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# *Glomus patagonicum* sp. nov. (Glomerales), a new arbuscular mycorrhizal fungus from Argentina

by

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With 2 figures and 1 table

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**Abstract**: *Glomus patagonicum* sp. nov. was found in the rhizosphere of *Bromus setifolius* near El Calafate in Santa Cruz province, Argentina. The species is distinguished by the presence of large ornamented warts on the outer surface of the spore and on the walls of the subtending hyphae. The fungus colonised roots of *Lycopersicon esculentum* and *Trifolium repens* and formed Paris-type mycorrhiza.

**Key words**: *Bromus setifolius*, Glomerales, Paris-type mycorrhiza, Patagonia.

## Introduction

Spores of an unidentified species of the genus *Glomus* Tul. & C. Tul. were recovered in the southern province of Santa Cruz, during a survey of arbuscular mycorrhizal colonisation of *Bromus setifolius* J. Presl associated with *Mulinum spinosum* (Cav.) Pers. (Neneo). The characteristics of the species do not match any published species from of *Glomus* because of the presence of large warts on the outer surface of the spore and subtending hypha. We describe it here as a new species.

### Materials and methods

Five rhizosphere samples of native *Bromus setifolius* associated with *Mulinum spinosum* were collected at depths of 20-30 cm in a field located at 50° 24' 18" S and 72° 44' 33" W in Santa Cruz

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Province, Argentina. The site is close to the *Nothofagus* forest domain, in the southern part of the Andes. It is characterised by annual precipitation that exceeds 300 mm and strong winds. The soil was a sandy loam of pH 5.5, with 6.52% C, 0.525% N, 10.9% P, 16.8% $Ca^{++}$, 2.7% $Mg^{++}$, 0.05% $Na^{+}$, and 2.0% $K^{+}$.

Soil samples were mixed and kept at 5°C until used. Spores were extracted from soil by wet-sieving and decanting (Gerdemann & Nicolson 1963) and were used for morphological description, plant inoculation and germination assays. Descriptions were based on approximately 50 spores, uniform in colour, shape and size, selected from 236 spores with similar morphology and collected from field samples. They were mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG) (Koske & Tessier 1983) or PVLG and Melzer's reagent (1:1 v/v). Spores were photographed with a MC 100 Zeiss camera connected to an Axioskop Zeiss microscope.

Spore-wall characteristics and terminology are those suggested by Walker (1983). Classification follows that of Morton & Benny (1990). Spore colour was determined according to Munsell's colour table (1954). Voucher specimens were preserved in (PVLG) and deposited in the herbarium of the Department of Biological Sciences, School of Exact and Natural Sciences, University of Buenos Aires (BAFC) and in the Herbarium LPS at the "Instituto de Botánica Carlos Spegazzini", La Plata, Buenos Aires Province.

Multispore cultures were set up using tomato *(Lycopersicon esculentum* Mill.) and white clover (*Trifolium repens* L.) as hosts. Plants were grown in 5 cm diam. Petri dishes with autoclaved vermiculite (120° C, 20 min). Seeds were surface-sterilised with 10% hypochlorite solution in water for 2 min and sown on the vermiculite. The Petri dishes were incubated in a greenhouse at 20-25°C and watered three times per week with deionised water and Hewitt solution (Hewitt 1952). Spore inoculation was carried out using 20-30 morphologically similar spores from field-collected samples. Spores were applied to a hole made in the surface of the autoclaved vermiculite (Sieverding 1991). Mycorrhizal colonisation was observed on root pieces excised from cultivated plants after three months of culture and from field collected *B. setifolius* plants. Roots were stained with Trypan blue (Phillips & Hayman 1970) and mounted in PVLG.

Some spores, collected from field samples, were surface-sterilized with 50 ml of sterilized water plus 1 g of chloramine T together with 20 g of streptomycin and a trace of surfactant (Mosse 1962). Surface-sterilised spores were transferred to a 5 cm diam. Petri dish containing 0.4% Gel-Gro in 10 mM 2-(N-morpholin) ethanesulphonic acid (MES) buffer (pH 7), incubated at 25°C and observed periodically for germination under a binocular microscope.

Since spores did not germinate we were unable to determine the ontogenetic sequence of wall layer formation.

