

“*Quercirhiza tomentellostellata*”

+ *Quercus suber* L.

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Short description

Mycorrhizal systems of “*Quercirhiza tomentellostellata*” are monopodial-pinnate to -pyramidal in shape and dark brown to black in colour. They form outer mantle layers with roundish to angular cells which bear groups of globose cells with conical wall outgrowths, and become elongated and star-like arranged in middle mantle layers. Inner layers are plectenchymatous with cells varying in shape but mostly elongated, distributed with no discernible pattern alternating with portions of ring-like arrangement. Rhizomorphs are slightly differentiated and produce thin and densely twisted peripheral hyphae. The mycosymbiont of this ectomycorrhiza was identified as a member of the genus *Tomentella* by nrDNA ITS sequence analysis.

Morphological characters (Fig. 1): Mycorrhizal systems dark brown to black, densely arranged in the soil, up to 10 mm long, monopodial-pinnate to -pyramidal and often covered with soil particles. – *Main axes* 0.5 mm diam. – *Unramified ends* straight, slightly bent, some strongly bent, cylindrical, 0.7–3 mm long and 0.25–0.4 mm diam. – *Surface of unramified ends* densely grainy, distinct, mantle not transparent, cortical cells not visible; emanating hyphae colourless, infrequent and not specifically distributed. – *Rhizomorphs* not very frequent, dark brown, smooth, round in cross-section, connected with the mantle surface in a distinct point and ramified at restricted points. – *Cystidia* lacking. – *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Fig. 2): Mantle hyphae contents and cell walls brownish, walls and septa 0.5 µm thick; blue granules and gelatinous matrix absent. – *Outer mantle layers* (Fig. 2a) built by roundish to angular cells, rosette-like arranged, with regions bearing groups of globose cells with conical warts (type K, according to AGERER 1987–2006, AGERER & RAMBOLD 2004–2007), cells of mounds 20–30 x 30–45 µm, walls 0.4 µm thick, outer mantle cells 7–12 x 18–21 µm; surface smooth but in some regions amorphous yellowish brown exuded material with adhering soil particles present. – *Middle mantle layers* pseudoparenchymatous with angular cells, rosette-like arranged, 13–22 x 11–29 µm. – *Inner mantle layers* plectenchymatous with no discernible pattern but in some areas with ring-like pattern; hyphal cells variable in shape: (i) cylindrical and slightly constricted at septum (most frequent), (ii) irregularly shaped, (iii) roundish (least frequent); cells of cylindrical hyphae 28–84 µm long and 5–7(8) µm diam., irregularly shaped hyphae 6–12 µm diam., roundish cells 4–9 µm diam.; clamps present. – *Very tip* with similar features as remaining mantle areas.

Anatomical characters of emanating elements (Figs. 1, 3): *Rhizomorphs* slightly differentiated in structure, with 2–3 central thicker hyphae, non-vessel-like, surrounded by cylindrical undifferentiated hyphae, and by thin and densely twisted peripheral hyphae (type C, AGERER 1987–2006); nodia and ramifications with conical young side-branches present, with one or two side branches at nodium, hyphal contents and walls yellowish brown; central non-vessel-like hyphae 4.6–5.2 µm diam., straight; surrounding hyphae 2.2–3.4 µm diam., sinuous but becoming rather straight with depth; peripheral, twisted hyphae 1–2 µm diam.; hyphal cells of peripheral hyphae shorter than the hyphal cells of central non-vessel-like hyphae and of those surrounding the central ones. – *Emanating hyphae* on mantle and rhizomorph surface 2.4–3 µm diam., 20–140 µm long, clamped, plasmatically yellowish to light brown, straight to bent, slightly constricted at septa, with simple cylindrical apical ends, walls and septa even in thickness, 0.3–0.7(1) µm thick; simple septa present but infrequent, clamps oval in dorsal view, in lateral view as a semicircle, as broad as their hypha, reversely oriented clamps not observed; ramifications Y-shaped with one side branch, backwards oriented ramifications not observed; surface smooth, without any crystals or other appositions. *Cystidia* lacking. – *Chlamydospores* lacking.

