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LYOPHYLLUM MALEOLENS AND *LYOPHYLLUM AEMILIAE*
TWO SIMILAR BUT DISTINCT SPECIES

Abstract

The independence of L. maleolens from L. aemiliae is sanctioned through the molecular analysis of the typus and clarified some correct and incorrect interpretations present in the literature. An English version of the article is attached to this issue.

Riassunto

Viene stabilita l'indipendenza di L. maleolens da L. aemiliae tramite l'analisi molecolare dei typus e chiarite alcune interpretazioni corrette e non, presenti nella letteratura. Al presente numero viene allegata una versione in inglese dell'articolo.

Key words Agaricales, Lyophyllaceae, *Lyophyllum*, *L. aemiliae*, *L. maleolens*, taxonomy.

Introduction

Lyophyllum maleolens Melis & Contu and *Lyophyllum aemiliae* Consiglio are two species which, on the basis of their macro- and micromorphological characteristics, belong to *Lyophyllum* sect. *Semitalina* (Singer) Consiglio & Contu for smooth spores and basidia on average longer than 30 μm and subsect. *Semitalina* Consiglio & Contu, for the ellipsoidal or subfusiform spores and for the not yellow gills. Within the subsection they are characterized by the lamellas assuming a yellow to orange-reddish color to the touch before turning black. The species in question are little known and sometimes misinterpreted. To prevent them from being synonymised or forgotten, the authors sequenced the ITS and rpb2 regions of the rDNA of the species in question using two collections of *L. aemiliae* (including an isotypus) and one of *L. maleolens* (*epitypus*).

This need emerged given that some authors interpreted *L. maleolens* differently.

BELLANGER (2016) refers to a *L. maleolens* ss. Corriol and a *L. maleolens* ss. Bidaud who in the phylogenetic tree proposed by him and previously by BELLANGER *et al.* (2015) place themselves in the clades Va 13 and Va 11, respectively.

In this work the macroscopic, microscopic, environmental and molecular characteristics of the two taxa are compared.

Well the *typus* of *L. maleolens* corresponds to the collection present in the clade Va13 (ss. CORRIOL FR2014066) while the *typus* of *L. aemiliae* to that present in the clade Va 11 (ss. BIDAUD FR2014018).

Materials and methods

Macroscopic and microscopic description

For the macroscopic description, see the original works (CONSIGLIO 1998, MELIS & CONTU 2001). Microscopic observation, was carried out in this study and compared with that of the original works. It was performed on dried material of the *typus*, (including the *holotypus* of *L. maleolens* of which it was possible to study the microscopy but it was not possible to obtain the genetic sequences). The dried material was rehydrated with 3% Ammonium hydroxide. The preparations were observed in anionic Congo red or in water with OPTIKA microscopes. Spore size data is the result of at least 32 measurements for each collection. Each spore was measured using the piximeter software on images obtained from cameras dedicated to the microscopes used.

The data relating to the sporal size, length and width, was obtained following the statistical method proposed by HEINEMANN & RAMMELOO (1985), the data obtained is composed of a first number in parentheses which represents the smallest spores, a second number without parentheses which represents the smallest value obtained from the standard deviation, a third number, underlined, which indicates the average value, a fourth number which identifies the largest value obtained from the standard deviation and finally the last value is that relating to the largest spores. The QM (Average Quotient) is given by the arithmetic mean of the quotients of the individual spores. The quotient of a spore is the ratio of length to width. The apiculus is excluded from the measurements.

Choice of champignons

The microscopic and molecular characters of the *isotypus* of *L. aemiliae* and of another collection of the same species, both kindly provided by prof. Giovanni Consiglio, author of the species (CONSIGLIO 1998). The *holotypus* collection of *L. maleolens* was also reanalysed microscopically, unfortunately, the various attempts to extract the DNA failed. A collection made by Mario Melis, co-author of the species and collector of the *holotypus*, in the same place as the original collection, which here is designated as *epitypus*, was therefore examined.

Molecular analysis

The total DNA was extracted and amplified by the Alvalab laboratory in Oviedo (Spain) according to the laboratory's standardized method. (ALVARADO *et al.* 2010, 2012). After having obtained the sequences of the stretch comprising partial 18S, ITS1, 5.8S and total ITS2, partial 28S, the rpb2 marker was analysed. The sequences were compared using the Blast software (ALTSCHUL *et al.* 1990) of the NCBI website with similar sequences present in the web database, Unite and GenBank.

