

***Glomus intraradices* and *Pacispora robiginia*, species of arbuscular mycorrhizal fungi (Glomeromycota) new for Poland**

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Morphological characters of spores and mycorrhizae of *Glomus intraradices*, as well as spores of *Pacispora robiginia*, arbuscular mycorrhizal fungi of the phylum Glomeromycota, were described and illustrated. Additionally, the known distribution of these species in both Poland and other regions of the world was presented. Both the species were not so far recorded in Poland and this paper is the second report of the finding of *P. robiginia* in the world.

Key words: *Glomus intraradices*, Glomeromycota, *Pacispora robiginia*, distribution

INTRODUCTION

Investigations of the occurrence of arbuscular mycorrhizal fungi of the phylum Glomeromycota in Poland revealed *Glomus intraradices* N.C. Schenck et G.S. Sm. and *Pacispora robiginia* Sieverd. et Oehl. Both fungi were not so far recorded in Poland and this paper is the second report of the finding of *P. robiginia* in the world.

The aim of this paper is to present morphological characters of the *G. intraradices* and *P. robiginia* specimens found, as well as the known distribution of these fungi in the world.

MATERIALS AND METHODS

Establishment and growth of trap and one-species cultures, extraction of spores, and staining of mycorrhizae. Spores examined in this study came from both pot trap and one-species 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 (Stutz, Morton 1996). The method used to establish trap cultures, their growing conditions, and the method of spore extraction were previously described (Błaszowski et al. 2004).

One-species cultures were also generally established and grown as given in Błaszowski et al. (2004), with two exceptions. First, instead of marine sand, their growing medium was an autoclaved commercially available coarse-grained sand (grains 1.0-10.0 mm diam. - 80.50%; grains 0.1-1.0 mm diam. - 17.28%; grains < 0.1 mm diam. - 2.22%) mixed (5:1, v/v) with clinophtholite (Zeocem, Bystré, Slovakia) of grains 2.5-5 mm. Clinophtholite is a crystalline hydrated aluminosilicate of alkali metals and alkaline earth metals having, e.g., a high ion exchange capability and selectivity, as well as a reversible hydration and dehydration. pH of the sand-clinophtholite mixture was 7.3. Second, the cultures were kept in transparent plastic bags, 15 cm wide and 22 cm high as suggested by Walker and Vestberg (1994), rather than open pot cultures (Gilmore 1968). To prevent contamination of the cultures with other AMF but still to allow exchange of gases, we left an opening, *ca.* 1 cm wide, in the centre of the upper part of each bag, while the edges on both sides were closed with small plastic clips. The cultures were watered with tap water once a week, harvested after five months when spores were extracted for study. To reveal mycorrhizae, root fragments located *ca.* 1-5 cm below the upper level of the growing medium were cut off with a scalpel. The host plant used in both trap and one-species cultures was *Plantago lanceolata* L.

Microscopy survey. Morphological properties of spores and their wall structure were determined based on examinations of at least 100 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar, Bollan, Heather 1979) and a mixture of PVLG and Melzer's reagent (1:1, v/v). Spores at all developmental stages were crushed to varying degrees by applying pressure to the cover slip and then stored at 65°C for 24 h to clear their contents from oil droplets. These were then examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded on a Sony 3CDD color video camera coupled to the microscope.

Terminology of spore structure is that suggested by Stürmer and Morton (1997) and Walker (1983, 1986). 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. The authors of the fungal names are those presented at the Index Fungorum website <http://www.indexfungorum.org/AuthorsOf-FungalNames.htm>. Specimens were mounted in PVLG on slides and deposited in the Department of Plant Pathology, University of Agriculture, Szczecin, Poland.

Colour microphotographs of spores and mycorrhizae of the fungi presented here can be viewed at the URL <http://www.agro.ar.szczecin.pl/~jblaszkowski/>.

DESCRIPTIONS OF THE SPECIES

Glomus intraradices N.C. Schenck et G.S. Sm.

Spores occur in aggregates or singly in the soil, and frequently are formed inside of roots (Figs 1 and 2). *Aggregates* pale yellow (3A3) to greyish yellow (2B5); of a different shape, usually ovoid 0.3-1.8 x 1.0-3.0 mm; containing from 2 to more than 100

