Structure and in situ development of the microlichen *Gyalectidium paolae* (Gomphillaceae, Ascomycota), an overlooked colonist on palm leaves in southwest Florida[1]

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Premise of the study: The rarely reported lichen *Gyalectidium paolae* can be locally abundant on palm leaves in southwest Florida, where it may reproduce when as small as 0.15 mm diameter. We examined structural and developmental features to better understand the lifestyle of this extreme ephemeral.

Methods: Blocks containing resin-embedded thalli were sectioned and examined with TEM and SEM-BSE. Propagule development was studied with light microscopy applied to inoculated and naturally colonized plastic coverslips placed in the field.

Key Results: Thallus areolae showed a heterogeneous covering that varied from cellular cortex to a simpler structure derived from fungal wall materials and sparse fungal cells of reduced diameter. Plates of crystalline deposits seemed to interrupt thallus structure, elevating the surface layer. No organized algal layer was present. Symbiont interactions were limited to appositional wall contacts with no haustorial penetration observed. Symbiotic propagules germinated promptly, but relative growth of fungal versus algal components varied considerably. Smaller photobiont cells released from sporangia were present at the periphery of the thallus, or escaped to some distance. Fully formed hyphophores with abundant propagules appeared within five months, although there was evidence that propagule formation in *Gyalectidium* might occur much sooner.

Conclusions: *Gyalectidium paolae* builds relatively simple thalli with limited fungal structure, prioritizing rapid formation of asexual propagules. Co-dispersal of algal symbionts permitted propagules to develop directly into thalli, but microenvironmental conditions may strongly influence survival and developmental equilibrium between the two symbionts necessary for success as a lichen.

INTRODUCTION

Lichen-forming fungi associate with specific algal symbionts to produce a wide variety of collaborative constructions. Their composite thalli include some of the most complex vegetative structures produced by fungi, often with well-differentiated tissue layers and distinct organs (Honegger, 2012). Many lichens are conspicuous, long-lived components of their communities; some fruticose species, such as the lace lichen (*Ramalina menziesii*) or Methuselah's beard (*Usnea longissima*), may reach several meters in length (Herre, 1904; Esseen and Renhorn, 1998). At the other end of the spectrum, there are a number of diminutive, much simpler lichens that are relatively short-lived and commonly overlooked. As weaker competitors, they tend to make use of transient, unstable substrata, and reproduce in relatively short time (Poelt and Vězda, 1990; Scheidegger, 1995). In their itinerant existence they resemble many non-symbiotic fungi that disperse from one

ephemeral food source to the next. For these lichen fungi, however, it is the physical substratum rather than the organic carbon source that is of limited duration.

Perhaps the greatest diversity of ephemeral lichens is found among the specialized colonizers of non-deciduous leaf surfaces in tropical and subtropical environments. The foliicolous (epiphyllous) lichen fungi have been the subject of two extensive monographs, and currently encompass over 800 known species of phylogenetically diverse ascomycetes (Santesson, 1952; Lücking, 2008). Showing relatively little preference for particular host plant species (Lücking, 1998), these lichens share similar adaptations for completion of their life cycle within the time span of their leaf substratum (Lücking, 2001), which most typically lasts for about two to three years (Coley, 1988; Lücking, 1998; Sanders, 2014b). Most foliicolous lichens build crustose thalli that proceed to asexual or sexual reproduction while still quite small. Although available anatomical details are meager, the thallus appears to be more simply constructed than in many other lichens. Those that utilize the discoid, multicellular *Phycopeltis* as photobiont often consist of little more than a network of fungal hyphae that overrun and penetrate beneath one to several adjacent plates of radiating algal filaments (Grube and Lücking, 2002; Sanders, 2002). However, a large percentage of foliicolous lichens house unicellular green algal populations, and in such cases it is the lichen fungus that must organize and structure the thallus. In one such group (Asterothyriaceae), a plate of radiating, tightly branched fungal filaments – resembling the alga *Phycopeltis* – comprises a cortex overlying the unicellular algal symbiont (Henssen and Lücking, 2002), but this arrangement appears to be exceptional. More commonly reported is a simpler "corticiform layer" (Lücking, 2008), although further information about this structure is lacking. Because the minute foliicolous thalli are difficult to section effectively and fresh material may not be readily available to researchers in temperature regions, their anatomical organization and the interactions between the symbionts have been little studied. And while early stages of lichen establishment have been observed in a foliicolous community (Sanders and Lücking, 2002) and the life cycles of two members documented (Sanders 2002, 2014a), for most of these diverse associations the patterns of thallus formation and development remain unknown.

During the course of field studies in southwestern Florida, the presence of the foliicolous lichen *Gyalectidium paolae* was briefly noted (Sanders, 2014a,b). This distinctive taxon was previously known only from a few sites in the rain forests of east-central Mexico (Herrera-Campos and Lücking, 2003); its occurrence in Florida, where it can be locally abundant, suggests a considerably broader distribution. Most likely, *G. paolae* is not rare but overlooked. Its tiny thalli are barely visible to the unaided eye and not easily distinguished with an ordinary hand lens. Under the higher magnifications of the dissecting microscope, however, their characteristic asexual reproductive structures (hyphophores) are readily recognized. Despite its size, often miniscule even by foliicolous standards, this lichen forms discrete thalli of one to several areolae mottled with large, irregular, raised patches of crystalline material. At their margins, the thallus areolae bear striking hyphophores that resemble eyelids fringed with black lashes (Figs. 1-4). Developing beneath these structures

are the propagules known as diahyphae: bunches of sausage-like chains of conidia that are dispersed as a unit with associated photobiont cells (Fig. 30). As a distinctive example of an ephemeral lichen reproductive at extremely small size, *Gyalectidium paolae* provides an intriguing subject for study of the structural and developmental adaptations associated with its specialized niche. The present work applies light and electron microscopy to investigate thallus structure, type of symbiont interactions, and early stages of propagule establishment and development in this remarkable lichen.

MATERIALS AND METHODS

Electron microscopy – Hand-sections of lichen and leaf substratum collected from the FGCU campus were fixed in 3% glutaraldehyde in phosphate buffer, post-fixed with osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Spurr's low-viscosity resin (de los Ríos & Ascaso, 2002). Ultrathin sections were stained with lead citrate (Reynolds, 1963) and examined in a Zeiss Leo 910 transmission electron microscope. After ultrathin sectioning, the sectioned surfaces of specimen blocks were stained with lead citrate and coated with carbon. The blocks were then affixed to SEM stubs and the cut surfaces examined with a FEI INSPECT scanning electron microscope using backscattered electron imaging mode.

