

“*Quercirhiza tomentelloccumulata*”

+ *Quercus suber* L.

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Short description

Mycorrhizae blackish, rough, with soil particles sticking to the mantle. Emanating hyphae dark brown, distributed unevenly. Mantle in outer and middle layers pseudoparenchymatous, with roundish cells. Inner layers with undifferentiated hyphae distributed with no discernible pattern alternating with ring-like arranged regions. Outer mantle layers with roundish cells overlaying groups of 2–6 cells. Emanating hyphae densely warty.

Morphological characters (Fig. 1): Mycorrhizal systems monopodial-pinnate to monopodial-pyramidal, 0–1 orders of ramification, 4–7(11) mm long, with ca. 16–25 side-branches per 10 mm, usually not dense and infrequent. – Main axes (0.3)0.4–0.6(0.65) mm diam. – Unramified ends blackish, cylindrical, up to 2.5(3) mm long, 0.25–0.35 mm diam.; not carbonizing. – Surface of unramified ends rough, distinct, not transparent, cortical cells not visible; soil particles sticking to the mantle; emanating hyphae dark brown and not specifically distributed. – Rhizomorphs lacking. – Cystidia lacking. – Sclerotia not observed.

Anatomical characters of mantle in plan views (Fig. 2): Outer and middle mantle layers pseudoparenchymatous, both with roundish cell walls and plasmatically brownish, clamps, exudates and gelatinous matrix absent. – Outer mantle layers with roundish cells, with groups of cells overlaying the outer layer (type K according to AGERER 1987-2006, AGERER & RAMBOIN 2004-2007) (Figs. 2b, c); mantle cells 15.5–25(30) µm long and 10–22(27) µm diam., with brownish contents, walls 0.5–1 µm thick and septa with dolipore like structures. – Middle mantle layers smooth and denser, with roundish cells 15–30 µm long, 19–26(35) µm diam., more irregular shaped in depth (Fig. 2d) and uniformly distributed; walls and septa 0.5–0.7 µm thick, brownish contents present. – Inner mantle layers plectenchymatous with cylindrical and undifferentiated hyphae, distributed with no discernible pattern, alternating with ring-like arranged hyphae, isolated roundish cells present (Fig. 2e); hyphal cells 37–97 µm long and 4–5.3 µm diam., walls and septa 0.5 µm thick, isolated roundish cells up to 5–7.5 µm diam. – Very tip with features as main areas.

Anatomical characters of emanating elements (Figs. 2a): Rhizomorphs lacking. Emanating hyphae (Fig. 2a) straight, 6.5–7.5(8) µm diam., plasmatically and cell walls brownish, walls 1 µm thick, apical ends simple and roundish; with clamps and infrequent simple septa, clamp in dorsal view thinner than its hypha, oval shape; intrahyphal hyphae not observed; anastomoses

abundant and diverse: 1) open with a long bridge (up to 42 µm long), 2) closed by a septum with long bridge, and 3) open or closed with very short bridge; ramifications abundant; backwards oriented ramifications not observed; surface densely warty, younger hyphae less frequently rough. – Cystidia lacking. – Chlamydospores lacking.

Colour reaction with different reagents: Mantle 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 ectomycorrhizae on cork oak roots was extracted using the EZNA Fungi DNA miniprep kit (Omega Biotek, Doraville, USA) according to MARTÍN & GARCÍA-FIGUERES (1999). DNA was resuspended in pre-warmed Sterile Ultra Pure Water (Q-BIOgene, France). ITS nrDNA was amplified by nested PCR using the primer pair ITS1F/ITS4B (GARDES & BRUNS 1993) followed by ITS1/ITS4 (WHITT et al. 1990). Amplification reactions were done using individual reactions to a final volume of 25 µl with Ready-To-Go PCR Beads (Amersham Biosciences, Little Chalfont Buckinghamshire, England) as mentioned in WISKA et al. (1998). The PCR amplifications were performed in a Perkin-Elmer GeneAmp PCR System 9700 (Applied Biosystem, Foster City, California, USA). Amplification products were cleaned using QIAquick Gel PCR purification kit (QIAGEN, Valencia, California, USA). When more than 20 ng/µl were obtained, both strands were sequenced separately using primers ITS1F or ITS1 and ITS4, with an ABI PRISM 3700 and the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase (Applied Biosystem) at the DNA Automatic Sequencing Service (SSAD, CIB-CSIC, Madrid, Spain). Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA) was used to identify the consensus sequence from the two strands. The new sequences have been lodged in the EMBL database with the accession number AM92140.

