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Note

Two *Glomus* species from rhizosphere soil of a bent grass nursery in Japan

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This paper reports two *Glomus* spp. isolated from rhizosphere soil in a bentgrass nursery of a golf course in Hyogo Prefecture, western Japan. One was identified as *G. etunicatum*, new to Japan, while the other remained unidentified because of lack of information.

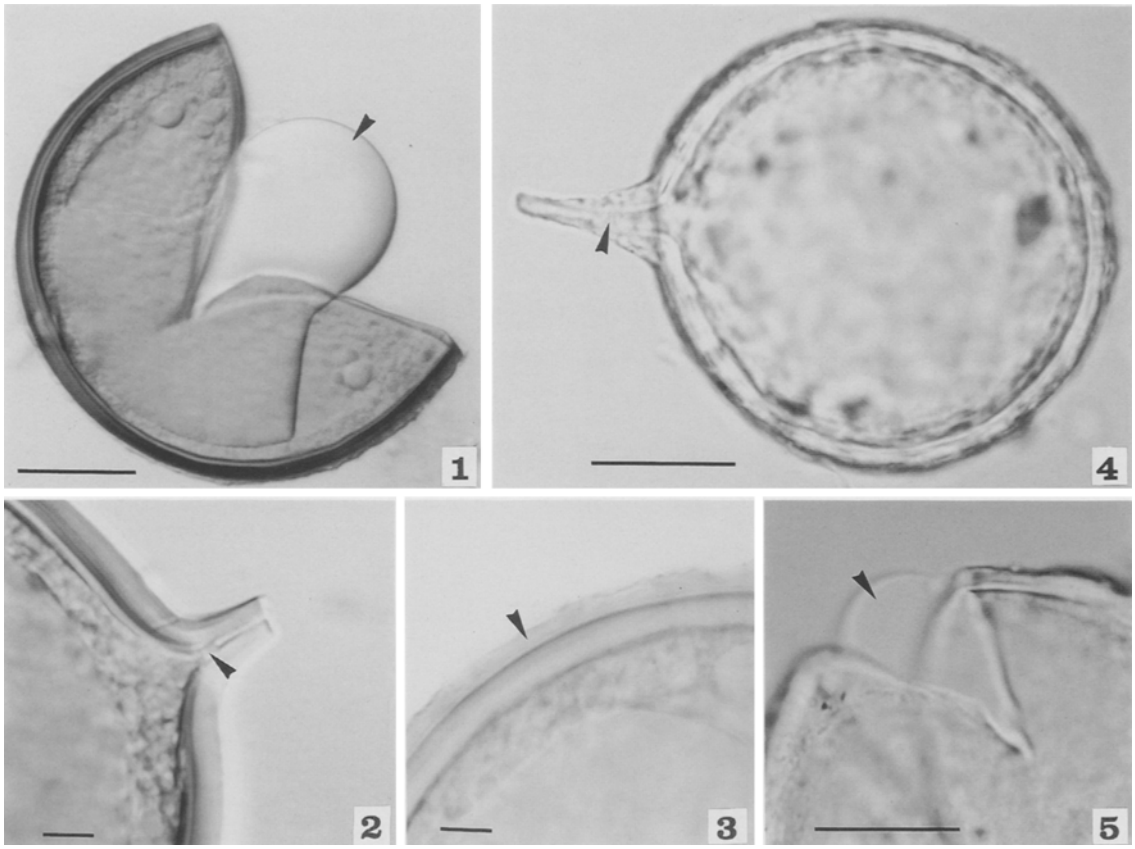
Key Words——arbuscular mycorrhizae; bent grass nursery; *Glomus etunicatum*.

During an investigation of arbuscular mycorrhizal fungi in a bent grass nursery, chlamydospores of two *Glomus* species were obtained. This study describes the morphological characteristics of these fungi. A part of this work has been reported elsewhere (Murakoshi et al., 1995).

