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Glomus halonatum Rose & Trappe (Glomeromycota) in South America: comments on the morphological characteristics of the species

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RESUMO – (*Glomus halonatum* Rose & Trappe (Glomeromycota) na América do Sul: comentários sobre a morfologia das espécies). Glomerosporos ornamentados de um fungo micorrízico arbuscular foram coletados em uma área de Floresta Atlântica em Goiana (Pernambuco, Brasil). A área tinha sido cultivada com *Coffea canephora* Pierre ex. Froehner [=*Coffea robusta* (L.) Linden)] e *Bixa orellana* L., abandonada, e hoje está coberta por espécies herbáceas invasoras. Após análise taxonômica usando critérios descritivos e terminológicos atuais, a espécie foi identificada como *Glomus halonatum*. São apresentados descrição, ilustrações e comentários sobre a espécie.
Palavras-chave: *Glomeraceae, Glomus*, diversidade, taxonomia

ABSTRACT – (*Glomus halonatum* Rose & Trappe (Glomeromycota) in South America: comments on the morphological characteristics of the species). Ornamented glomerospores of an arbuscular mycorrhizal fungus were collected from an area of Atlantic Forest in Goiana (Pernambuco, Brazil). The area had been cultivated with *Coffea canephora* Pierre ex. Froehner [=*Coffea robusta* (L.) Linden)] and *Bixa orellana* L., then left fallow and today is covered by herbaceous raider species. After taxonomic analysis using current descriptive and terminological criteria, the species was identified as *Glomus halonatum*. A description, illustrations and comments about the species are presented.
Key words: *Glomeraceae*, *Glomeromycota*, *Glomus*, diversity, taxonomy

Introduction

Arbuscular mycorrhizal fungi are widely distributed in terrestrial ecosystems and can be found in both natural and agricultural areas. The documentation of new patterns of species distribution is necessary for the accurate estimation of the diversity and distribution of this important group of symbiotic fungi.

Some morphological characteristics used for the identification of AMF species, such as color and size of spores vary and can be affected by intrinsic or environmental factors (Bentivenga *et al*. 1997; Walker & Vestberg 1998). Thus, isolates of a single species collected from different localities can present variations in color and size, but, although serving as useful morphological support, these differences should not be used on their own to identify a species (Silva *et al*. 2005).

Descriptions of AMF species became more detailed when Walker (1983) proposed the use of terminology based on separation and types of spore walls. Today, with particular emphasis given to the ontogenesis of the spore wall layers most researchers have adopted this terminology (Morton 1995; Stürmer & Morton 1997, 1999; http://invam.caf.wvu.edu).

The purpose of this work was to record the occurrence of *Glomus halonatum* in Brazil, to describe, illustrate, and compare its description with those of similar species, providing additional information on the morphological aspects of this AMF. This species was earlier recorded in England and Mexico, where it was originally described (Rose & Trappe 1980) and, recently, in a list of AMF species found in the rhizosphere of *Eucalyptus*, in Bahia (Carrenho *et al*. 2008).

Material and methods

Soil samples were collected (0–20 cm deep) at random from an area of Atlantic Forest located at the Experimental Station of Itapirema (07°34'00''S; 35°00'00''W) belonging to IPA (Instituto Agronômico de Pernambuco), Goiana Municipality, Pernambuco state, Northeast Brazil). The area had been cultivated with *Coffea canephora* Pierre ex. Froehner [=*Coffea robusta* (L.) Linden], and *Bixa orellana* L., then left fallow, and today is covered by a variety of herbaceous raider species. The local soil has the following characteristics: 3 mg P dm^{-3} of soil; pH in water 6.20; 0.09 (K), 0.00 (Al), 1.55 (Ca) and 0.60 (Mg) $cmol_c$ dm^{-3}; 64% (coarse sand), 24% (fine sand) 2% (silt), and 10% (sandy loam).

Spores of AMF were extracted from soil by wet sieving and sucrose centrifugation (Gerdemann & Nicolson 1963; Jenkins 1964), separated and mounted with PVLG (alcohol polivinyl lactoglycerol) or PVLG + Melzer. Pot cultures were mounted using *Panicum miliaceum* L. and *Sorghum bicolor* L. as plant hosts, during two cycles of four months, though without success on spore multiplication. For the description of the species the terminology proposed by Morton was adopted (http://invam.caf.wvu.edu) and, for spores, that suggested by Goto & Maia (2006).

Results and discussion

Glomus halonatum Rose & Trappe Mycotaxon 10: 413, 1980.

Fig. 1, 2, 3 and 4.

