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Geosiphon pyriforme, an Endosymbiotic Association of Fungus and Cyanobacteria: the Spore Structure Resembles that of Arbuscular Mycorrhizal (AM) Fungi

A. Schüßler^{1,3}, D. Mollenhauer², E. Schnepf¹, and M. Kluge³

¹ Zellenlehre, Universität Heidelberg, FRG

² Forschungsinstitut Senckenberg, Frankfurt a. M., FRG

³ Institut für Botanik, Technische Hochschule Darmstadt, FRG

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Abstract

The zygomycete *Geosiphon pyriforme* is the only known endocyanosis of a fungus. The *Nostoc* spp. filaments are included in photosynthetically active and nitrogen fixing, multinucleated bladders, which grow on the soil surface. The spores of the fungus are white or slightly brownish. They are about 250 µm in diameter and develop singly on hyphal ends or, less frequently, intercalarily. The wall of the spores consists of a thin innermost layer, a laminated inner layer with a thickness of about 10–13 µm, and an evanescent outer layer. The laminated layer is composed of helicoidally arranged microfibrils, and is separated from the evanescent outer layer by a thin electron-dense sub-layer. Polarisation microscopy indicates the occurrence of chitin. Shape and wall ultrastructure of the *Geosiphon* spores and their cytoplasm resemble that of *Glomus* spores, but are different from that of other genera of the Glomales and Endogonales. Germination occurs by a single thick hyphal outgrowth directly through the spore wall. Like various AM forming fungi, *Geosiphon pyriforme* contains endocytic bacteria-like organisms, which are not surrounded by a host membrane. Our observations indicate that *Geosiphon* is a potential AM fungus.

Key words

Geosiphon pyriforme, endocyanosis, taxonomy, AM fungi, *Glomus*.

Abbreviations and Symbols

AM: Arbuscular mycorrhiza
BLO: bacterium like organism
SG: structured globule

Introduction

Geosiphon pyriforme is the only known symbiosis of a fungus with endosymbiotic cyanobacteria. This consortium was first recognised by von Wettstein (1915), who described it as a siphonal alga, but also suggested the presence of chitin. Knapp (1933) recognised *Geosiphon* as a fungus with endosymbiotic cyanobacteria and described it as an intracellular phycomycetal lichen. Today it is clear that the fungal partner is a zygomycete, and the cyanobacteria usually *Nostoc punctiforme*, but other *Nostoc* species are also able to take part in this symbiosis (Kluge et al., 1993).

The fungus lives together with *Nostoc* on the surface and in the uppermost layer of damp, loamy, and nutrient-deficient soils, together with some typical bryophytes in a synusia classified under the community of subatlantic dwarf plants of the Centunculo-Anthocerotetum W. Koch 1926 (Mollenhauer, 1988, 1992). The mycelium consists of syncytial hyphae (diameter 2–8 µm), with septa in senescing or dead hyphae, and may give rise to white to slightly brownish spores with a diameter of about 250 µm. The symbiotic consortium develops by the endocytosis of *Nostoc* filaments through a hyphal tip and the formation of a multinucleated, siphonal bladder with a length of up to more than 1 mm (Knapp, 1933; Mollenhauer, 1988, 1992). During the growth of the bladder, the symbiotic *Nostoc* cells multiply and increase 10-fold in volume, compared with the free-living ones. Heterocysts are formed within the bladders in a similar relation to the vegetative cells in free-living *Nostoc*. The apical two thirds of the mature bladders contain the *Nostoc* filaments, which are located in a single, sack-formed, peripheral compartment, and many centrally located vacuoles. Fungal cytoplasm in the basal portion contains many lipid droplets, but no cyanobacteria (Kluge et al., 1993).

The biology of *Geosiphon pyriforme* has been reviewed by Mollenhauer (1988, 1992) and Kluge et al. (1993), the ultrastructure was investigated by Schnepf (1964). The latter study contributed substantially to the formulation of Schnepf's theorem of compartmentation of the eucaryotic cell and provided strong arguments in favour of the endosymbiosis theory of cell evolution. Since it is possible to culture *Geosiphon pyriforme* in the laboratory (Mollenhauer and Mollenhauer, 1988), it could be

shown that the bladders are photosynthetically active (Kluge et al., 1991) and fix N_2 (Kluge et al., 1992).

The fungal partner of the *Geosiphon* symbiosis forms big, globose spores which are filled with reserve substances (Knapp, 1933). We investigate the shape and ultrastructure of these spores in order to explore the systematic position of the fungus, which is currently uncertain. It turned out that they resemble those of the AM forming genus *Glomus* (Glomales, Zygomycetes). The validity of the spore shape as a character for taxonomic classification was recently confirmed by small subunit (SSU) rRNA sequence analysis (Simon et al., 1993).

Materials and Methods

Cultures: *Geosiphon pyriforme* was cultured on soil of its natural habitat as described by Mollenhauer and Mollenhauer (1988). The spores were harvested from 5–6 months old cultures out of the upper soil layer and germinated in Petri dishes. The germination medium was composed of 0.5 mM $Ca(NO_3)_2$, 0.5 mM $CaSO_4$, 0.5 mM $MgSO_4$, 1 mM NaCl, 1 mM KCl, 10 μ M KH_2PO_4 , 40 μ M H_3BO_3 , 2 μ M $ZnSO_4$, 10 μ M $MnSO_4$, 50 μ M FeNa-EDTA, and 0.1 μ M Na_2MoO_4 . Germination was promoted by adding an axenic *Funaria hygrometrica* protonema of about 1 cm in diameter onto the liquid surface (see also Mollenhauer, 1988, 1992). The presence of the moss strongly increased germination of the spores.

