MYCOTAXON

Volume LXXVI, pp. 187-195 October-December 2000

GLOMUS MINUTUM, A NEW SPECIES IN GLOMALES (ZYGOMYCETES) FROM POLAND

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Abstract:

An ectocarpic species of the genus *Glomus, G. minutum* (Glomales, Zygomycetes), was discovered in rhizosphere soils of plants colonizing maritime sand dunes of northwestern Poland. *Glomus minutum* is characterized by the formation of small, hyaline spores with a wall consisting of two components: a smooth, permanent outer component and a laminate inner component. None of them stains in Melzer's reagent. Mycorrhizae formed by *G. minutum* in one-species cultures with *Plantago lanceolata* consisted of arbuscules, intra- and extramatrical hyphae.

Key Words: Glomales, mycorrhizae, arbuscular fungi, Zygomycetes

Introduction

Examination of field-collected soils and those resulting from pot trap cultures from maritime sand dunes of northwestern Poland

revealed an undescribed species of arbuscular mycorrhizal fungi of the genus *Glomus*. This fungus is described here as *G. minutum*. *Glomus minutum* is characterized by the formation of small, hyaline spores that differ from those formed by other unpigmented members of the genus by wall structure and a clustering habit.

Materials and Methods

Collection of soil samples, establishment of trap and single-species pot cultures, and growth conditions are as 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-H_20 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. The cultures were harvested after 4-12 mo. Spores were extracted by wet sieving and decanting (Gerdemann and Nicolson, 1963). Mycorrhizas were revealed by the Phillips and Hayman (1970) method, using 0.1% trypan blue.

Morphological properties of spores and their subcellular structures were determined based on at least 100 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier, 1983) and a mixture of PVLG and Melzer's reagent (1:1, v/v). The spores represented all stages of differentiation of the fungus. Terminology of spore structure is that suggested by Walker (1983) and Walker et al. (1998). Spore color 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), University of Agriculture, Szczecin, Poland, and in the herbarium at Oregon State University (OSC) in Corvallis, Oregon, USA.

