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Community of Endophytic Fungi from Alpine Conifers on Mt. Seorak

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ABSTRACT
Endophytic fungi occupy various ecological niches, which reinforces their diversity. As few studies have investigated the endophytic fungi of alpine conifers, we focused on four species of alpine conifers in this study—*Abies nephrolepis*, *Pinus pumila*, *Taxus cuspidata* var. *nana*, and *Thuja koraiensis*—and examined them for endophytic fungi. A total of 108 endophytic fungi were isolated. There were four taxa in *A. nephrolepis*, 12 in *P. pumila*, 18 in *T. cuspidata* var. *nana*, and 17 in *T. koraiensis*; these were divided into five classes: Agaricomycetes (3.2%), Dothideomycetes (29.0%), Leotiomycetes (15.0%), Sordariomycetes (41.9%), and Orbiliomycetes (1.6%). The most prevalent fungi were *Sydowia polyspora* (22.7%) and Xylariaceae sp. (22.7%) in *P. pumila*, *Phomopsis juglandina* (16.1%) in *T. cuspidata* var. *nana*, and *Thuja*-endophytes sp. 1 (70.0%) in *T. koraiensis*. However, there was no dominant species growing in *A. nephrolepis*. Some host plants were analyzed using next-generation sequencing. We obtained 4618 reads for *A. nephrolepis* and 2268 reads for *T. koraiensis*. At the genus level, the top three endophytic fungi were Ophiostomataceae_uc (64.6%), Nectriaceae_uc (15.5%), and unclassified organism (18.0%) in *A. nephrolepis* and Nectriaceae_uc (41.9%), Ophiostomataceae_uc (41.8%), and Magnaporthaceae_uc (9.2%) in *T. koraiensis*. Our results show that there are different communities of endophytic fungi among different host plants, even if the host plants are in the same region. Such ecological niches are important in terms of the ecological restoration of alpine conifers.

1. Introduction

Endophytic fungi are nonpathogenic fungi that inhabit tissues such as the leaves and stems of host plants [1]. They are mainly composed of Ascomycota and Basidiomycota [2]. Endophytic fungi can be found in all plants on the planet and have a positive effect on the growth and maintenance of host plants. However, endophytic fungi do not necessarily form symbiotic relationships but rather neutral ones that neither positively nor negatively affect the host plant, although some can opportunistically develop into pathogens or saprophytes depending on the health of the host plant. Taken together, endophytic fungi are found in all healthy plants and form various relationships with their host plants, including neutrality, symbiosis, parasitism, and pathogenicity. Furthermore, they can affect the growth and maintenance of host plants [3].

In general, endophytic fungi receive nutrients and shelter from the host plants, and in return, promote the growth and defense mechanisms of the host plant. Unlike mycorrhizal fungi, which form a rhizome that envelops plant roots, endophytic fungi exist entirely within plant tissues, and mycelium may also develop in the intercellular space of the host plant [4]. Numerous studies of endophytic fungi have shown that these fungi enhance host plant health and biomass, and lead to accumulation of secondary metabolites and reduction of water loss in host plants [5,6]. Host plants are exposed to various biotic and abiotic stressors, and endophytic fungi play essential and diverse roles in the ecosystem to promote host plant growth and stress tolerance. Consequently, both mycologists and chemists have shown great interest in these fungi [7,8].

In the Korean Peninsula, regions approximately 1000 m above sea level are generally defined as subalpine regions [9]. Of particular note, these regions are currently directly affected by the decline of alpine conifers. Among them, Mt. Seorak, which is about 1708 m above sea level, was designated as the first biosphere reserve in Korea by the United Nations Educational, Scientific and Cultural Organization (UNESCO) in 1982. It is a forest ecosystem with excellent conservation value in which approximately 1000 taxa of plants are distributed [10].

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On Mt. Seorak, there are approximately 15 conifer species including Japanese red pine (*Pinus densiflora* Siebold et Zucc.), which is distributed throughout the Korean Peninsula. Among these species, four are alpine conifers.

