Lichens

6

Martin Grube

Abstract

Lichenized fungi initiate their symbiotic structures from microscopic stages after recognition of compatible algae. The partnerships ultimately emerge as complex macroscopic phenotypes which are unrivaled in the fungal kingdom by their resilience and durability. This chapter presents an overview of lichen symbioses and covers the morphology and systematics of the fungal phenotypes, as well as their associations with diverse photobionts. This is followed by a coarse overview of ecophysiology and the secondary chemistry. A special focus is given to the diversity of and the interactions with additional microorganisms. Finally, a few comments on the effects of pollution and environmental change point to the usefulness of lichens as bioindicators.

Keywords

Symbioses · Morphology · Mycobiont · Photobiont · Microbiome

6.1 Introduction

Lichens are a unique group of organisms in many ways. They are perhaps the only fungal life forms that can be recognized when looking down through the windows of a flying airplane. Lichen symbioses can develop ground-covering carpets in landscapes where plant vegetation is open or absent. In the boreal tundra, along rocky coasts, in alpine habitats, or in coastal deserts they may represent the dominating life form (Fig. 6.1). Although it is impossible to get a precise figure, and only a rough estimate claims 8% coverage of the Earth's land surface (Larson 1987), lichens contribute substantially to biosphere processes and food chains (Elbert et al. 2012; Asplund and Wardle 2017).

In earlier days of systematics, lichens were classified as a separate taxonomic group: "Lichenes," but the etymology of this term is not entirely clear. The Greek word *λειχην* (as it appears in Dioscorides' works), possibly derived from λειχω (Latin lingo), means to lick up or "eating around itself," whereas the word λιχεin has the meaning of "poor" or "little." Whether Linnaeus encapsulated this association when calling lichens "rustici pauperrimi" ("peasants") of vegetation is unclear, but he may have appreciated lichens more had he known that each of them represents a pocket-sized ecosystem.

M. Grube (✉)
Institute of Biology, University of Graz, Graz, Austria
e-mail: martin.grube@uni-graz.at

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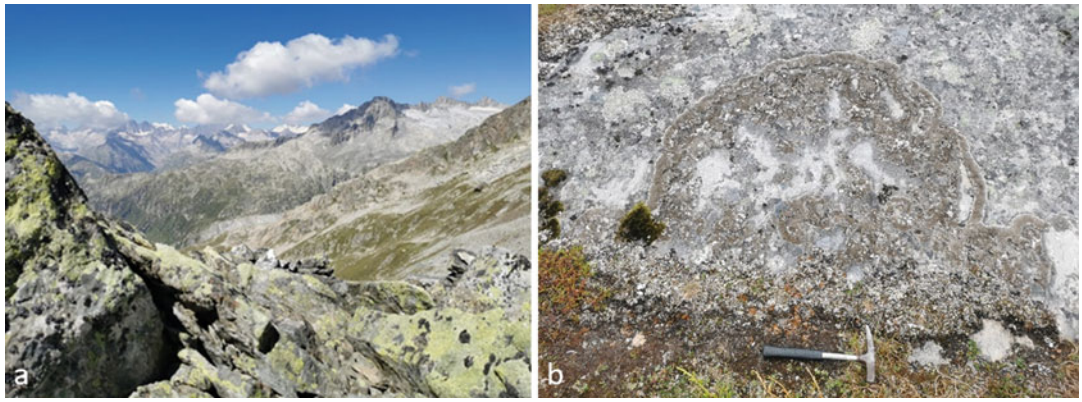

Fig. 6.1 (**a**) Alpine landscape tinged by lichens. Switzerland; (**b**) large thallus (c. 1 m diam.) of *Hypogymnia physodes*, Sweden. Photographs: M. Grube

6.2 What Are Lichen Symbioses?

The recognition of lichens as a dual association between a fungus and an alga is attributed to Simon Schwendener, who initially announced his discovery in a talk for the annual general meeting at the Swiss Natural History Society in 1867, and later summarized it in his publication (Schwendener 1869). He concluded that lichen-forming fungi are "parasites, although with the wisdom of statesmen," with their algal partners as "helotes," the class of slaves in ancient Sparta. The term symbiosis (as "Symbiotismus") was later introduced to biology by Albert Bernhard Frank (1876), with lichens as an example. After vigorous rejection of the symbiotic hypothesis by many contemporary lichenologists, it took decades until lichens were classified as fungal life forms and arranged within the taxonomic system of fungi. In textbooks, they are commonly described as a partnership of one fungus with an algal partner (considering also cyanobacteria within the algal lifestyle). While this view has not changed much, evidence shows that lichens evolved as open systems and can be interpreted as miniature ecosystems, which include a variety of organisms operating at different trophic levels (Farrar 1976). A lichen can therefore be regarded as a self-sustaining ecosystem formed by the interaction of a fungus (mycobiont) and one or more microbial photosynthetic partners (photobionts) and an indeterminate number of other microscopic organisms living in association with the fungus and algae (Hawksworth and Grube 2020). In most cases, the fungus acts as the main partner to provide structure. The symbiotic phenotype results from the interaction with the microbial photosynthetic partner, required for the development of fungal sexual structures. Nevertheless, the participating fungus may grow separately under certain conditions—including axenic cultivation (and the same holds true for the algal partners). We may call the fungus a physiologically facultative biotroph, while the resulting symbiotic "lichen" phenotype results from ecologically obligate biotrophy of the lichen-forming fungus (Honegger 2012). As the classification of lichen-forming fungi is fully integrated into the fungal systematics as a whole, we consider the lichen symbiosis as a fungal lifestyle. Therefore, lichens also can be described as fungi that form self-sustained ecosystems containing an extracellular arrangement of one or more microbial photosynthetic partners and an indeterminate number of other microscopic organisms. All organisms within the symbiosis retain their independent taxonomic names, and the collective association itself has no separate name.

Various cases of algal–fungal interactions are not recognized as lichen symbioses, including cases where photoautotrophs are harbored inside fungal cells. For example, *Mortierella elongata* (Mucoromycota), which takes up algae of *Nannochloropsis oceanica* (Du et al. 2019) in its hyphae or *Geosiphon pyriforme* (Glomeromycota) containing *Nostoc* cyanobacteria in bladder-like swollen fungal cells (Schüßler and Kluge 2001). Other examples of fungal–algal associations are harder to distinguish from lichens as defined above. Loose fungal–algal associations have sometimes been dubbed "primitive" or "borderline lichens" (Kohlmeyer et al. 2004). In such cases, the fungal partners do not develop a well-differentiated cortical layer or other fungal structures considered characteristic of lichen thalli, but they develop ascomata and conidiomata following non-pathogenic growth on certain types of algae (as in e.g., *Collemopsidium* spp., *Mastodia tessellata*, *Trizodia acrobia*). Others can live either as saprotrophs on bark or as primitive lichens depending on the details of substrate conditions. This phenomenon has been named "optional lichenization" by Wedin et al. (2004). Also in these cases, characteristic thallus structures are hardly developed. Comparing the genomes of such poorly developed lichen-like life forms with fully developed lichen phenotypes could provide insights into the genomic architecture required for forming lichens.

It has often been claimed that lichens are very old fungal lifestyles. Whether fossils found in c. 600 Ma phosphorites of the Doushan Formation are primitive lichens is unclear (Yuan et al. 2005). It has been suggested that they represent fungal interactions with benthic marine cyanobacterial colonies. Using time-calibrated phylogenies of ascomycete fungi and algae, focusing on lineages with lichen symbionts, Nelsen et al. (2020) estimated ages of several interacting clades and found out that fungal origins of lichenization must have occurred soon after the emergence of land plants (tracheophytes). Clear fossil evidence for this step, which may inform hypotheses of how lichen thalli evolved from unspecific fungal–algal associations, is missing. The younger (micro-) fossils of lichens from the Lower Devonian Welsh Borderland are approximately 415 Ma old (Honegger et al. 2013). They already show leaf-like thalli with characteristic internal stratification. This level of differentiation is also found in younger fossils of lichens, some of which can even be determined to genus level (cited in Honegger 2012).

6.3 The Lichen Phenotype

The formation of more or less sealed, hydroelastic layers of vegetative mycelium appears to be a key evolutionary step for developing a lichen phenotype. Usually, the fungus tightly wraps algal colonies, which enforces functional coherence and lichen thallus integrity. A tight closure of mycelial structures is achieved when fungal hyphae glue together by their more or less gelatinizing outer cell walls, which creates a structure where cells are embedded in a joint matrix. The gelatinizing outer cell wall material has been named variably by different authors "Kittsubstanz" (cementing substance) by Peveling (1970), "gelatinous matrix" by Ahmadjian (1993), "conglutinate zone" by Honegger (1991), or "extracellular interaction matrix" (Spribille et al. 2020). The latter term included the potential roles of additional microorganisms in building this structure. Spribille et al. (2020) provided a comprehensive summary about the current state of knowledge about polysaccharides in lichens. It still needs to be determined whether proteins targeting cell wall polysaccharides could eventually play a role in the functioning of the intercellular matrix (e.g., in cell wall remodeling or as carbohydrate-binding proteins). The tissue-like functional integration of fungal plectenchyma, by conglutination by polysaccharides of the outer cell walls, is otherwise well known from the excipula of fungal sporocarps. Since such sporocarp structures are known from groups which share ancestry with those that contain complex lichen thalli (Díaz-Escandón et al. 2022), it is likely that basic ontogenetic processes evolved before lichenization

and progressively adapted to form thalline cortex structures as well.

The proper formation of the extracellular "glue" to establish lichen thalli requires interaction with compatible photobionts. Resynthesis experiments have therefore been conducted to better understand the processes of interplay between algae and fungi. These experiments showed that associations with other than the original algae lead to only loose or temporary attachment of fungi (e.g., Ahmadjian et al. 1980; Schaper and Ott 2003). Resynthesis experiments in agar plates must nevertheless be interpreted with caution, because apart from the identity of the photobiont, medium composition and growth conditions also play a substantial role in the ontogeny of lichens. Mycobionts tend to develop only irregular cell clusters in standard culture media, and even co-culture with the native photobiont does not generally lead to the development of native thallus structures. As an alternative to growth on agar media, Stocker-Wörgötter (2001) used sterilized soil to successfully re-establish the native phenotype of other lichens starting with axenically cultured symbionts. However, combined with appropriate culturing conditions (e.g., under nutrient poor or changing light conditions) the formation of layered thallus structures is possible in certain species. Recently, Kono et al. (2020) managed to grow the native phenotype of *Usnea hakonensis* axenically to study upregulated sets of fungal and algal genes in a symbiotic state. They found evidence for various processes involved in symbiotic establishment, including cell wall remodeling, production of hydrophobins (which seal an apoplastic continuum between interacting cells of fungi and algae) and symbiosis-specific nutrient flow (including polyol transporters).

The required sequential process of lichenization involves five distinguishable steps: (1) the "pre-contact stage," (2) the "contact stage," (3) "envelopment" of algal cells by the fungus, (4) their "incorporation" into a pre-thallus, and (5) "differentiation" into a thallus. It was originally proposed that mycobionts recognize compatible photosynthetic partners by the shape of their cells (Ahmadjian and Jacobs 1981; "thigmotropism hypothesis"). Joneson and Lutzoni (2009) found no support for this hypothesis using glass beads as algal "dummies." Based on these results, Joneson et al. (2011) introduced a "signalling hypothesis" after they found that between 11 and 28% of mycobiont and photobiont genes, including three fungal lipases, are upregulated in the "pre-contact" and the "contact stage" prior to physical contact of the symbionts. Fungal genes upregulated in cocultures of *Cladonia grayi* with its algal partner *Asterochloris glomerata* include membrane transporters, secreted hydrolases, and small proteins, as well as a specific ribitol transporter (Armaleo et al. 2019). The latter is of relevance, as it has been shown that the polyol ribitol enhances both growth of mycobionts and their mucilage production (Meeßen et al. 2013). Interestingly, ribitol is not secreted in aposymbiotic cultures, suggesting more complex upstream signaling processes. Further research of ontogenetic processes ought to consider the role of fluctuating hydric conditions in the natural habitat, since it has been shown that such fluctuations contribute to algal cell wall remodeling (González-Hourcade et al. 2020), and possibly alter the transmission of signaling metabolites as well.

Current literature suggests that certain groups of molecules (including fungal lectins and algal cyclic peptides) are correlated with early contact of symbionts, while others (e.g., phytohormones and molecules involved in carbon exchange) are important throughout all stages of lichen synthesis. In the fully formed thallus, specialized (traditionally called "secondary") lichen metabolites and mineral nutrition (via substrate, microbial, or airborne) stabilize the thallus functionality (Pichler et al. 2023). Poorly water-soluble specialized metabolites can accumulate as substantial amounts of crystalline extracellular deposits on the surface of hyphae, particularly in the peripheric cortex where they act as light filters, among other functions.

