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# *Glomus trufemii* (*Glomeromycetes*), a new sporocarpic species from Brazilian sand dunes

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Abstract — *Glomus trufemii,* which forms large aggregates (≤ 850 × 1200 µm) in the rhizosphere of a herbaceous plant community in NE Brazilian sand dunes, is described as new. Its subglobose glomerospores measuring 72–92 × 79–105 µm have two spore wall layers: an evanescent hyaline 0.3–0.8 µm thick outer layer and a laminate orange brown to dark red brown 7.4–15.5 µm thick inner layer. The pigmentation of the subtending hypha is similar but often much lighter than that of the laminate spore wall layer. The spore size and color, spore wall structure, and features of the spore base and subtending hyphae separate this species from similar yellow-brown to brown spored species like *G. badium*, *G. glomerulatum,* and *G. brohultii*.

Key words — morphology, *Glomeromycota*, *Glomerales*, restinga

## Introduction

The Tulasne brothers described the genus *Glomus* in 1844 for *G. macrocarpum* (the type species according to Clements & Shear 1931). Since then many other fungi with glomoid spores like those of *G. macrocarpum* have been named, so that now *Glomus* comprises >60 species. For many years *Glomus* was considered monophyletic based solely on morphological evidence. Morton & Redecker (2001), based on morphological and molecular data, erected the genera *Paraglomus* (*Paraglomeraceae*) and *Archaeospora* (*Archaeosporaceae*), whose members also formed glomoid spores. Schwarzott et al. (2001) inferred from phylogenetic SSU rDNA molecular analyses that *Glomus* is non-

monophyletic. Schüßler et al. (2001) subsequently erected a new phylum, *Glomeromycota*, in which *Glomus* was represented by three phylogenetically different groups: A, B, and C. Oehl & Sieverding (2004) transferred glomoid species with two spore walls (with the inner wall serving as a germinal wall) to their new genus *Pacispora*. Thereafter Walker & Schüßler (2004) transferred *Glomus spurcum* C.M. Pfeiff. et al. to the newly established genus *Diversispora* and order *Diversisporales*.

To date, glomoid glomerospores can be found in species representing *Archaeosporales, Diversisporales, Glomerales,* and *Paraglomerales,* making their identification difficult in the absence of molecular data. Morphological identification has been facilitated recently in a thorough review of glomoid spore-forming species (Oehl et al. 2011), which introduced helpful suggestions based on molecular data associated with morphological differences among the major phylogenetic clades in the *Glomeromycota*. Our description of *G. trufemii* is based exclusively on the new key morphological criteria, and the species fits the Oehl's et al. (2011) revised concept of *Glomus*.

## Material & methods

### Study area and sites

Samples were collected in the Parque Estadual das Dunas de Natal "Jornalista Luiz Maria Alves," the first Conservation Unit created in the Municipality of Natal, Rio Grande do Norte State and currently one of the largest urban conservation areas with dune vegetation in Brazil. The site is located at 5°46′S 35°12′W with soils analyzed as pH ($H_2O$) = 5.5–5.75, P = 2–4 mg $kg^{-1}$, 2,86 g $dm^3$ of organic matter. The climate is tropical rainy (type Am of Köppen) with a short four-month dry period, with a 25.5 °C mean annual temperature and 1191 mm mean annual precipitation. In the sand dune ecosystem, the typical 'restinga' vegetation varies from herbaceous to shrubs and trees (Oliveira-Filho 1993, Oliveira-Filho & Carvalho 1993). The park is composed of mainly represented by tree species of the *Anacardiaceae, Bignoniaceae*, *Fabaceae*, *Myrtaceae*, and *Rubiaceae* (*Anacardium occidentale*, *Caesalpinia echinata*, *Campomanesia dichotoma*, *Chamaecrista ensiformis*, *Eugenia ligustrina*, *Guettarda platypoda*, *Myrcia guianensis*, *Myrciaria tenella*, *Tabebuia roseoalba*, *Tocoyena sellowiana*) and herbaceous species of *Poaceae*, *Cyperaceae, Asteraceae, Araceae,* and *Rubiaceae*.

### Morphological analyses

Glomerospores and sporocarps were separated from the soil samples by sucrose gradient in plate dishes (Błaszkowski et al. 2006). Sporocarps and glomerospores were placed in dishes with water and separated under a dissecting microscope. Sporocarps were fragmented to study spore organization. Glomerospores were mounted on microscope slides either in water (to check unmodified characteristics of spore wall components and spore colour; Spain 1990) or permanently in PVLG (Omar et al. 1979) or PVLG with Melzer's reagent (Koske & Tessier 1983).