In situ development from naturally dispersed propagules – Plastic netting was cut into strips of approximately 3 x 25 cm; vinyl microscope cover slips were then attached by fitting their corners into diagonal slits made in the strips. The strips were tied with synthetic cord onto the surfaces of *Sabal palmetto* leaves on the FGCU campus where foliicolous lichen colonization was evident. At varying intervals, some cover slips were removed, the bottom surface wiped clean, then placed onto a drop of water on a glass microscope slide. Another drop of water was placed on the upper surface of the colonized cover slip, a fresh glass cover slip was fitted over it, and the surface microorganisms were examined under an Olympus BX-51 compound microscope.

In situ development of inoculated propagules – A simplified version of Larsen's propagule-sowing approach (2010) was adopted. Leaves of *Sabal palmetto* and *Serenoa repens* (saw palmetto) bearing foliicolous lichens were collected from the FGCU campus, and leaf segments bearing thalli of *G. paolae* were removed with scissors. The thalli were covered with a drop of water and allowed to soak for about one minute. Masses of diahyphal propagules were then transferred with a fine forceps to single drops of water placed at the center of vinyl cover slips fitted into mesh strips as described above. The strips were allowed to dry for 24 h, then placed over sabal palm leaves within foliicolous communities near to where the source lichens had been collected. At intervals, individual cover slips were removed and examined microscopically as described above.

RESULTS

Electron microscopy – Examination of the thallus of *G. paolae* with electron microscopy revealed an outer covering layer of fungal origin that was not continuously cellular (Fig. 5). In some areas, particularly where the thallus was thicker and algal cells proliferated in abundance, a fungal cortex 1-2 (-3) cells deep was present (Figs. 6-7). These cells were roundish with only modestly thickened walls (Fig. 7). In other areas, the covering layer appeared to consist of cell wall material extending from and continuous with sparsely distributed fungal cells whose lumina were much smaller than elsewhere in thallus (Fig. 12). Below the covering layer, larger fungal cells were interspersed with scattered algal cells; no discrete algal layer could be discerned. Significant spaces occurred within the thallus (S in Fig. 5), likely facilitating gas exchange, although such spaces were considerably reduced in places where algal cell division was abundant (Figs. 6-7). Larger cavities of jagged outline were also observed, particularly where the overlying surface layer appeared elevated (asterisks in Fig. 8); these spaces appeared to correspond to sites of mineral crystal deposition (compare Figs. 3-4), although the crystals themselves were not evident in the electron micrographs. Toward the periphery of the areolae, the thickness of the thallus decreased considerably (Fig. 9), except where hyphophores developed. Photobionts divided into packets of spores (Fig. 10). Fungal cells made close wall-to-wall contact with one to several algal cells (Figs. 13, 17); they often showed some degree of appressoria-like flattening against the algal wall that increased the surface area of contact, and a fine outer layer uniting the symbionts could sometimes be distinguished (arrows in Fig. 13). However, no evidence of algal wall penetration or initiation of haustorial outgrowths from fungal cells was visible in any of the symbiont contact zones examined.

Near to hyphophores, the thallus became thicker, with cell division and cell density notably greater. The hyphophores appeared as raised margins with a profusion of fungal and algal cells beneath (Fig. 11). The darkly pigmented hyphophore scale was composed of elongate cells with extremely thick, electron-dense cell walls (Fig. 14). These specialized cells arose directly from fungal cells of normal appearance (Fig. 15); their cytoplasm appeared to senesce with maturity, although poor penetration of fixative through their massive walls might be responsible for this impression. Conidia emerging from below the hyphophore scale showed budding at both ends (Fig. 16), and frequently made wall-to-wall contact with nearby algal cells, often partially encircling them (Fig. 17). The fungal cells of the conidial chains were readily distinguished cytologically from vegetative cells of the thallus by their rich content of oil droplets (Figs. 16-17).

In situ development – Propagules of *G. paolae* sown onto plastic cover slips showed sequential stages of development into organized thalli, although mortality was high. Germination of many propagules was observed within 5 d after placement in the field. Hyphae emerged from the tips of the terminal conidial segments and extended radially outward (Figs. 18-19). Many of the sown propagules, however, failed to germinate (Fig. 20); some showed loss of pigment in the co-dispersed algal symbiont (Fig. 21). Among those propagules that successfully germinated, subsequent development was highly variable. In a number of cases, algal symbionts at the periphery of the propagule

proliferated abundantly while extension of fungal hyphae was still relatively limited (Figs. 22-23). In other cases, vigorous growth and branching of fungal hyphae took place before any multiplication of photobiont cells was apparent (Fig. 24). Some of the recent products of algal cell division – visible as cells of considerably smaller size (Fig. 22) – were clearly not held firmly by the lichen fungus and could be observed free of the developing thallus (arrows in Fig. 23). Some propagules appeared to have developed vigorously toward an organized thallus, but were moribund when observed after several weeks (Fig. 25). Successful organization of the thallus seemed to involve the spreading of algal cells more or less uniformly over the periphery of the primordium, presumably assisted by oriented growth of the fungal hyphae beneath (Figs. 26-27). The alga-containing primordia developed into thallus areolae, with fungal hyphae extending well beyond these lichenized portions to form a prothallus (Figs. 27-29). Reproductive stages were not seen on the inoculated cover slips, but did occur on some of those colonized naturally. Formation of the characteristic vegetative propagule of *Gyalectidium* was observed on an incipient hyphophore developing on colonizing thalli only 36 d after substrate placement (Fig. 28). Fully developed hyphophores characteristic of *G. paolae*, bearing abundant propagules, occurred on cover slips retrieved about five months after placement (Figs. 29-30). The smallest areolae observed with mature hyphophores extruding propagules were less than 150 µm in diameter. Granules of crystalline material developed with an initially scattered distribution, appearing to accrete gradually into larger continuous patches (Fig. 31).

DISCUSSION

Construction of the thallus – Although not present in all lichens, the cortex is often the most differentiated and characteristic layer of the thallus; it may be represented by any of a diverse range of tissue types (Henssen and Jahns, 1974; Hale, 1983; Büdel and Scheidegger, 2008). According to Ferraro et al. (2001), a cortex is characteristic of the foliicolous genus *Gyalectidium*. In *G. paolae*, it is evident as an overlying layer of approximately isodiametric fungal cells (Figs. 6-7), but only some portions of the thallus areolae are corticated. Elsewhere one finds a sparsely cellular covering of fungal wall material and cell lumina of reduced diameter, a simpler structure that may better correspond to a corticiform layer (Lücking, 2008). Irregular patches of white crystalline material can also occupy a significant proportion of the upper region of the thallus (Figs. 1-4). The crystals were not directly visible in the electron microscope images, suggesting that these components may be removed in processing the specimens for electron microscopy. The localized elevations of the corticiform layer and irregularly jagged spaces below these regions (Fig. 8) indicate that crystal deposition may be particularly concentrated in places where the cellular cortex is not developed, perhaps substituting for the protective function of the cortex. Although not investigated chemically, the white crystals are likely to be calcium oxalate hydrates, as observed in diverse species of *Gyalectidium* (Ferraro et al., 2001) and other foliicolous members of the Gomphillaceae (de Oliveira et al., 2002), as well as many other lichens (Wadsten and Moberg, 1985; Giordani

