American Journal of Botany 84(8): 992–1004. 1997.

A CYANOLICHEN FROM THE LOWER DEVONIAN RHYNIE CHERT[1]

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The 400 million-year-old Rhynie chert has provided a wealth of information about various types of fungal interactions that existed in this Early Devonian paleoecosystem. In this paper we report the first unequivocal evidence of a lichen symbiosis from the Rhynie chert. Specimens of a new genus, *Winfrenatia,* consist of a thallus of superimposed layers of aseptate hyphae and, on the upper surface, numerous uniform depressions. Extending into the base of each depression are hyphae that form a three-dimensional netlike structure. Enclosed within each of the net spaces is a coccoid cyanobacterium, each cell of which is surrounded by a thick sheath. These photobiont cells divide in three planes, resulting in cell clusters of up to perhaps 64 individuals. The photobiont is parasitized by the fungus in the base of each net as new cyanobacterial cells are formed distally. Reproduction is by endospores and soredia. Affinities of the mycobiont appear closest to members of the Zygomycetes, while the photobiont is most similar to coccoid cyanobacteria of the *Gloeocapsa* and *Chroococcidiopsis* types. We speculate that this cyanobacterial symbiosis was well adapted to exploit and colonize new ecological niches, especially in the periodically desiccated environment postulated for the Rhynie chert paleoecosystem.

Key words: cyanobacteria; Devonian; lichen; mycobiont; parasitism; photobiont; symbiosis; zygomycete.

Lichens are symbiotic associations that involve a mycobiont, typically an ascomycete, and a green alga or cyanobacterium as the photobiont; some lichens possess multiple photobionts (Hawksworth, 1988). Approximately 90% of living lichen photobionts belong to the Chlorophyceae, and of these trebouxioid and trentepohlioid genera are the most common (Ahmadjian, 1993). Among cyanolichens *Nostoc* is the most common photobiont. Although lichens encompass a continuum of physiological interactions from parasitic to mutualistic symbioses (Honegger, 1992), all photobionts represent extracellular endosymbionts of their fungal host. Modern lichens occupy a wide range of ecological niches and inhabit sites from the tropics to polar regions, and from deserts to rain forests.

Although some lichen thalli are delicate and would appear to be poor candidates for preservation, most living forms are constructed of sturdy cells and tissue systems that would suggest they could easily be preserved as fossils. There are, however, few reports of fossil lichens (Taylor and Taylor, 1993), and none of these provides conclusive proof of a symbiotic association. For example, *Thuchomyces lichenoides* has been interpreted as a Precambrian lichen (Hallbauer, Jahns, and Beltmann, 1977). It consists of what appear to be hyphal filaments, but evidence of the photobiont is lacking. *Pelicothallos* is a Tertiary structure believed to represent a lichen encased in amber (Garty, Giele, and Krumbein, 1982), offers even less convincing evidence of a symbiotic association. What are interpreted as apothecia on a lichen thallus have also been described from Triassic sediments (Ziegler, 1992). However, based on the limited micrographs that are presented, it is difficult to evaluate the nature of this material. More recently, Stein, Harmon, and Hueber (1993) suggested that the enigmatic Devonian organism *Spongiophyton* may be a lichen thallus, but in this example there is nothing known about either biont. Still others have postulated the existence of lichens based on sediment structures (caliche) that are sometimes associated with modern lichen communities (e.g., Klappa, 1979). The Precambrian Ediacaran organisms and other vendobionta have recently been suggested as representing lichens based on how certain structures might be preserved as fossils (Retallack, 1994), however, this hypothesis has no biological basis. Finally, the fact that there are no bona fide examples of fossil lichens is also a reflection of the general unfamiliarity with these organisms and the very small number of people actively looking for fossil specimens, a situation that was commented on $>$ 100 yr ago (Lindsay, 1877–1879)!

In our opinion, a fossil lichen must clearly demonstrate the presence of both bionts and a thallus that is different from that of either symbiont alone. In addition, there should be some evidence that the two bionts interacted physiologically. In this paper we report the first cellular evidence of a fossil lichen from the Rhynie chert in which both symbionts are preserved in a consistent and unique juxtaposition, indicating they were physiologically interrelated during life.

MATERIALS AND METHODS

The Rhynie chert locality contains more than ten plant-bearing beds that are preserved as siliceous sinters (Trewin and Rice, 1992). The age of the chert based on palynomorph assemblages is generally regarded as Pragian (Richardson, 1967). The fossils were studied by means of

[1] Manuscript received 1 August 1996; revision accepted 15 November 1996.

This study was initiated during the tenure of a Senior U.S. Fellowship from the Von Humboldt Foundation awarded to the first author. Financial support provided by the Deutsche Forschungsgemeinschaft (Re 200/16–2) and National Science Foundation is gratefully acknowledged.

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petrographic thin sections prepared by cementing a thin slice of the chert block to a microscope slide and subsequently grinding the rock down to a thickness of 50–150 μm. Photomicrographs were made using oil emersion objectives directly on the rock surface. Slides are a component of the W. Remy collection deposited in the Geologische-Paläontologische Institut und Museum, Abteilung Paläobotanik, Westfälische Wilhelms-Universität Münster, Münster, Germany. Slide numbers are included with figure captions.

RESULTS

Systematics—*Winfrenatia* Taylor, Hass et Kerp

Generic diagnosis—Thallus of thin mycelial mats constructed of superimposed horizontal layers of interwoven aseptate hyphae; upper layers vertically folded to form numerous ridges and depressions; mycobiont hyphae extending into depressions to form three-dimensional network; photobiont of spherical coccoid unicells or cell clusters, each surrounded by extracellular sheath; reproduction by endospores and soredia.

Winfrenatia reticulata Taylor, Hass et Kerp

Specific diagnosis—Thallus with irregular margin, at least 10 cm long and 2 mm thick, individual hyphal layers 2–6 hyphae thick, depressions circular to elliptical, 0.3–0.8 mm in diameter and up to 1 mm deep, hyphae 1.0–4.0 μm in diameter with granular surface, hyphae in depression extensively branched to form net with uniform lacunae each 20–30 μm in diameter, hyphae of net sometimes forming vertical plates that subdivide larger depressions; each lacuna occupied by a single unicell 10–16 μm in diameter and surrounded by an approximately 6-μm thick sheath, photobiont unicellular and small in base of depression, with increasing cell divisions and larger diameters distally, initial divisions form a plate of four cells with subsequent divisions resulting in an aggregation of up to 32, perhaps 64 cells; vegetative reproduction by soredia and clusters of endospores, each 1.6–3.2 μm in diameter.

Holotype—Specimen represented by petrographic thin section slides PB 1210, PB 1211, PB 1213, PB 1238, PB 1241, PB 1308, PB 1310, PB 1323, PB 1329, PB 1351, PB 1354, PB 1357, PB 1374, PB 1376, PB 1377, PB 1380, PB 1382, PB 1384, PB 1386, PB 1388, PB 1396, PB 1397, PB 1406, PB 1414, PB 1604 in the W. Remy collection permanently deposited in the Forschungsstelle für Paläobotanik, Westfälische Wilhelms-Universität, Münster.

Type locality—Rhynie, Aberdeenshire, Scotland. National Grid Reference NJ 494276 (Edwards, 1986).

Age—Early Devonian.

Stratigraphic position—Pragian.

Etymology—The generic name *Winfrenatia* is proposed as a combination of the given names of Winfried and Renate Remy for their numerous scholarly contributions on the Rhynie chert flora. Their meticulous work on Lower Devonian floras has opened new vistas in plant evolution, and provided critical details about the life history biology of early land plants. The specific epithet *reticulata* refers to the net-like organization formed by the mycobiont.

