Diversity of bacteria associated with lichens in Mt. Yunmeng in Beijing, China

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Lichens host highly diverse and complex microbial communities, which may perform essential functions in these symbiotic micro-ecosystems. In this study, sequencing of 16S rRNA was used to investigate the bacterial communities associated with lichens of two growth forms (foliose and crustose). Results showed that Pseudomonadota, Actinomycetota and Acidobacteriota were dominant phyla in both types of lichens, while Acetobacterales and Hyphomicrobiales were the dominant orders. Alpha diversity index showed that the richness of bacteria hosted by foliose lichens was significantly higher than that hosted by crustose ones. PCoA results showed a significant difference between beta diversity of the foliose lichen-associated bacterial communities and those of crustose lichen-associated ones. PICRUSt2 predictions of gene function showed most functions, annotated by the lichen-associated bacteria, to be related to metabolism, suggesting that related bacteria may provide nutrients to their hosts. Generally, our results propose that microbial communities play important roles in fixing nitrogen, providing nutrients, and controlling harmful microorganisms, and are therefore an integral and indispensable part of lichens, forming a self-sufficient micro-ecosystem.

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Abstract

Lichens host highly complex and diverse microbial communities, which may perform essential functions in these symbiotic micro-ecosystems. In this research, sequencing of 16S rRNA was used to investigate the bacterial communities associated with lichens of two growth forms (foliose and crustose). Results showed that Pseudomonadota, Actinomycetota and Acidobacteriota were dominant phyla in both types of lichens, while Acetobacterales and Hyphomicrobiales were the dominant orders. Alpha diversity index showed that the richness of bacteria hosted by foliose lichens was significantly higher than that hosted by crustose ones. PCoA results showed a significant difference between beta diversity of the foliose lichen-associated bacterial communities and those of crustose lichen-associated ones. PICRUSt2 predictions of gene function showed most functions, annotated by the lichen-associated bacteria, to be related to metabolism, suggesting that related bacteria may provide nutrients to their hosts. Generally, our results propose that microbial communities play important roles in fixing nitrogen, providing nutrients, and controlling harmful microorganisms, and are therefore an integral and indispensable part of lichens, forming a self-sufficient micro-ecosystem.

Introduction

Lichens, a symbiotic complex that consists of two partner organisms, i.e., a fungal partner (mycobiont) and a photoautotrophic partner (a green algae or cyanobacterium, photobiont), are considered among the most endurable organisms on earth (*Nash, 2008*). They can thrive in most terrestrial environments, including the Antarctic, the Arctic, deserts, etc., where environmental conditions are too harsh to be tolerable for most plants (*Øvstedal & Smith, 2001; Printzen, 2008*). Especially, lichens that dwell on rocks accelerate the weathering process of their substrates and facilitate pedogenesis through mechanical and chemical processes (*Gayathri & Swamy, 2012;*

Swamy, Gayathri & Devaraja, 2016), therefore creating suitable environment for other organisms, such as mosses, microfauna, or other microbes. (*Grube & Berg, 2009; Grube et al., 2009; Bates et al., 2011*).

Microbial communities hosted in lichen thalli have been researched by various authors using cultural approaches (*Grube et al., 2009*), molecular fingerprinting methods (*González et al., 2005*), fluorescence in situ hybridization staining (*Erlacher et al., 2015*), and, recently, next-generation sequencing approaches (*Lee et al., 2014; Hodkinson et al., 2012; Swamy & Gayathri, 2021*). Grube *et al.* (2015) concluded that lichen-associated microbial communities may play important roles in the health, growth, and fitness of their hosts (*Bosch & McFall-Ngai, 2011*). Subsequently, a growing number of researchers urges to redefine lichens as holobionts and as self-sufficient ecosystems that contain a dominant fungus, one or more photosynthetic partners, and a plethora of other microorganisms (*Allen & Lendemer, 2022; Hawksworth & Grube, 2020; Simon et al., 2019*). Factors that affect the composition of lichen-associated microbial communities are yet to be determined. Hodkinson *et al.* (2012) suggested that the composition of bacterial communities depends mainly on the availability of fixed carbon and nitrogen, as well as on the selective pressure induced by mycobionts through the production of secondary metabolites with antimicrobial activity. Other researchers proposed that either host species, growth form, substrate and geography might be putative factors shaping the microbial community (*Park et al., 2016; Fernández-Brime et al., 2019; Alonso-Garcia & Villarreal, 2022*). When we mapping the lichens biodiversity of Beijing Municipality, a heavily urbanized ecosystem with a degraded lichens diversity, we also collect materials from Mt. Yunmeng, an area that was gradually restored in recent years (http://www.yunmengshan.org.cn/ymsjqjs). Here we had the rare opportunity to evaluate the diversity of lichen-associated bacteria communities in an ecosystem during its restoration process, and therefore enhance our understanding of the maintenance and dynamics of lichen-associated microbial communities and the relationship with their hosts.

Materials & Methods

Study Area

Lichen samples were collected at Mt. Yunmeng (40°26’N - 40°38’N, 116°30’E - 116°50’E), which is located in the northwest of Miyun District, Beijing. The climate of this area is characterized by warm temperate semi-humid continental monsoon. The average annual temperature is 10.9°C, the average annual precipitation ca. 700mm, with 76% of the precipitation occurring from June to August. The original vegetation was severely destroyed by human activities, but the government classified Mt. Yunmeng and its adjacent areas as a nature reserve and a national forest park in the early 1990s. Since then, a secondary temperate deciduous broadleaf forest developed and became dominant, with planted coniferous forest interspersing some areas. Today about 90% of the Mt. Yunmeng area is covered by forest (http://www.yunmengshan.org.cn/ymsjqjs).

While most parts of Mt. Yunmeng were classified as nature reserve, its eastern part has developed into a series of scenic spots which attract large numbers of tourists. One of these spots is the Black Dragon Pool (160 to 380 m a.s.l), which is a 4.5 km long canyon, with a small creek and a riverine

forest on both sides. Tourist activities may have destroyed or negatively affected the local lichen communities.

Collection of Materials

Lichen samples were collected in October starting from the Black Dragon Pool, crossing the border between the scenic spot and the nature reserve, and ending at the heart of nature reserve at about 1130 m a.s.l. Lichen samples were identified refer to Brodo *et al* (2001) and Nash *et al* (2007). In total, twenty samples were obtained with three from the scenic spot (F1-F3) and seventeen from the nature reserve (F4-F10, C1-C10), most of which were saxicolous (growing on granites), while only two were epiphytic (growing on *Quercus* sp.). The number of crustose (C1-C10) and foliose (F1-F10) lichens were equivalent, while no fruticose lichens were found at all (for detailed information see in Table 1). Lichen samples (one entire thalli of each individual) were collected using sterile blade and forceps and placed in sterile polythene bags, sealed and labelled. Samples were transported to the laboratory using an ice box and then stored at -80℃ for further processing. Lichen samples were washed with ultra-pure laboratory grade water to remove dirt and debris.