et al., 2003). Various possible biological roles have been proposed for such crystals in lichens, including chemical storage of water (Wadsten and Moberg, 1985), photoprotective reflection of excessive radiation (Lücking, 1999), or concentrative reflection of suboptimal light within the algal layer (Modenesi et al., 2000). Calcium oxalate depositions are also known to offer plants protection from herbivory (Franceschi and Nakata, 2005), although invertebrates that feed on foliicolous lichens are not necessarily deterred (Lücking and Bernecker-Lücking, 2000). Within an economically constructed foliicolous thallus, calcium oxalate, which is metabolically inexpensive to produce (Giordani et al., 2003), may represent an alternative shielding material that saves on the energetic costs required to maintain a complete cortex of respiring fungal cells. However, the highly irregular distribution of the crystalline plates in *G. paolae* is not so easily reconciled with any of the functional roles proposed for them.

In situ development of propagules – Co-dispersal of lichen algae, particularly in the form of vegetative propagules, is widely held to be a common strategy among lichens (Bowler and Rundel, 1975). Co-dispersal may be especially important in facilitating colonization of newly exposed leaf surfaces, which are unlikely to harbor a diverse pool of potential photobionts (Sanders, 2014a). The propagules of *G. paolae* were very similar in morphology and mode of germination to those of another species of *Gyalectidium* that colonized cover slips placed in a neotropical lowland forest (Sanders and Lücking, 2002). The subsequent developmental stages reported here provide further insight into the functionality of co-dispersal as an establishment strategy in this lichen. Because development was not studied under controlled conditions, other organisms, including additional lichen propagules, were also able to colonize the inoculated coverslips. However, the cohort of *G. paolae* propagules, sown within a restricted area of the coverslip, could be distinguished with a fair degree of confidence, at least in the first several weeks after placement in the field. Most notable was the range of variability in development, with many propagules showing photobiont degeneration before germination (Fig. 21) or after substantial development (Fig. 25). Conceivably, some of the propagules may have been immature when taken for inoculation, although it is not clear what sort of additional maturation process might be required after propagules are fully formed morphologically. While genetic differences among propagules cannot be entirely ruled out (the source thalli were not necessarily a single clone), the highly variable results suggest that microenvironmental heterogeneities in light, moisture, and/or nutrient availability may strongly impact development at a very fine scale. The remarkable variability observed in the relative rates of fungal/algal development in the early stages of growth (Figs. 22-23 vs. 24) complements the findings of laboratory culture and resynthesis studies, where different regimes of moisture, nutrients and light often favored growth of one symbiont over the other, impeding formation or maintenance of the lichen symbiosis (Thomas, 1939; Scott, 1960; Ahmadjian, 1962; Bertsch and Butin, 1967; Pearson 1970). It would be interesting to follow the eventual fates of developmental stages in each case, but unfortunately the destructive sampling applied in the present study did not allow sequential observation of the same propagules over time. The appearance of mature

hyphophores characteristic of *G. paolae* on the uninoculated cover slips by 5 months after placement is consistent with the observations of Larsen (2010), who noted hyphophore formation in an unidentified *Gyalectidium* species within a comparable time frame in a Costa Rican forest. Another foliicolous lichen, *Calopadia puiggarii*, developed thalli with mature asexual reproductive structures in slightly less time, about 3-4 months (Sanders, 2014a). With *Gyalectidium*, however, there was evidence in the present study that asexual propagules could form on thalli as young as five weeks old, before a recognizable hyphophore scale has even developed (Fig. 28). With asexual cycles completed in less than 5 months, these lichens might, with favorable timing and conditions, conceivably grow and reproduce on deciduous leaves. However, perpetuation would only be ensured if some propagules managed to disperse to a more enduring substratum before leaf fall. On sabal palm leaves, which last for about 3 years at the field site (Sanders, 2014b), *G. paolae* could complete numerous asexual generations on the same leaf under favorable conditions. Sexual stages (apothecia) have not yet been reported in this species.

Contacts between symbionts – The variety of fungal-algal contacts in numerous, mainly crustose lichens was first discerned with light microscopy in the remarkable studies of Tschermak (1941) and Plessl (1963). The general pattern of their findings was that simpler crustose thalli without distinct tissue differentiation tended to show fine, peg-like fungal structures penetrating the algal cell wall and protruding into the lumen, whereas anatomically more complex lichens with distinct tissue layers exhibited more limited penetration pegs that entered the algal cell wall without fully traversing it (intraparietal); intermediates were also observed. Subsequent application of electron microscopy largely confirmed the light microscopic observations of those pioneering studies (Galun et al., 1970; Honegger, 1984, 1986). In the present study of *Gyalectidium paolae*, we saw only wall-to-wall apposition between symbionts without haustorial penetration, a situation more commonly reported in anatomically more complex lichens (Honegger, 1988). By contrast, in *Strigula*, a foliicolous lichen examined in previous TEM studies, well-developed haustoria commonly penetrate the cells of its ulvophycean photobiont (Chapman, 1976; Matthews et al., 1989). The varying composition and structural properties of the cell wall in different photobiont taxa may influence the type of symbiont contacts that develop. Sporopollenin-like polymers highly resistant to degradation have been identified in the walls of lichen algae such as *Coccomyxa* and *Elliptochloris*, and correlated with the absence of haustorial penetration by the lichen fungi that house them (Honegger, 1984). On the other hand, the photobionts *Myrmecia* and *Dictyochloropsis* lack such wall polymers but are usually not penetrated by mycobiont haustoria (Brunner and Honegger, 1985). Whether resistant polymers occur in the photobiont of *G. paolae* is not known. The *Trebouxia*-like algae associated with the numerous foliicolous species of Pilocarpaceae and Gomphillaceae including *G. paolae* have not yet been identified, and are currently under study.

Although the penetrative contacts between lichen symbionts are known as haustoria, they are not directly involved in transfer of metabolites, which appear to simply leak from the symplast of the alga in symbiosis (Collins and Farrar, 1978). The lichen

haustorium, most notably the intraparietal type, seems to have evolved into a more strictly physical means of symbiont attachment that might play a role in coordinating symbiont distribution to produce anatomically complex thalli (Honegger, 1996). An attachment function for these penetrative contacts is particularly evident in the marine lichen *Wahlenbergiella* (=*Verrucaria*) *tavaresiae*, where haustoria expand laterally to form an anchoring flange inserted between wall layers of its phaeophycean photobiont (Sanders et al., 2004). Similarly, the intraparietal haustorial complexes formed by *Mastodia tessellata* have been interpreted as a possible mechanism for coordinating mycobiont growth with that of its multicellular green photobiont (Pérez-Ortega et al., 2010). In *Gyalectidium paolae*, algal distribution within the developing areolae appears to be achieved without penetrative attachments between symbionts, although it should be noted that a relatively low level of anatomical organization is achieved.

