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Glomus multiforum and *G. verruculosum*, two new species from Poland

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Abstract: Two new vesicular-arbuscular mycorrhizal fungal species, *Glomus multiforum* and *G. verruculosum* (Glomales, Zygomycetes) coming from northwestern Poland are described and illustrated. *Glomus multiforum* produces spores singly in the soil. The deep yellow to brown spores are globose to subglobose, (170–)215(–230) µm diam or ovoid, 150–220 × 180–310 µm and have a single wall consisting of three layers. Outermost layer 1 is mucilaginous, hyaline, and stains reddish in Melzer's reagent. Layer 2 of young spores is hyaline and ornamented with ingrowths filling the pits of layer 3; it sloughs with spore age. Layer 3 is laminated, colored and ornamented with evenly distributed pits. *Glomus verruculosum* forms spores singly in the soil. Spores are yellow to orange, (150–)189(–265) µm diam. Their single wall consists of two layers: a hyaline, semiflexible outer layer and a yellow to orange, laminated inner layer ornamented with warts projecting inward from the innermost lamina. None of these layers stain in Melzer's reagent. Both *G. multiforum* and *G. verruculosum* formed spores and vesicular-arbuscular mycorrhizae in single-species pot cultures with *Plantago lanceolata* and *Sorghum sudanense.*

Key Words: Glomales, mycorrhizae, ontogeny, vesicular-arbuscular fungi, Zygomycetes

INTRODUCTION

In continuing investigations of arbuscular mycorrhizal fungi of cultivated and uncultivated soils of Poland, two undescribed *Glomus* species with ornamented spores were found. The pitted spores of the first species and the warts present on the inner surface of the laminated spore wall layer of the second fungus distinguish them from other members of the genus *Glomus*. Hence, these fungi are described here as *G. multiforum* sp. nov. and *G. verruculosum* sp. nov.

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MATERIALS AND METHODS

Roots and attached soil of sample plants were collected from a depth of 5–30 cm, air dried for 2 wk and subsequently refrigerated at 4 C until processing. Trap cultures were established from the field-collected mixtures of soil and roots. These mixtures were placed in four 15-cm plastic pots (1350 cm^3). Two pots were seeded with *Plantago lanceolata* L. and two with *Sorghum sudanense* (Staph.) Piper. About 60 seeds of each plant were added to each pot. Plants were grown in a greenhouse at 18–30 C with supplemental 16-h lighting provided by one SON-T AGRO sodic lamp (Philips Lighting Poland S. A.) placed 1 m above pots. The maximum light intensity was 180 µE $m^{-2}s^{-1}$ at pot level. Plants were watered 2–3 times a wk. Trap cultures were harvested at approximately 1-mo intervals, beginning 4 mo and ending 11 mo after plant emergence. Spores were extracted by wet sieving and decanting (Gerdemann and Nicolson, 1963), and roots were stained in 0.05% trypan blue (Phillips and Hayman, 1970) and examined for the presence of mycorrhizae.

Establishment of single-species pot cultures was made by placement of 10 to approximately 30 newly formed spores on roots of 10–14-d-old seedlings of *P. lanceolata* and *S. sudanense.* The plants were grown in 8-cm plastic pots (250 cm^3) for 3–12 mo. The growth medium was an autoclaved sand coming from maritime dunes adjacent to Świnoujście (*G. verruculosum*; pH 6.7, 12 and 26 mg L^{-1} P and K, respectively) and a mixture of soil representing a good rye complex and maritime sand (*G. multiforum*; pH 6.5, 52.1 and 84.5 mg L^{-1} P and K, respectively). Stages of differentiation of each fungus were determined based on newly formed spores extracted from both trap and single-species cultures, the latter being harvested at ca 20–30-d intervals.

Morphological investigations were conducted based on at least 100 spores of each species mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier, 1983) and Melzer's reagent, respectively. Specimens for scanning electron microscopy examination were fixed in 3% glutaraldehyde in a 0.02 M phosphate buffer (pH 6). They were then dehydrated in a graded ethanol series: 30, 50, 80 and 100%, critical point dried, mounted on aluminum stubs, and coated with gold. Wall characteristics of

spores and terminology are those suggested by Franke and Morton (1994), Spain et al. (1989), Stürmer and Morton (1997), and Walker (1983). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names are from Kornerup and Wanscher (1983). Specimens have been mounted in PVLG on slides and deposited in the Department of Plant Pathology (DPP), Academy of Agriculture, Szczecin, Poland, and in the herbarium at Oregon State University (OSC), USA. Nomenclature of other fungi mentioned here follows Walker and Trappe (1993). The classification is that of Morton and Benny (1990).

