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Co-occurrence patterns of wood-decaying fungi and ants in dead pines of South Korea

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ABSTRACT

Interaction between fungi and insects such as ants, beetles, wasps and termites inhabiting dead pine trees has significant ecological implication in the forest as they can decompose wood debris and add nutrients to the soil; however, only scarce information is available regarding the interaction between wood-decaying fungi and ants. We investigated wood-decaying fungi co-occurring with ants in dead pine trees of South Korea. A total of 57 pairs of wood-decaying fungi and ants were collected from 11 localities. 30 species of wood-decaying fungi and 14 species of ants were identified based on morphology and molecular analysis. Fungal species belonging to *Trichaptum*, *Xylodon*, *Hyphodontia*, and *Ceriporia* were dominant and co-occurred with common ant species of *Lasius*, *Camponotus*, *Pristomyrmex*, and *Crematogaster* across most of the sampling sites. This study provides a new baseline in unravelling the complex interaction between wood-decaying fungi and ants in forest ecosystems.

Introduction

Wood-decaying fungi are saprotrophs distributed across several orders of class Agaricomycetes (Basidiomycota) (Hibbett et al., 2007). They decompose wood into simple sugars, oligosaccharides and humic substances which are subsequently added to soil (Carlile et al., 2001). Through decomposition of fallen dead wood, these fungi provide habitats for many other organisms and regenerate forests (Sippola et al., 2005; Heilmann-Clausen et al., 2014; Fukasawa et al., 2015).

Habitats and nutrients generated by wood-decaying fungi are sufficient factors to attract various invertebrates (Johansson et al., 2006; Warren and Bradford, 2011). Some of them have also developed symbiotic relationships with fungi (Hågvar, 1999; Mueller et al., 2005; Schigel, 2011a; Li et al., 2015). Ants have also exhibited strong co-evolutionary dynamics with fungi (Masiulionis et al., 2014). The most widely known and explored ant-fungus mutualism is between the Attine ants (tribe Attini), best known as "leaf cutter ants" (*Atta* and *Acromyrmex* genera) and their fungal cultivars, Leucocoprineae and Pterulaceae, in Southern and Central America and southern parts of the USA (Hinkle et al., 1994; Mehdiabadi and Schultz, 2010; Masiulionis et al., 2014). Masiulionis et al (2014) reported that ants can cultivate the fungi in the nest and protect the fungi from alien microorganism such as bacteria and other invading fungi and, in return, these fungi provide nutrients to the ants in the form of specialized inflated hyphal tips called gongylidia. Ants of diverse genera have been observed as common visitors of wood-decaying fungi as well (Lewis and Worthen, 1992; Orr and Charles, 1994; Mueller et al., 2001). Recently, Epps and Penick (2018) scrutinized mushroom visiting ants (*Aphaenogaster* spp.) and reported that these ants with a generalist diet fed on wood-decaying fungus, *Pleurotus ostreatus*.

In South Korea, where forests are mostly coniferous, several species of ants are observed, such as *Aphaenogaster*, *Camponotus japonicas*, and *Formica japonica* from various altitudes (Kwon et al., 2012; Kwon et al., 2013). Pine debris is susceptible to various wood-decaying fungi such as *Athelia epiphylla*, *Fomitopsis pinicola*, *Gloeophyllum sepiarium*, *Oligoporus placentus*, *Phanerochaete filamentosa*, and *Trichaptum abietinum* (Son et al., 2011). These wood-decaying fungi attract insects such as woodborers (Park et al., 2014; Floren et al., 2015) and wasps (Pažoutová and Šrůtka, 2007). However, there has not been a comparable study of mutualistic associations between ants and wood-decaying fungi. To understand their relationship, investigation of co-occurrence patterns of ants and fungi is required first. In this study, we investigated these co-occurrence patterns in dead pine trees. We further assessed which ant-fungi pair had the highest co-occurrence.

