Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae)

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**Summary**

- Ectomycorrhizal (ECM) fungi form highly diverse communities in temperate forests, but little is known about their community ecology in tropical ecosystems.
- Using anatomotyping and rDNA sequencing, ECM fungi were identified on root tips of the introduced *Eucalyptus robusta* and *Pinus caribea* as well as the endemic *Vateriopsis seychellarum* and indigenous *Intsia bijuga* in the Seychelles.
- Sequencing revealed 30 species of ECM fungi on root tips of *V. seychellarum* and *I. bijuga*, with three species overlapping. *Eucalyptus robusta* shared five of these taxa, whereas *P. caribea* hosted three unique species of ECM fungi that were likely co-introduced with containerized seedlings. The thelephoroid (including the anamorphic genus *Riessiella*), euagaric, boletoid and hymenochaetoid clades of basidiomycetes dominated the ECM fungal community of native trees. Two species of Annulatascaceae (Sordariales, Ascomycota) were identified and described as ECM symbionts of *V. seychellarum*.
- The low diversity of native ECM fungi is attributed to deforestation and long-term isolation of the Seychelles. Native ECM fungi associate with exotic eucalypts, whereas co-introduced ECM fungi persist in pine plantations for decades.

**Key words:** community structure of ectomycorrhizal (ECM) fungi, DNA barcoding, exotic forest plantations, host specificity, *Intsia bijuga* (Caesalpiniaceae), island biogeography, *Vateriopsis seychellarum* (Dipterocarpaceae).


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**Introduction**

Ectomycorrhizal (ECM) fungi comprise an estimated 7000–10 000 species worldwide (Taylor & Alexander, 2005), most of which remain undescribed. The majority of these undescribed species likely inhabit poorly studied tropical ecosystems and/or form inconspicuous or no fruit-bodies (e.g. ascomycetes and members of the thelephoroid, sebacinoid and athelioid clades of basidiomycetes). ECM fungi with such inconspicuous fruit-bodies dominate fungal communities in boreal and temperate forest ecosystems (Horton & Bruns, 2001). In temperate forests of the northern and southern hemispheres, ECM fungi form highly diverse communities of up to a few hundred species per site (May & Simpson, 1997; Horton & Bruns, 2001; Bastias et al., 2006; Tedersoo et al., 2006). *Pseudotsuga menziesii* harbors c. 2000 species of ECM.
fungi throughout its range (Trappe, 1977), whereas a single
tree may host more than 15 species (Saari et al., 2005). Such
a high fungal species richness most likely results from
differential preference for hosts and soil conditions (Bruns,
1995). Some recent studies indicate that ECM fungi deliver
species- and even strain-specific benefits to their host plants
(Wong et al., 1989; van der Heijden & Kuyper, 2003). Such
functional diversity, especially in enzymatic activities (Courty
et al., 2005) and nutrient acquisition strategies (van der
Heijden & Kuyper, 2003), results in greater biomass and
production of host plants with increasing symbiont richness
(Baxter & Dighton, 2005).

In tropical ecosystems, the ecology and function of ECM
fungi have remained poorly understood, and much of the
information on community composition relies on fruit-body
surveys. Despite some ecological limitations, fruit-body surveys
indicate that tropical rainforests and savannas support highly
diverse communities of ECM fungi (Henkel et al., 2002;
Verdeken & Buyck, 2002; Watling et al., 2002; Lee et al.,
2003). A few preliminary below-ground studies support these
findings in Diterocarpaceae (Sirikantaramas et al., 2003;
Moyersoen, 2006) and closely related Sarcofoaceae (Ducousso
et al., 2004). By contrast, Pisonia grandis R. Br. (Nyctaginaceae)
hosts a few thelephoroid ECM symbionts in Australia (Chambers
et al., 2005), whereas neotropical members of Nyctaginaceae
(including Pisonia, Neoa and Guatipus spp.) seem to be associated
with additional fungal lineages (Moyersoen, 1993; Lodge,
1996; Haug et al., 2005).

