INTRODUCTION

In obligate symbioses, co-evolution of the partners often drives gene loss that results in complementarity of the symbionts’ metabolic capacities (e.g., Bublitz et al., 2019). Lichens are a diverse group of fungal-algal symbioses composed of at least one phototrophic partner (a green alga or a cyanobacterium) and at least one fungus. The fungus is currently assumed to be obligatorily associated with the phototroph. However, despite early suggestions for complementarity between fungal and phototroph gene products (Ahmadjian, 1993), evidence for this has been lacking. In 2018, Pogoda and colleagues were the first to report ostensible gene loss and complementarity in the lichen symbiosis. Based on analysis of mitochondrial genomes of several lichen-forming lecanoromycete fungi, Pogoda et al. (2018) reported that ATP9, a gene encoding one subunit of ATP synthase, from a few mitochondrial genomes. Here, we show that while these fungi indeed have lost the mitochondrial ATP9, each retain a nuclear copy of this gene. Our analysis reaffirms that lichen fungi produce their own ATP.
given that ATP would need to move through the cell walls and membranes of both of the partners involved in the exchange.

We therefore hypothesized that the ATP9 gene was present in the genomes but overlooked during the analysis. By replicating Pogoda et al. (2018) analysis on the species of interest, and then applying a series of stress tests, we were able to detect a putative homologue ATP9 in all four fungi.

2 | RESULTS AND DISCUSSION

2.1 | Pogoda et al. (2018) results replicated

For the four lichens the lecanoromycete fungi of which were reported to lack the ATP9 gene, we generated new metagenomes (Table S1), and from them assembled and binned near-complete lecanoromycete genomes. Using these data, we replicated the results of Pogoda et al. (2018): with a tBLASTn search, we located homologues of two other mitochondrial genes, ATP6 and ATP8, but obtained no hit for ATP9 above the threshold (bit score >100) (Box 1, Supporting Information).

2.2 | ATP9 gene in the nuclear genomes

To test the hypothesis that the four species which Pogoda et al. (2018) reported as lacking ATP9 in fact retain it, we searched for the gene in the recently published lecanoromycete genome of Alectoria sarmentosa (Tagirdzhanova et al., 2021), a close relative of A. fallacina, one of the four fungi reportedly lacking ATP9. We identified one putative ATP9 homologue, ASARMPREDX12_000654, in the A. sarmentosa lecanoromycete nuclear genome. This gene was assigned to Inteproscan accession IPRO00454 (ATP synthase, F0 complex, subunit C), and pfam accession PF00137 (ATP synthase subunit C). When blasted against the NCBI Protein database, it aligned with other fungal ATP9 (Table S2).

Using ASARMPREDX12_000654 as a query, we located putative ATP9 homologues in all four lecanoromycete genomes we extracted from the metagenomes (Box 1, Supporting Information). Each of these ATP9 homologues showed up in the tBLASTn search we ran replicating Pogoda et al. (2018; see the previous section), but had a bit score below the threshold (ranging from 35 to 48). Next, using the newly found ATP9 homologues, we searched metagenomic data from the original publication. This reaffirms that, as expected, the fungi

2.3 | Endosymbiotic gene transfer was followed by gene loss in the mitochondrion

The conclusion that these ATP9 genes come from the nuclear genomes is also supported by the ATP9 phylogeny we constructed (Box 2). In the phylogeny, the lecanoromycete nuclear ATP9 genes grouped together with known nuclear ATP9 from other ascomycete fungi. The nuclear ATP9 clade was nested within the mitochondrial ATP9 (mtATP9) clade, which in turn was nested within the alphaproteobacterial ATP9 clade, supporting the hypothesis that the nuclear ATP9 under consideration originated in a transfer from the mitochondrion to the nucleus. Given the fact that nuclear ATP9 occur in several other classes of Ascomycota (Box 2), the transfer dates back to at latest before the diversification of Pezizomycotina.