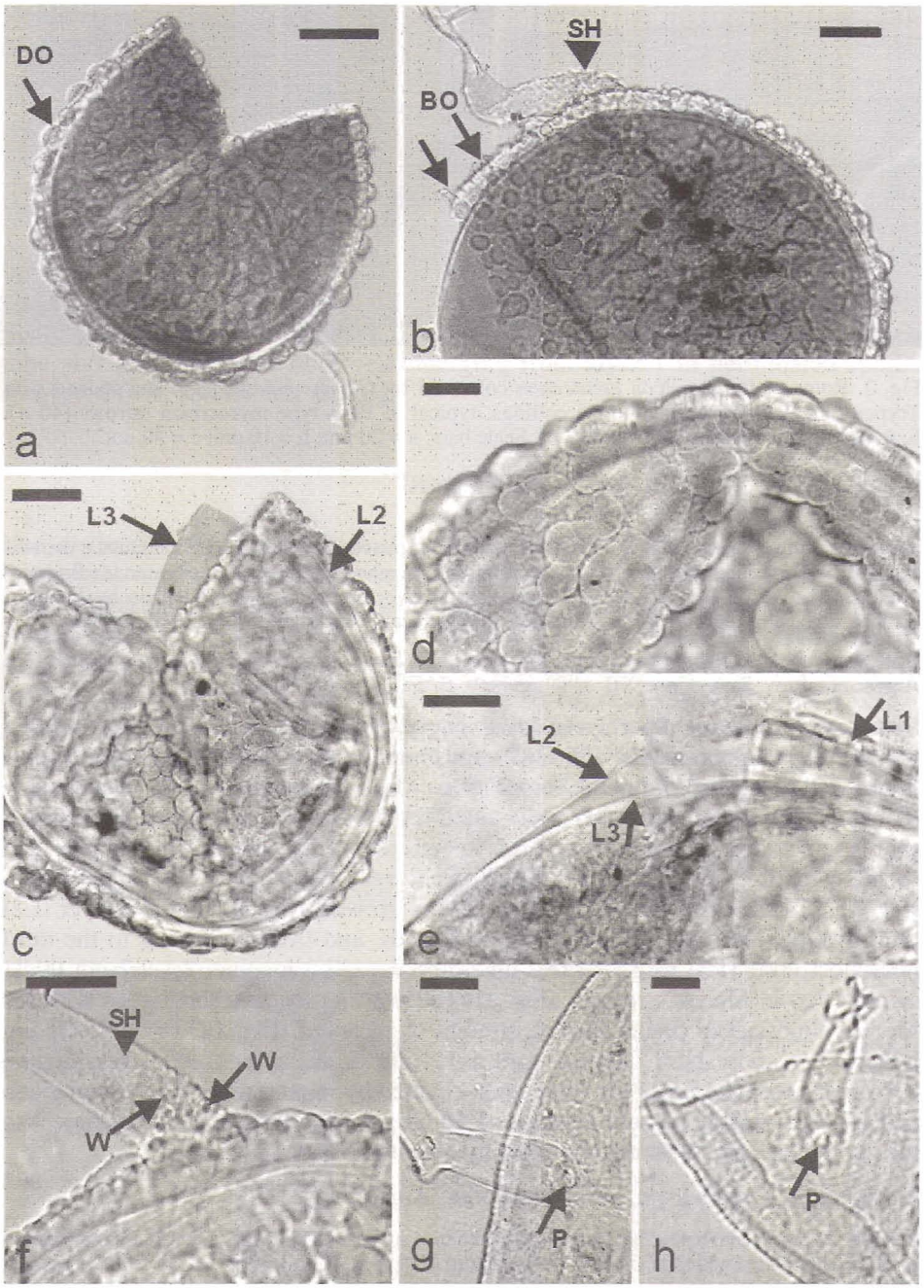
## Results

### Taxonomy

**Glomus patagonicum** Novas et Fracchia, sp. nov. Fig. 1

Sporocarpia ignota. Sporae hyalinae vel pallide luteae, singula in solo, globosae vel subglobosae, (98-)136(-187) µm diam., ornamentatione inclusa.

