Smith, G. S. & Schenck, N. C. (1985), Two new dimorphic species in the Endogonaceae: *Glomus ambisporum* and *Glomus heterosporum*. Mycologia 77(4), 566-574. Reproduced with permission from *Mycologia*.

TWO NEW DIMORPHIC SPECIES IN THE ENDOGONACEAE: *GLOMUS AMBISPORUM* AND *GLOMUS HETEROSPORUM*

GEORGE S. SMITH

Department of Plant Pathology, University of Georgia, Athens, Georgia 30602

AND

N. C. SCHENCK

Department of Plant Pathology, University of Florida, Gainesville, Florida 32611

ABSTRACT

Two new vesicular-arbuscular (VA) mycorrhizal fungi (Endogonaceae) from Florida, *Glomus ambisporum* and *G. heterosporum*, differ from all previously described species in the production of two distinct spore types. Black sporocarps or sporocarp aggregates of *Glomus ambisporum* are produced in the soil and hyaline spores are formed within host tissues. The outer wall of sporocarpic spores is subhyaline with a faint reticulum of hexagonal plates. *Glomus heterosporum* has light to dark brown sporocarps or sporocarp aggregates produced externally to host tissues and forms hyaline spores within host tissues. The outer wall of the sporocarpic spores is hyaline, smooth, and evanescent. Sporocarps of both species have spores radially arranged around a central hyphal plexus which resemble sporocarps of *Sclerocystis rubiformis*. This is the first report of dimorphism in the genus *Glomus*.

Key Words: vesicular-arbuscular mycorrhizae, sporocarp, taxonomy.

Two new species of mycorrhizal fungi in the genus *Glomus* (Endogonaceae) are described from Florida. Both species are dimorphic, producing sporocarps in the soil as well as spores or sporocarps within plant tissues. Sporocarps produced in the soil consist of an ordered arrangement of chlamydospores radiating from a central plexus of hyphae. Sporocarps produced within plant tissues consist of an unordered cluster of chlamydospores.

SPECIES DESCRIPTIONS

Glomus ambisporum Smith & Schenck, sp. nov. FIGS. 1; 2; 4A, B; 5A

Sporae sporophoris continuae, dimorphae: brunneae crasse tunicatae aut hyalineae, tenuiter tunicatae. Sporae brunneae in solo in sporocarpis atrobrunneis, subglobosis vel variabiles, 315–690 × 424–776 μm, radiatim ordinatae, globosae, 85–157 μm vel subglobosae 98–166 × 93–157 μm, cum 3 parietibus: paries intimus membranaceus, minus quam 1 μm crassus; medius laminatus, atrobrunneus vel ater, 3–14 μm; extimus reticulatus, subhyalinus 2–4 μm, evanescens. Sporae hyalinae in texturis hospitis dispersae, globosae, subglobosae, vel ellipsoideae 54–197 × 44–163 μm, cum 2–3 parietibus hyalinis; paries intimus membranaceus, 0.4–1 μm crassus; medius laminatus, 2–4 μm; extimus usquae ad 4 μm, evanescens.

Sporocarps dark brown to black, subglobose to highly variable in shape, 315–690 × 424–776 μm, consisting of a single layer of chlamydospores originating from a central core of thick interwoven hyphae. Peridium absent. Two spore types produced. Chlamydospores produced solely in sporocarps in soil or aggregated around roots, dark brown to black, predominantly globose and 85–157 μm diam to occasionally subglobose and 98–166 × 93–157 μm. Sporocarp aggregates up

to 18 × 12 × 2 mm. Sporocarps originating from and connected by a broad (20 μm) thick-walled (3 μm) hypha. Spores in sporocarps mature asynchronously by budding from a sporogenous cell. Spores with three walls. Inner wall membranous, less than 1 μm thick. Middle wall laminate, dark brown to black, 3–14 μm thick, confluent with hyphal attachment. Outer wall reticulate, ephemeral, 2–4 μm thick, subhyaline, and extending for entire length of hyphal attachment to center of sporocarp. Reticulum an ordered arrangement of hexagonal plates 3–9 μm wide. Outer wall often fracturing near the point of hyphal attachment on detached spores resulting in a collar circumscribing the attachment. Hyphae at the point of attachment 10–24 μm wide. Spores rarely with two adjacent attachments measuring 31–34 μm wide. Hyphal attachment frequently branched. Spore contents hyaline, non-globular, and separated from hyphal attachment by inner membranous wall or an occluded pore. Second spore type formed singly or in unordered sporocarps in roots or dead host tissue. Spores subhyaline to hyaline, globose to subglobose, ellipsoid or highly variable in shape, 54–197 × 44–163 μm with two or occasionally three walls. Inner wall membranous, 0.4–1 μm thick. Middle wall laminate, hyaline to subhyaline, 2–4 μm thick. Outer wall hyaline, evanescent, up to 4 μm thick. Hyphae at point of attachment 5–10 μm wide. Reaction to Melzer's reagent negative. Forming typical vesicular-arbuscular mycorrhizae.

