Symbiotic Cyanobacteria in Lichens

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Abstract

Cyanolichens are obligate symbioses between fungi and cyanobacteria. They occur in many types of environments ranging from Arctic tundra and semi-deserts to tropical rainforests. Possibly even a majority of their global species diversity has not yet been described. Symbiotic cyanobacteria provide both photosynthate and fixed nitrogen to the fungal host and the relative importance of these functions differs in different cyanolichens. The cyanobiont can either be the sole photosynthetic partner or a secondary symbiont in addition to a primary green algal photobiont. In addition, the cyanolichen thallus may incorporate a plethora of other microorganisms. The fungal symbionts in cyanolichens are almost exclusively ascomycetes. *Nostoc* is by far the most commonly encountered cyanobacterial genus. While the cyanobacterial symbionts are presently not readily identifiable to species, molecular methods work well on the generic level and offer practical means for identifying symbiotic cyanobacterial genotypes. The present diversity of lichen cyanobionts may partly reflect the evolutionary effects of their lichen-symbiotic way of life and dispersal.

1. Introduction

Cyanobacteria are ancient monophyletic lineage of unicellular and multicellular prokaryotes that possess chlorophyll *a* and are capable of oxygenic photosynthesis. Prokaryotic fossils morphologically resembling modern cyanobacteria have been found from Archean deposits and it is generally believed that cyanobacterial photosynthesis raised oxygen levels in the atmosphere around 2.5–2.3 billion years ago, hence establishing the basis for the evolution of aerobic respiration. Recent findings suggest that the non-photosynthetic ancestors of cyanobacteria were anaerobic, motile and obligately fermentative (Di Rienzi *et al.*, 2014).

The first appearance of multicellular cyanobacteria may have coincided with the beginning of the rise of atmospheric oxygen and three clades, representing the majority of extant cyanobacteria, which seem to have evolved soon after (Schirrmeister *et al.*, 2011, 2012). Later, through endosymbiosis, some cyanobacteria evolved into the plastids of photoautotrophic eukaryotes about 900 million years ago (Deusch, 2008; Shih and Matzke, 2013), and the nitrogen fixation of diazotrophic cyanobacteria continues to play a crucial role in the nutrient cycles of many aquatic and terrestrial ecosystems (Zehr, 2011; Elbert *et al.*, 2012; Rousk *et al.*, 2013).

Thousands of species of lichen-forming fungi have cyanobacteria as photosynthetic symbionts and are therefore collectively referred to as "cyanolichens" (Rikkinen, 2002). Many of these, quite possibly even the majority, have not yet been described (e.g., Lumbsch *et al.*, 2011; Lücking *et al.*, 2014; Moncada *et al.*, 2014a, 2014b). Symbioses with cyanobacteria have been established repeatedly in different lineages of Fungi, and convergent evolution has often resulted in similar symbiotic morphologies in distantly related lineages (Muggia *et al.*, 2011). The morphological and physiological characteristics of lichen symbioses tend to be highly specialized, and involve extensive molecular crosstalk between the symbionts (Chua *et al.*, 2012; Junttila *et al.*, 2013; Kampa *et al.*, 2013; Wang *et al.*, 2014).

Within a cyanolichen thallus, the cyanobacterial symbiont or "cyanobiont" provides sugar (glucose) and/or fixed atmospheric nitrogen (as ammonium) to the fungal host or "mycobiont". The mycobiont provides its symbiotic partners water, carbon dioxide, and a relatively stable environment that is relatively well protected from environmental extremes and predation (e.g., Rikkinen, 1995, 2002).

2. Morphology and Functional Organization

On the basis of thallus structure, lichens have traditionally been divided into foliose, fruticose, and crustose species. This division is obviously artificial and convergent forms have evolved in many lichen lineages. Most cyanolichens are foliose, i.e., their growth is predominately horizontal, and the thallus is typically dorsiventral, flat, and lobate (Fig. 1a). Fruticose cyanolichens have upright, shrubby thalli with cylindrical lobes that are often attached to the substrate by a relatively narrow base (Fig. 1b). Crustose cyanolichens have relatively undifferentiated thalli and often grow tightly attached to the substrate (Fig. 1c).

