

**SCUTELLOSPORA ARENICOLA AND GLOMUS TRIMURALES:
TWO NEW SPECIES IN THE ENDOGONACEAE**

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San Miguel Island is the westernmost member of the Channel Islands and is situated 42 km off the coast of southern California. This small island (37 km²) is transversed by three major sand dune fields (Johnson, 1980) that account for ca 15% of the habitable island surface. Examination of soil samples collected from around the roots of plants growing in the dunes revealed two undescribed species, one each in *Scutellospora* and *Glomus*. Spores of the latter previously had been found in sand dunes of the eastern United States seaboard (Koske, 1987).

Spores were recovered from soil by sucrose centrifugation (Walker *et al.*, 1982). Descriptions and observations reported in this study are from specimens mounted in a polyvinyl alcohol/lactic acid/glycerol (PVLG) mounting medium (Koske and Tessier, 1983) or in Melzer's reagent. Wall descriptions and terminology follow those suggested by Walker (1983, 1986) and Morton (1986). Except where noted, all stated dimensions are based on analysis of ca 150 spores of each species. Holotypes have been deposited in the herbarium at Oregon State University (OSC), and isotypes have been deposited at the Farlow Herbarium (FH) and at Kew (K).

***Scutellospora arenicola* Koske & Halvorson, sp.
nov.** FIGS. 1, 2, 4

Sporae lucentes, translucetes, pallide hepaticae vel cinnamomeae (interdum luteae), subglobosae vel irregulares, (160–)240(–360) × (120–)220(–310) μm. Tunicae sporarum quinque in turmis duabus (A et B).

Turma A tunica 1 laevi vel parum aspra, hepatica vel cinnamomea, 0.8–1.5 μm crassa, ad tunicam 2 pallidam, (3.5–)8(–12) μm crassam, in solutione Melzeri porphyrascentum arcte adhaerenti. Turma B tunicis tribus: tunica 3 membranacea, hyalina vel luteola, 0.8–1.5 μm crassa, cum tunica 4 coriacea, 1–2 μm crassa contigua; tunica 5 amorphia, hyalina, 1–2 μm crassa, in solutione Melzeri purpurascens, ad tunicam 4 arcte adhaerens. Scutellum pallide hepaticum, cordatum vel irregulare, 120–140 × 120–140 μm. Cellula suspensoriformis hepatica vel cinnamomea, pallidior quam spora, (35–)41(–47) μm lata, tunica 1.5–2 μm crassa, ad basem sporae 2.5–7 μm crassa. Cellulae auxiliares 2–7 in fasciculo pallide hepaticae, pyriformes, turbinateae vel subglobosae, 20–41 × 20–40 μm.

Spores formed singly in the soil or in roots, terminally on a bulbous suspensor-like cell; glistening, translucent, pale yellow-brown to orange-brown (occasionally yellow); subglobose to irregular; (160–)240(–360) × (120–)220(–310) μm. Spore wall structure (murograph, FIG. 4) of five walls (1–5) in 2 groups (A, B). Group A consisting of a unit wall (wall 1) tightly adherent to a laminated (wall 2). Wall 1 smooth or slightly roughened, brittle, yellow-brown to orange-brown, 0.8–1.5 μm thick. Wall 2 lighter in color than wall 1, (3.5–)8(–12) μm thick, staining dark red-brown in Melzer's reagent. Group B consisting of two closely adjacent walls (walls 3, 4) and an amorphous wall (wall 5) adherent to wall 4. Wall 3 membranous, hyaline to pale yellow, 0.8–1.5 μm thick. Wall 4 coriaceous, hyaline to pale yellow, 1–2 μm thick, usually thicker than wall 3. Wall 5 hyaline, amorphous, of variable thickness in PVLG; 1–3 μm thick and staining dark reddish-

