FULL PAPER

Anatomical and ITS rDNA-based phylogenetic identification of two new West African resupinate thelephoroid species

Nourou Soulemane Yorou · Atsu Kudzo Guelly ·
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Abstract An anatomical approach coupled with molecular phylogeny of 84 sequences of thelephoroid taxa have been used to describe two new West African resupinate Thelephorales, namely, *Tomentella agereri* and *Tomentella maroana*. *T. agereri* presents a maximal sequence similarity of 94% with its genetically closest species, *Tomentalla pilosa*, according to a Blastn search in public GenBanks. By molecular phylogenetics, it is nested within the *T. pilosa* complex, a well-supported (bootstrap support of 100%) monophyletic clade composed of cystidiate and differentiated rhizomorphic species, although it presents contrasting anatomical features including the lack of cystidia, the presence of undifferentiated rhizomorphs, and basidiospores with very short aculei, up to 0.5 μm. *Tomentalla maroana* is close, by molecular phylogenetic study, to *T. ellisii, T. pisoniae*, and *T. hjortstamiana*. The phylogenetic proximity between *T. maroana* and *T. ellisii* is supported by morphological characters between the two species, namely, a crustose adherent basidiocarp, a differentiated sterile margin, and a granular hymenium. The two species deviate from each other by 11.38–12.37% with regard to the ITS rDNA sequences, whereas the intraspecific genetic distances vary from 1.68% to 2.9% among the three specimens assigned to *T. maroana*. Discriminating characters as well as genetic distance between the new species and the closely related species are discussed in detail.

Keywords Anatomy · Genetic distance · Phylogenetic position · Taxonomy · Tropical Africa

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Introduction

Thelephorales encompass a total of about 180 accepted species (Kirk et al. 2008) that are accommodated in 14 genera and two families: the Bankeraceae and the Thelephoraceae (Donk 1964; Jülich 1981; Stalpers 1993). The family Bankeraceae has a predominantly temperate distribution and is frequently reported from Europe and North America (Harrison 1964, 1968; Maas Geesteranus 1975; Baird 1986a, b; Harrison and Grund 1987; Arnolds 1989, 2003; Pegler et al. 1997; Parfitt et al. 2007). However, representatives of the genera *Hydnellum* P. Karst., *Sarcodon* Quél. ex P. Karst., and *Phellodon* P. Karst. have been reported from tropical and subtropical Asia (Maas Geesteranus 1971). In contrast, Thelephoraceae have a worldwide distribution (Patouillard 1897; Malençon 1952, 1954; Wakefield 1966; Corner 1968; Hjortstam and Ryvarden 1988, 1995; Stalpers 1993; Kõljalg 1996; Martini and Hentic 2005; Yorou 2008), although they have been frequently reported from Europe, North America, and temperate Asia with the highest species richness in coniferous forests (Larsen 1964, 1968, 1974; Wakefield 1966, 1969; Kõljalg 1996).

Taxonomically, members of the family Thelephoraceae in general and the resupinate species in particular display a limited number of anatomical characters, often overlapping, making their delimitation very difficult (Kõljalg 1996). However, the size, shape, and type of ornamentation

of the basidiospores coupled with the presence or absence of cystidia have been traditionally considered as the most discriminating features (Larsen 1968, 1974; Stalpers 1993; Kõljalg 1996; Dämmrich 2006). The taxonomic relevance of rhizomorphs was for a long time underestimated in characterizing them simply as either mono- or dimitic (Kõljalg 1996; Dämmrich 2006), although consistent anatomical features at the species level have been confirmed for rhizomorphs and detailed anatomical patterns have proven to play a paramount discriminating role, mostly within closely related species (Yorou and Agerer 2008, 2011a).

The tropical African resupinate Thelephorales are particularly difficult to identify, not only because of the scarcity of available, scientifically reliable documentation but mostly because there is a limited number (still with no clear hiatus) of discriminating anatomical characters between specimens of different or contrasting biogeographic origins (Yorou et al. 2007; Yorou and Agerer 2007a). In such cases, the combination of traditional anatomically and morphologically based taxonomy and molecular techniques (barcoding threshold and phylogenetic inferences) were invaluable for an unambiguous discrimination and identification of tropical African thelephoroid taxa and in tracing their affinities with temperate or boreal taxa (Yorou et al. 2007; Tedersoo et al. 2007; Yorou and Agerer 2007a, 2008).

The present paper is part of a series exclusively devoted to tropical African resupinate Thelephorales and their ectomycorrhizae. Nine new species were described in previous investigations (Yorou et al. 2007; Yorou and Agerer 2007a, 2008; Yorou et al. 2011a, b), using a combination of anatomo-morphological and molecular data. The present article aims at anatomical and morphological characterization of two additional new tropical African thelephoroid species and addressing their phylogenetic placement with regard to temperate and boreal species.

Materials and methods

Specimen sampling

Specimens were collected in various vegetation types in the Northern Guinean seasonal forests of West Africa (White 1983). Specimens were dried using a propane gas-heated field dryer (De Kesel 2001). Preliminary notes were recorded using fresh material. Color codes of the dried basidiocarps are given according to Kornerup and Wanscher (1978). All specimens used for descriptions and the holotypes are deposited in M (Holmgren et al. 1990) with the following herbarium labels: SYN878, SYN879, SYN892, and RA13792.

Light microscopy and SEM investigations

We refer to Yorou and Agerer (2007a,b, 2008) for the light microscopy protocol. For scanning electron microscopy (SEM) investigations, small hymenial parts taken from dried basidiocarps were fixed in 300–400 µl glutaraldehyde–cacodylate buffer and afterward treated as follows: 60 min in 2.5% glutaraldehyde, gradual washing (5, 15, 30, 60 min) in a neutral cacodylate buffer (75 mM cacodylate, 2 mM $MgCl_2$, 100 µl H_2O, pH 7), 1–2 h incubation in 1% OsO_4 buffer (2.5 ml OsO_4, 7.5 ml H_2O), with subsequent washing in distilled water. The samples were then gradually dehydrated in a series of acetone solutions as follows: 10, 20, 40, 60, 80% (each 15 min), and 2 × 100% (15 and 30 min). Samples were then stored in 100% acetone overnight followed by critical point drying (Anderson 1951). The samples were sputter-coated with gold (using argon gas, under 0.05 mbar) for 3 h and 30 min, until a layer of approximately 15 nm was obtained. SEM was then carried out using a JEOL 5800 LV with a tension of 25 kV and working distance of 10–12 mm. Digital SEM images were captured using Orion V (vers. 5.22) Image Management System.

DNA extraction and sequencing

DNA was extracted from dried basidiocarps after Gardes and Bruns (1993) using a Quiagen DNeasy plant Mini Kit (Quiagen, Hilden, Germany), according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was performed for internal transcribed spacers ITS1, ITS2, and for the 5.8S region of the nuclear ribosomal DNA, using fungi-specific primer ITS1F and basidiomycete-specific primer ITS4B. PCR amplification was performed using Ready To Go beads (Amersham Pharmacia Biotech, Piscataway, NJ, USA), with 24 µl PCR solution (125.2 µg ddH_2O, 20 µl buffer, 6.00 µl MgCl, 20 µl dNTP mix, 10 µl ITS1F, 10 µl ITS4B, 0.8 µl Taq polymerase at 5 U/µl) and 1 µl extracted DNA. The PCR was programmed as follows: 94°C for 3 min, 60°C for 1 min, 72°C for 1 min (1 cycle), and consecutively 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min 30 s (28 cycles), 94°C for 1 min, 60°C for 1 min, and 72°C for 10 min (1 cycle). Amplified PCR products (2 µl) were run with bromophenol blue (2 µl) on 1% agarose gels for 30 min at 95°C, then stained in ethidium bromide for 10 min and afterward in ddH_2O for 1 min. PCR products were then visualized under UV light. Successful DNA bands were purified using the QIAquick-PCR purification Kit (Qiagen) according to manufacturer's instructions. DNA sequencing was performed by the sequencing service of Department of Biology I (Ludwig-Maximilians-Universität, München, Germany), using BigDye Terminator

Ready Reaction Cycles Sequencing Kit v 3.1 (Applied Biosystems, Foster City, CA, USA). Sequencing was performed on 1 μl purified DNA probes plus 0.3 μl ITS1F (forward primer) and 0.3 μl ITS4B (reverse primer). Four sequences of both new species were generated and were deposited in GenBank NCBI with accession numbers EF507250, EF507251, EF507252, and EF538424.

