

Phylogenetic placement and anatomical characterisation of two new West African *Toментella* (Basidiomycota, Fungi) species

Nourou S. Yorou · Moussa Diabate · Reinhard Agerer

Received: 27 September 2010 / Revised: 4 January 2011 / Accepted: 7 January 2011 / Published online: 10 February 2011
© German Mycological Society and Springer 2011

Abstract *Toментella amyloapiculata* and *T. agbassaensis* are described as new species within the genus *Toментella* based on materials we collected in the West African, northern Guinean seasonal forests. We used a combination of anatomical characters, sequence analyses and phylogenetic inference of 71 ITS rDNA sequences to characterise the two new species. Anatomically, *T. amyloapiculata* is characterised by simple septate brown to dark brown, thick-walled subicular and subhymenial hyphae and triangular to slightly lobed brown basidiospores (in frontal view), with isolate aculei of 1–2 µm. Phylogenetically, *T. amyloapiculata* forms a sister species of *T. fuscocinerea* with a moderate bootstrap support of 70%. *T. amyloapiculata* deviates from *T. fuscocinerea* by 10.07–11.73% in their sequence similarities. As far as *T. agbassaensis* is concerned, it clusters phylogenetically together with *T. bryophila* with a strong bootstrap support of 99%. The species is characterised by slightly differentiated rhizomorphs with yellowish hyphae, clamped, thick-walled and yellow to dark yellow subicular hyphae and pale yellow, small basidiospores of 6–8(8.5) µm with aculei of up to 0.5 µm. Both new species deviated from each other by 11.0–11.60% with regard to the ITS rDNA nucleotides.

Keywords Genetic distance · Morphology · SEM micrographs · *Toментella*, Thelephorales · Benin

N. S. Yorou (✉) · R. Agerer
Department Biology I, Division of Organismic Biology: Mycology,
University of München,
Menzinger Str. 67,
80638 München, Germany
e-mail: n.s.yorou@bio.lmu.de

M. Diabate
Conservateur Herbier SERG IRAG, CRRA Sérédou / IRAG,
BP: 1523,
Conakry, Guinea

Introduction

The genus *Toментella* belongs to the order Thelephorales Corner ex. Oberw. which encompasses worldwide distributed species (Köljalg 1996; Larsen 1964, 1968, 1974; Stalpers 1993; Yorou and Agerer 2008). Due to the paucity of strong and evident inter-specific discriminating anatomical features, the species concept has been unstable for a long time within the genus *Toментella*, as attested by numerous synonymies and combinations (Köljalg 1996; Larsen 1974; Wakefield 1966, 1969). The recent application of molecular systematics and DNA barcoding (Tedersoo et al. 2008, 2009) brought more insights into the understanding of species limits within *Toментella* (Köljalg et al. 2000, 2001; Yorou and Agerer 2007a, 2008; Yorou et al. 2007). Within the genus *Toментella*, however, it is common that the search for a query identity in public databanks (NCBI, UNITE) results in a great number of best matches comprising different morphological species with an equal identity rate to the query. Though the similarity of a given tomentelloid species can be easily checked in public databanks such as UNITE (Köljalg et al. 2005), it is still difficult to assign a specific name to a query with absolute certainty without referring to anatomo-morphological characters of either the basidiocarp (Köljalg 1996; Larsen 1974; Stalpers 1993) or the ectomycorrhizae (Agerer 1987–2008). This problem is particularly recurrent when dealing with species/queries with different geographic origins (and consequently different mycorrhiza hosts) for which anatomical descriptions are lacking.

In this paper, two new tropical African species are described using a combination of anatomo-morphological characters and molecular data. The aim of the present and previous investigations (Yorou and Agerer 2007a, 2008, 2011a; Yorou et al. 2007) has been to contribute with our

knowledge of species concepts within *Tomentella* by means of anatomical and molecular data.

Materials and methods

Specimen sampling

Specimens were collected in various vegetation types in the Northern Guinean seasonal forests of West Africa (White 1983) in the summers of 2005, 2006 and 2009. Preliminary notes were recorded using fresh materials that were afterwards dried using a propane gas heated field dryer (De Kesel 2001). Colour 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).

Light microscopy and SEM investigations

We refer to Yorou and Agerer (2007a, 2007b, 2008, 2011a, 2011b, 2011c) for protocols of light microscopy and scanning electron microscopy.

DNA extraction and sequencing

DNA was extracted from dried basidiocarps. DNA extraction and PCR amplification are detailed in Yorou and Agerer (2008, 2011a). DNA sequencing was performed by the sequencing service of the Department Biology I (Ludwig-Maximilians-Universität, München), using BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1 (Applied Biosystems, Foster City, CA, USA). Sequencing was performed on 1 µl purified DNA probes plus 0.3 µl ITS1F (forward) and 0.3 µl ITS4B (reverse). Three sequences of the new species have been generated and deposited in GenBank (NCBI) under accession numbers EF507257, EF507262, EF507263.

Sequence editing and similarity test between studied specimens

The sequences were edited with BioEdit v7.0.5 (Hall 2005). A rapid similarity test was run in BioEdit v7.0.5 in order to test the anatomo-morphological discrimination we made for the specimens. In addition, sequences of anatomo-morphologically close species were used to estimate the genetic distance between the studied specimens and species already described. Sequences were first aligned using “ClustalW Multiple Alignment” and the options “full multiple alignment”, “bootstrap NJ tree” and the “number

of bootstrap=1,000” activated. The 5’ and 3’ ends were excluded in order to get fully aligned segments. Identity/similarity of generated sequences was calculated using the “Pairwise alignment, calculation of the similarity/identity” option of BioEdit v7.0.5.

