

Glomus corymbiforme, a new species in Glomales from Poland

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Abstract: *Glomus corymbiforme* sp. nov. is described and illustrated. This fungus was found associated with roots of different plant species colonizing maritime sand dunes adjacent to Swinoujście in northwestern Poland. *Glomus corymbiforme* is distinctive in forming corymbiform sporocarps with spores enveloped individually by a hyphal mantle composed of dichotomously branched hyphae. Spores are pastel yellow to orange, globose to subglobose, (50–)142(–220) μm in diameter or ovoid to pyriform, 110–125 \times 120–200 μm . Spore wall structure consists of an outermost unit wall tightly adherent to a middle laminated wall and of a separable innermost membranous wall. *Glomus corymbiforme* produced spores and vesicular-arbuscular mycorrhizae in single-species pot cultures with *Sorghum vulgare*.

Key Words: Glomales, *Glomus corymbiforme*, new species, occurrence, Poland, Zygomycetes

During studies on arbuscular fungi (Glomales, Zygomycetes) associated with roots of plants colonizing maritime sand dunes of Poland, spores of an undescribed species of the genus *Glomus* Tul. & Tul. were recovered. Because of the corymbiform organization of the spores in sporocarps, this species is described as *G. corymbiforme*, sp. nov.

Roots and attached soil of sample plants were collected from a depth of 5–30 cm and subsequently refrigerated at 4 C until processing. Pot cultures were established by mixing field-collected soils and roots with autoclaved coarse-grained sand (1:2, v/v; pH 6.6, 13 and 12 mg L⁻¹ P and K, respectively). This mixture was placed in 15-cm plastic pots (1350 cm³) and seeded with *Sorghum sudanense* (Staph.) Piper. Plants were grown in a greenhouse at 18–30 C with 12-h photoperiod (combined incandescent and cool white fluorescent light) and watered two to three times a week. Pot cultures were harvested after 4–5 months, spores extracted (Gerdemann and Nicolson, 1963), roots

stained with 0.05% trypan blue (Phillips and Hayman, 1970), and examined for the presence of mycorrhizae.

To establish single-species pot cultures, 50–80 newly formed spores were placed on roots of 10- to 14-day-old seedlings of *Sorghum vulgare* Pers. The seedlings were grown in 10-cm plastic pots (400 cm³) for 3–5 months. The pot medium was an autoclaved mixture of coarse-grained sand and sandy loam soil (1:2, v/v; pH 6.5, 15 and 12 mg L⁻¹ P and K, respectively).

Morphological investigations were conducted based on spores extracted from both field soil samples and pot cultures. At least 100 and 20 spores were mounted in polyvinyl alcohol/lactic acid/glycerol, PVLG (Koske and Tessier, 1983) and Melzer's reagent, respectively, and examined. Spore wall characteristics and terminology are those suggested by Bentivenga and Hetrick (1991), Spain et al. (1989), 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 preserved on slides mounted in PVLG 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 fungi noted here follows Almeida and Schenck (1990) and Walker and Trappe (1993); the classification follows that of Morton and Benny (1990).

Glomus corymbiforme Błaszowski, sp. nov.

FIGS. 1–9

Sporae in sporocarpis corymbiformis, raro singulatim in solo, e sporophoris paniculatis vel rectis efformatae. Sporocarpi globosa vel subglobosa; (180–)336(–490) μm diam; aliquando irregulare; 180–350 \times 210–500 μm ; sporis 2–13 in tunica hypharum. Tunica (20–)47.5(–90) μm crassa, hyphis leptotichis, hyalinis vel luteolo-albis, septatis, 3- vel 4-plo ramosis formata. Sporophorum nonseptatum vel parce septatum; hyalinum vel luteum; (10.3–)14.7(–17.5) μm latum; pariete (1.5–)1.6(–1.7) μm crasso; 2- vel 3-plo ramoso monopodialibus, raro rectum; ramis principalibus 1–10-plo ramificationibus monopodialibus secundariibus. Sporae pallidae luteae vel aurantiacae; globosae vel subglobosae; (50–)142(–220) μm diam; aliquando ovoideae vel piriformae; 110–125 \times 120–200 μm ; hypha subtenda solitaria. Tunica sporae tristratosa (stratis 1–3) autem in una ("A") consociata; strato "1" rigido, hyalino vel intense luteo, (0.7–)1.1(–1.7) μm crasso; strato "2" laminato, pallide luteo vel aurantiaco, (3.9–)7(–