spores (Fig. 1). Spores origin blastically at the tip of either branched hyphae (when in aggregates) or non-branched hyphae (when single) continuous with mycorrhizal extraradical hyphae. *Spores* hyaline, when juvenile, pale yellow (3A3) to greyish yellow (2B5), frequently with a greenish tint, when mature; globose to subglobose; (30-)92(-120) μm diam; occasionally ovoid to irregular; 46-90 x 62-120 μm (Figs 1 and 2). *Subcellular structure of spores* consists of a spore wall comprising three layers (layers 1-3; Figs 3-6). Layer 1, forming the spore surface, mucilaginous, (0.7-)1.4 (-2.5) μm thick, always highly deteriorated or completely sloughed in mature spores. Layer 2 semipermanent, semiflexible, hyaline, (2.2-)3.0(-3.9) μm thick, more or less deteriorating with age and either retaining as a granular structure or completely sloughed at maturity. Layer 3 laminate, pale yellow (3A3) to greyish yellow (2B5), (2.0-)6.7(-11.0) μm thick, consisting of one (in juvenile spores) to more than 20 sub-layers (laminae), each ca. 0.5-1.0 μm thick, usually easily separating from each other in crushed spores. The spore colour darkens with the increasing number and thickness of the laminae during differentiation of the spore wall. In Melzer's reagent, only layer 1 stains bluish red (12A8) to cerise (12C8; Figs 3-6). *Subtending hypha* pale yellow (3A3) to greyish yellow (2B5); straight or curved; cylindrical or slightly flared, occasionally slightly constricted at the spore base; (13.7-)15.5(-18.4) μm wide at the spore base (Fig. 6). *Wall of subtending hypha* pale yellow (3A3) to greyish yellow (2B5); (2.7-)5.1(-6.6) μm thick at the spore base; composed of three layers continuous with spore wall layers 1-3 (Fig. 6); layer 1 extends up to 25 μm below the spore base, and layer 2, when young, develops along the whole subtending hypha and also is a component of the wall of both the branched hyphae of aggregates and the non-branched hyphae continuous with mycorrhizal extraradical hyphae. *Pore* 3.4-8.1 μm wide, open (Fig. 6). *Germination*. Not observed by the authors of this paper. According to Morton (2002) and Stürmer and Morton (1997), spores of *G. intraradices* appear to germinate by a germ tube arising from the innermost sublayer (lamina) of the spore wall layer 3. Then, the germ tube emerges from the lumen of the subtending hypha. Additionally, in some specimens, a germ tube arises from broken ends of hyphal fragments some distance from the spore base. This behaviour probably accounts for the high infectivity of hyphal fragments of this species.

MYCORRHIZAE. In one-species pot cultures with *P. lanceolata* as the host plant, mycorrhizae of *G. intraradices* consisted of arbuscules, vesicles, as well as intra- and extraradical hyphae (Figs 7 and 8). Arbuscules were very numerous and evenly distributed along the root fragments examined. They consisted of a trunk grew from a parent hypha and many branches with very fine tips (Fig. 7). Vesicles occurred sporadically and were widely dispersed along the axis of the root fragments (Fig. 8). They were ellipsoid; 20.0-32.0 x 27.5-60.0 μm . The intraradical hyphae usually extended parallel to the root axis and were (2.0-)4.0(-5.6) μm wide. They sometimes formed Y- or H-shaped branches and frequently coils (Fig. 8). The coils were ellipsoid; 15.0-20.0 x 40.0-97.5 μm ; rarely circular; 35.0-45.0 μm diam; when observed in a plane view. The extraradical hyphae were (1.7-)2.5(-4.4) μm wide and occurred abundantly. In 0.1% trypan blue, arbuscules stained pale violet (16A3) to royal purple (16D8), vesicles light lilac (16A5) to royal purple (16D8), intraradical hyphae violet white (16A2) to reddish violet (16A8), coils violet white (16A2) to reddish violet (16C8), and extraradical hyphae violet white (15A2) to reddish violet (16A8; Figs 7 and 8).

PHYLOGENETIC POSITION. Results of molecular analyses have accommodated *G. intraradices* in subclade b of *Glomus* group A in the family *Glomeraceae* Piroz. et Dalpé of the order Glomerales J.B. Morton et Benny, also comprising *G. clarum* Nicol. et N.C. Schenck, *G. coremioides* (Berk. et Broome) D. Redecker et J.B. Morton, *G. fasciculatum* (Thaxt.) Gerd. et Trappe emend. C. Walker et Koske, *G. manihotis* R.H. Howeler, Sieverd. et N.C. Schenck, *G. proliferum* Dalpe et Declerck, *G. sinuosum* (Gerd. et B.K. Bakshi) R.T. Almeida et N.C. Schenck, and *G. vesiculiferum* (Thaxt.) Gerd. et Trappe (Schwarzott, Walker, Schüßler 2001). Unfortunately, the phylogenetic distance between *G. intraradices* and *G. aggregatum* N.C. Schenck et G.S. Sm. emend. Koske, *G. antarcticum* M. Cabello, *G. aureum* Oehl et Sieverd., *G. cerebriforme* McGee, *G. glomerulatum* Sieverd., *G. invermaium* I.R. Hall, and *G. pallidum* I.R. Hall, species compared below as well, remains unknown to date.

DISTRIBUTION AND HABITAT. In Poland, the authors of this paper found spores of *G. intraradices* in 25 samples of rhizosphere soils and roots. Of them, five came from under *Ammophila arenaria* (L.) Link, *Artemisia campestris* L. and *Petasites spurius* (Retz.) Rchb. colonizing maritime sand dunes adjacent to Świnoujście (53°55'N, 14°14'E) in October 1993 and June 1997, three from under *P. lanceolata* growing in the Tuchola Forest (53°46'N, 17°42'E-53°40'N, 17°54'E) in September 1996, four from under *Festuca rubra* L. s. s. and *Holcus mollis* L. colonizing the inland sand dunes of the Błędowska Desert (50°22'N, 19°34'E) in June 1997, five from under *A. arenaria* and *Agrostis stolonifera* L. growing in maritime sand dunes of the Słowiński National Park (54°45'N, 17°26'E) in August 1996, one from under *Hieracium* sp. growing in an arsenic heap near Żłoty Stok (50°26'N, 16°52'E) and sampled in July 2002, four from under *Taxus baccata* L. growing in the Central Cemetery in Szczecin (53°26'N, 14°35'E) in May 2001, and four from under *Juncus conglomeratus* L. em. Leers colonizing the bank of the Puck Bay (54°42'N, 18°28'E) in August 2001.

