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Genetic diversity of ectomycorrhizal Basidiomycetes from African and Indian tropical rain forests

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Abstract Ectomycorrhizal (ECM) fungi have a worldwide distribution. However, the ecology of tropical ECM fungi is poorly documented, limiting our understanding of the symbiotic associations between tropical plants and fungi. ECM Basidiomycete diversity was investigated for the first time in two tropical rain forests in Africa (Western Upper Guinea) and in Asia (Western Ghats, India), using a fragment of the mitochondrial large subunit rRNA gene to type 140 sporocarps and 54 ectomycorrhizas. To evaluate taxonomic diversity, phylogenetic analyses were performed, and 40 sequences included from identified European specimens were used as taxonomic benchmarks. Five clades were recovered corresponding to six taxonomic groups: boletoids, sclerodermatoids, russuloids, thelephoroids, and a clade grouping the Amanitaceae and Tricholomataceae families. Our results revealed that the Russulaceae species display a great diversity with several putative new species, especially in Guinea. Other taxonomic issues at family/section levels are also briefly discussed. This study provides preliminary insights into taxonomic diversity, ECM status, and biogeographic patterns of ECM fungi in tropical two rain forest ecosystems, which appear to be as diverse as in temperate and boreal forests.

Keywords Ectomycorrhizal Basidiomycetes · Tropical rain forests · Mitochondrial LrRNA gene

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Introduction

Despite their prominent role for tree growth, studies on ectomycorrhizal (ECM) fungi are almost exclusively focusing on temperate and boreal ecosystems. More than 5,000–6,000 species of ECM fungi have been described in these ecosystems (Molina et al. 1992), whereas diversity and distribution of tropical ECM fungi remain poorly known, available data being mostly restricted to taxonomical inventories in South America, Southeast Asia, and West Africa (Singer and Araujo 1979; Thoen and Bâ 1989; Smits 1992; Watling and Lee 1995; Buyck et al. 1996; Lee et al. 1997; Sanon et al. 1997; Natarajan et al. 2005). Large areas of tropical and subtropical forests are dominated by ECM trees (Redhead 1980; Alexander and Högberg 1986), suggesting a key role of these symbioses in the functioning of some tropical forest ecosystems (Onguene and Kuyper 2001; Rivière et al. 2005). For instance, the Dipterocarpaceae family in Southeast Asia comprises 470 tree species that largely dominate tropical rain forests and represent a major source of commercial timber (Maury-Lechon and Curtet 1998). These tree species have been shown to be associated with various fungal genera such as *Russula*, *Boletus*, *Cortinarius*, *Lactarius*, *Laccaria*, *Pisolithus*, *Amanita*, *Scleroderma*, *Suillus*, *Strobilomyces*, and *Cantharellus* (Smits 1992; Watling and Lee 1995; Natarajan et al. 2005). In African dry woodlands and tropical rain forests, the dominant group of ECM trees includes caesalpinioid legumes (12 genera in the *Amherstieae* and one genus, *Afzelia*, in the Detarieae) and Phyllanthaceae (one genus, *Uapaca*; Newbery et al. 1997; Thoen and Bâ 1989; Sanon et al. 1997; Onguene and Kuyper 2001), and associated ECM fungi belong to several genera, mainly *Russula*, *Lactarius*, *Amanita*, *Boletus*, and *Cantharellus* (Buyck et al. 1996; Eberhardt and Verbeken 2004). ECM trees can form local patches that contribute from 45 to 70% of the basal rain forest area, such as in the Korup National Park in Cameroon where three species *Microberlinia bisulcata*, *Tetraberlinia bifoliolata*, and *Tetraberlinia moreliana* are dominant (Newbery et al. 1997).

Investigations on ECM fungi are delicate, as morphological characters of ectomycorrhizas are usually not sufficient for species recognition (Gardes and Bruns 1993; Bruns et al. 1998). This has long represented a considerable limitation to our understanding of ECM fungi diversity and functioning (Debaud et al. 1999; Kretzer et al. 2003), as sporocarp description only provides an incomplete picture of ECM communities (Egger 1995; Gardes and Bruns 1996; Jonsson et al. 2000; Grogan et al. 2000), and although sporocarps are necessarily associated with ectomycorrhizas, a fungus-forming ectomycorrhiza may not always form sporocarps (Horton and Bruns 2001).

Molecular tools developed during the last decade have proved to be of great help for ECM fungi systematic purposes, therefore considerably improving our knowledge of the diversity and ecology of these pivotal organisms (Horton and Bruns 2001). Such approaches are not only complementary to the morphological analysis of sporocarps (White et al. 1990; Gardes et al. 1991; Baura et al. 1992), but they also enable identification of ECM fungal genera or species through analyses conducted on DNA extracted from ectomycorrhizas. The latter approach is often achieved through comparisons with database DNA sequences of ribosomal internal transcribed spacer (ITS) or mitochondrial sequences (Gardes and Bruns 1993; Bruns et al. 1998; Kõljalg et al. 2005). A combination of morphological and molecular analyses thus appears the most valuable approach to identify sporocarps and unidentified ectomycorrhizas (Gardes et al. 1991; Bruns et al. 1998, Miller and Buyck 2002). Unfortunately, investigations on tropical ECM fungi have so far been based solely on the morphological description of sporocarps.

In this study, we focus on tropical rainforests that are classified as hotspots of biodiversity notably because of their wide variety of endemic plants (Myers et al. 2000). We have examined species diversity of ECM Basidiomycetes from one African and one Indian tropical forest using both morphological and molecular identification of sporocarps and/or ectomycorrhizas. Genetic analyses were based on the ML5/ML6 region of the mitochondrial large subunit rRNA gene. Even if this conservative DNA region may be not suitable for identification at the species level, its use is justified, as specific primers can be applied not only to sporocarp DNA but also to DNA extracted from ectomycorrhizas, avoiding amplification of the associated tree genomic DNA (Gardes et al. 1991). We retrieved 198 DNA mitochondrial sequences that represent, to our knowledge, the first samples in the DNA-based identification database for tropical ECM fungi (Kõljalg et al. 2005). To propose species identification through molecular typing of sporocarps vs ectomycorrhiza, 40 reference sequences from available databases were included in our phylogenetic analyses.

Materials and methods

Study sites and sampling schemes

Study sites were located in Southern Guinea and in Western India. Southern Guinea is one of the last West African areas where primary tropical rain forests still subsist. These remnant forests cover hills and mountains ranging in altitude from 500 m in the Ziama forest (8°51′N, 9°31′W) to 1,752 m on the Mount Nimba forest (7°60′N, 8°49′W). They are typical evergreen or semi-evergreen rain forests with a mean annual rainfall of 2,500–3,000 mm and a dry season from January to March. Based on our own observations, these Guinean forests shelter ECM trees belonging to the Caesalpiniaceae (*Afzelia bella*, *Paramacrolobium coeruleum*, *Anthonotha fragans*, *A. macrophylla*, *Cryptosepalum tetraphyllum*, *Pelligriniodendron diphyllum*, and *Gilbertiodendron limba*), and the Phyllanthaceae (*Uapaca heudelotii*, *U. esculenta*, *U. guineensis*, and *U. chevalieri*). Two additional species, *U. somon* and *Afzelia africana*, were found in the dryer and lower woodlands bordering the Mount Nimba rain forest.

The Kadamakal Reserve Forest is located in the Western Ghats, India, in the district of Kodagu (Karnataka) near the village of Uppangala (12°30′N; 75°39′W). Its altitude ranges from 400 to 600 m. Annual rainfall is about 5,200 mm with a marked dry season from December to March. Vegetation is a dense moist evergreen forest dominated by three species, *Dipterocarpusindicus*, *Kingiodendron pinnatum*, and *Humboltia brunonis* (Pascal and Pélissier 1996). Two ECM Dipterocarpaceae dominate the high canopy, *Vateria indica* and *D. indicus*, which together represent 41.2% of the basal area (Pélissier et al. 1998). To optimize the assessment of the floristic diversity, the Uppangala forest was sampled following a previously designed transect (three plots from 180 to 370 m long and 20 m wide; see Pélissier et al. 1998).