Molecular-phylogenetic analysis

The most similar sequences of each generated consensus sequence were searched for in UNITE (Kõljalg et al. 2005, <http://unite.ut.ee>) using the “Blastn” search option. Sequences of the top 10 most similar taxa identified to species level were downloaded. Similarly, we downloaded the top 10 best matches of the new generated sequences in Genbank NCBI (<http://www.ncbi.nlm.gov>) using the “Megablast search option” (Zhang et al. 2000). We excluded sequences of uncultured/environmental samples. The downloaded sequences were complemented with sequences used and published during previous investigations on tropical African resupinate Thelephorales (Yorou and Agerer 2007a, 2008, 2011a; Yorou et al. 2007). In addition, we added thelephoroid sequences from the Seychelles (Suvi et al. 2010; Tedersoo et al. 2007) to the dataset. All sequences were automatically aligned in BioEdit v7.0.5. The alignment was manually checked, improved and ambiguous columns that we could not align with absolute certainty were excluded. The final dataset included a total of 71 ITS rDNA thelephoroid sequences with a length of 506 characters. We used the final dataset to search for the most likely tree using RAXML (Stamatakis 2006). The GTR model of substitution was applied having Maximum Likelihood as optimal criterion. Robustness of individual branches was estimated using 1,000 bootstrap replicates (Stamatakis 2006). Simultaneously, the best scoring tree was obtained by running a fast bootstrap search of 100 pseudoreplicates in the program RAXML v.7.0.4 (Stamatakis et al. 2008). In addition, the most parsimonious tree was searched for by executing batch files generated by PAUPRat (Sikes and Lewis 2001) in PAUP* v4.0 (Swofford 2002), in order to check the topology of the most likely tree we obtained with the program RAXML (Stamatakis 2006; Stamatakis et al. 2008). The heuristic search was set with parsimony as the optimality criterion, multistate taxa interpreted as uncertainty, Zero-length branches set not to collapse, Maxtrees setting=600 and Tree-Bisection-Reconnection (TBR) as branch-swapping algorithm. All characters are unordered, of equal weight and gaps were treated as missing data. Consistency (CI) and Retention indices (RI) (Farris 1989) were calculated in order to estimate the amount of homoplasy.

Results

ITS rDNA-based differences between the new species

Specimen SYN 893 (accession number EF507263) deviates from SYN 930 (accession number EF507262) by 1.83%. Both specimens are accommodated under the new species *Tomentella amyloapiculata*. Higher deviation rates ranging from 11.00 to 11.60% could be calculated between *T. agbassaensis* (SYN 981; accession number EF507257) and above mentioned specimens.

According to “Blastn” search in UNITE, the most similar species of *Tomentella amyloapiculata* are *T. fuscocinerea* (Pers.) Donk (UDB003300) and *T. cinerascens* (P. Karst.) Höhn. & Litsch. (UDB003309) with identity rates ranging from 90 to 91%. Four different species, namely *T. stuposa* (Link) Stalpers, *T. bryophila* (Pers.) M.J. Larsen, *T. lapida* (Pers.) Stalpers and *T. ramosissima* (Berk. & M.A. Curtis) Wakef. present highest identity 91% with *T. agbassaensis*, according to the Megablast search (NCBI).

Phylogenetic placement of the new species

Phylogenetic inference of the ITS rDNA sequence dataset generated a total of 4,341 most parsimonious trees with equal length of 572 steps (CI=0.568, RI=0.876). Of 506 unambiguously aligned characters, 199 were variable and 173 were parsimonious informative. All the most parsimonious trees were similar in topology. The likelihood of the most likely tree found is $-5,215.909571$ (tree length=2.118876).

The topology of the trees generated by means of both RAxML and PAUP is quite similar. Thus, only the most likely tree obtained from RAxML is presented (Fig. 1). In general, the topology and bootstrap support of different clades of the most likely tree found is similar to the ones reported from previous studies (Kõljalg et al. 2000, Kõljalg et al. 2001; Yorou and Agerer 2007a; Yorou et al. 2007). However, in this study, the clade comprising *T. punicea* and *T. botryoides* form a sister clade of the *T. stuposa* complex, rather than forming a sister clade of *T. ferruginea* and *T. umbrinospora*.

In both RAxML and PAUP, *T. agbassaensis* clusters together with *T. bryophila* with a very strong bootstrap support of 99%. The clade comprising *T. agbassaensis* and *T. bryophila* forms a sister clade to *T. lapida* with a strong support of 93%. All three species, *T. agbassaensis*, *T. bryophila* and *T. lapida*, form a moderately supported (74% bootstrap) sister group of *T. stuposa* whose five representatives in this study cluster together with 91% bootstrap

support. The phylogenetic placement of *T. agbassaensis* as sister species of *T. bryophila*, coupled with different anatomo-morphological characters (see below), support the erection of *T. agbassaensis* as a new species.

As far as *T. amyloapiculata* is concerned, it clusters together with *T. fuscocinerea* with a moderately bootstrap support of 70%. A sister clade of this group is a still undescribed *Tomentella* species from Benin (West Africa). These species together form a sister group to a clade comprising *T. lilacinogrisea* Wakef., *T. cinerascens* and *T. subclavigera* Litsch. with no bootstrap support at deeper roots. *T. amyloapiculata* is described here as new species based on sequence analyses and morpho-anatomical characters (see below).

Description of the two new species

Tomentella agbassaensis Yorou sp. nov. (Mycobank: MB 517983) Genbank NCBI, accession number EF507257

