

A taxonomically broad metagenomic survey of 339 species spanning 57 families suggests cystobasidiomycete yeasts are not ubiquitous across all lichens

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PREMISE: Lichens are fungi that enter into obligate symbioses with photosynthesizing organisms (algae, cyanobacteria). Traditional narratives of lichens as binary symbiont pairs have given way to their recognition as dynamic metacommunities. Basidiomycete yeasts, particularly of the genus *Cyphobasidium*, have been inferred to be widespread and important components of lichen metacommunities. Yet, the presence of basidiomycete yeasts across a wide diversity of lichen lineages has not previously been tested.

METHODS: We searched for lichen-associated cystobasidiomycete yeasts in newly generated metagenomic data from 413 samples of 339 lichen species spanning 57 families and 25 orders. The data set was generated as part of a large-scale project to study lichen biodiversity gradients in the southern Appalachian Mountains Biodiversity Hotspot of southeastern North America.

RESULTS: Our efforts detected cystobasidiomycete yeasts in nine taxa (*Bryoria nadvornikiana*, *Heterodermia leucomelos*, *Lecidea roseotincta*, *Opegrapha vulgata*, *Parmotrema hypotropum*, *P. subsumptum*, *Usnea cornuta*, *U. strigosa*, and *U. subgracilis*), representing 2.7% of all species sampled. Seven of these taxa (78%) are foliose (leaf-like) or fruticose (shrubby) lichens that belong to families where basidiomycete yeasts have been previously detected. In several of the nine cases, cystobasidiomycete rDNA coverage was comparable to, or greater than, that of the primary lichen fungus single-copy nuclear genomic rDNA, suggesting sampling artifacts are unlikely to account for our results.

CONCLUSIONS: Studies from diverse areas of the natural sciences have led to the need to reconceptualize lichens as dynamic metacommunities. However, our failure to detect cystobasidiomycetes in 97.3% (330 species) of the sampled species suggests that basidiomycete yeasts are not ubiquitous in lichens.

KEY WORDS Biodiversity inventory; endophyte; lichenicolous; metagenome; obligate symbiont; parasite.

Lichens are a remarkable, evolutionarily heterogeneous assemblage of fungi that have converged on similar symbiotic strategies involving partnerships of fungi with photosynthetic algae or cyanobacteria (Lutzoni et al., 2001; Gueidan et al., 2008; Schoch et al., 2009). Taxonomic diversity of these fungi is estimated to be high, with more than 50,000 species described to date (Hawksworth, 1991; Hawksworth and Lücking, 2017). This diversity is distributed across multiple lineages of both ascomycetes and basidiomycetes (Lücking et al., 2016) and across terrestrial biomes worldwide (Brodo et al., 2001; Arcadia, 2013), wherein lichens serve numerous important

ecological roles (Slack, 1988; Seaward, 1996; Belnap and Lange, 2005; Cornelissen et al., 2007; Asplund and Wardle, 2017).

For more than a century, lichen symbioses have been thought to comprise individual fungal species that associate with either a single green alga or cyanobacterium (Ahmadjian, 1967; Lorch, 1988; Honegger, 1991, 1992; Mitchell, 2007). In a limited number of lineages (3–4%; Yoshino et al., 2019), individual fungal species associate with both types of photosynthesizing organisms, either as separate individuals or together in the same individual (James and Henssen, 1976; Jahns, 1988; Goffinet and Bayer, 1997; Magain and

Sérusiaux, 2014). Such lichens, however, were classically considered exceptions to the general pattern of lichen symbiont associations (James and Henssen, 1976; Jahns, 1988). This traditional view of lichens, as symbioses formed by one fungus and one photosynthesizing partner, has evolved substantially during the last decade. Numerous studies have yielded a growing body of evidence that lichens and the communities they form are far more complex and dynamic than previously thought (U'Ren et al., 2010, 2012; Chagnon et al., 2015; Spribille et al., 2016; Tuovinen et al., 2019). Indeed, these studies have demonstrated that lichens have diverse and spatially partitioned microbiomes (Grube and Berg, 2009; Hodkinson and Lutzoni, 2009; Hodkinson et al., 2012; Aschenbrenner et al., 2016, 2017), that individual lichens host multiple genotypes of a given photosynthetic partner (Piercey-Normore, 2006; Muggia et al., 2013), and that there is extensive sharing of symbionts and microbial communities (Lücking et al., 2009; Belinchón et al., 2015; Cornejo et al., 2016; Onuț-Brännström et al., 2018). The conceptual transformation of lichens from binary symbiont pairs to intricate metacommunities was catalyzed by the recent discovery of specialist basidiomycete yeasts in the cortices (upper or outer layers) of certain ascomycete lichens (Spribille et al., 2016). This transformation was further advanced by the demonstration that at least some of those basidiomycetes, long thought to be parasites of ascomycete lichen fungi, instead form lichen-like associations with the algal partners (Tuovinen et al., 2019).

