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Fine Structures of Foliicolous Lichens and Their Lichenicolous Fungi Studied by Epifluorescence

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Abstract

The investigation of foliicolous lichens and their lichenicolous fungi using epifluorescence microscopy reveals valuable details of their thallus structures. In foliicolous lichens with a trentepohlioid photobiont, algal cells are usually arranged in a single layer. Hyphal structures above and below this algal layer can be used to differentiate morphologically similar sterile thalli of certain species. The growth and outline of the lichens with trentepohlioid photobionts is determined by the algal symbiont. Specific growth patterns of these algae suggest that foliicolous lichens have a high specificity for their trentepohlioid symbionts. In species with chlorococcoid green algae, the thallus is composed of irregularly branched and anastomosing hyphae of varying thickness. The thicker hyphae form a net-like layer on the substrate, enclosing a basal layer of groups of algal cells, while the thinner hyphae form tree-like structures adhering to algal cells above this layer. The infection strategies in lichenicolous fungi on foliicolous lichens were also studied and it was found that members of the Arthoniales, which are non-lichenized lichen parasymbionts, take advantage of the trentepohlioid symbionts of their lichen hosts.

Keywords: Epiphylls, foliicolous lichens, epifluorescence, thallus anatomy, lichenized fungi, lichenicolous fungi

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1. Introduction

In most lichen associations, the fungal partner is responsible for the outline of the lichen thallus. There, the mycobiont may form complex, multilayered structures to shape the lichen thallus and to host the algal partner. Notable exceptions are some gelatinous and filamentous lichens. Anatomical details of thallus structures are well-known in lichens on a range of substrata, however, information is still not complete for a highly specialized group, the foliicolous lichens. Lichens growing on leaves are adapted to smooth surfaces, to the ephemeral nature of their substratum, and, in tropical rainforest to a low light / high temperature / high humidity regime. As a consequence, their thalli are usually thin and exhibit comparatively short life-cycles (Lücking, 2001).

Some information exists on the anatomy of special features of foliicolous lichens, although isidia or soredia, as organs for vegetative dispersal, are uncommon (Santesson, 1952; Vezda, 1975; Sérusiaux 1985; Lücking and Matzer, 1996), except for the disc-shaped isidia produced by a number of unrelated taxa (Lücking, 2001). On the other hand, asexual reproduction via pycnidia or campylidia (Sérusiaux, 1986; Vezda, 1986) and hyphophores (Vezda, 1979; Vezda and Poelt, 1987), is characteristic of many groups. In addition, hair-like structures on the thalli can be formed by single fungal hyphae, whether hyaline (*Mazosia pilosa*; Kalb and Vezda, 1988) or pigmented (*Microtheliopsis winkleri*; Lücking, 1994a). These can develop into sterile, white or black setae composed of agglutinated hyphae (*Tricharia*; Vezda, 1979; Lücking, 1997a), or can even be composed of short, erect algal threads surrounded by fungal hyphae (*Coenogonium epiphyllum* and *C. ciliatum*; Lücking and Kalb, 2000). Many species contain crystals (mostly calcium oxalate), distributed over the thalli or localized in warts and ribs. The thallus may have a smooth or farinose appearance or consist of goniocysts (Sérusiaux, 1985), i.e. aggregates of algal cells surrounded by a single layer of paraplectenchymatic fungal cells, and may form a cortex of varying anatomical nature, composed of rectangular cells in *Asterothyrium* and *Psorotheciopsis*. However, apart from ultrastructural studies of the hypothallus in *Fellhanera bouteillei* (Modenesi et al., 1986) and calcium oxalate deposition in *Actinoplaca strigulacea* (de Oliveira et al., in press), practically no information exists on anatomical structures of the mycobiont or its contact with the algal symbiont.

The problem of identifying foliicolous lichens by vegetative characters has been addressed to some extent with keys to sterile foliicolous lichens (Lücking, 1992), and by a list of 38 species with their diagnostic vegetative characters (Lücking, 1994b). This is important because foliicolous lichens are ecological indicators of tropical forest habitats and microhabitats, but often occur in the sterile state (Lücking, 1997b, 2001). Colonization patterns, particular

adaptations, and life strategies of foliicolous lichens would be better understood if details of their thallus anatomy was available. Here, we present anatomical observations of vegetative thallus features in various groups of foliicolous lichens, including some of their lichenicolous fungi, and discuss the taxonomic and ecological implications.

2. Material and Methods

Light and epifluorescence microscopy was carried out using a Zeiss Axioskop compound microscope with an Hg-lamp as U.V. light source. The dyes used include Calcofluor White and Cotton Blue, all as 1% aqueous solution. For Calcofluor White we used filter set no. 09 from Zeiss.

Because it is difficult to obtain cross-sections of foliicolous lichens, and because such sections are of limited use to observe hyphal patterns, we observed most structural details in surface view. Thalli were either detached directly with a forceps or embedded in a film of nail-varnish prior to detachment (Grube, 2001). To visualize the hyphae, we applied Calcofluor White (Sigma, 1% aqueous solution) in combination with an epifluorescence optics. The dye was applied for 5 min. After staining with Calcofluor White, thalli were rinsed with 5% KOH to help reduce the background fluorescence and this also resulted in a swelling of the tissue. Furthermore, the algal cell walls appeared bright yellowish-green after such treatment, whereas the fungal walls fluoresced bright blue. This color difference made it easier to observe minute details of both symbionts.