Sporocarps belonging to Basidiomycete families that were typically ECM were collected each August during four successive years in Guinea. In India, samples were collected during two successive years, at the beginning of the monsoon season (May–June). In each spot, fruitbodies were harvested to cover most of the morphological variation observed in the field. When technically possible, fine roots were also collected under sporocarps as well as from young trees by excavating superficial roots all the way from the trunk to the ultimate fine roots. Sporocarps were dried at 45°C and then morphologically identified, vouchered, and stored in the Museum National d'Histoire Naturelle, France, and in the herbarium of the Center of Advanced Study in Botany, India. A small portion of the flesh of each sporocarp was placed separately on a cotton layer into tubes half-filled with silica gel (Prolabo) for rapid-drying and stored at room temperature for subsequent DNA extraction. Fine roots with ectomycorrhizas were gently washed under tap water and placed in tubes with silica gel. ECM status of the tree species was determined by morphological and molecular identification of ectomycorrhizas sampled on the roots. In an attempt to culture some of the fungi collected, small internal pieces of fresh sporocarps were deposited and, when fungal growth was observed, maintained on modified Melin–Norkrans agar medium (Marx 1969).

Molecular analyses

DNA was extracted from dried sporocarp flesh, mycelial cultures, or from mycorrhizas, using a DNeasy Plant Mini kit following the manufacturer's recommendations (Qiagen, France). An approximately 500-bp fragment of the mtLrRNA gene was amplified using the specific primers ML5 (5′-CTCGGCAAATTATCCTCATAAG-3′) and ML6 (5′-CAGTAGAAGCTGCATAGGGTC-3′; White et al. 1990). Polymerase chain reaction (PCR) reactions were performed in a total volume of 25 µl, containing aliquots of 1 µl of genomic DNA, 1 µM of each primer, 1.5 U of Taq DNA Polymerase (Amersham Pharmacia Biotech), 200 µM of each dinucleotide triphosphate, 10 mM Tris–HCl, 50 mM KCl, and 1.5 mM $MgCl_2$. Amplification was performed with a DNA thermal cycler (GenAmp PCR System 2400, Perkin Elmer) as follows: one cycle for 5 min at 95°C followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min 30 s, and extension at 72°C for 7 min. PCR products were separated by electrophoresis in 1% (wt/vol) agarose gels in 1× Tris–acetic acid–ethylenediaminetetraacetic acid with ethidium bromide at 10 mg/ml in the running buffer. Each fragment was purified using a QIA quick gel extraction kit (Qiagen). Both forward and reverse sequences were obtained with ML5–ML6 primers using an ABI Prism BigDye Terminator Cycle sequence kit (Applied Biosystems, Foster City, CA) and then analyzed on an Applied Biosystems model 310 DNA sequencer (Perkin-Elmer).

Sequences were aligned on Sequence Navigator version 1.0.1 (PE Applied Biosystems). A total of 198 sequences were edited and assembled using Autoassembler (Perkin-Elmer). In addition, 40 reference fragments from known boreal and temperate species were downloaded from National Center for Biotechnology Information databank. These data were used as external taxonomic benchmarks, as well as references to investigate any phylogeographic pattern. Most of these sequences were retrieved from the Bruns et al. (1998) database (http://plantbio.berkeley.edu/~bruns/). Five sequences (*Tulasnella irregularis*, *Sebacina* sp., and three *Cantharellus* spp.) were used as outgroups (Bruns et al. 1998). Alignment were obtained using Clustal X 1.60 (Thompson et al. 1994) and then manually corrected with Genedoc software (Nicholas et al. 1997).

Phylogenies were reconstructed with the maximum likelihood method using PAUP 4.0b5 (Swofford 2001). The best-fitting models of molecular evolution for each dataset were selected using the WinModeltest program (Posada and Crandall 1998) on the basis of the likelihood ratio test (LRT, Huelsenbeck and Rannala 1997). Node support is evaluated through 100 replications bootstrap analyses (Felsenstein 1985).

Results

ECM status of tropical trees

The presence of ectomycorrhizas was observed on six caesalpinioid legume tree species (*A. africana*, *A. bella*, *A. fragans*, *A. macrophylla*, *G. limba*, and *P. coeruleum*) and five species of the genus *Uapaca* (*U. guineensis*, *U. esculenta*, *U. heudelottii*, *U. somon*, and *U. chevalieri*) growing in Guinean forests, as previously reported (Thoen and Bâ 1989). Furthermore, we report here for the first time the ECM status of two Caesalpinioideae species of the Amherstieae tribe; that is, *C. tetraphyllum* and *P. diphyllum* were observed for the first time. In India, sporocarps were collected under the two species *V. indica* and *D. indicus* that were already known as ECM tree species (Natarajan et al. 2005; Rivière et al. 2005).

Ectomycorrhizas vs sporocarps and assessment of diversity

In Guinea, 213 sporocarps and 100 ectomycorrhizas were sampled under the eight Caesalpiniaceae and the five Phyllanthaceae species mentioned above. From these, 119 and 55 sequences were obtained, respectively. Additionally, two pure fungal cultures were successfully established from fresh sporocarps of one *Boletus* sp. (M332) and one *Scleroderma* sp. (M296). In Uppangala, the soil layer is characterized by fragmented rocks and small stones that make excavation of superficial roots hardly possible; 25 different DNA sequences were retrieved from collected sporocarps.

Morphological characterization showed that sporocarps could be assigned to five families (Amanitaceae, Russulaceae, Sclerodermataceae, Tomentellaceae, and Tricholomataceae) and one superfamilial group (boletoids). No sporocarp within the Thelephoraceae was collected. These results were all confirmed by our genetic investigations. However, no ectomycorrhiza could be clearly assigned to any species or even genus using morphological identification methods (based on sheath color and texture, pattern of ramification, and presence or absence of mycelial strands; Smith and Read 1997). All DNA sequences obtained could be accurately compared to benchmarks sequences, thus allowing unambiguously reference of them to a precise genus and sometimes species.

Sequences analyses, taxonomic assignations

A total number of 201 sequences of the ML5/ML6 fragment, ranging from 319 to 463 bp, were obtained. As already reported by Bruns et al. (1998), four sequences from Guinean sporocarps included large insertions and made them difficult to amplify, which prevented obtaining the whole stretch. They were therefore discarded. Of the 197 remaining sequences, 140 were derived from sporocarps, 55 from ectomycorrhizas, and 2 from mycelial cultures (see here above). Because of high variation, the 5′ region of the ML5/ML6 fragment had to be discarded, thus leading to a 340-bp long matrix. One hundred and nineteen different genotypes (i.e., with at least 1 bp difference between them) were identified, and the corresponding sequence deposited in Genbank (see Table 1, AM117605–AM117723). The HKY85+γ+Inv model selected with the LRT criteria as implemented in the WinModeltest was applied. Despite the relatively short length and conservative sequences considered in this study, the overall phylogeny based on the 340 nucleotides was reasonably supported by bootstrap values (Fig. 1).

The most represented family was the Russulaceae (75 samples of both sporocarps and ectomycorrhizas), followed by the Amanitaceae (26), the boletoids and Chalciporus group (32, including the genera *Boletus*, *Xerocomus*, *Leccinum*, *Tubosaeta*, *Strobilomyces*, and *Chalciporus*), the Sclerodermataceae (32), the tricholomatoids(10), and the telephoroids (21, of which only one sporocarp recognized as a *Tomentella* sp.). Within the Guinean samples, 27 ectomycorrhiza sequences were identical to those obtained from sporocarps (noted on the same branch in Figs. 2, 3, 4, and 5, see Table 1).

Four subsequent phylogenies were reconstructed to independently focus on the four previously identified clades in Fig. 1, i.e., (1) Russulaceae, (2) boletoids, (3) *Scleroderma*, and (4) Amanitaceae plus Tricholomataceae groups (Figs. 2, 3, 4, and 5). Aligned matrices were, respectively, 391, 375, 375, and 321 bp long, depending on the confidence of the alignment of the 5′ portion.

