First Report on Fungal Symbionts of *Lycopodiaceae* Root from Mount Gede Pangrango National Park Indonesia

Yusuke Takashima[1,2], Kazuhiko Narisawa[2], Iman Hidayat[3] and Gayuh Rahayu[1]*

[1] Department of Biology, Faculty of Mathematics and Natural Sciences,
Bogor Agricultural University, Dramaga Campus, Bogor 16680, Indonesia
[2] Graduate School of Agriculture, Ibaraki University,
3−21−1 Chuo, Ami-machi, Inashiki-gun, Ibaraki, 300−0393, Japan
[3] Research Center for Biology, Indonesian Institute of Sciences (LIPI),
Jl. Raya Jakarta-Bogor km 46, Cibinong 16911, West Java, Indonesia

Association of arbuscular mycorrhizal fungi (AMF) with plant roots is often stated as critical to the successful establishment and diversification of terrestial plants. Information on AMF associations with *Lycopodiaceae*, ancient lineages of terrestrial plants, contributes to the understanding of terrestrial colonization of plants. While diversity of AMF and other fungal endophytes of some agricultural plants in Indonesia is extensively studied, terresterial *Lycopodiaceae* are not well documented. In this study, colonization status of AMF and root endophytic fungi (REF) in sporophytes of terresterial *Lycopodiaceae* (*Huperzia selago, H. serrata* and *Lycopodium clavatum*) inhabiting Mt. Pangrango at different altitudes were investigated on the basis of root microscopic observations and culture-dependent methods, respectively. As results, AMF colonization was observed in *H. selago* collected from near summit (NS, 2998 m asl) and *H. serrata* collected from Kandang Badak (KB, 2440 m asl) and Cibereum Water Fall (CF, 1728 m asl), but not in *L. clavatum* from Mount Pangrango Summit (PS, 3019 m asl). These results indicate that the colonization rate of AMF is affected by differences in altitude. REF isolates consisting of seven genera including *Cadophora, Cladophialophora*, *Cryptosporiopsis*, *Leohumicola*, *Leptodontidium*, *Phialocephala*, *Pseudoclathrosphaerina* (putatively similar to *Meliniomyces*), and ecologically uncertain *Helotiales* were obtained. These isolates contained putative dark septate endophytes (DSE) and ericoid mycorrhiza and were more diverse around sub-alpine areas (NS, KB and PS sites) than in motane areas (CF site). The presence of these REF may be affected by a complex and mixed plant vegetation in the transition zone between the montane and sub-alpine areas.

Key words: arbuscular mycorrhizal fungi, dark septate endophytes, Indonesia, *Lycopodiaceae*, mycoheterotrophic plants

----

Introduction

Lycopodiaceae are an ancient group of herbaceous and sister clade to all other extant vascular plants (Raubeson and Jansen, 1992). Most species are epiphytes on trees in the tropics (Wikström *et al*., 1999), and evergreen montain forests of South America and South East Asia (Wikström *et al*., 1999). Terresterial *Lycopodiaceae* are known to be distributed in the high altitude mountains in the neotropics (Wikström *et al*., 1999). In Indonesia, terresterial *Lycopodiaceae* are found at the sub-alpine summit of Mount Gede Pangrango National Park (Sadili *et al*., 2009).

Members of *Lycopodiaceae* (*Huperzia, Lycopodium,* and *Lycopodiella*) are characterized by a life cycle with chlorophyllous sporophytes and long-lived (several years) gametophyte phases (Merckx, 2013).

The long-lived gametophytes of *Lycopodiaceae* are mycoheterotrophs (Winther and Friedman, 2008; Merckx, 2013). The occurence of root endophytic fungi (REF), especially arbuscular mycorrhiza fungi (AMF) (Schmidt and Scow, 1986; Zhao, 2000; Winther and Friedman, 2008; Fernández *et al*., 2008, Schmidt *et al*., 2008; Kessler *et al*., 2010; Horn *et al*., 2013) and dark septate endophytes (DSE) (Fernández *et al*., 2008; Schmidt *et al*., 2008; Zubek *et al*., 2010; Horn *et al*., 2013) have been reported from *Lycopodiaceae* inhabiting tropical regions such as Central and South America, Galapagos, South East Asia and temperate Europe. Eventhough the presence of AMF and DSE from South East Asian *Lycopodiaceae* has been reported, these reports cover only three species (*H. serrata*, *Huperzia* sp. and *Lycopodium cernuum*) from Peninsular Malaysia and Sulawesi Island (Indonesia) (Kessler *et al*., 2010), and 1 species (*L. japonicum*) from tropical and subtropical areas of Yunnan, China (Zhao, 2000). Therefore, in this study, colonization status of AMF and DSE in sporophytes of *L. clavatum*, *H. selago* and *H. serrata* inhabiting Mt. Pangrango were investigated to understand the diversity of their fungal symbionts. This information will enrich and fill gaps in the information on fungal symbionts of tropical mycoheterotropic plants.