Sequence edition and estimation of similarities and identities among studied specimens

The sequences were edited and the consensus sequence of each investigated specimen was executed in BioEdit v. 7.0.5 (Hall 2005) using the Cap. Contig. Assembl. Program option. The generated sequences were then submitted to a local similarity analysis to check to what extent they deviate from each other and to test if our anatomo-morphological discrimination is supported by molecular data. To do this, generated sequences were automatically aligned using ClustalW Multiple Alignment (BioEdit v. 7.0.5). We activated the "full multiple alignment," "bootstrap NJ tree," and "number of bootstrap = 1,000" options. As sequences have unequal lengths, the automatic alignment resulted in many insertion or deletion gaps. For anatomically close specimens, we included gaps in the similarity tests to highlight the maximal deviation rate. For the opposite condition, gaps are excluded in the calculation of similarity between evidently different anatomo-morphological specimens to estimate the minimal sequence deviation. Identity/similarity was calculated using the "pairwise alignment, calculation of the similarity/identity" option of BioEdit v. 7.0.5. In the second step, we compared the newly generated sequences with ITS sequences deposited in public databanks. The most similar sequences were searched for in UNITE (Kõljalg et al. 2005; Abarenkvov et al. 2010; http://unite.ut.ee) using the "Blastn" search option. Sequences of the top ten similar taxa identified to species level were downloaded. Unknown or unidentified *Tomentella* or *Thelephora* species were disregarded. In addition, most similar sequences were searched for in GenBank NCBI (http://www.ncbi.nlm.gov) using the Megablast (Zhang et al. 2000) search option. We deactivated the "uncultured/environmental sample sequences" search option to avoid acquiring sequences of taxonomically uninformative/unidentified taxa.

Molecular phylogenetic analysis

The most similar ITS sequences were downloaded from the public GenBanks (UNITE and NCBI). Additional sequences addressed during recent studies on tropical African resupinate Thelephorales (Yorou and Agerer 2007a, 2008; Yorou et al. 2007) and from the Seychelles (Tedersoo et al. 2007; Suvi et al. 2010) were added to the dataset. All sequences were automatically aligned in BioEdit v. 7.0.5. The alignment was manually checked and optimized. Ambiguous columns that we could not align with absolute certainty were excluded. The final dataset include a total of 84 ITS rDNA sequences with an alignment length of 500 characters. The most likely tree was searched for using the program RAxML v.7.0.4 (Stamatakis 2006). The best tree was obtained by executing 100 rapid bootstrap inferences and thereafter a thorough search for the most likely tree using one distinct model/data partition with joint branch length optimization (Stamatakis et al. 2008). The generalized time-reversible (GTR) model of substitution was applied having maximum likelihood as the optimal criterion. In addition, the most parsimonious trees were searched for by executing batch files generated with PAUPRat (Sikes and Lewis 2001) in PAUP* v4.0 (Swofford 2002), with weighting mode set to multiplicative. The parsimony analysis is set as follows: heuristic search option, addition sequence random, tree-bisection-reconnection (TBR) swapping, all characters unordered, of equal weight, and gaps are treated as missing data. A consensus tree was calculated of all trees with equal minimal length, and posterior probabilities were recorded for each branch.

Results

ITS rDNA-based differences between the new species

Specimens SYN878 (accession number EF507250), SYN879 (accession number EF507251), and SYN892 (accession number EF507252) deviate from each other by 1.68–2.9% with regard to their ITS rDNA sequences. All three specimens are accommodated under the new species *Tomentella maroana*. The sequence (accession number EF538424) obtained from the specimen RA13792 deviates from those of *T. maroana* by 18.28–18.70%. It is described here as the new species *T. agereri*.

The top five best matches of *T. maroana* are composed of *Tomentella sublilacina* (Ellis & Holw.) Wakef. according to Blastn search in UNITE. Sequences similarity between both species ranges from 88% to 91%. In contrast to *T. maroana*, for which the top five best matches are *T. sublilacina*, four different species with sequences identities varying from 90% to 94% are most similar to *T. agereri* according to "Megablast" search in NCBI. *Tomentella agereri* is 94% similar to *T. atroarenicolor* Nikol. and *T. pilosa* (Burt) Bourdot & Galzin, whereas lower identity rates (90% and 92%) are obtained with *T. umbrinospora* M.J. Larsen and *T. ferruginea* (Pers.) Pat., respectively. Differing from "Blastn" search in UNITE, the "Megablast" search in NCBI shows more diversity in

the best matches of both new species. However, both searches generated *T. sublilacina* as the closest species to *T. maroana* whereas *T. pilosa* and *T. atroarenicolor* are most similar to *T. agereri*.

Phylogenetic position of the two new species

A total of 4,341 most parsimonious trees (shortest tree length = 972, CI = 0.568, RI = 0.876) were generated from 10,020 trees. Of 500 reliably aligned characters of the ITS rDNA, 199 were variable and 173 characters were parsimony informative. The final alignment has 234 distinct alignment patterns, and the proportion of gaps and completely underestimated characters in the alignment is estimated at 0.13%. The likelihood of the most likely tree found is −5295.424348 (tree length = 4.510765). The following substitution rates were estimated by RAxML: A ↔ C, 1.476325; A ↔ G, 8.882025; A ↔ T, 1.025856; C ↔ G, 0.498660; C ↔ T, 8.280101; G ↔ T, 1.000000; base frequencies were Freq. (A) = 0.224580, Freq. (C) = 0.247659, Freq. (G) = 0.242133, and Freq. (T) = 0.285627; gamma shape parameter = 0.898260; and proportion of invariable sites = 0.451289. In general, the topology is very similar in all trees generated by RAxMl and PAUP. Therefore, we present only the most likely tree obtained from RAxML (Fig. 1). The major clades of the most likely tree and their constitutive species are similar to those generated during previous molecular phylogenetic investigations on resupinate Thelephorales (Kõljalg et al. 2000, 2001; Yorou and Agerer 2007a; Yorou et al. 2007).

In all phylogenetic trees generated, *Tomentella agereri* forms a sister species to *Tomentella pilosa*, whose representatives all cluster together with 100% bootstrap support. The terminal clade comprising *T. agereri* and *T. pilosa* is well supported by a high bootstrap value of 98%. Both species form, together with *T. capitata* Yorou & Agerer, *Tomentella brunneocystidia* Yorou & Agerer, and *Tomentella atroarenicolor*, a strongly supported monophyletic clade with a bootstrap value of 100%.

So far as *T. maroana* is concerned, all three investigated specimens cluster together with a bootstrap support of 100%. *T. maroana* forms a sister species of the group comprising *Tomentella ellisii* (Sacc.) Jülich and Stalpers, *Tomentella pisoniae* Suvi & Kõljalg, and *Tomentella hjortstamiana* Suvi & Kõljalg, with, however, no bootstrap support. The sister group of all four species (*T. ellisii*, *T. maroana*, *T. pisoniae*, *and T. hjortstamiana*) is composed of *Tomentella terrestris* (Berk. & Broome) M.J. Larsen and *Tomentella sublilacina*, whose representatives cluster all together with 98% bootstrap support. There is, however, no bootstrap support between the *T. sublilacina* clade and that composed of *T. maroana*, *T. ellisii*, *T. pisoniae*, and *T. hjortstamiana*.

Description of the new species

Tomentella agereri Yorou sp. nov.   Figs. 2–4
Mycobank MB560122

Basidiocarpi resupinati, tenues, ad 0.5 μm alti, a substrato pro partibus minoribus separabiles, arachnoidei, continui; rhizomorphis margine sterili absentibus. Hymenophorae laeves, continues; hymeniis brunneis usque ad rufo-brunneis; subiculis tenuissimis, arachnoideis, flavis, margine strerili determinato, aurantiaco-flavo, quam hymenio pallidiore. Rhizomorphae in subiculo presentes, sub stereomicroscopio flavae, in aqua et in 2.5% KOH flavae, monomiticae; omnibus hyphis similibus, uniformibus, laxis, in aspectu plano paene paralleliter dispositis, fibuligeris, septis simplicibus deficientibus, 4–7 μm diametro, tenuitunicatis, in aqua et in 2.5% KOH incoloratis usque ad pallide flavis, in aqua incrustatissimis, sed in 2.5% KOH incrustatione dissolventi, non cyanescentibus, subcongophilis, subcyanophilis, non amyloideis. Hyphae subiculi fibuligerae, septis simplicibus deficientibus, 3.5–6(–7) μm latae, frequenter regulares, interdum inflatae ad 7–8 μm, in 2.5% KOH infrequenter sinuosae, cruciformibus ramificationibus raris, anastomosibus infrequentibus, in aqua incrustatissimae, in 2.5% KOH incrustatione dissolventi, in aqua et in 2.5% KOH incoloratae usque ad pallide flavae, non cyanescentes, subcongophilae, subcyanophilae, non amyloideae. Hyphae subhymenii fibuligerae, 2.5–4.5(–6) μm diametro, cellulis interdum brevissimis sed numquam inflatis, tenuitunicatae (0.2–0.5 μm), in aqua incrustatissimae, incrustatione in 2.5% KOH dissolventi, hyphae in reagente Melzeri laeves, in aqua et in 2.5% KOH pallide flavidae, non cyanescentes, subcongophilae, subcyanophilae, non amyloideae.

