GLOMUS ARENARIUM, A NEW SPECIES IN GLOMALES (ZYGOMYCETES)

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ABSTRACT

A new ectocarpic arbuscular mycorrhizal fungal species, *Glomus arenarium* (Glomales, Zygomycetes), was recovered from maritime sand dunes of northern Poland. *Glomus arenarium* forms spores with a narrow and hyaline subtending hypha. Spores are orange to raw umber, globose to subglobose, (55-)97(-120) µm diam or ovoid, 65-105 × 95-140 µm. Their wall consists of three layers: a hyaline outermost layer present only in very young spores, a semiflexible, hyaline middle layer rarely present in mature spores, and a permanent, laminate, orange to raw umber innermost layer. No spore wall layers of *G. arenarium* stain in Melzer's reagent. This fungus formed spores and arbuscular mycorrhizae in single-species pot cultures with *Plantago lanceolata*.

KEY WORDS: Glomales, mycorrhizae, ontogeny, arbuscular fungi, Zygomycetes.

INTRODUCTION

Glomus arenarium sp. nov. was found in maritime sand dunes of northern Poland. This fungus sporulated in the field soils, as well as in trap and single species pot cultures. Spores of this fungus highly resemble those of *G. etunicatum* Becker & Gerd. in size and colour, but have a 3-layered wall structure compared with 2-layered of the latter fungus. *Glomus arenarium* is described here based on morphology and ontogenetic patterns of spore differentiation.

MATERIALS AND METHODS

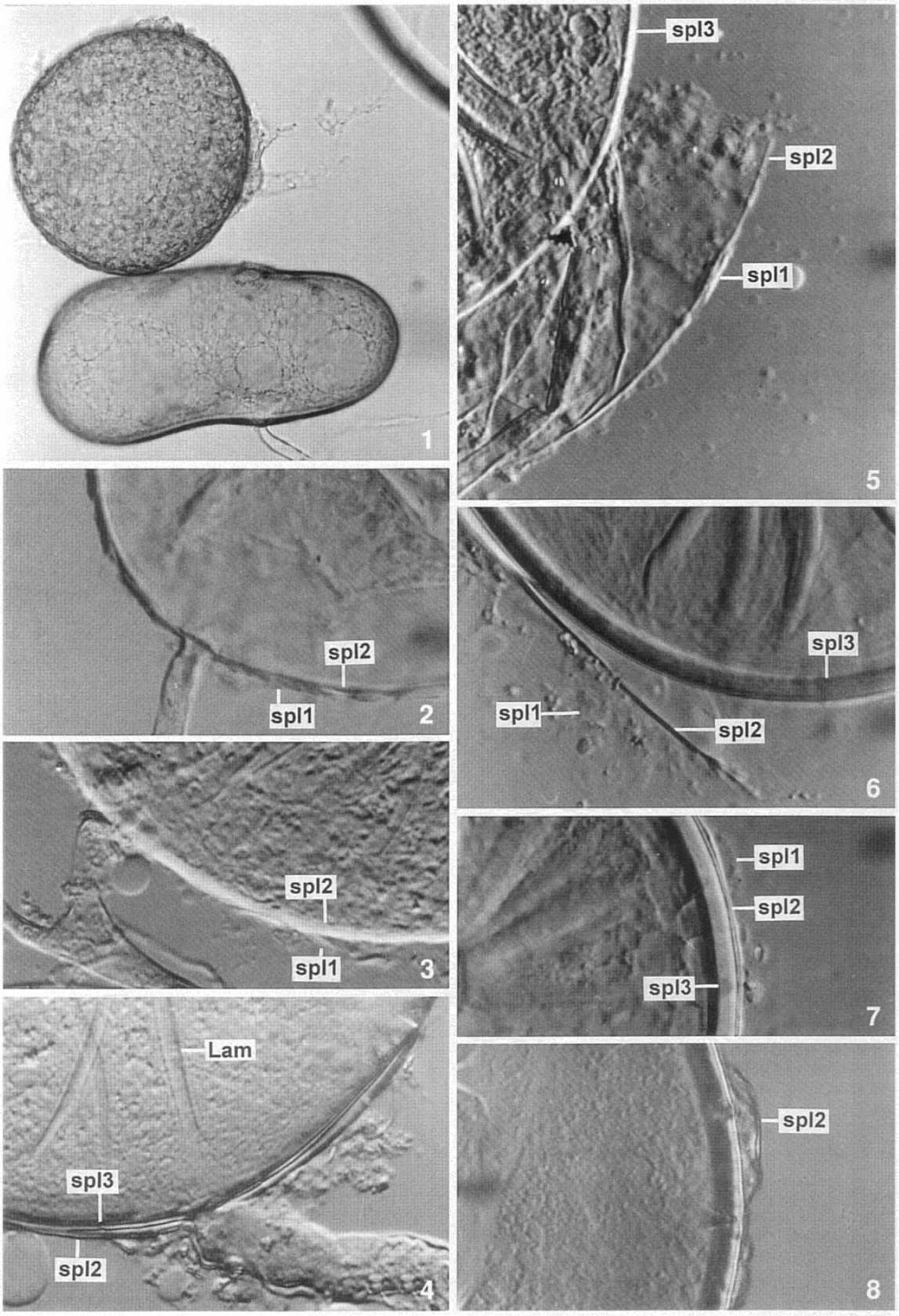
Collection of soil samples, establishment of trap and single-species pot cultures, as well as growth conditions are as those described previously (Błaszkowski and Tadych 1997). The growth medium of single-species pot cultures was an autoclaved sand of maritime dunes adjacent to Świnoujście (pH 6.7; 12 and 26 mg L^{-1} P and K, respectively). The host species used in both trap and single-species cultures was *Plantago lanceolata* L. Trap and single-species pot cultures were harvested at approximately 1-month intervals, beginning 2 months and ending 11 months after plant emergence. Spores were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963). Mycorrhizae were revealed following clearing and staining root fragments according to Phillips and Hayman (1970). Stages of differentiation of the fungus were determined from newly formed spores extracted from both trap and single-species cultures.