Calcofluor White (excitation at 437 nm, emission at 490 nm) has a high affinity with certain linear polysaccharides (Wood, 1980): β-conformation at the C1, presence of CH_2OH-6-moiety, and equatorial arrangement of hydroxyl groups at C2 and C4.

The following taxa and specimens were used in this study: *Amazonomyces farkasiae* (Lücking) Lücking et al. [Costa Rica, II. 2000, Lücking 00-163, hb. Lücking]; *Ampullifera foliicola* Deight. [Madeira, R. Sant., Fung. lichenicoli exs. 51, on *Byssoloma subdiscordans*]; *Arthonia accolens* Stirt. [Costa Rica, VII. 1992, Lücking 92-5490; Ecuador, V. 1996, Lücking 96-464]; *Arthonia orbygniae* (H.B.P. Upadhyay) Matzer [Costa Rica, Lücking, Lich. Fol. exs. 3, GZU; Costa Rica, VII. 1997, Lücking 97-1632; Brazil, VIII. 1993, Kalb & Kalb s.n., both hb. Lücking]; *Arthonia trilocularis* Müll. Arg. [Guyana, II. 1996, Lücking 96-3720; Brazil, V. 1995, Lücking 95-279, both hb. Lücking]; *Bacidia brasiliensis* (Müll. Arg.) Zahlbr. [Costa Rica, II. 2000, Lücking 00-285, hb. Lücking]; *Bapalmuia palmularis* (Müll. Arg.) R. Sant. [Costa Rica, II. 2000, Lücking 00-173, hb. Lücking]; *Chroodiscus coccineus* (Leight.) Müll. Arg. [Costa Rica, XI. 1999, Grube, GZU; Costa Rica, II. 2000, Lücking 00-165, hb. Lücking]; *Echinoplaca*

pellicula (Müll. Arg.) R. Sant. [Costa Rica, VI. 1997, Lücking 97-1123; Ecuador, V. 1996, Lücking 96-722, both hb. Lücking]; *Fellhanera paradoxa* (Vezda) Vezda [Costa Rica, II. 2000, Lücking 00-303, 00-347, 00-415, all hb. Lücking]; *Hemigrapha tenellula* (Müll. Arg.) R. Sant. ex Matzer [Costa Rica, VII. 1997, Lücking 97-1722 (on *Porina lucida*), 97-1724 (on *P. lucida*), both hb. Lücking)]; *Mazosia adelphoparasitica* Matzer [Brazil, V. 1995, Lücking 95-277, hb. Lücking]; *Mazosia melanophthalma* (Müll. Arg.) R. Sant. [Guyana, II. 1996, Lücking 96-3597; Ecuador, V. 1996, Lücking 96-455, both hb. Lücking]; *Mazosia phyllosema* (Nyl.) Zahlbr. [Costa Rica, II. 2000, Lücking 00-115, hb. Lücking]; *Mazosia rotula* (Mont.) Massal. [Costa Rica, VII. 1992, Lücking 92-4153, GZU, Costa Rica, II. 2000, Lücking 00-123, 00-358, both hb. Lücking]; *Microtheliopsis uleana* Müll. Arg. [Costa Rica, IV. 1997, Lücking 97-437; Ecuador, V. 1996, Lücking 96-593, both hb. Lücking]; *Opegrapha mazosiae* Matzer [Costa Rica, 1.VIII. 1991, Matzer 1400 & Pelzmann; holotype]; *Opegrapha porinicola* Matzer [Ecuador, V. 1996, Lücking 96-479 (on *Phyllophiale alba*), hb. Lücking]; *Pocsia borhidii* (Vezda & Farkas) Lücking & Kalb [Costa Rica, II. 2000, Lücking 00-136, 00-431, and s.n., all hb. Lücking]; *Porina epiphylloides* Vezda [Tanzania, III. 1989, Farkas 89100, hb. Lücking]; *Porina leptosperma* Müll. Arg. [Costa Rica, II. 2000, Lücking 00-354, 00-434, both hb. Lücking]; *Porina limbulata* (Kremp.) Vain. [Costa Rica, II. 2000, Lücking 00-138, 00-176, both hb. Lücking]; *Porina radiata* Kalb et al. [Costa Rica, II. 2000, Lücking 00-118; Guyana, II. 1996, Lücking 96-3867, both hb. Lücking]; *Porina rubentior* (Stirt.) Müll. Arg. [Costa Rica, XI. 1999, Grube, GZU]; *Porina rubescens* (Lücking) Hafellner & Kalb [Costa Rica, XI. 1999, Grube, GZU]; *Strigula phyllogena* (Müll. Arg.) R.C. Harris [Costa Rica, II. 2000, Lücking 00-146, 00-262, both hb. Lücking].

3. Results

Thallus structure in lichens with trentepohlioid photobiont

In foliicolous lichens with *Phycopeltis* (Trentepohliaceae), the photobiont usually forms a continuous layer of rectangular cells in radiate plates or angular-rounded cells in irregular plates with interspaces (Thompson and Wujek, 1997). More or less disc-like, radiate plates were observed in *Arthonia accolens*, *Mazosia rotula*, *Microtheliopsis uleana*, *Porina limbulata*, *Porina rubentior* (Fig. 1), *Porina rubescens* (Fig. 2) and *Chroodiscus coccineus*. The radiating filaments can form elongate branching systems, as in *Arthonia orbygniae* (Fig. 3). In other species, the interspaces were particularly distinct, and the branching angles of the algal filaments are rather wide. Interestingly, this is also true for some morphotypes of *Chroodiscus coccineus*, which are

involved in lichenicolous interactions (Fig. 4). This phenomenon of a photobiont switch is treated in detail by Lücking and Grube (2002).

