Miscellaneous Contributions to the Anatomy and Molecular Phylogeny of tropical African resupinate Thelephorales

Dissertation
Zur Erlangung des Doktorsgrades der Naturwissenschaften
(Dr. rer. nat.)
Der Fakultät für Biologie der Ludwig-Maximilians-Universität München

Vorgelegt von

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München, February 2008

Datum der mündlichen Prüfung: 26. Februar 2008
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To my wife Alice YOLLOU YOROU who endured a 3-year period of physical separation. To my daughter Astride Magnoutewa Dolorès whose early childhood I very much missed.

Acknowledgments

The current project would have neither started nor successfully been completed without the constant financial support of the German Academic Exchange Service (DAAD). My DAAD contact people, e.g. Mrs. Leistritz and Mrs. Basu (from 2006 on), played paramount liaison roles during countless exchanges with the DAAD. I would like to express my sincere thanks to both contact persons. My special thanks go to Dr. Roland Weiß, the present chief of the Subdivision 413 Africa/Sub-Sahara and my scholarship donor DAAD. Additional financial support was given by the International Foundation for Science (IFS) and the African Forestry Research Network (AFORNET) to whom I address my best thanks.

I am deeply indebted to my supervisor Prof. Dr. Reinhard Agerer for his unparalleled guidance throughout this project. In 2002, although we had not met before, he invited me to his laboratory on a short-term exchange. After this visit he arranged a laboratory space for me, committed himself to supervising my PhD studies, and supported the grant application I submitted to the DAAD. During our initial discussions about the fascinating but daunting subject of *Tomentella* and its allies, I doubted whether I was capable of conducting a taxonomic study of such a difficult fungal group. Despite my previous mycological monographing experience, Prof. Agerer taught me much about how to make quality microscope preparations and gave me invaluable instruction in the art of producing scientific line drawings. During his time as my supervisor, he not only fulfilled the role of scientific advisor, but acted as my spiritual and moral mentor, helping build my trust and self confidence in my own work. I also acquired invaluable professional skills.

The DAAD scholarship was offered to me on an annual basis. In this context, progress reports, coupled with references regarding my previous performance, ability and skills, were prerequisites for renewing the scholarship. Dr. Peter Döbbeler never hesitated to write a reference letter for me each time I approached him. He always showed interest in my achievements and often inquired about my scientific progress. For this friendly and collegial attention and support, I extend my sincere gratitude to him.

I had been a student for three years when I took part in my first mycological expedition in Benin in 1997. Taking part in this expedition was perceived by me as a student job. I never imagined that I would still be working on tropical fungi ten years on. Such interest in mycology would have not developed without the encouragement of Prof. Brice Sinsin. Prof. Sinsin teaches tropical ecology at the University of Abomey-Calavi in Benin (West Africa), and although his personal area of expertise is not fungal, he was able to put me in touch with the Belgian mycologists Prof. Rammeloo and Dr. De Kesel, with whom he co-supervised my Master studies in 1999-2000. Two years later he contacted Prof. Agerer with whom he discussed the possibility of my studying for a PhD. The present work is the result of this initiative. Later on, Prof. Sinsin facilitated all collection trips I undertook in Benin and always made laboratory space available to me. I would like to express my deepest thanks to him on two counts: firstly for the basic role he played, and secondly for his commitment to the promotion of young Beninese scientists in general.

During the course of this project, I benefited a lot from lively discussions with my laboratory colleagues. Much advice was dispensed during the monthly meetings of

the mycological working group, as I reported the progress of my investigations. Drs. Ludwig Beenken, Thassilo Franke, Stefan Raidl, Christoph Hahn, Philomena Bodensteiner, Eva Facher and Alex Kocyan are thanked for their advice with regard to taxonomic and microscopic investigations, SEM studies and molecular analyses. The constant assistance of Rita Verma and Philomena Bodensteiner are scanned in my memory forever. Many thanks to Sebastian Gardt, who helped me to overcome stress, induced by long working days, and my colleagues Erika Di Marino and Jie Wei with whom I often stayed in the laboratory till late into the night.

Special thanks are due to Robert Sieglstetter, Alexander Hofmann, Eva Schmidbauer Marion Hartl and Miriam Voll who greatly alleviated social difficulties I faced during my stay in Munich. I would have been in big trouble, and it would have not been possible to successfully complete this project without your infallible social assistance.

I address my deepest thanks to my field guide Salomon Boko and the population of the Wari-Maro village (Central Benin). Despite the harsh tropical African sun and/or intense rains, Salomon Boko always agreed to guide me for several hours at a time. We turned logs and lifted bark in every corner of the Wari-Maro forest reserve, despite the high risk of a face-to-face encounter with poisonous reptiles. I would like to thank him for taking such dangerous endeavours.

Last but not least, I address my warm thanks to my parents and Beninese friends whom I have greatly missed during the last 4 years.

Content

1. List of original publications

This dissertation is based on the following publications, which are refereed to in the text by their Roman numeral (**I-VI**, in a chronological order):

I. **Yorou SN,** Kõljalg 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. Mycological Progress 6: 7-18.

II. **Yorou SN**, Agerer R. 2007. *Tomentella furcata*, a new species from Benin (West Africa) with basidia forming internal hyphae. Mycological Progress 6: 239-247.

III. **Yorou SN,** Agerer R. 2007. *Tomentella africana*, a new species from Benin (West Africa) identified by morphological and molecular data. Mycologia (in press, accepted on 17.09.2007).

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

V. **Yorou SN**, Agerer R, Raidl S. 2008. *Afzeliaerhiza beninensis*. + *Afzelia africana* Smith. Descriptions of Ectomycorrhizae 11 (accepted).

VI. **Yorou SN**, Agerer R, Raidl S. 2008. *Uapacaerhiza wariensis*. + *Uapaca guineensis* Mül. Arg. Descriptions of Ectomycorrhizae 11 (accepted).

Table 1. Author´s contribution to each paper (%)

	I	**II**	**III**	**IV**	**V**	**VI**
Collecting trips and specimen sampling	100	100	100	100	100	100
Microscopic investigations and line drawings	80	80	90	90	90	90
Isolation and morpho/anatomotyping of ectomycorrhizae	n.a.*	n.a.	n.a.	n.a.	90	90
DNA extraction and PCR	50	100	100	100	100	100
Sequences analyses and phylogenetic studies	40	80	80	80	100	100
Drafting and improvement of Manuscripts	80	90	90	90	60	60

* n.a., not applicable

2. Introduction

2.1. Current state of knowledge on Thelephorales

2.1.1. Diversity, taxonomic position and important anatomical features

Thelephorales Corn ex. Oberw. are members of the Agaricomycetes Class (Basidiomycota, Fungi). The thelephoroid fungi number over 177 accepted species (Kirk *et al*. 2001) that are accommodated within 14 genera and 2 families (Donk 1961, Jülich 1981, Stalpers 1993): the Bankeraceae and the Thelephoraceae (Table 1). Bankeraceae was originally separated from Thelephoraceae to accommodate species with ornamented colourless spores with regular outline, and the occurrence of basidiocarps emitting an odour of fenugreek (Donk 1961). It included *Bankera* Coker & Beers ex Pouzar, and *Phellodon* P. Karsten. This original definition of Bankeraceae is controversial, however, since the above mentioned diagnostic features occur in many other thelephoroid genera (Stalpers 1993). To provide more reliable diagnostic features, Jülich (1981) enlarged the original definition of Bankeraceae and included all genera with typical fenugreek odour, with pileate and stipitate fruit bodies, hydnoid to spinose hymenophores with brown and lobed as well as colourless and evenly ornamented basidiospores. Currently, Bankeraceae comprises 5 distinct genera: *Bankera*, *Hydnellum* P. Karsten, *Phellodon*, *Sarcodon* Quél. ex P. Karst and *Boletopsis* Fayod. The family Thelephoraceae comprises genera with effuse, effuso-reflex, resupinate, apodal pileate and/or spathulate, pleuropodal pileate to clavaroid fruit bodies and colourless to strongly pigmented, warted to typically echinulate basidiospores with an uneven outline (Stalpers 1993, Kõljalg 1996, Corner 1968). In general, Thelephorales consist of species with strongly ornamented, non-amyloid spores with a large apiculus and often dark-coloured fruit bodies. The presence of thelephoric acid, that turns blue-green in KOH, seems to be characteristic for the group (Donk 1964, Bresinsky & Rennschmid 1971, Gill & Steglich 1987, Oberwinkler 1977). Thelephoric acid is a therphenylquinone (diphenylbenzoquinone) of the shikimic acid pathways. Its derivatives and partially unstable components determine the colour of the basidiomata. Most species have darkly coloured basidiomata. However, ochraceous, golden yellow, reddish-brown, pinkish, greenish, and orange-brown to dark brown fruit bodies have been reported (Corner 1968, Stalpers 1993, Maas Geesteranus 1971, 1975, Pegler et al 1997).

Microscopically, Thelephorales comprise species with either monomitic or dimitic to trimitic hyphal systems. Skeletals are mostly associated to rhizomorphs of species of *Hydnellum*, *Pseudotomentella*, *Tomentella* and *Thelephora* (Larsen 1974, Kõljalg 1996, Stalpers 1993). Binding-like hyphae have been also reported within some species of *Tomentella* (Melo *et al.* 2002). Hyphae are usually regular but inflations, with distinctive swellings between septa, are recorded for *Thelephora fragilis* Corner, for species of *Sarcodon*, *Hydnellum*, *Bankera* and in some *Tomentella* species. The presence/absence of clamps seems to be of major taxonomic value. There are indeed species with clamps on all primary septa. Some species are almost clampless whilst others present a combination of both situations. For some species, subicular hyphae have brown to blackish granular, discoid or amorphous incrustations that sometimes completely dissolve during treatment with potassium hydroxide.

Table 2: Subdivisions of Thelephorales according to Stalpers (1993)

Order	Families	Genera	Number of species	Ecology
Thelephorales	*Bankeraceae* Donk	*Bankera*	6	EcM
		Boletopsis	5	EcM
		Hydnellum	38	EcM
		Phellodon	16	EcM
		Sarcodon	36	EcM
	Thelephoraceae Chevall.	*Amaurodon* J. Schröt	6	?
		*Botryohypochnus** Donk	4	?
		Lenzitopsis Malençon & Bertault	1	?
		Pseudotomentella Svrček	15	EcM
		Polyozellus Murril	1	EcM
		Thelephora Ehrhart ex Willdenow	49	EcM
		Tomentella Persoon ex Patouillard	75	EcM
		Tomentellago Hjortstam & Ryvarden,	1	?
		Tomentellopsis Hjortstam	5	EcM
		*Tylospora** Donk	2	EcM

* *Amaurodon* is not recognised by Stalpers (1993) as a thelephoroid genus. Kõljalg (1996) and Larsson et al. (2004) confirmed its affiliation to Thelephorales. The accommodation of *Botryohypochnus* and *Tylospora* into Thelephorales is still controversial. EcM = ectomycorrhizal

Unlike in many fungal orders, cystidia are rare in Thelephorales. They do occur scantily in few species of *Hydnellum*, *Phellodon*, *Thelephora* and *Tomentella* (Stalpers 1993). Cystidia have been shown to play important roles in species discrimination within the genus *Tomentella* (Kõljalg 1996). If present, cystidia are either capitate with distinctive distal apex, capitate, subcapitate, clavate, hyphoid or acuminate (**paper I**). As far as basidia are concerned, they vary from narrow-clavate to utriform, either with or without basal clamps. They are usually 4-sterigmate. However, species with up to 8-spored basidia are reported from the genus *Thelephora*, and some *Tomentella* species have 2-spored basidia (Corner 1968, Stalpers 1993).

For some species, subicular hyphae usually evolve into rhizomorphs that play major taxonomical importance (Kõljalg 1996, Stapers 1993). Rhizomorphs of Thelephorales show great diversity of patterning and a relatively complex structure in some species. Dimitic rhizomorphs are typical for the genus *Pseudotomentella.* Monomitic rhizomorphs are reported for many *Tomentella* species. Based on size differences between central and peripheral rhizomorphal hyphae, a character that commonly occurs in rhizomorphs of many tomentelloid and *Thelephora* species, Agerer (1999) defined the “thelephoroid rhizomorph type” (or rhizomorph type C, Agerer 1987-2006, 1999) that is supposed to be slightly differentiated in comparison to types A (uniform-loose) and B (uniform compact). “Thelephoroid rhizomorphs” (Agerer 1987-2006) can have nodes and conical structures at their ramification points. Interestingly, many species with the thelephoroid rhizomorph type (with nodes at ramification points) possess, in older ontogenetic stages, irregularly shaped, clamped or simple septate, multiple branched thin hyphae on the rhizomorphal surface (Raidl & Müller 1996, Jakucs & Agerer 1999, 2001, **see also paper I and III**). Even if the somewhat differentiated rhizomorphs are termed “thelephoroid type”, this should not be regarded as a synapomorphic feature for Thelephorales in general.

Rhizomorphs with loosely arranged uniform hyphae ("uniform-loose type", Agerer 1999) are present in *Tomentella radiosa* (Agerer & Bougher 2001, **see also paper IV**) and in *Tomentella albomarginata* (Bourdot & Galzin) M.P. Christ. (Agerer 1996). Uniform-compact rhizomorphs (Agerer 1999) have been reported for *Bankera fuligineo-alba* (J.C. Schmidt) Coker & Beers (Agerer & Otto 1997), *Phellodon niger* (Fr.) P. Karst. (Agerer 1992a), *Hydnellum peckii* Banker (Agerer 1993), and *Tomentellopsis submollis* (Svrček) Hjortstam (Agerer 1998) whilst "phlegmacioid rhizomorphs" are present in *Boletopsis leucomelaena* (Pers.) Fayod (Agerer 1992b) and *Sarcodon imbricatus* (L.) P. Karst. (Agerer 1991a).

Chlamydospores have been reported for some species. Within the genus *Pseudotomentella*, chlamydospores are present on the rhizomorphs of *P. rhizopunctata* E. C. Martini & Hentic (Martini & Hentic 2003), *P. vepallidospora* M. J. Larsen (Kõljalg 1996), and *P. atrofusca* M. J. Larsen (Kõljalg 1996; Melo *et al*. 2002). With exception of *Tomentella guadalupensis* E. C. Martini & Hentic (Martini & Hentic 2005), chlamydospores are unknown in *Tomentella* (Kõljalg 1996). They are however known in other thelephoroid genera such as *Phellodon* (Agerer 1992a), *Sarcodon* (Agerer 1991a) and *Hydnellum (*Agerer 1993).

Although the Thelephorales display a limited number of anatomical features that commonly overlap, many authors have adopted a narrow species concept (Kõljalg 1996, Larsen 1968, 1974, Wakefield 1969, Stalpers 1993, Corner 1968). Within Bankeraceae, minor differences in the colour of fresh fruit bodies, precipitation and staining of the flesh in alkaline or KOH, the type of tissue as well as the morphology of spore warts (rounded, flattened or bifurcate) have been used as "reliable" delimitation criteria (Arnolds 2003, Maas Geesteranus 1971, 1975, Pegler et al 1997, Stalpers 1993, Dickson 2000, Baird 1986a,b, Harisson & Grund 1987). Parfitt et al. (2007) report high sequence variability between specimens previously assigned to the same morphological species. The authors highlighted the need to redefine species concepts within Bankeraceae by means of molecular and morphological data. In the Thelephoraceae, the size, shape and ornamentation type of the basidiospores and, to some extent, the presence/absence of cystidia have been assumed to be the most discriminating features (Kõljalg 1996, Larsen 1968, 1974, Stalpers 1993, Dämmrich 2006). Rhizomorphs have been used to group species into sections (Kõljalg 1996). However, basidiospores of Thelephoraceae display a continuum of shape, whilst irregularly-shaped spores may be observed within the same species (see Kõljalg 1996, Dämmrich 2006).

2.1.2. Shape, size and ontogeny of basidiospores of Thelephorales

Basidiospores of Thelephorales present a variety of shape and ornamentations. Several assumptions have been considered regarding ontogeny of basidiospores and their ornamentations. The plesiomorphic spore shape is considered to be subglobose (or ellipsoid), either smooth or with simple and/or short warts (Stalpers 1993). The first hypothesis about the ontogeny of basidiospores (and subsequently including ornament arrangements) was published by Malençon (1958). According to the author, young spores are smooth. They develop asymmetrically to the apiculus and produce early humps or lobes that are symmetrically arranged at base, apex and lateral parts of the basidiospores. In some species, lobules (or second degree lobes) may evolve from these major humps, on which tertiary lobes

may emerge, solitarily or clustering in two or three, sometimes even more (Stalpers 1993). Secondary and/or tertiary lobes may be either very short und slightly round (warts) or becoming conical while maturing (spines). Warts and spines in Thelephorales are centrifugal outgrowths of the spores (Malençon 1958). The number of primary and secondary lobes varies from 3 to 8. An alternative assumption regarding spore ontogeny suggests that the young basidiospore (as stated by Stalpers 1993 for some *Pseudotomentella* species) is already three or four-lobed and becomes more or less regular while maturing. Stalpers (1993) defined five types of ornamentation within Thelephorales, namely: (1) the regular warted type (*Thelephora ramarioides* D.A. Reid, *Th. nigricans* Stalpers, *Phellodon niger*, *Lenzitopsis oxycedri* Malençon & Bertault); (2) the regular bifurcately warted type (*Tomentella crinalis* (Fr.) M.J. Larsen, *Pseudotomentella atrocyanea* (Wakef.) Burds. & M.J. Larsen, *P. mucidula* (P. Karst.) Svrček, *P. tristis* (P. Karst.) M.J. Larsen), (3) the regular spiny type (*Tomentella ellisii* (Sacc.) Jülich & Stalpers, *T. lapida*, *T. macrospora* Höhn. & Litsch, *T. stuposa* (Link) Stalpers, *T. bryophila* (Pers.) M. J. Larsen, *Tomentellopsis zygodesmoides* (Ellis) Hjortstam), (4) the irregular spiny type (*Thelephora* spp, *Tomentella lateritia* Pat., *T. botryoides* (Schwein.) Bourdot & Galzin, *T. punicea* (Alb. & Schwein.) J. Schröt, *T. neobourdotii* M.J. Larsen), and (5) the irregular coarsely warted to cresty type (*Sarcodon imbricatus*, *Boletopsis leucomelaena*, *Hydnellum spongiosipes* (Peck) Pouzar). According to this classification, no distinction with regard to ornamentation types could be made between thelephoroid genera, although many *Pseudotomentella* species fall under the regular bifurcately warted type.

2.1.3. Molecular investigations and phylogenetic positions of Thelephorales

Thelephoroid fungi have been regarded as a monophyletic group though their exact delimitation remains unresolved. Donk (1964) excluded species with colourless basidiospores from the Thelephorales. Larsson et al (2004) suggested the exclusion of *Tylopsora* (with colourless but ornamented basidiospores) from the Thelephorales, a genus that Stalpers (1993) included on account of the presence of rhizomorphs and its ectomycorrhizal status. Based on phylogenetic evidence, the same authors (Larsson et al. 2004) confirmed the affiliation of the genus *Amaurodon* as a distinct monophyletic genus within Thelephorales. In recent molecular studies, thelephoroid fungi unambiguously emerge as a monophyletic group (Larsson *et al.* 2004, Binder *et al.* 2005, Binder & Hibbett 2002, Hibbett & Thorn 2001, Hibbett 2006) with bootstrap supports varying from 84 to 97%. In all above mentioned studies, Bankeraceae and Thelephoraceae cluster together in a monophyletic group (thelephoroid). Kõljalg (1996) was the first to address lower-level morphological phylogeny within Thelephoraceae, focusing mostly on the so-called resupinate Thelephorales (including *Amaurodon*, *Pseudotomentella*, *Tomentellospis* and *Tomentella*). Within Thelephoraceae, the author was able to highlight *Amaurodon* and *Pseudotomentella* as monophyletic genera, whilst monophyly of *Tomentellopsis* and *Tomentella* are questionable according to the method used to infer the trees (Kõljalg 1996).

Although there is currently no doubt that Thelephorales form a monophyletic group, evolutionary trends within this order and its phylogenetic affiliation with other groups have been hardly addressed. Hibbett (2006) reported Thelephorales to be a sister clade of Polyporales with, however, a moderate bootstrap support. Unlike many other homobasidiomycetous clades such as “Boletes” (Binder & Hibbett 2006),

"Cantharelloid" (Moncalvo *et al.* 2006), "Gomphoid-phalloid" (Hosaka et al. 2006), detailed phylogenetic analysis and evolutionary trends within Thelephorales are rare and still remain ambiguous. In the work of Larsson *et al*. (2004), resupinate Thelephorales are basal whilst species with erect fruit bodies form terminal clades. Unlike Larsson et al. (2004), the analysis of Binder et al. (2005) suggested multiple transformations between resupinate and erect fruit-bodied species within the thelephoroid clade, since the *Bankeraceae* are nested within *Thelephoraceae* in the resultant tree.

2.1.4. Ecology and distribution of Thelephorales

There is now evidence that many representatives, if not all, of thelephoroid fungi form ectomycorrhizal associations with forest trees in temperate areas. Weir (1921) was probably the first to report on Thelephorales (*Thelephora terrestris* Ehrh.) as ectomycorrhizal symbionts. Many other studies confirmed later the ectomycorrhizal status of more conspicuous Thelephoroid genera such as *Thelephora* (Agerer 1988, Agerer & Weiß 1989, Ingleby *et al* 1990, Raidl 1997), *Boletopsis* (Agerer 1992b), *Bankera* (Agerer & Otto 1997), *Hydnellum* (Agerer 1993), *Sarcodon* (Agerer 1991a, Raidl & Agerer 1992), and *Phellodon* (Agerer 1992a). The assumption that resupinate fruit-bodied Thelephorales (*Tomentella*, *Tomentellospis* and *Pseudotomentella*) also form ectomycorrhizae was postulated by Danielson *et al*. (1984). Indeed, until recently, the ectomycorrhizal status of fungal species has been traditionally elucidated through examination of conspicuous, epigeous or hypogenous fruit bodies that grow directly under forest trees. Investigations of Agerer (1991b, 1987-2006) were among the first to provide pioneering descriptions of ectomycorhizae through close examination of fruit bodies and root-tips. These works supplied reliable evidence of ectomycorrhizal formation between given fungal species and forest trees. Following this method, it has been possible to confirm the ectomycorrhizal status of many resupinate Thelephorales in temperate forests (Agerer 1994, 1996, Agerer & Bougher 2001, Jackus and Agerer 1999, Jackus *et al.,* 2005, Raidl & Muller 1996). Recent applications of molecular tools in community studies of ectomycorrhizae have increased our understanding of the ecological role of Thelephorales in general. Molecular techniques ease the analysis and identification of EcM-fungi by comparing either the RFLPs-patterns or DNA sequences of EcM-root tips and identified sporocarp material. DNA sequencing and phylogenetic analysis have been used to trace the phylogenetic position of known and unknown EcM fungi directly from root tips (Fransson *et al.* 2000, Vrålstad *et al.*, 2000, 2002). Such methods have provided evidence that the Thelephorales, mostly resupinate ones, are not only common EcM-formers (species richness) but account for a large percentage (20-45%) of below-ground ectomycorrhizal communities of boreal and temperate forests (Erland & Taylor 1999, Kõljalg *et al*., 2000, 2002, Tedersoo *et al.*, 2006, Horton & Bruns 2001; Sirikantaramas *et al*. 2003).

Thelephoroid fungi are cosmopolitan (Corner 1968, Stalpers 1993, Kõljalg 1996, Maas Geesteranus 1971, 1975). Specifically, the Bankeraceae seem to have predominantly temperate distribution and are frequently reported from Europa and North America (Pegler *et al.* 1997, Arnolds 1989, 2003, Maas Geesteranus 1975, Baird 1986a, 1986b, Harrison 1964, 1968, Harrison & Grund 1987, Parfitt et al. 2007). Maas Geesteranus (1971) reported however the presence of *Bankera*,

Hydnellum, *Sarcodon* and *Phellodon* in tropical and subtropical Asia. Thelephoraceae seem to have a worldwide distribution, though they have been frequently reported from Europa, North America and temperate Asia with highest species richness in coniferous forests (Kõljalg 1996, Kõljalg *et al.* 2000, Wakefield 1966, 1969, Larsen 1964, 1968, 1974). Very little is known about Theleporaceae from the tropics. However, in his monograph of *Thelephora* species, Corner (1968) reported two species (T. *brunneoviolacea* Beeli and *T. cerberea* Corner) from tropical Africa and about 10 from tropical and subtropical Asia. A few other papers (Corner 1968, Wakefield 1966, Malençon 1952, 1954, Patrouillard 1897) mentioned one to two resupinate Thelephorales from tropical Africa. Except the present dissertation, recent additional documentations on Thelephorales from tropical Africa are those of Martini & Hentic (2002) and Tedersoo et al. (2007). Wakefield (1966), Hjortstam & Ryvarden (1988, 1995), Martini & Hentic (2005), and Corner (1968) reported few other neotropical species. Thelephoroid fungi from tropical America and Africa are poorly documented in general.

2.2. Scientific background and objectives of the present dissertation

At the African level, taxonomic investigations on larger fungi are rather scarce and reduced to description of few species within genera and families. Complete monographs within target genera or families are rare. *Termitomyces*, *Russula* and *Lactarius* are undoubtedly the most documented fungal group in tropical Africa. With recent contributions by Heim (1977), Morris (1986, 1987), Mossebo *et al* (2002) and Turnbull & Watling (1999), *Termitomyces* ranges among the most known fungal genera in tropical Africa. The *Russulaceae* (*Lactarius* and *Russula*) have been investigated through various contributions of Verbeken (1995; 1996a,b, 2000), Verbeken et al. (2000a,b) and Buyck (1993, 1994, 1997), especially from the Zambesian Centre of Endemism (Burundi, Congo Democratic Republic, Zimbabwe, Zambia) and Guineo-Congolean Centre of Endemism (Cameroon, Gabon and Central African Republic). Our taxonomic knowledge of both genera comes mainly from Zambesian and Guineo-Congolean Centre of Endemism. Investigation on *Lactarius* from Soudanian Centre of Endemism are given only recently by van Rooij *et al* (2003) who monographed 22 *Lactarius* species in Benin. A monograph of tropical African species of *Marasmius* and its allied genera is completed recently by Antonín (2007). Fragmented documentation is given for many fungal groups, among others the genus *Amanita* (Pegler & Shah-Smith 1997, Beeli 1935).

The overall goal of the present dissertation is to provide baseline taxonomic and molecular documentations for a continuous monograph of tropical Africa Thelephorales. So far, only few tropical African fungal groups with rather more conspicuous fruit bodies have been investigated.

3. Methodology

3.1. Specimen sampling

Fruit bodies of resupinate Thelephorales were collected in northern Guinean seasonal forests in central and northern parts of Benin (West Africa) during collection trips undertaken in 2003, 2004, 2005 and 2006. Geographic coordinates of collection sites were recorded using a Global Positioning System (GPS). Preliminary morphological features of collected specimens were recorded from fresh material. Specimens were then dried using a propane gas-heated field dryer (De Kesel 2001). Dried specimens were labelled and conserved in plastic "minigrip" bags for further microscopic investigations. Colour codes of dried fruit bodies were assigned according to Kornerup & Wanscher (1978). All studied specimens, including type material of new species, are deposited in M (Holmgren et al. 1990). In addition to specimens we collected, type material of some resupinate Thelephorales was borrowed from herbaria H, BPI and PH (Holmgren et al. 1990).

Ectomycorrhizae sampling was conducted by taking soil samples (10 x 10 x 10 cm) beneath representative Thelephorales sporophores. Subsequently, the native tree species from under which the soil samples were collected was recorded. Soil samples were briefly examined in the field and ectomycorrhizal root tips were roughly cleaned using a field dissection microscope. Isolated EcM were conserved in formol vapour. Back from the field, the roughly cleaned EcM were cleaned again, and depending on colour, occurrence and abundance of cystidia and emanating elements, sorted into morphotypes (see Agerer 1987-2006), and preserved in FEA and formol vapour. In addition, single root tips of every morphotype were kept in 200 – 300 µl CETAB DNA extraction buffer (100 mM tris-HCl pH 8, 1.4 M NaCl, 20 mM EDTA, 2% cetyl trimethyl ammonium bromide) and stored at room temperature for DNA extraction. Reference numbers ensured an unequivocal assignment of the split samples.

3.2. Microscopic investigations

For microscopic investigations, fine sections through the basidiocarp were made using a razor blade under a stereomicroscope and mounted in water and afterwards in 2.5% potassium hydroxide (KOH), in Congo Red, in Cotton Blue and in Melzer's reagent (Kreisel & Schauer 1987). Line drawings and measurements were made at X 1000 magnification using a Leica microscope (Leica DM LB2) fitted with a drawing tube. Measurements of basidiospores do not include the apiculus and ornamentation. Measurements of basidia exclude sterigmata. Species descriptions follow criteria compiled by Kõljalg (1996) that present general morphological information on specimens and detailed anatomical features of specific elements such as rhizomorphs, subicular hyphae, basidia, cystidia and basidiospores. Author names and nomenclatural aspects of taxa are given according to the "Name indices for Fungi and Lichens (http://www.mycology.net/)".

The microscopic descriptions of EcM follow Agerer's Method (Agerer 1987-2006). Mantle preparations were taken from root tips, mounted in tap water. Longitudinal section (20-25 µm thick) of rhizomorphs were made by means of a cryotome and mounted in lactic acid. All preparations were examined using a light

microscope Leica DM LB2. Line drawings were also made at magnification X 1000 using a drawing tube. Measurements follow recommendations of Agerer (1987-2006). Colour reactions of the mantle preparations were tested by mounting in cotton blue, lactic acid, Melzer's reagent, and sulpho-vanillin.

3.3. Molecular investigations

3.3.1. DNA Extraction, target genes, primers, and PCR amplification

DNA of fruit bodies was extracted from dried specimens. For collected EcM, DNA was isolated from root tips previously conserved in CETAB DNA extraction buffer. Ribosomal DNA was extracted from 3-4 specimens of each species using the Qiagen DNeasy plant Mini Kit according to the manufacturer's instructions. PCR amplification was performed for ITS rDNA regions (ITS1, ITS2, 5.8S) using either universal primer pairs ITS1 (5´-TCCGATGGTGAACCTGCGG-3`) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) (white et al. 1990), or commonly fungi-specific primer ITS1F (5´-CTTGGTCATTTAGAGGAAGTAA-3´) in combination with the basidiomycetes specific primer ITS4B (5´-CAGGAGACTTGTACACGGTCCAG-3´) (Gardes & Bruns 1993). PCR amplification using primer pairs ITS1F and ITS4B has been successfully used to amplify Thelephorales (Kõljalg *et al.* 2000, 2001). The ITS regions of rDNA seem to be the best choice for infrageneric discrimination of *Tomentella* species.

PCR products were purified using the QIAquick-PCR purification Kit according to manufacturer's instructions. The PCR was performed on 25 µl solution composed of 24 µm Master mix and 1 µl of extracted DNA. 24 ml Master mix are composed of 15 µl H_2O, 2.5 µl of 10x PCR-puffer (MBI), 1.8 µl of 25 mM MgCl2 (MBI), 1 µl of 100 pM ITS1 (respectively ITS1F), 1 µl of 100 pM ITS4 (respectively ITS4B), 2.5 µl dNTP-Mix (2 mM/Base, MBI) and 0.2 µl Taq-polymerase (5 U/µl, MBI). If not otherwise mentioned, PCR program was planned as follow: 94 C for 3 min, 60 C for 1 min, 72 C for 1 min (1 cycle), 94 C for 1 min, 60 C for 1 min, 72 C for 1 min and 30s (28 cycles), 94 C for 1 min, 60 C for 1 min and 72 C for 10 min (1 cycle). Two (2)µl PCR products 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 ddH2O for 2 min. PCR products were visualised under the UV light. PCR products were then purified using the QIAquick-PCR purification Kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. DNA sequencing was performed by the sequencing service of the Institute for Genetics, 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 µm DNA probes plus 0.3 µm ITS1 (or ITS1F as forward primer) and 0.3 µm ITS4 (respectively ITS4B as reverse primer). All sequences are deposited in the NCBI GenBank.

3.3.2. Sequence edition and phylogenetic analysis

Sequences were edited with BioEdit v7.0.5 (Hall 2005). There exist many sequences of resupinate Thelephorales in various public Genbanks such as the European Molecular Biology Laboratory (EMBL), the National Centre for

Biotechnology Information (NCBI) and UNITE (Kõljalg et al. 2005). Prior to phylogenetic analyses, sequences were submitted to BLASTn searches against nr fungal sequences databases of either UNITE, EMBL and/or NCBI. Close sequences were then downloaded and aligned against our newly generated sequences. Sequence alignment was performed through the option ClustalW Multiple Alignment of BioEdit v7.0.5 (Hall 2005). In some cases, pair-wise base differences between close species were calculated using the "Identity/similarity" option of BioEdit v7.0.5. If not specify otherwise, phylogenetic analysis was performed using PAUP version 4.0b10 (Swofford 2002). The heuristic search option, ten replications of random-taxon entry and tree bisection reconnection (TBR) swapping were selected. Gaps were treated as missing values. Bootstrap analysis was performed with 1000 replicates under the heuristic search (Felsenstein 1985). All molecular characters were assessed as independent, unordered, and of equal weight using Fitch parsimony (Fitch 1971).

4. Results and discussions

4.1. Specimens

A total of three collection trips were undertaken during 2004, 2005 and 2006. We collected over 800 specimens of resupinate Thelephorales, among them more than 600 were microscopically examined. Over 100 representative specimens are illustrated, either through line drawings and colour photographs taken from the light microscope or SEM pictures. DNA was successfully extracted from 125 representative specimens, of which 75 purified PCR products are deposited at the host laboratory (Section Mycology, Department of Biology I and GeoBio Centre, LMU, München). A total of 26 good sequences was obtained and deposited at the NCBI GenBank with Accession numbers DQ848610 to DQ848613, EF507250 to EF507264 and EF538418 to EF538424 (See appendices 1). We collected over 60 EcM samples of which 6 were anatomically examined.

4.2. Anatomical features of tropical African Thelephorales

The first 4 papers (**papers I-IV**) addressed anatomical characters of resupinate Thelephorales.

- The first paper **(paper I)** focussed on the relevance of cystidia in the taxonomy of resupinate Thelephorales;
- In the second paper **(paper II)**, the limitation of basidiospore ornamentation in demarcation between *Pseudotomentella* and *Tomentella* was highlighted;
- In the third paper (**paper III**), anatomic characters of rhizomorphs were discussed along with their role in species discrimination within non-cystidioid species showing similar basidiospores;
- In **paper IV**, an anatomical re-evaluation is focused on already described species. Important and constant anatomical features for each species are outlined. In this paper, the value of providing detailed anatomical descriptions and faithful line drawings of each element is highlighted.
- In **papers V and VI**, the ectomycorrhizal formation between resupinate Thelephorales and tropical African native forest trees was confirmed. Both

papers provided detailed anatomical and morphological features of the ectomycorrhizae.

Tropical African resupinate Thelephorales display interesting anatomical features still unknown or scantily documented. The most important features reported during these studies are the commonness of irregularly shaped thin hyphae on the rhizomorphal surface of some species (**paper I and III**), basidiospores with distinctly forked spines and the occurrence of internal hyphae within basidia of some species (**paper II**). Until recently, irregularly shaped thin hyphae on the rhizomorph surface of some thelephoroid species were scantily documented (Raidl 1997, Raidl & Müller 1996, Jakucs & Agerer 1999). Through these investigations, the presence of irregulalry shaped thin surface hyphae is confirmed, and fundamental differences between such hyphae and skeletals are highlighted (**see paper III** for detailed discussions on this matter). Up to now, the taxonomical relevance of such surface hyphae has been disregarded. In general, it seems that African resupinate Thelephorales lack distinctive skeletals, but instead many species have rather irregularly shaped thin hyphae on their rhizomorphs. Of 600 specimens we examined so far, we were not able to see skeletals and dimitic hyphal systems for any specimen. Except *P. armata* (Martini & Hentic 2002), there is till now no report on skeletals and dimitic hyphal systems for tropical African Thelephorales.

Two tropical African species, namely *Pseudotomentella armata* Martini & Hentic and *Tomentella furcata* Yorou & Agerer, have been described as having forked spore ornamentations. Detailed microscopic analyses revealed fundamental differences between the ornamentation type of both species and those from temperate and boreal *Pseudotomentella* species (known to show bi- to trifurcate ornaments). In most *Pseudotomentella* species, bi- or trifurcate ornaments consist of short spines or aculei/warts that cluster in groups of two or three on the first or second degree lobes (Malençon 1958). Such structural arrangement of a short lobe and its clustering aculei has often been regarded as bi- or trifurcation (Kõljalg 1996, Stalpers 1993, Larsen 1964). Spore ornamentation in *P. armata* and *T. furcata* actually consists of distinctly long conical spines (up to 3 µm) that end in forks. Light microscope and SEM investigations confirmed the absence of lobes and there is no clustering of spines/aculei in either species.

Cystidia have been reported in two tropical African species, namely *T. capitata* and *T. brunneocystidia* (**see paper I**). Both species have capitate cystidia that differ in length from capitate cystidioid temperate species. Of all tropical species investigated so far, one of the most interesting anatomical features is the presence of internal hyphae within the basidia of *T. furcata* (**paper II**), a character previously unrecorded within Thelephorales. In conclusion, there is no doubt that many other interesting characters of significant relevance for the evolutionary interpretation of Thelephorales, are still to be reported from tropical African thelephoroid fungi.