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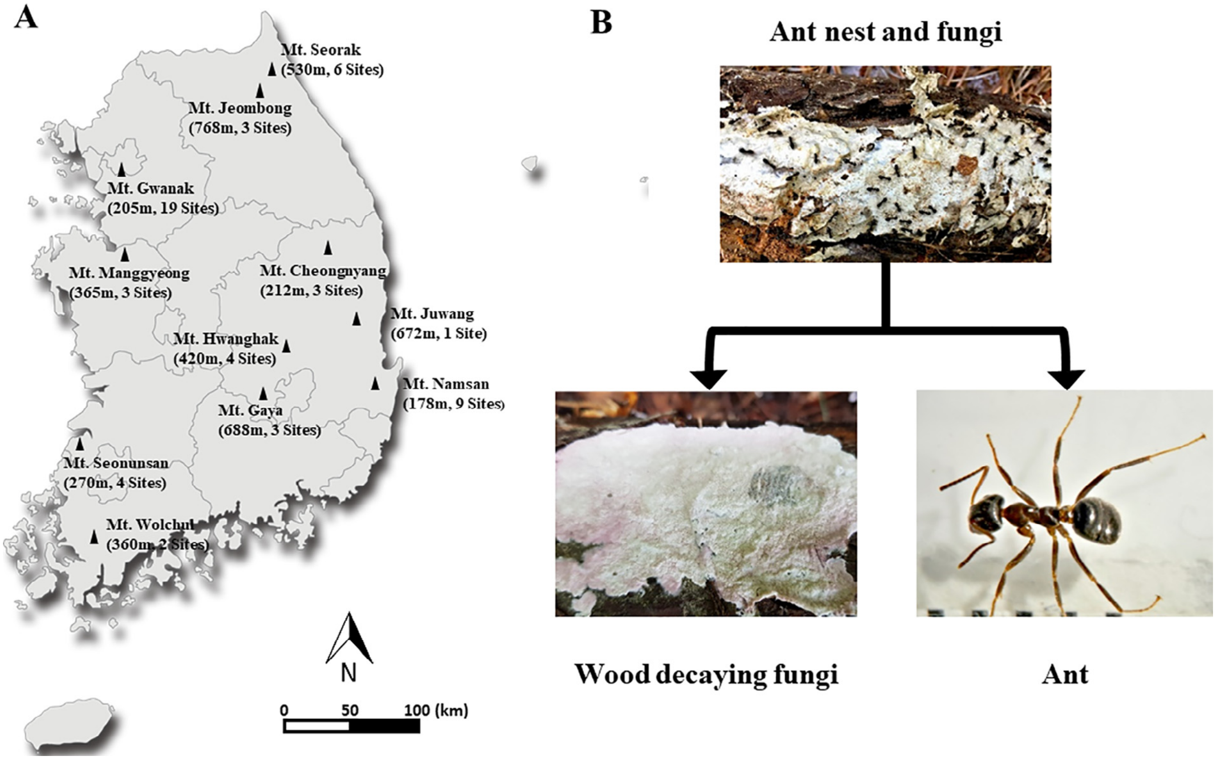

Fig. 1. Sampling locations and strategy. A, Map of South Korea showing all sampling locations, altitudes of mountains and number of sampling sites. **B,** After confirming co-occurrence of ant and fungi, they were collected in pairs.

Materials and methods

Sampling sites and specimen collection

We conducted sampling of wood-decaying fungi and co-occurring ants on dead pine trees (*Pinus densiflora*) between mid-March to September 2018 across 57 sites of 11 locations in Korea (Fig. 1A). They were collected only after we confirmed that (1) wood-decaying fungi and ants were both present on a dead pine tree (Fig. 1B) and (2) an ant nest was found within the tree trunk. Based on these criteria, we avoided collecting specimens when ants simply foraged wood-decaying fungi without a nest underneath. All sites were of lower to higher altitude mountainous regions with moderate understory vegetation to completely covered litter layers. At all sites, vegetation was dominated by *Pinus densiflora*.

Fungi collection and identification

Whole fruiting bodies inhabiting deadwoods were collected by using knife and chisel depending on the size, dried and deposited in the Seoul National University Fungus Collection (SFC). They were identified via morphological and molecular approaches. Classical methodology was achieved by macro- and micro-morphological observations using taxonomic guides (Largent and Thiers, 1977; Breitenbach and Kränzlin, 1986)

Genomic DNA was extracted from fruiting bodies using a modified Cetyl trimethyl ammonium bromide (CTAB) extraction protocol of Rogers and Bendich (1994). The internal transcribed spacer (ITS) region was amplified using combinations of the forward primers (ITS1F and NSI1) and reverse primers (ITS4B, LB-W, and NLB4) (White et al., 1990; Gardes and Bruns, 1994; Martin and Rygiewicz, 2005; Tedersoo et al., 2008). PCR amplification was performed as described by Park et al. (2013). PCR products were visualized on a 1% agarose gel and purified using the Expin PCR purification kit (GeneAll Biotechnology, Seoul, South Korea). Sanger sequencing was performed at Macrogen (Seoul, South Korea) on an automated DNA sequencer (ABI Prism 3730XL analyzer; Applied Biosystems, Foster City, California) using the aforementioned PCR primers.

Newly generated sequences were proofread and edited using MEGA 5 (Tamura et al., 2011). Molecular identification was conducted with ITS region using BLAST against GenBank database. Species level identification was achieved through the sequence similarity with 98–100%. Specimens that had sequence similarity of 95 to 97% or no species information in open database remained at the genus or higher level (Table S1). All sequences were deposited at GenBank under accession numbers MK992816-MK992868 (Table 1).

