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Glomus insculptum, a new arbuscular mycorrhizal species from Poland

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Abstract–A new ectocarpic arbuscular mycorrhizal fungal species, *Glomus insculptum* (Glomerales, Glomeromycota), was found in inland sand dunes of southern Poland. *Glomus insculptum* produces yellow-colored spores that are globose to subglobose, (50-) 71(-85) μm diam, or ovoid, 55—60 x 80—85 μm, and have a spore wall composed of two layers. The outer layer is permanent and the inner layer is laminate and ornamented with round pits evenly distributed on its inner surface. *Glomus insculptum* forms arbuscular mycorrhizae in single-species pot cultures with *Plantago lanceolata*.

Key Words–Glomeromycota, mycorrhizae, new species

Introduction

The largest inland sandy area of Poland is the Błędowska Desert (Szczypek & Wika 1984). It is located in the eastern part of Silesia Upland (50°22' N, 19°34'E) and occupies 30 km^2. The unique plant communities of the Błędowska Desert are phytocenosis of inland sandy dunes and xerothermic swards with original psammophytic species. *Corynephorus canescens* (L.) P. B., *Elymus arenarius* L., and *Koeleria glauca* (Schkuhr) DC. are the dominant plant species in the dunes (Mrozik & Wika 1993).

Apart from 20 described species of arbuscular mycorrhizal fungi of the phylum Glomeromycota, examination of rhizosphere soil samples collected under plants of the Błędowska Desert in the years 1995-1997 revealed a new species of *Glomus* forming spores with pitted inner surface of their laminate spore wall layer (Błaszkowski et al. 2002). Subsequent culturing of this fungus in both trap and single-species cultures confirmed the uniqueness of this as a new species. The fungus is described here as *Glomus insculptum*.

Materials and Methods

Collection of soil samples, establishment of trap and single-species pot cultures, as well as growth conditions generally are as those described previously (Błaszkowski & Tadych 1997). Briefly, rhizosphere soils and roots of sampled plants were collected from a depth of 5-30 cm using a small garden shovel. In the laboratory, about 200-g subsamples were taken from each sample to determine the species of arbuscular fungi sporulating in the field. Then, the remaining soil-root mixtures were either air dried for 2 weeks and subsequently refrigerated at 4ºC or directly used to establish trap cultures. Trap cultures were established to obtain a great number of living spores of different developmental stages and to initiate sporulation of species that were present but not sporulating in the field collections. The growing substrate of the trap cultures was the field-collected material mixed with an autoclaved coarse-grained sand coming from maritime dunes adjacent to Świnoujście (pH 6.7; 12 and 26 mg L^{-1} P and K, respectively; Błaszkowski 1995). These mixtures were placed in 9x12.5-cm plastic pots (500 cm^3) and thickly seeded with *Plantago lanceolata* L. Plants were grown in a greenhouse at 15-30ºC with supplemental 8-16-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 week. No fertilizer was applied during the growing period. Trap cultures were harvested at approximately 1-month intervals, beginning three months and ending five to seven months after plant emergence. Spores were extracted by wet sieving and decanting (Gerdemann & Nicolson 1963). Presence of mycorrhizae was determined following clearing and staining of roots (Phillips & Hayman 1970) modified as follow: tissue acidification with 20% HCl instead of 1%, and trypan blue concentration 0.1% instead of 0.05% (Koske, pers. comm.).

Single-species pot cultures were established from about 50 to 100 newly formed spores stored before inoculation in water at 4ºC for 24 h. After removal of soils debris, spores were collected in a pipette and transferred onto a compact layer of mycorrhizae-free roots of 10-14-day-old seedlings of *P. lanceolata* placed at the bottom of a hole of *ca*. 1 cm wide and 4 cm deep formed in a wetted growing medium filling 8-cm plastic pots (250 cm^3). The growing medium was an autoclaved sand of maritime dunes adjacent to Świnoujście with chemical properties listed above. Subsequently, the spores were covered with another layer of roots attached to 4-6 additional host plants, and the roots and sandwiched spores were buried in the growing medium. Finally, three to six seeds of *P. lanceolata* were placed on the surface of the growing substrate and covered with a thin layer of autoclaved sand. The cultures were harvested after 4-8 months and spores extracted. The

effectiveness of the method of establishment of one-species cultures described above usually exceeded 90% (Błaszkowski et al. 2002).