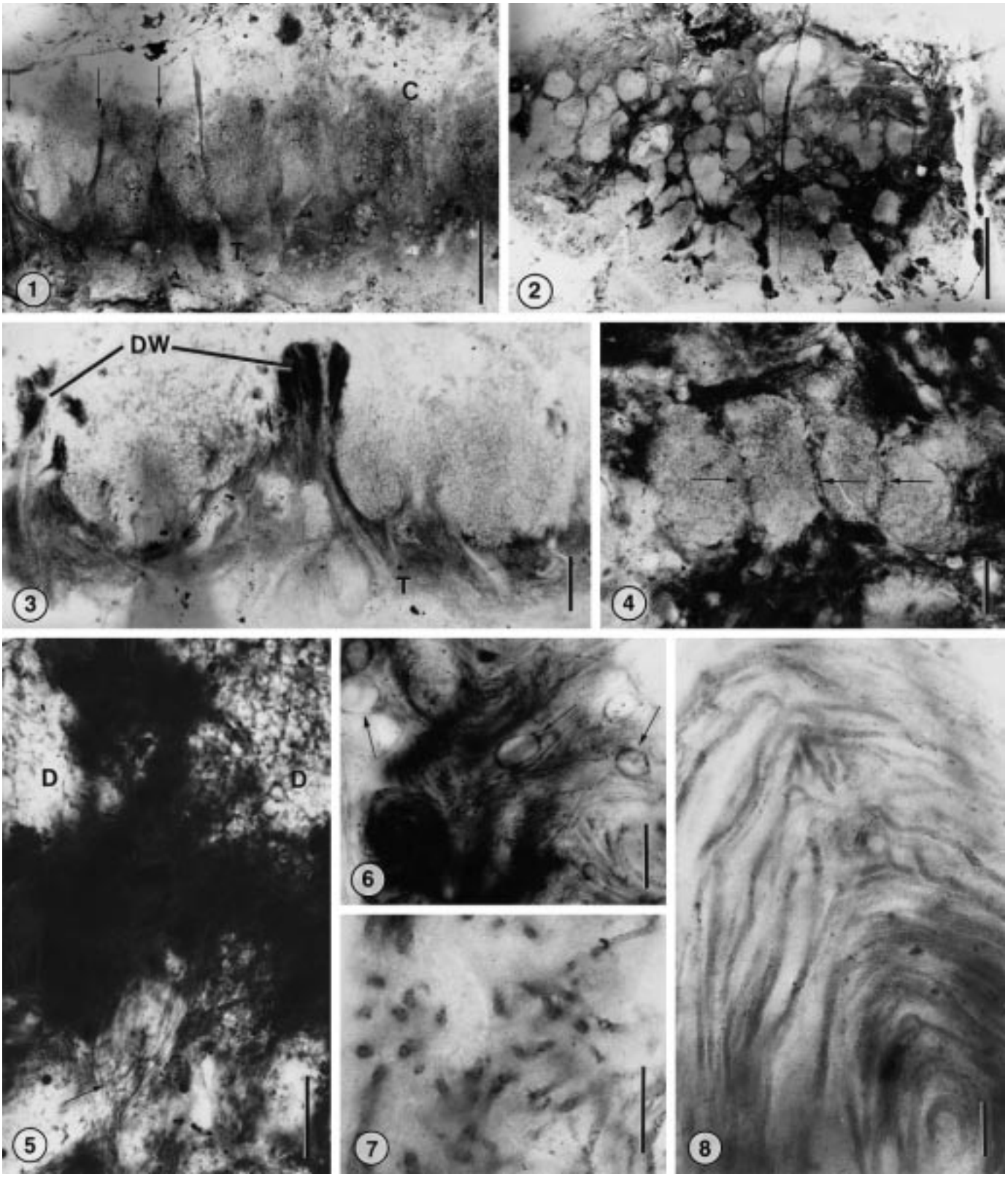
RESULTS

General morphology—The lichen is represented by a single, incomplete specimen that extends approximately 6.0 cm throughout a chert block (Fig. 1). Although the size and overall morphology of the thallus are difficult to determine accurately, we speculate that the total lateral extent of the thallus probably exceeded 10 cm. The margin of the thallus is irregular and can be distinguished by alternating dark and light regions in the chert (Fig. 2). Axes of the charophyte *Palaeonitella* extend through some areas of the thallus (Fig. 6). Some cells of the charophyte contain hyphae (Fig. 6) that are identical to those of the mycobiont, suggesting that the lichen thallus has developed around *Palaeonitella* during growth.

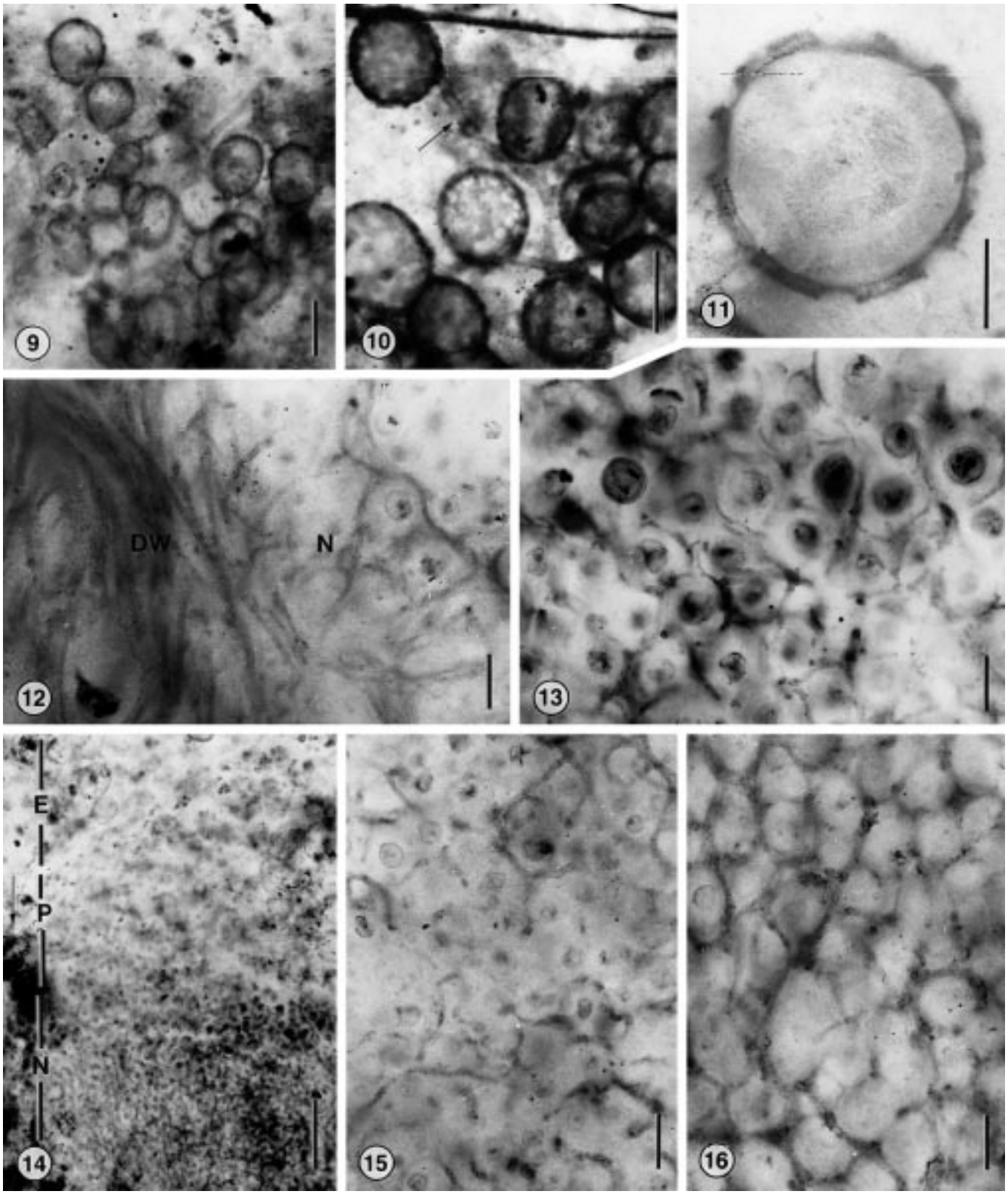
The thallus of *Winfrenatia* is best described as heteromerous, a condition in which there are two distinct regions of the thallus. Most of the thallus consists of a thin mycelial mat, 1–2 mm thick. Each mat is formed by 2–4 superimposed layers of parallel hyphae, each layer 2–6 hyphae thick. The uppermost two layers are vertically oriented and folded into loops (Fig. 8) that form a pattern of ridges and up to 40 shallow, circular to elliptical depressions or indentations on the thallus surface, each 0.5–1.0 mm deep (Figs. 1–4). Throughout the thallus the depressions appear to be somewhat uniform and vary from 0.3 to 0.8 mm in diameter (Fig. 2). Most are open, but in a few areas the tightly matted hyphae of the ridges extend over the depression to form a partial canopy. The second component of the lichen thallus is represented by the depressions on the surface. Here loosely organized hyphae from the mat extend inward and fill the depression with a netlike structure (Fig. 12). In other regions of the thallus vertically oriented plates of hyphae in the form of septations subdivide each of the depressions (Fig. 4); other depressions are completely filled with hyphae (Fig. 2). We interpret the differences seen in each of the depressions as representing specific stages in the growth and development of the lichen thallus. These stages are discussed in a later section.