10) μm crasso; strato "3" membranaceo, hyalino, (0.5–)0.9(–1.2) μm crasso. Hypha subtenda crenea vel intense aurantiaca; recta vel recurva, infundibuliforma, aliquando coliga; (9.8–)21(–31.1) μm lata ad basim sporae; pariete cremo vel intense aurantiaco; (2.2–)6.5(–13.7) μm crasso, stratis 1, 2 sporae continuo. Porus e septo strati "3" sporae vel e septo continuo strati "2" sporae efformata. Vesicular-arbusculares mycorrhizae formans.

HOLOTYPE. Poland, Świnoujście, infra *Ammophila arenaria* Link, 6 Oct. 1993, Błaszowski, J., 2022 (DPP).

Spores occurring in corymbiform sporocarps when formed from branched sporophores, rarely single in the soil when produced from straight sporophores. *Sporocarps* globose to subglobose; (180–)336(–490) μm diam; sometimes ovoid; 180–350 \times 210–500 μm ; composed of 2–13 (av. 6) spores enveloped individually by hyphal mantle (FIG. 1). *Mantle* (20–)47.5(–90) μm thick; consisting of a network of hyphae branching dichotomously three to four times more or less at right angles; hyphae thin-walled, hyaline to yellowish white (3A2), septate (FIGS. 4, 5); length and diameter of branches diminishing with each successive dichotomy; initial hypha (9.8–)17.7(–27.2) μm long, (4.2–)4.9(–6.9) μm wide; developing from spore wall 1; final branch (13.5–)19.4(–25) μm long, (1.2–)1.8(–2.9) μm wide; distance between septa (14.5–)25.7(–31.4) μm ; mantle frequently absent in mature spores (FIG. 2). *Sporophore* (FIGS. 2, 3) coenocytic to sparsely septate; hyaline to yellow (5A6); (10.3–)14.7(–17.5) μm wide; with a wall (1.5–)1.6(–1.7) μm thick; usually with two to three main monopodial branches, rarely straight; main branches frequently with 1–10 (av. 4) monopodial second branches sometimes branched monopodially one to two times (FIGS. 2, 3); main, second and third branches slanted at 30–45° towards their parent hyphae; straight and branched sporophores bearing spores by swelling hyphal tip. *Spores* (FIGS. 1, 2) pastel yellow (3A4) to orange (6A8); globose to subglobose; (50–)142(–220) μm diam; sometimes ovoid or pyriform; 110–125 \times 120–200 μm ; with a single subtending hypha. *Spore wall structure* (FIGS. 6, 9) of three walls (1–3) in one group (A). Wall 1 unit, smooth, hyaline to deep yellow (4A8), (0.7–)1.1(–1.7) μm thick, closely attached to wall 2. Wall 2 laminated, pastel yellow (3A4) to orange (6A8), (3.9–)7(–10) μm thick. Wall 3 membranous, hyaline, (0.5–)0.9(–1.2) μm thick, usually tightly adherent to wall 2. *Subtending hypha* (FIGS. 7, 8) cream (4A3) to deep orange (5A8); straight or recurvate; funnel-shaped, sometimes cylindrical or constricted; (9.8–)21(–31.1) μm wide at the spore base. *Wall of subtending hypha* cream (4A3) to deep orange (5A8); (2.2–)6.5(–13.7) μm thick; continuous with spore walls 1 and 2. *Pore* (FIGS. 7, 8) occluded by ingrowth of spore wall 3, a septum, ca 0.7 μm wide, continuous with the innermost lamina of spore wall 2, and occa-