Additionally, Turnau et al. (2001) detected *G. intraradices* in roots of *Fragaria vesca* L. colonizing a 20-year-old Zn waste located in Chrzanów (50°08'N, 19°24'E) in southern Poland using a nested polymerase chain reaction with taxon-specific primers, although no spores of this fungus were found.

The holotype of *G. intraradices* has been selected from spores extracted from pot-cultured *Paspalum notatum* Flugge initiated from a sample originally isolated from among roots of *Citrus* sp. cultivated in Orlando, Florida, U.S.A. (Schenck, Smith 1982). Schenck and Smith (1981, 1982) found this species to be one of the most common *Glomus* species occurring in Florida, where it was associated with roots of many plant species.

Literature data and investigations of the authors of this paper indicate *G. intraradices* to have a worldwide distribution. Apart from Poland and Florida, this fungus has also been encountered in many other regions of the U.S.A., e. g., in California (Bethlenfalvay, Dakessian, Pacovscky 1984; Koske, Halvorson 1989), Kentucky (An et al. 1993), Massachusetts (Błaszowski, unpubl. data), Texas (Stutz, Morton 1996) and Hawaii (Koske, Gemma 1996), as well as in Canada (Dalpé 1989; Klironomos et al. 2001), Portugal (Błaszowski, unpubl. data), Bornholm (Denmark; Błaszowski, unpubl. data), Switzerland (Jansa et al. 2002; Oehl et al. 2005), Africa (Błaszowski, unpubl. data; Stutz et al. 2000), Israel (Błaszowski, Czerniawska 2006), Turkey and Cyprus (Błaszowski, unpubl. data), China (Gai et al. 2006; Zhang, Wang 1992), and India (Mohankumar et al. 1988).

Our investigations of field-collected mixtures of rhizosphere soils and roots and those sampled from trap cultures established from a part of the field mixtures revealed that the arbuscular fungi co-occurring with *G. intraradices* were *Acaulospora lacunosa* J.B. Morton, *A. mellea* Spain et N.C. Schenck, *A. morrowiae* Spain et N.C. Schenck, *Archaeospora trappei* (R.N. Ames et Linderman) J.B. Morton et D. Redecker emend. Spain, *Entrophospora baltica* Błaszk., Madej et Tadych, *G. aggregatum*, *G. arenarium* Błaszk., Tadych et Madej, *G. clarum*, *G. constrictum* Trappe, *G. corymbiforme* Błaszk., *G. deserticola* Trappe, Bloss et J.A. Menge, *G. etunicatum* W.N. Becker et Gerd., *G. fasciculatum*, *G. gibbosum* Błaszk., *G. insculptum* Błaszk., *G. macrocarpum* Tul. et C. Tul., *G. microcarpum* Tul. et C. Tul., *G. lamellosum* Dalpé, Koske et Tews, *G. pustulatum* Koske, Friese, C. Walker et Dalpé, an undescribed *Glomus* sp., *Paraglomus laccatum* (Błaszk.) C. Renker, Błaszk. et F. Buscot, *Scutellospora armeniaca* Koske et Halvorson, and *S. dipurpureus* J.B. Morton et Koske.

The spore abundance of *G. intraradices* in the field samples ranged from 1 to 30 in 100 g dry soil, and the proportion of spores of this fungus in spore populations of all the arbuscular fungi isolated ranged from 0.7 to 33.3%.

COLLECTIONS EXAMINED. Africa: Tunisia, Sousse (35°50'N, 10°38'E), under *Amophila arenaria* (L.) Link, 20 Sept. 2006, J. Błaszkowski, 2733 (DPP); Cyprus: near Larnaca (34°55'N, 33°38'E), among roots of *Oenothera drummondii* Hook., 23 Oct. 2003, J. Błaszkowski, unnumbered collection (DPP); Denmark: Bornholm, near Vestern Sømarken (55°0'N, 14°58'E), in the rhizosphere of *A. arenaria*, 3. Oct. 2004, J. Błaszkowski, 2730 (DPP); Israel: near Tel-Aviv (32°4'N, 34°46'E), around roots of *O. drummondii*, 15 June 2000, J. Błaszkowski, unnumbered collection (DPP); Poland: Świnoujście, under *A. arenaria* and *Petasites spurius* (Retz.) Rchb., 6 Oct. 1993, under *Artemisia campestris* L., 13 June 1997, J. Błaszkowski, 2717-2729 and 2731-2732 (DPP); Tuchola Forest, among roots of *P. lanceolata*, 21 Sept. 1996, J. Błaszkowski, unnumbered collection (DPP); the Błędowska Desert, in the rhizosphere of *Festuca rubra* L. s. s. and *Holcus mollis* L., 26 June 1997, J. Błaszkowski, unnumbered collection (DPP); Słowiński National Park, associated with roots of *Agrostis stolonifera* L. and *A. arenaria*, 2 and 3 Aug. 1996, J. Błaszkowski, unnumbered collection (DPP); Złoty Stok, under *Hieracium* sp., at the end of June 2002, unnumbered collection (DPP); Szczecin, among roots of *Taxus baccata* L., 2 May 2001, J. Błaszkowski, unnumbered collection (DPP); Osłonino, in the rhizosphere of *Juncus conglomeratus* L. em. Leers, 1 Sept. 2000, J. Błaszkowski, unnumbered collection (DPP); Portugal: Faro (37°1'N, 7°36'W), among roots of *A. arearia*, 10 June 2001, J. Błaszkowski, unnumbered collection (DPP); Turkey: near Karabucak-Tuzla (36°43'N, 34°59'E), in the rhizosphere of *A. arenaria*, 10 June 2001, J. Błaszkowski, unnumbered collection (DPP); U.S.A.: Massachusetts, under *A. breviligulata* Fern., 15 Oct. 2002, J. Błaszkowski, unnumbered collection (DPP).