There is limited information on the biogeography of
ECM fungi, but most fungal genera are globally distributed,
suggesting their antiquity and/or effective long-distance dis-
persal. Tiny spores of fungi and other cryptogams are carried
by wind thousands of kilometers (Hallenberg & Kueffer, 2001;
Muñoz et al., 2004). For example, both Leptospermoideae
(Myrtaceae) and associated Pisolithus spp. most probably
colonized New Zealand from the Australian continent via
long-distance dispersal (Moyersoen et al., 2003). In addition
to dispersal, continental drift and climatic oscillations have
driven vicariance events and facilitated migration of entire
plant communities and their associated microbes (Wilkinson,
1998; Halling, 2001). More recently, humans have unintention-
tially facilitated the spread of pathogenic and symbiotic
fungi by importing forestry products and establishing exotic
forest plantations (Mikola, 1969; Wingfield et al., 2001). The
introduced pathogens have switched hosts and affected the
local plant communities and populations detrimentally
(Wingfield et al., 2001). There is little such information on
the invasion ecology of ECM fungi, because the natural
ranges of individual species are poorly documented. A few
review studies, however, suggest that two well-known fly agar-
ics, Amanita muscaria and A. phalloides, can become invasive
in native communities (Orlovich & Cairney, 2004; Pringle
& Vellinga, 2006). Establishment of exotic forest plantations
with pregrown tree seedlings is the main source of cointroduc-
tion of mycorrhizal fungi (Mikola, 1969). Such cointroduced
fungi, in turn, facilitate invasion of natural communities by
Eucalyptus spp. in Spain (Diez, 2005). In addition, exotic
fungi may deplete soil resources (Chapela et al., 2001). Native
organisms of small, isolated islands are especially vulnerable to
exotic pathogens and invasive species (Lomolino et al., 2006).

The granitic islands of the Seychelles represent mountain
tops of the largely submerged Mahé microcontinent, which
was separated from the Indian subcontinent (Deccan plate)
c. 65 million yr ago (mya; Briggs, 2003). Long-term isolation
of the Seychelles has resulted in the development of endemic-
rich biota. Fleischmann et al. (2003) considered 34% of the
native higher plant species endemic. Such a high degree of
endemicity, substantial deforestation and the small area render
the Seychelles highly vulnerable to invasive species. After
human settlement in 1770, up until the early 20th century,
native forests were gradually cut down and replaced mainly by
introduced cinnamon (Cinnamomum verum), which was
spread by birds. Coconut plantations (Cocos nucifera) replaced
low-altitude forest during much of the 19th and 20th centuries
as the main economic crop. Further deforestation occurred in
the early 20th century when commercial distillation of cinnamon
oil required huge amounts of wood fuel. Other introduced
trees, shrubs and lianas have since invaded the indigenous
plant communities. Native vegetation still exists in remote, less
accessible mountain regions and small uninhabited islands,
providing invaluable sources for taxonomists and biogeographers.
Among the native plants of the Seychelles, representatives of
three families, Diterocarpaceae, Caesalpiniaeae and Nyctagi-
naeae form ECM associations (Alexander & Lee, 2005). On
roots of P. grandis (Nyctaginaceae), two unidentified species of
ECM fungi were previously documented in the Seychelles
(Asford & Allaway, 1985).

The aims of this study were to document the above- and
below-ground diversity of ECM fungi of both the indigenous
Intsia bijuga (Caesalpiniaeae) and the endemic Vateriopsis
seychellarum (Diterocarpaceae) as well as the introduced
Eucalyptus robusta and Pinus caribea in the Seychelles. Using
molecular sequence data, it is shown that ECM communities
of the native plants are species-poor and overlap with symbionts
of the introduced E. robusta, but not P. caribea.

Materials and Methods

ECM host plants

Intsia bijuga (Colebr.) Kuntze is an indigenous, highly valued
timber tree that inhabits particularly the coastal areas (up to
c. 200 m) of the granitic islands of the Seychelles. Intsia bijuga
is distributed from Southeast Asia to the Mascarenes,
Tanzania, New Guinea, Fiji and Samoa (ILDIS, 2001). Several
isolated patches of a few clumped trees have remained in
the Seychelles. Vateriopsis seychellarum Heim, a Seychelles
endemic hardwood, was virtually extinct by the early 20th
century as a result of extensive logging. Later, a small natural stand was discovered at L’Abondance in a less accessible mountainous area. From this site, several seedlings were collected and outplanted at Casse Dent, where a single tree has survived. More recently, seedlings from this single tree were collected, pregrown in nurseries, and outplanted in mountain slopes at Le Niol and Sans Soucis, but with little success.

To prevent erosion and to increase timber production, experimental plantations of exotic forestry trees were established in 1977. Seeds of *Eucalyptus robusta* Sm. were introduced from Australia and sown in containers in the Seychelles. Containerized *Pinus caribea* L. seedlings were imported from Kenya, where they most likely received their ECM symbionts. Seedlings of both the introduced and native trees were raised in a nursery at Sans Soucis Forestry Station (W. Andre, pers. comm.). Subsequently, a few small plantations of *E. robusta* and *P. caribea* were established in eroded mountain slopes. Both species form declining stands and display no evidence of naturalization.

**Sampling**

Details of study sites and sample size are given in Table 1. ECM fungi associated with *V. seychellarum* and *I. bijuga* were studied in most major native stands in Mahé (Anse Major, L’Abondance and North Point) and Praslin islands (Vallée de Mai) (Supplementary Material, Fig. S1). To estimate the continuity of ECM fungal communities, a few experimental plantations of *V. seychellarum* (Le Niol, Sans Soucis) were sampled. In addition, a single planted *V. seychellarum* adult and surrounding seedlings were studied in a tea plantation at Casse Dent. The introduced *P. caribea* and *E. robusta* were sampled in the largest plantation at Le Niol Forestry Station to document potential host shifts of ECM fungi between the native and introduced trees.

