ISSN 2785-4124

Volume 16

2026-08-26

MYCOLOGICAL OBSERVATIONS

Gruppo Micologico e Naturalistico DLF Verona

MYCOLOGICAL OBSERVATIONS

open access, electronic journal

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Volume 16 **2026-08-26**

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Article received 4 April 2026, accepted 27 May 20265

Segnalazione di *Chromosera viola* nell'Italia nordorientale

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Parole chiavi / Key words:
Agaricales
Hygrocybaceae
Hygrocybe
photographs
microscopy
rare mushrooms

Riassunto: La specie *Chromosera viola* viene segnalata per la prima volta in Italia nordorientale. Il lavoro è corredato da originali e inedite fotografie a colori, proponendo una descrizione completa e dettagliata delle caratteristiche macro- e microscopiche della specie.

Abstract: La *The species Chromosera viola is reported for the first time in northeastern Italy*. The paper is supplied with original and unpublished color photographs, providing a detailed complete description of the macro- and microscopic features of the species.

INTRODUCTION

Nel 1990 Boertmann istituì all'interno del genere *Hygrocybe* (Fr.) Kumm la sezione *Oreocybe* Boertm. (Boertmann 1990), comprendente tutte le specie caratterizzate da un portamento imbutiforme onfalinoide e dalla crescita generalmente muscicola, tipicamente in ambienti freddi (Candusso 1997). Con poche eccezioni, le specie presentano cappello e gambo entrambi viscosi e sfumature violacee in qualche punto del carpoforo, caratteristica che permette di riconoscerle agevolmente già dal punto di vista macroscopico.

La filogenesi molecolare dimostrò successivamente che *Hygrocybe*, almeno come inteso classicamente, era un genere polifiletico, rendendo necessario lo spostamento di svariate specie in generi nuovi. *Hygrocybe* sottogenere *Oreocybe* (Boertm.) Beis. si rivelò essere filogeneticamente vicino ad un'altra specie onfalinoide, peraltro molto simile anche nei colori, *Omphalina cyanophylla* Fr. Quel. (Vizzini & Ercole 2012). Quest'ultima specie era stata inclusa in un genere appositamente creato, *Chromosera* Redhead, Ammirati & Norvell (Redhead, Ammirati & Norvell 1985), in cui sono conseguentemente confluite tutte le specie della sezione *Oreocybe*.

In questo genere, *Chromosera viola* (Geesink & Bas) Vizzini & Ercole (basionimo in Arnolds 1985) risulta probabilmente la più appariscente, per via della vistosa colorazione viola lilacea ovunque nello sporoforo, caratteristica unica fra le specie segnalate nel territorio europeo (Grootmyers *et al.* 2025). La sua rarità rende le segnalazioni della sua presenza molto circoscritte nel territorio italiano. Conseguentemente, le fotografie della specie in questione sono molto limitate. Inoltre, immagini degli elementi microscopici degli sporofori sono quasi assenti in letteratura.

In questo lavoro, si vuole presentare un recente ritrovamento in territorio italiano, per la prima volta nell'Italia nordorientale, più specificamente nel territorio di Borgo Valbelluna in provincia di Belluno. La specie risulta infatti assente nell'elenco fornito dal Censimento dei Funghi del Triveneto. Le descrizioni dei caratteri morfologici macroscopici e microscopici della specie vengono riviste alla luce dei campioni raccolti. Sono incluse svariate immagini di dettagli inediti, sia macroscopici, sia microscopici.

MATERIALI E METODI

Gli esemplari sono stati fotografati da G. Isotton e G. B. Carollo sul campo e in studio. I dati e le immagini di microscopia sono stati ottenuti da G. Pobbe a partire dalle exiccata reidratate in acqua, colorate in rosso congo e osservate in KOH 2 %, utilizzando un microscopio Zeiss con obiettivo Zeiss 1000x.

TASSONOMIA

Chromosera viola (J. Geesink & Bas) Vizzini & Ercole [Figure 1–6]
Micol. Veg. Medit. 26(1): 97 (2012) [2011]

Basionimo: *Hygrocybe viola* J. Geesink & Bas, in Arnolds, *Persoonia* 12(4): 478 (1985)

Figura 1. *Chromosera viola* nel suo tipico habitat

Figura 2. *Chromosera viola* in terreno battuto, in prossimità di briofite

Caratteri macroscopici

Portamento igrociboide-onfalinoide; aspetto delicato e ceraceo.

Cappello diametro 0,5–1,2 cm; inizialmente emisferico e convesso, quindi appianato con leggera depressione centrale negli esemplari maturi; margine sottile, spesso ondulato e crenulato; forma marcatamente floriforme; su perficie pileica liscia o finemente pruinosa, di colore violaceo-lilacino, striato per trasparenza, con sfumature

dal rosa malva pallido fino a un lilacino più saturo o rosato-violaceo verso il disco centrale e in corrispondenza delle lamelle sottostanti; negli esemplari secchi tende a sbiadire verso toni rosati-lilacei opachi.

Lamelle piuttosto spaziate, in numero di 20-21 per esemplare; con lamellule pliciformi di diversa lunghezza, regolarmente disposte e con anastomosi nei seni interlamellari; inizialmente bianche, poi crema, infine a macchie ocracee; filo intero, concolore; inserzione sul gambo da decorrente a marcatamente decorrente.

Gambo 8–15 × 2–3 mm, a volte irregolare; esile e spesso ricurvo, leggermente ingrossato verso il cappello, con sezione più circolare alla base e progressivamente più ellittica verso l'alto; consistenza fibrosa, flessibile, ma comunque molto fragile; superficie glabra e lucida, concolore al cappello, a volte con lievi variegature cromatiche, con riflessi satinati-sericei e quasi iridescenti in luce radente. Assenti anello, cortina o zone particolarmente differenziate.

Figura 3. *Chromosera viola*: dettaglio di gambo e imenoforo, che permette di apprezzare le numerose anastomosi presenti nei seni interlamellari

Carne quasi assente; di consistenza ceracea, acquosa; violacea; sapore non distintivo, odore debolmente fungino o assente.

Caratteri microscopici

Basidiospore 6.5–10(11) × 5–6.5 µm, in media 7.9 × 5.6 µm, Q = 1.1–2.2, Q_m = 1.41.

Basidi 35–45 (55) × 8–10 (11) µm.

Trama lamellare irregolare, per brevi tratti quasi subregolare, costituita da ife catenulate, da globose a cilindriche, anche con depressioni, di spessore 5–25 µm.

Filo lamellare fertile con basidi, cheilocistidi assenti.

Pleurocistidi assenti.

Pileipellis di tipo cutis con qualche ifa emergente a terminale arrotondato, ife larghe 3–12 (18) µm.

Cortex del gambo con ife appressate tipo cutis, 2–8 µm, con qualche ifa emergente ad apice arrotondato.

Giunti a fibbia presenti.

Habitat: in mezzo al muschio e su terra battuta in prossimità, in bosco di *Fagus sylvatica*, in innumerevoli esemplari.

Materiale studiato: Italia, Veneto, Belluno, Borgo Valbelluna, località Valmaor, coordinate geografiche N 46° 01' 27.3" E 12° 05' 58.1'', ca. 670 m s.l.m., in un bosco di *Fagus*, 15 ottobre 2025, *leg. G. Isotton*. Gli exsiccata saranno depositati presso FGRV–Fungarium Certificato Regione del Veneto.

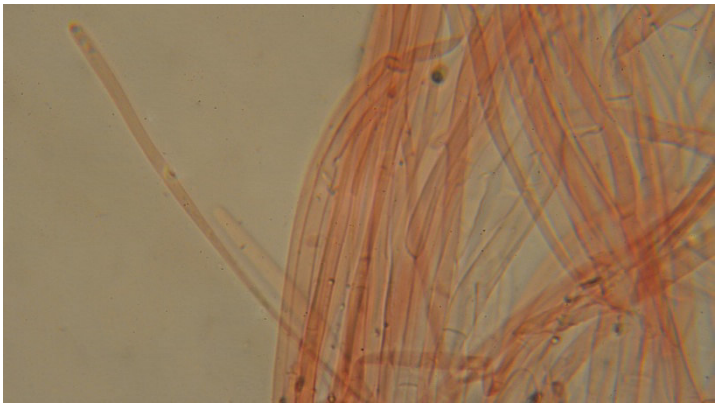

Figura 3. *Chromosera viola*: cortex del gambo con ife appressate tipo cutis, con qualche ifa emergente ad apice arrotondato (ingrandimento 400x, osservazione in rosso congo)

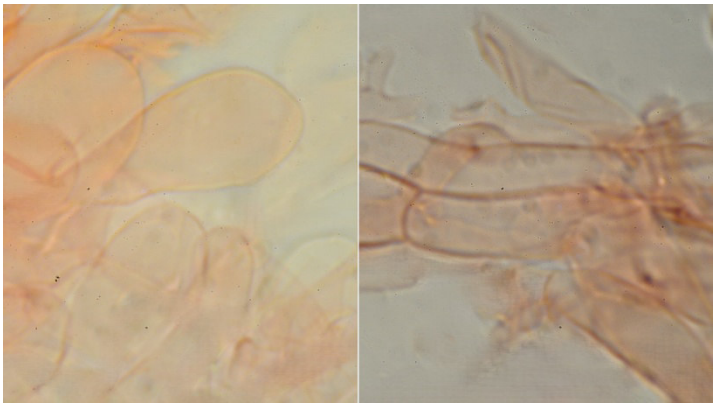

Figura 4. *Chromosera viola*: trama lamellare costituita da ife globose irregolari (a sinistra), a tratti subregolari (a destra) (ingrandimento 1000x, osservazione in rosso congo)

NOTE

Rispetto alla descrizione originale, gli esemplari esaminati presentano dimensioni leggermente maggiori (il cappello supera il centimetro di diametro), mentre gli altri caratteri macroscopici sono pienamente concordi (Arnolds 1985). Sotto il profilo microscopico si segnala una peculiare trama lamellare irregolare, a tratti subregolare, e la stipitipellis del gambo avente qualche ifa emergente ad apice arrotondato. L'habitat di crescita rispecchia perfettamente quanto riportato nella descrizione originale.

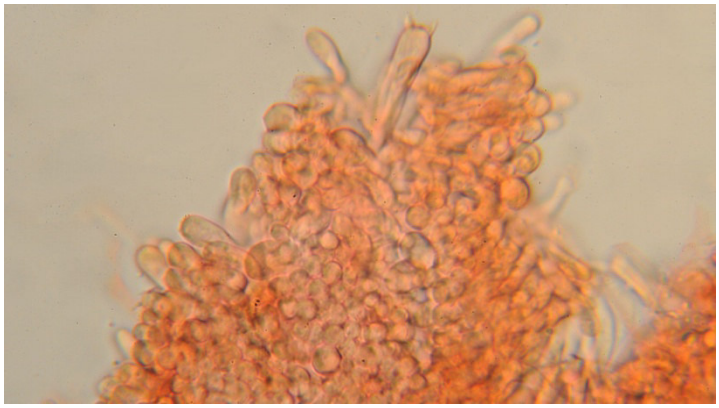

Figura 6. *Chromosera viola*: filo lamellare (ingrandimento 400x, osservazione in rosso congo)

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MycolObs - Mycological Observations Vol. 16: 5-9 2026-08-26

Article received 15 June 2026, accepted 26 July 20265

Gloiocephala cerkezii (*Basidiomycota*, *Physalacriaceae*) in Bosnia and Herzegovina

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Key words:
Agaricales
Marasmiineae

Abstract: The first record of *Gloiocephala cerkezii* from Bosnia is presented, accompanied by a detailed description and imagery of its macro- and microcharacters.

INTRODUCTION

The genus *Gloiocephala* Massee (Massee 1892), which was for a long period included in *Marasmius* Fr. as *Marasmius* subgen. *Gloiocephala* (Massee) Bon and recently re-elevated to generic level following phylogenetic analyses, contains only five species in Europe (Tkalčec & Mešić 2008). One of their most peculiar morphological characteristics among the genera of the suborder *Marasmiineae* is a vein-like hymenium or, when sufficiently developed, extremely few lamellae.

Gloiocephala cerkezii Tkalčec & Mešić was described in 2008 from a series of collections in neighbouring Croatia, around Zagreb (Tkalčec & Mešić 2008). More recently, Tkalčec, Mešić, Ainsworth & Jordal (2019) assessed its rarity, reporting that it was still only known '*from two small localities in Croatia; a small grassland and a disused orchard, the latter of which is now destroyed*'. Consequently, this Bosnian collection expands its range, although only by less than 150 km to the southeast (town of Prijedor, locality Raškovac). The species range beyond the north-western Balkans still remains to be certified.

MATERIALS AND METHODS

Basidiomes were photographed in the field using Canon Ixus 185 and Canon Ixus 285 HS cameras. Microscopy data and images were obtained from fresh material stained with Congo red and observed in water; spores were also observed in Melzer's reagent.

TAXONOMY

Gloiocephala cerkezii Tkalčec & Mešić
Mycologia 100(2): 320 (2008)

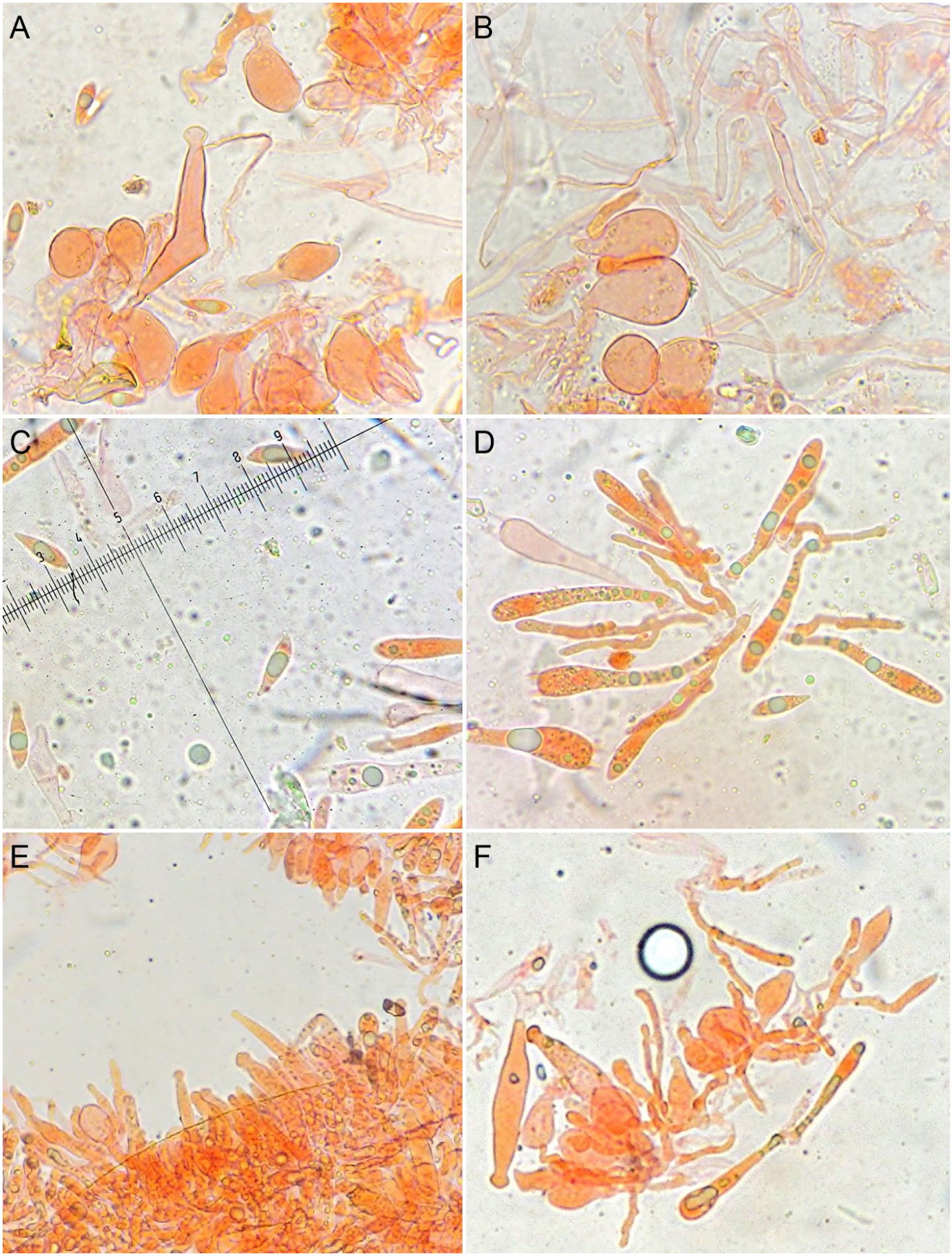
Macroscopic characters

Pileus 3–5 mm broad, convex, sometimes irregular to somewhat lobed; whitish to beige with a concolourous or slightly darker centre, often strewn with brownish dots, sometimes brownish areas irregularly cover portion of pileus; margin thin.

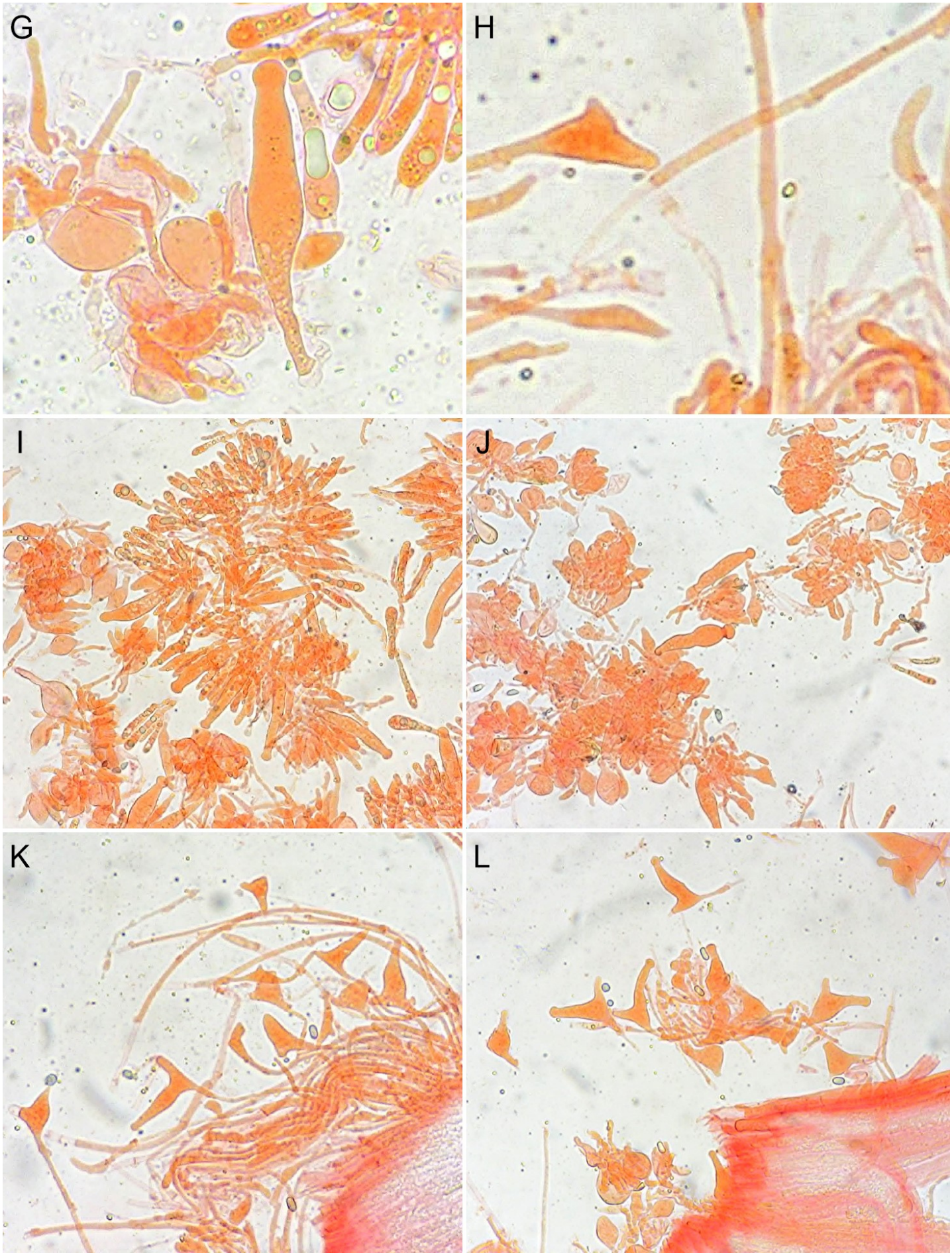
Hymenium composed of very distant wrinkles or scarcely developed and forked to intervenose lamellae, rarely reaching the margin of pileus, whitish.

Stipe 13–18 mm long, up to 1 mm broad, cylindrical, base somewhat bulbillose, central; white above, dark brown to blackish below, with a central zone of progressive passage between the two extreme colour tints, the dark portion more and more expanded upwards while basidiomes age; pubescent; insititious.



A-B: Pileipellis and gelified subpellis; C: Spores; D: Basidia; E-F: Cheilocystidia



G: Pleurocystidium; H: Clamp connections; I-J: Hymenial overview; K-L: Caulocystidia

Microscopic characters

Spores 14.0–17.5 × 3.8–4.2 µm; very narrow, in front view oblong, in side view subamygdaliform, in both views base tapering and apex somewhat prolonged; usually with a large guttula, hyaline; congophilous, inamyloid, indextrinoid; without germ pore.

Basidia 4-spored, narrowly clavate.

Cheilocystidia of three intermixed types: (a) capitate and narrowly fusiform-utriform to narrowly fusiform or cylindraceous, sometimes narrowly lageniform to utriform and non-capitate; wall thin to slightly thickened, numerous; (b) irregularly filiform and sometimes forked, diffuse; (c) claviform and similar to those in the pileipellis, occasional.

Pleurocystidia similar to the type (a) of the cheilocystidia, numerous.

Pileipellis a hymeniderm of clavate to ellipsoid or thickly subutriform elements intermixed with pileocystidia similar to cheilocystidia of the type (a); subpellis a hyphal layer immersed in a strongly gelified matrix.

Caulocystidia mostly similar to the type (a) of the cheilocystidia but often with an added lateral projection.

Clamp connections present everywhere.

Collections examined: Bosnia and Herzegovina, Republika Srpska, Prijedor, Raškovac, in a flooded meadow along the Sana river bank, among *Lolium* sp., 11 and 12 June 2026, legit D. Trivič (pers. herb.).

NOTES

The Bosnian collections differ slightly from the original description as the gill edge seems to occasionally have clavate cystidia which resemble the pileal cells. However, all other characteristics align well.

