

Ambispora brasiliensis*, a new ornamented species in the arbuscular mycorrhiza-forming *Glomeromycetes

BRUNO TOMIO GOTO^{1*}, LEONOR COSTA MAIA¹ & FRITZ OEHL²

^{1*}brunogoto@hotmail.com

¹*Departamento de Micologia, CCB, Universidade Federal de Pernambuco Av. Prof. Nelson Chaves s/n, Cidade Universitaria, 50670-420, Recife, PE, Brazil*

²*Zurich-Basel Plant Science Center, Institute of Botany, University of Basel Hebelstrasse 1, 4056 Basel, Switzerland*

Abstract — A new dimorphic fungal species in the arbuscular mycorrhiza-forming *Glomeromycetes* was found in the Cerrado biome in Minas Gerais (Southeast Brazil) and is here described from acaulosporoid glomerospores under the epithet *Ambispora brasiliensis*. Glomoid glomerospores of this species were found only once, connected to a germinating acaulosporoid glomerospore. The acaulosporoid glomerospores are formed on a pedicel branching from the neck of a sporiferous saccule. They are hyaline to pale yellow in color, globose, 62–95 µm in diameter, to subglobose to oval, 59–88 × 69–100(–118) µm, and with three spore walls. Crowded irregular pustules, 2.4–7.0(–10.0) µm high and 4.2–9.8(–15.0) µm wide, are formed on the surface of the outer wall. The glomoid glomerospores are hyaline to subhyaline, globose, 25–30 µm in diameter, and have a bi-layered spore wall without ornamentation. The acaulosporoid glomerospores of the new fungus can easily be distinguished from all other species in the genus *Ambispora* by spore size and by the distinct pustulate ornamentation.

Key words — *Glomeromycota*, *Ambisporaceae*, bimorphic, germination

Introduction

Species of the genus *Ambispora* C. Walker et al. of the arbuscular mycorrhiza (AM) forming *Ambisporaceae* C. Walker et al. (*Glomeromycetes*) can form acaulosporoid and glomoid glomerospores (Spain et al. 2006, Walker et al. 2007a, Walker 2008). For some species only the acaulosporoid morph is known (e.g. *A. jimgerdemannii* (N.C. Schenck & T.H. Nicolson) C. Walker), for others only the glomoid morph (e.g. *A. leptoticha* (N.C. Schenck & G.S. Sm.) C. Walker et al. and *A. callosa* (Sieverd.) C. Walker et al.), while at least *A. appendicula* (Spain et al.) C. Walker, *A. gerdemannii* (S.L. Rose et al.) C. Walker et al. and *A. fennica* C. Walker et al. definitely form both morphs (Spain et al. 2006, Walker et al. 2007a, Walker 2008). There has been some confusion regarding the valid taxonomic nomenclature of *Ambisporaceae* and its type genus *Ambispora* in the

recent literature resulting from past incorrect re-combinations and synonymies (see review by Spain et al. 2006), or obviously illegitimate names attributed by Spain et al. (2006) at the genus level (see Walker et al. 2007a,b, 2008).

Acaulosporoid glomerospores of *Ambispora* can quite easily be recognized by their morphology (Spain et al. 2006) whereas the glomoid morph might be mistaken with glomerospores of the *Glomeraceae*. Indeed, Walker et al. (2007a) identified *Glomus callosum* Sieverd. as belonging to *Ambispora* only using molecular biological techniques.

Acaulosporoid glomerospores of an undescribed *Ambispora* species were detected in rhizospheric soil from a natural ecosystem of the Brazilian Cerrado biome in Serra do Cipó (Minas Gerais State, Southeast Brazil). The species, characterized by a distinct pustulate ornamentation on the outer spore wall, is presented here under the epithet *Ambispora brasiliensis*.

Material and methods

Soil sampling and culturing of AM fungi

Soils were sampled in the Cerrado biome of Serra do Cipó (Minas Gerais State, Brazil) from the rhizosphere of typical native plant vegetation, mainly consisting of *Vellozia caruncularis* Mart. ex Seub., in July 2004. The site is located at 19° 06'S and 43° 35'W. Soil pH (H₂O) was 4.7.

