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Entoloma ochreoprunuloides from Italy, with notes on its geographical distribution and allied species

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Abstract—An Italian collection of *E. ochreoprunuloides* [≡ *E. prunuloides* var. *obscurum*] is described. The specimen was identified by means of morphology, and by the analysis of its nrITS sequence. The European distribution of the species is also discussed. The sequence from a single Italian specimen of *E. luteobasis* suggests that *E. luteobasis* and *E. ochreoprunuloides* may be conspecific.

Key words—*Basidiomycota*, *Agaricomycetes*, tricholomatoid clade, taxonomy

Introduction

Entoloma (Fr. ex Rabenh.) P. Kumm. is a large genus of *Agaricomycetes*, with worldwide distribution; more than 1500 taxa have been described (Morgado et al. 2013). Until a few years ago, the systematics of *Entoloma* was based only on morphological data (Noordeloos 1981, 1992, 2004), but recent studies based on DNA sequences have started to appear in the literature (e.g., Co-David et al. 2009; Baroni et al. 2011; Kokkonen 2015). The consequence of these investigations is that the infrageneric subdivision is continuously evolving, but this process is just at the beginning. For example, Noordeloos (1981, 1992, 2004) treats *Entoloma* as a single genus subdivided into several subgenera, while Largent (1994) recognizes *Entoloma* sensu stricto and many segregate genera,

most corresponding to the subgenera used by Noordeloos. Recently Baroni et al. (2011) proposed *Entocybe* T.J. Baroni et al. as a new genus that includes a few select species from *Entoloma* subg. *Entoloma* and a few other anomalous species previously placed in *Rhodocybe* sect. *Rhodophana* (Kühner) Singer. These taxa produce bumpy spores reminiscent of those found in *Rhodocybe* and abundant clamp connections that are not typical of *Rhodocybe. Entocybe* was erected for a distinct clade in *Entoloma* subg. *Entoloma* based on both morphological and molecular data.

At the species level, even some well-known species appear far more complex when investigated at the molecular level. For example, Morgado et al. (2013) focused on the subgenus *Entoloma* using a multigene approach (mtSSU, nrLSU, rpb2, nrITS) to find that *E. bloxamii* (Berk. & Broome) Sacc., *E. prunuloides* (Fr.) Quél., and *E. sinuatum* (Bull.) P. Kumm. each represent species complexes containing different taxa when considered from a worldwide perspective. Kokkonen (2015), who examined nrITS, nrLSU and rpb2 sequences of several *Entoloma* taxa mainly in *E.* sect. *Rhodopolia* (Fr.) Noordel. (actually elevated at subgeneric level), also proposed several novel taxa and combinations.

The present contribution concerns the *E. prunuloides* complex in *E.* sect. *Entoloma*, a section characterised by small, somewhat isodiametric and obscurely angular spores and pileipellis hyphae arranged as a simple cutis or ixocutis. From a phylogenetic point of view, the species of this section form a clade that appears basal to the bulk of the species in the *Entoloma* sensu lato clade. Morgado et al. (2013) demonstrated that at least three different species have been covered under the name "*E. prunuloides*"—(1) *E. pseudoprunuloides* Morgado & Noordel., for a Canadian collection; (2) typical *E. prunuloides*, probably with an exclusively European distribution; and (3) *E. ochreoprunuloides* [≡ *E. prunuloides* var. *obscurum*], from France, Germany, and United Kingdom.

We here describe the finding of an Italian collection of *E. ochreoprunuloides*, whose nrITS sequence was examined and confirmed as conspecific with the holotype collection.

Materials & methods

Morphology

Fresh basidiomata were photographed in the field using a Nikon D80 digital camera. Microscopic features were examined using a Nikon Eclipse E200 light microscope and observations were made on mounts in Congo red, followed by 5% NH_3 solution.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from two herbarium specimens (*E. ochreoprunuloides* TO 3327; *E. luteobasis* WU 17842) with NaOH (Osmundson et al. 2013); 2 mg of dry sample were homogenized in 250 µl of soda at 0.5M with a pestle. After 5 minutes to allow for

sedimentation, 5 µl of the extract were removed and diluted in 195 µl of 100mM Tris-HCl at pH 8.0, and 1 µl of the dilution was used as template DNA. The nrITS region was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR was performed in 25 µl reaction volumes following Gardes & Bruns (1993).

PCR products were purified and sequenced by IGA Technology Services (Udine, Italy).