ETYMOLOGY: Latin: ambi = both; sporum = spore; referring to the dimorphic nature of this fungus.

TYPE: Spores from a pot culture of Bahia grass (*Paspalum notatum* Flugge) initiated from soil collected at 1219 N.W. 43 Ave., Gainesville, Fla. HOLOTYPE OSC No. 44,289; ISOTYPES FH; FLAS No. F-54259.

Observations.—This species is known only from a single collection of an unidentified grass species in a home garden in Gainesville, Florida. In Bahia grass pot cultures, 6–12 months are required for sporocarp formation. Hyaline spores have been recovered only when roots or dead leaf sheaths were macerated mechanically prior to wet-sieving. For these reasons, this species may be easily overlooked and be more widely distributed than our observations indicate. While it is possible that these hyaline spores are merely an immature stage of the darker spore, the hyaline spores were observed only inside host tissue whereas the darker spore type was found only in sporocarps external to host tissue. Bahia grass pot cultures have been established from single pigmented sporocarps or hyaline spore clusters of *G. ambisporum* and maintained for up to 24 months. Both spore types are always produced, and they retain their original appearance and characteristics. In greenhouse studies, *G. ambisporum* forms typical VA mycorrhizae on Bahia grass (*P. notatum*), cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max* (L.) Merr.].

Glomus heterosporum Smith & Schenck, sp. nov. FIGS. 3A–F; 4C, D; 5B

Sporae ex sporophoris septatis separatae, dimorphae: brunneae crasse tunicatae aut hyalinae tenuiter tunicatae. Sporae brunneae in solo in sporocarpis pallide brunneis vel atrobrunneis, globosis vel subglobosis, 242–726 × 242–641 μm radiatim ordinatae, obovoideae, globosae vel ellipsoideae, 99–206 × 61–201 μm, cum 2 parietibus: paries intimus laminatus, 3–10 μm; extimis levis, hyalinus, 2–7 μm, evanescens. Sporae hyalineae in texturis hospitis solitariae vel in sporocarpis inordinalitep aggregatae, globosae, valde variabiles, 31–102 × 27–68 μm, cum 3 parietibus hyalinis; paries extimus membranaceus, usquae ad 1 μm crassus; medius 1–2.6 μm; extimus minus quam 1 μm, evanescens.

Sporocarps light to dark brown, globose to subglobose, 242–726 × 242–641 μm, consisting of a single, ordered layer of chlamydospores originating from a central core of thick interwoven hyphae. Peridium absent. Two spore types produced. Chlamydospores produced in sporocarps, light to dark brown, obovoid to

