

論文の内容の要旨

論文題目: Ectomycorrhizal fungal communities of secondary *Tristaniopsis* forests in
Indonesia

(インドネシアにおけるトリスタニオプシスの優占する二次林の外生菌根菌群集)

氏 名 ヘルベルト

Introduction

Dominant trees in many forests ecosystems are associated with ectomycorrhizal (ECM) fungi and depend on their survival. In fact, the ECM fungi and their composition could be the most significant determinant of seedling establishment in disturbed areas, under boreal, temperate, and subtropical regions. However, there is no previous study documenting ECM fungal communities in heavily disturbed tropical areas. Available data of ECM fungi in Southeast Asia are mostly from undisturbed Dipterocarpaceae forests, largely because this dominant ECM host is often replaced by fast growing arbuscular mycorrhizal host trees after disturbance. However, in some part of Southeast Asia, potentially ECM trees belonging to Myrtaceae become dominant in disturbed sites, although we know nothing about their ECM colonization and ECM fungal communities.

Myrtaceae included both arbuscular mycorrhizal and ECM host lineage. The latter includes *Eucalyptus*, on which ECM fungi have been documented in native areas like Australia and been introduced to other areas. *Tristaniopsis* is another ECM host lineage from Myrtaceae, distributed in part of Southeast Asia, Australia and Oceania. Some of *Tristaniopsis* species appear in secondary forests in Southeast Asia, and we do not know the ECM community and composition associated with this tree. In this study we would like to understand the ECM fungal community characteristics in secondary *Tristaniopsis* forests. Our objectives were (1) to confirm the ECM colonization of *Tristaniopsis* under secondary tropical forest settings, (2) to characterize ECM fungal diversity and species composition, (3) to clarify how many ECM fungal species are shared with other tree species, especially with Dipterocarpaceae, and (4) to investigate the environmental effect on ECM fungal communities.

Materials and Methods

Samples were collected from nine secondary *Tristaniopsis* forests located in Bangka (four sites) and Kalimantan (five sites). We randomly collected 100 soil samples (5cm x 5cm to

10cm depth) from Bangka and 150 soil samples from Palangkaraya. Each sampling point was selected within a few meters from a *Tristaniopsis* tree to have a better chance of collecting its ECM roots. The interval between samples was at least 5 m apart to avoid collecting the same ECM clone in different samples. Collected samples were stored at ambient temperature until the next procedure. Within four days after collection, roots were carefully separated from soil aggregate and cleaned in tap water. The ECM roots were examined under a stereomicroscope (Olympus ZS40).

ECM root tips in the soil samples were classified into morphotypes, from each of which three tips were subjected to DNA extraction separately. PCR and sequencing were performed targeting ITS regions on ribosomal DNA. Obtained sequences were grouped into molecular operational taxonomic units (MOTUs) based on $\geq 97\%$ similarity threshold. Each MOTU (species hereafter) identity was assigned based on BLAST results in INSD (International Nucleotide Sequence Database). Frequency of individual ECM fungal species were pooled and used for community analysis. The relative importance of soil parameter, climate, and geographic distance in structuring ECM fungal communities were also analyzed.

Results and Discussion

Of 250 soil samples, 186 (74.4%) contained ECM tips from which 1750 ECM root tips were used for molecular identification. Sequences were successfully obtained from 853 root tips (48.7%). In 127 ECM fungal species (18 families) were identified from 853 high-quality sequences (48.7%) with ITS similarity threshold of 97%: 56 and 79 ECM fungal species from Bangka and Palangkaraya, respectively. Only 18 ECM fungal species were found in five or more soil samples, while 81 ECM were singletons (i.e., were found only once) and 12 ECM were doubletons. The most species-rich ECM fungal families were Thelephoraceae (26 species), Russulaceae (25 species), and Boletaceae (13 species). The most frequently observed species was Thelephoraceae sp.1, which was found in 39 of 250 soil samples, followed by *Russula* sp.1 (29 soils) and *Thelephoraceae* sp.12 (19 soils). The jackknife2 richness estimated there are 145 species across the nine sites. The rarified species accumulation curve and Jackknife2 estimate continued rising with increasing sampling effort. It indicates that more species would be found if more samples were taken.