Unlike the thallus organization in most modern lichens, there is no protective layer over the upper surface of *Winfrenatia*. In addition we find no evidence of a specialized layer of hyphae on the lower surface of the thallus. Rather, there are what appear to be bundles of hyphae that extend from the lower surface of the thallus into the matrix (Fig. 5). These may represent some type of rhizine or cilia, however the preservation in this region of the thallus is generally poor.

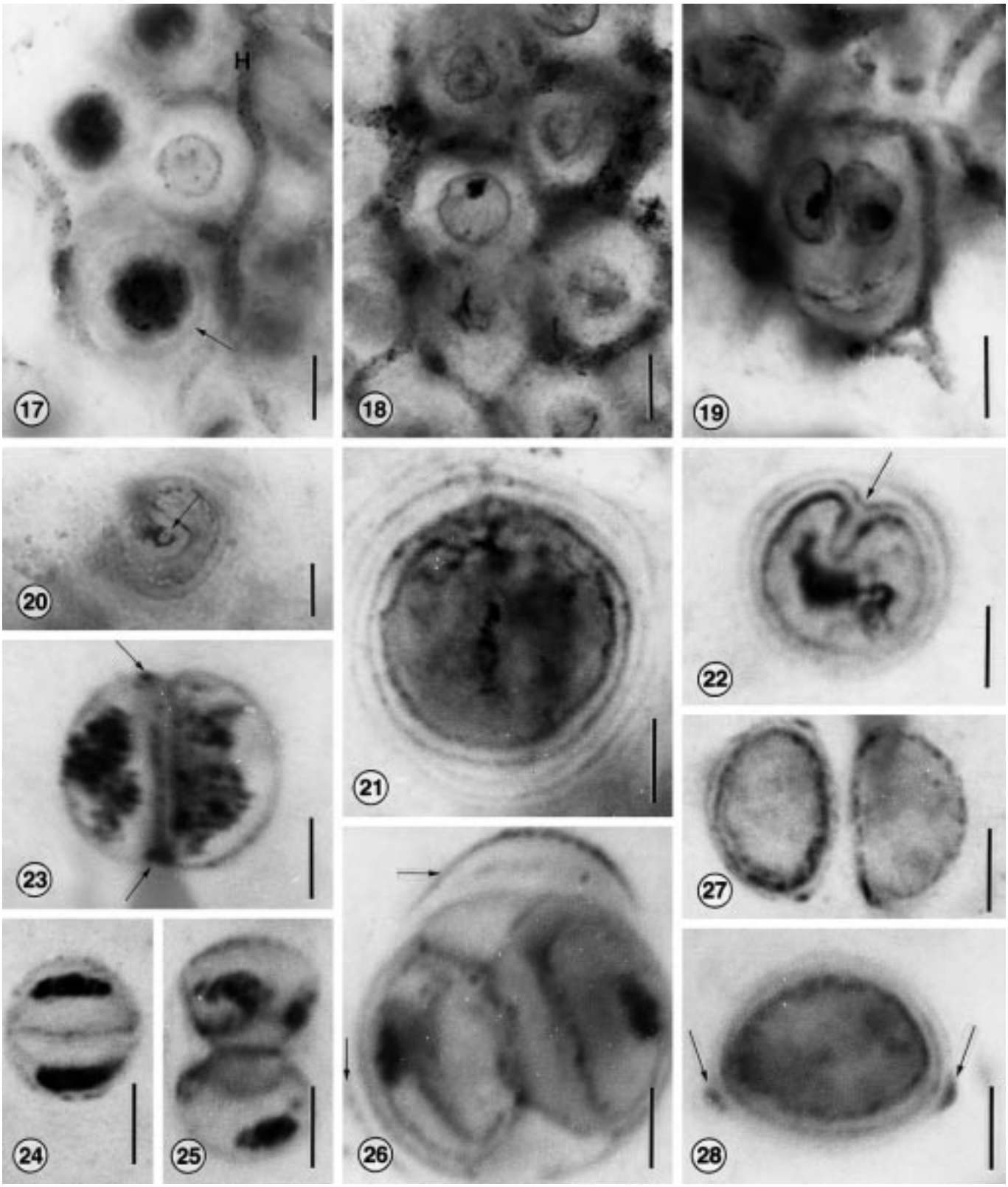
Mycobiont—Each of the thallus depressions contains aseptate hyphae. These hyphae, unlike those that make up the main portion of the thallus, are more loosely organized into a three-dimensional, netlike structure (Figs. 13, 15, 16). As a result of repeated anastomoses hyphae form circular to hexagonal lacunae, each ranging from 20 to 30 μm in diameter (Figs. 17, 18). Along the inner margin and base of each depression these hyphae are



Figs. 1–8. Thallus morphology of *Winfrenatia reticulata*. **1**. Longitudinal section of thallus (T) showing walls of depressions (arrows). Note clusters of chlamydospores (C). P 1414. Bar = 500 μm. ×30. **2**. Transverse section of thallus showing depressions (light regions) and walls of depressions (opaque zones). P 1384. Bar = 1.0 mm. ×15. **3**. Detail of depression showing wall (DW) and portion of thallus (T). P 1323. Bar = 200 μm. ×50. **4**. Transverse section of thallus showing wall of depression (opaque) and initial stages in septation formation (arrows). P 1604. Bar = 200 μm. ×50. **5**. Section of thallus showing two depressions (D) in fungal thallus. Arrow at base indicates a bundle of hyphae extending from the lower surface of the thallus. P 1386. Bar = 100 μm. ×150. **6**. Axes of the charophyte *Palaeonitella* (arrows) growing through the fungal thallus. P 1604. Bar = 100 μm. ×120. **7**. Hyphae in section view. P 1604. Bar = 20 μm. ×800. **8**. Hyphae in region of depression wall showing loop configuration. P 1391. Bar = 20 μm. ×500.



Figs. 9–16. Mycobiont of *Winfrenatia reticulata*. Bar = 20 μm unless otherwise noted. **9**. Several thick-walled spores associated with thallus. P 1376. Bar = 50 μm. ×200. **10**. Thick-walled spores terminally attached to hyphae (arrow). Note uniform reticulate wall. P 1310. Bar = 50 μm. ×300. **11**. Section of spore like that in Fig. 10 showing detail of reticulate wall. P 1374. ×800. **12**. Section through inner edge of depression wall (DW) showing loosely arranged hyphae that form net (N). P 1391. ×500. **13**. Detail of net and relationship to cells of the photobiont. P 1386. ×500. **14**. Longitudinal section of depression showing topographic relationship between basal net (N), region in which the largest photobiont cells occur (P) and zone of empty sheaths (E). Arrow at left indicates top of depression wall. P 1386. Bar = 100 μm. ×120. **15**. Section of depression near middle showing the incomplete organization of the hyphal net. P 1388. ×500. **16**. Section of depression near base showing the organized nature of the hyphal net. Compare with Fig. 15. P 1386. ×500.



