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Polish Glomales

XI. Glomus pustulatum

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Abstract. The morphological features of spores of *Glomus pustulatum* found in Poland are described and illustrated, and the worldwide occurrence of this species is characterized. Spores of *G. pustulatum* from Poland do not differ from those originally found in Canada and the United States of America. They were found in only one of more than 300 soil samples taken from over 100 localities in Poland. *G. pustulatum* was associated with roots of *Ammophila arenaria* colonizing maritime sand dunes of the Słowinski National Park, with a mean spore density of 12 in 100 g dry soil. Associated species of *G. pustulatum* were *Acaulospora dilatata* and *Scutellospora dipurpurascens*. *G. pustulatum* was found in Poland for the first time and is probably a species new to Europe.

Key words: Glomales – Glomus – Taxonomy – Poland

Introduction

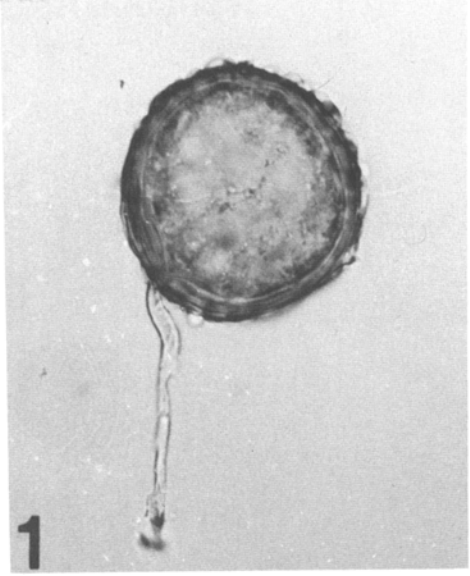
As part of a study of the occurrence of arbuscular fungi (Glomales) in Poland, soil samples were taken from around plants colonizing maritime sand dunes in the Słowinski National Park (54°45′N, 17°26′E). Among the numerous species recovered was *Glomus pustulatum*, a fungus so far not recorded in Poland. The aim of the work reported in this paper was to describe and illustrate the morphological features of *G. pustulatum* spores from Poland and to characterize the worldwide occurrence of this species.

Materials and methods

Soils were sampled from a depth of 5–30 cm using a small shovel. After storage in a refrigerator at 4° C for 6 months, spores were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963). Pot cultures were established in 200-cm containers filled with an autoclaved soil:sand (1:2 v/v) mixture. *Trifolium repens* L. was the host plant. Plants were grown in a greenhouse at 18–25° C with a 12-h photoperiod and watered once a day. Ten- to 15-day-old clover seedlings were inoculated with 30 field-collected spores sandwiched between two layers of roots. After 1 year, pot cultures were harvested and the soils wet sieved. The roots were stained (Phillips and Hayman 1970) and examined for the presence of mycorrhizae. Fifty field-collected spores were mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG) (Koske and Tessier 1983) and examined. The spore wall characters and terminology are those suggested by Walker (1983). Spore colour was examined under a dissecting microscope with fresh specimens immersed in water and colours determined according to the Methuen Handbook of Colour (Kornerup and Wanscher 1983). Specimens preserved in 5% formalin and PVLG were deposited in the Department of Plant Pathology (DPP), Academy of Agriculture, Szczecin, Poland. The spelling of scientific names is according to Almeida (1989) and Walker (1991), and the classification is that of Morton and Benny (1990).