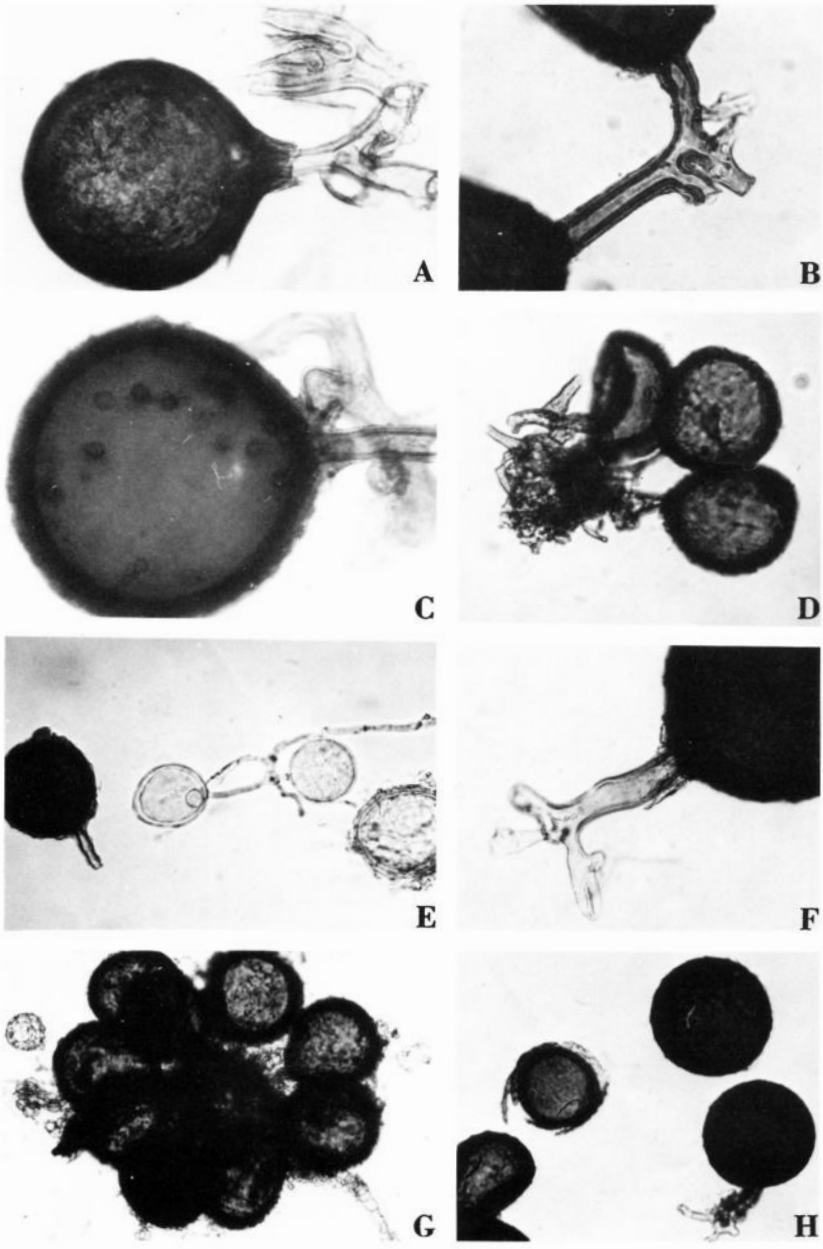


FIG. 1A–H. *Glomus ambisporum*. A. Sporocarpic globose chlamydospore with branched attachment and outer wall forming a collar around the attachment, ×300. B. Two sporocarpic chlamydospores with outer wall extending down subtending hyphae, ×300. C. Sporocarpic chlamydospore with two hyphal attachments; the globoid structures within the spore are probably hyperparasites, ×400. D. Thick, interwoven hyphae at center of sporocarp, ×175. E. Hyaline, thin-walled chlamydospores from host tissues adjacent to a dark brown, sporocarpic chlamydospore, ×150. F. Sporocarpic chlamydospore with a tripodal attachment, ×370. G. Sporocarp, ×130. H. Sporocarpic chlamydospore with outer wall fractured, × 150.