Terminology follows Oehl et al. (2011) and Furrazola et al. (2011). The spore denomination of Goto & Maia (2006) was used. Zeiss Axioskop compound microscopes with or without

Nomarski differential interference contrast (DIC) were used for detailed spore observations; digital images were taken with either an Axiocam camera and AxioVision (v. 3.1 software at 1300 × 1030 dpi) or Canon digital cameras.

#### Arbuscular mycorrhizal cultures

Bait cultures for the soils from sand dunes (Parque das Dunas de Natal) with spores and sporocarps were established in the greenhouse of the Universidade Federal do Rio Grande do Norte with *Zea mays* L. as host plant, using sterilized soil and 0.5 L pots.

Pure cultures of the new fungus were attempted by inoculating *Zea mays* seedlings germinated in autoclaved quartz sand with *G. trufemii* sporocarps isolated from sand dunes. After filling 0.5 L pots with sterilized sand dune soil, we formed a small hole in the center using a glass shaker and discharged spores and sporocarps into the hole using Pasteur pipettes. Then a *Z. mays* seedling was placed into the hole, covered with sand, and grown for one cycle of 3–4 months. The fungus did not produce spores in either bait cultures grown even for 9–12 months or pure cultures.

## Taxonomy

***Glomus trufemii*** B.T. Goto, G.A. Silva & Oehl, **sp. nov.** Figs. 1–9

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Differs from *G. brohultii* by more regularly shaped subtending hyphae that are pigmented further below the spore bases and darker colored orange to red brown spores with slightly thicker walls.

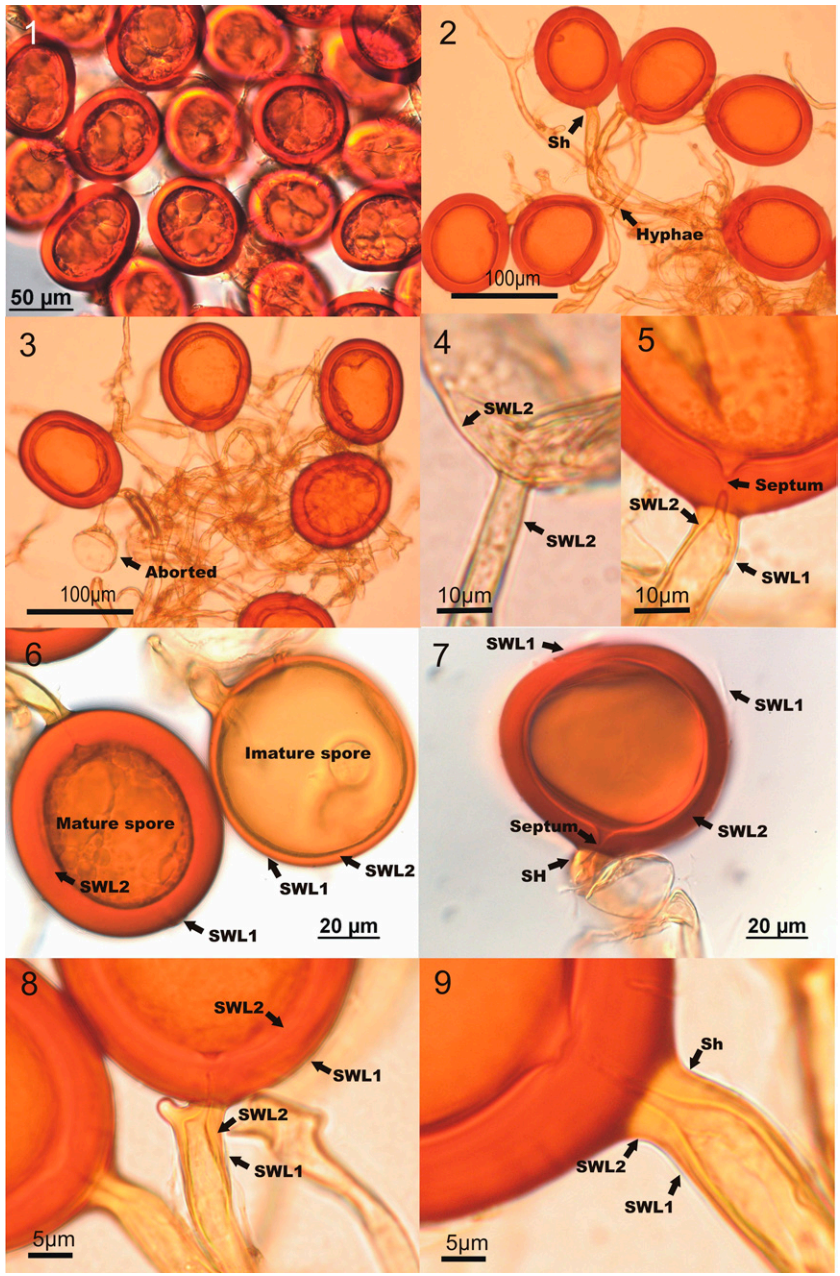
Type: Brazil, Rio Grande do Norte, near Natal, Parque das Dunas de "Natal", sand dunes with herbaceous plant community on a convex hillside, 15.Feb.2010, B.T. Goto, in permanent slide (PVLG) (**Holotype**: UFRN1482; **Isotype**: UFRN1483, ZT Myc 15118).