DNA extraction, 16S rRNA gene amplification and sequencing

HiPure Soil DNA Kit was used to extract total genome DNA according to manufacturer's protocols (Magen Biotechnology Co., Ltd, China). DNA concentration was monitored by Qubit® dsDNA HS Assay Kit. 20-30ng of DNA was used to generate amplicons containing V3-V4 hypervariable regions using a MetaVX Library Preparation kit (GENEWIZ, Inc., South Plainfield, USA). The forward primer contained the sequence 5′-CCTACGGRRBGCASCAGKVRVGAAT-3′ and the reverse primer, 5′-GGACTACNVGGGTWTCTAATCC-3′, which were designed by GENEWIZ (Suzhou, China). The polymerase chain reaction (PCR) system contained 2.5μl of TransStart buffer, 2μl of dNTPs, 1μl of each primer, 0.5μl of TransStart Taq DNA polymerase, 20ng template DNA, and ddH2O which was added to obtain the total volume of 25 μl. The PCR ran as follows: 3min of denaturation at 94℃, 24 cycles of 5s at 95℃, 90s of annealing at 57℃, 10s of elongation at 72℃, and a final extension at 72℃ for 5min. Indexed adapters were added to the ends of the amplicons by limited cycle PCR. Finally, the library was purified with magnetic beads. Paired-end (PE) sequencing was carried out with the Illumina Miseq Platform (Illumina, San Diego, USA) at Genewiz, Inc.

Bioinformatic analysis

PRINSEQ (0.20.4) was used for sequence quality control. After quality filter (reads with length <200 bp) and removal of chimeric sequences, all sequences were grouped into operational taxonomic units (OTUs) using VSEARCH (1.9.6) (sequence similarity was set to 97%) against the SILVA v138 database. The OTUs were then classified into different taxonomic levels using the Ribosomal Database Project (RDP) classifier (2.13). The names of annotated bacteria follow the List of Prokaryotic names with Standing in Nomenclature (LPSN) (*Parte et al., 2020*). Alpha diversity indices (Chao 1 and Shannon), which indicate bacterial community richness and

diversity, were calculated using Mothur software (1.35.1). Principal Co-ordinates Analysis (PCoA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on the Bray-Curtis distance matrices were employed to determine beta diversity and cluster samples, respectively. Analysis of Similarities (ANOSIM) was applied to test the differences between bacterial communities associated with foliose and crustose lichens. Linear discriminant analysis Effect Size (LEfSe) was conducted using the Galaxy framework online tool (https://huttenhower.sph.harvard.edu/galaxy/), which can find microorganisms with significant differences between groups by calculating the contribution of each microbial abundance to the overall difference. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was conducted to predict the function of lichen-associated microbiota based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Clusters of Orthologous Groups (COG) databases. All statistical analyses were performed with SPSS 22.0 (IBM Corp., Armonk, NY). Differences in phylum, order and genus relative abundances are presented as Means ± SE. Alpha diversity indices were calculated by using the Independent-sample t-test. A P-value < 0.05 was considered statistically significant, while a P-value < 0.01 indicated that differences were highly significant. The raw data obtained in this study have been submitted to the NCBI Sequence Read Archive under Bioproject number PRJNA972522 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA972522).

Results

16S rRNA gene sequencing data statistics

After filtration of low-quality regions and removal of chimeras, a total of 1,232,513 effective sequences were obtained, with 36,494-79,179 for each sample and an average length of 442.04bp (Table 2). Using cluster analysis, we identified 426 OTUs at 97% similarity level, including 16 phyla, 30 classes, 61 orders, 83 families and 125 genera.

The rarefaction curve (Figure 1a) shows that the number of OTUs in the sample increases with sequencing depth, the curve finally flattens, indicating that the sequencing had covered most bacteria in the samples. Overall, the observed OTUs which stand for species richness in foliose lichens was higher than that of crustose lichens. The rank abundance curve (Figure 1b) shows two aspects of diversity, namely richness and evenness. In general, the evenness and richness of the bacteria associated with foliose lichens was higher than that with crustose lichens.

Composition of bacteria associated with foliose and crustose lichens

At the phylum level, the most dominant phylum of bacteria associated with foliose and crustose lichens was Pseudomonadota, with a relative abundance of 55.88 ± 4.45% and 69.76 ± 5.48%, in foliose and crustose, respectively (Figure 2). The phyla Actinomycetota (foliose: 13.44 ± 2.61%; crustose: 9.46 ± 2.70%) and Acidobacteriota (foliose: 13.63 ± 1.83%; crustose: 12.15 ± 2.72%) were also present with a relatively high abundance. At the order level (Figure 3), the dominant orders of bacteria were Acetobacterales and Hyphomicrobiales, with relative abundance of 4.44 ± 1.02%, 6.13 ± 0.71% for foliose lichens and 6.62 ± 2.11%, 4.39 ± 1.08% for crustose lichens. At

the genus level (Figure 4), *Sphingomonas* (foliose: 5.44 ± 1.09%; crustose: 3.14 ± 1.16%) and *Acidiphilium* (foliose: 2.5 ± 0.54%; crustose:3.99 ± 1.14%) had higher relative abundance in both groups of lichens.

Diversity of bacterial communities associated with foliose and crustose lichens

Alpha diversity results showed that the chao1 indices of foliose and crustose lichens (Figure 5a) were 246.57 ± 20.42 and 151.40 ± 30.38, respectively (P=0.02). The Shannon indices of bacterial communities associated with foliose and crustose lichens (Figure 5b) were 3.69 ± 0.55 and 2.71±0.56 respectively but did not show significant differences between the two groups (P=0.43). The UPGMA tree (Figure 6) and the PCoA (Figure 7) unraveled the microbial composition between different samples. Performing ANOSIM analysis, we found a significant difference between the community structures of bacteria associated with foliose and crustose lichens (P=0.02).

Taxon differences of lichens-associated bacteria between foliose and crustose lichens

According to phylogenetic map (Figure 8), nine bacterial taxa showed significant differences between foliose and crustose lichens. Among the bacteria associated with foliose lichens, the relative abundance of Chloroflexi, Bacteroidia, Bacteroidota, Micromonosporae, Micromonosporales, Chitinophagae, Chitinophagales, Blastocellaceae, Blastocellales was significantly higher than that of bacteria associated with crustose lichens. No taxon was found to have a significantly higher relative abundance with crustose lichens than foliose lichens.

PICRUSt2 gene function predictions

Functional annotations were based on KEGG and COG databases. Most of the bacterial gene functions were related to metabolism (Figure 9a), such as the metabolisms of carbohydrates, amino acids, vitamins, and cofactors. The abundance of genes related to amino acid transport and metabolism, function unknown, etc. were high (Figure 9b). No significant difference in the functional abundance between associated bacteria of foliose and crustose lichens was found.

Discussion

The diversity and composition of bacterial communities associated with foliose and crustose lichens in Mt. Yunmeng were examined using 16S amplification sequencing. Our results showed that the dominant phyla of bacteria associated with these two types of lichens were Pseudomonadota, Acidobacter, Actinomycetota, which were similar to those described by other studies on lichens (*Swamy & Gayathri, 2021; Grube et al., 2015; Bjelland et al., 2011*). Acetobacterales and Hyphomicrobiales were the most abundant orders of lichen-associated bacteria, which were also reported by other studies (*Bates et al., 2011; Hodkinson & Lutzoni, 2009*).

Our results suggest that the growth form of lichens has an impact on the bacterial communities associated with lichens, which is similar to results published by Park *et al* (*2016*). Firstly, alpha

diversity indices showed that the richness of associated bacteria in foliose lichens was significantly higher than that in crustose lichens (Figure 5), which may be attributed to the more complex structure of the thalli in foliose lichens compared to that of crustose lichens (*Nash, 2008*). The fact that the powdery and the most poorly structured crustose lichen *Lepraria* sp. (C4) clustered at the most outer branch of the UPGMA tree (Figure 6) also supports this suggestion. Secondly, foliose and crustose lichens were grouped into several different clades respectively in the cluster analysis (Figure 6). On the UPGMA tree, five distinct clades can be recognized, with Clade I and III mainly comprising crustose lichens, while Clade II, IV and V comprising foliose lichens. Thirdly, PCoA results showed a significant difference between beta diversity of foliose lichen-associated bacterial communities and that of crustose lichen-associated communities (Figure 7). Combining, these findings suggest that the growth form of lichens had a strong influence on the bacterial community composition.

