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Acaulospora scrobiculata and *Glomus versiforme* (*Glomeromycota*), newly and second time, respectively, found in Poland

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Morphological properties of spores and mycorrhizae of *Acaulospora scrobiculata* and *Glomus versiforme*, arbuscular fungi of the phylum *Glomeromycota*, were described and illustrated. The two species were revealed in trap cultures containing root-rhizosphere mixtures of plants colonizing maritime dunes of the Baltic Sea located in north-western Poland and then propagated in one-species cultures to characterize properties of their mycorrhizae. *Acaulospora scrobiculata* had not previously been found in Poland, and the only earlier finding of *Gl. versiforme* in this country comes from the year 1912. The known distribution of the two fungal species in the world is also presented.

Key words: arbuscular fungi, *Glomeromycota*, mycorrhizae

INTRODUCTION

Wet sieving and decanting of root-rhizosphere soils taken from trap cultures representing maritime dunes of the Baltic Sea revealed two species of arbuscular fungi of the phylum Glomeromycota (Schüßler, Schwarzott and Walker 2001), of which *Acaulospora scrobiculata* Trappe had not previously been recorded in Poland and *Glomus versiforme* (Karsten) Berch had been found only once in this country (Bucholtz 1912). Spores of the two fungal species were subsequently used to establish one-species cultures to recognize the morphological characteristics of their mycorrhizae.

The main aims of this paper were to describe and illustrate the morphological properties of spores and mycorrhizae of *Ac. scrobiculata* and *Gl. versiforme* found in Poland. These fungi were also compared with species producing spores of the most

similar morphological properties. Additionally, the known distribution of the two fungi in the world was presented.

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 and 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 4°C or directly used to establish trap cultures. Trap cultures were established to receive a great number of living spores of different developmental stages and to initiate sporulation of non-sporulating species in the field conditions. 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). 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 15-30°C 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 $\mu E\ m^{-2}s^{-1}$ at pot level. Plants were watered 2-3 times a week. No fertilization 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 and Nicolson 1963). Presence of mycorrhizae was determined following clearing and staining of roots. Based on the experience of the authors of this paper and suggestions of Professor R. E. Koske, Rhode Island University, U. S. A., two changes were introduced to the original method given by Phillips and Hayman (1970). First, roots earlier cleared in 10% KOH were acidified in 20% HCl instead of 1% HCl as in the original procedure. Second, the concentration of trypan blue was increased from 0.05% to 0.1%.

Single-species pot cultures were established from about 50 to 100 newly formed spores stored before inoculation in water at 4°C for 24 h. After removing soil debris, they were collected in a pipette and transferred onto a compact layer of 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 of chemical properties given above. Subsequently, the spores were covered with another layer of roots coming from 4-6 plants of the host, 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.

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 and Tessier 1983) and a mixture of PVLG and Melzer's reagent (1:1, v/v). The spores represented all stages of differentiation of the fungus. Spores were crushed to varying degrees by applying pressure to the coverslip and then stored at 65°C for 24 h to clear their contents from oil droplets. Such prepared spores were examined under an Olympus compound microscope equipped with differential interference contrast optics. Microphotographs were captured in a Sony 3CDD colour video camera coupled to the microscope.

Terminology of spore structure is that suggested by Spain et al. (1989), Stürmer and Morton (1997), and Walker (1983). Spore colour was examined under a dissecting microscope on fresh specimens immersed in water. Colour names are from Kornerup and Wanscher (1983). Nomenclature of fungi and plants is that of Walker and 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.

Acaulospora scrobiculata Trappe Figs 1-10

Sporocarps unknown. *Spores* borne singly in the soil; produced laterally on the neck of a sporiferous saccule (Fig. 1). Mature spores yellowish white (3A2) to pale yellow (3A3); globose to subglobose; (90-) 120 (-135)µm diam (Fig. 1). *Subcellular structure of spores* consists of a spore wall and two inner germination walls (Figs 2 and 3). Spore wall composed of three layers (layers 1-3). Outer layer 1 evanescent, hyaline, (1.0-) 1.7 (-2.5) µm thick, tightly adherent to layer 2 (Fig. 2), continuous with the wall of a sporiferous saccule neck (Fig. 1), frequently completely sloughed in mature spores (Fig. 3). Layer 2 laminate, yellowish white (3A2) to pale yellow (3A3), (3.2-) 5.5 (-8.8) µm thick, ornamented with evenly distributed pits (Figs 2-6); pits circular to ellipsoid, 1.2-2.5 µm diam when seen in a plan view and 0.5-1.0 µm deep in a cross-sectional view. Layer 3 flexible, hyaline, (0.4-) 0.7 (-0.8)µm thick, sometimes slightly separating from layer 2 (Figs 2 and 3). Layers 1-3 do not react in Melzer's reagent.

Germination wall 1 consists of two, flexible, hyaline layers, tightly adherent to each other in moderately crushed spores, but usually separating in vigorously crushed spores (Figs 2 and 3); each layer ca. 0.5 µm thick. None of these layers stains in Melzer's reagent.

