Journal of Asia-Pacific Biodiversity 15 (2022) 613–622

Contents lists available at ScienceDirect

Journal of Asia-Pacific Biodiversity

journal homepage: http://www.elsevier.com/locate/japb

Original Article

Diversity and species distribution of lichens in Gwangneung Forest☆

Jung Shin Park[a], En-Mi Sun[b], Jung-Jae Woo[c], Sang-Kuk Han[a], Soon-Ok Oh[a,*]

[a] Division of Forest Biodiversity, Korea National Arboretum, Pocheon 11186, South Korea
[b] Baekdudaegan National Arboretum, Bonghwa 36209, South Korea
[c] Korean Lichen Research Institute, Sunchon National University, Sunchon 57922, South Korea

ARTICLE INFO

Article history:
Received 6 February 2022
Received in revised form
8 April 2022
Accepted 11 April 2022
Available online 9 May 2022

Keywords:
Gwangneung Forest
Lichen
Phylogenic analysis
Taxonomy

ABSTRACT

Gwangneung Forest is a forest area that has been in existence for more than 550 years with a long history of natural and artificial forests in harmony. Lichens are not only a single organism but also a group of basic organisms formed from a stable symbiotic association between a fungus and algae and/or cyanobacteria. They are closely associated with forests and have formed an axis through Gwangneung Forest. From 2016 to 2021, a survey was conducted to investigate the lichen flora distribution by species, focusing on the viewing area within the Korea National Arboretum and the undisclosed research area centered in the Suribong Peak area. As a result of collecting and identifying 246 specimens, 68 species of lichens were identified in 11 orders, 22 families, and 39 genera. The tree with the largest number of lichens was *Koelreuteria paniculata*, followed by *Sophora japonica*, *Ginkgo biloba*, and *Malus sieboldii*. In trees belonging to *Aceraceae*, crustose lichens rather than foliose lichens were mainly found. Through this survey, three unreported species were discovered: *Lepraria cupressicola*, *Leprocaulon nicholsiae*, and *Graphis pinicola*. *Lepraria cupressicola* and *Leprocaulon nicholsiae* were distributed throughout the arboretum viewing area and *Graphis pinicola* was identified only in one viewing area.

Introduction

Gwangneung Forest was built as the tomb of King Sejo, the seventh King of Joseon, and Queen Jeonghui, and has maintained a unique ecosystem without fire or disturbance for over 550 years. Today, it is designated as a biosphere reserve and is managed and conserved by the government (Jo et al. 2020a). Gwangneung Forest consists of natural forests accounting for 602.34 ha (54%) and artificial forests accounting for 462.95 ha (42%) (Jo et al. 2020b). Natural forests include the colonies of *Quercus serrata* and *Carpinus laxiflora* centered in Suribong, and artificial forests include the special broad-leaved trees such as *Pinus koraiensis*, *Abies holophylla*, and *Robinia pseudoacacia* mainly in lowlands, as well as *Pinus rigida*, *Pinus koraiensis*, and *Abies holophylla* planted in 1970 (Jo et al. 2020b). In Gwangneung Forest, the average annual temperature has been 11.8 °C for 60 years with an average annual precipitation of 1,387.3 mm. The precipitation is the highest in July and August with the lowest precipitation in December and January (Jo et al. 2020b).

Research in Gwangneung Forest has been mainly conducted on flora and climate, as well as the distribution of various species of insects, birds, mushrooms, and reptiles in the forest. Surveys on lichens in Gwangneung Forest have only partially identified the lichen flora distribution; however, no report on the entire lichen flora in Gwangneung Forest has been made (Byun et al. 2005, 2007; Lee et al. 2006; Kwon et al. 2008, 2009, 2012; Kim et al. 2011; Kim et al. 2016, 2017; Wang et al. 2017).

Lichens are symbiotic organisms consisting of fungi and algae. In the 2000s, Korean and foreign researchers started researching lichens in South Korea, and a list of lichens in South Korea was published for 510 species in 2005, and 788 species in 2013 (Hur et al. 2005; Moon 2013). Lichens are classified into crustose, foliose, and fruticose according to their forms, and each form is slightly different depending on the environment in which the lichen grows. As is the case in most fungi, the vast majority of lichenized ascomycetes have a sexual (generative reproduction; apothecia, perithecia) and an asexual (vegetative reproduction; soredia, isidia) life cycle (Nash 2008). In addition, algae determine

☆ Given his role as Subject Editor, Sang-Kuk Han had no involvement in the peer-review of this article. Full responsibility for the editorial process for this article was delegated to Bong-Kyu Byun.

* Corresponding author. Tel.: +82 31 540 8842
E-mail address: okkass15@korea.kr (SO Oh)

Peer review under responsibility of National Science Museum of Korea (NSMK) and Korea National Arboretum (KNA).

https://doi.org/10.1016/j.japb.2022.04.008
pISSN2287-884X eISSN2287-9544/

the nutrition of lichens in the presence of light and water, and exposure to pollutants leads them to die. However, toxitolerant species can survive in polluted environments better than other plants. For this reason, lichens are used as indicators of environmental pollution and are important species that can predict visible changes in the environment (Abas 2021). According to the Gwangneung Forest Survey Report published in 2020, a total of 23 families, 26 genera, and 36 species were identified as a result of surveying the lichens in Gwangneung Forest in 2010 and 2014 (Jo et al. 2020a, b). In 2010, 21 species in 16 families and 18 genera were reported, and species mainly found in the lowland forests of South Korea, such as *Bacidia subincompta*, *Lepraria lobificans*, *Lepraria texta*, *Myelochroa aurulenta*, *Phaeophyscia adiastola*, and *Porpidia albocaerulescens* were identified. In 2014, 30 species in 17 families and 20 genera were identified, and tropical lichens such as *Coenogonium luteum* and forest health indicator species such as *Myelochroa aurulenta* were identified (Jo et al. 2020a, b). These two surveys mainly focused on some viewing areas within the National Arboretum, and no detailed information about tree species or collection areas has been obtained. However, this survey aimed to provide the data accumulated from 2016 to 2021, detailed information listed from the collection area and tree species, the species distribution of lichens in Gwangneung Forest, and their relationship with the tree species. The goal was to investigate the environment and distribution of lichens in Gwangneung Forest in various ways, such as identifying structures of fruit bodies and types of propagules to investigate the reproductive activity of lichens in Gwangneung Forest and examining types of algae coexisting with the fungi that make up lichens. In this study, a survey was conducted on the entire area from the undisclosed research area around Suribong to the viewing area within the arboretum to monitor the health of Gwangneung Forest and promote efficient conservation and management of the forest through lichens (which are referred to as environmental indicator species) by identifying the lichen flora of Gwangneung Forest. The overall lichen flora in Gwangneung Forest was investigated as the first of a series of research goals.