It also seems that the lichen species itself may affects the bacterial community it is hosting. Among the materials we collected, there were three species with at least two samples (Table 1), i.e., *Phaeophyscia* sp. (F1, F2, F6, F7), *Aspicilia cinerea* (C1, C3, C10) and *Candelaria asiatica* (F4, F5). Two samples of each species clustered together in the UPGMA tree, namely F2 and F6 for *Phaeophyscia* sp., C1 and C10 for *A. cinerea*, and F4 and F5 for *C. asiatica* (Figure 4). Based on the existing data, since the composition of microbial communities shows some species specificity, we speculate that it is likely to be affected by the host lichen. However, not all the samples of the same lichen species clustered together. For example, though belonging to the same species *Phaeophyscia* sp., F1 and F7 were located at branches far from the clade formed by F2 and F6 (Figure 4), suggesting that the species specificity of bacterial communities is not robust. Most of the lichens we collected were saxicolous, and only two samples were epiphytic (F8 and F9), which were scattered among the others in the UPGMA tree (Figure 6). Based on the existing data, we therefore speculate that the substrates may have even less impact on the lichen-associated bacteria, a result however, that requires further research.

Tourists have been considered to impose great pressure on the biodiversity at scenic spots (*Yang & Xu, 2003*). As the scenic spots and the nature reserve were both sampled, we expected a measurable difference in the biodiversity of lichens and their associated bacteria between these two areas. Our results indeed show a measurable difference in the lichen diversity between the two areas while their associated bacteria composition shows no significant difference. Based on the existing data, the presence of tourists seems has an impact on the diversity of lichens in two areas, but has little impact on the lichen associated microbiota. Among the collected samples, only three were obtained from the Black Dragon Pool, much less than collected from the nature reserve (Table 1). As shown in the UPGMA tree (Figure 6), samples from the scenic spots (F1-F3) were scattered among those from the nature reserve, and it seems no significant differences between the composition of lichen-associated microbial communities were detected. Other researchers proposed that host species, growth form, substrate and geography might be putative factors that affect bacterial communities in lichen thalli (*Bates et al., 2011; Park et al., 2016; Fernández-Brime et al., 2019; Alonso-Garcia & Villarreal, 2022; Sierra et al., 2020*). According to our

results, it can only be inferred that the growth form has an impact on bacterial communities associated with lichens.

Bacteria in lichen-hosted microbial communities were considered to have ecological and physiological roles and are indispensable for the lichen thallus (*Grube et al., 2009*). In this study, most of the gene functions annotated by the lichen-associated bacteria were related to metabolism (Figure 9), which suggests that the related bacteria may contribute to improve the nutritional balance of their hosts. The associated bacteria orders, Hyphomicrobiales and Acetobacterales are well known to be able to fix nitrogen in microbe-plant interactions (*Garrity, Bell & Lilburn, 2005; Saravanan et al., 2008*). They were also detected in samples investigated by other studies and were recognized as an important symbiotic component that may provide fixed nitrogen to lichen ecosystems (*Bates et al., 2011; Hodkinson & Lutzoni, 2009*). Besides, these two orders of bacteria are also known to perform other crucial functions supporting the symbiosis, such as providing auxin and vitamins, and thus helping to protected the lichens from physiological stress (*Erlacher et al., 2015; Cernava et al., 2017*). Gonzales *et al.* (*2005*) studied the culturable Actinomycetes isolated from lichens and found half of the isolates could produce antibacterial substances. Moreover, a strain of Actinomycete isolated from *Cladonia uncialis* was found to be able to produce a new compound with strong antibacterial activity (*Davies et al., 2005*). In addition, Bhatti *et al.* (*2017*) showed that Actinomycetes play a role in eliminating harmful microorganisms in soil, and it was proposed that they might play a defensive role in lichens. Therefore, most of these lichen-associated bacteria may play an irreplaceable and important role during the colonization and growth of lichens and their micro-ecosystems on different substrates.

Conclusions

The relationship between the microbes and their lichen hosts has intrigued deliberate rethinking of the concept of lichen symbiosis (*Allen & Lendemer, 2022*). Are the microbes only tenants living in the rooms provided by lichens, paying a certain rent, or are they part of lichen itself? In other words, should we continue to regard lichen as a dual system with a mycobiont and a photobiont described by Schwendener in 1867 (*Honegger, 2000*), or do they rather represent a multilateral system involving not only the mycobiont and photobiont, but also their associated microbes? We should notice that some bacteria were persistently found to co-exist with lichens in a series of studies and might therefore play an essential role in the microbial community. In this study, we found that the growth form of lichens has a great effect on the composition of their microbial communities. But why should we not take another perspective and consider the possibility that the microbial communities play a key role to determine the growth form of their host? After all, in some cases it was reported that the same species of lichens assume different growth forms (*Takahashi et al., 2006*). Although we have not yet investigated this particular microbial community, this possibility is worth to be explore in future studies. Therefore, it is reasonable to view some lichen-associated bacteria, if not all, as integrated and inseparable members of the symbiotic ecosystem that play a significant role sustaining this ecosystem.

References

Allen JL, Lendemer JC. 2022. A call to reconceptualize lichen symbioses. *Trends in Ecology & Evolution* 37(7): 582-589 DOI: 10.1016/j.tree.2022.03.004.

Alonso-Garcia M, Villarreal AJC. 2022. Bacterial community of reindeer lichens differs between northern and southern lichen woodlands. *Canadian Journal of Forest Research* 52(5): 662-673 DOI: 10.1139/cjfr-2021-0272.

Bates ST, Cropsey G, Caporaso JG, Knight R, Fierer N. 2011. Bacterial communities associated with the lichen symbiosis. *Applied and Environmental Microbiology* 77(4): 1309-1314 DOI: 10.1128/AEM.02257-10.

Bhatti AA, Haq S, Bhat RA. 2017. Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis* 111: 458-467 DOI: 10.1016/j.micpath.2017.09.036.

Bjelland T, Grube M, Hoem S, Jorgensen SL, Daae FL, Thorseth IH, Øvreås L. 2011. Microbial metacommunities in the lichen-rock habitat. *Environmental Microbiology Reports* 3(4): 434-442 DOI: 10.1111/j.1758-2229.2010.00206.x.

Bosch TC, McFall-Ngai MJ. 2011. Metaorganisms as the new frontier. *Zoology* 114(4): 185-190 DOI: 10.1016/j.zool.2011.04.001.

Brodo IM, Sharnoff SD, Sharnoff S. 2001. Lichens of north america. Yale university press.

Cernava T, Erlacher A, Aschenbrenner IA, Krug L, Lassek C, Riedel K, Grube M, Berg G. 2017. Deciphering functional diversification within the lichen microbiota by meta-omics. *Microbiome* 5(1): 82 DOI: 10.1186/s40168-017-0303-5.

Davies J, Wang H, Taylor T, Warabi K, Huang XH, Andersen RJ. 2005. Uncialamycin, a new enediyne antibiotic. *Organic Letters*. 7(23): 5233-5236 DOI: 10.1021/ol052081f.