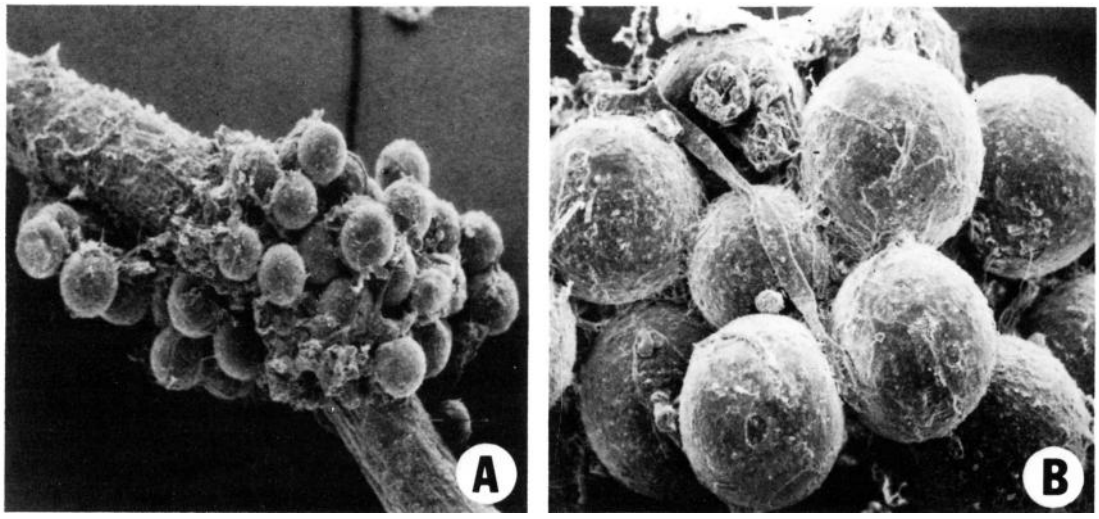


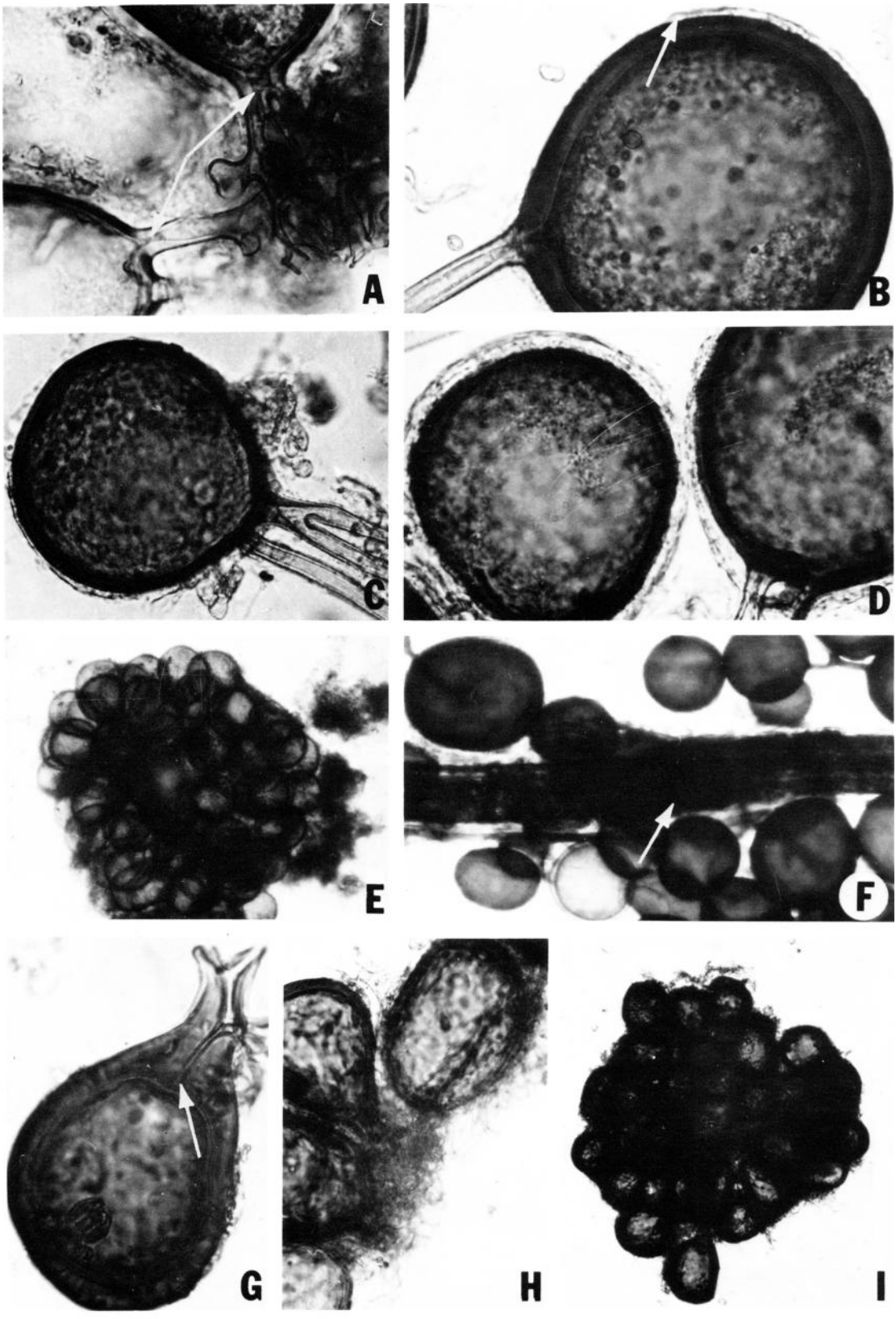
FIG. 2A, B. *Glomus ambisporum*. Scanning electron micrographs. A. Sporocarp forming around Bahia grass root, ×75. B. Outer walls of sporocarpic spores, ×220.

