An Easy, Rapid, and Cost-Effective Method for DNA Extraction from Various Lichen Taxa and Specimens Suitable for Analysis of Fungal and Algal Strains

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Abstract  Lichen studies, including biodiversity, phylogenetic relationships, and conservation concerns require definitive species identification, however many lichens can be challenging to identify at the species level. Molecular techniques have shown efficacy in discriminating among lichen taxa, however, obtaining genomic DNA from herbarium and fresh lichen thalli by conventional methods has been difficult, because lichens contain high proteins, polysaccharides, and other complex compounds in their cell walls. Here we report a rapid, easy, and inexpensive protocol for extracting PCR-quality DNA from various lichen species. This method involves the following two steps: first, cell breakage using a beadbeater; and second, extraction, isolation, and precipitation of genomic DNA. The procedure requires approximately 10 mg of lichen thalli and can be completed within 20 min. The obtained DNAs were of sufficient quality and quantity to amplify the internal transcribed spacer region from the fungal and algal lichen components, as well as to sequence the amplified products. In addition, 26 different lichen taxa were tested, resulting in successful PCR products. The results of this study validated the experimental protocols, and clearly demonstrated the efficacy and value of our KCl extraction method applied in the fungal and algal samples.

Keywords  Lichens, Fungi, Algae, Genomic DNA, rRNA, Sequencing

Over 19% (approximately 14,000 species) of all known fungi are lichenized [1], and it is estimated 8% of terrestrial ecosystems are covered by lichens [2]. Lichens are mutualistic organism; a composite of a fungus and photosynthetic partner, which can be an alga, cyanobacterium, or both. The organisms exhibit a broad geographic and ecological distribution, from tropical to polar regions worldwide [3]. Lichens have shown utility in the medical community. Metabolites produced by some species exhibit broad-spectrum antibiotic, anti-tumor, and antioxidant activities [4]. Molecular biology approaches are now well-developed, and applied to address a broad range of scientific questions. Morphology-based lichen identification for biodiversity, conservation, phylogenetic, and population level studies, among others is required in conjunction with studies using molecular marker-based information, including internal transcribed spacer (ITS), large subunit RNA (LSU), small subunit RNA (SSU), and other regions that evolve at desired evolutionary rates [5-8]. Therefore, PCR and DNA sequencing are routinely employed to examine sequence information among lichen taxa [9].

However, obtaining genomic DNA from lichens has been challenging due to high protein and polysaccharide levels, and tough cell walls [10]. Furthermore, because lichens typically grow very slowly, inadequate fresh material cannot always be field collected for some species. A number of different DNA isolation methods have been developed for from herbarium and fresh lichen material [6, 11-14]. In general, highly sophisticated methods for lichen cell wall disruption, and expensive commercial kits have been applied to obtain genomic DNA. However, for identification purposes, PCR-quality genomic DNA should be suitable for amplification, which ranges from 500 bp to 1,500 bp.

For these purposes, a rapid, easy, and inexpensive method to extract PCR-quality DNA from various lichen taxa...
and specimens (fresh and preserved) is necessary. In a previous study, we developed a DNA extraction method for fungi, including *Magnaporthe oryzae*, *Fusarium* spp., and *Phytophthora* spp., which we successfully applied to five lichen species [15]. Although the method is relatively easy and applicable, only a small number of lichens were examined. In the present study, we improved the method, which we applied to 12 herbarium species, represented by 113 samples, and 26 field collected fresh lichen species. The objective of this study was to develop a rapid, reliable, and inexpensive protocol for DNA extraction suitable for PCR and DNA sequencing in a range of lichen taxa. A critical step in this procedure was breakage and cell lysis due to the rigidity of lichen cell walls.

**MATERIALS AND METHODS**

**Specimens: fresh and preserved lichen collections.** All the lichen thalli applied in this study were obtained from the Korean Lichen Center (KoLRI) at Sunchon National University (Sunchon 540-742, Korea). Material from 12 different species, i.e., 113 samples representing the species was obtained from herbarium specimens (Supplementary Table 1). In addition, twenty-six different species was obtained from fresh material (Supplementary Table 2).