purple in Melzer's reagent. A pale yellow-brown, cardioid to irregular germination shield $120\text{--}140 \times 120\text{--}140 \mu\text{m}$ forms on wall group B. *Suspensor-like cell* borne terminally on a sparsely septate subtending hypha; $(35\text{--})41\text{--}(47) \mu\text{m}$ broad, yellow-brown to orange-brown, paler than the spore; walls $2.5\text{--}7 \mu\text{m}$ thick at the base, thinning to $1.5\text{--}2 \mu\text{m}$ thick distally; with one stout projection $15\text{--}30 \times 6\text{--}12 \mu\text{m}$. *Auxiliary cells* in soil borne in loose clusters of 2–7; pale yellow-brown; pyriform, turbinate, to subglobose, $20\text{--}41 \times 20\text{--}40 \mu\text{m}$; produced on coiled hyphae $1.8\text{--}2.5 \mu\text{m}$ diam, concolorous with auxiliary cells.

DISTRIBUTION AND HABITAT: Known only from sand dunes and sandy soils on San Miguel Island, California and the adjacent mainland (Koske and Halvorson, 1988). Spores of *S. arenicola* occurred much more frequently in stabilized sand dunes than in older dune-derived soils or in mobile dunes.

MYCORRHIZAL ASSOCIATIONS: Associated in the field with roots of *Abronia maritima* Nett. ex Wats., *Ambrosia chamissonis* var. *bipinatisecta* (Less.) Greene, *Astragalus miguelensis* Greene, *Calystegia macrostegia* ssp. *macrostegia* (Greene) Brummitt, *Carpobrotus aequilaterus* (Haw.) N. E. Brown, *Distichlis spicata* (L.) Greene, *Duddleya greenei* Rose, *Eriogonum grande* ssp. *rubescens* (Greene) Munz, *Happlopappus detonsus* (Greene) Raven, *Malacothrix incana* (Nutt.) T. & G., *Poa douglasii* Nees, and *Syrinchium bellum* Wats. Accounts of the natural history and vegetation of San Miguel Island are available in two recent publications (Johnson, 1980; Philbrick and Haller, 1988).

ETYMOLOGY: Latin, *arenicola* = dwelling on sand; referring to the known habitat of the species.

COLLECTIONS EXAMINED: HOLOTYPE: CALIFORNIA, SANTA BARBARA CO., San Miguel Island, among roots of *Poa douglasii*, *Carpobrotus aequilaterus* and *Malacothrix incana* (5.Aug.88, Koske 2374)/OSC. ISOTYPES: FH, K.

OTHER MATERIALS EXAMINED: CALIFORNIA, San Miguel Island, 5–6.July.84; Koske 580, 581 (OSC), 588–591, 593–596, 600, 606; 26.July.85; Koske 616; 13.Nov.85; Koske 2147 and 2148; 7.Aug.88; Koske 2371 and 2399; Oxnard, 31.July.85; Koske 731.

Spores of *Scutellospora arenicola* are readily distinguished from all other described species of the genus by their wall structure and pigmentation. Wall 1 is not easily separable from the thicker wall 2 to which it adheres (FIG. 1A), although cracks that appear in wall 1 in crushed spores provide evidence for its presence (FIG. 1G). Much of the color of the spores is concentrated in wall