Colour reaction with different reagents: *Mantle preparations:* Melzer’s reagent: amyloid reaction of some wall patches of mantle hyphae; guaiac: n.r. (= no reaction); iron (II) sulphate: n.r.; KOH 15%: n.r.; lactic acid: n.r.; sulpho-vanillin: n.r.; toluidine blue: n.r.

Autofluorescence: Not tested.

DNA-Analysis: For methods compare “*Quercirhiza tomentellostellata*” (Azul et al. 2008). The sequence has been lodged in the EMBL database with the accession number AM924147. The sequence of “*Quercirhiza tomentellostellata*” showed the highest similarity to the sequences of Thelephoraceae. It clustered together (E-value 0.0) with the *Tomentella stuposa* clade of the UNITE database (KOLJALG et al. 2005), differing by 0.5% (3 of 600 sites) from the sequence UDB002428 with the alignment score of 1070 base pairs, and differing by 0.4% (2 of 556 sites) from both sequences UDB00245 and UDB00244, with the alignment score of 952 base pairs.

Reference specimen for *Quercus ectomycorrhiza*: Portugal, Distrito de Évora, Concelho de Montemor-o-Novo, Freguesia de Lavre, Herdade Freixo do Meio (latitude N 38°41'10", longitude W 8°20'23"), in managed oak woodland dominated by *Quercus suber* L., soil core exc. and myc. isol. AM Azul, 13.11.2004, AAM 1123/04 (in COI). Etymology: the epitheton *tomentellostellata* refers to the genus *Tomentella* and the star-like arrangement of cells in the middle mantle layers.

Discussion: This ectomycorrhiza reveals similarity to “*Quercirhiza tomentelloflexuosa*” on *Quercus suber* L. (AZUL et al. 2006), “*Quercirhiza cumulosa*” on *Quercus ilex* L. (DE ROMAN et al. 2002) and *Tomentella stuposa* (Link) Stalpers on *Quercus cerris* L. (JAKUCS et al. 2005), regarding the structure of outer mantle layers, the presence of clamped emanating hyphae and rhizomorphs.

“*Q. tomentelloflexuosa*” exhibits angular to irregularly shaped outer mantle cells but does not form heaps of cells on the mantle surface, has a transient organization between plectenchymatous and pseudoparenchymatous in middle layers and a plectenchymatous inner mantle layer with hyphae mostly cylindrical and ring-like distributed. In contrary, “*Q. tomentellostellata*” forms an outer mantle layer with roundish to angular cells composing rosettes, which bear groups of globose cells on the mantle surface, and the middle mantle layer is pseudoparenchymatous also with rosette-like arranged angular cells. The rhizomorphs of “*Q. tomentelloflexuosa*” are slightly differentiated and possess distinctly sinuous hyphae below the peripheral thin and branched hyphae, whereas in “*Q. tomentellostellata*” the central hyphae are covered by thin, densely twisted ones.

In contrast to "*Q. tomentellostellata*", the second similar type, "*Q. cumulosa*", forms emanating hyphae with hemispherical warts, its rhizomorphs have enlarged, thick-walled central hyphae, but do not possess peripheral twisted or curled thin hyphae, mantle hyphae have pseudoparenchyma organization, with angular cells of 9-15 µm diam., and mounds of flattened cells of 9-16(18) µm diam., clearly thicker walls, 0.5-1 µm thick, and the inner mantle layers are mostly pseudoparenchymatous or with a transitional character to a plectenchyma.

