Exploring the limits of morphological variability and ecological preferences of *Entoloma albotomentosum*

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The first and so far only known collection of *Entoloma albotomentosum* from Slovakia is described and illustrated. Because of discrepancies between various published descriptions of the species, our collection is compared with the material originating from the type collecting site in Austria. According to our observations, both studied specimens are similar in having a thin pileipellis made up of dispersed and narrow hyphal terminations arranged in a cutis or a cutis with transitions to a trichoderm, presence of coralloid hyphae in the pileipellis and spores no shorter than 9 µm. The delimitation of the species, variously interpreted in the literature, is discussed. The knowledge of the occurrence, ecology and threat of *E. albotomentosum* in Europe is also summarised.

Key words: Agaricales, morphology, ecology, Slovakia, Europe.

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Uvádzame opis a ilustrácie prvého a dosiaľ jediného zberu *Entoloma albotomentosum* zo Slovenska. Vzhľadom na nezrovnalosti v publikovaných opisoch druhu, porovnávame náš zber s materiálom z typovej lokality v Rakúsku. Podľa našich pozorovaní sa obe študované položky podobajú tenkou pokožkou klobúka tvorenou rozptýlenými a úzkymi ukončeniami hýf, ktoré formujú kutis alebo kutis prechádzajúci do trichodermu, prítomnosťou koraloidných hýf v pokožke klobúka a spórami nie kratšími ako 9 µm. Diskutujeme o vymedzení druhu, ktoré sa v literatúre rôzni. Sumarizujeme tiež poznatky o výskyte, ekológii a ohrozenosti *E. albotomentosum* v Európe.

INTRODUCTION

Entoloma albotomentosum Noordel. & Hauskn. was described based on material collected in Austria on decaying grass leaves (Noordeloos & Hausknecht 1989). With its small (pileus diameter of up to 10 mm) white basidiomata, usually growing hidden in the grass, it is difficult to find in the field. The stipe is also very small (about $5 \times 0.5 \text{ mm}$), but distinct with its mostly eccentric (as also described

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in the original paper), less frequently lateral or central insertion (Noordeloos 1992, 2004).

More than 20 years after its description, E. albotomentosum has been recorded in various European countries (e.g. Arnolds & Veerkamp 2008, Ebert et al. 1992, Halama 2011, Krieglsteiner 2003, Rocabruna & Tabarés 2001). In Slovakia, we only know E. albotomentosum from our single specimen collected on decaying grasses in 2005, but it has not been published to date.

Our morphological observations on the Slovak collection seem to fit the description in the protologue (Noordeloos & Hausknecht 1989), which is provided with a rather brief description of micromorphological characters but without illustrations of the pileipellis structure. In later publications by the senior author (Noordeloos 1992, 2004), however, the description has not been much improved.

There are two recent publications with detailed morphological descriptions, and both modifying the known limits of the species' variability. Ebert et al. (1992) described distinctly more inflated hyphal terminations in the pileipellis (11–26 μ m wide) and Halama (2011) distinctly shorter (8.86–9.05 μ m) and broader spores (Q = 1.27–1.29). In both cases, these values exceed the variability outlined in the protologue (Noordeloos & Hausknecht 1989) (Tab. 1). Because of these discrepancies in the species profile, doubts arose if our collection with a pileipellis of narrow hyphal terminations (4.5–7.5 μ m wide) arranged in almost a cutis pattern and relatively narrow spores (Q = 1.37–1.64) really represented *E. albotomentosum*.

In our effort to verify the identity of the Slovak collection as well to acquire more information on the morphological variability of *E. albotomentosum*, we opted to study the type material.

MATERIAL AND METHODS

To prove the identity of our single Slovak collection (SLO 798), we asked for the type of *Entoloma albotomentosum*: "Holotypus: Austria, Niederösterreich, Teichholz prope Irnfritz, 8. 10. 1987, A. Hausknecht 2035.2 (WU 7052)" (Noordeloos & Hausknecht 1989). In return, we did not receive the type (as the basidiomata are very small, the holotype is not at disposal for loan due to the high risk of destroying the material), but the following specimen: "Österreich, NÖ, Irnfritz, Teichholz, 7259/3, an *Calamagrostis*, 1989-09-17, A. Hausknecht (WU 8055)". The quadrant of the Central European grid mapping system (MTB) "7259/3" is the same as for the type locality (Noordeloos & Hausknecht 1989). We were also enabled to study two Polish specimens (WRSL 348, WRSL 349) described as *Entoloma albotomentosum* by Halama (2011). The herbarium acronyms follow Thiers (on-line).

