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Entrophospora schenckii and *Pacispora franciscana*, arbuscular mycorrhizal fungi (Glomeromycota) new for Europe and Poland, respectively

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Morphological properties of spores of *Pacispora franciscana*, as well as spores and mycorrhizae of *Entrophospora schenckii*, arbuscular fungi of the phylum Glomeromycota found for the first time in Poland and Europe, respectively, are described and illustrated. Additionally, the known distribution of the two fungi is presented.

Key words: arbuscular fungi, Glomeromycota, mycorrhizae, distribution

INTRODUCTION

Arbuscular mycorrhizal fungi commonly associate with vascular land plants growing in different sites of the world (Błaszkowski 2003; Gianinazzi and Gianinazzi-Pearson 1986). They increase nutrition of plants and their resistance to different abio- and biotic stresses (Griffioen and Ernst 1989; Schönbeck 1978; Turnau and Haselwandter 2002).

At present, arbuscular fungi are classified in the phylum Glomeromycota (Schüßler, Schwarzott and Walker 2001). Ca. 170 described fungal species forming arbuscular mycorrhizae are distributed in nine genera. Of them, the five-species genus *Entrophospora* R. N. Ames et R. W. Schneid. comprises *E. schenckii* Sieverd. et Toro, a species recorded only in two sites of the world (Mohankumar et al. 1988; Sieverding and Toro 1987), and the recently erected genus *Pacispora* Sieverd. et Oehl consists of seven species, including *P. franciscana* Sieverd. et Oehl so far found only in Italy and Switzerland (Oehl and Sieverding 2004).

Examination of soil samples coming from both the field and trap cultures showed the presence of *E. schenckii* and *P. franciscana* in soils of Poland. Additionally, spores of *P. franciscana* were encountered in Turkey.

The aim of this paper was to describe morphological properties of *E. schenckii* and *P. franciscana* found by the authors of this paper and to present the known distribution of these fungi.

MATERIALS AND METHODS

Establishment of trap cultures and one-species cultures. 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 100-g subsamples were taken from each sample to determine the species of arbuscular mycorrhizal 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 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 15-30°C with supplemental 8-16-h lighting provided by one SON-T AGRO sodium 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 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 and Nicolson 1963). Presence of mycorrhizae was determined following clearing and staining of roots (Phillips and 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 4°C 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 *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 were extracted. The effectiveness of the method of establishment of one-species cultures described above usually exceeded 90% (Błaszkowski, Adamska and Madej 2002).

Microscopical survey. Morphological properties of spores and their subcellular structures were determined based on at least 100 spores mounted in polyvinyl al-

cohol/lactic acid/glycerol (PVLG; Koske and 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, Sieverding and Schenck (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.

Colour microphotographs of spores and mycorrhizae of *E. schenckii*, as well as spores of *P. franciscana* can be viewed at the URL http://www.agro.ar.szczecin.pl/~jblaszkowski/.

DESCRIPTIONS OF THE SPECIES

Entrophospora schenckii Sieverd. et Toro

Spores single in the soil (Fig. 1); develop inside the neck of a sporiferous saccule (Fig. 2); hyaline; globose to subglobose; (45-)50(-75) μm diam; sometimes ovoid to pear-shaped (Fig. 1); 45-55 × 50-80 μm. Subcellular structure of spores composed of a spore wall with three layers (swl1-3; Figs 3 and 4). Layer 1 evanescent, hyaline, (0.5-)0.6(-0.7) μm thick, continuous with the wall of a sporiferous saccule (Fig. 3), usually absent or highly deteriorated in mature and older spores. Layer 2 semipermanent, smooth, (0.5-)0.6(-0.9)μm thick, slowly degrades with age, usually present in mature spores (Figs 3 and 4). Layer 3 semiflexible; (1.1-)2.5(-4.0) μm thick (Figs 3 and 4). None of the spore wall layers stains in Melzer's reagent. *Cicatrix*. Two circular scars are present (Fig. 2). A scar proximal to the saccule is 5-12 μm diam when observed in a plane view. A scar distal to the saccule is 2-3.5 μm diam; it rarely was present in the spores examined. *Sporiferous saccule* hyaline, globose to subglobose, 45-70 μm diam, occasionally ovoid (Figs 3 and 4), 45-55 × 50-70 μm, usually becomes detached in mature spores. Wall of sporiferous saccule composed of one layer, 0.5-0.7 μm thick, continuous with spore wall layer 1. Germination not observed.

