

Lactarius splendens, a second species with white latex in *Lactarius* section *Deliciosi*

Jorinde Nuytinck, Annemieke Verbeken, Irja Saar, Herman Lambert, Jean Bérubé, and Andrus Voitk

Abstract: An uncommon, small, orange-capped *Lactarius* Pers. with white latex was confirmed, using molecular studies of the type specimens, to be *Lactarius splendens* Hesler & A.H. Sm. Phylogenetic analysis revealed that the species falls within *Lactarius* section *Deliciosi* (Fr.) Redeuilh, Verbeken & Walley, very closely related to *L. rubrilacteus* Hesler & A.H. Sm. and *L. porninsis* Rolland. Hitherto *L. porninsis* was the only white-latex species known in the otherwise orange-latex section *Deliciosi*; both are associated with *Larix* Mill.

Key words: latex colour, phylogeny, type study, *Lactarius rubrilacteus*, *Lactarius porninsis*.

Résumé : L'identité d'un petit *Lactarius* Pers. à chapeau orangé et peu fréquent qui produit un latex blanc a été confirmée en tant que *Lactarius splendens* Hesler & A.H. Sm. par des études moléculaires réalisées sur les spécimens types. L'analyse phylogénétique a révélé que l'espèce fait partie de *Lactarius* section *Deliciosi* (Fr.) Redeuilh, Verbeken & Walley, étroitement apparentée à *L. rubrilacteus* Hesler & A.H. Sm. et *L. porninsis* Rolland. À ce jour, *L. porninsis* était la seule espèce à latex blanc connue de la section *Deliciosi* qui produit autrement un latex orangé; les deux sont associées à *Larix* Mill. [Traduit par la Rédaction]

Mots-clés : couleur du latex, phylogénie, étude de type, *Lactarius rubrilacteus*, *Lactarius porninsis*.

Introduction

Attempts to identify a small, orange-capped *Lactarius* Pers. with white latex, found three times during 12 years in the Canadian province of Newfoundland and Labrador (NL) under *Larix* Mill. in *Sphagnum* L. late in the season, eventually led to a similar collection from Quebec, tentatively identified as *Lactarius splendens* Hesler & A.H. Sm. by one of the authors (A.M.V.). Since then, a second, more recent collection from Quebec was added. Initial molecular studies revealed that the three specimens from Newfoundland and Labrador (NL) and the two from Quebec were conspecific, and fell within *Lactarius* section *Deliciosi* (Fr.) Redeuilh, Verbeken & Walley. To date, only one species with white latex is known in the orange-latex section *Deliciosi* (Nuytinck and Verbeken 2007): *L. porninsis* Rolland, which is a medium-sized European species with orange caps 4–13 cm in diameter and a zonate margin, common

throughout the montane forests of central Europe, also found under *Larix* (Rolland 1889).

Lactarius splendens is an uncommon, and therefore, little-known species from northeastern North America, seldom described in books or recorded in lists. Since its original description (Hesler and Smith 1979), it has been featured in Milk Mushrooms of North America (Bessette et al. 2009), mentioned and illustrated in Ectomycorrhizae of Maine 2, a list of *Lactarius* species with their various hosts (Homola and Czapowskyj 1981), and keyed out, but not described, in Mushrooms of Northeastern North America (Bessette et al. 1997). Apart from the holo- and para-types in MICH, we found three collections in MIN, and two in DAOM (herbarium acronyms following Thiers 2017).

The purpose of this communication is to confirm the identity of our collections by molecular studies of the type specimens, confirm its ranking in *L.* section *Deliciosi*, and give an extended and up-to-date description of this little

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Table 1. Collections of *Lactarius* sp. from which new sequences have been generated for this study.