The Amanitaceae phylogeny was reconstructed under the best-fitted F81+γ+Inv model, whereas the three others were reconstructed using the HKY85+γ+Inv model. For each of these phylogenies, only a few of the internal nodes were well supported by high bootstrap values (Figs. 2, 3, 4, and 5). The Russulaceae family, with 45 sequences plus nine references taxa, was the largest group sampled in our study. Russulaceae includes two genera *Lactarius* and *Russula*. *Lactarius* samples did not group together and appeared polyphyletic. At a lower taxonomical level, the *Eurussula* subgenus appeared to be polyphyletic too, whereas the subgenus *Compactae* and each section *Heterophylla* or *Foetentinae* were found monophyletic. In the *Scleroderma* phylogeny, 32 sequenced have been obtained including 16 ectmomycorrhizas, which represent the highest mycorrhizas vs sporocarps ratio in our sample. The boletoid group includes the genera *Boletus*, *Boletellus*, *Xerocomus*, *Leccinum*, *Tubosaeta*, and *Strobilomyces*. All *Leccinum* sequences as well as the two *Strobilomyces*

Table 1 Taxonomic designations of the species described in the present study

Taxon	Herbarium number	Collection site	Accession number[a]
Albatrellus flettii	–	–	AD001540
Albatrellus skamanius	–	–	AD001542
Amanita annulatovaginata	C72	Guinea	AM117709
Amanita calyptrata	–	–	AD001545
Amanita cf. *lanosa*	C49	Guinea	AM117686
Amanita franchetii	–	–	AD001546
Amanita pachycolea	–	–	AD001550
Amanita phalloides	–	–	AD001552
Amanita silvicola	–	–	AD001553
Amanita sp.	C601, E19	Guinea	AM117697
Amanita sp.	C342, C330, C324	Guinea	AM117668
Amanita sp.	C294	Guinea	AM117651
Amanita sp.	C314	Guinea	AM117657
Amanita sp.	C322, C348	Guinea	AM117659
Amanita sp.	C352	Guinea	AM117669
Amanita sp.	C378, C377, C19	Guinea	AM117682
Amanita sp.	C44	India	AM117685
Amanita sp.	C68	India	AM117703
Amanita sp.	C95	India	AM117720
Amanita sp.	C99	India	AM117721
Amanita sp.	C6	India	AM117705
Amanita sp.	C17	India	AM117637
Amanita sp.	C21	India	AM117642
Amanita sp.	C288	Guinea	AM117647
Amanita sp.	C315, C606	Guinea	AM117658
Amanita sp.	C291	Guinea	AM117648
Amanita sp.	C173	Guinea	AM117636
Boletellus ananas	–	–	AD001558
Boletellus russellii	–	–	AD001560
Boletoid sp.	E160	Guinea	AM117622
Boletoid sp.	E2	Guinea	AM117627
Boletoid sp.	E319	Guinea	AM117628
Boletus edulis	–	–	AD001562
Boletus flaviporus	–	–	AD001563
Boletus satanas	–	–	AD001566
Boletus sp.	C39	Guinea	AM117683
Boletus sp.	C364	Guinea	AM117675
Boletus sp.	C661	Guinea	AM117701
Boletus sp.	C510	Guinea	AM117689
Boletus sp.	M332, C332	Guinea	AM117635
Boletus sp.	C170	Guinea	AM117625
Boletus viridiflavus	–	–	AD001569
Cantharellus cibarius	–	–	AD001573
Cantharellus cinnabarinus	–	–	AD001574
Cantharellus tubaeformis	–	–	AD001575
Chalciporus piperatoides	–	–	AD001576
Chalciporus sp.	C365	Guinea	AM117676
Gyroporus cyanescens	–	–	AD001591
Hydnum rufescens	–	–	AY293257
Lactarius aff. *gymnocarpus*	C329	Guinea	AM117664
Lactarius aff. *medusae*	C841	Guinea	AM117716
Lactarius aff. *pulchrispermus*	C158	Guinea	AM117613
Lactarius annulatoangustifolius	C360, C62	Guinea	AM117673
Lactarius cf. *brunnescens*	C63	Guinea	AM117699
Lactarius gymnocarpus	C842	Guinea	AM117717
Lactarius piperatus	–	–	AD001603

Table 1 (continued)

Taxon	Herbarium number	Collection site	Accession number[a]
Lactarius ruvubuensis	C305, C185, C8	Guinea	AM117654
Lactarius sp.	C194, C151, C152	Guinea	AM117640
Lactarius sp.	C703, E25, E340, E321, E318	Guinea	AM117706
Lactarius sp. nov. *Plinthogali*	C13, E13	Guinea	AM117608
Lactarius sp.	C49	India	AM117687
Lactarius volemus	–	–	AD001604
Leccinum duriusculum	–	–	AF484444
Leccinum sp.	E223, C573, E10, C640, C723, E341, C367	Guinea	AM117626
Leccinum sp.	C32, E32, E14, C46	Guinea	AM117665
Leccinum sp.	C570, C355, C349	Guinea	AM117693
Leccinum sp.	C3	India	AM117684
Leccinum sp.	C59	Guinea	AM117696
Pisolithus arhizus	–	–	AD001620
Pluteaceae sp.	C70	Guinea	AM117707
Russala brevipes	–	–	AF156913
Russula aff. *annulata*	C189	Guinea	AM117639
Russula aff. *annulata*	C66, C356, C154	Guinea	AM117702
Russula aff. *azurea*	C36	India	AM117679
Russula aff. *delica*	C27	India	AM117645
Russula aff. *emeticella*	C64	India	AM117700
Russula aff. *parasitica*	C728, C568	Guinea	AM117708
Russula aff. *pectinata*	C81	India	AM117715
Russula aff. *pectinatoides*	C1	India	AM117641
Russula aff. *pruinata*	C312, C192	Guinea	AM117656
Russula aff. *pseudodelica*.	C93	India	AM117718
Russula aff. *rosea*	C73	India	AM117710
Russula aff. *senecis*	C94	India	AM117719
Russula aff. *subfoetens*	C74	India	AM117711
Russula burkei	–	–	AY010269
Russula cellulata	C373	Guinea	AM117681
Russula cf. *radicans*	C51	Guinea	AM117690
Russula compacta	–	–	AF393148
Russula congoana	C14, C65, C74, E20	Guinea	AM117609
Russula discopus	C293, C371	Guinea	AM117650
Russula earlei	–	–	AF518722
Russula exalbicans	–	–	AY293269
Russula liberiensis	C183	Guinea	AM117638
Russula meleagris	C292, C50, C68, C639, C155, C375	Guinea	AM117648
Russula parasitica	C2, C191	Guinea	AM117652
Russula rosacea	–	–	AD001633
Russula sp.	C597, C598, C629, C715, C75	Guinea	AM117695
Russula sp.	C357, C190	Guinea	AM117671
Russula sp.	C11	Guinea	AM117606
Russula sp.	C621	Guinea	AM117698
Russula sp.	C372	Guinea	AM117680
Russula sp.	C7, E121	Guinea	AM117713
Russula sp.	C353	Guinea	AM117670
Russula sp.	C6	Guinea	AM117704
Russula sp.	C334, E334	Guinea	AM117667
Russula sp.	C4	India	AM117688
Russula sp.	C76	India	AM117712
Russula sp. nov. *Archaeina*	C53	Guinea	AM117691
Russula sp. nov. aff. *sesenagula*	C366	Guinea	AM117677
Russula xerampelina	–	–	AY323507
Russuloid sp.	E18	Guinea	AM117623
Scleroderma citrinum	–	–	AF393149

Table 1 (continued)