The thallus is clearly 3-layered in many species. On the upper side, above the algal layer, a net of strongly branched and anastomosing hyphae is developed in *Mazosia* (Fig. 5). Sometimes, thicker (c. 2 µm) primary and thinner (c. 1 µm) secondary hyphae can be distinguished and recall a street map (Fig. 6). In *Amazonomyces farkasiae*, bundles of more or less parallel hyphae grow above the algal layer (Fig. 7). On the lower side of species with closely spaced photobiont filaments forming disc-like colonies or elongate branch systems, there is a thin layer of hyphae growing below the walls of the algal cells and following exactly the radiating photobiont cell pattern (Figs. 1 and 2). These hyphae are connected by more or less frequent anastomoses, which may form an almost reticulate structure in *Porina limbulata* (Fig. 8), which is even more conspicuous in *Arthonia trilocularis*.

Mazosia rotula and *Porina radiata* have very similar vegetative thalli with radiate crystalline ridges and a radiate photobiont. However, epifluorescence clearly reveals anatomical differences. First, the upper hyphal layer is formed by richly branched hyphae of varying thickness with small interspaces, which recall a street-map in the *Mazosia* species. In contrast, *Porina radiata* has thinner, more irregularly arranged hyphae of equal thickness (Fig. 9). Second, in *Porina radiata* the crystalline ridges on the lower side are enclosed by a rim of short, angular cells (Fig. 10), which are not found in *Mazosia*.

The hyphae of many investigated taxa form papillose appendages that seem to grow downwards onto the leaf surface and might represent attachment structures (Fig. 8). In *Mazosia dispersa*, the papillae are rather large and globose (c. 4–5 µm in diam). A histochemical study of the mucilage layer of the hypothallus in *Fellhanera bouteillei* did not mention such papillae (Modenesi et al., 1986), which coincides with our observations in foliicolous lichens with chlorococcoid green algae as photobionts, and which suggest that papillae are missing here. The cells of the lower layer may also have different shapes and can be more or less irregularly thickened in different taxa, e.g. in *Arthonia orbygniae*, where almost globose cells grow in a moniliform pattern. Furthermore, in *Arthonia accolens*, *A. trilocularis*, *Microtheliopsis uleana*, *Porina rubentior* and *P. limbulata*, the lower layer is conspicuous, while the upper layer is often reduced more or less exposing the photobiont.

In species with irregular photobiont growth, such as *Mazosia phyllosema* (Fig. 6) and *Porina leptosperma*, the structure is essentially the same, but the shape of the lower side hyphal layer is different due to the differently shaped algal cells. In species with interspaces, such as *Strigula phyllogena*, the hyphae of the lower side are closely adnate to the algal cells, thus not filling the interspaces (Fig. 11). Species with *Trentepohlia*, e.g. *Amazonomyces*

farkasiae (Fig. 12), have the same structure as those with irregularly shaped *Phycopeltis*.

Thallus structure in lichens with chlorococcoid photobiont

In Gomphillaceae (e.g. *Echinoplaca pellicula*), the *Trebouxia*-like algal cells form a single, rather homogeneous layer (Fig. 13) which is enclosed by a thick hyphal layer above and below. Calcium oxalate crystals are often embedded in the upper hyphal layer. A *Coccomyxa*-like photobiont was found in *Fellhanera paradoxa* (Fig. 14), *Bapalmuia palmularis*, and *Bacidia brasiliensis*. In the Pilocarpaceae, e.g. *Fellhanera*, the algal cells are usually arranged in loose groups and separated by algal-free zones or areas with less algal cells. The thallus is not layered but consists of hyphae of different thickness. The thicker hyphae form a loose net on the leaf surface and surround the individual algal groups or form tree-like structures from which thinner hyphae originate. These are connected to individual algal cells by appressoria. The whole structure recalls a tree with attached fruits. The algal agglomerations are several cell-layers high and connected to the leaf surface by their lowest layer. A similar structure is seen in *Pocsia borhidii* (Fig. 15), where the hyphae of the basal layer densely entangle the photobiont cells.

Figs. 1–6. See next page.

Figure 1. *Porina rubentior* from below. The mycobiont hyphae extend beyond the algal colony (Grube s.n.). Bar = 20 µm.

