

## GLOMUS SPINOSUM SP. NOV. IN THE GLOMACEAE FROM TAIWAN

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**Abstract:** *Glomus spinosum* forms yellowish brown to dark red brown spores in sporocarps or single in the soil. The chlamydo spores are 40-90 µm in diam, and have a 4-10 µm thick wall which consists of a thin pale yellow-brown outer wall covered with a hyaline to light yellow dense mass of flexible hairy spines, up to 15 µm long and each up to 3 µm in diameter, a thick dark brown laminated middle wall with warts on the surface, up to 1 µm high, and a thin yellowish brown inner wall in one wall group. *Glomus spinosum* forms typical vesicular-arbuscular mycorrhizae with *Cunninghamia lanceolata* (Lambert) Hooker and other plant species.

**Key words:** *Cunninghamia lanceolata*, Glomaceae, *Glomus*, sporocarp, vesicular-arbuscular mycorrhizae.

### INTRODUCTION

During the survey of hypogeous fungi in the rhizosphere of *Cunninghamia lanceolata* spores of a new species of Glomales described here as *Glomus spinosum* were found. The description is based on observation of intact and crushed spores mounted in water, polyvinyl alcohol-lactic acid glycerol (PVLG) and Melzer's reagent examined at 400× with a phase-contrast and bright-field microscope. In addition, observations of spores in scanning electron microscopy were carried out to define the wall characteristics of this new species. For wall terminology, Walker's concept (1983) is followed.

### MATERIALS AND METHODS

Field-collected spores of the here described new species were inoculated to the rhizosphere of one month old non-infected *Allium porrum* L., *A. sativum* L., *Festuca elatior* var. *arundinacea* L. and one-year old *C. lanceolata* seedlings which had been planted in an autoclaved sandy medium. After three months to one year culturing in the glasshouse, chlamydo spores were extracted by wet sieving and decanting (Gerdemann and Nicolson, 1963). One hundred wet-sieved spores were examined under the stereomicroscope against white and black backgrounds for color determination. One hundred segments of 1 cm long roots were randomly taken and stained in 0.05% cotton blue to examine the presence of vesicular-arbuscular mycorrhizae (Phillips and Hayman, 1970). At least 300 chlamydo spores were cleaned in water and provided for scanning electron microscopic (SEM) observation. For SEM observation, spores and rootlets were fixed in 2.5% glutaraldehyde solution buffered with 0.05M Na-cacodylate at least for one day. The fixed samples were washed with 0.05M Na-cacodylate buffer solution at pH 7.2 four times, each time 15 minutes, dehydrated with a series of acetone from 30% to 100%, post-fixed in 1% OsO<sub>4</sub>, washed again with 0.05M

Na-cacodylate buffer solution four times, dried by critical point dryer using liquid CO<sub>2</sub>, mounted on an aluminum stub, coated with gold, and then observed under JSM-6300 scanning electron microscope at 20 KV.

### DESCRIPTION

GLOMUS SPINOSUM Hu sp. nov. (Fig.3 and 4)

Sporocarpia irregularia, ad 5 mm, pallide brunneola. Peridium destitutum. Gleba pallide brunnea, cum sporis, hyphis, radicibus, at arena immixta. Chlamydo spores singulae vel laxe gregariae, in solo efformatae, globosae vel subglobosae, 40-90 µm diam. Spores cortex 4-10 µm crassus. Spores stratum primum hyalium vel pallide hepaticum, 0.5 µm crassum cum villis ad 15 µm longis, stratum iterum cinnamomeum, porphyreum vel obscure bruneum, vel subfuscum, 3-6 µm crassum, nodulis congestis ornatis, stratum tertium luteo-brunnum, 0.5 µm crassum. Hyphae subtendentes 7-16 µm diam.; formans mycorrhiza cum vesiculis et arbusculis.

