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## Contributions to the revision of the genus *Entoloma* (Basidiomycota, Agaricales) in Europe: six new species from subgenus *Cyanula* and typification of *E. incarnatofuscescens*

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**Abstract:** In anticipation of a phylogenetically revised monograph of *Entoloma* in Europe, six new species of subgenus *Cyanula* are described here. *Entoloma cistocruentatum* is associated with *Cistus* in Spain, *E. dislocatum* occurs in montane regions in Catalonia (Spain) and Tuscany (Italy), *E. indikon* is known from Denmark and three species are mainly distributed in the Nordic countries in Europe: *E. calceus*, *E. perchalybeum* and *E. praecipuum*. *Entoloma incarnatofuscescens*, from the /Rusticooides clade is neotypified. A fully amended description is given based on molecular evidence, which includes the recently described *E. violaceoparkensis* and *E. klofacianum* which became later synonyms.

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## INTRODUCTION

This study is part of a large-scale molecular phylogenetic and morphological revision of the /*Cyanula* clade of the genus *Entoloma* in Europe to be published in due course (Dima *et al.* in prep.) and a new, completely revisited monograph of all European species of the /*Cyanula* clade (Noordeloos in prep.). The /*Cyanula* clade is here defined in a wide sense, including all clampless, often vividly coloured species, formerly included in subgen. *Leptonia* but shown to be phylogenetically quite distant from the clamped *Leptonia s. str.* taxa (Morozova *et al.* 2014). The material in the present study comes from various sources. In the Nordic countries, much work has been done on *Entoloma*, in the framework of the Norwegian *Entoloma* project and studies of the alpine mycota in Sweden resulting in a constant flow of publications in recent years (Brandrud *et al.* 2018, 2019, Crous *et al.* 2021, Dima *et al.* 2021, Haelewaters *et al.* 2021, Noordeloos *et al.* 2018, 2020). Jordi Vila and collaborators studied the mycota of Spain, Catalonia (Caballero & Vila 2013, Vila & Caballero 2007, 2009, Vila & Llimona 2010, Vila *et al.* 2013, 2014, 2021) which yielded some of the proposed new taxa.

The phylogenetic position of the new species described here will be dealt with in depth in a forthcoming study based on a world-wide sampling of the subgenus *Cyanula* (Dima *et al.* in prep.).

## MATERIAL AND METHODS

### Morphology

All collections studied were photographed in the field and attention was paid in observing the surrounding vegetation and putative ecology for each collection based on above-ground observations. The material was described after collecting to document the ephemeral macroscopic characters (especially colours) and dried and stored in the respective fungaria. Microscopic characters were studied with standard light microscopy methods. Spores, basidia and cystidia were observed in squash preparations of small parts of the lamellae in 5 % KOH or 1 % Congo Red in concentrated NH<sub>4</sub>OH. The pileipellis was examined on a radial section of the pileus in water. Basidiospore

dimensions are based on observing 40 spores in side view, while cystidia and basidia dimensions are based on observing at least 10 structures per collection. Basidia were measured excluding sterigmata and the spores excluding hilum. Spore length to width ratio is reported as 'Q' and average length to width ratio is reported as 'Qav'. All studied material is stored in the herbaria of Oslo (O), Gothenburg (GB), or Leiden (L), unless otherwise indicated.

### DNA extraction and sequencing

Total DNA of most of the samples was extracted from 15–30 mg of dry material, using a NucleoSpin Plant II Mini Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Final elutions were done in a total volume of 100 µL elution buffer. The internal transcribed spacer (ITS) was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). Polymerase chain reaction (PCR) protocols were following Dima *et al.* (2016) and Papp & Dima (2018). The successful PCR amplification was checked on a 1 % agarose gel stained with Ethidium bromide. Sanger sequencing was performed by LGC Genomics (Berlin, Germany) with the same primers used in the PCR amplification. Molecular study of some material was performed in the Norwegian Barcode of Life (NorBOL) or followed Alvarado *et al.* (2015). Chromatograms were checked and edited with the CodonCode Aligner package (CodonCode Corp., Centerville, Massachusetts, USA). Sequence comparison with public and own databases followed Noordeloos *et al.* (2017). Our dataset is composed of 117 nrDNA ITS sequences, carefully selected after an initial analysis using published and all our unpublished ITS sequences (data not shown). Newly generated sequences were submitted to GenBank. The dataset was aligned in MAFFT online v. 7 (<http://mafft.cbrc.jp/alignment/server>) choosing the E-INS-I strategy (Kato & Standley 2013). The alignment was checked and edited in SeaView v. 5 (Gouy *et al.* 2021). Maximum Likelihood (ML) phylogenetic reconstruction was performed in PhyML v. 3.1 (Guindon *et al.* 2010) using the non-parametric, Shimodaira-Hasegawa version of the approximate likelihood-ratio test (SH-aLRT) (Anisimova *et al.* 2011) with the following settings: GTR+I+G model of evolution, gamma distribution of 10 rate categories and tree topology search as SPR. The resulting phylogenetic tree (Fig. 1) was edited in MEGA 7 (Kumar *et al.* 2016) and Adobe Illustrator CS4.

## RESULTS

### Phylogeny

We used a total of 115 *Entoloma* and two *Clitopilus* sequences (as outgroup) for our analysis. The ITS alignment comprises 915 characters including gaps. The resulting phylogenetic tree from the PhyML analysis is shown in Fig 1. All of the species described as new to science or typified in this study received high statistical support in our analysis. Altogether 19 ITS barcode sequences were newly generated for this study (Fig. 1).

### Taxonomy

*Entoloma cistocruentatum* Vila, Noordel. & Dima, *sp. nov.* MycoBank MB 840817 Fig. 2A, D.

*Etymology:* The epithet refers to the morphological similarity with *Entoloma cruentatum* and the habitat under *Cistus*.