Basidia fibuligera, (30–)35–55(–60) μm longa, apice (6–)6.5–7.5(–8) μm lata, basi (5–)5.5–6.5(–7) μm lata, utriformia, non stipitata, semper sinuosa, infrequenter cum septis transversibus, in aqua et in 2.5% KOH incolorata, non cyanescentia, fere semper congophila sed in Congo red nonnulla basidia incolorata si basidia septata et in partibus distalibus distincte congophila, cyanophila, non amlyoidea, 4-sterigmatica, sterigmatibus 3–7(–7.5) μm longis, basi 1.5–2.5 μm latis. Basidiosporae (6–)6.5–7(–8) × (5–)5.5–6.5(–7) μm in aspectu frontali, (6–)6.5–7.5(–8) × (5–)5.5–6(–6.5) μm in aspectu laterali, in aspectu frontali triangulares, parte proxima dilatata usque ad lobata, ejus latitudine basidiosporae longitudine aequanti, ellipsoideae in aspectu laterali, semper guttulatae, verrucosae usque ad echinulatae, aculeis brevissimis, 0.2–0.5 μm altis, interdum binis ternisve aggregatis et primo aspectu 2- vel 3-furcatis, in aqua incoloratae usque ad pallide subflavae, in 2.5% KOH pallide flavae, non cyanescentes, non congophilae, non cyanophilae, non amyloideae. Chlamydosporae absentes.

Fig. 1 Maximum likelihood tree showing placement of the two new species among *Tomentella* species. Bootstrap values higher than 50% are shown *above the branches*. GenBank (UNITE or NCBI) accession numbers and country of origin of selected species are indicated after species names. Both new species are highlighted in *bold*. Some clades have been collapsed to reduce the span of the tree
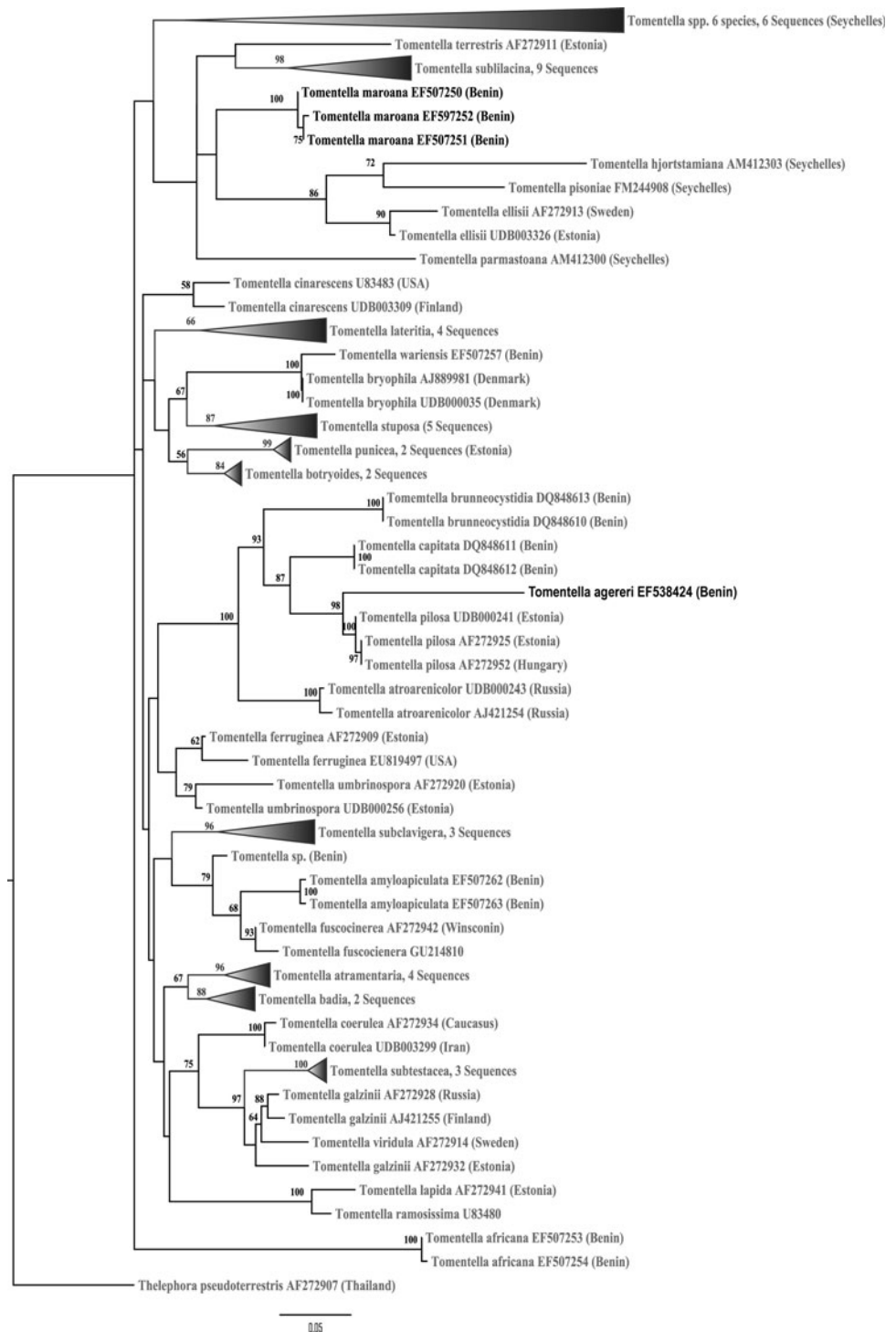
Basidiocarp resupinate, thin, up to 0.5 mm thick, separable from the substrate as small plaques, arachnoid, continuous. Rhizomorphs absent at the sterile margin. Hymenophore smooth, continuous, hymenium brown (7E6) to reddish brown (8E6), subiculum very thin, arachnoid, yellow, sterile margin determinate, paler than the hymenium, orange yellow. Rhizomorphs present in the subiculum, yellow under stereo microscope, yellow in water and in 2.5% KOH, monomitic; rhizomorphs undifferentiated (Fig. 2), uniform-loose (Agerer 1999), or of type A (according to Agerer 1987–2008), nearly parallel arrangement in plane view, clamped, simple septa absent, 4–7 μm wide, thin walled, colorless to pale yellow in water and in 2.5% KOH, heavily encrusted in water, encrustation dissolving in 2.5% KOH, not cyanescent, slightly congophilous, slightly cyanophilous, not amyloid. Subicular hyphae
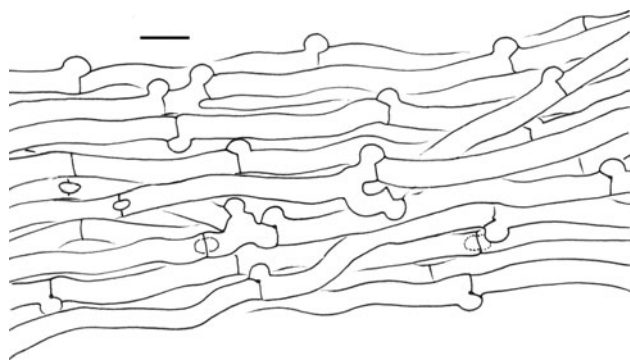
Fig. 2 *Tomentella agereri*. Optical section through the rhizomorph. *Bar* 10 μm