Light microscopy: The spores were studied with a ZEISS IM 35 microscope and Normarski optics, either in water or in the water soluble ZEISS W15 embedding medium with a refractive index of $n = 1.515$. For fluorescence microscopy we used the following filter combinations: UV-light: 365 nm excitation, long-pass 420 nm emission; blue light: 450–490 nm excitation, long-pass 520 nm emission.

Electron microscopy: Conventionally fixed and embedded spores were badly preserved. It was not possible to cut ultrathin sections, because the cytoplasm broke out of the spore wall during trimming or cutting. Better results were achieved by fixation with 2.5 % glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) and vacuum infiltration (4 times for 5 minutes), followed by 20 h at 4 °C in the same fixant or with the aid of a microwave oven. Hereby the sample was put into an Eppendorf vial (1.5 ml) filled with 2.5 % glutaraldehyde in 0.05 M phosphate buffer and placed in a cool water bath. It was then transferred into a microwave oven for 3 min at 120 watts. This procedure was repeated 5 times. Changing the water bath kept the fixation temperature below 30 °C. The spores were then left for another 3 h in the same glutaraldehyde solution. After rinsing they were postfixed for 2 h with 2 % OsO_4 in the same buffer, dehydrated in an acetone series (10, 25, 50, 75, 90, 100, 100 %, each step 30 minutes), and infiltrated with a series of Spurr's resin (Spurr, 1969) (30, 50, 75, 100 %), with 12 h steps at 50 % and 75 % for a better resin penetration (other steps 1 h). They were then twice incubated in 100 % Spurr's resin for 12 h. The samples were cut with a diamond knife on a Reichert-Jung Ultracut. The sections were post-stained with uranyl acetate and lead citrate and examined with a Philips CM 10 or EM 400 at 100 kV.

To better visualise the wall microfibrils, some spores were extracted in a saturated aqueous KOH solution for 3 h at room temperature after glutaraldehyde fixation (Bonfante-Fasolo and Vian, 1984). Then they were post-fixed with OsO_4 and treated as described before.

Results

Light microscopy

Geosiphon pyriforme forms single, white or slightly brownish spores with a diameter of about 250 μ m (Fig. 2); 80 % of the spores have diameters between 220 and 280 μ m (Fig. 1). They are formed singly at hyphal ends or, less frequently, intercalarly (Figs. 2 and 14). During germinating a single thick hyphal outgrowth emerges directly from the spore wall. It branches immediately into thinner hyphae (Fig. 3).

The spores contain many lipid droplets of different size and "structured globules" (SGs, diameter 3–6 μ m). The latter consist of a highly refracting, homogeneous outer and a granular interior part (Fig. 6). The SGs show a strong blue fluorescence when excited with UV light (Fig. 8), and a slight green fluorescence when excited with blue light. They swell within a few minutes after they have been released in water by squeezing the spore. The inner granular appearance is then lost and many SGs eventually fuse (Fig. 7). In W15 embedding medium no granular inner structures are seen.

The spore wall consists of three layers (terminology after Bonfante-Fasolo and Vian, 1984): an innermost one, followed by the thick, laminated inner layer, and an evanescent outer one, which is sometimes lost (Figs. 4 and 9). The innermost layer becomes visible when the spore is squeezed (Fig. 4). It then separates from the inner layer and is flexible. The inner layer is generally about 10–13 μ m thick and rigid. Often it reveals a laminate appearance which is usually more distinct in the outer part, sometimes giving the impression that this inner layer consists of two different portions. The outermost part of the inner layer consists of a highly refracting sublayer. It is especially visible when the outer evanescent layer is absent (Fig. 10). It resists treatment with concentrated H_2SO_4 and is whitish autofluorescent at 365 nm excitation (green-yellow at 450–490 nm excitation). The innermost layer is also relatively resistant to H_2SO_4 . The outer, evanescent layer is irregular in appearance and rather dark (Fig. 9). It extends for some micrometers along the subtending hy-