Gloiocephala cerkezii has a clear set of morphological characteristics which facilitate its identification among the only four other species so far known from Europe.

Its elongate stipe is unique, as the stipe of the other four European species is very short to almost absent [not more than 6(10) mm long].

Gloiocephala menieri (Boud.) Singer also differs by a lateral or strongly eccentric stipe, and wider spores (5.5–7.5 mm); *G. cornelii* (Læssøe & Noordel.) E. Horak has a usually spathuloid pileus; *G. caricis* (P. Karst.) Bas has predominantly 2-spored basidia; and *G. pseudocaricis* (Noordel.) Tkalčec & Mešić has a pinkish-ochraceous pileus and lacks capitate pileocystidia.

Based on this and the Croatian findings *Gloiocephala cerkezii* grows both on herbal culms and tree twigs, in humid to dry habitats.

ACKNOWLEDGMENTS

Warm thanks to Z. Tkalčec and A. Mesic for confirming the correct morphological identification of the Bosnian collections, and the journal editorial staff for arranging the manuscript.

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Article received 8 June 2026, accepted 5 July 2026, additional images received 22 July 2026

Crepidotus alsaticus, a new species of subgenus *Dochmiopus*

(versione italiana a pag. 24)

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Keywords:
Fungi
Basidiomycota
Agaricomycetes
Agaricales
Agaricineae
Crepidotaceae
ITS
phylogenetic analysis

Abstract: During fungal diversity surveys in the Alsace region of France (near Largitzen), a new taxon *Crepidotus alsaticus* was discovered. Through a combination of morphological analysis and molecular phylogenetics, this species is classified within *Crepidotus* subgenus *Dochmiopus*. It exhibits distinct features that separate it from closely related taxa. This paper provides a comprehensive morphological description of *C. alsaticus*, details of its ecology and habitat, and comparisons with morphologically similar species within the same subgenus.

INTRODUCTION

According to recent monographs (Senn-Irlet 1995; Consiglio & Setti 2008; Hausknecht & Krisai-Greilhuber 2010), the genus *Crepidotus* (Fr.) Staude is represented in Europe by approximately 22–27 taxa, including both species and varieties. This genus has a relatively low species count and a predominantly saprophytic ecology. Most species colonise moderately decayed woody substrates—typically twigs, branches, or small logs of deciduous trees and less frequently of conifers—or they grow on plant debris, the bark of living trees, or directly on the soil. A notable exception is the rare mycoparasite *Crepidotus fungiphilus* Hauskn. & Krisai.

Macroscopically, species of the genus *Crepidotus* are characterised by a very distinct morphological structure that is easily identified visually: ranging from almost exclusively shell-shaped or fan-shaped to spatula-shaped. Colours vary from pure white to creamy or ivory white; in some species, they show isabelline-cream or apricot-brown (*Crepidotus ehrendorferi* Hauskn. & Krisai), scarlet red (*C. cinnabarinus* Peck), or beautiful pinkish (*C. roseoornatus* Pöder & Ferrari) hues. The cap surface—generally lacking a veil—has a felt-like or velvety texture; it may be finely pubescent or covered in a dense, uniform fleece (sometimes barely perceptible), though it rarely appears smooth or coated with a thin gelatinous layer. Some species feature surface ornamentation in the form of appressed, interwoven fibers or a distinctly squamulose (scaly) structure. The gills range from moderately spaced to crowded; they are usually whitish, later turning ochre-brown, or they match the colour of the cap—for instance, yellowish in *Crepidotus carpaticus* Pilát, yellow-orange in *C. crocophyllus* (Berk.) Sacc., reddish-brown in *C. cinnabarinus*, and pinkish in *C. roseoornatus*. The stipe is either barely present or entirely absent.

Microscopic characteristics can vary significantly among species. From a diagnostic standpoint, they facilitate the identification of the vast majority of species with relative ease. European species exhibit a hymenophoral trama ranging from regular to sub-regular, and clamp connections may be present or absent. Spore shape ranges from elliptical to broadly elliptical, suboval, subcylindrical to subfusiform, subglobose, or spherical. Under a light microscope, they may appear smooth, punctate, rugulose, verrucose, or spinose-echinulate (with the latter type being truncate at the apex). Electron microscopy reveals that they generally lack a germ pore, or—in very rare, isolated cases—show only a very faint hint of one; chemically, they are inamyloid and indextrinoid. Hymenial cystidia are found only on the gill edge (cheilocystidia) and are extremely variable in shape, with at least 30–40 different structural types observed.

The genus *Crepidotus* has been extensively documented in Europe. Authors who have contributed significantly to the understanding of the genus include Favre (1935); Pilát (1948, 1949); Orton (1960); Clémençon (1977); Watling & Gregory (1989); Norstein (1990); Stangl, Kriegelsteiner & Enderle (1991); Senn-Irlet (1991, 1992, 1995); Ludwig (2001); Consiglio & Setti (2008); and Hausknecht & Krisai-Greilhuber (2010).

In the annals of mycological history, the name *Crepidotus* was originally introduced by Fries (1821) as a subgenus in his comprehensive *Systema Mycologicum*. It was subsequently elevated to the rank of genus by Staude (1857) and placed within the family *Crepidotaceae* (S. Imai) Singer by Singer (1951, 1986). Over the years, Singer included up to nine different genera in this family, comprising both agaricoid forms [*Crepidotus*, *Melanomphalia* M.P. Christ., *Pleurotellus* Fayod, *Simocybe* P. Karst., *Tubaria* (W.G. Sm.) Gillet] and cyphelloid forms (*Chromocyphella* De Toni & Levi, *Episphaeria* Donk, *Pellidiscus* Donk, *Phaeosolenia* Speg.). However, that earlier classification was based strictly on morphology, whereas recent molecular studies have significantly altered the taxonomic framework. Analyses of various diagnostic biomarkers have revealed striking molecular inconsistencies; consequently, species or genera previously assigned by classical mycology to a specific or exclusive group have—following current phylogenetic assessments—been transferred to other genera or even new families. Thus, the taxonomic placement of the genera Singer originally assigned to the *Crepidotaceae* has also been revised.

Vizzini (in Consiglio & Setti 2008) holds that only three genera should currently be assigned to the family *Crepidotaceae*: *Crepidotus*, *Simocybe*, and the cyphelloid genus *Pellidiscus*. Furthermore, Vizzini (2008) transferred the genus *Tubaria* to the new family *Tubariaceae* Vizzini, while regarding the remaining genera as occupying an uncertain taxonomic position that requires further phylogenetic study.

Upon initial visual inspection in the field, our collections showed no distinctive features and were misidentified as the very common *Crepidotus cesatii* (Rabenh.) Sacc. or *C. variabilis* (Pers.) P. Kumm. However, upon detailed examination of the collection, we were surprised by its mealy odour and consequently directed our investigation toward the genus *Clitopilus*—specifically *C. hobsoni* (Berk.) P.D. Orton, which shares similar macromorphological and organoleptic characteristics. Yet, microscopic analysis revealed features that were unusual for the genus *Clitopilus*: the spores were broadly elliptical to subamygdaliform and appeared smooth (lacking ornamentation) under light microscopy and the cheilocystidia were extremely variable, comprising over 20 types, with some exceeding 100 µm in length. Most notably, the non-gelatinised pileipellis contained striking swollen-vesiculose hyphae—ranging from fusiform and ampulliform to even spherical—that strongly resembled those of *Singerocybe phaeophthalma* (Pers.) Harmaja. Ultimately, phylogenetic analysis confirmed our initial assessment regarding the genus *Crepidotus*.

MATERIALS AND METHODS

Morphology

The basidiomes were photographed in their natural habitat using a Samsung Galaxy Note 10 Plus digital camera, while a Nikon Coolpix 4100 was used for microscopy images. Microscopic analysis was performed using a Leica DME binocular microscope equipped with achromatic objectives. Fresh material was examined in aqueous solution or 3% KOH, or stained with Congo Red. Melzer's reagent was used to test for amyloid or dextrinoid reactions; sulfobenzaldehyde solutions were used to detect laticiferous hyphae; Cresyl Blue was used to check for metachromatic reactions in spores and hymenial tissues; and Toluidine Blue was used to highlight the presence of gelatinised layers in the pileipellis (Clemençon 1977a, b).

The code LUG refers to the Fungarium of the Cantonal Natural History Museum of Lugano (Switzerland). All images and tables are by E. Musumeci.

Phylogeny

DNA extraction, amplification and sequencing

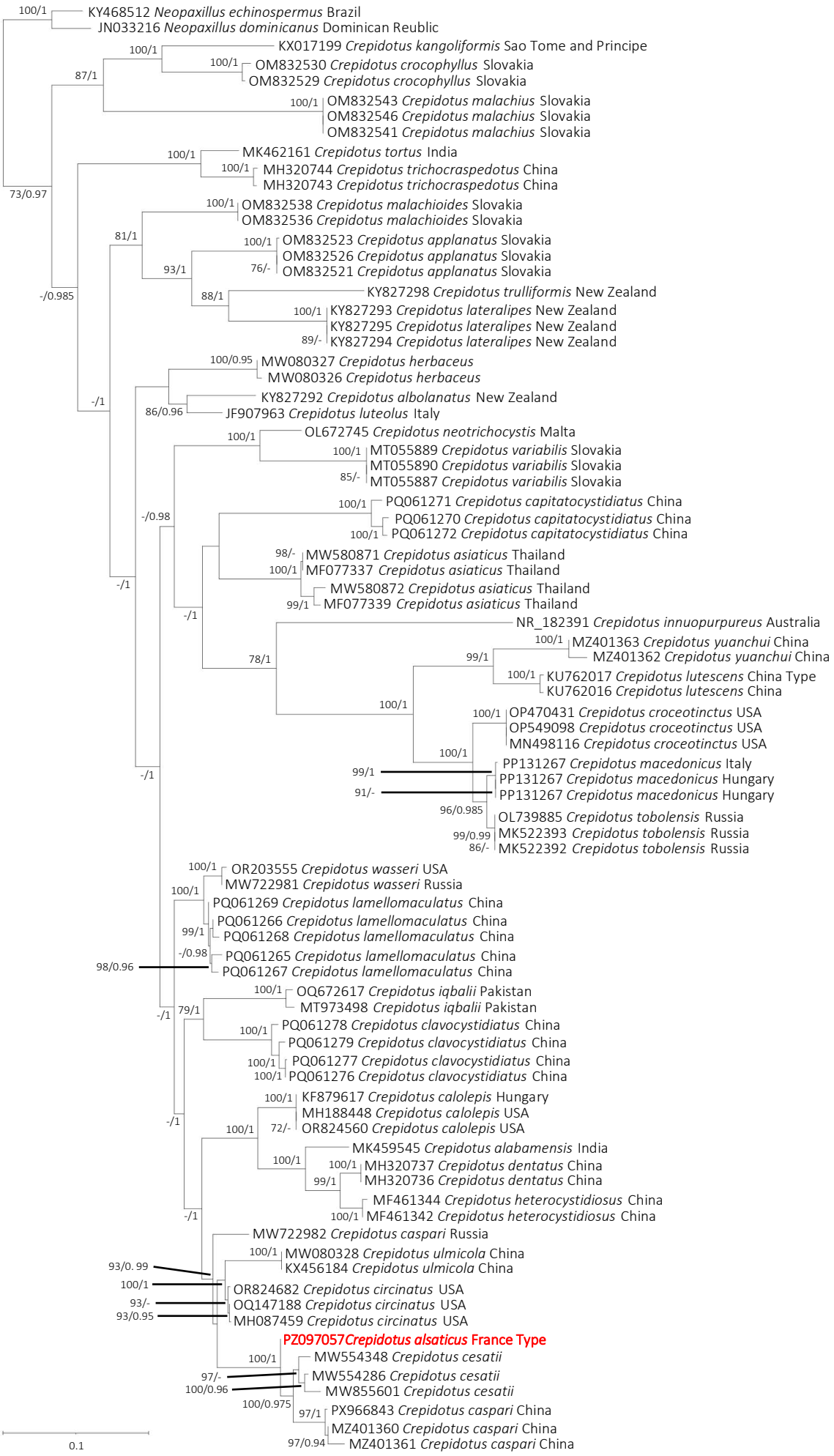
Genomic DNA was extracted from dried material using a standard CTAB protocol modified for fungal samples (Doyle & Doyle 1987). The ITS region of the nuclear ribosomal DNA was amplified via PCR using the primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). Amplification reactions were performed in a standard final volume containing reaction buffer, $MgCl_2$, dNTPs, primers, and Taq DNA polymerase. The resulting PCR products were verified by agarose gel electrophoresis, purified, and subsequently sequenced with the Sanger method in both directions by a commercial sequencing service. The obtained sequences were assembled and manually corrected by comparing forward and reverse chromatograms, then aligned with reference sequences retrieved from GenBank using MAFFT v7 (Katoh & Standley 2013).

Species / Specie	Collection ID / ID raccolta	Origin / Origine	ITS
Crepidotus alabamensis	TBGT15610	India	MK459545
Crepidotus albolanatus	PDD:72865	New Zealand	KY827292
Crepidotus alsaticus	LUG 20999	France	PZ097057
Crepidotus applanatus	SLO 2534	Slovakia	OM832521
Crepidotus applanatus	SLO 2539	Slovakia	OM832523
Crepidotus applanatus	SLO 2551	Slovakia	OM832526
Crepidotus asiaticus	TJB9995ITS	Thailand	MF077337
Crepidotus asiaticus	TJB10018ITS	Thailand	MF077339
Crepidotus asiaticus	FFAAS0336		MW580871
Crepidotus asiaticus	FFAAS0338		MW580872
Crepidotus calolepis	WU 28902	Hungary	KF879617
Crepidotus calolepis	314298	USA	MH188448
Crepidotus calolepis	OMDL iNat # 142786935	USA	OR824560
Crepidotus capitatocystidiatus	FFAAS1310	China	PQ061270
Crepidotus capitatocystidiatus	FFAAS1311	China	PQ061271
Crepidotus capitatocystidiatus	FFAAS1312	China	PQ061272
Crepidotus caspari	LE 227999	Russia	MW722982
Crepidotus caspari	FFAAS0343	China	MZ401360
Crepidotus caspari	FFAAS0342	China	MZ401361
Crepidotus caspari	S8	China	PX966843
Crepidotus cesatii	110116MFBPL0352		MW554286
Crepidotus cesatii	110116MFBPL0379		MW554348
Crepidotus cesatii	MK966515		MW855601
Crepidotus circinatus	MushroomObserver.org/307011	USA	MH087459
Crepidotus circinatus	iNAT:66988897	USA	OQ147188
Crepidotus circinatus	OMDL iNat # 141511315	USA	OR824682
Crepidotus clavocystidiatus	FFAAS1316	China	PQ061276
Crepidotus clavocystidiatus	FFAAS1317	China	PQ061277
Crepidotus clavocystidiatus	FFAAS1318	China	PQ061278
Crepidotus clavocystidiatus	FFAAS1319	China	PQ061279
Crepidotus croceotinctus	iNat31834012	USA	MN498116
Crepidotus croceotinctus	iNaturalist # 126570732	USA	OP470431
Crepidotus croceotinctus	iNaturalist 131145003	USA	OP549098
Crepidotus crocophyllus	SLO 2433	Slovakia	OM832529
Crepidotus crocophyllus	SLO 2588	Slovakia	OM832530
Crepidotus dentatus	HMJAU37097	China	MH320736
Crepidotus dentatus	HMJAU37161	China	MH320737
Crepidotus herbaceus	HMJAU37025		MW080326
Crepidotus herbaceus	HMJAU37009		MW080327
Crepidotus heterocystidiosus	HMJAU37054	China	MF461342
Crepidotus heterocystidiosus	HMJAU37034	China	MF461344
Crepidotus innuopurpureus	MEL:2503290	Australia	NR_182391
Crepidotus iqbalii	LAH36654	Pakistan	MT973498
Crepidotus iqbalii	MU248	Pakistan	OQ672617
Crepidotus kangoliformis	BAP 664 (Holotype, SFSU)	São Tome and Principe	KX017199

Crepidotus lamellomaculatus	FFAAS1305	China	PQ061265
Crepidotus lamellomaculatus	FFAAS1306	China	PQ061266
Crepidotus lamellomaculatus	FFAAS1307	China	PQ061267
Crepidotus lamellomaculatus	FFAAS1308	China	PQ061268
Crepidotus lamellomaculatus	FFAAS1309	China	PQ061269
Crepidotus lateralipes	PDD:72508	New Zealand	KY827293
Crepidotus lateralipes	PDD:72571	New Zealand	KY827294
Crepidotus lateralipes	PDD:98270	New Zealand	KY827295
Crepidotus luteolus	16834	Italy	JF907963
Crepidotus lutescens	HMJAU 21976	China	KU762016
Crepidotus lutescens	HMJAU 37002	China	KU762017
Crepidotus macedonicus	PV773	Hungary	MH780921
Crepidotus macedonicus	DB3859	Hungary	MH780922
Crepidotus macedonicus	MB19102501	Italy	PP131267
Crepidotus malachioides	SLO 2391	Slovakia	OM832536
Crepidotus malachioides	SLO 2578	Slovakia	OM832538
Crepidotus malachius	SLO 2091	Slovakia	OM832541
Crepidotus malachius	SLO 2530	Slovakia	OM832543
Crepidotus malachius	SLO 2541	Slovakia	OM832546
Crepidotus neotrichocystis	CS1150	Malta	OL672745
Crepidotus tobolensis	TCSS UB RAS9477	Russia	MK522392
Crepidotus tobolensis	LE 287655	Russia	MK522393
Crepidotus tobolensis	LE313671	Russia	OL739885
Crepidotus tortus	TBGT17194	India	MK462161
Crepidotus trichocraspedotus	HMJAU37138	China	MH320743
Crepidotus trichocraspedotus	HMJAU37250	China	MH320744
Crepidotus trulliformis	PDD:98274	New Zealand	KY827298
Crepidotus ulmicola	-	China	KX456184
Crepidotus ulmicola	HMJAU37027	China	MW080328
Crepidotus variabilis	SLO 2423	Slovakia	MT055887
Crepidotus variabilis	SLO 2021	Slovakia	MT055889
Crepidotus variabilis	SLO 2018	Slovakia	MT055890
Crepidotus wasseri	LE 287679	Russia	MW722981
Crepidotus wasseri	MO500187	USA	OR203555
Crepidotus yuanchui	FFAAS0340	China	MZ401362
Crepidotus yuanchui	FFAAS0341	China	MZ401363
Neopaxillus dominicanus	MCVE 26928	Dominican Republic	JN033216
Neopaxillus echinospermus	AH45884	Brazil	KY468512

Table 1. All samples analysed in this study are listed in alphabetic order. The new species is written in red and bold / Tabella 1. Tutti i campioni analizzati in questo studio sono elencati in ordine alfabetico. La nuova specie è indicata in rosso e in grassetto.

Fig. 1. Phylogenetic tree inferred from ITS sequence data using Maximum Likelihood (ML) and Bayesian Inference (BI). Node support values are shown as ML bootstrap support (BS)/Bayesian posterior probability (PP). For clarity, only strongly supported nodes (BS ≥ 70% and PP ≥ 0.90) are labelled / Albero filogenetico ottenuto dall'analisi delle sequenze della regione ITS mediante Massima Verosimiglianza (ML) e Inferenza Bayesiana (BI). I valori di supporto riportati ai nodi sono espressi nel formato supporto bootstrap (BS)/probabilità posteriori bayesiane (PP). Per una migliore leggibilità sono mostrati esclusivamente i valori di supporto elevati (BS ≥ 70% e PP ≥ 0,90).


100/1 KY468512 Neopaxillus echinospermus Brazil
JN033216 Neopaxillus dominicanus Dominican Reublic
100/1 KX017199 Crepidotus kangoliformis Sao Tome and Principe
100/1 OM832530 Crepidotus crocophyllus Slovakia
87/1 OM832529 Crepidotus crocophyllus Slovakia
100/1 OM832543 Crepidotus malachius Slovakia
OM832546 Crepidotus malachius Slovakia
OM832541 Crepidotus malachius Slovakia
100/1 MK462161 Crepidotus tortus India
100/1 MH320744 Crepidotus trichocraspedotus China
73/0.97 MH320743 Crepidotus trichocraspedotus China
100/1 OM832538 Crepidotus malachioides Slovakia
OM832536 Crepidotus malachioides Slovakia
81/1 100/1 OM832523 Crepidotus applanatus Slovakia
OM832526 Crepidotus applanatus Slovakia
93/1 76/- OM832521 Crepidotus applanatus Slovakia
-/0.985 88/1 KY827298 Crepidotus trulliformis New Zealand
100/1 KY827293 Crepidotus lateralipes New Zealand
KY827295 Crepidotus lateralipes New Zealand
89/- KY827294 Crepidotus lateralipes New Zealand
100/0.95 MW080327 Crepidotus herbaceus
MW080326 Crepidotus herbaceus
-/1 KY827292 Crepidotus albolanatus New Zealand
86/0.96 JF907963 Crepidotus luteolus Italy
100/1 OL672745 Crepidotus neotrichocystis Malta
100/1 MT055889 Crepidotus variabilis Slovakia
MT055890 Crepidotus variabilis Slovakia
85/- MT055887 Crepidotus variabilis Slovakia
100/1 PQ061271 Crepidotus capitatocystidiatus China
-/0.98 PQ061270 Crepidotus capitatocystidiatus China
100/1 PQ061272 Crepidotus capitatocystidiatus China
98/- MW580871 Crepidotus asiaticus Thailand
-/1 100/1 MF077337 Crepidotus asiaticus Thailand
MW580872 Crepidotus asiaticus Thailand
99/1 MF077339 Crepidotus asiaticus Thailand
-/1 NR_182391 Crepidotus innuopurpureus Australia
100/1 MZ401363 Crepidotus yuanchui China
99/1 MZ401362 Crepidotus yuanchui China
78/1 100/1 KU762017 Crepidotus lutescens China Type
KU762016 Crepidotus lutescens China
100/1 100/1 OP470431 Crepidotus croceotinctus USA
OP549098 Crepidotus croceotinctus USA
MN498116 Crepidotus croceotinctus USA
100/1 PP131267 Crepidotus macedonicus Italy
-/1 99/1 PP131267 Crepidotus macedonicus Hungary
91/- PP131267 Crepidotus macedonicus Hungary
96/0.985 OL739885 Crepidotus tobolensis Russia
99/0.99 MK522393 Crepidotus tobolensis Russia
86/- MK522392 Crepidotus tobolensis Russia
100/1 OR203555 Crepidotus wasseri USA
100/1 MW722981 Crepidotus wasseri Russia
PQ061269 Crepidotus lamellomaculatus China
99/1 PQ061266 Crepidotus lamellomaculatus China
PQ061268 Crepidotus lamellomaculatus China
-/0.98 PQ061265 Crepidotus lamellomaculatus China
98/0.96 PQ061267 Crepidotus lamellomaculatus China
100/1 OQ672617 Crepidotus iqbalii Pakistan
-/1 79/1 MT973498 Crepidotus iqbalii Pakistan
100/1 PQ061278 Crepidotus clavocystidiatus China
PQ061279 Crepidotus clavocystidiatus China
100/1 PQ061277 Crepidotus clavocystidiatus China
100/1 PQ061276 Crepidotus clavocystidiatus China
100/1 KF879617 Crepidotus calolepis Hungary
MH188448 Crepidotus calolepis USA
-/1 100/1 72/- OR824560 Crepidotus calolepis USA
MK459545 Crepidotus alabamensis India
100/1 100/1 MH320737 Crepidotus dentatus China
99/1 MH320736 Crepidotus dentatus China
MF461344 Crepidotus heterocystidiosus China
-/1 100/1 MF461342 Crepidotus heterocystidiosus China
MW722982 Crepidotus caspari Russia
100/1 MW080328 Crepidotus ulmicola China
93/0. 99 KX456184 Crepidotus ulmicola China
100/1 OR824682 Crepidotus circinatus USA
93/- OQ147188 Crepidotus circinatus USA
93/0.95 MH087459 Crepidotus circinatus USA
PZ097057Crepidotus alsaticus France Type
100/1 MW554348 Crepidotus cesatii
97/- MW554286 Crepidotus cesatii
100/0.96 MW855601 Crepidotus cesatii
100/0.975 97/1 PX966843 Crepidotus caspari China
MZ401360 Crepidotus caspari China
97/0.94 MZ401361 Crepidotus caspari China
0.1

Phylogenetic analysis

Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. The ML analysis was performed using 1,000 bootstrap replicates to assess nodal support via IQ-TREE (Nguyen *et al*. 2015). The Bayesian analysis was carried out using MrBayes v.3.2 (Ronquist *et al*. 2012) with Markov chain Monte Carlo (MCMC) runs for a total of 1,000,000 generations, sampling trees at regular intervals and discarding the initial fraction as burn-in. The resulting trees were visualised and edited using FigTree v1.4.4 and iTOL (Letunic & Bork, 2021).