Results

Glomus pustulatum Koske, Friese, Walker & Dalpé (Figs. 1–10). *Sporocarps* unknown. *Spores* formed singly in the soil or in roots, terminally on a coenocytic or sparsely septate hypha; deep orange (5A8); globose to slightly ovoid; (85–) 100 (–110) μm in diameter. *Spore wall structure* comprises three walls (1–3) in one group. Wall 1 unit, greyish-yellow (1B4) to butter yellow (4A5), with a basic thickness of (1.0–) 1.7 (–2.7) μm, ornamented with blistery, cup- or irregularly shaped outgrowths, 2.0–5.9 μm high, 3.0–11.8 μm wide at the base, circular, elliptic to irregular in plan view, unevenly distributed on the spore surface. Wall 2 laminated, deep orange (5A8), (3.2–) 5.4 (–10.3) μm thick. Wall 3 membranous, hyaline, 0.8–1.0 μm thick, tightly adherent to wall 2. *Subtending hypha* pale yellow (3A3) at the spore base, lighten to hyaline distally, straight or curved, cylindric or funnel shaped, (5.4–) 6.5 (–7.1) μm wide at the spore base, with a wall (0.2–) 1.3 (–2.0) μm thick, continuous with spore wall 2; pore closed by thickening of spore wall 2 or by a curved septum. No spore wall reaction with Melzer's reagent.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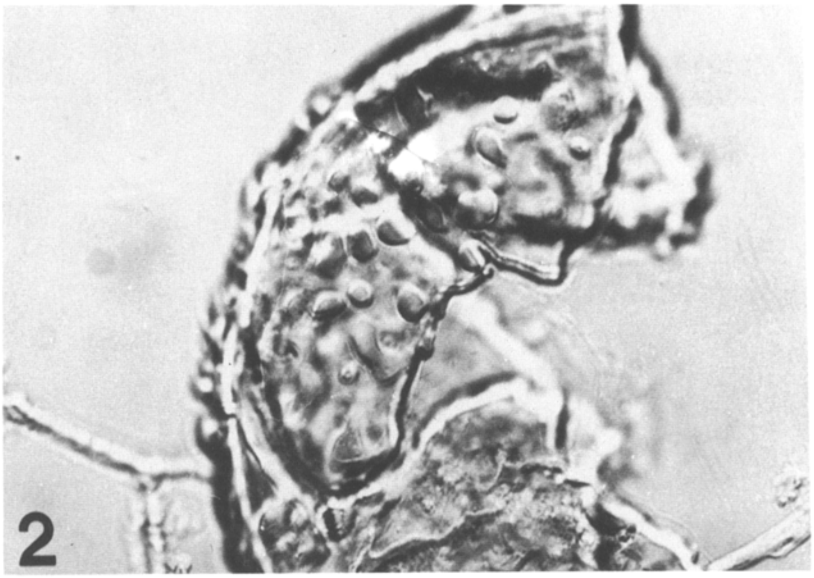