The macromorphological characters were observed on fresh material. Dried specimens were used to study micromorphological characters. Microscopic mounts

were prepared in ammoniacal Congo red after a short pre-treatment in a 3% aqueous solution of KOH and observed under an Olympus CX41 or BX41 microscope with an oil-immersion lens at a magnification of $1000\times$. Drawings of all microscopic structures were made with a camera lucida using an Olympus U-DA drawing attachment at a projection scale of $2000\times$. Statistics for measurements of micromorphological characters are based on 20 measurements of basidia and 30 measurements of all other micromorphological characters per specimen. They are given as minimum, maximum (in parentheses), average +/- standard deviation and average (av.) values. Abbreviations: L = number of lamellae reaching the stipe, l = number of lamellulae between each pair of lamellae, Q = ratio of length and width of spores. Descriptive terminology follows Vellinga (1988) and Noordeloos (1992).

Both Slovak and Austrian specimens are in poor condition and do not allow for DNA extraction. They consist of fragments of basidiomata (up to 1 mm in size) and a lens is needed to recognise the individual parts, such as pileus, stipe and hymenophore.

The nomenclature of vascular plants is based on the work by Marhold (1998), of bryophytes by Kubinská & Janovicová (1998).

RESULTS

Entoloma albotomentosum Noordel. & Hauskn., Z. Mykol. 55(1): 32, 1989.

Figs. 1–5

Holotypus: Austria, Niederösterreich, Teichholz prope Irnfritz, 8.10.1987, A. Hausknecht 2035.2 (WU 7052); Paratypus in L.

Description of Slovak collection of Entoloma albotomentosum

Macroscopic characters (Fig. 1). Basidiomata growing in small groups (altogether six basidiomata). Pileus 3–8 mm in diam., circular to irregularly circular, plano-convex; margin straight, undulate to eroded with age, translucently striate when wet; white, later pinkish by maturing spores and lamellae showing through; surface fibrillose, shiny; slightly hygrophanous. Stipe 4–6 \times 0.3–0.5 mm, eccentric, cylindrical or tapering upwards, white, glabrous, at base dirty white and tomentose. Context very thin, whitish, smell indistinct, taste not recorded. Lamellae L = 8–12, l = 1, up to 1 mm wide, also slightly intervenose, linear to subventricose, adnate, white when young, pinkish with age, edge entire, concolorous. Spore print pinkish.



Fig. 1. Entoloma albotomentosum: basidiomata (Slovakia, Laborecká vrchovina Mts., Military Open-Air Museum, 12 Oct 2005, SLO 798). Photo S. Jančovičová.

Microscopic characters (Figs. 2, 3). Spores $(9.2)10.1-11.5(12.2) \times$ (6.2)6.7 - 7.7(8.1) µm, av. 10.8×7.2 µm, Q = (1.23)1.37 - 1.64(1.74), av. Q = 1.51, in side view angular (with 5–7 angles) and often with a suprahilar depression, with pinkish hue in 10% KOH, cell walls indistinctly or distinctly thickened, with a large, but not clearly delimited central vacuole. Basidia (27)28.4–33.2(35) × (10)10.6-12.2(13) µm, av. 30.8×11.4 µm, broadly clavate, 4-spored, at base 3.5–5.5 µm wide. Subhymenium ca. 15–20 µm deep, dense, pseudoparenchymatic, composed of 3–10 µm wide cells, sharply delimited from gill trama hyphae. Marginal cells on gill edge mostly similar to basidioles but shorter, cheilocystidia not clearly differentiated, dispersed, lageniform or fusiform, rarely broadly ellipsoid, occasionally with lateral nodules, measuring $(8)10.5-19.2(22) \times (4)4.4-8.6(14.5)$ μ m, av. 14.8 × 6.5 μ m. Pleurocystidia absent. Pileipellis near margin of cap a cutis, composed of a thin layer of sparse repent, long and narrow thin-walled hyphae, with terminal cells usually very long, ca. 4.5–7.5 µm wide, with obtuse, sometimes subcapitate tips, often branched or nodulose, without any visible pigments; sharply delimited from distinctly wider (7-25 µm) hyphae in the trama; in some