Collections examined. POLAND. The Błędowska Desert, under pot-cultured *P. lanceolata*, 4 March 1998, Błaszkowski J., 2460-2488 (DPP).

Distribution and habitat. In Poland, spores of *E. schenckii* were found only in one trap culture with a soil and root mixture taken from under *Juniperus communis* L. growing in the Błędowska Desert located in southern Poland (50°22'N, 19°34'E). No spores of arbuscular fungi were found in the field soil. The arbuscular fungi co-occurring with *E. schenckii* in the trap culture were *Glomus insculptum* Błaszk. and *G. mosseae* (Nicol. et Gerd.) Gerd. et Trappe.

The type of *E. schenckii* has been isolated from a pot culture of tropical kudzu established with soil coming from a rose nursery located in Melecio Ospina, Co-

lombia, South America (Sieverding and Toro 1987). The only other literature report of the presence of *E. schenckii* is that from India, where the fungus has been found associated with plants growing along the Madras sea coast (Mohankumar et al. 1988).

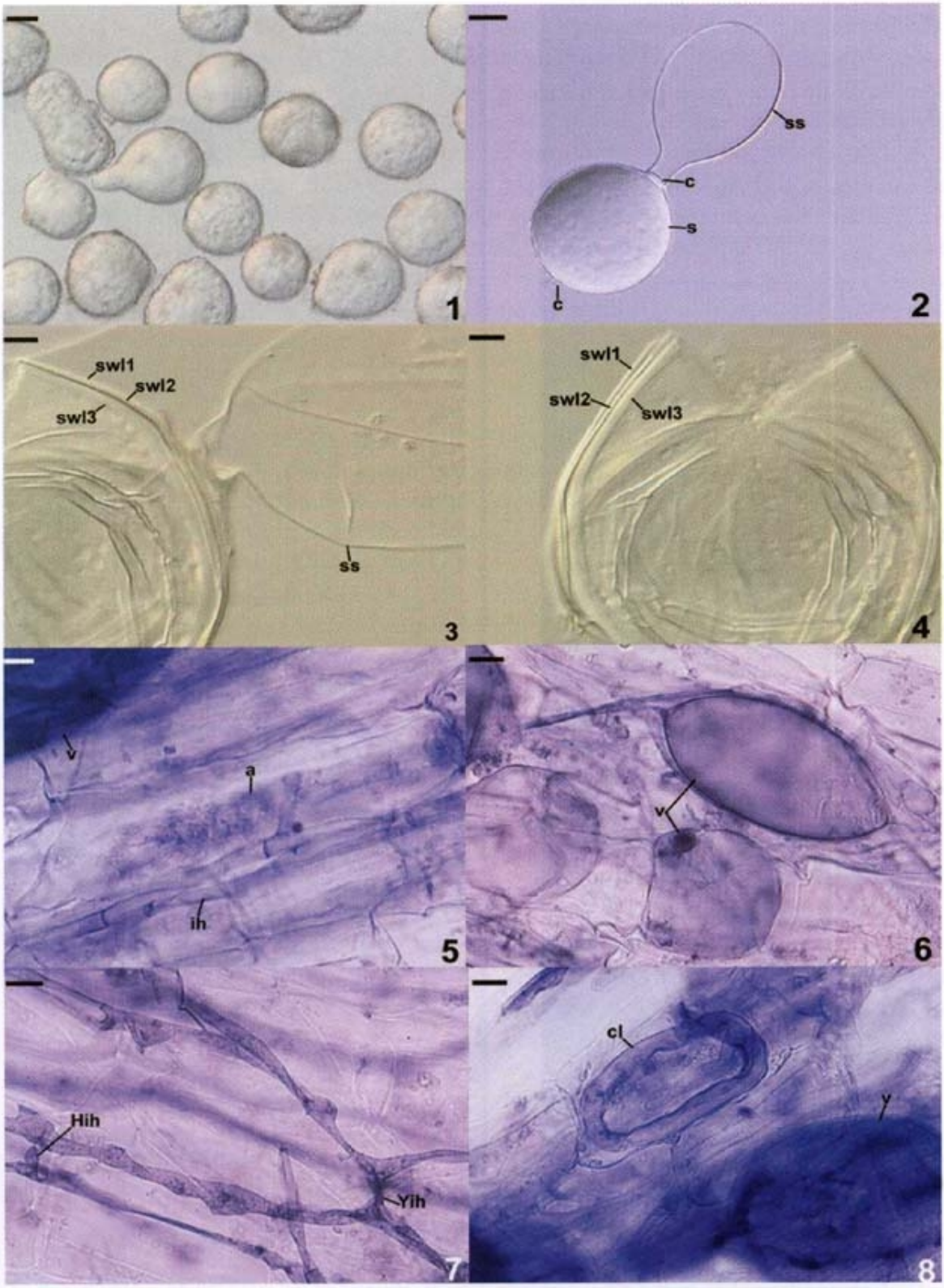
Mycorrhizal associations. In the field, *E. schenckii* was associated with arbuscular mycorrhizal roots of *J. communis* growing in inland sand dunes of the Błędowska Desert, as a trap culture with spores of this fungus indicated. However, the field soil used to establish this culture did not contain spores of *E. schenckii*. Many species of arbuscular fungi do not sporulate in the field at all or their sporulation is seasonal (Gemma and Koske 1988; Stutz and Morton 1996). Additionally, *E. schenckii* produces thin-walled and delicate spores that may have been completely decomposed by soil microorganisms before the collection of the soil sample. Lee and Koske (1994) revealed many soil microorganisms parasitizing spores of arbuscular fungi.

