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Glomus herrerae, a new sporocarpic species of *Glomeromycetes* from Cuba

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Abstract

A new species forming glomoid spores in large sporocarps (500–900 × 780–1500 µm), two spore wall layers, being swl1 thin (0,3–0,8 µm), semi-persistent and hyaline to light yellow; swl2 thick (12–30 µm), laminated, orange brown to dark red brown was found in semi-natural ecosystems in Cuba and is herein described as *Glomus herrerae*.

Key words – *Glomerales* – morphology – rain forest – taxonomy

Introduction

During many years the taxonomy and classification of arbuscular mycorrhizal fungi (AMF) was almost exclusively based in spore morphology (Thaxter 1922, Gerdemann & Trappe 1974, Morton & Benny 1990) with different pattern of spore development being used to discriminate taxa at the generic level (Gerdemann & Trappe 1974, Ames & Schneider 1979). Species with glomoid development represent a wide group representing approximately 50% of AMF described (Oehl et al. 2011a; Goto & Jobim 2017).

Many new species with glomoid spore development was described during 90's years, exclusively based on available data sets, but this was considered a very difficult task based on limited morphological characters available in glomoid species (Goto et al. 2012a). In 2001, new evidences based on molecular level, showed a larger diversification on AMF evolution, mostly in glomoid species (Schwarzott et al. 2001), culminating in the proposition of *Glomeromycota* phylum (Schuessler et al. 2001).

Ten years later, the taxonomy and classification of arbuscular mycorrhiza (AM) is undergoing a revolution, with concomitant morphological and molecular data being used to improve, classes, orders, families and genera (Goto et al. 2012b, Oehl et al. 2011a,b,c,d, Schuessler Walker 2010), mostly to accommodate glomoid species. Oehl et al. (2011d) was able to show useful morphological features to characterized species groups with glomoid spore development.

Most of the sporocarpic fungi are glomoid species represented in the order *Glomerales* (65 species), whereas the other comprises the orders *Diversisporales* (11) and *Archaeosporales* (1).

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These species are taxonomically neglected in the studies, due to the adoption of the methodology standardized by Gerdemann & Nicolson (1963) for the extraction of glomerospores from rhizospheric soil samples. These species require an active search in areas above the more superficial layers of the soil given to the ecological habit of the semi-hypogeous or epigeous, therefore, the sampling of these species has been neglected in most diversity inventories (Goto et al. 2016, Furrazola et al. 2016). In an effort to access the diversity of sporocarpic species in Cuba, an inventory were conducted in semi-natural and disturbed savannah ecosystems, where having been found rare species (Furrazola et al. 2016) and a new fungus forming glomoid spores in large sporocarps, herein described as *Glomus herrerae* based in recently morphological data available.

Materials & Methods

Study area

The studied area was located at Floristic Managed Reserve (FMR) San Ubaldo-Sabanalamar, located in Pinar del Río Province, south western Cuba. This reserve has 5212 ha and constitutes an uncommon ecotype at country. It is classified like a coastal marine fluvial accumulative flatness, particularly deltaic and lacustrian, and with soils classified like Arenosols, Fluvisols, carbonated. The flora is composed of 321 species belonging to 87 botanical families, with 11 local endemics, distributed in five vegetable formations on white sands. The climate is the Termoxerochimenic, type fairly dry according with Vilamajó (1989), with 3–4 dry months and 1200–1400 mm mean annual month.

Two places were selected where were collected soil samples. The selected place were: A semi-natural savannah with certain degree of disturbance, product of a cattle low-intensity activity at close zones (N 22 08 40,4'; W 83 58 35,2'), dominated by *Scoparia dulcis* L., *Cynodon dactylon* (L.) Pers., *Sida brittonii* León, *Portulaca pilosa* L., *Tephrosia cinerea* L. Pers. and *Stylosanthes* sp. The second one constitutes it a savannah in recuperation (N 22 09 14,5 '; W 83 57 41,6 '), right after 8 years without no kind of agricultural intervention where predominated *Panicum* sp., *Rhynchelitrum repens, Sida cordifolia* L., *Alysicarpus vaginalis* (L.) DC, *Cynodon dactylon* (L.) Pers. and *Portulaca oleraceae* L. with soils analyzed as pH (H2O) = 5.0, P = 5 mg kg^{-1}, 2.31 g dm^3 of organic matter in natural savannahs and pH (H2O) = 5.2, P = 3.3 mg kg^{-1}, 1.6 g dm^3.

