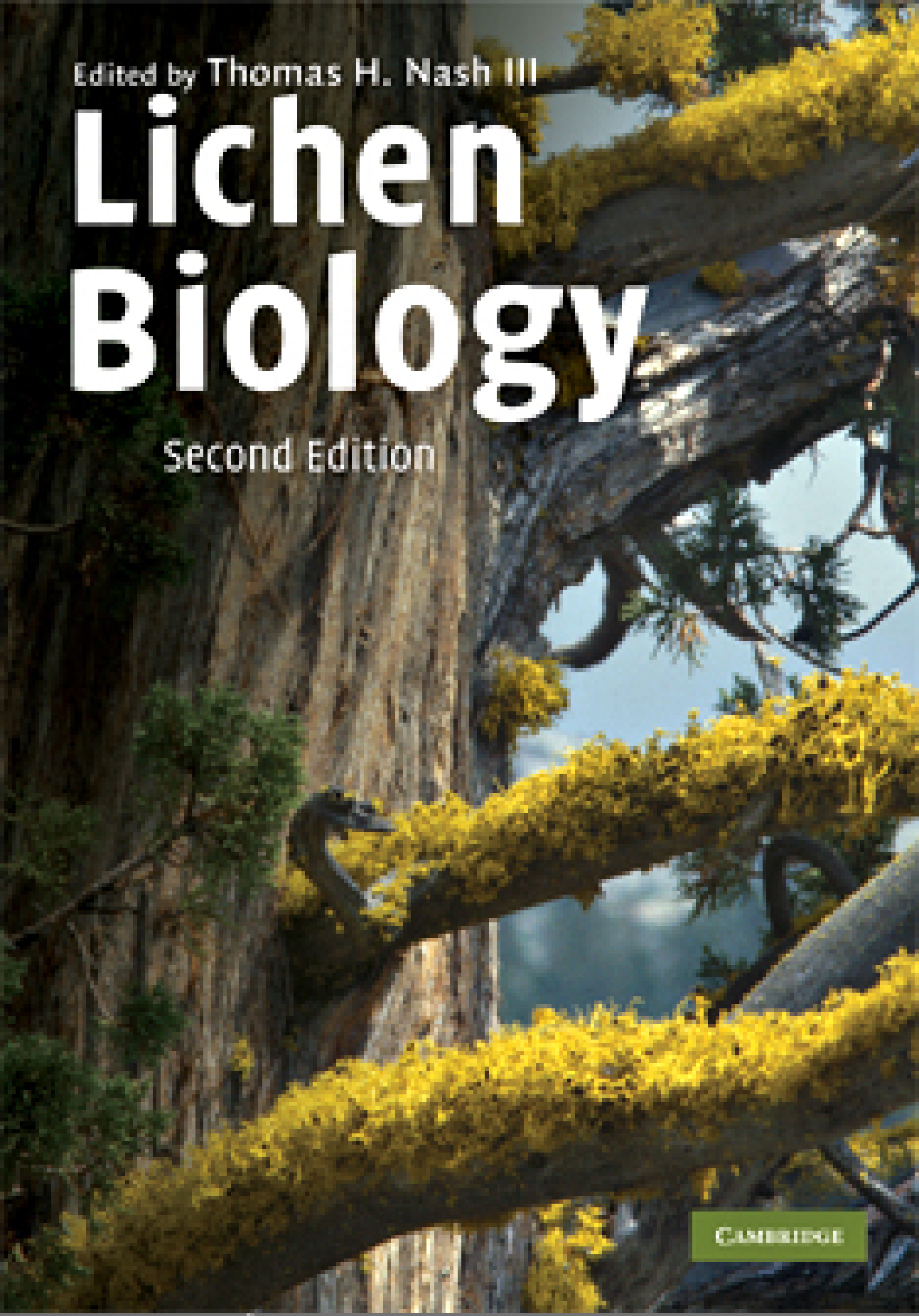

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Lichen Biology

Lichens are symbiotic organisms in which fungi and algae and/or cyanobacteria form an intimate biological union. This diverse group is found in almost all terrestrial habitats from the tropics to polar regions. In this second edition, four completely new chapters cover recent developments in the study of these fascinating organisms, including lichen genetics and sexual reproduction, stress physiology and symbiosis, and the carbon economy and environmental role of lichens. The whole text has been fully updated, with chapters covering anatomical, morphological and developmental aspects; the chemistry of the unique secondary metabolites produced by lichens and the contribution of these substances to medicine and the pharmaceutical industry; patterns of lichen photosynthesis and respiration in relation to different environmental conditions; the role of lichens in nitrogen fixation and mineral cycling; geographical patterns exhibited by these widespread symbionts; and the use of lichens as indicators of air pollution. This is a valuable reference for both students and researchers interested in lichenology.

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Lichen Biology

Second Edition

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CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo

Cambridge University Press
The Edinburgh Building, Cambridge CB2 8RU, UK
Published in the United States of America by Cambridge University Press, New York
www.cambridge.org
Information on this title: www.cambridge.org/9780521871624

First published in print format 2008

ISBN-13 978-0-511-41082-6 eBook (Adobe Reader)

ISBN-13 978-0-521-87162-4 hardback

ISBN-13 978-0-521-69216-8 paperback

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Preface to the second edition

Twelve years ago the first edition of *Lichen Biology* was published, and brought a new synthesis to the field of lichenology. In the meantime, rapid advances in many areas, particularly in molecular biology, have expanded our horizons and added depth to our knowledge of areas already under investigation. Consequently, it is appropriate that a second edition has now been consummated.

The original edition had 13 chapters, but this edition has 17 chapters and has added an appendix on lichen culturing, which is becoming prominent in the expanding biotechnology area. New chapters include one on sexual reproduction (Chapter 6), summarizing knowledge not available in 1996. As prominent examples of stress-tolerant organisms, lichens have developed a variety of strategies that allow them to occupy both extremely cold and hot environments; consequently, these investigations were meritorious of a chapter of their own (Chapter 8). In addition, a chapter on growth (Chapter 10), a topic briefly covered in the original photosynthesis chapter, is now expanded to cover much new information and the major advances over the past decade. Although many aspects of the ecology of lichens were covered in the first edition, a number of important areas were omitted. This has been rectified in Chapter 14. Of the remaining chapters, the chapter titles remain the same from the first edition, but all chapters have been revised to a greater or lesser degree. For example, the chapter on the individual (Chapter 13) and air pollution (Chapter 15) bear little resemblance to their original counterparts. Altogether 13 additional people have contributed substantially to this edition.

As with the first edition, this book should be of interest to the specialist, whether amateur or professional lichenologist. Furthermore, the book will provide an essential reference for many other people, such as anyone interested in the phenomenon of symbiosis, ecologists interested in the role of lichens in ecosystems, or a land manager charged with assessing the effects of air pollution on natural systems. We also hope it will stimulate the next generation of students and young scientists to advance our knowledge of these wonderful organisms.

1

Introduction

T. H. NASH III

1.1 The symbiosis

Lichens are by definition symbiotic organisms, usually composed of a fungal partner, the mycobiont (Chapter 3), and one or more photosynthetic partners, the photobiont (Chapter 2), which is most often either a green alga or cyanobacterium. Although the dual nature of most lichens is now widely recognized, it is less commonly known that some lichens are symbioses involving three (tripartite lichens) or more partners. The potential relationships of mycobionts and photobionts may in fact be quite complex (Chapter 4), and a rigorous classification of many types of relationships was developed by Rambold and Triebel (1992). In general, lichens exist as discrete thalli and are implicitly treated as individuals in many studies (but see Chapter 13), even though they may be a symbiotic entity involving three kingdoms! From a genetic and evolutionary perspective, lichens can certainly not be regarded as individuals and this fact has major implications for many areas of investigation, such as developmental and reproductive studies (Chapter 5).

The nature of the lichen symbiosis is widely debated and deserves further investigation. Most general textbooks and many researchers refer to lichens as a classical case of mutualism, where all the partners gain benefits from the association. Alternatively, lichens are regarded as an example of controlled parasitism, because the fungus seems to obtain most of the benefits and the photobiont may grow more slowly in the lichenized state than when free-living (Ahmadjian 1993). In fact, the relationships may be much more complex, especially when additional lichenicolous fungi (Lawrey and Diederich 2003) occur on/in lichens. These are different fungi from the dominant mycobiont, and they may have a parasitic, commensalistic, mutualistic or saprophytic/saprobic

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relationship to the lichen (Rambold and Triebel 1992). Parasitic symbiotic fungi may cause extensive damage, resulting in localized necrotic patches or in complete death of the thallus. On the other hand, commensalistic symbiotic fungi apparently share the photosynthetically derived products from the photobiont with the mycobiont of the existing symbiosis. Such secondary fungi are assumed not to benefit their hosts, and they do not appear to damage them, although they may lead to the formation of gall-like growths, the morphology and physiology of which are, as yet, little understood. A few cases are being discovered where the secondary parasitizing fungus progressively eliminates the primary mycobiont, taking over the photobiont to produce a new thallus of its own; the stability of such a union can be fragile, since in some cases it has been discovered that the original photobiont can be exchanged for another preferred photobiont after the takeover.

Within the realm of what we call lichens, the degree of lichenization varies tremendously from a few photobiont cells that seem to be almost haphazardly associated with a fungus (e.g. some Caliciales) to the more typical well-integrated thallus, in which a distinct photobiont layer is found beneath cortical fungal tissue (Chapters 4 and 5). In most of the latter cases, the lichen bears little morphological similarity to the bionts that form it. Because of the differences in degree of lichenization, no single definition may adequately cover the full range of relationships found within lichens.

The morphology of the lichenized thallus is strongly influenced by the photobiont and its direct contact with the mycobiont (Chapters 4 and 5). In nature there are at least a few cases where the same mycobiont, as ascertained with molecular techniques, is able to form two very different, interconnected thalli with respectively a cyanobacterium and a green alga (Armaleo and Clerc 1990). These different morphotypes are called photosymbiodemes, and their occurrence implies ontogenetic control by the photobiont. In culture, unlichenized mycobionts remain relatively amorphous, but initiate thallus development when they first come in contact with their photobiont (Ahmadjian 1993; Chapter 5). Subsequently the mycobiont may completely envelop the photobionts and, particularly in the case of green algae, penetrate the surface of the photobiont with structures called haustoria. Because haustoria are sometimes associated with dead photobiont cells and because parasitic fungi frequently form haustoria, Ahmadjian (1993) interprets lichenization as an example of controlled parasitism. Although there is limited experimental evidence, these haustoria are assumed to facilitate carbohydrate transfer from the photobiont to the mycobiont. In the future it would be interesting to determine whether haustoria can also facilitate nutrient delivery from the mycobiont to the photobiont.

Certainly there is variation in the degree to which the symbiosis is an obligate one for the partners involved. The green algal *Trebouxia*, which occurs in approximately 20% of all lichens, has rarely been found free-living (Chapter 2). In contrast, other photobiont genera, such as *Gleocapsa*, *Nostoc*, *Scytonema*, and *Trentepohlia*, occur commonly both in lichenized and free-living states. In at least some cases, both free-living and lichenized populations occur in the same habitat, such as free-living *Nostoc* and *Scytonema* in desert soils and their lichenized counterparts respectively in the terricolous lichens *Collema* and *Peltula*. The degree to which the same photobiont species occurs in both free-living and lichenized states (Beck 2002) is not well established, because relatively few lichen algae have been definitively identified to species, and more generally, the systematics at the species level of many cyanobacteria and unicellular green algae are not well resolved (Chapter 2). Nevertheless, it appears that most lichens are highly specific in their choice of photobiont (Beck *et al.* 1998; Rambold *et al.* 1998). In contrast, the systematics of the mycobiont is well known. Because isolated mycobionts grow so slowly, they are unlikely to survive well in the free-living state due to competition with other fungi or consumption by other organisms. Thus, most mycobionts are assumed to have an obligate relationship to lichenization, although the specificity of the mycobiont for a particular photobiont may not be as great as one might assume. In addition to the photosymbiodeme example cited above, more than one species of *Trebouxia* have been isolated from the same thallus (Friedl 1989*b*; Ihda *et al.* 1993).

Overall, the lichen symbiosis is a very successful one as lichens are found in almost all terrestrial habitats from the tropics to polar regions (Chapter 14). Certainly as a result of the symbiosis, both photobiont and mycobiont have expanded into many habitats, where separately they would be rare or non existent. For example, most free-living algae and cyanobacteria occur in aquatic or at least very moist terrestrial habitats, but as part of lichens they occur abundantly in habitats that are frequently dry as well. Not only may the fungus enhance water uptake due to its low water potential (see below), but also it substantially reduces the light intensity to which the photobiont is exposed (Ertl 1951). High light intensity adversely affects the photobiont (Demmig-Adams *et al.* 1990), and hence lichenization is one mechanism by which photobionts may expand into high light environments. Thus, there may well be benefits to lichenization from the perspective of the photobiont. Overall, it may be less important to evaluate lichenization from a strict cost/benefit perspective than to recognize it as a prominent example of a successful symbiosis. Additional studies will doubtlessly help to elucidate further our understanding of the symbiosis.

1.2 Systematics

Lichens are classified as fungi (Chapter 17), and estimates of the number of species vary from 13 500 (Hawksworth and Hill 1984) to approximately 17 000 (Hale 1974). Because many regions of the world have been poorly collected, the higher number may well be more reasonable. By far the largest number of lichens are Ascomycetes and in fact almost half of the described Ascomycetes are lichenized (Chapter 17). In addition, there are a few lichenized Basidiomycetes and Deuteromycetes (= Fungi Imperfecti). The latter group is an artificial class, in which sterile species are placed. If fruiting structures are eventually found, then these lichens may in due course be classified as either Ascomycetes or Basidiomycetes. In addition, in the Actinomycetes, Mastigomycetes and Myxomycetes, there are a few symbiotic associations with some properties similar to lichens, but in general these are excluded from lichen classifications.

