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Discovering neglected lichen diversity with DNA-based inventories: metabarcoding lichen-forming fungi in Bryce Canyon National Park, Utah, USA

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Abstract. National parks and other federally designated natural areas play critical roles in preserving unique habitats, communities, and biodiversity. However, in the United States, it is estimated that 80–90% of species diversity in national parks is presently unknown. Therefore, contemporary biodiversity inventories are critical for conservation, management and establishing baselines for future comparisons. Ongoing efforts to characterize lichen diversity highlight diverse and robust communities in a number of national parks in the USA. In arid regions of the western USA facing ecological transformations, lichens can play a pivotal role for monitoring these changes. Lichen diversity in Bryce Canyon National Park (BRCA) in southern Utah, USA remains nearly completely uncharacterized, despite nearly 100 years as a federally protected area. Our study aims to provide a critical perspective into the lichen diversity of BRCA. Using a metabarcoding community sampling approach, we documented 215 candidate lichen-forming fungal species distributed across ecologically distinct sites in BRCA. At each sampled site, species richness ranged from 104 to 133 species, with no more than 20% shared species among the three sites. The limited overlap between collection sites suggests that BRCA harbors greater diversity than initially thought. We document a number of sensitive lichens, particularly *Usnea* spp. and *Ramalina sinensis*, that should be monitored as air pollution, land use, and impacts of climate change affect biological communities in the park. The inventory also includes unknown species and other species that have not been documented in the western USA. While our DNA-based inventory highlights strikingly rich lichen diversity, future voucher-based collections will be essential for robust taxonomic determinations.

Key words: bulk sampling, DNA barcoding, fungal ITS, high-throughput amplicon sequencing, operational taxonomic units (OTUs), vouchers

Introduction

One of the goals of the national parks system in the United States is to preserve biodiversity by conserving natural resources and protecting wildlife habitats (Westman 1990; Bukovnik 2022). Upon establishment of the National Park Service in 1916, the fundamental purpose of a park is to "conserve the scenery and the natural and historic objects and the wild life [sic] therein", preserving these resources for the future (Bukovnik 2022). Thus, national parks are often considered as refuges for regional biodiversity, and developing strategic plans to design and conduct biological inventory programs are critical to bring parks to an acceptable level of resource awareness (Stohlgren et al. 1995).

The Colorado Plateau in the southwestern United States hosts a wide range of habitats due to altitudinal gradients, diverse geological formations and soils, and variable precipitation patterns (Uhey et al. 2020). These habitats support diverse lichen communities which have been used to monitor ecological and disturbance gradients (Rushforth et al. 1982; St. Clair et al. 2002, 2007; Shrestha & St. Clair 2009; Leavitt et al. 2021; McCune et al. 2022). Characterizing lichen diversity in this complex regional landscape remains challenging, and a substantial proportion of the diversity remains unknown (St. Clair et al. 1993; Henrie et al. 2022; Munger et al. 2022), including lichen diversity in national parks. On the Colorado Plateau, partial lichen surveys have been compiled for Zion National Park (Rushforth et al. 1982), Capitol Reef National Park (Yearsley 1993), Glen Canyon National Recreation Area (Munger et al. 2022), and Cedar Breaks National Monument (Smith 2000). Despite

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these partial surveys, lichen diversity in national parks on the Colorado Plateau remains poorly known (Bennett & Wetmore 2005). To date, there has been no formal attempt to characterize lichen diversity in Bryce Canyon National Park (BRCA).

Located in Garfield and Kane counties on the eastern Paunsaugunt Plateau in southern Utah, BRCA received its initial designation as a national monument in 1923 to preserve its "unusual scenic beauty, scientific interest, and importance" (National Park Service, 2022). Sedimentary deposits in BRCA vary in age and origin, and include sandstones, siltstones, conglomerates, shale, and the iconic Eocene limestones that form Bryce's pink cliffs (Lundin 1989). Wind, rain, freeze and thaw cycles, and chemical weathering have formed the hoodoos, spires, and rock fins that attract tourists every year (Lundin 1989). Ecosystems representative of the Upper Sonoran, Transitional, and Canadian life zones all occur within BRCA, with a mosaic of microenvironments juxtaposing montane and desert ecosystems (Bowers 1991; Fertig & Topp 2009). Elevations range from 1,860 to 2,374 meters above sea level (m a.s.l.), and the park spans multiple level IV ecoregions, from "High Plateaus" to "Escarpments" to "Semiarid Benchlands and Canyonlands". Four main vegetation types have been characterized within the park: a pinyon-juniper woodland belt on badland slopes, a submontane forest belt on moderate slopes with better soil development, montane forests above 2,590 m a.s.l., and some areas of riparian and wetland vegetation (Spence & Buchanan 1993; Fertig & Topp 2009). The variation in elevation, geology, and vegetation contributes to the overall biodiversity in BRCA.

In addition to general surveys of vegetation types that have been made in the park, a comprehensive inventory of the vascular plant flora of BRCA has been completed in recent years (Fertig & Topp 2009). Birds and mammals in the park have also been documented relatively well in BRCA (National Park Service, 2022), but other organismal groups remain relatively unknown.

Evidence of human occupation in BRCA dates from the Paleoindian/early Archaic period through the middle and late archaic periods, with most archeological sites representing short-term residential locales or special use areas (Wenker 2004). Even with the more recent Euro-American settlements in the mid-19th century, little development occurred within what would ultimately become BRCA, except for highways and a tourist lodge. Now, BRCA hosts more than 1.5 million visitors annually (National Park Service, 2021). The high volume of visitors inevitably has an impact on the park and its ecosystems (Call et al. 1981), and one of the main negative effects may be the emissions from vehicles that travel on roads through the park (Steuer 2010). Assessing the human impact on biological communities in BRCA will be critical for meeting the fundamental purpose of a national park, i.e., conserving the scenery and the natural and historic objects for the future.

Lichens may be used as biomonitors, including elemental analysis to measure the effects of air quality and other factors of ecosystem health (Henderson-Sellers & Seaward 1979; Conti & Cecchetti 2001; Nimis et al. 2002; Will-Wolf et al. 2017). Moreover, lichen community composition can indicate the degree to which human land use is impacting ecosystems (Chuquimarca et al. 2019).

Relatively comprehensive lichen inventories have typically required coordinated efforts among taxonomic experts (Lendemer et al. 2013; Spribille et al. 2010, 2020; McCune et al. 2020), and the inventories are based on the identifications of vouchered specimens. However, DNA metabarcoding studies can facilitate a powerful perspective into fungal diversity that has been, to some degree, unattainable using traditional phenotype-based approaches (DeSalle & Goldstein 2019; Nilsson et al. 2019; Baldrian et al. 2021; Tedersoo et al. 2022). While taxonomic identification of many fungal samples remains challenging with DNA metabarcoding studies (Nilsson et al. 2019), incorporating taxonomic expertise and consideration of physical specimens may improve the accuracy of specimen identification (Cao et al. 2016; Sheth et al. 2017; Pappalardo et al. 2021). For lichen-forming fungi (LFF), DNA metabarcoding studies have revealed unprecedented taxonomic diversity in the southwestern USA. In some cases, DNA has been derived from vouchered specimens, documenting higher levels of diversity than what was inferred from phenotype-based identifications alone (Wright et al. 2019; Leavitt et al. 2021; Munger et al. 2022). In other cases, LFF inventories based on metagenomic data alone highlighted a high level of species level diversity in arid regions of the Colorado Plateau, although taxonomic determinations remained ambiguous in many cases (Henrie et al. 2022).