ellipsoid, occasionally globose, 99–206 × 61–201 μm. Sporocarp aggregates occasionally formed. Spores in sporocarps maturing asynchronously by budding from a sporogenous cell. Spores with two distinct walls. Inner wall laminate, brown, 3–10 μm thick. Outer wall smooth, evanescent, hyaline, 2–7 μm thick, frequently absent on aged spores. Hypha at point of attachment 5–31 μm wide. Spores frequently with multiple hyphal attachments in a 43:15:1 ratio of single, dual, and triple attachments. Hyphal attachments frequently branched. Spore contents hyaline, non-globular, and separated from hyphal attachment by a septum. Second spore type formed singly or in unordered sporocarps in host tissues, testa, or *Rhizobium* nodules. Spores hyaline, globose to highly variable in shape, 31–102 × 27–68 μm diam. Spore with three hyaline walls. Inner wall membranous, up to 1 μm thick. Middle wall unit, 1–2.6 μm thick. Outer wall evanescent, less than 1 μm thick. Hypha at point of attachment 5–7 μm wide. Reaction to Melzer's reagent negative. Forming typical vesicular-arbuscular mycorrhizae.

ETYMOLOGY: Greek: hetero = different; Latin: sporum = spore; referring to the dimorphic nature of this fungus.

TYPE: Spores from a single-spore pot culture of Bahia grass initiated from sporocarps collected from soybean microplots at the Agricultural Research Center, Live Oak, Florida. HOLOTYPE OSC No. 44,288; ISOTYPE FH; FLAS No. F-54260.

Observations.—*Glomus heterosporum* was collected initially from soil associated with roots of soybean (*G. max*) near Live Oak, Florida, and an unidentified grass species at the Agronomy Farm, University of Florida, Gainesville. In greenhouse studies it forms typical VA mycorrhizae on Bahia grass (*P. notatum*); sweetgum (*Liquidambar styraciflua* L.); and cotton (*G. hirsutum*). Single spore cultures have reproduced both spore types. *Glomus heterosporum* has been maintained in Bahia grass pot culture for up to 24 months and it retains its original appearance and characteristics. In Bahia grass or sorghum pot cultures, 4–8 months are usually required for sporocarp formation. The smaller, hyaline spores found within roots, testas, or *Rhizobium* nodules, develop within 4 months and thereafter occur concomitantly with the sporocarpic spores. Sporocarps of the hyaline spore type do not contain an ordered arrangement of chlamydospores.


A
B
C
D
E
F
G
H
I

DISCUSSION

Four ontological and morphological features warrant the assignment of *G. ambisporum* and *G. heterosporum* to the genus *Glomus* even though both possess the spore-free central plexus which is the single most critical morphological character of the genus *Sclerocystis*. These features include: (1) the loose aggregation of chlamydospores in the sporocarp as contrasted with the tightly appressed spores of species in the genus *Sclerocystis*, (2) the thick, coarse, loosely interwoven hyphae composing the central plexus of the sporocarp as contrasted with the tightly interwoven, fine hyphae characteristic of *Sclerocystis* species, (3) the presence of a second spore type that clearly belongs in the genus *glomus*, and (4) the apparent asynchronous spore ontogeny within a single sporocarp. Gerdemann and Trappe (**4**) stated, "*Sclerocystis* is very closely related to *Glomus*, differing from it only in the orderly arrangement of chlamydospores within the sporocarp. In *Sclerocystis* the spores are tightly arranged in a single layer surrounding a central plexus of sterile hyphae . . . synonymizing the two genera would serve no useful purpose at this time." Hall (**6**) reported that *S. rubiformis* Gerd. & Trappe sporocarps often aggregated into mats up to 20 mm diam, incorporating varying amounts of soil. An aggregate comprising over 300 individual sporocarps has been observed once in *G. heterosporum*, and aggregation is a frequent phenomenon in *G. ambisporum*. Clearly these three species share numerous similar macroscopic characters (FIGS. 1G; 3E, I). However, the absence of a septum delimiting spore contents, wall structure, spore size, multiple attachments, and the loose, thick hyphae comprising the central plexus clearly separates *G. ambisporum* and *G. heterosporum* from *S. rubiformis*.

