Chapter 4
Cyanobacteria and Algae of Biological Soil Crusts

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4.1 Introduction

Cyanobacteria are the oldest photoautotrophic component of biological soil crusts (biocrusts) known (see figure on front page). The oldest record of a fossil soil structure that may be interpreted as a biological soil crust is reported from as early as 2.6 billion years ago, and it presumably was composed of cyanobacteria

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(Watanabe et al. 2000; Beraldi-Campesi 2013, see also Chap. 3 by Beraldi-Campesi and Retallack). Fossil records suggest that eukaryotic algae first occurred on land 480–460 million years ago, while molecular clock estimates suggest an earlier colonization of about 600 million years ago. It is also hypothesized that this colonization of land by eukaryotes was facilitated by a partnership between a photosynthetic organism and a fungus (Heckman et al. 2001). Some of the early eukaryotic algae were able to form biocrusts (see Sect. 4.2.2). In this chapter, we will enumerate, as far as possible, cyanobacterial and eukaryotic algal species that are known so far to either form or occur in biocrusts. We will also discuss appropriate methods to assess their diversity and discuss ecological functions of the cyanobacterial and algal diversity.

4.2 Cyanobacterial and Eukaryotic Algal Diversity

4.2.1 Role and Diversity of Biocrust Cyanobacteria

From a functional point of view, cyanobacteria of biocrusts can be divided into three different groups: (1) Filamentous cyanobacteria, such as *Microcoleus*, that stabilize soils by gluing soil particles together and thus form soil aggregates due the presence of extracellular matrix (ECM; Figs. 4.1, 4.2, 4.3, and 4.4; see also Chap. 9 by Colesie et al. and Chap. 13 by Garcia-Pichel et al.). Those cyanobacteria are responsible for biocrust formation and are also the most abundant cyanobacteria species in the biocrusts. The formation of filaments in cyanobacteria is an essential feature that enables them to colonize physically unstable environments and to act as successful pioneers in the biostabilization process (Garcia-Pichel and Wojciechowski 2009). Due to the fact that the ECM remains over many years after the trichomes have either moved out of their sheath envelopes or died, the soil-stabilizing effect remains. (2) Cyanobacteria that prefer to live in the biocrust environment, enhancing the ecological role of biocrusts, e.g., through their contribution to C- and N-cycling. Examples are the unicellular *Chroococcidiopsis* (Fig. 4.5), the filamentous *Scytonema* (Fig. 4.4) and *Stigonema* (Figs. 4.6 and 4.7). (3) Cyanobacteria that only stochastically occur in biocrusts and may originate from other habitats, such as the aquatic environment or lichen symbiosis (e.g., *Chroococcus* Fig. 4.8, *Gleocapsa*, *Gloeocapsopsis*, *Cylindrospermum*, many *Phormidium* species, *Tolypothrix* Fig. 4.8).

In the first volume of the Ecological Studies series dealing with biocrusts (Belnap and Lange 2003), Büdel (2003) was not able to compare cyanobacteria at the species level at the scale of continents and subcontinents because only 35 cyanobacterial genera from biocrusts were known at that time. Since then, knowledge has increased considerably, and now more than 320 species in over 70 genera are known to occur in biocrusts worldwide. Many studies have included the cyanobacterial species diversity of biocrusts, identified either by morphological

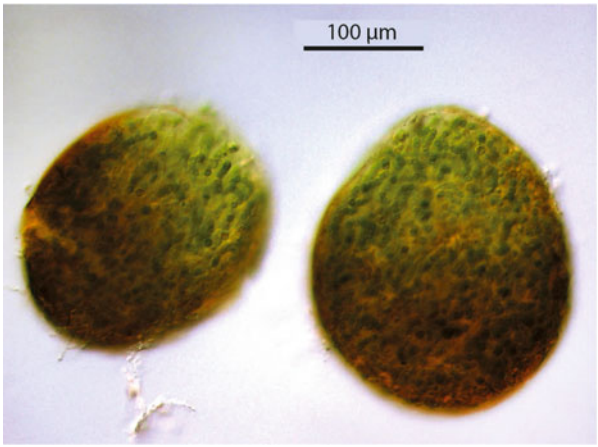

Fig. 4.1 *Nostoc microscopicum*, biocrust of Southern Tunisia

Fig. 4.2 *Nostoc commune*, trichomes inside a young colony, Negev Desert, Israel

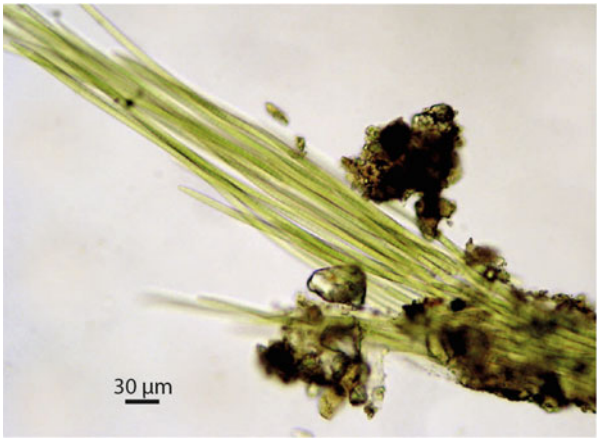

Fig. 4.3 *Microcoleus vaginatus*, biocrust of the Negev Desert, Israel

Fig. 4.4 *Scytonema* cf. *ocellatum*, biocrust of Israel

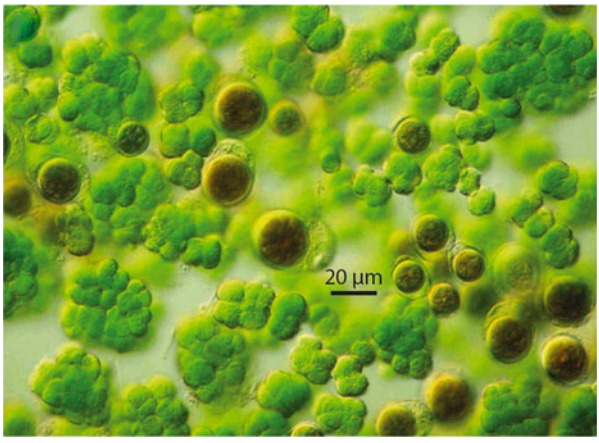

Fig. 4.5 *Chroococcidiopsis* sp., biocrust of Western Cape region, South Africa

(e.g., Langhans et al. 2009; Lewis and Flechtner 2002; Deb et al. 2013) or molecular methods (e.g., Gundlapally and Garcia-Pichel 2006) alone or, ideally, by both methods combined using the so-called "polyphasic approach" (e.g., Dojani et al. 2014). Of the 320 cyanobacteria species reported for biocrusts so far, only about 80 have been reported from at least two of the seven geographical regions distinguished here, while the majority (235 species) are listed only once (see Table 4.1 in the supplementary online material at http://extras.springer.com/2016/978-3-319-30212-6). These numbers also include the hypolithic cyanobacterial diversity (see also Chap. 11 by S. Pointing et al.). On a continental scale, biocrust cyanobacteria are relatively well investigated with no major gaps (Fig. 4.9a). However, comparing the diversity of different continents, it immediately becomes obvious that the second smallest continent, Europe, has the highest species number, even though Asia and Africa are the two largest continents with considerably larger

