

***Entoloma trichomarginatum*, a new species of subgenus *Leptonia* (Entolomataceae) from Spain**

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Abstract — *Entoloma trichomarginatum* sp. nov. is described from Catalonia (Spain). It belongs to subgenus *Leptonia* and is characterized by the deep blue to almost blue-black tomentose pileus, bluish lamellae sides, and the sterile lamellar edge composed of almost 350 µm long cheilocystidia filled with blue intracellular pigment. A comparison with other close taxa is given, as well as photographs and drawings of the macroscopical and microscopical characters.

Key words — macrofungi, Basidiomycetes, Agaricales, biodiversity, taxonomy

Introduction

The mycobiota of Spain, and especially Catalonia, is fairly well known, and several species of *Entoloma* (Fr.) P. Kumm. have been described up to date (Maire 1933, 1937; Singer 1947, Noordeloos et al. 1992, Esteve-Raventós & De La Cruz 1998, Wölfel & Noordeloos 2001, Esteve-Raventós & Ortega 2003, Noordeloos 2004, Vila & Caballero 2007). However, in the course of a study of the macrofungi of the Natural Park of Cadí-Moixeró (Catalonia, Spain) between 2002 and 2008, we gathered a species of subgenus *Leptonia* that could not be named with the existing selected literature. The species is here proposed as a new based on morphological and ecological features.

The Natural Park of Cadí-Moixeró, mainly calcareous, is located in the north of Catalonia and belongs to the mountain range of the Pre-Pyrenees; it is dominated by a mediterranean climate in the lowlands and a warm continental climate in the highlands. Both climatological and lithological factors favor a wide range of different habitats with a high mycofloral diversity, among which some interesting findings have already been published (Llorens van Waveren & Llistosella 2004).

Materials and methods

The material studied was collected by both authors during some of their visits to the Natural Park of Cadí-Moixeró in 2006, and was photographed *in situ* using a Nikon D-100 camera.

Macroscopical description and extensive notes were made on fresh fruit bodies before they were dried. Colour notations in parenthesis have been taken from Kornerup & Wanscher (1981).

Microscopical analysis and measurements of the micromorphological structures were studied in slides mounted in Congo Red, 3% KOH and 15% NH₄OH, with a Nikon Labophot microscope, using the standard techniques. All line drawings were made with the aid of a drawing tube, and were reproduced with a digital Deltapix infinity X camera. The method used to calculate spore ranges is the one proposed by Heinemann & Rammeloo (1985) where Qav corresponds to the range of the mean of Q (length/width ratio) of n spores.

Mycological terminology follows Kirk et al. (2001) and Josserand (1952).

The material studied is deposited in BCN herbarium of the Centre de Documentació de Biodiversitat Vegetal (CDOcBiv) of the Universitat de Barcelona.