There are approximately 600 conifer species worldwide, with many of them being endangered or vulnerable to recent environmental changes. In particular, the decline of conifers distributed over the subalpine zone is remarkable, and their global distribution area is shrinking [11]. The decline of alpine conifers is not limited to these species and severely impacts overall biodiversity loss in these regions. Therefore, it is essential to conserve the diversity of endophytic fungi associated with endangered conifers. However, few basic ecological studies investigating alpine conifers and their endophytic fungi have been conducted. This is because the distribution area of alpine conifers is small compared with the national land area, they are poorly accessible, and the economic feasibility of alpine conifer forests is low compared with that of other forests [12].

Therefore, in this study, we investigated four alpine conifers—*Abies nephrolepis* (Trautv.) Maxim., *Pinus pumila* (Pall.) Regel, *Taxus cuspidata* var. *nana* Rehder, and *Thuja koraiensis* Nakai—mainly distributed near the summit of Mt. Seorak. We isolated and identified endophytes from these tree species in an attempt to investigate the community structure of endophytic fungi in relation to their host plants.

2. Materials and methods

2.1. Samples

The needle leaves of conifers were collected from Mt. Seorak (38° 07′ 47.53″N, 128° 27′ 47.53″E) spanning Inje-gun and Sokcho-si, Gangwon-do, in September 2013. From each of the four alpine conifer species, namely, *Abies nephrolepis*, *Pinus pumila*, *Taxus cuspidata* var. *nana*, and *Thuja koraiensis*, we sampled 10 branchlets. Needle leaves were stored and transported in a sealable zipper bag, and endophytic fungi were isolated within 24 h.

2.2. Isolation of foliar endophytic fungi

The collected needle leaves were washed with tap water to remove surface matter, prepared to a size of about 1 cm, and surface sterilized. The surface sterilization conditions were 1% sodium hypochlorite (NaOCl) for 3 min, followed by 96% ethyl alcohol (C_2H_5OH) for 2 min. Finally, samples were washed twice using sterile water. The needles were placed on potato dextrose agar (PDA, MBcell, Seoul,

Korea) medium and then cultured in an incubator for four weeks at 25 °C in the dark to isolate endophytic fungi. The isolated mycelium was cultured using PDA and used for the pure culturing of endophytic fungi to observe colony morphology and to extract total genomic DNA [13].

2.3. Analysis of endophytic fungi by DNA sequences

2.3.1 DNA barcoding by PCR

The isolated endophytic fungi were selected and total genomic DNA was extracted using the Plant SV mini kit (GeneAll, Seoul, Korea) according to manufacturer's instructions. For polymerase chain reaction (PCR), the fungal-specific primers ITS1F and ITS4 were used to selectively amplify the internal transcribed spacers (ITS), ITS1 and ITS2, as well as 5.8S ribosomal DNA regions [14]. PCR conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min, and a final elongation at 72 °C for 5 min. Electrophoresis was performed using 1.5% agarose gels to check the amplification result and then stored at 10 °C [13]. Nucleotide sequence analysis was performed by SolGent (Daejeon, Korea), and analyzed nucleotide sequences were matched using BLAST at NCBI (https://www.ncbi.nlm.nih.gov/) to identify the highest similarity taxa of fungal species. Nucleotide sequences were aligned using MEGA10.0.5. The reliability was then evaluated *via* 1000 bootstrap analysis based on the Kimura-2 parameter distance model, and a phylogenetic tree was created by the neighbor-joining method [15]. The finally confirmed fungal taxa were compared with host plants using the species diversity index (H') [16].

2.3.2 Next-generation sequencing

Next-generation sequencing (NGS) was performed based on needle samples of *A. nephrolepis* and *T. koraiensis*. First, PCR amplification was performed of the ITS2 region as part of a fungal DNA barcode using the primers UTS3-Mi and ITS-Mi and the following conditions: initial denaturation at 95 °C for 3 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final elongation at 72 °C for 5 min [17]. Secondary amplification was performed using the primers index i5 and i7 to attach Illumina Nextera barcodes under the same thermocycling conditions but for eight cycles. Amplification results were evaluated using a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA) and DNA 7500 chip. Mixed amplicons were collected and NGS was performed using a

MiSeq Sequencing System (Illumina, Singapore) according to the manufacturer's protocol. Sequence reads were identified and analyzed using the CL community software 3.42 (ChunLab, Seoul, Korea). The number of sequences was analyzed, and the observed diversity richness (operational taxonomic units; OTUs), estimated alpha diversity index (ACE, Shannon), and the metagenomic compositions were visualized using Krona charts [18]. These were defined using the Mothur program package [19], which applies a cutoff value of 97% similarity of the ribosomal DNA sequences [20].