For culturing the native AM fungal communities, 50 g of the field soil were mixed with 400 g autoclaved sand-vermiculite substrate (1:1 w/w, pH 5.5) and filled into 500 ml pots. *Sorghum bicolor* (L.) Mönch was used as bait plant. The culture pots were maintained at the greenhouse of the Department of Mycology, Universidade Federal de Pernambuco, Recife. Additionally, multiple glomerospores of the new species were separated and used as infective propagules in single species cultures using the same pot size and sand-vermiculite culture substrate as described above. So far, all trials failed to establish a successful symbiosis with the new AMF species both in bait cultures and in single species cultures.

Morphological analyses

Acaulosporoid glomerospores were extracted from field soils and the culture substrates by wet sieving (Gerdemann & Nicolson 1963) and sucrose centrifugation (Jenkins 1964). The spores were thereafter isolated under a stereo microscope and mounted in polyvinyl-lacto-glycerol (PVLG), PVLG + Melzer's reagent (Brundrett et al. 1994) and in water, respectively. For the description of the glomerospores and the spore walls, the terminology of Spain et al. (2006) recently presented for species of the genus was used. We use the term glomerospore for all spores formed by species of the *Glomeromycetes*, as proposed by Goto & Maia (2006).

Description of the new species

Ambispora brasiliensis B.T. Goto, L.C. Maia & Oehl, sp. nov.

Figs. 1–9

MYCOBANK MB 511612

Sporocarpia ignota. Sporae dimorphae; spora acaulosporoideae hyalinae vel subhyalinae, globosae (62–95 μm in diametro) vel subglobosae vel ovaes (59–88 \times 69–100(–118) μm , cum ternibus tunicis stratis pluribus, in solo singulatim formatae appendice lateraliter ex hypha terminata in sacculo sporangifero; spora cum tunica exterior, tunica media et tunica interior: tunica exterior tunica mediaque coniuncta sacculi et hyphae; tunica exterior stratis tribus in totum 2.7–3.8 μm crassa, stratum exterius tunicae exterioris hyalinum, evanescens, 0.4–0.7 μm crassum; stratum secundum hyalinum at flavo-pallidum, 2.2–3.2 μm crassum, pustulibus irregularibus ornatum altis 2.4–7.0(–10) μm et 4.2–9.8(–15) μm latis ornatum; stratum interius tunicae exterioris hyalinum at flavo-pallidum, subtile; tunica media duobus stratis hyalinis in totum 2.8–5.2 μm crassa; tunica interior de novo formans stratis duobus vel tribus, in totum 2.2–2.9 μm crassa. Spora glomoidae hyalinae vel subhyalinae, 25–30 μm in diameterum, stratis duobus. Holotypus hic depositus 78879: URM.

ETYMOLOGY: *brasiliensis* referring to Brazil, the country where the species was found.

GLOMEROSPORE FORMATION. Sporocarp formation is unknown. The species is dimorphic producing acaulosporoid and glomoid morphs. Acaulosporoid glomerospores form singly in soils upon a short pedicel arising laterally on the tapering hyphal neck of a sporiferous saccule (Figs. 1–3). The acaulosporoid glomerospores form three spore walls: an outer wall (ow), a middle wall (mw) and an inner wall (iw) (Figs. 4–9). Glomoid glomerospore formation was observed once on a hypha connected to a germinating acaulosporoid morph.

SPORIFEROUS SACCULE OF ACAULOSPOROID GLOMEROSPORES are hyaline to light yellow, globose to subglobose, 65–115 μm in diameter and formed terminally on a hypha (Figs. 1–2). The saccule wall generally is bi-layered with a hyaline to light yellow, rapidly degrading, evanescent outer layer (0.5–0.9 μm thick) and a hyaline to subhyaline, semi-persistent inner layer (0.6–1.5 μm thick). The hyphal neck of the sporiferous saccule is 10–17 μm wide at the saccule base tapering to 8–12 μm at the point where the pedicel of the acaulosporoid glomerospore branches, narrowing further to 5–8 μm at a distance of 40–120 μm from the pedicel. A septum arises in the tapering hyphal neck distal to the saccule where the pedicel forms. The sporiferous saccule generally collapses and detaches from the mature spore while the pedicel often persists on the glomerospore.