Germination wall 2 composed of two tightly adherent hyaline layers (Figs 2 and 3). Layer 1 flexible, (1.7-) 2.2 (-2.7) µm thick, covered with a granular material, frequently scattering in crushed spores. Layer 2 flexible, 0.5-1.2 µm thick and 5.5-12.0 µm thick in spores crushed in water and PVLG, respectively, staining reddish white (8A2) in Melzer's reagent (Figs 2 and 3).

Sporiferous saccule hyaline; globose to subglobose; 110-140 µm diam; sometimes ovoid; 110-120 x 120-130 µm (Fig. 1). Neck 78-85 µm long, tapering from 10.0-25.0 µm diam at the saccule to 12.5-22.5 µm diam at the spore and to 5.5-8.0 µm diam in the region of its development from a thin-walled parent hypha (Figs 1 and 5). Saccule wall consists of a hyaline, single, 1.7-2.5 µm thick layer, not reacting in Melzer's reagent. Saccule collapsing at maturity because of the transfer of its content into the spore. In most mature spores, sporiferous saccule is detached (Figs 1 and 6).

The cicatrix remaining after saccule detachment is flat or slightly raised, resembling a low collar when seen in a cross-sectional view (Fig. 5); circular to ovoid, 7.5-11.0 µm diam when observed in a plan view (Fig. 6), with a centrally positioned pore, through which the content of a sporiferous saccule is transferred to a developing spore. At maturity, the pore is closed due to deposition of a material coming from the cytoplasm of a spore.

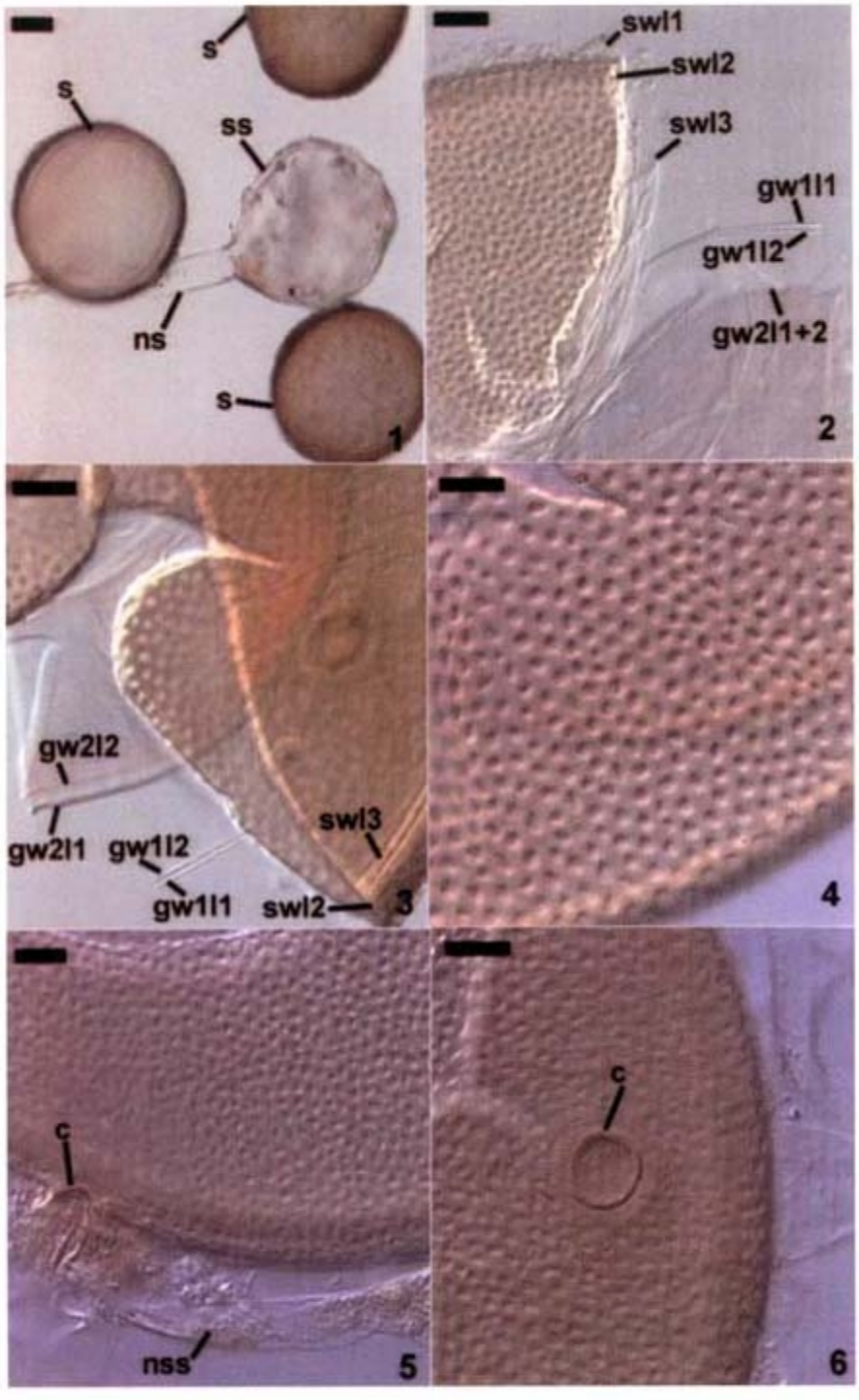
Collection examined. Poland. Szczecin, spores and mycorrhizal root fragment from one-species cultures with *P. lanceolata* as the plant host, 24 Oct. 2002, Błaszkowski J., 2388-2396, DPP.