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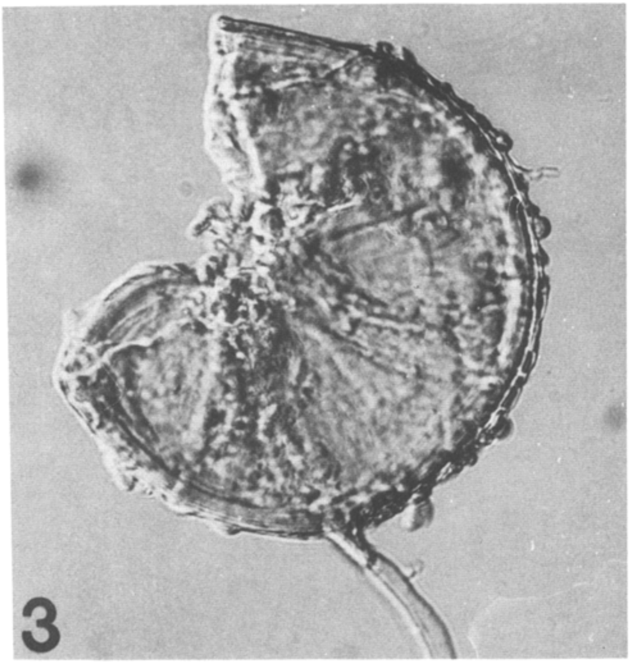
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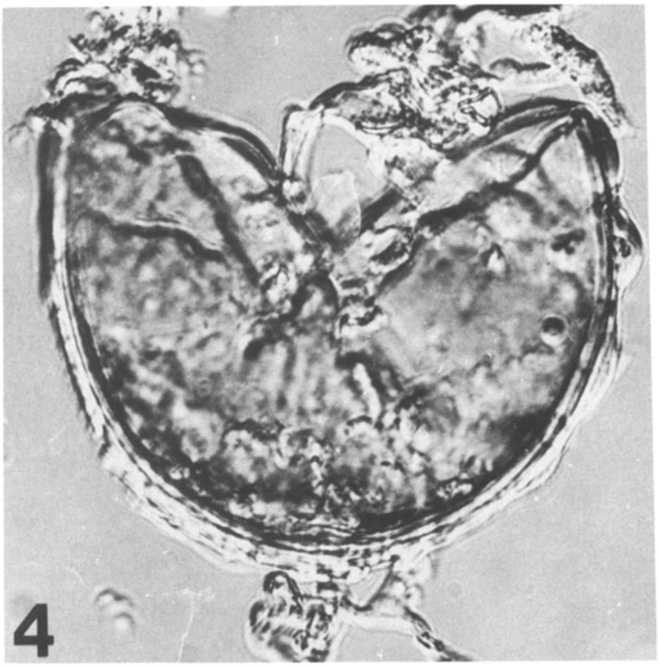
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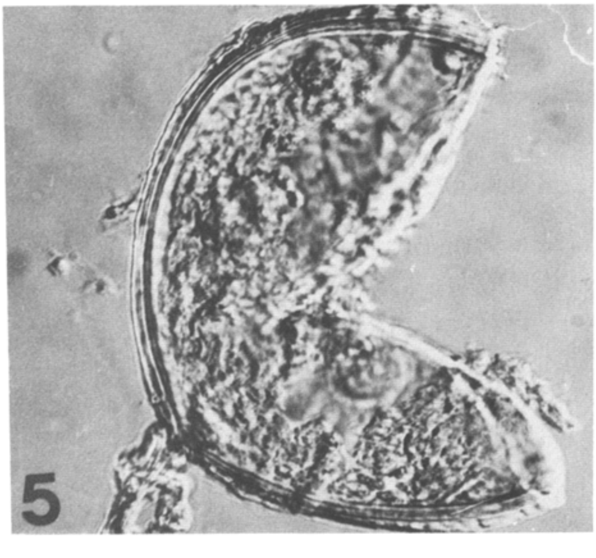
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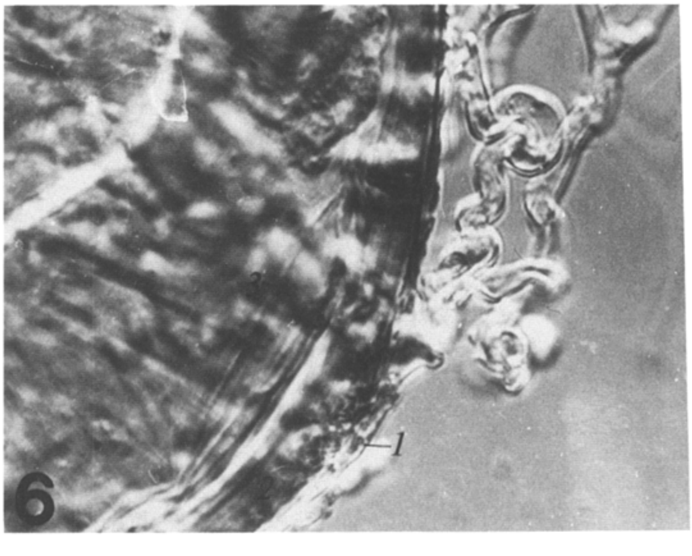
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Spores were found in only one of over 300 soil samples examined, associated with roots of *Ammophila arenaria* growing in a sandy foredune positioned ca. 100 m from the shore of the Baltic Sea. The mean spore density of this species was 12 in 100 g dry soil. Associated species of arbuscular fungi were *Acaulospora dilatata* Morton and *Scutellospora dipurpurascens* Morton & Koske. The pH of the soil sample was 6.1 and concentrations of ions were NO_3 10, P 10, K 0, Ca 0, Mg 16 and Cl 33 mg/l dry soil.