ACAULOSPOROID GLOMEROSPORES form singly on a short hyphal pedicel (Fig. 3). The spores are globose (62–95 μm in diameter) to subglobose to oval, 59–88 \times 69–100(–118), hyaline to subhyaline in water and generally become light yellow to ochre-yellow in PVLG-based mountants. The two wall layers of the saccule continue on the glomerospore differentiating into two separate glomerospore walls: a two to three layered outer wall (ow) and a bi-layered middle wall (mw).

A two-to-three layered inner wall (IW) forms *de novo*, presumably after the spore pore has been closed by the inner layer of the middle wall (MWL2) which differentiates from MWL1. The IW functions as a germinal wall.

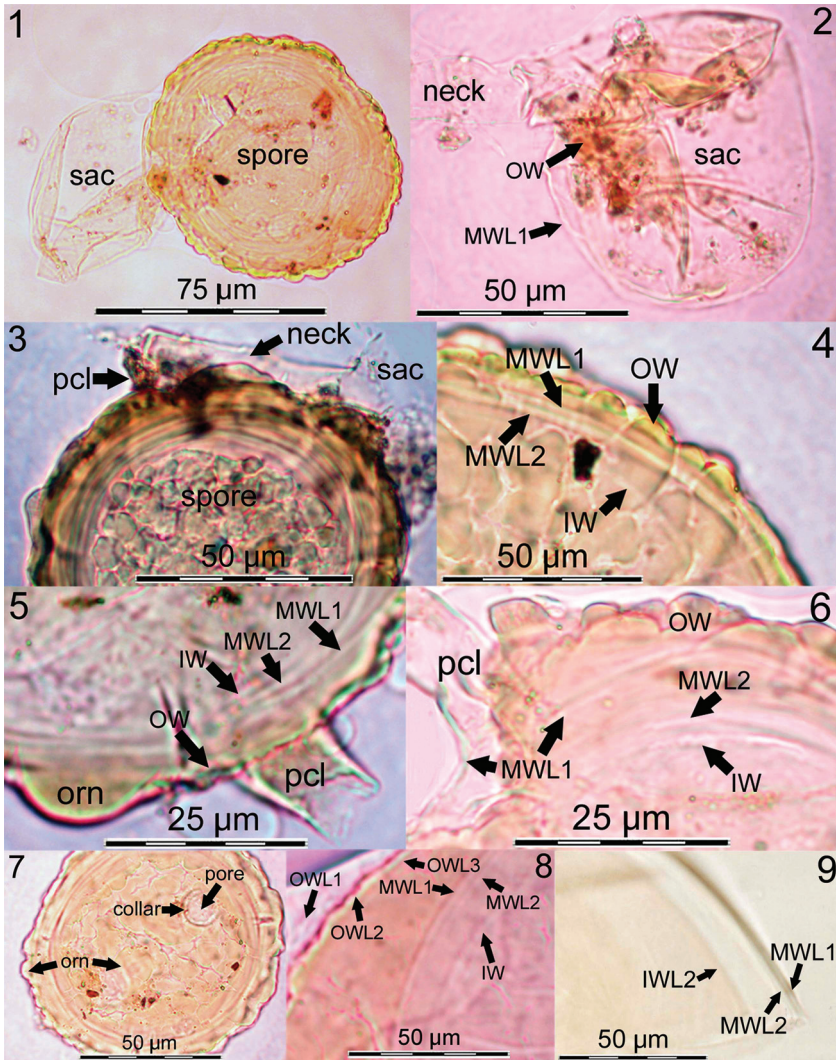
OUTER WALL generally consists of three layers (Figs. 4–8). The outer layer (OWL1) is hyaline to subhyaline, evanescent, 0.4–0.7 μm thick, and often is difficult to detect, as it tightly adheres to OWL2 (Fig. 8). OWL2 is hyaline to light yellow and in total 2.2–3.2 μm thick and crowded with conspicuous, convex pustules (Fig. 3–8). The pustules vary greatly in shape and size and are generally 2.4–7.0(–10) μm wide and 4.2–9.8(–15) high (Figs. 4–7). The inner layer (OWL3) is thin (0.3–0.6 μm), hyaline to subhyaline and tightly attached to OWL2 making it difficult to detect. In older spores, the outer wall might degrade completely, but usually some fragments remain on the surface of the middle wall.

