

## NEW ULTRASTRUCTURAL AND PHYSIOLOGICAL FEATURES OF THE THALLUS IN ANTARCTIC LICHENS

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The paper describes anatomical and physiological features of photobionts and mycobionts in *Bryoria forsteri* Olech & Bystrek, *Caloplaca regalis* (Vain.) Zahlbr., *Cetraria aculeata* (Schreb.) Fr., *Ramalina terebrata* Hook f. & Taylor, *Sphaerophorus globosus* (Huds.) Vain. and *Usnea antarctica* Du Rietz, collected in the Antarctic under varied weather conditions. Green algae from the genera *Lobosphaera* and *Trebouxia* were gathered in depressions of the cortex under the more resistant mycobiont hyphae. In photobiont cells a large amount of highly osmiophilic electron-dense PAS-negative material, lipid-like in character, was of particular interest. Similar material also filled certain areas of the aerial apoplast. A star-shaped chromatophore with central and lateral pyrenoids encompassed most of the photobiont protoplast in all the studied species. Regularly arranged thylakoids with evenly widened lumina along their entire length and osmiophilic lipid droplets adhering to their outer surfaces were visible within the pyrenoid. Inside the chloroplast, large protein inclusions tightly joined with the thylakoids were observed. The mycobionts were closely attached to each other and with the photobionts by means of an outer osmiophilic wall layer, and formed intramural haustoria. Their protoplasts were filled with PAS-positive polysaccharides and a large amount of lipid-like substances. The photobionts were physiologically active and produced a large amount of electron-dense osmiophilic material, and PAS-positive starch grains were visible around their pyrenoids in the thalli collected in different weather conditions. The permanent reserves of nutritive materials deposited in the thalli enable these organisms to quickly begin and continue indispensable physiological processes in the extreme Antarctic conditions.

**Key words:** Antarctic lichens, *Bryoria forsteri*, *Caloplaca regalis*, *Cetraria aculeata*, *Ramalina terebrata*, *Sphaerophorus globosus*, *Usnea antarctica*, morphology, ultrastructure.

### INTRODUCTION

Lichens are organisms with exceptional features. The combination of the fungi's adaptation skills and the algae's high productivity gives rise to an organism with new, better, unique characteristics which its components do not possess. These new features adapt lichens to the most extreme conditions. That is why they are the most species-rich group of organisms in the Antarctic (Smith, 1995; Kappen, 2000; Øvstedal and Lewis Smith, 2001; Olech, 2004). Antarctic lichens are adapted to life under extremely low temperature, dehydration and solar radiation (Kappen et al., 1987; 1995). They carry out photosynthesis with the minimum of light (Brown et al.,

1988), at temperatures below 0°C or even below the temperature of ice nucleation in the cellular fluids of the thallus. Lichens stimulate ice deposition in intercellular spaces, just as vascular plants do. They transform freezing, loosely bound water into tightly bound water which does not freeze, in order to avoid freezing of the intracellular water (Harańczyk et al., 2003; Harańczyk et al., 2008). They tolerate temperatures as low as -196°C (Kappen and Lange, 1970). Antarctic lichens are capable of photosynthesis even at -20°C in laboratory conditions (Kappen, 2000).

Most lichen compounds strongly absorb UV-B, and colored compounds such as parietin absorb some of the photosynthetically active radiation. A few very common compounds are in the lichen cor-

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tex at high concentrations, forming a screen above the photobiont, whereas the majority are located in the photobiont layer of the upper part of the medulla (Fahselt and Alstrup, 1997). Lichens are characterized by distinctive morphological and physiological plasticity which depends on water availability and light intensity (Valladares et al., 1994, 1996, 1997; Valladares and Sancho, 1995; Pintado et al., 1997) and is connected with seasonal variation (Fiechter and Honegger, 1988; Hovengen, 2000).

Entire lichen thalli as well as their individual components visibly and relatively rapidly respond to factors unfavorable to their metabolism such as ozone, acid rain or deposition of heavy metals; the response includes changes in protoplast ultrastructure (Tarhanen et al., 1997; Tarhanen, 1998).

We studied the ultrastructure of cell walls and protoplasts of cells in lichen thalli and determined the location of insoluble polysaccharides, lipids and other metabolites in six species of Antarctic lichens collected in varied weather conditions: on a warm sunny and a cool cloudy day from the same places on King George Island, near the Henryk Arctowski Antarctic Station. We present the anatomical and physiological characteristics of photo- and mycobionts of these species, which differ slightly in thallus structure and which have not been examined previously in that regard.

## MATERIAL AND METHODS

### LICHEN COLLECTIONS

Lichen thalli of *Bryoria forsteri* Olech & Bystrek, *Caloplaca regalis* (Vain.) Zahlbr., *Cetraria aculeata* (Schreb.) Fr., *Ramalina terebrata* Hook f. & Taylor, *Sphaerophorus globosus* (Huds.) Vain. and *Usnea antarctica* Du Rietz were collected in the vicinity of the Polish H. Arctowski Antarctic Station (62°09.41'S, 58°28.10'W) on King George Island (South Shetland Islands) during austral summer in January 2007. Lichen thalli were collected twice from the same sites and at about the same time of day (1–2 p.m.) in varied weather conditions: on 13 Jan. 2007, which was sunny and warm with temperatures at +9–11°C, and on 31 Jan. 2007, which was cloudy and cool with temperatures at -1–0°C.

### LIGHT AND ELECTRON MICROSCOPY

4–6 mm fragments of the thallus of each species from the two sampling times were sectioned for fixation in 3.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 10 h at room temperature for anatomical examinations by light microscopy (LM) as well as ultrastructural observations with a transmission electron microscope (TEM). After brief rinsing in 0.1 M phosphate buffer and water, the mate-

rial was post-fixed in a 2.5% water solution of osmium tetroxide. The post-fixed material was rinsed again, dehydrated in a graded ethanol series and transferred to mixtures with increasing ratios of Poly-Bed 812 epoxide resin. Semithin and ultra-thin sections were prepared with a Leica ultramicrotome (Ultracut R) using diamond knives. Ultra-thin sections (60–90 nm) were placed on copper grids (300 mesh) and contrasted with a saturated aqueous solution of uranyl acetate and lead citrate, following Reynolds (1973). Observations and electronograms were made with a JEOL JEM 100S TEM. The semithin sections (1.5–2.0 µm) were stained with toluidine blue with azure B and then observed and photographed with Olympus and Nikon Optiphot II light microscopes. The PAS reaction was conducted on fragments fixed in Carnoy's fixative after rinsing, and the time of incubation in periodic acid and Schiff's reagent was prolonged to 1 h. Similarly, 4–6 mm fragments of the thallus of each species from the two sampling times were fixed in Carnoy's fixative for the PAS reaction for insoluble polysaccharides, following Pearse (1962). After the PAS reaction, the fragments were dehydrated and embedded in Poly Bed 812 resin. Then semithin sections were prepared for observations and LM photography. For each of the six species we used six or seven fragments from different lichen thalli.

