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TRIIN SUVI

Mycorrhizal fungi of native and introduced trees in the Seychelles Islands

TARTU UNIVERSITY
PRESS

Department of Botany, Institute of Ecology and Earth Sciences,
Faculty of Science and Technology, University of Tartu, Estonia

Dissertation was accepted for the commencement of the degree of *Doctor philosophiae* in botany and mycology at the University of Tartu on January 8, 1010 by the Scientific Council of the Institute of Ecology and Earth Sciences.

Supervisors: Prof. Urmas Kõljalg, University of Tartu, Estonia
Dr. Leho Tedersoo, University of Tartu, Estonia

Opponent: Prof. John Cairney, University of Western Sydney

Commencement: Room 1020, 14A Ravila Street, Tartu, on 17 February 2010 at 10.15 a.m.

Publication of this thesis is granted by the Institute of Ecology and Earth Sciences, University of Tartu and by the Doctoral School of Earth Sciences and Ecology created under the auspices of European Social Fund.

ISSN 1024–6479
ISBN 978–9949–19–297–7 (trükis)
ISBN 978–9949–19–298–4 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 8

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers that are referred to in the text by Roman numerals:

I. Suvi T, Tedersoo L, Abarenkov K, Gerlach J, Beaver K, Kõljalg U. 2010. Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. *Mycologia* 102: in press.

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

III. Tedersoo L, Suvi T, Beaver K, Saar I. 2007. Ectomycorrhizas of *Coltricia* and *Coltriciella* (Hymenochaetales, Basidiomycota) on Caesalpiniaceae, Dipterocarpaceae and Myrtaceae in Seychelles. *Mycological Progress* 6: 101–107.

IV. Tedersoo L, Suvi T, Larsson E, Kõljalg U. 2006. Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research* 110: 734–748.

Authors contribution to each paper

	I	II	III	IV
Idea and design	+	+	–	+
Sampling	+	+	+	+
Morpho/anatomotyping	–	–	–	+
Molecular analyses	+	+	+	+
Data analyses	+	–	–	+
Writing	+	+	+	+

INTRODUCTION

Mycorrhiza is a mutualistic relationship between plants and fungi (Read & Perez-Moreno, 2003; Smith & Read, 2008). Based on the morphology and anatomy of the mycorrhizal root, different types of mycorrhiza have been described. The most common and widespread is arbuscular mycorrhiza (AM), which is formed by the majority of the land plants (Smith & Read, 2008). In AM, fungi from the phylum Glomeromycota form vesicles and highly branched structures – arbuscules – inside the plant root cells. Another common type of mycorrhiza, ectomycorrhiza (EcM) is formed by the species of Basidiomycota, Ascomycota and Zygomycota. There are approximately 6000 EcM plant species and 20 000 – 25 000 EcM fungal species in the world (Rinaldi *et al.*, 2008; Brundrett, 2009). Ectomycorrhiza evolved many times independently and mostly from saprotrophic fungi (Hibbett *et al*., 2000; Tedersoo *et al*., 2010a). The oldest fossil evidence of EcM dates back to Eocene (LePage *et al*., 1997). In EcM associations, fungi form a sheath (fungal mantle) around the root tip and Hartig net between the cortical cells, where the nutrient exchange takes place. In addition, particularly EcM fungi may have abundant emanating hyphae in soil that take up and transport nutrients and water. Ectomycorrhiza is widespread and can be found in many ecosystems, but it is mostly characteristic of boreal and temperate forests.

In natural conditions, mycorrhiza is usually beneficial to both partners – fungi provide mineral nutrients (N, P) to the host plant that, in return, transfers carbohydrates fixed via photosynthesis to the fungus. In addition to nutrition, mycorrhiza provides other benefits to the plant, for example protection against pathogens (Whipps, 2004), heavy metals (Adriaensen *et al.*, 2003). Plants also benefit from the common mycelial networks (CMN), which are formed by mycorrhizal fungi that associate with several host plants simultaneously. Transfer of carbohydrates between green plants via CMN has been demonstrated (Simard *et al.*, 1997). These networks are particularly useful to the seedlings (Selosse *et al.*, 2006) that may benefit from fungi without contributing their own carbon as fungal partners receive it from the neighbouring adult trees.

Detection of EcM colonization in plant roots is relatively easy, but the identification of mycobionts has been intricate for a long time. Before molecular methods became available, the EcM fungal communities were studied based on sporocarp surveys and/or root tip morphology/anatomy. Unfortunately, both approaches have their shortcomings: sporocarp surveys are effective to spot species with large fruit-bodies like Russulales, Agaricales and Boletales. However, taxa forming resupinate (Thelephorales, Sebacinales) and hypogeous fruit-bodies (Hysterangiales) or no fruit-bodies at all (*Cenococcum*) were hardly ever recorded, biasing the results towards certain basidiomycete taxa and severely underestimating the richness below ground. Morphotyping/ anatomotyping was more effective in terms of discovering different groups, but species identification based on this data was complicated. To reliably identify EcM species, hyphal connections between colonized root tips and fungal sporocarps

need to be tracked. Alternatively, mantle anatomy of EcM root tips can be compared to that of already established descriptions. Unfortunately, variation of morphological and anatomical features of EcM of different species often overlaps, leading to lumping of closely related taxa and hence underestimation of actual species richness.

Rapid establishment and development of molecular tools has substantially facilitated identification of mycobionts and understanding of mycorrhizal ecology. The most extensively used and precise molecular method is sequencing of certain DNA regions. The Internal Transcribed Spacer (ITS) of the nuclear ribosomal RNA genes is most commonly used for identification of EcM fungi from roots and soil (Peay *et al.*, 2008). It is highly variable and thus enables to differentiate among fungal species. Sequences obtained from roots, dead wood, soil, etc. are queried against public sequence databases and identified based on their similarity to previously deposited sequences from correctly identified material. The main shortcoming of this approach involves the lack of reliable and well annotated reference data that is particularly severe in groups with no recent taxonomic treatment. For confident species-level identification, a (nearly) perfect match to the database sequence is anticipated. Since there is interspecific sequence variation, particularly among geographically distant populations, microbial ecologists have defined a DNA barcoding threshold to group sequences into species or, better – Operational Taxonomic Units (OTUs). Most EcM studies have utilized 97–98% similarity criterion to define species. Unfortunately, EcM fungal lineages strongly differ in the best suitable barcoding threshold and ideally, optimal thresholds should be used for each group. Since lineage-level information is very scant, I believe that using 97–98% similarity threshold is acceptable.

Majority of the EcM studies have been carried out in temperate regions, probably because both the highest abundance and diversity of EcM trees and researchers are concentrated in this area. Therefore, tropical regions received less attention and, largely due to logistic problems, are quite poorly studied. Recently, researches have started to pay attention to tropical EcM communities and reports from tropical areas have started to accumulate (Sirikantaramas *et al.*, 2003; Chambers *et al*., 2005, Haug *et al*., 2005; Yuwa-Amornipitak *et al.*, 2006). It is known that EcM plants form a minority in tropical areas compared with AM plants and their distribution is sparse creating challenging conditions for their symbiotic fungi (Alexander & Lee, 2005; Tedersoo *et al*., 2010b). The first tropical EcM studies have revealed controversial results. A Southeast Asian dipterocarp forest supports a high diversity of EcM fungi (Peay *et al.*, 2010), but Australian and South American Nyctaginaceae family hosts only a few fungal partners (Chambers *et al*., 2005; Haug *et al*., 2005, but see Tedersoo *et al*., 2010b). In general, it seems that tropical regions also support high EcM fungal richness promising new and exciting findings in the future. It is interesting to note that fungal taxa that are species-rich in temperate ecosystems (Thelephorales, Russulales, Boletales) are also common in tropical rain forests (Tedersoo & Nara, 2010).