To date, published studies that screened lichens for basidiomycete yeasts have emphasized sampling in the family Parmeliaceae (Spribille et al., 2016; Tuovinen et al., 2019). Basidiomycete yeasts have also been detected in several other families of Lecanoromycetes (Spribille et al., 2016), the most species-rich lineage of lichen-forming fungi. Nonetheless, their ubiquity across a much wider diversity of lichen lineages remains to be tested. Here, we present the results of a broad survey for basidiomycete yeasts through analysis of a new metagenomic data set spanning 339 lichen species representing 57 families and 25 orders, generated as part of a large-scale study of lichen biodiversity in the Southern Appalachian Biodiversity Hotspot of temperate eastern North America (Lendemer et al., 2013, 2017; Allen and Lendemer, 2016; Tripp and Lendemer, in press).

MATERIALS AND METHODS

Tissue collection for metagenomics data set

Fresh material of 413 lichen thalli spanning 339 species, 57 fungal families, and 25 fungal orders of ascomycetes (Appendix S1) was collected from sites throughout the southern Appalachian Mountains as part of a system-wide investigation into drivers of lichen biodiversity and distributions in this region (Tripp et al., 2019). Samples were collected and identified by J. C. Lendemer and E. A. Tripp between December 2016 and January 2018, with the goal of obtaining a taxonomically comprehensive set of taxa representing every lichen species encountered at the project sites and representing diverse lichen growth forms and ecologies. Vouchers were deposited in the herbaria of the New York Botanical Garden (NY) and University of Colorado, Boulder (COLO) (Appendix S2). Vouchers were collected following standard protocols in the field (Brodo et al., 2001), and subsamples for molecular study were removed within 24 h of collection. For macrolichens, ca. 1 × 1 cm of tissue was removed from the thallus margins and lobes; for

microlichens, tissue was scraped from substrates using sterile razor blades. Tissue samples were air dried in a laminar flow hood for 24 h then frozen at −20°C until transport to University of Colorado for DNA extraction and subsequent sequencing.

DNA extraction and whole-genome shotgun sequencing

Dried tissue samples were pulverized using tungsten carbide bearings in a 96-well plate shaker (Qiagen, Germantown, MD, USA). Genomic DNA was then extracted with a Qiagen DNeasy 96 plant kit. Individual samples were transferred from 1.5 mL microcentrifuge tubes to the provided 96-well plates. Following Pogoda et al. (2018), the Qiagen protocol was modified to include a 10 min 65°C incubation step for the disrupted tissue in lysis buffer and a 100% ethanol wash before final drying of the membrane before elution. Extracted samples were then stored at −20°C before library preparation.

Samples were prepared for sequencing using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA), optimized for 1 ng of input DNA. Each sample was uniquely identified by the dual index adapters Nextera i5 and i7. Libraries that passed quality control were sequenced for paired-end 151 base pair reads on an Illumina NextSeq and utilized PhiX v.3 as a control.

Metagenomic data set and BLAST queries

Libraries were filtered with Trimmomatic-0.36 to trim adapters from reads, implementing the parameters “LEADING:3 TRAILING:3 MINLEN:100” (Bolger et al., 2014). Filtered reads were then assembled using SPAdes 3.9.0 with parameters “-careful -k 21,33,65,81” (Bankevich et al., 2012). These parameters were selected based on our prior studies that have found them highly effective in assembling high-copy loci within the lichen metagenome, including the mitochondrial genomes of both the mycobiont and photobiont (Brigham et al., 2018; Funk et al., 2018; Pogoda et al., 2018, 2019), the nuclear ribosomal DNA of the mycobiont and photobiont and cyanobiont (K. G. Keepers et al., unpublished manuscript), as well as the photobiont chloroplast genome (K. G. Keepers, unpublished data).