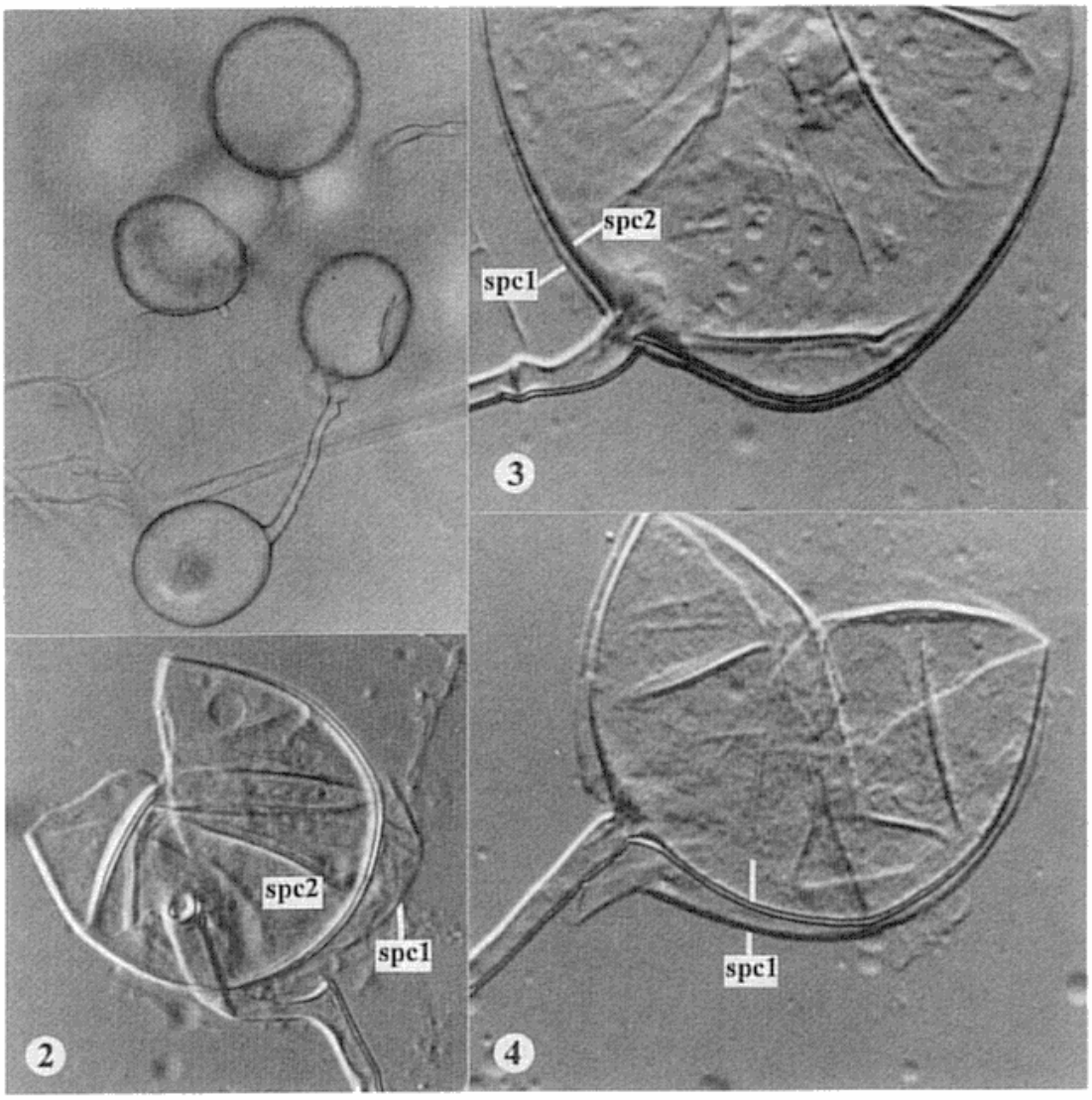
Descriptions and Discussions

Glomus minutum J. Błaszkowski, M. Tadych et T. Madej, sp. nov. Figs. 1-4

Sporocarpia ignota. Sporae laxae gregaria, e sporophoris rectic efformatae. Sporophorum nonseptatum vel parce septatum; hyalinum vel pallide aurantiacum; (2.5-)3.0(-3.4) µm latum; pariete (0.5-)0.6(-0.7) µm crasso; rectum. *Sporae* hyalinae; globosae vel subglobosae; (18-)39(-65) µm diam; aliquando ovoideae; 18-30 x 40-80 µm; hypha subtenda solitaria. *Tunica sporae* e startis duobus (strati 1-2); strato "1" semielastico, hyalino, (0.2-)0.6(-0.7) µm crasso; strato "2" laminato, hyalino, (0.5-)0.8(-1.2) µm crasso. *Hypha* hyalina; recta vel recurva; cylindrica vel infundibuliforma, raro coliga; (4.2-)5.7(-8.1) µm lata ad basim sporae; pariete hyalino; (0.7-)1.0(-2.7) µm crasso, stratis 1-2 sporae continuo. *Porus* e septo continuo strati 2 sporae efformata. Arbuscular mycorrhizae formans.

HOLOTYPUS. POLAND, Szczecin, infra *Plantago lanceolata* L., 10 Jan. 1999, *Błaszkowski, J., 2246* (DPP).

Sporocarps unknown. Spores formed singly in the soil or in loose aggregates associated with roots (Fig. 1). *Spores* hyaline; globose to subglobose; (18-)39(-65) µm diam; sometimes ovoid; 18-30 x 40-80 µm; with a single subtending hypha (Figs. 1-4), rarely two. Subcellular structure of spores consists of one wall (Figs. 2-4) with two hyaline components (components 1 and 2). Outermost component 1 smooth, semiflexible, (0.2-)0.6(-0.7) µm thick, tightly adherent to component 2 (Figs. 2-4), flexible in young spores (Fig. 2), semiflexible in mature spores, continuous with two components of subtending hypha (Fig. 4). Component 2 laminate, smooth, (0.5-)0.8(-1.2) µm thick, formed by gradual synthesis of very thin, <0.5 µm thick, laminae in the spore and its subtending hypha. Components 1 and 2 do not react in Melzer's reagent. *Subtending hypha* hyaline; straight or recurved; cylindrical or slightly flared (Figs. 2-4), rarely constricted; (4.2-)5.7(-8.1) µm wide at the spore base. *Wall of subtending hypha* hyaline; (0.7-)1.0(-2.7) µm thick at the spore base; continuous with spore wall components 1-2 (Fig. 4). *Pore* occluded by a septum, 2.0-4.9 µm wide, continuous with the innermost lamina of spore wall component 2. Spore contents of oil droplets.


spc2
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Collections examined. HOLOTYPE. POLAND. Szczecin, associated with roots of pot-cultured *P. lanceolata*, 10 Jan. 1999, *Błaszkowski, J., 2246* (DPP); ISOTYPES: *Błaszkowski, J., 2247-2258* (DPP) and two slides at OSC.