*Basidiocarps* collybioid. *Pileus* 15–20 mm diam, convex to plano-convex, flattened to somewhat depressed at centre, not umbonate; not hygrophanous, not translucently striate or only slightly at margin of younger specimens, very dark blue-black, barely fading when aging; entirely finely fibrillose to subsquamulose, especially with age. *Lamellae* unequal with few lamellulae, adnate, relatively distant, somewhat thick, pale greyish blue then with pink tinges; with a slightly paler, entire, or somewhat irregular, concolourous edge, particularly in immature specimens. *Stipe* 25–35 × 3–4 mm, cylindrical, often twisted, concolourous with pileus or paler, finely fibrillose to subsquamulose, especially towards the apex; with white tomentose base. *Context* pale grey blue. *Taste* and *smell* not noted. *Basidiospores* 8–9.6 × 5.9–6.9 µm, av. 9.0 × 6.4 µm; Q = 1.16–1.62, Qav = 1.4, heterodiametrical, (5–)6(–7)-angled in side view. *Basidia* 32–38 × 8.5–10 µm; 4-spored, almost cylindrical. *Lamella edge* fertile. *Cheilocystidia* absent. *Pileipellis* a cutis with transitions to a trichoderm, with elements 3.7–9 µm wide. *Subpellis* with broader cylindrical elements, up to 24 µm. *Pigment* blue, intracellular, abundant in pileipellis. *Clamp-connections* absent in all tissues.

*Habitat and distribution:* In Mediterranean vegetation with *Cistus salviifolius* and *C. monspeliensis*, on siliceous soils. Known only from Spain.

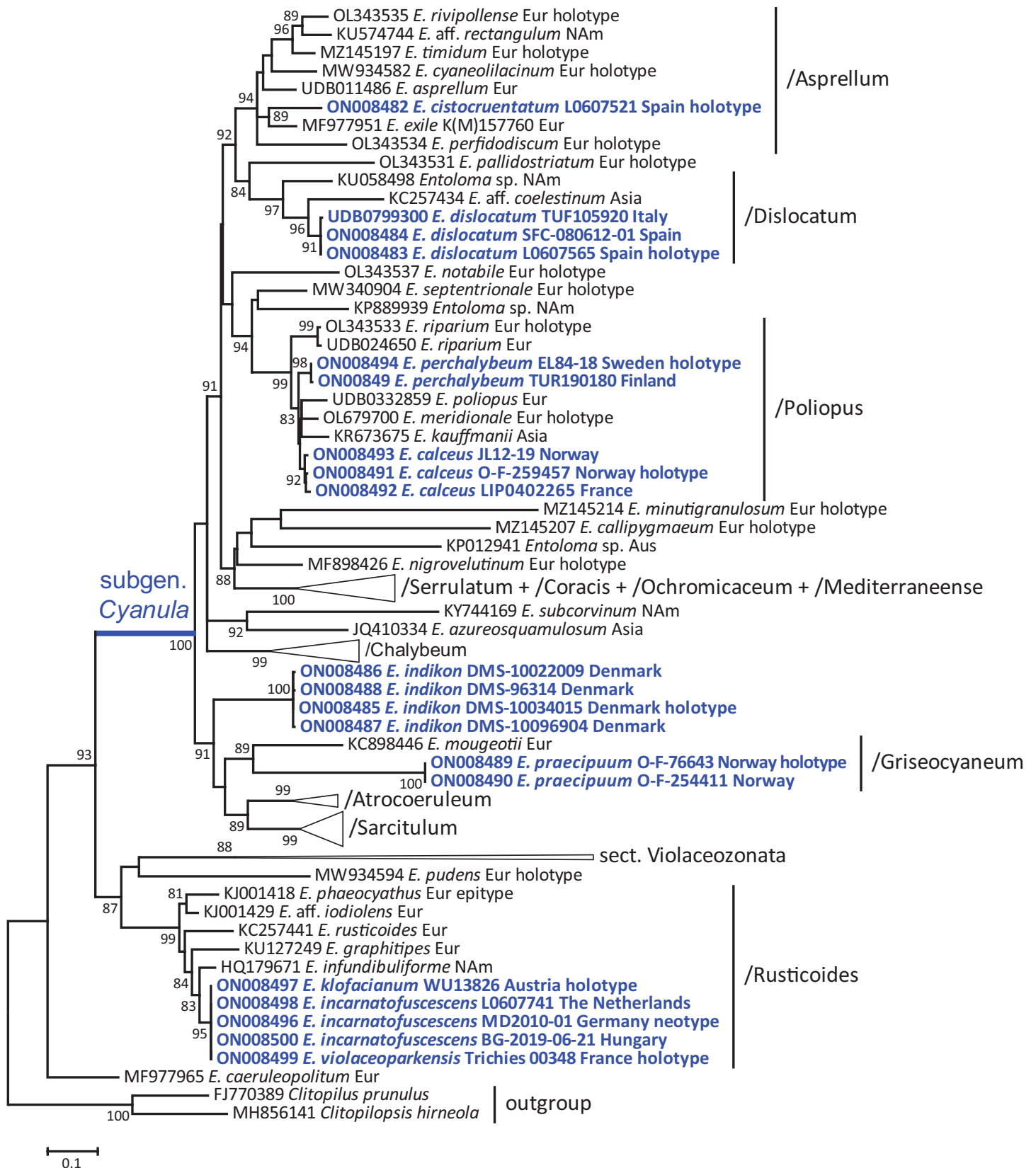
*Typus:* **Spain**, Catalonia, Selva, Tossa de Mar, Serra d'Aiguafina, alt. 80 m a.s.l., under *Cistus salviifolius* and *C. monspeliensis* on siliceous soil, 17 Dec. 2002, X. Llimona, J. Vila & E. Ballesteros (**holotype** L-0607521, **isotype** JVG 1021217-2) – ITS sequence, GenBank ON008482.

*Notes:* *Entoloma cistocruentatum* nests within the *Asprellum* clade and is distinctive on account of its very dark blue-black basidiocarps, fibrillose-squamulose stipe, fertile lamella edge and relatively small spores. *Entoloma asprellum* is very different, usually with a brown, rarely blue, translucently striate pileus and much larger spores. *Entoloma chalybeum*, in the *Chalybeum* clade, it can also have dark blue tones but is differentiated by its microscopy (spores, lamella edge) and habitat on grasslands and the recently described *E. caeruleopinophilum*, of the same clade, has less dark blue colours, striate pileus and larger spores. It also resembles of *E. cruentatum* and *E. pseudocruentatum* because of the blue tinged basidiocarps, small spores and fertile lamella edge. However, both these species are phylogenetically very distant and belong to different clades in subgenus *Cyanula* (Crous *et al.* 2021). *Entoloma cistocruentatum* is morphologically most easily distinguished from *E. cruentatum* and *E. pseudocruentatum* by the non-translucently striate pileus and Mediterranean habitat.