We queried the assembled metagenomes using default command-line BLASTn search parameters and seven rDNA sequences of cystobasidiomycete yeasts that have been shown to associate with ascomycete lichens, as follows. Two BLAST queries were performed with members of *Cyphobasidium* Millanes, Diederich & Wedin s.s. Millanes et al. (2016). One of these (KU587705) was a partial 18S reference sequence for *C. hypogymniicola* (Diederich & Ahti) Millanes, Diederich & Wedin, the type species of *Cyphobasidium*, generated by Millanes et al. (2016). The other (KU948882) was a partial 26S sequence of an unidentified cystobasidiomycete yeast from *Hypogymnia physodes* (L.) Nyl. recovered as sister to *C. hypogymniicola* by Spribille et al. (2016: S13, isolate T114). The remaining BLAST queries were performed with cystobasidiomycete yeast sequences published by Spribille et al. (2016) and that were recovered in other cystobasidiomycete clades outside of the *Cyphobasidium*. These additional sequences were KU948747 [18S, ITS1, 5.8S, ITS2, and partial 26S from *Thamnotia vermicularis* (Sw.) Schaer. recovered as sister to *Microsporomyces* Q.M.Wang, F.Y.Bai, M.Groenew. & Boekhout (Spribille et al., 2016: S12, isolate T1402)]; KU948755 [ITS1, 5.8S, ITS2, and partial 26S from *Parmotrema crinitum* (Ach.) M.Choisy recovered as sister to *Symmetrospora* Q.M.Wang, F.Y.Bai,

M.Groenew. & Boekhout (Spribille et al., 2016: S12, isolate T1509)]; KU948887 [partial 26S from *Parmeliopsis ambigua* (Wulfen) Nyl. recovered in a clade sister to all sequences of *Cyphobasidium* (Spribille et al., 2016: S12, isolate 1579)]; KU948863 [18S from *Nephromopsis endocrocea* Asahina recovered within *Bucklelyzma* Q.M.Wang, F.Y.Bai, M.Groenew. & Boekhout (Spribille et al., 2016: S14, isolate T1656)]; KU948865 [partial 18S from *Brodoa oroarctica* (Krog) Goward recovered in a clade with other lichen-derived samples that was recovered as sister to *Microsporomyces* (Spribille et al., 2016: S14, isolate T1667)].

BLAST tables were first filtered to include only BLAST hits of 100 bp or longer, and then all hits were subsequently sorted by percentage identity. The top 100 BLAST hits constituting contigs with $\geq 95\%$ percent identity were identified using a web-blast search against the NCBI non-redundant database (Appendices S3 and S4). The 100 sequences returned from the above two queries are available at Zenodo (<https://doi.org/10.5281/zenodo.3240665>).

We implemented multiple BLAST queries with different reference sequences for two reasons. First, if the community of yeasts in or on a lichen thallus was sufficiently diverse, community-level variation within the rapidly evolving ITS1 and ITS2 regions may have prevented those loci from assembling into contigs large enough to satisfy our detection heuristics. Consequently, we selected reference sequences of *Cyphobasidium* from more slowly evolving coding regions of the rDNA complex (18S and 26S; Schoch et al., 2012; Yahar et al., 2016). Second, given that Spribille et al. (2016) recovered lichen-associated yeasts in multiple, highly divergent fungal lineages within the cystobasidiomycetes, we queried the data set using additional sequences outside of *Cyphobasidium* in an effort to detect any cystobasidiomycete taxa with sequences too divergent from *Cyphobasidium* to be detected and identified using our BLAST workflow.

The single-copy coverage of the mycobiont nuclear genome was then estimated in the assemblies containing BLAST hits to cystobasidiomycetes. The SPAdes v3.9.0 assembler includes the coverage estimate for each contig in the contig's header, which allowed for rapid identification of the coverage of the mycobiont single-copy genome. Histograms of the depths of the one thousand largest contigs per assembly were constructed to visualize the distribution of nuclear genome contigs. Contigs that fell within the normal distribution of depths were identified as fungal using a web-blast search against the NCBI non-redundant database. The coverage values for the individual contigs identified as belonging to cystobasidiomycetes were derived directly from their respective headers.

RESULTS

In total, we detected cystobasidiomycete yeasts in 2.7% of the taxa and 2.2% of the samples in our data set (nine species, nine samples). Command-line BLAST searches of the metagenomic data set using an 18S reference sequence for *Cyphobasidium hypogymnicola*, the type species of *Cyphobasidium*, returned only 13 contigs with a match of 100 bp or greater, and these were derived from seven different samples (Appendix S3). Recognizing the divergence between basidiomycete yeast sequences found by Spribille et al. (2016; supplemental figs. 12–14), we also carried out command-line BLAST searches of the metagenomic data set using six additional sequences from lichen-associated yeasts recovered in clades within cystobasidiomycetes that were phylogenetically distant from

KU587705, including additional representative loci. These searches cumulatively returned only a three additional cystobasidiomycete sequences (Appendix S4).