Sampling and morphological analyses

Sporocarps were extracted from the soil samples as describing in Furrazola et al. (2011). Subsequently, the analysis of sporocarps and spores were similar to described in recent published papers (Furrazola et al. 2011, Goto et al. 2012c). Spores were mounted on microscope slides either in water, to check unmodified characteristics of spore wall components (Spain 1990) and colour, or permanently in in polyvinyl-alcohol–lacto–glycerin (PVLG), PVLG + Melzer's reagent (Brundrett et al. 1994).

The terminology used to species description was Oehl et al. (2011a), Furrazola et al. (2011) and Goto et al. (2012c). To spore denomination Goto & Maia (2006) was used. Zeiss Axioskop compound microscopes with or without Nomarski differential interference contrast (DIC) were used for observations and digital images were taken with an Axiocam camera and AxioVision (v. 3.1 software at 1300 x 1030 dpi), or with Canon digital cameras.

Arbuscular mycorrhizal cultures

Bait cultures with soils from studied areas were established in greenhouse at Ecology and Systematics Institute on *Sorghum bicolor* (L.) Moench and *Plantago major* L. as host plants, as described by Furrazola et al. (2011) and Torres-Arias et al. (2017).

Results

Glomus herrerae Torres-Arias, Furrazola & B.T. Goto sp. nov ..... Figs 1–9

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Etymology – in honor to Ricardo Herrera, taxonomist that for a long time grouped many researchers in arbuscular mycorrhizal studies in Cuba and South America.

Sporocarps formed in large aggregates (500–900 × 780–1500µm), green brown to dark brown (Fig. 1) adherents in live or dead roots with thousand or hundred spores. Peridium absent. Spores formed in fasciculate arrangement forming a large sporcarp or fascicules free on soil forming spore aggregates. Hypha of the gleba with interwoven arrangement forming many spore aggregates around a central plexus (Figs 1–2). Spores green brown to dark red brown (Figs 1–4) formed terminally or intercalary in subtending hypha (Fig. 3). Spores subglobose (82–110 × 140–210 µm) rarely globose (100–208 µm in diameter).

Spores formed terminally or intercalary on hypha (Figs 3–9), in maturity (Figs 1–9), and may slightly darken to dark brown when ageing in soils. The spores are globose (100–208 µm in diameter) to subgloboses (82–110 × 140–210 µm) rarely elliptic.

Spore wall is 12–30 µm thick in total and consists of two layers (Figs 2–3). The first layer (swl1) is hyaline to light yellow, thin (0.3–0.8 µm), semi-persistent observe in young and mature spore (Figs 2–3). The second layer (swl2) is pigmented orange brown to dark red brown, thick (12–28 µm) laminated, smooth (Figs 2–3). Spore wall layers continuous with subtending hypha layers (Fig. 8). The pigmentation of swl2 is continuous with subtending hypha wall (Figs 4–9). Melzer reaction deep pink to red purple and present only in laminated layers in young spores recently extract from cultures (Fig. 2–3). Mature spores do not present Melzer reaction and young spores lose Melzer's reaction after one week.

Subtending hypha (sh) generally present, single or double, straight, cylindrical to sharply curved (Figs 7–9). The colour of subtending hypha is orange brown to dark brown. The colour of spore is continuous in subtending hypha, generally acquiring light yellow colour after 100–150 µm of spore base. Subtending wall 11–30 µm width (mean-16.7 µm) at the point of attachment, wall 4.0–15 µm thick (mean 7.2 µm) near the spore base, tapering to approx. 1.5–2.5 µm distally; occlusion by septum formed by spore wall (swl2) thickening (Fig. 6). Occlusion of the spore contents is by ingrowths of the laminated spore wall component (Figs 4–9). Germination was not detected and arbuscular mycorrhiza formation is known to *Sorghum bicolor* L.

Spore development was deduced from identified spores in several aggregates found in different developmental stages. The hyaline hyphal wall layer differentiates into a hyaline, semi-persistent spore wall layer (swl1) and then a laminate layer (swl2) that becomes pigmented with increasing numbers of developing sublaminae (8 laminae). After the spores mature, their pore is closed by introverted thickening of swl2 and an additional bridging septum arising from the laminate wall layer.

Known distribution – Only detected in Cuba.

Material examined – Cuba, Floristic Managed Reserve (FMR) San Ubaldo-Sabanalamar, located in Pinar del Río province, south western Cuba, on soil, 15 Jul 2009, Torres-Arias, UFRN Fungos - 2800, URM 90070, holotype – ex-type culture in CUBA.