Habitat and distribution in Poland. Spores of *Ac. scrobiculata* were revealed in a trap culture containing a mixture of roots and rhizosphere soil of *Corynephorus canescens* (L.) P. Beauv. growing in dunes of the Baltic Sea adjacent to Świnoujście (53°55'N, 14°14'E) in north-western Poland. The field mixture was collected on 13 June 1997 and did not contain any spore of *Ac. scrobiculata*. Seasonal sporulation has been observed in arbuscular fungi (Gemma et al. 1989). The arbuscular fungi found in the field-collected root-soil sample were *Entrophospora baltica* Błaszk., Madej et Tadych, *Glomus arenarium* Błaszk., Tadych et Madej, *Gl. corymbiforme* Błaszk., *Gl. fasciculatum* (Thaxter) Gerd. et Trappe emend. Walker et Koske, *Gl. gibbosum* Błaszk., and *Scutellospora pellucida* (Nicol. et Schenck) Walker et Sanders. The fungal species accompanying *Ac. scrobiculata* in the trap culture were *Gl. arenarium*, *Gl. gibbosum*, and *Scu. pellucida*.

General distribution. *Acaulospora scrobiculata* has originally been described from spores collected in Mexico (Trappe 1977). This fungus probably has a worldwide distribution, despite most literature reports of its finding come from the U.S.A. Literature data and the results of long-term investigations of the first author of this paper suggest that *Ac. scrobiculata* highly prefers sandy soils, especially maritime dunes. The maritime dune sites found to contain spores of *Ac. scrobiculata* have been those located in the U.S.A. (Friese and Koske 1991; Gemma and Koske 1989; Gemma, Koske and Carreiro 1989; Koske 1987, 1988; Koske and Gemma 1996, 1997; Koske and Halvorson 1981; Koske and Tews 1987; Schenck and Smith 1982; Sylvia 1986; Sylvia and Will 1988; Tews and Koske 1986), Canada (Dalpé 1989), Brazil (Stürmer and Bellei 1994), Spain (including Majorca), Greece, Israel (Błaszkowski, pers. observ.), Italy (Paccioni and Puppi 1988; Puppi et al. 1986), and Australia (Koske 1975). This fungus has also been isolated from lacustrine dunes (Koske and Tews 1987). The reports of the presence of *Ac. scrobiculata* in non-dune soils come from the U.S.A. (Miller, Domoto and Walker 1986; Walker, Mize and McNabb 1982), Mexico (Trappe 1977), Cameroon (Musoko, Last and Mason 1994), Finland (Vestberg 1995), China (Zhang, Wang and Huang 1992), Taiwan (Wu and Chen 1986), and Japan (Saito and Vergas 1991).