Although one might hypothesize that cyanobacteria, green algae, and fungi evolved from lichens, it is generally assumed that lichenization occurred subsequent to development of these organisms. In the fossil record there is limited evidence for the occurrence of lichens, but this may be more due to lack of preservation than their absence from earlier eons. In fact, several quite old fossils have recently been interpreted as being lichens (Chapters 5 and 16). The diversity of lichenized fungi and the fact that some groups contain both lichenized and free-living fungi has led to the inference that lichenization and delichenization have occurred more than once and in fact may have occurred several times (Gargas *et al.* 1995; Lutzoni *et al.* 2001). The initial inference is supported by the occurrence of lichens in different classes of fungi, and, within the Ascomycetes, by the fact that lichenization occurs exclusively in only five of the 16 orders, in which lichenization has thus far been found (Hawksworth 1988*a*). If lichenization has occurred multiple times, then in an evolutionary sense lichens cannot be regarded as one group or, as a phylogeneticist would say, lichens are polyphyletic (Chapter 17).

1.3 Diversity and ecological domain of lichens

Among the terrestrial autotrophs of the world, lichens exhibit intriguing morphological variation in miniature (Chapter 4). In color they exhibit a fantastic array of orange, yellow, red, green, gray, brown, and black (Wirth 1995; Brodo *et al.* 2001). Lichens vary in size from less than a mm^2 to long, pendulous forms that hang over 2 m from tree branches (Chapter 4). Almost all lichens are perennials, although a few ephemerals (e.g. *Vezdaea*) are known. At the other extreme some lichens are estimated to survive well over 1000 years and may be

useful in dating rock surfaces (Beschel 1961; Section 10.7). Linear growth varies from imperceptible to many millimeters in a year.

Lichens occur commonly as epiphytes on trees and other plants, and in some ecosystems epiphytic lichen biomass may exceed several hundred kg ha^{-1} (Coxson 1995). In addition, they frequently colonize bare soil, where they are an important component of cryptogamic soil crusts in arid and semi-arid landscapes (Evans and Johansen 1999; Belnap and Lange 2003). Furthermore, lichens occur almost ubiquitously on rocks with the most obvious ones occurring as epiliths, either growing over the surface or embedded within the upper few millimeters. A few lichens even occur endolithically within the upper few millimeters of the rock, such as occurs in Antarctica (Friedmann 1982). In the tropics and subtropics, some rapidly growing lichens even colonize the surface of leaves as epiphylls (Lücking and Bernecker-Lücking 2002). Although most lichens are terrestrial, a few occur in freshwater streams (e.g. *Peltigera hydrothyria*) and others occur in the marine intertidal zone (e.g. *Lichina* spp. and the *Verrucaria maura* group).

Lichens occur in most terrestrial ecosystems of the world, but their biomass contribution varies from insignificant to being a major component of the whole ecosystem (Kershaw 1985; Chapter 14). In many polar and subpolar ecosystems, lichens are the dominant autotrophs (Longton 1988). In addition, lichens are conspicuous components of many alpine, coastal and forest ecosystems, such as the temperate rain forests of the southern hemisphere (Galloway 2007) and taiga of the northern hemisphere (Kershaw 1985). Because most lichens grow relatively slowly, their primary productivity contribution is fairly small in most ecosystems (Chapter 10). On the other hand, the more rapidly growing species may increase their biomass by 20–40% in a year and these species may play an important role in the mineral cycling patterns of their ecosystems (Section 12.10), particularly if cyanolichens are the dominant component (Chapter 11).

1.4 Lichens as poikilohydric organisms

Most flowering plants and conifers have developed the capacity to maintain the water status of their leaves or needles at fairly constant levels and hence are referred to as homiohydric organisms. In contrast, lichens are prominent members of poikilohydric organisms, whose water status varies passively with surrounding environmental conditions (Chapter 9). Other poikilohydric organisms include the bryophytes, some ferns and other primitive vascular plants. All of these organisms become desiccated relatively rapidly and, as a consequence, water availability is of prime importance for their

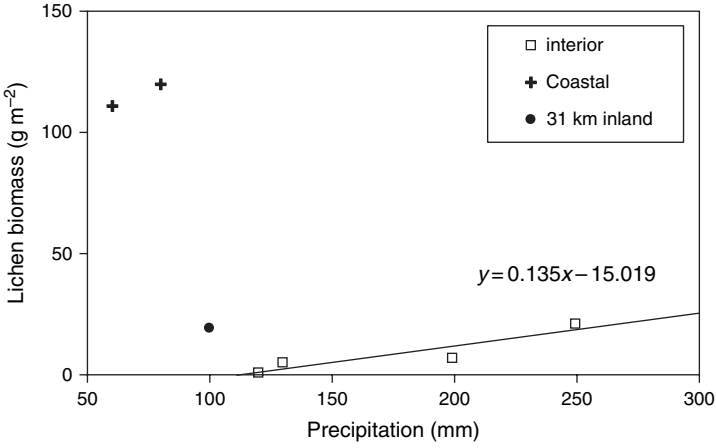

Fig. 1.1 Relationship of biomass of lichen communities within the Sonoran Desert region to mean annual precipitation (Nash and Moser 1982).

survival and in explaining their patterns of occurrence (Chapter 9). One might assume that poikilohydric organisms are highly dependent on precipitation, primarily in the form of rain. Certainly this is true for many lichens, as can be seen for the lichen biomass relationship among interior desert sites (Fig. 1.1, the straight line). On the other hand, lichen biomass near the Pacific Ocean in the western part of the Sonoran Desert vastly exceeds values that would be predicted based on precipitation alone (Fig. 1.1, crosses). This illustrates the ability of lichens to utilize other water sources, such as fog and dew. In addition, lichens have the remarkable ability to extract some moisture from non saturated air under conditions of low temperatures and high humidities. This is essentially the reverse of transpirational water flow occurring through vascular plants and is due to the low osmotic values of lichen thalli. However, under intermediate to high temperatures and intermediate to low humidities, the water potential gradient from the lichen to the atmosphere is reversed and evaporation occurs.

1.5 Practical applications

Many of the secondary products formed by lichens are unpalatable and may serve as defensive compounds against herbivores as well as decomposers (Rundel 1978; Chapter 14). As a consequence, it is not surprising that these secondary products are frequently used by the pharmaceutical industry as antibacterial and antiviral compounds. In addition, lichens have long been used as a source of natural dyes and in the making of perfumes. In both cases the secondary products provide the chemical basis for these applications (Chapter 7).

The differential sensitivity of lichens to air pollution has been recognized for over a century and a half, and the application of lichenological studies to biomonitoring of air pollution is now well developed (Chapter 15). For example, patterns in lichen communities may be correlated with sulfur dioxide levels in the atmosphere (Hawksworth and Rose 1970). In recent years sulfur dioxide levels have been reduced, either by improved controls on emissions or by more efficient dispersion strategies, and, as a consequence, lichens are now reinvading areas from which they had previously disappeared (Rose and Hawksworth 1981; Bates *et al.* 1990). However, the recolonization is incomplete because other factors, such as high nitrate deposition, modify lichen community composition as well. Finally, lichens are efficient accumulators of metals and persistent organic pollutants and are frequently used as surrogate receptors for documenting deposition of these pollutants (Chapter 12).

1.6 Lichens as self-contained miniature ecosystems

The lichen thallus is a relatively stable and well-balanced symbiotic system with both heterotropic and autotrophic components. From this perspective, the lichen can be regarded as a self-contained miniature ecosystem (Farrar 1976*c*; Seaward 1988), particularly if one considers the parasitic lichenicolous fungi colonizing lichens as this ecosystem's decomposers. The lichen fungus undoubtedly benefits enormously by obtaining its nutrition from the photobiont, but the photobiont's gain from the association is less obvious. Fundamentally, the photobiont gains protection from high light, temperature extremes and to some extent drought, but the premise that the alliance between free-living algae or cyanobacteria and the fungal partner enables them to live together in inhospitable areas where they could not do so independently cannot be fully justified. Pushed to its ultimate limit, this train of thought leads to the fallacy that lichens are the only form of life possible on other planets – a false assumption, because, even supposing that the environment there was capable of supporting life as we know it, then representatives of both symbiotic partners would have to be present in the first instance. However, lichens have recently been put to the test in terms of their ability to cope with extreme conditions of outer space, even Martian conditions, the symbiotic system and germination capacity proving remarkably resistant to UV radiation and vacuum exposure (de Vera *et al.* 2003, 2004).

The lichen symbiosis typically involves a close physiological integration. The usually dominant mycobiont is, of course, a heterotrophic organism that derives its carbon nutrition from the photobiont (Chapter 3). The flux of carbohydrates, as polyols in the case of green algal lichens and glucose in the case of

cyanolichens, from the photobiont to the mycobiont is well established (Smith and Douglas 1987). This is a necessary benefit for the mycobiont and is the result of the photobiont's cell walls being more permeable to carbohydrate loss in the lichenized than nonlichenized state (Hill 1976). In addition, the mycobiont gains a nitrogen source in the case of cyanolichens, in which nitrogen fixation occurs in the photobiont (Chapter 11). No comparable flux of nutrients from the mycobiont to the photobiont has been demonstrated. However, the recent demonstation of recycling of nitrogen and phosphorus in a mat lichen is an exciting first step to providing such documentation (Hyvärinen and Crittenden 2000; Ellis *et al*. 2005). Does the fungus in general serve as a reservoir of inorganic nutrients for the photobiont through the haustoria? Certainly other fungi facilitate nutrient uptake in other symbiotic relationships, such as occurs in mycorrhizae and rhizospheric fungi. Another result of close physiological integration is the occurrence of a wide range of secondary products, many of which occur as crystals extracellularly within the lichens (Chapter 7). Most of these are unknown in free-living fungi (or other organisms) and hence their occurrence adds to the uniqueness of the lichen symbiosis.

From an ecological perspective, lichens may be even more complex, as free-living bacteria and non symbiotic fungi may be found associated with an "individual" (Section 13.4) and, as a consequence, some authors regard a lichen as a miniature ecosystem. Further support for accepting the lichen as an ecosystem is provided when one considers the range of other benign or harmful microorganisms associated with one or more of the above bionts; these include fungi and bacteria found both on the surface and within thalli, or in the microenvironment generated beneath thalli or within lichen-weathered substrata (Bjelland and Ekman 2005), and also invertebrates which graze upon them, or seek protection from predators through crypsis or by sheltering beneath thalli; the intimate relationship between the lichen and its substratum in the case of epiphytic, lignicolous and foliicolous species adds to the complexity of the microhabitat generated.

2

Photobionts

T. FRIEDL AND B. BÜDEL

2.1 Major differences in cyanobacteria versus algae

Nearly 40 genera of algae and cyanobacteria have been reported as photobionts in lichens (Tschermak-Woess 1988; Büdel 1992). Three genera, *Trebouxia*, *Trentepohlia*, and *Nostoc*, are the most frequent photobionts. The genera *Trebouxia* and *Trentepohlia* are of eukaryotic nature and belong to the green algae; the genus *Nostoc* belongs to the oxygenic photosynthetic bacteria (cyanobacteria). Eukaryotic photobionts are also referred to as "phycobionts" while cyanobacterial photobionts are sometimes called "cyanobionts." The vast majority of eukaryotic photobionts belongs to the green algae (phylum Chlorophyta) which share many cytological features and their pigmentation, e.g. the presence of chlorophylls *a* and *b*, with the land plants (Bold and Wynne 1985; van den Hoek *et al.* 1993). Only two genera of eukaryotic photobionts containing chlorophylls *a* and *c* (phylum Heterokontophyta *sensu* van den Hoek *et al.* 1993) have thus far been reported: *Heterococcus*, Xanthophyceae, and *Petroderma*, Phaeophyceae (Tschermak-Woess 1988; Gärtner 1992).

Cyanobacteria are of prokaryotic nature and lack chloroplasts, mitochondria, and a nucleus, all of which are found in eukaryotic algae. In cyanobacteria, thylakoids lie free in the cytoplasm, often more or less restricted to the periphery. The circular DNA is not associated with histones and is concentrated in areas of the cytoplasm free of thylakoids which sometimes are called "nucleoplasm."

Metabolite transfer from the autotrophic photobiont to the heterotrophic mycobiont depends on the type of photobiont involved. In lichens with green algal photobionts, the carbohydrates are sugar alcohols; in lichens with cyanobacteria it is glucose (Feige and Jensen 1992; Section 10.2.1). The mode of

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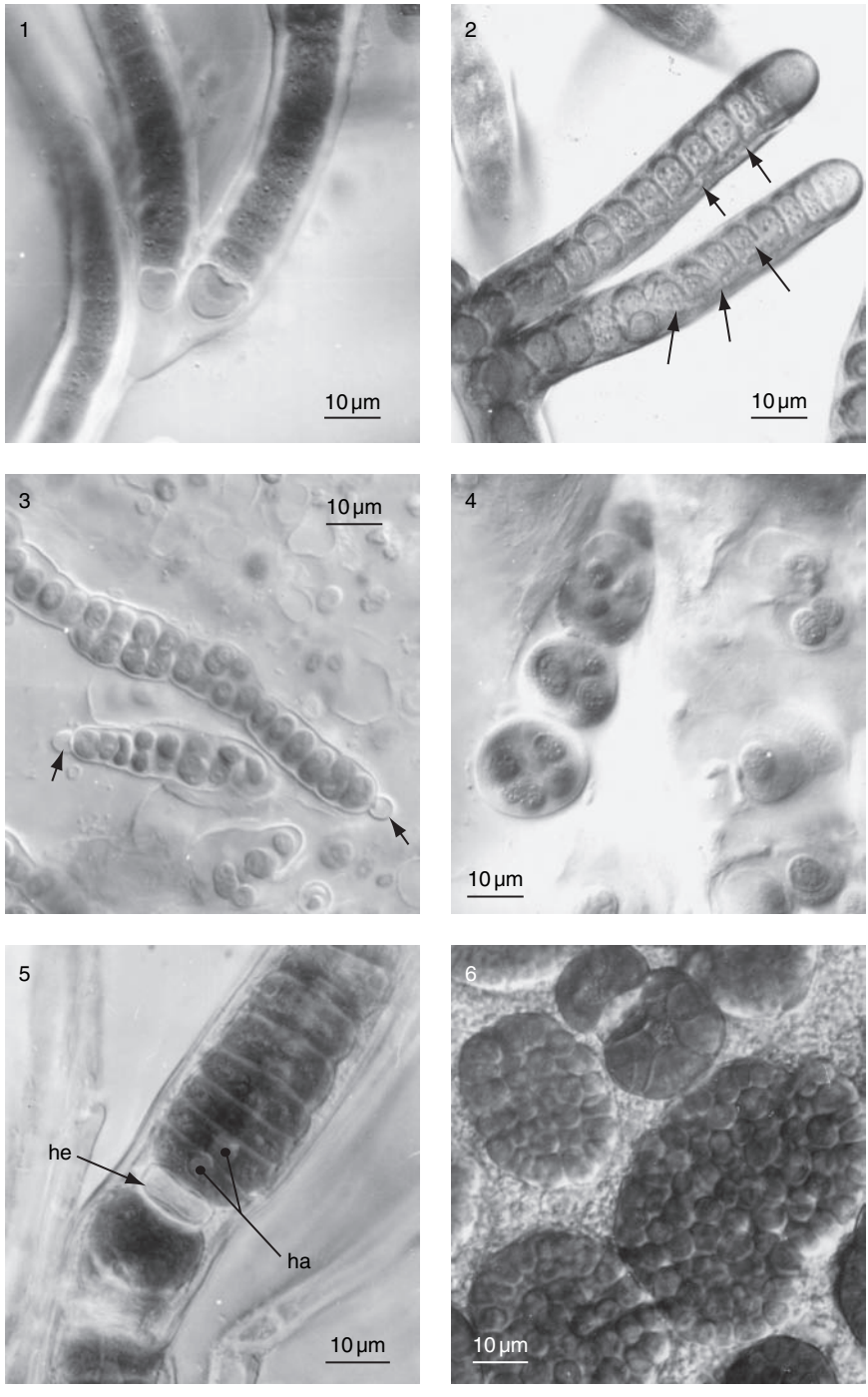
activation of CO_2 uptake is another basic feature that varies depending on whether the photobiont is prokaryotic or eukaryotic. In many green-algal lichens, positive net photosynthesis is possible after water vapor uptake alone. In contrast, in cyanobacterial lichens no measurable gas exchange occurs because the water content level required to activate photosynthesis is higher and liquid water is needed to obtain such levels (Lange *et al.* 1986).