Material studied

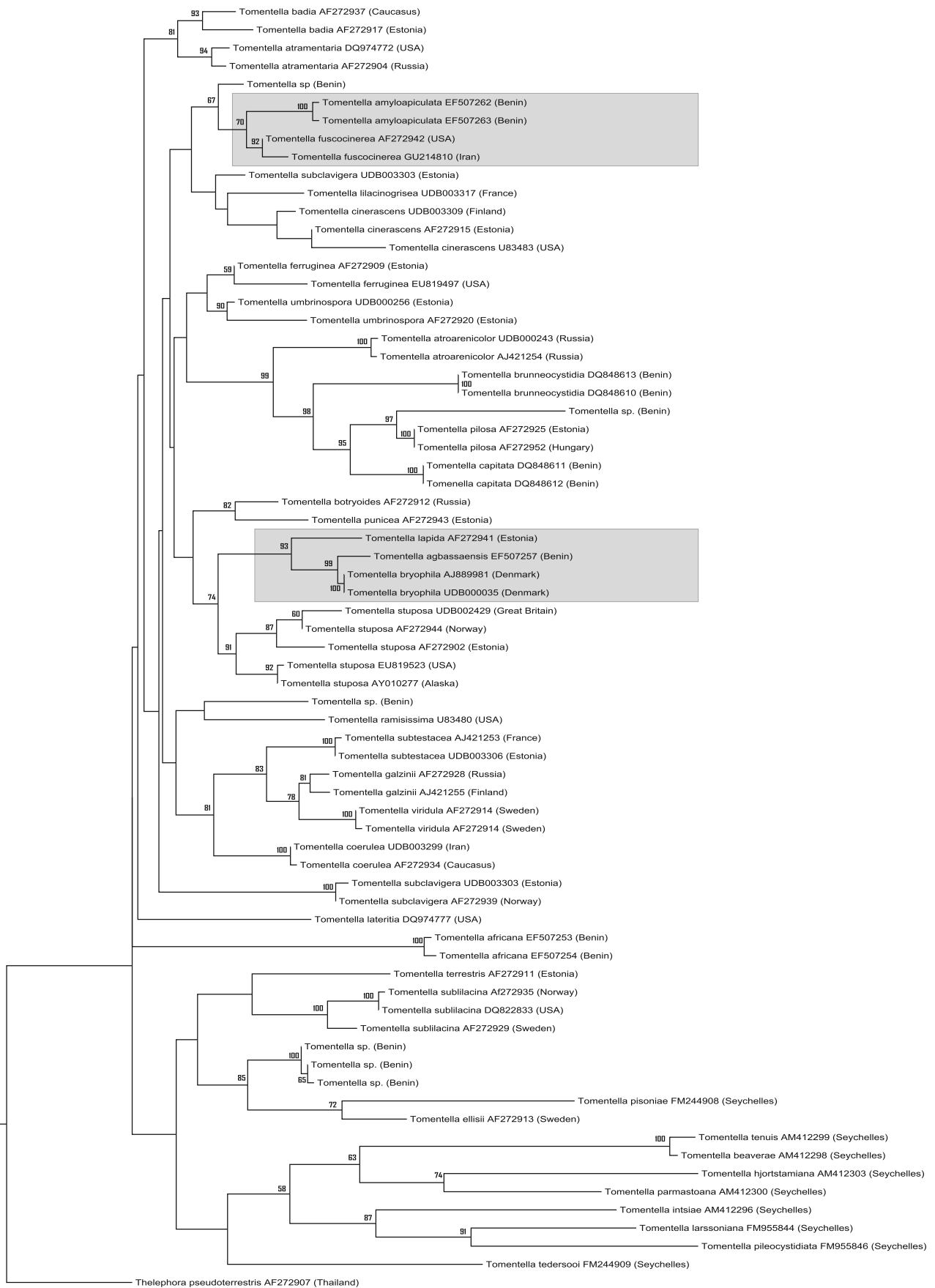
Benin, central part, Borgou Province, region of Agbassa, forest reserve of Wari-Marou, 08°50'06.5"N, 002°17'15.9"E, leg and det. NS Yorou, 19.08.2006, herb. SYN 981 (M), holotype,

Etymology

In reference to Agbassa, one locality in the forest reserve of Wari-Marou where the type material has been collected.

Description

Basidiocarpis resupinatis, tenuibus, usque ad 0.5 mm altis, separabilibus, pelliculosis, continuis. Subiculo tenui, arachnoideo, pallidiore quam hymenium, flavo, marginibus sterilibus indeterminatis. Rhizomorphae in subiculo presentes, rhizomorphae crassiores in aqua et in 2.5% KOH distincte ochraceae, monomiticae; hyphis centralibus dilatatis, fibuligeris, raro septis simplicibus, 4–6(7) μm in diametro, crassitunicatis (0.5–1 μm), in marginibus cum hyphis tenuibus, tortuosis; hyphae distincte presentes ad conicae ramificationes laterales rhizomorpharum, 1–3 μm in diametro, interdum fibuligeris deficientibus. Hyphae subiculi fibuligerae, 4–6(–6.5) μm in diametro, in 2.5% KOH interdum sinuosae, crassitunicatae (0.5–1 μm). Hyphae subhymenii fibuligerae, septa simplicia absentia, 3–5 (–7) μm in diametro, crassitunicatae (0.5–1 μm). Cystidia absentia. Basidia fibuligera, 25–45 μm longa, apicibus 7–



◀ **Fig. 1** The Maximum Likelihood tree showing the placement of the new species within *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. The clades in which both new species fall are indicated in grey

9 μm , basalibus 5.5–7 μm , clavata, 4-sterigmatica, sterigmatibus 3–5(–7) μm longis, basaliter 1.5–2.5 μm latis. Basidiosporae (6–)6.5–8(–8.5) \times (4.5)5–7(–7.5) μm in aspectu frontali, (5–)6–7(–7.5) \times (5–)5.5–6.5(–7) μm in aspectu laterali, triangulares et partibus proximalibus dilatatis in aspectu frontali, ellipsoideae in aspectu laterali, echinulatae, aculeis plerumque isolatis, interdum duo vel plus quam duo, brevissimis (0.2–0.5 μm altis), in aqua et in 2.5% KOH flavidae, non cyanescentes, non congophilae, non cyanophilae, non amyloideae. Chlamydosporae absentes.

Basidiocarp resupinate, thin, up to 0.5 mm, separable from the substrate, pelliculose, continuous. Hymenophore smooth to finely granular, continuous, hymenium brown (6E4 to 6E5), subiculum thin, arachnoid, paler than the hymenium and yellowish, sterile margin indeterminate.

