

***GLOMUS AGGREGATUM* EMENDED: A DISTINCT TAXON IN THE *GLOMUS FASCICULATUM* COMPLEX**

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ABSTRACT

The description of *Glomus aggregatum* is emended, broadening the range of spore characteristics encompassed to include mature spores possessing one or two walls and forming inner spores by internal proliferation from subtending hyphae. *Glomus aggregatum* is one of several species in the *G. fasciculatum* "complex." The species is very similar to a fossil species, *Rhizophagites butleri*, that had been placed in synonymy with *Glomus fasciculatum* by Gerdemann and Trappe. *Glomus aggregatum* is frequently recovered from maritime and lacustrine dunes where it sporulates in the soil, in roots, in dead spores of other VA species, in decaying organic matter, and between the cleavage planes of mica particles.

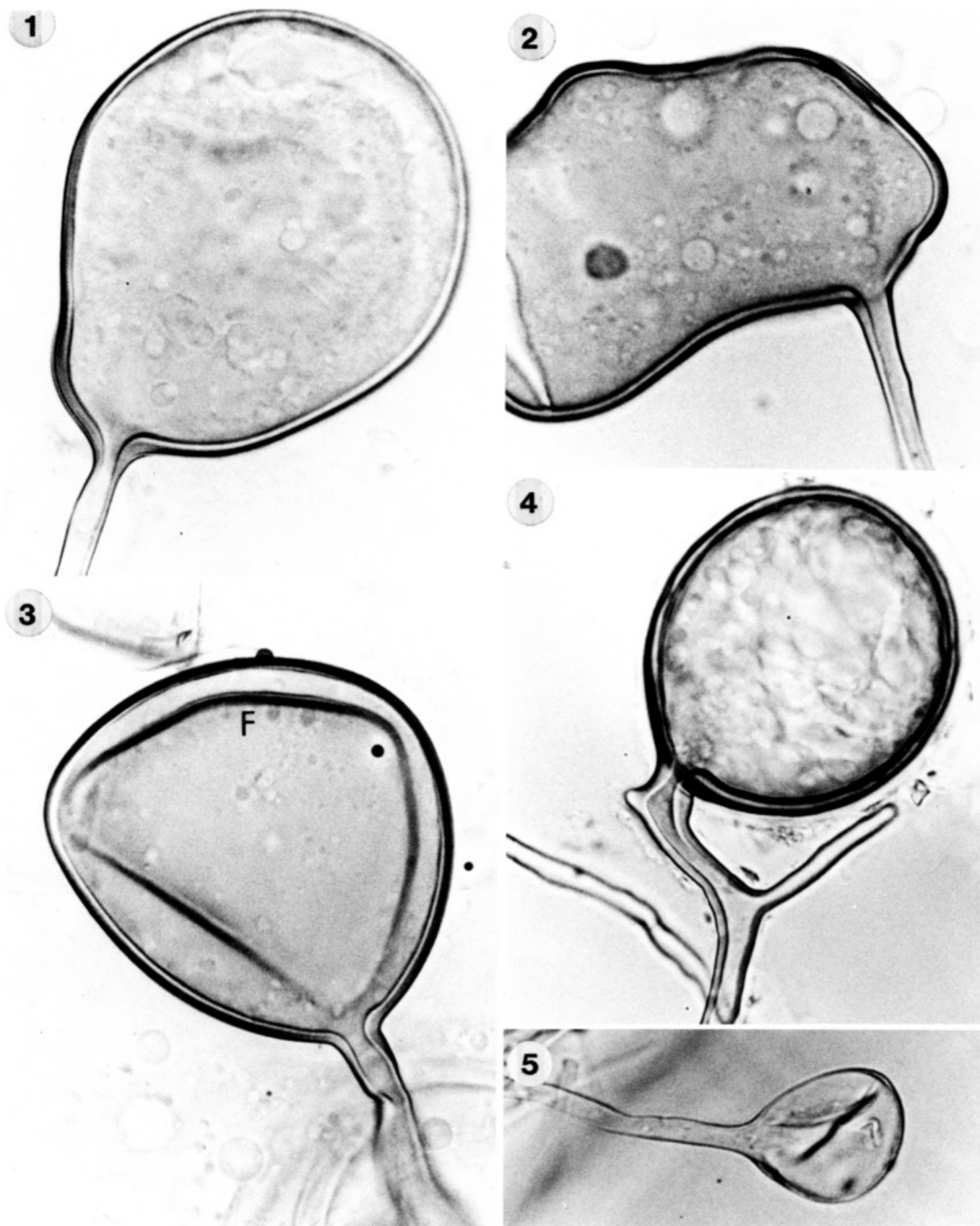
Key Words: VA mycorrhizae, *Glomus fasciculatum*, *Rhizophagites*, sand dunes.

In 1943, Rosendahl described a new fossil genus and species of endogonaceous fungus, *Rhizophagites butleri* Rosendahl, based on collections from Pleistocene deposits in Minnesota and from peat and glacial clays from Alberta. Butler (1939) had illustrated and described but not named the species earlier, assigning it to the extant genus *Rhizophagus*. The species was characterized by spores that measured 73–103 × 79–124 μm, possessed one or two separable walls, and formed spores by internal proliferation.

