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GLOMUS MAJEWSKII, A NEW SPECIES OF ARBUSCULAR MYCORRHIZAL FUNGI (GLOMEROMYCOTA)*

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Abstract. A new arbuscular mycorrhizal fungal species (Glomeromycota) of the genus *Glomus*, *G. majewskii* is described and illustrated. Spores of *G. majewskii* form singly in the soil, are pale yellow (3A3) to light yellow (3A5), globose to subglobose, (52–)60(–73) µm diam., sometimes ovoid to irregular, 49–62 × 58–84 µm. Their spore wall consists of a permanent, smooth, hyaline, (1.0–)1.4(–2.3) µm thick outermost layer (layer 1); a laminate, smooth, pale yellow (3A3) to light yellow (3A5), (1.8–)4.1(–6.9) µm thick middle layer (layer 2); and a flexible, hyaline, (0.6–)0.8(–1.0) µm thick innermost layer (layer 3). Layers 2 and 3 stain intensively in Melzer's reagent. In the field, *G. majewskii* was associated with roots of *Ammophila arenaria* colonizing maritime dunes of Bornholm (Denmark). Many attempts to grow the species in single-species culture failed.

Key words: arbuscular mycorrhizal fungi, Glomeromycota, new species

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota are among the world's most commonly occurring soil fungi, coexisting in symbiotic associations with *ca* 70–90% of land plants (Wang & Qiu 2006; Smith & Read 2008; Brundrett 2009).

The soils especially favoring the development of AMF are maritime sand dunes (Koske 1987; Dalpé 1989; Tadych & Błaszkowski 2000) because of their low nutrient and organic matter content (Nicolson & Johnston 1979; Koske 1988), as well as the absence of parasites of AMF (Koske *et al.* 2004). A plant species commonly colonizing European maritime sand dunes and harboring abundant and diverse populations of AMF is *Ammophila arenaria* (L.) Link (Tadych & Błaszkowski 2000; Błaszkowski 1993a, b).

Of the *ca* 220 described species of the Glomeromycota, at least 37 have originally been found in maritime dunes and many others have been associated with roots of dune plants (Sridhar & Beena 2001; Błaszkowski *et al.* 2010; www.agro.ar.szczecin.pl/~jblaszkowski/). However, the literature data suggest that the vast majority of AMF remain undescribed and that most of the undescribed species belong to the genus *Glomus* (Helgason *et al.* 2002; Fitter 2005; Hijri *et al.* 2006; Kovács *et al.* 2007; Öpik *et al.* 2009). These species remain unknown for various reasons: AMF are not sampled or rarely sampled in most regions; there are few specialized and experienced mycologists dealing with morphology of members of the Glomeromycota; and sporulation of many AMF in field conditions is seasonal, rare or absent (Gemma *et al.* 1989; Stürmer & Bellei 1994; Stutz & Morton 1996).

An effective way to force production of spores of AMF hidden inside the roots of their host plants is to cultivate field-collected mixtures of rhizosphere soil and root fragments of these plants in successive (Stutz & Morton 1996) or long-term (Oehl *et al.* 2004) pot trap cultures. However, species producing spores in multi-species trap cultures frequently do not sporulate in single-species culture (J. Błaszkowski, pers. observ.). The factor most limiting establishment of an AMF in single-species culture seems to be the

* This paper is dedicated to Professor Tomasz Majewski on the occasion of his 70th birthday.

lack of microorganisms of the soil from which the fungus originated. Soil microorganisms have been demonstrated to hasten mycorrhizal fungal spore germination (Azcon-Aguilar *et al.* 1986) and root colonization (Azcon-Aguilar & Berea 1985).

Examination of trap cultures with rhizosphere soils and roots of *A. arenaria* growing in maritime sand dunes of Bornholm (Denmark) revealed spores of an undescribed species of Glomeromycota forming glomoid spores. This fungus is described here as *G. majewskii* sp. nov.

Materials and methods

Establishment and growth of trap cultures and extraction of spores. Spores examined in this study were derived only from pot trap cultures. Many attempts to grow the fungus in single-species cultures failed. Trap cultures were established to obtain a large number of living spores and to initiate sporulation of species that were present but were not detected in the field collections (Stutz & Morton 1996). The method used to establish trap cultures, their growth conditions and the spore extraction methods were as described previously (Błaszkowski *et al.* 2006). The host plant of the cultures was *Plantago lanceolata* L.