Figs. 17–28. Photobiont of *Winfrenatia reticulata*. Bar = 5 μm unless otherwise noted. **17**. Detail of ramifying net-forming hyphae (H) and photobiont cells. Note material surrounding photobiont cell (arrow). P 1211. Bar = 10 μm. ×1 200. **18**. Detail of hyphal net and central position of each photobiont cell. P 1386. Bar = 10 μm. ×1 200. **19**. Hyphal net containing two cells just after division. P 1391. Bar = 10 μm. ×1 500. **20**. Scar (arrow) on photobiont cell that may represent some form of apposition or intergelatinous protrusion. P 1382. ×2 000. **21**. Cell showing remnants of several sheaths. P 1351. ×3 000. **22**. Cell with invagination (arrow) interpreted as early stage in cell division. Note outer sheath. P 1238. ×3 000. **23**. Two daughter cells prior to separation. Note thickened areas (arrow). P 1213. ×3 000. **24**. Two daughter cells with lenticular cell

more tightly appressed so that lacunae are smaller (Fig. 12); toward the center of each depression hyphae are more loosely organized, resulting in incomplete nets (Fig. 15).

Hyphae range from 1.0 to 4.0 μm in diameter (Figs. 7, 8), with the wall often appearing granular or with irregularly thickened regions. What appear as constrictions along the length of a hypha represent regions where twisting and/or collapse has occurred (Fig. 8). Although distinct septa have not been clearly resolved, the netlike organization suggests that cross walls may be present where the hyphae branch, or that the lacunate structure is the result of closely spaced hyphal anastomoses.

Photobiont—The photobiont of *Winfrenatia* almost always occurs as a solitary cell within each of the net-lacunae formed by the mycobiont (Figs. 17, 18), however this generally depends upon where in the depression the photobiont cells are located (e.g., base, midlevel, or distal). In a few cases a net-lacuna may contain a pair of daughter cells (Fig. 19). Individual cells of the photobiont range from 10 to 16 μm in diameter with smaller cells found near the base of the depression. Typically unicells are larger toward the center of the depression and in more distal regions (Fig. 13). We interpret the difference in cell diameter as a function of both position in the depression and level of cyanobacterial cell development (Fig. 14). Cell diameter is also influenced by the degree of physiological interaction with the mycobiont since the cells near the base of the depression are interpreted as being parasitized and often appear moribund.

Each cell or cell cluster is surrounded by an extracellular sheath that morphologically corresponds to the gelatinous or mucoid envelope present in extant cyanobacteria (Fig. 21). The sheath ranges up to 6 μm in thickness and serves to position the photobiont in the center of the hyphal net lacuna (Figs. 17, 18). Some cells possess one to several extracellular sheaths that represent the successive encapsulated cell walls of daughter cells (Fig. 21). These are typically preserved as concave lenses that may be highly variable in thickness (Figs. 39, 40). They occur around cells that were presumably alive at the time of fossilization since in moribund cells the sheaths are often fragmented. In other regions aggregations of empty sheaths are all that remain of the photobiont (Fig. 41). They occur in the matrix above the depression and probably represent the remnants of cells from which endospores were released (Fig. 14). This pattern is somewhat similar to that found in some extant cyanolichens in which a distinct stratification of photobiont sheaths occurs in the peripheral regions of the thallus (Tschermak-Woess, 1988). The surface of the sheath in *Winfrenatia* often appears differentially thickened (Fig. 42).

Some photobiont cells possess small lenticular structures outside of the cell wall, in the region typically occupied by the gelatinous sheath (Figs. 37, 38). They vary from 0.5 to 3.0 μm long and are up to 1.4 μm wide. Except for the larger size they superficially appear similar to thickened regions in the wall of cyanobacterial cells immediately following cell division (Fig. 28). One possible explanation is that these lenticular structures represent accumulations of deteriorated cell wall or sheath material, mineral material that was trapped in the sheath when the cells were alive, or crystals of lichen secondary metabolites (Smith and Douglas, 1987). Such structures may also constitute gas or water bubbles that were trapped in the envelope at the time of preservation.

Within each depression of the lichen thallus is a sufficiently large population of photobiont cells to demonstrate all stages of division. Cell division appears to follow a consistent pattern within each of the depressions, and also appears to be uniform in relationship to the degree of interaction with the mycobiont. Cells are slightly lenticular immediately following cell division (Fig. 27); a few cells are folded and distorted as a result of the separation of wall components. Almost all cells contain dark inclusions (Fig. 24) that most probably represent organic debris that has become aggregated during cell senescence (Golubic and Barghoorn, 1977).

The first stage in cell division of the photobiont begins with a slight invagination on either side of the parent cell (Figs. 22, 23) that continues until two daughter cells are formed (Figs. 24, 25). Some daughter cells completely fill the parent cell wall (Fig. 26), while others appear to have undergone shrinkage prior to preservation. A second division plane takes place at right angles to the first and results in a plate of four cells (Fig. 29). Some daughter cells demonstrate nonsynchronous development in which only one of the pair divides (Fig. 31). Some of these cell clusters appear as tetrahedral tetrads (Fig. 30), however the typical arrangement of four daughter cells is isobilateral (Fig. 29). The spatial arrangement of the cells in a tetrahedron has been reported in other fossil coccoid cyanobacteria (e.g., Schopf and Blacic, 1971), but is now known to represent the mechanical rearrangement of the individual cells rather than tetrads formed via meiosis (Golubic and Barghoorn, 1977). Subsequent divisions at right angles to the first two planes produce clusters of eight (Figs. 29, 32) and 16 coccoid cells (Fig. 34). As in modern cyanobacteria, the polyhedral packing of the cells results in a solid cellular aggregation. Because of cell packing and the limited resolution within the chert we are unable to determine the maximum number of coccoid cells and groups of cells in each cluster (Fig. 35), although 16 appears common. There are a few coccoid cells in the matrix that are invested by multiple sheaths (Figs. 21, 33), providing indirect evidence that the total number of cells per aggregation may be higher, perhaps 32 or 64 (Fig. 36). It is impossible to count the number of cells in large aggregations because of the opaque nature of the material. While reducing the thickness of the preparation increases optical resolution, it has the adverse effect of eliminating a portion of the cell aggregation.

The division pattern in the photobiont of *Winfrenatia*

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contents. P 1384. ×3 000. **25**. Two daughter cells just prior to separation. P 1380. ×3 000. **26**. Two daughter cells still within a common cell wall. Note remnants of outer sheath (arrows). P 1238. ×3 000. **27**. Two daughter cells just after separation. Note flattened juxtaposed cell surfaces. P 1351. ×3 000. **28**. Daughter cell showing thickened corners (arrows) and bilayered wall. P 1377. ×3 000.

results in several types of cell aggregations, each characterized by a specific size range relative to the number of completed divisions. For example, the largest cells (15 μm) are present after the first division, while each cell at the tetrad stage is 11 μm in diameter. This decrease in cell size continues (eight-cell stage [7 μm], 16-cell stage [5 μm], to the production of endospores (32- and 64-cell stages) where each cell is 1.6–3.2 μm in diameter. We interpret this as a normal progression in the development of both vegetative cells and endospores. It is more difficult to determine what happens to individual cells once they become separated from the sheath. We assume that some enlarge and function as vegetative cells; others undoubtedly remain small and function as endospores. Endospores range from 1.6 to 3.2 μm in diameter, and are often aggregated into clusters in the matrix above the depression (Fig. 43). Some possess two opaque dumbbell-shaped inclusions 0.5 μm in diameter (Fig. 44).