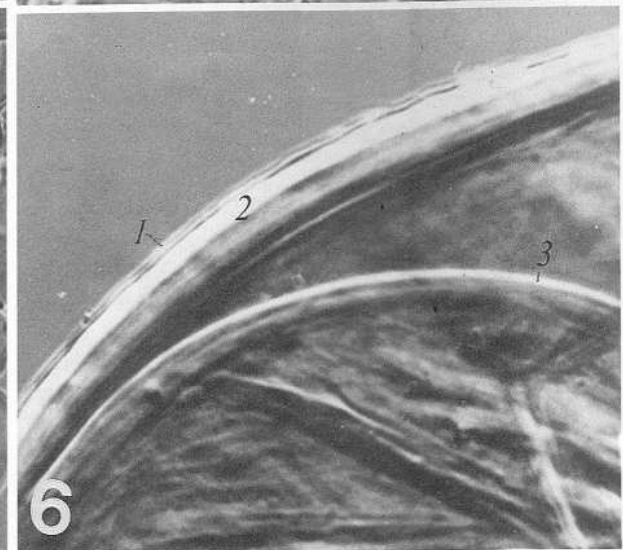
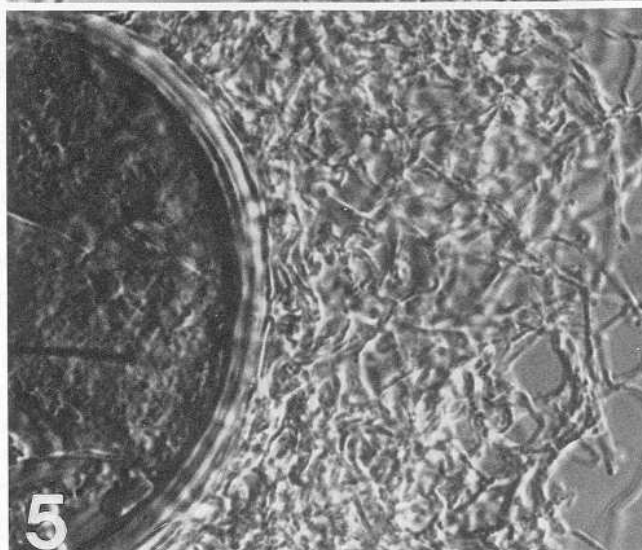
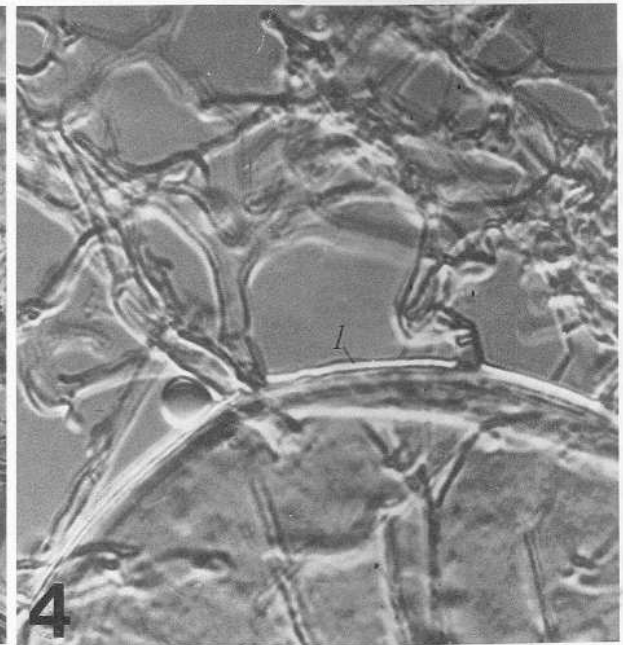
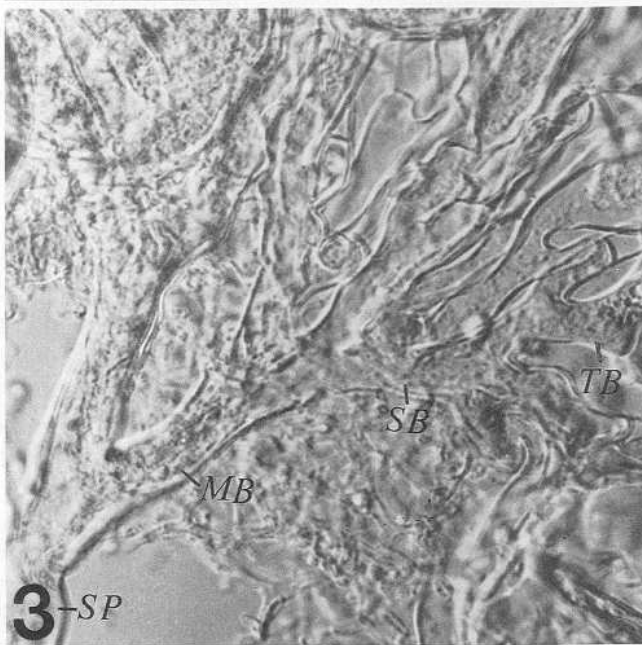
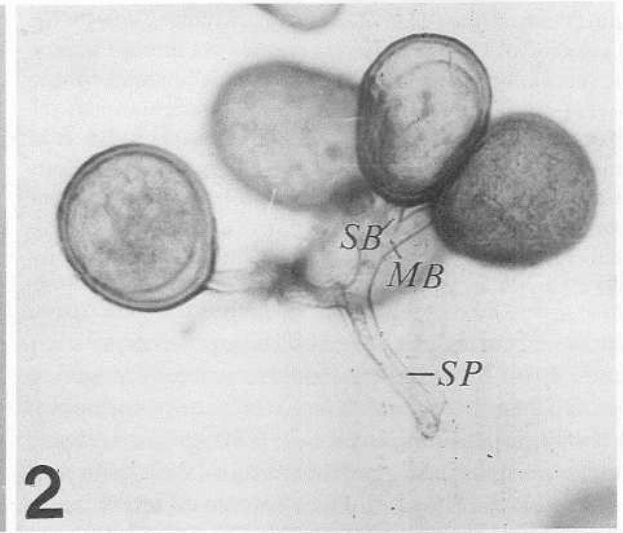
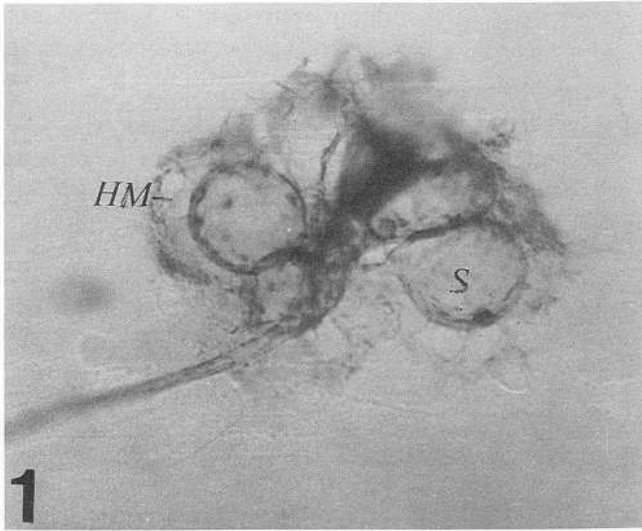
sionally by thickening of spore wall 2. Spore contents of oil droplets. Mantle and spores not reacting in Melzer's reagent.