Erlacher A, Cernava T, Cardinale M, Soh J, Sensen CW, Grube M, Berg G. 2015. Rhizobiales as functional and endosymbiontic member in the lichen symbiosis of *Lobaria pulmonaria L*. *Frontiers in Microbiology* 6: 53 DOI: 10.3389/fmicb.2015.00053.

Fernández-Brime S, Muggia L, Maier S, Grube M, Wedin M. 2019. Bacterial communities in an optional lichen symbiosis are determined by substrate, not algal photobionts. *FEMS Microbiology Ecology* 95(3): fiz012 DOI: 10.1093/femsec/fiz012.

Garrity GM, Bell JA, Lilburn T. 2005. "Class I. Alphaproteobacteria class. nov.," in Bergey's Manual of Systematic Bacteriology, Vol. 2, eds Garrity G. M., Bren-ner D. J., Krieg N. R., Staley J. T. (New York, NY: Springer), 1

Gayathri D, Swamy CT. 2012. Lichens: a novel and potential source as antimicrobials for human use. *Journal of Phytology* 4: 38-43

González I, Ayuso-Sacido A, Anderson A, Genilloud O. 2005. Actinomycetes isolated from lichens: evaluation of their diversity and detection of biosynthetic gene sequences. *FEMS Microbiology Ecology* 54(3): 401-415 DOI: 10.1016/j.femsec.2005.05.004.

Grube M, Berg G. 2009. Microbial consortia of bacteria and fungi with focus on the lichen symbiosis. *Fungal Biology Reviews* 23(3): 72-85 DOI: 10.1016/j.fbr.2009.10.001.

Grube M, Cardinale M, de Castro JV, Muller H, Berg G. 2009. Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *The ISME Journal* 3(9): 1105-

1115 DOI: 10.1038/ismej.2009.63.
Grube M, Cernava T, Soh J, Fuchs S, Aschenbrenner I, Lassek C, Wegner U, Becher D, Riedel K, Sensen CW, Berg G. 2015. Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *The ISME Journal* 9(2): 412-424 DOI: 10.1038/ismej.2014.138.
Hawksworth DL, Grube M. 2020. Lichens redefined as complex ecosystems. *The New Phytologist* 227(5): 1281-1283 DOI: 10.1111/nph.16630.
Hodkinson BP, Gottel NR, Schadt CW, Lutzoni F. 2012. Photoautotrophic symbiont and geography are major factors affecting highly structured and diverse bacterial communities in the lichen microbiome. *Environmental Microbiology* 14(1): 147-161 DOI: 10.1111/j.1462-2920.2011.02560.x.
Hodkinson BP, Lutzoni F. 2009. A microbiotic survey of lichen associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49: 163-180 DOI: 10.1007/s13199-009-0049-3.
Honegger R. 2000. Great discoveries in bryology and lichenology - Simon Schwendener (1829-1919) and the dual hypothesis of lichens. *Bryologist* 103: 307-313. DOI: 10.1639/0007-2745(2000)103[0307:SSATDH]2.0.CO;2
Lee YM, Kim EH, Lee HK, Hong SG. 2014. Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. *World Journal of Microbiology & Biotechnology* 30(10): 2711-2721 DOI: 10.1007/s11274-014-1695-z.
Nash III, T.H. 2008. Lichen biology. 2 ed. Cambridge University Press.
Nash III TH, Ryan BD, Diederich P, Gries C, Bungartz F. 2007. Lichen Flora of the Greater Sonoran Desert Region. American Bryological and Lichenological Society.
Øvstedal DO, Smith RIL. 2001. Lichens of Antarctica and South Georgia. Cambridge University Press, Cambridge.
Park CH, Kim KM, Kim OS, Jeong G, Hong SG. 2016. Bacterial communities in Antarctic lichens. *Antarctic Science* 28(6): 455-461 DOI: 10.1017/S0954102016000286.
Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. 2020. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology* 70(11): 5607-5612 DOI: 10.1099/ijsem.0.004332.
Printzen C. 2008. Uncharted terrain: the phylogeography of arctic and boreal lichens. *Plant Ecology & Diversity* 1(2): 265-271 DOI: 10.1080/17550870802328702.Saravanan VS, Madhaiyan M, Osborne J, Thangaraju M, Sa TM. 2008. Ecological occurrence of *Gluconacetobacter diazotrophicus* and nitrogen-fixing Acetobacteraceae members: their possible role in plant growth promotion. *Microbial Ecology* 55(1): 130-140 DOI: 10.1007/s00248-007-9258-6.
Sierra MA, Danko DC, Sandoval TA, Pishchany G, Moncada B, Kolter R, Mason CE, Zambrano MM. 2020. The microbiomes of seven lichen genera reveal host specificity, a reduced core community and potential as source of antimicrobials. *Frontiers in Microbiology* 11: 398 DOI: 10.3389/fmicb.2020.00398.
Simon JC, Marchesi JR, Mougel C, Selosse MA. 2019. Host-microbiota interactions: From

holobiont theory to analysis. *Microbiome* 7(1): 5 DOI: 10.1186/s40168-019-0619-4.
Swamy CT, Gayathri D. 2021. High throughput sequencing study of foliose lichen-associated bacterial communities from India. *Molecular Biology Reports* 48(3): 2389-2397 DOI: 10.1007/s11033-021-06272-6.
Swamy CT, Gayathri D, Devaraja TN. 2016. Antibacterial activity of lichens *Parmotrema tinctorum* and *Pyxine sorediata* and their secondary metabolites. *International Journal of Advanced Life Sciences* 9: 373-380
Takahashi K, Wang LS, Tsubota H, Deguchi H. 2006. Photosymbiodemes *Sticta wrightii* and *Dendriscocaulon* sp. (Lichenized Ascomycota) from Yunnan, China. *Journal of The Hattori Botanical Laboratory* 100: 783-796 DOI: 10.1002/jpln.200690016.
Yang X, Xu M. 2003. Biodiversity conservation in Changbai Mountain Biosphere Reserve, northeastern China: status, problem, and strategy. *Biodiversity & Conservation* 12: 883-903 DOI: 10.1023/A:1022841107685.

Figure 1

Figure1. (a) Rarefaction curves and (b) rank abundance curve.

