CYANOBACTERIAL LICHENIZED FUNGI AND THEIR PHOTOBIONTS IN VIETNAM

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Zusammenfassung

Über die Diversität der Flechtenflora von Vietnam ist nur sehr wenig bekannt. Zum einen bedingt durch fehlende einheimische Wissenschaftler auf diesem Gebiet, nahezu alle vor 1970 durchgeführten Studien wurden von ausländischen Wissenschaftlern durchgeführt, zum anderen bedingt durch die langen Kriegsjahre der späten Sechziger und Siebziger Jahre. Zudem haben frühere Studien die Flechten, welche in Flachländern, vor allem in Aufschlüssen sowie in Sandünen vorkommen, oft vernachlässigt, obwohl derartige Standorte in Vietnam weit verbreitet sind. Es wird daher erwartet, das entsprechende Untersuchungen zur Verbreitung und Vielfalt von Cyanolichenen und deren Cyanobionten in Vietnam einen erheblichen Beitrag zur Flechtenflora von Vietnam leisten können.

Im Rahmen der vorliegenden Dissertation wurden mehrere Sammlungsreisen von Cyanobakterienflechten in den Flachländern Zentral- und Südvietnams durchgeführt um die Vielfalt und Verbreitung zu untersuchen. Darüber hinaus wurden deren Photobionten isoliert und kultiviert, sowie morphologisch wie auch molekularbiologisch charakterisiert und bestimmt (polyphasic approach).

Insgesamt wurden 66 Cyanobakterienflechtenarten gefunden, 50 davon erwiesen sich als neu für Vietnam, womit die Zahl bekannter Arten in Vietnam verdoppelt wurde. Eine Art, *Pyrenopsis melanophthalma* stellt eine neue Art dar und wird neu beschrieben. Zwei weitere Flechten sind bisher nicht identifizierbar und stellen höchstwahrscheinlich neue ebenfalls Arten dar.

Eine bemerkenswerte Trennung von Habitaten wurde für verschiedene geographische Regionen gefunden: saxicole (felsbewohnende) Arten der Ordnung der Lichinales dominieren in küstennahen Aufschlüssen, während lecanorale

Cyanobakterienflechten und cyanobakterielle Basidioflechten überwiegend in Waldgebieten vorkamen. Die gefundene Diversität korreliert negativ mit zunehmender Niederschlagmenge, was auf ein kompetitives Verhältnis von Cyanobakterienflechten und Gefäßpflanzen, hindeutet. In Gebieten mit eingeschobener Trockenphase sind Cyanobakterienflechten der Ordnung Lichinales zunehmend konkurrenzstärker.

Elf Cyanobakterienstämme, einschließlich acht Baoecyten-bildende Stämme der Gattung *Chroococcidiopsis* und drei Heterocyten-bildende Arten der Gattungen *Nostoc* und *Scytonema* wurden aus Flechten erfolgreich isoliert.

Phylogenetische und morphologische Analysen zeigten, dass *Chroococcidiopsis*-Arten vermutlich die einzigen Photobionten in vietnamesischen *Peltula*-Arten sind. Neue morphologische Merkmale wurden in zwei aus Flechten isolierten *Chroococcidiopsis* Stämmen gefunden: (1) das Pink gefärbte Cytoplasma eines Photobiontenstammes isoliert aus einer neuen Lichinaceae und (2) die pseudofilamentöse Anordnung von Zellen welche aus seriellen binären Teilungen eines Stammes aus der Flechte *Porocyphus dimorphus* isoliert wurde, hervorgehen.

Das fädige, heterocytenführende Cyanobacterium *Scytonema* sp. wurde als Photobiont der Ascomycetenflechte *Heppia lutosa* (Lichinaceae) bestätigt. Erstmals wurde das intrazelluläre haustoriale System der Basidiomycetenflechte *Cyphellostereum* und ihrem fädigen Photobionten, *Scytonema* sp., untersucht und vorgestellt. Die *Scytonema*-Photobionten der Basidiomycetenflechten (*Cyphellostereum, Dictyonema*) wurden im Flechtenthallus detailiert untersucht und die Unterschiede dargestellt.

Die phylogenetischen Analysen der Photobiontenstämme *Nostoc* aus *Pannaria tavaresii* und *Parmeliella brisbanensis* weisen darauf hin, dass eine hohe Selektivität in *Parmeliella brisbanensis* aus verschiedenen Regionen der Erde vorliegt, während eine deutlich niedrigere Selektivität in *Pannaria tavaresii* aus verschiedenen geographischen Regionen zu verzeichnen ist.

Damit hat die vorliegende Arbeit deutlich zur Erweiterung unseres Kenntnisstandes über Cyanobakterien-Flechten und deren Cyanobionten in Vietnam beigetragen.

Abstract

The biodiversity of the cyanobacterial lichen flora of Vietnam is chronically understudied. Previous studies often neglected the lichens that inhabit lowlands especially outcrops and sand dunes that are common habitats in Vietnam.

A cyanolichen collection was gathered from lowlands of central and southern Vietnam to study their diversity and distribution. At the same time, cultured photobionts from those lichens were used for polyphasic taxonomic approach.

A total of 66 cyanolichens were recorded from lowland regions in central and southern of Vietnam, doubles the number of cyanolichens for Vietnam. 80% of them are new records for Vietnam in which a new species *Pyrenopsis melanophthalma* and two new unidentified lichinacean taxa were described.

A notably floristic segregation by habitats was indicated in the communities. Saxicolous Lichinales dominated in coastal outcrops that corresponded to 56% of lichen species richness. Lecanoralean cyanolichens and basidiolichens were found in the lowland forests. Precipitation correlated negatively to species richness in this study, indicating a competitive relationship.

Eleven cyanobacterial strains including 8 baeocyte-forming members of the genus *Chroococcidiopsis* and 3 heterocyte-forming species of the genera *Nostoc* and *Scytonema* were successfully isolated from lichens.

Phylogenetic and morphological analyses indicated that *Chroococcidiopsis* was the unique photobiont in *Peltula*. New mophological characters were found in two *Chroococcidiopsis* strains: (1) the purple content of cells in one photobiont strain that was isolated from a new lichinacean taxon, *and* (2) the

pseudofilamentous feature by binary division from a strain that was isolated from *Porocyphus dimorphus*.

With respect to heterocyte-forming cyanobiont, *Scytonema* was confirmed as the photobiont in the ascolichen *Heppia lutosa* applying the polyphasic method. The genus *Scytonema* in the basidiolichens *Cyphellostereum* was morphologically examinated in lichen thalli. For the first time the intracellular haustorial system of basidiolichen genus *Cyphellostereum* was noted and investigated.

Phylogenetic analysis of photobiont strains *Nostoc* from *Pannaria tavaresii* and *Parmeliella brisbanensis* indicated that a high selectivity occurred in *Parmeliella brisbanensis* that were from different regions of the world, while low photobiont selectivity occurred among *Pannaria tavaresii* samples from different geographical regions.

The herewith presented dissertation is therefore an important contribution to the lichen flora of Vietnam and a significant improvement of the actual knowledge about cyanolichens in this country.

1

Introduction

1.1 General overview in cyanobacterial lichens and their photobionts

Lichens are a symbiosis between a mycobiont that can either be an ascomycete or a basidiomycete fungus, with one or two photobionts, an alga or a cyanobacterium in bipartite lichens, or both in tripartite lichens. About 10% of all lichens are bipartite cyanobacterial lichens (cyanolichens in the running text) and 3-4% are tripartite lichens in which the cyanobacterium is the secondary photobiont (Friedl and Büdel, 2008; Honegger, 2008).

About 1500–1550 cyanolichens were found in different lineages of the lichen systematics including the ascomycete orders Agyriales, Arthoniales, Lecanorales, Lichinales, Ostropales, Pertusariales, Pyrenulales, and the basidiomycete order Agaricales and Corticiales (Jørgensen, 2007; Tehler and Wedin, 2008; Oberwinkler, 2012; Rikkinen, 2013). Cyanolichens mainly belong to the orders Lecanorales and Lichinales. Only 10% of basidiomycete cyanolichens of the basidiomycete order Agaricales, less than 50 species, were known in a previous study (Parmasto,

1978); but recent studies found that a single species of this family, *Dictyonema glabratum* really contained 126 different species (Lücking et al., 2014a; Chaves et al., 2004). These authors even predicted a number of 452 species in this morphospecies (a species circumscribed by its morphology) using the coalescent-based species recognition method.

Cyanolichens occur in diverse environments. Lecanoralean cyanolichens distribute mainly in forests with a moderate light intensity and a suitable humidity (Rikkinen et al., 2002). They are mainly foliose lichens containing photobionts of the genera *Nostoc* and *Rhizonema* such as lichens in ascomycetes families Collemataceae, Lobariaceae, Pannariaceae and Coccocarpiacae, or lichens of the basidiomycete family Hygrophoraceae. Lichinalean cyanolichens inhabit all climatic and orographic zones but prefer arid and semi-arid regions (Büdel et al., 1994; Büdel, 1999; Schultz et al., 2000; Schultz and Büdel, 2002). Photobionts in the order Lichinales are more diverse than those in Lecanorales, commonly including species of the coccoid cyanobacterial genera *Chroococcidiopsis, Gloeocapsa, Myxosarcina* as well as species of the filamentous heterocytous genera *Scytonema, Stigonema*, and *Calothrix* (Henssen, 1963; Büdel and Henssen, 1983; Schultz and Büdel, 2002; Friedl and Büdel, 2008).

There are 13 cyanobaterial morphogenera associated with lichenzed fungi that were cultured and isolated (Büdel and Schultz, 2002), comparing to about 40 cyanobacterial genera that were described as photobionts from lichens. While only *Nostoc* and *Stigonema* can be recognized in lichen thalli, the others often expose a modified morphology in the lichen thallus due to the influence of fungal hyphae (Henssen, 1963; Ahmadjian, 1967, 1993; Tschermak-Woess, 1983). For instance, the filamentous cyanobacteria *Rhizonema*, *Scytonema* and *Calothrix* loose their natural arrangement and form clusters or short filaments in lichen thalli, as for example in the lichen genera *Coccocarpia*, *Heppia*, *Spilonema*, *Acantholichen*, and *Lichinodium* (Henssen, 1963; Marton and Galun, 1976; Ahmadjian, 1993; Oberwinkler, 2012). Comparing to the free-living state, they rarely keep their original morphology, as for example the genus *Scytonema* in lichens of the genera *Thermutis*, *Lichenothrix*, and *Dictyonema* s. lat. (Henssen, 1963; Galun et al., 1970; Marton and Galun, 1976; Tschermak-Woess, 1983; Oberwinkler, 2012). In addi-

tion, many cyanobionts do not express all division modes within a thallus, like baeocyte-forming cyanobacterial photobionts.

Baeocyte-forming cyanobacteria are unicellular species whose cells reproduce by two different modes: binary division and multiple fission. Binary division results in two equally large cells after each division; while cells in multiple fission reproduce many smaller cells called baeocytes (Komárek and Anagnostidis, 1998). The baeocyte-forming cyanobacteria genera *Chroococcidiopsis* and *Myxosarcina*, photobionts in many lichens in order Lichinales, undergo the baeocyte formation only in culture media (Büdel and Henssen, 1983, 1988; Bubrick and Galun, 1984; Henssen et al., 1988; Donner, 2013). Therefore, cyanobiont isolation is a necessary step for a positive identification (Ahmadjian, 1967, 1993; Komárek, 2006; Friedl and Büdel, 2008).

Modern cyanobacterial taxonomy requires a polyphasic approach in which a taxon is determined by its phylogenetic position based on 16S rRNA sequences, its morphological characters, and its ecological properties (Komárek, 2006; Kauff and Büdel, 2011; Komárek et al., 2014). Revisions in cyanobacterial morphogenera from previously traditional taxonomy basing on this criterion effectively discriminated new genera from similar morphogenera. *Chroococcidiopsis* and *Rhizonema* are two interesting examples in cyanobacterial photobiont studies that were discriminated from related morphogenera by phylogenetic analyses (Fewer et al., 2002; Lücking et al., 2009).

Chroococcidiopsis has been primarily found as a photobiont of the genus *Peltula* (Bubrick and Galun, 1984; Büdel, 1999; Büdel et al., 2000; Fewer et al., 2002; Friedl and Büdel, 2008). Other morphogenera such as *Myxosarcina*, *Entophysalis* and *Gloeocapsa* were also found as photobionts in *Peltula* (Bubrick and Galun, 1984). Moreover, these authors found that a *Peltula* species, which was distributed throughout various geographical regions, could associate with different photobionts.

Chroococcidiopsis and *Myxosarcina* differ from each other by non-motile baeocytes in *Chroococcidiopsis*, compared to motive baeocyte in *Myxosarcina* (Waterbury and Stanier, 1978). However, this character appears to depend on environ-

mental factors (Komárek and Anagnostidis, 1998). Molecular analysis can help to discriminate between these two genera. Phylogenetic analysis of 16S rRNA sequences indicated that *Chroococcidiopsis* separates from the order Pleurocapsales and is a sister group to the filamentous heterocytous cyanobacteria of the order Nostocales (Fewer et al., 2002; Donner, 2013). These results were strongly supported by multigene, protein and metagenomic analysis (Seo and Yokota, 2003; Donner, 2013; Shih et al., 2013; Howard-Azzeh et al., 2014; Komárek et al., 2014). Consequently, the genus *Chroococcidiopsis* was placed as the family Chroococcidiopsidaceae, within the order Chroococcidiopsidales (Büdel and Kauff, 2012; Komárek et al., 2014).

The second example is *Scytonema*-like photobionts associate with the ascolichens *Coccocarpia*, and the basidiolichens *Dictyonema* sensu lato and *Acantholichen*. The morphogenus *Scytonema* was given to cyanobacterial photobionts in the ascomycete families Coccocarpiaceae, Lichinaceae, Stereocaulaceae and the basidiomycete family Hygrophoraceae (Henssen, 1963; Marton and Galun, 1974, 1976; Oberwinkler, 1980, 1984, 2012; Arvidsson, 1982; Büdel and Henssen, 1983; Lücking et al., 2007). Photobionts in thalli of *Dictyonema* and *Cyphellostereum* are characterized by filamentous morphology and heterocytes similar to those in free-living *Scytonema* (Slocum and Floyd, 1977; Slocum, 1980; Oberwinkler, 2012; Lücking et al., 2013, 2014a,b,c). However, a phylogenetic analysis of partial 16Sr RNA sequences of these photobionts indicated that they belong to the new clade *Rhizonema*, separate from the *Scytonema*-clade (Lücking et al., 2009). There is evidence that true-branching filmaments observed in thalli of *Dictyonema coppinsii* belong to the new cyanobacterial genus *Rhizonema* (Büdel and Kauff, 2012; Lücking et al., 2014a).

1.2 Study on cyanolichens in Vietnam

Overview of lichen studies in Vietnam

Comparing to about 12000 higher plant species so far known in Vietnam (Pham, 1999), the knowledge of lichens in Vietnam is still too meager. Most

of earlier records of lichens were conducted in the two last centuries by Europeans. In an earlier period, Krempelhuber (1873) recorded lichens not only in Saigon (in Cochinchina – the south part of Vietnam today) but also in Hongkong, Shanghai and Guadong, China. Harmand (1928) studied lichens of Indochina including three countries: Laos, Vietnam, Cambodia. In the north of Vietnam (once named Tonkin), Müller (1891) sampled mostly crustose species and Vězda (1977) focused on foliicolous lichens. In the centre of Vietnam (old name is Annam), Abbayes (1964); Schmid (1974); Tixier (1966) recorded mostly macrolichens that were collected in the highland at elevations from 800 m to 1200 m. The study of Vietnam lichens was disrupted after the 1970's by wars, and then by the lack of attention on these small objects.

How many lichen species occur in Vietnam? Aptroot and Sparrius (2006) estimated about 1000 species. A checklist of 275 lichen species was provided by these authors. They found 122 new species for Vietnam in total during their short trip in Hanoi, northern Vietnam, in 2004. Another note of 81 macrolichens found in central highland of Vietnam, two third of which were recorded for the first time for Vietnam by Võ (2009). The studies of Nguyen et al. (2011a,b), and Jayalal et al. (2013) on foliicolous and foliose lichens recorded 15 new species in the central highland of Vietnam. Studies on crustose lichens in Vietnam carried out in families Graphidaceae, Verrucariaceae of the karst mountains in the north and central highland of Vietnam revealed 3 new species belonging to Graphidaceae (Sparrius et al., 2006; Joshi et al., 2013, 2014; Gueidan et al., 2014). In summary, these studies resulted in new additions of about 100 records to the lichen flora of Vietnam in 8 years after the revision by Aptroot and Sparrius (2006). This is a valuable contribution but it is still far to reach the estimation of 1000 lichen species mentioned by these authors.

Cyanolichens in Vietnam

Less than 50 cyanolichens are known from Vietnam from the earlier investigations (Aptroot and Sparrius, 2006; Võ, 2009; Jayalal et al., 2013). Most of them are lichenized fungi associated with photobiont *Nostoc* and *Rhizonema* such as foliose lichens *Coccocarpia, Collema, Leptogium, Lobaria, Pseudocyphellaria*, and *Sticta*.

They often inhabit forests under humid and moderate light conditions. Lichens in habitats with poor vegetation coverage of coastal land includes outcrops, low hills, and sand dunes along the eastern territory of Vietnam have never been noted. The author found that a coastal orographic region in central Vietnam, extending from the Ninh Thuan province to the Binh Thuan province, with a savannah climate known to be preferred by lichens of the families Lichinaceae, and Peltulaceae (Büdel, 1987; Schultz et al., 2000; Schultz and Büdel, 2002; Jørgensen, 2007). This is a potential area to discover new cyanolichens for the lichen diversity of Vietnam.

1.3 Motivation and research questions

A demand in further understanding the lichen flora of Vietnam, particularly cyanolichens and their photobionts motivated the author to conduct this study. From previous studies on Vietnamese lichens, it can be concluded that there is a good probability of cyanobacterial lichens, especially of the class Lichinomycetes occuring in poor habitats along the coastline. Moreover, an opportunity to focus on lichens with diverse growth forms than only foliose cyanolichens were known before in Vietnam. It was my intention to improve knowledge on diversity of cyanolichens from lowland habitats in the centre and south of Vietnam.

This study has focussed simultaneously on the taxonomy of cyanobacterial photobionts whose taxonomy can not be recognized from lichen thalli. These photobionts mostly belong to the baeocyte-forming cyanobacteria and to filamentous heterocyte-forming cyanobacteria.

Firstly, the baeocyte-forming cyanobacteria *Chroococcidiopsis* and *Myxosarcina* are exclusive photobionts of lichens of the order Lichinales (Friedl and Büdel, 2008). These photobionts rarely express the baeocyte-forming stage when lichenized (Büdel and Henssen, 1983; Bubrick and Galun, 1984). *Chroococcidiopsis* was proved to be the common photobiont of lichens belonging to the family Lichinaceae (Büdel and Henssen, 1983; Donner, 2013; Fewer et al., 2002). In the family Peltulaceae, the genera *Chroococcidiopsis* and *Myxosarcina* were morphologically studied, but only the photobiont strain *Chroococcidiopsis* of the lichen

species *Peltula euploca* origining from Africa was confirmed by phylogenetic analyses (Fewer et al., 2002; Donner, 2013). Although some genera of the Lichinales such as *Anema*, *Gloeoheppia*, and *Lichinella* associate with *Chroococcidiopsis* as their photobiont only (Bubrick, 1978; Donner, 2013), the genus *Peltula* apparently has a low selectivity since 4 different photobiont genera were identified. *Peltula polyspora* (= *Peltula patellata*) occurring in distant geographical regions (Bubrick and Galun, 1984; Bubrick, 1978) was found to associate with distinct photobiont genera. For this context, two research questions arise: 1- Which cyanobacterial lichens associate with baeocyte-forming cyanolichens in Vietnam? *and* 2- What is the photobiont diversity of the Peltulaceae in Vietnam?

Secondly, the scytonematoid photobionts of the families Coccocarpiaceae and Hygrophoraceae were recently revised belonging to the genus *Rhizonema* when applying the phylogenetic analysis (Lücking et al., 2009). Its morphology was characterized by true branching within lichen thalli (Lücking et al., 2014a). However, before these studies, the photobiont genus *Scytonema* was confirmed by culturing photobionts isolated from *Heppia* (Marton and Galun, 1976; Ahmadjian, 1967). Moreover, the photobiont *Scytonema* is morphologically recognized by false-branching heterocytous filaments in loose thallus parts of *Dictyonema irpicinum* (Slocum, 1980). This cyanobiont genus needs to be verified by molecular analyses to find out whether it belongs to the family Scytonemataceae.

1.4 Aims of this thesis

In summary, the aims of this thesis are:

1)- Cyanolichen taxonomy and diversity in lowland habitats of central and southern Vietnam. In the taxonomic context, I focus on the taxonomy of the ascolichen family Lichinaceae and the basidiolichen family Hygrophoraceae.

2)- Photobiont taxonomy and diversity of Vietnamese cyanolichens. In this context, the baeocyte-forming on the one hand and the filamentous heterocyte-forming cyanobacterial genera on the other hand are hard to recognize in lichen

thalli will be studied by isolating and cultuvating them. Their morphological features will be combined with phylogenetic characters.

2

Material and methods

2.1 Natural history of Vietnam: geographic, climatic characterization, and vegetation formations

Vietnam is located in the eastern Indochina Peninsula with a diverse topography and climate. The main backbone of this S-shaped territory is the Annamite Range which forms a natural border between Vietnam and the two countries, Lao and Cambodia. Mountains and highlands mainly lie in the north and west of Central Vietnam. Toward the eastern territory, two main deltas of Red river and Mekong river, located in the north and south, respectivelly; coastal narrow plains located in the centre, are dissected by portions of the Annamite range reaching the sea.

The climate in Vietnam varies, caused by the diverse geomorphology associated with a latitudinal extent of 14 degrees (from 8 ° to 23 °N). Orographic factors and monsoon winds influence the climate and its seasonality. The northern and highland regions are influenced by seasonal variations much more than the south-

ern and lowland areas. There are three main climate types from the north to the south of Vietnam according to the Köppen-Geiger climatic zones (Peel et al., 2007): temperate dry and hot summer climate (Csa) in the north western mountain Vietnam, tropical monsoon climate (Am) in the northern region limited at Bach Ma mountain at a latitude of 16 °N and a small part in southern Vietnam, and tropical savannah climate (Aw) in the lower half of central and southern Vietnam. In general, the annual temperature is 25–27 °C in the south and gradually decreases towards the north with altitude, to 17–18 °C. Summer precipitation varies in climate types with the annual precipitation being more than 1200 mm. The influence of seasonal factors decreases gradually southward. In the south, the climate exhibits dry and rainy seasons with a small temperature change throughout the year. The climate can be seen exceptionally severe in a region in central Vietnam, where precipitation is less than 800 mm and the dry period lasts 7–8 months.

Climate and geomorphology play an important role in vegetation formations of Vietnam. According to the modified Whitmore forest classification (Whitmore, 1998; Ghazoul et al., 2010), different vegetation formations can be distinguished based on the four key climatic criteria (e.g., water, drainage, soil and elevation):

- *Montane rainforests*, mostly located in the northern mountains at altitudes $\geq$ 800 m, and the central highland, at altitudes $\geq$ 1000 m. The forests are composed of conifers, Fagaceae, Lauraceae, and Magnoliaceae as floristic key elements (Blasco et al., 1996; Averyanov et al., 2003; Thái, 1978).

- *Non-seasonal lowland rainforests*, existing only in central Vietnam, in the lower zone of Bach Ma mountain and adjacent regions with a high annual precipitation (>3000 mm; Nguyen et al. (2000)).

- A conspicuous forest formation–the *seasonal rainforest*, today mostly limited to protected areas such as national parks and nature reserves in the central and southern Vietnam. This forest type is recognized as evergreen and semi-evergreen forests following Blasco's forest classification which is frequently used in Vietnam (Blasco et al., 1996).

- *Monsoon rainforest*, including moist and dry deciduous forests that cover large areas in the north and in the south parts of Vietnam.

In addition, Vietnam harbours a number of forest formations of minor relevance in some regions. These are caused by particular configurations of geological, geomorphological and local weather conditions. For instance, seasonal rain forests over limestone landscapes with less available water and poor soil, or dry forests are limited to two adjacent provinces, Ninh Thuan and Binh Thuan.

2.2 Sampling sites

In order to address the extremely poor status of lichenological knowledge in Vietnam, the present study aimed at a broad species inventory, and selected to cover a diverse set of geographical, environmental, and vegetation-related conditions. Therefore, local cyanolichen communities were sampled at 11 study locations across a large latitudinal range of Central and South Vietnam, spanning six latitudinal degrees from 11 ° to 17 °N and elevational zones lower than 1000 m, as well as a representative variety of Vietnamese lichen habitats from sand dunes, rocky outcrops to epiphytic habitats in lowland forests (Fig. 2.1, Table 2.1). More precisely, lichens of two types from sand dune habitats were sampled: (i) the coastal sand dunes of Cam Ranh and Qui Nhon, and (ii) the inland sand dunes in Phong Dien. The vegetation of coastal fixed dunes is characterized by the dominant tree species *Vatica tonkinensis, Shorea* spp., *Irvingia malayana, Parinari annamense*, and shrubs such as *Buchanaria siamensis, Memecylon edule, Manilkaria* sp., *Grewia asiatica, Connarus cochinchinensis* (Fig. 2.2 E). On the other hand, the inland sand dunes are mainly dominated by shrubs such as *Combretum quadrangulare, Melaleuca cajeputi, Opuntia* sp.

The two rocky outcrop habitats were situated at coastal sites in Qui Nhon and Ninh Thuan. Both were sparsely inhabited mainly by tree species *Opuntia dillenii, Calotropis gigantea, Streblus asper*, and rarely *Largerstroemia* sp. and *Pandanus odorifer* (Fig. 2.2 A).

The lowland forest habitats, based on Whitmore forest classification (Whitmore, 1998) can be assigned to three categories: (i) none-seasonal forest, (ii) seasonal forest, and (iii) monsoon forest. None-seasonal forests in Bach Ma and Phong Dien are located in a region with the highest precipitation and the shortest dry

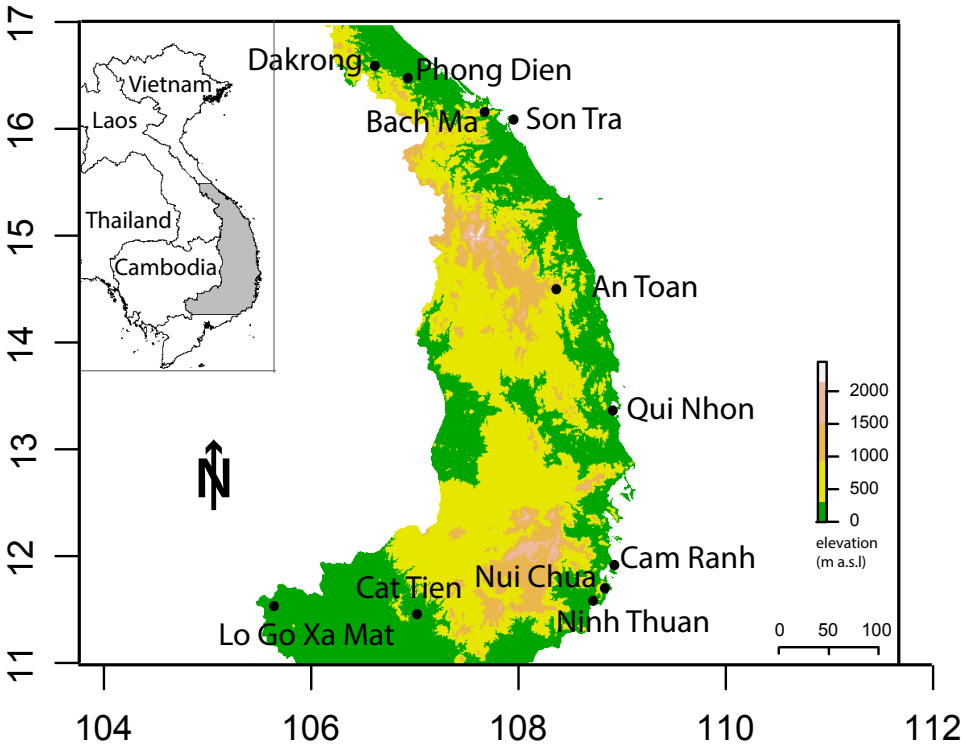

Figure 2.1: Locations of lichen collecting sites across Central and Southern Vietnam. The eleven sites visited during the present study are indicated by bold dots. The map provides latitudinal and longitudinal coordinates and color-coded elevational zones (m a.s.l.).

Figure 2.2: Typical habitats at studied sites
A, outcrop in Ninh Thuan – the main habitat of lichens of the order Lichinales; **B**, rock polls on a rapid in Cat Tien, habitat of *Peltula clavata* and *P. obcurans*; **C** and **D**, dry forest in Nui Chua at ca. 400 m asl (**C**) and 700 m asl (**D**) where inhabited by many cyanolichen species of both orders Lichinales and Lecanorales; **E**, a sand dune in Nha Trang, next to Ninh Thuan, only epiphytic chlorolichens grow on shrubs; **F**, mountain forest in An Toan, the stream where genera *Pannaria, Coccocarpia* and *Leptogium* occur on the boulders

period of Vietnam (Table 2.1). Five sites were selected in the seasonal forests at different latitudes and landscapes in Dakrong, Son Tra, An Toan, Cat Tien, Lo Go Xa Mat (Fig. 2.1). The forests in three first sites are located on hills and mountains in central Vietnam, and forests in the latter are situated in plain regions in southern Vietnam. The dry forest is a subtype of the monsoon forest located in the driest region in central Vietnam, characterized by dipterocarps and shrubs on soil and mixed forests in a mountain in Nui Chua.

According to the Köppen-Geiger climate classification (Peel et al., 2007) two basic climate types can be distinguished within the set of sampling sites: (i) forests under the influence of a tropical monsoon climate (Am), (ii) outcroups in Qui Nhon and Ninh Thuan, and the forests in Nui Chua and Lo Go Xa Mat, all are shaped by a tropical savannah climate (Aw). This climate classification seems to be more practical than the classification commonly used by researchers in Vietnam based on Gaussen et al. (1967). The dry period is determined by the number of months, at which precipitation (mm) less than two times the temperature (°C) (Table 2.1) (Gaussen et al., 1967; Nguyen et al., 2000)

2.3 Lichen sampling and handling

Lichen specimens were collected during two field trips in the dry season in 2012 and 2013. The field campaigns took place during the dry season facilitating the specimen collection and transportation. The lichen specimens were gathered from three major substrates: open soil, rock surface, and tree trunks. Sampling of epiphytic lichens on tree trunks was restricted from 0–1.6 m height. In one case, however, some of the species listed in the checklist were collected from a big fallen tree in An Toan. Specimens were dried over silica gel at room temperature, curated and stored in the Pham Hoang Ho herbarium (PHH) at the Department of Ecology–Evolutionary Biology, University of Science–Vietnam National University, Ho Chi Minh city. Duplicates are deposited in Herbarium Berlin-Dahlem (B) and Herbarium Hamburgense (HBG).