Results

The ITS sequence of our Italian collection (TO 3327; GenBank KU984720) shared 99% (582/583) of nucleotides with the German holotype of *E. ochreoprunuloides* (L [E. Arnolds 01-142]; GenBank KC710092) and 100% homology with two independent sequences from an Italian collection labelled as *E. luteobasis* (WU 17842; GenBank KU984721 [this paper], LN850613 [Kokkonen 2015]).

Taxonomy

Entoloma ochreoprunuloides Morgado & Noordel., Persoonia 31: 171. 2013.
FIGS 1A, C–F

≡ *Entoloma prunuloides* var. *obscurum* Arnolds & Noordel., Fungi Europaei vol. 5a: 838. 2005 [non *Entoloma obscurum* Hesler 1967].

SELECTED ICONOGRAPHY: Noordeloos (2004; Pl. 1b, p. 1166), Morgado et al. (2013; Fig. g 1–2, p. 168)

Description of the Tuscan collection (TO 3327)

PILEUS 4–6.5 cm broad, convex, or even conico-convex when young, then more applanate but with a consistently broad (sometimes acute) central umbo; surface fibrillose (even strongly so), greasy; colour sepia brown to ochre, darker in the center, lighter and more brown-yellow at the periphery; hygrophanous; margin irregular, fimbriate, strongly involute, striate. LAMELLAE emarginate to almost adnate, crowded, at first whitish or light grey, then pink. STIPE 5–9 × 0.5–1.4 cm, cylindrical, base ending in a distinctly tapered tip; longitudinally fibrillose, concolourous with the pileus, whitish towards base. CONTEXT rather thin in the pileus, thicker near the umbo, whitish; SMELL intensely mealy (farinaceous), TASTE mealy.

SPORES 6.0–7.6 × 5.4–7.1 µm, av. 6.8 × 6.4 µm, Q = 1.0–1.17(–1.26), Q_{av} = 1.08, somewhat isodiametric, 4–5(–6)-angled, angles blunt under the light microscope. BASIDIA 27–40 × 7–11 µm, 4-spored, clavate. HYMENOPHORAL TRAMA made up of cylindrical to fusoid, parallel hyphae, 35–60 × 4–8 µm. PILEIPELLIS three-layered, with the outermost layer (suprapellis) an ixocutis of very narrow (1.5–2.5 µm diam) hyphae dispersed in a gelatinous matrix, the middle layer (mediopellis) a cutis of parallel cylindrical hyphae (4–6 µm

diam), and the bottom layer (subpellis) of broad, inflated hyphae measuring 25–50 × 15–25 µm. Pigments pale, intracellular, diffuse. Stipitipellis a cutis of narrow (3–5 µm diam) cylindrical hyphae. Caulocystidia absent. Clamp-connections frequent in all tissues.

Habitat densely gregarious to sub-cespitose in a broad-leaved wood composed mainly of *Quercus cerris* L. (Turkey oak; *Fagaceae*) and *Carpinus betulus* L. (hornbeam; *Betulaceae*).

Material studied: *Entoloma ochreoprunuloides*: **ITALY**, **Tuscany**, **Grosseto**, Scansano, Monte Auto, under *Quercus cerris* and *Carpinus betulus*, 6.11.2013, legit M. Clericuzio (TO 3327; GenBank KU984720).

Additional material studied: *Entoloma luteobasis*: **ITALY**, **Apulia**, **Foggia**, Vieste, Val del Tesaro, under *Q. cerris*, 16.11.1997, legit A. Hausknecht & F. Reinwald (WU 17842; GenBank KU984721)

Discussion

The Italian collection of *E. ochreoprunuloides* was made by one of us (MC) in Tuscany (Italy) in a mixed hardwood forest. The micro-morphologic data revealed small, rather rounded isodiametric spores averaging ca. 7 µm; pileipellis hyphae arranged in an ixocutis; abundant clamp-connections; and broad inflated hyphae in the subpellis and hymenophoral trama. All these data supported it as a species of *Entoloma* sect. *Entoloma* (sensu Noordeloos 1981, 1992, 2004). From a macro-morphological point of view, the basidiomes recalled the *E. prunuloides* group but differed from those of a typical *E. prunuloides* not only in pileus colour—sepia ochraceous rather than whitish—but also for their slender aspect (quite unlike the stout and fleshy basidiomes of *E. prunuloides;* Noordeloos 1992, 2004). This collection was therefore assigned to *E. prunuloides* var. *obscurum*, a variety described in 2004 and later raised to species rank and renamed as *E. ochreoprunuloides* (Morgado et al. 2013).