Fruit-body surveys and root tip sampling were performed simultaneously once or twice in each stand between 28 February and 15 March, 2006. To find resupinate and hypogeous fruiting taxa, dead wood and litter were carefully turned over. To study the effect of microtopography on ECM fungal communities, a few experimental plantations of *V. seychellarum* (Le Niol, Sans Soucis) were sampled. In addition, a single planted *V. seychellarum* adult and surrounding seedlings were studied in a tea plantation at Casse Dent. The introduced *P. caribea* and *E. robusta* were sampled in the largest plantation at Le Niol Forestry Station to document potential host shifts of ECM fungi between the native and introduced trees.

Molecular typing

At least two root tips of each anatomic type per study site and pieces of selected fruit-bodies (Table 2) were subjected to DNA extraction using a High Pure PCR Template Preparation Kit for Isolation of Nucleic Acids from Mammalian Tissue (Roche Applied Science, Indianapolis, IN, USA). The rDNA internal transcribed spacer (ITS) and nuclear large subunit (nLSU) were amplified as described in Tedersoo et al. (2006) using primers ITS1F (5′-cttggtgataggaattac-3′) and TW13 (5′-gtcctcgtttcaagag-3′). DNA extracts producing multiple PCR products were reamplified using the primer ITS1F and a newly designed homobasidiomycete-specific primer ITS4B1 (5′-caaggrcttacggrcctca-3′), which is a modification from ITS4B (Gardes & Bruns, 1996). PCR products were purified using Exo-Sap enzymes (Sigma, St Louis, MO, USA). Sequencing was performed using Exonuclease and T7 sequencing primers (5′-actatcctgagggaaacttc-3′) and/or ITS5 (5′-ggaagtaaaggctcgaaccg-3′) for the ITS region; and cb6 (5′-gtcataactagggagcgg-3′) and/or TW14 (5′-gcatatcaggaggaacctc-3′) for the nLSU. Contigs were assembled using Sequencher 4.0 (GeneCodes Corp., Ann Arbor, MI, USA). A value of 97.0% ITS region identity (excluding flanking 18S and 28S rDNA sequences) was used as a DNA barcoding criterion (Tedersoo et al., 2003). All unique sequences were submitted to EMBL (accession nos AM412253-AM412304). BlastN and fasta3 searches were performed against public sequence databases NCBI, EMBL and UNITE (Kõljalg et al., 2005) to provide at least tentative identification for the ECM fungi.

Phylogenetic analyses

To determine the phylogenetic affinities of two ECM ascomycetes from the Seychelles, 75 nLSU sequences of identified fruit-bodies or cultures were downloaded from the NCBI database. In addition, an nLSU sequence of *Nothofagus* ECM from Tasmania (Tedersoo et al., 2007) was included. The sequences were aligned using Mafft ver. 5.681 (Katoh et al., 2005) and corrected manually. The final matrix comprised 78 sequences and 1442 characters. Parsimony analyses were performed using PAUP ver. 4.0b81 (Swofford, 2002) with 100 heuristic search replicates, tree bisection-reconnection (TBR) branch swapping and gaps as missing characters. Representatives of Orbiliomycetes and Pezizomycetes were used as outgroup taxa. Bootstrap support was calculated based on 10 random sequence addition replicates and 500 bootstrap replicates. Bayesian analyses were performed as implemented in MrBayes version 3.1.1 (Ronquist & Huelsenbeck, 2003) with 200 000 generations, GTR + I + G substitution model (as retrieved from MrModeltest ver. 2.2; Nylander, 2004) and 20 000 as the ‘burn in’ value.
Table 1 Characteristics of study sites in the Seychelles (ectomycorrhizal hosts are indicated in bold)