Sporocarps not observed. Glomerospores usually globose (325-) 202.5×200 (-297.5) µm from yellow to orange brown (Fig. 1). Spore wall with three layers. The first evanescent, hyaline, mucilaginous, varying from 12.5–27.5 µm in thickness and in some cases presenting

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a tenuous Melzer's reaction (Fig. 2 and 3). This layer is normally smooth in young spores and granulose or with wide depressions at maturity. In senescent spores it can be absent or totally covered by soil debris. The second layer is laminated (17.5–25 µm), yellow brown to orange brown, with 1.0–2.4 µm tall spines on the external surface (Fig. 4). Occasionally this second layer can be divided, giving the impression that two laminated layers are present. The third layer is hyaline, with membranous appearance (< 2.0 µm) and apparently forms a septum in the insertion of the subtending hypha. One subtending hypha per spore, with a septum near the insertion, yellow to yellow brown wall (17.5–37.5 µm), formed by two layers: an external one continuous with the evanescent layer, hyaline to mucilaginous, and a laminated layer similar to that of the spore and ornamented. This ornamentation is observed for approximately 10 µm in the subtending hypha. No layer produces a distinct Melzer reaction, but an ill-defined reaction on the surface of the evanescent wall was observed. Germinal tube not observed.

Distribution: Mexico and England (Rose & Trappe 1980). In Brazil, spores were found in the rhizosphere of *Eucalyptus* in the state of Bahia (Carrenho *et al*. 2008) and isolated from soil at Goiana, Pernambuco state.

Examined Material: Pernambuco: Municipality of Goiana, *G. halonatum* Rose & Trappe. (X/2002), *Costa***,** URM 45715; isolated from soil in area covered by herbaceous vegetation located at the Experimental Station of Itapirema GERMANY. **Vogtsburg**: *Glomus spinuliferum* Oehl & Sieverding, (VI/2002), *Oehl*, URM 45714 isotype, isolated from areas covered by semi-natural grassland in Vogelsang Pass, near Vogtsburg (Natural Reserve of Kaiserstuhl), Germany.

The morphological characteristics (color, size, and form and wall structure) of glomerospores of *G. halonatum* found in Pernambuco are similar to those described in the protologue (Rose & Trappe 1980), except for the absence of sporocarps and by the thickness of the first spore wall layer. In the examined material this layer was hyaline and evanescent and slightly thicker (12.5–27.5 µm) than that mentioned in the original description (8–20 µm).

Glomus halonatum can be mistaken for *Glomus monosporum* Gerdemann & Trappe due to the similarity of color and ornamentation of the glomerospores (Gerdemann & Trappe 1974), but the latter have only two wall layers, while *G. halonatum* has three spore wall layers.

Glomerospores of *G. halonatum* can also be mistaken for those of *G. pansihalos* Berch & Koske*, G. spinosum* Hu,