Materials and Methods

Sample Collection

Totally 38 terrestrial sporophytes of 3 *Lycopodiaceae* consisting of 5 samples of *L. clavatum* (Fig. 1A), 11 samples of *H. selago* (Fig. 1B), and 22 samples of *H. serrata* (Fig. 1C) were collected from Mt Pangrango at Gunung Gede Pangrango National Park, West Java (Table 1). All samples were taken from their habitats at the slopes beside the trail at 4 localities, *H. serrata* from near the Cibereum Water Fall (CF) and Kandang Badak (KB), *H. selago* from near the Summit (NS) and *L. clavatum* from Pangrango Summit (PS). The sampling sites have different altitudes (S 06°46.2′ −06°75.2′, E 106°57.9′ −106°98.9′) and soil properties (Table 1). Gametophytes were not found in the root of any sporophytes at all localities. A part of samples were kept in the Herbarium Bogoriense (BO) as voucher specimens.

Colonization of AMF

Roots of collected samples were gently washed with tap water to remove soil particles and cut into ap-

Fig. 1. *Lycopodiaceae* at Mt. Pangrango. A) *Lycopodium clavatum*, B) *Huperzia selago*, C) *Huperzia serrata*

proximately 1 cm segments. Segments of each sample were separately cleared by heating at 90℃ for 15 min in 10% (w/v) KOH and continuously treated with 5% H_2O_2 at room temperature for 5 min except for PS, then rinsed with tap water and acidified with 1N HCl and stained with 0.05% trypan blue in lactophenol for 30 min at room temperature. The percentage of arbuscular mycorrhizal colonization was determined by the grid line intersect method (Giovannetti and Mosse, 1980). Stained root segments were randomly distributed into a 9 cm petri dish with the grid line and the number of AMF colonized intersections counted with a stereo microscope (SZX16; Olympus, Tokyo, Japan) at magnification x40. Stained root segments were redistributed and the number of AMF colonized intersections counted. This process was continued until the

Table 1. Samples and enviromental parameters of the sampling sites at Mt. Pangrango.

| Name of species | No. samples | Sampling Site (Code) | Elevation (m) | Soil properties | | | | | |
				pH_{H_2O}	pH_{KCl}	P_2O_5 (mg/100 g dried soil)	C%	N%	C/N
Lycopodium clavatum	5	The summit of Mt. Pangrango (PS)	3019	5.4	4.4	1.9	32.8	1.33	24.8
Huperzia selago	11	Near the summit (NS)	2998	5.8	5.1	1.2	33.4	1.77	18.9
Huperzia serrata	12	Kandang badak (KB)	2440	4.9	4.2	1.9	23.9	1.43	16.6
Huperzia serrata	10	Near the Cibereum water fall (CF)	1728	5.0	3.8	3.7	17.9	1.21	14.8

total number of intersections reached over 500. The percentage of the number of AMF colonized intersections in the total number of intersections was calculated. Segments of roots that were used for determining AMF colonization rates were mounted on slide glasses suspended in lactophenol, and then covered with cover slips. Morphological characteristics of AMF such as arbuscules, vesicles, spores and coiled hyphae in the root cells were observed under a light microscope (BX53; Olympus, Tokyo, Japan) equipped with a CCD camera (DP21; Olympus, Tokyo, Japan).

Isolation of REF

At least 15 randomly chosen root segments per sample were washed three times with a 0.005% solution of Tween 20 (Wako Pure Chemicals, Osaka, Japan), followed by three rinses with sterilized distilled water. Segments air-dried overnight in a laminar were plated onto 9 cm petri dishes (3 segments per petri dish, 5 replicates) with 1/2 corn meal agar medium (8.5 g corn meal agar (infusion form, Difco) and 7.5 g Bacto agar (Difco) in 1 L distilled water). During incubation up to 30 days at approximately 23℃, small agar pieces containing visible fungal colonies from roots were transferred to LcA medium (Miura medium) (0.2 g yeast extract (Difco), 1 g glucose (Merck), 2 g NaNO₃ (Merck), 1 g KH₂PO₄ (Merck), 0.2 g KCl (Merck), 0.2 g MgSO₄·7H₂O (Merck), 18 g Bacto agar (Difco) in 1 L distilled water) (Miura and Kudo, 1970). This isolation step was carried out routinely during the in-

cubation.