Taxonomic description

Entoloma trichomarginatum Llorens van Wav. & Llistos., sp. nov. FIGS 1–7

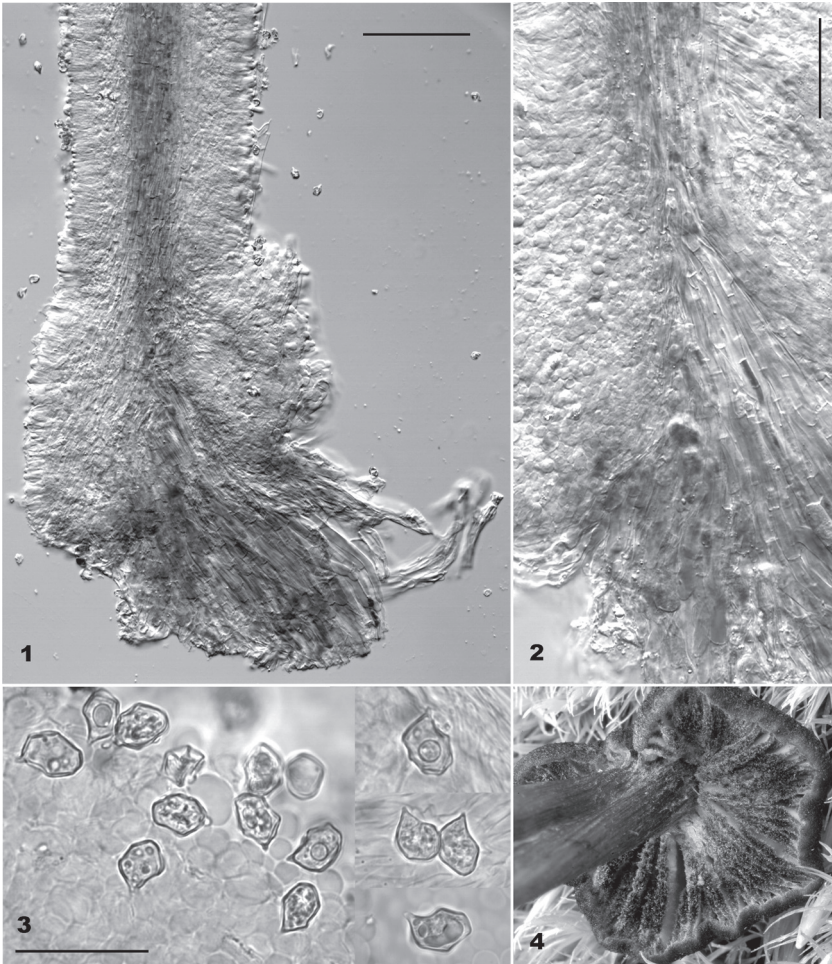
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Pileus 15–25 mm, a convexo ad planoconvexum parum depressus haud hygrophanus nec trans lumen striatus; coeruleo-nigrescens vel obscure coeruleus, totus tomentosus vel squamulosus. Lamellae adnatae, coeruleo cinerascens; acies lamellarum flocculosa, nigro-coerulea. Stipes 40–50 mm longus 3–5 mm latus, cylindraceus, apicem versus coeruleo pruinoso, versus basim fibrilloso-squamuloso. Caro cinereo vel coeruleo pallens; odor leviter farinaceus, sapore indistincto. Sporae 9–11.5 µm longae, 6.5–8.5 µm latae, 5–7 angulatae. Basidia tetrasporigena, efibulata. Acies lamellarum steriles; cheilocystidia usque ad 350 µm longa, 7–15 µm lata, contento caesio. Pileocutis sicut trichoderma, versus centro hymenoderma, elementis late clavatis 15–25 µm latis. Pigmentum intracellulare coeruleo. Fibulae absentes.

TYPE: *Inter muscus, in Pinus mugo subsp. uncinata silvam in solo calcareo crescens, in loco dicto Pla de Prat, prope Josa i Tuixén, in Catalaunia (Hispania), a L. Llorens van Waveren lectus, 17/X/2006, in herbario Universitatis Barcinonensis (BCN-LLVW1230, holotypus) servatus.*