Sods (11 cm in diam) were collected from the nursery of bentgrass *Agrostis palustris* Huds. cv. Penncross at Takarazuka Golf Club, Hyogo Prefecture, in June 1992. Spores were collected from the rhizosphere of bent grass by the wet-sieving method (Gerdemann and Nicolson, 1963) combined with sucrose density gradient centrifugation (Jenkins, 1964). The recovered spores and sporocarps of *Glomus* spp. and *Sclerocystis* sp. were used as inocula for spore proliferation on bent grass seedlings by the soil funnel technique (Menge and Timmer, 1982). The seedlings were kept in a growth chamber at 20°C and exposed to continuous light (5,300 lx). *Sclerocystis* sp. neither infected nor grew after incubation for 4 mo. *Glomus* spp. were able to grow under these conditions, and their spores were extracted from the soils and used for morphological observation. Each spore was mounted on a slide in polyvinyl alcohol-lactic acid-glycerol solution (Koskey and Tessier, 1983), then crushed, after which its wall structure was examined under a compound microscope with Nomarsky interference equipment. The reaction of spore walls to Melzer's reagent was also examined (Morton, 1986). Murographs depicting the wall structure followed the guidelines proposed by Walker (1983).

Two types of spores of the genus *Glomus* were isolated. Chlamydospores of the first type were formed singly in soil, mostly globose to subglobose and rarely irregular. They ranged 57–155 (av. 105) μm in diam. They were yellow under reflected light, and they contained hyaline oil droplets (Fig. 1). A pore, 4–8 μm in diam, was formed in subtending hypha and occluded by a thin septum (Fig. 2) at or under the point of attachment. Spore walls consisted of an ephemeral, hyaline outer layer 0.5–4 μm thick, and a persistent yellow laminate inner wall layer 4–8 μm thick. The outer wall layer stained pale red in Melzer's reagent (Fig. 3). This species was identified as *G. etunicatum* Becker & Gerdemann from the murograph and its spore features (Becker and Gerdemann, 1977; Morton, 1989). This is the first report of the species from Japan.

Chlamydospores of the second type formed singly in the soil. They were globose or subglobose and ranged 55–123 (av. 82) μm in diam. They were pale-yellow under reflected light and had an open pore with a thin septum (Fig. 4) occluding under the attachment of spore. Chlamydospores contained a hyaline oil droplet (Fig. 5). Spore wall, 3–8 μm thick, extends into the subtending hypha, 3–7 μm wide. The subtending hypha was single and 4–14 μm wide at the base. The wall did not react with Melzer's reagent. From the morphological features such as size and color of chlamydospores (Nicolson and Schenck, 1979), this fungus appears to be related to *G. clarum*. However, *G. clarum* spore has two wall layers with hyaline globule contents of variable size (Nicolson and Schenck, 1979). This fungus also resembles *G. occultum* in the range of size, color and retaining laminated wall layer of chlamydospores (Walker, 1982). However, chlamydospores of this fungus were not observed in the cortex of roots after incubation for 4 mo, unlike *G. occultum* described by Walker (1982). Ueda et al. (1992) isolated *G. occultum* in Japan, which produced spores with three thin wall layers and never formed intraradical spores. From its similarity to the laminated wall pointed out by Walker (1982), and the ectocarpic strucuture observed by Morton (1985) and Ueda et al. (1992), this fungus seems to fall within the range of intraspecific vari-



Figs. 1–3. Spores of *Glomus etunicatum*.
1. Crushed spore with an oil droplet (arrowhead).
2. Septum (arrowhead) at the spore attachment.
3. Wall structure in Melzer's reagent. Only outer wall layer (arrowhead) staining pale red.
All scales = 25 μm.

Figs. 4, 5. Spores of *Glomus* sp.
4. Intact spore, showing a septum (arrowhead) at the spore attachment.
5. Crushed spore with an oil droplet (arrowhead).
All scales = 25 μm.

ation of *G. occultum*. More detailed investigations are needed to warrant such identification.

Further investigations are also necessary to reveal the range of distribution of the *Glomus* spp. reported here in this country.

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