**Reagents and solution.** KCl extraction buffer: 100 mM Tris·HCl (pH 8.0), 10 mM EDTA (pH 8.0), 1 M KCl; 1× TE buffer: 10 mM Tris·HCl (pH 8.0), 1 mM EDTA (pH 8.0); Chloroform; Isopropanol (chilled); Ethanol 70%; 3 M NaCl.

**DNA isolation.** Schematic diagram was presented in Fig. 1.

1) Place 10–100 mg of lichen thallus in a XXTuff Reinforced Microvial (Biospec, Bartlesville, OK, USA). Add three or four 2.5-mm sterile glass beads (Daihan Scientific, Seoul, Korea) to the sample; put the microvial in liquid nitrogen; disrupt the sample with a Mini-Beadbeater-24

![Fig. 1. Schematic diagram of the DNA extraction method.](image-url)
(Biospec Products) for 30 sec; repeat the procedure, i.e., put the microvial in liquid nitrogen to the sample; and repeat sample disruption until the sample forms a fine powder.

2) Add 300 µL of KCl extraction buffer to the sample, and invert strongly by hand approximately 20 times; add 300 µL chloroform, and invert gently approximately 20 times.

3) Centrifuge sample for 1 min at 12,000 rpm at room temperature (RT). Transfer upper aqueous layer to a new 1.5-mL microcentrifuge tube, and add 180 µL (60% total volume) of chilled isopropanol. Mix by very gentle inversion.

4) Centrifuge for 1 min at 12,000 rpm at RT. Discard the supernatant. Dry the pellet at 50~65°C dry oven or heat block for 5 min. Resuspend the pellet in 100 µL of TE buffer (1×) at 50~65°C dry oven or heat block for 5 min. Purified DNA may be stored at 4°C or −20°C.

Optional step to remove high-concentration polysaccharides: Add 1 M NaCl (final concentration) to 100 µL of resuspended DNA. Mix by gentle inversion. Add 60% total volume of chilled isopropanol. Mix by very gentle inversion. Proceed with step 4.

Determination of DNA yield and quality. The quality and quantity of extracted DNAs were measured using an Epoch Multi-Volume Spectrophotometer System (BioTek, Winooski, VT, USA).

PCR amplification and DNA sequencing. We tested the efficacy of various rRNA regions, which were amplified from the extracted DNAs and primer pairs listed in Supplementary Table 3 using the i-StarMAXII PCR master mix system (iNTRON Biotechnology, Seongnam, Korea), or the AccuPower PCR PreMixs (Bioneer, Seoul, Korea). A Takara PCR thermal cycler MP (Takara, Tokyo, Japan), or a 96-well GenAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) was used in a regular 30 cycle PCR reaction. Amplified PCR products were purified using MEGAquick-spin Total Fragment DNA Purification Kit (iNTRON Biotechnology) prior to sequencing, and forward and reverse strands were sequenced using the same PCR amplification primers. The generated sequences of fungi and algae from lichen were submitted to GenBank (http://www.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

Twelve lichen species were selected as representatives to compare DNA yield and quality between the KCl method we developed for lichens, and a general commercial DNA extraction kit (DNeasy Plant Mini Kit; Qiagen, Valencia, CA, USA). Genomic DNA yield and quality is provided in Table 1. Our method resulted in relatively high DNA concentrations, ranging from 54~566 ng/mg (average, 192.3 ng/mg) in lichen samples, whereas comparatively lower DNA concentrations were detected from the commercial kit, ranging from 11 to 500 ng/mg (average, 102.6 ng/mg) (Table 1). Therefore, our method outperforms standard commercial DNA extraction kits, resulting in higher DNA yield from small amounts of lichen tissue. The highest DNA concentration was obtained from Punctelia subflava using our method, which was coincidentally one of the two lowest generated using the commercial kit (11 ng/mg and 12 ng/mg, respectively) (Table 1). Moreover, the A260/280 ratio ranged from 1.5 to 2.1 using both methods, indicating low protein contamination. However, the average A260/280 ratio for our manual method showed slightly higher quality (1.88) compared to the commercial kit (1.78) (Table 1).