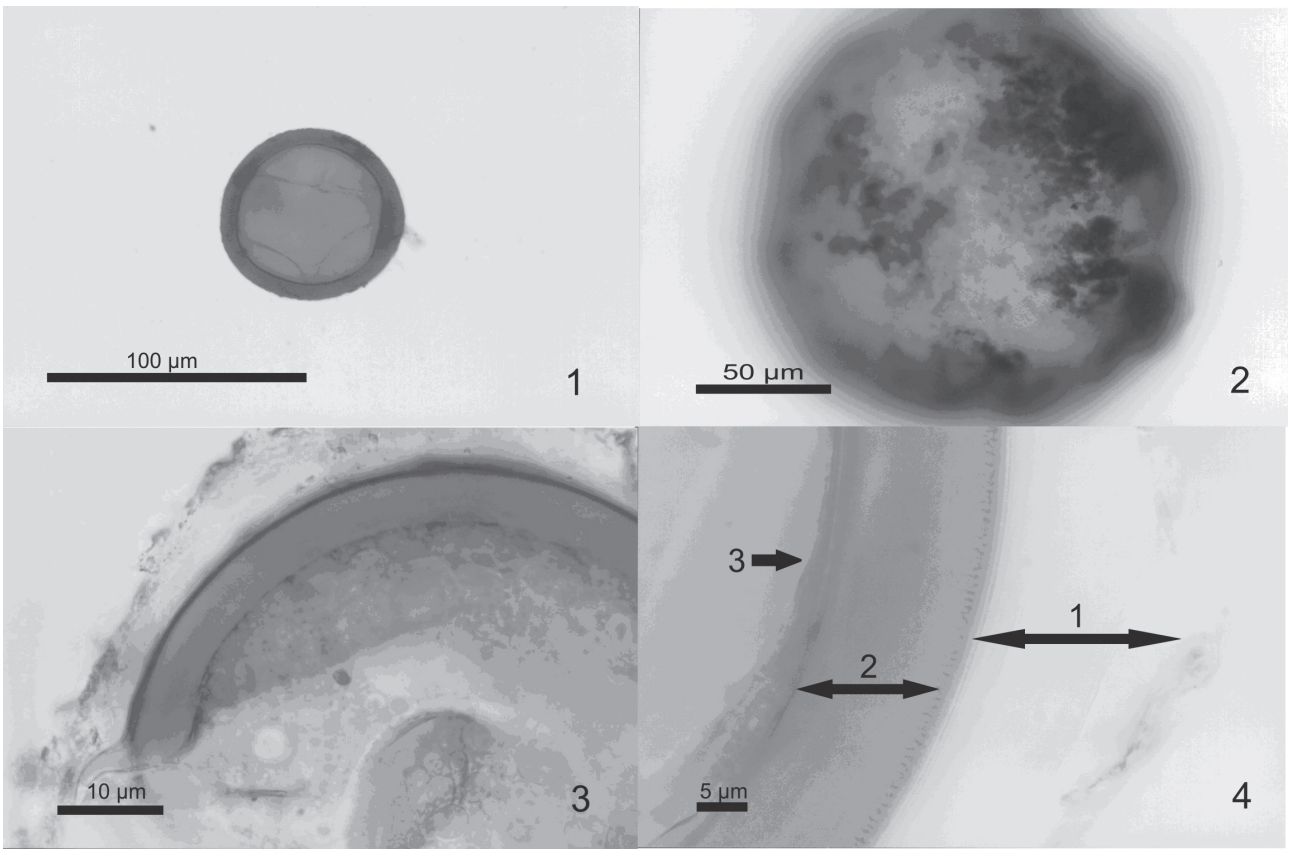

Figure 1–4. *Glomus halonatum* Rose & Trappe. 1- General aspect of a glomerospore in PVLG; 2 – Detail of the evanescent spore wall surface reacting in Melzer; 3 – Detail of the structure of the spore wall showing the thick evanescent wall; 4 – Detail of the three layered wall structure (1, 2, 3) with ornamentation of the second layer (2) immersed in the evanescent layer (1).

G. verruculosum Blaszkowski and *G. spinuliferum* Sieverd. & Oehl. For example, *Glomus pansihalos, G. spinosum* and *G. halonatum* present the same number of spore wall layers and in all of them a hyaline, thick wall layer is present. However, in *G. pansihalos* the external layer presents radial striations in columns when in PVLG (Berch & Koske 1986), while in *G. halonatum* no striations are found. Moreover, *G. halonatum* presents homogenously distributed spine ornamentation, whereas *G. pansihalos* and *G. spinosum* present sparser ornamentation, similar to warts (Berch & Koske 1986; Hu 2002).

The glomerospores of *G. halonatum* have three wall layers whereas those of *G. verruculosum* have only two. Moreover, the latter have round tip ornamentation (warts) (Blaszkowski & Tadych 1997) whereas in *G. halonatum* the ornamentation has spiny tips.

Glomerospores of *G. halonatum* are similar to those of *G. spinuliferum* in color and spore wall structure. Both species have spores with three wall layers, one of which with spines immersed in the evanescent, hyaline and mucilaginous layer. However, *G. halonatum* has bigger spores than *G. spinuliferum* and sporocarps covered by a peridium of hypha loosely arranged (Rose & Trappe 1980), while in *G. spinuliferum* no sporocarps were observed at all (Oehl *et al.* 2003). Rose & Trappe (1980) mentioned one, two or three glomerospores loosely grouped in sporocarps of *G. halonatum*. The technique for extraction of spores from soil can disturb the sporocarps, contributing to the non-observation of such structures in the examined material. Up to now, spore cultures of *G. halonatum* and *G. spinuliferum* were not successful, so the descriptions are based on glomerospores isolated directly from the field (Rose & Trappe 1980; Oehl *et al*. 2003).

Glomus halonatum is also distinguished from *G. spinuliferum* by the thick evanescent layer that forms a halo similar to a ring in most spores. Oehl *et al*. (2003) stated that the external spore wall of *G. spinuliferum* (1-2 µm) can occasionally expand up to 50 µm in thickness in PVLG. This effect has also been described for *Glomus pansihalos* Berch & Koske, *Glomus macrocarpum* Tul. & Tul., *Glomus coronatum* Giovannetti and lately in *Glomus caesaris* Sieverding & Oehl (Berch & Koske 1986; Berch & Fortin 1983; Giovannetti *et al*. 1991; Oehl *et al.* 2002), as discussed by Morton (1986 a,b). It was not possible to observe the expansion of the external wall layer in glomerospores of *G. spinuliferum* probably due to the small number of spores present in the isotype examined. The absence of other distinctive characteristics shows the need for better examination of *G. halonatum* and *G. spinuliferum* isolates, to look for more differences between these species, considering that both are, in some aspects, morphologically similar.

In the same way, none of the species discussed in comparison to *G. halonatum* present this thick, evanescent wall layer, forming a distinct halo around the glomerospore. Thus, *G. halonatum* differs from all other *Glomus* species by presenting this distinctive morphological feature.

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