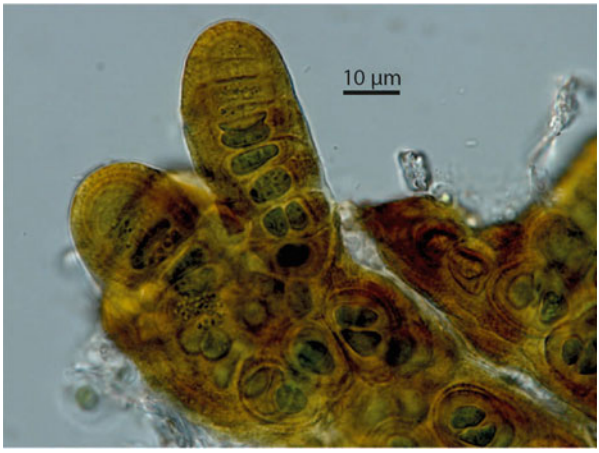

Fig. 4.6 *Stigonema turfaceum*, biocrust of the high Arctic tundra, Canada

Fig. 4.7 Moss associated *Stigonema* cf. *hormoides*, Antarctic biocrust

amounts of arid and semiarid landscapes (Fig. 4.9b). This certainly does not reflect true biogeography but is rather an effect of the number of group-specialized scientists and the time they have worked on a certain continent. It is also obvious that there is a need for much more biodiversity assessments of biocrusts in the Americas. Knowledge about the actual distribution of cyanobacterial species and their abundance is of a great importance for understanding recent and ancient environmental dynamics. Cyanobacterial diversity influences ecosystem processes by changing the environment on a micro- and macroscale. Also the cyanobacterial diversity in biocrusts, as well as in any other environments, is regulated by many factors, including anthropogenic activities across temporal and spatial scales.

The two filamentous species, the heterocyte-bearing *Nostoc commune* (Fig. 4.2) and *Microcoleus vaginatus* (Fig. 4.3), have been reported for biocrusts of all continents. These may be the core ecosystem "engineers," forming the early

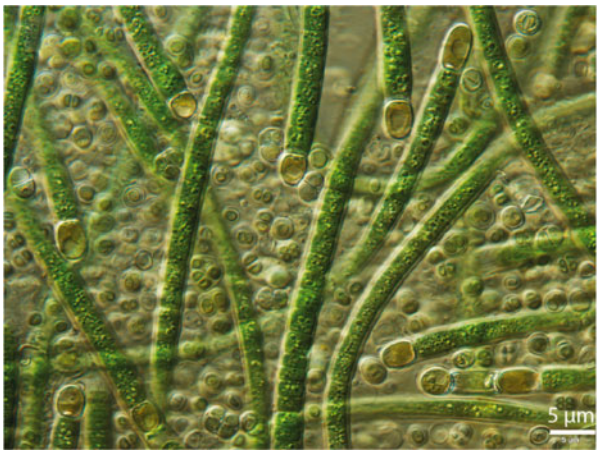

Fig. 4.8 *Tolypothrix* sp. and *Chroococcus* sp., biocrust from loess sediment, Serbia

biocrusts and contributing the initial soil carbon and nitrogen inputs. Another two filamentous species found in biocrusts of all continents (except Antarctica) are *Coleofasciculus chthonoplastes* and *Trichocoleus sociatus*; both were formerly classified in the genus *Microcoleus*. Also the unicellular genus *Chroococcidiopsis* has been reported from biocrusts of all continents except Europe. Another three species are reported from biocrusts of five continents, i.e., *Nostoc microscopicum* (Fig. 4.1), *Schizothrix calcicola*, and *Scytonema myochrous*. The first two of them do not occur in the harsh climate of Antarctica. Ten species have been reported from biocrusts of four continents, i.e., *Aphanothece saxixola*, *Aphanothece muscicola*, *Calothrix parietina*, *Hassallia bysoidea*, *Microcoleus paludosus*, *Nostoc muscorum*, *Nostoc punctiforme*, *Scytonema hofmani*, and *Stigonema ocellatum* (Fig. 4.4). Twenty-four species have been reported from three continents and 48 species from two continents. A list of all biocrust cyanobacteria species worldwide, compiled from the literature, can be found in Table 4.1 of the online supplement of this book, which can be downloaded from http://extras.springer.com/2016/978-3-319-30212-6.

4.2.2 Role and Diversity of Eukaryotic Algae in Biocrusts

Based on their presumed role in biocrusts, the eukaryotic algae associated with biocrusts may be distinguished into four functional groups. (1) Crust-forming algae which may actively support the formation of crusts by entrapping soil particles due to their filamentous nature and/or secretion of mucilage, e.g., *Klebsormidium* (Fig. 4.10) and *Zygogonium*. Crust-forming algae occur in lower diversity, but may produce relatively high biomass. (2) Algae which are attached to soil particles and to the crust-forming algae. They are highly diverse and occur mostly in low abundances (Büdel et al. 2009), e.g., *Spongiochloris* (Fig. 4.11), *Neochlorosarcina*