Given the contemporary ecological changes occurring on the Colorado Plateau (Munson et al. 2011; Finger-Higgens et al. 2023), we aimed to expedite the documentation of diversity in lichen communities in BRCA using DNA metabarcoding. Specifically, we (1) created a baseline inventory of LFF occurring at three sites in BRCA, (2) compared LFF community composition among the sampled sites, and (3) identified rare and unknown species that merit additional attention in subsequent voucher-based investigations. To accomplish these aims, we collected bulk community samples and characterized LFF diversity using amplicon-based high-throughput sequencing and DNA metabarcoding. We also completed broad, general surveys at other sites to identify potential sensitive indicator lichens. This initial lichen inventory establishes an essential biodiversity baseline and provides information to inform conservation efforts in a nationally protected area that is experiencing significant ecological changes.

Materials and methods

Site selection and field methods

To represent distinct, but typical, habitats found in BRCA, we selected three sites for lichen community sampling (Fig. 1; Table 1). "Aspen Trough" represented relatively mesic habitat on the eastern edge of the Paunsaugunt Plateau. The site was in a heavily forested broad ravine at a high elevation (2540 m a.s.l.) within BRCA. Here,

Figure 1. General habitat at the three sites sampled for DNA metabarcoding. A – "Mossy Cave", a site with exposed, eroded Claron limestone typical for BRCA in a mixed mountain shrubland community (2110 m a.s.l.); B – "Hat Shop", an exposed, arid habitat in a mixed mountain shrubland dominated by Straight Cliffs sandstone and mudstone, rather than the Claron limestone typical at the other two sites (2160 m a.s.l.); C – "Aspen Trough", a relatively mesic site on the eastern edge of the Paunsaugunt Plateau in a mixed conifer forest (2540 m a.s.l.).

the forest was dominated by a *Abies concolor* and *Quercus gambelii* complex, with *Populus tremuloides* (Tendick et al. 2011; Survey 2022) occurring on Claron limestone. The second site, the "Hat Shop", represented a more exposed, arid habitat at a lower elevation within the park (2160 m a.s.l.). The site extended below the "Hat Shop", a unique geological formation, to the Right Fork of Yellow Creek. This site was dominated by a mixed mountain shrubland complex with *Pinus ponderosa* and *Pinus edulis*, *Quercus gambelii*, and various *Juniperus* species (Tendick et al. 2011). The geological substrate was dominated by Straight Cliffs sandstone and mudstone, rather than the Claron limestone typical at the other two sites (Survey 2022). Finally, the "Mossy Cave" site comprised rocky substrates and geological formations, including the dominant exposed, eroded Claron limestone with more limited erratic mudstone and conglomerates as well (2110 m a.s.l.). The sampling area included a mixed mountain shrubland complex with *Pinus ponderosa* and *P. edulis*, *Quercus gambelii*, and various *Juniperus* species. One of the only perennial streams in BRCA occurs within the "Mossy Cave" sampling area. Each sampling area covered ca. two hectares.

Our previous work suggests that combined community sampling efforts (i.e., samples collected by multiple people) capture higher levels of lichen diversity in metabarcoding surveys of lichen-forming fungi than individual collections (Henrie et al. 2022). Therefore, for sampling at BRCA, lichens were collected by a team of six researchers, composed of five minimally trained technicians and one professional lichenologist (SDL), using an "intuitive meander" sampling approach targeting microhabitats and substrates with the highest lichen diversity. Aided with a 10× hand lens, samples were taken from lichens growing

Table 1. Summary of the three sites in Bryce Canyon National Park sampled for this study in the summer of 2022. The number of short reads, species counts, and cluster counts shown are for lichen-forming fungi only.

Sample area	Habitat type	Geographic coordinates	Altitude [m a.s.l.]	Short reads	Clusters/ species
"Aspen Trough"	Mesic fir/oak woodland in highland plateau; Claron limestone	37.5826°, –112.2262°	2540	55387	410/130
"Hat Shop"	Arid shrubland; Straight Cliffs sandstone and mudstone	37.5589°, –112.1462°	2160	35761	459/133
"Mossy Cave"	Arid shrubland; Claron limestone	37.6639°, –112.1143°	2110	21919	377/104