SPOROCARPS (Fig. 3 B) pale brown, irregular, up to 5 mm diam. formed by loose interwoven pale yellowish brown hyphae; hyphae consisting of 3 different sizes, (1) 0.5-1.5 µm wide with walls 0.2-0.3 µm thick; (2) 3.6-4.8 µm wide with walls 0.5 µm thick; (3) 5.0-6.0 µm wide with walls 0.5 µm thick; walls yellow with greenish tint, cytoplasm light yellowish; hyphae mostly nonseptate. Peridium absent. Within the sporocarps the spores and hyphae are often intermixed with roots and sand.

CHLAMYDOSPORES formed in sporocarps, or single in soil, or in dead roots, globose to subglobose, 40-90 µm diam. with the majority of spores in the range of 50-70 µm diam.(Fig.1); spores with roughened surface when observed under low magnification power microscope.

SPORE WALL 4-10 µm thick without outer ornamentation. Spore wall (see micrograph, Fig. 2 ) consisting of three wall layers in one group. Wall 1 a hyaline to subhyaline to pale yellow-brown unit wall, ca 0.5 µm thick covered with a mass of subhyaline to yellowish hairy protuberances, up to 15 µm long and each 1.5-3 µm in diam. Protuberances often covered on their surface with mucilageous granular-like material (Fig. 4). Wall layer 2, orange-brown, red-brown to dark brown, or nearly black, laminated, 3-6 µm thick, ornamented with subhyaline warts, 1.5-3 µm high, 0.7-1.3 µm in diam. which grow into wall 1 (Fig. 4 A). Wall 3 yellowish brown, membranous, 0.5 µm thick. Spore walls do not react with Melzer's reagent.

SUBTENDING HYPHA single, straight or slightly recurved, at the point of spore attachment 7-16 µm wide, with yellowish brown to brown or dark brown wall; wall is continuous of wall layer 2 and generally as thick as the spore wall layer 2 but often unevenly thickened, up to 65 µm from attachment; hyphal attachment wall is not covered with protuberances of wall layer 1. The attachment point of the subtending hypha with the spore is often occluded by the innermost wall layer 3, or by a septum in the subtending hypha in some distance from the attachment point.

FORMS VESICULAR ARBUSCULAR MYCORRHIZAE.

GERMINATION TUBE emerges through the subtending hypha.

ETYMOLOGY: Latin, *spinosus* "spiny", in reference to the spore wall ornamentation.

DISTRIBUTION AND HABITAT: In the rhizosphere of *C. lanceolata* stands, Nan-Tou county,

Taiwan, altitude 700-2000 m. Forming vesicular-arbuscular mycorrhizae with seedlings of *C. lanceolata*, *A. porrum* and *A. saivum* in sand pot culture.

COLLECTION EXAMINED: Type: Nan-Tou county, National Taiwan University Forest, Taiwan, from rhizosphere in the Ah-horizon of *C. lanceolata* stands. 02. Jan. 2000, Hu-0102 (slide); Forest Soil and Mycorrhiza Laboratory, Department of Forestry, National Taiwan University. Holotype: Maintained in pot culture of *C. lanceolata*, Hu 3212(NTU).

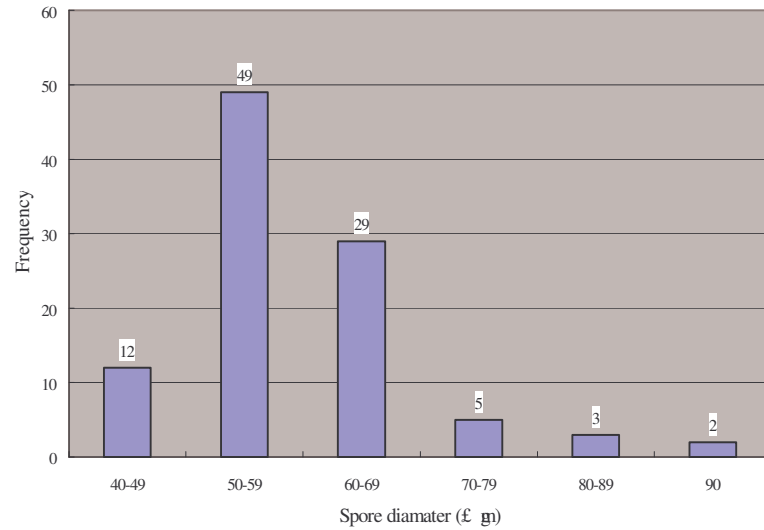


Fig.1 *Glomus spinosum* spore diameter frequency from a random selection of 100 mature spores. For non-globose spores, the average diameter of cross and long direction was measured.