The sequence of “*Q. tomentelloccumulata*” differed by 1% from *Tomentella badia* (UDB001656) (2 of 535 sites), by 5% from *Tomentella badia* (UDB000961) (5 of 519 sites) and by 5% from *Tomentella atramentaria* (UDB000235) (8 of 521 sites). UNITE database (<http://unite.ut.ee/>).

Reference specimen for *Quercus ectomycorrhiza*: Portugal, Concelho de Santiago do Cacém, (N38°03'11", W8°33'24"), in managed oak woodland dominated by *Quercus suber* L., soil core exc. AM Azul, 17.03.2000, myc. isol. AM Azul, mycorrhizae AAM 134/00 (in COI). – **Further material studied:** 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. AM Azul, 20.07.2000, myc. isol. AM Azul, mycorrhizae AAM 134/00-II (in COI) and soil core exc. AM Azul, 03.05.2004, myc. isol. AM Azul, mycorrhizae AAM 369/04 (in COI).

Etymology: the epitheton *tomentelloccumulata* is related to the outer mantle layers with roundish cells overlaying groups of 2–6 cells and to the suggested fungal partner of the genus *Tomentella*.

Discussion: “*Quercirhiza tomentelloccumulata*” was identified by morphological and anatomical features as an ectomycorrhiza formed by a *Tomentella* species, which was confirmed by nrDNA ITS sequencing analysis.

“*Quercirhiza tomentelloccumulata*” reveals high similarity with “*Quercirhiza cumulosa*” on *Quercus ilex* L. subsp. *ballota* (DE ROMAN et al. 2002a), particularly due to pseudoparenchymatous outer and middle mantle layers, presence of hemispherical warts on emanating hyphae and absence of cystidia. However, the two morphotypes are very likely two different *Tomentella* species, because “*Q. tomentelloccumulata*” shows pseudoparenchymatous outer mantle layers with roundish cells and mounds of roundish cells (Figs. 2b, c), while the outer mantle layers of

Quercirhiza cumulosa is described as pseudoparenchymatous with angular cells and mounds of flattened cells (type O, AGERER 1987-2006).

The genus *Tomentella* (order Thelephorales) has a worldwide distribution but is still largely unexplored. There are more than forty *Tomentella* mycorrhizae described, associated with gymnosperms, woody angiosperms and orchids (AGERER 1996, AZUL et al. 1999, AZUL et al. 2006 a-g, AZUL et al. 2008 a-d, BRAND 1991, DANIELSON & PRUDEN 1989, DE ROMÁN et al. 2002 a, b, JAKUCS et al. 1997, JAKUCS et al. 2005, JAKUCS & AGERER 2001, KÖLJALG et al. 2000, KÖLJALG et al. 2001, KÖLJALG et al. 2002, RAIDL & MÜLLER 1996). Most of the *Tomentella* ECM described so far are dark brown, but exhibit high diversity regarding mantle and emanating element structure and organization. The main features to distinguish *Tomentella* ectomycorrhizae were extensively described by AGERER (2006).

A great diversity regarding mantle and emanating element structure and organization was found among the thelephoroid mycorrhizae observed in European Mediterranean oak woodlands. Sixteen tomentelloid mycorrhizae have been described in detail on the Iberian Peninsula, 14 associated with *Quercus suber* L. and two associated with *Quercus ilex* L. Table 1 summarizes the diagnostic characteristics reported for tomentelloid mycorrhizae observed in European Mediterranean oak woodlands. Rhizomorphs, when formed on ectomycorrhizae, reveal important information. In case of the tomentelloid ectomycorrhizae, the rhizomorphs described so far exhibit a slightly differentiated structure (type C, according to AGERER 1987-2006). Some structural differences are reported for the peripheral hyphae, which vary from a few loosely entwined and multiply-branched e.g., "*Q. tomentelloflexuosa*" (AZUL et al. 2006f) to densely entwined and multiply-branched, e.g., "*Q. nodulosomorpha*" (AZUL et al. 1999), *Q. tomentellofuniculosa* (AZUL et al. 2006g) and "*Q. tomentellostellata*" (AZUL et al. 2008a).

