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## Descriptions of Ectomycorrhizae

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# Entoloma nitidum Quel. plus Carpinus betulus L

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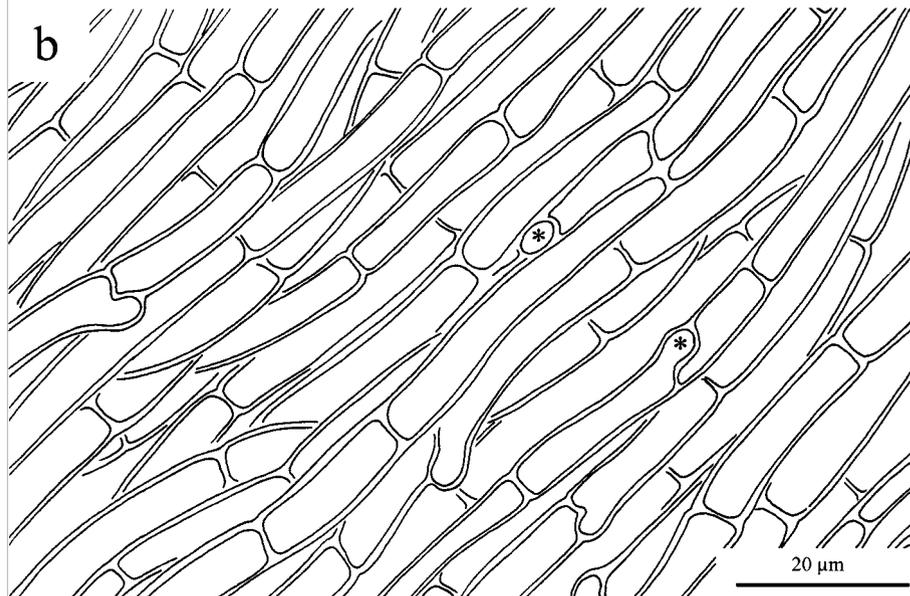
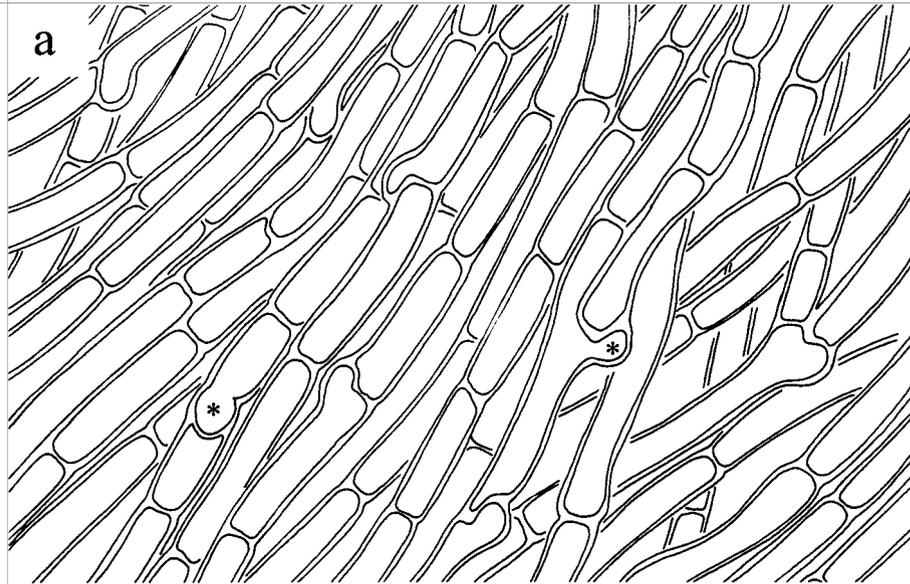


Fig. 1 – *Entoloma nitidum* Quél.+ *Carpinus betulus* L.