RESULTS

Phylogenetic analyses

Phylogenetic analysis conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches revealed broad topological congruence between the two methods. In both reconstructions, the taxon identified as *Crepidotus alsaticus* was recovered as sister to a clade comprising *Crepidotus caspari* and *Crepidotus cesatii*, exhibiting high support values for both ML bootstrap support (up to 100) and Bayesian posterior probability (up to 1.00).

Despite close phylogenetic proximity, *C. alsaticus* forms a distinct, stable, and autonomous lineage, exhibiting no topological intermixing with related taxa. This evolutionary independence is robust, even when based solely on the ITS marker. While the genetic differences are modest, they are highly structured and consistent, supporting the interpretation of *C. alsaticus* as a distinct species rather than an intraspecific variant.

However, the limited variability of the ITS region cannot fully resolve relationships among closely related taxa, suggesting a recent divergence. Therefore, establishing *C. alsaticus* as a new species relies on distinctive morphological traits alongside ITS molecular evidence. Future multilocus analyses (e.g., LSU, RPB2, TEF1) will be necessary to provide finer phylogenetic resolution and definitively confirm its distinct systematic status.

Taxonomy

Crepidotus alsaticus Musumeci & Maraia ***sp.nov***. [MB 863569]

Fig. 1. *Crepidotus alsaticus* LUG 20999 - Typus

Fig. 2. *Crepidotus alsaticus* LUG 20999 - Typus

Fig. 3. *Crepidotus alsaticus* LUG 20999 - Typus

Original Diagnosis

Pileus 5–15 mm diam., from the earliest stage plane to slightly expanded, later semicircular and finally distinctly conchiform to expanded; margin involute when young, becoming sinuous-lobate at maturity. Surface densely pubescent, tomentose to finely villose (visible under a hand lens). Colour initially pure white, later becoming grey-cream. Lamellae moderately distant to spaced, with lamellulae; lamellar edge slightly eroded; initially whitish, later grey-cream and finally with brownish tones. Stipe usually absent or only rudimentary. Context thin, inconspicuous; odour faintly farinaceous. Basidiospores 6.5–8.5 × 5–6 µm, smooth, no ornamentation visible under the optical microscope, pluriguttulate inside, yellowish in 3% KOH; ellipsoid to broadly ellipsoid-subovoid in frontal view, ellipsoid to slightly amygdaliform in lateral view; wall slightly thickened; apiculus short; germ pore absent. Basidia 25–33 × 7–9 µm, subclavate, 4-spored. Hymenophoral trama regular; hyphae 3–25 µm wide, long cylindrical-filamentous; in the inner trama variably shaped, subphysaloid to suballantoid, broadly ellipsoid to subglobose; hyaline, non-pigmented, non-encrusted. Cheilocystidia 25–102 × 7–30 µm, very abundant on the lamellar edge, extremely polymorphic, at least 20 morphotypes observed; shapes including sublageniform, cylindrical, moniliform, fusiform, subclavate, irregularly ovoid, often with lateral protuberances, nodulose or coralloid; apex sometimes sinuous, forked, subcapitate or spatulate. Pleurocystidia absent. Pileipellis a non-gelatinised epicutis, composed of irregularly interwoven hyphae 3–30 µm wide, hyaline to slightly pigmented, some strongly yellow-olive pigmented; hyphae cylindrical filamentous intermixed with larger elements broadly ellipsoid-subphysaloid, fusiform, ampulliform to sphaeriform, reminiscent of vesiculose hyphae in Singerocybe phaeophthalma; surface often covered by a fine, uniformly distributed encrustation. Clamp connections present.

Habitat: growing on decaying twigs of deciduous trees (probably Alnus) in very humid marshy habitats.

Type: France, Alsace, Haut Rhin, Largitzen, in a flat hilly area, 420 m asl, temperature 9°-12°, humidity 100-87%, 06.10.2025, E. Musumeci 095827-25,Holotype deposited in the Fungarium of the Cantonal Natural History Museum of Lugano (Switzerland), LUG 20999.

Etymology: the epithet alsaticus refers to the Alsace region (France), where the type specimen was collected.

Macroscopic characters

Pileus 5 – 15 mm, from the earliest stage applanate-expanded, becoming semicircular, and finally distinctly conchiform to expanded; margin involute, becoming sinuous-lobate at maturity; surface covered with dense fine hairs (visible under a hand lens), tomentose or finely villose; colour uniformly white, later grey-cream.

Lamellae moderately distant to spaced, with lamellulae; edge slightly eroded; initially whitish, then grey-cream, finally brownish.

Stipe usually absent or very short.

Context inconspicuous; odour farinaceous when rubbed.

Microscopic characters

Basidiospores 6.5–8.5 × 5–6 µm, yellowish in 3% KOH, smooth (no ornamentation visible under light microscopy), containing multiple oil droplets, elliptical to broadly elliptical-suboval in face view, elliptical to slightly amygdaliform in side view; thin-walled, apiculus faintly visible, germ pore absent.

Basidia 25–33 × 7–9 µm, subclavate, 4-spored.

Hymenophoral trama regular; hyphae 3–25 µm wide, long cylindrical-filamentous; in the inner trama variably shaped, subphysaloid-suballantoid to broadly ellipsoid or subglobose; hyaline, non-pigmented, non-encrusted.

Pleurocystidia not observed. *Cheilocystidia* 25–102 × 7–30 µm, extremely polymorphic with at least 20 morphotypes observed: sublageniform, subcylindrical, submoniliform, fusiform, subclavate, irregularly ovoid, and other shapes; often with lateral protuberances to nodulose or coralloid; apex sometimes sinuous, forked, subcapitate or spatulate; numerous.

Pileipellis a non-gelatinised epicutis, composed of irregularly interwoven hyphae: a) 3–30 µm wide, irregularly intertwined, hyaline to slightly pigmented or sometimes strongly yellow-olive pigmented, subcylindrical-filamentous, b) larger and more voluminous hyphae broadly ellipsoid-subphysaloid, subfusiform, ampulliform to sphaeriform, reminiscent of the vesiculose hyphae in *Singerocybe phaeophthalma*; wall surface often covered by a fine, uniformly distributed encrustation.

Clamp connections present.

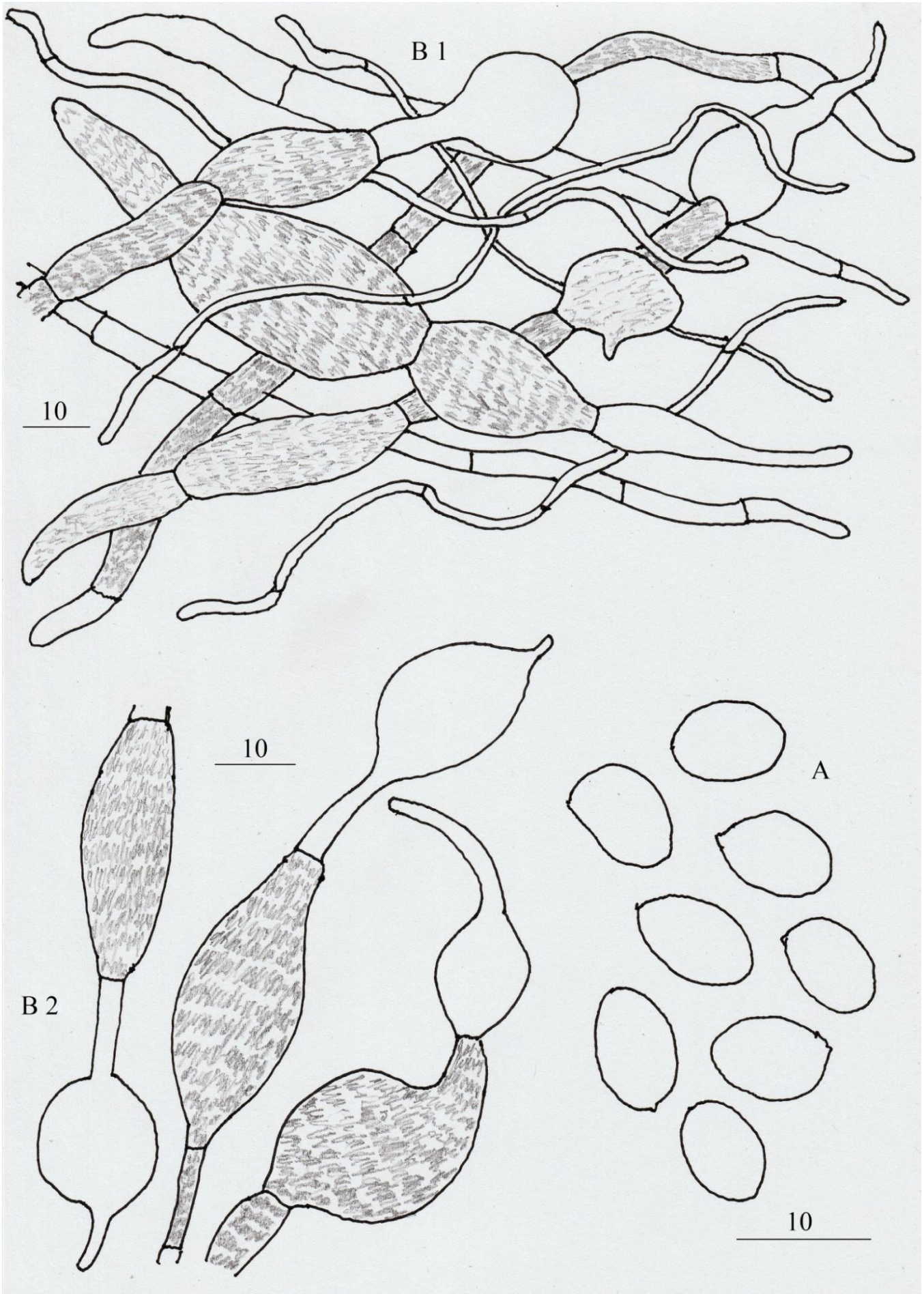


Table 2. A: spores; B1: pileipellis; B2: details of the fusiform-ampullaceous pileipellis hyphae / Tavola 2. A: spore; B1: rivestimento pileico; B2: particolare delle ife fusiformi-ampollacee del rivestimento pileico

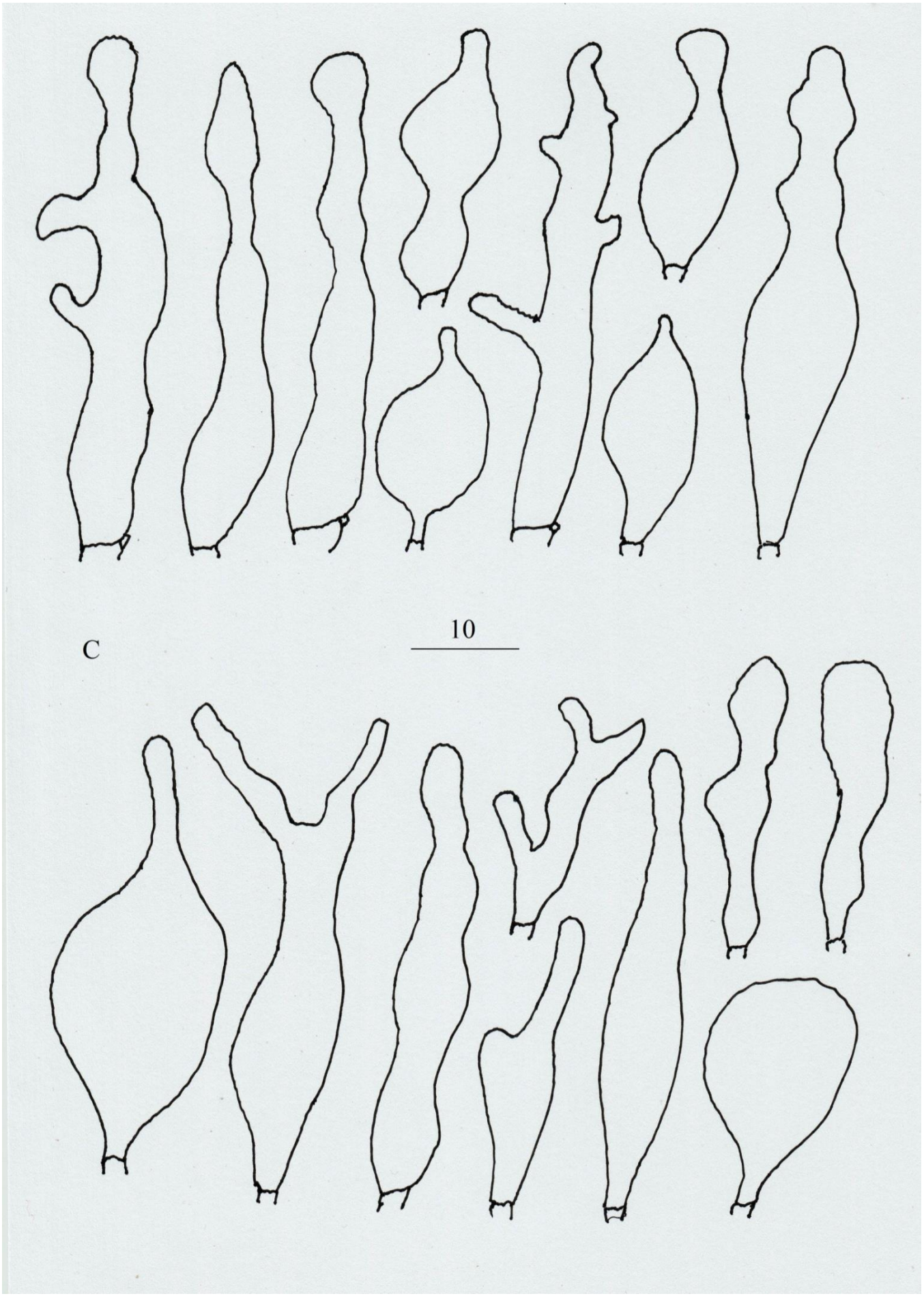


Table 3. C: Cheilocystidia / Tavola 3. C: Cheilocistidi

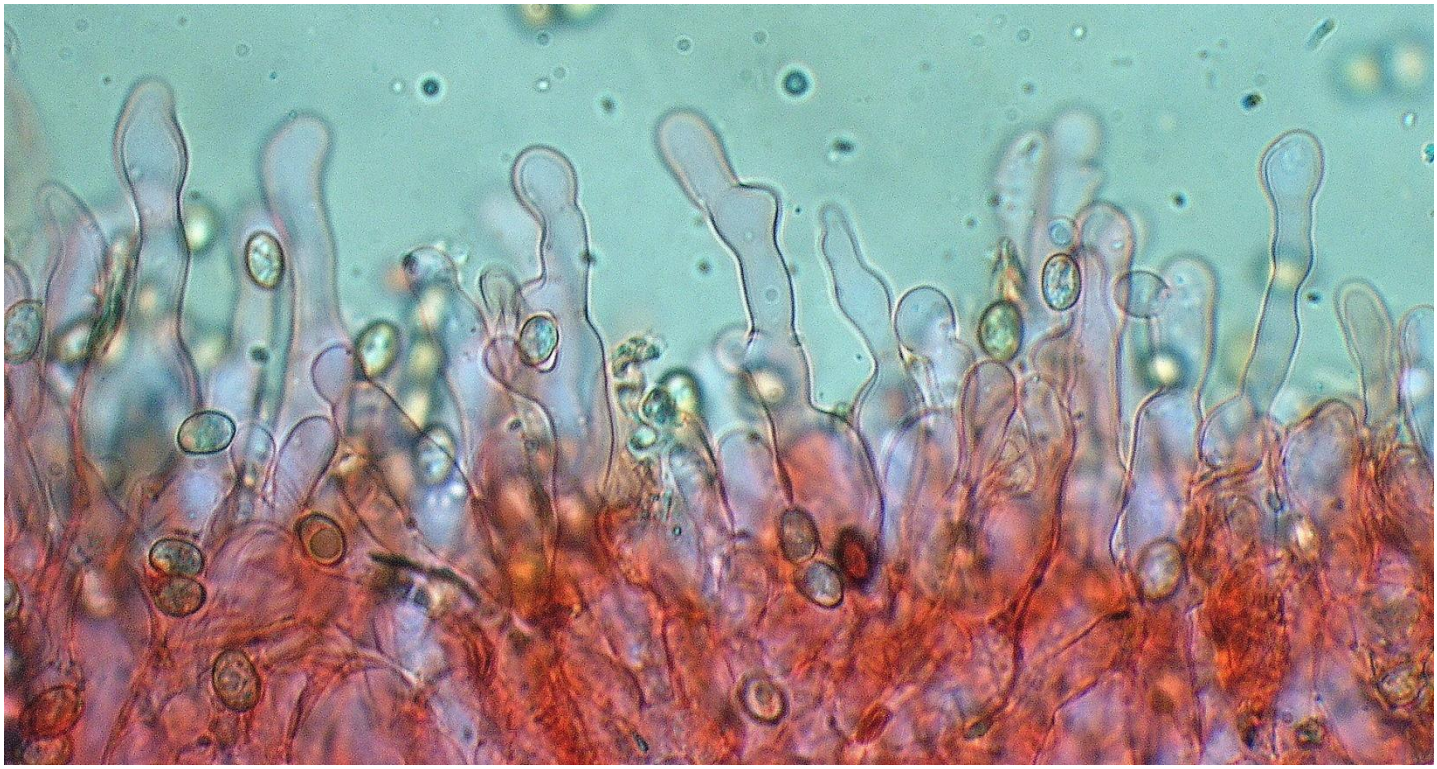


Fig. 4. *Crepidotus alsaticus*. Typus. Cheilocystidia / cheilocistidi

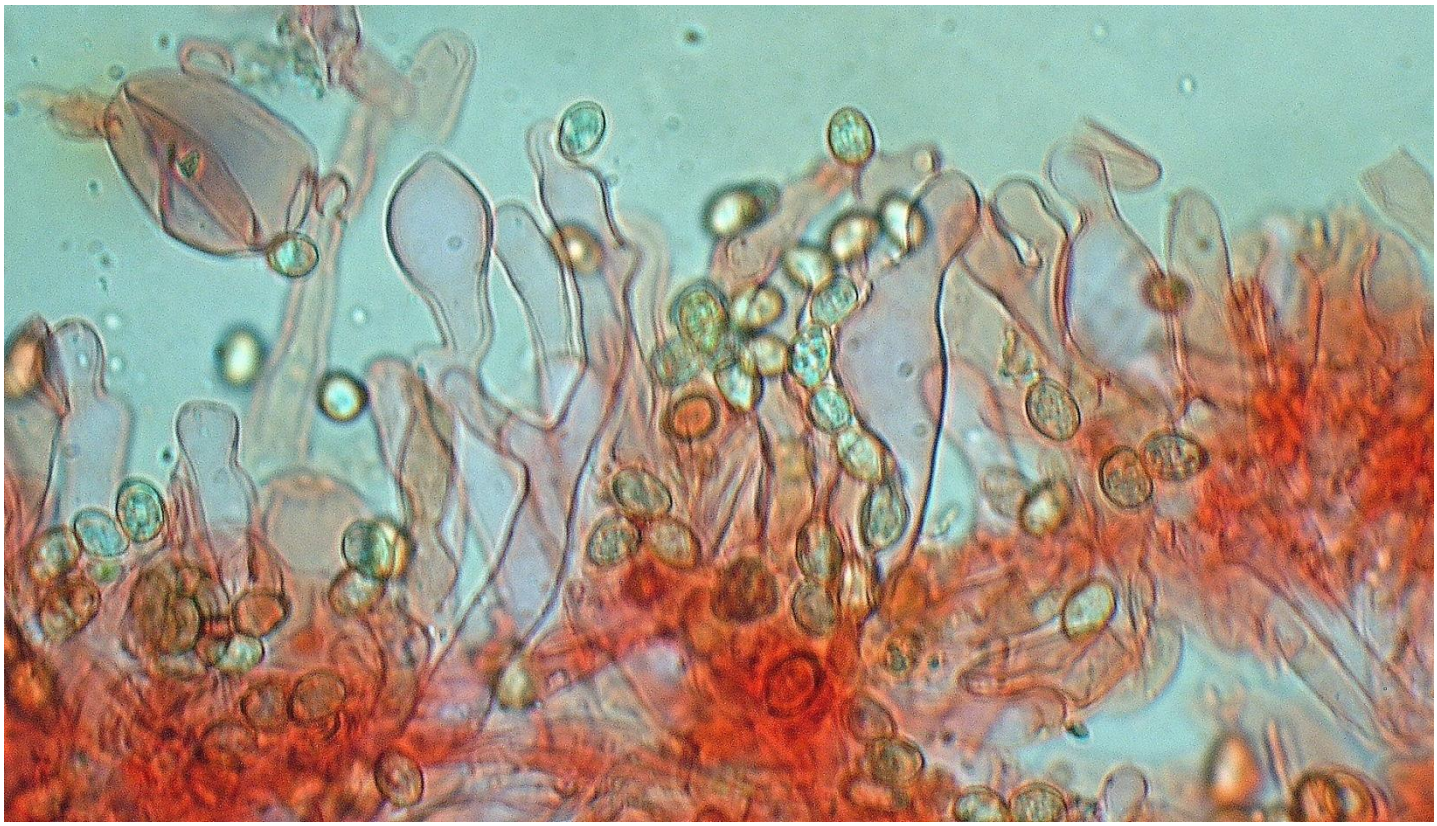


Fig. 5. *Crepidotus alsaticus*. Typus. Cheilocystidia / cheilocistidi

Habitat: basidiomes gregarious on decaying twigs of *Alnus glutinosa* (?) in a wooded area—specifically a well-lit area near a pond belonging to the sport fishing club (SFV Dreiländereck)—where *Alnus glutinosa*, *Quercus rubra*, *Carpinus betulus*, *Fagus sylvatica*, and various wetland plants are present; on very damp and marshy, calcareous soil with a clay or carbonate-rich clayey substrate. Other species found nearby: *Cystolepiota moelleri* Knudsen, *Cystolepiota sistrata* (Fr.) Singer ex Bon & Bellù, *Galerina clavata* (Velen.) Kühner, *Alnicola* sp.