Identification of REF

Only slow growing isolates with colonial diameter sizes less than 3 cm after 30 days incubation were chosen for identification. Morphological characteristics on LcA and PDA were observed followed by molecular identification using the internal transcribed spacer (ITS) region. Genomic DNA was extracted from mycelia grown on media overlaid with a sterile cellophane membrane with the Prepman Ultra Sample Preparation Reagent (Applied Biosystems, CA, USA). The extracted DNA was purified by PCI (Phenol: Chloroform: Isoamyl alcohol=25:24:1 (v/v/v)) treatment, followed by ethanol precipitation. Dried DNA pellets were resuspended with TE buffer (pH 8.0), and then the DNA solution used as template DNA for polymerase chain reactions (PCR). ITS5 (5′ -GGAA-GTAAAAGTCGTAACAAGG-3′) and ITS4 (5′ -TC-CTCCGCTTATTGATATGC-3′) (White *et al.*, 1990) primers were used to amplify the fungal ITS region by PCR. The reaction mixture (total 50 μL) contained 5 μL of 10x Ex *Taq* buffer (TaKaRa Bio, Shiga, Japan), 2.5 μL of each primer, 4 μL of dNTP mixture, 34.85 μL of MilliQ water, 0.15 μL of TaKaRa Ex *Taq* DNA polymerase (TaKaRa Bio, Shiga, Japan), and 1 μL of template DNA. PCR was performed using a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio, Shiga, Japan) as follows: 95℃ for 10 min, 35 cycles of 94℃ for 20 s, 47℃ for 30s, 72℃ for 40 s, followed by a final 7 min

extension at 72℃ (Toju *et al.*, 2012). For cycle sequencing, a mixture of sequencing reaction solution (10 μL) containing 0.5 μL of the BigDye® Terminator v3.1 Cycle Sequencing Ready Reaction Premix (Applied Biosystems, CA, USA), 1.5 μL of the BigDye® Terminator v3.1 Cycle Sequencing Buffer (5x) (Applied Biosystems, CA, USA), 0.32 μL of ITS5 primer (forward), 6.68 μL of MilliQ water, and 1 μL of purified PCR product was prepared. Cycle sequencing reactions were performed using a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio, Shiga, Japan) as follows: 96℃ for 2 min, 30 cycles of 96℃ for 30 s, 50℃ for 15 s, 60℃ for 3 min. Reaction products were purified by ethanol precipitation, after drying, then the pellet was resuspended in 20 μL of Hi-Di Formamide, and processed on an ABI PRISM® 3100 DNA Analyzer (Applied Biosystems, CA, USA). Then, ITS sequences were compared with similar ITS sequences retrieved from the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/) using the BlastN algorithm.

Results

AMF colonization

Associations of AMF were observed with roots of *H. selago* and *H. serrata* collected from the CF, KB and NS sites, but not with roots of *L. clavatum* collected from the PS site. The colonization rate of AMF in *Huperzia* spp. varied depending on the host and the sampling sites. The average AMF colonization rate in *H. serrata* was commonly higher than *H. selago* (9.77±7.96%). Furthermore, *H. serrata* roots collected from the KB site hosted more AMF (14.4±8.60%) than those collected from the CF site (11.6±10.7%) (Table 2).

Under the light microscope, Paris type colonization patterns such as hyphal coils (Fig. 2a, b, c and e), arbusculate coils (Fig. 2g, h and j) and Arum type colonization patterns such as arbuscules with inter-cellular hyphae (Fig. 2f, k and l) in roots of *Huperzia* spp. were observed. Not only these typical characteristics, but also unique morphologies such as rough hyphae (Fig. 2d), aborted hyphae (Fig. 2o), hyphal branching (Fig. 2e, m and o), and swelling hyphae (Fig. 2h, i and o) were also observed. No typical mycorrhizal structures were found in roots of *L. clavatum* (Fig. 2p). Only one intercellular AMF spore (spore size: ca. 189 μm, width of sporogenous cell: ca. 38 μm) without a germination shield was observed in the roots of *H. serrata* at the KB site (Fig. 2n). On the other hand, typical morphologies of DSE such as dark pigmented hyphae and microsclerotia were not found.

