

Mycologia, 91(6), 1999, pp. 1083–1093.

© 1999 by The Mycological Society of America, Lawrence, KS 66044-8897

Glomus eburneum and *G. luteum*, two new species of arbuscular mycorrhizal fungi, with emendation of *G. spurcum*

Linda J. Kennedy

Jean C. Stutz¹

Department of Plant Biology, P.O. Box 1601-87,
Arizona State University, Tempe, Arizona 85287-1601,
USA

Joseph B. Morton

Division of Plant and Soil Sciences, 401 Brooks Hall,
P.O. Box 6057, West Virginia University,
Morgantown, West Virginia 26505-6057, USA

Abstract: Two species of arbuscular mycorrhizal fungi, *Glomus eburneum* and *G. luteum*, are described and the description of *G. spurcum* is emended. All species produce spores singly in soil. *Glomus eburneum* spores are hyaline or white to cream, usually irregular (40×60 to $140 \times 160 \mu\text{m}$ diam) and more rarely globose (40 – $140 \mu\text{m}$ diam). The spore wall consists of two adherent permanent hyaline layers, neither of which reacts in Melzer's reagent. The outer layer is thin ($<1.2 \mu\text{m}$), and the inner layer is finely laminate. Spores of *G. luteum* are globose to subglobose, 60 – $180 \mu\text{m}$ diam and pale yellow to dark yellow with a brownish tint. The spore wall consists of four layers, the two outer layers often degrading at maturity. The outer layer is mucilaginous and stains pinkish-red in Melzer's reagent. The second layer is hyaline and semirigid. Rigid, pale yellow to brownish yellow laminae comprise the third layer. Mature spores exhibit a fourth layer that is thin, flexible and may separate from the laminate layer under pressure. Both species were identified from pot cultures established with soil and root fragments from a semiarid giant sacaton (*Sporobolus wrightii*) grassland and subsequently cultured on sudangrass, *Sorghum sudanense*. Both species formed arbuscular mycorrhizae in pot cultures with corn (*Zea mays*) as the host.

Key Words: arid, classification, Glomales, semiarid, taxonomy, vesicular-arbuscular mycorrhizae, Zygomycetes

INTRODUCTION

In several studies of mycorrhizal fungal communities along rivers and streams of southeastern Arizona, two

undescribed species were found associated with *Sporobolus wrightii* Monro ex Scribn., a perennial tallgrass of southwestern North America. The species from Arizona grouped with reference isolates of undescribed species in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and, therefore, represent true phylogenetically delimited species (Morton et al 1993). We describe these species, *Glomus eburneum* and *G. luteum*, based on developmental patterns and morphological properties of mature spores as defined for the genus *Glomus* by Morton (1996) and Stürmer and Morton (1997).

MATERIALS AND METHODS

Samples consisting of soil and root fragments were collected from the base of giant sacaton, *Sp. wrightii*. All samples were transported in insulated carriers to laboratory facilities at Arizona State University (ASU) and stored at 5.6 C . A subsample of 500 cm^3 from each sample was mixed with #12 and #20 silica sand (1:1:1 v/v) that had been autoclaved for 1 h at 120 C , placed in grower's pots (3 L) and overseeded (60 – 100 seeds) with sudangrass, *Sorghum sudanense* (Piper) Staph. Cultures were maintained in a glasshouse at day and night temperatures of 26 C and 15 C respectively with natural lighting of $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. After 14 wk, pot contents were harvested and stored at 5.6 C until examination.

Spores were separated from each pot culture sample by wet sieving and decanting followed by centrifugation in a 20–60% sucrose density gradient (Daniels and Skipper 1982). Spores of each morphotype were collected from trap cultures and pipetted onto roots of 10–12 d old sorghum (*Sorghum bicolor* L.) seedlings which were then transplanted into $4 \times 21 \text{ cm}$ cone-tainers (Stuewe and Sons, Corvallis, Oregon) and grown for 120 d in a growth room at West Virginia University (WVU) with a temperature range of 21 – 28 C , $225 \mu\text{mol m}^{-2} \text{ s}^{-1}$ light intensity at pot level and a 14-h photoperiod.

Of the two species described in this paper, only monospecific cultures of *G. eburneum* were successfully established, and these cultures were transplanted to 750 cm^3 deepots (Stuewe and Sons, Corvallis, Oregon). Sudangrass (*S. sudanense*) seed was sown into the surrounding soil and grown for 4 mo to increase inoculum and verify purity. Cultures of *G. luteum* from other living accessions in INVAM were grown for taxonomic study concurrently with those of *G. eburneum* using standardized methods (Morton et al 1993).

Morphological properties of spores from each fungal spe-

Accepted for publication July 7, 1999.

¹ Corresponding author, email: jstutz@asu.edu

cies were examined and measured using a Nikon E600 Infinity microscope equipped with differential interference contrast optics. Selected images were captured using a Sony CCD video camera and printed using a Tektronic Phaser Model 450 dye-sublimation printer. Color versions of the photographs in this paper can be viewed on INVAM's World Wide Web site at <http://invam.caf.wvu.edu>.

Color of spores was determined under reflected light from a two-branch fiber optic illuminator (color temp 3400 K) to co-illuminate spores and a printed INVAM color chart (available from JBM). Colors on the chart are described numerically as percentages of cyan, magenta, yellow, and black.

Whole spores were collected, washed and stored in 0.05% sodium azide at 4 C for preservation. Permanent slides were prepared by mounting spores in polyvinyl-lactic acid-glycerin (Koske and Tessier 1983) alone and 1:1 (v/v) with Melzer's reagent. Vouchers are deposited in INVAM; at ASU; Oregon State University (OSC), Corvallis, Oregon; and Farlow Herbarium (FH), Harvard University, Cambridge, Massachusetts.

To examine mycorrhizal morphology, two *Zea mays* L. seeds were placed in actively growing deepots of *G. eburneum* AZ420A, *G. luteum* SA112, and *G. spurcum* NB125 cultures and maintained at WVU under conditions described above. Roots of corn plants were removed after 60 and 90 d and stained in 0.05% direct blue using the procedure of Koske and Gemma (1989).

TAXONOMY

Glomus spurcum is similar to one of the new species, *G. eburneum*. Spores more closely resemble those of *G. eburneum* than the protologue would suggest and a formal emendation therefore is necessary for an accurate comparison.

Glomus spurcum Pfeiffer, Walker et Bloss emend. Kennedy, Stutz et Morton Figs. 1–4

Spores formed singly in soil; usually globose to subglobose, rarely irregular; white when newly formed with clear centers (Fig. 1), becoming pale yellow-brown with more opaque contents with age or after prolonged storage. Amongst seven isolates from three continents, spores showed a much greater size range (40–120 μm) than described by Pfeiffer et al (1996). Spore populations of three isolates in culture (INVAM accessions AZ420B, CU126, and SC151) did not exceed 80 μm diam, whereas those of other isolates (INVAM accessions HA567, NB102A, NB106A, and TX144) produced less than 20% of spores smaller than 80 μm . Spore wall consists of two adherent hyaline layers (Figs. 2–4). The outer layer (1) is as described by Pfeiffer et al (1996): hyaline, <1 μm thick, flexible, and frequently separating (sometimes completely) from the remainder of the spore wall (Fig. 2). Since this outer layer does not react in Melzer's reagent, spores are unlikely to have a mucilaginous coating as described; rather it is an accumulation of organic matter. A mucilaginous layer is found on spores of many *Glomus* species, and it can be detected unmistakably