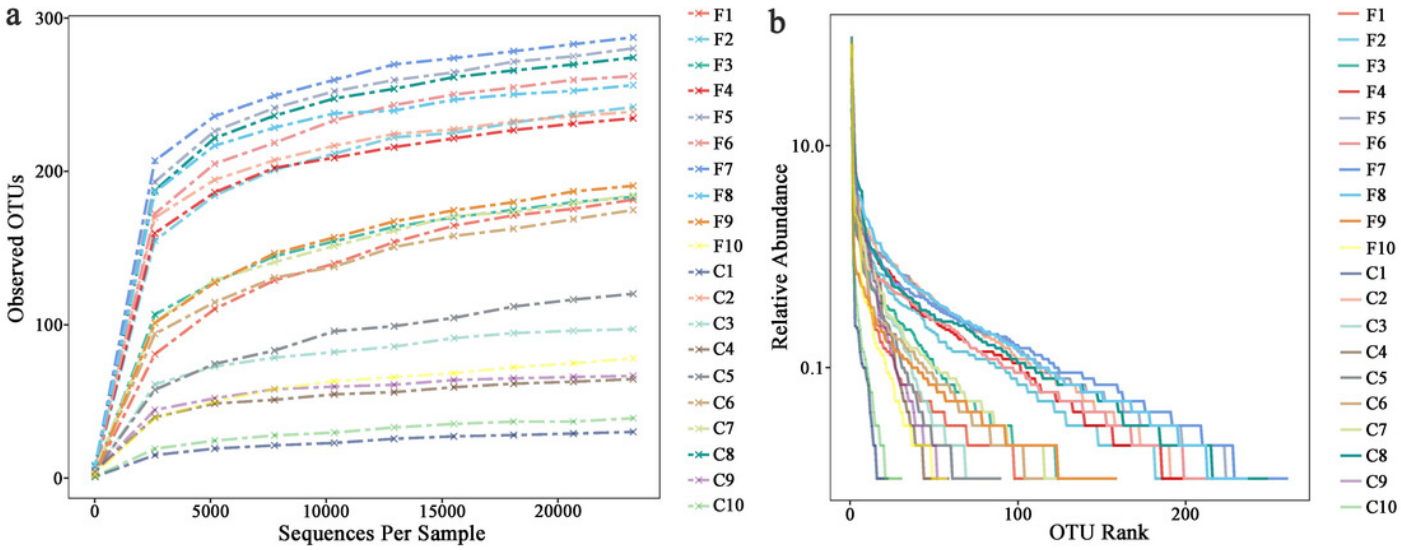

Figure 2

Figure 2. Distribution of bacteria at the Phylum level associated with foliose and crustose lichens. (a) for groups, (b) for each sample.

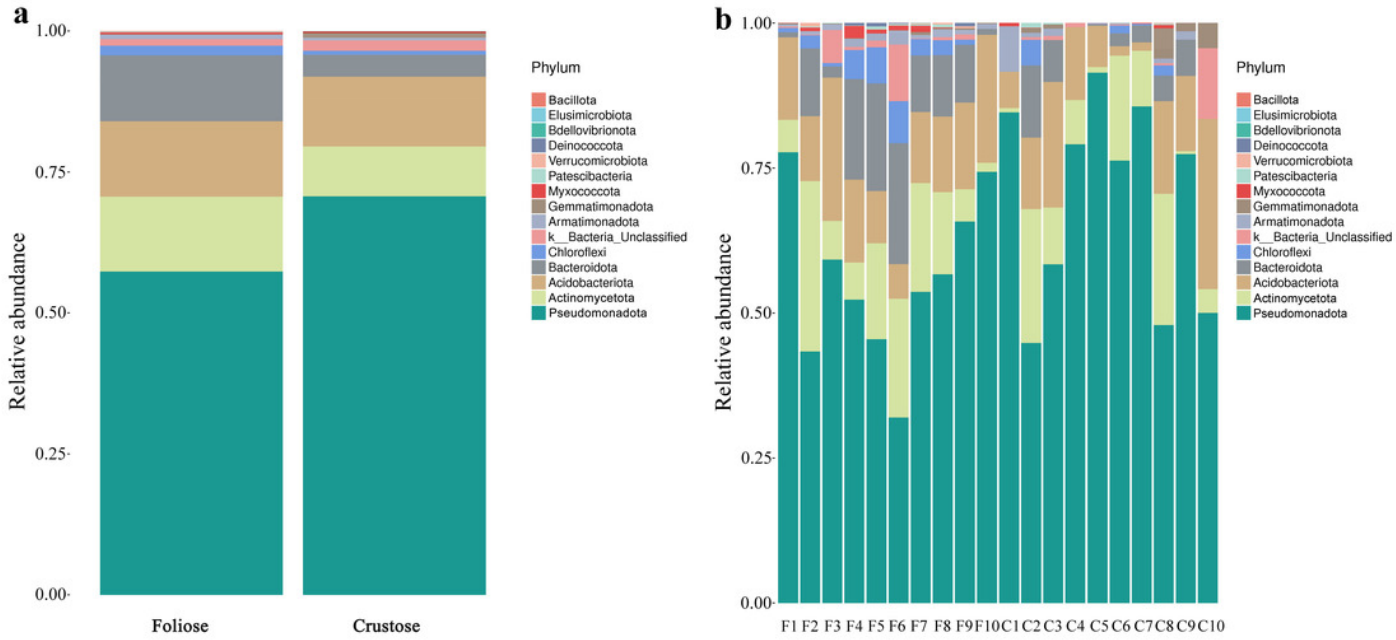

Figure 3

Figure 3. Distribution of bacteria at the Order level associated with foliose and crustose lichens. (a) for groups, (b) for each sample.

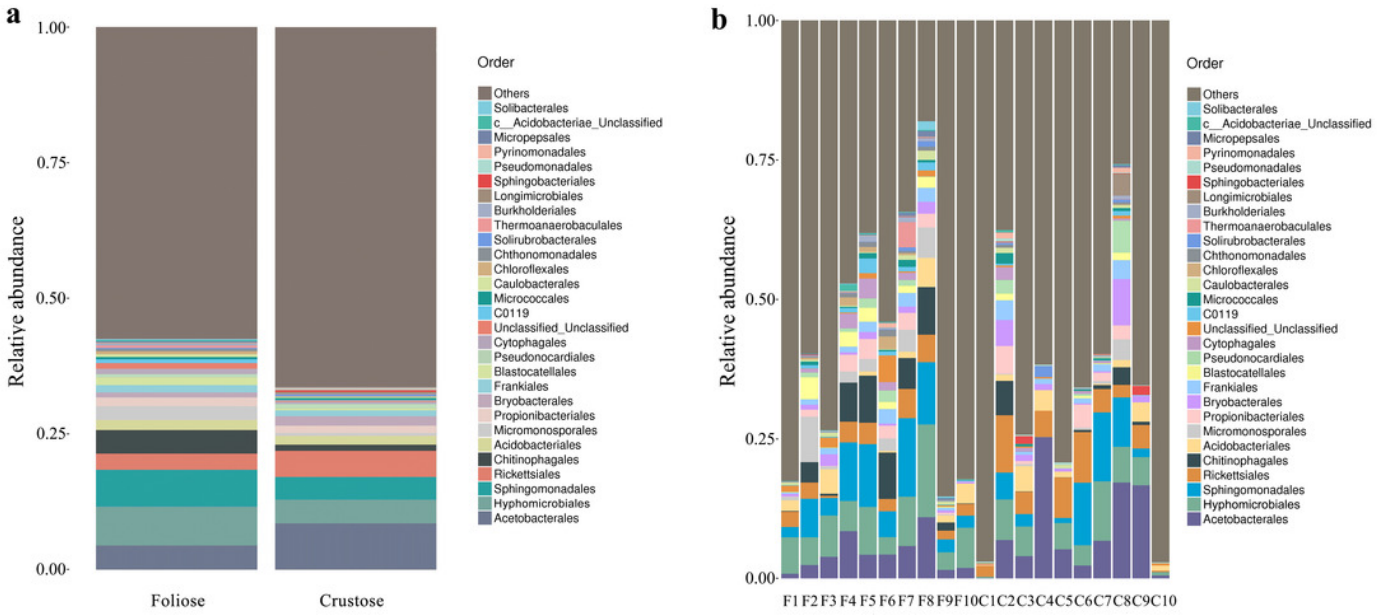

Figure 4

Figure 4. Distribution of bacteria at the Genus level associated with foliose and crustose lichens. (a) for groups, (b) for each sample.

