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GLOMUS SPURCUM: A NEW ENDOMYCORRHIZAL FUNGUS FROM ARIZONA

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SUMMARY

A new species of endomycorrhizal fungus (*Glomus spurcum*) is described and illustrated. The fungus is known to form arbuscular mycorrhizae with at least eight species of plants. Intraradical vesicle formation by *G. spurcum* has not been observed. Intraradical hyphae can be enlarged and lobed in a manner similar to that described for a species of *Acaulospora*. The mycorrhizae formed by *G. spurcum* are similar to that of some species of *Acaulospora* but the gross morphology of the spores and spore development place it in *Glomus* as that genus is currently defined.

Spores of *Glomus spurcum* were found originally with *Acaulospora delicata* Walker, Pfeiffer & Bloss in a greenhouse bed of sand used for the propagation of various ornamental plants from stem cuttings. Cultures were obtained by extracting spores and mixing them with sterile sand which was then sown with seeds of *Sorghum sudanense* (Piper) Staph. (Walker, Pfeiffer and Bloss, 1986). Plants were maintained in a growth chamber (27°C day: 15 °C night), illuminated with a mixture of fluorescent and incandescent light at approximately 96 µE m^{-2} s^{-1}. Additional pot cultures were later established with other grasses and some herbaceous annual plants, some of which were maintained for five years with no evidence of morphological change to the spores, regardless of plant or soil type. The species was also identified from natural environments in Arizona, Mexico and Hawaii.

The species description is made from light microscopy of specimens mounted in water, polyvinyl alcohol lactophenol (PVL) or polyvinyl alcohol lactoglycerol (PVLG) to which stains and reagents were added as required. The color of intact spores was determined

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from specimens in water, viewed at 20-50 X under a dissecting microscope with reflected light from a tungsten-halogen source having a color temperature of 3200 °K. The same light source was used to illuminate the color chart (Anon, 1969) for color-matching. Colors of individual walls are as viewed through a compound microscope with a color temperature of 3200 °K, matched with the color chart illuminated as for the dissecting microscope. Numbers in parenthesis after color descriptions refer to color numbers on the chart. Mycorrhizae were cleared and stained by a slightly modified Phillips and Hayman (1970) technique.

Spores were also examined with a scanning electron microscope. Spores were fixed for 3 d in 1.5% glutaraldehyde-phosphate buffer, pH 7.0. After fixation, they were placed in a drop of water on a microscope slide and crushed gently by application of pressure to a glass cover slip. The broken spores then were removed and washed in distilled water, processed through an acetone series, 50-100% with a minimum of 15 mm per dilution bath, placed in absolute ethanol for 1 h, and air dried. Sputter coating was for 120 s with gold-palladium (60:40). Coated spores were attached to the microscope stage with double sided adhesive tape after which a small sliver of adhesive tape was used to hinge open broken spores, thus allowing observation of the fractured walls.

We have used the term wall in the species description only as an aid for the description. Berch (1986) was correct in her criticism of the use of the term wall in describing species. Her proposal to substitute the word layer for wall is equally flawed, since the true nature of the walls and layers in spores of members of the Glomales is not yet completely understood. The terminology used by Morton (1995) for walls or wall layers in fruiting structures of members of *Scutellospora* moves towards clarifying this debate. With *G. spurcum*, we have been unable to determine the ontogenetic sequence of wall or wall layer production and have consequently retained the terminology of Walker (1983). Names of species in the Glomales follow Walker and Trappe (1993).

GLOMUS SPURCUM Pfeiffer, Walker & Bloss sp. nov. Figs. 1-3.

Sporocarpia ignota. Chlamydosporae singulae vel laxe fasculatae in solo (57-)75-110(- 130) x (40-)75-110(-115) µm, globosae vel subglobosae interdum ovoideae vel obovoideae, hyalinae, sporarum tunica tristratis. Hypha affixa obscura. Sporae et hyphae cum mucosa exteriore.

SPOROCARPS unknown.