clamped, simple septa absent, 3.5–6(–7) μm wide, usually regular, sometimes inflated, then up to 7–8 μm wide, rarely sinuous in 2.5% KOH, cross-shaped branching and anastomoses rare, heavily encrusted in water, encrustation dissolving in 2.5% KOH, colorless to pale yellow in water and in 2.5% KOH, hyphae not cyanescent, slightly congophilous, slightly cyanophilous, not amyloid. Subhymenial hyphae clamped, 2.5–4.5(–6) μm wide, the cells sometimes very short but never inflated (Fig. 3), thin walled (0.2–0.5 μm), heavily encrusted in water, encrustation dissolving in 2.5% KOH, hyphae smooth in Melzer's reagent, colorless to very pale yellow in water and in 2.5% KOH, not cyanescent, slightly congophilous, slightly cyanophilous, not amyloid. Cystidia absent. Basidia clamped at base, (30–)35–55(–60) μm long, (6–)6.5–7.5(–8) μm at apex, (5–)5.5–6.5(–7) μm at base, utriform, not stalked, always sinuous, rarely with transverse septa, colorless in water and in 2.5% KOH, not cyanescent, usually congophilous, some basidia remaining colorless in Congo red, if septate then the upper part distinctly congophilous, cyanophilous, not amyloid, 4-sterigmate, sterigmata 3–7(–7.5) μm long and 1.5–2.5 μm at base. Basidiospores (6–)6.5–7(–8) × (5–)5.5–6.5(–7) μm in frontal face, (6–)6.5–7.5(–8) × (5–)5.5–6(–6.5) μm in lateral face, in frontal view triangular to lobed, with a widened proximal part, proximal part as large as the length of the basidiospores, ellipsoid in lateral view (Fig. 4), always with oil drops, warted to echinulate, aculei very short, 0.2–0.5 μm, sometimes grouped in two or three, giving an impression of bi- or trifurcation, basidiospores colorless to very pale yellow in water, pale yellow in 2.5% KOH, not cyanescent, not congophilous, not cyanophilous, not amyloid. Chlamydospores absent.

Material studied

Benin, central part, Borgou Province, Sinendé region, forest close to Fô-Bouko village, 10°8′46.6″N, 002°15′6.2″E, leg.

R. Agerer, 22.08.2003, det. N.S. Yorou, herb. RA13792 (M), holotype. Genbank NCBI, accession number EF538424.

Etymology

The epithet is proposed in honor of Prof. Dr. Agerer, who collected the type material.

Habitat

On the underside of dead bark, in woodlands and forests dominated by Euphorbiaceae (*Uapaca togoensis* Pax), Caesalpiniaceae (*Isoberlinia doka* Craib & Stapf, *Isoberlinia tomentosa* Craib & Stapf, *Burkea africana* Hook. F., *Afzelia africana* Smith).

Tomentella maroana Yorou sp. nov.   Figs. 5–7
Mycobank MB560123

Basidiocarpi resupinati, tenues, ad 0.5 mm alti, adherentes, crustosi, continues, fusco-brunnei usque ad obscure rubro-brunnei. Hymenophorae laeves usque ad granulosae, continuae; hymeniis pallidiore, brunneis; subiculis arachnoideis obscuro-badio usque ad fere nigro, margine sterili determinato, cinereo-brunneo, pallidiore quam hymenio. Rhizomorphae in subiculo presentes, sub stereomicroscopio brunneae, tenues (usque ad 20 μm diametro) in aqua et in 2.5% KOH brunneae, crassiores (quam 20 μm) in aqua et in 2.5% KOH usque ad obscuro-brunneae, monomiticae, omnibus hyphis similibus, compactis, uniformibus, fibuligeris, plano aspectu compacte fere paralleliter dispositis, septis simplicibus presentibus, 4–6(–7) μm diametro, semper crassitunicatis (1.5–2.5 μm), in aqua et in 2.5% KOH non incrustatis, in aqua et in 2.5% KOH brunneis usque ad obscuro-brunneis, non cyanescentibus, non congophilis, non cyanophilis, non amyloideis. Hyphae subiculi fibuligerae, septis simplicibus infrequentibus, 4–6(–7) μm diametro, crassitunicatae (1.5–2.5 μm crassae), muribus flavis, regulares, in aqua et in 2.5% KOH frequenter tortuosae; hyphae marginis sterilis regulares, 4–5 μm diametro; hyphae subiculi infrequenter inflatae et infrequenter tortuosae, anastomosibus cruciformibus, non incrustatae, in aqua et in 2.5% KOH brunneae usque ad obscuro-brunneae; hyphae marginis sterilis in aqua et in 2.5% KOH brunneae, non cyanescentes, non congophilae, non cyanophilae, non amyloideae. Hyphae subhymenii fibuligerae, 4–7(–8) μm diametro, cellulis non brevibus, interdum inflatis (usque ad 10 μm), crassitunicatae (1–2 μm), muribus flavis, in aqua et in 2.5% KOH non incrustatae, interdum cyanescentes, non congophilae, non cyanophilae, non amyloideae. Cystidia absentia. Basidia fibuligera, (40–)45–65(–70) μm longa, apice (8–)9.5–11(–12) μm lata, basi (5.5–)6–7.5(–8) μm lata, clavata, non stipitata, interdum sinuosa, infrequenter cum septis transversibus; basidiis et
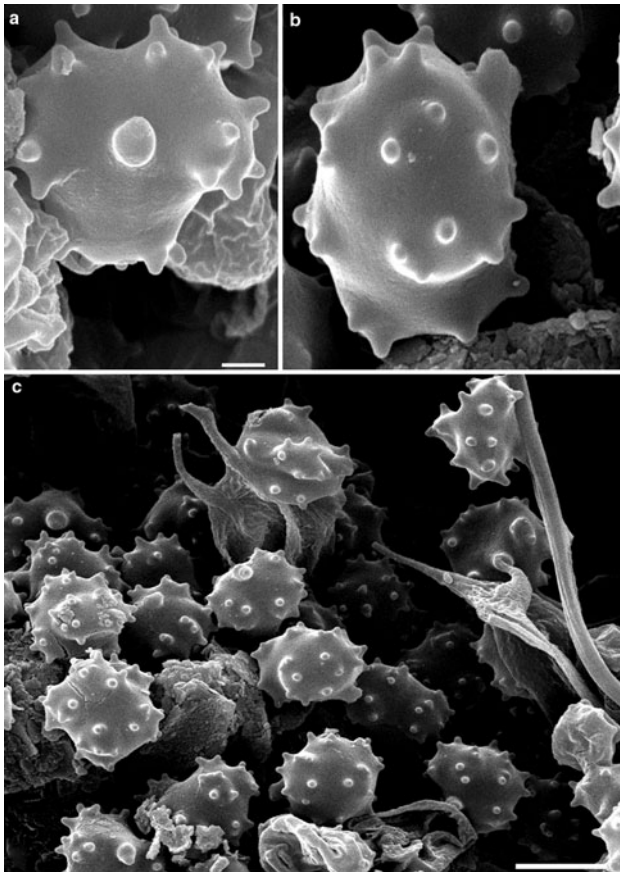
Fig. 3 *Tomentella agereri.*
a Basidiospores in lateral view.
b Basidiospores in frontal view.
c Section through the
basidiocarp. *Bars* 10 μm
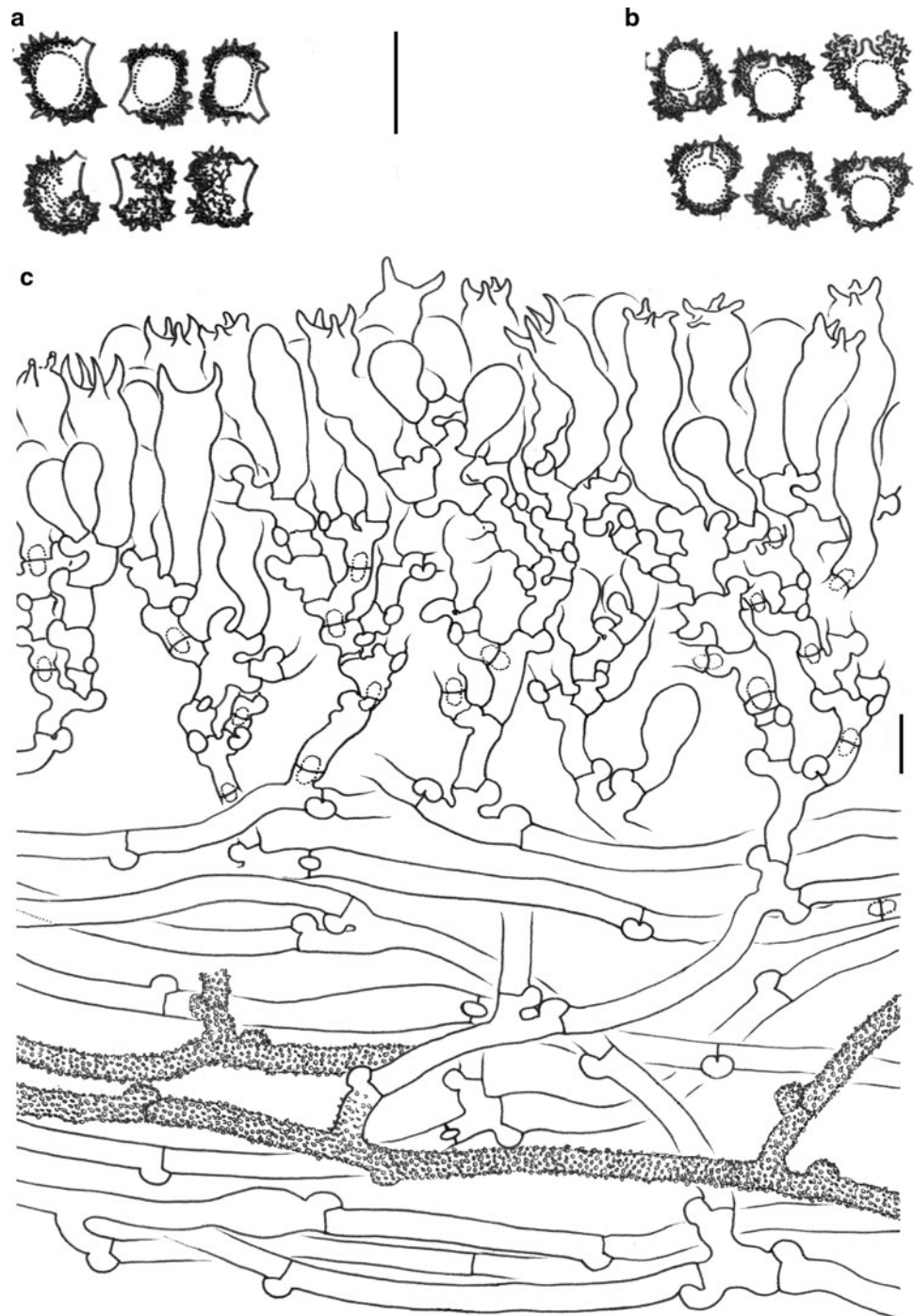
basidiolis marginis basidiocarpi interdum distincte crassit-
unicatis (1–1.5 μm); basidiis in aqua et in 2.5% KOH in-
coloratis usque as pallide brunneis, interdum cyanescentiis,
congophilis, subcyanophilis; basidiis juvenilibus distincte
cyanophilis, non amyloideis, 4-sterigmatica, sterigmaticis
5–9(10) μm longis, basi 1.5–3 μm latis. Basidiosporae (8–)
8.5–11(–12) × (7–)7.5–8.5(–9) μm in aspectu frontali, (8–)
8.5–11(–11.5) × (7–)7.5–8(–8.5) μm in aspectu laterali,
triangulares et parte proxima dilatata usque ad sublobata in