*Entoloma dislocatum* Vila, Dima & Noordel., *sp. nov.* MycoBank MB 840819 Fig. 2B, E.

*Etymology:* Named after its remarkable phylogenetic position in an essentially extra-European clade within *Cyanula*.

*Basidiocarps* collybioid. *Pileus* 10–40 mm diam, convex to conico-convex then applanate, sometimes depressed to umbilicate at the centre, not hygrophanous, not translucently striate or up



**Fig. 1.** Phylogenetic tree derived from Maximum Likelihood (PhyML) analysis based on nrITS1-5.8S-ITS2 data. ML bootstrap support (BS) values are shown at the nodes (BS > 80 %). Species treated in this study are marked with blue.

to half the radius in well hydrated specimens, vivid blue (Italian collection) to dark blue or blue grey, almost black at centre, paler, more grey-tinged in old specimens, especially at the margin, finely fibrillose-tomentose, more squamulose towards the centre. *Lamellae* adnate to subdecurrent, relatively distant and thick, sometimes ventricose, rarely anastomosing, white to

pale grey then with pink tinges, rarely with blue tinges (Italian collection), with entire, concolourous edges, or sometimes with grey-blue tones in old specimens. *Stipe* 25–40(–60) × 3–4 mm, central, cylindrical, concolourous with pileus or paler, glabrous to slightly tomentose or with scattered fibrils, white tomentose at base. *Context* thin, pale greyish. *Taste* and *smell*



**Fig. 2.** **A, D.** *Entoloma cystocruentatum*. **A.** Habit in situ. **D.** Spores. **B, E.** *Entoloma dislocatum*. **B.** Habit in situ. **E.** Spores. **C, F, G.** *Entoloma indikon*. **C.** Habit in situ. **F.** Spores. **G.** Lamella edge. Scale bar = 10 µm. All figures from the respective holotypes.

indistinct. *Basidiospores* 8.2–10.7 × 5.7–7.6 µm, av. 9.5 × 6.6 µm, Q = 1.27–1.8, Qav = 1.46, heterodiametrical, 6–7(–8)-angled, rarely with a subnodulose aspect in side view. *Basidia* 27–35 × 12–14 µm, 4-spored, narrowly clavate. *Lamella edge* sterile to heterogeneous. *Cheilocystidia* 40–65 × 12–16 µm, subcylindrical to clavate, hyaline or with diffuse blue-grey intracellular pigment. *Pileipellis* a cutis of cylindrical hyphae, 10.5–16.5 µm

wide, with transitions to a trichoderm at the centre and clavate terminal elements, up to 18 µm wide. *Pigment* grey to blue greyish, intracellular in pileipellis. *Clamp-connections* absent in all tissues.

*Habitat and distribution:* Terrestrial in deciduous mixed forest (*Fagus sylvatica*) on calcareous soil; in mixed deciduous forest

(*Castanea sativa*) on acid soil and in a forest of *Quercus ilex*, *Q. humilis* and *Pinus pinea*, under *Hedera helix* and *Rubus ulmifolius* on acid soil. Known from Spain and Italy.

**Typus:** Spain, Catalonia, Osona, Vidrà, near Collfred, alt. 1 340 m a.s.l., under *Fagus sylvatica*, on calcareous soil, 17 Sep. 2008, J. Vila & X. Llimona (**holotype** L-0607565, **isotype** JVG 1080917-4) – ITS sequence, GenBank ON008483.

**Additional materials examined:** Italy, Monti Sabatini, Bracciano-Martignano Regional Natural Park (Rome), alt. 530 m a.s.l., on the road through a *Castanea sativa* forest, 2 Nov. 2020, A. Knijn & A. Ferretti, TUF105920 – ITS sequence, UNITE UDB0799300. Spain, Catalonia, Vallès Oriental, Santa Maria de Martorelles Serra de Marina, alt. 360 m a.s.l., in a forest of *Quercus ilex*, *Q. humilis* and *Pinus pinea*, under *Hedera helix* and *Rubus ulmifolius* on acid soil, 12 Jun. 2008, F. Caballero, SFC 080612-01 – ITS sequence, GenBank ON008484.

**Notes:** At first sight, *Entoloma dislocatum* is similar to *E. chalybeum* with its dark blue, opaque pileus and stipe. However, the lamellae lack blue tinges, and the lamella edge is concolourous or vaguely tinged blue, whereas that of *E. chalybeum* is dark brown to bluish black. Also, the spores of *E. dislocatum* are distinctly smaller. And finally, the habitat in deciduous forest is different. Both *E. dislocatum* and the recently published *E. pallidostriatum* (Vila *et al.* 2021) do not belong to the /Chalybeum or /Poliopus clades, but take a rather isolated position in a small, but distinct clade with some poorly known extralimital species (Dima *et al.* in prep.). Knijn *et al.* (2021) described an unnamed blue *Cyanula* species from Italy, which corresponds with *E. dislocatum* after a comparison of the ITS sequences.

***Entoloma indikon*** Kehlet, Noordel. & Dima, **sp. nov.** MycoBank MB 842932 Fig. 2C, F, G.

**Etymology:** ἰνδικῶν = dark blue, referring to the stipe colour. The name derives from the Greek word ‘*indikon*’ which simply means ‘from India’ or indigo, a blue dye shipped from India to Europe.