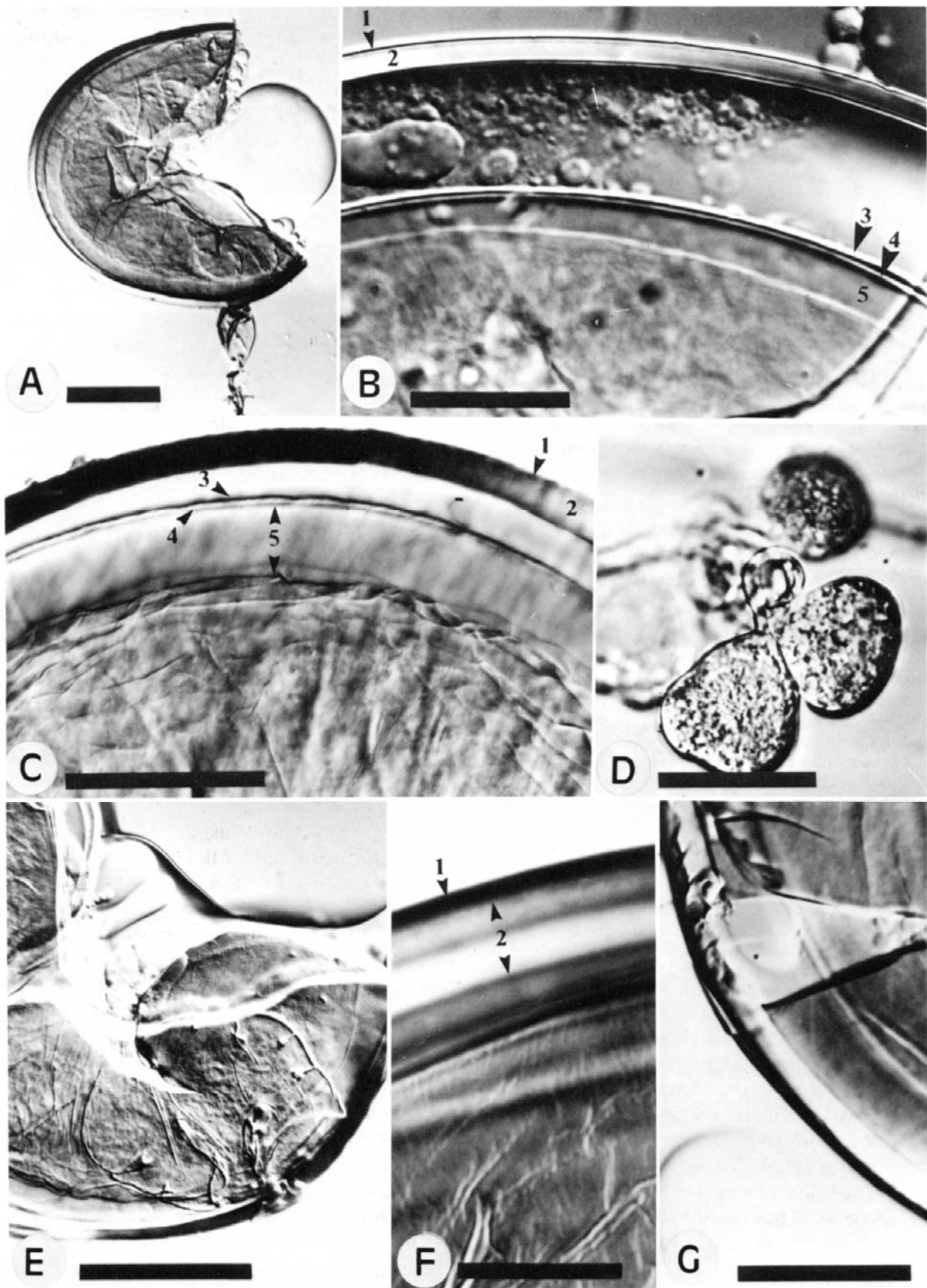
1 (FIG. 1F), and when this wall is eroded by soil microorganisms or broken away during crushing, lighter patches of the underlying wall 2 show through (FIG. 1G). The darker pigmentation of this wall imparts an dark "outlined" effect to the translucent spores when they are observed with the dissecting microscope.

Wall 2 of spores of *S. arenicola* often is of uneven thickness, with some areas of the wall of a single spore measuring $6 \mu\text{m}$ thick and others areas of the same spore measuring up to $11.5 \mu\text{m}$ thick. The laminations are very fine, similar to those in the thick outer wall of *S. erythropha* (Koske & Walker) Walker & Sanders. The laminations are not easily observed unless spores are forcibly crushed.

The two outermost walls of wall group B (walls 3, 4) remain appressed even in crushed spores, and they may be difficult to resolve separately. Wrinkling of the membranous wall 3 is apparent in most specimens (FIG. 1F). In addition to the coarse folding, this wall bears numerous fine wrinkles that impart an irregular profile to the wall when viewed on edge at $400\times$ (FIG. 1C, F). The germination shield (FIG. 1D) forms on wall group B, but its association with particular walls could not be determined.

