

Commentary

News from 'black belt' masters of symbiosis

The reputation of lichens as extremotolerant organisms is built on their occurrence in habitats where most other eukaryotes find it difficult to survive. Lichens withstand recurrent cycles of desiccation and re-wetting in their environments by reverting to a state without active signs of life during periods of drought, yet they are able to resume metabolism quickly with appropriate levels of hydration. In this stage, a substrate's conditions, including chemistry, pH level, and water availability, determine the composition of lichen species in different habitats. Their sensitivity in metabolically active stages also explains the use of lichens as fine-tuned bioindicators of environmental conditions.

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The intertidal zones along extratropical rocky coasts represent unique habitats for lichens. These are characterized by periodic inundation with sea water (and rainwater or heat and drought, depending on aerial meteorological conditions), in addition to variations in temperature and mechanic forces. Lichens adapted to this habitat form a clear belt-like zonation along rocky coasts of temperate to cold latitudes. Especially where the intertidal range is high, these belts are recognizable from a distance by the characteristic colors of the lichen thalli (Fig. 1). The lowermost belt of the littoral fringe has a black color, which even to the unexperienced eye can be distinguished from remnants of an oil spill due to its uniformity. Most lichens of this community are crusts attached with their entire surface to the rocks (often dominated by *Hydropunctaria maura* (Wahl.) Keller & Gueidan and related species). By contrast, the genus *Lichina* is well-distinguished by shrub-like growth.

Lichina pygmaea (Lightf.) C. Agardh (Lichinomycetes), which develops dark flattened bands (Fig. 2), has been the focus of early research on osmoregulation (Feige, 1972, 1973, 1975). The species from the Northern Hemisphere is widely known, whereas recent work showed that Southern Hemisphere samples need to be distinguished as a different species, *Lichina intermedia* (C. Bab.) M. Schultz (Schultz, 2017; Garrido-Benavent *et al.*, 2023). In a 2021

study on *L. pygmaea*, Christmas *et al.* (2021) found that in addition to its known cyanobacterial photobiont *Rivularia* (Nostocales), thalli of *L. pygmaea* regularly contain other cyanobacteria, in particular *Pleurocapsa* (Pleurocapsales), along with other cyanobacteria and eukaryote algae in minor amounts. Few metagenomic and transcriptomic studies on lichens have revealed the roles of either mycobiont or photobiont partners (Juntilla *et al.*, 2013; Wang *et al.*, 2015; Steinhäuser *et al.*, 2016; Armaleo *et al.*, 2019), or focused on the associated bacterial microbiome (Cernava *et al.*, 2017, 2019). A new paper by Christmas *et al.*, recently published in *New Phytologist* (2023, doi: [10.1111/nph.19320](https://doi.org/10.1111/nph.19320)) provides novel insights, by using rRNA based metabarcoding and metatranscriptomic analyses, to investigate how two prominent cyanobacteria present in the thalli react by differential activity across inundation scenarios. The authors sampled this species at low- and high-tide. The analyses were consistent with their hypothesis, showing that *Rivularia* was more active when thalli were inundated at high tide, whereas the activity of *Pleurocapsa* dominated at low tide. The differences were observed both in the ribosomal RNA and in mRNA levels. These differences were generally less pronounced when thalli were hydrated from recent rainfall at low tide, which suggests that higher salt levels significantly contribute to photobiont task splitting.

The study also revealed evidence for a wider ability of lichen mycobionts to process carbohydrates produced by the hosted photobionts, including their polysaccharides. It is worth noting that the high abundance of trehalose metabolism-related genes in *Rivularia* could be correlated with a response to seawater stress. Both cyanobacterial photobionts produce disaccharides (trehalose in *Rivularia*; sucrose in *Pleurocapsa*). These appear to be exported as components of the extracellular polysaccharide matrix. Careful analysis of the meta-transcriptome suggested that the disaccharides are hydrolyzed by fungal enzymes in the extracellular matrix before they can be taken up by appropriate monosaccharide transporters, as disaccharide transporters are missing. These new results suggest a shift from the typical textbook understanding, mostly based on lichens involving eukaryotic algae, which claims that specific polyols produced by algae and glucose of cyanobacterial photobionts are the sole transport metabolites for feeding the fungal symbionts. The extracellular pre-processing of more complex carbohydrates originating from the photosynthetic partners indicates that lichen mycobionts have additional possibilities to control their carbon budget.