2.2 Identification, reproduction, and taxonomy of photobionts

2.2.1 Cyanobacteria

Identification of cyanobacterial photobionts in the intact lichen thallus is often impossible since the morphology of the photobiont is changed by the influence of the fungal partner. Filamentous forms may be deformed to such a degree that their originally filamentous organization cannot be recognized within the lichen thallus, e.g. in the genus *Dichothrix* (Fig. 2.1). Only the truly branched filamentous cyanobacterial genus *Stigonema* (Fig. 2.2) and the nonbranched genus *Nostoc* (Fig. 2.3) can often be identified within the lichen thallus. Furthermore, cyanobacteria do not show all characteristic stages of their life cycles in the lichenized state. Because it is essential to know these stages for positive identification of cyanobacteria, even at the genus level (Komárek and Anagnostidis 1998, 2005), isolation and cultivation of the cyanobacterial photobiont are necessary steps for positive identification. The mode of vegetative cell divisions is also important in the delimitation of many unicellular cyanobacteria at the genus level. However, using molecular techniques, at least determination on the genus level is possible directly from the lichen thallus, using specific primers for cyanobacterial 16S rDNA (e.g. Lohtander *et al.* 2003; O'Brien *et al.* 2005).

Cyanobionts with heterocysts like *Nostoc* (Fig. 2.3) increase heterocyst frequency up to five times when lichenized compared with the free-living state (Feige and Jensen 1992). Also, cell size of cyanobacterial photobionts may be increased compared with cultured or free-living material, as has been reported for the genera *Gloeocapsa* (Fig. 2.4) and *Chroococcidiopsis* in the lichen genera *Lichinella*, *Peccania*, *Psorotichia*, *Synalissa*, and *Thyrea* (Geitler 1937; Büdel 1982). Increase of cell size can be a result of a very close mycobiont–photobiont contact, as in the deeply penetrating haustoria. This can be seen well in the vegetative trichome cells of *Scytonema* sp. within the lichen *Dictyonema sericeum* (Fig. 2.5). Unicellular cyanobacterial photobiont genera, e.g. *Chroococcidiopsis* and *Myxosarcina*, very rarely show their specific mode of reproduction when lichenized, but frequently show these stages when cultured. For instance, *Chroococcidiopsis* (Fig. 2.7) and *Myxosarcina* (Fig. 2.6) are characterized in culture



Figs. 2.1–2.6 Light microscopy of cyanobacterial photobionts. Fig. 2.1. *Dichothrix* sp. isolated from *Placynthium nigrum*, showing the characteristic branching mode of the genus. Fig. 2.2. *Stigonema ocellatum*, free-living sample at an early stage of lichenization; fungal hyphae indicated by arrows. Fig. 2.3. *Nostoc* sp. from *Peltigera canina*;

by multiple fission following one or two binary divisions (Waterbury and Stanier 1978; Büdel and Henssen 1983).

Various cyanobacterial genera have been found forming lichens, either as the primary or secondary photosynthetic partner. Their characteristic organization is shown in Figs. 2.1–2.7. Diagnostic features as well as systematic position according to the new system suggested by Anagnostidis and Komárek (1985, 1988, 1990) and Komárek and Anagnostidis (1998, 2005) and the bacteriological approach (Castenholz and Waterbury 1989) are summarized in Table 2.1. Other genera are mentioned in the literature, but they are not included here because their identification has not yet been based on cultured material. The taxonomy of cyanobacteria found thus far in lichens is summarized in Büdel (1992). The heterocyst-containing genus *Nostoc* (Fig. 2.3) is the most common cyanobacterial photobiont, closely followed by the unicellular genera *Gloeocapsa* (Fig. 2.4) and *Chroococcidiopsis* (Fig. 2.7).

At present, taxonomy of cyanobacteria at the species level is in a state of flux. Identification of cyanobacterial isolates from lichens at the species level is, at least for many unicellular taxa, almost impossible, since the species concepts in use (e.g. Geitler 1932; Komárek and Anagnostidis 1998, 2005) are basically defined on ecological features. Although the close relationship of cyanobacteria (formerly called blue-green algae) and bacteria has been known for more than a century (Cohn 1853), classification of the cyanobacteria was mainly based on their morphology. As in many other groups of organisms, problems arise in applying morphological criteria to systematics because considerable variation of morphological features occurs in relation to different environmental conditions. Recently, however, sequence comparisons of the small ribosomal subunit RNA (16S rRNA) have led to a revised view on the systematics and phylogeny of cyanobacteria (Wilmotte and Golubic 1991; Turner *et al.* 2001; Fewer *et al.* 2002; Gugger and Hoffmann 2004; Henson *et al.* 2004; Svenning *et al.* 2005; Tomitani *et al.* 2006). These data support the modern concept of using such morphological criteria as mode and planes of division, cell differentiation and morphological

Caption for Figs. 2.1–2.6 (cont.)
primordia of colonies with the typically apically attached primary heterocytes (arrows). Fig. 2.4. *Gloeocapsa sanguinea* in and at the margin of *Synalissa symphorea*. Fig. 2.5. *Scytonema* sp. in the thallus of *Dictyonema sericeum*; vegetative cells penetrated by mycobiont haustoria (ha), arranged in the center of cyanobiont cells along the longitudinal axis of the filament, heterocyte (he) not penetrated. Fig. 2.6. *Myxosarcina* sp. isolated from *Peltula euploca*, just after the cyanobiont was liberated from the thallus; colonies with numerous nanocytes are surrounded by bacteria, typical for early stages of the isolation procedure.

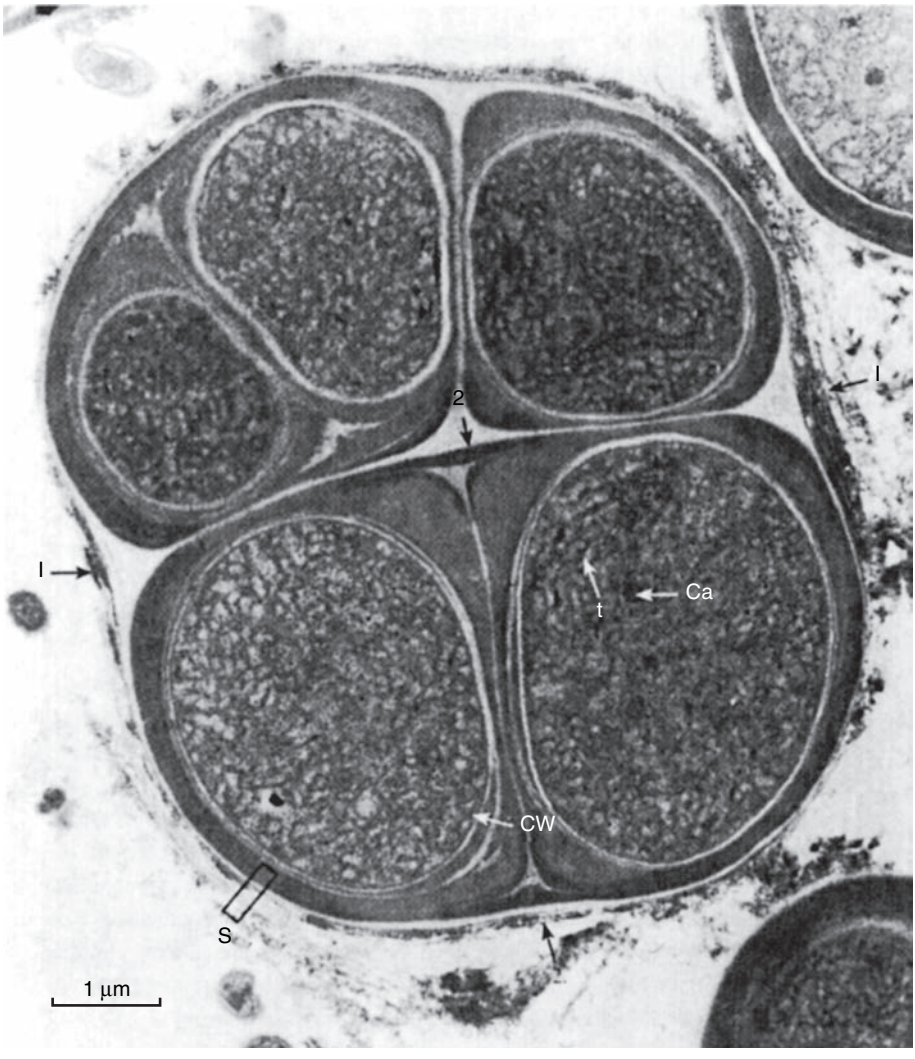


Fig. 2.7 Ultrastructure of *Chroococcidiopsis* sp., isolated from *Psorotichia columnaris*. Colony of daughter cells after two binary divisions (arrows), followed by irregular subsequent multiple fission in the upper part. S, striated sheath, CW, cell wall (outer lipoprotein bilayer, inner murein or petidoglucan layer), t, thylakoids, Ca, carboxysomes.

complexity, and differences in developmental stages in the life cycle as diagnostic features. In the 16S rRNA phylogenies, cyanobacteria with one cell type and binary division in one plane only are polyphyletic (i.e. do not have a common ancestor). The morphologically more complex groups of genera with cell division in several planes and producing nanocytes ("baeocytes" according to Waterbury and Stanier 1978) form two distinct clusters, placing

Table 2.1. *Genera of cyanobacteria identified from lichens arranged according to taxonomic characters*

Taxonomical character	"Botanical" system	"Bacteriological" system
1. Unicellular		
1.1 Binary division only	Chroococcales[a]	Chroococcales
Gloeocapsa, Chroococcus, Cyanosarcina, Entophysalis		
1.2 Binary division + multiple fission	Chroococcales[a]	Pleurocapsales
Nanocytes immotile		
Chroococcidiopsis		
Nanocytes motile		
Myxosarcina, Hyella		
2. Filamentous with heterocytes		
2.1 Nonbranched		Nostocales
Nostoc		
2.2 False branching, no tapering trichomes		Nostocales
Scytonema		
2.3 False branching, tapering trichomes		Nostocales
Calothrix, Dichothrix		
2.4 True branching		Stigonematales
Stigonema, Hyphomorpha		

[a] The order Chroococcales is subdivided into seven families in the "botanical" system.
Source: Büdel (1992).

the unicellular *Chroococcidiopsis* cluster as the closest living relatives to the heterocyst-forming filamentous cyanobacteria with a high statistical support. The *Pleurocapsa* cluster itself forms a well-supported sister group to the Chroococcales (Fewer *et al.* 2002). The 16S rDNA data confirm that heterocyst-forming cyanobacteria are monophyletic. Within that cluster, the Nostocales are divided into three groups (Fig. 2.8). One is well supported and includes the filamentous, nonbranched, and heterocyst-containing taxa (e.g. *Nostoc*). The second, also well supported, cluster includes the filamentous, heterocyst-containing taxa with false branching (e.g. *Scytonema*). The third cluster is not supported and contains filamentous, heterocyst-forming taxa with false branching and/or tapering trichomes (e.g. *Tolypothrix*, *Calothrix*). The morphologically most complex group of cyanobacteria, the former Stigonematales, with true branching, heterocyst-forming trichomes (e.g. *Fischerella*, *Hapalosiphon*, and *Stigonema*) have low statistical support and are polyphyletic. This has been conclusively demonstrated earlier (Gugger and Hoffmann 2004).