6.4 Morphologies and Growth Types

Lichens display diverse morphological shapes and growth styles. Here the focus is given to ascomycetous lichens, with thalli that may be coarsely classified as brush-like, crust-like, or leaf-like forms (Grube and Hawksworth 2007). This is an arbitrary grouping, since there is much greater diversity when studying the details of lichen growth forms. In the following, I would prefer to primarily distinguish lichen growth types which are surface-attached (including those penetrating the substrate surface) and the remainder, which develops surface-detached forms of thalli. Basidiomycetous lichens have thalli which are constructed in simpler ways and lack the typical stratification found in many ascomycetous lichens (Oberwinkler 2012). Most could be assigned to crustose types of thalli and consist of small clusters of algal cells surrounded by fungal hyphae. In other cases (*Dictyonema*), the thallus is apparently derived from the basidiocarps, and thus superficially resembles foliose thalli (Dal-Forno et al. 2013).

6.5 Surface-Attached: Crustose Lichens

Crustose thalli (Fig. 6.2 a–e) include both, more or less mycelial forms that develop within the substrate and those with the main body mass appearing on top of the substrate, where they often develop a closed upper fungal layer (upper cortex). Species developing within the substrate, such as rocks (mostly calcareous rocks) or bark, usually lack such an upper cortex. Crustose thalli attach directly to the substrate with their entire lower surface and usually develop over or inside more or less stable substrates. Microstructure, chemical cues, mechanical parameters, and water holding capacity could be factors determining which species can colonize the substrate. Episubstratic crustose lichens develop typical growth pattern as their symbiotic structures are prone to poikilohydric conditions and recurrent shear forces of swelling and shrinking caused by changing water content. These forces commonly induce the development of fissures in the thalli, which give rise to the formation of areoles. These small thallus patches can become physiologically independent (removal of an areole does not influence the vitality of neighboring areoles). The distinct fissures between the areoles seen in dry stages usually close tightly when thalli of these types are irrigated. The formation of crustose thalli with areole formation is correlated with the development of a specifically composed cortex layer. This type of fungal layer contains the remnants of algal cell walls in the intercellular matrix between living or decaying fungal cells (phenocortex, e.g. in *Rhizocarpon geographicum*, Fig. 6.3a, c, e). Algal cell walls are pushed toward and into the upper cortex from the algal layer, where new algal cells are produced by autospore formation.

The anchoring of crustose and squamulose thalli in the substrate is accomplished either by hyphae produced over the whole lower surface (such as a rhizohyphal felt, which is produced by individual hyphae extending into the soil substrate) or by central rhizinae (when hyphae bound together in a root-like structures).

6.6 Surface-Detached Thalli: Foliose and Fruticose Lichens

While crustose lichens still contain decaying algal cell walls in the cortex, surface-detached lichens exclude algal cells from their upper cortex, and the uniform extracellular matrix apparently represents a mechanical reinforcement allowing them to grow in three dimensions away from the substrate or as flat branches over the substrate surface. Such growth strategies are found in unrelated lineages of lichenized fungi and have also evolved among closely related groups of lichens (Grube and Arup 2001). The upper cortex layer in surface-detached lichens seems more coherent because fragmentation into areoles does not occur (e.g., in *Rusavskia elegans*, Fig. 6.3b, d, f). By exclusion form the cortex, the walls of algal

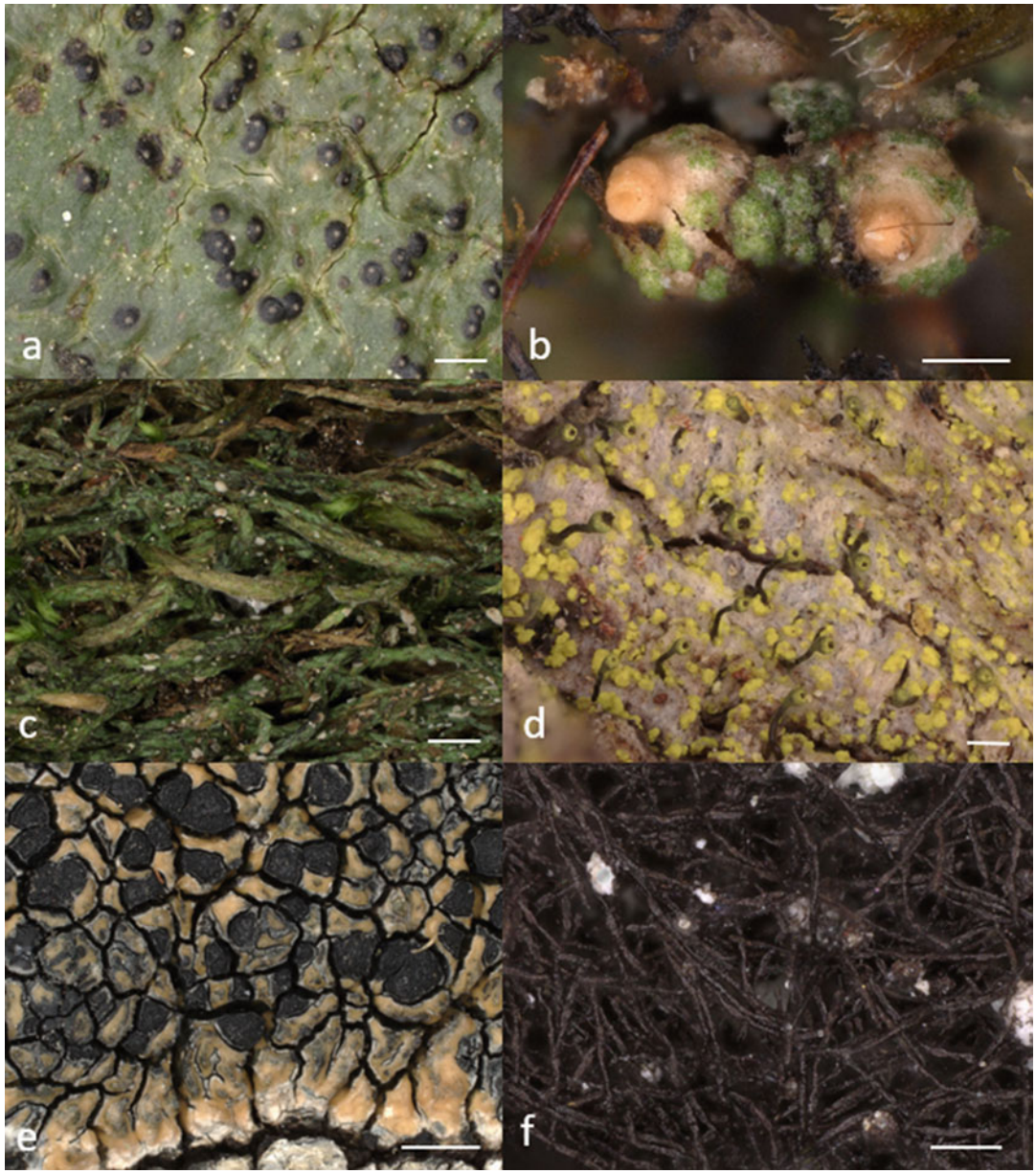

Fig. 6.2 Crustose (**a**–**e**) and microfilamentous (**f**) lichens: (**a**) *Pyrenula nitida* (Austria), bar = 500 µm; (**b**) *Leucocarpia biatorella* (Sweden), bar = 250 µm, (**c**) *Vezdaea aestivalis* (Austria), bar = 1 mm (**d**) *Chaenotheca chrysocephala* (Austria), a pin lichen, bar = 250 µm, (**e**) *Sporastatia testudinea* (Austria), bar = 1 mm, (**f**) *Cystocoleus ebeneus* (Austria), bar = 1 mm. Photographs: M. Grube

cells often keep their shape in the algal layer even after the cell content decayed, reminiscent of a sort of cell "ghosts" cells in that layer. It is still unknown whether the persistence of algal cell walls is due to loss or down-regulation of fungal carbohydrate metabolizing genes.

With increased stability and flexibility, the eucortex facilitates the functional integrity needed

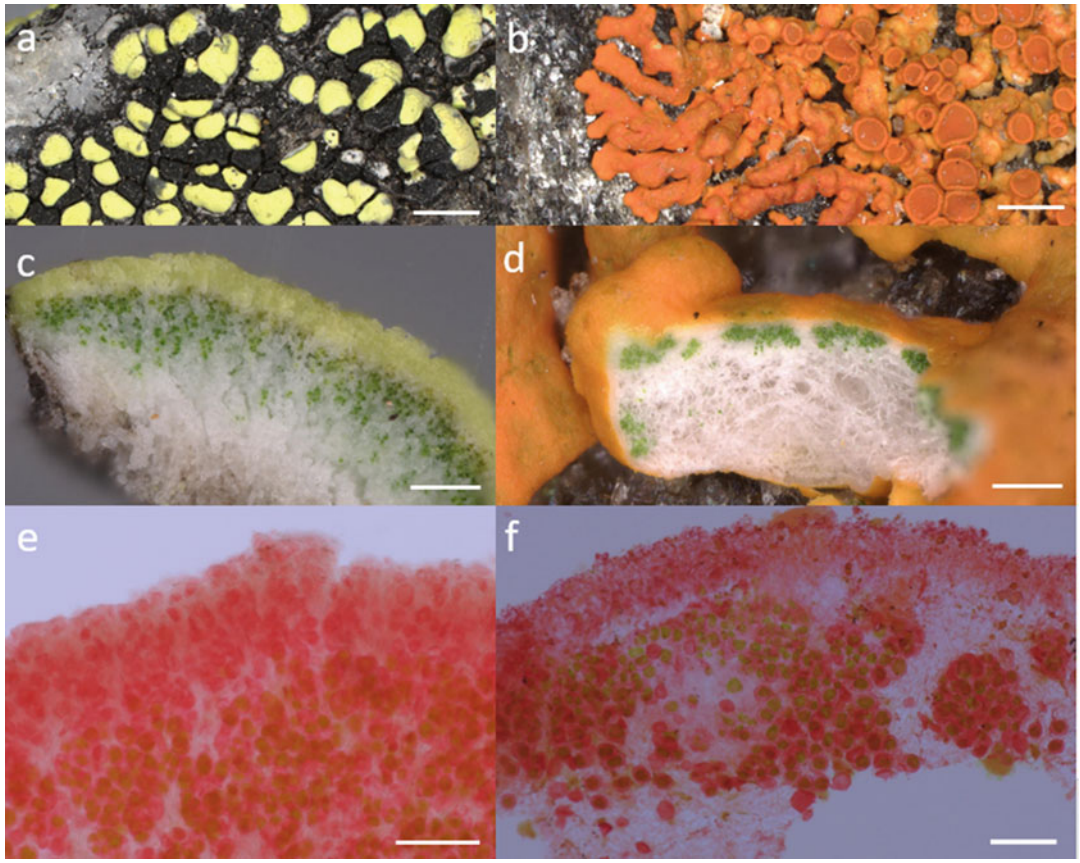

Fig. 6.3 From surface-attached to surface-detached lichens, *Rhizocarpon geographicum* (**a**: habit, **c**: section, **e**: section stained in alkaline Congo red) and *Rusavskia elegans* (**b**: habit, **d**: section, f: section stained in alkaline Congo red) *R. geographicum* is attached to the rock with the entire lower surface and has a phenocortex, whereas *R. elegans* develops a cortex on the lower side, has an eucortex on the upper side, and detaches from the surface with its lobes, **a**, **b**: bar = 1 mm, **c**: bar = 100 μm, **d**: bar = 250 μm, **e**, **f**: bar = 50 μm. Photographs: M. Grube

for development of the larger thalli of morphologically diversified foliose (leaf-like) and fruticose (shrub-like) macrolichens. Many of these lichens also have internal hydrophobic layers to facilitate gas exchange. There are also morphological intermediates between foliose and crustose growth, notably lichens that develop placodioid thalli. Their lower sides partially detach from the substrate at the thallus periphery where they also develop cortex-like plectenchyma on the lower side upper cortex of such transitional morphologies is composed of a mosaic of adjacent portions with either pheno- or eucortex."