Table 2.1: Climate and habitat at sampling sites.

Habitat clasification based on Whitmore (1998), climate type based on Köppen-Geiger climate classification (Peel et al., 2007)

Sampling site	Precipitation (mm)	Dry months	Köppen climate symbol	Habitat
Dakrong	2079	3	Am	Seasonal rain forest
Phong Dien-Hue	2936	0	Am	Non-seasonal lowland rain forest, Inland sand dune
Bach Ma	3442	1	Am	Non-seasonal lowland rain forest
Son Tra	2041	3	Am	Seasonal rain forest, Outcrop
An Toan	2002	3	Am	Seasonal rain forest
Qui Nhon	1697	3	Aw	Outcrop, Coastal sand dune
Cam Ranh	1324	7	Aw	Coastal sand dune
Nui Chua	761	7	Aw	Dry forest
Ninh Thuan	761	7	Aw	Outcrop
Cat Tien	2469	4	Am	Seasonal rain forest
Lo Go Xa Mat	1813	4	Aw	Seasonal rain forest

Am: tropical monsoon climate, Aw: tropical savannah climate

2.4 Morphology studies

Lichens and cyanobacterial photobionts were first identified based on the refering morphological criteria of different species. For lichen identification, morphological features and anatomical structures of thalli and ascomata were studied. Regarding photobiont identification, baeocyte-forming cyanobacteria were investigated for their (i) division patterns, (ii) binary division and/or multiple fission, and (iii) vegetative cell size measured on agar cultures. Morphology of lichens and cultured photobionts were investigated and documented using a stereomicroscope (Zeiss, Oberkochen) and a microscope (Axiokop, Zeiss, Oberkochen) equipped with AxioCamMRc 5 digital camera (Zeiss, Oberkochen). Cross sections, 18-30 µm thick, were prepared using a freezing microtome (Reichert-Jung 1206) and mounted in water or Lactophenol Cotton Blue. KOH 10% and Lugol's solution were used to test the apothecial reaction (turning red, blue or no reaction).

Low-Temperature Field-Emission Scanning Electron Microscopy (LT-SEM) was used to examine the microstructural details of lichens and photobionts. Fully turgescent samples were fixed onto a copper specimen-holder. The loaded specimen-holder was submerged in liquid nitrogen and then transferred to a high vacuum preparation chamber of a cryo-unit (K1250X Cryogenic Preparation System, Quorum Technologies Ltd, Ashford, Great Britain) at -130 °C. After sublimation at -80 °C for 30 mins, the sample was sputter-coated (layer 25 nm) with gold-palladium and transferred to the LT-SEM (SUPRA 55 VP, Carl Zeiss NTS, Oberkochen, Germany) and viewed at -130 °C temperature and 5 kV accelerator voltage.

2.5 Lichen identification and taxonomy

Lichen identification was carried out by using specific identification keys, lichen floras, revisions and doctoral theses (Table 2.2), and by comparison with voucher specimens from the herbarium at Department of Plant Ecology and Systematics, University of Kaiserslautern; Herbarium Hamburgense; and Herbarium Berlin-Dahlem. Helpful guides were also received from experts Burkhard Büdel, Matthias Schultz, Harrie Sipman and Robert Lücking for the taxonomic groups found in my cyanolichen collections.

The taxonomy of species was assesed and updated from recent results by phylogenetic analyses. The two families Collemataceae and Hygrophoraceae were revised recently – and these results were taken into regard in this study. For the species of family Hygrophoraceae, I followed the updated key by Lücking et al. (2009, 2014a) – in which the outside hyphae, haustoria and photobiont characters were used to discriminate two genus *Dictyonema* and *Cyphellostereum*.

2.6 Photobiont studies

2.6.1 Isolation, culture, and maintenance

Obtaining pure photobiont strains from lichens is a necessary step for a safe determination, because the developmental stages are only expressed in the free liv-

Table 2.2: Literatures used for cyanolichens identification

Family	References used to identify
Coccocarpiaceae	Arvidsson (1982); Henssen and Tønsberg (2000); Lücking et al. (2007); Spribille et al. (2014)
Collemataceae	Degelius (1974); Swinscow and Krog (1988); Verdon et al. (1992); Jørgensen (2007); Otálora et al. (2014)
Lichinaceae	Henssen (1965); Wetmore (1970); Henssen et al. (1988); Schultz et al. (2000); Schultz (2000); Schultz and Büdel (2002); Schultz (2007a,b,c,d, 2008); Thüs and Schultz (2008); Tretiach and Schultz (2008)
Lobariaceae	Galloway (1994, 1998, 2001); Galloway et al. (2001)
Pannariaceae	Henssen (1965, 1999); Jørgensen and Galloway (1992); Jørgensen and Henssen (1999); Jørgensen (2003, 2010)
Peltulaceae	Wetmore (1970); Büdel (1987, 2001); Marques et al. (2013)
Hygrophoraceae	Henssen (1963); Slocum and Floyd (1977); Slocum (1980); Oberwinkler (1980, 1984, 2012); Yánez et al. (2012) Lücking et al. (2013, 2014a); Dal-Forno et al. (2013)

ing state, i.e., the culture medium. Morever, these isolates then supply material for phylogenetic analyses. I used the modified protocol of Büdel and Henssen (1983) to isolate and culture photobionts from selected lichens of the family Lichinaceae, Peltulaceae and Pannariaceae. Starting material for the isolation process of the cyanobiont was a thallus washed in sterile water. Briefly, the washed thalli were cut and placed on agar 1.5% (10 ml/petri) and liquid (50ml/bottle) of medium BG11 (Waterbury and Stanier, 1978). Every lichen species was placed on three agar petri dishes and into three liquid bottles. All samples were incubated in a culture room at 17 °C under a 14:10 hours light-dark regime with a light intensity of 10–40 photons $*m^{-2}$ $*s^{-1}$ provided by daylight cool white fluorescent lamps (Radium Spectralux NL-T8 58 W/840; Radium Lampenwerk). The cross sections were observed weekly with a binocular to avoid culturing epiphytic cyanobacteria

other than the photobiont. After 6–8 weeks, the first colonies had grown beyond the thalli in the agar medium and were investigated by light microscopy. The photobiont colonies were mechanically purified from bacteria and other contaminants (rolling over sterile agar), and transferred to fresh medium every 2–4 weeks. The samples in liquid media were monitored and studied after 4–7 months depending on the growth of the various species. All the photobiont strains were numbered from BB15.01 to BB15.11 (Table 3.4) and maintained in a culture room (Department of Plant Ecology and Systematics, University of Kaiserslautern, Germany). The strains maintenance is necessary for further reference and phylogenetic analyses.

2.6.2 Molecular work

The small subunit of the ribosomal RNA (16S rRNA) gene is a common molecular maker that combines two morphological and ecological characters through the polyphasic approach for the classification of cyanobacteria (Komárek, 2006; Komárek and Mareš, 2012; Komárek et al., 2014). This universal and conservative gene was used alone or in combination with other genes in taxonomical and diverse studies of cyanobionts (Mioa et al., 1997; Paulsrud et al., 1998; Fewer et al., 2002; Rikkinen et al., 2002; Summerfield et al., 2002; O'Brien et al., 2005; Lücking et al., 2009; Fedrowitz et al., 2011; Donner, 2013; Rikkinen, 2013). Accordingly, the abundant database of 16S rRNA sequences of cyanobacterial strains in GenBank facilitates was used to determine the cyanobiont strains in this study.

A modified CTAB method (Cubero and Crespo, 2002) was used to extract DNA from selected lichens that were used for the photobiont isolation, and from the cultured photobionts itself. For the lichens with little available thallus material or with slow growing photobionts, the modified microslide PCR (Wolinski et al., 1999) was used. This method allowed me to run PCR directly from specimens instead of DNA extracts. Briefly, a 1 mm X 10 mm microslide was immersed in gelatin/chromalum solution (0.25% gelatin, 0.025% chromium III potassium sulphate) for 5 seconds, and air dried under sterile conditions in a laminar flow cabinet. About 5–7 cross sections of washed lichen thalli or a piece of cultured photobiont were/was attached to the microslide and adhered to the surface by

drying on a heat block (HLC Heating-ThermoMixer) at 45 °C for 5 min, then in a microwave (Moulinex FM411) on the defrost function for 10 min. These microslides were used as DNA extract in PCR protocol.

The 16S rRNA gene sequences were amplified using the primers Wil1 and Wil18 (Wilmotte et al. 1993). A volume of 50 µl PCR reactions contained 25 µl of Taq PCR Mastermix (QIAGEN), 2 µl of each primer at 1 pmol/µl, 19 µl PCR water and 2 µl of DNA with concentrations vary from 25 to 100 ng/µl. The PCR cycle was as follows: initial denaturation step at 94 °C for 3 mins, 30 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C, and the final elongation at 72 °C for 10 mins. All PCR amplifications were performed in the Effendorf Mastercycler Thermo Cycler AG 22331.

PCR products were examined by gel electrophoresis on 1% agarose gel labelled with GelStar® Nucleic Acid Gel Stain (Lonza Rockland, Inc. USA) and successful PCRs were cleaned up using "Nucleo Spin Extract II" kit (Macherey-Nagel, Düren, Germany). DNA concentration was measured, standardized to ca. 100 ng/µl, and directly sequenced by a commercial provider (SeqIT GmbH, Kaiserslautern, Germany) with both forward and reverse primers, Wil1 and Wil18 (Wilmotte et al., 1993).

For the strain *Scytonema* BB15.09 isolated from *Heppia lutosa*, low quality sequence was obtained from both direct PCR amplification from DNA extract and microslide PCR. This result was probably caused by either other free-living cyanobacteria growing together with this *Scytonema* in the culture medium, or by a thick mucilaginous sheath effected to DNA extract and PCR (Garcia-Pichel et al., 2001; Mareš et al., 2013). Hence, we used denaturing gradient gel electrophoresis (DGGE) method based on Muyzer et al. (1993) to obtain clean sequence of this strain. This approach effectivity separates same length fragments of 16S rDNA sequences of a microbial population on the DGGE gel based on decreased electrophorentic mobility of melted DNA sequences in gels that contain linear gradient DNA denaturants (Muyzer, 1999). Each separate band on the DGGE gel corresponds to a 16S rDNA sequence of a species in the microbial population.

Briefly, DNA template used in DGGE was obtained after two PCR reactions. Reaction mixture for the first PCR – including 1 µl each of primers 27F1 and 149RC, 3 µl $MgCl_2$, 1 µl DNA – was firstly amplified in Mastercycler (Effendorf) following by initial preheating at 95 °C for 3 mins; 30 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C; and the final elongation at 72 °C for 10 mins. The following PCR was implemented with reation mixture containing 1 µl each of primers CYA781F, CYA359R, 25 µl Taq, 20 µl PCR water, 1 µl previous PCR with the similar PCR cycle to the first PCR. DGGE of the PCR products was performed on an 8 % (w/v) polyacrylamide gel with urea and formamide as denaturants with a gradient of 50–65%. About 800 ng of each PCR product from the second PCR and gene ruler were mixed to 3 µl loading dye, and loaded into the wells of the prepared DGGE gel. Electrophoresis was performed in 1 X TAB buffer at 60 °C at a voltage of 100 V for 17 hours. DGGE gel bands were visualized by staining with SYBR gold nucleic acid gel stain. Clean bands were cut out with a sterile blade and incubated for 24 hours in 100 µl of diffusion buffer. The acrylamide gel was filtered out and the resulting solution was cleaned using the cleanup kit NucleoSpin Gel and PCR (Macherey-Nagel, Düren, Germany). DNA was reamplified using the primer pair CYA361F and CYA785R (Mühling et al., 2008), which was found to reamplify the bands more successfully than the primers used for the initial PCR. Conditions were as stated above, except for the annealing temperature of 59 °C.

2.6.3 Phylogenetic analysis

Partial 16S rRNA sequences in this study were compared to those from GenBank by using blastn section in the BLAST program v. 2.2.32+ (Altschul et al., 1990) at website: http://blast.ncbi.nlm.nih.gov/Blast.cgi. Studied sequences were obtained from GenBank were used for phylogenetic analyses (Table 6.1). The data set was aligned by MAFFT v.7 at website: http://mafft.cbrc.jp/alignment/server/, L-INS-i algorithm (Katoh et al., 2002; Katoh and Standley, 2013), trimmed to remove poor aligned stretches by trimAl with method automated 1 (Capella-Gutiérrez et al., 2009) inplementated through the web server Phylemon 2 (Sánchez et al., 2011). The best model GTR+G+I for the alignment data was inplemented

by jModelTest 2 v. 2.1.1 (Guindon and Gascuel, 2003; Darriba et al., 2012) using the Akaile imformation substitution criterion AIC (Akaike, 1973).

I performed maximum likelihood analyses with the computer program RAxML v7.2.8 (Stamatakis et al., 2008) to infer the phylogeny of baeocyte-forming photobionts using 1000 bootstrap replicates. For heterocyte-forming photobionts, the ML bootstraping with 500 replicates was performed in RAxML v7.2.6 (Stamatakis, 2006) at Trex server (http://trex.uqam.ca/) (Boc et al., 2012).

Bayesian analysis was performed with MrBayes v.3.2.5 (Huelsenbeck et al., 2001). Four runs with four chains were run simultaneously for 1 milion generations, with the temperature parameter of 0.1. Chains were sampled every 100 generations. The posterior probabilities were performed from the tree that were sampled after the burnin interval 250000 generations.

Two data sets were used for phylogenetic analyses. A data set of baeocyte-forming cyanobacterial photobionts included 34 GenBank sequences and 14 sequences of *in situ* photobiont and isolates, obtained from species of Lichinales in this study. A data set of the heterocyte-forming photobionts included 41 GenBank sequences and 4 sequences obtained from thallus of *Coccocarpia erythroxyli*, and isolated photobionts of two pannarioid lichens and *Heppia lutosa*. The outgroup taxon *Gloeobacter violaceus* PCC 7421 obtained from GenBank were used for phylogenetic analyses.

Isolates need to be verified since cyanobacteria, being a thallus epiphyte rather than the photobiont, could have emerged in culture medium (Kardish et al., 1990; Mioa et al., 1997; Summerfield et al., 2002). As a result, photobionts were verified by at least two of the following steps: 1- manipulating the very first colonies grown beyond the lichen thallus cross sections (Büdel and Henssen, 1983), 2- finding the presence of haustoria in photobiont colonies during the very first stage of development, and 3- the molecular comparison of *in situ* photobionts and isolates (Kardish et al., 1990; Mioa et al., 1997; Summerfield et al., 2002). The last step (step 3) was based on the identity of two sequences, the *in situ* photobiont and the isolate from a lichen using a comparison tool, blast two sequences (bl2seq) at website: http://blast.ncbi.nlm.nih.gov/Blast.cgi. This blast-based tool searches

the similarity of two sequences through pairwise alignment, then derives their identity percent (Tatusova and Madden, 1999).

2.7 Assessment of biodiversity and community structure

Local lichen biodiversity was expressed as species richness obtained across 57 study plots at 10 sites covering an estimated area of 12 ha. To evaluate the sampling completeness, I generated a species accumulation curve applying the model explained by Colwell et al. (2004) and estimated an extrapolated species richness using Chao 2 estimator (Chao, 1987) based on the total observed species richness and species incidence data.

The cyanolichen communities at the sites were assessed with regard to growth forms, floristic similarity, and environmental variables including (1) precipitation which was obtained from the nearest metereological stations (Nguyen et al., 2000), (2) light which was categorized in shady vesus exposed at each plot, and (3) elevation which were measured by GPS. The resemblance between cyanolichen communities was evaluated by quantifying the Jaccard's dissimilarity index (D) among cyanolichen communities based on the Jaccard similarity index (S) in the fomular: D = 1 – S (Legendre and Legendre, 2012). A clustering analysis used this dissimilarity matrix to draw hierarchical relationship between the communities onto a ordination diagram (Legendre and Legendre, 2012). The analyses were implemented in R using functions *specpool, specaccum, vegdist, hclust* in the vegan package applied to a species incidence matrix and environmental factors (Tables A.2–A.4).

Correlation analysis was used to determine the strength of relationship between species richness with environmental variables. Correlation coefficients were calculated by Pearson's product-moment correlation. The regression analysis described the form of these relationships. These analyses were also implemented by using R.

3

Results

3.1 Cyanolichens

3.1.1 Cyanolichens diversity

Overall, 800 lichen specimens were collected during two field trips (Map 2.1). From these, 264 specimens belonged to the cyanolichens. In total, I found 66 different cyanolichen species in which 50 species were new records for Vietnam. Of those, 53 species were identified at species level (Tables 3.2), 11 taxa were identified at genus level and 2 remained unidentified (Table 3.3). The cyanolichen community compositions are treated in detail in the section 3.3.

Most species were ascolichens, only 8% of them belonged to the basidiolichens. Of the ascolichens, 34% of taxa were foliose cyanolichens belonging to order Lecanorales, while 56% of the taxa belonged to the crustose-squamulose Lichinales. The genera *Peltula* (Peltulaceae, Lichinales) and *Leptogium* (Collemataceae, Lecanorales) were found to be common genera (Table 3.1). The family Lichinaceae was the most diverse with 10 genera recorded. The family Pannariaceae was represented by five genera. The five basidiolichens discussed in this study were discovered in forests

belonging to the genera *Dictyonema* and *Cyphellostereum*. *Cyphellostereum* spp. is characterized by intracellular hautoria and false-branching photobionts that were not mentioned in previous studies.

Table 3.1: Clasification of genera treated in this study

Phylum	Order	Families	Genus
Ascomycota	Dothideales	*incertae sedis*	*Collemopsidum*
	Peltigerales	Coccocarpiaceae	*Coccocarpia, Spilonema*
		Collemataceae	*Collema, Leptogium*
		Lobariaceae	*Pseudocyphellaria, Sticta*
		Pannariaceae	*Ramalodium, Pannaria*
			Parmeliella, Physma, Staurolemma
	Lichinales	Lichinaceae	*Heppia, Lemmopsis, Metamelanea*
			Pecania, Porocyphus, Psorotichia
			Pterygiopsis, Pyrenocarpon
			Pyrenopsis,Thermutis
		Peltulaceae	*Peltula*
Basidiomycota	Agaricales	Hygrophoraceae	*Cyphellostereum, Dictyonema*

The lichens of the three families Lichinaceae, Peltulaceae and Hygrophoraceae, were all tiny and often difficult to identify. Hence, they are described in detail below.

Table 3.2: Checklist of taxa identified to the species level

	Species	Location	Herbarium number
*	*Coccocarpia erythroxyli* (Spreng.) Swinsc. & Krog	AT, NC, CT	124041, 13113f, 122246
*	*Coccocarpia glaucina* Kremp.	AT, NC	122236, 13118n, 122244c, 122250
*	*Coccocarpia microphyllina* Lücking & Aptroot	AT	13118i, 13112e
	Coccocarpia palmicola (Spreng.) Arv. & D.J. Galloway.	AT, NC, ST, DR	13118k, 122238, 122252, 13117f
*	*Coccocarpia pellita* (Ach.) Müll. Arg.	CT	124025, 124029, 13113h, 13112f
	Collema furfuraceum (Arnold) Du Rietz	AT, NC	122201, 13117j, 13112d, 13115f
	Collema pulcellum var. *subnigrescens (Müll. Arg.) Degel.*	NC	122189
*	*Dictyonema moorei* (Nyl.) Henssen	AT	13112b3a, 13112h
*	*Heppia lutosa* (Ach.) Nyl.	NT	122058, 122270
*	*Lemmopsis arnoldiana* (Hepp) Zahlbr.	NT	122211
*	*Leptogium austroamericanum* (Malme) C. W. Dodge.	NC	122160, 122177
	Leptogium azureum (Swartz) Mont.	DR, PD, NC	122263, 122268, 13502m13803d
*	*Leptogium cochleatum* (Dicks.) P.M.Jørg.& P.James.	DR, ST, AT, NC	122259, 122264, 122265, 13111k
*	*Leptogium coralloideum* (Meyen & Flot.) Vain.	AT	122178, 13111j
	Leptogium cyanescens (Pers.) Körb.	DR, PD, ST, AT, CT, NC	124004, 122240, 13111h, 13111c
*	*Leptogium denticulatum* F. Wilson	DR, PD, BM, ST, AT, CT, NC	124005, 122174, 13205d, 13414k
*	*Leptogium marginellum* (Sw.) Gray	DR, H, AT, NC	122179, 122180, 13111a, 13112c
*	*Leptogium poliophaeum* Verdon	CT	124017, 124042
*	*Metamelanea umbonata* Henssen	QN	13109b-1, 13109b-2, 13109b
*	*Pannaria tavaresii* P.M. Jørg.	AT	13115h-1, 13115h-2
	Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J. Galloway	DR, AT, NC	122254, 122262,13117c,122163
	Parmeliella nigrocincta (Mont.) Müll. Arg.	AT, NC	122232, 122260, 13117e-5
*	*Peccania tiruncula* (Nyl.) Henssen	NT	122075

Continued on next page

Table 3.2: Checklist of taxa identified to the species level (continued from previous page)

	Species	Location	Herbarium number
*	*Peltula bolanderi* (Tuck.) Wetmore	QN, NC	122050, 122001, 122004, 123013a
*	*Peltula clavata* (Kremp.) Wetmore	CT	124030, 124038, 124048, 124051
*	*Peltula euploca* (Ach.) Poelt	QN	123020
*	*Peltula impressa* (Vain.) Swinscow & Krog	ST, NT	122013, 122014, 122029, 13205a-2
	Peltula obscurans (Nyl.) Gyel.	CT, NT, NC	124034, 124035, 122118, 122120
*	*Peltula omphaliza* (Nyl.) Wetmore	NT	122209
*	*Peltula placodizans* (Zahlbr.) Wetmore	ST, NT	122045, 122048, 122030, 13205a-1
	Physma byrsaeum (Ach.) Tuck.	AT	13115k, 13112b1, 13112b3, 13115d
*	*Physma pseudoisidiatum* Aptroot & Sipman	AT	13117h2, 13117g
*	*Porocyphus coccodes* (Flot.) Körb.	NT	122074, 122076
*	*Porocyphus dimorphus* Henssen	QN, NT	122124, 123018
*	*Pseudocyphellaria argyracea* (Delise) Vainio	AT	13115j, 13118c
*	*Pseudocyphellaria aurata* (Ach.) Vainio.	AT	13113a, 13115c1
*	*Pseudocyphellaria ardesiaca* D.J.Galloway	AT	13115c2, 13112g-1, 13112h
*	*Psorotichia americana* s. lat.	NT	122230
*	*Pterygiopsis guyanensis* Schultz	NT	122041, 122042
*	*Pyrenocarpon thelostoma* (Ach. ex J. Harriman) Coppins & Aptroot	NT	122132, 122138a
*	*Pyrenopsis polycocca* (Nyl.) Tuck.	NC	122011, 122012
*	*Pyrenopsis portoricensis* Zahl.	QN, NC	13108e, 122199
*	*Pyrenopsis subareolata* Nyl.	NT	122140
**	*Pyrenopsis melanophthalma* Büdel & Schultz & Võ	QN	123013, 123014, 123015
*	*Pyrenopsis triptococca* Nyl.	NT	122100, 122106
*	*Ramalodium neocalendonicum* (Räs) Henssen	AT	13118d
*	*Spilonema americanum* (Henssen & Tonsberg) T. Sprib., Tønsberg & Muggia	NC	122244a

Continued on next page

Table 3.2: Checklist of taxa identified to the species level (continued from previous page)

	Species	Location	Herbarium number
*	*Staurolemma* cf. *perforatum* P.M.Jørg.	AT	13117a
*	*Sticta marginifera* Mont.	NC	122231, 122237, 122241a, 122258,
*	*Sticta cyphellulata* (Müll.Arg.) Hue	AT	13118a2, 13118a3
*	*Sticta duplolimbata* (Hue) Vain	AT	13118a1
*	*Thermutis velutina* (Ach.) Flotow	NT	122138b

*: new species for Vietnam; **: new species; AT: An Toan, BM: Bach Ma, CT: Cat Tien, DR: Dakrong, J: Hue, near to Phong Dien, LGXM: Lo Go Xa Mat, NC: Nui Chua, NT: Ninh Thuan, PD: Phong Dien, QN: Qui Nhon, ST: Son Tra. *Ramalodium neocaledonicum* and *Staurolemma* cf. *perforatum* were collected at the height 12 m on a fallen tree

Table 3.3: Checklist of taxa identified to the genus level or unidentified

	Species	Location	Herbarium number
	Collema sp.	NC	122200
*	*Collemopsidum* sp.	NT	122301, 122021
*	*Cyphellostereum* sp. 1	LGXM	13112g-2
*	*Cyphellostereum* sp. 2	AT	13118e
*	*Cyphellostereum* sp. 3	CT, LGXM	124054, 121032
*	*Dictyonema* sp.	NC	122244b
	Pannaria sp.	AT, NC	122242, 13118g-2, 122159, 13117e
*	*Peltula* sp.	NC	122040, 122272
*	*Porocyphus* sp.	AT	13802t-2
*	*Pterygiopsis* sp.	NT	122052
	Leptogium sp.	NC, AT	13116e, 122244e
**	Lichinaceae A	NC	122229, 122150, 122151
**	Lichinaceae B	DR	13802f
	cyanolichen 123016	QN, ST	123016,13204b

*: new records for Vietnam, **: new species. AT: An Toan, CT: Cat Tien, DR:Dakrong, LGXM: Lo Go Xa Mat, NC: Nui Chua, NT: Ninh Thuan, QN: Qui Nhon

3.1.2 Lichens of the family Lichinaceae

Twenty percent of the species treated in this study are members of the family Lichinaceae and were also new records for the lichen flora of Vietnam. Most of them occurred on exposed rocks at coastal outcrops. More than 70% of these species were collected in Ninh Thuan Province, which is the driest region of Vietnam. Some species were found on old pieces of corals or shells collected at the beach and on ancient coral ridges in the tidal zone. The saxicolous lichens grew either on granite or on calcareous rocks, the latter originating from corals. Only one terricolous lichen, *Heppia lutosa*, was found in stable sandy soil next to the beach or soil from weathered rock.

***Heppia lutosa* (Ach.) Nyl., Syn. Lich. 2: 45 (1863)**

Ref.: (Marton and Galun, 1974; Büdel, 1987; Henssen, 1994; Büdel and Schultz, 2002)

Fig. 3.1 D–F

Thallus: squamulose to peltate, olive brown or grayish black, lobate, almost immersed in or closely attached to subtrate, subgelatinous; lobes aggregate or simple, up to 1–2.5 mm diameter, 110–150 µm thick, margin often deep black; surface rough, rimose, flat or concave. Thallus anatomy: homoiomerous, loose structure by perpendicularly fungal hyphae, fungal cells globose or elongate: 8–10 x 4 µm; upper side: phenocortex not continuous, lower side: ecorticate; attachment: single rhizohyphae, 6 µm wide, thick walled, 2 µm thick in lumina. Photobiont: *Scytonema*, clustered in thallus, (7.5)–12(–17) µm. Ascomata: immersed apothecia, several per thallus, urceolate, disc red brown, about 0.2–0.25 mm diam; exciple: ca. 25 µm thick, composed of loose fungal hyphae, hymenium I+ pale blue then red, K/I+ blue, about 150 µm tall; subhymenium 24–36 µm thick; asci: cylindrical, 8-spored, cylindrical/obvoid, asci wall I+ blue; ascospores hyaline, simple, elliptic, 15–26 x 7.5–8.5 µm. Conidiomata: pycnidia spherical, immersed in thallus, about 110 µm diameter, inside wall convoluted, conidia baciliform, 2.5–3 x 1–1.5 µm.

Ecology and distribution: This species grew together with *Scytonema* on soil on rocky outcrops, next to the beach in the Vinh Hai commune, Ninh Thuan Province, Vietnam.

Studied specimens No.: 122270, 122058

Notes: The studied specimens show a thin thallus (110–150 µm thick) compared to the same species from Africa (170–380 µm thick) (Büdel, 1987) and in North America (>200 µm thick) (Büdel and Schultz, 2002); the phenocortex was found along the upper surface and around magin of aged thalli. This layer is composed of both, cyanobiont and mycobiont cells (hyphae) that is compacted and thicker in aged thalli. The cyanobiont cells spread to the upper surface as in the description of *Heppia echinulata* (Marton and Galun, 1974) in two specimens. In old thalli, the dead photobiont cells were pale yellow or brownish. In the lower thallus parts, we observed the lichenization process in which the fungal hyphae change the arrangement of the photobiont filaments into a cluster of photobiont cells, where the filamentous structure of the photobiont could no more longer be

recognized (Fig. 3.1 E). This was also be observed in fresh cultures, where the photobiont was still not independent from the fungal haustoria (Fig. 3.1 F).

***Lemmopsis arnoldiana* (Hepp) Zahlbr., Natürl. Pflanzenfam. Teil 1: 171 (1906)**.

Ref: (Schultz and Büdel, 2002; Jørgensen, 2007)

Fig. 3.1 G

Thallus: crustose-areolate, poorly developed, blackish; areoles scattered, thin, 70–80 µm thick, small, up to 0.1 mm in diameter. Thallus anatomy: homoiomerous, paraplectenchymatous. Photobiont: chroococcoid cyanobacterium. Ascomata: apothecia, 0.15–0.2 mm in diameter, urceolate, prominent, thallus margin at the beginning cover all apothecia, then recede, exposing brown disc, more/less flat or concave; exciple proper: conspicuous, composed interwoven hyphae, narrow at base, enlarge at tip to 30 µm wide; hymenium hyaline, K/I+ blue, 110–120 µm high, paraphyses septate, often anatomosing at base, conglutinate and branched at tips, apical cells thickened; asci narrow clavate, K/I+ blue, 8-spored; ascospores simple, hyaline, oval, 11.5–13.5 x 5–6 µm. Conidiomata: not observed.

Ecology and distribution: Found on exposed rock in a shallow stream effected by anthropogenic impacts in Ninh Thuan Province.