SPORES formed singly or aggregated in soil, terminally on thin-walled, coenocytic or sparsely septate hyphae; globose to subglobose (rarely ovoid to obovoid or irregular), (57-)75-110(-130) x (40-)75-110(-115) µm. Infrequently formed in the cortical cells of roots. At maturity, usually becoming detached from the sporogenous hyphae. Spore color in water with reflected light, hyaline with a “frosted” appearance caused by the roughened nature of a mucilaginous material covering the outer wall. Spores often appearing dirty and adopting the color of the substrate because of adherent soil particles (Fig. 2A).

SPORE WALL STRUCTURE of three walls (walls 1-3), in three groups (A, B and C), each with a single wall (Fig. 1, Fig. 2F). Wall group A of a single, hyaline to very pale

yellowish cream (3) somewhat flexible unit wall (wall 1), 0.25-0.75 m thick, covered in older spores by a mucilaginous material, 0.5-4 µm thick, which appears to be formed by overlapping plate-like structures when examined with the scanning electron microscope (Fig. 2H). When some specimens are mounted in water, PVL or PVLG, wall 1 with its mucilaginous covering rapidly separates from wall 2, producing a balloon-like effect (Fig. 2B); the thickness of wall 1 and its mucilaginous covering remain unchanged. Wall group B of a single, laminated wall (wall 2), hyaline to lightly colored, (l.5-)2.0-4.0(-5.0) µm thick with up to 10 subequal laminations that may be so thin as to be difficult to detect (Figs. 2F & 2G). Wall group C of a single, very thin hyaline membranous wall (wall 3), less than 0.5 µm thick, often adherent to wall 2, and thus difficult to detect (Fig. 2F). Wall 3 reacting quickly in Melzer's reagent to become greenish yellow (57), whereas the colors of other walls and the mucilaginous material are unchanged in this reagent. The mucilaginous material and walls 1 & 3 cyanophilous in cotton blue; wall 2 not staining. Walls 2 & 3 sometimes appearing, by light microscopy, to form an endospore (Fig. 2E).

SUBTENDING HYPHAE straight (3.5-)4.0-5.0(-6.0) m diameter, parallel-sided, not thickened at the spore base. Walls 0.5-1.0 thick, continuous with wall 1 of the spore and the mucilaginous outer material, often with adherent soil particles (Figs. 2A & 2C). The hypha shrivels and collapses at spore maturity and becomes difficult to see. Spore occlusion apparently by continuation of wall 2 at the spore base, giving the appearance of a septum (Fig. 2E). A thin septum may occur in the subtending hypha 17-35 µm from the spore base (Fig. 2C).

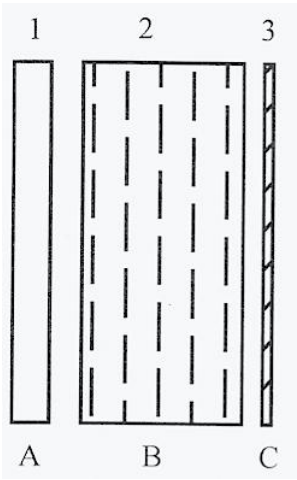


Fig. 1. Murograph (Walker, 1983) of *Glomus spurcum*. Spore wall structure of three groups (A B & C) each with a single wall (walls 1-3). Walls illustrated as: Unit wall 1, unshaded; laminated wall 2, broken lines; membranous wall 3, diagonal lines. The mucilaginous material covering spores is not illustrated in the murograph. Walls are numbered sequentially from outer to inner walls.

SPORE GERMINATION difficult to discern and not yet conclusively determined. Apparently by the development of a germination tube from wall 3, emerging directly through the outer walls. Details have been obscured by debris which adheres to the mucilaginous covering and wall degradation.

ETYMOLOGY: Latin - spurcum (dirty), referring to debris which adheres to the mucilaginous outer covering of spores and mycelium.