Microscopy survey. Morphological properties of spores and their wall structure were determined based on examination of at least 100 spores mounted in water, lactic acid, polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar *et al.* 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 of oil droplets. They were then examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded with a Sony 3CDD color video camera coupled to the microscope.

Spore structure terminology follows Stürmer and Morton (1997) and Walker (1983). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names follow Kornerup and Wanscher (1983). Nomenclature of fungi and plants follows Walker and Trappe (1993) and Mirek *et al.* (1995) respectively. The authors of the fungal names are those presented on the Index Fungorum website http://www.indexfungorum.org/AuthorsOfFungalNames.htm. Voucher specimens were mounted in PVLG and a mixture of PVLG and Melzer's reagent (1:1, v/v) on slides and deposited in the Department of Plant Protection (DPP), West Pomeranian University of Technology, Szczecin, Poland. Color microphotographs of spores of the new species can be viewed at http://www.agro.ar.szczecin.pl/~jblaszkowski/.

Taxonomy

Glomus majewskii Błaszkowski, *sp. nov.*

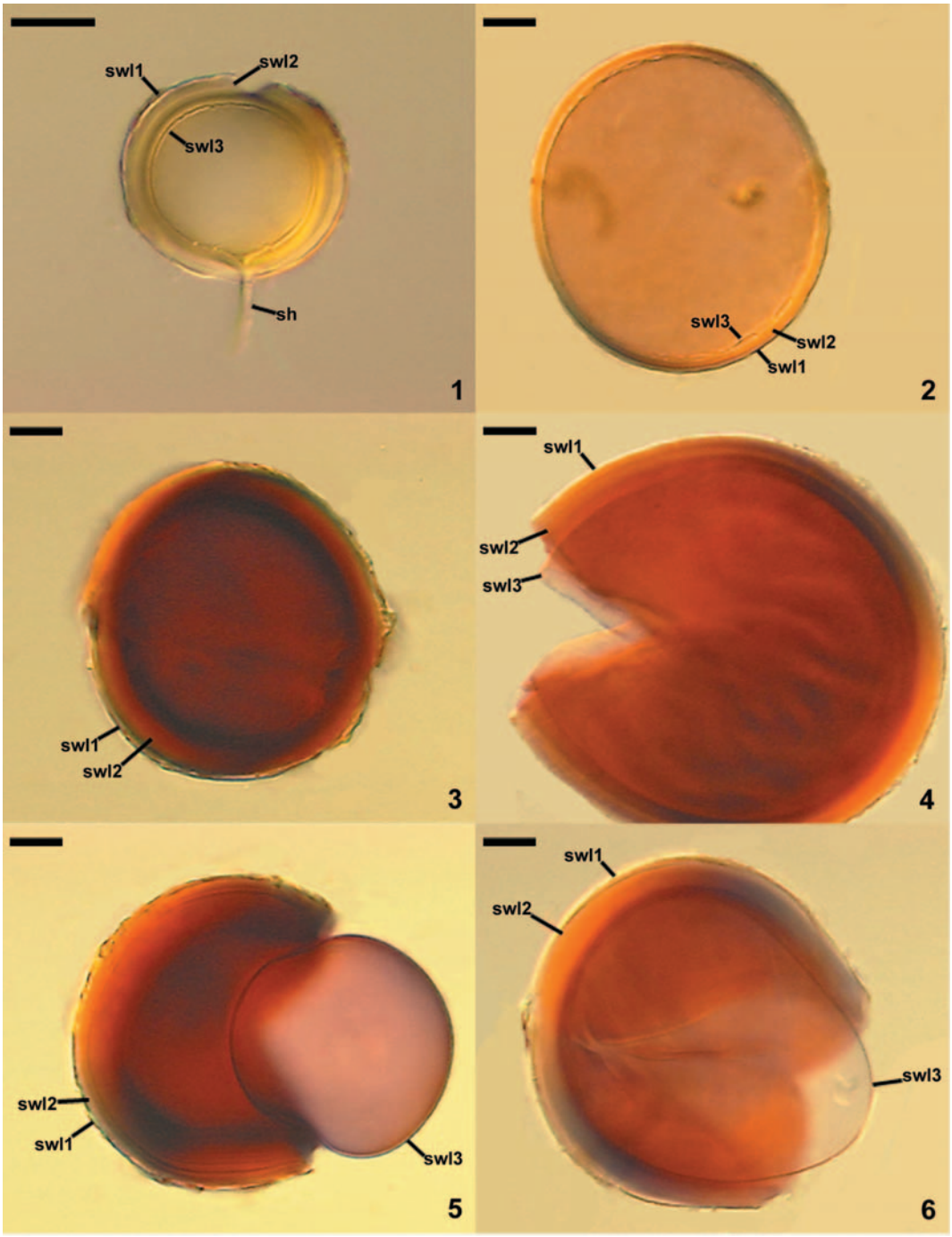
Figs 1–6

MycoBank No. MB 518762

Sporocarpia ignota. Sporae singulatim in solo efformatae. Sporae pallide luteae; *globosae vel subglobosae*; *(52–)60(–73) µm diam.*; *raro ovoideae, oblongae vel irregulares*; *49–62 × 58–84 µm. Tunica sporae stratis tribus (strata 1–3)*; *stratum '1' diuturnum*, *glabrum*, *hyalinum*, *(1.0–)1.4(–2.3) µm crassum*; *stratum '2' laminatum*, *glabrum*, *pallide luteum*, *(1.8–)4.1(–6.9) µm crassum*; *stratum '3' hyalinum*, *glabrum*, *elasticum*, *(0.6–)0.8(–1.0) µm crassum. In solutione Melzeri stratum '2' atroviolaceum*, *stratum '3' erythrum. Hypha sporifera pallide lutea*; *recta vel recurvta*; *cylindrica vel infundibuliformis*; *(2.8–)3.0(–3.4) µm lata ad basim sporae*; *pariete pallide luteo*, *0.6–0.9 µm crasso*, *stratis 1–3 in parietem sporae continuantibus. Porus hyphae 0.5–0.9 diam.*

Type: POLONIA: Sedinum (Szczecin), infra *Plantago lanceolata*, 10 March 2008, *J. Błaszkowski 3167* (holotype, DPP).