Studied specimen No.: 122211.

Lichinacean species A

Fig. 3.1 A–C

Thallus: crustose-areolate, dark green to black; cracked areoles angular, surface smooth, thin toward margin, 0.15–0.4 mm wide, (30–)55–80 µm thick. Thallus anatomy ecorticate, homoiomerous, paraplectenchymatous, hyphae cells 3–5.5 µm; photobionts cells in clusters; attachment hyphal rhizines. Photobionts mainly *Gloecapsa*-like cyanobacterium, cells 2.5–3.7(–4.3) µm diameter, brown-yellow sheath, but also the green alga *Trebouxia* occurs. Ascomata: of the apothecia type, immersed, then prominent, thallus margin receded, pale proper margin

exposed, up to 0.2–0.25 µm diameter; dics flat, reddish brown, 0.1–0.2 mm diameter, flat then concave; proper exciple thin, 20–25 µm wide apically, subparaplectenchymatous of moniliform hyphae; hymenium K/I+ blue, 40–60(–90) µm high, divided by sterile hyphae; paraphyses thick, moniliform, branched, apical cells 4.5–7 x 3–3.5 µm; asci narrow clavate, apical wall thickened, rostrate, 8-spored, K/I+ dark blue in amyloid cap; ascospores hyaline, simple, subglobose, 11.5–14.5 x 7–8.5 µm, thick-walled 1 µm. Conidiomata: pycnidia ovoid, immersed in thallus, wall convoluted, hyaline; conidia bacilliform, 3.5–4 x 1 µm.

Ecology and distribution: Found on boulders beside the stream that could be inundated in the rainy season, Ninh Thuan Province. This species was found on both shady and exposed habitat.

Studied specimen 122229, 122150, 122151.

Note: This is an unknow genus that is close to *Lemmopsis* and *Euopsis*. Characteristics are prototunicate asci, ascoma developing from ascogones arising in tangles of generative hyphae in the thallus, paraphyse moniliform, two photobionts: green algae *Trebouxia* and a *Gloecapsa*-like cyanobacterium (Fig. 3.1 B) suggest genus *Euopsis*. Howerver, the ascus is lecanoralean-type (Fig. 3.1 C) instead of *Euopsis*-type that was found in *Euopsis* (Henssen et al., 1988). In another respect, the ascoma appearance, exciple, thallus structure, and hymenium divide by sterile hyphae resemble *Lemmopsis*.

Lichinacean species B

Fig. 3.12 (page 67)

Specimens No. 13802f, 13802c-2

This species is still unidentified. Thallus: morphology black brown, foliose; narrow distended lobes, lobe tips round or branched, sometimes raised up; attached by rhizines. Thallus anatomy: homoiomerous, cartilaginous; hyphae reticular, meshes photobiont in groups. Photobiont: *Chroococcidiopsis*, cell content purple or pale blue green, clusters of 2, 4 or many cells. Ascomata: not mature, develop from a tangle of generative hyphae. Conidiomata: pycnidia spherical, immersed, white pore, convoluted wall, conidia bacillar, 2 µm long.

Ecology and distribution: This lichen was collected from a outside wall of a limestone cave in Dakrong.

Remarks: This unknown species was characterized by pycnidia with a convoluted wall and a bright ostiole; the thallus structure of the fan-shaped thallus (Fig. 3.12 C) is similar to *Pterygiopsis* (Henssen, 1963; Schultz and Büdel, 2002; Jørgensen, 2007; Schultz, 2007a) but ascomata ontogeny starts from a tangle of generative hyphae; photobiont is a purple colored species of *Chroococcidiopsis* (Fig. 3.12 E–I, page 67). New findings from this *Chroococcidiopsis* strain were mentioned in detail in the sections 3.2.1 (page 62), and 4.2.1 (page 84).

***Metamelanea umbonata* Henssen, Lichenologist 21(2): 105 (1989)**

Ref.: (Henssen, 1989)

Fig. 3.1 H

Thallus: crustose, areolate, reddish brown, gelatinous when wet; areoles diffuse, rough, 0.05–0.4 mm broad. Thallus anatomy: ecorticate, loose structure, with clustered photobiont aggregates, reddish brown towards surface. Photobiont: a chroococcoid cyanobacterium, reddish sheath. Ascomata: apothecia, immersed in the thallus, apothecia aggregated, later forming on big apothecia, disc umbonate; exciple composed of slender hyphae, loosely interwoven, branching, hyaline; hymenium hyaline, K/I+ blue, 50–90 µm high; paraphyses septate, branching, apical cell clavate 5 x 3 µm; subhymenium hyaline, ca. 20 µm high; asci clavate, 8-spored; ascospores eliptic, simple, hyaline, 6–8 x 3–7 µm. Conidiomata: pycnidia, immersed in thallus, conidia elliptic, 2 x 1 µm.

Ecology and distribution: Found on rock in the coastal region in Phuong Mai Peninsula, Qui Nhon City.

Studied material: 13109b

***Peccania tiruncula* (Nyl.) Henssen, Lichenologist 22(2): 143 (1990)**

Ref.: (Brown et al., 2002; Tretiach and Schultz, 2008)

Fig. 3.1 I

Thallus: dwarf fruticose, in scattered cushions, black, glossy, gelatinous, swollen and brownish yellow or greenish olive when wet; cushions small, usually less than 1 mm; lobes cylindrical, branched, 0.35–0.45 mm long, 60–80 µm in diameter when wet, with rounded tips. Thallus anatomy: ecorticate, heteromerous, gelatinous with a loosely reticular matrix; hyphal strands at the lower side. Photobiont: a chroococcoid cyanobacterium, 4.5–6.5 µm in diameter, with concentric gelatinous layers. Ascomata: apothecioid, sessile, 0.3–0.35 mm in diameter; thalline margin prominent; disc black, flat; hymenium reddish brown in upper parts, 70–85 µm high, K/I+ blue; paraphyses septate, branched at tip; asci elongate, thick walled at tip, 8-spored; ascospores simple, hyaline, broad elliptic, 8.5–11 x 6–7 µm. Conidiomata: pycnidia immersed in lobes, conidia filiform 25–30(–35) µm.

Ecology and distribution: on coral in sand dunes in Thai An village, next to the beach in Ninh Thuan Province.

Studied material: 122075.

Porocyphus coccodes (Flot.) **Körb., Syst. Lich. Germ.: 426 (1855)**

Ref.: (Henssen, 1963)

Fig. 3.2 A

Thallus: crustose-areolate, black or brown, hyaline olive green when wet; areoles: granular or coralloid. Thallus anatomy: ecorticate, homoiomerous, fungal hyphae cells round or elongate, arranged in clade-like; photobiont cells single or in pairs, in gelatinous sheath, hyaline in thallus centre, yellowish brown in thallus surface; attached by thick-walled rhizines. Photobiont a chroococoid cyanobacterium. Ascomata: apothecia, laminal, one to several per areole, immersed, 100–200 µm diameter; juvenile disc convex, later on expanded, flat to concave, reddish brown; exciple of loosely interwoven hyphae; hymenium KI+ blue, ca. 50 µm high; hypothecium ca. 17 µm high; paraphyses septate, branching at tips; asci thin walled, 8-spored; ascospores hyaline, elliptic, (12–)16–20 x 5–7 µm. Conidiomata: pycnidia, pyriform, immersed; conidia bacillar or subglobose 1 µm diameter, embeded together.

Ecology and distribution: Found on a piece of coral in sand dunes along the beach, Ninh Thuan Province.

Studied material: 122076, 122074

Note: The coralloid areoles with tiny horizontal and cylindrical lobes can only be seen at a high magnification. The ascomata in this species are laminal instead of terminal in *P. dimorphus*. Moreover, *P. coccodes* is attached to the substrate by rhizines while the horizontal part of *P. dimorphus* is attached by a gelatinous layer.

***Porocyphus dimorphus* Henssen, Symb. Bot. Ups. 18 (1): 123 (1963)**

Ref.: (Henssen, 1963; Schultz, 2007c)

Fig. 3.1 J–K

Thallus: dark olive (specimen 123018) or shiny black (specimen 122124), basal lobes horizontal, effigurate marginally, slender, 22–44 µm wide, 0.15–0.22 mm long; vertical lobes: arised from basal lobes or from the thalline margin of old ascoma, cylindrical, thickened at tip, 20–50 µm wide, 166–280 µm high when wet. Thallus anatomy: ecorticate, homoiomerous, hyphae cells arrange in fountain-like pattern. Photobiont: a filamentous cyanobacterium, cells 4.3–6.5 µm diameter Ascomata: pycnoascocarpia, subglobose, at tips of vertical lobes, 0.9–1.1 mm diameter; disc punctiform, brown; hymenium K/I+ blue, 110–130 µm high; asci cylindrical, thin wall, bring 8 spores uniseriate; ascospores globose to broad-ellipsoid, hyaline, simple, 8–13 x 5.5–6.5 µm. Conidiomata: pycnidia, globose, immersed in thallus, conidia elliptic, 2.2–2.4 x 1.2 µm.

Ecology and distribution: grew together with *Scytonema* and *Peltula* on exposed rocks of an outcrop in Binh Dinh Province, and Ninh Thuan Province. Studied material: 123018, 122124.

***Porocyphus* sp.**

Fig. 3.2 B

Thallus: crustose, areolate, blackish brown, surface smooth, cracked-areoles flat or uneven. Thallus anatomy: ecorticate, paraplectenchymatous, 100–120 µm thick; hyphal cells round or elongate, thallus separated partly by vertical hyphae trands; photobiont cells in clusters. Photobiont: a chroococcoid cyanobacterium, 2.5–3.5(–4.5) µm diameter. Ascomata: pycnoascocarpia, develop beneath pycnidia, immersed in thallus first, then pale yellow exciple exposed, ascoma reach to 0.4 mm diameter; hymenium K/I+ blue, paraphyses simple, slender; asci cylindrical, 8-spored, 6–7 x 5–5.5 µm, asci wall K/I+ blue; ascospores simple, hyaline, 16.5–17.5 x 7–8 µm. Conidiomata: pycnidia, immersed, broad oval, conidia bacillar 1.7–2.3 µm long.

Ecology and distribution: on shady limestone with *Leptogium*, Chinh Cave located in Dakrong Nature Reserve, Quang Tri Province.

Studied material: 13802t

Notes: The studied specimen is morphologically similar to *Porocyphus ruttneri*. However, it differs from *P. ruttneri* by its short bacillary conidia (2 µm long, in *P. ruttneri* 10 µm long; Henssen 1963). The thallus is not effigurate as in *P. effiguratus* or *P. kenmorensis* in the group of placoid thalli of the genus *Porocyphus* (Henssen, 1974).

***Pterygiopsis guyanensis* M. Schultz, Porembski et Büdel Pl. Biol. (Stuttgart) 2: 489 (2000)**

Ref.: (Schultz et al., 2000)

Fig. 3.2 C

Thallus: dark red or dark olive, crustose, areolate, marginal areoles effigurate, central areoles smaller, angular, isidia-like or granular-like structure on surface. Thallus anatomy: paraplectenchymatous, hyphae cells and photobiont cells fan-like in cross-section; fungal cells 3.75–5 µm diameter. Photobiont: chroococcoid cyanobacteria in red sheath, cells 5–6.25 µm diameter in marginal thallus, 7.5–12.5 µm diameter in central thallus. Ascomata: pycnoascocarp, julvenile. Conidiomata:

pycnidia, immersed in thallus, pointed, spherical, wall convoluted, 85–100 µm diameter, conidia cylindirical 3–4 x 0.5–0.8 µm.

Ecology and distribution: on exposed rock in rocky mountain in the semi-arid area, Ninh Thuan Province.

Studied material: 122041, 122042.

***Pterygiopsis* sp.**

Fig. 3.2 D

Thallus: red-brown, crustose-areolate, marginal areoles effigurate, central areoles usually granular numerously, 0.15–0.25 µm wide. Thallus anatomy: ecorticate, paraplectenchymatous, 50–115 µm thick, hyphae and photobiont cells arrange in fan-shape. Photobiont: chroococcoid cyanobacterium, 6.5–9.5 µm diameter, red sheath toward surfaces. Ascomata: apothecia, one per areole, immersed, disc black, still young. Conidiomata: pycnidia, immersed in thallus, spherical, wall convoluted; conidia bacilliform, 1.7–2.5 µm.

Ecology and distribution: on rock, with *Peltula bolanderi* on an outcrop with sparse shrub vegetation, *Opuntia dillenii* and *Lagerstroemia lecomtei*, in Ninh Thuan Province.

Studied specimen No. 122052.

Notes: This species is characterized by its separate areoles with numerous obvious granules. The habitus resembles those of *Pyrenopsis triptococca* but the thallus anatomy is fan-shape and there are sterile hyphae in the hymenium.

***Pyrenocarpon thelostomum* (Ach. ex J. Harriman) Coppins & Aptroot, Lichenologist 40(5): 372 (2008)**

Ref.: (Coppins et al., 1992; Jørgensen, 2007; Thüs and Schultz, 2008)

Fig. 3.2 E

Thallus: crustose-areolate, brownish black, areoles sparsely, irregular, to 0.15 mm wide, thin, surface flat, gelatinous when wet; 100–180 µm thick. Thallus

anatomy: ecorticate, homoiomerous, paraplectenchymatous, epinecral layer 18–24 µm thick, fungal hyphae reticulate, cells irregular (globose, angular, or cylindrical) 1.7–2.6 µm thick; photobiont cells in cluster, enclosed in brownish yellow sheaths. Photobiont: unicellular cyanobacterium *Chroococcidiopsis*. Ascomata: apothecia immersed in thallus when young, then prominent in hemispherical or disc-shaped; disc pale yellow/brownish, convex, thallus margin persistent; exciple proper paraplectenchymatous of moniliform cells, exciple 20 µm wide at tips; hymenium KI+ blue, divided by sterile hyphae; paraphyses slender, septate, branched at tips, anatomosing, apical cells moniliform, 1.2–1.5 x 5–6 µm; asci thin wall, 8-spored; ascospores simple, hyaline, 9–12.5 x 4.5–6.5 µm. Conidiomata: pycnidia immersed to thallus, inner wall convoluted, oval ca. 200 x 300 µm wide; conidia globose 1.5–1.8 µm diameter, or broad elliptic 1.5 x 2.5 µm.

Ecology and distribution: grew with *Thermutis velutina* on shady rocks along a perennial stream in Cong Hai commune, Ninh Thuan Province.

Studied material : 122138a

Notes: In this species, the epihymenium was covered by released spores and thallus particles. Lichenized photobiont clusters could be seen adjacent to sterile hyphal strands in the hymenium. Probably originated from parts of the thallus between ascoma initials. An aggregated apothecium developed, that appeared as a homogeneous hymenium. The cyanobacterial photobiont *Chroococcidiopsis* was isolated and grown in culture medium. It reproduced by both, binary fission in the first stage, then multiple fission when colonies grew indepently.

***Psorotichia americana* s. lat.**

Fig. 3.2 F

Thallus: crustose-areolate, dark green or olive green, areoles minute, irregular, 0.1–0.2(–0.5) µm, marginal areoles thin and appressed, central areoles cracked; surface smooth. Thallus anatomy: ecorticate, homoiomerous, paraplectenchymatous, (50–)110–145 µm thick, hyphae cells round to elongate, 3–3.5 µm wide, 5.5–7 µm long, vertical arrangement, photobiont cells in irregular clusters. Photobiont: chroococcoid cyanobacterium, 2.3–3(–3.5) µm diameter, brown yellow sheath; at-

tachment by short interwoven hyphae. Ascomata: apothecia, immersed in thallus, disc-shaped, broad at base, 0.1–0.2 µm diameter, thallus margin flat, not prominent; disc brown, hyaline when wet, flat, 60–100 µm diameter; proper exciple thin, subparaplectenchymatous of moniliform hyphal cells, 20–25 µm wide apically, hyaline; epihymenium colourless; hymenium hyaline, K/I-, 88–110 µm high; subhymenium 10–25 µm thick; paraphyses septate, slender, anatomosing and branching, apical cells 1 x 2.3–3.5 µm; asci narrow clavate, thin wall, 8-spored, K/I-; ascospores simple, hyaline, elliptic, thin wall, (15.5–)18–20 x 6–7 µm, K/I+ pale blue then fading. Conidiomata: pycnidia, immersed in thallus, oval, 140 x 90 µm, yellow in wall and pore; conidia oblong, 5.5 x 3 µm.

Ecology and distribution: on boulder on bank of stream which can be inundated in the rainy season; Tien Spring, Ninh Thuan Province.

Studied specimen: 122230

Notes: ascomata in this species were almost embedded and immersed in the green thallus, with a broad base.

***Pyrenopsis melanophthalma* Büdel, Schultz & Võ, sp. nov.**

Ref.: (Schultz et al., 2000); corresponding to *Pyrenopsis* spec. e

Fig. 3.2 K

Thallus: crustose-areolate, brownish red, dull; areoles minute, thin, angular, 0.05–0.1 mm wide. Thallus anatomy: paraplectenchymatous, loosely structure, broken easily. Photobiont: a unicellular cyanobacterium, with red sheath. Ascomata: apothecia, flat, distinct, immersed in thallus, thalline margin prominent, 0.17–0.35 mm diameter, proper exciple about 18 µm, composing of loose hyphae; disc black, flat, 0.1–0.25 mm diameter; hymenium thin 80–100 µm high, K/I+ blue, paraphyses moniliform; asci 8-spored, clavate, thick wall (ca. 2 µm), thick gelatinous outermost layer at tip K/I+ blue, inner amyloid cap K/I+ dark blue; ascospores simple, elliptic to broad elliptic, hyaline, 14–22 x 6–8 µm. Conidiomata: pycnidia immersed in areoles, elliptic, 60 x 80 µm; conidia narrow elliptic, 2–3 x 1 µm.

Ecology and distribution: on exposed rock of an outcrop in Bai Rang, Qui Nhon city.

Studied material: 123013, 123014 and 123015

This species was mentioned by Schultz et al. (2000) from Venezuela, voucher species Büdel 24067a. The type in Vietnam is characterized by numerous, large, conspicuous apothecia with black discs that are embedded in a dull brownish red thallus, rostrate asci with a rather thick outermost gelatinous cap, uniformly thin thallus.

The species was not regularly described as a new species Schultz et al. (2000). Therefore, we suggest the name *Pyrenopsis melanophthalma* implying the neat apothecia with jet black discs well contrasting with the surrounding dark brown red thallus.

The holotype (123014) and an isotype (123015) were deposited to HBG, an isotype (123013) was kept in PHH.

***Pyrenopsis polycocca* (Nyl.) Tuck., Syn. North Am. Lich. I: 136(1882)**

Fig. 3.2 G

Thallus: reddish brown, swollen when wet, crustose-areolate, areoles angular, central areoles 0.1–0.3 µm broad, 35–60 µm thick, marginal areoles effigurate, 0.3–0.5 x 0.1–0.25 µm, thick 75–100 µm; upper surface granular, or isidioid, lower side attached to substrate by hyphae strands in gelatinous layer. Thallus anatomy: ecorticate, homoiomerous, paraplectenchymatous, photobiont coccoid cyanobacterium with red sheath. Photobiont: chroococcoid cyanobacterium. Ascomata: pycnocarp, immersed in thallus, one per thallus, disc flat, black; epihymenium brownish yellow, hymenium K/I+ blue, 65–90 µm high, asci cylindrical to subclavate, ca. 16-24 spores; ascospore hyaline, globose, 3.8–4.5 µm or subglobose 3.5 x 5.5 µm. Conidiomata: not observed.

Ecology and distribution: grew together with *Peltula* on rocks in an outcrop in Khanh Hai district, Ninh Thuan Province.

Studied material: 122011, 122012

***Pyrenopsis portoricensis* Zahlbr., Mycologia 22(2): 72 (1930)**

Fig. 3.2 H

Thallus: crustose, areolate, reddish brown; areoles, irregular, angular, separated, 0.1–0.4 mm diameter, granular on surface. Thallus anatomy: homoiomerous with loosely interwoven hyphae, 90–110 µm thick. Photobiont: a chroococcoid cyanobacterium with red sheaths. Ascomata: apothecia one per areole, immersed in thallus, expanded gradually, thallus margin flat; dics black, rough, umbonate, up to 200 µm diameter; epihymenium brown, hymenium divided by sterile hyphae, K/I+ blue; paraphyses moniliform, branching, anatomosing; subhymenium elongated as stipe; asci clavate, 8-spored, K/I+ blue; ascospores simple, hyaline, ellipsoid, 5–9 x 2.5–3.5 µm. Conidiomata: pycnidia, immersed in thallus, ovoid, conidia bacilliform, ca. 3 µm long.

Ecology and distribution: On exposed rock in Eo Gio, Nhon Ly Commune, Qui Nhon city. Studied material : 13108e

***Pyrenopsis subareolata* Nyl., Not. Sällsk. Fauna Fl. Fenn. Förh. 5: 27 (1861)**

Fig. 3.2 I

Thallus: crustose-areolate, dark brown; areoles irregular, rimose, uneven, 160–250 µm wide, marginal areoles about 60 µm thick, central areoles 0.15–0.2 mm thick. Thallus anatomy: homoiomerous, paraplectenchymatous. Photobiont: chroococcoid cyanobacterium 5–6.5 µm diameter, in clusters with red sheath. Ascomata: apothecia one per areole, immersed, disc pointed, dark-redish brown; proper exciple thin, hymenium 80–90 µm, K/I+ blue, subhymenium 35–40 µm, asci 8-spored; ascospores ovoid, hyaline, 13 x 5.5 µm. Conidiomata: not observed.

Ecology and distribution: on exposed boulder along the bank of a spring in Den village, Ninh Thuan Province.

Studied specimen 122140

***Pyrenopsis triptoccocca* Nyl., Flora 64: 2 (1881)**

Ref.: (Schultz, 2007d)

Fig. 3.2 J

Thallus: crustose-areolate, darkish brown, warty on surface; areoles angular, tiny, 0.1–0.3 mm wide, 60–85 µm thick, marginal areoles smooth, effigurate, central areoles granular on surface, granules 50–70 µm diameter. Thallus anatomy: homoiomerous, paraplectenchymatous. Photobiont: *Gloeocapsa*-like cyanobacterium, 9.5–11.5 µm diameter with reddish brown sheath. Ascomata: pycnoascocarps developed beneath pycnidia, sessile, urceolate, 0.2–0.3 mm diam, thalline margin prominent and crenulate, disc flat, yellowish then brown; exciple lacking; hymenium K/I+ blue, 100 µm high; paraphyses septate, branched, moniliform, cells 1.5 µm wide at base, 2.8–3.4 µm wide at tip; subhymenium rather thick, ca. 70 µm high; asci rostrate, 8-spored, elongate, thick outermost gelatinous cap, K/I+ blue, amyloid inner cap K/I+ dark blue; ascospores simple, hyaline, broad elliptic, 12–16 x 8.5–10 µm. Conidiomata: pycnidia, spherical, immersed in thallus, conidia bacilliform, 3.5–4.5 x 0.7–0.9 µm.

Ecology and distribute: on rock in a grassland next to an outcrop in Lang Me Village, Ninh Thuan Province.

Studied material: 122100

Notes: This species is characterized by a spherical shape of the prominent apothecia with crenulate thalline margin on a warty thallus, and with a thick subhymenium.

***Thermutis velutina* (Ach.) Flot., Linnaea 23: 170 (1850)**

Ref.: (Henssen, 1963)

Fig. 3.2 L

Thallus: filamentous in cushion, shiny black; filaments vertical, 15–17 µm diameter. Thallus anatomy: fungal hyphae slender, 1.6–2 µm wide. Photobiont: filamentous cyanobacterium *Scytonema* with a reddish sheath, cells 10 x 3.5–4

µm, sparsely surrounded by fungal hyphae. Ascomata: not observed. Conidiomata: pycnidia lateral, brownish yellow, oval 45–48 µm wide, conidia globose, 1–1.5 µm diameter.

Ecology and distribution: This species grew together with *Pyrenocarpon thelostoma* on shady rocks along a perennial stream in Cong Hai commune, Ninh Thuan Province.

Specimen 122138b

Notes: The habitus of this species resembles the free living cyanobacterium *Scytonema* when not all photobiont filaments of the cushions are lichenized. If lichenized, the fungal hyphae sparsely surrounded the photobiont. A densely wrapped layer of the mycobiont only surrounds the photobiont filaments where pycnidia are present. The cells of photobiont are more or less larger in the adjacent area of pycnidia.

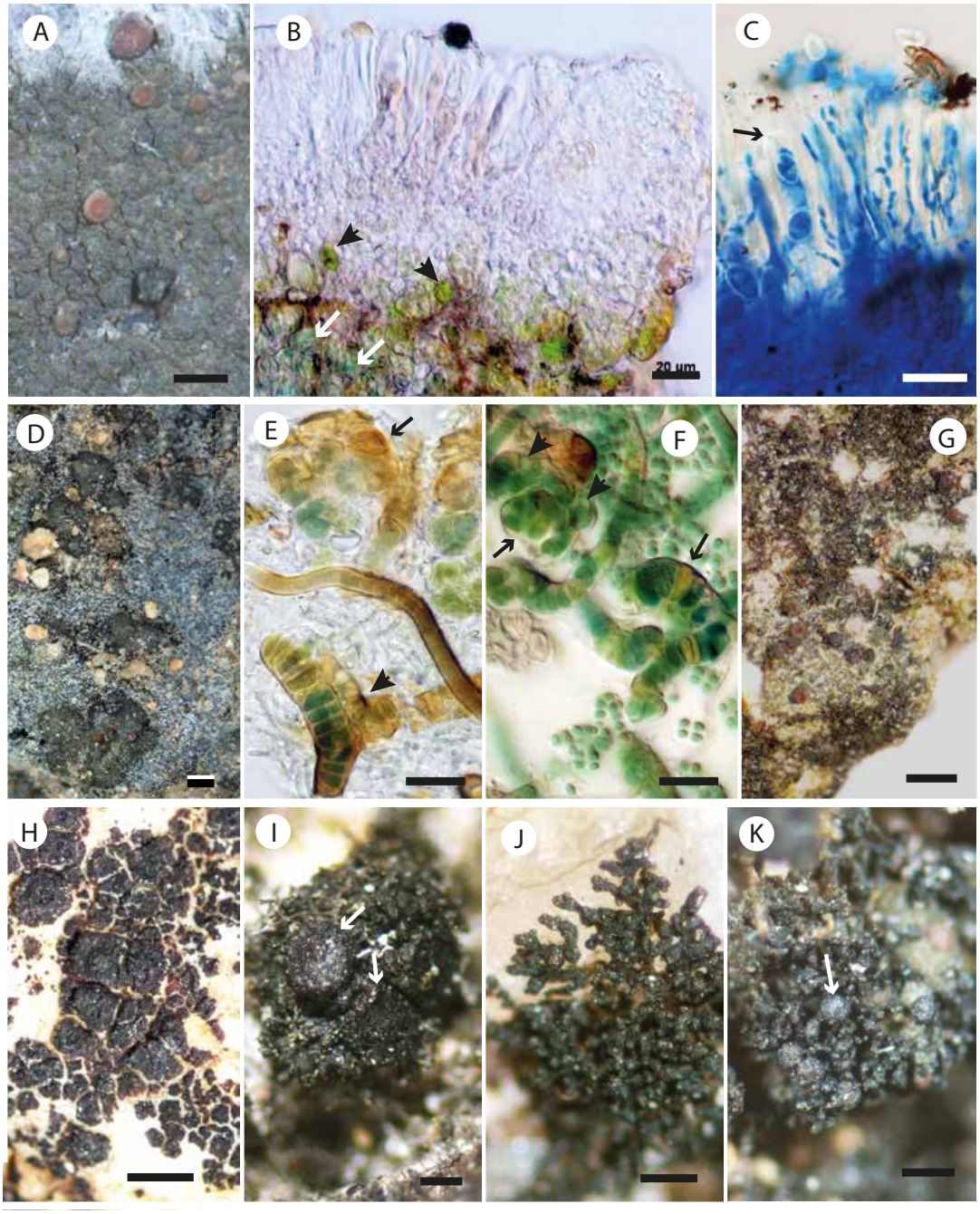

Figure 3.1: Morphology and anatomy of Lichinacean species A, *Heppia lutosa, Lemmopsis arnoldiana, Metamelanea umbonata, Peccania tiruncula*, and *Porocyphus dimorphus*

A-C: species of the Lichinacean species A, **A**, morphology, **B**, two photobionts in a cross section of the ascoma, green algae (arrow heads) and cyanobacterium (arrows), **C**, lecanoralean ascus (arrow) and moniliform paraphyses in Lactophenol Cotton Blue; **D-F**: *Heppia lutosa*, **D**, habit, **E**, thallus cross section with phenocortex at the upper surface (arrow) and structures of lichenization between mycobiont and photobiont *Scytonema* in the lower part of the thallus (arrow head), **F**, parts of lichenized photobiont filaments in culture medium (arrows) and photobiont cells penetrated by haustoria (arrow heads); **G**, *Lemmopsis arnoldiana*; **H**, *Metamelanea umbonata*; **I**, *Peccania tiruncula* with ascomata (arrows); **J**–**K**: *Porocyphus dimorphus*, **J**, vertical thallus, **K**, horizontal thallus with pycnoascocarps at the end of thallus lobes (arrow). Scales: A, D, G and H: 500 µm; I–K: 200 µm; C, E and F: 20 µm

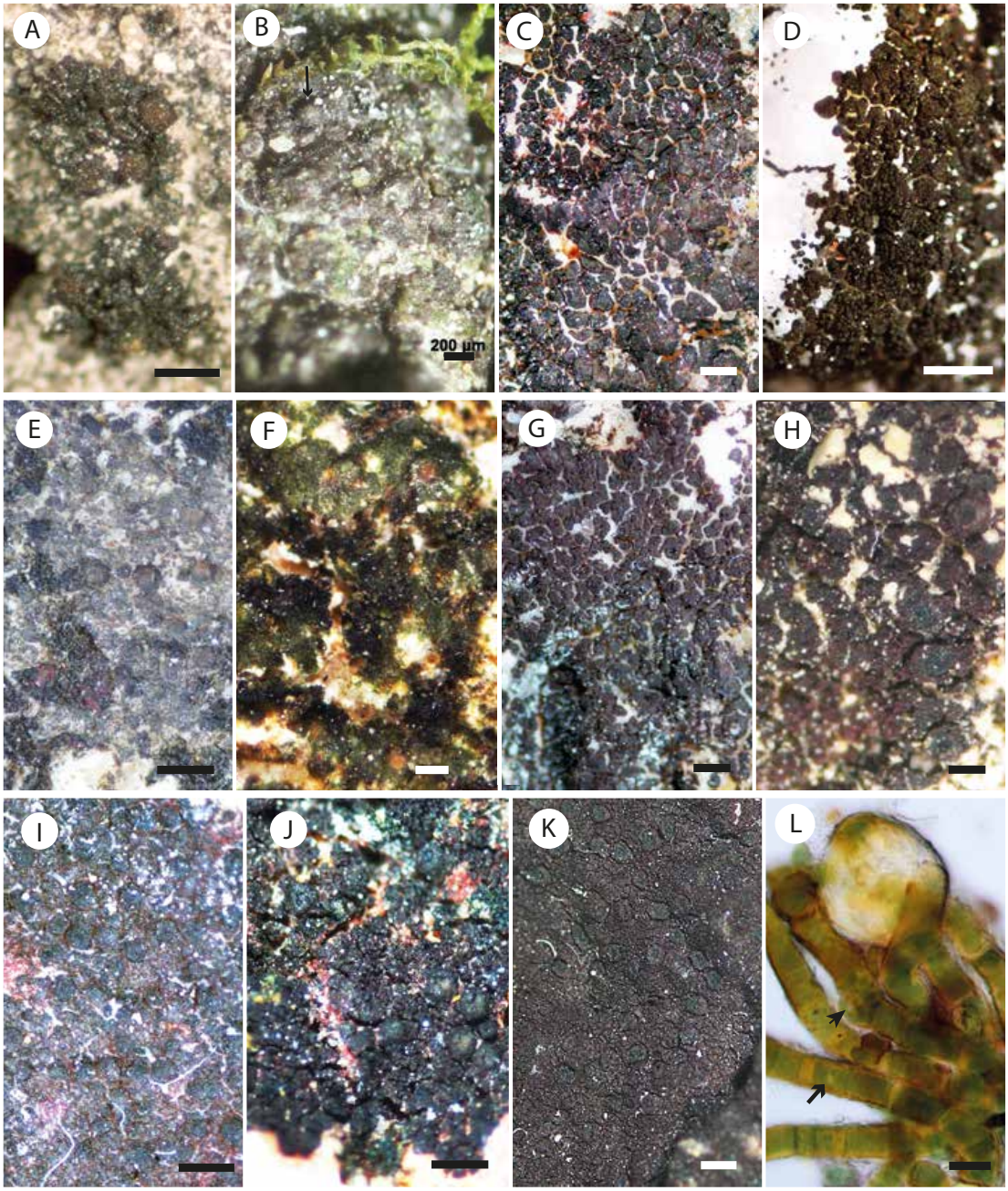

Figure 3.2: **A**, *Porocyphus coccodes*; **B**, *Porocyphus* sp. (ascoma indicated by arrow); **C**, *Pterygiopsis guyanensis*; **D**, *Pterygiopsis* sp.; **E**, *Pyrenocarpon thelostomum*; **F**, *Psorotichia americana* s. lat; **G**, *Pyrenopsis polycocca*; **H**, *P. portoricensis*; **I**, *P. subareolata*; **J**, *Pyrenopsis triptococca*; **K**, *Pyrenopsis tenuis*; **L**, *Thermutis velutina*, filamentous photobiont *Scytonema* loosely lichenized with haustoria (arrow) and a cluster of rearranged photobiont cells (arrow head).
Scales: A, B and F: 200 µm; B–E, G–K: 500 µm; L: 20 µm

3.1.3 Lichens of the family Peltulaceae

The genus *Peltula* was represented with seven species found in Vietnam: *Peltula bolanderi, P. clavata, P. euploca, P. impressa, P. obscurans, P. omphaliza* and *P. placodizans*. Six of them are new records for the Vietnamase lichen flora. Only *P. euploca* was exceptionally found on soil in the vertical rock crevices of a coastal outcrop, the others occurred on rock of two different habitats. The first habitat were coastal rocky outcrops harbouring the species *P. bolanderi, P impressa, P. obcurans, P. omphaliza* and *P. placodizans*. The second habitat, were rocks on a rapid bank at Cat Tien forest, inhabited by *P. clavata* and *P. obscurans*. The two species *P. euploca* and *P. omphaliza*, were considered rare species when compared to the distribution of *Peltula bolanderi, P. impressa* and *P. obscurans*.