Until this work, I am aware of only a single published study on EcM fungi in the Seychelles. Ashford & Allaway (1982) studied roots of *Pisonia grandis* and found two different anatomotypes that they considered to be formed by one species. They demonstrated extremely narrow EcM fungal partners range of *P. grandis* and hypothesized that the high rate of N and P amendment to the soil as bird guano may result in such specificity. Mycorrhizal relations and fungal partners of other Seychelles' plants have not been addressed.

The Seychelles archipelago consists of more than one hundred granitic, coral and sand islands that are situated in Indian Ocean just south of the equator. The granitic islands of Seychelles represent mountain tops of the largely submerged Mahé microcontinent that was separated from Gondwana approximately 65 million years ago and evolved in isolation thereupon (Briggs, 2003). Long-term isolation of the Seychelles from other continents and the fact that human settlement was established as recently as 1770 A.D. has been favorable for the evolution of endemic species. Fleischmann *et al.* (2003) considered 34% of the native higher plant species endemic. However, most of the natural vegetation has been lost in the Seychelles. After human settlement, majority of the forests were rapidly cut to establish plantations of cinnamon (*Cinnamomum verum* J. Presl), coco-nut (*Cocos nucifera* L.) and some oil plants. Native forest has been preserved only in remote, hardly accessible mountainous regions, but even these areas suffer from establishment of invasive species. In addition to these nearly natural regions, new protected areas have been established to promote re-establishment of indigenous vegetation. In the Seychelles, three plant families – Dipterocarpaceae (*Vateriopsis seychellarum* Heim.), Caesalpiniaceae (*Intsia bijuga* (Colebr.) Kuntze) and Nyctaginaceae (*Pisonia grandis* R. Br., *Pisonia sechellarum* F. Friedmann) (Fig. 1) were suspected to form ectomycorrhiza based on previous reports from these species, genera or families elsewhere. Besides these native plants, the introduced Pinaceae (*Pinus caribea* Morelet) and Myrtaceae (*Eucalyptus robusta* Sm.) also form mycorrhiza with EcM fungi.

Aims of the present study

1. Detection of EcM fungal partners from the roots of five different EcM plants in Seychelles – *I. bijuga*, *V. seychellarum*, *E. robusta*, *P. caribea* and *P. grandis* (I, II, III).
2. Comparison of EcM fungal communities between native and introduced plant species (II).
3. Description of new species of EcM fungi based on collected fruit-bodies (I).
4. Detection of mycorrhizal status of the endemic *P. sechellarum* from Silhouette Island (I).

MATERIALS AND METHODS

Study sites and EcM host plants

Sampling was carried out in four granitic islands in the Seychelles archipelago – Mahé, Praslin, Cousin and Silhouette. Six different plant species were studied – *P. grandis*, *P. sechellarum*, *I. bijuga*, *V. seychellarum* (Fig. 1), *P. caribea* and *E. robusta*. Eight study sites were selected on Mahé island to sample *I. bijuga*, *V. seychellarum, P. caribea* and *E. robusta* (II)**.** A single study site was selected on Praslin, Cousin and Silhouette to sample *I. bijuga* (II), *P. grandis* and *P. sechellarum* (I), respectively (Fig. 2)**.** EcM host plants never grew mixed at any of the sites.

Sample collection

Soil samples (15 x 15 cm to 5 cm depth) were collected at each site by use of a sharp knife. The number of samples varied between sites (n = 5–24), depending on the number of trees at the site. Samples were processed on the same day or kept in the refrigerator overnight. Roots were carefully separated from the substrate and cut into ca 3 cm fragments. Twenty randomly selected fragments per root sample were put into 1% CTAB extraction buffer (100 mM Tris-HCl [pH 8.0], 1.4 M NaCl, 20 mM EDTA, 1% cetyl trimethyl ammonium bromide) until further analyses. Roots were studied under dissecting microscope and light microscope and separated into morpho- and anatomotypes based on their morphological and anatomical features (Agerer, 1991).

Description of EcM roots included the following steps: first, roots were assessed visually or using a dissecting microscope to detect EcM root tips. Ectomycorrhizas were distinguished by the presence of a conspicuous hyphal sheath and altered morphology of the root tip. Usually, EcM root tips have a larger diameter compared to the non-mycorrhizal tips; some fungal species cause elongation or ramification of the root tips. After separating non-EcM roots, morphological features were studied in more detail to divide EcM root tips into different morphotypes (within a sample) by use of a dissecting microscope. The most informative features include the colour and texture of the surface of the fungal sheath, presence or absence and macromorphology of emanating hyphae, rhizomorphs and cystidia. After dividing EcM root tips into the morphotypes, anatomical features were studied under the light microscope at 1,000x magnification. The fungal sheath includes several layers that usually possess different hyphal shape and arrangement patterns. In addition, the anatomy of hyphae, rhizomorphs and cystidia were determined if present. Based on these features, a single morphotype could be further separated into several anatomotypes.

