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NEWS AND VIEWS

COMMENT

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Lichen fungi do not depend on the alga for ATP production: A comment on Pogoda et al. (2018)

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Abstract

Lichen fungi live in a symbiotic association with unicellular phototrophs and most have no known aposymbiotic stage. A recent study in *Molecular Ecology* postulated that some of them have lost mitochondrial oxidative phosphorylation and rely on their algal partners for ATP. This claim originated from an apparent lack of *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.

KEYWORDS
ATP synthase, *ATP9*, gene loss, mitochondrial genome, symbiosis

1 | 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 F_1F_0 ATP synthase subunit C, one of the key proteins involved in oxidative phosphorylation, was missing from several fungal mitochondrial genomes (see also Funk et al., 2018; Pogoda et al., 2019; Stewart et al., 2018). For some of these species, the authors were able to find a copy of this gene in the nuclear genome (a gene transfer phenomenon known from many eukaryotes, including ascomycetes, see Déquard-Chablat et al., 2011). For four lichen symbioses—*Alectoria fallacina*, *Gomphillus americanus*, *Heterodermia speciosa*, and *Imshaugia aleurites*—they did not detect any copy of the fungal *ATP9* gene. The authors concluded that in these symbioses, the fungus may rely on the alga for ATP production. This result has been since cited as evidence of obligate dependence of lichen fungi on their algal partners (e.g., Funk et al., 2018; Puri et al., 2021).

Several lines of evidence make this scenario improbable:

1. The complete loss of oxidative phosphorylation would inevitably be reflected in massive change in the mitochondrial genome (e.g., Heinz et al., 2012). The fact that all but one of the analysed mitochondrial loci were found in all the genomes suggests that the function of mitochondria remains intact.
2. Fungal sexual reproduction via ascospores is intact in all four species; *G. americanus* reproduces only sexually. No vertical transmission is associated with this route. The ascospore has to be autonomous in order to germinate and find a compatible alga.
3. Close relatives of some of these species have been isolated in axenic cultures (e.g., *Heterodermia pseudospeciosa* and *Alectoria ochroleuca*; Crittenden et al., 1995; Yoshimura et al., 2002). They, therefore, are autonomous in ATP production.
4. All known instances of symbionts importing host ATP are from intracellular endosymbioses (e.g., Haferkamp et al., 2006). In lichens, the transfer would require sophisticated new mechanisms,

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 Interproscan accession IPR000454 (ATP synthase, F_0 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, and found highly similar genomic regions in all of them. For *A. fallacina* and *G. americanus* the putative *ATP9* genes were identical in our assemblies and the assemblies from Pogoda et al. (2018); in *H. speciosa* and *I. aleurites* the sequences were >98% identical with bit score >1000.

Analysis of coverage suggests that the putative *ATP9* copy was located in the nuclear genome (Box 1). In all four cases, contig coverage was similar to other contigs assigned to their respective nuclear genomes and much less than that of the mitochondrial contig.