**Basidiocarps** collybioid. **Pileus** 10–35 mm diam, campanulate to convex then plano-convex with acute or slightly flattened to truncate centre, with straight, later somewhat crenulate margin, medium to dark reddish brown with darker, blackish brown centre (“eye”), vaguely translucently striate when fresh, then distinctly and deeply translucently striate, minutely granulose all over at first, later covered with fine fibrillose patches, more or less glabrous towards margin. **Lamellae** L = about 36, l = 3–5, adnate-emarginate to deeply emarginate, ventricose, whitish or creamy-pink, then brownish pink, with slightly irregular, concolourous edge. **Stipe** 30–70 × 2–4 mm, cylindrical or gradually broadened towards base, rather pale blue to dark blue-violaceous all over, not glabrous but with scattered whitish longitudinal fibrils, base white tomentose. **Basidiospores** 8.5–10(–11) × 6.5–8.4 μm, av. 9.5–9.6 × 7.0–7.4 μm, Q = 1.1–1.45, Qav = 1.3–1.35, heterodiametrical to subisodiametrical, 5–6 angled in sideview with pronounced angles. **Basidia** 34–50 × 9.5–12.5 μm, 4-spored, clavate. **Lamella edge** heterogeneous to entirely sterile, made up of scattered or densely packed hyphae with tufts of subcylindrical to fusiform terminal elements, 6–15 μm wide, without or

with pale blue, intracellular pigment. **Pileipellis** a cutis with transitions to a trichoderm, with clavate terminal elements, 100–140 × 20–50 μm. **Pigment** brown, intracellular. **Stipitipellis** a cutis of cylindrical hyphae, 4.5–12 μm, wide, with clusters of clavate to subcylindrical terminal elements (“caulocystidia”). **Clamp-connections** absent in all tissues.

**Habitat and distribution:** Terrestrial in damp humus under *Alnus* and *Frangula*. Only known from two localities in central Sjælland, Denmark, including the type locality, where it has been observed during several years.

**Typus:** Denmark, Sjælland, Lejre, Helvigstrup Skov, terrestrial in damp humus, with *Alnus glutinosa*, *Frangula alnus* and ferns in undergrowth, 14 Sep. 2019, T. Kehlet (**holotype** DMS-10034015, C) – ITS sequence, GenBank ON008485.

**Additional materials examined:** Denmark, Sjælland, Lejre, Helvigstrup Skov, 10 Sep. 2019, T. Kehlet, DMS-10022009 (C) – ITS sequence, GenBank ON008486; *ibid.*, 19 Sep. 2020, T. Kehlet, DMS-10096904 (C) – ITS sequence, GenBank ON008487; Sjælland, Strandskov, near Englerup, 12 Jun. 2010, T. Læssøe, DMS-96314 (C) – ITS sequence, GenBank ON008488.

**Notes:** *Entoloma indikon* is a sister species of *E. phaeodiscum* (Vila & Caballero 2007) from which it differs in colour of the pileus and stipe, the pileus often becoming deeply translucently striate, the more distinctly fibrillose stipe surface and the heterogeneous to sterile lamella edge. Both species share small, iso- to heterodimmetrical spores and belong to the small /Phaeodiscum clade, sister to the /Griseocyaneum clade.

***Entoloma praecipuum*** J.B. Jordal, Noordel. & Dima, **sp. nov.** MycoBank: MB 842929 Fig. 3A–D.

**Etymology:** *praecipuus* (Lat.) = special, extraordinary.

**Basidiocarps** collybioid. **Pileus** 10–25 mm broad, campanulate to convex, expanding with age with umbilicate center and undulating margin, not distinctly hygrophorous, deeply translucently striate when moist, very dark sepia at centre, moderately dark brown on limb and paler brown at margin and between the striae, rather smooth when young, then innately fibrillose breaking up in scattered, irregular and relatively coarse squamules with age. **Lamellae** rather crowded, deeply emarginate, ventricose, initially pale grey then pale pink with coarsely serrate, concolourous edge. **Stipe** 50–70 × 2–5 mm, cylindrical, gradually broadened towards base, almost white, hyaline, covered in white, innate fibrils lengthwise, very brittle and easily splitting lengthwise. **Smell and taste** not known. **Basidiospores** 7.5–10(–10.5) × 5.0–7.0 μm, av. 8.5–10 × 5.5–6.0 μm, Q = 1.0–1.3, Qav = 1.1–1.15, subisodiametrical to shortly heterodiametrical, mostly 5, rarely 6-angled in side-view, with fairly sharp angles. **Basidia** 20–27 × 7–10 μm, 4-spored, clampless. **Lamella edge** sterile with densely clustered, subcylindrical-flexuous cheilocystidia, septate, with terminal elements of 20–34 × 5–11 μm, without pigment. **Hymenophoral trama** regular, made up of cylindrical to inflated hyphae, 4–15 μm wide. **Pileipellis** a cutis to a trichoderm of cylindrical to inflated hyphae, 10–20 μm wide with clavate terminal elements, up to 25 μm wide. **Pigment** pale brown, intracellular in pileipellis. Brilliant granules scarce in trama of pileus and lamellae. **Stipitipellis** a cutis of narrow, cylindrical



**Fig. 3.** A–D. *Entoloma praecipuum*. A. Habit *in situ*. B. Spores. C. Lamella edge. D. Pileipellis (all from holotype). E–H. *Entoloma calceus*. E, F. Habit *in situ*. G. Spores. H. Lamella edge (E from PAM00092901, all other figures from holotype). Scale bar = 10  $\mu\text{m}$  (spores); 20  $\mu\text{m}$  (lamella edges); 40  $\mu\text{m}$  (pileipellis).

hyphae, 4–11  $\mu\text{m}$  wide, with a few loose terminal endings, especially at apex. *Clamp-connections* absent in all tissues.

*Habitat and distribution:* In semi-natural grasslands, rich in *Hygrocybe* and *Entoloma* species. So far only known from the type locality and a nearby locality, both in Norway.

*Typus:* Norway, Møre og Romsdal, Sunndal, Jordalsgrend, Jordalsøra, in semi-natural meadow, southern boreal zone, 30 Jul. 2020, J.B. Jordal (holotype O-F-76643; isotype L-0607467) – ITS sequence, GenBank ON008489.

*Additional material examined:* Norway, Møre og Romsdal, Sunndal, Jordalsgrend, Jordalsvøttu, in semi-natural meadow, southern boreal

zone, 28 Aug. 1994, G. Gaarder & J.B. Jordal, O-F-254411 – ITS sequence, GenBank ON008490.

*Notes:* *Entoloma precipuum* belongs to the /Griseocyaneum clade, where it takes a rather isolated position (data not shown). It differs from most species in this clade by the less distinctly squamulose, but rather innately fibrillose, translucently striate pileus, complete lack of blue tinges and the sterile lamella edge. *Entoloma kedrovense* from the Russian Far East (Noordeloos & Morozova 2008) is somewhat similar but has a squamulose pileus and larger spores.