Rhizomorphs present in the subiculum, rare at the sterile margins, dark-brown under stereomicroscope, young rhizomorphs (up to 20 μm) yellow, old rhizomorphs deep yellow-brown in water and in 2.5% KOH, monomitic, slightly differentiated, of type C (according to Agerer 1987–2008; Fig. 2), composed of central large hyphae, covered by thinner peripheral ones, central individual hyphae clamped, simple septa rare, peripheral hyphae commonly tortuous mostly at the conical side branches, all hyphae yellow to deep yellow in water and in 2.5% KOH, central hyphae 4–6 (–7) μm in diam., thick-walled (0.5–1 μm), peripheral hyphae thinner, 1–3 μm , some clampless.

Subicular hyphae clamped, simple septa rare, 4–6(–6.5) μm wide, regular, not inflated, commonly with sidebranch outgrowths, cross-shaped branches and closed anastomoses common (Fig. 3), sometimes sinuous in 2.5% KOH, thick-walled (0.5–1 μm), yellow to deep-yellow in water and in 2.5% KOH, without encrustations in water and in 2.5% KOH.

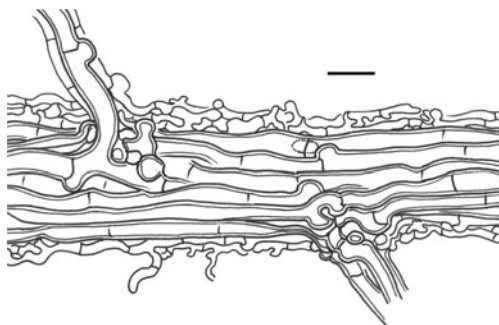


Fig. 2 *T. agbassaensis*. Optical section through a rhizomorph (from SYN 981). Bar 10 μm

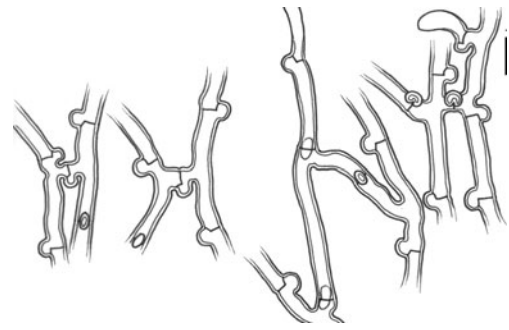


Fig. 3 *T. agbassaensis*. Closed anastomoses of the subicular hyphae (from SYN 981). Bar 10 μm

Subhymenial hyphae clamped, simple septa absent, 3–5 (–7) μm wide, neither short nor inflated (Fig. 4), thin-walled (0.5–1 μm), colourless to pale yellow in water and in 2.5% KOH.

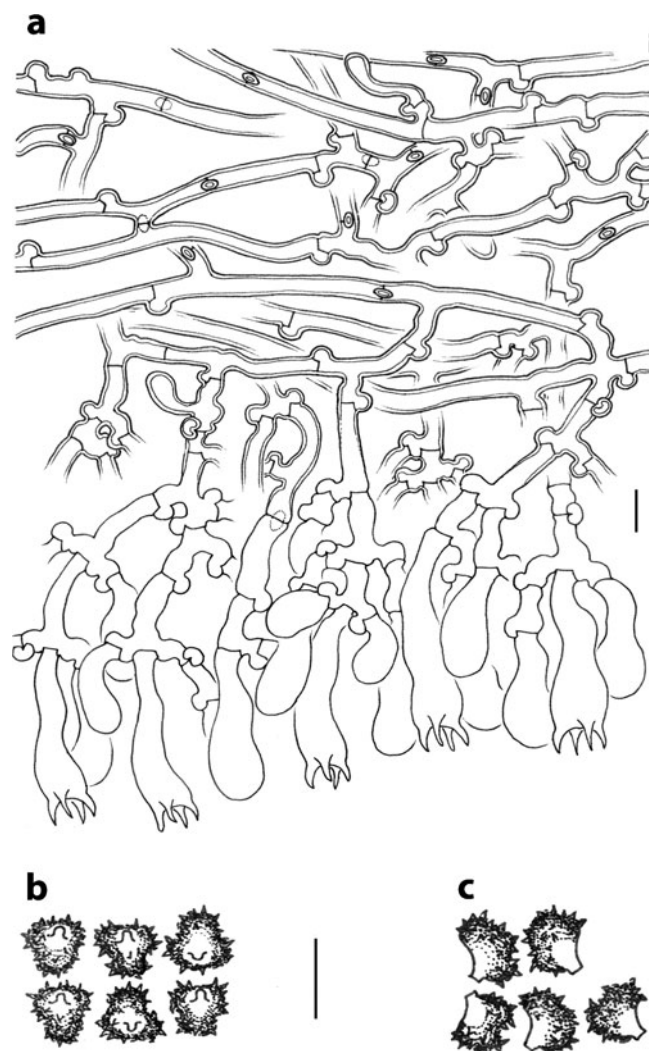


Fig. 4 *Tomentella agbassaensis*. **a** Section through the basidiocarp, **b** basidiosporae in frontal view, **c** basidiosporae in lateral view (from SYN 981). Bar 10 μm

Cystidia absent.

Basidia clamped at base, 25–45 μm long, 7–9 μm at apex, 5.5–7 μm at base, clavate, not stalked, sometimes sinuous, without transverse septa, colourless to very pale yellow in water and in 2.5% KOH, slightly congophilous, slightly cyanophilous, 4-sterigmate, sterigmata 3–5(–7) μm long and 1.5–2.5 μm at base.