MYCORRHIZAE: sparse, poorly staining in cotton blue, trypan blue or acid fuchsin red; with broad intraradical hyphae and coarse arbuscules. No distinct intraradical vesicles have been observed, and no differences have been noted among mycorrhizae with different plant species (Figs. 3A-F).

MYCORRHIZAL ASSOCIATIONS: known to form a type of arbuscular endomycorrhiza with *Helianthus annuus* L., *Lycopersicon esculentum* Mill., *Medicago sativa* L., *Paspalum notatum* Flügge, *Plantago lanceolata* L., *Sorghum saccharatum* Moench., *S. sudanense*, and *Zea mays* L.

COLLECTIONS EXAMINED

HOLOTYPE: ARIZONA, Pima County, Tucson; from a pot culture with *S. sudanense* Walker (W989), (E); Isotypes OSC, ARIZ. The origin of the sand in the greenhouse at the University of Arizona (Building 42-2R) from which the original spores were extracted is unknown.

OTHER COLLECTIONS: ARIZONA, Pima County, Molino Basin, Santa Catalina Mountains, in the Encinal Region (Niering and Whittaker 1963); extracted from soil under mixed grasses (W9l2). ARIZONA, Pima County, Tucson; from a pot culture with *P. notatum* (W1659) and P. lanceolata (W1658) started with spores from the holotype culture. HAWAII, Kauai, Hanapepe; from the root zone of *Leucaena*

Fig. 2. Light micrographs (LM) and scanning electron micrographs (SEM) of spores of *Glomus spurcum*.

A. Spore mounted in PVL with intact subtending hypha (sh) with adherent debris (arrows) on the mucilaginous covering (LM).

B. Wall 1 and the mucilaginous covering "ballooning" when the spore was mounted in PVL on a microscope slide with cover slip. Innermost walls 2 & 3 unchanged (LM).

C. Intact subtending hypha with a septum (arrow) (LM).

D. Outer wall (wall 1), and the mucilaginous covering separated from walls 2 & 3 upon crushing the spore on a microscope slide with a coverslip (LM).

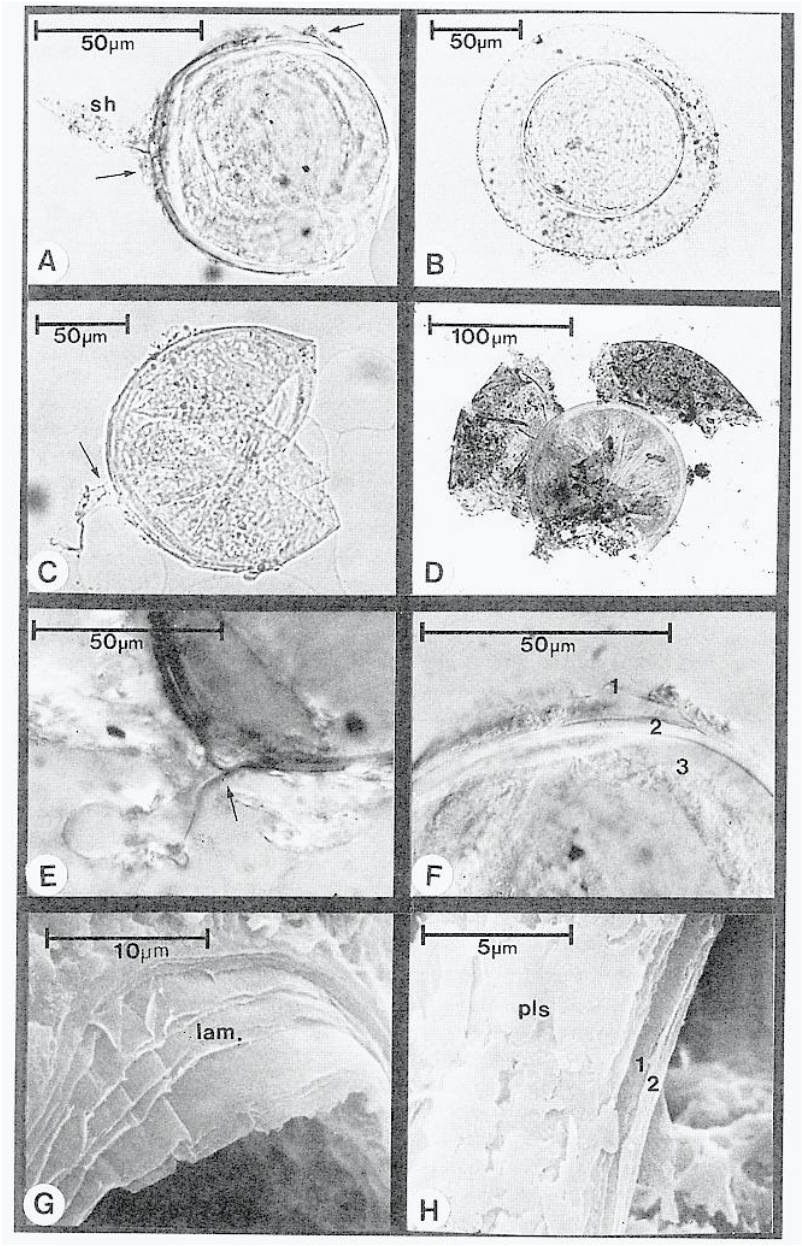
E. Wall 2 invaginated (arrow) in the subtending hypha giving the appearance of walls 2 & 3 forming an endospore (LM).