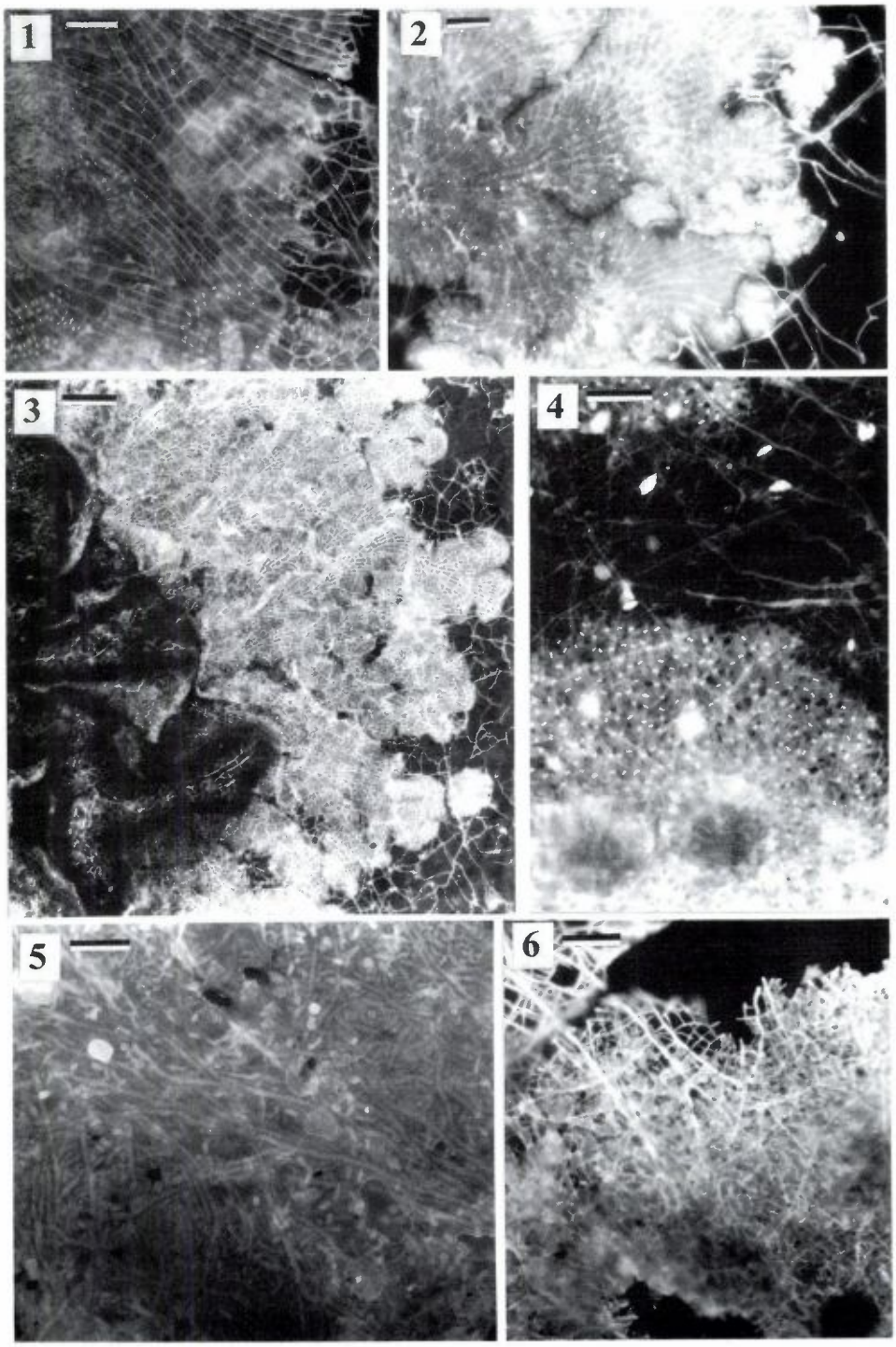
Figure 2. *Porina rubescens* from below. The mycobiont hyphae grow between the algal threads, but do not form an association with adjacent *Phycopeltis* colonies of another species (Grube s.n.). Bar = 20 µm.

Figure 3. *Arthonia orbygniae* seen from below. Note the irregularly branched ascomata with ascogenous hyphae and dark excipuloid layer, and the thallus of elongated plates of *Phycopeltis* (Lücking 97-1632). Bar = 50 µm.

Figure 4. *Chroodiscus coccineus* morphotype with loosely branched photobiont. The septa of the photobiont filaments are strongly stained (Grube s.n.). Bar = 50 µm.

Figure 5. *Mazosia rotula* from above, with densely packed strongly branched hyphae (Lücking 92-4153). Bar = 20 µm.

Figure 6. *Mazosia phyllosema* from above, the street-map like hyphae extend over the photobiont margin. Note the loosely branched photobiont (Lücking 00-115). Bar = 20 µm.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Photobiont delimitation and thallus margins

Species with sharply delimited photobiont areas are mainly found in taxa associated with Trentepohliales. Sometimes, the trentepohlioid colonies are bordered by a few surrounding hypha of the mycobiont, but more often, the hyphae which convey the algal filaments extend far beyond the algal growth margin to form a hyaline prothallus, which is indistinct under the stereomicroscope. This prothallus can be rather wide and connect several algal colonies (=thallus initials), but usually only of the same algal species (see below). The hyphae of the lower and upper layers may fuse at the thallus margin. Slight differences have been observed in the branching and anastomosing pattern of prothallus hyphae. In *Mazosia phyllosema* we observed irregularly thick hyphae; the thicker hyphae (c. 2 µm) form longer stretches, often with two or three hyphae in parallel, while the thinner hyphae branch off in a 90° angle (Fig. 6). A 90° angle of side branches is also found in prothalli of other species, but further details may differ (Figs. 1 and 2). In *Arthonia orbygniae*, the prothallus hyphae grow in a more irregular pattern (Fig. 3).

Species with chlorococcoid algae frequently have diffuse borders of algal growth, and small algal colonies are often found at the periphery of the thalli. In most species, the mycobiont forms an extensive network of prothalline hyphae. These may inter-connect initial thalli of the same species.

Figs. 7–12. See next page.

Figure 7. *Amazonomyces farkasiae* seen from above. Groups of more or less parallel hyphae extend over the photobiont layer (Lücking 00-163). Bar = 10 µm.