Based on symbiont composition, cyanolichens can be divided into two artificial groups: bipartite and tripartite cyanolichens (Fig. 2a). In most bipartite lichens, the cyanobiont forms a more or less continuous layer immediately below the upper cortex of the thallus (Fig. 2b). The thalli of tripartite cyanolichens, on the other hand, house both green algal and cyanobacterial photobionts (Fig. 2c). In these symbioses, the cyanobacteria tend to represent a small proportion of total photobiont biomass and are usually restricted to special structures called cephalodia (Figs. 1b and 3a–3d). However, there are also some tripartite lichens in which the fungus has two "primary" photosynthetic photobionts both contributing substantially to the photosynthesis of the same thallus (e.g., Henskens et al., 2012). In addition, some green algal lichens establish ephemeral associations with neigh-boring cyanobacteria or cyanolichens, presumably in order to access a supply of fixed nitrogen (Poelt and Mayhofer, 1988; Rambold and Triebel, 1992). The mycobionts of some tripartite lichens can produce different thallus morphologies in symbiosis with compatible green algae and cyanobacteria, respectively. Chimeroid lichens with green algae and cyanobacteria as primary photobionts in different parts of the same thallus are called photosymbiodemes. The two differ-ent morphotypes may either combine into a compound thallus or live separate lives (James and Henssen, 1976; Brodo and Richardson, 1979; Tønsberg and Holtan-Hartwig, 1983; Goffinet and Bayer, 1997; Heiðmarsson et al., 1997; Paulsrud et al., 1998; Stenroos et al., 2003; Aptroot and Schumm, 2009; Magain et al., 2012; Moncada et al., 2013; Magain and Sérusiaux, 2014).

Lichen-symbiotic cyanobacteria can deliver both photosynthate and/or fixed nitrogen to their fungal partners. The relative importance of these two activities varies between bi- and tripartite lichens. The cyanobionts of bipartite lichens tend to show lower heterocyst frequencies and lower rates of nitrogen fixation than those of tripartite species. In tripartite cyanolichens, the cyanobionts

typically exhibit relatively high rates of nitrogen fixation, while the green algal photobiont typically delivers most of the photosynthate (e.g., Nash, 2008).

While lichens have traditionally been perceived as pairwise interactions between one fungal host and one or two photosynthetic symbionts, many of them actually involve several photobiont species and can also incorporate a plethora of other microorganisms. Recent studies have shown that lichens host diverse and specialized communities of non-phototrophic bacteria, which are likely to play important roles in the symbiosis (Grube *et al.*, 2009; Hodkinson and Lutzoni, 2009; Bates *et al.*, 2011; Hodkinson *et al.*, 2012; Sigurbjörnsdóttir *et al.*, 2014). Also the diversity of obligately lichenicolous fungi is remarkable, both on lichen surfaces and hidden within the inner layers of the thalli (Lawrey and Diederich, 2011; U'Ren *et al.*, 2012; Werth *et al.*, 2013).

Most cyanolichen symbioses are obligate in the sense that the fungal hosts cannot survive without their photosynthetic partners and the cyanobionts do not seem to commonly establish independent free-living populations outside lichen thalli. In many cases, the cyanobionts are vertically transmitted within specialized vegetative propagules and maintained through host generations, insuring a close and long-term symbiosis (e.g., Rikkinen, 2003).

3. Symbiont Diversity

Cyanobacteria occur abundantly in aquatic environments and almost in all types of terrestrial ecosystems. A small minority of them form symbiotic associations with eukaryotic organisms such as lichen-symbiotic fungi. Among the Fungi, cyanobacteria form symbioses almost exclusively with ascomycetes (Ascomycota), especially with Lecanoromycetes (Miadlikowska *et al.*, 2014) and Lichinomycetes (Schultz *et al.*, 2001). Comparable symbioses with other types of fungi are rare, with the prominent exceptions of *Dictyonema* (Fig. 4d) and related basidiomycetes (Dal-Forno *et al.*, 2013; Lücking *et al.*, 2014) and the glomeromycete *Geosiphon* (Kluge *et al.*, 2002). Only about 10% of presently known lichen-forming fungi associate with cyanobacteria. The others establish their symbioses with green algae (Chlorophyta), especially with trebouxiophytes (Škaloud and Peksa, 2010; Ruprecht *et al.*, 2012; Muggia *et al.*, 2013; Dal Grande *et al.*, 2014; Sadowska-Deś *et al.*, 2014) and species of Trentepohliales (López-Bautista, 2006; Rindi *et al.*, 2009), or more rarely with other types of eukaryotic algae (Thüs *et al.*, 2011).

At present, lichen-symbiotic cyanobacteria cannot usually be identified to clearly delimited species. This is partly due to fundamental problems in the species level taxonomy of cyanobacteria. From early 19th century to the late 20th century, many genera and species of "blue-green algae" were described on the basis of morphology and life-history traits (e.g., Geitler, 1932). While cyanobacteria are now known to belong to Eubacteria, cyanobacterial taxa can still be described following two sets of rules, i.e., those of the Botanical and the Bacteriological Code of Nomenclature, respectively (Oren, 2004, 2011; Oren and Tindall, 2005; Komárek, 2006, 2010a, 2011). At the genus level the standardized molecular method currently used for identifying cyanobacteria is 16S rRNA gene sequencing. While many traditional Geitlerian genera are confirmed, the species cannot usually be delimited on the basis of 16S rRNA sequences alone (Komárek, 2010b). An additional problem is that the phenotypic and genotypic features of many symbiotic cyanobacteria are drastically modified in symbiosis (e.g., Ran *et al.*, 2010).