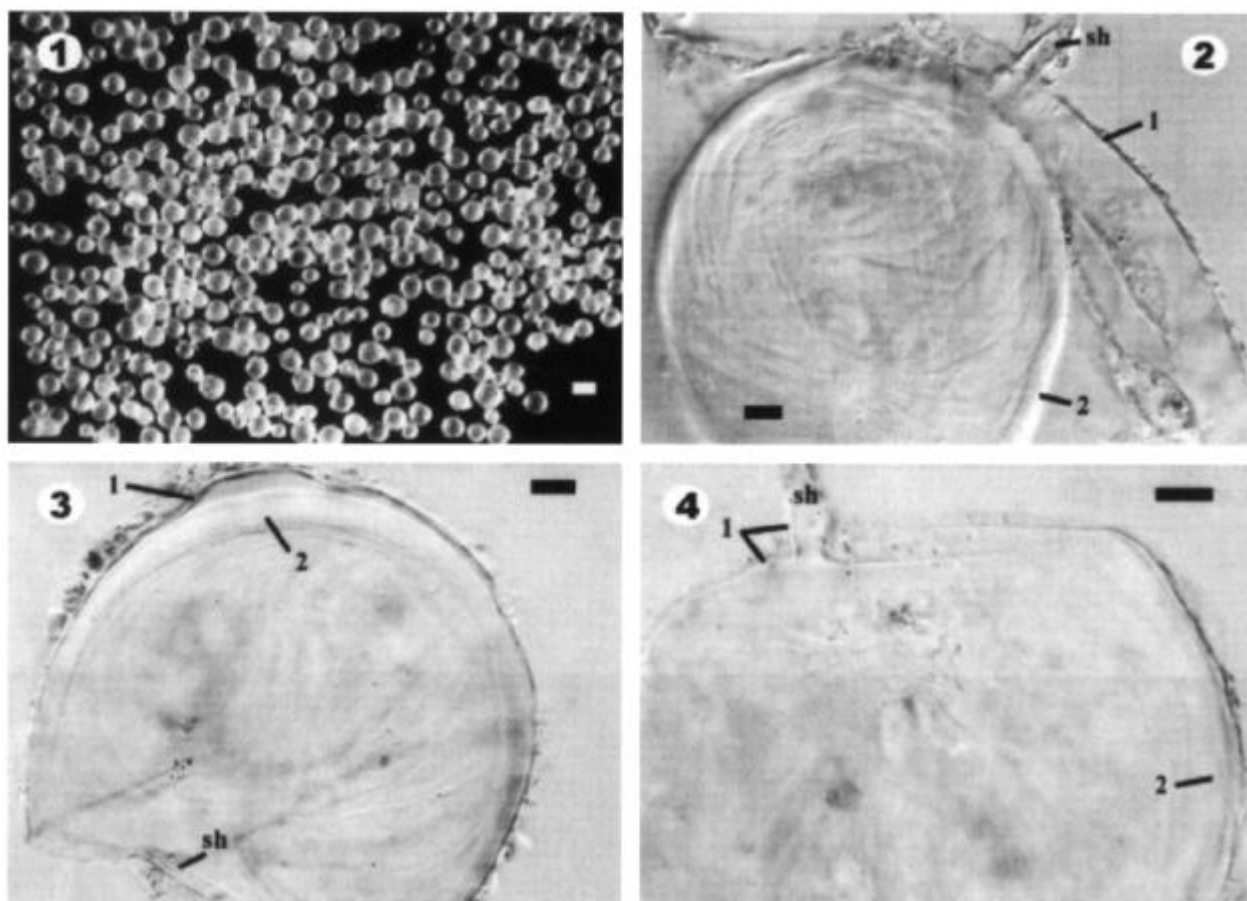
in Melzer's reagent, where it produces a pale to dark dextrinoid reaction, depending on thickness (see Figs. 17, 27). Pfeiffer et al (1996) describe two additional "walls" (layers in this paper), whereas we detected only one in all isolates examined. This layer (2) is laminate and is flexible enough to produce numerous folds on its inner surface when broken and pressure applied (Figs. 2, 3). These folds could easily be misinterpreted as a thin flexible membranous layer, but no phenotype could be discerned which indicated a distinct structure. No part of the laminate layer reacted in Melzer's reagent. Subtending hyphae were found on less than 5% of spores on any slide. As described by Pfeiffer et al (1996), it is straight and thin-walled (< 1 μm thick), and continuous with the outer layer of the spore wall (Fig. 4). There is no gradual thinning of the spore to hyphal wall, as is common in spores of most *Glomus* species. The laminate layer of the spore wall abruptly thins at the spore base to the extent that there is no visual evidence that it continues as an inner layer of the hyphal wall.

Collections examined. UNITED STATES. ARIZONA: University of Arizona pot culture, C. M. Pfeiffer, INVAM Voucher #849, 23 Jun 1990. Cochise County. From lower floodplain terrace, 17 Jun 1998, J. Stutz and L. Kennedy, INVAM culture AZ420B. TEXAS: Near Tornillo, 30 May 1992, J. Stutz and C. Martin, INVAM culture TX144. SOUTH CAROLINA: Huntington State Park beach dune, 31 Jul 1998, B. Thomas, INVAM culture SC151. HAWAII: Island of Kauai, 7 Mar 1994, R. Koske, INVAM culture HA567. CUBA: Site unknown, 9 Oct 1995, R. Herrera, INVAM culture CU126. NAMIBIA. Dune transect, 4 Jun 1993, C. Klopatek, INVAM cultures NB102A, NB106A.

Glomus eburneum Kennedy, Stutz et Morton, sp. nov. Figs. 5–13

Mycorrhizas arbusculares formans. Sporae singulatae eiformatae; hyalinae vel eburneae; globosae 40–140 μm in diam vel irregulares 40 \times 60–140 \times 160 μm in diam. Parietis sporis stratis duobus; stratum exterius hyalinis, <1–1.2 μm crassum; stratum interius laminare, flexibilis, hyalinis, 1.2–3.8 μm crassum. Hypha subtendentes 3–6.5 μm in diam; septum recurvatus, 1–2 μm crassum, 1–10 μm sub spora.

Spores formed singly in soil; mostly irregular, 40 \times 60 to 140 \times 160 μm diam; more rarely globose, 40–140 μm diam (mean = 83 μm , n = 160) (Fig. 5A); bright white when newly formed (Fig. 6), becoming a pale ivory color (0-0-10-0) in old spores or those stored for long periods, less frequently hyaline. The spore wall consists of two adherent hyaline layers (Figs. 7–9). The outer layer (1) is < 1–1.2 μm thick, semiflexible, and usually remains intact long after the spore has matured. The inner layer (2) also is semiflexible (forming folds in broken spores) and finely laminate, 1.2–3.8 μm thick (mean = 2.3 μm , n = 87). Neither layer reacts in Melzer's reagent. At the point of attachment to the spore, the subtending hypha (Figs. 7, 9) is cylindrical to slightly flared, 3.0–6.5 μm (mean = 4.9 μm , n = 40). In mature spores,



FIGS. 1–4. *Glomus spurcum* (accession HA567A). Whole spores in water; broken spores in PVLG and Melzer's reagent (1:1 v/v). All micrographs of mounted spores were taken using differential interference contrast optics. 1. Whole spores in water are consistently globose or subglobose in shape and have transparent centers due to fusion of oil globules. 2. Spore wall with separating outer hyaline layer (1) from the semiflexible inner laminate layer (2). The subtending hypha (sh) stays with the outer layer. 3. Spore with outer layer (1) more adherent to the inner laminate layer (2) which is spreading to varying degrees with differentially applied pressure, and an attached subtending hypha (sh). 4. Spore showing continuity between the outer spore wall layer (1) and the only visible layer of the subtending hypha (sh). Note absence of the inner spore wall layer (2) along the length of the hypha. Bars: 1 = 100 μm ; 2–4 = 10 μm .

a recurved septum, 1.0–2.0 μm thick, forms from a continuation of the laminate layer of the spore wall, positioned 1.0–10 μm in the hyphal lumen. The subtending hyphal wall consists of a continuation of both layers of the spore wall to the septum, where it is 1.0–2.0 μm thick, after which it consists of only the outer layer of the spore wall and is <1.0 μm thick.