F. Details of wall structure. Wall 1; outer, unit wall with mucilaginous covering and adherent debris. Wall 2; laminated wall. Wall 3; membranous wall often difficult to detect

(LM).

G. Laminae (lam.) in wall 2 illustrated (SEM).

H. Apparent plate like structure (pls) of the mucilaginous material covering spores, walls 1 & 2 illustrated (SEM).


50µm
sh
A
50µm
B
50µm
C
100µm
D
50µm
E
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1
2
3
F
10µm
lam.
G
5µm
pls
1
2
H

leucocephala (Lam.) de Wit in sand dunes (W1574). MEXICO, west of Ciudad Obregon, Santa Maria Beach; from beach sand collected beneath *Abronia maritima* Nutt. Pot cultures were established from the Hawaiian collection (R. E. Koske and J. N. Gemma, University of Rhode Island - personal communication) and the Mexican collection (Pfeiffer).

DISCUSSION

Glomus spurcum is a widespread species whose hyaline spores, wall structure, narrow hyphal attachment and mucilaginous covering of the outer wall readily separate this species from nearly all other members of the genus Glomus. A newly described species, *Glomus viscosum*, Walker et al. (Walker et al., 1995), has hyaline spores with an outer mucilaginous covering and a spore size similar to *G. spurcum*. Unlike *G. spurcum*, *G. viscosum* possesses a single wall with two layers and a persistent subtending hypha. The single wall and mucilaginous covering of *G. viscosum* is not flexible and does not balloon when mounted on microscope slides in water, PVL or PVLG. The spore size of *G. spurcum* and *G. viscosum* overlap; however, *G. spurcum* spores are generally larger, the maximum length being 130 m compared with a maximum length of 97µm for *G. viscosum*. Spores of *Glomus albidum* Walker & Rhodes appear similar to those of *G. spurcum* at maturity but *G. albidum* is much larger, up to 198 x 177 µm compared with a maximum of 130 x 115 m for *G. spurcum* and, *G. albidum* lacks an inner, membranous wall (Walker and Rhodes, 1981). Spores of *Glomus diaphanum* Morton & Walker, *Glomus laccatum* Blaszkowski, *Glomus lacteum* Rose & Trappe, *Glomus occultum* Walker and *Glomus scintillans* Rose & Trappe may appear superficially similar to those of *G. spurcum*, but all have spores with more prominent subtending hyphae, different wall structures and lack the persistent mucilaginous covering (Morton and Walker, 1984; Blaszkowski, 1988; Rose and Trappe, 1980; Walker, 1982). The unusual ballooning of wall 1 and attached mucilaginous material when some spores of *G. spurcum* are placed in mountants has not been recorded for any other member of the genus *Glomus*.