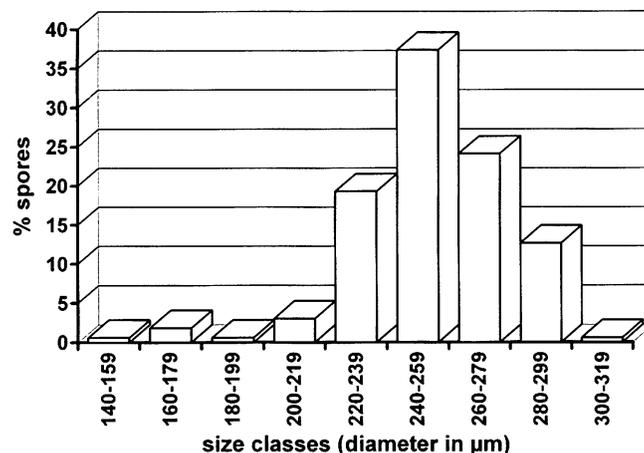
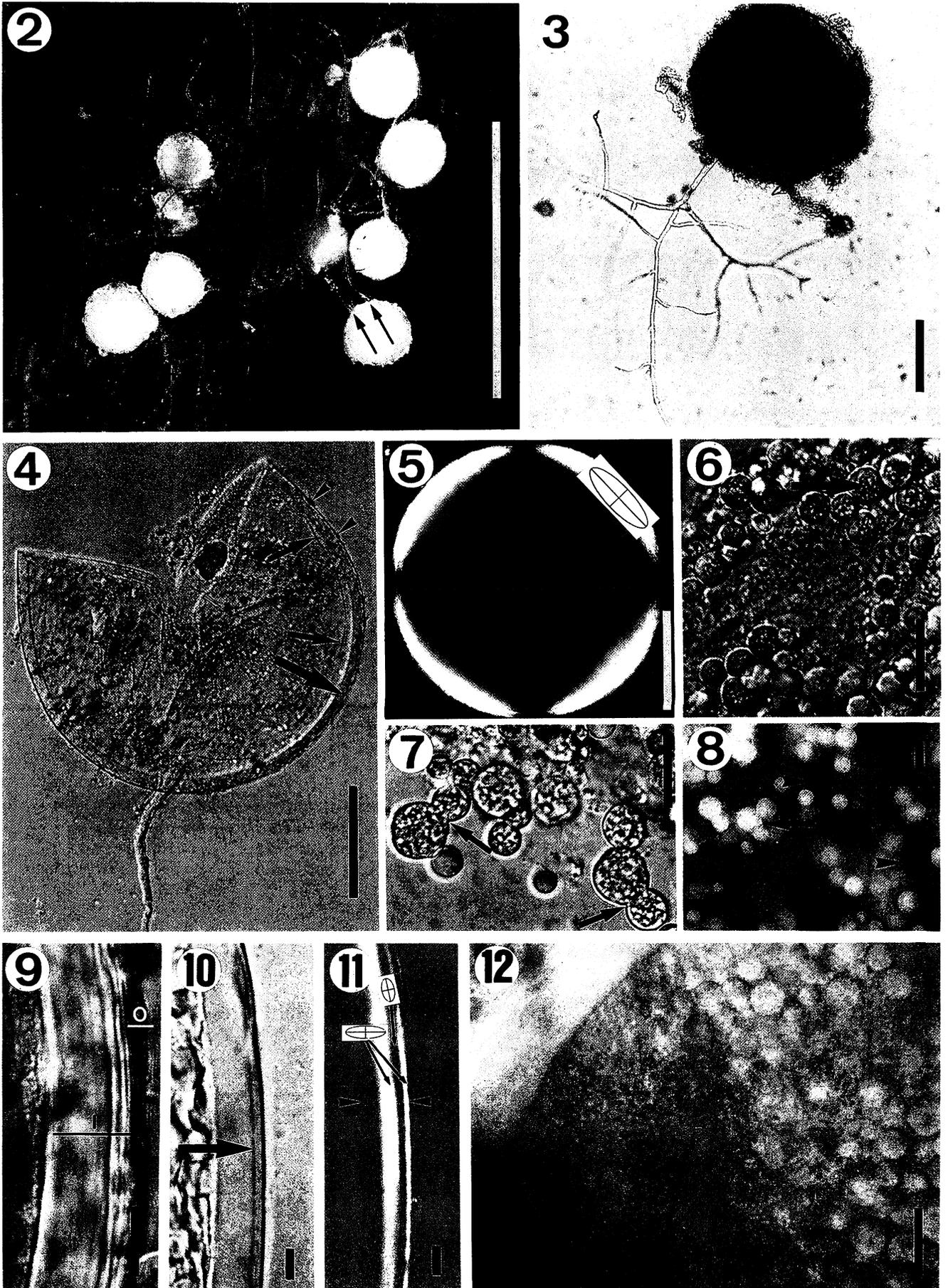


Fig. 1 Size distribution of *Geosiphon pyriforme* spores (measurement of 166 spores, $M = 253.5$, $SD = 23.9$).



Figs. 2–12 *Geosiphon* spores, light microscopy.

pha. This proximal part of the hypha is closed by thin septum-like structures and a long plug comprising the "septa" (Figs. 13 and 14). This plug also continues through the laminated wall of the spore. Inside the hypha it often ends at a thick septum (Fig. 13).

The spore wall is strongly birefringent under the polarising microscope (Fig. 5). In water the relative optical character of all wall layers is positive. In contrast, after incubation with the W 15 medium the relative optical character of the inner layer becomes negative, with the exception of the dark outer sublayer. The birefringence of the inner layer continuously decreases towards the innermost layer (Figs. 10 and 11).

Before germination of the spore, a "cytoplasmic pole", free of SGs and big lipid droplets, develops (Fig. 12). In this region a single thick hyphal outgrowth emerges directly through the spore wall. Germination is distinctly promoted by the presence of *Funaria* protonemata (germination rate after 3 weeks without *Funaria* 6%, with *Funaria* 68%).

Outgrowing hyphae allowed confirmation of the identity of the spores. By addition of *Nostoc punctiforme* new *Geosiphon pyriforme* bladders were reconstituted (Fig. 15).

Electron microscopy

The preservation of the cytoplasm within the spore is insufficient in general and varies, depending on the preparation method. Nevertheless, important details can be observed. The main components of spore contents are big and small lipid droplets (diameter up to 15 μm) and the SGs (Figs. 16 and 17). They measure, in general, 4–6 μm in diameter, are osmiophilic, and included within a vesicle. The space between the SG and the vesicle membrane is filled with a coarse, flocculent material (Figs. 17 and 18). At high magnification the dense matrix of the SGs is shown to contain relatively electron-translucent slightly bent rods in paracrystalline arrays. The rods have a diameter of about 6 nm and are packed hexagonally with a center-to-center spacing of about 8 nm (Figs. 18–20).