## RESULTS

### CYTOLOGICAL ANALYSIS

Fruticose thalli of the studied lichen species were brown-black or black (*Bryoria forsteri*), orange-yellow (*Caloplaca regalis*), brown or red-brown (*Cetraria aculeata*), yellow or yellow-brown (*Ramalina terebrata*), orange or orange-brown with white parts (*Sphaerophorus globosus*) or multicolored grey and green or yellow and green with numerous black areas (*Usnea antarctica*). The intensively branched thalli clung or lay closely to the substrate (e.g., *Caloplaca regalis*, *Bryoria forsteri*) or were erect. All of them showed heteromeric structure with distinct cortical and algal layers (Figs. 1a–c, 4a,b,e,f,g, 5a,b). The cortex of all six species was compact. The cortical layer consisted of several mycobiont layers with cell walls of equal thickness, and lumina circular in cross section (Fig. 2a, b). The hyphae forming the outermost layer had brown or black cell walls, as in *Bryoria forsteri* (Fig. 1a). In the remaining part of the thallus the mycobiont walls stained weakly both with toluidine blue with azure B (Figs. 1d, 4b, 5a) and in the PAS reaction (Figs. 1b, 4f,g, 5b–d). The whole cortical layer of *Bryoria forsteri*, *Sphaerophorus globosus* and *Usnea antarctica* stained uniformly in the PAS reaction (Figs. 4f,g, 5b). In the *Caloplaca regalis* thallus

the areas of the mycobiont layer staining most in the PAS reaction were mainly on the underside (Fig. 1b). *Cetraria aculeata* and *Ramalina terebrata* thalli had only 2–4 outermost mycobiont layers with PAS-positive cell walls, as after toluidine blue with azure B (Fig. 4b). Diverse materials were observed inside the fungus cells of all the examined thalli: PAS-positive components, PAS-negative components, and osmiophilic material (Figs. 1d, 4g). In all six species the inner part of the lichen, the loosely built algal layer, was surrounded by a compact thallus cortex. The mycobionts of the algal layer were connected with the hyphae of the external thallus part in many places. Inside the thallus the algal cells and fungal hyphae were not evenly distributed but formed more or less compact clusters (Figs. 1a–c, 4a,b,e,f,g, 5a,b). Both single photobionts and those occurring in clusters were entwined with closely clinging hyphae. Intercellular spaces of different sizes, sometimes filled with osmiophilic material, were visible between the mycobionts and myco- and photobionts (Fig. 1e,f). Photobiont cells in the algae from *Lobosphaera* and *Trebouxia* were oval or circular in cross section. Thin algal cell walls stained with toluidine blue and were PAS-positive (Figs. 4f, 5b,d). PAS-negative osmiophilic material was found in the peripheral parts of algal cell protoplasts, in the form of inclusions and drops of various sizes (Figs. 1a–d, 4g, 5d). These drops often fused, as can be seen in *Sphaerophorus globosus*, and formed thick black sheaths (Fig. 4g).

#### ULTRASTRUCTURAL FEATURES OF PHOTOBIONTS AND MYCOBIONTS

The photobiont cell walls of *Lobosphaera* and *Trebouxia* were relatively thin and only slightly osmiophilic in all the studied lichen species (Figs. 2d,e, 4h). Electron-opaque lipid material was observed in the protoplast in the majority of algal cells of the examined Antarctic lichens. This material varied in the degree of osmiophily and was deposited in different amounts (Figs. 2c,e, 4c,d).