In one-species cultures with *P. lanceolata* as the plant host, *E. schenckii* produced mycorrhizae with arbuscules, vesicles, as well as with intra- and extraradical hyphae (Figs 5-8). Arbuscules were not numerous and highly dispersed along the root fragments examined. Their branches and tips were delicate and difficult to see (Fig. 5). Vesicles (Figs 6 and 8) were numerous, unevenly distributed, ellipsoidal, 25-70 × 50-160 μm, to prolate, 20-25 × 80-130 μm, frequently with depressions located at one of their ends. Intraradical hyphae were (2.5-)4.1(-6.9) μm wide and grew pararell to the root axis (Fig. 5). They were straight or slightly curved, sometimes formed H- or Y-shaped branches (Fig. 7), and coils (Fig. 8), 15.0-30.5 × 25.0-56.3 μm. Extraradical hyphae measured (3.8-)4.2(-4.4) μm wide, and their abundance was rather low. In 0.1% trypan blue, arbuscules stained violet white (16A2) to pastel violet (16A4), vesicles pale violet (16A3) to reddish violet (16C8), intraradical hyphae pastel violet (16A4) to deep violet (17D8), and extraradical hyphae lilac (16B4) to violaceous (16C5).

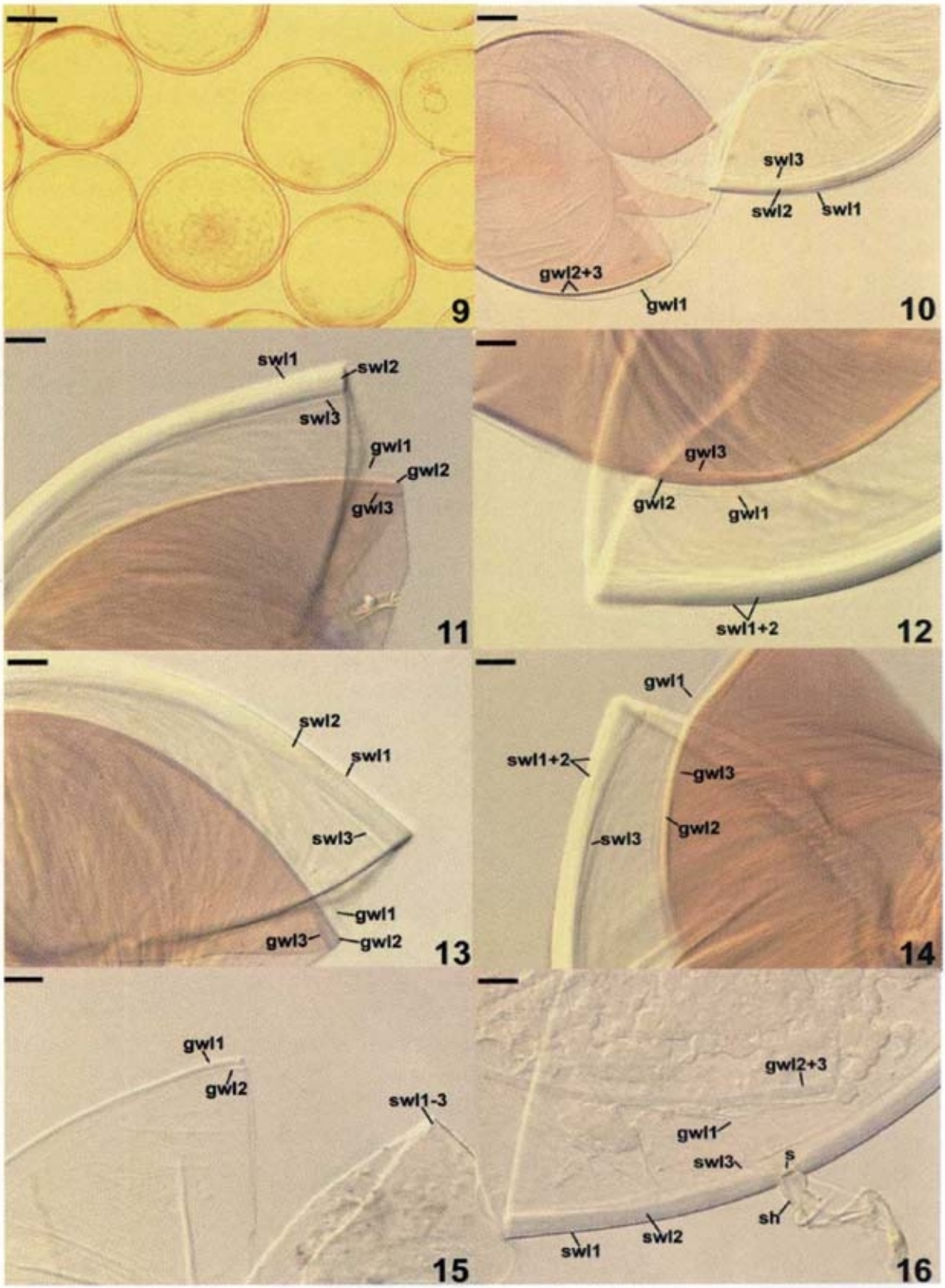
Discussion. When spores of *E. schenckii* lack the sporiferous saccule, they are almost indistinguishable from those of *Archaeospora trappei* (Ames et Linderman) Morton et Redecker. Spores of the two fungi are colourless and overlap in size range (Błaszkowski 2003). Moreover, the spore wall structure of the two species is identical in both the number of layers and their phenotypic properties. The important indicators suggesting the affinity to *E. schenckii* are the pear-shaped spores with the swellings formed in the region of their contact with the neck of the sporiferous saccule (Fig. 1).