aspectu frontali, ellipsoideae in aspectu laterali, non gut-
tulatae, verrucosae usque ad echinulatae, aculeis brevissi-
mis, 0.2–0.5 μm altis, interdum binis ternisve aggregatis, in
aqua et in 2.5% KOH pallide brunneae, interdum cyanes-
centes, non congophilae, non cyanophilae, non amyloi-
deae. Chlamydosporae absentes.

Basidiocarp resupinate, thin, up to 0.5 mm thick,
adherent to the substrate, crustose, continuous, dark brown
(7F7) to reddish dark brown (8F7). Hymenophore smooth

Fig. 4 *Tomentella agereri*. Scanning electron microscopy (SEM) of basidiospores. **a** Basidiospore in nearly proximal view. **b** Basidiospore in lateral view. **c** General view of basidiospores. *Bars* **a**, **b** 1 μm; **c** 5 μm
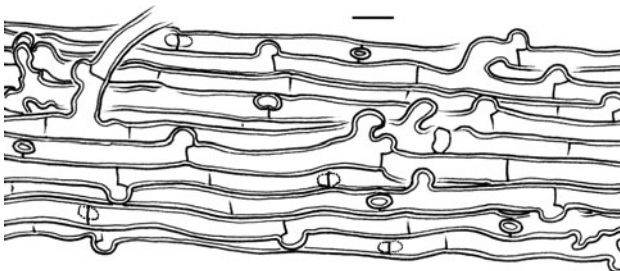
Fig. 5 *Tomentella maroana*. Optical section through rhizomorph. *Bar* 10 μm

to granular, continuous, hymenium paler, brown (6F4), subiculum arachnoid, dark brown to almost black, sterile margin determinate, paler than the hymenium, greyish brown. Rhizomorphs present in the subiculum, brown under stereo microscope, brown (young rhizomorphs up to 20 μm) to dark brown (old rhizomorphs thicker than 20 μm) in water and in 2.5% KOH, monomitic, undifferentiated (Fig. 5), uniform compact (Agerer 1999), or of type B (according to Agerer 1987–2008), with a parallel compact arrangement in plane view, individual hyphae
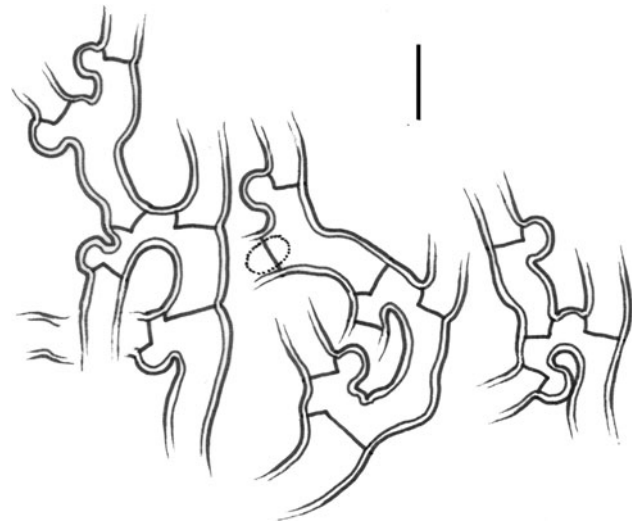

Fig. 6 *Tomentella maroana*. Anastomoses of subicular hyphae. *Bar* 10 μm

clamped, simple septa present, 4–6(–7) μm wide, always thick walled (1.5–2.5 μm), walls yellowish, without encrustation in water and in 2.5% KOH, brown to dark brown in water and in 2.5% KOH, not cyanescent, not congophilous, not cyanophilous, not amyloid. Subicular hyphae clamped, simple septa rare, 4–6(–7) μm wide, always thick walled (1.5–2.5 μm), walls yellowish, hyphae usually regular in outlines, commonly tortuous in 2.5% KOH, hyphae from the sterile margin regular, 4–5 μm wide, rarely inflated and rarely tortuous, cross-shaped branching and anastomoses present (Fig. 6), without encrustation, brown to dark brown in water and in 2.5% KOH, hyphae from the sterile margin brown in water and in 2.5% KOH, not cyanescent, not congophilous, not cyanophilous, not amyloid. Subhymenial hyphae clamped, 4–7(–8) μm wide, never short, sometimes inflated (up to 10 μm) (Fig. 7), thick walled (1–2 μm), walls yellowish, without encrustation in water and in 2.5% KOH, brown to dark brown in water and in 2.5% KOH, sometimes cyanescent, not congophilous, not cyanophilous, not amyloid. Cystidia absent. Basidia clamped at base, (40–)45–65(–70) μm long, (8–)9.5–11(–12) μm at apex, (5.5–)6–7.5(–8) μm at base, clavate, not stalked, sometimes sinuous, rarely with transverse septa, some basidia and basidioles from the sterile margin distinctly thick walled (1–1.5 μm), basidia colorless to pale brown in water and in 2.5% KOH, partly cyanescent, congophilous, slightly cyanophilous, young basidia distinctly cyanophilous, not amyloid, 4-sterigmate, sterigmata 5–9(–10) μm long and 1.5–3 μm at base. Basidiospores (8–)8.5–11(–12) × (7–)7.5–8.5(–9) μm in frontal face, (8–)8.5–11(–11.5) × (7–)7.5–8(–8.5) μm in lateral face, triangular with a widened proximal part to slightly lobed in frontal view, ellipsoid in lateral view, oil drops absent, warted to echinulate, aculei very short,