Glomus ambisporum and *G. heterosporum* were isolated originally within 10 km of each other. They are similar in sporocarp morphology, and both are dimorphic. However, these two species have been maintained in nearly adjacent pot cultures over a period of 4 yr under identical conditions of host, soil type, and environment. Sufficient characters persist to distinguish the two species. Whereas sporocarps and spores are black or nearly so in *G. ambisporum* and light to dark brown in *G. heterosporum*, these two species are most easily distinguished by wall morphology. In *G. ambisporum* the outer wall on sporocarpic spores is faintly reticulate, rarely as thick as the inner wall, and never hyaline. The wall on the hyaline spore is very similar to the sporocarpic spore wall, differing primarily in its dimensions and absence of melanized pigments (FIG. 5A). Sporocarpic spores of *G. heterosporum* possess a laminate inner wall with a smooth, hyaline, evanescent outer wall. The outer wall may become as thick as the inner laminate wall. The wall on the hyaline spore differs from the sporocarpic spore wall in the presence of the inner membranous wall and the lack of laminations or melanized pigments (FIG. 5B). In addition, the hyphal contents in *G. heterosporum* are delimited by a septum whereas an occluded pore or membranous wall occurs in *G. ambisporum*.

←

FIG. 3A–F. *Glomus heterosporum*. A. Thick, interwoven hyphae at center of sporocarp connecting two chlamydospores; arrows point to septa delimiting spore contents, ×340. B. Immature chlamydospore with outer wall in initial developmental stages, ×390. C. Chlamydospore with dual attachment, one branched, ×300. D. Two chlamydospores with hyaline outer wall highlighted, ×395. E. Sporocarp lacking a peridium, ×75. F. Hyaline spores in cortex of Bahia grass root stained with trypan blue in lactophenol; arrow denotes region of root stele, ×210. FIG. 3G–I. *Sclerocystis rubiformis* paratype #2150 OSC herbarium. G. Mature chlamydospore with single wall; pore (arrow) connects spore contents with hyphal attachment, ×500. H. Three chlamydospores enmeshed in central plexus of fine interwoven hyphae, ×300. I. Sporocarp without peridium, ×125.