Other materials examined. POLAND. Spores from trap cultures established based on rhizosphere soils of *Ammophila arenaria* Link, *Corynephorus canescens* (L.) P. B., *Festuca rubra* L., *F. polesica* Zap., *Galium ? aparine* L., *Hieracium pilosella* L., *H. umbellatum* L., *Petasites spurius* (Retz.) Rchb. and *Potentilla ? anserina* L. colonizing maritime dunes adjacent to Świnoujście (53°55'N, 14°14'E), *Błaszkowski, J., unnumbered collection* (DPP).

Etymology. minutum, referring to the small spores formed by the fungus. We wish to dedicate this species to Prof. Dr. Habil. Marian Piech, Department of Biometry, University of Agriculture, Szczecin, a prominent scientist and the former Rector of the Szczecin University of Agriculture.

Distribution and habitat. Glomus minutum sporulated abundantly in 14 of the 79 trap cultures containing rhizosphere soils collected on 13 June 1997. The soils came from under dune plants growing near Świnoujście in northwestern Poland (53°55'N, 14°14'E). However, none of the field-collected soils contained spores of this fungus. The lack of spores of *G. minutum* in the field soils may have resulted from three reasons. First, *G. minutum* produces delicate, thin-walled spores that are probably quickly decomposed by soil microorganisms. Parasitic microorganisms may significantly reduce populations of spores of AMF in the field (Lee and Koske 1994). Second, the new fungal species might not have been sporulating at the time of sampling. Seasonal dependence has been observed in sporulation of AMF (Gemma et al. 1989).

Figs. 1-4. *Glomus minutum.* **1.** Cluster of intact spores of different shape, bright field microscopy (BFM), x 513. **2.** Most juvenile spore with a very thin, flexible spore wall component (spc) 1 and a thicker spore wall component 2, differential interference contrast (DIC), x 1428. **3.** Tightly adherent spore wall components (spc) 1 and 2 of a crushed mature spore, DIC x 1428. **4.** Vigorously crushed spore with separated spore wall components (spc) 1 and 2; note the separated subtending hyphal wall components, DIC, x 1428.

Third, *G. minutum* perhaps is a rarely or not sporulating fungus in the field conditions. A high proportion of non-sporulating fungi has been found in different ecosystems (Brundrett et al. 1999; Stutz and Morton 1996).

The plant species associated with *G. minutum* in the field were *A. arenaria*, *C. canescens*, *F. rubra*, *F. polesica*, *G. ? aparine*, *H. pilosella*, *H. umbellatum*, *P. spurius* and *P. ? anserina*. The arbuscular mycorrhizal fungi accompanying *Glomus minutum* in the field and trap cultures were *Acaulospora trappei* Ames & Linderman, *G. constrictum* Trappe, *G. corymbiforme* Błaszk., *G. lamellosum* Dalpé et al., *G. macrocarpum* Tul. & Tul., *G. microcarpum* Tul. & Tul., *G. pustulatum* Koske et al., undescribed *Glomus* 111, *Glomus* 122, *Glomus* 133, *Scutellospora dipurpurescens* Morton & Koske and *S. persica* (Koske & Walker) Walker & Sander.

The soil chemical properties of the Świnoujście dunes were: pH($H_2$0), 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).

Mycorrhizal associations. *Glomus minutum* 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* were composed of intramatrical hyphae, (3.4-)5.9(-9.1) µm wide, growing parallel to the root axis. These hyphae sometimes formed coils, 12.5-27.5 x 17.5-55.0 µm, short branches swollen at their tip or short, perpendicular branches connected with the neighbouring, parallel hyphae. Arbuscules were numerous and had fine branches that were difficult to see clearly. No vesicles were present in roots of plants even when the cultures were 6 month old. Extramatrical hyphae were 3.2-3.7 µm wide. In 0.1% trypan blue, intramatrical hyphae stained bluish white (22A2), arbuscules bluish white (22A2) to violet white (15A2) and extramatrical hyphae violet white (18A2).