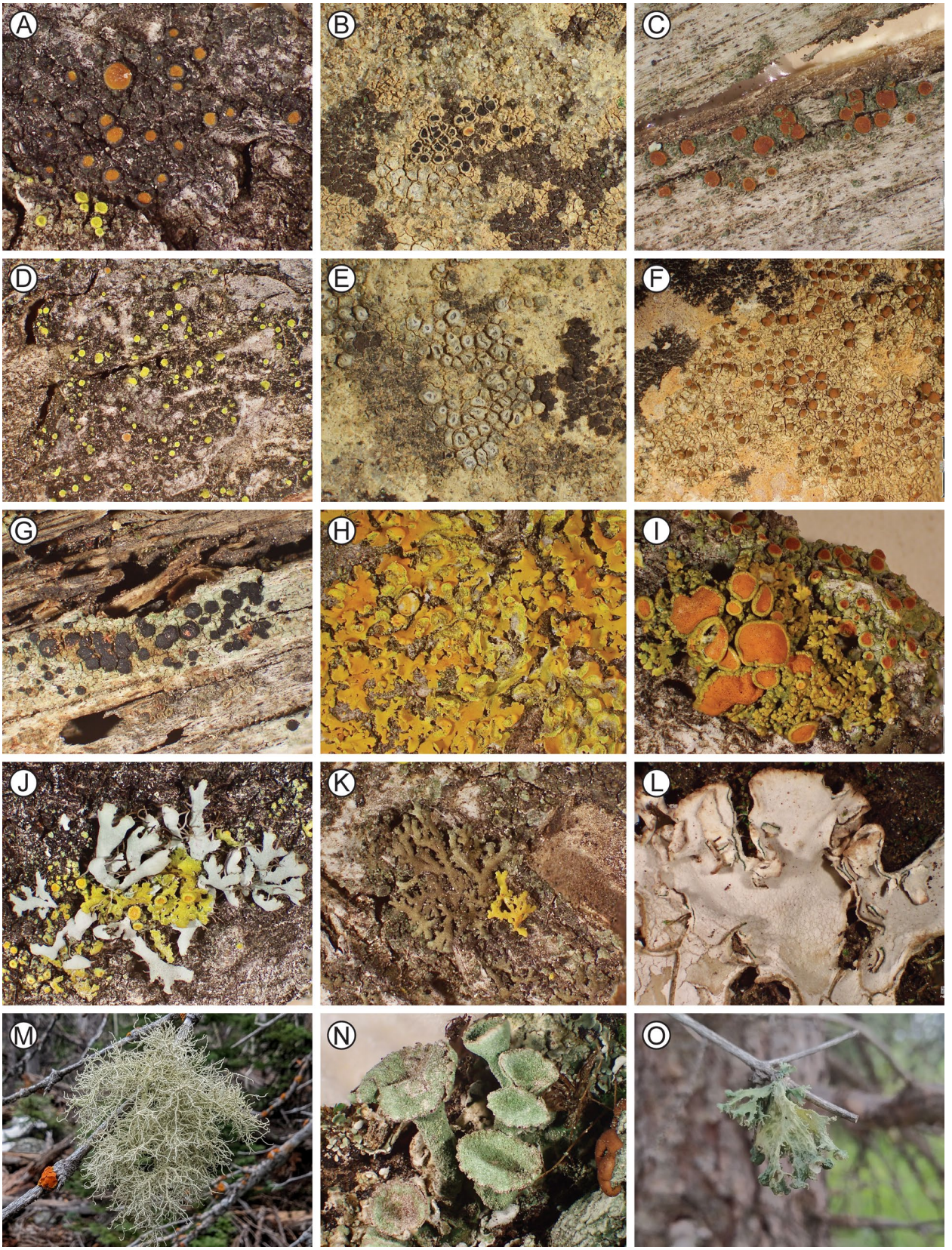

Figure 2. Examples of lichens occurring in BRCA collected for bulk community samples used for DNA metabarcoding. A – *Caloplaca* cf. *chlorina*; B – *Pyrenodesmia* sp.; C – *Caloplaca* s.lat. sp.; D – *Candelariella* cf. *antennaria*; E – *Circinaria* sp.; F – *Protoblastinia* aff. *rupestris*; G – *Lecidella euphorea*; H – *Xanthomendoza fallax*; I – *Xanthomendoza montana*; J – *Physcia adscendens*; K – *Phaeophyscia nigricans*; L – *Peltigera neorufescens*, M – *Usnea perplexens*; N – *Cladonia pocillum*; O – *Ramalina sinensis.*

on cliff faces, boulders and smaller rocks, soils, woody vascular plants, and detritus (Figs 2 & 3). Small, similarly sized portions of lichen thalli were picked or scraped off with sterilized forceps from all potentially different lichens for bulk, metagenomic analyses. At each site, the dry, bulk samples were placed directly into a sterile Nasco Whirl-Pak 18 oz. collecting bag (Nasco, Fort Atkinson, WI, USA). Each member of the team sampled lichens

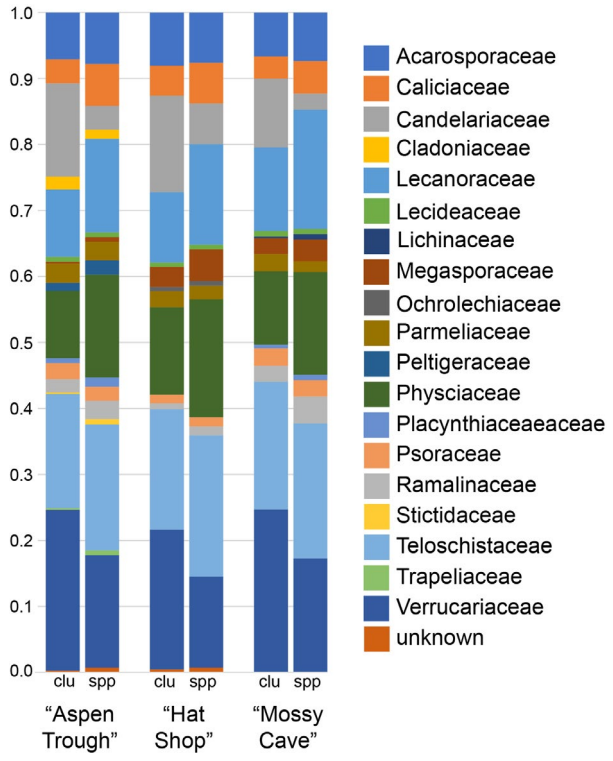

Figure 3. Proportion of lichen-forming fungal species by family at the three sites sampled for DNA metabarcoding – "Aspen Trough", "Hat Shop", and "Mossy Cave". The bar plots represent family diversity at each of the three sampling sites. Both cluster counts ("clu") and species ("spp") counts are included for each site.

for two hours or until 15 minutes had gone by since the last tentative new lichen was observed and collected. All samples were returned to the lab within four hours of collecting and kept at –20°C until DNA extractions were performed.

A limited number of opportunistic lichen collections were made throughout the park to explore potential diversity beyond the three selected sites.

Molecular laboratory methods

DNA was extracted from each of the three bulk community samples (Table 1). Community samples were homogenized using sterilized mortar and pestles, and DNA was extracted from two to four g of homogenized material from each sample using the PowerMax Soil DNA Isolation Kit (Qiagen). To characterize the range of lichen-forming fungal diversity in each meta-community DNA extraction, we amplified the hypervariable ITS2 region using polymerase chain reaction (PCR) with primers ITS3F (GCATCGATGAAGAACGCAGC) and ITS4R (TCCTCCGCTTATTGATATGC) (Op De Beeck et al. 2014). PCR products were sequenced at RTL Genomics (Lubbock, TX, USA), using 2x300 paired-end sequencing on the Illumina MiSeq platform. The complete RTL Genomics amplification and sequencing protocol is described in Nagarkar et al. (2021).

For the samples collected opportunistically at other locations in the park, total genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Sequence data were generated from the entire ITS region (ITS1, 5.8S, ITS2) using the primer pair ITS1f with ITS4. The temperature profile for PCR amplifications of this region followed Leavitt et al. (2018), and PCRs were performed using Cytiva PuReTaq Ready-To-Go PCR Beads (Thermo Fisher Scientific). PCR products were visualized on 1% agarose gel and cleaned using ExoSAP-IT (USB), following the manufacturer's recommendations. Complementary strands were sequenced with the same primers used for PCR amplifications, and sequencing reactions were performed using BigDye 3.1 (Applied Biosystems). Products were run on an ABI 3730 automated sequencer (Applied Biosystems) at the DNA Sequencing Center at Brigham Young University, Provo, Utah, USA.

Short-read processing and analyses

FROGS v3.2 (Find, Rapidly OTUs with Galaxy Solution) was used to analyze ITS2 amplicon metabarcoding data (Escudié et al. 2017; Bernard et al. 2021). We followed the protocol outlined in Bernard et al. (2021). In short, paired-end reads for each sequence in the data were merged, primers were trimmed, and unmatched sequences were discarded in the FROGS v3.2 preprocessing step. Merged reads were then filtered using the FROGS v3.2 swarm clustering tool, and the clusters were formed with the aggregation distance clustering set to 1. Subsequently, chimeric sequences were removed using the chimera removal tool, implementing default parameters. The FROGS v3.2 filtering tool was used to remove low abundance clusters by setting the minimum proportion of sequences to keep OTUs to 0.000005 (from ~6000 total clusters), following Bernard et al. (2021). All remaining clusters were filtered using the ITSx tool to ensure that clusters met requirements for the ITS2 region in preparation for the taxonomic affiliation step. Initial taxonomic assignment of the clusters was completed by comparing the clusters passing filters to the UNITE 8.3 database using the RDP probabilistic classifier (Cole et al. 2013) and BLAST comparisons (Nilsson et al. 2019). All analyses were performed on the Migale Galaxy Server. All non-lichen-forming fungi were excluded from subsequent downstream analyses.

Refining taxonomic assignments

Representative sequences for each cluster representing LFF from the three bulk community samples, inferred using FROGS, were combined with Sanger sequences derived from opportunistic collections made at other sites in BRCA. Following Henrie et al. (2022), family-level multiple sequence alignments (MSAs) were generated from genetic clusters from the ITS2 short read data from BRCA and aligned with ITS sequences from BOLD project LIMW (https://v4.boldsystems.org/index.php/MAS_Management_DataConsole?codes=LIMW; Henrie et al. 2022; Kerr & Leavitt 2023) using the program MAFFT v7 (Rozewicki et al. 2017). We implemented the G-INS-i alignment algorithm and '1PAM / K=2' scoring matrix, with an offset value of 0.1, the 'unalignlevel' = 0.4, and the remaining parameters were set to default values.