DISCUSSION

Development of Winfrenatia—Each of the depressions represents a specific developmental stage in the growth of the lichen thallus and level of interaction between the bionts. What we interpret as developmental stages in the lichen thallus are illustrated in Fig. 46. In Stage 1 there is a distinct size gradation among the coccoid cells of the photobiont in which small cells are located near the base of the depression. Cells toward the center of the depression possess larger diameters with those more distal appearing in various stages of division. At this stage the mycobiont has only partially invaded a thallus depression and consists of elongate hyphae that are not well organized into nets.

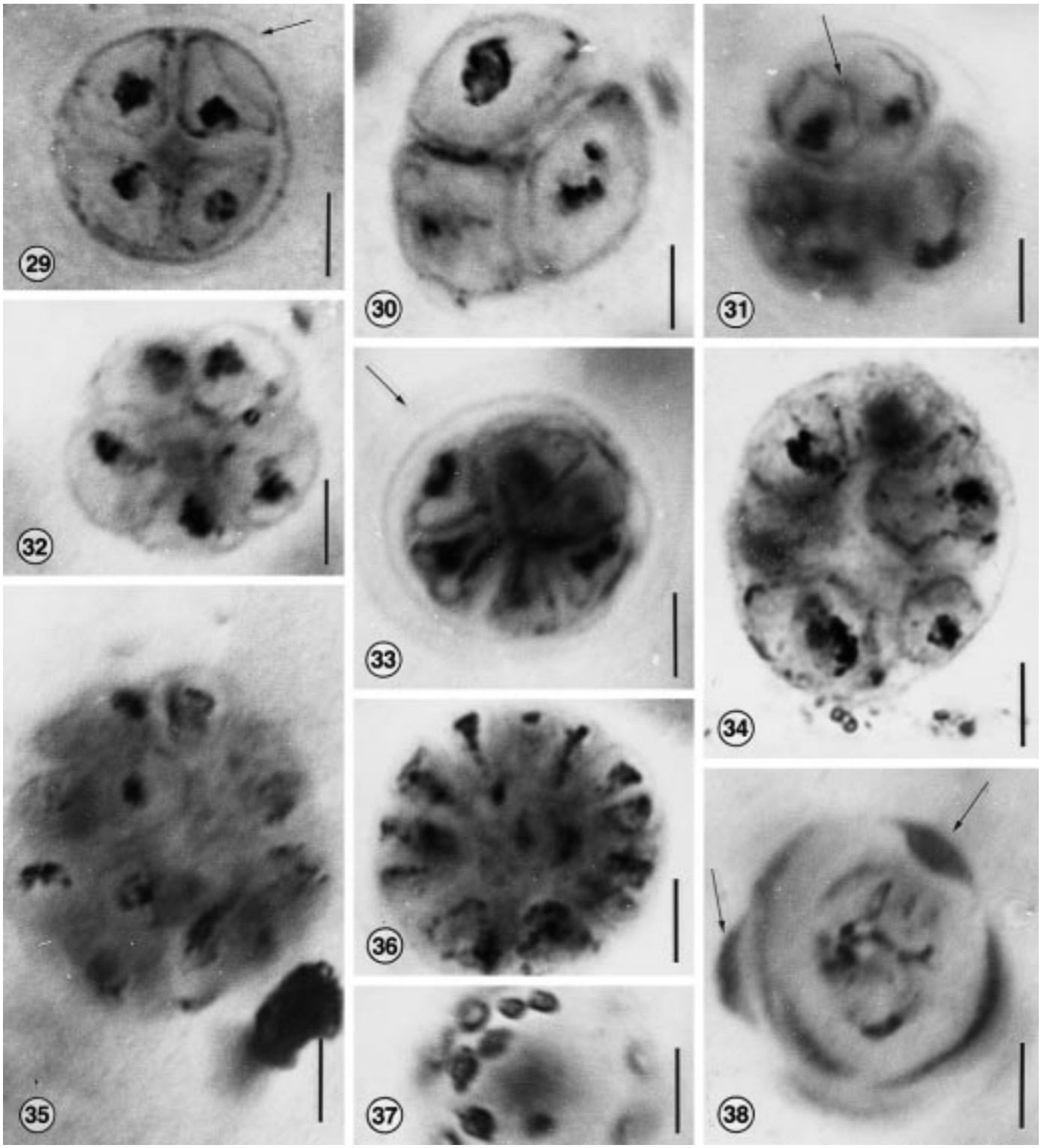
Stages 2 and 3 illustrate the increased interrelationship of the bionts in which each of the coccoid cells is now enclosed by the hyphal net. Plates of parallel hyphae are conspicuous (Stage 4), as are moribund photobiont cells at the base of the depression. We speculate that as more cells of the photobiont are parasitized, the depression becomes completely invested with hyphae (Stage 4a). Cells near the top of the depression show the largest number of divisions, and it is also in these regions that the cells are not completely invested by hyphae. Some of these cell aggregations and fragments of hyphae are commonly separated from the thallus at this stage (5), and are interpreted as soredia (Fig. 45). It is probable that soredia develop into new (Stages 6–7) thalli that ultimately form depressions containing photobiont cells surrounded by hyphae (Stage 7).

Increasing the number of depressions on the lichen thallus may have also been accomplished by the formation of endospores. Stage 8 depicts a depression in which some of the vegetative cells at the distal margin are free from the mycobiont. These coccoid cells that land on a region of the fungal thallus either in the form of endospores or vegetative cells (Stage 9) may have the capacity of influencing the development of ridges on the thallus surface (Stage 10). If this scenario is accurate it means that at one level the fungus is responsible for inducing the photobiont to form new cells that are later parasitized. Cyanobacterial cells that were not in direct contact with the hyphae continue to form new vegetative cells and endospores, and thus maintain the photobiont population. Cells near the distal region of the depression are typically larger and possess all stages of cell division. This is perhaps a reflection of available light, or the fact that these cells are not in contact with the mycobiont. Soredia serve as the mechanism for the lichen to exploit new habitats. The parasitic interactions of the mycobiont result in the necessity of forming additional depressions on the thallus in order to accommodate photobiont cells. These were formed by vegetative cells and endospores. Such a system provides a mechanism to increase cells of the photobiont, and may constitute an adaptive strategy that is critical to the maintenance of the fungus.