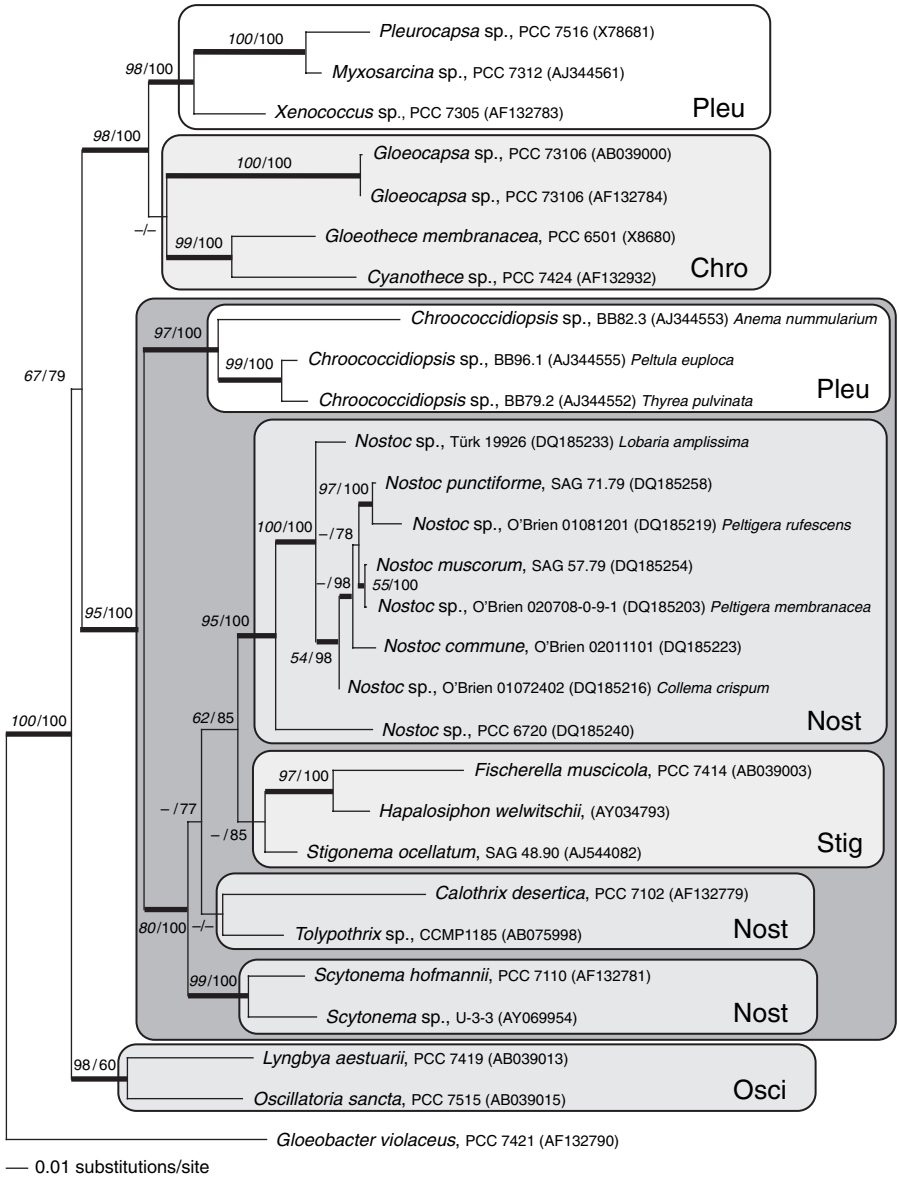
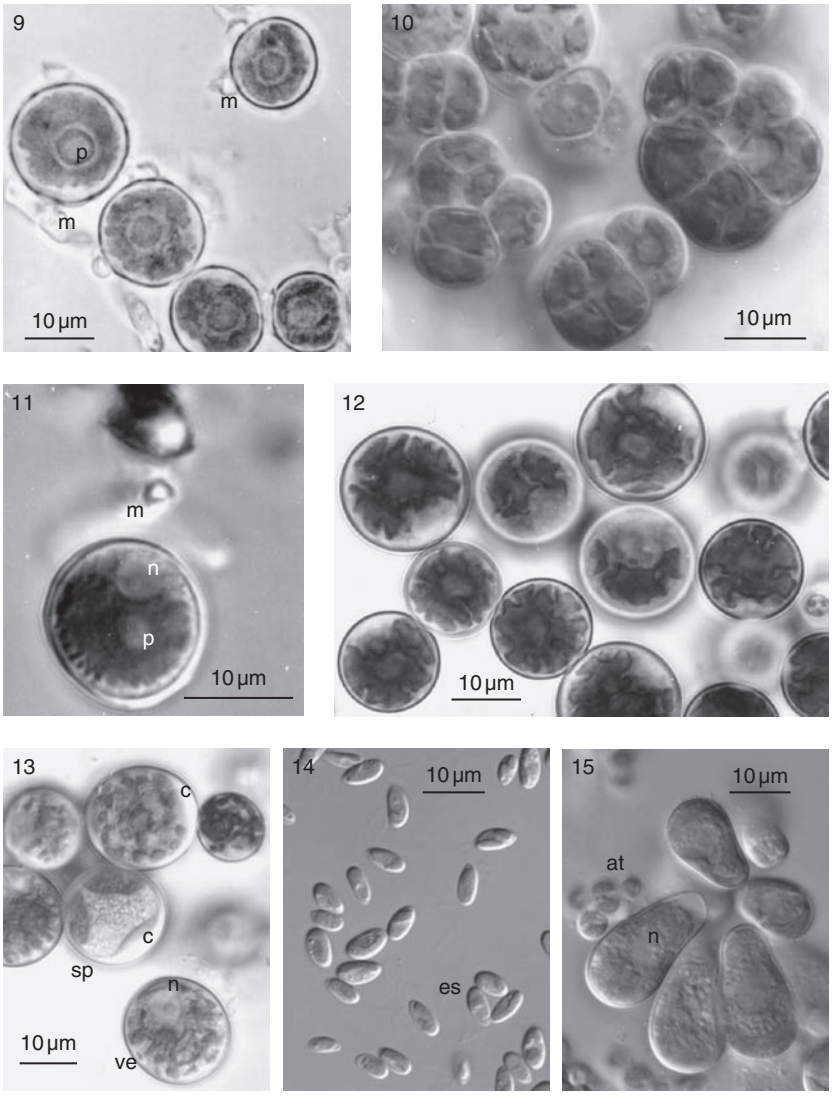


Fig. 2.8 16S rDNA phylogeny of cyanobacterial genera that also occur as cyanobionts (F. Kauff and B. Büdel, unpublished data). *Gloeobacter violaceus* was added as outgroup. Chro, Chroococcales; Nost, Nostocales; Osci, Oscillatoriales; Pleu, Pleurocapsales; Stig, Stigonematales. Bootstrap frequencies (number in italics) and posterior probabilities are indicated above horizontal branches. Phylogram generated with RAxML-HPC-2.1.2 Stamatakis (2006) out of 200 replicates and a GTRMIX model. Bootstrap proportions calculated with 500 replicates. Posterior probabilities estimated with MrBayes3.1.1 Huelsenbeck and Ronquist (2001), generating 20 000 000 generations using a GTR model with gamma distribution and a proportion of invariable sites. GeneBank accession number given in brackets.

2.2.2 *Green algae*

The organization of green-algal photobionts is simple: only coccoid (Figs. 2.9–2.15), sarcinoid or filamentous (Figs. 2.16–2.21) forms are known. No flagellates are known from lichens. Furthermore, filamentous forms are often reduced to short filaments (Fig. 2.20) or even to unicells (Fig. 2.17) within

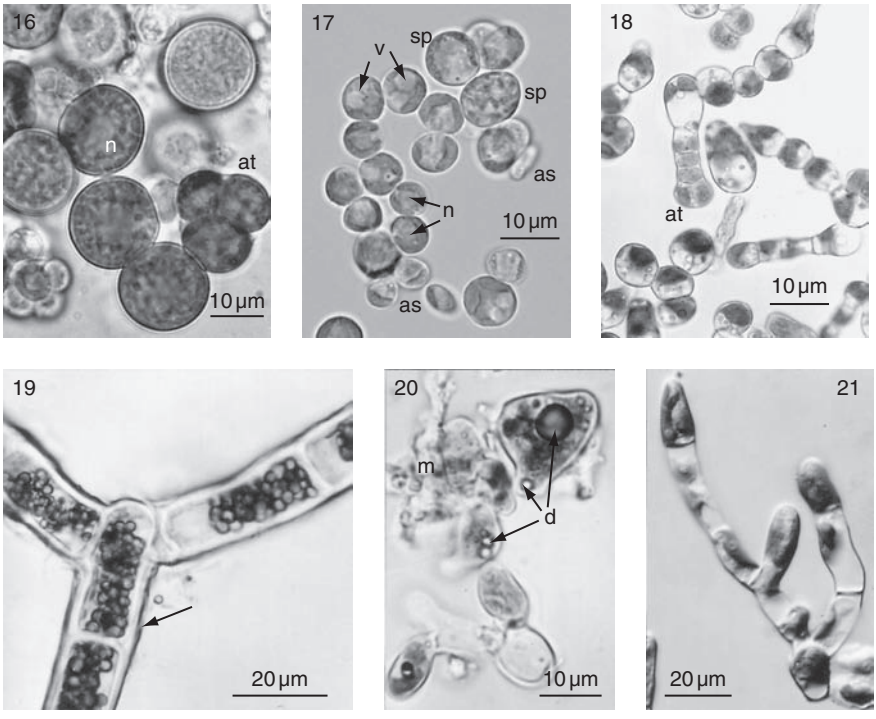


Figs. 2.9–2.15 *Trebouxia* spp. and other coccoid species, lichenized and in culture. Fig. 2.9. *Trebouxia gigantea* within a thallus of *Xanthomaculina hottentotta*. Unicellular stages attached to mycobiont hyphae (m). Note prominent pyrenoid (p) in the center of the algal chloroplast. Fig. 2.10. *Trebouxia gigantea* in culture, isolated from

lichen thalli. In lichens, green algae, except those belonging to the order Trentepohliales, reproduce only asexually by sporulation (i.e. forming motile zoospores with flagella and/or immotile autospores which lack emergent flagella) or true cell division (for terminology see Sluiman *et al.* 1989). If flagellated stages (zoospores) can be formed (e.g. in *Trebouxia*), they are released frequently in culture, but in the lichen thallus they may be observed only occasionally (Slocum *et al.* 1980). Identification of green lichen algae at the genus level is often possible without culturing, e.g. by simple squash preparations of the algal layer. For identification of the species, cultures are essential because important features such as chloroplast morphology or certain stages of the life cycle may be reduced or absent in the lichenized state. Most green lichen algae are only facultative photobionts, i.e. they are also occur independently as epiphytes, endoliths or as soil algae.

In modern taxonomic concepts of the Chlorophyta (e.g. Mattox and Stewart 1984; van den Hoek *et al.* 1993) the classes of green algae are regarded as different evolutionary lineages with each identified by a unique type of flagellated stage (zoospores or gametes). In contrast to traditional taxonomies, the former single class Chlorophyceae is now split into several separated new classes. The class Pleurastrophyceae with the order Pleurastrales (Mattox and Stewart 1984) and, independently, the new order Microthamniales (Melkonian 1990), have been established to classify the most common green lichen photobiont *Trebouxia* and some other morphologically diverse green algae (e.g. *Pleurastrum terrestre*, *Microthamnion kuetzingianum*) which share, however, unique

Caption for Figs. 2.9–2.15 (cont.)
Xanthomaculina hottentotta. Autospore packages are dominant in the culture four weeks after the alga was liberated from the mycobiont. Fig. 2.11. *Trebouxia arboricola* within a thallus of *Omphalora arizonica*, m, mycobiont hypha. Pyrenoid (p) in the center of the chloroplast, nucleus (n) located externally in an invagination of the chloroplast. Fig. 2.12. *Trebouxia gelatinosa*, vegetative cells in culture. Note crinkled chloroplast. Fig. 2.13. *Asterochloris* sp. isolated from *Diploschistes albescens* in culture. Vegetative cells (ve) with deeply incised and crinkled chloroplast, nucleus (n) located externally in an invagination of the chloroplast. Smooth chloroplast appressed to cell wall in early stage of sporangium development (sp). Sporangia with typical cap-like wall thickening (c). Fig. 2.14. Vegetative cells of *Coccomyxa subellipsoidea* isolated from *Botrydina vulgaris* in culture (strain SAG 216–13). Vegetative cells elongated and of irregular shape with a flat chloroplast appressed to the cell wall and without pyrenoids. (es) empty walls of autosporangia. Photograph taken by I. Kostikov. Fig. 2.15. Vegetative cells of *Myrmecia biatorellae* isolated from *Lobaria linita* in culture (strain SAG 8.82). Chloroplast smooth and attached to the cell wall, nucleus (n) with nucleolus located centrally; at, autospore package. Photograph taken by T. Darienko.



Figs. 2.16–2.21 Fig 2.16. *Dictyochloropsis reticulata* isolated from *Brigantiaea ferruginea* in culture (strain SAG 2150). Reticulate chloroplast attached to the cell wall, nucleus (n) located in the center of the cells. at, autospore package. Fig. 2.17. *Elliptochloris bilobata* in culture, isolated from subaerial habitats (strain SAG 245.80). Flat chloroplast appressed to cell wall, center of the cell with nucleus (n) and several large vacuoles (v). Sporangia at different developmental stages (sp). Note autospores (as) of different shape which is characteristic for the genus, elongated (right) and more spherical (middle). Fig. 2.18. Filamentous stages of *Leptosira obovata* in culture, isolated from freshwater (strain SAG 445–1). at, autosporangium. Fig. 2.19. Branched filament of *Trentepohlia* sp., free-living sample. Cells filled with carotenoid droplets covering the chloroplasts. Note lamellate cell wall (arrow). Fig. 2.20. Short and unbranched filament of a lichenized *Trentepohlia* sp. within a thallus of *Enterographa subpallidella*. Note carotenoid droplets (d) and mycobiont particle (m). Fig. 2.21. Young branched filament of *Dilabifilium arthropyreniae* in culture, isolated from *Arthropyrenia kelpii* (strain SAG 467–2).

ultrastructural features of their zoospores and their mitosis/cytokinesis patterns (Melkonian and Peveling 1988; Melkonian 1990). The taxonomic position of the order Microthamniales, however, is uncertain. It is regarded as a distinct lineage of green algae closely related to the class Chlorophyceae *s. str.* (Melkonian 1990) or as an order of the class Ulvophyceae (Sluiman 1989).