Family-level ITS MSAs were analyzed under a maximum likelihood (ML) criterion as implemented in IQ-TREE v2 (Nguyen et al. 2014), with 1,000 ultra-fast bootstrap replicates (Hoang et al. 2017), and the best-fitting substitution model for the entire ITS region was selected using ModelFinder (Kalyaanamoorthy et al. 2017). Trees were visualized using FigTree v1.4.4 (Rambaut 2008). BLAST searches against GenBank were performed to identify the most similar sequences and potentially inferred taxonomic identity in cases where sequences were not recovered within monophyletic candidate species represented in the custom regional database from BOLD.

In addition to characterizing DNA-based diversity at each site, comparisons of species/species hypotheses were made among each site and visualized using the web-based tool InteractiVenn (Heberle et al. 2015).

Results

Illumina ITS2 amplicon short reads generated for this project are available in NCBI's Sequence Read Archive under PRJNA977639, and 82 Sanger sequences generated for this study were deposited in GenBank under accession numbers OR083144–OR083227. Photographs of some lichens observed in BRCA are provided in supplementary file S1.

Although lichen thalli were specifically targeted during field sampling, 53.3% of the clusters were inferred to originate from non-lichenized fungi in the taxonomic assignment step. However, lichen-forming fungi represented 67% of the total read count (supplementary file S2). Most lichen-forming fungal clusters were successfully identified to the genus level, but two clusters remained unidentified at the family level.

Our amplicon-based, metacommunity DNA barcoding approach revealed high diversity of lichen-forming fungi in Bryce Canyon National Park. From the three sampled sites, a total of 544 clusters were inferred to be derived from lichen-forming fungi; and these represented 206 species/species hypotheses in 21 families (supplementary file S2). Of the 206 species hypotheses (SHs), 64 were not represented in a custom regional DNA barcode reference library for lichen-forming fungi of the Intermountain West, USA (Kerr & Leavitt 2023). With the addition of a limited number of opportunistic collections made in BRCA beyond the three sites sampled using metagenomic DNA barcoding, a total of 215 SHs were documented (Table 2). As expected, based on observations during field sampling, most clusters represented crustose lichens, with more limited representation of foliose and fruticose lichens (Fig. 2). The highest lichen-forming fungal species and cluster diversity was inferred in the family *Teloschistaceae*, comprising 18% of the lichen-forming fungal species across all sites (Fig. 3). *Lecanoraceae*, *Physciaceae*, and *Verrucariaceae* also ranked among the best represented families in the three sites. *Lecideaceae*, *Lichinaceae*, *Ochrolechiaceae*, *Stictidaceae*, and *Botryolepraria* (family incertae sedis) were each represented by only a single cluster (Fig. 3). Clusters representing the mycobiont from macrolichens were relatively rare in our results, apart from a limited number of clusters representing *Cladonia*, *Peltigera*, and *Usnea* species at "Aspen Trough".

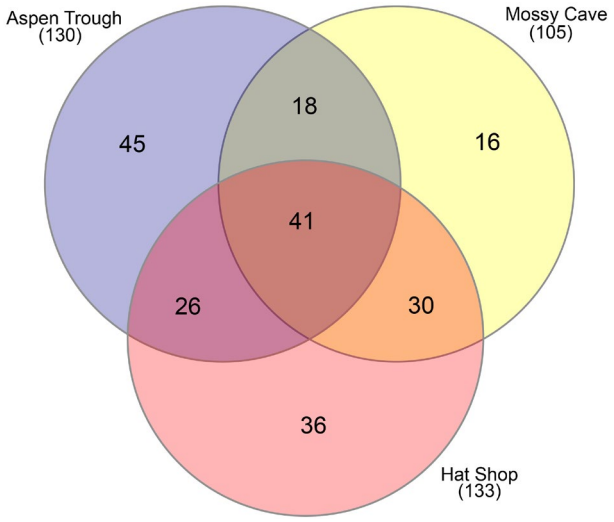

Figure 4. Venn diagram comparison of lichen-forming fungal species among the three sites sampled for DNA metabarcoding – "Aspen Trough", "Hat Shop", and "Mossy Cave". Counts refer to the number of species, rather than clusters or reads. The total species number for each site is included under the site name in parentheses.

Of the three sampled sites, "Hat Shop" and "Aspen Trough" had the highest species-level diversity, with 133 and 130 SHs, respectively (459 and 410 clusters, respectively; Table 1; supplementary file S2). At "Mossy Cave", 105 SHs were inferred representing 377 clusters. While the number of inferred species at each site was relatively similar, there was little overlap in species composition among sites (Fig. 4). Of the 206 SHs inferred, only 41 (~20%) were present at all three sites in BRCA. "Aspen Trough" had the most unique species (45) of the three sites, while "Mossy Cave" had the fewest (16).

A total of 82 sequences were generated using Sanger sequencing from samples collected at other areas in BRCA. These sequences represented a total of 21 SHs, nine of which were not sampled in bulk community samples. In addition to the "Aspen Trough" site, fruticose lichens, *Ramalina sinensis* and *Usnea* spp., were also observed in a mixed conifer/aspen forest along Podunk Creek, northwest of Yovimpa Pass on the south side of BRCA. Family level alignments comprising sequences from the Intermountain West Bold custom reference library, representative ITS2 sequences from each cluster inferred using FROGS, and full ITS sequences from opportunistic sampling, along with the resulting family-level topologies, are provided in supplementary file S3.

A preliminary species list of LFF for BRCA is reported in Table 2.

Discussion

Despite being designated as a national park for nearly a century, the lichen diversity in Bryce Canyon National Park (BRCA) had only superficially been documented. Here, we show that BRCA harbors unique and a previously unrecognized high level of lichen diversity, with

Table 2. Preliminary inventory of lichen-forming fungi (LFF) in Bryce Canyon National Park. The inventory is based on DNA community barcoding at three sites – "Mossy Cave", "Aspen Trough", and "Hat Shop", supplemented with Sanger sequence data generated from limited opportunistic sampling at other sites. LFF species are organized alphabetically by family; "family" rows are subheadings and summarize the total number of DNA clusters (number of species in parentheses) documented to date and the number of species documented at each site. "+" signs indicate whether a species is present at each of the three sites. The 'Sanger data' column includes GenBank accession numbers for ITS sequence data generated from opportunistically collected voucher specimens, with collection numbers from the Herbarium of Non-Vascular Cryptogams in brackets. Taxa in red text indicate lichens represented only from voucher specimens, and not from bulk community samples. Species with provisional names in the custom BOLD regional DNA reference library are indicated by "BOLD LIMW", rather than a taxonomic authority; Candidate species only known from metagenomic data and not linked to known species hypotheses – "spBRCAXX" – do not include any taxonomic authority. In the 'Known distribution' column 'regional' indicates the species/species hypothesis is known to occur in the Colorado Plateau, and 'na' indicates that the extent of distribution of the species/species hypothesis is not currently known. Sampled lichenicolous fungi not shown in the table include: *Corticifraga* sp. (OR083227 [v003]), *Didymocyrtis epiphyscia* (OR083212 & OR083213[v093]), *Rhinocladiella* sp. (OR083217 [v086]), and an unknown lichenicolous fungal species (OR083218 [v104]).