<table>
<thead>
<tr>
<th>Study sitea</th>
<th>Geocode, altitude (m asl)</th>
<th>Vegetation</th>
<th>Soil type</th>
<th>nb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anse Major steep coastal shrubland</td>
<td>4°37.3'S, 55°23.5'E; 40 m</td>
<td><em>Intsia bijuga</em> (Colebr.) Kuntze (six scattered trees, 20–50 yr), <em>Nephrolperma vanhoutteana</em> (Wendl. ex van Houtt) Balf., <em>Chrysobalanus icaco</em> L., <em>Alstonia macrophylla</em> Wallich ex Don, <em>Dicanopteris linearis</em> (Burm.)</td>
<td>Humus patchily accumulated in cracks of granite bedrock                                           6 (10)</td>
<td></td>
</tr>
<tr>
<td>Casse Dent tea plantation</td>
<td>4°39.3'S, 55°26.4'E; 410 m</td>
<td><em>Camellia sinensis</em> (L.) Kuntze; <em>Vateriaopsis seychellarum</em> Heim (a single 40-yr-old tree with tens of seedlings), no herb layer by recalcitrant leaves of <em>V. seychellarum</em></td>
<td>Ploughed soil (formerly yellow laterites) patchily covered                                         Old tree, 10 (10), seedlings, 5 (5)</td>
<td></td>
</tr>
<tr>
<td>L'Abondance undisturbed submontane forest</td>
<td>4°41.3'S, 55°29.1'E; 480 m</td>
<td><em>V. seychellarum</em> (c. 15 trees, 20–200 yr), <em>Pandanus</em> spp., <em>Northea homei</em> (MM Hartog) Pierre, <em>Cinnamomum verum</em> J. Presl, abundant mosses</td>
<td>Thin litter and humus layer on coarse quartz sand                                                   16 (16)</td>
<td></td>
</tr>
<tr>
<td>Le Niol eucalypt plantation</td>
<td>4°37.5'S, 55°25.9'E; 210 m</td>
<td><em>Eucalyptus robusta</em> Sm. (c. 200 trees, 29 yr), <em>C. verum</em>, <em>Dillenia ferruginea</em> Heim, <em>C. icaco</em>, <em>D. linearis</em></td>
<td>Strongly eroded yellow lateritic soils with a thin humus horizon                                 10 (12)</td>
<td></td>
</tr>
<tr>
<td>Le Niol pine plantation</td>
<td>4°37.5'S, 55°25.8'E; 180 m</td>
<td><em>Pinus caribea</em> L. (c. 1000 trees, 29 yr), <em>Sandoricum koetjape</em> Merrill, sparse herb layer</td>
<td>Yellow lateritic soils with a thin humus horizon and thick litter layer                           11 (12)</td>
<td></td>
</tr>
<tr>
<td>Le Niol <em>Vateriaopsis</em> plantation</td>
<td>4°37.6'S, 55°25.8'E; 180 m</td>
<td><em>S. koetjape</em>, <em>V. seychellarum</em> (c. 20 trees, 2–20 yr), <em>Pandanus</em> spp., <em>Paraserianthes falcataria</em> (L.) Niels, <em>C. verum</em>, sparse herb layer</td>
<td>Eroded brown soil with patches of organic matter                                                 Saplings, 9 (12) seedlings, 5 (5)</td>
<td></td>
</tr>
<tr>
<td>North Point coastal forest</td>
<td>4°33.7'S, 55°26.3'E; 30 m</td>
<td><em>I. bijuga</em> (five clumped trees, 25–50 yr), <em>Albizia lebbeck</em> (L.) Benth., <em>C. verum</em>, <em>A. macrophylla</em>, <em>Calophyllum inophyllum</em> L., <em>Cocos nucifera</em> L.</td>
<td>Eroded yellow to yellow-brown soils with patchy humus layer                                       18 (24)</td>
<td></td>
</tr>
<tr>
<td>Sans Soucis forestry plantation in steep slope</td>
<td>4°38.4'S, 55°26.9'E; 300 m</td>
<td><em>S. koetjape</em>, <em>V. seychellarum</em> (two trees, 15 yr)</td>
<td>Eroded red-brown soils with little organic matter                                               5 (10)</td>
<td></td>
</tr>
<tr>
<td>Vallée de Mai, Unesco World Heritage Site</td>
<td>4°20.0'S, 55°44.0'E; 180 m</td>
<td><em>Lodoicea maldivica</em> (JF Gmelin) Persoon, <em>Pandanus</em> spp., <em>I. bijuga</em> (14 trees in 5 clumps, 25–80 yr), <em>Psidium cattleianum</em> Sabine. For more details, see Fleischmann et al. (2005)</td>
<td>Yellow to red lateritic soils with patchy humus and litter layers                               19 (24)</td>
<td></td>
</tr>
</tbody>
</table>

*aAll sites are on Mahé island, except Vallée de Mai, which is on Praslin; bnb, number of root samples that included living ectomycorrhizas. The number of root samples actually taken is indicated in parentheses.
Species Herbarium code Locality EMBL accession no.
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Boletellus sp. a TU105073 Vallée de Mai, Praslin AM412293
Coltricia dependens b TU103611 Casse Dent, Mahé AM412254
Coltricia aff. oblectans b TU105089 Vallée de Mai, Praslin AM412245
Coltricia aff. oblectans c TU103621 Le Niol, Mahé AM412246
Scleroderma sp. h, c TU103614 Le Niol, Mahé AM412302
Thelephoraceae sp. e TU105081 Danzil, Mahé AM412302
Tomentella fibrosa TU105118 Vallée de Mai, Praslin AM412301
Tomentella sp. h TU103690 Anse Major, Mahé AM412294
Tomentella sp. b TU105090 Vallée de Mai, Praslin AM412295
Tomentella sp. b TU105130 Vallée de Mai, Praslin AM412296
Tomentella sp. b TU103995 North Point, Mahé AM412297
Tomentella sp. b TU105060 North Point, Mahé AM412298
Tomentella sp. b TU105058 North Point, Mahé AM412299
Tomentella sp. b TU103582 North Point, Mahé AM412300
Tomentella sp. TU103641 North Point, Mahé AM412303

*TU, acronym for the Herbarium of the Natural History Museum of the University of Tartu, Estonia.
*Also found below ground.
*Found only in a nonnative stand.