The foliose and fruticose lichens, often called "macrolichens," develop the most complex vegetative structures in the kingdom of fungi. Leaf-like (foliose) thalli develop flattened branches of diverse shape (Fig. 6.4a). In most cases, these grow at the tips of the branches, while older parts tend to cease growth and sometimes degenerate at their rear ends (e.g., *Arctoparmelia centrifuga*, *Brodoa intestiniformis*, and others which can form concentric rings). Foliose lichens represent the typical stratified anatomy of lichens illustrated in textbooks with an upper cortex protecting the algal layer below, a loose medulla delimited by a closed lower cortex layer, which connects to the substrate by rhizines as holdfast structures. Besides rhizines extending from the lower surface

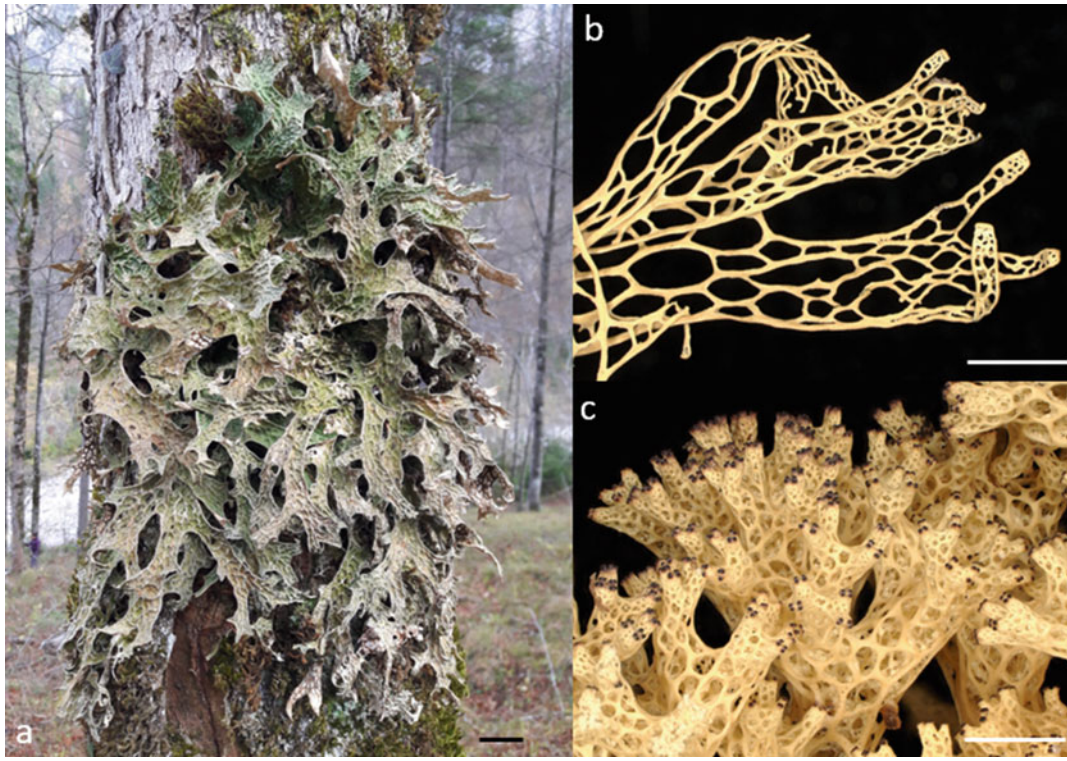

Fig. 6.4 Foliose and terete, surface-detached lichens, (**a**) foliose "lung lichen" *Lobaria pulmonaria* (Austria), bar = 5 cm; (**b**) foliose-terete "California state lichen" *Ramalina menziesii* with typical net-like expansions (California), bar = 5 mm; (**c**) fruticose-terete lichen *Cladia retipora* with net-like growth (New Zealand), bar = 5 mm. Photographs: M. Grube

of the thalli, many other structures of taxonomic value can be formed at the periphery (e.g., cilia which are thick hairs formed by hyphal strands emanating from the thallus margins) or on the surface of thalli, ranging from hair felts to diverse structures for vegetative dispersal, such as isidia (Fig. 6.5a, b) or soredia (Fig. 6.5c, d). Also, special structures for gas exchange, such as pseudocyphellae (patches on upper or lower surfaces where loose hyphae replace the tight cortex for improved gas exchange in many genera), cyphellae (distinct holes on the lower surfaces as in *Sticta* spp.), or holes in the upper surfaces of the thallus (e.g., *Hypogymnia* spp.) are known.

Umbilicate forms (*Umbilicaria* species) represent a special case of foliose growth. Umbilicate thalli differ by being connected to the substrate with a single central, rather rigid holdfast structure, the umbilicus, which is made of strongly conglutinated fungal hyphae. Thalli of *Dermatocarpon* are often also called umbilicate, yet they rather extend laterally from a holdfast and are better called monophyllous. In contrast to most other foliose and terete lichens, the growth of the thalli in Umbilicariaceae can be more or less irregular or "diffuse," (e.g., *Lasallia pustulata*) with its patchy thallus protuberances; growth is, however, inconspicuous at the thallus tips, where thalli may break up or form lacunae and gaps. Sometimes the term intercalary is used synonymously for this type of growth, but this implies discrete non-apical growth and does not apply here.

Terete (shrubby) growth forms vary from upright, brush-like forms to pendant beard-like forms (*Usnea*). They usually lack upper and lower side of the thalli, and their thalli look rather similar from all sides. Typically, developed terete

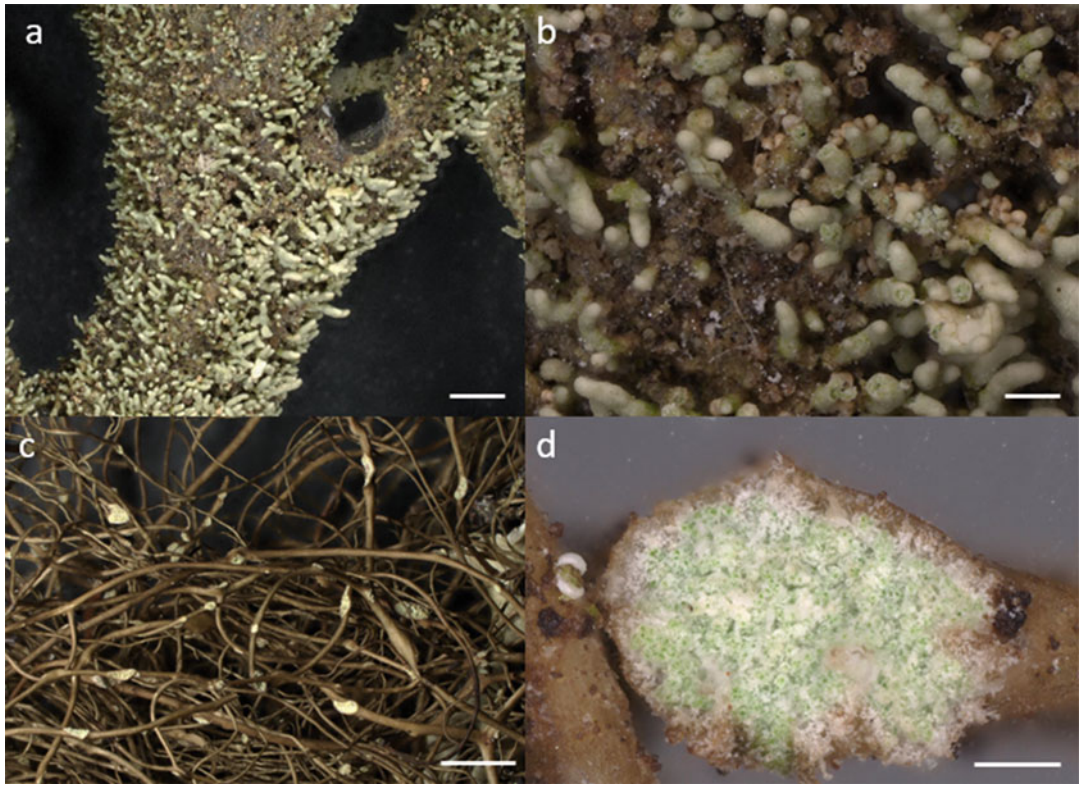

Fig. 6.5 Vegetative dispersal units, (**a**) *Pseudevernia furfuracea* (Austria), foliose thallus surface with isidia, bar = 1 mm; (**b**) isidia detail, bar =200 μm; (**c**) *Bryoria fuscescens* (Austria), soralia on surface of terete thallus, bar = 5 mm; (**d**) soralium with tiny soredia, bar = 150 μm. Photographs: M. Grube

forms are found, e.g., in the genera *Alectoria*, *Bryoria*, *Usnea*, etc. In certain genera, the thalli may also become more leaf-like, and then the distinction between terete and foliose growth is blurred (e.g., *Ramalina*). Perhaps the most spectacular thalli in that genus are formed by *Ramalina menziesii*, occurring along the North American Pacific coast and perhaps the fastest-growing lichen species (Fig. 6.4b). It has flat branches composed of net-like units that develop by diffuse expansion of perforated tissue produced at the growing branch apex (Sanders and Ascaso 1995). Spectacular net-like growth patterns are also known from the southern hemispheric genus *Cladia* (see Fig. 6.4c). More complex thalli are known from *Cladonia* species, which are dimorphic and composed of a scaly leaf-like primary thallus and terete outgrowths called podetial.

6.7 Jelly Lichens

Lichen thalli are rather brittle when dry and flexible when wet. A special case is the jelly lichens, which become slippery and increase significantly in size when irrigated (Fig. 6.6a, b). Most of them (Collemataceae) are associated with cyanobacteria and most of their gelatinous intercellular matrix originates from the photobionts. Jelly lichens generally do not have hyphae with hydrophobic surfaces and lack internal air-filled cavities in the thalli. In contrast to typical green algal lichens, they often lack a distinct functional layering. *Leptogium* species develop a cortex of a single layer of fungal cells, but the thalli of *Collema* lack such a cortex. Although the fungal hyphae merely extend between the predominant algal polysaccharide matrix, the mycobiont has an

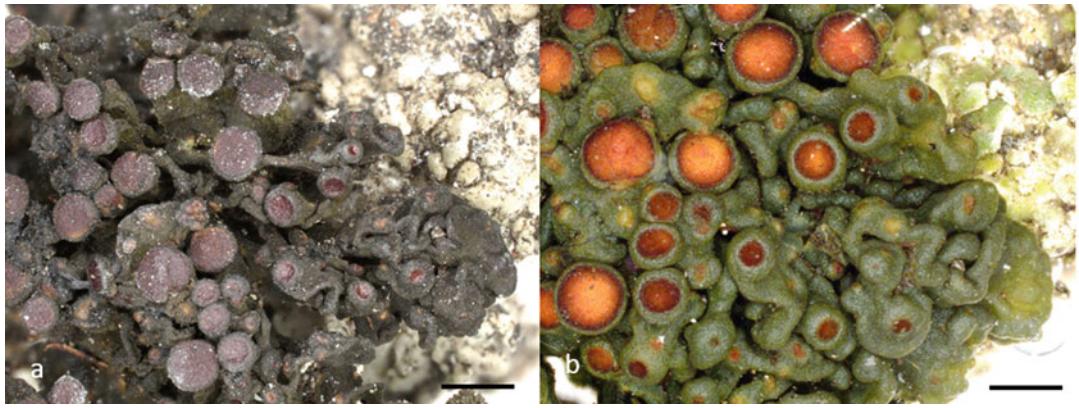

Fig. 6.6 Massive swelling of a jelly lichen, *Enchylium polycarpon* (Austria) with cyanobacterial photobiont (*Nostoc* sp.), when wet, (**a**) dry stage, (**b**) wet stage, bars = 1 mm. Photographs: M. Grube

influence on the overall thallus shape, which differs from free-living cyanobacterial colonies. Apart from these cyanobacterial forms, there are a few more or less jelly-like green algal lichens, but these form thin crusts on soil, mosses, or other lichens (e.g., genera *Epigloea*, *Thrombium*).

6.8 Other Growth Types: Microfilamentous, Microglobose, Leprose, and Byssoid Lichens

Apart from the types presented before, there are other forms which are composites of microscopic thallus structures. The shapes of microfilamentous lichens (Fig. 6.2f) are determined by the photobiont partners which are filamentous forms of cyanobacteria (e.g., *Scytonema* in *Pyrenothrix*, *Scytonema* in *Ephebe*) or trentepohlioid green algae (*Trentepohlia* in *Cystocoleus*, *Racodium,* or many *Coenogonium* species). Microglobose lichens are composed of globose colonies of green algae packed with a coherent, usually single layer of fungal cells (*Micarea* or *Vezdaea*). Similar surface-attached forms are also developed by certain basidiomycetous lichens (e.g., *Lichenomphalia umbellifera* and *Multiclavula mucida*). These mostly hydrophilic thalli contrast with leprose lichens, which have a woolly or powdery appearance, due to loosely interwoven and hydrophobic hyphae developing around small colonies of green algae. These lichens are often sterile and are dispersed by regular fragmentation of the thallus (which usually allows for vegetative dispersal). Thalli that generally do not fragment for dispersal but which have a hydrophobic "fluffy" internal structure without an upper cortex are called byssoid. Such thallus types are frequently found in the tropics (e.g., *Cryptothecia*, *Sagenidiopsis*, *Tania*). Occasionally they break apart into irregular clusters and are then referred to as floccose thalli.

Thallus morphology has always played an important role in lichen classification. Molecular phylogenetic analyses, however, data revealed many cases of convergent evolution of similar growth styles, either by emergence of surface-detached foliose or terete forms from different crustose ancestors within closely related lineages (Grube and Hawksworth 2007), or as similar morphologies that evolved in unrelated lineages (Grube and Kantvilas 2006; Muggia et al. 2011).

6.9 Vegetative Dispersal

Numerous lichens can propagate by specialized vegetative diaspores, which combine fungal and algal symbionts in tiny structures which may easily separate from the parental thallus. This form of

propagation keeps the partners together and allows them to start thallus reproduction without a risky search for germinated fungal spores of compatible algal symbionts in the environment. Because vegetative dispersal units are heavier than fungal spores alone, they are predominantly involved in local dispersal (Walser 2004), where successful combinations of symbionts in parental thalli can easily reproduce in the same habitat.

The various types of vegetative propagules are classified by their morphological details, such as isidia (mostly pin-like propagules with terete organization), schizidia (scaly propagules with dorsoventral organization), soredia or other, more special types. Basically, soredia are formed by undifferentiated hyphae which bind a small number of algal cells, whereas many other forms have a covering fungal cortex layer and usually a larger population of algal or cyanobacterial cells. Certain propagules of foliicolous lichens, such as the stalked discs of *Phyllophiale alba*, or the shallow discs of *Chroodiscus mirificus* are unique types of vegetative propagules.