DNA suitability for PCR amplification was verified by examining several primer sets. The PCR products generated using LSU and SSU are shown in Fig. 2A and 2B, respectively.

| Table 1. Yield and quality of DNA from twelve representative lichen species |
|-----------------------------|---------------------|---------------------|
| KoRLI No. | Lichen species | KCl method DNA conc. (ng/mg) | A\text{260/280} | Commercial kit DNA conc. (ng/mg) | A\text{260/280} | GenBank accession No. |
| | | | | | |
| 007239 | Flavoparmelia caperata | 76 | 1.5 | 16 | 1.5 | KM207200 KM207205 |
| 007742 | Heterodermia diademata | 94 | 2.0 | 35 | 1.8 | KM207201 KM207206 |
| 000978 | Heterodermia hypoleuca | 60 | 1.9 | 63 | 1.7 | - - |
| 009659 | Lobaria discolor | 58 | 1.8 | 83 | 1.7 | - - |
| 008278 | Lobaria retigera | 314 | 2.1 | 213 | 1.8 | - - |
| 001899 | Myelochroa entotheciaochroa | 70 | 1.8 | 57 | 1.8 | - - |
| 011648 | Myelochroa irregosa | 92 | 1.9 | 100 | 1.5 | KM207202 KM207207 |
| 011592 | Parmotrema tinctorum | 54 | 1.8 | 70 | 1.9 | - - |
| 016180 | Peltigera polydactylon | 354 | 2.0 | 508 | 1.8 | - - |
| 016294 | Peltigera praetextata | 364 | 2.0 | 71 | 1.9 | - - |
| 007637 | Punctelia subflava | 566 | 2.0 | 12 | 1.8 | KM207203 KM207208 |
| 007349 | Umbilicaria esculenta | 206 | 1.8 | 11 | 2.1 | KM207204 KM207209 |
| Average | | 192.3 | 1.88 | 102.6 | 1.77 | |

*Not performed sequencing analysis after amplification of internal transcribed spacer regions.*
DNA was extracted from lichen thalli, which is composed of a fungus and its photobiont alga. Piercey-Normore and DePriest [16] developed specific primer sets to selectively amplify fungal and algal ITS regions, respectively. We adopted specific primer sets to compare our KCl method, and the commercial kit. Identical PCR products were obtained from both methods in fungal (Fig. 2C, upper) and algal ITS regions (Fig. 2C, lower).

Furthermore, five amplified PCR products generated by the KCl method were selected for sequencing fungal and algal ITS regions (asterisks, Fig. 2C lower left). Successful sequencing results were obtained in the fungus and alga through direct sequencing without cloning. These sequences were registered in GenBank (Supplementary Table 1).

During DNA isolation, gelatinous DNA pellets were obtained from many species, which indicates high polysaccharide concentrations. All 12 DNAs from *Umbilicaria esculenta* (Supplementary Table 1) yielded gelatinous pellets, which were hard to dissolve in TE. Previous studies reported high-salts, such as NaCl, effectively remove plant polysaccharides [17, 18]. Therefore, polysaccharides were removed by applying 1 M NaCl (final concentration) to DNA dissolved in 100 mL TE; and subsequent DNA precipitation by addition of 0.6 volumes of isopropanol. Results showed
most polysaccharides were effectively removed; purified DNA was easily dissolved in TE, leaving the amplified target region for PCR. This result showed NaCl effectively remove lichen polysaccharides.

We also successfully applied this method to over 113 additional isolates, which included 12 different taxa (genera, species, and subspecific taxa) collected from 2002 to 2010 (Supplementary Table 1). The specific fungal and algal ITS region was successfully amplified (Fig. 3). In addition, our KCl method was applied to examine 50 samples from 26 different lichen taxa (Supplementary Table 2), amplifying a larger PCR product, which included the ITS region plus 26S rRNA (> 1.0 kb); successful PCR products were obtained (Fig. 4). Collectively, these results indicated that the KCl method was applicable to herbarium and field collected lichen specimens (Supplementary Table 1), but also varied lichen taxonomic levels (Supplementary Table 2, Fig. 4).