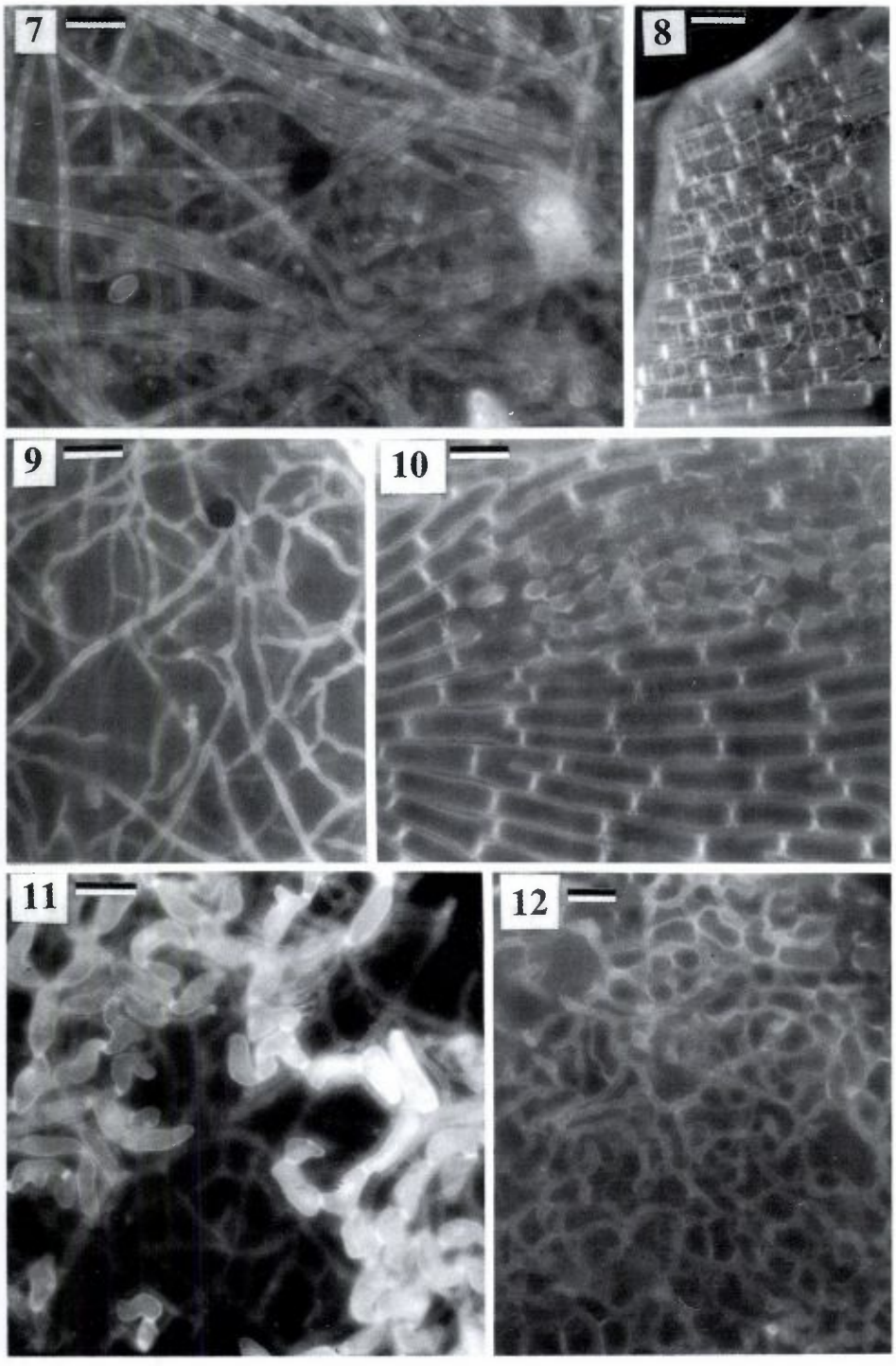
Figure 8. *Porina limbulata* from the lower side. The mycobiont grows in the lower angles between the algal cells, the hyphae are connected by crossing anastomoses. Brightly stained knobs are papillae, developed at the lower side of the hyphae (Lücking 00-138). Bar = 20 µm.

Figure 9. *Porina radiata* seen from above. An irregularly branched and anastomosed network of hyphae covers the photobiont layer (Lücking 00-118). Bar = 10 µm.

Figure 10. *Porina radiata* seen from below. Short, angular cells enclosing crystals at the lower side (Lücking 00-118). Bar = 10 µm.

Figure 11. *Strigula phyllogena* from below. Hyphae are adnate to the irregularly branched photobiont and do not fill the interspaces (Lücking 00-262). Bar = 10 µm.

Figure 12. *Amazonomyces farkasiae* seen from below. A network of mycobiont hyphae is developed between the irregularly branched photobionts (Lücking 00-163). Bar = 10 µm.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Individual chlorococcoid algal cells are often found attached to the hyphae of the prothallus in species which are associated with a chlorococcoid photobiont. This could indicate that chlorococcoid algal cells might enter symbiosis immediately by being attached to a pre-existing web of hyphae, whereas trentepohlioid algae are contacted after they have established multicellular colonies on a leaf surface. Sanders (2001) suggested that motile algal zoospores could be chemically attracted by the mycobiont, which could explain the observed pattern, but so far we failed to observe zoospores.

Selection of and contacts with photobionts

By studying leaves which are sparsely covered with lichens but feature abundant growth of *Phycopeltis* colonies, we found that non-lichenized colonies of *Phycopeltis* generally do not differ significantly from adjacent colonies associated with fungal hyphae. On the other hand, on leaves which harbour lichens with various types of photobionts, adjacent free-living *Phycopeltis* colonies with differing growth pattern are usually not accepted as photobionts of lichen species with a particular hyphal and photobiont branching system (Fig. 2).

Figs. 13–20. See next page.