Etymology: *trufemii* = in honor of Sandra Farto Botelho Trufem, taxonomist, who long taught many young students and researchers in arbuscular mycorrhizal fungal taxonomy.

Sporocarps formed in aggregates (500–850 × 780–1200 µm), orange brown to dark red brown adherent to living or dead roots with hundreds to thousands of glomerospores; rarely singly in soils. Melzer's reaction not observed. Peridium absent. Sporocarpic hyphae hyaline (3.8–)7.6–10.4(–12.8) µm without a septum and with interwoven arrangements.

Glomerospores formed terminally on hyphae, subglobose to elliptical (72–92 × 79–105 µm) or rarely globose (72–96 µm diam.), orange brown when young to dark red brown at maturity, perhaps darkening slightly to dark brown when ageing in soil.

Spore wall 7.4–15.5 µm thick overall, consisting of two layers (swl1, swl2); swl1 thin (0.3–0.8 µm), hyaline and difficult to observe in mature spore, but generally visible in the subtending hyphal wall; swl2 thick (7.4–15.5 µm), pigmented orange brown to dark red brown, laminated, smooth. Spore wall layers continuous with subtending hyphal wall layers. The pigmentation of swl2 continues into the subtending hyphal wall, but this layer is often lighter


1
50 µm
2
Sh
Hyphae
100µm
3
Aborted
100µm
4
SWL2
SWL2
10µm
5
Septum
SWL2
SWL1
10µm
6
Imature spore
Mature spore
SWL2
SWL1
SWL2
SWL1
20 µm
7
SWL1
SWL1
Septum
SWL2
SH
20 µm
8
SWL2
SWL1
SWL2
SWL1
5µm
9
Sh
SWL2
SWL1
5µm

in color in the subtending hypha. SWL1 and SWL2 do not stain when exposed to Melzer's reagent.

SUBTENDING HYPHA (SH) generally present, single, straight or constricted, cylindrical to sharply curved, light yellow to dark yellow; 5.1–12.7 µm diam. (mean-8.8 µm) at the spore base, the hyphal wall 2.6–5.1 µm thick (mean 4.2 µm) near the spore base and tapering to approx. 1 µm at 15 µm from the base; occlusion formed by introverted SWL2 thickening and often through an additional bridging septum arising from SWL2, but sometimes the pore appearing partly open.

ABORTED GLOMEROSPORES frequently observed and forming terminally on hyphae, generally collapsed or subglobose to elliptical (35–56 × 40–46 µm), light yellow to dark yellow, possibly darkening slightly to light brown when aging in soils. Spore wall layer (SWL2) (<3.8 µm) not staining in Melzer's reagent.

GERMINATION structures unknown.

GLOMEROSPORE DEVELOPMENT was deduced from positively identified spores in aggregates found in different developmental stages. The hyaline hyphal wall layer differentiates into a hyaline, evanescent spore wall layer (SWL1) and then a laminate layer (SWL2) that becomes more pigmented with increasing numbers of developing sublaminae. After the glomerospores mature, their pore is closed by introverted thickening of SWL2 and an additional bridging septum arising from the laminate wall layer.

ARBUSCULAR MYCORRHIZA FORMATION remains unknown.

DISTRIBUTION — So far, the new fungus was detected only in Brazil. Known in soil from sand dunes mainly harbouring herbaceous species of *Araceae, Asteraceae, Cyperaceae, Poaceae* and *Rubiaceae* (e.g.: *Acroceras zizanioides* (Kunth) Dandy, *Anthurium affine* Schott, *Aspilia procumbens* Baker, *Centratherum punctatum* Cass., *Cyperus maritimus* Poir., *C. meyenianus* Kunth, *Mitracarpus eichleri* K.Schum., *Raddia brasiliensis* Bertol., *Richardia grandiflora* (Cham. & Schltdl.) Steud.) growing in native, ecologically unstable restinga ecosystems in Parque das Dunas, Natal (Brazil).