DESCRIPTIONS OF THE SPECIES

Glomus multiforum Tadych & Błaszkowski, sp. nov. FIGS. 1–6

Sporocarpia ignota. Sporae singulatim in solo, e sporophoris rectic efformatae. Sporophorum nonseptatum vel parce septatum; hyalinum vel pallide aurantiacum; (9.0–)11.6(–13.0) μm latum; pariete (0.5–)0.9(–1.2) μm crasso; rectum. Sporae intense luteae vel brunneae; globosae vel subglobosae; (170–)215(–230) μm diam; aliquando ovoideae; 150–220 × 180–310 μm; hypha subtenda solitaria. Tunica sporae e startis tribus (strati 1–3); strato "1" mucilaginoso, hyalino, 0.5–1.7 μm crasso, in solutione Melzeri rubicoso; strato "2" semielastico, hyalino, (0.5–)1.3(–2.9) μm crasso, cum verrucis ordinatis; strato "3" laminato, intense luteo vel brunneo, (5.6–)7.4(–9.6) μm crasso, cum cavernis ordinatis, 2.0–3.2 μm diam vel ovatis, 2.7–2.9 × 3.4–4.9 μm et 1.5–2.7 μm profundis. Hypha subtenda intense lutea vel brunnea; recta vel recurva; infundibuliforma, raro coliga; (24.0–)27.2(–31.9) μm lata ad basim sporae; pariete intense luteo vel brunneo; (7.6–)8.7(–10.3) μm crasso, stratis 1–3 sporae continuo. Porus e septo continuo strati 3 sporae efformata. Vesicular-arbusculares mycorrhizae formans.

HOLOTYPUS. POLAND, Szczecin, infra *Sorghum sudanense* (Staph.) Piper, 2 Jan. 1996, *Błaszkowski, J., 2108* (DPP).

Sporocarps unknown. Spores borne singly in the soil (FIG. 1); produced from straight sporophores. *Sporophore* coenocytic to sparsely septate; hyaline to light orange (5A2); (9.0–)11.6 (–13.0) μm wide; with a wall (0.5–)0.9(–1.2) μm thick; bearing spores by swelling at hyphal tips. *Spores* deep yellow (4A8) to brown (6E8); globose to subglobose; (170–)215(–230) μm diam; sometimes ovoid; 150–220 × 180–310 μm; with a single subtending hypha (FIG. 1). Spores with one wall (FIGS. 4–6) composed of three layers (layers 1–3). Outermost layer 1 mucilaginous, smooth, hyaline, 0.5–1.7 μm thick before disintegration, closely adherent to layer 2 (FIGS. 4, 5), staining reddish (12C5) in Melzer's reagent, usually absent in mature spores. Layer 2 hyaline, (0.5–)1.3(–2.9) μm thick, ornamented with ingrowths filling the pits of layer 3 (FIGS. 4, 5), sloughing with age. Layer 3 laminated, deep yellow (4A8) to brown (6E8), (5.6–)7.4(–9.6) μm thick, evenly pitted with round, 2.0–3.2 μm diam, rarely ovate, 2.7–2.9 × 3.4–4.9 μm and 1.5–2.7 μm deep depressions, separated by ridges, 1.5–6.6 μm wide (FIGS. 2–5). *Subtending hypha* deep yellow (4A8) to brown (6E8); straight or recurvate; funnel-shaped (FIGS. 1, 6), rarely constricted; (24.0–)27.2(–31.9) μm wide at the spore base. *Wall of subtending hypha* deep yellow (4A8) to brown (6E8); (7.6–)8.7(–10.3) μm thick; continuous with spore wall layers 1–3 (FIG. 6). *Pore* occluded by a septum (FIG. 6), 2.0–2.5 μm wide, continuous with the innermost lamina of spore wall layer 3. Spore contents of oil droplets. Germination by regrowth through the subtending hypha.

Collections examined. HOLOTYPE. POLAND. Szczecin, associated with roots of pot-cultured *S. sudanense*, 2 Jan. 1996, *Błaszkowski, J., 2108* (DPP); ISOTYPES: *Błaszkowski, J., 2109–2121* (DPP) and two slides at OSC. A living culture of *G. multiforum* was deposited in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (West Virginia University, USA).

Other materials examined. POLAND. Izdebki (53°17′N and 17°19′E, Pilá voivodeship), from the root zone of a mixture of *Plantago major* L. and *Poa trivialis* L., 18 June 1995, *Błaszkowski, J., 2122–2147* (DPP).

Etymology. multiforum referring to the pitted spores. We wish to dedicate this species to Prof. Dr. Tadeusz Madej, Department of Plant Pathology, Academy of Agriculture, Szczecin.

