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Glomus rubiforme, an arbuscular mycorrhizal fungus new to the mycota of Poland

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Glomus rubiforme is described and illustrated, as well as its occurrence in Poland and in the world is presented. *Glomus rubiforme* forms pale yellow to light brown spores arranged in blackberry-like sporocarps. The spores develop from a centrally positioned, inflated, thick-walled cell. The spore wall consists of two layers: a sloughing, hyaline outer layer adherent to a coloured, laminated layer. *Glomus rubiforme* is a new arbuscular mycorrhizal fungus to the mycota of Poland.

Key words: hypogeous fungi, *Glomus rubiforme*, arbuscular mycorrhiza.

INTRODUCTION

Glomus rubiforme (Gerd. et Trappe) Almeida et Schenck has originally been described as *Sclerocystis rubiformis* Gerd. et Trappe (Gerdemann and Trappe 1974). Almeida and Schenck (1990) transferred all but one, *S. coremioides* Berk. et Broom, *Sclerocystis* species to the genus *Glomus* based on spore ontogeny. Spores of *S. coremioides* are formed individually on a subtending hypha that does not branch to form a new sporophore near the spore base, as was found in the other *Sclerocystis* species and some sporocarpic species of the genus *Glomus*. Although Wu (1993) did not accept Almeida and Schenck's (1990) proposition, the current opinion of most taxonomists dealing with this group of fungi agree with the latter authors.

Reports of the occurrence of *G. rubiforme* are relatively not numerous (Bhattacharjee et al. 1980; Dalpé 1989; Dalpé et al. 1986; Gerdemann and Trappe 1974; Grandi and Trufem 1991;

Grandi et al. 1987; Hall 1977; Hamel et al. 1994; Johnson 1977; Miller et al. 1985; Mosse and Bowen 1968; Musoko et al. 1994; Nicolson and Schenck 1979; Ragupathy and Mahadevan 1993; Sieverding 1989; Wu 1993; Wu and Chen 1986) and this fungus has not so far been noted in Poland.

The aim of this paper is to describe and illustrate the specimens of *G. rubiforme* found in Poland. Additionally, this paper presents the distribution of this fungus in Poland and in the world.

MATERIALS AND METHODS

Collection of soil and root samples, as well as establishment of trap and single-species pot cultures were made as previously described (Błaszkowski 1997). The host plants used were *Plantago lanceolata* L., *Sorghum sudanense* (Staph.) Piper, and *S. vulgare* Pers. Plants were grown in a greenhouse at 18–30°C with supplemental 16-h lighting provided by one SON-T AGRO sodic lamp (Philips Lighting Poland S. A.) placed 1m above pots. The maximum light intensity was 180 μE $m^{-2}s^{-1}$ at pot level. Plants were watered 2–3 times a week. Trap cultures were harvested at ca 1-month intervals, beginning 5 months and ending 12 months after plant emergence, spores extracted by wet sieving and decanting (Gerdemann and Nicolson 1963), roots stained in 0.05% trypan blue (Phillips and Hayman 1970), and examined for the presence of mycorrhizae.

Because single-species cultures failed, morphological investigations of *G. rubiforme* were conducted based on field-collected specimens and those coming from trap cultures. At least 100 sporocarps mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier 1983) and 20 sporocarps mounted in PVLG mixed with Melzer's reagent (1:1, v/v) were examined. Wall characteristics of spores and terminology are those suggested by Franke and Morton (1994), Spain et al. (1989) 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). Specimens have been mounted on slides in PVLG and deposited in the Department of Plant Pathology (DPP), Academy of Agriculture, Szczecin, Poland. Nomenclature of other fungi follows Walker and Trappe (1993). Nomenclature of plants with which *G. rubiforme* was associated in Poland is according to Mirek et al. (1995). The classification is that of Morton and Benny (1990).

Explanation of the abbreviation used: Bł. – J. Błaszkowski.