Taxon	Herbarium number	Collection site	Accession number[a]
Scleroderma hypogaeum	–	–	AF114468
Scleroderma sp.	C24	India	AM117644
Scleroderma sp.	C7	India	AM117714
Scleroderma sp.	C55	India	AM117692
Scleroderma sp.	C156, E119, E84	Guinea	AM117611
Scleroderma sp.	C157, C361, E361, E17, E83, E127, E136, E142, E143, E150	Guinea	AM117612
Scleroderma sp.	C302	Guinea	AM117653
Scleroderma sp.	C22, E22	Guinea	AM117643
Scleroderma sp.	C109, C408	Guinea	AM117605
Scleroderma sp.	M296, C296	Guinea	AM117634
Scleroderma sp.	C27	India	AM117646
Scleroderma sp.	C12	India	AM117607
Scleroderma sp.	C153, C320	Guinea	AM117610
Sclerodermatoid sp.	E9, E29	Guinea	AM117633
Sclerodermatoid sp.	E124, E81	Guinea	AM117723
Sclerodermatoid sp.	E137	Guinea	AM117618
Sebacina sp.	–	–	AD001635
Strobilomyces floccopus	–	–	AD001640
Strobilomyces sp.	C363, E53	Guinea	AM117674
Thelephora terrestris	–	–	AD001647
Thelephoroid sp.	E21	Guinea	AM117624
Thelephoroid sp.	E42	Guinea	AM117629
Thelephoroid sp.	E01, E02, E03, E05	Guinea	AM117722
Thelephoroid sp.	E128	Guinea	AM117615
Thelephoroid sp.	E51	Guinea	AM117630
Thelephoroid sp.	E138	Guinea	AM117618
Thelephoroid sp.	E130, E132, E135, E06, E07	Guinea	AM117616
Thelephoroid sp.	E139, E140	Guinea	AM117620
Thelephoroid sp.	E134	Guinea	AM117617
Thelephoroid sp.	E148	Guinea	AM117621
Thelephoroid sp.	E55	Guinea	AM117631
Thelephoroid sp.	E82	Guinea	AM117632
Tomentella atrorubra	–	–	U86858
Tomentella sp.	C30	Guinea	AM117655
Tricholoma sp.	C331, C347	Guinea	AM117666
Tricholoma pardinum	–	–	AD001654
Tricholoma sp.	C327	Guinea	AM117662
Tricholoma sp.	C572	Guinea	AM117694
Tricholomatoid sp.	C324	Guinea	AM117661
Tricholomatoid sp.	C369	Guinea	AM117678
Tricholomatoid sp.	C323	Guinea	AM117660
Tricholomatoid sp.	C328, C317	Guinea	AM117663
Tubosaeta brunneosetosa	C16	Guinea	AM117614
Tulasnella irregularis	–	–	AD001656
Xerocomus chrysenteron	–	–	AD001659
Xerocomus sp.	C358, C567	Guinea	AM117672
Xerocomus subtomentosus	–	–	AD001660

C for sporocarps, *E* for ectomycorrhizas, *M* for mycelium (pure culture)

[a] The sequence from Guinea and India are available on GenBank Database under the accession numbers listed above.

sequences were grouped within a single clade. All other sequences were intermingled in the phylogeny, including mostly *Boletus* and *Xerocomus* species. The single *Chalciporus* sequence was located close to *Chalciporuspiperatoides* and roots the rest of the boletoid group sensu stricto. Finally, in the fourth analysis, all Amanitaceae cluster together in a highly supported monophyletic clade (Fig. 5), whereas Tricholomataceae (including Plutaceae, *Hydnum*, and *Tricholoma* genera) were paraphyletic. It should be noted that *Hydnum* is known to belong to the Cantharellales,

