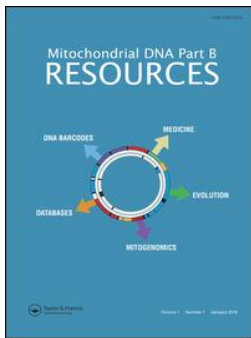

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Genomic insights into the mitochondria of 11 eastern North American species of *Cladonia*

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ABSTRACT

Cladonia is among the most species-rich genera of lichens globally. Species in this lineage, commonly referred to as reindeer lichens, are ecologically important in numerous regions worldwide. In some locations, species of *Cladonia* can comprise the dominant groundcover, and are a major food source for caribou and other mammals. Additionally, many species are known to produce substances with antimicrobial properties or other characteristics with potentially important medical applications. This exceptional morphological and ecological variation contrasts sharply with the limited molecular divergence often observed among species. As a new resource to facilitate ongoing and future studies of these important species, we analyse here the sequences of 11 *Cladonia* mitochondrial genomes, including new mitochondrial genome assemblies and annotations representing nine species: *C. apodocarpa*, *C. caroliniana*, *C. furcata*, *C. leporina*, *C. petrophila*, *C. peziziformis*, *C. robbinsii*, *C. stipitata*, and *C. subtenuis*. These 11 genomes varied in size, intron content, and complement of tRNAs. Genes annotated within these mitochondrial genomes include 15 protein-coding genes, the large and small ribosomal subunits (mtLSU and mtSSU), and 23–26 tRNAs. All *Cladonia* mitochondrial genomes contained *atp9*, an important energy transport gene that has been lost evolutionarily in some lichen mycobiont mitochondria. Using a concatenated alignment of five mitochondrial genes (*nad2*, *nad4*, *cox1*, *cox2*, and *cox3*), a Bayesian phylogeny of relationships among species was inferred and was consistent with previously published phylogenetic relationships, highlighting the utility of these regions in reconstructing phylogenetic history.

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Cladonia; genome; lichen; mitochondrion; symbiosis

Introduction

Cladonia is a charismatic genus of lichen-forming ascomycetes in the order Lecanorales (Miadlikowska et al. 2014). Globally, the genus contains upwards of 300 species (Ahti 1998; Stenroos and DePriest 1998; Stenroos et al. 2002; Kirk 2017), most of which are characterized by dimorphic thalli that consist of a squamulose primary thallus and a fruticose secondary thallus (Ahti 1998). In some groups, such as the true reindeer lichens that were historically classified in *Cladina*, the primary thallus is ephemeral and crustose (Thomson 1967; Ahti 1998). In other groups, including a clade of three eastern North American endemic species (*C. apodocarpa*, *C. petrophila*, and *C. stipitata*), the fruticose secondary thallus is reduced to a short stipe or is entirely lacking (Lendemer and Hodkinson 2009).

This morphological variation is mirrored in the chemical diversity within the genus, which spans a wide variety of secondary metabolites (Rundel 1969; Ahti 1998; Nash et al. 2002),

many among which have been inferred to have pharmacological importance (Lauterwein et al. 1995; Zhang et al. 2012; Kosanić et al. 2014; Ramos et al. 2014). The visually striking thalli of *Cladonia* frequently comprise the dominant groundcover in diverse habitats including deserts, temperate forest glades, and boreal forests. In the latter, *Cladonia* are the major food source for ungulates such as reindeer, solidifying their role as a vital component of ecosystem function (Ahti 1998; Stenroos and DePriest 1998; Stenroos et al. 2002; Kirk 2017). Members of the genus grow in a range of moisture and light conditions, reflecting the broad ecological amplitude of the genus as a whole (Cringan 1957; Ahti and Hepburn 1967; Corns 1983; Meades and Moores 1989).