While the species level taxonomy of symbiotic cyanobacteria remains a challenge, DNA methods now offer a practical way for accurately identifying cyanobacterial genotypes from both fresh lichen specimens (Rikkinen, 2013) and old herbarium material (Palinska *et al.*, 2006). As yet, the genotypic identity of the cyanobiont has only been determined from a minute fraction of all cyanolichen species. This refers not only to the cyanobionts of rare, inconspicuous, or cryptic taxa, but also to those of many common and widely distributed species.

Nostoc is by far the most commonly encountered genus of cyanobacteria in lichen symbioses (Rikkinen, 2013). Many *Nostoc* genotypes are symbiotic, either serving as a source of fixed carbon and nitrogen, as in cyanolichens (Figs. 4a and 4b), or solely as a source of nitrogen, as in plant symbioses. Also other nostocalean and stigonematalean genera, like the recently circumscribed *Rhizonema* (Lücking *et al.*, 2009, 2013; Dal-Forno, 2013), are known to include lichen cyanobionts (Figs. 4c and 4d). For example, *Calothrix*, *Dichothrix*, and *Stigonema* are thought to include lichen-symbiotic forms (e.g., Tschermak-Woess, 1988, Voytsekhovich *et al.*, 2011a, 2011b), but their occurrences remain to be confirmed by molecular methods (Komárek, 2010b). Lichenomycetes are believed to associate with *Gleocapsa*, *Anacystis*, and other genera distantly related to nostocalean and stigonematalean cyanobacteria (Tschermak-Woess, 1988; Voytsekhovich *et al.*, 2011a, 2011b). Also their identities remain to be confirmed and studied with molecular methods.

Many recent studies have firmly established that lichen-symbiontic *Nostoc* genotypes are closely related to plant symbiotic and free-living forms of the same genus (Rikkinen, 2013). They have shown that the mycobionts of cyanolichens tend to be highly selective with respect to their

cyanobionts (e.g., O'Brien *et al*., 2013; Miadlikowska, 2014b; Magain and Sérusiaux, 2014). In most cases only one or a few closely related cyanobacterial genotypes serve as the appropriate symbiotic partner for individual fungal taxa, while all bipartite cyanolichen species so far analyzed have had only one cyanobiont genotype within each thallus, some of them can house different cyanobionts in different thalli. Furthermore, some cyanobacterial genotypes are shared by several unrelated fungal species. This shared specificity may give rise to facilitative interactions between different cyanolichens. For example, the establishment of an ascospore-dispersed cyanolichen may be effectively facilitated by the prior establishment of another species that distributes the shared cyanobiont within its symbiotic propagules. In any case, the unequal availability of appropriate cyanobionts is likely to explain many interesting patterns of cyanolichen species occurrence (Rikkinen, 1995, 2003, 2013).

4. Ecological Perspectives

Cyanolichens occur in many types of terrestrial environments ranging from Arctic tundra and semi-deserts to tropical rainforests. Whereas green algal lichens can achieve positive net gas exchange when exposed to high atmospheric humidity alone, a corresponding effect in cyanolichens requires direct exposure to liquid water. Their diversity tends to be highest in humid and relatively cool climates characteristic of many tropical mountains (Fig. 5a) and maritime regions of higher latitudes (Fig. 5b). In the temperate zone, many epiphytic species are restricted to old growth forests and have been adversely affected by logging as well as acid precipitation and human-induced changes in air quality. Under favorable conditions, cyanolichens contribute significantly to epiphyte biomass and the nitrogen cycle of the ecosystem (e.g., Elbert *et al*., 2012). Recently, Hodkinson *et al*. (2014) demonstrated the presence and transcription of genes encoding enzymes for two different nitrogen fixation pathways (molybdenum- and vanadium dependent systems) in lichen-associated *Nostoc*. Such flexibility may be very significant for lichens, which often grow on nutrient-poor substrates and get most of their mineral nutrition through aerial deposition (Fig. 5e). Some cyanolichens are important components of biological soil crusts in savannas, semi-deserts, deserts, and disturbed sites, where they help to stabilize the soil and contribute to its fertility (Pietrasiak *et al*., 2013; Büdel *et al*., 2014). In some regions their diversity is seriously threatened by overgrazing and climate change.

One emerging theme in cyanolichens is the possible role of toxic cyanobacterial metabolites in the ecology of cyanolichens. Recent studies have demonstrated that some lichen-symbiotic cyanobacteria produce significant amounts of microcystin and other toxic peptides *in situ* (Oksanen *et al*., 2004; Kaasalainen *et al*., 2009, 2012, 2013). Microcystins are more familiar from cyanobacterial blooms in aquatic ecosystems, where they have caused animal poisonings around the world. In terrestrial ecosystems, cyanolichens represent a potential source of hepatotoxins for grazers. Many molluscs and arthropods, but also mammals feed on lichens (Fig. 5d) and mollusc grazing has been identified as the limiting ecological factor for cyanolichens in some humid forest ecosystems (e.g., Gauslaa, 2008). Many lichen-forming fungi produce toxic secondary substances as a defense against herbivores. It is possible that the cyanobionts of some lichens contribute to the defense by producing toxic compounds.