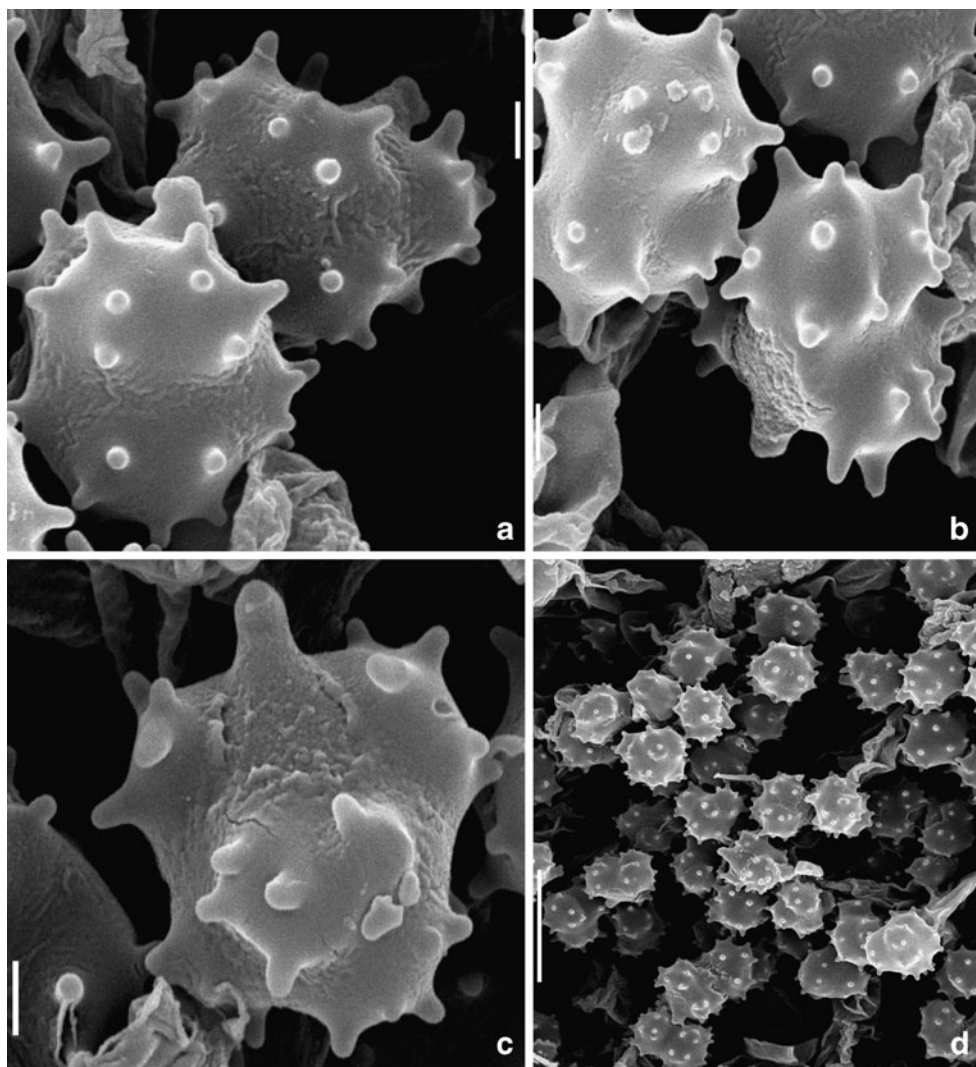
Basidiospores (6–)6.5–8(–8.5) \times (4.5–)5–7(–7.5) μm in frontal face, (5–)6–7(–7.5) \times (5–)5.5–6.5(–7) μm in lateral face, triangular with widened proximal part in frontal, ellipsoid in lateral view, (Fig. 5), oil drops absent, echinulate, aculei usually isolated, sometimes grouped in 2 or more, very short (0.2–0.5 μm), basidiospores pale yellow in water and in 2.5% KOH.

Chlamydoconidia absent.

Chemical reactions

No structure was found to be cyanescent, amyloid, congophilous or cyanophilous if not specifically indicated.

Fig. 5 *Toментella agbassaensis*. SEM of basidiospores (from SYN 981). **a** Basidiospore in lateral view, **b** basidiospores in dorsal view, **c** basidiospores in frontal view, **d** general view of basidiospores in different faces. Bars (**a–c**) 1 μm , (**d**) 10 μm



Habitat

On the undersides of dead logs and barks, under native Caesalpiniaceae (*Isobertinia doka* Craib and Stapf, *I. tomentosa* Craib & Stapf, *Burkea africana* Hook. F. & *Afzelia africana* Smith) and Euphorbiaceae (*Uapaca togoensis* Pax).

Toментella amyloapiculata Yorou sp. nov. (Mycobank: MB 517984) GenBank NCBI, accession numbers EF507263 (holotype), EF507262.

Material studied

Benin, central part, Borgou Province, region of Wari-Marou, forest reserve of Wari-Marou, 08°22'20.7"N, 002°48'32.5"E, leg and det. NS YOROU, 06.08.2005, herb. SYN 893 (M), holotype, Genbank NCBI, accession number EF507263. — Benin, central part, Borgou Province, region of Wari-Marou, forest reserve of Wari-Marou, 08°23'19.8"N, 002°47'25.6"E,

09.08.2005, herb. SYN 930 (M), Genbank NCBI, accession number EF507262. — Guinea, upper Guinea, Province of Kakan, Prefecture of Kouroussa, Forest reserve of Baro, 10°57'09.2"N, 009°01'45.0"W, on dead logs, leg. and det. NS. Yorou, 18.07.2009, herb. SYN 2291 (M). — Guinea, upper Guinea, Province of Kakan, Prefecture of Kouroussa, on the road between Kouroussa and Dabola, forest reserve of Moussaya, 10°00'56.7"N, 009°59'47.1"W, on dead logs, leg. and det. NS. Yorou, 19.07.2009, herb. SYN 2325 (M).

Etymology

In reference to the typically amyloid apiculi.

Description

Basidiocarpis resupinatis, tenuibus, ad 0.5 mm altis, adhaerentibus usque ad separabilibus, crustosis, continuis. Rhizomorphae in subiculo et in margine absentes. Hyphae subiculi septis simplicibus, 4–6(–7) μm in diametro. Hyphae subhymenii septis simplicibus, 4–7(–8) μm in diametro, crassitunicatae (0.5–1 μm). Cystidia absentia. Basidia septis simplicibus, 40–65 μm longa, apicibus 7.5–13 μm , basibus 6.5–8 μm , utriformia, 4-sterigmatica, sterigmatibus 7–10(–11) μm longis, basaliter 2–3 μm latis. Basidiosporae (8–)8.5–11(–12) \times (7.5–)8–9(–10) μm in aspectu frontali, (8–)8.5–11.5(–12) \times (7–)7.5–8.5(–9) μm in aspectu laterali, triangulares, partibus proximale dilatatis usque ad sublobatis in aspectu frontali, ellipsoideae in aspectu laterali, echinulatae, aculeis isolatis, 1–2 μm longis, in aqua et in 2.5% KOH pallide brunneae usque ad brunneae, non congophilae, non cyanophilae, apiculo distincte amyloideo. Chlamydosporae absentes.