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

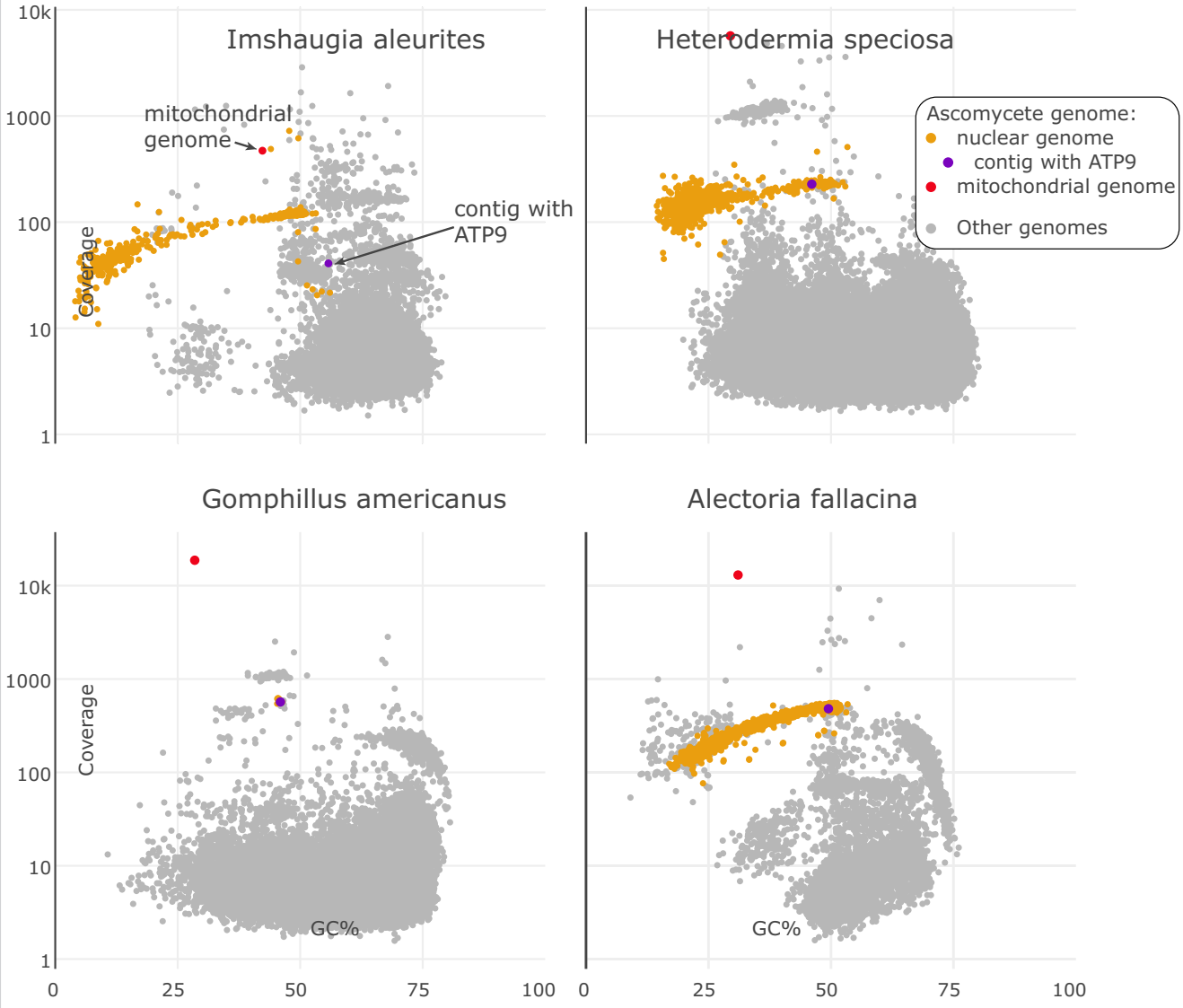
All four putative *ATP9* contained at least one intron (Table S3). The dN/dS ratios between the nuclear *ATP9* from *Lecanoromycetes* ranged from 0.007 to 0.249 indicating that the gene is under purifying selection and is not a nonfunctional transfer from the mitochondrial to nuclear genome (see Richly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

BOX 1 Coverage analysis

GC-coverage plots for the four metagenomes produced in this study. Dots representing contigs are positioned according to their GC content and coverage. Metagenomic data analysed using the metaWRAP pipeline (Uritskiy et al., 2018). We assembled metagenomes de novo, binned them using coverage and kmer distribution statistics, and identified lecanoromycete metagenome-assembled genomes (Supporting Information). In each metagenome, we located the contig corresponding to the mitochondrial genome (red dots): With a tBLASTn search, we located homologues of two mitochondrial loci, *ATP6* and *ATP8*; in each case they resided together in a single high-coverage contig, which we assigned to the mitochondrial genome. The contig containing putative nuclear *ATP9* (purple dots), by contrast, had a lower coverage than the mitochondrial contig and similar to that of other contigs assigned to the nuclear genomes (orange dots).