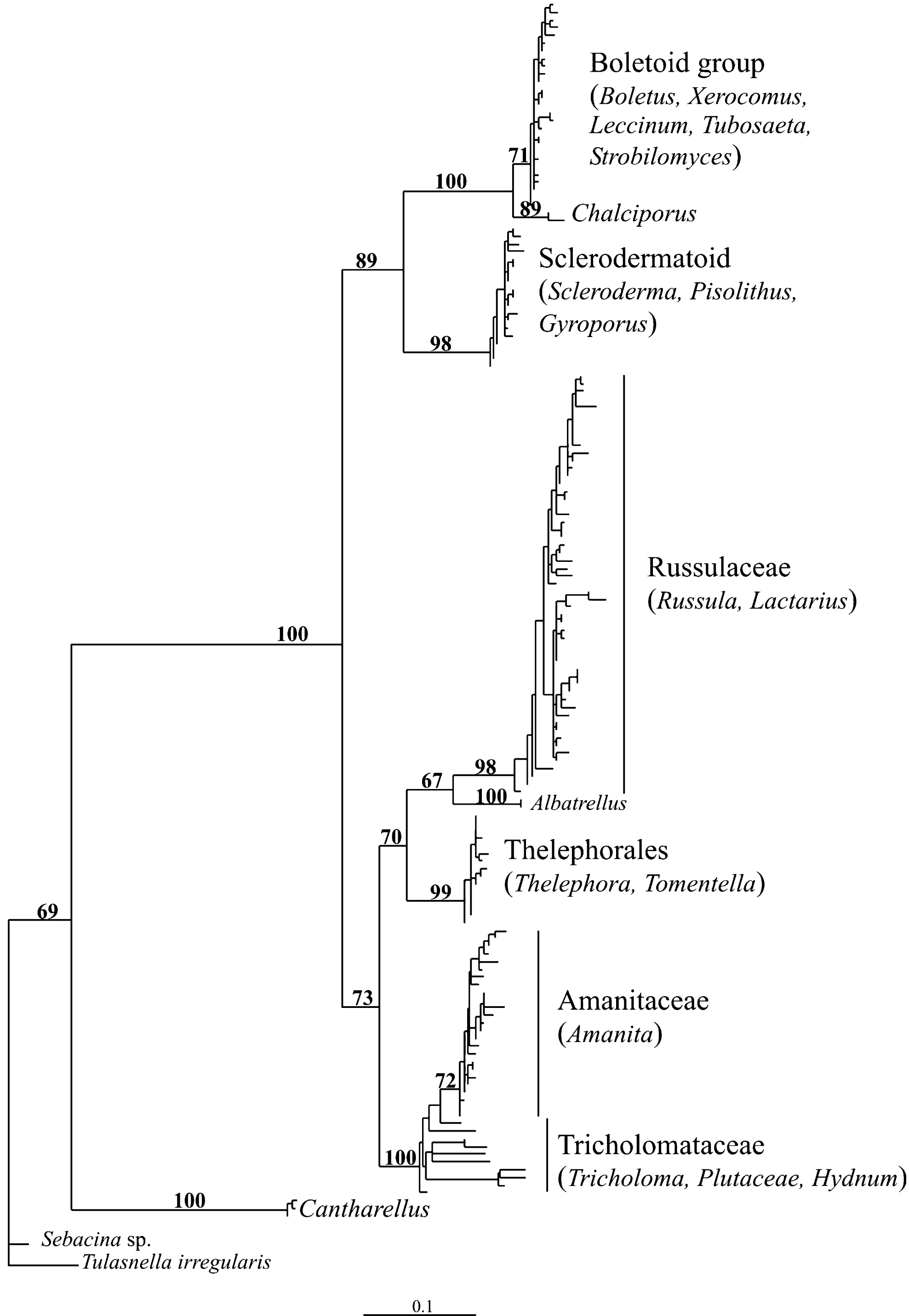


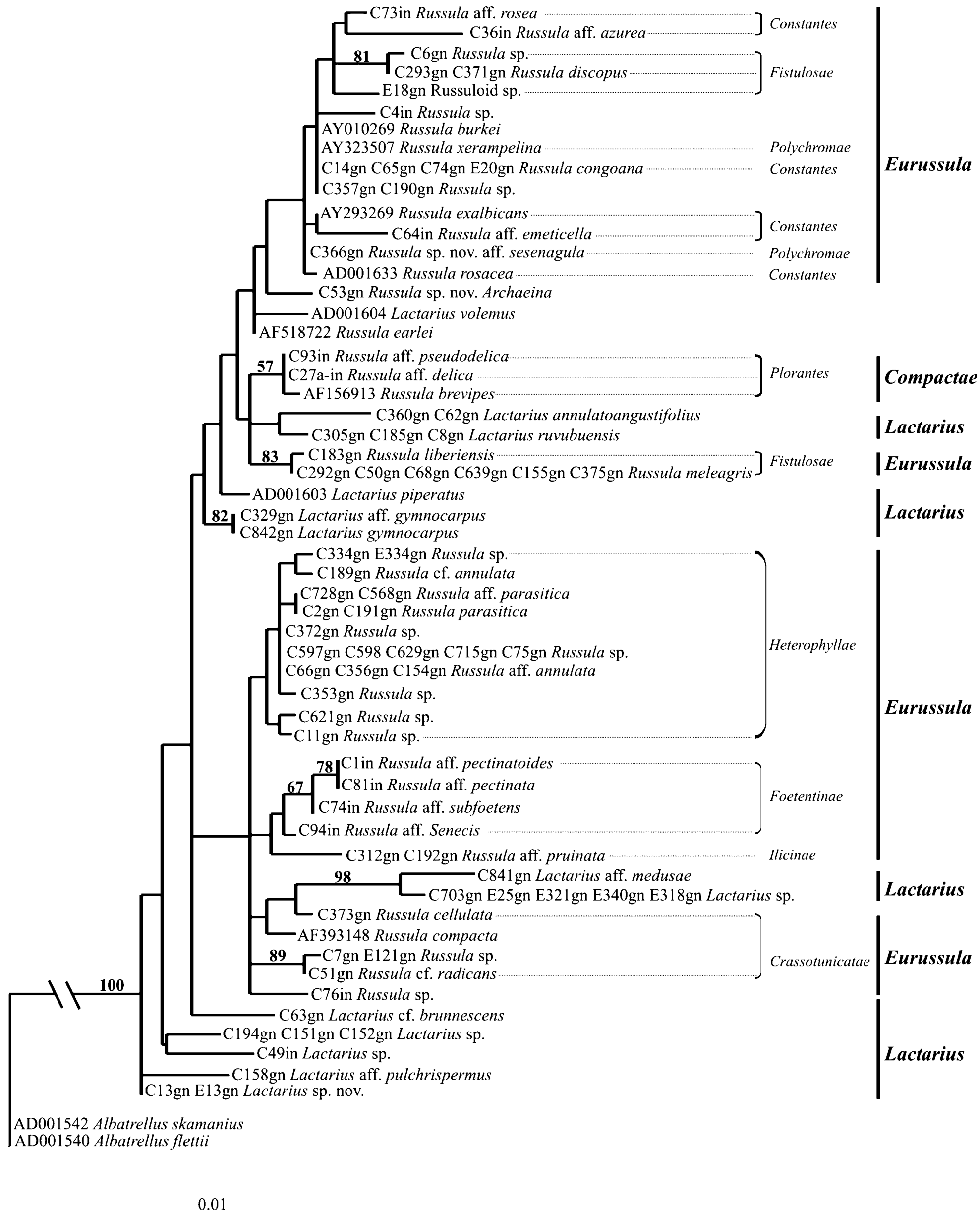
Fig. 1 Maximum likelihood ML5–ML6 using a HKY85+ γ+Inv model (α=0.5684, proportion of invariable sites=0.3421, rate categories=4) for 160 sequences and 389 sites. Bootstrap support values greater than 50% are indicated at the relevant nodes. Main taxonomical groups included in the phylogeny are indicated. *Sebacina* sp. and *Tulanesnella irregularis* were chosen as out-groups according Bruns et al. (1998)

not Tricholomataceae. The "misplacement" of this genus in our tree (*Hydnum rufescens*, AY293257) is most probably due to a misidentification in the Genbank database.

Discussion

Bruns et al. (1998) claimed that the ML5/ML6 region often provides identical sequences for closely related species, thus limiting the interest of this conservative DNA fragment at the species level. They proposed that the ITS fragment, on the contrary, is more accurate for species identification. We chose to focus on ML5/ML6 sequences for three main reasons: (1) With regards to the high morphological diversity of sporocarps and ectomycorrhizas found in the field, it first appeared pivotal to obtain rapid but nonambiguous results at the genus level; (2) as recommended by Gardes and Bruns (1993), we previously sequenced the ITS

Fig. 2 Russulaceae maximum likelihood ML5–ML6, using a HKY85+γ+Inv model (α=0.5897, proportion of invariable sites=0.6539, rate categories=4) for 57 different sequences and 391 sites. Bootstrap support values greater than 50% are indicated at the relevant nodes. Identical sequences are included in the same terminal node. The brackets to the right of the tree indicate the clades including species of the same section, and vertical lines indicate sections of the same subgenera (*in bold*). Names and grouping follow Singer (1986), Romagnesi (1985), and Miller and Buyck (2002) classifications. *gn* Sample from Guinea, *in* Indian sample. Equality between numbers means perfect homology between their sequences



fragment of 37 Guinean *Russula* and 7 Indian *Russula* specimens, but sequences were too variable to be unambiguously aligned, and none of the sequences matched with known species (Rivière 2004); and (3) we consistently observed that ITS primers often amplify plant DNA from ectomycorrhizas, whereas the ML5/ML6 primers never did.

Our molecular analyses allowed genera-level identification of all sporocarps and otherwise unidentified ectomycorrhizas. Interestingly, among the 55 ECM sequences, a strict correspondence between sporocarps and ectomycorrhizas was obtained for only 27 of them. This is most probably due to the fact that many but not all ECM fungi produce fruitbodies. Moreover, sporocarp production also varied over the season, thus escaping sampling (Kõljalg et al. 2000; Erland et al. 1999). For instance, 20 ectomycorrhizas, but no sporocarps, were found to be closely related to thelephoroid taxa. It is well known that sporocarps are seldom produced by Telephorales, but they commonly form mycorrhizas on roots of young trees (Kõljalg et al. 2000). Our study has once again confirmed that belowground fungal diversity is dissimilar from that of aboveground sporocarps (Gardes and Bruns 1996; Dahlberg et al. 1997; Jonsson et al. 1999), thus underlying the importance of molecular analyses for the assessment of ECM fungal diversity.

Some differences were noted in the fungal richness between the two sites, as well as between the tropical and more northern areas, as described in the literature. Russu-