Inseparable from the coriaceous wall (wall 4) is the amorphous wall (wall 5) (FIG. 1B, C). This wall is most distinguishable in spores that have been crushed in Melzer's reagent, in which it stains reddish-purple. It also shows some plasticity when spores are crushed in PVLG, allowed to become infiltrated by the acidic PVLG solution, and then crushed again (Morton, 1986). Following this "double crushing" technique, the amorphous wall expands in thickness and the innermost surface of the wall exhibits fine wrinkles (FIG. 1C). The bulk of the amorphous wall then lies between the wrinkled zone and the coriaceous wall (FIG. 1B, C). In spores that have been "double crushed," the amorphous wall could be interpreted incorrectly as a space between an apparently wrinkled membranous wall (actually the innermost part of the amorphous wall) and the coriaceous wall. See Morton (1986, 1988) for a complete description of the features of the amorphous wall and methods for identifying it.

FIG. 1. A–G. *Scutellospora arenicola*. A. Crushed spore with suspensor-like attachment. Bar = $100 \mu\text{m}$. B, C. Walls 1–5 are indicated in these crushed spores. Note roughness of wall 1 and extensive wrinkling of inner surface of the amorphous wall 5 in FIG. C. Bar = $30 \mu\text{m}$. D. Cluster of auxiliary cells on coiled hyphae. Bar =



50 μm . E. Germination shield in crushed spore. Bar = 90 μm . F. Coarse and fine wrinkles on membranous wall (wall 3). Dark wall 1 and lighter, thicker wall 2 also are apparent. Bar = 25 μm . G. Crushed spore in which outer pigmented wall has split ("v"-shaped crack), revealing the lighter colored walls below. Bar = 30 μm .

Spores of *S. arenicola* are likely to be confused with those of *S. erythropha* because of similarities in color of the inner and outermost wall groups. Spores of the latter fungus typically are larger (FIG. 2), darker, and have a more complex wall structure. In addition, spores of *S. erythropha* lack the innermost amorphous wall of *S. arenicola* and have a darker margin around their germination shield.

Pale specimens of *S. arenicola* are yellow to yellow-brown, and the yellow ones superficially resemble spores of *S. calospora* (Nicol. & Gerd.) Walker & Sanders and *S. aurigloba* (Hall) Walker & Sanders in this respect. Presence of the thicker outer wall group (walls 1, 2) and the finely wrinkled innermost side of the amorphous wall are features that readily distinguish lightly pigmented spores of *S. arenicola* from these two species.

Spores of *S. arenicola* that have lost their suspensor-like cell could be mistaken for those of *Acaulospora laevis* Gerd. & Trappe because of gross resemblance in color, size, and texture of the outermost wall (smooth). However, spores of *A. laevis* have a thinner outer wall and two easily separating inner membranous walls.

Glomus trimurales Koske & Halvorson, *sp. nov.* FIGS. 3, 4

Sporae singulae in solo efformatae, luteolae vel ochraceae, globosae, subglobosae, ellipsoideae, pyriformes vel irregulares, saepe lenticulares, (60–)121(–130) × (70–)118(–200) μm. Tunica sporarum parietibus tribus. Paries 1 lamellatus, luteolus vel hepaticus, 0.8–2.5(–3.5) μm crassus. Paries 2 luteolus vel ochroleucus, 1–3 μm crassus. Paries 3 lamellatus, hyalinus vel luteolus (ochraceus in sporis veteribus), (1–)5–11(–15) μm crassus. Hyphae sustentantes hyalinae vel luteolae, 4.5–8.5 μm latae, tunicis 1–1.5(–2) μm crassis. Porus obturamento granulato clausus.