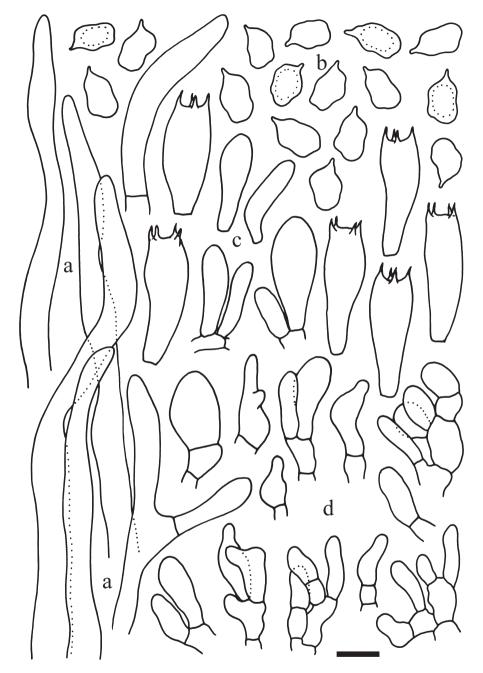


Fig. 2. Entoloma albotomentosum: $\bf a$ – hyphal terminations in stipitipellis, $\bf b$ – spores, $\bf c$ – basidia and basidioles, $\bf d$ – marginal cells and cheilocystidia on gill edge (Slovakia, Laborecká vrchovina Mts., Military Open-Air Museum, 12 Oct 2005, SLO 798). Scale bar equals 10 μ m. Del. S. Adamčík & S. Jančovičová.



Fig. 3. Entoloma albotomentosum: coralloid hyphal terminations in pileipellis (above) and vertical section of pileipellis (cutis) (Slovakia, Laborecká vrchovina Mts., Military Open-Air Museum, 12 Oct 2005, SLO 798). Scale bar equals 10 μm. Del. S. Adamčík & S. Jančovičová.

places (near cap centre?) with coralloid, shorter and more densely packed terminal cells. Stipitipellis composed of repent, cylindrical hyphae with narrowed (4–8 μm) and ascending tips, towards base usually wider, occasionally with lateral branches. Trama of gills, cap and stipe composed of parallel, sparsely branched and anastomosed, ca. 10–35 μm wide and mostly >150 μm long hyphae without incrustations or thickened cell walls. Clamp connections absent in all tissues.

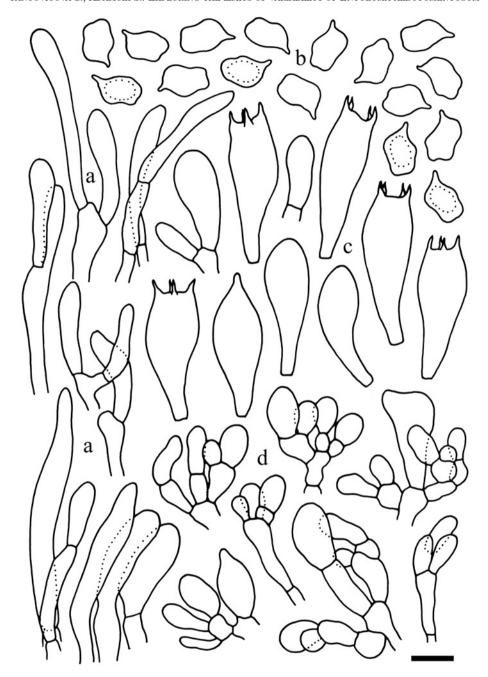


Fig. 4. Entoloma~albotomentosum: a – hyphal terminations in stipitipellis, b – spores, c – basidia and basidioles, d – marginal cells and cheilocystidia on gill edge (Austria, Niederösterreich, Irnfritz, Teichholz, 17 Sep 1989, WU 8055). Scale bar equals 10 μ m. Del. S. Adamčík & S. Jančovičová.

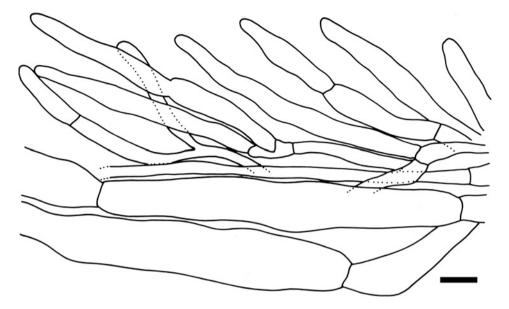


Fig. 5. Entoloma albotomentosum: vertical section of pileipellis (cutis with transitions to a trichoderm) (Austria, Niederösterreich, Irnfritz, Teichholz, 17 Sep 1989, WU 8055). Scale bar equals 10 μm. Del. S. Adamčík & S. Jančovičová.