NOTES. When observed under a dissecting microscope, three groups of species of the genus *Glomus* known to form spores both singly and in aggregates more or less resemble *G. intraradices*. The group of species most similar in colour and size of spores to those of *G. intraradices* is represented by *G. aggregatum*, *G. antarcticum*, *G. fasciculatum*, *G. pallidum*, *G. proliferum*, and *G. vesiculiferum*.

Considering the phenotypic and biochemical properties of the components of the spore wall of these species observed under a light microscope, *G. intraradices* is

most closely related to *G. aggregatum*. The number and the types of layers forming the spore wall of these species and their reactivity in Melzer's reagent are identical (Błaszowski 2003; pers. observ.). The only character distinguishing these fungi is the formation of spores inside of their parent spores by internal proliferation in *G. aggregatum* (Koske 1985), a phenomenon not found in *G. intraradices* (Błaszowski, pers.observ.; Stürmer, Morton 1997). Morton (2002) hypothesized these species to be synonymous. Thus, results of molecular analyses of both fungi are urgently needed to explain this supposition.

Similarly as in *G. intraradices*, the spore wall of *G. antarcticum* and *G. fasciculatum* is 3-layered (Błaszowski 2003; Cabello, Gaspar, Pollero 1994; Walker, Koske 1987). However, of these layers of *G. antarcticum*, only the outermost one, forming the spore surface, sloughs with age. In contrast, in *G. intraradices*, two outer spore wall layers are of the type of sloughing layers (Figs 3-6). Moreover, the spore wall of *G. intraradices* lacks the innermost, flexible layer of the *G. antarcticum* spore wall. Finally, while the outermost spore wall layer of *G. intraradices* stains intensively in Melzer's reagent (Figs 3-6), none of the wall layers of *G. antarcticum* spores reacts in this reagent.

As mentioned above, *G. fasciculatum* also produces spores of a 3-layered wall, of which all are permanent, however (Błaszowski 2003; Walker, Koske 1987; vs. two outer layers slough with age in *G. intraradices*; Figs 3-6). Similarly as in *G. antarcticum*, the distinctive component of the spore wall of *G. fasciculatum* is an innermost flexible, colourless layer, which is lacking in the wall of spores of *G. intraradices*. Still other important difference between these fungi regards the reactivity of their spores in Melzer's reagent. While the structure of spores of *G. intraradices* staining in this reagent is only their outermost spore wall layer (Figs 3-6), two outer wall layers of spores of *G. fasciculatum* are reactive in Melzer's reagent, including its laminate layer, not staining in any other known species of the genus *Glomus* (Błaszowski, pers. observ.). Additionally, *G. intraradices* probably is much more plastic ecologically than *G. fasciculatum*. The former fungus has successfully been used in many experiments (Gopi, Douds, Douds 2000). Although *G. fasciculatum* has been one of the most frequently cited species of arbuscular fungi in papers describing the influence of arbuscular fungi on plants, Walker and Koske (1987) concluded this fungus to have certainly been confused with other species of the Glomeromycota. Many attempts to grow *G. fasciculatum* in one-species cultures made by one of the authors of this paper (J. Błaszowski) failed. In the literature, there is no convincing evidence of the properties of mycorrhizae of *G. fasciculatum* from a one-species culture.

Although *G. proliferum* has originally been described to form hyaline spores (Declerck et al. 2000), pictures obtained from Dr. C. Walker, U. K., also show yellow-coloured spores of this fungus, deceptively similar to those of *G. intraradices*. However, in respect of size, only the largest spores of the former species attain the lower size range of spores of the latter fungus (Błaszowski, pers. observ.; Declerck et al. 2000). The spore wall of *G. proliferum* has originally been characterized to consist of four permanent layers, but examination of this fungus (culture: MVCL 41827) obtained from Prof. S. Declerck, Université catholique de Louvain, Mycothèque de l'Université catholique de Louvain, Unité de microbiologie, Belgium, revealed only three layers of phenotypic and biochemical properties identical to those of *G. intraradices*.

At least three morphological characters separate *G. intraradices* and *G. pallidum*. First, Hall (1977) characterized spores of the latter species to be whitish, and not yellow-coloured as most mature spores of the former fungus (Fig. 1). Second, spores of *G. pallidum* generally are smaller than those of *G. intraradices* [32-78 x 28-68 μm diam according to Hall 1977; vs. (30-)92(-120) μm diam or (60-)80-120(-160) μm diam as the authors of this paper and Stürmer and Morton (1997) determined, respectively]. Third, in contrast to the 3-layered spore wall of *G. intraradices* (Figs 3-6), only two layers build the spore wall of *G. pallidum*. Among them, the middle, semi-flexible wall layer of *G. intraradices* spores is lacking.