The class Lecanoromycetes includes fungi with either nuclear or mitochondrial ATP9, and sometimes both (Box 2). The two known nuclear ATP9 homologues, ATP9-5 and ATP9-7 were both represented in the lecanoromycete genomes. The patchwork of three ATP9 homologues (mtATP9, ATP9-5, and ATP9-7) can be explained by an ancestral transfer followed by gene loss. Most notably in the context of this study, several groups of Lecanoromycetes have lost mtATP9 and retained only a nuclear copy. Gene loss also affected nuclear ATP9 homologues. None of the 10 surveyed Lecanoromycetes retained both ATP9-5 and ATP9-7. Cladonia macilenta had neither (while retaining mtATP9), the other species had either one or the other. Members of subclass Lecanoromycetidae, other than Cladonia, retained ATP9-5, while Gomphillus, the only member of Ostropomycetidae surveyed, retained ATP9-7. Further research will map the nuclear ATP9 across the lecanoromycete fungi and check how the new data points alter our understanding of the evolutionary history of this gene.

2.4 | A nonfunctional transfer can be ruled out

All four putative ATP9 contained at least one intron (Table S3). The dN/dS ratios between the nuclear ATP9 from Lecanoromycetes ranged from 0.007 to 0.249 indicating that the gene is under purifying selection and is not a nonfunctional transfer from the mitochondrial to nuclear genome (see Richly & Leister, 2004).

2.5 | Conclusion

Pogoda et al. (2018) hypothesized that some lichen fungi rely on other members of the symbiosis for ATP production based on the apparent lack of the ATP9 gene in four Lecanoromycetes. We were able to find a putative ATP9 homologue in all four genomes, both in new data produced for this study and in metagenomic data from the original publication. This reaffirms that, as expected, the fungi
postulated to lack ATP9 retain a nuclear copy of the gene, as in many other fungi.

Our reanalysis underlines that the apparent lack of any one gene does not automatically translate into the loss of biological function, especially when the rest of the pathway is maintained. While ATP9 indeed appears missing from mitochondrial genomes of some Lecanoromycetes, this result by itself is not sufficient to back the claim that lichen fungi lost oxidative phosphorylation.
BOX 2  Phylogenetic tree of ATP9

Phylogenetic tree of $F_{1}F_{0}$ ATP synthase subunit C across fungi and bacteria. To assemble the data set, we used three types of sequences: putative ATP9 homologue from the four genomes generated for this study (in bold), ATP9 homologues from publicly available lecanoromycete genomes (Supporting Information), and published sequences of $F_{1}F_{0}$ ATP synthase subunit c from a variety of fungi and bacteria (Table S4). We aligned the sequences and reconstructed a phylogeny with IQTree (Nguyen et al., 2015). The four ATP9 homologues in question grouped together with known nuclear ATP9 from Ascomycota (classes Eurotiomycetes, Sordariomycetes, and Dothideomycetes). The remaining lecanoromycete sequences were either in the same nuclear ATP9 clade, or grouped together with mtATP9, with one species (Ramalina intermedia) having both a nuclear and a mitochondrial homologue. All but one lecanoromycete nuclear ATP9 were assigned to the ATP9-5 clade; these fungi were from Lecanoromycetes subclass Lecanoromycetidae. The only member of subclass Ostropomycetidae, Gomphillius americanus, had ATP9-7.
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Gulnara Tagirdzhanova, Toby Spribille, and John P. McCutcheon designed the study and wrote the manuscript. Gulnara Tagirdzhanova gathered and analysed the data and produced figures.

DATA AVAILABILITY STATEMENT

Raw metagenomic data, metagenomic assemblies, and genome annotations: European Nucleotide Archive (PRJEB42325). Full description of the analysis, custom scripts, and data files have been made available at the Dryad repository at https://doi.org/10.5061/dryad.xgxd2

REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.


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