Notes – So far, the new fungus was only detected in rhizosphere of *S. dulcis, C. dactylon, S. brittonii, S. cordifolia, P. pilosa, T. cinerea, R. repens,* and *A. vaginalis,* from native savannah ecosystems.

Key to AMF species forming large dark brown sporocarps

1. Sporocarps produce dimorphic spores.....2
1'. Sporocarps produces monomorphics spores 3
Sporocarps dark brown to black, forming large aggregates, (315–690 × 424–776 µm). Spores globoses (85–157 µm) to subgloboses (98–166 × 93–157 µm). The first morphotype is dark brown to black, consisting of three layers in the spore wall: swl1 is evanescent, 2–4 µm thick, presenting a reticulate surface; swl2 is laminate, 3-14 µm thick and L3 is membranous (< 1µm thick). Hypha at the point of attachment 10-24 µm wide frequently branched. The second morphotype is hyaline to

subhyaline and it consists of three layers: swl1 is evanescent, 4 µm thick, swl2 is laminated, 2–4 µm thick and swl3 is membranous, 0.4–1 µm thick. Hypha at point of attachment 5–10 µm wide ..... *Glomus ambisporum*

2.’ Sporocarps light to dark brown, forming medium to large aggregates (242–726 × 242–641 µm). Spores globoses to subgloboses. The first morphotype is light to dark brown, large (99–206 × 61–201 µm) and consisting of two layers: swl1 is evanescent (2–7 µm) and swl2 laminate (3–10 µm). Hypha at point of attachment 5–31 µm wide, spores presenting multiple hyphae attached. The second morphotype is hyaline, small (31–102 × 27–68 µm) and consisting of three layers: swl1 evanescent (< 1 µm), swl2 unitary (1–2.6 µm) and swl3 up to 1 µm thickness. Hypha at point of attachment 5–7 µm wide ..... *Glomus heterosporum*

3. Sporocarps produce spores consisting of three layers. Sporocarps pale yellow to brown, small to medium (190–270 × 290–380 µm), producing spores globose to subglobose (30–35 × 40–65 µm), frequently surrounded by branched and convoluted hyphae. It consists of two layers: L1 evanescent, (0.5–)0.8(–1.0) µm thick, L2 laminate (2.7–)3.9(–4.9) µm thick and swl3 (semi)flexible. Hypha straight to recurvate, funnel-shaped, sometimes cylindrical or constricted; (6.1–)7.9(–9.3) µm wide at the spore base ..... *Glomus fuegianum*

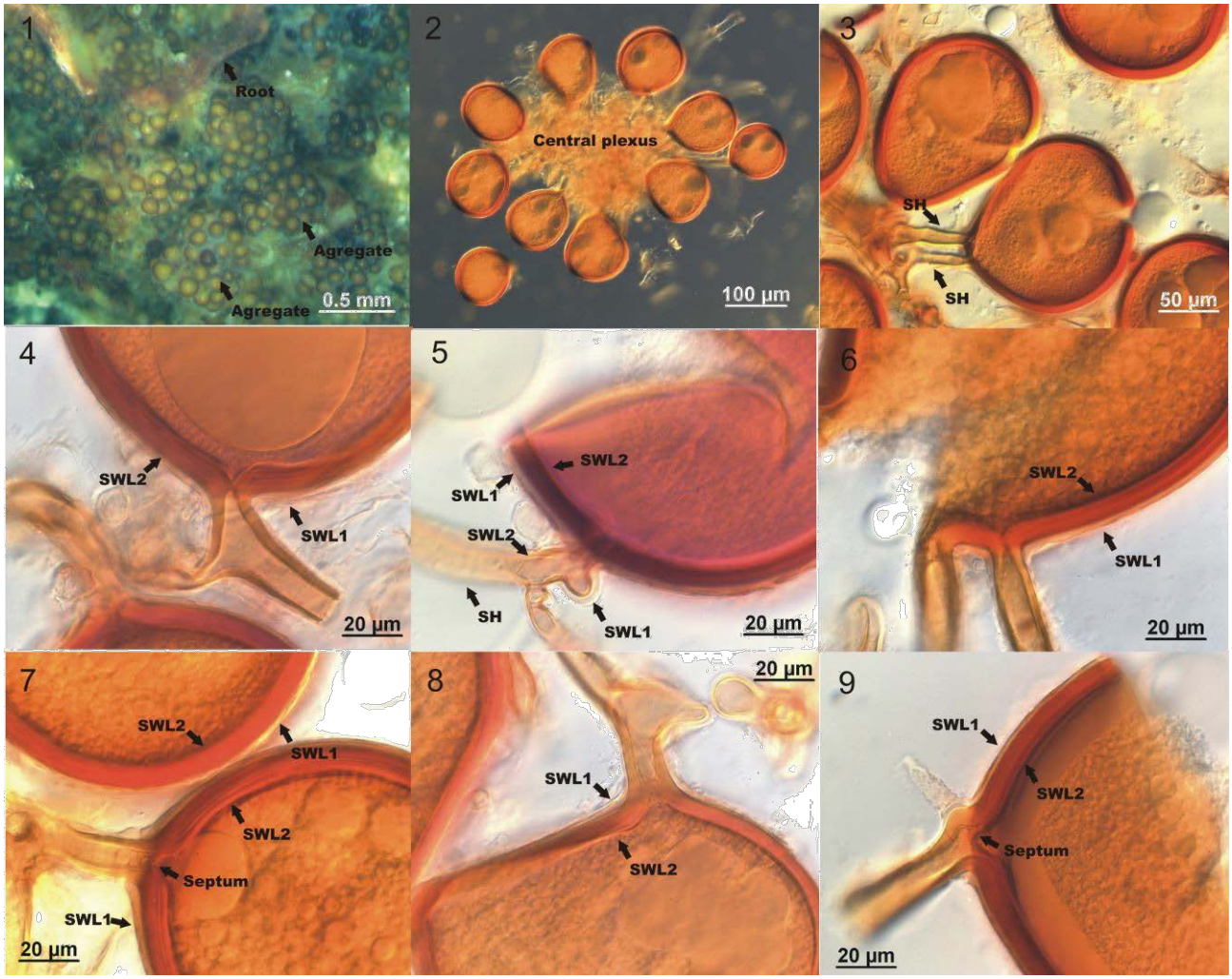
3.’ Sporocarps produces spores consisting of two layers 4