The spores were associated in the field with vesicular-arbuscular mycorrhizal roots of *Ammophila arenaria,* but failed to form mycorrhizae with *T. repens* in pot cultures.

The collections examined were 1596–1613, J. Błaszkowski, among and in roots of *Ammophila arenaria,* 1. 08. 1989, Słowinski National Park, Łeba, Poland (DPP).

Discussion

Identification

The distinctive feature of *G. pustulatum* is the ornamentation of the outermost spore wall. The predominate structures forming the ornamentation are blister-like outgrowths (Figs. 2–5). In cross view, they are most frequently circular or arched. The size of these structures seems to be inversely related to their frequency on the spore surface. Cup-shaped and irregular outgrowths occur rarely and mainly on spores densely covered with blister-like thickenings. In a few specimens, both the number of pustules and their size are very low and hence such spores are almost smooth. The pustules are solid, not fatty, and do not disappear when spores are mounted in lactic acid. The latter feature contrasts with the finding of Koske et al. (1986). Wall 2 easily separates into single laminae or their groups, especially in vigorously crushed spores. The outer laminae are usually darker than the inner ones, which are sometimes almost hyaline. Wall 3 is most difficult to see as it always adheres tightly to wall 2 and is hyaline. Even vigorous crushing of spores did not separate it completely from the laminated wall. However, numerous wrinkles on the inner surface of wall 2 and small areas of this wall separated from wall 2 (Fig. 6) (seen most frequently at the edge of spore crack) support its presence.

Most spores of *G. pustulatum* recovered by the author possess a straight and cylindric or slightly funnel-shaped subtending hypha (Figs. 7–9), which is curved only in a few specimens (Fig. 10). Its wall is thin and usually lighter coloured than the spore. However, subtending hyphae of some specimens 2.5–12.5 μm down the spore base are coloured similarly to spore wall 2, with which the subtending hyphal wall is continuous. The pore in the subtending hypha is usually closed by the thickened spore wall 2; in a few spores a curved septum connecting the innermost lamina of wall 2 is also present (Fig. 10). According to Koske et al. (1986), the pore in *G. pustulatum* is closed only by ingrowth of wall 2. Alternate occlusion of a subtending hyphal pore either by wall thickening or a septum has been described in other species of the genus *Glomus* (Morton 1988). The spore base of many specimens is obscured by germ hyphae growing most often near the subtending hypha (Figs. 7–9), although germinating spores with germ tubes developed at the opposite of the subtending hypha are also present (Fig. 6). The germ tubes grow through a small hole seen in wall 1, but there no germination structure resembling that found in members of *Acaulospora* is seen (Błaszkowski 1994; Morton and Benny 1990). The emergence of germ tubes from the wall of a spore of the genus *Glomus* was seen in *G. albidum* Walker & Rhodes (Walker and Rhodes 1981), *G. dominikii* Błaszk. (J. Błaszkowski, unpublished work), *G. pallidum* Hall (Hall 1977), and *G. scintillans* Rose & Trappe (Rose and Trappe 1980). No spore germinating by regrowth of the subtending hypha was found. This kind of germination occurs most frequently in *Glomus* spp. (Morton and Benny 1990).

A number of species of the genus *Glomus* are comparable to *G. pustulatum.* Ornamented spores are formed by *G. callosum* Sieverding, *G. dominikii,* and *G. scintillans,* but spores of these species are lighter coloured, covered with warts (versus mostly blister-like outgrowths in *G. pustulatum*), and have a different wall structure (Błaszkowski 1988; Rose and Trappe 1980; Sieverding 1988). In addition, except for *G. dominikii,* the spores of the other species are much larger than those of *G. pustulatum.* The muronyms of spores of *G. callosum* and *G. scintillans* are A(UoL) (Sieverding 1988) and A(Uo) B(UU) (Schenck and Pérez 1987), respectively. Spores of *G. dominikii* were originally described as 3-walled with an outermost unit wall and two membranous walls (Błaszkowski 1988). Examination of spores of this species under a light microscope equipped with Nomarski unterference contrast, however, revealed that spore group A consists of a thin outermost unit wall tightly adherent to a laminated wall, and, in agreement with the reinterpretation of flexible walls in arbuscular fungi (Morton 1989), group B consists of a thin unit wall and a relatively thick but flexible inner coriaceous wall, staining beetroot purple (13D8) in Melzer's reagent.