GenBank No.	Herbarium No.	Collecting No.	Name	Year	Location	Collector
KY024219	MICH11209 HOLOTYPE	Homola 5796	<i>L. splendens</i>	1973	USA, Maine, Penobscot County, Milford	Richard L. Homola
KY024220	MICH37366 PARATYPE	Homola 6307	<i>L. splendens</i>	1975	USA, Maine, Penobscot County, Milford	Richard L. Homola
KY513922	DAOM 740081	08.08.15-LS-119 (lat23)	<i>L. splendens</i>	2008	Canada, NL, Labrador, Konrad Brook Pond	Esteri Ohenoja
KY513925	DAOM 740079	09.10.10.av13	<i>L. splendens</i>	2009	Canada, NL, GMNP, Baker's Brook fen	Andrus Voitk
KY513924	GENT AV2013-006	AV2013-006	<i>L. splendens</i>	2013	Canada, QC, Rimousky-Neigette	Annemieke Verbeken
KY513923	DAOM 740080	14.09.25.av01	<i>L. splendens</i>	2014	Canada, NL, GMNP, Stuckless Pond fen	Maria Voitk
KY513921	QFB-28541	HL 1443, Anticosti 2015-84	<i>L. splendens</i>	2015	Canada, QC, Anticosti Island	Herman Lambert
KY513929	GENT JN2008-034	JN2008-034	<i>L. rubrilacteus</i>	2008	USA, WA, Mt Rainier-Snoqualmie National Forest, Camp Sheppard trailhead	Jorinde Nuytinck
KY513930	GENT JN2008-030	JN2008-030	<i>L. rubrilacteus</i>	2008	USA, WA, North Cascades National Park, Goodell Creek campground	Jorinde Nuytinck
KY513928	GENT JN2002-023	JN2002-023	<i>L. porninsis</i>	2002	Italy, Trentino, Maso Postel	Roberto Flores
KY513926	GENT JN2002-025	JN2002-025	<i>L. porninsis</i>	2002	Italy, Trentino, Maso Postel (Pergine Valsugama)	Jorinde Nuytinck
KY513927	GENT DS09-660	DS09-660	<i>L. porninsis</i>	2009	France, Mercantour, Lac les Mesches (close to Casterino)	Dirk Stubbe

Note: NL, Newfoundland and Labrador; GMNP, Gros Morne National Park; QC, Quebec; WA, Washington.

known species as the second member of the section with white latex.

Materials and methods

We used standard field techniques, and the macroscopic descriptions are based on in-situ observations. Spores were measured from fresh deposits, and other microscopic examination from exsiccatae, staining the preparations with Congo red and using 1000× magnification (oil) and an Olympus Ortholux II microscope. Fresh spores were examined in distilled water and exsiccatae from squash preparations rehydrated with 10% ammonium hydroxide. Molecular methods for type specimens follow Saar and Voitk (2015); new sequences generated for this study are listed in Table 1. ITS sequences were aligned using the online version of MAFFT version 7 (Kato and Toh 2008), using the E-INS-I strategy. Trailing ends of the alignment were trimmed in BioEdit version 7.0.9.0 (Hall 1999). Maximum Likelihood (ML) analyses were conducted with RAxML version 8.0.24 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010).

Results

Molecular analysis (Fig. 1) showed that the NL and Quebec collections were conspecific with the types of *L. splendens*. As the preliminary analysis (Nuytinck and Voitk 2016) suggested, this larch associate with white latex fell in the orange-latex section *Deliciosi*. It nested in a paraphyletic group together with *L. rubrilacteus* Hesler & A.H. Sm. and

L. porninsis. Raw sequence signals revealed an intraspecific variation in the *L. splendens* ITS region of eight nucleotide positions. The interspecific variation between the three species also averaged the same number of nucleotides. There were no consistent insertions/deletions that distinguished the three species. Longer branch lengths of some of the *L. splendens* and *L. rubrilacteus* accessions (e.g., *rubrilacteus* EU526010, *rubrilacteus* EF685085, *rubrilacteus* EF685084, and *splendens* KY513921) are caused by differences in sequence quality rather than giving a real phylogenetic signal. None of the branching patterns within these paraphyletic species receive significant bootstrap support, but differences in morphology, habitat, tree associates, and distribution served to distinguish these three species more readily (Table 2).

Description

Lactarius splendens Hesler & A.H. Sm. (Fig. 2)
MB 116116

MACROMORPHOLOGY: Pileus 18–48 mm in diameter, convex, margin decurved, plane with age; centre slightly depressed, often with small central umbo; surface viscid, especially over the disc, with gelatinous layer seen when moist; opaque, yellowish orange, darker in centre; edge sometimes flaky-powdery. Lamellae close to moderately spaced, straight to sinuous, adnate with small decurrent tooth, light yellow, turning more orange with maturity; edge very minutely jagged; lamellulae 0–3. Stipe 15–40 mm × 3–8 mm,

Fig. 1. Phylogeny of *Lactarius splendens* and related species. The Canadian collections cluster together with the two types from MICH in a paraphyletic nested group in sect. *Deliciosi*, together with the red-latex *L. rubrilacteus* from western North America, and the European *L. porninsis*, another white-latex *Larix* associate. Multilocus analysis is required to resolve the relationship of these three species.