Table 2 List of fruit-bodies found in the Seychelles and sequenced for this study

Statistical analyses

A computer program EstimateS, version 7. (Colwell, 2004), was used to calculate species accumulation (rarefaction) curves and the minimum richness estimators, Chao2 and Jackknife2 (Gotelli & Colwell, 2001). These functions were used to predict the sufficiency of sample size and to estimate the proportion of unseen species at different sites and in the Seychelles in general, using root samples and sites as sampling units, respectively. The sampling units were sampled randomly without replacement using 1000 replications. Confidence intervals were calculated for the rarefaction curve at the whole community level as implemented in Colwell (2004).

To study the relative effect of soil microsites on ECM fungal community composition, detrended correspondence analysis (DCA) was performed using individual root samples, binary-transformed species and downweighted rare species. The effects of host species, host population size, soil type, altitude and geographical distance on the ECM fungal community composition were determined at the site level. Above- and below-ground data were pooled for the latter analysis and binary-transformed. Both ordination analyses were performed in PC-Ord (McCune & Mefford, 1997).

Results

Diversity of ECM fungi

Anatomotyping integrated with sequencing revealed 30 species of ECM fungi on root tips of the Seychelles’ native trees (Table 3). Only Scleroderma sp1 and sp3 (North Point) were indistinguishable based on anatomical characters. Additional fruit-body surveys in native forests revealed 13 species (including eight Tomentella spp.), of which Tomentella sp. (TU103641) and Tomentella fibrosa (Berk. & M.A. Curtis) Kõljalg remained undetected below ground (Table 2). V. seychellarum and I. bijuga hosted 18 and 15 fungal species on root tips, respectively, whereas the introduced E. robusta and P. caribea were associated with seven and three species, respectively (Table 4). P. caribea shared no ECM fungal species with other hosts, whereas E. robusta had three species in common with V. seychellarum and two species in common with I. bijuga. Despite greater sampling effort, the two native hosts shared only three fungal species. All species associated with native trees had ITS sequence identities of less than 90.4% to any published sequences in databases (Table 3).

The minimum richness estimators Chao2 and Jackknife2 predicted that 51.2 and 57.4 species, respectively, of ECM fungi are associated with native trees of the Seychelles (Fig. 1). The Chao2 estimator curve was descending, whereas the Jackknife2 curve was steeply rising when all plots were sampled in random order. Because of the large variation in Chao2 richness estimates, probably resulting from a relatively small number of samples and species, its values are considered less reliable and hence are not reported further. The only natural stand of V. seychellarum at L’Abondance supported the highest number of ECM fungal species (15 spp. in 16 samples; Table 4). Jackknife2 estimated the minimum richness of 29.5 species for this site. Jackknife2 and rarefaction curves were steeply rising with increasing numbers of samples at L’Abondance (Fig. 2), indicating insufficient sampling effort. At other sites, however, the observed species richness ranged from one to eight, resulting in the leveling off of both rarefaction and estimator curves (Fig. 2).

Small plantations of V. seychellarum were established by replanting current-year seedlings from Casse Dent. At this site, seedlings were colonized by four species of ECM fungi in total (mean, 1.4; range, one and two species per seedling).

including the three most common species from the mother
tree (five species in 10 samples; Table 4). Saplings replanted at
isolated sites were colonized by the same fungal species
present at Casse Dent, except for hymenochaetoid sp3, which
was shared with L’Abondance, and thelephoroid sp8, a unique
species. However, the replanted saplings had retained only
one to three ECM fungal species per plantation, indicating
local extinction of several taxa.

