.

This name was traced by KORF (1996) in Teng's notes. Comments by KORF (1996): ‘This species was not treated in the Chinese version, *Chung-kuo Ti Chen-chun*, 1963. Teng added it in the revised key (above), but the portion of this manuscript containing the description of the fungus and any references as to where it was collected or reported from China has (have) been lost.'

- ***Lactarius deliciosus* var. *indicus*** Atri, Saini & D.K. Mann, Geobios, New Reports 10: 110 (1991), as 'indica'

  ATRI *et al.* (1991) reported four varieties of *L. deliciosus* from northwest India: *L. deliciosus* var. *deliciosus*, var. *olivaceosordidus*, var. *areolatus* and var. *indicus*. The latter variety is described as new (as *L. deliciosus* var. *indica*) and is reported to be very similar to *L. deliciosus* var. *deliciosus*. It is different in possessing an azonate pileus, larger spores (9–10.5 × 7.5–9 µm in var. *indicus* versus 7–9 × 6–7 µm in var. *deliciosus*) and a white spore deposit. We neither examined Indian material of *L. deliciosus* var. *indicus* nor from the other varieties reported to occur in this country. It seems necessary to compare Indian material to both European and Asian type or reference collections as the boundary between the European and Asian mycota is unclear or overlapping. The occurrence of American varieties of *L. deliciosus*, for which it has been shown that they are not conspecific whith the Eurasian *L. deliciosus*, seems highly improbable however.

- ***Lactarius sanguifluus* var. *asiaticus*** Dörfelt, Kiet & A. Berg, Feddes Repert. 115: 169 (2004)

  Type material from this species was requested but not yet received. The original description is too incomplete to come to a sure conclusion, but this taxon, described from Vietnam, seems to be similar to *L. hatsudake* or *L.* sp. 4

Acknowledgements

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Chapter 9

Descriptions of the ectomycorrhizae formed by European species in *Lactarius* sect. *Deliciosi*

Abstract

The ectomycorrhizae of six of the ten European species in *Lactarius* sect. *Deliciosi* are described and illustrated morphologically and anatomically. All identifications were verified by comparing the ITS sequence of the ectomycorrhizae with those of the basidiocarps growing nearby. The descriptions of the ectomycorrhizae are compared mutually and with the available literature data. We conclude that the resemblance between the ectomycorrhizae is too high to allow a precise identification of all species based on morphological or anatomical features, but the strong host specificity and the preference for certain environmental conditions might aid in identification.

1. Introduction

The absorption rootlets of many trees in the families Pinaceae, Fagaceae, Betulaceae, Salicaceae, etc. in the temperate and cold climate zones are colonized by ectomycorrhizal (ECM) fungi. These mutualistic fungi form a sheath or mantle of pseudoparenchymatic or plectenchymatic tissue that encloses the rootlets. From this mantle a labyrinthine inward growth of hyphae between the epidermal and cortical cells develops, called the Hartig net. In the opposite direction, an outwardly growing, external mycelium (in the form of hyphae and sometimes rhizomorphs) is present that colonizes the soil and efficiently exploits this soil extending beyond the depletion zones that develop around the roots. In this way, the fungi improve the uptake and transfer of water and mineral nutrients to their hosts while they are dependent on the plant for photosynthetic sugars (SMITH & READ, 1997).
A large number of fungal species have been recorded to form ectomycorrhizae, the majority of which are basidiomycetes. Mycorrhizal symbionts with diverse plant hosts have evolved repeatedly from saprotrophic precursors but there have also been multiple reversals to a free-living condition. Ectomycorrhizae in basidiomycetes should thus be considered as unstable, evolutionary dynamic associations (HIBBETT *et al.*, 2000). Certain ectomycorrhizal fungi can, to a limited extend, act saprotrophically in the soil (LEAKE & READ, 1997) but the large majority are obligate symbionts. Host trees under natural conditions are also strongly dependent on their ectomycorrhizal fungi. The percentage of rootlets infected in healthy trees belonging to the Fagaceae and Pinaceae is close to 100% (COLPAERT, 1994). The majority of the ectomycorrhizal fungi have a broad host range, but specificity at the level of plant genus, is found in a diverse range of fungi, hosts and habitats (SMITH & READ, 1997). *Lactarius* sect. *Deliciosi* forms a striking example of this host specificity.

The ectomycorrhizae of ± 40 *Lactarius* species have been described. A synthesis of the ectomycorrhizal types and most important distinguishing features per subgenus and section in the genera *Lactarius* and *Russula* is given in EBERHARDT (2000). A difference between the *Lactarius* and *Russula* root tips is the presence or respectively absence of lactifers, in parallel with what is observed in the basidiocarps. The next most taxonomically important feature is the cellular structure of the mantle in plan view. The mode of ramification, dimensions and shape of the ectomycorrhizae are in the first place determined by the host tree and by the properties of the substratum. Emanating hyphae are in general inconspicuous and uncommon in the Russulaceae (EBERHARDT, 2000).

In ecological studies focussing on the ectomycorrhizal community, problems consistently arise in the identification of ectomycorrhizal tips (HORTON & BRUNS, 2001). Several studies have proven that the morphological characterisation of root tips might be very helpful in identification (HORTON, 2002;

SAKAKIBARA, 2002). Morphological identification is also the easiest and cheapest way and it is thus useful to collect as many descriptive data on ectomycorrhizae as possible.

The ectomycorrhizae of several species in *Lactarius* sect *Deliciosi* have already been described more or less elaborately. Table 1 lists all references that describe *Lactarius* sect *Deliciosi* ectomycorrhizae. This provides an excellent opportunity to thoroughly compare the morphology of ectomycorrhizae in a group of closely related species. Moreover, we describe the ectomycorrhizae of two additional, undescribed species (*L. quieticolor* and *L. semisanguifluus*) here and compare our personal descriptions of *L. deliciosus*, *L. deterrimus*, *L. porninsis* and *L. salmonicolor* with the existing literature data. We also examine whether the ectomycorrhizae of the different species differ enough to allow identification on the species level. Furthermore we examine whether the descriptive features can provide phylogenetic information.

Table 1: Available literature on ectomycorrhizae formed by members of *Lactarius* sect. *Deliciosi*. Because the identification of American isolates as *L. deliciosus* is doubtful, we put them between quotation marks.

	Species	Host	Reference
1.	*L. akahatsu*	*Pinus densiflora*	YAMADA *et al.* (2001b)
2.	"*L. deliciosus*"	*Abies lasiocarpa*	KERNAGHAN *et al.* (1997)
3.	*L. deliciosus*	*Pinus silvestris*	UHL (1988)
4.	*L. deliciosus*	*Pinus taeda*	HUANG (1990, 1996)
5.	"*L. deliciosus*"	several coniferous trees	MOLINA & TRAPPE (1982a)
6.	"*L. deliciosus*"	*Arctostaphylos uva-ursi* and *Arbutus menziesii*	MOLINA & TRAPPE (1982b)
7.	"*L. deliciosus*"	*Abies concolor*	ACSAI & LARGENT (1983)
8.	*L. deterrimus*	*Picea abies*	MÜNZENBERGER *et al.* (1986)
9.	*L. deterrimus*	*Picea abies*	AGERER (1986b)
10.	*L. hatsudake*	*Pinus densiflora*	YAMADA *et al.* (2001b)
11.	*L. hatsudake*	*Pinus taeda*	HUANG (1990, 1996)
12.	*L. paradoxus*	*Pinus banksiana*	DANIELSON (1984)
13.	*L. porninsis*	*Larix decidua*	TREU (1990)
14.	*L. rubrilacteus*	*Pseudotsuga menziesii*	EBERHART & LUOMA (1997)
15.	*L. salmonicolor*	*Abies alba*	PILLUKAT (1996b)
16.	*L. salmonicolor*	*Abies alba*	EBERHARDT *et al.* (2000)
17.	*L. sanguifluus*	*Arctostaphylos uva-ursi*	ZAK (1976)
18.	*L.* sp.? (*deliciosus*)	*Pinus ponderosa* and *Pseudotsuga menziesii*	MASSICOTTE *et al.* (1999)

2. Materials & Methods

2.1. Morphology

The ectomycorrhizae described here were collected in Belgium and Italy. Collection data are listed with the descriptions. Voucher specimens of basidiocarps and mycorrhizae are deposited in GENT as dried and fixed material, together with slides. For the sampling and describing of the ectomycorrhizae, we largely followed the methods and terminology as explained by AGERER (1991; 1987-2002). Soil cores were excavated from underneath sporocarps and soaked overnight in water. The soil debris was cautiously washed away and ectomycorrhizal roots were carefully separated under a dissecting microscope. Several tips were stored in FAA (5% formaldehyde, 45% ethanol, 5% acetic acid) and CTAB-buffer (2% w/v CTAB, 100 mM Tris, 1.4 M NaCl and 20 mM EDTA) for subsequent morphological and molecular study.

Macroscopical descriptions were carried out in daylight on freshly isolated material. Colour codes are according to KORNERUP & WANSCHER (1962). Photographs were taken with a Nikon DXM1200 digital camera and a Nikon SMZ800 stereoscopic microscope using the Eclipse Net 1.16.4 software. Observations of the mantle in plan view were made on freshly isolated material from which mantle fragments were detached with a fine needle. Preparations were studied in both H_2O and 0.05% toluidine blue. Mycorrhizal tips were embedded in Technovit ® using a Technovit ® 7100 kit (Kulzer). Five µm thick longitudinal and cross-sections were made with a Reichert-Jung Supercut 2050 microtome and stained with 0.05% toluidine blue. For taking photographs we used a Nikon DXM1200 digital camera and an Eclipse E600 microscope.

2.2. Molecular identification

The identity of the mycorrhizal tips was checked by comparing the ITS sequence of the ectomycorrhizae with those of the basidiocarps. Following methods were used:

DNA preparation

DNA was extracted using a modified CTAB procedure. Fresh material (ectomycorrhizal tips or small pieces of basidiocarps) was ground in CTAB buffer at room temperature and incubated for 1 h at 65°C. Proteins were removed by two chloroform/iso-amylalcohol extractions. DNA was precipitated by adding 500 µl cold isopropanol and centrifuging for 10 min. at 5000 rpm. Pellets where washed with 70% ethanol and dissolved in 200 µl TE.

PCR and direct sequencing of the ITS region

Part of the 18S rDNA, the ITS1 region, the 5.8S rDNA part, the ITS2 region and part of the 28S rDNA were sequenced. Primers ITS1-F and ITS4-B (GARDES & BRUNS, 1993) were used for the PCR reaction. The touchdown PCR profile as described in chapter 3 was used. The obtained PCR products were purified using ExoSAP (USB, USA). DNA sequencing reactions were performed with the ABI PRISM® BigDye^{TM} Terminators v3.0 Cycle Sequencing Kit using primers ITS1-F, ITS2, ITS3 and ITS4-B (GARDES & BRUNS, 1993; WHITE *et al.*, 1990) on an ABI PRISM® 377 DNA Sequencer.

Data analysis

The Phred and Phrap Software (EWING *et al.*, 1998; EWING & GREEN, 1998) was used to process the raw data. ITS sequences were aligned in ClustalX (THOMPSON *et al.*, 1997) and compared in BioEdit 5.0.9 (HALL, 1999).

3. Descriptions

3.1. *Lactarius deliciosus* (L.: Fr.) Gray

+ *Pinus sylvestris* L.

Fig. 1

***Morphological characters* (Fig 1 A-C):** *Mycorrhizal systems* up to 5.5 mm long, strongly branching dichotomously to almost coralloid, organised in small clusters. – *Main axes* 0.4–0.6 mm diam. – *Unramified ends* straight, cylindric, often starting to divide dichotomously at the apex, up to 1.5 mm long and 0.5 mm diam.; colour orange (6B7) to brownish orange (6C7), not transparent, very tips paler, whitish to yellowish (4A3), older parts becoming brownish and locally greenish; lactifers with bright orange latex visible through the outer mantle; mantle secreting orange latex when injured; cut surfaces discolouring reddish. – *Surface of unramified ends* smooth, no emanating hyphae present, root tissues not shining through. – *Rhizomorphs* rather scarce, up to 80 µm diam., ± rounded in cross-section, mainly originating from the main axes, sometimes almost perpendicular to the surface; colour pale orange.

***Anatomical characters of the mantle in plan view*:** *Outer mantle layers* plectenchymatous, hyphae irregularly and very densely arranged, no clear gelatinous matrix present (mantle type B according to AGERER, 1991); hyphae more or less straight but often branching, 2–3 µm diam., thin-walled; colour pale yellowish; lactifers absent. – *Middle mantle layers* plectenchymatous, no special pattern discernable, not gelatinised (mantle type B according to AGERER, 1991); hyphae 2.5–6 µm diam., thin-walled; colour yellowish; lactifers present but rather scarce, straight, often branching, up to 8 µm diam. – *Inner mantle layers* plectenchymatous to locally almost pseudoparenchymatous, with very densely arranged hyphae, 3–4 µm diam. – *Very tip* plectenchymatous, hyphae irregularly arranged, slightly more widely spaced; hyphae thin walled, up to 4.5 µm diam.; lactifers rare.

***Anatomical characters of emanating elements*:** *Rhizomorphs* undifferentiated to sometimes slightly differentiated (rhizomorph type B to C according to AGERER, 1991), margin smooth, hyphae compactly arranged, central hyphae sometimes somewhat enlarged (rhizomorph type B or C according to AGERER, 1991); hyphae 2–4 µm diam. – *Emanating hyphae* absent. – *Cystidia* absent.

***Anatomical characters, longitudinal section* (Fig. 1 D-E):** *Mantle* 20–40 µm thick, different layers not clearly discernable, almost entirely densely plectenchymatous, no clear gelatinous matrix present; outermost hyphae very compactly arranged, 2–3.5 µm diam., lactifers lacking; middle layer with wider hyphae, up to 7 µm diam., containing lactifers; innermost layer locally almost pseudoparenchymatous, hyphae up to 4 µm diam; lactifers present; mantle of the very tip up to 70 µm thick, lactifers present but scarce; no clear calyptra remnants visible. – *Tannin cells* arranged in 1 row, thin, tangentially elongated, irregularly shaped to collapsed, 15–80 µm tangentially, 4–10 µm radially. – *Cortical cells* rounded to irregular, 30–60 µm tangentially, 15–50 µm radially. – *Hartig net* in section surrounding 2 of the 3 rows of cortical cells, forming 1–2 rows of rather irregularly shaped cells, up to 5 µm diam.; Hartig net in plan view forming palmetti-like lobes, 2–7 µm diam.

***Anatomical characters, cross-section* (Fig. 1 F):** *Mantle* different layers not discernable, almost entirely densely plectenchymatous, no clear gelatinous matrix present. – *Tannin cells* arranged in a single row, up to 25–40 × 3-5 (10) µm. – *Cortical cells* rounded to square, 15–60 × 15–50 µm.

***Examined specimen*:** Belgium, Close to Ave-et-Auffe, 244 m a.s.l., N50°07.060' E005°08.025', under *Pinus sylvestris*, 26.10.2003, J. Nuytinck 2003-047 (GENT).

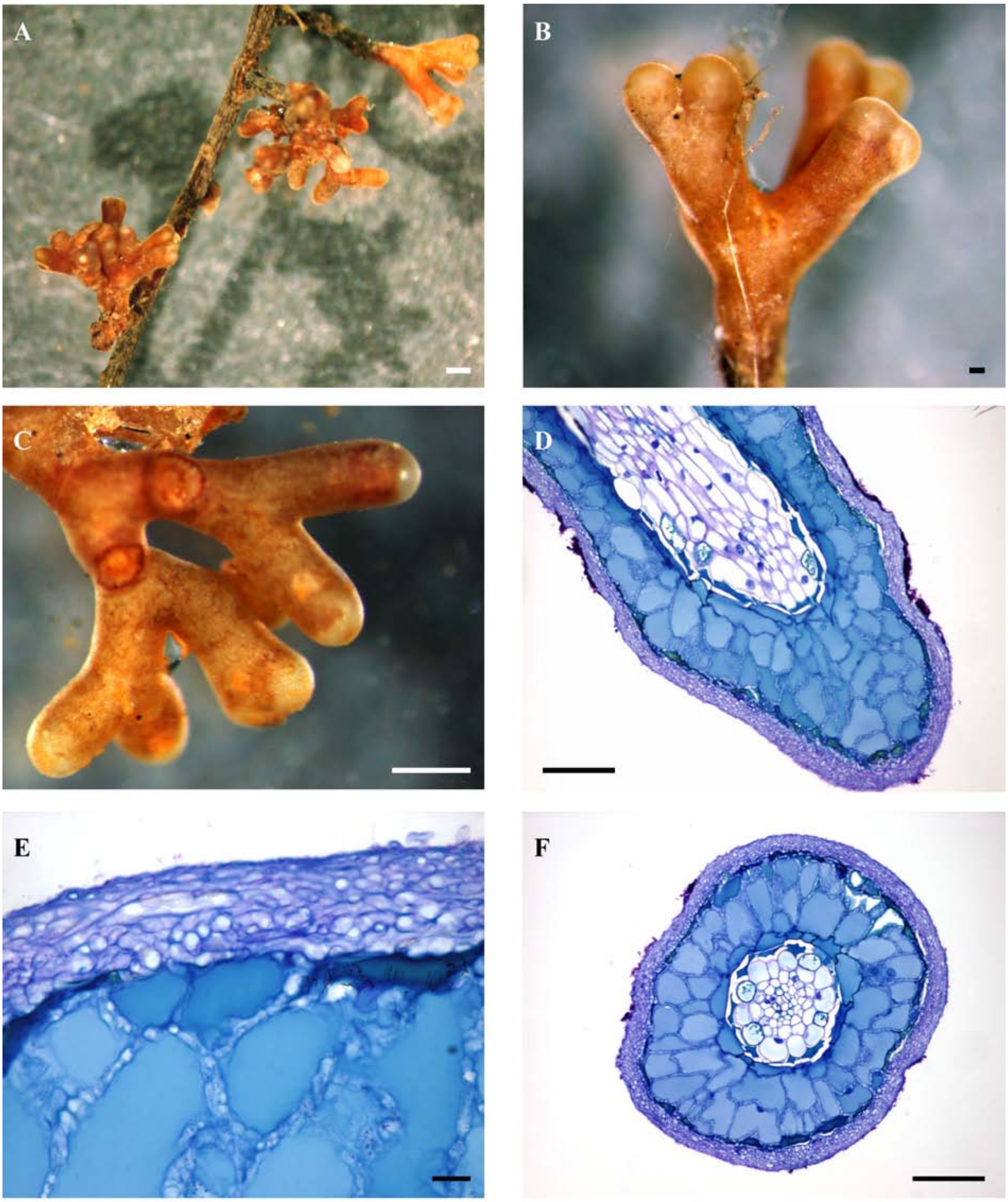

Fig. 1: *L. deliciosus* ECM **A.** Small clusters of strongly dichotomously branching ectomycorrhizal systems (scale bar = 1 mm); **B.** Dichotomously branching root tips with rhizomorph (scale bar = 0.1 mm); **C.** Mantle surface showing lactifers with orange latex and cut surfaces discolouring reddish (scale bar = 1 mm); **D.** Overview longitudinal section (scale bar = 100 µm); **E.** Detail longitudinal section (scale bar = 10 µm); **F.** Overview cross-section (scale bar = 100 µm).

3.2. *Lactarius deterrimus* Gröger

+ *Picea abies* (L.) Karst.

Fig. 2

***Morphological characters* (Fig. 2 A-B):** *Mycorrhizal systems* up to 15 mm in length, irregular to monopodial-pinnate, forming dense clusters. – *Main axes* 3–6 mm long, straight to sometimes curved or rarely strongly tortuous. – *Unramified ends* straight and regularly cylindric or curved to tortuous, 0.5–1.5 mm long, 0.4–0.65 mm diam., orange coloured (6B/C8), very tips paler in colour but not transparent, discolouring brownish and greenish when old; mantle secreting orange latex when injured. – *Surface of unramified ends* smooth, no emanating hyphae found, root tissues not shining through. – *Rhizomorphs* rare, up to 70 µm diam., more or less round in cross-section, orange or green coloured.

***Anatomical characters of the mantle in plan view* (Fig. 2 C-D):** *Outer mantle layers* plectenchymatous, hyphae irregularly arranged but mainly in the longitudinal direction, no clear gelatinous matrix present (mantle type B according to AGERER, 1991); hyphae (2) 3–4 µm broad, thin-walled; colour pale yellowish; no lactifers present. – *Middle mantle layers* plectenchymatous, hyphae irregularly arranged (mantle type B according to AGERER, 1991); hyphae 3–6 µm diam., not gelatinised, thin-walled; colour yellowish; lactifers present but scarce, straight but frequently branching, up to 9 µm diam. – *Inner mantle layers* plectenchymatous, hyphae irregularly and densely arranged (mantle type B according to AGERER, 1991), 2–5 µm diam.; lactifers scarce – *Very tips* plectenchymatous, hyphae irregularly arranged and more widely spaced, 2–4 µm thick, lactifers up to 7 µm diam., rather scarce.

***Anatomical characters of emanating elements*:** *Rhizomorphs* undifferentiated, hyphae more or less parallel, no thicker central hyphae observed (rhizomorph type B according to AGERER, 1991); hyphae 2.5-3.5 µm diam., thin-walled; colour slightly yellowish. – *Emanating hyphae* not observed. – *Cystidia* absent.

***Anatomical characters, longitudinal section* (Fig. 2 E):** *Mantle* up to 45 µm thick, no distinct layers recognisable, entirely plectenchymatous, no gelatinous matrix present; outermost hyphae 2–3.5 µm diam., no lactifers present; middle mantle layer with scarce lactifers, up to 9 µm diam., innermost layer with densely arranged hyphae, 2–6 µm diam., lactifers present. – *Tannin cells* arranged in 1, 2 or 3 rows, strongly flattened tangentially, irregularly shaped and often collapsed, 30–90 µm tangentially, 6–12 µm radially, inner tannin cell layer up to 15 µm diam. – *Cortical cells* elongated tangentially, often irregularly shaped, 35–120 µm tangentially, 14-20 (35) µm radially. – *Hartig net* in section surrounding cortical cells up to the endodermis or leaving half a row of cortical cells free, arranged in 1 row of rounded to angular cells, 3.5–5.5 µm diam., Hartig net in plan view forming palmetti-like lobes, 2–6 µm diam.

***Anatomical characters, cross-section* (Fig. 2 F):** *Mantle* no layers recognisable, entirely densely plectenchymatous, most hyphae orientated longitudinally, no clear gelatinous matrix present. – *Tannin cells* in 1–3 rows, narrow, longitudinally orientated, 30–40 × 5–10 µm. – *Cortical cells* tangentially elongated, 30–60 × 15–35 µm.

***Examined specimen*:** Italy, close to Castel del Monte, N42°23.062' E013°45.365', 1433 m a.s.l., small mixed conifer forest on slope, under *Picea abies*, 1.11.2001, J. Nuytinck 2001-099/100 (GENT).

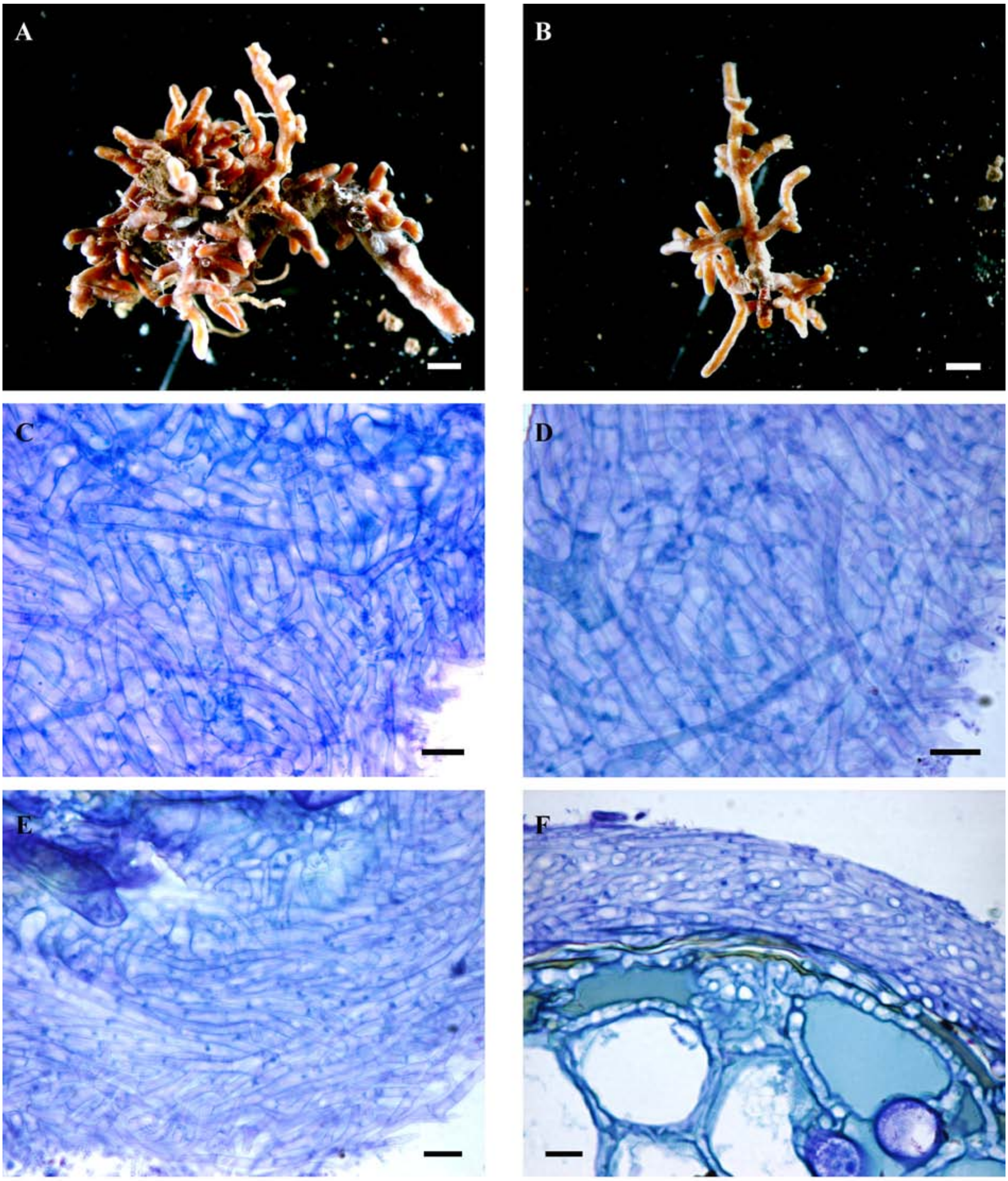

Fig. 2: *L. deterrimus* ECM **A.** Densely clustered mycorrhizal systems (scale bar = 1 mm); **B.** Irregularly branching mycorrhizal system (scale bar = 1 mm); **C.** Plan view of the outer surface of the mantle (scale bar = 10 µm); **D.** Plan view of the middle to inner mantle layer with lactifers (scale bar = 10 µm); **E.** Detail of a longitudinal section through the very tip (scale bar = 10 µm); **F.** Cross-section (scale bar = 10 µm).

3.3. *Lactarius porninsis* Rolland

+ *Larix decidua* Mill.

Fig. 3

Morphological characters **(Fig. 3 A-C):** *Mycorrhizal systems* up to 10 (12) mm long, mostly very regularly monopodial-pinnate. – *Main axes* 0.5–0.7 µm diam., 5–9 µm long, mostly straight. – *Unramified ends* straight, regularly cylindric, 0.5–4 mm long, 0.4–0.5 µm diam.; colour orange to brownish orange (6/7C8) or brown (7D8), older parts brown, very tips somewhat paler and more transparent. – *Surface of unramified ends* smooth, no emanating hyphae present, root tissues not shining though. – *Rhizomorphs* present, rather common, up to 90 µm diam, round in cross-section, not originating from specific places; colour pale yellowish to white.

Anatomical characters of the mantle in plan view**:** *Outer mantle layers* plectenchymatous, no pattern discernable, with an unclear gelatinous matrix or matrix absent (mantle type B to C according to AGERER, 1991); hyphae relatively rarely branching, 2–4 µm diam., thin-walled; colour yellowish; lactifers absent. – *Middle mantle layers* plectenchymatous, without pattern, no gelatinous matrix present (mantle type B according to AGERER, 1991); hyphae 2–5 µm diam., thin-walled; colour yellowish; lactifers rather scarce, straight, often branching, up to 12 µm diam. – *Inner mantle layers* plectenchymatous, without gelatinous matrix (mantle type B according to AGERER, 1991); lactifers very scarce. – *Very tips* plectenchymatous, similar to rest of mantle.

Anatomical characters of emanating elements**:** *Rhizomorphs* undifferentiated (rhizomorph type B according to AGERER, 1991), margin smooth, hyphae compactly arranged, 2–4 µm diam. – *Emanating hyphae* absent. – *Cystidia* absent.

Anatomical characters, longitudinal section**:** *Mantle* 25–35 µm thick, different layers not discernable, plectenchymatous, no clear gelatinous matrix present; outermost hyphae compactly arranged, 2–4 µm diam.; lactifers lacking; middle layer with hyphae up to 7 µm diam.; lactifers up to 11 µm diam.; innermost hyphae 2–5 µm diam.; lactifers very scarce; calyptra remnants clearly visible and brown. – *Tannin cells* arranged in 1–3 rows, elongated and orientated parallel to the root axis, irregular in shape and often collapsed, 40–100 µm tangentially, 4–20 µm radially. – *Cortical cells* strongly elongated tangentially, 40–140 µm tangentially, 10–35 µm radially. – *Hartig net* in section surrounding 4–5 rows of cortex cells but never reaching up to the endodermis, forming 1 or more rarely 2 rows of rounded to rather square or rectangular shaped cells, 3.5–5.5µm broad, Hartig net in plan view forming palmetti-like lobes.

Anatomical characters, cross-section **(Fig. 3 D-F):** *Mantle* different layers not discernable, entirely densely plectenchymatous, no clear gelatinous matrix visible. – *Tannin cells* arranged in 2–3 rows, 10–30 × 7–25 µm. – *Cortical cells* rounded to rectangular, 14–40 × 20–40 µm.

Examined specimen**:** Italy, Trentino, N46°01.555' E011°13.035', 773 m a.s.l., in mixed forest, under *Larix decidua*, calcareous soil, 2.10.2002, J. Nuytinck 2002-019 (GENT).

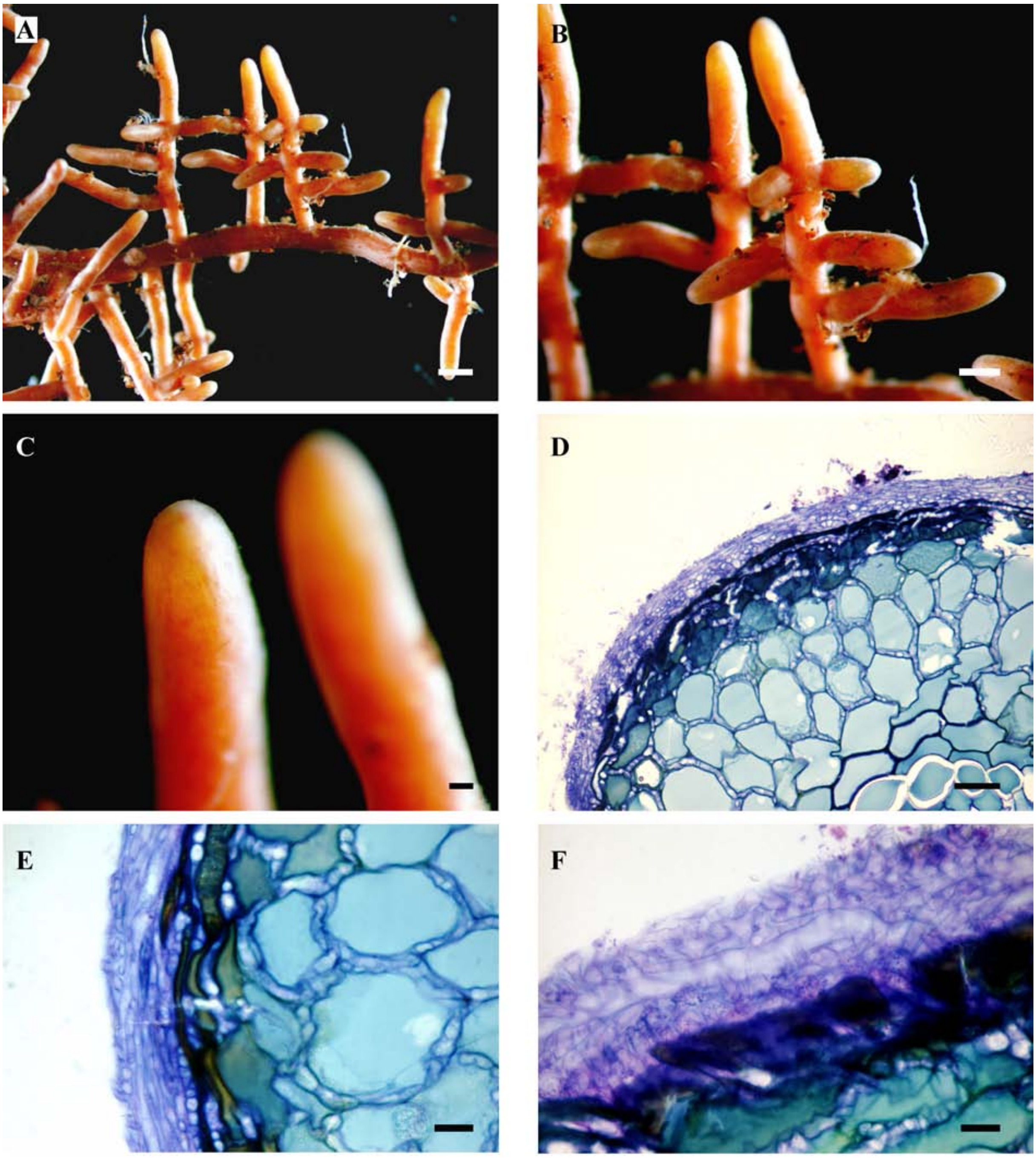

Fig. 3: *L porninsis* ECM **A.** Mycorrhizal systems with whitish rhizomorphs (scale bar = 1 mm); **B.** Regularly monopodial-pinnate mycorrhizae with a rhizomorph (scale bar = 0.5 mm); **C.** Unramified ends (scale bar = 0.1 mm); **D.** Cross-section showing the tannin cells and Hartig net (scale bar = 50 µm); **E.** Cross-section through the plectenchymatous mantle (scale bar = 10 µm); **F.** Detail of the mantle in longitudinal section showing a lactifer in the middle mantle layer (scale bar = 10 µm).