Material and methods

Study site and morphological examination

The survey area included the 1,065 ha of land managed and operated by the National Arboretum, and survey and collection were conducted in the viewing and undisclosed areas of the arboretum and around Suribong (536 m) (Figure 1). Gwangneung Forest is geographically located in the central part of the Korean Peninsula (latitude of 37° 42′ to 37° 45′ north and longitude of 126° 00′ to 126° 02′ east), about 4 km from east to west, about 7 km from north to south, centered on Bongseonsacheon Stream, including Suribong Peak and Jukyeopsan Mountain, forming a gentle slope (Jo et al. 2020b). For the distribution survey and species identification, sampling was performed by visual inspection and a total of ten surveys were conducted: four surveys in 2016 (May, June, and August), one in 2018 (October), two in 2020 (May and September), and three surveys in 2021 (May, June, and July). As lichens grew with their thallus fixed on a tree or rock, they were collected by removing the lichen from the substrate using a bladed tool. Crustose lichens were mainly collected with a focus on their apothecium containing spores rather than the thallus, as the main features

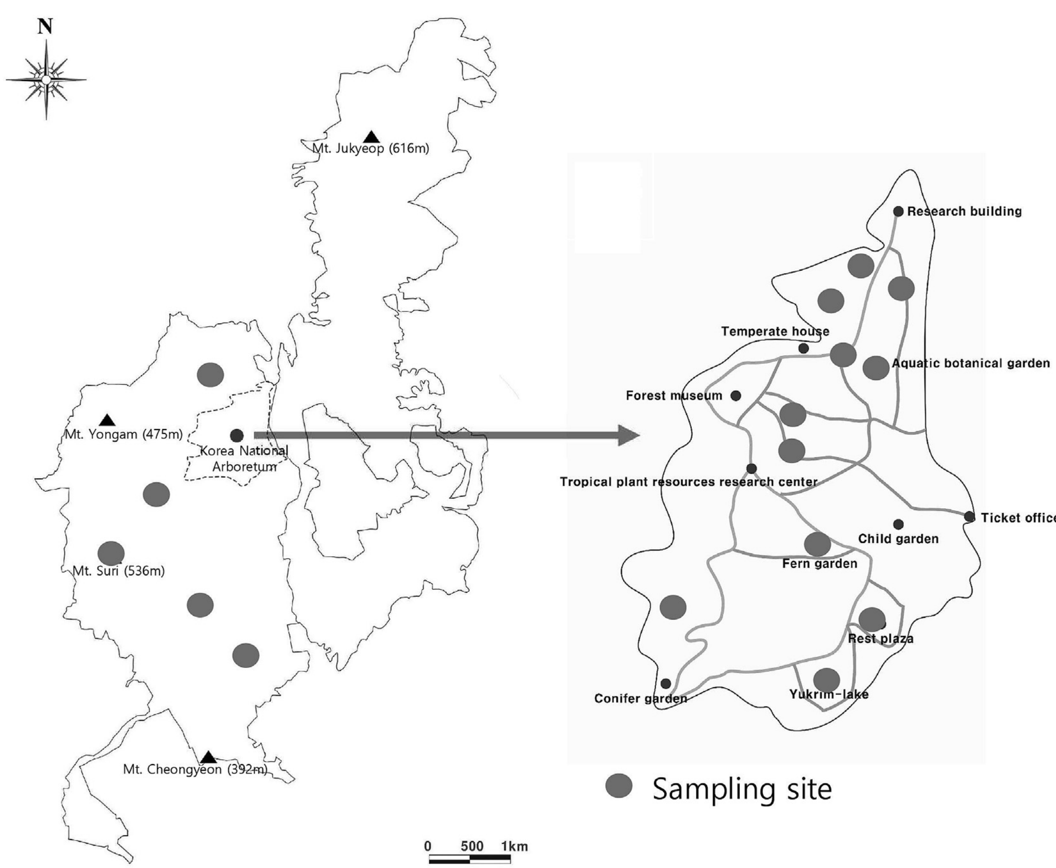

Figure 1. A map of Gwangneung Forest. The arrow shows a detailed map of Korea national arboretum. The gray circle indicates the sampling site.

for identification were contained in the apothecium. Foliose lichens were collected along with the substrate to prevent the overall shape from being scattered considering the structure of the lobe or the shape of the thallus. The collected voucher specimens are stored in the Korea National Arboretum Herbarium (KH). The Flora of Microlichen and Macrolichen in Korea was primarily used for lichen identification (Hur et al. 2016, 2017). As for the species that were suspected to be unreported, articles on each genus (Lauri et al. 2009; Luecking et al. 2009; Joshi et al. 2010, 2013; Tripp and Lendemer 2019) or lichen classification books (Brodo et al. 2001; Smith et al. 2009) were used to identify the species. A stereomicroscope (Olympus SZX7; Olympus, Tokyo, Japan) was used to examine the lichen morphology and a compound microscope (Olympus CX22LED; Olympus) was used to inspect the mycelial structure inside the apothecium or thallus. In order to identify the internal substances of each lichen, K (5% potassium hydroxide), C (aqueous solution of calcium hypochlorite), and P (paraphenylene diamine) solutions were used to examine the color reaction (chemical color test) (Orange et al. 2001). As for the genera *Lepraria* and *Leprocaulon*, which have the key for identification only in their secondary products, chemical identification was performed by thin-layer chromatography (TLC) (Orange et al. 2001; Elix 2014).

DNA extraction and PCR amplification

DNA extraction was performed mainly by using lichen thalli with apothecial discs. Samples were ground and extracted using the DNeasy plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. PCR amplifications were conducted using Amplitaq DNA polymerase (ThermoFisher, Waltham, MA, USA). The following primers were used for PCR amplifications: internal transcribed spacer (ITS) for ITS4 (Kretaer et al. 1996) and ITS5 (White et al. 1990). The following program was used for the amplification of ITS: initial denaturation for 4 min at 94 °C, followed by 30 cycles at 94 °C for 40 s, 52 °C for 40 s, 72 °C for 50 s, and then a final extension step at 72 °C for 8 min. Amplified DNA was concentrated and purified using a PCR quick-spin PCR Product Purification Kit (INTRON Biotechnology, Inc. Sungnam City, Korea) for sequencing analysis.