Diagnostic features	Ectomycorrhizae	Host	Reference
pseudoparenchymatous, with blue granules, clamps and cystidia	<i>Q. ateracusrugosa</i>	<i>Q. suber</i>	AZUL et al. 2006a
	<i>Q. nodulosomorpha</i>	<i>Q. suber</i>	AZUL et al. 1999
pseudoparenchymatous, with clamps and cystidia present	<i>Tomentella galzinii</i> Bourd.	<i>Q. suber</i>	AZUL 2002
	<i>T. pilosa</i> (Burt) Bourdot & Galzin	<i>Q. suber</i>	AZUL 2002
	<i>Q. auraterocystidiata</i>	<i>Q. suber</i>	AZUL et al. 2006b
	<i>Q. tomentellocumulata</i>	<i>Q. suber</i>	this study
pseudoparenchymatous, with clamps and cystidia lacking	<i>Q. summatriangularis</i>	<i>Q. suber</i>	AZUL et al. 2006d
	<i>Q. tomentelloflexuosa</i>	<i>Q. suber</i>	AZUL et al. 2006f
	<i>Q. cumulosa</i>	<i>Q. ilex</i>	DE ROMÁN et al. 2002a
	<i>Q. stellata</i>	<i>Q. ilex</i>	DE ROMÁN et al. 2002b
	<i>Q. tomentellostellata</i>	<i>Q. suber</i>	AZUL et al. 2008a
	<i>Q. flavocystidiata</i>	<i>Q. suber</i>	AZUL et al. 2006c
plectenchymatous with ring-like to star-like arranged hyphae, clamps, cystidia and rhizomorphs present or lacking	<i>Q. tomentellocystidiata</i>	<i>Q. suber</i>	AZUL et al. 2006e
	<i>Q. tomentellofuniculosa</i>	<i>Q. suber</i>	AZUL et al. 2006g
	<i>Q. tomentelloreticulata</i>	<i>Q. suber</i>	AZUL et al. 2008b
	<i>Q. tomentelloepidermoidea</i>	<i>Q. suber</i>	AZUL et al. 2008c