Community composition of ECM fungi
On a fine scale, the dominant fungal species occurred in most
root samples of a particular site, independent of microtopo-

<table>
<thead>
<tr>
<th>Species</th>
<th>EMBL accession no.</th>
<th>Best ITS match in public sequence databases</th>
<th>% identities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hymenochaetoid sp1</td>
<td>AM412255</td>
<td>Coltricia perennis DQ234559</td>
<td>77.0b</td>
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<tr>
<td>Hymenochaetoid sp2</td>
<td>AM412260</td>
<td>Coltricia perennis DQ234559</td>
<td>78.9b</td>
</tr>
<tr>
<td>Hymenochaetoid sp3</td>
<td>AM412258</td>
<td>Coltricia perennis DQ234559</td>
<td>72.1b</td>
</tr>
<tr>
<td>Hymenochaetoid sp4</td>
<td>AM412257</td>
<td>Boletellus mirabilis AF335451</td>
<td>73.3b</td>
</tr>
<tr>
<td>Boletellus sp.a</td>
<td>AM412261</td>
<td>Boletellus mirabilis AF335451</td>
<td>73.8</td>
</tr>
<tr>
<td>Boletoid sp3</td>
<td>AM412262</td>
<td>Boletus aereus DQ131619</td>
<td>75.9b</td>
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<tr>
<td>Boletoid sp5</td>
<td>AM412263</td>
<td>Boletus erythropus DQ131634</td>
<td>76.4b</td>
</tr>
<tr>
<td>Boletoid sp6</td>
<td>AM412264</td>
<td>Clavulina cristata A8899929</td>
<td>79.7</td>
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<tr>
<td>Cantharelloid sp1</td>
<td>AM412265</td>
<td>Cortinarius teratagus AF389151</td>
<td>89.8</td>
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<tr>
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<td>AM412266</td>
<td>Cortinarius teratagus AF389151</td>
<td>90.9</td>
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<tr>
<td>Cortinarius sp3</td>
<td>AM412267</td>
<td>Cortinarius acutovelatus AY083175</td>
<td>90.3</td>
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<tr>
<td>Cortinarius sp4</td>
<td>AM412268</td>
<td>Cortinarius caninus U56024</td>
<td>83.2</td>
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<tr>
<td>Cortinarius sp5</td>
<td>AM412269</td>
<td>Ramaria sp. DQ36508</td>
<td>76.8b</td>
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<tr>
<td>Gomphoid sp1</td>
<td>AM412270</td>
<td>Inocybe lanuginosa DQ357905</td>
<td>78.8</td>
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<tr>
<td>Inocybe sp1</td>
<td>AM412271</td>
<td>Pisolithus tinctorius AF374634</td>
<td>85.7</td>
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<tr>
<td>Pisolithus sp1</td>
<td>AM412272</td>
<td>Rhizopogon fuscorubens AF058313</td>
<td>98.5</td>
</tr>
<tr>
<td>Rhizopogon sp1</td>
<td>AM412273</td>
<td>Gymnomyces fallax AY239349</td>
<td>90.1</td>
</tr>
<tr>
<td>Rhizopogon sp2</td>
<td>AM412274</td>
<td>Scleroderma cepa DQ453694</td>
<td>75.7</td>
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<tr>
<td>Russuloid sp1</td>
<td>AM412275</td>
<td>Scleroderma cepa DQ453694</td>
<td>87.1</td>
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<tr>
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a Also found as fruit-bodies.
b Based on partial ITS match.
c Based on nLSU match.
frequent taxa on *I. bijuga* at North Point, Vallée de Mai and Anse Major, respectively. *Pisolithus* sp1 was the most frequent species on root tips of *P. caribea*. In general, thelephoroid clade was the most species-rich taxon on *I. bijuga* and *V. seychellarum* (seven and six spp., respectively), followed by the boletoid clade on *I. bijuga* (five spp.) and the euagaric and hymenochaetoid clades on *V. seychellarum* (three spp. for both). *V. seychellarum* hosted two species of ascomycetes, which had a thin, black pseudoparenchymatous mantle (Fig. S2, Text S1). Several root tips produced identical sequences, and phylogenetic analyses placed these taxa within Sordariomycetes, particularly Annu-

<table>
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<th>Vallée de Mai</th>
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<sup>a</sup>Found also as fruit-bodies.

<sup>b</sup>F, found only as fruit-bodies.
variation among sites (mean, 0.23%; range, 0–1.70%). In particular, the ITS sequences of ECM fungi in the *E. robusta* plantation were invariable (up to 0.18% sequence variation) and always identical to the ITS sequences of conspecific taxa associated with native trees, providing further evidence for their common origin.

**Discussion**

Low ECM fungal diversity was demonstrated in the Seychelles compared with temperate forests (Horton & Bruns, 2001) and tropical forests of Dipterocarpaceae (Sirikantaramas et al., 2003; Moyersoen, 2006) and Sarcolaenaceae (Ducousso et al., 2004) that potentially support hundreds of fungal species. Neither *I. bijuga* nor *V. seychellarum* formed ECM associations with narrow fungal lineages, in contrast to *P. grandis*, which inhabits extremely nutrient-rich coral cays (Chambers et al., 2005). DCA suggested that host species, soil type, altitude and longitude affected the community composition of ECM fungi most strongly at the site level. However, these variables were strongly related, rendering the actual causal mechanisms uncertain. In this study, we were unable to detect micro-habitat preference among ECM fungi, which is in contrast to

![Fig. 3](image-url)
boreal forests (Goodman & Trofymow, 1998; Tedersoo et al., 2003). This further indicates that specialist fungi are uncommon in the Seychelles. The lack of niche differentiation supports the coexistence of fewer species (Lomolino et al., 2006). The low observed alpha diversity (one to 15 species per site) probably results from low overall gamma diversity (species pool), which could be related to the restricted area of host populations and the Seychelles’ long-term isolation from neighboring continents as predicted by the equilibrium theory of island biogeography (MacArthur & Wilson, 1967; Lomolino et al., 2006).