3. Results

3.1 Isolation and culture of endophytic fungi

A total of 108 endophytic fungal strains were isolated from the four alpine conifer species distributed on Mt. Seorak. As a result of molecular identification of these strains, 35 taxa of endophytic fungi were identified as follows: four strains isolated from *A. nephrolepis*, 11 strains from *P. pumila*, 11 strains from *T. cuspidata* var. *nana*, and 12 strains from *T. koraiensis* (Table 1). The nucleotide sequence of each fungal strain was analyzed in the ITS region, and BLAST analysis revealed a similarity of 84%–100% depending on the taxon. Furthermore, at the class level, excluding *incertae sedis*, all endophytic fungi were divided into one of the following six groups: Agaricomycetes (3.1%), Dothideomycetes (34.4%), Exobasidiomycetes (3.1%), Leotiomycetes (28.1%), Orbiliomycetes (3.1%), and Sordariomycetes (25.0%). A few endophytic fungal strains were found at the same ratio in *A. nephrolepis*, so it was not possible to state that any one taxon was dominant, whereas *Sydowia polyspora* and Xylariaceae sp. codominated *P. pumila* at about 23% each. In *T. cuspidata* var. *nana*, *Phomopsis* sp. was dominant at about 26%, and in *T. koraiensis*, an unidentified taxon, *Thuja*-endophytes sp. 1 was dominant at about 70%. No common endophytic fungi were isolated from all host plants, but *S. polyspora* was common in the three conifer species *A. nephrolepis*, *P. pumila*, and *T. koraiensis*. In addition, *Pezicula neosporulosa* was found in both *T. cuspidata* var. *nana* and

Table 1. Molecular identification of endophytic fungi from the leaves of four subalpine conifers in this study.

Strain	Accession No.	Closest GenBank Taxa	Similarity (%)	AN	PP	TC	TK
		Results of BLAST search on NCBI		Relative abundance[1]			
13E075	LC168780.1	*Nemania* sp.	99	0.25			
13E076	GQ412719.1	*Sydowia polyspora*	99	0.25	0.23		0.04
13E308	KM108362.1	*Darkera picea*	99	0.25			
13E309	MG905603.1	*Coleophoma parafusiformis*	98	0.25			
13E082	MK396572.1	*Sphaerulina berberidis*	99		0.05		
13E084	JN850999.1	*Tilletiopsis albescens*	99		0.05		
13E093	AB741589.1	Xylariaceae sp.	94		0.23		
13E097	KF274296.1	*Pinus*-endophyte sp. 1	99		0.05		
13E098	AB926079.1	*Moellerodiscus pinicola*	99		0.05		
13E099	MH857842.1	*Dactylella microaquatica*	95		0.05		
13E312	KJ406797.1	*Phaeothecoidea* sp.	84		0.05		
13E314	JQ761451.1	Sordariomycetes sp.	99		0.05		
13E313	LC014891.1	Rhytismataceae sp.	98		0.09		
13E315	JF332166.1	*Lophodermium nitens*	95		0.14		
13E105	MF029133.1	*Phomopsis* sp.	99			0.26	
13E108	DQ474117.1	*Phoma macrostoma*	98			0.03	
13E109	KU837233.1	*Phomopsis juglandina*	99			0.19	
13E114	MG813226.1	*Paraphaeosphaeria neglecta*	99			0.04	
13E115	KT004568.1	Ascomycota sp.	94			0.08	
13E118	KJ817299.1	*Phialocephala* sp.	100			0.03	
13E122	MK311336.1	*Emmia lacerate*	98			0.03	
13E317	MH734785.1	*Lachnellula* sp.	98			0.03	
13E320	KY522950.1	Helotiales sp.	100			0.03	
13E326	FJ025260.1	*Taxus*-endophyte sp. 1	98			0.01	
13E123	EF419921.1	*Thuja*-endophyte sp. 1	99				0.70
13E128	LC163510.1	*Glomerella* sp.	100				0.02
13E137	FJ025230.1	*Neocucurbitaria cava*	98				0.02
13E138	LC163508.1	*Lophiostoma* sp.	100				0.02
13E144	MH118270.1	*Alternaria* sp.	99				0.06
13E149	KM265763.1	*Diaporther nobilis*	99				0.02
13E151	JX290142.1	*Mycosphaerella* sp.	96				0.02
13E156	MK907770.1	*Valsa ceratophora*	99				0.06
13E175	M279619.1	*Steccherinum* sp.	99				0.02
13E176	JX073098.1	*Botryosphaeria dothidea*	98				0.02
13E332	KR859231.1	*Pezicula neosporulosa*	99			0.16	0.02
Total Number of Isolates				4	22	31	51
Species Richness				4	11	11	12
Species Evenness				1	0.90	0.85	0.53
Shannon Diversity Index (H′)				1.39	2.15	2.03	1.32