Fig. 2 Toplight photograph of *Geosiphon* spores, one spore with two subtending hyphae, showing its intercalary formation (arrows). Scale bar = 1 mm.

Fig. 3 Germinated spore with branched germination hypha. Scale bar = 100 μm .

Fig. 4 Mature spore, crushed out: flexible innermost wall layer separated from the inner wall layer (small arrows), inner wall layer (big arrow), and rest of the evanescent wall layer (arrowheads). Scale bar = 100 μm .

Fig. 5 Polarisation microscopic photograph of a spore in water, the relative optical character is shown by an index ellipse. Scale bar = 100 μm .

Fig. 6 Structured globules (SGs), immediately after crushing out of the spore. Note the homogeneous outer part (arrowheads) and the granular interior (arrow). Scale bar = 10 μm .

Fig. 7 Liberated SGs after a few minutes in water, they begin to swell, fuse (arrows), and lose their granular appearance. Scale bar = 10 μm .

Fig. 8 Epifluorescence with UV excitation: the liberated SGs (arrows) are strongly autofluorescent (light blue), the lipid droplets appear as black globules (arrowheads). Scale bar = 10 μm .

Fig. 9 Part of the spore wall, the outer evanescent wall layer (O) and the inner laminated wall layer (I) are seen, innermost layer not separated from the inner wall. Scale bar = 10 μm .

Fig. 10 Part of a spore wall without evanescent wall in W 15 embedding medium: the highly refracting outer sublayer of the inner wall layer is seen (arrow). Scale bar = 10 μm .

Fig. 11 Same part as Fig. 10, polarised light with gypsum Red I plate: the region above the dark sublayer retained a weak positive relative optical character, the other parts have a strong negative relative optical character. The birefringence is much stronger in the outer part of the inner wall than in the inner part. The thickness of the spore wall is marked by arrows. Scale bar = 10 μm .

Fig. 12 Part of the cytoplasmic pole of a spore just before germination (left side) and storage granules (SGs and lipid droplets) at the right side. Scale bar = 10 μm .

Fig. 13 Subtending hypha: septum-like structures (small arrowheads), thick closing septum (big arrowhead), plug inside the hypha (small arrow) and connection with the spore wall (big arrow). Scale bar = 50 μm .

Fig. 14 A spore with two subtending hyphae (big arrows), showing its intercalary formation. Inside one hypha the plug is visible (small arrow). Scale bar = 50 μm .

Fig. 15 A *Geosiphon* spore (asterisk) with outgrown, highly branched hyphae and many young *Geosiphon* bladders, containing endosymbiotic *Nostoc* filaments. Scale bar = 500 μm .

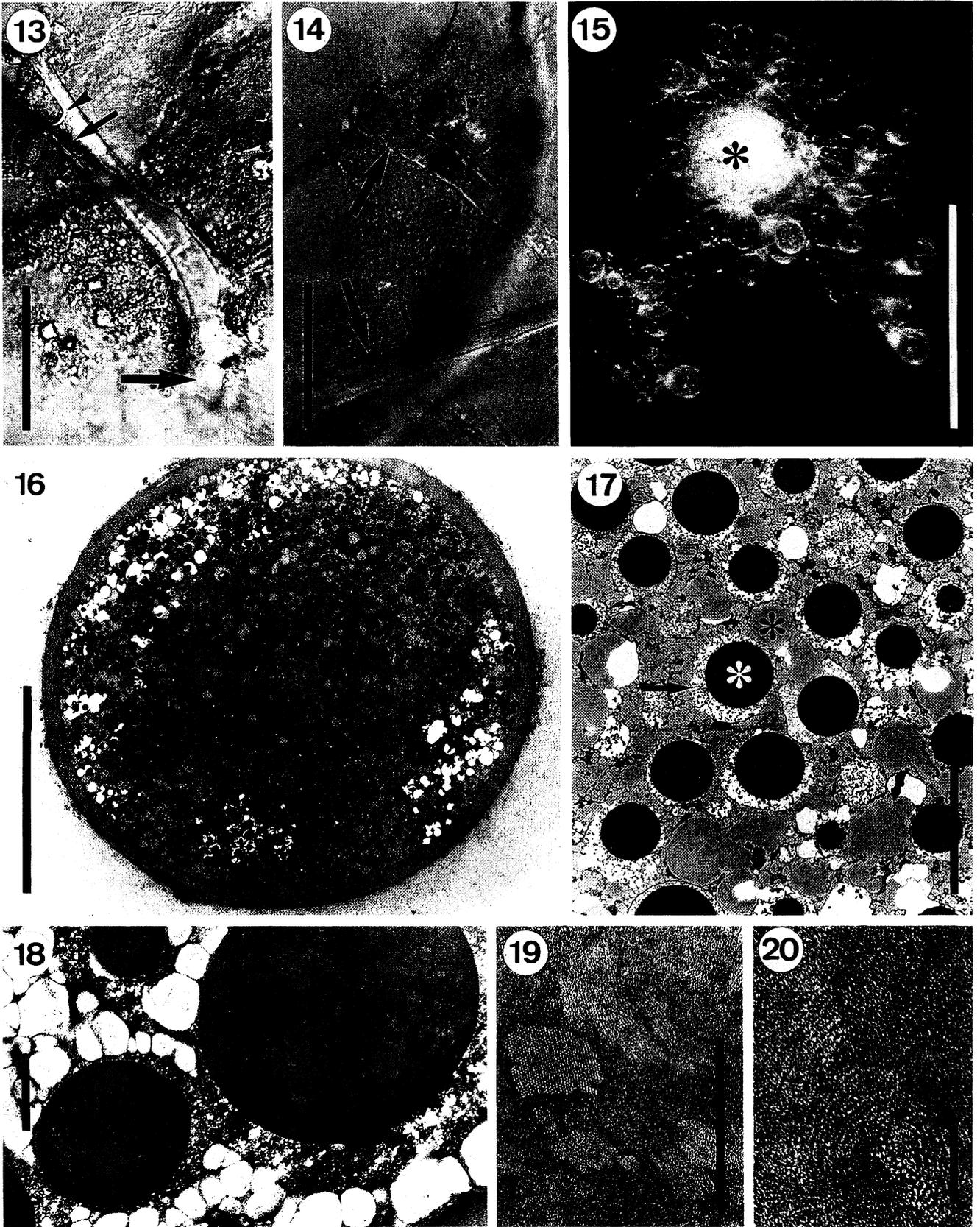
Fig. 16 Spore in median section with many SGs and lipid droplets; white areas: some storage granules artificially broken. Scale bar = 100 μm .

