

"Quercirhiza flavocystidiata"

+ *Quercus suber* L.

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Short description

This ectomycorrhiza shows, a cover of bright yellow cystidia (appearing fluorescent in surface light), causing a short-spiny surface, on a dark brown mantle. Mantle plectenchymatous in all layers, outer and middle layers star-like, inner layers plectenchymatous with sinuous hyphae. Cystidia similar to normal hyphae, but fusiform and clamped at the base. Rhizomorphs frequent, slightly differentiated, occurring on mycorrhizal systems and in the soil, brown to dark brown, ramified at restricted points, conical-young side branches present, cystidia on rhizomorphs lacking. Sclerotia lacking.

Morphological characters (Fig. 1): Mycorrhizal systems monopodial-pyramidal, up to 18 mm long, 0-2 orders of ramification, usually dense, with ca. 42-50 side-branches per 10 mm, sometimes covered with few soil particles. – Main axes 0.35-0.5(0.6) mm diam. – Unramified ends almost straight, infrequently bent, cylindrical, not infl-

shaped cells: filamentous cells (7) 1-27 µm long and 3-5.2 µm diam., walls and septa 0.5 µm thick, clamps not observed. - Very tip plectenchymatous like the other parts of the mantle, not gelatinous, with hypha-like cystidia.

Anatomical characters of emanating elements (Fig. 3): - Rhizomorphs up to 45 µm diam., slightly differentiated, with 1-2 central hyphae non vessel-like (type C according to AGERER 1987-2002, AGERER & RAMBOLD 1998) and non-gelatinous matrix; conical young side-branches not found, hyphal cells plasmatically brownish and membranaceously yellowish brown, walls 0.3-0.5 µm thick; young rhizomorph undifferentiated and loosely organized, up to 17 µm diam., with 15-18 hyphae, hyphal cells 2.5-3.5(5) µm diam. and 13-38(49) µm long, hyphae rather sinuous and loosely distributed; older rhizomorphs 25-45 µm diam., more sinuous in structure and slightly differentiated, with 1-2 central hypha, 3.5-5(6) µm diam. and remaining central hypha 2.5-4(5) µm diam., rather irregularly sinuous distributed; surface smooth but occasionally with soil particles adhered; crystals, contents or drops of exuded pigment lacking; emanating hyphae not very frequent. - *Emanating hyphae* on the mantle and rhizomorphs, straight to bent, cylindrical, with elbow-like structures, 2-3 µm diam., cells 57-94 µm long, plasmatically and membranaceously yellowish brown, walls and septa 0.5 µm thick, clamps present, secondary septa lacking, apical ends simple, cylindrical, and with walls similarly thick walls as in remaining parts of the hyphae; ramifications mostly close to the septa, forming an angle of approximately 90° and 1-2 side-branches below the septum, backwards oriented ramifications present, intrahyphal hyphae absent; surface smooth, contents or drops of exuded pigment lacking. - *Cystidia* all similar in structure and similar to normal hyphal ends, cystidia 36-47 µm long, 3-3.4 µm diam. at the base and 2.8-3.1 µm diam. at the tip, slightly fusiform, clamped at the base (type F according to AGERER 1987-2002, AGERER & RAMBOLD 1998); walls up to 0.4 µm thick. - *Sclerotia* lacking. - *Chlamydospores* not observed.

Colour reaction with different reagents: *Mantle and rhizomorph preparations*: Melzer's reagent: n.r. (= no reaction); guaiac: n.r.; 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: DNA of the mycorrhizae on cork oak roots was extracted using the E.Z.N.A Fungal DNA MiniPrep Kit (Omega Biotech, Doraville, USA). ITS nrDNA was amplified by PCR using the primer pair ITS1F and ITS4 (WHITE *et al.* 1990, GARDNER & BRUNS 1993). PCR reactions were done using individual reactions to a final volume of 25 µl with Ready-To-Go PCR Beads (Amersham-Pharmacia Biotech) as mentioned in Winka *et al.* (1998) with a final DNA concentration around 0.25 ng/µl. The PCR product was purified using QIAquick Gel PCR purification kit (QIAGEN Inc., Chatsworth, CA, USA) according to the manufacturer's instructions. Fragments were sequenced using the same primers mentioned above at the DNA Automatic Sequencing Service (SSAD, CIB-CSIIC, Madrid, Spain).

Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA) was used to recognize the consensus sequence from the two strands of the ITS nrDNA. The new sequence has been lodged in the EMBL database with the accession number AJ972894. A nucleotide-nucleotide search (BLAST) was done at the National Center of Biological Information (NCBI) using BLAST (ALTSCHUL *et al.* 1997). The sequence of "*Quercirhiza flavocystidiata*" (AJ972894) matched perfectly with more than 100 Thelephoraceae sequences (E-value 0.0), which means the probability of the match appearing by chance ensure that this ectomycorrhiza belongs to Thelephoraceae family. The higher alignment score, 787 in this situation, occurs with sequence AJ534914, *Tomentella* sp. J54 (TEDERSOO *et al.* 2003).

Reference specimen for *Quercus ectomycorrhiza*: Portugal, Distrito de Setúbal, Concelho de Alcâcer do Sal, Freguesia de St. Susana, Herdade Serra Mendes de Cima (latitude 38°32'02", longitude 8°23'17"), on

cork oak forest, on *Quercus suber* L., soil core exc. AM Azul, 16.05.2000, myc. isol. AM Azul, mycorrhizae AAM 162/00 (in COI). - *Etymology*: the epitheton *flavocystidiata* is due to the colour of the cystidia.

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Discussion: "*Quercirhiza flavocystidiata*" is the only known dark brown ectomycorrhiza with the following combination of features: bright, fluorescent-like yellow, hypha-like cystidia on a plectenchymatous mantle with ring-like hyphal arrangement, pseudoparenchymatous middle mantle layer with star-like arranged angular cells and emanating hyphae with clamps (AGERER & RAMBOLD 2004-2005). Most of the tomentelloid species described until now are brownish or blackish brown (AGERER & RAMBOLD 2004-2005; JAKUCS *et al.*, 2005), but with great differences concerned to the mantle pattern and rhizomorphs organization. The morphological and anatomical features resemble ectomycorrhizae formed by tomentelloid species. The DNA-analysis revealed that "*Q. flavocystidiata*" sequence matched perfectly with more than 100 Thelephoraceae sequences (E-value 0.0). Thus it can be assumed "*Q. flavocystidiata*" is new ectomycorrhiza formed by different *Tomentella* species.

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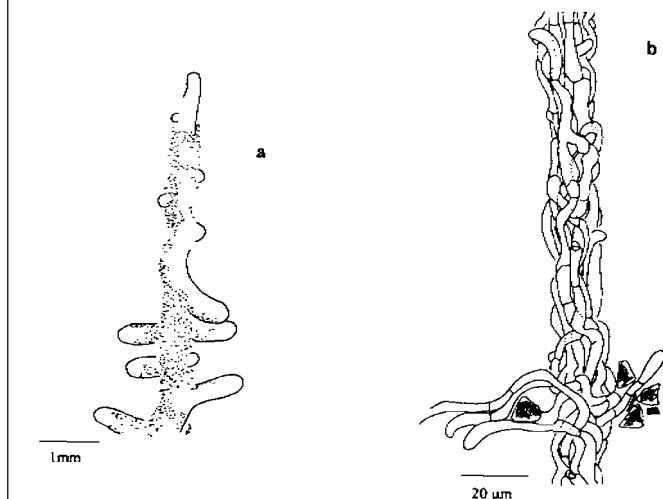


Fig. 1 - "*Quercirhiza flavocystidiata*" + *Quercus suber* L.

48

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49

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50

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51

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