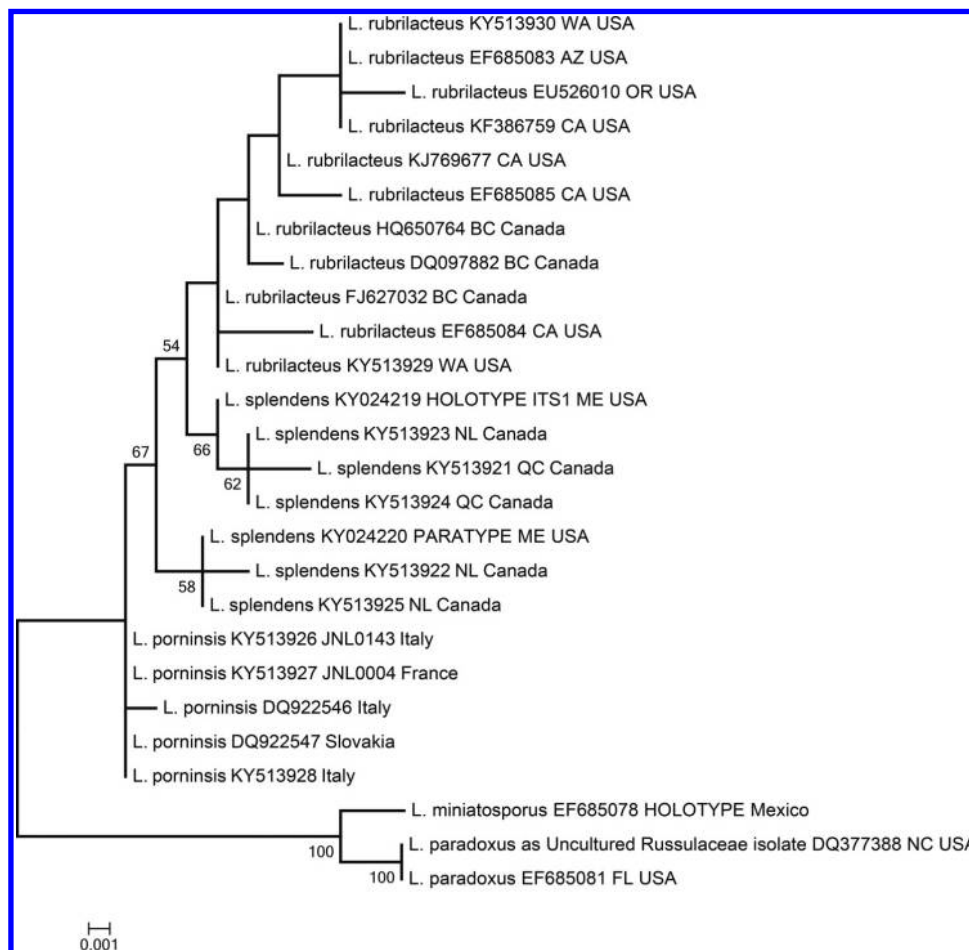


Table 2. Differing morphological, ecological, and distributional characters of *Lactarius porninsis*, *L. rubrilacteus*, and *L. splendens*.

	<i>L. porninsis</i>	<i>L. rubrilacteus</i>	<i>L. splendens</i>
Cap			
Avg. diam. (cm)	7	8	3.5
Zonation	Some	Conspicuous	None to minimal
Colour	Orange	Yellowish brown	Orange
Latex colour	White	Red	White
Flesh staining	None	Green	None
Habitat	Montane forest	Montane forest	Wetland with <i>Sphagnum</i>
Tree associate	<i>Larix</i>	<i>Abies</i> , <i>Pinus</i>	<i>Larix</i>
Distribution	Central Europe	Western North America	Eastern North America