Fig. 17 Higher magnification of the spore content with SGs (white asterisk), SG-vesicle membrane (arrow), and lipid droplets (black asterisk). Scale bar = 10 μm .

Fig. 18 SGs with paracrystalline regions. Scale bar = 1 μm .

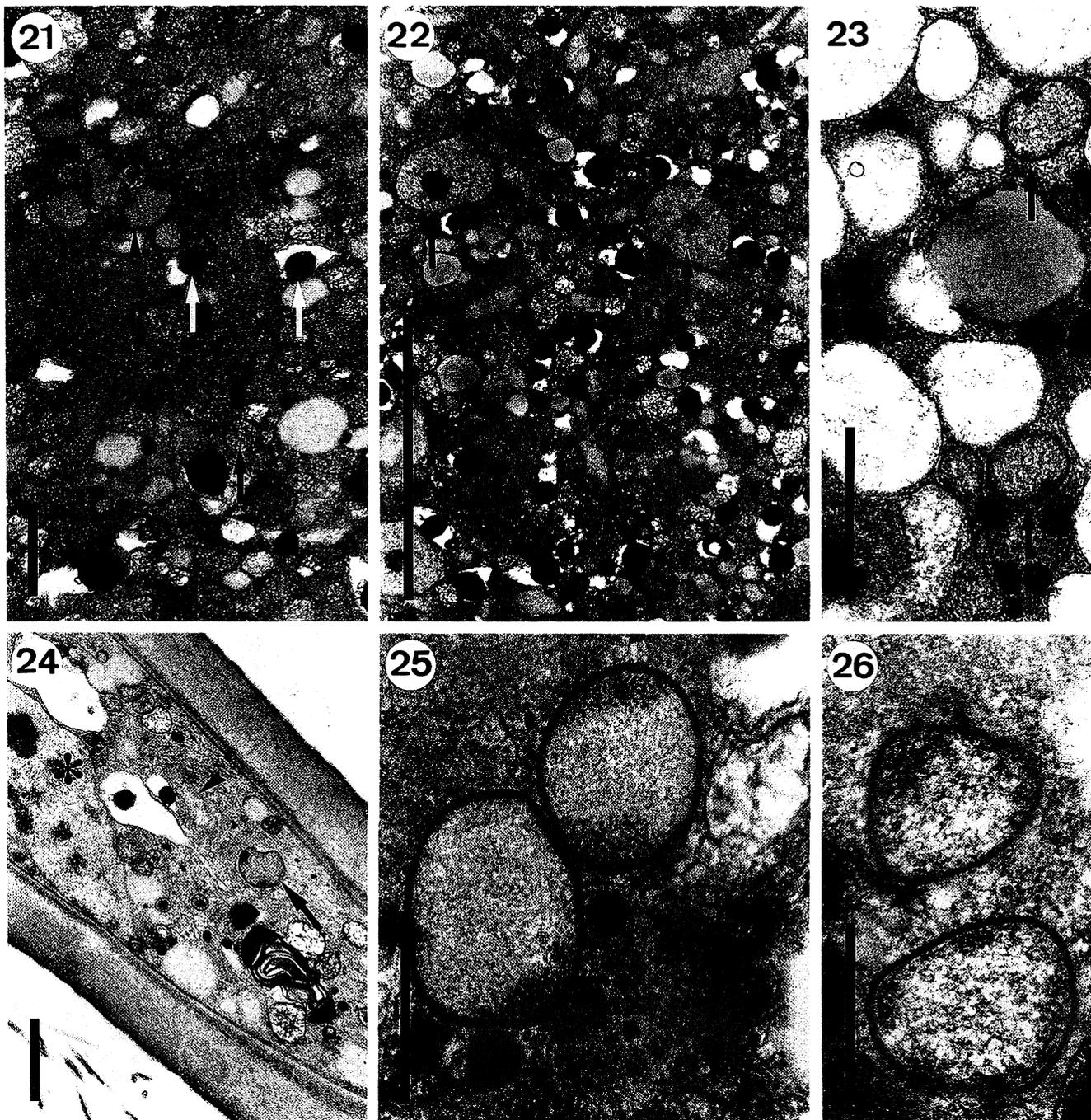
Fig. 19 Paracrystalline regions composed of slightly bent rods in hexagonal arrangement. Scale bar = 0.5 μm .