Pacispora franciscana Sieverd. et Oehl

Spores borne singly in the soil (Fig. 9); origin blastically at the tip of extrardical hyphae of mycorrhizal roots. Spores hyaline to white, glistening; globose to subglobose; (80-)130(-175) μm diam; sometimes ovoid; 80-120 × 90-170 μm; with a single subtending hypha (Fig. 16). Subcellular structure of spores consists of a spore wall and one inner germination wall (Figs 10-16). Spore wall composed of three layers (layers 1-3). Layer 1 permanent, semiflexible, smooth, hyaline, (0.5-)0.9(-1.1) thick, usually tightly adherent to layer 2, occasionally slightly separating from layer 2 (Fig. 10), especially in forcibly crushed spores. Layer 2 laminate, smooth, hyaline to



Figs 1-8. Morphology of intact spores, subcellular structure of crushed spores, and mycorrhizae of *Entrophospora schenckii* in *Plantago lanceolata* roots stained in 0.1% trypan blue. Fig. 1. Intact spores. Fig. 2. Spore (s) within the neck of sporiferous saccule (ss); two cicatrix (c) are visible. Fig. 3. Spore wall layers 1-3 (swl1-3) and sporiferous saccule (ss). Fig. 4. Spore wall layers 1-3 (swl1-3). Fig. 5. Arbuscule (a), vesicle (v), and intraradical hyphae (ih). Fig. 6. Vesicles (v). Fig. 7. H-(Hih) and Y-(Yih)branched intraradical hyphae. Fig. 8. Coil and vesicle. Figs 1, 5-8, bright field microscopy; Figs 2-4, differential interference contrast. Figs 1, 2, in lactic acid; Figs 3, 4, in PVLG+Melzer's reagent; Figs. 5-8, in PVLG. Bars: Figs 1, 2=20 μm; Figs 3-8=10 μm.