3.4. *Lactarius quieticolor* Romagn.

+ *Pinus nigra* Arn.

Fig. 4

***Morphological characters* (Fig. 4 A-B):** *Mycorrhizal systems* up to 5.5 µm long, simple to dichotomously branching, not organised into dense clusters. – *Main axes* 2–5 mm long and 0.4–0.6 mm diam., straight. – *Unramified ends* straight, regularly cylindric, 2–3 mm long and up to 0.5 mm diam.; colour orange (6B6/7) to buff-orange (5B7), older parts discolouring brownish, not transparent; very tips paler and slightly transparent; exuding orange latex when injured. – *Surface of unramified ends* smooth, no emanating hyphae present, root tissues not shining through. – *Rhizomorphs* up to 80 µm diam., rounded in cross-section, mainly originating from the basal parts of the mycorrhizal systems; colour whitish to yellowish.

***Anatomical characters of the mantle in plan view* (Fig. 4 C):** *Outer mantle layers* plectenchymatous, hyphae irregularly arranged to orientated parallel to the axis of the root, an unclear gelatinous matrix present (mantle type C according to AGERER, 1991); hyphae straight, frequently branching, 2–3 µm diam., thin-walled; lactifers lacking. – *Middle mantle layers* plectenchymatous, hyphae mainly orientated parallel to the axis of the root, gelatinous matrix present (mantle type C according to AGERER, 1991); hyphae up to 6 µm diam., thin-walled; lactifers present, often branching, up to 8 µm diam. – *Inner mantle layers* plectenchymatous, hyphae irregularly and densely arranged, 3–4 µm diam. – *Very tips* plectenchymatous, hyphae more widely spaced, up to 5 µm diam.; lactifers present.

***Anatomical characters of emanating elements*:** *Rhizomorphs* undifferentiated (rhizomorph type B according to AGERER, 1991), margin smooth, hyphae compactly arranged, up to 4 µm diam. – *Emanating hyphae* absent. – *Cystidia* absent.

***Anatomical characters, longitudinal section* (Fig. 4 D-E):** *Mantle* up to 50 µm thick, different layers not discernable, plectenchymatous, hyphae mainly orientated parallel to the axis of the root; outermost hyphae compactly arranged, 2–3.5 µm diam.; lactifers lacking; middle layer with hyphae up to 6 µm diam.; lactifers up to 9 µm diam., scarce; innermost hyphae 2–5 µm diam.; lactifers scarce; no clear calyptra remnants visible. – *Tannin cells* arranged in 1–2 rows, elongated and orientated parallel to the root axis, irregular in shape and often collapsed, 35–80 µm tangentially, 4–35 µm radially. – *Cortical cells* rounded, 35–70 µm tangentially, 40 – 52 µm radially. – *Hartig net* in section surrounding cortical cells up to the endodermis, forming 1 row of square to rectangular shaped cells, 2.5–4 µm broad, Hartig net in plan view forming palmetti-like lobes up to 6 µm diam.

***Anatomical characters, cross-section* (Fig. 4 F):** *Mantle* different layers not discernable, densely plectenchymatous, gelatinous matrix not clearly visible. – *Tannin cells* arranged in 1–2 rows. – *Cortical cells* rounded to square, 25–60 × 40 – 52 µm.

***Examined specimen*:** Belgium, Wachtebeke-Moerbeke, Heidebos, under *Pinus nigra*, in grassy place, 11.11.2002, J. Nuytinck 2002-048 (GENT).

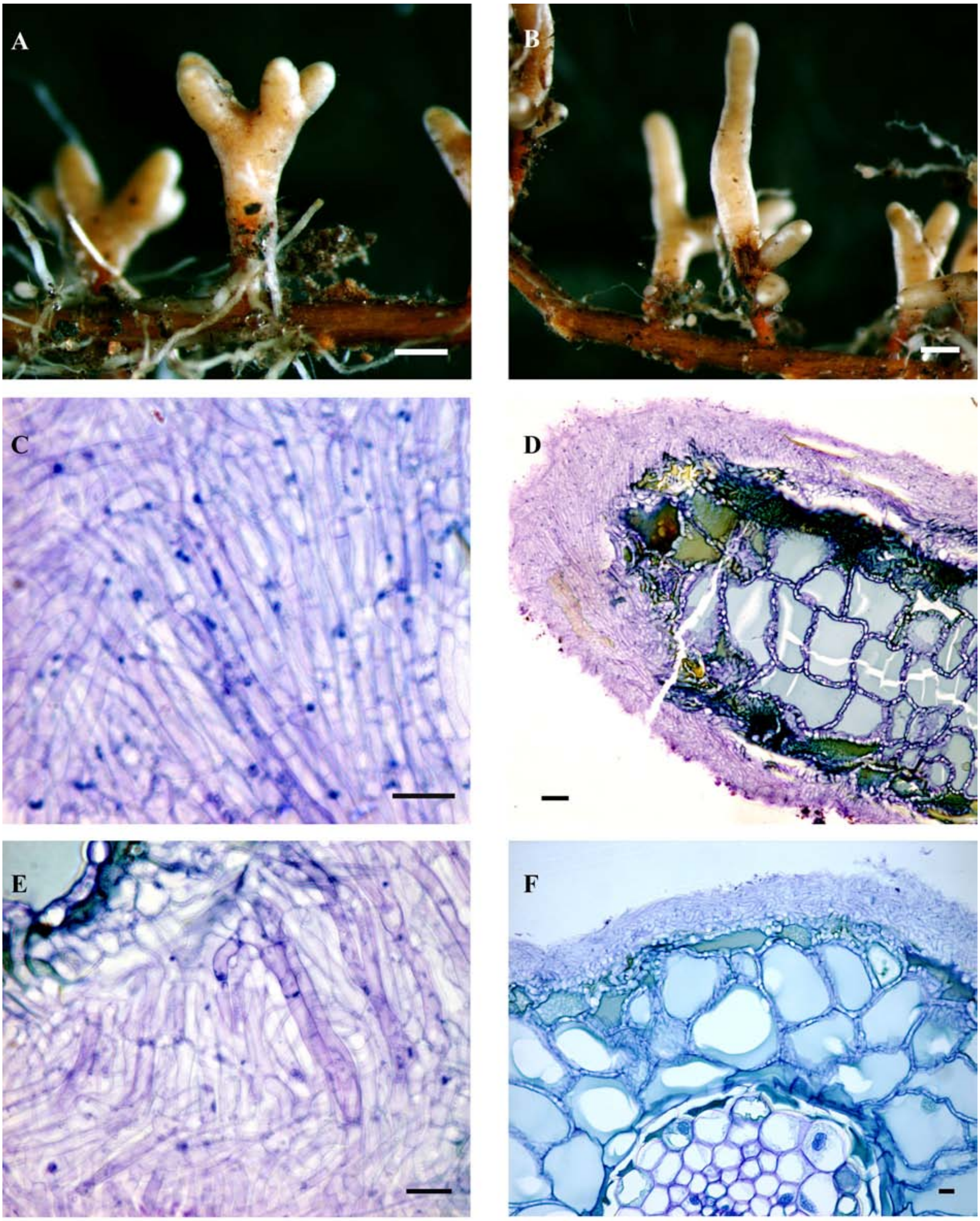

Fig. 4: *L. quieticolor* ECM **A.** Dichotomously branching ectomycorrhizal tip (scale bar = 0.5 mm); **B.** Unramified ends (scale bar = 0.5 mm); **C.** Detail of the outer plectenchymatous mantle in plan view (scale bar = 10 µm); **D.** Longitudinal section through the very tip (scale bar = 50 µm); **E.** Detail of the mantle in longitudinal section close to the very tip (scale bar = 10 µm); **F.** Cross-section (scale bar = 10 µm).

3.5. *Lactarius salmonicolor* R. Heim & Leclair

+ *Abies alba* Mill.

Fig. 5

***Morphological characters* (Fig. 5 A-B):** *Mycorrhizal systems* monopodial-pinnate or simple, up to 15 mm long, often arranged in dense clusters but sometimes only small and unramified ends ectomycorrhizal. – *Main axes* 3–10 mm long and 0.5–0.8 mm diam. – *Unramified ends* straight to bent, cylindrical to slightly irregularly shaped, up to 3 mm in length and 0.4–0.55 mm diam., orange to brownish (8C/D8), older parts becoming rather deep brownish (7E8) and discolouring green, very tips lighter and slightly transparent; lactifers containing orange latex visible through the outer mantle; mantle secreting orange latex when injured. – *Surface of unramified ends* smooth or becoming slightly granular with age, cortical cells not visible through the mantle; no emanating hyphae present. – *Rhizomorphs* rare, emanating from the middle of basal part of the ectomycorrhizae, up to 0.1 mm diam., round in cross-section, surface smooth, pale orange.

***Anatomical characters of the mantle in plan view* (Fig. 5 C):** *Outer mantle layers* densely plectenchymatous, no pattern discernable, no clear gelatinous matrix present (mantle type B according to AGERER, 1991); hyphae rather straight, sometimes with hyphal protrusions, 2–4 µm diam., thin-walled. – *Middle mantle layers* less densely plectenchymatous than outer mantle layers, no pattern discernable (mantle type B according to AGERER, 1991); hyphae up to 5.5 µm diam.; lactifers abundant, 5.5–9 µm diam., with a clear yellowish-orange content. – *Inner mantle layers* densely plectenchymatous (mantle type B according to AGERER, 1991); lactifers abundant. – *Very tips* hyphae more densely agglutinated than in the subapical region, thinner, 1.5–3.5 µm diam.; lactifers more rare.

***Anatomical characters of emanating elements*:** *Rhizomorphs* undifferentiated (rhizomorph type B according to AGERER, 1991), margin smooth; hyphae 2-5 µm diam., thin-walled, frequently branching, side-branches growing parallel to the axis of the rhizomorph. – *Emanating hyphae* not observed. – *Cystidia* not observed.

***Anatomical characters, longitudinal section* (Fig. 5 D-E):** *Mantle* up to 60 µm thick, densely plectenchymatous, different layers not clearly distinguishable, no clear gelatinous matrix present; outermost hyphae very compactly arranged, 2–4 µm diam.; lactifers absent; middle mantle layer with hyphae up to 6 µm diam., more widely spaced; containing rather abundant lactifers, up to 9 µm diam.; innermost hyphae densely arranged; lactifers rather abundant; mantle of the very tip up to 80 µm thick, lactifers more scare and thinner; brownish calyptra remnants sometimes visible. – *Tannin cells* arranged in 1–2 rows, tangentially elongated, often collapsed, 35-90 µm tangentially, 7–15 µm radially. – *Cortical cells* rectangular, mostly regularly-shaped; elongated tangentially, 60–100 µm tangentially, 20–30 µm radially. – *Hartig net* in section surrounds 3–4 of the 4–5 rows of cortical cells, forming 1–2 rows of irregular to square cells, up to 5 µm thick; Hartig net in plan view forming palmetti-like lobes 2–8 µm diam.

***Anatomical characters, cross-section* (Fig. 5 F):** *Mantle* different layers hardly discernable, entirely plectenchymatous, no clear gelatinous matrix visible. – *Tannin cells* arranged in 1–2 rows, 20–35 × 3–6 µm. – *Cortical cells* rounded, square to more irregular, 20–50 × 20–45 µm.

***Examined specimen*:** Belgium, Rochefort, Les Fèches, under *Abies alba*, in young plantation 30.10.2002, J. Nuytinck 2002-046 (GENT).

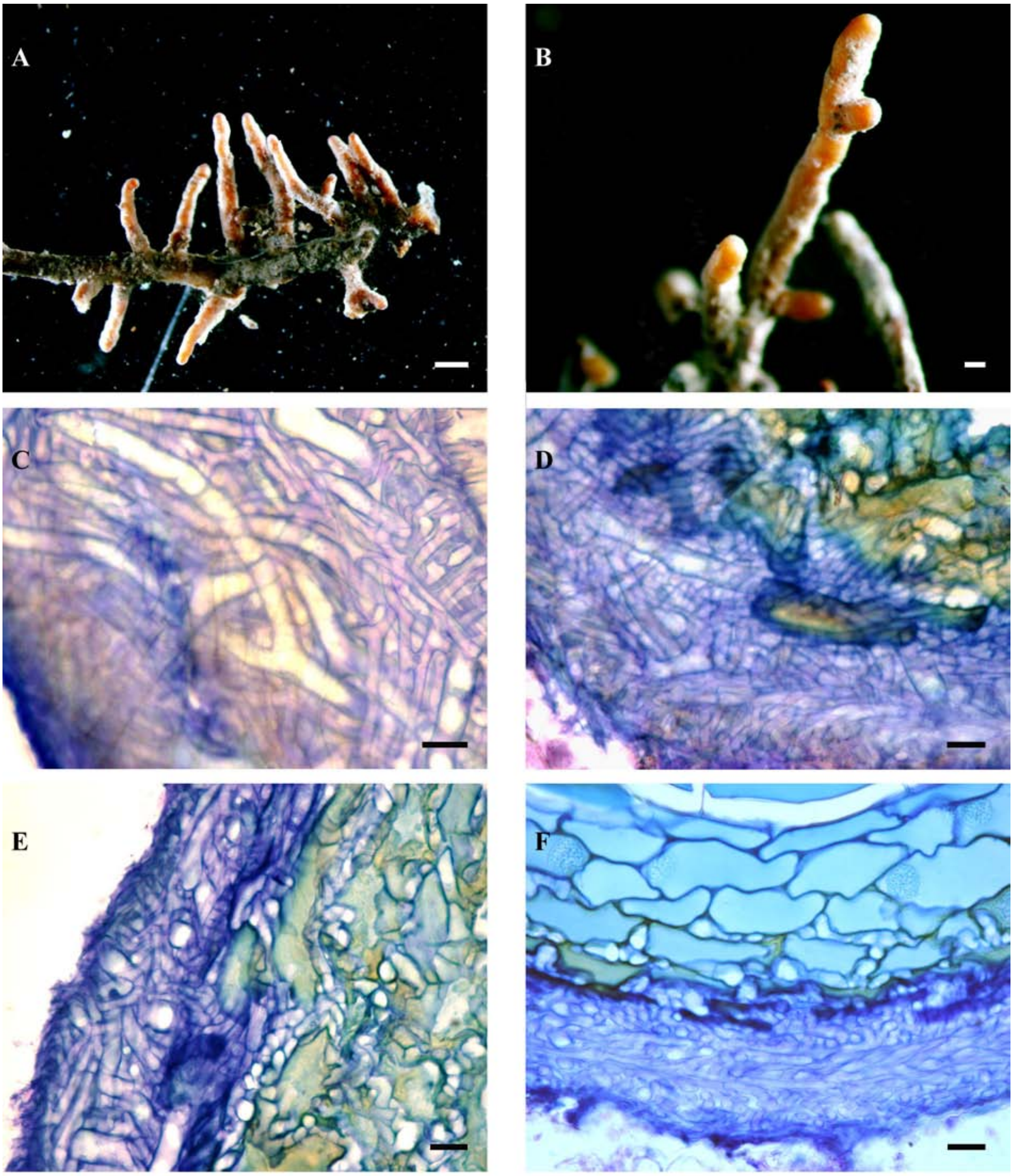

Fig. 5: *L. salmonicolor* ECM **A.** Ectomycorrhizal system, small parts of the root are ectomycorrhizal (scale bar = 1 mm); **B.** Detail of a root tip (scale bar = 0.5 mm); **C.** Plan view of the inner mantle layer showing very wide lactifers (scale bar = 10 µm); **D.** Detail of the mantle of the very tip in longitudinal section (scale bar = 10 µm); **E.** Longitudinal section through the plectenchymatous mantle (scale bar = 10 µm); **F.** Cross-section (scale bar = 10 µm).

3.6. *Lactarius semisanguifluus* R. Heim & Leclair

+ *Pinus nigra* Arn.

Fig. 6

***Morphological characters* (Fig. 6 A-B):** *Mycorrhizal systems* simple to irregularly branching, up to 9 mm long, not arranged in dense clusters. – *Main axes* 0.4–0.6 mm diam, 4–9 mm long. – *Unramified ends* straight or curved, cylindric to slightly irregularly cylindric, short, mostly 1–2 mm long; colour orange (6B6/7), not transparent; very tips paler and somewhat transparent; lactifers visible through the outer mantle; mantle exuding orange latex when injured. – *Surface of unramified ends* smooth, becoming slightly granular, no emanating hyphae present, root tissues not shining through. – *Rhizomorphs* not observed.

***Anatomical characters of the mantle in plan view* (Fig. 6 C-D):** *Outer mantle layers* plectenchymatous, hyphae irregularly and densely arranged, no gelatinous matrix present (mantle type B according to AGERER, 1991); hyphae often branching, 2–3 µm diam., thin-walled; colour pale yellowish; lactifers very scarce. – *Middle mantle layers* plectenchymatous, hyphae mainly arranged parallel to the root axis, less dense than the outer mantle layer, no clear gelatinous matrix present (mantle type B according to AGERER, 1991); hyphae up to 6.5 µm diam., thin-walled; colour yellowish; lactifers mostly straight, branching, up to 15 µm broad. – *Inner mantle layers* plectenchymatous to almost pseudoparechymatous, with very densely arranged hyphae, 3–5.5 µm diam., lactifers present but scarce. – *Very tips* plectenchymatous, hyphae sometimes arranged in a net-like structure, rather widely spaced; hyphae thin-walled, up to 4.5 µm diam.; lactifers scarce.

***Anatomical characters, longitudinal section* (Fig. 6 E):** *Mantle* up to 50 µm thick, different layers hardly discernable, densely plectenchymatous, no clear gelatinous matrix present; outermost hyphae densely arranged, 2–3 µm diam., lactifers lacking; middle layer with more widely spaced and wider hyphae, up to 6.5 µm diam.; lactifers rather abundant to rather scarce, up to 15 µm broad; innermost layer locally almost pseudoparenchymatous; hyphae very densely arranged and up to 4 µm diam., lactifers rather scarce; mantle of the very tip up to 60 µm thick; lactifers scarce; no clear calyptra remnants visible. – *Tannin cells* arranged in 1 (2) rows, thin and often collapsed, irregularly shaped, tangentially elongated, 20–70 µm tangentially, 4–9 µm radially. – *Cortical cells* rounded to irregularly shaped, 30–70 µm tangentially, 15–50 µm radially. – *Hartig net* in section surrounding 2–3 of the 5–6 rows of cortical cells, forming one row of rounded cells, 2–4.5 µm diam.; Hartig net in plan view forming palmetti-like lobes.

***Anatomical characters, cross-section* (Fig. 6 F):** *Mantle* different layers almost discernable, entirely plectenchymatous but outer layer more densely and inner layer almost pseudoparenchymatous, no clear gelatinous matrix visible. – *Tannin cells* arranged in 1–2 rows, 25–30 × 4–20 µm . – *Cortical cells* rounded to square, 15–50 × 15–35 µm.

***Examined specimen*:** Italy, Chiarino (Passo delle Capannelle), N42°30.376' E013°24.679', 981 m a.s.l., small *Pinus nigra* stand on slope with deciduous trees, 29.10.2001, J. Nuytinck 2001-092 (GENT).

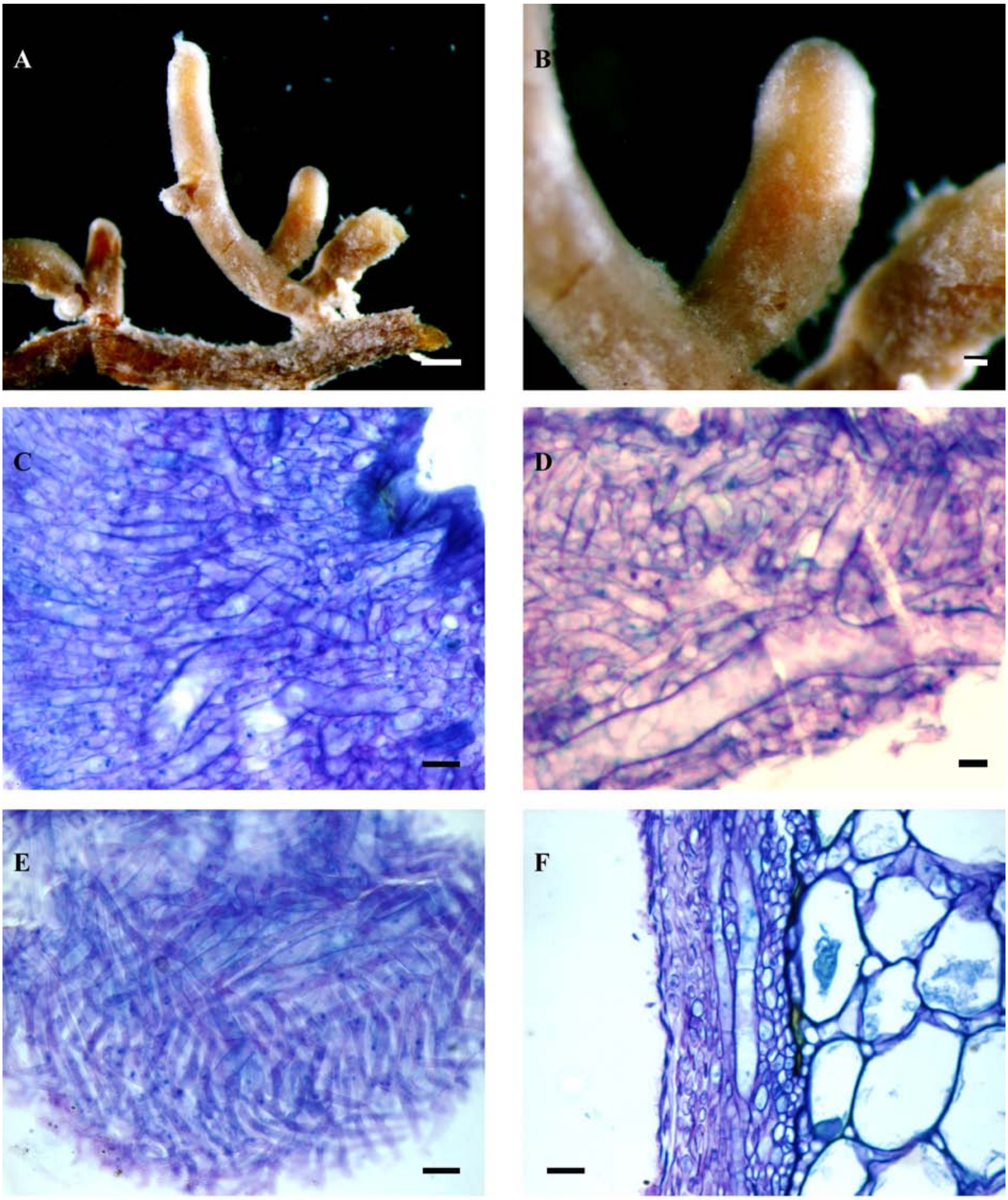

Fig. 6: *L. semisanguifluus* ECM **A.** Irregularly branching ectomycorrhizal tip (scale bar = 0.5 mm); **B.** Detail of an unramified end (scale bar = 0.1 mm); **C.** Plan view of the outer to middle mantle (scale bar = 10 µm); **D.** Plan view of the middle to inner mantle (scale bar = 10 µm); **E.** Longitudinal section of the mantle at the very tip (scale bar = 10 µm); **F.** Cross-section (scale bar = 10 µm).

4. Discussion

Both molecular and morphological approaches are currently being used to describe ectomycorrhizal communities. In many cases, a combined approach employs morphological sorting of the ectomycorrhizal root tips with molecular identification using RFLP analysis or sequencing of the nrDNA ITS region. In most studies involving molecular identification there is a poor correspondence between fungi that appear dominant as sporocarps and those that appear dominant on ectomycorrhizal roots; the species richness belowground is higher than the one found above ground (HORTON & BRUNS, 2001). As a consequence, many fungi from root tip samples remain unidentified (HORTON, 2002). Ectomycorrhizal root tips are less ephemeral structures than basidiocarps and although their turnover might be considerable, it is reasonable to assume that their species composition is more stable than that of sporocarps (KÅRÉN, 1997). Although morphological descriptions of ectomycorrhizal roots have provided useful data for identifying the fungi below ground (AGERER, 1987-2002; GOODMAN *et al.*, 1996-1998; INGLEBY *et al.*, 1990), only relatively few species have been described by this method. Sporocarp surveys and morphological descriptions continue to lay a solid foundation from which ectomycorrhizal fungal diversity can be assessed and this foundation increases the utility of molecular techniques in studies of ectomycorrhizal fungal diversity and ecology (HORTON, 2002). In addition, SAKAKIBARA *et al.* (2002) conclude that the detailed morphological classification of root tips is very useful as the primary method of ectomycorrhizal classification, when used in conjunction with molecular techniques.

In this chapter, we describe the ectomycorrhizae of six out of ten European species in *Lactarius* sect. *Deliciosi*. All identifications were verified by comparing the ITS sequence of the ectomycorrhizae with those of the basidiocarps growing nearby. ITS sequences of ectomycorrhizae and basidiocarps were identical in four cases, but differed up to 4 bp in *L. quieticolor* and *L. porninsis*. This difference however, falls within the intraspecific and even intragenomic variation observed in species in *Lactarius* sect *Deliciosi* (see chapter 4).

Lactarius ectomycorrhizae most typically have a smooth mantle, just like the ectomycorrhizae formed by *Russula* spp., but other surface types also occur (AGERER, 1991; EBERHARDT, 2000; TREU, 1990). They consist of hyphae without clamp-connections that have cell walls without surface ornamentation. Emanating hyphae are generally rare and inconspicuous. Rhizomorphs, especially highly differentiated ones, are more frequently observed in *Lactarius* species than in *Russula* (EBERHARDT, 2000). The most important distinguishing feature between *Lactarius* and *Russula* ectomycorrhizae is the presence or absence respectively of lactifers. This provisional distinction however needs to be confirmed as the ectomycorrhizae of more *Lactarius* and *Russula* species are described. In addition, the mantle of *Lactarius* mycorrhizae often exudes a small amount of latex upon injury.

Several features of ectomycorrhizae are influenced by the host tree. The shape and ramification pattern are in the first place determined by the host tree genus but can also be influenced by the fungal symbiont (AGERER, 1991). *Pinus* ectomycorrhizae for example are widely known to be dichotomously forked and repeated ramification can result in dense clusters or coralloid ectomycorrhizal systems (AGERER, 1991). Monopodial systems are common in most tree genera. The branching pattern of the *L. semisanguifluus* + *Pinus* ectomycorrhizae described here seems deviating in that view but too little material was available to be sure. Anatomical features that are mainly determined by the host are the deepness of the Hartig net, the presence of tannin cells and the shape of the cortical cells in longitudinal sections. These features allow a clear distinction between Angiosperm and Gymnosperm hosts.

Within *Lactarius* subgenus *Piperites* ectomycorrhizae with an entirely plectenchymatous mantle are common but ectomycorrhizae with a partially pseudoparenchymatous mantle also occur. Two clearly defined and different mantle types can be recognised: several species, divided over three different sections are characterised by a maze- or labyrinth-like plectenchymatous mantle, while species in

Lactarius sect. *Deliciosi*, including *L. porninsis*, posses a typical entirely plectenchymatous mantle (EBERHARDT, 2000).

Most species in *Lactarius* sect. *Deliciosi* have several other features in common, such as the generally orange to orange-brown coloured mantle and the colour change to green upon bruising or in age. Lactifers generally occur most abundantly in the middle mantle layers. The lactifers are often nicely visible through the outer mantle layer but mostly rather rare in anatomical preparations. Rhizomorphs seem to be common in *Lactarius* sect. *Deliciosi*.

The colours of the basidiocarps and their ectomycorrhiza are not always identical or even similar. Striking is that the ectomycorrhizae formed by *L. salmonicolor* show a strong greenish discolouration while this is almost absent in the basidiocarps. Moreover, *L. indigo,* with its blue coloured basidiocarps, also forms orange coloured ectomycorrhizae (FLORES *et al.*, 2002).

Most authors stress the similarity of the ectomycorrhizae formed by different members of *Lactarius* sect. *Deliciosi* (e.g. KERNAGHAN *et al.*, 1997; YAMADA *et al.*, 2001b). UHL (1988) compares the ectomycorrhizae of *L. deliciosus* and *L. deterrimus* and finds only significant differences in the reaction to certain reagentia (formaldehyde and $FeSO_4$). FLORES *et al.* (2002) describe and compare the artificially synthesised ectomycorrhizae of *L. deliciosus* (strain collected in Spain) and *L. indigo* (several strains collected in Guatemala) with both European and Mesoamerican pine species. Mycorrhizae of both species were found to be very similar but could be differentiated by their final colour when old, which was green in *L. indigo* but rusty tawny in *L. deliciosus*. Slight differences in the thickness and deepness of the Hartig net between the cortex cells depended on the used *L. indigo* strain. HUANG (1996) who describes and compares the ectomycorrhizae of *L. deliciosus* and *L. hatudake* collected in China also finds that they are very similar; only the colour of the very tips differs slightly and the colour of the exuded latex is orange in *L. deliciosus* while it is red in *L. hatsudake*, similarly to the basidiocarps. The hyphae with clamp connections that are illustrated in HUANG (1996) are most probably due to contamination.

When comparing the ectomycorrhizae described here, the similarity is indeed striking. Macroscopically *L. porninsis* has a more brownish coloured mantle surface and *L. quieticolor* a more buff-orange colour while the other species have a characteristic bright orange coloured mantle. Furthermore *L. porninsis* possesses white latex and does not show any greenish discolouration. The structure of the mantle is entirely plectenchymatous in all described species but, due to the very densely arranged hyphae, seemed locally almost pseudoparenchymatic in *L. deliciosus* and *L. semisanguifluus*. DANIELSON (1984) describes *L. paradoxus* with a pseudoparenchymatous-epidermoid outer mantle layer. This would mean *L. paradoxus* forms an exception in this for the rest very homogeneous section. As indicated by EBERHARDT (2000), this observation should be treated with extreme care since the same author (in BRADBURY *et al.*, 1998) also describes the mantle of "*L. deliciosus*" as epidermoid while all other authors (KERNAGHAN *et al.*, 1997; UHL, 1988; this study) describe it as plectenchymatic[*].

The hyphae in *L. salmonicolor* show a stronger tendency to form hyphal protrusions than the other species; these protrusions are also described and illustrated in PILLUKAT (1996a,b). We never observed a very clear interhyphal gelatinous matrix in this study although it is reported to be present in several species in *Lactarius* sect. *Deliciosi* (*L. akahatsu*, *L. salmonicolor* (only in PILLUKAT, 1996b), *L. pornisis* and *L. deterrimus*). This matrix is explicitly stated to be absent in *L. rubrilacteus* and *L. salmonicolor* (EBERHARDT *et al.*, 2000). The presence or absence of this matrix is probably not a reliable feature for identification, in part because of the difficulties to observe it clearly. Emanating hyphae were sometimes observed but are very rare in this section (KERNAGHAN *et al.*, 1997; MASSICOTTE *et al.*, 1999; YAMADA *et al.*, 2001b). It seems evident however that mycelium is present to help in the uptake of water and mineral nutrients. Emanating hyphae were indeed observed in the in vitro synthesised ectomycorrhizae of *L. akahatsu* and *L. hatsudake* (YAMADA *et al.*, 2001b). The

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[*] Remark: due to the misapplication of the name *L. deliciosus* in North America, see chapter 5, it is not always clear which taxon "*L. deliciosus*" refers to.

mycelium is described as pale ochre to pale ochre-orange, smooth and mat to shiny. Emanating hyphae break off very easily (AGERER, 1986b) and this could happen during cleaning of the tips.