Despite widespread occurrence and exceptional morphological and ecological variation among species of *Cladonia* (Osyczka et al. 2014; Pino-Bodas et al. 2015), relatively few genetic resources have been developed for the genus (e.g. *Cladonia grayi*; Joneson et al. 2011). Here, we present annotated mitochondrial genomes of nine species of *Cladonia*:

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C. apodocarpa, *C. caroliniana*, *C. furcata*, *C. leporina*, *C. petrophila*, *C. peziziformis*, *C. robbinsii*, *C. stipitata*, and *C. subtenuis*. Of these, six (*C. apodocarpa*, *C. caroliniana*, *C. leporina*, *C. petrophila*, *C. robbinsii*, and *C. stipitata*) are endemic to eastern North America where they (and the remaining three species) are especially abundant in the southern Appalachian Mountains (Fink 1935; Thomson 1967; Hale 1969; Ahti 1984; Brodo et al. 2001; Lendemer and Hodkinson 2009; Lewis 2014; Tripp and Lendemer 2019 [in press]; Tripp et al., in review). To enable future comparative evolutionary and genomic analyses within genus *Cladonia*, we analysed differences in genome size and protein coding gene content, characterized intron-encoded retrotransposable elements, and conducted Bayesian analyses of five mitochondrial genes to assess efficacy of using these loci in phylogenetic reconstruction.

Methods

Samples of *Cladonia apodocarpa* (voucher: Lendemer 48789; Lat/long 35.0253, -83.1747; NCBI Accession MG958507), *C. caroliniana* (voucher: Lendemer 49894; Lat/long 34.3291, -87.5092; NCBI Accession MG708277), *C. furcata* (voucher: Tripp 6423 & Lendemer; Lat/long 34.9555, -85.9996; NCBI Accession MG711314), *C. leporina* (voucher: Tripp 6543 & Lendemer; Lat/long 34.3291, -87.5092; NCBI Accession MG725377), *C. petrophila* (voucher: Lendemer 49138 & Tripp; Lat/long 34.4717, -86.0511; NCBI Accession MG941021), *C. peziziformis* (voucher: Tripp 6281 & Lendemer; Lat/long 34.4559, -85.5845; NCBI Accession MG686615), *C. robbinsii* (voucher: Lendemer 49897 & Tripp; Lat/long 34.3291, -87.5092; NCBI Accession MG725618), *C. stipitata* (voucher: Tripp 6060 & Lendemer; Lat/long 35.4835, -83.8148; NCBI Accession MG851822), and *C. subtenuis* (voucher: Lendemer 49895 & Tripp; Lat/long 34.3291, -87.5092; NCBI Accession MG949117) were collected by J.C. Lendemer, E.A. Tripp, K.G. Keepers, K.H. White, C.R. Anderson Stewart, J.R. Hoffman, and C.M. McCain from the Southern Appalachian Mountains of North Carolina and Alabama, U.S.A. between 2015 and 2017. Museum voucher specimens are housed at the New York Botanical Garden (NY Herbarium) and University of Colorado (COLO Herbarium).

Genomic DNA was extracted using a Qiagen DNeasy 96 plant kit, with the protocol modified to include a 10 minute 65°C incubation step for the ground material in lysis buffer and a 100% ethanol wash before final drying of the membrane prior to elution. Genomic libraries were prepared using Nextera® XT DNA library prep kits (Illumina®) and each sample was barcoded by the unique dual index adapters Nextera® i5 and i7. Samples that passed QC were processed for paired-end 150 base pair reads on the Illumina NextSeq® sequencer at the University of Colorado's BioFrontiers Institute Next-Generation Sequencing Facility in Boulder, Colorado. Genome assemblies and annotations were conducted by undergraduate and graduate students enrolled in N. Kane's Genomics class at the University of Colorado, Boulder, with additional assistance and careful verification performed by more experienced graduate students and faculty.

Genomic reads were trimmed using Trimmomatic-0.36 with the parameters: 'ILLUMINACLIP:NexteraPE-PE.fa:2:20:10 MINLEN:140 LEADING:20 TRAILING:20' (Bolger, et al 2014). Reads were assembled *de novo* using SPAdes v. 3.9 (Bankevich et al. 2012), annotated using DOGMA (Wyman et al. 2004) and Chlorobox (Tillich et al. 2017), and finished in Sequin v. 15.10 from NCBI (Bethesda, MD) for final submission to GenBank.