Sequence alignments and phylogenetic analysis

The obtained sequences were aligned with ClustalW ver. 1.83 (Thomson et al. 1994) and edited using the Bioedit program. Phylogenetic analyses were conducted using MEGA 7. In addition, we also performed a Maximum likelihood analysis using MEGA 7 with 1,000 bootstrap values was applied. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). We selected an outgroup for phylogenetic analysis according to previous reports (Lendemer 2020; Barcenas-Peña et al. 2021).

Results and discussion

Of the 246 specimens collected from Gwangneung Forest, 68 species of lichens were identified in 11 orders, 22 families, and 39 genera (Appendix A). Among them, foliose lichens, which were collected the most, accounted for 57% with 139 specimens, crustose lichens accounted for 41% with 102 specimens, and fruticose lichens accounted for 2% with five specimens (Figure 2A). The northern area within the viewing of the arboretum happened to be where most of the collection took place, where trees with a height of 4 to 5 m or less were planted to create a growing environment. Lichens were most commonly found on *Acer takesimense* and *Broussonetia x kazinoki* trees. In *Acer takesimense*, only crustose lichens (*Arthonia* sp., *Lecanora imshaugii*, and *Lecidella euphorea*) were distributed. In *Broussonetia* x *kazinoki*, crustose lichens (*Lecanora imshaugii* and *Lecidella euphorea*) as well as foliose lichens (*Candelaria asiatica*, *Parmotrema margaritatum*, *Phaeophyscia adiastola*, *Phaeophyscia limbata*, *Puntelia subrudecta*, and *Pyxine sorediata*) were distributed. Crustose and foliose lichens were also distributed in rocks and trees such as *Quercus serrata*, *Euonymus alatus*, *Euonymus alatus f. ciliatodentatus, Pourthiaea villosa*, and *Acer triflorum*.

In areas other than the viewing area, lichens were mainly distributed near Suribong Peak and the observatory. Suribong Peak has an altitude of 536 m, which is a highland of Gwangneung Forest, where 17 specimens were collected and 11 species were identified. Because this area is characterized by abundant light and high altitude, *Anaptychia isidiza*, which was collected from the highlands of Korea, and *Punctelia rudecta*, known as a species affected by the atmospheric environment, were distributed (Crespo et al. 2004). Almost no lichens were found in the northeast to south areas of the National Arboretum, which were undisclosed viewing areas, and lichens were distributed only in areas characterized by a low canopy and high airflow, such as the area near the observatory with few trees. In this area, *Phaeophyscia limbata*, a foliose lichen with good fertility due to its soredia, which can also be found in urban areas, was identified, along with seven types of crustose lichens. Almost no lichens were identified inside Gwangneung Forest, which was an area isolated for 550 years and had not been artificially reforested. Naturally grown trees forming a dense forest with a high canopy seemed to contribute to the scarcity of lichens. Such areas, with a stagnant flow of atmospheric environment, are considered unsuitable for the growth of lichens that need light for nutrient activity. However, although viewing areas have been through artificial reforestation, various lichens were found as a result of smooth airflow, low density, and low canopy in these areas.

As a result of comparing the previous survey with the list of previous reports, 10 species of lichens (*Bacidia schweinitzii*, *Biatora longispora*, *Cladonia subconistea*, *Myelochroa aurulenta*, *Parmotrema reticulatum*, *Phaeophyscia adiastola*, *Phaeophyscia exornatula*, *Phaeophyscia limbata*, *Porpidia albocaerulescens*, *Punctelia rudecta*) were identified as those that had been identified in 2010 and 2014. On the contrary, eight species (*Acarospora* sp., *Bacidia circumspecta*, *Bacidia subincompta*, *Caloplaca flavorubescens*, *Candelaria concolor*, *Cladonia chlorophaea*, *Myelochroa coreana*, *Phaeophyscia hirtella*) that had been collected in 2010 and 2014, were not identified in this study. However, the identification of *Bacidia circumspecta* and *Bacidia subincompta* may vary depending on the color change of the epihymenium during the identification process, and the identification of *Myelochroa coreana* and *Phaeophyscia hirtella* may be completely different depending on the location and presence of soredia. For *Candelaria concolor*, in particular, most of the individuals collected in South Korea are classified as a new species from 2018, *C. asiatica*, based on the classification and identification method using DNA with a high possibility of misidentification (Liu and Hur 2018). Through this survey, 54 new species were added to the list and various types of lichens were found to be distributed in Gwangneung Forest (Appendix B).

Lichen distribution by morphology and their reproductive strategy

In terms of reproduction, among crustose lichens, 56 individuals of 22 species reproduced using apothecia, and seven individuals of four species reproduced using perithecia. There were 39 individuals of eight species that underwent asexual reproduction using soredia or isidia (Figure 2B). In Gwangneung Forest, lichens reproducing by soredia accounted for more than 90% of the crustose lichens