REF in the roots of Lycopodiaceae

Totally 420 fungal isolates were obtained from 435 root segments. The slow growing fungal group within the REF community that had less than 3 cm diameter colonies within 30 days incubation were low (112 isolates; 26.7%). The relative abundance of slow growing fungi was only 11.5%, 32.5%, 31.9% and 28.6% of the total isolates at the CF, KB, NS and PS sites, respectively (Fig. 3).

Due to most slow growing isolates not sporulating on LcA and PDA media, these isolates were then identified by comparing their ITS sequences with similar ITS sequences retrieved from the NCBI nucleotide database using the BlastN algorithm. As a result, these isolates were from seven genera including *Cadophora, Cladophialophora, Cryptosporiopsis, Leohumicola, Leptodontidium, Phialocephala, Pseudoclathrosphaerina* (putatively similar to *Meliniomyces*), *Helotiales* related isolates and others (Fig 3).

The distribution of slow growing isolates were different from AMF. Isolates of *Cryptosporiopsis, Leohumicola* and *Pseudoclathrosphaerina* included in ericoid mycorrhiza were more frequently isolated from *Lycopodiaceae* inhabiting the sub-alpine areas (>2400

Table 2. AMF colonization in the root of *Lycopodiaceae* from Mt. Pangrango.

Site code	No. Samples observed	Host Species	AMF colonization rate (%)
PS	4	*Lycopodium clavatum*	0
NS	10	*Huperzia selago*	9.77±7.96
KB	10	*Huperzia serrata*	14.4±8.60
CF	6	*Huperzia serrata*	11.7±10.7
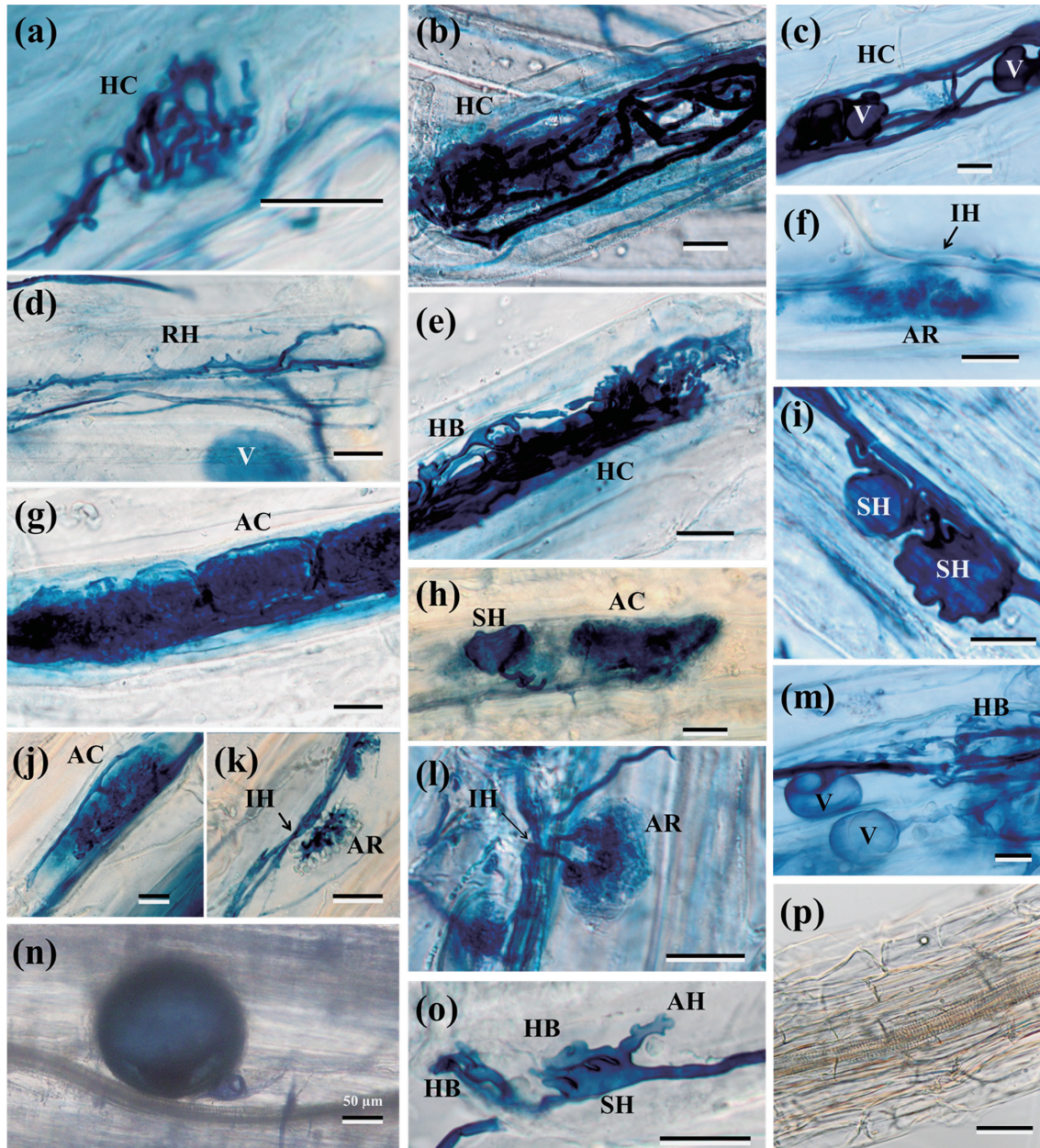
Fig. 2. AMF in the roots of *Huperzia* spp. at CF (a, d, g, j, k), KB (b, e, h, l, o, n), and NS (c, f, i, m). Hyphal coil (HC: a, b, c, e); arbuscule (AR) from intercellular hyphae (IH) (f, k, l); Arbusculate coil (AC: g, h, j); vesicle (V: c, d, m); rough hyphae (RH: d); aborted hyphae (AH: o); hyphal branching (HB: e, m, o) and swelling hyphae (SH: h, i, o). An intercellular spore found in the root of *H. serrata* at the KB site (n) and sterile root of *Lycopodium clavatum* at the PS site (p). Scale bar; a–m, o, p: 20 μm, n: 50 μm.