Studies of symbiont contacts in other lichens have suggested that no photobionts are free within the lichen thallus (Honegger, 1996); fungal hyphae penetrate the spore packets of dividing trebouxioid algal symbionts, establishing attachments to the still-contained daughter cells that will facilitate their distribution by subsequent fungal growth (Greenhalgh and Anglesea, 1979; Honegger, 1987). By contrast, examination of wet-mounted, living foliicolous thalli growing on cover slips suggests that the nascent unicellular photobionts of these lichens are not initially attached to any fungal hyphae. One can occasionally observe the release of small algal cells from the autosporangia at the periphery of the thallus; the exiting spores immediately position themselves alongside the larger neighboring algal cells (Figs. 22-23). Presumably they are held there by surface interactions until extending mycobiont cells establish appositional contact, sealing an apoplastic conduit between symbionts with secreted wall materials (Fig. 13) as elucidated by Honegger (1984, 1986, 1988, 1996). The released algal cells can also escape the thallus entirely (Fig. 23), where they might be subsequently recaptured by prothallic hyphae, or give rise to (perhaps transient) free-living populations available to other lichen fungi of the foliicolous community (Sanders, 2014a). On an ephemeral substratum with limited time and capacity for a diverse pool of photobionts to develop, these thallus fugitives may be important sources of symbiotic partners for the many fungal propagules dispersed by foliicolous lichens without accompanying algae.

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FIGURE LEGENDS

1-4. Light microscope images of *G. paolae* colonizing sabal palm leaves on the FGCU campus. 1. Thallus of several areolae. 2. Tiny areolae (brackets) barely distinguishable against green and white leaf background; hyphophores (arrows) already developed at this stage. 3. Single thallus areola. 4. Larger, continuous thallus with several marginal hyphophores and scattered crystalline deposits. Scale bars = (Figs. 1, 2, 3, 4) 200 µm.

5-9. SEM-backscattering images showing cut surfaces of resin blocks within which *G. paolae* has been embedded. 5. Thallus with only localized development of cellular cortex (arrowheads); fungal wall material without distinguishable cell lumina forms much of uppermost layer (arrow). Many large spaces (S) are present among fungal and algal cells. 6. Thallus showing much more extensive development of a cellular fungal cortex (c). Below, symbiont cells are densely packed with fewer spaces between them. This image might represent a thallus zone adjacent to a hyphophore. 7. Detail of 6 showing fungal cortex (c). 8. Thallus with large jagged spaces (asterisks) possibly corresponding to mineral crystals. 9. Edge of thallus areola showing gradually declining thickness. Scale bars = (Figs. 5, 7) 10 µm; (Figs. 6, 8, 9) 20 µm.

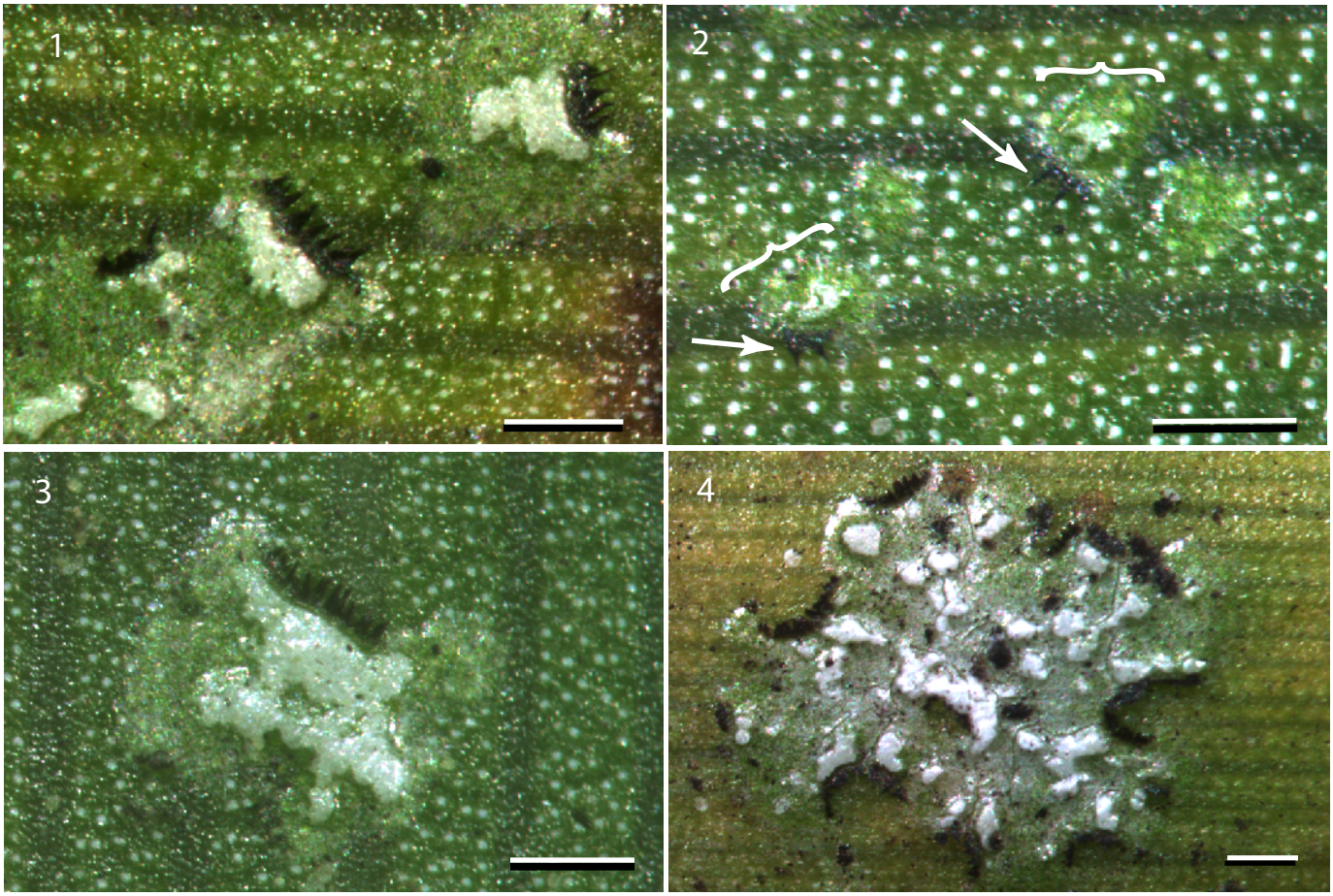
10-11. SEM-backscattering images showing cut surfaces of resin blocks within which *G. paolae* has been embedded. 10. Interior of thallus with dividing packet of algal cells (arrows). 11. Edge of thallus with hyphophore (h) and masses of propagules (p) composed of conidial chains (electron-bright due to osmiophilic lipid content) and photobiont cells. Scale bars = (Fig. 10) 5 µm; (11) 10 µm.