Other collections examined: same site as the type, 12.10.2025, *E. Musumeci 009106-25*.

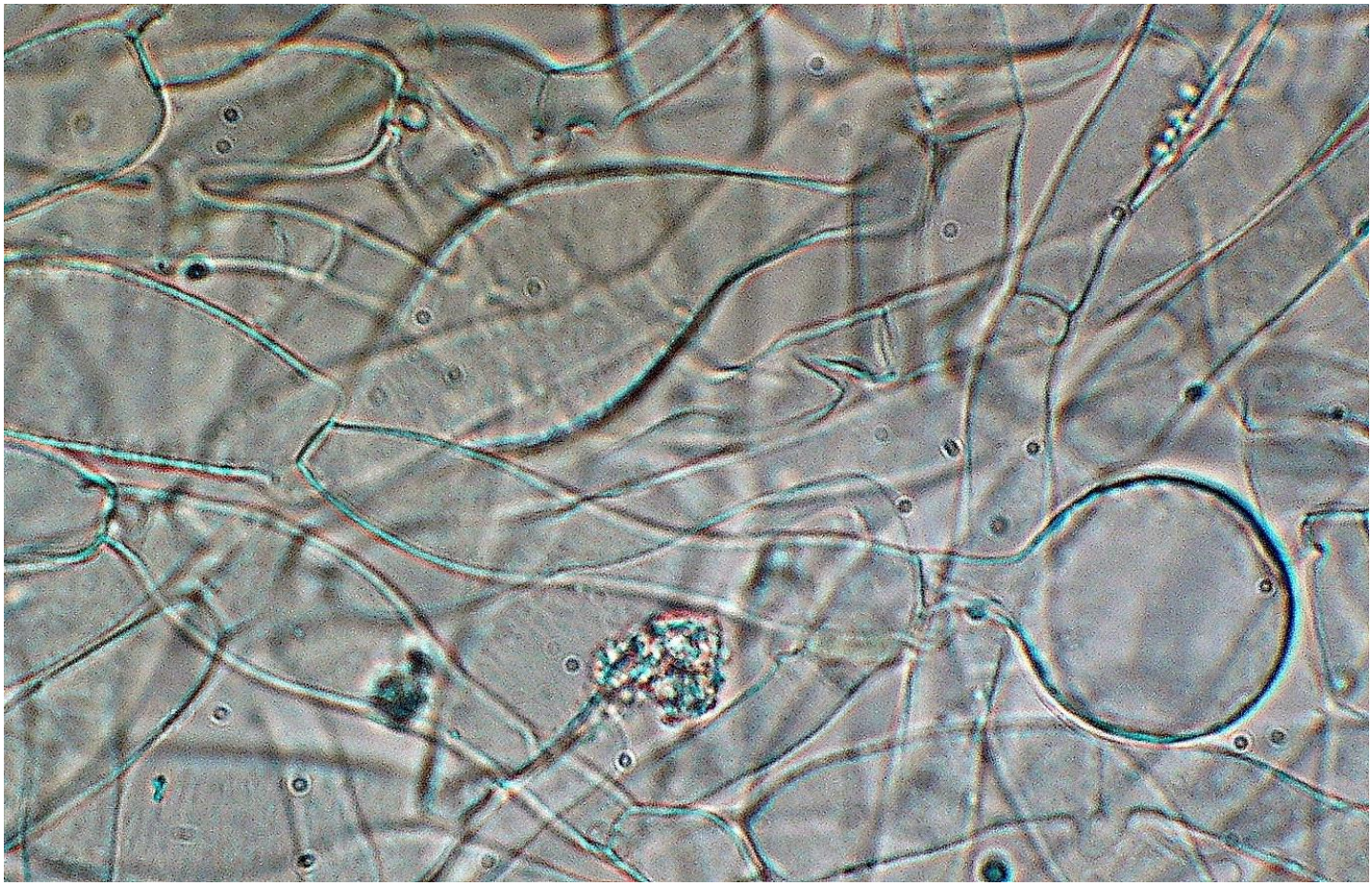


Fig. 6. *Crepidotus alsaticus*. Typus. Pileipellis

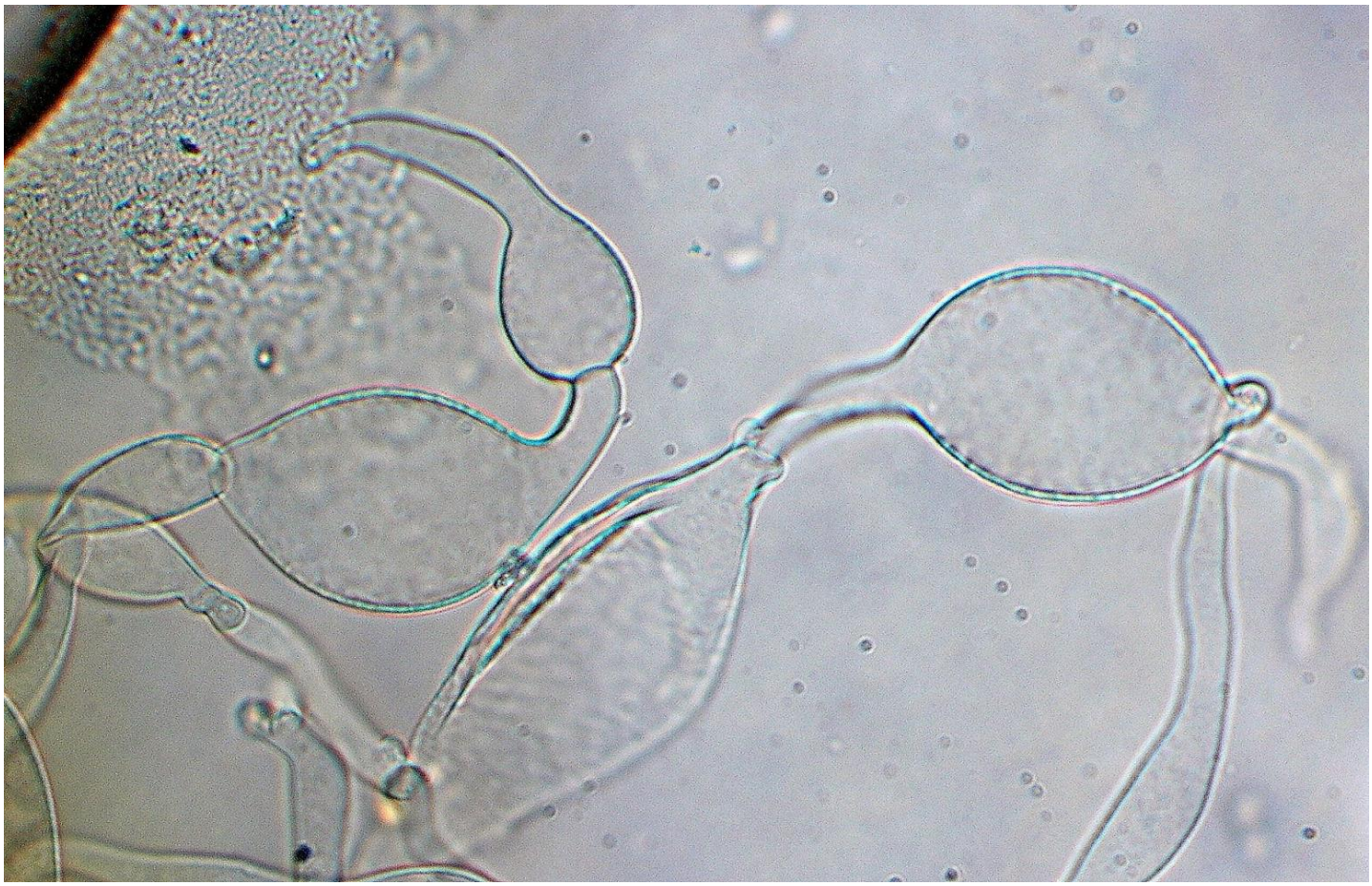


Fig. 7. *Crepidotus alsaticus*. Typus. Pileipellis

NOTES

Based on the presence of clamped hyphae and a pileipellis lacking a gelatinous layer, *Crepidotus alsaticus* is classified within *Crepidotus* subgenus *Dochmiopus* (Pat.) Pilát, in accordance with the classifications of Senn-Irlet (1995) and Consiglio & Setti (2008). Because its spores appear smooth under light microscopy, it is placed in *Crepidotus* subgen. *Dochmiopus* sect. *Autochtoni* (Senn-Irlet) Consiglio & Setti (Consiglio & Setti 2008). However, its unusual swollen, ampulliform pileipellis hyphae clearly distinguish it from all other taxa in the subgenus, suggesting that the creation of a new monospecific section could be considered.

Crepidotus autochthonus J.E. Lange, the section type species, is distinguished by cylindrical cheilocystidia, a significantly high presence of pileocystidia, and the absence of smell.

Another species with which *Crepidotus alsaticus* shares some affinity is *C. caspari* Velen.—included in *Crepidotus* subgen. *Dochmiopus* sect. *Dochmiopus* ser. *Caspari* (Consiglio & Setti 2008)—which differs in having rough-verrucose spores and entirely different pileipellis structures lacking ampullaceous or subfusiform-sphaerocyst-like hyphae. Additionally, it has smaller cheilocystidia (subutriform to claviform, up to 42 µm long) and lacks a farinaceous smell.

All other species within *Crepidotus* subgen. *Dochmiopus* are characterised by having distinctly verrucose spores.

ACKNOWLEDGMENTS

We thank Luis Parra (Spain); we would also like to express our gratitude to the MycolObs editorial team for reviewing, editing and translating the manuscript into English.

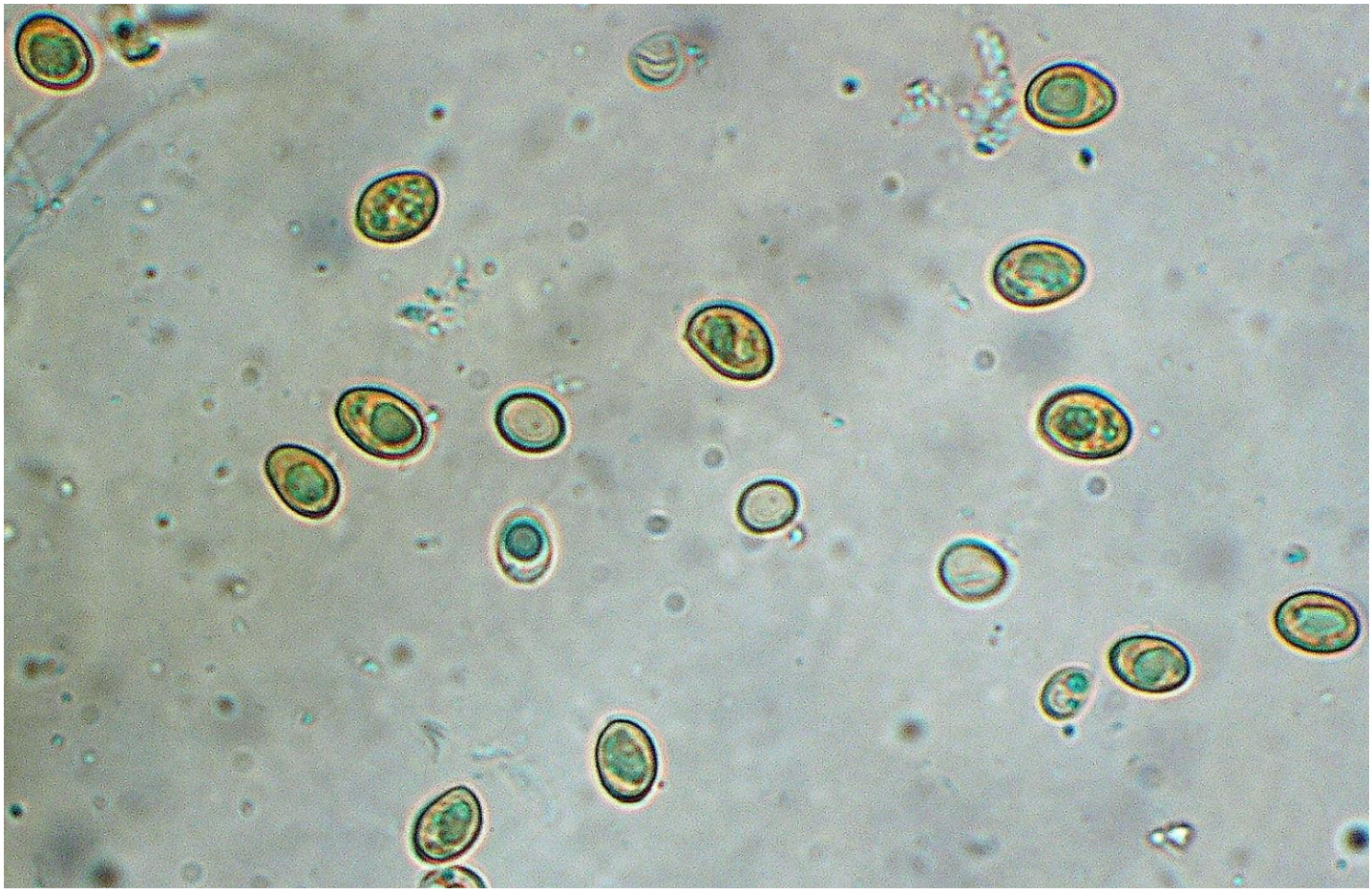


Fig. 8. *Crepidotus alsaticus*. Typus. Spores / spore

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Crepidotus alsaticus, una nuova specie del sottogenere *Dochmiopus*

Parole chiave:
Fungi
Basidiomycota
Agaricomycetes
Agaricales
Agaricineae
Crepidotaceae
ITS
analisi filogenetica

Riassunto: Viene descritta una nuova entità del genere *Crepidotus* rinvenuta durante alcune escursioni esplorative sulla diversità fungina nel territorio alsaziano nella località di Largitzen. La specie è stata indagata su base morfologia e studi di biologia molecolare. *Crepidotus alsaticus* è caratterizzata da notevoli peculiarità e viene agevolmente differenziata dalle sue consimili. Nella classificazione sistematica, per via dei caratteri specifici viene collocata in *Crepidotus* Sottogenere *Dochmiopus*. Della nuova specie vengono proposti i caratteri morfocromatici e microscopici nonché la descrizione ecologica dell'ambiente di crescita con relativo microclima e la comparazione con le specie simili collocate nel medesimo sottogenere.

INTRODUZIONE

Attualmente in Europa il genere *Crepidotus* (Fr.) Staude secondo le recenti monografie (Senn-Irlet 1995; Consiglio & Setti 2008; Hausknecht & Krisai-Greilhuber 2010) viene rappresentato, fra specie effettive e varietà, da circa 22–27 specie. Questa cifra non è eccessiva considerando la natura prevalentemente saprofita del genere molte delle cui specie amano insediarsi su substrato legnoso moderatamente degradato, di solito su ramoscelli, aste, tronchetti di latifoglie, di rado su conifere, raramente su residui vegetali, su corteccia di alberi viventi, direttamente su terreno o, caso davvero unico, parassita su altri funghi (*Crepidotus fungiphilus* Hauskn. & Krisai).

Dal punto di vista morfocromatico le specie del genere *Crepidotus* sono caratterizzate da una struttura morfologica di tipologia assai ben delineata e di facile determinazione visiva: quasi prettamente conchiliforme, flabelliforme fino a spatuliforme. Le colorazioni variano dal bianco puro al bianco-crema o bianco-avorio, in alcune specie volgono al crema-isabellino, bruno-albicocca (*Crepidotus ehrendorferi* Hauskn. & Krisai) al rosso scarlatto (*C. cinnabarinus* Peck), a bellissime colorazioni rosate (*C. roseoornatus* Pöder & Ferrari).

La superficie del cappello, generalmente priva di velo, ha una struttura feltrata-vellutata, finemente pubescente o con una folta peluria uniforme a volte appena percettibile, di rado appare liscia o con un sottile strato gelatinoso. In alcune specie sono presenti delle decorazioni sotto forma di fibrosità appressate-intrecciate o formanti una architettura marcatamente squamulosa. Le lamelle variano da moderatamente spaziate a fitte, sono solitamente biancastre, poi bruno-ocra oppure sono concolori con il cappello, per esempio giallastre in *Crepidotus carpaticus* Pilat, gialle-aranciate in *C. crocophyllus* (Berk.) Sacc., bruno-rossastre in *C. cinnabarinus*, rosate in *C. roseoornatus*. Il gambo è molto poco accennato o assente.

I caratteri microscopici possono variare sensibilmente a seconda della specie e dal punto di vista diagnostico contribuiscono a identificare la grande maggioranza delle specie con relativa facilità. Le specie europee presentano una trama imenoforale da regolare a subregolare e i giunti a fibbia possono essere presenti o assenti.

Le spore variano nella forma da ellittiche a largamente ellittiche, subovaliformi, subcilindriche-subfusiformi, subglobose, sferiche; al microscopio ottico possono apparire lisce, punteggiate, rugulose, verrucose o spinose-echinulate, queste ultime tronche all'apice; se osservate al microscopio elettronico, di solito non possiedono poro germinativo o in rarissimi casi isolati esso è molto debolmente accennato; ai reattivi chimici si rivelano inamiloidi e indestrinoidi. I cistidi imeniali sono presenti solo sul filo lamelle (cheilocistidi) e sono estremamente variabili nella forma. Se ne possono osservare almeno 30-40 diverse tipologie strutturali.

In Europa il genere *Crepidotus* è stato ampiamente documentato. Tra gli autori che hanno contribuito significativamente alla conoscenza del genere ricordiamo Favre (1935), Pilát (1948, 1949), Orton (1960), Clémençon (1977), Watling & Gregory (1989), Norstein (1990), Stangl, Kriegelsteiner & Enderle (1991), Senn-Irlet (1991, 1992, 1995), Ludwig (2001), Consiglio & Setti (2008), Hausknecht & Krisai-Greilhuber (2010).

Consultando gli annali della storia micologica, il nome *Crepidotus* in origine viene introdotto da Fries (1821) come sottogenere nel suo elaborato *Systema Mycologicum*; venne in seguito elevato al rango di genere da Staude (1857) e inserito nella famiglia *Crepidotaceae* (S. Imai) Singer da Singer (1951, 1986) che nel corso degli

anni vi introdusse fino a 9 diversi generi, sia agaricoidi [*Crepidotus*, *Melanomphalia* M.P. Christ., *Pleurotellus* Fayod, *Simocybe* P. Karst., *Tubaria* (W.G. Sm.) Gillet] sia cyphelloidi (*Chromocyphella* De Toni & Levi, *Episphaeria* Donk, *Pellidiscus* Donk, *Phaeosolenia* Speg). Tuttavia, questa catalogazione dell'epoca era costruita unicamente su base morfologica, mentre recenti studi di natura molecolare hanno portato notevoli cambiamenti nell'organigramma sistematico. I risultati delle analisi dei vari biomarcatori diagnostici hanno evidenziato delle eclatanti incongruenze a livello molecolare a tal punto che specie o generi ritenute dalla micologia classica attenenti ad un gruppo specifico o esclusivo dopo gli attuali accertamenti filogenetici sono state trasferite in altri generi oppure in nuove famiglie. Pertanto, anche la posizione sistematica dei generi inseriti da Singer nelle *Crepidotaceae* è stata rielaborata e riconfigurata. Vizzini (in Consiglio & Setti 2008, nelle note relative alla posizione tassonomica del genere *Crepidotus*), ritiene che attualmente siano da attribuire alla famiglia *Crepidotaceae* solamente 3 generi: *Crepidotus*, *Simocybe* e il genere cyphelloide *Pellidiscus*. Inoltre, Vizzini (2008) ha trasferito il genere *Tubaria* nella nuova famiglia *Tubariaceae* Vizzini mentre considera i rimanenti generi in una posizione tassonomica ancora incerta che necessita di ulteriori studi filogenetici.

Le nostre raccolte, in un primo approccio visivo sul campo, non mostravano alcuna peculiarità ed erano state misidentificate con i comunissimi *Crepidotus cesatii* (Rabenh.) Sacc. o *C. variabilis* (Pers.) P. Kumm. Elaborando in dettaglio la raccolta però siamo rimasti sorpresi dal suo odore di farina e avevamo allora indirizzato l'indagine verso il genere *Clitopilus*, in particolare *C. hobsoni* (Berk.) P.D. Orton che ha simili strutture morfocromatiche e organolettiche. Durante le analisi in laboratorio tuttavia sono emerse delle peculiarità inusuali per il genere *Clitopilus*. Le spore risultavano essere largamente ellittiche e subamigdaliformi, e prive di ornamentazioni al microscopio ottico; i cheilocistidi erano estremamente variabili, con oltre 20 diverse tipologie e alcuni lunghi oltre 100 µm. Ma soprattutto nel rivestimento pileico, non gelificato, erano presenti eclatanti ife rigonfie-vescicolose, a tratti fusiformi, ampollacee o perfino sferiche che ricordano molto quelle di *Singerocybe phaeophthalma* (Pers.) Harmaja. L'analisi filogenetica ha infine confermato l'impressione iniziale del genere *Crepidotus*.