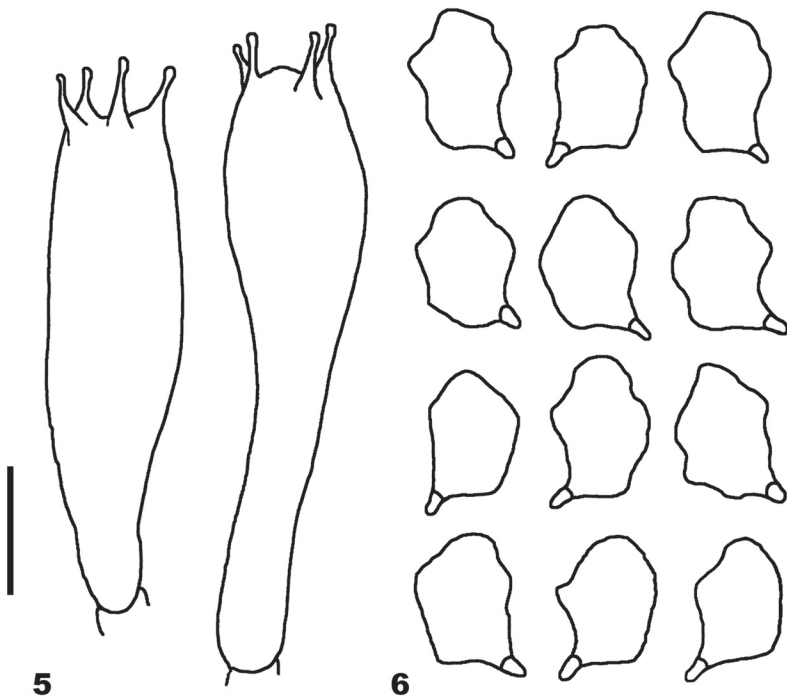
ETYMOLOGY: *tricho* (Greek) = hair; *margino* (Latin) = border, margin; referring to the hairy-flocculose lamella edge.

BASIDIOMATA collybioid in stature. **PILEUS** 15–25 mm diam., convex when young, expanding to plano-convex at maturity, with rounded or slightly depressed centre, rarely infundibuliform or with small papilla; surface not distinctly hygrophanous, not translucently striate; dark blackish blue to intense blue (F4 21), uniformly coloured, entirely tomentose at first, later on breaking up in radially arranged squamules, especially dense in central part; margin decurved at first, then straight, at times lobed or irregularly undulate.



FIGS 1-4. *Entoloma trichomarginatum*. 1. Cross section of the lamellar edge to show the hymenium and the blue-pigmented hymenophoral trama and cheilocystidia. Bar = 100 μ m. 2. Detail of the hymenophoral trama to show the tramal origin of the cheilocystidia. Bar = 50 μ m. 3. Mature spores. Bar = 25 μ m. 4. Macroscopical detail of the hairy-flocculose lamellar edge.

LAMELLAE, L = 15–35, l = 1–2, moderately crowded, adnate, thick; colour pale blue or greyish blue when young, then grey–blue with a pink tinge, with blue–black hairy–flocculose edge. STIPE 40–50 \times 3–5 mm, central, cylindrical or compressed, often gradually distinctly broadened towards base; dark bright blue coloured, paler than pileus, fading with age especially in the lower part, apex blue pruinose, downwards more polished but with blue scattered fibrils,



FIGS 5-6. *Entoloma trichomarginatum*. 5. Basidia 6. Spores. Bar = 10 μ m.

covered with whitish mycelium at extreme base, fistulose with age. CONTEXT blue-grey in cortex, whitish with greyish tinges in the inner parts, not changing colour when bruised. SMELL weakly farinaceous, especially when cut. TASTE mild.

BASIDIOSPORES 9–11.5 \times 6.5–8.5 μ m, on average 10.11 \times 7.41 μ m, (Q = 1.24–1.58, Q_{av} = 1.38), heterodiametrical, 5–7-angled in side view with pronounced angles. BASIDIA 35–50 \times 11–14 μ m, clavate, four-spored, clampless. HYMENOPHORAL TRAMA regular, of long cylindrical hyphae with abundant intracellular blue pigment. LAMELLAR EDGE sterile, composed of long sterile strands of cylindrical hyphae all along the edge of the lamellae. CHEILOCYSTIDIA numerous, up to 350 \times 7–15 μ m, cylindrical, apex rounded-clavate, often septate, filled with abundant blue intracellular pigment. PLEUROCYSTIDIA absent. PILEIPELLIS consisting of a trichoderm with transitions to a hymeniderm at centre, made up of inflated terminal elements, up to 15–25 μ m wide, filled with intracellular blue pigment. STIPITPELLIS with pigment blue or grey-blue, exclusively intracellular. CLAMP CONNECTIONS absent from all tissues.