Ants collection and identification

Ants in contact with the fungus and those nesting inside the deadwood were collected into plastic bag and then transfer to 1.5 mL Eppendorf tubes containing 70% ethanol by employing hand collection method and transported to the laboratory. At least 20 ants were sampled per deadwood and nesting. They were identified by morphological and molecular approaches. For morphological approach, we observed ants under Nikon SMZ1500 stereo microscope and identified according to the taxonomic keys (Japanese Ant Image Database, 2010 (http://ant.edb.miyakyo-u.ac.jp/); Kwon et al., 2012; Dong, 2017).

Molecular approach was performed as follows. An individual ant was placed in a 1.5 mL Eppendorf tube after the removal of gaster which is known to contain a substance inhibiting the PCR (Feldhaar et al., 2003). Genomic DNA was extracted by using AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, South Korea) according to the manufacturer's protocol. For PCR amplification and sequencing, we targeted the insect barcode mitochondrial cytochrome *c* oxidase subunit I (COI) gene which is the basis of ants' molecular identification (Wilson, 2012). COI was amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994). PCRs were conducted in the C1000 thermal cycler (Bio-Rad, Hercules, CA, USA) using the AccuPower PCR Premix (Bioneer Co., Daejeon, Korea) in a final volume of 20 μL

Table 1
List of collected wood-decaying fungi and their co-occurring ant species across sampling sites.