The unique structures of *G. vesiculiferum* are its thin-walled vesicles associated with a peridium-like layer covering sporocarps of this fungus (Gerdemann, Trappe 1974). Additionally, spores of *G. vesiculiferum* generally are smaller (49-85 μm diam when globose or up to 100 x 70 μm when ovoid to irregular; Gerdemann and Trappe 1974) than those of *G. intraradices* (see above) and have only a 2-layered wall (Gerdemann, Trappe 1974 vs. 3-layered in *G. intraradices*; Figs 3-6).

The second group of species compared here represents only *G. cerebriforme*, whose spores partly overlap in size with those of *G. intraradices* [25 x 25-65 x 80 μm diam after McGee (1986) vs. (30-)92(-120) μm diam or (60-)80-120(-160) μm diam according to Błaszkowski et al. (pers. observ.) and Stürmer and Morton (1997), respectively], but remain hyaline throughout their entire life cycle, and, thereby, are similar only to the juvenile spores (Błaszkowski et al., pers. observ.) of the species discussed here. Moreover, the distinctive character of the former species is the formation of its spores on racemose hyphae, and not on hyphae irregularly branched as in the latter fungus.

The next diametrical differences between these species occur in the number, the phenotypic characters, and the spatial distribution of layers of their spore wall. The spore wall of *G. cerebriforme* consists of a thick, laminate outer layer and a thin, flexible inner one (McGee 1986). Thus, the structural layer of this wall is an outermost layer, forming the spore surface, and not an innermost one as in the spore wall of *G. intraradices*, which is covered with two impermanent layers (Figs 3-6), but does not overly a flexible layer as in *G. cerebriforme*. Additionally, compared with *G. intraradices*, the subtending hypha of *G. cerebriforme* spores is much narrower [3-7 μm wide after McGee (1986) vs. (13.7-)15.5(-18.4) μm wide as presented here].

The third group of species superficially resembling *G. intraradices* comprises *G. aureum*, *G. glomerulatum*, and *G. invermaium*. Compared with the relatively large (92-120 μm diam when globose) and pale yellow (3A3) to greyish yellow (2B5) mature spores of *G. intraradices* (Błaszkowski et al., pers. observ.; Fig. 1), globose spores of all the other species are smaller and darker-coloured [(27-)40-60 μm diam, light orange (5A4) to orange (5A7) in *G. aureum*; Błaszkowski et al., pers. observ.; Oehl et al. 2003; 40-70 μm diam, light orange (5A5) to golden yellow (5B8) in *G. glomerulatum*; Błaszkowski et al., pers. observ.; Sieverding 1987; 50-75 μm diam, light brown to brown in *G. invermaium*; Hall 1977]. Except for *G. intraradices* having a 3-layered spore wall (Figs 3-6), that of all these species is 2-layered. Moreover, the layers forming the spore surface of *G. glomerulatum* and *G. invermaium* are laminate and unit *sensu* Walker (1983), respectively, thus, they are permanent, whereas the outermost spore wall layer of *G. intraradices* lives shortly and usually is completely sloughed or at most occurs patchily as a highly decomposed structure at maturity (Fig. 4).

The distinctive character of spores of *G. glomerulatum* also is that the innermost layer of their wall is a thin, flexible, membranous, uniform structure, and not a rigid layer composed of many sublayers (laminae) as in the other three species and that *G. glomerulatum* produces only intercalary spores, which, thereby, always have two subtending hyphae (Błaszowski et al., pers. observ.; Sieverding 1987). An intercalary mode of spore origination has also been observed in *G. intraradices* and many other species of the Glomeromycota (Błaszowski et al., pers. observ.), but such spores usually constituted a small part of all the spores produced.

The last morphological character distinguishing species of this group is the width of the subtending hyphae of their spores. It is widest in *G. intraradices* [(13-7)15.5 (-18.4) μm wide; Błaszowski et al., pers. observ.], intermediate in *G. invermaium* (6-13 μm wide; Hall 1977), and narrowest in *G. glomerulatum* (6-10 μm wide; Błaszowski et al., pers. observ.; Sieverding 1987).

As presented in the section "Phylogenetic position", apart from species compared above, *G. intraradices* is molecularly also related to *G. clarum*, *G. coremioides*, *G. manihotis*, and *G. sinuosum* (Schwarzott et al. 2001).

Although there is no formal decision, morphological characters and results of molecular analyses of spores of *G. clarum* and *G. manihotis* have suggested these fungi to be synonymous (Morton 2002; Schwarzott et al. 2001). Morphologically, *G. clarum* differs markedly from *G. intraradices* in colour and size of spores, as well as in phenotypic properties of the components of their wall. Spores of the former fungus may be darker [to yellow-brown; Morton 2002; vs. pale yellow (3A3) to greyish yellow (2B5) or hyaline to greenish yellow after Błaszowski et al., pers. observ. and Stürmer and Morton 1997, respectively], much larger when globose [(120-)180-200(-280) μm diam; Stürmer and Morton 1997; vs. (30-)92(-120) μm diam or (60-)80-120(-160) μm diam after Błaszowski et al., pers. observ. and Stürmer, Morton 1997, respectively], and have two laminate layers in their 3-layered wall (vs. only one such layer in *G. intraradices*; Figs 3-6).