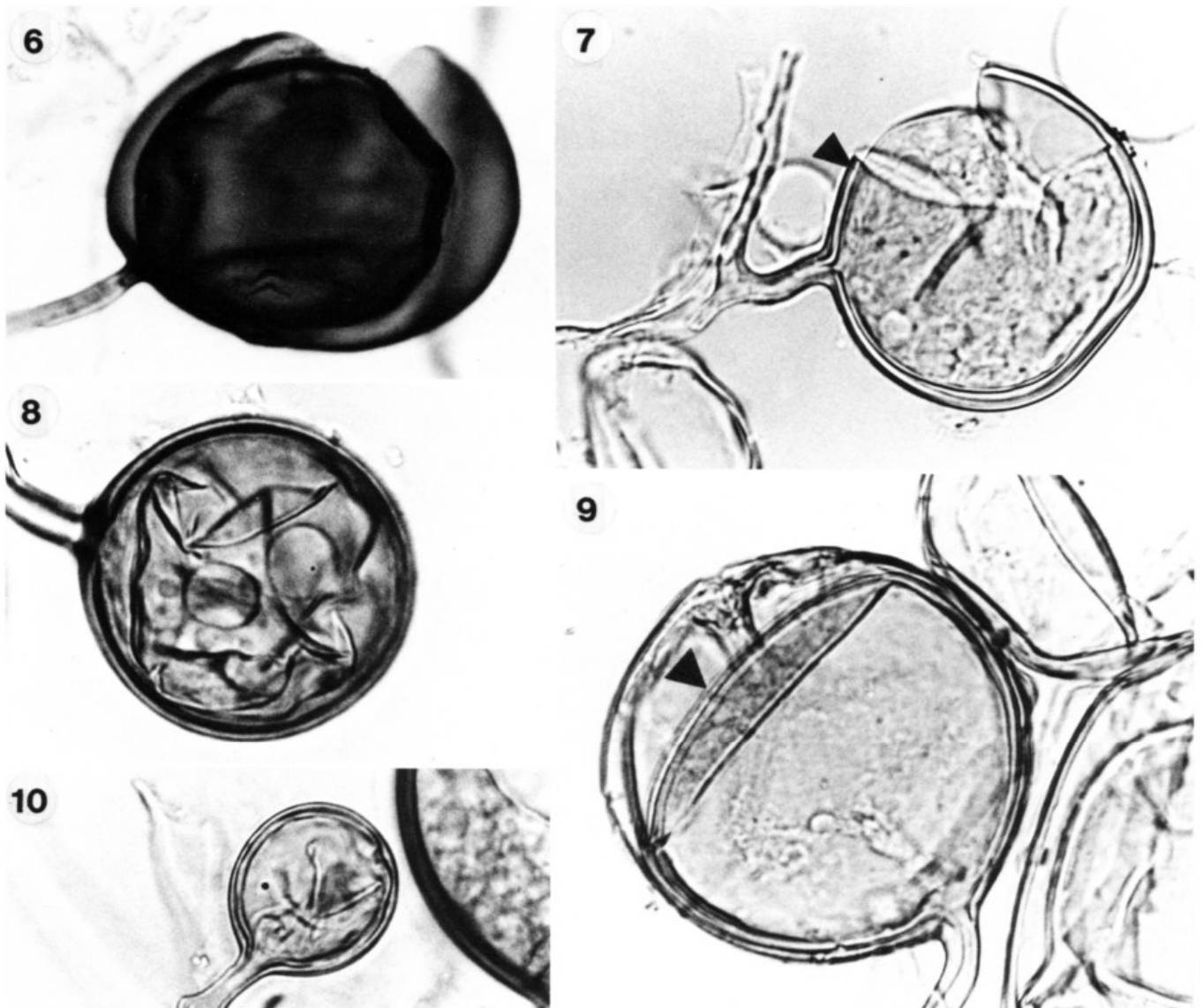
Spores of a fungus similar to those of *R. butleri* have been isolated frequently during investigations of the vesicular-arbuscular mycorrhizae (VAM) associated with dune-colonizing plants of Lake Huron, Lake Michigan, Hawaii, and the Atlantic Coast of the U.S. and Canada. Spores typically were bounded by a single wall 1–3 μm thick, sometimes by two walls, and occasionally contained spores formed by the same type of internal proliferation as described for *R. butleri*. Attempts to obtain type material of *R. butleri* for comparison were unsuccessful. Until Walker's recent study of the type material of *Glomus fasciculatum* (Thaxter) Gerd. & Trappe (Walker, pers. comm.), the spores of the *R. butleri*-like species from the dunes were referable to *G. fasciculatum* (*sensu lato*), which included among its synonyms *R. butleri* (Gerdemann and Trappe, 1974).

Schenck and Smith (1982) recently described a small-spored VAM species, *G. aggregatum* Schenck & Smith, from a citrus nursery in Florida. Although the original description mentioned only spores with two thin walls, examination of the type material revealed that many spores in the collection possessed but a single wall and that some were formed by internal proliferation. Schenck (pers. comm.) believed that the formation of the second wall was a late stage in the development of all spores of the species and represented the wall structure of the mature spore. Thus, he and Smith described the two-walled condition as a characteristic of the species. Rosendahl had earlier reached a similar conclusion regarding the spore wall of *R. butleri*.

Examination of many collections of the *R. butleri*-like fungus from the dunes and of the type material of *G. aggregatum* indicated that both were of the same species and that the majority of spores in many collections possess just a single wall. Accordingly, the description of *G. aggregatum* is emended to indicate the



FIGS. 1-5. *Glomus aggregatum*. Spores with single walls. 1-3. Mature spores. Note open channel and laminations in wall. Spore in FIG. 3 has collapsed, and the folding (F) of the wall is visible, $\times 800$. 4. Mature spore, $\times 650$. 5. Immature spore with thin single wall, $\times 800$.



FIGS. 6–10. *Glomus aggregatum*. Spores with two walls. 6. Spore with flexible inner wall, stained in trypan blue, $\times 620$. 7. Spore with a thin inner wall. Note gap (arrow) between two walls, $\times 760$. 8. Intact spore with wrinkles and folds of inner wall visible. Stained in trypan blue, $\times 760$. 9. Spore with two walls of approximately equal thickness. Inner wall has collapsed near top of spore (arrow), $\times 760$. 10. Immature spore with two subequal walls, $\times 760$.