Sporocarps unknown. Spores formed singly in the soil; pale yellow to pale brownish-yellow; globose, subglobose, ellipsoid, pyriform, or irregular, often lenticular; (60–)121(–130) × (70–)118(–200) μm. Spore wall structure (see micrograph, FIG. 4) of three walls (1–3) in one group. Wall 1 laminated, pale yellow to yellow-brown, 0.8–2.5(–3.5) μm thick. Wall 2 a unit wall, pale yellow to brownish-yellow, 1–3 μm thick. Wall 3 laminated, hyaline to pale yellow (brownish-yellow in older spores), (1–)5–11(–15) μm thick. Attachment hypha hyaline to pale yellow, straight or slightly constricted at the point of attachment, 4.5–8.5 μm wide, wall 1–1.5(–2) μm thick at point

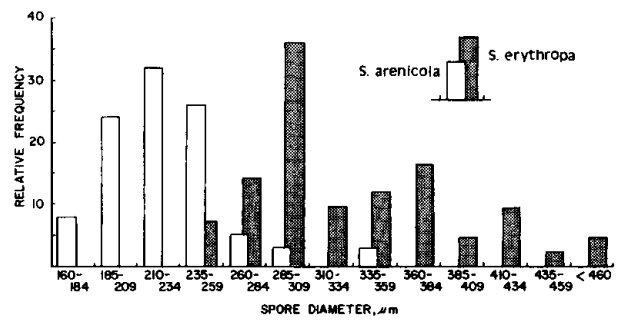


FIG. 2. Spore diameter frequency diagram comparing *Scutellospora arenicola* and *S. erythropha*. Based on 86 spores of *S. arenicola* and 64 spores of *S. erythropha*. For nonglobose spores, only the largest diameter was measured.

of attachment, tapering to <1 μm thick 5–10 μm below spore base. Pore usually closed by a granular plug. None of the walls reacting with Melzer's solution.

ETYMOLOGY: Latin, *tri* = three, *murus* = wall; named for the three walls of the spore.

MYCORRHIZAL ASSOCIATIONS: Associated in the field with roots of *Abronia maritima*, *Ambrosia chamissonis*, *Ammophila breviligulata* Fern., *Carpobrotus aequilaterus*, *Distichlis spicata*, *Eriogonum grande* var. *rubescens*, *Malacothrix incana*, and *Syrinchium bellum*.

DISTRIBUTION AND HABITAT: Known from sand dune soils in California, New Jersey, Maryland, and Virginia (Koske, 1987). Soils ranged in pH from 5.2–8.5. *Glomus trimurales* was not recovered from sandy loam soils in California in sites located less than 1 km from dune sites that contained spores in densities of up to 95 spores/100 cc.

COLLECTIONS EXAMINED: HOLOTYPE: CALIFORNIA, SANTA BARBARA CO., San Miguel Island, among roots of *Abronia maritima* and *Ambrosia chamissonis* (5.July.84; Koske 565)/OSC. ISOTYPES: FH, K.

OTHER MATERIALS EXAMINED: CALIFORNIA, SANTA BARBARA CO., San Miguel Island, 5–6.July.84; Koske 554, 562 (PARATYPE/OSC), 569–571, 576, 583, 602–603; 27–29.July.85; Koske 616, 620, 621, 640, 643, 646, 648, 684, 697, 701, 706, 709; MARYLAND, WORCESTER CO., Assateague Island, 14.Mar.82; Koske 432; NEW JERSEY, OCEAN CO., Seaside, 13.Mar.82; Koske 369; Ocean City, 14.Mar.82; Koske 379; Beach Haven, 14.Mar.82; Koske 385; VIRGINIA, ACCOMACK CO., Assateague Island, 15.Mar.82; Koske 465; Virginia Beach Township, Seashore State Park, 23.April.83; Koske 504.

Glomus trimurales is easily distinguished from other *Glomus* species by its spore wall structure, especially the thick, hyaline to pale yellow innermost wall (FIG. 3A–D), and by the appearance of the narrow, thin-walled attachment hypha (FIG.