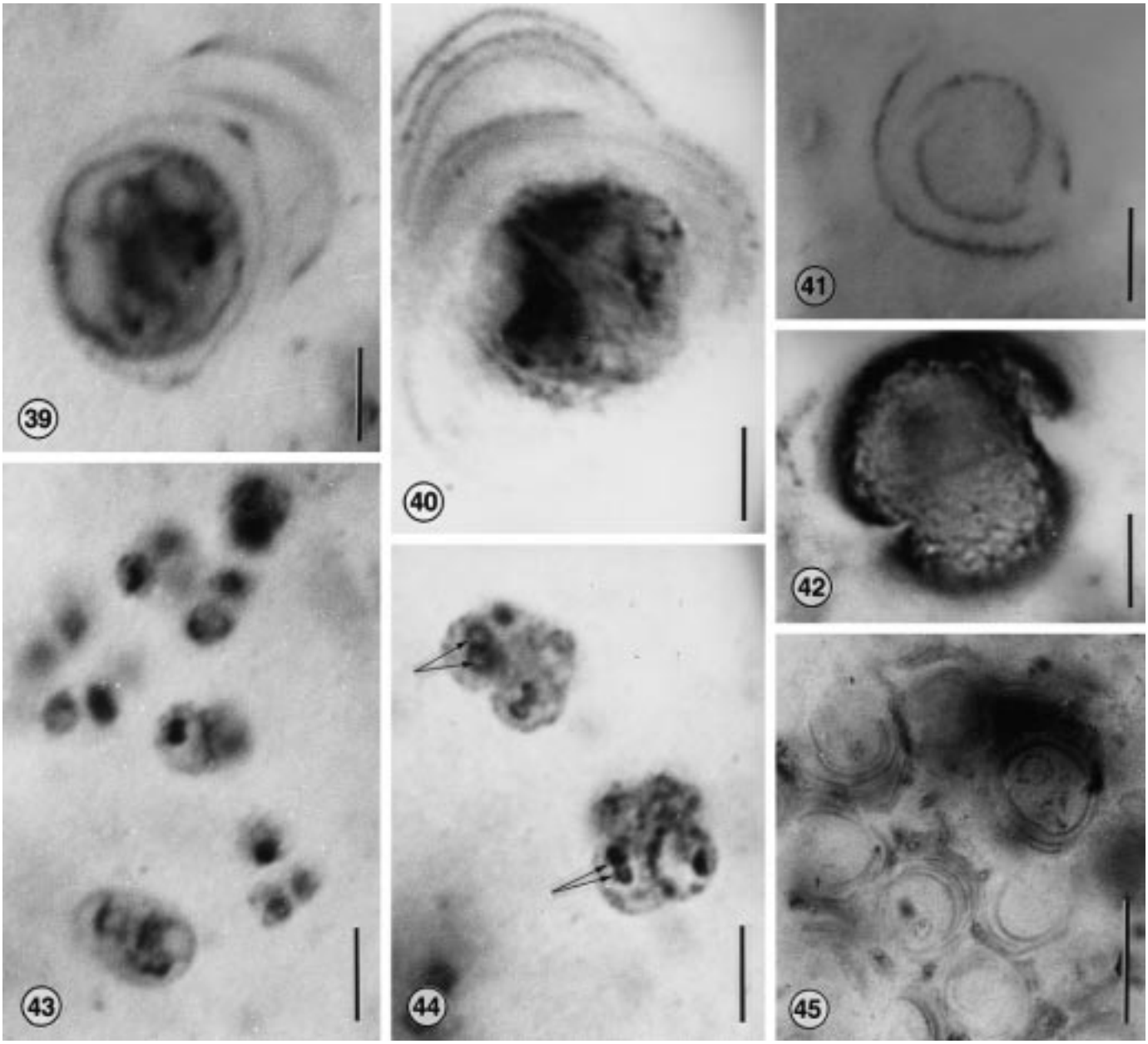
The relatively uniform size of each thallus depression and the formation of hyphal septations when depressions become large suggest that there may be some optimum number of photobiont cells for each depression relative to interaction with the mycobiont. This may be related to some physiological constraints such as hyphal length, movement of carbon to the mycobiont, and/or chemistry of the extracellular gelatinous material surrounding each cyanobacterial cell. Some thallus depressions that are nearly filled with hyphae generally contain only moribund cyanobacterial cells, suggesting that these regions constitute older, perhaps nonfunctional portions of the lichen thallus (Stage 4a).

Physiological interaction—The consistent association of hyphae around each of the coccoid cyanophyte cells provides compelling evidence of the physiological interaction between the two bionts in *Winfrenatia*. Additional evidence of this physiological interaction includes the uniformity in the size of the thallus depressions and the consistent spatial relationship of cell stages within each of the depressions. In modern cyanolichens the movement of materials from the photobiont to the mycobiont does not require direct cell to cell contact. Rather, appositions and the transfer of carbon may be associated with the gelatinous material around the cell (Honegger, 1992). We have observed a few cyanobacterial cells with possible apposition sites on the cell wall (Fig. 20).

Numerous authors have commented on the difficulty of determining the level of physiological interaction between the bionts of extant lichens, and such interrelationships can never be conclusively documented with fossils. Nevertheless, there are several examples of symbiosis in other Rhynie chert fossils that clearly demonstrate that complex physiological interactions had evolved by Early Devonian time. One of these is the presence of arbuscules in the specialized cortical cells of *Aglaophyton* that are morphologically identical to those of modern arbuscular endomycorrhizae (Remy et al., 1994; Taylor et al., 1995). Also present in the Rhynie chert are several examples of mycoparasites (Hass, Taylor, and Remy, 1994). In one type of mycoparasitism chlamydospores parasitized by chytrids formed papillae on the inner surface of the spore wall. Such structures constitute a host response in which new wall material is synthesized by the spore protoplast, presumably as a defense against the invading parasite (Lee and Koske, 1994). These examples underscore that a variety of complex physiological interactions existed more than 400 million years ago in the Rhynie chert ecosystem, and thus it should not be surprising that biolog-



Figs. 29–38. Photobiont cells of *Winfrenatia reticulata*. Bar = 5 μm. **29**. Eight-cell stage within a common wall in which the focal plane shows only four cells. Arrow indicates a thin zone that may represent the outer limit of the gelatinous sheath. PB 1308. ×3000. **30**. Tetrad of photobiont cells. P 1351. ×3000. **31**. Tetrad of photobiont cells in which one daughter cell has divided (arrow). P 1396. ×3000. **32**. Eight-cell stage as a result of the third division in which five cells are visible; three others at another focal plane. PB 1210. ×3000. **33**. Eight–16 cell stage in which six cells are visible. Note the common cell wall and outer sheath (arrow). PB 1396. ×3000. **34**. Eight–16 cell stage within a common wall. Note the size and shape difference when compared with same stage in Fig. 33. P 1213. ×3000. **35**. Probable 16-cell stage. P 1354. ×3000. **36**. Probable 16–32 cell stage in which the cells are positioned around the periphery and are wedge-shaped. P 1351. ×3000. **37**. Surface of photobiont cell showing lenticular thickened areas. P 1211. ×3000. **38**. Cell showing different configurations of sheath material (arrows) in section view. P 1406. ×3000.



Figs. 39–45. Photobiont of *Winfrenatia reticulata.* Bar = 5 μm unless otherwise noted. **39**. Cell surrounded by remnants of two sheaths. Note small lenticular thickening (arrow). P 1238. ×3000. **40**. Cell surrounded by five sheath layers. P 1239. ×3000. **41**. Empty sheaths. P 1382. ×3000. **42**. Irregularly thickened surface of sheath. P 1351. ×3000. **43**. Possible endospores in matrix above depression. P 1241. ×3000. **44**. Groups of probable endospores. Note spherical cell contents arranged in pairs (arrows). P 1241. ×3000. **45**. Possible soredium. P 1351. Bar = 25 μm. ×800.

ical interactions in the form of lichen symbioses were present at the same time as well.

Affinities of mycobiont—The affinities of the mycobiont in *Winfrenatia* remain unknown since no spore-producing structures have been identified within the lichen thallus. There are, however, numerous thick-walled spores associated with some of the depressions (Figs. 9–11), and these are unlike the chlamydospores commonly found in plant tissues and in the matrix of the chert. These unusual spores are nearly spherical, range up to 65 μm in diameter, and are ornamented by uniform depressions on the outer surface (Figs. 10–11). Although ascomycetes are believed to have evolved by Late Silurian time (Sherwood-Pike and Gray, 1985), the thick-walled spores associated with the lichen thallus bear no resemblance to the spores of any modern ascomycetes. The presence of these fossil spores associated with the lichen thallus, together with aseptate hyphae, suggests that perhaps the mycobiont has affinities with some zygomycetous group, although both characters can be found in other fungal groups as well. Perhaps these thick-walled sculptured spores associated with the thallus of *Winfrenatia* represent the zygospores of the mycobiont. Strengthening the zygomycete affinities of the *Winfrenatia* mycobiont is the fact that zygomycetes were a common el-