Morphological properties of spores and their subcellular structures were determined based on at least 70 and 30 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier 1983) and a mixture of PVLG Melzer's reagent (1:1, v/v), respectively. Terminology of spore structure is that suggested by Franke and Morton (1994), 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. Color names are from Kornerup and Wanscher (1983). Specimens were mounted in PVLG on slides and deposited in the Department of Plant Pathology (DPP), Academy of Agriculture, Szczecin, Poland, and in the herbarium at Oregon State University (OSC) in Corvallis, Oregon, USA.

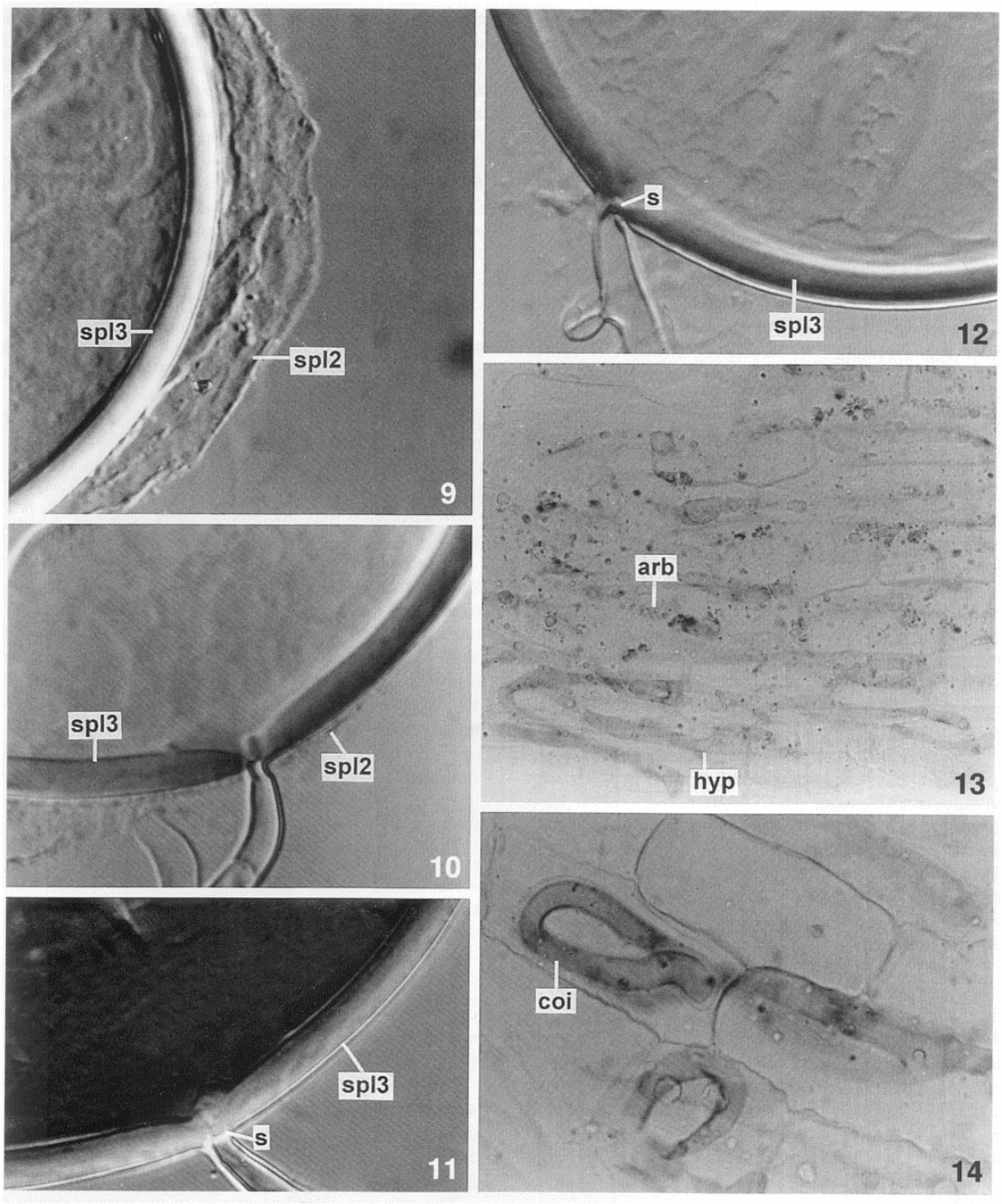
DESCRIPTION OF THE SPECIES

Glomus arenarium J. Błaszkowski, M. Tadych et T. Madej, sp. nov. Figs 1-14.

Sporocarpia ignota. Sporae singulatim in solo vel in radici, e sporophoris rectic efformatae. Sporophorum nonseptatum vel parce septatum; hyalinum vel pallide aurantiacum; (4.5-)5.2 (-12.7) µm latum; pariete (0.5-)0.7(-1.2) µm crasso; rectum. Sporae aurantiacae vel ochraceae; globosae vel subglobosae; (55-)97(-120) µm diam; aliquando ovoideae; 65-105 × 95-140 µm; hypha subtenda solitaria. Tunica sporae e startis tribus (strati 1-3); strato "1" mucilaginoso, hyalino, (0.5-)0.9(-1.5) µm crasso; strato "2" semielastico, hyalino, (0.7-)1.1(-1.5) µm crasso; strato "3" laminato, laevigato, aurantiaco vel ochraceo,



Captions to Figs 1-8 see next page



Figs 1-14. Spores and mycorrhizae of *Glomus arenarium*.