[1]Relative abundance: The percentage of isolates at the study sites out of the total number of isolates.
AN: Abies nephrolepis; PP: Pinus pumila; TC: Taxus cuspidata var. nana; TK: Thuja koraiensis.
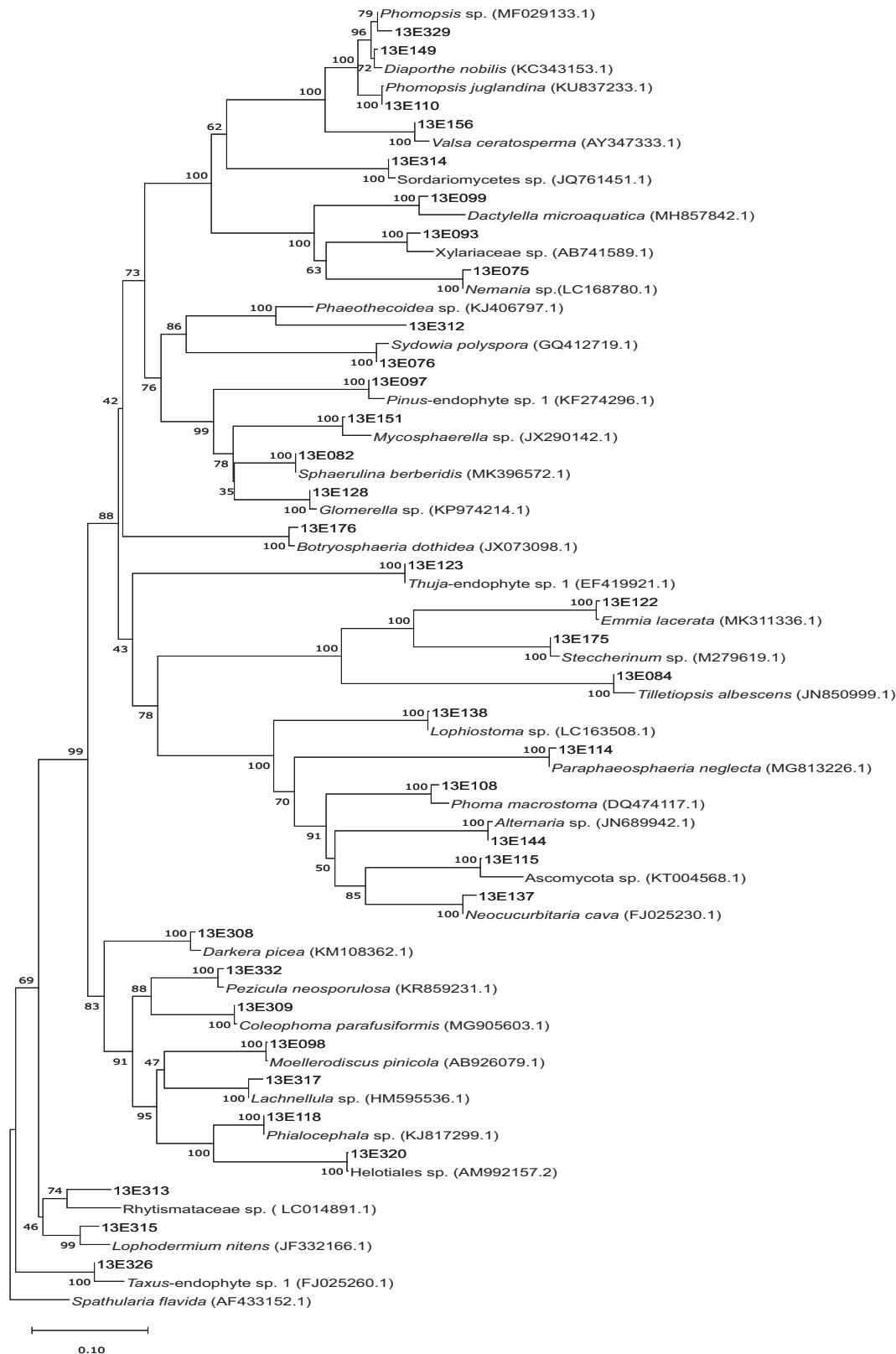
Figure 1. Phylogenic tree of endophytic fungi from four subalpine conifers on Mt. Seorak in Korea. ITS1, ITS2, and 5.8S rDNA regions were used for sequence analysis to confirm the topological appropriation of fungal isolates. *Sphatularia flavida* was used as an out-group and bootstrap values are shown at the branches (1,000 replicates).