Spain (1990) made a plea for spore diagnoses of members of the Glomales to be made from spores mounted in water. Her arguments were largely based on the way that wall types are characterized. Certain types of walls are changed, microscopically and possibly chemically, dependent upon the pH or other chemical properties of different media. Because most of the more recent descriptions have been made from specimens mounted in polyvinyl alcohol-based, acidic mounting media, we have chosen to adhere

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Fig. 3. *Glomus spurcum* mycorrhizas with *Plantago lanceolata* (Pt) or *Sorghum sudanense* (Ss) and extra-radical mycelium; light micrographs.

A & B. Coarse arbuscule-like structures (arrows) in cortical cells of P1.

C. Intercellular hyphae forming 'H-connections' (arrow) in root of P1.

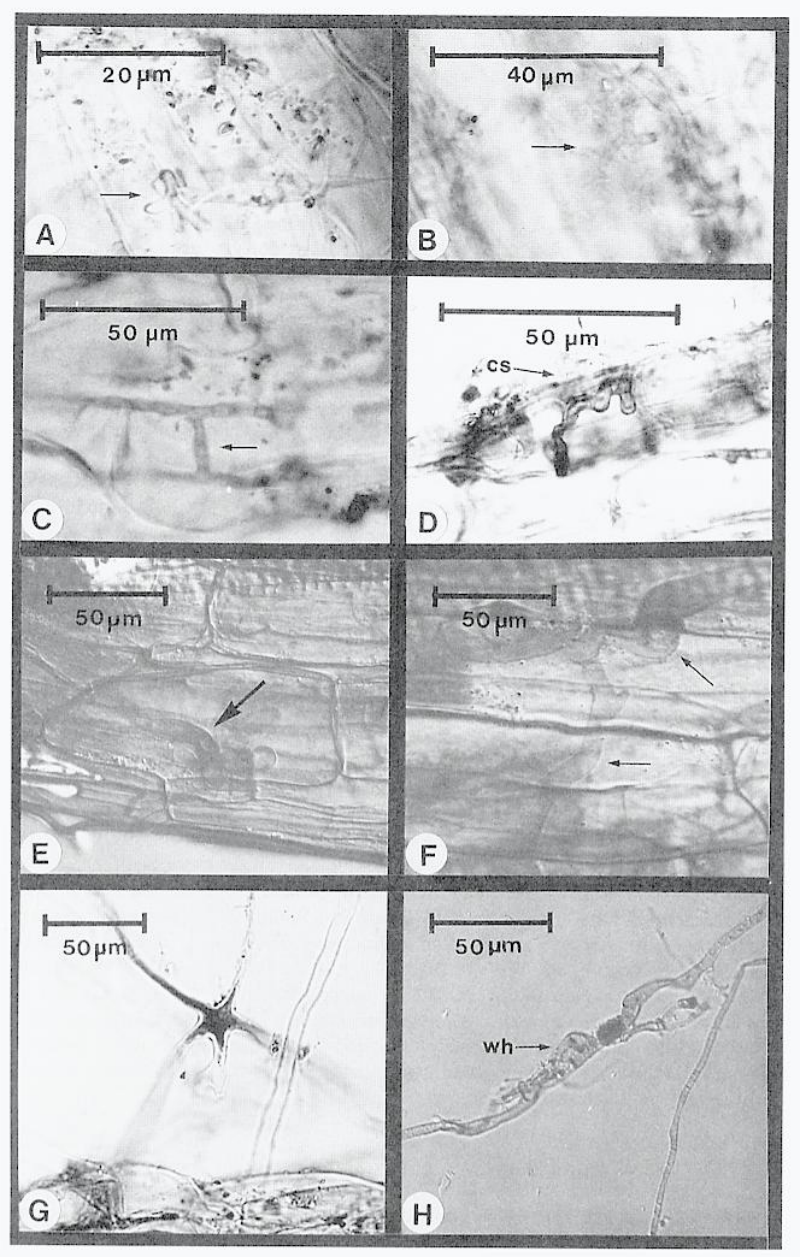
D. Colonization structure (cs) on root surface of P1.