Mycorrhizae. Fungal structures are detectable in roots, but are often hard to see because of variable staining intensity in direct blue. Densely branched arbuscules are darkest and become less visible as they senesce (FIGS. 10, 11). Arbuscules develop predominantly from intracellular spread of hyphae. Coiled hyphae, many of which are clustered near entry points, are 3.0–10 μm wide (FIG. 12) and often are localized within cortical cells. Straight hyphae are 2.0–5.0 μm in width and usually stain lightly (FIG. 13). No vesicle formation was observed in corn roots

from 4-month-old pot cultures, even after plant senescence. This mycorrhizal morphology is not typical for most *Glomus* species, but is shared by a few species in both *Glomus* and *Acaulospora*, namely *G. occultum*, *G. gerdemannii*, *A. gerdemannii*/*G. leptotrichum*, and *A. trappei* (Morton et al 1997, Morton unpubl). *Glomus spurcum* was described as forming similar mycorrhizae on *Plantago lanceolata* (Pfeiffer et al 1996), but its mycorrhizae on corn appear to be more typical (continuous overlapping infection units, darkly staining arbuscules and hyphae) except for higher frequencies of intraradical hyphal coiling. Purity of the test isolate of *G. spurcum* (NB125) was not verified because the parent culture died, so comparisons among other cultured isolates are needed to define the conserved phenotype.

HOLOTYPE. UNITED STATES. ARIZONA: Cochise County (Sect.31, T22S, R22E). 10 Oct 1997, *J. Stutz* and *L.*

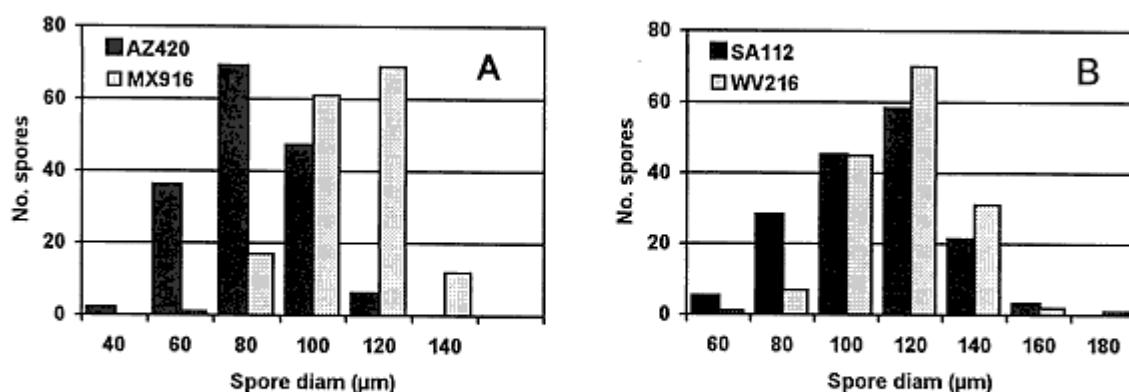
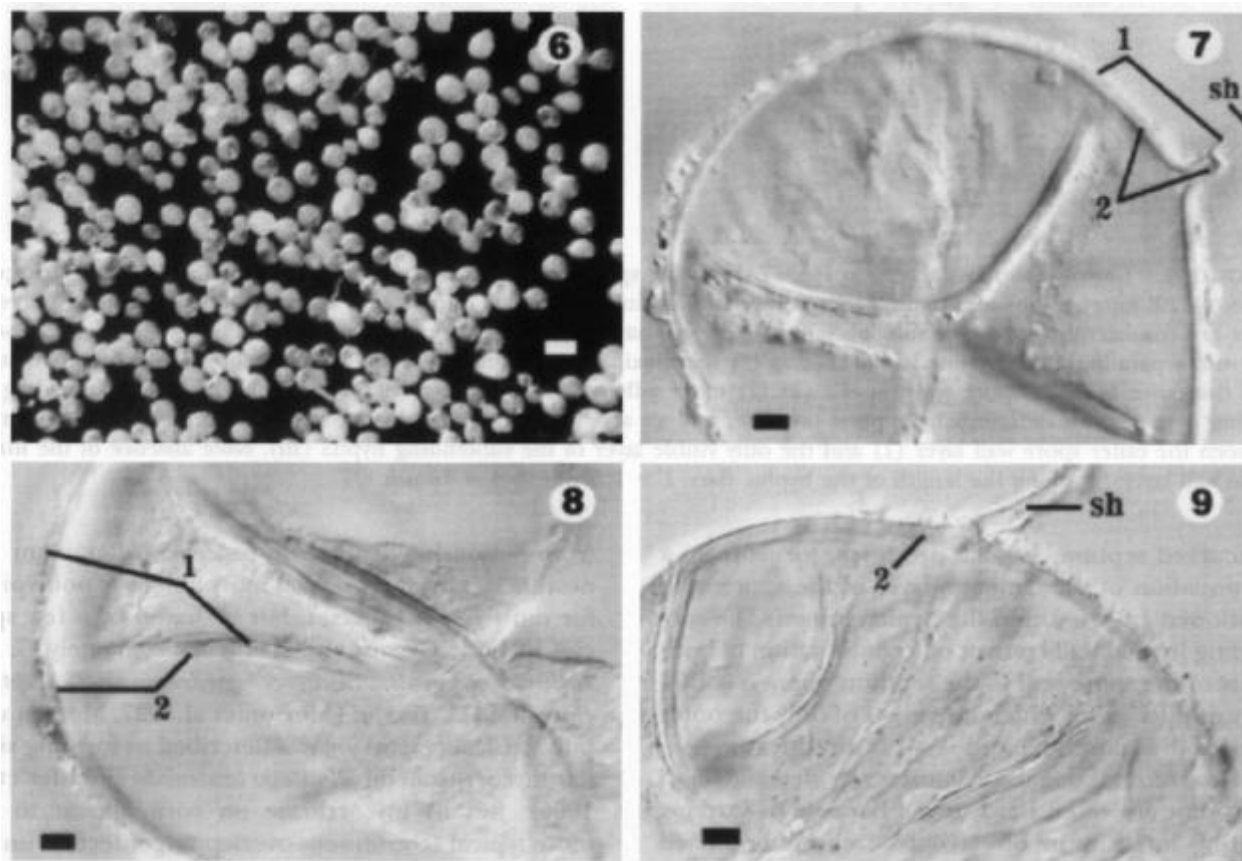
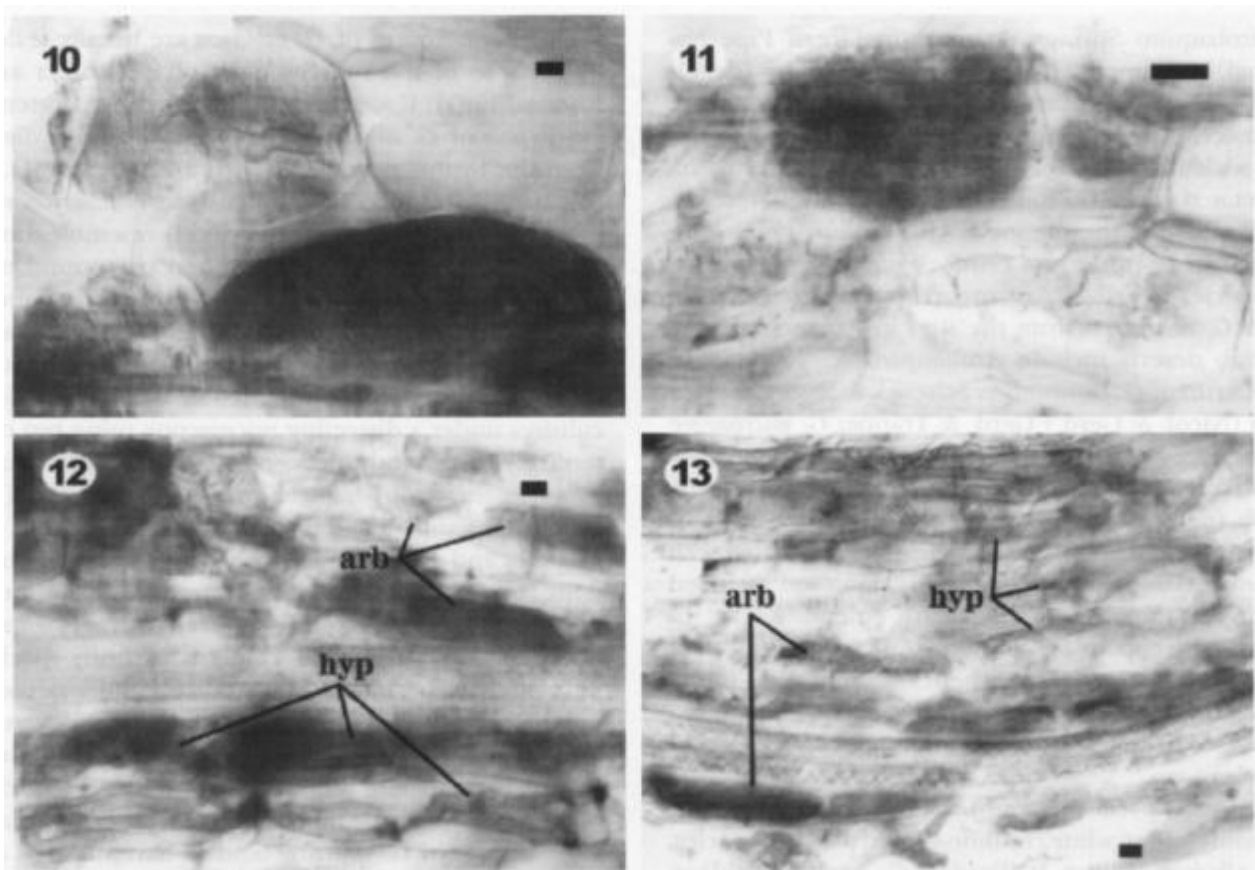