MATERIALI E METODI

Morfologia

I corpi fruttiferi sono stati fotografati in habitat con un dispositivo digitale Samsung Galaxy Note 10 Plus, per le immagini di laboratorio e microscopia è stata utilizzata una NIKON Coolpix 4100. Per le analisi di microscopia è stato utilizzato un microscopio binoculare Leica DME con obiettivi acromatici; Il materiale fresco è stato osservato in soluzione acquosa, KOH 3% o colorato con Rosso Congo; il reattivo di Melzer è stato utilizzato per saggiare eventuali reazioni di amiloidia e/o di destrinoidia; soluzioni di sulfobenzaldeide per verificare la presenza di ife laticifere; il Blu cresile per verificare la reazione metacromatica nelle spore e nei tessuti imeniali; il Blu di toluidina è stato utilizzato per evidenziare la presenza di strati gelificati nel rivestimento pileico (Clemençon 1977a, b).

Il codice d'erbario LUG si riferisce al Fungarium del Museo di Storia Naturale Cantonale di Lugano (Svizzera). Tutte le immagini e le tavole sono di E. Musumeci.

Filogenetica

Estrazione DNA, amplificazione e sequenziamento

Il DNA genomico è stato estratto da materiale essiccato utilizzando un protocollo standard CTAB modificato per campioni fungini (Doyle & Doyle 1987). La regione ITS del DNA ribosomiale nucleare è stata amplificata mediante PCR utilizzando i primers ITS1F e ITS4 (White *et al*. 1990; Gardes & Bruns 1993). Le reazioni di amplificazione sono state eseguite in un volume finale standard contenente tampone di reazione, $MgCl_2$, dNTPs, primers e DNA polimerasi Taq. I prodotti PCR ottenuti sono stati verificati mediante elettroforesi su gel di agarosio, purificati e successivamente sequenziati col metodo Sanger in entrambe le direzioni presso un servizio commerciale di sequenziamento. Le sequenze ottenute sono state assemblate e corrette manualmente mediante confronto dei cromatogrammi forward e reverse, quindi allineate con sequenze di riferimento recuperate da GenBank utilizzando il software MAFFT v7 (Katoh & Standley 2013).

Analisi filogenetica

Le analisi filogenetiche sono state condotte mediante approccio di Massima Verosimiglianza (ML) e Inferenza Bayesiana (BI). L'analisi ML è stata eseguita utilizzando 1000 repliche bootstrap per la valutazione del supporto nodale mediante IQ-TREE (Nguyen *et al*. 2015). L'analisi bayesiana è stata effettuata con MrBayes v.3.2 (Ronquist *et al*. 2012) mediante catene Markov Monte Carlo (MCMC) per un totale di 1.000.000 di generazioni, campionando gli alberi a intervalli regolari e scartando la frazione iniziale come burn-in. Gli alberi risultanti sono stati visualizzati e modificati con FigTree v1.4.4 e iTOL (Letunic & Bork, 2021).

RISULTATI

Analisi filogenetiche

L'analisi filogenetica condotta mediante approccio di Massima Verosimiglianza (ML) e Inferenza Bayesiana (BI) ha evidenziato un'ampia congruenza topologica tra i due metodi. In entrambe le ricostruzioni, il taxon identificato come *Crepidotus alsaticus* risulta collocato in stretta associazione con *Crepidotus caspari* e con *Crepidotus cesatii*, mostrando valori di supporto elevati sia in termini di supporto bootstrap ML (fino a 100) sia di posterior probability bayesiana (fino a 1.00).

Nonostante tale prossimità filogenetica, *C. alsaticus* mostra una chiara individualità filogenetica, emergendo come linea autonoma e costantemente distinta all'interno del complesso analizzato. La sua posizione risulta infatti stabile in entrambe le analisi, senza fenomeni di mescolamento topologico con i taxa affini, evidenziando quindi una indipendenza evolutiva riconoscibile anche sulla base del solo marker ITS. Le differenze genetiche osservate, pur relativamente contenute, appaiono coerenti e sufficientemente strutturate da sostenere l'esistenza di un'entità filogeneticamente separata, interpretabile come specie indipendente piuttosto che come semplice variante intraspecifica.

Tuttavia, la limitata variabilità della regione ITS non consente una completa risoluzione delle relazioni tra taxa strettamente correlati, suggerendo che la divergenza osservata possa essere relativamente recente o non pienamente rappresentata da questo singolo marker. Pertanto, il riconoscimento di *C. alsaticus* come nuova specie non può basarsi esclusivamente sui dati molecolari ITS, ma trova ulteriore e importante supporto nell'integrazione con caratteri morfologici distintivi e, in prospettiva, richiede conferma attraverso analisi multilocus (es. LSU, RPB2, TEF1), in grado di fornire una risoluzione filogenetica più fine. Questo approccio integrato consente di interpretare correttamente la posizione sistematica del taxon, riconoscendone l'autonomia specifica e la sua indipendenza filogenetica pur nel contesto di un complesso di specie geneticamente affini.

Tassonomia

Crepidotus alsaticus Musumeci & Maraia *sp.nov*.

Diagnosi Originale (tradotta dall'inglese)

Cappello 5–15 mm diam., fin dai primi stadi da piano a leggermente espanso, in seguito semicircolare e infine distintamente conchiforme o espanso; margine involuto da giovane, divenente sinuoso-lobato a maturità. Superficie densamente pubescente, tomentosa o finemente villosa (visibile con lente d'ingrandimento). Colore inizialmente bianco puro, poi divenente grigio-crema. Lamelle da moderatamente rade a spaziate, con lamellule; filo lamellare leggermente eroso; inizialmente biancastre, poi grigio-crema e infine con toni brunastri. Gambo solitamente assente o solo rudimentale. Carne sottile, incospicua; odore debolmente farinaceo. Basidiospore 6.5–8.5 × 5–6 µm, lisce, nessuna ornamentazione visibile al microscopio ottico, internamente pluriguttulate, giallastre in KOH al 3%; da ellissoidali a largamente ellissoidali-subovoidali in vista frontale, da ellissoidali a leggermente amigdaliformi in vista laterale; parete leggermente ispessita; apicolo corto; poro germinativo assente. Basidi 25–33 × 7–9 µm, subclavati, 4-sporici. Trama imenoforale regolare; ife larghe 3–25 µm, lungamente cilindriche-filamentose; nella trama interna variabilmente conformate, da subfisaliformi a suballantoidi, a largamente ellissoidali o subglobose; ialine, non pigmentate, non incrostate. Cheilocistidi 25–102 × 7–30 µm, molto abbondanti sul filo lamellare, estremamente polimorfici, almeno 20 morfotipi osservati; queste morfologie includono sublageniformi, cilindrici, moniliformi, fusiformi, subclavati, irregolarmente ovoidali, sovente con protuberanze laterali, nodulosi o coralloidi; apice a volte sinuoso, forcato, subcapitato o

spatolato. Pleurocistidi assenti. Pileipellis una epicutis non gelificata, composta di ife irregolarmente intrecciate larghe 3–30 µm, da ialine a leggermente pigmentate, alcune fortemente pigmentate giallo-oliva; ife cilindriche filamentose intermiste con elementi più grandi, largamente ellissoidali-subfisaliformi, fusiformi, ampolliformi o sferiformi, richiamanti le ife vescicolose in Singerocybe phaeophthalma; superficie sovente ricoperta da una fine incrostazione uniformemente distribuita. Giunti a fibbia presenti.

Habitat: crescente su rametti in decomposizione di alberi decidui (probabilmente Alnus) in ambienti paludosi molto umidi.

Tipo: Francia, Alsazia, Alto Reno, Largitzen, in un'area collinare piana, 420 m slm, temperatura 9°-12°, umidità 100-87%, 06.10.2025, E. Musumeci 095827-25 (LUG 20999), Olotipo depositato nel Fungarium del Museo Cantonale di Storia Naturale di Lugano (Svizzera), LUG 20999.

Etimologia: l'epiteto alsaticus si riferisce alla regione Alsazia (Francia), dove gli esemplari del typus sono stati raccolti.

Caratteri macroscopici

Cappello 0.5–1.5 cm, sin dal primo stadio di crescita aperto-disteso poi semicircolare infine marcatamente conchiliforme-espanso, margine involuto, negli esemplari maturi sinuoso lobato; superficie rivestita da una folta peluria (lente !), tomentoso o finemente villoso; di colore bianco uniforme poi grigio-crema.

Lamelle poco fitte o spaziate con presenza di lamellule, filo lamelle leggermente eroso, biancastre poi grigio-crema infine con tonalità brune.

Gambo solitamente assente o molto debolmente accennato.

Carne poco rilevante, alla frizione odore di farina.

Caratteri microscopici

Spore 6.5–8.5 × 5–6 µm, gialline in KOH 3 %, lisce (nessuna ornamentazione visibile al microscopio ottico) pluriguttulate all'interno, ellittiche, largamente ellittiche-subovaliformi in proiezione frontale, ellittiche fino a lievemente amigdaliformi in proiezione laterale, con parete poco spessa, apicolo brevemente accennato, poro germinativo assente.

Basidi 25–33 × 7–9 µm, subclaviformi, tetrasporici.

Trama imenoforale regolare, ife larghe 3–25 µm, lungamente cilindriche-filamentose, nella trama interna diversamente conformate, da subfisaloidi-suballantoidi, largamente ellittiche fino a subglobose; ialine, non pigmentate e non incrostate.

Cheilocistidi 25–102 × 7–30 µm, numerosi sul filo lamelle, estremamente variabili nella forma con almeno 20 diverse tipologie: sublageniformi , subcilindrici, submoniliformi, fusiformi, subclaviformi irregolarmente ovoidali o diversamente conformati, sovente con protuberanze laterali, nodulosi o coralloidi; apice a volte sinuoso, forcato, subcapitulato o spatuliforme.

Pleurocistidi non osservati.

Epicute non gelificata, composta da due tipi frammisti di ife: a) larghe 3–30 µm, irregolarmente intrecciate, da ialine a lievemente pigmentate o a volte marcatamente giallo-oliva, subcilindriche-filamentose, b) più grossolane e voluminose, largamente ellittiche-subfisaloidi, subfusiformi, ampolliformi fino a sferici che ricordano molto le ife vescicolose di *Singerocybe phaeophthalma*. Superficie epiparietale rivestita sovente da una fine incrostazione uniforme regolarmente diffusa.

Giunti a fibbia presenti.

Habitat: Esemplari gregari su ramoscelli in decomposizione di *Alnus glutinosa* (?) in tratto boschivo interno in areale luminoso nelle vicinanze di uno stagno della sede del Club di pesca sportiva (SFV Dreiländereck) con presenza di *Alnus glutinosa*, *Quercus rubra*, *Carpinus betulus*, *Fagus sylvatica*, e diverse piante palustri; su terreno calcareo, substrato argilloso o di tessitura argillosa ricca di carbonati; suolo molto umido acquitrinoso. Altre specie rinvenute nelle vicinanze: *Cystolepiota moelleri* Knudsen, *Cystolepiota sistrata* (Fr.) Singer ex Bon & Bellù, *Galerina clavata* (Velen.) Kühner, *Alnicola spec.*

Altra raccolta esaminata: stesso sito del typus, 12.10.2025, *E. Musumeci 009106-25*.

NOTE

Per via delle ife con unioni a fibbia e la trama del cappello priva di strato gelatinoso *Crepidotus alsaticus* trova collocazione in *Crepidotus* sottogenere *Dochmiopus* (Pat.) Pilát seguendo le classificazioni infrageneriche proposte da Senn-Irlet (1995) e Consiglio & Setti (2008); ma si potrebbe anche considerare la creazione di una nuova sezione monospecifica per via delle strane ife ingrossate-ampolliformi riscontrare nel suo rivestimento pileico che la differenziano nettamente da tutte le altre entità del sottogenere. Seguendo Consiglio & Setti (2008) *Crepidotus alsaticus* viene collocato in *Crepidotus* subgen. *Dochmiopus* sez. *Autochtoni* (Senn-Irlet) Consiglio & Setti per via delle spore lisce al microscopio ottico.

Crepidotus autochthonus J.E. Lange, la specie eponima, si distingue per i cheilocistidi cilindracei e la presenza rilevante di pileocistidi, oltre all'assenza di odore. Un'altra specie con la quale *Crepidotus alsaticus* potrebbe avere qualche affinità è *C. caspari* Velen. che è inclusa in *Crepidotus* subgen. *Dochmiopus* sez. *Dochmiopus* ser. *Caspari* (Consiglio & Setti 2008) e differisce per avere spore ruvide-verrucose; cheilocistidi piccoli, subutriformi-claviformi, e grandi fino a 42 µm; ife della pileipellis totalmente differenti, priva di ife ampollacee o subfusiformi-sferociformi; e, dal punto di vista organolettico, per l'assenza di odore farinaceo. Tutte le altre specie del sottogenere si differenziano per avere spore nettamente verrucose.

RINGRAZIAMENTI

Ringraziamo Luis Parra (Spagna); desideriamo altresì ringraziare il team editoriale di MycolObs per aver revisionato, curato e tradotto il manoscritto in inglese.

Article received 16 April 2026, accepted 21 July 2026

Fungal diversity and its ecological role in probiotic-enhanced aquaculture ponds

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Key words:
Aspergillus
Macrobrachium rosenbergii
Economic analysis
sustainability
Fungal diversity
Growth performance
Probiotics
Soil quality

Abstract: Two aquaculture ponds of freshwater prawn (*Macrobrachium rosenbergii*) near Vishnuvakkam, Tamil Nadu, India, were studied to analyse how probiotics affect fungi and how they together contribute to improve soil condition, development of culture and ecosystem balance. One pond received no additives; the other was treated with regular doses of beneficial microbes. In the probiotic-enhanced pond, fungal species diversity increased to 35 species, whereas the untreated water hosted only 28, increasing diversity by a quarter. We observed, also using standard statistical controls, that the number of fungi increased on average by 365.45% ± 17.736% under the influence of probiotics. Among fungal groups, *Aspergillus* dominated the control pond; the probiotic setting encouraged a wider mix of fungi. Better silt levels - reaching 35.09% - alongside 29.59% sand appeared alongside this richer community. Nutrient access improved under these conditions, supporting stronger prawn growth. Survival stayed total in the treated group, where growth measured 35.54, nearly double the control's 19.37, and output climbed to 372 kg against just 237 kg without intervention. Financial outcomes followed the same trend: returns outweighed costs by 1.785 times, far above the control's 1.355. Profit landed at INR 2,06,758, dwarfing the INR 57,971 seen elsewhere. Fungi, shaped by probiotics, clearly shape system performance; they are not merely present but pivotal in lasting aquaculture setups.

INTRODUCTION

Fungi rank high in ecological flexibility across the planet. Because they tolerate wide pH ranges they establish easily in varied habitats. While found in oxygen-rich settings, some flourish where air is limited. These traits let them dominate early stages of freshwater community development (Manoharachary & Ramarao 1983). Far from being mere residents, fungal populations drive change within fish farming waters. Through breaking down dead material, nutrients shift forms under their influence. Soil texture improves when their networks spread underground.

Manoharachary & Ramarao (1983) found 47 species of fungi living in just two muddy freshwater ponds in Hyderabad - evidence that small water bodies can host dense fungal communities. Although limited to one region, their work highlighted biodiversity potential tucked inside quiet wetlands. Flowing waters tell a different story; Singh & Wadhwani (1989) showed this clearly when they compared fungi in moving rivers versus still pools. Where water moves slowly or fast appears to guide which fungi settle where. Because movement alters conditions beneath the surface, it also shifts what grows below.

Further studies have broadened the understanding of fungal life from other regions. In Nigeria, Okaemo & Olufemi (1997) mapped fungi in tilapia farming waters; and Okpokwasilli, Olufemi & Okaemo (1998) explored similar communities in catfish aquaculture systems. Indian researchers contributed findings too - Girivasan,

Kumar & Sathish (1998) reported on fungus species living in peat-based earths. Then came discoveries from hidden places: Koilraj, Marimuthu & Muthulakshmi (1999) pulled distinct fungal biomes out of cavern ecosystems in 1999, showing survival where nourishment is scarce and sunlight absent.

Among the most recent studies, Paulraj (2002a) identified twelve species of mesophilic fungi alongside seven thermophilic ones within *Macrobrachium rosenbergii* farming systems, offering one of the first fungal inventories in such environments. Shifting focus slightly, this work probes deeper: instead of just listing species, it explores whether introducing probiotics alters these fungal networks, then traces any shifts toward changes in pond ecology or harvest outcomes.

MATERIALS AND METHODS

Pond Location

The *Macrobrachium rosenbergii* culture farm used for this study is located in Vishnuvakkam, approximately 56 km from Chennai, in the Thiruvallur District of Tamil Nadu, India. The farm comprises two ponds: a control pond and a probiotic experimental pond, each covering an area of 0.603 hectares (length 298 m, width 213 m). Both ponds have a depth of approximately 1.5 m. The control pond is separated from the probiotic experimental pond by a bund measuring 80-95 cm in width, and all other sides of the ponds are similarly bordered. The ponds are surrounded by agricultural fields and feature a sluice gate (2 m × 1.5 m) for water drainage. Three screens with mesh sizes of 0.5, 1.0, and 1.5 cm are installed at the sluice gate to prevent the escape of animals during water drainage. Additionally, two 8 inch diameter emergency pipes with valves facilitate water release during the rainy season. To prevent cannibalism, shelters (such as country tiles and coconut leaves) were placed within the ponds.

Postlarval Stocking and Acclimatization

Postlarvae (60,000) were sourced from Aqua Nova hatchery (P) Ltd., located in Kannathur, Chennai, approximately 106 km from the culture farm. Five hundred healthy and active postlarvae (PL-15), with a mean length of 12.8 ± 1.1 mm and a mean weight of 1.2 ± 0.2 mg, were packed in polythene bags (40 × 80 cm) containing two liters of water, which were inflated with oxygen and sealed with rubber bands, Brine shrimp nauplii were added as food during transportation.

The post-larvae were transported after sunset using a van. Upon arrival, the polythene bags were placed in the ponds for about one hour to acclimatize. The bags were then opened with minimal disturbance, allowing pond water to gradually enter. The postlarvae were slowly released into both ponds, resulting in a stocking density of 1.3 individuals/m^2.

Soil Fungal Analysis

Mesophilic fungi were isolated from soil samples using different culture media at varying temperatures. For this study, a Czapek-Dox-Agar (CDA) medium was employed for mesophilic fungi isolation. The composition of the CDA medium per 1000 ml of distilled water is as follows:

Sodium nitrate: 2.0 g
Potassium dihydrogen phosphate: 1.0 g
Magnesium sulfate: 0.5 g
Potassium chloride: 0.5 g
Ferrous sulfate: 0.01 g
Agar: 20.0 g
Sucrose: 30.0 g

One gram of soil sample was thoroughly dispersed in 10 ml of sterile distilled water to create a stock solution. Sequential dilutions were prepared: 1 ml of the stock solution was transferred to 9 ml of sterile water, mixed well and this process was repeated. From this dilution, 1 ml was pipetted into sterile petri plates containing antibiotic-amended CDA medium (10^3 dilutions), with streptomycin sulfate used to inhibit bacterial growth.

The Petri plates were incubated at 29 ± 1°C and 21± 1°C for one week, with six replicates maintained for each soil sample. Fungi were stained with lacto-phenol cotton blue and observed under a light microscope.

Identification was performed using standard manuals (Cooney & Emerson 1964; Gilman 1967; Barnett & Hunter 1972; Onions, Bridge & Sutton 1981). Percentage contribution and colony-forming units (CFU) were calculated using the following formulas:

Percentage Contribution (PC):

$$\frac{\text{Total no. of colonies of a species}}{\text{Total no. of colonies of all species}} \times 100$$

Colony Forming Unit (CFU):

$$\frac{\text{Average no. of colonies / plates}}{\text{Volume plated}} \text{ dilution factor}$$

Harvesting and Growth Assessment

The total length (cm) and body weight (g) of harvested prawns in both ponds were measured four times monthly during the harvest period. Growth metrics, including specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), benefit-cost ratio (BCR), and feed efficiency (FE), were calculated according to the methods outlined by Sweilum (2006):

Specific Growth Rate (SGR):

$$\frac{\text{(Final weight - Initial weight)}}{\text{Culture period in days}} \times 100 \text{ days}$$

Feed Conversion Ratio (FCR):

$$\frac{\text{Quantity of feed consumed}}{\text{Total weight gained}}$$

Protein Efficiency Ratio (PER):

$$\frac{\text{Weight gain (g) x No. of prawns}}{\text{Protein intake}}$$

Feed Efficiency (FE):

$$\frac{\text{Total weight gain (g)}}{\text{Quantity of food consumed}}$$

Harvesting Process

The harvest occurred on the 119th day of culture, with partial harvests performed in subsequent months. before harvesting, the water level was lowered to 0.5 m. The complete harvest was conducted over four days (December 15-20, 2018) between 5:00 am - 10:30 am and 3:00 pm - 6:30 pm, using drag nets and hand-picking to ensure 100% harvest efficiency. Fish populations were also collected. Post-harvest, the animals were weighed, sorted by size, and packed in ice. Stunted prawns were segregated and cultured in a separate pond for further growth analysis.

Economic Analysis

Harvested prawns were sold in the Chennai export market, while fish were sold in the local market. Costs associated with seed, feed, fertilizers, power, labour, harvesting, and trial netting were recorded and compared for both control and probiotic experimental ponds. Operational costs, net income, and profits were calculated, excluding the cost of pond leasing. Production costs were based on wholesale market prices for inputs from the 2018-2019 periods.

Statistical Analysis

Statistical analysis was performed on data regarding soil texture, water parameters, fungal populations, and plankton counts using the t-test (Systat version 10.0). Dissolved oxygen (DO), pH, and temperature were analyzed using correlation, regression, and ANOVA at a 5% significance level. Growth relationships (positive/negative) between the control and experimental pond-cultured prawns were assessed using SPSS Inc. (2010).

RESULTS (FUNGAL DIVERSITY AS THE PRIMARY ECOLOGICAL DIFFERENTIATOR)

Comparative Fungal Genera Richness

Among results, one stands out clearly: fungal variety differed sharply across the two ponds. In the probiotic-treated water body, researchers identified 35 separate fungal groups; meanwhile, the untreated counterpart hosted just 28 - marking a gain of 25% in classification count due to added microbial supplements. Measurements showed fungal levels in the treated pond reached an average of 365.45%, with a margin of error at ±17.736%, notably surpassing those in the standard pond under statistical testing ($P < 0.05$). Such a rise in fungus numbers suggests that introduced beneficial microbes - especially strains within the *Bacillus* family - helped shape underground conditions favouring greater fungal settlement.