6.10 Conidium Formation

Campylidia, commonly present in Pilocarpaceae and Ectolechiaceae (but also found in Arthoniaceae and Monoblastiaceae), are hood-shaped conidiophores, which may also release algae, but there is no tight connection of the photobionts with the macroconidia (Sanders 2014). Beside the joint dispersal of symbionts there are also purely fungal forms of mitotic propagation, ranging from simple formation of conidia by hyphae at the thallus margins (e.g., thallospores, not uncommon in various crust lichens from arid zones) to more specialized types. Diverse shapes of highly specialized conidium-producing synnemata are characteristic of the Gomphillaceae (Ferraro 2004). While many Gomphillaceae also produce ascospores, there are species in various major lineages, which are only known from sterile stages (e.g., species of *Cheiromycina*, *Dictyocatenulata*, *Reichlingia*, *Sporodochiolichen*).

6.11 Lichen Mycobionts and Their Systematics

The historical development of lichen systematics has been led by methodological advances, which initiated periods of taxonomic rearrangements. In the early days of lichen taxonomy, only bare eyes and simple lenses helped to classify lichens by external characters, while compound microscopes facilitated the observation of anatomical characters—particularly spore characters—in the second half of the nineteenth century. Analyses of secondary chemistry, by thin layer chromatography in particular, were introduced in the second half of nineteenth century, while the analysis of molecular sequence data was introduced in the 1990s to achieve an even more coherent phylogenetic framework. After algal-excluding ("fungal") primers were designed (Gargas and Taylor 1992), it was also possible to use extracts from entire thalli for phylogenetic approaches, avoiding the cumbersome axenic cultivation of mycobionts. At all times, technological advancements were sparked by the hope of better understanding the relationships of lichens and achieve a taxonomy based on their evolutionary history.

The number of independent origins of lichens in the fungal kingdom was one of the prominent questions when molecular methods became popular. In early analyses with limited sampling of lichenized lineages, Gargas et al. (1995) suggested only two lichenization events in the Ascomycota and three such events in Basidiomycota. With a more extended sampling, Lutzoni et al. (2001) found that even a single lichenization event could not be excluded and suggested that important non-lichenized lineages may have had lichenized ancestors (including the molds in Eurotiales).

The total number of lichenized species is given as 19,387 species, which are classified in 39 orders of fungi (Lücking et al. 2016). The number of lichenized species in the fungal classes is unevenly distributed in the fungi. The largest classes Lecanoromycetes (15,131 species), Arthoniomycetes (1541), but also the smaller Lichinomycetes (390), Candelariomycetes (76),

and Coniocybomycetes (31) contain primarily lichenized species, whereas other classes such as Eurotiomycetes (1203), Dothideomycetes (812), Agaricomycetes (172), or Sordariomycetes (1) contain a majority of non-lichenized species (Lücking et al. 2016). Recently, the Lichinomycetes have been expanded to include Candelariomycetes and Coniomycetes, as well as some previously unclassified non-lichenized lineages (Díaz-Escandón et al. 2022). The large number of lichenized species in Lecanoromycetes and Arthoniomycetes correlates positively with the phenotypic complexity of their thallus organization, which will be discussed below in greater detail. Although these classes are only distantly related and possibly evolved the lichenized habit independently, there are parallels in the construction of the symbiotic phenotype which suggest that certain general functional constraints play a role in the evolution of the lichen thallus. The similarities involve general anatomical structure and the involvement of the same secondary metabolites in these unrelated lineages.

The remainder of lichens are found in few lineages of the primarily non-lichenized basidiomycetes, in the orders Atheliales, Cantharellales, Corticiales, and Agaricales. Species diversity in this groups still seems to be underestimated. Lücking et al. (2014) provided evidence that *Dictyonema glabratum*, once thought to consist of a single species, is much more diverse than expected. They distinguished at least 126 species and predicted more than 400.

Molecular data have elucidated the large-scale evolutionary relationships of lichens, yet reconciling phylogenetic insights with taxonomy became challenging, in particular at the level of genera, which were previously circumscribed by growth form and other externally visible characters. Many were shown to be non-monophyletic, which required segregation and renaming of lineages. In addition, analyses of traditionally species-rich genera resulted in substantial taxonomic splitting. Both lead to a recent, perhaps too ambitious, proliferation of genus names. Large traditional genera, such as *Lecanora*, *Arthonia*, *Caloplaca*, or *Graphis*, are now split into dozens of smaller entities. For example, starting with a few genus names (Arup et al. 2013), the family Teloschistaceae now contains more than 100 genera.

Technological advances also influenced the way in which species of lichenized fungi are recognized. Early concepts considered variation in the thallus shapes and colors, as well as anatomical characters of the thallus and fruitbody (including spore measurements) for a phenotypic species concept. Simple histochemical differences using Lugol's solution on the thallus have also played a role in some lineages (Lugol staining variation in the ascus walls became very important for recognizing higher categories in Lecanorales by the 1980s), and various chemical spot tests are still quick indicators for recognizing species (e.g., using potassium hydroxide, hypochloride, or p-phenylenediamine). Beside phenotypic characters, which remain important to classify lichen species, chemical compounds were included in species circumscriptions since the 1960s in the framework of chemosystematics. Chemical variation, however, is not trivial to interpret, as the compounds involved have a varying degree of phylogenetic conservation in lichens. Compounds present in the upper cortex are often more conserved than those located below the algal layer. The compounds below the algal layer are often chemically related. Variation in compound presence was often considered as species-specific, or specific for subspecific taxa. However, when chemical patterns were in conflict with inference from phenotypic or molecular sequence data, classification remained controversial.

Soon after first attempts to detect genetic variation using DNA hybridization (Blum and Kashevarov 1986), PCR methodology and DNA sequencing were applied to study the relationships of lichenized fungal species. In the early studies, however, the low number of individuals sampled with only one marker (usually ITS) was not sufficient to clearly resolve species relationships. With the analysis of several independent loci, a gene genealogical approach helped to recognize species in the morphologically variable species complex of *Letharia vulpina* (Kroken and Taylor 2001). Another

approach used large numbers of samples from numerous populations in a population genetic approach to draw attention to the slow genetic drift in lichens (Printzen et al. 2003). The resulting ancestral polymorphisms can be problematic for application of sequence-based species recognition (e.g., DNA barcoding). For practical reasons, the systematics of species in lichenized fungi still relies on a combination of phenotypic and genotypic characters. In a few recent cases, the analysis of whole fungal genomes used to address the relationships of closely related species has shown substantial variation across genomes and evidence for hybridization in lichen fungi (Keuler et al. 2020). Hybridization and introgression may be largely underestimated factors in shaping complexes of closely related lineages of lichen species in which the separation of clear-cut species has always been a challenge (Fernandez-Mendoza et al. 2023).

6.12 Lichen Photobionts and Their Systematics

About 90% of the lichenized fungi are associated with eukaryotic algae, and of these, almost all belong in equal numbers in the classes Trebouxiophyceae and Ulvophyceae of the Chlorophyta. Exceptions are the partners of several lichens known or suspected to be members of Chlorophyceae (Sanders and Masumoto 2021). Photobionts of the Trebouxiophyceae are usually unicellular and rarely observed in free-living stages; they are relatives of free-living unicellular algae, except for *Prasiola*, a multicellular, leafy photobiont of the borderline lichen *Mastodia tessellata*. On the other hand, photobionts belonging to Ulvophyceae generally form thread-like or branched thalli in free-living stages. The most prominent genus of Ulvophyceae is *Trentepohlia*, but delimitation of species and genera is not clear (Nelsen et al. 2011). Some of the photobionts are assigned to the closely related genus *Printzina* (Hametner et al. 2014a, b), those of foliicolous lichens are usually assigned to *Cephaleuros* and *Phycopeltis*. The growth form of these algae in lichenized stages on the phylloplane is multicellular and similar to those of nearby free-living forms. The growth type of *Trentepohlia* remains thread-like in members of the lichen genus *Coenogonium* found on bark in the understory of tropical rainforests. In other tropical species, the thread-like morphology of *Trentepohlia* may be modified by the fungus (e.g., variations between thallus and isidia in the foliicolous *Chroodiscus mirificus*, Lücking and Grube 2002). Multicellularity is otherwise more or less absent in the lichenized state in bark- or rock-inhabiting or in surface-detached lichens with *Trentepohlia* symbionts. In these cases, only few concatenated or individual cells remain as remnants of multicellularity. Notably, Resl et al. (2022) found more genes for carbohydrate-active enzymes (CAZymes) in fungal genomes of lichens with *Trentepohlia* than in those associating with unicellular green algae. Could the larger number of CAZymes be linked to an ability to gain control over the algal growth architecture?

Compared with their fungal partners, algal partners are much less diversified morphologically patterns. In their review, Sanders and Masumoto (2021) included about 50 genera of algal and cyanobacterial genera as lichen partners. Since lichen algae are largely microscopic organisms without distinctive features, phenotypic classification has always been difficult. Moreover, the algae lack sexuality and change their ultrastructure in the lichenized state with variations depending on the fungal partner. Phenotypically based classification was therefore impossible without axenic cultures under standardized conditions. Meanwhile, molecular sequence data have massively improved the knowledge about photobiont diversity. Recent work in different algal lineages estimated substantial hidden genetic diversity (e.g., Muggia et al. 2014, 2018, 2020 in *Trebouxia*, Gustavs et al. 2017 in *Coccomyxa/Elliptochloris*, or Grube et al. 2017, Kosecka et al. 2020, Borgato et al. 2022 in Trentepohliaceae). Nevertheless, we are still far from describing diversity with species names.

6.13 Cyanobacteria

Approximately 10% of lichens contain cyanobacteria as their primary photobiont (Rikkinen 2017). Cyanobacteria are primary symbionts in about 50 genera and 1000 species of lichens and secondary symbionts in about 20 genera and 500 species (Rai 2002). Most of the cyanobacterial lichens include strains of the genus *Nostoc*, *Rhizonema*, and to lesser extent, lineages of *Calothrix*, *Fischerella* (syn. *Stigonema*), *Scytonema*, or the unicellular *Gloeocapsa*, *Chroococcus*, *Chroococcidiopsis*, and *Anacystis*, all of which are capable of fixing nitrogen. Like eukaryotic photobionts, the cyanobacteria are also modified in the lichenized state and seem to be regulated by the fungal partner both in growth form and in the frequency of heterocysts (Hyvärinen et al. 2002). Whether unculturable strains are also unable to grow free-living in nature still needs to be studied.

6.14 Cyanobacteria as Secondary Symbionts

When occurring as secondary photobionts in addition to green algae, in the so-called tripartite lichens, cyanobacteria are often contained within specialized structures called cephalodia. Different cephalodia of the same thallus may contain the same of different strains of related cyanobacteria. Cephalodiate lichens are an example of division of labor, since the green algal photobionts produce the gross amount of photosynthates whereas the cyanobionts in the cephalodia focus on nitrogen fixation (Nash 2008), which agrees with higher heterocyst frequencies and higher rates of N_2 fixation than found in bipartite cyanolichens. In many species, the cephalodia are external and gall-like, and then more or less scattered on the upper or lower surface of the thallus. Sometimes they are hidden internally in small packets (e.g., *Lobaria* and *Sticta*), while they form a more or less closed layer beneath the green algal layer in *Solorina crocea*.

6.15 Photobiont Flexibility

For a long time, the association of a fungus with an alga was supposed to be stable in a species. Molecular studies with both crustose species (e.g., Blaha et al. 2006) and foliose species (e.g., Fernández-Mendoza et al. 2011; dal Grande et al. 2018; Garrido-Benavent et al. 2020), however, have shown that lichens are able to associate with different strains of closely related algae or cyanobacteria, respectively, depending on the niche, elevation, and geographic location. Similar habitat-adapted patterns of symbiosis have also been observed in basidiomycetous lichens (Gasulla et al. 2020). These results suggest that many widespread lichen species can adapt to local conditions by association with a locally ideal partner. Nevertheless, specificity for the photoautotrophic partner varies among species. There are also species which have high specificity for their algal partners, for example *Lobaria pulmonaria*, which always is found associated with *Dictyochloropsis reticulata*. Whether high specificity coincides with ecological specialization of lichens still needs to be explored. Škvorová et al. (2022) showed that the promiscuity of *Cladonia* mycobionts with their algae is limited by climatic factors and soil chemistry, since most mycobionts analyzed in their study could switch only between algae with similar ecological preferences. The variable extent of photobiont specificity could also influence the patterning of lichen communities (photobiont-mediated guilds; Kaasalainen et al. 2021, Peksa et al. 2022).

Although it has been suspected that an individual thallus is not uniform with respect to the photobiont (e.g., by isolation of several strains from the same lichen, Friedl 1989), the co-occurrence of two algae has been shown in the thallus by Casano et al. (2011) using a combined molecular and microscopic approach. This strategy may increase the ecological flexibility of lichen symbioses. The significance of this phenomenon remains under studied, and it is not known if this is a transient process, a passive phenomenon, or an actively regulated pattern of

co-occurrence. Co-occurrence of algal strains has been studied in few species so far but has been found to occur with very different photobionts. Henskens et al. (2012) found that cyanolichens may also incorporate green algae in their thalli occasionally.