FIG. 5. Distribution of spore diameters in two geographic isolates of *Glomus eburneum* and *G. luteum*. A. *Glomus eburneum* spore populations from accessions AZ420 (■) and MX916 (□). B. *Glomus luteum* spore populations from accessions SA112 (■) and WV216 (□).



FIGS. 6–9. *Glomus eburneum* reference accession AZ420A. Whole spores were photographed in water; broken spores were mounted in PVLG unless otherwise stated. All micrographs of mounted spores were taken using differential interference contrast optics. 6. Whole spores in water are variable, obovoid or “tear-drop” in shape. Spores are bright white when contents are dense and opaque. 7. Spore in Melzer’s reagent; spore wall consisting of two hyaline layers, the outer layer (1) persistent even in aged spores and the inner layer (2) finely laminate. In the subtending hypha (sh), spore wall layer 2 forms a septum and layer 1 continues as part of the hyphal wall. 8. Spore wall layers (1, 2) form folds in crushed spores indicating some flexibility. 9. Subtending hypha (sh) showing continuity of hyphal wall layers and spore wall layers. The septum is formed from the laminate spore wall layer (2). Bars: 6 = 100 µm; 7–9 = 5 µm.



FIGS. 10–13. Mycorrhizae of *Glomus eburneum* AZ420A in roots of 2-month-old *Zea mays* seedlings, stained with 0.05% direct blue and mounted in PVLG. 10. Arbuscules with variable staining intensity. 11. Darkly stained arbuscule with dense branches, and lightly stained arbuscule after senescence, with only the trunk hypha persisting. 12. Arbuscules (arb) and coiled intracellular hyphae (hyp). 13. Spatial distribution of arbuscules (arb) and hyphae (hyp) in a mycorrhiza. Bars: 10 = 5 μm ; 11–13 = 10 μm .

Kennedy, from INVAM culture AZ420A. Deposition at OSC of preserved spores in sodium azide and broken spores mounted permanently on a glass slide. PARATYPE. MEXICO. Zacatecas. 20 Dec 1994, *E. Davies*, INVAM culture MX916A. Deposited at FH. CULTOTYPE. INVAM culture AZ420A.

Other materials examined. NAMIBIA. From a dune transect, 10 Jul 1992, *C. Klopatek*, INVAM slide #1823. UNITED STATES. ARIZONA: Cochise County. From lower floodplain terrace, 10 Oct 1997, *J. Stutz* and *L. Kennedy*, INVAM culture AZ414A. Cochise County (Sect. 4, T24S, R22E). From upper and lower floodplain terraces, 9 and 25 Mar 1997, *L. Kennedy* and *R. Tiller*, ASU slides PB-H 38 and PB-C 7, respectively. Santa Cruz County. From sacaton grassland near O'Donnell Creek, 2 Jul 1997, *L. Kennedy* and *R. Tiller*, ASU slide AR-B 97. Quitobaquito Springs (31°56'N, 113°01'W). From a ciénega, 11 Jan 1995, *J. Stutz*, *S. Hosier* and *C. Martin*, ASU slide 124DW. WEST VIRGINIA: Mingo Co. near Logan, revegetated coal strip mine site, 26 Sep 1996, *K. Heldreth*, INVAM culture WV216B.

Etymology. Latin, *eburneum*, refers to the opaque bright white color of spores in reflected light.

Distribution and habitat. This species appears to be

found in predominately arid and semiarid habitats, but was recovered from a revegetated coal mine site in West Virginia. Spores of *G. eburneum* were associated with roots of giant sacaton, *Sp. wrightii*, a native species of grass found only along rivers and streams of the semiarid regions of southwestern North America. Spores were found in soil and pot cultures from giant sacaton growing in near monoculture on upper floodplain terraces and from sacaton growing on lower floodplain terraces and from sacaton growing on lower floodplain terraces with a cottonwood (*Populus fremontii* Wats.) overstory along the San Pedro River in Arizona. Soils were typically alkaline (pH 7.8–8.2), with low organic matter (1.2–2.9%), low phosphorus (6–29 mg kg⁻¹), and low EC (1–2.8 dS/m). Soil textures of lower floodplain terraces were loam or silty loam and upper terrace soils were silty clay. This species also was found in samples from a giant sacaton grassland along O'Donnell Creek, Arizona; in cultures from soil and root fragments of screwbean mesquite (*Prosopis pubescens* Benth.) growing in soil with low organic matter (1%) and low P (3 mg kg⁻¹) at

Quitobaquito Springs, Arizona, in Organ Pipe National Monument, from a semiarid region (details unknown) near Zacatecas, Mexico; and from a dune transect in the Namib desert. Spores of *G. eburneum* were identified in soil and root fragments from a revegetated coal strip mine in Mingo County, West Virginia. Flora of the site included *Trifolium pratense* L., *Trifolium repens* L. and *Festuca elatior* L.

Species of arbuscular mycorrhizal fungi associated with *G. eburneum* from the sites in Arizona and the Namib deserts include *Acaulospora trappei* Ames & Linderman; *G. intraradices* Schenck & Smith; *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe; *G. microaggregatum* Koske, Gemma & Olexia; *G. spurcum*; *G. occultum*; and several undescribed species. *Glomus luteum* (described below) was also associated with *G. eburneum* in the Arizona sites. Species associated with *G. eburneum* in the West Virginia mine soil included *G. aggregatum* Schenck & Smith emend. Koske; *G. brasilianum* Spain & Miranda; *G. clarum* Nicolson & Schenck; *G. claroideum* Schenck & Smith; *G. intraradices* Schenck & Smith; *G. luteum*; *A. morrowiae* Spain & Schenck; and several undescribed *Glomus* species.