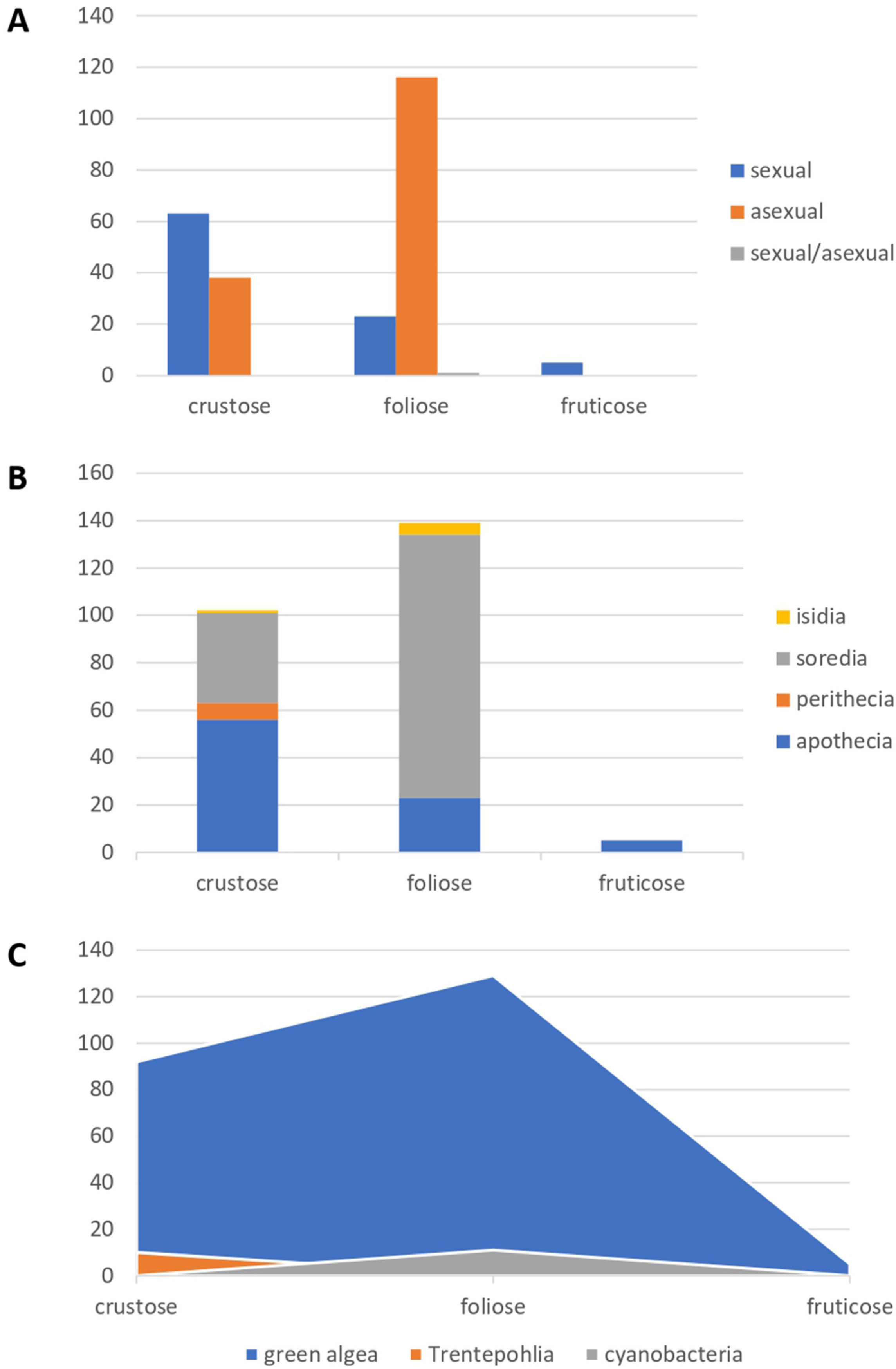

Figure 2. A, The number of specimens by lichen type; B, The number of specimens by reproductive strategy; C, The number of specimens by photobiont type. X-axis: lichen type, Y-axis: number of specimens.

reproducing by soredia or isidia, and all of them had green algae as symbiotic algae (Figure 2C). Among foliose lichens, 23 individuals of nine species reproduced using apothecia and 115 individuals of 20 species reproduced using soredia or isidia, suggesting that most of the foliose lichens in Gwangneung Forest reproduced by using soredia or isidia by simply spreading the mycelia mixed with algae, rather than using apothecia (Figure 2B). Among them, only five individuals of three species were identified as lichens that reproduced using isidia, and most of them were lichens that reproduced using soredia and had green algae. In addition, during asexual reproduction using isidia, two species had green algae as symbiotic algae, and only one species had cyanobacteria as symbiotic algae. Most of the foliose lichens in Gwangneung Forest were identified to have symbiotic algae like crustose lichens (Figure 2C). There were four species of fruticose lichens in the single genus of *Cladonia*, and all of them reproduced using ascomycetes and had green algae as their symbiotic algae (Figure 2B–C). Reproduction using soredia or isidia is one of the methods enabling rapid reproduction of lichens with thallus that has already been made, and it is often observed in lowlands, shaded areas, and urban areas (Moon 2012). Lichens adopting this reproductive strategy were the most commonly found throughout the entire viewing areas within the National Arboretum as well as the entire Gwangneung Forest area up to Suribong Peak including the undisclosed areas from the northeast to the south.

Based on these results, most crustose or foliose lichens of Gwangneung Forest have been found to reproduce using soredia or isidia, which calls for further discussions about the inflow of the lichens in the form of soredia at the beginning of the Gwangneung Forest and the environment of Gwangneung Forest in the future.

Lichen distribution by species

The most abundantly distributed and the most frequently collected species in Gwangneung Forest was *Candelaria asiatica* (12%) followed by *Phaeophyscia limbata* (10%) and *Myelochroa aurulenta* (9%), which were all foliose lichens (Figure 3). Among crustose lichens, *Leprocaulon nicholsiae* (6%) was the most abundant followed by *Lepraria cupressicola* (4%) and *Lecanora imshaugii* (3%). There were only four fruticose lichen species in the single genus *Cladonia*, among which *Cladonia subconistea* (1%) was the most abundant. In Gwangneung Forest, 10 species of lichens (*Candelaria asiatica* (29), *Phaeophyscia limbata* (24), *Myelochroa aurulenta* (21), *Leprocaulon nicholsiae* (16), *Phaeophyscia adiastola* (10), *Lepraria cupressicola* (9), *Lecanora imshaugii* (8), *Bacidina egenula* (7), *Anaptychia isidiata* (6), and *Bacidia arceutina* (5)) accounted for 55% of the total lichen flora distribution, with the rest 58 species accounting for 45%. All three lichens, *Candelaria asiatica, Phaeophyscia limbata,* and *Myelochroa aurulenta*, which accounted for the largest part of the flora distribution, were found to reproduce using soredia. Foliose lichens (57%) accounted for most of the lichens spread in this way, and it was the selective reproductive method of lichens that was observed most commonly in Gwangneung Forest.

Lichen distribution by tree

The tree with the largest number of lichens was *Koelreuteria paniculata* (5% with 12 species), followed by *Sophora japonica*, *Ginkgo biloba*, and *Malus sieboldii* (4% each with 11 species). Many lichens were observed in these trees, and *Candelaria asiatica*, *Myelochroa aurulenta*, and *Phaeophyscia limbata*, which were most widely distributed in arboretums, were also identified. In addition, more crustose lichens than foliose lichens were distributed on trees belonging to *Aceraceae* such as *Acer palmatum* (4%), *Acer takesimense* (4%), and *Acer pseudosieboldianum* (3%), and *Lecanora imshaugii* was the most common species among the collected lichens. This species is known to be found on *Cornus controversa* and *Quercus robur*, but in Gwangneung Forest, almost all individuals had been found on trees belonging to *Aceraceae*, as well as *Ilex serrata* and *Liriodendron tulipifera* (Brodo 1984). Most studies on lichens and trees have been conducted by examining the kind of lichens growing in a certain forest and their relationship (Uliczka and Angelstam 1999; Loppi and Frati 2004; Johansson et al. 2007). In particular, the water-