Fig. 7 *Tomentella maroana.*
a Basidiospores in lateral view.
b Basidiospores in frontal view.
c Section through basidiocarp.
Bars 10 μm
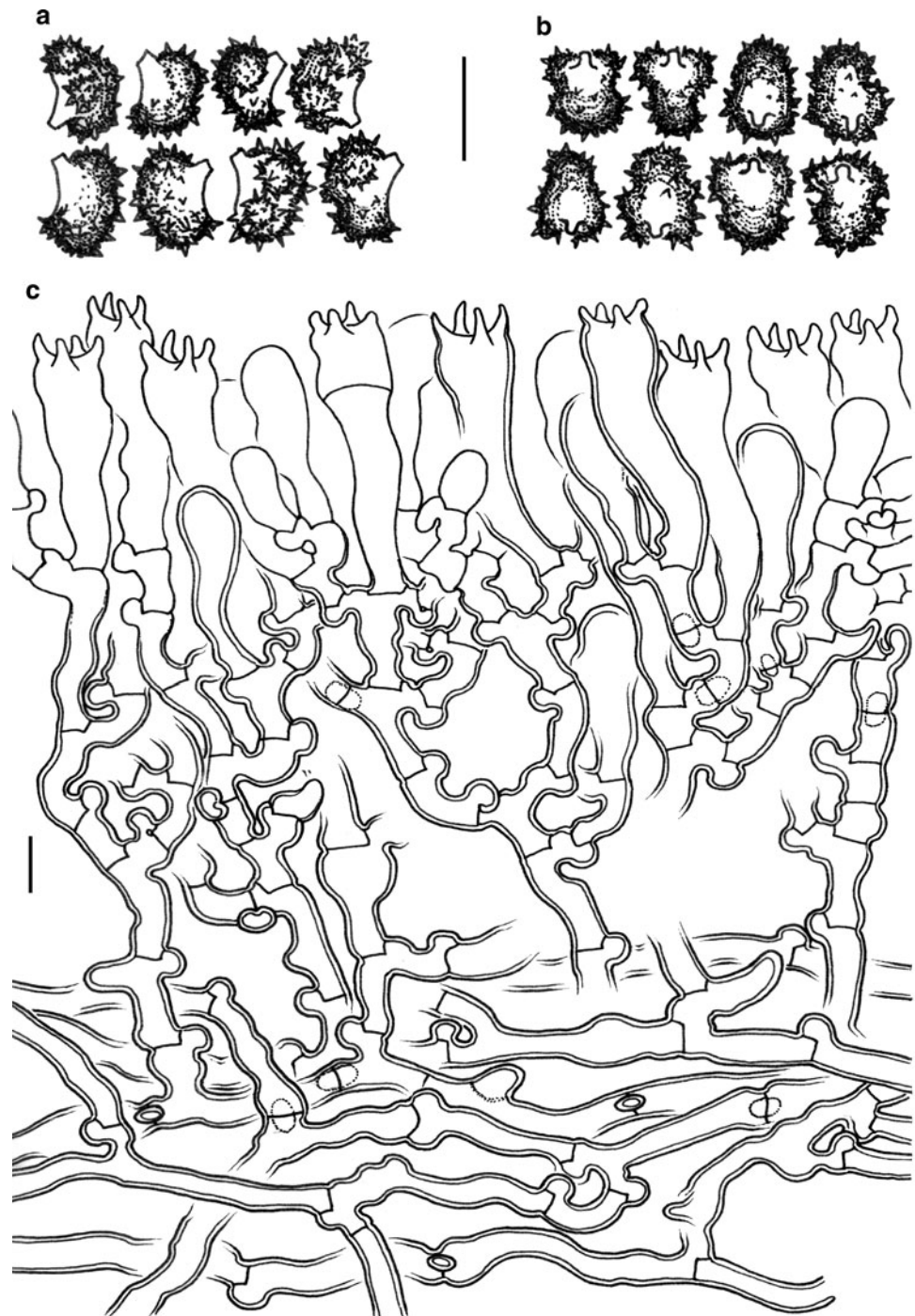
0.2–0.5 μm, sometimes in groups of two or three, basidiospores pale brown in water and in 2.5% KOH, partly cyanescent, not congophilous, not cyanophilous, not amyloid. Chlamydospores absent.

Materials studied

Benin, central part, Borgou Province, Wari-Maro region, forest reserve of Wari-Maro, 08°20′21.7″N, 002°49′32.6″E, leg and det. N.S. Yorou, 06.08.2005, herb. SYN878 (M), holotype, GenBank NCBI, accession number EF507250.—Benin, central part, Borgou Province, Wari-Maro region, forest reserve of Wari-Maro, 08°20′22.1″N, 002°49′32.9″E, leg and det. N.S. Yorou, 06.08.2005, herb. SYN879 (M), GenBank NCBI, accession number EF507251; SYN892 (M), GenBank NCBI, accession number EF507252.—Togo, central western part, Sotouboua Prefecture, Fazao-Malfakassa National Parc, 08°42′58.6″N, 000°46′22.2″E, leg. and det. N.S. Yorou, 05.06.2008, herb. SYN1677 (M).

Etymology

The epithet is given in reference to Wari-Maro, the collecting site of the type material.

Habitat

The forest reserves of Wari-Maro and Fazao-Malfakassa are dominated by Caesalpiniaceae (*Isoberlinia* spp., *Afzelia africana*…) and Euphorbiaceae (*Uapaca* spp.). Specimens of *T. maroana* were collected on dead logs and barks under *A. africana* individuals.

Discussion

Tomentella agereri is characterized by the complete lack of cystidia, the presence of uniform loose rhizomorphs, and small basidiospores not longer than 7(–8) μm with very short aculei (0.2–0.5 μm). By molecular phylogenetics, it falls in the clade formed by *Tomentella pilosa* and its allied species. The monophyly of the clade formed by *T. pilosa*, *T. capitata*, *T. brunneocystidia*, and *T. atroarenicolor*, in which *T. agereri* is placed, has been constantly confirmed in various molecular phylogenetic studies on resupinate Thelephorales and their ectomycorrhizae with strong bootstrap support of 84% (Kõljalg et al. 2001), 83% (Yorou and Agerer 2008), 98% (Yorou et al. 2007), 90% (Yorou and Agerer 2007a), and 99% (Jakucs et al. 2005). This placement of *T. agereri* is in accordance with a Blastn search undertaken in UNITE and NCBI. Anatomically, this clade is composed, except for *T. agereri*, of cystidiate species (Stalpers 1993; Kõljalg 1996; Dämmrich 2006; Melo et al. 2006; Yorou et al. 2007), which present either capitate cystidia (*T. pilosa*, *T. capitata*, *T. brunneocystidia*) or hyphoid cystidia (*T. atroarenicolor*). In this clade, cystidia are present on rhizomorphs of *T. pilosa*, *T. capitata*, and *T. atroarenicolor* (Melo et al. 1998, 2006; Jakucs and Agerer 1999; Yorou et al. 2007). Further investigation confirmed the presence of capitate cystidia on the ectomycorrhizal mantle of *T. pilosa* also (Jakucs and Agerer 1999). Anatomical description of the ectomycorrhizae of other species of this clade (namely, *T. capitata* and *T. brunneocystidia*) is not available for comparison, but the shape of the cystidia present in the hymenium is one of the anatomical features that support the clustering of *T. pilosa*, *T. capitata*, and *T. brunneocystidia* in a well-supported clade (bootstrap support of 93%). The separation of *T. atroarenicolor* as a sister species (with a strong bootstrap support of 100%) of these three capitate cystidiate species is justified by the hyphoid shape of the cystidia in the latter species. All members of this well-supported clade (except for *T. agereri* with uniform rhizomorphs) are

characterized by slightly differentiated rhizomorphs, composed of central, wider hyphae that are covered by smaller, entwined and tortuous, multi-branched peripheral hyphae forming a kind of dense rind around the rhizomorphs (Jakucs and Agerer 1999; Agerer 2006; Yorou et al. 2007). The clustering of *T. agereri* in the anatomically and molecular phylogenetically well-defined clade (*T. pilosa* complex) is astonishing because of the lack of cystidia, the uniform loose rhizomorphs, and tiny aculei, but unambiguous evidence is provided that it is a new species within this clade. Although this clade is robust and supported by a bootstrap value of 100%, strong anatomical similarities that could explain the placement of *T. agereri* within cystidiate species are currently lacking, except the common triangular to slightly lobed shape and the yellowish color of the basidiospores. *Tomentella agereri* could have lost the capacity to form both cystidia and differentiated rhizomorphs.