***Entoloma calceus*** Noordel., Bendiksen, Brandrud, P.-A. Moreau & Vila, *sp. nov.* MycoBank MB 842930 Fig. 3E–H.

*Misapplied name:* *Entoloma atromarginatum* (Romagn. & J. Favre) Zschiesch. *sensu* Moreau in Noordeloos, 2004: 1333 (photo).

*Etymology:* From Latin *calceus* = shoe, referring to the shape of the lake at the type-locality: “Skotjern”, which means lake in shape of a shoe.

*Basidiocarps* collybioid. *Pileus* 10–25(–30) mm diam, conico-campanulate then hemispherical, with small but usually marked depression, sometimes truncate-conical to convex, initially not hygrophanous and hardly translucently striate, becoming translucently striate-grooved, entirely blackish-blue to deep violaceous blue at first, then fading to pale mouse-grey, sometimes however, retaining the blackish-blue colour at centre, finally discolouring whitish to pale pinkish with age. *Lamellae* about 35 reaching stipe, 1–2 series of lamellae, rather distant, ventricose almost free, remaining white for a relatively long time, then pale pink; edge serrulate, concolourous with sides or black especially towards margin of pileus. *Stipe* 60–80 × 1–2 mm, smooth, deep blue when young, later hyaline grey-blue, paler at apex, white towards base. *Context* very thin and brittle, deep bluish when young. *Smell* none. *Basidiospores* (10–)10.9–12.5(–14) × (7.5–)8–9.5 μm, av. 10.5–11.8 × 7.9–8.8 μm, Q = (1.2–)1.3–1.6, heterodiametrical, with 6–7 angles. *Basidia* 22–30 × 8.5–10.5 μm, cylindrical, mostly 4-spored (a few 2-spored, generating macrospores up to 13.8 × 9.6 μm), clampless. *Lamella edge* sterile of the serrulatum type, made of an alternance of clusters of cheilocystidia 7–15 μm broad, some septate, clavate to lageniform, some irregularly shaped almost tibiiform, issued from radially arranged hyphae, more abundant towards insertion of stipe, with or without dark brown intracellular pigment. *Hymenophoral trama* made of parallel, mostly slender hyphae 3.5–9 μm wide, cylindrical to inflate towards septa, smooth, pale, mixed with numerous vascular hyphae 5–6 μm wide, strongly branched and forming a reticulum. *Pileipellis* a cutis with transitions to a trichoderm of somewhat gelatinized cylindrical hyphae 3.5–5.5 μm wide, with clavate terminal elements, 10–25 μm wide. *Pigment* dark blue, intracellular in pileipellis. *Stipitipellis* a simple cutis without hairs or caulocystidia. *Stipititrampa* with numerous lactiferous hyphae. *Clamp-connections* absent.

*Habitat and distribution:* Terrestrial, sphagnophilous, in mires, fens and peat-bogs in boreal-montane biomes, such as a swamp area in *Picea abies* forest, and in a few with *Carex rostrata*. Also found in pioneer vegetation in an inundated zone of hydropower plant. Known from Norway and France.

*Typus:* **Norway**, Oppland, Lunner, Skotjernfjellet og Snellingsrøysene Nature Reserve, in swamp *Picea abies* forest margin along small mire stripe (7 m broad, dominated by *Carex rostrata* in *Sphagnum* sp. (*cf. russowii*), 580 m a.s.l., 11 Aug. 2018, E. Bendiksen & T.E. Brandrud, TEB 051-18 (**holotype** O-F-259457) – ITS sequence, GenBank ON008491.

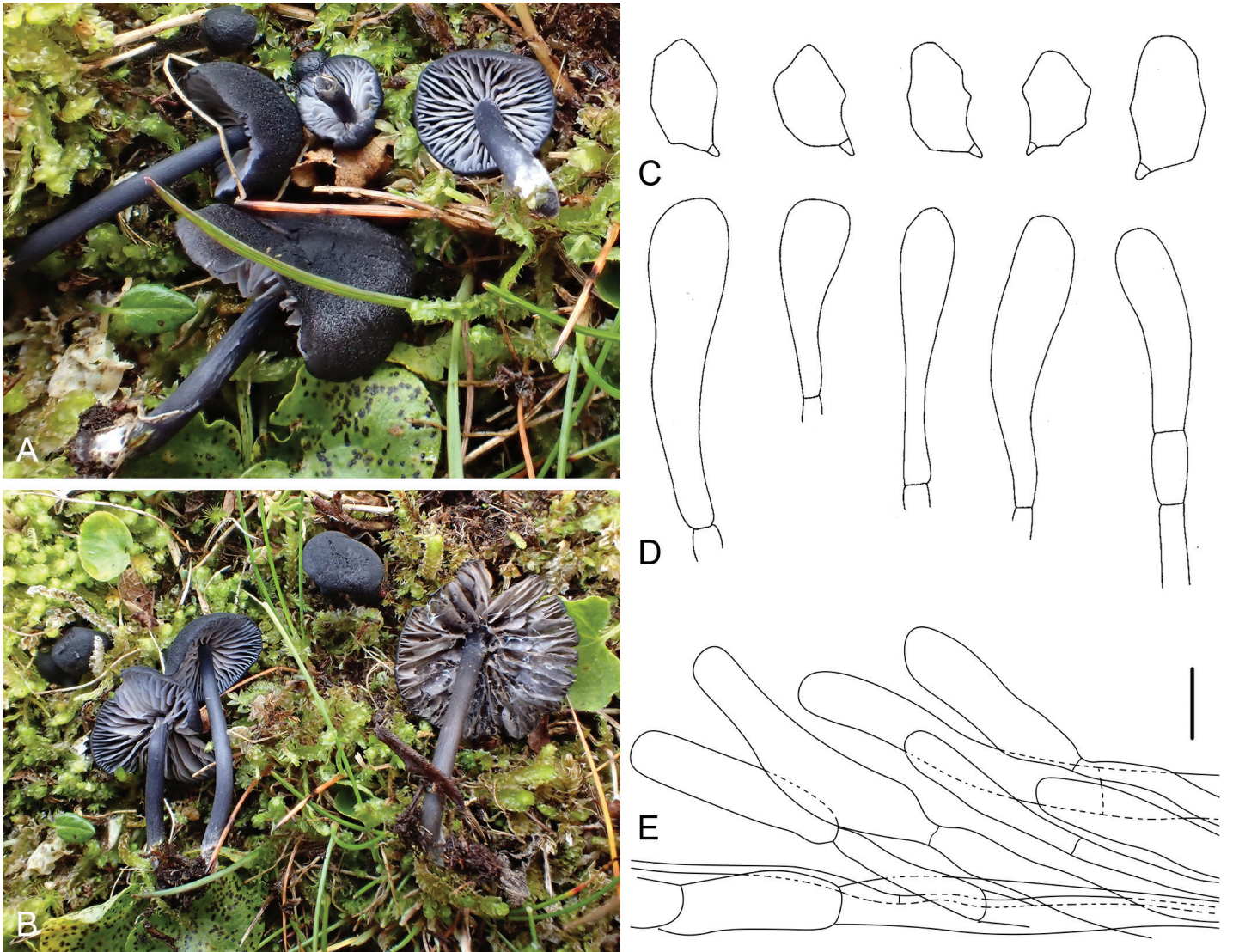
*Additional material examined:* **France**, Isère, Séchilienne, lac Luitel, floating peat bog, attached to *Sphagnum magellanicum*, 29 Sep. 2000, P.-A. Moreau, PAM00092901 (LIP 0402265) – ITS sequence, GenBank ON008492. **Norway**, Nordland, Hattfjelldal, Røsvassholmen, in *Picea abies* forest with short-grown and sparse vegetation in the water regulation zone, 22 Aug. 2019, J. Lorås, JL12-19 – ITS sequence, GenBank ON008493.