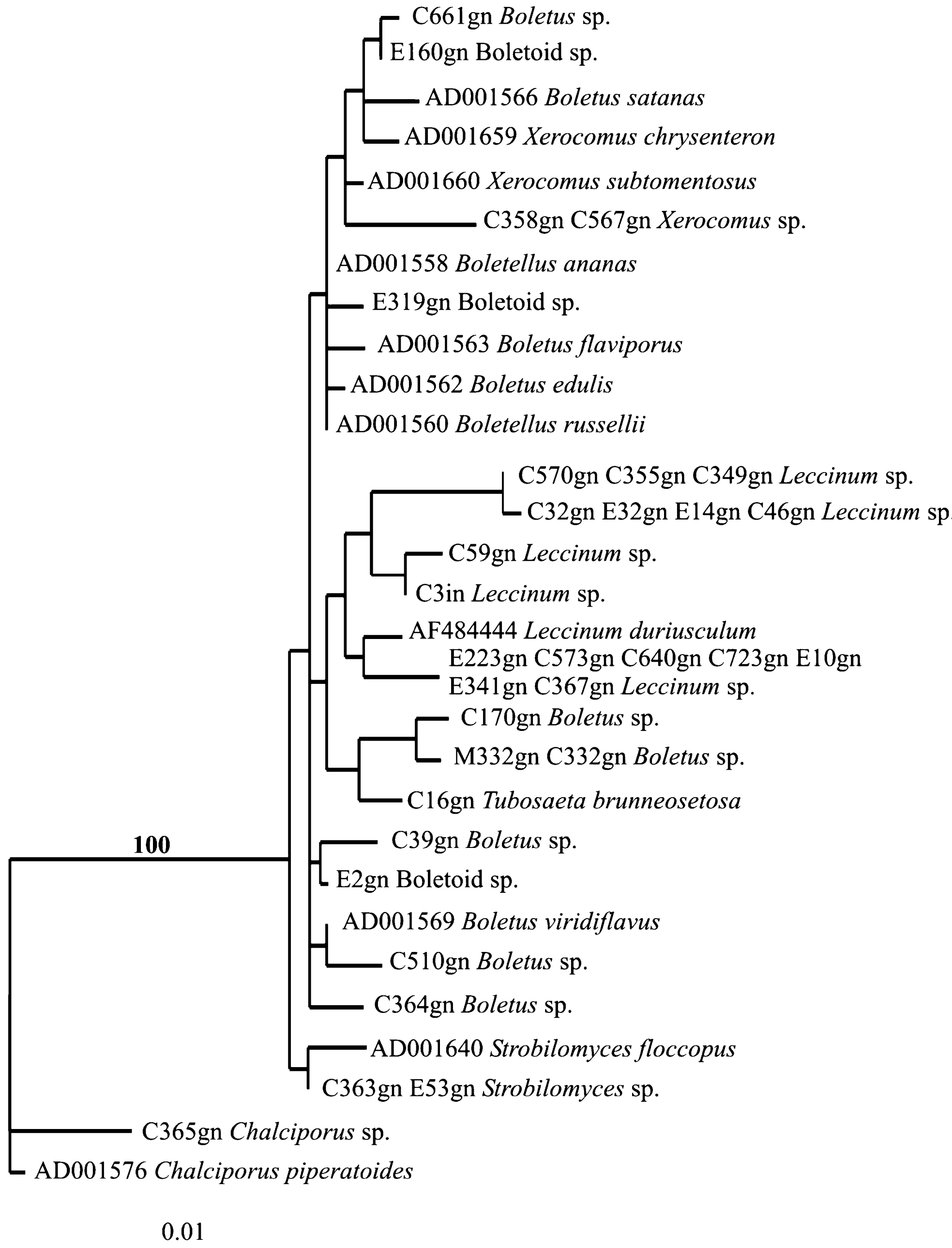


Fig. 3 Boletoids maximum likelihood ML5–ML6, using a HKY85+γ+Inv model (α=0.8894, proportion of invariable sites=0.6875, rate categories=4) for 29 different sequences and 375 sites. Bootstrap support values greater than 50% are indicated at the relevant nodes. Identical sequences are included in the same terminal node. *gn* Guinean sample, *in* Indian sample. Equality between numbers means perfect homology between their sequences

laceae genotypes sequenced from the Guinean site, which include vouchered new species, are in accordance with the observations of Buyck (1994a,b, 1997) suggesting a high species diversity of this family in Africa. However, no species from India have been found to be common with those of Guinea. This lack of shared species (or sequences) between Africa and India suggests that there has been no recent gene flow between the two continents. Berbee and Taylor (1993, 2001) suggested that the divergence within ECM fungi occurred around 180 Mya, whereas separation of the Indian–Malagasy block from Africa is usually dated around 120 Mya. Based on morphological identifications, Russulaceae described in the Uppangala forest site may be related to known species from Europe (Natarajan et al. 2005). However, none of the *Russula* nor *Lactarius* species collected in India share the same sequence with already known African or European taxon. Molecular phylogeography of tropical species of the genus *Russula* based on nuclear sequences is urgently needed to resolve such an interesting ECM group.

A single Boletaceae sample was recovered from India (identified as *Leccinum* sp.), whereas 17 genotypes (and so species sensu Bruns et al. 1998) were found in Guinea. Accordingly, Natarajan et al. (2005) described only two

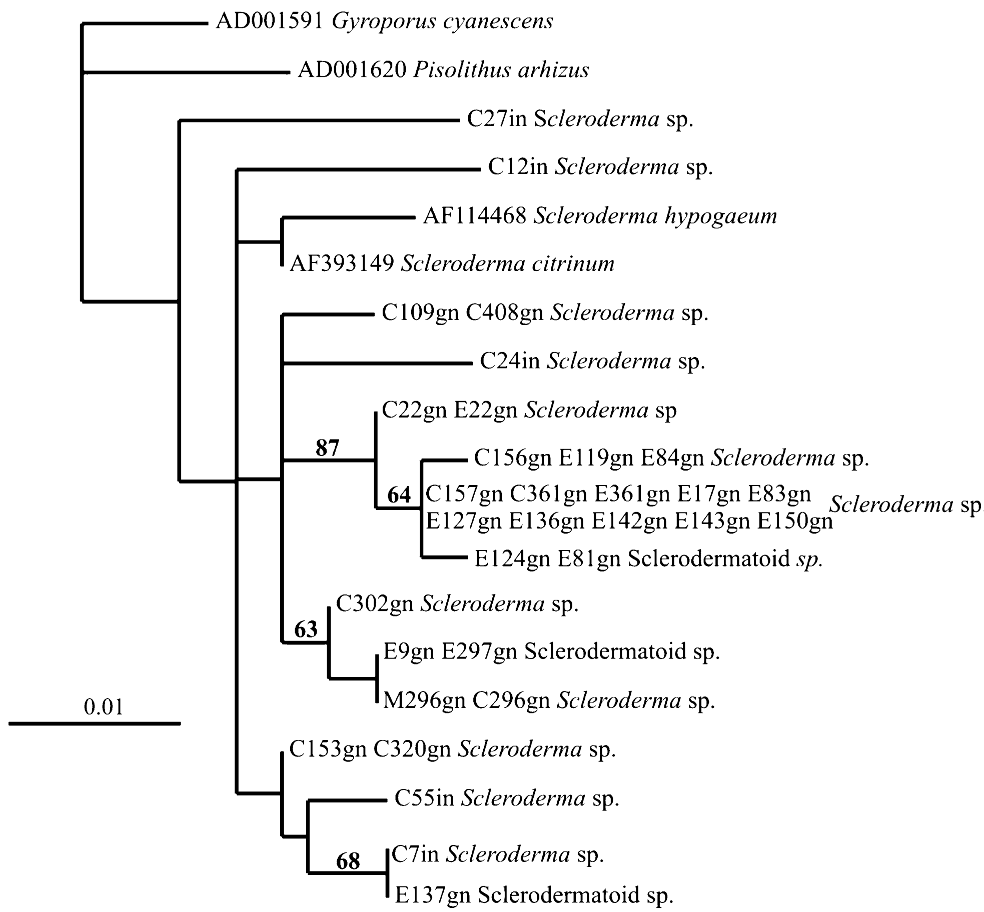


Fig. 4 *Sclerodema* maximum likelihood ML5–ML6, using a F81+γ+Inv model (α=1.391, proportion of invariable sites= 0.6401, rate categories=4) for 19 different sequences and 391 sites. Bootstrap support values greater than 50% are indicated at the relevant nodes. Identical sequences are included in the same terminal node. *gn* Guinean sample, *in* Indian sample. Equality between numbers means perfect homology between their sequences

Suillus and one *Strobilomyces* species in India. In addition, very few Cortinariaceae specimens or species belonging to the Suilloid group have been found so far in tropical forests (e.g., *Cortinarius* in Cameroon and in India; Onguene and Kuyper 2001; Natarajan et al. 2005). Our study supports this trend, as none of the sequences available in Genbank database and used here as genetic benchmarks fell within Cortinariaceae or Suilloids. Nevertheless, tropical forests are still undersampled relative to northern boreal forests, and further surveys are clearly needed to confirm this result.

The maximum likelihood tree topology provides well-supported relationships at the family level. The placement of all collected sporocarps is in agreement with the morphological determinations at the family or genus level. At lower systematic levels, the situation is not as clear. Apart from the discovery of a new species in Uppangala, *Pisolithus* viz. *P. indicus*, numerous Basidiomycete sporocarps collected were morphologically identified as closely related to already known European species (Natarajan et al. 2005). In Guinea, a great diversity of sequences belonging to the Russulacae family was obtained. Morphological taxonomies of this family (Romagnesi 1985; Singer 1986; Buyck 1994a,b, 1997) are rarely congruent, reflecting the ambiguities of characters used in the classification of this group. In a similar way, some of the sporocarps collected were morphologically identified as already described species, but many others could not be linked to already known morphotypes. The latter may represent putative new species, and further studies are required (species identification in progress, all specimens housed at the Muséum National d'Histoire Naturelle and Centre of Advanced Study in Botany). So far, three have been formally identified as new species, *Russula* sect. *Archaeinae* sp. nov. (C53), *Russula* sp. nov. aff. *sesenagula* (C366), and *Lactarius* nov. (C13). However, recognition of the remaining ones as new taxa would be premature at this stage.