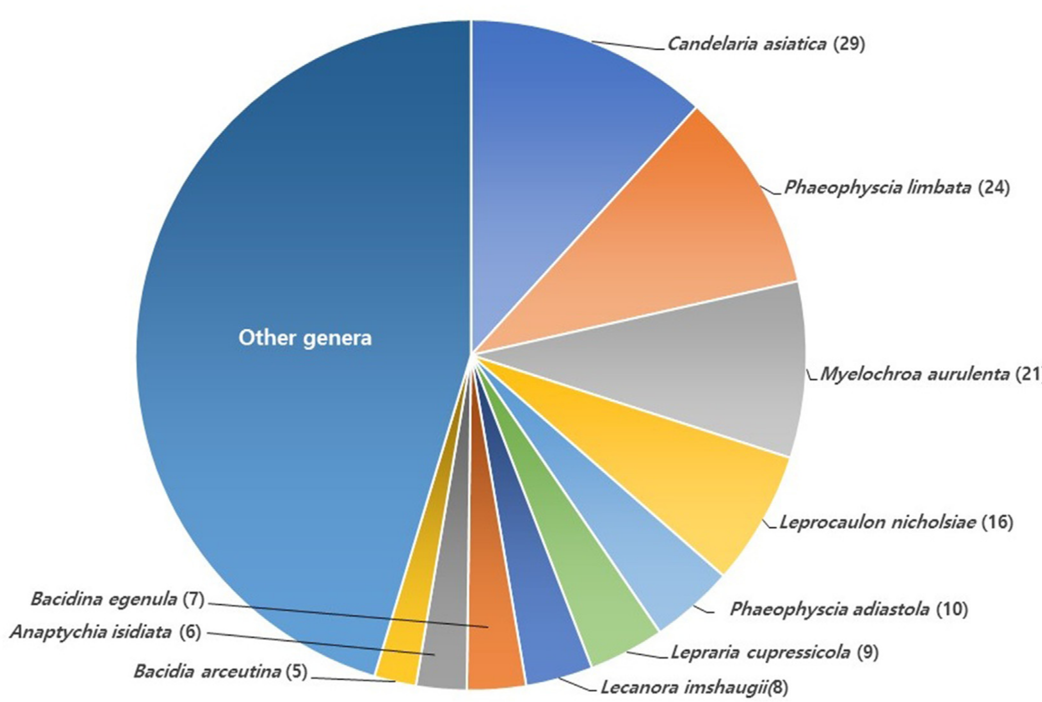

Figure 3. proportion of collected species in Gwangneung Forest.

holding capacity and pH of a tree are known as the factors that greatly affect the species and number of lichens in a tree (Loppi and Frati 2004). The trees with many lichens in the arboretum may have had such characteristics affecting the growth of lichens. However, because most of these studies have been conducted with only trees growing in Europe, it is difficult to apply these results to identify the relationship between lichens and native trees in South Korea. In future studies, research on the relationship between the growth of trees and the number of lichens may be conducted.

New records of Gwangneung Forest

In this study, three species were first discovered in Gwangneung Forest (Figure 4). In particular, the genus *Lepraria*, with 19 species reported in South Korea, is one of the most distributed lichens on rocks and trees in urban areas and lowlands. It is difficult to identify these lichens belonging to the genus *Lepraria* as they have undetermined shapes and are made up of soredia. As their identification keys are mostly classified as substances, their species names have not been properly identified. Through the survey of Gwangneung Forest in this study, one unreported species was identified as it did not match the classification and identification keys for the species in South Korea. In addition, it has been thought that *Leprocaulon* belongs to the fruticose lichen and can be easily distinguished morphologically due to its podetia. However, this survey revealed that what was once identified as *Lepraria* based on its structure turned out to be *Leprocaulon*, a crustose lichen, not *Lepraria* based on the classification and identification method using DNA, and it was observed to be distributed throughout the viewing areas in Gwangneung Forest. Crustose lichens belonging to the genus *Graphis* are classified according to their lirella shape and carbonized internal structure. In this Gwangneung Forest survey, one unreported species of the genus *Graphis* was found, and the descriptions of the three unreported species are as follows:

For *Lepraria* and *Leprocaulon*, from which ITS sequencing results could be obtained, an interspecies comparison was performed using the ITS barcode to determine the relationship of the unreported species with these species (Appendix C). *Lepraria cupressicola* had a very close relationship with *L. harrisiana*, and they were actually classified based on the absence of lecanoric acid (Barcenas-Peña et al. 2021). As species may change depending on the presence or absence of certain substances, the differences between species can also be identified by molecular biological methods (Figure 5). *Leprocaulon nicholsiae* is a relative of *Leprocaulon beechingii*. While both species contain usnic acid and zeorin, there are significant morphological differences between them, as *L. nicholsiae* has a

Figure 4. A, Habitat of *Leprocaulon nicholsiae*; B, An enlarged thallus of *L. nicholsiae*; C, Habitat of *Lepraria cupressicola*; D, An enlarged thallus of *L. cupressicola*; E, Habitat of *Graphis pinicola*; F, An enlarged thallus of *G. pinicola* <scale bar: 0.5mm (A); 2mm (B–C)>