4.3. Diversity and ecology of resupinate Thelephorales in Benin (West Africa)

It seems that, with the exception of the genus *Tomentella*, other resupinate Thelephoroid genera, e.g. *Pseudotomentella*, *Tomentellopsis* and *Amaurodon*, are absent, or very poorly represented in Benin (West Africa), at least from the northern

Guinean seasonal forests we have so far investigated. None of the 600 collections examined is reminiscent of *Pseudotomentella*, *Tomentellopsis*, or *Amaurodon*. Some specimens had anatomical features that resembled those of the genus *Pseudotomentella* and *Tomentellopsis*. Molecular studies however suggest that these specimens belong either to the genus *Tomentella* (**see paper II**) or *Tylospora* (unpublished data). The absence of the genera *Tomentellopsis* and *Pseudotomentella* in the seasonal forests of West Africa could be significant in the understanding of evolutionary relationships between tropical African and temperate species, as both genera are considered to be more primitive than *Tomentella* (Kõljalg 1996). However, as many tropical African areas still remain completely unexplored mycologically, no reliable conclusion about the presence/absence of *Tomentellopsis* and *Pseudotomentella* could be made at this moment.

All 600 examined specimens in this study can be sorted into 19 different *Tomentella* species, of which 9 are new. Four new species are described in **papers I, II and III** and presented in this PhD thesis. Additional descriptions will be delivered later.

In Benin, ecological plasticity of resupinate Thelephorales seems to be confined to the Ceasalpinioid-dominated seasonal forests located in the Guineo-Soudanian transition zones and in the Soudanian Centre of Endemism (White 1983). Despite repeated excursions, we were not able to collect Thelephorales in the relict Guineo-Congolean semi-deciduous and rain forests (White 1979, 1983) located in southern part of Benin. The presence of Thelephoroid specimens in Ceasalpinioid-dominated forests of the Soudanian Centre of Endemism and their absence in Guinean forests of South Benin provide strong evidence that they form ectomycorrhizae with various native trees of the Soudanian Centre of Endemism. However, the presence of their fruit bodies in these areas is not common. Except the common *T. africana* (**paper III**), all other species display small pieces of fruit bodies on rather scarce and fragmented substrates. To build their down-facing fruit bodies, many resupinate Thelephorales use either logs, dead barks in decomposition, coarse woody debris, or leaf litter as substrate from which they are directly linked to the fine roots of their tree partners (Kõljalg 1996, Kõljalg et al. 2000, 2002, Tedersoo et al. 2003). In this context, the frequency of the fruit bodies of resupinate Thelephorales (and wood-inhabiting fungi in general) depends upon the availability of substrates. The Soudanian and Zambesian Centres of Endemism are characterised by a frequent occurrence of bushfire (Goldammer 1990, Stott 1991, Laris 2002, Baker 2000). Due to the annual occurrence of bushfire, logs, dead bark and leaf litter are entirely or partly burnt, thus hampering the development of fruit bodies of wood-inhabiting fungal taxa (including resupinate Thelephorales).

4.4. Diversity and anatomo-morphological characterisation of tropical African ectomycorrhizae with emphasis on *Afzelia africana* Smith and *Uapaca guineensis* Müll. Arg.

Through the anatomical and molecular investigations of root tips we sampled (**papers V and VI**), we were able to confirm the ectomycorrhizal formation between thelephoroid species and native tropical African forest trees, namely *A. africana* (Ceasalpiniaceae) and *Uapaca guineensis* (Euphorbiaceae). Both **papers V** and **VI** are among the first studies providing anatomo-morphological characterisation of

tropical African ectomycorrhizae. Up to now, anatomical descriptions existed for only seven tropical African ectomycorrhizae (Inglebey 1999, Moyersoen 1996a,b,c, Beenken 2004). **Paper V**, presented here is the first to provide an anatomical illustration of ectomycorhizae formed by *A. africana*. Beenken (2004) provided an anatomo-morphological and molecular characterisation of ectomycorrhizae formed by *Russula* spp. on *Uapaca guineensis* and *Uapaca staudtii* Pax. in Cameroon. However, both *A. africana* and *U. guineensis* have been repeatedly reported to form ectomycorrhizae with a range of fungal species (see below).

The first reports of ectomycorhizal formation on tropical African native trees were provided by Payronel and Fassi (1957) on *Gilbertiodendron deweri* (De Wild.) Léonard. Later on, many studies (Högberg, 1982, Högberg & Nylund 1981, Högberg & Piearce 1986) addressed the taxonomic distribution of tropical African ectomycorrhizal trees. Most observations (Alexander 1985, 1987, 1989, Alexander & Högberg 1986, Högberg & Nylund 1981, Högberg 1982) reported the commonness of ectomycorrhizal symbiosis within the Amherstieae tribe of the Cesalipiniaceae, in the Dipterocarpaceae and Euphorbiaceae. Ectomycorrhizae have been also reported on exotic tree members of Casuarinaceae (Bâ et al. 1987), Pinaceae (Lesueur & Ducousso 1995), Myrtaceae and Mimosaceae (Ducousso 1991). In the Soudanian and Zambesian Centres of Endemism, where ectomycorrhizal associations are common, tree partners are not very diverse, but those trees are predominant in vegetation structure, accounting for more than 70% of basal area (Malaisse 1978), thus playing an important ecological and economical role. However, among the approximately 70 tropical African ectomycorrhizal forest trees (Fassi & Moser 1991), *A. africana* and *U. guineensis* remain the most investigated species with regard to their symbiotic relationship with fungi. Redhead (1968) was probably among the first to report on the ectomycorrhizal status of *A. africana*. Since then, various studies have been undertaken, either to assess the diversity and variability of its putative fungal partners (Sanon et al 1997, Thoen & Bâ, 1989, Thoen & Ducousso 1989) or to test its colonisation patterns and/or growth response to various fungal strains in controlled environments (Bâ *et al.*, 1991, 1994, 1999, 2002, Diedhiou et al. 2004). Ducousso et al. (1999) reported a total number of 70 fungal taxa associated to *A. africana* in the Côte D'Ivoire. In Senegal, Thoen & Bâ (1989) reported a total of 34 and 43 associated fungal taxa for *A. africana* and *U. guineensis* respectively. In most cases, putative fungal partners refer to species with more conspicuous fruit bodies, such as representatives of the Boletales and Agaricales (Ducousso et al 2002, Verbeken et Buyck 2002, Sanon et al 1997, Thoen & Bâ, 1989, Thoen & Ducousso 1989). Ectomycorrhizal fungal taxa with inconspicuous fruit bodies have been disregarded. **Papers V and VI** are among the first to address the ectomycorrhizal importance of resupinate fungal taxa. Tedersoo et al. (2007) reported Thelephorales as one of the most species-rich ectomycorrhizal taxon associated with native Dipterocarp and Ceasalpinioid forests in the Seychelles. Up to 9 Thelephoroid ectomycorrizal specimens have been also recorded from forest soils in Senegal (Diedhiou et al. 2004). They reported the Thelephoroid specimens as early-stage ectomycorrhizal taxa of *A. africana*. However, this study provided insufficient anatomo-morphological illustrations of the morphotypes assigned to Thelephorales.

4.5. Divergence of the ITS rDNA regions and phylogenetic positions of tropical African Thelephorales

In the **papers I, II, and III,** the divergence of ITS rDNA sequences within tropical African specimens and between these specimens and temperate ones was partly adressed. Many African species presented very little divergence of their ITS regions, ranging from 0.0 to 2.68% (see Appendix 2). Specimens with low variability of the ITS regions cluster together in all phylogenetic analyses. Genetic distance between our specimens and the nearest described temperate ones range between 4.3% (between *T. capitata* and *T. pilosa*) to 12.9 % (between *T. africana* and *T. umbrinospora*, see Appendix 3). In agreement with our results, high divergence of the ITS regions (10.5 to 14.4 %) between tropical African thelephoroid specimens (Seychelles Islands) and temperate ones have been previously reported by Tedersoo et al. (2007). In DNA sequence-based analyses of fungal and microbe communities, various authors used sequence identity higher than 3 ± 1 as phylogenetic species limits (Izzo et al. 2005; O´Brien *et* al., 2005, Parrent et al. 2006). However, there is evidence of ITS divergence greater than 4% within the same morphological species (Tedersoo et al. 2006, 2007). Ishida et al. (2007) suggested a limit of 99% as a phylogenetic species criterion. Within the specimens used in this study, species delimitation was effected using a dual approach. In addition to DNA barcoding threshold, anatomical comparison with closely related species contributed important information. Phylogenetically, except in the case of *T. furcata* versus *T.* cf. *furcata*, all specimens with an ITS deviation < 3% clustered together in monophyletic groups with strong bootstrap support (**see paper I, II and III**).

Globally, tropical African species fall into various clades within the genus *Tomentella*. In some cases (**paper I**), they cluster together with morphologically close temperate species. However, morphologically convergent specimens may be phylogenetically very divergent, and only detailed anatomical observations could explain such divergence (see **paper III** on *T. africana* and *T. umbrinospora*). **Paper II** on *T. furcata*, also highlights how anatomy can be phylogenetically misleading.

Unlike many other genera that present similar ITS regions, such as *Cortinarius* and *Hebeloma* (Aanen et al. 2000, Frøslev et al. 2005), Thelephorales range among the fungal lineages with a very strong divergence of their ITS regions. As a consequence, the resolution of the deeper relationships within the genus falls when a large data set (higher than 50 sequences) is used for phylogenetic analysis. In such cases, we found a very high homoplasy index (between 0.75-0.80%) indicating many character state convergences (tree and data not shown here). The homoplasy index dropped to -0.50% when we used small data set (lower than 50 sequences, **see paper III**). The Blast search in the public GenBank (NCBI, EMBL) using a thelephoroid sequence as query resulted in an unlimited number of unknown uncultured EcM sequences as best matches. The number of unknown sequences was limited when the search was undertaken in UNITE. In any case, the resolution of deeper relationships between species and the overall quality of the phylogenetic results where enhanced when a smaller data set was used.

5. Summary

The Thelephorales (Basidiomycota, Fungi) form a monophyletic group with approximately 177 accepted species. The Thelephorales are cosmopolitan and encompass mainly ectomycorrhizal species. Unlike many fungal lineages, evolutionary trends within and between members of Thelephorales still remain incompletely assessed. Additionally, most phylogenetic investigations on fungi have failed to include representative samples from tropical Africa. In the present study started four years ago, we have assessed and documented some of the diversity of tropical African Thelephorales. It represents a part of a future broader but continuous project aiming for a complete monograph, and highlighting the anatomical and molecular relevance of tropical species in the evolutionary interpretation of Thelephorales in general. The study was started in the northern Guinean seasonal forests, located from central to north Benin (West Africa). Northern Guinean seasonal forests are characterised by a low specific plant richness, but predominated by a few ectomycorrhizal trees of the Caesalpiniaceae (*Isoberlinia doka* Craib & Stapf *Isoberlinia tomenteosa* (Harms) Craib & Stapf, *Burkea africana* Hook., and *Afzelia africana* Smith), Dipterocarpaceae (*Monothes kerstingii* Gilg.) and Euphorbiaceae (*Uapaca guineensis* Müll. Arg.). Four collecting trips were undertaken during the rainy seasons of 2003, 2004, 2005 and 2006. Specimens of Thelephorales and soil cores were randomly sampled under native ectomycorrhizal trees. The collecting trips yielded over 800 specimens of Thelephorales and 60 EcM samples in total. Taxonomic investigations and species concepts of the specimens were assessed using a combination of both molecular and anatomo-morphological approaches.

All examined specimens were sorted into 19 morphologically different species. In this dissertation four new species are described and illustrated. A full description is provided for each species, together with reliable line drawings and, where possible, with SEM micrographs. Anatomic studies have confirmed and emphasised the commonality of irregularly shaped thin hyphae on the rhizomorph surfaces of many African species. Novel anatomical features hitherto unknown within Thelephorales, and rarely recorded for Hymenomycetes in general, are recorded. Detailed anatomical comparison with type species has enabled us to depict fundamental arguments about the discrimination of thelephoroid genera. We report fundamental differences in spore ornamentation between African and temperate tomentelloid species.

Using molecular PCR methods, we confirmed the high divergence rate of the ITS regions of thelephoroid fungi. Within morphologically close specimens, the ITS rDNA sequence deviation generally ranges between 0.0 to 2.68%. However, genetic distance between some specimens (e.g.*Tomentella furcata* Yorou & Agerer and *T.* cf. *furcata* nom. prov.) illustrates how morphologically convergent specimens may be greatly divergent with regard to their ITS rDNA. Phylogenetically, tropical African species of Thelephorales are either basal or terminal within various clades of investigated temperate, boreal and tropical species. Generally, they highly deviate from temperate and boreal closest species by 4.3 to 12.9% with regard to the ITS rDNA sequences.

Northern Guinean seasonal forests harbour a great diversity of Thelephorales that are, however, hard to detect due to the down-facing growth of fruit bodies of

most resupinate Thelephorales, and the annual burning of required substrates. Only one species, *Tomentella africana* Yorou & Agerer, is widespread and commonly recorded. Many other species occur scarcely on fragmented substrates. Though the Thelephorales are cosmopolitan, we failed to record species that are reported to have a worldwide distribution. Notably, representatives of the resupinate thelephoroid genera *Pseudotomentella* Svrček and *Tomentellopsis* Hjortstam were missing, as well as species of the mainly temperately distributed family Bankeraceae. In this study, we provide evidence of the ectomycorrhizal formation between Thelephorales and native tropical African forest trees. Anatomical and molecular characterisation of ectomycorrhizae formed between Thelephorales and native West African forest trees (namely *Afzelia africana* and *Uapaca guineensis*) are provided for the first time.

The present studies are based on original material collected in some Ceasalpinioid /Euphorbiaceae-dominated vegetation types found from central to north Benin. The Zambesian Centre of Endemism, located in South-East Africa, also harbours a variety of different ectomycorrhizal trees including *Brachstegia* spp, *Julbernadia* spp, and *Isoberlinia* spp. Stands dominated by monospecific ectomycorrhizal forest trees are also present in the rain forests of South Cameroon. The variety of ectomycorrhizal tree species present in tropical Africa implies a greater species richness of Thelephorales than that currently reported from Benin.

The present thesis represents a keystone study and provides baseline data for a continuing monograph of Thelephorales in tropical Africa. Intensive monographic and taxonomical investigations will undoubtedly reveal many interesting, and probably plesiomorphic, anatomical features from tropical Thelephorales. In this context, detailed anatomical investigations integrated with DNA sequence analyses and phylogenetic inferences are promising tools for discriminating thelephoroid species in general, and tropical ones in particular.

Taxonomic novelties in this study:

6. References

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Appendices

Appendices 1: Voucher specimens and DNA sequences of tropical resupinate Thelephorales and allied genera deposited in GenBank NCBI.

Vouchers	Published (as deposited in Genbank) and working names	Genbank accession numbers	Collectors
RA 13793 (M)	*Tomentella* sp.	EF538424	Agerer
RA 13799 (M)	*Tomentella brunneocystidia*	DQ848610	Agerer
RA 13785 (M)	*Tomentella capitata*	DQ848611	Agerer
SYN 860 (M)	*Tomentella capitata*	DQ848612	Yorou
SYN 839 (M)	*Tomentella brunneocystidia*	DQ848613	Yorou
SYN 878 (M)	*Tomentella coffeina* nom prov	EF507250	Yorou
SYN 879 (M)	*Tomentella coffeina* nom prov	EF507251	Yorou
SYN 892 (M)	*Tomentella coffeina* nom prov	EF507252	Yorou
SYN 945 (M)	*Tomentella africana*	EF507253	Yorou
SYN 991 (M)	*Tomentela africana*	EF507254	Yorou
SYN 1007 (M)	*Tomentella africana*	EF507255	Yorou
SYN 890 (M)	*Tomentella africana*	EF507256	Yorou
SYN 981 (M)	*Tomentella* sp.	EF507257	Yorou
SYN 965 (M)	*Tomentella* cf. *furcata* nom prov	EF507258	Yorou
SYN 929 (M)	*Tomentella* cf. *furcata* nom prov	EF507259	Yorou
SYN 997 (M)	*Tomentella* cf. *furcata* nom prov	EF507260	Yorou
SYN 924 (M)	*Tomentella furcata*	EF507261	Yorou
SYN 930 (M)	*Tomentella* cf. *cinnereoumbrina*	EF507262	Yorou
SYN 893 (M)	*Tomentella* cf. *cinereoumbrina*	EF507263	Yorou
SYN 1000 (M)	*Tomentella* sp1	EF507264	Yorou
SYN 925 (M)	Corticiaceae sp.	EF538418	Yorou
SYN 936 (M)	Corticiaceae sp.	EF538419	Yorou
SYN 963 (M)	Corticiaceae sp.	EF538420	Yorou
SYN 970 (M)	Tomentella sp.	EF538421	Yorou
SYN 989 (M)	Corticiaceae sp.	EF538422	Yorou
SYN 990 (M)	Corticiaceae sp.	EF538423	Yorou
SYN 738 (M)	Ectomycorrhizae	EU334438	Yorou
SYN 906 (M)	Ectomycorrhizae	EU334439	Yorou

Appendice 2 : Pair wise base differences between specimens of different tropical African resupinate Thelephorales.

Species names (published and working names)	Accession numbers (NCBI)	Pair wise base difference (%)
Tomentella brunneocystidia Yorou & Agerer	DQ848610, DQ848613	0.0 – 0.1
Tomentella capitata Yorou & Agerer	DQ848611, DQ848612	0.0 – 0.2
Tomentella sp. nom. prov.	EF507250-EF507252	0.71 - 1.26
Tomentella africana Yorou & Agerer	EF507253- EF507256	0.0 - 2.2
Tomentella furcata Yorou & Agerer *Tomentella* cf. *furcata*	EF507258-EF507261	2.68 - 18.49
Tomentella cf. *cinereoumbrina*	EF507262-EF507263	1.26

Appendice 3: Pair wise base differences between specimens of different tropical African resupinate Thelephorales and morphologically close temperate species.

Species names	Morphologically close species	Pair wise base differences (%)
Tomentella capitata (DQ848611, DQ848612)	*Tomentella pilosa* (AF272925)	4.3 – 5.7
Tomentella brunneocystidia (DQ848610, DQ848613)	*Tomentella pilosa* (AF272952)	6.5 – 7.0
*Tomentella africana (*EF507253- EF507256)	*Tomentella umbrinospora* (AF272920)	12.1 – 12.9
	Tomentella lateritia (AF272926)	11.2 – 11.5
Tomentella furcata (EF507261)	*Tomentella bryophila* (AF272908)	12.3
Tomentella cf. *furcata* (EF507258-EF507260)	*Tomentella bryophila* (AF272908)	11.88 – 17.25
Tomentella sp. (EF507250-EF507252)	*Tomentella sublilacina* (AF272935)	10.59 – 10.92
	Tomentella ellisii (AF272913)	13.56 – 14.1
Tomentella cf. *cinereoumbrina* EF507262-EF507263	*Tomentella fuscocinerea* (AF272942)	10.13 – 10.59

Publications

Mycological Progress **6**: 7-18

Mycol Progress (2007) 6:7–18
DOI 10.1007/s11557-006-0519-4

ORIGINAL ARTICLE

Studies in African thelephoroid fungi: 1. *Tomentella capitata* and *Tomentella brunneocystidia*, two new species from Benin (West Africa) with capitate cystidia

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Received: 2 June 2006 / Revised: 30 October 2006 / Accepted: 3 November 2006 / Published online: 22 December 2006

Abstract This paper presents *Tomentella capitata* and *Tomentella brunneocystidia* as new species based on molecular data and anatomical features. Both *T. capitata* and *T. brunneocystidia* form sister species with *Tomentella pilosa*. All three taxa are well supported by bootstrap values. Anatomically, *T. capitata* and *T. brunneocystidia* are very close and are similar in shape, size, ornamentation of basidiospores, and size and colour of subicular hyphae. Monomitic rhizomorphs sometimes covered by irregularly shaped thin hyphae are present in both species. Shape and pigmentation of the cystidia are the most discriminating features between *T. capitata* and *T. brunneocystidia*. The cystidia of *T. capitata* are maximum 35 µm long, show a distinctive globose apex and are sometimes covered with dark brown pigmentation and/or encrustation, whereas cystidia of *T. brunneocystidia* are bigger, up to 55 µm long, with a sub-capitate shape and dark blue to dark green contents all over their length. The differences to species, already described as having capitate and clavate cystidia, are discussed. A key for the identification of cystidioid *Tomentella* species is given.

Taxonomical novelties: *Tomentella capitata* Yorou & Agerer, *Tomentella brunneocystidia* Yorou & Agerer.

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Introduction

Donk (1964) defined corticioid fungi as an assemblage of extremely diverse organisms. The corticioid fungal group includes indeed many paraphyletic genera (Parmasto 1995) and has been considered as the outcome of a regressive evolution of erect basidiomata (Corner 1954), whilst Heim (1948) regarded them as primitive forms of the current erected basidiomes. The last assumption is supported by recent phylogenetic analysis (Larsson et al. 2004) as corticioid fungi have proven to occupy basal clades within almost all major identified lineages, among them the thelephoroid one.

Thelephoroid fungi are characterised by the presence of thelephoric acid (Bresinsky and Rennschmid 1971; Gill and Steglich 1987) and the strongly ornamented, often dark brown basidiospores. The group is rather diverse regarding shape of the basidiomes, and includes species with either poroid, irpicoid, lamellate, hydnoid or odontoid hymenophores (Stalpers 1993).

Tomentella Persoon ex. Pat. is a member of Thelephorales (Stalpers 1993). It has been previously described under several generic names including *Caldesiella* Sacc., *Hypochnus* Fr. per Fr., *Odontia* Pers., *Corticium* subg. *Tomentella* Pers., and *Thelephora* Pers. with *Thelephora ferruginea* (Pers.) Pers. as type species. The generic name *Tomentella* is conserved and *Tomentella ferruginea* (Pers.) Pat. is quoted as type species (Larsen 1974).

Microscopically, the genus *Tomentella* comprises species with either monomitic or dimitic hyphal systems, clamped or simple septate hyphae (Stalpers 1993; Kõljalg 1996),

basidia and cystidia (if present) of various shape and for some species relatively complex rhizomorphs. The most recent monograph of this genus (Kõljalg 1996) based the delimitation of *Tomentella* from *Tomentellopsis* Hjortstam and *Pseudotomentella* Svrček on the size of basidiospores, their ornamentation and their colour in 3% KOH and in water. In this context, members of the genus *Tomentellopsis* possess simple septate hyphae, colourless subicular and sub-hymenial hyphae, and colourless echinulate basidiospores not exceeding 8 µm in size. The species of *Pseudotomentella* hold colourless, yellowish to brownish (in 3% KOH) spores with bi- or tri-furcate ornamentations, whereas *Tomentella* covers species with spores different from those of *Tomentellopsis* and *Pseudotomentella*. Within the genus *Tomentella*, Larsen (1974); Stalpers (1993) and Kõljalg (1996) based the species delimitation on the presence or absence of rhizomorphs and cystidia but also on the size, shape and ornamentation of spores.

If present, cystidia are either hyphoid, acuminate, clavate or capitate (Kõljalg 1996) and with or without encrustation and pigmentation.

In this study, we describe *T. capitata* and *T. brunneocystidia* as new species. Both species have capitate cystidia that do not resemble cystidia of already described *Tomentella* species.

There are many papers which include one to few species of resupinate thelephoroid fungi found in Africa (Wakefield 1966; Malençon 1952, 1954; Patouillard 1897; Martini and Hentic 2002). This paper is a first of a series exclusively devoted to tropical African resupinate thelephoroid species and their ectomycorrhizae with native trees.

Materials and methods

Collections and specimens

The study is based on specimens collected during the rainy season of 2003, 2004 and 2005 in woodlands and dry forests of the Soudanean Endemism Centre (White 1983). Specimens are collected under native trees and dried using a propane gas heated dryer (De Kesel 2001). Preliminary morphological descriptions are made using both fresh and dried material. Colour patterns are given according to Kornerup and Wanscher (1978). All specimens used for descriptions and the holotypes are deposited in M (Holmgren et al. 1990). Isotypes of both new species are deposited in TU (Holmgren et al. 1990).

Light microscopy and drawings

Microscopic structures are described using dried specimens. Fine sections through the basidiocarp were made using a razor blade under a stereomicroscope (WILD Heerbrugg M5, Switzerland) and mounted in water and afterwards in 2.5% KOH. Microscopic studies were performed using a light microscope Leica DM LB2. Measurements were made at a magnification of ×1,000. Measurements of basidia and basidiospores do not include sterigmata, apiculus and ornamentation. Line drawings were made at magnification ×1,000 using a drawing tube. Observations were also made in Cotton blue, Congo Red and Melzer's reagent (Kreisel and Schauer 1987). The format for descriptions follows Kõljalg (1996).

Molecular methods and analyses

DNA from fungal fruit bodies were extracted and ITS regions sequenced following methods described in Kõljalg et al. (2000). Sequences are kept in Genbank NCBI with accession numbers DQ848610–DQ848613. ITS sequences of two specimens of *Tomentella capitata* and two of *T. brunneocystidia* were obtained and aligned manually against ITS data matrix used in Kõljalg et al. (2001). This paper (Kõljalg et al. 2001) studied *Tomentella* species which form cystidia and these data are therefore vital in molecular recognition of new cystidioid taxa. In addition, two unpublished ITS sequences of *Tomentella pilosa* (Burt) Bourdot & Galzin from Costa Rica and USA and one *T. pilosa* sequence from ectomycorrhizae (GenBank no AY874385) were included into data matrix.

Distance neighbour joining analyses with Hasegawa–Kishino–Yano (HKY85) substitution model, pairwise base differences and bootstrap support were calculated by using the beta version 4.0d81 of PAUP (Swofford 2003).

Results

Sequence analyses

Neighbour-joining tree demonstrating the position of *T. capitata* and *T. brunneocystidia* sequences is shown on Fig. 1. The two specimens of *T. capitata* have identical sequences and cluster together. Specimens of *T. brunneocystidia* cluster together too. Both *T. capitata* and *T. brunneocystidia* are close to *T. pilosa* with which they form a monophyletic group with a high bootstrap value. Sequence differences between all three species are given in Table 1.

T. capitata Yorou & Agerer, sp. nov.

Description

Basidiocarpis resupinatis, separabilibus, pelliculosis, continuis. Hymenio ochraceo-brunneo usque ad atro-brunneo,

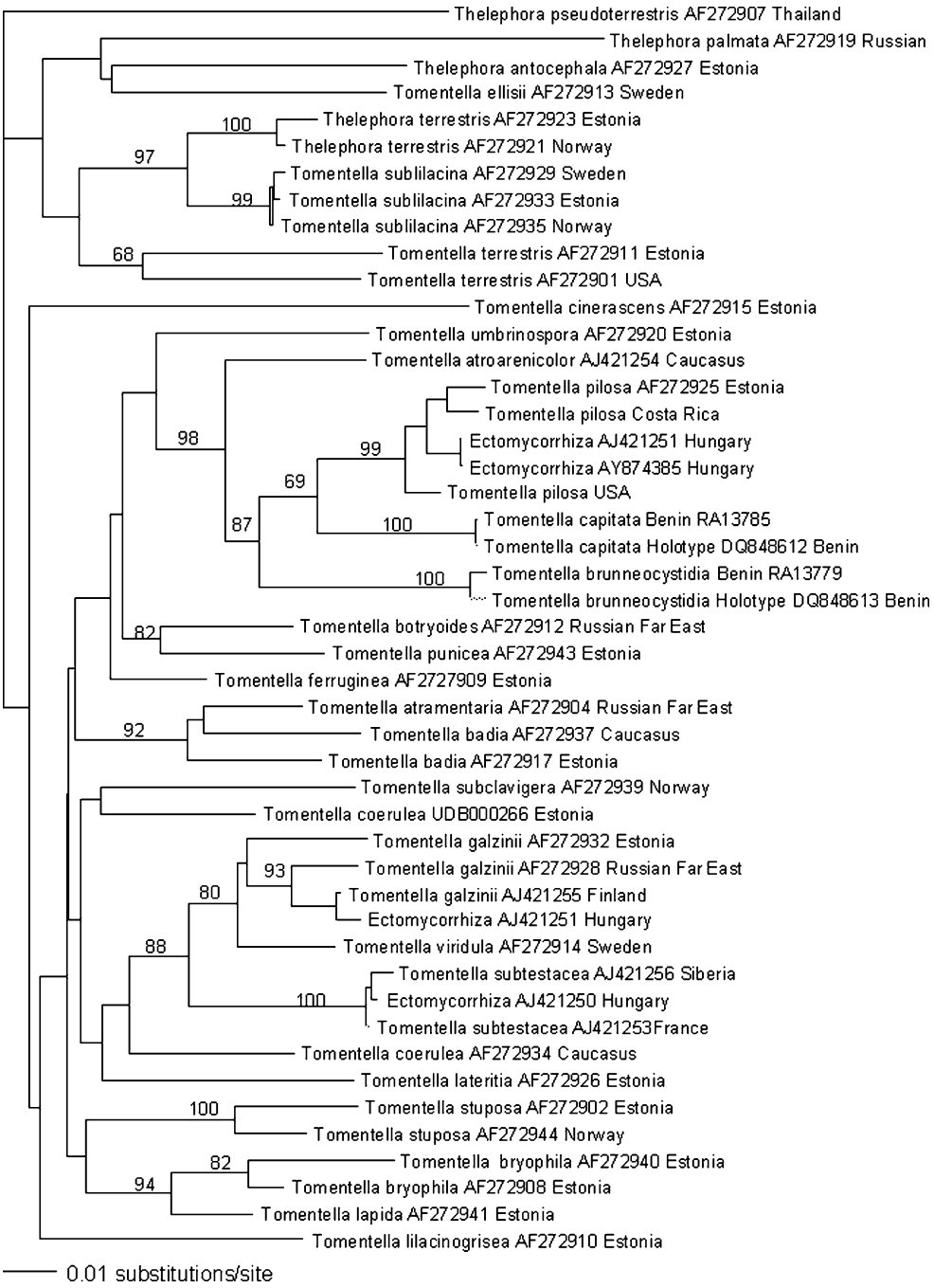

Fig. 1 The neighbour-joining tree showing the placement of *Tomentella capitata* and *T. brunneocystidia* among *Tomentella* and *Thelephora* species. Bootstrap values are shown above the branches. The GenBank, EMBL or UNITE codes of samples are indicated after the species name

Table 1 Pairwise base difference between *Tomentella capitata*, *Tomentella brunneocystidia* and *Tomentella pilosa*

	Tomentella capitata (%)	*Tomentella brunneocystidia* (%)
Tomentella pilosa	4.3–5.7	6.5–7.0
Tomentella brunneocystidia	7.5	

interdum viride-brunneo, granuloso vel farinoso, subiculo concolorato. Marginibus indeterminatis.

Rhizomorphae in subiculo et in margine, atro-brunneae usque ad atro-umbrinae, monomiticae, leves, interdum cystidiis capitiformibus; cystidiae brunneae usque ad atro-brunneae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, nec amyloideae; hyphis in superficie tenuis, 1–2 μm in diametro, defibulatis, irregulariter ramificatis; hyphae internae fibuligerae, 0.5–1 μm crassitunicatae, brunneae usque ad atro-brunneae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, nec amyloideae;

hyphae sub superficio rhizomorpharum 4–6 μm in diametro, hyphae in centro rhizomorpharum 4–7 μm in diametro.

Hyphae subiculi fibuligerae, septa simplicia deficientia, (3.5)4–5.5(6)μm in diametro, 0.5–1 μm crassitunicatae, non incrustatae, pallide brunneae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, muribus in parte amyloideis.

Hyphae subhymenii fibuligerae, 3–5 μm in diametro, tenuitunicatae usque ad 0.5 μm crassitunicatae, cellulis brevibus, inflatis, incoloratae usque ad pallide brunneae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, nec amyloideae, hyphae juveniles hymenio et subhymenio in parte muribus hyphis subamyloideis.

Cystidia rhizomorpharum capitiformia, base fibuligera, 20–48(50) μm longa, apice 4–6 μm in diametro, base 3–4.5 μm, incolorata in 2.5% KOH et in aqua, rare incrustata; cystidia hymenii late capitiformia, 25–35 μm longa, apice 9–14(16) μm in diametro, base 4–6.5 μm, non septata, interdum flavo-brunnea, plerumque incolorata vel capitibus incrustatis atro-brunneis.

Basidia 25–45 μm longa, 8–12 μm in diametro, base 6–8 μm, basibus fibuligeris, suburniformia, non stipitata, rare sinuosa, septa transversa deficientia, 4-sterigmatica, sterigmatibus 5–7 μm longis, basaliter 1–1.2 μm latis, incolorata in 2.5% KOH et in aqua, cyanophilae et congophilae.

Basidiosporae 7–9(9.5)× 7–9(9.5) μm in aspectu frontali, (7.5)8–9(9.5)× 7–8(9) μm in aspectu laterali, lobatae in aspectu frontali, ellipsoideae in aspectu laterali, echinulatae, aculeis usque ad 2 μm longis, guttulis frequentibus, pallide brunneae usque ad brunneae in 2.5% KOH et in aqua, nec cyanophilae, nec congophilae, nec amyloideae.

Chlamydosporae absentes

Basidiocarp—resupinate, separable from the substrate, pelliculose, is continuous. Hymenium is light brown (6D3), brown (6F4) to dark brown (7F4), granulose/farinose, concolorous with subiculum; sterile margin indeterminate.

Rhizomorphs—present in subiculum and at the margins, dark brown to dark umber under a dissection microscope, dark brown in water and in 2.5% KOH, monomitic, young rhizomorphs (thinner than 30 μm) smooth, rarely with irregularly shaped thin hyphae on the surface (Fig. 2), older rhizomorphs (thicker than 30 μm), patchily covered by irregularly shaped thin hyphae (Fig. 3), sometimes with cystidia emerging from the surface (Fig. 3), cystidia colourless in 2.5% KOH and in water, sometimes with encrustation, neither congophilous nor cyanophilous, nor amyloid; superficial thin hyphae 1–2 μm diameter, emerging from thicker hyphae (Fig. 4), frequently branched, exceptionally with clamps, frequently with simple septa, some without septa and recalling then binding hyphae of polypores; internal hyphae (hyphae below surface plus central hyphae) clamped, thick-walled (0.5–1 μm thick), brown to dark brown in 2.5% KOH and in water, not congophilous, not cyanophilous, not amyloid; hyphae below surface 4–6 μm, few hyphae repeatedly simple septate; central hyphae wider, 4–7 μm, simple septa infrequent.

Subicular hyphae—consistently broadly clamped (Fig. 5), simple septa lacking, (3.5)4–5.5(6) μm, thick-walled (0.5–1 μm), without encrustation, pale brown in 2.5% KOH and water, not congophilous, not cyanophilous, walls amyloid at patches.

Subhymenial hyphae—clamped, 3.5–5 μm diameter, thin- to thick-walled (0.5 μm), cells not short and inflated, colourless to pale brown in water and in 2.5% KOH, neither congophilous nor cyanophilous, walls of young hyphae of hymenium and subhymenium slightly amyloid.

Cystidia—from rhizomorphs capitate, clamped at base, aseptate along their length, 20–48(50) μm long, 4–6 μm at apex and 3–4.5 μm at base, colourless in 2.5% KOH and in water, rarely with encrustation, neither congophilous nor cyanophilous, nor amyloid, cystidia of the hymenium broadly capitate, clamped at base, aseptate along their length, 25–35 μm long, 9–14 (16) μm at apex and 4–6.5 μm at base, sometimes with yellow–brown contents, often colourless but then with dark brown pigment and/or encrustation covering the inflated apex.

Basidia—25–45 μm long, 8–12 μm at apex and 6–8 μm at base, clamped at base, suburniform, not stalked, rarely sinuous, without transverse septa, colourless in 2.5% KOH and in water, cyanophilous and congophilous, 4-sterigmate, sterigmata 5–7 μm long and 1–1.2 μm at base.

Basidiospores—7–9(9.5)×7–9(9.5) μm in frontal face and (7.5)8–9(9.5)×7–8(9) μm in lateral face, lobed in

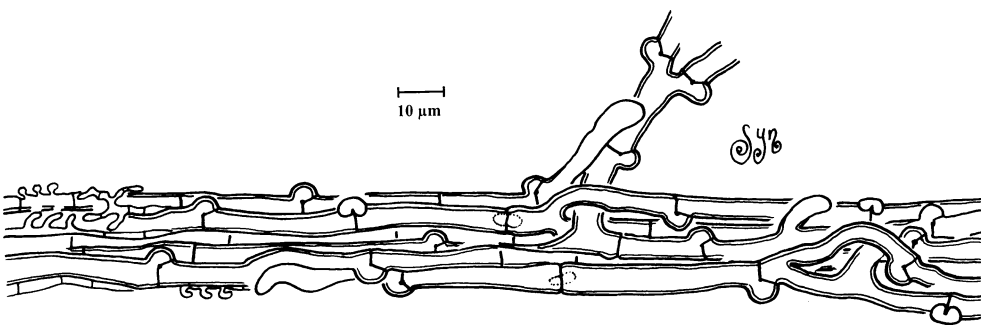

Fig. 2 Young rhizomorph of *Tomentella capitata* Yorou & Agerer

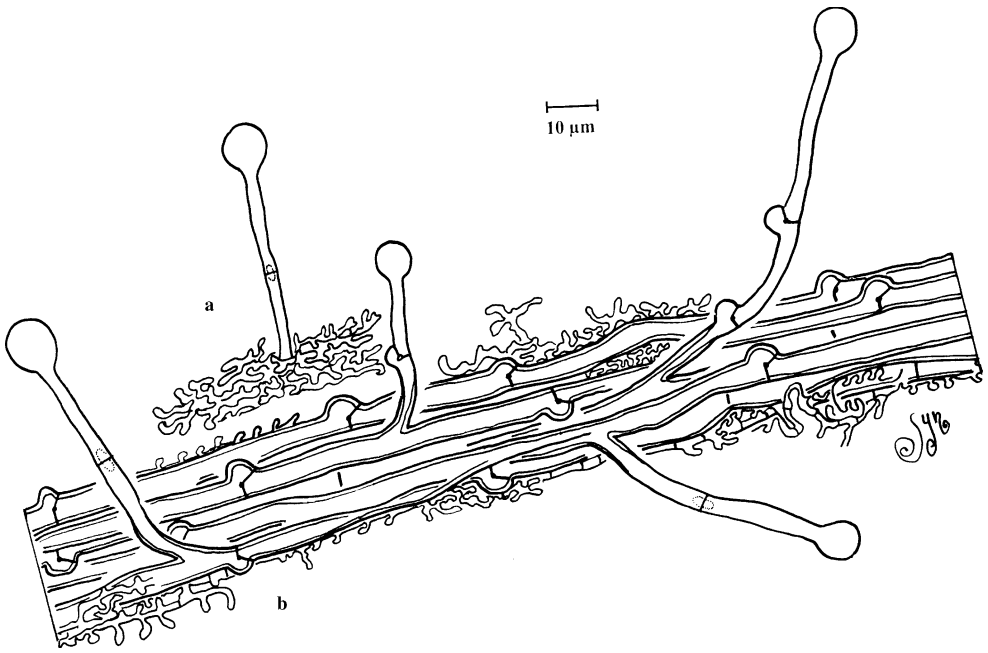

Fig. 3 Rhizomorphs of *Tomentella capitata*, **a** capitate cystidium with irregularly shaped thin hyphae and **b** optical section through the rhizomorph

frontal view, ellipsoid in lateral view, echinulate, aculei relatively long, 1–2 µm, oil drops common, pale brown to brown in 2.5% KOH and in water, not cyanophilous, not congophilous, not amyloid.