## Discussion

*Glomus* species are very common in sand dunes (Błaszkowski 2010; Błaszkowski et al. 2009a,b, 2010a,b) and several new species have been described recently (*G. africanum* Błaszk. & Kovács, *G. achrum* Błaszk. et al.,

FIGS. 1–9. *Glomus trufemii.* 1. Sporocarps in PVLG; note the numerous glomerospores in this part of sporocarp. 2–3. Fragment of sporocarp with healthy glomerospores and aborted spores. 4. Aborted spores with only one spore wall layer (SWL2). 5. Septum formed by SWL2. 6. Spores in different developmental stages. 7. Spore wall layers in mature spore (SWL1&2). 8–9. Spore wall layers (SWL1&2) continuous with subtending hyphal wall (SW) that form distinct pigmentation.

*G. bistratum* Błaszk. et al., *G. indicum* Błaszk. et al., *G. iranicum* Błaszk. et al., *G. majewskii* Błaszk., *G. perpusillum* Błaszk. & Kovács). *Glomus trufemii* is readily distinguished from previously described sporocarpic *Glomus* species by glomerospore size and colour, spore wall structure, (including that at the spore base), and characters of the subtending hypha. Most of these species form colourless or pale-coloured spores singly or in loose aggregates (Błaszkowski et al. 2009a,b; 2010a,b) and thus are easy to distinguish from the sporocarpic dark-spored *G. trufemii*. Sporocarps of *G. trufemii* slightly resemble spore aggregates of *G. aggregatum* N.C. Schenck & G.S. Sm. and *G. fasciculatum* (Thaxt.) Gerd. & Trappe (Schenck & Smith 1982, Gerdemann & Trappe 1974). However, the *G. fasciculatum* SWL2 stains in Melzer's reagent (vs. no reaction in *G. trufemii*). Spores of *G. aggregatum* generally are irregular in shape (vs. globose to subglobose) and have much thinner spore and subtending hyphal walls. The spore wall of *G. trufemii* is one of the thickest among the known glomeromycotan species with glomoid spores.

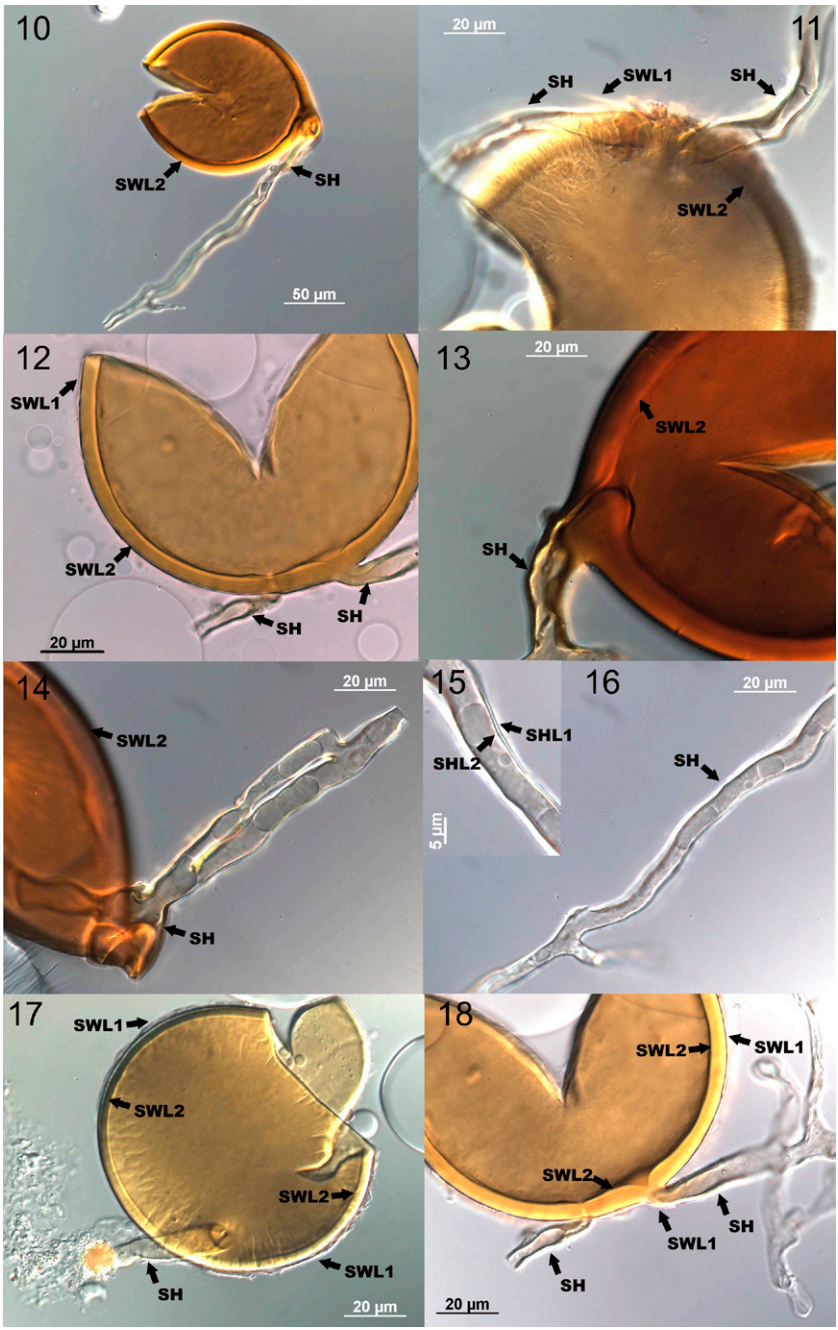
Several *Glomus* spp. form sporocarps without a peridium with similarly sized and coloured spores similarly distributed in a sporocarp: *G. ambisporum* G.S. Sm. & N.C. Schenck, *G. atrouva* McGee & Pattinson, *G. badium* Oehl et al., *G boreale* (Thaxt.) Trappe & Gerd., *G. botryoides* F.M. Rothwell & Victor, *G. brohultii* Sieverd. & R.A. Herrera, *G. flavisporum* (M. Lange & E.M. Lund) Trappe & Gerd., *G. glomerulatum* Sieverd., *G. heterosporum* G.S. Sm. & N.C. Schenck, *G. invermaium* I.R. Hall, and *G. macrocarpum* Tul. & C. Tul. (Oehl et al. 2011). However, of these only *G. badium*, *G. brohultii,* and *G. glomerulatum* also form spores with subtending hyphae generally clearly lighter-coloured than the spore wall (Sieverding 1987, Herrera-Peraza et al. 2003, Oehl et al. 2005, Oehl et al. 2011). In the other species, the wall color of both spore and subtending hyphae is similar or identical (Oehl et al. 2011).