Morphological properties of spores and their subcellular structures were determined based on at least 100 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske & Tessier 1983) and a mixture of PVLG and Melzer's reagent (1:1, v/v). Spores in all stages of development were crushed to varying degrees by applying pressure to the coverslip and then stored at 65°C for 24 h to clear their contents of oil droplets. These were examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were captured in a Sony 3CDD color video camera coupled to the microscope.

Terminology of spore structure is that suggested by Spain et al. (1989), Stürmer & Morton (1997), and Walker (1983). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names are from Kornerup & Wanscher (1983). Nomenclature of fungi and plants is that of Walker & Trappe (1993) and Mirek et al. (1995), respectively. Specimens were mounted in PVLG on slides and deposited in the Department of Plant Pathology (DPP), University of Agriculture, Szczecin, Poland, and in the herbarium at Oregon State University (OSC) in Corvallis, Oregon, USA.

Color microphotographs of spores and mycorrhizae of *G. insculptum* can be viewed at the URL http://www.agro.ar.szczecin.pl/~jblaszkowski/.

Descriptions of the species

Glomus insculptum J. Błaszkowski, sp. nov. **Figs. 1-10**

Sporocarpia ignota. Sporae singulatim in solo vel in radici, e sporophoris rectic efformatae. Sporophorum nonseptatum vel parce septatum; hyalinum; (3.7-)4.8(-5.6) µm latum; pariete 0.3-0.5 µm crasso; rectum. Sporae pallide luteae vel aureae; globosae vel subglobosae; (50-)71(-85) µm diam; aliquando ovoideae; 55-60 x 80-85 µm; hypha subtenda solitaria. Tunica sporae e startis duobus (strati 1-2); strato "1" rigido, diuturno, glabro, hyalino vel pallide luteo, (0.9-)1.1(-1.5) µm crasso; strato "2" laminato, pallide luteo vel aureo, (3.2-)4.4(-6.1) µm crasso cum superficie interia cum orbicularis cavernis ordinatis, (1.2-)1.6(-2.0) µm diam, 1.2—2.0 µm profundis. Hypha hyalina; recta vel recurva; cylindrica vel infundibuliformа, raro coliga; (2.9-)5.2(-6.9) µm lata ad basim sporae; pariete hyalino; (0.7-)1.3(-2.9) µm crasso, stratis 1-2 sporae continuo. Porus e septo continuo strati 2 sporae efformata. Arbuscular mycorrhizae formans.

Sporocarps unknown. Spores borne singly in the soil (Figs. 1, 2); produced from straight sporophores (Figs. 1-7). *Sporophore* coenocytic to sparsely septate; hyaline; (3.7-)4.8(-5.6) μm wide; with a wall 0.3—0.5 μm thick; bearing spores by swelling at hyphal tips (Fig. 1). *Spores* yellowish white (2A2) to golden yellow (5B8); globose to subglobose; (50-)71(-85) μm