Most recent approaches to green-algal systematics also include molecular data (Figs. 2.24, 2.25). Gene sequence comparisons of the small ribosomal

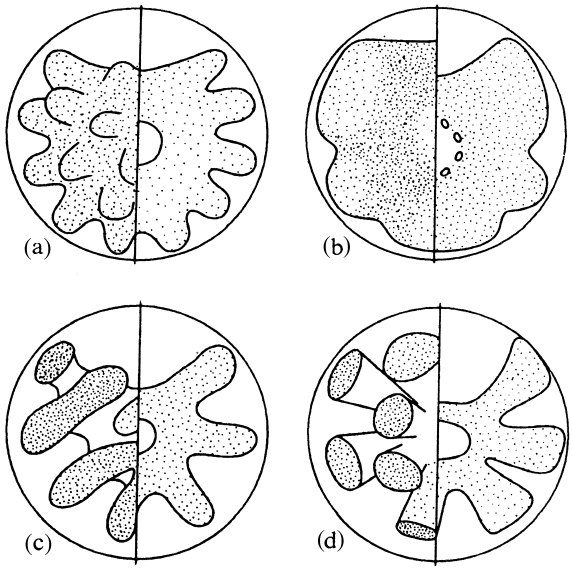


Fig. 2.22 Schematic drawings of some chloroplast types in *Trebouxia*. Left part of cells, surface view; right part of cells, optical section. (a) *T. crenulata*, (b) *T. corticola*, (c) *T. jamesii*, (d) *T. irregularis* (from S. Takeshita, unpublished data).

subunit RNA (18S rRNA) support the ultrastructural findings that *Trebouxia* forms together with *Pleurastrum terrestre* and *Microthamnion kuetzingianum* a distinct group of green algae, the Microthamniales (Friedl and Zeltner 1994). This order is evolutionarily distinct from the Ulvophyceae, but shares a sister group relationship with the Chlorophyceae *s. str.*, while the Pleurastrophyceae are polyphyletic in their rRNA phylogenies (Steinkötter *et al.* 1994). Based on these data, *Trebouxia* forms together with other coccoid lichen and soil algae (*Myrmecia biatorellae*, *Friedmannia israelensis*) an independent lineage, the "Lichen Algae Group" within the Microthamniales (Friedl and Zeltner 1994).

A great variety of morphologically diverse green algae has been identified from lichens. A complete list of all green-algal taxa found as photobionts so far was presented by Tschermak-Woess (1988). Examples of green-algal photobionts are given below.

Trebouxia spp. (Figs. 2.9–2.12, 2.22, 2.23) are the most common green photobionts. Within lichen thalli, only nonmotile stages with a reduced chloroplast occur (Figs. 2.9, 2.11). In culture, different patterns of autospore formation (e.g. autospore packages present [Fig. 2.10], cell cycle A; or autospore packages absent, cell cycle B; Friedl 1993) and motile stages (zoospores) are formed for reproduction. The nucleus is located in an invagination of the chloroplast (Figs. 2.11, 2.12). Vegetative cells exhibit a central massive or star-shaped chloroplast that is

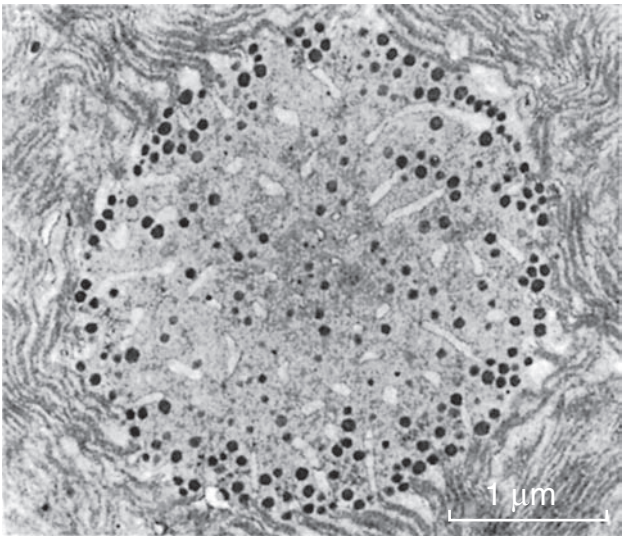


Fig. 2.23 Electron microscopy of a pyrenoid of *Trebouxia impressa*, lichenized phycobiont within a thallus of *Parmelia sulcata*.

wrinkled in different ways (Figs. 2.11, 2.12, 2.22) and contains several pyrenoids with a diverse ultrastructure (Fig. 2.23; Friedl 1989*a*). Several distinct patterns of chloroplast lobes are formed (Ettl and Gärtner 1984), and these are used to identify species of *Trebouxia* (Gärtner 1985). Twenty-five species are recognized by Gärtner (1985), and 16 species by Friedl (1989*b*). *Trebouxia* was formerly split into two genera, *Trebouxia* and *Pseudotrebouxia*, based on differences seen in the mode of reproduction. Cell packages that occur only in some species ("*Pseudotrebouxia* spp.") but not in others ("*Trebouxia* spp.") were believed to be the result of true cell division and therefore fundamentally different from autospores and zoospores (Archibald 1975; Hildreth and Ahmadjian 1981). However, this separation has not been supported by recent studies of autospore formation patterns (Ettl and Gärtner 1984; Friedl 1993), chloroplast characters (Gärtner 1985; Friedl 1989*a*), and zoospore ultrastructure (Melkonian and Peveling 1988). Gene sequence comparisons of the large ribosomal subunit (25S rDNA) support chloroplast morphology (Ettl and Gärtner 1984) as an important character for the distinction of *Trebouxia* species (T. Friedl and C. Rokitta, unpublished). In molecular phylogenies based on gene sequences of the small and large ribosomal subunits, *Trebouxia* appears as a paraphyletic genus (Friedl and Zeltner 1994; T. Friedl and C. Rokitta, unpublished). This supports the division of *Trebouxia* into at least two genera, but these findings do not coincide with the concept of *Pseudotrebouxia sensu* Archibald (1975). It is not yet clear whether *Trebouxia* is an obligate symbiotic genus of green algae or whether it also occurs free-living (Ahmadjian 1988, 1993).

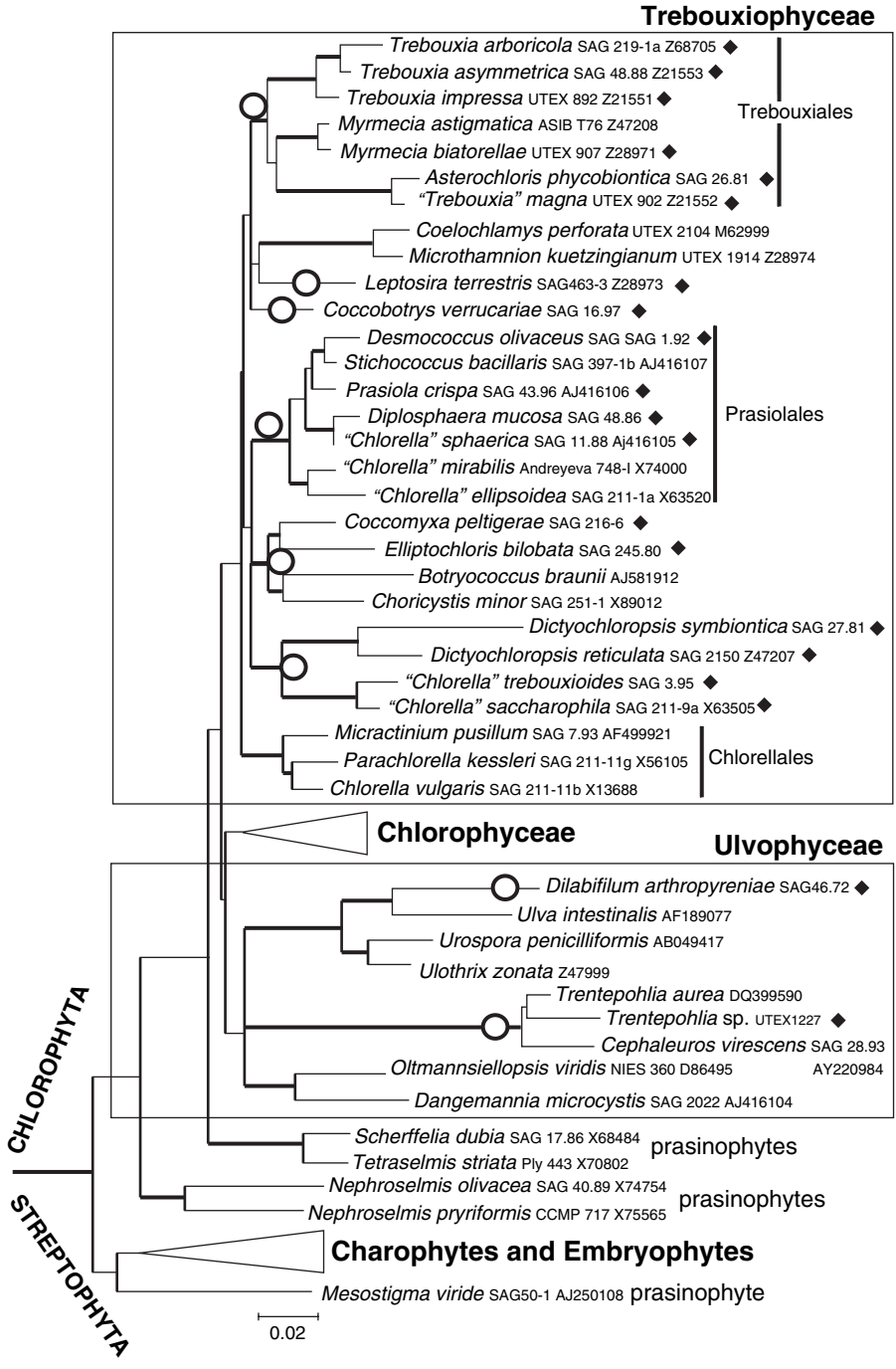


Fig. 2.24 Phylogeny of green-algal photobionts and nonlichenized green algae inferred from 18S rDNA sequence analyses. Species representing lichen photobionts are marked with a filled bar. A circle indicates a lineage of green algae in which lichen symbionts have evolved. Lichen photobionts are closely related to nonlichenized

The small colonies that have been observed (e.g. Tschermak-Woess 1978) on bark and soil a few times could also have escaped from damaged lichen thalli.

Myrmecia biatorellae differs from *Trebouxia* spp. by the cup-shaped chloroplast that lacks pyrenoids and occupies a parietal position, and the nucleus is located centrally (Fig. 2.15). Species of *Dictyochloropsis* are characterized by their reticulate chloroplast (Fig. 2.16). A close relationship of *Myrmecia biatorellae* with *Trebouxia* spp. is suggested by ultrastructural (Deason 1989) and molecular data (Friedl and Zeltner 1994) while the taxonomic position of *Dictyochloropsis* spp. is still unclear. Species of *Myrmecia* and *Dictyochloropsis* are also found as soil algae and on bark (Nakano *et al.* 1991).

In several other coccoid green photobionts, flagellated stages are unknown even in culture and reproduction is performed by autospores exclusively. Thus, a proper classification of these autosporic taxa remains unclear. Autosporic phycobionts include species of *Chlorella* and several other *Chlorella*-like algae, e.g. *Coccomyxa* (Fig. 2.14), *Elliptochloris*, *Diplosphaera* and *Nannochloris* (Tschermak-Woess 1988). Cells of *Chlorella* and *Chlorella*-like algae have a spherical or ellipsoidal shape, are often minute in size (some are less than 10 μm in diameter), contain a simple parietal chloroplast (Fig. 2.14) and some produce a gelatinous matrix (e.g. *Coccomyxa*). Due to their small size and lack of easily studied characters, taxomomy of these algae is only poorly understood. Differences seen in the formation of autospores may be a distinctive feature of important taxonomic value (Gärtner 1992). *Chlorella* is polyphyletic (Huss and Sogin 1990). *Diplosphaera* and *Nannochloris* may be closely related to each other (Gärtner 1992). *Chlorella* spp.

Caption for Fig. 2.24 (cont.)
species. Note that there are several independent origins for the symbiotic lifestyle within the classes Trebouxiophyceae and Ulvophyceae. The phylogeny shows the deep division of green algae into two clades, Chlorophyta and Streptophyta. The green-algal class Chlorophyceae and the group comprising charophytes and embryophytes which do not contain lichen photobionts are shown as diamonds, which stand for sequences not shown in the graphic but are used for the calculation of the tree. The code next to a species name is the accession number for a culture strain when available from a public culture collection. The other code refers to the GenBank accession number of the sequence; sequences without GenBank accession numbers are still unpublished. A distance phylogeny (neighbor-joining method; Hasegawa *et al.* [1985] model) is shown, on which statistical support ($>$ 70% in bootstrap tests, $>$ 0.7 a priori probabilities) using a maximum-likelihood model in conjunction with a minimum evolution distance approach, maximum parsimony and maximum likelihood (Bayesian inference; Huelsenbeck and Ronquist 2001) is indicated by thick internal branches. The phylogeny was rooted by two species of Glaucophyta which have been pruned away from the graphic.

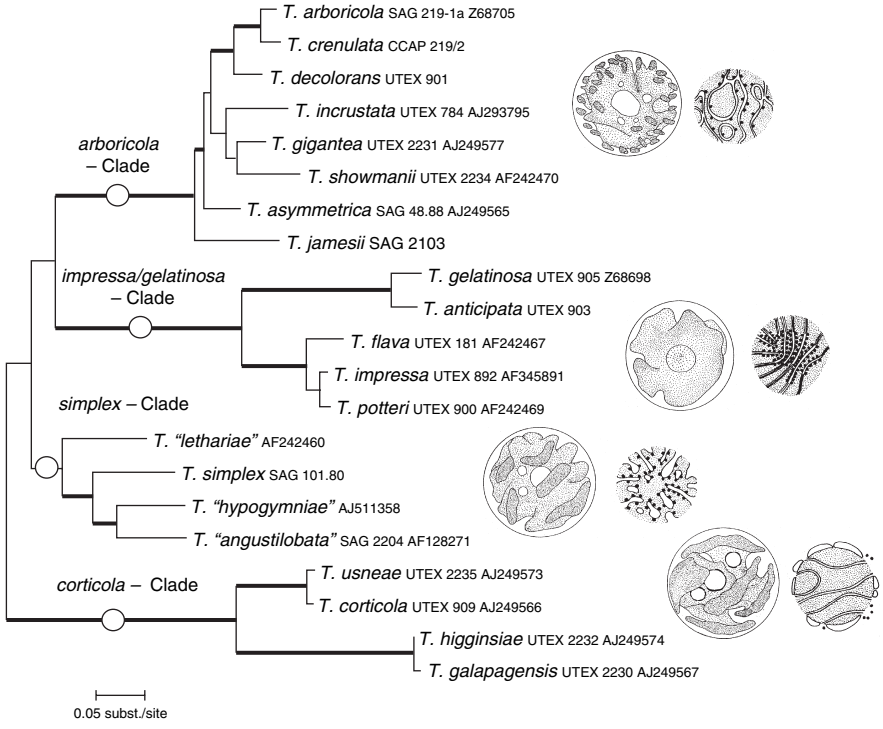


Fig. 2.25 Phylogeny of species of the green-algal photobiont *Trebouxia* from culture inferred from ITS rDNA sequence analyses. Four deeply diverging clades of species (marked by circles on internal branches) are resolved and are named according to a certain species from the clade. Schematic drawings for chloroplast morphology as viewed by light microscopy (left) and pyrenoid ultrastructure (right; Friedl 1989*a*) are shown next to each clade; these features define clades within *Trebouxia*. Only sequences from authentic cultures were used for this phylogeny. Authentic cultures are valuable as references; they represent the culture material on which the taxonomic description of a species was based. Species names in quotation marks indicate that a formal taxonomic description for this species is still pending. The code given next to a species name represents the accession numbers for a culture strain if available from public culture collections. The other code refers to the GenBank accession number of the sequence if available from public sequence data bases. Sequences without GenBank accession numbers are still unpublished. An unrooted maximum likelihood phylogeny is shown on which statistical support ($>70\%$ in bootstrap tests, >0.7 a priori probabilities) using a maximum-likelihood model in conjunction with a minimum evolution distance approach, maximum parsimony and maximum likelihood (Bayesian inference; Huelsenbeck and Ronquist 2001) is indicated by thick internal branches.

and *Chlorella*-like algae are well known from diverse aquatic and aerophytic habitats; *Chlorella* spp. are also common as endosymbionts of invertebrates.

