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Glomus epigaeus sp.nov., a useful fungus for vesicular–arbuscular mycorrhizal research[1]

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A new endomycorrhizal species, ***Glomus epigaeus***, produces abundant chlamydospores in sporocarps on the soil surface. This species has been maintained for more than 1 year in pot culture on various hosts and produces 8–15 sporocarps monthly from each pot. A simple technique of shaking sporocarps in water with broken glass chips is described for the separation of spores from sporocarps.

The epigeous habit, narrow, thin-walled, inserted hyphal attachment, and bright yellow to yellow–brown wall color in transmitted light separate *G. epigaeus* from *Glomus macrocarpus*, which normally does not fruit epigeously and has a much broader, thick-walled, noninserted hyphal attachment and brown wall color in transmitted light.

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Le ***Glomus epigaeus***, une nouvelle espèce endomycorhizienne, produit à la surface du sol des sporocarpes contenant d'abondantes chlamydospores. Cette espèce a été maintenue par culture en pot sur divers hôtes et produit de 8 à 15 sporocarpes par mois dans chaque pot. Les auteurs décrivent une technique pour séparer les spores d'un sporocarpe en les agitant dans l'eau en présence de fragments de verre.

L'habitat épigé, l'hyphe support inséré étroit et à paroi mince, ainsi que la paroi jaune brillant à jaune brunâtre en lumière transmise distinguent le *G. epigaeus* du *Glomus macrocarpus*: ce dernier ne fructifie généralement pas en position épigée, il possède un hyphe support beaucoup plus large, non inséré et à paroi plus épaisse, alors que la paroi de la spore est brune en lumière transmise.

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Introduction

Gerdemann and Trappe (1974) reported epigeous fruiting of members of the Endogonaceae on the soil surface of potted plants in greenhouses. In fall 1976, sporocarps of a chlamydosporic vesicular–arbuscular mycorrhizal fungus were discovered on the soil surface of pots containing Norfolk Island pine (*Araucaria excelsa* R. Br.) in the Oregon State University botany greenhouse (Fig. 1). Subsequently, 8–15 sporocarps have been harvested monthly from each pot except during dry months. Meanwhile, reexamination of some collections of Endogonaceae from Mexico and Ecuador revealed a similar-appearing fungus. We judge the fungus to be undescribed and sufficiently distinct from *G. macrocarpus* to propose the name ***Glomus epigaeus*** for it.

To determine if *G. epigaeus* would fruit epigeously in pot cultures, sporocarps were harvested from the potted *Araucaria* and inoculated into pots containing sand–soil–vermiculite mix seeded to either *Sorghum vulgare* Pers. or *Asparagus officinalis* L. These pot cultures were raised in the United States Department of Agriculture – Science and Education Administration (S.E.A.) Ornamental Plants Research Laboratory greenhouse and irrigated automatically by a mist system. After 4 months, epigeous sporocarps were formed in both asparagus and sorghum pot cultures. Sporocarp production continued even after the sorghum was removed from mist irrigation to a bench and watered manually. Epigeous fruiting appears, however, to require maintenance of relatively humid conditions.

These pots have been in production for more

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than a year. During this period, weekly applications of 150 mL of either one-eighth strength Hoagland's solution or full-strength Long Ashton solution (Hewitt 1966) with one-quarter strength phosphorus have worked well. Each pot produces up to 15 sporocarps per month with little seasonal fluctuation.

Although sporocarps fruit directly on surface roots of Norfolk Island pine (Fig. 1) and sorghum, they often form among moss (*Funaria hygrometrica* Hedw.) which grows abundantly on the soil surface. We have not noticed vesicular–arbuscular mycorrhizal infection of moss rhizoids. Whether or not rhizoids are colonized by the fungus, the moss probably helps maintain relatively high humidity near the soil surface and thereby promotes fruiting of *G. epigaeus*.