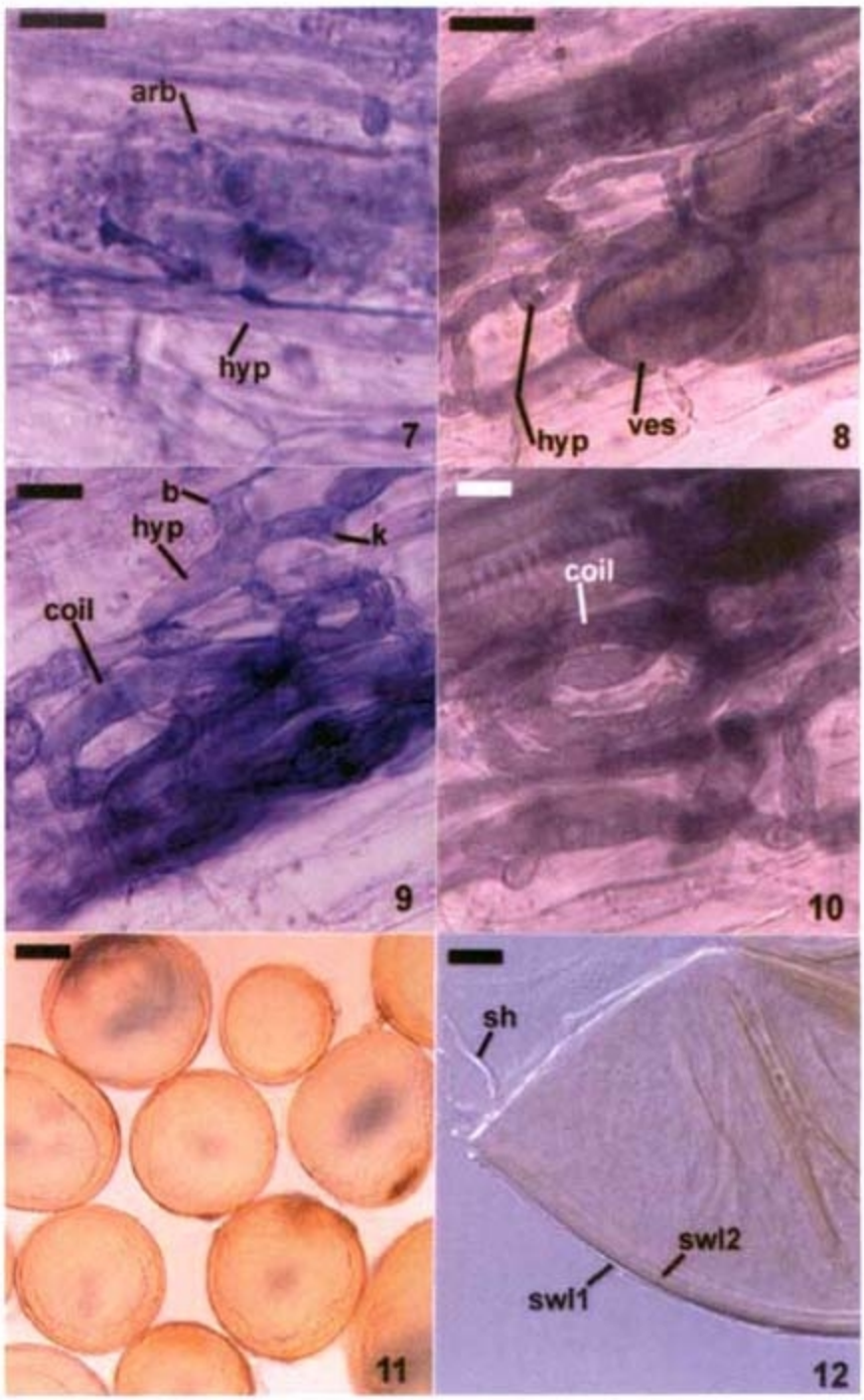
Mycorrhizal associations. In the field, *Ac. scrobiculata* existed in vesicular-arbuscular mycorrhizal roots of *C. canescens*.

The mycorrhizae of *Ac. scrobiculata* produced in single-species cultures with the plant host *P. lanceolata* consisted of arbuscules, vesicles, as well as intra- and extraradical hyphae (Figs 7-10). Arbuscules were not numerous and usually were unevenly distributed along the roots (Fig. 7). Vesicles usually were ellipsoid, 7.5-35 x