T. koraiensis (Figure 1). As a result of comparing the species diversity indices for endophytic fungi isolated from each host plant, the species diversity index (*H'*) of the endophytic fungi was 1.39 in *A. nephrolepis*,

2.15 in *P. pumila*, 2.03 in *T. cuspidata* var. *nana*, and 1.32 in *T. koraiensis*.

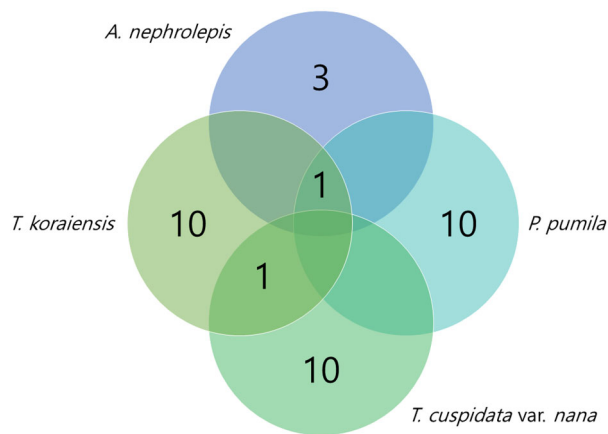The topological appropriation of the phylogenetic tree for endophytic fungi isolated from alpine coni-

Figure 2. Venn diagram showing the number of endophytic fungal taxa isolated from the needles of four alpine conifers on Mt. Seorak in Korea.

Table 2. Summary of next-generation sequencing data of community of endophytic fungi from leaves of *Abies nephrolepis* and *Thuja koraiensis* in this study.

Diversity indices		
Alpha diversity	*Abies nephrolepis*	*Thuja koraiensis*
ACE	147.00	254.00
Chao1	147.00	254.00
Jackknife	147.00	254.00
NPShannon	2.80	4.53
Shannon	2.76	4.41
Simpson	0.14	0.04
Phylogenetic Diversity	21.00	30.00
Relative abundance of endophytic fungi at the genus level		
Genus level	*Abies nephrolepis* (%)	*Thuja koraiensis* (%)
Fusarium	0.74	3.79
Magnaporthaceae_uc	1.26	9.17
Nectriaceae_uc	15.46	41.93
Ophiostomataceae_uc	64.57	41.80
Russulaceae_uc	–	0.09
Unclassified organisms	17.97	3.22

fers was confirmed by the neighbor-joining method. The sum of branched branch lengths for the optimal phylogenetic tree was 5.19. All ambiguous positions were removed and 803 datasets were involved in the final analysis, among which approximately 71 nucleotide sequences played a central role in determining their topology (Figure 2).

3.2 NGS

Through NGS analysis, 4618 reads were obtained from *A. nephrolepis* and 2268 reads from *T. koraiensis*. These results are supported by Good's Lib. The coverage was >0.99 and was sufficient to identify the entire endophytic fungal community. A total of 147 OTUs were identified for *A. nephrolepis* and 254 OTUs were identified for *T. koraiensis*. We determined that the Chao1 index was 147.00 in *A. nephrolepis* and 254.00 in *T. koraiensis*, whereas the Shannon diversity index was 2.76 and 4.41, respectively. The Simpson diversity index was 0.14 and 0.04 in *A. nephrolepis* and *T. koraiensis*, respectively. Lastly, regarding phylogenetic diversity, we found that the diversity of endophytic fungi was high in *T. koraiensis* at 30 and 21 in *A. nephrolepis* (Table 2).