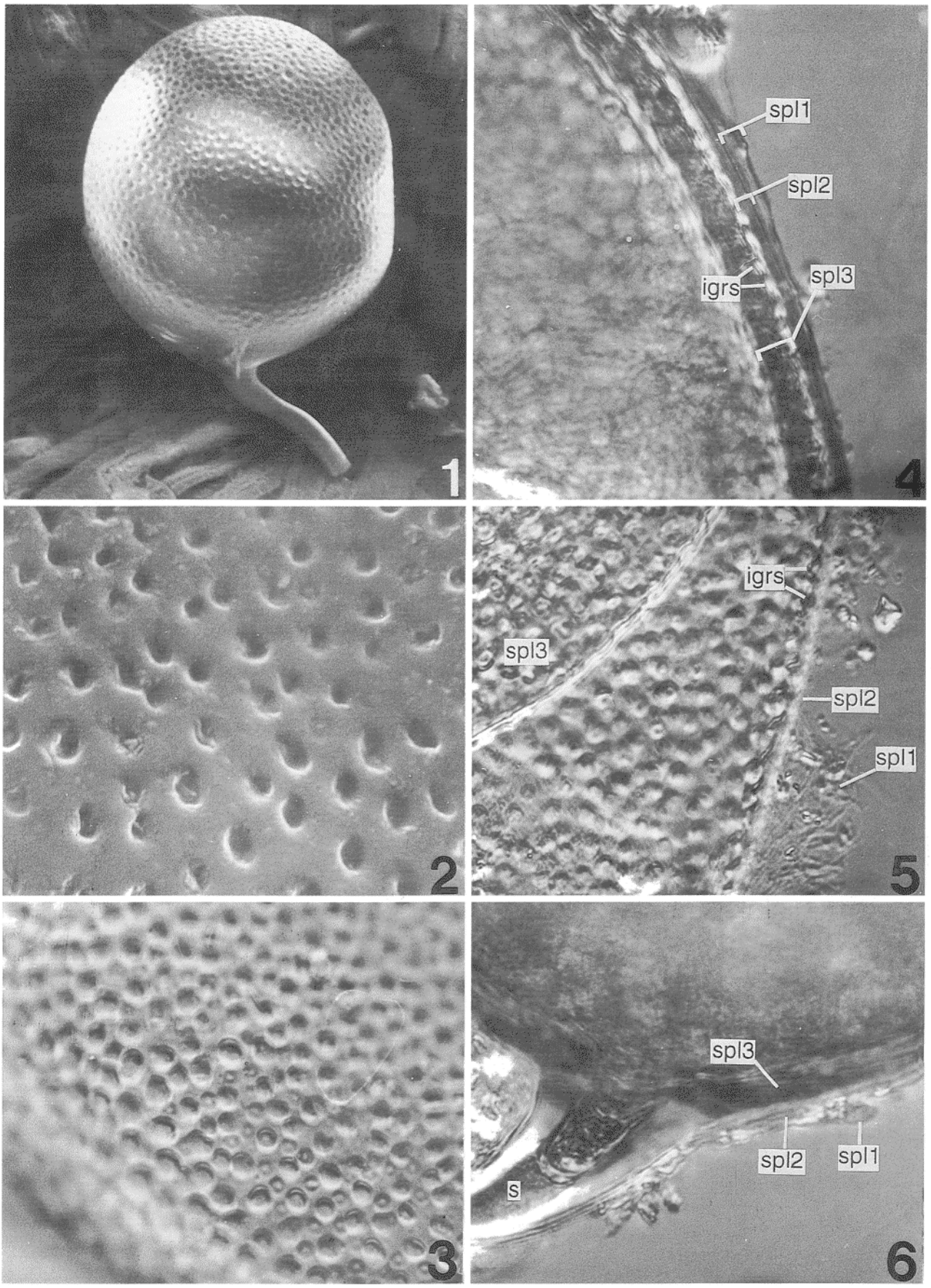
Distribution and habitat. Glomus multiforum was found only in one of almost 1000 soil samples collected in 165 localities of Poland. This fungus occurred among roots of a mixture of *P. major* and *P. trivialis* growing on a meadow. The spore density of *G. multiforum* in the field-collected soil was 23 in 100 g dry soil. The proportion of spores of this new species in the spore population of all arbuscular fungi isolated was 26.4%. The species of arbuscular fungi accompanying *G. multiforum* were *G. deserticola* Trappe, Bloss & Menge, *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske, *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, and two undescribed *Glomus* spp.

The chemical properties of the soil sample containing *G. multiforum* spores were: pH 6.6; N, 2551.8 mg L^{-1}; P, 1326.9 mg L^{-1}; K, 56.8 mg L^{-1}; organic C, 2.9%.

Mycorrhizal associations. Glomus multiforum was associated in the field with vesicular-arbuscular mycorrhizae of *P. major* and *P. trivialis*. This fungus formed vesicular-arbuscular mycorrhizae in pot cultures with *P. lanceolata* and *S. sudanense*.