DESCRIPTION AND DISCUSSION

Glomus rubiforme (Gerd. et Trappe) Almeida et Schenck

Spores occurring in sporocarps in the soil. *Sporocarps* pale yellow (3A3) to light brown (6D8), globose, 200–260 μm diam. or ovoid, 100–200 × 190–360 μm, without a peridium; with 3 to 18 spores (Fig. 1). *Spores* pale yellow (3A3) to light brown (6D8); globose to subglobose; (40.0–) 52.0 (–70.0) μm diam. or ovoid to prolate; 35–45 × 50–70 μm; with a single subtending hypha; developed from a thick-walled, inflated hypha; spores arranged in a hemispherical layer in young sporocarps or radially to form a blackberry-like sporocarp when mature (Fig. 1, 2, 3 and 4). *Spore wall structure* composed of one wall comprising two layers (layers 1 and 2, Fig. 3 and 4). Layer 1, forming the spore surface, sloughing, smooth, hyaline, (0.3–) 0.5 (–0.8) μm thick before disintegration, closely adherent to layer 2. Layer 2 laminated (Fig. 2, 3 and 4), pale yellow (3A3) to light brown (6D8), (2.7–) 3.1 (–3.7) μm thick. Subtending hypha pale yellow (3A3) to light brown (6D8), straight to recurvate; funnel-shaped, sometimes cylindrical or constricted; (8.8–) 10.7 (–14.2) μm wide at the spore base (Fig. 1, 2, 3 and 4). *Wall of subtending hypha* pale yellow (3A3) to light brown (6D8), (2.9–) 4.8 (–6.6) μm thick, continuous with spore wall layers 1 and 2 (Fig. 3). *Pore* (1.5–) 2.2 (–2.9) μm wide, occluded by a septum, ca 2.0 μm wide, continuous with the innermost lamina of wall layer 2, and occasionally by thickening of spore wall layer 2. Spore contents of oil droplets.

Polish collection examined. Pobrzeże Kaszubskie–Żelistrzewo, under *Pinus sylvestris* + an unrecognized grass, 11.1985, Bł. 1029 (DPP); Jastrzębia Góra, under *P. sylvestris* + an unrecognized, 11.1985, Bł. 1030–1032; Jastrzębia Góra, under *Lolium multiflorum*, 11.1986, Bł. Mierzeja Helska – Hel, under *Chamaecyparis lawsoniana*, 7.1989, Bł. Garb Tarnogórski – Pustynia Błędowska, under *Potentilla* sp., 7.1995, Bł. 1033–1036; Pustynia Błędowska, under *Verbascum phlomoides*, 7.1995, Bł.; Bolesław, under vascular plant, 9.1989, Bł. Wybrzeże Słowińskie – Słowiński National Park, under *Ammophila arenaria*, 6.1994, Bł. Równina Pyrzycka – Lipnik, under *Triticum aestivum*, 7.1992, Bł.; Przelewice, under *Agropyron repens* + *Picea abies*, 5.1985, Bł. Uznam and Wolin – Świnoujście, under *Petasites spurius*, 10.1992, Bł. Równina Wkrzańska – Police, under *Calamagrostis arundinacea*, 7.1992, Bł.

Distribution and habitat. Of the over 1300 soil samples so far collected in ca 160 localities of Poland, *G. rubiforme* was found in 13 soils coming from 7 physiographic regions (Fig. 5). The soils represented both cultivated and uncultivated sites, the letter including forests, heaps, maritime and inland dunes. The spore abundance of *G. rubiforme* in 100g dry soil ranged from 1 to 160 (av. 57.9). The proportion of spores of this fungus in spore