12-17. TEM images of *G. paolae*. 12. Upper layer of thallus composed of wall materials (arrows) continuous with sparse fungal cells of reduced diameter (Compare fungal cells within covering layer at upper left of figure with those below layer at lower right). 13. Fungal cell making intimate wall contact with three algal cells. 14. Thick-walled fungal cells composing hyphophore scale; less specialized fungal cell nearby. 15. Thick-walled cell of hyphophore scale arising from less specialized fungal cell. 16. Conidial cell of diahyphal propagule, with cells budding off at both ends (arrows); oil droplets occupy much of cytoplasm. 17. Conidial cell with budded proliferations (arrows), making close contacts with adjacent algal cell. *Abbreviations*: a, algal cell; c, conidium; f, fungal cell; h, fungal cell of hyphophore.; o, oil globule. Scale bar = (Figs. 12, 13, 14, 15, 16, 17) 1 µm.

18-26. Light microscope images of lichen development in situ on cover slips inoculated with *G. paolae* propagules. 18. Germinating propagule after 5 d with hyphae emerging from tips of terminal conidia (arrows). 19. Developing propagule (13 d) with radiating fungal hyphae and proliferating algal cells. 20. Propagule showing no signs of germination after 5 d. 21. Ungerminated propagule (5 d) showing loss of pigment in algal cells (arrows). 22. Germinated propagule with algal cells (arrows) proliferating at margin but relatively limited extension of fungal hyphae (13 d). 23. Extensive proliferation of algal symbionts relative to modest development of fungal prothallus; some algal cells (arrows) apparently liberated from thallus (13 d). 24. Germination of a group of propagules, showing extensive

development of fungal hyphae but no proliferation of algal cells (arrow) at this stage (13 d). 25. Young thallus moribund after abundant proliferation of algal cells (arrows), now discolored, and extensive fungal prothallus development (arrowhead). 26. Developing thallus showing relatively uniform distribution of algal cells elevated above substratum (55 d). 27. Detail of developing thallus showing algal cells at periphery, some dividing (arrow) into autospores; 55 d. Scale bars = (Figs. 18, 20, 21) 10 μm; (19, 22, 25, 27) 20 μm; (23, 24, 26) 50 μm.

