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Bulbospora minima, a new genus and a new species in the Glomeromycetes from semi-arid Northeast Brazil

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A new genus, and new fungal species of the Scutellosporaceae (Gigasporales), named *Bulbospora minima*, was found in the National Park Vale do Catimbau, located within the semi-arid 'dry white savanna forest' biome, called 'Caatinga', in Northeastern Brazil. The fungus resembles *Orbispora pernambucana* since it forms triple walled spores on sporogenous cells (= bulbi, suspensors), and a mono-lobed, hyaline to rarely light yellow germination orb on the inner, germination wall. However, the small spores and the size of the sporogenous cells (62–95 × 58–87 and 18–26 × 13–22 µm, respectively) render this fungus unique within the Gigasporales, since no other species of this order differentiates consistently spores < 100 µm and sporogenic cells < 25 µm in diameter. Phylogenetically, the new fungus forms an ancestral gigasporalean clade next to the *Orbispora* and *Scutellospora*, which supports the placement of the fungus into the new genus *Bulbospora*, and into the Scutellosporaceae. It is the tenth Gigasporales species described during the last decade from NE Brazil, suggesting that this tropical region is a 'hot-spot' of gigasporalean biodiversity.

Keywords: Arbuscular mycorrhizal fungus, Glomeromycota, Gigasporales, Scutellosporaceae, gen. nov., sp. nov.

The order Gigasporales (Oehl *et al.* 2011 c, Silva *et al.* 2012) has the largest spore sizes hitherto observed in the fungi kingdom (e.g. Błaszkowski 2012). Especially Gigasporaceae, Dentiscutataceae, and Racocetraceae comprise several species with 350–750(1050) µm spore diameters, e.g. *Gigaspora gigantea*, *Dentiscutata nigra*, *D. reticulata*, *Racocetra coralloidea* (Nicolson & Gerdemann 1968, Nicolson & Schenck 1979, Souza *et al.* 2005, Oehl *et al.* 2008). Also in the Scutellosporaceae, spores are rather big, (105)150–520 µm,

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when compared to most other glomeromycotan species belonging to other orders (Oehl *et al.* 2008, Pontes *et al.* 2013). In Glomerales, Diversisporales, Archaeosporales and Paraglomerales, spore sizes are, with some genus-, and also species-specific variability, often between (20)45–180(380) µm (Oehl *et al.* 2011 a, Błaszkowski 2012, Mello *et al.* 2013). Of these latter orders, only *Funneliformis*, *Acaulospora* and *Ambispora* species have a few species with similar spore sizes of 180–380(520) µm as Gigasporales (e.g. *F. caledonius*, *Acaulospora laevis*, *A. foveata*, *Ambispora appendicula*; Gerdemann & Trappe 1974, Janos & Trappe 1982, Schenck *et al.* 1984, Spain *et al.* 2006). In the Gigasporales, however, species with spore sizes < 100 µm have never been reported.

Gigasporales species form their spores on sporogenous cells that arise from sporogenous hyphae (Morton & Benny 1990). These cells, (25)31–80(140 µm) in diameter, have sizes known for spores of many Glomeraceae (Schenck & Pérez 1990), Archaeosporaceae or the majority of Acaulosporaceae species (Błaszkowski 2012). So far, sporogenous cell sizes of regularly less than 25 µm in diameter have never been reported.

In the frame of diversity studies on arbuscular mycorrhizal (AM) fungi in the National Park do Catimbau of the Caatinga biome of Northeast (NE) Brazil, a unique gigasporalean species was detected that regularly forms spores < 100 µm diameter, and sporogenous cells that are regularly < 25 µm in diameter. This fungus differentiates triple walled spores on sporogenous cells, and hyaline to subhyaline (to rarely slightly yellow), mono-lobed germination orbs, suggesting that this fungus belongs to the Scutellosporaceae. The aim of the present study was to thoroughly analyze and present this fungus using concomitant spore morphological, molecular and phylogenetic analyses. It is hereafter described within a new genus under the epithet *Bulbospora minima* due to its spore wall characteristics, the diagnostic small spore and sporogenous cell sizes, and due to its unique phylogeny.