Fig. 1. *Glomus patagonicum* spore. a. Crushed spore mounted in PVLG showing ornamentation and wall structure (40 ×). Arrow pointing to dome-shaped outgrowth (DO). b. Spore mounted in PVLG and Melzer's reagent, showing ornamented subtending hypha (SH); arrows pointing to the bottle-shaped outgrowths (BO) (40 ×). c. Crushed spore showing wall structure; arrows pointing to layers 2 (L2) and 3 (L3) (40 ×). d. Crushed spore showing ornamentation (40 ×). e. Spore showing wall layers (L1, L2 and L3) (100 ×). f. Subtending hypha showing minute warts (W) continuous with L1 of spore wall (100 ×). g-h. Pore (P) not occluded (100 ×). Scale bars: a = 30 µm; b, c = 20 µm; d = 10 µm; e = 5 µm; f = 20 µm; g = h = 15 µm.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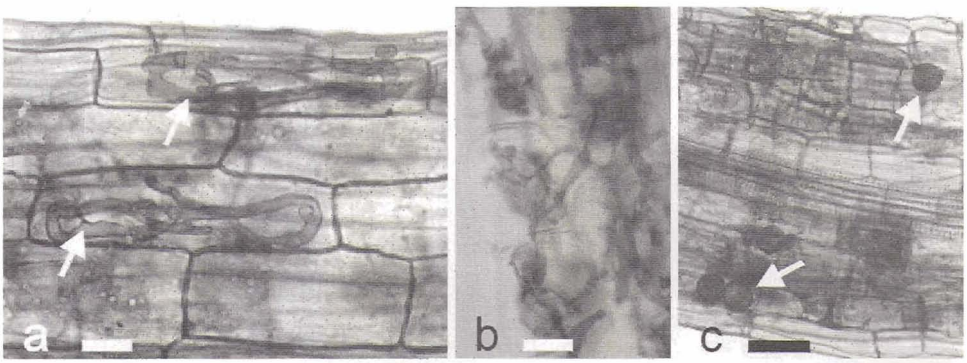


Fig. 2. Roots of *Lycopersicon esculentum* colonised by *Glomus patagonicum* and stained with Trypan blue. a. Intracellular fungal coilings, typical of Paris-type mycorrhiza (arrow) (40 ×). b. Arbuscules. c. Vesicles (arrows) (20 ×). Scale bars: a = 20 µm; b = 10 µm; c = 40 µm.

Paries sporarum tristratosus (1-3). Tunica externa 3.5-4.5 µm crassa, hyalina, rigida, laminata, verrucis exiguis praedita; tunica media ad 0.5 µm crassa, hyalina, membranacea, tunicae internae arcte adhaerens; tunica interna 2.4-2.7 µm crassa, hyalina, amorpha, iodi ope rubra. Hypha subtendens hyalina, non occlusa, ad basim sporarum 15.5-21 µm lata. Mycorrhizam vesiculari-arbuscularem formans.

Holotypus: ex solo rhizosphaerico ad *Bromum setifolium* iuxta *Mulinum spinosum* (Neneo), in Argentinia, Prov. Santa Cruz, XII. 1999, in herb. BAFC N° 50704 conservatus.

Sporocarps unknown. Spores occurring singly in the soil, hyaline to light yellow; globose to subglobose (98-)136(-187) µm diam., with a single subtending hypha. Spore wall composed of three layers (Fig 1e). Outermost wall layer (L 1) consisting of a hyaline, two-laminated and ornamented layer, 3.5-4.5 µm thick, which in young spores is covered by minute warts 0.5-1.3 µm diam. that increase in diameter and form in mature spores dome-shaped structures, (10.0-)10.5(-15.5) µm diam. (Fig. 1a, 1d) or bottle-shaped 1-4 µm high outgrowths (Fig 1b). Middle layer (L2) thin, hyaline, less than 0.5 µm thick, with folds, and firmly adhering to the third innermost layer (L3) (Fig. 1c, 1e). Innermost layer L3 hyaline, 2.4-2.7 µm thick, staining red in Melzer's reagent (Fig. 1c). Subtending hypha concolorous with the spore, cylindrical to funnel-shaped, straight or recurved (Fig. 1a, 1b); 15.5-21 µm wide at the spore base. Hyphal wall 1-1.5 µm thick, composed of two layers, continuous with the L1 of the spore wall, covered by minute warts 0.5-1.3 µm diam. and 1.0-1.5 µm high up to a distance of 50-60 µm from the point of attachment to the spore (Fig. 1f). Pore not occluded by a septum (Fig. 1g, 1h).

Etymology: *patagonicum*, from Patagonia, Argentina.