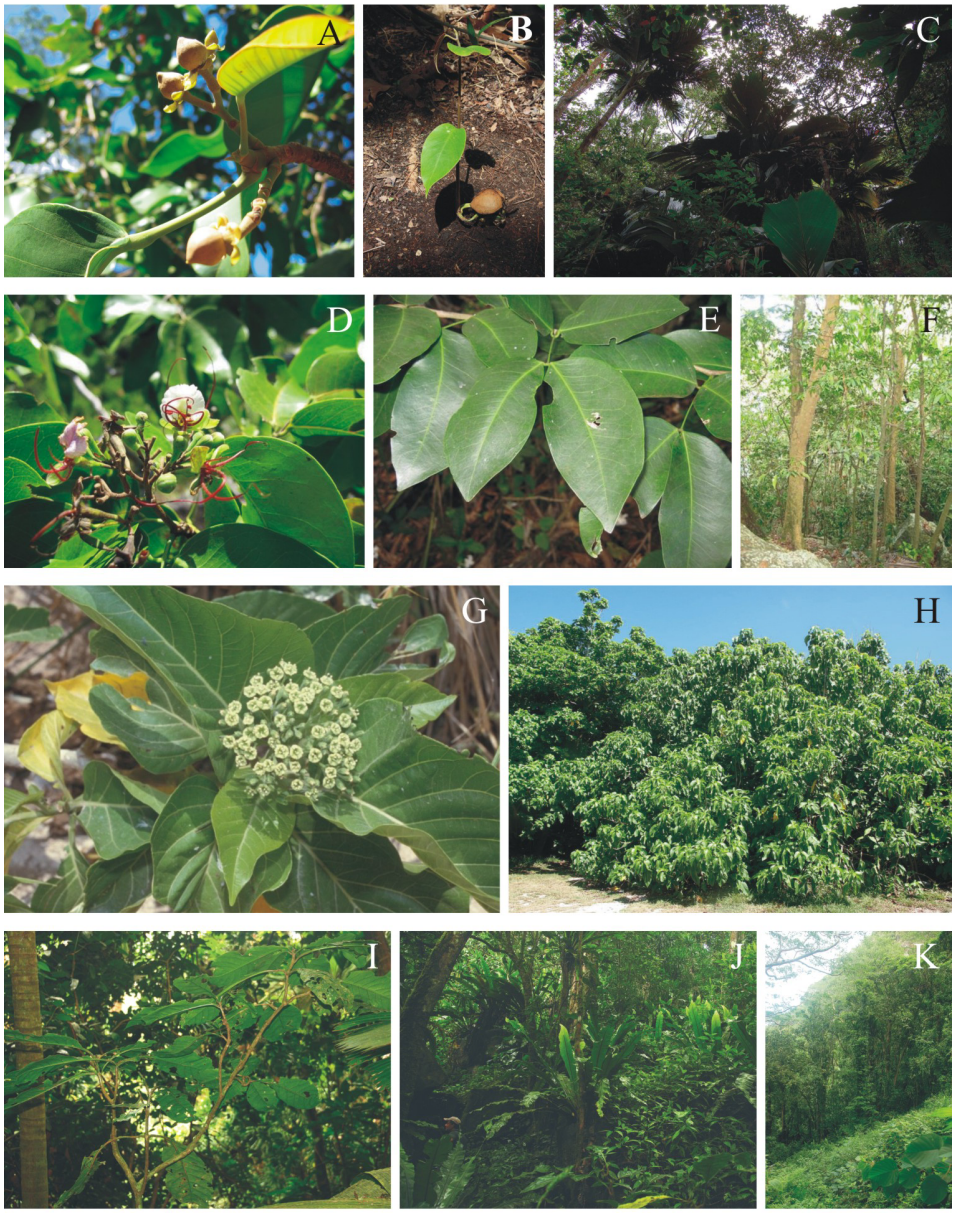

Fig. 1. Studied plant taxa. **A–C** *Vateriopsis seychellarum*: **A** fruits, **B** seedling, **C** habitat; **D–F** *Intsia bijuga*: **D** flowers, **E** leaves, **F** habitat; **G–H** *Pisonia grandis*: **G** flowers and leaves, **H** habitat; **I–K** *Pisonia sechellarum*: **I** leaves, **J–K** habitat. Photos made by Aline Finger (**A–B**), Charlotte Klank (**C**), Katy Beaver (**D–F**), Forest and Kim Starr (**G**), Leho Tedersoo (**H**) and Bruno Senterre (**I–K**)

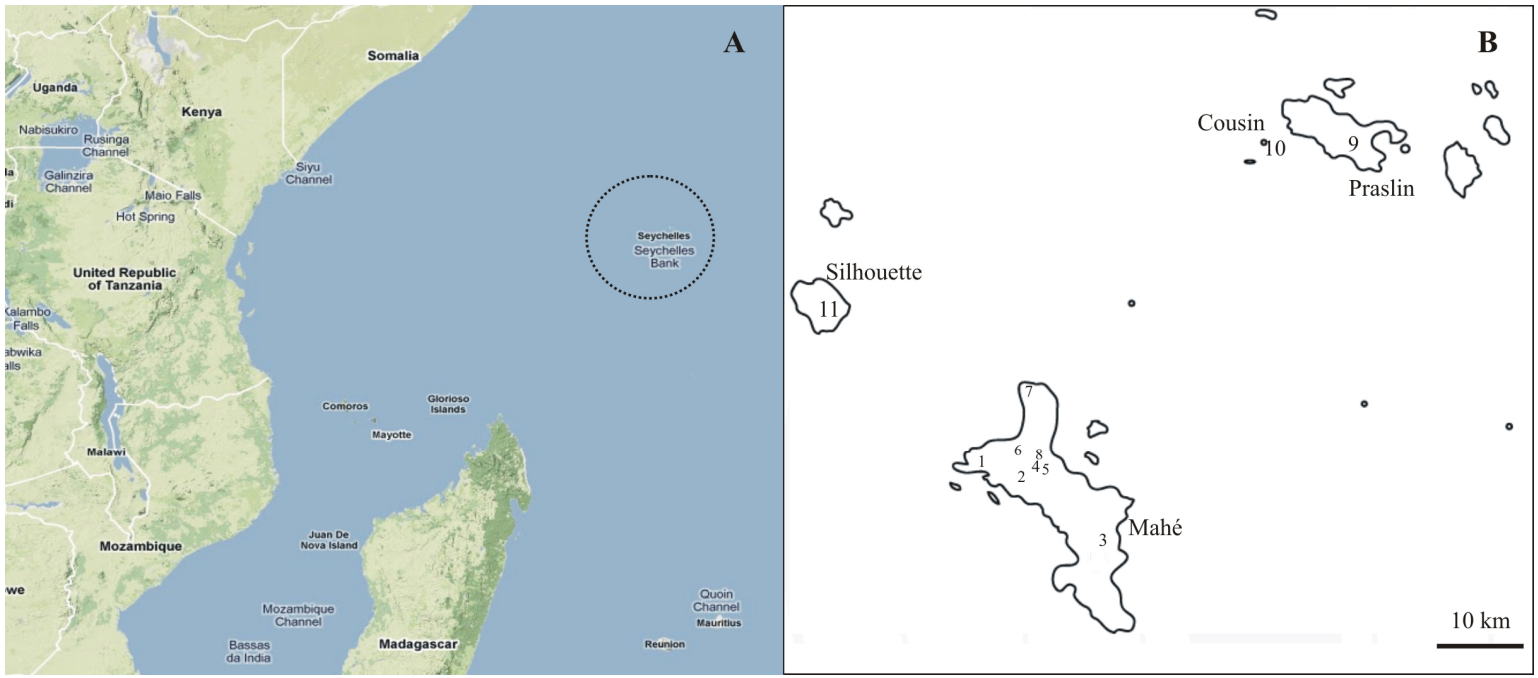

Fig. 2. A Location of the Seychelles archipelago marked with the circle in Indian Ocean. **B** Inner islands of Seychelles and the sampling sites in four islands: **1** Anse Major coastal shrubland (*I. bijuga*); **2** Casse Dent tea plantation (*V. seychellarum*); **3** L'Abondance undisturbed submontane forest (*V. seychellarum*); **4** Le Niol eucalypt plantation (*E. robusta*); **5** Le Niol pine plantation (*P. caribea*); **6** Le Niol *Vateriopsis* plantation (*V. seychellarum*); **7** North Point coastal forest (*I. bijuga*); **8** Sans Soucis forestry plantation (*V. seychellarum*); **9** Valeé de Mai, Unesco World Heritage Site (*I. bijuga*); **10** Coastal *Pisonia* woodland (*P. grandis*); **11** *Pisonia sechellarum* forest (*P. sechellarum*).

Fungal fruit-bodies were collected from all sites. To find resupinate and hypogeous sporocarps, litter and dead wood was carefully turned over. All fruit-bodies were air-dried at +30° C and packed in air-tight mini-grip plastic bags to avoid moisture and pest attack.

Molecular analyses

Molecular analyses were used to determine fungi from the roots and fruit-bodies (see IV for molecular methods). For the molecular analyses, at least two EcM root tips of each anatomotype per study site, root sections of *P. sechellarum* and pieces of fruit-bodies were used (I, II, III). DNA was extracted using the High Pure PCR Template Preparation Kit for Isolation of Nucleic Acids from Mammalian Tissue (Roche Applied Science) following manufacturer's instructions. DNA extracted from root tips and sporocarps was amplified using primers ITS1F (5'-cttggtcatttagaggaagtaa-3) and TW13 (5'-ggtccgtgtttcaagacg-3'). Primer pair AM1 (5'-gtttcccgtaaggcgccgaa-3')/NS31 (5'-ttggagggcaagtctggtgcc-3') was used to amplify AM fungi. The primer ITS1F in combination with a universal primer ITS4 (5'-tcctccgcttattgatatgc-3'), a basidiomycete-specific LB-W (5'-cttttcatctttccctcacgg-3') or an ascomycete-specific LA-W (5'-cttttcatctttc gatcactc-3') were used to detect other root-associated fungi from non-EcM root tips of *P. sechellarum*.

PCR included an initial 3 min at +95° C, followed by 35 cycles of 30 sec at +95° C, 30 sec at +55° C and 1 min at +72° C (2 sec increment time for each following cycle; final cycle, 10 min). PCR products (2 µl) were run with bromophenol blue (1 µl) on 1% agarose gel with ethidium bromide for ca 1 hour and visualized under the UV light.