Species producing roughened spores and having an innermost membranous wall that are most likely to be confused with those of *G. pustulatum* are *G. claroides* Schenck & Smith (Schenck and Smith 1982), *G. hoi* Berch & Trappe (Berch and Trappe 1985), *G. fistulosum* Skou & Jakobsen, and *G. flagilistratum* Skou &

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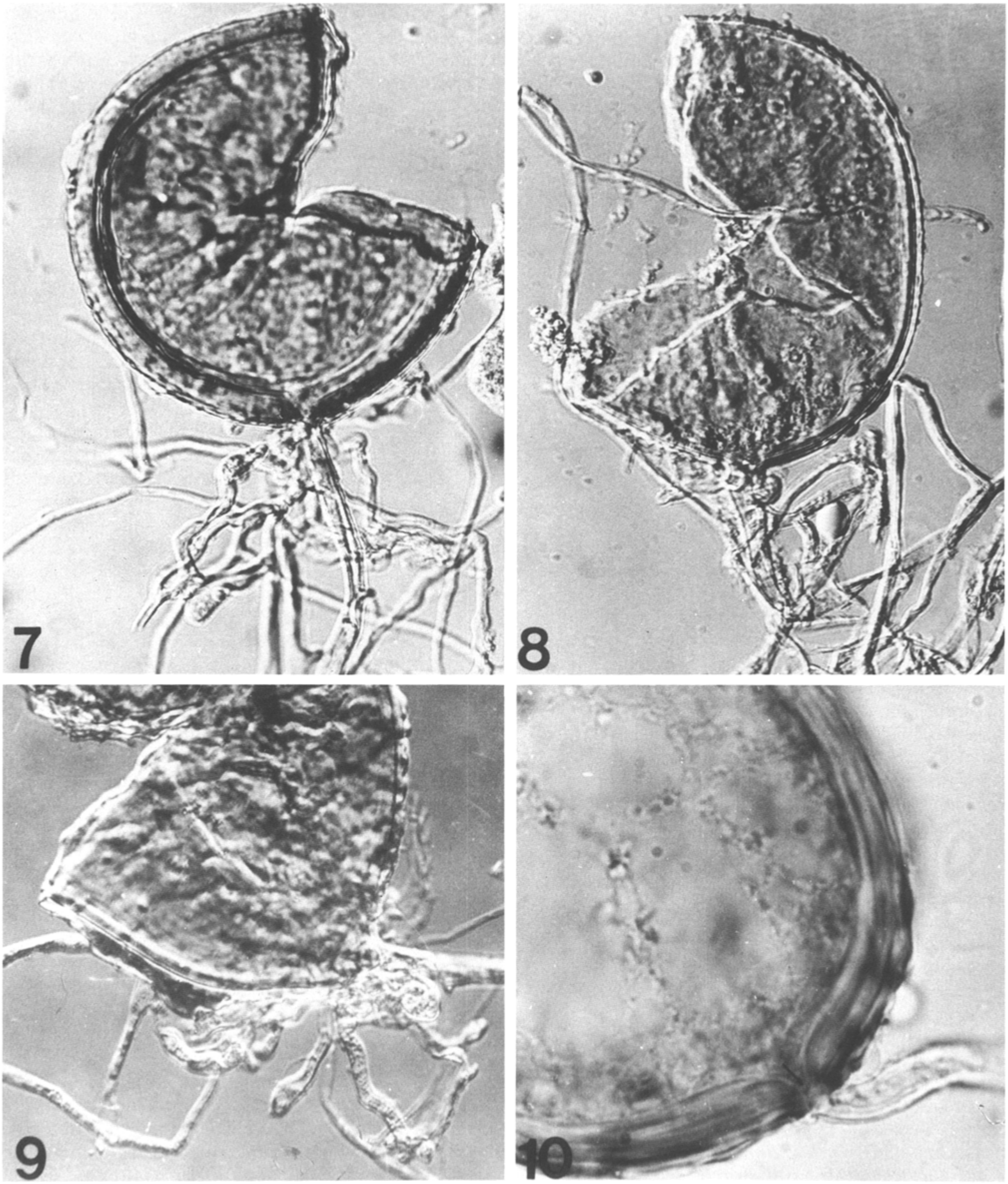
Figs. 1–6. *Glomus pustulatum. Numbers* indicate spore wall arrangement. **Fig. 1.** Intact spore, bright-field microscopy (BFM). ×330. **Fig. 2.** Blister-like thickening on spore surface in a plan view, Nomarski interference contrast (NIC). ×780. **Figs. 3–5.** Ornamentation of crushed spores in a cross view, NIC. ×650. **Fig. 6.** Wall structure and germ tube growing from spore wall, NIC. ×923