Kaasalainen *et al*. (2012) proposed that the high diversity of microcystin variants and related genes in lichen-symbiotic cyanobacteria could partly reflect the effects of their lichen-symbiotic way of life and dispersal. When packaged into propagules of symbiotically dispersing lichens, the population size of the cyanobionts is reduced and they invariably experience a severe genetic bottle-neck. At the same time, the close symbiotic association with the fungal partner can promote the evolution of different traits from those commonly seen in free-living cyanobacteria (e.g., Ran *et al*., 2010). The recurrent bottlenecks and other population-shaping-effects may have been important in generating the genetic and chemical diversity, now seen in the cyanobacterial symbionts of lichens.

5. Conclusions

We are still only beginning to understand the complex network of biological interactions and evolutionary processes in which symbiotic cyanobacteria and their fungal partners evolve. Many recent findings indicate that we have been crudely underestimating the biological diversity hidden within cyanolichens and other lichen symbioses. DNA techniques offer practical methods for studying the genetic diversity of symbiotic cyanobacteria *in situ*, and for making accurate comparisons between lichen cyanobionts and their non-symbiotic relatives. In the future, we can expect many intriguing new results concerning the cyanobionts of previously ignored lichen lineages and unexplored ecological settings. They can significantly improve our understanding of the biology lichens and of symbiosis as a whole.