Wood-decaying fungi	Specimen code	Co-occurring ant	Specimen code	Locality	GenBank Acc. Nos.	
					Fungal ITS	Ants COI
Amylostereaceae						
Amylostereum areolatum	SFC20180314-02	*Camponotus atrox*	SFC20180314-A01	Mt. Jeombong	MK992817	MN010595
Atheliaceae						
Athelia epiphylla	SFC20180314-01	*Camponotus atrox*	SFC20180314-A01	Mt. Jeombong	MK992816	–
Bondarzewiaceae						
Heterobasidion ecrustosum	SFC20180919-03	*Pheidole fervida*	SFC20180919-A03	Mt. Seorak	MK992864	MN010636
Botryobasidiaceae						
Botryobasidium sp. 1	SFC20180710-21	*Lasius japonicus*	SFC20180710-A10	Mt. Hwanghak	MK992837	MN010611
Botryobasidium sp. 2	SFC20180609-01	*Lasius japonicus*	SFC20180609-A01	Mt. Gwanak	MK992824	MN010603
	SFC20180710-16	*Lasius spathepus*	SFC20180710-A05	Mt. Namsan	MK992832	MN010609
Botryobasidium sp. 3	SFC20180710-22	*Lasius japonicus*	SFC20180710-A11	Mt. Hwanghak	MK992838	MN010612
Fomitopsidaceae						
Dacryobolus sp. 1	SFC20180907-154	*Pheidole fervida*	SFC20180907-A03	Mt. Gaya	MK992862	MN010633
Dacryobolus sp. 2	SFC20180907-153	*Aphaenogaster japonica*	SFC20180907-A02	Mt. Gaya	MK992861	MN010632
Postia hirsuta	SFC20180822-19	*Camponotus nipponensis*	SFC20180822-A03	Mt. Gwanak	MK992857	MN010627
Hydnodontaceae						
Trechispora confinis	SFC20180710-18	*Crematogaster teranishii*	SFC20180710-A06	Mt. Namsan	MK992834	–
	SFC20180710-23	*Polyrhachis lamellidens*	SFC20180710-A12	Mt. Hwanghak	MK992839	MN010613
Meruliaceae						
Ceriporia alachuana	SFC20180710-15	*Lasius japonicus*	SFC20180710-A04	Mt. Namsan	MK992831	MN010608
	SFC20180810-01	*Lasius japonicus*	SFC20180810-A01	Mt. Gwanak	MK992849	MN010621
	SFC20180810-03	*Nylanderia flavipes*	SFC20180810-A03	Mt. Gwanak	MK992851	MN010623
C. nanlingensis	SFC20180710-17	*Lasius japonicus*	SFC20180710-A05	Mt. Namsan	MK992833	MN010609
	SFC20180822-20	*Vollenhovia emeryi*	SFC20180822-A04	Mt. Gwanak	MK992858	MN010628
Phlebia acanthocystis	SFC20180704-91	*Camponotus vitiosus*	SFC20180704-A01	Mt. Wolchul	MK992826	MN010605
P. acerina	SFC20180711-01	*Pristomyrmex punctatus*	SFC20180711-A01	Mt. Cheongnyang	MK992841	MN010615
Phanerochaetaceae						
Phlebiopsis gigantea	SFC20180711-02	*Pristomyrmex punctatus*	SFC20180711-A01	Mt. Cheongnyang	MK992842	–
Polyporaceae						
Perenniporia koreana	SFC20180719-04	*Lasius japonicus*	SFC20180719-A04	Mt. Gwanak	MK992847	MN010619
Trichaptum abietinum	SFC20180531-03	*Pheidole fervida*	SFC20180531-A03	Mt. Seonun	MK992820	MN010598
	SFC20180531-04	*Pheidole fervida*	SFC20180531-A04	Mt. Seonun	–	MN010599
	SFC20180710-12	*Crematogaster teranishii*	SFC20180710-A01	Mt. Namsan	MK992828	MN010606
	SFC20180710-13	*Pristomyrmex punctatus*	SFC20180710-A02	Mt. Namsan	MK992829	MN010607
	SFC20180710-14	*Crematogaster teranishii*	SFC20180710-A03	Mt. Namsan	MK992830	–
	SFC20180719-05	*Aphaenogaster japonica*	SFC20180719-A05	Mt. Gwanak	MK992848	MN010620
	SFC20180822-21	*Crematogaster teranishii*	SFC20180822-A05	Mt. Gwanak	–	MN010629
	SFC20180919-01	*Camponotus kiusuensis*	SFC20180919-A01	Mt. Seorak	–	MN010634
	SFC20180928-35	*Camponotus atrox*	SFC20180928-A01	Mt. Juwang	–	MN010640
Rickenellaceae						
Peniophorella pubera	SFC20180601-02	*Lasius japonicus*	SFC20180601-A02	Mt. Manggyeong	MK992822	MN010601
P. subpraetermissa	SFC20180719-01	*Vollenhovia emeryi*	SFC20180719-A01	Mt. Gwanak	MK992844	–
Resinisium furfuraceum	SFC20180822-18	*Pristomyrmex punctatus*	SFC20180822-A02	Mt. Gwanak	MK992856	MN010626
	SFC20180907-152	*Lasius japonicus*	SFC20180907-A01	Mt. Gaya	MK992860	MN010631
	SFC20180919-02	*Camponotus kiusiuensis*	SFC20180919-A02	Mt. Seorak	MK992863	MN010635
	SFC20180919-06	*Lasius japonicus*	SFC20180919-A06	Mt. Seorak	MK992867	MN010639
	SFC20180929-32	*Camponotus atrox*	SFC20180929-A01	Mt. Jeombong	MK992868	MN010641
Schizophyllaceae						
Schizophyllum commune	SFC20180704-92	*Camponotus vitiosus*	SFC20180704-A01	Mt. Wolchul	MK992827	–
Schizoporaceae						
Xylodon chinensis	SFC20180710-20	*Pristomyrmex punctatus*	SFC20180710-A09	Mt. Namsan	MK992836	MN010610
	SFC20180818-36	*Camponotus nipponensis*	SFC20180818-A02	Mt. Gwanak	MK992854	–
X. flaviporus	SFC20180710-24	*Lasius japonicus*	SFC20180710-A13	Mt. Hwanghak	MK992840	MN010614
X. ovisporus	SFC20180531-01	*Pristomyrmex punctatus*	SFC20180531-A01	Mt. Seonun	MK992818	MN010596
	SFC20180531-02	*Lasius japonicus*	SFC20180531-A02	Mt. Seonun	MK992819	MN010597
	SFC20180719-03	*Lasius japonicus*	SFC20180719-A03	Mt. Gwanak	MK992846	MN010618
	SFC20180810-02	*Lasius spathepus*	SFC20180810-A02	Mt. Gwanak	MK992850	MN010622
	SFC20180822-22	*Vollenhovia emeryi*	SFC20180822-A06	Mt. Gwanak	MK992859	MN010630
X. nespori	SFC20180601-03	*Pristomyrmex punctatus*	SFC20180601-A03	Mt. Manggyeong	MK992823	MN010602
	SFC20180822-17	*Camponotus quadrinotatus*	SFC20180822-A01	Mt. Gwanak	MK992855	MN010625
Tapinellaceae						
Pseudomerulius aureus	SFC20180818-35	*Pheidole fervida*	SFC20180818-A01	Mt. Gwanak	MK992853	–
Tubulicrinaceae						
Hyphodontia pallidula	SFC20180601-01	*Pristomyrmex punctatus*	SFC20180601-A01	Mt. Manggyeong	MK992821	MN010600
	SFC20180710-19	*Crematogaster teranishii*	SFC20180710-A07	Mt. Namsan	MK992835	–
	SFC20180711-03	*Lasius japonicus*	SFC20180711-A02	Mt. Cheongnyang	MK992843	MN010616
	SFC20180719-02	*Lasius japonicus*	SFC20180719-A02	Mt. Gwanak	MK992845	MN010617
	SFC20180810-04	*Camponotus nipponensis*	SFC20180810-A04	Mt. Gwanak	MK992852	MN010624
Tubulicrinis sp.	SFC20180919-05	*Lasius japonicus*	SFC20180919-A05	Mt. Seorak	MK992866	MN010638
Unidentified family						
Auriculariales sp.	SFC20180919-04	*Lasius japonicus*	SFC20180919-A04	Mt. Seorak	MK992865	MN010637