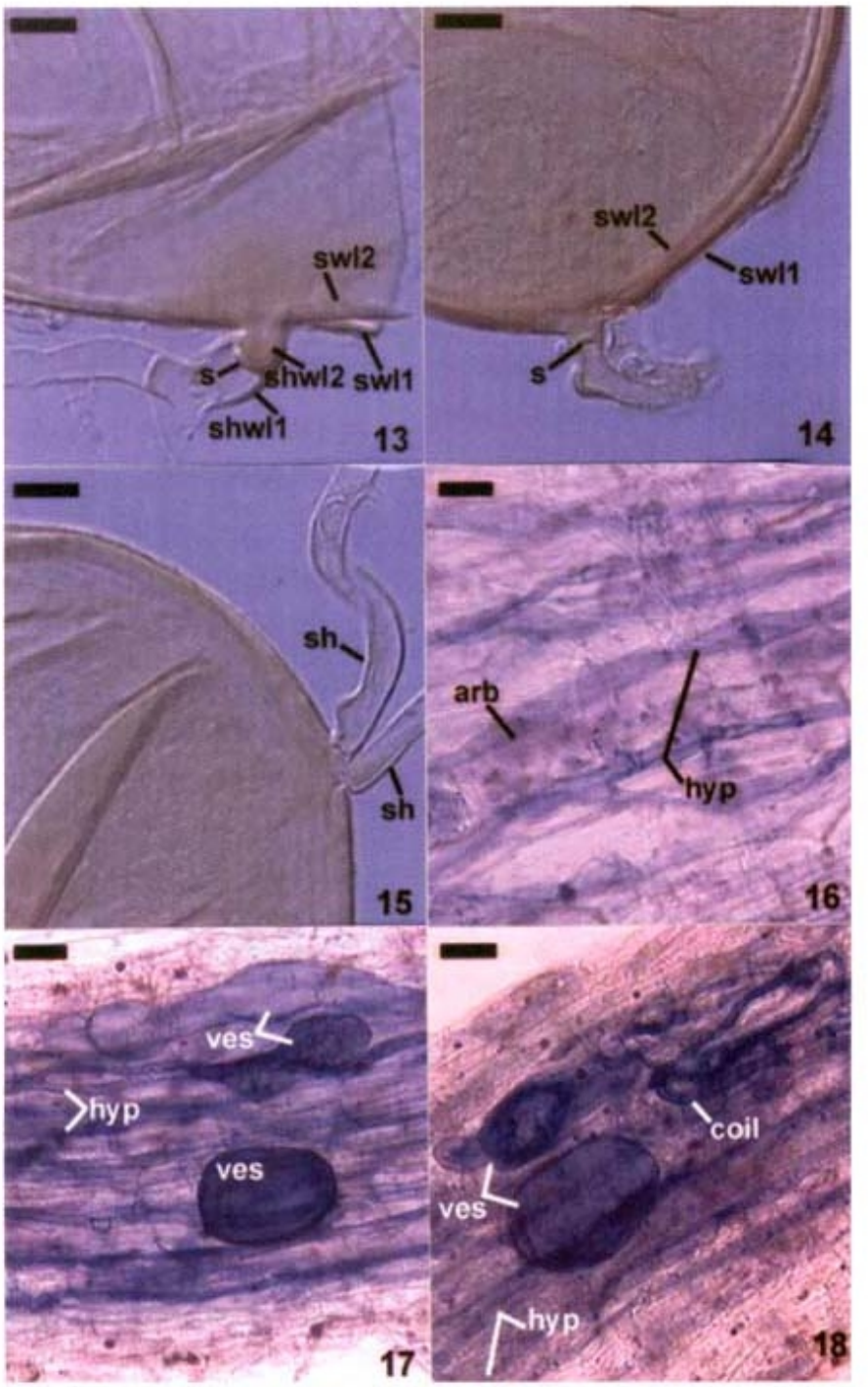


Figs 1-6. Morphology of intact spores, subcellular structure of crushed spores, and mycorrhizae of *Acaulospora scrobiculata* in *Plantago lanceolata* roots stained in 0.1% trypan blue. Fig. 1. Spores (s) with and without sporiferous saccule (ss); neck of sporiferous saccule (ns) is visible. Figs 2 and 3. Spore wall layers (swl) 1-3, layers 1 and 2 of germinations walls 1 (gw1l1 and 2) and 2 (gw2l1 and 2) of a spore crushed in Melzer's reagent; in Fig. 3, swl1 is completely sloughed. Fig. 4. Pitted spore wall layer 2. Fig. 5. Cicatrix (c) and neck of sporiferous saccule (nss) in a cross-sectional view.

Fig. 6. Cicatrix (c) in a plan view. Fig. 1. bright field microscopy; Figs 2-6, differential interference contrast. Bars: Fig. 1 = 20 μm, Figs 2-6 = 10 μm.



Figs 7-12. Morphology of intact spores, subcellular structure of crushed spores, and mycorrhizae of *Glomus versiforme* in *Plantago lanceolata* roots stained in 0.1% trypan blue. Fig. 7. Arbuscule (arb) developed from hypha (hyp). Fig. 8. Vesicle (ves) and intraradical hyphae (hyp). Figs 9-10. Coil (coil) and intraradical hyphae with short branches (b) and knobs (k). Fig. 11. Intact spores. Fig. 12. Spore wall layers (swl) 1 and 2.
Figs 7-11, bright field microscopy; Fig. 12, differential interference contrast.
Bars: Fig. 8 = 20 μm, Figs 7, 9, 10 = 10 μm.



Figs 13-18. Morphology of intact spores, subcellular structure of crushed spores, and mycorrhizae of *Glomus versiforme* in *Plantago lanceolata* roots stained in 0.1% trypan blue. Fig. 13. Spore wall layers (swl) 1 and 2 continuous with subtending hyphal wall layers (shwl) 1 and 2; recurved septum (s) is visible. Fig. 14. Spore wall layers (swl) 1 and 2 and transverse septum (s) of subtending hypha.

Fig. 15. Spore with two subtending hyphae (sh). Fig. 16. Arbuscule (arb) and intraradical hyphae (hyp).

Fig. 17. Vesicles (ves) and intraradical hyphae (hyp). Fig. 18. Vesicles (ves), intraradical hyphae (hyp), and coil (coil). Figs 11, 16-18, bright field microscopy; Figs 12-14, differential interference contrast. Bars: Figs 13-17 = 10 µm, Fig. 8 = 20 µm.

10-52.5 μm, rarely globose, 30-38 μm, and occurred irregularly (Fig. 8). Intraradical hyphae developed parallel to each other and to the root axis, rarely had Y-shaped branches, sometimes had short lateral branches or knobby swellings, and were 2.5-7.4 μm wide (Figs 7-9). The hyphae frequently formed coils, 15-20 x 30-40 μm (Figs 9 and 10). Extraradical hyphae infrequently occurred and were 2.7-2.9 μm wide. In 0.1% trypan blue, arbuscules, vesicles, coils, intra- and extraradical hyphae stained pale violet (17A3) to greyish violet (17C4), pastel violet (17B4) to light violet (17B5), violet (17A6) to greyish violet (17B5), pale violet (17A3) to greyish violet (18B5), and greyish violet (17B3-C6), respectively (Figs 6-10).

The soil chemical properties of the Świnoujście dunes were: pH(H_2O), 3.8-6.7; NO_3(mgL^{-1}), 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 (Błaszkowski 1995).

Disscusion. Apart from *Ac. scrobiculata*, other species of this genus forming pitted spores are *Ac. cavernata* Błaszk., *Ac. dilatata* Morton, *Ac. foveata* Trappe et Janos, *Ac. lacunosa* Morton, *Ac. paulineae* Błaszk. and *Ac. undulata* Sieverding.