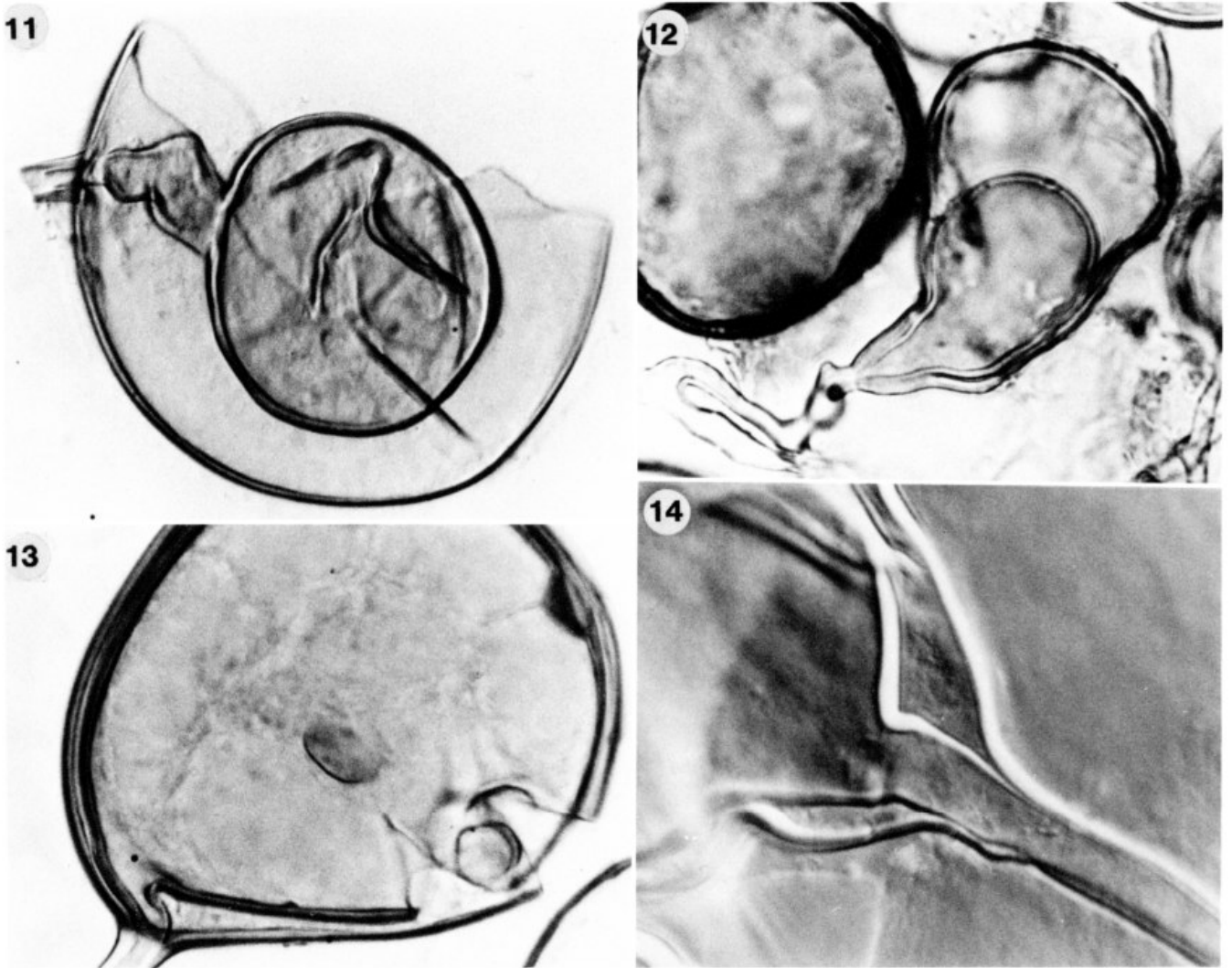
variability in wall structure to allow investigators to identify accurately this apparently common species from sandy soils.

Specimens have been deposited in the herbarium of Oregon State University (OSC), the Farlow Herbarium (F), and at Kew (K). Wall descriptions and terminology are those suggested by Walker (1983).

GLOMUS AGGREGATUM Schenck & Smith emend. Koske

FIGS. 1–30

Sporocarps $200\text{--}1800 \times 200\text{--}1400 \mu\text{m}$, lacking a peridium, of loosely aggregated spores. Spores formed in sporocarps, in roots, or free in the soil, smooth, globose to subglobose, pyriform to very irregular; globose to subglobose spores $(20\text{--})40\text{--}85(-120) \times (20\text{--})40\text{--}85(-120) \mu\text{m}$, irregular spores $(30\text{--})40\text{--}120(-210) \times$



FIGS. 11–14. *Glomus aggregatum*. Spores formed by internal proliferation. 11. Outer spore crushed open. Note unusually wide attachment hypha of inner spore, $\times 650$. 12. Irregularly shaped spore with inner spore, $\times 530$. 13. Walls of inner spore and outer spore give the appearance of a single spore with two walls. Note attachment hypha of inner spore, $\times 650$. 14. Base of spore illustrating the regrowth of a hypha from the original attachment hypha, $\times 1310$.

(20–)40–90(–120) μm ; very pale yellow to yellow-brown to orange-brown, often with a greenish tint in transmitted light. Spore wall structure (murograph, FIG. 30) of one or two separable, colored, laminated walls, each 1–3(–5) μm thick, thicker near point of attachment; if two walls present, the outer is thicker. Spores forming inside of spores by internal proliferation. Subtending hyphae straight, curved, occasionally infundibuliform, constricted, swollen or irregular, up to 12 μm wide at the spore base when funnel-shaped, (4–)6–10 μm wide otherwise; walls 1–3.5 μm thick; some spores with two subtending hyphae. Pore usually open, 1.5–4(–9) μm diam or closed by a thin, curved septum in the subtending hypha, by an inner wall near the spore base, or occasionally by a cytoplasmic plug or spore wall thickening, not occluded by hyphal wall thickening.

DISTRIBUTION AND HABITAT: Common in maritime sand dunes of the Atlantic Coast of the U.S. and Canada and lacustrine dunes of Lake Huron and Lake Michigan. Also known from an abandoned railroad bed in Kalamazoo, Michigan, from sand dunes in Hawaii, and from a citrus nursery near Haines City, Florida.