Glomus coremioides and *G. sinuosum* are morphologically completely unlike *G. intraradices*. The former two fungi produce compact sporocarps with a peridium (Błaszowski et al., pers. observ.; Gerdemann, Trappe 1974; Morton 2002; vs. single spores or in loose aggregates without a peridium in *G. intraradices*), in which spores are organized in a single layer and develop from a central plexus of hyphae (vs. randomly distributed spores when in aggregates and develop terminally from branched hyphae; Fig. 1). Moreover, spores of the former two species are (1) ovoid to clavate (vs. usually globose to subglobose in *G. intraradices*), (2) darker-coloured [brown and orange-brown, respectively, after Gerdemann, Trappe 1974 and Morton 2002, respectively; vs. pale yellow (3A3) to greyish yellow (2B5) in *G. intraradices*; Błaszowski et al., pers. observ.], and (3) their wall consists of only one layer (Błaszowski et al., pers. observ.; Gerdemann, Trappe 1974; Morton 2002; vs. 3-layered in *G. intraradices*; Figs 3-6).

***Pacispora robiginia* Sieverd. et Oehl**

Spores produced singly in the soil, blastically at the tip of mycorrhizal extraradical hyphae. *Spores* pale orange (5A3) to golden yellow (5B7); globose to subglobose; (100-)125-155(-161) μm diam; rarely ellipsoidal; 95-135 x 135-165 μm ; with a single subtending hypha (Figs 9-14). *Subcellular structure of spores* consists of a spore wall

and an inner germination wall (Figs 9-14). *Spore wall* consists of three layers (layers 1-3; Figs 9-14). Layer 1, forming the spore surface, permanent, of a smooth upper surface, unit, pale orange (5A3) to golden yellow (5B7), 2.5-3.5(-5.0) μm thick, tightly adherent to layer 2. Layer 2 laminate, light orange (5A4-5), 4.0-6.5 μm thick. Layer 3 permanent, concolorous with layer 2, <0.5 μm thick, usually tightly adherent to the lower surface of layer 2 in even vigorously crushed spores and, hence, very difficult to observe. *Germination wall* includes three hyaline layers (layers 1-3; Figs 9-14). Layer 1 flexible, 0.4-0.8 μm thick, usually separates from layer 2 in crushed spores. Layer 2 coriaceous, 2.2-3.0 μm thick. Layer 3 flexible, <1.0 μm thick, usually tightly adherent to the lower surface of layer 2, sometimes wrinkles in spores vigorously crushed in PVLG. In Melzer's reagent, only layer 2 of the germination wall stains pare red (9A3; Fig. 13). *Subtending hypha* light orange (5A4-5) at the spore base and for some distance from the spore base; usually straight, sometimes slightly curved; cylindrical; 8-15 μm wide at the spore base, 12-25 μm at a some distance from the spore base (Fig. 14). *Wall of subtending hypha* light orange (5A4-5) for some distance from the spore base, then gradually lightens up to hyaline; 2.6-3.8 μm thick at the spore base, <1 μm thick 30-80(-100) μm below the spore base, composed of two layers continuous with spore wall layers 1 and 2 (Fig. 14). *Pore* closed at the spore base by a transverse septum formed by spore wall layer 2 and by adherent spore wall layer 3. *Germination shield*. Not found. *Germination*. Unknown.

MYCORRHIZAE. *Pacispora robiginia* has been associated with mycorrhizal roots of plants of grasslands (Oehl, Sieverding 2004). However, attempts to grow this fungus in one-species cultures failed and, hence, the properties of mycorrhizae of *P. robiginia* remain unknown.

DISTRIBUTION. The type of *P. robiginia* has been selected from spores isolated from a calcareous Lithic Leptosol in the High Alpines at the Haldensteiner Calanda (9°27'E, 46°53'N) at 2800 m above the sea level, near Chur (Kanton Graubünden), Switzerland (Oehl, Sieverding 2004). The same scientists have also found this fungus among roots of plants of High Alpine grasslands located in the Gotthard region (Kanton Wallis), Central Switzerland.

In Poland, one of the authors of this paper (Sz. Zubek) isolated spores of *P. robiginia* from under *Soldanella carpatica* Viehr. growing in Tatra Mountains.

COLLECTIONS EXAMINED. Poland: Tatra Mountains, Kozi Grzbiet (49°14'N, 19°53'), under *S. carpatica*, 5 Sept. 2004, J. Błaszowski, 2734 and 2735 (DPP); Switzerland: Dr. F. Oehl's specimens: T12701, 33211005; MZ2690, 11031004; DS2483, 15280904; Ax2701, 26220704; AX2700, 21220704; DS2537, 05280904; TI2704, Aloenis14071004; TI2701, 07071004; MJ2690, 14031004; MJ2690, 12031004; DS52537, 06280904; TI2701, 32211005; MJ2690, 20041004; Ax2665, 35071004; IC2888, 03240504; IC2890, 01240504; IC2800, 13240504; MJ2690, 13031004; Ax2675, 36071004; IC2800, 17240504; HC2600, 26130704; GR2600, 20220304; GR2600, 09220304.