Discussion. *Glomus minutum* is unique due to its very small, hyaline spores with a wall consisting of two permanent

components, *i. e.*, components present in both the most juvenile and fully mature specimens (Figs. 2-4).

The ontogenetical development of *G. minutum* spores expresses in relatively low increases in size of spores and the thickness of their wall. The changes in wall thickness mainly result from the synthesis of additional laminae in the laminate component 2.

When observed under a stereoscope microscope, spores of *G. minutum* resemble those of *G. diaphanum* Morton & Walker, *G. laccatum* Błaszk., *G. occultum* Walker, *G. spurcum* Pfeiffer et al. and *G. viscosum* T. H. Nicolson (Błaszkowski 1988; Kennedy et al. 1999; Morton 1998; Morton and Walker 1984; Pfeiffer et al. 1996; Walker 1982; Walker et al. 1995). All species form hyaline spores and the largest spores of *G. minutum* attain the lower level of the spore size range of the other species listed above. However, although *G. viscosum* produces spores in loose clusters, *G. minutum* produces them in much tighter clusters that might be thought of as primitive spore bodies.

Examination of *G. minutum* spores under a compound microscope readily facilitates separation of this fungus from other hyaline glomalean species. While the spore wall of *G. minutum* consists of two components (Figs. 2-4), that of *G. diaphanum* and *G. occultum* comprises three. The outermost component of *G. minutum* spore wall is permanent, semiflexible in mature spores and adheres to the innermost laminate component (Figs. 3-4). In contrast, the outermost spore wall component of *G. diaphanum* and *G. occultum* sloughs with age and is usually absent in mature spores. Additionally, this sloughing component is associated with either a middle permanent component (*G. occultum*) or a middle laminate component (*G. diaphanum*). *Glomus minutum* also lacks the innermost semiflexible component of *G. diaphanum* and the innermost permanent component of *G. occultum*.

Glomus spurcum produces spores with two permanent wall components, of which, however, the outer one may be darker colored (hyaline to pale yellow-brown) and thicker (0.4-1.2 µm) than that of *G. minutum* remaining hyaline and being not thicker than 0.7 µm. Additionally, the outer component of *G. spurcum* spores easily wrinkles and separates from the neighbouring

laminate component (vs. it usually tightly adheres to a laminate component in *G. minutum*), which is much thicker than that of the new fungus described here (1.4-4.2 µm vs. 0.5-1.2 µm thick).

Glomus viscosum also has a spore wall structure with two components. However, its outer spore wall component is thicker, more plastic and separates more readily from the laminate component. Additionally, the outer component of *G. minutum* spores does not exude a mucigel-like substance as found in *G. viscosum*.

The features distinguishing *G. minutum* and *G. laccatum* are properties of their spore wall and subtending hypha. The outermost spore wall component of the latter species sloughs with age and usually is absent in mature specimens, and its laminate component is composed of easily separating and thick (ca 0.5-2.2 µm) laminae (vs. inseparable and very thin in *G. minutum*). The subtending hypha of *G. laccatum* compared with that of *G. minutum* is much wider (7.4-12.9 µm vs. 4.2-8.1 µm), and its wall is less compact, because it is formed from loose laminae continuous with those of the laminate spore wall component.

The mycorrhizal colonization of *G. minutum* most resemble those of *G. spurcum* and *G. viscosum* in consisting of only hyphae and arbuscules with no vesicles (Morton 1998; Pfeiffer et al. 1996), which are present in mycorrhizal structures of *G. diaphanum* and *G. occultum* (Morton 1998; Morton and Walker 1984; Walker 1982). However, the mycorrhizae of *G. minutum* stain very lightly in trypan blue, whereas those of *G. spurcum* and *G. viscosum* may be intensively stained. Morphological properties of mycorrhizae of *G. laccatum* are unknown.

Acknowledgment

We would like to thank Doctor C. Walker, Biological Research and Imaging Laboratory, 2 Penny Hedge, New Milton, Hampshire, UK, for examination of *G. minutum* and valuable comments on the manuscript. This study was supported in part by Committee of Scientific Researches, grant no. 6.P04C.015.13.

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