PCR products were purified using Exo-Sap enzymes (Sigma, St Louis, MO, USA). Sequencing was performed using primers ITS4 and/or ITS5 (5'-ggaagtaaaagtcgtaacaagg-3') for the ITS region and ctb6 (5'-gcatatcaa taagcggagg-3') and/or TW14 (5'-gctatcctgagggaaacttc-3') for the LSU rDNA. Primers AM1 and NS31 were used to sequence 18S region of rDNA of AM-forming fungi. Sequences were processed and contigs were assembled using Sequencher 4.0 software (GeneCodes Corp., Ann Arbor, MI, USA). All unique sequences were submitted to the European Molecular Biology Laboratory (EMBL) database. Blastn and fasta3 searches were performed against International Nucleotide Sequence Databases (INSD) and UNITE (Kõljalg *et al.*, 2005) to provide approximate identification of mycorrhizal fungi.

Phylogenetic analyses

To determine phylogenetic placement of fungi detected from the Seychelles, phylogenetic analyses were performed for the /tomentella-thelephora (I), /coltricia (III) and /sordariales (II) lineages. For these taxa, additional sequences derived from fruit-bodies or EcM root tips were downloaded from INSD (International Nucleotide Sequence Databases). The sequences were aligned using Mafft ver. 5 (Katoh *et al.*, 2005) or Mafft ver. 6 (Katoh *et al.*, 2008). Phylogenetic analyses were performed using PAUP* ver 4.0 (Swofford, 2002)

and Mr. Modeltest ver. 2.2 (Nylander, 2004) or Modeltest 3.7 (Posada & Crandall, 1998).

Statistical analyses

It is almost impossible to find all EcM species represented in the community, because of their patchy and uneven distribution. To predict the sufficiency of sample size and to estimate the proportion of unseen species at different sites and in the Seychelles in general, program EstimateS ver. 7 (Colwell, 2004) and the minimum richness estimators Chao2 and Jacknkife2 (Gotelli & Coldwell, 2001) were used (II). Data from *P. grandis* was not included because it is known to associate with very narrow range of symbiotic fungi that are specific to *P. grandis* and have never found to associate with other plant species.

Different biotic and abiotic factors affect EcM fungal communities. To determine factors affecting the most EcM communities associated with *I. bijuga*, *V. seychellarum*, *P.caribea* and *E. robusta* Detrended Correspondence Analysis (DCA) in PC-Ord (McCune & Mefford, 1999) was performed (II).

RESULTS

Diversity of EcM fungi

All sampled plants possessed EcM root tips, except *P. sechellarum*. Molecular analyses revealed three AM fungal species from *P. sechellarum* roots in addition to endophytes and saprobes (I).

Altogether 37 species of EcM fungi were detected from all hosts (Table 1) (I, II). Native *P. grandis*, *V. seychellarum* and *I. bijuga* hosted three, 17 and 15 EcM fungal species, respectively. From introduced *E. robusta* and *P. caribea* roots seven and three fungal species were revealed, respectively. Inquiries against different sequence databases showed that all fungal species associated with native trees had less identity than 90.4% with published sequences, except partners of *P. grandis*.

The minimum richness estimators Chao2 and Jackknife2 predicted that there are 51,2 and 57,4 EcM species, respectively, associated with the native trees in Seychelles (excluding data from *P. grandis* that seems to have narrow fungal specificity). Insufficient sampling effort was indicated by Jackknife2 and rarefaction curves only for L'Abondance site that was supporting the highest EcM species richness per site. For the other sites, both curves were leveling off (II).

EcM fungal community composition

Community composition for *I. bijuga*, *V. seychellarum*, *P. caribea* and *E. robusta* at the site level was most affected by EcM host species, soil type, altitude and geographical distance (as coordinates). These variables were correlated with the main axes of DCA (II).

EcM fungi were shared between host plants. Only *P. caribea* (II) and *P. grandis* (I) possessed fungi distinctive from the others. They both hosted three EcM fungal species. From *P. caribea* roots we detected two species from the /suillus-rhizopogon lineage and one species from the /pisolithus-scleroderma lineage. *P. grandis* was associated only with members of the /tomentella-thelephora lineage. *I. bijuga*, *V. seychellarum* and *E. robusta* had several common EcM fungal species (II). *Coltriciella dependens* (Berk. & M.A.Curtis) Murrill was found from habitats of all three hosts, although form *I. bijuga* site it was detected only as a fruit-body. Other common species were shared only between two different hosts. Introduced *E. robusta* revealed species common with both *V. seychellarum* and *I. bijuga*. At the same time two native plant species shared three fungal partners – *Tomentella beaverae*, /tomentella-thelephora sp3 and /coltricia sp2.

Table 1. Ectomycorrhizal fungal species detected from the roots of native and introduced plant species. The sites were species occurred are given with the numbers according to Fig. 2.

Species	EMBL accession no. of ectomycorrhiza	Study site
Coltriciella dependens	AM412255	1º, 2*, 3, 4
/coltricia sp1	AM412260	1, 4
Coltricia aff. oblectans	AM412258	3, 6
/coltricia sp2	AM412257	3, 9*
/boletus sp1	AM412261	1, 9º
/boletus sp2	AM412262	1
/boletus sp3	AM412263	3
/boletus sp4	AM412264	9
/clavulina sp1	AM412265	3
/cortinarius sp1	AM412266	1, 9
/cortinarius sp2	AM412267	4
/cortinarius sp3	AM412268	3
/cortinarius sp4	AM412269	3
/ramaria-gautieria sp1	AM412270	4
/inocybe sp1	AM412271	2
/suillus-rhizopogon sp1	AM412273	5
/suillus-rhizopogon sp2	AM412274	5
/russula-lactarius sp1	AM412275	2, 3, 4
/pisolithus-scleroderma sp1	AM412272	5
/pisolithus-scleroderma sp2	AM412276	7
/pisolithus-scleroderma sp3	AM412277	2, 4*, 6
/pisolithus-scleroderma sp4	AM412278	7
/sordariales sp1	AM412279	3
/sordariales sp2	AM412280	3
/tomentella-thelephora sp1	AM412281	3
Tomentella tenuis	AM412288	7*
Tomentella parmastoana	AM412289	1*, 7º, 9*
/tomentella-thelephora sp2	AM412290	8
/tomentella-thelephora sp3	AM412291	2, 3, 6, 8, 9
Tomentella larssoniana	AM412283	1º, 4, 9*
Tomentella pileocystidiata	AM412284	7*, 9*
Tomentella intsiae	AM412285	9*
/tomentella-thelephora sp4	AM412286	3
Tomentella beaverae	AM412287	2, 3, 6, 7*
/tomentella-thelephora sp5	FM244913	10
Tomentella tedersooi	FM244912	10*
Tomentella pisoniae	FM244910	10*

º found only as a fruit-body
* found also as a fruit-body

Table 2. New *Tomentella* species and their occurrence as fruit-bodies in the study sites (site numbers respond to the Fig. 2)

New species	EMBL accession no. of holotype	Study site
Tomentella intsiae	AM412296	9
Tomentella beaverae	AM412298	7
Tomentella parmastoana	AM412300	1, 7, 9
Tomentella hjortstamiana	AM412303	7
Tomentella tenuis	AM412299	7
Tomentella pileocystidiata	FM955846	7, 9
Tomentella larssoniana	FM955844	1, 9
Tomentella tedersooi	FM244909	10
Tomentella pisoniae	FM244908	10

The most species rich study site was L'Abondance – a natural stand of *V. seychellarum*. The stand was dominated by /russula-lactarius sp1 that was also dominating on *E. robusta* (II). For *I. bijuga,* the most species rich site was Vallée de Mai that is also a natural stand dominated by a mystic palm, Coco de Mer (*Lodoicea maldivica* (J.F.Gmelin) Persoon). At this site, *Tomentella larssoniana* was the most common, but this fungus was also found on roots of *E. robusta* (II). In general, species of the /tomentella-thelephora lineage were dominating on roots of *I. bijuga* in different study sites (II). For *V. seychellarum,* species from different lineages dominated in different habitats. The /tomentella-thelephora lineage was the most species-rich on *I. bijuga*, *V. seychellarum* and *P. grandis*, followed by the /boletus lineage on *I. bijuga* (three spp.) and the /coltricia lineage on *V. seychellarum* (three spp.) and *I. bijuga* (three spp.) (I, II).