Discussion. The unique feature of *G. multiforum* spores is their ornamentation consisting of evenly distributed pits in wall layer 3 (FIGS. 1–5). All the spores recovered from the original soil sample were pitted. However, spore populations isolated from single-species pot cultures of this new fungus always contained from 5% to 10% of mature spores with no or poorly visible pits.

Immature spores are hyaline and have a wall composed of two, thin layers. Because these layers tightly



adhere to each other, they are frequently seen as a one-layered structure. At times, layer 1 thickens and gradually deteriorates. Layer 2 thickens and produces evenly distributed ingrowths. It is rigid and resembles a unit wall sensu Walker (1983). In the next stage of spore development, layer 1 further decomposes and begins to slough, although almost all spores possess this layer. Layer 2 remains unchanged. The laminated layer is gradually synthesized from thin, colored laminae, of which some outer ones form pits due to the tight adherence to the ingrowths of layer 2 (FIGS. 4, 5). Only layer 1 stains reddish (12C5) in Melzer's reagent (FIG. 4). Two sloughing layers of which the outer one stains in Melzer's reagent also occur in spores of, e.g., *G. clarum* Nicol. & Schenck, *G. intraradices* Schenck & Smith, *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (Błaszkowski, 1994, pers. observ.; Morton, 1989; Stürmer and Morton, 1997), and *Entrophospora infrequens* (Hall) Ames & Schneider (Błaszkowski, pers. observ.). In the next stage, layer 1 may be completely sloughed. Layer 2 is more or less decomposed. In many spores, layers 1 and 2 resemble a structure composed of many layers, of which the innermost one does not stain in Melzer's reagent. Layers 1 and 2 of older spores may by completely sloughed, although most spores coming from both the field and pot cultures possess layer 2.

Differentiation of spore wall layers in *G. multiforum* proceeded similarly as in *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd. (Morton, 1996). The innermost laminated layer 3 of this new fungus began to form when the outer layers 1 and 2 completed their phenotypic properties. The best evidence of such a sequence in the development of these spore wall layers are the pits of layer 3, which form based on the ornamentation of spore wall layer 2. The presence in one-species cultures of *G. multiforum* of both spores with very shallow pits and those without them suggests that formation of layer 3 proceeds after a pause. During this period layer 2 probably undergoes decomposition that causes its ingrowths to become too soft to impress clear or any pits in the laminated layer 3.

When seen under a dissecting microscope, spores of *G. multiforum* most closely resemble those of *G. mosseae* and *G. verruculosum* Błaszk., the latter species described in this paper. Both species form spores similar in size, shape, color, and have a relatively broad, most frequently funnel-shaped subtending hypha occluded by a curved septum. However, *G. mosseae* spores frequently occur in sporocarps with a peridium, whereas those of *G. multiforum* and *G. verruculosum* are ectocarpic. Additionally, most mature spores of *G. multiforum* usually are darker than those of *G. mosseae*.

Examination of spores under a light microscope readily separates *G. multiforum* from *G. mosseae* and *G. verruculosum*, as well as from all the other known species of the genus *Glomus*. Only *G. multiforum* produces pitted spores. Smooth spores of *G. multiforum* may be distinguished from those of *G. verruculosum* with no ornamentation on the inner wall surface based on their reaction in Melzer's reagent. The outermost spore wall layer of the former fungus stains reddish, whereas spores of the latter species are nonreactive in this reagent. Smooth spores of *G. multiforum* and *G. mosseae* may be indistinguishable due to the similarity in appearance and spore wall structure with the outermost mucilaginous layer staining in Melzer's reagent. However, the spore wall of the former fungus usually is much thicker (6.6–14.2 μm) than that of the latter species (2–7 μm; Gerdemann and Trappe, 1974; Błaszkowski, pers. observ.).

Spores of *G. multiforum* examined under a light microscope are most similar to those of *Acaulospora gerdemannii* Schenck & Nicol. The latter fungus produces spores with a *Glomus*-like pedicel and has the exact same ornamentation pattern on wall layers 2 and 3 as does the former species (Morton, 1989). However, *A. gerdemannii* forms spores laterally on the neck of a sporiferous saccule, whereas those of *G. multiforum* develop terminally by swelling hyphal tip. The sporiferous saccule of fungi of the genus *Acaulospora* frequently becomes completely detached (Walker, 1987), and hence their spores, especially those of *A. gerdemannii* with its persistent pedicel, may be confused with spores of *Glomus* spp. having a fragile subtending hypha, as, e.g., in *G. albidum* Walker & Rhodes (Walker and Rhodes, 1981; Błaszkowski, pers. observ.). However, the subtending hypha of *G. multiforum* is much longer than that of *A. gerdemannii*. Additionally, compared with *G. multiforum* spores possessing a single wall comprising three layers: two sloughing layers and a laminated layer, those of *A. gerdemannii* have two walls: an outer wall composed of a sloughing layer adherent to two