*Notes:* *Entoloma calceus* is an attractive species, with initially bright, deep violaceous blue pileus and stipe. The pileus, however, changes dramatically with age to mouse-grey and becomes slightly translucently striate. The lamella edge has a structure similar to that of *E. serrulatum* and can be pigmented or not. Our current studies in subgen. *Cyanula*, to be published in due course (Dima *et al.* in prep.) make clear, that a coloured lamella is not of great diagnostic value in *Cyanula*. The collection from France (PAM00092901) has a blackish-brown lamella edge contrasting with the pale colours of pileus and stipe and was accordingly identified as *E. atromarginatum* (Noordeloos 2004). This taxon, described from peat bogs in the French Jura (Romagnesi & Favre 1938), was described and illustrated as a pale brown species, which may resemble discoloured specimens of *E. calceus* (Fig. 3E). Our original identification of the French collections differed mainly by larger spores and persistent light blue colours; this pale colour could have escaped J. Favre’s colour-blind eyes. Our observations of the lectotype (M.E. N.) confirm the spore dimensions provided by Romagnesi & Favre (12–15 × 7–9 μm); *E. calceus* has distinctly shorter spores, 10.5–11.8 × 7.9–8.8 μm. The lectotype of *R. atromarginatus* appeared unsuitable for the extraction of DNA and no modern collection strictly matching the protologue is known to us at this time.

***Entoloma perchalybeum*** Noordel., J.B. Jordal & Dima, *sp. nov.* MycoBank MB 842931 Fig. 4.

*Etymology:* *per* (Lat.) = resembling, referring to the likeness with *E. chalybeum*.

*Basidiocarps* collybioid. *Pileus* 10–25 mm diam, hemispherical to convex, finally expanded, with blunt, subumbilicate centre, not hygrophanous, initially not translucently striate, but becoming deeply translucently striate to centre with age, very dark blackish blue and entirely tomentose at first, then paler between the striate and at margin, more purplish brown, with fine, dark blue, pointed squamules all over. *Lamellae* moderately distant, adnate, white with blue tinge, with entire, concolourous edge. *Stipe* 20–40 × 2–4 mm, cylindrical, dark blue like pileus, smooth, polished, base white tomentose. *Basidiospores* 9.0–12.5 × 6.4–9.2 μm; average 10.1–10.8 × 7.4–7.9 μm, Q = 1.2–1.5; Qav. = 1.20–1.37, heterodiametrical, with 6–7 rather blunt angles. *Basidia* 25–36 × 9.5–12.5 μm, subclavate, remarkably constricted, round base, 4-spored. *Lamella edge* sterile. *Cheilocystidia* 25–58 × 5.5–19 μm, cylindrical, subclavate, hyaline (type). *Hymenophoral trama* of cylindrical hyphae 3.5–15 μm wide. *Pileipellis* a cutis of cylindrical, inflated hyphae, 9–35 μm wide, terminal cells 30–50 × 12–16 μm, short clavate, brown intracellular pigment in ammonia. *Pigment*



**Fig. 4.** *Entoloma perchalybeum*. **A, B.** Habit *in situ*. **C.** Spores. **D.** Cheilocystidia. **E.** Pileipellis (all from holotype). Scale bar = 10 µm (spores, cystidia); 20 µm (pileipellis).

brown grey, intracellular vacuolar and granular (in ammonia). *Stipitipellis* a cutis of cylindrical hyphae, 3.5–9 µm wide, pale brown intracellular pigment, hyaline, surface with some short terminal cells 25–40 × 11–17 µm. *Clamp-connections* absent in all tissues.

**Habitat and distribution:** Terrestrial in groups, calciphilous, in alpine heaths and boreal, damp mixed forest with *Picea abies*, *Pinus sylvestris*, *Betula* spp. and *Alnus incana*. Subarctic, found in Northern Fennoscandia only.

**Typus:** Sweden, Pite Lappmark, Arjeplog, west of Nuorta Krapesvarre, in rich alpine vegetation, low alpine zone, 12 Aug. 2018, J.B. Jordal, E. Larsson & J. Vauras (**holotype** GB-0209474, **isotype** L-0608235) – ITS sequence, GenBank ON008494.

**Additional material examined:** Finland, Regio kuusamoensis, Kuusamo, Valtavaara Nature Reserve, damp forest with *Picea abies*, *Pinus sylvestris*, *Betula* spp. and *Alnus incana*, 30 Aug. 2005, J. Vauras, TUR190180 – ITS sequence, GenBank ON008495.