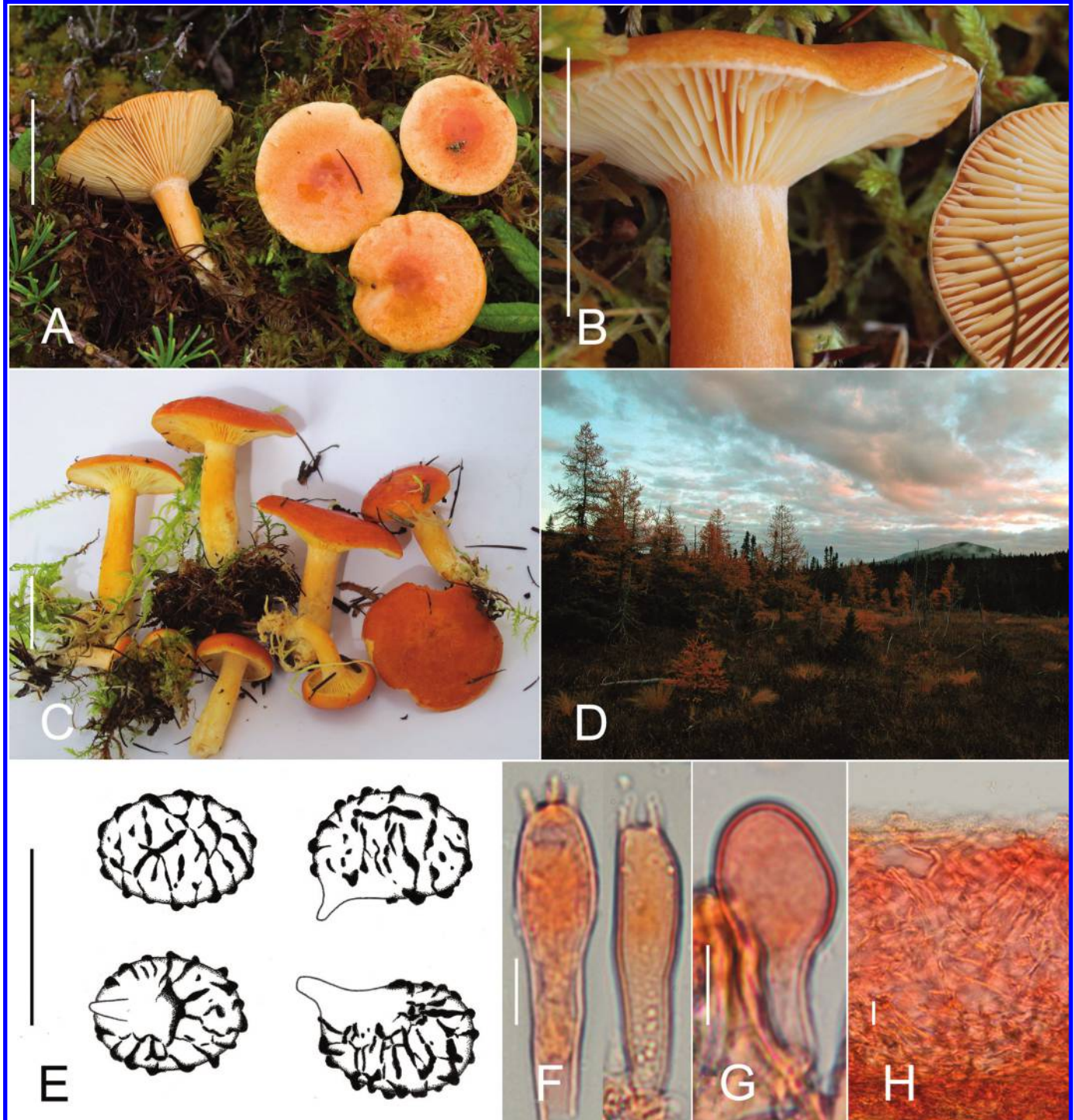
even, ascrobiculate, smooth, orange-yellow; solid to pithy, then hollowing. Context yellowish, unchanging; smell nonspecific; taste disagreeable, distinctly stale. Latex: sparse, white to whey coloured; no colour change or

staining, unchanging on white tissue. Spore print is yellowish white.

MICROMORPHOLOGY: Spores of current collection (91 spores, 1 collection, 1 sporocarp): (6.7)7.3–8.6(9.2) $\mu\text{m} \times$ (5.1) 5.2–6.2 (6.8) μm , $Q = 1.3\text{--}1.5(1.7)$, ellipsoid, ornamented with warts and rounded edges up to 0.5 μm high, isolated or forming a partial reticulum. Spores of sequenced basidiocarps from the holo- and para-type collections (40 spores, 2 collections, 2 sporocarps), examined by a different observer, were similar in appearance, measuring (6.9)7.8–8.9(9.4) $\mu\text{m} \times$ (5.6)6.0–7.0(7.2) μm , $Q = 1.2\text{--}1.5$ (Hesler and Smith's 1979 report of the same material was 7–9 \times 6–7). Basidia measured 37–50 $\mu\text{m} \times$ 9–13 μm , were 4-spored and clavate. Pleurocystidia 40–60 $\mu\text{m} \times$ 15–20 μm , clavate, very rare. Cheilocystidia not seen. The suprapellis is an isolattice composed of branched thin-walled hyphae, which are 3–6 μm in diameter, arising from an interwoven layer of hyphae without incrustation. Clamp connections absent. **HABITAT:** Small scattered groups in fens and bogs in *Sphagnum* under *Larix*.