*Glomus glomerulatum* produces intercalary yellow brown glomerospores having 2–4 subtending hyphae (vs only terminal spores with one subtending hypha in *G. trufemii*). Moreover, *G. trufemii* spores are darker (orange-brown to dark reddish brown) and have a significantly thicker wall.

*Glomus badium* forms irregularly sized 3-layered spores around an intrasporocarpic gleba, while *G. trufemii* generally produces regularly sized subglobose 2-layered spores in sporocarps lacking glebas.

*Glomus trufemii* and *G. brohultii* spores have a similar size and wall structure. Also, although *G. brohultii* was not originally described as forming sporocarps

FIGS. 10–18. *Glomus brohultii.* Bi-layered spores (SWL1&2). 10. Glomerospores in PVLG; note the subtending hypha (SH) which is pigmented up to ca. 50 µm below the spore base before becoming subhyaline to hyaline. 11–12. Spores frequently have two subtending hyphae. 13. Mature spore with SWL2 thickened at the spore base. 14. Irregular subtending hypha becoming hyaline in a rather short distance from the spore base (20–30 µm). 16. Hyaline subtending hypha with two layers (SHL1&2). 17–18. Crushed spores with two spore wall layers (SWL1&2).


10
SWL2
SH
50 µm
11
20 µm
SH
SWL1
SH
SWL2
12
SWL1
SWL2
SH
SH
20 µm
13
20 µm
SWL2
SH
14
20 µm
SWL2
SH
15
SHL1
SHL2
5 µm
16
20 µm
SH
17
SWL1
SWL2
SWL2
SWL1
SH
20 µm
18
SWL2
SWL1
SWL2
SH
SWL1
SH
20 µm

or aggregates, sporocarps have been observed recently (Oehl, pers. obs.) during AMF diversity studies in cultivated white and yellow yam sites and adjacent natural savannas in West Africa (Tchabi et al. 2009), ruling out sporocarp or aggregate formation as a diagnostic character separating *G. trufemii* and *G. brohultii*. However, *G. brohultii* spores are paler and lack orange or red ones— yellow-brown to brown, not orange-brown to dark red-brown — and its spore walls are generally thinner. Furthermore, in *G. trufemii* the subtending hyphal pigmentation extends further below the spore base, both in spores formed in sporocarps or singly in the soil. Finally, the shape of the subtending hyphae is much more irregular in *G. brohultii* (Herrera-Peraza et al. 2003: this paper, Figs. 10–18).

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