Fig. 1. Two spores of different shape, bright field microscopy (BFM), ×515. Fig. 2. Most juvenile spore with spore wall layers (spl) 1 and 2, differential interference contrast (DIC), ×1428. Fig. 3. Slightly older spore with spore wall layer (spl) 1 and thickened layer 2, DIC, ×1428. Fig. 4. Juvenile spore with spore wall layers (spl) 2 and 3 and wrinkled lamina (lam), DIC ×1428. Fig. 5. Spore wall layers (spl) 1-3 of a young spore, DIC, ×1428. Figs 6-7. Spore wall layers (spl) 1-3 of mature spores, DIC, ×1428. Fig. 8. Swollen layer 2 of a mature spore; layer 1 is completely sloughed, DIC, ×1428.

Fig. 9. Deteriorating spore wall layer (spl) 2 separated from spore wall layer 3 of a mature spore, DIC, ×1428; layer 1 is sloughed, DIC, ×1428.

Fig. 10. Highly deteriorated spore wall layer (swl) 2 adherent to spore wall layer 3 of a mature spore, DIC, ×1428.

Fig. 11. Narrow subtending hypha occluded by a septum (s) of a mature spore with layers 1 and 2 sloughed and the laminate layer 3 remaining, DIC, ×1428.

Fig. 12. Mature spore with only spore wall layer 3 and a recurved subtending hypha occluded by a septum (s), DIC, ×1428. *Plantago lanceolata* mycorrhizae with intraradical hyphae and arbuscules stained lightly in 0.1% trypan blue, BFM, ×840.

(2.0-)6.1(-8.8) µm crasso. Hypha hyalina; recta vel recurva; cylindrica vel infundibuliforma, raro coliga; (3.7-)4.9(-8.1) µm lata ad basim sporae; pariete hyalino; (0.7-)1.1(-1.7) µm crasso, stratis 1-3 sporae continuo. Porus e septo continuo strati 3 sporae efformata. Arbuscular mycorrhizae formans.