Fig. 20 Paracrystalline-arranged rods appear hollow (arrowhead). Scale bar = 0.1 μm .



Figs. 13–15 *Geosiphon* spores, light microscopy.

Figs. 16–20 *Geosiphon* spores, electron microscopy.



Figs. 21–26 *Geosiphon*, electron microscopy.

Fig. 21 Part of the cytoplasmic pole of a germinated spore: BLOs (arrowheads), vesicles with flocculent contents (black arrows), and small vacuoles with a highly electron-dense precipitate (white arrows). Scale bar = 1 μm .

Fig. 22 Same spore as in Fig. 21, part of the cytoplasmic pole, with nuclei (arrow) and mitochondria (arrowheads). Scale bar = 5 μm .

Fig. 23 BLOs (arrows) within the base of a *Nostoc*-containing *Geosiphon* bladder, the upper one with a median constriction. Scale bar = 1 μm .

Fig. 24 Part of the primary germination hypha of a spore, containing a myelin figure, a BLO (arrow), a nucleus (asterisk), and a mitochondrion (arrowhead), thick cell wall. Scale bar = 1 μm .

Fig. 25 BLOs in the cytoplasmic pole of a spore. Scale bar = 0.5 μm .

Fig. 26 BLOs in the base of a *Nostoc*-containing bladder. Scale bar = 0.5 μm .

The cytoplasmic pole of germinating spores contains, besides nuclei and mitochondria, mainly small lipid droplets, small vacuoles with an electron dense precipitate which does not completely fill the vacuole, and vesicles with coarse, flocculent contents (Figs. 21 and 22). In addition, in the spores (Figs. 21, 23 and 25) as well as in the outgrown hyphae (Fig. 24) and in the bases of the *Nostoc*-containing bladders (Figs. 23 and 26), bacteria-like organisms (BLOs) are found. They measure about 0.5 μm in diameter and are ovoid, frequently with a median constriction (Fig. 23). A surrounding host membrane is lacking. The BLOs have a relatively thick cell wall, covering the plasma membrane. An "outer membrane" is not present. The large ribosome-free central area contains fine fibrils, presumably representing the DNA (Fig. 26).

The inner, laminated wall layer is the most prominent part of the spore wall, as also seen with the light microscope. Its laminated structure is especially distinct in its outer part (Figs. 27 and 28). Suitable sections at high magnification give the impression of a regular, arc-like substructure (Fig. 33), which is due to the helicoidal pattern in the sense of Bonfante-Fasolo et al. (1986) and Vian et al. (1993). This pattern becomes clearer when the cell walls are pre-treated with KOH (Figs. 29 and 32). At the outside of the inner wall layer, there is an approximately 0.2 μm thick, electron dense sublayer (Figs. 27 and 28). The evanescent outer wall layer is relatively electron dense, irregular, and seems to be packed more loosely than the inner wall (Fig. 27).

The innermost wall layer is about 0.4 μm thick and delimited against the inner wall layer by a thin electron-dense zone (Figs. 28–31). This wall layer has a laminated substructure which is, however, not visible everywhere (Fig. 30). This substructure is visualised as thin, electron dense lines (Fig. 31).

Discussion

The results of our studies indicate various similarities between the spores of *Geosiphon pyriforme* and those of AM fungi, especially some *Glomus* species. Polarisation microscopy reveals a strong birefringence, as in *Glomus* spores (Bonfante and Bianciotto, 1994), and a relative optical character of the inner laminated wall layer which is positive in water but negative in the highly refractive W 15 embedding medium. That indicates a chitin-like molecule character. Chitin is shown to be the main component of spore walls of some *Glomus* species (Bonfante-Fasolo et al., 1986; Weijman and Meuzelaar, 1979). Von Wettstein (1915) reported evidence for chitin on the photosynthetically active *Geosiphon* bladders. The different positive optical character of the outermost region of the laminated wall layer cannot be explained. It is probable that this layer is the highly refractive portion and comprises the electron-dense outer sublayer. This region also contains the H_2SO_4 -resistant part of the wall and the auto-fluorescing portion. These observations fit well to those of Bonfante-Fasolo and Grippiolo (1984), who described the occurrence of a sporopollenin and melanin-containing layer in *Glomus epigaeum* at the same location (see also Grippiolo and Bonfante-Fasolo, 1984).

Likewise the arrangement of the putative chitinous microfibrils in the *Geosiphon* spore wall resembles that of *Glomus*. The laminated inner wall layer of the *Geosiphon* spores has the same "arched" appearance as in *Glomus fasciculatum* (Bonfante-Fasolo, 1982; Bonfante-Fasolo and Schubert, 1987), *Glomus versiforme* (Bonfante-Fasolo and Grippiolo, 1984; Bonfante-Fasolo and Vian, 1984; Bonfante-Fasolo et al., 1986), *Glomus macrocarpum* and *Glomus caledonium* (Bonfante-Fasolo and Schubert, 1987). This pattern is due to a helicoidal arrangement (in the sense of Livolant et al., 1978 and Vian et al., 1993), frequently occurring in cell walls which show strong extension during growth. The microfibrils are deposited parallel in sheets and change their orientation continuously from sheet to sheet (see the model published by Bonfante-Fasolo and Grippiolo, 1984; Bonfante-Fasolo et al., 1986). Such a helicoidal organisation was never described for fungal spores except for those of the group of AM fungi (Bonfante and Bianciotto, 1994), and it is not ubiquitous in the genus *Glomus* (Bonfante-Fasolo and Schubert, 1987; Meier and Charvat, 1992). The spore wall of *Geosiphon* resembles most that of *Glomus versiforme*, which also shows the evanescent outer wall layer, the laminated helicoidal organised inner wall layer, and the innermost wall layer with its lamination, caused by the thin electron dense lines. Although *Glomus versiforme* forms sporocarps, these features indicate a relatively close relationship between these two organisms.