Dominant Fungal Genus: *Aspergillus* P. Micheli ex Haller

Despite its strong presence in both environments, *Aspergillus* showed reduced dominance in the probiotic pond where new fungal groups appeared alongside it. The control pond, hosted only a few species making *Aspergillus* stand out clearly. This shift suggests external inputs helped broaden microbial range. Because these fungi play key roles in organic breakdown and nutrient cycling, such changes may influence ecosystem function directly. Breaking down tough organics like cellulose, starch, or proteins depends on enzyme activity.

Besides the shift in numbers, one pond showed greater fungal variety than the other. With probiotics, it hosted 35 separate fungal groups; without them, just 28 - meaning a quarter more types appeared where supplements were used. Fungal levels there reached 365.45% ±17.736%, clearly exceeding the second site, confirmed by statistical testing at P less than 0.05. That rise points to beneficial bacteria, especially *Bacillus* strains, shaping underground conditions favouring fungi.

Fungal Diversity and Soil Texture: A Bidirectional Relationship

The soil in the probiotic pond was found to be different when examined - its makeup carried 35.09% silt and 29.59% sand, levels that exceeded those found in the untreated plots. Because fungi worked beneath the surface, tiny threads wove through grains, locking bits together into stable clusters. Where these networks grew denser, moisture stayed longer, held within microscopic pockets. Complexity emerged not from size but variety: broader species of fungi built more intricate webs. With each new strand, the ground gained strength, shaped by unseen branching.

Beyond microbial shifts, chemical markers tell a clear story: nitrogen levels rose notably where fungi thrived (probiotic: 41.09 ± 1.423; control: 29.454 ± 1.485). Phosphorus followed a similar trend, higher in treated conditions (4.654 ± 0.228 compared to 3.945 ± 0.166). Potash displayed an even starker contrast, reaching 129.272 ± 8.543 against 105.909 ± 8.182. These jumps trace back to active fungal processes boosting elemental release. As nutrients increase below, life above benefits just as much - the prawn population gains support through enriched ground chemistry.

Fungal Contributions to Prawn Growth and Pond Productivity

While fungi do not directly feed the prawns, their contributions to the trophic web of the pond are profound. Fungi start the breakdown of dead leaves, leftover food, and waste, returning essential elements to the water. Following this process, tiny animals like zooplankton feed on the fungal growth, bridging energy flow toward *Macrobrachium rosenbergii* through natural consumption patterns. Some fungi can suppress waterborne diseases through competition for space and nutrients, thereby limiting the establishment and spread of pathogenic fungi such as *Chytridiomyces* (Paulraj 2012). Where fungal threads form dense layers,

oxygen moves better through mud, helping bottom-dwelling shrimp find food. These changes happen gradually, shaped by how species interact beneath the surface.

The outcome of this enriched fungal ecology was a Specific Growth Rate (SGR) of 35.54 in the probiotic pond versus 19.37 in the control — an 83.5% improvement — alongside 100% survival rate and a total yield of 372 kg compared to 237 kg in the control.

DISCUSSION (RETHINKING AQUACULTURE THROUGH A FUNGAL LENS)

Among ponds treated with probiotics, a 25% increase in fungal genera stand out. Ecosystem resilience often reflects fungal diversification. For example, in the event of heat peaks or excess waste, the system with 35 genera adapts more smoothly than its counterpart which contains only 28. Stability in prawn habitats improves when fungi multiply, as they support the balance through their layered presence.

In both ponds, *Aspergillus* predominates; a pattern which is also echoed in other studies (Paulraj 2002b; Manoharachary & Ramarao 1983; Okaemo & Olufemi 1997). Found everywhere in tropical pond soils, these fungi adapt quickly due to broad metabolic abilities. Their status as leading organisms points clearly toward central involvement in breaking down organic material within such environments.

Soil nutrient gains - especially nitrogen, phosphorus and potassium - run alongside each other suggesting that fungi are key to breaking down organic matter within the probiotic pond. Instead of passive bystanders, these organisms likely drove nutrient recycling by transforming complex materials into usable forms. Moreover, evidence from Wang, Zhang & Liu (2005), together with findings from Rengpipat, Ratanachai & Khamput (1998), points to microbial life as a driving force behind sediment nutrition shifts. Behind the scenes, tiny biological networks appear responsible for freeing locked-up resources. Rather than acting alone, microbes functioned as a linked system enabling sustained availability of vital elements.

Looking at the numbers, the probiotic pond showed a benefit-cost ratio of 1.785, whereas the control sat at 1.355. Net earnings reached Rs. 2,06,758 in contrast to Rs. 57,971 without treatment. Although these improvements are often tied to probiotics generally, they depend heavily on the support provided by fungal activity within the system. Such benefits emerge alongside shifts driven by microbial interactions made possible through probiotic use.

CONCLUSION

Fungi play a silent but vital role in probiotic fish farming. Evidence shows that adding probiotics to ponds where *Macrobrachium rosenbergii* is raised doesn't just shift bacterial life - it triggers wider changes among fungi, boosting the number of fungal species by one quarter while also raising overall fungus levels noticeably ($P < 0.05$).

What stands out is how *Aspergillus* dominates in both settings, forming the core of fungal activity and keeping basic nutrient flows running. However, resilience emerges differently - where the probiotic pond hosts 35 genera, interactions grow deeper, supporting stronger prawn development, better soil structure, and higher profits over time.

These findings position fungal diversity as a key indicator of aquaculture pond health and advocate for mycological monitoring as a standard component of sustainable aquaculture management. Future research should investigate specific fungal taxa responsible for biocontrol and nutrient transformation functions within probiotic ponds, and explore targeted fungal inoculants as complements to established probiotic regimes.

Fungi are not background organisms in aquaculture — they are foundational architects of pond ecology.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors have declared no conflict of interest.

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Article received 6 August 2026, accepted 23 August 2026

Clitopilus perexiguus sp. nov. from Nordio Forest and *Clitopilus dilectissimus* Part II

(versione italiana a pag. 46)

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Key words:
Agaricales
Entolomataceae
new species
Italy
ITS
LSU
Mediterranean
taxonomy
Venice

Abstract: The new species *Clitopilus perexiguus* is described from the Nordio Forest Nature Reserve (Italy). Morphological and phylogenetic analyses support the taxonomical novelty status of *C. perexiguus* which is characterised by smooth spores and very small, white basidiomes with an umbilicate pileus and a central, fully developed stipe. An expanded taxonomic treatment and phylogenetic analysis of *C. dilectissimus* is also provided, which was recently described from the same forest. *C. dilectissimus* is compared with morphologically similar taxa and is recovered in a consistent (though only marginally supported) clade containing numerous North American collections provisionally identified as *C. scyphoides*-IN01, together with several unnamed or misidentified *Clitopilus* collections. On the basis of their high ITS similarity and phylogenetic placement, these collections are interpreted here as *C. dilectissimus*, substantially extending the known geographical and ecological range of the species. Additional loci will nevertheless be required to resolve relationships within this assemblage more robustly.

INTRODUCTION

The Bosco Nordio (Nordio woodland) is a protected wooded area located in proximity to the Adriatic coastline in northern Italy, approximately 30 km south of Venice. It is mainly characterised by an ash-ilex grove formation (*Fraxino orni-Quercion ilicis*) also with presence of *Populus alba*, *Populus tremula*, *Quercus robur*, *Quercus pubescens*, *Pinus pinaster*, *Juniperus communis*, grown on ancient dunes (relict dunes) and having a calcareous sandy substrate (Voto 2021).

The genus *Clitopilus* (Fr. ex Rabenh.) P. Kumm. contains 354 or 390 names worldwide (fide Index Fungorum and MycoBank, respectively), including autonyms, synonyms and infrageneric taxa. It comprises diverse gilled species in the family *Entolomataceae* Kotl. & Pouzar with a saprotrophic ecology. The spore print is pink; mature gills are usually decurrent and pinkish; the stipe can be central to eccentric or absent to reduced; spores are usually angular, mostly featuring distinct longitudinal ridges; and the basidiomes of many species have a farinaceous odour. *Clitopilus* has undergone major modern taxonomic shifts driven by molecular phylogenetic studies which have reshaped its boundaries, merging several taxa previously placed in *Rhodocybe* Maire (Co-David, Langeveld & Noordeloos 2009). Its type species, *Clitopilus prunulus* (Scop.) P. Kumm. (basionym *Agaricus prunulus* Scop.), is also present in the Nordio forest (found once by P. V.). A fourth species of the genus new to the same forest will be reported in a next contribution.

MATERIALS AND METHODS

Morphological study

Macroscopic descriptions were obtained on fresh material immediately after collection. Photographs were taken with a Nikon Coolpix A10 camera. All micromorphological measurements were taken from fresh material mounted in water or Congo red. Spore measurements are reported using the following formula: (lowest length) 10th percentile–90th percentile (highest length) × (lowest width) 10th percentile–90th percentile (highest width). In the case of *Clitopilus perexiguus*, since a notable difference was noted between the measurements of

spores from the hymenium and the stipe, only spores measured on the stipe are included in the description. All images by P.V.

GB stands for GenBank accession number, while iNat and MO stand for iNaturalist and Mushroom Observer observation numbers, respectively.

Molecular study

DNA extraction, PCR amplification and Sanger sequencing were performed by commercial facilities to generate an LSU sequence (GB PZ782068) from the holotype collection of *Clitopilus dilectissimus* (PAD H0062396) and an ITS sequence (GB PZ782067) from the holotype collection of *C. perexiguus* (PAD H0064020).

Comparative sequences representing 96 additional collections were obtained from GenBank, iNaturalist and Mushroom Observer. Related sequences were identified using NCBI BLAST (Altschul *et al*. 1990) and MycoMap's MycoBLAST (https://mycomap.org/mycoblast). The dataset also included the previously published ex-type ITS sequence of *C. dilectissimus* (PV661559; Voto 2025). Five similarity searches were conducted: NCBI BLAST searches using the ITS sequences of *C. dilectissimus* and *C. perexiguus* and the LSU sequence of *C. dilectissimus* as queries, and MycoBLAST searches using the ITS sequences of *C. dilectissimus* and *C. perexiguus* as queries. Two *Lyophyllum* P. Karst. sequences were used as the outgroup. The final concatenated dataset comprised 98 collections, represented by 98 ITS and 48 LSU sequences. Missing regions were represented by gaps. The ITS1, 5.8S and ITS2 regions were extracted from the ITS sequences using ITSx v. 1.1.3 (Bengtsson-Palme *et al*. 2013). These three regions, together with the LSU region, were aligned separately using MAFFT v. 7 (Katoh & Standley 2013) with the L-INS-i algorithm. The alignments were inspected and trimmed using UGENE v. 53.1 (Okonechnikov *et al*. 2012) and then concatenated into a single partitioned dataset. The combined dataset contained 1563 characters, including gaps. The dataset was concatenated and partitioned by locus (ITS1: 1–258; 5.8S: 259–416; ITS2: 417–633; LSU: 634–1563).

Maximum-likelihood (ML) analysis was performed using IQ-TREE version 3 (Wong *et al*. 2026). The best-fitting substitution models (TVM+G4 for ITS1, F81 for 5.8S, TPM2u+G4 for ITS2 and HKY+F+R3 for LSU) were selected using IQ-TREE's inbuilt ModelFinder (Kalyaanamoorthy *et al*. 2017; Wong *et al*. 2026). Branch support was assessed using 1,000 ultrafast-bootstrap replicates. Bayesian inference (BI) was performed using MrBayes version 3.2.7 (Ronquist *et al*. 2012). ITS1, 5.8S and ITS2 were combined and treated as a single ITS partition, while LSU constituted the second partition. Each partition was assigned an independently parametrised GTR+G. The analysis comprised two independent runs, each with four Markov chains, for 2,000,000 generations. Trees were sampled every 1,000 generations, with the first 25% discarded as burn-in. The resulting trees were visualised in iTOL (Letunic & Bork 2019) and the ML tree annotated in Canva (Canva Pty Ltd.).

RESULTS

Phylogenetic analyses

The topologies of the ML and BI trees were largely congruent, so only the ML tree is given with BI values mapped against the corresponding nodes. Branches with bootstrap support (BS) and posterior probabilities (PP) values ≥70% and ≥0.90, respectively, are shown at the nodes.

Both ML and BI recovered *Clitopilus dilectissimus* within a clade containing numerous collections assigned the provisional species code *C. scyphoides*-IN01, together with two collections named *C. scyphoides* f. *reductus* Noordel., one named *C. hobsonii* (Berk.) P.D. Orton and two unnamed *Clitopilus* collections. The subtending branch uniting *C. dilectissimus* with these collections received only marginal support in each analysis (BS 67%, PP 0.80); however, the same relationship was recovered across the final ML and BI analyses as well as multiple prior exploratory analyses, where different models of evolution and alignment and sampling strategies were used. Furthermore, the *Clitopilus scyphoides*-IN01 ITS sequences are very similar (some 100%) to the *C. dilectissimus* ITS ex-type sequence. Taken together, this indicates that the allegiance of *C. dilectissimus* to this clade is likely real despite the marginal support in our phylogenetic analysis. The marginal support may reflect insufficient resolution within the ITS+LSU dataset. Additional protein-coding loci, particularly *rpb2* and *tef1*-α,

would allow this relationship to be tested more robustly. Overall, we suggest that *Clitopilus scyphoides*-IN01 is *C. dilectissimus*.

Clitopilus perexiguus was recovered as sister to a clade comprising two unnamed North American collections, assigned the provisional species code '*Clitopilus sp.* CA04'(HAY-F-003655 and MYCO-1010094). The clade comprising *C. perexiguus* and the two *C*. sp. CA04 collections received maximal support in both analyses (BS 100%, PP 1.00). This clade was in turn sister to two unnamed Chinese collections (FJAU75563 and FJAU75564), with maximal support (100%/1.00). Furthermore, *C. perexiguus* was recovered within a broader clade that also contained *C. albominutellus* (E. Ludw.) Musumeci & Contu and *C. giovanellae* (Bres.) Singer, species assigned to *Clitopilus* sect. *Omphaloidei* G. Moreno, Contu, A. Ortega, Platas & Peláez (Musumeci & Contu 2018).

Taxon	Collection code	iNat/MO	Country	ITS	LSU
Clitopilus albidus H	CAL 1319	-	India	MF926596	MF926595
C. albominutellus	114928-22	-	France	PQ662633	PQ662635
C. albominutellus	5936-12	-	France	PQ662632	PQ662634
C. austroprunulus	MEN2009001	-	Australia	KC139084	-
C. austroprunulus	MEN2009062	-	Australia	KC139085	-
C. baronii H	AMB 18363	-	Italy	NR_176131	-
C. baronii	K (M) 179703	-	UK	MN855362	-
C. baronii	AMB 18362	-	Italy	MN855368	-
C. baronii	AMB 18378	-	Italy	MN855370	-
C. baronii	KUN-HKAS 145333	-	China	PQ793166	PQ781610
C. baronii	KUN-HKAS 145334	-	China	PQ793167	PQ781611
C. brunneiceps H	KUN-HKAS 104510 (HYJ95)	-	China	MN061295	MN065684
C. chichawatniensis	LAH37432	-	Pakistan	ON980766	ON980763
C. chichawatniensis	LAH37431	-	Pakistan	ON980767	ON980764
C. cretoalbus	LAH37017	-	Pakistan	OM935685	OM934826
C. cretoalbus	LAH 35709	-	Pakistan	NR_185709	ON229505
C. crispus	KUN-HKAS 90506 (Zhao2632)	-	China	MN061312	MN065702
C. crispus	KUN-HKAS 102670 (530828MF055)	-	China	MN061313	MN065704
C. crispus	KUN-HKAS 84667 (Han371)	-	China	MN061314	MN065705
C. dilectissimus H	**PAD H0062396**	-	**Italy**	PV661559	**PZ782068**
C. fasciculatus H	A. v. Zaayen, 15-V-1979	-	Netherlands	OQ845966	-
C. fasciculatus	297071	MO_297071	USA (California)	MG551863	-
C. fusiformis	KUN-HKAS 104513 (JSP207)	-	China	MN061297	MN065686
C. fusiformis	KUN-HKAS 104515 (JSP209)	-	China	MN061300	MN065690
Clitocybe giovanellae	S.F.14368	-	Spain	EF413030	EF413027
C. hobsonii E	K(M) 122842	-	UK	NR_182819	-
C. hobsonii	CBS 270.36	-	UK	MH855797	-
C. hobsonii	K (M) 167650	-	UK	MN855371	-
C. hobsonii	QYL10	-	-	OK652826	OK655769
C. hobsonii	iNat_271630334	iNat_271630334	USA (California)	-	-

Taxon	Collection code	iNat/MO	Country	ITS	LSU
C. aff. hobsonii	K:M195388	-	UK	MN855375	-
C. kamaka H	PDD 96106	iNat_1057573	New Zealand	NR_137867	-
C. cf. kamaka	KA12-0364	-	South Korea	KR673433	-
C. lampangensis H	CMU:JK0147	-	Thailand	NR_175631	MK764935
C. lampangensis	SDBR-CMUNK0047	-	Thailand	MK764934	MK773856
C. orientalis	CAL 1613	-	India	MG345134	MG321558
C. parasiticus H	KUN-HKAS145336	-	China	PQ793169	PQ781613
C. parasiticus	KUN-HKAS145335	-	China	PQ793168	PQ781612
C. parasiticus	KUN-HKAS145337	-	China	PQ793170	PQ781614
C. passeckerianus	P73	-	South Korea	KY962489	KY963073
C. passeckerianus	P78	-	South Korea	KY962494	KY963078
C. passeckerianus	CBS 299.35	-	Austria	FJ770386	MH867198
C. passeckerianus	K:M134571	-	UK	MN855376	-
C. perexiguus H	PAD H0064020	-	Italy	PZ782067	-
C. pinsitus	CBS 623.70	-	UK	FJ770403	MH871665
C. reticulosporus	WU27150	-	Austria	KC885966	-
C. rhodotrama I	MICH:10218	-	Argentina	PZ579810	-
C. scyphoides	CBS 127.47	-	France	MH856181	MH867707
C. "scyphoides-IN01"	CM24-07037	iNat_229278366	USA (Indiana)	-	-
C. "scyphoides-IN01"	DR08	iNat_130008778	USA (Indiana)	OP743453	-
C. "scyphoides-IN01"	iNAT_16635123	iNat_16635123	USA (New York)	MZ198224	-
C. "scyphoides-IN01"	5089	iNat_136703753	USA (Pennsylvania)	OP749260	-
C. "scyphoides-IN01"	IN25-03115	iNat_318059823	USA (Indiana)	-	-
C. "scyphoides-IN01"	20180810TEMP000	iNat_145242172	USA (Indiana)	-	-
C. "scyphoides-IN01"	MYCO-1017459	iNat_178439013	USA (Pennsylvania)	-	-
C. "scyphoides-IN01"	AM128; 78930	iNat_31559053	USA (Indiana)	-	-
C. "scyphoides-IN01"	20180818TEMP000	iNat_144024893	USA (Indiana)	-	-
C. "scyphoides-IN01"	TENN-F-075953	iNat_7003045	USA (Ohio)	-	-
C. "scyphoides-IN01"	2944	iNat_242415850	USA (Missouri)	-	-
C. cf. scyphoides	KUN-HKAS 104511 (Zhu1775)	-	China	MN061329	MN065720
C. scyphoides f. reductus	CM2019-00985	iNat_32147800	USA (Ohio)	-	-
C. sinoapalus H	KUN-HKAS 101191 (Yang6002)	-	China	MN061322	MN065711
C. sinoapalus	KUN-HKAS 102807 (530828MF263)	-	China	MN061319	MN065710
C. sinoapalus	KUN-HKAS 82230 (Lifang1403)	-	China	MN061320	MN065712
C. sinoapalus	KUN-HKAS 77037 (Wu865)	-	China	MN061321	MN065713
C. sp.	LF01346	-	Thailand	OP680442	-
C. sp.	FRFTM-501	iNat_334423848	Peru	-	-
C. sp.	676	iNat_308605908	USA (Michigan)	-	-
C. sp.	KUN-HKAS 104512 (JSP140)	-	China	MN061330	MN065721

Taxon	Collection code	iNat/MO	Country	ITS	LSU
C. sp.	FJAU75564	-	China	PX475680	PX482116
C. sp.	FJAU75563	-	China	PX474882	PX482115
C. sp.	RA716-5	-	USA (Arkansas)	MK217427	-
C. sp.	GNP2	MO_231731	Canada (Saskatchewan)	MW183928	MW183928
C. sp.	RA715-5	-	USA (Arkansas)	MK217426	-
C. sp.	RA712-18	-	USA (Arkansas)	MK234227	-
C. sp.	KA17-0568	-	South Korea	MN294896	-
C. sp. "CA04"	HAY-F-003655	iNat_167291390	USA (California)	OR860203	-
C. sp. "CA04"	MYCO-1010094	iNat_171428792	USA (Nebraska)	-	-
C. sp. "ID01"	RO26-02465	iNat_325787616	USA (Idaho)	-	-
C. sp. "IN07"	OSF2022-0243	iNat_130280165	USA (Indiana)	OP549132	-
C. sp. "kamaka-PNW02"	HAY-F-007977	iNat_197270552	USA (California)	PQ484105	-
C. sp. "kamaka-PNW02"	42578	iNat_128490733	USA (Oregon)	-	-
C. sp. "MX01"	iNat_230226661	iNat_230226661	Mexico	-	-
C. sp. "sp-IN07"	JK425	iNat_121499598	USA (Tennessee)	-	-
C. subalbidus H	GDGM72219	-	China	ON963951	NG_243733
C. subalbidus	GDGM72229	-	China	ON963952	ON963946
C. subscyphoides	CAL 1326	-	India	MF927542	MF946580
C. subscyphoides	KUC20230817-12	-	South Korea	PQ619324	PQ619357
C. subscyphoides	GDGM72683	-	China	ON963953	ON963947
C. subscyphoides	KUC20230817-08	-	South Korea	PQ619323	PQ619356
C. subscyphoides	GDGM73056	-	China	ON963954	ON963948
C. umbilicatus	KUN-HKAS 80289 (Han58)	-	China	MN061323	MN065715
C. umbilicatus	KUN-HKAS 80310 (Han79)	-	China	MN061324	MN065716
C. umbilicatus	KUN-HKAS 80370 (Han140)	-	China	MN061325	MN065717
C. velutinus	CORT014618	-	Dominican Republic	MN784991	-
Lyophyllum decastes	Lyophyllum_decastes_ZHU	-	-	OR488578	-
L. semitale	CBS369.47	-	-	AF357048	-
"Rhizoctonia sp."	ATT213	-	USA (Texas)	HQ607889	-

Table 1. Collections and sequences included in the phylogenetic analyses assessing the placement of *Clitopilus perexiguus* and *C. dilectissimus*. Taxon names, type status, collection or voucher codes, iNaturalist or Mushroom Observer observation numbers (where applicable), geographical origins and GenBank accession numbers for ITS and LSU sequences are provided. Where no collection or voucher code is clear from an iNaturalist or Mushroom Observer observation that was not uploaded to GenBank, the respective observation number is used instead. A dash in the ITS or LSU columns indicates that no GenBank accession number was available. E, I and H stand respectively for epitype, isotype and holotype /

Tabella 1. Raccolte e sequenze incluse nelle analisi filogenetiche per esaminare la collocazione di *Clitopilus perexiguus* e *C. dilectissimus*. Vengono forniti i nomi dei taxa, lo status di typus, i codici di raccolta o di campione, i numeri di osservazione iNaturalist o Mushroom Observer (dove applicabili), le origini geografiche e i numeri di accesso GenBank per le sequenze ITS e LSU. Qualora da un'osservazione su iNaturalist o Mushroom Observer non depositata in GenBank, non risulti chiaro alcun codice di raccolta o di campione, viene utilizzato al suo posto il rispettivo numero di osservazione. Un trattino nelle colonne ITS o LSU indica che nessun numero di accesso a GenBank è disponibile. E, I e H stanno rispettivamente per epitipo, isotipo e olotipo.