A simple and cost effective protocol for extracting lichen genomic DNA was proposed in this study. The KCl DNA extraction method described here permits extraction from a wide range of lichen species (Supplementary Tables 1 and 2), and amplification of primary molecular clock-related genes [9], including ITS, LSU, and SSU (Fig. 2). The KCl based DNA extraction method was originally designed as an easy and rapid method to PCR amplification in plants [19] and has since been widely employed in fungal DNA extraction, including successful use in *Magnaporthe* and *Fusarium* [15]. Unlike former protocols, we included a chloroform extraction step to increase DNA quality, which was effective in most lichen taxa (data not shown). Moreover, the addition of 1 M NaCl for high polysaccharide concentrations in lichens resulted in notable polysaccharide removal, and also increased DNA quality (data not shown).

Compared to existing methods [11-13], our KCl extraction approach exhibits several advantages; a very low learning curve, and cost effectiveness, which include materials and time. First, the KCl method does not involve complicated processes, expensive reagents, and low temperature centrifugation. Therefore, the method is easy to learn, and applicable for a general laboratory. Second, the time required for DNA extraction is short; for example, it is possible to process over 100 samples in 1–2 hrs. This is primarily due to the absence of an incubation time with lysis buffer, and a short centrifugation time. Furthermore, it is possible to pause (or prolong) the isolation if necessary after the addition of isopropanol, for example overnight or longer. Third, the method requires a small amount of lichen thalli (< 10 mg). DNA yields (54–566 ng/mg) were higher than those previously obtained by sodium dodecyl sulfate (7–12 ng/mg), or cetyltrimethylammonium bromide (CTAB; 15–25 ng/mg), or defined CTAB method for lichen (> 25 ng/mg) [11]. The average DNA quality was also slightly higher than that observed from the commercial kit (Supplementary Table 3). Finally, our KCl method can reduce contamination potential because it excludes contact with contaminants such as mortar, pestle, and other equipment.

In summary, our KCl DNA extraction method demonstrated high efficacy in PCR amplification and sequence analysis from herbarium and fresh lichen materials across a wide range of lichen species, including the fungal and algal components. Therefore, the methodology provides a simple, cost effective, and readily available protocol suitable for use with various lichen materials to examine fungal and algal population dynamics, phylogenetic relationships, biodiversity, and conservation concerns.

**ELECTRONIC SUPPLEMENTARY MATERIAL**

Supplementary data including three tables can be found with this article online at http://www.mycobiology.or.kr/src/sm/mb-42-311-s001.pdf.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the Korea National Research Resource Center Program through the National Research Foundation of Korea (2012M3A9B8021726), Forest Science and Technology Projects (project S111212L030100) provided by the Korea Forest Service, the National Research Foundation of Korea grant funded by the Ministry of
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An Easy, Rapid, and Cost-Effective Method for DNA Extraction from Various Lichen Taxa and Specimens Suitable for Analysis of Fungal and Algal Strains

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http://www.mycobiology.or.kr/src/sm/mb-42-311-s001.pdf.

Supplementary Table 1. List of fungal taxa and herbarium collections from 2003 to 2012 used in this study