asl.; KB, NS and PS sites).

Discussion

In this study, all sporophytes of *Huperzia* spp. collected were found associated with AMF, but not with *L. clavatum* in Mt. Pangrango. Nevertheless, AM-like associations by *Mucoromycotina* with the subterranean gametophytes of *L. clavatum* (Schmid and Oberwinkler, 1993) and AMF associations with the chlorophyllous sporophytes of *L. clavatum* were reported previously (Winther and Friedman, 2008). In *L. cernuum*, AMF and AM-like fungi were also
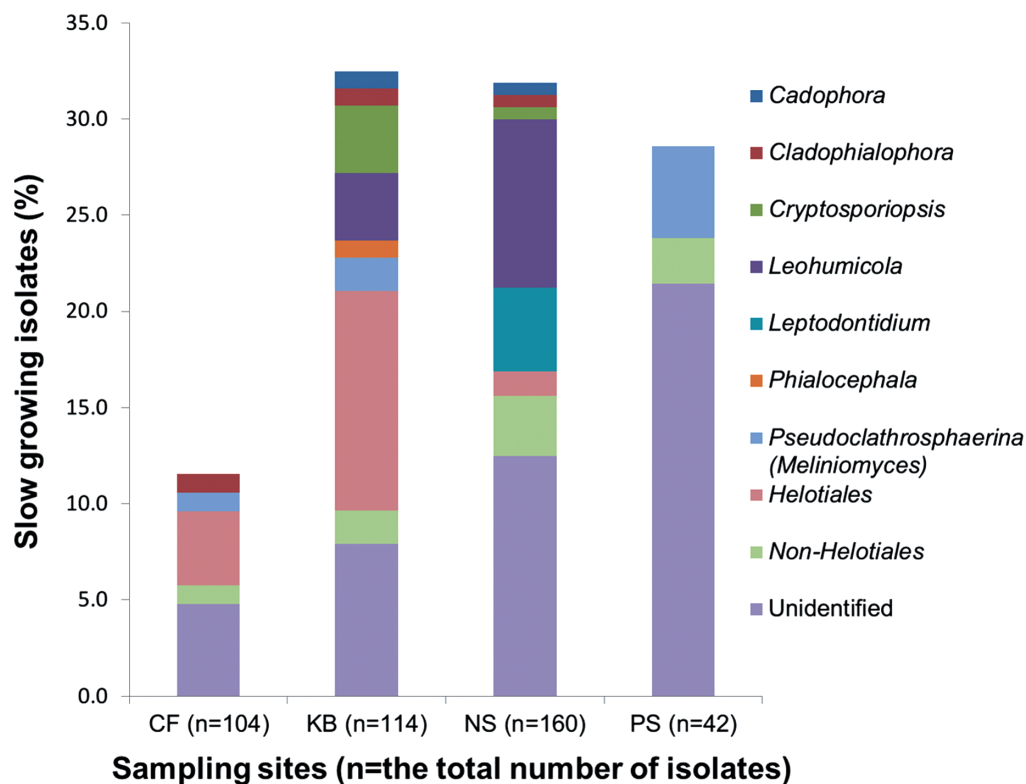
Fig. 3.   Isolation rates of slow growing fungi isolated from *Lycopodiaceae* inhabiting in Mt. Pangrango.