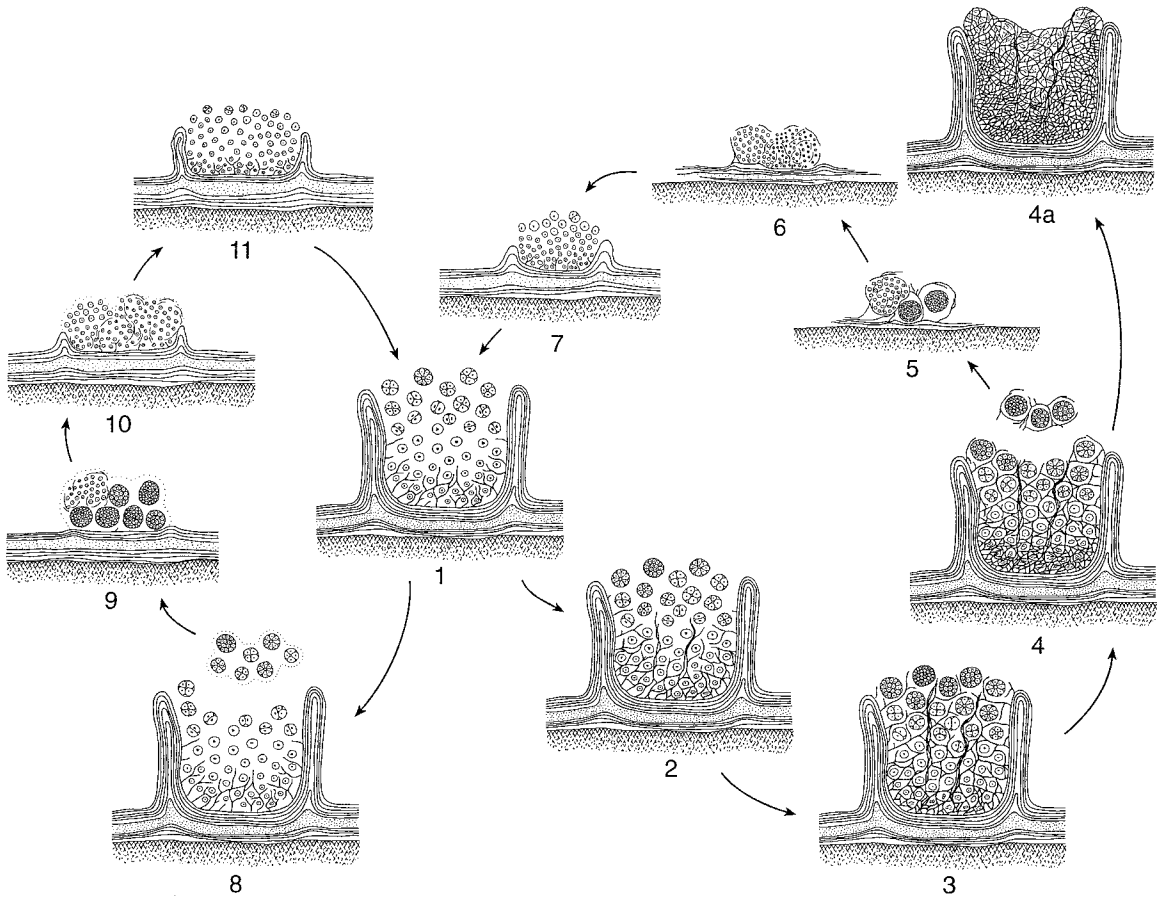


Fig. 46. Suggested stages in the life history of *Winfrenatia.* See text for details.

ement of the Rhynie chert ecosystem (Taylor et al., 1995). Since this group of fungi possessed the ability to enter into mutualistic symbioses in the form of endomycorrhizae by Early Devonian time, the potential for a physiological interaction with a cyanobacterium at this time is equally plausible. Although the mycobiont of modern lichens is typically an ascomycete, or rarely a basidiomycete, at least one zygomycetous fungus (*Geosiphon pyriforme*) is symbiotically associated with the cyanobacterium *Nostoc* (Kluge, Mollenhauer, and Mollenhauer, 1991; Mollenhauer, 1992). As more is learned about fossil lichens it will be interesting to see whether the mycobionts in other early symbioses also involved zygomycetes.

Affinities of photobiont—The taxonomic affinities of the photobiont of *Winfrenatia* are more easily resolved. Based on general size and shape of the cells and the thickness and organization of the sheath, the fossil photobiont is most similar to the extant coccoid cyanobacteria *Gloeocapsa, Chroococcus,* and *Chroococcidiopsis* (Büdel, 1992). In addition, all of the daughter cell division patterns that occur in these modern coccoid cyanobacteria are present within the population of fossil cells.

Cyanobacteria of the *Gloeocapsa* type have a long geologic history (e.g., Foster, Reed, and Wicander, 1989), with some forms extending well back into the Proterozoic, and possibly earlier in the Archean (Schopf, 1992a). The presence of well-preserved sheaths in many of these forms plotted against cell diameter has been an important character used to suggest affinities among coccoidal microorganisms (Schopf, 1992b). Preservation of many of these Precambrian gloeocapsid forms is excellent, with some containing examples of endospores (Zhang, 1988).

Are there older lichens in the fossil record?—Although well-preserved examples of cyanobacteria are at least 3.5 billion years old, it is not known whether any of the complex Precambrian microbial communities consisted of physiological symbioses of any type. Moreover, there is no record of fungi from any of these older deposits. There are, however, several interesting Precambrian fossils that may in fact represent some level of lichen symbiosis in slightly younger rocks. For example, the enigmatic genera *Chuaria* and *Tawuia* are now interpreted as colonies of cyanobacteria (Sun, 1987), and several Late Proterozoic organisms thought to represent multicellular thallophytes from South China superficially resemble the structure of *Winfrenatia* (Zhang and Yuan, 1992). In addition, there are several interesting fossil biostructures that are common in shallow-water carbonate deposits (see Riding, 1991 and papers therein for review). One of these is *Wetheredella,* a common Paleozoic form (Kazmierczak and Kempe, 1992). This taxon consists of various-sized tubes, some containing pores(?), which grow concentrically around what is termed a foreign