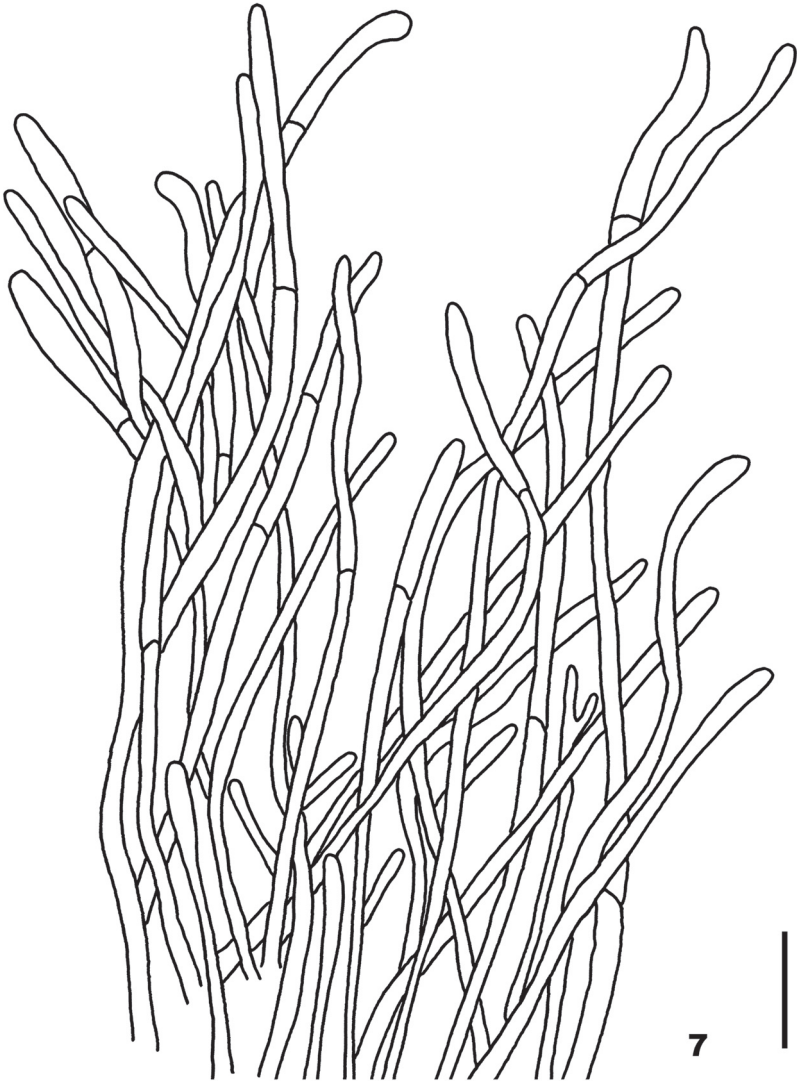


FIG 7. *Entoloma trichomarginatum*. Cheilocystidia. Bar = 40 μ m.

HABITAT: In a clearing of a *Pinus mugo* subsp. *uncinata* forest with *Juniperus communis* and *Buxus sempervirens*, in mossy soil, at 1800 m above sea level; calcareous soil.

KNOWN DISTRIBUTION: type locality.

Discussion

Entoloma trichomarginatum is referred to subgenus *Leptonia* based on its collybioid habit, typically trichodermoid pileipellis (demonstrated by the minute scales at least at centre), blue colours on pileus and stipe, and absence of clamp-connections. The combination of the strongly fimbriate structure and blue-black pigmentation of the lamellar edge, entirely composed of dense clusters of cylindrical to clavate sterile hyphae, are characteristic of stirps *Serrulatum*.