***Peltula bolanderi* (Tuck.) Wetmore, Ann. Mo. Bot. Gard. 57: 168 (1971)**

Fig. 3.3. A

Thallus: often squamulose, peltate, single, surface rough or smooth, olive green ±undulate, variable size up 1.7–1.8 mm diam., margin sorediate; soredia marginal, linear or orbicular, grey; thallus attached to the substratum by an umbilicus. Thallus anatomy: 180–220 µm thick, upper surface ecorticate, epinecral layer yellow, thin, ca. 10 µm; photobiont layer 95–105 µm. Photobiont: *Chroococcidiopsis*, cells 5–8 µm diam., in clusters; medulla composed of interwoven and elongated hyphae 95–130 µm thick; lower cortex brown, pseudoparenchymatous, 15–25 µm thick; lower surface pale yellowish. Ascomata: 1 per thallus, immersed in thallus, raised rim when mature, disc red, concave, 90–135 µm diam.; epihymenium pale yellow, hymenium I+ red, 110–140 µm high, asci: clavate, unitunicate-rostrate, K/I+blue, >100 ascospores; ascospores simple, hyaline, globose to oblong, 4.5–7 X 2.4–3.5 µm, paraphyses branched at tip by shorter and wider cells; subhymenium 45–70 µm. Conidiomata: pycnidia unknown.

Ecology and distribution: often with *P. placodizans* or *P. impressa* and cyanobacteria, on granite rocks in coastal outcrops in Ninh Thuan, Qui Nhon (Binh Dinh) and Son Tra.

Studied material: 122002, 122283, 123013

Remarks: This species is known with single squamules or a polyphyllous thallus composed of many smaller squamules (Wetmore, 1970; Marques et al., 2013). The studied specimens were almost singlular squamules with a thick margin, immersed apothecia a small rim (Fig. 3.3 A). The specimens with polyphyllous thallus were less frequent and characterized by thin thalli and without ascomata.

***Peltula clavata* (Kremp.) Wetmore, Ann. Mo. Bot. Gard. 57: 168 (1971)**

Fig. 3.3. B

Thallus: suffructicose, black; squamules club-shaped, up to 2 mm long, isidiate. Thallus anatomy: ecorticate, epinecral layer thin, dark brown, 3–6 µm thick; photobiont: *Chroococcidiopsis*-like, cells 3–7 µm diam., in group of 2 to 4 cells; medulla almost hollow, very loosely elongate hyphae. Ascomata and conidiomata unknown.

Material: 124051, 124048

Ecology and distribution: grew with *Peltula obscurans* and *Scytonema* on seasonal inundated rock in a rapid bank at Ben Cu, Cat Tien (Fig. 2.2 B, page 13).

Remarks: *P. clavata* was recognized by black clavate squamules with a hollow medulla. The studied specimens were often covered by clay, resulting from annual floods during the rainy seasons. Vietnamese species inhabited similar habitats to those of Africa, in which *P. clavata* grew on vertical wall of a rock pool next to the water level or on rocky surfaces of river banks. This species has a wide ecological range and grows next to the water level in arid habitat (Büdel, 1987, 2001; Büdel and Nash III, 2002).

***Peltula euploca* (Ach.) Poelt, Acta Rer. Nat. Mus. Slov. 13: 8 (1967)**

Fig. 3.3. C and D

Thallus: peltate, sometimes polyphyllous, squamules then composed of many subsquamules, rosette-like; subsquamules lobate, margins downrolled, 5.5–7 mm diam.; soredia orbicular, black, marginal or laminal; attached by a holdfast. Thallus anatomy: 265–285 µm thick, upper surface ecorticate, epinecral layer yellow, 7.5–10 µm thick; photobiont layer ca. 115–130 µm thick, photobiont *Chroococcidiopsis*, cells 7.5–11 µm diam., in 2- or 4-celled groups; medulla composed of loosely and elongate hyphae; lower cortex paraplectenchymatous, 50–60 µm thick, hyphae cells anticlinally arranged; lower surface yellow to brown, rough with irregular ridges. Ascomata and conidiomata unknown.

Material: 123020

Ecology and distribution: on soil in vertical rock crevices in a coastal outcrop, Bai Rang, Qui Nhon, Binh Dinh.

Remarks: *P. euploca* was recognized by mainly single peltate squamules, but three thallus varieties were also mentioned: polyphyllous form, small form close to *P. bolanderi*, and effigurate forms (Marques et al., 2013). The polyphyllous form of *P. euploca* in this study is clearly separated from to *P. bolanderi* with single peltate thalli. Additionally, this study expanded the known habitat of this species from rock to soil.

***Peltula impressa* (Vain.) Swiscow & Krog, Norw. J. Bot. 26: 219 (1979)**

Fig. 3.3. E

Thallus: placodioid, esorediate, olive-green; central areoles convex, or flat, up to 2 mm diam.; marginal thalli effigurate, ribbon-like, 0.5–1 .2 X 0.2–0.3 mm; attached by a central umbilicus. Thallus anatomy: (60 –) 200–270 µm thick, upper side ecorticate, epinecral layer yellow; photobiont layer 90–100 µm, photobiont *Chroococcidiopsis*, cells 7–11 µm diam.; medulla composed of loosely interwoven hyphae leaving hollow spaces, hyphal cells roundish or elongate; lower cortex paraplectenchymatous. Ascomata: one each central areole, disc red, expanded and concave in mature or old ascomata; epihymenium brown, hymenium K/I+ blue,

95–150 µm thick, asci clavate, >64 ascospores; ascospores simple, hyaline, globose, 3.5 -6 µm diam. Conidiomata: unknown.

Studied material: 122010, 122014, 13205a-2

Ecology and distribution: on rock of coastal outcrops in Ninh Thuan, Son Tra.

Remarks: this species is characterized by a placodioid morphology with central areoles that are convex or flat, carrying one ascoma per areole, with a ribbon-like margin. Ascomata: immersed with a expanded disc. In the specimen 13205a-2, the hymenium of big/old ascomata were divided by sterile hyphae.

***Peltula obscurans* (Nyl.) Gyeln., Rep. Spec. Nov. Regn. Veg. 38: 308 (1935)**

Fig. 3.3. F

Thallus squamulose, scatter, olive-green; squamules up to 2 mm diam., margin deeply lobate, attach by central umbilicus. Thallus anatomy: ca. 200 µm thick, upper surface ecorticate, epinecral layer brown; photobiont layer ca. 150 µm thick, photobiont *Chroococcidiopsis*-like, cells 7–10 µm diam., in clusters; medulla composed of roundish hyphal cells; lower surface brown. Ascomata: one per squamule, disc red, expanded up to 2 mm diam., raised rim in the mature ascoma; asci: clavate or obclavate, >100 ascospores; ascospore: hyaline, simple, globose to ellipsoid, 4.5 X 2.5–3 µm diam. Conidiomata: pycnidia, ovoid; conidia bacilliform ca. 3 µm.

Studied material: 122016, 124034

Ecology and material: on rock in coastal outcrops in Ninh Thuan and in rapid bank in Cat Tien. The specimens in Cat Tien grew together with *Peltula clavata*.

Remarks: this species was also recorded from in Northern Vietnam (Aptroot and Sparrius, 2006). It was recognized by scattered and lobate squamules, apothecia with a raised rim.

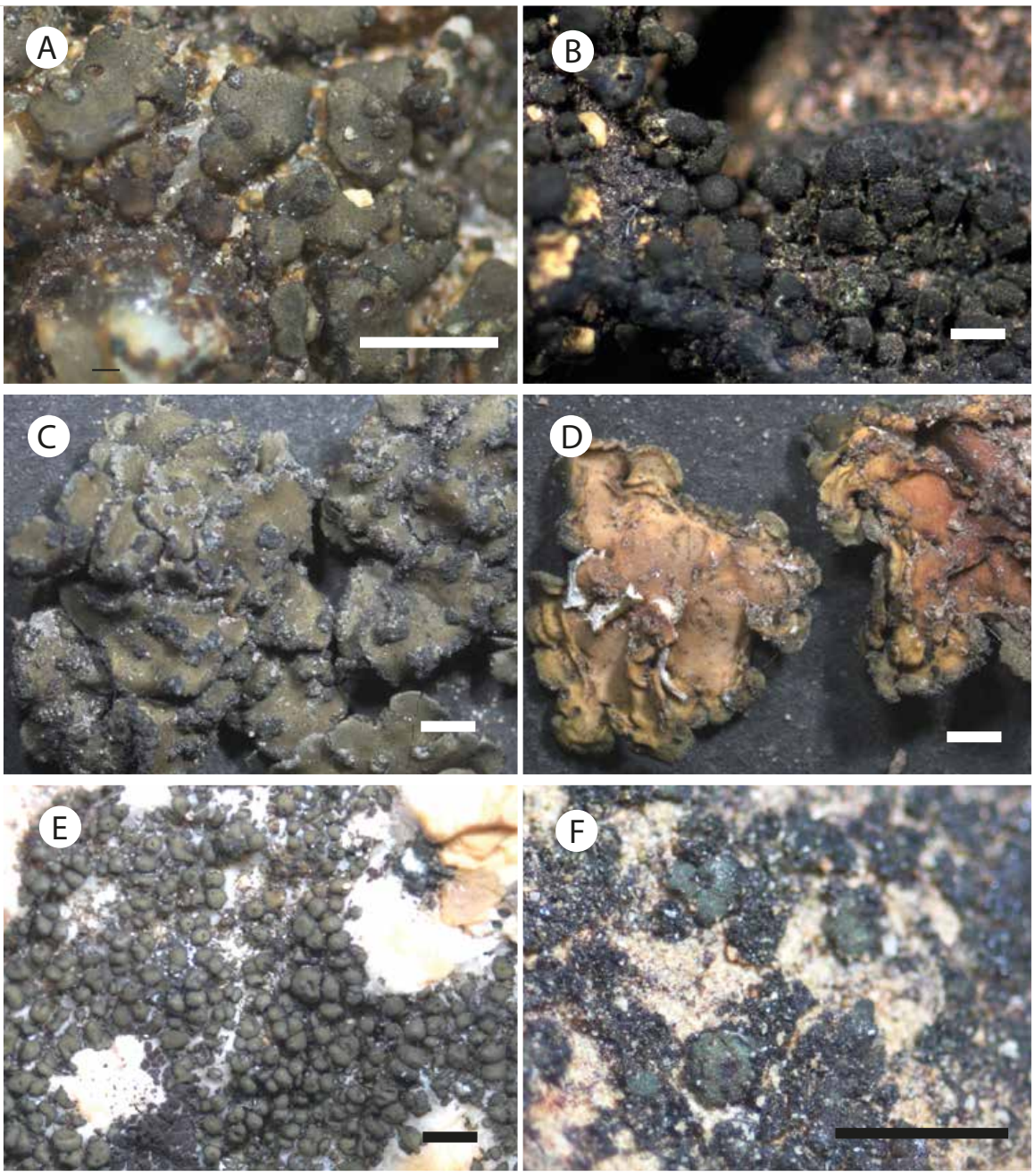

Figure 3.3: **A**, *Peltula bolanderi*; **B**, *P. clavata*; **C and D**, *P. euploca*, upper surface (**C**) and lower surface (**D**); **E**, *P. impressa*; **F**, *P. obscurans.*
Scale: 1 mm

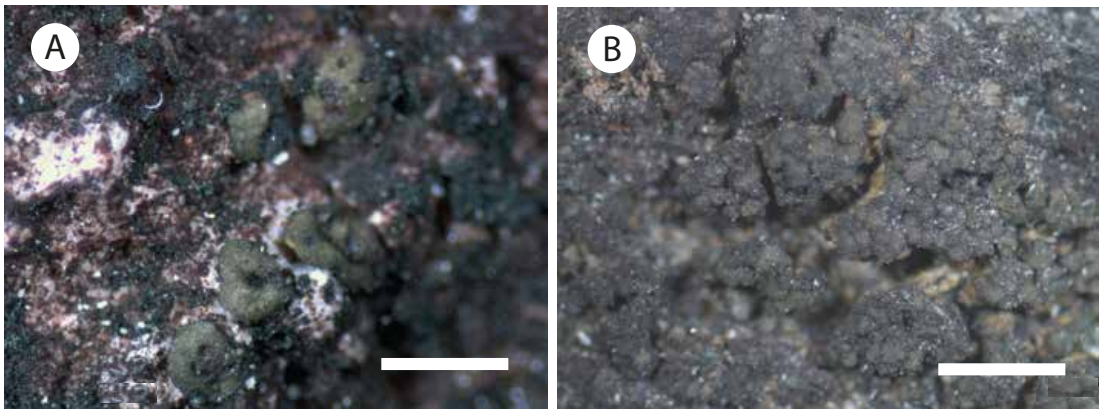

Figure 3.4: **A**, *Peltula omphaliza*; **B**, *P. placodizans.*
Scale: 1 mm

Peltula omphaliza **(Nyl.), Wetmore Ann. Mo. Bot. Gard. 57: 168 (1971)**

Fig. 3.4. A

Thallus: peltate, scattered, olive-green; squamules: 0.5–1.5(–2) mm diam., roundish, convex, margin entire, not sorediate, attached by a central umbilicus. Thallus anatomy: 55–70 µm thick; ecorticate, epinecral layer yellow, ca. 10 µm thick, photobiont layer 75–85 µm thick, photobiont *Chroococcidiopsis*-like, cells 6–10 µm diam., in 2- to 4-celled groups; medulla loosely interwoven and elongate hyphae; an epinecral layer brown in upper surface, no lower cortex. Ascomata: immersed, adnate, 2–5 per squamule, disc red, expanded in mature ascoma; epihymenium yellow; hymenium ca. 130 µm high, K/I+ blue; asci clavate, >100 ascospores; ascospore simple, hyaline, globose to broad ellipsoid, 5–7 X 3–4 µm; subhymenium ca. 15 µm. Conidiomata: unknown.

Studied material: 122029

Ecology and distribution: grew together with the cyanobacterium with *Gloeocapsa* on rock in a coastal outcrop in Ninh Thuan.

Remarks: this species was recognized by single and roundish, convex squamules with many ascomata per squamule that grew sparsely on rocks, squamules esorediate.

***Peltula placodizans* (Zahlbr.), Wetmore Ann. Mo. Bot. Gard. 57: 168 (1971)**

Fig. 3.4. B

Thallus: crustose, olive-green, areolate in centre and effigurate at margin; central areoles flat, 1.5–3 mm diam.; marginal lobes irregular or elongate, branched at tips, 0.4–1 X 0.2–0.3 mm; soredia punctiform, black, attached to substrate by all lower surface. Thallus anatomy: 95–120 µm thick, ecorticate, epinecral layer brown; photobiont layer rather thick, more than two third of thallus, photobiont *Chroococcidiopsis*, cells 4.5–10.5 µm diam.; medulla composed of loosely interwoven hyphae, and hyphal cells roundish; lower cortex thin, paraplectenchymatous composed of roundish cells, pseudoparaplectenchymatous. Ascomata: in central areoles, one per areole, immersed, disc red; epihymenium yellow; hymenium 120–160 µm thick, K/I+ blue, asci clavate, >100 ascospores; ascospore globose to oblong, 2–3 X 3.5–6(–7) µm. Conidiomata: pycnidia immersed in thallus, obpyriform, pycnidia bacilliform, 2–3 µm.

Studied material: 122003, 122020, 13205a-1.

Ecology and distribution: often grew together with *P. bolanderi* on rock in coastal outcrops at Son Tra, Ninh Thuan and Nui Chua.

Remarks: this species is characterized by an areolate-placodioid form, with flat areoles. Soredia were rarely seen in the specimens of Ninh Thuan. Moreover, the thallus morphology of the specimens studied were very variable. Areoles of the specimen 13205a-1 were rather thick and sorediate while others were esorediate with a thin thallus similar to *P. impressa*.

3.1.4 Basidiomycete lichens of the family Hygrophoraceae

Five species of two genera *Cyphellostereum* and *Dictyonema* were found in Vietnam. Three different species of *Cyphellostereum*, the species *Dictyonema moorei*, as well as another *Dictyonema* species were recognized based on their morphological and anatomical characters, and their distribution.

The species were determined to the genus level, except *Dictyonema moorei* (Nyl.) Henssen. All of them were characterised by thallus fibrils that were loosely attached to the substrate. The amount of sample material was too low for molecular analysis, hence I describe them here as species types that differ in morphology and distribution, and provide a key for identification. The photobionts of these *Cyphellostereum* species were the morphogenus *Scytonema*, differing from the photobiont *Rhizonema* within the same lichen genus from the Philippines, and from Neotropical America which are treated in detail in section 3.2.2.

3.1.4.1 Basidiolichens species

***Cyphellostereum* sp. 1**

(Fig. 3.5 D–G, and Fig. 3.8)

Thallus: filamentous crustose, bright green-blue, with a fibrilose thallus dominated by the structure of the scytonematoid photobiont, bundles of fibrils intertwining. External hyphae 1.8–2.5 µm broad, sparsely in thallus at early developmental stage, densely in old thallus, no clamp connection, enveloped filamentous photobionts but leaving interspaces; intracellular hyphae were born from external hyphae, penetrating through the center of photobiont cells except heterocytes. Photobiont: scytonematoid, trichome cells more or less square-shaped, 4–8(–10) µm wide and 4–8 µm long, false-branching; heterocytes frequent. Basidiomata: white, erect in the upper side; basidiospores not observed.

Ecology and distribution: the species grew on the bark along a *Shorea* trunk in mixed forest Lo Go Xa Mat, Tay Ninh Province; another cyanolichen found in the same habitat was *Spilonema* sp.

Studied material: 13112g-2

***Cyphellostereum* sp. 2**

(Fig. 3.6 A–C)

Thallus: filamentous crustose, dark olivaceous green; fibrils appressed to substrate. External hyphae 3.5–5 µm broad, no clamp connection; intracellular hyphae present, penetrating through photobiont cells except heterocytes. Photobiont: scytonematoid, trichome cells 12–14 µm wide and (4–)5–7(–10) µm long; heterocytes rarely; false branches. Basidiomata: not observed.

Ecology and distribution: this species covered on the lichen *Coccocarpia* spp. growing on rock in a stream in An Toan forest. Studied material: 13118e

***Cyphellostereum* sp. 3**

(Fig. 3.6 D–F)

Thallus filamentous, appressed, olivaceous green, interwoven and swollen. External hyphae 3–4 µm wide; hyphal sheath composed of irregular hyphae, leaving interspaces; intracellular hyphae sometimes not seen in fibrils at early developmental stage. Photobiont: scytonematoid, trichome cells (9–)10–11.5 µm wide and 4.5–6.5 µm long, false branching, heterocytes present. Basidiomata: not observed.

Ecology and distribution: this species grew together with mosses on tree trunk in Cat Tien National Park and Lo Go Xa Mat National Park.

Studies material: 124054 and 121032

Dictyonema moorei (Nyl.) Henssen, Symb. Bot. Upsal. 18: 1 (1963)

(Fig. 3.7 and Fig. 3.9)

Thallus appressed-fibrilose, black olivaceous; fibrils often composed of two parallel photobiont filaments, ending in a loop in early developmental stages, or by clavate tips in which the photobiont cells cluster irregularly, fibrils raised from a basal fibril or a swollen tip of old fibrils. Hyphae 5–7 µm thick, clamp connections, associate with hyphae sheath; closed hyphal sheath is composed of hyphae

cells with undulate lateral walls; intracellular hyphae penetrated photobiont cells. Photobiont: a scytonematoid cyanobacterium; width of photobiont cells in filaments varies widely, from (6–)9–12(–18) µm, depending on the developmental stage, length of photobiont cells 4–8 µm.

Ecology and distribution: The studied material grew together with mosses and the cyanolichen *Physma byrsaeum* on the trunk at about 12m high of a fallen tree, in An Toan Nature Reserve, Binh Dinh Province, 14 °32 N, 108 °43 E.

Studied material: 13112b3a, 13112h.

Remarks: This species was characterized by two photobiont filaments in each thallus fibril, the single photobiont filaments (Fig. 3.7 G) only seen in early developmental stages. Triseriate photobiont filaments in thallus fibrils was also mentioned (Parmasto, 1978; Lücking et al., 2013) although was not observed in this investigation. The species also occurs in Japan, Papua New Guinea, Hawaiian Islands, New Zealand, Australia, Chile (Henssen, 1963; Galloway and Fife, 1992; Hoffmann and Büdel, 1992; Elix and McCarthy, 1998; Lepp, 2004).

***Dictyonema* sp.**

(Fig. 3.5 A–C)

Thallus filamentous crustose, dark blue-green; prothallus white, distinct; fibrils solitary, horizontal, parallel, 60–85 µm long, 12–17 µm wide. Closed hyphal sheath composed of undulate-walled cells, enveloping filamentous photobiont; intracellular haustoria tubular; photobiont scytonematoid, false branching; hyphae in prothallus thick walled, with clamp connections. Photobiont: a scyotnematoid cyanobacterium, trichome cells 12–15 µm wide, 3–7 µm long; photobiont filaments constricted at heterocytes. Basidiospores not observed.

Ecology and distribution: The species grew on a boulder with *Coccocarpia*, *Spilonema americana*, *Pannaria* and other chlorolichens, along an understorey spring in mountain forest of Nui Chua National Park, 700 m altitude; 11 °43 N, 109 °08 E.

Studied material: 122244b

Remarks: This species is related to the species of the group that are characterized by "fibrils distinctly combed" in the key of *Dictyonema* s. str. (Lücking et al., 2013). The studied material resembled *D. schenkianum* with its combed appearance and the obvious white prothallus.

3.1.4.2 Key to basidiolichen species

1 Thallus with a hyphal sheath composed of elongated cells irregularly wrapping the *Scytonema* filaments leaving interspaces; tips of photobiont filaments emerge out of the hyphae **2**

- Thallus with hyphal sheath composed of paraplectenchymatous, jigsaw-puzzle-shaped cells wraping all *Scytonema*-like filaments **4**

2 Photobiont filament narrow, 4–8 µm wide, photobiont cells square-shaped ***Cyphellostereum*** sp. 1

- Photobiont filaments more than 10 µm wide, photobiont cells rectangular . . **3**

3 Growing on boulders along streams in forests at an elevation of ca. 900 m ***Cyphellostereum*** sp. 2

- Growing on bark, in plain forests . . . . . ***Cyphellostereum*** sp. 3

4 Branching fibrils composed of two photobiont filaments in parallel arrangement, rarely one filament, fibril tips often swollen, photobiont cells at tips in clusters, on tree trunks . . . . . ***Dictyonema moorei***

- Thallus composed of solitary fibrils, parallel arrangement, composed of one photobiont filament, white prothallus distinctly, on rock . . . . . ***Dictyonema*** sp.

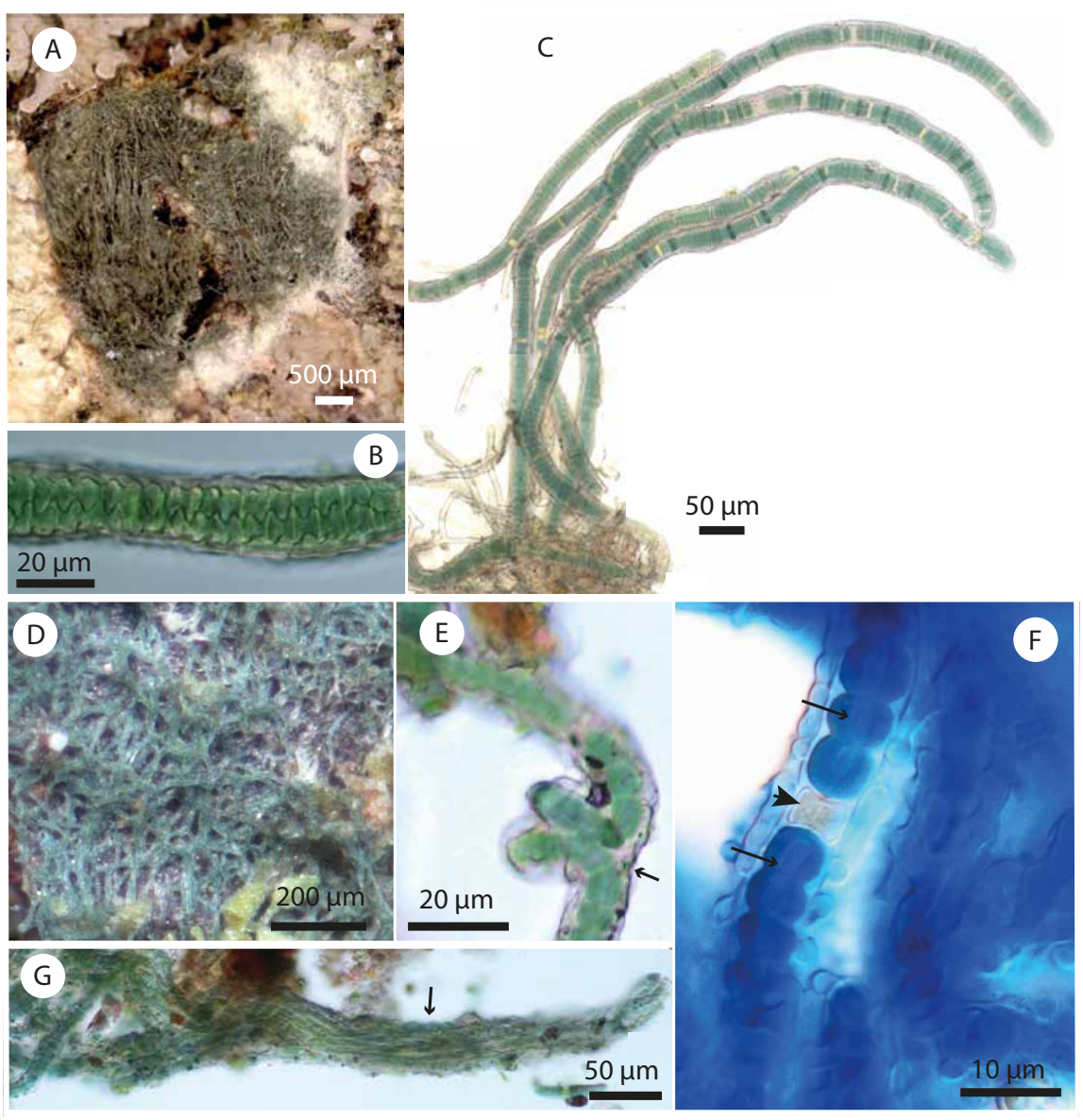

Figure 3.5: Basidiolichens *Dictyonema* sp. and *Cyphellostereum* sp. 1

A–**C**, *Dictyonema* sp.: **A**, habitat, fibrils appressed unidirectional; **B**, closed hyphal sheath formed by undulate-walled cells; **C**, long and separate fibrils with fungal hyphae at base. **D**–**G**, *Cyphellostereum* sp. 1: **D**, habitat; **E**, false branching of *Scytonema* photobionts in fibrils (arrow); F, tubular intracellular haustoria in a filbril (arrows) end before a heterocyte (arrowhead); G, a bundle of intertwined fibrils (arrow).