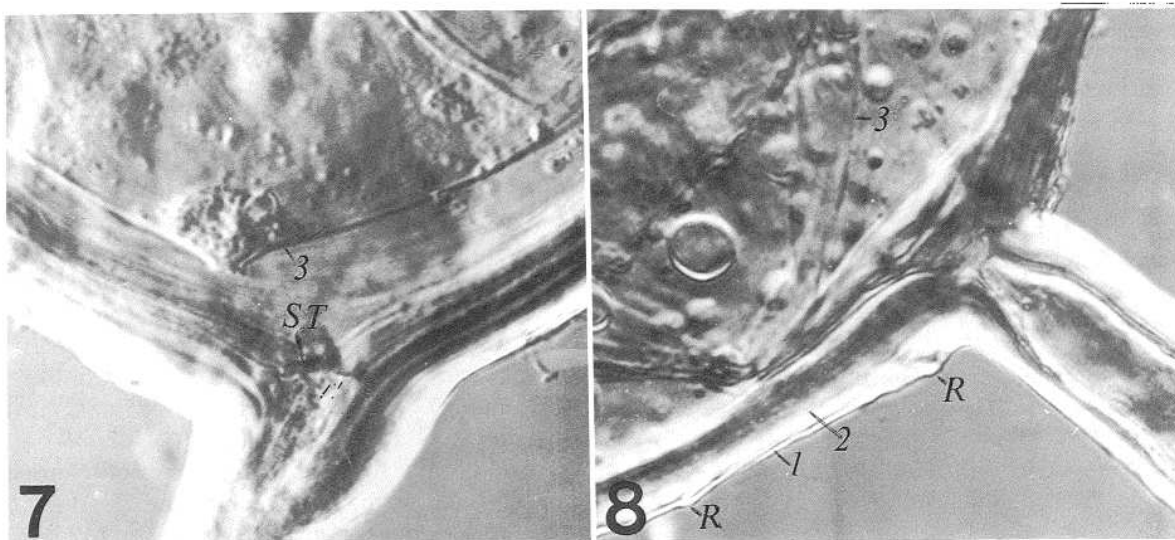
Collections examined. HOLOTYPE. POLAND. Świnoujście, under *Ammophila arenaria* Link, 6 Oct. 1993, Błaszowski, J., 2022 (DPP); ISOTYPES: Błaszowski, J., 2023–2025 (DPP) and two slides at OSC.