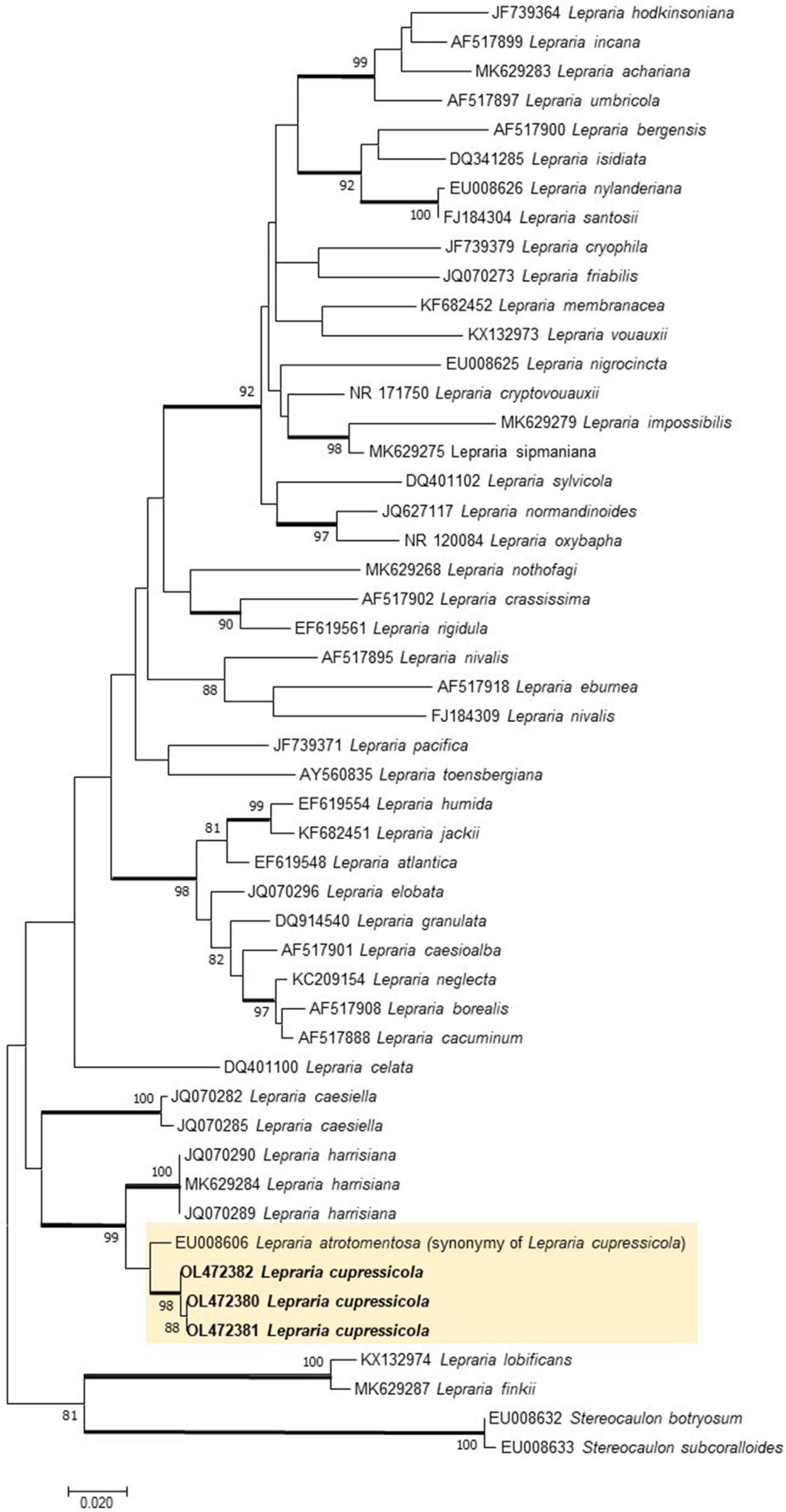

Figure 5. A phylogenetic tree is generated from internal transcribed spacer sequences (ITS). The numbers at the nodes represent the percentage of their occurrence in 1,000 bootstrap replicates. Only nodes supported by 80 or more bootstraps are shown. The clade of *Lepraria cupressicola* is marked by the yellow box.

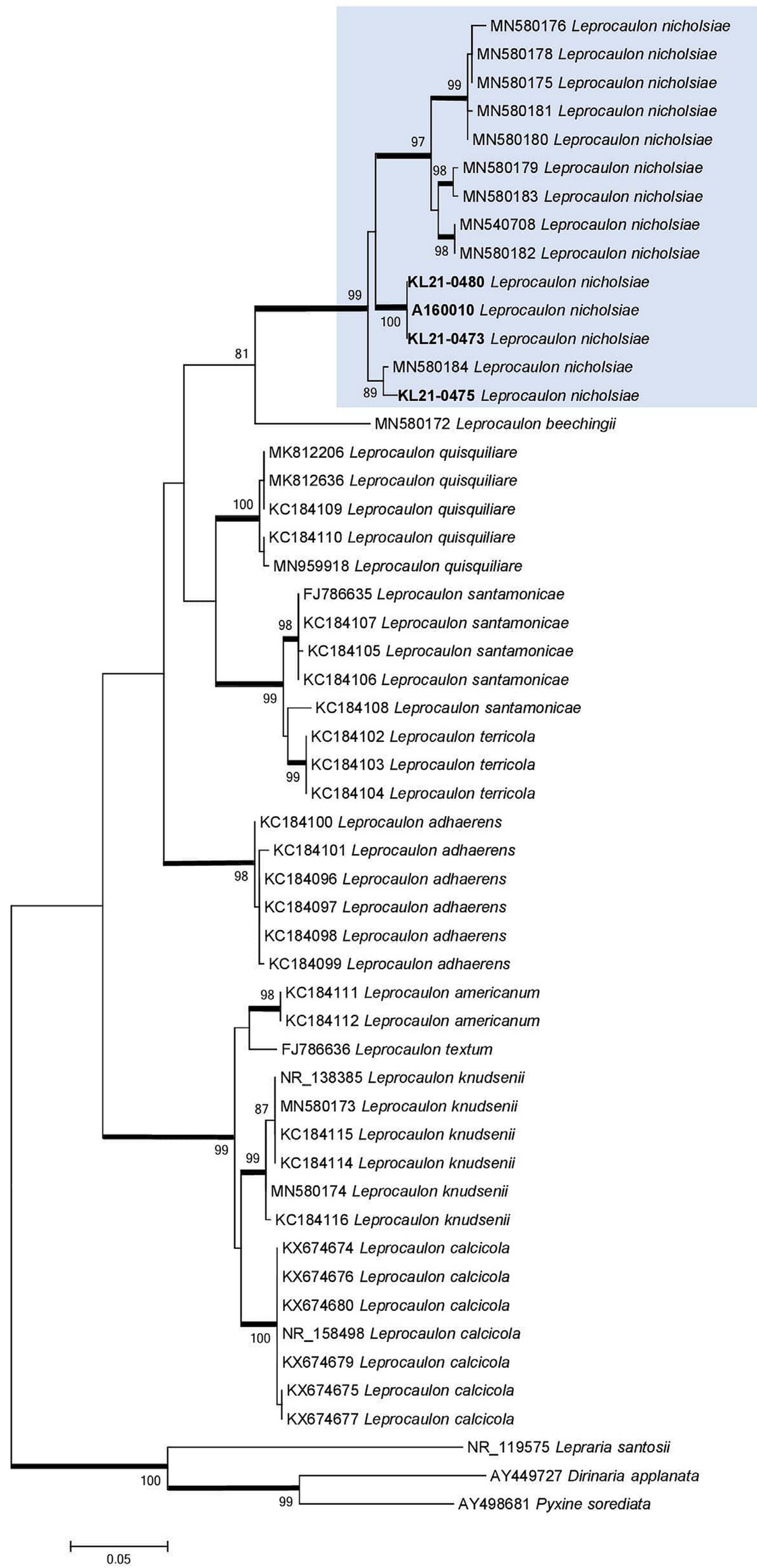

Figure 6. A phylogenetic tree is generated from internal transcribed spacer sequences (ITS). The numbers at the nodes represent the percentage of their occurrence in 1,000 bootstrap replicates. Only nodes supported by 80 or more bootstraps are shown. *Leprocaulon nicholsiae* is marked by the blue box.

placodioid thallus with distinctly crisped margins, whereas *L. nicholsiae* has dispersed piles of granules (Lendemer 2020). A molecular biological comparison clearly revealed that the two species are physically similar but morphologically different (Figure 6).