Figs 9-16. Morphology of intact spores and subcellular structure of crushed spores of *Pacispora francisana*. Fig. 9. Intact spores. Figs 10-15. Spore wall layers 1-3 (swl1-3) and germination wall layers 1-3 (gwl1-3); germination wall layers 2 and 3 are stained in spores crushed in PVLG+Melzer's reagent. Fig. 16. Spore wall layers 1-3 (swl1-3), germination wall layers 1-3 (gwl1-3), and subtending hypha (sh) closed by septum (s). Fig. 9, bright field microscopy; Figs 10-16, differential interference contrast. Fig. 9, in lactic acid; Figs 10-14, in PVLG+Melzer's reagent; Figs 15, 16, in PVLG. Bars: Fig. 9=50 μm; Fig. 10=20 μm; Figs 11-16=10 μm.

white (A1), (2.0-)3.8(-7.5) μm thick. Layer 3 flexible to semiflexible, hyaline to white (A1), 0.5-0.8 μm thick, usually tightly adherent to layer 2. *Germination wall* comprises three hyaline layers (gwl1-3). Layer 1 flexible, hyaline, (0.3-)0.5(-0.9) μm thick, easily separating from layer 2. Layer 2 flexible, coriaceous, hyaline, (0.9-)1.9(-3.1) μm thick. Layer 3 flexible, hyaline, less than 0.5 μm thick, usually tightly adherent to layer 2, difficult to see. Germination wall layers 2 and 3 stain dull red (9B4) to deep red (11B8) in Melzer's reagent (Figs 10-14). *Subtending hypha* (Fig. 16) hyaline; straight or slightly curved; cylindrical, sometimes constricted at the spore base; (6.2-)8.5(-12.5) μm wide at the spore base. *Wall of subtending hypha* hyaline; (1.0-)1.5(-1.8) μm thick at the spore base, gradually thinning up to 0.5 μm thick distally; composed of two layers continuous with spore wall layers 1 and 2. *Pore* open or closed by a transverse septum positioned at the level of spore wall layer 2 (Fig. 16). *Germination* not observed.

Collections examined. POLAND. THE WESTERN POMERANIA PROVINCE, Nowogard, from under *Lupinus luteus* L., 5 Aug. 1985, Błaszkowski J., 1045-1048 (DPP); Kamień Pomorski, from the root zone of *Pisum sativum* subsp. *arvense* (L.) Asch. et Graebn., 25 July 1985, B. J., unnumbered collection (DPP); Brzozowo, from among roots of *Triticum aestivum* L., 25 July 1985, B. J., unnumbered collection (DPP); Przybiernów, from under *Secale cereale* L., 25 July 1985, B. J., unnumbered collection; Przelewice, from the rhizosphere of *Thuja occidentalis* L., 1 Oct. 1986, B. J., unnumbered collection (DPP); Dziadowo, from among roots of *Malus domestica* Borkh., 15 Sept. 1986, B. J., unnumbered collection (DPP); Lipnik, from under *T. aestivum*, 10 Aug. 1986, B. J., 2489-2506 (DPP); Lipnik, from among roots of *Hordeum vulgare* L., 3 June 1988, B. J., 2507-2512 (DPP); Lipnik, from under *T. aestivum*, 6 July 2003, B. J., 2513-2520 (DPP); THE LUBLIN PROVINCE, Zwierzyniec, from the rhizosphere of *Festuca rubra* L. s.s., 18 Sept. 1985, B. J., 2521-2527 (DPP); Chełm, from among roots of *Zea mays* L., 19 Sept. 1986, B. J., unnumbered collection (DPP). TURKEY. Near Karabucak-Tuzla (36°43'N, 34°59'E), from under *Ammophila arenaria* (L.) Link, 7 June 2001, B. J., unnumbered collection (DPP).

Distribution and habitat. *Pacispora franciscana* probably has a worldwide distribution. This fungus has originally been described from spores isolated from a grassland with olive trees growing in Umbria, Italy (Oehl and Sieverding 2004). The same mycologists also encountered this fungus in the High Alpines of Eastern Switzerland.

Pacispora franciscana has probably earlier been recorded as the "white reticulate spore" by Mosse and Bowen (1968) in Australia and as the "white smooth-walled azygospore" in Libyan soils and in the Negev Desert, Israel, by El Giahmi, Nicolson and Daft (1976) and Dodd and Krikun (1984), respectively.

In Poland, *P. franciscana* has for the first time been found associated with roots of *L. luteus* cultivated in Lipnik (north-western Poland; 51°44'N, 15°41'E) in 1985. Later, spores of this fungus have been isolated from 12 other rhizosphere soil samples coming from under eight cultivated and uncultivated plant species growing in different regions of Poland (Błaszkowski, pers. observ.). Additionally, this fungus occurred among roots of *A. arenaria* colonizing sandy dunes of the Mediteranean Sea located near Karabucak-Tuzla, Turkey.