NOTES. Under a dissecting microscope, spores of *P. robiginia* resemble yellow-coloured spores of many species of the genus *Glomus*. Examination of the subcellular structure of spores of the former fungus under a compound microscope readily reveals its main generic character, i. e., the inner complex and relatively thick germination wall (Figs 9-14) resembling a germination wall of spores of fungi of the genus *Scutellospora* C. Walker et F.E. Sanders (Błaszowski 2003). However, spores of *Pacispora* spp. form at the tip of more or less cylindrical hyphae (Fig. 14), and thus

identically to those of *Glomus* spp., whereas spores of the genus *Scutellospora* origin from a bulbous sporogenous cell.

Of the known species of the genus *Pacispora*, only *P. boliviana* Sieverd. et Oehl forms spores of a similar colour to that of spores of *P. robiginia* (Oehl, Sieverding 2004). However, the upper surface of the structural laminate wall layer of spores of *P. robiginia* is smooth (Figs 9-14), and that of spores of *P. boliviana* is ornamented with shallow, usually pentagonal pits (Oehl, Sieverding 2004). Spores of the other species of this genus are colourless (Błaszowski 2003; Oehl, Sieverding 2004).

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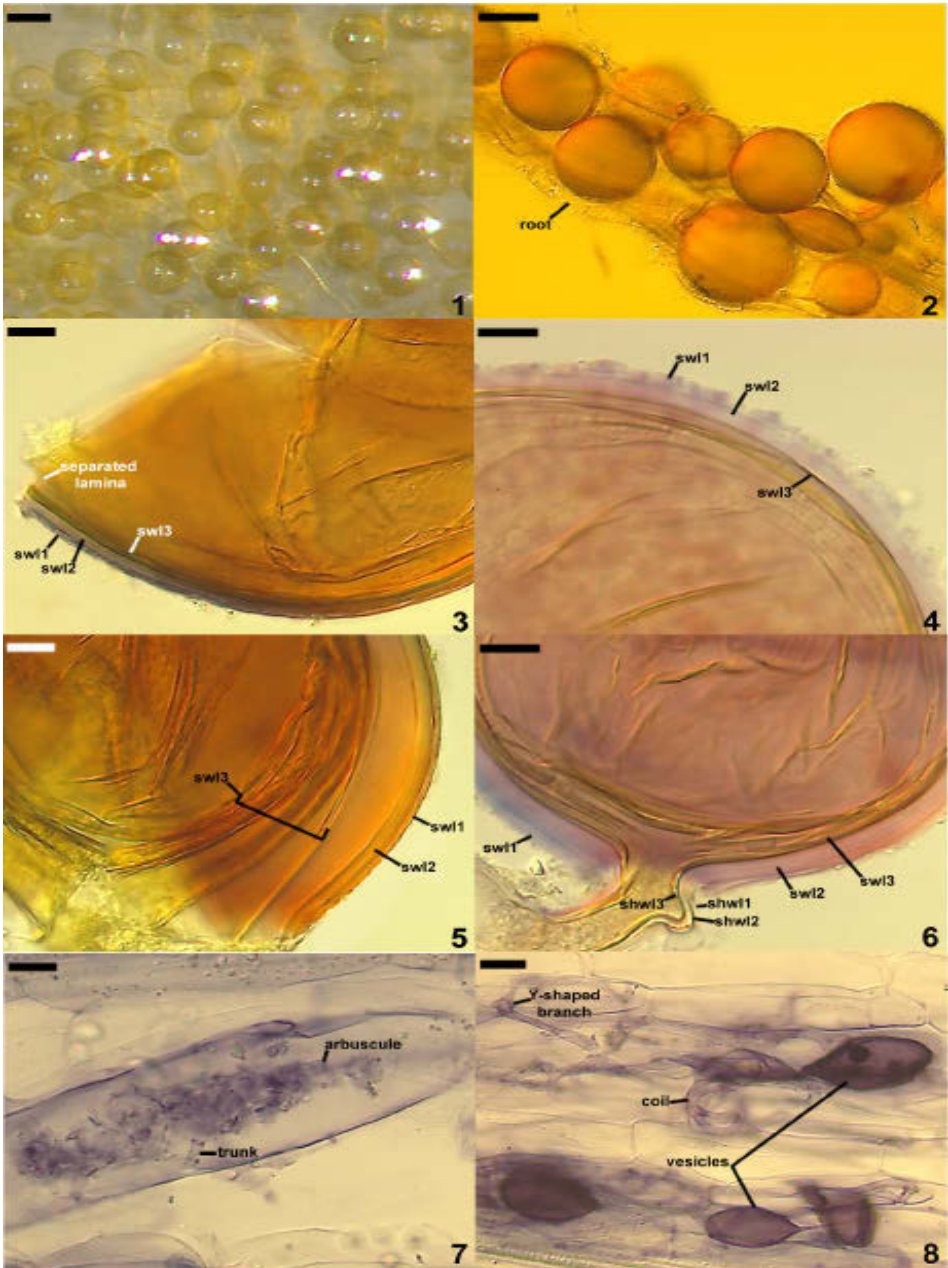
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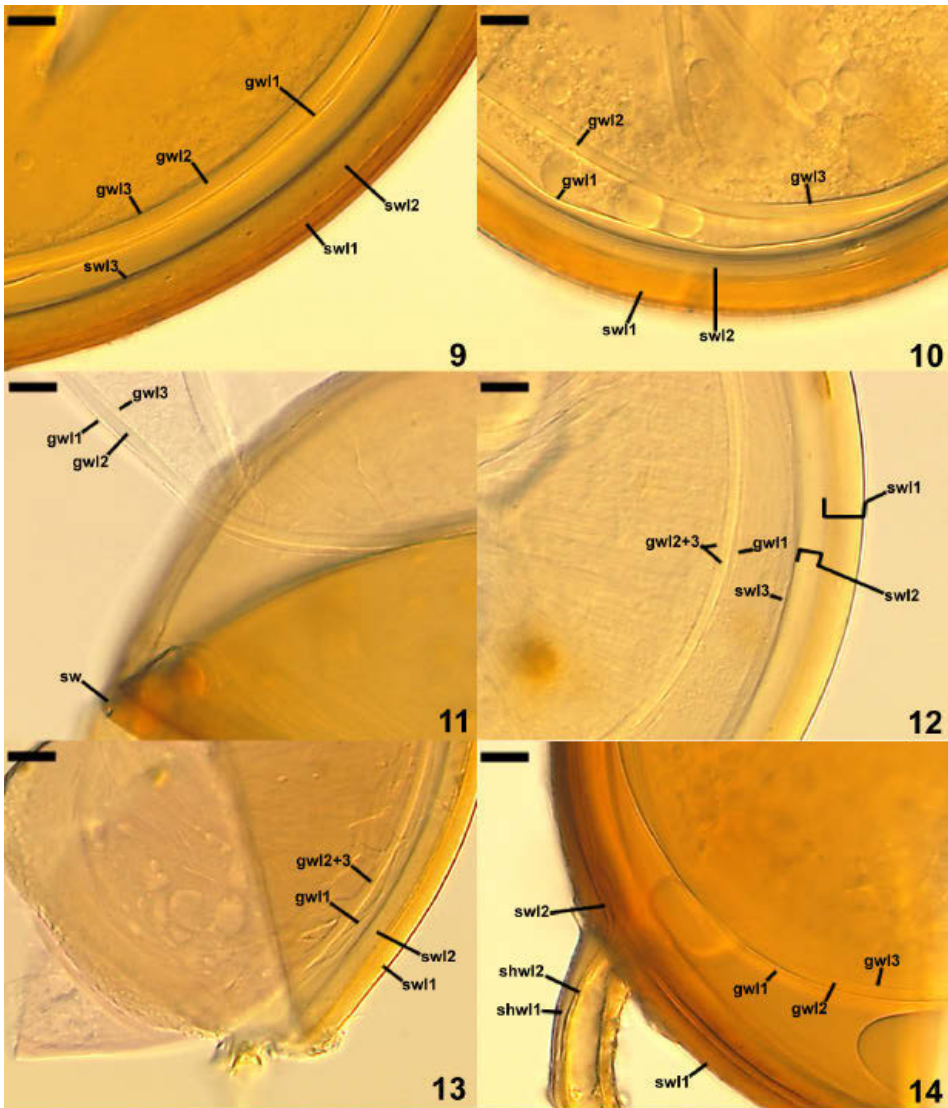
Glomus intraradices i *Pacispora robiginia*, nowe dla Polski gatunki mikoryzowych grzybów arbuskularnych (Glomeromycota)