Although spores of *Ac. paulinae* and *Ac. undulata* resemble in colour those of *Ac. scrobiculata*, spores of the former two fungi are much smaller [(60-)72(-95) μm diam, 55-85 μm diam, respectively; Błaszkowski 1988; Sieverding 1988] than those of the latter species [(90-)120(-135) μm diam]. Additionally, while the subcellular structure and the biochemical properties of spores of *Ac. scrobiculata* and *Ac. paulinae* are identical, *Ac. undulata* has only a 2-layered spore wall and one, 1-layered germination wall staining yellowish orange in Melzer's reagent [vs. a 3-layered spore wall and two 2-layered germination walls with the innermost layer of the second wall staining reddish white (Błaszkowski et al., pers. observ.) to light purplish pink to light purple (Morton 2000) in Melzer's reagent in *Ac. scrobiculata*].

The main property distinguishing *Ac. scrobiculata* from *Ac. cavernata*, *Ac. dilatata*, *Ac. foveata*, and *Ac. lacunosa* is spore colour. Spores of *Ac. scrobiculata* are yellowish white to pale yellow, and the colour of spores of the other species ranges from shadows of yellow (*Ac. dilatata*, *Ac. lacunosa*; Błaszkowski 1990, 1994; Morton 1986, 2000) to shadows of brown (*Ac. cavernata*, *Ac. foveata*; Błaszkowski 1989; Janos and Trappe 1982; Morton 2000).

Additionally, the mean diameter of *Ac. foveata* spores is over 2-fold greater than that of spores of *Ac. scrobiculata*. The spore size of the other species compared more or less overlaps. Finally, the unique property of *Ac. cavernata* is the structure and phenotypic properties of the innermost germination wall. In most species of the genus *Acaulospora*, this wall consists of two layers with the outer layer covered with a granular material, frequently dispersing in crushed spores. In contrast, the second germination wall of *Ac. cavernata* is composed of three layers: (1) a flexible, hyaline, up to 0.5 μm thick layer ornamented with small knobs (not granules), (2) a flexible, hyaline, (1.5-) 1.7 (-2.9) μm thick middle layer, and (3) a plastic, hyaline layer, 3.1-8.0 μm thick in PVLG and staining beetroot purple in Melzer's reagent. The knobby layer resembles a plastic covering of the inner surface of post envelopes.

When devoid of a sporiferous saccule, spores of *Ac. scrobiculata* are almost indistinguishable from those of *Entrophospora kentinensis* Wu et Liu. The only difference is the lack of the third spore wall layer of the former species in the spore wall of the latter fungus (Błaszkowki, pers. observ.; Morton 2000; Wu et al. 1995).

The morphological properties of the *Ac. scrobiculata* mycorrhizae described here generally agree with those presented in the only earlier description of mycorrhizae of this fungus (Morton 2000). The short lateral branches and knobby swellings formed by hyphae of *Ac. scrobiculata* inside roots of *P. lanceolata* (Fig. 9) were not observed by Morton (2000) in roots of *Zea mays* L. Colonization pattern of an arbuscular fungal species may be influenced by host plant (Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983; Hetrick, Bloom and Feyerherm 1985).

In Schüessler's et al. (2001) classification of arbuscular fungi, *Acaulospora* spp. are included in the family *Acaulosporaceae* Morton et Benny of the order *Diversisporales* C. Walker et Schuessler.

Glomus versiforme (Karsten) Berch Figs 11-18

Sporocarps unknown. *Spores* borne singly in the soil (Fig. 11); produced from straight sporophores. *Sporophore* coenocytic to sparsely septate; hyaline; (3.8-) 4.4 (-4.7) µm wide; with a wall (0.5-) 0.7 (-0.8) µm thick; bearing spores by swelling at hyphal tips. Mature spores pale yellow (3A3) to deep yellow (4A8); globose to subglobose; (80-) 106 (-150) µm diam; sometimes ovoid; 80-105 x 85-150 µm; usually with a single subtending hypha (Figs 11, 13, and 14), sometimes with two to three subtending hyphae (Fig. 15). *Subcellular structure* of spores consists of one wall with two layers (layers 1 and 2; Figs 12-14). Outer layer 1 semipermanent, hyaline, (0.7-) 1.0 (-1.2) µm thick, tightly adherent to layer 2 (Figs 12-14). Layer 2 laminate, pale yellow (3A3) to deep yellow (4A8), smooth, (2.7-) 4.1 (-5.4) µm thick (Figs 12-14). Layers 1 and 2 do not react in Melzer's reagent. Most juvenile spores with wall layer 1 only. Layer 2 begins to form when layer 1 is completely differentiated. *Subtending hypha* pale yellow (3A3) to deep yellow (4A8); straight or recurvate; cylindrical or flared, rarely constricted or irregular; (4.9-) 6.3 (-7.8) µm wide at the spore base (Figs 11, and 13-15). Wall of subtending hypha pale yellow (3A3) to deep yellow (4A8); (1.6-) 1.8 (-2.0) µm thick at the spore base; composed of two layers continuous with spore wall layers 1 and 2 (Fig. 13); layer 2 extends up to 8.5 µm below the spore base; when not deteriorated, layer 1 further develops below the end of layer 2 (Fig. 13) and is continuous with the wall of a sporophore. *Pore* occluded by a curved septum continuous with the inner sublayers of spore wall 2 (Fig. 13) or by a transverse septum (Fig. 14).