DISCUSSION

The cystobasidiomycete sequences detected in our metagenomic data set derived from tissue samples of *Bryoria nadvornikiana* (Gyeln.) Brodo & D.Hawksw., *Heterodermia leucomelos* (L.) Poelt, *Lecidea roseotincta* Coppins & Tønsberg, *Opegrapha vulgata* (Ach.) Ach., *Parmotrema hypotropum* (Nyl.) Hale, *P. subsumptum* (Nyl.) Hale, *Usnea cornuta* Ach., *U. strigosa* (Ach.) A.Eaton, and *U. subgracilis* Vain. Of these nine species, seven are foliose and have a leafy morphology (*H. leucomelos*, *P. hypotropum*, *P. subsumptum*) or fruticose and have a shrubby morphology (*B. nadvornikiana*, *U. cornuta*, *U. strigosa*, *U. subgracilis*). All but one of those seven belong to the family Parmeliaceae as have the majority of hosts in which cystobasidiomycete fungi previously detected (Spribille et al., 2016). The seventh species, *Heterodermia leucomelos*, belongs to the Physciaceae, which is another family where cystobasidiomycete sequences have previously been detected (Spribille et al., 2016). *Lecidea roseotincta* is a crust-forming lichen that belongs to the family Lecanoraceae (Schmull et al., 2011). *Opegrapha vulgata* is a crustose member of the Arthoniomycetes, a class of fungi from which cystobasidiomycetes have not previously been reported.

Notably, we failed to detect cystobasidiomycete fungi in 97% (330 of 339 species) of the taxa sampled in this study. It is possible that the failure to detect yeasts in 330 of the 339 species may be attributable to sequencing at an insufficient depth. However, the same amount of raw sequence data (2 Gb) was used as input into each de novo assembly, therefore eliminating the possibility of a detection bias due to variable DNA input into the assemblies. Further, we did detect cystobasidiomycetes in nine samples, and in several samples, the coverage of the cystobasidiomycete was comparable to, or higher than, that for the single-copy nuclear genome of the primary lichen mycobiont (Fig. 1 and Table 1; average cystobasidiomycete to mycobiont ratio of coverages 0.51 ± 0.36 , ratio range 0.16–1.55).

It is also possible that detections were false positives due to sensitivity of WGS sequencing to microscopic contaminants of lichens that grow on the same substrates and in close association with the sampled species (K. G. Keepers et al., unpublished manuscript). Such contamination cannot be ruled out for any data derived from direct sampling of lichen thalli. Nonetheless, it is unlikely in our data set given that we detected cystobasidiomycetes in only 2.2% of samples and all but two of these detections were in lichens from lineages where the yeasts have previously been detected independently by others.

Given our results, the extreme skew in our analyses toward non-detection of yeasts suggests low occurrence, low abundance, and/or uneven distribution of cystobasidiomycetes in natural communities of lichens. The latter is supported by fluorescent in situ hybridization (FISH) staining of cystobasidiomycetes in lichen thalli that showed yeasts to be restricted to the lichen cortex (Spribille et al., 2016) and hence spatially restricted to a small area in proportion to the overall thallus. However, the majority of lichens sampled produce cortical tissues (exceptions are ecorticate taxa such as *Lepraria* Ach.), and our tissue sampling protocol captured the lichen cortex in all cases when present. Nonetheless, low occurrence, low abundance, and/or spatially restricted occurrence could explain why,