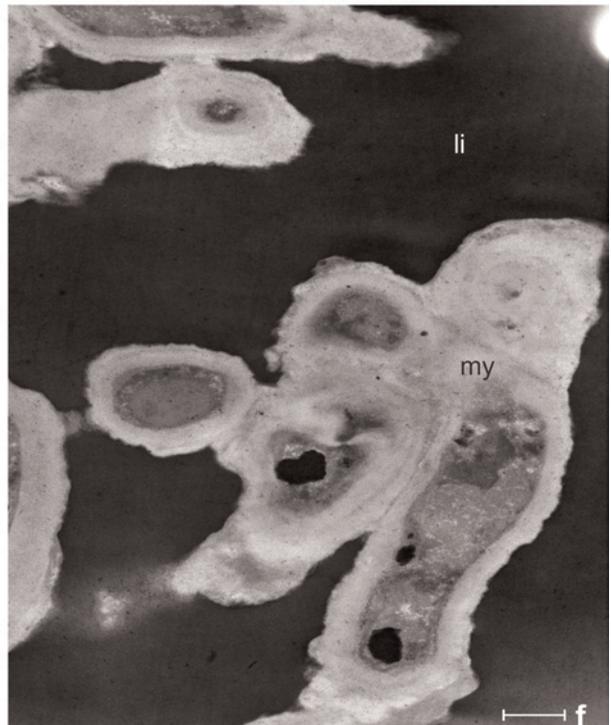
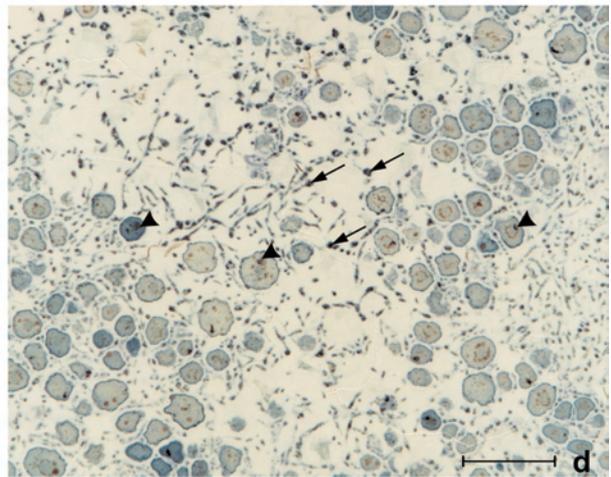
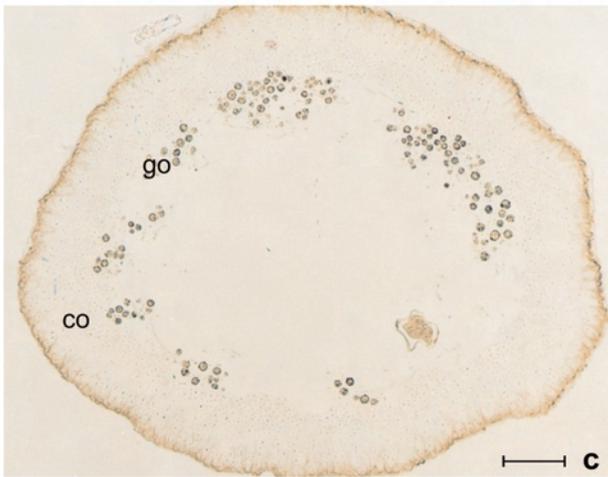
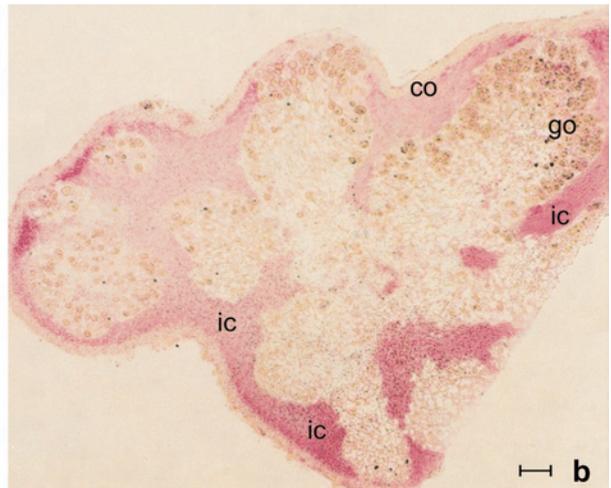
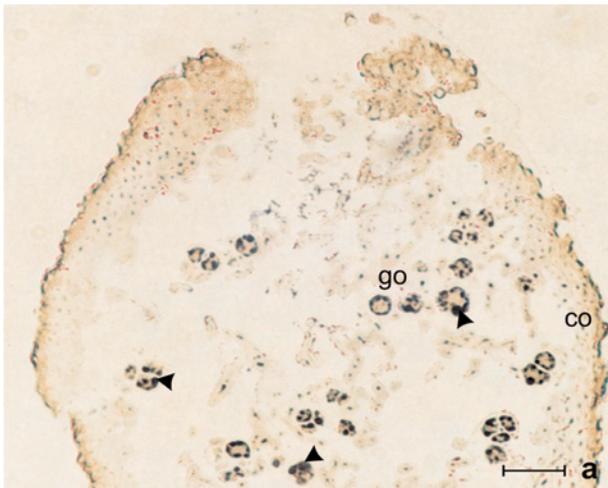
The external part of the protoplast was undulated, with many concavities and convexities. Drops and inclusions with osmiophilic lipid material, of different sizes, were visible in the peripheral cytoplasm of certain cells (Figs. 3a,b, 4c,d,h, 5e). These drops were surrounded by a distinctly lighter layer (Figs. 4h, 5e). Sometimes they were evenly and concentrically arranged (Fig. 4h) or fused into large areas (Fig. 5e). The biggest part of the photobiont protoplast in all the species was filled with a star-shaped chromatophore (Fig. 3a–d), the surface of which was irregular and undulated, with numerous protuberances and concavities containing cytoplasm with organelles, mainly mitochondria (Fig. 3a–d). Numerous spherical or oval mitochondria most often adhered to the surface of the plastid or were lodged in its depressions (Fig. 3a,c,d).

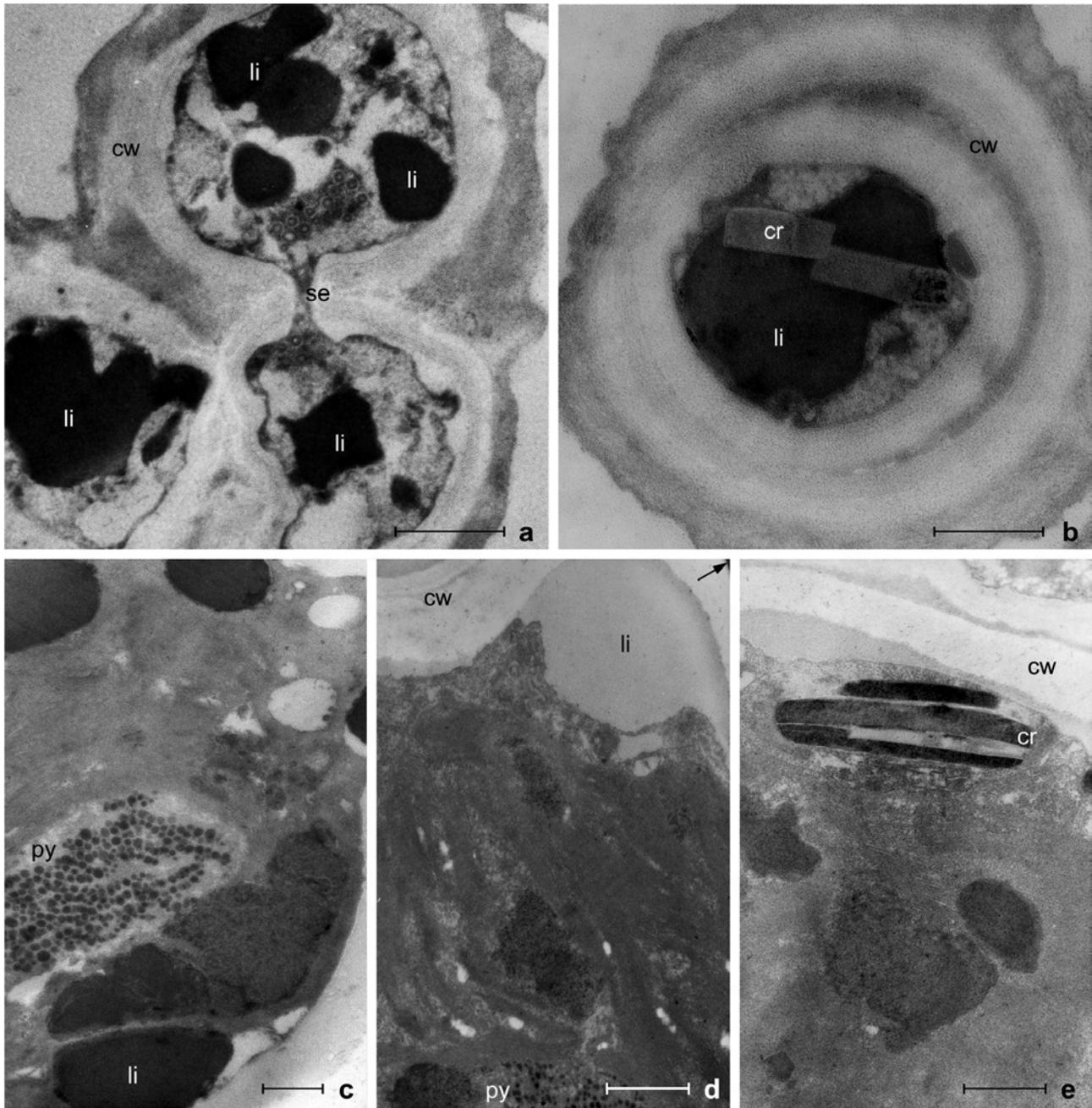
The lamellar system of the chromatophore was well-developed, with evenly arranged chloroplast lamellae. Protein inclusions of different sizes, tightly connected with thylakoid groups, occurred in the photobiont protoplasts from thalli collected on both warm sunny and cool cloudy days. In the pyrenoid region, fine osmiophilic lipid droplets and single or fusing pyrenoglobuli were always visible (Figs. 2c,d, 3a,b, 4c,h). Six to ten evenly spaced thylakoids with wide, regular lumina were very often observed in the protein region of the pyrenoid (Figs. 3a,b, 4c). Fine osmiophilic pyrenoglobuli were attached to their outer surfaces. Starch grains of different sizes were concentrically distributed around the pyrenoid (Fig. 4d,h).