reported from chlorophyllous gametophytes and root nodules of sporophytes of *L. cernuum* found in the Peninsular Malaysia (Duckett and Ligrone, 1992). In contrast, no such associations were found from the chlorophyllous gametophytes of *L. cernuum* var. *salakense* from Mt. Salak (Treub, 1888), and sporophytes of *L. flabelliforme* and *L. tristachyum* from mixed woodlands of Southern Ontario, Canada (Berch and Kendrik 1982). This suggests that AMF associations with the roots of some species of *Lycopodium* are probably habitat dependent.

The morphologies of AMF in *Huperzia* spp. commonly are similar to Paris type AMF characterized by coiled hyphae (Fig. 2a, b, c and e) and arbusculate coils (Fig. 2g, h and j) or Arum type AMF characterized by arbuscules with intercellular hyphae (Fig. 2f, k and l) (Smith and Read, 2008). On the other hand, the morphological characteristics of intercellular spores found in the roots of *H. serrata* at the KB site (Fig. 2n) are possibly similar to spores of *Gigasporaceae* based on the spore size and the width of sporogenous cells compared with descriptions in the International Culture Collection of Arbuscular Mycor-

rhizal Fungi (INVAM) (http://invam.wvu.edu/). Additionally, unique morphologies of AMF such as rough hyphae, aborted hyphae, hyphal branching and swelling hyphae were observed (Fig. 2d, e, h, i, m and o). In these unique morphologies, aborted hyphae (Fig. 2o) and hyphal branching (Fig. 2m and o) were similar to the morphologies observed in colonization patterns of *Glomus versiforme* with a reduced mycorrhizal colonization (rmc) mutant of tomato (Gao *et al.*, 2001). Rough hyphae (Fig. 2d), hyphal branching (Fig. 2e), and swelling hyphae (Fig. 2h, i and o) were similar to the AMF morphotypes found in roots of *Alzatea verticillata* (*Alzateaceae*) in South Ecuador (Beck *et al.*, 2007). To understand whether these unique morphologies in the roots of *H. serrata* and *H. selago* at CF, KB and NS sites are specially found in these habitats, further investigations on AMF associated with *H. serrata* and *H. selago* in different locations and identification of these AMF species are needed.

In the REF isolation, slow growing isolates consisted of seven genera including *Cadophora, Cladophialophora, Cryptosporiopsis, Leohumicola, Lepto-*

dontidium, *Phialocephala*, and *Pseudoclathrosphaerina* (Fig 3). Of these genera, *Leohumicola* was described as a hyphomycetes included in ericoid mycorrhizal species, but the phylogenetic clade is sister to *Tricladium splendens* included in order *Helotiales* (Hambleton *et al*., 2005b). Species of *Cryptosporiopsis* are mainly known as anamorphs of *Pezicula* or *Neofabraea* species (*Helotiales*) (Verkley, 1999) and also included in ericoid mycorrhizal species (Sigler *et al*., 2005). The ITS sequences of *Pseudoclathrosphaerina* obtained in this study showed 96% similarity with *P. spiralis* (accession number EF029231), a *Pseudoclathrosphaerina* species described by Cooper (2005) based on morphology. The ITS sequences of *Pseudoclathrosphaerina* and *P. spiralis* were phylogenetically nested in the *Meliniomyces* clade (Hambleton *et al*., 2005a), included in ericoid mycorrhizal species (unpublished data). The taxonomical position of *P. spiralis* needs to be reviewed. Four other genera namely *Cadophora, Cladophialophora*, *Leptodontidium* and *Phialocephala* are known to be DSE related to *Helotiales*.