Spores are separated easily from sporocarps by immersing them in 20–30 mL of distilled water with 30–50 g of broken glass (shards 2–5 mm in diameter) in a 250-mL Erlenmeyer flask. The flask is shaken on a reciprocal shaker at two to three cycles per second for approximately 30 min or until spores are separated.

Glomus epigaeus is extremely useful for vesicular–arbuscular mycorrhizal research because it regularly produces thousands of clean, uniform, easily harvested spores for experimental inoculum. Pot cultures need not be dismantled to obtain spores. Its sporocarps have not been found to include spores of other mycorrhizal fungi, so it appears to be dependably free of such contaminants. The quantity of inoculum used in an experiment can be easily determined as weight or number of spores. It germinates readily under proper conditions and has been used successfully in extensive germination studies (Daniels, unpublished data). Such studies otherwise would have been either excessively time consuming or reduced to dangerously small numbers of test spores. *Glomus epigaeus* can be useful in many other types of studies on spores and mycorrhizal fungus–host interactions.

An occasionally observed hyperparasite of *G. epigaeus* closely resembles *Rhizidiomycopsis stomatosa* Sparrow, a hyperparasite of several endomycorrhizal fungi (Schenck and Nicolson 1977).

Glomus epigaeus Daniels and Trappe, sp.nov. (Figs. 1–3)

Chlamydosporae singillatim in terra vel in sporocarpiis epigaeis enatae. Sporocarpia ochracea vel brunnea, irregularia, 2–8 × 3–15 mm. Peridium destitutum. Sporae globosae, subglobosae, vel late ellipsoideae, (60–)75–140(–165) × 95–140 μm, laeves, juventute albae, deinde pallide ligneae vel pallide hepaticae; tunicae 5–8(–10) μm crassae, tunica exteriore subhyalina, 0.5–1 μm crassa, tunica interiore maxime crassa, aurea. Hyphae affixa typice 4–6 μm diametro, saepe in tunicam sporae inserta, plerumque ad paginam sporae ad 6–10 μm expansa. Hyphae sporocarpiorum 3–15(–30) μm diametro, subinde tumoribus irregularibus, hyalinae, eburneae, vel pallide brunneae. Holotypus Trappe 5174.

Sporocarps irregular, 2–8 × 3–15 mm, sometimes fused into larger masses, dull brownish yellow to brown, arising from a basal pad of pale grayish yellow, loose mycelium with a few interspersed spores; peridium lacking, but hyphal aggregations sometimes extended from the basal pad over lower sporocarp sides. Spores also borne singly or in clusters in soil.