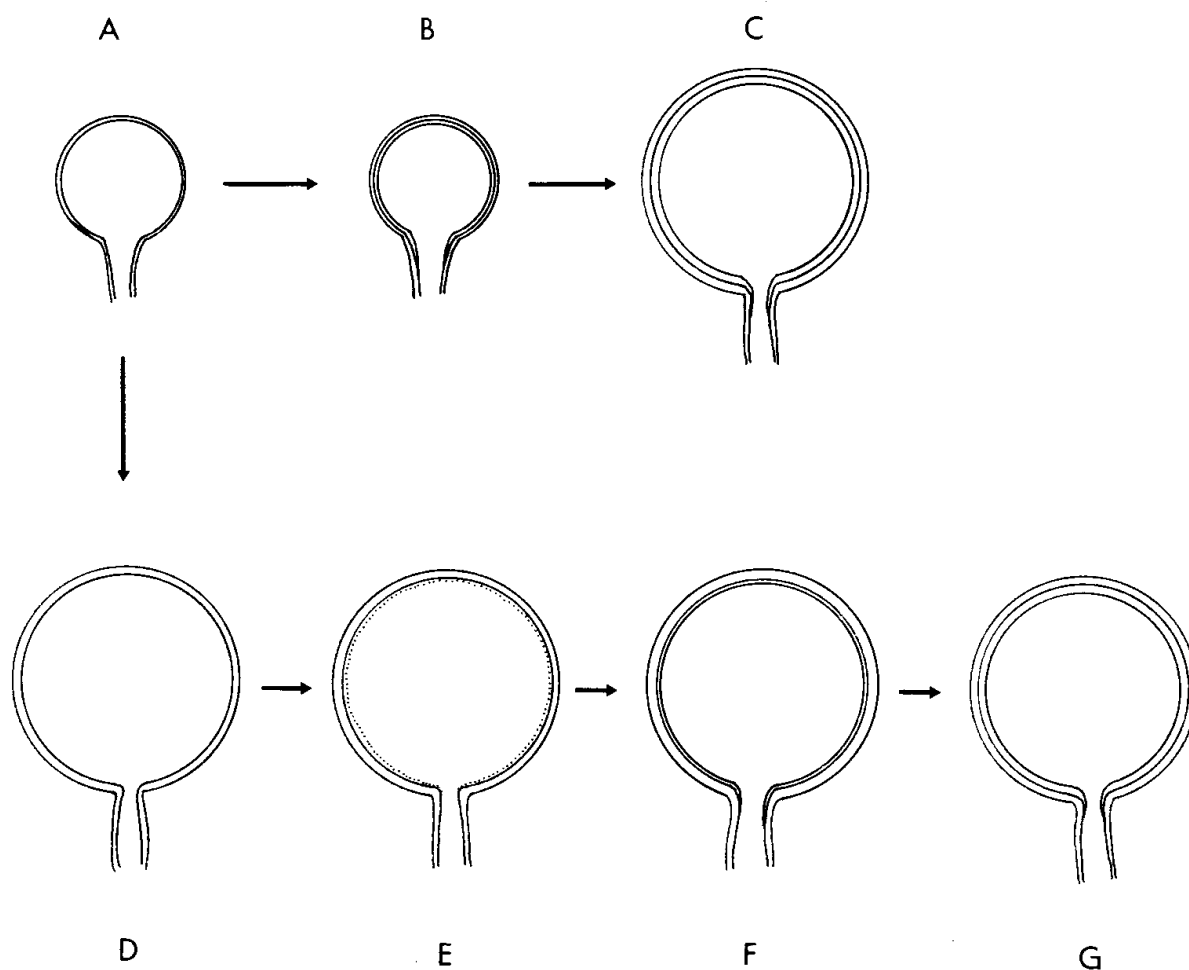


FIG. 15. *Glomus aggregatum*. Possible developmental sequences of spores that result in mature spores with one or two walls. A–C. Two walls form in young spores, persist as spores expand. A, D. Young spore with single wall expands to produce a mature spore with a single wall. D–G. Full-sized spore with a single wall forms a thin inner wall that increases in thickness, leading to the two-walled mature spore.

MYCORRHIZAL ASSOCIATIONS: Associated, but not proven mycorrhizal with *Ammophila breviligulata* Fern., *Solidago sempervirens* L., *Uniola paniculata* L., *Calamovilfa longifolia* (Hook) Scribn., *Equisetum arvense* L., *Scaevola taccada* (Gaertn.) Roxb., *Citrus sinensis* × *Poncirus trifoliata*, and an unidentified dune grass from Nova Scotia. Forming typical VA mycorrhizae in pot cultures with bahia grass, *Paspalum notatum* Flugge.

COLLECTIONS EXAMINED: USA: FLORIDA, type material of *Glomus aggregatum* Schenck & Smith at the Farlow Herbarium; HAWAII, Kailua, 17 July 1984, Koske 802; MARYLAND, Assateague Island, 14 Mar. 1982, Koske 431; MASSACHUSETTS, South Dartmouth, 29 Apr. 1984, Koske 542; MICHIGAN, Kalamazoo, 11 Sept. 1983, Olexia 10B; NEW JERSEY, Cape May Point, 14 Mar. 1982, Koske 402; Sea Isle City, 14 Mar. 1982, Koske 397; RHODE ISLAND, Moonstone Beach, 29 June 1979, Koske 229, 19 Nov. 1980, Koske 268, 269, 18 Feb. 1983, Koske 491, 1 May 1984, Gemma 1043, 17 May 1984, Koske 541; Sand Hill Cove, 23 Mar. 1981, Koske 286; Block Island, 8 April 1983, Friese 76; VIRGINIA, Assateague Island, 15 Mar. 1982, Koske 436, 458; Ocean Beach State Park, 23 April 1983, Koske 518, 521, 524; CANADA: NOVA SCOTIA, Cape Breton Highlands, 18 July 1983, leg. J. Cooke, Koske 534; ONTARIO near Ipperwash Provincial Park, 21 July 1973, Koske 26LH.