Other materials examined. POLAND. All from Świnoujście, under *A. arenaria*, 1 Oct. 1991, Błaszowski, J., 2004–2010 (DPP); under *A. arenaria*, 22 Sept. 1992, Błaszowski, J., 2011–2015 (DPP); under *A. arenaria*, 13 May 1993, Błaszowski, J., 2016–2018 (DPP); under *Petasites spurius* (Retz.) Rchb., 6 Oct. 1993, Błaszowski, J., 2019 (DPP); under *Hieracium umbellatum* L., 6 Oct. 1993, Błaszowski, J., 2020–2021 (DPP); under *A. arenaria*, 6 Oct. 1993, Błaszowski, J., unnumbered collections (DPP).

Etymology. Latin, *corymbiforme*, referring to the clustered organization of spores in sporocarps.

Distribution and habitat. *Glomus corymbiforme* occurred in 20 of the almost 600 examined soil samples collected in 157 localities of Poland. Of the 158 soil samples representing cultivated sites, none contained spores of this new species (Błaszowski, 1993). This fungus was associated only with roots of plants colonizing maritime dunes adjacent to Świnoujście. Examination of soils of other maritime dune sites (Błaszowski, 1994) and those coming from inland dunes (Błaszowski, 1992) did not reveal *G. corymbiforme* spores. The plant species under which *G. corymbiforme* spores occurred were *A. arenaria*, *H. umbellatum*, and *P. spurius*. The plant most frequently investigated was *A. arenaria* (16 samples). The spore density of *G. corymbiforme* ranged from 3 to 829 (av. 150.1) in 100 g dry soil. The proportion of spores of this species in the spore populations of all arbuscular fungi recovered ranged from 2.3 to 100% (av. 53.9%). The species density of arbuscular fungi in samples containing *G. corymbiforme* ranged from 1 to 6 (av. 3.1) in 100 g dry soil. The species accompanying *G. corymbiforme* were *Acaulospora elegans* Trappe & Gerd. (= *A. bireticulata* Rothwell & Trappe) (Morton et al., 1993; Walker, pers. comm.) (present in 10% of soils with *G. corymbiforme*); an undescribed *Entrophospora* sp. (15%); *G. aggregatum* Schenck & Smith emend. Koske (20%); *G. clarum* Nicol. & Schenck (5%); *G. constrictum* Trappe (5%); *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske (15%); *G. intraradices* Schenck & Smith (5%); *G. macrocarpum* Tul. & Tul. (5%); *G. pustulatum* Koske et al. (50%); three undescribed *Glomus* spp. (25%); *Scutellospora armeniaca* Błazsk. (10%); *S. dipurpureus* Morton & Koske (45%); and *S. pellucida* (Nicol. & Schenck) Walker & Sanders (10%). Of the species most frequently co-occurring with *G. corymbiforme*, the mean proportions of *G. aggregatum*, *G. pustulatum*, and *S. dipurpureus* in the spore populations isolated





FIGS. 7-8. *Glomus corymbiforme*. 7. Funnel-shaped subtending hypha of crushed spore occluded by septum (ST); wall 3 enclosing spore content is arrowed. DIC, $\times 1220$. 8. Spore with slightly constricted subtending hypha; spore walls are arrowed; roughnesses (R) of wall 1 are seen. DIC, $\times 1220$.

from 100 g dry soil were 24.7% (range: 1.4-78.2%), 26.5% (1.0-78.9%), and 16.0% (0.7-40.3%), respectively.