Tomentella maroana is characterized by a brown to dark brown crustose basidiocarp. Important anatomical features are the distinctly thick-walled subicular and subhymenial hyphae and the triangular to slightly lobed (in lateral view) pale brown basidiospores with short aculei of 0.2–0.5 μm. Although the most similar species is *T. sublilacina* according to Blastn search in both UNITE and NCBI, it is molecular phylogenetically close to *T. ellisii*, *T. pisoniae*, and *T. hjortstamiana*. The molecular phylogenetic proximity between *T. ellisii* and *T. sublilacina* has been repeatedly reported in many molecular phylogenetic studies (Kõljalg et al. 2000, 2001; Yorou and Agerer 2007a; Yorou et al. 2007). From all aforementioned molecular studies, the delimitation between *T. ellisii* and *T. sublilacina* is consistent; *T. sublilacina* is more closely related to *T. terrestris* than to *T. ellisii*, which appears to be closely related to *T. maroana*, *T. pisoniae*, and *T. hjortstamiana* in this study. In this group, some species have been reported to present undifferentiated rhizomorphs (Agerer 1987–2008, 1999), namely, *T. sublilacina* (Agerer 1996 under *T. albomarginata* (Bourdot & Galzin) M.P Christ. and *T. radiosa* (Agerer and Bougher 2001; Yorou and Agerer 2007b). Morphologically, *T. pisoniae* deviates from *T. maroana*, *T. ellisii*, and *T. hjortstamiana* by its arachnoid separable basidiocarp and the dark grey hymenophore (Suvi et al. 2010) with an indeterminate sterile margin. All three species—*T. maroana*, *T. ellisii*, and *T. hjortstamiana*—present crustose adherent basidiocarps with a differentiated sterile margin, but *T. hjortstamiana* deviates morphologically from both *T. ellisii* and *T. maroana* by the smooth grey yellowish hymenium (Suvi et al. 2010). Morphologically, *T. ellisii* is the closest species to *T. maroana*. Anatomical dissimilarities could be highlighted between both species. *Tomentella ellisii* differs by having thin-walled, colorless to only very pale brown, subicular hyphae (Kõljalg 1996; Dämmrich 2006; Yorou and Agerer

2011b), whereas *T. maroana* presents constantly brown to dark brown thick-walled (1.5–2.5 μm) hyphae. Short and inflated, thin-walled subhymenial hyphae up to 12 μm are common in *T. ellisii* (Dämmrich 2006; Yorou and Agerer 2011b), whereas those of *T. maroana* are never short or inflated but always thick walled (1.5–2.5 μm). Also, reniform (in lateral view) basidiospores are reported for *T. ellisii* (Dämmrich 2006; Yorou and Agerer 2011b), but we could not observe such basidiospores in all three specimens of *T. maroana* examined. In addition, basidiospores of *T. ellisii* present aculei of up to 1 μm, whereas those of *T. maroana* never exceed 0.5 μm. In contrast to *T. ellisii*, in which rhizomorphs are absent (Yorou and Agerer 2011a) or infrequent (Kõljalg 1996; Dämmrich 2006), *T. maroana* is characterized by the presence of undifferentiated (uniform compact) rhizomorphs with thick-walled brown to dark brown individual hyphae. The molecular phylogenetic placement of *T. maroana* is thus supported by rhizomorph anatomy because some species in this clade present undifferentiated rhizomorphs (see above).

The barcoding threshold has become a tool for a rapid screening of morphologically close specimens and the detection of cryptic species (Tedersoo et al. 2008, 2009). DNA barcoding has been widely used in ectomycorrhizal community studies to obtain the most similar species of a given environmental sample (Tedersoo et al. 2003; Izzo et al. 2005; O'Brien et al. 2005; Parrent et al. 2006). However, the ITS rDNA-based identification of thelephoroid species in public GenBanks (UNITE, NCBI, or EMBL) commonly results in either a large number of insufficiently identified environmental samples as best matches, or in a large number of structurally different species with, however, equal similarity percentage to the query (Nilsson et al. 2006, 2009; personal observation). On one hand, it may be a consequence of unequal sequence length and query coverage, which results in an overestimation of sequence similarity (see explanation in Tedersoo 2007; Nilsson et al. 2009) but also insufficient taxonomic annotation of specimens in the public GenBank (Nilsson et al. 2006). In such cases, the barcoding threshold loses its value, and only anatomical comparison can ensure a reliable identification to species level of the query. In resupinate Thelephorales, specimens (either fruit bodies or environmental samples) accommodated in one and the same species and clustering together in a well-supported terminal clade actually present sequence deviations that are generally less than 5% (Kõljalg et al. 2000; Yorou et al. 2007; Yorou and Agerer 2008). Sequence deviations higher than 6–7% suggest the presence of at least two different species in the clade (Kõljalg et al. 2000). Presently, however, a 3–4% threshold is discussed or accepted for discerning species (Izzo et al. 2005) to simplify interpretations of environmental samples, a procedure still waiting for justification (Nilsson et al. 2008). In the present study, all three specimens assigned to *T. maroana* deviate only by 1.68–2.9%; they cluster together in a terminal clade supported by 100% bootstrap. All three specimens of *T. maroana* deviate from those of *T. ellisii*, *T. pisoniae*, and *T. hjortstamiana* by at least 11.38–12.37%, 13.29–13.69%, and 14.03–15.52%, respectively. By molecular phylogenetics, *T. agereri* deviates from the closest species, *T. pilosa*, by at least 10.4–11.3%.

References

Abarenkvov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T, Liimatainen K, Peintner U, Kõljalg U (2010) The UNITE database for molecular identification of fungi-recent updates and future perspectives. New Phytol 186:281–285

Agerer R (1987–2008) Colour atlas of ectomycorrhizae. 1st–14th delivery. Schwäbische Gmünd, Einhorn

Agerer R (1996) Ectomycorrhizae of *Tomentella albomarginata* (Thelephoraceae) on Scots pine. Mycorrhiza 6:1–7

Agerer R (1999) Never change a functional successful principle: the evolution of Boletales s. 1. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendteria 6:5–91

Agerer R (2006) Fungal relationship and structural identity of their ectomycorrhizae. Mycol Prog 5:67–107

Agerer R, Bougher NL (2001) *Tomentella subamyloidea* sp. nov. and *Tomentella radiosa* (Thelephorales, Hymenomycetes, Basidiomycota) from Australia. Aust Syst Bot 14:607–614

Anderson TF (1951) Techniques for the preservation of three dimensional structures in preparing sequences for the electron microscope. Trans NY Acad Sci II 12:130–134

Arnolds E (1989) Former and present distributions of stipitate hydnaceous fungi (Basidiomycetes) in the Netherlands. Nova Hedwigia 48:107–142

Arnolds E (2003) The hydnoid fungi of Netherlands and Belgium. Coolia 46:1–96

Baird RE (1986a) Type studies of North American and other taxa of stipitate hydnums: genera *Bankera*, *Hydnellum*, *Phellodon*, *Sarcodon*. Bibliotheca Mycol 103:1–89

Baird RE (1986b) Study of the stipitate hydnums from the southern Appalachian mountains: genera *Bankera*, *Hydnellum*, *Phellodon*, *Sarcodon*. Bibliotheca Mycol 104:1–156

Corner EJH (1968) A monograph of *Thelephora* (Basidiomycetes). Beih Nova Hedwigia 27:1–110

Dämmrich F (2006) Studien der tomentelloiden Pilze in Deutschland-Unter besonderer Berücksichtigung der Zeichnungen von Frau Dr. H. Maser aus den Jahren 1988–1994. Teil 1: Die Gattung Tomentella. Z Mykol 72:167–212

De Kesel A (2001) A mushroom dryer for the travelling mycologist. Field Mycol 2:131–133

Donk MA (1964) A conspectus of the families Aphyllophorales. Persoonia 3:199–324

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizas and rusts. Mol Ecol 2:113–118

Hall T (2005) BioEdit: biological sequence alignment editor for Win95/98/NT/2K/XP. Ibis Therapeutic, Carlsbad, CA

Harrison KA (1964) New or little known North American stipitate hydnums. Can J Bot 42:1205–1233

Harrison KA (1968) Studies on the hydnums of Michigan I. Genera *Phellodon*, *Bankera*, *Hydnellum*. Mich Bot 7:212–264