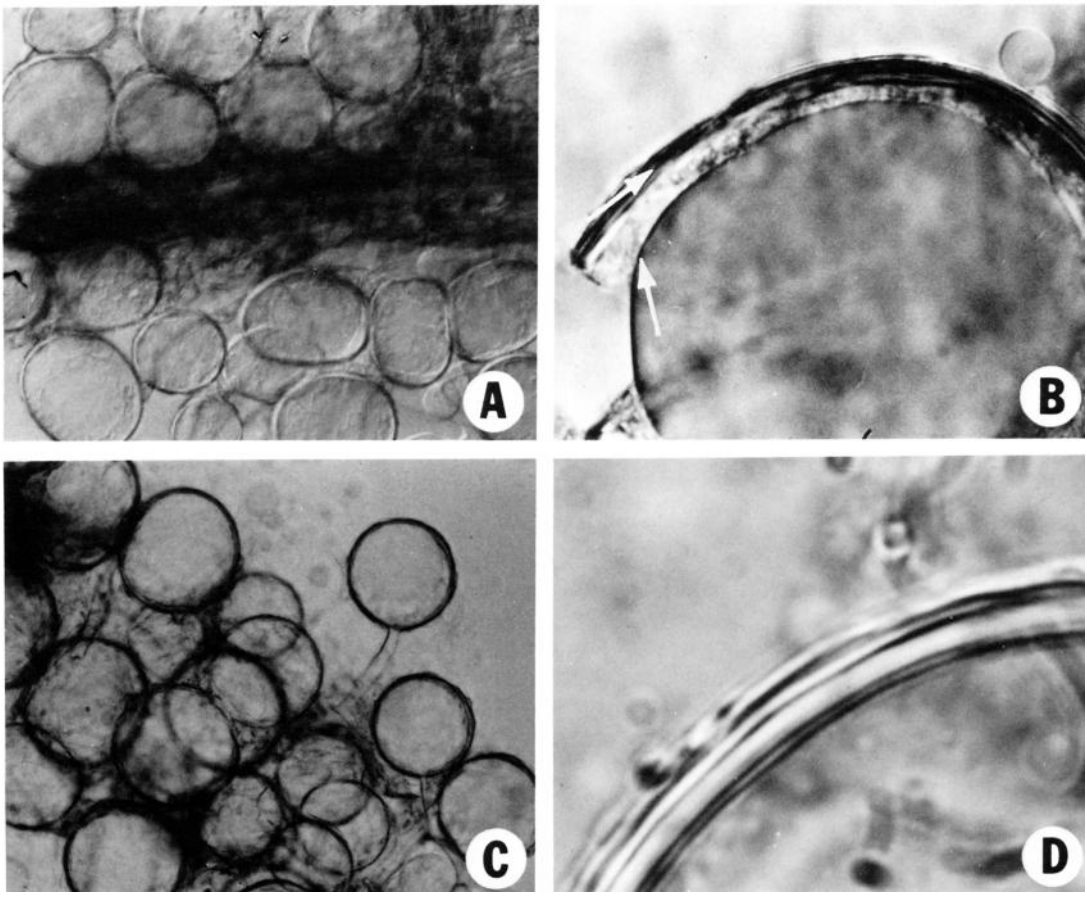


FIG. 4A, B. *Glomus ambisporum*. A. Hyaline spores clustered in dead parenchyma tissue of Bahia grass leaf sheath, ×150. B. Hyaline spore with wall fractured; arrows point to inner membranous wall and outer wall, ×675. FIG. 4C, D. *Glomus heterosporum*. C. Sporocarp of hyaline spores extracted from Bahia grass testa, ×120. D. Three walls of hyaline spore, ×2500.

Glomus ambisporum sporocarps superficially resemble *S. rubiformis* but can be distinguished from that species by the large and thick-walled chlamydospores which have a reticulate outer wall and also by the lack of a central plexus consisting of tightly interwoven, thin hyphae. The reticulum present on the outer wall is extremely difficult to detect with the light microscope even at high magnification. Scanning electron micrographs also fail to resolve the reticulum (FIG. 2) suggesting that it is destroyed by the fixation procedure or is present within the outer wall. Sporocarps recovered from soil are frequently coated with debris which seems to obscure the reticulum, and on these spores the outer wall appears to be slightly roughened. On spores recovered from aggregated sporocarps, the reticulum appears as an ordered arrangement of hexagonal plates on the outer wall. *Glomus ambisporum* could also be mistaken for *Glomus melanosporum* Gerd. & Trappe (**4**) but can be distinguished from it by the lack of exuding latex from cut sporocarps and the absence of a color gradation in the chlamydospore laminate wall. Single chlamydospores of *G. ambisporum* that become dislodged from sporocarps would resemble most closely *Glomus geosporum* (Nichol. & Gerd.) Walker (**8**). Spores of *G. ambisporum* can be separated from those of *G. geosporum* by their generally

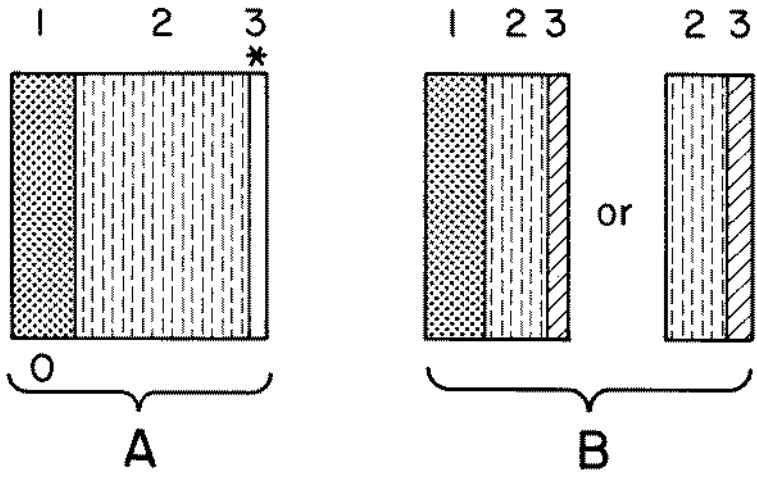


Figure 5a. *Glomus ambisporum*

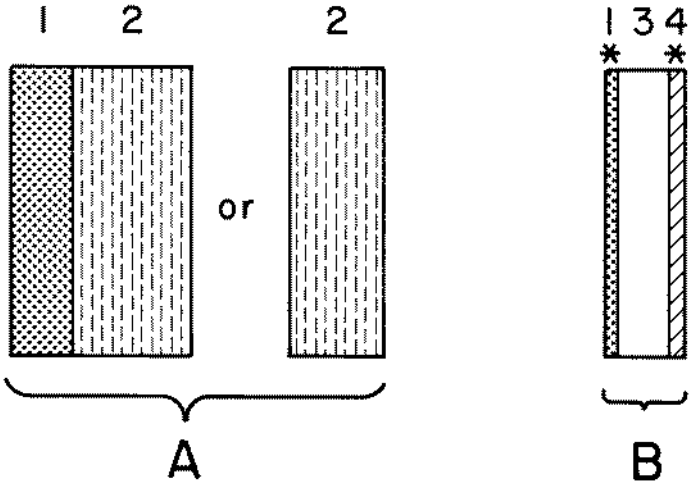


Figure 5b. *Glomus heterosporum*

FIG. 5A, B. Murographs of *Glomus ambisporum* and *Glomus heterosporum*. A. Wall structure of sporocarpic spore (A) and hyaline spore (B). Wall 1 is evanescent, wall 2 is laminate, wall 3 is membranous. B. Wall structure of sporocarpic spore (A) and hyaline spore (B). Wall 1 is evanescent, wall 2 is laminate, wall 3 is unit, and wall 4 is membranous. * = wall difficult to detect; O = wall sometimes ornamented.