DISCUSSION

Identity of Entoloma albotomentosum

Our observations on the Austrian specimen (WU 8055; Figs. 4–5) from the type collecting site (topotype) mostly agree with the description of Entoloma~albotomentosum in the protologue (Noordeloos & Hausknecht 1989) and with our observations on the Slovak collection (SLO 798; Figs. 2–3) (Tab. 1). Particularly the characters used for delimitation of the taxon (e.g. Noordeloos 2004) are similar: white basidiomata with eccentric stipe, growth on decaying grass remnants, hyphae without clamp connections, spores ca. $10-11 \times 6.5-8~\mu m$, av. Q = 1.3-1.5 and pileipellis almost of the cutis type made up of 4–12 μm wide hyphae.

The gill edge is according to the original description "fertile or with scattered coralloid hairs in some specimens". We have confirmed the presence of scattered nodulose elements in our Slovak specimen (Fig. 2d), but not in the Austrian one (Fig. 4d). Both specimens have the gill edge covered by (besides scattered basidia) marginal cells (are similar to the basidioles on the gill surface, but shorter) and by dispersed larger ellipsoid, lageniform, pyriform or fusiform elements which might be interpreted as cheilocystidia. The presence and frequency

of coralloid cells might depend on the distance of the observed gill fragment from the cap margin – as demonstrated in some species of the genus *Pseudobaeospora* Singer (Adamčík et al. 2007).

The species epithet "albotomentosum" refers to its white coloration and fibrillose-tomentose covering of the pileus and stipe (Noordeloos & Hausknecht 1989). This macroscopic aspect corresponds to the microscopic structure of the pileipellis of both Slovak and Austrian specimens: it is formed by hyphae of up to 8.5 µm wide which are long repent and loosely arranged contrasting to the voluminous wider hyphae in the trama (7–25 µm). In both studied specimens we also observed coralloid structures similar to those on the gill edge in some parts of the pileipellis (probably in the cap centre) (Fig. 3). The collections differ in the form of hyphal terminations: the topotype bears more densely arranged hyphae with shorter terminal cells (mostly up to 100 µm long) with ascending tips (Fig. 5), while the Slovak collection has filiform and very long terminal cells (often longer than 100 µm) which are repent and sometimes branched or have nodules (Fig. 3). These discrepancies in the pileipellis structure are probably caused by the distance of the studied fragment of the pileipellis from the cap centre, but also by the maturing of the basidiomata and expanding of the pileipellis – a similar pattern has been described in Pseudobaeospora pillodii (Quél.) Wasser (Ronikier & Moreau 2007).

Our observations of two Polish specimens (WRSL 348, WRSL 349), described as Entoloma~albotomentosum by Halama (2011), showed a smaller spore size; our spore measurements are (7.6)8.3–9.9(11.8) × (5.3)5.8–6.9(8.2) µm, av. 9.1 × 6.4 µm, Q = (1.1)1.33–1.55(1.7), av. Q = 1.44. We also observed a similar pileipellis structure as described by the author. The dispersed hyphal terminations are mostly repent or ascending, sometimes occurring in small fascicules, forming a cutis or a cutis with transitions to a trichoderm in some places. The terminal cells of the pileipellis hyphae are mostly distinctly shorter and often clavate, measuring ca. 20–45 × 5–9 µm, which does not agree with our observations on Slovak and Austrian material (Tab. 1). Clear evidence that the Polish collections do not represent E.~albotomentosum is provided by the clamp connections present on the bases of basidia and basidioles which were reported to be missing by Halama (2011).

Comparing published data, we could not confirm some characters described by Ebert et al. (1992), which are also different from the original description of *E. albotomentosum* (see Introduction). The hyphal terminations in the pileipellis described by Ebert et al. (1992) are too inflated and have an inverse aspect compared to the trama hyphae, which are illustrated as being narrower (Tab. 1). This publication is very brief in the description of the pileipellis and there is no mention of presence of coralloid structures. It is also possible that Ebert et al. (1992) described a taxon different from *E. albotomentosum*. However, considering the very subtle structure of the basidiomata of the species with a pileipellis and trama

Tab. 1. Selected microscopic characters observed on studied specimens of Entoloma albotomentosum compared with published data.