The mycobiont hyphae were tightly linked with photobiont cells. In some places a layer of electron-dense and PAS-positive material surrounded the fungal hyphae that were in contact with algal cells. Under this layer an electron-light mycobiont wall layer was visible. In the mycobiont cell walls were several layers, usually 3–5, of material showing different degrees of osmiophily (Figs. 1e,f, 2a,b). Significant amounts of dense lipid-like material (Figs. 2a, b) and electron-light vacuole regions were visible in the mycobiont protoplasts of all the thalli

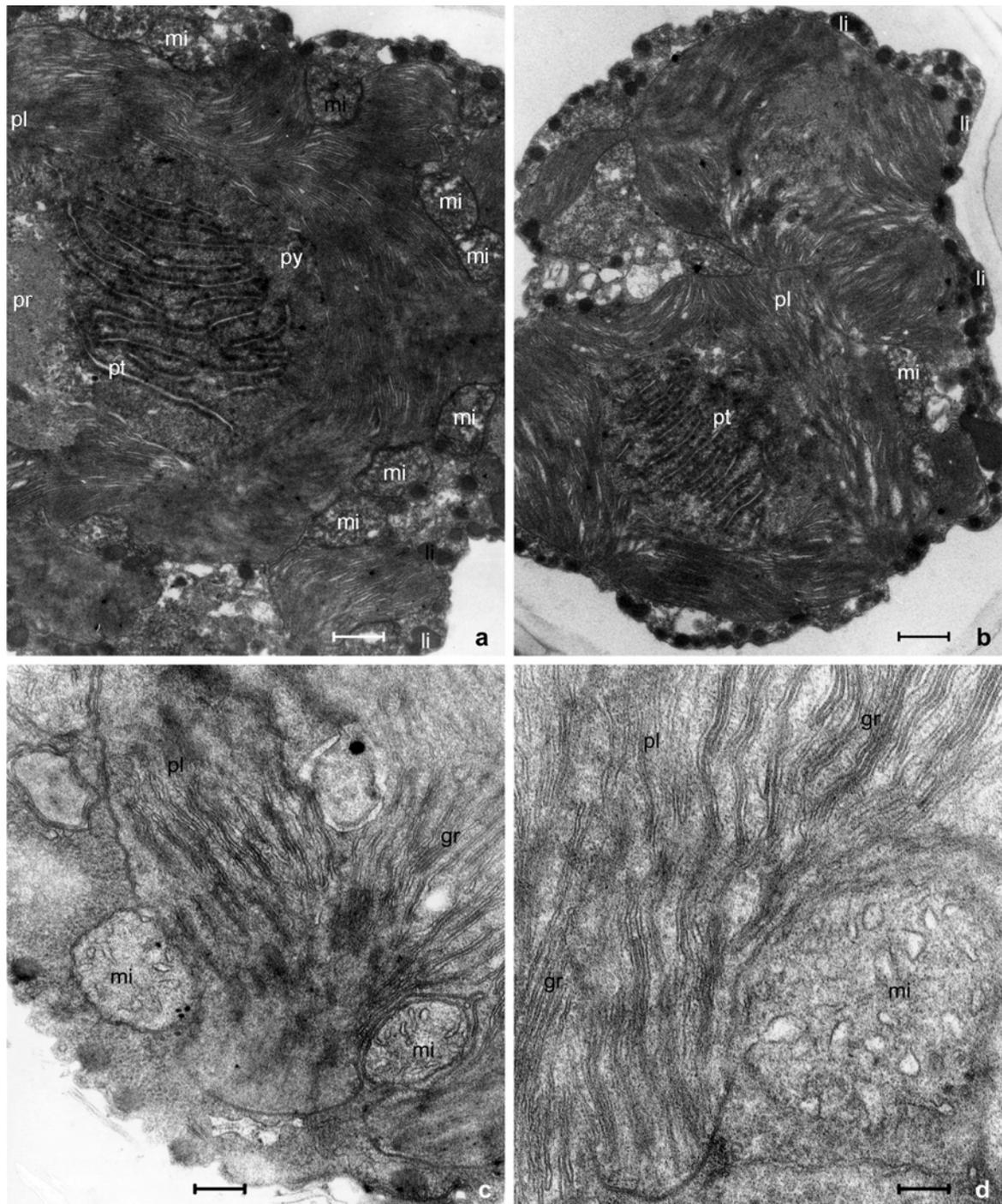
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**Fig. 1.** Anatomy of the thallus of *Bryoria forsteri*, *Caloplaca regalis* and *Cetraria aculeata* lichens, and intercellular spaces between fungal hyphae. (a) In cross section from discontinuous, torn cortical layer (co) of *Bryoria forsteri* lichen thallus collected on a sunny day, abundant osmiophilic droplets (arrowheads) are visible in young, mature and older photobiont cells (go, green alga *Lobosphaera*). Bar = 100  $\mu\text{m}$ , (b) Anatomy of *Caloplaca regalis* lichen thallus collected on a sunny day, with PAS-stained insoluble polysaccharides. Algal layer (go) with *Trebouxia* cells in upper section of thallus under cortical layer (co). In lower part of thallus, mycobiont areas with large amount of insoluble polysaccharides (ic). Bar = 300  $\mu\text{m}$ , (c) Anatomy of *Cetraria aculeata* thallus collected on a cool cloudy day. External part of thallus cortical layer visibly stained yellow-brown (co). Under the cortical layer is the gonidial layer (go) with algal (*Trebouxia*) cells. Bar = 300  $\mu\text{m}$ , (d) Algal layer fragment of *Caloplaca* thallus presented in photograph 1b. Osmiophilic material occurs in both young and mature *Trebouxia* cells (arrowheads) and in fungal hyphae (arrows). Bar = 100  $\mu\text{m}$ , (e, f) Fungal hyphae (my) in algal layer of *Caloplaca* thallus collected on a cool cloudy day. Mycobiont cell walls consisting of a few layers and numerous concentric bodies (arrows) in their protoplasts. Among the hyphae are electron-emptly intercellular spaces (e, is) and spaces filled with strongly osmiophilic lipids (f, li). Bars = 10  $\mu\text{m}$ .