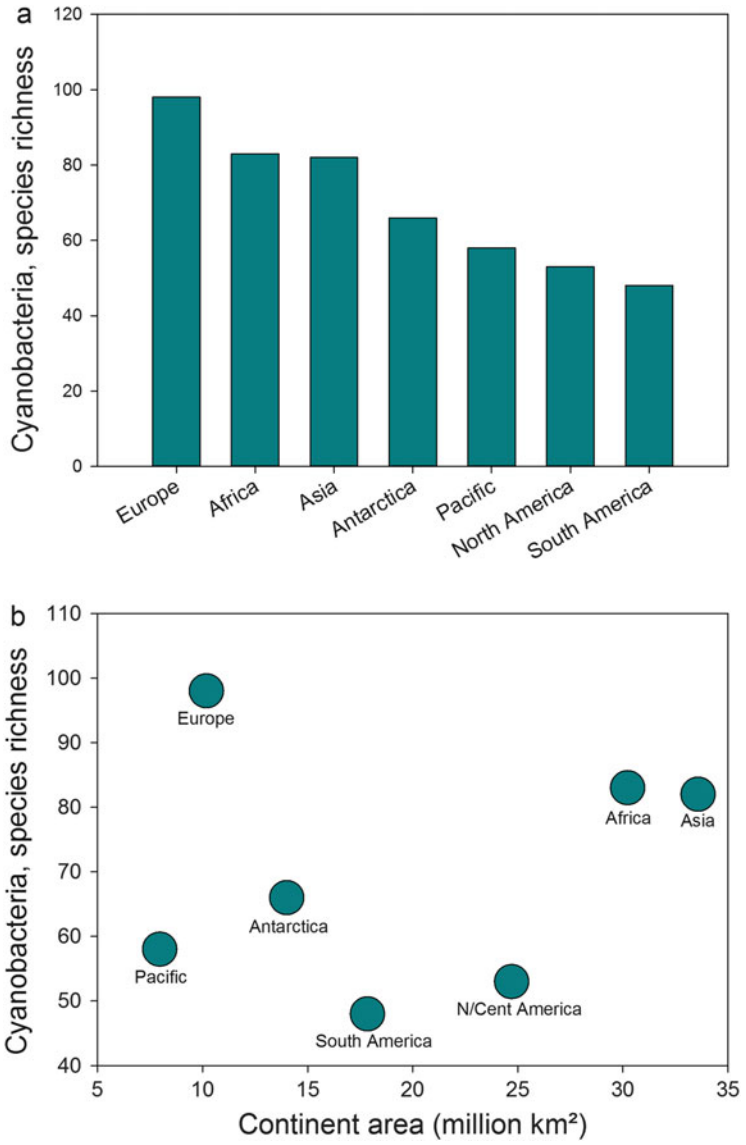

Fig. 4.9 Cyanobacterial species richness on a continental scale (**a**) and related to continental size (**b**); the region "Pacific" includes Australia and New Zealand

(Fig. 4.12), and most diatoms (Figs. 4.13, 4.14 and 4.15). (3) A smaller group of green algae that occurs within lichens as symbionts (photobionts), free living within the biocrusts, and/or living epiphytically on lichens, e.g., *Myrmecia* (Fig. 4.16) and *Stichococcus* (Fig. 4.17) (4) Freshwater algae which originate from aquatic habitats, but may occur in the soil, as it can be a "wet" habitat with many aqueous niches,

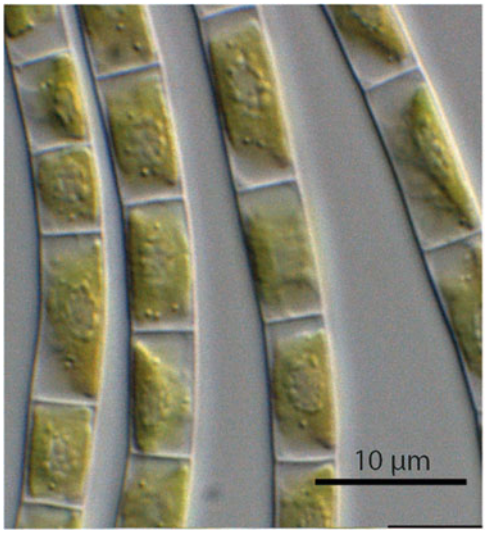

Fig. 4.10 *Klebsormidium flaccidum* (Kützing) P.C. Silva, K.R. Mattox & W.H. Blackwell

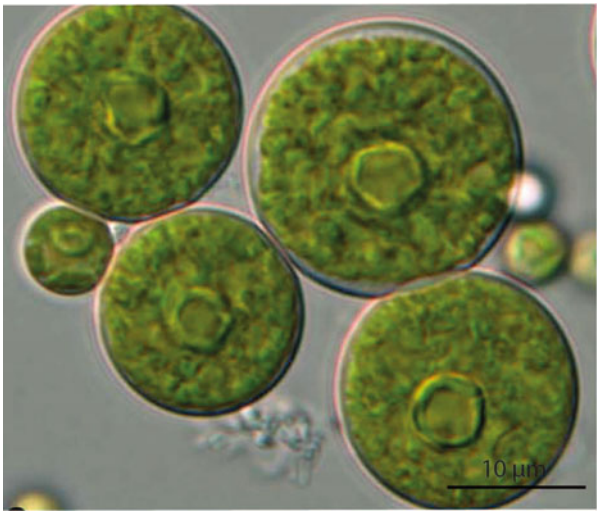

Fig. 4.11 *Spongiochloris minor* Chantanachat & H.C. Bold

e.g., *Chlorococcum*, *Chlamydomonas* s.l., *Scenedesmus* s.l. and *Mychonastes* (Fig. 4.18). In biocrusts as well as desert soils, these algae may often be associated with bryophytes because of their higher water content, or they may be present as dormant resting stages.

There are no eukaryotic algae exclusively found in biocrusts; rather, they represent various algal lineages with different levels of ecological specialization. Eukaryotic algae are probably the least studied phototrophic component of biocrusts. The reasons may be that eukaryotic algae are rarely crust-forming species and they exhibit simple morphologies with distinguishing features only expressing

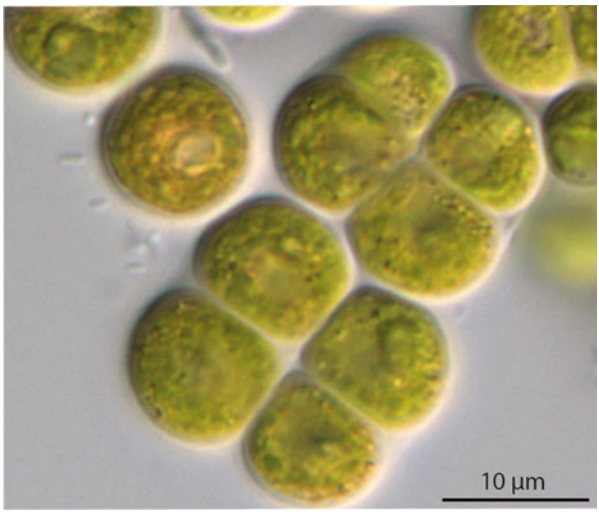

Fig. 4.12 *Neochlorosarcina negevensis* (Friedmann & Ocampo-Paus) S. Watanabe

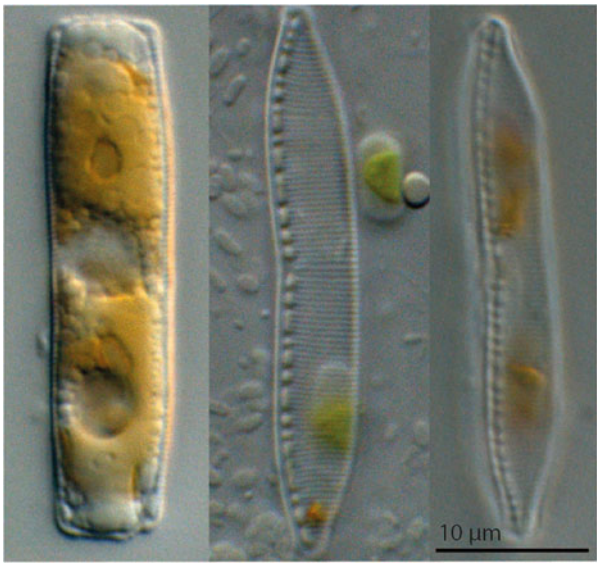

Fig. 4.13 *Hantzschia amphioxys* (Ehrenberg) Grunow

in unialgal cultures. Using direct microscopy, algal forms other than the filamentous ones are hard to detect because they occur in low abundance or may be present as dormant resting stages, particularly in dry biocrusts. Also, early studies on eukaryotic biocrust algae were based on direct microscopy and, as they encountered many resting stages, may have recorded low algal diversity. Other sources of uncertainty in assessing the eukaryotic algal diversity of biocrusts may come from the fact that soil phycologists often do not pay attention whether the algae were found in "bare soil" or within biocrusts. In addition, molecular methods have