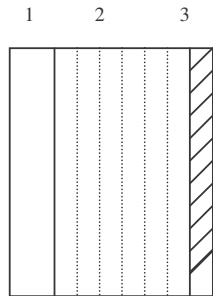


Fig.2. Murograph of *Glomus spinosum*. Spore wall structure (after Walker, 1983) is of three walls in one group. Arabic numerals indicate each wall in order from outer to inner wall; wall 1 and 2 are ornamented, wall 1, 2 and 3 are unit, laminated and membranous, respectively.

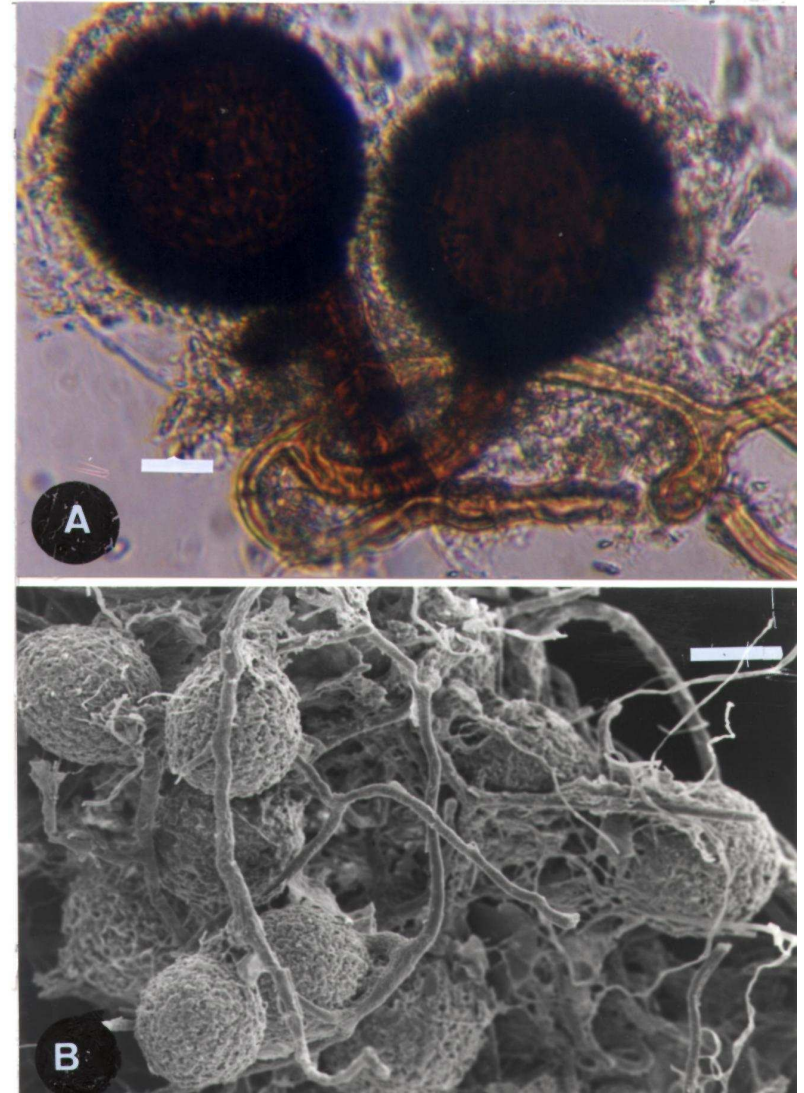


Fig. 3. *Glomus spinosum*. A. Two mature spores mounted in PVLG by phase-contrast microscopy show the spines of wall 2 surrounded by the protuberances of wall 1; bar = 15µm. B. Sporocarp by scanning electron microscopy (SEM), bar = 30µm.