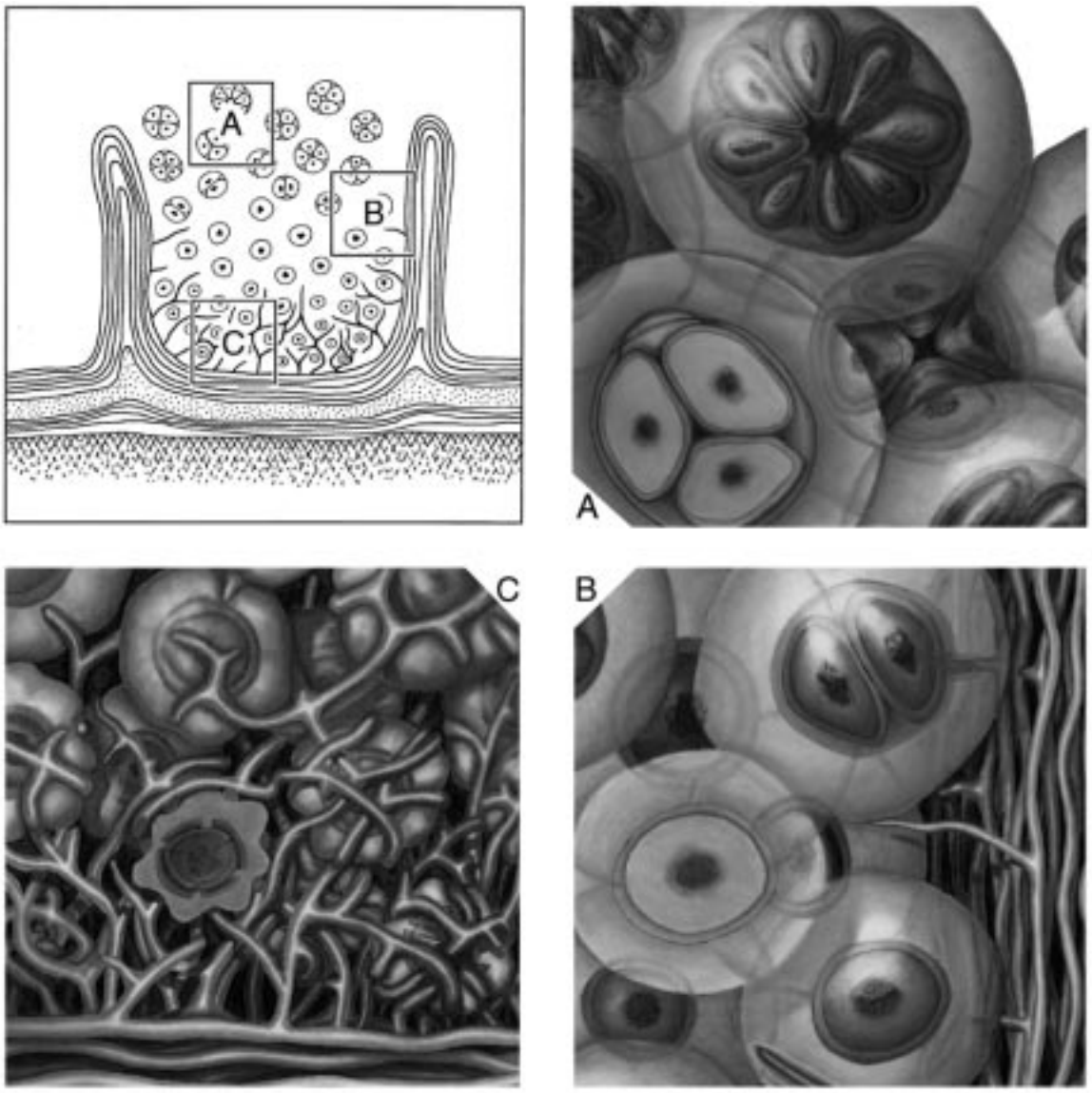


Fig. 47A–D. Diagrammatic reconstruction of *Winfrenatia*. (A) Longitudinal section showing relationships between photobiont and mycobiont within one depression. (B) Dividing photobiont cells with thickened extracellular sheaths. (C) Detail of photobiont cells close to depression wall with hyphae beginning to invade depression. (D) Photobiont cells near base of depression showing extensive net-like organization of mycobiont.

body. A similar-appearing structural organization thought to represent a modern analogue is found in the mildly alkaline crater lake on Satonda Island, Indonesia. It consists of mats of coccoid cyanobacteria of the *Pleurocapsa* type growing in crevices between thalli of red algae and foraminifera. This biological organization consists of vesicles and tubes of various size, in addition to the remnants of coccoid cyanobacteria sheaths. If the tubes in these fossil structures represent a fungus physiologically interacting with cyanobacteria, then perhaps the fossil *Wetheredella*-like biostructures (Cambrian–Cretaceous) represent some form of lichen symbiosis.

Evolution of the lichen symbiosis—In the absence of a reliable fossil record ideas about the evolution of the lichens have relied on the extant thallus morphology, chemistry, and the nature of the symbionts. Based on the coevolution of fungi and algae, and modern distributions based on the ancient position of continents, Hawksworth (1988) suggested that lichens may have evolved as early as Permian time. Of particular interest is his mention of Precambrian cyanobacteria as the potential photobionts of even earlier, but as yet undiscovered, lichen symbioses.

It is interesting that *Winfrenatia* demonstrates at least one type of early lichen symbiosis that may have involved controlled parasitism. This idea was initially used to explain modern lichens (Schwendener, 1869), and later supported by experimentally based resynthesis studies (Ahmadjian and Jacobs, 1981). Also, some extant endolithic cyanobacterial communities in Antarctica may offer a modern analogue for the initial evolution of the *Winfrenatia*-type lichen. In certain of these microbial communities unicellular green algae enter into intermittent lichen associations with fungal hyphae in porous rocks

(Friedmann, 1982). This cryptoendolithic lichen association appears to be loosely organized since the algae and fungi do not always appear to be physically associated. Coccoid cyanobacteria (e.g., *Chroococcidiopsis* and *Gloeocapsa*) are also present in the community, but apparently are not symbiotically associated with fungal hyphae. The fact that these Antarctic epilithic and endolithic communities contain few cyanolichens (Vincent, 1988) may be related to the necessity of the cyanobiont having liquid water for photosynthesis. Modern cyanolichens require liquid water for photosynthesis, while lichens with a green alga photobiont can obtain net photosynthetic rates using water vapor (Lange et al., 1989; Büdel and Lange, 1991). It is hypothesized that the cyanobacterial wall provides a high diffusion resistance to water vapor (Ott and Schieleit, 1994), or perhaps water uptake is somehow influenced by the cell sheath. If these physiological constraints are extended back to *Winfrenatia,* then the extensive mucoid sheaths surrounding the photobiont cells in the fossil might represent an adaptation to retain liquid water. However, thallus structure also directly influences the water relations in modern lichens (Schroeter, Jacobsen, and Kappen, 1991), and in this context it is difficult to evaluate thallus anatomy in *Winfrenatia* in relation to that of modern lichens. For example, there is no evidence of a protective cortical layer in the fossil that covers the upper surface, and nothing suggestive of aeration pores, structural features that might be expected within a highly desiccating environment. Perhaps the thallus anatomy of *Winfrenatia* is related to high heat stress that could be tolerated only because of the ability of the thallus to rapidly desiccate. This is consistent with the Rhynie chert paleoenvironment, which varied from semiarid to more pluvial based on sedimentological evidence (Rice et al., 1995).

While *Winfrenatia* lacks the structural organization of the modern lichen thallus, this symbiosis was certainly capable of exploiting new ecological niches that were unavailable to either biont alone. Since the lichen life style has evolved several times and has involved various combinations of algal, cyanobacterial and fungal symbionts, there are no doubt multiple advantages to each of the biont associations depending upon both the ecological and biological interactions of the organisms. In the case of *Winfrenatia,* like that of modern lichens, the increase in fitness to the fungus appears obvious. The selective advantages to the cyanobacterium, however, are less clear. These may be related to a more stable environment for the cyanobacterial cells in the thallus depressions, and/or somehow associated with available light. An equally important advantage to the photobiont in *Winfrenatia* may lie in the production of secondary substances in the form of lichen acids, which may have served to deter herbivores in the Rhynie chert ecosystem. The absence of these compounds in the cyanobacterial colonies, which were certainly rich in carbohydrates, would have made them more likely to have been a food source than in their symbiotic association with a fungus. Possibly the initial physical association between the two bionts is related to high ultraviolet levels that were deleterious to the cyanobacterial cells. If our speculation about the growth and development of *Winfrenatia* is accurate, it underscores the current belief that many modern symbioses constitute a variety of interactions that range from parasitic to mutualistic, and that these levels of interdependence are continually changing in relation to everchanging ecological and biological factors. For example, we hypothesize that perhaps the cyanobacterial colonies were ''protected'' by their symbiotic partnership that discouraged herbivory, but also suggest a scenario that involves the controlled parasitism of a limited number of photobiont cells.

Certainly one potential adaptative advantage in forming a symbiotic association with a cyanobacterium would be a reliable source of nitrogen. Although heterocyst-forming species of cyanobacteria are the most common nitrogen fixers, unicellular coccoid cyanobacteria, including *Gloeocapsa,* also fix nitrogen (Rippka et al., 1971). We do not know whether the cyanobacterial cells in *Winfrenatia* were capable of fixing nitrogen, however it would appear that a nitrogen-fixing photobiont, especially in a habitat of reduced nutrients like the Rhynie chert ecosystem, would offer a selective advantage in an early symbiosis. If this is accurate, one might ask why are there so few modern cyanolichens? The answer to this question far exceeds the scope of this paper, but in the case of *Winfrenatia,* the answer may be related to the fact that the mycobiont was possibly a zygomycetous fungus. In modern lichens excessive water reduces $C0_2$ diffusion to the photobiont (Palmqvist, 1995). In this context perhaps the aseptate nature of zygomycete hyphae contributed to excessive water uptake and thus decreased net photosynthesis. The ability to better control excessive water and therefore maintain a high level of $C0_2$ to the photobiont may be related to a different hyphal structure (e.g., septa and pores) found in the Ascomycetes. Moreover, the ability of Ascomycetes to form symbiotic partnerships with more than a single type of photobiont may have provided a selective advantage over other fungal groups in the Early Devonian ecosystem, which was rapidly increasing in biological diversity.

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