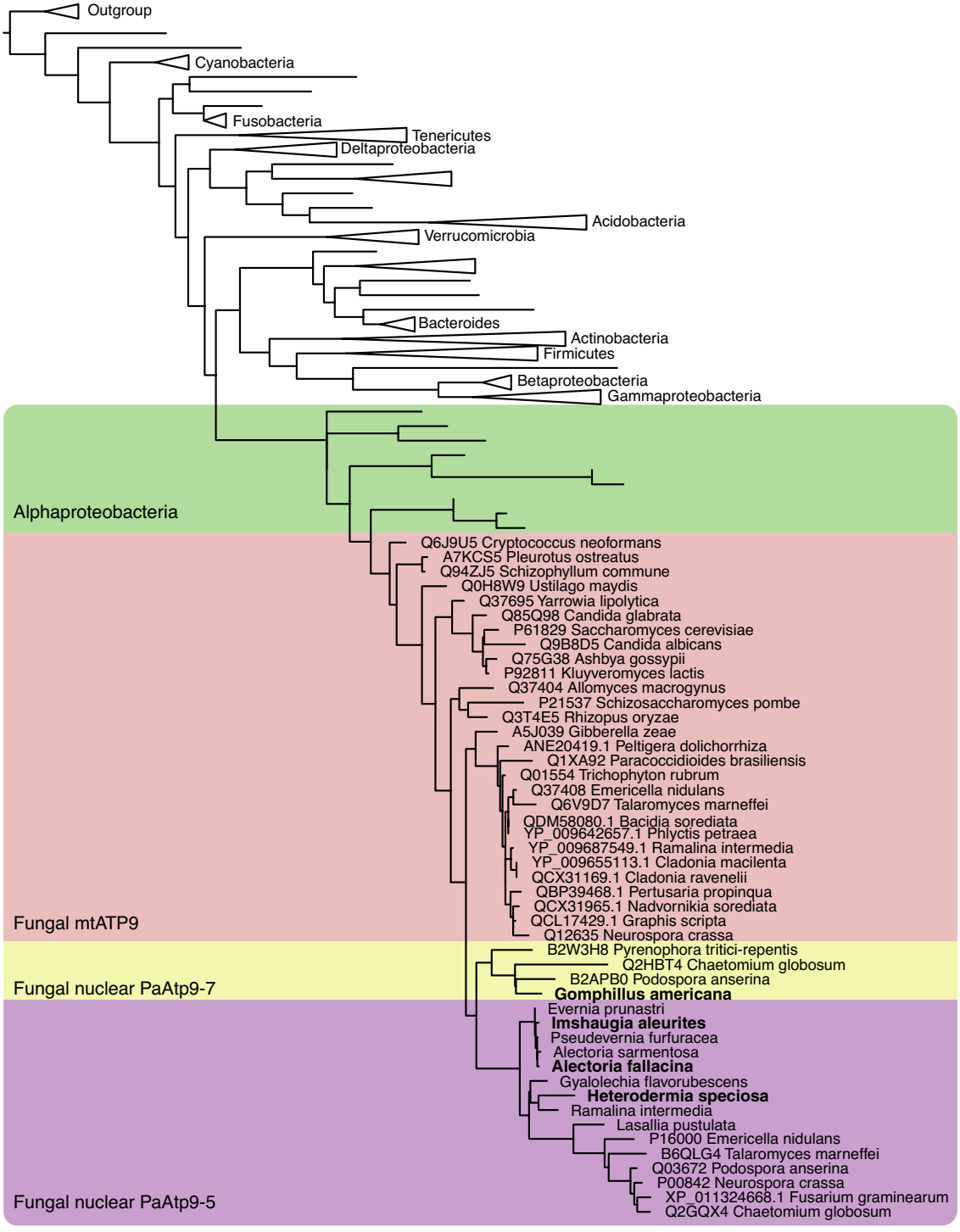


postulated to lack *ATP9* retain a nuclear copy of the gene, as in many other fungi.

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_1F_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_1F_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*, *Gomphillus 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.xgxd254gd and https://github.com/metalichen/Lichen-fungi-do-not-depend-on-the-alga-for-ATP-production.

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SUPPORTING INFORMATION

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

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