Chlamydospores—absent.

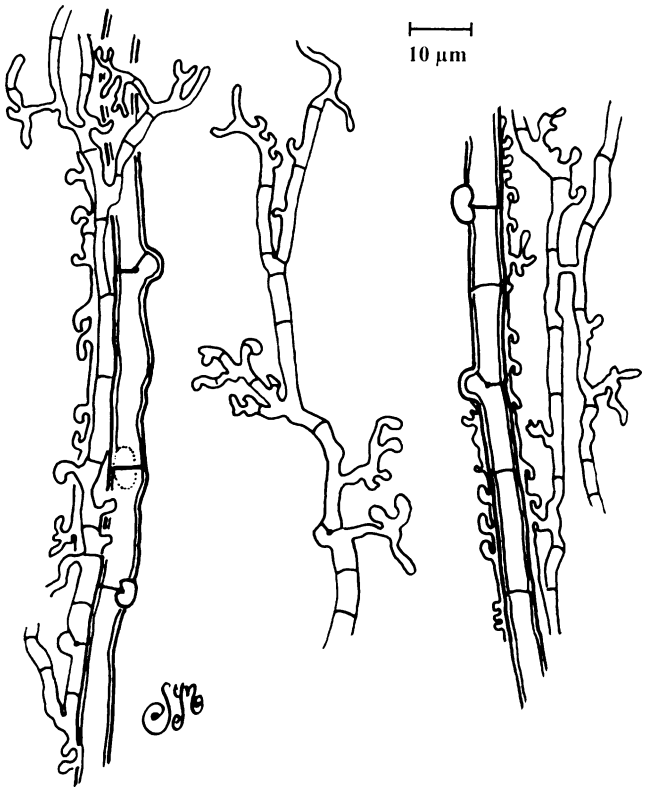

Fig. 4 Surface view of rhizomorph of *T. capitata* showing the connection between irregularly shaped thin hyphae and generative ones

Type material

Benin, central part, Borgou Province, reserved forest of Wari–Maro, Wari–Maro site, 08° 49′19.0″ N, 002° 16′ 32.4″ E, on dead bark and log, leg. N. S. Yorou, 05.08.2005, herb SYN 860. Holotype in M; isotype in TU. Genbank NCBI, accession number DQ848612.

Etymology

The epithet is proposed in reference to the distinctly capitate cystidia.

Material studied

Benin, central part, Borgou Province, Sinendé region, forest close to Fo–Bouko Village, 10° 8′46.6″ N, 002° 15′6.00″ E, on dead bark and log, leg. R. Agerer, 22.08.2003, herb RA 13781 (M). Benin, central part, Borgou Province, reserved forest of Wari–Maro, Agbassa area, 08° 53′31.5″ N, 002° 19′08.8" E, on dead bark and log, leg. N. S. Yorou, 14.07.2004, herb SYN 631 (M). Benin, central part, Borgou Province, reserved forest of Wari–Maro, Wari–Maro area, 09°00′47.1″ N, 002° 01′36.9″ E, on dead bark and logs of native trees, leg. N. S. Yorou, 05.08.2005, herb SYN 862 (M), SYN 856 (M), SYN 846 (M), SYN 848 (M), SYN 841 (M), SYN 844 (M), and SYN 855 (M).

Habitat

In woodlands and dry forests of the Soudanean Endemism Centre (White 1983) dominated by *Ceasalpiniaceae*,

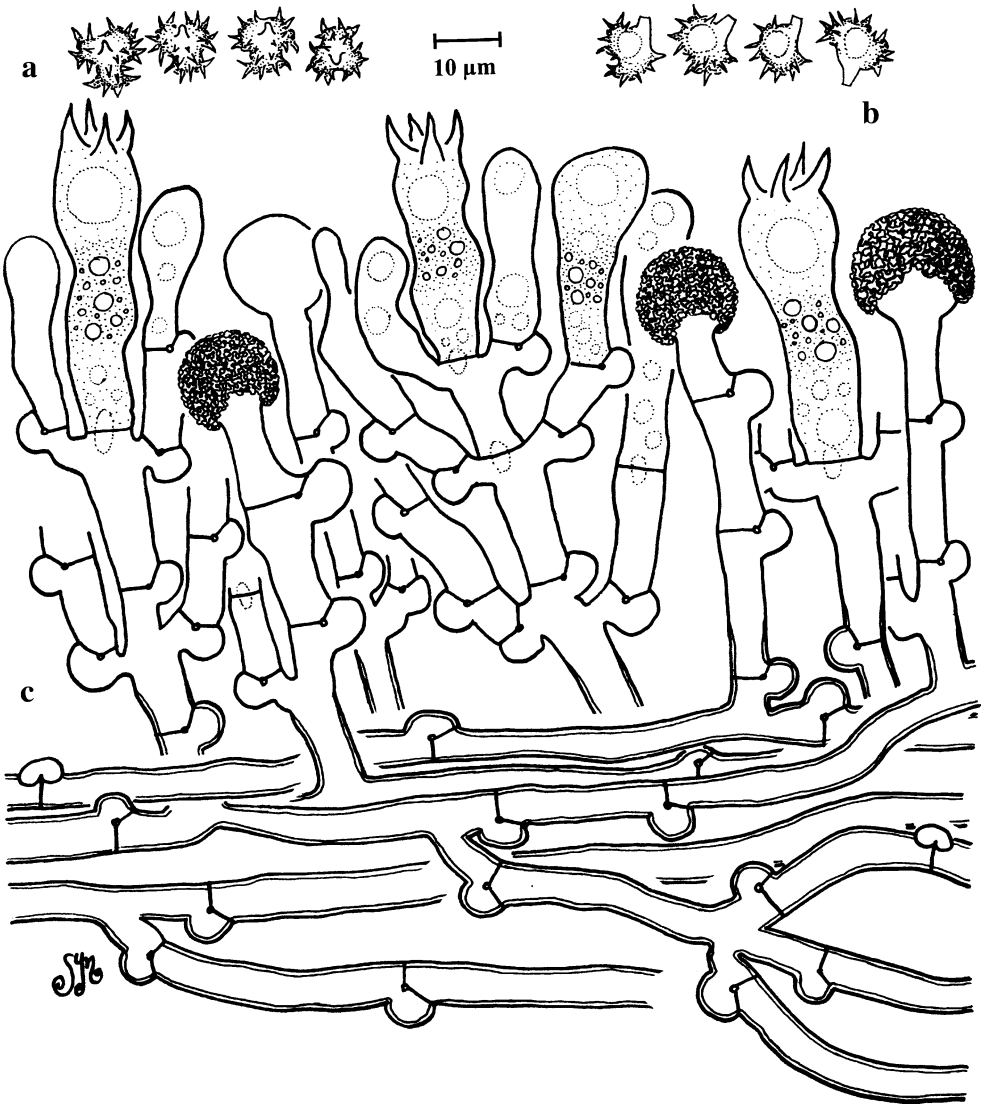

Fig. 5 *Tomentella capitata* Yorou & Agerer, **a** basidiospores in frontal view, **b** basidiospores in lateral view, and **c** section through the basidiocarp

Euphorbiaceae and *Dipterocarpaceae*. On dead bark and log, under native trees such as *Afzelia africana* Smith, *Isoberlina doka* Craib & Stapf, *Isoberlina tomentosa* (Harms) Craib & Stapf, *Monothes kerstingii* Gilg, *Uapaca togoensis* Pax, *Burkea africana* Hook. F. and *Detarium microcarpum* Guill. & Poir. Sporophores growing on dead bark and logs of various trees, always at the interface between soil and logs.

Tomentella brunneocystidia Yorou & Agerer, sp. nov

Description

Basidiocarpis resupinatis, separabilibus, arachnoideis, continuis. Hymenio aureo-brunneo usque ad atro-brunneo, granuloso, subiculo concolorato. Marginibus indeterminatis.

Rhizomorphae in subiculo et in margine, frequentes, frequenter ramificantes, atro-brunneae, monomiticae, leves, cystidiis deficientibus; hyphis in superficie tenuis infrequentibus, 1–2 µm in diametro, defibulatis, irregulariter ramificatis; hyphae internae fibuligerae, 0.5–1 µm crassitunicatae, subbrunneae usque ad brunneae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, nec amyloideae; hyphae sub superficio rhizomorpharum 3–4 µm in diametro, frequentibus hyphis multis septis simplicibus, hyphae in centro rhizomorpharum 3–6 µm in diametro, septis simplicibus raris vel deficientibus.

Hyphae subiculi fibuligerae, septa simplicia deficientia, (3)4–6(6.5) µm in diametro, 0.5–1 µm crassitunicatae, incrustatae granulis brunneis, pallide flavae vel flavae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, nec amyloideae.

Hyphae subhymenii fibuligerae, 4–7 µm in diametro, tenuitunicatae usque ad 0.5 µm crassitunicatae, cellulis brevibus, inflatibus deficientibus, flavae in 2.5% KOH et in aqua, nec congophilae, nec cyanophilae, nec amyloideae.

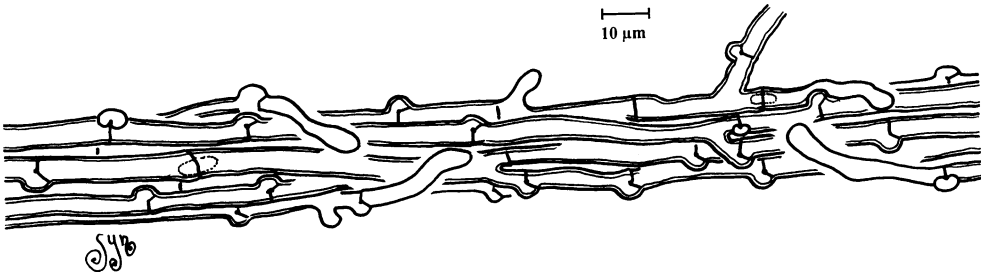

Fig. 6 Optical section through young rhizomorph of *T. brunneocystidia* Yorou & Agerer

Cystidia ab hyphae subiculae formantes, rhizomorphis cystidiis deficientibus, subcapitiformia, base fibuligera, (22) 25–50(55) µm longa, apice 7–10 µm in diametro, base 5–7 µm, non septata, non incrustata, frequenter contento atro-brunneo, in 2.5% KOH brunneo-atro, interdum incolorata.

Basidia (20)22–38(40) µm longa, 6–10 µm in diametro, base 5–8 µm, basibus fibuligeris, clavata, non stipitata, frequenter sinuosa, septa transversa deficientia, 4-sterigmatica, sterigmatibus 5–8 µm longis, basaliter 1–1.2 µm latis, frequenter incolorata in 2.5% KOH et in aqua sed interdum subflava, cyanophilae et congophilae, non amyloidea.

Basidiosporae 7.5–8× 7–8 µm in aspectu frontali, 6.5–7 (8) µm in aspectu laterali, triangulares vel lobatae in aspectu frontali, ellipsoideae in aspectu laterali, echinulatae, aculeis usque ad 1 µm longis, guttulis frequentibus, pallide brunneae usque ad subflavae in 2.5% KOH et in aqua, nec cyanophilae, nec congophilae, nec amyloideae .

Chlamydosporae absentes

Basidiocarp—resupinate, separable from the substrate, arachnoid, continuous. Hymenium is golden brown (5D7) to dark brown (6F5), granulose, and concolorous to the subiculum. Sterile margin is indeterminate.

Rhizomorphs—present in the subiculum and at margin, in great numbers and repeatedly branched, dark under a dissection microscope, pale brown to brown in 2.5% KOH and in water, without cystidia, monomitic; young rhizomorphs (thinner than 40 µm) smooth (Fig. 6), sometimes peripheral clamped hyphae with many simple septa (Fig. 7); older rhizomorphs (thicker than 40 µm) commonly patchily covered with dense irregularly shaped thin hyphae (Fig. 8); superficial thin hyphae 1–2 µm diameter, emerging from commonly simple septate hyphae (Fig. 9), multiply and densely branched, clampless, some without septa then similar to binding hyphae of polypores, sometimes growing along and around the thicker hyphae (Fig. 10); internal hyphae (hyphae below surface plus central hyphae) clamped, thick-walled (0.5–1 µm), yellow in 2.5% KOH and in water, not congophilous, not cyanophilous, not amyloid; hyphae below surface 3–4 µm, many hyphae with several simple septa; central hyphae wider, 3–6 µm, simple septa infrequent.

Subicular hyphae—clamped (Fig. 11), (3)4–6(6.5) µm; thick-walled (0.5–1 µm), simple septa lacking, pale yellow to yellow in 2.5% KOH and in water, not cyanophilous, not congophilous, not amyloid, with red–brown encrustation that are observable in water and Melzer's reagent, rapidly dissolving in KOH.

Subhymenial hyphae—clamped, 4–7 µm in wide, cells not short and inflated, thin to thick-walled (0.5 µm), yellow in water and in 2.5% KOH, not congophilous, not cyanophilous, not amyloid.

Cystidia—sub-capitate, arising from subicular hyphae, clamped at base, aseptate along their length, (22)25–50(55) µm long, 7–10 µm at apex and 5–7 µm at base, frequently with dark green or dark blue contents all over their length, without encrustation, sometimes colourless.

Basidia—(20)22–38(40) µm long, 6–10 µm at apex and 5–8 µm at base, clamped at the base, clavate, not stalked, often sinuous, without transverse septa, often colourless but sometimes light yellow in 2.5% KOH and in water, cyanophilous and congophilous, not amyloid, 4-sterigmate, sterigmata 5–8 µm long and 1,5–2 µm at base.

Basidiospores—7.5–8×7–8 µm frontal face and 6.5–7 (8) µm lateral face, triangular to lobed as seen in frontal view and ellipsoid in lateral view, echinulate, aculei short, not exceeding 1 µm, oil drops common, pale brown to pale yellow in 2.5% KOH and in water, not cyanophilous, not congophilous, not amyloid.

Chlamydospores—absent.

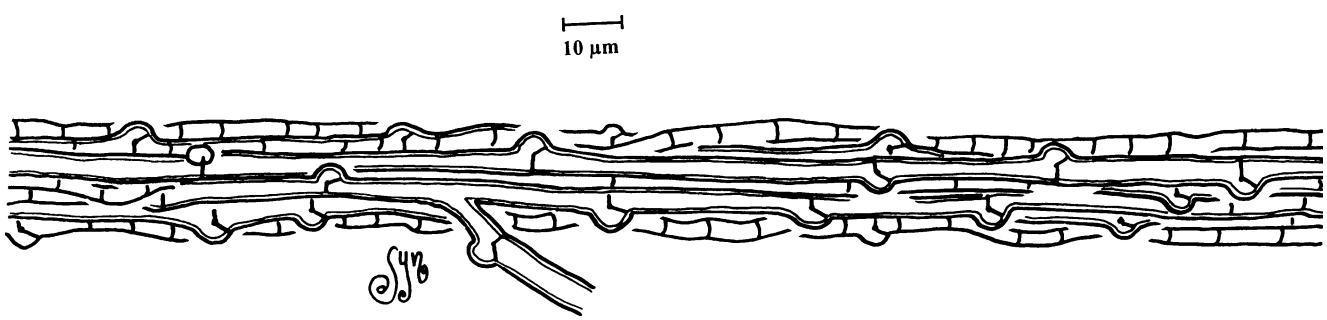

Fig. 7 Optical section of young rhizomorph of *T. brunneocystidia* showing mostly simple septate peripheral hyphae

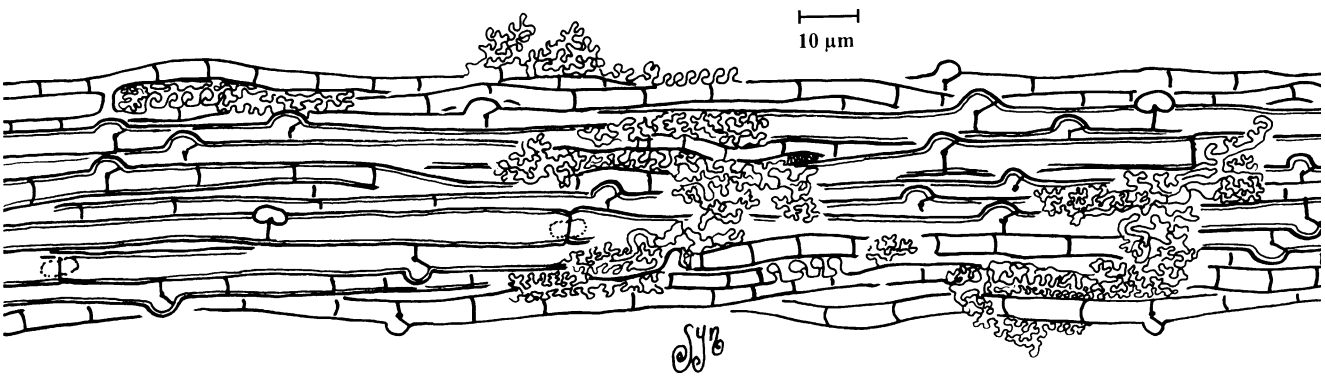

Fig. 8 Optical section through an old rhizomorph of *T. brunneocystidia*

Type material

Benin, central part, Borgou Province, reserved forest of Wari–Maro, Wari–Maro area, Wari–Maro, 09° 00′47.3" N, 002° 52′36.9" E, on dead barks and logs of native trees, leg. N. S. Yorou, 05.08.2005, herb. SYN 839. Holotype in M; isotype in TU. Genbank NCBI, accession number DQ848613.

Etymology

The epithet is proposed in reference to the brown to dark green content of the cystidia.

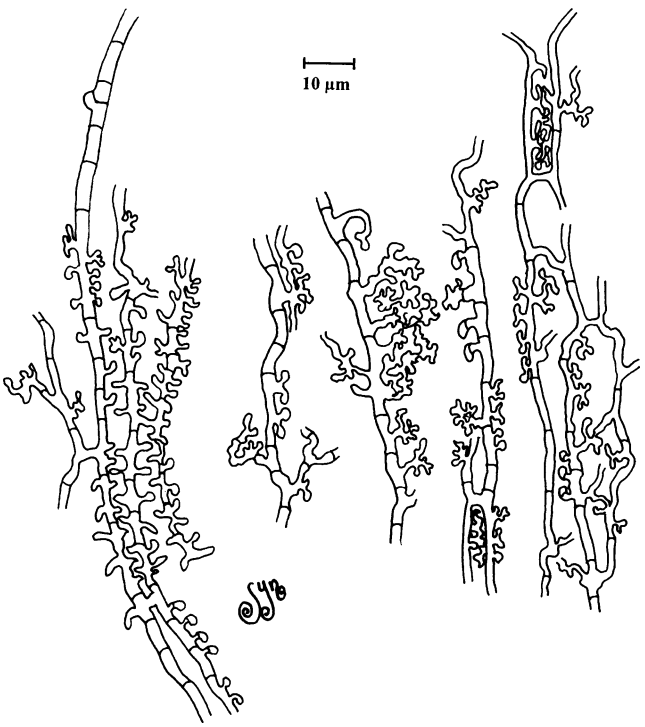

Fig. 9 Surface view of the rhizomorph of *T. brunneocystidia* showing the connection between surface hyphae and generative ones

Material studied

Benin, central part, Borgou Province, reserved forest of Ouémé Supérieur, Arboretum of Kpessou–Samari, 09° 00′ 47.3″ N, 002° 52′36.9″ E, on dead bark and logs of native trees, leg. R. Agerer, 23.08.2003, herb. RA 13815 (M), RA 13779 (M), RA 13823 (M). Benin, central part, Borgou Province, reserved forest of Wari–Maro, Wari–Maro area, 9°00′7.7″ N, 002° 00′18.8″ E, on dead bark and logs, leg N. S. Yorou, 05.08.2005, herb. SYN 859 (M), SYN 863 (M). Benin, central part, Borgou Province, forest of Wari–Maro, Wari–Maro area, Wari–Maro, 08° 12′25.6″ N, 002° 47′31.8″ E, leg. N. S. Yorou, 06.08.2005, herb SYN 865 (M), SYN 868 (M), SYN 883 (M), SYN 885 (M), SYN 889 (M), SYN 894 (M), SYN 897 (M).

Habitat

Collected in woodlands and dry forests of various native trees such as *Isoberlinia doka*, *I. tomentosa*, *U. togoensis* and *A. africana*. On dead bark and logs, either at the interface between soil and bark or on free bark.

Discussion

Both *T. capitata* and *T. brunneocystidia* are comparatively different regarding the ITS sequences (4.3–5.7 and 6.5–7.0% respectively) with *T. pilosa*. All three species form a monophyletic group with *T. atroarenicolor* Nikol. as a sister species. The last taxon is also clearly deviating from *T. capitata* and *T. brunneocystidia* by 5.9 and 7.2%, respectively.

T. capitata and *T. brunneocystidia* are anatomically very similar. Both species are separable from the substrate, form granulose to farinose hymenophores and present dark rhizomorphs under a dissection microscope. Microscopic similarities of these species include the thick-walled (0.5–1 µm) subicular hyphae of 4–6 µm (rarely 7–8 µm), the dark

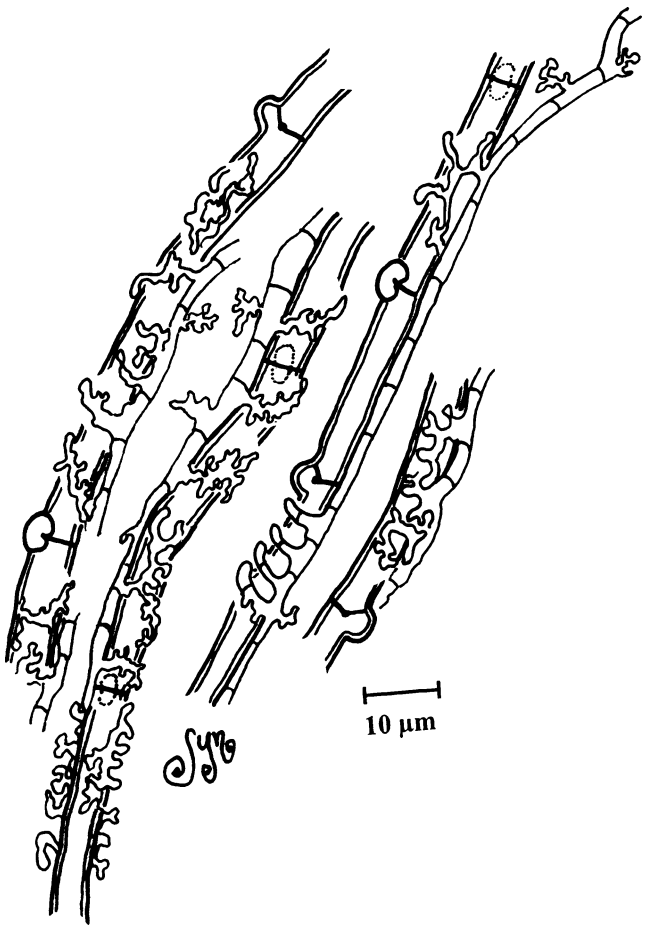

Fig. 10 Surface view of rhizomorph of *T. brunneocystidia* showing thin hyphae growing along and around generative hyphae

brown (in water and in 2.5% KOH) rhizomorphs that form irregularly shaped thin hyphae on their surface and the triangular to lobed basidiospores in frontal view and ellipsoid in lateral view. The size of the basidiospores is slightly dissimilar, ranging from 7–9.5 µm in *T. capitata* and 6.5–8 µm in *T. brunneocystidia*. However, the aculei of the basidiospores of *T. capitata* are distinctly longer (1–2 µm) than those of the basidiospores of *T. brunneocystidia* that are at most 1 µm. The species also differ in shape, colour, and to some extent, the contents of cystidia. The cystidia in *T. capitata* are 25–35 µm and have a distinctly capitate apex 9–14 (16) µm in diameter. They commonly present a dark brown pigmentation and/or encrustation covering the inflated head (Fig. 5). The cystidia in *T. brunneocystidia* are longer, ranging from 25 to 50 (55) µm have a subcapitate apex of 7–10 µm diameter (Fig. 11) and brown, dark blue to dark green contents all over their length. However, colourless cystidia are sometimes present in *T. brunneocystidia*. Other differences include hyphal dimension, presence of cystidia on rhizomorph and the colour of subhymenial hyphae in water, 2.5% KOH and Melzer's reagent. In *T. capitata*, hyphae are pale brown to brown (in water and in 2.5% KOH) slightly amyloid in patches. An amyloid reaction is also present in young hyphae of the hymenium and the subhymenium. In *T. brunneocystidia*, subicular and subhymenial hyphae are pale yellow to yellow (in water and in 2.5% KOH) and no positive reaction is observed with Melzer's reagent. The subicular hyphae of *T. brunneocystidia* have encrustation that quickly dissolves in KOH. No encrustations are observed on subicular hyphae of *T. capitata*. Subhymenial hyphae of *T. capitata* are 3.5–5 µm wide while those of *T. brunneocystidia* range from 4 to 7 µm diameter. In *T. capitata*, old rhizomorphs have capitate cystidia that emerge from the surface. No cystidia are observed on rhizomorphs of *T. brunneocystidia*.

Among the described *Tomentella* species, *T. pilosa* and *Tomentella viridula* Bourdot & Galzin have capitate cystidia (Kõljalg 1996; Melo et al. 1998). The cystidia of *T. pilosa* are 50–120 µm and of 6.5–13 µm diameter at apex. Those of *T. viridula* are 40–80 µm long and have a diameter of 5–8 (12) at apex. Furthermore, *T. virudula* lacks rhizomorphs and the rhizomorphs in *T. pilosa* are dimitic with distinctive skeletal hyphae of 2–3 µm (Kõljalg 1996). Both *T. capitata* and *T. brunneocystidia* present brown to dark brown rhizomorphs that are monomitic and show on their surface rather irregularly shaped and repeatedly branched thin hyphae of 1–2 µm diameter. Distinctive skeletal hyphae have not been observed.

Clavate cystidia within the genus *Tomentella* are known from *Tomentella muricata* (Ellis & Everh.) Wakel., *Tomentella clavigera* Litsch. and *T. subclavigera* Litsch. (Kõljalg 1996). In *T. clavigera*, cystidia are 80–140 µm long and 6–8 µm wide at apex (Kõljalg 1996). Those of *T. subclavigera* are 105–145 µm long and 7–11 µm at apex (Kõljalg 1996; Melo et al. 1998), and cystidia in *T. muricata* are 50–105 µm long and 6.5–8.5 µm diameter at apex. Cystidia in all three species are therefore longer and narrower than cystidia of *T. brunneocystidia*. Neither *T. clavigera* nor *T. subclavigera* has rhizomorphs in contrast to *T. brunneocystidia*.

The presence of irregularly shaped thin hyphae on the surface of rhizomorphs has only rarely been reported for thelephoroid fungi. Raidl and Müller (1996) and Raidl (1997) mentioned the presence of repeatedly branched thin hyphae on the surface of rhizomorphs of ectomycorrhizae formed by *T. ferruginea* (Pers.) Pat. on *Fagus sylvatica* L. Similar narrow surface hyphae have been shown for rhizomorphs of ectomycorrhizae formed by *T. subtestacea* Bourdot & Galzin on *Populus alba* (Jakucs and Agerer 2001) where they are described as highly specialised, tortuous, repeatedly ramified, densely entwined, glued and of dark yellow to brown colour. In both, *T. capitata* and *T. brunneocystidia*, the thin hyphae on the rhizomorphs are tortuous, too, densely and repeatedly branched, mostly clampless but they are brown to dark brown in water and in 2.5% KOH. According to Raidl

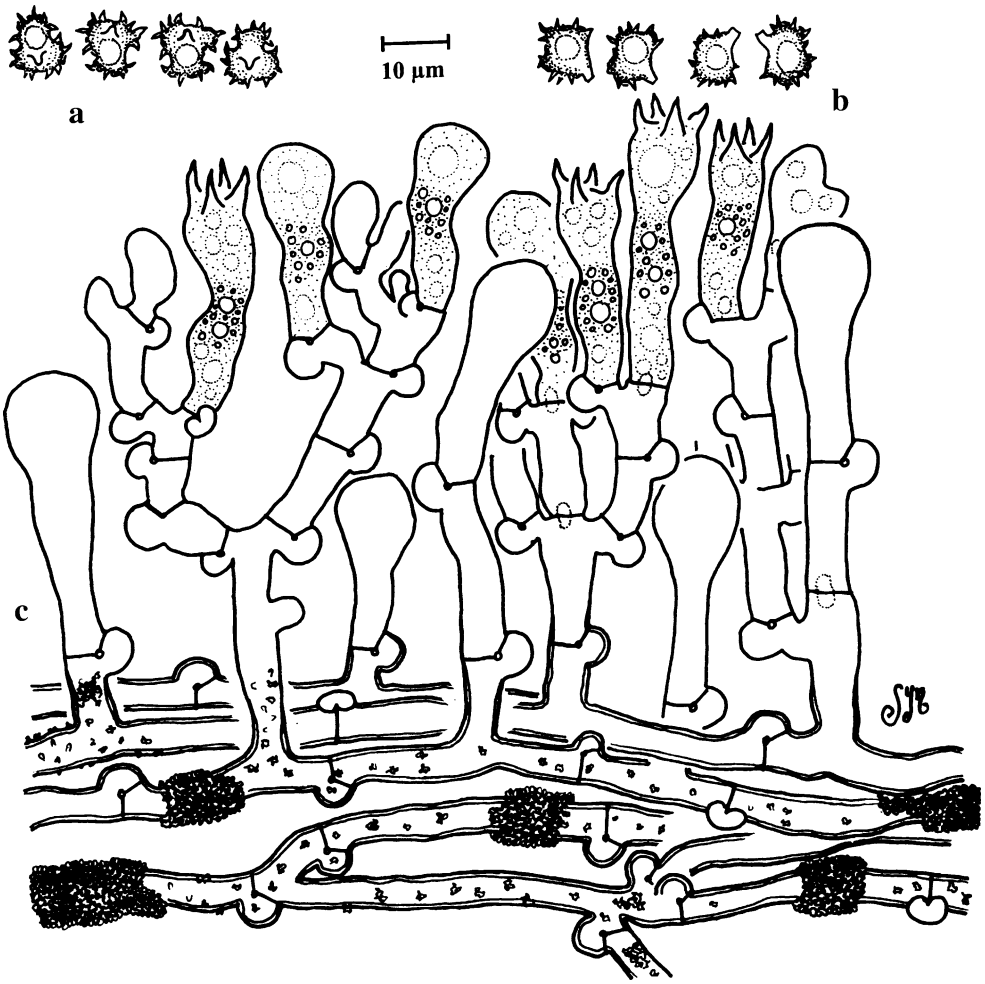

Fig. 11 *Tomentella brunneocystidia* Yorou & Agerer, **a** basidiospores in frontal view, **b** basidiospores in lateral view, and **c** section through the basidiocarp

(1997), the surface narrow hyphae occur at a late stage of the rhizomorphs' ontogeny. We agree with this statement because young rhizomorphs (under 35 µm diameter) of our specimens commonly lack such hyphae.

All studied specimens are collected in woodlands and dry forests of the Soudanean Endemism Centre (White 1983). Woodlands and dry forests in Benin are dominated by *Ceasalpiniaceae* (mainly *I.doka* and *I. tomentosa*, *B.*

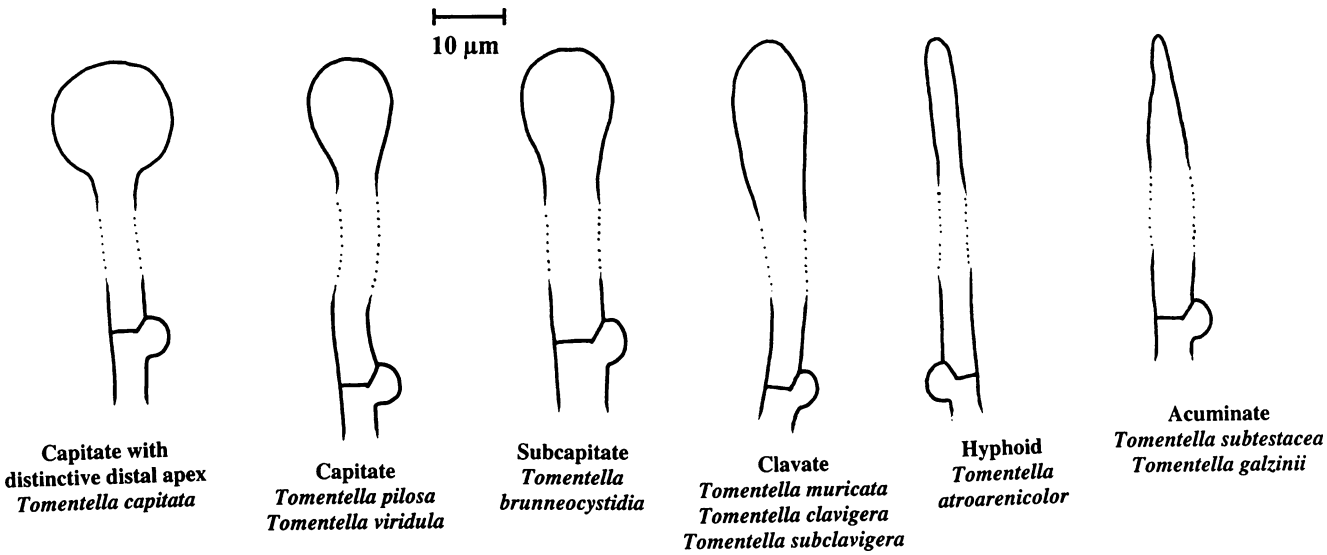

Fig. 12 Shape of cystidia within *Tomentella* species

africana, *D. microcarpum*, few specimens of *A. africana* are also recorded); *Euphorbiaceae* (*U. togoensis*, *Uapaca guinneensis*,) *Fabaceae* (*Pterocarpus erinaceus*) and one single member of *Dipterocarpaceae* (*Monotes kerstingii*). All mentioned species are putative ectomycorrhizae formers (Sanon et al. 1997; Ducousso et al. 2002). Sporophores of *T. capitata* were always found at the underside of logs or bark. Sporophores of *T. brunneocystidia* have also been collected on dead trunks that had no contact with the soil. In temperate and boreal forests, members of resupinate telephoroid fungi (including *Tomentella*) are proved to be ectomycorrhizal symbionts (Agerer 1996; Agerer and Bougher 2001; Jakucs and Agerer 1999; Jakucs et al. 2005; Kõljalg et al. 2000, 2001). It is very likely that both *Tomentella* species form ectomycorrhizal symbiosis with native trees.

Key for the identification of cystidioid *Tomentella* species

(Shapes of cystidia are illustrated in Fig. 12)

1. Cystidia subcapitate to capitate
2. Rhizomorphs absent, cystidia capitate, 40–80 µm long, 5–9 (12) µm at apex, colourless, often with encrustation around the head. *T. viridula*
   2*. Rhizomorphs present
3. Rhizomorphs dimitic, with distinctive skeletal hyphae, cystidia capitate, up to 130 µm long. *T. pilosa*
   3*. Rhizomorphs monomitic, covered by irregularly shaped, tortuous and repeatedly ramified narrow hyphae similar to binding hyphae of *Polyporaceae*
4. Cystidia with distinctive distal apex, 25–35 µm long and 9–14 µm at apex, old rhizomorphs sometimes with distinctive capitate cystidia of 20–48(50) µm long and 4–6 µm at apex, aculei 1–2 µm long. *T. capitata*
   4*. Cystidia subcapitate, 22(25)–50(55) long and 7–10 µm at apex, often with dark brown to blue–green content over their length, aculei 0.5–1 µm long. *T. brunneocystidia*
   1*. Cystidia of other shape
5. Cystidia clavate
6. Rhizomorphs present, cystidia 50–105 µm long. *T. muricata*
   6*. Rhizomorphs absent
7. Cystidia 80–140 µm long, basidia sometimes greenish in KOH, basidiospores triangular to lobed in frontal view. *T. clavigera*
   7*. Cystidia 105–145 µm long, basidia never greenish, basidiospores ellipsoid to globose in frontal view. *T. subclavigera*
   5*. Cystidia acuminate or hyphoid
8. Cystidia acuminate, with brown deposits at apices, rhizomorphs absent,
9. Hymenophore greenish, basidiospores 7–8.5 µm long in frontal and lateral faces, light brown in KOH. *T. galzinii*
   9*. Hymenophore brownish, basidiospores 6.5–8.5 µm long in frontal and lateral faces, light brown to reddish–brown in KOH. *T. subtestacea*
   8*. Cystidia hyphoid, rhizomorphs present. *T. atroarenicolor*

Acknowledgement Financial supports were provided by the German Academic Exchange Service (DAAD) through the grant no. A/03/15106. Field works and equipment were financially supported by the African Forests Research Network (AFORNET) with the grant no. 02/2005 and the International Foundation for Science (IFS) under grant no. D/4033-1. Leho Tedersoo and Triin Suvi are also much thanked for their daily assistance during the visit of the first author at the Institute of Botany and Ecology, University of Tartu in Estonia.