Morphologically and anatomically "*Quercirhiza tomentellostellata*" closely resembles *Tomentella stiposa* on *Quercus cerris* L. described by JAKUČS et al. (2005). Both ectomycorrhizae possess pseudoparenchymatous outer mantle layers bearing heaps of globose cells that form minute conical cell wall outgrowths, rather irregular middle layers with elongated angular cells organized in star-like nests, plectenchymatous inner layers, clamped emanating hyphae and they both lack cystidia. This similarity is also confirmed by nrDNA ITS sequencing analysis, since "*Q. tomentellostellata*" and *T. stiposa* clustered together (see the information on DNA analysis above). However being closely related to *T. stiposa*, "*Q. tomentellostellata*" ectomycorrhizae might be formed by different species because of the differences in the structure of rhizomorphs: in case of "*Q. tomentellostellata*" they are slightly differentiated with 2-3 central thicker hyphae, surrounded by cylindrical undifferentiated hyphae, and by thin and densely twisted peripheral hyphae, while *T. stiposa* shows rhizomorphs internally not differentiated (type B, AGERER 1987-2006). Blue granules have also been observed in *T. stiposa* ectomycorrhizae but were absent in case of "*Q. tomentellostellata*". *T. stiposa* is apparently very homogeneous regarding fruitbody features, but perhaps highly variable with respect to ectomycorrhizae (JAKUČS et al. 2005, AGERER & RAMBOLD 2004-2008). New information is necessary to clarify the identity of the mycobiont forming "*Q. tomentellostellata*".

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Captions: Fig. 1. - Habit; mycorrhizal system monopodial-pyramidal with branched rhizomorphs occurring on the main axis and connected distinctly with mantle surface. Fig. 2. - a. Plan view of outer mantle layer; roundish to angular hyphal cells bearing globose cells on mantle surface. - b. Plan view of middle mantle layer with angular cells; hyphae rosette-like arranged. - c. Plan view of inner mantle layer; cylindrical hyphae with regions ring-like arranged. - Fig. 3. Plan views of rhizomorphs. - a. Ramification region, surface view. - b. Ramification region, as seen from the opposite side in comparison to 'a'. - c. Young rhizomorph surface with thin and densely twisted peripheral hyphae. - d. Young rhizomorph, from middle layers to surface illustrating a central thicker, non-vessel-like hypha, cylindrical undifferentiated hyphae and the transition to the thin and densely twisted peripheral hyphae. All figs. from AAM 1123/04 (in COI). Bar for Fig.1: 2 mm, bar for Fig. 2 and 3: 10 µm.