HOLOTYPUS. POLAND, Szczecin, infra *Plantago lanceolata* L., 10 Jan. 1998, *Błaszkowski J., 2182* (DPP).

Sporocarps unknown. Spores borne singly in the soil (Fig. 1) or sometimes in roots; produced from straight sporophores. *Sporophore* coenocytic to sparsely septate; hyaline; (4.5-)5.2 (-12.7) µm wide; with a wall (0.5-)0.7(-1.2) µm thick; bearing spores by swelling at hyphal tips. *Spores* orange (5B8) to raw umber (5F8); globose to subglobose; (55-)97(-120) µm diam; sometimes ovoid; 65-105 × 95-140 µm; with a single subtending hypha (Fig. 1), rarely two. Subcellular structure of spores consists of one wall (Figs 4-7) with three layers (layers 1-3). Outermost layer 1 hyaline, (0.5-)0.9(-1.5) µm thick before disintegration, closely adherent to layer 2 (Figs 2, 3, 5-7), smooth in juvenile spores, gradually deteriorating and sloughing at the end of formation of layer 2, always absent in mature spores (Figs 8-12). Layer 2 hyaline, smooth, (0.7-)1.1(-1.5) µm thick, semiflexible (folding when separated from the laminate layer 3), sloughing with age, rarely present in mature spores (Figs 2--10). Layers 1 and 2 are continuous with a two-layered subtending hypha of juvenile spores. Layer 3 laminate, smooth, orange (5B8) to raw umber (5F8), (2.0-)6.1(-8.8) µm thick in mature spores (Figs 6-12), formed by gradual synthesis of very thin, approximately 0.5 µm thick, laminae in the spore and its subtending hypha; the first lamina is highly flexible (Fig. 4) and frequently separates from layer 2 in crushed spores. Spore wall layers not reacting in Melzer's reagent. Layers 1 and 2, when not deteriorated, swell in lactic acid-based mountants. Layer 3 then appears to be covered with blisters (Fig. 8) or surrounded with an aureola. *Subtending hypha* hyaline to yellowish white (4A2); straight or recurvate; cylindrical or funnel-shaped (Figs 1, 2, 10-12), rarely constricted; (3.7-)4.9(-8.1) µm wide at the spore base. *Wall of subtending hypha* hyaline to yellowish white (4A2); (0.7-)1.1(-1.7) µm thick at the spore base; continuous with spore wall layers 1-3 in juvenile spores, then consisting of a single layer continuous with spore wall layer 3 (Figs 10-12). *Pore* occluded by a septum, 2.3-4.7 µm wide, continuous with the innermost lamina of spore wall layer 3 (Figs 11, 12). Spore contents of oil droplets.

Collections examined. HOLOTYPE. POLAND. Szczecin, associated with roots of pot-cultured *P. lanceolata*, 10 Jan. 1998, *Błaszkowski J., 2182* (DPP); ISOTYPES: *Błaszkowski J., 2183-2201* (DPP) and two slides at OSC. A living culture of *G. arenarium* was deposited in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (West Virginia University, USA); accession number: INVAM#PL101.

Other materials examined. POLAND. Świnoujście (53°55'N, 14°14'E), from the root zone of *Artemisia campestris* L. and *Petasites spurius* (Retz.) Rchb., 6 Nov. 1993, under *Ammophila arenaria* Link and *Helichrysum arenarium* (L.) Moench., 19 July 1996, *Błaszkowski J., unnumbered collection* (DPP). Słowiński National Park (54°45'N, 17°26'E), under *A. arenaria*, 2 Aug. 1995, *Błaszkowski J., unnumbered collection* (DPP).

Etymology. Arenarium, referring to the sandy habitat from which this fungus was isolated.

Distribution and habitat. Spores of *G. arenarium* were recovered from eight soil samples representing maritime sand dunes of the Baltic Sea. Seven soil samples were collected from dunes adjacent to Świnoujście in northwestern Poland (53°55'N, 14°14'E), and one came from the 12/195 deflation hollow of the Słowiński National Park (54°45'N, 17°26'E). The plant species associated with *G. arenarium* were *A. arenaria*, *A. campestris*, *H. arenarium*, *P. spurius*, and *Senecio* sp. The spore abundance of *G. arenarium* in the field-collected samples ranged from 1 to 56 (mean 22.8) in 100 g dry soil. The arbuscular mycorrhizal fungal species richness in the soil samples containing *G. arenarium* ranged from 1 to 3 (mean 1.9) in 100 g dry soil. The fungi co-occurring with *G. arenarium* in the field were *Entrophospora baltica* Błaszk. et al., *G. constrictum* Trappe, *G. corymbiforme* Błaszk., *G. etunicatum*, *G. gibbosum* Błaszk., *G. intraradices* Schenck & Smith, *Scutellospora dipurpurescens* Morton & Koske, *S. pellucida* (Nicol. & Schenck) Walker & Sanders, and *S. persica* (Koske & Walker) Walker & Sanders.