The phylogenetic tree was thus obtained: two datasets were processed containing some sequences of the ITS and rpb2 regions relating to species belonging to the genus *Lyophyllum*, clade Va according to BELLANGER *et al.* (2015) taken from the GenBank database, including also the sequences of the *typus* collections of *L. aemiliae* and *L. maleolens*; the sequences related to *Tephrocycbe anthracophila*, GenBank KP192640 (ITS region), KP192522 (rpb2) with outgroup function have also been added.

The sequences of the two databases were separately aligned using the Muscle application of the MEGA 6 software (TAMURA *et al.* 2013); they were combined using the MESQUITE software (MADDISON & MADDISON 2017) and, after obtaining the file with the .phy extension, the Maximum Likelihood system was used using the RAXMLGUI 2.0 program (EDLER *et al.*, 2020) setting as parameters bootstrap 1000 and model GTRGAMMA. The phylogenetic tree thus obtained from the combined analysis was processed with the ThreeGraph 2 software.

The "nexus for Mr. Bayes" file was also obtained from the Mesquite software, which made it possible to calculate the posterior probability using the Mr. Bayes program version 3.2.7 (RONQUIST *et al.* 2012), using the following parameters: 10 million generations, sample rate 1000 and finally the 25% value for the sumt and sump burnin. Also in this case the phylogenetic tree obtained was processed with the ThreeGraph 2 software through which the ML values were transferred to the corresponding branches of the tree obtained with M. Bayes.

TAXONOMY

Lyophyllum aemiliae Consiglio, *Journal of Mycology* LXI (2): 99-104 (1998)

Collections studied GC 92218 – AE01 (*isotypus*): Parco La Martina (Monterenzio, BO), 14.11.1992, *leg.* G. Consiglio & S. Spisni, GenBank ITS region OQ195776, rpb2 OQ286519. GC 92219 – AE02 is a second collection made on the same day at the same station. GenBank ITS region OQ195773.

Macroscopic description (CONSIGLIO 1998)

Pileus: 4-7 cm, flat-convex with wide obtuse umbo and wavy-lobed margin, slightly streaked.

Pileic coat: lubricated, hygrophanous, uneven grey-brown, irregularly spotted with large darker, blackish-brown patches.

Lamellae: rather dense, adnate or marginalized whitish or a little greyish, turning to a rusty-reddish yellow to the touch, a little orange, more or less persistent, and then blackening.

Stipe: 50-80 × 4-8 mm., rather slender, regular, cylindrical or slightly attenuated below, sometimes slightly curved, greyish-brownish in colour, paler in the cap, underneath very fine whitish, longitudinal, silky fibrils, with the shaggy base for a cottony, whitish mycelial felt.

Context: firm, whitish, graying when cut, weak, pleasant, herbaceous smell and taste.

Microscopic description (redone in this work)

Spores (7.69) 7.93-8.66-9.4 (10.47) × (4.6) 4.67-5.08-5.49 (6.07) μm, Q = 1.61-1.81, QM = 1.71, ellipsoid-fusiform, smooth, often with a large central guttula.

Basidia 36-46 × 11-13.5 μm clavate, tetrasporic.

Pleurocystidia absent.

Marginal cells not observed.

Pileipellis cutis with gelled hyphae, parallel cylindrical, thick 2.5-6 μm.

Lyophyllum maleolens Melis & Contu, *Micologia e Vegetazione Mediterranea* vol. 15 (2): 101-105 (2001) [2000].

Collections studied *holotypus*: Italy, Sardinia, Domusnovas (SU) Marganai forest, loc. Sa Duchessa January 13th, 2001, *leg.* Mario Melis.

Here designated *epitypus*, because after various attempts, it was not possible to obtain the sequence of the *holotypus*, as deteriorated, furthermore the species is interpreted by various authors in a non-univocal way. The *epitypus* comes from Domusnovas (SU), loc. Sa Duchessa, collected by Mario Melis on December 1st, 2012, was deposited in the CAG herbarium (HOLMGREN *et al.* 1990) with number C/13.9.6 a, GenBank: Regione ITS OQ195779, rpb2 OQ286518.

The *holotypus* was deposited by Mario Melis in the herbarium of the Science Department and the Environment section Botany of the University of Cagliari listed in the *Index Herbariorum* with the acronym CAG (HOLMGREN *et al.* 1990) to which the code C/13.9.6 was assigned.