In the case of *A. nephrolepis*, except for unidentified taxa, about 99.95% of endophytic fungi were found to be Ascomycetes, whereas in the case of *T. koraiensis*, except for unidentified taxa, Ascomycetes accounted for 99.47% and Basidiomycetes for 0.17%. At the class level, the group commonly dominant in both host plants was Sordariomycetes, accounting for >90% in both host plants. We also found Agaricomycetes and Lecanoromycetes. At the order level, Ophiostomatales accounted for >69.0% in *A. nephrolepis* and Hypocreales was dominant with >46.5% in *T. koraiensis*. At the family level, Ophiostomataceae

accounted for >64.5% in *A. nephrolepis* and Nectriaceae accounted for >45.7% in *T. koraiensis*. At the genus level, Ophiostomatace_uc accounted for about 64.6% in *A. nephrolepis* and 41.8% in *T. koraiensis*, followed by Nectriaceae_uc at 15.5% and 41.9%, respectively. Except for *Fusarium*, Magnaportheaceae_uc and Russulaceae_uc were also identified (Figure 3).

4. Discussion

In this study, we determined that Basidiomycetes and Ascomycetes accounted for approximately 3.2% and 96.8%, respectively, of endophytic fungi identified in the four species of alpine conifers through the isolation and culture methods. Among precedent studies on endophytic fungi by isolation and culture methods about conifers in Korea, conifer trees distributed on Mt. Minjuji (N 36° 02′, E 127° 50′, 1,241.7 m) and Mt. Oseor (N 38° 27′, E 126° 39′, 790.7 m), Basidiomycetes accounted for about 3% and Ascomycetes accounted for approximately 97% [13,21]. Therefore, the biodiversity of endophytic fungi isolated from conifers distributed in the Korean peninsula did not differ significantly in the composition ratio of Basidiomycetes or Ascomycetes regardless of altitude or region. In addition, the composition of endophytic fungi isolated from the leaves of *Panax ginseng* C.A. Mey. was approximately 3% Basidiomycetes and approximately 97% Ascomycetes [22]. The composition of endophytic fungi isolated from the leaves of *Scirpus* spp. comprises approximately 2% Basidiomycetes and approximately 98% Ascomycetes [23]. The composition of endophytic fungi isolated from perennial plants such as *P. ginseng* and *Scirpus* spp. did not show a significant difference from those isolated from conifers. These results point out that regardless
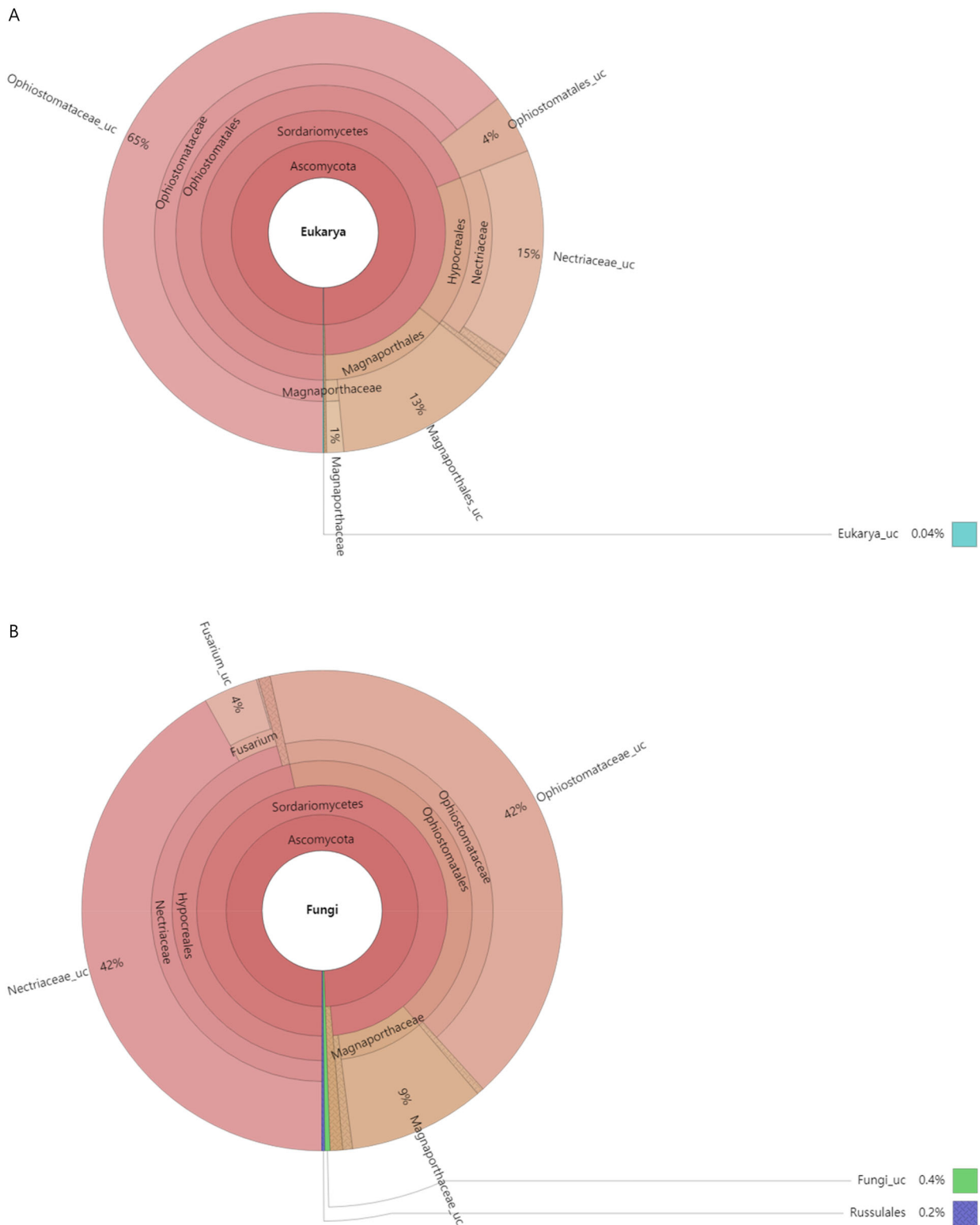
A