The *Serrulatum* group seems to be microscopically monotonous. In most species the heterodiametrical spores average $8\text{--}11.5 \times 6\text{--}8 \mu\text{m}$, show 5–7 angles in side view, and have a 1.2–1.7 Q ratio range. The lamellar edges is composed of inflated terminal elements of sterile hyphae (cheilocystidia) averaging $20\text{--}100 \times 3\text{--}20 \mu\text{m}$ and containing bluish, blackish or brownish intracellular pigments. Thus most species in this stirps are distinguished mainly by macroscopical characters, such as fruitbody colour and chromatic variability during development, or habitat preference.

Nevertheless, *E. trichomarginatum*, with its uniformly deep blue to almost black basidiocarp, is mainly characterised by two microscopical characters: the blue pigmented hyphae of the hymenophoral trama and the blue pigmented cheilocystidia that are more than three times longer than the average in this stirps. In this species, cheilocystidia consist of clavate to cylindrical chains of sterile hyphae that originate from within the hymenophoral trama and extend beyond the lamellar edge, giving it an extraordinarily flocculose appearance. According to the literature (Horak 1978, Noordeloos 1992, Vesterholt 2002, Noordeloos 2004) no other described *Entoloma* species produces cheilocystidia of this size. Furthermore, *E. trichomarginatum* exudes a weakly farinaceous odor, contrasting with the lack of odor noted for all species in stirps *Serrulatum*. It should be noted, however, that several species in subgenus *Leptonia* (e.g., *E. dichroum* (Pers.) P. Kumm., *E. placidum* (Fr.) Noordel., *E. violaceozonatum* Noordel. & Liiv, *E. juniperinum* Barkman & Noordel.) do have this characteristic farinaceous smell.

Similar blue-coloured species with pigmented cheilocystidia include *E. chalybeum* (Pers.) Noordel., *E. serrulatum* (Fr.) Hesler, *E. caesiocinctum* (Kühner) Noordel., and *E. gomerense* Wölfel & Noordel. *Entoloma chalybeum* resembles *E. trichomarginatum* in basidiocarp colour, habit, and pale blue to greyish lamellae (most easily observed in young specimens) but differs in having much shorter cheilocystidia with an irregular brown intracellular pigments. The cosmopolitan *E. serrulatum*, with a characteristic bright blue pileus and stipe and an irregular lamellar edge with blue-pigmented cheilocystidia, differs lacking the blue tinges on the lamellar faces and serrulate, less flocculose lamellar edge.

However, *E. serrulatum* var. *nigrovenosum* Courtec., which shares almost the same macroscopical characters with *E. trichomarginatum*, can be recognized by its more rounded, rugulose pileus that is much darker, almost black, and with a slight blue tinge that does not fade with age (Courtecuisse 1993). *Entoloma trichomarginatum* has been found in a habitat similar to that of *E. serrulatum*, which is common in grasslands and grassy vegetations in temperate, boreal and subalpine regions (Noordeloos 1984, Noordeloos & Gulden 1989), but it also occurs in alpine and arctic regions. *Entoloma caesiocinctum* can be distinguished from *E. serrulatum* by the brown pileus that is tinged slightly blue at the margin and less tomentose-squamulose. The completely translucent, striate pileus and $\leq 50 \mu\text{m}$ long cheilocystidia depicted in the description and illustration of *E. gomerense* by Wölfel & Noordeloos (2001) are additional distinguishing characters.

Most other members of the stirps differ mainly by the colour of pileus and stipe, by which it is possible to distinguish the following species: *E. querquedula* (Romagn.) Noordel. with olivaceous tinges, *E. carneogriseum* (Berk. & Broome) Noordel. with pale pinkish brown or yellow-brown colours, *E. callirhodon* Hauskn. & Noordel. with a striking pink reddish colour, *E. violaceoserrulatum* Noordel. with violaceous tinges, and *E. xanthoserrulatum* Noordel. & Vauras with predominantly yellow colours. Moreover, *E. linkii* (Fr.) Noordel., which has blue colours only at the lamellar edge, is always found on wood remains of *Fagus sylvatica*. Lastly, *E. brunneoserrulatum* Eyssart. & Noordel., has a brown pileus, brown lamellar edge, and larger, 11–13 μm long basidiospores.