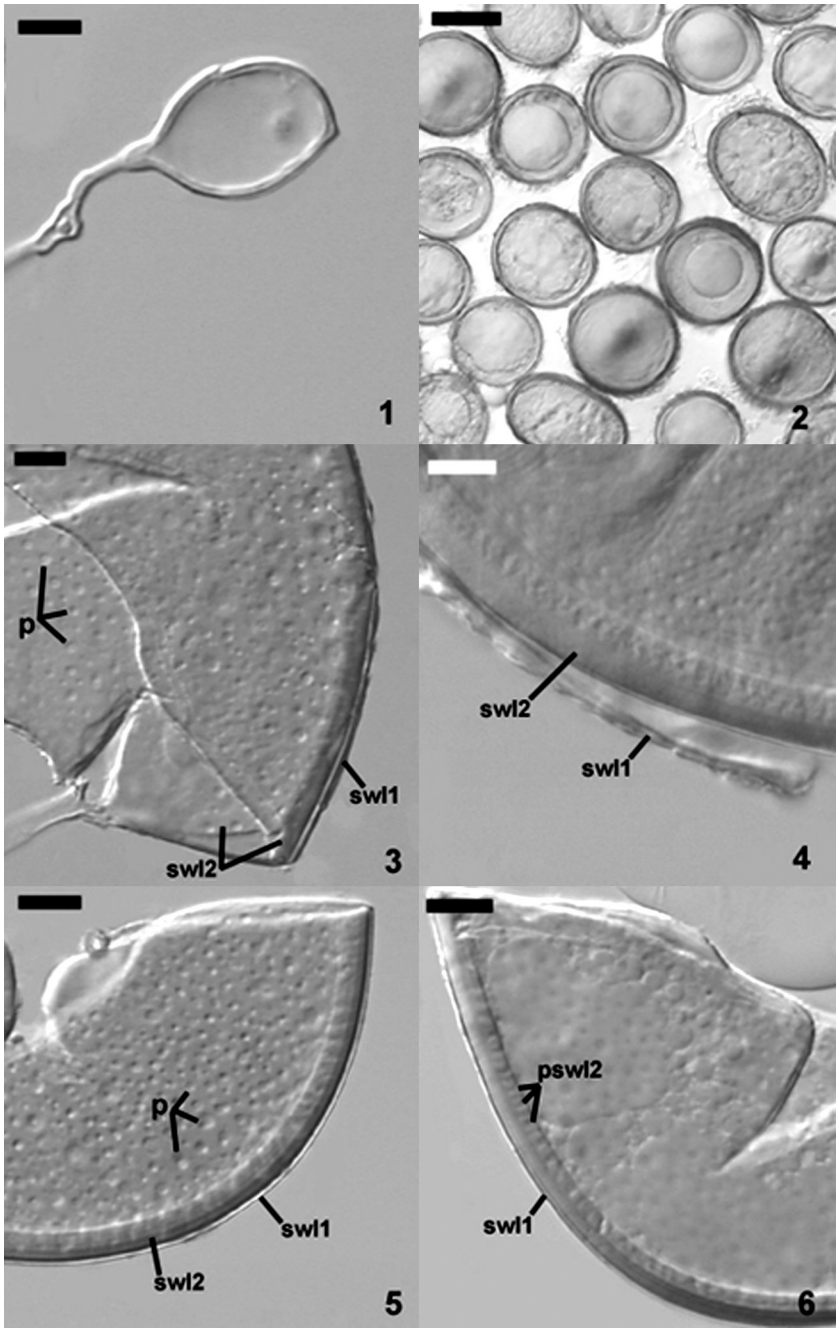
diam; sometimes ovoid; 55-60 x 80-95 μm; with a single subtending hypha (Fig. 1-3, 7). Subcellular structure of spores consists of one wall with two layers (swl 1-2; Figs. 3-7). Outermost layer 1 permanent, smooth or with a slightly roughened outer surface, hyaline, ca. 0.5 μm thick, continuous with a one-layered subtending hypha of the most juvenile spores (Fig. 1), then darkening to pale yellow (3A3) and thickening to (0.9-)1.1(-2.7) μm (Figs. 3-7). Layer 2 laminate, yellowish white (2A2) to golden yellow (5B8), (3.2-)4.4(-6.1) μm thick, with a smooth outer surface and an evenly pitted inner surface (Figs. 3-6); pits round, (1.2-)1.6(-2.0) μm diam (Figs. 3-5), 1.2-2.0 μm deep, separated by ridges, (0.7-)1.1(-1.7) μm wide (Figs. 4, 6); in the most juvenile spores, the inner surface is frequently smooth; in young (immature) spores, it contains shallow pits that may be difficult to see (Figs. 6, 7). No spore wall layers react in Melzer's reagent.