B

Figure 3. Krona chart of endophytic fungi represented by internal transcribed spacer (ITS) 2 region recovered from the needle leaves of two alpine conifers, (A) *Abies nephrolepis*, (B) *Thuja koraiensis*.

of the host plant and habitat, Ascomycetes predominate as endophytic fungi in most host plants, presumably performing certain ecological functions.

In general, the higher the species diversity, the more complex and diverse the interactions between individuals within a community [24]. Our findings indicate that more diverse interactions exist since the species diversity of endophytic fungi was high in

the order of *T. koraiensis*, *A. nephrolepis*, *T. cuspidata* var. *nana*, and *P. pumila*, but there is a limitation to further ecological interpretations of the species diversity index alone. However, it is possible to consider the surrounding vegetation associated with the host plant as a factor influencing the species diversity of endophytic fungi [25]. Host plants are not grouped such that there is only one species

in a certain area, but share the same space and time with other surrounding plants. In particular, depending on the type or health of the host plant, the type of endophytic fungi present will differ [26]. Therefore, the dispersal of endophytes distributed inside the plant itself affects each other and becomes the basis for the isolation and identification of other endophytic fungi as a result depending on the host plant.

In addition, we found that isolated endophytic fungal communities differed depending on the host plant, identifying 100% of singleton species in *A. nephrolepis*, approximately 63% in *P. pumila*, 54% in *T. cuspidata* var. *nana*, and approximately 66% in *T. koraiensis*. Although a singleton species can have a special ecological relationship with the host plant, the presence of a singleton species may indicate a biased result due to the collection time and tissue of the host plant or reflect opportunistic fungal infection. In particular, considering that the endophytic fungal communities formed differently even though the four host plants were collected at the same time and from the same mountain area, the premise is that the composition of endophytic fungi is primarily determined by which host plants exist [23]. Finally, since the existence of singleton species cannot be confirmed in the short term, periodic monitoring over many years is required. In view of previous studies, the existence of singleton species must be investigated through multiyear monitoring and bacterial isolation because endophytic fungi isolated from host plants differ according to season [27].