| Fungal taxa               | Year | KoLRI No.* | GPS                | Altitude (m) | Substratum |
|---------------------------|------|------------|--------------------|--------------|------------|
| *Flavoparmelia caperata*  | 2003 | 000146     | -                  | -            | -          |
|                           | 2003 | 000150     | -                  | 400          | Bark       |
|                           | 2003 | 000156     | -                  | 510          | Rock       |
|                           | 2004 | 002103     | 38°04'05.6" N, 128°26'58.8" E | 480          | Bark       |
|                           | 2004 | 002154     | 38°03'31.6" N, 128°26'41.6" E | 865          | Bark       |
|                           | 2004 | 002321     | 38°11'16.4" N, 128°21'42.7" E | 450          | Bark       |
|                           | 2007 | 007239     | 37°02'23.9" N, 128°42'55.3" E | 1,224        | Rock       |
|                           | 2007 | 007377     | 36°58'00.9" N, 128°30'31.1" E | 1,385        | Rock       |
|                           | 2010 | 012044     | 35°36'33.5" N, 127°39'65.8" E | 917          | Rock       |
|                           | 2010 | 012122     | 35°43'73.6" N, 127°46'07.8" E | 1,205        | Rock       |
|                           | 2010 | 012394     | 37°54'54.3" N, 127°58'57.7" E | 704          | Bark       |
| *Heterodermia diademata*  | 2003 | 000361     | -                  | 680          | Rock       |
|                           | 2003 | 000367     | -                  | 690          | Rock       |
|                           | 2003 | 000384     | -                  | 690          | Rock       |
|                           | 2004 | 002041     | 36°52'01.3" N, 128°06'34.3" E | ?-           | Rock       |
|                           | 2004 | 002121     | 38°03'46.7" N, 128°26'44.6" E | 680          | Bark       |
|                           | 2004 | 002304     | 38°11'16.4" N, 128°21'42.7" E | 450          | Rock       |
|                           | 2007 | 007741     | 34°45'39.1" N, 128°02'55.7" E | 251          | Rock       |
|                           | 2007 | 007742     | 34°45'39.1" N, 128°02'55.7" E | 251          | Rock       |
|                           | 2007 | 007744     | 34°45'39.1" N, 128°02'55.7" E | 251          | Rock       |
|                           | 2010 | 012611     | 37°40'51.7" N, 126°21'47.2" E | 287          | Rock       |
|                           | 2010 | 012639     | 37°14'68.5" N, 126°28'94.1" E | 5            | Rock       |
|                           | 2010 | 012650     | 37°14'68.5" N, 126°28'94.1" E | 5            | Rock       |
| *Heterodermia hypoleuca*  | 2003 | 000129     | -                  | 1,050        | Rock       |
|                           | 2003 | 000138     | -                  | 1,000        | Rock       |
|                           | 2003 | 000139     | 36°57'14.5" N, 128°29'18.8" E | -            | Rock       |
|                           | 2004 | 000968     | 35°48'28.5" N, 128°07'00.6" E | 965 (916)    | Rock       |
|                           | 2004 | 000978     | 35°48'54.6" N, 128°06'55.1" E | 1,145 (1,097) | Bark       |
|                           | 2004 | 000979     | 35°48'54.6" N, 128°06'55.1" E | 1,275 (1,236) | Rock       |
|                           | 2007 | 007289     | 36°52'52.5" N, 128°25'47.0" E | 1,106        | Rock       |
|                           | 2007 | 007296     | 36°52'38.4" N, 128°25'39.6" E | 1,163        | Rock       |
|                           | 2007 | 007328     | 36°50'45.5" N, 128°27'08.6" E | 1,008        | Rock       |
|                           | 2010 | 012364     | 37°54'79.7" N, 127°59'09.9" E | 685          | Rock       |
|                           | 2010 | 012385     | 37°54'93.1" N, 127°59'00.8" E | 714          | Rock       |
|                           | 2010 | 012404     | 37°54'93.0" N, 127°58'62.1" E | 672          | Rock       |
| *Lobaria discolor*        | 2003 | 000458     | 37°06'11.4" N, 128°55'55.8" E | 1,254        | Rock       |
|                           | 2003 | 000573     | 37°06'04.3" N, 128°57'04.3" E | 1,420        | Rock       |
|                           | 2003 | 000611     | -                  | 1,345        | Rock       |
|                           | 2004 | 001223     | 37°47'17.6" N, 128°33'10.6" E | 1,280 (1,265) | Rock       |
|                           | 2004 | 001234     | 37°47'24.4" N, 128°32'57.9" E | 1,410 (1,387) | Rock       |
|                           | 2004 | 001246     | 37°47'40.0" N, 128°32'42.7" E | 1,545 (1,535) | Rock       |
|                           | 2008 | 009656     | -                  | 800          | Bark       |
### Supplementary Table 1. Continued