Sporocarps unknown. Spores formed singly in the soil (Figs 1–6), developing blastically at the tip of sporogenous hyphae continuous with mycorrhizal extraradical hyphae. Spores pale yellow (3A3) to light yellow (3A5), globose to subglobose, (52–)60(–73) µm diam., sometimes ovoid to irregular, 49–62 × 58–84 µm, with one subtending hypha (Figs 1–6). Spore wall consists of three layers (Figs 1–6). Layer 1, forming the spore surface, semi-flexible, permanent, smooth, hyaline, (1.0–)1.4(–2.3) µm thick, sometimes slightly folding in vigorously crushed spores (Figs 1–6). Layer 2 laminate, smooth, pale yellow (3A3) to light yellow (3A5), (1.8–)4.1(–6.9) µm thick (Figs 1–6). Layer 3 flexible, hyaline, (0.6–)0.8(–1.0) µm thick (Figs 1, 2 & 4–6). In Melzer's reagent, only layers 2 and 3 stain light orange (6A4) to brownish



Figs 1–6. *Glomus majewskii* Błaszkowski *sp. nov*. 1 – spore with spore wall layers (swl 1–3) and subtending hypha (sh); 2 – spore wall layers (swl) 1–3 of intact spore; 3 – spore wall layers (swl) 1 and 2; swl 3 not visible (note intensively stained swl 2); 4–6 – spore wall layers (swl) 1–3 (note nonreactive swl 1 and stained swl 2 and swl 3). 1 – spore in PVLG, 2–6 – spores in PVLG + Melzer's reagent. (1–6, differential interference microscopy). Scale bars: 1 = 20 µm; 2–6 = 10 µm.

violet (11E8) and orange white (6A2) to dull red (11B4; Figs 2–6). Subtending hypha pale yellow (3A3) to light yellow (3A5), straight or recurved, cylindrical to funnel-shaped, (2.8–)3.0(–3.4) µm wide at spore base (Fig. 1). Wall of subtending hypha pale yellow (3A3) to light yellow (3A5), 0.6–0.9 µm thick at spore base, continuous with spore wall layers 1 and 2. Pore *ca* 0.5–0.9 µm diam., occluded by a curved septum continuous with spore wall layer 3 and sometimes additionally with some innermost laminae of spore wall layer 2. Germination unknown.

MYCORRHIZAL ASSOCIATIONS. In the field, *G. majewskii* was associated with roots of *Ammophila arenaria*.

In trap cultures, *G. majewskii* was associated with roots of *Plantago lanceolata*. However, many attempts to grow this fungus in single-species culture failed. Therefore the properties of its mycorrhizal structures remain unknown.

PHYLOGENETIC POSITION. Unknown.

ADDITIONAL SPECIMENS EXAMINED. POLAND. Szczecin, 5 June 2010, *J. Błaszkowski 3220* (DPP).