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II

Mycological Progress **6**: 239-247

Mycol Progress (2007) 6:239–247
DOI 10.1007/s11557-007-0543-z

ORIGINAL ARTICLE

Tomentella furcata, a new species from Benin (West Africa) with basidia forming internal hyphae

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Received: 26 April 2007 / Revised: 18 July 2007 / Accepted: 20 July 2007 / Published online: 15 August 2007

Abstract In this study, a new tropical African resupinate thelephoroid species, namely *Tomentella furcata*, is described. Microscopically, it is characterised by mostly simple septate and thick-walled (1–2 μm) subicular and subhymenial hyphae, suburniform basidia with transverse septa and/or infrequently with an internal hypha, large sterigmata of 2–4 μm at base, and distinctly yellow, subglobose to globose basidiospores (both in frontal and lateral view) with long (1–2 μm) and dense, simple or forked spines. Phylogenetically, *T. furcata* falls within terminal clades of investigated *Tomentella* species. We discuss ornamentation types of basidiospores for discrimination of resupinate thelepohoroid genera and confirm the limitation of ornament bifurcation as a diagnostic feature of *Pseudotomentella*. Anatomical dissimilarities between *T. furcata* and close species are given.

Introduction

Thelephoroid fungi represent one of the homobasidiomycetous clades identified by Larsson et al. (2004), Binder et al. (2005) and Hibbett and Thorn (2001). Microscopically, the group comprises species with mostly pigmented, strongly ornamented non-amyloid spores. Thelephoroid fungi include a total of over 177 accepted species that are accommodated in 12 genera (Kirk et al. 2001) and two families (Stalpers 1993): the *Bankeraceae* Donk (mostly with colourless basidiospores) and the *Thelephoraceae* Chevall. (mostly with brown to yellowish basidiospores). The so-called resupinate Thelephorales encompass four genera (Kõljalg 1996), namely *Amaurodon* J. Schröter, *Tomentellopsis* Hjortstam, *Pseudotomentella* Svrček and *Tomentella* Pers. ex Pat., and include about 100 species (Kirk et al. 2001). *Pseudotomentella* was originally described as *Tomentella* sect. *Tomentellastrum* Bourd. & Galz. (Bourdot and Galzin 1924). The section was then raised to generic level by Svrček (1958). Traditionally, the delimitation between *Tomentella* and *Pseudotomentella* is mainly based on the ornamentation of basidiospores, the presence/absence of clamps on subicular hyphae and of rhizomorphs, and the shape of basidia (Kõljalg 1996, Larsen 1968, 1974, Stalpers 1993). Typical (but not always consistently present) characters of *Pseudotomentella* are the presence of dimitic rhizomorphs, of simple septate and thick-walled hyphae (Svrček 1958, Larsen 1968, Stalpers 1993), the absence of cystidia (Kõljalg 1996; Stalpers 1993, Svrček 1958), utriform and stalked basidia, and colourless to yellowish, rarely brown (in 3% KOH) basidiospores with bi- or trifurcate ornaments (Larsen 1968, Kõljalg 1996, Stalpers 1993). In contrast to *Pseudotomentella*, the genus *Tomentella* is characterised mostly by clamped subicular hyphae, clavate basidia, and brown basidiospores (Larsen 1971, 1974, Stalpers 1993, Kõljalg 1996). Currently, no typical synapomorphies can be defined for the genus *Tomentella* with regard to basiodiospore ornaments. Though all described *Pseudotomentella* species have bi- or trifurcate spore ornaments (Kõljalg 1996; Kõljalg and

Taxonomical novelty: *Tomentella furcata* Yorou & Agerer.

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Dunstan 2001; Kõljalg and Larsson 1998; Larsen 1971, Martini and Hentic 2002, 2003, Melo et al. 2002, Stalpers 1993), this character cannot be regarded as a synapomorphic feature for the genus *Pseudotomentella* because some species of *Tomentella* and *Thelephora* also have bi- to trifurcate spore ornamentation (Kõljalg 1996, Stalpers 1993, Dämmrich 2006).

In this paper, we report a new tropical resupinate thelephoroid species that has simple septate thick-walled subicular hyphae, distinctly yellow basidiospores with isolated, forked and unforked conical spines. Simple septate subicular hyphae, coupled with forked spore ornaments, are reminiscent of the genus *Pseudotomentella*. However, the complete lack of rhizomorphs contradicts the placement of the new species in the genus *Pseudotomentella*. Molecular data and phylogenetic analysis support the placement of the new species within the genus *Tomentella*, thus confirming the limitation of spore ornamentation in the demarcation between *Tomentella* and *Pseudotomentella*.

Material and methods

Specimen sampling, light microscope studies and line drawings

Specimens of *T. furcata* were collected in woodlands and dry forests in the central part of Benin during the rainy season of 2003 and 2005. Collections were made under native trees and dried using a propane gas heated dryer (De Kesel 2001). Colours of dried basidiomata are given according to Kornerup and Wanscher (1978). Dried specimens were used for light microscopic studies. Fine sections through the basidiocarp were made using a razor blade under a stereomicroscope and mounted in water and afterwards in 2.5% KOH, in Congo Red, in Cotton Blue and in Melzer's reagent (Kreisel and Schauer 1987), respectively. Microscopic studies were performed using a light microscope Leica DM LB2. Measurements were made at 1,000× magnification. Measurements of basidiospores do not include the apiculus and ornamentation. Measurements of basidia exclude sterigmata. Line drawings were made at 1,000× magnification using a drawing tube. Descriptions are made following criteria given by Kõljalg (1996). Studied materials including holotype of *T. furcata* are deposited in herbarium **M** (Holmgren et al. 1990).

DNA extraction, amplification and sequencing

DNA was extracted from dried basidiocarp using a Quiagen DNeasy plant Mini Kit (Quiagen, Hilden, Germany), according to the manufacturer's instructions, and eluded in 100 µl of supplied elution buffer. PCR amplification (Gardes and Bruns 1993) was performed for internal transcribed spacers ITS1, ITS2, and for the 5.8S region of the nuclear ribosomal DNA, using basidiomycete specific primer pairs ITS1F (5′-cttggtcatttagaggaagtaa-3′) and ITS4B (5′-caggagacttgtacacggtccag-3′). Successful amplifications resulted in single bands on electrophoresis gels (agarose 1%). DNA was successfully extracted from the holotype of *T. furcata*. Amplified DNA was purified using the QIAquick-PCR purification Kit (Qiagen) according to manufacturer's instructions. DNA sequencing was performed through BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1 (Applied Biosystems, Foster City, Calif., USA) at the sequencing service of the Institute for Genetics, Department Biology I (Ludwig-Maximilians-Universität, München, Germany). One sequence of *T. furcata* is deposited in Genbank NCBI with accession numbers EF507261.

Sequences analysis and test for their generic position

Forward and reverse nucleotide sequences were edited with BioEdit v7.0.5 (Hall 2005). During a first step analysis, sequences were submitted to BLASTn searches against nr fungal sequences databases of UNITE (Kõljalg et al. 2005) and of the National Centre for Biotechnology Information (NCBI). Some thelephoroid sequences from the Seychelles Islands (Tedersoo et al. 2007) were also checked and added to the data set. ClusterW Multiple Alignment (of BioEdit v7.0.5) was then used to align our sequences against those published by Yorou et al. (2007) and Kõljalg et al. (2000, 2001).

Phylogenetic analysis

The BLASTn searches in both UNITE and NCBI highlighted the placement of *T. furcata* in the genus *Tomentella*. As we did not expect this taxonomic placement (due to the presence of forked ornaments), the phylogenetic position of the investigated specimen within different genera of Thelephorales was tested a second time. To do this, sequences of all representatives of the thelephoroid clade (Larsson et al. 2004) and 2 sequences from the more basal gomphoid-phalloid clade, namely *Kavinia himantia* (Schwein.) J. Erikss. and *Ramaria corrugata* (P. Karst.) Schild, were added to the data set. Recent molecular investigations have highlighted great difficulties in unambiguously aligning the ITS regions of different thelephoroid genera. As it was not possible to make reliable alignment using ITS sequences (Kõljalg et al. 2000, 2001), a tree was inferred using alignment of the 5.8S region only, with *K. himantia* and *R. corrugata* as outgroups. Again, this phylogenetic analysis confirmed the placement of *T. furcata*

Table 1 Sequences producing significant alignments with *T. furcata* (EF507261) using BLASTn search option in UNITE. The 10 first alignments are *Tomentella* species. For simplicity, only the top 5 alignments are presented

Best matches	Accession numbers	% Similarity	Score (bits)	E-value
Tomentella lilacinogrisea	UDB000953	92	488	e-138
Tomentella lateritia	UDB000963	92	484	e-137
Tomentella bryophila	UDB000253	93	468	e-132
Tomentella badia	UDB000961	89	462	e-130
Tomentella ferruginea	UDB000256	92	462	e-130

within the *Tomentella* clade (tree not shown). Specifically for the present study, phylogenetic analysis was then performed using both ITS (ITS1 and ITS2) and 5.8S regions of *Tomentella* species from the previous data set plus four *Thelephora* species. Phylogenetic analysis was performed using PAUP version 4.0b10 (Swofford 2002). We used the heuristic search option, ten replications of random-taxon entry and tree bisection reconnection (TBR) swapping. Gaps were treated as missing values. Bootstrap analysis was performed with 1,000 replicates under the heuristic search (Felsenstein 1985). All molecular characters were assessed as independent, unordered, and of equally weight using Fich parsimony (Fich 1971). Uninformative characters were excluded from the analysis. Similar studies have used *Thelephora pseudoterrestris* as the outgroup (Yorou et al. 2007, Kõljalg et al. 2000, 2001). The same taxon is also used as the outgroup in the present analysis.

Results

Phylogenetic position of *T. furcata*

The BLASTn search option of UNITE clearly highlights the placement of *T. furcata* within the genus *Tomentella*. The first five best alignments are given in Table 1. Using the NCBI database; the top 50 best matches are either unknown thelephoroid ectomycorrhizae or *Tomentella* species. Table 2 presents the top 5 best matches using BLASTn option of NCBI. Phylogenetically, *T. furcata* occurs within terminal clades of all studied *Tomentella* species (Fig. 1). It forms a monophyletic group together with unknown tropical African *Tomentella* species (accession numbers EF507258–EF507260) with a bootstrap support of 100%. This monophyletic group is clustered together with *T. lilacinogrisea* Wakef. with a weak bootstrap support (56%). *T. lateritia* Pat., *T. bryophila* (Pers.) M. J. Larsen, *T. ferruginea* (Pers.) Pat. and *T. badia* (Link) Stalpers, which ranged among the best 5 matches (according to the BLASTn search of UNITE), fall in more basal clades.

Description

Tomentella furcata Yorou & Agerer, sp. nov.

Basidiocarpis resupinatis, adherentibus, pelliculosis, continuis. Hymenio brunneo usque ad atro-brunneo, remanenti, granuloso, subiculo atro-brunneo. Marginibus indeterminatis.

Rhizomorphae absentes.

Hyphae subiculi plerumque simplice septati, (4.5)5–7(8) μm in diametro, 1–2 μm crassitunicatae, non incrustatae, brunneae usque ad atro-brunneae in 2.5% KOH et in aqua, sed muribus luteis, nec congophilae, nec cyanophilae, nec amyloideae.

Hyphae subhymenii simplice septatae, interdum fibuligerae, 5–8(10) μm in diametro, crassitunicatae, muribus 1–1,5 μm, incoloratae usque ad pallide brunneae in 2.5% KOH et in aqua, interdum exudatis venetis, subcongophilae, nec cyanophilae, nec amyloideae.

Cystidia absentia.

Basidia 25–40(45) μm longa, 9–12(13) μm in diametro, base 5–9 μm, basibus simplice septatis, interdum fibuligeris, suburniformia, non stipitata, interdum sinuosa, septa transversa vel hyphae internae presentes; hyphae internae

Table 2 Best matches of *T. furcata* (EF507261) using BLASTn search option in NCBI. The first 50 best matches are either unknown Ectomycorrhizae (EcM) or *Tomentella* species. For simplicity, only the top 5 five matches are presented

Best matches	Accession numbers	% similarity	Query coverage (%)	E value
Thelephoraceae sp.	U83467	87	66	0.0
Uncultured EcM	EF101772	86	68	0.0
Tomentella sp.	AJ534912	85	68	0.0
Uncultured EcM	AY634144	86	66	0.0
Uncultured EcM	AJ633588	90	62	0.0

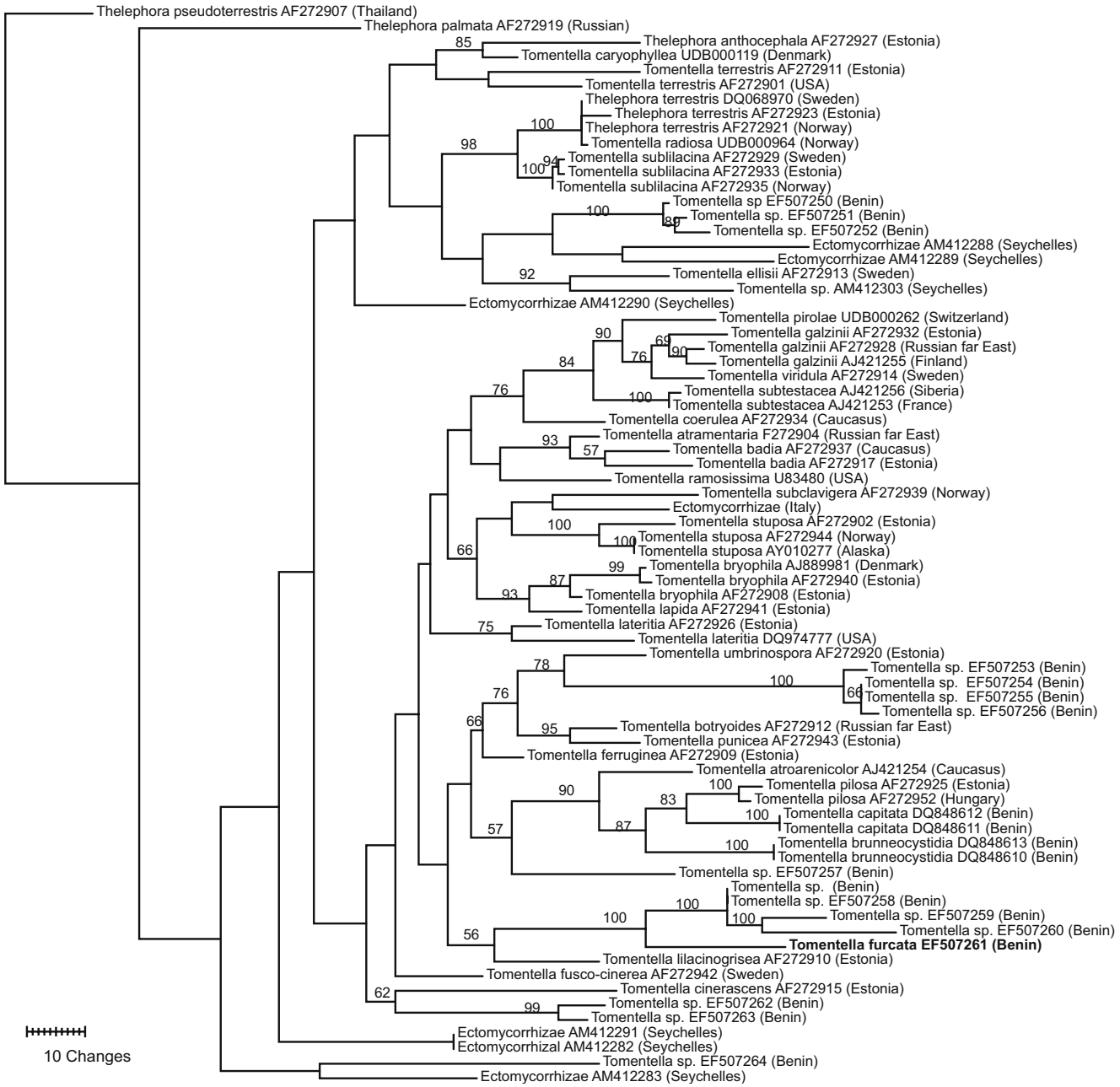

Fig. 1 One of the most parsimonious trees showing the placement of *Tomentella furcata* within *Tomentella* and *Thelephora* species. Bootstrap values are shown above branches. The Genbank (NCBI or UNITE) codes as well as the origin of the vouchers are indicated after species names

simplice septatae, 0.5–1 μm crassitunicatae, laeves, incoloratae, rarissime hyphae internae unum sterigmatem externum basidiarum formatae; basidia plerumque tenuitunicata, basidia hyphis subiculis formatae, 1–2.5 μm crassitunicata, plerumque incolorata in 2.5% KOH et in aqua, interdum pallide brunneae, plerumque contentis venetis usque ad atro-caeruleis, 4-sterigmatica, sterigmatibus 8–10 μm longis, basaliter 2–4 μm latis; basidia incolorata subcongophila, subcyanophila, non amyloidea.

Basidiosporae (7.5)8–12(13)× (7.5)8–11(13) μm in aspectu frontali, (7)8–11(13)× (7.5)8–9(12) μm in aspectu laterali, globosae in aspectu frontali, subglobosae in aspectu laterali, flavae usque ad stramineae in 2.5% KOH et in aqua, 0.1–1μm crassitunicatae, echinulatae, aculeis simplicibus vel bifurcatis, 1–2 μm longis, guttulis magnis frequentibus, nec cyanophilae, nec congophilae, nec amyloideae.

Chlamydosporae absentes.

Basidiocarp resupinate, crustose, adherent to the substrate, continuous, 0.1–0.5 mm thick. *Hymenophore* brown to dark brown (7F6), sometimes with olivaceous tinges, smooth to granulose, not changing colour, subiculum dark brown, sterile margin indeterminate.

Rhizomorphs absent.

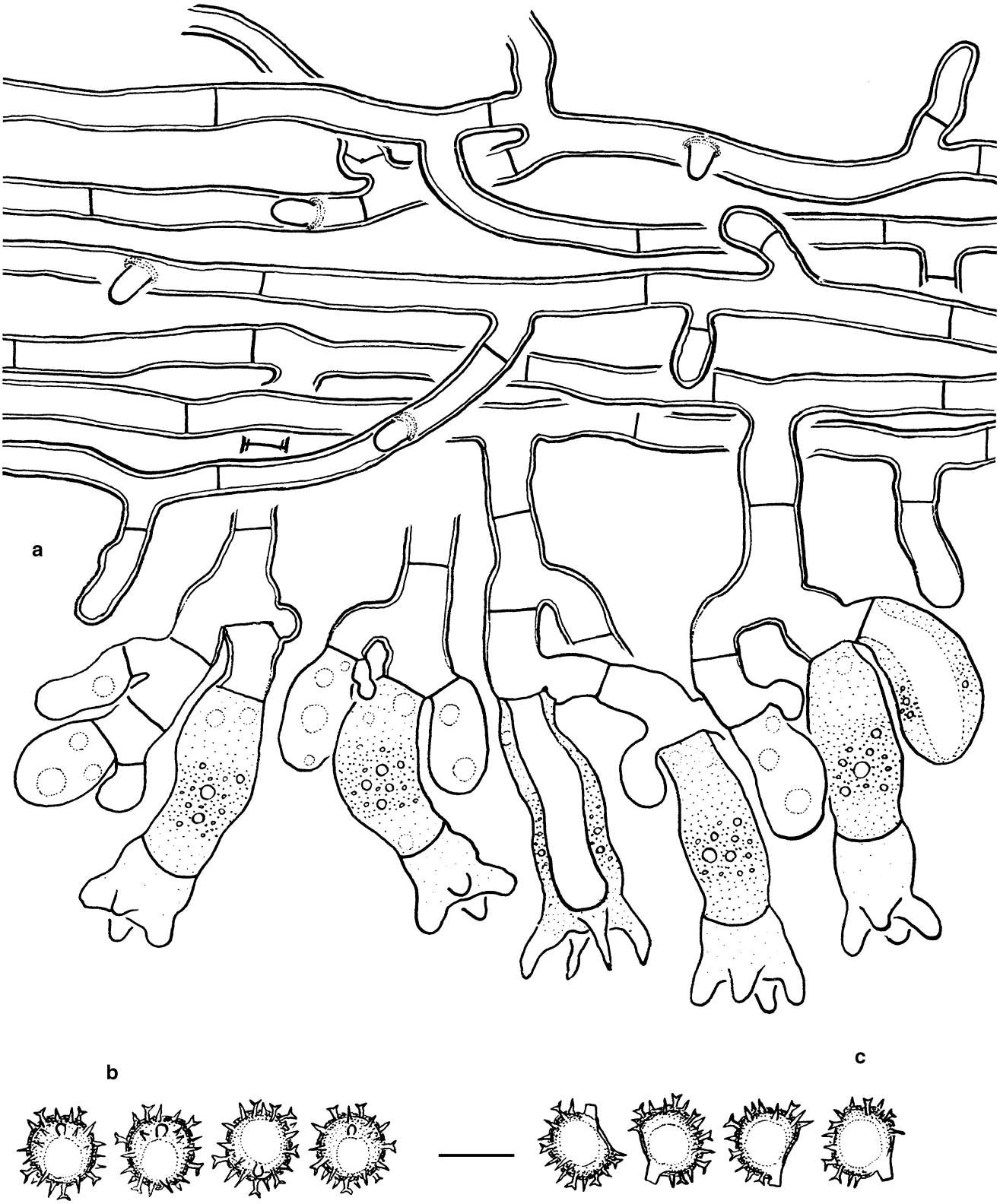

Fig. 2 *Tomentella furcata*. **a** Section through the basidiocarp; **b** basidiospores in frontal view; **c** basidiospores in lateral view. *Scale bar*=10 μm

Subicular hyphae simple septate, (4.5) 5–7 (8) μm wide; clamps rare, thick-walled (1–2 μm); without encrustations, total hyphae brown to dark-brown (in 2.5 % KOH and in water), but walls yellow-brown, not congophilous, not cyanophilous, not amyloid, cross-shaped branching absent.

Subhymenial hyphae simple septate, clamps rare, 5–8 (10) μm wide, thick-walled (1–1.5 μm), colourless to light brown (in 2.5% KOH and in water), sometimes associated with blue-green exudates, slightly congophilous, not cyanophilous, not amyloid.

Cystidia absent.

Basidia 25–40 (45) μm long, 9–12 (13) μm wide at apex and 5–9 μm at base (Fig. 2), simple septate at base, sometimes clamped, suburniform, not stalked, sometimes sinuous, often with transverse septa, sometimes with an internal hyphae starting at the basal septum and growing into the basidia; intra-basidial hyphae simple septate (Fig. 3), thick-walled (0.5–1 μm) at the base, smooth and colourless, sometimes present already within young basidia. Rarely, intrabasidial hyphae endings may be acuminate, emerging through the apex of the basidium. While basidia are thin-walled, immature basidum-like structures that are very thick-walled (1–2.5 μm) emerge from subicular hyphae; walls yellowish. Basidia often colourless (in 2.5 % KOH and in water), sometimes pale brown, sometimes with blue-green to dark-blue contents, colourless, basidia slightly congophilous, slightly cyanophilous, not amyloid, 4-sterigmate, sterigmata 8–10 μm long and very wide (2–4 μm) at base.

Basidiospores (7.5) 8–12 (13)×(7.5)8 –11 (13) μm in frontal face and (7) 8–11 (13)×(7,5) 8–9 (12) μm in lateral face, subglobose to globose in both frontal and lateral views, deep yellow (in 2.5% KOH and in water), thick-walled (0.5–1 μm), not cyanophilous, not congophilous, not amyloid, with large oil drops, oil drops turning dark-blue in Melzer reagent, with prominent apiculus of 1–2×1–2 μm, apiculus not amyloid, echinulate, aculei conical, 1–2 μm long and 0.5 μm at base, commonly forked, forks up to 0.5 μm.

Chlamydospores absent.

Type material

BENIN, central part, Borgou province, forest reserve of Wari-Maro, Wari-Maro area, 08°12′25.6″N, 002°47′31.8″E, on dead logs, leg. N. S. Yorou, 05.08.2005, herb. SYN 924 (**M**), isotype at TU, Genbank NCBI, accession number EF507261.

Etymology

The epithet is given in reference to the ornamentations of the basidiospores. Ornamentations consist of isolated spines that are commonly forked.

Habitat

On dead bark and logs of native trees, in woodlands and dry forests dominated by *Ceasalpiniaceae* (*Isoberlinia doka* Craib & Stapf, *Isoberlinia tomentosa* Craib & Stapf, *Burkea africana* Hook. F. and a few individuals of *Afzelia africana* Smith), by *Euphorbiaceae* (*Uapaca togoensis* Pax) and *Dipterocarpaceae* (*Monotes kerstingii* Gilg).

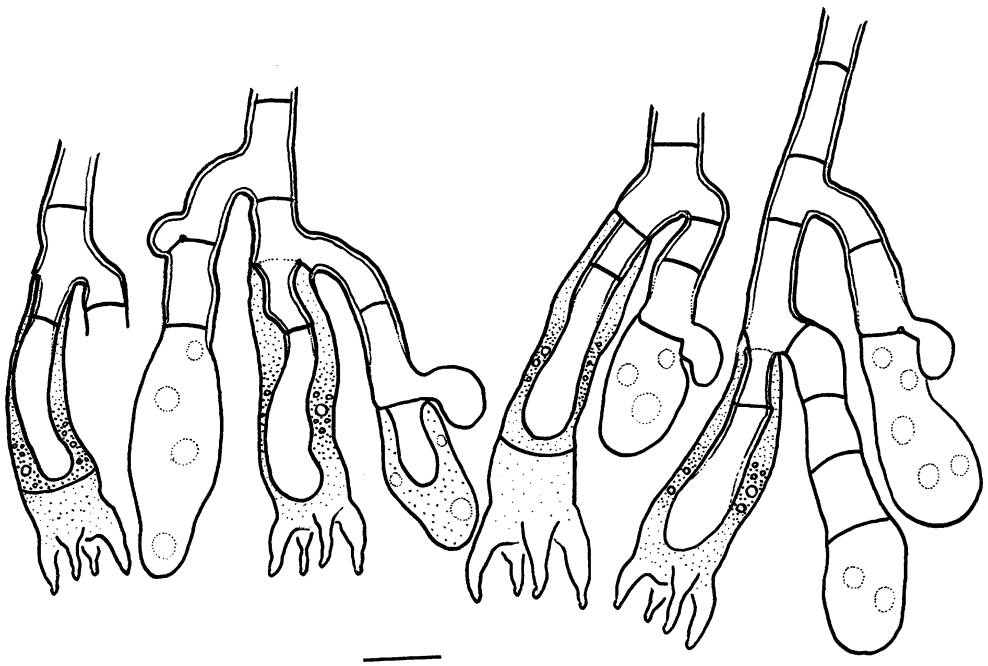

Fig. 3 *Tomentella furcata*. Optical section of basidia showing intra-basidial hyphae. *Scale bar*=10 μm

Discussion

The delimitation of *Pseudotomentella* and *Tomentella* is mostly based on the ornamentation types of basidiospores (Larsen 1971, 1974, Kõljalg 1996, Stalpers 1993). In the genus *Pseudotomentella,* spore ornamentations consist of bi- to trifurcate warts and/or spines. In the present study, both BLASTn (in UNITE) and phylogenetic analysis clearly place *T. furcata*, a species with isolated, simple and forked spines, within the genus *Tomentella* rather than in *Pseudotomentella*. Bifurcate ornaments have been also reported for other *Tomentella* species, namely *T. italica* (Sacc.) M. J. Larsen, *T. crinalis* (Fr.) M. J. Larsen, *T. radiosa* (P. Karst.) Rick and *T. fibrosa* (Berk & M. A. Curtis) Kõljalg (Kõljalg 1996, Dämmrich 2006) and for some *Thelephora* species (Stalpers 1993). The presence of bi- or trifurcate ornaments within three different genera (*Thelephora*, *Pseudotomentella* and *Tomentella*) provides evidence that this character is rather unreliable for the discrimination of thelephoroid genera.

Another African thelephoroid species with forked ornaments was described by Martini and Hentic (2002) as *Pseudotomentella armata*. In both *P. armata* and *T. furcata*, spore ornamentation consist of long (1.5–3 μm), isolated simple and forked spines. However, basidiospores of *P. armata* are colourless to very light yellow (in water and in 2.5% KOH), and strongly cyanophilous. With sizes ranging from 6.5 to 8.5 μm in both frontal and lateral faces (Martini and Hentic 2002), they are far smaller than those of *T. furcata*. Furthermore, in contrast to *T. furcata*, *P. armata* shows dimitic rhizomorphs. It presents colourless but congophilous, clamped and thin-walled subicular and subhymenial hyphae, and very long (40–90 μm length) clavate basidia.

Within the genus *Tomentella*, distinctly yellow subglobose basidiospores are reported for *Tomentella bryophila* (Kõljalg 1996). *T. bryophila* differs from *T. furcata* by the absence of forked spines (spines are never forked in *T. bryophila*), the consistently clamped subicular and subhymenial hyphae, the absence of a cyanescent reaction, and the amyloid reaction of

Table 3 Detailed anatomical comparison between *Tomentella furcata*, *Tomentella bryophila* and *Pseudotomentella armata*

Structure	Characters	*P. armata*	*T. furcata*	*T. bryophila*
Basidiocarp		Separable	Adherent	Adherent to separable
Hymenophore		Light brown (7D4 to 7D5)	Dark brown (7F6)	Brown-orange (7C6-7D6), rust to brown-orange (8D7)
Rhizomorphs		Present, dimitic, highly differentiated	Absent	Absent
Subicular and sub-hymenial hyphae		Clamped, simple septa occasional, thin-walled, colourless,	Simple septate, clamps rare, thick-walled, brown to dark brown,	Regularly clamped, thick-walled, partly encrusted, pale yellow to yellow, sometimes inflated,
Basidia	Length (μm)	40–85 (95)	25–40 (50)	40–70 (72)
	Shape	Clavate	Suburniform,	Suburniform to clavate,
	Septa and internal proliferations	Transverse septa absent, internal proliferation absent	Transverse septa present, intra-basidial hyphae sometimes present,	Rarely with transverse septa, rarely with intra-basidial hyphae,
	Reaction	Not cyanescent, congophilous, slightly cyanophilous,	Cyanescent, not congophilous, slightly cyanophilous,	Not cyanescent, congophilous, cyanophilous,
Sterigma	Length x width at base (μm)	4.5–7(9)×1–1.5	8–10×2–4	6–10×1–3
Basidiospores	Size: frontal view	5(6)–8×6–7.5	(7.5)8–12(13)×(7.5) –11(13)	(7.5)8–11×8–10
	Siz: lateral view	6–8×6.5–8.5	(7)8–1113)×(7.5)8–9(12)	8–12×7.5–8.5(9)
	Shape	Irregularly ellipsoid in frontal and lateral views,	Sub-globose to globose in frontal and lateral views	Sub-globose to globose, rarely ellipsoid,
	Colour	Colourless to very pale yellow,	Yellow	Yellow
	Reactions	Strongly cyanophilous, apiculus not amyloid	Not cyanophilous, apiculus not amyloid,	Not cyanophilous, apiculus amyloid,
	Aculei	2–3.5 μm, commonly forked,	1–2 μm, commonly forked,	1.5–3.5 μm, never forked,

apiculus of its basidiospores. Detailed anatomical dissimilarities between *T. furcata*, *T. bryophila* and *P. armata* are given in Table 3.

The presence of internal hyphae within basidia of *T. furcata* is a new feature, not only within the genus *Tomentella*, but generally within the Thelephorales (Corner 1968; Kõljalg 1996; Larsen 1971, 1974; Maas Geesteranus 1975; Stalpers 1993). Although the intrabasidial hyphae are reminiscent of repetobasidia, the latter were not found." Intra-basidial proliferations were first reported by Eriksson (1958) as repetobasidia. Repetobasidia are formed due to an internal replacement of an old basidium by a young one through the basal septum, leading to a succession of several generations of basidia. Repetobasidia are a rather rare phenomenon within the Hymenomycetes. It is only known from few corticioid genera such as *Galzinia* Bourdot, *Repetobasidium* J. Eriksson, *Repetobasidiellum* J. Eriksson & Hjortstam, *Conferticium* Hallenberg (Hjortstam et al. 1987) and in *Hyphodontia* J. Eriksson (Hallenberg and Hjortstam 1996). While the presence of repetobasidia is the diagnostic feature for *Repetobasidium* and *Repetobasidiellum* (Eriksson et al. 1981), they only occur occasionally in the genus *Galzinia*—except for *G. pedicellata* Bourdot where they are common (Eriksson and Ryvarden 1975)—and in *Conferticium* (Hallenberg 1980). In *Repetobasidium* and *Repetobasidiellum*, the formation of repetobasidia occurs at short intervals in the same place, often resulting in a dense sheath of several basidial cell wall remnants at the base of the basidia (Eriksson et al. 1981). In all above-mentioned genera, residual walls of old basidia surrounding the new young basidia are commonly observed. In *T. furcata*, neither mature basidia emerging from the intra-basidial proliferations nor residual walls of old basidia were observed. An extremely rare phenomenon that has been observed is the formation of a sterigma-like structure on the apex of the intra-basidial hyphae that emerges through the basidial apex. A spore has not been observed yet on such a sterigma.

Acknowledgements The German Academic Exchange Service financially supported this study through the grant A/03/15106. Field works and equipment were supported by the African Forests Research Network with the grant no. 02/2005 and the International Foundation for Science (grant D/4033-1). We are much indebted to Dr. Alexander Kocyan and Alison Davies (Institute for Systematic Botany, LMU-München) for their valuable assistance with phylogenetic analyses and linguistic checking of the manuscript respectively.

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III

Mycologia 100 (1): 68-80

Mycologia, 100(1), 2008, pp. 68–80.

Tomentella africana, a new species from Benin (West Africa) identified by morphological and molecular data

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Abstract: A common resupinate thelephoroid fungus was collected in northern Guinean seasonal forests in central and north of Benin (West Africa). The species is reminiscent of *Tomentella umbrinospora* with respect to the color and thickness of basidiomata and rhizomorphs, the shape of basidiospores in frontal view and the size of subicular hyphae. Both species fall phylogenetically within two clades. Based on detailed anatomical comparison (mostly of rhizomorphs and basidiospores) with the holotype of *T. umbrinospora* and phylogenetic analyses including ITS rDNA sequences of 40 *Tomentella* species, *T. africana* is described as a new species. Genetic distance between the newly described species and *T. umbrinospora* is 12.1–12.9%, based on ITS rDNA sequences. *T. africana* is characterized anatomically by yellow-brown thick (0.3–0.8 mm) monomitic rhizomorphs that are commonly covered by irregularly shaped thin hyphae, thin- to thick-walled subicular hyphae of two size ranges, clavate and clamped basidia of 30–60 µm and light yellow to pale brown echinulate basidiospores with irregular shape in frontal view. Detailed anatomical and molecular dissimilarities between *T. africana* and close species are discussed. Differences between irregularly shaped surface thin hyphae and skeletal ones are highlighted. We stress the relevance of rhizomorphal structures in the discrimination of resupinate thelephoroid fungi.

Key words: anatomy, hyphal system, ITS rDNA sequences-based phylogeny, rhizomorphal structure, *T. Africana*, *T. umbrinospora*, tropical Africa

INTRODUCTION

The use of shape and ornamentation of basidiospores in the discrimination of resupinate thelephoroid fungi (Kõljalg 1996) presents some limitations because basidiospores of most species have irregularly shaped outlines. The shape of basidiospores is similar in many different species (Stalpers 1993). In such cases cystidia (Yorou et al 2007) and rhizomorphs respectively help in species delimitation. Rhizomorphs are indeed one of the most important discriminative elements within resupinate thelephoroid fungi (Kõljalg 1996, Stalpers 1993). Rhizomorphs have been defined as ''multi-hyphal linear aggregates'' (Cairney et al 2001), independent of their ontogenetical, anatomical or functional patterns. However their ontogeny and anatomical structures are of remarkable taxonomical value (Raidl 1997, Agerer 1987–2006) and have been used successfully to trace relationships within and between various fungal groups (Agerer 1999, 2002, 2006, Agerer and Iosifidou 2004, Iosifidou and Agerer 2002). Many resupinate thelephoroid species present constantly dimitic rhizomorphs composed of generative and skeletal hyphae, namely *Tomentella ferruginea* (Pers.) Pat., *T. botryoides* (Schwein.) Bourdot & Galzin, *Pseudotomentella rhizopunctata* E.C. Martini & Hentic and *P. armata* E.C. Martini & Hentic (Kõljalg 1996, Stalpers 1993, Loci 1997, Martini and Hentic 2002, 2003, Melo et al 1998). Some species, such as *Tomentella radiosa* (P. Karst.) Rick, *T. ellisii* (Sacc.) Jülich & Stalpers, *T. italica* (Sacc.) M.J. Larsen and *T. sublilacina* (Ellis & Holw.) Wakef. (Kõljalg 1996), show monomitic rhizomorphs. Both dimitic and monomitic rhizomorphs of Thelephorales may form chlamydospores (Agerer 1991, 1992, 1993, Martini and Hentic 2003, 2005) or irregularly shaped thin hyphae on their surface (Yorou et al 2007, Raidl and Müller 1996, Jakucs and Agerer 1999, 2001).