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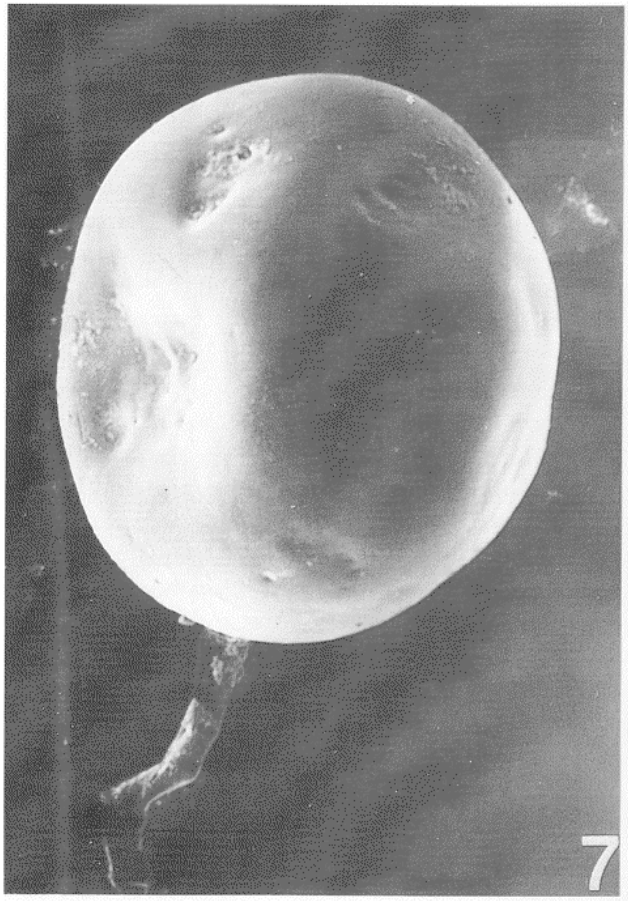
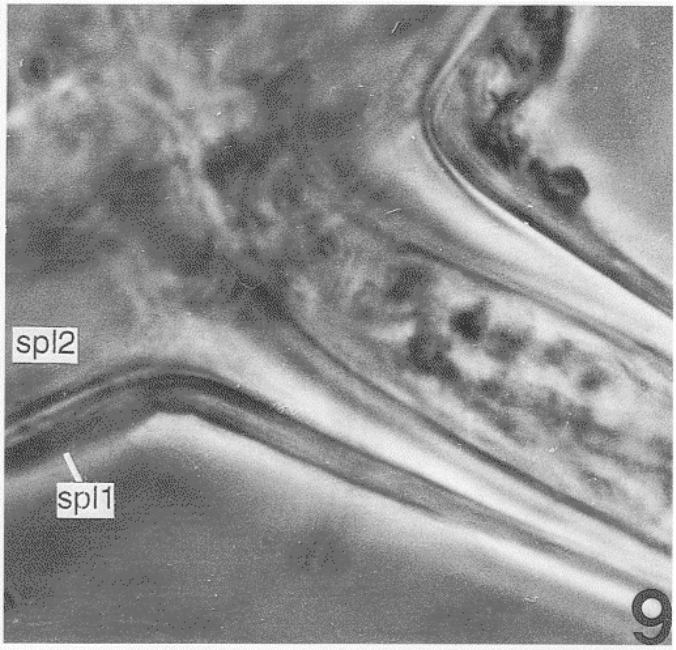
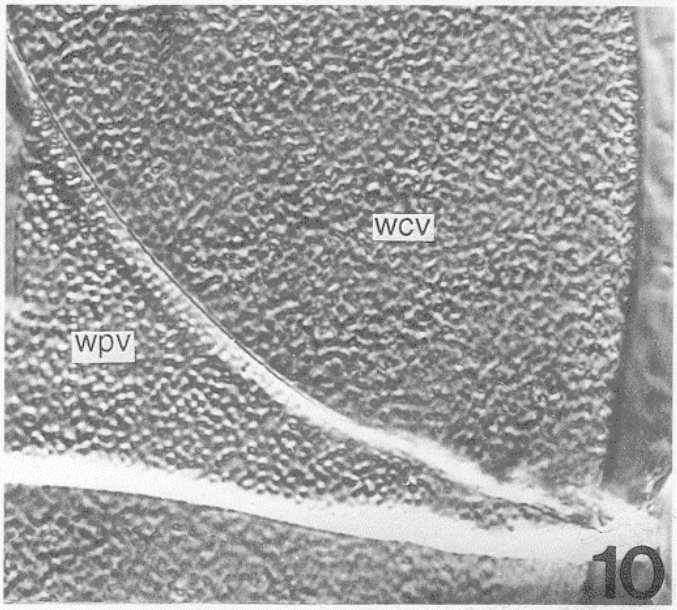
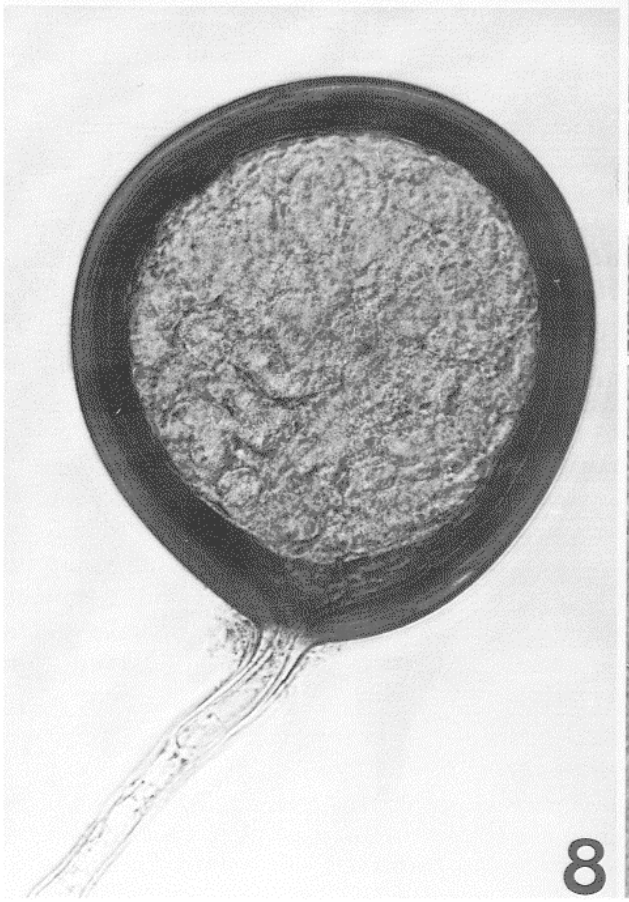
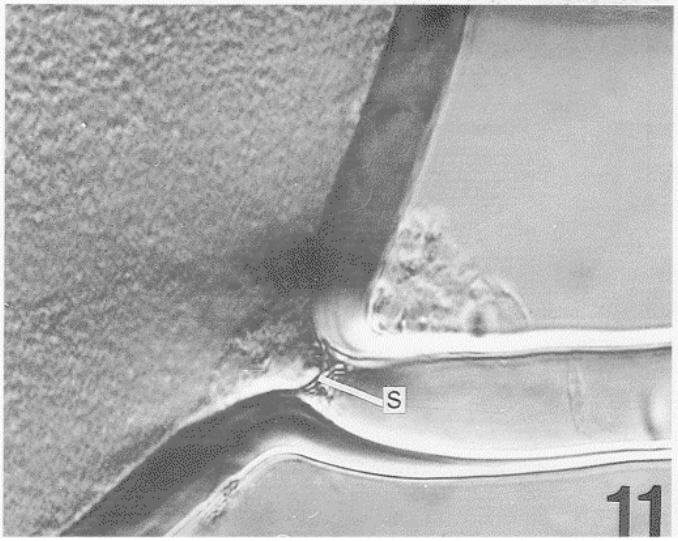
FIGS. 1–6. *Glomus multiforum*. 1. Intact spore. Scanning electron microscopy (SEM), × 270. 2 and 3. Pits of spore wall layer 3. SEM and differential interference contrast (DIC), respectively, × 1620 and × 1428, respectively. 4 and 5. Three layers of spore wall (spl 1–3) with ingrowths (igrs) of spore wall layer 2 are visible. Both DIC, both × 1428. 6. Funnel-shaped subtending hypha with a curved septum (s); spore wall layers (spl) 1–3 are seen. DIC, × 1428.