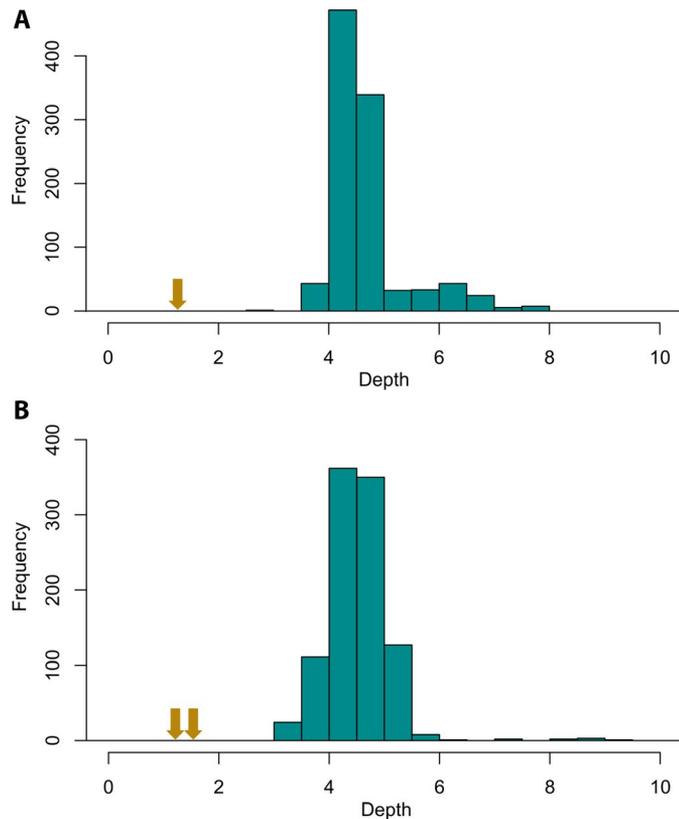


FIGURE 1. Examples of contig depth distributions from (A) *Usnea cornuta* and (B) *Heterodermia leucomelos* used to estimate single-copy depth of the mycobiont nuclear genome that show the cystobasidiomycete contigs (yellow arrows) fall near, or within, one order of magnitude of the distribution of the 1000 largest contigs in the metagenome (blue bars).

in the absence of PCR and amplicon sequencing of targeted molecular markers, we did not detect basidiomycete yeasts in a larger number of Parmeliaceae lineages where they have previously been demonstrated to occur. Taken together, our results call into question whether *Cyphobasidium* and other related yeasts previously

detected in lichen thalli are obligately associated with all individuals of every lichen species in which they have been detected. In fact, members of the cystobasidiomycetes, to which *Cyphobasidium* belongs, are widely characterized by their fungal parasite lifestyles (Diederich, 1996; Bauer et al., 2006; Millanes et al., 2011, 2016). Hence, these yeasts may function as parasites that infect specific host lichens when conditions are conducive to infection, as has been recently asserted (Hawksworth et al., 2016; Oberwinkler, 2017). Experimental studies and additional genomic work targeting detection of these yeasts in more samples and through additional sequencing techniques would help elucidate their role in the lichen microcosm.

CONCLUSIONS

The diverse metacommunities of lichen thalli have become the focus of considerable study, especially from the perspective of biotic constraints on the development and distribution of symbioses in nature. The discovery of lichen-associated basidiomycete yeasts has been particularly noteworthy, catalyzing a call to reconceptualize lichens as dynamic metacommunities. Lichen-associated basidiomycete yeasts have been implicated as previously overlooked members of the lichen symbiome, at both phylogenetic and spatial scales. However, here we have shown that analysis of metagenomic data from phylogenetically broad sampling of hundreds of lichen species within a lichen biodiversity hotspot detected very few occurrences of *Cyphobasidium* or other cystobasidiomycete yeasts. These results suggest that such yeasts may not be as ubiquitous as previously thought.

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TABLE 1. NCBI GenBank accessions for cystobasidiomycete yeast sequences and their host lichens detected during this study, cross-referenced with study isolate numbers (Appendix S2), yeast sequence depth, and mycobiont rDNA sequence coverage.

Isolate	Cystobasidiomycete GenBank accession	Mycobiont rDNA GenBank accession	Host lichen taxon	Cystobasidiomycete rDNA depth	Lichen mycobiont single-copy rDNA coverage range	Ratio
FEN_313	MN037399	MK092093	<i>Heterodermia leucomelos</i>	1.43	4.5	0.32
FEN_271	MN037400	MN038162	<i>Usnea strigosa</i>	3.54	6	0.59
FEN_107	MN037401	MK106005	<i>Bryoria nadvornikiana</i>	1.04	5.5	0.19
FEN_107	MN037402	MK106005	<i>Bryoria nadvornikiana</i>	1.47	5.5	0.27
FEN_368	MN037403	MG720066	<i>Usnea subgracilis</i>	1.24	7.5	0.17
FEN_313	MN037404	MK092093	<i>Heterodermia leucomela</i>	1.24	4.5	0.28
FEN_85	MN037405	MN038163	<i>Lecidea roseotincta</i>	1.36	4	0.34
FEN_117	MN037406	KY315997	<i>Opegrapha vulgata</i>	1.93	2.5	0.77
FEN_262	MN037407	MN038164	<i>Parmotrema hypotropum</i>	1.18	3	0.39
FEN_117	MN37408	KY315997	<i>Opegrapha vulgata</i>	2.12	2.5	0.85
FEN_117	MN037409	KY315997	<i>Opegrapha vulgata</i>	2.90	2.5	1.16
FEN_259	MN032489	MN038165	<i>Parmotrema subsumptum</i>	2.03	4.5	0.45
FEN_63	MN032112	KY100278	<i>Usnea cornuta</i>	1.26	4.5	0.28
FEN_271	MN032113	MN038162	<i>Usnea strigosa</i>	1.55	6	0.26

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AUTHOR CONTRIBUTIONS

J.L. and K.K. wrote the manuscript and collaborated on data presentation. J.L. and E.T. led field and genomic sampling components of the study, with collaboration from C.M. C.P. led wet lab components with guidance from N.K. K.K. led data analysis with guidance from NK and input from J.L. J.L., E.T., N.K. and C.M. obtained support and developed the southern Appalachian lichen project. All authors reviewed and revised drafts of the manuscript.