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update: 2007-05-02]. DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de, München, Germany. – AGERER R (1996) Ectomycorrhizae of *Tomentella albomarginata* (Thelephoraceae) on Scots pine. Mycorrhiza 6: 1-7. – AGERER R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progr 5: 67-107. – AZUL AM, AGERER R, FREITAS H (1999) "*Quercirhiza nodulosomorpha*" + *Quercus suber* L. Descri Ectomyc 4: 103-108. – AZUL AM, AGERER R, FREITAS H (2006a) "*Quercirhiza ateracusrugosa*" + *Quercus suber* L. Descri Ectomyc 9/10: 75-79. – AZUL AM, MARTIN MP, AGERER R, FREITAS H (2006b) "*Quercirhiza auraterocystidiata*" + *Quercus suber* L. Descri Ectomyc 9/10: 81-86. – AZUL AM, MARTIN MP, AGERER R, FREITAS H (2006c) "*Quercirhiza flavocystidiata*" + *Quercus suber* L. Descri Ectomyc 9/10: 93-97. – AZUL AM, AGERER R, MARTIN MP, FREITAS H (2006d) "*Quercirhiza summatriangularis*" + *Quercus suber* L. Descri Ectomyc 9/10: 111-114. – AZUL AM, AGERER R, FREITAS H (2006e) "*Quercirhiza tomentellocystidiata*" + *Quercus suber* L. Descri Ectomyc 9/10: 115-119. – AZUL AM, AGERER R, FREITAS H (2006f) "*Quercirhiza tomentelloflexuosa*" + *Quercus suber* L. Descri Ectomyc 9/10: 121-126. – AZUL AM, MARTIN MP, AGERER R, FREITAS H (2006g) "*Quercirhiza tomentellofuniculosa*" + *Quercus suber* L. Descri Ectomyc 9/10: 127-134. – AZUL AM, AGERER R, MARTIN MP, FREITAS H (2008a) "*Quercirhiza tomentellostellata*" + *Quercus suber* L. Descri Ectomyc 11/12: 141-146. – AZUL AM, AGERER R, MARTIN MP, FREITAS H (2008b) "*Quercirhiza tomentelloreticulata*" + *Quercus suber* L. Descri Ectomyc 11/12: 135-139. – AZUL AM, AGERER R, MARTIN MP, FREITAS H (2008c) "*Quercirhiza tomentelloepidermoidea*" + *Quercus suber* L. Descri Ectomyc 11/12: 131-134. – BRAND F (1991) *Ektomykorrhizen an Fagus sylvatica*. Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. Libri Botanici vol 2. IHW, Eching. – DANIELSON RM, PRUDEN M (1989) The ectomycorrhizal status of urban spruce. Mycologia 81: 335-341. – DE ROMÁN M, AGERER R, DE MIGUEL A (2002a) "*Quercirhiza cumulosa*" + *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. Descri Ectomyc 6: 13-18. – DE ROMÁN M, AGERER R, DE MIGUEL A (2002b) *Quercirhiza stellata* + *Quercus ilex* subsp. *ballota* (Desf.) Samp. Descri Ectomyc 6: 19-24. – GARDIN M, BRUNS TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molec Ecol 2: 113-118. – JAKUCS E, AGERER R, BRATEK Z (1997) "*Quercirhiza fibulocystidiata*" + *Quercus spec.* Descri Ectomyc 2: 67-71. – JAKUCS E, AGERER R (2001) *Tomentella subtestacea* Bourdot & Galzin + *Populus alba* L. Descri Ectomyc 5: 213-219. – JAKUCS E, KOVÁCS GM, AGERER R, ROMICS C, ERŐS-HONTI Z (2005) Morphological-anatomical characterization and molecular identification of *Tomentella stiposa* ectomycorrhizae and related anatomotypes. Mycorrhiza 15: 247-258. – KÖLJALG U, DAHLBERG A, TAYLOR AFS, LARSSON E, HALLENBERG N, STENLID J, LARSSON K-H, FRANSSON PM, KAREN O, JONSSON J (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Mol Ecol 9: 1985-1996. – KÖLJALG U, JAKUCS E, BOKA K, AGERER R (2001) Three ectomycorrhizae with cystidia formed by different *Tomentella* species as revealed by rDNA ITS sequences and anatomical characteristics. Folia Cryptog Estonia Facs 38: 27-39. – KÖLJALG U, TAMMI H, TIMONEN S, AGERER R, SEN R (2002) rDNA sequence-based phylogenetic analysis of *Tomentellopsis* species from boreal and temperate forests, and the identification of pink-type ectomycorrhizas. Mycol Progress 1: 81-92. – MARTIN MP, GARCIA-FIGUERES F (1999) *Colletotrichum acutatum* and *C. gloeosporioides* cause anthracnose on olives. European J Plant Pathol 105: 733-741. – RAIDL S, MÜLLER WR (1996) *Tomentella ferruginea* (Pers.) Pat. + *Fagus sylvatica* L. Descri Ectomyc 1: 161-166. – WHITE TJ, BRUNS T, LEE S, TAYLOR J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for polygenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. New York, USA: Academic Press, 315-322. – WINKA K, AHLBERG C, ERIKSSON OE (1998) Are there lichenized Ostropales? Lichenologist 30 (4-5): 455-462.

Captions: Fig. 1. – Habit. Fig. 2. – a. Emanating hypha with warty surface – b. Outermost mantle layer; illustrating the groups of roundish cells on the surface of the mantle. – c. Outer mantle layer; pseudoparenchymatous with roundish cells overlaying groups of cells. – d. Middle mantle layer; cells more irregular in shape. – e. Inner mantle layer; plectenchymatous with cylindrical hyphae ring-like arranged but also isolated roundish cells. All figs. from AAM 569/04 (in COI).