The morphologically closest species to *E. ochreoprunuloides* is *E. luteobasis* Ebert & E. Ludw. (described from Germany and Italy), which differs mainly by its slightly to distinctly yellow or ochraceous stipe base (Ebert et al. 1992, Noordeloos & Hausknecht 1998, Noordeloos 2004). Because the holotype of *E. luteobasis* is missing from the Leiden herbarium (L; Nicolien Sol, pers. comm.), we turned to an Italian collection of *E. luteobasis* (WU 17842) described in Noordeloos & Hausknecht (1998) and Noordeloos (2004). Our ITS sequence analyses revealed that this collection is conspecific with *E. ochreoprunuloides*. This suggests that *E. luteobasis* (proposed in 1992) might be considered an earlier name for *E. ochreoprunuloides* (published in 2013). However, additional collections and sequences of both taxa and further phylogenetic analyses will be required to test this potential synonymy.

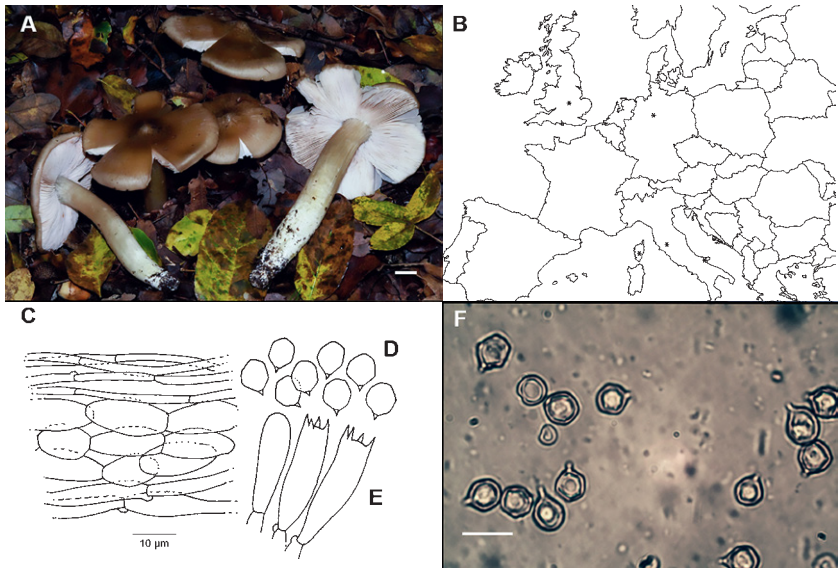

Fig. 1. *Entoloma ochreoprunuloides* (TO 3327). A. Habit. B. Map of *E ochreoprunuloides* distribution. C. Pileipellis. D. Spores: E. Basidia. E. Spores (in water). Scale bars: A = 1 cm; C–F = 10 µm.

From a morphological point of view, delimiting species in the *E. prunuloides-bloxamii* group is no easy task. Morgado et al. (2013) published a box plot of spore dimensions for all the species of the group that showed *E. ochreoprunuloides* spores as among the smallest in the whole section. However, our Tuscan collection falls within the limits of spore size and shape for the species (5.9–7.1 × 5.7–7.2 µm, Q = 1.0–1.16, Q_{av} = 1.04; Morgado et al. 2013). Our data also agree well with the WU 17842 (*E. luteobasis*) figure and spore dimensions in Noordeloos & Hausknecht (1998; 6.6–7.6 × 6.2–7.5 µm, av. 7.0 × 6.6 µm, Q_{av} = 1.05). Spore dimensions can be used to distinguish *E. ochreoprunuloides* from *E. prunuloides* (with a spore average ca. 1 µm larger; Morgado et al. 2013). Typically, *E. prunuloides* tends to be more robust and to have pale (whitish to light ochraceous) tinges on the pileus (Noordeloos 1992, 2004), but it should be stressed that pileus colours are rather variable and should be used only in connection with other characters. In contrast, our collection showed sepia brown ochraceous colours, which are probably the most typical for the species; Noordeloos & Hausknecht (1998) reported a brown (from chocolate brown through orange brown) pileus with a greyish

margin. Morgado et al. (2013) also described a new form (*E. ochreoprunuloides* f. *hyacinthinum*) with violet or pink tinges on both pileus and stipe. DNA sequences from two collections of this form, however, show an almost complete identity with the typical form (Morgado et al. 2013).

With its dark pileus colors and small spores, the Canadian species *E. pseudoprunuloides* is morphologically closer to *E. ochreoprunuloides* than to *E. prunuloides*. However, the phylogenetic analysis by Morgado et al. (2013) placed it as sister to *E. prunuloides*.

Our report widens the distribution of *E. ochreoprunuloides* to include Italy, in addition to Germany (NordRhein-Westfalen, Ibbenbüren, holotype), UK, and France (Corsica). A map with all of the localities is presented in Fig. 1B.

Acknowledgments

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