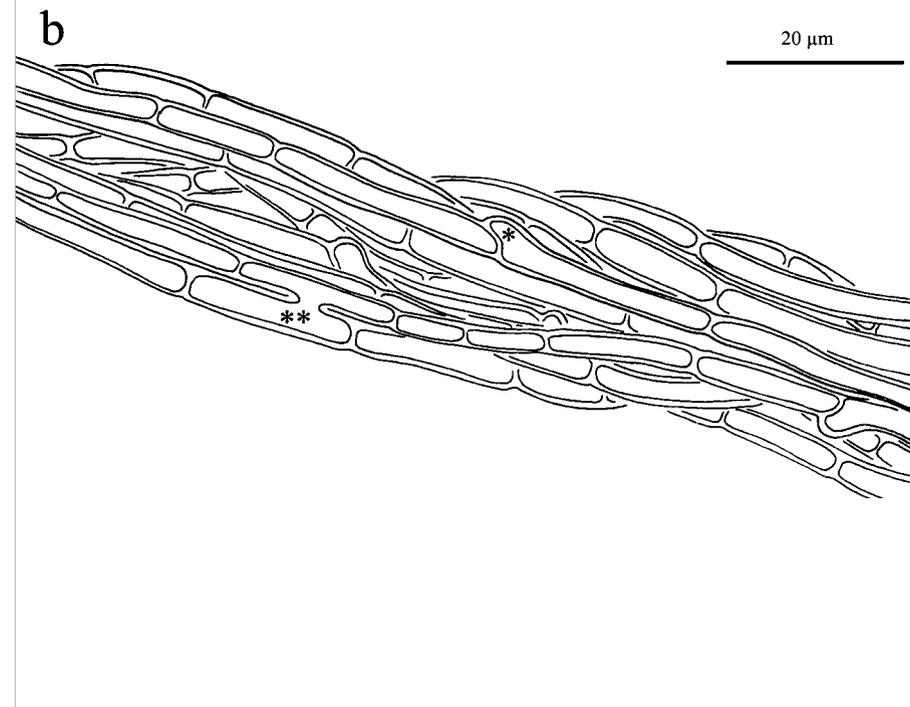
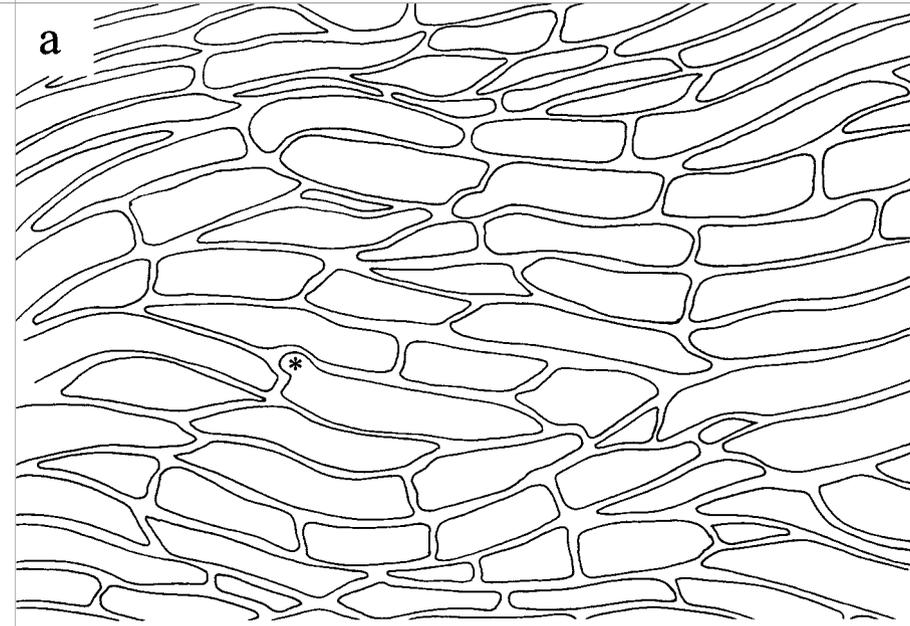


Fig. 1 – *Entoloma nitidum* Quél.+ *Carpinus betulus* L.

***Entoloma nitidum* Quél.  
 + *Carpinus betulus* L.**

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**Short description**

The mycorrhiza is characterized by whitish to yellowish monopodial-pyramidal systems with smooth to loosely woolly surface, plectenchymatous outer mantle layers with clamped and irregularly arranged hyphae, undifferentiated rhizomorphs with smooth margins and paraepidermal Hartig net. These features are similar to those reported for other ectomycorrhizal *Entoloma* species.