New species of *Tomentella*

Based on morphological and molecular data derived from fruit-bodies, we described nine new *Tomentella* species from Seychelles: *Tomentella pisoniae* Suvi & Kõljalg, *Tomentella tedersooi* Suvi & Kõljalg**,** *Tomentella parmastoana* Suvi & Kõljalg, *Tomentella hjortstamiana* Suvi & Kõljalg, *Tomentella tenuis* Suvi & Kõljalg, *Tomentella beaverae* Suvi & Kõljalg, *Tomentella pileocystidiata* Suvi & Kõljalg, *Tomentella larssoniana* Suvi & Kõljalg, *Tomentella intsiae* Suvi & Kõljalg (Table 2) (I). Two of them were found from *P. grandis* habitat and the other seven from different *I. bijuga* sites. We did not detect fruit-bodies of new species in *V. seychellarum* habitat, although *T. beaverae* was found as EcM also on *V. seychellarum* roots. There was only one species (*T. hjortstamiana*) detected solely as a fruit-body. Other eight new species were additionally found on root tips.

DISCUSSION

Fungal species composition

The overall species richness of EcM fungi in the Seychelles was low compared to temperate forests (I, II). For example, we detected 172 EcM species from a temperate wooded meadow in Estonia (IV). Besides climate, the main difference between the Tagamõisa wooded meadow and study sites in the Seychelles is the number of different host species per site. In the wooded meadow, many EcM host species grow intermixed, while the Seychelles' study sites always possessed a single EcM plant species. Nevertheless Douglas *et al*. (2005) detected 81 EcM species from a monodominant *Pinus contorta* Douglas stand, which is substantially more than the 37 species of the Seychelles. Therefore, it seems that in addition to lack of host species variety in sites there are other factors leading to a low EcM species richness in the Seychelles such as isolation (Peay *et al*., 2007) and the tropical climate (Tedersoo & Nara, 2010).

When taking into account all detected EcM species in the Seychelles, the most species rich taxa were Thelephorales, Agaricales and Boletales (although this division varied according to plant species). These taxa are distributed all over the world and are often the most species rich groups in different EcM communities in temperate regions (Kõljalg *et al.*, 2000), but also in the tropics (Yuwa-Amornpitak *et al.*, 2006; Peay *et al.*, 2010). Despite the fact that there are similarities with the other EcM communities in the world on the higher taxon level, the species found from the Seychelles were highly divergent. For the most species detected from the root tips the ITS sequence similarity with previously established species was usually below 90%. This may be due to the isolation of the Seychelles from the continents for more than 65 million years, enabling species to diverge from their ancestors. This may be, however, artefactual because when performing this study, no other sequences from tropical ecosystems were available. The more recent queries against INSD suggest that more similar species are present in SE Asia, Continental Africa and even Australia.

Dipterocarpaceae is one of the few plant groups in the tropics that forms EcM (Alexander & Lee, 2005). Dipterocarpaceae comprises many species, including the endemic *V. seychellarum* that are all ectomycorrhizal. *V. seychellarum* hosted the most EcM fungal species in the Seychelles (II). It was sampled from four sites that revealed altogether 17 EcM fungal partners. Dipterocarpaceae has been shown to host diverse EcM fungal communities (Sirikantaramas *et al.*, 2003; Moyersoen, 2006; Yuwa-Amornpitak *et al.*, 2006). Peay *et al.* (2010) found 105 EcM fungi from a highly diverse dipterocarp forest and a maximum of 26 species per 0.4 ha plot in Borneo. I believe that besides differences in host diversity, the long-time isolation and extensive deforestation that resulted the loss of habitats of *V. seychellarum* are the primary reasons for the observed richness differences. Another EcM host *I. bijuga* shared three fungal partners with *V. seychellarum*. It has been shown by Alexander *et al.* (1992) that seedlings of *Intsia palembanica* Miq. became colonized by the same

fungi that inhabited the neighbouring adult dipterocarps. This indicates that sharing of fungal partners is common for these plant taxa in general. In addition, the introduced *E. robusta* readily associated with fungi colonizing roots of *V. seychellarum* and *I. bijuga* (II). It seems that *E. robusta* received its fungal associates from these native trees, because the detected species have never been found to be associated with eucalypts in any exotic plantations or in Australia. More recent studies of my research group confirm these findings.

Pinus caribea (II) and *P. grandis* (I) did not share their EcM fungal partners with the other EcM plants, although there was no physical barrier to do so. *P. caribea* is exotic to the Seychelles and was introduced from Kenya as seedlings that were probably infected with EcM fungi before being planted at degraded sites in the Seychelles. Much higher species richness has been reported from other exotic pine plantations world-wide (Dunstan *et al.*, 1998). At least two of the fungi (*Rhizopogon* spp.) are specific to pines and therefore it is unlikely that *P. caribea* got them from native plants of the Seychelles or via long-distance spore dispersal. *P. caribea* has maintained its fungal partners for almost 30 years and at these fungi have not evidently shifted hosts to other EcM trees. A similar situation has been reported from other exotic pine plantations, where non-inoculated seedlings cannot survive in the new environment (Mikola, 1970) and usually fruit-bodies of the fungi co-introduced with the pines are not found in native plant communities. This may not be true with all introduced EcM fungi of pines, because there are reports of host shifts of *Amanita muscaria* (L.:Fr.) Lam. to the native *Nothofagus* of New Zealand (Orlovich & Cairney, 2004). The disability to associate with native fungal species and the non-invasiveness of pine EcM fungi have restricted the natural regeneration and distribution of *P. caribea* in the Seychelles and probably prevent it from becoming invasive. It has to be emphasized that in spite of sharing of mycobionts with the native trees, *E. robusta* has not yet become invasive.

Root associated fungi of *P. grandis*

Pisonia grandis is also native to the Seychelles. It belongs to the Nyctaginaceae family that is considered to be predominantly non-mycorrhizal with a few exceptions – some species are known to form arbuscular mycorrhiza or EcM (Wang & Qiu, 2006; Brundrett, 2009). Only Pisonieae tribe includes EcM members that include *Neea* (St. John, 1980), *Guapira* (Haug *et al.*, 2005) and *Pisonia* (Ashford & Allaway, 1982). Distribution of *Pisonia* includes tropical islands in the Atlantic, Pacific and Indian oceans in addition to South American continent. *P. grandis* is colonizing small islands, often called "bird islands" across the tropical Indo-Pacific (St. John, 1951; Burger, 2005; Douglas & Manos, 2007). It is dispersed by the sea birds when sticky fruits attach to the birds' feathers (Walker, 1991; Turner, 2001). Birds, in turn, use branches of *P. grandis* as roosting and nesting sites.