Figure 1. Maximum-likelihood phylogram showing the phylogenetic placement of *Clitopilus perexiguus* and *C. dilectissimus*, inferred from the concatenated ITS1, 5.8S, ITS2 and LSU dataset. Branch-support values are shown above the branches as BS/PP. Values below 70% BS and 0.90 PP are not shown. The *C. dilectissimus* label is shown in green and that of *C. perexiguus* is shown in red. Bayesian posterior probabilities were mapped onto the corresponding nodes of the ML tree. Two *Lyophyllum* sequences were used as the outgroup. The scale bar indicates the expected number of substitutions per site /

Figura 1. Filogramma di massima verosimiglianza che mostra il posizionamento filogenetico di *Clitopilus perexiguus* e *C. dilectissimus*, dedotto dall'insieme di dati concatenato di ITS1, 5.8S, ITS2 e LSU. I valori di supporto dei nodi sono riportati sopra i rami come BS/PP. I valori inferiori a 70% per BS e 0,90 per PP non sono mostrati. Il nome di *C. dilectissimus* è evidenziato in verde e quello di *C. perexiguus* in rosso. Le probabilità posteriori bayesiane sono state poste sui nodi corrispondenti dell'albero ML. Due sequenze di *Lyophyllum* sono state utilizzate come gruppo esterno. La barra di scala indica il numero atteso di sostituzioni per sito.

Taxonomy

Clitopilus perexiguus Voto sp. nov. [MB 864869] (Fig. 2-3)

Holotype. Italy, Veneto, Venice, S. Anna di Chioggia, Bosco Nordio, gregarious among moss on sandy substrate, 6 November 2024, *P. Voto* (PAD H0064020*)*, GB PZ782067 (ITS).

Etymology. The name refers to the very small size of the basidiomes and comes from the Latin adjective *perexiguus*, composed from the intensive prefix *per* ("very", "completely") and the adjective *exiguus* ("scanty", "small").

Figure 2. *Clitopilus perexiguus*. Holotypus in habitat / Figura 2. *Clitopilus perexiguus*. Olotipo in habitat

Macroscopic characters

Pileus 2.5–3.7 mm broad, convex with an up to 0.6 mm deep umbilicate centre, extreme margin sulculate; wholly white, subglabrous.

Lamellae 21–23, usually intermixed with 1 lamellula, arcuate-decurrent, white; edge concolorous.

Stipe 4.7–5.3 × 0.35–0.4 mm, cylindric, bent upward due to geotropism, white, subglabrous.

Context not analysed.

Microscopic characters

Spores (7.5)7.6–8.8(9.3) × (3.2)3.5–4.3(4.9) µm, on average 8.3 × 3.9 µm, Q = (1.93)1.98–2.33(2.51), on average 2.17; in front view oblong to elliptic, in side view more or less narrowly amygdaliform; hyaline-pale, ridges not or only doubtfully perceptible under the light microscope; apiculus distinct, germ pore absent.

Basidia 20–30 × 7–9 µm, clavate to cylindric-clavate, 4-spored, sterigmata up to 5 µm long.

Hymenial trama irregular.

Melzer's reagent on spores, basidia and hymenial trama yellowish green.

Cheilocystidia and *Pleurocystidia* not found.

Pileipellis a cutis of somewhat interwoven to flexuous hyphae 2.5–6.0 µm broad, with occasional diverticules or nodules up to 4 µm long; pigment inconspicuous; hyphae locally covered with roundish to elongate crystals; present some pileocystidioid, cylindraceous to tapering, emerging elements 12–21 × 5–5.5 µm.

Caulopellis a cutis of parallel hyphae 3.5–4.0 µm broad, locally covered with roundish to elongate crystals.

Caulocystidia 15–50 × 2.0–7.5 µm, mostly cylindrical to flexuously cylindraceous, sometimes lageniform.

Clamp connections absent.

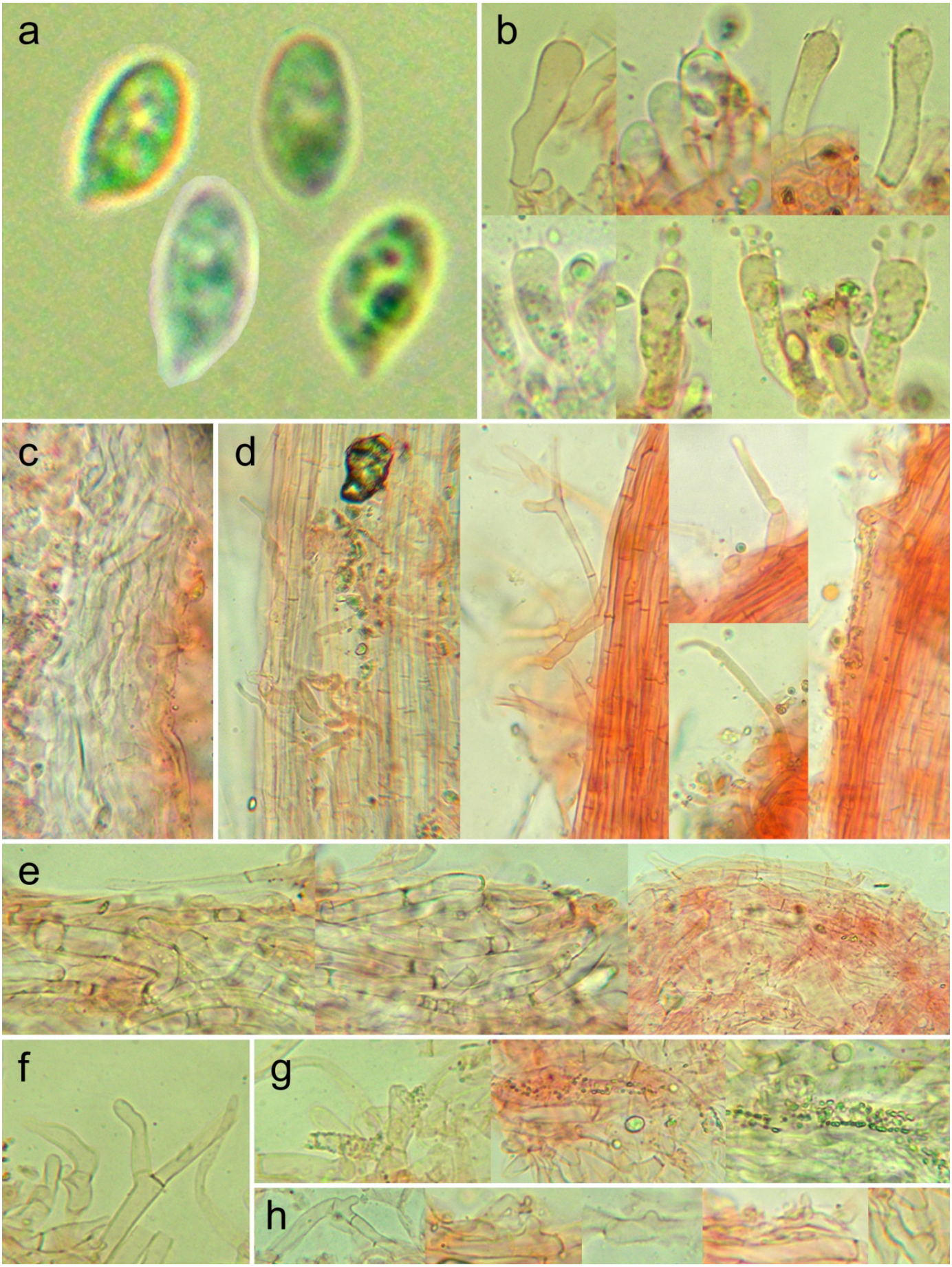

Figure 3. *Clitopilus perexiguus*. a) spores, b) basidia, c) hymenial trama, d) caulopellis and caulocystidia, e) pileipellis, f) pileocystidioid elements, g) crystals on pileipellis, h) nodules and diverticules on pileipellis /
Figura 3. *Clitopilus perexiguus*. a) spore, b) basidi, c) trama imeniale, d) caulopellis e caulocistidi, e) pileipellis, f) elementi pileocistidioidi, g) cristalli nella pileipellis, h) noduli e diverticoli in pileipellis

NOTES

The discovery of two new species of *Clitopilus* (Fr. ex Rabenh.) P. Kumm. within a relatively small wooded area (70 ha of the woodland is open to the public, while the remaining 43 ha is closed to the public and completely undisturbed) is noteworthy. It may indicate that small and inconspicuous members of the genus remain under-recorded in Mediterranean habitats.

Ridgeless to scarcely ridged spores (no spore could be observed in polar view), a centrally well-developed stipe and an umbilicate pileus, together with extremely small basidiomes, make *Clitopilus perexiguus* peculiar within the genus. Even so, some taxa need to be compared with it. In particular, we refer to the species belonging to *Clitopilus* sect. *Omphaloidei*, a section characterised by smooth spores and an omphalioid habit ('*Species sporis laevibus habitusque omphaloideus praeditis*') and currently comprising, in Europe, *Clitopilus giovanellae* and *Clitopilus albominutellus* (Moreno *et al*. 2007).

Clitopilus giovanellae is distinguished by somewhat larger basidiomes (pileus 5–15 mm broad, stipe 10–20 × 1–2 mm) and somewhat shorter spores [5–7.5(8) × 3–4 µm, on average 6.4 × 3.8 µm] with a consequently lower Q value, in the range 1.6–2.0, on average 1.8 (Moreno *et al.* 2007).

Clitopilus albominutellus, notwithstanding its name, has somewhat larger basidiomes (pileus 4–12 mm broad, stipe 8–18 × 0.5–1.5 mm) and shorter spores (4.8–7.0 × 3.5–4.2 µm) with a Q value which, although not provided by the authors, can be inferred to be lower based on the reported spore dimensions (Musumeci & Contu 2018).

Among the older species still lacking representative sequences, we found *Clitopilus minimus* (Murrill) Murrill 1942, described from Florida (USA) on a lawn, and *Clitopilus minutus* Herp. 1912, described from Germany on potting soil in a garden, whose names evoke basidiomes of a very small size. The former is reported, as *Pleurotus minimus* Murrill, with smaller, ovoid or ellipsoid spores (6 × 4 µm) with a consequently lower Q value (Murrill 1942); while the latter has a dull yellow to fuliginous ('*e sordido-flavo fuliginoso*') pileus and broader (7–8 µm), angular-spherical ('*anguloso-sphaerici*') spores (Herpell 1912).

Our phylogenetic analyses recovered *Clitopilus perexiguus* as sister to a clade containing two North American collections provisionally identified as *Clitopilus* sp. CA04. The clade comprising all three collections received maximal support. Although the analyses support *C. perexiguus* as a distinct lineage relative to the named species included in the dataset, it was represented by only a single collection and a single ITS sequence. Its monophyly and intraspecific sequence variation therefore could not be assessed. Furthermore, because only ITS sequences were available for *C. perexiguus* and the two North American *Clitopilus* sp. CA04 collections, their strongly supported relationship is based solely on this locus.

The collection *Clitopilus* sp. CA04 HAY-F-003655 (GB OR860203; iNat 167291390), from California, was reported from a rock crevice on bare ultramafic soil, with each basidiome attached to a small piece of rock embedded in red clay. This nutrient-poor habitat may indicate some ecological similarity to the mossy, sandy substrate of *C. perexiguus*. The basidiomes are bright white, with minutely fuzzy or scurfy pilei, ruffled margins at maturity, white wavy-decurrent lamellae and white, central stipes with slight basal tomentum. However, no measurements were provided, and the pileus shape cannot be assessed confidently from the available images.

The second collection, *Clitopilus* sp. CA04 MYCO-1010094 (iNat 171428792), from Nebraska, was reported as locally abundant on woodland debris in open mixed oak–hickory woodland. The photographs show small, white, centrally-stiped basidiomes, several with apparently depressed pilei, while the accompanying notes describe decurrent lamellae, undulating pileus margins in older specimens and strong, elastic stipes.

No information on spore ornamentation or size is available for either collection.

These North American collections may represent *C. perexiguus* or a closely related taxon, but further macro- and micromorphological data, together with sequences from additional loci, are required to determine their taxonomic identity.

Clitopilus dilectissimus Voto *MycolObs* 12:46 (2025) (Fig. 4-5)

In the protologue (Voto 2025), the pileus is erroneously described as 7–1.8 mm wide, whereas the correct width is 7–18 mm.

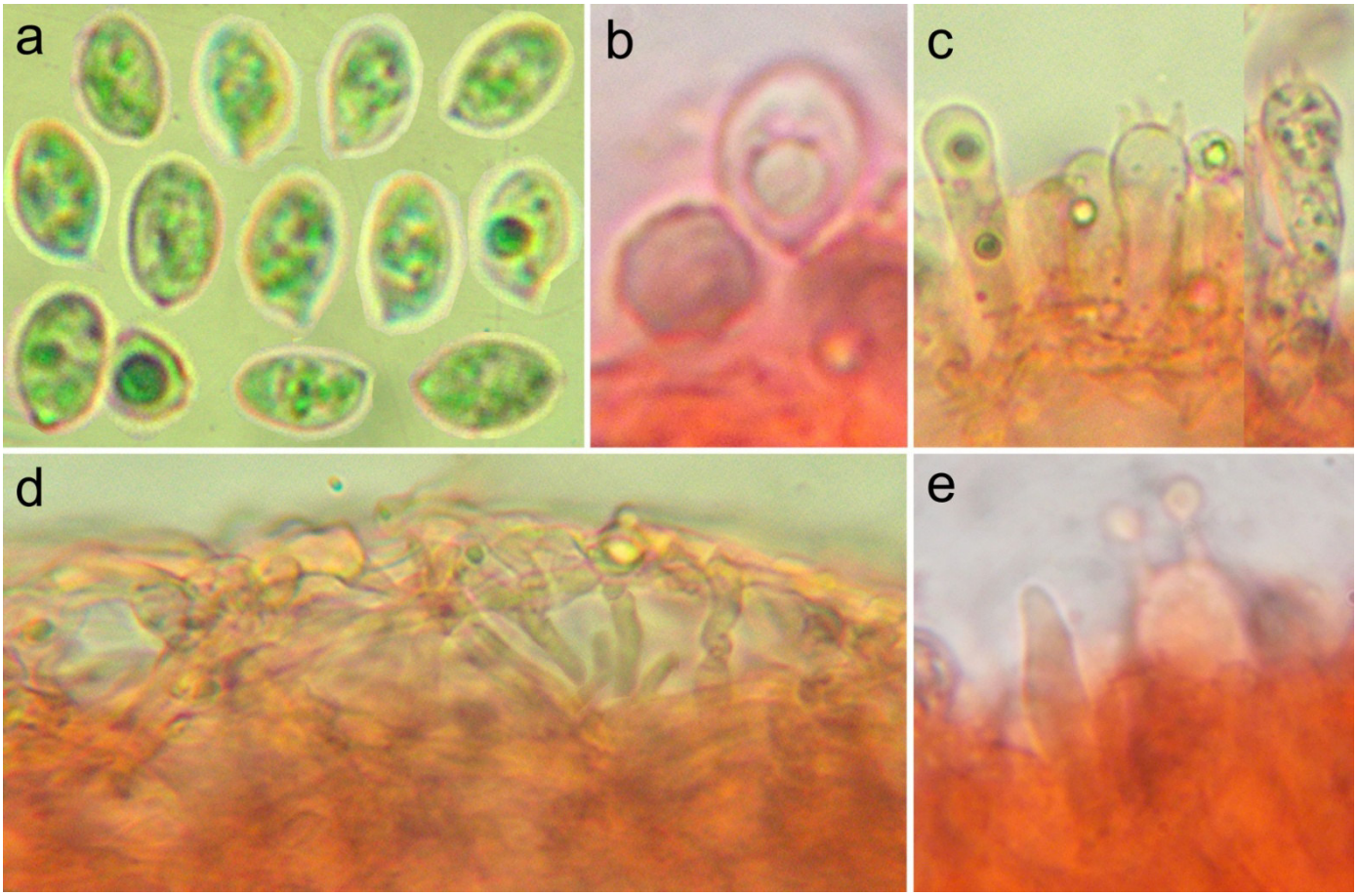

Figure 4. *Clitopilus dilectissimus*. a) spore, b) a spore viewed in polar projection, c) basidia, d) pileipellis, e) an occasional fusiform-lageniform cystidiole / **Figura 4**. *Clitopilus dilectissimus*. a) spore, b) spora in proiezione polare, c) basidi, d) pileipellis, e) un occasionale cistidiolo fusiforme-lageniforme

Our phylogenetic analyses further support the novel status of *C. dilectissimus* proposed by Voto (2025) principally because it is not nested among collections assigned to any single formally named species represented in the dataset, but rather in a clade predominantly containing numerous collections assigned the provisional species code *C. scyphoides*-IN01 (and collections given two other names, which can be differentiated on morphological grounds, as shown below).

In the original description (Voto 2025), the identification of the species was considered to be facilitated by its coprophilous ecology which made it comparable with the only other coprophilous species then known in the genus, *Clitopilus passeckerianus* (Pilát) Singer. This taxon, originally described as *Pleurotus passeckerianus* Pilát, has somewhat larger basidiomes with the pileus 8–40 mm large (Pilát 1935). In the present analyses, *C. dilectissimus* was phylogenetically distinct from the well-supported clade formed by collections identified by Dr. Passecker as *C. passeckerianus*.

Several of the sequence-matched North American observations (code *C. scyphoides*-IN01) resemble *C. dilectissimus* in their small, white, pleurotoid basidiomes with reduced lateral stipes. They differ ecologically in having been reported principally as mycophagous from polypore basidiomes [*Cerioporus squamosus* (Fr.) Quél., twice; *Ganoderma tsugae* Murrill; *Meripilus sp*.? and *Trametes sp*. among the identified hosts] or wood rather than dung. Unless future studies refute the conspecificity we propose, these observations indicate broader preferences and geographical distribution than originally documented for *C. dilectissimus*.

Clitopilus scyphoides (Fr.) Singer, which according to our phylogenetic analyses is quite distinct from *C. dilectissimus* and other closely related collections, has a well-developed, though possibly eccentric, stipe, and a generally terrestrial growth; while *Clitopilus scyphoides* var. *intermedius* (Romagn.) Noordel., which has a fruity odour, exhibits a central stipe and longer (7.0–10.0 × 3.5–5.0 µm) spores (Noordeloos 1988).

Clitopilus scyphoides f. *reductus*, though sharing a strongly reduced and lateral stipe, differs in having 6–8-angled, shorter spores [6.0–7.0(8.1) × 3.5–4.2 µm] with a lower Q value, in the range 1.4–2.0, on average 1.7(Noordeloos 1984).

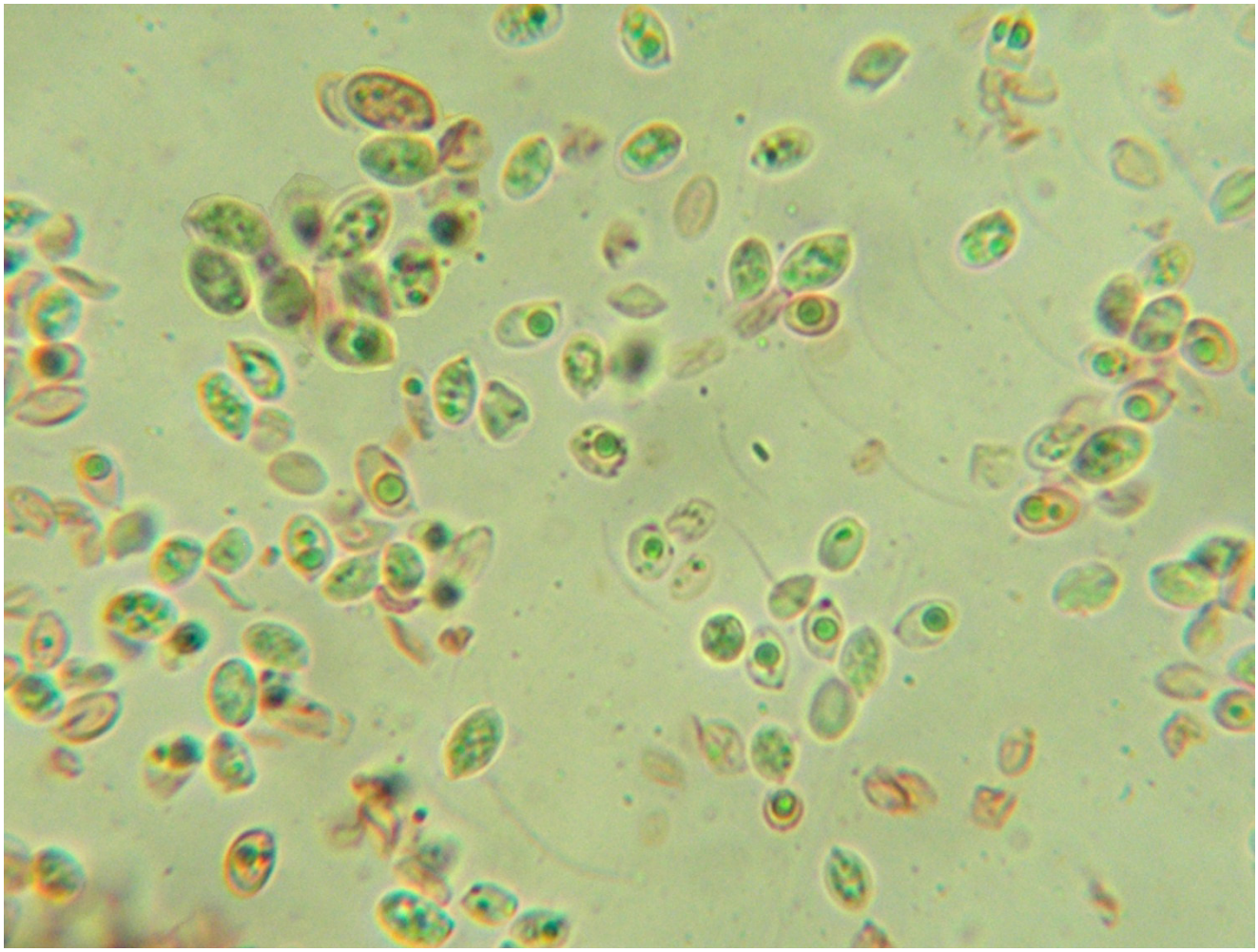

Figure 5. *Clitopilus dilectissimus*. Overview of spores / **Figura 5**. *Clitopilus dilectissimus*. Panoramica delle spore

Clitopilus hobsonii, otherwise macromorpholgically similar, differs in having broader spores, 5.0–5.5(6.0) µm (Noordeloos 1988).