4. Spores stain in Melzer's reagent. Sporocarps forming large aggregates (500–900 × 780–1500µm), green brown to dark brown, producing spores greenish brown to reddish brown, subglobose (82–110 × 140–210 µm), rarely globose (100–208 µm). It consists of two layers, swl1 evanescent (0.3–0.8µm) and swl2 laminated, 12–30 µm thick. Hypha single or double, straight, cilindrical to sharply curved, 11–30 µm wide ..... *Glomus herrerae*

4’. Spores do not stain in Melzer's reagent. Sporocarps forming large aggregates (500–850 x 780–1200 µm), orange brown to dark red brown. Spores subglobose to elliptic (72–92 × 79–105 µm) or rarely globose (72–96 µm). Spore wall consisting of two layers: swl1 evanescent, 0.3–0.8 µm thick, and swl2 laminated, 7.4–15.5 µm. Hypha single, straight or constricted, cylindrical to sharply curved, 5.1–12.7 µm wide ..... *Glomus trufemii*

Discussion

Glomus herrerae is readily distinguished from previously described sporocarpic *Glomus* species by spore colour, size and spore wall. Sporocarps of *G. herrerae* are similar with that *G. ambisporum* G.S. Sm. & N.C. Schenck, *G. fuegianum* (Speg.) Trappe & Gerd., *G. heterosporum* G.S. Sm. & N.C. Schenck and *G. trufemii* B.T. Goto, G.A. Silva & Oehl (Oehl et al. 2011d; Goto et al. 2012c). *Glomus herrerae*, *G. ambisporum* and *G. heterosporum* produce large dark brown sporocarps, but only *G. ambisporum* and *G. heterosporum* produce dimorphic spores. Furthermore, the spores of *G. ambisporum* have three layers in the spore wall, and *G. herrerae* present only two layers. *G. heterosporum* also produce a morphotype with a spore wall composition similar to *G. herrerae*, but the laminated layer (swl2) is notably smaller 3-10µm (Smith & Schenck 1985). *Glomus fuegianum* may be distinguished from *G. herrerae* mainly by the presence of three layers on the spore wall, furthermore, its sporocarps occasionally present a peridium and it produces pale yellow spores, differing to *G. herrerae* that vary from orange brown to dark red brown. *G. trufemii* presents very similar sporocarps in color and size, however, the spores size (72–92 × 79–105µm) and laminated layer (7.4–15.5µm) are smaller and has not Melzer’s reaction in the second layer (Goto et al. 2012c).



Figs 1–9 – *Glomus herrerae*. 1, 2, 3 General aspects of sporocarp and spore arrangement from trap cultures samples. 4, 5, 6, 7, 8, 9 spore wall layers (SWl1 and SWl2). – Bars = 20 µm.

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