Certain lichens have the ability to form thalli with entirely different types of photobionts, the so-called photosymbiodemes. Photosymbiodemes have been long known from certain large leaf-like lichens in the family Peltigeraceae. The photobionts involve cyanobacteria (*Nostoc*) or green algae as primary photobiont (in the latter case *Nostoc* colonies may remain present in cephalodia). The thallus shapes of photosymbiodemes may look fairly similar (*Pseudocyphellaria rufovirescens*—green algal, vs. *P. murrayi*—cyanobacterial) irrespective of the involved photobiont, or completely different. Their representatives were even classified in different genera of lichens (e.g. *Lobaria amplissima*—green algal, vs. *Dendriscocaulon umhausense*—cyanobacterial). The mycobiont switches between different kingdoms of photobiont correlate with ecological preferences of the photobionts involved (usually with cyanobacterial morphs under wetter conditions) and their ability to cope with light stress. Green algal morphs are equipped with a photoprotective xanthophyll cycle that is missing in the cyanobacterial morph (Demmig-Adams et al. 1990b). Also, there are generally differences in the sexual strategy of the mycobiont depending on the photobiont type. In phycosymbiodemes involving cyanobacteria and coccoid green algae, it is usually the green algal morph which develops fungal sporocarps. A fascinating new type of photosymbiodeme was uncovered by Ertz et al. (2018), which is an example of a mycobiont associated with either members of Trebouxiophyceae or Trentepohliaceae, including a switch of its reproduction strategy and phenotypic dimorphism. *Lecanographa amylacea* (Arthoniomycetes) is sexual and lacks soredia with a trentepohlioid photobiont, whereas the same fungus can form a soredial photomorph with *Trebouxia* sp. (formerly known as *Buellia violaceofusca*). These patterns of photobiont flexibility raise fundamental questions of how partnerships and interactions are genetically controlled in different lineages of lichenized fungi and how flexible the variation might be. It also raises the question of how different photobionts influence the morphology of the lichen symbioses and to what extent this could be taxonomically relevant. Steinová et al. (2022) demonstrated that the photobiont species involved in closely related *Cladonia* species was a better marker for species phenotype than the molecular marker of the fungal symbiont, distinguishing a species without soredia from those that do. Again, the specific photobiont seems to influence the reproductive strategy of the fungal partner.

6.16 The Interface of Fungi With Algae

Complex haustorial structures found in certain fungal–plant interactions are missing in lichen fungi, and the contact of fungi with their photobionts is fairly simple; in many cases the algal cell wall is not even penetrated. The main flux of solutes in the apoplast seems to be driven by wetting and drying cycles, whereas active uptake of carbohydrates is presumable via the immediate mycobiont and photobiont contact sites (Honegger 2012).

Simpler, finger-shaped, transparietal (intracellular) haustoria are commonly formed in surface-attached (crustose) lichens, whereas surface-detached lichens commonly develop intraparietal haustoria (Honegger 2012). Three types can be distinguished: type 1 is characterized by appressoria and short infection pegs, which do not penetrate the algal cell wall. Type 2 has short infection pegs sheathed by the algal cell wall, and type 3 develops very short infection pegs with thin fungal cell walls at the contact site within the outer cell walls of still growing algal cells. Honegger (2012) noticed coordinated growth of haustoria with the developing algal cells and suggested this growth pattern shifts algal cells over short distances in the algal layer in Teloschistaceae and Parmeliaceae. Softness and

more pronounced hygroscopic swelling of young thallus parts (in contrast with older parts; Grube unpublished) would fit with some flexibility of algal assortment in growing thalli. Appressoria seem more commonly developed in lichens with an eucortex and hydrophobic medullar layer. The interfaces of lichens with trentepohlioid algae have been less well studied, but early reports suggest both intraparietal and transparietal haustoria in an age-dependent pattern, suggesting that early intraparietal stages develop into transparietal haustoria in aging algal cells (Matthews et al. 1989).

Additional interaction structures can be observed in lichen interactions of basidiomycetes. Most of the fungi wrap their algal partner in small globules, but they do not develop distinctly developed algal layers. Basidiomycetes associated with the filamentous cyanobacterial genus *Rhizonema* form haustoria from the so-called mantle hyphae and may penetrate the cyanobacterial cell chains longitudinally with a central hypha (e.g., *Dictyonema*). Basidiomycetes associated with green algae wrap hyphae around clusters of algal cells and may eventually develop appressoria. The rather aggressive mycelia of *Athelia* species, which overgrow and attack a wide range of lichens and algal colonies, develop haustoria within the parasitized algal cells.

6.17 Ecophysiology of Lichens

One the most fascinating aspects of lichen biology is the physiological integration of entirely unrelated organisms, which react in a synchronized manner to environmental conditions. The algae (and cyanobacteria) are kept under fungal control in lichen thalli to sustainably produce more photosynthates than the algae alone require, and which are sufficient to sustain the entire symbiotic system. This is astonishing as the photobiont contributes a minor fraction to the total lichen biomass, sometimes less than 10%. It appears that lichens are—by evolutionary optimization—highly efficient photosynthetic "machines." Growth of the lichen symbiosis, depending on positive carbon gain, depends on a finely balanced share of carbon between the photobiont and mycobiont in excess of respiration (most of which can be attributed to the fungus). Photosynthates taken up by the fungi are called transfer sugars. In lichens with green algae, transfer sugars comprise acyclic polyalcohols such as ribitol, sorbitol, and erythritol, whereas mycobionts in association with cyanobacteria receive glucose from their partner. To finely tune this system, lichenized fungi evolved morphological adaptations to regulate access to light and water, and gas exchange for the algal partner. Widespread species with broad ecological preferences may optimize their water relations *via* anatomical variations within species (Colesie et al. 2017). Such anatomical differences can also lead to different organization of algal layers, as shown by Vondrak and Kubásek (2013). They observed that in morphs from arid or mountain regions, algal cells are organized in thick stacks (algal stacks) separated by vertical channels of likely light-transmitting fungal hyphae (fungal stacks).

Light is the energy source for photosynthesis, while water acts as an electron acceptor molecule and carbon dioxide as carbon source. Light access, as well as protection from excess light, is regulated through positioning of the photobiont beneath a protective fungal cover, and gas exchange in the layer algal beneath is facilitated by water-repelling fungal hyphae. Water transfer is also supported by various hyphal structures, such as the distinctive rhizines and veins on the lower surface of *Peltigera* species. Variations in theses phenotypic structures depend on the ecological circumstances. Unsurprisingly, similar morphologies may emerge convergently in unrelated groups of lichens. For instance, unrelated epiphytic and epiphyllous lichens in the understory of lowland tropical rainforests usually limit investment in fungal biomass under the prevailing conditions of dim light and high temperatures that challenge the balance of carbon fixation of the algae against respiration of the entire lichen. Hence, the thalli of epiphyllous lichens from different lineages (e.g., *Arthonia* and *Porina*) are sometimes confined to a single layer of hyphae running along edges of neighboring algal threads.

Lichens are known for their persistence under environmental conditions that are hostile for most other life forms. Depending on fungal species, lichens are able to survive periodic extremes of heat or cold, and notably, desiccation. The pronounced desiccation tolerance of many lichens is a key feature for surviving such extremes (Kranner et al. 2008). Likely, genomic traces evolved to correlate with desiccation tolerance. For instance, Armaleo and Chiou (2021) found a fascinating link between growth rate, desiccation tolerance, and ribosome biogenesis. Ribosome biogenesis requires splicing of group I introns present in the ribosomal RNA genes. Most introns populating lichen rDNA are unable to self-splice and their insertion into yeast ribosomal RNA genes caused the yeast strains to grow 4.4–6 times slower, and made them 40–1700 times more desiccation tolerant depending on intron position and number. Fast growth rates would indeed poorly reconcile with the effects of recurrent drying on metabolism.

The primary mechanisms of desiccation tolerance must, however, act directly at the cellular level. Desiccation tolerance may generally be considered as the ability to restore metabolism from the air-dried state, or, in a more precise definition, the capability to survive drying at relative humidities below 65% (absolute water content equal to or below 0.1 g H_2O g^{-1} dry mass and a water potential of ≤ -100 MPa; Oliver et al. 2020). The mechanisms conferring desiccation tolerance (Kranner et al. 2008; Pichler et al. 2023), although still not fully understood, are believed to comprise molecules that support "vitrification," i.e., the formation of a "glassy state." For example, non-reducing sugars may substitute for water, maintaining the spacing between and within macromolecules, avoiding molecular crowding and cellular collapse, likely in conjunction with LEA-like proteins ("Late Embryogenesis Abundant" dehydrins). Moreover, a potent antioxidant machinery appears to be essential for desiccation-tolerant organisms (Kranner et al. 2005), serving to protect from oxidative damage by reactive oxygen species produced as inevitable by-products or disrupting electron transport chains upon desiccation. Furthermore, proteins need to be protected in the dry state to retain sufficient molecular order to revive upon rehydration. Osmotic stress is ameliorated by the production of osmoprotectants acting as osmolytes. Various types of small molecules serve this purpose, including various sugars, sugar alcohols (similar or the same as the transfer sugars), and other low-molecular-weight molecules with neutral charge and low or no toxicity at high concentrations, such as certain amino acids and their derivatives, for example mycosporines, and betaines (Gostinčar et al. 2012).

Lichens can resume full metabolic activity within minutes, apparently with proteins already in place and thus not dependent on their complete resynthesis by the ribosomal machinery. Upon rehydration, at least the side groups of carbon backbones must be able to move for enzymatic activity, as is the case in a "rubbery," but not yet a "glassy" state. Transcription, translation, and other more complex processes require transition to the liquid state with full carbon backbone mobility of proteins (Candotto Carniel et al. 2021; Farrant and Hilhorst 2021). Expressed as a percentage of dry weight, lichen water contents can range from 2–15% in a dry state to 100–300% under hydrated conditions, and some cyanobacterial jelly lichens may even take up 2000%. Most of the water is kept in the strongly swollen cell walls (mostly fungal in green algal lichens or cyanobacterial in jelly lichens). Suitable levels of humidity are of profound importance to run photosynthesis. Between 70 and 150% water relative to dry weight appears to be optimal, although lichens may start with net photosynthesis at c. 20% water weight. Productivity decreases with higher amounts of water due to the low solubility of CO_2 leading to gas exchange inhibition. To lower this type of inhibition, the anatomical structure of lichens and particularly the hydrophobicity of certain internal parts of stratified lichen thalli play a significant role to ensure gas exchange for efficient photosynthesis. The hydrophobicity of fungal structures relies on secondary lichen compounds covering the surface of the fungal cell walls, as well as a hydrophobic cell wall surface layer. Being highly significant

with regard to translocation of solutes between the partners, this layer covers both mycobiont and photobiont cells as it creates an apoplastic continuum for both symbionts. The sealing is achieved by the self-assembly of hydrophobins, a family of cysteine-rich surface-active proteins forming a continuous rodlet-layer on the symbiont cells. Class 1 hydrophobins have been characterized in *Xanthoria* species (Scherrer et al. 2000; Scherrer and Honegger 2003) and in *Dictyonema glabratum* (dikaryotic hyphae, three different hydrophobins; Trembley et al. 2002).

Many lichens are able to maintain photosynthesis despite wide variation in water content. Photosystem II (Φ, quantum yield) efficiency often used as a proxy of photosynthetic activity, gives a good approximation, but under certain conditions does not directly relate to actual CO_2 uptake considering alternative electron transport routes (e.g., photorespiration, Mehler reaction; Green et al. 1998). High rates of area-related photosynthesis and chlorophyll contents have been reported, in the range of those known from leaves of deciduous trees or evergreen forests (Green et al. 2008). Nevertheless, there seems to be substantial variation of rates among or within thalli of a species and with time. Generally, young thallus parts are much more active than older parts, which agrees well with patterns of microscopically assessed cell vitality and chlorophyll fluorescence (Grube, unpublished observations).

Excess light can cause photoinhibition, i.e., the decrease in photochemical efficiency experienced in response to irradiation exceeding the energy used for photosynthetic ATP and NADPH production and/or photodestruction, i.e. the light-induced damage to the photosynthetic apparatus. Sensitivity of lichens to photoinhibition is species-specific and depends on the present type of algal or cyanobacterial photobiont (Demmig-Adams et al. 1990a) and their capacity for non-photochemical quenching (NPQ), including xanthophyll cycles (Demmig-Adams et al. 1990b; García-Plazaola et al. 2012).

6.18 Nitrogen

Growth of lichens depends on the availability of nitrogen as an essential macronutrient for synthesis of proteins and nucleic acids, among other important roles. For the approximately 90% of green algal lichen species, it is assumed that fixed nitrogen is provided by various forms of deposition from the atmosphere, including aerosols but also in more robust manner by bird manure. It remains to be studied, whether surface-attached bacteria potentially contribute—either dead or alive—to the nitrogen budget of lichens.