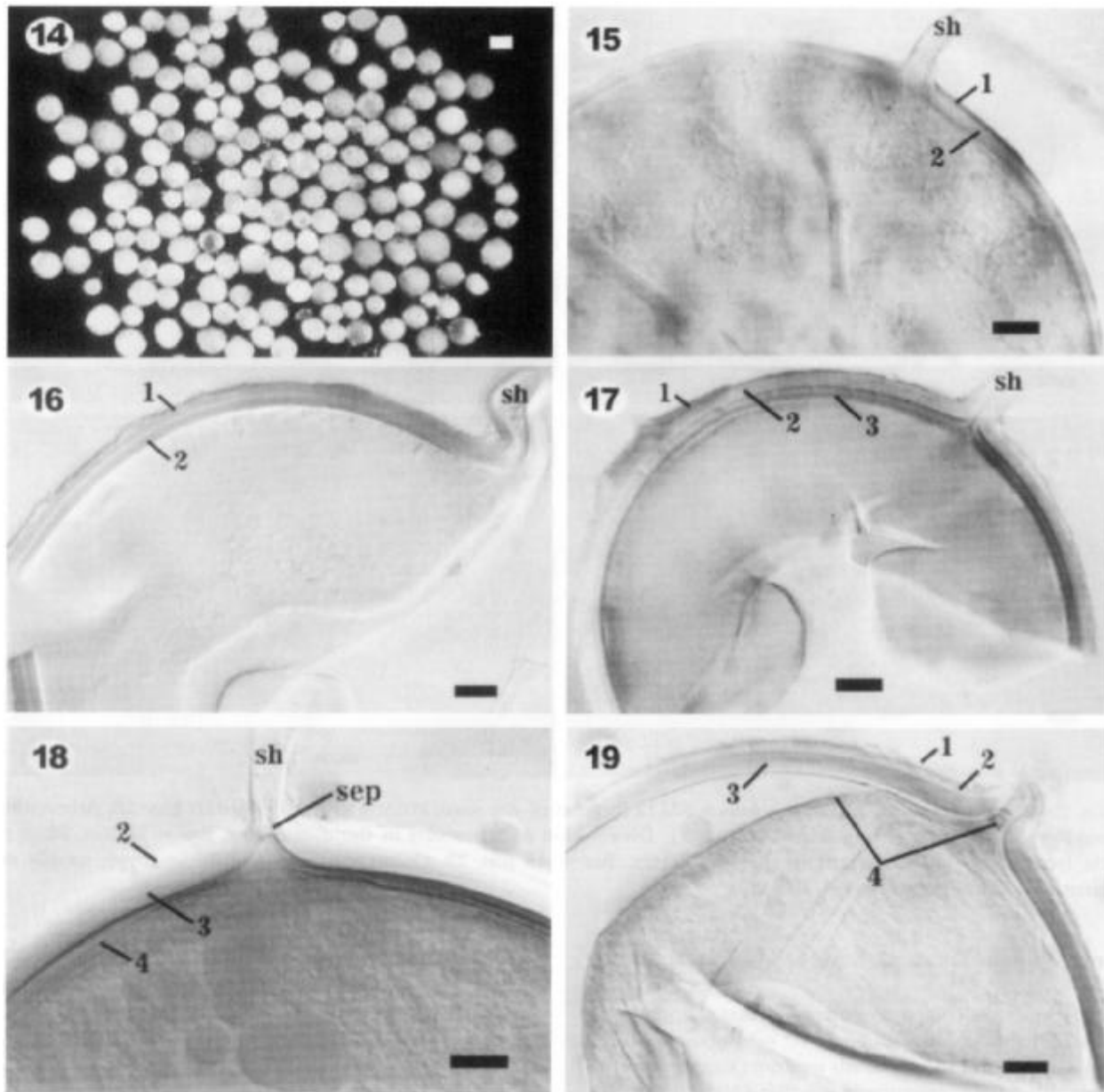
Discussion. *Glomus eburneum* bears some similarity to some other white/hyaline-spored *Glomus* species, *G. albidum* Walker & Rhodes, *G. occultum* Walker, and *G. viscosum* Nicolson under a dissecting microscope, but sufficient features exist to separate them. Spores of *G. occultum* (INVAM reference isolate CL700) are as variable in shape as *G. eburneum*, but spore contents rarely are dense so spores mostly are bright hyaline, regardless of age. Spore wall structure of *G. occultum* also is distinctive, with three layers of nearly equal thickness that sometimes separate to varying degrees (Morton 1985) rather than two tightly adherent layers of unequal thickness as found in spores of *G. eburneum*. The subtending hypha of *G. occultum* rarely is occluded with a recurved septum, which is evident in many spores of *G. eburneum*.

Spores of *G. viscosum* are hyaline, but differ from *G. eburneum* by forming in small loose aggregates rather than singly. Spore wall structure is of similar organization (two layers), but the outer layer is thicker, more plastic, and separates more readily from the laminate layer (Walker et al 1995). *Glomus viscosum* also has a persistent subtending hypha, but it does not form a prominent septum from the laminate layer of the spore wall, as does *G. eburneum*.

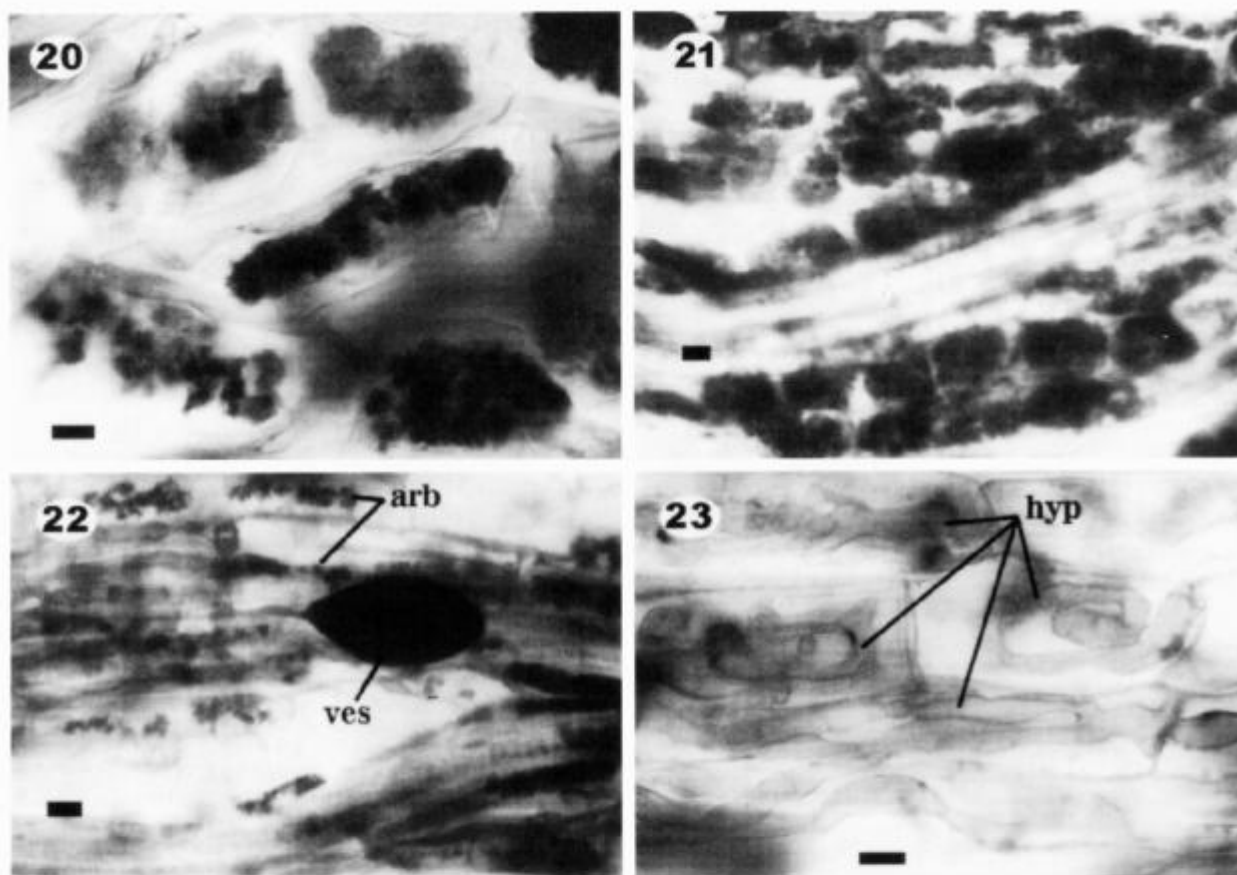
No live culture of *G. albidum* was available for comparison, and the paratype material at OSC contains spores resembling *G. intraradices*. Mature *G. albidum* spores stain dull orange to yellow and juvenile spores are pink to orange-red in Melzer's reagent (Walker and Rhodes 1981), whereas *G. eburneum* spores show