Future DNA sequence-based studies will help circumscribe the different species and establish a more sound taxonomical vision of this difficult *Serrulatum* group.

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Literature cited

Courtecuisse R. 1993. Macromycetes intéressants, rares ou nouveaux (VI) *Entolomataceae*. Documents Mycologiques 23: 1–38.

- Esteve-Raventós F, De La Cruz M. 1998. *Entoloma exiguum*, a new species of subgenus *Claudopus* (*Entolomataceae*, *Agaricales*) from Spain. *Persoonia* 17: 141–144.
- Esteve-Raventós F, Ortega A. 2003. *Entoloma alliodorum*, a new species of subgenus *Claudopus* with a garlic odour. *Mycotaxon* 86: 227–232.
- Heinemann P, Rammeloo J. 1985. De la mesure des espores et son expression. *Agarica* 6: 366–380.
- Horak E. 1978. *Entoloma* in South America. I. *Sydowia* 30: 40–111.
- Josserand M. 1952. La description des Champignons Supérieurs. Lechevalier. Paris.
- Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the Fungi. 9th Edition. CAB International. Wallingford, UK.
- Kornerup A, Wanscher JH. 1981. Taschenlexikon Der Farben – 1440 Farbnuancen und 600 Farbnamen. Muster-Schmidt Verlag. Zürich. Göttingen.
- Llorens van Waveren L, Llistosella J. 2004. Contribució a la flora dels Fongs del Parc Natural del Cadí-Moixeró (Catalunya). I. El gènere *Entoloma* (Fr.) P. Kumm. *Revista Catalana de Micologia* 26: 165–176.
- Maire R. 1933. Fungi Catalaunici. Contributions à l'étude de la Flore Mycologique de la Catalogne. *Treballs del Museu de Ciències Naturals de Barcelona* 15: 1–120.
- Maire R. 1937. Fungi Catalaunici. Contributions à l'étude de la Flore Mycologique de la Catalogne. *Publicacions de l'Institut Botànic* 3(4): 1–128.
- Noordeloos ME. 1984. *Entolomataceae* (*Agaricales*, *Basidiomycetes*) in Greenland. I. The genus *Entoloma*. *Persoonia* 12: 263–305.
- Noordeloos ME. 1992. *Entoloma* s.l. Fungi Europaei. vol. 5. Giovanna Biella. Saronno, Italia.
- Noordeloos ME. 2004. *Entoloma* s.l. (Supplemento). Fungi Europaei. vol. 5a. Massimo Candusso, Alassio, Italia.
- Noordeloos ME, Gulden G. 1989. *Entoloma* (*Basidiomycetes*, *Agaricales*) of alpine habitats on the Hardangervidda near Finse, Norway, with a key including species from Northern Europe and Greenland. *Canadian Journal of Botany* 67: 1727–1738.
- Noordeloos ME, Tabarés M, Rocabrana A. 1992. A new species of *Entoloma* subgenus *Pouzarella* from Spain. *Persoonia* 15: 123–125.
- Singer R. 1947. Champignons de la Catalogne. Espèces observées en 1934. *Collectanea Botanica* 1: 199–246.
- Vesterholt J. 2002. Contribution to the knowledge of species of *Entoloma* subgenus *Leptonia*. *Fungi non delineati* 21: 1–63.
- Vila J, Caballero F. 2007. *Entoloma* nuevos o interesantes de la Península Ibérica. *Fungi non delineati* 38: 63.
- Wölfel G, Noordeloos ME. 2001. Neue older bemerkenswerte *Entoloma*- Arten der Kanarischen Inseln. *Österr. Zeitschr. f. Pilzk.* 10: 185–200.