Species	# Clusters	Mossy Cave	Aspen Trough	Hat Shop	Sanger data	Known distribution
Acarosporaceae	45 (19)	9 spp.	11 spp.	11 spp.	1 sp.	
Acarospora americana H. Magn.	7			+		regional
Acarospora aff. *glaucocarpa* (Ach.) Körb.	2		+			regional
Acarospora aff. *strigata* 1 BOLD LIMW	1			+		regional
Acarospora aff. *strigata* 2 BOLD LIMW	4		+	+		regional
Acarospora aff. *strigata* 3 BOLD LIMW	2		+	+		regional
Acarospora tintickiana St. Clair, Newberry & S. Leavitt	1	+				regional
Acarospora 'spBRCA01'	2	+		+		na
Acarospora s.lat. sp. 1 BOLD LIMW	4	+	+	+		regional
Polysporina gyrocarpa 2 BOLD LIMW	4	+		+		regional
Polysporina leavittii K. Knudsen & Hollinger	5	+	+	+		regional
Polysporina 'spBRCA02' BOLD LIMW	1		+			regional
Polysporina 'spBRCA03' BOLD LIMW	5	+	+	+		regional
Sarcogyne aff. *bernardinensis* K. Knudsen, J.N. Adams, Kocourk. & Y. Wang	1		+			new to region
Sarcogyne aff. *hypophaea* (Nyl.) Arnold	1			+		regional
Sarcogyne wheeleri K. Knudsen, J.N. Adams, Kocourk. & Y. Wang	1	+				regional
Sarcogyne 'spBRCA04'	2	+	+			na
Sarcogyne 'spBRCA05'	2		+	+		na
Sarcogyne 'spBRCA06'	1	+	+			na
Sarcogyne 'spBRCA07'	NS				OR083201 (v009)	na
Caliciaceae	23 (12)	6 spp.	9 spp.	9 spp.	2 spp.	
Amandinea s.lat. sp. 1 BOLD LIMW	2		+	+		regional
Amandinea s.lat. sp. 2 BOLD LIMW	2	+	+	+		regional
Amandinea s.lat. 'spBRCA08'	3	+	+	+		na
Buella 'spBRCA09'	1		+	+		na
Buella 'spBRCA10'	2	+	+	+		na
Buellia 'spBRCA11'	1	+	+	+		na
Buellia s.lat. 'spBRCA12'	2	+	+	+		na
Diplotomma venustum (Körb.) Körb.	5			+		regional
Tetramelas chloroleucus (Körb.) A. Nordin	NS				OR083192 (v074), OR083193 (v076), OR083194 (v085), OR083195 (v107), OR083196 (v082)	regional
Tetramelas insignis (Nägeli) Kalb group	1		+			regional
Tetramelas 'spBRCA13'	1		+		OR083197 (v114)	na
Tetramelas aff. *pulverulentus* (Anzi) A. Nordin & Tibell	3	+		+		new to region
Candelariaceae	67 (9)	3 spp.	5 spp.	9 spp.	NS	
Candelariella '*antennaria* clade' Räsänen	14	+	+	+		regional
Candelariella '*aurella* clade' (Hoffm.) Zahlbr.	23	+	+	+		regional
Candelariella '*rosulans* clade' (Müll.Arg.) Zahlbr.	18		+	+		regional
Candelariella '*vitellina* clade' (Hoffm.) Müll.Arg.	1			+		regional
Candelariella 'spBRCA15'	1			+		na
Candelariella 'spBRCA16'	2		+	+		na
Candelariales (incertae sedis) sp. 1 BOLD LIMW	5			+		regional
Candelariales (incertae sedis) 'spBRCA13'	2	+		+		na
Candelariales (incertae sedis) 'spBRCA14'	1		+	+		na
Cladoniaceae	8 (3)	NS	1 sp.	NS	1 sp.	

Table 2. Continued.

Species	# Clusters	Mossy Cave	Aspen Trough	Hat Shop	Sanger data	Known distribution
Cladonia cariosa agg. (Ach.) Sprengel	7		+		OR083144 (v001), OR083145 (v057), OR083146 (v058), OR083147 (v072), OR083148 (v089)	regional
Cladonia fimbriata (L.) Fr.	1		+		OR083152 (v002), OR083149 (v059)	regional
Cladonia pocillum (Ach.) O.J. Rich.	NS				OR083150 (v060), OR083151 (v0730	regional
Lecanoraceae	57 (30)	22 spp.	20 spp.	22 spp.	6 spp.	
Lecanora aff. *albellula* 1 BOLD LIMW	1	+	+		OR083171 (v099)	regional
Lecanora anopta Nyl.	1		+	+		new to region
Lecanora aff. *chlarotera* Nyl.	1	+		+		regional
Lecanora polytropa '56' BOLD LIMW	1			+		regional
Lecanora prolificans in ed.	1		+		OR083169 (v079), OR083170 (v095)	regional
Lecanora saligna (Schrader) Zahlbr.	1	+	+	+		regional
Lecanora s.lat. 'spBRCA17'	1	+	+			na
Lecanora s.lat. 'spBRCA18'	1	+	+	+		na
Lecanora s.lat. 'spBRCA19'	1		+	+		na
Lecanora s.lat. 'USCRNA2'	2	+	+	+	OR083168 (v077)	na
Lecidella elaeochroma (Ach.) M. Choisy	2	+	+	+	OR083198 (v083)	regional
Lecidella euphorea (Flörke) Nyl.	2	+	+	+	OR083199 (v103), OR083200 (v110)	regional
Lecidella stigmatea agg. (Ach.) Hertel & Leuckert	15	+	+	+	OR083172 (v081), OR083173 (v100), OR083173 (v097)	regional
Myriolecis altaterrae in ed.	2	+	+	+		regional
Myriolecis flowersiana (H. Magn.) Śliwa, Zhao Xin & Lumbsch	2	+	+	+		regional
Myriolecis aff. *flowersiana* (H. Magn.) Śliwa, Zhao Xin & Lumbsch	1	+		+		regional
Myriolecis aff. *semipallida* 1 BOLD LIMW	1		+			regional
Myriolecis aff. *semipallida* 2 BOLD LIMW	2			+		regional
Myriolecis wetmorei (Śliwa) Śliwa, Zhao Xin & Lumbsch	2	+	+	+		regional
Myriolecis aff. *zosterae* 1 BOLD LIMW	5	+		+		regional
Myriolecis 'spBRCA20'	2	+	+	+		na
Myriolecis 'USCRNA1' BOLD LIMW	1		+			regional
Myriolecis myrio sp. 1 BOLD LIMW	1	+	+	+		regional
Myriolecis myrio sp. 3 BOLD LIMW	1	+		+		regional
Myriolecis sp. 1 BOLD LIMW	1			+		regional
Myriolecis sp. 2 BOLD LIMW	2	+	+	+		regional
Myriolecis sp. 3 BOLD LIMW	1	+	+			regional
Myriolecis sp. 4 BOLD LIMW	1	+				regional
Rhizoplaca melanophthalma (DC.) Leuckert & Poelt	1	+				regional
Rhizoplaca s.lat. 'spBRCA21'	1	+		+		na
Lecideaceae	3 (1)	1 sp.	1 sp.	1 sp.	NS	
Lecideaceae (genus identity unknown) 'spBRCA22'	3	+	+	+		na
Lichinaceae	1 (1)	1 sp.	NS	NS	NS	
Lichinales 'spBRCA23'	1	+				na
Megasporaceae	15 (8)	4 spp.	1 sp.	7 spp.	1 sp.	
Aspicilia determinata (H. Magn.) J.C. Wei	2			+		regional
Aspicilia diploschistiformis McCune & J. Di Meglio	1		+		OR083175 (v066), OR083176 (v067)	new to region
Circinaria calcarea (L.) Mudd	5	+		+		regional
Circinaria cf. *calcarea* (L.) Mudd	2			+		regional
Lobothallia 'spBRCA24'	1			+		na
Megaspora rimisorediata Valadbeigi & A. Nordin	1	+		+		regional
Megaspora verrucosa (Ach.) Hafellner & V. Wirth	1	+		+		regional
Teuvoa junipericola Sohrabi & S. Leavitt	2	+		+		regional
Ochrolechiaceae	3 (1)	NS	NS	1 sp.	NS	

Table 2. Continued.