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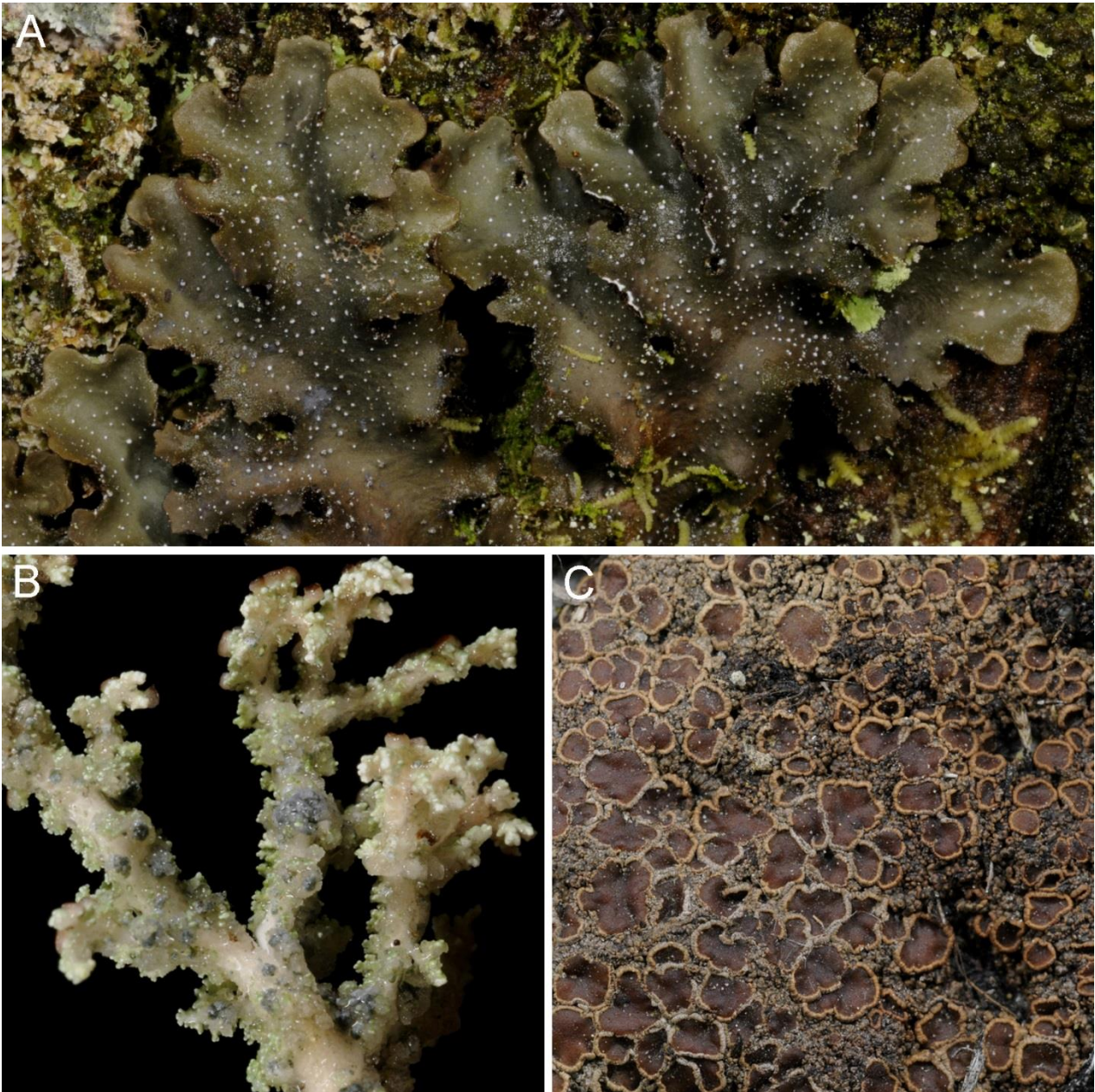
Figure 1. Cyanolichen growth forms. (a) Foliose bipartite cyanolichen (*Pseudocyphellaria* sp., Peltigerales). The cyanobacterial symbiont (*Nostoc*) forms a continuous layer just below the upper cortex of the lichen thallus. (b) Fruticose tripartite cyanolichen (*Stereocaulon* sp., Lecanorales). The cyanobacterial symbiont (*Rhizonema*) is housed in wart-like cephalodia, while the green algal symbiont forms the photobiont layer. (c) Crustose bipartite cyanolichen (*Psoroma hypnorum*, Peltigerales). The cyanobacterial symbiont (*Nostoc*) forms a continuous layer just below the upper cortex of the lichen thallus. The brown disks are fungal apothecia (fruiting-bodies).
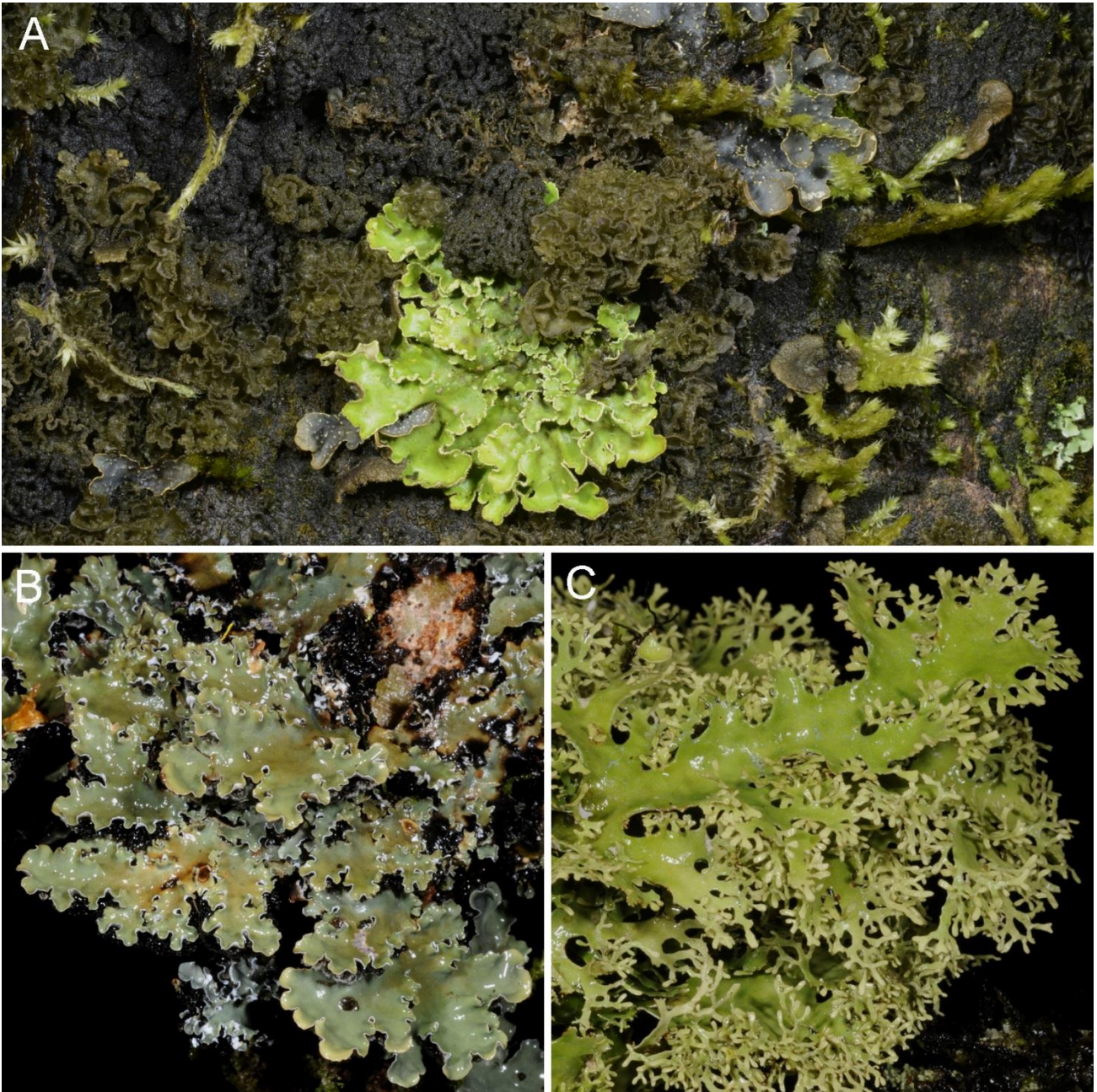