Previous studies revealed two EcM fungal partners on the roots of *P. grandis*. One of the studies was carried out in Cousin Island in the Seychelles, were EcM fungi were detected only based on morphology and anatomy of the

colonized root tip (Ashford & Allaway, 1982, 1985). By using molecular techniques, Chambers *et al.* (2005) showed that two EcM fungi colonizing roots of *P. grandis* in the Great Barrier Reef were members of the /tomentella-thelephora lineage and they hypothesized that these mycobionts might be the same species found in the Seychelles because of morphological similarities. We detected three different EcM species from the roots of *P. grandis* in Cousin Island (I). Two of them were closely related to the species detected by Chambers *et al.* (2005). However, no fungal species were shared between *P. grandis* and other Seychelles' EcM plants. In fact, sequence comparisons have revealed no association of fungi isolated from *P. grandis* roots and any other hosts. It seems that *P. grandis* is associated only with certain *Tomentella* species throughout its distribution area. This is quite an unusual situation, because most EcM plants host many different fungal partners from different taxa. Other EcM plant species in Nyctaginaceae do not support such a narrow host range (Tedersoo *et al.*, 2010b). The exceptionally restricted mycobiont range of *P. grandis* may be attributable to the unique environment in "bird islands". The high rate of simultaneous N and P amendment in the guano habitat of relatively saline and alkaline coral sand soils provide a unique environment for the fungi and plants (Allaway & Ashford, 1984). For EcM fungi that are usually distributed in nutrient-poor soils, such fertile soil conditions may prove particularly challenging (Chambers *et al.*, 2005). Allaway & Ashford (1984) found that the input of N and P to the soil in "bird islands" may reach 1000 and 220 kg ha^{-1} y^{-1}, respectively. Nitrogen deposition may reduce the above- and below-ground diversity of EcM fungi (Brandrud & Timmermann, 1998; Wallenda & Kottke, 1998; Lilleskov *et al.*, 2002; Avis *et al.*, 2003; Edwards *et al.*, 2004), EcM colonization (Baum & Makeschin, 2000; Treseder, 2004) and the quantity of extramatrical mycelium (Nilsson & Wallander, 2003; Hendricks *et al.*, 2006). The impact of P fertilization on EcM fungi is poorly understood, but the present knowledge indicates that enhanced levels of soil P affect the EcM fungal community (Baum & Makeschin, 2000; Hedh *et al.*, 2008) and reduce EcM colonization (Bougher *et al.*, 1990). High levels of nutrient deposition may offer a selective advantage to certain *Tomentella* species among other fungi in *P. grandis*. Sharples & Cairney (1997, 1998) demonstrated that a mycobiont isolated from *P. grandis* roots was able to utilize organic nitrogen compounds. At the same time, this fungus had a poor ability to utilize inorganic nitrogen, suggesting adaptation of the fungus to rapidly mobilize organic nitrogen in environmental conditions where addition of large amount of nitrogen is coupled with high rates of leaching.

New detected relationships and fungal species

We demonstrated EcM lifestyle of four different species of the /coltricia lineage that belong to the order Hymenochaetales. Hymenochaetales comprise predominantly wood-inhabiting fungi that are saprobes or weak parasites, causing white rot (III). Some members possess biotrophic associations with mosses. There were a few dubious reports on the synthesis of arbutoid mycorrhiza and

EcM with species of *Coltricia* (see Tedersoo et al. 2010a) In addition, Thoen & Ba (1989) tracked the mycelial connections of *Coltricia cinnamomea* (pers.) Murr. on *Uapaca guineensis* Müll. Arg roots and reached brown bristly EcM root tips. But still there was no solid evidence for EcM lifestyle of /coltricia *in situ*. In the Seychelles, four different /coltricia species were detected from *V. seychellarum*, *I. bijuga* and *E. robusta* roots. Two of them were also detected as fruit-bodies. Our findings complement that of Tedersoo *et al.* (2007), where *Coltricia perennis* (L.) Murrill possessed rations of stable ^{13}C and ^{15}N isotope concentrations similar to that of most EcM basidiomycetes and clearly different from saprobes.

Pisonia sechellarum is endemic to the Seychelles and occurs only in Silhouette Island. There was no previous information about its mycorrhizal status and therefore one of the aims of this study was to uncover the mycorrhizal relations of this rare plant. The genus *Pisonia* includes species that are non-mycorrhizal or form AM or EcM (Wang & Qiu, 2006; Brundrett, 2009). We detected three AM fungal partners from *P. sechellarum* roots, but found no evidence for EcM formation (I). Based on this information, we can conclude that *P. sechellarum* is AM plant.

In addition to the new mycorrhizal relationships, we also found and described nine new *Tomentella* spp. (I). Fruit-bodies of two of them were collected from the *P. grandis* habitat in Cousin Island and the others form different habitats of *I. bijuga*. All these species, except *T. hjortstamiana*, were also detected from plant roots. Describing new species form the tropics is essential to learn more about their ecology and also obtain reference sequences for the database to improve species identification from the environmental samples in the future.

CONCLUSIONS

Ectomycorrhizal fungal diversity is low in the Seychelles compared to other temperate and tropical ecosystems, probably due to long-term isolation and extensive removal of natural vegetation. Although we did not detect close relationships with previously detected EcM species, we cannot exclude the possibility that we missed it because there are not many studies about EcM communities in the tropics and information on tropical EcM fungal taxonomy is restricted. The most species rich taxa in the Seychelles were Thelephorales, Agaricales and Boletales, that are also the most common and diverse in other tropical and temperate fungal communities.

Many EcM fungal species overlapped on roots of native trees and the introduced *Eucalyptus robusta*. On the contrary, the introduced *Pinus caribea* did not share its fungal partners that were co-introduced with the seedlings. Although *E. robusta* was associated with the local fungi it still has not become invasive, yet. The role of EcM fungi and suitable niches for the introduced plants in becoming invasive needs further investigation.

Pisonia grandis is native to the Seychelles, but it did not share its fungal partners with other native or introduced EcM trees. The unique environmental conditions of *P. grandis* habitat may be responsible for the lack of species overlap. High rates of nutrient amendment to the soil as bird guano create challenging conditions for EcM fungi that are more common in nutrient-poor soils. In addition, it seems that these extreme conditions allow *P. grandis* to associate with only few species of *Tomentella* that are specialized to this environment, throughout the distribution of *P. grandis*. Another species of Nyctaginaceae, *Pisonia sechellarum*, was forming AM and not EcM.

Description of new fungal species is important for the future EcM community studies, because we need DNA sequences from the well-annotated fruit-bodies to detect and name species derived from environmental samples. We described nine new *Tomentella* species from the Seychelles, eight of them were also found on the roots of different trees. This shows that despite limited sampling period, the overlap between fruit-bodies and EcM can be remarkable. In conclusion, more attention should be paid on concomitant collection of the fruit-bodies during EcM studies.

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SUMMARY IN ESTONIAN

Pärismaiste ja võõrpuuliikidega seotud mükoriisaseened Seišelli saartel

Enamik maismaataimi moodustavad koos seentega ühiseid struktuure mida nimetatakse seenjuureks ehk mükoriisaks. Antud kooseluvorm on üldjuhul kasulik mõlemale osapoolele. Seen varustab taime mineraalainete (lämmastik, fosfor) ning taim omakorda seent suhkrutega. Lisaks aitab seen taimel üle elada põuaseid aegu, varustades taime veega ning pakub kaitset erinevate haigusetekitajate ja raskemetallide vastu. Seenjuure väliste ja anatoomiliste tunnuste põhjal jagatakse see erinevatesse tüüpidesse. Kõige levinum seenjuure tüüp on arbuskulaarne mükoriisa, mille korral seeneniidid tungivad taimerakkudesse, moodustades seal vesiikuleid ja dihhotoomselt harunedes põõsakujulisi arbuskuleid. Ektomükoriisa korral moodustavad seeneniidid juuretipu ümber seenmantli ning juurerakkude vahele Hartigi võrgustiku, mille kaudu toimub partneritevaheline toitainete liikumine. Ektomükoriisa on samuti levinud kogu maailmas, seda moodustavad ligikaudu 6000 taime- ja 20 000–25 000 seeneliiki. Vaatamata sellele, et ektomükoriisa on valdavaks seenjuure tüübiks parasvöötmes, omab ta suurt tähtsust ka troopilistel aladel. Viimasel ajal on hakatud üha enam tähelepanu pöörama troopilistele ektomükoriisaseente kooslustele ja on leitud, et nende liigirikkus on kohati võrreldav parasvöötmes esinevatega.