The present paper reports a yellow-brown to red-brown resupinate thelephoroid species we collected frequently in woodlands of central to north Benin (West Africa). The specimens assigned to this species represent more than 50% of our collections and occur either on soil or on the undersides of burned logs and bark. Macroscopic and some microscopic features of the specimens resemble those of *Tomentella umbrinospora* M.J. Larsen, a member of thelephoroid fungal group with putative worldwide distribution (Larsen 1968, 1974, Kõljalg 1996).

Accepted for publication 17 September 2007.
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Microscopic observations of the holotype of *T. umbrinospora* however revealed distinct anatomical dissimilarities regarding the structure of rhizomorphs, the size of basidiospores and of basidia. Anatomical features as well as molecular phylogenetic studies support the description of *T. africana* as a new species. This paper is the third part of a series dedicated to tropical African resupinate thelephoroid fungi.

MATERIALS AND METHODS

Specimen sampling, light microscope studies and line drawings.—Specimens were collected in various woodlands and savannahs in central and northern parts of Benin (West Africa) during the rainy seasons of 2003, 2004 and 2005 and dried with a propane field dryer (de Kesel 2001). The holotype of *T. umbrinospora* was loaned from the herbarium of the New York State Museum. Color codes of dried basidiocarps are given according to Kornerup and Wanscher (1978). Herbaria names follow Holmgren et al (1990). We refer to Yorou et al (2007) and Yorou and Agerer (2007) for microscopic studies of specimens and line drawings. Descriptions follow criteria compiled by Kõljalg (1996). All studied specimens of *T. africana* and its holotype are deposited in M. An isotype is deposited in TU.

Scanning electron miscroscope (SEM) studies.—Small samples taken from fresh fruit bodies were fixed in 300–400 µL glutaraldehyde-cacodylate buffer and washed with tap water as follows: 60 min in 2.5% glutaraldehyde, gradual washing (5 min, 15 min, 30 min, 60 min) in a neutral cacodylate buffer (75 mM cacodylate, 2 mM MgCl2, 100 µL H2O, pH 7), 1–2 h incubation in 1% O_sO_4 buffer (2.5 ml O_sO_4, 7.5 mL H_2O) with subsequent washing in distilled water. The samples were dehydrated gradually in a series of acetone solution as follows: 10% × 15 min, 20% × 15 min, 40% × 15 min, 60% × 15 min, 80% × 15 min, 100% × 15 min, 100% × 30 min. Samples were stored in 100% acetone overnight followed by critical point drying (Anderson 1951). Dehydrated samples were mounted on aluminium stubs by means of adhesive tape and then sputtered with platinum (60 s at 20 C and 20 mA) using a BALTEC SCD 050 sputter coater (BAL-TEC AG, Balzers, CH). Samples were examined with a LEO 438 VP E scanning electron microscope (LEO Electron Microscopy Inc., USA).

DNA extraction, amplification and sequencing.—DNA was extracted from fruit bodies according to Gardes and Bruns (1993) with a QIAGEN DNeasy plant Mini Kit (QIAGEN Inc., Hilden, Germany), according to the manufacturer's instructions. PCR amplification was performed for internal transcribed spacers ITS1, ITS2 and for 5.8S region of the nuclear ribosomal DNA, using fungi-specific primer ITS1F (5′ cttggtcatttagaggaagtaa 3′) and basidiomycete-specific primer ITS4B (5′ caggagacttgtacacggtccag 3′). PCR amplification was performed with Ready To Go™ beads (Amersham Pharamacia Biotech., Piscataway, New Jersey), with 24 µm of PCR solution (composed of 180 µm ddH2O, 30 µm buffer, 21.6 µm MgCl, 12 µm ITS1F, 12 µm ITS4B, 30 µm dNTP-Mix and 2.4 µm Taq-Polymerase) and 1 µm 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), 94 C for 1 min, 60 C for 1 min, 72 C for 1 min and 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 µm) were run with bromophenol blue (2 µm) on 1% agarose gels for 30 min at 95 C, then stained in ethidium bromide for 10 min and in ddH2O for 1 min. PCR products were viewed under UV light. Successful DNA bands were purified with the QIAquick-PCR purification Kit (QIAGEN GmbH, Hilden, Germany) according to manufacturer's instructions. DNA sequencing was performed by the sequencing service of the Institute for Genetics, Department Biology I (Ludwig-Maximilians-Universität, München), with BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1 (Applied Biosystems, Foster City, California). Sequencing was performed on 1 µm DNA probes plus 0.3 µm ITS1F (forward) and 0.3 µm ITS4B (reverse). Four sequences of the new species are deposited in GenBank NCBI with accession Nos. EF507253, EF507254, EF507255 and EF507256.

Phylogenetic analysis.—The contiguous nucleotide sequences were edited with BioEdit v7.0.5 (Hall 2005). The sequences were submitted to BLAST and/or FASTA against nuclear ribosomal fungal sequence databases of UNITE (Kõljalg et al 2005) and of the National Centre for Biotechnology Information (NCBI) to test to what extent they match with ITS rDNA sequences of existing thelephoroid fungi. All selected sequences fall within the genus *Tomentella*. Sequences showing high identities score after BLAST were downloaded. Additional sequences published by Yorou et al (2007) and Kõljalg et al (2000, 2001) also were checked and added to the dataset. Alignment was performed with Clustal W Multiple (BioEdit v7.0.5) alignment and manually improved. Identity/similarity of sequences of closest species was calculated with the PAIRWISE ALIGNMENT option of BioEdit v7.0.5, after sequences were aligned and ambiguous regions deleted. Phylogenetic analyses were performed with PAUP version 4.0b10 (Swofford 2002). For maximum parsimony (MP) analysis we used the heuristic search option; starting tree(s) obtained via stepwise addition, 10 replications of random-taxon entry and tree bisection reconnection (TBR) swapping were selected. Gaps were treated as missing values. MASTREES reset to 10 000 and MULTREES option effective, steepest descent option not in effect, zero length branches collapsed. A neighbor joining (NJ) analysis was performed with the Kumira 2-parameter model (Kimura 1980). Bootstrap analysis was performed with 500 replicates under the heuristic search (Felsenstein 1985). All characters were assessed as independent, unordered and of equal weight with Fitch parsimony (Fitch 1971).

RESULTS

Both BLASTn and FASTA highlighted the placement of all African sequences within the genus *Tomentella*. With BLAST sequences of all four specimens assigned to *T. africana* showed great similarities with uncul-

TABLE I. Pairwise base differences (%) within specimens of *T. africana* and among *T. africana*, *T. umbrinospora* and *T. lateritia*

	T. africana (EF507254)	*T. africana* (EF507256)	*T. africana* (EF507255)	*T. umbrinospora* (AF272920)	*T. lateritia* (AF272926)
T. africana (EF507253)	1.7	2.2	1.6	12.9	11.5
T. africana (EF507254)		0.5	0.0	12.1	11.2
T. africana (EF507256)			0.5	12.3	11.3
T. africana (EF507255)				12.1	11.2

tured ectomycorrhizae or unknown *Tomentella* sequences (data not shown). Sequence identities with best matches are 89–92%. However the closest known species is *T. lateritia* Patouillard. *T. africana* deviates from *T. lateritia* by 11.2–11.5% in regard to the ITS rDNA sequences. Genetic distance between all four specimens assigned to *T. africana* is low, at 0.0–2.2% (TABLE I).

After exclusion of unknown EcM sequences the final dataset included a total of 40 *Tomentella* sequences with an alignment length of 607 characters (including gaps). With parsimony analysis, 336 characters were constant and 202 parsimony informative. The heuristic searches uncovered 201 most parsimonious trees of equal length 828 steps; with consistency, retention, homoplasy and rescaled consistency of 0.459; 0.629; 0.541 and 0.289 respectively. Both parsimony and neighbor joining analyses generated trees with similar topology, except that by neighbor joining *T. lateritia* clustered together with *T. africana* with a bootstrap support lower than 50%. In all generated trees all four specimens assigned to *T. africana* cluster together in a monophyletic group with a strong bootstrap support of 100% (FIG. 1). In all analyses *Tomentella umbrinospora*, a species morphologically close to *T. africana*, falls within a different clade. Genetic distance between *T. africana* and *T. umbrinospora* were 12.1–12.9% according to the ITS rDNA sequences.

TAXONOMY

Tomentella africana Yorou & Agerer, sp. nov. FIGS. 2–10

Basidiocarpis resupinatis, separabilibus, arachnoideis, continuis. Hymenio atro-brunneo usque ad castaneo, granuloso vel cavernoso, ubi vivo guttulis rufo-brunneis, subiculo flavo; marginibus indeterminatis. Rhizomorphae in subiculo et in margine, frequentes, 0.3–0.8 mm diam, flavo-brunneae, monomiticae, rhizomorphae tenuae laeves, rhizomorphae crassae (crassiores quam 20 µm), flavae vel flavo-brunneae, semper hyphis in superficie tenuis, 0.5–1.5(–2) µm diam, frequenter irregulariter ramificatis, defibulatis, hyphae internae fibuligerae, multis septis simplicibus in distantiis brevibus, hyphae sub superficio rhizomorpharum 1–2.5 µm diam, hyphae in centro rhizomorpharum 2.5–6(–7) µm diam, flavidae usque ad flavobrunneae non amyloideae. Hyphae subiculi fibuligerae, septis simplicibus vulgaribus, flavidae usque ad flavae hyphis dissimile diametro presentibus, hyphae tenuitunicatae 2.5–4.5(–5) µm diam et hyphae subcrassitunicatae, 1–1.5(2) µm diam. Hyphae subhymenii fibuligerae, septa simplicia absentia, 3.5–6(–7) µm diam, tenuitunicatae, flavidae, non amyloideae. Cystidia absentia. Basidia (30–)35–58(–60) µm longa, 7.5–10 µm diametro, base 6–8 µm, basibus semper fibuligeris, clavata, non stipitata, rare sinuosa, septa transversa deficientia, 4-sterigmatica, incolorata usque ad flavida, cyanophila et congophila. Basidiosporae 7.5–9(–9.5) × 7.5–8.5(–9.5) µm in aspectu frontali, 7.5–9(–9.5) × 7.5–8(–9) µm in aspectu laterali, irregulariter formatae, interdum subglobosae et triangulare lobatae in aspectu frontali, ellipsoideae in aspectu laterali, flavidae usque ad subbrunneae in 2.5% KOH et in aqua, echinulatae, aculeis densis, 0.5–1.5 µm altis, guttulis infreqeuntibus, nec cyanophilae, nec congophilae, nec amyloideae.

Chlamydosporae absentes.

Holotypus. SYN 890 (M), GenBank NCBI, accession No. EF507256

Basidiocarp resupinate, separable from the substrate, arachnoid, continuous, 0.5–1.5 mm thick. Hymenophore dark-brown (6E7) to chestnut (6F7) in mature basidiocarps, granulose to cavernous, with red brown drops in fresh condition, subiculum yellowish, paler than hymenophore, sterile margin mostly indeterminate.

Rhizomorphs present in subiculum and at the margins, abundant, thick and sometimes visible with the naked eye, 0.3–0.8 mm thick, compact (FIG. 2), yellow-brown under a dissection microscope, yellow to pale brown in water and in 2.5% KOH, monomitic, of type C (Agerer 1999, Agerer 1987–2006); young rhizomorphs (thinner than 20 µm) smooth (FIG. 3), colorless to light yellow (in water and in 2.5% KOH), old rhizomorphs (thicker than 20 µm) yellow to yellow brown (in water and in 2.5% KOH), covered by dense and irregularly shaped thin hyphae (FIG. 4); superficial thin hyphae 0.5–1.5(–2) µm diam (FIG. 5), emerging from generative hyphae (FIG. 6), frequently branched, simple septate, entwined, plectenchyma-

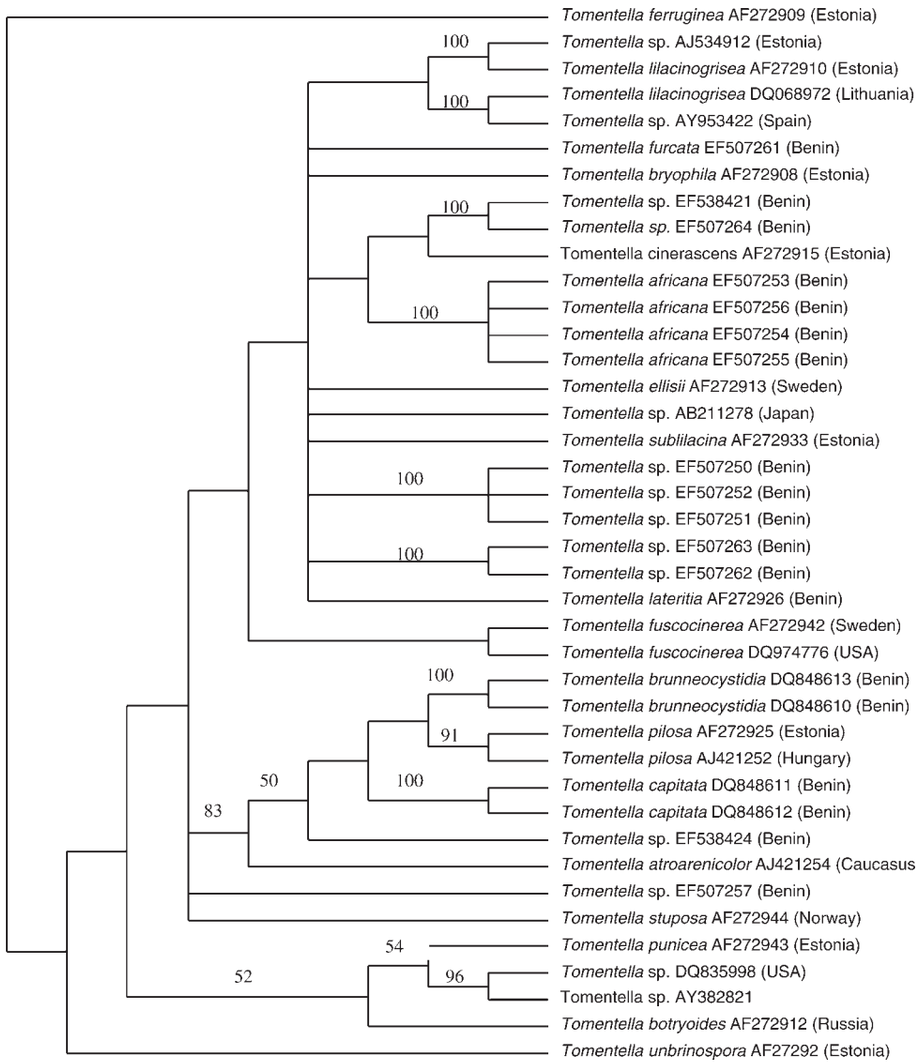

FIG. 1. Strict consensus of 201 most parsimonious trees generated from heuristic searches in PAUP 4b10 based on ITS rDNA sequences of 40 *Tomentella* taxa (tree length = 848 steps, CI = 0.459; RI = 0.629; HI = 0.541 and RC = 0.289). Bootstrap values from 500 replicates greater than 50% are given above branches. GenBank accession numbers as well as the origin of vouchers are given after speciesô names.

tous in surface, sometimes growing along and around generative hyphae; internal hyphae (hyphae below surface and central hyphae) clamped, simple septa common (FIG. 7) and occurring in short intervals (5–10 µm), thin- to thick-walled (0.2–0.5 µm), slightly yellow to yellow-brown (in water and in 2.5% KOH), not congophilous, not cyanophilous, not amyloid, hyphae below surface 1–2.5 µm, central hyphae wider 2.5–6(7) µm.

Subicular hyphae usually clamped, simple septa common, light yellow to yellow (in water and 2.5% KOH), of two size ranges (FIG. 8), some with 1–1.5(–2) µm diam, thin-walled, others with 2.5–4.5(–5) µm diam, thin- to thick-walled (0.2–0.5 µm), rarely with brown drops (in water and 2.5% KOH) on their surface, neither congophilous, nor cyanophilous, nor amyloid.

Subhymenial hyphae always clamped, simple septa absent, 3.5–6(7) µm wide, thin-walled (0.2 µm), yellowish (in water and in 2.5% KOH), slightly congophilous and slightly cyanophilous, not amyloid.

Cystidia absent.

Basidia (30–)35–58(–60) µm long, 7.5–10 µm wide at apex and 4–6.5 µm wide at base, consistently clamped at base, clavate, not stalked, rarely sinuous, transverse septa absent, colorless to light yellow (in water and in 2.5% KOH), slightly congophilous and slightly cyanophilous, not amyloid, sometimes with

FIG. 2. Old rhizomorph of *Tomentella africana* (from SYN 991). Bar = 2 μm.

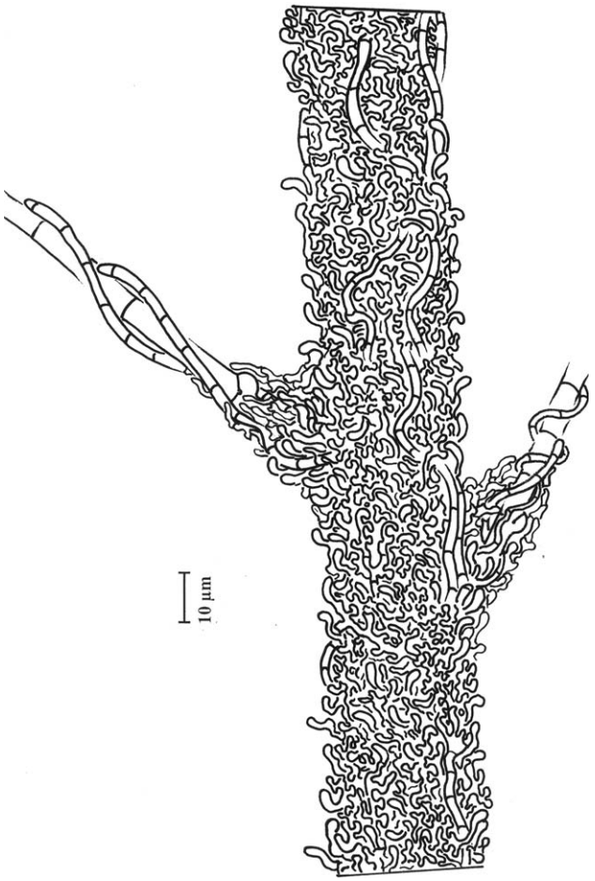

FIG. 4. *Tomentela africana* (from SYN 890). Surface view of a thick rhizomorph showing dense, multiply branched, irregularly shaped, thin hyphae and conical structures at the point of ramification.

granular content then neither congophilous nor cyanophilous, 4-sterigmate, sterigmata 7–9 μm long and 2–2.5 μm wide at base. Basidiospores 7.5–9(–9.5) × 7.5–8.5(–9) μm in frontal face and 7.5–9(9.5) × 7.5–8(9) μm in lateral face, with irregular shape in frontal view, sometimes subglobose, sometimes triangular to lobed (FIG. 9), ellipsoid in lateral view (FIG. 10), light yellow to pale brown (in water and in 2.5% KOH), echinulate, aculei dense, irregular in size (0.5–1,5 μm), oil drops infrequent, not congophilous, not cyanophilous, not amyloid.

Chlamydospores absent.

Type material. Benin, central part, Borgou province,

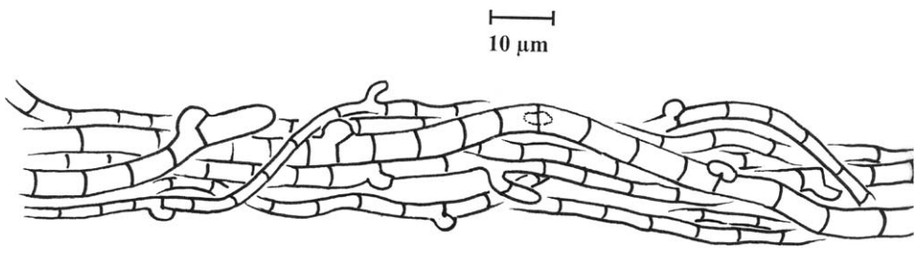

FIG. 3. *Tomentella africana* (from SYN 890). Thin rhizomorph from the margin of basidiocarp.

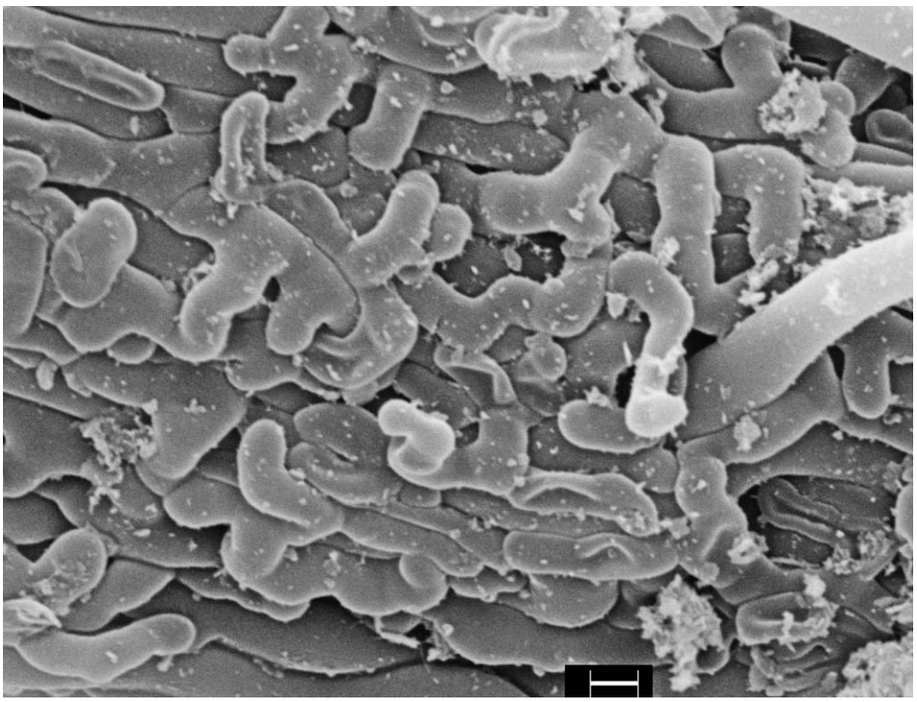

FIG. 5. *Tomentela africana* (from SYN 991). Details of irregularly shaped surface thin hyphae showing a plectenchymatous arrangement. Bar = 1 μm.

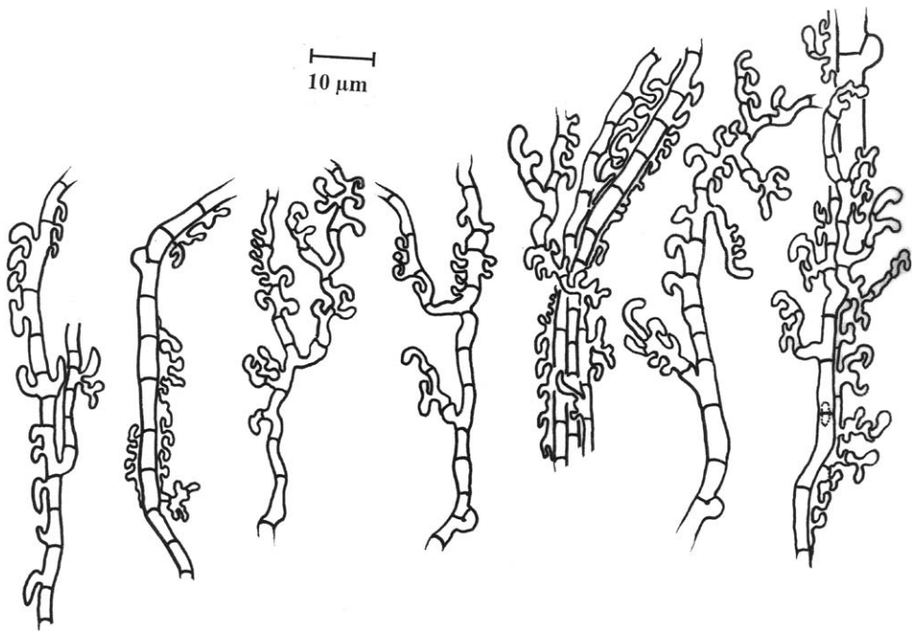

FIG. 6. *Tomentella africana* (from SYN 945). Surface hyphae of rhizomorphs showing the connections between the thin surface hyphae and hyphae growing below them in the rhizomorphs outer parts.

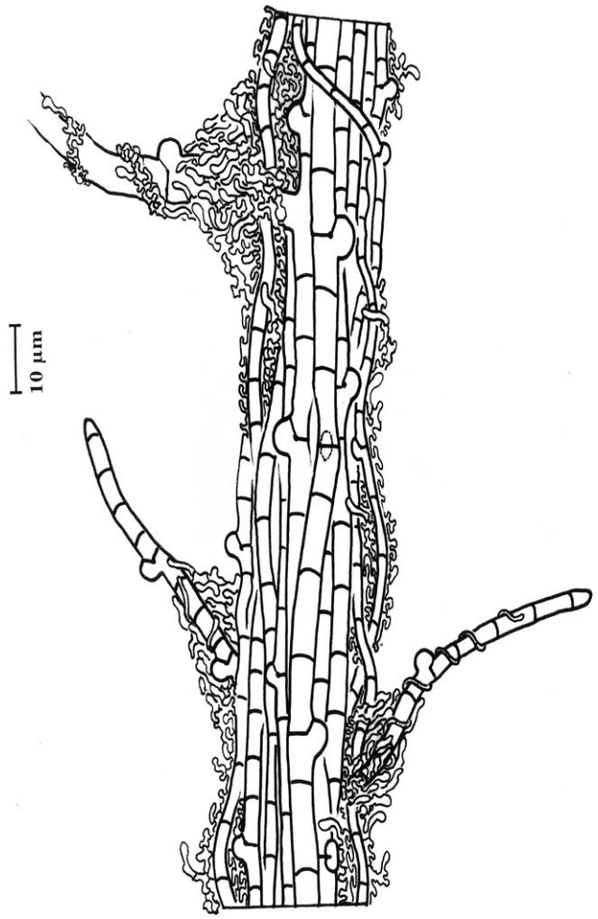

FIG. 7. *Tomentella africana* (from SYN 1007). Optical section through a thicker rhizomorph showing superficial thin hyphae, below surface hyphae and central wider hyphae.

forest reserve of Wari-Maro, Wari-Maro region, 08°12′25.6″N, 002°47′31.8″E, on the undersides of dead bark, 06 Aug 2005, leg. NS Yorou, SYN 890 (M), Holotype in M, isotype in TU. GenBank NCBI, accession No. EF507256.

Additional material studied: Benin, Borgou province, Sinendé region, forest close to Fô-Bouko village, 10°8′46.6″N, 002°15′6.0″E, on logs, 22 Aug 2003, leg. R. Agerer, RA 13780 (M); RA 13802 (M). Benin, Borgou province, reserved forest of Wari-Maro, Agbassa region, 08°55′44.5″N, 002°20′45.1″E, on logs, 23 Aug 2003, leg. R. Agerer, RA 13831 (M); on burned bark and logs, 16 Jun 2004, leg. NS Yorou, SYN 655 (M). Benin, Borgou province, reserved forest of Wari-Maro, Wari-Maro region, 09°00′47.1″N, 002°01′36.9″E, on soil, leaf litter, logs and dead bark, 05 Aug 2005, leg. NS Yorou, SYN 840 (M); SYN 843 (M); SYN 866 (M); SYN 871 (M); Wari-Maro region, 08°12′25.6″N, 002°47′31.8″, on soil, leaf litter, logs and dead bark, 06 Aug 2005, leg. NS Yorou, SYN 882 (M); SYN 888 (M); SYN 891 (M); 18 Aug 2006, leg. NS Yorou, SYN 945 (M), GenBank NCBI, accession No. EF507253. Benin, Atacora province, forest reserve of Alibori supérieur, Ouassa Pehunco region, 10°08′14.67″N, 2°19′31.70″E, on soil, under leaf litter, 20 Aug 2006, leg. NS Yorou SYN 991 (M) GenBank NCBI, accession No. EF507254; SYN 1007 (M), GenBank NCBI, accession No. EF507255.

Habitat and ecology. Basidiocarps occur in abundance as continuous but thick films on the soil surface, just under leaf litter of native trees or on the undersides of dead and/or partly burned barks and logs. *Tomentella africana* is common in woodlands dominated by *Isoberlinia doka* Craib & Stapf and *Isoberlinia. tomentosa* (Harms) Craib & Stapf (*Ceasal-Ceasalpiniaceae*).

Etymology. The epithet refers to the origin of the holotype and the commonness of the species in woodlands and savannahs of Benin (West Africa).

Tomentella umbrinospora M. J. Larsen FIGS. 11–13

Holotype. USA, New York, Greenbush, herb. NYS 2648.

Basidiocarp resupinate, separable from the substrate, arachnoid, continuous, 0.5–1 mm thick. Hymenophore brown (6D3-6E3), dark-brown (6E7) to chestnut (6F7) in mature basidiocarps, granulose, subiculum yellowish, paler than hymenophore, sterile margin byssoid, concolorous with subiculum.

Rhizomorphs present in subiculum and at the margins, thick, visible already at 10× under dissection microscope, yellow to light brown (in water and 2.5% KOH), dimitic, of type C, always covered by skeletal hyphae (FIG. 11), skeletal hyphae abundant, simple septa common, 0.5–1.5(–2) µm, colorless to light yellow (in water and 2.5% KOH), not congophilous, neither cyanophilous nor amyloid; hyphae below surface clamped, simple septa present (FIG. 12), thin-walled, colorless (in water and 2.5% KOH), congophilous, slightly cyanophilous, not amyloid; adjoining inner hyphae 2–4.5 µm, thin-walled (0.3 µm), sometimes strongly encrusted, encrustation observable in water and in cotton blue, rapidly and completely dissolving in 2.5% KOH and partly in Congo red; central hyphae 6–8(–9) µm. Subicular hyphae clamped, simple septa rare, 2.5–4(–5) µm diam, light yellow to pale brown (in water and 2.5% KOH), thin-walled (0.2–0.3 µm), strongly encrusted (observable in water and in cotton blue), encrustation rapidly and completely dissolving in 2.5% KOH leading to yellow-green to yellow-brown solution that is observable with the naked eye, partly dissolving in Congo red, skeletal hyphae sometimes present in the subiculum, 0.5–1.5(–2) µm diam, subicular hyphae colourless to light yellow, slightly congophilous, slightly cyanophilous, not amyloid.

Subhymenial hyphae clamped; 2.5–4(–6) µm diam, simple septa absent, thin-walled (0.3 µm), strongly encrusted (in water and in Congo red), encrustation

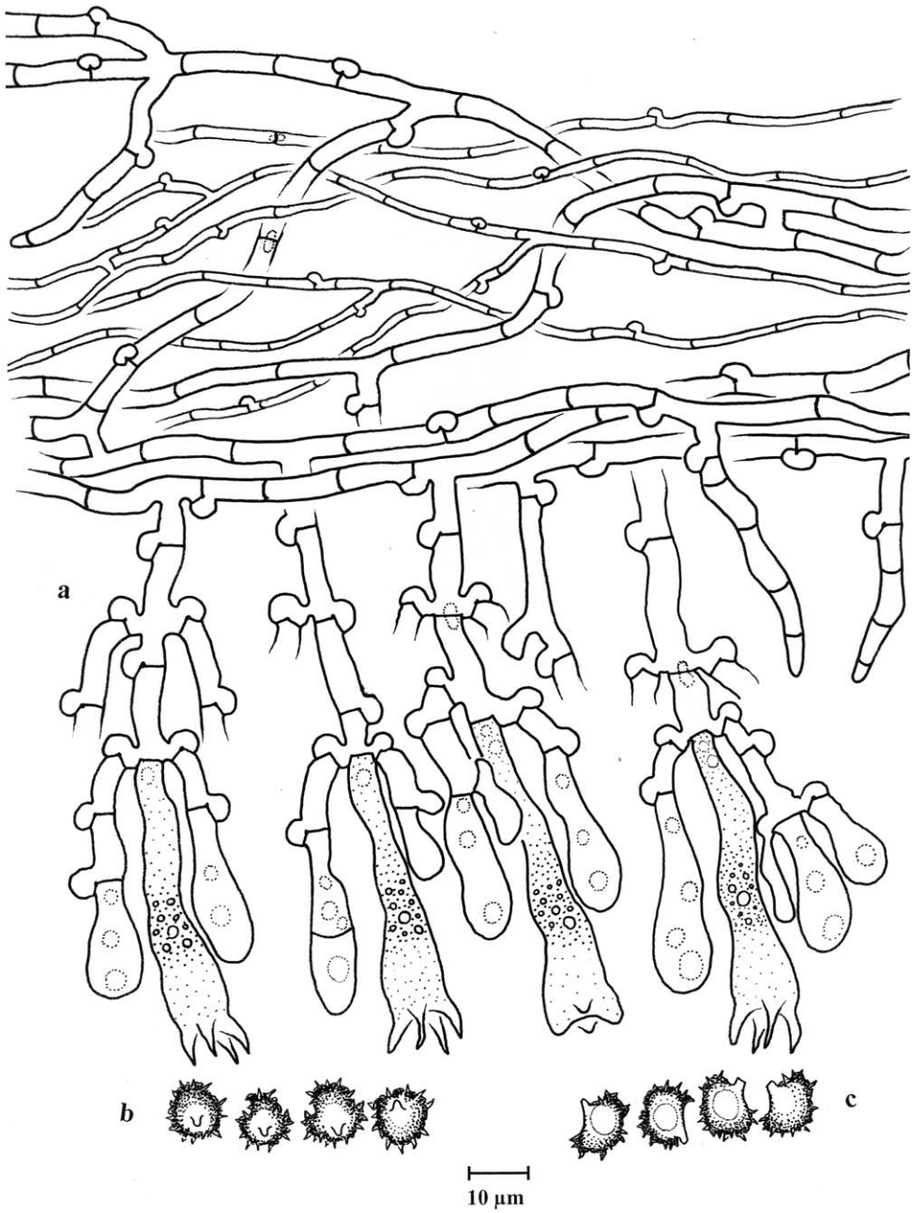

FIG. 8. *Tomentella africana* (from SYN 890). a. Section through the basidiocarp. b. Basidiospores in frontal view. c. Basidiospores in lateral view.

rapidly dissolving in 2.5% KOH, colorless to light yellow (in water and in 2.5% KOH), slightly congophilous and slightly cyanophilous, not amyloid.

Cystidia absent.

Basidia (35–)40–60(–65) µm long, (3.5)4–7(9) µm wide at apex and 3–4 µm wide at base, clamped at base, narrow clavate to clavate (FIG. 13), not stalked, sometimes sinuous, sometimes with transverse septa, colorless to light yellow, sometimes ochraceous (in water and in 2.5% KOH) with granular contents, slightly congophilous, not cyanophilous, not amyloid, 4-sterigmate, sterigmata 4–6 µm long and 1–1.5 µm wide at base, sterigmata rarely with transverse septa.

Basidiospores (5.5–)6–7.5(–8.5) × (5.5–)6–7(–8) µm in frontal face and 6–7.5(–8) × 5.5–7(–8) µm in lateral face, with irregular shape in frontal view, sometimes subglobose to triangular, rarely lobed; ellipsoid in lateral view, thin to thick-walled (0.5 µm), light yellow to pale brown (in water and 2.5% KOH), echinulate, aculei short, 0.1–0.8(–1) µm, oil drops infrequent, not congophilous, not cyanophilous, not amyloid.

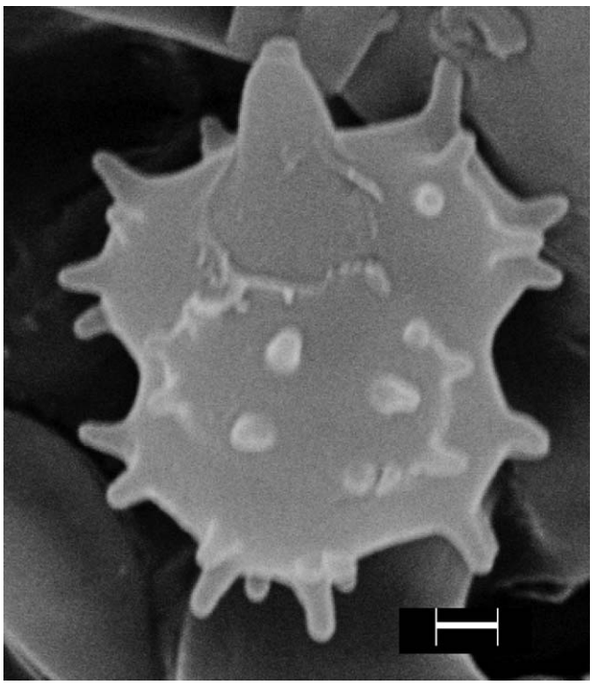

FIG. 9. *Tomentela africana* (from SYN 890). Basidopsores in frontal view. Bar = 1 μm.