Rhizomorphs are commonly observed on the ectomycorrhizae formed by members of *Lactarius* sect. *Deliciosi*. In only few studies they are mentioned to be absent (e.g. in "*L. deliciosus*" described by ACSAI & LARGENT, 1983 and in MASSICOTTE *et al.*, 1999). Although we did not observe rhizomorphs on the ectomycorrhizae of *L. semisanguifluus*, we expect them to be present when more material will be examined. The rhizomorphs we observed on the five other species belong to rhizomorph type B according to AGERER (1991), meaning that they are undifferentiated (all hyphae are of uniform diameter), have a smooth margin and that the hyphae are rather compactly arranged. Only in *L. deliciosus* rhizomorphs with slightly wider hyphae were observed, but lactifers were lacking. Undifferentiated to slightly differentiated rhizomorphs are also observed in *L. deliciosus* var. *areolatus* (KERNAGHAN *et al.*, 1997), *L. deterrimus* (AGERER, 1986b; MÜNZENBERGER *et al.*, 1986) and *L. deliciosus* (UHL, 1988). Several authors however, do mention strongly differentiated rhizomorphs with centrally large vesicular hyphae (e.g. in *L. deliciosus* and *L. hatsudake*, HUANG (1990, 1996); *L. akahatsu* and *L. hatsudake*, YAMADA *et al.* (2001b) and *L. salmonicolor*, PILLUKAT (1996b)). A possible explanation for these different observations (undifferentiated versus strongly differentiated hyphae) is that rhizomorphs are not always present in their fully developed stage on all ectomycorrhizae. The diameters of the rhizomorphs we observed are also significantly smaller (up to 100 µm) than what is mentioned for e.g. *L. salmonicolor* (up to 300 µm). The structure of the rhizomorphs observed by YAMADA *et al.* (2001b) on *L. akahatsu* differs form the other species in that it has a differentiated surface-layer of hyphae with a distinctly thickened cell wall.

As already concluded by EBERHARDT *et al.* (2000) mycorrhizal morphology and anatomy can be used to distinguish mycorrhizae formed by *Lactarius* species of the different sections from one another. Within sections, morphological differences tend to be slight. The resolution provided by morphological characters is sometimes greater however than that of RFLP analysis (EBERHARDT *et al.*, 2000). It seems impossible to produce an identification key to the ectomycorrhizae of *Lactarius* sect. *Deliciosi* because of the great similarity and the insufficient knowledge of the eventual intraspecific variability of the few slightly differing characters. The strong host specificity and the preference for certain environmental conditions do allow, to a certain extent, the identification of the ectomycorrhizae in this section (EBERHARDT *et al.*, 2000).

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Ornella Comandini and Andrea Rinaldi are thanked for the invitation to visit the lab in L'Aquila and the useful explanation on ectomycorrhizal techniques. Lieselotte Van Laethem is thanked for the help with collecting and describing the ectomycorrhizae.

Chapter 10

Characterization of *Lactarius tesquorum* ectomycorrhizae on *Cistus* sp. and molecular phylogeny of related European *Lactarius* taxa

Adapted from:
NUYTINCK J., VERBEKEN A., RINALDI A.C., LEONARDI M., PACIONI G. & COMANDINI O. (2004) – Characterisation of *Lactarius tesquorum* ectomycorrhizae on *Cistus* sp., and molecular phylogeny of related European *Lactarius* taxa. *Mycologia* **96**: 272-282.

Abstract

Lactarius is one of the larger genera of ectomycorrhizal Basidiomycota, with about 400 species recognized worldwide. The ectomycorrhizae formed by *Lactarius tesquorum* on *Cistus* sp., one of the most common and ecologically relevant shrubs present in the semi-arid regions in the Mediterranean basin, are described here in terms of morphological, anatomical and molecular features. An ITS rDNA sequence-based phylogenetic analysis was performed on the related European *Lactarius* taxa (*L. mairei*, *L. pubescens*, *L. scoticus*, *L. spinosulus*, *L. torminosulus* and *L. torminosus*) currently classified together with *L. tesquorum* in *Lactarius* subgenus *Piperites* section *Piperites*. *Lactarius* sect. *Piperites* s.s. can be divided into two main clusters; *L. mairei* and especially *L. spinosulus* are less closely related to the other taxa. This study is part of a broader efford to at extending our knowledge of the distribution, phylogeny, and ectomycorrhizal biology of *Lactarius* species in selected ecosystems.

1. Introduction

Lactarius is a cosmopolitan, ectomycorrhizal genus of agaricoid basidiomycetes, placed with *Russula*, some gasteroid and hypogeous taxa (e.g. *Zelleromyces*, *Macowanites*, *Arcangeliella*) and some former Aphyllophorales (e.g. *Auriscalpium*, *Bondarzewia*, *Hericium*, *Peniophora*) in the order Russulales. About 400 *Lactarius* species are currently known worldwide, but since some large geographic regions, rich in ectotrophs, remain underexplored, the actual number of species is presumably significantly higher (VERBEKEN, 2001). Members of the genus have been reported in ectomycorrhizal association with numerous trees and shrubs, and their important ecological role as late-stage root colonizers in a range of ectotrophic plant communities is largely appreciated (HUTCHISON, 1999).

Despite a longstanding tradition of morphological taxonomy studies (focusing primarily on European and North American species), significant differences exist among the various classification systems, especially concerning the delimitation between different infrageneric groupings and the placement of certain taxa (BASSO, 1999a; BON, 1980, 1983; HEILMANN-CLAUSEN *et al.*; 1998; HESLER & SMITH, 1979). A comprehensive natural classification of *Lactarius*, supported by molecular phylogenetic analysis, is still lacking. Very recently however, several investigations have been carried out that provided us with useful insight into the phylogeny of some species groups within *Lactarius* and other members of the Russulales (EBERHARDT, 2002; EBERHARDT *et al.*, 2000; HENKEL *et al.*, 2000; MILLER & BUYCK, 2002; MILLER *et al.*, 2001; PETER *et al.*, 2001).

In the past few years, we have initiated a long-term study to extend our knowledge on the distribution, ecology, phylogeny and ectomycorrhizal biology of *Lactarius* species occurring in selected ecosystems in Europe. The main idea is to explore the *Lactarius* ectomycorrhizal diversity of host trees or shrubs that characterize a given ecologically valuable ecosystem. Relevant *Lactarius* ectomycorrhizal types are fully characterized from a morpho-anatomical and molecular point of view, and the anatomical characters of the ectomycorrhiza are compared to those known from related taxa. At the same time, morphological and molecular data obtained for closely related *Lactarius* taxa, living either in the same habitat or linked to other hosts, are used to clarify the systematic position of the concerned species. The combination of morphological characters of sporocarp and mycorrhiza and their molecular features thus contributes to a reliable taxonomy of the genus.

This method has been applied successfully for the *Lactarius* symbionts of silver fir (*Abies alba*), mainly in central Italy, and related *Lactarius* species (COMANDINI *et al.*, 1998; EBERHARDT *et al.*, 2002). Here, we focus on the *Lactarius* associations of *Cistus* spp., a group of about twenty woody, evergreen or semideciduous shrub species found in wide semi-arid areas from the Canary Islands throughout the Mediterranean region to the Caucasus. *Cistus* species are obligate seeding, early colonizers that follow disturbance, particularly fire, in low maquis-type Mediterranean ecosystems, rendering their ectomycorrhizal ecology particularly interesting. In the frame of this research we identified the ectomycorrhizae formed by *Lactarius tesquorum* Malençon on *Cistus* sp. In addition, an ITS rDNA sequence-based phylogenetic analysis of the related European *Lactarius* taxa currently classified together with *L. tesquorum* in *Lactarius* subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman sect. *Piperites* (Fr. ex J. Kickx f.) Burl., was carried out to clarify relationships at species-level and the infrageneric classification. Species in *Lactarius* section *Piperites* are characterized by a sticky pileus with a tomentose, hairy or bearded margin, white and unchanging milk (in contrast to the yellowing milk of the species in *Lactarius* subsect. *Scrobiculati* Hesler & A.H. Sm., which are placed in the same section by HESLER & SMITH (1979) and BASSO (1999a)), scattered to numerous macrocystidia, an ixocutis-structure of the pileipellis and mostly subreticulate spores ornamented with rounded ridges. Described on the basis of material originating from Morocco (MALENÇON, 1979), *L. tesquorum* was subsequently recognized as a commonly occurring species in maquis-type habitats of many Mediterranean countries (CONTU, 1986; LALLI & PACIONI, 1988).

In addition to *L. tesquorum*, the section includes six more, generally accepted European taxa. Two well-known, common birch-associated species that are widely distributed in Europe, *L. torminosus* (Schaeff.: Fr.) Pers. and *L. pubescens* Fr. belong to this section. *Lactarius scoticus* Berk. & Broome is a small mimic of *L. pubescens*, growing in arctic-alpine birch vegetations (for a detailed comparison between *L. pubescens* and *L. scoticus* – as *L. favrei* H. Jahn – see JAHN, 1982). KNUDSEN & BORGEN (1994) described *L. torminosulus* Knudsen & T. Borgen, a dwarf version of *L. torminosus* and an arctic species associated with *Betula nana* or *B. glandulosa. Lactarius mairei* Malençon differs from the other representatives by the association with *Quercus*. *Lactarius spinosulus* Quél., by most modern authors placed in *Lactarius* section *Colorati* (Bataille) Hesler & A.H. Sm. because of the resemblance to *L. lilacinus* (Lasch: Fr.) Fr., has been placed in *Lactarius* sect. *Piperites* by NEUHOFF (1956), HEILMANN-CLAUSEN *et al.* (1998) and BASSO (1999a). It shares most characteristics with *L. mairei* (spore ornamentation and squamulose, zonated pileus) and shares the host tree, *Betula*, with most other *Piperites*. PEARSON (1950) furthermore described a variety of *L. mairei*, *L. mairei* var. *zonatus* A. Pearson, differing from the type variety by a brighter colour, a distinctly zonated pileus and smaller spores. Another variety, *L. mairei* var. *ilicis* Lalli & Pacioni, was proposed by LALLI & PACIONI (1988) and differs by the smaller size and the more gracile habitus, a shorter stipe and shorter hairs on the pileus. The same authors described *L. tesquorum* var. *splendidus* Lalli & Pacioni characterized by a vividly orange, zonated pileus and growing with *Quercus ilex*.

2. Materials & Methods

2.1. Fungal collections

Table 1: Collection data and GenBank accession numbers of the taxa studied. Vouchers: GENT, University of Ghent; AQUI, University of L'Aquila; SIENA, University of Siena. Abbreviations: ECM, ectomycorrhiza; ns, not sequenced; AV, A. Verbeken; LVL, L. Van Laethem; RW, R. Walleyn.

Species	var.	Collection data		GenBank accession number
		Date	Origin/source (voucher)	
subgenus *Piperites*				
sect. *Piperites*				
L. mairei		04/07/1986	Italy, Roio, 86/8 (AQUI)	ns
L. mairei		01/11/2000	Sardinia, Tempio Pausania, AV 2000-105 (GENT)	AY336952
L. mairei		03/11/2000	Sardinia, Calangianus, AV 2000-118 (GENT)	AY336950
L. mairei	*ilicis*	02/11/1980	Italy, Garfa, 367 (AQUI)	ns
L. mairei	*ilicis*	01/11/1998	Italy, Arbuzzo, (AQUI)	ns
L. mairei	*ilicis*	19/11/2000	Italy, Volterra, 5525 (SIENA)	ns
L. mairei	*ilicis*	24/11/2001	Italy, Murlo, 5526 (SIENA)	AY336951
L. pubescens		16/09/1996	Norway, Nannenstad, AV 96-931 (GENT)	AY336958
L. scoticus		21/08/1999	Sweden, Borgsjö, RW 1579bis (GENT)	AY336956
L. scoticus		26/08/2001	Sweden, Borgsjö, RW 2084 (GENT)	AY336957
L. spinosulus		04/10/1998	France, Fôret de Dorans, AV 98-05 (GENT)	ns
L. spinosulus		25/08/2001	Sweden, Borgsjö, RW 2070 (GENT)	AY336961
L. spinosulus		23/09/2002	Belgium, Waulsort, LVL 2002-016 (GENT)	AY336960
L. tesquorum		09/12/2001	Sardinia, Is Cannoneris, 2001-15 (AQUI)	AY336955
L. tesquorum ECM		09/12/2001	Sardinia, Is Cannoneris, 2001-15M (AQUI)	
L. tesquorum	*splendidus*	09/10/1979	Italy, Circeo, 235 (AQUI)	ns
L. torminosulus		27/08/1999	Sweden, Borgsjö, RW 1607 (GENT)	AY336953
L. torminosulus		28/08/2001	Sweden, Rotnåset, RW 2129 (GENT)	AY336954
L. torminosus		26/08/2001	Sweden, Borgsjö, RW 2074 (GENT)	ns
L. torminosus		23/09/2002	Belgium, Waulsort, LVL 2002-013 (GENT)	AY336959
sect. *Zonarii*				
L. scrobiculatus		-	-	AF140262
L. intermedius		-	-	AF140256
subgenus *Russularia*				
L. hepaticus		-	-	AF096989
L. quietus		-	-	AJ272247
outgroup				
Russula brevipes		-	-	AF349714

The collections used are indicated, with herbarium number and place of origin, in Table 1. All collections are deposited in the herbaria of the universities of Ghent (GENT), L'Aquila (AQUI) and Siena (SIENA). Sporocarps and ectomycorrhizae of *L. tesquorum* were harvested at a single site in Sardinia, in the territory of Domus de Maria (Cagliari, Italy), about 250 m above sea level. Collected fungal specimens were found under *Cistus* spp. in a maquis-type vegetation growing along roads. Several *Cistus* species (*C. monspeliensis*, *C. salvaefolius*, *C. incanus*) were present densely packed on the collection spot, so that it was not possible to identify the host(s) of *L. tesquorum* at species level. Soil cores were excavated from beneath sporocarps and immersed overnight in water, and ectomycorrhizal roots were carefully separated under a dissecting microscope. Several tips were immediately transferred to 50% EtOH and stored at - 20°C for DNA analysis. The general methodology and terminology used to characterize ectomycorrhizae followed AGERER (1986a, 1987-2002, 1991). Munsell Soil Color Charts (1975) were used as reference for the descriptions of the colours of the ectomycorrhizae. Voucher specimens of mycorrhizae are deposited in AQUI as dried and fixed/preserved material (4% glutaraldehyde or EtOH 50%) respectively, together with slides.

2.2. Microscopy

Mantle preparations of fresh ectomycorrhizae were fixed on slides with polyvinyl lactophenol for both light and confocal laser scanning microscopy (CLSM). For light microscopy, observations were made with a Zeiss Axioplan 2 microscope and photographs were taken with Ilford Panf Plus 50 film. For longitudinal sections (2.5 µm thick), ectomycorrhizae were embedded in LR White resin (Multilab), cut with a Leica Ultracut R ultramicrotome and stained with toluidine blue in 1% sodium borate for 15 s at 60°C. CLSM observations were made using a Molecular Dynamics Sarastro 2000 microscope equipped with a Nikon Optiphot fluorescence microscope (Planapo 60/1.4 lens) and an Argon gas laser (25 mW). Laser excitation wavelength was fixed at 488 nm.

2.3. Molecular analysis

DNA preparation

DNA was extracted by a CTAB procedure (DOYLE & DOYLE, 1990), modified as follows. Dried material was first ground in liquid N_2 (about 50 mg). One ml extraction buffer (0.1 M Tris-HCl pH 8, 0.5 M NaCl, 0.05 M EDTA and 0.01 M β-mercapto-ethanol) and 50 µl 10% SDS were added and the mixture was incubated for 30 min. at 65°C. This extract was centrifuged for 10 min. at 14000 rpm (Eppendorf centifuge). An equal volume of isopropanol was added to the supernatant and, after mixing the solution, it was centrifuged again as above. The pellet was washed with 70% ethanol, air dried and dissolved in 400 µl dH_2O. Then 400 µl CTAB buffer (2% w/v CTAB, 0.2 M Tris-HCl pH=7.5, 2 M NaCl and 0.05 M EDTA) was added and after incubation for 15 min. at 65°C, the mixture was extracted twice with chloroform/iso-amylalcohol (24:1). Two volumes of 96% ethanol were added and the mixture was centrifuged again as above to pellet the DNA. The pellet was washed with 70% ethanol and dissolved in 100 µl TE (10mM Tris-HCl, pH 8 and 0.1 mM EDTA).

PCR and direct sequencing of the ITS region

For the PCR reaction primers ITS1-F and ITS4-B (GARDES & BRUNS, 1993) were used. The following touchdown PCR profile was used: 95°C during 2 min., then 10 cycles of denaturation at 95°C (during 15 s), primer annealing at 63°C (during 20 s) with the temperature decreasing with 1°C at each cycle, polymerisation during 1 min. at 72°C; followed by 35 cycles with the same profile for denaturation and polymerisation but with a constant annealing temperature of 53°C; polymerisation was completed by an incubation of 2 min. at 72°C. The PCR products were purified with ExoSAP (USB, USA). DNA sequencing reactions were performed with the ABI PRISM® BigDye^{TM} Terminators v3.0 Cycle Sequencing Kit using primers ITS1-F, ITS2, ITS3 and ITS4-B (GARDES & BRUNS, 1993; WHITE *et al.*, 1990) on an ABI PRISM® 377 DNA Sequencer. The Phred and Phrap Software (EWING *et al.*, 1998; EWING & GREEN, 1998) was used to process raw data.

Data analysis

ITS sequences were aligned in ClustalX (THOMPSON *et al.*, 1997) and the alignment was manually corrected where necessary. The alignment was deposited in TreeBASE (ID-number SN1521-4590). Phylogenetic analysis of the ITS sequences was performed with PAUP*4b10 (SWOFFORD, 2002). A maximum parsimony analysis was performed with all sites treated as unordered and unweighted and with gaps treated as missing. For tree searching the branch and bound algorithm was used. Support for the internodes of the most parsimonious trees was estimated by 10000 bootstrap replicates with a heuristic search with 10 random-addition sequences for each replicate. Sequences were deposited in GenBank (Table 1).

3. Results

3.1. Descriptions of ectomycorrhizae

Morphological characters

Mycorrhizal systems simple or monopodial-pinnate, (1.3) 1.5–2.7 (3.2) mm long and 0.3 to 0.4 mm diam. Unramified ends straight to bent, (0.4) 0.7–1.2 (1.3) mm long and 0.2–0.3 mm diam. Surface of unramified ends smooth, rather transparent; cortical cells often visible, frequently covered with soil particles. Colour pale yellow (2.5YR 7/4) to brownish-yellow (10YR 6/6); very tips with the same colour or sometimes with slightly darker tones; ageing mycorrhizae dark yellowish brown (10YR 4/6) to dark brown (10YR 4/3). Emanating hyphae not observed. Rhizomorphs rare, 30–70 µm diam., sometimes ramified, connected to the mantle at restricted points, mainly at the base, with smooth margins, concolorous with the mantle. Mantle secretes white latex when injured.

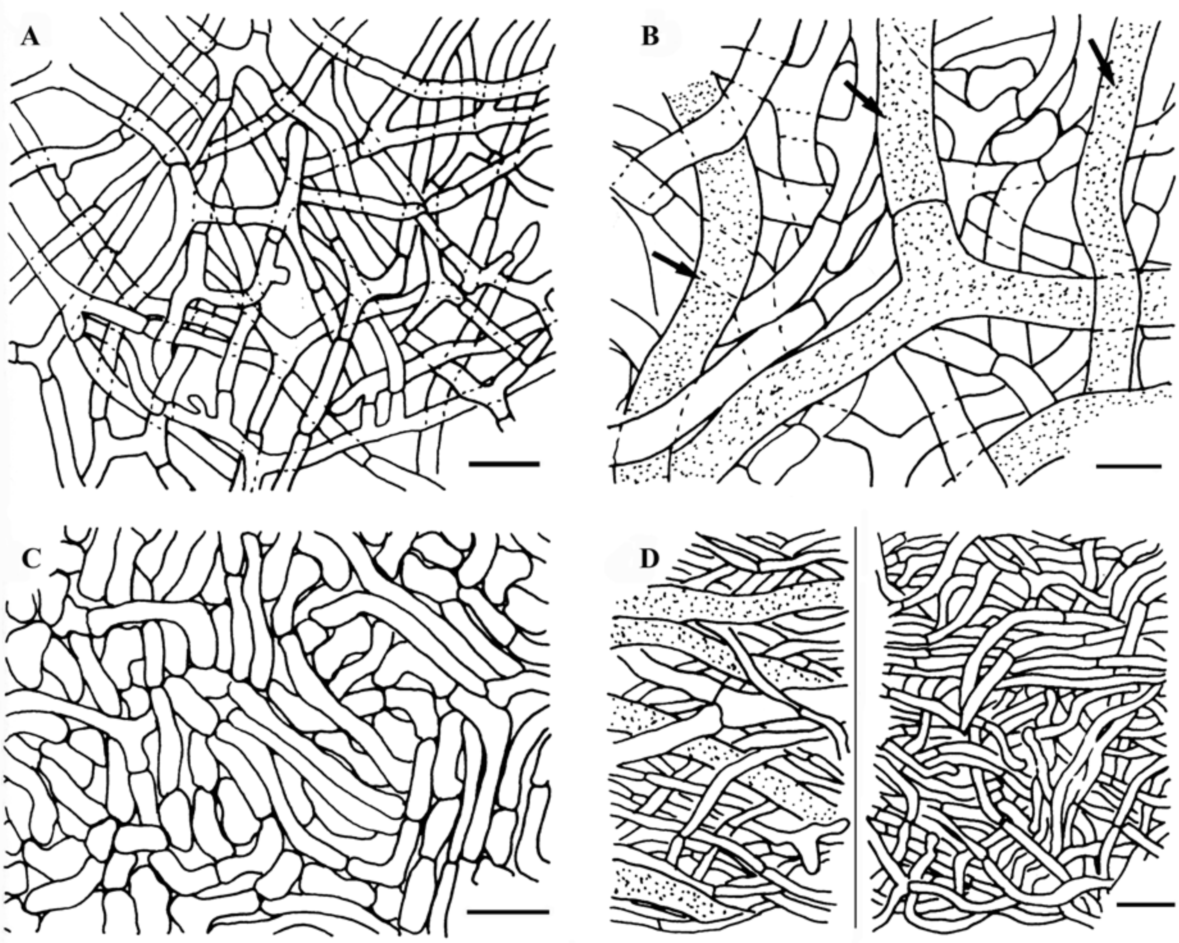

Fig. 1: Anatomical characters of *L. tesquorum* ectomycorrhizae. **A.** Outer mantle layer with a plectenchymatous structure formed by a loose net of hyphae; **B.** Middle mantle layer with a densely plectenchymatous to nearly pseudoparenchymatous structure and abundant lactifers (arrows); **C.** Inner mantle layer with a densely plectenchymatous structure; **D.** Surface view of thicker rhizomorphs, showing losely woven hyphae (right side) and surface view of the middle layer of rhizomorphs, with a highly differentiated structure (left side). Scale bars = 10 µm.

Anatomical characters of mantle in plan views

Very outer mantle layers plectenchymatous (Figs. 1A, 2a), formed by a loosening net of hyphae, 2–2.5 (3) µm diam., hyaline, clampless, frequently branched, sometimes straight, sometimes wavy, frequently septated, elbow-like protrusions present. Middle mantle layers densely plectenchymatous to nearly pseudoparenchymatous (Figs. 1B, 2b), hyphae arranged without special pattern, 4–5 µm diam.; laticifers abundant, 5–8 µm diam., thin walled, frequently branched, septated. Inner mantle layers densely plectenchymatous to nearly pseudoparenchymatous (Figs. 1C, 2c), in some parts hyphal cells 8–10 µm long and 5–7 µm diam., in others hyphae densely arranged and 3–4 µm diam. Very tips organized like other parts of the mantle.

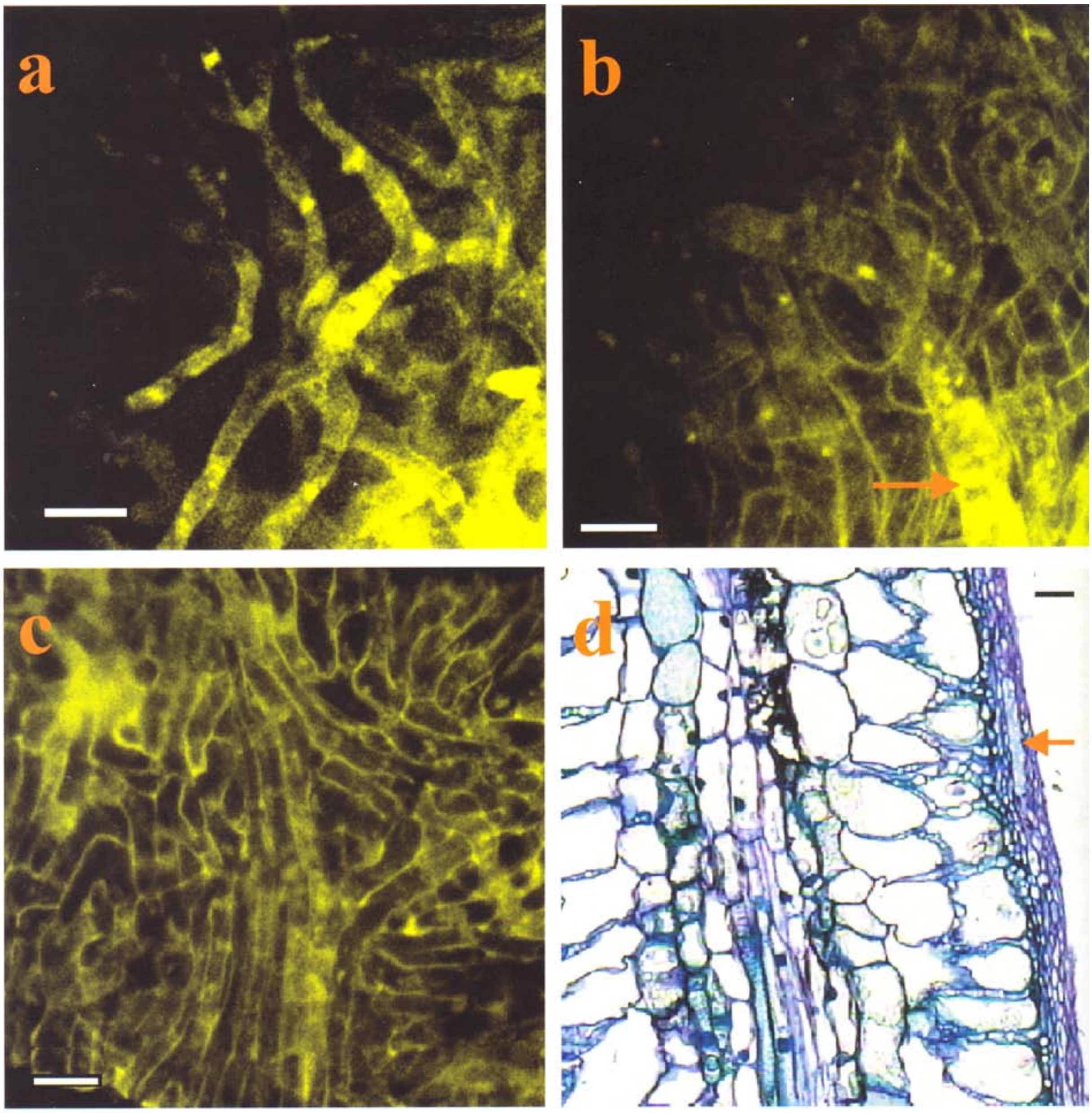

Fig. 2: Anatomical characters of *L. tesquorum* ectomycorrhizae. **a.** Outer mantle layer viewed with CLSM; bright fluorescent spots on the hyphae are due to latex spilled from lactifers during preparation of the mantle; **b.** Middle mantle layer viewed with CLSM; arrow indicates a lactifer containing brightly fluorescent latex remnants; **c.** Inner mantle layer viewed with CLSM; **d.** Longitudinal section viewed with an optical microscope; arrow indicates a lactifer in the middle part of the mantle. Scale bars = 10 µm.

Anatomical characters of emanating hyphae

Rhizomorphs highly differentiated with vessel-like central hyphae [rhizomorph type F according to AGERER (1987-2002)]; vessel-like hyphae 5–8 µm diam., cell walls less than 1 µm thick, very long, septated, distance between septa (17) 22–40 (45) µm, frequently filled with abundant whitish content (like laticifers) (Fig. 1D, left side); peripheral hyphae 2.5–3 µm diam., thin-walled, similar to those of the outer mantle, rather loosely interwoven in the thicker parts of rhizomorphs (Fig. 1D, right side). Thinner rhizomorphs only slightly differentiated, with more compactly arranged hyphae.

Anatomical characters, longitudinal section

Mantle 10–15 µm thick, densely plectenchymatous, with very long laticifers, 5–8 µm thick, in the middle part (Fig. 2d); no differentiation into different layers; hyphae 4–10 µm long and (1) 2–5 µm thick. Tannin cells mostly thin, collapsed, 3–4 µm thick. Cortical cells in 2 (3) rows, generally radially rectangular or with an irregular shape, 35–47 µm radially, 13–22 µm tangentially. Hartig net surrounds 1 (2) rows of cortical cells, forming palmetti-like lobes of 2–2.5 µm in diam. in plan view, and 1 row of rectangular, 1.5–3 µm thick cells in section.

3.2. DNA analysis

The ITS sequences of the sporocarps of *L. tesquorum* from Sardinia and of the ectomycorrhizae found beneath them were identical, thus confirming the identity of the collected mycorrhizal structures.

The sequence alignment contained 728 characters of which 146 variable basepairs were parsimony-uninformative and 72 were parsimony-informative. *Russula brevipes* Peck (AF349714) was chosen as outgroup. *Lactarius hepaticus* Plowr. (AF096989) and *L. quietus* (Fr.: Fr.) Fr. (AJ272247) from *Lactarius* subgenus *Russularia* (Fr. ex Burl.) Kauffman sect. *Russularia* Fr. ex Burl. (nom. illeg.) were included in the alignment and two *Lactarius* species belonging to *Lactarius* section *Zonarii* (Quél.) Bon (nom. inval.) subsect. *Scrobiculati*, namely *L. scrobiculatus* (Scop.: Fr.) Fr. (AF140262) and *L. intermedius* (Krombh. →) Berk. & Broome (AF140256), were included to assess the relationships of this group with *Lactarius* sect. *Piperites*. An ITS sequence from *L. tesquorum* was available from GenBank (AF096986) and added to the phylogenetic analysis. The maximum parsimony analysis of the ITS sequences resulted in three shortest trees (Fig. 3) with a length of 301 steps, CI 0.82, RI 0.73 and RC 0.60. These trees differ only in the arrangement of the species within the clade of *L. tesquorum*, *L. scoticus* and *L. pubescens*. Main observations from this phylogenetic analysis are: (i) *Lactarius* sect. *Piperites* is probably not monophyletic (bootstrap < 50%); (ii) the majority of species fall into two well-supported groups (bootstraps 83% and 97%): on one side *L. torminosus* and *L. torminosulus* (clade A in Fig. 3) and on the other side *L. tesquorum*, *L. scoticus* and *L. pubescens* (clade B in Fig. 3). They group together with a bootstrap support of 83%; (iii) *L. mairei* and *L. spinosulus* seem more distantly related to this core group of *Piperites*; (iv) the species belonging to *Lactarius* subsect. *Scrobiculati* included in the analysis clearly form a distinct group (supported by a bootstrap value of 89%) separated from *Lactarius* sect. *Piperites*.

3.3. Morphological analysis of *L. mairei* and *L. tesquorum*

As mentioned above, LALLI & PACIONI (1988) described a variety of *L. tesquorum*, *L. tesquorum* var. *splendidus*, from central Italy and reported its association with *Quercus ilex*. The same authors also recognized three varieties of *L. mairei*, one of which (*L. mairei* var. *ilicis*) associated with *Q. ilex* and occurring sympatrically with *L. tesquorum* var. *splendidus* (LALLI & PACIONI, 1988). The type material of both *L. tesquorum* var. *splendidus* and *L. mairei* var. *ilicis*, collected in 1979 and 1980 respectively, is conserved at AQUI. An attempt to amplify the ITS region from the DNA extracted from these samples failed; this confirms the difficulties encountered by other researchers (Eberhardt, pers. comm.) trying to analyze the DNA of relatively old *Lactarius* herbarium samples. More recent collections of *L. mairei* var. *ilicis* (from AQUI and SIENA, see Table 1), described morphologically by ANTONINI & ANTONINI (2002), were included in our molecular analysis. We combined morphological and molecular data to get insights on the status of these taxa. The most important spore characters are illustrated in Fig. 4. In addition to the size and the shape of the spores, important features can be observed in the amyloidity of the plage and in the type of ornamentation: the height of the ornamentation, the composing elements (warts/ridges/spines) and the degree of reticulation. Fig. 4 C shows spores that are distinctly more elongate and narrower than Fig. 4 A, B and D. The ornamentation in Fig. 4 C is almost completely reticulate and composed of rather low and regular ridges, while the ornamentation in Fig. 4 B and D consists of abundant, isolated, short ridges and warts and shows a spiny aspect because of the irregular height. Fig. 4 A also shows a spiny aspect, but the ridges are much more connected and thus forming a more reticulate ornamentation than in Fig. 4 B and D. In all spores, the plage is inamyloid or at most very slightly distally amyloid.