| Fungal taxa | Year | KoLRi No.* | GPS | Altitude (m) | Substratum |
|-------------|------|-------------|-----|--------------|------------|
| **Lobaria retigera** | 2003 | 000419 | 35°29'44.3" N, 126°53'27.6" E | 1,000 | Bark |
| | 2003 | 000568 | 35°17'16.9" N, 128°28'47.1" E | 1,394 | Rock |
| | 2003 | 000606 | 36°56'53.7" N, 128°28'11.0" E | 1,325 | Rock |
| | 2004 | 001065 | 35°18'22.6" N, 127°34'49.6" E | 1,565 | Rock |
| | 2004 | 001070 | 35°18'41.6" N, 127°35'26.7" E | 1,560 | Rock |
| **Peltigera praetextata** | 2009 | 010382 | 37°51'29.2" N, 128°31'52.2" E | 706 | Soil |
| | 2009 | 010387 | 37°51'29.2" N, 128°31'52.2" E | 706 | Soil |
| **Myelochroa entotheiocha** | 2003 | 000509 | 35°17'36.2" N, 127°34'16.1" E | - | Rock |
| | 2003 | 000528 | - | - | Wood |
| | 2003 | 000540 | 36°56'45.5" N, 128°30'06.6" E | 1,006 | Rock |
| | 2004 | 001041 | 35°17'45.5" N, 127°33'02.9" E | 1,450 | Rock |
| **Myelochroa irugans** | 2003 | 000530 | - | - | Bark |
| | 2003 | 000532 | - | - | Bark |
| | 2004 | 001041 | 35°17'35.2" N, 127°32'49.9" E | 1,450 | Bark |
| | 2004 | 001047 | 35°17'45.5" N, 127°33'02.9" E | 1,440 | Bark |
| | 2004 | 001875 | - | - | Bark |
| | 2004 | 001899 | - | - | Bark |
| | 2007 | 007255 | 36°57'23.4" N, 128°25'47.3" E | 872 | Rock |
| | 2007 | 007281 | 36°52'52.9" N, 128°25'47.3" E | 1,110 | Rock |
| | 2007 | 007287 | 36°52'52.9" N, 128°25'47.4" E | 1,115 | Rock |
| **Parmotrema tinctorum** | 2003 | 000192 | - | - | Bark |
| | 2003 | 000193 | - | - | Bark |
| | 2004 | 000780 | 34°59'27.9" N, 127°20'01.8" E | 210 | Bark |
| | 2004 | 000822 | 35°29'40.4" N, 126°35'01.5" E | 37 | Bark |
| | 2007 | 007756 | 34°45'38.6" N, 128°02'54.0" E | 262 | Rock |
| | 2007 | 007757 | 34°45'38.3" N, 128°02'53.6" E | 280 | Rock |
| | 2010 | 011593 | 34°08'46.4" N, 126°33'00.3" E | 289 | Rock |
| | 2010 | 011648 | 34°08'46.4" N, 126°33'00.3" E | 368 | Rock |
| | 2010 | 011655 | 34°08'44.3" N, 126°32'85.0" E | 407 | Rock |
| **Peltigera polydactylon** | 2009 | 009875 | 33°22'77.5" N, 126°33'74.9" E | 1,200 | Moss |
| | 2009 | 009894 | 33°22'77.5" N, 126°33'74.9" E | 1,270 | Moss |
| | 2009 | 009962 | 33°22'77.5" N, 126°33'74.9" E | 1,200 | Moss |
| | 2012 | 016180 | 33°27'06.0" N, 126°32'02.0" E | 1,709 | Bark |
| | 2012 | 016184 | 33°27'06.0" N, 126°32'02.0" E | 1,709 | Bark |
| **Punctelia subflava** | 2004 | 000783 | 34°59'27.9" N, 127°20'01.8" E | 210 | Bark |
| | 2004 | 000868 | 35°29'40.4" N, 126°35'01.5" E | 37 | Bark |
| | 2007 | 007756 | 34°45'38.6" N, 128°02'54.0" E | 262 | Rock |
| | 2007 | 007757 | 34°45'38.3" N, 128°02'53.6" E | 280 | Rock |
| | 2010 | 011576 | 34°08'46.4" N, 126°33'00.3" E | 289 | Rock |
| | 2010 | 011592 | 34°08'46.4" N, 126°33'00.3" E | 368 | Rock |
| | 2010 | 011631 | 34°08'46.4" N, 126°33'00.3" E | 351 | Rock |
### Supplementary Table 1.