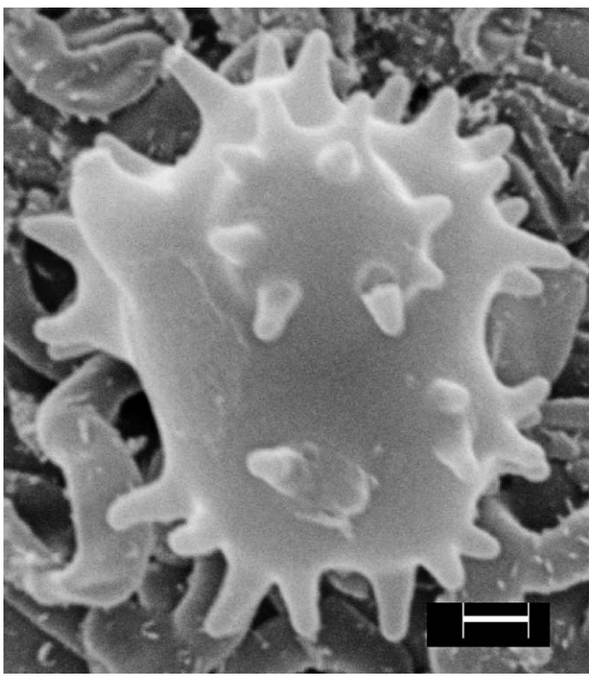

FIG. 10. *Tomentela africana* (from SYN 890). Basidiospores in lateral view. Bar = 1 μm.

Chlamydospores absent.

Material studied. Holotype of *T. umbrinospora*, received from USA, New York, Greenbush, on hardwood, leg. C.H. Peck, herb. 2648 (NYS). (Protolog of *T. umbrinospora*: Forestry Syracuse Univ., Tech. Publ. 93:61. 1968).

DISCUSSION

Various studies in some ectomycorrhizal communities used a pairwise base difference >3% as species limit (Tedersoo et al 2003, O'Brien et al 2005). Pairwise base differences among *T. africana*, *T. umbrinospora* and *T. lateritia*, coupled with differential anatomorphological characteristics (see below) strongly suggest *T. africana* as a new separate species. *T. africana* has many macroscopic and microscopic similarities to *T. umbrinospora*. Macroscopic similarities include the thickness of the basidiocarp (0.5–1.5 mm for *T. africana* and 0.5–1 mm for *T. umbrinospora*), the yellow-brown thick rhizomorphs, the yellowish subiculum and the dark-brown to chestnut hymenium. A common microscopic feature between both species is the basidisopores with irregular shape in the frontal view. Basidiospores of *T. umbrinospora* however are smaller, with shorter aculei than those of *T. africana*. Furthermore basidia of *T. umbrinospora* are narrower than those of *T. africana*. Transverse septa are present sometimes in basidia and sterigmata of *T. umbrinospora*. An interesting feature that has been reported only recently within resupinate thelephoroid fungi is the presence of irregularly shaped thin hyphae on the rhizomorph surface of some species (Yorou et al 2007, Raidl 1997, Raidl and Müller 1996, Jakucs and Agerer 1999, 2001). The ontogeny of such thin surface hyphae has been addressed by Raidl (1997). In this paper, we address the difference between such thin hyphae and skeletal ones.

Skeletal hyphae are described as regularly cylindrical, long but often sinuous and rarely septate thick-walled hyphae (Hartig 1885, Falck 1912, Corner 1932). Clemençon (1997) supplemented the description of skeletals by mentioning the scarcity of branches and incrustation. Skeletal hyphae are reported to be nonamyloid, noncyanophilous but slightly congophilous (Clémençon 1997). Simple septa like those in generative hyphae lack. However adventitious or false septa resulting from dried protoplasm (Ryvarden and Johansen 1980) can be frequent exclusively at the tips of hyphae (Clémençon 1997). Skeletal hyphae are common within *Polyporaceae* where they play a key role in the delimitation of genera and species (Ryvarden 1978, 1991, Ryvarden and Gilbertson 1987, 1993, 1994, Ryvarden and Johansen 1980). They also have been reported in few corticioid genera such as *Amylostereum* Boidin,

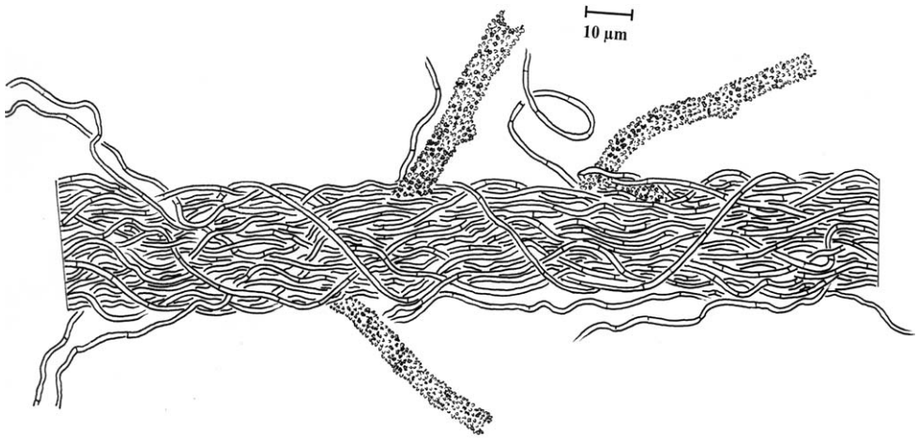

FIG. 11. *Tomentela umbrinospora* (from the holotype). Surface view of a thick rhizomorph showing skeletal hyphae.

Mycoaciella J. Eriksson & Ryvarden (Eriksson et al 1978), *Cystostereum* Pouzar, *Dacryobolus* Fries (Eriksson and Ryvarden 1975), *Pseudotomentella*, *Tomentella* Persoon ex Patrouillard and *Thelephora* Ehrhart ex Willdenow (Kõljalg 1996, Larsen 1968, 1974, Stalpers 1993) to name but a few. However, within thelephoroid fungi, thin, often only slightly thick-walled, multiseptate hyphae are designated skeletals. They often are associated with rhizomorphs. Their presence in the subiculum of tomentelloid fungi has been rarely reported. However, due to their multiseptate status and the mostly only slightly thick walls and their frequently present cytoplasm, it is questionable whether it is justified to designate these hyphae as skeletals in Falck's (1912) sense, Corner's (1932) definition and Clémençon's (1997) re-evaluation.

Dimitic rhizomorphs with so-called skeletal hyphae 1–2 µm wide have been reported for *Tomentella umbrinospora* (Melo et al 1998, Larsen 1974, Kõljalg 1996, Losi 1997). However Kõljalg (1996) stated that rhizomorphs collected below the subiculum may be momomitic, lacking thus skeletal hyphae, while Stalpers (1993) mentioned the scarcity of skeletal hyphae in this species. The holotype of *Tomentella umbrinospora* we examined presented dimitic rhizomorphs. Skeletal hyphae 0.5–1.5(–2) µm diam with numerous simple septa are common on the surface of all examined rhizomorphs of *Tomentella umbrinospora*. This is in accordance with the original descriptions of *T. umbrinospora* (Larsen 1968, 1974), which mentioned dimitic hyphal systems for this species. Unlike *T. umbrinospora* that presented a dimitic hyphal system, *T. africana* presented a monomitic hyphal system. Skeletal hyphae were not observed in the subiculum or on rhizomorphs. All subicular hyphae are clamped and present also numerous simple septa. The smaller subicular hyphae of *T. africana* resemble skeletal hyphae of *T. umbrinospora*.

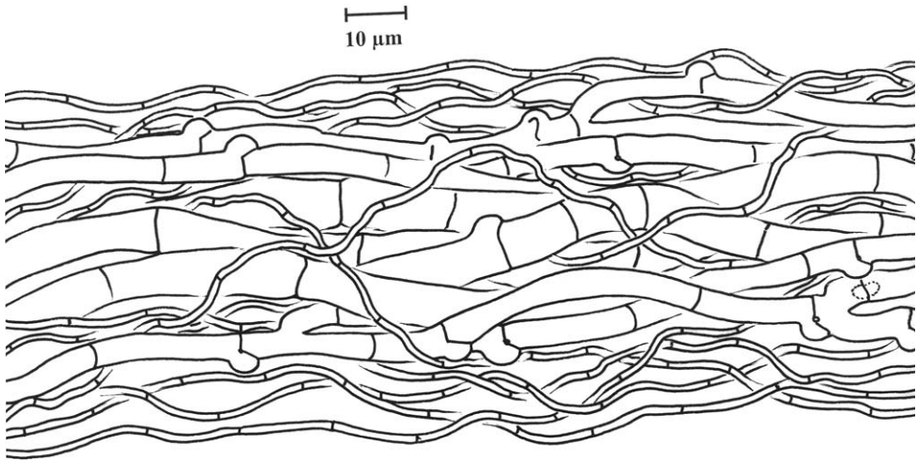

FIG. 12. *Tomentella umbrinospora* (from the holotype). Optical section through a thicker rhizomorph.

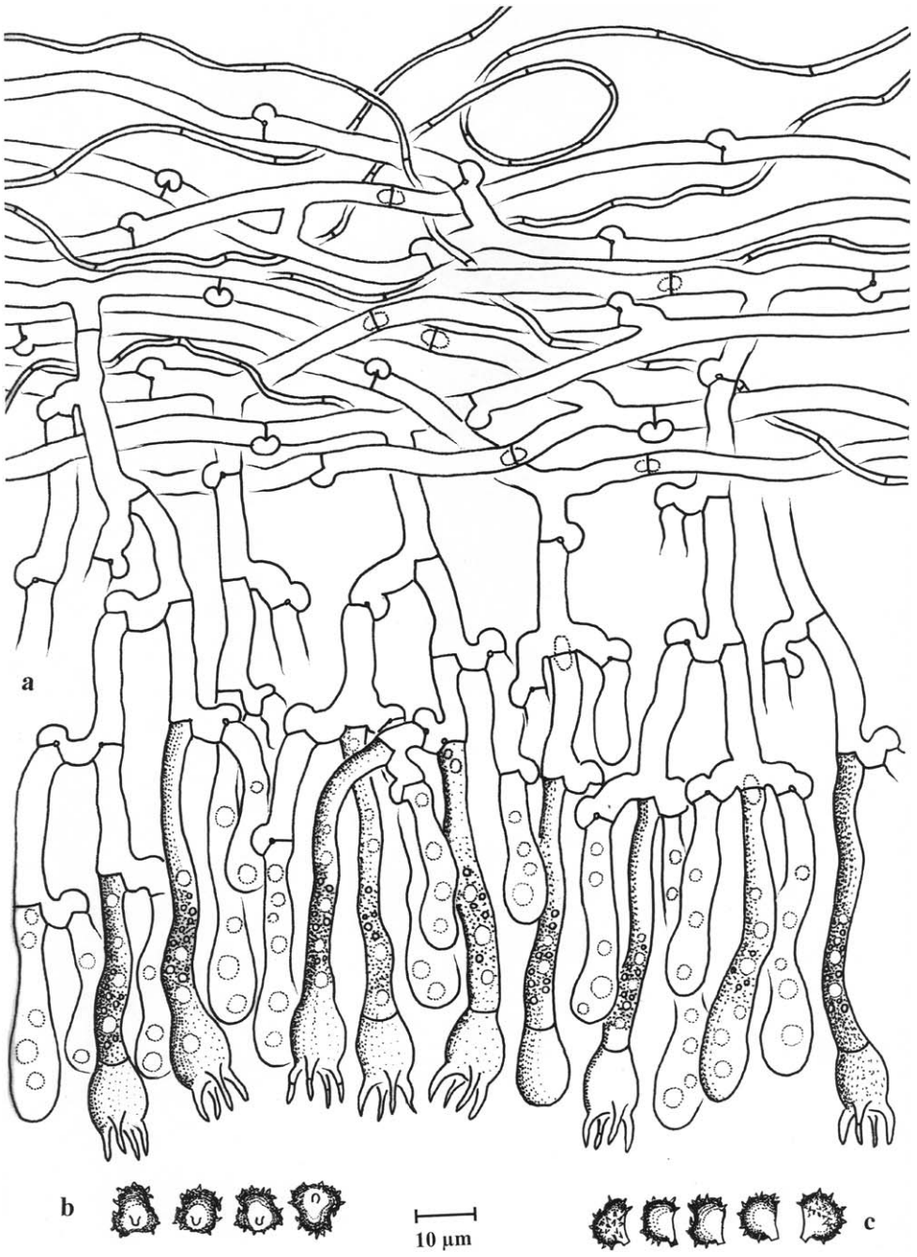

FIG. 13. *Tomentella umbrinospora* (from the holotype). a. Section through the basidiocarp. b. Basidiospore in frontal view. c. Basidiospores in lateral view.

However they differ considerably in the presence of clamps and therefore cannot be regarded as skeletals, particularly because they possess only thin walls. In later developmental stages all rhizomorphs of *T. africana* are covered by dense, irregularly shaped thin hyphae. Unlike so-called skeletal hyphae, the irregularly shaped thin hyphae are short, tortuous, multiply branched and intermingling, resulting in a dense plectenchymatous cover of the rhizomorph surface.

Many resupinate thelephoropid species we collected in Benin woodlands showed only small pieces of basidiocarps on fragmented substrate. This is partly due to the annual occurrence of bush fires in woodlands and savannahs of tropical Africa, which regularly burn substrates (litter, dead bark and logs), thus jeopardizing the development of basidiocarps of resupinate, lignicolous fungi. However one interesting ecological feature of *T. africana* is the frequency

of its basidiocarp in these areas. Basidiocarps of *T. africana* occur in abundance on soil mainly under *Isoberlinia doka* and *Isoberlinia tomentosa*, or on the underside of dead and/or burned bark and logs. *T. africana* is undoubtedly the most abundant resupinate thelephoroid fungus in woodlands and savannahs of the Soudanian Centre of Endemism. The absence of this species in dense semideciduous relic forests lacking ectomycorrhizal trees of southern Benin, coupled with its abundance in woodlands and seasonal forests of central to north Benin, suggests that it is an ectomycorrhizal former with native trees.

ACKNOWLEDGMENTS

We are much indebted to the German Academic Exchange Service (DAAD) for financial support (grant No. A/03/15106). The African Forests Research Network (AFORNET) through grant No. 02/2005 and the International Foundation for Science-IFS-(grant D/4033-1) jointly financed the collection trips and equipments. We also thank Dr Scott Kroken (associate editor) and Dr Clovis Douanla-Meli (University of Kassel, Germany) for their valuable advice with molecular studies. Drs Eva Facher and Thassilo Franke are much thanked for their technical help with SEM.

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IV

Nova Hedwigia **85**: 521-539

Nova Hedwigia **85** 3—4 521—539 Stuttgart, November 2007

Type studies of three tomentelloid species (Basidiomycota, Thelephorales): *Tomentella radiosa*, *Tomentella cinereoumbrina* and *Tomentella punicea*

by

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With 14 figures and 1 table

Yorou, N.S. & R. Agerer (2007): Type studies of three tomentelloid species (Basidiomycota, Thelephorales): *Tomentella radiosa*, *Tomentella cinereoumbrina* and *Tomentella punicea.* Nova Hedwigia 85: 521-539.

Abstract: This paper presents additional anatomical descriptions of *T. radiosa*, *T. cenereoumbrina* and *T. punicea* based on their holotype. For each species, original iconographies and anatomical features are presented and discussed in details. Similarities and differences between closest species are highlighted. ITS rDNA sequences´ similarities and phylogenetic relationship between *T. radiosa* and *Thelephora terrestris* are reported. This paper confirms the presence of skeletals and binding-like hyphae on the rhizomorphs of *T. punicea*.

Key words: Anatomy, rhizomorphal structures, *Tomentella radiosa*, *T. cinereoumbrina*, *T. punicea.*

Introduction

Many species of resupinate Thelephorales have been considered as conspecific, resulting in the adoption of a wide species concept, at least for some species (e.g., *T. radiosa* (P.Karst.) Rick in Kõljalg 1996), whilst on the over hand same species has been repeatedly described as new, resulting in widespread synonymy (comp. Larsen 1965, 1966, 1968, 1970, 1974, Kõljalg 1996, Wakefield 1960). To avoid such confusion, it is advisable to retrieve detailed informative anatomical data from type specimens. Such comparative analyses have already helped discriminate

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DOI: 10.1127/0029-5035/2007/0085-0521 0029-5035/07/0085-0521 $ 4.75

tomentelloid species previously considered as synonymous, as in the case of *Tomentella lilacinogrisea* Wakef. and *Tomentella neobourdotti* M.J.Larsen (Martini and Hentic 2005). Detailed anatomical descriptions highlighting notable features, coupled with reliable, informative and faithful illustrations are invaluable for the correct species delimitation. The tropical African thelephoroid fungi can be particularly difficult to identify due to a lack of easily available, scientifically reliable literature. Anatomical comparisons with type species have enabled the identification of new species (Yorou & Agerer 2007a,b). Herewith, we aim at providing additional anatomical characters of previously published descriptions and to highlight important discriminative features for each type.

Materials and methods

Measurements and drawings were made from microscopical preparations. Fine sections of fruit bodies were mounted in water and afterwards in 2.5% KOH, in Congo Red, in Cotton Blue and in Melzer's reagent (Kreisel & Schauer 1987), respectively. Microscopic studies were performed using a light microscope Leica DM LB2. Measurements were made at magnification X 1000 and did not include the apiculus, ornamentation of basidiospores and sterigmata. Line drawings were made at magnification X 1000 using a drawing tube. Colour codes of dried basidiocaps are given according to Kornerup and Wanscher (1978). Descriptions follow criteria compiled by Kõljalg (1996). Herbarium abbreviations follow Holmgren et al (1990). We refer to Yorou & Agerer (2007a, b) for protocols of molecular and phylogenetic studies.

Results

Tomentella radiosa (P.Karst.) Rick, Broteria 2 (Ser. 3): 79. 1934. Figs 1-5

BASIDIOCARP resupinate, adherent to the substratum, pelliculose, continuous, 0.5- 0.8 mm thick. Hymenophore crustose to granulose, brown (6E5), continuous, subiculum pale to yellow brown, sterile margin determinate, fimbriate, consisting of agglutinated parallel hyphae, whitish to concolorous with subiculum.

RHIZOMORPHS present in subiculum, common at the margins, colourless, pale yellow to brown under dissection microscope, pale yellow to pale brown in water and in 2.5% KOH, 20-70 µm wide, undifferentiated, uniform loose (Agerer 1999), of type A (Agerer 1987-2006), margin smooth, individual hyphae uniform and loosely arranged (Fig. 1), 4-6(7) µm diameter, clamped, simple septa also present, rarely inflated, thin to thick-walled (0.5-1 µm), colourless, sometimes pale yellow to pale brown in water and in 2.5% KOH.

SUBICULAR HYPHAE broadly clamped, simple septa infrequent, clamps commonly thick-walled (2-3 µm), mostly with a large (1-2 µm diam.) hole, hyphae 4-7(8) µm wide, sometimes inflated, then up to 11 µm diam., exceptionally with a balloon-like inflation (20 µm) (Fig. 2), thick-walled (0.5-1.5 µm), walls light yellow, cross-shaped ramifications absent, anastomosis common (Fig. 3), elbow-like outgrowths or short side-branches common (Fig. 4). Subicular hyphae light brown to brown in water and in 2.5% KOH. Hyphae from the sterile margin loosely arranged and parallel, never isolated, clamped, simple septa common, thin-walled (0.2-0.6 µm), without

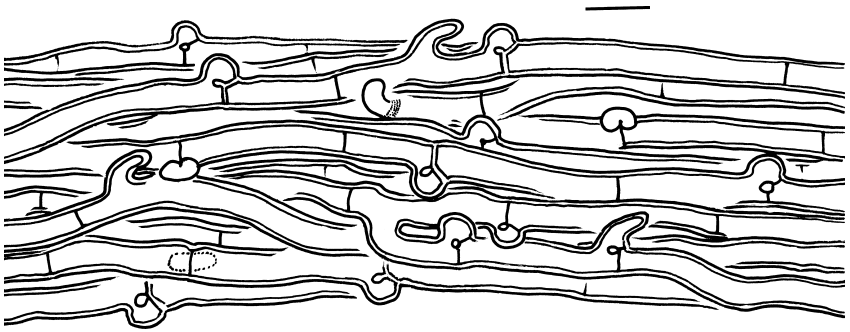

Fig. 1: *Tomentella radiosa*. Optical section through the rhizomorph (from the holotype). Scale bar = 10 µm.

encrustations, colourless to very pale yellow in water and in 2.5% KOH, congophilous, cyanophilous, rarely patchily amyloid especially at septa and tips of the hyphae.

SUBHYMENIAL HYPHAE clamped, 5-8 µm wide, often short and inflated (Fig. 5), thin to thick-walled (0.5-1 µm), always thick-walled at their base, colourless to pale brown in water and in 2.5% KOH, strongly congophilous, cyanophilous, not amyloid.

CYSTIDIA absent.

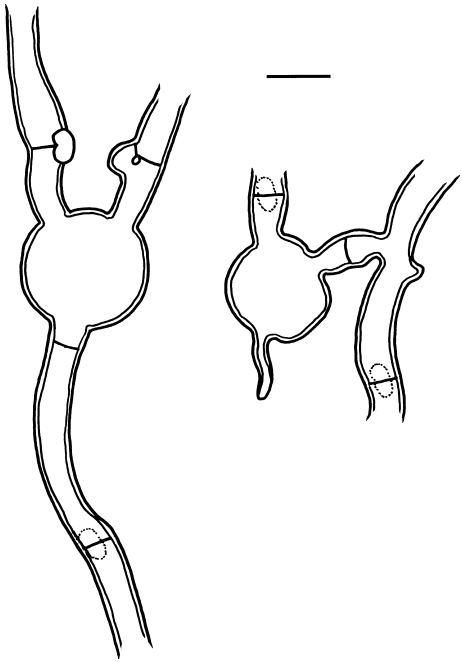

Fig. 2: *T. radiosa*. Optical section of subicular hyphae showing balloon-like inflation (from the holotype). Scale bar = 10 µm.

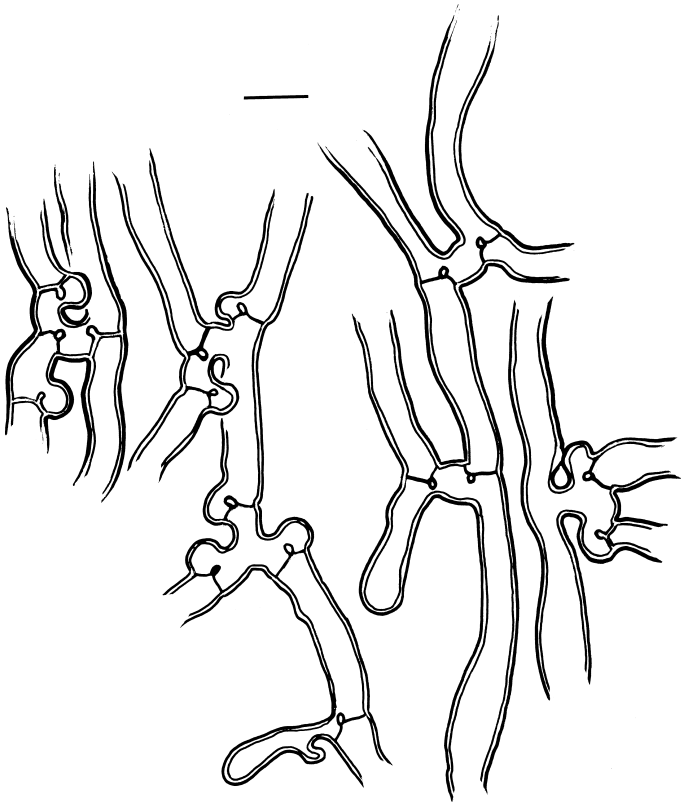

Fig. 3: *T. radiosa*. Optical section of subicular hyphae showing the H-like anastomosis (from the holotype). Scale bar = 10 µm.

Basidia 40-55 µm long, 8-10 µm at apex, 6-8 µm at base, clavate, clamped at base, not stalked, not sinuous, colourless to rarely light yellow in water and in 2.5% KOH, 4-sterigmate, sterigmata 4-7 µm long and 1-1.5 µm at base.

Basidiospores of two types, first type (6)6.5-7.5 × 6-8 µm in frontal face, 6-7.5 × 6-8 µm in lateral face, ellipsoid to lobed in frontal view, ellipsoid in lateral view, second type 7.5-11.5 (13) × 7-9 µm in frontal face, 7.5-10 (12) × 7.5-9 µm in lateral face, triangular in frontal view, ellipsoid to reniform in lateral view, echinulate, aculei very short (0.2-0.4 µm), dense and irregular, pale brown in water and in 2.5% KOH, slightly congophilous, cyanophilous, not amyloid.

Chlamydospores absent.

Material studied: Holotype: *Hypochnus fuscus* (Pers.: Fr.) P.Karst. var. *radiosus* P.Karst., Medd. Soc. Fauna Fl. Fenn. 9: 71. 1882: Finland, Helsingfors, on dead wood, W.Nylander, X 1858 (H).

Remarks: The presences of large, broadly clamped, thick-walled subicular hyphae with large holes at clamps and thickened septa, as well as triangular and reniform pale brown basidiospores with very short aculei, are typical anatomical features for

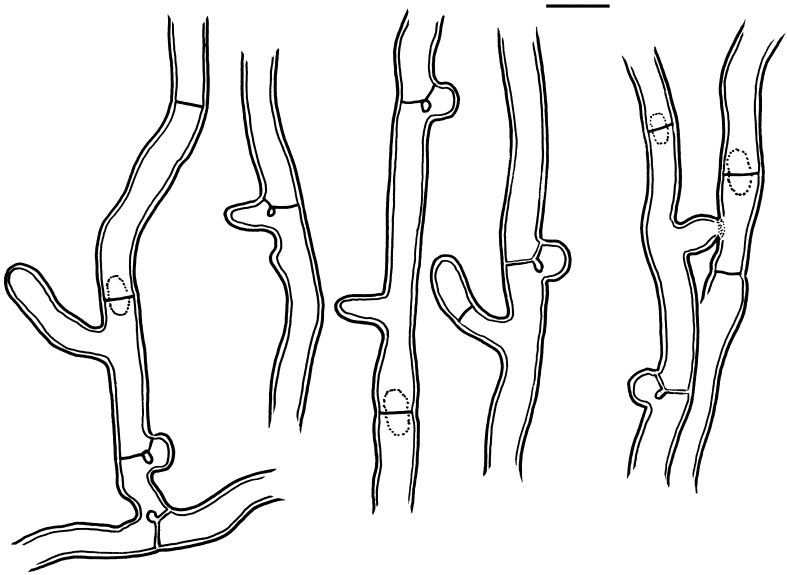

Fig. 4: *T. radiosa*. Optical section of subicular hyphae showing elbow-like outgrowths (from the holotype). Scale bar = 10 µm.

T. radiosa. Previous descriptions of *T. radiosa* (Agerer & Bougher 2001, Dämmrich 2006, Kõljalg 1996, Melo et al. 2003) already reported a wide variability of basidiospore shapes and size. *Tomentella ellisii* (Sacc.) Jülich & Stalpers present also somewhat reniform basidiospores in lateral view (Dämmrich 2006). Both species present basidiospores with size ranging between 6-13 µm (Dämmrich 2006, Kõljalg, 1996, Melo et al. 2003). Furthermore, *T. ellisii* reveals subicular and subhymenial hyphae with the same size and inflations as those of *T. radiosa*. However, *T. ellisii* is characterised by a cyanescent reaction of its basidia in KOH (a character that *T. radiosa* lacks) and the more prominent basidiospores ornaments of up to 1 µm long aculei.

The presence of elbow-like outgrowths of subicular hyphae and the rarely slightly amyloid hyphae in *T. radiosa* have been reported only recently by Agerer & Bougher (2001). Amyloidy is currently a rarely documented character within tomentelloid fungi. Amyloidy occurs patchily and only very slightly either on the subicular hyphae of a few species such as *Tomentella subamyloidea* Agerer and *T. radiosa* (Agerer & Bougher 2001), or on the apiculus of basidiospores of *Tomentella lapida* (Pers.) Stalpers and *Tomentella bryophila* (Pers.) M. J. Larsen (Dämmrich 2006, Yorou & Agerer 2007a).

According to Larsen (1974), *T. radiosa* presents two kinds of subicular hyphae. He reports that some hyphae are 3-5.5 µm in diameter, frequently clamped, thick-walled, and pale to medium brown, and that other hyphae are 5.5-7 µm in diameter, simple septate, mostly thin-walled, and pale yellowish to brown. The holotype we examined

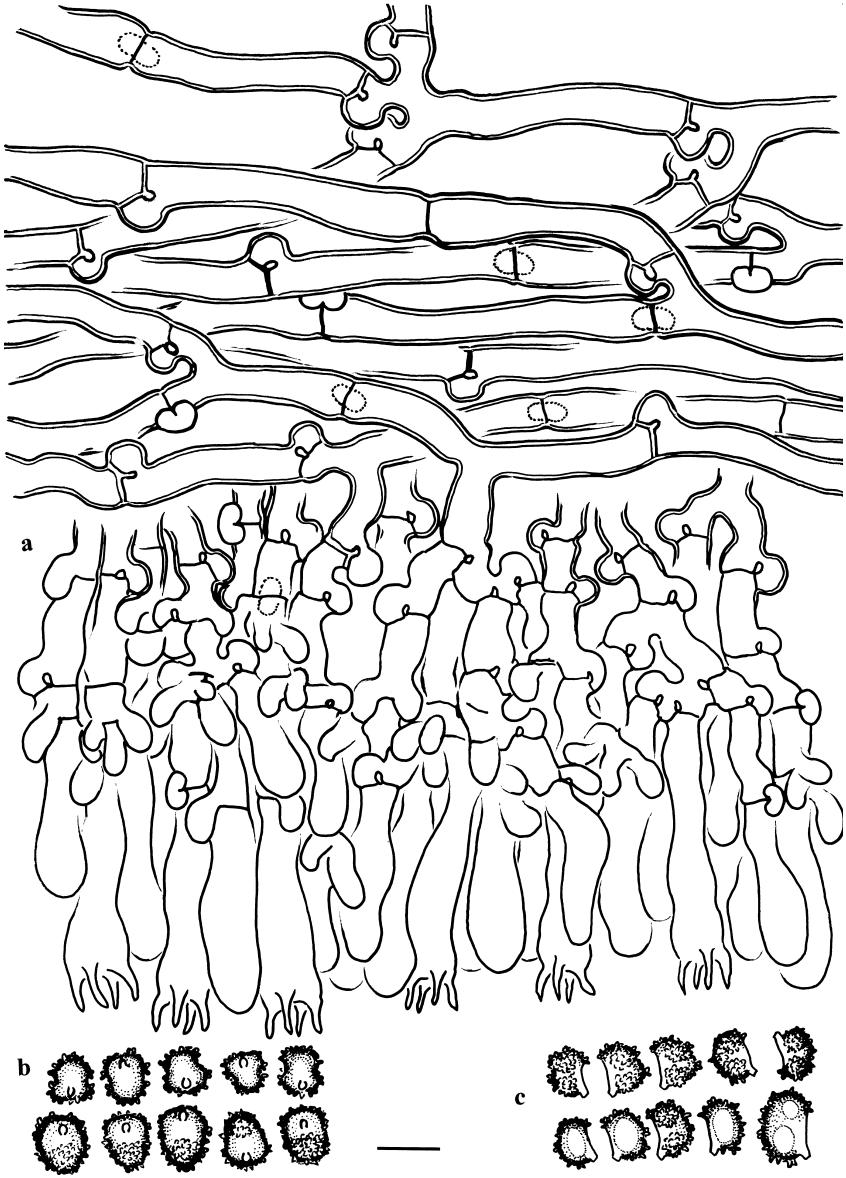

Fig. 5: *Tomentella radiosa.* a. Section thought the basidiocarp, b. Basidiospores in frontal view, c. Basidiospores in lateral view (from the holotype). Scale bar = 10 µm.

reveals clamped hyphae of similar size ranges without a distinct hiatus in dimensions. Differences are found only in the colour, thickness and the frequency of clamps, rather than their width. Subicular hyphae are thick-walled (0.5-1.5 µm), light brown to brown, regularly clamped, whilst hyphae from the sterile margin of fruit body are

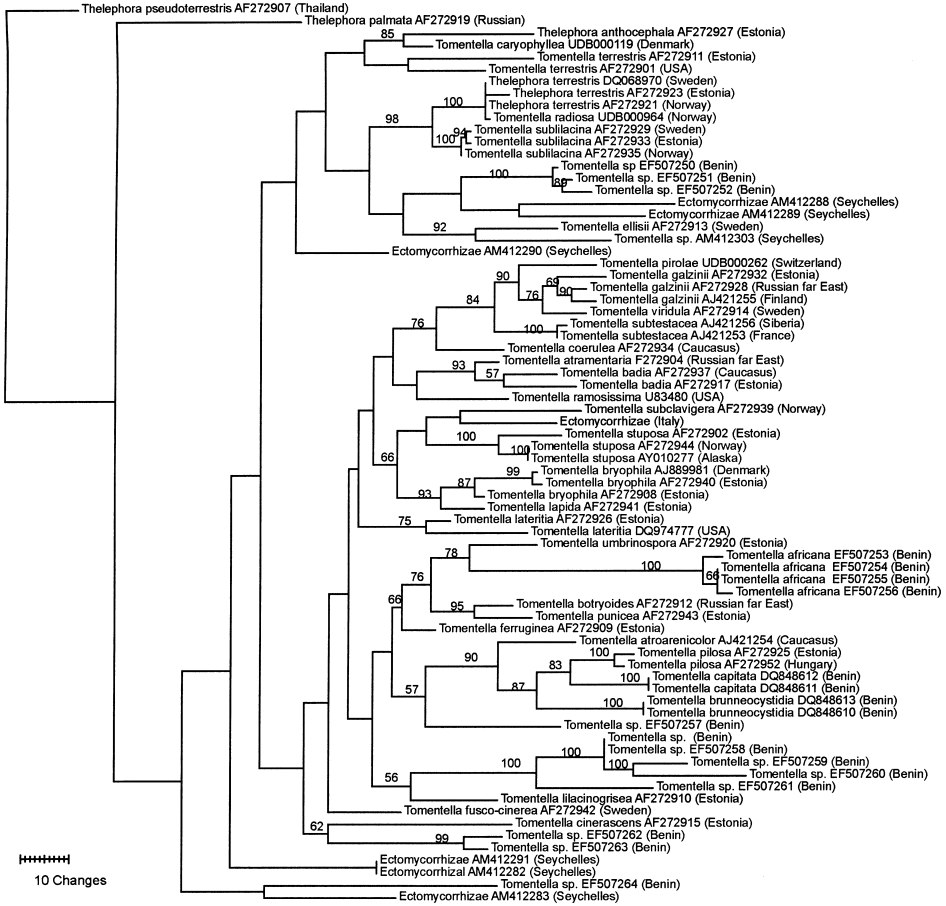

Fig. 6. One of the most parsimonious trees showing the grouping of *Tomentella radiosa* and *Thelephora terrestris* in the same clade. Bootstrap values are shown above branches. The Genbank (NCBI, EMBL or UNITE) codes as well as origin of the vouchers are indicated after species names.

rather thin-walled (0.2-0.6 µm), colourless to light yellow, clamped and simple septate. Clampless hyphae as mentioned by Larsen (1974) are absent.

Kõljalg (1996) adopted a broad species concept for *T. radiosa*, including specimens with quite resupinate fruit bodies and those with clavaroid processes at their margins. According to Kõljalg (1996), *T. radiosa*, *T. oligofibula* M.J.Larsen, *T. purpurea* Wakefield, and *T. carbonaria* M.J.Larsen are conspecific. He highlighted great similarities between *T. radiosa* and *Thelephora terrestris* Ehrhart, a species with pileate to sub-sessile, infundibuliform to imbricate fruit bodies (Corner 1968). Anatomical differences between *T. radiosa* and *Th. terrestris* refer to basidiospore shape and ornaments, and as a consequence, both species are considered by Kõljalg (1996) as different, thus applying a narrow species concept. In addition to basidiospores shape and ornaments, rhizomorphal structure shows important dissimilarities between both

Table 1. ITS rDNA sequences' similarities (%) between *Tomentella radiosa* and *Thelephora terrestris.*

	Th. terrestris (AF272923)	*Th. terrestris* (DQ068970)	*Th. terrestris* (AF272921)
T. radiosa (UDB000964)	98.91	99.82	99.82
Th. terrestris (AF272921)	99.1	100	

species. Like *T. radiosa*, *Th. terrestris* has undifferentiated rhizomorphs (Agerer 1988) with loosely arranged uniform hyphae. However, they differ from those of *T. radiosa* by having cystidia (Agerer & Weiß 1989). Contrary to Kõljalg (1996), Dämmrich (2006) adopted a broad species concept and suggested *T. radiosa* as the resupinate form of *Th. terrestris* (= *Thelephora terrestris* f. resupinata (Bourdot & Galzin) Donk). We didn't examine the holotype of *Th. terrestris*. However, in recent molecular investigations (Yorou & Agerer 2007a, b, see also Fig. 6), a specimen identified as *T. radiosa* presents almost identical sequences with those of *Th. terrestris*. Genetic distance between *T. radiosa* and *Th. terrestris* ranges from 0.18 to 1.09% regarding ITS rDNA sequences (Table 1). Phylogenetically, *T. radiosa* (accession number UDB000964, Genbank UNITE) clusters together with *Th. terrestris* s. str. (accession numbers AF272921, AF272923 and DQ068970, Genbank NCBI) with a very strong bootstrap support (100%). Based on cited specimens, molecular data support the synonymy suggested by Dämmrich (2006). However, to make reliable conclusions about taxonomical relationships between both species, molecular investigations should be undertaken using all specimens described as *T. radiosa* (Agerer & Bougher 2001, Dämmrich 2006, Kõljalg 1996, Melo et al, 2003, Larsen, 1965, 1974, Wakefield 1966, 1969) and compared to *Th. terrestris* s. str. It is likely that specimens described as *T. radiosa* encompass representatives of *Th. terrestris* f. resupinata (sensu Dämmrich 2006) and those of *T. radiosa* (sensu Kõljalg 1996).