Species	# Clusters	Mossy Cave	Aspen Trough	Hat Shop	Sanger data	Known distribution
Ochrolechiaceae 'spBRCA25'	3			+		na
Parmeliaceae	12 (5)	2 spp.	4 spp.	3 spp.	3 sp.	
Melanohalea elegantula (Zahlbr.) Essl.	1	+	+	+		regional
Melanohalea subolivaceae (Nyl.) Essl.	9	+	+	+	OR083156 (v070), OR083157 (v113), OR083158 (v121)	regional
Melanohalea aff. *subolivacea* (Nyl.) Essl.	1		+			regional
Usnea perplexans Stirt.	1		+	+	OR083153 (v010), OR083154 (v064a)	regional
Usnea hirta (L.) Weber ex F.H. Wigg.	1				OR083155 (v064b)	regional
Peltigeraceae	5 (3)	–	3 spp.	–	3 spp.	
Peltigera monticola Vitik.	2		+		OR083225 (v005)	regional
Peltigera neorufescens Goward & Manoharan-Basil	2		+		OR083219 (v004), OR083220 (v006), OR083224 (v008), OR083222 (v054), OR083223 (v055), OR083221 (v056)	regional
Peltigera ponojensis Gyelnik	1		+		OR083226 (v007)	regional
Pertusariales	2 (1)	NS	NS	1 sp.	NS	
Pertusariales s.lat. 'spBRCA64'	2			+		na
Physciaceae	69 (33)	19 spp.	22 spp.	26 spp.	3 spp.	
Phaeophyscia 'spBRCA29'	1			+		na
Phaeophyscia aff. *hirsuta* (Mereschk.) Essl.	1	+	+	+	OR083189 (v080), OR083190 (v088), OR083191 (v096)	regional
Phaeophyscia cf. *chloantha* (Ach.) Vain.	4	+	+	+		regional
Phaeophyscia cf. *hirsuta* (Mereschk.) Essl.	3			+		regional
Phaeophyscia nigricans (Flörke) Moberg	1		+			regional
Phaeophyscia orbicularis (Necker) Moberg	1		+			regional
Physcia adscendens (Fr.) H. Olivier	1	+	+	+	OR083186 (v116), OR083187 (v122), OR083188 (v119)	regional
Physcia aff. *biziana* (A. Massal.) Zahlbr.	2	+	+		OR083178 (v101), OR083179 (v102), OR083184 (v105), OR083181 (v106), OR083180 (v108), OR083185 (v109), OR083182 (v115), OR083183 (v118)	regional
Physcia caesia (Hoffm.) Furnr.	1			+		regional
Physcia dimidiata (Arnold) Nyl.	4	+	+	+		regional
Physcia dubia (Hoffm.) Lettau	1	+				regional
Physcia magnussonii Frey	1		+			regional
Physconia enteroxantha (Nyl.) Poelt	1		+	+		regional
Rinodina s.lat. *juniperina* Sheard	2	+	+	+		regional
Rinodina s.lat. aff. *lobothalloides* in ed.	1			+		regional
Rinodina s.lat. *luridata* 2 BOLD LIMW	10			+		regional
Rinodina s.lat. *riparia* Sheard	2	+		+		regional
Rinodina s.lat. *straussii* J. Steiner	4	+	+	+		regional
Rinodina s.lat. 'spBRCA27'	4	+	+	+		na
Rinodina s.lat. 'spBRCA28'	1			+		na
Rinodina s.lat. 'spBRCA30'	2	+		+		na
Rinodina s.lat. 'spBRCA31'	1	+	+			na
Rinodina s.lat. 'spBRCA32'	1		+	+		na
Rinodina s.lat. 'spBRCA33'	1	+	+	+		na
Rinodina s.lat. 'spBRCA34'	1	+		+		na
Rinodina s.lat. 'spBRCA35'	3		+	+		na
Rinodina s.lat. 1 BOLD LIMW	4	+		+		regional
Rinodina s.lat. 'GCNRA' BOLD LIMW	1		+	+	OR083177 (v066)	regional
Rinodina s.lat. 'USCRNA' BOLD LIMW	1	+	+	+		regional

Table 2. Continued.

Species	# Clusters	Mossy Cave	Aspen Trough	Hat Shop	Sanger data	Known distribution
Rinodina bischoffii (Hepp) A. Massal.	2	+	+	+		regional
Rinodina grandilocularis in ed.	3	+	+	+		regional
Rinodina aff. *obnascens* (Nyl.) Oliv.	1		+			regional
Rinodina zwackhiana (Krempelh.) Körb.	2	+	+	+		regional
Placynthiaceae	3 (2)	1 sp.	2 spp.	NS	NS	
Placynthium aff. *nigrum* 1 BOLD LIMW	2	+	+			regional
Placynthium aff. *nigrum* 2 BOLD LIMW	1		+			regional
Psoraceae	12 (6)	3 spp.	3 spp.	2 spp.	NS	
Protoblastinia aff. *rupestris* group	NA				OR083166 (v011), OR083167 (v015)	na
Protoblastinia aff. *rupestris* 1 BOLD LIMW	1		+			regional
Psora 'spBRCA36'	1	+		+		na
Psora aff. *elenkinii* 1 BOLD LIMW	4	+	+			regional
Psora cerebriformis W.A. Weber	1		+			regional
Psora montana Timdal	5	+	+	+		regional
Ramalinaceae	9 (6)	5 spp.	4 spp.	2 spp.	2 spp.	
Bibbya vermifera (Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman	1	+	+	+		regional
Lecania clairi in ed.	1	+				regional
Ramalina sinensis Jatta	NA				OR083211 (v062)	regional
Thalloidima candidum (Weber) A. Massal.	3	+	+			regional
Toninia s.lat. 'spBRCA38'	1	+				na
Toniniopsis 'spBRCA37'	3	+	+	+		na
Stictidaceae	1 (1)	NS	1	NS	NS	
Stictidaceae 'spBRCA39'	1		+			na
Teloschistaceae	94 (40)	25 spp.	27 spp.	31 spp.	3 spp.	
Athallia aff. *cerinella* (Nyl.) Arup, Frödén & Søchting	1	+	+	+		new to region
Athallia sp. BOLD LIMW	1	+	+	+		regional
Blastenia 'spBRCA40'	1	+	+	+		na
Blastenia furfuracea (H. Magn.) Arup, Søchting & Frödén	NA				OR083207 (v079), OR083208 (v087)	regional
Calogaya biatorina (A. Massal.) Arup, Frödén & Søchting	1	+	+	+		regional
Calogaya decipiens (Arnold) Arup, Frödén & Søchting	1			+		regional
Calogaya ferrugineoides (H. Magn.) Arup, Frödén & Søchting	2	+		+		regional
Calogaya saxicola (Hoffm.) Vondrák	2	+	+	+		regional
Caloplaca chlorina (Flotow) H. Olivier	1	+	+	+		regional
Gyalolechia epiphyta Lynge	2	+		+		regional
Parvoplaca 'spBRCA41'	3	+	+	+		na
Polycaulina 'USCRNA 1' BOLD LIMW	5		+	+		regional
Polycaulina 'USCRNA 2' BOLD LIMW	NA				OR083160 (v063), OR083161 (v091)	regional
Pyrenodesmia 'spBRCA42'	1		+	+		na
Pyrenodesmia 'spBRCA43'	6	+	+	+	OR083165 (v067)	na
Pyrenodesmia 'spBRCA44'	1			+		na
Pyrenodesmia 'spBRCA45'	1			+		na
Pyrenodesmia 'spBRCA46'	1			+		na
Pyrenodesmia 'spBRCA47'	1	+	+			na
Pyrenodesmia aff. *atroalba* 1 BOLD LIMW	7	+		+	OR083203 & OR083206 (v066), OR083204 (v068)	regional
Pyrenodesmia aff. *atroalba* 2 BOLD LIMW	1	+	+	+		regional
Pyrenodesmia cf. *albovariegata* B. de Lesd.	1		+		OR083205 (v067)	regional
Pyrenodesmia cf. *atroalba* (Tuck.) I.V. Frolov & Vondrák	1		+			regional
Pyrenodesmia variabilis s.lat. 1 BOLD LIMW	10	+	+	+		regional
Pyrenodesmia variabilis s.lat. 2 BOLD LIMW	3		+			regional
Rusavskia elegans 1 BOLD LIMW	2	+	+	+		regional
Rusavskia elegans 2 BOLD LIMW	3	+	+	+		regional
Rusavskia elegans 3 BOLD LIMW	1	+	+	+		regional
Rusavskia sorediata (Vain.) S.Y. Kondr. & Kärnefelt	1		+			regional