Ten percent of lichen species live together with cyanobacteria (mostly *Nostoc*) as the primary symbiont and about 3–5% have specialized organs called cephalodia to grow cyanobacteria in addition to the primary green algal partner (tripartite lichens). Comparison with primarily cyanobacterial lichens suggested a separation of tasks in tripartite lichens. By assessing the number of heterocysts, the cells in which nitrogen fixation takes place, Hyvärinen et al. (2002) found that heterocyst frequency in bipartite lichens ranges from 2 to 8%, and in tripartite lichens between 10 and 55%. Several, mostly soil-inhabiting, green algal lichens with squamulose morphology are known to grow either facultatively or obligately near free-living blue-green algae, mainly *Stigonema.* This little studied phenomenon is known as cyanotrophy (Poelt and Mayrhofer 1988), which represents a form of ecological facilitation. Some of the species occur only on very poor, acidic rocks on *Stigonema*, whereas they may occur independent of *Stigonema* in habitats with high nutrient availability. Nitrogen fixation by cyanobacteria yields NH_4^+, which is metabolized to glutamine, part of which is then transferred to the mycobiont. The mycobiont catalyzes glutamine to glutamate and other amino acids, which can be directed to the green algal photobiont. Supplying the photobiont with higher levels of nitrogen could

potentially increase the carbon gain capacity, whereas directing more nitrogen into fungal tissue may increase the rate of maintenance respiration (Lambers 1985; Reich et al. 1998). Thus, a finely tuned balance likely contributed to adjusting growth control of the photobiont and the overinvestment in fungal structures.

Nitrogen fixation requires higher levels of moisture than respiration and photosynthesis, and apparently do not start below water contents of 80% dry weight, while maximal fixation rates were found at 200-400% ODW in species with saturation at 500–800% oven dry weight (ODW) (Kershaw 1985).

Biological fixation occurs via nitrogenase, which uses the relatively rare element molybdenum as a cofactor. Alternatively, nitrogenases also may use vanadium or iron in place of molybdenum in the active site, and genes for those enzymes have been detected in the *Nostoc* symbionts of *Peltigera* species (Hodkinson et al. 2014) in addition to standard molybdenum-dependent nitrogenases. Thus, these alternative possibilities for nitrogen fixation provide flexibility of metabolic processes and play a more prominent role for lichens in habitats where molybdenum is limited. Consistent with this thought, the acquisition of vanadium is strongly regulated by the abundance of molybdenum according to Darnajoux et al. (2017), who also found evidence for the activity of alternative vanadium-based nitrogenase in the *Nostoc* cyanobiont of *Peltigera aphthosa* s.l.

6.19 Phosphorous

The requirements of controlled resource allocation in a symbiotic system extends to phosphorous, which in contrast to nitrogen may be lost by sedimentation. Lichens cope with this limitation by recycling phosphorous, as shown in *Cladonia* podetia by Hyvärinen and Crittenden (2000). Similar recycling was also shown for nitrogen in the reindeer lichen *Cladonia portentosa* by Ellis et al. (2005), who suggested this ability as a reason for the ecological success and landscape-tinging presence of *Cladonia* mats in subarctic regions. Johansson et al. (2011) showed varied effects of simulated phosphorous deposition in dependence of nitrogen deposition, as P supply can both mitigate and intensify the negative effects of excess nitrogen depending on the (epiphytic) lichen species, and apparently allocation to the symbionts.

Transcriptomic analysis of resynthesized *Usnea hakonensis* thalli showed upregulation of photobiont genes involved in photosynthate transport and mycobiont genes involved in nitrogen and phosphorus transport, when compared to isolated and cultured myco- and photobionts (Kono et al. 2020). In this species, genes for acid phosphatase and phosphate transport are upregulated in the mycobiont, but are absent in the photobiont, which instead upregulates genes for ATPase, suggesting dependence of the photobiont from fungal phosphorous supply.

6.20 Secondary Lichen Products

Lichens make the fungal world more colorful with their secondary metabolites. For practical reasons, the term secondary metabolites will be used here, although these compounds fulfill important ecological functions. The compounds, produced by the fungal partner are a metabolic sink for excess carbon but more importantly they serve in the biology of lichen symbioses as photoprotectants against intense radiation, as defense against feeders, or as factors mediating metal homeostasis and pollution tolerance of lichen thalli. For their bioactive properties they are also of interest for biotechnology, while they have long served as taxonomic characters.

Secondary metabolites are deposited as microscopic crystals in the intercellular spaces or are present as insoluble amorphous to melanin-like pigments in hyphal walls or outside of the walls (e.g., as pigment caps or as deposits between fungal cells). The crystallized compounds tend to be water-insoluble and accumulate in the thallus, where they are unevenly distributed. Some are only known as compounds present in the upper cortex. Others may be present in layers below the algal layer, either as medullary

compounds or—when a lower cortex is missing—as compounds possibly shielding the lower surface against the environment or chelating ions used during substrate exploitation. The gross distribution of compounds within the thalli could be studied merely by localizing spot reactions, or by fluorescence microscopy (Kauppi and Verseghy-Patay 1990). Progress in mass spectrometry-based imaging methods now resolves metabolic patterns at high spatial and chemical resolution, which makes it possible to visualize the microscopic distribution of each individual compound (Gadea et al. 2020; Garg et al. 2016).

The most widespread lichen substances are depsides and depsidones, followed by representatives of different compound classes, such as yellow-greenish usnic acids, reddish-orange anthraquinones, and bright yellow pulvinic acids (Fig. 6.7a). Of the depsides, atranorin seems to be the most common compound turning the lichens grayish-white (present in perhaps a third of lichen species). The genomes of lichen fungi sequenced so far suggest the presence of dozens of biosynthetic pathway gene clusters, but only a few compounds are produced in a single thallus; these, however, may accumulate in massive amounts in the upper cortex (sometimes up to 30% of dry weight). Only rarely, a higher number of compounds is present in a single thallus. One notable exception is *Lecanora elixii*, which contains more than 10 compounds, most of which are xanthone derivatives. In addition to filtering different wave lengths of light, the upper cortical compounds seem to function as a light switch depending on water content: More light can pass through the cortex to the photosynthetic algal layer beneath when the crystals are more widely spaced in swollen hydrated thalli (Dietz et al. 2000). The enhanced shielding by light reflection from closely spaced crystals in dry stage may prevent the detrimental effects of photoinhibition and radical formation.

Different biochemical pathways lead to a vast diversity of more than 1000 known lichen compounds. The three most important ones are the acetyl-polymalonyl pathway (polyketides), mevalonate pathway (isoprenyls), and shikimic acid pathway (shikimates). The compounds can be classified in major classes of lichen secondary metabolites (summarized in Table 6.1). Some of the compounds can also be dimerized or are able to form heterodimers such as bisanthraquinones, dimeric xanthones, or heterodimers of xanthones with chromones (Nguyen et al. 2020; Tuong et al. 2020). Otherwise, visually detectable compounds present in low amounts (e.g., in ascomata) of many, particularly tropical lichens have poorly been explored (Fig. 6.7b, c; Yamamoto et al. 2002) and still await their structural discovery.

Major compounds can be characteristic of lineages at various taxonomic levels, rarely so at the order level (e.g., most representatives of orange colored Teloschistales produce anthraquinones), but more commonly at the genus level (e.g., the ubiquitous yellowish-green colored species in *Xanthoparmelia* or *Usnea* in Parmeliaceae). Chemistry, however, not always reconciles clearly with phylogeny, as some lineages are chemically more variable than others with respect to their main cortical compounds. Chemical variation of medullary compounds has been most commonly used in the classification of species, either by presence of single compounds or by variation in the patterns of chemically related substances (chemosyndromatic variation).

Because most of the lichen compounds belong to the class of polyketides, their corresponding building enzymes, the polyketide synthases (PKS) raised interest during the early exploration of lichenized fungal genomes. Schmitt and Lumbsch (2009) found that those genes of class 1 polyketide synthases (PKS1) in lichens coding for methylsalicylic acids (MSAS) are more closely related to similar genes from bacteria than to other fungal PKS1 genes. They concluded that horizontal gene transfer is responsible for the capability of lichenized fungi to produce these phenolic compounds. Since then, it became a challenge to find out which of the genes are responsible for the production of the compounds accumulating in the thalli. In an early attempt, Brunauer et al. (2009) characterized the transcript of a polyketide synthase gene (PKS) from the cultured mycobiont of *Xanthoria elegans*

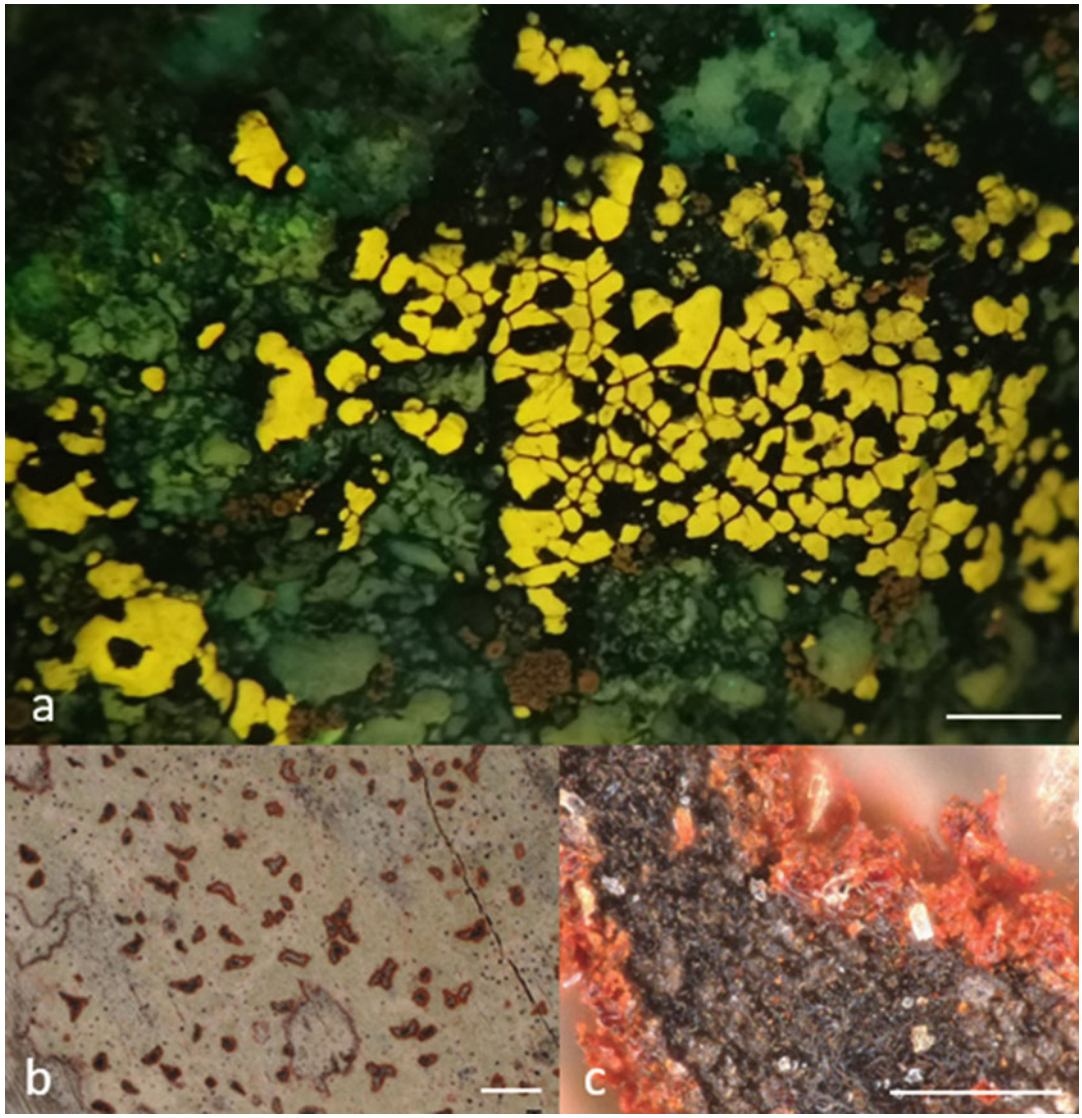

Fig. 6.7 Secondary metabolites in lichens, (**a**) Fluorescence of rhizocarpic acid in thalli of *Rhizocarpon geographicum* under illumination with UV360-light, bar = 2 mm; (**b**) *Coniocarpon rubrocinctum* fruitbodies with crimson margin, bar = 1 mm; (**c**) Crimson crystals (quinoid compounds, likely isofuranonaphthoquinones) on ascomatal margins of *C. rubrocinctum*, bar = 100 µm. Photographs: M. Grube

(XePKS1) using SMART-rapid amplification of cDNA ends (RACE) cDNA synthesis and sequencing of cloned cDNA. They chose conditions under which the cultures exclusively produced anthraquinones to suggest that the detected gene transcript was responsible for the synthesis of anthraquinones. Later, comparative analyses revealed gene clusters producing other major lichen compounds, such as grayanic acid (Armaleo et al. 2011), usnic acid (Abdel-Hameed et al. 2016), atranorin (Kim et al. 2021), or gyrophoric acid (Singh et al. 2022). With the present knowledge about the putative usnic acid biosynthesis cluster, Pizarro et al. (2020) conducted a broader analysis of genomes from Parmeliaceae and found that this gene cluster is

Table 6.1 Survey of the major classes of specialized metabolites in lichens

Acetyl-polymalonyl pathway	Secondary aliphatic acids, esters and related derivatives		
	Polyketide-derived aromatic compounds	Mononuclear phenolic compounds	
		Di- and tri-aryl derivatives of simple phenolic units	Depsides, tridepsides, and benzyl esters
			Depsidones and diphenyl ethers
			Depsones
			Dibenzofurans, usnic acids and derivatives
		Anthraquinones and biogenetically related xanthones	
		Chromones	
		Naphthaquinones	
		Xanthones	
Mevalonate pathway		Di-, sester-, and triterpenes	
		Steroids	
Shikimic acid pathway		Terphenylquinones	
		Pulvinic acid derivatives	

ancestral in Parmeliaceae. All species producing usnic acid contained this gene cluster, but all species without usnic acid apparently lost synthesizing genes quickly during evolution.