Fig. 4.14 *Luticola mutica* (Kützing) D.G. Mann

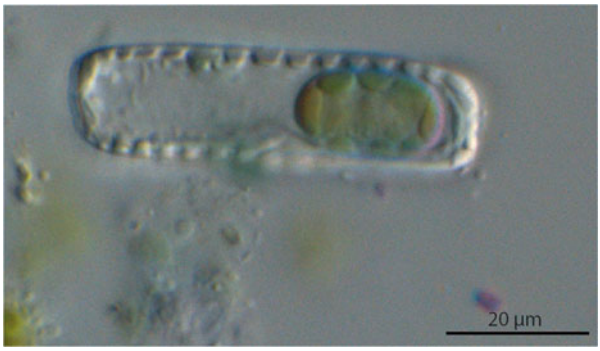

Fig. 4.15 *Pinnularia borealis* Ehrenberg

shown that many morpho-species and genera of soil algae are actually of different phylogenetic entities (species and genera), which makes their correct identification using microscopy even more difficult (e.g., Fucíková et al. 2011; Fucíková and Lewis 2012a, b).

The list of species (Table 4.2 in the supplementary online material at http://extras.springer.com/2016/978-3-319-30212-6) represents the most current compilation of the literature on eukaryotic algae detected within, or clearly associated with, biocrusts. An overview of the eukaryotic algal diversity reported in this compilation is shown in Figs. 4.19 and 4.20. Eukaryotic algae that are reported from biocrusts were either green algae from both phyla, the Chlorophyta and Streptophyta, or members of one of three lineages of stramenopiles, i.e., the diatoms (Bacillariophyceae), xanthophytes (Xanthophyceae), or eustigmatophytes (Eustigmatophyceae). The identifications used in those studies have almost exclusively been based on morphological criteria observed by microscopy and from material in unialgal cultures. The geographical distribution of eukaryotic biocrust algae is only poorly understood, and there is still an ongoing debate whether microalgae exhibit biogeography or not. Due to their small size and desiccation resistance as well as other harsh environmental conditions, most terrestrial eukaryotic algae may

Fig. 4.16 *Myrmecia bisecta* Reisigl

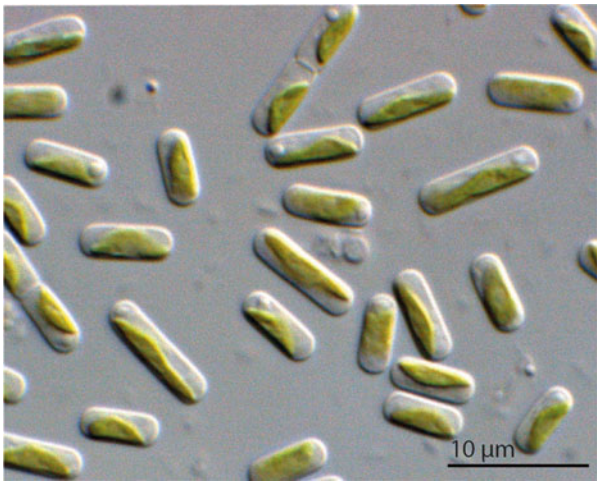

Fig. 4.17 *Stichococcus bacillaris* Nägeli

be easily distributed, e.g., by wind currents, and therefore one may anticipate an ubiquitous distribution for most species. In addition, when compared on a continental scale, the diversity of biocrust-associated eukaryotic algae as compiled from the literature is erratic (Figs. 4.19a, b and 4.20). This is probably reflecting the amount of work spent on samples from a certain continent rather than actual numbers of species diversity and geographic distribution. For example, the eukaryotic biocrust algal

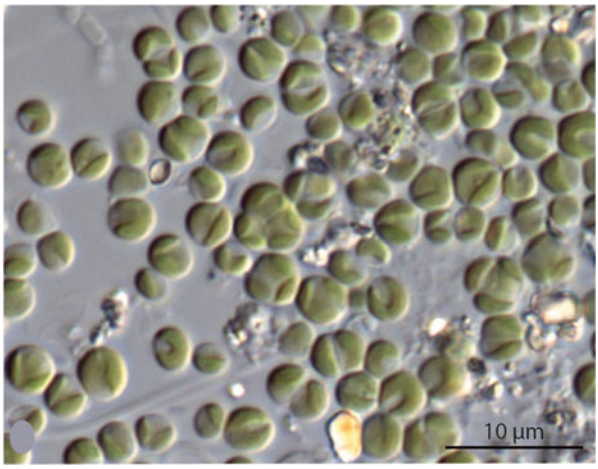

Fig. 4.18 *Mychonastes homosphaera* (Skuja) Kalina & Puncochárová

diversity appears much higher in Antarctica than in the Pacific region (Australia and New Zealand) or South America, as the latter two regions have been clearly neglected so far and require more attention (Fig. 4.19a).

Based on current compilations of literature, i.e., Table 4.2 in the supplementary online material (http://extras.springer.com/2016/978-3-319-30212-6) the filamentous species of *Klebsormidium* (Klebsormidiophyceae, Fig. 4.10) and *Zygogonium* (Zygnematophyceae), both from the Streptophyta, are most commonly encountered in biocrusts, especially in sandy soils. The unicellular zygnematophyte *Cylindrocystis* (Fig. 4.21) is the most common unicellular streptophyte green alga (Zygnematophyceae) in biocrusts, probably contributing to crust formation as it forms mucilage. Less frequent is the unicellular genus *Interfilum* (Fig. 4.22).

Biocrust Chlorophyta belong to three classes, the Chlorophyceae, Trebouxiophyceae, and Ulvophyceae; the systematics of the green algae as presented in this chapter follows Friedl and Rybalka (2012). Most biocrust green algae may not actively support biocrust formation but are associated with biocrust components in various ways (e.g., lichen photobionts).