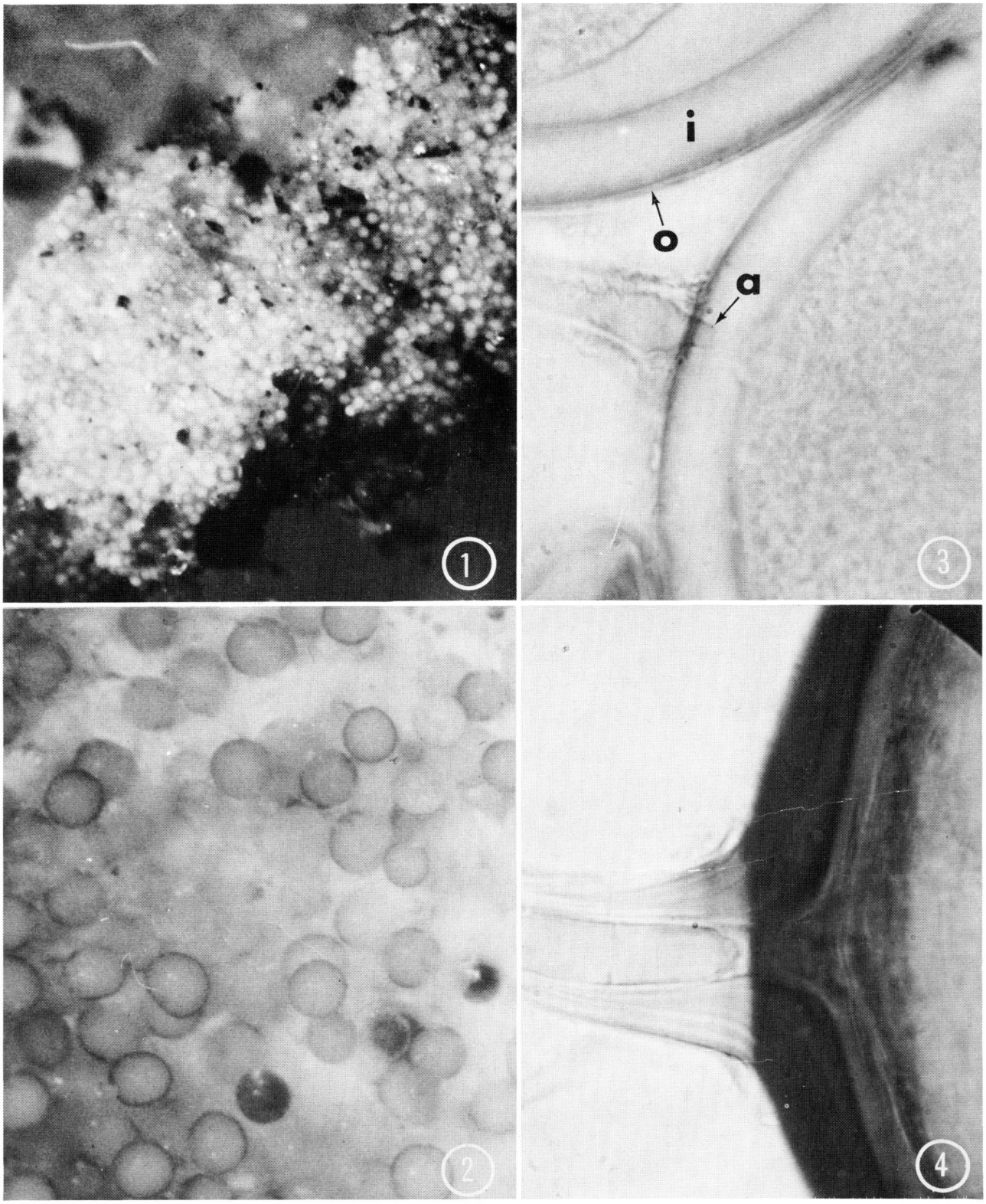
Chlamydospores globose to subglobose or broadly ellipsoid, (60–)75–140(–165) × 95–140 μm, smooth, white in youth, by maturity light yellowish brown to light brown, as mounted in H_2O, KOH, or lactophenol golden yellow to bright brownish yellow. Spore walls 5–8(–10) μm thick, including a subhyaline outer layer 0.5–1 μm adhering to a thick inner layer that ranges from yellow throughout to light orange – brown at the outer surface grading to yellow at the inner surface, in Melzer's reagent orange, in cotton blue – lactophenol cyanophilous in youth but acyanophilous at maturity. Hyphal attachment typically 4–6 μm diameter, often inserted into the spore wall and occluded septum-like at the inner part of the wall, the subtending hypha usually expanded abruptly to 6–10 μm diameter at the spore surface and with thin or only slightly thickened, hyaline to pale yellow or pale brownish pink walls. Spore contents of uniformly small, hyaline oil globules 2–5 μm diameter in sporocarpic spores. Sporocarp hyphae 3–15(–30) μm diameter, much branched, with occasional, irregular swellings, the walls hyaline to pale yellow or brown and thin to ± 1 μm thick, cyanophilous in cotton blue – lactophenol.

DISTRIBUTION AND HABITAT: Subtropical forests of central Mexico and Ecuador; on and in soil of potted plants in greenhouses at Corvallis, Oregon, and Arcata, California.