Materials and methods

Study site

Soils were sampled in the semi-arid Caatinga biome at two sites in the National Park of 'Catimbau' (Municipality of Buique), Pernambuco State. The samples were taken in May and September 2012, and in March 2013 from the rhizosphere (0–20 cm depth) of two different vegetation types: a sandy Caatinga with a thorny scrub vegetation in sandy sediment (Lemos & Rodal 2001, Andrade *et al.* 2004) and in a rocky outcrop, with depressions containing soil islands between the rocks, with plant communities associated with and bounded by rocky surface (Conceição *et al.* 2007, Melo 2012). The Park covers 62'296 ha, and the collection sites are located at 08° 32' 25.9" S, 037° 15' 02"W and 08° 31' 55.8" S, 037° 15' 06.2"W, respectively, at about 900 m above sea level. The soils were characterized by 28.8 and 38.2 g kg^{-1} or-

ganic carbon, while pH (H_2O) was each 4.5, and available phosphorus 5.4 and 12.1 mg P kg^{-1} (extracted after Mehlich; Nelson *et al.* 1953), respectively. The climate is semi-arid hot (type Bsh of Köppen) with a dry summer, high (30–40 °C) daytime temperatures and lower (15 °C) nighttime temperatures. The mean annual precipitation is 610 mm (Goto *et al.* 2009).

AMF bait cultures

Soil from Catimbau was placed in nine 2 L pots under greenhouse conditions at the Department of Mycology, Universidade Federal de Pernambuco (Recife), as described in Mello *et al.* (2012), with the objective to cultivate and reproduce the native AMF communities. Corn (*Zea mays*), sorghum (*Sorghum bicolor*), peanuts (*Arachis hypogaea*), and sunflower (*Helianthus annuus*) were planted as host plant mixtures in the same pots during three months. Additionally, multiple spores of the species were separated and used as infective propagules in single species cultures on *S. bicolor*. The new species has not yet been propagated successfully in bait cultures or single species cultures.

Morphological analyses

About 200 spores were extracted from the field soils by wet sieving and sucrose centrifugation (Sieverding 1991). The spores were mounted in polyvinyl-alcohol–lactic acid–glycerin (PVLG), in PVLG + Melzer's reagent, and in water, and microscopically examined. The terminology of Oehl *et al.* (2011 a) and Silva *et al.* (2012) was followed for morphological spore and germination characters of gigasporalean species. Voucher specimens were deposited in the herbaria of the Eidgenössische Technische Hochschule, ETH in Zürich, Switzerland (Z+TT), and of the Universidade Federal de Pernambuco, Recife, Brazil (URM).

Molecular analyses

DNA was extracted from single spores. Individual spores were placed on a slide in a drop (5–10 µl) of ultrapure water, crushed with a needle, and used directly in the PCR reactions. The extracts served as templates for a semi-nested PCR using primers ITS3 (White et al. 1990) – 28G2 (Silva *et al.* 2006) and LR1 (Van Tuinen *et al.* 1998) – 28G2 consecutively. The template for the second PCR reaction was a 1:50 dilution of the first product. PCR reactions were carried out in a volume of 50 µl, containing 75 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.01 % Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1 µM of each primer and 2 units of DreamTaq™ DNA polymerase (Thermo Scientific, Maryland, USA); cycling parameters were 5 min at 95 °C (1 cycle), 45s at 94 °C, 1 min at 56 °C, 1 min at 72 °C (40 cycles), and a final elongation of 7 min at 72 °C followed the last cycle. The final amplicons (~690 bp) were purified with a Wizard SV Gel and PCR Clean-up System (Promega, Madison, USA),

sequenced directly or cloned with a pGEM®-T Easy Vector Systems (Promega, Madison, USA) following the manufacturer's instructions and sequenced. Sequencing was provided by the Central Laboratory of the CCB/UFPE (Recife, Brazil).