Glomus aggregatum is distinguishable from other species by its small, smooth, thin-walled spores of various shapes, the pale yellow to yellow-brown color, the generally open pore, the presence of mature spores with one or two thin walls, and, frequently, by the production of spores by internal proliferation. Spore shape, wall structure, and the morphology of the subtending hypha are highly variable (FIGS. 1–4, 6–9, 18–20, 26, 28, 29) but a representative range can be found in

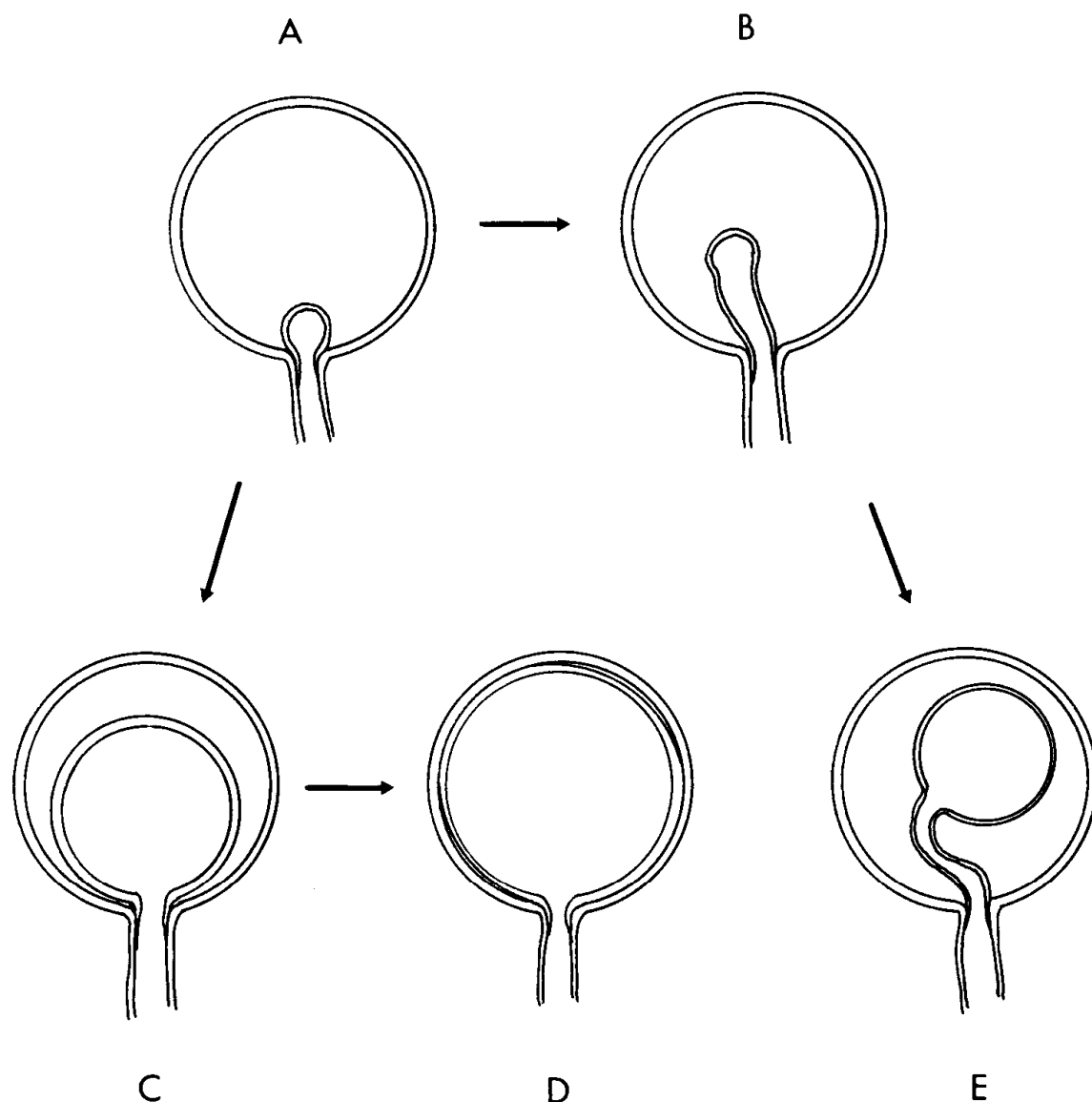
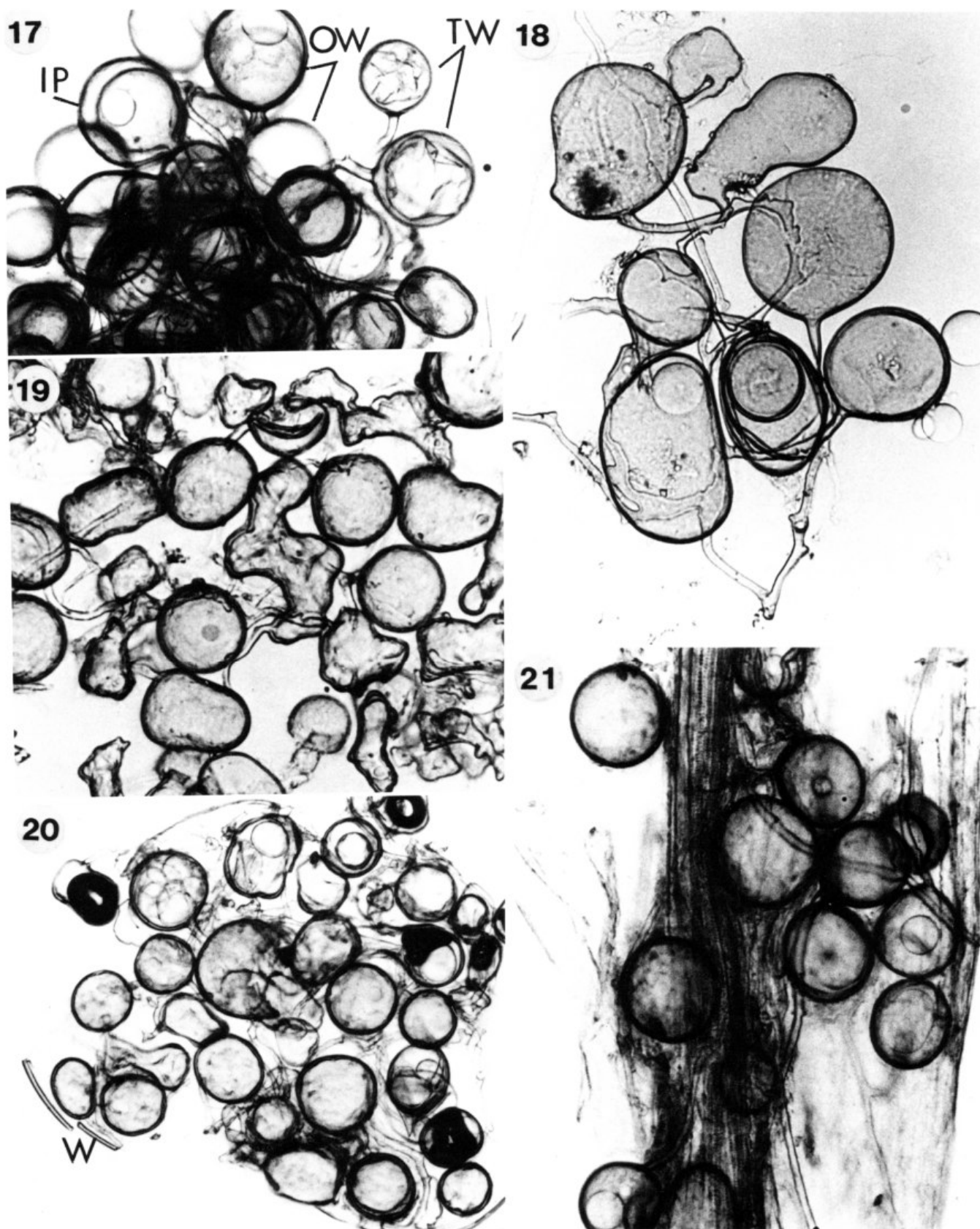