Figure 13. *Trebouxia*-like photobiont of *Echinoplaca pellicula*, the well developed upper mycobiont layer of this species is poorly stained in Calcofuor White (Lücking 96-722). Bar = 10 µm.

Figure 14. *Coccomyxa*-like photobiont of *Fellhanera paradoxa* (Lücking 00-347). Bar = 10 µm.

Figure 15. *Pocsia borhidii* seen from below. A loose branching system of hyphae is developed on the leaf surface, knob-like appressoria contact the coccale photobionts (Lücking 00-431). Bar = 10 µm.

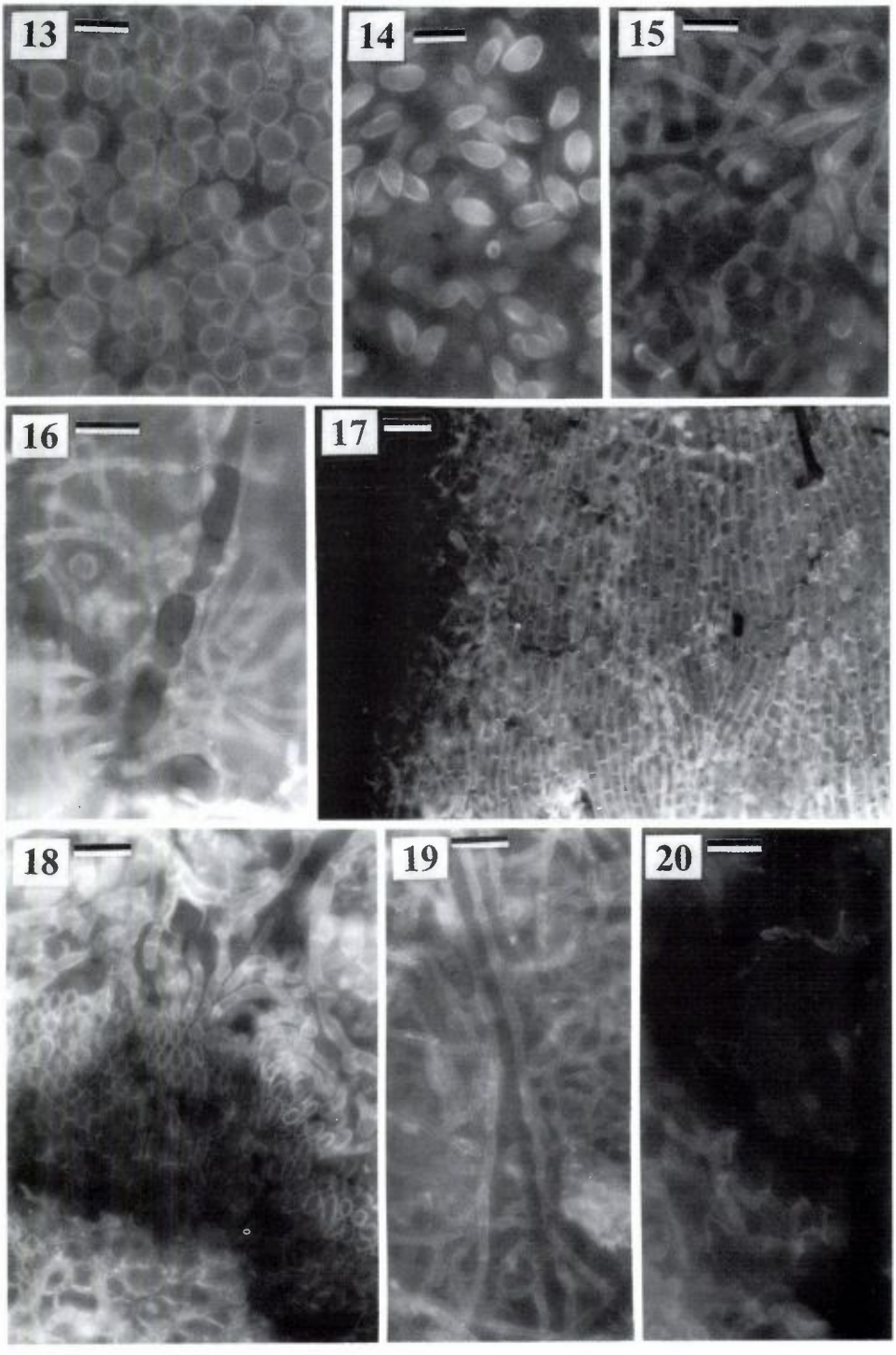
Figure 16. Dark coloured hypha of the lichenicolous *Opegrapha porinicola*, entangled by brightly stained host hyphae (Lücking 96-479). Bar = 10 µm.

Figure 17. The lichenicolous *Opegrapha mazosiae* with brightly stained hyphae extending from the ascomata to the left (holotype). The parasitic hyphae are thicker than those of the host and grow into the angles between the algal filaments. Bar = 20 µm.

Figure 18. The lichenicolous *Hemigrapha tenellula* seen from below, note the ascogenous hyphae and vegetative hyphae extending into the host thallus (Lücking 97-1724). Bar = 10 µm.

Figure 19. The lichenicolous *Hemigrapha tenellula* hyphae at the upper surface paralleled by brightly stained host hyphae (Lücking 97-1724). Bar = 10 µm.