The isolation rates of slow growing isolates were relatively higher in sub-alpine areas such as PS, NS and KB sites, when compared to the montane areas such as the CF site. Although *H. serrata* were collected from both CF and KB sites, the isolation rate of slow growing fungi at the KB site was six times higher than the CF site. This is probably because of the presence of an *Ericaceae* plant community that is usually associated with DSE and ericoid mycorrhiza in the sub-alpine areas in Mt. Pangrango. A study of the floristic composition of Mt. Pangrango conducted by Yamada (1976) reported indigenous *Ericaceae* species at the KB and PS sites, and the author also speculated the KB site was originally a forest located in the transition zone between montane (1500−2400 m asl.) and sub-alpine (＞2400 m asl.) areas. This author's speculation came from a complex and mixed plant vegetation in the KB site. The highest isolation rate of slow growing isolates including DSE and ericoid mycorrhiza were found in the KB site. Especially, DSE are known to have a broad host range. Therefore, the presence of various DSE species in the KB site of this study may be partly because of the complex and mixed plant vegetation in the KB site. To ascertain that the *Ericaceae* plant community in the sub-alpine area is essential for the presence of DSE and ericoid mycorrhiza in *Lycopodiaceae*, further research on

hyphal networks of the *Lycopodiaceae* and *Ericaceae* is needed.

Most slow growing isolates obtained in this study were related to *Helotiales*. Recently, ascomyceteous endophytes related to *Helotiales* sequences were found from gametophytes of *L. alpinum* in Germany (Horn *et al*., 2013). Our results partly support the evidence of *Helotiales* related fungal endophytes are possible fungal partners of the *Lycopodiaceae*. In this study, choosing a culture-dependent investigation of REF provide an opportunity for mycorrhizal symbiotic experiments using these *Helotiales* related isolates and *Lycopodiaceae*. Previously, a *Helotiales* related DSE, *Phialocephala fortinii,* showed a positive effect on seed germination and protocorm development of *Dactylorhiza praetermissa*, a mycoheterotrophic terrestrial orchid, even though *P. fortinii* is not the main symbiont of *D. praetermissa* (Zimmerman and Peterson, 2007). Likewise, the initial growth of *Lycopodiaceae* may be promoted by inoculation of *Helotiales* related isolates obtained in this study. Depending on the results of the inoculation experiments, these *Helotiales* may potentially be used as the culturable fungal agent instead of AMF to solve cultivation difficulties of *Lycopodiaceae*.

Conclusion

The sporophytes of *Lycopodiaceae* in Mt. Pangrango, except for *L. clavatum* at the PS site, were associated with AMF. Some morphologies of AMF observed in this study were unique and have not been reported previously. Moreover, the culture-dependent method revealed that the sporophytes of the investigated *Lycopodiaceae* were also associated with *Helotiales* related REF including DSE and ericoid mycorrhiza.

Acknowledgements

References

Beck, A., Haug, I., Oberwinkler, F., Kottke, I., 2007. Structural characterization and molecular identification of arbuscular mycorrhiza morphotypes of *Alzatea verticillata* (*Alzateaceae*), a prominent tree in the tropical mountain rain forest of South Ecuador. Mycorrhiza 17, 607–625.

Berch, S.M., Kendrick, B., 1982. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-allies. Mycologia 74, 769–776.

Cooper, J.A., 2005. New Zealand hyphomycete fungi: additional records, new species, and notes on interesting collections. New Zeal. J. Bot. 43, 323–349.

Duckett, J.G., Ligrone, R., 1992. A light and electron microscope study of the fungal endophytes in the sporophyte and gametophyte of *Lycopodium cernuum* with observations on the gametophyte-sporophyte junction. Can. J. Bot. 70, 58–72.

Fernández, N., Messuti, M. I., Fontenla, S., 2008. Arbuscular mycorrhizas and dark septate fungi in *Lycopodium paniculatum* (*Lycopodiaceae*) and *Equisetum bogotense* (*Equisetaceae*) in a Valdivian temperate forest of Patagonia, Argentina. Am. Fern. J. 98, 117–127.

Gao, L.L., Delp, G., Smith, S.E., 2001. Colonization patterns in a mycorrhiza-defective mutant tomato vary with different arbuscular-mycorrhizal fungi. New Phytol. 151, 477–491.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

Hambleton, S., Sigler, L., 2005a. *Meliniomyces*, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (≡ *Hymenoscyphus ericae*), *Leotiomycetes*. Stud. Mycol. 53, 1–27.