The helicoidal organisation of microfibrils also exists in spores of *Gigaspora* spp. (Mosse, 1986; Sward, 1981a) and *Acaulospora laevis* (Mosse, 1970c; Mosse, 1986), but their spore wall structure clearly differs in the succession and organisation of wall layers from that of the genus *Glomus* (Mosse, 1970b, c; Sward, 1981a, b, c). These differences are seen also light microscopically and serve to determine the different genera and species of the

Fig. 27 Spore wall: outer evanescent wall layer (O), inner laminated wall layer (I), dark sublayer (D), and innermost wall layer (IM). Scale bar = 5 μm .

Fig. 28 Spore wall with remainder of the outer evanescent wall layer (O). The region containing the dark sublayer (D) and parts of the inner laminated wall layer are encrusted with electron dense material. Also the innermost wall layer (IM) is seen. Scale bar = 5 μm .

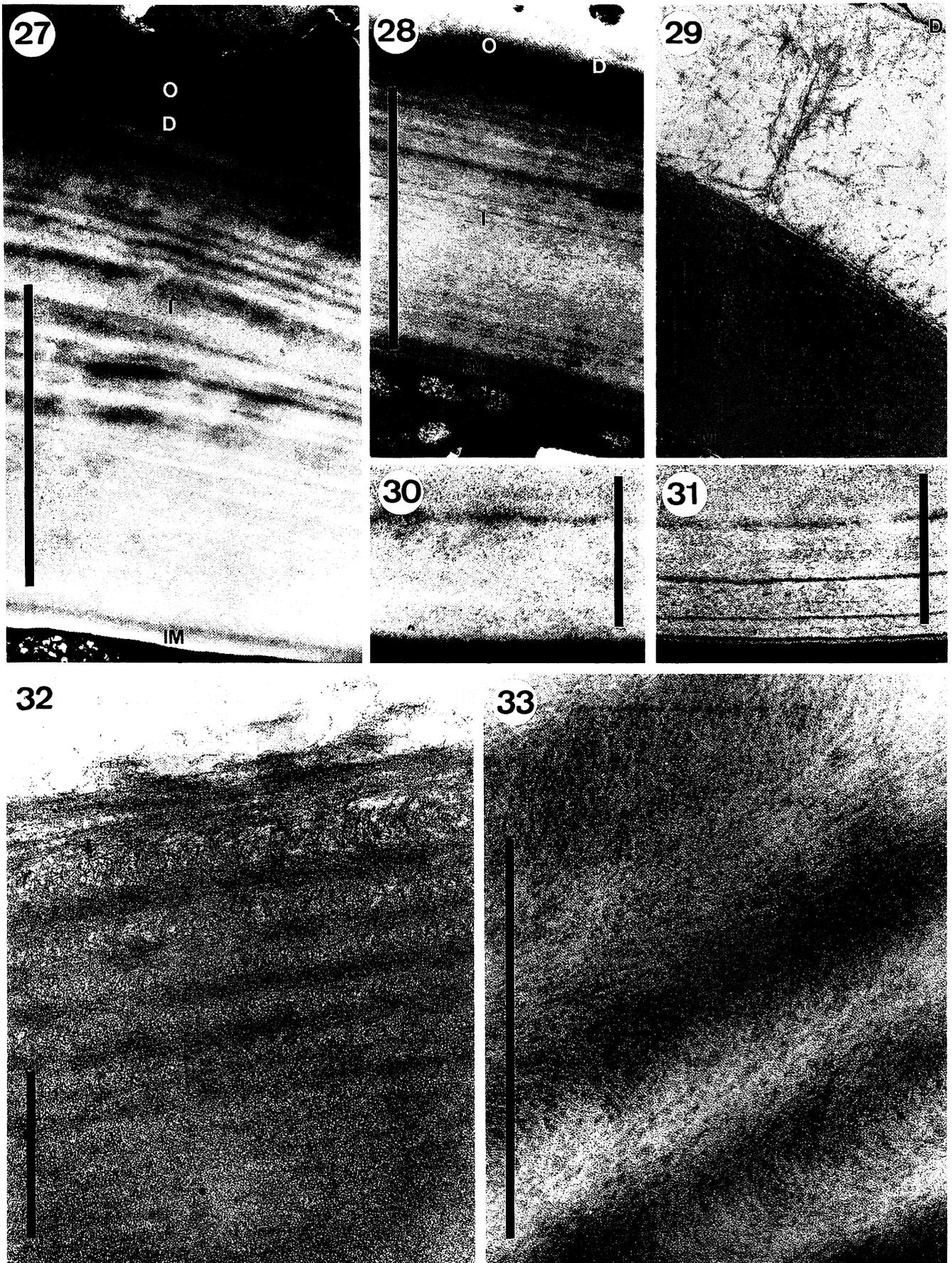
Fig. 29 KOH-extracted spore wall. The region with the electron-dense incrustations, seen in Fig. 28, is strongly swollen, the laminations of the inner wall layer (I) and the innermost wall layer (IM) are more distinct. Scale bar = 5 μm .