The chemical properties of 11 randomly selected soil samples containing *G. corymbiforme* were: pH, 3.8-6.7; NO_3 (mg/L), 20-72; P, 5-12; K, 2-26, Mg, 10-41; Na, 4-23; Cl, 15-25; KCl, 0.1-0.6; organic C (%), 0.1-1.1.

Mycorrhizal associations. *Glomus corymbiforme* was associated in the field with vesicular-arbuscular mycorrhizae of *A. arenaria*, *H. umbellatum*, and *P. spurius*. The levels of mycorrhizal colonizations in *A. arenaria*, *H. umbellatum*, and *P. spurius* were 2.1-35.6% (av. 27.0%), 5.7-74.1% (av. 42.3%), and 8.1-49.8% (av. 46.3%), respectively, of root length (Giovannetti and Mosse, 1980). This fungus formed vesicular-arbuscular mycorrhizae in pot cultures with *S. vulgare*.

The distinctive features of *G. corymbiforme* are the hyphal mantle enveloping individual spores and the clustered, corymbiform organization of spores in sporocarps.

Spores of *G. corymbiforme* are initiated from thin-walled vesicles produced terminally from straight or branched sporophores swelling at their tips. At times, the vesicle wall becomes thicker due to the formation

of a laminated wall first and then an innermost membranous wall. Straight, thin-walled hyphae grow from the vesicles and the outermost wall of immature spores. Small pores connecting the spore inside with the hyphal lumen are visible in young but mature specimens when seen in a plan view. These hyphae branch dichotomously and become thinner with each successive dichotomy. The hyphae of each spore intertwine with those of neighboring spores, forming a common mantle enveloping a sporocarp. This mantle detaches from mature spores.

The surface of most mature spores seen in a cross-sectional view is somewhat roughened, although walls 1 and 2 tightly adhere to each other. These roughnesses probably are a result of deformations of walls 1 and 2 originated during both the development of initial mantle hyphae and the process of occlusion of the pores connecting these hyphae with the spore contents. The latter likely interrupts physiological unity first and then physical unity between the spore and hyphae. Formation of occlusion also is a reason of detachment of hyphal sporiferous saccule from spore in many species of the genus *Acaulospora* (Morton, 1988).

Spore wall 1 may be difficult to see in mature spec-

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FIGS. 1-6. *Glomus corymbiforme*. Numbers indicate spore wall arrangement (see FIG. 9). 1. Young sporocarp containing spores (S) with hyphal mantle (HM). Bright-field microscopy (BFM), $\times 120$. 2. Mature sporocarp with spores arising from main (MB) and second (SB) branches of sporophore (SP). BFM, $\times 150$. 3. Main (MB), second (SB) and third (TH) branches of sporophore (SP). Differential interference contrast (DIC), $\times 1180$. 4. Dichotomously branched hyphae developed from spore wall 1. DIC, $\times 1180$. 5. Spore with abundant interwoven hyphae of hyphal mantle. DIC, $\times 1080$. 6. Walls of spore crushed in PVLG. DIC, $\times 1220$.

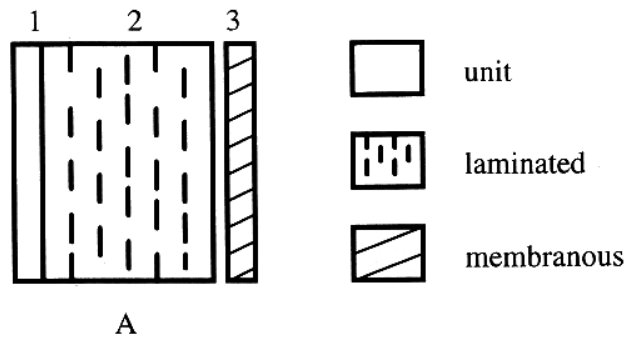


FIG. 9. Murograph of the wall structure of *Glomus corymbiforme* (after Walker, 1983). Arabic numerals indicate each wall in order from outer to inner wall; letter indicates wall group.