**Fig. 2.** Anatomy and ultrastructure of hyphae in *Caloplaca regalis* thallus collected on a sunny day, and ergastic materials in algal cell protoplasts of *Caloplaca* thallus collected on a cool cloudy day. **(a)** Fungal hyphae in algal layer of thallus shown in photogram 1d. Cell wall (cw) consisting of a few layers and a cytoplasmic connection between two neighboring hyphae (se) visible in cross section of mycobionts. In protoplasts of both fungal cells, distinctively arranged concentric bodies in vicinity of cytoplasmic channel. A considerable part of the volume of protoplasts in fungal hyphae is occupied by lipid areas (li). Bar = 3  $\mu$ m, **(b)** Mycobiont cell from thallus presented in Fig. 1d, with 5-layer cell wall (cw), lipids (li) and crystalloids (cr). Bar = 3  $\mu$ m, **(c)** Protoplast fragment of mature *Trebouxia* cell with large amount of materials varying in osmiophily. The largest part of the protoplast is occupied by lipids (li). In the pyrenoid area (py), thylakoid lamellae with lipid droplets. A pale but osmiophilic periplasmic space is visible under the electron-light algal cell wall. Bar = 5  $\mu$ m, **(d)** In peripheral region of protoplast, directly under cell wall (cw), is a space probably formed in a place previously occupied by lipids. At exactly the same height an osmiophilic lipid substance adheres to the external surface of the cell wall (arrow). Bar = 5  $\mu$ m, **(e)** In peripheral cytoplasm, large crystalloids (cr) are under cell wall (cw). Bar = 5  $\mu$ m.



**Fig. 3.** *Trebouxia* cell ultrastructure in *Caloplaca regalis* thallus collected on a warm sunny day. (a) Centrally located chloroplast (pl) with well-differentiated thylakoids and large pyrenoid (py) in center. In pyrenoid area are thylakoids (pt) with evenly wide lumina and lipid droplets. Protein complexes (pr) of various sizes joined with thylakoid membranes. Numerous mitochondria (mi) tightly adhering to the chloroplast are concentrically arranged in its depressions and between its branches (patches). Lipid droplets (li) of various sizes in peripheral region of cell protoplast. Bar = 5  $\mu\text{m}$ , (b) *Trebouxia* protoplast with pyrenoid in lateral part of chloroplast (pl); regular arrangement of thylakoid system and lipid droplets (li). Between chloroplast patches are mitochondria (mi) and cytoplasm areas with grains and vesicles. Numerous small lipid droplets (li) in peripheral cytoplasm. Bar = 5  $\mu\text{m}$ , (c) Fragment of peripheral part of *Trebouxia* protoplast, with tightly connected organelles: chloroplast (pl) and mitochondria (mi). Bar = 2.5  $\mu\text{m}$ , (d) Chloroplast fragment (pl) with well-developed granal system (gr) and mitochondrion (mi) closely attached to plastid. Bar = 1.5  $\mu\text{m}$ .

studied. In the mycobiont hyphae, commonly found were concentric bodies, fine structures regular and spherical in shape with an osmiophilic rim and transparent center (Figs. 1e, 2a) and secondary metabolites in the form of crystals (Fig. 2b).

Distinctive crystals also occurred in the peripheral parts of protoplasts of mature and ageing photobiont cells (Fig. 2e). Electron-empty intercellular spaces, spaces with the remains of osmiophilic material (Fig. 1e) and entire spaces tightly filled with electron-dense osmiophilic lipids were observed between the hyphae (Fig. 1f).

## DISCUSSION

Our anatomical and ultrastructural studies of the thalli of six Antarctic lichen species showed new features of photo- and mycobionts not previously described. These features may prove to be adaptations to the extreme environmental conditions of the Maritime Antarctic.

### SPECIFIC ANATOMICAL FEATURES OF ANTARCTIC LICHENS

Intense insolation, especially UV-B radiation (280–320 nm), is a ubiquitous stress factor, above all for photosynthesizing organisms. Due to depletion of the ozone layer this is a particularly serious problem in the Antarctic (Solhoug et al., 2003). All of the lichen species we studied showed massive accumulation of pigment substances in the outer part of the cortical layer. Of the six species, the hyphae in the outermost layer of the cortex of *Bryoria forsteri* thallus contained the most black-