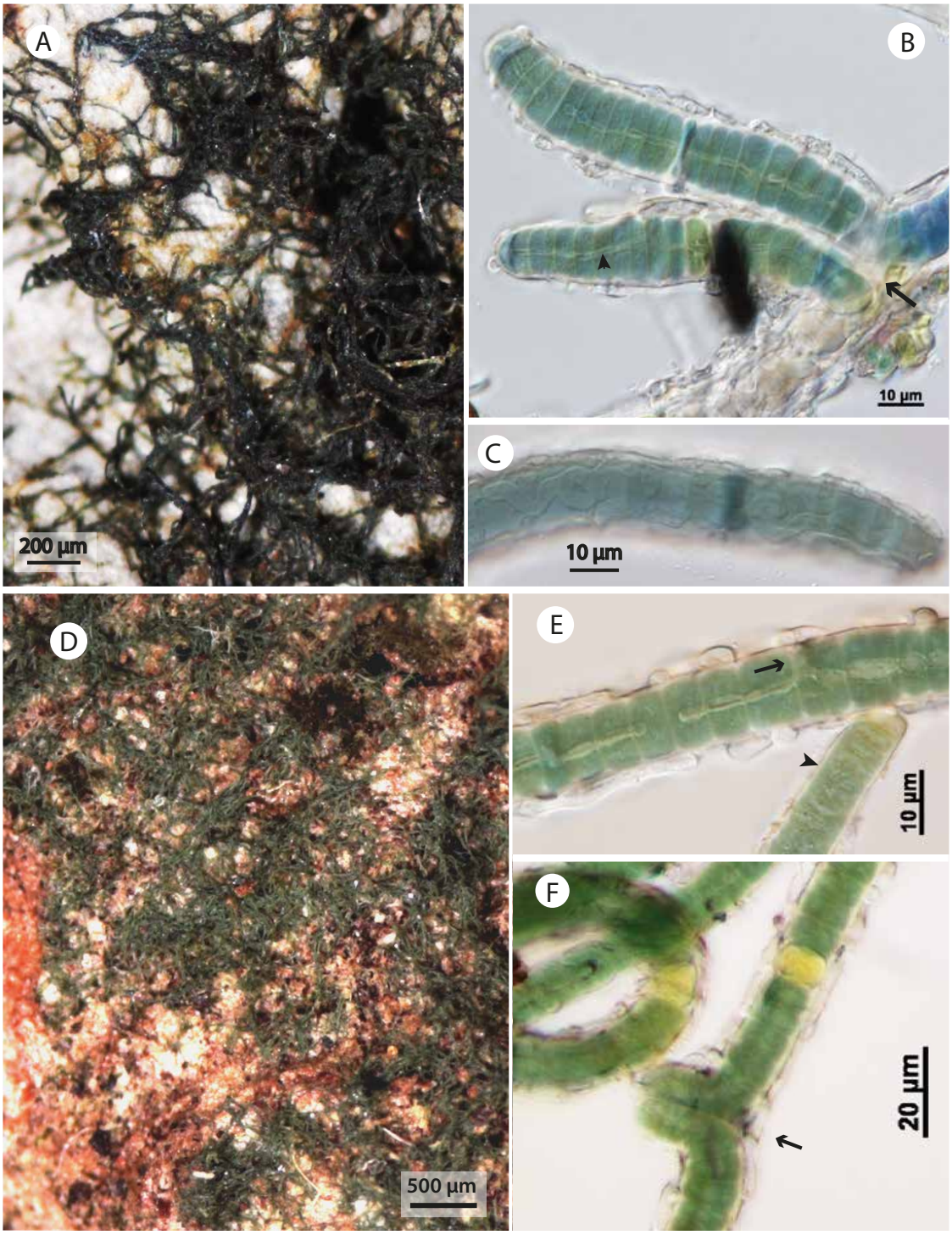

Figure 3.6: *Cyphellostereum* sp. 2 and *Cyphellostereum* sp. 3

A–**C**, *Cyphellostereum* sp. 2: **A**, habitat; **B**, false branching (arrow) of photobiont *Scytonema* with tubular haustorium (arrow head); **C**, hyphal sheath irregular, leaving interspaces. **D**–**F**, *Cyphellostereum* sp. 3: **D**, habitat; **E**, haustorial system with connection (arrow) between external hyphae and intracellular haustorium, photobiont tip free, growing out of hyphae (arrow head); **F**, false branching of the *Scytonema* photobiont (arrow)

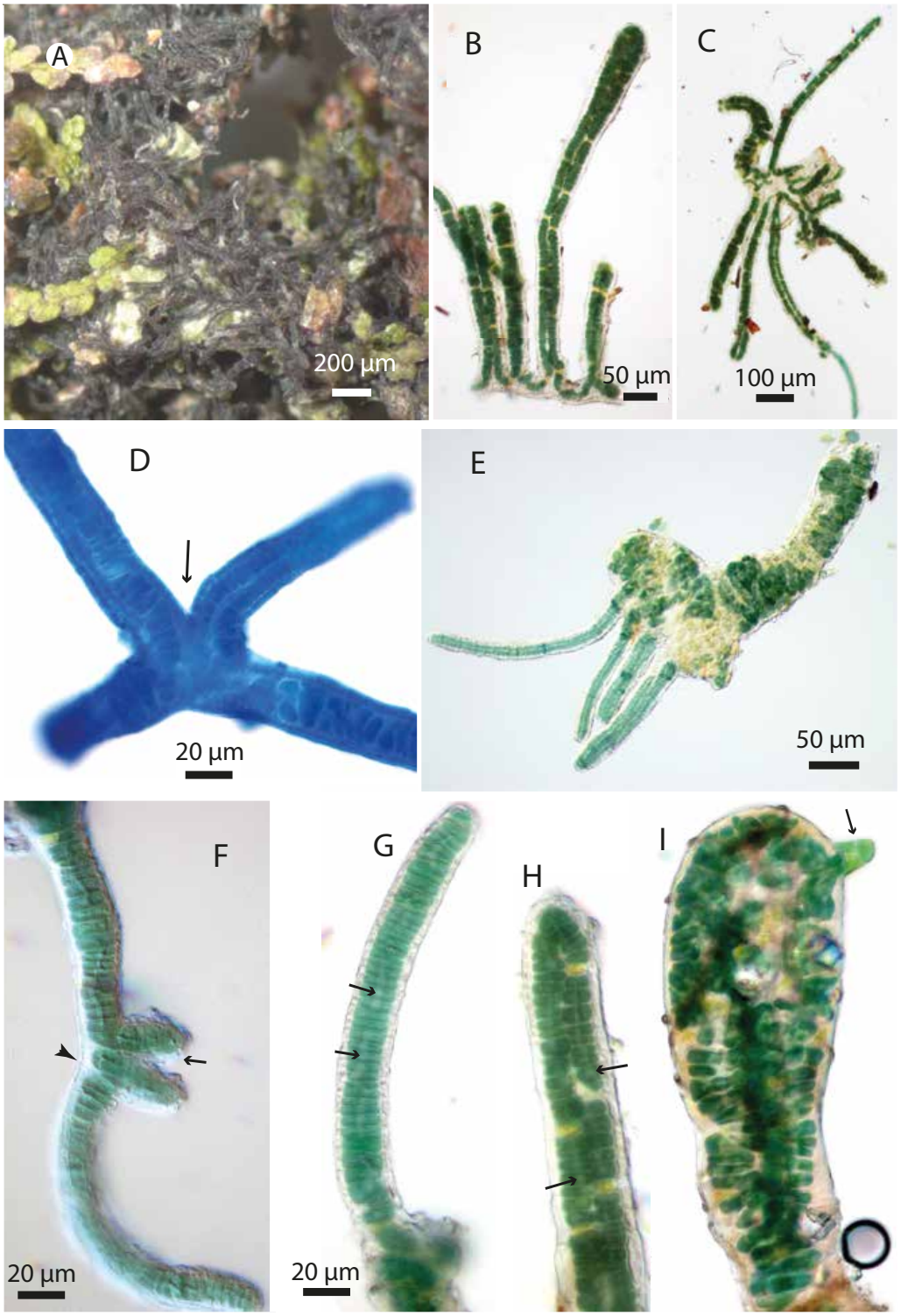

Figure 3.7: Morphology and photobiont of *Dictyonema moorei*.

A, habitat. **B** and **C**, appearance of fibrils. **D** and **F**, false-branching-like structure (arrows) with a break next to the false branching (arrow head). **E**, new fibrils grow from a clavate tip of an old fibril. **G**–**I**, fibril features, **G**, a fibril composed of one photobiont filament with tubular haustoria (arrows); **H**, two photobiont filaments with tubular haustoria (arrows); **I**: a clusters of photobiont cells with a new growing fibril (arrow of I)

3.1.4.3 Intracellular haustorium in *Cyphellostereum*

This genus has the most incoherent relationship between mycobiont and photobiont cells in *Dictyonema* s.lat. The *Cyphellostereum* species presented here have filamentous thalli. The association level depended on the development stage. At the base of the fibril layer, photobiont fibrils were tightly wrapped by sinuous hyphae (Fig. 3.8). In contrast, the uppermost photobiont filaments were covered by loose hyphae with interspaces, and the photobiont filament tips were even free outside the mycobiont (Fig. 3.6 B and E).

The intracellular haustorial system was observed in all studied *Cyphellostereum* specimens by light microscopy (Fig. 3.5 F, Fig. 3.6 B and E) and in *Cyphellostereum* sp. 1 with electron microscopy in addition (Fig. 3.8 B). They were very similar to the intracellular haustorial system in *Dictyonema moorei* (Fig. 3.7 G–H, and Fig. 3.9) and *Dictyonema* spp. in this study, and also known from previous studies (Roskin, 1970; Slocum and Floyd, 1977; Slocum, 1980; Oberwinkler, 1980, 1984; Tschermak-Woess, 1983). The tubular haustorial septate hyphae of *Cyphellostereum* rising from the hyphal sheath pierced to the centre of photobiont filaments, haustorial trichome without heterocytes (Fig. 3.6 E). The loose hyphae sheath of *Cyphellostereum* often left the photobiont tips freely and became dense in older fibrils. This hyphal envelope corresponds with that of *Cyphellostereum* mentioned in recent studies (Yánez et al., 2012; Lücking et al., 2013; Dal-Forno et al., 2013). However, the intracellular haustoria of the studied *Cyphellostereum* species differed from the appressorial haustoria of these former studies.

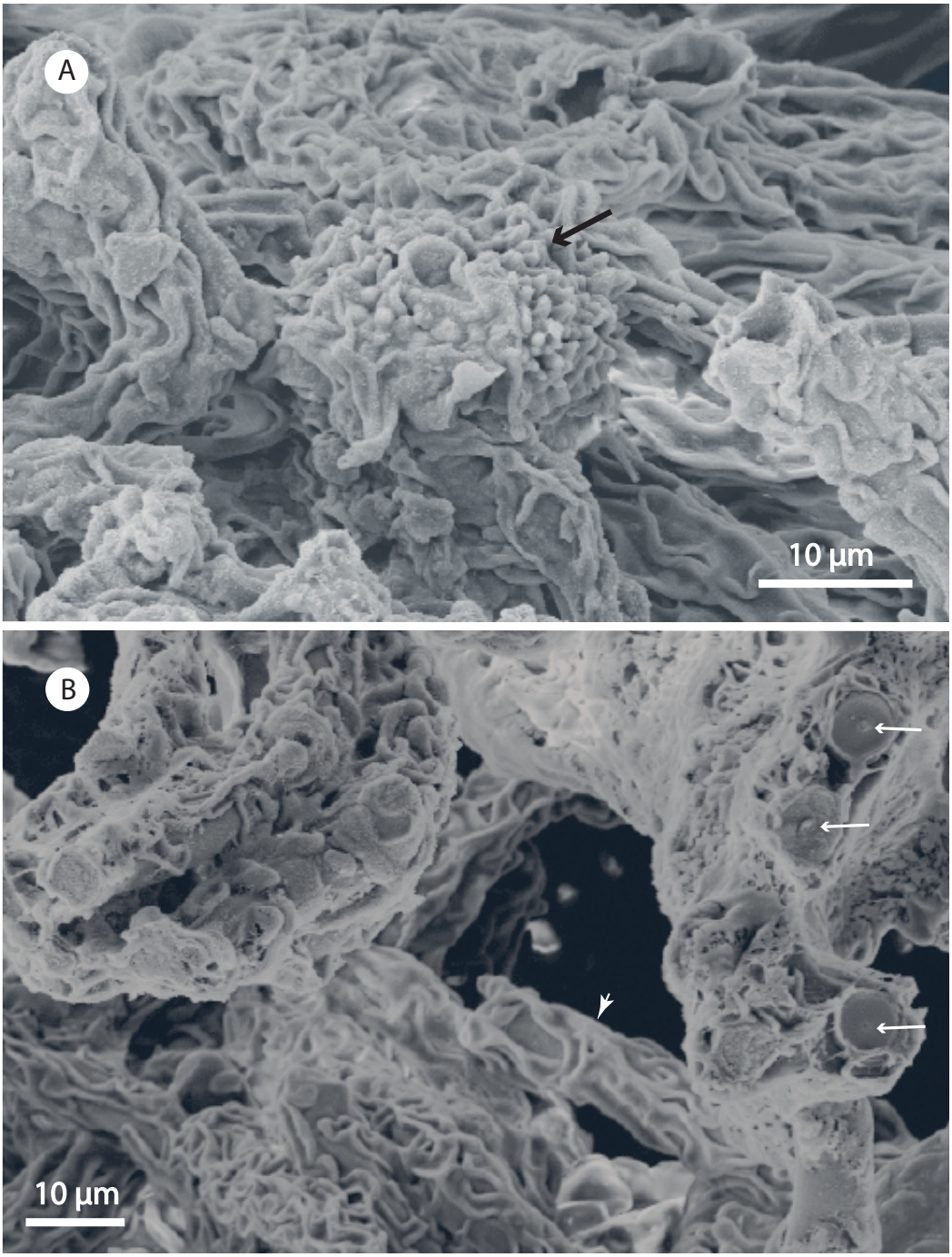

Figure 3.8: LT-SEM photos of *Cyphellostereum* sp. 1

A, basidioma on upper thallus surface (arrow); **B**. Thallus fibril: photobiont filament enveloped by irregular shaped mycobiont hyphae (arrow head), intracellular haustoria in the centre of the photobiont filaments (arrow)

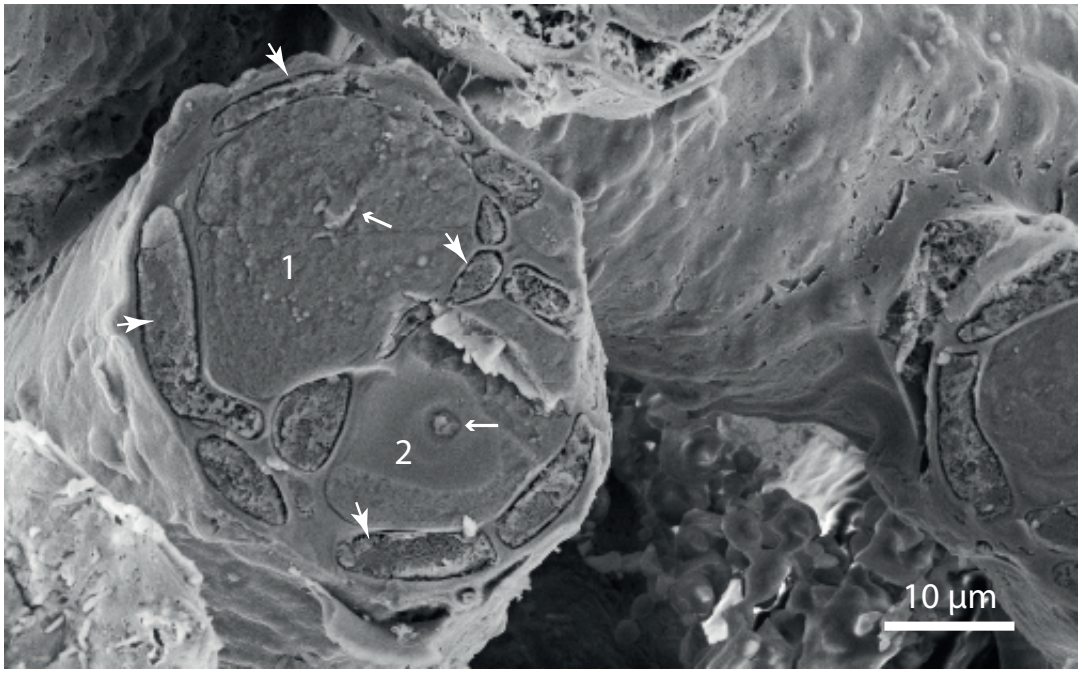

Figure 3.9: LT-SEM photo of intracellular haustoria in *Dictyonema moorei*.
Two cyanobiont filaments (1 and 2) wrapped by compacted hyphal cells (arrow heads), and intracellular haustoria in centre of photobiont cells (arrows)

3.2 Cyanobionts

A total of 11 cyanobacterial strains were isolated from cyanolichens, 8 baeocyte-forming cyanobacteria and 3 heterocyte-forming filamentous cyanobacteria (Table 3.4). All baeocyte-forming cyanobacteria and one heterocyte-forming filamentous cyanobacterium were obtained from lichinalean members of genera *Peltula, Heppia, Porocyphus, Pyrenocarpon* and the Lichinacean sepecies (no. 13802f). Two heterocyte-forming filamentous cyanobacteria were isolated from two pannariod species *Pannaria tavaresii* and *Parmeliella brisbanensis* (Table 3.4).

Table 3.4: Lichens and their photobionts used in studying photobiont morphology and phylogenetic analyses of 16S rRNA sequences

Lichen	Isolated strain	Herbarium no.	Substrate	Distribution
**Coccocarpia erythroxyli*	-	122246	S	5
Cyphellostereum sp. 1	-	13112g-2	C	4
Cyphellostereum sp. 2	-	13118e	S	1
Cyphellostereum sp. 3	-	124054, 121032	C	4
Dictyonema moorei	-	13112b3a,13112h	C	1
Dictyonema sp.	-	122244b	S	5
Heppia lutosa	*BB15.09 *Scytonema*	122270	T	6
Pannaria tavarensii	*BB15.10 *Nostoc*	13115h-1	C	1
Parmeliella brisbanesis	*BB15.11 *Nostoc*	13117e-3	C	1
**Peltula bolanderi* (PEB2)	-	123013a	T	7
**P. bolanderi* (PEB1)	BB15.01 *Chroo.*	122002	S	6
**P. clavata* (PEC)	*BB15.02 *Chroo.*	124051	S	2
**P. euploca* (PEE)	*BB15.03 *Chroo.*	123020	T	7
* *P. impressa* (PEI)	*BB15.04 *Chroo.*	13205a-2	S	8
* *P. obscurans* (PEO)	-	124034	S	2
**P. placodizans* (PEP1)	-	122010	S	6
**P. placodizans* (PEP2)	*BB15.05 *Chroo.*	13205a-1	S	8
Porocyphus dimorphus	*BB15.06 *Chroo.*	123018	S	7
Pyrenocarpon thelostomum	BB15.07 *Chroo.*	122138a	S	6
Lichinaceae	*BB15.08 *Chroo.*	13802f	S	3

*Samples were used in studying 16S rRNA sequences; Chroo.: *Chroococcidiopsis*; C: corticulous, S: saxicolous, T: terricolous; 1: An Toan, 2: Cat Tien, 3: Dakrong, 4: Lo Go Xa Mat, 5: Nui Chua, 6: Ninh Thuan, 7: Qui Nhon, 8: Son Tra

3.2.1 Baeocyte-forming cyanobacterial photobionts

3.2.1.1 Morphology

Baeocyte-forming cyanobacteria were isolated from 5 species *Peltula* (Peltulaceae) and 3 species of Lichinaceae (Fig. 3.10, 3.11, and 3.12), numbered from BB15.01 to BB15.08. Six isolates grew on both, agar and liquid media; only two isolates (BB15.02) from *Peltula clavata* and (BB15.06) from *Porocyphus dimorphus* grew only in the liquid medium.

The isolates underwent both binary and multiple division patterns in the culture medium. The very first cells divided by binary division and could be enveloped by a thick mucilage sheath as in *P. placodizans* and *P. euploca*. Mature cells of 5 strains grew on agar and their size varied from ca. 3.2 µm in strains BB15.02 out of *Peltula clavata* and BB15.06 out of *Porocyphus dimorphus*, to 6.7–8.8 µm in strains BB15.01, BB15.03, BB15.04 out of *P. bolanderi, P. euploca, P. impressa*,

respectively. Haustoria were present in 6 cultured photobionts on agar for a long time, that is, more than 5 months. An exception was found in the isolated colonies of *P. placodizans*, where haustoria were present even after 18 months from the isolation date. In this case, haustoria continued to modify the photobiont colonies (Fig. 3.10 D, F, H, and Fig. 3.11 A, C).

The isolates grew in culture showed simultaneous or successive multiple fission or both of these patterns, to form and release baeocytes. Four isolates from *Peltula* spp. underwent successive multiple fission in which the daughter cells after some binary fissions divided in multiple planes. Strains BB15.06 and BB15.07 divided by simultaneous multiple fission in which, the vegetative cells increased their size before dividing in different planes. Strains BB15.02 and BB15.08 divided by both patterns, successive and simultaneous multiple fission. Especially, enlarged cells with thick walls and the bright content in the strains BB15.02 from *Peltula clavata* were observed in cultures. (Fig. 3.10 B).

The strains BB15.06 and BB15.07 exposed new morphological features that were mentioned in detail below.

A new character of the cyanobacterial baeocyte-forming strain BB15.06: binary division in parallel planes in successive generations

This new feature was observed in the strain BB15.06 isolated from the lichen species *Porocyphus dimorphus*. Initial binary divisions (1-2) with rectangular division planes are knew from all baeocyte-forming cyanobacteria (Komárek and Anagnostidis, 1998; Büdel and Kauff, 2012). Only cells of the strain BB15.06 in liquid medium divided in parallel planes in the binary division mode resulting in short pseudofilaments embedded in a mucilaginous matrix, easily losing its shape (Fig. 3.11 F, G, and H). The simultaneous multiple fission occurred only when the isolate was transfered to agarized medium.

The purple cyanobacterial baeocyte-forming strain BB15.08

This strain was isolated from the Lichinaceaean species (13802f) living on a shaded wall of a limestone mountain in Dakrong. The cell content of this strain was dark purple, rarely pale blue green within the lichen thallus. The photo-

biont retained the purple color for 7–9 months in culture medium, then gradually changed to pale blue green. The cells in colonies divided by binary division for 5–6 months before undergoing multiple fission patterns. During binary division, the cells divided in perpendicular planes resulting in cubic, spherical or fan-shaped colonies (Fig. 3.11 F, H). The cell size varied from 4–6 µm. Multiple fission occurred inside a mucilage envelope and resulted in baeocyte formation (Fig. 3.11 I) by either successive or simultaneous multiple fissions (Fig. 3.11 G–I).

Table 3.5: Morphology characters of baeocyte-forming strains *Chroococcidiopsis*

Culture	Agar medium	Liquid medium	Haustorium	Binary division	Multiple division	Cell content	Cell size [µm]
BB15.01	+	+	+	various planes	successive	blue green	6.73 ± 1.35
BB15.02	-	+	-	various planes	both modes	blue green	3.21 ± 0.31
BB15.03	+	+	+	various planes	successive	blue green	7.65 ± 1.26
BB15.04	+	+	+	various planes	successive	blue green	8.79 ± 1.67
BB15.05	+	+	+	various planes	successive	blue green	-
BB15.06	-	+	-	parallel planes	simultaneously	blue green	3.24 ± 0.38
BB15.07	+	+	+	various planes	simultaneously	blue green	-
BB15.08	+	+	+	various planes	both modes	dark purple to blue greenish	-

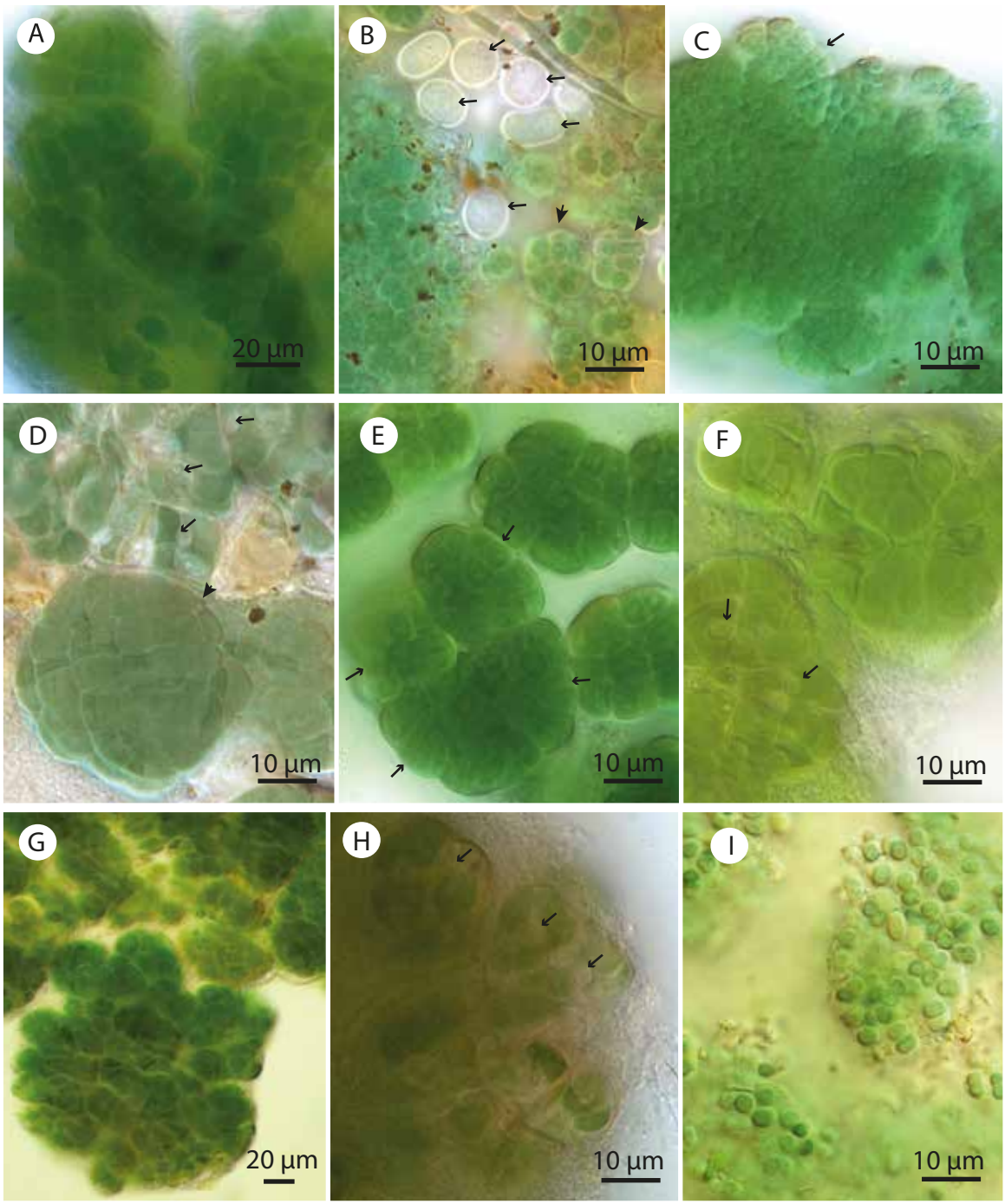

Figure 3.10: Isolates from cyanolichens *Peltula clavata, P. euploca, P. impressa* and *P. placodizans*

A–**C**, strain BB15.02 from *Peltula clavata*: **A**, cells in binary division on agar; **B**, thick wall cells enlarge before multiple fission (arrows) and baeocytes are formed by successive multiple fission (arrow head); **C**, agglomeration with cells at margin in binary division mode with radial planes (arrow) (B and C in liquid medium). **D**–**E**, photobiont BB15.03 from *P. euploca*: **D**, photobiont cells in a spherical colony (arrow) and haustoria in photobiont cells (arrows); **E**, multiple fission occurs in 4 sister cells (arrows). **F**–**G**, photobiont BB15.04 from *P. impressa* (on agar): **F**, haustoria in photobiont cells of colonies; **G**, multiple fission agglomerations. **H**–**I**, photobiont BB15.05 from *P. placodizans*, **H**, haustoria in photobiont colonies (arrows); **I**, cells released from a mucilage sheath