no reaction. Spores of *G. albidum* are usually sealed by collapse of the subtending hyphae (Walker and Rhodes, 1981). If a septum is present in the subtending hypha of *G. albidum*, it is found 5–20 μm distal from the hyphal pore (Walker and Rhodes 1981), a distance which occurs infrequently in *G. eburneum*.

Spores of *G. eburneum* most closely resemble those of *G. spurcum* under dissecting and compound microscopes. To complicate matters, both species often are found together, at least in arid and semiarid plant communities of Arizona, Texas, and Mexico. Spores could be separated for production of monospecific cultures under a dissecting microscope using two criteria: (i) consistently globose for *G. spurcum* and obovoid to tear-drop shaped for *G. eburneum* and (ii) transparent with a fused central oil globule for *G. spurcum* and bright white with dense, opaque contents for *G. eburneum*. These differences were most dramatic in actively growing cultures; with age, spores lost their brightness and contents of both species were highly variable in appearance. Under a compound microscope, differences in spore wall structure are not distinctive in lightly broken spores. Both species have a spore wall with two hyaline layers, the outer layer much thinner than the inner laminate layer. Spores of *G. spurcum* tend to have more of an organic matter coating, but this feature is highly variable and thus of little diagnostic value. Differences are in properties of the layers, and these become evident only in heavily crushed spores. In these spores, the outer layer of *G. spurcum* often separates from the laminate layer (sometimes completely), whereas it remains adherent in *G. eburneum*. The laminate layer in *G. spurcum* is much more flexible than that of *G. eburneum*, often spreading in regions where most pressure was applied and forming abundant folds on the inner surface (FIGS. 3, 4). The laminate layer of *G. eburneum* is not brittle, but it lacks the plasticity of the same layer in *G. spurcum* spores (FIGS. 7–9). The most dramatic difference between species is in the structural transition from spore to subtending hyphal wall. Spores of *G. spurcum* rarely show the subtending hypha because the hyphal wall is a continuation of the thin outer layer of the spore wall, which often separates, and there is no structural support provided by continuation of the thicker laminate layer into the hypha (FIG. 4). In contrast, the subtending hyphal wall of *G. eburneum* thins more gradually from the spore base because it is mainly a continuation of the laminate layer of the spore wall (FIGS. 7, 9). It also has a robust septum at variable distances from the spore (FIG. 7), two traits which result in retention of the subtending hypha on most broken spores.



FIGS. 14–19. *Glomus luteum* reference accession SA112. Whole spores were photographed in water; broken spores were mounted in PVLG and Melzer's reagent (1:1 v/v). All micrographs were taken using differential interference contrast optics. 14. Whole spores in water. 15. Juvenile spore with a spore wall consisting only of an outer layer (1), staining pinkish-red in Melzer's reagent, and early stages of an inner layer (2). Wall layers of subtending hypha (sh) are continuous with spore wall layers. 16. A more differentiated juvenile spore shows partial degradation of the outer spore wall layer (1) and thickening of the hyaline inner layer (2) with added sublayers, or laminae. Wall layers of subtending hypha (sh) show similar changes in differentiation. 17. Spore with three distinct layers (1, 2, 3) of spore wall that continue into the wall of the subtending hypha (sh). 18. A mature spore in which the innermost thin flexible layer (4) has been synthesized and remains attached to the subtending hyphal wall and septum. The outer layer (1) has sloughed, the second layer (2) is partially degraded, and the third yellow-brown layer (3) is finely laminate. A septum (sep) has formed from laminate layer in the lumen of the subtending hypha (sh). 19. Mature spore with all four spore wall layers visible; the outermost hyaline layer (1) mostly degraded, the second hyaline layer (2) still intact, the third layer (3) finely laminate and rigid, and the innermost layer (4) thin, flexible and still attached to the spore wall in the region of the septum of the subtending hypha. Bars: 14 = 100 μ m; 15–19 = 10 μ m.



FIGS. 20–23. Mycorrhizae of *Glomus luteum* SA112 in roots of *Zea mays*, stained with 0.05% direct blue 20. Arbuscules of various ages, all staining darkly. Bar = 10 μ m. 21. Distribution of arbuscules in the root cortex. Bar = 10 μ m. 22. A rare vesicle (ves) and arbuscules (arb) in the root cortex. Bar = 15 μ m. 23. Coiled intracellular hyphae (hyp), usually most frequent near entry points. Bar = 15 μ m.

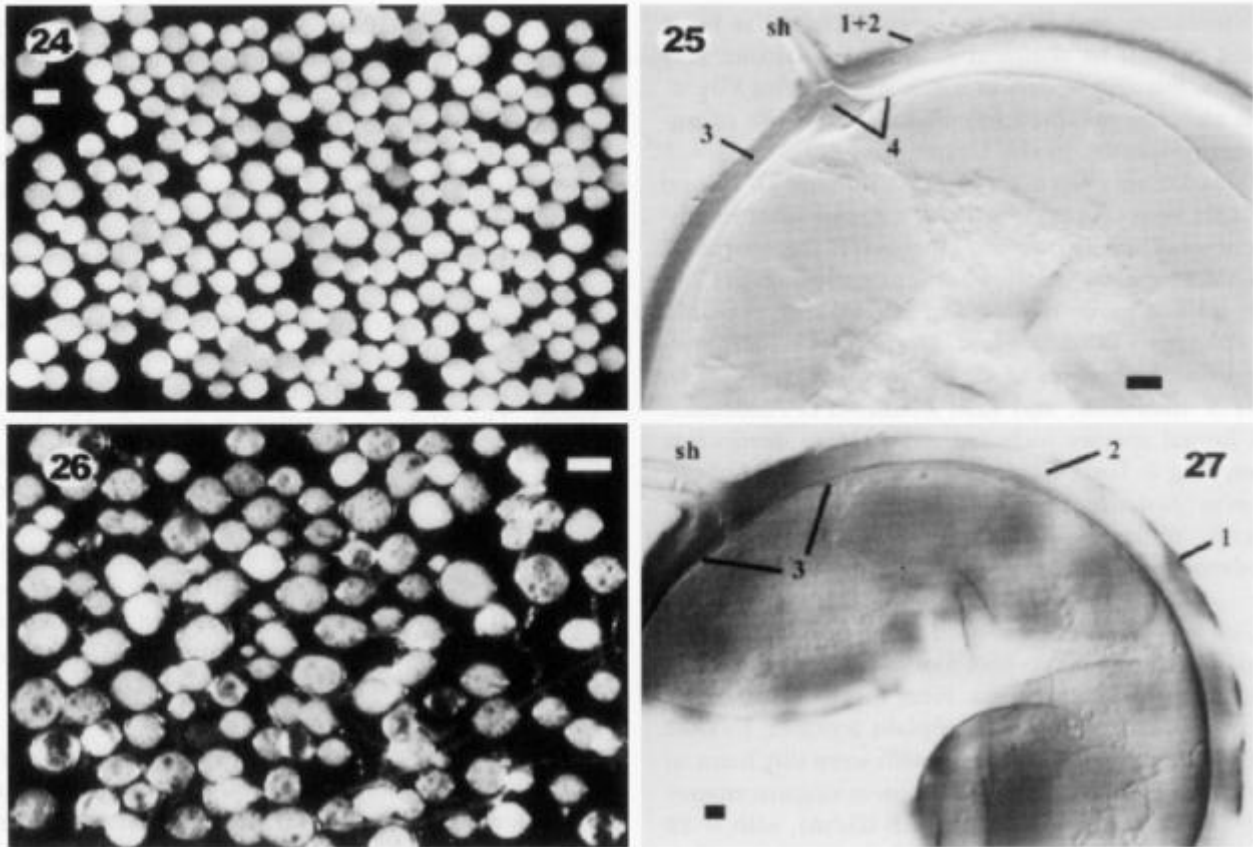
***Glomus luteum* Kennedy, Stutz et Morton, sp. nov.**