ETYMOLOGY. Latin, *majewskii*, in honor of Professor Tomasz Majewski, Department of Phytopathology, Warsaw University of Life Sciences – SGGW, Warsaw, a long-time student of fungi and distinguished mycologist.

DISTRIBUTION AND HABITAT. Using traditional methods for finding AMF (not molecular), *G. majewskii* was so far found in one trap culture containing a mixture of the rhizosphere soil and root fragments of *A. arenaria* growing in maritime dunes adjacent to Dueoddle (54°59′N, 15°04′E) located on Bornholm Island belonging to Denmark.

Spores of *G. majewskii* were not found in either *ca* 3000 field-collected soils or *ca* 2500 pot trap cultures representing different regions of Africa and Europe as well as Asia and the U.S.A. (J. Błaszkowski, pers. observ.). The AMF co-occurring with *G. majewskii* in the trap culture were *Acaulospora lacunosa* J. B. Morton, *G. aggregatum* N. C. Schenck & G. S. Sm. *emend.* Koske, and *Scutellospora dipurpurescens* J. B. Morton & Koske.

NOTES. *Glomus majewskii* is distinguished by its pale-colored, small spores formed singly in the soil and by having a spore wall consisting of three permanent layers, of which the laminate layer 2 and the flexible innermost layer 3 stain intensively in Melzer's reagent (Figs 1–6).

Of the described species forming glomoid spores of a 3-layered spore wall in which the innermost layer 3 is flexible, morphologically *G. majewskii* is most closely related to *G. fasciculatum* (Thaxt.) Gerd. & Trappe *emend.* C. Walker & Koske. Both species form similarly colored spores, and all components of their spore wall are phenotypically identical (Walker & Koske 1987; Błaszkowski 1990, 2003; Morton 2002). However, while *G. majewskii* produces spores only singly in the soil (Fig. 1), those of *G. fasciculatum* occur singly and in clusters. Additionally, globose spores of the species described here generally are markedly smaller [(52–)60(–73) µm diam.] than those of *G. fasciculatum* [(50–)105(–130) µm diam.].

The most evident differences between the species compared here reside in the biochemical properties of their spore wall components. In *G. majewskii*, spore wall layers 2 and 3 stain in Melzer's reagent (Figs 2–6), and in *G. fasciculatum* the reactive spore wall components in this reagent are layers 1 and 2.

Of the group of fungi characterized above, other species with glomoid spores that may be confused with *G. majewskii* are *G. drummondii* Błaszk. & Renker, *G. lamellosum* Dalpé, Koske & Tews, and *G. walkeri* Błaszk. & Renker.

Spore wall layer 1 of *G. drummondii* is impermanent and usually completely sloughed in mature spores (vs. permanent and always present even in old spores of *G. majewskii*; Figs 1–6), and layer 2 of this wall does not stain in Melzer's reagent (vs. staining intensively in *G. majewskii*; Błaszkowski *et al.* 2006). Additionally, the subtending hypha of *G. majewskii* spores is less uniform in shape (cylindrical to funnel-shaped; Fig. 1) than that of *G. drummondii* spores (cylindrical to slightly flared).

Spores of *G. lamellosum* generally are markedly larger [(60–)100(–140) µm diam. when globose], their spore wall layer 1 is impermanent

(vs. permanent in *G. majewskii*; Figs 1–6) and much thicker [(2.2–)5.3(–10.5) µm thick when intact], and layer 2 of this wall does not react in Melzer's reagent (vs. staining intensively in *G. majewskii*; Figs 2–6; Dalpé *et al.* 1992; Błaszkowski *et al.* 2002; Błaszkowski 2003). Moreover, the subtending hypha of *G. lamellosum* spores is more uniform in shape (cylindrical to flared vs. cylindrical to funnel-shaped in *G. majewskii*; Fig. 1) and much wider [(8.3–)13.0(–15.2) µm wide at the spore base vs. (2.8–)3.0(–3.4) µm wide at the spore base in *G. majewskii*].

Three characters separate *G. majewski* and *G. walkeri*. First, while spore wall layer 1 of the former species is permanent and does not stain in Melzer's reagent (Figs 1–6), that of the latter fungus is a semi-permanent structure and stains intensively in Melzer's reagent (Błaszkowski 2003; Błaszkowski *et al.* 2006). Second, spore wall layer 3 of both species is flexible, but it stains in Melzer's reagent only in *G. majewskii* (Figs 4–6). Third, the subtending hypha of spores of the fungus discussed here is much narrower than that of *G. walkeri* spores [(7.4–)9.1(–12.7) µm wide].

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