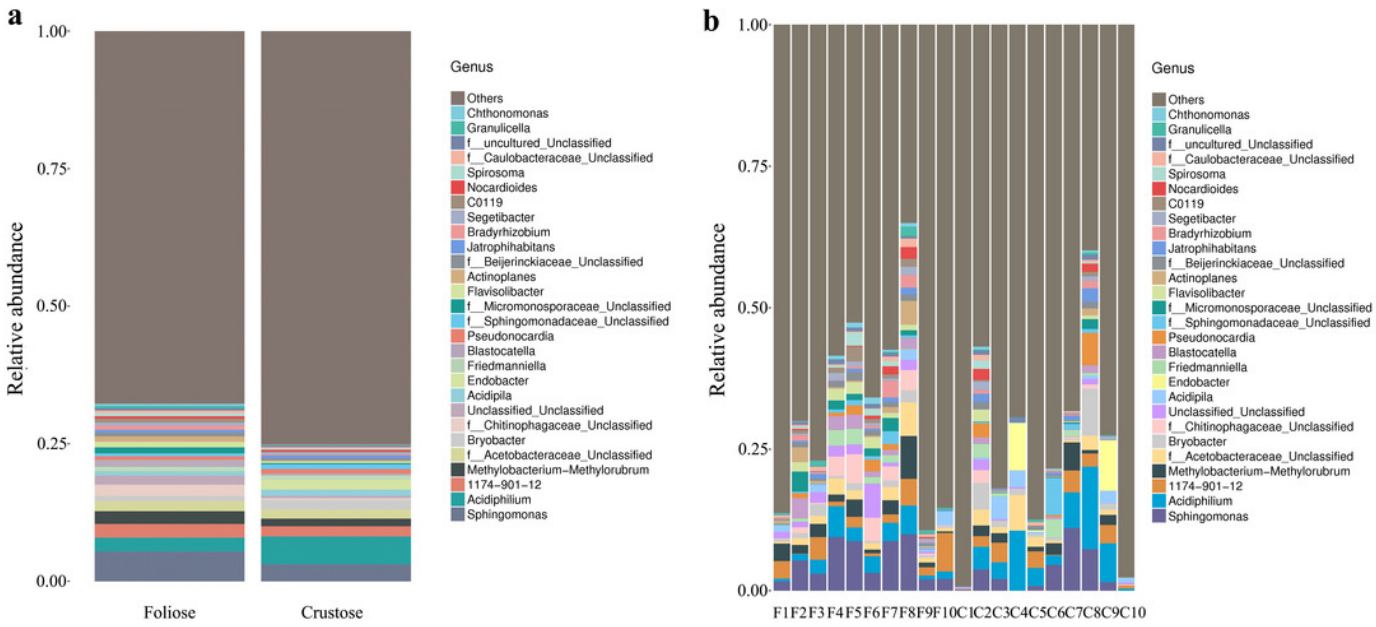

Figure 5

Figure5

Figure 5. Boxplot of (a) Chao1 and (b) Shannon diversity indices refer to the bacterial communities associated with foliose and crustose lichens. Boxes represent the interquartile range (IQR; between 25th and 75th percentiles), horizontal line inside the box defines the median, outliers greater than 1.5 and less than 3 times the IQR.

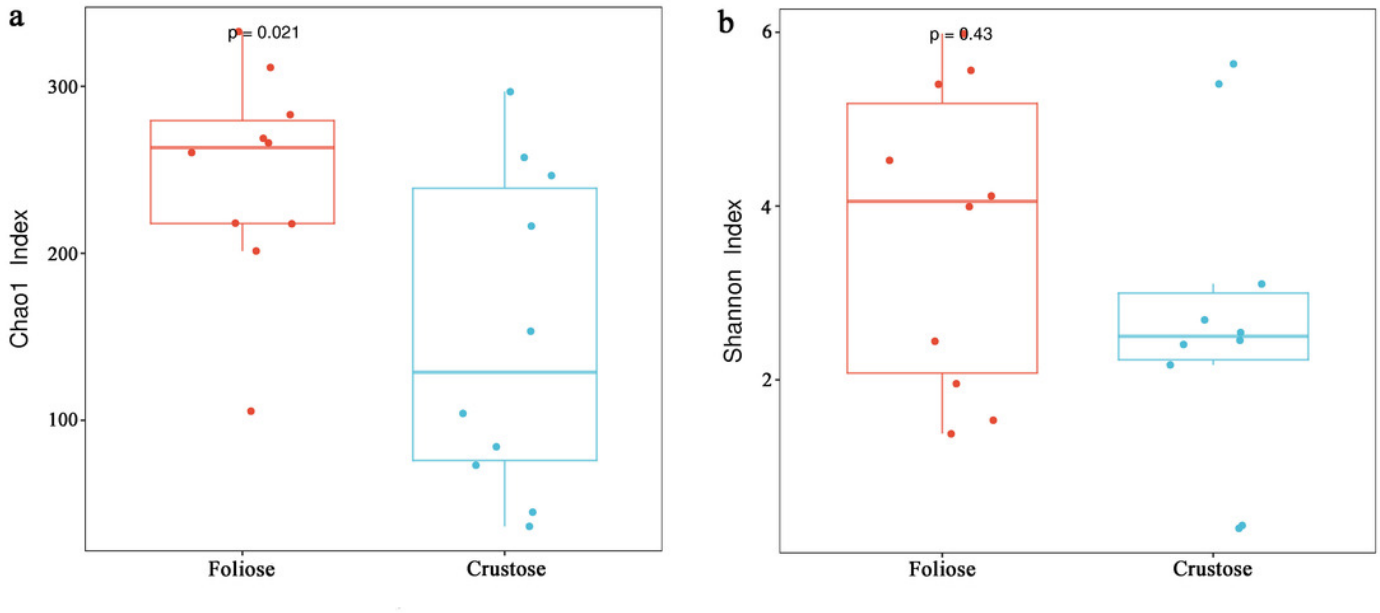

Figure 6

Figure 6. UPGMA cluster analysis based on Bray-Curtis distances with five clades being recognized.