Collection examined. Poland. Szczecin, spores and root fragments from one-species cultures with *P. lanceolata* as the plant host, 15 Nov. 2002, Błaszkowski J., 2397-2417, DPP.

Habitat and distribution in Poland. As culturing of the field-collected root-rhizosphere soil in a trap culture indicated, *Gl. versiforme* characterized here was associated in the field with *Viola tricolor* L. colonizing the Baltic Sea dunes adjacent to Świnoujście, although this fungus did not sporulate when the plant species was sampled, i. e., on 13 June 1997. The spores of arbuscular fungi recovered from the field sample came from *Gl. arenarium*, *Gl. corymbiforme*, *Gl. microcarpum* Tul. et Tul., and *Scu. persica* (Koske et Walker) Walker et Sanders. Apart from *Gl. versi-*

forme, the arbuscular fungi sporulating in the trap culture were *Gl. arenarium*, *Gl. minutum* Błasz., Tadych et Madej, and *Scu. persica*.

The chemical properties of the Świnoujście dune soils were as those presented in the section "Distribution and habitat" of *Ac. scrobiculata*.

The only earlier finding of *Gl. versiforme* in Poland is that from the vicinity of Wrocław (Bucholtz 1912). However, no detailed localization of the finding was given.

General distribution. Despite the relatively not numerous literature reports of the occurrence of *Gl. versiforme*, the existing data suggest that this fungus has a worldwide distribution and is adapted to different environmental conditions, including those of desert, subtropical, and temperate areas, as well as those of greenhouses (Bethlenfalvay, Dakessian and Pacovsky 1984; Karsten 1984; Mayo, Davis and Motta 1986; Simpson and Daft 1990; Walker, Mize and McNabb 1982).

Glomus versiforme has originally been described from spores collected from the surface of potting soil harboured in a greenhouse of the botanical garden at Helsinki, Finland (Karsten 1884). Then, this fungal species has been encountered in different states of the U.S.A. (Bethlenfalvay et al. 1984; Daniels and Trappe 1979; Gerdemann and Trappe 1974; Mayo, Davis and Motta 1986; Thaxter 1922; Walker, Mize and McNabb 1982), in Canada (Talukdar and Germida 1993), Mexico and Ecuador (Daniels and Trappe 1979), Italy (Bucholtz 1912; Bonfante-Fasolo and Vian 1984), Poland, Lithuania (Bucholtz 1912), Sweden (Kers 1985), Tasmania (Berch and Fortin 1983), India (Simson and Daft 1990), and Australia (McGee 1986).

Mycorrhizal associations. In the field, *Gl. versiforme* was associated with vesicular-arbuscular mycorrhizae of *V. tricolor*.

The mycorrhizae of *Gl. versiforme* formed in single-species pot cultures with *P. lanceolata* as the plant host consisted of arbuscules, vesicles, as well as intra- and extraradical hyphae (Figs 16-18). Arbuscules were not numerous, patchily distributed along the roots (Fig. 16). Vesicles were globose, 38-45 µm diam, or ellipsoid, 13-30 x 45-125 µm (Figs 17 and 18). Intraradical hyphae grew parallel to each other and to the root axis, sometimes had Y- or H-shaped branches, and were 2.2-9.8 µm wide (Figs 16-18). The hyphae frequently formed coils, 20-43 x 30-110 µm (Fig. 18). Extramatrical hyphae were abundant and 1.7-3.7 µm wide. In 0.1% trypan blue, arbuscules, vesicles, coils, intra- and extraradical hyphae stained violet white (19A2), pale violet (19A3) to violet blue (19 C 8), pale violet (19A3) to deep blue (19D8), violet white (19A2) to violet blue (19 C 8), and violet white (19A2) to violet blue (19C8), respectively (Figs 16-18).

Discussion. When observed under a dissecting microscope, spores of *Gl. versiforme* are most similar to those of *Gl. arenarium*, *Gl. caledonium* (Nicol. et Gerd.) Trappe et Gerd., *Gl. claroideum* Schenck et Smith, *Gl. etunicatum* Becker et Gerd., *Gl. geosporum* (Nicol. et Gerd.) Walker, and *Gl. macrocarpum* Tul. et Tul. Spores of all these species are yellow-coloured and have a similar appearance and size range.

The main properties distinguishing *Gl. versiforme* from the other species listed above are the number, as well as the phenotypic and biochemical properties of components of wall structure of its spores.