	personal observations of the Slovak specimen (SLO 798)	personal observations of the Austrian specimen (WU 8055)	Noordeloos & Hausknecht (1989), Noordeloos (1992)	Ebert et al. (1992)	Halama (2011)
spore length (µm)	(9.2)10.1–11.5(12.2)	(9.2) 9.9–11.2(11.9)	9-12.5	(9)9.5–12(12.5)	(7.23)8.86–9.05(12.14)
spore width (µm)	(6.2)6.7-7.7(8.1)	(6.3)7-8.1(8.6)	6.5–8	(6.5)7.5–9.5	(5.89)6.95 - 7.09(8.87)
ð	(1.23)1.37 - 1.64(1.74)	(1.24)1.32–1.47(1.56)	1.1–1.5	-	(0.97)1.27 - 1.29(1.64)
av. Q	1.51	1.4	1.3	I	ı
stipe	eccentric	-	eccentric	mostly central, rarely eccentric	eccentric
cheilocystidia	not clearly differentiated, dispersed, lageniform or fusiform, occasionally with lateral nodules	not clearly differentiated, dispersed, ellipsoid, pyriform or fusiform	absent or with scattered coralloid hairs in some specimens	absent	absent
pileipellis	cutis made up of a thin layer of narrow and long repent hyphae, terminal cells long, ca. 4.5–7.5 µm wide, often branched or nodulose, in some places shorter and coralloid	cutis with transitions to a trichoderm made up of dispersed repent hyphae with ascending tips, terminal cells cylindrical, (25)32.5–92.5(170) × (5)6.5–8.5(10) µm, in some places shorter and coralloid	cutis with transitions to a trichoderm made up of cylindrical to inflated, 4–12 pm wide hyphae	cutis with transitions to a trichoderm, made up of cylindrical hyphae with narrowly ellipsoid, some- times slightly constricted or narrowly balloon- shaped hyphal ends, 11–26 µm wide	cutis with transitions to a trichoderm made up of cylindrical to inflated, (3.41)7.32–8.13(12.01) µm wide hyphae
clamp connections	absent	absent	absent	not observed	absent

together hardly exceeding 100 µm in depth, we can confirm from our own experience how difficult it is to recognise the correct position and orientation of hyphal structures of this species.

Specimens examined

Entoloma albotomentosum

Austria. NÖ (Niederösterreich), Irnfritz, Teichholz, 7259/3, an *Calamagrostis*, 17 Sep 1989, leg. A. Hausknecht (WU 8055).

Slovakia. Laborecká vrchovina Mts., Military Open-Air Museum, near the village of Vyšný Komárnik, 49°24'17.84" N, 21°41'57.89" E, alt. ca. 420 m, grassland, on debris of decaying grasses (*Poaceae*), 12 Oct 2005, leg. S. Jančovičová (SLO 798).

Entoloma sp.

Poland. Domaszowice (Oleśnica Plain), $51^{\circ}02'59.15''$ N, $17^{\circ}52'42.44''$ E, alt. 163 m, abandoned rural land with scattered Betula sp., with Calamagrostis sp., Rubus sp. and Solidago gigantea in the herb layer, on damp rotten leaf-sheaths and debris of herbaceous plants (Calamagrostis sp.), 14 Aug 2010, leg. M. Halama (WRSL 348, as Entoloma albotomentosum); ibid., 17 Aug 2010 (WRSL 349, as Entoloma albotomentosum).

Distribution, ecology and threat of Entoloma albotomentosum in Europe

By doing a literature search, browsing the internet and based on personal communication, we have gathered data on the occurrence of *Entoloma albotomentosum* in several European countries. As discussed above, identification of the species is tricky and we think that some reports might concern another taxon. Such reports are either misidentifications or based on misinterpreted characters. Considering that a taxonomic revision of *E. albotomentosum* collections is needed, the presented distributional and ecological data are only preliminary.

The species was described based on material from Austria (three collections, including the holotype) and Germany (one collection). The material was collected on dead (also living?) parts (leaves and roots) of *Calamagrostis epigejos* and probably other grasses but also of sedges (*Carex*) in moist stands (riparian vegetation of lakes and ponds), in September and October (Noordeloos & Hausknecht 1989). Since then, other collections of *Entoloma albotomentosum* have been published, e.g. two collections from Austria (both from the type locality) (Noordeloos & Hausknecht 1993) and four from Germany (all from moist stands, e.g. from a stand with *Marchantia polymorpha*, but never with *Sphagnum*, from August to October) (Ebert et al. 1992, Krieglsteiner 2003). The Austrian specimen included in this study, collected on *Calamagrostis* sp. at the type locality in September (WU 8055), has probably not been published yet. In Austria, *E. albotomentosum* is a red-listed species (Krisai-Greilhuber 1999).