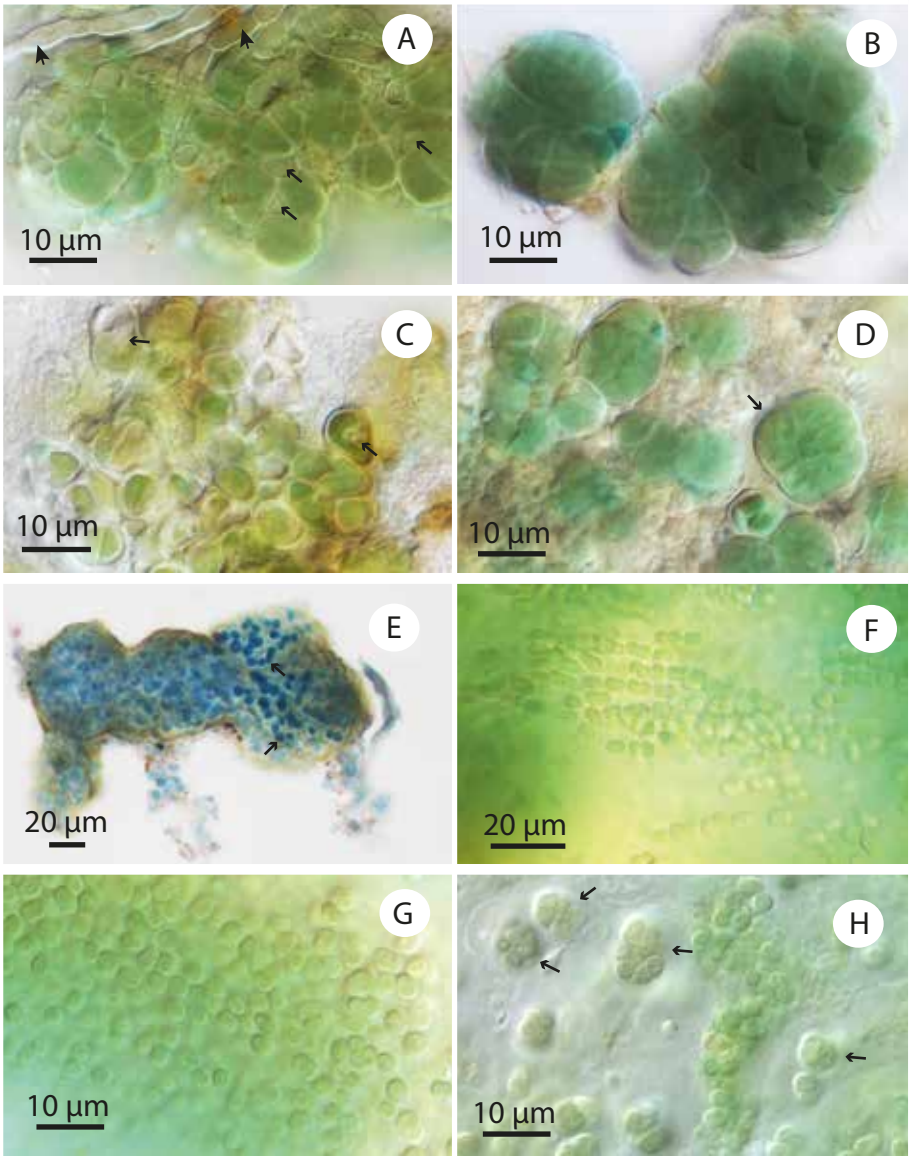

Figure 3.11: Isolates from cyanolichens *Peltula bolanderi, Pyrenocarpon thelostomum* and *Porocyphus dimorphus*

A–**B**, photobiont BB15.01 from *Peltula bolanderi* (liquid medium); **A**, photobiont cells with penetrating haustoria (arrows) originating from hyphae (arrow heads); **B**, colonies of photobiont cells in binary division. **C**–**D**, photobiont BB15.07 from *Pyrenocarpon thelostomum*; **C**, photobiont cells growing from thallus, haustoria penetrating cells (arrows); **D**, colonies of photobiont in multiple division. **E**–**H**, *Porocyphus dimorphus* and isolate BB15.06: **E**, thallus cross section, photobiont cells in short chains (arrows); **F**, isolate on agar medium, cells in pseudofilaments, binary division in mucilage envelope; **G**, isolated cyanobacteria in liquid medium, cells of variable sizes; **H**, isolated cyanobacteria on agar medium, multiple fission in mucilage envelope. (E in Lactophenol cotton blue)

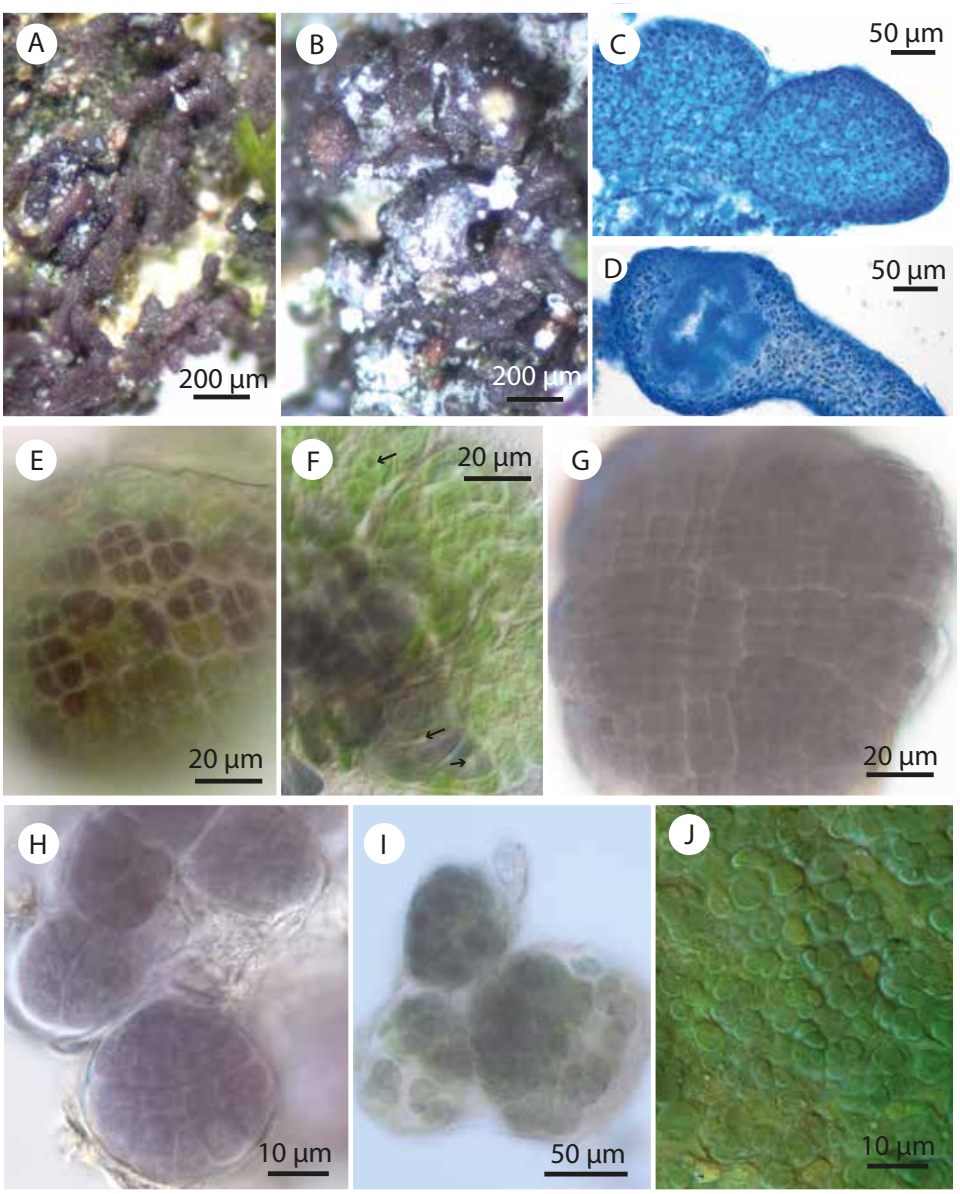

Figure 3.12: Lichinacean species B (no. 13802f) and its purple photobiont, *Chroococcidiopsis* BB15.08

A–**B**, morphology; **C**, thallus cross section; **D**: cross section of a pycnidium showing convoluted inner wall; **E**, thallus cross section, purple photobiont cells in 4-celled clusters; **F**, initially purple cells (centre) turned blue-green at margin, haustoria penetrate photobiont cells (arrows); **G**, supercolony derived by successive multiple fission; **H** and **I**, stages of multiple fission, cells release from mucilage layer; **J**, simultaneous multiple fission, single cells in variable sizes, change blue-green after 9 months in culture. (C and D in Lactophenol cotton blue).

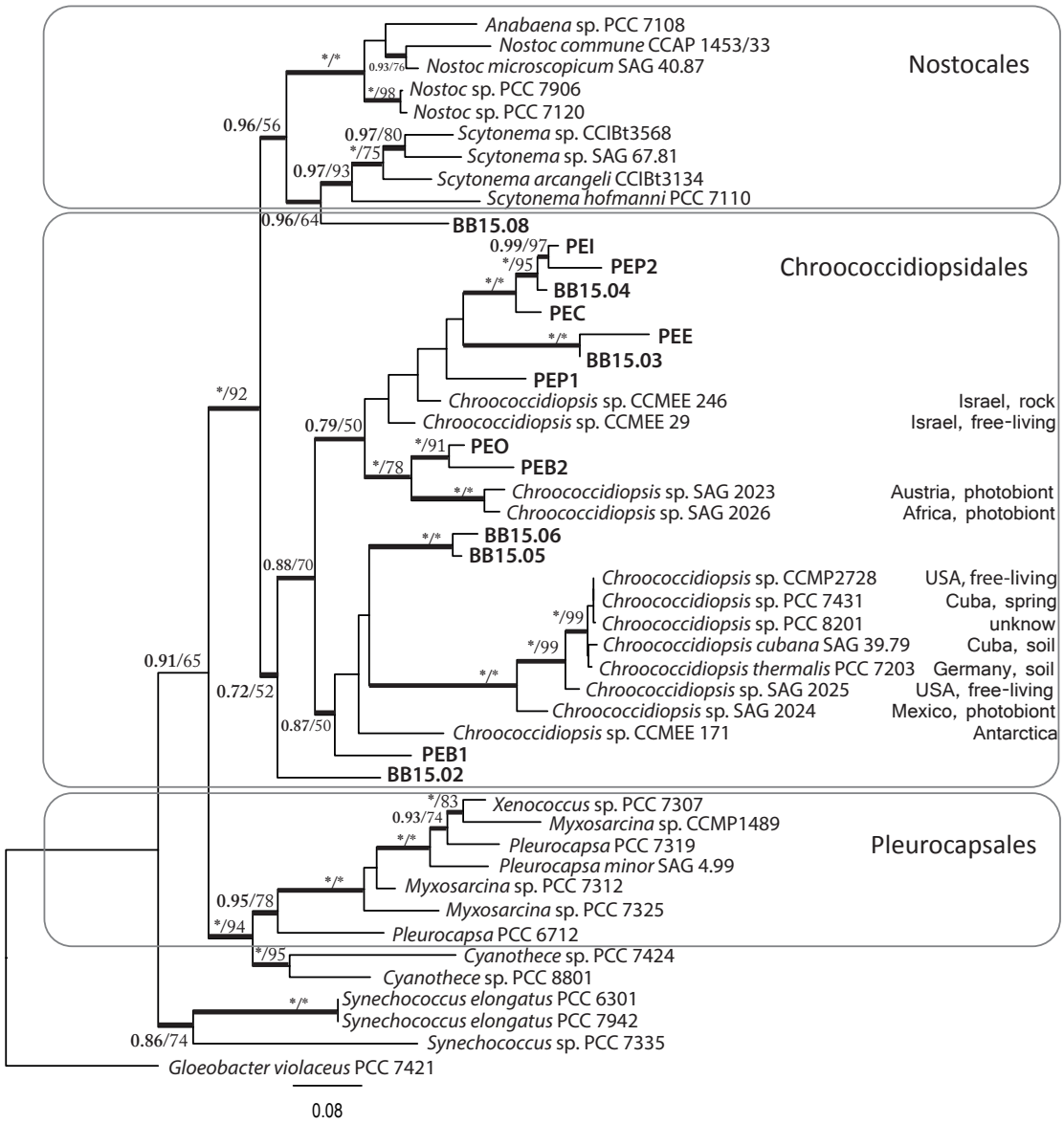

Figure 3.13: Phylogenetic tree of baeocyte-forming cyanobionts based on 16S rRNA data.

The 16S rRNA sequences obtained from refering lichens as well as their isolated photobiont, and 36 related sequences required from GenBank. Best tree was estimated with posterior probabilities (on the left) obtained from MrBayes and boostrap support (on the right) obtained from RAxML. * indicates 1 for posterior probability and 100 for bootstrap value

3.2.1.2 Taxonomy and phylogeny

Photobiont verification

The partial 16S rRNA sequence of *in situ* photobionts of lichen species (*Peltula clavata, P. euploca, P. impressa*, and *P. placodizans*) and their isolates were compared to verify the photobionts identity (Table 3.6). The two photobiont strains BB15.03 and BB15.04 isolated from *P. euploca* (PEE) and *P. impressa* (PEI) respectively, are equivalent to each other due to high similarity found in the blast search for two sequences (99% identity) (Table 3.6), and their close positions in the phylogenetic reconstruction (Fig. 3.13). In contrast, *P. clavata* and *P. placodizans* and their isolates, BB15.02 and BB15.05, shared 92% and 91% identical, respectively. These identities were far from the threshold of identical sequences of strains that were from 97% ((Stackebrandt and Goebel, 1994)) and recently from 98.5% ((Stackebrandt and Ebers, 2006; Stackebrandt, 2009, 2011; Kim et al., 2014)). The other isolates, BB15.01, BB15.06, BB15.07, BB15.08 and corresponding lichens were not compared since the microslide PCR applying to these lichens or the small amount of their photobionts was not successful.

Table 3.6: Photobiont confirmation by identity between *in situ* photobionts and isolates

Isolate	Lichen	Identity
BB15.02	*Peltula clavata* (PEC)	92
BB15.03	*P. euploca* (PEE)	99
BB15.04	*P. impressa* (PEI)	99
BB15.05	*P.placodizans* (PEP2)	91

Phylogenetic analysis of partial 16S rRNA sequences of *in situ* cyanobionts and isolated cyanobionts indicate that these photobionts are *Chroococcidiopsis* species

Total 14 sequences from six isolated strains of baocyte-forming cyanobacteria and eight *in situ* cyanobionts of *Peltula* spp. with variable lengths from 700 bp to 1400 bp were used for phylogenetic analyses (Table 3.4). Among of the isolated strains, five of them were obtained from *Peltula* spp. (BB15.02 to BB15.05), and one of them (BB.15.08) were required from the Lichinacean species B (no. 13802f).

Maximum likelihood analysis resulted in a best phylogenetic tree using GRT+G+I model with a score of optimization Likelihood: -11477.647823, propotion of invariable sites = 0.546823, $\alpha = 0.640374$, nucleotid frequencies estimated (A =0.257762, C = 0.228296, G = 0.318571, T = 0.195372), a rate matrix of substitutions (A-C = 0.836235, A-G = 1.589002, A-T = 0.939389, C-G = 0.551576, C-T = 2.777791, G-T = 1.000000). The trees from Bayersian runs were used to compute the posterior probability values. The topology of Bayesian consensus tree was was consistent with that of maximum likelihood. The maximum likelihood tree represented with bootstrap supports (BS) and posterior probability values (PP) were indicated on the nodes (Fig. 3.13).

All 14 sequences from isolated strains and *in situ* cyanobacteria grouped in the same clade as other *Chroococcidiopsis* and Nostocalean members from GenBank with statistical support (PP = 1, BS = 92) and were different from the Pleurocapsales clade (PP = 1, BS = 94) (Fig. 3.11). Two main subclades of *Chroococcidiopsis* were identified in the phylogenetic tree: (i) a subclade of 9 sequences from 7 *in situ* photobionts and 2 isolated strains of *Peltula* spp. in this study, photobionts of Lichinales from Africa and Austria, and free-living *Chroococcidiopsis* from Israel, (ii) a subclade of 3 sequences from 2 isolates, an *in situ* photobiont in this study, and an operational taxonomic unit that contained the type strain *Chroococcidiopsis thermalis* PCC 7203. Two sequences of the isolate BB15.02 and the purple strain BB15.08 were placed apart from the two subclades mentioned above. The strain BB15.02 clustered at the base of two *Chroococcidiopsis* subclades. Particularly, the purple strain BB15.08 grouped to the clade of free-living *Scytonema* (Nostocales) with good statistical support by the Bayersian analysis(PP = 0.96).

3.2.2 Heterocyte-forming cyanobacterial photobionts

3.2.2.1 Morphological characters of photobionts and their modifications in symbiotic association

Three heterocyte-forming cyanobiont strains were isolated from lichens, *Nostoc* strains BB15.10 and BB15.11 from two pannariod species, and a *Scytonema* strain BB15.09 from *Heppia lutosa* (Table 3.4). The photobiont culture of scy-

tonematoid photobionts from the genus *Coccocarpia* and from basidiolichens were not successful.

Scytonematoid photobionts of the basidiolichen genera *Cyphellostereum* and *Dictyonema* were studied directly from the thalli because of loose associations between the two symbionts and hence photobionts almost maintained their filamentous characters.

Nostoc in pannarioid lichens

Nostoc were tighly packed in pseudocolonies with a mucilage envelope in thalli of the lichen species, *Pannaria tavaresii* and *Parmeliella brisbanensis*. Isolated photobionts from these lichens were characterized by non-branching filaments of spherical vegetative cells with intercalary heterocytes surrounded by a mucilage layer. This gelatinous layer was thinner and less solid in the BB15.11 isolated from *Parmeliella brisbanesis* than that of strain BB15.10 from *Pannaria tavaresii*. The filament width varied from 6–7.5 µm in strain BB15.11 to 5.5–6.2 µm in strain BB15.10.

Photobiont *Scytonema* in *Heppia lutosa*

The photobiont strain *Scytonema* BB15.11, isolated from *Heppia lutosa* expressed the characteristic features of the species such as isopolar and false branching filaments not seen in its symbiotic association (Fig. 3.14). Cells were cylindrical, 7–9 µm wide and 5–7 µm long; yellow terminal cells, equal or longer than wide; heterocytes intercalary, variable in lenght, 6.8–13 µm; filaments had yellowish sheath. A lichenization process was observed in culture medium after 3.5 months in which fungal hyphae with its haustoria modified the arrangement of photobiont filaments into clusters of photobiont cells. The photobiont filaments became wider and gradually clustered in groups (Fig. 3.14 B). The filamentous structure of the photobiont can no longer be recognized such as photobiont clusters in the lichen thalli.

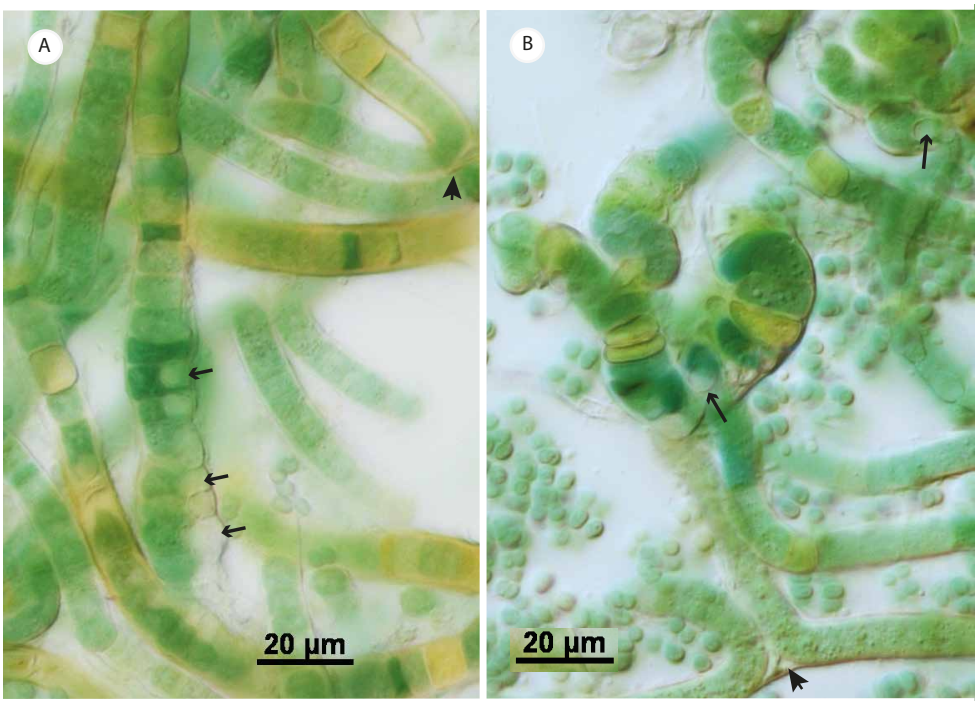

Figure 3.14: Photobiont *Scytonema* from *Heppia lutosa* developed after 3.5 months. False branches of free photobiont growing in culture medium (arrow heads), and fungal haustoria continue to penetrate and modify photobiont filaments (arrows)

Scytonema in *Cyphellostereum* and *Dictyonema*

The *Scytonema*-like photobionts of the three *Cyphellostereum* investigated here did not change their morphological feature in the lichen thallus. They are characterized by narrow trichomes from (5–)6–11–(14) µm, false-branching, yellow intercalary heterocytes and a round terminal cell (Fig. 3.5. E, and Fig. 3.6. B and F). Heterocytes were square-shaped, and as wide as vegetative cells. The width of photobiont filaments in *Cyphellostereum* sp. 1 was narrower than in two others species, only 5–8 µm in the species *Cyphellostereum* sp. 1 comparing to 9–10(–14) µm in the others. Photobiont cells in the species *Cyphellostereum* sp. 1 were square or almost round and different from the rectangular cells of the photobionts in *Cyphellostereum* sp. 2 and *Cyphellostereum* sp. 3. Photobiont reproduced at the ends of the filaments with the terminal part of the filaments more or less liberated out of the mycobiont hyphae; hence false-branches were observed in the lichen thalli such as those in free-living *Scytonema*.

The normally filamentous photobionts *Scytonema* in the two basidiolichens of the genus *Dictyonema* could be modified somehow by fungal hyphae. Their trichomes were often longer and wider than those in *Cyphellostereum* and constricted at heterocytes. For the photobiont in *Dictyonema* sp., cells were (9–)12–17 µm wide and 5–7 µm long, heterocytes 7–11 µm wide and 3–7 µm long (Fig. 3.5 C); for the photobiont in *Dictyonema moorei* with its uniseriate/biseriate trichomes, cells were (8–)12–17.5 µm wide and (4–)5–8 µm long, heterocytes were 8–14 µm wide and 3–6 µm long (Fig. 3.7 F–H). The heterocytes in photobionts of *Dictyonema* were narrower than vegetative cells; hence the trichomes often were constricted at heterocytes. The photobiont trichomes were completely wrapped by the mycobiont hyphae. Trichomes of the photobiont in *Dictyonema moorei* often broke and were coiled in irregular cell clusters at swollen parts of thalli.

The photobionts in *Dictyonema* variably reproduced through different developmental stages of the lichen. False branching of *Dictyonema moorei* cyanobiont were modified by the mycobiont (Fig. 3.7. F). Some cells at the bifurcated point of the false branchings irregularly divided. For *Dictyonema* sp., the false-branching feature was unclear with Y-shaped and T-shaped structures from two photobiont trichomes (Fig. 3.15. C and D), in which one trichome grew better than the other. Irregular growth of photobiont trichomes was observed at swollen parts of old thalli in *Dictyonema moorei*.The first photobiont cells grew beyond the swollen parts of lichen thalli (Fig. 3.7. I) and then developed a new fibrilous thallus with one or two photobiont filaments in a fibril (Fig. 3.7. C and E). The irregular division of cells in thallus to form swollen parts were observed in both species of *Dictyonema* in which the cyanobiont trichome broke and then coiled in clusters (Fig. 3.7. I, 3.15. A and B).

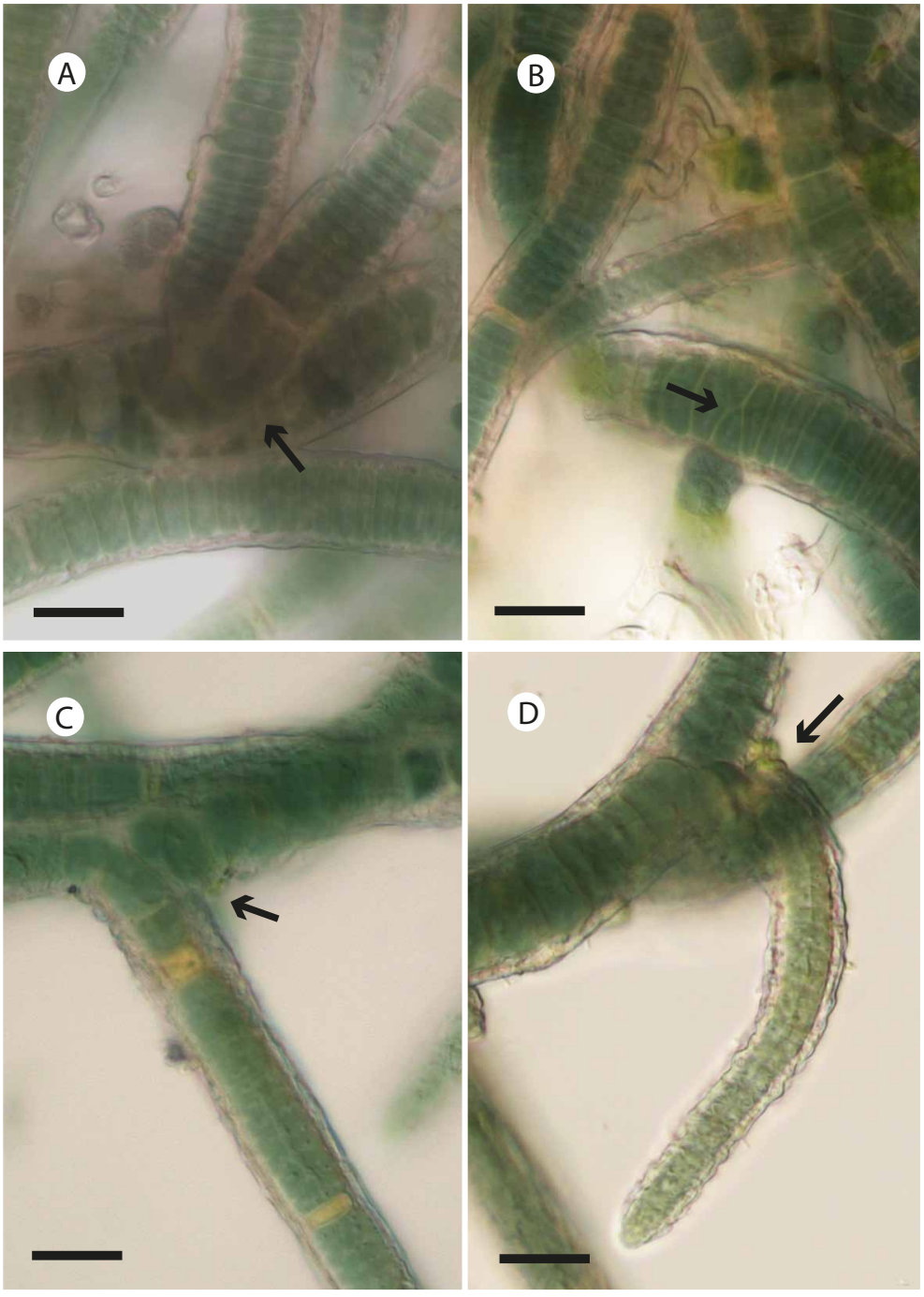

Figure 3.15: Photobiont growth in thallus of basidiolichen *Dictyonema* sp.
A; trichome raise from a swollen thallus part (arrow), **B**; cells in a trichome divide irregularly (arrow); **C**–**D**, Y-shaped and T-shaped false-branches were modified by lichenized fungi. Scale 20 µm.

3.2.2.2 Taxonomy and phylogeny

The 16S rRNA sequences obtained from heterocyte-forming photobionts had different lengths, from 570 bp for the photobiont strain *Scytonema* BB15.09 from *Heppia lutosa*, to 800–1183 bp for two trains *Nostoc* BB15.10 and BB15.11 from pannarioid lichen species (*Pannaria tavaresii* and *Parmeliella brisbanesis*) and an *in situ* photobiont *Rhizonema* of species *Coccocarpia erythroxyli*.

Maximum likelihood analysis at TREX server resulted in a best phylogenetic tree using GRT+G+I model with a score of optimization Likelihood: -5887.576135, nucleotid frequencies estimated (A = 0.257762, C = 0.228296, G = 0.318571, T = 0.195372), a rate matrix of substitutions (A-C = 0.771035, A-G = 1.379027, A-T = 1.026587, C-G = 0.509928, C-T = 2.779088, G-T = 1.000000). Bayesian runs converged after 10 000 generations and the consensus trees were used to calculate the posterior probabilities. The maximum likelihood tree represented with bootstrap supports (BS) and posterior probability values (PP) from Bayersian analyse were indicated on the nodes (Fig. 3.16).

Two *Nostoc* strains were clearly separated in the phylogenetic tree. The *Nostoc* strain BB15.11 from *Parmeliella brisbanensis* was placed in a clade including the same species from *P. brisbanensis* collected in Thailand and Reunion, with well-supported data (PP = 1, BS = 97), and was the sister clade of a cluster of free-living *Nostoc* strains.

In contrast, the *Nostoc* strain BB15.10 isolated from *Pannaria tavaresii* was placed in a clade composed of *Nostoc* strains of other pannarioid lichens (*Pannaria* spp. and other *Parmeliella* spp.) and *Peltigera* spp. This clade included two subclades, *Nostoc* A group and *Nostoc* B group. The first subclade corresponds to the "*Nephroma* guild" including *Nostoc* strains from pannarioid lichens while the latter (*Nostoc* B group) represents to the "*Peltigera* guild" (Rikkinen et al., 2002; Elvebakk et al., 2008). The *Nostoc* strains (Ch and BB15.10) from *Pannaria tavaresii* from Chile and Vietnam nested in each of these subclades, and hence they are distant on the phylogenetic tree. The difference here related to the substratum of the lichen specimens, the saxicolous species from Chile (Rikkinen et al., 2002) and the corticolous species from Vietnam.