Phylogenetic analyses

Querying the National Center for Biotechnology Information databases with the BLASTn program, we verified that the sequences obtained from the new fungus were affiliated with the Gigasporales (Glomeromycota). The AMF sequences (partial LSU rRNA) obtained were aligned with other glomeromycotan sequences from GenBank using the program ClustalX (Larkin *et al.* 2007). The mismatches in the alignment were corrected manually using BioEdit program (Hall 1999). The sequences were deposited at GenBank under the accession numbers KJ944321–KJ944325.

Maximum parsimony (MP) analysis with 1000 bootstrap replications was performed using the Phylogenetic Analysis Using Parsimony (PAUP) program version 4 (Swofford 2003). Bayesian (two runs over 2×10^6 generations with a sample frequency of 200 and a burnin value of 25 %) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The model of nucleotide substitution (GTR + G) was estimated using Topali 2.5 (Milne *et al.* 2004). Two sequences from *Pacispora scintillans* (FM876831, FM876832) were used as outgroup.

Results

Taxonomy

***Bulbospora* Oehl & G. A. Silva, gen. nov.**
MycoBank no.: 809231

Diagnosis. – Differs from *Orbispora* by small spore (< 100 µm in diam.) and small sporogenous cell (< 25 µm in diam.) sizes.

Typus generis. – *Bulbospora minima* Oehl, Marinho, B. T. Goto & G. A. Silva, Sydowia 66(2): p. 316.

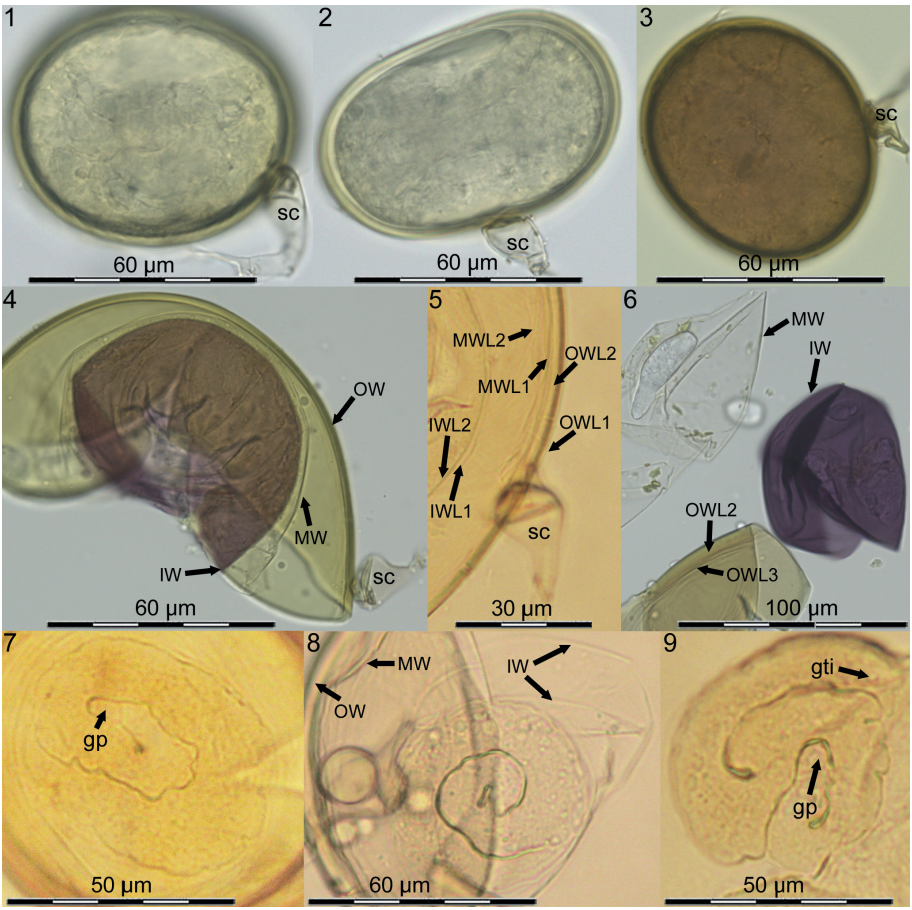
Etymology. – From Latin *bulbus*, referring to the onion-like, small sporogenous cells at the spore bases.