MYCORRHIZAL ASSOCIATIONS: Field associations unknown; in greenhouses, associated with potted plants of *Angiopteris evecta*, *Araucaria excelsa*, *Asparagus officinalis*, *Passiflora* sp., *Philodendron* spp., and *Sorghum vulgare*.

ETYMOLOGY: Latin, *epigaeus* (growing upon the ground).

COLLECTIONS EXAMINED: Holotype: U.S.A.: Oregon, Benton Co., Corvallis, United States Department of Agriculture – S.E.A. Ornamental



FIGS. 1–3. *Glomus epigaeus*. Fig. 1. Sporocarp. × 15. Fig. 2. Spores. × 70. Fig. 3. Spore walls, showing thin outer layer (*o*) and thick inner layer (*i*) and insertion of hyphal attachment (*a*) into the spore wall. × 1000. FIG. 4. *Glomus macrocarpus*. Laminated spore wall and typical hyphal attachment. × 1000.

Plants Laboratory greenhouse, on surface of soil of potted asparagus, leg. B. Daniels, 13 December 1977 (Trappe 5174, OSC).

PARATYPES: U.S.A.: Oregon, Benton Co., Oregon State University botany greenhouse, leg. J. W. Gerdemann, October 1969 (Trappe 2074, OSC); leg. J. Trappe, 12 March 1970 (Trappe 2133, OSC). California, Humboldt Co., Humboldt State University greenhouse, leg. Robert Ames, 10 November 1974 (Trappe 4028, OSC). MEXICO: Morelos, 10 km north of Cuernavaca along Carretera Vieja Cuernavaca – Mexico on soil under humus in subtropical *Pinus–Quercus* forest, leg. J. Trappe, 12 September 1972 (Trappe 3470, OSC and ENCB). ECUADOR: Morona, 13 km from Limón (General Plaza Gutierrez) on the Limón–Mendéz road, leg. K. P. Dumont, S. E. Carpenter, and P. Buriticá, 3 August 1975 (Dumont EC-2165, NY and OSC).

The earlier collections of *Glomus epigaeus* (Trappe 2074 and 2133) were included with "small-spored" *Glomus macrocarpus* Tul. & Tul. by Gerdemann and Trappe (1974). Reexamination of these and the other collections cited has, however, revealed some constant differences between these two species. *Glomus epigaeus* characteristically forms epigeous sporocarps; the spore walls are bright yellow to bright yellowish brown in transmitted-light microscopy; the spore contents are of uniformly small oil globules; and, perhaps most obviously, the hyphal attachments are nearly always 10 μm or less in diameter, with little or no wall thickening of attached hyphae and with the spore wall occluded by an inner, septum-like deposit that leaves an insertion of the attached hypha into the outer spore wall. *Glomus macrocarpus*, in contrast, normally does not form epigeous sporocarps; its spore walls are yellowish brown to dark brown in transmitted-light microscopy; its spore contents include oil globules ranging from small to nearly filling the spore; and its hyphal attachments are usually larger than 10 μm diameter, with walls of attached hyphae greatly thickened to occlude the opening (Fig. 4). The spore diameter of *G. epigaeus* ranges from that typical of *G. fasciculatus* (Thaxter) Gerd. & Trappe into that of 'small-spored' *G. macrocarpus*. In characters other than spore size, *G. fasciculatus* resembles *G. macrocarpus* rather than *G. epigaeus*.

Other 'tropical' Endogonaceae have been found in northern greenhouses: *Sclerocystis coremioides* Berk. & Br. and *S. dussii* (Pat.) von Höhn (Gerdemann and Trappe 1974). At the same time, many species of Endogonaceae occur from tropical to temperate and subalpine habitats (Gerdemann and Trappe 1974; Trappe 1977). *Glomus epigaeus* has been designated as '*G. macrocarpus*' in the past, so it may have been misidentified in collections from temperate zones. Reexamination of such collections in light of our data may reveal a much broader distribution for *G. epigaeus* than is now known.

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