Tomentella cinereoumbrina (Bres.) Stalpers, Stud. Mycol. 35: 96. 1993. Figs 7-8

Basionym: *Hypochnus cinnereoumbrina* Bres., Stud. Trent. (Ser. 2, Sci. Nat. Econom.) 7: 62. 1926.

Basidiocarp resupinate, adherent to the substratum, arachnoid to crustose, continuous, hymenophore grey to light brown (7D4), smooth to granular, sterile margin indeterminate.

Rhizomorphs absent.

Subicular hyphae mostly simple septate (Fig. 7), 3-6(6.5) µm, thick-walled (0.5-1 µm), walls yellowish, sometimes tortuous, lateral protuberances common, without encrustations, colourless to brown in water and in 2.5% KOH, rarely cyanescent, congophilous, cyanophilous, not amyloid.

Subhymenial hyphae simple septate, clamps present, more common than in subicular hyphae, 3-6 µm wide, thin-walled, without encrustations, colourless to light brown in water and in 2.5% KOH, sometimes cyanescent, congophilous, cyanophilous, not amyloid.

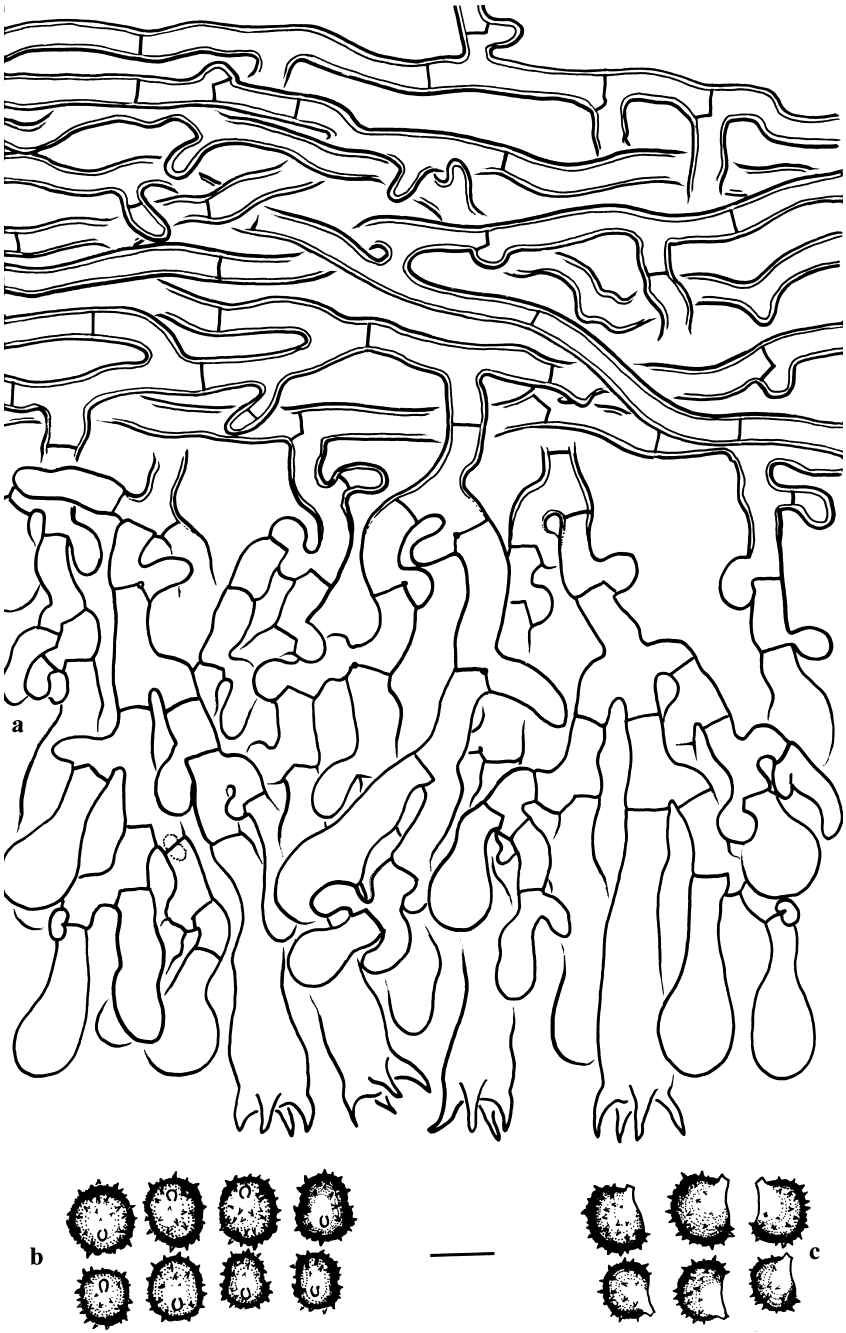

Fig. 7: *Tomentella cinereoumbrina*. a. Section through the basidiocarp; b. Basidiospores in frontal view; c. Basidiospores in lateral view (from the lectotype). Scale bar = 10 µm.

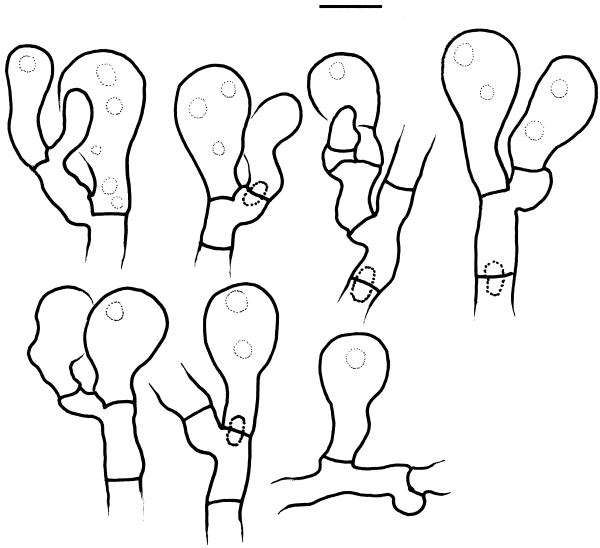

Fig. 8: *T. cinereoumbrina*. Young basidia showing distal apice (from the lectotype). Scale bar = 10 µm.

CYSTIDIA absent.

BASIDIA 35-70 µm long, 8-11(12) µm at apex, 4-8 µm at base, clamped at base, clavate to utriform, sinuous, not stalked, colourless to very light brown in water and in 2.5% KOH, sometimes cyanescent, young basidia with distinctive distal apex (Fig. 8), 4-sterigmate, sterigmata 6-10 × 2-4 µm, basidia congophilous, cyanophilous, not amyloid.

BASIDIOSPORES (8.5)9-11(12) × (7.5)8-9(11) µm in frontal face, (7.5)8-10(11) × (7)8-9 µm in lateral face, irregularly ellipsoid to broadly ellipsoid in frontal view, broadly ellipsoid in lateral view, echinulate, aculei dense and very short, 0.4-1 µm, pale brown in water and in 2.5% KOH, very slightly congophilous, not cyanophilous, not amyloid.

CHLAMYDOSPORES absent.

MATERIAL STUDIED: LECTOTYPE: Italy, ad truncos arb. frond., Gocciadoro, J.Bresadola, VI 1901 (BPI)!

REMARKS: *T. cinereoumbrina* is easily identifiable by the mostly simple septate subicular and subhymenial hyphae, the young basidia with distinct distal apex and the broadly ellipsoid pale brown basidiospores with very short aculei. Simple septate subicular hyphae are known from only few *Tomentella* species including *Tomentella fibrosa* (Berk. & M.A.Curtis) Kõljalg, *Tomentella fuscocinerea* (Pers.) Donk, *Tomentella badia* (Link) Stalpers (Kõljalg 1996, Melo et al. 2000, Dämmrich 2006) and *Tomentella furcata* Yorou & Agerer (Yorou & Agerer 2007a). Infrequent clamps on subicular hyphae have been also reported for *T. oligofibula*. The first three species are completely clampless whilst clamps occur occasionally on subicular and more frequently on subhymenial hyphae of *T. cinereoumbrina*, *T. oligofibula* and *T. furcata*. From this

last group, *T. furcata* is easily recognised by its distinctly yellow subglobose to globose basidiospores that bear simple, and/or distinctly forked spines, and by the presence of rather short suburniform basidia with infrequent transverse septa and/or intra-basidial hyphae. *T. cinereoumbrina* differs from *T. oligofibula* by its broadly ellipsoid basidiospores with rather short spines. Basidiospores of *T. oligofibula* sometimes have bifurcate ornaments and are reminiscent of those of *T. radiosa* (Larsen et al. 1994, Melo et al 2003, Kõljalg 1996).

Tomentella punicea (Alb. & Schwein.) J.Schröt. In Cohn, Krypt.-Fl. Schles. 3: 420. 1889. Figs 9-14

Basionym: *Thelephora punicea* Alb. & Schwein.: Fr. Albertini J.B. & Schweinitz L.D.Consp. Fung. Lusat. 278. 1805

Basidiocarp resupinate, separable from the substrate, arachnoid to membranous, continuous, up to 1 mm thick, hymenophore olive brown (4E4) to yellow-brown (5E4 to 5E5), granulose, subiculum yellow brown, sterile margin determinate, byssoid to fimbriate, brownish yellow.

Rhizomorphs present in subiculum and at margins, dark brown under the dissection microscope, thick and visible already at X6, yellow brown in water and in 2.5% KOH, dimitic to trimitic (Figs. 9-10), slightly differentiated (type C or thelephoroid rhizomorph type according to Agerer 1987-2006, 1999), individual hyphae colourless, sometimes yellowish to very pale brown in water and in 2.5% KOH, strongly congophilous, slightly cyanophilous, not amyloid, central hyphae wide (Fig. 11), 6-10 µm diam., thin-walled, of two kinds, some usually clamped, but simple septa common, some completely clampless, then tortuous; below surface hyphae similar to subicular ones, 3-4.5 µm wide, clamped, simple septa common, resulting in short cells, surface hyphae consisting mostly of skeletals that commonly end in binding-like hyphae; skeletals (Fig. 12) 2-4 µm wide, thick-walled (0.5-2 µm), clampless, simple septa rare, rarely branched, tortuous and commonly bent; yellowish, sometimes filled with yellow-brown content; binding-like hyphae (Fig. 13) emerging from skeletals and below surface generative hyphae, 1-2(2.5) µm diam., thin-walled, dendroid, tortuous, clampless, simple septa rare.

Subicular hyphae clamped, 2-4.5(5) µm diam., thin-walled, with shiny reddish to yellow-brown particles/granules (in water) (Fig. 14) that rapidly dissolve in 2.5% KOH resulting in a citrine (yellow-green to yellow-brown) solution (observable even with naked eyes), colourless to pale yellow in water and in 2.5% KOH, congophilous, slightly cyanophilous, not amyloid;

Subhymenial hyphae clamped, 3-4 µm wide, thin-walled, with yellow-brown to red shiny particles/granules (observable in water), particles rapidly dissolving in 2.5% KOH, colourless to pale yellow in water and in 2.5% KOH, congophilous, slightly cyanophilous, not amyloid,

Cystidia absent.

Basidia (35)40-60 µm long, 5.5-7.5 µm wide at apex and 3.5-5 µm wide at base, clamped at base, clavate, sinuous, sometimes with transverse septa, sometimes with

Fig. 9: *Tomentella punicea*. Views of an old rhizomorph. a. Surface view; b. Optical section (from the lectotype). Scale bar = 10 µm.

yellow-brown to red-brown particles (observable only in water) on their surface, colourless to pale yellow, congophilous, slightly cyanophilous, not amyloid, 4-sterigmate, sterigmata 4.5-7 µm long and 1-1.5 µm wide at base.

BASIDIOSPORES (6)6.5-8(8.5) × 6(6.5)8 µm in frontal face, (6)6.5-8(8.5) × 6(6.5)8 µm in lateral face, triangular with widened proximal part (proximal part as wide as the

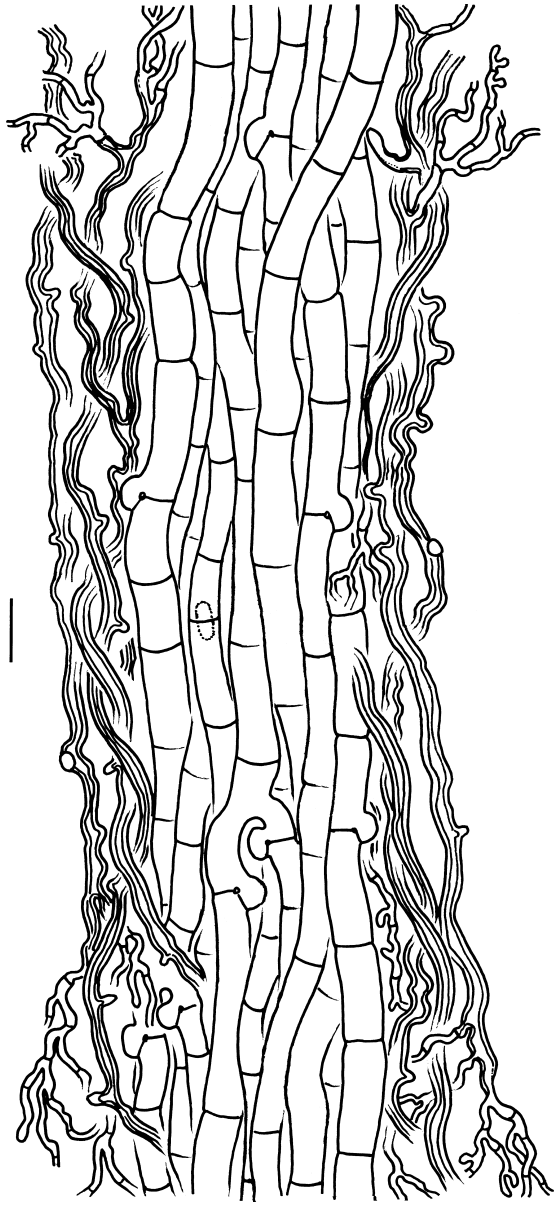

Fig. 10: *T. punicea*. Optical section through an old rhizomorph. Note the presence of distinctive skeletals that ending into binding-like hyphae. Scale bar = 10 µm.

length of the spores) to lobed in frontal view, ellipsoid in lateral view, thick-walled (0.5-1 µm), pale yellow to pale brown, slightly congophilous, cyanophilous, not amyloid, commonly with oil drops, oils drops turning dark-brown in Melzer's reagent,

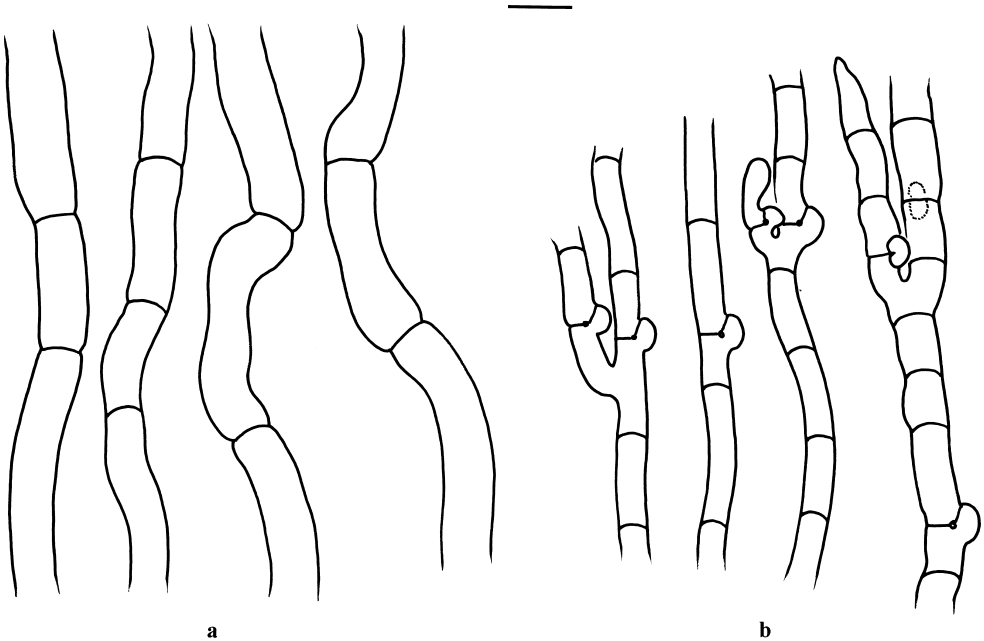

Fig. 11: *T. punicea*. Surface view of central hyphae of a rhizomorph. a. Hyphae simple septate; b. Hyphae clamped and simple septate. Scale bar = 10 µm.

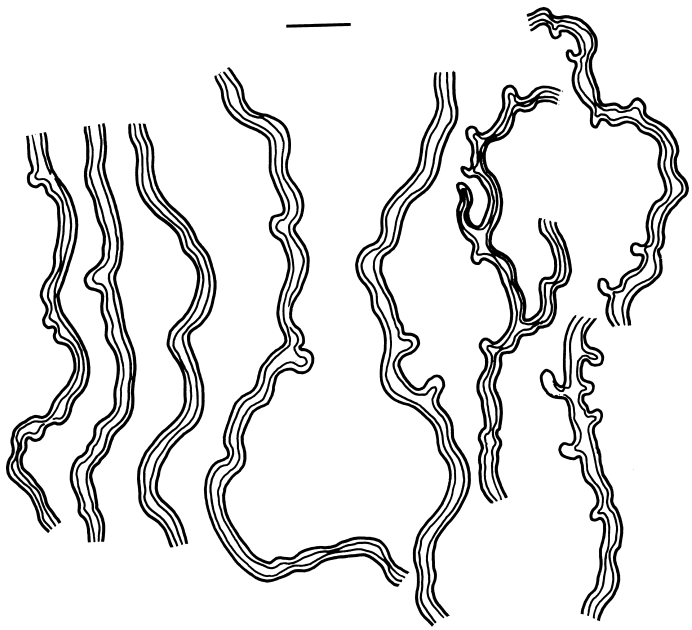

Fig. 12: *T. punicea*. Details of skeletals on rhizomorphs (from the lectotype). Scale bar = 10 µm.

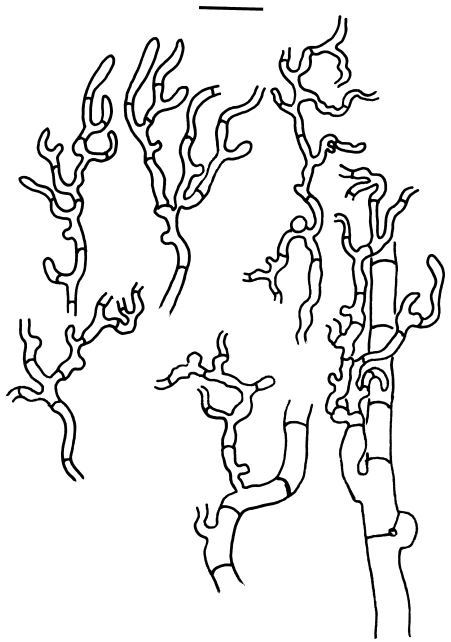

Figure 13: *T. punicea*. Details of binding-like hyphae on the surface of rhizomorphs (from the lectotype). Scale bar = 10 µm.

echinulate, aculei short (0.5-1 µm), densely arranged giving an impression of bifurcation.

CHLAMYDOSPORES absent.

MATERIAL STUDIED: LECTOTYPE: USA, the Academy of Natural Sciences of Philadelphia, Bethlehem, Schweinitz herbarium, herb. 676 (PH)!

REMARKS: The presence of skeletals and binding-like hyphae on the rhizomorphs is typical for this species. In addition, shiny red-brown granules (in water) that dissolve and produce a citrine solution in KOH make the identification of *T. punicea* quiet easy.

Melo et al (2003) highlighted the presence of two kinds of generative hyphae, but did not comments on central enlarged hyphae and whether the rhizomorphs are differentiated. According to Agerer (1999, 1987-2006), rhizomorphs of *T. punicea* belong to the thelephoroid type (rhizomorph type C or slightly differentiated). According to Dämmrich (2006), *T. punicea* shows monomitic rhizomorphs. Kõljalg (1996) mentioned the presence of both monomitic and dimitic rhizomorphs, but provided rather scanty information about the features of the hyphae. Dimitic to trimitic hyphal systems with binding-like hyphae have been reported for *Tomentella rubiginosa* (Bres.) Maire and *Tomentella atroarenicolor* Nikol. (Melo et al 2003, 2006). *Tomentella atroarenicolor* is recognisable by the presence of hyphoid cystidia

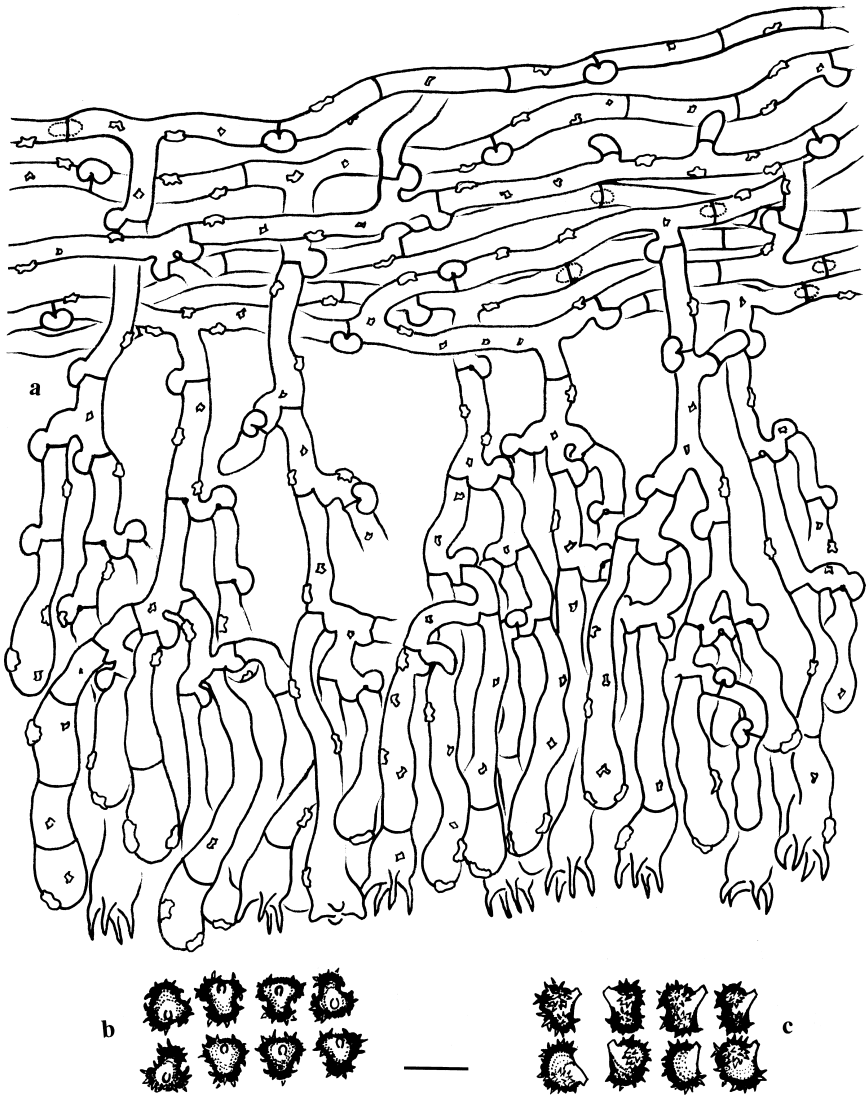

Fig. 14: *Tomentella punicea*, a. Section through the basidiocarp, b. Basidiospores in frontal view, c. Basidiospores in lateral view (from the lectotype). Scale bar = 10 µm.

in the hymenium and on rhizomorphs. Kõljalg (1996) and Dämmrich (2006) considered *T. rubiginosa* to be synonymous to *T. punicea*, while Melo et al (2003) and Stalpers (1993) regarded both species as different due to the presence of longer and narrower clavate basidia in *T. rubiginosa*.

Among the species we have examined so far, *T. punicea* is the unique tomentelloid species that presents skeletal hyphae in the sens of Hartig (1885), Falck (1912),

Corner (1932) and Clemençon (1997). All other tomentelloid fungi reported to show dimitic rhizomorphs, have, in addition to generative hyphae, rather exclusively very thin (1-2 µm), thin to only slightly thick-walled, sinuous, sometimes branched and multi-septate hyphae.

Only two Tomentelloid fungi produce a citrine (yellow-green to yellow-brown) solution when encrustations are dissolved in KOH. To date, except *T. punicea*, such a reaction is only known from *Tomentella umbrinospora* M.J.Larsen (Larsen 1974, Losi 1997, Yorou & Agerer 2007b). The latter species differs from *T. punicea* by the red-brown to chestnut colour (6D3-6E3) of its hymenophore and the presence of numerous, thinner, only slightly thick-walled multi-septate skeletals and the complete absence of binding-like hyphae (Yorou & Agerer 2007b).

Tomentella punicea is anatomically close to *Tomentella ferruginea* (Pers.: Pers.) Pat. (Kõljalg 1996) and macroscopically to *Tomentella botryoides* (Schw.) Bourd. & Galz. (Dämmrich 2006). A similarity between *T. punicea* and *T. botryoides* is supported by various DNA-sequence analyses where both species form a monophyletic group with a bootstrap support of 71% (Kõljalg et al. 2000), 82% (Yorou et al. 2007), and 95% (Yorou & Agerer 2007a). Studies by Yorou & Agerer (2007a) indicate that *T. ferruginea* is a sister species to the group comprising *T. punicea*, *T. botryoides*, *Tomentella africana* Yorou & Agerer and *T. umbrinospora*. However, *T. punicea* differs from both, *T. botryoides* and *T. ferruginea,* by the lack of cyanescent reactions in KOH and the presence of distinctive skeletals in the sense of Hartig (1885), Falck (1912) and of binding-like hyphae.

Acknowledgements

Type materials were made available to us by curators of the herbaria H, BPI and PH to whom we address our sincere thanks. The German Academic Exchange Service (DAAD) is also thanked for the grant no. A/03/15106 offered to the first author. We ware much indebted to Dr. Alison Davies (Institute for Systematic Botany, LMU-Munich) for her valuable help with linguistic checking of the manuscript.

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Received 21 May 2007, accepted in revised version 30 June 2007.

Descr. Ectomyc. **11**: #-# (2008)
(Accepted)

Agerer R, Danielson RM, Egli S, Ingleby K, Luoma D, Treu R (eds)
DESCRIPTIONS OF ECTOMYCORRHIZAE 11: #-# (2008)

Afzeliaerhiza beninensis
+ Afzelia africana Smith

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Short description

The ectomycorrhizae of *Afzeliaerhiza beninensis* belong to the genus *Tomentella*. They are light brown when young and brown to dark-brown at maturity, irregularly monopodial-pinnate to monopodial-pyramidal. The outer layer of the mantle is plectenchymatous with no discernable patterns and is formed by rather long, branched, intermingling and irregularly inflated hyphae. Outer mantle shows adherent soil particles and crystals that are locally embedded in a gelatinous matrix. The middle mantle is pseudoparachymatous with distinctive epidermioid cells of irregular shapes, or being either round or occasionally angular. The inner mantle is distinctly plectenchymatous consisting of compact parallel to bent hyphae. Rhizomorphs are yellow to brown, smooth, covered by soil particles and without cystidia. Rhizomorphs are slightly differentiated, consisting of mostly parallel uniform central hyphae and peripheral thinner ones. All hyphae show clamps and simple septa. Uni- or bilateral conical ramifications are present on the rhizomorphs.

Morphological characters: *Mycorrhizal systems* (Fig. 1a), regularly or irregularly monopodial-pinnate to monopodial-pyramidal, with 1-2 orders of ramification, up to 7 mm long. - *Main axes* 0.4 to 0.8 mm diam. - *Unramified ends* (Fig. 1b) bent to tortous, up to 3 mm long and 0.3-0.5 mm diam., tips rounded. - *Surface of unramified ends* smooth to grainy, sometimes with soil particles, light brown, toward the basis brown to dark brown, commonly with emanating hyphae. - *Rhizomorphs* present, quite compact, brown, up to app. 0.2 mm diam., frequently and repeatedly branched, smooth, often originating at the very base of the mycorrhizal systems and mostly running along the main axis, origin at mantle distinct to fan-like. - *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figs. 1c, 2): *Mantle surface* (Fig. 1c) plectenchymatous, without any pattern, and with portions of gelatinous matrix between the hyphae (mantle type C, according to AGERER 1987-2006 and AGERER & RAMBOLD 2004-2007), showing sometimes short, commonly very long, branched or somewhat inflated to roundish hyphae; hyphae 3-4.5 µm diam., locally embedded in a yellowish gelatinous matrix, hyphae membranaceously light yellow to yellow, simple septate, clamps sometimes visible, surface smooth, thin to slightly thick-walled (up to 0.5 µm); matrix with adherent soils particles; mantle mostly covered with a loosely arranged hyphal net, cells 25-70 µm long, 3-4 µm diam., commonly branched, occasinally somewhat inflated, then 6-8 µm, mostly simple septate, sometimes clamped. - *Middle mantle layers* (Fig. 2a) compactly pseudoparenchymatous with no distinctive pattern, consisting of epidermoid to irregular lobed, sometimes rounded to occasionally angular cells; cells (3.5)5-8(9) µm diam., membranaceously yellow, thin to slightly thick-walled (up to 0.5 µm), surface smooth. - *Inner mantle layers* (Fig. 2b) densely plectenchymatous; hyphae arranged mostly in parallel, cells

straight to bent, 10-20 (30) µm long and 4-8 µm diam., thin to slightly thick-walled (up to 0.5 µm), no clamps visible, cell walls light yellow. - *Very tip* with the same structural characters as in outer and middle parts of the mantle, but plectenchymatous inner part with hyphae arranged mostly in parallel not found

Anatomical characters of emanating elements: - *Rhizomorphs* (Fig. 3) up to 120 µm diam., yellow to light brown, margin smooth, without emanating hyphae, covered by soil particles that are embedded in a yellowish to yellow-brown gelatinous surface matrix, undifferentiated to slightly differentiated (Fig. 3a; Type C according to AGERER 1987-2006 and AGERER & RAMBOLD 2004-2007), central hyphae almost parallel, (3.5)4-7(9) µm diam., sometimes bent and curled to twisted, intermingled, mostly clamped, but simple septa present, colourless to light yellow, thick-walled (0.5-1 µm), walls yellowish; peripheral hyphae almost parallel, considerably thinner than inner ones but not like sceletal hyphae (according to LARSEN 1974 and KÖLJALG 1996), 2.5-3.5 µm diam., simple septa common, clamps rare, thin-walled, yellow-brown, irregularly shaped and tortuous, surface smooth; often with adherent soil particles, nodia and conical side branches present, ramification with one or two branches at nodia (Fig. 3b) - *Emanating hyphae* only present on the mantle, 3-3.5 µm, colourless to light yellow, thin to slightly thick-walled (up to 0.5 µm), mostly with clamps, simple septa rare, surface smooth. - *Cystidia* not observed.

Anatomical characters, longitudinal section: *Mantle* (20)25-40(50) µm thick, surface yellowish due to the gelatinous matrix layer, differentiated in two distinct layers; outer part 15-30(35) µm thick, loosly plectenchymatous at the very surface and becoming denser to the middle layer, comprising oval to roundish to somewhat elongate hyphae of 3-4 µm diam. that are embedded in a yellowish matrix, hyphae thin to slightly thick-walled (up to 0.5 µm), cell walls yellow to light brown; inner part 15-30 µm, consisting of more elongated hyphal elements often growing in parallel, cells 5-15(30) µm long and 3-6(8) µm diam., hyphae thin to slightly thick-walled (up to 0.5 µm), membranaceously yellowish to very light brown, mantle of the very tip up to 25-30 µm thick, in opposite to mature parts no layers discernable, hyphae oval to roundish to somewhat elongated, mostly 3-5 µm diam., occassionally inflated up to 8 µm, membranaceously yellowish, thin to slightly thick-walled. - *Tannin cells* lacking. - *Rhizodermal cells* tangentially elongated to cylindrical, app. twice as long as broad, tangentially 25-40 (50) µm, radially 10-20 µm. - *Hartig net* periepidermal, hyphae mostly in 1 row, rarely in 2 rows, 2-4(5) µm diam., with portions of yellowish matrix between the hyphae.

Colour reaction with different reagents: *Mantle and rhizomorph preparations from formol fixed material:* cotton blue: n. r. (= no reaction); ethanol 70%: n. r.; FEA: n. r.; formol 40%: n. r.; guaiac: n. r.; KOH 15%: n. r.; lactic acid: n. r.; Melzer' s reagent: n. r.; sulpho-vanillin: n. r.

Autofluorescence: *Whole mycorrhiza:* UV 254 nm: lacking; UV 366 nm: lacking. - *Mantle in section*: UV-filter 340-380 nm: lacking; blue filter 450-490 nm: lacking; green filter 530-560 nm: lacking.

Reference specimen for Afzelia africana ectomycorrhiza: Africa, Benin, central part, Borgou Province, forest reserve of Wari-Maro, Wari-Maro site, N 08° 49´19.0´´, E 02° 16´32.4´´, in ceasalpinioid forests dominated by *Isoberlinia doka* Craib & Stapf and *Isoberlinia tomentosa* (Harms) Craib & Stapf in mixture with *Monotes kerstingii* Gilg, *Afzelia africana* Smith, *Burkea africana* Hook., and *Uapaca guineensis* Müll. Arg. Mycorrhiza SYN 738 (in M): leg., soil core exc., myc. isol. N. S. Yorou, 05.08.2005. Identification of mycorrhiza as a member of the genus *Tomentella* by DNA-analysis and comparison (see below) with fruitbody SYN 1000 (in M): Benin, central part, Borgou province, Wari-Maro region, forest reserve of Wari-

Maro, N 08°55´24.4´´, E 02° 44´40.7´´, forest dominated by *Isoberlinia doka, I. tomentosa, Uapaca guineensis*, and *Burkea africana*, leg. et det. N. S. Yorou, 20.08.2006.
Identification of tree partner of mycorrhiza through PCR and sequencing the chloroplast tRNA introns of root tips using primer pairs trnl-c/trnl-d (TABERLET ET AL. 1991), sequences 99 % identical (e-value = 0.0) to *Afzelia africana* (AF365129 in NCBI); query coverage = 100 %.

DNA-analysis: Identification of mycorrhiza as a member of the genus *Tomentella* by comparison of ITS rDNA sequences (PCR and sequencing of the ITS rDNA regions using basidiomycete specific primer pairs ITS1F-ITS4B according to GARDES & BRUNS 1993) of both ectomycorrhiza SYN 738 (Accession number EU334438, Genbank NCBI) and fruitbody SYN 1000 of *Tomentella* spec. (EF507264, GenBank NCBI, compare YOROU & AGERER 2007a and b) with 98 % similarity. Fruitbody SYN 1000 could neither be determined with the genus keys for *Tomentella* (KÖLJALG 1996, DÄMMRICH 2006), nor connected to a certain species in GenBank NCBI (YOROU & AGERER 2007a and b) and may represent a new species not described so far.

Discussion: Up to now about 39 types and species of *Tomentella* and *Tomentella*-like ectomycorrhizae are described with several coniferous or broad leafed tree species, but not with *Afzelia africana*. There are 32 mostly very short descriptions (including 2 *Tomentella*-like ones) that were not determined to the fungal species (summarized by DE ROMAN et al. 2005 and another recent description by YOROU et al. 2008). The following seven mycorrhizae could be connected to fruitbodies of a certain species (AGERER 2006, DE ROMAN et al. 2005): *Tomentella brunneorufa* M.J. Larsen (AGERER & BOUGHER 2001), *T. ferruginea* (Pers.) Pat. (RAIDL & MÜLLER 1996), *T. galzinii* Bourdot (JAKUCS et al. 1997, KÖLJALG et al. 2001 sub nomine *Quercirhiza fibulocystidiata*), *T. pilosa* (Burt) Bourdot & Galzin (JAKUCS & AGERER 1999), *T. stuposa* (Link) Stalpers (JAKUCS et al. 2005a), *T. sublilacina* (Ellis & Holw.) Wakef. (AGERER 1996 sub nomine *T. albomarginata* (Bourdot & Galzin) M.P. Christ.), and *T. subtestacea* Bourdot & Galzin (JAKUCS & AGERER 2001). Further, several dark brown mycorrhizae have been identified as formed by *Tomentella* due to anatomical features or molecular analysis (e.g. AZUL et al. 1999, DE ROMAN & DE MIGUEL 2005, GOLLDACK et al. 1999, JAKUCS et al. 2005 a, b, MLECZKO 2004 a, b).
According to AGERER (2006), *Tomentella* ectomycorrhizae reveal a high structural diversity regarding mantle (plectenchymatous to pseudoparenchymatous), rhizomorphs (absent, undifferentiated, or differentiated), hyphae (thin or thick-walled, clamped or simple septate), and cystidia (present or absent). The persence of blue granules or a blueish to greenish staining reaction with KOH may also be an important feature for some mycorrhizae as it is true for the distinction of fruitbodies (see LARSEN 1974, KÖLJALG 1996, DÄMMRICH 2006). In general, different combinations of these characters make it possible to characterize and distinguish fruitbodies or mycorrhizae.
However, common features of most *Tomentella* mycorrhizae described so far are brown mycorrhizae (from light brown to dark brown or almost black) formed by yellowish to brownish hyphae. The species described here with *Afzelia africana* shows a plectenchymatous outer mantle with a gelatinous matrix but a pseudoparenchymatous middle mantle, no cystidia and slightly differentiated rhizomorphs of the thelephoroid type (compare AGERER 1999, 2006) with both clamps and simple septa. However, a reaction of fresh material with KOH could not be tested, because only fixed material was available for this description. According to the presence of these rhizomorphs also at the margin of the fruitbody, this species belongs to the subgenus *Tomentella* (KÖLJALG 1996), in opposite to the subgenus *Alytosporium* that commonly lacks rhizomorphs.