## DISCUSSION

*Glomus spinosum* is morphologically easy distinguished from spores of other species in the genus *Glomus* by its dark red brown colored chlamydospores, by having long protuberances on the outer wall, warts on the second spore wall, and by the formation of spores in sporocarps. Spores of *G. spinosum* resemble those of *G. aggregatum* Schenck and Smith (Koske, 1985), when observed under low magnification power stereomicroscope in having similar size or color. However, *G. spinosum* can easily be differentiated from them by the spines when observed at higher magnification. Other ornamented species of the genus *Glomus* are hyaline to subhyaline or are not formed in sporocarps, e.g. *G. scintillans* Rose & Trappe (Rose and Trappe, 1980), *G. chimonobambusae* Wu & Liu (Wu et al., 1995), or *G. verruculosum* (Blaszkowski and Tadych, 1997).

*Glomus spinosum* infected the roots of *C. lanceolata* at high rates with more than 70% infected roots, of *A. porrum* at medium rates (25-30%) and *A. sativum* also at medium rates (30-40%). However, the roots of *F. elatior* var. *arundinacea* were not infected in the greenhouse sand pot culture after inoculation with *G. spinosum*. Hence, it may be that *G. spinosum* is not compatible with all plant species.

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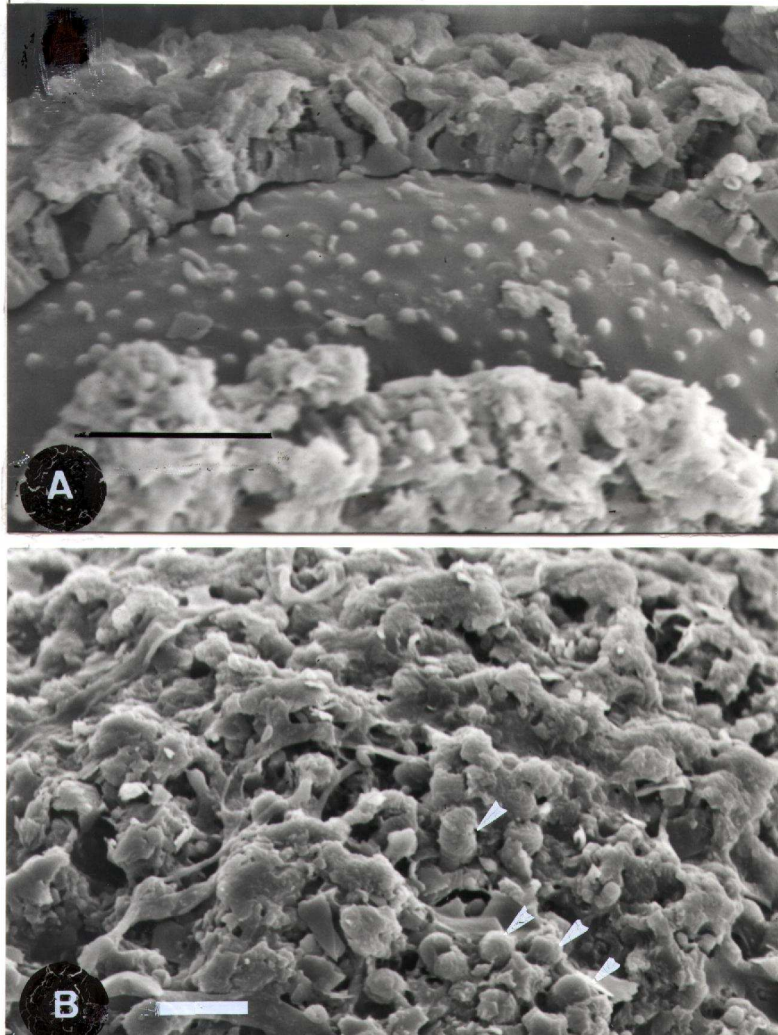


Fig. 4. *Glomus spinosum* A. SEM detail of the crowded protuberances on wall 1 and warts on the outer surface of wall 2, bar= 10 µm. B. SEM detail of the ornamentation shows the mucilage-like granular material covering the protuberances of wall layer 1 (arrows), bar=5 µm.