The largest group of biocrust green algae are the Chlorophyceae (21 species, see Table 4.2 in supplementary online material at http://extras.springer.com/2016/978-3-319-30212-6). They are recorded from two or more geographic regions. All biocrust Chlorophyceae are unicellular but phylogenetically diverse within the class. They belong to several distinct phylogenetic lineages of the class, the orders Volvocales (syn. Chlamydomonadales) and Sphaeropleales (also called the "DO group") and are even further distributed on various lineages within these orders. Several biocrust Volvocales form cell packages and may produce mucilage, thus somehow supporting crust formation, e.g., *Chlorosarcinopsis*, *Tetracystis*, *Neochlorosarcina* (Fig. 4.12), and *Borodinellopsis*. Other genera are either inconspicuous tiny coccoids (e.g., *Mychonastes*, Fig. 4.18) or form large unicells, e.g., *Macrochloris* and *Spongiochloris* (Fig. 4.11). Some coccoid members of Volvocales easily form flagellated stages (e.g., *Chlorococcum*); other Volvocales

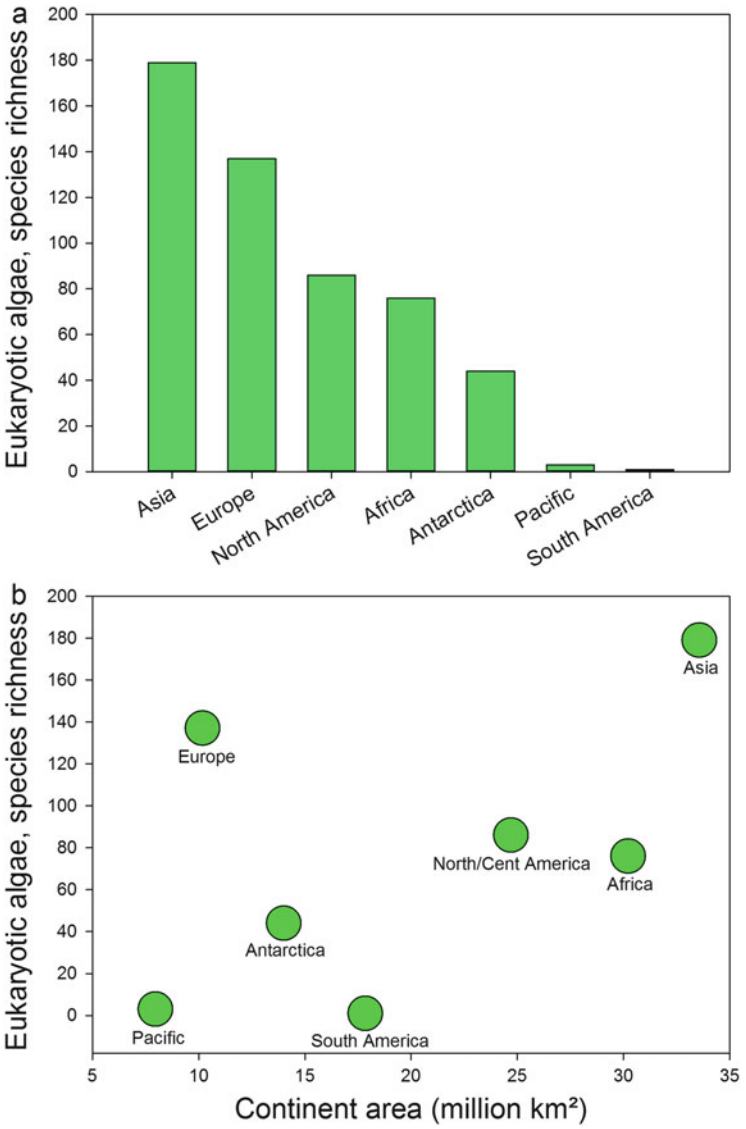

Fig. 4.19 Eukaryotic algal species richness on a continental scale (a) and related to continental size (b); the region "Pacific" includes Australia and New Zealand

are flagellates in their vegetative stages, e.g., *Chlamydomonas* and *Chloromonas*. The latter occur in immotile stages and are drought resistant by mucilage formation. Just a few members (five species) of the chlorophycean order Sphaeropleales, are common in biocrusts and are widely distributed. Unicellular coccoid *Bracteacoccus* species (Fig. 4.23) are most common in biocrusts, as well as representatives of the

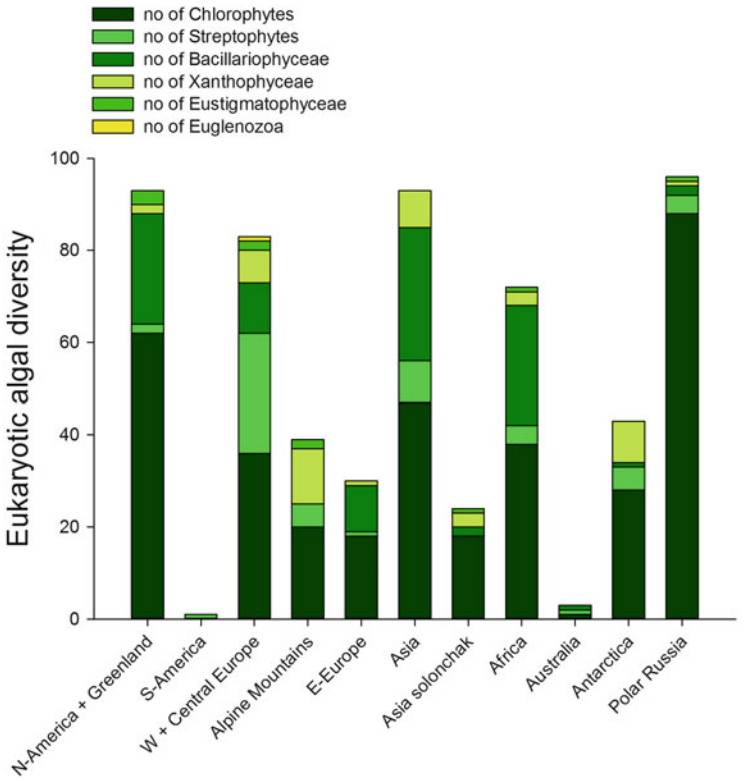

Fig. 4.20 Eukaryotic algal diversity related to class and higher taxonomic ranks and larger eco-regions

family of colony forming Scenedesmaceae, i.e., species of *Acutodesmus*, *Coelastrella*, and *Scenedesmus*.

Members of the Trebouxiophyceae form the second largest group of the Chlorophyta, i.e., 15 genera are reported and are widely distributed. Species of *Chloroidium*, *Coccomyxa* (incl. *Pseudococcomyxa*), *Muriella*, *Myrmecia* (Figs. 4.16 and 4.24), and *Chlorella*-like algae, including the "true" *Chlorella*, *Chlorella vulgaris* (Fig. 4.25), and *Elliptochloris* (Fig. 4.26) were found in almost every type of soil crust and from all geographic regions. However, *Chlorella* in its traditional taxonomic circumscription is of multiple origins and in fact represents several genera, separated from *Chlorella* s.str. only recently. The second most encountered trebouxiophytes are those which form cell packages, i.e., *Apatococcus*, *Desmococcus*, and *Diplosphaera* (Figs. 4.27 and 4.28). The filamentous trebouxiophyte *Prasiola* forms green turf and therefore may also contribute to crust formation in regions with maritime climate. However, it has been reported only from the Alps and Antarctica. Species of *Asterochloris*, *Chloroidium*