imens due to the similarity in color to wall 2. Disclosure of this wall facilitates examination of young spores in which wall 1 is hyaline (vs. pastel yellow wall 2) and continuous with the initial hyphae of the hyphal mantle. Additionally, in mature spores, this wall is thicker than the laminae of wall 2 and occasionally somewhat detaches at the spore base, when spores are mounted in lactic acid for a few days. Wall 2 is a typical laminated wall whose recognition improves its observation under a light microscope equipped with Nomarski differential interference contrast optics. The membranous wall 3 is very thin and usually tightly adheres to the laminated wall 2 in immature spores and, therefore, may easily be omitted. At times, this wall becomes thicker and somewhat more rigid, and hence easily separates from wall 2 in mature spores. Wall 3 is a complete endospore with an invagination extending up to 7 μm into the lumen of the subtending hypha. The subtending hyphae of most of the specimens examined are funnel-shaped, and only ca 25% of spores have cylindrical or weakly constricted subtending hyphae.

Apart from *G. corymbiforme*, other species of the genus *Glomus* forming spores enveloped by a hyphal mantle are *G. convolutum* Gerd. & Trappe, *G. globiferum* Koske & Walker, *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. mortonii* Bent. & Hetrick, *G. pubescens* (Sacc. & Ellis) Trappe & Gerd., *G. sinuosa* (Gerd. & Bakshi) Almeida & Schenck, and *G. tortuosum* Schenck & Smith. The ontogeny of the hyphal mantle of *G. corymbiforme* is most similar to that of the mantle of *G. globiferum* spores. Both species form a mantle from initial hyphae growing from thin-walled vesicles or young spores (Wu and Sylvia, 1993). However, in *G. globiferum*, these hyphae bear vesiculate swelling that are absent in *G. corymbiforme*. According to Koske and Walker (1986), the mantle hyphae of *G. globiferum* also are darker (yellow-brown vs. hyaline to yellowish white

in *G. corymbiforme*) and wider [5–50 μm vs. (1.2–)1.8–4.9(–6.9) μm]. Additionally, spores of the former species occur singly in the soil, whereas the latter fungus mainly produces spores in sporocarps. The sinuous mantle hyphae of *G. mortonii*, *G. sinuosa*, and *G. tortuosum* (Bentivenga and Hetrick, 1991; Gerdemann and Bakshi, 1976; Schenck and Smith, 1982) readily distinguish the three species from *G. corymbiforme* with its mantle composed of dichotomously branched hyphae. The hyphae enclosing spores of *G. convolutum*, *G. mosseae*, and *G. pubescens* are irregularly branched (Gerdemann and Trappe, 1974) and, thereby, unlike those enveloping *G. corymbiforme* spores. Additional features separating *G. corymbiforme* from *G. pubescens* and *G. sinuosa* are the size and shape of spores (Gerdemann and Bakshi, 1976; Gerdemann and Trappe, 1974). Spores of *G. corymbiforme* are larger (av. 142 μm diam; range 50–220 μm diam) than those of both *G. pubescens* (20–48 \times 18–45 μm) and *G. sinuosa* (45–118 \times 30–83 μm). *Glomus sinuosa* produces obovate to clavate spores, whereas most *G. corymbiforme* spores are globose to subglobose.

Regardless of whether the hyphal mantle is present, the uniquely clustered (corymbiform) organization of *G. corymbiforme* sporocarps readily distinguishes this new species from all other described species of *Glomus*.

Single mature spores of *G. corymbiforme* may be confused with those of *G. globiferum* in which the hyphal mantle is occasionally poorly developed and difficult to see (Koske and Walker, 1986). Both species have spores similar in size, shape, and wall structure. However, spores of the latter fungus are darker (orange-brown to red-brown or occasionally black) than those of the former fungus, being typically orange. Other fungal species producing spores with three walls of the same types as in *G. corymbiforme* are *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske, *G. maculosum* Miller & Walker, and *G. pustulatum* Koske et al. (Koske et al., 1986; Miller and Walker, 1986; Walker and Koske, 1987). However, spores of *G. fasciculatum* are lighter (pale yellow to yellow-brown vs. pastel yellow to orange), smaller [(50–)60–95(–149) \times 55–90(–149) μm vs. (50–)142(–220) μm diam], and stain in Melzer's reagent (vs. not reacting in *G. corymbiforme*). *Glomus maculosum* and *G. pustulatum* have spores with ornamented walls 3 and 1, respectively, whereas all walls in *G. corymbiforme* are smooth.

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