Genomic comparison indeed shows considerable gene content dynamics even at low taxonomic levels. Singh et al. (2021) detected biosynthetic gene cluster variation across *Lasallia pustulata* populations from Mediterranean and cold-temperate climate zones. Although all populations produced gyrophoric acid and to minor extent lecanoric and hiascic acid irrespective of the sampling location, the population genomic analyses indicated that *U. pustulata* contains three clusters that are highly differentiated between the Mediterranean and cold-temperate populations, with one entire cluster exclusively present and a putatively dysfunctional second cluster in cold-temperate populations. In a third gene cluster, variation is fixed in all cold-temperate populations. The authors suggested this pattern is shaped by both positive and hitchhiking selection. The pattern of expressed and detected metabolites seems to be unaffected from genomic variation in this case, but the results are of considerable interest for chemosystematics. If pathway genes are present in genomes within a species, the taxonomic significance of chemical differences needs to be critically reassessed when species are distinguished solely by such characters. However, genomic variation of biosynthetic genes not directly reflected by the expressed chemotypes may raise questions about unknown roles of the gene products and unstudied correlations with other characters.

6.21 Additional Interactions: Lichenicolous Lichens, the Lichen Microbiome, and the Lichens as Holobionts

Being widespread and slow-growing organisms with persistent thalli, lichens might be a readily available source of nutrition for associated microorganisms, primarily bacteria and fungi (Fig. 6.9a–d). Past studies about lichen-associated microorganisms have focused on the diversity of lichen-associated fungi, which have been characterized by their phenotypes, while bacterial diversity was studied more thoroughly only in the last two decades by molecular methods.

In a strict sense, lichenicolous fungi live exclusively on lichens, and they may include both, non-lichenized and lichenized fungi, which are also known as lichenicolous lichens (Fig. 6.8a, b). The distinction between these and free-living

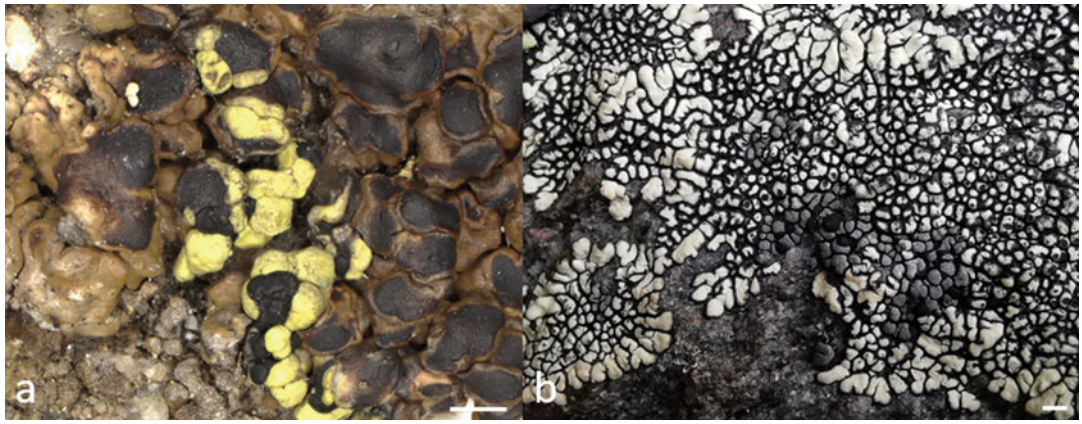

Fig. 6.8 Examples of lichenicolous lichens, (**a**) *Rhizocarpon dinothetes* on *Protoparmelia badia* (Switzerland), (**b**) *Rhizocarpon renneri* on *Dimelaena oreina* (Switzerland), bars = 1 mm. Photographs: M. Grube

lichens can sometimes be difficult when they are parasitic on other lichens only in juvenile stages to become independent later (Hafellner 2018) and then develop faster than the initial host thallus. More commonly, lichenicolous lichens grow slowly and remain dependent on the host thallus. Lichenicolous lichens take advantage of their host's algae and may maintain them in the parasitic thallus or, alternatively, switch to another, secondarily acquired photobiont (De Los Ríos et al. 2002b; Wedin et al. 2016; Moya et al. 2020). The take-over of the already lichenized host algae by another fungus requires physiological adjustment for symbiosis with another fungus and was recently called "translichenization" (Pichler et al. 2023).

6.22 Non-lichenized Lichenicolous Fungi and More: The Fungal Microbiome

Fungi have been recognized as lichen inhabitants long before the symbiotic nature of the biology of the host has been revealed. Since the mid-nineteenth century, researchers observed high specificity of many lichenicolous (=living on/in lichens) fungi for their host lichens (Berkeley 1844; Nylander 1857). Zopf (1897) had already referred to them as an additional part of the lichen symbiosis, forming a "parasymbiosis." 2319 species of mostly obligate lichenicolous fungi have been named (Diederich et al. 2018), but their true diversity is still not known. Species may develop local necroses or brain-like outgrowths (galls; Fig. 6.9c) on thalli of their specific hosts but most other species develop more or less without symptoms. The architecture of the overall lichen structure in these cases remains as originally determined by the principal lichen mycobiont.

Since lichens are composite organisms, lichenicolous fungi can have varied affinities to the partners of lichens. Some of the species are clearly associated with the algal partners of the host, whereas others have a mycoparasitic lifestyle, or are preferentially found in the intercellular gels produced by either the fungal partner of the host, or by both fungal and algal partners (Fig. 6.10; De Los Ríos et al. 2002a). Moreover, the cortical layers of lichens are frequently inhabited by dark-septate fungi, with unknown affinity to any partner of the host.

In addition to fungi developing diagnostic spore-producing structures, many asymptomatic fungi can be cultured from crushed lichen thalli (Petrini et al. 1990). According to DNA-sequence analyses, these endolichenic fungi largely belong to families and genera also known as endophytes of plants (Tripathi and Joshi 2019). Using cultivation techniques, up to 48 different fungi were recorded from a single lichen species (U'Ren

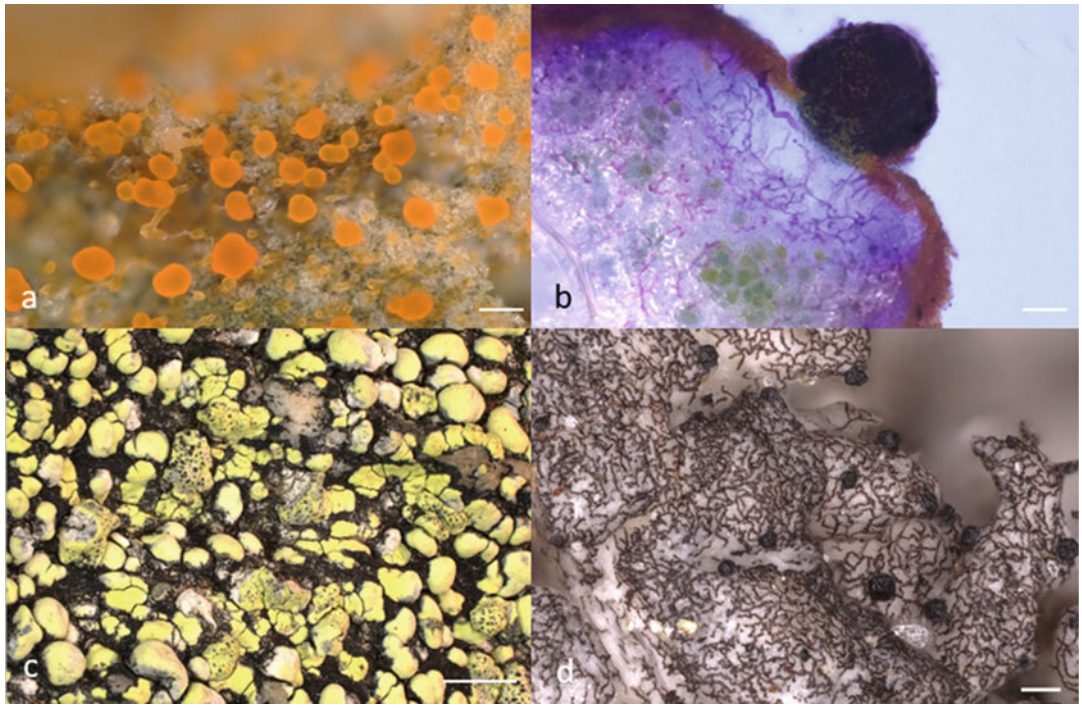

Fig. 6.9 Lichenicolous habit: (**a**) *Melittangium lichenicola* on *Xanthoria parietina*, bar = 50 µm (**b**) *Zwackhiomyces coepulonus* on *Rusavskia elegans*, bar = 50 µm (**c**) *Cercidospora cephalodiorum* on *Rhizocarpon geographicum*, bar = 2 mm (**d**) *Sphaerellothecium contextum* on *Ochrolechia frigida,* bar = 100 µm. Photographs: M. Grube

et al. 2012). Little is known about their role and abundance in their hosts, as they could include trapped resting spores or those with very limited growth, respectively. Microscopic techniques, which differentially visualize endolichenic fungi to distinguish them from the lichen mycobiont, are required to gain more insight into the roles of these associated fungi.

The idea of a unitary role of one main fungal partner in shaping lichen thalli was challenged by the discovery of basidiomycetous yeasts (genus *Cyphobasidium*, Cystobasidiomycetes, Pucciniomycotina). Spribille et al. (2016) visualized these yeasts for the first time in the thallus cortex of *Bryoria fremontii*, a pendent lichen in Parmeliaceae. Those specimens with an abundance of yeast cells had a yellow color, due to vulpinic acid in the branches. These individuals have earlier been classified as a separate species, *Bryoria fremontii*, until molecular work revealed they belong to the same species (Velmala et al. 2009). Further tests need to confirm whether the presence of yeasts or their chemistry could alter the physical properties of the cortex or correlate with long pseudocyphellae developing along infected thalli. Such differences could indicate modified patterns of gas exchange and thus a change in ecological requirements. Because Spribille et al. (2016) detected representatives of this basidiomycetous yeast lineage in a variety of other lichens (particularly Parmeliaceae) using a sensitive PCR assay with highly specific primers, they suggested an integral role for these fungi in the upper cortex of lichens. Their suggestion of a high degree of specificity of these fungi was put in perspective by Mark et al. (2020). It appears that these and other yeasts have a widespread occurrence in lichens (e.g., Fernández-Mendoz et al. 2017; Tuovinen et al.

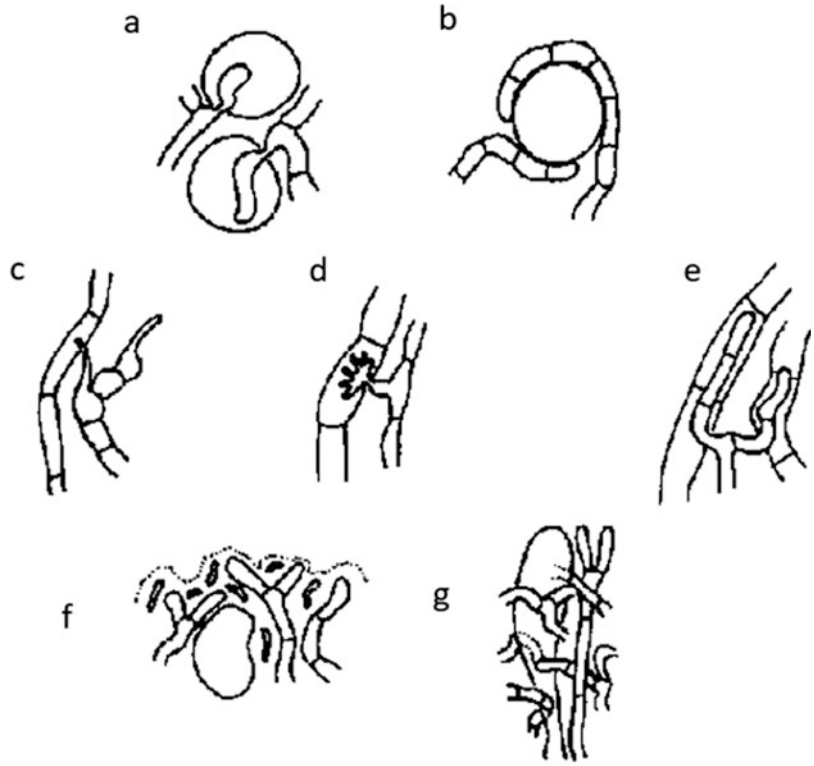

Fig. 6.10 Interaction types of lichenicolous fungi with their hosts, (**a**) haustoria formed in the algal partner of the host, (**b**) appressoria attaching to the algal partner of the host, (**c**) simple haustoria in the host hyphae, (**d**) complex haustoria in the host hyphae, (**e**) intrahyphal hyphae in the host, (**f**) exploitation of the intercellular gels of the host, (**g**) aggressive penetration of host structures. Drawings by M. Grube

2021), while their hyphal and fertile states develop only in certain host species.