28-31. Development from naturally dispersed propagules on uninoculated cover slips. 28. Margin of young areola with developing hyphophore (arrowheads) that is already producing a propagule (arrow) 36 d after cover slip exposure. 29. Areola with fully formed hyphophore (arrowheads) and extensive surrounding prothallus (p); 151 d after exposure. 30. Detail of hyphophore with several propagules (arrows); 151 d after exposure. 31. Areola with hyphophore, showing accretion of crystalline deposits (arrows); 151 d after exposure. Scale bars = (Figs. 28, 30) 20 μm; (Figs. 29, 31) 50 μm.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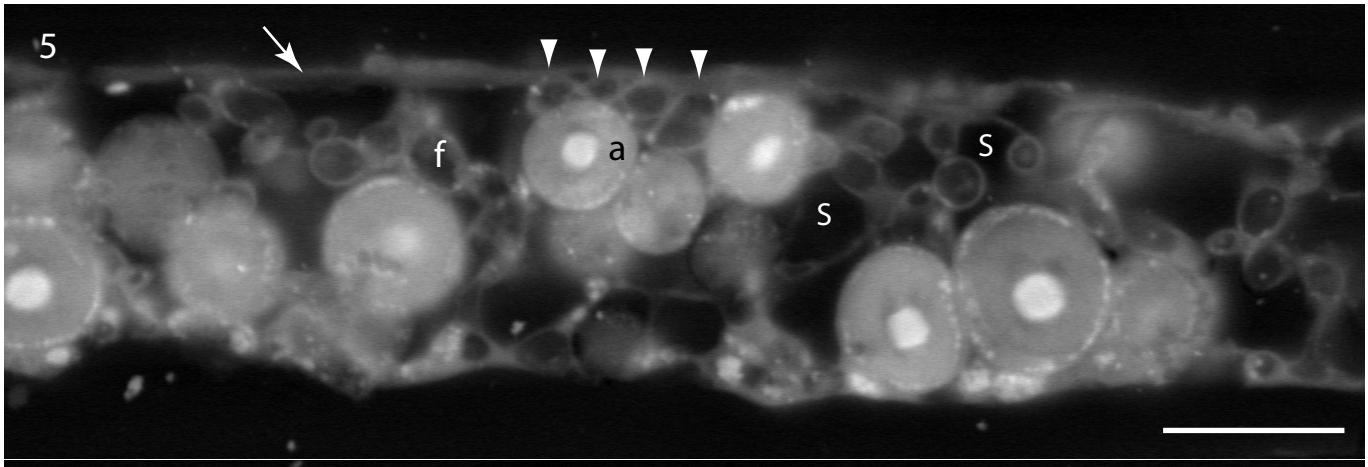