Subtending_hypha hyaline to pale yellow (3A3); straight or recurved; cylindrical or slightly flared (Figs. 3, 7), rarely constricted; (2.9-)5.2(-8.5) μm wide at the spore base, consisting of two layers (shwl 1 and 2) continuous with spore wall layers 1 and 2 (Fig. 7); layer 2 of spore wall is highly thinned at the spore base and then either further thins to 0.7 μm or forms a recurved septum, 1.5-2.7 μm wide and 0.5—0.7 μm thick, positioned 1.0—6.0 μm in the hyphal lumen (Fig. 7).

Etymology. Latin, *insculptum*, referring to the pitted inner surface of the laminate spore wall layer 2.

Specimen examined: HOLOTYPE: Poland. Szczecin, associated with roots of pot-cultured *P. lanceolata*, 25 June 1999, *Błaszkowski, J., 2271* (DPP); ISOTYPES: *Błaszkowski, J.*, 2272-2309 (DPP) and two slides at OSC.

Figs. 1-10. Spores and mycorrhizae of *Glomus insculptum* in roots of *Plantago lanceolata* stained in 0.1% trypan blue. **1.** Young spore. **2.** Mature spores. **3.** Spore wall layers 1 (swl1) and 2 (swl2), pits (p) on the inner surface of spore wall layer 1, and subtending hypha (sh). **4.** Spore wall layer 1 (swl1) separated from pitted spore wall layer 2 (swl2). **5.** Spore wall layers 1 (swl1) and 2 (swl2) with pits (p) seen through the spore surface. **6.** Spore wall layer 1 (swl1) and pits of spore wall layer 2 (pswl2) seen in a cross-sectional view. **7.** Spore wall layers 1 (swl1) and 2 (swl2) continuous with subtending hyphal wall layers 1 (shwl1) and 2 (shwl2); septum (s) of the subtending hypha is visible. **8.** Arbuscule (arb) with its poorly visible trunk (tr). **9.** Coils of intraradical hyphae. **10.** Coiled (chyp) and straight (shyp) intraradical hyphae. Figs. 1, 3, 4, 6, 7, spores crushed in PVLG + Melzer's reagent. Fig. 2, spores in water. Fig. 5, spore crushed in PVLG. Figs. 8-10, roots in PVLG. Figs. 1 and 3-10, differential interference contrast; Fig. 2, bright field microscopy. Bars: Figs. 1 and 3-10=10 μm, Fig. 2=50 μm.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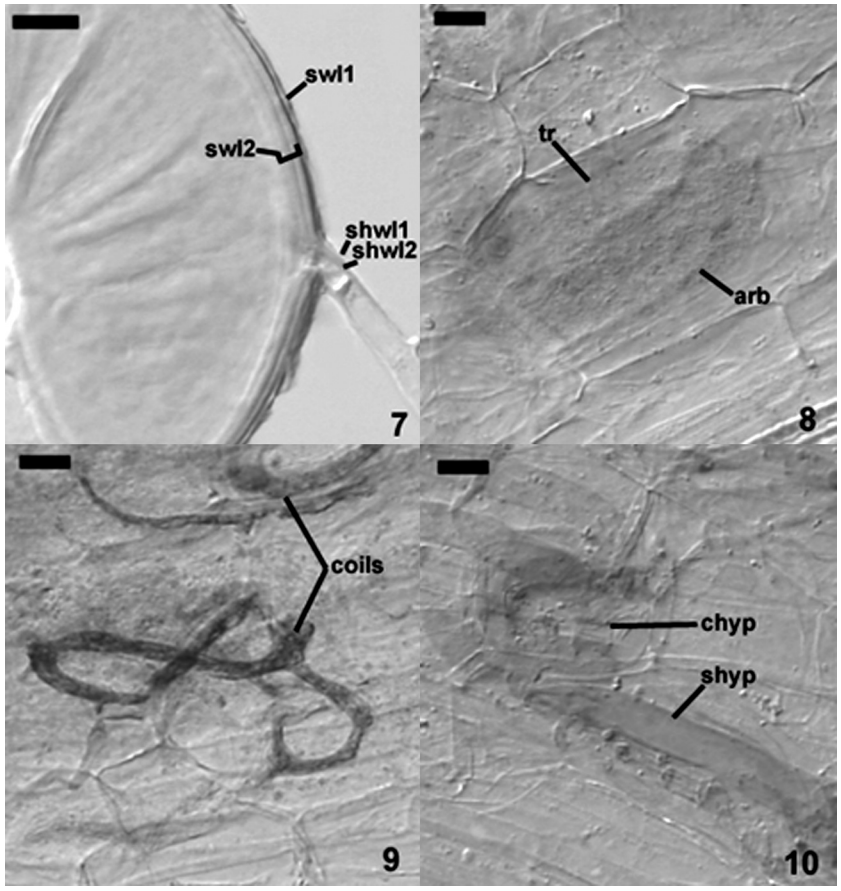