FIGS. 5, 14–23

Mycorrhizas vesicular-arbusculares formans. Sporae singulatae efformatae; lutea; globosae vel subglobosae, 60–180 μ m in diam. Parietis sporae stratis quatuor; stratum exterius hyalinis, 1.3–5.0 μ m crassum, in solutione Melzeri roseoescens; stratum secundus hyalinis, 1.3–6.5 μ m crassum; stratum tribus lamelliforme, 2.5–10 μ m crassum, luteus; stratum interius flexibilis, <0.5 μ m crassum. Hypha subtendentes 9.0–19 μ m in diam.

Spores formed singly in soil; globose to subglobose; 60–180 μ m diam (mean = 109 μ m, n = 160) (FIG. 5B); becoming pale yellow (0-0-20-0) to dark yellow with a brownish tint (0-10-60-0). Spores have a thin halo under reflected light when all layers are present (FIG. 14). The spore wall consists of four phenotypically discrete layers; the outer two layers degrading following maturation, a permanent pigmented laminate layer, and an innermost layer separating to resemble an endospore. Only the outer two layers are evident during early development (FIGS. 15, 16). The outer layer (1) is mucilaginous and stains pinkish-red (0-40-20-0) in Melzer's reagent when intact.

This layer degrades and sloughs unevenly, thus varies in thickness (1.3–5.0 μ m, n = 70), even on the same spore. The second layer (2) is hyaline, semirigid, 1.3–6.5 μ m thick (mean = 2.5 μ m, n = 154), produces no reaction in Melzer's reagent, and also degrades and sloughs to varying degrees on developing spore (FIGS. 18, 19). A third layer (3) forms de novo simultaneously in both the spore and subtending hyphal wall, thickening by synthesis of finely adherent sublayers (or laminae), pale yellow (0-0-20-0) to brownish yellow (0-10-60-0) at maturity, 2.5–10 μ m thick (mean = 5.5 μ m, n = 130) (FIG. 17). The innermost layer to form (4) is thin, <0.5 μ m, and is flexible enough to form folds and wrinkles in broken spores (FIGS. 18, 19). This layer may separate completely from the spore wall in broken spores, but it often remains attached in the region of the septum occluding the hyphal lumen (FIGS. 18, 19). The subtending hypha of *G. luteum* often is broken off at the spore wall and thus may be difficult to detect. When present, it is cylindrical to slightly flared, 9.0–19 μ m wide at the spore. A septum formed from the pig



FIGS. 24–27. Spores of two fungal species similar to *G. luteum*. Whole spores were photographed in water; broken spores were mounted in PVLG and Melzer's reagent (1:1 v/v). 24. Whole spores of *G. claroideum* reference accession SC186. Bar = 100 μm . 25. Broken mature spore of *G. claroideum* SC186. The outer layers (1, 2) of the spore wall are mostly degraded. The third layer (3) is laminate and pigmented. The thin innermost layer (4) is still attached to the subtending hyphae (sh). Bar = 5 μm . 26. Whole spores of *G. clarum* reference accession BR147A. Bar = 200 μm . 27. Broken mature spore of *G. clarum* BR147A. The outer layer (1) of the spore wall is partially degraded and shows a darkly dextrinoid reaction to Melzer's reagent. The second layer (2) is hyaline, rigid and persistent. A thin, laminate layer (3) is present in mature spores. Bar = 10 μm .

mented laminate layer (3) of the spore wall layer often occludes spore contents (FIG. 18).

Mycorrhizae. Within cortical cells of corn, mycorrhizal colonization is easily detected because structures stain darkly in direct blue. Arbuscules consist of trunks 1.5–4.0 μm wide with branch hyphae usually thinning incrementally (FIGS. 20, 21). Vesicles formed infrequently in corn roots after three months (FIG. 22), usually ellipsoid, 32 \times 75 μm to 59 \times 95 μm diam. Hyphae at entry points usually are coiled, 2.0–5.0 μm in width (FIG. 23). Hyphae in the root cortices are of similar width when growing straight and parallel to the root axis, or 4.0–9.0 μm wide when forming coils distributed in localized patches (most from entry points).

HOLOTYPE. CANADA. SASKATCHEWAN: 10 Jun 1997, L. Xavier, INVAM culture SA112. Deposition at OSC of preserved spores in sodium azide and broken spores mounted permanently on a glass slide. **ISOTYPE.** INVAM culture

SA112. Deposited at FH. **CULTOTYPE.** INVAM culture SA112.

Other materials examined. UNITED KINGDOM. Origin unknown, 17 Feb. 1995, R. Francis, University of Sheffield, INVAM culture UK134. UNITED STATES. WEST VIRGINIA: Near Jane Lew. From 2-yr-old stand of *Trifolium pratense* under cultivation, 14 Sep 1994, J. Morton, INVAM culture WV944. Mingo County, near Logan. From mixed vegetation at a coal strip mine site (see *G. eburneum*, above), 26 Sep 1996, K. Heldreth, INVAM culture WV216A. ARIZONA: Cochise County, near Sierra Vista. From *Sp. wrightii* on upper and lower floodplain terraces, 10 Oct 1997, J. Stutz and L. Kennedy, INVAM cultures AZ420A and AZ414B. Cochise County, near Cascabel. From *Sp. wrightii* on lower floodplain terrace, 22 Apr 1997, L. Kennedy and R. Tiller, ASU slide BC-B 72. CANADA. SASKATCHEWAN: See Talukdar and Germida (1993a). From wheat (*Triticum aestivum* L. cv. Katepwa), 10 Nov 1992, N. Talukdar, INVAM culture SA101.

Etymology. Latin, *luteum*, refers to the bright yellow color of many spores under reflected light.

Distribution and habitat. *Glomus luteum* has been found in arid to semiarid habitats in Arizona and Canada, but also occurs in mesic sites in West Virginia. It also was obtained in culture from a site of unknown properties in the United Kingdom.

Talukdar and Germida (1993a) isolated *G. luteum* from six sites cropped to wheat (*T. aestivum*) in Saskatchewan. Soil at one site (Outlook), the source of INVAM accession SA101, was slightly alkaline, pH 7.6, with 2.1% organic matter and 10 mg kg⁻¹ available phosphorus. Talukdar and Germida (1993b) cultured various isolates of *G. luteum* on *Z. mays*, *S. bicolor*, *S. sudanense*, and *Lens esculenta* L. Associated AM fungal species included *Acaulospora denticulata* Sieverding & Toro, *Gigaspora decipiens* Hall & Abbott, *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske, *G. etunicatum* Becker & Gerdemann, *G. mosseae*, and one undescribed species.