DATA ACCESSIBILITY

Taxon sampling and voucher metadata are provided in Appendices S1 and S2, respectively. Voucher data are available via the C.V. Starr Virtual Herbarium at NY and the Consortium of North American Lichen Herbaria (CNALH). Sequence data of BLAST results aimed at detecting cystobasidiomycete yeasts are available via Zenodo (<https://doi.org/10.5281/zenodo.3240665>). Cystobasidiomycete yeast sequences detected in this study and rDNA of the host lichens have been deposited in NCBI (Table 1).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Taxon sampling for the metagenomic data set used in this study.

APPENDIX S2. Molecular voucher metadata for metagenomic samples included in this study.

APPENDIX S3. Top 100 hits from BLAST search of the metagenomic data set using the reference sequence (KU587705.1).

APPENDIX S4. Top 100 hits from BLAST search of the metagenomic data set using six different reference sequences.

LITERATURE CITED

- Ahmadjian, V. 1967. The lichen symbiosis. Blaisdell, Waltham, MA, USA.
- Allen, J. L., and J. C. Lendemer. 2016. Climate change impacts on endemic, high-elevation lichens in a biodiversity hotspot. *Biodiversity and Conservation* 25: 555–568.
- Arcadia, L. 2013. Lichen biogeography at the largest scales. *Lichenologist* 45: 565–578.
- Aschenbrenner, I. A., T. Cernava, G. Berg, and M. Grube. 2016. Understanding microbial multi-species symbioses. *Frontiers in Microbiology* 7: 180.
- Aschenbrenner, I. A., T. Cernava, A. Erlacher, G. Berg, and M. Grube. 2017. Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. *Molecular Ecology* 26: 2826–2838.
- Asplund, J., and D. A. Wardle. 2017. How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews* 92: 1720–1738.
- Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Leslin, et al. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19: 455–477.
- Bauer, R., D. Begerow, J. P. Sampaio, M. Weiß, and F. Oberwinkler. 2006. The simple-septate basidiomycetes: a synopsis. *Mycological Progress* 5: 41–66.
- Belinchón, R., R. Yahr, and C. J. Ellis. 2015. Interactions among species with contrasting dispersal modes explain distributions for epiphytic lichens. *Ecography* 38: 762–768.
- Belnap, J., and O. L. Lange. 2005. Lichens and microfungi in biological soil crusts: community structure, physiology, and ecological functions. In J. Dighton, J. F. White, and P. Oudemans [eds.], *The fungal community, its organization and role in the ecosystem*, 3rd ed., 117–138. CRC Press, Taylor & Francis, Boca Raton, FL, USA.
- Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.
- Brigham, L. M., L. M. Allende, B. R. Shipley, K. C. Boyd, T. J. Higgins, N. Kelly, C. R. Anderson Stewart, et al. 2018. Genomic insights into the mitochondria of 11 eastern North American species of *Cladonia*. *Mitochondrial DNA, B* 3: 508–512.
- Brodo, I. M., S. Duran Sharnoff, and S. Sharnoff. 2001. *Lichens of North America*. Yale University Press, New Haven, CT, USA.
- Chagnon, P.-L., J. M. U'Ren, J. Miadlikowska, F. Lutzoni, and A. E. Arnold. 2015. Interaction type influences ecological network structure more than local abiotic conditions: evidence from endophytic and endolichenic fungi at a continental scale. *Oecologia* 180: 181–191.
- Cornejo, C., P. R. Nelson, I. Stepanchikova, D. Himelbrant, P. M. Jørgensen, and C. Scheidegger. 2016. Contrasting pattern of photobiont diversity in the Atlantic and Pacific populations of *Erioderma pedicellatum* (Pannariaceae). *Lichenologist* 48: 275–291.
- Cornelissen, J. H. C., S. I. Lang, N. A. Soudzilovskaia, and H. J. During. 2007. Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany* 99: 987–1001.
- Diederich, P. 1996. The lichenicolous heterobasidiomycetes. *Bibliotheca Lichenologica* 61: 1–198.
- Funk, E. R., A. N. Adams, S. M. Spotten, R. A. Van Hove, K. T. Whittington, K. G. Keepers, C. S. Pogoda, et al. 2018. The complete mitochondrial genomes of five lichenized fungi in the genus *Usnea* (Ascomycota: Parmeliaceae). *Mitochondrial DNA, B* 3: 305–308.
- Goffinet, B., and R. J. Bayer. 1997. Characterization of mycobionts of photomorph pairs in the Peltigeraceae (lichenized Ascomycetes) based on internal transcribed spacer sequences of the nuclear ribosomal DNA. *Fungal Genetics and Biology* 21: 228–237.
- Grube, M., and G. Berg. 2009. Microbial consortia of bacteria and fungi with focus on the lichen symbiosis. *Fungal Biology Reviews* 23: 72–85.
- Gueidan, C., C. R. Villaseñor, G. S. de Hoog, A. A. Gorbushina, W. A. Unteriner, and F. Lutzoni. 2008. A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Studies in Mycology* 61: 111–119.
- Hawksworth, D. L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research* 95: 641–655.
- Hawksworth, D. L. 2016. Transcriptomics leads to discovery of basidiomycete yeasts on lichen cortices – and speculation as to function. *IMA Fungus* 7: 65–66.
- Hawksworth, D. L., and R. Lücking. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology Spectrum* 5(4): FUNK-0052-2016.
- Hodkinson, B. P., and F. Lutzoni. 2009. A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49: 163–180.
- Hodkinson, B. P., N. R. Götzel, C. W. Schadt, and F. Lutzoni. 2012. Photoautotrophic symbiont and geography are major factors affecting highly structured and diverse bacterial communities in the lichen microbiome. *Environmental Microbiology* 14: 147–161.
- Honegger, R. 1991. Fungal evolution: symbiosis and morphogenesis. In L. Margulis and R. Fester [eds.], *Symbiosis as a source of evolutionary innovation*, 319–340. MIT Press, Cambridge, MA, USA.
- Honegger, R. 1992. Lichens: mycobiont–photobiont relationships. In W. Reisser [ed.], *Algae and symbioses: plants, animals, fungi, viruses, interactions explored*, 255–275. Biopress, Bristol, UK.