Käesolev töö viidi läbi Seišellidel – India ookeanis paikneval saarestikul, mis on mandritest olnud edaldatud ligikaudu viimased 65 miljonit aastat. Inimasustus saabus saarele alles 1770. aastal, millega kaasnes peagi ulatuslik metsade mahavõtmine, et rajada istandusi erinevatele õlitaimedele, kaneelile ja kookospalmile. Seetõttu hävis suurematel saartel enamus looduslikust taimestikust, säilides vaid raskesti ligipääsetavates kohtades. Paljud sissetoodud taimed olid võimelised saartel edukalt kasvama, levima ning uusi kasvukohti asustama, seades ohtu allesjäänud kohalike liikide elupaigad. Kahekümnenda sajandi alguses hakati tähelepanu pöörama looduse säilitamisele ja seetõttu on praeguseks loodud mitmeid looduskaitsealasid ning tingimusi kohaliku taimestiku taastumiseks.

Seišellidel esineb viis ektomükoriisat moodustavat taimeliiki viiest erinevast sugukonnast – tsesalpiinialised (*Caesalpiniaceae; Intsia bijuga*), kaksiktiibviljakulised (*Dipterocarpaceae; Vateriopsis seychellarum*), imelillelised (*Nyctaginaceae; Pisonia grandis*), mürdilised (*Myrtaceae; Eucalyptus robusta*) ja männilised (*Pinaceae; Pinus caribea*). Lisaks kasvab Silhouette saarel liik *Pisonia sechellarum*, mida ei leidu kuskil mujal maailmas (on endeemne liik). Enne seda uuringut ei olnud teada, millist seenjuure tüüpi antud taimeliik moodustab, oli vaid teada, et perekond *Pisonia* sisaldab liike, mis on ektomükoriissed, arbuskulaarmükoriissed või ei moodusta seenjuurt. Lisaks *P. sechellarumile* on Seišellidele endeemne ka *V. seychellarum*. *I. bijuga* on kohalik liik, kuid *P. caribea* ja *E. robusta* sissetoodud istanduste rajamiseks.

Töö eesmärgid oli määrata seenjuurt moodustavad seened *V. seychellarum, I. bijuga, P. grandis, E. robusta* ja *P. caribea* juurtest. Võrrelda seenekooslusi sissetoodud ning kohalike taimede vahel. Kindlaks määrata, millist tüüpi seenjuurt moodustab *P. sechellarum*. Korjata erinevates proovipaikadest ektomükoriisete seente viljakehi ning uutele liikidele koostada kirjeldused. Selles töös kasutatav proovivõtu ning molekulaarsete ja statistiliste analüüside metoodika töötati välja eelneva uuringuga Tagamõisa puisniidul. Väljatöötatud meetodid andsid ka troopilistes taimekooslustes väga hea tulemuse.

Proovivõtualad asusid neljal erineval saarel – Mahé (taimeliigigd *I. bijuga, V. seychellarum, E. robusta, P. caribea*), Praslin (*I.bijuga*), Cousin (*P. grandis*) ja Silhouette (*P. sechellarum*). Erinevad ektomükoriisat moodustavad taimeliigid ei kasvanud kunagi koos ühel proovivõtualal. Juureproovid (15x15 cm, sügavusega 5cm) korjati noa abil. Proovide arv oli vastavalt alale 5–24, olenedes peremeestaime arvukusest antud kohas. Lisaks korjati proovialadelt seente viljakehad, pöörates erilist tähelepanu liibuvaid ning maa-siseseid viljakehi moodustatavatele liikidele. Seente poolt asustatud juuretipud jagati väliste ja anatoomiliste tunnuste põhjal nn. morfotüüpidesse. Kogutud viljakehad kuivatati ja määrati liigi või perekonna tasemel. Nii mükoriissetest juuretippudest kui viljakehadest eraldati seene DNA ning määrati ribosoomi DNA ITS1, 5.8S ja ITS2 piirkondade nukleotiidsed järjestused. Saadud DNA järjestusi kasutati liikide määramiseks erinevate avalike andmebaaside (EMBL, NCBI, UNITE) abil ning ka fülogeneesi analüüside läbiviimiseks.

Erinevate peremeestaimede juurtelt leidsime kokku 37 ektomükoriisat moodustavat seeneliiki. Võrreldes parasvöötme metsadest ning ka troopilistest taimekooslustest kirjeldatud ektomükoriisaseente liigirikkusega, on Seišellidelt leitud liikide arv madalam. Peamiseks põhjuseks on tõenäoliselt asjaolu, et Seišellid on olnud mandritest eraldatud ligikaudu 65 miljonit aastat. Lisaks saarte väiksusele ja isoleeritusele võis liikide väljasuremist põhjustada ka looduslike metsade suureulatuslik maharaiumine, mistõttu kadus enamus seentele sobivaid elupaiku.

Kõige rohkem ektomükoriisseid seeni oli seotud saartele looduslike liikidega *V. seychellarum* (17 seeneliiki) ja *I. bijuga* (15 seeneliiki). Introdutseeritud *E. robusta* ja *P. caribea* juurtest leiti vaid kolm seeneliiki. *V. seychellarum*, *I. bijuga* ja *E. robusta* jagasid ektomükoriisseid seeni, samal ajal kui liigiga *P.caribea* seotud seeni me ei leidnud ühegi teise taime juurtelt. Tõenäoliselt on viimased sisse toodud koos männiistikutega. *P. grandis* oli seotud kolme ektomükoriisse seeneliigiga, mida samuti ei esinenud kuskil mujal saarestikus. Kõige liigirikkamad olid seeneseltsid *Thelephorales*, *Agaricales* ja *Boletales*. Antud seltsid on avaldatud uurimistööde põhjal ühed liigirikkamad ning sagedamini esinevad ektomükoriisa taksonid nii parasvöötme kui ka troopilistes metsades. Vaatamata sellele oli Seišellidelt leitud seeneliikide ning varasemates uuringutes täheldatud liikide vaheline sarnasus väike. DNA ITS nukleotiidsete järjestuste kontrollimine erinevate DNA järjestuste andmebaaside vastu tuvastas enamatel juhtudel suurimaks järjestustevaheliseks sarnasuseks 90%. Seente puhul loetakse liigi lävieväärtuseks tavaliselt 97–98% ITS järjestuste sarnasust. Seetõttu võib arvata,

et tegu on kas teadusele kirjeldamata taksonitega või avalikest andmebaasidest puuduvad vastavate seeneliikide ITS järjestused. Arvestades saarte pikaajalist isoleeritust ning endeemsete taimeliikide kõrget arvu saab oletada, et ka seeneliigid, mis nende taimedega seenjuuri moodustavad on arenenud uuteks liikideks.

Liigi *P. sechellarum* juuri asustasid arbuskulaarmükoriissed seened, ühtegi ektomükoriisset juuretippu ei leitud. Samasse perekonda kuuluv liik *P. grandis*, moodustas samal ajal ektomükoriisat kolme erineva *Tomentella* liigiga. Juba varasemate andmete põhjal oli püstitatud hüpotees, et liigiga *P. grandis* on seotud ainult perekonna *Tomentella* liigid, kuigi taim levib nii India kui ka Vaikse ookeani troopilistel saartel. Antud uurimistöös leitud andmed kinnitavad seda hüpoteesi kusjuures leitud *Tomentella* kolm liiki ei moodustanud seenjuurt ühegi teise peremeestaimega, kuigi proovivõtupaikade vahelised kaugused olid väikesed. Taolist omapärast seotust vaid kindla seeneperekonna liikidega saab seletada *P. grandis* kasvupaiga eripäraga. Nimelt on ta levinud saartel, millel pesitseb palju linde, kelle lämmastiku- ja fosforirikkad väljaheited muudavad mullastiku toitainete rikkaks. Vaatamata suurele toitainete lisandumisele, toimub ka nende kiire välja leostumine mullast. Mõningad uuringud on andnud alust arvata, et liigiga *P. grandis* seotud seened on võimelised kiiresti omastama lämmastikku, muutes nad spetsialistideks antud keskkonnas. Praeguseks on veel teadmata, kuidas toimub antud spetsialist-seeneliikide levik, kuid tõenäoliselt on levitajateks linnud, kes levitavad ka *P. grandis* seemneid.