**Morphological characters:** Mycorrhizal systems (Fig. 1) abundant, dense, (4)7-46(70) mm long, with monopodial-pyramidal shape; (0)3(5) side branches per 10 mm, 1-2(3) orders of ramification and feebly mild taste. Main axes (0.3)0.5-0.8(0.9) mm in diam. Unramified ends up to 12 mm long, 0.2-0.4(0.5) mm in diam., usually sinuous, rarely straight, bent or tortuous, with cylindric, rarely tapering tip; mycorrhiza yellowish, very tip whitish-yellowish, older parts ochre to yellowish brown. Surface of unramified ends usually smooth, sometimes covered with soil particles, rarely loosely woolly in small areas, with distinctly visible mantle surface, mantle transparent to semi-transparent due to mantle thickness, epidermal cells visible through mantle; emanating hyphae and cystidia lacking. Rhizomorphs abundant, of no specific origin, distinctly connected to mantle, concolourous to mantle, with smooth margins, frequently ramified at restricted points; short mycorrhiza-like outgrowths with blunt tips lacking; dimorphism not observed. Sclerotia not observed.

**Anatomical characters of mantle in plan views** (Figs. 2, 3): Mantle without brownish contents and matrix. Outer mantle layers (Fig. 2a) plectenchymatous, hyphae irregularly arranged, often growing longitudinally with regard to root orientation (type B; AGERER & RAMBOLD 1998); hyphal shape cylindrical, not constricted at septa or ampullate at both sides of septum, cells 1.8-4.1(4.4) µm in diam., (9)12-54(105) µm long, with walls 1.2-1.5 µm thick, smooth with few soil particles, colourless to membranaceously yellow; clamps frequent, simple septa as thick as remaining walls, angles between junctions usually less than 45°; anastomoses open with a short smooth bridge or bridge almost absent, bridge as thick as hyphae, with walls as thick as remaining walls. Middle mantle layers (Fig. 2b) plectenchymatous with broad streaks of parallel hyphae oriented 0-45° compared to those of the outer mantle, hyphae (2.4)6.1(6.8) µm in diam., (20)25(78) µm long, with walls 0.6-1.1 µm thick, smooth, colourless to membranaceously yellowish; clamps frequent, simple septa frequent. Inner mantle layers (Fig. 3a) plectenchymatous with streaks of parallel hyphae oriented as the middle mantle ones, (3.3)3.6-7.9(8.8) µm in diam., (14)39(89) µm long, walls 0.6-1.1 µm thick, colourless to membranaceously yellowish; clamps frequent, simple septa frequent. Very tip: mantle layers arranged like other parts of mantle, cells 1.8-6 µm in diam.

**Anatomical characters of emanating elements** (Fig. 3): Rhizomorphs (Fig. 3b) up to 57.7 µm

thick, undifferentiated with hyphae of uniform diameter, margins rather smooth with hyphae often compactly arranged (Type B; AGERER & RAMBOLD 1998); peripheral hyphae not specialized, (1.6)1.9-2.8(3.6)  $\mu\text{m}$  in diam., (4)9-59  $\mu\text{m}$  long, with walls 0.5-1.1  $\mu\text{m}$  thick, colourless to membranaceously yellowish-brownish, smooth with soil particles mainly concentrated at ramification points; central hyphal cells (1.5)1.9-3.2(3.7)  $\mu\text{m}$  in diam., (6)10-75  $\mu\text{m}$  long, walls 0.8-1.3  $\mu\text{m}$  thick with indistinct pores and septa thick as walls. Clamps in dorsal view oval, as broad as its hypha, in lateral view a semicircle or less, thinner than its hypha, not constricted at contact point to the subtending hyphal cell; simple septa as thick as remaining walls. Crystals lacking. *Emanating hyphae* not observed. *Cystidia* lacking. *Chlamydospores* not observed.