E. Coarse arbuscule like structure (arrow) in cortical cell of Ss.

F. Swollen internal mycelial structures and broad hyphae in root of Ss.

G. Extra-radical mycelium with a star-shaped hyphal junction.

H. Extra-radical mycelium showing a type of 'wound-healing" (wh) response.


20 µm
A
40 µm
B
50 µm
C
50 µm
cs
D
50 µm
E
50 µm
F
50 µm
G
50 µm
wh
H

to that convention. Nevertheless, we are sympathetic to Spain's arguments, and consequently, have studied spores mounted in water. Spores of *G. spurcum* in water show only minor differences from those in PVL or PVLG. Wall 1 balloons more in PVL or PVLG than it does in water, though the phenomenon does not occur consistently in any mountant. The innermost flexible wall (wall 3) is easier to observe in water than in PVL or PVLG. Future species descriptions should perhaps include discussion of spores mounted in water as well as other media such as PVLG.

The genus *Glomus* Tulasne & Tulasne, as currently defined (Gerdemann and Trappe, 1974; Morton and Benny, 1990) is poorly delimited, and consists of an assemblage of species that are unlikely to be monophyletic. At least one species, *Glomus leptotichum* Schenck & Smith is considered to be a morph of *Acaulospora gerdemannii* Schenck & Nicolson (Nicolson and Schenck, 1979) (J. B. Morton, personal communication). *Glomus spurcum* differs in several ways from more typical species in the genus such as *Glomus macrocarpum* Tul. & Tul. It has the spore contents bounded by a membranous wall in a manner similar to that reported for *G. leptotichum* (Schenck and Smith, 1982). Spores appear to germinate by direct growth of a germ tube through the spore walls similar to that described for *G. albidum* (Walker and Rhodes, 1981) and *G. scintillans* (Rose & Trappe, 1980) rather than by regrowth through the subtending hypha. Details of germ tube formation and growth through walls of *G. spurcum* are difficult to discern because of debris which adheres to the mucilaginous covering and wall degradation.

The extra-radical mycelium of *Glomus spurcum* is typically coenocytic and has frequent coarse hyphal junctions with up to five branches (Fig. 3G). Occasionally, a type of wound-healing response, reminiscent of that found in members of the Gigasporaceae or in *Acaulospora nicolsonii* Walker, Reed & Sanders can be found (Fig. 3H) (Walker, Reed and Sanders, 1984). The mycorrhizae formed by this fungus are unusually sparse and poorly-developed for a species of *Glomus* (Figs. 3A-F). In a sample of roots from a pot culture with *M sativa*, only 14 entry points were observed in 23.5 mm of root. In one pot culture with *P. lanceolata*, sporulation was abundant, but neither root staining nor observation by fluorescence microscopy revealed mycorrhizae. However, in another pot culture with *P. lanceolata*, mycorrhizae which stained very weakly with cotton blue were found. Vesicles have not been observed, and although arbuscules are produced, they consist of rather coarse hyphal elements; not typical of those described for *Glomus*, *Scutellospora* or *Gigaspora* spp. The mycorrhizae formed by *G. spurcum* are similar to those described for G. leptotichum (Schenck and Smith, 1982) and also resemble those illustrated for Abbott's isolate of an *Acaulospora* similar to *A. laevis* Gerdemann & Trappe from Badgingarra, Western Australia (Abbott, 1982; Figs 22 & 23). In the key to colonization patterns (Abbott, 1982), mycorrhizae of G. spurcum key out as members of the genus *Acaulospora*. *Glomus spurcum* may fulfil the criteria propounded by Morton (1990) for an "early progenitor species". Its hyphae are compatible with the plant roots in which they develop and it sporulates abundantly in pot culture from a poorly developed mycorrhizal base.

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