Glomus luteum was cultured from rhizosphere soil of giant sacaton at two sites along the San Pedro River in southeastern Arizona. Near Sierra Vista, Arizona, on upper and lower floodplain terraces, INVAM cultures AZ420A and AZ414B, soils were silty loam to silty clay, alkaline (pH 7.8–8.2), low in organic matter (1.2–2.9%), and low in EC (1–2.8 dS/m), with 6–29 mg kg⁻¹ available P. The second site was near Casabel, Arizona (Kennedy et al 1997), a sandy loam soil, with pH of 8.0, 1.3% organic matter, and 6 mg kg⁻¹ P. Associated AM fungal species were *A. trappei*; *A. delicata* Walker, Pfeiffer & Bloss; *G. eburneum*; *G. macrocarpum*; and *G. spurcum* and one undescribed species.

In West Virginia, a culture containing *G. luteum* (INVAM accession WV944) was obtained from the rhizosphere of 2-yr-old red clover (*Trifolium pratense*) in a cultivated field near Jane Lew. Soil was a loamy sand, pH 5.9, with 13 mg kg⁻¹ P. The only associated AM fungal species was *G. occultum*. *Glomus luteum* spores (INVAM accession WV216A) were also found in soil and root fragments of *Festuca elatior*, *Trifolium repens* and *Trifolium pratense* hydroseeded onto a revegetated coal strip mine site near Logan. This species was one of eleven species of arbuscular mycorrhizal fungi present (see species composition from site containing *G. eburneum* described above).

Discussion. Spores of *G. luteum* are within the size and color range of many *Glomus* species, but they most closely resemble those of *G. clarum* and *G. claroideum*. In fact, the holotype of this species is identical to another accession from the same region (SA101) that has been reported as *G. clarum* isolate NT4 (Talukdar and Germida 1993a, b, Walley and Germida 1996). To clearly establish the differences between these species, spores from Saskatchewan iso-

lates (SA112, SA101), Arizona (AZ414B) and the United Kingdom (UK134) were examined and compared to spores of *G. claroideum* (INVAM accessions AZ225D, BR147A, PL115, SC186, SF119, UT171) (FIGS. 24, 25) and *G. clarum* (INVAM cultures AU402B, AZ151A, BR147B, CL883A, FL979A, NC112A) (FIGS. 26, 27).

The outermost spore wall layer (1) of all three species is mucilaginous (staining pale to dark reddish-brown in Melzer's reagent), of similar thickness when intact, and subject to degradation with age due to decomposition by bacteria (Walley and Germida 1996). The Melzer's reaction is most darkly dextrinoid in *G. clarum* spores (FIG. 27). The second spore wall layer (2) is hyaline in all three species, but differs in rigidity, width, and permanence. In both *G. claroideum* (FIG. 25) and *G. luteum* (FIG. 18) spores, this layer degrades as spores age. However, it rarely persists on spores of the former and often is present on over 80% of spores in mature cultures of the latter, probably because it is much thinner (0.6–2.0 µm) in *G. claroideum* than in *G. luteum* (1.3–6.3 µm). In *G. clarum* spores, the second layer is rigid (often breaking with only the slightest pressure), permanent, laminate, and thickest (9–14.5 µm). Thickness and retention of this layer gives spores of *G. luteum* a halo appearance under reflected light (FIG. 14), which is even more pronounced on spores of *G. clarum* (FIG. 26). The third layer (3) in all three species is laminate and pigmented, varying from pale yellow to brownish yellow in color. This layer is thickest in *G. luteum* (mean = 5.0 µm) (FIG. 18, 19), of intermediate thickness in *G. claroideum* (mean = 3.8 µm) (FIG. 25), and thinnest in *G. clarum* (mean = 2.5 µm) (FIG. 27). The greater thickness of this layer in *G. luteum* accounts for the darker yellow appearance of many spores in comparison to those of *G. claroideum*. Similarly, thickness in *G. clarum* correlates with spore color (<1 µm in white spores, >2 µm in yellow spores). Spores of *G. luteum* and *G. claroideum* also have a thin flexible innermost layer (4) of the spore wall (FIGS. 19, 25) synonymous with a "membranous wall" (Walker 1983). Even though this layer is thin and flexible, it is not homologous with flexible inner walls in spores of *Acaulospora*, *Entrophospora*, and *Scutellospora* species because it forms as part of the spore wall rather than independent of it (Morton et al 1995). This connection is evident in many broken spores of *G. luteum* and *G. claroideum*, where L4 either has a small projections indicating insertion into the pore of the subtending hypha or it remains attached to the spore wall where it is continuous with the subtending hyphal wall.

ACKNOWLEDGMENTS

This work was supported by a fellowship from the Environmental Protection Agency U 914993-01-3 for L.J.K, Arizona Water Protection Fund grant 95-018WPF to J.C.S. and National Science Foundation grant DBI-9600699 to J.B.M. We thank Richard Koske for helpful discussions following review of the manuscript.

LITERATURE CITED

- Daniels BA, Skipper HD. 1982. Methods for the recovery and quantitative estimation of propagules from the soil. In: Schenck NC, ed. *Methods and principles of mycorrhizal research*. St. Paul, MN: APS Press. p 29-35.
- Kennedy LJ, Stutz JC, Tiller RL. 1997. Arbuscular mycorrhizal fungi associated with *Sporobolus wrightii* in riparian ecosystems of the desert southwest. *Inoculum* 48: 18.
- Koske R, Gemma JN. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycol Res* 92:486-488.
- , Tessier B. 1983. A convenient, permanent slide mounting medium. *Mycol Soc Am Newslet* 34:59.
- Morton JB. 1985. Variation in mycorrhizal and spore morphology of *Glomus occultum* and *Glomus diaphanum* as influenced by plant host and soil environment. *Mycologia* 77:192-204.
- . 1996. Redescription of *Glomus caledonium* based on correspondence of spore morphological characters in type specimens and a living reference culture. *Mycorrhiza* 6:161-166.
- , Bentivenga SP, Wheeler WW. 1993. Germ plasm in the International Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation, and storage. *Mycotaxon* 48:491-528.
- , Bever JD. 1995. Discovery, measurement, and interpretation of diversity in symbiotic endomycorrhizal fungi (Glomales, Zygomycetes). *Can J Bot* 73(Suppl 1):25-32.
- , Bever JD, Pfeleger FL. 1997. Taxonomy of *Acaulospora gerdemannii* and *Glomus leptotichum*, synanamorphs of one anamorphic fungus in Glomales. *Mycol Res* 101: 625-631.
- Pfeiffer CM, Walker C, Bloss HE. 1996. *Glomus spurcum*: a new endomycorrhizal fungus from Arizona. *Mycotaxon* 59:373-382.
- Stürmer SL, Morton JB. 1997. Developmental patterns defining morphological characters in spores of four species in *Glomus*. *Mycologia* 89:72-81.
- Talukdar NC, Germida JJ. 1993a. Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Saskatchewan, Canada. *Can J Microbiol* 39:567-575.
- , ———. 1993b. Propagation and storage of vesicular-arbuscular mycorrhizal fungi isolated from Saskatchewan agricultural soils. *Can J Bot* 71:1328-1335.
- Walker C. 1983. Taxonomic concepts in the Endogonaceae: spore wall concepts in species descriptions. *Mycotaxon* 18:443-455.
- , Rhodes LH. 1981. *Glomus albidus*: a new species in the Endogonaceae. *Mycotaxon* 12:509-514.
- , Giovannetti M, Avio L, Citerinesi AS, Nicolson TH. 1995. A new fungal species forming arbuscular mycorrhizas: *Glomus viscosum*. *Mycol Res* 99:1500-1506.
- Walley FJ, Germida JJ. 1996. Failure to decontaminate *Glomus clarum* NT4 spores is due to spore wall-associated bacteria. *Mycorrhiza* 6:43-49.