Phylogenetic analysis

The phylogeny of nine species of *Cladonia* was inferred using sequences from the mitochondrial genes *cox3*, *nad2*, *nad4*, *cox1*, and *cox2* annotated in this study as per Funk et al. (2018). To test monophyly of these nine species of *Cladonia* against outgroups and to further place this lineage in the context of relatives, we downloaded sequences representing these genes from other taxa from GenBank, all of which derived from mitochondrial genomes that we and student colleagues assembled in earlier work, as follows: *Alectoria fallacina* [MG711470], *Arthonia susa* [MH015348], *Cladonia rangiferina* [KY460674], *Cladonia uncialis* [KY352404], *Coccocarpia palmicola* [NC_034332], *Gomphilus americanus* [NC_034790], *Graphis lineola* [KY315996], *Heterodermia speciosa* [KY328643], *Hypogymnia vittata* [KY362374], *Imshaugia aleurites* [KY352227], *Lepraria oxybapha* [KY348846], *Leptogium hirsutum* [NC_034928], *Menegazzia subsimilis* [KY352491], *Mycocalicium subtile* [KY348761], *Opegrapha vulgata* [KY315997], *Parmotrema stuppeum* [KY362439], *Pertusaria ostiolata* [KY346830], *Phlyctis boliviensis* [KY305663], *Pertusaria corallina* [NC_034779], *Usnea pensylvanica* [KY321923], *U. ceratina* [KX987159], and *U. cornuta* [KY100278]. The same five genes (*cox1*, *cox2*, *cox3*, *nad2*, and *nad4*) were extracted from the above accessions. Bayesian phylogenetic analyses were conducted on the individual trees and there was no conflict between any resultant trees (Mason-Gamer and Kellogg 1996). To improve support in the final tree, we concatenated the five genes using Muscle aligner (Edgar 2004) and then manually curated the alignment. Gaps were treated as missing data and regions in which more than one taxon was missing data were excluded from the alignment (available on Zenodo 1206280). The optimal model of GTR+ Γ +I was chosen using an Akaike information criterion (AIC) selected in ModelFinder (Kalyaanamoorthy et al. 2017), and applied to phylogenetic analysis. Bayesian topologies were inferred in MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), sampling trees over 75,000 MCMC generations (Nei and Kumar 2000) excluding 25,000 burn-in generations, with separate partitions specified for each of the five genes. Bayesian posterior probabilities were mapped onto a resultant 50% majority rule consensus Bayesian tree (Tamura and Nei 1993). This tree was rooted using *M. subtile* [Class Eurotiomycetidae] as well as *A. susa* and *O. vulgata* [Class Arthoniomycetidae] as outgroups.