Basidiocarp resupinate, thin, up to 0.5 mm, adherent to separable from the substrate, crustose, continuous. Rhizomorphs lacking. Hymenophore smooth to finely granular, continuous, hymenium dark brown (6F4), subiculum thin, arachnoid, dark brown to almost black, darker than the hymenium, sterile margin indeterminate.

Rhizomorphs absent.

Subicular hyphae simple septate, clamps completely absent, 4–6(–7) μm wide, usually regular, commonly with short side branch outgrowths, sometimes sinuous in 2.5% KOH, cross-shaped branching and anastomoses rare, thick-walled (0.5–1 μm), brown to dark-brown in water and in 2.5% KOH, without encrustations in water and in 2.5% KOH.

Subhymenial hyphae simple septate, 4–7(–8) μm wide, neither short nor inflated (Fig. 6), tortuous, thick-walled (0.5–1 μm), brown to dark-brown in water and in 2.5% KOH.

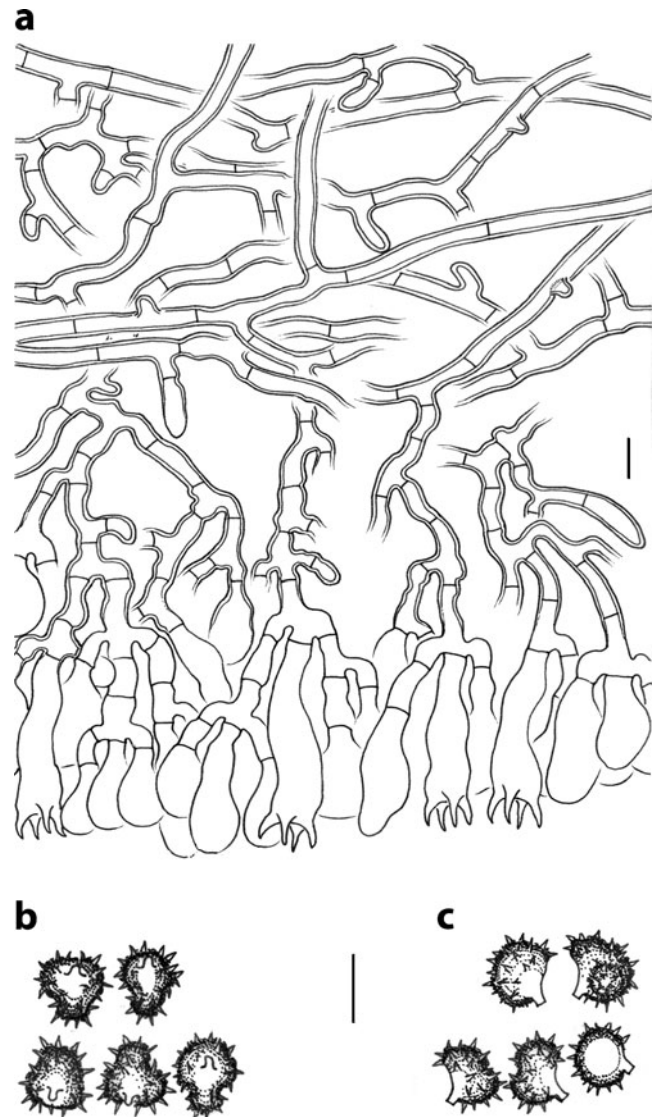


Fig. 6 *Tomentella amyloapiculata*. **a**. Section through the basidiocarp, **b**. Basidiospores in frontal view, **c**. Basidiospores in lateral view (From SYN 893). Bar=10 μm

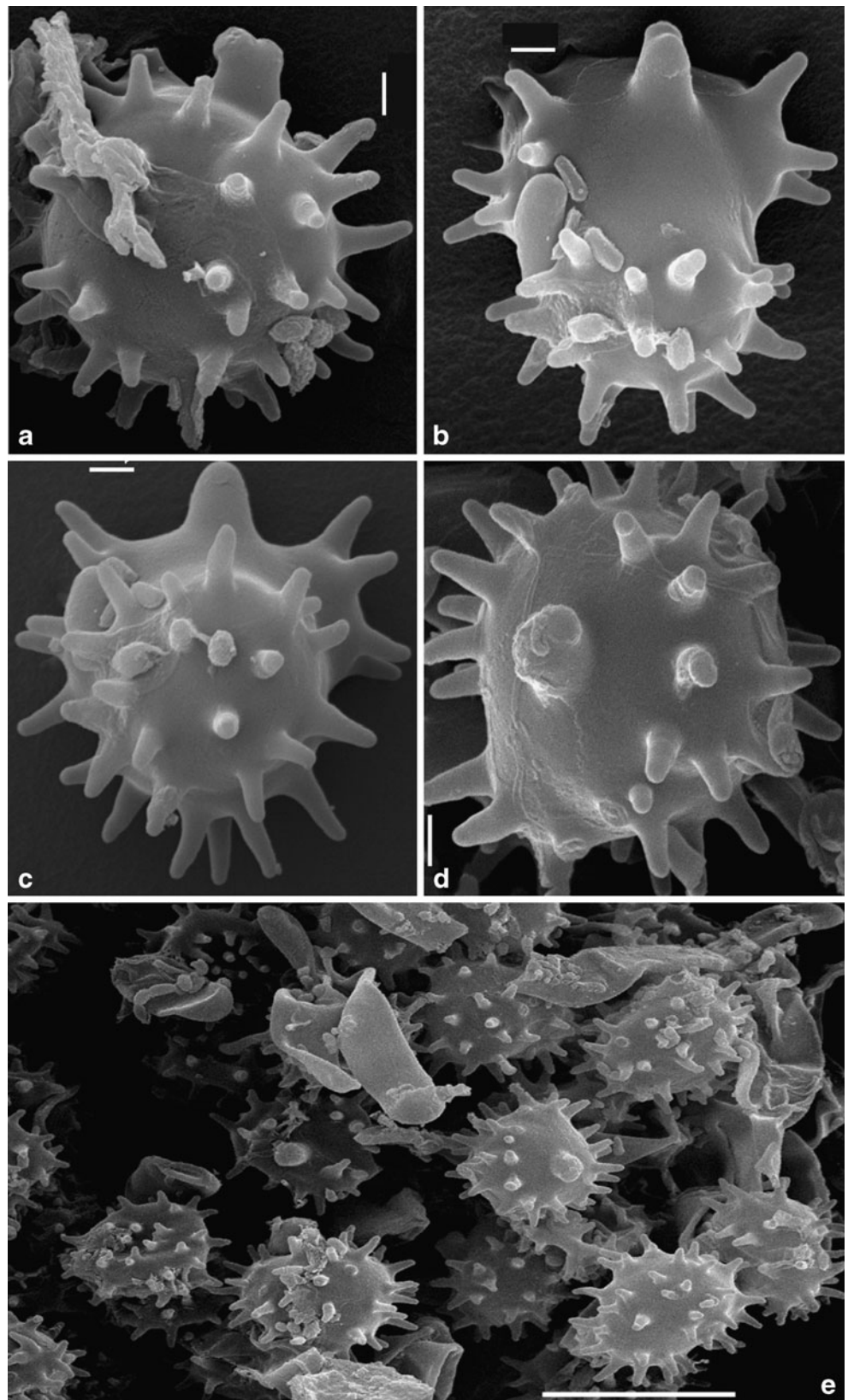
Cystidia absent.