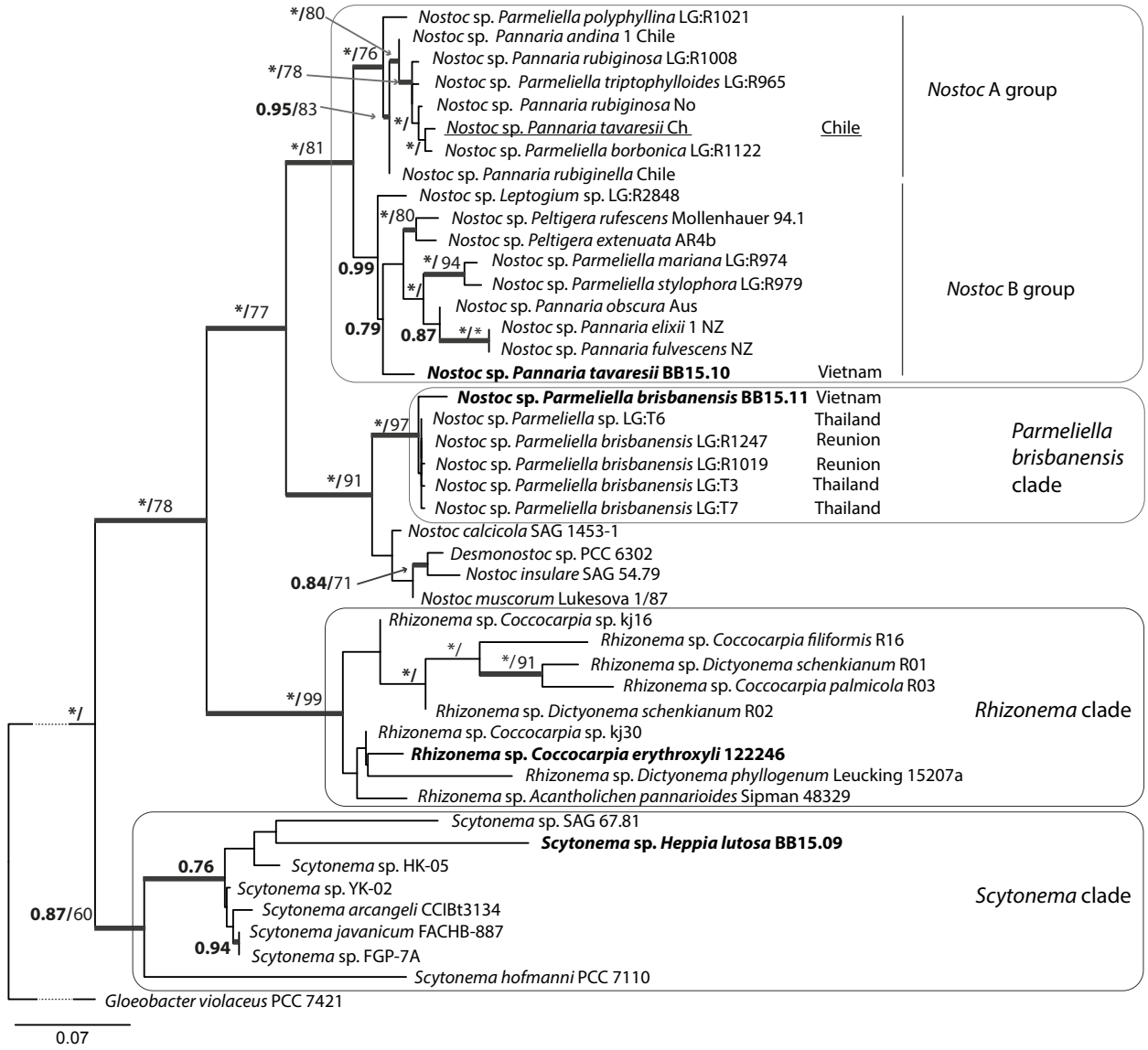

Figure 3.16: Phylogenetic tree of heterocyte-forming cyanobionts based on 16S rRNA data.

New sequences added in this study are indicated by boldfacing. Other sequences were obtained from GenBank. Best tree was estimated with posterior probabilities (on the left) obtained from MrBayes and boostrap support (on the right) obtained from RAxML. * indicates 1 for posterior probability and 100 for bootstrap value

For the scytonematoid photobionts, the phylogenetic analysis denoted the different genotypes of scytonematoid photobionts from studied lichens. The photobiont *Scytonema* BB15.09 isolated from *Heppia lutosa* clustered in a clade of free-living *Scytonema*, while the *in situ* photobiont from *Coccocarpia erythroxyli* 122246 was placed in the clade of the genus *Rhizonema*, a filamentous heterocyte-forming cyanobacterial genus with the photobiont species from the lichenized ascomycete genus *Coccocarpia* and the lichenized basidiomycete genus *Dictyonema* s. lat. (Lücking et al., 2009, 2014b).

3.3 Lichen community compositions

3.3.1 Sampling completeness

The species accumulation analysis revealed that the total number of species in all 57 study plots did not completely represented to the real species richness. The curve did not reach saturation (Fig. 3.18). Further analysis using the Chao 2 approach showed an estimation number of 90 species, which was 36% higher than the number of species collected (Fig. 3.17). Thus, the analysis also indicated that the real species richness of cyanolichen communities was considerably higher than expected from the observed number of species, and has not been fully assessed by the sampling effort in this study.

3.3.2 Floristic composition and similarity across habitats

The cyanolichen composition differed widely across the study locations with regard to dominant life forms, species richness, and floristic similarity (Fig. 3.19).

With respect to growth forms, four morphologies were recognized from studied lichens: crustose, foliose, filamentous and granular forms. Saxicolous crustose species occupied the outcrop sites at Ninh Thuan and Qui Nhon while epiphytic foliose lichens dominated the forest habitats. Filamentous and granular lichens were rare, only 5 filamentous basidiolichens of Hygrophoraceae occurred sparsely from the sites, and one granular species from Qui Nhon and Son Tra.

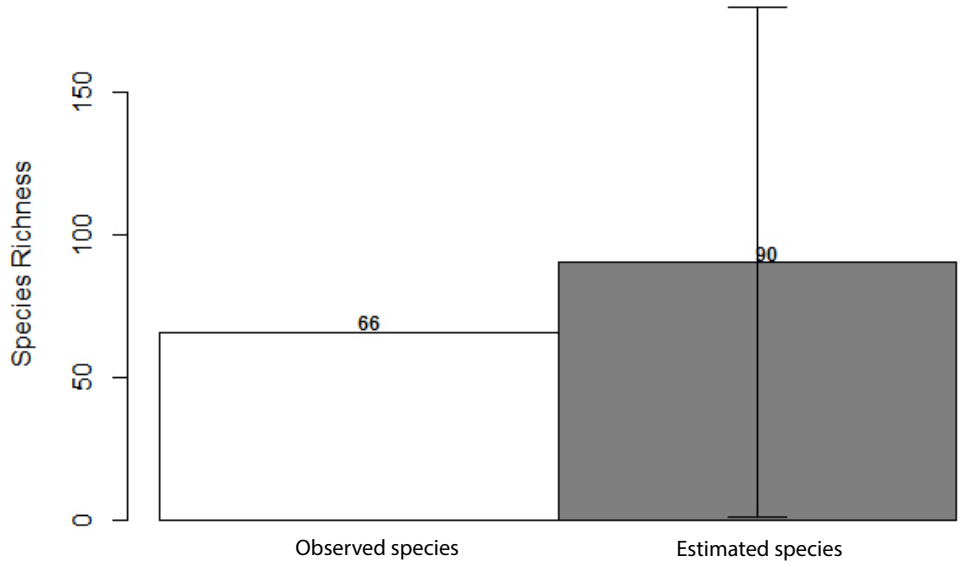

Figure 3.17: Mean observed and estimated species richness of cyanolichens across 57 study plots. Estimated species number refers to Chao 1. Error bars are standard deviations

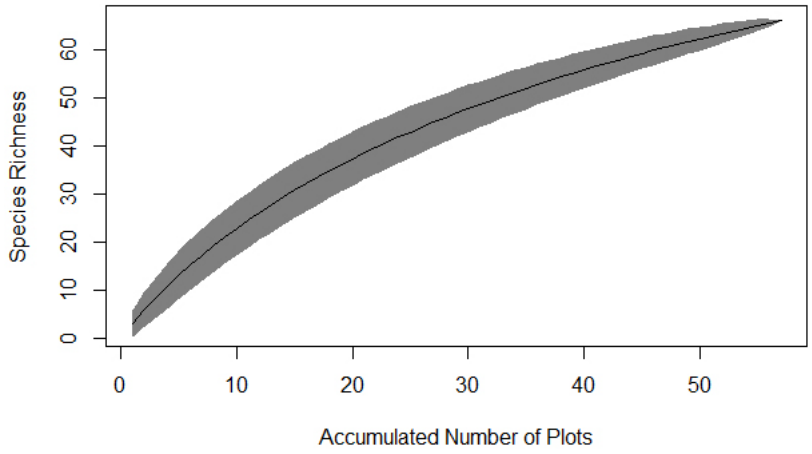

Figure 3.18: Species accumulation curve for cyanolichens collected across 57 study plots. The grey polygon represents 95% confidence intervals

Species richness was highest in the seasonal forests An Toan, followed by those in the dry forest Nui Chua and the outcrop Ninh Thuan. In contrast, the seasonal forest Lo Go Xa Mat and the non-seasonal forest Bach Ma contained the least number of cyanolichen species. The three sites, Bach Ma, An Toan and Nui Chua are habitats at an elevation (400 m a.s.l.), the other sites were below 400 m altitude. With the exception of Bach Ma, species richness was higher in forests with a high elevation than in those at lower elevations.

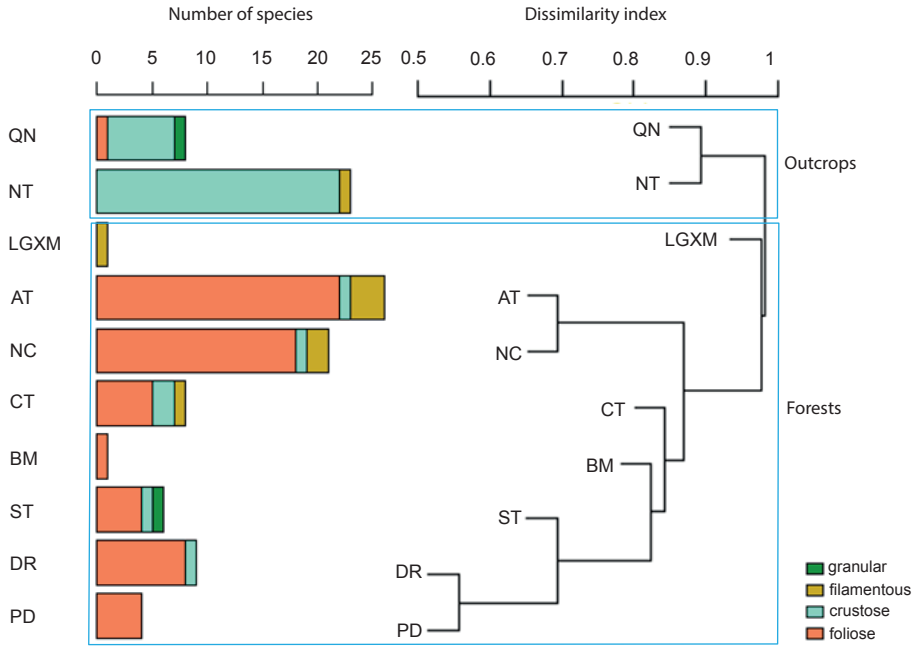

Figure 3.19: Stacked graph of species richness by growth forms at sites (on the left) and cluster dendogram basing on species dissimilarity between sites (on the right) (AT: An Toan, BM: Bach Ma, CT: Cat Tien, DR: Dakrong, LGXM: Lo Go Xa Mat, NC: Nui Chua, NT: Ninh Thuan, PD: Phong Dien, QN: Qui Nhon, ST: Son Tra)

Referring to floristic similarity, a cluster analysis indicated that the highest similarity occured in two geographically adjacent forests, Dakrong and Phong Dien (Fig. 3.19, Map at Fig. 2.1, page 12), and then the similarity decreased gradually at low-elevation sites toward the southern (Son Tra, Bach Ma, Cat Tien and Lo Go Xa Mat, see Map at Fig 2.1). The lowest similarity was seen in two outcrops at Qui Nhon and Ninh Thuan with the most crustose lichens differences between these sites.

I did not find any cyanolichen in sand dunes habitats. Only corticolous and terricolous chlorolichens were observed there.

3.3.3 Influence of precipitation on species richness

A correlation analysis addressing community variables and environmental factors revealed a significant negative relationship between mean annual precipitation and species richness of cyanolichen communities (Fig. 3.20). The data on the influence of two variables, light and elevation on species richness did not follow a normal distribution and thus could not be used for further analyses.

For precipitation, most of humid sites with >1600 mm rainfall per year harboured less than ten cyanolichen species, while the number of species more than doubled in dryer sites (<800 mm *yr^{-1}). This was also reflected by the coefficient of determination (R^2) indicating that approximately 46% of species richness variance could be explained by precipitation. The two localities, An Toan and Lo Go Xa Mat, were exceptions that located far from the regression line.

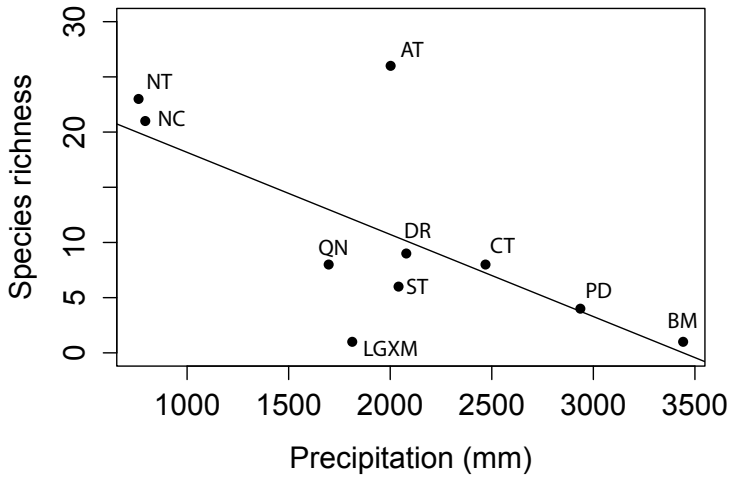

Figure 3.20: Relationship between species richness and precipitation
(t = -2.5896, df = 8, p-value = 0.03213,
95% CI: -0.91561313 – 0.07942943, r-value = -0.6752842
y= - 0.007x + 25.6, R^2 = 0.46
AT: An Toan, BM: Bach Ma, CT: Cat Tien, DR: Dakrong, LGXM: Lo Go Xa Mat, NC: Nui Chua, NT: Ninh Thuan, PD: Phong Dien, QN: Qui Nhon, ST: Son Tra)

4

Discussion

4.1 Cyanolichen diversity and composition

Prior to this investigation, only c. 50 cyanolichen species were identified from Vietnam. This study identified 55 species more, whereby doubling the number to over 100 species despite restrictions on sampling sites.

The species of the order Lichinales are all new records for the Vietnamese lichen flora. Most species in this study have been recorded from similar habitats throughout the world. For instance, 7 species of the genus *Peltula* are often recorded from arid and semi-arid habitats of the world (Schultz and Aptroot, 2008; Büdel, 1987; Büdel and Nash III, 2002; Büdel, 2001; Upreti and Budel, 1990; Wetmore, 1970; Büdel, 1999), *Porocyphus dimorphus* was recorded only from Africa (Henssen, 1963; Schultz and Aptroot, 2008). Some lichinacean species that had been found in limited regions are also found in coastal Central Vietnam. For instance, *Porocyphus dimorphus* is only known from Africa (Henssen, 1963; Schultz and Aptroot, 2008), *Lemmopsis arnoldiana* and *Pyrenocarpon thelostomum* are known to occur in the centre and north of Europe (Schultz and Büdel, 2002; Jørgensen, 2007; Thüs and Schultz, 2008), *Pyrenopsis melanophthalma* is found in Venezuela.

Cyanolichens of the order Lecanorales mostly inhabited forests. Some less frequent genera of this order were remarked from their habitats. *Spilonema americana* occurred on rocks neighboring other cyanolichens, *Coccocarpia* sp. and *Dictyonema* sp. These lichens associates to a photobiont genus – scytonematoid cyanobacteria. *Staurolemma* cf. *perforatum* was found on tree at heigh of about 17–20 m on a fallen tree while most *Staurolemma* species are known growing on rocks (Henssen, 1999; Jørgensen and Henssen, 1999; Jørgensen, 2010). In this study, three genera with simple spores *Ramalodium, Physma, Staurolemma* were placed in family Pannariaceae since molecular analyses confirmed that they phylogenetically clustered to the family Pannariaceae instead of Collemataceae (Otálora et al., 2013; Wedin et al., 2009; Otálora et al., 2014; Ekman et al., 2014).

Remarkable habitats in which the Vietnamese cyanolichens were found are (i) the amphibic habitat with lichen species such as *Lemmopsis arnoldiana, Peltula clavata, P. obscurans, Pyrenocarpon thelostomum,* and *Thermutis velutina*, that grew on boulders along streams where they can be seasonally inundated; (ii) shaded habitats of rocks in the limestone forest at Dakrong and in the savanna at Ninh Thuan in which two new saxicolous taxa of the family Lichinaceae were found resembling the genus *Pterygiopsis* (with purple cyanobiont *Chroococcidiopsis*), and are characterized by a lecanoralean ascus; (iii) rock crevices with soil was inhabited by *Peltula euploca*.

A notable floristic heterogeneity across the investigated habitats in Vietnam was observed in the cyanolichen species composition at the different sites. Communities of coastal outcrops were dominated by saxicolous lichens of the order Lichinales (Lichinaceae and Peltulaceae), while cyanolichen communities of forests were mostly dominated by epiphytic cyanolichens of the ascomycete order Lecanorales (Fig. 3.19). The highest species numbers were found in the mountain forest An Toan with species of the genera *Coccocarpia, Pseudocyphellaria, Sticta, Staurolemma,* and *Ramalodium*. There, I found species that were previously not known from Vietnam such as, *Coccocarpia microphyllina* known from Costa Rica, *Spilonema americanum* to date only known from north Pacific rim of North America, and *Staurolemma perforatum* so far only known from Japan and Korea.

The almost total absence of terricolous cyanolichens in lowland forests and of epiphytic cyanolichens in the forest Lo Go Xa Mat are probably a result of a thick leaf litter layer on the forest floor, combined with low light availability along the base of tree trunks. The poor cyanolichen diversity in Bach Ma is completely contrasted to the very high vascular plant diversity (more than 1400 species, Regalado Jr et al., 2005) in the region. However, the high precipitation value (ca. 3400 mm) and the lack of dry period in Bach Ma (Nguyen et al., 2000) is probably limiting the occurrence of cyanolichens.

Precipitation heterogeneity effected to the variability of cyanolichen species compositions across habitats (Fig. 3.20). The existing precipitation gradient of Vietnam relates to the geographic variance of the country, in which higher elevations are reached in the west with the Annamite Range, and become lower towards the east. Hence, the coastal regions are situated in the rain shadow caused by blocking off rainfall in the windward side of the mountain range. This effect creates a dry climate, particularly in Ninh Thuan province (Sterling et al., 2008; Gaussen et al., 1967). In the following part of the discussion this climatic variance was compared with the cyanolichens distribution patterns found in this study.

This study indicated a surprisingly negative correlation between precipitaion and lichen species richness, contrasting to the fact that precipitation increase is positively corelated to species richness in vascular plant communities (Gentry, 1982, 1986) and lichen communities in previous studies (Newmaster et al., 2003; Adams and Risser, 1971). The fact relates to (i) the specific ecophysiological features of cyanolichens regarding to their photosynthetic response to water and (ii) the interspecific competition with vascular plants. With respect to the first point, in the advantage of water availability, photosynthesis and metabolism of lichens are active. However, water suprasaturation does negatively influence on net photosynthesis as gases diffuse some 8600 times slower in water than in air (Cowan et al., 1992; Lange et al., 2001). As a result, cyanolichens, like most other lichens, show a strong depletion in CO_2 uptake. In the case of the rock inhabiting green algal lichen *Lecanora muralis*, even 38% of the total active (moist) period with in 15 months suffered from suprasaturation and thus low CO_2 uptake rates (Lange, 2002, 2003a,b). This is also the case for the cyanolichens *Collema tenax*

and two species of the soil inhabiting genus *Peltula* (Lange et al., 1998; Büdel et al., 2013). Vascular plants are homoiohydric, meaning that they regulate their water content and thus do not have problems with suprasaturation. Thus they are supreme competitors over poikilohydric organisms such as lichens. This might explain the second point, that better competitors such as vacular plants become dominant and somehow limit the growth of lichens through restraining them from resources such as light and substrate (i.e. soil) in forests. Contrastingly, on rock outcrops and soil, water avaiability is the limiting factor for the growth of vascular plants while the poikilohydric lichens can adapt well to that environment and then may become dominant organisms, especially the terricolous and saxicolous lichens of the order Lichinales.

4.2 Photobionts

The photobiont diversity found in the selected cyanolichens from Vietnam included baeocyte-forming cyanobionts and heterocyte-forming cyanobionts. Morphological studies and phylogenetic analysis of 16S rRNA sequences of photobionts denoted to *Chroococcidiopsis, Nostoc, Scytonema*, and *Rhizonema* from Vietnamese cyanolichens will be discussed below.

4.2.1 Baeocyte-forming cyanobacteria

Chroococcidiopsis is the unique photobiont in _Peltula_ species of Vietnam

In this study, the morphological features and phylogenetic analysis indicated that the baeocyte-forming genus *Chroococcidiopsis* was the unique cyanobiont in all *Peltula* species obtained from Vietnam. The reproduction of these photobionts is regularly performed by one or two binary division, and then followed by multiple fission. Baeocytes are formed by successive multiple fission from daughter cells of binary divisions, and colonies of these baeocytes are often adhered in clumps. With the phylogenetic analysis of partial 16s RNA sequences, all photobionts of *Peltula* were *Chroococcidiopsis*, and are completely separated from the clade of the

Pleurocapsales. They mostly clustered in a subclade as a sister to the subclade of *Chroococcidiopsis thermalis*.

Morphological features of the *Chroococcidiopsis* in culture strains can vary largely, especially in the initial stages when a culture establishes in the fresh medium. The *Gloeocapsa*-like feature with groups of two or four cells in thick mucilage sheaths in the strains BB15.01 and BB15.03 is similar to those in the *Chroococcidiopsis* strain 26391c PH from the lichen *Psorotichia* (Fig. 30, page. 373, Büdel and Henssen, 1998); or the *Entophysalis*-like habit with an enlarged margin of strain BB15.08 is similar to those in the strains of *Chroococcidiopsis* 26370a, a photobiont of the lichen *Peccania* sp. (Fig. 10, page 370, Büdel and Henssen 1998), strain Pn-7503-Pp from *Peltula polyspora* (Fig. 18, Bubrick and Galun, 1980), and strain Pn-7602-Po from *Peltula obscurans* (Fig. 19, Bubrick and Galun, 1980). These features belong to the binary division stage of the isolates and were rarely seen in stable fully grown cultures. Therefore, previous descriptions of the photobiont diversity in *Peltula* (Burbrick and Galun, 1984; Büdel, 1987) need to be interpreted carefully, with regard to the morphological variability that potentially overlies the genera diversity. It is necessary to re-examine the photobionts in *Peltula* distributed in different geographical regions by the polyphasic approach in which at least phylogenetic analyses can discriminate between photobiont genera in this lichen genus.

A total of 5 *Chroococcidiopsis* strains isolated from different *Peltula* species were determined by both morphological characteristics and molecular analysis. In this study, four photobiont strains (BB15.01, BB15.03, BB15.04 and BB15.05) were confirmed from *Peltula* spp. based on haustoria penetrated colonies during the isolation process. The strain BB15.02 isolated from *P. clavata* was grown in liquid medium and did not show haustoria in the early isolation/cultivation process. Sequence comparison between isolates and *in situ* photobionts of each *Peltula* species verified two photobiont strains BB15.03 and BB15.04. Sequences from isolates BB15.02, BB15.05 and *in situ* photobionts of *P. clavata* and *P. placodizans* failed to corroborate the identity of the photobionts. In the case of strain BB15.05 isolated from *P. placodizans*, the haustoria were seen in early cultivation colonies over a long period, up to 20 months, but the phylogenetic

comparison indicated that it was not the *in situ* photobiont. Therefore, I assume that the mycobiont hyphae caught an epiphytic *Chroococcidiopsis* of this *Peltula* that grew in the medium and then used it as a photobiont. In other words, free-living *Chroococcidiopsis* might serve as a potential associate or even photobiont of the lichen *Peltula placodizans*. This fact can also illuminate a previous question about a possible association between free-living cyanobacteria and lichenized fungi in Peltulaceae and Heppiaceae (Bubrick and Galun, 1984).

New features from *Chroococcidiopsis* strains of Lichinacean lichens

a- The purple Chroococcidiopsis BB15.08 isolated from the lichen no. 13802f.

This lichen was collected from the outside wall of a shady limestone cave. The strain BB15.08 resembles *Chroococcidiopsis* kashaii described from a limestone cave in Israel (Friedmann, 1961). The newly isolated strain BB15.08 and *C. kashaii* show similarities in the coloration, and in three kinds of division: binary division, successive fission, and simultaneous multiple fission. This coloration could be caused by an over-expression of the accessory pigment phycoerythrin as it was observed on cyanobacteria that grew under lowlight conditions in caves (Mulec et al., 2008). At higher light levels, such as the cultivation environment, physiological adaptation of the strain BB15.08 lead to a re-establishment of the blue-green color. This unicellular baeocyte-forming *Chroococcidiopsis* strain was especially closer to the clade of filamentous heterocyte-forming *Scytonema* (Nostocales) than to the clade of unicellular baocyte-forming *Chroococcidiopsis* (Chroococcidiopsidales) in the phylogenetic tree. The strain and species *C. kashaii* probably represents a shade-tolerant *Chroococcidiopsis* clade occurring in calcareous habitats.

The lichen No.13802f was placed in the family Lichinaceae based on its morphological characteristics. It is similar to *Pterygiopsis*, a genus in the family Lichinaceae, by its fan-shaped thallus (Fig. 3.12 C) and has pycnidia with a convoluted wall, has a bright ostiole that also characterizes members of the family Lichinaceae (Henssen, 1963; Schultz and Büdel, 2002; Jørgensen, 2007; Schultz, 2007a). Additionally, *Chroococcidiopsis*, the photobiont of this specimen is commonly known as an exclusive photobiont of the Lichinomycetes (Büdel and Henssen, 1983, 1986; Bubrick and Galun, 1984; Büdel et al., 1994, 1997, 2000; Büdel and Kauff, 2012;

Büdel, 1999). However, further investigations are needed, including specimens with ascomata before a clear assignment to a genus of the Lichinales is possible.

b- Binary division of the Chroococcidiopsis strain BB15.06 by a division plane being perpendicular to a same cell axis.

Chroococcidiopsis cells were only known to divide by perpendicular planes in succeeding generations in the binary division mode as in most *Chroococcidiopsis* strains of this study and from literature (Friedmann, 1961; Büdel and Henssen, 1983; Komárek and Anagnostidis, 1998; Büdel and Kauff, 2012; Donner, 2013; Komárek et al., 2014). Consequently, microscopic appearance of cell colonies from binary division is a cubic of powers of 2 of cells (4-8-16-). The strain *Chroococcidiopsis* BB15.06 that was confirmed by phylogenetic analysis (Fig. 3.13) and morphology (two division modes, binary division and simultaneous multiple fission (Fig. 3.11 F–H) indicated a new microscopic appearance, the short pseudofilamentous rows. This morphology was resulted from a new binary division mode in which cells divided by a plane that was perpendicular to a same cell axis in successive generations (Fig. 3.11 F). Consequently, daughter cells arranged into short pseudofilamentous rows that was easily disjoint in a mucilaginous layer. This binary division corresponds with the genera of family Synechococaceae, such as *Rhabdoderma* and *Synechococcus* (Komárek and Anagnostidis, 1998).

Strain BB15.06 was isolated from the lichen *Porocyphus dimorphus* where the photobiont was formally described to be the filamentous, heterocyte containing cyanobacterium *Calothrix* (Henssen, 1963; Schultz, 2007b). *Calothrix* is generally characterized by a heteropolar trichome with a heterocyte at the base. However, in the specimens of *P. dimorphus* in this study, the *in situ* photobiont cells were sometimes arranged in short pseudofilaments, but normally grew in the typical colony shape of the *Chroococcidiopsis*-type without heterocytes (Fig. 3.11 E). Although the appearance of the *in situ* photobiont and the isolated strain BB15.06 are similar each other (Fig. 3.11 E–F), the question remains of whether the strain BB15.06 is the photobiont of *Porocyphus dimorphus*. This can not be answered until we get verification by either the presence of haustoria in culture colonies, or by molecular analysis of the *in situ* photobiont.

4.2.2 Heterocyte-forming cyanobacteria

Two *Nostoc* photobiont strains of two pannarioid lichen species indicate a difference of photobiont selectivity.

The high similarity between *Nostoc* strains of *Parmeliella brisbanensis* thalli from distant geographical regions (Vietnam, Thailand and Reunion) in the phylogenetic analysis (Fig. 3.16) indicated the high specificity of the lichen species to distinct cyanobiont genotypes and the observed selectivity did not seem to change with geographical regions. In contrast, *Nostoc* strains from *Pannaria tavaresii* collected from distant geographical regions showed a low similarity and were placed in different clades (Fig. 3.16). This was probably related to habitat differences of the lichen specimens. While *P. tavaresii* from Vietnam grew on the bark of a tree, that from Chile grew on a rock surface (Elvebakk et al., 2008).

Is the isolated photobiont *Scytonema* from *Heppia lutosa* identical to the free *Scytonema* living in the same habitat to *Heppia lutosa*?

The photobiont *Scytonema* BB15.11 isolated from *Heppia lutosa* was confirmed by morphological characteristics and a phylogenetic analysis. Our results correspond with those of Marton and Galun (1976) and Ahmadjian (1967) who found cyanobacterial strains isolated from *Heppia echinulata* and *Heppia* sp., respectively, as belonging to the genus *Scytonema*. This heterocyte-forming photobiont genus differs from the photobiont *Rhizonema* from *Coccocarpia erythroxyli* by separated positions in 16S rRNA phylogenetic tree (Fig. 3.16). It ascertains that there are two different filamentous heterocyte-forming photobionts associated to lichenized fungi, *Scytonema* and *Rhizonema*.

H. lutosa in this study grew together with a free-living *Scytonema* species on soil. Moreover a lichenization process was observed several time at the lower side between hyphae in lichen thallus and this *Scytonema* (Fig. 3.1 E) in which the *Scytonema* filaments associated to mycobiont hyphae in the lichen thallus and their cells becoming modified to unicellular clusters with penetrating haustoria. The remaining part of these *Scytonema* filaments elongated beyond the lower side of the lichen thallus with its natural morphology. This fact raises a question whether

the photobiont *Scytonema* BB15.11 and the neighbor free-living *Scytonema* are a same strain? And if it is so, the lichenization process might indicate a development strategy of thallus that renewal from the lower side. Additionally, the accumulation of the old and dead photobiont layer at the upper surface supports the assumption of the upward growth of the thalli as observed in another lichinalean species, *Pseudopeltula necrocorticata* (Büdel and Schultz, 2011).

The photobiont *Rhizonema* was confirmed via phylogenetic analysis from thalli of *Coccocarpia erythroxyli*, and the photobiont *Scytonema* was observed directly from basidiolichens.