In total, from 11 host families identified only Myrtaceae (57.3%), Dipterocarpaceae (19.4%), Fagaceae (7.29%), Fabaceae (7.29%) and Gnetaceae (2.4%) were ECM host. The existence and dominance of Dipterocarpaceae indicating a regeneration of this host in secondary forests. This is a promising result, considering all sampling locations were highly disturbed areas.

The ECM fungal communities of co-existing host families (Myrtaceae and Dipterocarpaceae) were not significantly different ($F=1.06$, $R^2=0.07$, $p=0.38$). In fact, the

three most dominant ECM fungal species (*Russula* sp.2, Thelephoraceae sp.1, and Thelephoraceae sp.12) were shared among these host families, indicating their compatibility. However, sampling location affect the ECM fungal community ($F=1.94$, $R^2=0.14$, $p=0.02$). Moreover, in Palangkaraya, all the ECM fungal species that occurred more than 5 samples were found in both Myrtaceae and Dipterocarpaceae.

Most of ECM fungi species detected in this study had no similar (>97% similarity) records in INSD. Only 10 of 127 EMF had matches with deposited sequences. Eight of them (Agaricomycetes, *Amanita* sp.3, *Amanita* sp.4, *Atheliaceae* sp.3, Boletaceae sp.1, Cortinariaceae sp.1, *Russula* sp.2 and *Xenasmattella* sp.2) were likely the same species found in Lambir Hill National Park, Sarawak (Peay et al. 2010). Another species matched with the sequence of Thelephoraceae from Seychelles (Tedersoo et al., 2007); and *Heimioporus* sp. from Indonesia. None of EMF species in this study matched with sequences obtained from *Tristaniopsis* in New Caledonia (Waseem et al., 2017), *Eucalyptus*, and numerous ectomycorrhiza host trees in areas other than Southeast Asia.

Due to the lack of host diversity in Bangka, host and environmental effects on ECM fungal community were studied only in Palangkaraya. All soil properties (total C, total N, C/N ratio, and pH) have significant differences between the sampling locations. However, only pH ($F=3.6$, $P<0.001$) and total nitrogen ($F=3.9$, $P<0.001$) had a significant effect on the ECM fungal communities. This result corresponds with the forest's types from our sampling sites. Peat forests tend to have lower pH, while heath forests have lower Total N content. In contrast, climate variables did not have a significant effect on the composition of the fungal communities: annual average temperature ($F=1.56$, $p=0.154$) and precipitation ($F=1.29$, $p=0.262$). These results suggest that soil type has stronger role than climate on shaping ECM fungal community and supported by previous study (Essene et al., 2017).

Comparison between ECM fungi sequences obtained from this study reveal a ECM sharing pattern. Palangkaraya shared seven species with Bangka Island, five species with Lambir Hill and five species with Bukit Bangkirai. Bangka shared four ECM fungal species with Lambir Hill and three species with Bangkirai. There are no species sharing with New Caledonia. However, four ECM sequences from New Caledonia of them have >97% similarity with ECM from Australia. These results shows a geographical barrier between New Caledonia (Australasia) with Bangka and Palangkaraya (Indomalaya).

In phylogenetic analyses, many lineages in this study were related to ECM fungi from other Indomalaya, Australasia and Nearctic region. However, there some member of /amanita, /atheliaceae, /boletus, /laccaria, /scleroderma, /russula-lactarius and /tomentella-thelephora were clustered alone and did not related to the same lineage from other geographical region. This result indicating that most of ECM fungi associated with secondary *Tristaniopsis* forests were originated from Indomalaya, Australasia, and Nearctic, which some of this ECM were biogeographically isolated.

Conclusion

Secondary tropical forests dominated by *Tristaniopsis* trees in Bangka and Palangkaraya were found to harbor diverse ECM, many of which were new species that were not identified in previous studies. Some ECM fungi were shared between *Tristaniopsis* and other coexisting tree genera at the study sites. While many tropical rainforests become arbuscular mycorrhizal ecosystems after disturbance, secondary forests dominated by *Tristaniopsis* trees remain ECM ecosystems. Hence, they could function as refugia for ECM fungi that inhabit primary mixed dipterocarp forests in Southeast Asia