Coccobotrys and *Desmococcus* have sarcinoid growth habits or form multiseriate filaments (Zeitler 1954). Both genera are also known from endolithic habitats (Broady and Ingerfeld 1993). Taxonomically these genera have not yet been studied adequately.

One of the most common green photobionts is the filamentous genus *Trentepohlia* (Figs. 2.19, 2.20), which also grows as an epiphyte (e.g. on moist rocks or bark, even on tree leaves in the tropics). In these habitats, free-living *Trentepohlia* forms branched filaments of cylindrical cells with thick lamellate cell walls (Fig. 2.19) and several parietal chloroplasts. Zoospores and gametes are produced in specialized cells differing from vegetative cells. When grown as an epiphyte, the protoplast can be entirely filled by droplets of carotenoids (Fig. 2.19) and then *Trentepohlia* forms orange or reddish plant masses. The same pigmentation is present in lichenized *Trentepohlia* (Fig. 2.20), giving rise to an orange color of the algal layer. So, scratching a lichen thallus surface causes an orange appearance, and one can safely predict that *Trentepohlia* is the photobiont. Within lichen thalli, *Trentepohlia* forms only short and thin filaments (Fig. 2.20) or consists of unicellular stages. Taxonomic relationships of the Trentepohliales are uncertain; some ultrastructural zoospore characters are shared with the Ulvophyceae (Chapman 1984).

Other filamentous green photobionts are *Pleurastrum terrestre* and species of *Dilabifilium* (Fig. 2.21). *Pleurastrum terrestre* forms short uniseriate filaments of elongated cells with a cup-shaped chloroplast in liquid culture media, but is unicellular within lichen thalli. *Pleurastrum terrestre* is known as a photobiont only from the lichen genera *Vezdaea* and *Thrombium* (Tschermak-Woess and Poelt 1976; Tschermak-Woess 1988), but has often been isolated from soil samples (Tupa 1974). *Dilabifilium* (including *Pseudopleurococcus*) forms branched filaments of cylindrical cells and has been found in primitively organized crustose aquatic lichens (e.g. *Verrucaria* and *Arthropyrenia*; Tschermak-Woess 1976), but is also known as an epiphytic alga in marine habitats and freshwater (Johnson and John 1990; Ihda *et al.* 1993). *Pleurastrum terrestre* is a member of the Microthamniales and is synonymous with *Leptosira obovata*. The taxonomic position of the genus *Dilabifilium* is still unclear (Johnson and John 1990).

2.3 Occurrence within lichens

Most lichen species contain green algae as photobionts. Among the lichenized families of the Lecanorales (nomenclature of lichen orders according to Henssen and Jahns 1974), *Trebouxia* is the most frequent photobiont, while

Trentepohlia is more frequent in lichen genera of the Arthoniales (e.g. *Roccella*), Gyalectales (e.g. *Coenogonium*) and the Sphaeriales. Members of the Trentepohliales are also common green algal photobionts of epiphyllic lichens in the tropics. In the Ostropales (e.g. *Graphis*, *Diploschistes*) both *Trebouxia* and *Trentepohlia* are equally frequent. *Chlorella* and *Chlorella*-like algae are most frequent in the Caliciales, but also occur in some crustose lichens of the Lecanorales (e.g. *Lecidella*, *Micarea*, *Trapelia*). *Coccomyxa* is common in the families Baeomycetaceae and Peltigeraceae as well as in lichenized Basidiomycetes. In the Peltigeraceae and the Stictaceae, *Dictyochloropsis* is the most common green algal photobiont. *Myrmecia* is a common photobiont in the genus *Dermatocarpon* (including *Catapyrenium*, Verrucariales). The order Verrucariales is rather diverse with respect to their photobionts, as the green algae *Coccobotrys*, *Desmococcus* and *Dilabifilium* as well as the heterokonts *Heterococcus* (Xanthophyceae) and *Petroderma* (Phaeophyceae) have all been found as photobionts in species of *Verrucaria* species.

Furthermore, the same lichen species can contain different species of *Trebouxia*, i.e. one mycobiont can form morphologically identical thalli with different algal species. For example, three different species of *Trebouxia* (*T. arboricola*, *T. irregularis*, and *T. jamesii*) have been isolated from *Parmelia saxatilis* (Friedl 1989*b*). Other examples are known from species of *Anzia* (Ihda *et al.* 1993) and *Diploschistes* (Friedl and Gärtner 1988).

Only about 10% of all lichen species contain a cyanobacterium as the primary photobiont. The Collemataceae, Heppiaceae, Lichinaceae, Peltulaceae, and Placynthiaceae have cyanobacteria as the only photosynthetic partner. In contrast, lichens of the families Arthopyreniaceae, Coccocarpiaceae, Corticiaceae, Pannariaceae, Peltigeraceae, and Stictaceae may have either green algal or cyanobacterial photobionts.

Some lichen genera have a green alga as the primary photobiont and a cyanobacterium as a secondary one. In such cases the cyanobacterium is located either in external or internal cephalodia (Chapter 5). In addition to heterocyst-producing filamentous cyanobacteria, a number of strains of the genus *Chroococcidiopsis* are also capable of N_2-fixation under microaerobic or anaerobic conditions (Stewart 1980; Boison *et al.* 2004).

2.4 Isolation and maintenance of cyanobionts and phycobionts

Techniques for the isolation of photobionts have been described several times, and the interested reader is referred to special publications like the "Handbook of Lichenology" (Galun 1988*a*) or Ahmadjian (1967*b*) for reference. Here, we only can give some general instructions for the isolation procedures.

2.4.1 *Cyanobionts*

From a washed thallus, fragments of the cyanobacterial layer are transferred under sterile conditions to agar plates containing a mineral medium. The agar plates are then kept under low light intensities (10–30 μmol m^{-2} s^{-1} PPFD) and moderate temperatures (15–25 °C). After a few days or weeks, cyanobiont cells start to develop free from the mycobiont and then can be transferred to fresh agar plates under a dissecting microscope using a micropipette or a needle. For long-term cultivation, strains of isolated cyanobionts are kept on agar slants at low light intensities and temperatures at about 15–17 °C to keep growth at slow rates.

2.4.2 *Phycobionts*

Isolation of green-algal photobionts is similar to that of cyanobacteria. From washed lichen thalli, a squash preparation of the algal layer is made. From this suspension of algal cells and mycobiont fragments, either single algal cells are isolated under microscopical observation using micropipettes or the whole suspension is transferred onto agar plates. The "micropipette method" (Warén 1918–19) usually results in clonal and axenic cultures. The "whole suspension" method is easier and the chances that the algae will grow in culture are better, but the resulting algal colonies need further purification. Most green-algal photobionts grow easily in culture. They are best maintained on liquid or agarized mineral media (Bischoff and Bold 1963; Friedl 1989*a*). In comparison with their growth on a mineral medium alone, some green-algal photobionts (e.g. *Trebouxia*) grow much faster and in larger quantities after the addition of glucose and proteose peptone to the culture medium and consequently are considered facultative heterotrophs (Ahmadjian 1967*b*). Most green-algal photobiont cultures require low light intensities and moderate temperatures (about 10–30 μmol m^{-2} s^{-1} PPFD and 15 °C).

The following culture collections maintain a large variety of lichen photobionts: the "Culture Collection of Algae at the University of Texas at Austin (UTEX)", Austin, Texas, USA, the "American Type Culture Collection (ATCC)", Rockville, Maryland, USA (cyanobacterial photobionts only), the "Sammlung für Algenkulturen (SAG)", Göttingen, Germany, and the "Pasteur Culture Collection (PCC)", Paris, France (cyanobacterial photobionts only). Additional culture strains are available from other culture collections which are listed in Myachi *et al.* (1989).

3

Mycobionts

R. HONEGGER

Lichen-forming fungi (also termed lichen mycobionts) are, like plant pathogens or mycorrhizal fungi, a polyphyletic, taxonomically heterogeneous group of nutritional specialists (Tables 3.1 and 3.2) but otherwise normal representatives of their fungal classes. Long after the discovery of the dual nature of lichens by Schwendener (1867; Honegger 2000) and his proposal to include lichens in fungi, most biologists and even the majority of lichenologists considered lichens as a group of organisms that differ so fundamentally from all others that they had to be treated as a separate group, e.g. as a phylum "Lichenes"; this term is nowadays obsolete. Even in the early twenty-first century, many scientists consider lichens as plants, thus ignoring the fact that species names of lichens refer to the fungal partner, fungi forming a separate kingdom. It is the heterotrophic mycobiont of morphologically advanced lichens that mimics plant-like structures. In this chapter the similarities and differences between lichen-forming and nonlichenized fungi are discussed at the phylogenetic, morphological and cytological levels and also with regard to different nutritional strategies.

3.1 Lichenized versus nonlichenized fungi

3.1.1 Lichenization: a successful nutritional strategy

Fungi, as heterotrophic organisms, have developed various nutritional strategies for acquiring fixed carbon (Table 3.1). Lichenization, i.e. the acquisition of fixed carbon from a population of minute, living algal and/or cyanobacterial cells, is a common and widespread mode of nutrition. One out of five fungal species is lichenized (Table 3.1). Some lichen-forming fungi belong to

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Table 3.1. *Acquisition of fixed carbon by Fungi and fungus-like Protoctista*

Degradation of dead organic matter	
Saprobes	*c.* 45–50%
Symbioses with C-autotrophs or C-heterotrophs:	
Parasitic symbioses (biotrophic or necrotrophic) with	
Cyanobacteria	
Algae	
Plants	
Fungi (lichenized and nonlichenized)	*c.* 20%
Animals	
Humans	
Mutualistic symbioses	
Mycorrhizae (*c.* 8%)	
Lichens (*c.* 21%)	*c.* 30%
Mycetocytes, etc.	

Sources: Lewis (1973); Hawksworth *et al.* (1983).

Table 3.2. *Orders of Ascomycota and Basidiomycota, which include lichenized taxa*

Phylum Subphylum Class Subclass Order	**Nutritional strategies** (predominant form in bold characters)	**Thallus anatomy** in lichenized taxa[9]
Ascomycota[1,2,3]		
Pezizomycotina		
Arthoniomycetes		
Arthoniales	**l**, nl (sap, lp)	**ns**, s
Dothideomycetes		
Patellariales	**nl** (sap, lp), l	**ns**
Eurotiomycetes		
Pyrenulales	**nl** (sap), l	**ns**
Verrucariales	**l**, nl (sap, lp)	**ns**, s
Mycocaliciales	**nl** (sap), l	
Lecanoromycetes		
Acarosporomycetidae		
Acarosporales	**l**	**ns**
Ostropomycetidae		
Agyriales	**l**, nl (sap)	**ns**
Gyalectales	**l**	**ns**

Table 3.2. (*cont.*)

Ostropales	**nl** (sap, pp, lp), l	**ns**
Pertusariales	**l**	**ns**
Trichotheliales	**l**	**ns**
Lecanoromycetidae		
Lecanorales[8]	**l**, nl (sap, lp)	**ns**, **s**
Peltigerales	**l**	**s**, ns
Teloschistales	**l**, nl (lp)	**ns**, **s**
Lichinomycetes		
Lichinales	**l**	**ns**, s
Basidiomycota[4,5]		
Hymenomycetes		
Agaricales	**nl** (sap, myc, pp, lp), l	**ns**, s
Polyporales	**nl** (sap, myc, pp, f, lp), l	**ns**, s
Anamorphic fungi[6]	**nl**, l	**ns**
Sterile taxa with no known reproductive structures[7]		**ns**

Abbreviations: f, fungicolous; l, lichenized; lp, lichenicolous (lichen parasite); myc, mycorrhiza; nl, nonlichenized; ns, nonstratified; pp, plant pathogens; s, internally stratified; sap, saprotrophic.

[1] *c.* 98% of lichen-forming fungi are Ascomycetes.

[2] *c.* 42% of Ascomycetes are lichenized (*c.* 13 500 spp.), all belonging to subphylum Pezizomycotina.

[3] 15 out of 52 orders of Pezizomycotina include lichenized taxa, 5 of them being exclusively lichenized. *c.* 0.4% of lichen-forming fungi are Basidiomycetes.

[5] Only *c.* 0.3% of Basidiomycetes are lichenized (*c.* 50 spp.).

[6] *c.* 1.5% of lichen-forming fungi (*c.* 200 spp.) belong to the Anamorphic fungi.

[7] *c.*75 species of lichen-forming fungi are sterile (disperse via thallus fragmentation). With molecular data sets the taxonomic affiliation of some of these taxa will be identified.

[8] Lecanorales are the largest order, comprising *c.* 5500 species, >99% of them lichenized.