Hambleton, S., Seifert, K.A., Nickerson, N.L., 2005b. *Leohumicola*, a new genus of heat-resistant hyphomycetes. Stud. Mycol. 53, 29–52.

Horn, K., Franke, T., Unterseher, M., Schnittler, M., Beenken, L., 2013. Morphological and molecular analyses of fungal endophytes of achlorophyllous gametophytes of *Diphasiastrum alpinum* (*Lycopodiaceae*). Am. J. Bot. 100, 2158–2174.

Kessler, M., Jonas, R., Cicuzza, D., Kluge, J., Piątek, K., Naks, P., Lehnert, M., 2010. A survey of the mycorrhization of Southeast Asian ferns and lycophytes. Plant Biology 12, 788–793.

Merckx, V.S.F.T. (Ed.), 2013. Mycoheterotrophy, the Biology of Plants Living on Fungi. Springer, NY.

Miura, K., Kudo, M., 1970. An agar-medium for aquatic hyphomycetes (in Japanese). Trans. Mycol. Soc. Jpn. 11, 116–118.

Raubeson, L.A., Jansen, R.K., 1992. Chloroplast DNA evidence on the ancient evolutionary split in vascular land plants. Science 255, 1697–1699.

Sadili, A., Kartawinata, K., Kartonegoro, A., Soedjito, H.,

Sumadijaya, A., 2009. Structure and composition of subalpine summit habitats on Mt. Gede-Pangrango complex, Cibodas Biosphere Reserve, West Java, Indonesia. Reinwardtia 12, 391–404.

Schmid, E., Oberwinkler, F., 1993. Mycorrhiza-like interaction between the achlorophyllous gametophyte of *Lycopodium clavatum* L. and its fungal endophyte studied by light and electron microscopy. New Phytol. 124, 69–81.

Schmidt, S.K., Scow, K.M., 1986. Mycorrhizal fungi on the Galapagos Islands. Biotropica 18, 236–240.

Schmidt, S.K., Sobieniak-Wiseman, L.C., Kageyama, S.A., Halloy, S.R., Schadt, C.W., 2008. Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. Arct. Antarct. Alp. Res. 40, 576–583.

Sigler, L., Allan, T., Lim, S. R., Berbee, M., Berch, S., 2005. Two new *Cryptosporiopsis* species from roots of ericaceous hosts in western North America. Stud. Mycol. 53, 53–62.

Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis (3rd). Academic Press, San Diego.

Toju, H., Tanabe, A. S., Yamamoto, S., Sato, H., 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One. 7, e40863.

Treub, M., 1888. Etudes sur les lycopodiacees IV Le prothalle du *Lycopodium salakense*. Ann. Jard. Bot. Buitenzorg. 7, 141–148.

Verkley, G.J.M., 1999. A monograph of the genus *Pezicula* and its anamorphs. Stud. Mycol. 44, 1–180.

White, T.J., Bruns, T.D., Lee, S., Taylor, J.W., 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis MA, Gelfand DH, Sninsky J, White TJ (eds), PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.

Wikström, N., Kenrick, P., Chase, M., 1999. Epiphytism and terrestrialization in tropical *Huperzia* (*Lycopodiaceae*). Plant Syst. Evol. 218, 221–243.

Winther, J.L., Friedman, W.E., 2008. Arbuscular mycorrhizal associations in *Lycopodiaceae*. New Phytol. 177, 790–801.

Yamada, I., 1976. Forest Ecological Studies of the Montane Forest of Mt. Pangrango, West Java. II. Stratification and Floristic composition of the Forest Vegertation of the Higher Part of Mt. Pangrango. S. E. Asian Stud. 13, 514–534.

Zhao, Z.W., 2000. The arbuscular mycorrhizas of pteridophytes in Yunnan, southwest China: evolutionary interpretations. Mycorrhiza 10, 145–149.

Zimmerman, E., Peterson, R.L., 2007. Effect of a dark septate fungal endophyte on seed germination and protocorm development in a terrestrial orchid. Symbiosis (Rehovot) 43, 45–52.

Zubek, S., Tek, K.P., Naks, P. Heise, W., Wayda, M., Mleczko, P., 2010. Fungal Root Endophyte Colonization of Fern and Lycophyte Species from the Celaque National Park in Honduras. Am. Fern. J. 100, 126–136.