Basidia simple septate at base, 40–65 μm long, 7.5–13 μm at apex, 6.5–8 μm at base, utriform, not stalked, sometimes sinuous, without transverse septa, colourless to very pale brown in water and in 2.5% KOH, slightly congophilous, cyanophilous, 4-sterigmate, sterigmata 7–10 (11) μm long and 2–3 μm at base.

Basidiospores (8–)8.5–11(–12) \times (7.5–) 8–9(–10) μm in frontal face, (8–)8.5–11.5(–12) \times (7–)7.5–8.5(–9) μm in lateral face, triangular with widened proximal part to slightly lobed in frontal, ellipsoid in lateral view (Fig. 7), oil drops rare, echinulate, aculei isolated, 1–2 μm , basidiospores pale brown to brown in water and in 2.5% KOH, apiculus typically amyloid, turning dark blue in Melzer's reagent.

Chlamydosporae absent.

Fig. 7 *T. amyloapiculata*. SEM of basidiospores (from SYN 893). **a** Basidiospore in lateral view, **b** basidiospore in frontal view, **c** basidiospore in dorsal view, **d** basidiospore in proximal view, **e** basidiospores in different faces. Bars (**a–d**) 1 μm , (**e**)=10 μm



Reaction in chemicals

No structure was found to be cyanescent, amyloid, congophilous or cyanophilous if not specifically indicated.

Habitat

At the underside of dead bark and wood debris, in woodlands and forests dominated by Euphorbiaceae (*Uapaca togoensis*) and Ceasalpiniaceae (*Isoperlinia doka*, *I tomentosa*, *Burkea africana*, *Afzelia africana* and *Antho-nota crassifolia*).

Discussion

Tomentella agbassaensis is characterised by colourless to yellowish subicular and subhymenial hyphae and by yellowish triangular basidiospores of up to 8.5 µm in frontal view. Only a limited number of resupinate Thelephorales present yellowish hyphae and basidiospores (Dämmrich 2006; Kõljalg 1996; Stalpers 1993). Yellowish subicular/subhymenial hyphae and/or basidiospores have been reported for *T. italica* (Sacc.) M.J. Larsen (Dämmrich 2006; Kõljalg 1996), *T. furcata* Yorou & Agerer (Yorou and Agerer 2007a) and for *T. bryophila* (Dämmrich 2006; Kõljalg 1996; Yorou and Agerer 2011a). *T. italica* differs anatomically from *T. agbassaensis* by its irregularly globose bigger basidiospores of up to 10 µm with bifurcate ornaments, whilst those of *T. agbassaensis* are mostly triangular, up to 8(8.5) µm. *T. furcata* is reported from the same forest reserve but presents a crustose thin basidiocarp (Yorou and Agerer 2007a). It differs however by its simple septate brown to dark brown subicular hyphae, absence of rhizomorphs and distinctly yellow to deep yellow, bigger subglobose to globose basidiospores of up to 13 µm with longer bifurcate aculei. In a similar way, *T. bryophila* differs from *T. agbassaensis* by its bigger, distinctly globose basidiospores (up to 10 µm) with long, isolate and conical aculei of up to 2.5 µm.

Molecular-phylogenetically, *T. agbassaensis* is close to *T. bryophila*. One of the synapomorphic characters that could explain such proximity is the yellowish colour of the basidiospores in both species, though basidiospores of *T. bryophila* present amyloid apiculi (Dämmrich 2006; Yorou and Agerer 2011b). *Tomentella lapida* forms a sister species of both *T. agbassaensis* and *T. bryophila* (Fig. 1). In this group, *T. agbassaensis* presents quite different anatomical features, among others the smaller (up to 8.5 µm) triangular to slightly lobed basidiospores with short aculei (up to 0.5 µm) and the presence of slightly differentiated rhizomorphs, unlike both the previously mentioned species that lack them (Yorou and Agerer 2011c).