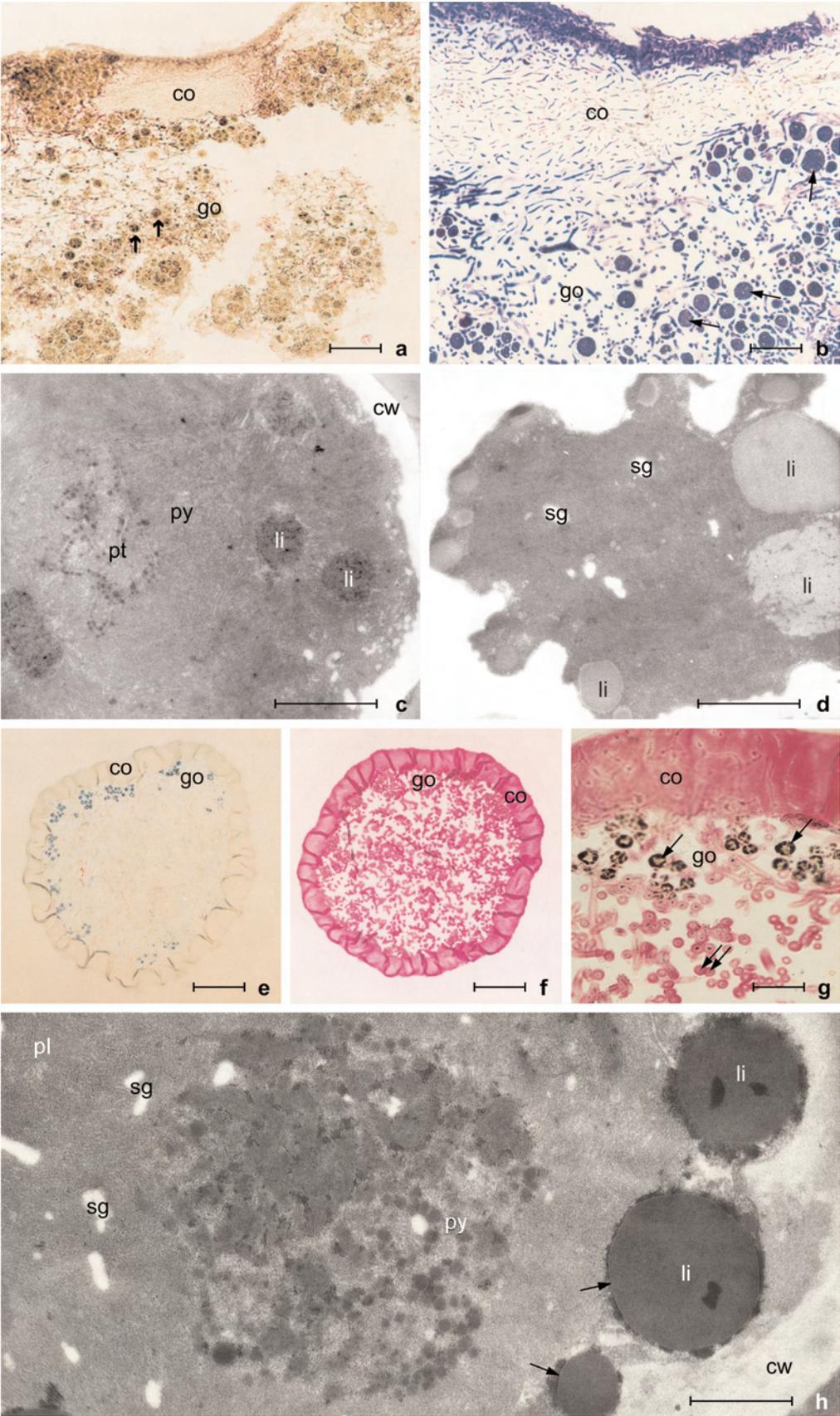
brown or black substances. According to Nybakken et al. (2004), certain secondary metabolites deposited in the cortical layer, such as usnic acid or melanin in *Cladonia arbuscula*, *C. rangiferina* and *C. stellaris*, are synthesized under the influence of UV-B radiation and may play a photoprotective role. UV-B radiation has been experimentally shown to induce synthesis of photoprotective compounds such as melanin in *Lobaria pulmonaria* but also parietin in *Xanthoria parietina* (Solhoug et al., 2003). The composition of secondary metabolites may change depending on environmental conditions (Murray, 1971).

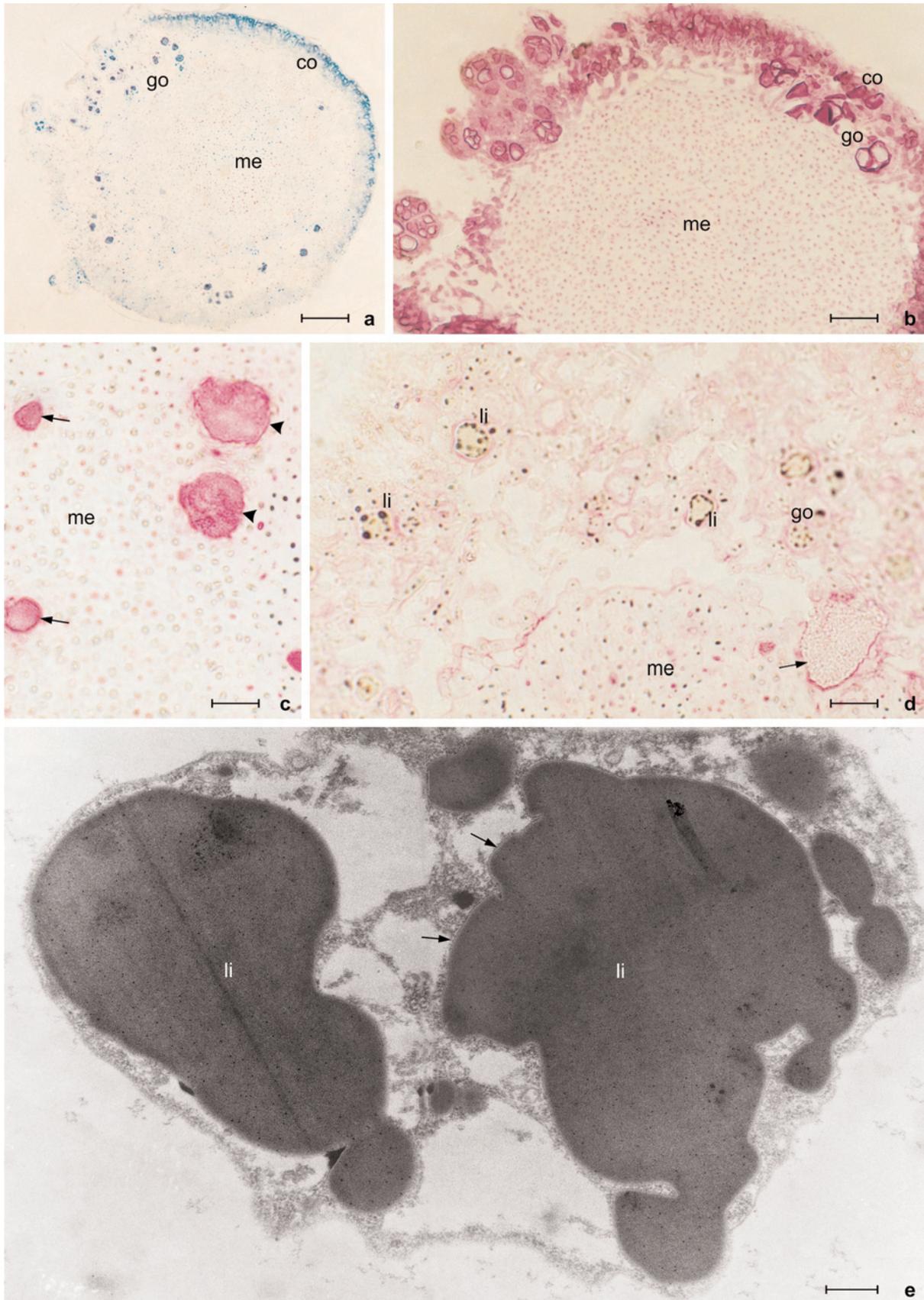
Staining of fungal cell walls with toluidine blue and the PAS reaction confirmed the varied chemical composition of hyphae in the six lichen species. The reaction results showed that the content of protein substances and reducing sugars in the mycobiont walls differed between the species. The cortical parts of the thalli in *Caloplaca regalis*, *Cetraria aculeata* and *Ramalina terebrata* clearly differed in pigment chemistry and content; the cortical layer in *Bryoria forsteri*, *Sphaerophorus globosus* and *Usnea antarctica* was relatively uniform in chemical content.