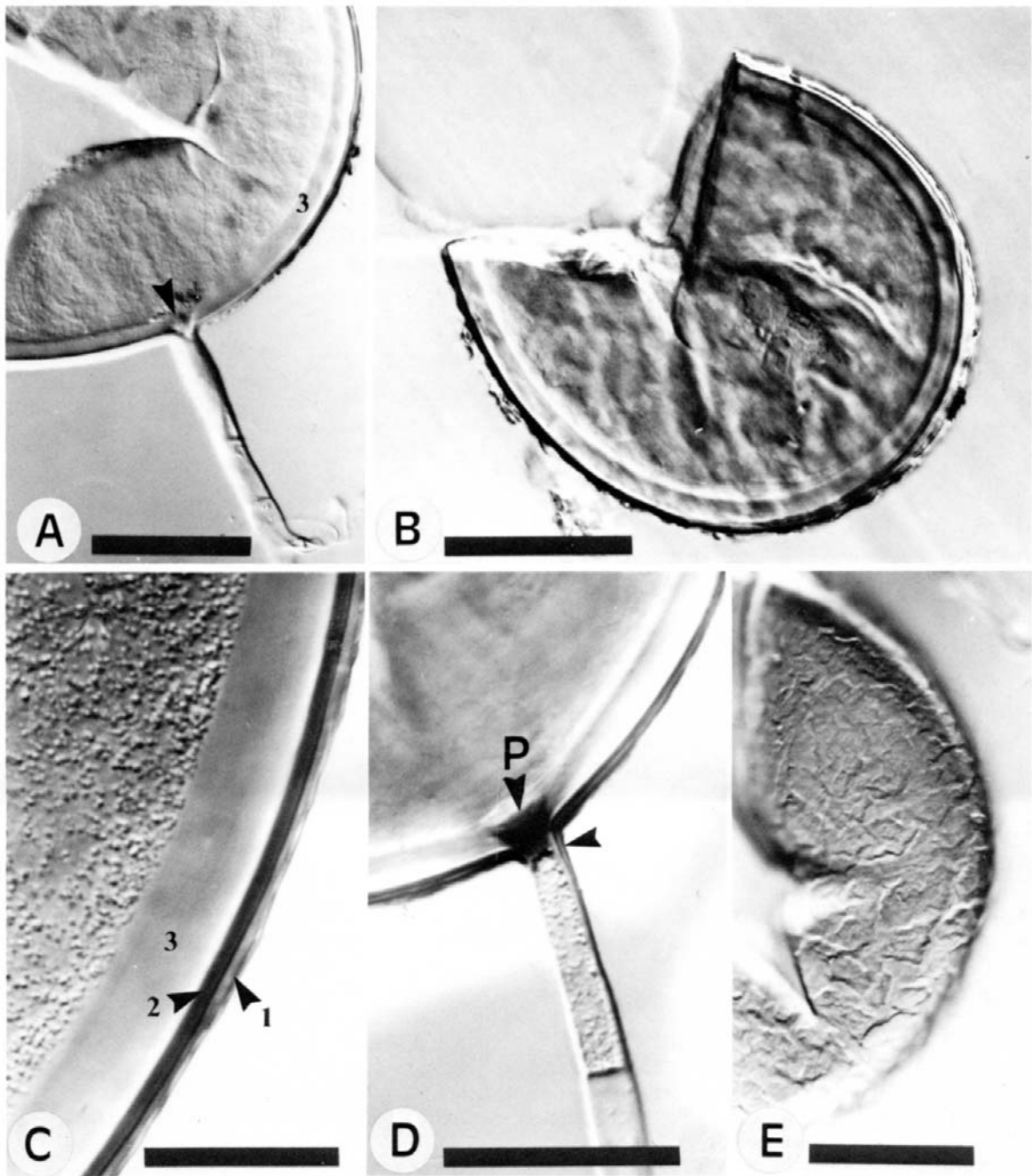


FIG. 3. A–E. *Glomus trimurales*. A. Crushed spore. Note plug (arrow) at point of attachment and relative thickness of wall 3 (“3”). Bar = 50 μm . B. Mosaic pattern on spore surface caused by fragmentation of lamination of outermost wall. Bar = 50 μm . C. Walls 1–3 are indicated in this crushed spore. Laminations are faintly visible in walls 1 and 3. Bar = 20 μm . D. Subtending hypha. Note continuation of walls of spore into subtending hypha (arrow) and septal plug (“P”). Bar = 40 μm . E. Surface of spore, showing fragmentation pattern of the outermost lamination of wall 1. Bar = 50 μm .