unit layers and a separable inner flexible, coriaceous wall *sensu* Walker (1983).

Other arbuscular fungi forming pitted spores similar in size, shape and color to those of *G. multiforum* are *Acaulospora cavernata* Błaszk., *A. excavata* Ingley & Walker, and *A. foveata* Trappe & Janos (Błaszkowski, 1989; Janos and Trappe, 1982; Ingleby et al., 1994). The presence of the persistent funnel-shaped subtending hypha in *G. multiforum* (vs. sessile spores in *A. cavernata, A. excavata,* and *A. foveata*) and inner walls in *A. cavernata, A. excavata,* and *A. foveata* (vs. no inner wall in *G. multiforum*) easily distinguishes the four fungi.

According to Morton (1990), the genus *Acaulospora* evolved from the genus *Glomus,* and *A. gerdemannii* is the most obvious transitional species. However, none of the earlier known *Glomus* spp. possess morphological properties which could indicate a presumable progenitor of *A. gerdemannii.* This fungus may be *G. multiforum.*

The high morphological variability of *G. multiforum* spores may cause difficulties in the recognition and, thereby, determination of the occurrence of this fungus, especially based on spores recovered from the field.

Glomus verruculosum Błaszkowski, sp. nov. FIGS. 7–11

Sporocarpia ignota. Sporae singulae in solo efformatae; luteae vel aurantiacae; globosae vel subglobosae; (150–) 189(–265) μm diam; aliquando ovoideae; 145–170 × 170–220 μm. Tunica sporae e stratis duabus (strati 1, 2); strato "1" semielastico, hyalino, (0.8–)1.3(–1.7) μm crasso; strato "2" laminato, luteo vel aurantiaco, (5.1–)9.8(–12.5) μm crasso, e verrucis, 0.8–1.7 μm altis, 0.5–0.7 μm diam ad basem. Hypha sustinentes luteae vel aurantiacae; rectae vel recurvate; subinfundibuliformes vel cylindricae; (15–) 21.3(–27.5) μm crassae; tunicae (4.8–)5.6(–6.9) μm crassae. Porus e curvo septo. Mycorrhizae cum vesiculis et arbusculis formans.

HOLOTYPUS. POLAND. Szczecin, infra *Sorghum sudanense* (Staph.) Piper, 15 May 1996, *Błaszkowski, J., 2148* (DPP).

Sporocarps unknown. Spores formed singly in the soil (FIGS. 7, 8); produced from straight sporophores. *Sporophore* coenocytic to sparsely septate; hyaline to yellowish white (4A2); (2.2–)3.5(–4.7) μm wide; with a wall (0.5–) 0.8(–1.1) μm thick; bearing spores by swelling at hyphal tip. *Spores* yellow (4A8) to orange (5B8); globose to subglobose; (150–)189(–265)μm diam; sometimes ovoid; 145–170 × 170–220 μm; with a single subtending hypha (Figs 7, 8). *Spore wall structure* (FIG. 9) of one wall with two layers (layers 1 and 2). Layer 1 semiflexible, hyaline, (0.8–)1.3(–1.7) μm thick, usually absent in field-collected specimens (FIGS. 9, 11). Layer 2 laminated, yellow (4A8) to orange (5B8), (5.1–)9.8(–12.5) μm thick, ornamented with evenly distributed warts projecting inward from the innermost lamina (FIG. 10); warts 0.8–1.7 μm high in cross view, 0.5–0.7 μm diam in plan view. *Subtending hypha* concolorous with spore wall layer 2; straight or recurved; funnel-shaped to almost cylindrical; (15–)21.3(–27.5) μm wide at the spore base (FIGS. 7–9, 11). *Wall of subtending hypha* yellow (4A8) to orange (5B8); (4.8–)5.6(–6.9) μm thick at the spore base; continuous with spore wall layers 1 and 2 (FIGS. 9, 11); layer 1 continuing distally for up to 25 μm along the subtending hypha. *Pore* closed by a curved septum, continuous with the innermost lamina of spore wall layer 2 (FIG. 11). Spore contents of oil droplets. Germination by regrowth through the subtending hypha.

Collections examined. HOLOTYPE. POLAND. Szczecin, under pot-cultured *S. sudanense,* 15 May 1996, *Błaszkowski, J., 2148* (DPP); ISOTYPES: *Błaszkowski, J. 2149–2163* (DPP) and two slides at OSC. Living cultures of *G. verruculosum* were deposited in the Banque Européene des Glomales (U. K.) and in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (West Virginia University, USA).

Other materials examined. POLAND. Szczecin, from the root zone of *G. aquatica,* 5 Aug. 1988, *Błaszkowski, J., 1198–1219;* Szczecin, under *E. hirsutum,* 25 Sept. 1989, *Błaszkowski, J., 1230–1233, 1271* (DPP).

Etymology. verruculosum referring to the warted spores. We wish to dedicate this species to Prof. Dr. Włodzimierz Songin, Department of Plant Cultivation, Academy of Agriculture, Szczecin.