**Anatomical characters, longitudinal section:** Mantle 19-40(46)  $\mu\text{m}$  thick, only outer mantle layers discernible. Outer mantle layers pseudoparenchymatous, (2)5-11(16)  $\mu\text{m}$  thick; hyphal cells (2)2.9-3.5(4.5)  $\mu\text{m}$  radially, (5)21-65  $\mu\text{m}$  tangentially; middle and inner mantle layers plectenchymatous, 12-25  $\mu\text{m}$  thick; hyphal cells 2.1-3.7(4.9)  $\mu\text{m}$  radially, (3.1)4.7-15.1  $\mu\text{m}$  tangentially; mantle at very tip 45-95  $\mu\text{m}$  thick. *Tannin cells* lacking. *Epidermal cells* rectangular to radially-elliptic, oriented obliquely, tangentially (7)9-23  $\mu\text{m}$ , radially (27)35-45  $\mu\text{m}$ ; ECt = 15.4  $\mu\text{m}$ , ECq = 0.26. *Hartig net* paraepidermal up to 2 rows, hyphal cells around epidermal cells roundish to cylindrical, in one row, around epidermal cells 2.3-4.2(5.1)  $\mu\text{m}$  thick; in plan view infrequently lobed, lobes up to 1.3  $\mu\text{m}$  wide. *Haustoria* lacking.

**Anatomical characters, cross-section:** Mantle structure and measures as in longitudinal sections if not otherwise indicated. Outer mantle layers plectenchymatous with hyphal cells tangentially 3-8(11)  $\mu\text{m}$ ; middle and inner mantle layers pseudoparenchymatous with hyphal cells tangentially 2.5-5.5  $\mu\text{m}$ . *Epidermal cells* round to radially-elliptic, round to tangentially-oval the inner ones, tangentially (7)12-31(33)  $\mu\text{m}$ , radially (5)11-23(25)  $\mu\text{m}$ ; ECt = 12.1  $\mu\text{m}$ , ECq = 0.75. *Hartig net* with hyphal cells around epidermal cells cylindrical, around epidermal cells 2.4-3.6(4.4)  $\mu\text{m}$  thick, in plan view lobes up to 2.4  $\mu\text{m}$  wide.

**Colour reaction with different reagents:** mantle preparations: cotton blue: blueish-green; guaiac: pale brown; iron(II)sulfate: no reaction (n.r.); KOH 15%: n.r.; lactic acid: n.r.; Melzer's reagent: n.r.; Sudan III: n.r.; sulfo-vanillin: n.r.; toluidine blue: lilac; trypan blue: pale blue.

**Autofluorescence:** Whole mycorrhiza (hand lamp): UV 254 nm: lacking; UV 366 nm: lacking. Mantle in section: UV-filter 340-380 nm: blue-pale yellow; blue filter 450-490 nm: pale yellow; green filter 530-560 nm: lacking.

**DNA-Analysis:** ITS regions from ribosomal DNA of both the exsiccated carpophore and the mycorrhizae amplified by PCR using ITS 1f and ITS 4 primers (GARDES & BRUNS 1993) show equal total base-pair lengths: 650 bp. Pattern of bands after restriction digest: Alu I: 325 bp, 175 bp, 90 bp, 60 bp; Hinf I: 350 bp, 210 bp, 100 bp (lengths rounded to the nearest 5 bp). Amplified products purified, sequenced and corrected (BUÉE et al. 2005). Differences between carpophore and ectomycorrhizae sequences < 1.54%. Carpophore sequence in direction 5'-3' as follows: TTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATAAACTAGTTGGGTTGTTGCTGGTTCTTAGGGACATGTGCACACCTGGCAAATGTTTTTAACCACCTGTGCACCTTTGTTAGATCTGAAATACTTCTTGAGGAAACTCAGTTTGAGGATTGCTGTGTGAAAA CCAGCTTTTCCTTGCAATTCAGGTCTATGTTTTATATATACTCCAAAAGCAAGTAATAG AATGTTATTATATGGGCCCTTTGAGCCTTTAAACAAATACAACCTTCAACAACGGATC TCTTGGCTCTCGCATCGATGAAGAACGACGACGAAATGCGATAAGTAATGTGAATTG CAGAATTCAGTGAATCATCGAATCTTTGTACGCACCTTGCGCTCCTTGGTATTCCGA GGAGCATGCCTGTTTGAGTGTATGAAATTCTCAACCTTTCTGGTTTTTATTAACCTA GTTTGGCTTGGATTGTGGGAGTTGCTGGCTTCTAAGAAGTCAGCTCTTCTTAAATG CATTAGCAAATCTTTGCTGACCATCTTTGGTGTGATAATTATCTACGTCATTGAG AATCAGCTTTTGTAGATTTAGCTTCTAATCGTCTTTCTTGAGACAACCTATGACAAT TTGACCTCAAATTCAGGTAGAG