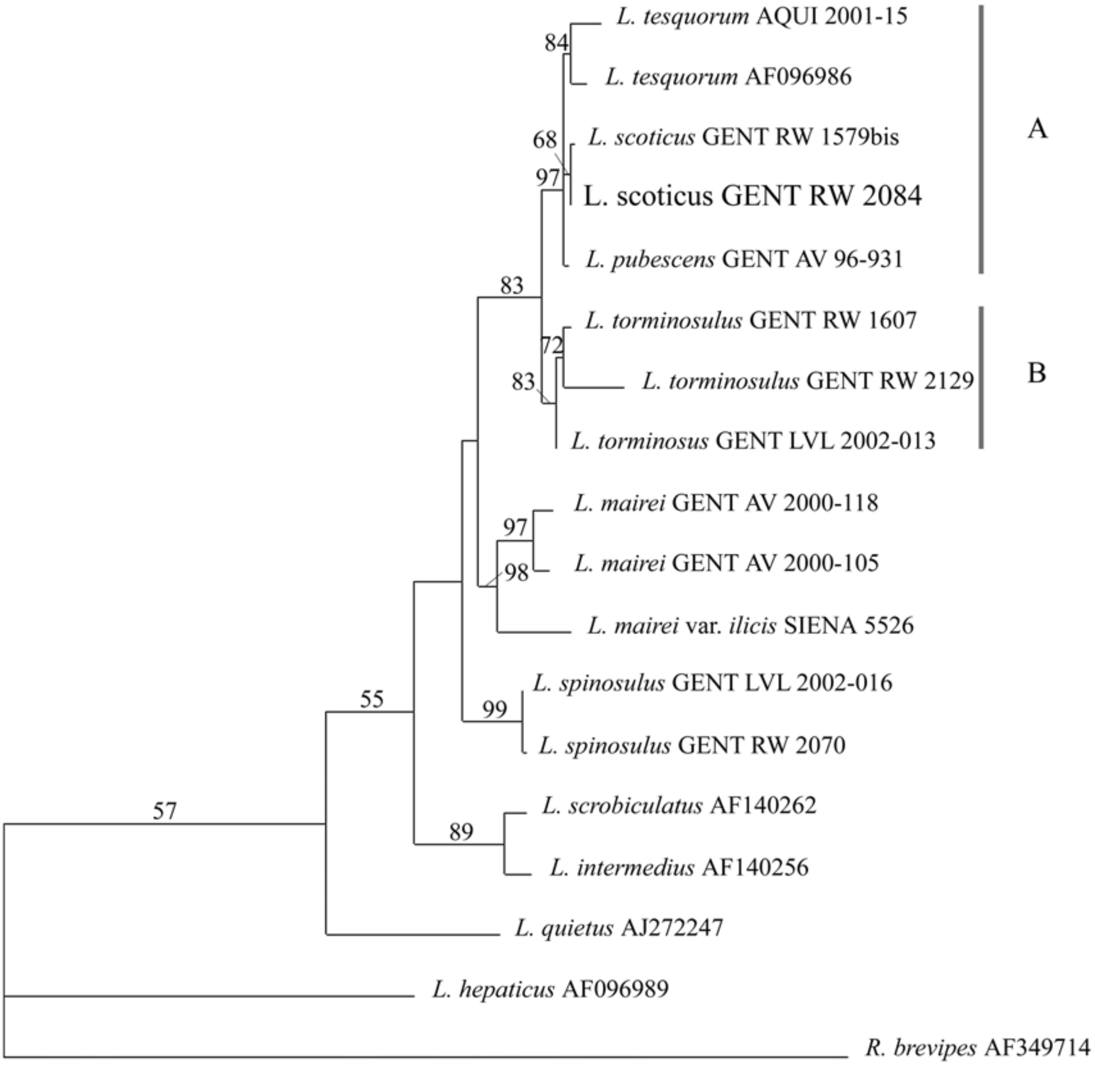

Fig. 3: One of three shortest trees (301 steps) resulting from maximum parsimony analysis of the ITS sequences. Bootstrap values higher than 50% are indicated above the branches.

4. Discussion

In spite of the ecological importance of *Cistus* spp. in Mediterranean-type ecosystems and the fairly large number of ectomycorrhizal macromycetes known (see for example CONTU, 1984; MALLOCH & THORN, 1985) to be linked to this host genus (e.g. *Pisolithus tinctorius*, *Russula cistoadelpha*, *R. monspeliensis*, *Hygrophorus pseudodiscoideus* var. *cistophilus*, *Amanita muscaria*, *A. cistetorum*, *Hebeloma erumpens*, *H. cistophilum*, *H. cylindrosporum*, *H. crustuliniforme* and *Leccinum corsicum*), only very few accounts of *Cistus* ectomycorrhizal types exist in the literature. To date, only the full characterization of the ectomycorrhizae formed by *Tuber* spp. on *Cistus incanus* (FONTANA & GIOVANNETTI, 1979; GIOVANNETTI & FONTANA, 1982) and by *Laccaria laccata* and *Boletus rhodoxanthus* on *Cistus ladanifer* (HAHN, 2001; TORRES *et al.*, 1995) are available. A number of other contributions have focused on the association of hypogeous ascomycetes such as *Tuber* and *Terfezia* with *Cistus* spp., both under natural conditions and in culture, but they usually resulted in preliminary and/or not exhaustive descriptions of morpho-anatomical details of relevant mycorrhizae (CHEVALIER *et al.*, 1975; FUSCONI, 1983; LEDUC *et al.*, 1986). It is of interest to note that *Cistus* spp., in analogy with other host plants, such as *Eucalyptus* spp., inhabiting fire-susceptible ecosystems, may also form vesicular arbuscular mycorrhizae. This plasticity being considered an adaptive trait to the cyclical pattern of accumulation and loss of organic resources due to fire (SMITH & READ, 1997).

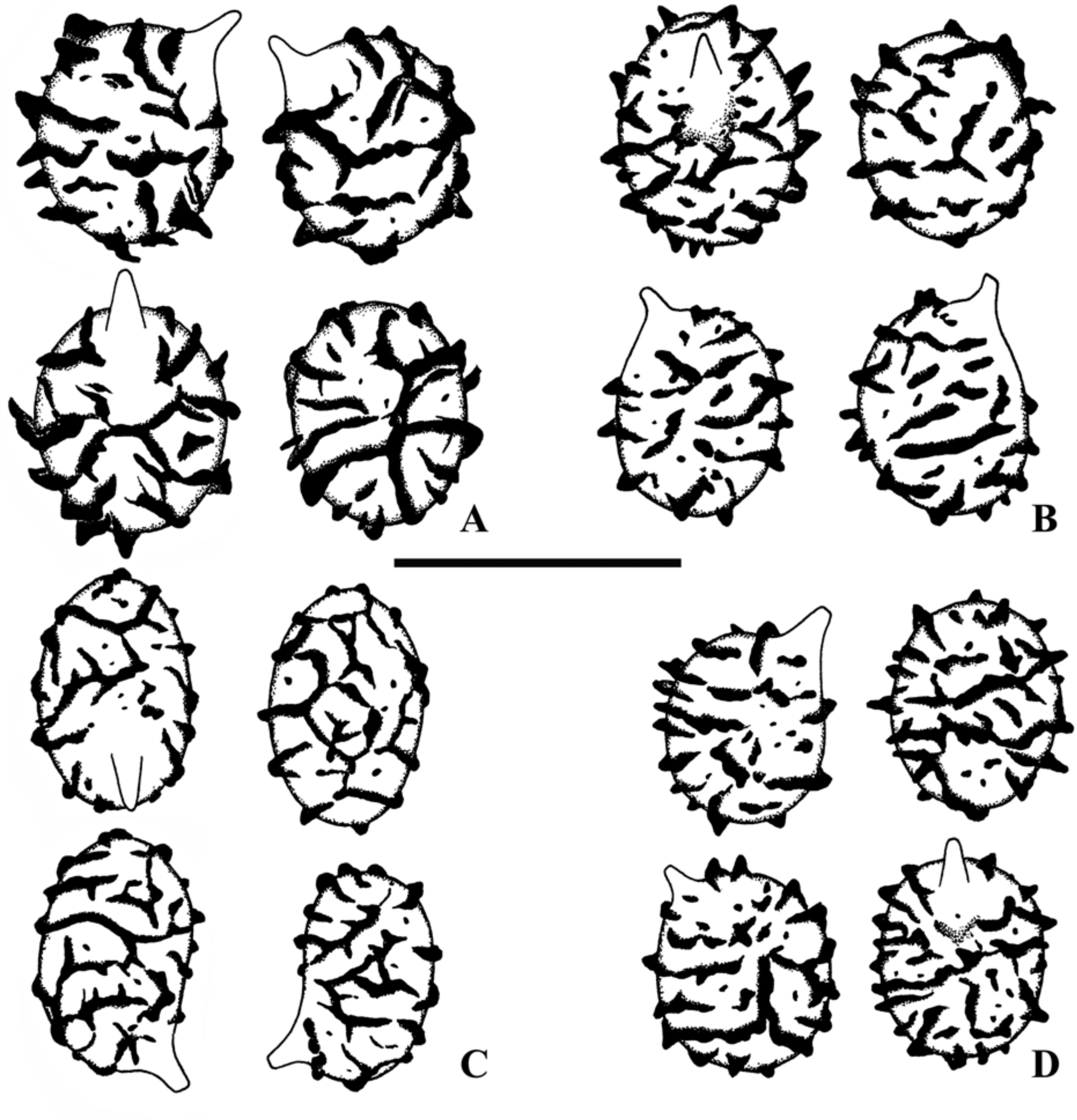

Fig. 4: Spores of **A** *L. mairei*, **B** *L. mairei* var. *ilicis*, **C** *L. tesquorum*, **D** *L. tesquorum* var. *splendidus*. For a description of salient features see Results. Scale bar = 10 µm.

Common host-dependent features of all ectomycorrhizal types described so far on *Cistus* spp., also present in our *L. tesquorum* samples, are: the simple or monopodial-pinnate ramification systems; the small diameter of the ectomycorrhizal tips (0.26 to 0.35 mm in *L. tesquorum*, up to 0.3 mm in *B. rhodoxanthus*); the thin mantle (10-20 µm in *L. laccata*, 10-15 µm in *L. tesquorum*, up to 18-25 µm in *B. rhodoxanthus*); cortical cells generally present in two rows, tangentially rectangular, mainly radially rectangular orientated in our samples, more rarely square to radially rectangular orientated (e.g. in *B.*

rhodoxanthus longitudinal sections); Hartig net generally uniseriate, surrounding 1(2) rows of cortical cells and rarely reaching the endodermis ("cortical Hartig net").

In addition to *L. tesquorum*, the only other member of *Lactarius* sect. *Piperites* for which descriptions of ectomycorrhizae have been published is *L. pubescens*, in association with *Betula pendula* and *Populus tremuloides* (GODBOUT & FORTIN 1985; INGLEBY *et al.*, 1990). Within the closely related *Scrobiculati*, the mycorrhizae of *L. scrobiculatus* on *Picea abies* and *Tsuga heterophylla* (AMIET & EGLI, 1991; KERNAGHAN & BERCH, 1997) and that of *L. intermedius* on *Abies alba* have been characterized (EBERHARDT *et al.*, 2000). A comparison of mycorrhizal appearance and structure (Table 2) clearly reveals that the mycorrhizae of *L. tesquorum* and *L. pubescens* share many structural features, whereas the *L. scrobiculatus* and *L. intermedius* mycorrhizae are rather dissimilar from those of both *L. tesquorum* and *L. pubescens*. This can be considered as a confirmation of the infrageneric classification proposed by HEILMANN-CLAUSEN *et al.* (1998), placing the groups *Piperites* and *Scrobiculati* in different sections within the subgenus *Piperites*, rather than the scheme of BASSO (1999a), placing the two groups together in the section *Piperites*. The assignment of the *Piperites* and *Scrobiculati* to different sections is also fully supported by the topology of the ITS-based phylogram depicted in Fig. 3.

Table 2: Comparison of structural features of the described mycorrhizae of *Lactarius* sect. *Piperites* and subsect. *Scrobiculati*. Abbreviations: SV, surface view; ML, middle layer; IV, inner view; RHIZ, rizomorphs; dpl, densely plectenchymatous; pl, plectenchymatous; ps, pseudaparenchymatous; undif, undifferentiated; hn, hyphal net; hd, highly differentiated; c, crystals; cy, cystidia.

Ectomycorrhizhal type	SV	ML	IV	RHIZ	Ref.
L. pubescens	dpl	ps	dpl	undif	GODBOUT & FORTIN, 1985; INGLEBY *et al.*, 1990
L. tesquorum	hn + dpl	dpl	dpl	hd	this study
L. intermedius	c + ps	pl	pl	undif	EBERHARDT *et al.*, 2000
L. scrobiculatus	cy + ps	ps	pl	undif	AMIET & EGLI, 1991; KERNAGHAN & BERCH, 1997

Despite the fact that only limited information is available so far on *Piperites* ectomycorrhizae, it is of interest to note that the results of the comparison of ectomycorrhizal morpho-anatomical features and those obtained from molecular analysis support each other, placing *L. tesquorum* and *L. pubescens* in the same group of closely related species within *Lactarius* sect. *Piperites*. This evidence spurs further investigation on the use of mycorrhizal characters to arrive at a reliable taxonomy for *Lactarius*.

An unexpected and, to some extent surprising, result of the molecular analysis is the distance of the morphologically similar *L. tesquorum* and *L. mairei*. Indeed, the two taxa are distinct within the *Piperites*, having a prevalently Mediterranean distribution and different ecological requirements with respect to the other species of the group, which occur more frequently or exclusively in northern European countries. *Lactarius mairei* is associated with *Quercus* spp. on calcareous soil, all other species (except *L. tesquorum*) are linked to *Betula* spp. In this context, it could be expected that the Mediterranean species *L. mairei* and *L. tesquorum* would form a separate cluster from the Nordic *Betula* taxa, but this is not supported by the ITS analysis.

It is difficult to formulate conclusions on the status of the intraspecific taxa *L. tesquorum* var. *splendidus* and *L. mairei* var. *ilicis* on the base of microscopical analysis as most indicated differences are macroscopical (see also ANTONINI & ANTONINI, 2002). But on examination of the spores of *L. tesquorum* var. *splendidus*, it becomes clear that this taxon should be a variety of *L. mairei*. The spores of *L. tesquorum* are somewhat longer and distinctly narrower and the ornamentation is more regularly reticulate, while in *L. mairei* the spore ornamentation is characterized by a spiny aspect and ridges that do not form a reticulum, but are often arranged somewhat in parallel (Fig. 4). Interestingly, LALLI &

PACIONI (1981) first regarded this taxon as a form of *L. mairei* before describing it as *L. tesquorum* var. *splendidus*. Molecular analysis of *L. mairei* var. *ilicis* (Fig. 3) confirms its status as a variety of *L. mairei*.

In our analysis, *L. spinosulus* is closely related to the members of *Lactarius* sect. *Piperites*. When a phylogenetic analysis is conducted with more taxa, the position of *L. spinosulus* is variable and a close relationship to *Lactarius* sect. *Piperites* is uncertain (unpublished data; EBERHARDT, 2000). Neither the placement of this species in *Lactarius* sect. *Piperites*, nor the placement in *Lactarius* sect. *Colorati* is confirmed by molecular analysis. The correct position of *L. spinosulus* in the genus consequently remains unclear.

In addition to *L. tesquorum*, only *L. cistophilus* Bon & Trimbach (*Lactarius* subgenus *Piperites*, sect. *Uvidi* (Konrad) Bon (nom. inval.)) is known to be linked specifically to *Cistus* spp. (BASSO, 1999a). Another species, *L. cyanopus* Basso (*Lactarius* subgenus *Piperites*, sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn), recently was described from northwestern Italy growing with the same host in nature (BASSO, 1998, 1999a, 1999b). However, it is more probable that it grows under *Pinus* (also present at the type locality), because this would match better the host specificity displayed by members of *Lactarius* sect. *Deliciosi* that are almost exclusively associated with Pinaceae. Moreover, the association of *L. rugatus* Kühner & Romagn. with *Cistus* has been claimed by some authors (BROTZU, 1998), although this species usually grows with *Quercus* in Mediterranean-type habitats. Work from our groups is currently underway to fully characterize these symbioses and results will form the object of another publication.

Acknowledgements

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Chapter 11

Systematics of European sequestrate lactarioid Russulaceae with spiny spore ornamentation

Adapted from:
NUYTINCK J., VERBEKEN A., DELARUE S. & WALLEYN R. (2003) – Systematics of European sequestrate lactarioid Russulaceae with spiny spore ornamentation. *Belgian Journal of Botany* **136**: 145-153.

Abstract

Molecular data confirm that *Arcangeliella borziana* Cavara (syn. nov.: *Arcangeliella volemoides* K. Mader & A. Mader) and *Zelleromyces stephensii* (Berk.) A.H. Sm. are 2 distinct *Lactarius* species. The taxonomic value of several morphological characters previously used in the systematics of Russulaceae (syn.: Elasmomycetaceae) is discussed. The new combinations *Lactarius borzianus* (Cavara) Verbeken & Nuytinck and *Lactarius stephensii* (Berk.) Verbeken & Walleyn are proposed. A lectotype is designated for *Hydnangium monosporum* Boud. & Pat. Its synonymy with *Lactarius stephensii*, as suggested by GROSS (1990), is confirmed.

1. Introduction

Sequestrate Russulaceae are very rare in Belgium. Up to now, only *Macowanites mattirolianus* (Cavara) T. Lebel & Trappe and *Zelleromyces stephensii* (Berk.) A.H. Sm. have been recorded for the country, both from *Querceto-Carpinetum* on limestone in southern Belgium (DE VRIES, 1977; THOEN, 1988). Recently, one of us collected a first sequestrate representative of Russulaceae in central Belgium, with evident latex production and spiny spores.

In Europe, three sequestrate species of lactarioid Russulaceae (accepted syn.: Elasmomycetaceae) characterised by spores with isolated acute warts or spines have been described: *Arcangeliella borziana* Cavara, *Z. stephensii* [syn.: *A. stephensii* (Berk.) Zeller & Dodge] and *A. volemoides* K. Mader & A. Mader. Considerable confusion remains in the current literature about the conspecificity of *Arcangeliella borziana* and *Zelleromyces stephensii* (LEBEL & TRAPPE, 2000). Furthermore, nothing has been written on *A. volemoides* since its description, except that many European records of *Z. stephensii* should be confirmed for possible confusion with this species (KREISEL, 2001). This encouraged us to clarify the taxonomic identity and systematic position of these taxa.

2. Material & Methods

2.1. Morphological analysis

This study is based on herbarium material from GENT and PC. Macroscopic characters are all based on fresh material. Colour-codes are from KORNERUP & WANSCHER (1978). Microscopic features were studied from fresh and dried material, mainly in Congo red in L4. Spore ornamentation is described and illustrated as observed in Melzer's reagent. Line drawings were made using a drawing tube at original magnifications 6000 × for spores, 3000 × or 1000 × for individual elements and 1000 × for sections. Basidia length excludes sterigmata length. Spores were measured in side view in Melzer's reagent, excluding the height of the ornamentation. Measurements are given as (MINa) [AVa-2×SD] – *AVa – AVb* – [AVb + 2×SD] (MAXb), with AVa = lowest mean value for the measured collections, AVb = greatest mean value and SD = standard deviation calculated for the measurements of one collection. Q corresponds with spore "length/width ratio" and is given as (MINQa) *Qa – Qb* (MAXQb) with Qa and Qb being the lowest, respectively the highest, mean ratio for the measured specimen.

Table 1: GenBank accession numbers and herbarium entries for the species used in the analysis of the phylogenetic relationships of *Zelleromyces stephensii*. All herbarium specimens are deposited in the herbarium of the Ghent University (GENT).

Species	GenBank accession/herbarium number
Arcangeliella borziana	AF286204
Lactarius acerrimus	AJ278139
Lactarius blennius	AY331015/L. Van Laethem 2002-033
Lactarius chrysorrheus	AF096983
Lactarius controversus	AJ272244
Lactarius deliciosus	AF249284
Lactarius fallax	AF335441
Lactarius fluens	AY331014/JN 2002-050
Lactarius fulvissimus	AF204679
Lactarius hepaticus	AF096989
Lactarius intermedius	AF140256
Lactarius mitissimus	AF157412
Lactarius pterosporus	AY331013/ L. Van Laethem 2002-019
Lactarius quietus	AJ272247
Lactarius repraesentaneus	AY331011/J. Nuytinck 2001-023
Lactarius scrobiculatus	AF140262
Lactarius serifluus s.l.	AY332558/R. Walleyn 1431
Lactarius subdulcis	AY331016/ L. Van Laethem 2002-034
Lactarius subsericatus	AF140254
Lactarius tabidus (as *L. theiogalus*)	AF349716
Lactarius tesquorum	AF096986
Lactarius trivialis	AJ534935
Lactarius uvidus	AJ534936
Russula delica	AF418605
Russula integra	AY061683
Zelleromyces giennensis	AF230900
Zelleromyces hispanicus	AF231913
Zelleromyces stephensii	AY331012/R. Walleyn 2930

2.2. Molecular analysis

DNA extraction

Dried material (about 50mg) was first ground in liquid N_2. One ml extraction buffer (0.1M Tris-HCl pH=8, 0.5M NaCl, 0.05 M EDTA and 0.01M β-mercapto-ethanol) and 50 µl 10% SDS were added and the mixture was incubated for 30 min at 65°C. This extraction was centrifuged for 10 min at 14000 rpm (Eppendorf centrifuge). An equal volume of isopropanol was added to the supernatant and after mixing the solution, it was centrifuged again as above. The pellet was washed with 70% ethanol, air-dried and dissolved in 400µl dH_2O. Then 400µl CTAB buffer (2% w/v CTAB, 0.2M Tris-HCl pH=7.5, 2M NaCl and 0.05M EDTA) was added and after incubation for 15 min at 65°C, the mixture was extracted twice with chloroform/iso-amylalcohol (24:1). Two volumes of 96% ethanol were added and the mixture was centrifuged again as above to pellet the DNA. The pellet was washed with 70% ethanol and dissolved in 100µl $T_{0.1}E$.

ITS amplification

Basidiomycete specific primers ITS1-F and ITS4-B (GARDES & BRUNS, 1993) were used to amplify the ITS region. The following touchdown profile was used: 95°C during 2 min, then 10 cycles of denaturation at 95°C (during 15 sec), primerannealing at 63°C (during 20 sec) with the temperature decreasing with 1°C at each cycle, polymerisation during 1 min at 72°C; followed by 35 cycles with the same profile for denaturation and polymerisation but with a constant annealing temperature of 53°C; polymerisation was completed by an incubation of 2 min at 72°C.

ITS sequencing

The obtained PCR products were purified using ExoSAP (USB, USA). DNA sequencing reactions were performed with the ABI PRISM® BigDye™ Terminators v3.0 Cycle Sequencing Kit using primers ITS1-F, ITS2, ITS3 and ITS4-B (Gardes & Bruns, 1993; White *et al.,* 1990) on an ABI PRISM® 377 DNA Sequencer. The Phred and Phrap Software (Ewing & Green, 1998; Ewing *et al.*, 1998) was used to process the raw data.

Data analysis

For our phylogenetic analysis, we combined the obtained ITS sequences of *Zelleromyces stephensii* and 6 *Lactarius* species with 21 ITS sequences of related *Lactarius*, *Zelleromyces, Arcangeliella* and *Russula* species, retrieved from GenBank (Table 1). Sequences were aligned in ClustalX (Thompson *et al.*, 1997) and the ambiguous regions in the alignment were excluded from the analysis (position 22–26, 56–69 and 506–524). For phylogenetic analyses PAUP*4b10 (Swofford, 2002) was used. A maximum parsimony analysis was performed with all sites treated as unordered and unweighted and with gaps treated as missing. A total of 1000 random-addition sequence replicates were implemented, 5 trees were held per step and the branch-swapping algorithm was TBR. Other settings were: random addition of taxa, MulTrees option in effect and no steepest descent. A bootstrap analysis with 1000 replicates and for each replicate 10 random addition sequences was performed to estimate the support for the internodes in the trees.

3. Results

3.1. Description of the sequenced collection of *Zelleromyces stephensii*

Fig. 1e-i, Fig. 2

Exsiccatum: Belgium, Berg, nature reserve ‘Tofbroek’, in leaf litter under *Populus* cf. *alba* and *Crataegus* at border of calcareous fen, 2 Dec 2002, *R. Walleyn* RW 2930 (GENT).

Basidiomata up to 15 mm diam., subglobose to oblong or slightly reniform, sometimes lobed, firm, with a small protruding sterile base. Peridial surface reddish brown (8DE8), darker (up to 8F8) when bruised or with age, not unicolourous but with a reddish, flamboyant aspect, smooth. Peridium 0.3–0.5 mm thick, persistent. Gleba paler than the peridium, orange (6B7) to brownish orange (6C7), darkening to orange brown (6D7) when long exposed, sienna (6E7) when dried, loculate; locules labyrinthuloid, ellipsoid to irregular, small, 2-4 per mm. An indistinct columella present in one fruitbody, absent in remaining ones, narrow (about 0.4 mm broad), branching, originating in the sterile base, concolourous with the peridium. Odour pleasant and fruity in mature specimens (reminding canned pine-apple). Latex present but scarce, white, mild tasting.

Basidiospores subglobose to broadly ellipsoid, mostly symmetric and orthotropic, 11.0–*12.3–13*–14 µm × 10.5–*11.5–11.8*–12.8 µm (Q = 1.04–*1.07–1.13*–1.21, n = 40); ornamentation weakly amyloid (brownish grey in Melzer’s reagent), composed of isolated, bunt spines, 0.5–1.0(1.3) µm long, cylindrical or somewhat irregular, sometimes curved; hilar appendix 1–6 × 1.5–2 µm, distinct, tapering to flattened near apex, hyaline, thin-walled. Basidia 1-spored, seldom 2-spored, 45–75 × 7–10 µm, some are remarkably 2(3)-septate with the upper part about 20–40 µm long, thin-walled, hyaline or with remarkable dark yellowish to brown content (forming a central plug); sterigmata eccentric, 4–6 µm long, irregular, tapering or slightly moniliform. Pseudocystidia very scarce, the few observed ones emergent (up to 30 µm), cylindrical, 5–7 µm broad, with yellowish brown oil-like content. Trama 60–90 µm broad, composed of mainly parallel arranged hyphae; hyphae thin-walled, 2–5 µm diam., often

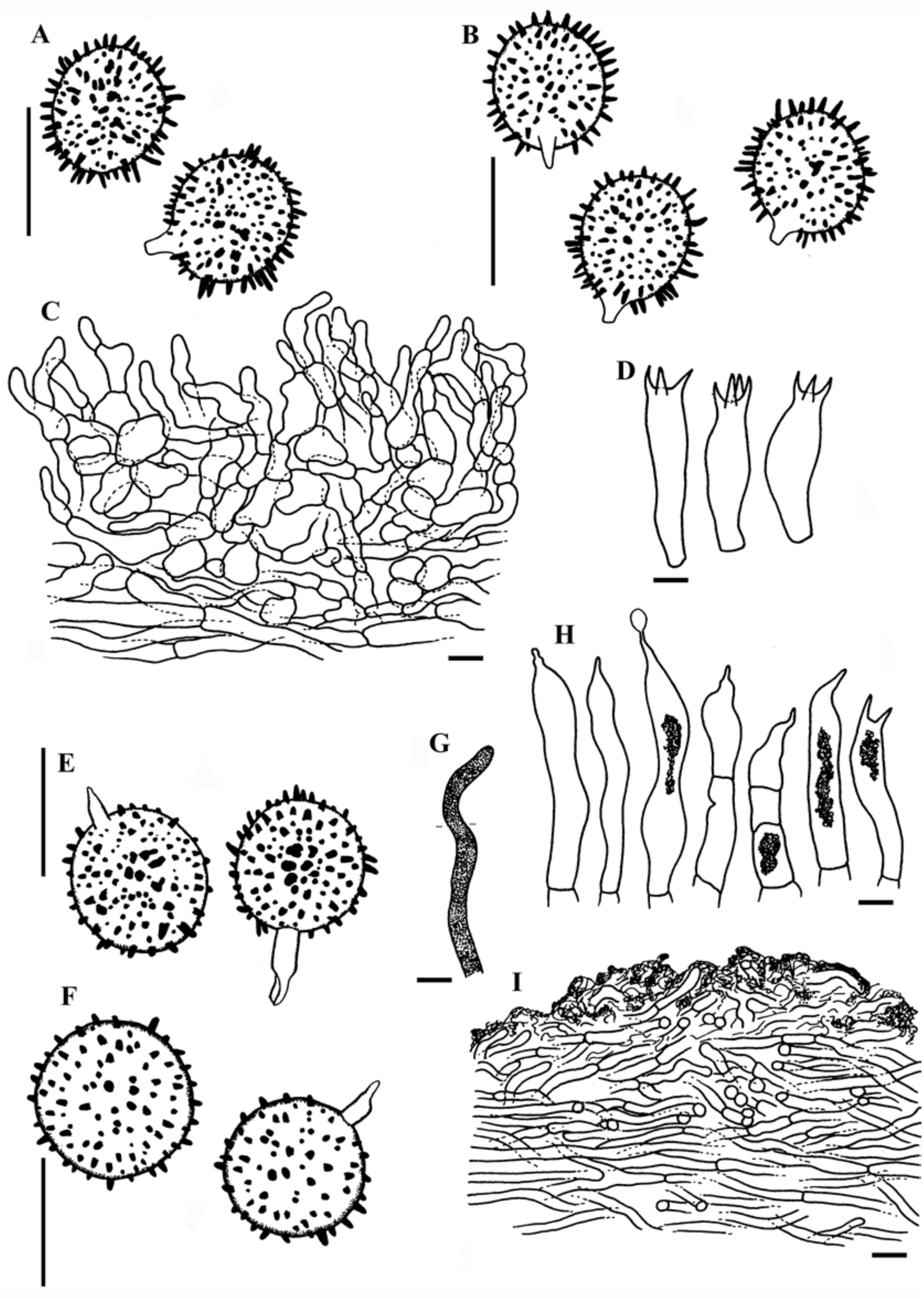

Fig. 1: ***Lactarius borzianus*** **A.** Spores (WSL 96-5-3344); **B.** Spores (typus *A. volemoides*); **C.** Section through the peridiopellis (WSL 96-5-3344); **D.** Basidia (WSL 96-5-3344) (bar = 10 µm) – ***Lactarius stephensii*** **E.** Spores (RW 2930); **F.** Spores (typus *Hydnangium monosporum*); **G.** Pseudocystidium (RW 2930), small bars indicate the height of the hymenium; **H.** Basidia (RW 2930); **I.** Section through the peridiopellis (RW 2930) (scale bar = 10 µm).

with small knobs and short side branches, frequently branched, embedded in a slime layer; abundant lactifers present. Peridiopellis an ixocutis to ixotrichoderm, composed of strongly interwoven hyphae embedded in a slime layer, 3–6 µm diam., locally with erect terminal elements, with a brownish layer (slimy, with abundant incrustations) on top.

other collections examined:
Belgium, Montquintin, under *Populus* x *canadensis*, 8/09/1992, *Thoen* 8241 (GENT, dupl. ex herb. D. Thoen). France, Nice, leg. Barla July 1885 in herb. Boudier (PC, **lectotype** of *Hydnangium monosporum*, **designated here**).

3.2. Description of sequenced collection of *Arcangeliella borziana*

Fig. 1a-d

The collection of *Arcangeliella borziana* used for molecular analysis (Switzerland, Fribourg, Cerniat, Parabock forest, Aug. 1996, WSL 96-05-3344, duplicate at GENT) is described by AYER (1998).

other collections examined:
Austria, Niederösterreich, Gföhl, leg. A. & K. Mader 12/09/1970 (WU-10875, type of *A. volemoides*).

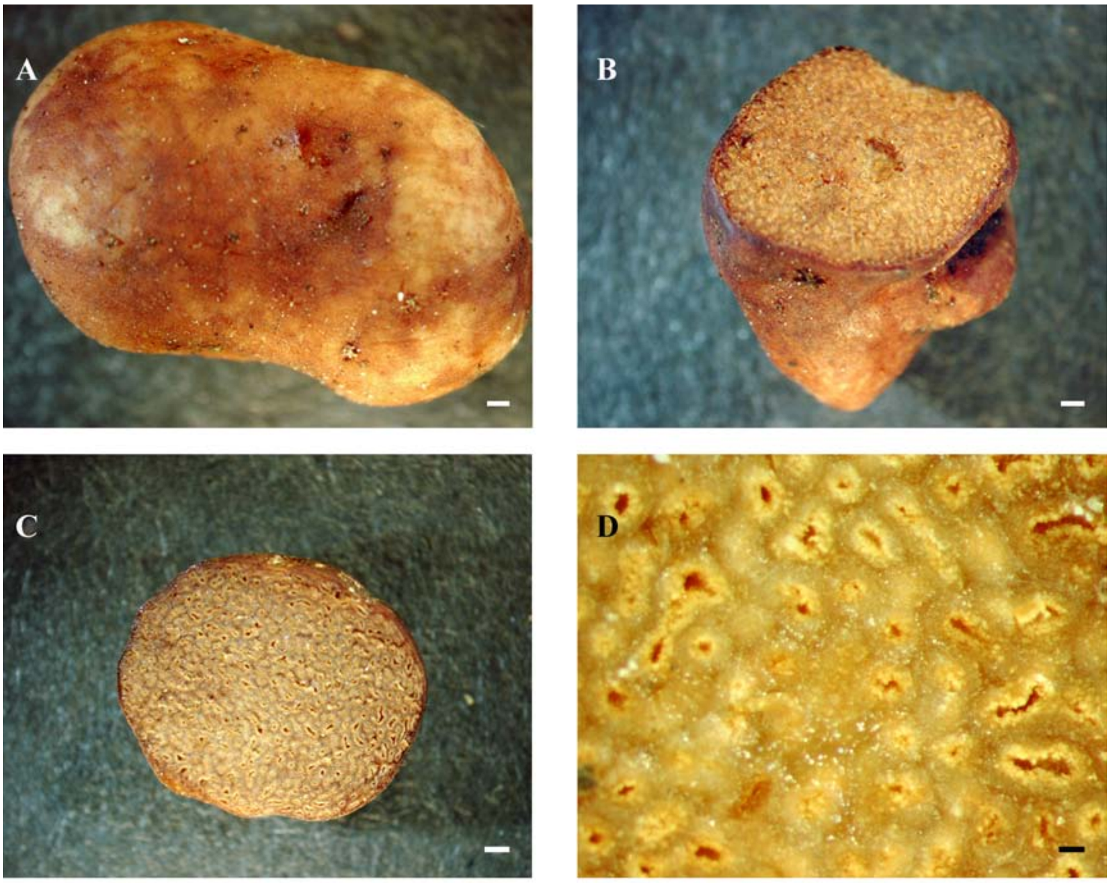

Fig. 2: ***Lactarius stephensii*** collection R. Walleyn 2930; **A.** Slightly reniform basidiome (scale bar = 1 mm); **B.** Cut fruiting body showing the orange brown, loculate gleba (scale bar = 1 mm); **C.** Cut fruiting body scarcely exuding white latex (scale bar = 1 mm); **D.** Detail of the loculate gleba (scale bar = 0.1 mm).