- Jahns, H. M. 1988. The establishment, individuality and growth of lichen thalli. *Botanical Journal of the Linnean Society* 96: 21–29.
- James, P. W., and A. Henssen. 1976. The morphological and taxonomic significance of cephalodia. In D. H. Brown, D. L. Hawksworth, and R. H. Bailey [eds.], *Lichenology: Progress and Problems*, 27–77. Academic Press, London, UK.
- Lendemer, J. C., R. C. Harris, and E. A. Tripp. 2013. The lichens and allied fungi of Great Smoky Mountains National Park: an annotated checklist with comprehensive keys. *Memoirs of The New York Botanical Garden* 104: i–viii, 1–152.
- Lendemer, J. C., C. R. Anderson Stewart, B. Besal, J. Goldsmith, H. Griffith, J. R. Hoffman, B. Kraus, et al. 2017. The lichens and allied fungi of Mount Mitchell State Park, North Carolina: a first checklist with comprehensive keys and comparison to historical data. *Castanea* 82: 69–97.
- Lorch, J. 1988. The true nature of lichens, a historical survey. In M. Galun [ed.], *CRC handbook of lichenology*, vol. 1, 3–32. CRC Press, Boca Raton, FL, USA.
- Lücking, R., J. D. Lawrey, M. Sikaroodi, P. M. Gillevet, J. L. Chaves, H. J. M. Sipman, and F. Bungartz. 2009. Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. *American Journal of Botany* 96: 1409–1418.
- Lücking, R., B. P. Hodkinson, and S. D. Leavitt. 2016. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – approaching one thousand genera. *Bryologist* 119: 361–416.
- Lutzoni, F., M. Pagel, and V. Reeb. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937–940.
- Magain, N., and E. Sérusiaux. 2014. Do photobiont switch and cephalodia emancipation act as evolutionary drivers in the lichen symbiosis? A case study in the Pannariaceae (Peltigerales). *PLoS ONE* 9: e89876.
- Millanes, A. M., P. Diederich, S. Ekman, and M. Wedin. 2011. Phylogeny and character evolution in the jelly fungi (Tremellomycetes, Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* 61: 12–28.
- Millanes, A. M., P. Diederich, and M. Wedin. 2016. *Cyphobasidium* gen. nov., a new lichen-inhabiting lineage in the Cystobasidiomycetes (Pucciniomycotina, Basidiomycota, Fungi). *Fungal Biology* 120: 1468–1477.
- Mitchell, M. E. 2007. Signposts to symbiosis: a review of early attempts to establish the constitution of lichens. *Huntia* 13: 101–120.
- Muggia, L., L. Vancurova, P. Skaloud, O. Peksa, M. Wedin, and M. Grube. 2013. The symbiotic playground of lichen thalli – a highly flexible photobiont association in rock-inhabiting lichens. *FEMS Microbiology Ecology* 85: 313–323.
- Oberwinkler, F. 2017. Yeasts in Pucciniomycotina. *Mycological Progress* 16: 831–856.
- Onuț-Brännström, I., M. Benjamin, D. G. Scofield, S. Heiðmarsson, M. G. L. Andersson, E. S. Lindström, and H. Johannesson. 2018. Sharing of photobionts in sympatric populations of *Thamnomlia* and *Cetraria* lichens: evidence from high-throughput sequencing. *Scientific Reports* 8: 4406.