Figure 20. The lichenicolous *Ampullifera foliicola* with small hyaline, brightly stained filaments emerging from dark brown hyphae (Sant., Fung. Lichenicoli Exs. 51). Bar = 10 µm.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Irrespective of the high specificity for trentepohlioid photobionts suggested by these findings, we have not observed specialized contact organs, in our samples. Penetration of the cell walls is generally not common in lichens with trentepohlioid symbionts, both in foliicolous lichens and those on other substrates. Exchange of nutrients is probably accomplished by the close attachment of hyphal and algal surfaces. This contrasts with species which are associated with a *Coccomyxa*-like chlorococcoid photobiont, where we observed small, knob-like appressoria (Fig. 15). In species with *Coccomyxa*-like photobionts, the appressoria contact the algal cells mostly at their shallow side.

Lichenicolous life style in primarily lichenized groups

We investigated infection structures in lichenicolous fungi belonging to primarily lichenized groups in the order Arthoniales. *Opegrapha porinicola* (growing on *Phyllophiale alba*), has ascomata which are erumpent and partly immersed in the host thallus. On the upper side, a loose net of brown vegetative hyphae spreads from the ascomata above the uppermost hyphal layer of the host thallus. These hyphae are irregularly articulate. The hyphae of the host grow attached to the lichenicolous hyphae and form abundant ring-shaped hyphal structures surrounding them (Fig. 16). The lichenicolous hyphae do not produce any lateral branches or appressoria which would contact the host thallus.

However, on the lower side, the ascomata of *Opegrapha porinicola* and *O. mazosiae* are directly attached to the leaf surface and do not grow over the host thallus. Expanding beyond the carbonized exciple, the ascogeneous hyphae and the lateral hypothecium are transformed into a rim of hyaline hyphae attached to the lower side of the host thallus. These hyphae extend underneath the host thallus and connect to adjacent algal cells of the host (Fig. 17). Thus, *Opegrapha* species are likely parasymbionts on the host photobionts.

In *Mazosia adelphoparasitica*, another member of the Arthoniales, growing on *Mazosia rotula*, we could not observe any vegetative hyphae originating from the ascomata and spreading over the surface of the host thallus. Instead, the ascomata are immersed, and the upper hyphal layer of the host laterally cover them without any visible hyphal connections. However, as in *Opegrapha* species, there is a layer of short parallel hyphae at the lower side, which connect the ascomata to adjacent algal cells. A similar situation was found in *Arthonia cinnabarinula*, whose hyphae connect the ascomata with parts of the host thallus and densely surround the host algae (de los Rios et al., 2002).

Lichenicolous life style in primarily non-lichenized groups

Hemigrapha tenellula on *Porina lucida* is at first glance similar to *Opegrapha porinicola* due to its elongate ascomata. However, the ascomata have a different structure: on the lower side, rather thick, branched and septate hyphae representing the ascogeneous hyphae form a dense layer (Fig. 18); on the upper side, the host thallus is infected with a rather dense net of brown vegetative hyphae which, contrary to *Opegrapha porinicola*, lack irregular constrictions and thus are not articulate (Fig. 19). However, as in the latter, these hyphae are found only in the upper hyphal layer of the host thallus and are accompanied by parallel hyphae of the host which almost enclose the parasitic hyphae. No short branches or appressoria can be detected. On the lower side, the ascomata have contact with the leaf surface, but in contrast to *Opegrapha*, we did not observe hyphae that connect with algal cells.

The vegetative, conidiogeneous hyphae of *Ampullifera* and *Dictyophrynella* form dense nets or star-like agglomerations on the host thalli. The hyphae are dark brown, often with an irregular to zig-zaggy shape, and densely provided with hyphopodia and conidia. In both *Ampullifera foliicola* on *Byssoloma subdiscordans* and *Dictyophrynella* sp. on *Flavobathelium epiphyllum*, the vegetative hyphae are raised over the upper hyphal layer of the host thallus. The hyphae form very thin, hyaline, strongly fluorescent, wavy connections at their tips to hyphae of the host thalli (Fig. 20). At their tips, these hyphae seem to form thin appressoria. Connections to algal cells or defence reactions by the host thallus could not be observed.

4. Discussion

These preliminary fluorescence microscopic studies revealed a surprising diversity of anatomical structures in foliicolous lichens, which were not previously recognized. Significant differences are observed between lichens with different photobionts, and the photobiont seems to be a more important parameter in the structure and life style of foliicolous lichens than in lichens on other substrata and of other growth-forms.

Trentepohlioid photobionts are typical in foliicolous members of Arthoniaceae, Opegraphaceae, Trichotheliaceae and Thelotremataceae (*Chroodiscus*). These lichens often have basal hyphal layers (when associated with *Phycopeltis*) and a hyphal structure which follow the photobiont branching pattern. Chlorococcoid photobionts are found chiefly in the Gomphillaceae, Asterothyriaceae, Pilocarpaceae, and Ectolechiaceae. The taxa are mostly *Trebouxia*-like, but a *Coccomyxa*-like photobiont was found in

some Pilocarpaceae (Fig. 14). In the latter, the algal colonies are nested within spaces formed by fungal hyphae.

Within these major growth types, individual differences between taxa have been observed. A conspicuous upper hyphal layer is discerned in species with a matt thallus, whereas nitidous thalli usually lack this upper layer. This is, however, not the case in species with a distinct cortex of rectangular fungal cells, as found in Asterothyriaceae. Differences in the hyphal texture of the lower layer, which connects to the substratum, are useful for taxonomic purposes. *Mazosia rotula* and *Porina radiata* cannot be separated by morphology alone when ascomata are absent, but differ in the structure of the upper hyphal layer and of the hyphae bordering the crystalline ridges (distinct in *Porina radiata*; Fig. 10). The same differences can be used to distinguish similar thalli of these genera with fine verrucae, such as *Mazosia melanophthalma* and *Porina epiphylloides*.