3.3. Phylogeny

Fig. 3

The alignment of 28 ITS sequences (part of 28S rDNA, ITS1, 5.8S rDNA, ITS2 and part of 18S rDNA, Table 1) contained 764 nucleotides, of which 407 nucleotides were constant and 191 nucleotides were parsimony-informative. Two *Russula* species (*R. delica* and *R. integra*) were used as outgroup. Maximum parsimony analysis produced 12 shortest trees of 735 steps (consistency index = 0.65, retention index = 0.6563, rescaled consistency index = 0.4295). The strict consensus tree is shown in Fig. 3, with associated bootstrap supports for the branches. In this tree, three subgenera of *Lactarius* form a distinct clade: subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman, subgenus *Russularia* (Fr. ex Burl.) Kauffman and subgenus *Plinthogali* (Burl.) Hesler & A.H. Sm. While *A. borziana* is closely related to *L. fulvissimus* and *L. subsericatus* in subgenus *Russularia*, *Z. stephensii* is included in subgenus *Piperites*. This dataset does not allow us to draw conclusions about which section *Z. stephensii* belongs to.

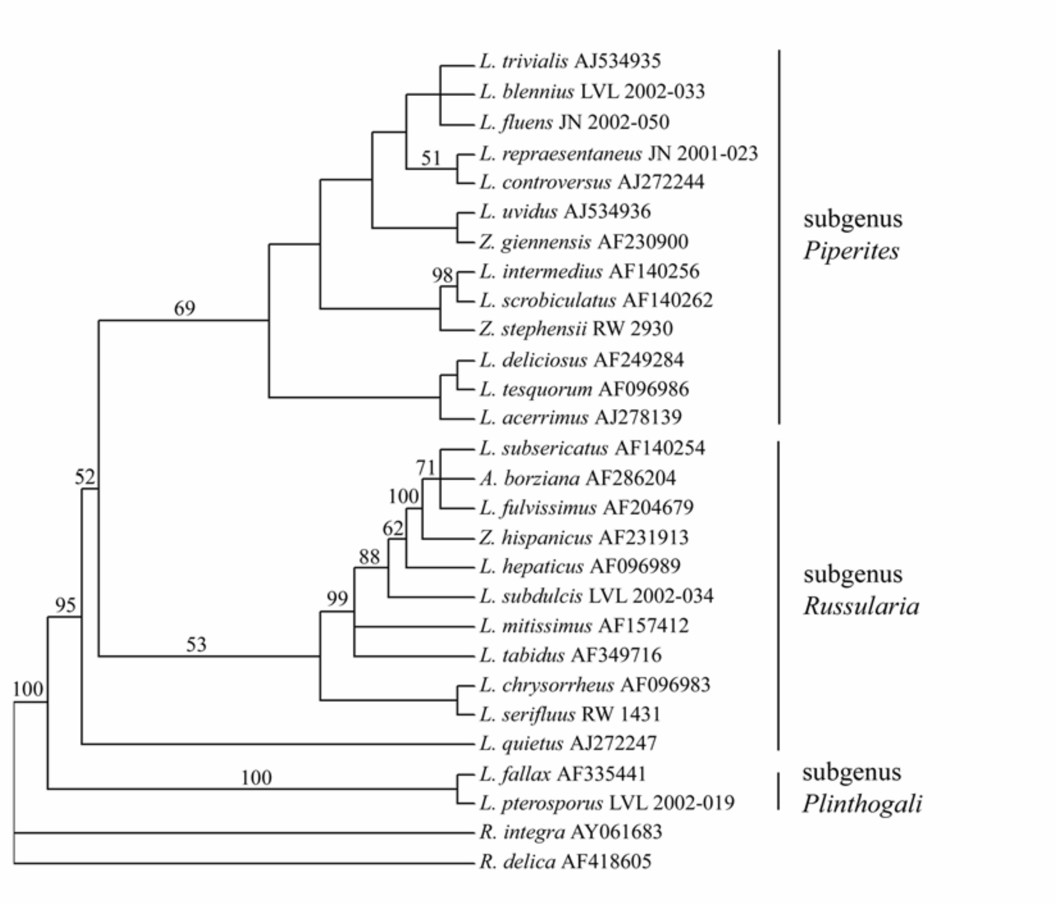

Fig. 3: Strict consensus of the 12 equally parsimonious trees resulting from maximum parsimony analysis of the ITS sequence data. Bootstrap values (1000 replicates) are shown above their respective branches. Abbreviations: A., *Arcangeliella*; L., *Lactarius*; R., *Russula*; Z., *Zelleromyces*.

4. Discussion

Up to now *A. borziana* was often synonymized with *Z. stephensii* (MONTECCHI & SARASINI, 1990; KRIEGLSTEINER, 1991b; AYER, 1998). GROSS (1990) distinguished three taxa in “this complex”, whereas recent important monographs on truffle-like fungi of Europe treat only one of both species, without discussing the distinctive characters (PEGLER *et al.*, 1993; MONTECCHI & SARASINI, 2000).

These literature data made LEBEL & TRAPPE (2000) conclude that much confusion still remains about the identity of these two taxa.

Both species can, however, easily be distinguished by morphological observations. *A. borziana* has carpophores with a distinct columella, a slightly exposed gleba at the rudimentary stiped base and asymmetric (putative ballistosporic) spores (Fig. 1a) with slender spines up to 1.5 (1.8) µm, produced on (3-) 4-spored basidia (Fig. 1d). The carpophores of *Z. stephensii* are entirely enveloped by the peridium, and produce symmetric, statismosporic spores (Fig. 1e) with warty, blunt spines up to 1 (1.3) µm, on 1- or 2-spored basidia (Fig. 1h). Their ecology seems also different. *A. borziana* forms ectomycorrhiza in submontane areas with *Picea* (PETER *et al.*, 2001), and maybe also with *Abies*, whereas *Z. stephensii* seems restricted to deciduous forests, also at lower altitudes.

The description of the new species *A. volemoides* (MADER & MADER, 1992), found under *Picea* and described as similar to *Z. stephensii* seemed confusing to us, because *A. borziana* was not mentioned at all in the discussion. Our study of the type confirmed our hypothesis that this species is synonymous with *A. borziana*. The descriptions of *Z. stephensii* based on the original material in the herbarium of Berkeley (K) mention 1-spored basidia (MADER & MADER, 1992) or 2-spored basidia (HAWKER, 1954; PEGLER *et al.*, 1993). Our examination revealed predominantly 1-spored basidia, easily mistaken for cystidia, although some scarce 2-spored basidia were observed. We assume that both spore numbers occur in the same species and that intermediate forms exist. GROSS (1990) suggested that monospored collections have been described as *Hydnangium monosporum* by BOUDIER & PATOUILLARD (1888). Examination of original material in the Boudier herbarium at PC (designated here as lectotype) confirms this hypothesis.

Good descriptions of *Z. stephensii* are given by DE VRIES (1977), HAWKER (1954) and PEGLER *et al.* (1993). For *A. borziana* we refer to the descriptions by LEBEL & TRAPPE (2000) and MONTECCHI & SARASINI (2000). Some literature records cannot be interpreted with certainty. The record of *Z. stephensii* in Switzerland by MILLER & MILLER (1986) e.g. refers to *A. borziana*, but the description given is probably based on both species, as they examined several other collections, including the type of *Z. stephensii*.

Several attempts have been made over the last decades to define the generic distinctions in the gasteroid Russulales (e.g. PEGLER & YOUNG, 1979; BEATON *et al.*, 1984; CALONGE, 1999; LEBEL & TRAPPE, 2000), mainly based on morphological characters such as hymenophoral trama anatomy, stipe-columella development, spore morphology and presence or absence of latex. In most cases, these genera were described as showing affinities with the agaricoid genera *Lactarius* or *Russula*. LEBEL & TRAPPE (2000) studied the morphological characters of the type species of all sequestrate genera in the Russulaceae, and reduced the number of gasteroid-russuloid genera to three, but left the taxonomic boundary of the gasteroid-lactarioid genera *Zelleromyces* and *Arcangeliella* over for future consideration. However, discoveries of new taxa often do not fit the generic descriptions (DESJARDIN, 2003). Meanwhile, molecular studies argument that the sequestrate members of Russulaceae have been derived from within *Russula* and *Lactarius* and that synonymy with these genera is acceptable (MILLER *et al.*, 2001). As a consequence, a new sequestrate species, with several conspicuous characters typical for *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn has already been described in the genus *Lactarius* (DESJARDIN, 2003). The classification of *L. rubriviridis* Desjardin in *Lactarius* sect. *Deliciosi* is now supported by molecular data (NUYTINCK & VERBEKEN, in preparation). PETER *et al.*, (2001) give molecular evidence for classification of *A. borziana* in the genus *Lactarius*, subgenus *Russularia*, close to *L. fulvissimus* Romagn., and our data support also the transfer of *Z. stephen*sii to this genus. Therefore, following combinations are proposed:

Lactarius stephensii (Berk.) Verbeken & Walleyn **comb. nov.**
≡ *Hydnangium stephensii* Berk., Ann. Mag. Nat. Hist., Ser. 1, 13: 352 (1844)
≡ *Octaviania stephensii* (Berk.) Tul. & C. Tul., Fungi hypogaei: 78 (1851)
≡ *Arcangeliella stephensii* (Berk.) Zeller & Dodge, Ann. Missouri Bot. Gard. 18: 463 (1931)
≡ *Zelleromyces stephensii* (Berk.) A.H. Sm., Mycologia 54: 635 (1962)
≡ *Martellia stephensii* (Berk.) K. Mader & A. Mader, Österr. Z. Pilzk. 1: 4 (1992)
= *Hydnangium monosporum* Boud. & Pat., J. Bot. (Morot) 2: 445 (1888) **syn. nov.**
= *Octaviania monospora* (Boud. & Pat.) Lloyd, Mycol. Notes 7(67): 1141 (1922)

= *Martellia monospora* (Boud. & Pat.) Astier & Pacioni, Doc. Mycol. 28(109-110): 9 (1998)

Lactarius borzianus (Cavara) Verbeken & Nuytinck **comb. nov.**
≡ *Arcangeliella borziana* Cavara, Nuov. Giorn. Bot. Ital. ser. 2, 7: 126 (1900)
≡ *Octaviania borziana* (Cavara) Svrcek, in Pilát, Gasteromycetes, Flora CSR B-1: 194 (1958)
≡ *Arcangeliella stephensii* var. *borziana* (Cavara) Krieglst., Z. Mykol. 57: 74 (1981)
= *Arcangeliella volemoides* K. Mader & A. Mader, Österr. Z. Pilzk. 1: 5 (1992) **syn. nov.**

Our molecular data demonstrate that *L. borzianus* and *L. stephensii* are less closely related than all literature data suggest, as *L. stephensii* seems a member of *Lactarius* subgenus *Piperites.* This is also confirmed by the micromorphological characters, in particular the peridiopellis that is a trichopalisade in *L. borzianus* (Fig. 1c) and an ixocutis to ixotrichoderm in *L. stephensii* (Fig. 1i).

The very distinct and thick ixocutis, present in most representatives of *Lactarius* subgenus *Piperites*, lacks in *L. stephensii*, which is not surprising in a hypogeous basidiocarp. The outer layer is very much encrusted (with soil particles), but slime is distinct among the underlying hyphae. The absence of acrid latex, another common feature in *Lactarius* subgenus *Piperites*, could also be explained as an adaptation to the hypogeous way of life, where spore dispersal depends on animal attraction.

Interestingly, both species co-evolved not only by the development of sequestrate basidomes but also formed a spiny spore ornamentation, which is rather unique among European representatives of the genus *Lactarius*. This confirms that several of the traditionally used characters are less valuable then previously accepted. It has been shown previously that the spore ornamentation in *Lactarius* can be heterogenous in the same section or subgenus (e.g. *Lactarius* subgenus *Lactiflui* (Burl.) Hesler & A.H. Sm.; VERBEKEN, 1998). When redefining the generic and infrageneric classification in Russulaceae, less (or no?) attention should be given to the basidiocarp development. For agaricoid as well as sequestrate taxa, the structure of pileipellis resp. peridiopellis, and the presence or absence of pseudocystidia seem reliable, phylogenetic characters.

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Chapter 12

General conclusions and future perspectives

1. *Lactarius* sect. *Deliciosi*: a state of the art

This study combines a molecular and a detailed morphological approach to clarify the taxonomy, nomenclature and phylogeny of a relatively small, but interesting and popular group of fungi: *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn. About 450 collections were studied, 230 of them were well-documented and often personally collected European specimens; most non-European collections were loans from different herbaria and were not or less well documented macroscopically. We recognise 31 species in this section on a worldwide scale; 29 of them are confirmed by both molecular and morphological data. Seven North American varieties, together with many Asian taxa, need more study to come to a final conclusion. That several taxonomic uncertainties were not solved here is mainly caused by an insufficient knowledge of the macroscopical characters that are indispensable for the delimitation of taxa in this section. From this point of view, it is not surprising that several insufficiently documented collections could not be identified. Four new species were discovered, but only two could be published as new because a detailed macroscopical description lacks for the other two species. More collecting and basic taxonomic work remains to be done on the underexplored Asian continent and also in North and Central American, especially in the complex of varieties in "*L. deliciosus*". Table 1 gives a final overview of the species and varieties we recognise here, divided over the three continents where they occur.

Furthermore, it should be remarked here that several species in this section are clearly distinct and can hardly be confused with any other species when found in fresh condition (e.g. *L. indigo, L. subpurpureus*, *L. semisanguifluus)*. The species with initially orange latex are the ones that cause most of the determination problems. Identifications with the aid of micromorphological characters, only based on herbarium specimens, will probably never be reliable and often even impossible if data on the collection location, host tree and latex colour are entirely lacking.

We included type material wherever possible in both our morphological and molecular study. The inclusion of type specimens in the molecular phylogenetic analyses was strongly hampered however by the difficulties of obtaining good quality DNA extracts from herbarium specimens more than 15 years old. An exact explanation for this phenomenon, that seems to be specific for several groups of fungi and is lacking in others, is not known.

When including *L. porninsis*, a species with white coloured latex, this section forms a monophyletic group in *Lactarius* subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman, as supposed by most current authors (BASSO, 1999a; HEILMANN-CLAUSEN *et al.*, 1998). *Lactarius porninsis* has always been an outlier in *Lactarius* sect. *Zonarii* (Quél.) Bon where it is traditionally classified and it is not entirely surprising that it is actually a member of *Lactarius* sect. *Deliciosi*. The mild tasting latex, the association with *Larix* and the general aspect of the pileus all point in that direction. The striking pigmentation of the latex in the different members of *Lactarius* sect. *Deliciosi* is largely caused by the same substances that are merely present in different proportions (SCHMITT, 1974). This might explain the easy switch from one latex colour to another during evolution. Red coloured latex for example originated at least five times independently.

The currently used definition of *Lactarius* sect. *Deliciosi* (see chapter 2) cannot be maintained. The following definition, taking into consideration that the white-milked *L. porninsis* and the hypogeous *L. rubriviridis* are members of the section, is proposed.

Emended definition of *Lactarius* sect. *Deliciosi*:

Basidiocarps epigeous and agaricoid or hypogeous and gasteroid; latex dirty yellow, orange, red, vinaceous brown, purplish, blue or rarely white (in one known species) coloured when first exuded; taste mild or very slightly acrid or bitter; injured tissues quickly to slowly staining green in most taxa; latex itself mostly not staining green; pileus and stipe not to distinctly viscid or slimy when fresh; pileus often zonate; stipe often scrobiculate; spores ornamented with an incomplete to (nearly) complete reticulum; pleuro- and cheilomacrocystdia mostly present; pileipellis an ixocutis or rarely a cutis; ectomycorrhizal root tips with and entirely plectenchymatous mantle.

Table 1: Overview of the taxa in *Lactarius* sect. *Deliciosi* accepted in this study.

Europe
L. cyanopus Basso
L. deliciosus (L.: Fr.) Gray
L. deterrimus Gröger
L. fennoscandicus Verbeken & Vesterh.
L. porninsis Rolland
L. quieticolor Romagn.
L. salmonicolor R. Heim & Leclair
L. sanguifluus (Paulet) Fr.
L. semisanguifluus R. Heim & Leclair
L. vinosus (Quélet→) Bataille
North and Central America
L. barrowsii Hesler & A.H. Sm.
L. chelidonium Peck var. *chelidonium* and var. *chelidonioides* (A.H. Sm.) Hesler & A.H. Sm.
L. indigo Schwein. var. *indigo* and var. *diminutivus* Hesler & A.H. Sm.
L. miniatosporus Montoya & Band.-Muñoz
L. paradoxus Beardslee & Burl.
L. pseudodeliciosus Beardslee & Burl. var. *pseudodeliciosus* and var. *paradoxiformis* (Murrill) Hesler & A.H. Sm.
L. rubrilacteus Hesler & A.H. Sm.
L. rubriviridis Desjardin, Saylor & Thiers
L. salmoneus Peck var. *salmoneus* and var. *curtisii* (Coker) Hesler & A.H. Sm.
L. subpurpureus Peck
L. thyinos A.H. Sm.
"*L. deliciosus*" var. *deterrimus* (Gröger) Hesler & A.H. Sm., var. *olivaceosordidus* Hesler & A.H. Sm., var. *areolatus* A.H. Sm. and var. *piceus* Smotl. (sensu HESLER & SMITH, 1979)
L. sp.3, an undescribed species form California, USA
Asia
L. akahatsu Tanaka
L. deliciosus (L.: Fr.) Gray
L. hatsudake Tanaka
L. laeticolor (S. Imai) Imazeki ex Hongo
L. subindigo Verbeken & E. Horak
L. thakalorum Bills & Cotter
L. sp. 1, an undescribed species from Hunan, China
L. sp. 2, an undescribed species from Hunan, China
L. sp. 4, an undescribed species from Java, Indonesia

It is hard to define the species concept that was finally used in this study; several considerations are listed here. First of all we tried as much as possible to base our species-level decisions on both molecular and morphological characters. Defining objective morphological criteria to delimit species is difficult. We tried to follow the criteria used in the Flora Agaricina Neerlandica (explained by KUYPER, 1988). It is stated there that at least two independent morphological characters that differ significantly should be found to recognise two taxa as different species. The inclusion of compatibility tests might have given us more insight into the reproductive boundaries between closely related pairs of species and the delimitation of biological species. Such an approach is however difficult or even not feasible in *Lactarius* because of the very slow growth rate of these fungi in culture and other practical reasons. Secondly, our species concept is probably not exactly the same in the different continents, which is a logical consequence of the different exploration rate of the different continents. The knowledge we have of the variability of the European species in *Lactarius* sect. *Deliciosi* is much better than that in the other continents. This might actually have lead to a somewhat larger species concept (e.g. in comparison to the concept used by many French mycologists) but also more accurate species concept for the European representatives. On the other hand, most of the non-European species were not studied in fresh condition by us, which means that we do neither have insights in their variability nor in the actual macroscopical differences between these non-European species. As a consequence, we often had to rely on microscopical features only (besides molecular data), and those are much less informative in this section. For this reason, the applied species concept is probably wider for at least the Asian taxa. It was, taking all of this into consideration, surprising and satisfying to find that almost all of our morphologically defined species were confirmed by both molecular datasets but it is to be expected that more sampling in Asia will reveal more taxa (e.g. in the *L. hatsudake* clade).

2. Results of the molecular approach

We used two DNA regions (ITS and a part of the *gpd* gene) in our phylogenetic analyses. Both have proved to be useful in solving species-level taxonomic problems and phylogenies. With both genes the resolution we obtained was very low in the basal clades of the of the *Lactarius* sect. *Deliciosi* branch, while most species were very well separated and some smaller clusters were well-supported. It seems not justified to further divide this section into subsections as has been attempted several times (BASSO, 1999; BON, 1980; HESLER & SMITH, 1979; SCHAEFER, 1970). The characters that intuitively would be used to make this eventual subdivision (e.g. the colour and colour change of the latex) are phylogenetically not very informative or even misleading. Convergent evolution is a very common phenomenon in the fungal kingdom and the plasticity of many of the important distinguishing characters in this section make it even more difficult to find morphological features that are phylogenetically informative.

The ITS sequence has proved its use as a tool for identification of fungal species and is being used in many ecological studies. An example is the identification of ectomycorrhizal root tips for studying the community structure of a forest (HORTON & BRUNS, 2001). The ITS region has many practical advantages such as the availability of specific and universal primer sequences, the ease with which it is amplified, the large number of ITS sequences already known etc. Most species in *Lactarius* sect. *Deliciosi* can indeed reliably be identified using this DNA region. Problems might arise however with very closely related species such as *L. vinosus* versus *L. sanguifluus* and *L. deterrimus* versus *L. fennoscandicus*.

3. Phylogeographic considerations

A very interesting aspect of the species in *Lactarius* sect. *Deliciosi*, which has received relatively low attention in fungi in general, is their phylogeography or how the current distribution of the species, keeping their phylogenetic relationships in mind, was accomplished. Most of the clades we revealed in our phylogenies, some of them very well supported, consist of Asian, American and/or European taxa. This means that ancestors of these clades must have existed when migration between these continents was still possible. The very recent migration seems improbable given the very low conspecificity between the continents.

Asia and America appear to have shared a land connection via the Bering land bridge (and possibly a periodic Aleutian bridge as well) from the Mesozoic (over 70 million years before present) until the late Miocene or early Pliocene, ~ 10 million years before present (FUJITA, 1978; BARRON *et al.*, 1981; MCKENNA, 1983; BRIGGS, 1987). A north Atlantic European-American land bridge is speculated to have existed from early Eocene (55 million years before present) until late Miocene, although it was possibly interrupted during the Oligocene, 30-40 million years before present (RAVEN & AXELROD, 1974; TIFFNEY, 1985a&b). However, disagreement about this North Atlantic land bridge abounds. Some geologists and biologists (KURTÉN, 1973; THIEDE, 1980; MCKENNA, 1983; BRIGGS, 1987) have contended that it existed from the Mesozoic only until the mid-Eocene (50 million years before present), in which case it would not have been a viable migration route during most of the Tertiary. The current distribution of the hosts of *Lactarius* sect. *Deliciosi* was undoubtedly strongly influenced by climate fluctuations. Periodic contact among the taxa of northern temperate forests was probably possible during warmer climate periods when northern land bridges were present (STANFORD *et al.*, 2000).

Well-preserved ectomycorrhizae have been found in approximately 50 million years old fossils from British Columbia (SMITH & READ, 1997). These fossil mycorrhizae are very similar to the ectomycorrhizae formed by members of the hypogeous genus *Rhizopogon*. These findings provide support for the molecular clock-based dating of the origin of ancestral epigeous basidiomycetes about 220 million years +/- 50 million years before present, more or less concurrently with the appearance of the Pinaceae (BERBEE & TAYLOR, 1993; SMITH & READ, 1997). This made also HALLING (2001) suppose that ectomycorrhizal homobasidiomycetes might have arisen ~200 million years ago.

It is not known when the ancestor of *Lactarius* sect. *Deliciosi* might have arisen and how the current biogeography of the taxa originated. Matsutake (*Tricholoma matsutake*) and close allies occur in an area roughly matching the distribution of coniferous genera such as *Pinus*, *Pseudotsuga*, *Tsuga*, *Picea*, *Cedrus* and *Abies* and thus have a very similar distribution to *Lactarius* sect. *Deliciosi*. An Eocene origin was proposed for the matsutake species-complex (CHAPELA & GARBELOTTO, 2004). A western North-American angiosperm-associated ancestor is assumed to have evolved into an increasingly specialized conifer symbiont. From these origins, matsutake appear to have followed migratory routes parallel to those of coniferous hosts. Patterns of vicariance between eastern North America and eastern Asia are well resolved and their origins were suggested to stem from migration through the Bering land bridge (CHAPELA & GARBELOTTO, 2004). The possibility that migration into Europe and Asia occurred through Atlantic bridges was rejected based on the analysis of genetic dissimilarity and geographical distance (CHAPELA & GARBELOTTO, 2004). Instead, African and European matsutake appear to be the most recent ends of a westward expansion of the area of these fungi form North America (CHAPELA & GARBELOTTO, 2004). Such a hypothesis could also be formulated for *Lactarius* sect. *Deliciosi* but a possible place of origin of this section cannot be inferred from our phylogenetic analyses. The ITS nrDNA data point towards a basal position of a clade containing *L. salmonicolor*, *L. laeticolor* and *L. thyinos* found in Europe, eastern Asia and eastern North America respectively. The analyses of the *gpd* sequences suggest that the Asian *L. subindigo* is basal to the rest of the section. *Lactarius* sp.2, an undescribed species found in Hunan, China and the eastern North American *L. salmoneus* are also basal taxa.

The low resolution we obtained in the basal clades of *Lactarius* sect. *Deliciosi* might give a clue on the history of the ancestors. A rapid speciation event, before migration became impossible, can explain that the relationships between the clades composed of species occurring on different continents are obscured. The ancestors that arose at that time must have been very similar to the extant species. Species in more distant clades of the inferred phylogenies are, after all, morphologically strikingly similar. This rapid speciation might, among other biological and analytical factors, also have caused the incongruence between the ITS and *gpd* signal.

A very different biogeographic pattern is for example observed in *Lactarius volemus* (Fr.: Fr.) Fr. a member of *Lactarius* subgenus *Lactiflui* (Burl.) Hesler & A.H. Sm. that is on morphological grounds believed to be a more ancient subgenus than *Lactarius* subgenus *Piperites* to which *Lactarius* sect. *Deliciosi* belongs (VERBEKEN, 1996). *Lactarius volemus* seems to occur in North America as well as Asia and Europe. No morphological differences are observed between the American, European and Asian specimens but it should be remarked that *L. volemus* is a morphologically very variable species. This conspecificity has not yet been examined molecularly or with compatibility experiments and thus we cannot exclude the existence of cryptic species. It anyway seems that speciation has not taken place to the same extent in *L. volemus* and close relatives as in *Lactarius* sect *Deliciosi*. We have no clue however on a possible explanation of this hypothesis. A switch of host tree for example seems to be one of the possible forces for a rapid speciation event (DEN BAKKER, 2004).

Another interesting aspect for that matter is the host specificity in *Lactarius* sect. *Deliciosi.* The well-documented host specificity of the European species has not been studied or noticed as much in America or Asia. *Lactarius indigo* is apparently associated with both *Pinus* and *Quercus*. Moreover, inoculation experiments with a Pacific Northwest American "*L. deliciosus*" isolate showed that ectomycorrhiza could be formed with a whole range of host tree genera (*Larix*, *Picea*, *Pinus, Pseudotsuga* and *Tsuga*; MOLINA & TRAPPE, 1982a). HESLER & SMITH (1979) and METHVEN (1997) mention both *Picea* and *Pinus* as hosts for the complex of varieties distinguished in the American "*L. deliciosus*". HUTCHISON & PICHÉ (1995) found that an isolate of *L. subpurpureus* could under in vitro synthesis conditions only develop ectomycorrhiza on its host *Tsuga canadensis*, and not with *Abies*, *Picea*, *Pinus*, *Larix*, *Betula* or *Alnus*.

4. Utility of morphological characters to establish natural relationships

As explained above, several striking features of members of *Lactarius* sect. *Deliciosi* that are useful for identification, might not be phylogenetically informative or might contain only very restricted information on the natural relationships between the taxa. Examples are the colour and discolouration of the latex on the context and the general appearance of the basidiomes (colour, zonation of the pileus, occurence of scobicules on the stipe, ...). Molecular data suggest that convergent and parallel evolution are very common processes in *Lactarius* sect. *Deliciosi* and fungi in general.

Another striking example of this phenomenon is that the change in morphology from agaricoid to gasteroid, including all transitional forms, has been achieved very frequently (and much more than until recently accepted) across all taxonomic levels in the homobasidiomycetes. In this thesis two examples of gasteroid representatives of *Lactarius* were examined. *Lactarius rubriviridis* with its red-coloured latex and greenish bruising is very reminiscent of the epigeous members of *Lactarius* sect. *Deliciosi*. Moreover, it is very similar in the size and ornamentation of the ballistospores to its closest relative, *L. barrowsii*. This picture is not so clear in the case of *L. stephensii* (Berk.) Verbeken & Walleyn. We show in this study that *L. stephensii* is a member of *Lactarius* subgenus *Piperites* and that *L. stephensii* and *L. borzianus* (Cavara) Verbeken & Nuytinck are less closely related than all literature data suggest. Micromorphological features, such as the structure of the peridiopellis, support this difference. A distinct ixocutis, present in most representatives of *Lactarius* subgenus *Piperites*, is absent in *L. stephensii*, but this is maybe not surprising in a hypogeous basidiocarp. The absence of acrid latex, another common feature in *Lactarius* subgenus *Piperites*, could also be explained as an adaptation to a hypogeous life style, where spore dispersal depends on animal attraction. Most strikingly, both *L. stephensii* and *L. borzianus* form spores with a spiny ornamentation, a very uncommon spore type among European representatives of the genus *Lactarius*. This, again, shows that many of the traditionally used morphological characters are probably less valuable phylogenetically then previously accepted.

5. Future perspectives

Lactarius and *Russula* are placed in the order Russulales together with some gasteroid and hypogeous taxa (e.g. *Zelleromyces*, *Macowanites*, *Arcangeliella*) and some former Aphyllophorales (e.g. *Auriscalpium*, *Bondarzewia*, *Hericium*, *Peniophora*; LARSSON & LARSSON, 2003). Most recent molecular approaches trying to elucidate the relationships in the genera *Russula* and *Lactarius* have not yet resulted in well-resolved phylogenies (EBERHARDT, 2002; MILLER *et al.*, 2001). The deeper relationships of other large genera, e.g. *Cortinarius* (PEINTNER *et al.*, 2001 & 2004), also remain unresolved using ITS and/or LSU sequences. Resolution and support in a phylogeny can be improved by increasing the number of characters and by increasing taxon sampling. In the case of large genera like *Russula* and *Lactarius* it seems appropriate to focus on smaller clades and include samples from a wide geographic range to resolve the taxonomic and phylogenetic problems step by step.

The sampling we did for this molecular study was very complete. All known species were included and for the majority of the species also several specimens were included. However, based on the information of two independent nuclear DNA regions, the deeper clades in the phylogenies we obtained are not well resolved. To obtain a more robust and reliable image of the relationships in *Lactarius* sect. *Deliciosi* more characters will have to be sampled. Examples of DNA regions that have been successfully applied to resolve relationships within genera are the largest subunit of RNA polymerase II (RPB1; MATHENY *et al.*, 2002), the intergenic spacer (IGS; VELLINGA, 2001) and the elongation factor 1-α (EF1-α).

In general, apart from the need of more molecular taxonomic studies on species level, including collections from a wide geographic range, there remains much basic taxonomical work to be done, also in well known species groups as *Lactarius* sect. *Deliciosi*. More sampling is needed from underexplored regions such as Asia and special attention needs to be paid to detailed macro- and micromorphological descriptions.

Summary

Lactarius section *Deliciosi* (Russulales, Basidiomycota) and its ectomycorrhiza: a morphological and molecular approach

The major part of this thesis deals with the taxa classified in *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.). Representatives of this section have bright coloured latex and basidiocarps. The latex is bright orange, yellowish, deep red or blue, the basidiocarps are often paler in colour and sometimes have more brownish to greyish tinges. Most taxa show a more or less slow discolouration to greenish when old or when bruised. One of the most important features for the identification of the species in this section is the rate of colour change of the latex on the context. Many species have orange latex at first that turns red and finally green. These striking colours and their discolouration can be explained by the presence of pigments with a guaiane sesquiterpenoid skeleton that are enzymatically modified when the basidiocarps are bruised (SCHMITT, 1974; STERNER & ANKE, 1995). It was shown during this study however, that *L. porninsis* Rolland, a European species with white latex traditionally classified in *Lactarius* sect. *Zonarii* (Quél.) Bon (nom. inval.), also belongs to this section. Moreover, *L. rubriviridis* Desjardin, Saylor & Thiers, a hypogeous species with red latex, is also a member of *Lactarius* sect. *Deliciosi*. This means the definition of the section has to be significantly broadened.
Most species form an ectomycorrhizal association with coniferous trees. This association is known to be very specific for the European species in *Lactarius* sect. *Deliciosi*. For *L. indigo* (Schwein.) Fr. and *L. subindigo* Verbeken & E. Horak, *Quercus* and *Castanopsis* (members of the Fagales) are a.o. reported as hosts (HESLER & SMITH, 1979; VERBEKEN & HORAK, 2000). Being obligatory associated with Pinaceae and/or Fagales, members of *Lactarius* sect. *Deliciosi* show a natural distribution that is mainly limited to the northern hemisphere. In many areas these species are considered popular edible mushrooms. This explains the recent interest to develop methods for the artificial inoculation of conifers with e.g. *L. deliciosus* (L.: Fr.) Gray or *L. sanguifluus* (Paulet) Fr. (GONZÁLEZ-OCHOA *et al.*, 2003; GUERIN-LAGUETTE *et al.*, 2000; PARLADE *et al.*, 2004).