The spore density of *P. franciscana* in the soil samples examined by the authors of this paper averaged 23.2 and ranged from 1 to 95 in 100 g dry soil. The participation

of spores of this fungus in the spore populations of all the arbuscular fungi isolated averaged 20.8% and ranged from 0.2 to 82.9%. The arbuscular fungi accompanying *P. franciscana* in the soils examined were *Acaulospora lacunosa* J. B. Morton, *G. aggregatum* N. C. Schenck et S. M. Sm. emend. Koske, *G. caledonium* (Nicol. et Gerd.) Trappe et Gerd., *G. constrictum* Trappe, *G. deserticola* Trappe et al., *G. etunicatum* W. N. Becker et Gerd., *G. fasciculatum* (Thaxt.) Gerd. et Trappe emend. C. Walker et Koske, *G. geosporum* (Nicol. et Gerd.) C. Walker, *G. macrocarpum* Tul. et C. Tul., *G. mosseae*, *G. przelewicensis* Błaszk., *Pacispora scintillans* (S.L. Rose & Trappe) Oehl & Sieverd., *Paraglomus occultum* (C. Walker) J. B. Morton et D. Redecker, *Scutellospora dipurpurescens* J. B. Morton et Koske, and *S. pellucida* (Nicol. et N. C. Schenck) C. Walker et F. E. Sanders.

Mycorrhizal associations. In Poland, *P. franciscana* was associated in the field with arbuscular mycorrhizae of *F. rubra*, *L. luteus*, *M. domestica*, *P. sativum* subsp. *arvense*, *S. cereale*, *T. occidentalis*, *T. aestivum*, and *Z. mays* growing in Brzozowo, Dziadowo, Kamień Pomorski, Lipnik, Nowogard, Przelewice, Przybiernów (the Western Pomerania province), Chełm, and Zwierzyniec (the Lublin province). In Turkey, this fungus occurred among arbuscular mycorrhizal roots of *A. arenaria*.

About 50 attempts to establish one-species cultures from both a single spore and many (ca. 20-50) spores of *E. schenckii* failed.

Discussion. When observed under a dissecting microscope, spores of *P. franciscana* most resemble those of *G. albidum* C. Walker et L. H. Rhodes, *G. diaphanum* J. B. Morton et C. Walker, *G. eburneum* L. J. Kenn. et al., and *G. viscosum* Nicol. All are hyaline to white, as well as more or less overlap in size and shape (Kennedy, Stutz and Morton 1999; Morton 1985; Morton and Redecker 2001; Walker et al. 1995; Walker and Rhodes 1981). Another fungus producing spores reminiscent of those of *P. franciscana* at low microscope magnifications is *Pac. scintillans*, especially its isolates with indistinctly ornamented spores (Błaszkowski 2003; Walker et al. 2004).

The method readily separating the fungal species listed above is examination of their subcellular spore structure under a compound microscope. Only *Pac. scintillans* and *P. franciscana* have spores with two walls: an outer spore wall and an inner germination wall (Figs 10-16). However, the outermost layer of the spore wall of *Pac. scintillans* is ornamented with warts, blunt spines or ridges and, thereby, dull (Walker et al. 2004), whereas that of *P. franciscana* is smooth and glistening. The diversity of all the other species compared here hides only in the spore wall.

Pacispora franciscana has originally been accommodated in the family Glomeraceae Piroz. & Dalpé (Oehl and Sieverding 2004). Recently, this fungus has been transferred to the newly erected family Pacisporaceae C. Walker et al., whose molecular properties showed an ancestry with the family Gigasporaceae J. B. Morton et Benny (Walker et al. 2004). Additionally, the frequent co-occurrence of *P. franciscana* and *P. scintillans* (Błaszkowski, pers. observ.) suggests these fungi to represent one dimorphic species. Molecular analyses of spores of *P. franciscana* are needed to confirm the supposition.

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Entrophospora schenckii i *Pacispora franciscana*, arbuskularne grzyby mikoryzowe (Glomeromycota) nowe odpowiednio dla Europy i Polski

Streszczenie

Opisano i zilustrowano cechy morfologiczne zarodników *Pacispora franciscana*, jak również zarodników i mikoryz *Entrophospora schenckii*, grzybów arbuskularnych z gromady Glomeromycota znalezionych po raz pierwszy odpowiednio w Polsce i Europie. Ponadto przedstawiono poznane rozmieszczenie obu tych gatunków.