Surprisingly few representatives of non-lichenized fungi on lichens are known as devastating pathogens, which suggests that the lichen biology is particularly well prepared to keep the colonizing organisms in balance. The poikilohydric lifestyle of many lichens may limit fast-growing pathogens requiring constant high humidity and instead support osmotolerant and slow-growing microbiota (while lichens may quickly mold when stored under humid conditions). Nonetheless, even in warmer habitats with continued levels of high humidity, which are better suited to promote pathogenic lifestyles, lichens seem to have control over their colonizers, either by producing bioactive compounds or because of extracellular production of reactive oxygen as a defense mechanism (Beckett et al. 2005). Osmotolerance of lichens could help to passively balance commensal associations that either do not express symptoms of disease or that produce localized infections and symptoms by species specialized on their hosts.

6.23 Bacterial Microbiome

Bacteria associated with lichens have been studied more thoroughly during the past two decades, although their presence was recognized long ago by light microscopy form dotted "sprinkles" of cells on the surface of sectioned thalli. The much larger bacterial spore-bearing structures ("fruiting bodies") of *Melittangium lichenicola* (Fig. 6.9a; Myxococcales, Deltaproteobacteria; commonly known as myxobacteria), however, led to the earliest description of a bacterial species on lichens (Thaxter 1892). Bacteria were later isolated from lichens and cultured (e.g., Cengia Sambo 1926; Henkel and Yuzhakova 1936). Their taxonomic affiliation relied on classic microscopic methods including physiological assays of cultivated strains, while classification

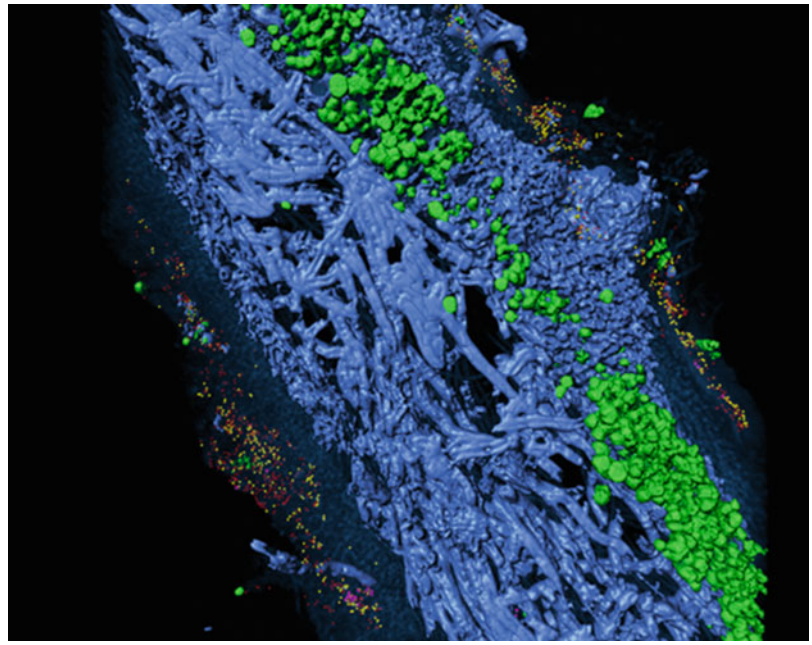

Fig. 6.11 Lichen thallus structure of lung lichen *Lobaria pulmonaria* in cross-section. 3D reconstruction of FISH image stacks. Eubacteria (red) and Alphaproteobacteria (yellow) on both, the upper and the lower cortex, Betaproteobacteria (pink) being less abundant and locally contained. Fungal hyphae (blue) and algae (green) visualized by autofluorescence (from Grube et al. 2015, with permission of Springer *Nature*)

of isolated bacteria by sequence data was accomplished much later (e.g., González et al. 2005; Cardinale et al. 2006; Liba et al. 2006). Counts of bacteria prepared by Cardinale et al. (2008) revealed about 6×10^7 bacteria per gram of lichen and showed that lichens are often densely covered by bacterial colonies in a biofilm-like manner. Using *In situ* hybridization with specific fluorescent probes for ribosomal RNA they also revealed that most bacteria belonged to the Alphaproteobacteria (Fig. 6.11). The diversity and specificity of lichen-associated bacterial communities was subsequently studied in more detail by culture-independent sequencing approaches and more sophisticated-omics technologies. Such studies demonstrated that lichens are furnished with a complex host-specific bacterial microbiome (Grube et al. 2009; Bates et al. 2011; Hodkinson et al. 2012; Aschenbrenner et al. 2014; Leiva et al. 2021). Further support for the specificity of the lichen community was demonstrated in a study of a lichenicolous lichen, when the bacterial community composition shifted dramatically with the onset of the invasion by the parasitic lichen (Wedin et al. 2016). In addition, the effect of thallus age and habitat as parameters driving the composition of bacterial communities was studied by Cardinale et al. (2012), showing that the relative abundance of Alphaproteobacteria "drops" in older thallus parts.

Lichen surfaces vary dramatically in their hydrophobicity/hydrophily that correlate with the numbers of bacteria observed on them: Hydrophobic surfaces generally bear fewer bacteria than hydrophilic surfaces. Microscopic observations also suggest that samples of widespread lichens from hot and dry habitats have fewer bacteria than samples of these lichens from cool and humid habitats (Grube, unpublished).

With complementary-omics approaches, such as metagenomics and metaproteomics, a better understanding of bacterial communities and their potential functions is possible (Grube et al. 2015). Potential vitamin and hormone production, and various other functions suggest that bacteria can be functionally important components of lichen symbioses. It has been suggested that they potentially contribute to the fitness of the lichen holobiont, similar to the situation of microbiomes of plants and animals. In addition, it is suggested that bacterial metabolites could also mediate defensive functions. Direct evidence for

production of bacterial defensive metabolites in the lichen thallus came from imaging mass spectrometry analyses of *Peltigera hymenina* by Garg et al. (2016), who were able to localize the production of secondary metabolites *in situ*.

According to metatranscriptomics data, the various bacterial groups also varied in their transcription patterns. Analyses of the metatranscriptome also revealed previously unrecognized groups, such as *Chthoniobacterales,* which contribute biotin and folate synthesis in *Lobaria pulmonaria* (Cernava et al. 2017). Moreover, the hundreds of different bacterial strains on a single thallus of this lichen respond to the poikilohydric conditions. Under dry conditions, upregulation of a specialized ketone metabolism indicated a switch to lipid-based nutrition (Cernava et al. 2019). With respect to the diversity of bacterial species present on lichen thalli, their varied physiological requirements suggest differential reactions under varying hygric conditions of their hosts.

6.24 Other (Eukaryotic) Colonizers of Lichens

Few reports exist on other eukaryotic organisms associated with lichens. Lakatos et al. (2004) reported on diatoms living in association with tropical representatives of the genus *Coenogonium*. Further microbial organisms seem to be regularly present in lichens, including plasmodial myxomycete amoebae, heterotrophic nanoflagellates, and naked and testate amoebae (Anderson 2014; Wilkinson et al. 2015). The influence of these organisms in the lichen symbiosis is unknown, because they seem to be transient.

6.25 Lichen-Associated Viruses

Beside microorganisms, viruses have been found in lichens, including single- (ssRNA) and double-stranded RNA (dsRNA) viruses, apparently algal origin due to their similarities with plant viruses (Petrzik et al. 2014, 2016). DsRNA viruses in the lichens *Chrysothrix chlorina* (Chrysothrix chrysovirus 1; CcCV1) and *Lepraria incana* (Lepraria chrysovirus 1; LiCV1) once classified in the genus *Alphachrysovirus*, rather have a relationship to chrysoviridae known from filamentous ascomycetous fungi (Petrzik et al. 2019). Interestingly, CcCV1 was not found in the lichen mycobiont but in the accompanying endolichenic fungus *Penicillium citreosulfuratum*. The symbiotic partners of lichens may contain several virus species independently and simultaneously: CaMV as well as the capsid protein gene of ApMV was detected in the photobiont of *Xanthoria parietina* and both, the plant cytorhabdovirus and the ApMV were found in *Usnea chaetophora* (Petrzik et al. 2014, 2015). Proteins assigned to rhabdoviruses and betaflexiviruses were found in the metaproteome of *Lobaria pulmonaria* (Eymann et al. 2017), and Grube et al. (2015) found bacteriophage sequences in the metagenome of the same lichen. The presence of bacteriophages in lichens is confirmed by occurrences of bacteriophage proteins assigned to the families Myoviridae and Siphoviridae, which infect Bacteria and Archaea (Eymann et al. 2017). Urayama et al. (2020) characterized the total dsRNA viral community of a lichen species using dsRNA-seq technology and revealed that partitiviruses were dominant and active. Sequences were classified into two genera including both plant- and fungi-infecting partitiviruses. While 17 of 65 OTUs were related to Partitiviridae, viruses related to seven dsRNA virus families (Amalgaviridae, Botybirnaviridae, Chrysoviridae, Endornaviridae, Megabirnaviridae, Picobirnaviridae, and Totiviridae), three ssRNA virus families (Gammaflexviridae, Hypoviridae, and Narnaviridae), and one unclassified RNA virus family (Polymycoviridae) were also identified from a single thallus of lichen (the host lichen species was not indicated, but possibly represents *Cladonia pyxidata* s.l. according to their Fig. 6.1).

These data suggest a high diversity of viruses associated with the different partners in lichens (i.e., mycobiont, photobiont, associated fungi, and bacteria). When Merges et al. (2021) found five novel viruses belonging to *Caulimoviridae*,

Myoviridae, *Podoviridae,* and *Siphoviridae* in metagenomic DNA of *Umbilicaria phaea*, they suggested that the Caulimovirus is associated with green algal photobionts (*Trebouxia*) of the lichen, as its abundance decreased with increasing elevation, reflecting the specific algal lineage hosting this virus. The remaining viruses were attributed to bacterial hosts, with patterns specific to the population of the host lichen. Functional annotation of sequences retrieved from cyanolichens indicate numerous viral-encoded auxiliary metabolic genes (AMGs, involved in amino acid, nucleotide, and carbohydrate metabolism, including AMGs for secondary metabolism and fatty acid biosynthesis). These results raise the question of whether viruses may modulate physiology and interactions within lichens (Ponsero et al. 2021), and thus affect the fitness of their hosts as well.

6.26 Lichens as Bioindicators of Pollution and Environmental Change

Deposition of toxic elements and molecules represents a major constraint for metabolically active lichens because they take up nutrients over their entire thallus surfaces. Hence, lichens seem generally more affected by gaseous emissions than many other organisms. Depending on the sensitivity of species, they respond to air pollution by structural changes and bleaching. Particularly damaging are oxides of sulphur and nitrogen, which affect basic metabolic processes (e.g., decreased electron flow through photosystem II, deactivation of nitrogenase, etc.) and consequently, sexual and asexual reproduction. Lichens have, therefore, been frequently used as bioindicators of air pollution (Conti 2008). A plethora of literature exists about this subject, which cannot be discussed in detail here. Amelioration from acid emissions since the 1980s led to recolonization of lichen-depleted locations, though not leading to a complete restitution of the original composition, but favorizing lichens characteristic for nutrient-rich, basic habitats (Wirth et al. 2013).

Lichens also accumulate pollutants. Particular focus was given to metal accumulation in the past, due to the interest in bioindication of toxic metals ("heavy metals"). Accumulation may occur via particulate entrapment in intercellular spaces of the thallus, by extracellular binding in the cell wall, or by intracellular uptake (Nash 2008). Various elements can be distributed differently in the thalli and accumulation also depends on other factors such as morphology, secondary metabolites, and not least on the species involved (Bačkor and Loppi 2009).

Lichens are affected by environmental change as well. A recent study revealed that climate change proceeds faster than the photobionts of lichens would be able to adapt (Nelsen et al. 2022). Cryophilic species of tundra habitats seem to retract from their southernmost occurrences. However, due to their high dependence on habitat conditions, any alteration in the lichen vegetation could result from a combination of accompanying factors as well. Lichens from exposed rock habitats seem to cope well with warmer temperatures, but more problems are expected for soil-inhabiting lichens, especially as these would be easily outcompeted by higher plant vegetation. Lichens adapted to cool and continuously humid sites are sensitive to climate change and especially to dry conditions. Many of these are also found in forests characterized by years of ecological continuity, such as old-growth forests. Unfortunately, these vulnerable forest habitats are threatened by economic interests as well. Lichen decline is not only a problem of climate change but perhaps more importantly a problem of habitat destruction and other anthropogenic impacts. For instance, lichens suffer from an excess of nitrogen deposition in areas massively influenced by agricultural activities. This impact seems responsible for the recent alterations observed in the lichen flora of both Alps (Nascimbene et al. 2019) and boreal forests (Esseen et al. 2022).

6.27 Concluding Remark

Just as lichens are a prominent example for the "living together" of unrelated organisms, the study of lichens has flourished by cooperation among researchers with different backgrounds. To gain new insights in lichen biology, research requires novel methodological approaches and a continued interest in interdisciplinarity. As such, the study of lichens will also move forward as a symbiotic activity of researchers in the future.

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