**Reference specimen:** Champenoux, France, lat 48°74' N; long 6°35' E, in a coeval, mixed *Quercus robur* and *Q. petraea* forest with *Carpinus betulus* understorey, superficial organic layer, pH 6.2, brown leached soil, 240 m asl; soil core exc. L. Montecchio, 10.02.03, myc. isol. L. Montecchio, 11.02.03; ectomycorrhiza in herb. L. Montecchio LMC1 in Padova; carpophore leg. A. Ferretti, det. M.E. Noordeloos in Baselga di Pinè, Italy, 04.10.02, confirmed by comparing *exiccata* sequence with Blast database (www.ncbi.nlm.nih.gov; AF335449; identity 99%), *exiccata* in herb. P.E. Courty PCEn1 in Champenoux.

**Discussion:** Up to now few investigations have been carried out on *Entoloma* mycorrhizae. The results of the observations reported here show that *E. nitidum* + *Carpinus betulus* has typical ectomycorrhizal features, as previously verified in *E. sericeum* + *Salix rotundifolia* (ANTIBUS et al. 1981), *E. alpicola* + *S. herbacea* (GRAF & BRUNNER 1996) and *E. sinuatum* + *Salix* spec. (AGERER 1997). Unfortunately, *E. erophilum* (ZEROVA & ROZHENKO 1996), *E. clypeatum* (ANDRUSZEWSKA & DOMINIK 1971; ZEROVA & ROZHENKO 1996) and *E. rhodopolium* (MODESS 1941) are too poorly illustrated to be definitely considered ectomycorrhizal, while the parasitic behaviour of *E. saepium* on root meristems of both *Rosa* and *Prunus* is well known (AGERER & WALLER 1993).

*Entoloma nitidum* has typical features in common with *E. sinuatum*, *E. sericeum* and *E. alpicola* such as the combination of systems monopodial-pyramidal, surface whitish-yellowish and smooth to woolly, outer mantle layers plectenchymatous with clamp connections, and Hartig net paraepidermal. Furthermore, as in *E. nitidum*, both *E. sinuatum* and *E. sericeum* have mantle layers with hyphae growing irregularly to parallelly, and *E. sinuatum*, belonging to the taxonomical section of *E. nitidum*, has both middle and inner mantles plectenchymatous, and rhizomorphs undifferentiated with margins smooth to loosely woven and hyphae compactly arranged.

Differently from the other *Entoloma* ectomycorrhizae described to date, *E. nitidum* + *Carpinus betulus* is characterized by epidermal cells visible through the mantle, very abundant rhizomorphs with no specific origin, and Hartig net infrequently lobed.