A previous study by Casano *et al.* (2011) suggested that two strains of the eukaryotic *Trebouxia* present in *Ramalina farinacea* may help the lichen adapt to a wider range of habitat conditions. Cultured algae showed that one of the strains had a higher performance at higher temperatures and irradiation than the other strain. These differential functions were, however, not studied in native thalli. The present work of Christmas *et al.* (2023) helps

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Fig. 1 Belt-like intertidal zonation of lichens on coastal rocks at low tide, with the black belt indicating the littoral zone (île de Landes, Brittany, France; Photograph: Martin Grube).



Fig. 2 *Lichina pygmaea* with dark band-shaped branches. Left: dry thallus, right: inundated thallus. Same scale for both pictures (Photograph: Martin Grube).

extend this hypothesis *in situ* to include adaptation to periodic variation within a single habitat, as *L. pygmaea* seems to perform better in fluctuating environmental conditions of the intertidal zone with two photobionts. Intra-thalline multiplicity of photobionts might indeed be more widespread than previously thought (de Carolis *et al.*, 2022). Such diversity also supports the view of lichens as forms of microbial ecosystems (Hawksworth & Grube, 2020), extending from a two-tier relation to encompass potential further photobionts as well as associated fungi and bacteria (e.g. in *Lichina*, West *et al.*, 2018). These results suggest that new studies, with a broader range of samples, are needed to determine whether photobiont multiplicity is a general adaptation mechanism of lichens under conditions with rapid environmental variations. While it would be plausible to restrict to a single partner

to lower costs by unified regulatory mechanisms, investing in intra-thalline niche-partitioning of photobionts seems to pay off when habitat conditions are highly variable.

A different type of habitat-driven partnership with photobionts is represented by photosymbiodemes. This symbiotic strategy generates two distinct photomorphs with either cyanobacteria or green alga as primary photobionts. A recent study on the lichen *Peltigera britannica* explored how gene expression in the fungus varied between thallus sectors with only cyanobacterial photobionts, and those with green algal photobionts (where photobionts are hosted as auxiliary partners only in small, separated organs called cephalodia), particularly under different temperature conditions (Almer *et al.*, 2022). Results of RNA-sequencing showed photobiont-mediated differences in fungal gene

expression. Genes associated with distinct biological processes are upregulated in association with specific symbionts, indicating that the type of symbiotic partner has a significant influence on the fungal gene expression. When examining the effects of temperature, Almer *et al.* (2022) found that higher temperatures caused an increase in gene expression related to heat shock response across all involved organisms. Both types of photobionts showed increased expression of photosynthesis-related genes at 15°C and 25°C. However, while the fungus and the cyanobacteria showed signs of thermal stress at 15°C, the green algae showed a stress response primarily at 25°C. This outcome suggested that the symbionts expressed responses to temperature changes, but more detailed differences of photobiont activities were not explicitly studied.


The data presented by Christmas *et al.* (2023) add a new piece to the puzzle of lichen biology. Being able to structure lichens as a minute ecosystem, lichen-forming fungi may: (1) switch between photobionts across their ecological and geographic range (Ortiz-Álvarez *et al.*, 2015); (2) sustain multiple photobionts (Casano *et al.*, 2011; Christmas *et al.*, 2023); (3) regulate their relative numbers in the thalli (Sun & Friedman, 2005); and also (4) regulate their activity, depending on the ecological and morphological context (Hyvärinen *et al.*, 2002). The evidence also suggests that lichenized fungi may (5) also display flexible usage of carbohydrates produced by the photobionts. These patterns may also occur in combination, which adds to the flexibility of symbiotic configuration in lichens. As emerging macroscopic life forms, emanating from the interaction of microbial partners, lichens continue to reveal new secrets about how to master a symbiotic lifestyle.

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