Fig. 30 Homogeneous innermost wall layer, same spore as in Fig. 31. Scale bar = 0.5 μm .

Fig. 31 Laminated innermost wall layer of the same spore as in Fig. 30. Scale bar = 0.5 μm .

Fig. 32 Outer part of the laminated wall layer after KOH extraction, the arc-like appearance of the microfibrils is clearly seen. Scale bar = 1 μm .

Fig. 33 Part of the laminated wall without pretreatment with KOH, arc-like substructure. Scale bar = 1 μm .



Figs. 27–33 *Geosiphon* spore walls, electron microscopy.

Glomales and Endogonales (Morton, 1988, 1990; Morton and Benny, 1990; Walker, 1983, 1987). The cytoplasmic organisation of the *Geosiphon* spores and hyphae is similar to that of other *Glomus* species (Bonfante and Bianciotto, 1994).

During germination of the spores no "germination compartment" is formed, which has been reported for *Acaulospora* (Mosse, 1970a, c; Mosse, 1986). The germination occurs directly through the spore wall, in contrast to the majority of *Glomus* species, which germinate through the subtending hypha (Gerdemann and Trappe, 1974). The direct germination is described for *Gigaspora margarita* (Sward, 1981c), *Glomus mossea* (Meier and Charvat, 1992), and some other *Glomus* species. The modes of *Glomus* spore-germination were recently suggested to be more plastic than has been noted previously (Meier and Charvat, 1992). Also, the formation of "germination compartments" as a character for taxonomic classification has become unclear recently (see Gibson et al., 1987; Mosse, 1986; Walker, 1987).

The formation of the *Geosiphon* spores is also different from that of *Acaulospora* and *Enteophospora*, which first form a "sporiferous saccule". *Gigaspora* and *Scutellospora* show a typical "bulbous suspensor cell" at the subtending hypha. The genus *Sclerocystis* is obligatory sporocarp-forming and does not form globose spores (Hall, 1987; Morton 1988, Morton and Benny, 1990; Trappe, 1982).

If the spore-characters of *Geosiphon* are compared with these used for the synoptic keys of Hall (1987), Trappe (1982), the cladogram of Morton (1990), and the revised classification of Morton and Benny (1990), clear evidence for the genus *Glomus* results.

The bacteria-like organisms (BLOs) are not surrounded by a host membrane. They seem to belong to the Gram-positive bacteria. Their ultrastructure is similar to the BLOs described in other members of the Glomales and Endogonales, like *Glomus mosseae* (Macdonald et al., 1982; Meier and Charvat, 1992), *Gigaspora heterogama*, an unidentified "white reticulate" AM fungus (Macdonald et al., 1982), *Glomus caledonium* (Macdonald and Chandler, 1981), *Acaulospora laevis* (Mosse, 1970b), *Gigaspora margarita* (Macdonald et al., 1982; Sward, 1981a, b, c), another unidentified AM fungus (Protzenko, 1974, 1975), *Endogone flammicorona* (Bonfante-Fasolo and Scannerini, 1977b), *Glomus versiforme* (Garriock et al., 1989), a member of the *Glomus fasciculatus* complex (Bonfante-Fasolo and Scannerini, 1977a; Scannerini and Bonfante-Fasolo, 1982), and in undetermined members of the Glomales, forming an AM-like association with hepatophytes (Ligrone and Lopes, 1989), anthocerophytes (Ligrone, 1988) and a *Lycopodium* species (Schmid and Oberwinkler, 1993) (for a review of BLOs in AM fungi see Scannerini and Bonfante-Fasolo, 1992).

We suggest that *Geosiphon pyriforme* represents a member of the genus *Glomus*. In consequence the question arises whether *Geosiphon* is an AM fungus. It is quite possible that it forms AM-like symbioses with mosses, liverworts or hornworts. The induction of hyphal

germination by moss exudates (see also Mollenhauer, 1988, 1992; Mollenhauer and Mollenhauer, 1988) supports this possibility. There are different liverworts, a moss (*Dicranella staphylina* Whitehouse) and an *Anthoceros* species, living in the natural habitat of *Geosiphon* (Mollenhauer, 1988, 1992). The hepatics and anthocerotetes have been known to form AM-like associations for a long time (Ligrone, 1988; Ligrone and Lopes, 1989; Nemeč, 1899; Pocock and Duckett, 1984; Stahl, 1949), and the moss *Funaria hygrometrica* also forms AM-like associations (Parke and Linderman, 1980).

Molecular biological investigations must show whether *Geosiphon* is indeed a *Glomus* spec. Small subunit rRNA sequence data for comparison are available now (Simon et al., 1992; Simon et al., 1993) and did confirm the taxonomic classifications based on spore shape. A method for detecting DNA polymorphism by random amplified polymorphic DNA (RAPD) analysis was reported recently (Wyss and Bonfante, 1993). *Geosiphon* could then be useful for various further studies on AM fungi.

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A. Schüßler

Institut für Botanik
der Technischen Hochschule Darmstadt
Schnittspahnstraße 10
D-64287 Darmstadt
Federal Republic of Germany