FIG. 16. *Glomus aggregatum*. Developmental sequences of spore formation by internal proliferation. A, C, D. Inner spore forms by expansion of a hyphal regrowth at the base of the spore. Note how the inner and outer spore complex in D could be interpreted as a single spore. Gap between spores is shown in D. A, B, E. Growth of hypha into a spore before inner spore formation occurs.

most collections. Indeed, the spores in a single sporocarp often display the wide morphological range of the species. Although some sporocarps may be composed almost entirely of globose or subglobose spores, many sporocarps also contain some very irregularly shaped spores (FIGS. 13, 18, 19). All walls in mature spores appear to be of the laminated type (Walker, 1983), with the innermost lamination often having a greenish color when viewed with bright-field illumination at 450–1000 \times . The events leading to various wall arrangements are illustrated in FIGS. 15 and 16. Spores originate as subglobose swellings bounded by a single wall (FIGS. 5, 15A). Many spores complete their development without forming additional walls and possess only the single, laminated wall when fully expanded (FIGS. 1–4, 15D).

An additional wall may form prior to full expansion of the spores (FIG. 15B) or after it (FIG. 15E, F), both events leading to a mature spore with two walls (FIGS. 9, 15C, F, G). Frequently, the innermost wall extends for a short distance down the subtending hypha. When spores are placed in a mounting medium such



FIGS. 17–21. *Glomus aggregatum*. 17–19. Sporocarps. 17. Note spores with two walls (“TW”), one wall (“OW”) and formed by internal proliferation (“IP”) in a single sporocarp, $\times 200$. 18. Note variation in spore morphology and inner spore, $\times 200$. 19. Single sporocarp with globose to irregular spores, $\times 200$. 20. Cluster of spores inside dead spore of *Gigaspora gigantea*. Wall of *Gigaspora* is indicated by “W,” $\times 140$. 21. Spores inside root of American beachgrass, *Ammophila breviligulata*, $\times 200$.

as polyvinyl alcohol (Walker, 1983) before the second wall has reached its full thickness (especially in those spores that produce the second wall after reaching full size), the newly forming wall collapses as the spore is plasmolyzed (FIGS. 6–8) and is clearly visible. The presence of the collapsible wall depends upon the age of the spores, and not all collections will necessarily contain this intermediate stage. At the stage of spore development when the collapsible inner wall is present, it is *ca.* 0.5 μm thick and is of the “membranous” type (Walker, 1983). As this wall thickens to *ca.* 1 μm , it develops the characteristics of the more rigid laminated wall type, although the laminations may not be visible at this stage. Additional deposition of laminae converts the thin rigid wall to a thicker (2–4 μm), more typical, laminated wall that is similar in appearance to the outermost wall. Because spores in intermediate stages of development are frequently recovered in soil samples, several possible wall structures are included in the micrograph (FIG. 30).

Inner spores may form inside of spores that themselves have one or two walls, and care must be taken to distinguish the wall structure of the newly formed spores from that of the spore in which they were formed. Inner spores can form by one or two processes: (1) a new hypha may grow from the old attachment hypha into the existing spore for some distance before expanding at the tip to form a new spore (FIGS. 11, 13, 14, 16A, B, E), or (2) a new spore can arise from the old attachment hypha at the spore base, expanding to fill all or part of the old spore (FIGS. 12, 16A, C). As shown in FIGS. 14 and 16D, a seemingly two-walled spore can result if the newly formed spore is closely appressed to the wall of the older spore.

Inner spores may themselves be the site of further internal proliferation, and both types of regrowth may occur sequentially in the same spore. Up to five nested spores have been observed in some collections.

Eight collections of *G. aggregatum* were examined to determine the percentage of spores of each different wall structure present in a typical collection. Spores with only a single wall comprised 30–95% of the total spores in a single collection. Spores with two walls comprised 0–70% of spores in a collection, and spores with inner spores formed by internal proliferation accounted for 0–12% of the spores in any collection. Darker, apparently older, sporocarps contained the higher percentage of spores with inner spores.