The photobiont *Rhizonema* was found to be the unique photobiont in cyanobacterial basidiolichens and ascolichens of the Coccocarpiaceae by a phylogenetic analysis (Lücking et al. 2009) of the partial 16S rRNA gene of the *in situ* photobiont of *Coccocarpia erythroxyli* in the present study (Fig. 3.16).

However, within the basidiolichens treated in this study, the photobionts were determined to belong to the genus *Scytonema* based on their morphological features, including cylindrical filaments with intercalary heterocytes, a round terminal cell and false-branching of the filaments. The cell size of the photobionts of the basidiolichen genera in this study were highly similar to that of *Rhizonema* photobionts (Dal-Forno et al., 2013; Lücking et al., 2013, 2014a; Yánez et al., 2012). The false-branching, an important feature for identification of the genus *Scytonema* was also observed by Slocum in *Dictyonema irpicinum* (Fig. 4 page 1009 by Slocum 1980). In the present study, this feature was more often exhibited in the genus *Cyphellostereum* than in *Dictyonema* since the photobiont was much less intensive surrounded by hyphae in *Cyphellostereum* compared to that in *Dictyonema*.

The cyanobacterium *Rhizonema* of the monogeneric family Rhizonemataceae was validated with the type species *R. interruptum* that was the photobiont of *Dictyonema coppinsii* by Lücking et al. (2009). The true branching feature of this species type is based on observations of the cyanobiont in the lichenized state (Lücking et al., 2009) and is the unique feature to discriminate *Rhizonema* from *Scytonema*. However, since *Cyphellostereum* cyanobionts are much less attacked

by the mycobiont free than in *Dictyonema*, it might be understood here that the feature of true branching is more common and easier to observe in *Cyphellostereum* than in *Dictyonema*.

The culture and microslide PCR did not work with scytonematoid photobionts associated with the basidiolichens and also the culture was failed with *Coccocarpia* spp., thus prevented me from further examination to find out the relationship between the *Scytonema* from basidiolichens (*Cyphellostereum* spp. and *Dictyonema* spp.) and *Heppia lutosa*, and *Rhizonema* from *Coccocarpia* in this study. Therefore, the following questions need to be examined for further progress relating to cyanobiont identification: (i) what are the genentic relationships between *Scytonema* from *Heppia* and *Scytonema* from basidiolichens (*Dictyonema* s.lat.), and between *Scytonema* from basidiolichens in this study with *Rhizonema* from basidiolichens in different regions and *Coccocarpia*; (ii) can the false-branching feature be shared by both genera, *Scytonema* and *Rhizonema*, such as the baeocyte-forming feature in the cyanobacteria of Chroococcidiopsidales and Pleurocapsales?

4.2.3 Mycobiont photobiont interaction in basidiolichens, and the tubular intracellular haustoria in the basidiolichens *Cyphellostereum*

Variable growth of the photobionts in *Dictyonema* indicate a transition of the photobiont morphology from filaments to cell clusters. Evolutionary progress of the cyanobacterial basidiolichen genera of Hygrophoraceae are confirmed from *Cyphellostereum, Dictyonema, Acantholichen, Corella,* to *Cora* in order, resulted from multigene phylogenetic analysis (Fig. 1 at page 4 by Dal-Forno et al., 2013). The *Cyphellostereum* spp. represents an earlier monophyletic clade that is a sister to a clade of remain genera. *Dictyonema* and *Cora* were younger polyphyletic clades. Additionally, these authors reconstructed ancestral character state characters of thallus morphology, photobiont morphology, hyphal sheath structure, and reproduction. Two characters of photobiont morphology and hyphal sheath structure were discussed here.

With respect to the photobiont morphology, the derived unicellular state observed in three last genera (*Acantholichen, Corella, and Cora*) were originated from ancestral genera (*Cyphellostereum* and *Dictyonema*) (Fig. 4.1). This variation was caused by the specific fungal morphogenetic effects on the photobiont (Lücking et al., 2009; Dal-Forno et al., 2013) and gradually derived through genera. However, in this study, both of these states were observed in *Dictyonema* sp and *D. moorei*. The filamentous state was seen in the photobiont of young thalli while unicellular morphology was observed in the photobiont of old thalli. It denoted that the photobiont morphology also was influenced by the tension/density of mycobiont hyphae to photobiont.

The hyphal sheath structure progressed from irregular hyphal sheath with appressoria for *Cyphellostereum* clade to compacted hyphal sheath by jigsaw-puzzle-shaped cells with tubular intracellular haustoria for the remaining genera of the phylogenetic tree including *Dictyonema, Acantholichen, Corella* and *Cora* (Dal-Forno et al., 2013) (Fig. 4.1). However, the haustoria of *Cyphellostereum* were not examined in detail by transmission electron microscopy (Oberwinkler, 1984), and were expected to be similar to the mycobiont-cyanobiont interaction in the basidiolichen genera *Cora* and *Dictyonema* (Oberwinkler, 2012). In this study, the tubular intracellular haustoria were observed in *Cyphellostereum* by both light and electron microscopy, and they resembled the haustorial system of *Dictyonema* in previous study as well as in this study (Slocum and Floyd, 1977; Oberwinkler, 1980, 1984; Slocum, 1980; Tschermak-Woess, 1983); hence it supports Oberwinkler's assumption. These findings contrast to recent studies in which only appressoria were found in *Cyphellostereum* (Dal-Forno et al., 2013; Lücking et al., 2013).

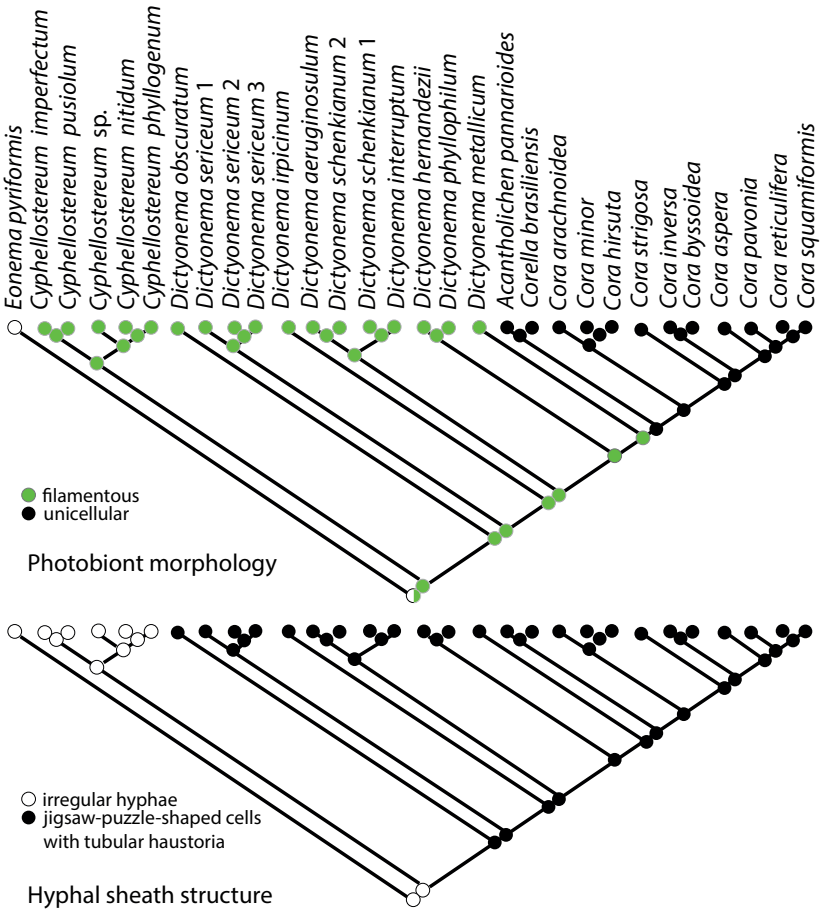

Figure 4.1: A part of Figure 2 in page 5 by Dal-Forno et al. (2013) exhibited the ancestral character state reconstruction of cyanobacterial basidiolichens of Hygrophoraceae based on the best tree from maximum likelihood and Bayesian analyses.

Two morphology characters, photobiont morphology and hyphal sheath structure, were extracted from the original figure. The photobiont morphology includes filamentous (ancestral state) and unicellular (derived state) appearance; the hyphal sheath structure includes irregular hyphae (ancestral state) and jigsaw-puzzle-shaped cells with tubular haustoria (derived state).

5

Conclusions

This study, on cyanolichens and their photobionts regarding their taxonomy and biodiversity, aims to contribute to the understanding to cyanolichens in lowland regions of Vietnam.

In total, 66 cyanolichens were identified for in Vietnam. Of those, 50 were new records for Vietnam. The lichens of the families Lichinaceae and Peltulaceae (Lichinales) are new for the lichen flora of Vietnam and mainly occurred in semi-arid coastal habitats. The results are extending the geographical range and habitats of these species in the tropical Asian region. One new species and two so far unidentified species in the family Lichinaceae point to the need for studies in such under collected tropical regions.

The intracellular haustorial system in *Cyphellostereum* resembled those of the genus *Dictyonema*. These two genera are discriminated from each other by their hyphal sheath morphology surrounding the cyanobiont. The mycobiont-photobiont interaction influenced photobiont features not only by genetic specificity but also by accumulated quantity of the mycobiont hyphae surrounding the photobiont. However, another kind of mycobiont-photobiont contact, the appressoria, was also observed in *Cyphellostereum* spp. occurring in Neotropical America and the Philip-

pines. Therefore, further studies of this genus are necessary to access the variability of its bionts through geographical regions.

Chroococcidiopsis was found to be the unique photobiont associated with *Peltula* spp. and three lichinacean species in this study. The results were supported by both morphological and phylogenetic characteristics from culture photobionts. Moreover, for the *Peltula* species, the free-living *Chroococcidiopsis* that lives in the vicinity of lichens could be a compatible photobiont for the lichenization process.

The important role of the photobiont culture was highlighted again in this study. Cultured photobionts expressed their developmental stages in culture medium, and then new characters could be discovered, such as pseudofilamentous features of a *Chroococcidiopsis* strain in this study. The morphology of isolated photobionts clarified their taxonomical position when the phylogenetic analysis was not sufficient.

The scytonematoid cyanobionts in lichens were confirmed in this study, including two genera, *Rhizonema* and *Scytonema*. The genus *Rhizonema* represented the photobiont of *Coccocarpia* while *Scytonema* was found in *Heppia lutosa*, *Cyphellostereum*, and *Dictyonema*. The photobiont *Scytonema* in basidiolichens from Vietnam differs from the photobiont *Rhizonema* from previous studies by false branching in *Scytonema* and true branching in *Rhizonema*.

The problem in studying scytonematoid photobionts is related to culture and the PCR procedure. Firstly, these photobionts from *Coccocarpia* spp. and basidiolichens were unculturable in BG11, at least in my approaches. With respect to the PCR of these photobionts, the sheath could be the main reason for DNA contamination, and hence for the low quality of PCR production. The DGGE can solve this issue but the short obtained sequences from this method influenced the resolution of phylogenetic tree. A method that was successfully applied by Mareš et al. (2015) and Komárek et al. (2013) should be considered with these photobionts for future studies.

Although this study significantly contributed to species richness of the lichen flora of Vietnam, the observed species diversity still does not reflect the real diver-

sity, as indicated by modeled estimates of species richness. In addition, the study indicated that the cyanobacterial lichen diversity and growth form were negatively affected by precipitation. The other ecological factors gave insignificant effect to species richness and community compositions. Therefore, to promote research results for further studies, some suggestions should be considered: plot-based sampling design including standardized assessment of explanatory variables such as relative humidity, light interception; and response variables such as community composition, functional group using for example the point-intercept method. Especially species identity and cover of species need to be taken into regard, and a sufficient number of sampling site replicates are needed to reach the necessary data size for statistically ecological analyses.

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6

Appendices

Supplementary tables

Table 6.1: Cyanobacterial strains from GenBank used in this study

Species	Origin	Author	Accession No.
Anabaena sp. PCC 7108	USA, intertidal zone	Lyra et al. 2004	AJ133162.1
Chroococcidiopsis cubana SAG 39.79	Cuba, dry soil	Cumbers & Rothschild 2014	JF810080.1
Chroococcidiopsis sp. CCMEE 171	Antarctica	Cumbers & Rothschild 2014	JF810071.1
Chroococcidiopsis sp. CCMEE 246	Israel, rock	Cumbers & Rothschild 2014	JF810073.1
Chroococcidiopsis sp. CCMEE 29	Israel	Cumbers & Rothschild 2014	JF810082.1
Chroococcidiopsis sp. CCMP2728	USA	Cumbers & Rothschild 2014	JF810075.1
Chroococcidiopsis sp. PCC 7431	Cuba, water, mineral spring	Seo & Yokota 2003	AB074506.1
Chroococcidiopsis sp. PCC 8201	Unknow, ***	Cumbers & Rothschild 2014	JF810081.1
Chroococcidiopsis sp. SAG 2023	Austria, *Thyrea pulvinata*	Fewer et al. 2002	AJ344552.1
Chroococcidiopsis sp. SAG 2024	Mexico, *Anema nummularium*	Fewer et al. 2002	AJ344553.1
Chroococcidiopsis sp. SAG 2025	USA	Fewer et al. 2002	AJ344554.1
Chroococcidiopsis sp. SAG 2026	Africa, *Peltula euploca*	Fewer et al. 2002	AJ344555.1
Chroococcidiopsis thermalis PCC 7203	Germany, soil	Ishida et al. 2001	NR_112108.1
Cyanothece sp. PCC 7424	Senegal, soil, rice field	Nubel et al. 1998	AJ000715.1
Cyanothece sp. PCC 8801	Taiwan, soil, rice field	Lucas et al. 2015	NR_074265.1
Desmonostoc sp. PCC 6302	_	Hrouzek et al. 2014	HG004582.1
Gloeobacter violaceus PCC 7421	saxicolous, Switzerland	Nakamura et al. 2003	NR_074282.1
Myxosarcina sp. CCMP1489*	Galapagos islands	Fewer et al. 2002	AJ344556.1
Myxosarcina sp. PCC 7312	Mexico, intertidal zone	Fewer et al. 2002	AJ344561.1
Myxosarcina sp. PCC 7325	Mexico, intertidal zone	Fewer et al. 2002	AJ344562.1
Nostoc calcicola SAG 1453-1	soil, Netherlands	Friedl et al. 2014	KM019926.1
Nostoc insulare SAG 54.79	_	Friedl et al. 2014	KM019927.1
Nostoc microscopirum SAG 40.87	Switzerland	Abdel-Bassetet. 2009	GQ287653.1
Nostoc muscorum CCAP 1453/22	freshwater	Day et al. 1990	HF678509.1
Nostoc muscorum Lukesova 1/87	soil, Czech Republic	Papaefthimiou et al. 2008	AM711523.1
Nostoc sp. *Leptogium* sp. LG:R2848	Reunion	Magain & Serusiaux 2014	KF704328.1
Nostoc sp. *Pannaria andina* 1 Ch	saxicolous, Chile	Elvebakk et al. 2008	EF536021.1
Nostoc sp. *Pannaria elixii* 1 NZ	terricolous, New Zealand	Elvebakk et al. 2008	EF174229.1

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Table 6.1: Cyanobacterial strains from GenBank used in this study (continued from previous page)

Species	Origin	Author	Accession No.
Nostoc sp. *Pannaria fulvescens* NZ	terricolous, New Zealand	Elvebakk et al. 2008	EF174231.1
Nostoc sp. *Pannaria obscura* Aus	corticolous, Australia	Elvebakk et al. 2008	EF174232.1
Nostoc sp. *Pannaria rubiginella* Ch	saxicolous, Chile	Elvebakk et al. 2008	EF536024.1
Nostoc sp. *Pannaria rubiginosa* LG:R1008	Reunion	Magain & Serusiaux 2014	KF704321.1
Nostoc sp. *Pannaria rubiginosa* No	corticolous, Norway	Elvebakk et al. 2008	EF174220.1
Nostoc sp. *Pannaria tavaresii* Ch	saxicolous, Chile	Elvebakk et al. 2008	EF174219.1
Nostoc sp. *Parmeliella borbonica* LG:R1122	Reunion	Magain & Serusiaux 2014	KF704320.1
Nostoc sp. *Parmeliella brisbanensis* LG:R1019	Reunion	Magain & Serusiaux 2014	KF704350.1
Nostoc sp. *Parmeliella brisbanensis* LG:R1247	Reunion	Magain & Serusiaux 2014	KF704347.1
Nostoc sp. *Parmeliella brisbanensis* LG:T3	Thailand	Magain & Serusiaux 2014	KF704351.1
Nostoc sp. *Parmeliella brisbanensis* LG:T7	Thailand	Magain & Serusiaux 2014	KF704352.1
Nostoc sp. *Parmeliella mariana* LG:R974	Reunion	Magain & Serusiaux 2014	KF704330.1
Nostoc sp. *Parmeliella polyphyllina* LG:R1021	Reunion	Magain & Serusiaux 2014	KF704327.1
Nostoc sp. *Parmeliella* sp. LG:T6	Thailand	Magain & Serusiaux 2014	KF704349.1
Nostoc sp. *Parmeliella stylophora* LG:R979	Reunion	Magain & Serusiaux 2014	KF704331.1
Nostoc sp. *Parmeliella triptophylloides* LG:R965	Reunion	Magain & Serusiaux 2014	KF704324.1
Nostoc sp. PCC 7120	Unknow	Kanebo et al. 2001	NR_074310.1
Nostoc sp. PCC 7906	fresh water	Arima et al. 2011	AB325908.1
Nostoc sp. *Peltigera extenuata* AR4b	Argentina	Kaasalainen et al. 2015	KF359707.1
Nostoc sp. *Peltigrera rufescens* Mollenhauer 94.1	terricolous, Canada	O'Brien et al. 2006	DQ185214.1
Pleurocapsa minor SAG 4.99	Namibia, quartz pebbe	Fewer et al. 2002	AJ344564.1
Pleurocapsa PCC 6712**	USA, freshwater	Ishida et al. 2001	AB039004.1
Pleurocapsa sp. PCC 7319	Mexico, intertidal zone	Ishida et al. 2001	AB039006.1
Rhizonema sp. *Acantholichen pannarioides* Sipman 48329	Costa Rica	Lücking et al. 2009	EU818947.1
Rhizonema sp. *Coccocarpia filiformis* R16	Costa Rica	Lücking et al. 2009	EU818948.1
Rhizonema sp. *Coccocarpia palmicola* R03	Costa Rica	Lücking et al. 2009	EU818949.1
Rhizonema sp. *Coccocarpia* sp. kj16		Kaasalainen et al. 2014	KF359678.1
Rhizonema sp. *Coccocarpia* sp. kj30		Kaasalainen et al. 2014	KF359679.1
Rhizonema sp. *Dictyonema phyllogenum* Lücking 15207a	Costa Rica	Lücking et al. 2009	EU818962.1

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Table 6.1: Cyanobacterial strains from GenBank used in this study (continued from previous page)

Species	Origin	Author	Accession No.
Rhizonema sp. *Dictyonema schenkianum* R01	Costa Rica	Lücking et al. 2009	EU818963.1
Rhizonema sp. *Dictyonema schenkianum* R02	Costa Rica	Lücking et al. 2009	EU818964.1
Scytonema arcangeli CCIBt3134	Brazil, periphyton	Komarek et al. 2013	KC682101.1
Scytonema arcangeli CCIBt3134	periphyton, Brazil	Komarek et al. 2013	KC682101.1
Scytonema hofmanni PCC 7110	Bermuda, cave limestone	Tomitani et al. 2006	AB075996.1
Scytonema hofmanni PCC 7110	Bermuda, cave limestone	Tomitani et al. 2006	AB075996.1
Scytonema javanicum FACHB-887	-	Zhu et al. 2013	JX872528.1
Scytonema sp. SAG 67.81	Germany, fresh water	Friedl et al. 2014	KM019951.1
Scytonema sp. FGP-7A	soil, USA	Yeager et al. 2007	DQ531698.1
Scytonema sp. HK-05	crust, Japan	Katoh 2015	AB694934.1
Scytonema sp. SAG 67.81	freshwater, Germany	Friedl et al. 2014	KM019951.1
Scytonema sp. YK-02	crust, Japan	Katoh 2015	AB694929.1
Synechococcus elongatus PCC 6301	USA, fresh water	Sugita et al. 2015	NR_074309.1
Synechococcus elongatus PCC 7942	USA, fresh water	Turner et al. 2010	AF132930.1
Synechococcus sp. PCC 7335	USA, fresh water	Honda et al. 1999	AB015062.1
Xenococcus sp. PCC 7305	USA, marine aquarium	Turner et al. 1999	AF132783.1
Xenococcus sp. PCC 7307	USA, rock chip	Seo & Yokota 2001	AB074510.1

*,**These strains named *Chroococcidiopsis* on GenBank was confirmed as *Myxosarcina* CCMP1489 and *Pleurocapsa* PCC 6712 by Fewer et al. (2002);***These strains were renamed to *Rhizonema* from the name *Scytonena* in GenBank following the discrimination the genus *Rhizonema* by Lücking (2014). The strains origin were gathered from websites of National Center for Biotechnology Information (NCBI), Culture Collection of Algae at the University of Göttingen, Germany (SAG), Catalogue of selected strains of Pasteur Culture Collection of Cyanobacteria (PCC), and Culture collection of Algae and Protozoa (CCAP)

Table 6.2: Species in plots

(full name of species in table 6.6)

plot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Coce	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0
Cocg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cocm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Coca	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	1
Cocp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
Cof	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
Cop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cosp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cms	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cys2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cys3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dim	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Dis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LichA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lez	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lea	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lec	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Ler	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Len	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1
Led	0	1	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	1
Leg	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Lep	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pasp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Prb	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Prn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Pcn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pei	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Peo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pep	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Phb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0
Php	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Poc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Psa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Psu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Ptg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptsp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pso	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pct	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pytr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyte	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Spa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stsp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Stc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Std	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Stm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thv	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LichB	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
spC	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Table 6.3: Species in plots (continued)

plot	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Coce	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cocg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cocm	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cocp	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cof	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Cop	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cosp	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cms	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Cyp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dim	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
LichA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lez	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Lea	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Lec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
Ler	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Len	0	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0
Led	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Leg	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lep	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meu	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pasp	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prb	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0
Prn	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Pcn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peb	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pee	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Peo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pep	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Phb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Php	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pod	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptsp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pso	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pct	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pytr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyte	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pyp	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Stsp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Std	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stm	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Thv	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LichB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
spC	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6.4: Species in plots (continued)

plot	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
Coce	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cocg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocp	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Cof	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cosp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cms	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyp1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Cys2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Dim	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LichA	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Hel	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Lem	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lez	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ler	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Len	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0
Led	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Leg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lep	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Meu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pasp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pcn	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Peb	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pec	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pei	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Peo	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Pel	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pep	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Phb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Php	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poc	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pod	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Psr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptg	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptsp	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pso	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Pct	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pytr	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pyte	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pys	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Ran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stsp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Std	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thv	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
LichB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
spC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6.5: Environment matrix

plot	site	lat	lon	light	sub	p	dsm	elevation	veg
1	DR	16.6	106.9	sha	Rock	2079	3	150	for
2	DR	16.6	106.9	sha	Rock	2079	3	185	for
3	DR	16.6	106.9	sha	Rock	2079	3	257	for
4	DR	16.6	106.9	exp	Rock	2079	3	250	for
5	DR	16.6	106.9	exp	Trunk	2079	3	293	for
6	PD	16.5	107.2	sha	Trunk	2936	0	41	for
7	PD	16.5	107.2	sha	Rock	2936	0	39	for
8	PD	16.2	107.8	sha	Rock	3442	0	808	for
9	ST	16.1	108.2	exp	Rock	2041	3	5	for
10	ST	16.1	108.2	exp	Rock	2041	3	69	for
11	ST	16.1	108.2	sha	Rock	2041	3	178	for
12	ST	16.1	108.2	sha	Rock	2041	3	74	for
13	ST	16.1	108.2	sha	Rock	2041	3	377	for
14	AT	14.5	108.7	sha	Trunk	2002	3	815	for
15	AT	14.5	108.7	exp	Trunk	2002	3	815	for
16	AT	14.5	108.7	sha	Rock	2002	3	824	for
17	AT	14.5	108.7	sha	Trunk	2002	3	824	for
18	AT	14.5	108.7	exp	Trunk	2002	3	824	for
19	AT	14.5	108.7	sha	Trunk	2002	3	840	for
20	AT	14.5	108.7	sha	Trunk	2002	3	907	for
21	AT	14.5	108.7	exp	Trunk	2002	3	907	for
22	AT	14.5	108.7	sha	Rock	2002	3	887	for
23	AT	14.5	108.7	sha	Trunk	2002	3	887	for
24	QN	13.7	109.2	exp	Rock	1697	3	18	out
25	QN	13.7	109.2	exp	Soil	1697	3	10	out
26	QN	13.7	109.2	exp	Rock	1697	3	94	out
27	QN	13.7	109.2	exp	Rock	1697	3	43	out
28	NC	11.7	109.2	sha	Rock	794	4	699	for
29	NC	11.7	109.2	sha	Rock	794	4	387	for
30	NC	11.7	109.2	exp	Rock	794	4	387	for
31	NC	11.7	109.2	sha	Rock	794	4	677	for
32	NC	11.7	109.2	sha	Trunk	794	4	636	for
33	NC	11.7	109.2	exp	Rock	794	4	442	for
34	NC	11.7	109.2	sha	Rock	794	4	393	for
35	NC	11.7	109.2	exp	Rock	794	4	393	for
36	NC	11.7	109.2	sha	Rock	794	4	742	for
37	NC	11.7	109.2	sha	Rock	794	4	715	for
38	NC	11.7	109.2	sha	Trunk	794	4	715	for
39	NC	11.7	109.2	exp	Rock	794	4	715	for
40	NT	11.6	109.2	sha	Rock	794	7	43	out
41	NT	11.6	109.2	exp	Rock	794	7	43	out
42	NT	11.6	109.2	sha	Soil	794	7	21	out
43	NT	11.6	109.2	exp	Rock	794	7	21	out
44	NT	11.6	109.2	exp	Rock	794	7	15	out
45	NT	11.6	109.2	exp	Rock	794	7	36	out
46	NT	11.6	109.2	exp	Rock	794	7	12	out
47	NT	11.6	109.2	exp	Soil	794	7	76	out
48	NT	11.6	109.2	exp	Rock	794	7	26	out
49	NT	11.6	109.2	sha	Rock	794	7	27	out
50	CT	11.4	107.4	sha	Trunk	2469	4	137	for
51	CT	11.4	107.4	exp	Rock	2469	4	137	for
52	CT	11.4	107.4	sha	Trunk	2469	4	167	for
53	CT	11.4	107.4	sha	Rock	2469	4	146	for
54	CT	11.4	107.4	exp	Trunk	2469	4	146	for
55	CT	11.4	107.4	exp	Rock	2469	4	145	for
56	CT	11.4	107.4	exp	Rock	2469	4	139	for
57	LGXM	11.6	105.9	sha	Trunk	1813	4	15	for

(lat: latitude, lon: longitude, lig: light, sha: shady, exp: exposed, sub: substrate, p: precipitation, dsm: dry preriod (month), veg: vegetation type, for: forest, out: outcrop)

Table 6.6: Abbreviation of species

Abbr.	Species	Abbr.	Species
Coce	*Coccocarpia erythroxyli*	Pec	*Peltula clavata*
Cocg	*Coccocarpia glaucina*	Pee	*Peltula euploca*
Cocm	*Coccocarpia microphyllina*	Pei	*Peltula impressa*
Coca	*Coccocarpia palmicola*	Peo	*Peltula obscurans*
Cocp	*Coccocarpia pellita*	Pel	*Peltula omphaliza*
Cof	*Collema furfuraceaum*	Pep	*Peltula placodizans*
Cop	*Collema pulcellum* var. *subnigrescens*	Phb	*Physma byrsaeum*
Cosp	*Collema* sp.	Php	*Physma pseudoisidiatum*
Cms	*Collemopsidum* sp.	Poc	*Porocyphus coccodes*
Cyp1	*Cyphellostereum* sp.1	Pod	*Porocyphus dimorphus*
Cyp2	*Cyphellostereum* sp.2	Psr	*Pseudocyphellaria ardesiaca*
Cyp3	*Cyphellostereum* sp. 3	Psa	*Pseudocyphellaria argyracea*
Dim	*Dictyonema moorei*	Psu	*Pseudocyphellaria aurata*
Dis	*Dictyonema* sp.	Ptg	*Pterygiopsis guyanensis*
LichA	Lichinaceae A	Ptsp	*Pterygiopsis* sp.
Hel	*Heppia lutosa*	Pso	*Psorotichia americana* s. lat.
Lem	*Lemmopsis arnoldiana*	Pct	*Pyrenocarpon thelostoma*
Lez	*Leptogium austroamericanum*	Pso	*Pyrenopsis triptococca*
Lea	*Leptogium azureum*	Pyte	*Pyrenopsis tenuis*
Lec	*Leptogium cochleatum*	Pyl	*Pyrenopsis polycocca*
Ler	*Leptogium coralloideum*	Pyp	*Pyrenopsis portoricensis*
Len	*Leptogium cyanescens*	Pys	*Pyrenopsis subareolata*
Led	*Leptogium denticulatum*	Ran	*Ramalodium neocalendonicum*
Leg	*Leptogium marginellum*	Spa	*Spilonemella americana*
Lep	*Leptogium poliophaeum*	Stsp	*Staurolemma perforatum*
Meu	*Metamelanea umbonata*	Stc	*Sticta cyphellulata*
Pasp	*Pannaria* sp.	Std	*Sticta duplolimbata*
Pat	*Pannaria tavaresii*	Stm	*Sticta marginifera*
Prb	*Parmeliella brisbanensis*	Thv	*Thermutis velutina*
Prn	*Parmeliella nigrocincta*	LichB	Lichiaceae B
Pcn	*Peccania tiruncula*	spC	a granular cyanolichen
Peb	*Peltula bolanderi*		

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Declaration

I hereby declare that this dissertation is based on my own work and has been generated by me as the result of my own original research.

I comfirm that all sources that I quoted from others works were cited in this thesis; all main help for my work were acknowledged.

Hiermit versichere ich, dass die vorliegende Dissertation von mir in allen Teilen selbstständig angefertigt wurde und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden.

Darüber hinaus erkläre ich, dass die Dissertationsschrift weder vollständig, noch teilweise einer anderen Fakultät mit dem Ziel vorgelegt worden ist, einen akademischen Grad zu erlangen.

Kaiserslautern, den