As in *Gl. versiforme*, the spore wall structure of *Gl. etunicatum* and *Gl. macrocarpum* also is two-layered with the outer layer sloughing with age (Becker and Gerdemann 1977; Berch and Fortin 1983; Błaszkowski 1993; Stürmer and Morton 1997). However, the outer layer of *Gl. etunicatum* spores stains dark pinkish red to reddish-purple in Melzer's reagent and usually is present only in young spores (Stürmer and Morton 1997), and that of spores of *Gl. versiforme* remains non-reactive in this reagent and frequently persists in mature spores (Figs 12-14; Błaszkowski et al., pers. observ.; Morton 2000). The behaviour of the sloughing outer layer of *Gl. macrocarpum* in Melzer's reagent is unknown. Additionally, the outer layer of *Gl. macrocarpum* spores swells in lactic acid-based mountants (Berch and Fortin 1983), a phenomenon not occurring in spores of *Gl. versiforme*. Finally, *Gl. macrocarpum* mainly is a hyphogeous fungus, where it most frequently produces spores in sporocarps enveloped in a peridium rather than singly in the soil (Błaszkowski 1993; Berch and Fortin 1983; Gerdemann and Trappe 1974). In contrast, spores of *Gl. versiforme* usually occur singly in the soil (Fig. 11; Błaszkowski et al., pers. observ.) and only sometimes in epigeous sporocarps lacking a peridium (Bonfante-Fasolo and Vian 1984; Morton 2000). *Glomus etunicatum* is an ectocarpic, underground fungus (Becker and Gerdemann 1977; Stürmer and Morton 1997).

Even when observed under a compound microscope, spores of *Gl. versiforme* may easily be mistaken for those of *Gl. arenarium*, *Gl. geosporum*, and *Gl. verruculosum*. Examination of many spores of different developmental stages readily separates the four species. While *Gl. versiforme* produces two-layered spores (Figs 12-14), the spore wall structure of the other three species consists of three layers. The laminate layer of *Gl. versiforme* spores is tightly associated with a hyaline, semipermanent outer layer (Figs 12-14), and that of spores of *Gl. arenarium* is enveloped in two hyaline layers: an evanescent outer layer adherent to a semiflexible inner layer (Błaszkowski, Tadych and Madej 2001). Both the layers balloons in lactic acid-based mountants and are usually completely sloughed in mature spores.

The laminate spore wall layer of *Gl. geosporum* and *Gl. verruculosum* is covered with a single evanescent layer that is usually completely sloughed in mature spores (Błaszkowski and Tadych 1997; Błaszkowski, pers. observ.; Morton 2000; vs. a semipermanent layer usually present in mature spores of *Gl. versiforme*; Figs 11-14). Additionally, the laminate layer of the two former species is associated with a permanent, coloured inner layer, which is lacking in *Gl. versiforme* spores. In *Gl. geosporum*, the third spore wall layer is smooth (Błaszkowski, pers. observ.; Morton 2000; Walker 1982), whereas it is ornamented with evenly distributed warts in spores of *Gl. verruculosum* (Błaszkowski and Tadych 1997).

Spores of *Gl. caledonium* resemble those of *Gl. versiforme* when the laminate, coloured structural layer of the former fungus is devoid of the hyaline outer cover consisting of three adherent layers: a mucilagenous outer layer, a rigid middle layer, and a granular inner layer (Morton 1996). However, examination of many spores of different maturity readily shows this unique complex of layers. Additionally, fully

developed spores of *Gl. caledonium* are markedly larger (180-320 μm, mean 259 μm diam; Morton 1996, 2000) than those of *Gl. versiforme* [(80-) 106 (-150) μm diam].

The only faint staining of arbuscules of *Gl. versiforme* mycorrhizae found in this study (Fig. 16) has also been revealed by, e. g., Hetrick et al. (1985) and Morton (2000). However, Morton (2000) did not find vesicles in roots of *Zea mays* L. In contrast, vesicles occurred in roots of *P. lanceolata* used in investigations of the authors of this paper (Figs 17 and 18) and those of nine plant species compared by Hetrick et al. (1985). In Hetrick's et al. (1985) experiments, *Gl. versiforme* formed vesicles only in roots of older plants, regardless of the host plant species used. In our studies, roots of *P. lanceolata* with vesicles of *Gl. versiforme* came from at least 6-month-old cultures.

In Schüßler's et al. (2001) classification of arbuscular fungi, *Gl. versiforme* is located in the order *Diversisporales* C. Walker et Schuessler.

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Acaulospora scrobiculata and *Glomus versiforme* (*Glomeromycota*), grzyby arbuskularne znalezione odpowiednio pierwszy i drugi raz w Polsce

Streszczenie

Opisano i zilustrowano cechy morfologiczne zarodników i mikoryz *Acaulospora scrobiculata* i *Glomus versiforme*, grzybów arbuskularnych z gromady *Glomeromycota*. Grzyby te ujawniono w kulturach pułapkowych zawierających mieszaniny korzeni i gleby ryzosferowej roślin zasiedlających wydmy nadmorskie Morza Bałtyckiego położone w północno-zachodniej Polsce. Następnie rozpropagowano je w kulturach jednogatunkowych w celu opisania własności ich mikoryz. *Acaulospora scrobiculata* nie była wcześniej notowana w Polsce. Natomiast jedyne doniesienie o znalezieniu *Gl. versiforme* w Polsce pochodzi z roku 1912. Przedstawiono również poznane rozmieszczenie obu tych grzybów w świecie.