**Notes:** *Entoloma perchalybeum* shares many characters with *E. chalybeum*, particularly when fresh on account of the very similar colour, non-translucently striate, squamulose pileus,

polished stipe and blue tinge in the lamellae, but the pileus becomes quickly translucently striate when maturing (*cf.* *Entoloma lazulinum*). Both *E. chalybeum* and *E. lazulinum* usually have brown instead of blue lamella edges and furthermore are phylogenetically very distant (Dima *et al.* in prep.).

#### Neotypification and emendation of *Entoloma incarnatofuscescens*

*Entoloma incarnatofuscescens* (Britzelm.) Noordel., *Persoonia* **12**: 461. 1985. MB 104243 Fig. 5.

**Basionym:** *Agaricus incarnatofuscescens* Britzelm., *Ber. naturhist. Augsburg* **8**: 6. 1894.

**Synonyms:** *Leptonia incarnatofuscescens* (Britzelm.) Sacc., *Syll. Fung.* **11**: 47. 1895.

*Entoloma klofacianum* Noordel. *et al.*, *Öst. Z. Pilzk.* **4**: 128. 1995.

*Entoloma violaceoparkensis* Noordel. & Trichies, in Noordeloos, *Entoloma* s.l., *Fungi Europaei* vol. 5 (Saronno) 5a: 1120. 2004.

? *Rhodophyllus leptonipes* Kühner & Romagn., *Rev. Mycol. (Paris)* **19**: 6. 1954.

**Emended description:** *Basidiocarps* omphalioid. *Pileus* 5–25(–40) mm broad, campanulate or conical then convex or plano-





**Fig. 5.** *Entoloma incarnatofuscescens*. Habit, spores and pileipellis. **A, B and D.** Neotype. **C.** Holotype of *E. violaceoparkensis*. E. L-0607741. **F.** Holotype of *E. klofacianum*. Scale bars = 10  $\mu$ m.

convex with involute then deflexed margin, usually with distinctly umbilicate centre to funnel-shaped, more rarely with slight depression or with small umbo, weakly to distinctly hygrophanous, when moist usually deeply translucently striate, rarely not, pinkish brown, yellowish brown to reddish brown, darker at centre and on striae, sometimes blue or violaceous-brown, slightly to distinctly pallescent on drying to greyish brown, minutely squamulose at centre, fibrillose towards margin or minutely tomentose-squamulose all over. *Lamellae* distant to

moderately crowded, adnate or emarginate with decurrent tooth, then decurrent, triangular to segmentiform, sometimes veined on sides, pale grey or brown then pinkish brown, rarely with bluish tinge, with concolourous or slightly darker edge. *Stipe* 15–20  $\times$  1–3 mm, cylindrical or compressed, often with bulbous base, dark to medium dark blue-grey, steel blue or violaceous-brown, smooth, glabrous, polished or with scattered longitudinal fibrils, white tomentose at base. *Context* brown in cortex of pileus, blue-grey in cortex of stipe, inner parts almost white. *Smell* none or

slightly farinaceous. *Basidiospores* 7.5–10.5(–11) × 6.0–8.5 µm, Q = 1.0–1.45(–1.6), Q<sub>av</sub> = 1.15–1.4, iso- to heterodiametrical, (4–) 5–7 angled in side view. *Basidia* 20–43 × 7–12 µm, clampless or rarely clamped. *Lamella edge* fertile. *Cystidia* absent. *Pileipellis* a trichoderm of strongly inflated clavate to spheropedunculate elements, up to 30 µm wide. *Pileitrama* regular, made up of strongly inflated hyphae, 4–20 µm wide. *Pigment* parietal, probably also pale intracellular in pileipellis; also rarely minutely encrusting in lower parts of pileipellis. Brilliant granules sparse. *Clamp-connections* absent or scarcely present.

**Habitat and distribution:** Terrestrial, saprotrophic, often on bare, preferably loamy, nutrient rich, damp soil in mixed forest, parks and gardens. Widespread and probably fairly common all over Europe.

**Typus:** **Germany**, Bayern, Kleinhartpenning, Hackensee, 16 Aug. 2010, M. Dondl (neotype MD 2010-01 (L), designated here) – MycoBank MBT 10005752, ITS sequence, GenBank ON008496.

**Additional materials examined:** **Austria**, Styria, Bad Gleichenberg, Kurpark (MTB 9161/1), 31 Aug. 1994, W. Klofac, WU 13826, holotype of *E. klofacianum* – ITS sequence, GenBank ON008497. **Germany**, Bayern, 86316 Friedberg, 12 Aug. 2019, T. Laschner, L-0607741 – ITS sequence, GenBank ON008498. **France**, Moselle, Moyeuve-Petite, 8 Aug. 2002, G. Trichies 00348, holotype of *E. violaceoparkensis* (L-0607466) – ITS sequence, GenBank ON008499. **Hungary**, Mecsek Mts, Kárász, 21 Jun. 2019, G. Benkő & K. Fábrics, BG-2019-06-19 (ELTE) – ITS sequence, GenBank ON008500.

**Notes:** This tiny, omphalinoid species, which in its typical form is easy to recognise on morphological characters, is often taken for a species of *Cyanula* because of the steel-blue, polished stipe, appears to belong to the /Rusticooides clade, which is rather distantly related to *Cyanula*. It appears to be rather variable in colour. Besides the normally pinkish brown to yellow brown pileus, also variants with blue and violaceous tinges are now included in this species as became clear from the molecular genetic study of *E. violaceoparkensis* and *E. klofacianum*. The shape of the spores varies considerably from subsodiametrical to distinctly heterodiametrical, often within one basidiocarp and includes also the variant with predominantly 4–5 angled, subsodiametrical spores, described as *E. klofacianum*. Clamp-connections are usually not found but can be present in the hymenium or pileipellis. Intracellular pigment is dominant, but sometimes also slight incrustations are found in the lower part of the pileipellis and in pileitrama. *Rhodophyllus leptonipes* probably represents a later synonym, based on the morphology (Kühner & Romagnesi 1954), but the lectotype (PC) appeared unsuitable for DNA extraction to confirm it.