Table 2. Continued.

Species	# Clusters	Mossy Cave	Aspen Trough	Hat Shop	Sanger data	Known distribution
Tayloriellina microphyllina (Tuck.) Søchting & Arup	2	+		+		regional
Variospora 'spBRCA48'	5	+	+	+		na
Variospora 'spSEIRO1' BOLD LIMW	2		+	+	OR083209 (v013), OR083210 (v014)	regional
Variospora dolomiticola (Hue) Arup, Søchting & Frödén	1			+		new to region
Xanthocarpia 'spBRCA49'	1		+			na
Xanthocarpia crenulatella 1 BOLD LIMW	1	+	+			regional
Xanthocarpia marmorata (Bagl.) Jatta	2	+	+	+		new to region
Xanthomendoza 'spUT' BOLD LIMW	1	+	+	+		regional
Xanthomendoza fallax (Arnold) Søchting, Kärnefelt & S.Y. Kondr.	2	+		+		regional
Xanthomendoza montana (L. Lindblom) Søchting, Kärnefelt & S.Y. Kondr.	13	+	+	+	OR083162 (v078), OR083163 (v094), OR083164 (v102)	regional
Trapeliaceae	1 (2)	-	1	-	1 sp.	
Xylographa 'spBRCA50'	1		+			na
Trapeliopsis_flexulosa (Fr.) Coppins & P. James					OR083159 (v065)	
family incertae sedis	1 (1)	-	1	-	NS	
Botryolepraria 'spBRCA16'	1		+			genus new to region
Verrucariaceae	113 (33)	21 spp.	24 spp.	20 spp.	2 spp.	
Dermatocarpon miniatum (L.) W. Mann	1		+			regional
Dermatocarpon taminium Heiðmarsson	1		+	+		regional
Dermatocarpon moulinsii (Mont.) Zahlbr.	1	+	+	+		regional
Endocarpon deserticola T. Zhang, X. L. Wei & J. C. Wei	1	+		+		regional
Heteroplacidium aff. *fusculum* (Nyl.) Gueidan & Cl. Roux	1			+		new to North America
Heteroplacidium aff. *zamenhofianum* (Clauzade & Cl. Roux) Gueidan & Cl. Roux	4		+	+		regional
Placidium rufescens (Ach.) A. Massal.	1	+				regional
Placidium pilosellum (Breuss) Breuss	1			+		regional
Placidium 'sp9V' BOLD LIMW	3	+		+		regional
Psoroglaena 'spBRCA51'	1		+			genus new to region
Staurothele 'clade1' BOLD LIMW	30	+	+	+	OR083214 (v067), OR083215 (v069)	regional
Staurothele elenkinii Oksner	3		+	+		regional
Staurothele monicae (Zahlbr.) Wetmore	3	+	+	+	OR083216 (v012)	regional
Verrucaria 'spK' BOLD LIMW	3	+	+	+		regional
Verrucaria bernardinensis Breuss	10	+	+	+		regional
Verrucaria muralis Ach.	4	+	+	+		regional
Verrucaria aff. *nigricans* (Nyl.) Zschacke	3	+	+	+		new to North America
Verrucaria s.lat. 'spBRCA52'	4	+	+	+		na
Verrucaria s.lat. 'spBRCA53'	1		+			na
Verrucaria s.lat. 'spBRCA54'	1	+				na
Verrucaria s.lat. 'spBRCA55'	1	+	+			na
Verrucaria s.lat. 'spBRCA56'	4		+	+		na
Verrucaria s.lat. 'spBRCA57'	1		+			na
Verrucaria s.lat. 'spBRCA58'	1		+			na
Verrucaria s.lat. 'spBRCA59'	1	+				na
Verrucaria s.lat. 'spBRCA60'	1	+		+		na
Verrucaria s.lat. 'spBRCA61'	1	+	+			na
Verrucaria s.lat. 'spBRCA62'	3	+				na
Verrucaria s.lat. 'spBRCA63'	1		+			na
Verrucaria s.lat. sp. 2 BOLD LIMW	9	+	+	+		regional
Verrucaria s.lat. sp. 3 BOLD LIMW	9	+	+	+		regional
Verrucariaceae sp. 1 BOLD LIMW	2	+	+			regional
Verruculopsis poeltiana (Clauzade & Cl. Roux) Gueidan, Nav.-Ros. & Cl. Roux	2	+	+	+		new to North America

a total of 215 species hypotheses (SHs) documented in this study. This is in line with the expectation that national parks are refuges for biodiversity (Beissinger et al. 2019). While our results revealed high lichen diversity in BRCA, the limited overlap between the three sampling sites within the park suggest that diversity is likely higher than our data show. We predict that other unsampled sites in BRCA with varying substrates, soils, vegetation types, and microclimates likely harbor other lichens not documented here (Sharon et al. 2002).

Given the paucity of lichen diversity data for many national parks in the western USA (Bennett & Wetmore 2005), DNA-based surveys provide a promising approach for more rapidly characterizing this overlooked component of biodiversity (Kelly et al. 2011; Wright et al. 2019; Henrie et al. 2022). Thorough lichen inventories are presently unavailable for most national parks on the Colorado Plateau in the southwestern USA, although a number of partial inventories suggest high levels of lichen diversity. For example, Rushforth et al. (1982) documented 104 lichens at seven sites in Zion National Park; 52 corticolous lichens were documented at 12 sites in Capitol Reef National Park (Yearsley 1993); Munger et al. (2022) found 100 lichen species at a single site in Glen Canyon National Recreation Area; and over 200 lichens have been documented from Cedar Breaks National Monument and other nearby sites on the western edge of the Markagunt Plateau, Iron County, Utah (Smith 2000). In comparison, our results revealed higher lichen diversity based on sampling limited to only three sites in BRCA. Similar DNA metabarcoding approaches could be systematically applied across national parks with incomplete data to establish lichen baseline data that would likely be unattainable in a reasonable timeframe using traditional inventories (Geml et al. 2014).