Fig. 4.21 *Cylindrocystis brebissonii* (Ralfs) De Bary

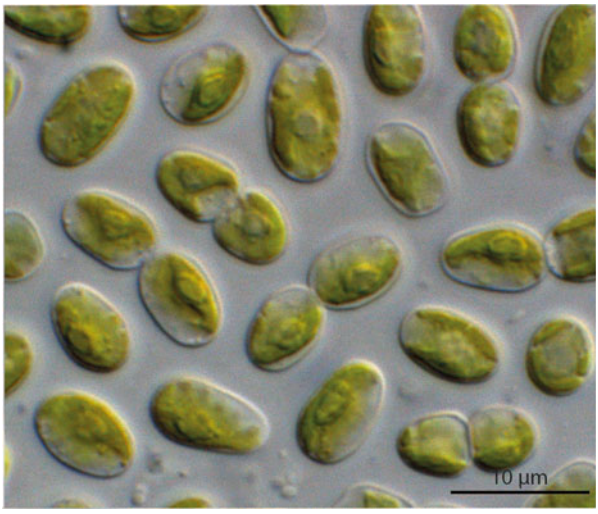

Fig. 4.22 *Interfilum terricola* (J.B.Petersen) Mikhailyuk, Sluiman, Massalski, Mudimu, Demchenko, Friedl, and Kondratyuk

(Fig. 4.29), and *Trebouxia* are frequently encountered as photobionts in lichens of biocrusts (Ruprecht et al. 2014).

The Ulvophyceae are the third green algal class commonly found in biocrusts, but just two inconspicuous unicellular or pseudofilamentous genera, *Planophila* and *Pseudendocloniopsis*, were reported from more than just a single geographic

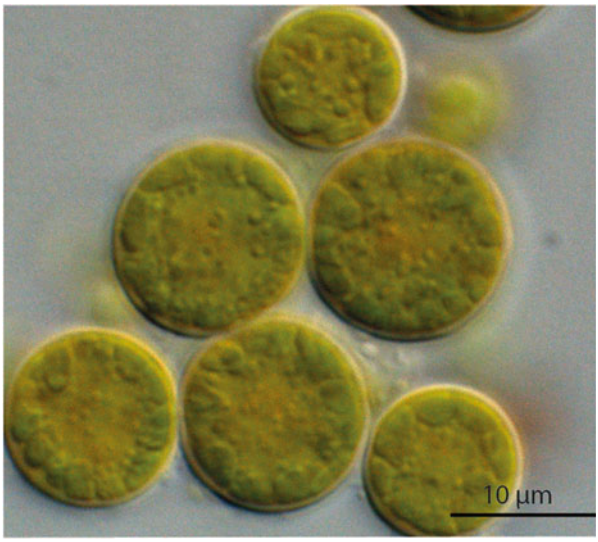

Fig. 4.23 *Bracteacoccus minor* (Chodat) Petrová

Fig. 4.24 *Myrmecia biatorellae* J.B. Petersen

region. The branched filamentous *Dilabifilum* is known as a crust-forming alga on different salty soils (solonchak or solonetz).

The second largest group of eukaryotic biocrust algae are the diatoms (e.g., Rumrich et al. 1989) with mostly pennate (elongated cells with bilateral symmetry) forms, i.e., the class Bacillariophyceae (Fig. 4.20). Inconspicuous species of the genera *Hantzschia* (Fig. 4.13), *Luticola* (Fig. 4.14), *Navicula* s.l. (incl. *Fistulifera*), *Nitzschia*, and *Pinnularia* (Fig. 4.15) were very common and may be found

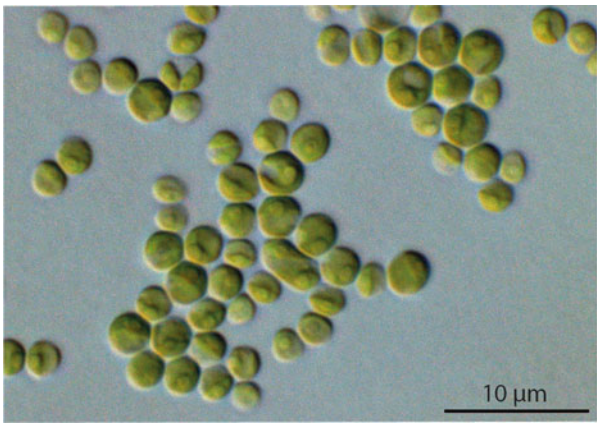

Fig. 4.25 *Chlorella vulgaris* Beyerinck

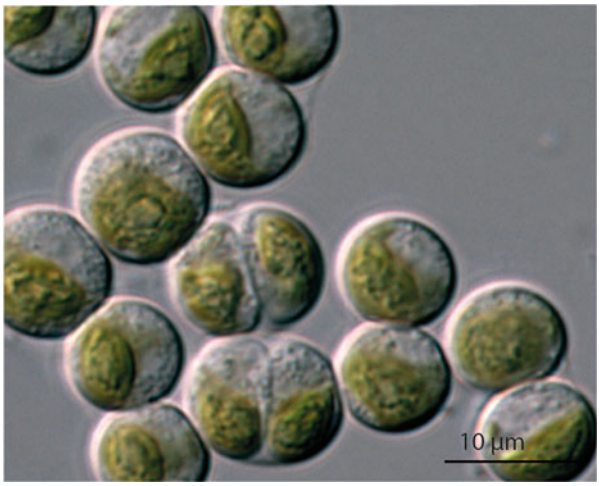

Fig. 4.26 *Elliptochloris subsphaerica* (Reisigl) Ettl & Gärtner

associated with almost every soil crust type. Two groups of Stramenopiles algae are common in terrestrial habitats and soils but may also be found in soil crusts.

Members of the class Xanthophyceae are probably restricted to cooler geographic regions with the unicellular coccoid species of *Botrydiopsis* being the most common, but also some filamentous (*Tribonema*, *Xanthonema*) or branched filamentous forms (*Heterococcus*) are frequently found (Fig. 4.20). Rarely also the coenocytic *Botrydium* has been recorded. Eustigmatophyceae have been reported from biocrusts, with only four unicellular genera recovered, i.e., *Ellipsoidion*, *Eustigmatos*, *Gloeobotrys*, and *Vischeria*.