The soil chemical properties of the Świnoujście dunes were: pH, 3.8-6.7; NO_3 (mg L^{-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). The chemical characteristics of the soil of the successional stage of the deflation hollow in which *G. arenarium* occurred were pH, 4.7; NO_3 (mg L^{-1}), 8.5; P, 1.0; K, 2.0; Mg, 5.0; Na, 0.0; Cl, 13.1; KCl, 35.0; organic C (%), 0.33.

Mycorrhizal associations. Glomus arenarium occurred among vesicular-arbuscular mycorrhizal roots of *A. arenaria*, *A. campestris*, *H. arenarium*, *P. spurius*, and *Senecio* sp. The mycorrhizae formed in a single-species culture of this fungal species with *P. lanceolata* as the host were composed of hyphae, (3.7-)6.1(-8.0) µm wide, growing parallel to the root axis (Fig. 13). They were straight or slightly curved, sometimes dichotomously branched and coiled. These coils were 18.6-27.3 × 29.7--37.7 µm (Figs 13, 14). Arbuscules were numerous and have fine branches difficult to see clearly. No vesicles were present in roots of plants even 6 month old. In 0.1% trypan blue, intramatrical hyphae stain bluish white (23A2) to pastel violet (17A4) and arbuscules violet white (17A2) to pale violet (17A3).

DISCUSSION

Glomus arenarium is characterized by the production of ectocarpic, small, orange to raw umber spores with a distinctive, narrow and hyaline or very light-colored subtending hypha (Figs 1, 10-12).

The pattern of spore wall differentiation in *G. arenarium* is similar to that of *Glomus* species investigated to data (Błaszkowski 1997; Błaszkowski and Tadych 1997; Morton 1996; Stürmer and Morton 1997), with discrete layers formed successively.

The only one described species of the genus *Glomus* resembling *G. arenarium* is *G. etunicatum*. The two fungi form spores similar in color, size and shape with a narrow subtending hypha, whose wall is much lighter-colored than the wall of its spore. Examination of the spore wall structure easily separates these fungi. The spore wall of *G. etunicatum* consists of a mucilaginous outer layer tightly adherent to a laminate inner layer (Stürmer and Morton 1997). In contrast, the wall of *G. arenarium* spores is composed of a sloughing outermost layer closely associated with a semiflexible middle layer that easily separates from a laminate innermost layer (Figs 5-10). Although the outermost layers of the two fungi are similar in appearance and

quickly slough, the mucilaginous layer of *G. etunicatum* spores stains dark pinkish to reddish purple and that of spores of *G. arenarium* does not react in this reagent. Additionally, the subtending hypha of *G. arenarium* spores is persistent and almost always present in mature spores (Figs 1, 10-12), whereas that of spores of *G. etunicatum* frequently breaks at the spore base, causing the spores to be similar to those of *Acaulospora* and *Entrophospora* spp. when observed at a low magnification.

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GLOMUS ARENARIUM, NOWY GATUNEK Z RZĘDU GLOMALES (ZYGOMYCETES)

STRESZCZENIE

Z wydm nadmorskich północnej Polski wyodrębniono nowy gatunek arbuskularnego grzyba mikoryzowego, *Glomus arenarium* (Glomales, Zygomycetes). *Glomus arenarium* tworzy zarodniki z wąskim, hialinowym trzonkiem. Zarodniki są pomarańczowe do koloru umbry, kuliste, (55-)97(-120) µm śr., lub jajowate, 65-105 × 95-140 µm. Ściana zarodników składa się z trzech warstw: najbardziej zewnętrznej warstwy obecnej tylko u bardzo młodych zarodników, giętkiej, hialinowej warstwy środkowej rzadko obecnej u zarodników dojrzałych i trwałej, pomarańczowej do koloru umbry warstwy wewnętrznej. Żadna z tych warstw nie barwi się w odczynniku Melzera. Grzyb ten tworzył zarodniki i mikoryzy arbuskularne w jednogatunkowych kulturach wazonowych z *Plantago lanceolata*.

SŁOWA KLUCZOWE: Glomales, mikoryzy, ontogeneza, grzyby arbuskularne, Zygomycetes.