During field sampling, species detection is not constant through time, and a small proportion of the species diversity will likely remain unsampled even with increasing effort, regardless of the sampling approach (e.g., voucher-based vs. metabarcoding). However, bulk sampling and DNA metabarcoding appear to capture the majority of LFF diversity more effectively within a site than vouchered-based inventories (Wright et al. 2019). Our voucher-based lichen inventory work in the Intermountain West has historically relied on a minimal team of expert lichenologists (one to two individuals), rather than a team of six researchers collecting bulk samples for metagenomic analyses, as used in this study. Including multiple field technicians and experts is critical to comprehensively characterize LFF diversity using metabarcoding approaches (Henrie et al. 2022). While the total sampling time (human hours) may be higher in the field, lichen diversity can be more thoroughly sampled using bulk sampling methods by eliminating the need for collecting physical vouchers. Similarly, time spent processing, curating and identifying specimens is eliminated, or minimized, with bulk sampling and metabarcoding. Once the field sampling component is completed for metabarcoding studies, standardized sequencing processing pipelines facilitate time- and cost-efficient data analyses (Bernard et al. 2021). However, this efficiency comes at a cost. The lack of permanent, physical specimens (vouchers) often results in taxonomic ambiguity. In cases of putative new species/new records, phenotype-based comparisons, etc., relocating and collecting the lichens of interest incurs additional costs.

Based on records available on the Consortium of Lichen Herbaria (CLH, https://lichenportal.org/; accessed 08 February 2023), only 24 lichens are listed for BRCA. Notably, several conspicuous lichens listed on CLH for BRCA were not observed in 2022, including *Xanthoparmelia* and *Letharia* species. *Xanthoparmelia* species rarely occur on limestone, and sporadic populations may occur in BRCA on the less dominant sandstone substrates in the park. However, the historic records of *Letharia* species in BRCA are unexpected, as members of this genus are nearly completely absent from the Colorado Plateau region. Rediscovering the *Letharia* population in the park should be prioritized and monitored to better understand this exceptional population.

Similarly, several soil crust lichens that have previously been seen in the park, including several *Psora* species and *Placidium squamulosum*, were not sampled for this study. We anticipate that future sampling targeting potential habitats supporting biological soil crust communities will likely reveal additional lichen diversity. Suitable habitat for biological soil crust communities has been observed in low elevation pinyon-juniper woodlands near Yellow Creek, on the plateau near East Creek meadow, and near a small tributary meadow near Swamp Creek overlook (T. Olstad [National Park Service], personal comm.).

While DNA-based inventories hold promise for more rapidly characterizing LFF diversity, the absence of vouchered specimens limits the taxonomic interpretation of this diversity (Mark et al. 2016), particularly with incomplete DNA reference libraries (Nilsson et al. 2019). Some species inferred from our data represent unexpected occurrences in the region, such as *Botryolepraria* sp., a saxicolous crustose lichen of uncertain familial placement previously observed only in the eastern USA. To verify the presence of unique species such as this, vouchered specimens are necessary (Bell et al. 2020). Furthermore, ~30% of the SHs documented at BRCA were not represented in the Intermountain West custom DNA reference library on BOLD (Kerr & Leavitt 2023). In the absence of vouchered specimens, these can only be treated with provisional names within the taxonomic level wherein they can be identified with confidence, e.g., order-, family-, or genus-level. Among the unidentified lichen-forming fungal SHs inferred at BRCA (Table 2), it is impossible to know if these are unique to BRCA or simply have not been previously sequenced and incorporated into our regional reference library.

Ongoing efforts to create regional databases, (e.g., Marthinsen et al. 2019; Kerr & Leavitt 2023) will be crucial for robust taxonomic assignments in DNA-based inventory research. In other cases, lichen inventories may be based on traditional voucher specimens coupled with

sequence data, linking known physical specimens directly with generated sequences. Recently, this approach has revealed higher levels of lichen diversity in the western USA than traditional inventory methods (Wright et al. 2019; Leavitt et al. 2021; Munger et al. 2022) . However, this integrative approach may introduce other unexpected taxonomic conundrums, such as revealing taxa that are unidentifiable in the field or otherwise genuinely cryptic, difficulties linking formally described species with the appropriate coinciding clade, and determining how to apply standardized taxonomic designations for less resolved identifications (Lücking et al. 2021).

Despite the lack of vouchered specimens, our DNA metabarcoding data is still valuable. Due to the lack of funding for biodiversity research and decreasing numbers of relevant taxonomic experts (Drew 2011; Grube et al. 2017; Stroud et al. 2022), standardized metabarcoding approaches provide a promising path forward to survey remaining unexplored areas. Representative ITS2 sequences are provided for all the candidate species (supplementary file S2), facilitating direct comparisons with other DNA-based studies. Furthermore, the short-read data are interoperable with other ITS2 amplicon-based datasets and can be integrated with other LFF metagenomic surveys (Wilkinson et al. 2016), including future research in BRCA. Similarly, because these data are reusable, taxonomic assignments can be easily revised in the future with improvements to DNA reference libraries.

From a conservation perspective, our lichen inventory at BRCA highlights potential bioindicator lichens to track ecological gradients. Corticolous lichens, particularly fruticose members of *Letharia* (not sampled in the present study, but historically documented in BRCA), *Ramalina*, and *Usnea* species are sensitive to climate and air quality change, and these restricted populations found in the park may be useful for biomonitoring in response to forecast climate change (Buckley & Foushee 2012; Copeland et al. 2017; Robison et al. 2022). The changing climate can affect species ranges, and may also increase drought and wildfire frequency, in addition to changes in monsoonal precipitation patterns (Munson et al. 2011), all of which can negatively impact biodiversity (Bellard et al. 2012).

Conclusions

All three sites in BRCA sampled for this study showed diverse and unique lichen communities. The fact that less than 20% of the LFF species were shared among all three sampling sites suggests that our data only show a portion of the park's diversity. Characterizing lichen diversity at other sites throughout BRCA should be prioritized, including relocating a population of *Letharia lupina* documented in 1976. Furthermore, a significant portion of the putative LFF species found in BRCA could not be assigned to formally described species, indicating that Bryce Canyon may harbor a unique component of lichen diversity in the Intermountain West. Voucher specimens should be collected to (1) verify presence of unexpected lichens, (2) assign samples inferred from sequence data to described species based on phenotypic data, and (3) identify and describe any species new to science. Park visitation and land use should be evaluated so that vulnerable populations of sensitive or rare lichens can be protected and monitored. "Aspen Trough" and the mixed conifer forest along Podunk Creek, northwest of Yovimpa Pass are presently the only known sites with fruticose lichens within the park, with *Ramalina sinensis* found so far only at the latter site. Continual updates to the lichen checklist for BRCA may provide insight into novel strategies for monitoring ecological disturbance and enhancing appreciation and deeper connections within the park (Halliwell et al. 2022).

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Supplementary electronic materials

File S1. Photos from a sample of lichen thalli observed across sites in Bryce Canyon National Park, Utah, USA. Download file

File S2. The complete list of ITS2 clusters generated using FROGS v3.2, and a filtered list of clusters representing lichen-forming fungi. Download file

File S3. Family-level ITS alignments and trees based on ITS2 clusters and Sanger sequencing data generated for this study and combined with sequences from BOLD project LIMW (https://v4.boldsystems.org/index.php/MAS_Management_DataConsole?codes=LIMW; Kerr and Leavitt 2023). Download file

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