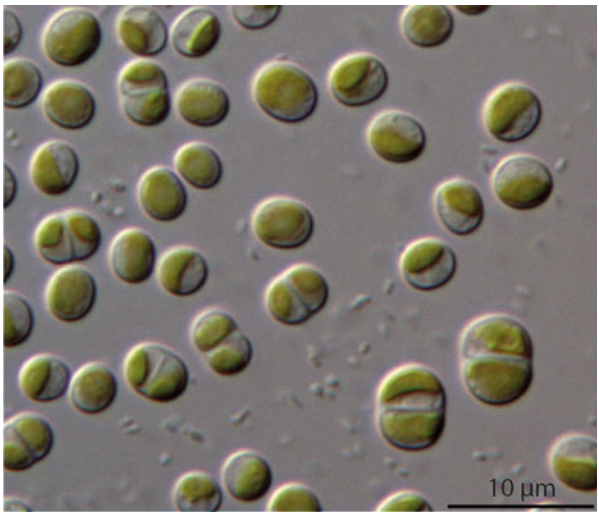

Fig. 4.27 *Diplosphaera chodatii* Bialosukniá

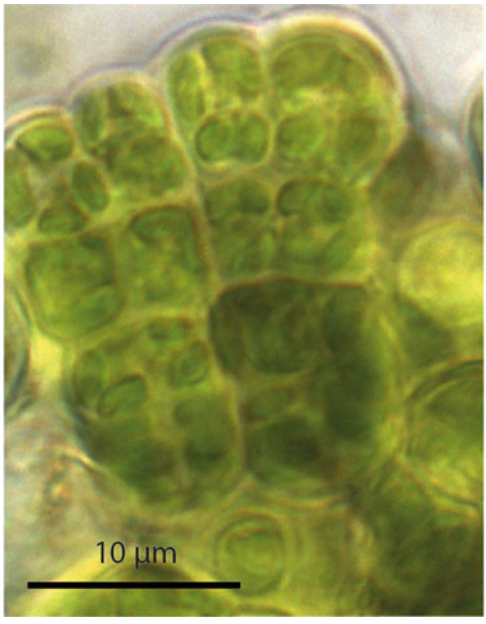

Fig. 4.28 *Desmococcus olivaceus* (Persoon ex Acharius) J.R. Laundon

Fig. 4.29 *Chloroidium ellipsoideum* (Gerneck) Darienko, Gustavs, Mudimu, Menendez, Schumann, Karsten, Friedl, and Pröschold

4.3 Methodological Aspects

4.3.1 Sampling

For any assessment of the biodiversity of cyanobacteria and eukaryotic algae in biocrusts, the samples should be as fresh as possible. An appropriate method which leaves the crust mostly undisturbed and yields sufficient material at the same time has recently been described by Büdel et al. (2009). A lower lid of a 10-cm petri dish is lined with several layers of cellulose paper and pressed into the upper 2 cm of the soil crust after which a trowel is pushed below the lid, lifted together with the sample from the surrounding soil, and turned around to carefully remove surplus soil from the sample. For optimal biodiversity assessment, a number of smaller samples [e.g., a sufficient number of samples (test with a saturation curve of species numbers) of 2 cm × 2 cm × 1 cm in size with visible soil crusts] should be pooled (e.g., Gollerbach and Shtina 1969; Kostikov et al. 2001; Novakovskaya and Patova 2013). Wet samples need to be carefully dried (e.g., on cellulose paper) before lab work in order to avoid fungal growth, but on the other hand, drying may hamper the growth of certain algal groups (e.g., Xanthophyceae).

4.3.2 Identification, Cultures, and Morphological Approach

For identification of biocrust cyanobacteria in the crust samples by microscopy, small amounts of crust material are soaked in water and separated into two subsamples. Using a dissecting microscope, the first subsample is transferred to a microscope slide and examined under a light microscope. The second subsample is pre-cultured under wet and low light conditions for 3–4 days followed by a light microscopy examination. This will allow growth of cyanobacteria so that they can

be better recognized and identified. In order to start cultures, a small amount of biocrust material soaked in water is checked by microscopy using sterile slides and then transferred to both liquid and agarized media, e.g., BG-11 medium (Waterbury and Stanier 1978), which are known to be well suited for cyanobacteria. Subsequently, they are kept at a temperature of about 20 °C under low light (50–-150 µmol photons m^{-2} s^{-1} photosynthetic active radiation) with a light-dark regime of 12:12 h. As soon as colonies are visible, they are checked by microscopy and then purified by transferring them several times onto fresh media. For developing cultures of biocrust cyanobacteria, many of the methods described below for eukaryotic algae can also be successfully employed. For identification of cyanobacteria using morphological criteria, the three volumes of the "Cyanoprokaryota" are recommended, i.e., Komárek and Anagnostidis (1998), Komárek and Anagnostidis (2005), and Komárek (2013).

For eukaryotic algae, direct microscopy of freshly collected soil samples can be useful for an initial survey of algal diversity. However, many eukaryotic algae in the crusts are present in untypical stages (e.g., resting cells), and feature characteristic for genus and species identification may only develop in culture. Therefore, a thorough microscopic investigation of eukaryotic biocrust algae involves several steps following microscopy of the sample, such as developing raw cultures using "wet chambers," agarized enrichment cultures, and, finally, unialgal cultures (Gollerbach and Shtina 1969). The methods do not differ from those used for soil algae except that the biocrust samples need mechanical disruption before starting cultures so that the developing colonies can be better separated from each other. Many studies have used this procedure to determine the biodiversity of soil, e.g., Novichkova-Ivanova (1980), Vinogradova and Darienko (2008), Kostikov et al. (2001). However, employing cultures generally has the risk that those species which may be hard to culture using standard procedures (and they could even be dominant) may be left undiscovered, whereas those species not actively participating in the crust communities (e.g., present just as dormant stages) may be amplified simply because they grow well in culture.

To establish cultures of eukaryotic biocrust algae, some authors use only agarized enrichment cultures (e.g., Peer et al. 2010; Hoppert et al. 2004) or even just direct microscopy of the samples (Büdel et al. 1994; Colesie et al. 2014; Kanda et al. 2002), but mostly a combination of both are applied (e.g., Flechtner et al 1998; Broady 1986; Broady and Weinstein 1998). Raw cultures using distilled water for wetting "wet chambers," introduced by Fritsch and John (1942) for the observation of soil diatoms, facilitate the observation of biocrust algae and induce their growth without selecting certain algae too much. It is important to consider that the nutrient composition of culture media introduces a considerable bias, as certain nutrient compositions will accelerate growth of certain species while inhibiting others. The biocrust is placed in a sterilized petri dish at air humidity up to 80 %. After 2–3 weeks of exposure to a light-dark 12:12 h regime, biofilms will appear on the surface of the biocrust sample to which several cover slips are slightly pressed. The sample surface should be uneven to leave space between the biofilm and the glass cover slip—this will form small chambers in which favorable microclimatic

conditions for algal growth on the cover slip surfaces will occur. The cover slips can be used for microscopy after some time (2–3 weeks) of incubation on the sample surfaces and even allow observation of a succession of algae if the cover slips are used for microscopy after consecutive time intervals. Cover slips can also be put into liquid or on the surface of agarized culture media to start cultures of the algae from these surfaces. Alternatively, small fragments of the biocrust are placed on agarized culture media which should not be too rich in nutrients and devoid of vitamins or any organic compounds to avoid the growth of fungi and bacteria. After incubation for 2–3 weeks, the first small colonies, often formed by a variety of different algae, can already be used for an initial estimate of the algal diversity by microscopy and also for establishing unialgal cultures after several purification steps.

For liquid cultures, fragments of the biocrust are placed in liquid media, e.g., 1–2 g of a crust sample is added to a 50–80 ml sterile medium in 100–150 ml Erlenmeyer flasks. One disadvantage of this method is that algae (e.g., resting stages of freshwater algae), which were in an inactive state in the biocrust and do not belong to the active algal community of the biocrust, can then easily develop in those cultures.