The cortical layer of all the studied lichens was characterized by high numbers of external hyphae, thick hyphal walls, and a large amount of a sticky, gelatinous, polysaccharide-like intercellular substance. Thanks to these features, the cortical layer, the layer most exposed to environmental stresses, retains its integrity and durability (Ahmadjian, 1993; Jacobs and Ahmadjian, 2007). Apart from its stabilizing role, the greater amount of insoluble polysaccharides in the bottom part of the thallus in *Caloplaca regalis* may function as a reservoir of

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**Fig. 4.** Thallus anatomy and location of insoluble polysaccharides and lipid substances in photo- and mycobiont cells in *Ramalina terebrata* and *Sphaerophorus globosus*. **(a)** Cross section through *Ramalina* lichen thallus collected on a warm sunny day. Thallus fragment with soredia can be seen. Fragments of compact mycobiont layer visible in cortical layer (co) and varied photobiont cells in gonidial layer (go). Lipid droplets in *Trebouxia* cells (arrows). Bar = 400  $\mu\text{m}$ . **(b)** Section through thallus collected on a cool cloudy day. External part of cortical layer (co) and photo- and mycobionts in algal layer (go) intensively stained with toluidine blue. Lipid droplets visible in *Trebouxia* cells (arrows). Bar = 180  $\mu\text{m}$ . **(c)** Electronogram shows fragment of photobiont cell from thallus in 4a. Osmiophilic inclusions (li) occur in vicinity of pyrenoid (pt, py) and in periphery of protoplast. Bar = 8  $\mu\text{m}$ . **(d)** Electronogram shows cell fragment from the thallus in 4b. Weakly osmiophilic lipid material visible in peripheral region of *Trebouxia* protoplast (li). Protoplast edge is in contact with large empty space, starch grains (sg) are visible. Bar = 8  $\mu\text{m}$ . **(e)** Cross section through *Sphaerophorus* radial lichen thallus collected on a warm sunny day. Mycobiont cortical layer (co) is of the same width on the whole thallus circumference. All fungal hyphae are tightly bound together. Algal layer (go) is found directly under the compact cortical layer. *Trebouxia* cells in the gonidial layer stained with toluidine blue with azure B, forming clusters of different sizes. Centrally located medulla is uniform in structure, composed of loosely but evenly arranged fungal hyphae. Bar = 200  $\mu\text{m}$ . **(f)** Cross section through the thallus collected on a cool cloudy day. Fungal hyphae of cortical layer (co), algal cells in gonidial layer (go) and medullar cells uniformly PAS-stained. Bar = 200  $\mu\text{m}$ . **(g)** Cross section of sample of the thallus presented in 4e. Insoluble polysaccharides shown by PAS reaction in mycobionts of cortical (co), between algal cells (go) and in medullar layer. Large amount of strongly osmiophilic material visible in *Trebouxia* cells in gonidial layer and lumina of fungal cells (arrows). Bar = 100  $\mu\text{m}$ . **(h)** Electronogram shows fragment of *Trebouxia* cell from thallus presented in 4e. Starch grains (sg) are scattered within and around the pyrenoid (py). Single and fusing pyrenoglobuli and large osmiophilic droplets (li, arrows) visible in peripheral region of cell protoplast. Bar = 3  $\mu\text{m}$ .





reserve materials, like the polysaccharide regions in the densely packed medulla and between the medulla and the gonidial layer in the thallus of *Usnea antarctica*. Under the compact cortical layer, a photobiont layer built of densely arranged algal cells entwined in fungal hyphae was found in all the examined species. In this way the sensitive and delicate algal cells were effectively protected by fungal hyphae, which are far more resistant to environmental stresses (e.g., light, water, mechanical). The soralia were the only places where the cortical layer was interrupted. These structures occurred in all the studied Antarctic lichen species except *Bryoria forsteri* (Olech, 2004; Olech and Bystrek, 2004). De Los Rios et al. (1999) described the restructuring of *Lasallia hispanica* and *Parmelia omphalodes* thalli under the influence of desiccation/hydration stress. Apparently the lichen thalli we examined cannot rearrange their anatomical structure to that extent in response to water stress, due to the very high mycobiont content in the cortical layer of Antarctic lichens.

#### ULTRASTRUCTURAL RESPONSES OF PHOTOBIONTS AND MYCOBIONTS TO STRESS FACTORS

The mycobiont hyphae surrounding the photobiont cells were closely connected to them. Numerous fungal hyphae, which formed intramural haustoria, adhered to the algal cell wall. By penetrating algal cell walls, the haustoria strengthen the thallus structure and prevent deformation of the sensitive and thin-walled photobiont during drought (Sanders et al., 2004). The hyphae clinging to the photobiont cells had a multilayer cell wall. The outermost electron-dense mycobiont wall layer also stretched onto the photobiont cell wall. Honegger (1986) described the same layer in representatives of Parmeliaceae and Lecanorales and stated that such a connection is very efficient because it is closer, whereas a wall-to-wall connection is simple and rarely found between lichen partners. Haustorial links, which we also observed in our material, most often occur between lichen components.

The cell wall of green algae of the genera *Lobosphaera* and *Trebouxia* consisted of a large amount of insoluble polysaccharides, visible in the PAS reaction in all the analyzed lichens. This is a feature observed in photobionts of the genus *Trebouxia* in all lichen species (Ahmadjian, 1993). In the cell walls of *Trebouxia* photobionts isolated from a *Ramalina gracilis* thallus and cultured in vitro, Cordeiro et al. (2006) identified  $\beta$ -galactofuranan; they also identified amylose as insoluble material obtained upon freeze-thawing of the alkaline extract. These polysaccharides were not found in the symbiotic thallus of *Ramalina gracilis*, which contained only water-soluble (isolichenan) and insoluble glucans (nigeran and laminaran) and galactomannan. Galactofuranan shares similarities with polysaccharides they found in some fungal cell walls.

In electronograms, wall layers varying in osmiophilily were observed in only a few *Trebouxia* cells. Ahmadjian (1993) stated that the cell wall of *Trebouxia* may be built of several more or less fibrous layers, sometimes even five of them. It might also consist of two: an outer electron-dense layer and an inner electron-light layer (Jacobs and Ahmadjian, 2007).