The endophytic fungi studied using the described isolation and culture methods were identified as about 35 taxa. Nevertheless, there were no endophytic fungi commonly found in all host plants, and only *Sydowia polyspora* (Bref. & Tavel) E. Müll. was identified in common among the three species of *A. nephrolepis*, *T. koraiensis*, and *P. pumila*. *S. polyspora* is mainly found in conifers of the genera *Pinus* and *Abies* [28,29]. Nevertheless, in this study, we confirmed that *S. polyspora* was also found in *T. koraiensis*, and it may be found in host plants belonging to other families and genera. Moreover, despite being isolated in the same medium, the number of commonly isolated endophytic fungi was rare, and these endophytic fungal communities were distinguishable to some extent depending on the host plant. Thus, these findings may provide indirect evidence of host preference or specificity according to the host plant.

Unrecorded species in Korea can be defined as species not found or included in official records in Korea. In Korea, 4683 fungal taxa have been discovered and recorded thus far. In particular, the research institutes under the Ministry of Environment are leading the discovery of unrecorded species. Since 2010, a catalog of species has been published in earnest as a result of several research projects. Korea is highly dependent on foreign biological resources overall and it has become important to efficiently manage its biological resources under the Convention on Biological Diversity (CBD) system [30]. In that context, five candidate unrecorded fungal taxa (*Darkera picea*, *Dactylella microaquatica*, *Phaeothecoidea* sp., *Paraphaeosphaeria neglecta*, and *Valsa ceratophora*) were identified through this study, and a taxonomic study is planned in the future. This work can be accepted as an ecological resource making important contributions not only to basic science but also to the development of ecological technology.

We provided for the first time a quantitative analysis by NGS of the endophytic fungal community of *A. nephrolepis* and *T. koraiensis*. The results of this study demonstrate that the most dominant endophytic fungi in both host trees belonged to the phylum Ascomycota, and at the class level, Sordariomycetes was identified. Furthermore, Ophiostomataceae_uc was identified as the most dominant genus in *A. nephrolepis* and Nectriaceae_uc in *T. koraiensis*. Although these host plants belong to different families, the dominance of Ascomycetes was the same and, although differences became more prominent as the sub-taxa descended, it was nevertheless difficult to confirm similar results even at the genus level compared with the results of the isolation and culture methods. Basically, there is a practical quantitative difference between fungi that can be isolated and cultured using a medium and those that cannot. Rather, the taxa identified using a specific medium may exist at a small proportion in actual plants and their ecological function may be considered low. Furthermore, a sizable number of singleton species is due to accidental infection of the host tree.

However, the resolution of nucleotide sequences produced by NGS is low compared with other microorganisms such as bacteria because NGS in fungi only uses the ITS2 region [31]. Consequently, it cannot be excluded that there may be other endophytic fungi that are also being isolated and cultured. Nevertheless, our results consistently show that the majority of Ascomycetes are dominant regardless the method used: conventional isolation and culture methods or NGS. Overall, taking into account their wide distribution and importance in providing resistance to both biotic and abiotic stresses, Ascomycete endophytic fungi should be further studied in terms of functional aspects.

5. Conclusion

This study identified 35 taxa from 108 endophytic fungal strains from four conifer species through isolation and culture methods. For *A. nephrolepis* and *T. koraiensis*, endophytic fungi were analyzed by NGS and 147 OTUs and 245 OTUs were identified, respectively. Most fungi belonged to Ascomycetes, and in the case of Basidiomycetes, approximately 3.2% were confirmed through isolation and culture methods and approximately 0.2% *via* NGS. The results of this study confirmed that Ascomycetes were dominant among endophytic fungi and differences between isolation and culture methods and NGS in sub-taxa were confirmed. Nevertheless, conifer species collected from the same area exhibited different endophytic fungal communities, which is a significant finding of the present study. Therefore, considering that they constitute different endophytic communities depending on the host plant, it can be seen that the distribution of the host plant is crucial for the existence of the endophytic fungi. Lastly, since climate change due to global warming has been increasing the death rate of alpine conifers in the forest ecosystem, it is necessary to prioritize monitoring alpine conifers and the excavation of endophytic fungi by isolation and culture methods. In conclusion, our study confirms the interaction between host plants and fungi in a restricted alpine area and contributes to a better understanding of the dispersal mechanism of host plants and endophytic fungi through mutual cooperation.

Disclosure statement

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