Figure 2. Bipartite and tripartite cyanolichens. (a) A diversity of bipartite and tripartite cyanolichens on tree trunk in tropical moist montane forest. (b) Bipartite cyanolichen (*Peltigera* sp.*,* Peltigerales) with cyanobacterial symbionts (*Nostoc*) in a layer just below the upper cortex of the stratified thallus. (c) Tripartite cyanolichen (*Pseudocyphellaria* sp., Peltigerales) with cyanobacterial symbionts (*Nostoc*) in cephalodia hidden inside the green algal thallus.
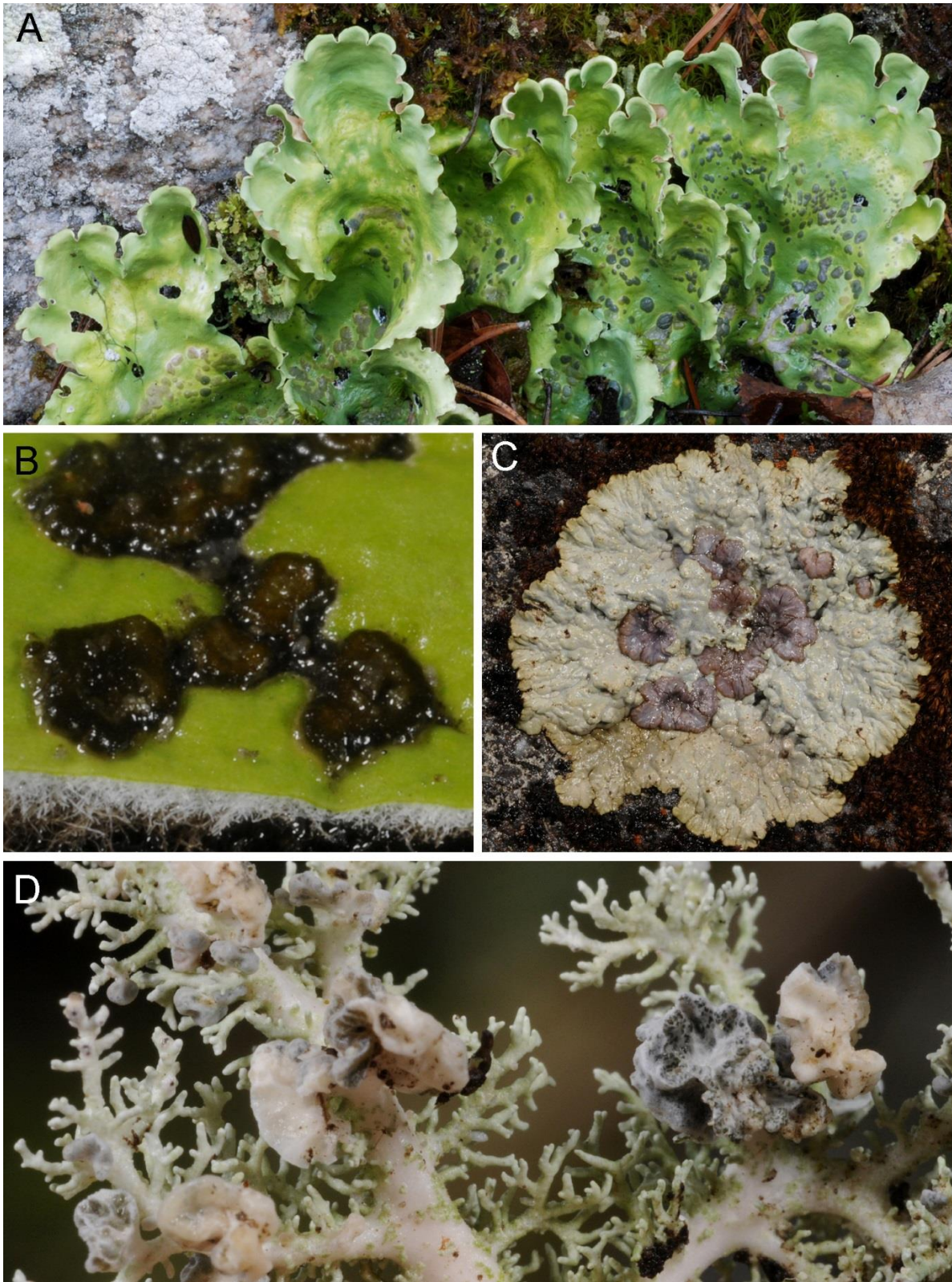
Figure 3. Cephalodia of tripartite cyanolichens. (a) Cephalodia with *Nostoc* on the upper surface of *Nephroma arcticum* (Peltigerales). The internal cephalodia are clearly visible through the upper cortex of the hydrated thallus. (b) Small external cephalodia with *Nostoc* on the upper surface of *Peltigera aphthosa* (Peltigerales). (c) Large external cephalodia with *Nostoc* on the upper surface of *Placopsis* species (Trapeliales). (d) Large sack-like cephalodia with *Rhizonema* on the main branches of *Stereocaulon ramulosum* (Lecanorales).