Published illustrations of photobionts in foliicolous lichens, e.g. by Lücking (1995), Lücking and Matzer (1996), or Vezda (1984), suggest that trentepohlioid algae, particularly *Phycopeltis*, have species-specific growth patterns, and the present observations confirm these results. By studying leaves which are sparsely covered with lichens, we found a) that non-lichenized colonies of *Phycopeltis* generally do not differ significantly from adjacent colonies which are associated with fungal hyphae and b) that adjacent free-living *Phycopeltis* colonies with different growth are usually not accepted as photobionts of a particular hyphal branching system. In agreement with the monograph of Thompson and Wujek (1997), this means a) that the growth patterns of *Phycopeltis* photobionts are characteristic for different algal species, and, from the viewpoint of photobiont selection, b) that foliicolous lichens with trentepohlioid symbionts could be rather specific for their photobiont partner (the particular situation in facultatively parasitic members of *Chroodiscus*, involving a photobiont switch, is discussed by Lücking and Grube, 2002). This photobiont specificity contrasts with results from lichens which are associated with *Trebouxia* growing on other substrata. There, growth patterns cannot be used for species recognition due to the chlorococcoid growth, but ultrastructural and molecular studies indicate that several species of *Trebouxia* may serve as photobiont for a fungal species (Friedl, 1989; Grube et al., in prep.). Due to the lack of morphological features, the photobiont specificity of foliicolous lichens with chlorococcoid algae is still unclear; however, a high specificity for trentepohlioid photobionts by mycobionts will be an interesting aspect in speciation and of coevolution of foliicolous lichens. In this context, the preference for a different photobiont by some populations of a lichen species could be an indicator of genetic differentiation.

Foliicolous lichens generally invest little resources in mycobiont structures, and the photobiont often dominates the shape of the lichen. Especially in

foliicolous lichens with *Phycopeltis* as photobiont, the externally visible thallus border corresponds to the circumference of the algal colony, and in species where the hyphal layer above the algae is reduced, the definition of a lichen as with exhabitant fungal partner does not fit well (Kirk et al., 2001). Anatomically, the algal partner is prominent and does not change significantly in morphology by the lichenization. Such lichens are ecologically more similar to those types exemplified by some gelatinous or filamentous lichens. Lichens with reduced fungal structures such as *Coenogonium* spp. are common in the lowland tropical rainforest understorey and support this reduction as a general adaptation to low light / high temperature conditions.

Lichenicolous fungi on foliicolous lichens can be divided into three 'eco-taxonomical' groups. The first group is represented by taxa that belong to otherwise lichenized groups, such as *Arthonia, Gyalideopsis, Mazosia* and *Opegrapha,* and which are usually visible on the host thallus by their conspicuous asco- or conidiomata. Lichenicolous life style in primarily lichenized groups, as found in the Arthoniales, mainly includes parasymbionts on foliicolous lichens with trentepohlioid algae. In the studied examples, the lichenicolous fungi contact the photobiont of the host, which does not seem to be damaged or altered in its growth. We have not observed close contacts of the parasite to the host hyphae or any indication of mycoparasitism. Nevertheless, the externally visible symptoms of lichenicolous fungi on foliicolous lichens are diverse, and further studies are required to understand details of the interactions. The principal phenomenon of algal parasitic behaviour in primarily lichenized groups was also shown by Grube and de los Rios (2001) and fits well with the hypothesis of Lutzoni et al. (2001), who suggest that parasitic habit on lichen photobionts is a first step of originally lichenized lineages towards other fungal life styles. In this respect, it is interesting that particularly in the chiefly lichenized Arthoniales, the proportion of lichenicolous species is relatively high, while other lichen groups with trentepohlioid photobiont, such as the Trichotheliales, do not include lichenicolous fungi but serve as hosts for many lichenicolous species.

A second group of lichenicolous fungi belong to primarily non-lichenized taxa, such as *Hemigrapha,* and reproduce sexually. A third group consists of hyphomycetes that form extensive vegetative and conidiogeneous mycelia on the host thallus, but very rarely develop perithecioid ascomata. *Ampullifera* (Fig. 20), *Dictyophrynella* and *Hansfordiellopsis* belong here.

We assume that species in the first group have a preference for the alga of the host and preferably to those algae that are related to those of the lichenized relatives of the parasite. It is interesting to note that none of the lichenicolous *Arthonia* species, which live on hosts with *Trebouxia,* e.g. the *Arthonia clemens* group (which could be related to lichenized *Arthonia* species with chlorococcoid photobionts), grow on foliicolous lichens with

trentepohlioid symbionts. Instead, only those species, which are related to lichenized forms with trentepohlioid algae, parasitize foliicolous lichens with trentepohlioid algae (Fig. 3). The formation of hyphae which densely entangle lichenicolous hyphae in the observed *Opegrapha* (Fig. 16), appears to be some kind of defense mechanism.

Hemigrapha tenellula (Fig. 19) is probably a parasite on the host hyphae or their gels. *Ampullifera* and *Dictyophrynella* are common hyphomycetes, which seem to be true parasites on the host mycobiont as they contact the fungal host cells by minute appressoria.

The amount and parameters of variation of thallus structures within a taxon remains to be discovered. Our impression is that specific features are always present in a species, and useful for vegetative recognition of foliicolous lichens. The density of hyphae may vary to a certain extent and is either correlated with ontogenetic or ecological factors. Comparative studies with more species will also reveal the degree of convergent evolution of thallus structures in different foliicolous lichen groups, especially when similar photobionts are involved.

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