We started this study with the investigation of the phylogeny of the European species in *Lactarius* sect. *Deliciosi* employing both molecular and morphological characters (chapter 4). The nrDNA ITS sequences and an 800 bp fragment of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*) were sequenced for this purpose. The inferred phylogeny confirms that the European species in *Lactarius* sect. *Deliciosi* form a monophyletic group within *Lactarius* subgenus *Piperites* (Fr. ex J. kickx f.) Kauffman. We show that the section contains nine, generally accepted species in Europe, eight of which are traditionally being placed in *Lactarius* sect. *Deliciosi*. *L. porninsis*, a species with white latex, also falls within in *Lactarius* sect. *Deliciosi*. Some recently described species are not confirmed here (*L. sanguineovirescens* Fillion) or their status remains doubtful because of a lack of collections (*L. cyanopus* Basso). AFLP results show a clear distinction between the closely related *L. deterrimus* Gröger and *L. fennoscandicus* Verbeken & Vesterh. *L. vinosus* (Quélet→) Bataille is often considered as a variety of *L. sanguifluus*. Morphological (especially macroscopical characters and spore-ornamentation) and molecular arguments (based on ITS sequences) are convincing enough to treat it as a separate species (chapter 3).

In chapter 5, a phylogenetic analysis of *Lactarius* sect. *Deliciosi* was performed based on collections of all known species. For most species, several samples were included, originating from a wide geographic range. The monophyly of the section and its position within *Lactarius* subgenus *Piperites*, as proposed in recent morphology-based classification schemes, is confirmed. The two DNA regions we used (ITS and a part of the gene encoding glyceraldehyde-3-phosphate dehydrogenase), show an incongruent phylogenetic signal. Much attention was paid to carefully observed macro- and micromorphological characters to draw taxonomic conclusions. We currently accept 38 taxa (31

species en 7 varieties) in *Lactarius* sect. *Deliciosi* on a worldwide scale; four species are new to science. More sampling is needed to resolve the status of the North American varieties, especially those of "*L. deliciosus*". Our knowledge of the Asian species in this section remains fragmentary. The intrasectional relationships do not coincide with the colour of the latex (as previously supposed). Intercontinental conspecificity is very low in general. The name *L. deliciosus* is wrongfully applied in North and Central America and only two species seem to occur in both Asia and Europe.

Elaborate macro- and micromorphological descriptions are presented for the representatives of *Lactarius* sect. *Deliciosi* in Europe, North and Central America and Asia (chapters 6-8), together with taxonomical and nomenclatural remarks, partly based on the previous molecular phylogenetic analyses. Ten European species, thirteen American species and nine Asian species are treated. Type specimens for most taxa were examined. An identification key is provided for the European representatives. Lectotypes are designated for *L. sanguifluus*, *L. semisanguifluus* R. Heim & Leclair and *L. vinosus*. Two new species, one from Java and one from California, will be described. Two other new species were discovered but lack detailed macroscopical notes. Several unidentified and insufficiently known taxa are briefly discussed. *L. deliciosus* var. *tenuis* Naveau is excluded from *Lactarius* sect. *Deliciosi*.

The ectomycorrhizae of six of the ten European species in *Lactarius* sect. *Deliciosi* are described and illustrated morphologically and anatomically in chapter 9. All identifications were verified by comparing the ITS sequence of the ectomycorrhizae with those of the basidiocarps growing nearby. The descriptions of the ectomycorrhizae are compared with each other and with the available literature data. We conclude that the resemblance between the ectomycorrhizae is too high to allow a sure identification of all species based on morphological or anatomical features, but the strong host specificity and the preference for certain environmental conditions might aid in identification.

Lactarius is one of the larger genera of ectomycorrhizal Basidiomycota, with about 400 species recognized worldwide. The ectomycorrhizae formed by *L. tesquorum* Malençon on *Cistus* sp., one of the most common and ecologically relevant shrubs present in the semi-arid regions in the Mediterranean basin, are described in terms of morphological, anatomical and molecular features in chapter 10. An ITS rDNA sequence-based phylogenetic analysis of the related European *Lactarius* taxa (*L. mairei* Malençon, *L. pubescens* Fr., *L. scoticus* Berk. & Broome, *L. spinosulus* Quél., *L. torminosulus* Knudsen & T. Borgen and *L. torminosus* (Schaeff.: Fr.) Pers.) currently classified together with *L. tesquorum* in *Lactarius* subgenus *Piperites* section *Piperites* (Fr. ex J. Kickx f.) Burl., was carried out. *Lactarius* sect. *Piperites* s.s. can be divided into two well-supported clusters: on one side *L. torminosus* and *L. torminosulus* and on the other side *L. tesquorum*, *L. scoticus* and *L. pubescens*. *L. mairei* and especially *L. spinosulus* seem to be less closely related to these taxa. This study is part of a broader framework, aiming at extending our knowledge of the distribution, phylogeny and ectomycorrhizal biology of *Lactarius* species in selected ecosystems.

Chapter 12 deals with an interesting collection of a hypogeous species in Belgium. Molecular data confirm that *Zelleromyces stephensii* (Berk.) A.H. Sm. and *Arcangeliella borziana* Cavara (syn. nov.: *Arcangeliella volemoides* K. Mader & A. Mader) are two distinct *Lactarius* species. It is shown that *Zelleromyces stephensii* is actually a member of *Lactarius* subgenus *Piperites.* The taxonomic value of several morphological characters previously used in the systematics of the Russulaceae (syn. Elasmomycetaceae) is discussed. The new combinations *Lactarius borzianus* (Cavara) Verbeken & Nuytinck and *Lactarius stephensii* (Berk.) Verbeken & Walleyn are proposed. A lectotype is designated for *Hydnangium monosporum* Boud. & Pat. Its synonymy with *Lactarius stephensii*, as suggested by GROSS (1990), is confirmed.

Samenvatting

Lactarius sectie *Deliciosi* (Russulales, Basidiomycota) en hun ectomycorrhiza: een morfologische en moleculaire benadering

Deze scriptie behandelt in hoofdzaak de melkzwammen die in *Lactarius* sect. *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walleyn (syn. sect. *Dapetes* (Fr. ex J. Kickx f.) Burl.) geklasseerd worden. Vertegenwoordigers van deze sectie bezitten opvallend gekleurde melk en vruchtlichamen. De melk kan helder oranje, gelig, donker rood of blauw gekleurd zijn; de vruchtlichamen zelf zijn vaak fletser gekleurd en vertonen dikwijls meer bruine en grijze tinten. De vruchtlichamen van de meeste taxa verkleuren in min of meerdere mate groen wanneer ze oud zijn of beschadigd worden. Eén van de belangrijkste kenmerken voor het op naam brengen van soorten in deze sectie is de snelheid waarmee de melk verkleurt op het vlees. Bij veel soorten is de melk eerst oranje, wordt dan rood en verkleurt uiteindelijk groen. De verklaring voor deze felle kleuren ligt bij de aanwezigheid van pigmenten met een sesquiterpenoid skelet; deze worden enzymatisch gemodificeerd bij beschadiging van de vruchtlichamen (SCHMITT, 1974; STERNER & ANKE, 1995). De resultaten van het voorliggend onderzoek wijzen er echter op dat *L. porninsis* Rolland, een Europese soort met witte melk die traditioneel in *Lactarius* sect. *Zonarii* (Quél.) Bon (nom. inval.) geplaatst wordt, ook tot *Lactarius* sect. *Deliciosi* behoort. Bovendien is ook *L. rubriviridis* Desjardin, Saylor & Thiers, een hypogeë soort met rood gekleurde melk, een lid van deze sectie. Dit betekent dat de definitie van *Lactarius* sect. *Deliciosi* moet herzien worden.
De meeste soorten uit deze boeiende sectie vormen ectomycorrhiza met naaldbomen. Deze symbiose is gekend als zeer specifiek voor de Europese soorten. Voor *L. indigo* (Schwein.) Fr. en *L. subindigo* Verbeken & E. Horak worden naast naaldbomen echter ook *Quercus* en *Castanopsis* (leden van de Fagales) als gastheer gerapporteerd (HESLER & SMITH, 1979; VERBEKEN & HORAK, 2000). Wegens de obligate symbiose met Pinaceae en/of Fagaceae is de natuurlijke verspreiding van *Lactarius* sect. *Deliciosi* hoofdzakelijk beperkt tot het noordelijk halfrond. In veel streken behoren deze soorten tot de populaire eetbare paddestoelen. Dit verklaart ook de vele recente inspanningen om naaldbomen artificieel te inoculeren met bvb. *L. deliciosus* (L.: Fr.) Gray of *L. sanguifluus* (Paulet) Fr. (GONZÁLEZ-OCHOA *et al.*, 2003; GUERIN-LAGUETTE *et al.*, 2000; PARLADE *et al.*, 2004).

In een eerste fase van dit onderzoek werden de evolutionaire verwantschappen van de Europese vertegenwoordigers van *Lactarius* sect. *Deliciosi* bestudeerd met behulp van zowel moleculaire als morfologische kenmerken (hoofdstuk 4). Hiervoor werden de nrDNA ITS regio en een 800-bp fragment van het gen dat codeert voor glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gesequeneerd. De resultaten bevestigen dat de Europese soorten in *Lactarius* sect. *Deliciosi* een monofyletische groep vormen binnen *Lactarius* subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman. Negen algemeen erkende Europese soorten behoren tot deze sectie: acht soorten die traditioneel in *Lactarius* sect. *Deliciosi* geplaatst worden en *L. porninsis* met witte melk. Verschillende, zeer recent beschreven soorten worden niet bevestigd in onze analyses (bvb. *L. sanguineovirescens* Fillion) of hun status blijft twijfelachtig door het gebrek aan collecties (*L. cyanopus* Basso). Resultaten van een AFLP studie tonen een duidelijk verschil tussen de dicht verwante *L. deterrimus* Gröger en *L. fennoscandicus* Verbeken & Vesterh. *L. vinosus* (Quélet→) Bataille wordt dikwijls als variëteit onder *L. sanguifluus* geplaatst. Morfologische (vnl. macroscopische en spore kenmerken) en moleculaire argumenten (ITS sequenties) tonen echter dat beide als aparte soorten moeten beschouwd worden (hoofdstuk 3).

In hoofdstuk 5 werd een fylogenetische analyse uitgevoerd die collecties van alle gekende soorten in deze sectie omvat. Voor de meeste soorten werden meerdere specimens, met een brede geografische verspreiding, geïncludeerd. Weer werd bevestigd dat *Lactarius* sect. *Deliciosi* een monofyletische groep vormt in *Lactarius* subgenus *Piperites*, zoals ook in de meest recente, op morfologie gebaseerde classificaties wordt aangenomen. De twee DNA regio's die gebruikt werden (ITS en *gpd*), tonen een incongruent fylogenetisch signaal. Voor het nemen van taxonomische conclusies werd voornamelijk rekening gehouden met waargenomen macro- en microscopische verschillen. We aanvaarden 38 taxa (31 soorten en 7 variëteiten) in deze sectie op een wereldwijde schaal; vier van hen zijn nieuw voor de wetenschap. Er moet meer ingezameld worden om de status van de Noord-Amerikaanse variëteiten op te helderen, voornamelijk deze binnen "*L. deliciosus*". Onze kennis van de Aziatische soorten blijft fragmentair. De kleur van de melk weerspiegelt niet correct de evolutionaire verwantschappen tussen de soorten zoals voordien vaak aangenomen. Er lijken zeer weinig soorten conspecifiek te zijn tussen de continenten. De naam *L. deliciosus* wordt onterecht gebruikt in Noord- en Centraal-Amerika en slechts twee soorten lijken zowel in Azië als Europa voor te komen.

De macro- en microscopie van de vertegenwoordigers van *Lactarius* sect. *Deliciosi* uit zowel Europa, Noord- en Centraal-Amerika als Azië worden uitgebreid beschreven en geïllustreerd in hoofdstuk 6 tot 8. Belangrijke nomenclatorische en taxonomische opmerkingen zijn voor een groot deel gebaseerd zijn op de eerdere moleculaire resultaten. Tien Europese, dertien Amerikaanse en negen Aziatische soorten worden behandeld. Een sleutel werd opgesteld voor de Europese soorten. De type specimens werden voor de meeste soorten bestudeerd. Er werden lectotypes aangeduid voor *L. sanguifluus*, *L. semisanguifluus* R. Heim & Leclair en *L. vinosus*. Twee nieuwe soorten, respectievelijk uit Java en Californië, worden beschreven. Twee andere Aziatische onbeschreven soorten zijn onvoldoende morfologisch gedocumenteerd om als nieuw te beschrijven. Verschillende niet geïdentificeerde en onvoldoende gekende taxa worden kort besproken. *L. deliciosus* var. *tenuis* Naveau wordt geëxcludeerd uit *Lactarius* sect. *Deliciosi*.

De ectomycorrhizae van zes van de tien Europese soorten in *Lactarius* sect. *Deliciosi* worden morfologisch en anatomisch beschreven en geïllustreerd in hoofdstuk 9. Identificatie van de ectomycorrhizae gebeurde door het vergelijken van de ITS sequentie van de ectomycorrhizae met deze van de nabij groeiende vruchtlichamen. De beschrijvingen van deze zes soorten worden onderling en met beschikbare literatuur gegevens vergeleken. Hieruit blijkt dat de gelijkenis tussen de ectomycorrhizae te groot is om een betrouwbare identificatie toe te laten enkel gebaseerd op morfologische en anatomische kenmerken. De sterke specificiteit voor een bepaalde gastheer en de voorkeur voor specifieke omgevingsparameters kunnen echter helpen bij de identificatie.

Lactarius is één van de grotere genera binnen de Basidiomycota; ongeveer 400 soorten zijn gekend wereldwijd. De ectomycorrhizae die *L. tesquorum* Malençon op *Cistus* sp. vormt worden beschreven in hoofdstuk 10. *Cistus* is één van de meest algemene en ecologisch relevante struikgewassen uit de semi-ariede gebieden in de Mediterrane regio. Een fylogenetische analyse werd uitgevoerd (m.b.v. ITS sequenties) op de verwante Europese taxa (*L. mairei* Malençon, *L. pubescens* Fr., *L. scoticus* Berk. & Broome, *L. spinosulus* Quél., *L. torminosulus* Knudsen & T. Borgen en *L. torminosus* (Schaeff.: Fr.) Pers.). *Lactarius* sect. *Piperites* (Fr. ex J. Kickx f.) Burl. kan opgedeeld worden in twee goed ondersteunde clusters. Deze groeperen enerzijds *L. torminosus* en *L. torminosulus* en anderzijds *L. tesquorum*, *L. scoticus* en *L. pubescens*. *L. mairei* en vooral *L. spinosulus* lijken minder dicht verwant te zijn aan de overige taxa. Deze studie kadert in een breder onderzoek dat zich richt op het verwerven van kennis over de verspreiding, fylogenie en ectomycorrhiza van *Lactarius*-soorten in specifieke ecosystemen.

In hoofdstuk 12 wordt een interessante Belgische vondst van een hypogeë soort besproken. Moleculaire data bevestigen dat *Zelleromyces stephensii* (Berk.) A.H. Sm. en *Arcangeliella borziana* Cavara (syn. nov.: *Arcangeliella volemoides* K. Mader & A. Mader) twee verschillende *Lactarius* soorten zijn. *Zelleromyces stephensii* hoort thuis in *Lactarius* subgenus *Piperites.* De taxonomische waarde van verschillende morfologische kenmerken die eerder gebruikt werden in de systematiek van de Russulaceae (syn. Elasmomycetaceae) wordt besproken. Twee nieuwe combinaties (*Lactarius borzianus* (Cavara) Verbeken & Nuytinck en *Lactarius stephensii* (Berk.) Verbeken & Walleyn) worden voorgesteld. Een lectotype wordt aangeduid voor *Hydnangium monosporum* Boud. & Pat. en de synonymie met *Lactarius stephensii*, zoals gesuggereerd door GROSS (1990), wordt bevestigd.

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Appendix

Nomenclator of *Lactarius* section *Deliciosi*

Jorinde Nuytinck & Ruben Walleyn

This nomenclator lists all the names we could find, that were used for taxa belonging to *Lactarius* sect. *Deliciosi*. It includes 74 names and does not embrace any taxonomic opinion. For each name the reference to the protologue is given plus its nomenclatural synonyms (where present), etymology, original description or diagnosis, type and type studies (where present). Under protologue we refer to the publication where everything associated with the name on its first publication is found, i.e. diagnosis, description, references, synonymy, geographical data, citation of specimens, discussion, comments, illustrations… For every name we mention whether or not (and where) it is indexed in one of four major works/nomenclators: IF (Index of Fungi, CABI), PL (Petrak's List), SF (Sylloge Fungorum, SACCARDO, 1882-1925 [-72]) or SO (Saccardo's Omissions, KIRK, 1985). All names have been published validly and legitimately, unless stated otherwise. Herbarium abbreviations are according to HOLMGREN *et al.* (1990).

Nomenclator

abietinus

- Protologue:

Lactarius deliciosus var. *abietinus* Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 219 (1947) [not indexed], nom. inval., nom. nud.

- Etymology:

Derived from its host tree genus: *Abies*

- Original description:

Sine diagn. Lat.

- Type:

Not typified

aeruginosus

- Protologue:

Lactarius deliciosus var. *aeruginosus* E. Bommer & M. Rousseau, Bull. Soc. Roy. Bot. Belgique 18: 94 (1879) [not indexed], nom. inval., nom. nud.

- Etymology:

aeruginosus (Lat.) = verdigris

- Original description:

Sine diagn.

- Type:

Not typified

akahatsu (Fig. 1)

- Protologue:

Lactarius akahatsu Tanaka, Bot. Mag. (Tokyo) 4: 394 (1890) [SF 14: 95, 19: 1023]

- Nomenclatural synonyms:

≡ *Lactarius hatsudake* var. *akahatsu* (Tanaka) Kawam., Bot. Mag. (Tokyo) 28: 525 (1914) [not indexed]

- Etymology:

Derived from the Japanese common name for this species: aka hatsu (take) (aka (Jap.) = red; hatsu (Jap.) = first; take (Jap.) = mushroom)

- Original description:

Pileus fleshy, 2-8 cm. broad, at first hemispherical, then expanded, umbilicate, viscid, obsolutely zoned, smooth, yellowish red, then pale; margin smooth, at first incurved, paler, at length slightly repand, rather thin and acute; stem 1-4 cm. long, nearly equal, curved, stuffed, then hollow, yellowish, pruinose; gills decurrent, orange, then greenish, rather thin (narrower than those of *L. Hatsudake*), broadest in front; juice orange, aromatic; spores echinulate, nearly spherical or avoid, subhyaline, 9-11 µ. long and 6-8 µ. in diameter.

- Type:

Not typified

areolatus

- Protologue:

Lactarius deliciosus var. *areolatus* A.H. Sm., Brittonia 12: 135 (1960) [IF 3: 11]

- Etymology:

areolatus (Lat.) = areolate, marked out into small, usually angular spaces

- Original description:

Pileus 5-12 cm latus, glaber, subviscidus, azonatus vel subzonatus, demum areolatus, obscure ruber vel rubro-aurantiacus; caro incisa deinde rubra, demum viridis; lamellae latae, confertae aurantiacae demum viridis; sporae 8-11 x 7-8 µ. Specimen typicum legit A. H. Smith (No. 46914) 27 Aug. 1954, Payette Lakes, Idaho, U.S.A.

- Type:

Holotypus: Smith 46914 (MICH!)
North America, USA: Idaho, Payette Lakes

- Type studies:

HESLER & SMITH (1979)

asiaticus

- Protologue:

Lactarius sanguifluus var. *asiaticus* Dörfelt, Kiet & A. Berg, Feddes Repert. 115: 169 (2004) [not indexed]

- Etymology:

asiaticus (Lat.) = originating from Asia

- Original description:

Basidiomata differunt varietate propria, cum minoribus basidiomatis et minoribus sporis.
Holotypus: specimen V 107, HAL fungi 1766, Isotypus Herbarium Universitatis Ha Noi, specimen Kiet Bm 38. 08.09.1999, Vietnam, Province Thua Thien, Hue, Bach Ma Nationalpark, Wegrand, ca. 1300 m üNN.

- Type:

Holotypus: V 107 fungi 1766 (HAL)
Asia, Vietnam: Prov. Thua Thien, Hue, Bach Ma National Park

atrovirens

- Protologue:

Lactarius deliciosus var. *atrovirens* J. Blum, Lactaires: 217 (1976) [IF 4: 476]

- Etymology:

atro- (Lat.) = black, dark; virens (Lat.) = green

- Original description:

a typo differt pileo minusculo, 3-4 cm, atrovirido sicut lamellis stipitoque. Récolte 1344, Dourdan (91-France).

- Type:

Holotypus: Blum 1344 (Type missing from PC)
Europe, France: Dourdan

barrowsii

- Protologue:

Lactarius barrowsii Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 74 (1979) [IF 5: 52]

- Etymology:

Named in honour of the collector: Mr. Barrows.

- Original description:

Pileus 3-10 cm latus, convexo-depressus, pallide incarnato-cinnamomeus, demum albus, viscidus; latex sanguifluus vel vinaceoruber; lamellae ochraceae tactu viridescens; stipes 2-4 cm longus, ± 2.5 cm crassus, siccus; sporae 8-10.5 x 6-7.5 μ; macrocystidia nulla. Specimen typicum in Herbarium University of Michigan conservatum est; legit prope Santa Fe, New Mexico, Barrows 35, September, 1969.

- Type:

Holotypus: Barrows 35 of 1969 (MICH!)
North-America, USA: New Mexico, near Santa Fe

brigantiacus

- Protologue:

Lactarius salmonicolor f. *brigantiacus* L. Remy, Bull. Trimestriel Soc. Mycol. France 80: 487 “1964” (1965) [not indexed], nom. inval., nom. nud.

- Etymology:

Described from the region of Briançon

- Original description:

Sine diagn. Lat.

- Type:

Not typified

canadensis

- Protologue:

Lactarius canadensis Winder, Mushrooms Canada, addenda [24] (1871) [SO: 35]

- Etymology:

Described from Canada

- Original description:

It is from 4 to 8 inches in diameter, the gills are crowded, and run down the ringless stem. The pileus, at first is convex, at length concave; the plant is firm and woody, and grows in autumn, in pine woods. When the plant is bruised, bright blue milk exudes, which soon changes to green. This plant is not edible.

- Type:

Not typified

carneus

- Protologue:

Lactarius rubrozonatus f. *carneus* Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003 ("2002")) [not indexed]

- Etymology:

carneus (Lat.) = flesh-coloured

- Original description:

A typo differt colore sporophororum tenuiori albido-rosato.
Typus: 29.10.93, L'Aquila, S. Giuliano, AQ (93/8), in Herbario Mycologico Aquilano (AQUI) servatus

- Type:

Holotypus: AQ 96/22 (AQUI)
Europe, Italy: L'Aquila, S. Giuliano

cheilidonioides

see chelidonioides

chelidonioides

- Protologue:

Lactarius chelidonioides A.H. Sm., Brittonia 12: 127 (1960) [IF 3: 11]

- Nomenclatural synonyms:

≡ *Lactarius chelidonium* var. *chelidonioides* (A.H. Sm.) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 84 (1979), as '*cheilidonioides*' [IF 5: 52]

- Etymology:

Named after *L. chelidonium,* -oides (Greek) = like, resembling

- Original description:

Pileus 3-8 cm latus, convexo-depressus demum subinfundibuliformis, glaber, viscidus, zonatus, subcaeruleus demum sordide aurantio-brunneus; lamellae confertae, decurrentes, sordide luteo-olivaceae; stipes siccus, sursum subcaeruleus, deorsum pallide sordide aurantio-luteus; sporae 7-9 x 5-6.5 µ; pleurocystidia nulla. Specimen typicum legit A. H. Smith (no. 62026), 12 Oct 1959, Dexter, Michigan, U.S.A.

- Type:

Holotypus: Smith 62026 (MICH!)
North America, USA: Michigan, Dexter

- Type studies:

HESLER & SMITH (1979)

chelidonium

- Protologue:

Lactarius chelidonium Peck, Rep. (Annual) New York State Mus. Nat. Hist. 24: 74 (1872) [SF 5: 439, 12: 978, 19: 1024]

- Etymology:

Named after (the juice of) the plant *Chelidonium* (*majus*)

- Original description:

Pileus fleshy, firm, centrally depressed, smooth, slightly viscid, of a grayish-green color with blue and yellow tints and a few narrow zones on the margin; lamellae close, narrow, forked and wavy at the base, sometimes anastomosing, grayish yellow; stem short, subequal, smooth, hollow, colored like the pileus; spores yellowish, 1/3000' in diameter.

Plant 2' high, pileus 2' broad, stem 4"-6" thick. Sandy soil about pine trees. Saratoga. October.

Taste mild, milk sparse, of a yellowish color resembling the juice of Celandine or the liquid secreted from the mouth of grasshoppers. The flesh when wounded is at first stained with a color like the milk, then changes to blue and finally to green. Closely allied to *L. deliciosus*, from which it differs in its more narrow lamellae, differently colored milk, smaller spores, etc.

- Type:

Holotypus: Peck, New York, Saratoga; lost (NYS)
North America, USA: New York, Saratoga

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)

curtisii

- Protologue:

Lactarius curtisii Coker, J. Elisha Mitchell Sci. Soc. 34: 41 (1918) [not indexed]

- Nomenclatural synonyms:

≡ *Lactarius salmoneus* var. *curtisii* (Coker) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 72 (1979) [IF 5: 53]

- Etymology:

Name in honour of the American mycologist M.A. Curtis (1808-1872)

- Original description:

Cap 1.5-6 cm., usually about 3.5-5 cm. wide, deeply depressed (umbelicate) in center, the margin inrolled until maturity or after, sometimes expanded in age, rounded or nearly plane, often irregular and with a deep sinus on one side; surface quite smooth, dry, and chalky looking azonate, chalk-white except for the brownish-yellow stains, turning light or orange salmon when bruised. Flesh quite firm and solid, about 8 mm thick near stem in large plants, turning a deep orange salmon near the gills when cut, light orange salmon elsewhere, in age not turning salmon; mildly peppery when young, hardly so when old. Milk scant, a beautiful deep orange salmon, mildly peppery, disappearing in age. Gills moderately close to somewhat distant, not forked, slightly decurrent, only 3 mm deep in large plants; color when young a fine orange salmon, after some time turning a smoky olive when bruised, fading to pallid ochraceous-buff with a tint of orange in age, smoky-olive in drying. Strem very short, only 0.6-1.5 cm long, and 7-10 mm thick at cap, tapering downward, quite smooth and even, light orange salmon with a whitish pellicle, hollow, often eccentric. Flesh like that of the cap, turning deep orange salmon near surface when cut. Spores (of No. 1845) cream color, subsphaerical to elliptic, distinctly tuberculate, a large oil drop, 6-6.5 x 7.5-8.7 µ [...] 1845. On top of hill southwest of Sparrow's Mill, in pasture, with small pines, September 19, 1915. Photo. Type.

- Type:

Holotypus: Coker 1845 (NCU)
North America, USA: North Carolina, hill southwest of Sparrow's Mill

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)

cyanopus

- Protologue:

Lactarius cyanopus Basso, Bull. Trimestriel Soc. Mycol. France 114: 67 (1998) [IF 6: 1094]

- Etymology:

cyano- (Greek) = blue; -pus (Greek) = -footed

- Original description:

Pileus 3-8 cm latus, modice carnosus, plano-convexus dein plano-depressus, novellus aequalis deinde usque ad undulatum lobatum. Cuticula aëre udo viscida, etiamque lucida in aëre sicco, colore livido tincta, eburneo-caerulescente, aurantio-caerulescente, interdum zonis signata plus minusve distinctis, caeruleo-virescentibus, aetate autem colore pallidiorem attingens. Lamellae e subdecurrentibus decurrentes, arcuatae, mediocri intervallo dispositae, fragiles, cremeo-lividae, pallide cremeo-aurantiacae vel cremeo-virescentes, fricatu colore cyaneo maculatae. Stipes cylindraceus, brevior quam pileus latus, 1,7-5 x 0,9-2,1 cm, initio plenus firmusque, mox cavus; colore caeruleo vel caeruleo-virescente superne tinctus, basi autem albido-caerulescens etiamque maculis ochraceis signatus. Nullo modo aut raro solum scrobiculatus. Caro mollis, mox ex insectorum larvis violata, sub cuticola albida, gradatimque caerulea (dein virescens) lamellarum iuncturam versus atque sub cortice usque ad dimidium fere stipitem; aurantiaca dein rubescens, denique viridi-cyanea in dimidia inferiore stipitis parte vel basi modo. Fructum redolens, sapore grato, nec acri neque amaro. Lac exiguus, in pileo et in stipite ex aliqua parte colore cyaneo-virescente tincto, basi tamen aurantiaco. Sporae late ellipticae, 8-9,5(-10) x (6-)7-8 µm, crestulis latiusculis praeditae, 0,5 µm altis, zebratim dispositis, aut parte reticulatae. Plaga suprahilaris non amyloides. Basidia subclaviformia, tetrasporica, 44-69 x 11-12 µm. Cystidia multa; cheilocystidia subfusiformia, summo attenuata, 22-39 x 5-7,5 µm; pleurocystidia haud vel parum eminentia, subcylindracea, in vertice obtuso-attenuata vel muricationibus instructa, 48-65 x 5-9 µm, ex sulfovanillina inertia. Epicutis strictim gelificata, hyphis fili instar implicatis sed laxius contexta, 2,3-3,5 µm latis, obtuse desinentibus, aequalibus vel etiam moniliformibus; in subcute hyphae adsunt, reticulatim compositae, 5-6 µm latae. Caulocutis cutiformis, hyphis filiformibus constans, intricatis at paulum disiectis, quae ex partibus suis extremis parvos caespites aliquando component extantes. Laticiferae plurimae, lac ochraceo-subviride continentes. Habitat: in silvis mediterraneis sub Cisto albido, ex aliqua parte infusus, autumno provecto. Holotypus lectus prope Civezza, Imperia, Liguria, a M. T. Basso et M. Candusso, 30/10/1998 in herbario «Museo Cantonale di Storia Naturale», Lucani (Helvetia) servatur, n° LUG 8630.

- Type:

Holotypus: 8630 (LUG)
Europe, Italy: Liguria, Imperia, Civezza

- Remarks:

Accompanying french description in BASSO (1999b)

deliciosus

- Protologue:

Agaricus deliciosus L., Species Pl.: 1172 (1753) [SF 5: 438, 15: 16]

- Nomenclatural synonyms:

≡ *Lactarius deliciosus* (L.: Fr.) Gray, Nat. Arr. Br. Pl. 1: 624 (1821) [SF 5: 438, 12: 979, 19: 1026, 20: 1261]
≡ *Lactifluus deliciosus* (L.: Fr.) Kuntze, Revisio Generum Plantarum, Pars II: 856 (1891) [not indexed]

- Etymology:

deliciae (Lat.) = pleasure, delight; -osus (Lat.) = full of
Linnaeus probably named this species after a more delicious species from Southern Europe (maybe what we call now *Lactarius sanguifluus*), thinking that the species from his own region was the same.

- Original description:

Agaricus stipitatus, pileo testaceo succo lutescente. Amanita lateritii coloris, croceo succo turgens. Dill. giss. 179. Fungus perniciosus, lateritio colore varians, succum acrem & croceum fundens. Mich. gen. 141, Hall. helv. 49. Habitat in Montibus, sterilibus, sylvosis.

- Type:

Not typified

deterrimus

- Protologue:

Lactarius deterrimus Gröger, Westfäl. Pilzbriefe 7: 10 (1968) [IF 3: 487]

- Nomenclatural synonyms:

≡ *Lactarius deliciosus* var. *deterrimus* (Gröger) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 94 (1979) [5: 52]

- Etymology:

deterrimus (Lat.) = the worst (refers to the edibility)

- Original description:

Pileus 4-12 cm latus, in statu iuvenili umbilicatus, postea depressus vel infundibuliformis, levis, humide haud glutinosus, sordide ochraceus, pallide aurantiacus, virescens, anguste zonatus. Lamellae confertae, intermixtae, adnexae vel subdecurrentes, usque ad 5 mm latae, ochraceae, postea aurantiacae, post laesionem viridibus maculis variegatis. Stipe cylindraceus, glaber vel vix scrobiculatus, valde cavus, pileo concolor, supra albo-pruinosus. Caro tenuis maxime in margine, alba vel ochracea-aurantiaca, lentissime (post 20-30 min.) purpurascens, acris. Lac coloratum ut Daucus carota, exiguum. Sporae oblongae, 7,5-10/6-7,5 µm, cum humilibus reticulatis verrucis. Habitat sub piceis parvis numerosissime vigens. Typum legi in monte "Krahnberg" prope Gotham, 12.9.1963, sub piceis, humi calcis concharum. *L. semisanguifluo* Heim et Leclair affinis, sed pileo minime carnoso, stipite graciliore, cavo, glabro vel affecto scrobiculis minutis vix notatis, carne colore lentissime purpurascente et ea re, quod sub piceis occurit diversus est. Typus in "Sachsisches Landesherbar" Dresden, DDR.

- Type:

Holotypus: requested information on type not received from DR
Europe, Germany: Krahnberg, near Gotha

diminutivus

- Protologue:

Lactarius indigo var. *diminutivus* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 69 (1979) [IF 5: 52]

- Etymology:

diminutus (Lat.) = made small, diminished

- Original description:

Pileus 3-5 cm latus, demum infundibuliformis, glaber, viscidus, caeruleus demum subviridis; latex caeruleus, odor nullus; sapor mitis; lamellae caeruleae demum olivaceae; stipes 15-25 cm longus, 5-8 mm crassus, caeruleus; sporae 7-9 x 6-7.5 µ. Specimen typicum in Herbarium University of Michigan conservatum; legit in Brazoria County, Texas, 26 Dec 1970, Hillhouse 249.