Specimen examined: Argentina. Santa Cruz Province, isolated from rhizospheric soil of *Bromus setifolius* associated with *Mulinum spinosum* (Neneo), Dec. 1999, coll. M. V. Novas, isol. S. Fracchia (Holotype: BAFC 50.704; Isotype: LPS 47216).

*Glomus patagonicum* formed vesicular-arbuscular mycorrhizae in single-species pot cultures with *Lycopersicon esculentum* and *Trifolium repens*. Extraradical hyphae were hyaline, 4.5-8.5 µm wide, and the intraradical hyphae were hyaline 1.0-1.8 µm wide.

Roots stained with Trypan blue revealed a Paris-type mycorrhiza (Fig. 2a). Mycorrhizal colonization of tomato and clover was mostly intracellular. At the penetration point, a hyphal swelling was produced intracellularly from which a wide (5-6 µm) hypha, grew and infected the neighbouring cell, forming extensive coils of intracellular hyphae in the cortex cells (Fig 2a). Arbuscules, with fine terminal hyphal branches, were observed intermingled with these coils (Fig 2b). Intracellular, globose (20-40 µm diam.) vesicles that stained dark in Trypan blue were also observed (Fig 2c). Paris-type mycorrhizae were also observed in field-collected roots of *B. setifolius*, but the assignment of this colonisation to *G. patagonicum* could not be confirmed.

Spores did not germinate in Gel-Gro after 15 days culture. However, a germination tube, 4-7 µm wide, was observed emerging from layer 1 in germinated spores recovered from soil by wet sieving.

## Discussion

The distinctive feature of *G. patagonicum* is the production of large, inflated outgrowths on the outermost spore wall layer. There are other species of *Glomus* with hyaline and ornamented spores but that clearly differ in the spore wall structure. Among them, *G. chimonobambusae* C. Wu & Y. Liu (Wu et al. 1995) has spores of similar size and remarkable ornaments on the walls of the spore and subtending hyphae. However, the ornaments are strikingly different in shape. The spores of *G. patagonicum* have domed outgrowths while those of *G. chimonobambusae* have longer, clavate projections. Other species of *Glomus* with ornamented and hyaline spores were compared, including *G. callosum* Sieverding (1988), *G. dominikii* Blaszkowski (1988) and *G. scintillans* Rose & Trappe (1980) (Table 1).

Both Arum- and Paris-types of AM were previously found in *Lycopersicon esculentum*, depending on the fungal species inoculated (Cavagnaro et al. 2001),

Table 1: Comparison of ornamentation (size, shape) and wall reaction between *G. patagonicum* and of other Glomerales species with ornamented spores.

| Species | Ornamentation | Reaction in Melzer's reagent |
|---|---|---|
| *Glomus callosum* | Crowded, minute warts | Information not available |
| *Pacispora chimonobambusae*** | Tiny warts and coarse, clavate projections up to 12.5 µm long | Wall 1 and wall 2 turning yellow, wall 4 pinkish red* |
| *P. dominikii*** | Fine warts 1.7-5.7 × 0.7-1.9 µm diam. | Wall 1 turns yellow, wall 3 staining red or orange-red* |
| *P. patagonicum*** | Minute warts increasing in diameter and forming dome-shaped or bottle-shaped outgrowths | L1 turning yellow, L3 turning red |
| *P. scintillans*** | Knobs 1-3 × 0.4-1.2(-3) µm | No reaction |

* from the original description
** after Oehl & Sieverding 2004

thus providing evidence that the morphology of AM is not solely under plant control but is also influenced by fungal identity.

The occurrence of Paris-type mycorrhizae observed in *Bromus setifolius* may be related with the cold climate, the low solar irradiance characteristic of high latitudes, or the partial shadow of the shrub *Mulinum spinosum*. It has been shown that the development of Paris-type AM is slower than that of Arum-type AM (Brundrett & Kendrick 1990; Cavagnaro et al. 2001). Brundrett & Kendrick (1990) suggested that the slower colonization of Paris type might be beneficial to the host plant, diverting only limited energy supply to the fungus, what might be desirable for plants growing slowly in a woodland environment.

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**Note added in proof**

An important reference concerning family Glomeraceae was published. Oehl & Sieverding (2004) created the genus *Pacispora* including *Glomus scitillans*, *G. doinikii* and *G. echimonobambusae* as species belonging to this genus.

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