In agreement with the low observed richness, the rarefaction curves suggest that most sites were exhaustively sampled, except for L’Abondance, where at least twice as many species probably occur. Similarly, the site-based rarefaction curve did not level off, indicating low fungal species overlap between

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**Fig. 4** Bayesian tree demonstrating the phylogenetic placement of three ectomycorrhizal (ECM)-forming Sordariomycete species (in bold) among euascomycetes. Parsimony bootstrap support (above branches; threshold, 70%) and Bayesian posterior probabilities (below branches; threshold, 95%) are indicated.
isolated stands. Based on recent, more widespread distribution of ECM host trees before deforestation and during periods of low sea level (Fleischmann et al., 2003), we hypothesize that many ECM fungi became extinct during deforestation in the 19th and early 20th centuries. Thus, we believe that the estimated value of 51–57 species likely represents a substantial underestimate of the historical species richness.

Certain lizard and snail taxa rapidly diversified in the granitic islands of the Seychelles during the loss of land connection in the Pleistocene (Radkey, 1996; Gerlach, 1999). Contrary to these findings, the ITS region provided no evidence for speciation of ECM fungal populations among islands and host plants, except for thelephoroid sp9, which had 1.70% ITS sequence difference in Mahé vs Praslin islands. The low intraspecific ITS diversity in the Seychelles’ ECM fungi is comparable to the low ITS variation in temperate woodlands of the northern and southern hemispheres at the local scale (Glen et al., 2001; Horton, 2002). Moreover, the interspecific ITS sequence variation was high (at least 12.0%) and sister relations among taxa were not detected. Notable exceptions include Sordariomycetes spp. (Fig. 4) and Scleroderma spp. (M. Binder, pers. comm.) that are, however, poorly covered with sequence data. We believe that the lack of speciation in ECM fungi is best explained by widespread distribution of host trees before the rise of sea level and deforestation. Alternatively, sister species may have gone extinct during the loss of habitat and decline in population size. The biogeographic relations and dispersal vs vicariance origin of the Seychelles’ fungi remain to be established when DNA sequence data accumulates from related continents.

Intsia bijuga and V. seychellarum shared only three fungal species, which may be attributed to either host or habitat effects. Namely, I. bijuga occupies coastal areas with seasonal climate up to c. 200 m above sea level, whereas V. seychellarum has persisted and is planted in more humid submontane habitats. Nevertheless, Alexander et al. (1992) demonstrated that ECM fungi from adult dipterocarps colonized and benefited the seedlings of Intsia palumbanica Miq. in Malaysia, indicating that at least some fungal species are functionally compatible with both plant families. The ECM Caesalpiniaeae, Dipterocarpaceae and Uapacaceae co-occur in miombos and seasonal forests of continental Africa (Lawton, 1978), where they have likely evolved and subsequently dispersed to other continents or drifted away during the break-up of Gondwana (Dayanandan et al., 1999; Lavin et al., 2005). Extremely poor dispersal abilities and presence of ECM symbiosis explain best the transatlantic disjunction of Dipterocarpaceae, suggesting their development in the Early Cretaceous (135 mya; Moyersoen, 2006). Based on dated phylogenies and fossil data, Caesalpiniaeae emerged in the Latest Cretaceous or Early Tertiary, whereas ECM lineages evolved around 30 mya and later (Lavin et al., 2005). Because of the shared habitats and at least some shared fungal species, we hypothesize that Caesalpiniaeae and Uapacaceae likely obtained most of their ECM fungi from dipterocarps in Africa.