- Type:

Holotypus: Hillhouse 249 (MICH!)
North America, USA: Texas, Brazoria County

fennoscandicus

- Protologue:

Lactarius fennoscandicus Verbeken & Vesterh., Cryptog. Mycol. 19: 87 (1998) [IF 6: 984]

- Etymology:

Named after the distribution area: Fennoscandia

- Original description:

L. deterrimus affinis, a quo imprimis differt pileo saepe valde zonato umbra lilacino-griseaque, sporis parvioribus (7.5-8.0 x 6.0-6.5 µm) et cheilocystidiis rarioribus parvioribusque. Holotypus: Sweden. Siljanfors, in rich mixed forest, under *Picea*, acid soil, with *Sphagnum* and *Vaccinum*, 31.08.97, leg. Morten Christiansen, Verbeken 97-530 (holotype GENT, isotypus C).

- Type:

Holotypus: Verbeken 97-530 (GENT!)
Europe, Sweden: Siljanfors

- Type studies:

HEILMANN-CLAUSEN *et al.* (1998)

flammeolus

- Protologue:

Agaricus flammeolus Pollini, Giorn. Fis. 9: 34 (1816) [SF 5: 450, 15: 20]

- Nomenclatural synonyms:

≡ *Lactarius flammeolus* (Pollini: Fr.) Fr., Epicr. Syst. Mycol.: 341 (1838) [SF 5: 450, 12: 979, 19: 1027]
≡ *Lactifluus flammeolus* (Pollini: Fr.) Kuntze, Revisio Generum Plantarum, Pars II: 856 (1891) [not indexed]

- Etymology:

flammeus (Lat.) = flame-coloured, fiery red, scarlet

- Original description:

Solitarius. Pileus colore flammeo, quandoque ad helvolum vel rubrum vergente, tres pollice latus, primum convexus, margine plano integro. Lamellae brevioribus intermixtae non decurrentes. Stipes cylindricus tres pollices circiter altus. Caro flavescens. Tota planta scatet succo rubo-croceo, leviter acri. In sylvis Baldi montis, praesertim in valle dell'Artillon atque ai Lavaci, nec non in jugis Lessiniis circa Chiesa nova et le Scandole saepe occurrit Junio, Julio et Augusto.

- Type:

Not typified

haemorrheus

- Protologue:

Lactarius haemorrheus Lowe, Grevillea 16: 121 (1888) [SF 9: 58, 12: 979]

- Etymology:

haema (Greek) = blood; rheô (Greek) = to flow

- Original description:

Pileo carnoso, convexo, demum centro depresso, saepe difformi, flavido-fusco, expallente, sericeo (2-3 unc. lat.). stipite brevi, obconico vel ventricoso, deorsum attenuato, albido, glabro, solido (1-1½ unc. long., ¾ unc. crass.). Lamellis subdistantibus, decurrentibus, incarnatis. Lacte copioso, sanguineo. On the ground. Madeira.

- Type:

Not typified

hatsudake (Fig. 1)

- Protologue:

Lactarius hatsudake Tanaka, Bot. Mag. (Tokyo) 4: 393 (1890) [SF 14: 95, 19: 1028]

- Etymology:

Derived from the Japanese common name for this species: hatsu dake or hatsu take (hatsu (Jap.) = first; dake or take (Jap.) = mushroom)

- Original description:

Pileus fleshy, 1-10 cm. broad, at first hemisphaerical, then expanded, umbilicate, viscid, zoned, smooth, dirty brown and slightly tinted with pink; margin smooth, at first incurved; stem stuffed, then hollow, pinkish, pruinose, subequal, short, 1-3 cm. long; gills decurrent, often branched, rather broad, broadest in the middle, purplish brown, then bluish; juice aromatic, dull pinkish brown; spores echinulate, nearly spherical or ovoid, subhyaline, 8-10 μ. in diameter.

- Type:

Not typified

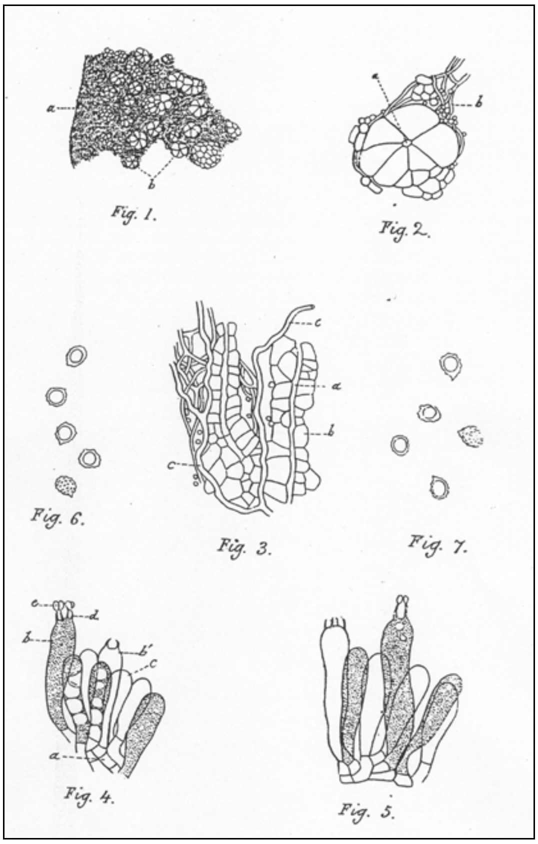

Fig. 1: Microscopic features of *L. akahatsu* and *L. hatsudake* form TANAKA (1890). Figs. 1-4 & 6: *L. hatsudake;* Figs. 5 & 7: *L. akahatsu.*

hemicyaneus

- Protologue:

Lactarius hemicyaneus Romagn., Rev. Mycol. (Paris) 23: 280 (1958) [IF 6: 148]

- Nomenclatural synonyms:

≡ *Lactarius deliciosus* var. *hemicyaneus* (Romagn.) Krieglst., Beitr. Kenntn. Pilze Mitteleuropas 7: 61 (1991) [IF 6: 211], nom. inval., no reference was made to the basionym

≡ *Lactarius deliciosus* var. *hemicyaneus* (Romagn.) Krieglst., Mitt. Arbeitsgem Pilzk. Niederrhein 9: 76 (1991) [IF 6: 148]

≡ *Lactarius quieticolor* var. *hemicyaneus* (Romagn.) Basso, Fungi Europaei 7. *Lactarius* Pers.: 275 (1999) [IF 6: 1094]

- Etymology:

hemi- (Greek) = half-; cyaneus (Greek) = dark blue

- Original description:

Pileo 2,5-6 cm. lato, carnoso, firmo, convexo-applanato, deinde expanso, medio depresso, margine involuta, e rufulo brunneo, ad instar L. quieti, etiamque fere in senectute e fusco rufo, zonato. Stipite 2,5-4 x 0,8-1,4 cm., firmo, pleno, inferne spongioso-cavo, saepe ad basim attenuato, ubi aurantiacus est, alibi e violaceo caeruleoque griseo, maculis viridibus demum variegato, pruinoso, interdum scrobiculato. Carne crassa, firma, lacte partim vivide caerulea (modo solum sub cute, modo in media parte superiore carpophori), sed semper in parte inferiorer saltem ex aurantiaca rubra, lente rubrovinosa aere facta. Lamellis stipatis, interdum crispatis, subdecurrentibus, angustis, modo e caeruleis pallide aurantiacis, si in lamellis ipsis lac caeruleum est, modo multo vividius aurantiacis, si id ibi aurantiacum, praeterea viridibus maculis senectude vel tactu variegatis. Sporis sordide ochraceis, 7,7-9,2 x 6,2-7,7 μ., subglobosis, ample reticulatis ac fere alveolatis cristis crassis latisque, specie eximia, simili priori. Cystidiis rarissimis. - In pinetis. Verisimiliter varietas prioris.

- Type:

Holotypus: 52-218 (PC)
Europe, France: Les Landes, Morcenx

- Type studies:

HEILMANN-CLAUSEN *et al.* (1998)
BASSO (1999a)

indicus

- Protologue:

Lactarius deliciosus var. *indicus* Atri, Saini & D.K. Mann, Geobios, New Reports 10: 110 (1991), as '*indica*' [not indexed]

- Etymology:

described from India

- Original description:

Carpophoris usque ad 5 cm longo. Pileo usque ad 10 cm lato, aurantio-rubro (8A6). Latex aurantiacus. Stipite usque ad 4 cm longo, 1.5 cm crasso, obclavato, aurantio-rubro (8A6). Sporis 9-10.5 x 7.5-9 μm, amyloideis, in cumulo albido. Macrocystidiis 60-67.5 x 5.25-6 μm; cheilocystidis 39-54 x 3.75-8.25 μm. India. Typus. PUN 1560, Daljit Kumar, September 21, 1989.

- Type:

Holotypus: PUN 1560 (PUN)
Asia, India: Himachal Pradesh, Dalhousie, on the way to Khajjiyar

indigo

- Protologue:

Agaricus indigo Schwein., Schr. Nat. Ges. Leipzig 1: 7 (1822) [SF 5: 438, 15: 23]

- Nomenclatural synonyms:

≡ *Lactarius indigo* (Schwein.) Fr., Epicr. Syst. Mycol.: 341 (1838) [SF 5: 438, 12: 979, 19: 1029]

≡ *Lactifluus indigo* (Schwein.) Kuntze, Revisio Generum Plantarum, Pars II: 857 (1891) [not indexed]

- Etymology:

indigo = deep blue

- Original description:

A. L. pileo umbilicato subzonato, lamellis stipite succoque in coeruleum vergente.
Solitarius sed non rarus inter folia. Major, affinis succoque habitu et magnitudine delicioso, sed stipite longiore. Pileus tempore humido colore omnino Indigo, sicciori expallescente, tum zonis ut indelicioso notatus. Lamellarum, stipites et succi color pulcherrime e coeruleo et Indigo mixtus.

- Type:

Not typified

indigoides

- Protologue:

Lactarius deliciosus var. *indigoides* Kavina nom. inval., unpublished herbariumname (PR), cited in Schaefer Z., Schweiz. Z. Pilzk. 48 (9): 139 (1970)

- Etymology:

indigo = deep blue; -oides (Greek) = like, resembling

- Original description:

Sine diagn. Lat.

- Type:

Not typified

japonicus (Fig. 2)

- Protologue:

Lactarius deliciosus var. *japonicus* Kawam., The Japanese fungi: 55 (1929) [IF 2: 346]

- Nomenclatural synonyms:

≡ *Lactarius japonicus* (Kawam.) Lar.N. Vassiljeva, Agarics and Boletes (Agaricales) of the Primorsk Region: 302 (1973) [IF 4: 278]

- Etymology:

Described from Japan

- Original description:

[orig. diagnose in Japanese]

- Type:

Not typified

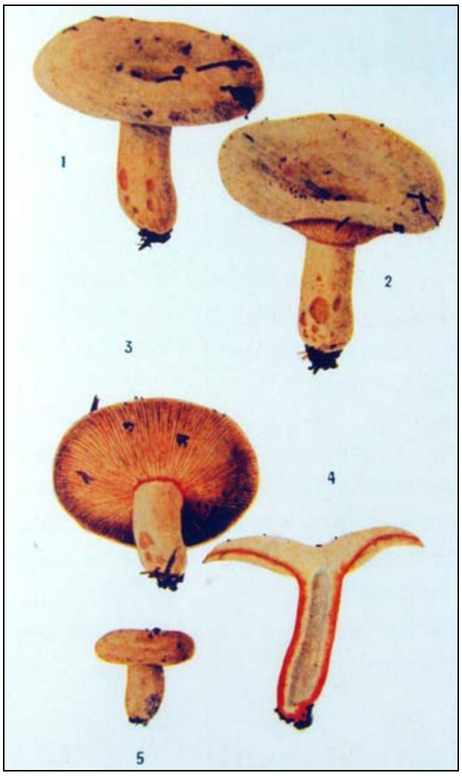

Fig. 2: KAWAMURA (1929) Fig. 1-5: *L. deliciosus* var. *japonicus* Kawam.

laeticolor

- Protologue:

Lactarius deliciosus f. *laeticolor* S. Imai, Bot. Mag. (Tokyo) 49: 607 (1935), as '*laeticolorus*' [PL 7]

- Nomenclatural synonyms:

≡ *Lactarius laeticolor* (S. Imai) Imazeki, Genshoku-Kinoko: 153 (1959), as '*laeticolorus*' [not indexed], nom. inval.

≡ *Lactarius laeticolor* (S. Imai) Imazeki ex Hongo, Acta Phytotax. Geobot. 18: 139 (1959), as '*laeticolorus*' [not indexed]

- Etymology:

laetus (Lat.) = cheerful, pleasant, bright; -color (Lat.) = -coloured

- Original description:

Vulnere non vel vix virescente.

- Type:

Holotypus: requested information on type not received from SAPS
Asia, Japan

laeticolorus

see laeticolor

lamelliporus

- Protologue:

Agaricus deliciosus var. *lamelliporus* Barla, Champ. Prov. Nice: 35 (1859) [not indexed]

- Nomenclatural synonyms:

≡ *Lactarius deliciosus* var. *lamelliporus* (Barla) Sacc., Sylloge Fungorum 5: 438 (1887)

- Etymology:

lamellatus (Lat.) = lamellate, composed of or arranged in layers or thin plates; porus (Lat.) = pore

- Original description:

Cette singulière variété de l'Agaric délicieux a les lamelles complètement recouvertes d'une poussière farineuse ou d'une matière blanche et feutrée. Lorsqu'on enlève cette matière, les lamelles paraissent comme des veines entrecroisées formant des espèces de pores ou d'alvéoles très inégales et de différentes grandeurs.
M. Venturi fait mention de cette variété (2), que Sauwerby avait déjà désignée sous le nom de Merulius helveloides, à cause de l'analogie que la membrane sporulifère de cet Agaric semble avoir avec celle des champignons du genre Mérule.
(2) Ventur., Stud. Micologici, p. 40
Fries (1) dit que cette altération (2) est produite par un petit cryptogame du genre *Sphaeria*, qui, en se développant sur les lamelles, les rend comme farineuses ou sapoudrées d'une pruine blanche et crayeuse.
Dans quelques provinces de l'Italie, on fait usage du champignon qui ofre cette particularité, et on le nomme *Lapacendro infarinato*; on l'apporte également sur notre marché, ou on le vend sous le nom de *Sanghin caussinat.* On peut le manger sans inconvénient, car la présence de cette substance blanche, qui d'ailleurs disparaît entièrement par un simple lavage et le moindre frottement, n'influe en aucune façon surs ses bonnes qualités.
Les individus dont les lamelles sont envahies par ces petits cryptogames à apparence pulvérulente, ont ordinairement le chapeau d'un fauve rougeâtre, déjeté, d'une forme moins régulière que ceux qui sont dans leur état normal, et le pédicule souvent très épais, court et aplati.
(1) Sphaeria lateritia innata, *Ag. deliciosus*, Fr., Syst. Myc. 2. p. 338. - Est *Lactarius deliciosus* a sphaeria incrustatus, Fr., Epicris., p. 532.
(2) Secretan (Myc. Suisse) fait mention de cette altération de la membrane sporulifère, et croit qu'elle dépend du développement, sur les lamelles, de la *Phlemorpha rufa*, Pers.

- Type:

Not typified

lateritius Blum

- Protologue:

Lactarius deliciosus var. *lateritius* J. Blum ex J. Blum, Lactaires: 216 (1976) [IF 4: 476]

- Based on :

Lactarius deliciosus var. *lateritius* J. Blum, Bull. Trimestriel Soc. Mycol. France 80: 293 (1964) [not indexed], nom. inval., nom. nud.

- Etymology:

lateritius (Lat.) = dark brick red, like old red tiles

- Original description:

a typo differt pileo ocraceo fulvo griseozonato. Récolte 62.2. A, Campredon, Catalogne (Espagne).

- Type:

Holotypus: Blum 62.2 (PC, no collection is available from PC with collection number 62.2)
Europe, Spain: Catalunya, Campredon

lateritius Pers.

- Protologue:

Lactarius lateritius Pers., Tent. Disp. Meth. Fung.: 64 (1797), as '*Lactaria lateritia*' [not indexed]

- Etymology:

lateritius (Lat.) = dark brick red, like old red tiles

- Original description:

Pileo umbilicato subaurantio: zonis virescentibus, lamellis fuccoque aurantiis.
Frequens in pinetis. Amanita lateritii coloris croceo fucco turgens. Dill. Cat. pl. giess. p. 174. Ag. deliciosus Schaeff. Fung. 174. & Linnaei? Ob fuccum sapore acerrimum non equidem deliciosa esse potest haecce species.

- Type:

Not typified

lividatus

- Protologue:

Lactarius lividatus Berk. & M.A. Curtis, Proc. Amer. Acad. Arts & Sciences 4: 119 (1860) [SF 5: 449, 12: 979]

- Etymology:

lividatus (Lat.) = made to appear livid (because of the colour of the gills)

- Original description:

Pileo leviter depresso stipiteque sursum incrassato rufis; lamellis angustis subconfertis e sordido helvolis lividis.
On hills, Japan. - Has somewhat the habit of *L. volemus*.

- Type:

Holotypus: 4260/80-7 (K)
Asia, Japan, Amami-Oshima Islands

- Type studies:

LALLI & PACIONI (1992)
NEDA (1992)

miniatosporus

- Protologue:

Lactarius miniatosporus Montoya & Band.-Muñoz, Mycotaxon 89: 48 (2004) [not indexed]

- Etymology:

miniatus (Lat.) = flame-scarlet red; -sporus (Lat.) = -spored; but the author probably wanted to refer to the small size of the spores

- Original description:

Pileus 10-110 mm latus, convexus, plano-convexus, demum depressus, azonatus vel subzonatus, viscidus, subroseus versus rubellus vel subroceo vinaceo pallidus, luteus decolorans; margien valde subroseo rubellus, striato, incurvato. Lamellae subdecurrentes, moderate distantes, vinaceo pallidus. Stipes 15-70 x (8-)10-20 mm, cilindricus, sordidus subroseus vel subroseo vinaceus. Caro alba, rubescens. Sporae 6.4-8(-8.8) x 4.8-6.4(-7.2) µm, ellipticae, sub-reticulatae vel reticulatae, ornamentum 0.6(-0.8) µm altis. Pleurocystidia absentia. Cheilocystidia 24-48 x 5.6-6;4 µm, sub-fusiformia. Pileipellis ixocutis, hyphis filiformis instructa. Holotypus: lectus prope MEXICO. Veracruz: Mpio. Xico, E Cofre Perote, Ejido Ingenio El Rosario, El Revolcadero, 3.VII.2002, Montoya 3830 (XAL).

- Type:

Holotypus: Montoya 3830 (XAL!)
North America, Mexico

ogasawarashimensis

- Protologue:

Lactarius ogasawarashimensis S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 16: 55 (1940) [IF 1: 6]

- Etymology:

Described from Chichishima island, the largest island of the Ogasawara islands (= Bonin Islands, 1000 kilometers South of Tokyo)

- Original description:

Solitarius vel gregarius, edulis. Pileo 3-10 cm lato, convexo dein plano, demum centro depresso, viscido, glabro, juvenile "congo-pink" dein pallescente, juvenile vix zonato, dein circulis "tilleul-buff" et obscuriore coloratis concentrice zonato, albo-lustrato, margine primo nudo; carne albida, crassa, lacte caerulescente, sapore odoreque nullo; lacte non copioso, "colombia-blue"; lamellis decurrentibus, confertis, "olive-buff", basi aliquando rugulosis, poste aliquando furcatis; stipite 2.5-6 cm longo, 6-15 mm crasso, subtus attenuato vel subaequali, "light sky-blue" vel "parula-blue", lustrato, cavo; sporis in cumulo "light-buff", subglobosis, 7.5-8.5 x 7-7.5 µ, verrucosis.
Hab. On the ground in woods of Pinus liuchuensis Mayr. (Liukiu-matsu). Chichishima: Ôgimura-Kuwanokiyama (Nov. 5, 1936); Ôgimura-Renjudani (Nov. 9, 1936). Edulis.

- Type:

Not typified

olivaceosordidus

- Protologue:

Lactarius deliciosus var. *olivaceosordidus* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 95 (1979) [IF 5: 52]

- Etymology:

olivaceus (Lat.) = olive-green; sordidus (Lat.) = dirty-looking, dingy, soiled

- Original description:

Pileus 4-8 cm latus, demum late infundibuliformis, olivaceosordidus, substriatus; latex sordide aurantiacus; sporae 8.5-10.5 x 6.5-8 µ; macrocystidia 42-63 x 3-5 µ. Specimen typicum in Herbarium University of Michigan conservatum est; legit prope Pacific City, Oregon, 15 November 1972, Smith 83831.

- Type:

Holotypus: Smith 83831 (MICH!)
North America, USA: Oregon, Pacific City

paradoxiformis

- Protologue:

Lactarius paradoxiformis Murrill, Lloydia 7: 304 "1944" (1945), as '*Lactaria paradoxiformis'* [IF 1: 121, 186]

- Nomenclatural synonyms:

≡ *Lactarius pseudodeliciosus* var. *paradoxiformis* (Murrill) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 82 (1979) [IF 5: 53]

- Etymology:

Named after *L. paradoxus*; -formis (Lat.) = -formed

- Original description:

Pileo convexo-plano-depresso, 6-8 cm lato, viscido, glabro, cremeo, subacrido; lamellis confertis, ochraceis, viridescentibus; sporis ellipsoideis, tuberculatis, 9 x 6 µ; stipite concolori, 2-5 x 1-1.5 cm.
Type collected by W.A. Murrill under red oak and hawthorn on Cherry Hill, South of Orange Lake, Marion Co., Dec. 7, 1941 (F 19250).

- Type:

Holotypus: Murrill, F19250 (FLAS!)
North America, USA: Florida, Marion Co., South of Orange Lake, Cherry Hill

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)

paradoxus

- Protologue:

Lactarius paradoxus Beardslee & Burl., Mycologia 32: 584 (1940) [IF 1: 17]

- Etymology:

paradoxus (Lat.) = not of the expected

- Original description:

Pileo carnoso, late convexo, demum centro depresso et infundibuliformi, caeruleo (232 ad 209), obscurioribus zonis, demum aetate pallidiore, udo viscosissimo, glabro, 5.2 ad 8 cm. lato; margine tenui, laevi; carne lacte colorato, inodoro; lacte fusco-rubro (335), subamaro aut astricto tum tarde acri; lamellis e luteolis (311) cinnamomeis (323) aut lacte rubescentibus, vulneratis virescentibus, inaequalibus, paucis ad stipitem furcatis, adnatis deinde decurrantibus, latis, fragilibus, lacte faciliter plurantibus; stipite supra livido (173), basi caeruleo

(209), aequali aut infra constricto et subradicante, 2-3 cm. x 1.2-1.5 cm.; sporis pallide luteis (66 ad 36 t-2), late ellipticis, latis lineis parvus tuberculis late reticulatis 6.87-7.5 µ. x 8-9 µ.

Type locality: Fort Christmas Land Development Co., hammock at "Old Faithful", near Fort Christmas, Florida.

Habitat: In somewhat grassy places under cabbage palmetto and live oaks in a fairly shady spot in rich soil, or in lawns having similar conditions.

- Type:

Lectotypus: Burlingham, 16-11-1939 (NY!) (designated in HESLER & SMITH, 1960)

North America, USA: Florida, Fort Christmas Land Development Co., hammock at "Old Faithful", near Fort Christmas

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)
MONTOYA *et al*. (1998)

piceae

- Protologue:

Lactarius deliciosus var. *piceae* Vassilkov, [Edible and poisonous fungi of central parts Europ.distr. U.S.S.R.]: 60 (1948), as '*picei*' [IF 2: 346], nom. inval., nom. nud.

- Etymology:

Collected under *Picea* trees

- Original description:

Sine diagn. Lat.

- Type:

Not typified

piceus

- Protologue:

Lactarius deliciosus var. *piceus* Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 218 (1947) [not indexed], nom. inval., nom. nud.

- Etymology:

piceus (Lat.) = black as pitch; but the author obviously wanted to refer to the host tree of this species: *Picea*

- Original description:

Sine diagn. Lat.

- Type:

Not typified

- Remarks:

Some confusion exists on the first date of publication of *Lactarius deliciosus* var. *piceus* Smotl. ROMAGNESI (1958) mentions a publication by SMOTLACHA (1912). BASSO (1999a) also considers var. *piceus* as validly published (and thus published before 01/01/1935). Fide Antonin (pers. comm.) the following text is written in Czech on p. 170 of SMOTLACHA (1912): "…*L. deliciosus* (L.) which is distinct by the orange saffron coloured milk, the fact that it is not acrid, its hollow stem and its context becoming green after touching. There are two varieties in our country: the first one larger, in grassy Pine forests, the second one smaller, under Spruce, esp. in young stands…". The name *Lactarius deliciosus* var. *piceus* is not mentioned here. *Lactarius deliciosus* var. *piceus* was mentioned for the first time in SMOTLACHA (1947) together with a description in Czech only.

pinastri

- Protologue:

Lactarius pinastri Romagn., Bull. Trimestriel Soc. Mycol. France 96: 308 (1980) [IF 5: 53]

- Etymology:

Collected under *Pinus pinaster*

- Original description:

Pileo 4-11,5 cm, crasso, compacto, duro, medio depresso, margine crassa, grosse involuta, primum e glauco griseo, tarde e rufulo brunneo, ambitu zonato, maculato. Stipite (2,3)-3-7 x 0,9-2,5 cm, primum e glauco griseo, dein ex aurantiaco brunneo, interdum scrobiculato, pruinoso, tactu virescente. Carne vividissime aurantiaca, acri. Lacte e rubro aurantiaco. Lamellis angustis, 2-8 mm, e rubellis aurantiacis, tactu virescentibus. Sporis cremeis, 7,5-9 x 6,3-7,7 µm, cristatis, reticulatis. Nonnullis laticiferis inter basidia. - Sub Pinis pinastris, in solo acido. Typus in Herb. Romagn. n° 71.214.

- Type:

Holotypus: Romagnesi 71-214 (PC!)
Europe, France: Manche, forêt communale de Pirou

- Type studies:

HEILMANN-CLAUSEN *et al.* (1998)
BASSO (1999a)

pini

- Protologue:

Lactarius deliciosus var. *pini* Vassilkov, [Edible and poisonous fungi of central parts Europ.distr. U.S.S.R.]: 60 (1948) [IF 2: 346], nom. inval., nom. nud.

- Etymology:

Collected under *Pinus* trees

- Original description:

Sine diagn. Lat.

- Type:

Not typified

pinicola

- Protologue:

Lactarius pinicola Smotl. ex. Z. Schaef., Schweiz. Z. Pilzk. 48: 141 (1970) [not indexed]

- Based on:

Lactarius deliciosus var. *pinicola* Smotl., Atlas hub jedlých a nejedlýck [Atlas of edible and inedible fungi]: 219 (1947) [not indexed], nom. inval., nom. nud.

- Etymology:

pinus (Lat.) = collected under *Pinus* trees; -cola (Lat.) = -dweller

- Original description:

Pileus firmus, crassus, 4-15 cm latus; convexus et involutus, dein anguste, sed profunde umbilicatus usque infundibuliformis, margine obtusus; subviscidus, mox siccus, aspere rugosus, sub lente tomentosus, in parte inferiori rugatum albido pruinosus, opacus, siccus, saepe subnitidus; pallide aurantiaco ruber, aurantiaco ochraceus, rubro aurantiaco maculatus, subzonatus, diu non virescit, sed in aetate saepe totus aerugineus. Lamellae confertae, fragiles, angustae usque angustissimae, subdecurrentes; pallide ochraceo aurantiacae, dein aurantiaceae, vulneratae paulum aerunascentes. Stipes firmus curtus, 3-6 x 1-3 cm, cylindricus, basi saepe attenuatus, solidus, deinde subcavus, siccus, maculatus et scrobiculatus, raro laevis, opacus; pilei concolor, saepe roseo tinctus, aetate aeruginascens. Caro firma, deinde fragilis, alba vel cremea, in ambitu statim a lacte carrote aurantiaca, dein aurantiaco rubra, iam immutabilis; sapore amarascens, subacris, odore penetrabilior quam L.deliciosus. Lac carrote aurantiacum, post 1 hora leviter rubescens, iam immutabile, aetate leniter aeruginascens. Sporae in cumulo F,7-9 x 6-7 µm, interrupte reticulatae; cystidia fusoidea, non copiosa. Nonnula carposomatorum iam in juventute sunt totabiliter aeruginea usque caerulea. Sub pinis, in aestate et autumno. Holotypus: Frýdstejn prope Jablonec n.N., Bohemia borealis, silva mixta, sub pini (18.10.1962, leg. Schaefer, PR 584024)

- Type:

Holotypus: PR 584024 (PRM)
Europe, Czech Rep.: Bohemia, Frýdstejn (near Jablonec)

porninae

see porninsis

porninsis (Fig. 3)

- Protologue:

Lactarius porninsis Rolland, Bull. Trimestriel Soc. Mycol. France 5: 168 (1889) [as '*porninae*' in SF 9: 57, 12: 980, 19: 1033]

- Etymology:

Named after the collector: Mrs. Pornin

- Original description:

Pileo carnoso, admodum firmo, superficiem rugosam, sed glabram, praebente, viscosam, pulchre aurantio-luteam, zonis subelevatis cinnabarrinis plerumque praeditam (Color cinnabarrinus ad velum generale spectare videtur), primitus convexo, dein applanato, tandem que infundibuliformi, irregulari, subumbonato, 0m,1 usque latitudine adaequante, margine levi, involuta. Lamellis luteis (Cucurbitae caro), pallentibus, demum ocraceis, lucem versus vibrantibus, angustis, confertis. Stipite firmo, elastico-corticato, medulla albida sat densa farcto, mox cavo, ocraceo, expallente, cylindrico, saepe curvato, radicoso, costato, striolato, glabro, diametrum pilei longitudine attingente, 0m,015 et ultra latitudine. Lacte persistenter albo, parco. Carne albida, primitus miti, denique subacri et amara, cetaria redolente, etiam prima aetate. Sporis asperis rotundis, 7,5 μ latis, ampla sporidiola nucleatis. Fungus simulat corium quiddam aurei Mali humi jacens. Chamonix, jusxta viam cataractae "Le Dard". Zermatt, clivis nemorosis Riffel montis. Augusto et Septembre mensibus, sub Laricibus copiose.

- Type:

Lectotypus: Bull. Trimestriel Soc. Mycol. France 5, pl. XIVbis: 2! (designated in HEIMANN-CLAUSEN *et al.*, 1998)
Europe, Switzerland, Chamonix

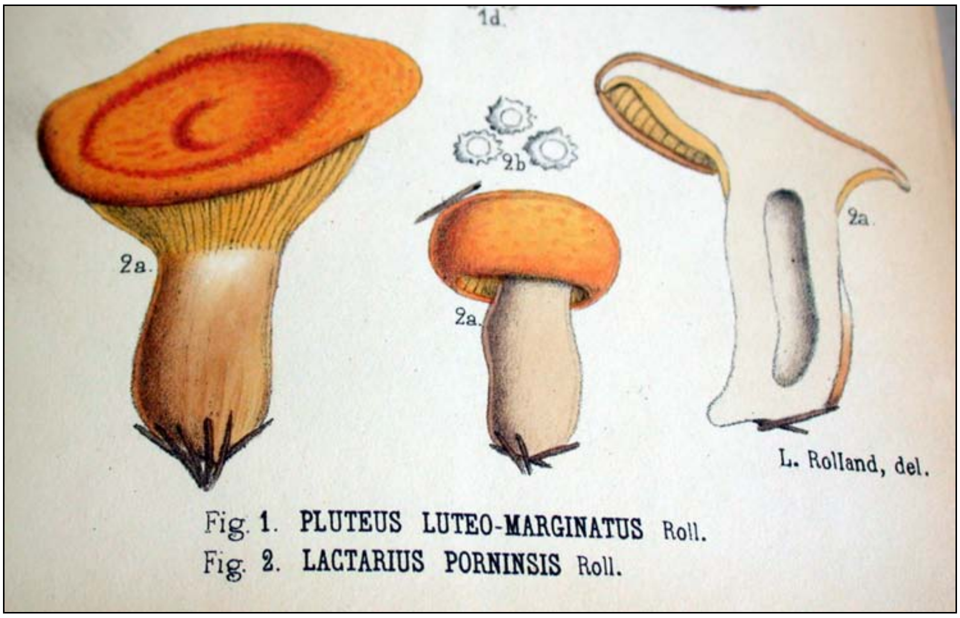

Fig. 3: Lectotype of *L. porninsis*

pseudodeliciosus

- Protologue:

Lactarius pseudodeliciosus Beardslee & Burl., Mycologia 32: 582 (1940), as '*Lactaria pseudodeliciosa*' [IF 1: 17]

- Etymology:

pseudo- (Greek) = false-

- Original description:

Pileo late convexo, profunde umbilicato, tum expanso, et infundibuliformi, 6-8.5 cm. lato, subalbo, disco e pallido ochroleuco, azono aut interdum leviter zonato, viscosissimo; margine tenui, minute fibrillis agglutinatis tomentoso; carne inodora, vulnerata virescente; lacte exiguo, aurantiaco, tarde acri; lamellis melleis, basi aurantiacis, inaequalibus, intervenosis, paucis basi cohaerentibus, adnatis aut adnato-decurrentibus; stipite isabellino, leniter scrobiculato, infra albo-tomentoso, curto, solido, 1.2 x 1 cm. ad 2.5 x 2 cm., basi unilatere acuto-constricto pseudo-radicanteque; sporis luteis (36 t 2-3), tuberculis et reticulatis, 6.87-7.5 μ. x 8.75-9 μ.
Type locality: Rock Springs, (Kelly Park), Orange Co., Florida.