Continued

| Fungal taxa             | Year | KoLRI No.¹ | GPS                        | Altitude (m) | Substratum |
|-------------------------|------|------------|----------------------------|--------------|------------|
| **Umbilicaria esculenta** | 2003 | 000145     | -                          | 1,050        | Rock       |
|                         | 2003 | 000201     | -                          | 1,218        | Rock       |
|                         | 2003 | 000224     | -                          | 705          | Rock       |
|                         | 2004 | 000967     | 35°48'28.5" N, 128°07'00.6" E | 965 (916)    | Rock       |
|                         | 2004 | 000969     | 35°48'28.5" N, 128°07'00.6" E | 965 (916)    | Rock       |
|                         | 2004 | 000991     | 35°49'11.3" N, 128°07'18.2" E | 1,440 (1,396)| Rock       |
|                         | 2007 | 007305     | 36°52'20.4" N, 128°25'53.0" E | 1,207        | Rock       |
|                         | 2007 | 007349     | 36°57'51.3" N, 128°30'32.6" E | 1,232        | Rock       |
|                         | 2007 | 007500     | 36°55'39.3" N, 128°27'47.0" E | 1,238        | Rock       |
|                         | 2010 | 012025     | 35°36'33.5" N, 127°39'65.8" E | 917          | Rock       |
|                         | 2010 | 012063     | 35°36'61.4" N, 127°39'62.4" E | 903          | Rock       |
|                         | 2010 | 012117     | 35°43'73.6" N, 127°46'07.8" E | 1,205        | Rock       |

¹Lichen collection number of Korean Lichen Research Institute (KoLRI).

²Not recorded.

### Supplementary Table 2.

List of fungal taxa and fresh lichen samples from 2013 used in this study

| Fungal taxa       | KoLRI No. | GPS                        | Altitude (m) | Substratum |
|-------------------|-----------|----------------------------|--------------|------------|
| **Anzia sp.**     | 017589    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Anzia sp.**     | 017597    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Anzia sp.**     | 017598    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Anzia sp.**     | 017599    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Anzia sp.**     | 017600    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Anzia sp.**     | 017601    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Anzia sp.**     | 017602    | 51°16'59.3" S, 072°50'27.7" W | 46           | Nothofagus sp. (trunk) |
| **Aspicilia sp.** | 017788    | 53°24'20.1" S, 071°15'46.0" W | 285          | Nothofagus sp. (trunk) |
| **Aspicilia sp.** | 017789    | 53°24'20.1" S, 071°15'46.0" W | 285          | Nothofagus sp. (trunk) |
| **Bryoria sp.**   | 017438    | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock, moss |
| **Bryoria sp.**   | 017461    | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock, moss |
| **Bryoria sp.**   | 017462    | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock, moss |
| **Cladonia corallifera** | 017431 | 51°34'36.8" S, 072°35'59.0" W | 133          | Moss |
| **Cladonia furcata** | 017580 | 51°16'59.3" S, 072°50'27.7" W | 46           | Soil |
| **Cladonia gracilis ssp. elongata** | 017532 | 51°21'46.8" S, 072°48'07.3" W | 64           | Soil |
| **Cladonia gracilis ssp. nigripes** | 017505 | 51°34'36.8" S, 072°35'59.0" W | 133          | Moss |
| **Cladonia pyxidata** | 017467 | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock, moss |
| **Cladonia sp.**  | 017513    | 51°33'30.1" S, 072°40'09.6" W | 120          | Soil |
| **Flavoparmelia sp.** | 017653 | 51°22'36.4" S, 072°45'18.8" W | 38           | Soil |
| **Pseudocyphellaria scabrosa** | 017701 | 51°58'54.0" S, 072°22'20.0" W | 140          | Nothofagus sp. (trunk) |
| **Pseudocyphellaria sp.** | 017702 | 51°58'54.0" S, 072°22'20.0" W | 140          | Nothofagus sp. (trunk) |
| **Pseudocyphellaria sp.** | 017745 | 52°03'06.1" S, 071°27'37.8" W | 193          | Soil |
| **Pseoroma sp.**  | 017545    | 51°21'46.8" S, 072°48'07.3" W | 64           | Soil |
| **Sticta cf. weigeli** | 017585 | 51°16'59.3" S, 072°50'27.7" W | 46           | Rock |
| **Unknown fruticose** | 017456 | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock, soil |
| **Usnea sp.**     | 017555    | 51°21'46.8" S, 072°48'07.3" W | 64           | Dead wood (trunk) |
| **Usnea sp.**     | 017694    | 52°01'30.8" S, 072°22'38.8" W | 195          | Nothofagus sp. (trunk) |
| **Usnea sp.**     | 017703    | 51°58'54.0" S, 072°22'20.0" W | 140          | Nothofagus sp. (trunk) |
| **Usnea sp.**     | 017450    | 51°34'36.8" S, 072°35'59.0" W | 133          | Nothofagus sp. (bark) |
| **Usnea sp.**     | 017499    | 51°34'36.8" S, 072°35'59.0" W | 133          | Nothofagus sp. (bark) |
| **Usnea sp.**     | 017728    | 51°33'30.1" S, 072°40'09.6" W | 120          | Rock |
| **Verrucaria sp.** | 017717 | 51°58'54.0" S, 072°22'20.0" W | 140          | Nothofagus sp. (trunk) |
| **Ramalina farinosa** | 017515 | 51°33'30.1" S, 072°40'09.6" W | 120          | Rock |
**Supplementary Table 2.** Continued