4.3.3 Molecular Approaches

Molecular markers may be most promising for assessing biocrust cyanobacteria and eukaryotic algal biodiversity. For cyanobacteria, it was revealed that due to their high physiological plasticity, which allows for their rapid adaptation to various environmental conditions, new morpho- and ecotypes may be developed within short time. This makes the discrimination of species using morphological and ecophysiological characters rather difficult and calls for molecular studies to define species. DNA sequences allow an unambiguous characterization and the comparison with reference sequences (as available from public databases), providing reliable estimates of genetic distances to infer the phylogenetic positions of algae and cyanobacteria. The first cyanobacteria 16S (SSU) rRNA gene sequences have already been determined almost 30 years ago (Giovannoni et al. 1988), followed by corresponding sequences (18S rRNA) for eukaryotic algae, and since then their numbers have ever been increasing up to several thousands in publicly available databases (e.g., NCBI). Due to the large number of available reference sequences, the SSU rRNA genes became the "golden standard," i.e., the molecular marker of choice for cyanobacteria as well as eukaryotic algae. There are many examples for studies on biocrust cyanobacteria which used 16S rRNA as molecular signatures (e.g., Abed et al. 2010; Zaady et al. 2010; Li et al. 2013; Dojani et al. 2014; Patzelt et al. 2014). However, SSU rRNA alone may not provide sufficient resolution for unambiguous distinction of species, and additional more variable markers (e.g., ITS rDNA) are required. For cyanobacteria, the 16S–23S spacer (ITS) has successfully been used, also because its secondary structure models bear valuable phylogenetic

information for species distinction (e.g., Boyer et al. 2002; Reháková et al. 2007; Siegesmund et al. 2008; Johansen et al. 2011). For eukaryotic algae, i.e., photobionts of lichens associated with soil crusts, the nuclear-encoded ITS rDNA in combination with the chloroplast-encoded intergenic spacer psbJ-L has recently been employed for species identification (Ruprecht et al. 2014).

There are two principal ways to apply molecular methods for assessing biocrust algal and cyanobacterial diversity: sequencing of cultured strains isolated from biocrusts and using DNA extracts directly from the biocrusts without culturing (culture-independent approach). For the latter approach, DNA is extracted directly from the fresh biocrust sample followed by PCR amplification, which will result in a mixture of PCR products from the various organisms present in the biocrust sample. They need to be separated by cloning before sequencing, and in order to assess the algal/cyanobacterial diversity most accurately, a larger number of clones need to be sequenced. The culture-independent approach requires PCR primers that selectively amplify certain groups of target organisms, e.g., PCR primers which preferentially amplify cyanobacteria (e.g., Nübel et al. 1997) or green algae (e.g., Hallmann et al. 2013), to enrich the clone libraries with sequences from the target organisms. Several recent studies used cyanobacteria-specific PCR primers for biocrust and/or soil cyanobacteria (Li et al. 2013; Dojani et al. 2014; Patzelt et al. 2014), whereas others studied biocrust cyanobacteria as part of the whole bacterial community using universal bacterial 16S rRNA PCR primers (Gundlapally and Garcia-Pichel 2006; Abed et al. 2010; Zaady et al. 2010; Zhang et al. 2012). Recently, next-generation DNA sequencing (NGS, e.g., using a Roche 454 FLX instrument with Titanium reagents, Steven et al. 2013a; Elliott et al. 2014) provided the ability to determine and read millions of DNA sequences in parallel, making them ideally suited for large-scale biodiversity analyses of environmental biocrust samples. There are already several studies which have analyzed biocrust cyanobacterial diversity based on 16S rRNA gene amplicons obtained with universal bacterial primers (Steven et al. 2013a, b; Elliott et al. 2014; Maier et al. 2014). For eukaryotic biocrust algae, almost all molecular studies have been based on unialgal cultured isolates established from biocrust samples; the culture-independent approach has been used not employing 18S rRNA but other conserved markers as plastid-encoded 16S or 23S rRNA genes (Maestre et al. 2006; Lin and Wu 2014).

As an alternative to the culture-independent DNA sequencing approach for the analysis of microbial biocrust communities, DNA fingerprinting based on PCR amplification has been used, i.e., DGGE (Gundlapally and Garcia-Pichel 2006; Zaady et al. 2010; Zhang et al. 2012), ARISA (Abed et al. 2012) and t-RFLP (Redfield et al. 2002), but so far only for cyanobacteria. DGGE profiling may be appropriate because the characteristic DGGE-banding patterns can easily be compared among many samples. If unique or different patterns are identified, a DGGE band representing a still unidentified species can also be sequenced and identified using sequence comparisons after excised from the gel, but this will yield only rather short sequences (Lin and Wu 2014; Maestre et al. 2006).

For species identifications, the molecular approach is often supplemented by microscopy of the cultures. The latter is still essential for correct identification when no sequences of closer relatives are available in public gene sequence databases (Lewis and Flechtner 2002, 2004; Büdel et al. 2009; Rindi et al. 2011; Flechtner et al. 2013). In general, in the culture-independent DNA sequencing approach, the PCR amplification step is crucial to the diversity assessment. The DNA of the most abundant or any other species may be preferentially amplified and mask the DNA of other less abundant or easy to amplify species and leave the latter species undiscovered. Shorter gene regions may be better amplified compared to longer ones—the lengths of amplicons depend on the type of PCR primers used or species present in the biocrust sample. In addition, biocrust samples may exhibit PCR-inhibiting compounds. It follows that also the culture-independent approach presents various biases. Culture-independent and culture-based approaches may therefore result in different diversities. Consequently, a combination of both approaches is required to assess the algal biodiversity as accurately as possible. In a recent example, results from the culture-dependent and culture-independent approaches were compared, and this pointed out the necessity of employing both techniques because several taxa could be recovered only via one or the other approach (Patzelt et al. 2014; Dojani et al. 2014).

4.4 Conclusion

Biocrust inhabiting cyanobacteria and eukaryotic algae are highly diverse, but only few of them are in fact responsible for crust formation. Most cyanobacteria and eukaryotic algae simply use the biocrust habitat but may enhance the biocrust functions by their presence. The main crust-forming cyanobacteria are *Microcoleus*, *Nostoc*, *Scytonema*, and *Stigonema*. In contrast, biocrusts formed by eukaryotic algae, i.e., *Klebsormidium* and *Zygogonium*, are relatively rare. Cyanobacteria from biocrusts are better studied compared to the corresponding eukaryotic algae. Data about the distribution of cyanobacteria and eukaryotic algae are fragmentary and strongly biased towards the intensity of work extended to samples from a certain continent. Also, there is an ongoing debate whether the cyanobacteria and eukaryotic algae exhibit biogeography at all. For microscopic identification of cyanobacteria and eukaryotic algae, it is recommended to develop cultures, but this has the risk that isolating procedures and culture conditions may lead to a strongly biased biodiversity. Therefore, the culture-independent molecular approach is recommended but so far has only been applied a few times, mostly for cyanobacteria. In order to assess the cyanobacterial/algal biodiversity as accurately as possible, the culture-independent and culture-based approaches need to be combined.

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