Sources: Hawksworth (1988a); Honegger (1992); Hawksworth and Honegger (1994); Kirk *et al.* (2001); Eriksson (2006*b*).

The majority of lichen-forming fungi (>55%) form nonstratified (crustose, microfilamentous, etc.) thalli, *c.* 20% form squamulose or placodioid thalli, and *c.* 25% form morphologically advanced, foliose or fruticose thalli with internal stratification.

orders with uniform nutritional strategies; others belong to orders with diverse strategies (Table 3.2).

A high percentage of lichen-forming fungi are ecologically obligate, but physiologically facultative biotrophs (organisms that obtain nutrients from a living host). In other words they can be cultured in the aposymbiotic

("free-living") state but in nature almost exclusively the symbiotic phenotype is found. Nonlichenized germ tubes or other free hyphae of lichen mycobionts certainly exist in natural ecosystems, but, due to their notoriously slow growth rates, they cannot be recovered with conventional isolation techniques.

Molecular phylogenies elucidate taxonomic relationships among lichenized and nonlichenized fungi. Until recently lichenization was thought to be an ultimate state. Among the most fascinating mycological discoveries of recent years is the finding that lichenization can be transient. Based on thorough analyses of large sets of molecular data from lichenized and nonlichenized ascomycetes using a Bayesian phylogenetic tree sampling methodology, combined with a statistical model of trait evolution, Lutzoni *et al.* (2001) demonstrated that (1) lichens evolved earlier than previously assumed, (2) gains of lichenization were distinctly less frequent during ascomycete evolution than previously assumed, and (3) lichen symbiosis was lost several times. Consequently, numerous taxa of nonlichenized ascomycetes, such as Eurotiomycetidae, derive from lichen-forming ancestors. This particular group comprises economically important taxa such as the genera *Penicillium* and *Aspergillus*, with numerous species used in biotechnology because of their interesting secondary metabolism, a trait shared with many of their ancestors. The "fungal branch of the tree of life" is currently under construction, and many new insights in phylogenetic relationships of lichen-forming and nonlichenized fungi are expected in the near future (Lutzoni *et al.* 2004).

3.1.2 Fossil records

Fossil records of lichens are extremely rare. Perhaps palaeontologists have not yet adapted their eyes to recognizing lichen-forming fungi and their photobionts. Two recent discoveries of lichen-like fossils support the view that lichenization might be a very ancient nutritional strategy. In marine phosphorites of the Doushantuo Formation (approx. 600 million years before present [MaBP]) from South China, and in the famous Early Devonian Rhynie chert beds in Scotland (approx. 480 MaBP), colonies of coccoid cyanobacteria or unicellular algae with mucilaginous extracellular sheaths were found, which are invaded by fungal hyphae (Taylor *et al.* 1995*b*, 1997; Yuan *et al.* 2005). This situation resembles the mycobiont–photobiont interface in various genera of Lichinaceae (Henssen 1963, 1986; Büdel 1987; Henssen 1995), all with cyanobacterial photobionts (examples in Honegger 2001), or in *Epigloea* spp. (ascomycetes *incertae sedis*), which are symbiotic with or at least live within the mucilaginous colonies of *Coccomyxa dispar*, a unicellular green alga forming massive gelatinous sheaths (Jaag and Thomas 1934; Döbbeler 1984; David 1987). *Winfrenatia reticulata*, the Early Devonian fossil, was assumed to be formed by a

zygomycete (Taylor *et al.* 1995*a*, *b*, 1997). As extant zygomycetes are not forming lichen symbioses, some investigators hesitate to interpret *Winfrenatia* as a lichen. However, lichenization can be lost in the course of time, as shown in ascomycete evolution (Lutzoni *et al.* 2001, 2004). Among the Early Devonian Rhynie chert bed fossils are beautifully preserved arbuscular mycorrhizae (Taylor *et al.* 1995*a*), strikingly similar to the ones which are nowadays symbiotic with more than 70% of higher plants. These arbuscular mycorrhizae represent a fungal symbiosis that had already reached an astonishing level of morphological and physiological complexity, although the early vascular plants (Rhyniales) were just starting to colonize terrestrial ecosystems. Terrestrial soil and rock surfaces had certainly been colonized by cyanobacteria and green algae long before the advent of vascular plants. With high probability groups of fungi have formed manifold interactions with these early photoautotrophic inhabitants of terrestrial ecosystems, ranging from parasitism to mutualism. The Protolichenes hypothesis of Eriksson (2005, 2006*a*) proposes the origin of the subphylum Pezizomycotina among nutritional specialists symbiotic with algae and/or cyanobacteria, so-called Protolichenes, whence extant lichenized and nonlichenized groups evolved. Fossil arbuscular mycorrhizae show that complex fungal interactions with photoautotrophic partners were already differentiated 480 Ma ago.

Presently known fossils of morphologically advanced, foliose or fruticose lichens come from Tertiary (65–1.5 MaBP) deposits, i.e. are comparatively young; older ones certainly exist but have not yet been discovered. An easily recognizable impression of a *Lobaria* thallus (resembling *L. pulmonaria*) was found in early to middle Miocene deposits (24–12 MaBP) of a humid conifer forest from Trinity County, California (MacGinitie 1937; Peterson 2000), a site which might harbour many more lichen fossils (Peterson 2000). Amber, fossilized tree resins from the Old World (Baltic amber; 55–35 MaBP) and New World (Dominican amber; 20–15 MaBP) contain extremely well-preserved organisms. Most investigators focused on vertebrate, invertebrate or higher plant fossils, but also a few well-preserved lichens were detected (Mägdefrau 1957). Recently two species of calicioid lichens were found in Baltic amber (Rikkinen 2003), and beautifully preserved thalli of two *Parmelia* species in Dominican amber (Poinar *et al.* 2000).

3.1.3 Cytological aspects

There is no evidence for any fundamental difference between lichenized and nonlichenized fungi. Cell wall structure and composition of lichen-forming ascomycetes occur within the range of variation observed in all Ascomycota (Honegger and Bartnicki-Garcia 1991). Lichen-forming ascomycetes

and basidiomycetes produce and secrete the same type of hydrophobic cell wall surface compounds, the hydrophobins, as nonlichenized fungal taxa (see Chapter 5; Scherrer *et al*. 2000; Trembley *et al*. 2002*a*, *b*).

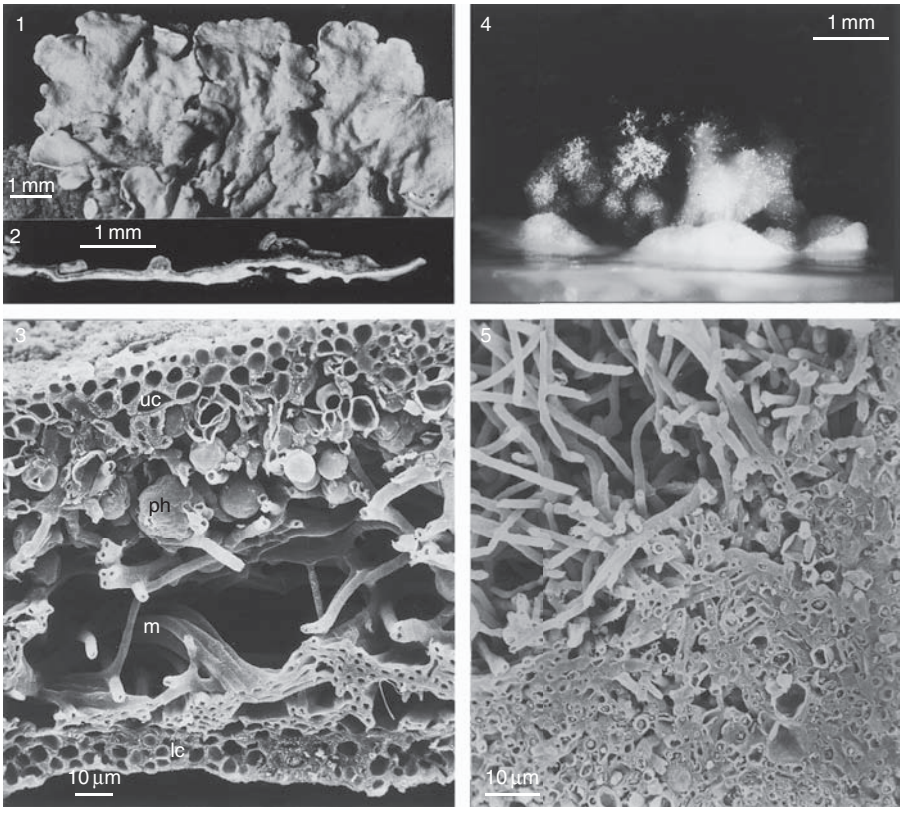
For quite a while lichen-forming ascomycetes were supposed to differ from nonlichenized taxa by the possession of concentric bodies, semicrystalline cell organelles (Figs. 3.6, 3.7) of approximately 0.3 μm diameter, comprising a proteinaceous, electron-dense shell around a gas-filled center. Concentric bodies are not membrane-bound and usually occur in clusters near the cell periphery. They are neither a peculiarity of lichen-forming ascomycetes nor of the symbiotic way of life, since they were found in a range of plant pathogens and saprobes, some of which occur in climatically extreme habitats in microbial communities of desert varnishes (Honegger 1993, 2001, 2006). Concentric bodies were found in the cytoplasm of all types of vegetative cells, in paraphyses, ascogenous hyphae, asci, and in mature ascospores. Longevity and a considerable desiccation tolerance seem to be the features shared by all ascomycetous cells that harbor concentric bodies; these were hypothesized to be remains of drought stress-induced cytoplasmic cavitation events (see Chapter 4), as regularly experienced by long-living fungal structures that are subjected to continuous wetting and drying cycles (Honegger 1995, 2001, 2006; Honegger *et al*. 1996).

Multiperforate septa, a rather unusual feature among ascomycetes, have been observed even by early light microscopists and later with electron microscopy techniques (Wetmore 1973) in medullary hyphae of some Peltigeraceae and Parmeliaceae (Figs. 3.8, 3.9). Only some, but not all, medullary hyphae of a thallus reveal this structural peculiarity. It remains unknown whether these particular hyphae are functionally different from the rest of the medullary hyphae.

3.1.4 *Symbiotic versus aposymbiotic phenotypes*

Aposymbiotic phenotypes in culture

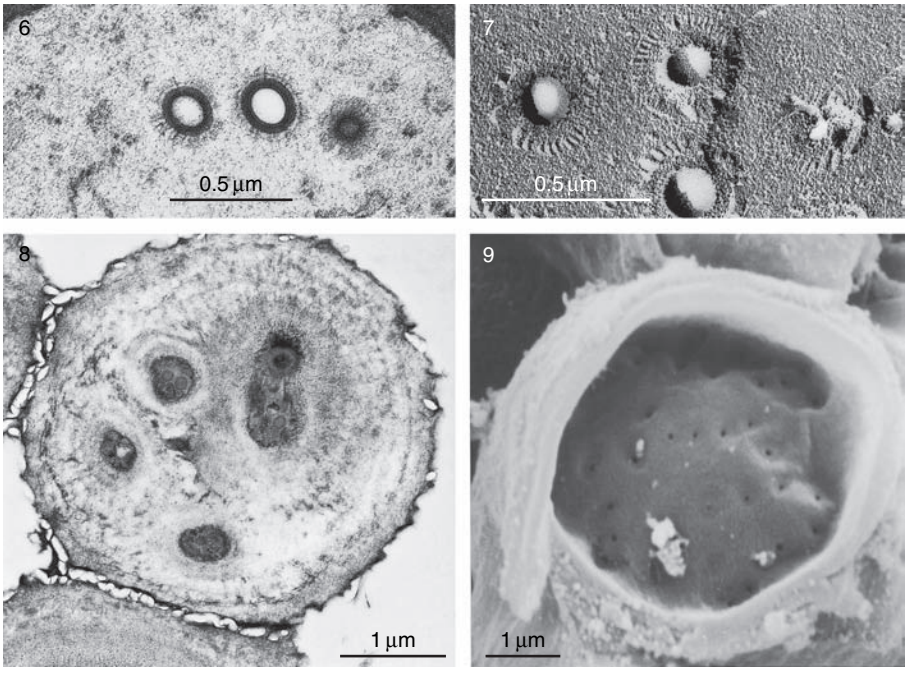
Lichen-forming fungi differ from nonlichenized taxa by their nutritional strategy and by their manifold adaptations to the cohabitation with a population of minute photobiont cells. In pure culture aposymbiotic lichen mycobionts form thallus-like colonies with no morphological resemblance to the symbiotic phenotype (Figs. 3.4, 3.5). The central, microaerobic part of such aposymbiotic thalli is usually composed of cartilaginous, conglutinate cell masses while filamentous growth, i.e. aerial hyphae, are formed at the periphery (Fig. 3.5; further examples in Ahmadjian, 1973; Honegger and Bartnicki-Garcia, 1991; Stocker-Wörgötter, 1995).



Figs. 3.1–3.5 Symbiotic and aposymbiotic phenotypes of the dorsiventrally organized macrolichen *Xanthoria parietina* (Teloschistales). Fig. 3.1. Laminal view, and Fig. 3.2. vertical cross section of the marginal lobes of the leaf-like thallus. Fig. 3.3. Detail of a vertical cross section: uc, conglutinate upper cortex; ph, photobiont layer harboring the globose cells of the green alga *Trebouxia arboricola*; m, gas-filled medullary layer built up by aerial hyphae; lc, conglutinate lower cortex. Fig. 3.4. Aposymbiotic phenotype on an agar medium. Fig. 3.5. Detail of the peripheral part of a cross section of the thallus-like fungal colony showing filamentous hyphal growth at the periphery and conglutinate zones in the microaerobic central part.