Some collections of *G. aggregatum* include spores with the “lignituber-like ingrowths” described from other *Glomus* species (Godfrey, 1957; Khan, 1971; Mosse, 1956; Mosse and Bowen, 1968) and spores of *Sclerocystis* (Gerdemann and Bakshi, 1977; Iqbal and Perveen, 1980). These ingrowths are characterized by having a narrow central channel (FIG. 26), apparently the result of invasion by another microorganism. A second type of localized thickening may occur in spore walls, similar to that reported in *G. maculosum* Miller & Walker *ined.*, and does not appear to be associated with microbial attack. Thickenings of this second type are subcircular to irregular in face view, 9–20 \times 18–20 μm , and hemispherical to irregular in side view, 4–10 μm high (FIGS. 24, 25). Spores with this type of localized thickening may have one or two such areas, and occasionally the pore leading to the attachment hypha is blocked by the thickening (FIG. 25).

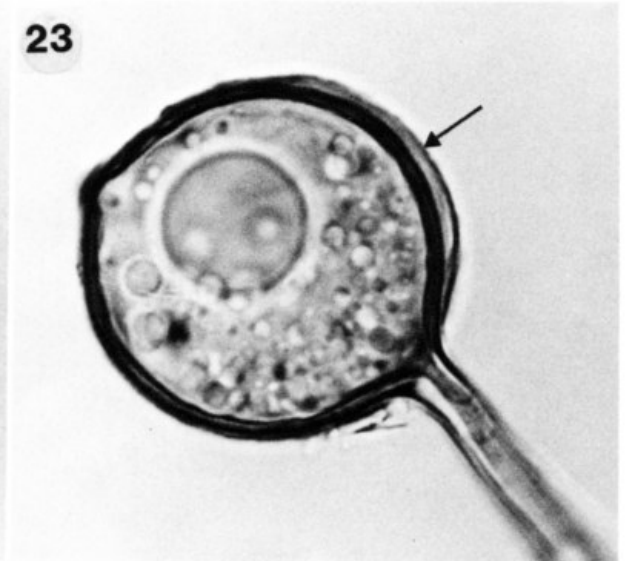
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FIGS. 22–29. *Glomus aggregatum*. 22. Spore with mucilaginous-like outer layer with folds and wrinkles, $\times 780$. 23. Spore with unevenly deposited mucilaginous-like layer (arrow), $\times 635$. 24, 25. Thickenings on spore walls. Note open spore in 24, $\times 780$. 26. Lignituber-like ingrowths in spore wall (stained with trypan blue), $\times 780$. 27. Intrahyphal hyphae. Outer (“O”) and the inner (“I”) hyphal walls are indicated, $\times 1580$. 28. Spore produced on short attachment hyphae, $\times 780$. 29. Constricted attachment hypha, $\times 635$.

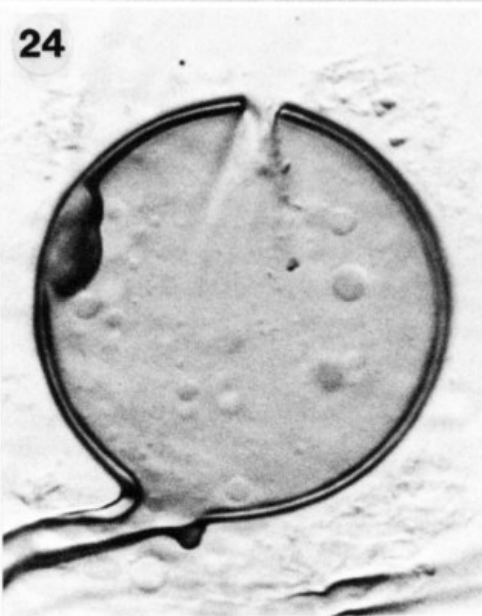
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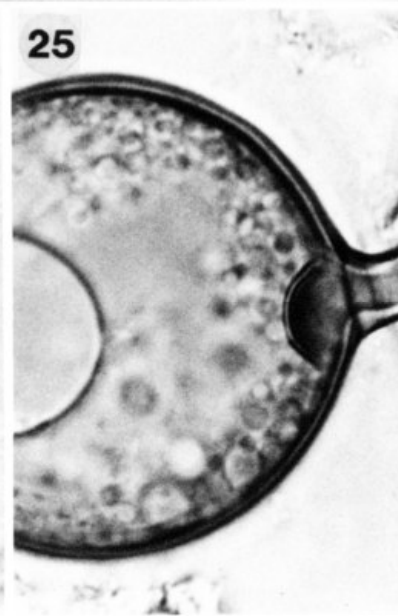
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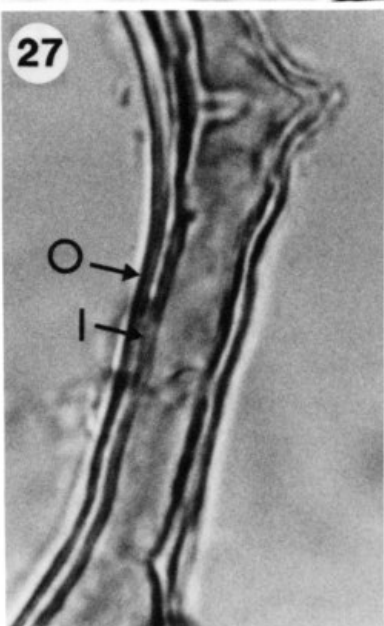
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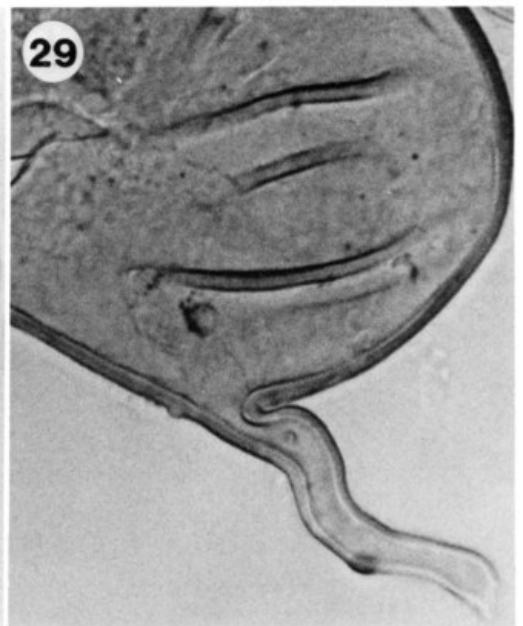
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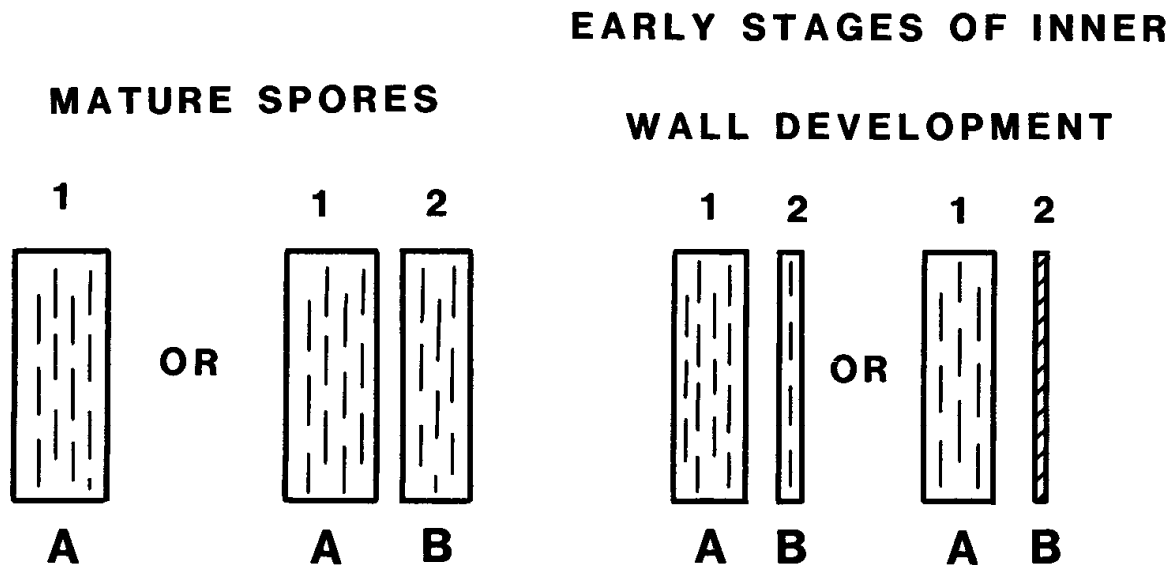