Several of the internal nodes exclusively grouped members of the same subgenus or section according to the morphological classification of sporocarps. Sections *Foetentinae*, *Heterophyllae*, and *Plorantes* (subgenus *Compactae*) each formed monophyletic groups. The three, *Foetentinae*, *Heterophyllae*, and *Plorantes*, sections are all well-defined taxa that are known to be well represented and highly diversified in tropical Africa, with most of the species being only encountered on this continent (Buyck 1997). It is noteworthy that the *Heterophyllae* form a single clade with large specific diversity. A major *Eurussula* clade includes all *Polychromae* and *Constantes* samples but also one clade of *Fistulosae*. *Polychromae*, and *Constantes* sections are intermingled. This pattern may be due to the

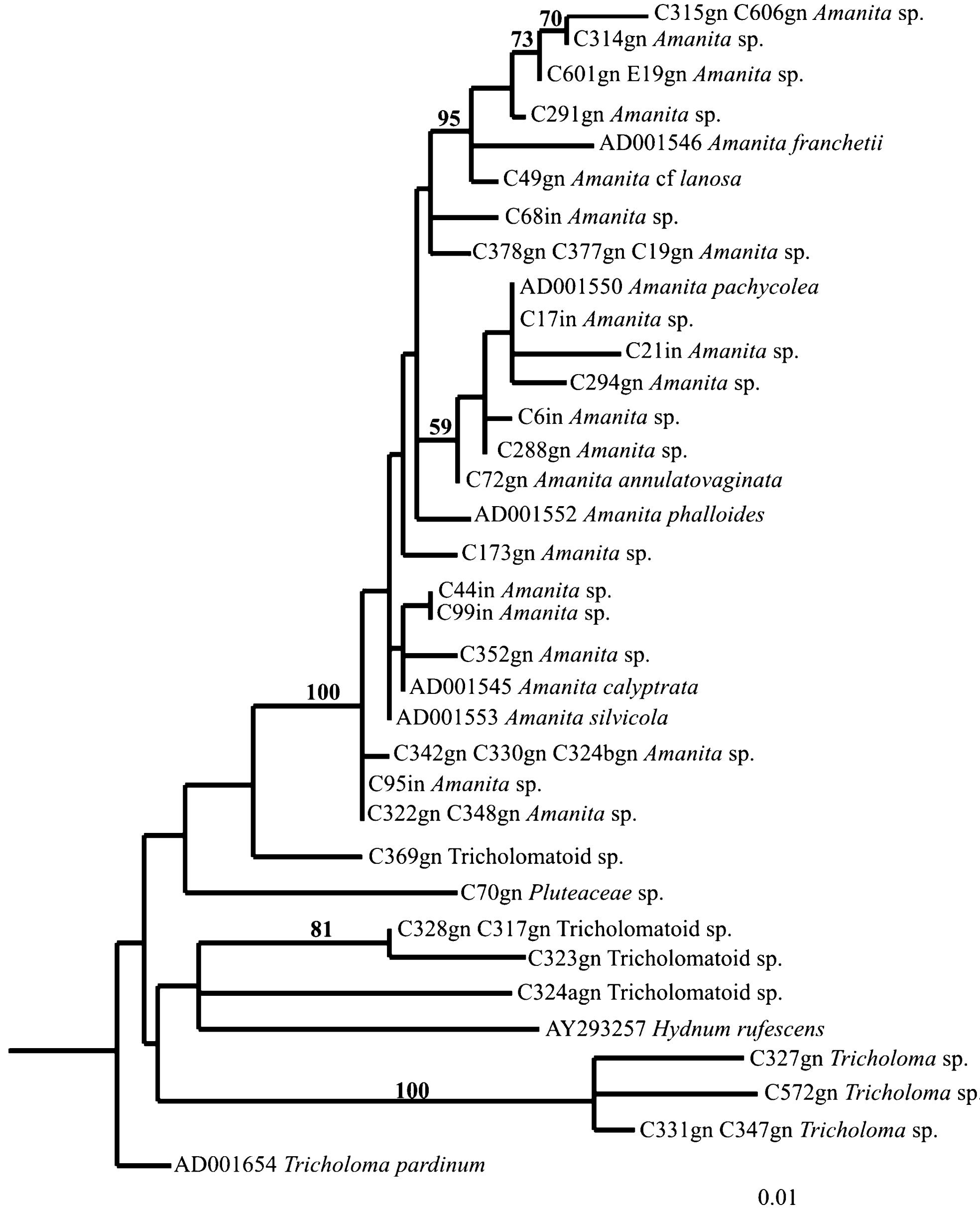


Fig. 5 Amanitaceae and Tricholomataceae maximum likelihood ML5–ML6, using a F81+γ+Inv model

limit of resolution of the ML5/ML6 marker. Finally, our phylogeny shows that *Fistulosae* diverged into two distinct clades that may represent two different sections. However, because of the low variability of DNA fragments, this groups would deserve to be reanalyzed using another nuclear marker (such as the large subunit [LSU] rDNA gene), to confirm these taxonomic issues.

Discrimination between *Russula* and *Lactarius* is based on the exudation upon flesh injuries and the extension of the lactiferous system into the hymenium in *Lactarius* and the lack of this character in *Russula* genus (Singer 1986). In our phylogenetic analyses, *Lactarius* appears as a paraphyletic group, whereas Shimono et al. (2004) supported the monophyly of all *Lactarius* species based on LSU rDNA. Once again, this discrepancy is probably due to the low variability of the ML5/ML6 fragment at this level.

Within the *Russula* genus, the subgenus *Eurussula* appears as a polyphyletic group, being split into four separated clades. This is not congruent with phylogeny based on nuclear regions (Eberhardt and Verbeken 2004; Eberhardt 2002; Miller and Buyck 2002). These differences can have several nonexclusive origins: (1) various resolution levels of the markers used among studies, because of different rates and modes of molecular evolution, (2) complex relationships between nuclear and mitochondrial genomes, or (3) the reduced level of resolution at the species level of the locus used in our study (Doyle 1992; Bull et al. 1993; Bruns and Szaro 1992). It is known that mitochondrial genomes evolved at least partially independently from the nuclear genome, thus sometimes leading to incongruent phylogenetic inferences (Moncalvo et al. 2000). Other potential sources of incongruence between these genomes are ancestral polymorphisms, horizontal transfers, etc. (Wall 2003). Such phenomena are not rare in plants and may obscure ECM Basidiomycete relationships as well. Unfortunately, too few molecular investigations have been performed so far to conclude (Hibbett et al. 2000; Moncalvo et al. 2000; Binder and Hibbett 2002; Miller and Buyck 2002, den Bakker et al. 2004).

The important diversity of the ECM fungi found in both the forests studied may be linked to tree species diversity, which is today endangered by strong human pressure that threatens tropical rain forests. The composition of ECM associations and the changes they undergo are still very poorly known in tropical regions. Thus, it has become urgent to improve our knowledge of the systematics and the ecology of tropical ECM symbiosis, as they constitute active partners of the forest ecosystems and may play a key role in forest regeneration.