- Type:

Lectotypus: Beardslee & Burlingham 22-12-1937 (NY!)
North America, USA: Florida, Orange Co., Rock Springs, (Kelly Park)

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)

quieticolor

- Protologue:

Lactarius quieticolor Romagn., Rev. Mycol. (Paris) 23: 280 (1958) [IF 6: 148, 211]

- Nomenclatural synonyms:

≡ *Lactarius deliciosus* var. *quieticolor* (Romagn.) J. Blum, Lactaires: 203 (1976), nom. inval., no reference was made to the basionym and the name was not indicated as a comb. nov.

≡ *Lactarius deliciosus* var. *quieticolor* (Romagn.) Kriegelst., Beitr. kenntn. Pilze Mitteleuropas 7: 68 (1991) [IF 6: 211], nom. inval., the page number was omitted in the reference to the basionym

- Etymology:

quieti = refers to *L. quietus*; -color (Lat.) = -coloured

- Original description:

Pileo (2,5)-5-6 cm. lato, carnoso, firmo, convexo-applanato, deinde expanso ac inaequaliter orbiculari, medio depresso, demum cyathiformi vel infundibuliformi, margine involuta, e brunneo subaurantiaco, deinde e rufulo brunneo ad instar L. quieti, paulum zonato ac pruinoso. Stipite 2,5-4,5 x 0,8-1,7 cm., brevi, subaequali, ampla caverna, ex aurantiaco brunneo, impolito, viridi colore vel maculis aerugineis tincto. Carne crassa, firma, lacte aurantiaca, aere virescente. Lacte ex aurantiaco rubro. Lamellis stipatis, tenuibus, angustis, arcuatis, deinde decurrentibus, sat vivide aurantiacis, sed brevi brunneo colore tinctis, maculis viridibus sordide variegatis. Sporis sordide ochraceis, subglobosis, 8-9,2 x 6,5-7,5 µ., ample reticulatis ac fere alveolatis cristis crassis latisque, specie eximia. Cystidiis angustissimis (5-6,5 µ.), paucioribus. - Sub Pinis silvestribus.

- Type:

Holotypus: Romagnesi 13-10-1957 (PC)
Europe, France: Seine-et-Oise, forêt de Rambouillet

- Type studies:

HEILMANN-CLAUSEN *et al.* (1998)
BASSO (1999a)

roseus

- Protologue:

Lactarius sanguifluus f. *roseus* Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003 (“2002”)) [not indexed]

- Etymology:

roseus (Lat.) = rosy

- Original description:

A typo differt colore sporophororum, pilei, lamellarum et stipitis amittenti tenui rosato.
Typus: 23.10.1996, L'Aquila, Roio, S. Lorenzo, AQ (96/30), in Herbario Mycologico Aquilano (AQUI) servatus.

- Type:

Holotypus: AQ (96/30) (AQUI)
Europe, Italy: L' Aquila, Roio, S. Lorenzo

rubescens

- Protologue:

Lactarius deliciosus f. *rubescens* J.A. Schmitt, Z. Pilzk. 39: 238 (1974) [IF 4: 278]

- Etymology:

rubescens (Lat.) = turning red, reddening

- Original description:

Carposomatibus magnis, spissis, carnosis, lacte primitus lateritio-crocato vulneribusque tarde decolorantibus primo obscuriore rubris (in una hora), demum virescentibus.
Typus E46 (Herb. J.A. Schmitt), 25.9.1967 Homburg/Rabenhorst

- Type:

Holotypus: Schmitt E46 (pers. herb. J.A. Schmitt)
Europe, Germany

rubrifluus

- Protologue:

Lactarius rubrifluus Gillet, Bull. Soc. Linn. Normandie II 4: 255 (1870) [not indexed]

- Etymology:

rubr- (Lat.) = red-; fluere (Lat.) = to flow

- Original description:

(p. 249-251:) Ce champignon se reconnaît aux caractères suivants:

Le chapeau, d'abord convexe, devient bientôt plan, déprimé, ombélique au centre, les bords plus mou moins repliés en dessous; il se creuse ensuite de plus en plus et arrive à être concave, les bords relevés sinueusement ou lobés; sa surface est lisse, humide, presque visqueuse, un peu luisante, entièrement couverte de petits poils appliqués et marquée de zones d'autant plus visibles qu'elles se rapprochent davantage de la marge qui est grise ou gris légèrement rosé; sa couleur est d'un jaune rougeâtre clair plus ou moins lavé de bleu ou de vert gris; quelquefois ces teintes se manifestent par plaques plus ou moins larges, le plus ordinairement elles sont à peu près uniformément répandues. Il n'est pas rare non plus de le trouver portant, sur les bords surtout, des taches d'un rouge vermillon assez vif; ces dernières se remarquent principalement quand le champignon est déjà un peu avancé en âge et quand sa surface est devenue entièrement d'un blanc sale ou jaunâtre. Son diamètre varie entre 5, 10 ou 12 centimètres; sa chair épaisse, compacte, pesante, jaune ou jaunâtre, devient subitement rouge ou rouge-orangé au contact de l'air, surtout au-dessus des feuillets et à sa réunion avec le pied.

Les feuillets, larges de 6-8 millim., sont inégaux, entiers, nombreux, concaves et décurrents; leur couleur est jaunâtre; ils présentent, lorsqu'ils sont vus sous un certain jour, un léger chatoiement carné; entamés, ils répandent un lait rouge ou rougeâtre.

Le pied long de 2-4 centimètres, et épais de 1 ou 2, est quelquefois égal, droit ou courbé et le plus souvent renflé au sommet; sa surface est lisse ou bien marquée de quelques taches irrégulières, enfoncées, rouges ou bleues; sa couleur est ordinairement celle du chapeau avec une ligne blanche assez bien dessinée à sa partie supérieure, à l'endroit même où les feuillets se réunissent à lui. Jeune, il n'est pas rare de le trouver entièrement d'un bleu clair.

La chair compacte est jaune-d'orangé clair ou rougeâtre vers les couches extérieures; l'intérieur, ordinairement, irrégulièrement creux, est blanc ou blanchâtre; on le trouve rarement plein.

Toute la plante est humide, pesante et fragile.

Sa saveur n'a rien de remarquable; son odeur, un peu plus pénétrante que celle de l'agaric délicieux, est plutôt désagréable qu'agréable. Enfin, toutes les parties blessées ne tardent pas à prendre des teintes d'un vert très-foncé.

.... (p. 255:) En attendant nous donnons à cette espèce le nom de rubrifluus, ...

- Type:

Not typified

rubrilacteus

- Protologue:

Lactarius rubrilacteus Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 76 (1979) [IF 5: 53]

- Etymology:

rubr- (Lat.) = red; lacteus (Lat.) = milky

- Original description:

Pileus 6-12 cm latus, late depressus, demum subinfundibuliformis, zonatus, viscidus; zonae aurantiae et subochraceae; contextu fragilis, luteoalbus, tarde aeruginosus; odor subaromaticus; sapor mitis. Latex triste sanguineus. Lamellae confertae, angustae, adnatae vel subdecurrentes. Stipes 2-6 cm longus, 10-30 mm crassus; saepe scrobiculatus sed siccus. Sporae 7.5-10 x 6.5-8 µ. Specimen typicum in Herb. Univ. Mich. conservatum est; legit Wm. B. Gruber n. 1200, prope Grants Pass, Oregon, 17 Nov. 1947.

- Type:

Holotypus: W.B. Gruber 1200 (MICH!)
North America, USA: Oregon, Grants Pass

rubriviridis

- Protologue:

Lactarius rubriviridis Desjardin, Saylor & Thiers, Mycologia 95: 148 (2003) [not indexed]

- Etymology:

rubr- (Lat.) = red; viridis (Lat.) = green

- Original description:

Gastrocarpia 25-60 mm diametro, 20-45 mm alta, irregulatim globosa vel ellipsoidia, fusca vel rubrofusca, tactu viridescens, alveolata. Peridium nullum maturitate. Gleba lacunosa, subflavida, tactu viridescens. Contextus viridescens ubi contusus. Latex parcus, laete ruber. Stipe-columella nulla. Columella praesens, conspicua. Odor et sapor haud distinctus. Basidiosporae (8-) 8.5-11 x (7-) 7.5-8.5 µm, ellipsoideae, reticulatae vel partiale reticulatae, ballistosporae, amyloideae. Trama hymenophoris hyphis filamentosis, sphaerocystis nullus. Peridiopellis nullus. In solo subter arboribus coniferis. Holotypus hic designatus: DED 7312 (SFSU), legit D.E. Desjardin, 14 June 2001, prope Cold Creek Campground, Sierra Co., California, USA.

- Type:

Holotypus: DED 7312 (SFSU!)
North America, USA: California, Sierra Co., Cold Creek Campground

- Remarks:

hypogeous

rubrozonatus

- Protologue:

Lactarius rubrozonatus Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003 (“2002”)) [not indexed]

- Etymology:

rubro- (Lat.) = red; zonatus (Lat.) = zoned, banded, marked circularly

- Original description:

Pileo e convexo-umbilicato, leviter subinfundibuliformi, lateritio, rubro-aurantiaco, evanescenti vinoso, manifeste zonato, viridi smaragdino maculato; lamellis rubro-aurantiacis, evanescentibus vinosis, intense viridi maculatis; stipite rubro-aurantiaco, primitus albo-pruinato, rubro-vinoso scrobiculato, carne alba aere evanescenti rubro-aurantiaca ad latera; sapore miti dein astringenti; lacte intense vinoso; sporis subglobosis, reticulatis (6.8)8.10 +- 0.56(9.6) x (5.2)6.32 +- 0.35(7.2) µm, L/l = 1.28.
Typus: 27.9.96, L'Aquila, Provvidenza, AQ (96/15), in Herbario Mycologico Aquilano (AQUI) servatus. ITS sequentia in GenBank deposita AY292987 numerata.

- Type:

Holotypus: AQ (96/15) (AQUI)
Europe, Italy: L' Aquila, Provvidenza

salmoneus

- Protologue:

Lactarius salmoneus Peck, Bull. Torrey Bot. Club 25: 369 (1898) [SF 16: 45]

- Homonym:

Lactarius salmoneus R. Heim & Leclair, Rev. Mycol. (Paris) 15: 79 (1950) [IF 2: 22, 57], was later renamed *L. salmonicolor* in HEIM (1953)

- Etymology:

salmoneus (Lat.) = salmon-pink

- Original description:

Pileus rather thin, convex, becoming nearly plane or slightly depressed in the center, dry, subvelvety, sometimes irregular, white, becoming reddish where bruised; milk bright salmon color, taste mild, slightly aromatic; lamellae narrow, close, adnate or decurrent, bright salmon color; stem short, solid, central or occasionally eccentric, velvety, white, salmon color within; spores subglobose, 7.5-9 µ long. Pileus 2.5-3.8 cm. broad; stem about 2.5 cm. long, 3-6 mm. thick. Cespitose, in wet swampy places, usually on naked ground that has been overflowed. August. Earle & Baker.

- Type:

Holotypus: Earle & Baker (NYS!)
North America, USA: Alabama, Vaughn's Mills

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)

salmonicolor

- Protologue:

Lactarius salmonicolor R. Heim & Leclair, Rev. Mycol. (Paris) 18: 221 (1953) [IF 3: 48]

- Nomenclatural synonyms:

≡ *Lactarius salmoneus* R. Heim & Leclair, Rev. Mycol. (Paris) 15: 79 (1950) [IF 2: 22, 57], illegitimate homonym of *Lactarius salmoneus* Peck (1898)

≡ *Lactarius subsalmoneus* Pouzar, Česká Mykol. 8: 44 (1954) [not indexed], superfluous nom. nov. for *L. salmoneus* R. Heim & Leclair

≡ *Lactarius deliciosus* var. *salmoneus* (R. Heim & Leclair) Kühner & Romagn., Flore Analytique des Champignons Supérieurs: 474 (1953) [not indexed]

- Etymology:

salmoneus (Lat.) = salmon-pink; -color (Lat.) = -coloured

- Original description:

Pileo usque ad 20 cm. lato, gibbero, deinde infundibuliformi, maxime inaequali, margine lobata, e salmoneo vel melleo ad pallide cremeum, neque viridi, neque rubro tincto, subtiliter zonato et secundum radiis venoso, viscoso, sicco lento. Stipite robusto, paulum radicato, interdum subexcentrico, ex aurantiaco fulvo, rugoso, parvis, ovatis aurantiacisque scrobiculis notato. Lamellis vivide auroreis, deinde pileo concoloribus, dente decurrentibus. Carne fragili, in pileo eburnea, in stipite circum intense aurantiaca sapore dulci, deinde leviter amaricante; odore Russulae pseudo-integrae. Lacte aurantiaco, maxime fluxo. Sporis 9,3-13 x 6,5-7,5 µ. (verrucis inclusis), reticulo interrupto, vario et complexo, verrucis eminentibus. - Sub Picea excelsis vigens.

- Type:

Lectotype: France, Bellême, no information available on the number of the type collection (PC), designated in HEILMANN-CLAUSEN *et al.*, 1998.

Europe, France: Bellême

sanguifluus (Fig. 4)

- Protologue:

Hypophyllum sanguifluum Paulet, Traité des champignons: 2 (9ed): pl. 81, fig. 3-5 (1811) [not indexed]

- Nomenclatural synonyms:

≡ *Lactarius sanguifluus* (Paulet) Fr., Epicr. Syst. mycol.: 341 (1838) [SF 5: 439, 14: 94, 12: 980, 19: 1035]

≡ *Lactifluus sanguifluus* (Paulet) Kuntze, Revisio Generum Plantarum, Pars II: 857 (1891) [not indexed]

- Etymology:

sanguis (Lat.) = blood; fluere (Lat.): to flow

- Original description:

name as label of plate 81, fig. 3-5

[text refering to this plate, also in first edition, vol. II, p. 186 (1793):]

Rougillon (pl. LXXXI, fig. 3,4,5). Cette plante, que je ne trouve ni d'écrite ni figurée nulle part, est très remarquablement.........

- Type:

Lectotypus: Traité des champignons: 2 (9ed): plate 81, fig. 3-5!

Europe, France

Epitypus: AQ (92/15) (AQUI)

Europe, Italy, L'Aquila, S. Guiuliano

- Type studies:

BASSO (1999a)

- Remarks:

The name 'Rougillon' is published earlier by Paulet (1793) but it is only in the 1811 edition that the name *Hypophyllum sanguifluum* is mentioned together with plate 81, figs. 3-5.

A neotype has been invalidly designated by LALLI & PACIONI (2003 ("2002")) (as original material – a plate – exists).

QUÉLET (1881) deservedly remarks that the description and plate by PAULET (1811) show strong differences with the more widely accepted view of *L. sanguifluus*: Paulet's drawing shows a bright purple mushroom with a filled stem, very different from *L. sanguifluus* (see Fig. 4). An epitype (AQ (92/15) AQUI) was for that reason designated by us (see chapter 6).

sanguinascens

see semisanguinascens

sanguineovirescens

- Protologue:

Lactarius sanguineovirescens Fillion, Bull. Trimestriel Féd. Mycol. Dauphiné-Savoie 29 (113): 21 (1989), as '*sanguineo-virescens*' [IF 5: 968]

- Etymology:

sanguineus (Lat.) = blood-red; virescens (Lat.) = becoming green

- Original description:

Pileus 3,5-8 (10) cm, convexo-applanatus, in centro depressus cum frequenti obtuso umbone; margine diu involuto; cute paulum viscosa, inaequaliter grumulosa, plus minusve evidenter zonata; ardosiaco-ravo-roseus cum griseo-malvaceis areis caesio-variantibus. Smaragdinis maculis totum carpophorum paulatim invaditur. Lamellae confertissimae, haud altae, ex arquatis leviter decurrentes, salmoneo-aurantiae, a pilei coloribus magnopere alienae. Stipes 4-8 x 1,5-2 cm, plerumque pilei diametro longior, basi paulum clavata; primum roseo-albus undatim malvaceus, dein omnino aurantiacus, irregularibus foveolis vivide salmoneo-aurantiis effossus. Caro in media cremea, sub cute viridi-caerulea, sub cortice caroticolor sed brevi tempore ex aurantio-rubro in sanguineum (5 min.) deinde purpureum (15 min.) colorem vergens. Odor sub tritu L. quieti instar. Sapor amarus, postea breviter sed distincte acer (post 2 min.). Sporae ovoideae vel subglobosae (6) 7-7,5 x (7,5) 8-9 µm, cristatae usque ad subreticulatas. Collectiones sub pinis in acido solo, in nemore ad Arlod supra Bellegarde (01). Holotypus in herbario R. Fillion n° 88-09-12.

- Type:

Holotypus: Fillion 8-9-12 (private herb. Fillion, isotypus: Bon 88127 (LIP!))
Europe, France: Bellegarde, Bois d'Arlot

- Type studies:

BASSO (1999a)

Fig. 4: Lectotype of *L. sanguifluus.*

sanguineus

- Protologue:

Lactarius sanguineus Teng, unpubl., cited in Fungi of China: 414 (1996) [not indexed], invalid, nom. nud.

- Etymology:

sanguineus (Lat.) = blood-red

- Original description:

Sine diagn. Lat.

- Type:

Not typified

- Remarks:

Name traced by KORF (1996) in Teng's notes. Comments by KORF (1996): 'This species was not treated in the Chinese version, *Chung-kuo Ti Chen-chun*, 1963. Teng added it in the revised key (above), but the portion of this manuscript containing the description of the fungus and any references as to where it was collected or reported from China has (have) been lost.'

semisanguifluus

- Protologue:

Lactarius semisanguifluus R. Heim & Leclair, Rev. Mycol. (Paris) 15: 79 (1950) [IF 2: 23]

- Etymology:

semi- (Lat.) = half-; sanguis (Lat.) = blood; fluere (Lat.): to flow

- Original description:

Pileo 10 cm. non superante, plerumque toto viridi, interdum ex aurantiaco roseo colore tincto. Stipite robusto, ex aurantiaco roseo, intense aurantiacis vel obscure viridibus scrobiculis notato. Lamellis e viridibus caeruleis vel ex aurantiacis roseis, saepe aliis intensius viridibus, aliis pallide aurantiacis, dente decurrentibus. Carne in pileo alba, sub cute viridi, deinde deorsum e violacea vinosa, in stipite aurantiaca, deinde in stipite e violacea fuscaque rubida; sapore dulci, deinde paulum acri; odore Russulae pseudointegrae. Lacte ex aurantiaco brunneo, deinde in violaceum vergente. Sporis 9-10,4 x 7-7,8 µ (verrucis inclusis), reticulo ex maculis angulatis et lineolis amyloideis satisque tenuibus constante, verrucis brevibus. - Sub Pinis silvestribus vigens.

- Type:

Lectotypus: Heim 1949-610 (PC!)
Europe, France: Bellême

- Type studies:

HESLER & SMITH (1979)
HEILMANN-CLAUSEN *et al.* (1998)

semisanguinascens

- Protologue:

Lactarius quieticolor f. *semisanguinascens* Bon, Rev. Mycol. (Paris) 38: 202 (1975) [IF 4: 382]

- Etymology:

semi- (Lat.) = half-; sanguinascens (Lat.) = beginning to bleed

- Original description:

A typo differt lacte plus minus rubescente, interdum tarde; in pinetis ad instar typi, sed in locis acidophilus (Callunetis) lectus, prope Boulogne-sur-Mer (Gallia), exs. in herbario Bon (Lille) N° 91078.

- Type:

Holotypus: Bon 91078 (pers. herb. M. Bon)
Europe, France: Boulogne-sur-Mer

- Remarks:

as '*sanguinascens*' in BON (1980)

subaustralis

- Protologue:

Lactarius subaustralis Hesler, Brittonia 12: 139 (1960) [IF 3: 11]

- Etymology:

sub- (Lat.) = somewhat, not completely, a little; australis (Lat.) = south, southern

- Original description:

Pileus 4 cm latus, convexo-depressus, albidus, azonatus, subvelutinus; lamellae pallidae; stipes albidus, subvelutinus, siccus; latex salmoneus. Specimen typicum legit R. L. Hesler (no. 12864), 30 Aug. 1958, Great Smoky Mountains National Park, Tennessee, U.S.A.

- Type:

Holotypus: Hesler 12864 (YRNN)
North America, USA: Tennessee, Great Smoky Mountains National Park

- Type studies:

HESLER & SMITH (1979)

- Remarks:

HESLER & SMITH (1979) excluded *L. subaustralis* from subgenus *Lactarius* (= sect. *Deliciosi*) and placed it in subgenus *Plinthogali*.

subindigo

- Protologue:

Lactarius subindigo Verbeken & E. Horak, Australian Syst. Bot. 13: 651 (2000) [IF 7: 245]

- Etymology:

sub- (Lat.) = somewhat, not completely, a little; indigo = deep blue

- Original description:

Pileus 70 mm, convexo-involutus dein umbilicatus, haud striatus, papilla nulla, conspicue zonatus, azureoviridis, aetate pallide ochraceus, viscidus, virescens tactu. Lamellae densae, arcuatodecurrentes, conspicue azureovirides, virescens tactu. Stipes 10-30 x 4-10 mm, cylindricus, pileo concolor, siccus, glaber. Caro conspicue azureoviridis, ad basim stipitis aurantiacas. Odor gratus. Sapor nullus. Latex azureus. Basidiosporae 6.6-7.5-8.3 x 5.4-5.9-6.5 µm, ellipsoideae, subreticulatae, amyloideae. Basidia 40-70 x 7-9 µm, tetraspora. Cheilo- et pleurocystidia nulla. Pileipellis ex hyphis hyalinis dense intertextis ixocutem formantibus. Fibulae nullae. Ad terram in silvis fagineis montanisque (*Castanopsis*, *Lithocarpus*). Nova Guinea: Papua, Morobe District, Bulolo, Watut, 21.x.1971, Horak 71-189 (holotypus CANB; isotypi GENT, ZH).

- Type:

Holotypus: Horak 71-189 (CANB), Isotypes (GENT!, ZH)
Asia, Papua New Guinea: Morobe District, Bulolo, Watut

subpurpureus

- Protologue:

Lactarius subpurpureus Peck, Rep. (Annual) New York State Mus. Nat. Hist. 29: 43 (1878) [SF 5: 439, 12: 980, 19: 1037, 20: 1261]

- Nomenclatural synonyms:

≡ *Lactifluus subpurpureus* (Peck) Kuntze, Revisio Generum Plantarum, Pars II: 857 (1891) [not indexed]

- Etymology:

sub- (Lat.) = somewhat, not completely, a little; purpureus (Lat.) = purple

- Original description:

Pileus at first convex, then expanded or depressed, smooth, subviscid, variegated with purplish and cinereous hues; lamellae dull-red or purplish; stem equal, colored like the pileus; milk sparse, dark-red.
Plant 2'-4' high, pileus 2'-3' broad, stem 3"-5" thick.
Mossy ground in swamps. Sandlake. August.
The species is related to *L. deliciosus*, but there are no orange hues either to the plant or the milk as in that species.

- Type:

Holotypus: Peck (NYS!)
North America, USA: New York, Sandlake

- Type studies:

HESLER & SMITH (1960)
HESLER & SMITH (1979)
MONTOYA & BANDALA (2004)

subsalmoneus

see salmonicolor

tenuis

- Protologue:

Lactarius deliciosus var. *tenuis* Naveau, Natuurw. Tijdschr. 5: 70 (1923) [PL suppl.: 83]

- Etymology:

tenuis (Lat.) = thin, fine, slender

- Original description:

Pileo aquoso-maculato, non virido. diam. 4 c. Stipes ad aëris contactum illico in colorem pallo-virescentem transit. 30 m/m x 8 m/m. Sp. 7-8 x 5.5-6.3 µ.
Muscinetum onder beuk. 26-9-20.

- Type:

Holotypus: BR-130746,87 (BR!)
Europe, Belgium: Peerdsbos (near Antwerp)

thakalorum

- Protologue:

Lactarius thakalorum Bills & Cotter, Mem. New York Bot. Garden 49: 193 (1989) [IF 5: 968]

- Etymology:

Named after the Thakalis, a group of people who reside in central Nepal and collect this mushroom for food.

- Original description:

Species *L. subpurpureo* similis. Pileus usque 8 cm latus, primum convexo-depressus, tum profunde depressus; superficies arida vel viscida, primum concentrico-zonata, tum azonata, rosea usque obscure vinaceo-rubra vel pallide rubello-pupurascens, deinde obscure flavidescens disperseque rubello-purpurascens tincta, denique maculas irregulares virides vel cinerascenti-virides formans; trama alba, cum latice disperse porphyrea, linea supra lamellas porphyrea notata. Latex porphyreus, non abundans. Lamellae decurrentes, subgriseo-roseae, subgriseo-aurantiacae usque dilute aurantiacae vel denique flavidae, sub vetustate vel vulneratae obscure virides. Stipes usque 6 cm altus, 1 cm latus; superficies arida, pruinam albo-canescentem producens, pileo concolor, sub vetustate areas virides tinctas formans. Basidiosporae flavae, 7-9 x 5.5-7.5 µm, subglobosae usque late ellipticae vel late obovatae; ornamentum usque 1 µm altum, e porcis crassis usque tenuibus interconjunctis et lineis gracilibus et verrucis particulisque paucis segregatis et reticulo paene perfecto compositum. Cystidia hymenii filamentosa 30-51 x 2-5 µm, flexuosa, inter basidia inclusa, hyphis laticiferis exorientia, contento refractivo, in sulfovanillin lividulo-griseo; vel cystidia hymenii 40-55 x 4-10 µm, hymenio vel subhymenio exorientia, subulata usque obclavata, sine vel aliquantum contento refractivo producentia. Pileus tramaque lamellae hyphas laticiferas abundantes efferens; hyphae laticiferae in sulfovanillin cineraceo-indicae usque purpurinae. Cuticula pilei ixocutis, stipitis non gelatinosa. Consociata *Pino wallichiana* et *Pino roxburghii*, in parte septentrionali Nepaliae centralis. Nepal, Dhaulagiri Zone, Mustang District, in flumine supra Khobang, 3 Sep 1985 VC 1313 (holotypus BPI).

- Type:

Holotypus: VC 1313 (BPI!)
Asia, Nepal: Dhaulagiri Zone, Mustang District

- Type studies:

MONTOYA & BANDALA (2004)

thyinos

- Protologue:

Lactarius thyinos A.H. Sm., Brittonia 12: 135 (1960) [IF 3: 11]

- Etymology:

HESLER & SMITH (1960) collected this species under *Thuja* trees (but *Thuja* is not ectomycorrhizal).

- Original description:

Pileus 3-9 cm latus, convexo-depressus, viscidus, glaber, subzonatus incarnato-aurantiacus; sapor mitis; lamellae subdistantes vel confertae; stipes viscidus; pleurocystidia 52-96 x 4-9 µ, elongata-fusoidea. Specimen typicum legit A. H. Smith (no. 22150), 20 Aug 1946, Reese's Bog, Cheboygan County, Michigan, U.S.A.

- Type:

Holotypus: Smith 22150 (MICH!)
North America, USA: Michigan, Cheboygan County, Reese's Bog

- Type studies:

HESLER & SMITH (1979)

vinosus

see violaceus

violaceocaeruleus

- Protologue:

Lactarius violaceocaeruleus Voglino, Boll. Soc. Bot. Ital. 1894: 122 (1894) [SF 22: 1505, 23: 127]

- Etymology:

violaceus (Lat.) = violet; caeruleus (Lat.) = blue

- Original description:

Pileus carnosus, umbilicatus, convexus, primitus margine involuto-rivulosus, azonus, glaber, violaceo-caeruleus, 8 cm. latus, caro albido-caerulea, lac copiosus, caeruleus, aromaticus; lamellae crassiusculae, subconfertae, adnatae vel decurrentes, caeruleae; basidia clavata, 4-sterigmica, 25 x 6-8; sporae sphaeroideae, leviter echinulatae, initio hyalinae demum albido-caeruleae, 8-9 µ diam.; stipes cilindricus, farctus demum cavus, brevis, 4-5 cm. longus, 1,5-2 cm. latus, pallide ochraceo-luteus.
Hab. in castanetis prope Busalla (Appennino ligure). Affine alla varietà violascens del *L. deliciosus*.

- Type:

Not typified

violaceus (Fig. 5)

- Protologue:

Agaricus deliciosus var. *violaceus* Barla, Tableau comparatif des champignons comestibles et vénéneux de Nice tav. 4, fig. 24 (1855) [IF 7: 164]

- Nomenclatural synonyms:

≡ *Lactarius sanguifluus* var. *violaceus* (Barla) Basso, Fungi Europaei 7. *Lactarius* Pers.: 308 (1999) [IF 7: 181]
≡ *Lactarius sanguifluus* var. *vinosus* Quél., Compt. Rend. Assoc. Franç. Avancem. Sci. 9: 668 (1881 (“1880”)) [SF 5: 439, 12: 980, 19: 1035], illegitimate nom. nov. for *Agaricus deliciosus* var. *violaceus* Barla
≡ *Lactarius vinosus* (Quél.→) Bataille, Fl. Monogr. Astérosporales: 28 (1908) [not indexed]
≡ *Lactarius sanguifluus* f. *vinosus* (Quél.→Bataille) Lalli & Pacioni, Micol. Veg. Medit. 17 (2): 121-132 (2003 (“2002”)), as “(Quél.) Lalli & Pacioni” [not indexed], invalid (as “stat. nov.” and reference to protologue wrong)

- Etymology:

violaceus (Lat.) = violet

- Original description:

(no description, only plate + name)
[description by BARLA (1859):
Agaricus deliciosus, Var. *violaceus*, Nob. (1)

Cette variété se fait constamment remarquer par sa couleur d'un rouge violacé, même pendant son premier développement. Les lamelles participent de cette couleur; elles ont souvent une teinte violâtre clair très prononcée.

Le pédicule est raide, assez court, souvent aminci à la base et comme couvert d'une légère poussière pruineuse et blanchâtre.

Ce champignon, lorsqu'on l'entame, laisse découler un suc lactescent d'un rouge vineux ou violacé. Dans nos montagnes, on le nomme Sanghin coulou de vin.

J'ai toujours remarqué que cette variété est plus tardive, et qu'elle se développe à l'approche de premiers froids, à la fin de l'automne. On l'apporte sur nos marchés, ainsi que l'Agaric délicieux ordinaire, dont elle a, du reste, le goût et les propriétés; sa chair est cependant plus compacte et exige une cocion assez prolongée.

(1) Je me réserve de publier la figure de cette variété dans les planches qui feront suite à cet ouvrage]

- Type:

Lectotypus: Tableau comparatif des champignons comestibles et vénéneux de Nice: tab. 4, fig. 24!
Europe, France: around Nice

- Type studies:

BASSO (1999a)

- Remarks:

A neotype has been invalidly designated by LALLI & PACIONI (2003 (“2002”)) (as original material – a plate – exists).

Fig. 5: Lectotype of *L. vinosus* (Fig. 24)

violascens

- Protologue:

Lactarius deliciosus var. *violascens* Panizzi, Comment. Soc. Crittog. Ital. 1: 174 (1862) [SF 5: 438]

- Etymology:

violascens (Lat.) = violet

- Original description:

L. Pileus carnosus, compactus, viscidus, umbilicatus, aurantio-pallidus, squamis obscurioribus zonatus. Stipes subconicus, albo-violaceus, scrobiculatus, intus spongiosus subcavus. Lamellae stipatae, perfragiles livido-violascentes. Lac primitus laccato-violaceum, arefactum virescens.

- Type:

Not typified

virescens

- Protologue:

Lactarius deliciosus f. *virescens* S. Imai, Bot. Mag. (Tokyo) 49: 607 (1935) [PL 7]

- Etymology:

virescens (Lat.) = becoming green

- Original description:

Vulnere virescente.

- Type:

Holotypus: requested information on type not received from SAPS
Asia, Japan

yvreus

- Protologue:

Lactarius sanguifluus var. *yvreus* C. Martin, Bull. Trav. Soc. Bot. Genève 7: 184 (1894) [SF 11: iii, 12: 980, 14: 94]

- Etymology:

Described from Bois d'Yvre

- Original description:

A l'entrée du bois d'Yvre, du côté d'Essery, sous des pins. Septembre à novembre.

Diffère de *L. deliciosus* Fr. par son aspect généralement plus massif, son pied proportionellement plus court, plus ramassé, moins régulièrement cylindrique, d'ordinaire atténué inférieurement; par le chapeau jaune d'ocre taché et panaché de rouge et de vert, ou jaune orangé taché de vert, ou entièrement vert taché de jaune, mais non couvert de zones concentriques; par les lames, qui vues d'ensemble par l'arête sont améthyste et vues par la face d'un rouge vineux orangé et tachées de vert; par le pied couleur améthyste taché de vert, souvent scrobiculeux, roux dans la vieillesse; par le lait, qui est, non rouge safrané, mais carmin foncé. Diffère de L. sanguifluus Fr. en ce que le pied est souvent creux et n'est pas rouge orangé, et en ce que les lames en sont pas crème puis orange rosé. Se rapproche de la var. *vinosus* Barla par les lames améthyste.

- Type:

Not typified

Note

An excellent web site that includes a very complete database of all the names published in the genus *Lactarius* can be found at http://www.mtsn.tn.it/russulales-news.