Distribution and habitat. Glomus verruculosum was found in seven of almost 1000 soil samples collected in Poland. All the soils containing this new species came from the root zone of plants growing along the banks of the river Odra. The spore density of *G. verruculosum* in 100 g dry soil ranged from 2 to 54 (mean 31.1). The participation of *G. verruculosum* spores in the total number of spores of arbuscular fungi recovered ranged from 3.1 to 83.3% (mean 43.9%). This fungus was isolated together with 1 to 2 other species of arbuscular fungi, including *G. aggregatum* Schenck & Smith emend. Koske, *G. constrictum* Trappe, *G. etunicatum* Becker & Gerd., *G. fasciculatum* (Thaxter) Gerd. & Trappe *emend.* Walker & Koske, and *G. mosseae.* The chemical properties of the soils harboring *G. verruculosum* ranged: pH 7.4–7.8; N, 24.9–42.9 mg L^{-1}; P, 24–57 mg L^{-1}; K, 39–215 mg L^{-1}; organic C, 0.42–1.71%.

Mycorrhizal associations. Glomus verruculosum was associated with vesicular-arbuscular mycorrhizae of

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FIGS. 7–11. *Glomus verruculosum.* 7 and 8. Intact spores. SEM and bright-field microscopy, × 349 and × 360, respectively. 9. Spore wall layers (spl) 1 and 2 at the spore base. Phase contrast microscopy, × 1900. 10. Warts of spore wall layer 2 in plan (wpv) and cross (wcv) views. DIC, × 1428. 11. Recurved subtending hypha occluded by a septum (s). DIC, × 1428.

Epilobium hirsutum L. and *Glyceria aquatica*. This fungus formed vesicular-arbuscular mycorrhizae in pot cultures with *P. lanceolata* and *S. sudanense*.

Discussion. The presence of warts on the inner surface of spore wall layer 2 (FIG. 10) is the most distinctive property of *G. verruculosum*. The warts are evenly distributed on layer 2. They are best seen at the edge of a crushed spore or on its inner surface observed in plan view using Nomarski interference contrast (FIG. 10). The warts occurred in all field-collected spores. However, spores coming from single-species pot cultures had the innermost lamina ornamented with warts only when these cultures were at least 8-mo-old.

Spores of *G. verruculosum* develop terminally by swelling hyphal tip. Very young spores of diameters of 40–60 μm have a wall composed of two thin tightly adherent hyaline layers, each 0.5–0.8 μm thick, continuous with the wall of subtending hypha. The outer layer is either rigid and resembles a unit wall sensu Walker (1983) or undergoes decomposition. None of these layers stain in Melzer's reagent. In the next stage of spore development, layer 1 slightly thickens, although the material constituting this layer simultaneously deteriorates and may slough (FIG. 9). Layer 2 thickens due to the increasing number of laminae. At the end of spore development, warts on the inner surface of the innermost lamina appear (FIG. 10). Layer 1 either is very thin or absent. The subtending hypha is always closed by a curved and smooth septum (FIG. 11), nevertheless, it is continuous with the innermost ornamented lamina of spore wall layer 2.

Spores of *G. verruculosum* most resemble those of *G. pansihalos* Berch & Koske in color, size, and in having a laminated wall ornamented with warts. However, the warts of the former project inward rather than outward as in the latter (Berch & Koske, 1986). Additionally, the outermost spore wall of *G. verruculosum* is evanescent, whereas that of *G. pansihalos* is expanding. *Glomus verruculosum* also lacks the innermost unit wall of *G. pansihalos*. Other species forming spores with warts are *G. callosum* Sieverding, *G. chimonobambusae* Wu & Liu, *G. dominikii* Blaszk., and *G. scintillans* Rose & Trappe. In all these species, the warts occur on the surface of hyaline to white spores of a different wall structure from that of *G. verruculosum* (Błaszkowski, 1988; Rose and Trappe, 1980; Sieverding, 1988; Wu et al., 1995).

Intact spores of *G. verruculosum* in early stages of development of warts on the inner surface of the laminated spore wall layer 2 may easily be confused with those of *G. geosporum* (Nicol. & Gerd.) Walker due to similarity in color, size, and properties of their subtending hyphae (Błaszkowski, pers. observ.; Miller and Jeffries, 1994; Walker, 1982). However, the laminated layer of both young and mature *G. geosporum* spores always is smooth. Additionally, young spores of this fungus stain deep red in Melzer's reagent (Błaszkowski, pers. observ.; Walker, 1982), whereas those of *G. verruculosum* remain nonreactive in this reagent through the entire life cycle.

Spores of *G. verruculosum* also resemble smooth *G. multiforum* spores. However, most mature spores of the latter fungus are pitted (vs. the smooth surface of *G. verruculosum* spores); the laminated layer of mature spores of the former species is always ornamented with warts extending into the lumen of the spore (vs. smooth the innermost lamina of the laminated spore wall layer in *G. multiforum*). Moreover, in contrast to spores of *G. multiforum*, neither young nor fully developed spores of *G. verruculosum* react in Melzer's reagent.

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