3A, D). Wall 3 was of uniform appearance in most specimens, typically measuring 5–11 (\bar{x} = 9) μm thick, and with indistinct laminations. Some spores had a single, dense lamination in this wall that had the appearance of a distinct wall. Such thick deposits in these laminated walls

may be analogous to the “false” growth rings in secondary xylem and could be the result of extreme environmental stress during the development of wall 3. The thickness of wall 3 apparently increases as spores develop. In apparently young, lightly colored spores (although of full size), wall

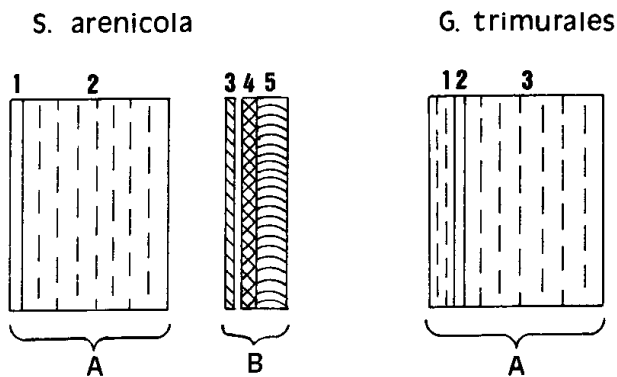


FIG. 4. Murographs of *Scutellospora arenicola* and *Glomus trimurales*. Wall structure of *S. arenicola* is: wall 1 (unit wall), 2 (laminated wall), 3 (membranous wall), 4 (coriaceous wall), 5 (amorphous wall). Wall structure of *G. trimurales* is: wall 1 (laminated wall), 2 (unit wall), 3 (laminated wall).

3 sometimes measured 1.0–1.5 μm thick, but was of similar color and texture as the typically thicker wall 3 of other spores. In old spores that had been stored in sand in plastic bags at 5 C for 4 years, wall 3 was brownish-yellow and measured up to 15 μm thick.

The outer wall (1) of *G. trimurales* consists of three to four laminae (FIG. 3C) that are readily apparent in some specimens. The outermost of these laminae appear to be stretched during expansion of the spore, resulting in their fragmentation into irregular plates. This is most noticeable in plan view of the spore surface (FIG. 3E) where the fragments can be seen separating from each other. The wall is persistent, however, and was present in spores stored at 5 C for 4 yr. None of more than 1200 spores recovered from field soils lacked wall 1. Because of the rough outer surface of wall 1, spores of *G. trimurales* have a matte appearance in reflected light. Wall 2 measured 0.8–1.5 μm thick in small spores that appeared to be young (long subtending hyphae still attached) and up to 3 μm thick in darker, older spores (including spores refrigerated for 4 years).

The three walls of the spore are continuous with the subtending hypha (FIG. 3D). Wall 3 continues for a distance of 5–10 μm below the base of the spore, and walls 2 and 3 can be observed as separate components of the subtending hypha for ca 20 μm below the spore base.

Glomus trimurales most closely resembles *G. claroideum* Schenck & Smith in size, color, and overall wall thickness of spores (Schenck and Smith, 1982). Spores of both species possess a

thick wall, but this wall is innermost (wall 3) in *G. trimurales* and outermost (wall 1) in *G. claroideum*. In addition, the thick wall of *G. trimurales* is laminated whereas the thick wall of *G. claroideum* is evanescent. Spores of *G. trimurales* also differ from those of *G. claroideum* in having a narrower attachment hypha (4.5–8.5 μm vs 7.5–15 μm).

Spores of *G. gerdemannii* Rose *et al.* resemble those of *G. trimurales* in that the innermost spore wall is laminated and thickens with age. However, spores of the former have a more complex wall structure consisting of 5 walls, some of which slough off as spores mature (Rose *et al.*, 1979).

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Key Words: *Scutellospora*, *Glomus*, VA mycorrhizae, sand dunes

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