Macroscopic description (MELIS & CONTU 2002)

Pileus: 2-6.5 cm, medium fleshy with a non-cartilaginous consistency, hemispherical then wider and finally flat, non-umbonate center, moist, bare, radially fibrillose but smooth. brownish-beige, deep brown or gray-brown, wavy margin with short and thin streaks even in the young.

Lamellae: medium dense unequal, adnate-marginate or hooked-adnate, beige-ochreous, relatively dark, instantly turning to rusty fawn to the touch, then markedly blackening, cut concolorous to the face.

Stipe: 5.8 × 1.5-2.5 cm, full then hollow, cylindrical, whitish, finely fibrillose, decorated at the base with evident white hairs up to 2 mm long.

Context: supple, reddening to the touch, darkening then blackening when cut, strong, floury-spermatoc odor, persistent and intensifying on drying, overall rather unpleasant; clearly floury taste then (after a few seconds) slightly astringent. Probably inedible.

Microscopic description (redone in this work)

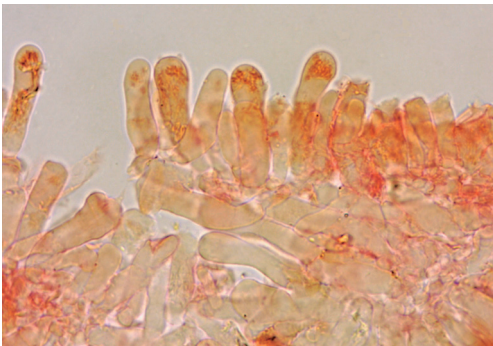
Spores (8.05) 8.22-8.78-9.34 (9.84) × (4.06) 4.56-4.88-5.2 (5.87) μm, Q = 1.69-1.92, QM = 1.8, ellipsoid-fusiform, also larmiform, smooth, often mono-oil droplets.



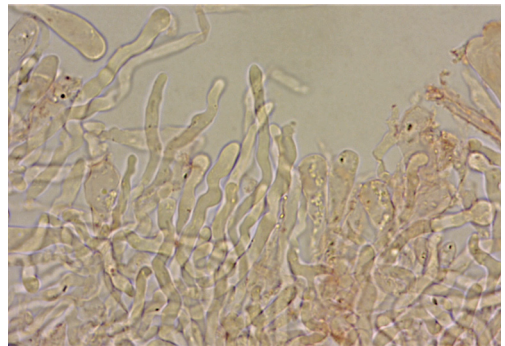
Lyophyllum aemiliae GC 92219. Photo by Giovanni Consiglio



Lyophyllum maleolens C 13.9.6a. Photo by Mario Melis



L. aemiliae. Lamellar edge without marginal hairs (1000×).
Photo by Giuseppe Porcu



L. aemiliae. Lamellar edge with numerous marginal hairs (1000×).
Photo by Giuseppe Porcu

Basidia clavate, tetrasporic, 35-40 × 9-15 μm with evident siderophilous granulations.

Pleurocystidia absent.

Marginal cells numerous, filiform, sometimes capitulated, rarely lageniform, 2.5-7 μm thick.

Pileipellis cutis with gelled, parallel and partly intertwined hyphae 2-5.5 μm thick.

Macroscopic and microscopic differences between the two taxa

Taken partly from our studies and partly from the original descriptions: *L. aemiliae* (CONSIGLIO 1998); *L. maleolens* (MELIS & CONTU 2001); and, for both, by CONSIGLIO & CONTU (2002).

Lyophyllum maleolens usually has a lighter, brownish-beige cap color and innate radial fibrils; gills initially turning to rust fawn and finally blackening; reddening then darkening finally blackening flesh, unpleasant smell and taste with a floury component; the presence of numerous marginal cells.

Lyophyllum aemiliae has a darker cap colour, grey-brown with blackish-brown patches, absence of fibrils; changing lamellae, initially with a yellowish, yellow-orange component, finally blackening; graying flesh, with a weak, pleasant herbaceous smell and taste; the absence of marginal cells.

The sporal differences between the two species are not significant.

Results

The proposed phylogenetic tree highlights the separation of the species referring to *L. aemiliae* and *L. maleolens* for which the interspecific diversity is confirmed, moreover it is attributed to *L. maleolens* ss. Corriol corresponds to the *typus*, while *L. maleolens* ss. Bidaud is to be attributed to *L. aemiliae*.