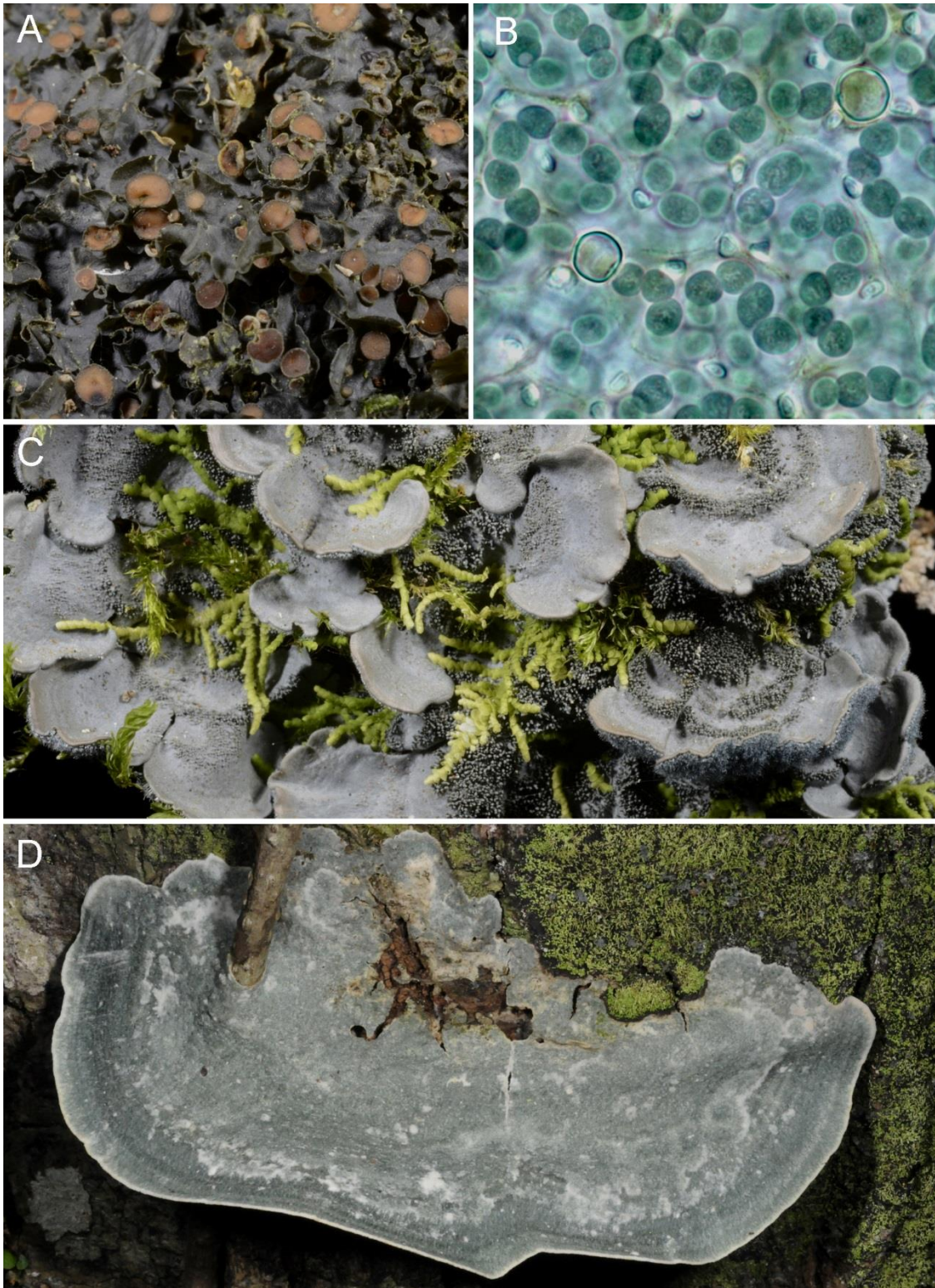
Figure 4. Cyanobacterial diversity in cyanolichens. (a) Gelatinous cyanolichen (*Leptogium* sp.) with symbiotic cyanobacteria (*Nostoc*) symbionts in non-stratified thallus; the brown disks are apothecia of the fungal symbiont. (b) The *Nostoc* cyanobionts of the same lichen photographed through the thin upper cortex of the hydrated thallus. The large clear cells are nitrogen-fixing heterocysts and the small translucent structures are fungal hyphae in optical cross-section. (c) Epiphytic species of *Coccocarpia* (Peltigerales, Ascomycota) with *Rhizonema* cyanobiont. (d) Epiphytic species of *Dictyonema* (Agaricales, Basidiomycota) with *Rhizonema* cyanobiont.
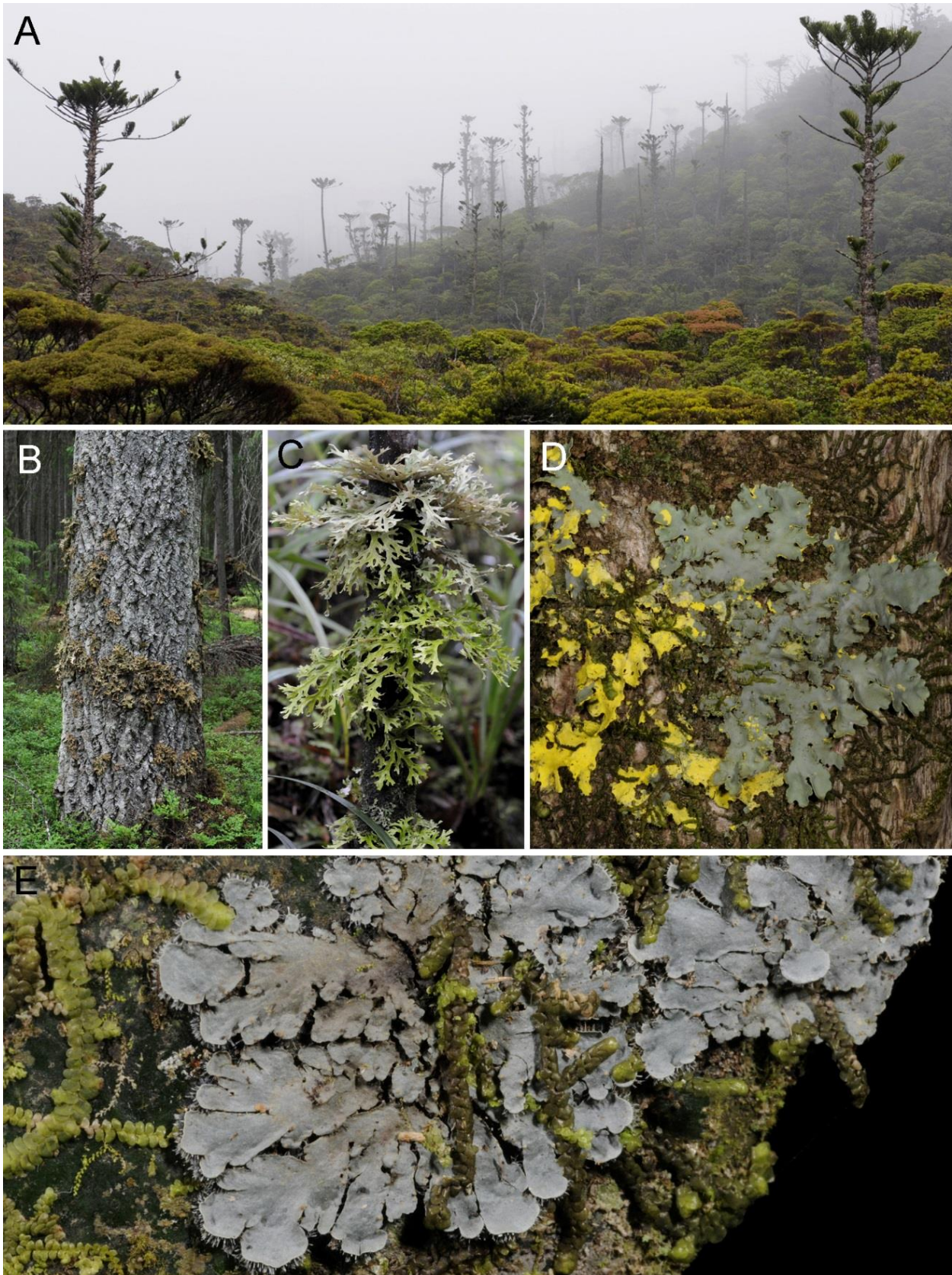
Figure 5. Habitat types rich in cyanolichen species. (a) Tropical moist montane forest on Mont Humboldt in New Caledonia; many cyanolichens (e.g., *Peltigera*, *Pseudocyphellaria, Sticta*, and *Coccocarpia* species) grow as epiphytes and among terricolous bryophytes. (b) Basal trunk of *Populus tremula* in boreal old-growth forest in central Finland; several *Peltigera* species grow on terricolous bryophytes while epiphytic

species of other genera (e.g., *Lobaria* and *Nephroma* spe-cies) are mainly confined to the basal trunks of deciduous trees. (c) Epiphytic cyanolichens contribute to nitrogen-fixation in many moist forest ecosystems. (d) Molluscs grazing on the upper cortex and photobiont layer of the cyanolichen have revealed the bright yellow medulla of the lichen. The medulla is unpalatable because of accumulation of toxic secondary metabolites produced by the fungus. (e) In tropical rainforests, small *Coccocarpia* species can grow on angiosperm leaves together with many folicolous liverworts, crustose lichens and fungi.