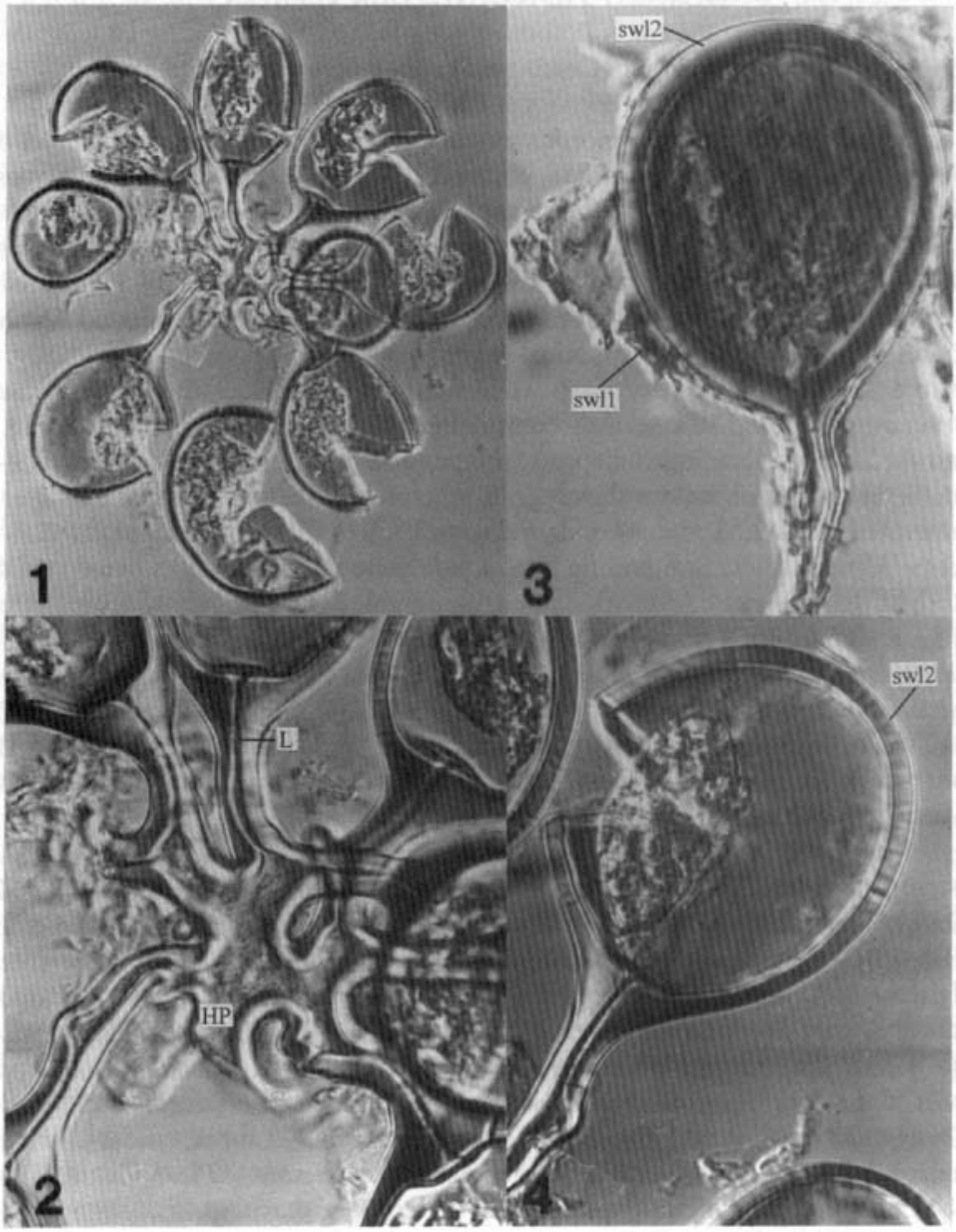

Fig. 1. Sporocarp with radially arranged spores, differential interference contrast (DIC), ×423. Fig. 2. Thick-walled hyphal plexus (HP). Highly thickened walls and narrow lumens (L) of subtending hyphae are seen, DIC, ×2083. Fig. 3. Spore wall layers 1 and 2 (swl 1, swl 2), DIC, ×1154. Fig. 4. Mature spore with laminated spore wall layer 2 (swl2) only, DIC, ×1154

Fig. 5. Distribution of *Glomus rubiforme* in Poland

populations of all arbuscular mycorrhizal fungi recovered ranged from 1.56% to 86.41% (av. 37.51%). The species riches of these fungi in soils in which *G. rubiforme* occurred ranged from 1 to 9 (av. 3.53). The plant species harbouring *G. rubiforme* were *A. arenaria*, *A. repens* growing near *P. sylvestris*, *C. arundinacea*, *Ch. lawsoniana*, *L. multiflorum*, *Potentilla* sp., *V. phlomoides*, *T. aestivum*, and unrecognized grasses. The fungi accompanying *G. rubiforme* were *Acaulospora lacunosa* Morton, *A. mellea* Spain et Schenck, *A. paulinae* Błaszk., *Complexipes moniliformis* Walker emend. Yang et Korf, *Endogone flammicorona* Trappe et Gerd., an undescribed *Entrophospora* sp., *Gigaspora gigantea* (Nicol. et Gerd.) Gerd. et Trappe, *G. aggregatum* Schenck et Smith emend. Koske, *G. caledonium* (Nicol. et Gerd.) Trappe et Gerd., *G. constrictum* Trappe, *G. corymbiforme* Błaszk., *G. deserticola* Trappe et al., *G. etunicatum* Becker et Gerd., *G. fasciculatum* (Thaxter) Gerd. et Trappe emend. Walker et Koske, G. ? *geosporum* (Nicol. et Gerd.) Walker, *G. laccatum* Błaszk., *G. macrocarpum* Tul. et Tul., *G. microcarpum* Tul. et Tul., *G. mosseae* (Nicol. et Gerd.) Gerd. et Trappe, unrecognized *Glomus* spp., *Scutellospora armeniaca* Błaszk. and *S. dipurpurescens* Morton et Koske. Except for *C. moniliformis*

known to form ectendo- and ectomycorrhizae with coniferous trees (W i l c o x et al. 1983) and *E. flammicorona* found to be an ectomycorrhizal fungus (T r a p p e and G e r d e m a n n 1972), the other fungi are arbuscular mycorrhizal producers (M o r t o n and B e n n y 1990).