Figs. 7–10. *Glomus pustulatum.* **Figs. 7–9.** Germinating spores with germ tubes developed near subtending hyphae, NIC. ×650. **Fig. 10.** Curved subtending hypha occluded by a septum (*arrow*), BFM. ×923

Jakobsen (Skou and Jakobsen 1989). However, the outermost spore wall in all these species is evanescent (versus unit in *G. pustulatum*), and *G. fistulosum* and *G. flagilistratum* have five- and six-walled spores, respectively, with two membranous walls in the intermost wall group. In addition, *G. ambisporum* Smith & Schenck (Smith and Schenck 1985), *G. dimorphicum* Boyetchko & Tewari (Boyetchko and Tewari 1986), and *G. globiferum* Koske & Walker (Koske and Walker 1986) form an inner membranous spore wall, but the two former species are dimorphic and produce sporocarps containing spores with an outermost evanescent wall. Spores of *G. globiferum* are, however, covered with a peridium and have one or two inner membranous walls or a single coriaceous wall.

An inner membranous wall also occurs in spores of *G. cerebriforme* McGee (McGee 1986), *G. diaphanum* Morton & Walker (Morton and Walker 1984), *G. fasciculatum* (Thaxter) Gerd & Trappe emend. Walker & Koske (Walker and Koske 1987), *G. glomerulatum* Sieverding (Sieverding 1987), and *G. maculosum* Miller & Walker (Miller and Walker 1986), but their outermost wall is always smooth and either unit (*G. fasciculatum* and *G. maculosum*) or laminated (the other species). In addition, the innermost membranous wall of *G. maculosum* contains a series of concentric discs increasing in diameter towards the inside of the spore.

Habitat and distributional ecology

G. pustulatum was originally found associated with roots of *Ammophila breviligulata* Fern. and *Honkenya peploides* (L.) Ehr. growing in maritime sands. Its spores have also been obtained in a pot culture with *Lathyrus maritimus* Bigel (Koske et al. 1986). Since that time, the only other record of this species is that in India (Mohankumar et al. 1988), also from sandy beach soils. The presence of spores of *G. pustulatum* among roots of *Ammophila arenaria* colonizing maritime sand dunes of the Słowinski National Park and their absence in more than 300 other soil samples taken from over 100 localities in Poland, as well as the literature data cited above (Koske et al. 1986; Mohankumar et al. 1988), suggest that this species has a relatively narrow ecological range of tolerance preventing its occurrence at other sites (Alexander 1971). Both chemical and physical as well as microbiological properties of soils probably delimit this range. Firstly, the soil from the site where *G. pustulatum* occurs in Poland is similar in chemical properties to those examined by Mohankumar et al. (1988) (pH 7.8–8.1, N 5.86, P 18.77, K 5.43 mg/kg dry soil), but apart from the pH it contains much less N, P, K, and Ca than other agricultural and uncultivated soils anywhere else. All these nutrients have been shown to limit the occurrence of arbuscular fungi (Anderson et al. 1984; Pfeifer and Bloss 1988). Secondly, *G. pustulatum* may prefer soils with a great amount of sand, as does *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe (Day et al. 1987). Thirdly, maritime sand dunes distinguish great diurnal and seasonal temperature changes (Gemma and Koske 1988; Koske 1987) which shorten spore dormancy periods (Gemma and Koske 1988; Louis and Lim 1988). Early root colonization by a particular species may limit the existence of another species, especially when they compete for the same site in the root (Arines et al. 1988; Wilson 1984). In addition, older roots are usually unsuitable for arbuscular fungi (Smith and Walker 1981), which are completely dependent on the host plant for energy (Stahl and Christensen 1982). Finally, maritime sands are relatively poor in other microorganisms that usually suppress the activity of arbuscular fungi (Hetrick and Wilson 1991).

Evolutionary relationships

Spores of *G. pustulatum* originally described by Koske et al. (1986) as well as those found by the author of this paper occurred singly in the soil. Thus, according to Morton (1990), *G. pustulatum* represents the most primitive group of taxa within the genus *Glomus*. This group also includes *G. clarum* Nicol. & Schenck, *G. etunicatum* Beck. & Gerd., *G. maculosum* Miller & Walker, *G. occultum* Walker, and *G. tortuosum* Schenck & Smith. However, the presence of an outer unit wall and an innermost membranous wall suggest closest evolutionary similarity to *G. maculosum.*

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