The protoplast structure of photobiont cells of all six Antarctic lichen species, both those collected on a warm sunny day and those collected when the weather was cool and cloudy, showed a large amount of electron-dense lipid material which accumulated at cell peripheries. Material in the form of droplets or large electron-opaque areas was limited to a thin light layer and remained in the peripheral parts of the cell. Sometimes this material filled more than half of the photobiont cell volume, visible even in semithin sections by light microscopy (e.g., in *Sphaerophorus globosus*; Fig. 4g). In the *Ramalina terebrata* thallus, lipids varying in osmiophilily were found, possibly indicating changes in their chemism. Bychek-Guschina (2002) consider lipid metabolism in lichen thalli to be an adaptation strategy of these organisms; according to them, a large amount of lipids deposited at cell peripheries in big



**Fig. 5.** Anatomy of radial thallus in *Usnea antarctica* and lipids in *Trebouxia* cell. (a) Fine dark layer on surface of compact cortical layer (co) visible in cross section of thallus collected on a warm sunny day. Fungal cells in external part of this layer stained with toluidine blue with azure B. Internal part of the cortical layer is weakly stained. Algal cells of gonidial layer stained both under compact cortical layer and within soralium (go). Medulla (me) is compact and fungal cells tightly bound together. Bar = 200  $\mu$ m, (b) From cross section of thallus collected on a cool cloudy day. Fungal and algal cell walls stained by PAS reaction within cortical layer (co) and soralium, and PAS-negative cell walls of compact medulla (me). Bar = 100  $\mu$ m, (c) Part of compact medulla (me) with distinctly PAS-positive small (arrows) and large (arrowheads) areas from thallus collected on a warm sunny day. Bar = 50  $\mu$ m, (d) From thallus collected on a cool cloudy day, with visible soralium and part of compact medulla (me). Lipid droplets (li) in algal cells in gonidial layer (go). In external part of compact medulla is a concentration of a substance granular in structure (arrow). Bar = 50  $\mu$ m, (e) Electronogram of surface section of *Trebouxia* cell protoplast from thallus shown in 5d. Fusing droplets of strongly osmiophilic material (li). Lipid material surrounded by fine light layer (arrows). Bar = 3  $\mu$ m.

or small droplets indicates that the organisms are well-adapted to the environmental conditions. Few or no lipid droplets were found in the thallus of *Hypogymnia physodes* collected in winter, whereas thalli collected in summer contained a considerable amount of lipids (Fiechter and Honneger, 1988). Brown et al. (1988) reported decreased content of lipid reserve materials under dark conditions in *Parmelia sulcata* and *P. laevigata*, and suggested that the lipid material gathered in photobionts is not the first to be exhausted in a situation unfavorable to lichens. The main metabolite used up in response to stress is starch. In the Antarctic lichens we studied, starch grains of various sizes occurred in the form of transparent areas around the pyrenoid or in the vicinity of mitochondria. In Brown et al.'s (1988) work, *Parmelia sulcata* thalli kept in the dark contained no starch supplies, while those under day/night conditions had a significant amount of starch. Tarhanen et al. (1997) observed a rise in the starch content of algal cells under increased ozone, and also suggested that lipid metabolism is facilitated in the presence of ozone. Lipids were rapidly used up under its influence but were resynthesized just as rapidly.

Numerous protein bodies of different sizes connected with chromatophores occurred in the algal cells of the lichens we examined. In view of their close contact with thylakoid membranes, they may be assumed to serve as a store of protective protein. Such proteins can be synthesized in a short time after a stress event, and effectively protect the thylakoid lamellae embedded in them. In the photobiont chloroplasts of *Lobosphaera* and *Trebouxia* a single pyrenoid was seen, most often in the center of the chromatophore.

The pyrenoid of the *Trebouxia* photobiont, classified as *gelatinosa* type (Friedl, 1989), was composed of a protein matrix with regularly arranged lamellae. The thylakoids were very often widened along their entire length and had osmiophilic pyrenoglobuli evenly spaced opposite one another, adhering to their external surface. The pyrenoglobuli were found in all the photobiont cells, although their number and osmiophily varied even in neighboring cells within the same thallus. Describing the ultrastructure of the *Trebouxia* photobiont in the thalli of ten lichen species examined in situ under conditions of thallus hydration and desiccation, Jacobs and Ahmadjian (2007) reported that starch grains occurred in all the chloroplasts, and lipid-containing globules occurred in both hydrated and desiccated conditions in the pyrenoids. The location of the pyrenoid and the electron-dense matrix can change during drought (Brown et al., 1988). According to Ahmadjian, (1993), the amount of pyrenoglobuli in the pyrenoid depends on light intensity: they are more numerous under low lighting. According to Brown et

al. (1988) and Ahmadjian (1993) they function as a type of reserve material and, depending on the light stress, time of year or hydration level of the thallus, can change their size, amount and location.

The spaces between algal cells and fungal hyphae in the thalli we studied were filled with electron-dense lipid materials in many places. Honneger (1993) described such areas as layers of secondary metabolites coming from the mycobiont, which might include substances hindering the growth of the photobiont. According to Palmqvist (2000) the intercellular substance contains large amounts of carbon anhydrase, which facilitates gas exchange between the lichen partners.

The interior of the mycobiont protoplasts was largely filled with lipid-like material, but optically electron-neutral areas were also visible. Ahmadjian (1993) described similar cytoplasm composition in mycobiont hyphae. Concentric bodies were common elements in the mycobiont hyphae. These fine spherical structures with an electron-dense rim and transparent medulla were distributed in the osmiophilic matrix. According to Peyeling et al. (1985) they remain in contact with the cell nucleus. They are built of a protein substance (Ahmadjian, 1993). The role of these structures is not known. It is suspected that they may be the equivalent of the Golgi apparatus. According to Brown and Wilson (1968) they constitute a 'membrane repair set'; that is, they are responsible for reconstruction of cytoplasmic membranes during cycles of hydration and desiccation.

Both the anatomical structure and ultrastructure of photobiont cells and mycobiont hyphae of Antarctic lichens vary greatly even within a small section of the thallus. Some of their features, such as accumulation of photoprotective substances in the cortical layer, large amounts of reserve materials deposited in various cell areas and intercellular spaces, and the close proximity of cell organelles, have been described in the cells of higher plants, including mesophyll cells of the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica* (Alberdi et al., 2002; Gielwanowska et al., 2005; Gielwanowska and Szczuka, 2005).

Our microscopy observations did not show any strong correlation between the atmospheric conditions (abiotic factors) that prevailed on the sampling days and intracellular thallus structure or the amount of deposited nutritive material. The *Lobosphaera* and *Trebouxia* photobionts were physiologically active and produced large amounts of electron-dense osmiophilic material, and PAS-positive starch grains were visible around their pyrenoids in the thalli collected on both the sunny warmer day and the cloudy cooler day. Inside the mycobionts and in the intercellular spaces of thalli there was a considerable amount of nutritive material in both cases. We suggest that the perma-

ment reserves of nutritive materials deposited in the symplast and apoplast of *Bryoria forsteri*, *Caloplaca regalis*, *Cetraria aculeata*, *Ramalina terebrata*, *Sphaerophorus globosus* and *Usnea antarctica* thalli enable these organisms to engage indispensable physiological processes rapidly and maintain them in the extreme conditions of the Antarctic.

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