Anatomically, the simple septate subicular and subhymenial hyphae and the triangular brown basidiospores of up to 12 µm with isolated aculei (of up to 2 µm) make the identification of *T. amyloapiculata* very easy. Among all the already described thelephoroid species, *T. fuscocinerea* is anatomically the closest. Like *T. amyloapiculata*, it presents an adherent crustose brown basidiocarp, simple septate subicular hyphae and brownish basidiospores with similar size. Anatomically, *T. fuscocinerea* is characterised by colourless to only very pale brown subicular hyphae of maximum 5 µm diam. (Dämmrich 2006; Kõljalg 1996), whilst those of *T. amyloapiculata* are wider (up to 7 µm) with brown to dark brown colour in water and in 2,5% KOH. According to Kõljalg (1996), *T. fuscocinerea* may present monomitic rhizomorphs in the subiculum. We were not able to observe rhizomorphs in both specimens of *T. amyloapiculata* we examined. Subhymenial hyphae of *T. fuscocinerea* are thin-walled, partly cyanescent (Dämmrich 2006; Kõljalg 1996; Melo et al. 2000). In addition, basidia of *T. fuscocinerea* may present a cyanescent reaction. Subhymenial hyphae of *T. amyloapiculata* are always thick-walled and no cyanescent reaction is observed neither in subhymenial hyphae nor in the basidia. Unlike *T. fuscocinerea*, apiculus of basidiospores of *T. amyloapiculata* are distinctly amyloid. Such a character has never been reported for *T. fuscocinerea*. The amyloid reaction is a rare character within resupinate Thelephorales (Dämmrich 2006). It is only known within a few species, namely on the hyphae of *T. subamyloidea* Agerer (Agerer and Bougher 2001). The apiculus of *T. bryophila* and *T. lapida* are partly amyloid (Dämmrich 2006; Yorou and Agerer 2011c). The two latter species differ by their typically globose basidiospores with long conical aculei from *T. amyloapiculata* (see above).

Molecular-phylogenetically, *T. amyloapiculata* forms a sister species of *T. fuscocinerea* with a moderate bootstrap support of 70%. Such phylogenetic proximity is supported by some synapomorphies that are highlighted in previous paragraphs.

Acknowledgment We are much indebted to the Deutsche Forschungsgemeinschaft (DFG) for financial support (project Ag7/19-1).

References

- Agerer R (1987–2008) Colour Atlas of Ectomycorrhizae, 1st–14th delivery. Schwäbische Gmünd, Einhorn
- Agerer R, Bougher NL (2001) *Tomentella subamyloidea* sp. nov. and *Tomentella radiosa* (Thelephorales, Hymenomycetes, Basidiomycota) from Australia. Aust Syst Bot 14:607–614
- 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
- Farris JS (1989) The retention index and rescaled consistency index. *Cladistics* 5:417–419
- Hall T (2005) BioEdit: Biological sequence alignment editor for Win95/98/NT/2 K/XP. Ibis therapeutic, Carlsbad, CA
- Holmgren PK, Holmgren NH, Barnet LC (1990) *Index Herbariorum*. Part I. Herbaria of the World. 8th edn. Regnum Vegetabile 120. New York botanical Garden, New York
- Köjljalg U (1996) *Tomentella* (Basidiomycota) and related genera in Temperate Eurasia. *Synop Fungorum* 9:1–213
- Köjljalg U, Dahlberg A, Taylor AFS, Larsson E, Hallenberg N, Stendil J, Larsson K-H, Fransson PM, Kårén O, Jonsson L (2000) Diversity and abundance of resupinate theleporoid fungi as ectomycorrhizal symbionts in Swedish boreal forests. *Mol Ecol* 9:1985–1996
- Köjljalg U, Jakucs E, Bóka K, Agerer R (2001) Three ectomycorrhizae with cystidia formed by different *Tomentella* species as revealed by rDNA ITS sequences and anatomical characteristics. *Folia Cryptogam Est* 38:27–39
- Köjljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjølner 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. Eyre 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. College of Forestry, Syracuse Univ., Tech. Publ 93:1–157
- Larsen MJ (1974) A contribution to the taxonomy of the genus *Tomentella*. *Mycol Mem* 4:1–145
- Melo I, Salcedo I, Telleria MT (2000) Contribution to the knowledge of tomentelloid fungi in the Iberian Peninsula. II. *Karstenia* 40:93–101
- 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 Algorithm for the RAxML Web-Servers. *Syst Biol* 75:758–771
- Suvi T, Tedersoo L, Abarenkov K, Beaver K, Gerlach J, Köjljalg 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, Sunderland, Mass.
- Tedersoo L, Suvi T, Beaver K, Köjljalg U (2007) Ectomycorrhizal fungi of the Seychelles: Diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Cesalpiniaceae) 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, Köjljalg 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 memoir to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa. Natural Resources Research, No. 20. Paris, UNESCO
- Yorou SN, Agerer R (2007a) *Tomentella furcata*, a new species from Benin (West Africa) with basidia forming internal hyphae. *Mycol Prog* 6:239–247
- Yorou SN, Agerer R (2007b) Type Studies of three tomentelloid fungi (Basidiomycota, Thelephorales): *Tomentella radiosa*, *Tomentella cinereoumbrina* and *Tomentella punicea*. *Nova Hedwig* 85:521–539
- Yorou SN, Agerer R (2008) *Tomentella africana*, a new species from Benin (West Africa) identified by morphological and molecular data. *Mycologia* 100:68–80
- Yorou SN, Agerer R. (2011a). Anatomical and ITS rDNA-based phylogenetic identification of two West African resupinate Thelephorales. *Mycoscience* (revised version submitted)
- Yorou SN, Agerer R (2011b) Non rhizomorphic resupinate Thelephorales from Italy. *Nova Hedwigia* (in press)
- Yorou SN, Agerer R (2011c) Rhizomorphic resupinate Thelephorales from Italy. *Nova Hedwigia* (in press)
- Yorou NS, Köjljalg U, Sinsin B, Agerer R (2007) Studies in African theleporoid fungi: 1. *Tomentella capitata* and *Tomentella brunneocystidia*, two new species from Benin (West Africa) with capitate cystidia. *Mycol Progress* 6:7–18
- Zhang Z, Schwarz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–214