Discussion

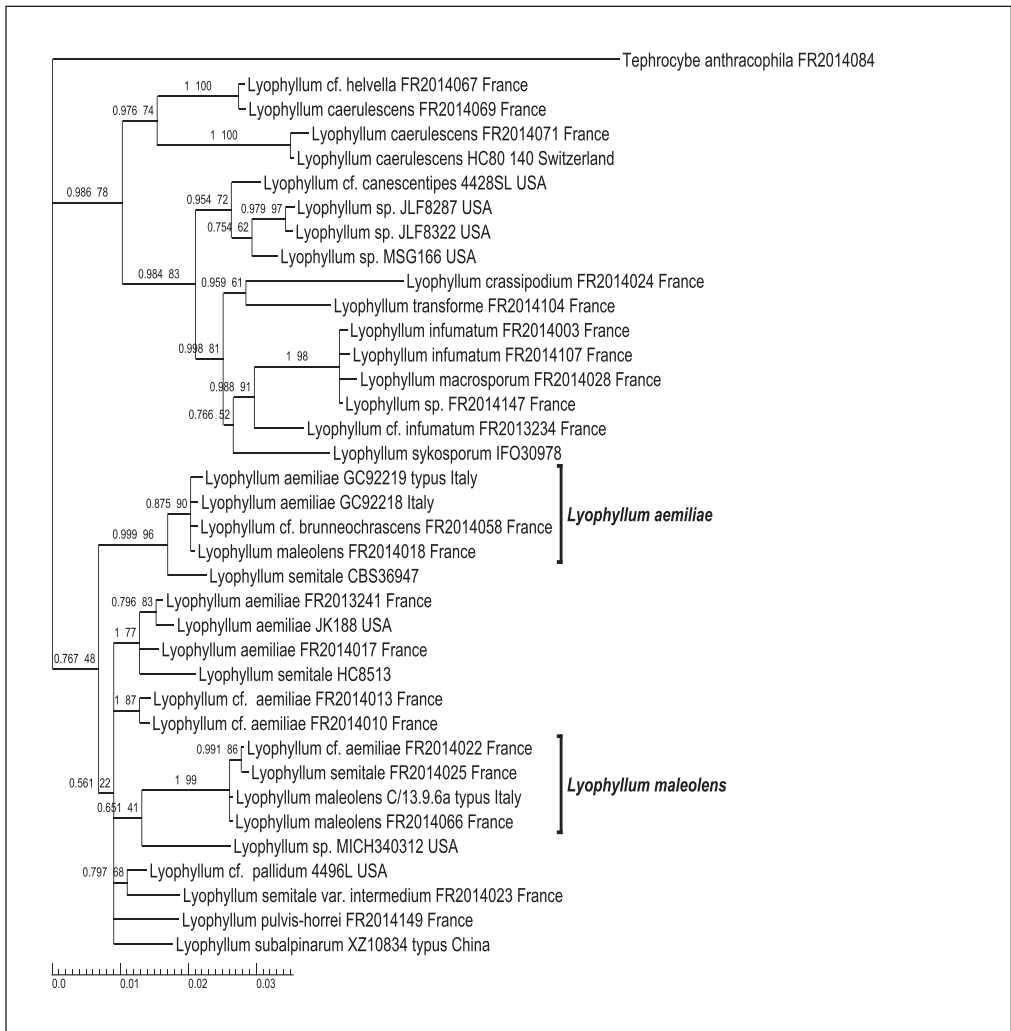
In relation to the French collections referred to Clades Va 13 and Va 11 in the phylogenetic tree proposed by BELLANGER (2016) and previously by BELLANGER *et al.* (2015) we can highlight the macro and micromorphological differences between *L. maleolens*, *L. aemiliae* and the other indicated species: *Lyophyllum brunneo-ochrascens* E. Ludwig is characterized by small-sized