Harrison KA, Grund DW (1987) Preliminary keys to the terrestrial stipitate hydnums of North America. Mycotaxon 28:419–426

Hjortstam K, Ryvarden L (1988) *Tomentellago* gen. nov. (Thelephoraceae, Basidiomycetes). Mycotaxon 31:39–43

Hjortstam K, Ryvarden L (1995) *Tomentella gigaspora* sp. nov. Mycotaxon 56:181–184

Holmgren PK, Holmgren NH, Barnet LC (1990) Index Herbarium. Part I. Herbaria of the world. 8th edn. Regnum Vegetabile 120. New York Botanical Garden, New York

Izzo A, Agbowo J, Bruns TD (2005) Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed conifer-broadleaf forests. New Phytol 166:619–629

Jakucs E, Agerer R (1999) *Tomentella pilosa* (Burt.) Bourdot & Galzin + *Populus alba* L. Descr Ectomyc 4:135–140

Jakucs E, Kovács GM, Agerer R, Romsics C, Erős-Honti Z (2005) Morpho-anatomical characterization and molecular identification of *Tomentella stuposa* ectomycorrhizae and related anatomotypes. Mycorrhiza 15:247–258

Jülich W (1981) Higher taxa of Basidiomycetes. Bibliotheca Mycol 85:1–485

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) The dictionary of fungi, 10th edn. CABI International, Wallingford

Kõljalg U (1996) *Tomentella* (Basidiomycota) and related genera in temperate Eurasia. Synop Fungorum 9:1–213

Kõljalg U, Dahlberg A, Taylor AFS, Larsson E, Hallenberg N, Stenlid J, Larsson K-H, Fransson PM, Kårén O, Jonsson L (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Mol Ecol 9:1985–1996

Kõljalg U, Jackus E, Bóka K, Agerer R (2001) Three ectomycorrhizae with cystidia formed by different *Tomentella* species as revealed by ITS rDNA sequences and anatomical characteristics. Folia Cryptogam Est 38:27–39

Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166:1063–1078

Kornerup A, Wanscher JH (1978) Methuen handbook of colour, 3rd edn. Methuen, London

Larsen MJ (1964) *Tomentella* and related genera in northern America. I. Studies of nomenclatural types of species of *Hypochnus* described by Burt. Can J Bot 43:1485–1510

Larsen MJ (1968) Tomentelloid fungi of North America. State Univ N Y Coll For Syracuse Univ Tech Publ 93:1–157

Larsen MJ (1974) A contribution to the taxonomy of the genus *Tomentella*. Mycol Mem 4:1–145

Maas Geesteranus RA (1971) Hydnaceous fungi of the eastern Old World. Vehr K Ned Akad Wet II 60:1–176

Maas Geesteranus RA (1975) Die terrestrichen Stachelpilze Europas. Vehr K Ned Akad Wet II 65:1–127

Malençon G (1952) Contribution à l'étude des champignons de la Kourimie. Bull Soc Bot Fr 99:33–52

Malençon G (1954) Prodrome d'une flora mycologique du Moyen-Atlas. Bull Soc Mycol Fr 77:117–156

Martini EC, Hentic R (2005) *Tomentella lilacinogrisea* et *T. guadalupensis* sp. nov. deux espèces de champignons tomentelloides des Caraibes. Bull Soc Mycol Fr 121:17–27

Melo I, Salcedo I, Tellería MT (1998) Contribution to the knowledge of tomentelloid fungi in the Iberian Peninsula. Folia Cryptogam Est 33:77–84

Melo I, Salcedo I, Tellería MT (2006) Contribution to the knowledge of tomentelloid fungi in the Iberian Peninsula. V. Nova Hedwigia 82:167–187

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Kõljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PloS ONE 1(1):e59. doi:10.1371/journal.pone.0000059

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evol Bioinform 4:193–201

Nilsson RH, Ryberg M, Abarenkov K, Sjökvist E, Kristiansson E (2009) The ITS regions as a target for characterization of fungal communities using emerging sequencing technologies. FEMS Microbiol Lett 296:97–101

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environment samples. Appl Environ Microbiol 71:5544–5550

Parfitt D, Ainsworth MA, Simpson D, Rogers HJ, Boddy L (2007) Molecular and morphological discrimination of stipitate hydnoids in the genera *Hydnellum* and *Phellodon*. Mycol Res 111:761–777

Parrent JL, Morris WF, Vilgalys R (2006) CO_2 enrichment and nutrient availability alter ectomycorrhizal fungal communities. Ecology 87:2278–2287

Patouillard N (1897) Catalogue raisonné des plantes cellulaires de la Tunisie. Imprimerie Nationale, Paris

Pegler DN, Robert PJ, Spooner BM (1997) British chanterelles and tooth fungi. An account of the British cantharelloid and stipitate hydnoid fungi. Royal Botanical Gardens, Kew

Sikes DS, Lewis PO (2001) PAUPRat: a tool to implement Parsimony Ratchet searches using PAUP*. (http://viceroy.eeb.uconn.edu/paupratweb/pauprat.htm)

Stalpers JA (1993) The aphyllophoraceous Fungi. I. Key to the species of the Thelephorales. Stud Mycol 35:1–168

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap alogarithm for the RAxML web-servers. Syst Biol 75:758–771

Suvi T, Tedersoo L, Abarenkov K, Beaver K, Gerlach J, Koljalg U (2010) Mycorrhizal symbionts of *Pisonia grandis* and *P. seychellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. Mycologia 102:522–533

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland

Tedersoo L (2007) Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Dissertationes Biologicae Universitatis Tartuensis 127, Tartu

Tedersoo L, Kõljalg U, Hallenberg N, Larsson K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol 159:153–165

Tedersoo L, Suvi T, Beaver K, Kõljalg U (2007) Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). New Phytol 175:321–333

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U (2008) Strong host preference of ectomycorrhizal

fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–490

Tedersoo L, Suvi T, Jairus T, Ostonen I, Põlme S (2009) Revisiting ectomycorrhizal fungi of the genus *Alnus*: differential host specificity, diversity and determinants of the fungal community. New Phytol 182:727–735

Wakefield EM (1966) Some extra-European species of *Tomentella*. Trans Br Mycol Soc 49:357–362

Wakefield EM (1969) Tomentelloideae of the British Isles. Trans Br Mycol Soc 53:161–206

White F (1983) The vegetation of Africa: a descriptive memoire to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa. Natural Resources Research, No. 20. UNESCO, Paris

Yorou NS (2008) Miscellaneous contributions to the anatomy and molecular systematic of tropical African resupinate Thelephorales. PhD thesis, University of Munich

Yorou NS, Agerer R (2007a) *Tomentella furcata*, a new species from Benin (West Africa) with basidia forming internal hyphae. Mycol Prog 6:239–247

Yorou NS, Agerer R (2007b) Type studies of three tomentelloid fungi (Basidiomycota, Thelephorales): *Tomentella radiosa*, *Tomentella cinereoumbrina* and *Tomentella punicea*. Nova Hedwigia 85:521–539

Yorou NS, Agerer R (2008) *Tomentella africana*, a new species from Benin (West Africa) identified by morphological and molecular data. Mycologia 100:68–80

Yorou NS, Agerer R (2011a) Rhizomorphic resupinate Thelephorales from Italy. Nova Hedwigia 92:177–204

Yorou NS, Agerer R (2011b) Non-rhizomorphic resupinate Thelephorales from Italy. Nova Hedwigia. doi:10.1127/0029-5035/2011/0092-0xxx (in press)

Yorou NS, Koljalg U, Sinsin B, Agerer R (2007) Studies in African thelephoroid fungi: 1. *Tomentella capitata* and *Tomentella brunneocystidia*, two new species from Benin (West Africa) with capitate cystidia. Mycol Prog 6:7–18

Yorou NS, Diabaté M, Agerer R (2011a) Phylogenetic placement and anatomical characterisation of two new West African *Tomentella* (Basidiomycota, Fungi) species. Mycol Prog. doi:10.1007/s11557-011-0739-0

Yorou NS, Gardt S, Guissou ML, Diabaté M, Agerer R (2011b) Three new *Tomentella* species from West Africa identified by anatomical and molecular data. Mycol Prog (in press). doi:10.1007/s11557-011-0760-3

Zhang Z, Schwarz S, Wagner L, Miller W (2000) A greedy alogarithm for aligning DNA sequences. J Comput Biol 7:203–214