DISTRIBUTION: Northeastern North America. Recorded as far west as Manitoba.

Fig. 2. *Lactarius splendens*. (A) Collection DAOM-740079 from Newfoundland in situ. (B) In situ close-up of collection DAOM-740081 from Labrador, showing scant white latex. (C) Voucher photo for collection GENT-AV2013-006 from Quebec. Scale bars = 2 cm. The long, thin, tapered and slightly curved needles are from *Larix*. (D) Habitat photo for collection DAOM-740079, fen beside trail to Baker's Brook Falls, Gros Morne National Park, Newfoundland and Labrador, Canada, 26 October 2010. (E) Microscopic drawings of basidiospores from GENT-AV2013-006; scale bar = 10 μm . (F–H) Micromorphology of collection QFB-28541 from Quebec in Congo red; scale bar = 10 μm . (F) Basidia. (G) Pleurocystidium. (H) Pileipellis. Original magnification: A–G, 1000 \times ; H, 400 \times .



Discussion

Lactarius splendens may be more common in its habitat, and awareness of its presence might spur more reports of this species. It bears a superficial resemblance to other

small orange *Lactarius* species with white latex, such as *L. alpinus* Peck, *L. aurantiacus* (Pers.) Gray, *L. lanceolatus* O.K. Mill. & Laursen, *L. luculentus* Burlingham, *L. luculentus* var. *laetus* Hesler & A.H. Sm., and *L. tabidus* Fr., but is phylo-

genetically distant from all of them. The differences between some of these taxa have been illustrated and discussed briefly in a preliminary report by Nuytinck and Voitk (2016). *Lactarius aurantiacus* is only known from Europe, and both varieties of *L. luculentus* are only known from western North America. *Lactarius alpinus* is an alder associate from ericaceous barrens, with a dry, scaly cap and a stem about twice the length of the cap diameter. *Lactarius lanceolatus* is associated with dwarf willow in the tundra, with a reddish-brown cap and a scrobiculate stem. *Lactarius tabidus* is a relatively common birch associate of boreal forested areas, with a somewhat larger cap that is more straw to tan coloured than yellow-orange.

Larix is an unusual associate for *Lactarius* — is it more than a coincidence that both the European and North American white-latex species in section *Deliciosi* share this host? Hesler and Smith (1979) reported *L. splendens* as a conifer associate, and Homola and Czapowskyj (1981) described it growing with *Thuja occidentalis* L., a tree that does not form ectomycorrhizal relationships. The three collections in MIN list tamarack (*Larix*) bog for one, pine and tamarack for another, and moss for the third. Photos are available for the last two collections, and both show *Sphagnum* and larch needles on and around the mushrooms. The two DAOM collections list cedar (presumably *T. occidentalis*) for one and *Picea* Mill. and *Larix* for the other. Both hemlock and cedar can be found in fens with larch, making it likely that larch was the associate for all. Larch grows in both dry areas, and bogs and fens.

All of our collections came from *Sphagnum* wetlands. The type collections mention *Sphagnum*, as does Homola and Czapowskyj, whereas other descriptions speak of moss in marsh, bog, or swamp, which are habitats where *Sphagnum* thrives. We were unable to deduce whether *Sphagnum* is an active symbiont to *L. splendens*, or a chance associate because it also thrives in such wetlands. The European *L. porninsis* does not have a preference for wetlands or *Sphagnum*. Apart from their association with *Sphagnum*, the two white-latex *Larix* associates also have other differences: *Lactarius porninsis* is a larger mushroom with more evident zonation on the cap, and, of course, grows an ocean and a continent away.

We were not able to resolve the phylogeny of *L. splendens*, *L. porninsis*, and *L. rubrilacteus* using ITS markers. Likely the region is not variable enough to distinguish these species, and multilocus analysis seems necessary. Their

marked morphological, ecological, and geographical differences delimit the species better than molecular studies with ITS alone.

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