Taxa voucher ITS rpb2

Taxa	voucher	ITS	rpb2
<i>Lyophyllum aemiliae</i>	GC 92219	OQ195773	OQ286519
<i>Lyophyllum aemiliae</i>	GC 92218	OQ195776	
<i>Lyophyllum aemiliae</i>	FR2013241	KP192562	
<i>Lyophyllum aemiliae</i>	FR2014017	KP192596	
<i>Lyophyllum aemiliae</i>	JK188	ON503032	
<i>Lyophyllum cf. aemiliae</i>	FR2014013	KP192591	
<i>Lyophyllum cf. aemiliae</i>	FR2014010	KP192582	KP192505
<i>Lyophyllum cf. aemiliae</i>	FR2014022	KP192597	KP192507
<i>Lyophyllum cf. brunneo-ochrascens</i>	FR2014058	KP192618	
<i>Lyophyllum cf. helvella</i>	FR2014067	KP192625	KP192516
<i>Lyophyllum caerulescens</i>	FR2014069	KP192626	
<i>Lyophyllum caerulescens</i>	FR2014071	KP192628	KP192517
<i>Lyophyllum caerulescens</i>	HC80/140	AF357052	EF421000
<i>Lyophyllum cf. canescentipes</i>	4428SL	KU574741	
<i>Lyophyllum crassipodium</i>	FR2014024	KP192608	KP192509
<i>Lyophyllum infumatum</i>	FR2014003	KP192584	KP192504
<i>Lyophyllum infumatum</i>	FR2014107	KP192655	KP192529
<i>Lyophyllum cf. infumatum</i>	FR2013234	KP192557	KP192500
<i>Lyophyllum macrosporum</i>	FR2014028	KP192612	
<i>Lyophyllum maleolens</i>	C/13.9.6 a	OQ195779	OQ286518
<i>Lyophyllum maleolens</i>	FR2014066	KP192624	KP192515
<i>Lyophyllum maleolens</i>	FR2014018	KP192607	KP192506
<i>Lyophyllum cf. pallidum</i>	4496SL	KU574739	
<i>Lyophyllum pulvis-horrei</i>	FR2014149	KP192665	KP192532
<i>Lyophyllum semitale</i>	CBS36947	AF357048	
<i>Lyophyllum semitale</i>	FR2014025	KP192598	
<i>Lyophyllum semitale</i>	HC85/13	AF357049	EF421002
<i>Lyophyllum semitale var. intermedium</i>	FR2014023	KP192604	KP192508
<i>Lyophyllum subalpinarum</i>	XZ10834	OP605492	
<i>Lyophyllum sykosporum</i>	IFO30978	AF357050	EF421003
<i>Lyophyllum transforme</i>	FR2014104	KP192653	KP192528
<i>Lyophyllum sp.</i>	JLF8287	ON259690	
<i>Lyophyllum sp.</i>	JLF8322	MT355555	
<i>Lyophyllum sp.</i>	MICH340312	OM985834	
<i>Lyophyllum sp.</i>	FR2014147	KP192664	KP192531
<i>Lyophyllum sp.</i>	MSG166	KU058499	KU138999
<i>Tephrocycbe anthracophila</i>	FR2014084	KP192640	KP192522

carpophores (the cap measures 2-4.5 cm) directly blackening in all their parts, the floury smell and taste, the ellipsoid spores and the absence of marginal cells (Ludwig 2001); *Lyophyllum semitale* (Fr.) Kühner is a strongly and directly blackening species in all its parts, with a faint floury smell and elongated fusiform spores; *Lyophyllum semitale* var. *intermedium* Romagn. it would differ from the previous one, with which it is currently synonymous, due to its small size (the pileus measures 2-4 cm) and smaller spores (Romagnesi 1987). *Lyophyllum pulvis-horrei* E. Ludw. & Koeck is characterized by its small size (pileus 1-3.8 cm), the carpophore and the gills which turn black if bruised, with a characteristic odor like that of barn dust, then floury when rubbed and spherical or largely elliptical spores lower (Ludwig 2001).

Recently (WEI *et al.* 2023) new species of *Lyophyllum* have been published from China, in particular *Lyophyllum subalpinarum* S.W., Wei, Q. Wang & Li which in the article appears



Bayesian phylogenetic tree inferred from the nrITS and rpb2 datasets of *Lyophyllum* clade Va (BELLANGER 2015). The branches show the Bayesian posterior probability (BPP) values and the corresponding Maximum Likelihood Bootstrap (MLB) support values. *Tephrocye anthracophila*, Genbank KP192640, was used as an outgroup taxon.

phylogenetically related to *Lyophyllum maleolens*, to *Lyophyllum pulvis-horrei* and *Lyophyllum semitale*. The new species *L. subalpinarum* differs from *L. maleolens* for the cap with yellowish, yellow-greyish colours, the lamellae directly blackening if bruised, the spores from rounded cylindrical to irregularly rhomboidal of smaller dimensions, and the absence of marginal cells.

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