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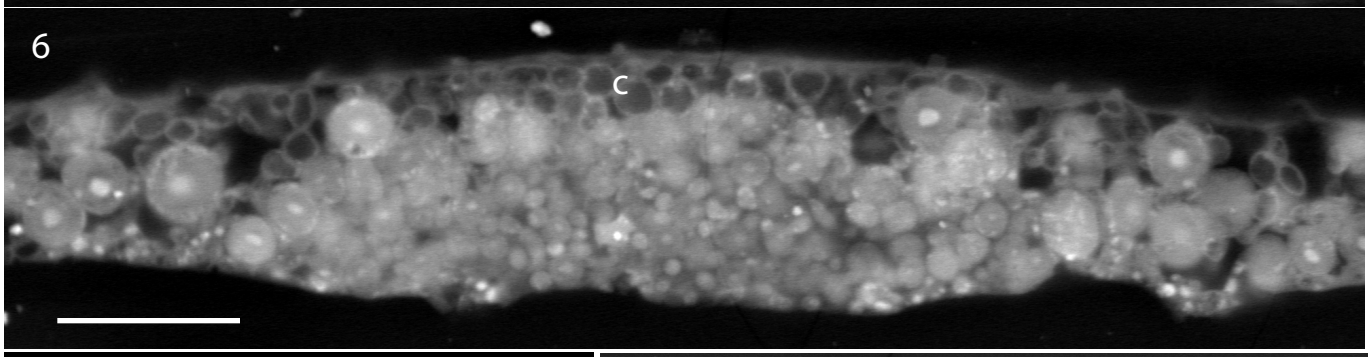
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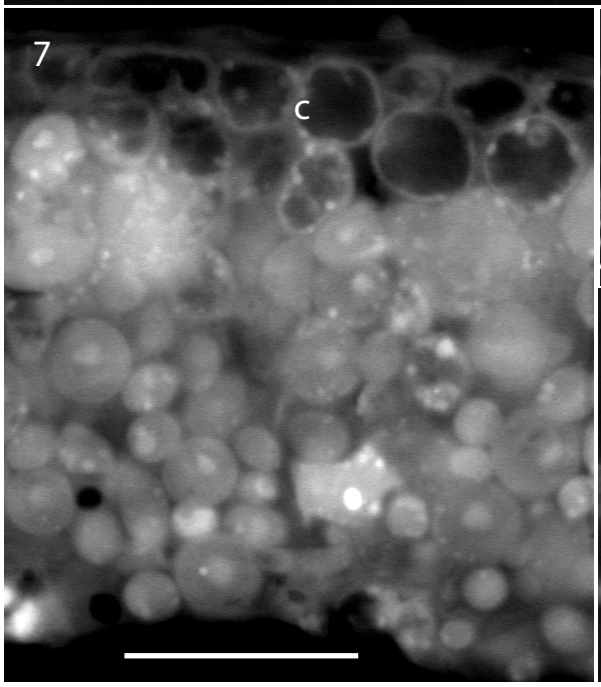
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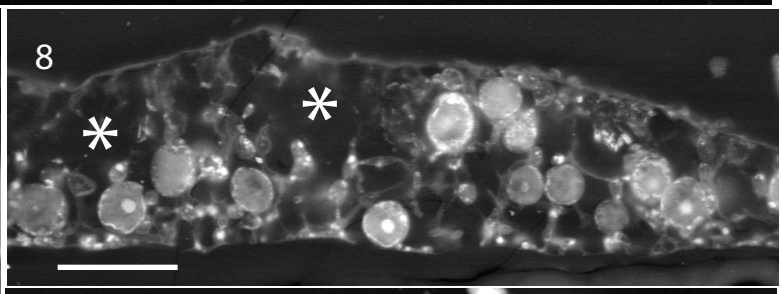
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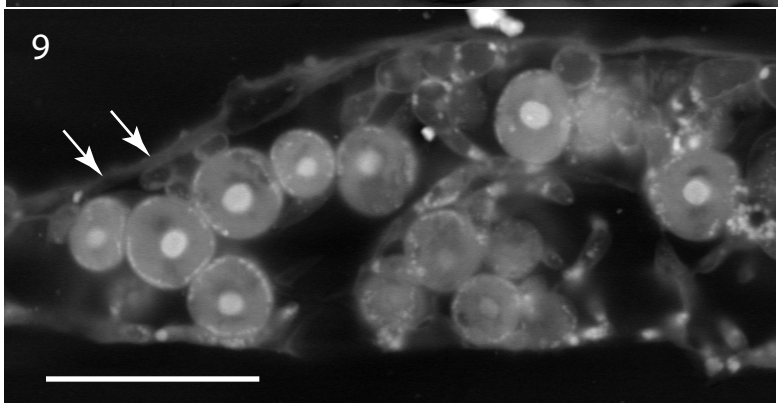
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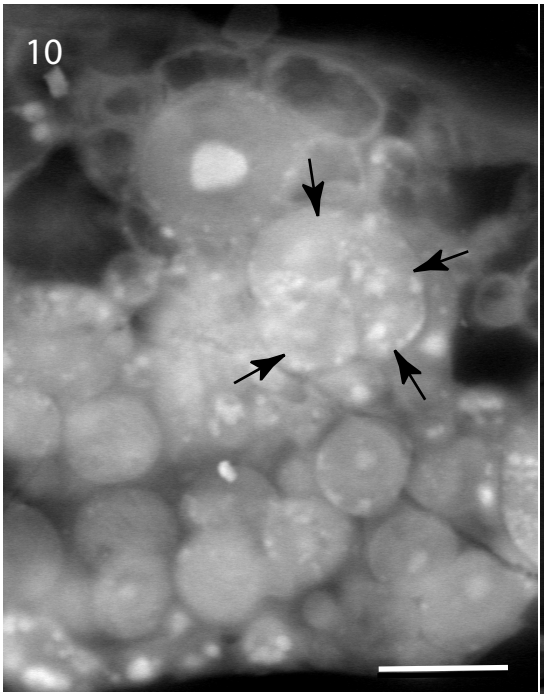
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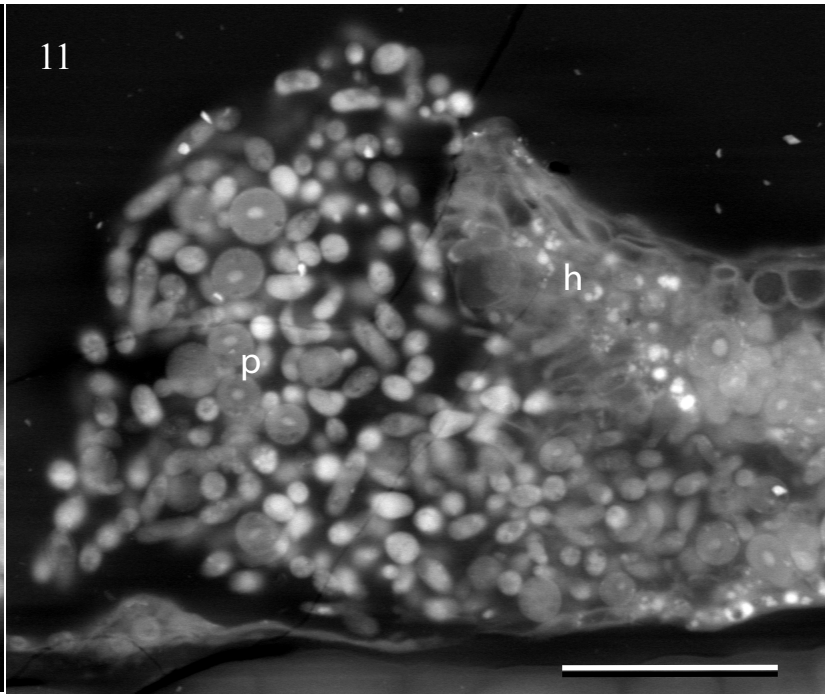