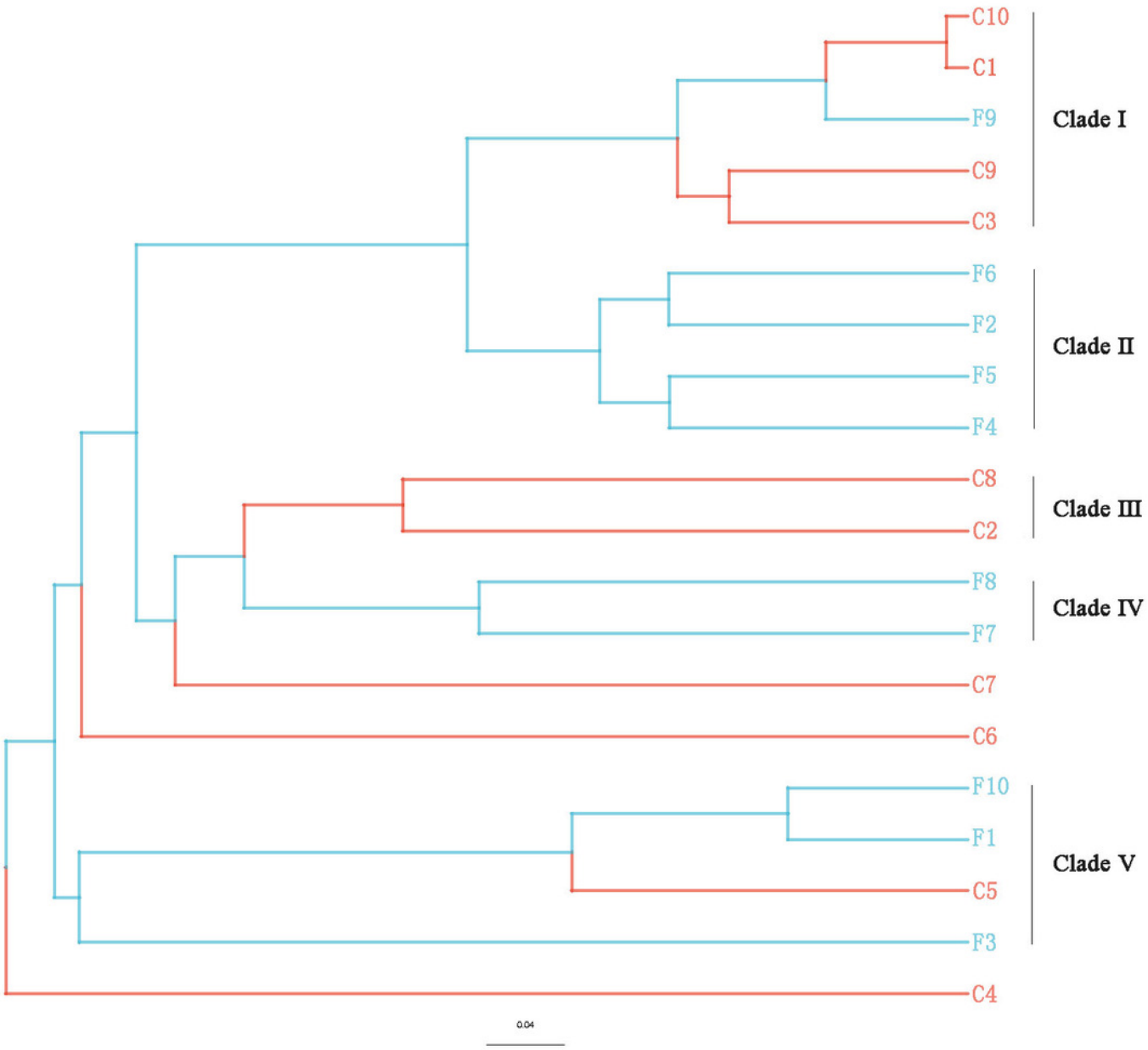

Figure 7

Figure 7. PCoA results for (a) PC1 vs PC2, (b) PC1 vs PC3, and (c) PC2 vs PC3. Percentages of total variation explained by each axis are shown in brackets.

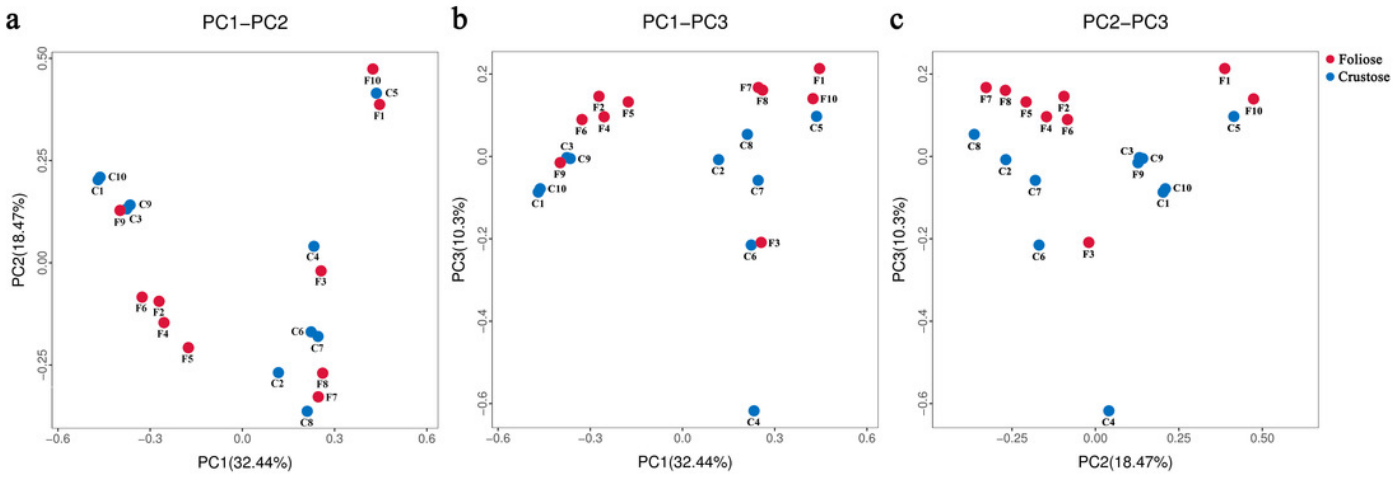

Figure 8

Figure 8. LefSe analysis.

(a) Cladogram, and (b) LDA value distribution histogram. From the inside to the outside: phylum, class, order, family, and genus level. Different color points in the phylogenetic tree represent bacteria which are significantly different between the two groups of lichens. Yellow points indicate bacteria that show no significant difference between the two groups. LDA score was set to be at 3.0.