(*continued on next page*)

Table 1 (*continued*)

Wood-decaying fungi	Specimen code	Co-occurring ant	Specimen code	Locality	GenBank Acc. Nos.	
					Fungal ITS	Ants COI
Hymenochaetales sp.	SFC20180609-02	*Vollenhovia emeryi*	SFC20180609-A02	Mt. Gwanak	MK992825	MN010604

- No sequence information.

containing 10 pmol of each primer and 1 μL of DNA under the following conditions: 95 °C for 5 min, followed by 35 cycles of 95 °C for 40 secs, 45 °C for 40 secs, and 72 °C for 1 min and a final extension step at 72 °C for 10 min.

Purification and sequencing of PCR product were conducted as described in the previous section. As reference sequences of ants native to East Asia are largely unavailable in GenBank, some specimens were identified based on the BLAST service of the National Institute of Biological Resources (https://species.nibr.go.kr) (Table S2). All sequences were deposited at GenBank under accession numbers MN010595-MN010641 (Table 1).

Results

Wood-decaying fungi inhabiting dead pine trees

Five fungal species were first identified morphologically based on their distinct shapes followed by molecular confirmation: *Ceriporia nanlingensis*, *Heterobasidion ecrustosum*, *Schizophyllum commune*, *Trichaptum abietinum*, and *Xylodon flaviporus*. Other species were identified first based on ITS sequence analysis and then confirmed by morphological characters. 48 specimens were identified to species level and seven to genus level while two to order level only. A total of 30 species of wood-decaying fungi was confirmed from 57 sites (Table 1).

Species belonging to Polyporaceae and Schizoporaceae were dominant followed by Meruliaceae, Rickenellaceae, Tubulicrinaceae, Botryobasidiaceae, and Fomitopsidaceae (Fig. 2A). Most of the remaining seven families were collected from single sites only (Table 1). *Xylodon* and *Trichaptum* were the most dominant genera followed by *Hyphodontia, Ceriporia*, and *Botryobasidium* (Table 1, Fig. 2A). In terms of species, *T. abietinum* had the highest collection frequency (9 sites) followed by *Xylodon ovisporus*, *Resinicium furfuraceum*, and *Hyphodontia pallidula* (each 5 sites). *Ceriporia alachuana* and *C. naglingensis* were collected from five sites. Some species such as *Amylostereum areolatum*, *Athelia epiphylla*, *Heterobasidion ecrustosum*, *Perenniporia koreana*, and *Pseudomerulius aureus* were collected only once (Table 1).

Ants nesting in dead pine trees

Ants were identified as 14 different species five tribes, namely Camponotini, Lasiini (Sub-family Formicinae), and Attini, Crematogastrini, Stenammini (Sub-family Myrmicinae) (Table 1, Fig. 2B). Species frequently observed were *Lasius japonicus* (17 sites) and *Pristomyrmex punctatus* (9 sites), followed by *Crematogaster teranishii* (5 sites), *Pheidole fervida* (5 sites), and *Vollenhovia emeryi* (4 sites). Ants from the genus *Camponotus* had the highest species diversity: *C. atrox*, *C. kiusuensis*, *C. nipponensis*, *C. vitiosus*, and *C. quadrinotatus*. Species *Aphaenogaster japonica* and *Lasius spathepus* were each collected twice while the least common species *Nylanderia flavipes* and *Polyrhachis lamellidens* were collected once (Table 1).

Co-occurrence patterns of wood-decaying fungi and ants

Ants' nests were deep inside dead pine trees or near the top layer of the dead pine tree harboring fruiting bodies or mycelia of fungi. At the family level for fungi and tribal level for ants, Polyporaceae co-occurred with all ant tribes while Meruliaceae, Rickenellaceae, Schizoporaceae, and Tubulicrinaceae co-occurred with three ant tribes Camponotini, Crematogastrini, and Lasiini. All of Botryobasidiaceae fungi co-occurred with tribe Lasiini (Fig. 3).