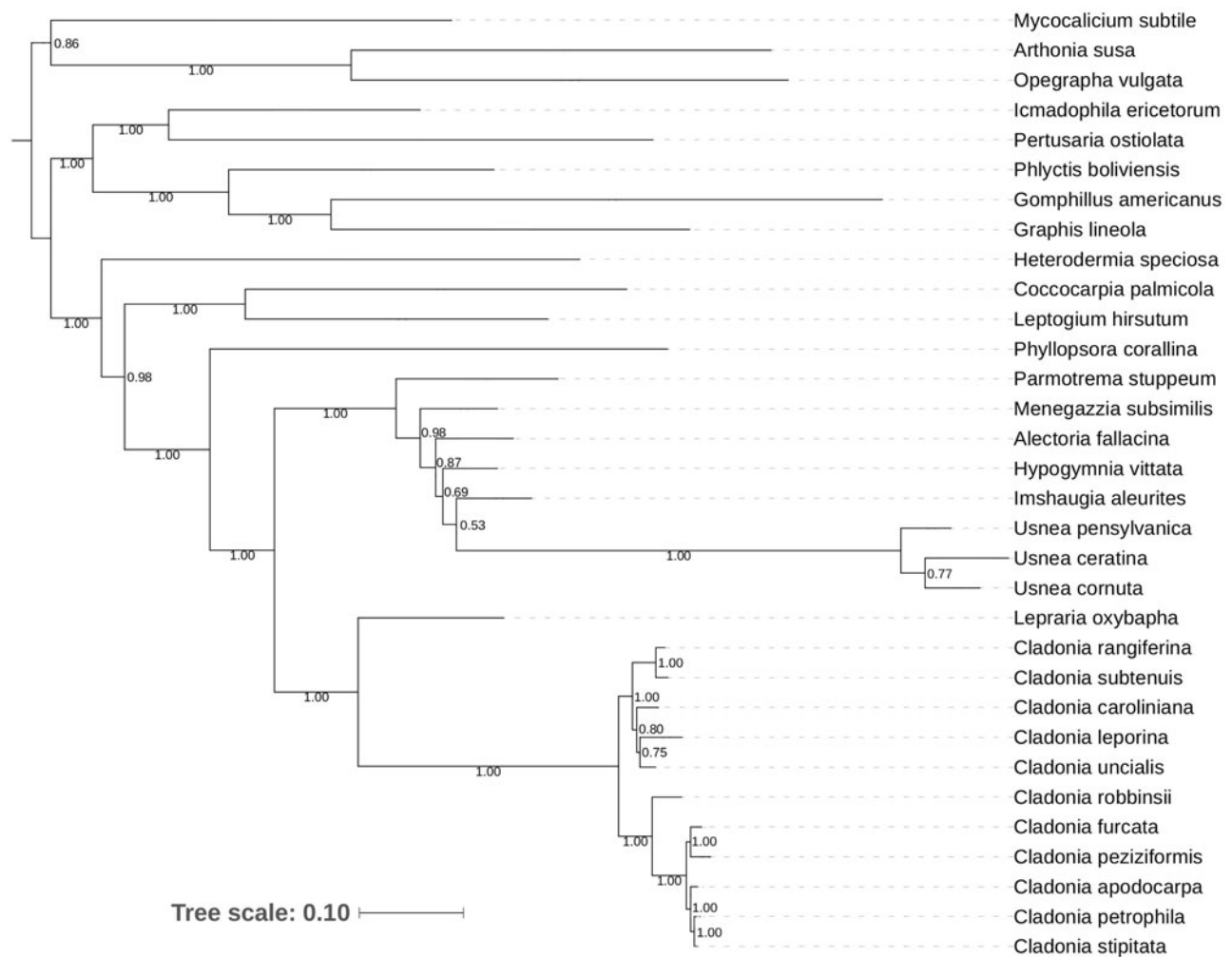


Figure 1. Majority rule consensus tree inferred from Bayesian analyses of five concatenated mitochondrial genes (*cox3*, *nad2*, *nad4*, *cox1*, *cox2*) from 11 *Cladonia* species (data herein assembled) plus several additional taxa whose sequences were downloaded from GenBank (and originally generated by the authors). Numbers indicate posterior probabilities. Branch lengths represent the number of substitutions per site per unit time (scale below). Tree was rooted using *M. subtile* [Eurotiomycetidae] as well as *A. susa* and *O. vulgata* [Arthoniomycetidae].

Results and discussion

Mitochondrial genome content and organization

The nine new genomes presented here broaden our understanding of mitogenome structure within *Cladonia* given that prior to this study there existed only two mitochondrial genomes from the genus (*C. rangiferina*, *C. uncialis*) available on GenBank. The size of these nine genomes varied from 45,312 bp (*C. peziziformis*) to 60,062 bp (*C. stipitata*) with a mean of 53,407 bp and standard deviation of 5307.8 bp. A large inversion block involving the genes *nad6*, *cox3*, *LSU*, *nad2*, and *nad3* was previously documented in *C. uncialis* (Pogoda et al. 2018), however, this inversion was not located in any of the newly analysed genomes. Similarly, no other genomic rearrangements were detected in the nine genomes, demonstrating a conserved synteny in these representatives of *Cladonia*. Each mitogenome contained between 23 and 26 tRNAs and their locations were consistent among the species analysed. An exception was *trnK*, wherein an additional copy of *trnK* was observed in *C. apodocarpa*, *C. petrophila*, and *C. stipitata*, located between the *cox1* and *nad1* genes. All nine mitogenomes contained 15 protein-coding genes (*cob*,

cox1, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *atp6*, *atp8*, *atp9*, and *rps3*). Notably, *atp9*, a key mitochondrial gene involved in energy production that has been lost in some lichen lineages, including members of the family Parmeliaceae (Pogoda et al. 2018), was detected in all nine *Cladonia*. The presence of *atp9* in our newly analysed mitogenomes is consistent with earlier results that detected this gene in two species of *Cladonia* (Pogoda et al. 2018).

Introns and intergenic spacer regions

Variation in intron position and size, particularly in *cox1*, accounted for the majority of variation in mitochondrial genome size. In all species except *C. peziziformis* that encoded *cox1* as a single exon, introns in *cox1* contained LAGLIDADG and GIY-YIG homing endonucleases. Presence of these selfish, parasitic elements in lichen mitochondrial genomes has been previously documented (Pogoda et al. 2018) and they are thought to aid in the mobility of their host introns (Guha et al. 2017). All species examined contained introns in *mtLSU*, and *C. stipitata* and *C. leporina* also contained introns in the *mtSSU*.