MIDDLE WALL is bi-layered and in total 2.8–5.2 μm thick (Figs. 4–6; Figs. 8–9). MWL1 is hyaline, 1.3–2.6 μm thick and continuous with the persistent inner layer of the pedicel. A septum arising in the pedicel from MWL1 during spore formation was rarely detected. MWL2 is also hyaline, 1.5–2.6 μm thick, tightly adherent to MWL1 and generally closes the pore of the pedicel.

INNER WALL is hyaline and three-layered but generally only the central layer (IWL2; Figs. 4–6; Fig. 8–9) or the inner two layers (IWL2 and IWL3) are visible. The outer layer IWL1 is 0.3–0.6 μm and difficult to detect, as it is tightly adherent to the central layer IWL2 which is 1.5–2.2 μm thick. The innermost layer is 0.4–0.8 μm thick and also tightly adherent to IWL2 but sometimes separates slightly showing several thin folds when pressure is applied.

PEDICEL OF ACAULOSPOROID GLOMEROSPORE branches from the hyphal neck of the sporiferous saccule in about 40–130 μm distance from the globose saccule terminus. The pedicel is (5–)7–16(–25) μm long, and 8–13 μm wide at the glomerospore base tapering to 5–9(–13) μm at the connection to the hypha. The two wall layers of the hyphal neck of the sporiferous saccule differentiate into the outer and the middle wall of the pedicel (ow and mw). On mature spores, the pedicel often persists resembling a subtending hypha of glomoid glomerospores (Fig. 5). Sometimes only the ow (Fig. 5) or the mw (Fig. 6) of the pedicel persist on the spore; rarely both pedicel walls are found on mature glomerospores. The ow sometimes forms a collar (Fig. 7) on the glomerospore (8.5–14 μm in diameter). The ow, if present on the pedicel, is 1.2–2.5 μm thick at the glomerospore base tapering to 0.5–0.9 μm towards the saccule neck. The mw is 1.3–2.1 μm at the glomerospore base tapering to 0.9–1.5 μm .

GLOMOID GLOMEROSPORES were detected only once on a connecting hypha about 250 μm from a germinating acaulosporoid glomerospore. The hyaline to subhyaline glomoid glomerospores were bi-layered and about 25–30 μm in diameter. The glomerospore wall was 1.9–2.5 μm thick in total, and the



Figs. 1–9: *Ambispora brasiliensis* forming acaulosporoid glomerospores with three walls (outer wall ow, middle wall mw and inner wall iw) upon a short pedicel arising laterally on the neck of a sporiferous saccule. Fig. 1. Acaulosporoid glomerospore and sporiferous saccule (sac) attached. Fig. 2. Saccule with two layers: evanescent outer layer continuous with ow of the spore and semi-persistent inner layer continuous with MWL1 of the acaulosporoid spore. Fig. 3. Pedicel (pcl) and degrading saccule. Figs. 4–6. Irregular pustulate ornamentation (orn) on middle layer of ow; MW has two layers (MWL1 + MWL2). Fig. 5. Pcl wall continuous with ow. Fig. 6. Pcl wall continuous with MWL1. Fig. 7. Glomerospore pore and collar formed on ow. Figs. 8–9. Spore wall structure: ow with three layers (evanescent OWL1, pustulate OWL2; OWL3 hardly detectable), MW bi-layered (MWL1 + MWL2); only central layer evident on iw.

subtending hypha was 2.0–3.1 μm wide at the base of the glomerospore, where a septum was not visible.

GERMINATION was observed once on an acaulosporoid glomerospore, where it initiated on the iw below the pedicel collar. Three germ tubes (2.4–4.0 μm wide) emerged from the center of a lobed germination structure, which was $34 \times 15 \mu\text{m}$ in diameter and consisted of 6–7 lobes that were each 12–15 μm long and 5–8 μm wide. The germ tubes had grown through the pedicel collar and branched in about 200–250 μm distance from the glomerospore. However, it was not clearly discernable if the lobed germination structure was formed between iw and mw or between the mw and the collar formed by the pedicel on the ow.