The introduced Eucalyptus and Pinus hosted few ECM symbionts in the Seychelles, although these genera associate with hundreds or even thousands of ECM fungi in their natural range (May & Simpson, 1997; Horton & Bruns. 2001). Dunstan et al. (1998) listed 99 species of ECM fungi associated with exotic pine plantations worldwide, but in the present study P. caribea host only three species of ECM fungi, two Rhizopogon and a Psilocibis species, that were never observed on other hosts in the Seychelles. P. caribea is most likely obtained these host-specific fungi (Molina & Trappe, 1982; Martin et al., 2002) from a nursery in Kenya. Based on fruit-body surveys, Rhizopogon spp. are among the most common introduced fungi in exotic pine plantations (Mikola, 1969; Dunstan et al., 1998; Giachini et al., 2004). With a few exceptions (Valenzuela et al., 1999; Orlovich & Cairney, 2004), fruit-bodies of pine-associated ECM fungi are not usually observed in native plant communities. Moreover, Pinus spp. seem unable to associate with native fungi in exotic habitats, resulting in death when cointroduced ECM fungi are lacking (Mikola, 1970). In contrast to pine, E. robusta most likely received all its ECM symbionts from the native trees in the nursery at Sans Soucis or from other native stands several kilometers away. Namely, E. robusta was introduced as seeds and its plantations shared most of the ECM fungal species with either V. seychellarum or I. bijuga in native and planted stands. In addition, none of the ECM fungi on E. robusta at Le Niol are known from other exotic eucalypt plantations (Bougher & Lebel, 2001; Giachini et al., 2004; Diez, 2005) or from Australian eucalypt woodlands (May & Simpson, 1997; Bougher & Lebel, 2001). Compared with pines, probably greater similarity in physiological features renders eucalypt more compatible with fungi native to dipterocarps and caesalpiniods. Associations with indigenous ECM fungi may potentially facilitate the invasion of eucalypts in native plant communities elsewhere, but this remains to be proven.

The thelephoroid, boletoid and euagaric clades were the most species-rich taxa on root tips of the Seychelles’ native plants. This supports the results of Sirikantaramas et al. (2003), who found thelephoroid fungi dominating on roots of Dipterocarpaceae in Malaysia. The high observed abundance of resupinate fruit-bodies of thelephoroid fungi at the beginning of the dry season suggests their relatively high drought tolerance. Because of the formation of resupinate fruit-bodies on the underside of debris, thelephoroid fungi are often overlooked. On the contrary, members of the boletoid and euagaric clades are strongly represented in tropical fruit-body surveys in Africa and Southeast Asia (Verbeke & Buyck, 2002; Watling et al., 2002), suggesting their commonness in the tropics. Among the euagarics, however, Cortinarius spp. are considered rare in tropical forests (Peintner et al., 2003), which is in contrast to the finding of four Cortinarius species in this study. The hymenochaetoid clade includes five species of Coltricia/Coltriciella in the Seychelles (Tedersoo et al., 2007). Although seldom reported in below-ground ECM fungal
communities, *Coltricia* and *Coltriciella* are common in the tropics, particularly in Southeast Asia and Australasia (Corner, 1991), but also in South America (Aime et al., 2003). The ECM mantle structure of two sordariomycete species resembled most the black unidentified ascomycetes found on *Salix* sp. in Washington state, USA (Trowbridge & Jumpponen, 2004; J. Trowbridge & A. Jumpponen, pers. comm.) and on *Shorea leprosula* in Malaysia (type T12; Lee et al., 1997). Surprisingly, the sebacinoid, atheloid and russuloid clades of basidiomycetes and *Cenococcum geophilum* (Ascomycota), which are common members of ECM fungal communities in the northern hemisphere, were rare or lacking in the Seychelles. In particular, the russuloid clade is most diverse in tropical forests based on fruit-body surveys (Verbeken & Buyck, 2002; Watling et al., 2002). We hypothesize that similarly to the low alpha and gamma diversity, peculiarities in fungal communities can be attributed to the long-term isolation of the Seychelles and small host populations that permit the persistence of the most easily dispersed, stress-tolerant and generalist species.

In conclusion, communities of the Seychelles’ ECM fungi are species-poor and the community composition is biased compared with other tropical and temperate ecosystems. This probably results from small host population sizes and the long-term isolation of the Seychelles. To conserve the biodiversity of ECM fungi and associated microbes, all native ECM stands should be protected. Attempts to propagate native ECM trees should focus on coinoculation of soil from small host stands to improve seedling establishment (Mikola, 1970). Low host specificity and high risk of cointroduction of soil microbes make native microbial community studies of ECM stands to improve seedling establishment (Mikola, 2002; Watling et al., 2002). We hypothesize that similarly to the low alpha and gamma diversity, peculiarities in fungal communities can be attributed to the long-term isolation of the Seychelles and small host populations that permit the persistence of the most easily dispersed, stress-tolerant and generalist species.

Functional compatibility and microevolution between introduced plants and native fungi (and vice versa) require further studies.

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movement of exotic forest fungi, especially in the tropics and the Southern

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Supplementary Material

The following supplementary material is available for this
article online:

**Fig. S1** Geographic position of granitic islands of the Seychelles
and location of study sites in Mahé and Praslin islands.

**Fig. S2** Ectomycorrhizas of Sordariomycetes on *Vateriopsis
seychellarum* at L’Abondance, the Seychelles.

**Text S1** Descriptions of Sordariomycete ectomycorrhizas.

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(This link will take you to the article abstract).

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