FIG. 30. *Glomus aggregatum*. Murograph. Mature spores possess one or two laminated walls separating into two wall groups (A and B) when spores are crushed. If a second spore wall forms, it may be visible first as a membranous wall, later thickening into a more rigid, laminated wall. Refer to FIG. 15 for the developmental sequence.

Occasional spores may possess an unevenly deposited mucilaginous-appearing outermost layer, 1–2(–13) μm thick surrounding all or part of the spore (FIGS. 22, 23). The origin of this layer is unknown, and a single sporocarp may contain spores with and without this layer. Because this layer is not a consistent feature of spores, it is not included in the murographs or description. A similar layer was reported on some spores of *G. clarum* Nicol. & Schenck (Nicolson and Schenck, 1979).

The loose sporocarps of *G. aggregatum* contain hyphae that often possess internal hyphae or have double walls similar to those of the spores (FIG. 27). Intra-hyphal hyphae have been reported for *G. fasciculatum* (Lim *et al.*, 1983) and have been observed in collections of the same species from Rhode Island (pers. observ.).

Spores of *G. aggregatum* frequently are recovered from sand dune soils, often as occupants of dead spores of other VAM fungi (Koske, 1984). Dead spores of species of *Gigaspora*, *Acaulospora*, and *Glomus* may be filled with the spores of *G. aggregatum* (FIG. 20). Clusters of spores also may occur free in the soil, in the cortex of roots (FIG. 21), in decaying leaves and other organic matter in the root zone, and between the cleavage planes of mica particles. Bethlenfalvay *et al.* (1984) observed a similar affinity for mica particles by an unidentified *Glomus* species in a California desert site.

The range of morphological variation, especially wall structure, encompassed by *G. aggregatum* is so great as to suggest that more than one species may be included in the emended taxon. However, because much of the range and many of the intermediate stages of development can frequently be found within single sporocarps, the integrity of this species appears intact.

Glomus aggregatum is one of the species of mycorrhizal fungi that is included in the *G. fasciculatum* complex. The single-walled *R. butleri*-like spores identified as *G. fasciculatum* in earlier sand dune studies (Koske, 1981; Koske and Halvorson, 1981) are more correctly referred to as *G. aggregatum*. Similarly, one of the "forms" of *G. fasciculatum* illustrated by Nemeč *et al.* (1981) and reported to be a frequent inhabitant of citrus orchard soils in Florida and California appears to be *G. aggregatum*. Spores of *G. fasciculatum* have a thin, hyaline, outer wall

appressed to a 5–15 μm thick, laminated inner wall. Spores of *G. deserticola* Trappe, Bloss & Menge (1984), another species in the *G. fasciculatum* complex, resemble those of *G. aggregatum* in size and wall structure (of single-walled *G. aggregatum* spores) but do not exhibit the wide range of shapes of *G. aggregatum* or form internal spores. Additionally, spores of *G. deserticola* are red-brown and do not occur in sporocarps, whereas those of *G. aggregatum* typically are yellow to yellow-brown and more frequently are recovered in the sporocarpic form.

The species of *Glomus* described and illustrated from England by Mosse (1959) appears to be *G. aggregatum*. She described the development of an inner (=second) wall in older spores, the pore typically open or occasionally closed by a basal plug, the change in color from pale yellow to darker with age, and the formation of "proliferating vesicle-like spores" in some collections. The *Glomus* reported from inside dead seeds by Taber (1982) and from inside nematode cysts (Tribe, 1977) may be *G. aggregatum*.

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