MYCORRHIZAL FORMATION — unknown.

DISTRIBUTION — Brazil: so far only found in the Cerrado biome of Serra do Cipó, Minas Gerais State.

SPECIMENS EXAMINED — BRAZIL. Minas Gerais. Serra do Cipó. HOLOTYPE (54-5401) and type specimens (54-5402) deposited at URM, Recife, Pernambuco, Brazil (accession numbers URM 78879 and 78880). Isotype (54-5402) specimens deposited at OSC, Corvallis, Oregon, USA (accession number OSC# 134501); and 54-5406 & 54-5407, deposited at Z+ZT, Zürich, Switzerland (accession number ZT MYC 159).

Discussion

The new fungal species, *Ambispora brasiliensis*, can easily be identified by its acaulosporoid morph and can be distinguished from all other *Ambispora* species forming acaulosporoid morphs by glomerospore size and by the distinct pustular ornamentation on the acaulosporoid glomerospore walls.

Acaulosporoid glomerospores of other genera in the *Glomeromycetes* — namely *Acaulospora*, *Archaeospora* and *Otospora* (Gerdemann & Trappe 1974, Morton & Redecker 2001, Palenzuela et al. 2008) — cannot be confused with *A. brasiliensis*. Spores of *Archaeospora* and *Otospora* have two walls (Palenzuela et al. 2008) contrasting with *Acaulospora* and *Ambispora*, which have three spore walls (Spain et al. 2006, Walker et al. 2007a, Palenzuela et al. 2007). Glomerospores of *Acaulospora* are not formed on a pedicel (= appendix) as known for the acaulosporoid glomerospore formation of *Ambispora*. Furthermore, in *Acaulospora* the middle spore wall forms de novo (e.g. Oehl et al. 2006) and is not continuous with the wall of the sporiferous saccule as noted for *Ambispora brasiliensis* and other *Ambispora* species (Spain et al. 2006, Walker et al. 2007a). Finally, only three *Acaulospora* species are known to form projections on the outer spore wall: *Ac. spinosa*, *Ac. tuberculata* and *Ac. denticulata*, which form — according to their names — either spiny, tuberculate or dentate ornamentations (Walker & Trappe 1981, Janos & Trappe 1982, Sieverding & Toro 1987) but not convex, irregular pustules as in *A. brasiliensis*.

We found a glomoid morph of this new species connected via hypha to a germinating acaulosporoid morph of *A. brasiliensis* only in one occasion. Such glomoid glomerospore formation during germination of acaulosporoid spores was also found for *A. appendicula* (Spain pers.comm. 2008; Goto & al. unpub. obs.). In addition to the glomoid morph, swollen hyphal tips on mycelia were also noted for *A. appendicula* (Schenck et al. 1984).

We were not able to culture *A. brasiliensis* but hope to conduct genetic analyses as soon as clean cultures are available. However, in the absence of genetic data, *A. brasiliensis* can be identified morphologically by observing its acaulosporoid glomerospores. As stated in the introduction, the glomoid morph of some of the *Ambispora* spp. is still unknown, and, in any case, *Ambispora* is more easily diagnosed by observing the acaulosporoid rather than the glomoid morph.

Acknowledgements

The authors acknowledge, in special, Dr. Ewald Sieverding (Institute of Plant Production and Agroecology in the Tropics, University of Hohenheim, Stuttgart, Germany) and Joyce L. Spain (Corvallis, Oregon USA), for reviewing the manuscript and making helpful comments and suggestions, and Dr. Nanuza Luiza de Menezes (Universidade de São Paulo, Brasil) for providing the opportunity to visit the Serra do Cipó, MG. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) that provided, respectively, a research grant to Leonor C. Maia and a PhD scholarship to Bruno T. Goto. This work was also supported by the Universidade Federal de Pernambuco, which provided a grant to F. Oehl as 'visiting professor'.

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