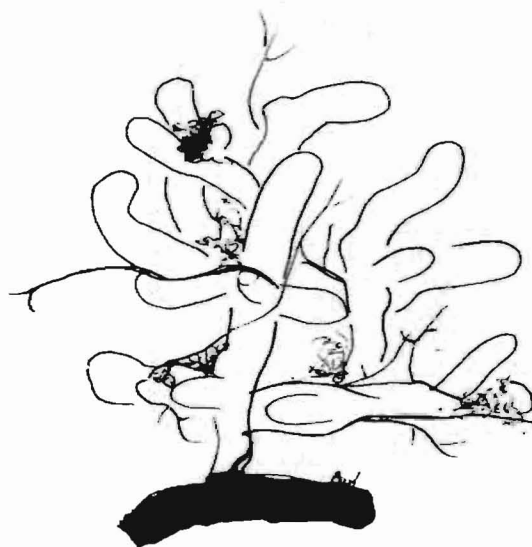


Fig. 1 - "*Quercirhiza tomentellostellata*" + *Quercus suber*

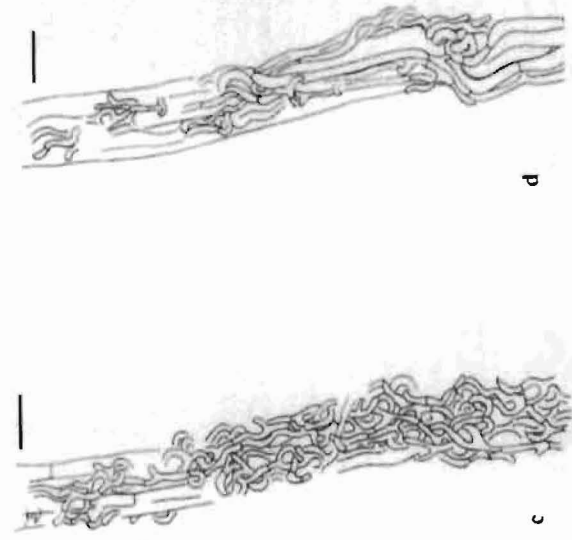
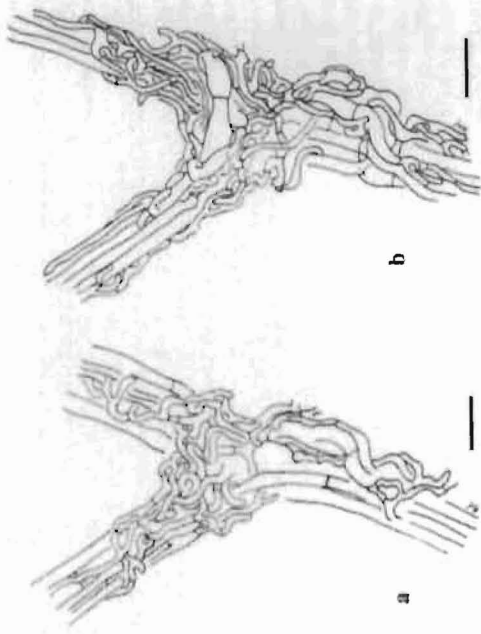
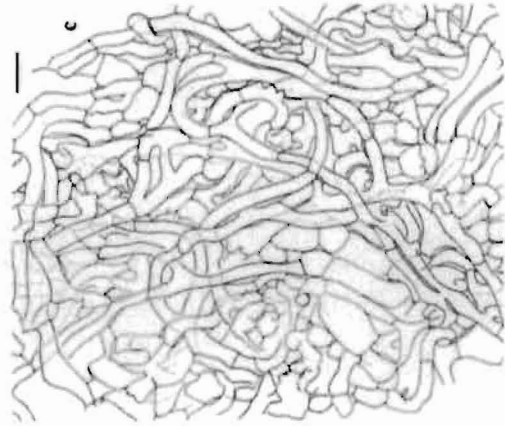
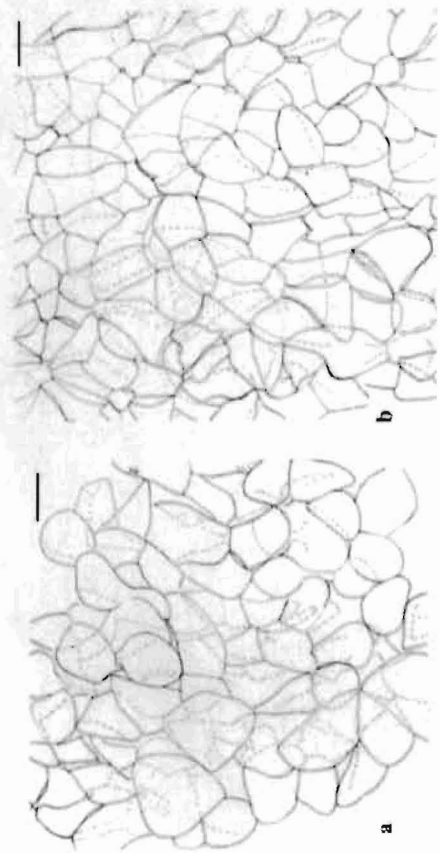


Fig. 2 - "*Quercirhiza tomentellostellata*" + *Quercus suber*

Fig. 3 - "*Quercirhiza tomentellostellata*" + *Quercus suber*