Trichaptum abietinum had the highest number of co-occurrence pairs with several ant species across the sampling sites: three pairs with *Crematogaster teranishii*, two pairs with *Pheidole fervida*, two pairs with *Camponotus* spp. and one pair each with *Aphaenogaster japonica* and *Pristomyrmex punctatus* (Fig. 3). Fungal species of *Xylodon* (*X. ovisporus*), *Botryobasidium* (*Botryobasidium* sp. 1, 2 and 3), *Ceriporia*, *Hyphodontia*

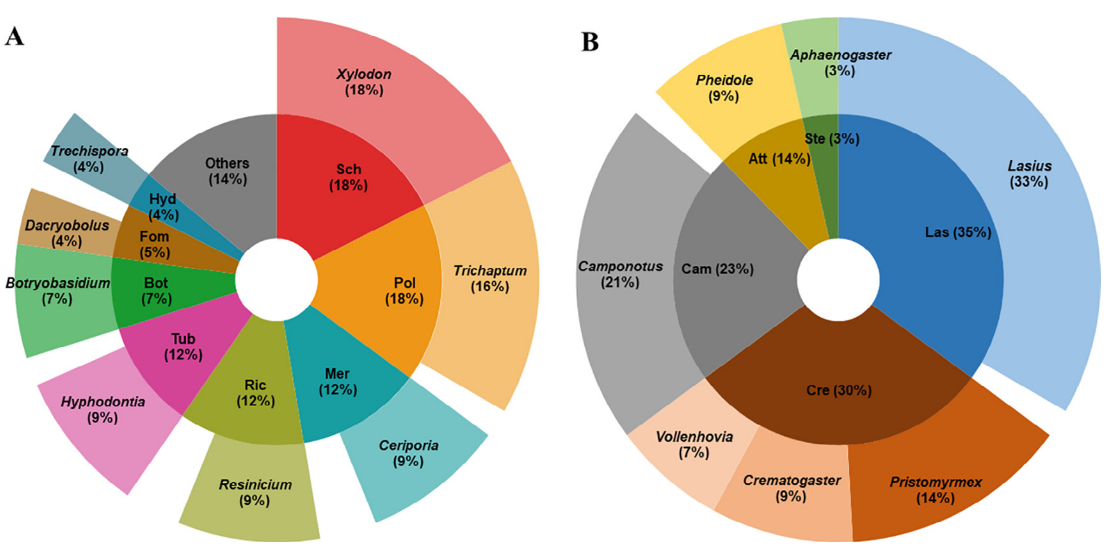

Fig. 2. Pie charts of dominant wood-decaying fungi and ants collected across sampling sites and their percentages. A, Inner circle represents fungal families: Sch-Schizoporaceae, Pol-Polyporaceae, Mer- Meruliaceae, Ric- Rickenellaceae, Tub-Tubulicrinaceae, Bot- Botryobasidiaceae, Fom- Fomitopsidaceae, and Hyd-Hydnodontaceae. Outer circle represents dominant fungal genera. **B,** Inner circle represents ants' tribes; Las- Lasiini, Cre- Crematogastrini, Cam- Camponotini, Att-Attini, and Ste-Stenammini. Outer circle represents dominant ant genera.