1. ***Lepraria cupressicola*** (Hue) Laundon, 2008
= *Lepraria atrotomentosa* Orange & Wolseley 2001
(Figures 4 and 5)

Description. Thallus crustose, leprose, powdery, diffuse, poorly delimited, greenish grey, diffused thallus having distinguished margin or poorly developed. Prothallus absent. Hypothallus, thin to thick, forming a thin continuous to discontinuous layer underneath the granules and extending outward from the edge of the margin. Rhizohyphae present, pale to black, Granules globose, aggregate, fine to medium, 50–80 μm diameter, abundant. Photobiont green, spherical, 7.5–12 μm diameter.

Material examined. Pocheon-si, Gyeonggi-do, 27 vii 2021 (SO Oh & JS Park), KL21-0472 (KHL0035543), KH.

Chemistry. K+ yellow, C+ pink, KC−, Pd+ pale yellow; atranorin, zeorin, lecanoric acid were detected in TLC.

Ecology and distribution. On siliceous rock or bark. Japan, China, Sri Lanka. New to Korea.

Remarks. Lepraria pallida is morphologically similar to this species and L. harrisiana also similar but does not produce lecanoric acid.

2. ***Leprocaulon nicholsiae*** Lendemer & Tripp, 2018
(Figures 4 and 6)

Description. Thallus crustose, leprose, aggregate-type, not stratified, initially composed of spherical granules that eventually merging to form a flat thin crust (0.1–0.2 mm thick), yellowish-green in color. Hyphae hyaline, 2–2.5 μm wide, septate. Prothallus persistent. Hypothallus absent. Rhizohyphae absent. Granules globose, 62.5–75 μm in diameter, ecorticate. Photobiont green, coccoid, cells globose, 7.5–12.5 μm wide.

Material examined. Pocheon-si, Gyeonggi-do, 2 v 2016 (SO Oh & SG Han), A160010 (KHL0035546), KH.

Chemistry. Thallus K−, C− KC+ yellow, P-; usnic acid, and zeorin were detected in TLC.

Ecology and Distribution. On siliceous rock or bark, USA, Canada. New to Korea.

Remarks. Leprose crustose species is morphologically similar to *Lepraria*. Recently, *Lepraria texum* was treated in *Leprocaulon* based on molecular data (Lendemer and Hodkinson 2013) as *Leprocaulon texum*. This species also has usnic acid, zeorin and atranoin, but *L. nicholsiae* differs by lacking atranorin.

3. ***Graphis pinicola*** Zahlbr., 1930
(Figure 4)

Description. Thallus corticolous, epiperidermal, crustose, continuous, smooth, dull, greyish-white, 100–120 μm thick in cross-section. Cortex layer is indistinct to 10–15 μm. Algal layer is 60–70 μm. The medulla is indistinct. Prothallus is indistinct. Ascomata lirelliform, erumpent. Lirellae is dispersed, 1–1.5 mm in length, black; covered by lateral thalline margin, short to elongate and irregularly branched, labia entire, epruinose. Disc open to closed, surrounded by a lateral thalline margin (*lineola*-morph). Proper exciple is laterally carbonized, 25–60 μm wide. Epihymenium is 20–25 μm high. Hymenium is clear, up to 100 μm high. Paraphyses 1–2 μm thick. Hypothecium hyaline, indistinct to 25 μm high. Ascus clavate, 8-spored. Ascospores hyaline, ellipsoidal, transversely 5–8 septate, 20–35 × 5–7 μm.

Material examined Pocheon-si, Gyeonggi-do, 21 ix. 2020 (JS Park), KL20-0567 (KHL0032390), KH.

Chemistry. Thallus K−, P−, C−, KC−; no lichen substances were detected in TLC.

Ecology and Distribution. On bark, China. New to Korea

Remarks. Graphis pinicola has short lirellae and sparsely to irregularly branched. This species is close to *G. scripta* but it has distinct pruinose on exposed disc.

Conclusions

As a result of collecting and identifying 246 specimens through 10 Gwangneung Forest surveys from 2016 to 2021, 68 species of lichens in 39 genera, 22 families, and 11 orders were identified. This study included an overall survey of undisclosed areas of Gwangneung Forest. Various types of lichens were investigated for their host tree species. Of the 246 lichens collected in Gwangneung Forest, 139 individuals were foliose lichens, and 116 individuals of 20 species were found to reproduce by using soredia. In Gwangneung Forest, 10 species of lichens accounted for 55% of the total, with most of them distributed in Gwangneung Forest, while 58 species of lichens made up the rest 45%. The tree with the largest number of lichens was *Koelreuteria paniculata*, followed by *Sophora japonica*, *Ginkgo biloba*, and *Malus sieboldii*. In trees belonging to *Aceraceae*, crustose lichens rather than foliose lichens were mainly found. Through this survey of Gwangneung Forest, three unreported species were found and a detailed description of each species was made. This survey was an omnidirectional survey of the entire Gwangneung Forest, and the first survey was conducted on the preservation of lichens in the Gwangneung Forest. Results of this survey may lead to further discussions on various topics regarding the conservation of Gwangneung Forest based on the changes in the number of species of lichens, changes in ecology, and changes in reproductive methods. Lichens have been used for a long time as environmental indicator species, and various aspects of lichens could be used as an axis of conservation in the preservation of Gwangneung Forest.

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.

Acknowledgments

The authors are grateful to Ji-seon Moon, Yun-ju Jo, and Jun-yeong Kim for assistance in DNA sequencing as well as Jeong-ju Seo for help in specimen collection. This work was conducted in accordance with “Research and classification of Higher fungi and lichens for conserving protected trees and stone cultural heritage” and funded by the Korea National Arboretum (KNA1-1-22, 17-2).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.japb.2022.04.008.

References

Abas A. 2021. A systematic review on biomonitoring using lichen as the biological indicator: A decade of practices, progress and challenges. *Ecological Indicators* 121:107–197.