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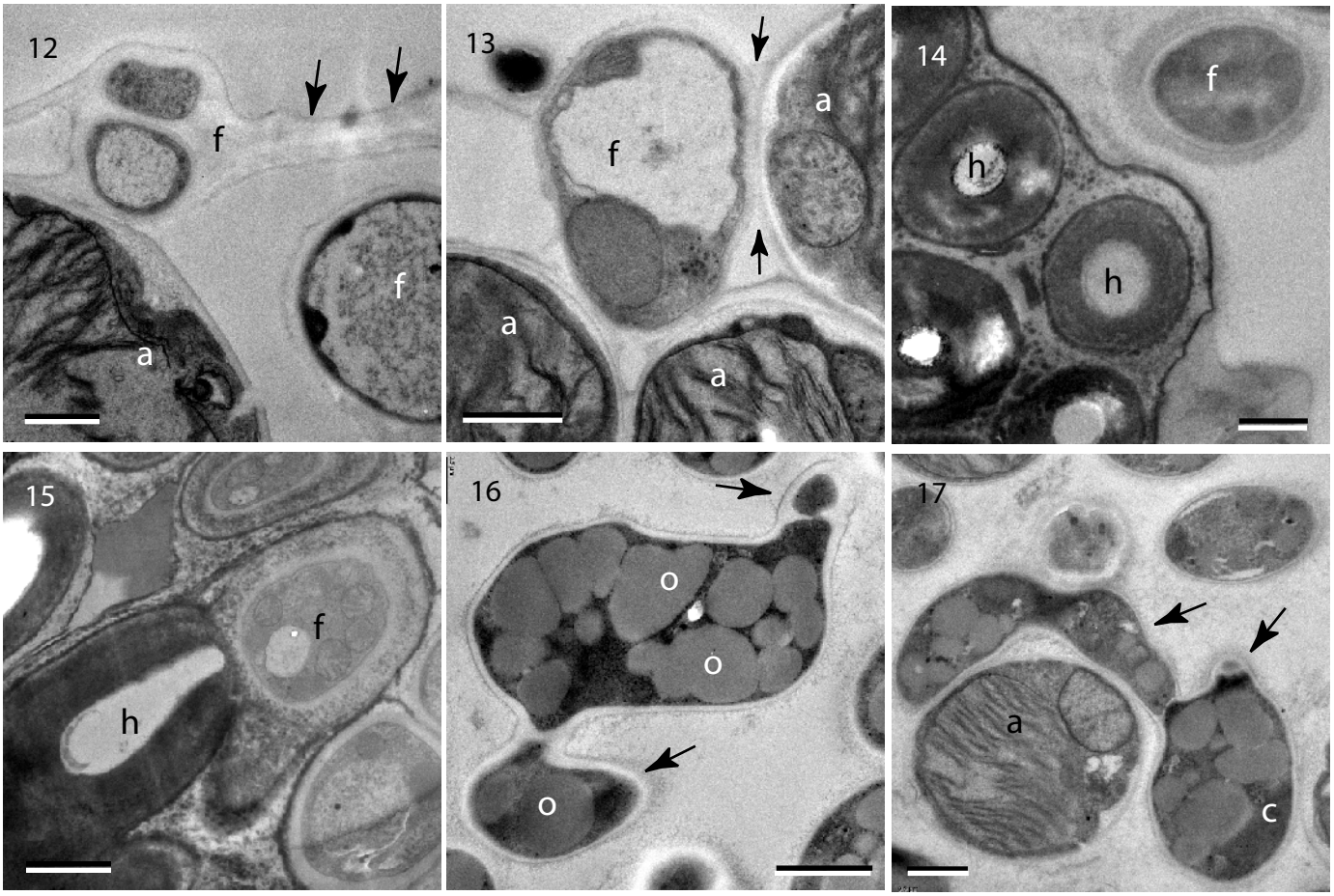
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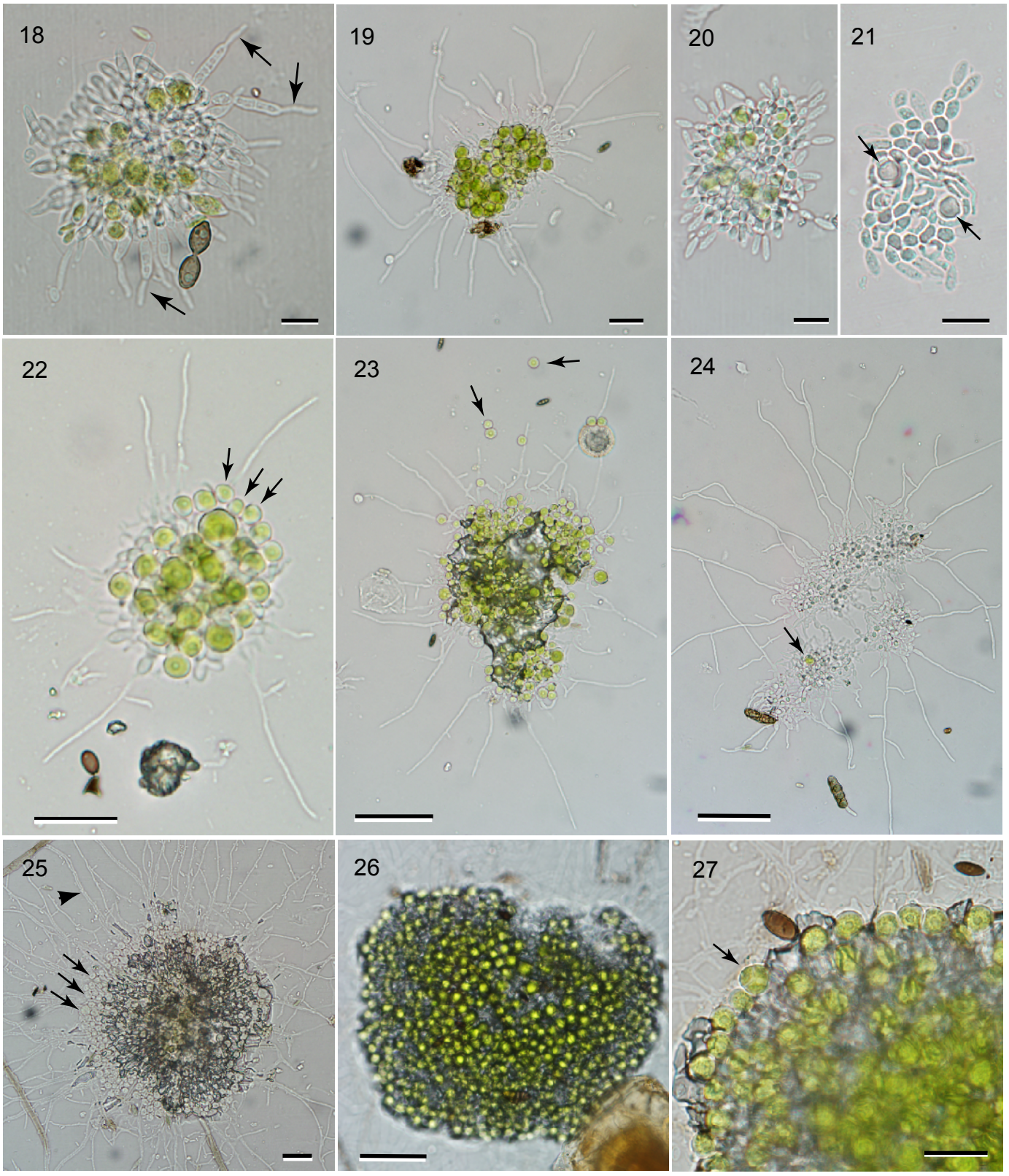
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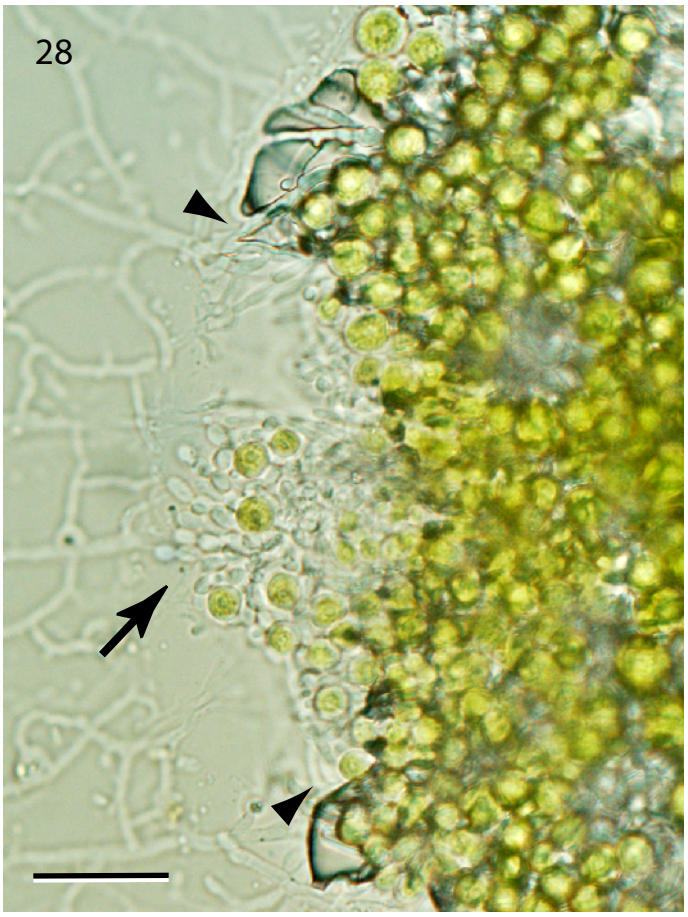
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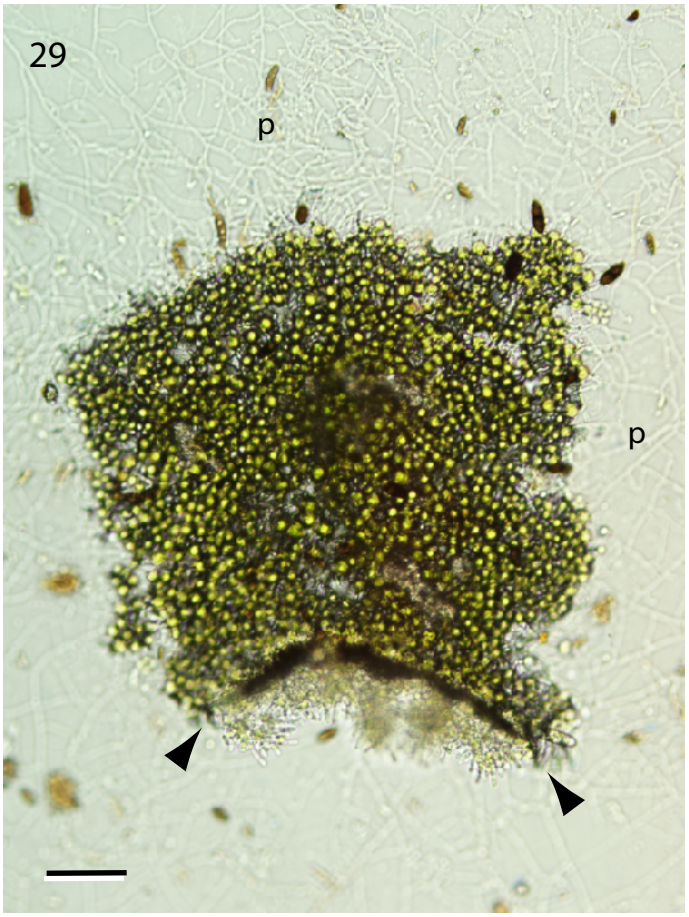
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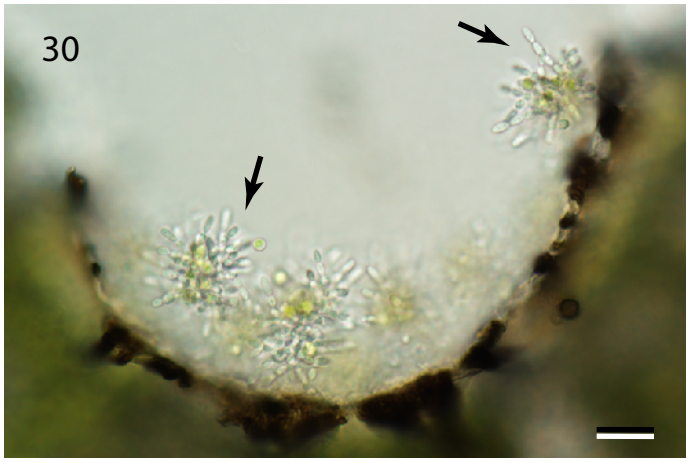
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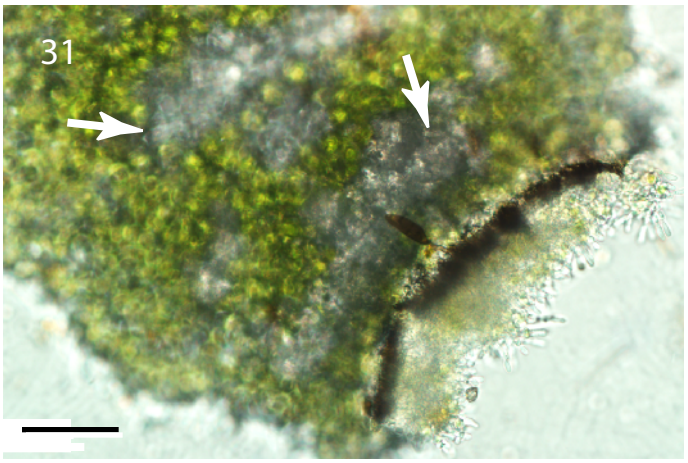
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