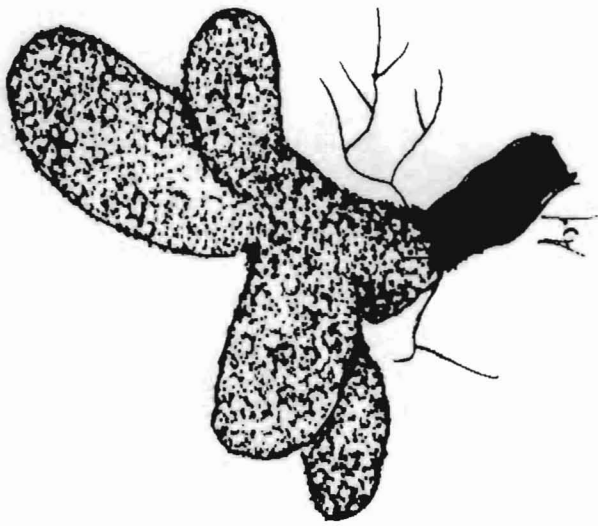


Fig. 1 - "*Quercirhiza tomentellocumulata*" + *Quercus suber*

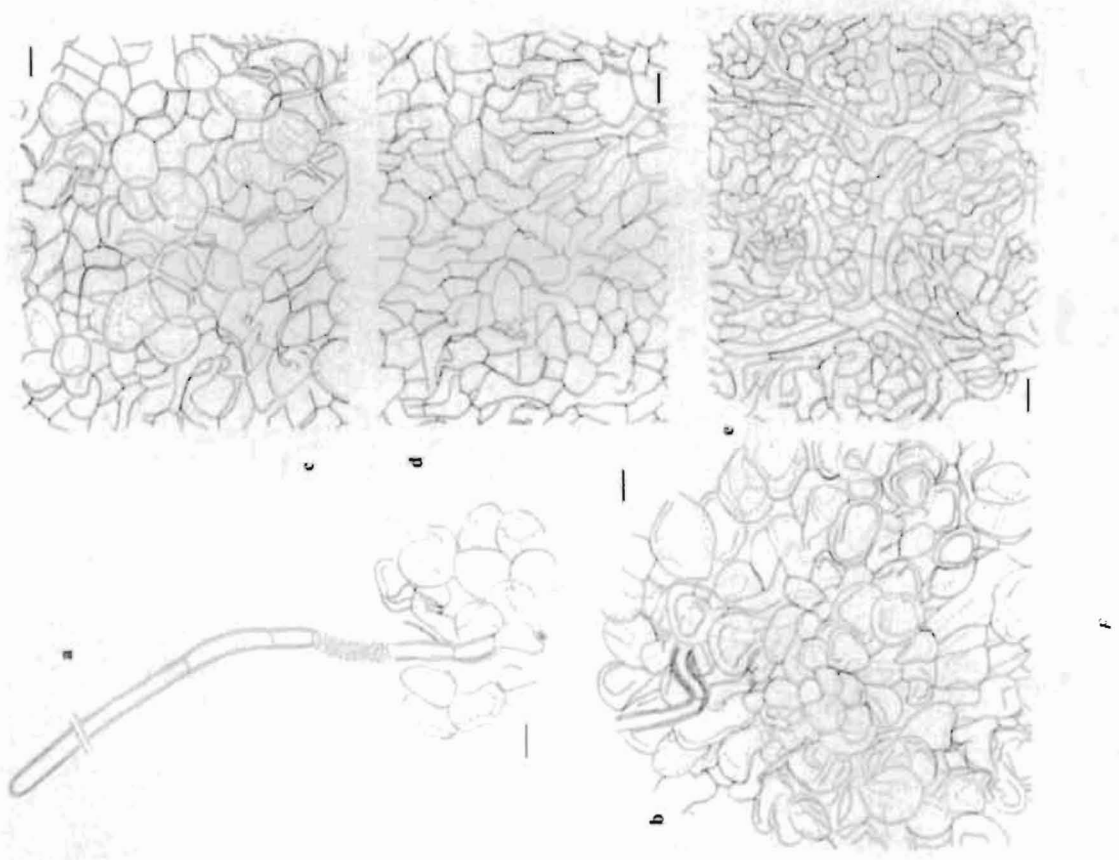


Fig. 2 - "*Quercirhiza tomentellocumulata*" + *Quercus suber*