This combination of features is not reported for any *Tomentella* mycorrhiza so far, although many of the descriptions mentioned above are quite short and do not refer to all the characters typical and important for *Tomentella* mycorrhizae. Pseudoparenchymatous mantles are described for *Tomentella stuposa* (JAKUCS et al. 2005a), and *T. sublilacina* (AGERER 1996) as well as for *T. galzinii* (JAKUCS et al. 1997), *T. pilosa* (JAKUCS & AGERER 1999), and *T. subtestacea* (JAKUCS & AGERER 2001). The latter 3 species belong to a group of light brown to greenish mycorrhizae that show typical so-called fibulocystidia (short cystidia with an intercalar clamp).
A plectenchymatous outer mantle and a pseudoparenchymatous inner one is reported for *T. ferruginea* mycorrhizae (RAIDL & MÜLLER 1996), but they differ clearly by the presence of well differentiated rhizomorphs (RAIDL 1997) with an outer layer of considerably thinner hyphae ("irregularly shaped thin hyphae" according to YOROU et al. 2007, or "skeletal hyphae" according to LARSEN 1974 and KÖLJALG 1996). Most similar in structure of mantle and rhizomorphs are *T. brunneorufa* mycorrhizae described by AGERER & BOUGHER (2001). They can be distinguished by the light orange color of the mycorrhizae (and whitish at their tips), the plectenchymatous middle mantle layer, and the lack of a gelatinous matrix at the mantle surface.
In conclusion, the *Tomentella* species with *Afzelia africana* described here can be distinguished by the combination of its mantle and rhizomorphs features. In the future, considerably more mycorrhizae have to be studied to obtain better information on occurrence and variability of these characters. This may possibly result in a natural classification of the genus *Tomentella* into different groups due to the features of their mycorrhizae (as stated by AGERER 2006).

Acknowledgements: Mr. E. Marksteiner`s skilful preparation of the sections is highly appreciated. The investigations were financially supported by the German Academic Exchange Service (DAAD, grant n° A/03/15106), the International Foundation for Science (IFS, grant D/4033-1), the African Forestry Research Network (AFORNET, grant n°002/05) and Deutsche Forschungsgemeinschaft (SFB 607/B7).

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Captions: Fig. 1 - a. Habit of ectomycorrhiza. - ***b.*** Unramified end of ectomycorrhiza. - ***c.*** Outer mantle layer in plan view. - ***Fig 2. - a.*** Middle mantle layer in plan view. - ***b.*** Inner mantle layer in plan view. - ***Fig 3. - a.*** Slightly differentiated mature rhizomorph in plan view (left) and optical section (right). - ***b.*** Section of a young rhizomorph with ramification. *All figs. from SYN 738 (in M).*

Fig. 1 - Afzeliaerhiza beninensis + Afzelia africana

Fig. 2 - Afzeliaerhiza beninensis + Afzelia africana

Fig. 3 - Afzeliaerhiza beninensis + Afzelia africana

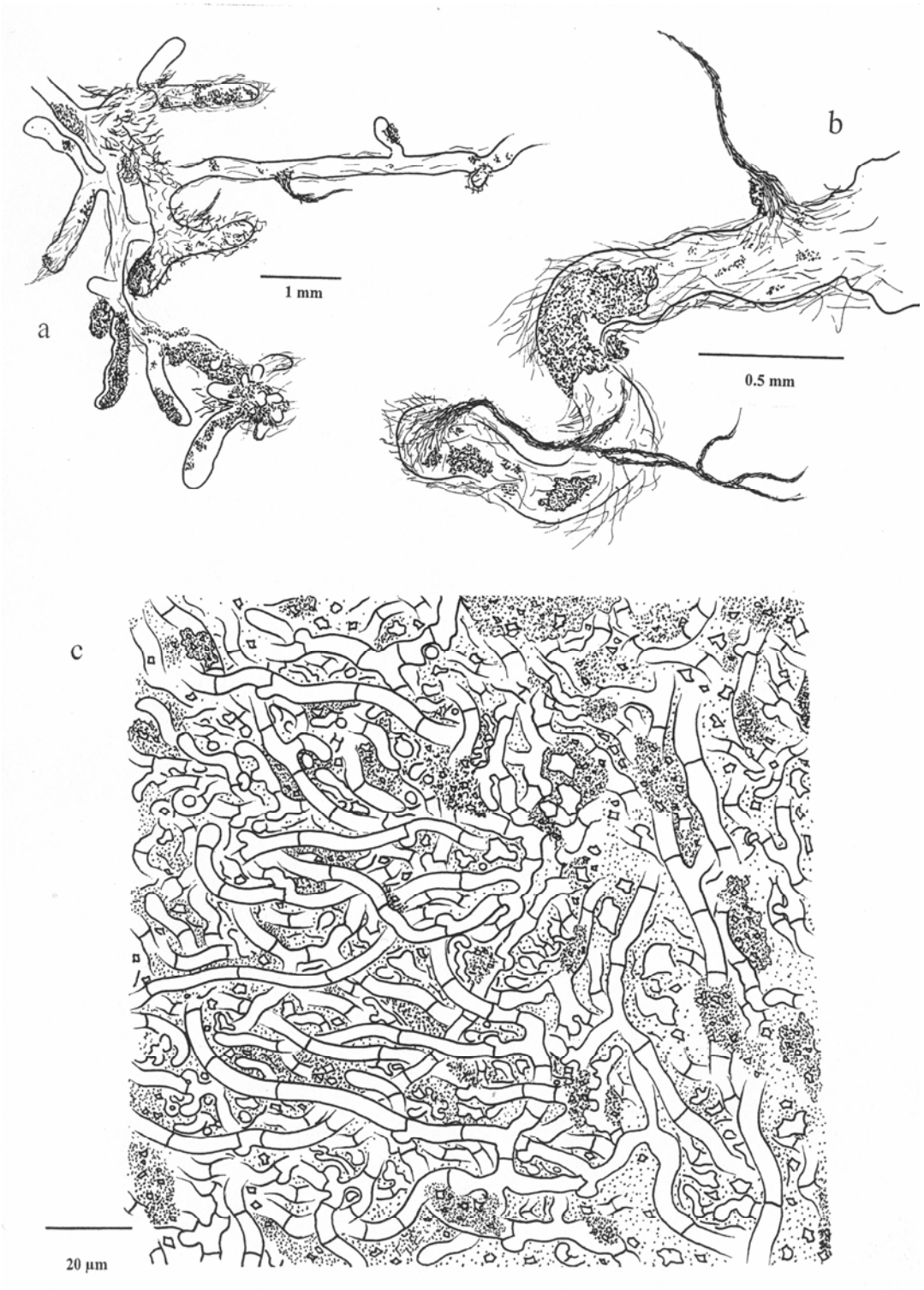

Fig. 1

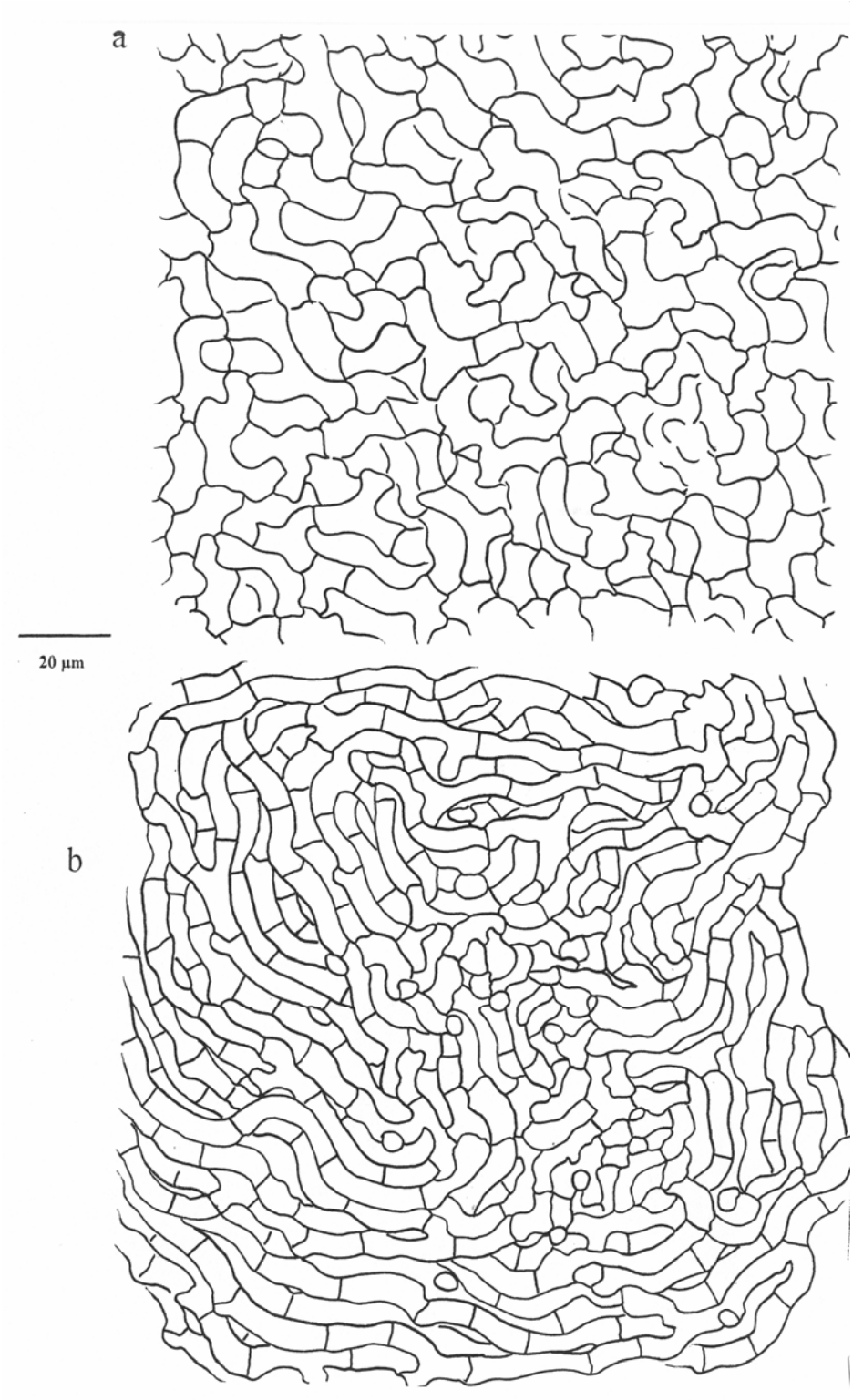

Fig. 2

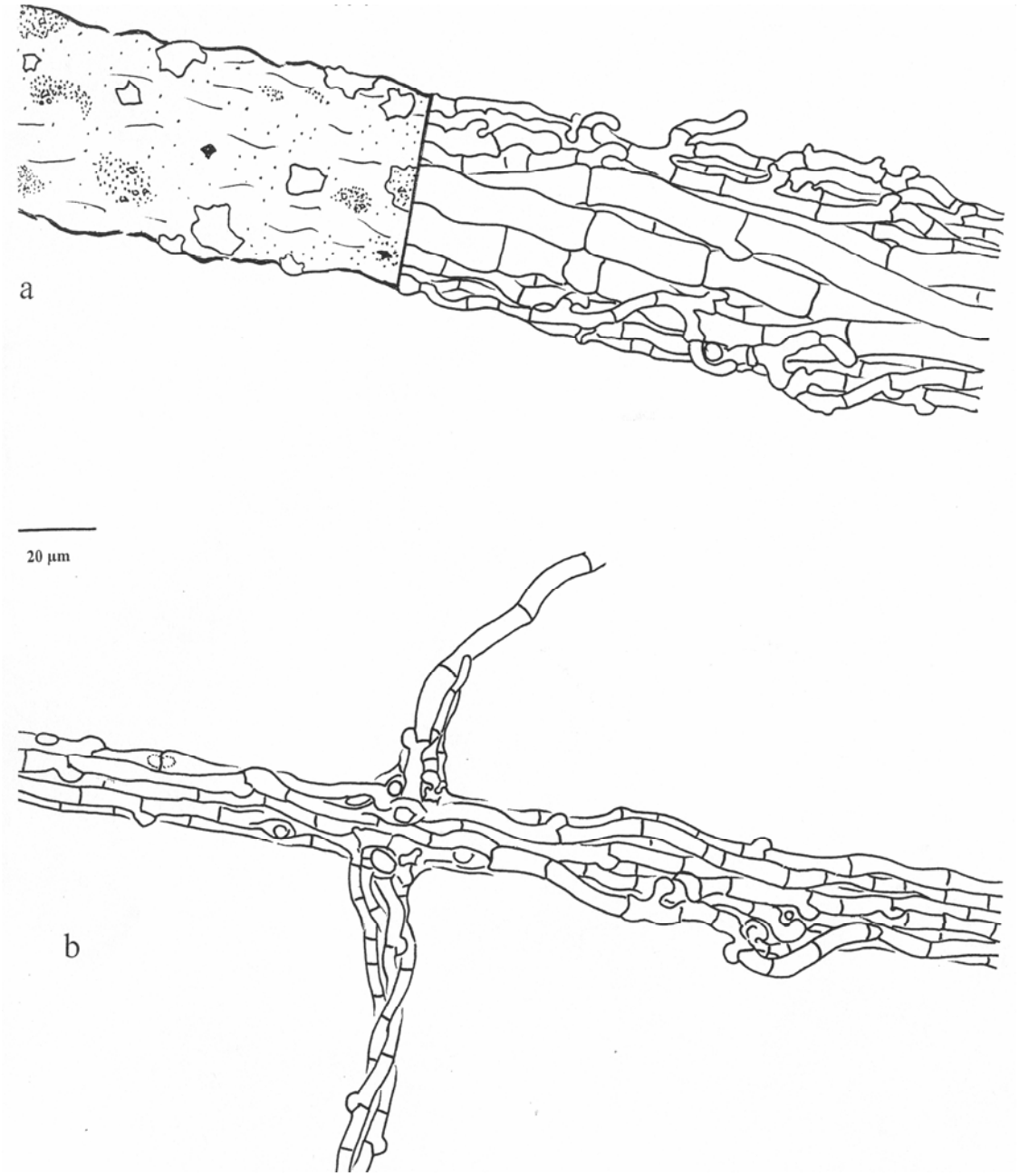

Fig. 3

VI

Descr. Ectomyc. **11**: #-# (2008)
(Accepted)

Agerer R, Danielson RM, Egli S, Ingleby K, Luoma D, Treu R (eds)
DESCRIPTIONS OF ECTOMYCORRHIZAE 11: #-# (2008)

Uapacaerhiza wariensis
+ Uapaca guineensis Müll. Arg.

NOUROU SOULEMANE YOROU, REINHARD AGERER, STEFAN RAIDL, Department Biology I and GeoBioCenter[LMU], Systematic Mycology, Ludwig-Maximilians-Universität München, Menzinger Straße 67, D-80638 München, Germany.

Short description

The ectomycorrhizae of *Uapacaerhiza wariensis* belong to the genus *Tomentella*. They are light brown to yellow brown at maturity, regularly to irregularly monopodial-pinnate. The outer layer of the mantle is plectenchymatous with discernable patterns consisting of cylindrical hyphae with ring-like to parallel arrangement. The outer mantle is densely covered by soil particles and crystals that are entirely embedded in a colourless to light yellow gelatinous matrix. The middle mantle is pseudoparenchymatous with irregularly round to elongate cells. The inner mantle consists of compact parallel to tortuous hyphae arranged in a dense, plectenchymatous layer. Rhizomorphs are colourless to light yellow, smooth, covered by soil particles and bear no cystidia, comprising uniform thin-walled hyphae growing more or less in parallel. Emanating hyphae of the rhizomorphs are mostly tortuous, clamped and simple septate and commonly covered by an amorphous gelatinous matrix.

Morphological characters (Fig. 1a): *Mycorrhizal systems* regularly or irregularly monopodial-pinnate, with 1-2 orders of ramification, up to 5 mm long, with distinct mantle surface, surface smooth, commonly with adherent soil particles. - *Main axes* up to 5 mm long 0.4-0.6 mm diam., mostly straight. - *Unramified ends* straight to bent, mostly up to 2 mm long, rarely up to 4 mm long when not ramified and 0.2-0.5 mm diam., cylindric, very tips rounded. - *Surface of unramified ends* smooth to slightly grainy, commonly with soil particles, yellow brown to red-brown toward the basis, rarely with emanating hyphae. - *Rhizomorphs* infrequent, up to app. 0.1 mm diam., distinctly connected to the mantle, originating from the mantle and running along the main axis, yellow-brown, concolorous or paler than the mantle, frequently branched, margin smooth. - *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figs. 1b, 2): *Mantle surface* (Fig. 1b) plectenchymatous and with portions of gelatinous matrix between the hyphae (mantle type C, according to AGERER 1987-2006 and AGERER & RAMBOLD 2004-2007); hyphae uniform cylindrical, 2.5-5 µm diam., cells 6-25 µm long, hyphae arranged more or less parallel to ring-like, mostly with simple septa, clamps infrequent and hardly discernable, colourless to light yellow, thin-walled, surface smooth, hyphae embedded in a strong yellowish gelatinous matrix, often with adherent soil particles, particles sometimes quite large (up to 15 µm diam.). - *Middle mantle layer* (Fig. 2a) more or less pseudoparenchymatous without any pattern, transition to plectenchymatous, consisting of irregularly roundish to short-celled hyphae, 6-9 µm diam., cell walls up to 0.5 µm, colourless to membranaceously yellow, with smooth surface. – *Inner mantle layer* (Fig. 2b) densely plectenchymatous, with distinctive pattern, consisting of more elongate hyphae often arranged in parallel, 2.5-4.5 (5) µm diam., cell walls up to 0.5 µm, no clamps visible, colourless to membranaceously yellowish, surface of cells

smooth. - *Very tip* with the same structural characters as older parts of the mantle. - *Cystidia* not observed. - *Chlamydospores* not observed.

Anatomical characters of emanating elements (Fig. 3): *Rhizomorphs* (Fig. 3a) smooth and undifferentiated with dictinct margin (Type B, according to AGERER 1987-2006 and Agerer & Rambold 2004-2007), colourless to very light yellow, up to 120 µm diam., nodia and conical side branches present but rare, surface smooth and covered by a yellowish gelatinous matrix, often covered by soil particles, some particles up to 14 µm diam.; consisting of uniform hyphae that grow more or less in parallel, hyphae membranaceously yellowish to light brown, 2.5-4 µm diam., thin-walled, with clamps and simple septa, surface smooth. - *Emanating hyphae* (Fig. 3b) straight, sometimes tortuous, 2.5-4 µm diam, colourless to membranaceously yellowish, thin-walled, clamped and simple septate, covered with patches of colourless to yellowish amorphous gelatinous material, with adherent soil particles. - *Cystidia* not observed.

Anatomical characters, longitudinal section: *Mantle* 25-40(50) µm thick, surface distinct with very few emanating elements and densely covered with adhering soil particles, no layers discernable; hyphae arranged very compact and embedded in a slightly yellowish matrix, single hyphae only hardly discernable; hyphae oval to roundish to somewhat elongated, 2-3 µm diam., hyphae thin-walled, membranaceously yellowish; mantle of the very tip thinner (10-20 µm), with no differences in structure to mature parts. - *Tannin cells* lacking. - *Rhizodermal cells* cylindrical to elongate, app. twice as long as broad, 30-40(50) µm x 15-20(25) µm. - *Hartig net* in section paraepidermal, around elongated rhizodermal cells and occassionally 1 row of cortical cells; hyphae in 1-2 rows, (1)1.5-3 µm diam., clampless, with portions of yellowish matrix between the hyphae.

Colour reaction with different reagents: *Mantle preparations from formol fixed material:* cotton blue: hyphae bluish, matrix deep blue; FEA: n. r. (= no reaction); lactic acid: n. r.; Melzer' s reagent: n. r.; sulpho-vanillin: n. r.

Autofluorescence: *Whole mycorrhizae:* UV 254 nm: lacking; UV 366 nm: lacking. - *Mantle in section*: UV-filter 340-380 nm: lacking; blue filter 450-490 nm: lacking; green filter 530-560 nm: lacking.

Reference specimen for Uapaca guineensis ectomycorrhizae: Mycorrhizae SYN 906 (in M): Benin, central part, Borgou Province, forest reserve of Wari-Maro, Wari-Maro site, N 08° 40´20.0´´, E 02° 14´33.3´´, in northern Guinean seasonal forests dominated by *Isoberlinia doka* Craib & Stapf and *Isoberlinia tomentosa* (Harms) Craib & Stapf with few representatives of *Afzelia africana* Smith, *Burkea africana* Hook., and *Uapaca guineensis*, leg., soil core exc., myc. isol. N. S. Yorou, 06.08.2006. Identification of mycorrhizae as a member of the genus *Tomentella* by DNA-analysis and comparison (see below) with fruitbody RA 13793 (in M): Benin, central part, Province Borgou, Sinende Region, forest close to Fô-Bouko Village, N 10°8´46.6´´, E 02° 15´6.0´´, forest with *Monothes kerstingii* Gilg, *Uapaca guineensis, Burkea africana, Isoberlinia* spec., *Daniella oliverii* (Rolfe) Stall. & Dalz., *Detarium microcarpum* Guill. & Perr., and *Afzelia africana*, leg. et det. R. Agerer, 22.08.2003.
Identification of tree partner of mycorrhiza through PCR and sequences of chloroplastic trnL genes using primer pair trnl-c/trnl-d (TABERLET ET AL. 1991). Sequence identity = 98 % with *Uapaca guineensis* (AY830287); query coverage = 100 %, e- value = 0.0.

DNA-analysis: Identification of mycorrhizae as a member of the genus *Tomentella* by comparison of ITS rDNA sequences (PCR and sequencing of the ITS rDNA regions using basidiomycetes specific primer pairs ITS1F-ITS4B according to GARDES & BRUNS 1993) of both ectomycorrhiza SYN 906 (Accession number EU334439, GenBank NCBI) and fruitbody RA 13793 of *Tomentella* spec. (EF538424, GenBank NCBI, compare YOROU & AGERER 2007a and b) with 98 % similarity. Fruitbody RA 13793 could either not be determined with the genus keys for *Tomentella* (KÖLJALG 1996, DÄMMRICH 2006), nor connected to a certain species in GenBank NCBI (YOROU & AGERER 2007a and b) and may represent a new species not described so far.

Discussion: Nearly 40 types and species of *Tomentella* and *Tomentella*-like ectomycorrhizae are described so far with several coniferous or broad leafed tree species, but not with *Uapaca guineensis*. There are about 32 mostly very short descriptions (including 2 *Tomentella*-like ones) that were not determined to the fungal species (summarized by DE ROMAN et al. 2005 and another recent description by YOROU et al. 2008), and other seven descriptions that could be connected to fruitbodies of a certain species (AGERER 2006, DE ROMAN et al. 2005). The common features and the high structural diversity of all *Tomentella* ectomycorrhizae described up to now are discussed in detail elsewhere (YOROU et al. 2008) with special respect to possibly distinguishing features.
The light brown *Uapacaerhiza wariensis* with *Uapaca guineensis* described here shows a plectenchymatous outer mantle with a gelatinous matrix and a pseudoparenchymatous inner one, no cystidia and undifferentiated rhizomorphs with both clamps and simple septa. According to the presence of these rhizomorphs also at the margin of the fruitbody, this species belongs to the subgenus *Tomentella* (KÖLJALG 1996), in opposite to the subgenus *Alytosporium* that lacks rhizomorphs.
Similar in many structural features are the mycorrhizae of *Afzeliaerhiza beninensis* with *Afzelia africana* (YOROU et al. 2008), but they differ in their dark brown colour and the slightly differentiated rhizomorphs of the thelephoroid type (type C according to AGERER 1999 and 2006). A plectenchymatous outer mantle and a pseudoparcnchymatous inner one is also reported for the dark brown mycorrhizae of *Tomentella ferruginea* (Pers.) Pat. (RAIDL & MÜLLER 1996), but they have well differentiated rhizomorphs with an outer layer of considerably thinner hyphae ("irregularly shaped thin hyphae" according to YOROU et al. 2007, or "skeletal hyphae" according to LARSEN 1974 and KÖLJALG 1996). Undifferentiated rhizomorphs of type B are also described from 3 other species: *Tomentella galzinii* Bourdot (JAKUCS et al. 1997), *T. stuposa* (Link) Stalpers (JAKUCS et al. 2005), and *T. sublilacina* (Ellis & Holw.) Wakef. (AGERER 1996). But all these species differ in their pseudoparenchymatous mantle surface. Further, *Tomentella galzinii* mycorrhizae are well characterized by typical so-called fibulocystidia that represent short cystidia with an intercalar clamp.
In conclusion, the *Tomentella* species with *Uapaca guineensis* described here can be distinguished by the combination of its mantle and rhizomorph features. In the future, considerably more mycorrhizae have to be studied to obtain more information on the occurrence and variability of these characters. This may possibly result in a natural classification of the genus *Tomentella* into different groups due do the features of their mycorrhizae (as stated by AGERER 2006).

Acknowledgements: We are much indebted to Mr. E. Marksteiner for his skilful preparation of the sections. The investigations were financially supported by the German Academic Exchange Service (DAAD) through the grant n° A/03/15106 and by Deutsche Forschungsgemeinschaft (SFB 607/B7). Additional financial supports were offered by the International Foundation for Science (IFS, grant D/4033-1) and by the African Forestry Research Networks (grant n°002/05).

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Captions: Fig. 1. - a. Habit of ectomycorrhizae. **- *b.*** Outer mantle layer in plan view. **- *2. - a.*** Middle mantle layer in plan view. **- *b.*** Inner mantle layer in plan view. **- *Fig. 3. - a.*** Mature rhizomorph in plan view (left) and in optical section (right) **- *b.*** Emanating hyphae. *All figs. from SYN 906 (in M).*

Fig. 1 - Uapacaerhiza wariensis + Uapaca guineensis

Fig. 2 - Uapacaerhiza wariensis + Uapaca guineensis

Fig. 3 - Uapacaerhiza wariensis + Uapaca guineensis

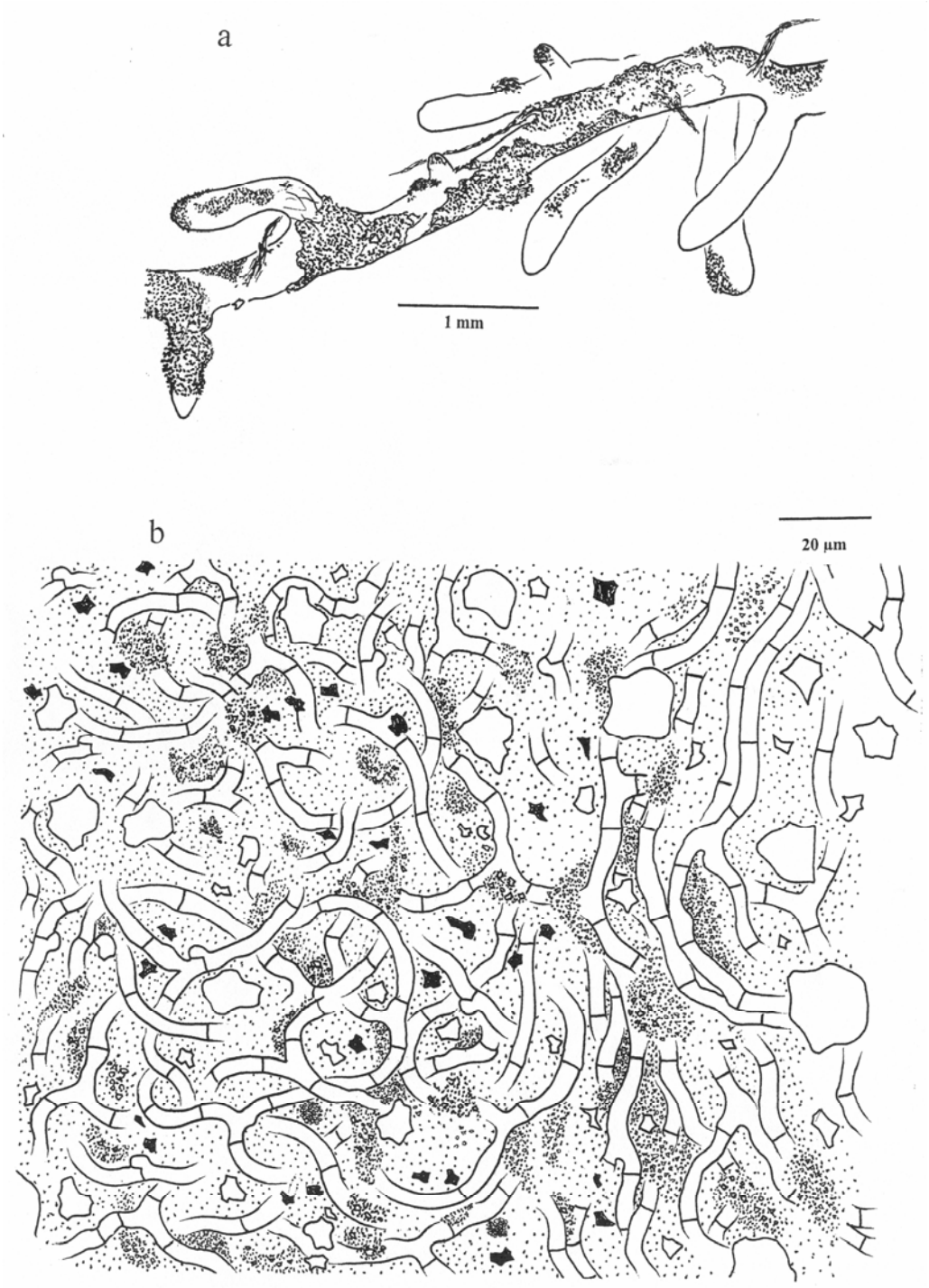

Fig. 1

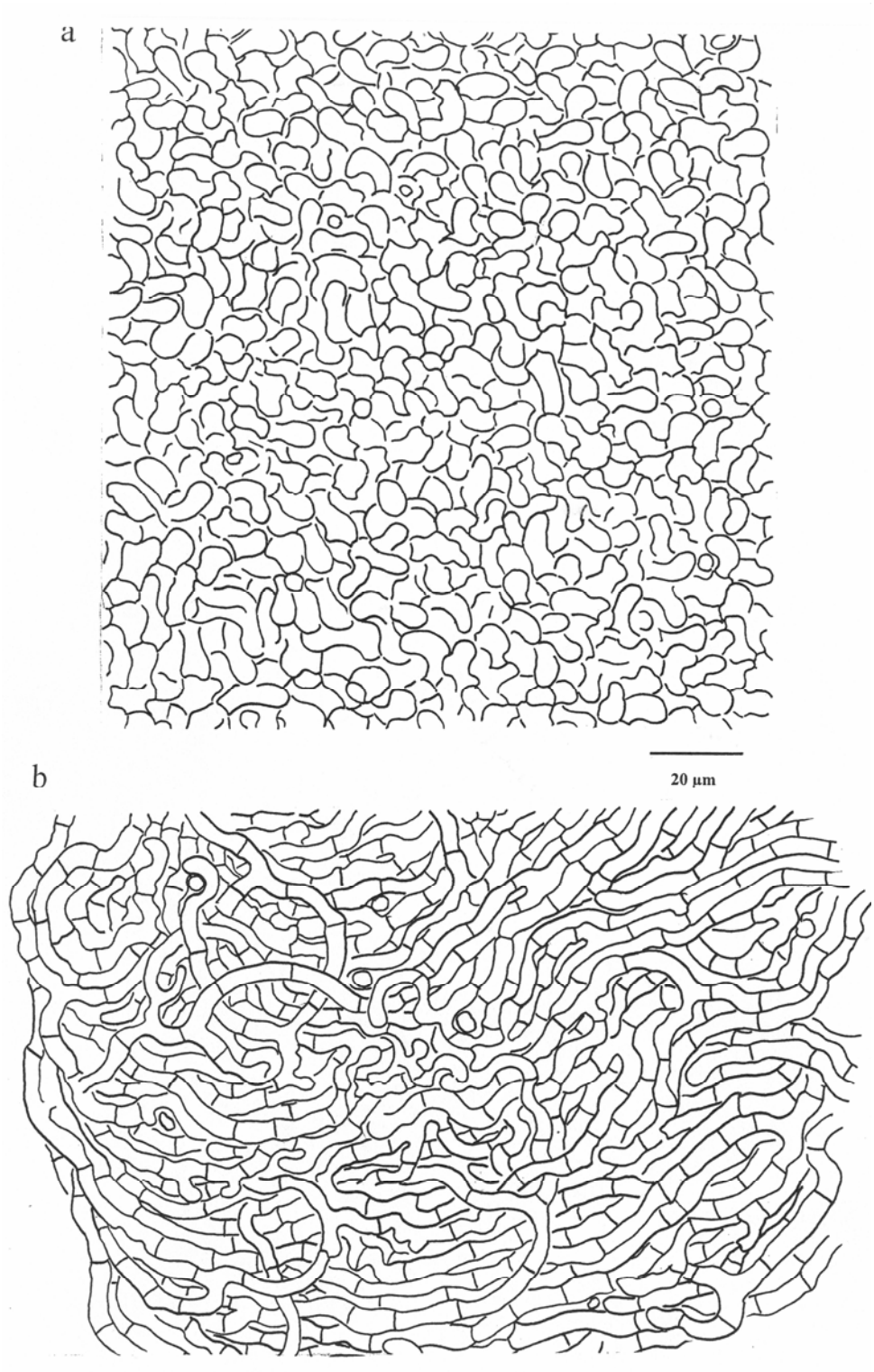

Fig. 2

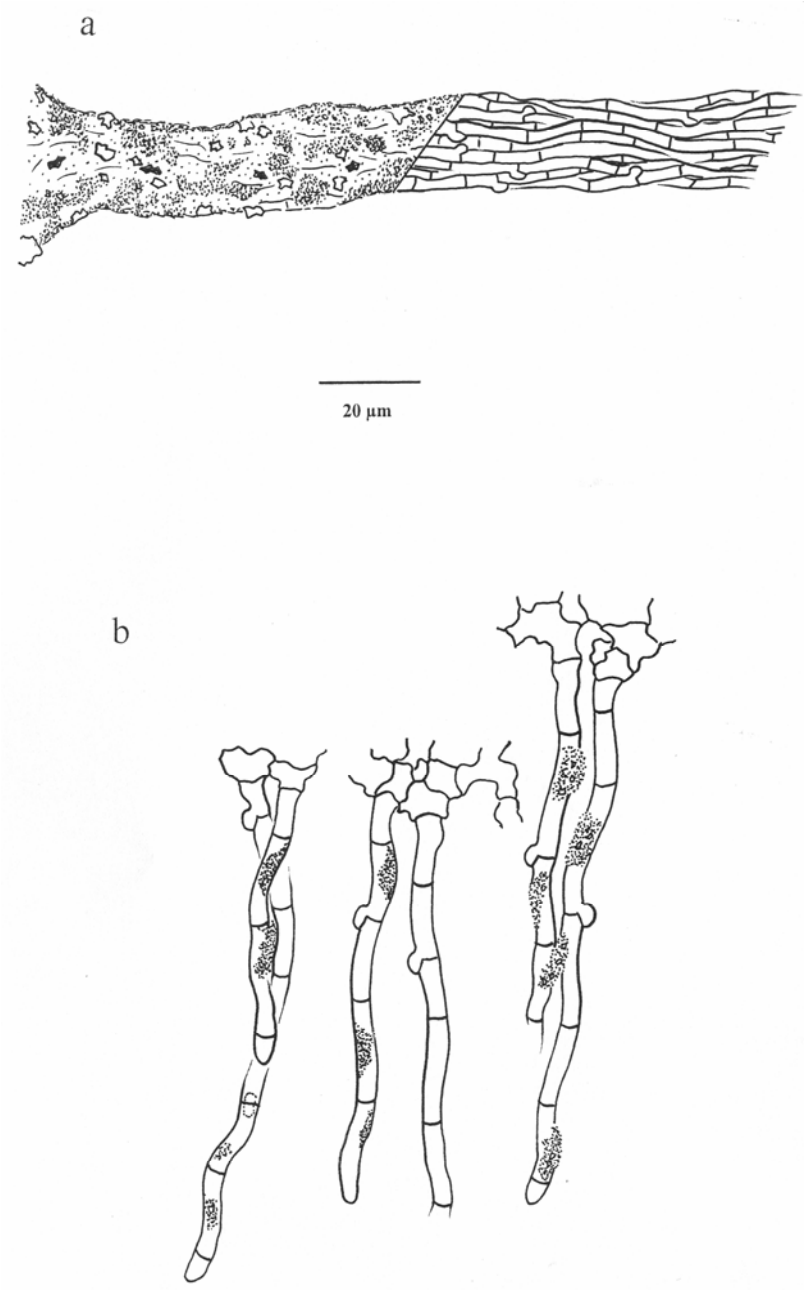

Fig. 3

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Selective publications

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Scholarships and research grants:

- **1994-2000:** Scholar of the Benin government,
- **2003-2007:** Scholar of the German Academic Exchange Service (DAAD),
- **2005:** Research grant from the African Forestry Research Network (AFORNET),
- **2006:** Research grant from the International Foundation for Science (IFS),
- **2006:** Travel grant from the International Foundation for Science (IFS),
- **2006:** Research grant from the British Ecological Society (BES),
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- African Mycological Association (AMA),
- German Mycological Society (DGfM).

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