Barcenas-Peña A, Dias R, Grewe F, et al. 2021. Contributions to the phylogeny of *Lepraria* (Stereocaulaceae) species from the Southern Hemisphere, including three new species. *The Bryologist* 124:494–505.
Brodo IM. 1984. The North American species of the *Lecanora subfusca* group. *Beihefte zur Nova Hedwigia*:63–185.
Brodo IM, Sharnoff SD, Sharnoff S. 2001. *Lichens of north America*. Yale University Press.
Byun BK, Lee YJ, Weon GJ, et al. 2005. Butterfly fauna of the Gwangneung forest, Korea. *Journal of Asia-Pacific Entomology* 8:199–210.
Byun BK, Kwon TS, Weon GJ, et al. 2007. Occurrence of *Callipogon relictus* Semenov (Coleoptera: Cerambycidae) in the Gwangneung forest, Korea with suggestions for the conservation. *Korean Journal of Applied Entomology* 46:19–25.
Crespo A, Divakar PK, Argüello A, et al. 2004. Molecular studies on *Punctelia* species of the Iberian Peninsula, with an emphasis on specimens newly colonizing Madrid. *The Lichenologist* 36 (5):299–308.
Elix JA. 2014. *A catalogue of standardized chromatographic data and biosynthetic relationships for lichen substances*. Canberra: The Author.
Hur JS, Koh YJ, Harada H. 2005. A checklist of Korea lichens. *The Japanese Society for Lichenology* 4:65–95.
Hur JS, Oh SO, Han SG. 2016. *Flora of Macrolichen in Korea*. Korea National Arboretum.
Hur JS, Oh SO, Park JS, et al. 2017. *Flora of Microlichens in Korea*. Korea National Arboretum.
Jo YC, Han SG, Kim IG, et al. 2020a. *Report on Plants, Animals, and Fungi of Gwangneung Forest*. Gwangneung Forest Biosphere Reserve Center, Korea National Arboretum.
Jo YC, Han SG, Jeong SH, et al. 2020b. *Annual report on Gwangneung forest and Experimental forest*. Gwangneung Forest Biosphere Reserve Center, Korea National Arboretum.
Johansson P, Rydin H, Thor G. 2007. Tree age relationships with epiphytic lichen diversity and lichen life history traits on ash in southern Sweden. *Ecoscience* 14: 81–91.
Joshi Y, Wang XY, Koh YJ, et al. 2010. The lichen genus *Lepraria* (Stereocaulaceae) in South Korea. *Mycotaxon* 112:201–217.
Joshi S, Jayalal U, Oh SO, et al. 2013. New Records and an Annotated Key for the Identification of *Graphis* Adans. in South Korea. *Mycobiology* 41:73–76.
Kim CS, Nam JW, Jo JW, et al. 2016. Studies on seasonal dynamics of soil-higher fungal communities in Mongolian oak-dominant Gwangneung forest in Korea. *Journal of Microbiology* 54:14–22.
Kim CS, Han SK, Nam JW, et al. 2017. Fungal communities in a Korean red pine stand, Gwangneung Forest, Korea. *Journal of Asia-Pacific Biodiversity* 10:559–572.
Kim DI, Lee JH, Kim IH, et al. 2011. Herpetofauna and distribution of each species in Gwangneung Forest. *Korean Journal of Herpetology* 3:1–9.
Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.
Kretaer A, Li Y, Szaro T, et al. 1996. Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications. *Mycologia* 88:776–785.
Kwon TS, Byun BK, Kang SH, et al. 2008. Analysis on Changes, and Problems in Phonology of Butterflies in Gwangneung Forest. *Korean Journal of Applied Entomology* 47:209–216.
Kwon TS, Byun BK, Lee BW, et al. 2009. Estimation of species richness of butterfly community in the Gwangneung forest, Korea. *Korean Journal of Applied Entomology* 48:439–445.
Kwon YS, Park SK, Hwang GY, et al. 2012. Bird distribution in relation to forest types in Gwangneung forest. *Journal of Forest and Environmental Science* 28:137–141.
Lauri S, Andres S, Randlane T. 2009. World survey of the genus *Lepraria* (Stereocaulaceae, lichenized Ascomycota). *The Lichenologist* 41:25–60.
Lee KH, Yeo KT, Jung SS. 2006. The distribution and life-form of ferns in Gwangneung forest. *Korean Journal of Plant Resources* 19:592–597.
Lendemer JC, Hodkinson BP. 2013. A radical shift in the taxonomy of *Lepraria* sl: molecular and morphological studies shed new light on the evolution of asexuality and lichen growth form diversification. *Mycologia* 105:994–1018.
Lendemer JC. 2020. *Leprocaulon beechingii* (Leprocaulaceae), a new species from the southern Appalachian Mountains of eastern North America. *The Bryologist* 123: 1–10.
Liu D, Hur JS. 2018. *Candelaria asiatica*, an ignored new species from South Korea. *Mycobiology* 46 (4):305–310.
Loppi S, Frati L. 2004. Influence of tree substrate on the diversity of epiphytic lichens: comparison between Tilia platyphyllos and Quercus ilex (Central Italy). *The Bryologist* 107:340–344.
Luecking R, Archer AW, Aptroot A. 2009. A world-wide key to the genus *Graphis* (Ostropales: Graphidaceae). *The Lichenologist* 41:363–452.
Moon KH. 2012. *What is lichen?*. Seoul: GEOBOOK.
Moon KH. 2013. *Lichen-forming and lichenicolous fungi of Korea*. National Institute of Biological Resources.
Nash TH. 2008. *Lichen biology*. Cambridge University Press.
Orange A, James PW, White F. 2001. *Microchemical methods for the identification of lichens*. British Lichen Society.
Smith CW, Aptroot A, Coppins BJ, et al. 2009. *Lichens of Great Britain and Ireland*. British Lichen Society.
Thomson JD, Hoggins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.
Tripp EA, Lendemer JC. 2019. Highlights from 10+ years of lichenological research in Great Smoky Mountains National Park: Celebrating the United States National Park Service Centennial. *Systematic Botany* 44:943–980.
Uliczka H, Angelstam P. 1999. Occurrence of epiphytic macrolichens in relation to tree species and age in managed boreal forest. *Ecography* 22:396–405.
Wang JH, Kim JE, Chol JK, et al. 2017. The fish community and habitat analysis in the Bongseonsa stream within Gwangneung forest, South Korea. *Korean Journal of Environmental Biology* 35:105–115.
White TJ, Bruns T, Lee SJWT, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*.