**Acknowledgements:** The authors are grateful to A. Ferretti and L. Lorenzon for supplying the *E. nitidum* exiccata.

**References:** AGERER R (1997) *Entoloma sinuatum* (Bull.:Fr.) Kummer + *Salix* spec. Descr. Ectomyc 2: 13-18. – AGERER R, RAMBOLD G (1998) DEEMY – a DELTA-based system for characterization and DEtermination od EctoMYcorrhizae, v.1.1. Institute for Systematic Botany, Section Mycologie, University of München. – AGERER R, WALLER K (1993) Mycorrhizae of *Entoloma saepium*: parasitism or symbiosis? Mycorrhiza 3: 145-154. – ANDRUSZEWSKA A, DOMINIK T (1971) Mycorrhiza of *Prunus cerasus* L. x *Rhodophyllus clypeatus* (Bull. ex Fr.) Quéf. Zeszyty Nauk Wyzsz Szkoły Roln Szczecin 37: 3-14. – ANTIBUS RK, CROXDALE JG, MILLER OK, LINKINS AE (1981) Ectomycorrhizal fungi on *Salix rotundifolia* III. Resynthesized mycorrhizal complexes and their surface phosphatase activities. Can J Bot 59: 2458-2465. – BUÉE M, VAIRELLES D, GARBAYE J. 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus sylvatica*) forest subjected to two thinning regimes. Mycorrhiza 15: 235-245. – GARDES M, BRUNS TD (1993) ITS primers with enhanced specificity for basidiomycetes - applications to the identification of mycorrhiza and rusts. Molecular Ecology 2: 113-118. – GRAF F, BRUNNER I (1996) Natural and synthesized ectomycorrhizas of the alpine dwarf willow *Salix herbacea*. Mycorrhiza 6: 227-235. – MODESS O (1941) Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. Symb Bot Upsal 5(1): 1-146. – ZEROVA MY, ROZHENKO GL (1996) *Entoloma erophilum* (Fr.) Karst. and *E. sericeum* (Bull.) Quéf. – Mycorrhizal symbionts of the oak. Ukr Bot Zk 23(4): 87-90.

**Captions:** Fig. 1 – Monopodial-pyramidal, smooth systems with rhizomorphs distinctly connected to mantle, and soil particles. – Fig. 2 – a. Plan view of the outer mantle layer. – b. Plan view of the middle mantle layer. – Fig. 3 – a. Plan view of the inner mantle layer. – b. Plan view of a rhizomorph. Single asterisk indicates a clamp; double asterisk indicates an open anastomosis. All figs. from LMC1 in PD.

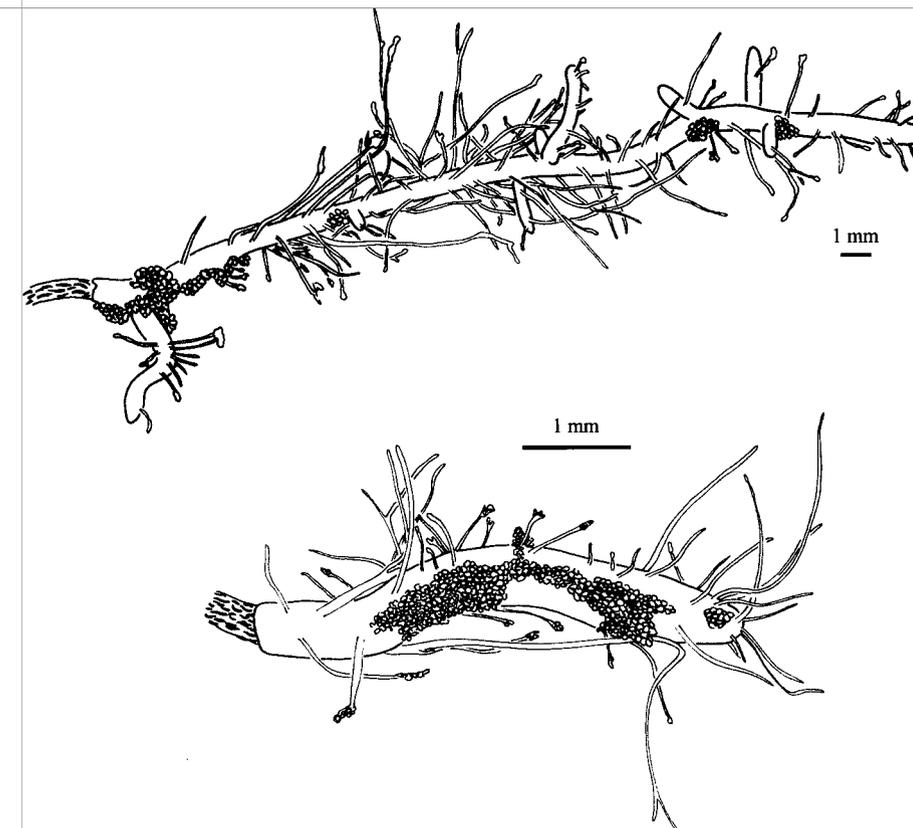


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