Phylogenetic history

Our molecular phylogenetic analyses (Figure 1) resolved all *Cladonia* species examined in this study as monophyletic (PP = 1.00) and furthermore, partitioned into two clades. In particular, taxa formerly ascribed to the genus *Cladina* together with species of *Cladonia* sect. *Unciales* (i.e. *C. subtenuis*, *C. rangiferina*, *C. caroliniana*, *C. leporina*, *C. uncialis*) were inferred to form a strongly supported clade ('Clade 1' herein; (PP = 1.00)) whereas species in *Cladonia* sensu stricto (i.e. species that are predominantly squamulose or podetiate including *C. robbinsii*, *C. peziziformis*, *C. apodocarpa*, *C. petrophila*, *C. stipitata*, *C. furcata*) formed a second strongly supported clade ('Clade 2' herein; (PP = 1.0)). Overall, relationships were concordant with trees produced using ribosomal DNA as well as chemical and morphological data (Stenroos et al. 2002), which included 10 of the 11 *Cladonia* represented here (*C. stipitata* was not included in that study).

Conclusions

The newly sequenced, assembled, and annotated *Cladonia* mitogenomes exhibited a moderate range in size, with intronic and intergenic regions of variable lengths associated with changes in genome size. These mitochondrial genomes all contained the same set of 15 protein-coding genes. Results from our study confirm earlier findings (Pogoda et al. 2018) that species of *Cladonia* have retained a key gene involved in energy production, *atp9*. Together, these data provide important genetic resources for these charismatic, morphologically variable and ecologically important lichens, as well as new resources for further study of lichen mitochondrial genome evolution and co-evolution with their obligate symbiotic partners (Tripp et al. 2017).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Ahti T, Hepburn TL. 1967. Preliminary studies on woodland caribou range, especially on lichen stands, in Ontario. Toronto (ON): Ontario

- Department of Lands and Forests, Research Branch (Ontario Dept. of Lands and Forests Res Rep; 74).
- Ahti T. 1984. The status of *Cladina* as a genus segregated from *Cladonia*. *Nova Hedwigia*. 79:25–61.
- Ahti T. 1998. The lichen family Cladoniaceae in the Neotropics. In: Marcelli MP, Seaward MRD, editors. *Lichenology in Latin America: history, current knowledge and applications*. Sao Paulo, Brazil: CETESB – Companhia de Tecnologia de Saneamento Ambiental – Estado de Sao Paulo; p. 109–115 [RLL List # 173/Rec.# 344].
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 19:455–477.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30:2114–2120.
- Brodo IM, Sharnoff SD, Sharnoff S. 2001. *Lichens of North America*. New Haven (CT): Yale University Press.
- Corns IGW. 1983. Forest community types of west-central Alberta in relation to selected environmental factors. *Can J Forest Res*. 13:995–1010.
- Cringan AT. 1957. History, food habits and range requirements of the woodland caribou of continental North America. *Trans N Am Wildl Conf*. 22:485–501.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 32:1792–1797.
- Fink B, Hedrick J. 1935. *Lichen flora of the United States*. Ann Arbor (MI): University of Michigan Press.
- Funk ER, Adams AA, Spotten SM, Van Hove RA, Whittington KT, Keepers KG, Pogoda CS, Lendemer J, Tripp EA, Kane NC. 2018. The complete mitochondrial genomes of five lichenized fungi in the genus *Usnea* (Ascomycota: Parmeliaceae). *Mitochondrial DNA B*. 3:305–308.
- Guha TK, Wai A, Mullineux ST, Hausner G. 2017. The intron landscape of the mtDNA *cytb* gene among the Ascomycota: introns and intron-encoded open reading frames. *Mitochondrial DNA A*. 1–10.
- Hale ME. 1969. *How to know the lichens*. Dubuque (IA): Wm. C. Brown.
- Huelsensbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*. 17:754–755.
- Joneson S, Armaleo D, Lutzoni F. 2011. Fungal and algal gene expression in early developmental stages of lichen-symbiosis. *Mycologia*. 103:291–306.
- Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermini LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods*. 14:587.
- Kirk PM. 2017. *Species Fungorum* (version Jan 2016). In: Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, et al., editors. *Species 2000 & ITIS Catalogue of Life*; Nov 28. Leiden, The Netherlands: Species 2000, Naturalis. ISSN 2405-8858. www.catalogueoflife.org/col
- Kosanić M, Ranković B, Stanojković T, Rančić A, Manojlović N. 2014. *Cladonia* lichens and their major metabolites as possible natural antioxidant, antimicrobial and anticancer agents. *LWT-Food Sci Technol*. 59:518–525.
- Lauterwein M, Oethinger M, Belsner K, Peters T, Marre R. 1995. In vitro activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid, and (–)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrob Agents Chemother*. 39:2541–2543.
- Lendemer JC, Hodkinson BP. 2009. The wisdom of fools: new molecular and morphological insights into the North American apodetiate species of *Cladonia*. *Opuscula Philolichenum*. 7:79–100.
- Lewis CJ. 2014. *Cladonia robbinsii* (A. Evans), a new lichen for Ontario and Manitoba, Canada. *Evansia*. 31:88–92.
- Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst Biol*. 45:524–545.
- Meades WJ, Moores L. 1989. *Forest site classification manual: a field guide to the Damman forest types of Newfoundland*. Forest resources development agreement report, vol. 3. St. John's (NL): Environment Canada.
- Miadlikowska J, Kauff F, Högnabba F, Oliver JC, Molnár K, Fraker E, Gaya E, Hafellner J, Hofstetter V, Gueidan C, et al. 2014. A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307

- fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Mol Phylogenet Evol.* 79:132–168.
- Nash TH, Ryan BD, Gries C, Bungartz F, editors. 2002. Lichen flora of the Greater Sonoran Desert region, vol. 1. Tempe (AZ): Arizona State University Lichen Herbarium.
- Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. Oxford: Oxford University Press.
- Osyczka P, Rola K, Lenart-Boroń A, Boroń P. 2014. High intraspecific genetic and morphological variation in the pioneer lichen *Cladonia rei* colonising slag dumps. *Open Life Sci.* 9:579–591.
- Pino-Bodas R, Burgaz AR, Martín MP, Ahti T, Stenroos S, Wedin M, Lumbsch HT. 2015. The phenotypic features used for distinguishing species within the *Cladonia furcata* complex are highly homoplasious. *The Lichenologist.* 47:287–303.
- Pogoda CS, Keepers KG, Lendemer JC, Kane NC, Tripp EA. 2018. Reductions in complexity of mitochondrial genomes in lichen-forming fungi shed light on genome architecture of obligate symbioses. *Mol Ecol.* 27:1155–1169.
- Ramos DDBM, Gomes FS, Napoleão TH, Paiva PMG, da Silva MDC, Barroso Coelho LCB. 2014. Antimicrobial activity of *Cladonia verticillaris* lichen preparations on bacteria and fungi of medical importance. *Chin J Biol.* 2014:219392.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics.* 19:1572–1574.
- Rundel PW. 1969. Clinal variation in the production of usnic acid in *Cladonia subtenuis* along light gradients. *Bryologist.* 72:40–44.
- Stenroos S, Hyvönen J, Myllys L, Thell A, Ahti T. 2002. Phylogeny of the genus *Cladonia* s. lat. (Cladoniaceae, Ascomycetes) inferred from molecular, morphological, and chemical data. *Cladistics.* 18:237–278.
- Stenroos SK, DePriest PT. 1998. SSU rDNA phylogeny of cladoniiform lichens. *Am J Bot.* 85:1548–1559.
- Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol.* 10:512–526.
- Thomson JW. 1967. The lichen genus *Cladonia* in North America. Toronto: University of Toronto Press.
- Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 45:W6–W11.
- Tripp EA, Lendemer JC. 2019 (in press). Field guide to the Lichens of Great Smoky Mountains National Park. Knoxville (TN): University of Tennessee Press.
- Tripp EA, Lendemer JC, McCain CM. in review. Impacts of disturbance on lichens—obligate symbiotic organisms—in a temperate biodiversity hotspot. *Divers Distrib.*
- Tripp EA, Zhang N, Schneider H, Huang Y, Mueller GM, Hu Z, Häggblom, Bhattacharya D. 2017. Reshaping Darwin's Tree: impact of the Symbiome. *Trends Ecol Evol (Amst).* 32:552–555.
- Wyman SK, Jansen RK, Boore JL. 2004. Automatic annotation of organelle genomes with DOGMA. *Bioinformatics.* 20:3252–3255.
- Zhang Y, Shi J, Zhao Y, Cui H, Cao C, Liu S. 2012. An investigation of the anti-diabetic effects of an extract from *Cladonia humilis*. *Pak J Pharm Sci.* 25:509–512.