Streszczenie

Opisano i zilustrowano cechy morfologiczne zarodników i mikoryz *Glomus intraradices* oraz zarodników *Pacispora robiginia*, mikoryzowych grzybów arbuskularnych z gromady Glomeromycota. Ponadto przedstawiono poznane rozmieszczenie tych gatunków zarówno w Polsce, jak i w innych regionach świata. Oba te gatunki nie były wcześniej podawane z Polski i niniejszy artykuł jest drugim doniesieniem o występowaniu *P. robiginia* w świecie.



Figs 1-8. *Glomus intraradices*. 1. Loose aggregate of intact spores. 2. Intraradical spores. 3-5. Spore wall layers 1-3 (swl1-3). 6. Spore wall layers 1-3 (swl1-3) continuous with subtending hyphal wall layers 1-3 (shwl1-3). 7. Arbuscule with trunk. 8. Vesicles and straight, coiled, and Y-branched hyphae. Fig. 1, spores in lactic acid. Figs 2-6, spores crushed in PVLG+Melzer's reagent. Figs 7 and 8, mycorrhizae stained in 0.1% trypan blue. Fig. 1, bright field microscopy; Figs 2-8, differential interference contrast. Bars: Figs 3-7=10 μ m; Fig. 8=20 μ m; Fig. 2=50 μ m; Fig. 1=100 μ m.



Figs 9-14. *Pacispora robiginia*. 9-13. Spore wall layers 1-3 (sw1-3) and inner germination wall layers 1-3 (gw1-3). 14. Spore wall layers 1 and 2 (sw1 and 2) continuous with subtending hyphal wall layers 1 and 2 (shw1 and 2). Figs 9-12 and 14, spores crushed in PVLG. Fig. 13, spore crushed in PVLG+Melzer's reagent. Figs 9-14, differential interference contrast. Bars: Figs 9-14=10 μ m.