ACKNOWLEDGMENTS

We would like to thank Joshua Birkebak for reviewing and providing helpful comments on the article.

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Clitopilus perexiguus sp. nov. dal Bosco Nordio e *Clitopilus dilectissimus* Parte II

Key words:

Agaricales
Entolomataceae
nuova specie
Italia
ITS
LSU
Mediterraneo
tassonomia
Venezia

Riassunto: Viene riportata la nuova specie *Clitopilus perexiguus* dalla Riserva Naturale del Bosco Nordio. Analisi morfologiche e filogenetiche supportano lo status di novità tassonomica di *C. perexiguus* che si caratterizza per le spore lisce e i basidiomi molto piccoli, bianchi, e provvisti di un cappello ombelicato e un gambo centrale pienamente sviluppato. Si espande inoltre il trattamento tassonomico e si fornisce un'analisi filogenetica di *C. dilectissimus*, che è stato recentemente descritto dallo stesso bosco. *C. dilectissimus* viene confrontato con taxa morfologicamente simili e viene collocato in un clado coerente (sebbene solo marginalmente supportato) che comprende numerosi campioni nordamericani provvisoriamente identificati come *C. scyphoides*-IN01, insieme a diverse raccolte di *Clitopilus* indeterminate o misidentificate. Sulla base della loro alta similarità ITS e del posizionamento filogenetico, queste raccolte vengono qui interpretate come *C. dilectissimus*, sostanzialmente estendendo l'intervallo geografico ed ecologico noto della specie. Saranno tuttavia necessari ulteriori loci per definire con maggiore robustezza le relazioni all'interno di questo raggruppamento.

INTRODUZIONE

Il Bosco Nordio è un area boschiva protetta localizzata in prossimità del litorale Adriatico in nord Italia, 30 km circa a sud di Venezia. Esso è principalmente caratterizzato da una formazione orno-lecceta (*Fraxino orni-Quercion ilicis*) con presenza anche di *Populus alba*, *Populus tremula*, *Quercus robur*, *Quercus pubescens*, *Pinus pinaster*, *Juniperus communis*, etc., cresciuta su dune fossili e basata su un substrato sabbioso calcareo (Voto 2021).

Il genere *Clitopilus* (Fr. ex Rabenh.) contiene 354 or 390 nomi nel mondo (fide Index Fungorum e MycoBank, rispettivamente), includendo autonimi, sinonimi e taxa infragenerici. Esso comprende diverse specie lamellate della famiglia *Entolomataceae* Kotl. & Pouzar con un'ecologia saprotrofa. La polvere sporale è rosa; le lamelle mature sono solitamente decorrenti e rosastre; il gambo può essere centrale o eccentrico oppure assente o ridotto; le spore sono solitamente angolose, esibendo per lo più distinte creste longitudinali; e i basidiomi di molte specie hanno un odore farinaceo. *Clitopilus* è stato oggetto di importanti cambiamenti tassonomici in epoca moderna determinati da studi filogenetici molecolari che ne hanno ridefinito la delimitazione, accorpando diversi taxa precedentemente attribuiti a *Rhodocybe* Maire (Co-David, Langeveld & Noordeloos 2009).

Anche la sua specie tipo, *Clitopilus prunulus* (Scop.) P. Kumm. (basionimo *Agaricus prunulus* Scop.), è presente nel Bosco Nordio (repertata una volta da P. V.). Una quarta specie del genere, nuova per lo stesso bosco, sarà documentata in un prossimo contributo.

MATERIALI E METODI

Studio morfologico

Le descrizioni macroscopiche sono state ottenute su materiale fresco appena dopo la raccolta. Le fotografie sono state scattate con una fotocamera Nikon Coolpix A10. Tulle le misurazioni micromorfologiche sono state effettuate su materiale fresco montato in acqua o rosso Congo. Le misure sporali sono riportate secondo la seguente formula: (lunghezza minore) 10° percentile–90° percentile (lunghezza maggiore) × (larghezza minore) 10° percentile–90° percentile (larghezza maggiore). Nel caso di *Clitopilus perexiguus*, avendo rilevato una notevole differenza tra le misurazioni sporali effettuate sull'imenio e sul gambo, vengono incluse nella descrizione solo le spore misurate sul gambo. Tutte le immagini di P.V.

GB sta per numero di accesso GenBank, mentre iNat e MO stanno per numeri di osservazione di iNaturalist e Mushroom Observer, rispettivamente.

Studio molecolare

L'estrazione del DNA, l' amplificazione della PCR e il sequenziamento Sanger sono stati eseguiti da laboratori commerciali per generare una sequenza LSU (GB PZ782068) dalla raccolta olotipica di *Clitopilus dilectissimus* (PAD H0062396) e una sequenza ITS (GB PZ782067) dalla raccolta olotipica di *C. perexiguus* (PAD H0064020).

Sequenze di confronto rappresentative di ulteriori 96 collezioni sono state ottenute da GenBank, iNaturalist e Mushroom Observer. Le sequenze affini sono state identificate tramite NCBI BLAST (Altschul *et al*. 1990) e MycoBLAST di MycoMap (https://mycomap.org/mycoblast). L'insieme di dati include inoltre la sequenza ITS del typus di *C. dilectissimus*, già pubblicata in precedenza (PV661559; Voto 2025). Sono state condotte cinque ricerche di similarità: tre tramite NCBI BLAST, utilizzando come ricerca (*query*) le sequenze ITS di *C. dilectissimus* e *C. perexiguus* e la sequenza LSU di *C. dilectissimus*, e due tramite MycoBLAST, impiegando come sequenza di ricerca le sequenze ITS di *C. dilectissimus* e *C. perexiguus*. Due sequenze di *Lyophyllum* P. Karst. sono state utilizzate come gruppo esterno (*outgroup*). L'insieme di dati concatenato finale comprende 98 raccolte, rappresentate da 98 sequenze ITS e 48 sequenze LSU. Le regioni mancanti sono state codificate come lacune (*gaps*). Le regioni ITS1, 5.8S e ITS2 sono state estratte dalle sequenze ITS mediante l'applicativo ITSx v. 1.1.3 (Bengtsson-Palme *et al.* 2013). Queste tre regioni, unitamente alla regione LSU, sono state allineate separatamente con MAFFT v. 7 (Katoh & Standley 2013) applicando l'algoritmo L-INS-i. Gli allineamenti sono stati ispezionati e ritagliati tramite UGENE v. 53.1 (Okonechnikov *et al.* 2012) e successivamente concatenati in un unico insieme di dati partizionato. L'insieme di dati combinato finale consta di 1563 caratteri, incluse le

lacune. L'insieme di dati è stato concatenato e partizionato per locus (ITS1: 1–258; 5.8S: 259–416; ITS2: 417–633; LSU: 634–1563).

L'analisi di massima verosimiglianza (ML) è stata eseguita utilizzando IQ-TREE versione 3 (Wong *et al*. 2026). I modelli di sostituzione ottimali (TVM+G4 per ITS1, F81 per 5.8S, TPM2u+G4 per ITS2 e HKY+F+R3 per LSU) sono stati selezionati tramite il ModelFinder integrato di IQ-TREE (Kalyaanamoorthy *et al.* 2017; Wong *et al*. 2026). Il supporto dei nodi è stato stimato mediante 1.000 repliche di *ultrafast-bootstrap*. L'inferenza bayesiana (BI) è stata condotta con MrBayes versione 3.2.7 (Ronquist *et al.* 2012). Le regioni ITS1, 5.8S e ITS2 sono state combinate e trattate come un'unica partizione ITS, mentre la regione LSU ha costituito la seconda partizione. A ciascuna partizione è stato assegnato un modello GTR+G parametrizzato in modo indipendente. L'analisi è stata impostata con due corse (*run*) indipendenti, ciascuna configurata con quattro catene di Markov e 2.000.000 di generazioni. Gli alberi sono stati campionati ogni 1.000 generazioni, scartando il primo 25% come *burn-in*. Gli alberi risultanti sono stati visualizzati in iTOL (Letunic & Bork 2019) e l'albero ML è stato annotato con Canva (Canva Pty Ltd.).

RISULTATI

Analisi filogenetiche

Le topologie degli alberi di massima verosimiglianza (ML) e di inferenza bayesiana (BI) sono ampiamente congruenti; pertanto, viene presentato esclusivamente l'albero ML con i valori di BI posti sui nodi corrispondenti. I rami con supporto di bootstrap (BS) e valori di probabilità a posteriori (PP) rispettivamente ≥70% e ≥0.90 sono indicati in corrispondenza dei nodi.

Entrambe le analisi ML e BI collocano *Clitopilus dilectissimus* all'interno di un clado contenente numerosi campioni assegnati al codice di specie provvisorio *C. scyphoides*-IN01, insieme a due campioni denominati *C. scyphoides* f. *reductus* Noordel., uno denominato *C. hobsonii* (Berk.) P.D. Orton e due campioni non identificati di *Clitopilus*. Il ramo basale che unisce *C. dilectissimus* a questi campioni ha ottenuto un supporto solo marginale in ciascuna analisi (BS 67%, PP 0.80); tuttavia, la medesima relazione è emersa sia nelle analisi finali ML e BI sia in molteplici analisi esplorative preliminari, condotte utilizzando differenti modelli evolutivi e di campionamento e strategie di allineamento. Inoltre, le sequenze ITS di *Clitopilus scyphoides*-IN01 sono altamente simili (fino al 100%) alla sequenza ITS del typus di *C. dilectissimus*. Nel complesso, ciò indica che l'appartenenza di *C. dilectissimus* a questo clado è verosimilmente reale, nonostante il supporto marginale riscontrato nella nostra analisi filogenetica. Tale supporto marginale potrebbe riflettere una risoluzione insufficiente all'interno dell'insieme di dati ITS+LSU. Ulteriori loci codificanti per proteine, in particolare *rpb2* e *tef1-α*, permetterebbero di testare questa relazione in modo più robusto. Complessivamente, suggeriamo che *Clitopilus scyphoides*-IN01 corrisponda a *C. dilectissimus*.

Clitopilus perexiguus è risultato affine (*sister*) a un clado comprendente due campioni nordamericani non denominati, assegnati al codice di specie provvisorio "Clitopilus *sp*. CA04" (HAY-F-003655 e MYCO-1010094). Il clado comprendente *C. perexiguus* e i due campioni di *C. sp.* CA04 ha ricevuto il massimo supporto in entrambe le analisi (BS 100%, PP 1.00). Tale clado è risultato a sua volta in posizione affine rispetto a due campioni cinesi non denominati (FJAU75563 e FJAU75564), con un supporto massimo (100%/1.00). Inoltre, *C. perexiguus* è risultato incluso in un clado più ampio comprendente anche *C. albominutellus* (E. Ludw.) Musumeci & Contu e *C. giovanellae* (Bres.) Singer, specie attribuite a *Clitopilus* sect. *Omphaloidei* G. Moreno, Contu, A. Ortega, Platas & Peláez (Musumeci & Contu 2018).

Tassonomia

Clitopilus perexiguus Voto sp. nov. (Fig. 2-3)

Olotipo. Italia, Veneto, Venezia, S. Anna di Chioggia, Bosco Nordio, gregario tra muschio su substrato sabbioso, 6 novembre 2024, *P. Voto* (PAD H0064020), GB PZ782067 (ITS).

Etimologia. Il nome si riferisce alla dimensione molto piccola dei basidiomi e deriva dall'aggettivo latino *perexiguus*, composto dal prefisso intensivo *per* ("molto", "completamente") e l'aggettivo *exiguus* ("scarso", "piccolo").

Caratteri macroscopici

Cappello 2.5–3.7 mm diam., convesso con un ombelico al centro profondo fino a 0.6 mm, estremo margine sulculato; completamente bianco, subglabro.

Lamelle 21–23, solitamente intramezzate da 1 lamellula, arcuate-decorrenti, bianche; filo concolore.

Gambo 4.7–5.3 × 0.35–0.4 mm, cilindrico, geniculato per geotropismo, bianco, subglabro.

Carne non analizzata.

Caratteri microscopici

Spore (7.5)7.6–8.8(9.3) × (3.2)3.5–4.3(4.9) µm, in media 8.3 × 3.9 µm, Q = (1.93)1.98–2.33(2.51), in media 2.17; in vista frontale da oblunghe a ellittiche, in vista laterale più o meno strettamente amigdaliformi; ialine-pallide, creste non o solo dubbiosamente percepibili al microscopio ottico; apicolo distinto, poro germinativo assente.

Basidi 20–30 × 7–9 µm, da clavati a cilindro-clavati, 4-sporici, sterigmi lunghi fino a 5 µm.

Trama imeniale irregolare.

*Reagent*e di *Melzer* su spore, basidi e trama imeniale verde giallastro.

Cheilocistidi e *Pleurocistidi* non trovati.

Pileipellis una cutis di ife un po' intrecciate o flessuose, larghe 2.5–6.0 µm, con occasionali diverticoli o noduli lunghi fino a 4 µm; pigmento incospicuo; ife localmente ricoperte di cristalli tondeggianti o allungati; presenti alcuni elementi emergenti pileocistidioidi, da cilindracei a rastremati, 12–21 × 5–5.5 µm.

Caulopellis una cutis di ife parallele larghe 3.5–4.0 µm, localmente ricoperte di cristalli tondeggianti o allungati.

Caulocistidi 15–50 × 2.0–7.5 µm, per lo più da cilindrici a flessuosamente cilindracei, a volte lageniformi.

Giunti a fibbia assenti.

NOTE

La scoperta di due nuove specie di *Clitopilus* (Fr. ex Rabenh.) P. Kumm. all'interno di un'area boschiva relativamente piccola (70 ettari del bosco sono aperti al pubblico, mentre altri 43 ettari sono inaccessibili e completamente indisturbati) è meritevole di nota. Potrebbe indicare che membri piccoli e incospicui del genere sono ancora poco rilevati negli ambienti mediterranei.

Spore prive di creste o scarsamente crestate (nessuna spora osservata in vista polare), il gambo centrale ben sviluppato e il cappello ombelicato, insieme a basidiomi estremamente piccoli, rendono *Clitopilus perexiguus* peculiare all'interno del genere. Anche così, alcuni taxa necessitano di essere confrontati con esso. In particolare, ci riferiamo alle specie appartenenti a *Clitopilus* sect. *Omphaloidei*, una sezione caratterizzata da spore lisce e un habitus omphalioide ('*Species sporis laevibus habitusque omphaloideus praeditis*') e che attualmente comprende, in Europa, *Clitopilus giovanellae* e *Clitopilus albominutellus*, (Moreno *et al*. 2007).

Clitopilus giovanellae si distingue per basidiomi un po' più grandi (cappello 5–15 mm diam., gambo 10–20 × 1–2 mm) e spore un po' più corte [5.0–7.5(8.0) × 3.0–4.0 µm, in media 6.4 × 3.8 µm] con un valore di Q conseguentemente più basso, nell'intervallo 1.6–2.0, in media 1.8 (Moreno *et al.* 2007).

Clitopilus albominutellus, malgrado il suo nome, ha basidiomi un po' più grandi (cappello 4–12 mm diam., gambo 8–18 × 0.5–1.5 mm), oltre a spore più corte (4.8–7.0 × 3.5–4.2 µm) con un valore di Q che, quantunque non fornito dagli autori, si deduce essere più basso in base alle dimensioni sporali riportate (Musumeci & Contu 2018).

Tra le specie di più vecchia data che ancora mancano di sequenze rappresentative, abbiamo trovato *Clitopilus minimus* (Murrill) Murrill 1942, descritta dalla Florida (USA) su un prato, e *Clitopilus minutus* Herp. 1912, descritta dalla Germania su terriccio da vaso in un giardino, i cui nomi evocano basidiomi di dimensione minuta. La prima è riportata, come *Pleurotus minimus* Murrill, con spore più piccole, ovoidali o ellissoidali (6 × 4 µm) e conseguentemente con un valore di Q più basso (Murrill 1942); mentre l'altra ha un cappello da giallo spento a fuligginoso ('*e sordido-flavo fuliginoso*') e spore più larghe (7–8 µm), angolose-sferiche ('*anguloso-sphaerici*') (Herpell 1912).

Nelle nostre analisi filogenetiche *Clitopils perexiguus* è risultato affine a un clado contenente due raccolte nordamericane provvisoriamente identificate come *Clitopilus* sp. CA04. Il clado comprendente tutte e tre le

raccolte ha ricevuto massimo supporto. Sebbene le analisi supportino *C. perexiguus* come una distinta linea evolutiva rispetto alle specie incluse nella tabella dati, esso è rappresentato solo da una singola raccolta e una singola sequenza ITS. Pertanto, non è stato possibile valutarne la monofilia e la variabilità intraspecifica di sequenza. Inoltre, poiché erano disponibili solo sequenze ITS per *C. perexiguus* e le due raccolte nordamericane 'Clitopilus sp. CA04', la loro relazione fortemente supportata si basa unicamente su questo locus.

La raccolta *Clitopilus* sp. CA04 HAY-F-003655 (GB OR860203; iNat 167291390), dalla California, è segnalata da una fessura rocciosa su suolo ultramafico nudo, con ciascun basidioma attaccato a un piccolo frammento di roccia inglobato in argilla rossa. Questo habitat povero di nutrienti potrebbe segnalare qualche similarità ecologica con il substrato muschioso e sabbioso di *C. perexiguus*. I basidiomi sono di colore bianco brillante, con cappelli finemente vellutati o squamulosi, margini ondulati a maturità, lamelle bianche e ondulate-decorrenti, e gambi bianchi e centrali con un leggero tomento basale. Comunque, non viene fornita alcuna misurazione, e la forma del cappello non può essere valutata con certezza attraverso le immagini disponibili.

La seconda raccolta, *Clitopilus* sp. CA04 MYCO-1010094 (iNat 171428792), dal Nebraska, è localmente abbondante su detriti forestali in boschi aperti misti *Quercus*-*Carya*. Le foto mostrano basidiomi piccoli, bianchi, a gambo centrale, diversi con cappelli apparentemente depressi; mentre le note di accompagnamento descrivono lamelle decorrenti, margini pileici ondulati in vecchi esemplari, e gambi solidi, elastici.

Nessuna informazione su ornamentazioni e dimensioni sporali è disponibile per nessuna delle due raccolte.

Queste raccolte nordamericane potrebbero rappresentare *C. perexiguus* o un taxon strettamente correlato, ma ulteriori dati macro- e micromorfologici, insieme a sequenze di ulteriori loci, sono necessari per determinare la loro identità tassonomica.

Clitopilus dilectissimus Voto *MycolObs* 12:46 (2025) (Fig. 4-5)

Nel protologo (Voto 2025), il cappello è erroneamente descritto largo 7–1.8 mm, mentre la larghezza corretta è di 7–18 mm.

Le nostre analisi filogenetiche forniscono ulteriore supporto allo status di nuova specie di *C. dilectissimus* proposto da Voto (2025) principalmente perché non è annidato tra raccolte assegnate ad alcuna specie formalmente valida rappresentata nella tabella dati, ma si annida in un clado principalmente contenente numerose raccolte cui è stato assegnato il codice di specie provvisorio *C. scyphoides*-IN01 (e altre raccolte cui sono stati dati due altri nomi e che possono essere differenziate su basi morfologiche, come discusso di seguito).

Nella descrizione originale (Voto 2025), l'identificazione della specie era ritenuta facilitata dalla sua ecologia coprofila che la rendeva comparabile solo con l'unica altra specie coprofila allora nota nel genere, *Clitopilus passeckerianus* (Pilát) Singer. Questo taxon, originariamente descritto come *Pleurotus passeckerianus* Pilát, ha basidiomi un po' più grandi, con cappello largo 8–40 mm (Pilát 1935). Nelle presenti analisi, *C. dilectissimus* risulta filogeneticamente distinto dal clado ben supportato formato da raccolte identificate dal Dr. Passecker come *C. passeckerianus*.

Diverse osservazioni nordamericane con sequenze corrispondenti (codice *C. scyphoides*-IN01) somigliano a *C. dilectissimus* nei loro piccoli basidiomi bianchi, pleurotoidi, con gambi laterali ridotti. Essi differiscono dal punto di vista ecologico per essere segnalati principalmente come micofagi su basidiomi di polipori [*Cerioporus squamosus* (Fr.) Quél., due volte; *Ganoderma tsugae* Murrill; *Meripilus sp*.? e *Trametes sp*. tra gli ospiti identificati] o su legno piuttosto che su sterco. A meno che studi futuri non confutino la conspecificità che proponiamo, queste osservazioni indicano per *C. dilectissimus* una più ampia preferenza ecologica e distribuzione geografica di quanto originariamente documentato.

Clitopilus scyphoides (Fr.) Singer, che in accordo con le nostre analisi filogenetiche è del tutto distinto da *C. dilectissimus* e altre raccolte strettamente correlate, ha un gambo ben sviluppato, sebbene possibilmente eccentrico, e una crescita generalmente terricola; mentre *Clitopilus scyphoides* var. *intermedius* (Romagn.) Noordel., che ha un odore fruttato, esibisce un gambo centrale e spore più allungate, 7.0–10.0 × 3.5–5.0 µm (Noordeloos 1988).

Clitopilus scyphoides f. *reductus*, sebbene condivida il gambo fortemente ridotto e laterale, differisce per avere spore con 6-8 angoli, più corte [6.0–7.0(8.1) × 3.5–4.2 µm] e con un valore di Q più basso, nell'intervallo 1.4–2.0, in media 1.7 (Noordeloos 1984).

Clitopilus hobsonii, altrimenti macromorfolgicamente similare, differisce per avere spores più larghe, 5.0–5.5(6.0) µm (Noordeloos 1988).

RINGRAZIAMENTI

Ringraziamo Joshua Birkebak per la revisione e i preziosi suggerimenti forniti sull'articolo.