- Piercey-Normore, M. D. 2006. The lichen-forming ascomycete *Evernia mesomorpha* associates with multiple genotypes of *Trebouxia jamesii*. *New Phytologist* 169: 331–344.
- Pogoda, C. S., K. G. Keepers, J. C. Lendemer, N. C. Kane, and E. A. Tripp. 2018. Reductions in complexity of mitochondrial genomes in lichen-forming fungi shed light on genome architecture of obligate symbioses. *Molecular Ecology* 27: 1155–1169.
- Pogoda, C. S., K. G. Keepers, A. Y. Nadiadi, D. W. Bailey, J. C. Lendemer, E. A. Tripp, and N. C. Kane. 2019. Genome streamlining via complete loss of introns has occurred multiple times in lichenized fungal mitochondria. *Ecology and Evolution* 9: 4245–4263.
- Schmull, M., J. Miadlikowska, M. Pelzer, E. Stocker-Wörgötter, V. Hofstetter, E. Fraker, B. P. Hodkinson, et al. 2011. Phylogenetic affiliations of members of the heterogeneous lichen-forming fungi of the genus *Lecidea sensu Zahlbruckner* (Lecanoromycetes, Ascomycota). *Mycologia* 103: 983–1003.
- Schoch, C., G.-H. Sung, F. López-Giráldez, J. P. Townsend, J. Miadlikowska, V. Hofstetter, B. Robbertse, et al. 2009. The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* 58: 224–239.
- Schoch, C. L., K. A. Seifert, C. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, and Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences, USA* 109: 6241–6246.
- Seaward, M. R. D. 1996. Lichens and the environment. In B. Sutton [ed.], *A century of mycology*, 293–320. Cambridge University Press, Cambridge, UK.
- Slack, N. G. 1988. The ecological importance of lichens and bryophytes. *Bibliotheca Lichenologica* 30: 23–53.
- Sprubille, T., V. Tuovinen, P. Resl, D. Vanderpool, H. Wolinski, M. C. Aime, K. Schneider, et al. 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492.
- Tripp, E. A., and J. C. Lendemer. In press. Field guide to the lichens of Great Smoky Mountains National Park. University of Tennessee Press, Knoxville, TN, USA.
- Tripp, E. A., J. C. Lendemer, and C. M. McCain. 2019. Habitat quality and disturbance drive lichen species richness in a temperate biodiversity hotspot. *Oecologia* 190: 445–457.
- Tuovinen, V., S. Ekman, G. Thor, D. Vanderpool, T. Sprubille, and H. Johannesson. 2019. Two basidiomycete fungi in the cortex of Wolf Lichens. *Current Biology* 29: 476–483.
- U'Ren, J. M., F. Lutzoni, J. Miadlikowska, and A. E. Arnold. 2010. Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. *Microbial Ecology* 60: 340–353.
- U'Ren, J. M., F. Lutzoni, J. Miadlikowska, A. D. Laetsch, and A. E. Arnold. 2012. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany* 99: 898–914.
- Yahr, R., C. L. Schoch, and B. T. M. Dentinger. 2016. Scaling up discovery of hidden diversity in fungi: Impacts of barcoding approaches. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 371: 20150336.
- Yoshino, K., K. Yamamoto, K. Hara, M. Sonoda, Y. Yamamoto, and K. Sakamoto. 2019. The conservation of polyol transporter proteins and their involvement in lichenized Ascomycota. *Fungal Biology* 132: 318–329.