Based on personal communication, *E. albotomentosum* was collected once in the Czech Republic on soil and debris of *Scirpus* in a wet meadow (*Calthion*), in September (Egertová, pers. comm.).

General information on the occurrence of the species in the Nordic countries Denmark and Norway is mentioned by Noordeloos (2012). Ludwig (2007) also included Sweden. Six concrete Danish collections from waterlogged stands, found on dead sedges (*Carex*) and grasses, in August and September are registered by the Danish Fungus Atlas (Heilmann-Clausen et al. on-line).

Following the fungal database of Britain and Ireland (Kirk & Cooper on-line), *E. albotomentosum* is known from England (13 collections) and Scotland (1 collection). It was recorded on rotting leaves, stem, roots and soil with *Holcus* sp., *H. mollis*, *H. lanatus*, *Deschampsia cespitosa*, other *Poaceae*, and *Pteridium aquilinum*, mostly in water-meadows, also in woodland edges and open grassy woodland; four collections are from rotten wood (underside of wood, fallen log, semi-buried stump) of *Castanea sativa*, *Acer pseudoplatanus* and other unspecified woody species; the records are from July to October.

Entoloma albotomentosum is registered as a red-listed species in the Netherlands (Arnolds & Veerkamp 2008).

In France, *E. albotomentosum* was collected on decaying culms of *Phragmites australis* in a mineral-rich peat bog, under a large decaying trunk of *Quercus* immersed in strongly rotten wood, and on dead twigs of *Rubus* subgen. *Rubus* (indicated as *Rubus fruticosus* by Moreau, pers. comm.) in a montane *Abies* forest; these 3 collections are from July (Moreau, pers. comm.)

Krieglsteiner (2003) also included Liechtenstein into the list of European countries where *Entoloma albotomentosum* occurs.

From Poland two collections from one locality were published, on abandoned rural land with scattered *Betula* and with *Calamagrostis*, *Rubus* and *Solidago* in the herb layer, on damp rotten leaf-sheaths and debris of herbaceous plants (*Calamagrostis* sp.), in August (Halama 2011). However, these collections belong to another *Entoloma* taxon (see Identity of *Entoloma albotomentosum*).

In Slovakia, our only known collection is from open grassland, found on debris of decaying grasses (*Poaceae*), in October.

From Spain, one collection of *Entoloma albotomentosum* was reported by Rocabruna & Tabarés (2001) growing on decaying plant debris in mixed woodland, associated with *Ilex aquifolium* and some planted conifers, in August. The occurrence of the species in Spain is also mentioned by González et al. (on-line).

Summarising the above-mentioned data, *Entoloma albotomentosum* has been recorded in 11 European countries: Austria, the Czech Republic, Denmark, France, Liechtenstein, the Netherlands, Norway, Slovakia, Spain, Sweden and the United Kingdom. In Austria and the Netherlands it is registered as a red-listed species. Growing saprotrophically, *E. albotomentosum* was recorded on herbaceous substrates, namely leaves, stems and roots, mostly of grasses such as *Calamagrostis* sp., *C. epigejos*, *Deschampsia cespitosa*, *Holcus* sp., *H. mollis*, *H. lanatus*, *Phragmites australis* and other unspecified *Poaceae*, also of sedges (*Carex*) and

the fern *Pteridium aquilinum*. A few records are from woody substrates, namely from rotten wood of *Acer pseudoplatanus*, *Castanea sativa*, *Quercus* sp., other unspecified woody plants, and also from twigs of *Rubus* sp. Until we summarised the ecology data on, we had thought that *Entoloma albotomentosum* was restricted to herbaceous plants (grasses, sedges). It was surprising for us to know about the British and French records from wood. However, as stated at the beginning, a taxonomic revision of the species is needed and the included ecological and distributional data are only preliminary. Because of the limited number of critically revised collections, we must conclude that the ecological preferences of *E. albotomentosum* are unknown. In general, *E. albotomentosum* was reported from grasslands, woodland edges and woodland and within these habitats nearly always on moist stands, e.g. riparian stands, peat-bogs, waterlogged stands. *Entoloma albotomentosum* seems to be a summer to autumn species, hitherto collected from July to October.

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