Other materials examined. Poland. The Błędowska Desert (50°22' N, 19°34' E), from the root zone of *Corynephorus canescens* (L.) P. Beauv., *Festuca rubra* L. s. s., *Holcus mollis* L. and *Juniperus communis* L., 26 June 1997, *Błaszkowski, J*., unnumbered collection (DPP). Spores from trap pot cultures established based on rhizosphere soils of the plant species listed above and from five other cultures with rhizosphere soils of the same plant species and *P. major* L., 2 Oct. 1999, *Błaszkowski, J*., unnumbered collection (DPP).

Distribution and habitat. Spores of *G. insculptum* were isolated from seven field-collected soil samples and 10 trap pot cultures that were established with rhizosphere soils of five plant species colonizing inland sand dunes of the Błędowska Desert (50°22' N, 19°34' E) in south of Poland. The plant species colonized by *G. insculptum* in the field were *C. canescens,*

F. rubra, H. mollis, J. communis and *P. major*. Spore abundance of *G. insculptum* in the field-collected samples ranged from 0 to 79 (mean 18) in 100 g dry soil. The arbuscular mycorrhizal fungal species richness in the soil samples containing *G. insculptum* ranged from 2 to 6 (mean 2.5) in 100 g dry soil. The fungi co-occurring with *G. insculptum* in the field were *Acaulospora lacunosa* Morton, *A. mellea* Spain & Schenck, *G. aggregatum* Schenck & Smith emend. Koske, *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske, *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, and *Scutellospora dipurpurescens* Morton & Koske. The fungi accompanying *G. insculptum* in trap cultures were *A. lacunosa, Archaeospora trappei* (Ames & Linderman) Morton & Redecker, *G. clarum* Nicol. & Schenck, *G. intraradices* Schenck & Smith, *G. lamellosum* Dalpé, Koske & Tews, *G. pustulatum* Koske, Friese, Walker & Dalpé, two undescribed *Glomus* species., *Paraglomus occultum* (Walker) Morton & Redecker, *S. armeniaca* Błaszk., and *S. dipurpurescens*.

The soil chemical properties of the Błędowska Desert dunes ranged: pH, 5.6-5.9; NO_3 (mg L^{-1}), 9.3-10.8; P, 3-5; K, 5-13; Mg, 7-23; Na, 0-1; Cl, 13.7-18.2; KCl (g L^{-1}), 0.060-0.075; organic C (%), 0.27-0.42.