| Fungal taxa                        | KoLRI No. | GPS                      | Altitude (m) | Substratum |
|------------------------------------|-----------|--------------------------|--------------|------------|
| Xanthoparmelia sp.                 | 017485    | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock       |
| Xanthoparmelia sp.                 | 017454    | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock       |
| Xanthoparmelia submougeotii        | 017517    | 51°33'30.1" S, 072°40'09.6" W | 120          | Rock       |
| Xanthoria candelaria               | 017650    | 51°22'36.4" S, 072°45'18.8" W | 38           | Rock       |
| Xanthoria cf. candelaria           | 017520    | 51°33'30.1" S, 072°40'09.6" W | 120          | Dead tree  |
| Xanthoria elegans                  | 017651    | 51°22'36.4" S, 072°45'18.8" W | 38           | Rock       |
| Xanthoria cf. elegans              | 017718    | 51°54'01.1" S, 072°27'13.0" W | 1            | Rock       |
| Xanthoria mendozae                 | 017459    | 51°34'36.8" S, 072°35'59.0" W | 133          | Rock       |
| Xanthoria polycarpa                | 017727    | 51°54'01.1" S, 072°27'13.0" W | 1            | Rock       |
| Xanthoria cf. polycarpa            | 017714    | 51°54'01.1" S, 072°27'13.0" W | 1            | Rock       |
| Xanthoria sp.                      | 017608    | 51°22'36.4" S, 072°45'18.8" W | 38           | *Nothofagus* sp. (trunk) |

**Supplementary Table 3.** Primers used in this study

| Target region | Primer name | Sequence (5'-3') | References |
|---------------|-------------|------------------|------------|
| SSU           | SR1R        | TACCTGGTTGATQCTGCCAGT | -          |
|               | SR7         | GTCCAACCTACGAGCTTTTTAA |            |
|               | LS1         | GTACCGGCTGAACCTAAGC | -          |
|               | LS5         | TCCTGAGGGAAACTTCG  |            |
| ITS           | ITS5        | GGAAGTAAAAAGTCTGAAACAAGG | White et al. (1990) [9] |
|               | ITS4        | TCCTCGCCTTTATGATGC | White et al. (1990) [9] |
| ITS region for | nu-SSU-1583-59 | CAACGAGGAATTT CCTAGT | DePriest (1993) [8] |
| initial amplification | ITS4-39 | TCTC TCCTGCTTATGATGC | White et al. (1990) [9] |
| Fungal specific | nr-SSU-1780-59 Fungal | CTGC GGGAAGGAT CATTATA | Piercey-Normore and DePriest (2001) [16] |
|               | nr-LSU-0012-39 Fungal | AGTTCAACGGG GTATCCTT | Piercey-Normore and DePriest (2001) [16] |
| Algal specific | nr-SSU-1780-59 Algal | CTGC GGGAAGGAT CATTGAT | Piercey-Normore and DePriest (2001) [16] |
|               | nr-LSU-0012-39 Algal | AGTTCAACGGG GTATCCTT | Piercey-Normore and DePriest (2001) [16] |
| ITS-LSU       | ITS1F       | CGTGTATTAGAGGAAGTAA | -          |
|               | LR5         | ATCCTGAGGGAAACTTC |            |

SSU, small subunit RNA; LSU, large subunit RNA; ITS, internal transcribed spacer.