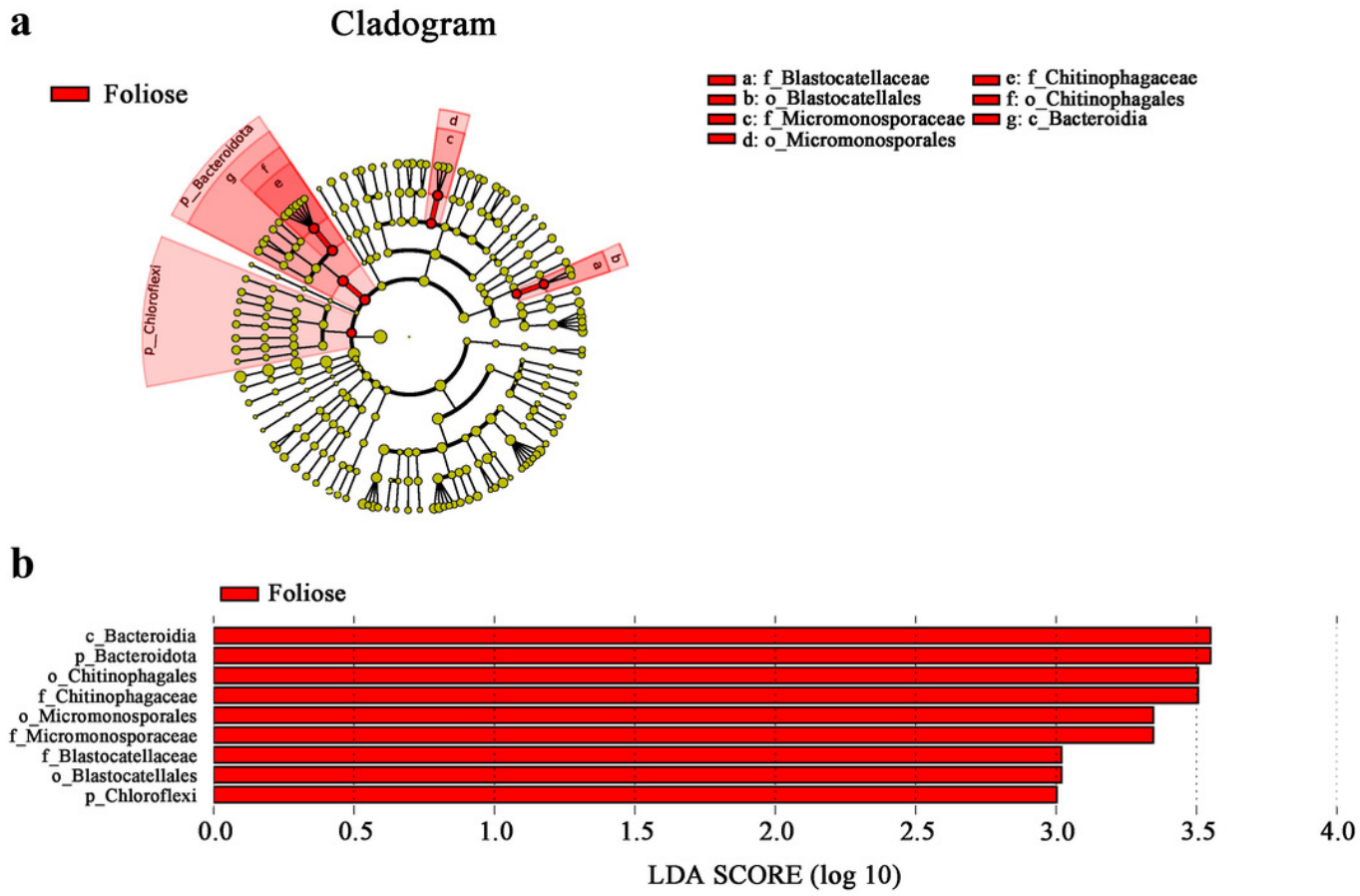

Figure 9

Figure 9. Functional annotation for non-redundant gene sets based on the functional classification of (a) KEGG (level 2) and (b) COG database.

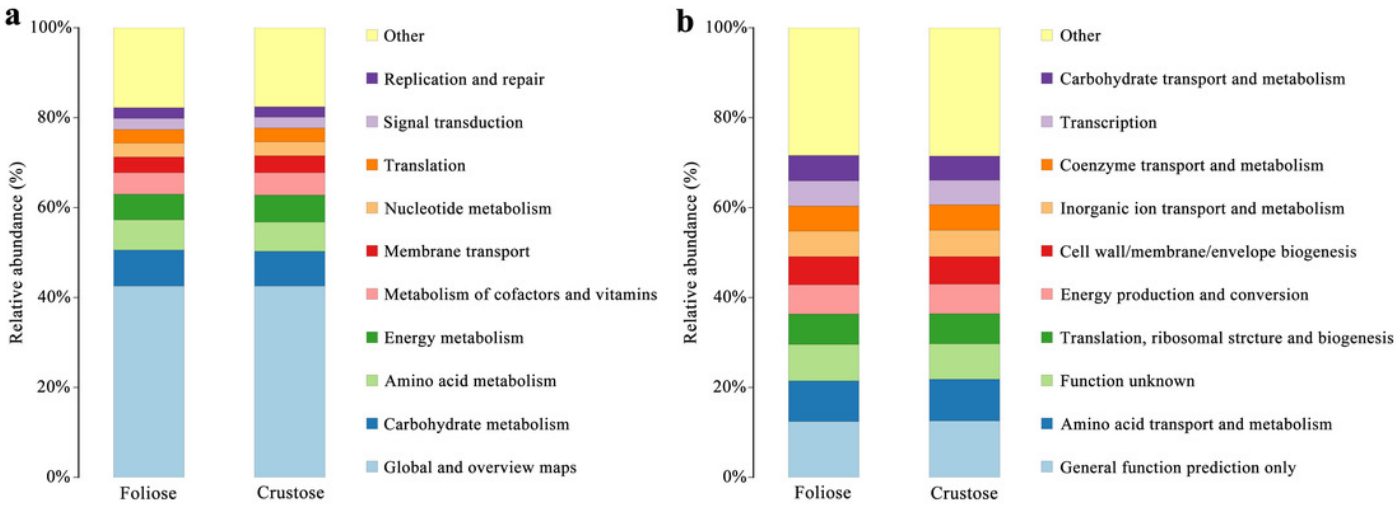

Table 1(on next page)

Table1. Information of lichen samples

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Sample ID	Lichen species	Growth form	Substrate	Sampling site	Altitude (m)
F1	*Phaeophyscia* sp.	Foliose	Granites	Scenic spot 116°7831′E,40°5611′N	283
F2	*Phaeophyscia* sp.	Foliose	Granites	Scenic spot 116°7832′E,40°5612′N	286
F3	*Canoparmelia* sp.	Foliose	Granites	Scenic spot 116°7832′E,40°5611′N	283
F4	*Candelaria asiatica*	Foliose	Granites	Nature reserve 116°7459′E,40°5677′N	753
F5	*Candelaria asiatica*	Foliose	Granites	Nature reserve 116°7392′E,40°5669	860
F6	*Phaeophyscia* sp.	Foliose	Granites	Nature reserve 116°7392′E,40°5668′N	857
F7	*Phaeophyscia* sp.	Foliose	Granites	Nature reserve 116°7349′E,40°5654′N	912
F8	*Parmelia* sp.	Foliose	*Quercus* sp.	Nature reserve 116°7266'E,40°5572′N	1115
F9	*Punctelia* sp.	Foliose	*Quercus* sp.	Nature reserve 116°7266'E,40°5572′N	1115
F10	*Ramalina* sp.	Foliose	Granites	Nature reserve 116°7255'E,40.5571′N	1130
C1	*Aspicilia cinerea*	Crustose	Granites	Nature reserve 116°763'E,40°5652′N	532
C2	*Aspicilia* sp.	Crustose	Granites	Nature reserve 116°7518'E,40°5678′N	705
C3	*Aspicilia cinerea*	Crustose	Granites	Nature reserve 116°7483′E,40.5671′N	721
C4	*Lepraria* sp.	Crustose	Granites	Nature reserve 116°7416′E,40.5678′N	813
C5	*Lecanora saxigena*	Crustose	Granites	Nature reserve 116°735′E,40°5654′N	912
C6	*Verrucaria funckii*	Crustose	Granites	Nature reserve 116°7348′E,40°5649′N	920
C7	*Bilimbia fuscoviridis*	Crustose	Granites	Nature reserve 116°7348′E,40°565′N	919
C8	*Buellia* sp.	Crustose	Granites	Nature reserve 116°7322′E,40°5629′N	958
C9	*Rhizoplaca* sp.	Crustose	Granites	Nature reserve 116°7255′E,40°5571′N	1123
C10	*Aspicilia cinerea*	Crustose	Granites	Nature reserve 116°7255′E,40°5571′N	1130

Table 2(on next page)

Table2. Statistics of the sequencing data after filtering of each sample

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Sample	PE_reads	Effective tags	AvgLen(bp)	GC(%)
C1	105344	76922	440.06	52.15
C2	86141	60796	441.5	53.45
C3	95084	68337	439.64	53.01
C4	94928	67785	445.24	53.33
C5	93315	66617	442.93	51.93
C6	100889	75061	441.09	50.72
C7	106163	79179	441.79	52.59
C8	79509	49814	444.23	55.31
C9	103014	69208	441.5	53.09
C10	81332	50249	439.71	52.46
F1	82675	45557	440.62	52.52
F2	78370	39679	444.18	54.12
F3	73996	36494	439.39	53.41
F4	82112	58093	441.96	53.71
F5	90903	62486	443.08	53.62
F6	98442	68661	443.19	53.76
F7	86286	58717	445.69	54.71
F8	81252	55099	444.29	54.65
F9	101652	70326	439.45	52.72
F10	113565	73433	441.27	52.10

PE (Paired-End) reads: Number of original PE reads; Effective tags: Number of valid sequences after chimera removal over the original number of PE reads; AveLen (bp): Average length of valid sequences; GC (%): GC content of valid data.