Mycorrhizal associations. In the field, *Glomus insculptum* occurred among vesicular-arbuscular mycorrhizal roots of *C. canescens*, *F. rubra*, *H. mollis*, *J. communis*, and *P. major*. Mycorrhizae of this fungus formed in one-species cultures with *P. lanceolata* as the plant host consisted of arbuscules, intra- and extraratrical hyphae. Arbuscules appeared as granular structures in cortical cells (Fig. 8). Fine branches were difficult to see. Arbuscules were numerous, but unevenly distributed in roots. The intraratrical hyphae were (4.2-)6.2(-7.4) μm wide and grew parallel to the root axis (Fig. 10). They were straight or slightly curved, sometimes dichotomously branched and frequently coiled (Figs. 9, 10); the coils were 20.8—34.6 x 10.5—22.3 μm. No vesicles were present in roots of plants up to 8-months-old. Extraradical hyphae were (1.7-)3.2(-3.5) μm wide and frequently associated with young and mature spores. In 0.1% trypan blue, arbuscules stained violet white (17A2), intramatrical hyphae violet white (17A2) to pastel violet (17A4), and extraradical hyphae pale violet (17A3).

Discussion

When observed under a dissecting microscope, spores of *G. insculptum* most resemble small-spored isolates of *G. aggregatum*, *G. arenarium* Błaszk. at al., *G. etunicatum* Becker & Gerd., *G. intraradices* Schenck & Smith, *G. pustulatum*, *G. trimurales* Koske & Halvorson, and *G. versiforme* (Karsten) Berch. All eight species form globose to subglobose and yellow-colored

spores, whose size range partly overlaps (Becker & Gerdemann 1977; Berch & Fortin 1983; Błaszkowski 1991; Błaszkowski et al. 2001, 2003; Koske 1985; Koske & Halvorson 1989; Koske et al. 1986; Morton 2000; Schenck & Smith 1982; Stürmer & Morton 1997).

Examination of subcellular structure and phenotypic properties of layers in the spore wall of specimens crushed in Melzer's reagent readily separates these species. Only *G. insculptum* forms spores in which the laminate spore wall layer is regularly pitted (Figs. 3-6). However, in young spores, the inner surface either is smooth or the pits are very shallow and difficult to see (Figs. 6, 7). Even then, *G. insculptum* is distinguishable from other species. Although *G. etunicatum*, *G. insculptum*, and *G. versiforme* have a spore wall composed of two layers, the outer layer of each differs. In *G. etunicatum* the layer sloughs as spores mature (Stürmer & Morton 1997), while in *G. versiforme* it is semi-permanent (Morton 2000) and in *G. insculptum* it is permanent (Figs. 3-7). Additionally, the outer spore wall layer of *G. insculptum* and *G. versiforme* (Morton 2000) is nonreactive in Melzer's reagent but stains dark pinkish red to reddish-purple in *G. etunicatum* (Stürmer & Morton 1997). *Glomus versiforme* also differs from *G. insculptum* in the occasional production of spores arranged in epigeous sporocarps (Berch & Fortin 1983; Morton 2000) vs. only single, hypogeous spores in *G. insculptum* and in that the mean diameter of globose spores of the former fungus is almost twice that of spores of the latter species. In contrast to the two-layered subcellular spore wall structure of *G. insculptum* (Figs. 3-7), that of *G. arenarium*, *G. pustulatum*, and *G. trimurales* consists of three layers.

The only other species of arbuscular fungi forming spores with an ornamented inner surface of their innermost wall layer are *G. kerguelense* Dalpé & Strullu and *G. verruculosum* Błaszk. However, compared with *G. insculptum*, spores of the two species are much larger (mean diameter = 71 μm in *G. insculptum* vs. 186.3 μm and 189.0 μm in *G. kerguelense* and *G. verruculosum*, respectively) and the ornamentation of the innermost layer of their wall consists of fine granules (*G. kerguelense*) or warts (*G. verruculosum*; Błaszkowski & Tadych 1997; Dalpé et al. 2002) vs. pits in *G. insculptum*.

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