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References

Alexander IJ, Högberg P (1986) Ectomycorrhizas of tropical angiospermous trees. New Phytol 102:541–549

Baura G, Szaro TM, Bruns TD (1992) *Gastrosuillus laricinus* is a recent derivative of *Suillus grevilleii*: molecular evidence. Mycologia 84:592–597

Berbee ML, Taylor JW (1993) Dating the evolutionary radiations of the true fungi. Can J Bot 71:1114–1127

Berbee ML, Taylor JW (2001) Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin RG, Lemke PA (eds) The Mycota VII. Part B. Systematics and evolution. Springer, Berlin, pp 229–245

Binder M, Hibbett DS (2002) Higher-level phylogenetic relationships of Homobasidiomycetes (Mushroom-forming fungi) inferred from four rDNA regions. Mol Phylogenet Evol 22:76–90

Bruns TD, Szaro TM (1992) Rate and mode differences between nuclear and mitochondrial small-subunit rRNA genes in mushrooms. Mol Biol Evol 9:836–855

Bruns TD, Szaro TM, Gardes M et al (1998) A sequence database for the identification of ectomycorrhizal Basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272

Bull JJ, Huelsenbeck PP, Cunningham CW, Swofford DL, Waddell PJ (1993) Partitioning and combining data in phylogenetic analysis. Syst Biol 42:384–397

Buyck B (1994a) *Russula* I (Russulaceae). Flore Illus Champignons Afr Cent 15:335–408

Buyck B (1994b) *Russula* II (Russulaceae). Flore Illus Champignons Afr Cent 16:411–542

Buyck B (1997) *Russula* III (Russulaceae). Flore Illus Champignons Afr Cent 17:545–597

Buyck B, Thoen D, Walting R (1996) Ectomycorrhizal fungi of the Guinea–Congo Region. Proc R Soc Edinb Sect B 104:313–333

Dahlberg A, Jonsson L, Nylund JE (1997) Species diversity and distribution of biomass above and belowground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. Can J Bot 75:1323–1335

Debaud JC, Marmeisse R, Gay G (1999) Intraspecific genetic variation and populations of ecomycorrhizal fungi. In: Varma AK, Hock B (eds) Mycorrhiza: structure, molecular biology and function. Springer, Berlin, pp 75–110

den Bakker HC, Zuccarello GC, Kuyper TH W, Noordeloos ME (2004) Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. New Phytol 163:201–215

Doyle JJ (1992) Gene trees and species trees: molecular systematics as one-character taxonomy. Syst Bot 17:144–163

Eberhardt U (2002) Molecular analyses of the agaricoid *Russulaceae*: correspondence with mycorrhizal and sporocarp features in the genus *Russula*. Mycol Prog 1(2):201–224

Eberhardt U, Verbeken A (2004) Sequestrate *Lactarius* species from tropical Africa: *L. angiocarpus* sp. nov. and *L. dolichocaulis* comb. nov. Mycol Res 108:1042–1052

Egger KN (1995) Molecular analysis of ectomycorrhizal fungal communities. Can J Bot 73:S1415–S1422

Erland S, Jonsson T, Mahmood S, Finlay RD (1999) Below-ground ectomycorrhizal community structure in two *Piceaabies* forests in southern Sweden. Scand J For Res 14:209–217

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

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

Gardes M, Bruns TD (1996) Community structure of ectomycorrhizal fungi in a *Pinusmuricata* forest: above and below-ground views. Can J Bot 74:1572–1583

Gardes M, White TJ, Fortin J, Bruns TD, Taylor JW (1991) Identification of indigenous and introduced symbiotic fungi in ectomycorrhizas by amplification of nuclear and mitochondrial ribosomal DNA. Can J Bot 69:180–190

Grogan P, Baar J, Bruns TD (2000) Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. J Ecol 88:1051–1062

Hibbett DS, Gilbert LB, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbioses in Basidiomycetes. Nature 407:506–508

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10:1855–1871

Huelsenbeck JO, Rannala B (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276:227–232

Jonsson L, Dahlberg A, Nilsson M-C, Kårén O, Zackrisson O (1999) Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinussylvestris* forests studied in comparing mycobiont diversity on seedlings and mature trees. New Phytol 142:151–162

Jonsson L, Dahlberg A, Brandrud T-E (2000) Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Piceaabies* forest subjected to experimental nitrogen addition: above and below-ground views. For Ecol Manag 132:143–156

Kõljalg U, Dahlberg A, Taylor AFS et al (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Mol Ecol 9:1985–1996

Kõljalg U, Larsson KH, Abarenkov K et al (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166:1063–1068

Kretzer AM, Dunham S, Molina R, Spatafora JW (2003) Microsatellite markers reveal the below ground distribution of genets in two species of *Rhizopogon* forming tuberculate ectomycorrhizas on Douglas fir. New Phytol 161:313–320

Lee SS, Alexander IJ, Watling R (1997) Ectomycorrhizas and putative ectomycorrhizal fungi of *Shorealeprosula* Miq. (Dipterocarpaceae). Mycorrhiza 7:63–81

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Maury-Lechon G, Curtet L (1998) Biogeography and evolutionary systematics of Dipterocarpaceae. In: Appanah S, TurnbullU MJ (eds) A review of dipterocarps taxonomy, ecology and silviculture. CIFOR, Jakarta, pp 5–44

Miller SL, Buyck B (2002) Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. Mycol Res 106:259–276

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbiosis: community—ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning. Chapman & Hall, London, UK, pp 357–423

Moncalvo JM, Drehmel D, Vilgalys R (2000) Variation in modes and rates of evolution in nuclear and mitochondrial ribosomal DNA in the mushroom genus *Amanita* (*Agaricales*, Basidiomycota): phylogenetic implications. Mol Phylogenet Evol 16:48–63

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

Natarajan K, Senthilarasu G, Kumaresan V, Rivière T (2005) Diversity in ectomycorrhizal fungi of a dipterocarp forest in Western Ghats. Curr Sci 88(12):1893–1895

Newbery DM, Alexander IJ, Rother LA (1997) Phosphorus dynamics in lowland African rain forest: the influence of ectomycorrhizal trees. Ecol Monogr 67:367–409

Nicholas KB, Nicholas HB, Deerfield DW II (1997) GeneDoc: analysis and visualization of genetic variation. Embnet News 4:1–4

Onguene NA, Kuyper TW (2001) Mycorrhizal associations in the rain forest of South Cameroon. For Ecol Manag 140:277–287

Pascal JP, Pélissier R (1996) Structure and floristic composition of a tropical evergreen forest in south-west India. J Trop Ecol 12:191–211

Pélissier R, Pascal JP, Houllier F, Laborde H (1998) Impact of selective logging on the dynamics of a low elevation dense moist evergreen forest in the Western Ghats (South India). For Ecol Manag 105:107–119

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Redhead JF (1980) Mycorrhiza in natural tropical forests. In: Mikola P (ed) Tropical mycorrhiza research. Clarendon, Oxford, pp 127–142

Rivière T (2004) Biodiversity, molecular ecology and phylogeography of tropical ectomycorrhizal symbiosis. Ph.D. thesis, Université de Montpellier II, France

Rivière T, Natarajan K, Dreyfus B (2005) Spatial distribution of ectomycorrhizal Basidiomycete *Russula* subsect. Foetentinae populations in a primary dipterocarp rainforest. Mycorrhiza 16:143–148

Romagnesi H (1985) Les Russules d’Europe et d’Afrique du Nord. J. Cramer, Lehre (reprint with supplement)

Sanon KB, Bâ AM, Dexheimer J (1997) Mycorrhizal status of some fungi fruiting beneath indigenous trees in Burkina Faso. For Ecol Manag 98:61–69

Shimono Y, Kato M, Takamatsu S (2004) Molecular phylogeny of *Russulaceae* (Basidiomycetes; Russulales) from the nucleotide sequences of nuclear large subunit rDNA. Mycoscience 45:303–316

Singer R (1986) The Agaricales in modern taxonomy, 4th edn. Koeltz Scientific, Koeningstein

Singer R, Araujo IJS (1979) Litter decomposition and ectomycorrhiza in Amazonian forests: 1. A comparison of litter decomposition and ectomycorrhizal Basidiomycetes in latosol–terra firme rain forest and white podzol Campinarana. Acta Amazon 9:25–41

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, San Diego, CA

Smits WTM (1992) Mycorrhizal studies in dipterocarp forests in Indonesia. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. Cambridge, pp 283–292

Swofford DL (2001) PAUP‡: phylogenetic analysis using parsimony (‡and other methods), ver. 4. Sinauer, Sunderland, MA

Thoen D, Bâ AM (1989) Ectomycorrhizas and putative ectomycorrhizal fungi of *Afzeliaafricana* Sm. and *Uapacaguineensis* Müll. Arg. in southern Senegal. New Phytol 113:549–559

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Wall JD (2003) Estimating ancestral population sizes and divergence times. Genetics 163:395–404

Watling R, Lee SS (1995) Ectomycorrhizal fungi associated with members of the Dipterocarpaceae in Peninsular Malaysia. J Trop For Sci 7:657–669

White TJ, Bruns TD, Lee SS, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelf DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and application. Academic, San Diego, pp 315–322