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## REFERENCES

- Alvarado P, Moreno G, Vizzini A, *et al.* (2015). *Atractosporocybe*, *Leucocybe* and *Rhizocybe*: three new clitocyboid genera in the Tricholomatoid clade (*Agaricales*) with notes on *Clitocybe* and *Lepista*. *Mycologia* **107**: 123–136.
- Anisimova M, Gil M, Dufayard J-F, *et al.* (2011). Survey of branch support methods demonstrates accuracy, power and robustness of fast likelihood-based approximation schemes. *Systematic Biology* **60**: 685–699.
- Brandrud TE, Bendiksen E, Jordal JB, *et al.* (2019). On some *Entoloma* species (*Tricholomatinae*, *Basidiomycota*) little known or new to Norway. *Agarica* **39**: 31–52.
- Brandrud TE, Bendiksen E, Jordal JB, *et al.* (2018). *Entoloma* species of the rhodopolioid clade (subgenus *Entoloma*; *Tricholomatinae*, *Basidiomycota*) in Norway. *Agarica* **38**: 21–46.
- Caballero F, Vila J (2013). *Entoloma* nuevos o interesantes de la Península Ibérica (3). Adiciones y correcciones. In: Vila J. *et al.* *Studies in Entoloma. Fungi non delineati* **66**: 63–85 + 136–145. Ed. Candusso, Alassio.
- Crous PW, Cowan DA, Maggs-Kölling G, *et al.* (2021). Fungal Planet description sheets: 1182–1283. *Persoonia* **46**: 313–528.
- Dima B, Lindström H, Liimatainen K, *et al.* (2016). Typification of Friesian names in *Cortinarius* sections *Anomali*, *Spilomei* and *Bolares*, and description of two new species from northern Europe. *Mycological Progress* **15**: 903–919.
- Dima B, Brandrud TE, Corriol G, *et al.* (2021). Fungal Systematics and Evolution: FUSE 7. *Sydowia* **73**: 271–340.
- Gardes M, Bruns TD (1993). ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**(2): 113–118.
- Gouy M, Tannier E, Comte N, *et al.* (2021). Seaview Version 5: a multiplatform software for multiple sequence alignment, molecular phylogenetic analyses and tree reconciliation. *Methods in Molecular Biology* **2231**: 241–260.
- Guindon S, Dufayard JF, Lefort V, *et al.* (2010). New algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.
- Haelewaters D, Dima B, Abdel-Hafiz BII, *et al.* (2020). Fungal Systematics and Evolution 6. *Sydowia* **72**: 231–356.
- Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Knijjn A, Ferretti A, Saar I (2021). Rare findings from the chestnut forest of Monte Rocca Romana (Latium, Italy). *Italian Journal of Mycology* **50**: 78–91.
- Kühner R, Romagnesi H (1954). Compléments à la 'Flore Analytique'. I. Espèces nouvelles ou critiques de *Rhodophyllus*. *Revue de Mycologie* **19**(1): 3–46.
- Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**: 1870–1874.
- Morozova OV, Noordeloos ME, Vila J (2014). *Entoloma* subgenus *Leptonia* in boreal-temperate Eurasia: towards a phylogenetic

- species concept. *Persoonia* **32**: 141–169.
- Noordeloos ME (2004). *Entoloma s.l.* (suppl.). *Fungi Europaei* vol. 5A, Ed. Candusso, Alassio.
- Noordeloos ME, Morozova OV (2010). New and noteworthy *Entoloma* species from the Primorsky Territory, Russian Far East. *Mycotaxon* **112**: 231–255.
- Noordeloos ME, Dima B, Weholt Ø, et al. (2017). *Entoloma chamaemori* a new, small-spored, boreal *Entoloma* species, with isolated phylogenetic position. *Phytotaxa* **298**: 289–295.
- Noordeloos ME, Lorås J, Eidissen SE, et al. (2020). Three new *Entoloma* species of the Cyanula clade from (sub)alpine habitats in Northern Norway and Sweden. *Sydowia* **73**: 185–196.
- Noordeloos ME, Weholt Ø, Bendiksen E, et al. (2018). *Entoloma aurorae-borealis* sp. nov. and three rare *Entoloma* species in the *Sinuatum* clade (subg. *Entoloma*) from northern Europe. *Sydowia* **70**: 199–210.
- Papp V, Dima B (2018). New systematic position of *Aurantiporus alborubescens* (*Meruliaceae*, *Basidiomycota*), a threatened old-growth forest polypore. *Mycological Progress* **17**: 319–332
- Romagnesi H, Favre J (1938). Quelques Rhodophylles nouveaux ou rares des hauts-marais jurassiens. *Revue de Mycologie* **3**: 60–77.
- Vila J, Caballero F (2007). *Entoloma* nuevos o interesantes de la Península Ibérica. *Fungi non Delineati* **38**. Ed. Candusso, Alassio.
- Vila J, Caballero F (2009). *Entoloma* nuevos o interesantes de la Península Ibérica (2). *Fungi non Delineati*. Pars XLV. Ed. Candusso, Alassio.
- Vila J, Carbó J, Caballero F, et al. (2013). A first approach to the study of the genus *Entoloma* subgenus *Nolanea sensu lato* using molecular and morphological data. *Fungi non Delineati*. **65**: 3–62, 93–135 (iconography). Ed. Candusso, Alassio.
- Vila J, Caballero F, Carbó J, et al. (2014). Preliminary morphologic and molecular study of the *Entoloma rusticoides* group (*Agaricales* - *Basidiomycota*). *Revista Catalana de Micologia* **35**: 65–99.
- Vila J, Llimona X (2010). Noves dades sobre el component fúngic de les comunitats de *Cistus* de Catalunya. III. Addicions, correccions i claus d'identificació. *Revista Catalana Micologia* **31**: 103–137.
- Vila J, Noordeloos ME, Reschke K, et al. (2021). New species of the genus *Entoloma* (*Basidiomycota*, *Agaricales*) from Southern Europe. *Österreichische Zeitschrift für Pilzkunde* **29**: 123–153.
- White TJ, Bruns T, Lee S, et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, et al., eds). Academic Press, New York, USA: 315–322.