smaller size and by the ease with which the spore walls separate (FIG. 1H). The separable outer wall is evanescent, but requires considerable aging before disintegration. The inner membranous wall is tightly appressed to the laminate middle wall and is not clearly distinguished by crushing the spores or immersion in hypertonic solution. Additionally, sporocarp aggregates may be connected in a funiform manner by a single, broad, thick-walled hypha. The presence of multiple sporocarps originating from a single fecund hypha has not been reported in the

Endogonaceae (7). Chlamydospore development within sporocarps is asynchronous in a manner similar to blastic conidial formation (3).

Glomus heterosporum most nearly resembles *S. rubiformis* (FIG. 3G–I), but its differentiation is based on the occurrence of two spore types, the presence of a septum delimiting the spore contents, the outer hyaline wall, generally larger spore size, and frequent occurrence of multiple attachments. Paratypes 3203 and 2874 of *S. rubiformis* from Trappe's collections bear a close resemblance to *G. heterosporum*. They and other paratypes in this collection are perhaps more closely related to *G. heterosporum* than to *S. rubiformis*. They possess two walls, a septum, and their spore diameter range is 34–112 × 31–95 μm. Spores of the *S. rubiformis* holotype 2108 and paratypes 2150 and 3141 have only one wall and their spore contents are delimited by an occluded pore. These differences warrant the erection of a new species distinct from *S. rubiformis*. Frequently, chlamydospores become dislodged from sporocarps and may lack the outer wall. These spores can be confused with either *Glomus macrocarpum* Tul. & Tul. (2, 5) or *Glomus etunicatum* Becker & Gerd. as their size ranges overlap and both possess pigmented laminate walls. *Glomus etunicatum* also has the hyaline, ephemeral outer wall that sloughs off with age (1). Clearly, intact sporocarps are needed to identify this species accurately.

ACKNOWLEDGMENTS

We thank Dr. E. S. Luttrell (University of Georgia), Dr. C. Walker (Forestry Commission, Scotland), and Dr. J. M. Trappe (USDA, Forest Service) for kindly reviewing this manuscript and for their helpful suggestions. Special thanks are extended to Dr. Luttrell for preparing the Latin descriptions. We also thank Vi Wilmot (University of Georgia) for preparing the SEM photographs.

LITERATURE CITED

1. **Becker, W. N., and J. W. Gerdemann.** 1979. *Glomus etunicatum* sp. nov. *Mycotaxon* **6**: 29–32.
2. **Berch, S. M., and J. A. Fortin.** 1983. Lectotypification of *Glomus macrocarpum* and proposal of new combinations: *Glomus australe, Glomus versiforme,* and *Glomus tenebrosum* (Endogonaceae). *Canad. J. Bot.* **61**: 2608–2617.
3. **Cole, G. T., and R. A. Samson.** 1979. *Patterns of development in conidial fungi.* Pitman Publishing Ltd., London. 190 p.
4. **Gerdemann, J. W., and J. M. Trappe.** 1974. *The Endogonaceae in the Pacific Northwest.* Mycologia Memoir No. 5, 76 p.
5. **Nicolson, T. H., and N. C. Schenck.** 1979. Endogonaceous mycorrhizal endophytes in Florida. *Mycologia* **71**: 178–198.
6. **Hall, I. R.** 1977. Species and mycorrhizal infections of New Zealand Endogonaceae. *Trans. Brit. Mycol. Soc.* **68**: 341–356.
7. **Trappe, J. M., and N. C. Schenck.** 1982. Taxonomy of the fungi forming endomycorrhizae. Pp. 1–9. *In: Methods and principles of mycorrhizal research.* Ed., N. C. Schenck. Amer. Phytopathol. Soc., St. Paul, Minnesota.
8. **Walker, C.** 1983. Species in the Endogonaceae: a new species (*Glomus occultum*) and a new combination (*Glomus geosporum*). *Mycotaxon* **15**: 49–61.

Accepted for publication December 17, 1984