The distinctive features of *G. rubiforme* are its sporocarps with relatively small, coloured spores originated from a centrally positioned hyphal plexus. When young, the sporocarps resemble a hemispherical layer. At times, they convert into blackberry-like structures due to the formation of subsequent spores radially developing. Single sporocarps sometimes are connected with hyphae in larger aggregates. The sporocarps are never enveloped in a peridium. The hyphal plexus of sporocarps consists of an inflated, thick-walled cell. Hyphal branches erect from this cell and swell at their tip, forming, thereby, spores of this species. At first, these branches are thin-walled. At times, they become thicker and more rigid due to the synthesis of further material in their wall.

The single wall of *G. rubiforme* spores is composed of two layers: a thin, sloughing, hyaline outer layer and a thicker, coloured, laminated inner layer. The outer spore wall layer is rarely present, especially in field-collected spores. None of these layers stains in Melzer's reagent.

The subtending hypha of *G. rubiforme* usually is funnel-shaped, although cylindric or constricted subtending hyphae were also found in specimens examined by the author of this paper. The subtending hyphal wall highly thickens with spore age due to the addition of material, mainly towards its lumen. This causes mature spores to usually have a subtending hypha with a narrow lumen connecting the spore inside with the hypha. The lumen in most mature spores is occluded by 1–3 thick septa. Young sporocarps usually contain spores with subtending hyphae without septa.

Species of arbuscular fungi most similar in appearance to *G. rubiforme* are *G. ambisporum* Smith et Schenck, *G. heterosporum* Smith et Schenck, and *G. taiwanense* (Wu et Chen) Almeida et Schenck. All the fungi produce spores in globular sporocarps. However, spores of *G. ambisporum* compared with those of *G. rubiforme* are larger [85–157 μm diam. vs. 27–125 × 29–87 (–110) μm (A l m e i d a and S c h e n c k 1990); 27.5–60.0 × 37.5–87.5 μm (W u 1993); (40.0–) 52.0 (–70.0) μm (Błaszkowski pers. observ.)], have a thicker wall [6–18 μm vs. 3.0–7.6, up to 13.5 μm at spore base (A l m e i d a and S c h e n c k 1990); 1.5–6.0 (–8.5 μm (W u 1993); (2.7–) 3.1 (–3.7) μm (Błaszkowski pers. observ.)] with a reticulate outermost layer (vs. the smooth layer in *G. rubiforme*). *Glomus heterosporum* also forms larger spores (99–206 × 61–201 μm) than *G. rubiforme*; they frequently possess many subtending hyphae (a single subtending hypha in *G. rubiforme*). Additionally, both *G. ambisporum* and *G. heterosporum* are dimorphic fungi, whereas *G. rubiforme* has one type of spores.

Although spores of *G. rubiforme* are in the same size range as those of *G. taiwanense*, the plexal hypha of the former fungus is a broad, thick-walled cell around which spores are produced, and that of the latter species is formed by fusion of more than one monohyphal stalk (Wu 1993).

Glomus rubiforme probably is a widely distributed fungus in the world. Its occurrence has been reported in Florida, Michigan, New York, Oregon and Washington of the USA (Gerdemann and Trappe 1974; Miller et al. 1985; Nicolson and Schenck 1979), in Canada (Dalpé 1989; Dalpé et al. 1986; Hamel et al. 1994), Brazil (Grandi and Trufem 1991; Grandi et al. 1987) and Colombia (Sieverding 1989), England and Wales (Mosse and Bowen 1968), Cameroon (Musoko et al. 1994), India (Bhattacharjee et al. 1980; Ragupathy and Mahadevan 1993), Taiwan (Wu 1993; Wu and Chen 1986) and New Zealand (Hall 1977; Johnson 1977; Mosse and Bowen 1968).

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Glomus rubiforme, arbuskularny grzyb mikoryzowy nowy dla Polski

Streszczenie

Opisano i zilustrowano oraz przedstawiono występowanie w Polsce i świecie *Glomus rubiforme*, arbuskularnego grzyba mikoryzowego. *Glomus rubiforme* tworzy jasnożółte lub jasnobrązowe zarodniki skupione w sporokarpach przypominających owoce jeżyny. Zarodniki te tworzą się z grubościennej, rozdętej komórki umiejscowionej w środku sporokarpu. Ściana zarodników składa się z dwóch warstw: złuszczającej się, hialinowej warstwy zewnętrznej przylegającej do zabarwionej warstwy zlaminowanej. *Glomus rubiforme* jest grzybem nowym dla mycota Polski.