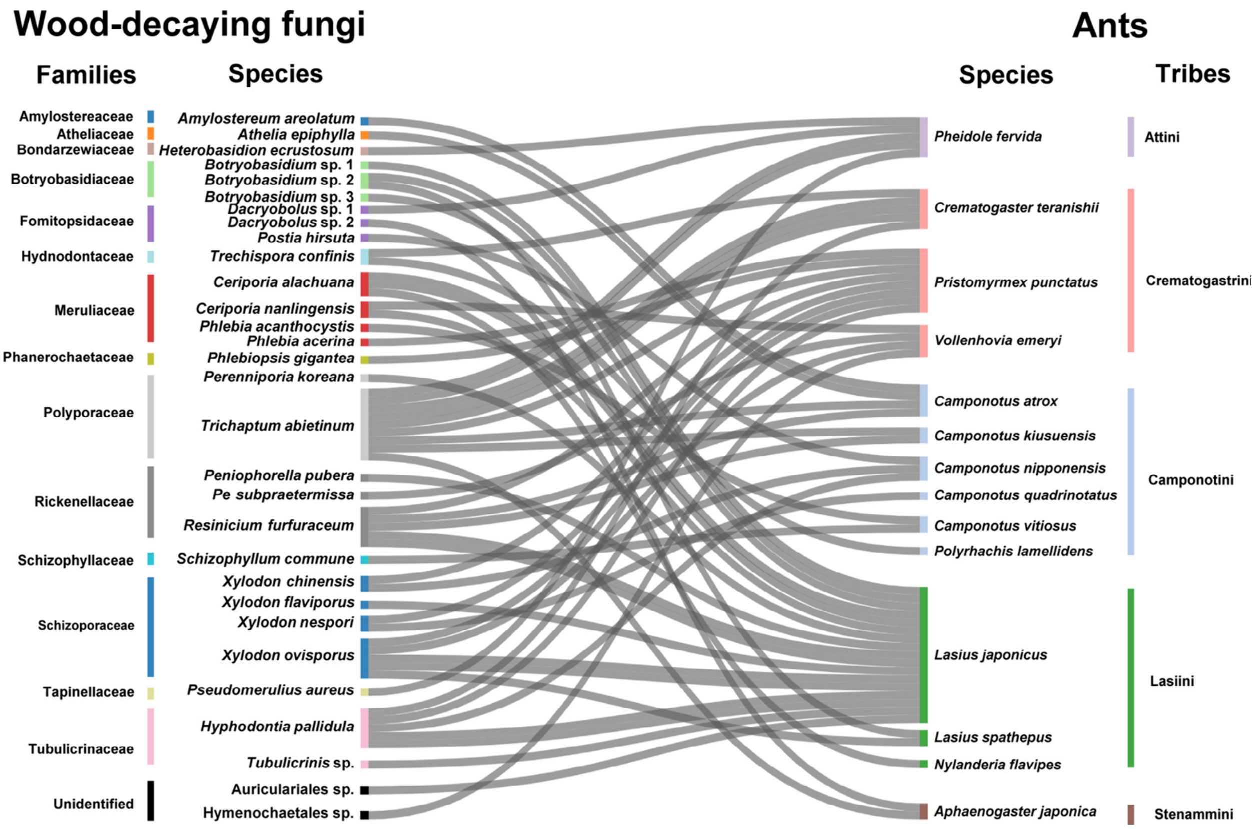

Fig. 3. A flow plot of co-occurring pairs of wood-decaying fungi and ants. The left side bars represent families and species of wood-decaying fungi; the right side bars represent the ants' tribes and species. The length of a bar next to the name of a species is proportional to its number of co-occurring pairs.

pallidula, and *Resinicium furfuraceum* followed in terms of co-occurrences pairs with multiple ant species across the sites, all of these fungi co-occurred with different species of ants with *Lasius japonicus* being the most common to all. *L. japonicus* ants had the highest number of co-occurrence pairs with wood-decaying fungi across the sampling sites: two pairs with *C. alachuana*, *H. pallidula*, *R. furfuraceum*, *X.ovisporus* and a single pair each with Auricularies sp., *Botryobasidium* species (sp. 1–3), *C. nanlingensis*, *Peniophorella pubera*, *Perenniporia koreana*, *Tubilicrinis* sp., and *X. flaviporus*. *Pristomyrmex punctatus* had the second highest number of co-occurrence pairs with multiple fungi species, the most being three pairs with *Xylodon* species.

Co-occurrence of some wood-decaying fungi and ants was observed by the tendency of ants displaying foraging-like movements on the surface of fruiting bodies. Ants from inside the deadwood and the deadwood's surroundings were observed to be displaying these movements. Pairs of wood-decaying fungi and ants involved in this tendency were *Ceriporia alachuana / Lasius japonicus*, *Heterobasidion ecrustosum / Pheidole fervida*, *Trichaptum abietinum / Crematogatser teranishii*, and *Xylodon ovisporus / Pristomyrmex punctatus*.

Discussion

To our knowledge, this is the first descriptive study addressing multiple species co-occurrences (wood-decaying fungi and ants) in dead pine trees of South Korea. Outside South Korea, many studies regarding wood-decaying fungi interactions with insects have only focused on various groups of fungivorous insects; the Czech Republic (Coleoptera, Hymenoptera and Lepidoptera) (Kula et al., 1999), the Fennoscandian countries (Coleoptera and Lepidoptera) (Hågvar, 1999; Thunes et al., 2000; Jonsell and Nordlander, 2004; Schigel, 2011b), China (Coleoptera and Diptera) (Komonen et al., 2003), Russia (Coleoptera) (Nikitsky and Schigel, 2004; Krasutskii, 2007), New Zealand (Diptera) (Osawa et al., 2011), Borneo (Coleoptera) (Yamashita et al., 2015), and Brazil (Diptera) (Valer et al., 2016).

Wood-decaying fungi are key players in forest ecosystems due to their ability of decomposing dead wood, recycling nutrients and initiating a successional dynamic for saproxylic arthropods (Jönsson et al., 2008; Lonsdale et al., 2008). A list of collected wood-decaying fungi from decaying pinewoods in this study was consistent with the relevant previous studies in Korea (Kim et al., 2009; Jang et al., 2014; Jang et al., 2016; Kim et al., 2017) and also outside Korea (Kubartová et al., 2012; Walker et al., 2014; Ottosson et al., 2015; Fukasawa and Matsuoka, 2015). *Athelia epiphylla* and *Schizophyllum commune* are known to be exclusive to deciduous trees (Allen et al., 2000; Takemoto et al., 2010), but rarely reported from coniferous trees such as pine (Kim et al., 2009). *Trichaptum abietinum*, which was frequently observed on pinewoods, is known as the first colonizer on dead pine trees in many forests (Weslien et al., 2011). *Hyphodontia* and *Xylodon* (specifically *Xylodon ovisporus*) were also dominant on decaying pinewoods across the sampling sites and have been reported to be common on deciduous forests as well (Gafforov et al., 2017). *Ceriporia* species which can cause decaying of both deciduous and coniferous wood (Wulandari et al., 2018) were also common. *Ceriporia alachuana* and *Ceriporia nanlingensis* are commonly isolated from pinewood (Dai, 2012). Other observed species were all commonly found in decaying pinewood (Lee and Jung, 2006; Wooding et al., 2013; Jang et al 2016).