Symbiotic phenotypes in nature

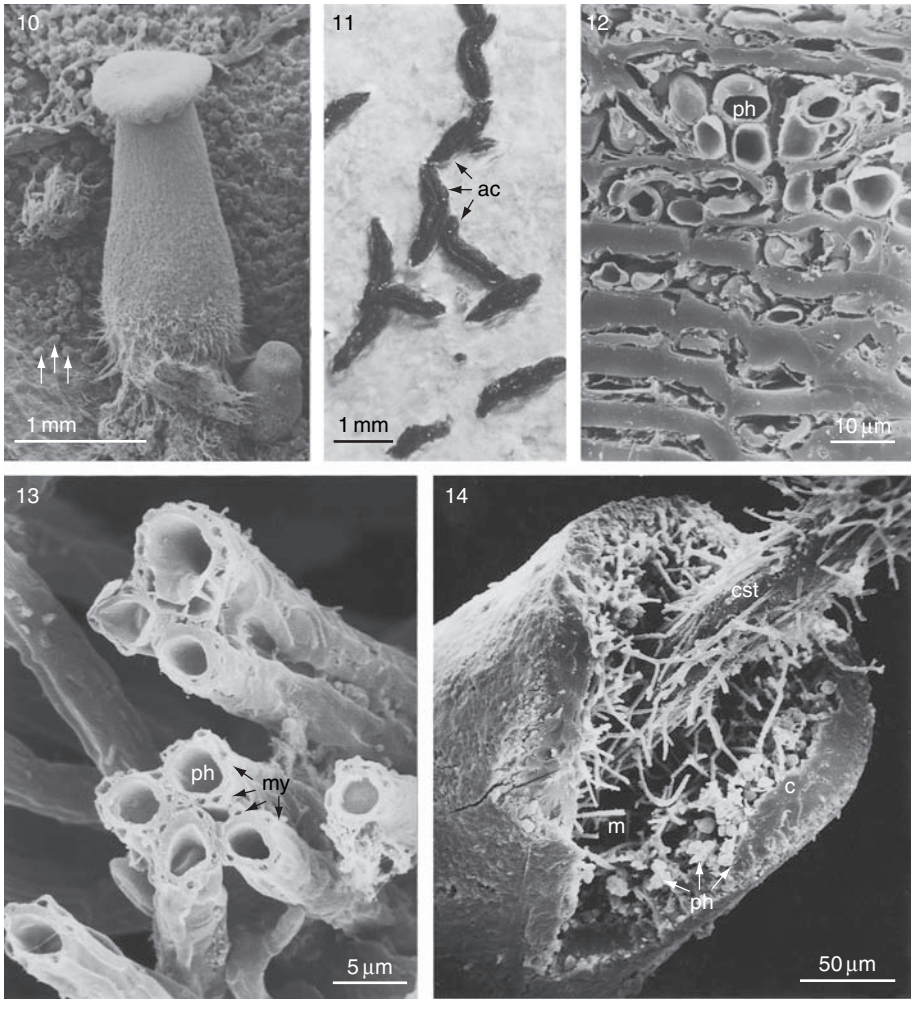
Most biologists consider lichens as being shaped like leaves (foliose; Fig. 3.1) or tiny, erect or pendulous shrubs (fruticose) and having an interesting internal stratification with the photobiont cell population incorporated in a thalline layer (e.g. Figs. 3.3 or 3.14). However, only one out of four lichen-forming fungi has such an impressive morphogenetic capacity. The majority of lichen mycobionts (Table 3.2) overgrow or ensheath photobiont cells on or



Figs. 3.6–3.9 Figs. 3.6–3.7. Concentric bodies, semicrystalline cytoplasmatic organelles of unknown origin and function in an ultrathin section and a freeze-fracture preparation of *Peltigera canina* (Peltigerales). Figs. 3.8–3.9. Multiperforate septa in medullary hyphae as observed in an ultrathin section of *Parmelia tiliacea* (Lecanorales) and a SEM preparation of *Peltigera canina*.

within the substrate and form microfilamentous (Fig. 3.13), microglobose (Fig. 3.10), or crustose thalli (Figs 3.11, 3.12), some of which are quite inconspicuous. About 20% of the lichen-forming fungi form squamulose or placodioid thalli with an internal stratification, but usually these thalli remain in close contact with the substrate. In only about 25% of lichen species do the mycobionts grow above the substrate and enter the third dimension by differentiating either a foliose or fruticose thallus with internal stratification (Figs. 3.3, 3.14; Table 3.2). These morphologically complex symbiotic phenotypes are formed by a range of functionally and morphologically different fungal cells (see Chapters 4 and 5).

The photobiont cell population is housed, maintained, and controlled within the fungal thallus. It is arranged similarly to the palisade parenchyma (i.e. the photosynthetically most active parts) in vascular plants: either in a plane, as in foliose lichens (Figs. 3.2, 3.3), or at the periphery of either erect (e.g. reindeer



Figs. 3.10–3.14 Structural and taxonomic diversity in lichen-forming fungi. Fig. 3.10. *Omphalina ericetorum* (Agaricales), a lichenized basidiomycete growing on detritus. Arrows point to microglobular lichenized structures (vivid green in fresh samples) on the surface of a decaying leaf. Figs. 3.11–3.12. *Graphis elegans* (Graphidales) produces its crustose, grayish thallus within the smooth bark of *Ilex europaeus*. ac, lirelliform ascomata; ph, coccoid green algal photobiont cells (*Trentepohlia* sp.). Fig. 3.13. Microfilamentous thallus of the tropical *Coenogonium subvirescens* (Gyalectales). The filamentous green-algal photobiont (*Trentepohlia* sp.) (ph) is ensheathed by mycobiont hyphae (my). Fig. 3.14. Cross section of *Usnea rubicunda* (Lecanorales), a radially organized, fruticose lichen with internally stratified thallus. c, conglutinate cortical layer; ph, photobiont cell population (*Trebouxia* sp.); m, gas-filled medullary layer; cst, conglutinate central strand.

lichens) or pendulous, radially organized structures (e.g. beard lichens; Fig. 3.14) analogous to the vegetative body of a variety of plants (e.g. *Ephedra*, rushes, many succulent plants in the Cactaceae, Stapeliaceae, etc.). In contrast to all other mutualistic symbioses of fungi and photoautotrophs, it is the fungal partner of these morphologically highly evolved lichens that secures adequate illumination and facilitates gas exchange of the photobiont (Honegger 1991*a*, 1992).

3.2 Specialized "lifestyles" of lichens and associated fungi

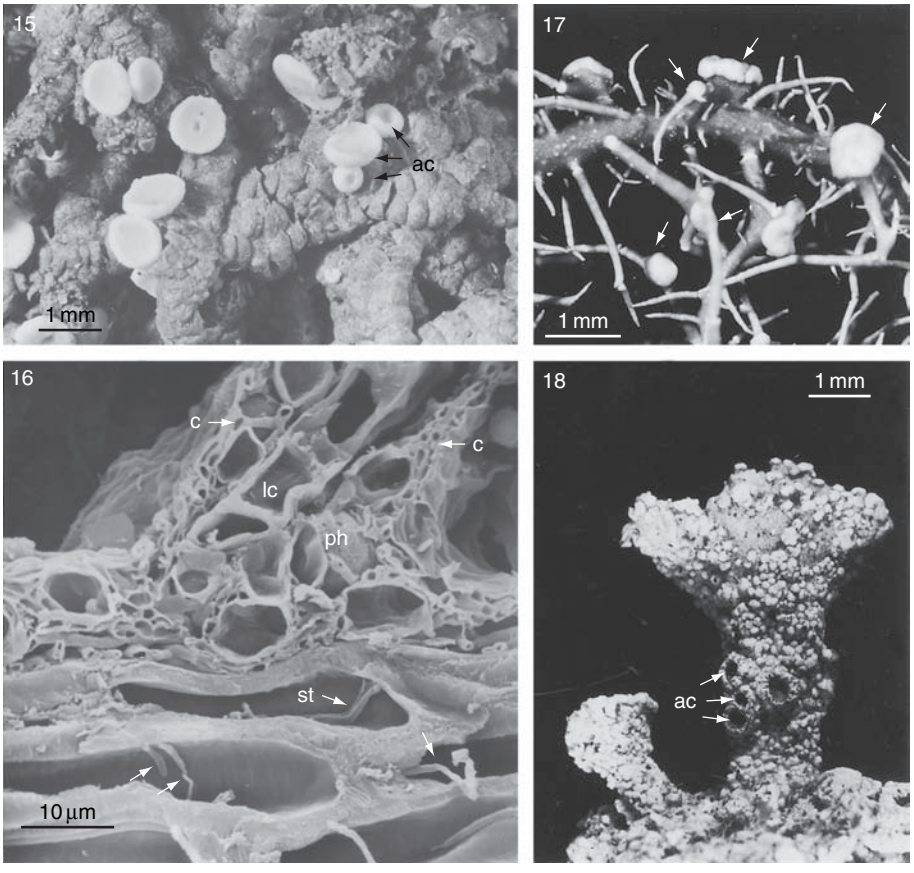
3.2.1 Parasitic lichens

Parasitic lichens are a group of obligately lichenized fungi that start their development on or within the thallus of another lichen species. Depending on the degree of colonization, the host thallus is either locally or completely overgrown and destroyed. Accordingly, parasitic lichens may either be confined to host thalli throughout their lifetime or outgrow their host and become independent. In the former case the species are readily recognized as parasites, but in the latter case, extensive observations on all developmental stages may be required before the species is recognized as a parasite.

Parasitic lichen-forming fungi acquire their photobiont either "by theft" from the host lichen or separately. In the latter case the compatible photobiont of the parasite is usually taxonomically not identical with the photoautotrophic partner of the host lichen (Poelt and Doppelbauer 1956; Hawksworth 1988*b*). A complicated mode of photobiont acquisition was observed in the crustose lichen *Diploschistes muscorum*. This parasitic lichen starts its development in the thallus squamules and podetia of *Cladonia* spp. (Fig. 3.18) that may be completely overgrown and destroyed. Juvenile *D. muscorum* associate with *Trebouxia irregularis*, the photobiont of the host lichen. However, large, independent thalli of *D. muscorum* were invariably found to have replaced *Trebouxia irregularis* by *T. showmanii* (Friedl 1987).

3.2.2 Bryophilous and foliicolous lichens

A large number of crustose lichens favor decaying bryophytes as a substrate (e.g. the *Buellietum olivaceobruneae* in the Antarctic; Kappen 1985), but a small, taxonomically heterogeneous group (approx. 40 species) is parasitic on live bryophytes that are overgrown or even intracellularly infected (Figs. 3.15, 3.16; Poelt 1985). Compatible photobionts are often found under the cuticle between leaf and stem cells (Fig. 3.16) or, in some combinations, even within leaf cells (Döbbeler and Poelt 1981).



Figs. 3.15–3.18 Examples of multiple symbioses. Figs. 3.15–3.16. *Dimerella lutea* (Gyalectales), a bryophilous lichen developing its inconspicuous crustose thallus between the cuticle (c) and leaf cells (lc) of the foliose liverwort *Frullania dilatata*. Leaf and stem cells of the hepatic are invaded by the lichen mycobiont (arrows). ac, ascomata (pale orange); ph, coccoid cells of the green-algal photobiont, a *Trentepohlia* sp.; st, central strand. Fig. 3.17. *Biatoropsis usnearum* (Tremellales), a cecidogenous lichenicolous fungus induces gall formations (arrows) on the fruticose thallus of *Usnea cornuta*. Fig. 3.18. The parasitic lichen *Diploschistes muscorum* starts its development in the thallus squamules and cup-shaped podetia of *Cladonia pyxidata* and acquires its green-algal photobiont (*Trebouxia irregularis*) by theft from the host lichen (for details see Friedl, 1987). ac, ascomata of the parasitic lichen.

In tropical and subtropical areas, the diverse, taxonomically heterogeneous group of foliicolous lichens (*c.* 600 species; Farkas and Sipman 1993, 1997) has attracted considerable interest in recent years. Their crustose, microfilamentous or squamulose thalli develop, usually quite unspecifically, on perennial leaves of a very wide range of vascular plants from sea level to montane areas,

with their greatest diversity being found in humid submontane rain forests (Gradstein and Lücking 1997; Lücking *et al.* 2003). Foliicolous lichens are bioindicators of microclimate (Lücking 1997). In one hectare of a submontane forest in Costa Rica *c.* 200 species of foliicolous lichens were detected (Gradstein and Lücking 1997). Because large numbers of economically important crops, such as many spice-producing shrubs and trees, *Camellia* (tea), various *Citrus* spp., *Coffea*, *Hevea* (rubber), *Theobroma* (cacao), etc., are colonized, these foliicolous lichens have attracted the interest of plant pathologists (Hawksworth 1988*c*). Most foliicolous lichen mycobionts are symbiotic with filamentous green algae of the genera *Cephaleuros* and *Phycopeltis* (both Trentepohliales, Ulvophyceae). Such algae occur abundantly in the aposymbiotic state on perennial leaves and are also considered as pests (Hawksworth 1988*c*). These potential photobionts grow either on the cuticle or below. Some of them even occur in the palisade parenchyma of the leaf. In association with lichen mycobionts, the growth rate of these foliicolous algae is reduced. Most epicuticular lichens probably use the leaf merely as a substrate and grow equally well on artificial substrates such as plastic tape or slides (Sipman 1994; Sanders 2001*a*; Sanders and Lücking 2002), but subcuticular ones are quite likely to benefit from nutrients of vascular plant origin.

3.2.3 Lichenicolous fungi and fungal endophytes of lichen thalli

A considerable number of fungi (approx. 1250 species in about 280 genera of ascomycetes and approx. 62 species in 10 genera of basidiomycetes; Hawksworth 1982, 1988*b*; Lawrey and Diederich 2003) gain their nutrition from lichens and a formal classification of relationships has been developed by Rambold and Triebel (1992). Beside these approx. 1300 lichenicolous species there are about 260 doubtfully and/or infrequently lichenicolous taxa. Some lichenicolous fungi are necrotrophic, i.e. have a devastating effect on either the lichen mycobiont (mycoparasites; Diederich 1996; de los Ríos and Grube 2000), or on the photobiont (algal parasites; Grube and Hafellner 1990) or on both (e.g. the widespread basidiomycete *Athelia arachnoidea*). Others sporulate abundantly without causing major damage to either the fungal or the photoautotrophic partner of the host thallus. In the literature the former group is referred to as parasites, the latter as "parasymbionts" or "commensalists." However, as very oligotrophic heterotrophs, these non-destructive inhabitants of lichens drain their nutrition from the thallus and thus are best regarded as mild parasites. Because experimental data are missing, it is often very difficult to interpret the biology of these multiple symbioses. With light and electron microscopic techniques it is often impossible to distinguish foreign hyphae within lichen thalli from the mycobiont proper. A few lichenicolous fungi are quite catholic