Ekspeditsiooni käigus koguti üheksa teadusele seni kirjeldamata *Tomentella* liigi viljakehad. Kasutades nii anatoomilisi kui ka molekulaarseid tunnuseid kirjeldati need uute liikidena: *Tomentella pisoniae* Suvi & Kõljalg, *Tomentella tedersooi* Suvi & Kõljalg**,** *Tomentella parmastoana* Suvi & Kõljalg, *Tomentella hjortstamiana* Suvi & Kõljalg, *Tomentella tenuis* Suvi & Kõljalg, *Tomentella beaverae* Suvi & Kõljalg, *Tomentella pileocystidiata* Suvi & Kõljalg, *Tomentella larssoniana* Suvi & Kõljalg, *Tomentella intsiae* Suvi & Kõljalg. Neist kaheksa liiki (v.a. *T. hjortstamiana*) leiti ka seenjuurtena. See tulemus näitab nn. maaaluse (seenjuur) ja -pealse (seene viljakeha) liigirikkuse suurt kokkulangevust. Siiani on varasemates uurimistöödes leitud, et maa-alune e. seenjuure liigirikkus on maapealsest märksa kõrgem. Antud uurimistöö põhjal võib oletada, et nn. maapealse liigirikkuse uuringutesse on suhtutud pealiskaudselt, sest liibuva viljakehaga seeneliike on mittespetsialistil keeruline avastada. Uute seeneliikide kirjeldamine kasutades nii morfoloogilisi kui ka molekulaarseid tunnuseid on väga oluline ka selleks, et keskkonnaproovidest leitud seente DNA-d oleks võimalik hiljem liigi tasemel määrata. Kuna usaldusväärse määrangu saamiseks on vaja kattuvust DNA järjestusega, mis pärineb korrektselt määratud seene viljakehast.

ACKNOWLEDGEMENTS

I am very grateful to my supervisors Urmas Kõljalg and Leho Tedersoo, who have helped me since I became a part of workgroup of mycologists.

I am particularly grateful to Kadri Põldmaa, Kadri Pärtel, Irja Saar, Anu Kollom, Merje Toome, Erast Parmasto, Ilmi Parmasto, Bellis Kullman, Indrek Sell, Kuulo Kalamees, Teele Jairus, Sergei Põlme and Mohammad Bahram for all the support and friendly attitude and of course I have to say special "thank you" to my room-mates Kessy Abarenkov and Heidi Tamm – I will never forget our tea breaks and stylish Wednesdays.

I am particularly grateful to Katy Beaver who was making all the arrangements in the Seychelles making it possible to carry out our study there and helped later with the papers. I thank Justin Gerlach who helped to collect root samples of *Pisonia sechellarum*. I am very grateful to Aline Finger, Charlotte Klank, Forest and Kim Starr and Bruno Senterre for providing me with the pictures of Seychelles' native trees.

I also thank my parents who have always believed in me even when it seemed that things go wrong and I am thankful to my darling Raul for supporting me during the last efforts of my PhD studies.

PUBLICATIONS

CURRICULUM VITAE

I. General

Name:	Triin Suvi
Date and place of birth:	29.05.1982, Antsla, Estonia
Citizenship and nationality:	Estonian
Language skills:	Estonian (mother tongue), English
Address:	Institute of Ecology and Earth Sciences, University of Tartu. 14A Ravila Street 50411 Tartu, Estonia. e-mail: triin.suvi@gmail.com
Current position	University of Tartu, Institute of Ecology an Earth Sciences, researcher

Education

1988–2000	Gymnasium of Põltsamaa
2000–2004	University of Tartu, Botany and Ecology, B.Sc.
2004–2005	University of Tartu, Botany and Mycology, M.Sc.
2005–2010	University of Tartu, PhD student in botany and mycology

II. Scientific and research activity

Research interests

Influence of human activity to ectomycorrhizal communities.
Ectomycorrhizal associates of Nyctaginaceae spp.
Ecology of species of Thelephoraceae.

Publications (CC)

Suvi T, Tedersoo L, Abarenkov K, Gerlach J, Beaver K, Kõljalg U. 2010. Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. *Mycologia*: accepted.

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Awards & Scholarships

Doctoral School of Ecology and Environmental Sciences PhD student scholarship, 2007

Ministry of Science and Education Best student PhD project, 2006 (2nd prize)

Doctoral School of Ecology and Environmental Sciences PhD student scholarship, 2006

Doctoral School of Ecology and Environmental Sciences PhD student scholarship, 2005

Other activities and memberships

2004 – Member of the Estonian Naturalists' Society

CURRICULUM VITAE

I. Üldandmed

Ees- ja perekonnanimi: Triin Suvi
Sünniaeg ja koht: 29.05.1982, Antsla, Eesti.
Kodakondsus: Eesti
Keelteoskus: eesti, inglise
Aadress, telefon, *e-mail*: Tartu Ülikool, Maateaduste ja Ökoloogia Instituut. Ravila 14A, 50411. triin.suvi@gmail.com
Praegune töökoht: Tartu Ülikool, Ökoloogia ja Maateaduste Instituut, erakorraline teadur.

Haridus

1988–2000 Põltsamaa Ühisgümnaasium
2000–2004 Tartu Ülikool, B.Sc. botaanika ja ökoloogia eriala
2004–2005 Tartu Ülikool, M.Sc botaanika ja mükoloogia eriala
2005–2010 Tartu Ülikool, doktorant botaanika ja mükoloogia erialal

II. Teaduslik ja arendustegevus

Peamised uurimisvaldkonnad.
Inimtegevuse mõju ektomükoriisaseente kooslustele
Perekonna *Nyctaginaceae* ektomükoriissed seened
Seeneperekonna *Thelephoraceae* liikide ökoloogia

Teaduspublikatsioonide loetelu:

Suvi T, Tedersoo L, Abarenkov K, Gerlach J, Beaver K, Kõljalg U. 2010. Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. *Mycologia*: accepted.

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Konverentsiettekanded:

Suvi T, Tedersoo L, Abarenkov K, Gerlach J, Beaver K, Kõljalg U. Ectomycorrhizal fungal symbionts of *Pisonia grandis* and mycorrhizal status of *Pisonia secheallrum* (Nyctaginaceae). 21st New Phytologist Symposium. The ecology of ectomycorrhizal fungi. Detsember 2008, Montpellier, Prantsusmaa.

Saadud uurimistoetused ja stipendiumid.

Ökoloogia ja keskkonnateaduste doktorikool, toetud doktorandi teadustöö finantseerimiseks 2007

II preemia bio-geoteaduste valdkonnas, doktoriõppe üliõpilaste astmes, Eesti üliõpilaste teadustööde riiklikul konkursil

Ökoloogia ja keskkonnateaduste doktorikool, toetud doktorandi teadustöö finantseerimiseks 2006

Ökoloogia ja keskkonnateaduste doktorikool, toetud doktorandi teadustöö finantseerimiseks 2005

Muu teaduslik organisatsiooniline ja erialane tegevus.

2004 – Eesti Looduseuurijate Seltsi liige

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