Ants associated with wood-decaying fungi in this study were of 14 species. Previous surveys of ant communities in South Korea have reported these species (Kim and Lyu, 2012; Kwon et al., 2013, 2014a, 2014b; Kwon, 2015a; Kwon, 2015b; Kwon and Lee, 2015; Kwon, 2016). *Lasius japonicus* was the most frequently detected ant species in this study. It is frequently found in Korean forests and dead pine tree is one of its preferred nesting place (Kwon and Lee, 2015). The least collected ant species in this study, *Nylanderia flavipes* also has been reported as one of the most common ant species in Korean pine forests (Kwon and Lee, 2015). Remaining ant species such as *Aphaenogaster japonica,* *Camponotus* spp., *Crematogaster teranishii*, and *Pheidole fervida* have been previously observed to nest inside decayed pinewood (Kwon et al., 2014b; Kwon, 2016).

For some wood-decaying fungi, interaction with insects is crucial for their establishment and colonization in dead woods (Strid et al., 2014). Insects such as bark beetles and wasps are one of the key players in dispersal of fungal spores to new dead trees (Floren et al., 2015). Most of the wood-decaying fungi found in this study were previously reported to be associated with beetles and wasps (Ševčík, 2003; Persson et al., 2011; Son et al., 2011; Olatinwo et al., 2013; Strid et al., 2014). *Amylostereum areolatum* and *Athelia epiphylla* are well known decaying fungi to have an interaction with wasp and beetles, respectively (Rajala et al., 2015; Seibold et al., 2019).

Although our study is limited with its small sample size, nevertheless our results showed that various wood-decaying fungi co-occurred with ants on dead pine trees. The most established form of feeding association between ants and fungi is that of Attine ants subfamily Myrmicinae and their fungal cultivars (Masiulionis et al., 2014), In our study we collected *Aphaenogaster japonica*, *Crematogaster teranishii*, *Pheidole fervida*, *Pristomyrmex punctatus*, and *Vollenhovia emeryi*, all of which belongs to sub-family Myrmicinae. Due to evolutionary processes, this interaction is mostly restricted to Attine ants (Mehdiabadi and Schultz, 2010), but it is crucial to study if remaining members within Myrmicinae have acquired comparable interactions with mushrooms.

Comparing to the sub-family Myrimicinae, interaction between ants of sub-family Formicinae and fungi are not well studied. *Lasius japonicus* and *Camponotus* species in sub-family Formicinae had the highest number of co-occurrence pair with wood-decaying fungi in this study. They also displayed foraging-like movements on basidiocarps. Similar feeding habits on wild mushrooms have been reported from ants species in sub-family Formicinae. In recent studies, ants of *Aphaenogaster* species exhibited interactions specifically with wood-decaying fungi (Epps and Penick, 2018). Some ants in sub-family Formicinae tribe Lasiini are also recognized as dietary specialist of wild-growing mushrooms (Beeren et al., 2014; À l'allemand et al., 2019)

Decomposing dead pine trees provides essential habitat for various ants, beetles, and wood-decaying fungi (Warren and Bradford, 2011). Since many wood-decaying fungi are associated with various beetles and wasps, it raises the question whether they also have any sort of association with ants. It is challenging to prove the specific relationship of wood-decaying fungi and co-occurring ants because there can be various explanations for such co-occurrences: e.g. i) wood-decaying fungi may indeed have a specific relationship with ants, ii) they may have accidentally occurred together, and iii) beetles may carry fungal spores and then ants use basidiocarps or mycelia as food source. More research is needed to uncover such relationships.

Conclusions

Our study provides a new baseline in unravelling the complex interaction between wood-decaying fungi and ants in forest ecosystems. Both wood-decaying fungi and ants are important in providing nutrients to the soil through decomposition of wood debris; yet, much is not known on how they interact with each other at this specific niche of dead pine tree. Though our study is limited with small sample size, it nonetheless showed diverse patterns of co-occurrence between wood-decaying fungi and ants. Based on our observations, future studies are needed to answer the following questions: Is there any specific interaction between wood-decaying fungi and ants? Do ants participate in dispersal of fungal spores? If so, how do the mechanisms happened by functional way?

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aspen.2019.10.009.

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