Characters. – Small spores (generally < 100 µm in diam.) formed on small sporogenous cells (< 25 µm in diam.), with triple walled spores, and single hyaline to subhyaline, mono-lobed yellow germination shields (= germ orbs).

***Bulbospora minima* Oehl, Marinho, B. T. Goto & G. A. Silva, sp. nov.** – Figs. 1–9
MycoBank no.: MB 809232

Diagnosis. – Differs from all other Gigasporales species by diagnostic small spore (< 100 µm in diam.) and small sporogenic cell (< 25 µm in diam.) sizes.

Holotype. – BRAZIL, Buique, Vale do Catimbau, Pernambuco State, 08° 32' 25.9" S, 037° 15' 02" W and about 900 m a. s. l. Specimen deposited at URM under the accession



Figs. 1–9. *Bulbospora minima*. **1–2.** White yellow to ochre spores formed on sporogenous cells (sc); spores mounted in PVLG. **3–6**. Uncrushed and crushed spores exposed to Melzer's reagent, consisting of three walls (OW, MW, IW) with multiple layers (OWL1-OWL3, MWL1-MWL2, IWL1-2). OW staining dark yellow to yellow brown; while IW stains purple to dark purple. **7–9.** Germination shields formed as mono-lobed germ orbs on the outer surface of IW, with one central germ pore (gp) and one peripheral germ tube initiation (gti) from where the germ tube(s) emerge during germination.

number URM 86413; collection date 1.5.2012, by Frederico Marinho. Isotype, and paratype specimens, which were also isolated from the national Park of Vale do Catimbau, at 08° 31' 55.8"S, 037° 15' 06.2"W. Isotype and paratype specimens were deposited at URM (URM86414 and URM86415) and at Z+ZT (ZT Myc 56951 and ZT Myc 56952), respectively.

E t y m o l o g y . – From Latin *minima*, referring to diagnostic small spore and sporogenous cell sizes of this new species, which so far have been unique in the Gigasporales.

C h a r a c t e r s . – Sporocarps unknown. Spores formed singly in soils and rarely in roots on sporogenous cells that arise from sporogenous hyphae.

The spores are whitish yellow to light ochre in Petri dishes under the dissecting microscope, and in water and PVLG in compound microscopes. They are elliptical, oval to ovoid or subglobose, rarely globose, and triple-walled. Spore dimensions are 62–95 × 58–87 µm.

The outer (OW) wall is triple-layered consisting of a thin (0.5–0.9 µm), evanescent to semi-persistent outer layer (OWL1), a uniform to finely laminate middle layer (OWL2; 2.1–3.5 µm) and a thin inner layer (OWL3; 0.5–1.0 µm) which becomes visible in crushed spores, when the middle wall (MW) readily separates from OW. OWL2 stains dark yellow to yellow brown in Melzer's reagent.

The middle wall is mono- to bi-layered, flexible, and in total 1.2–1.8 µm thick. Usually, the MW is visible only as one single layer.

The inner wall (IW) is bi- to triple layered, and also rather thin (1.9–2.9 µm). It consists of an outer layer (IWL1; 0.4–0.6 µm) which is hardly visible in crushed and uncrushed spores as tightly adherent to the next inner layer IWL2. The second layer is 1.5–2.3 µm, and stains purple to dark purple in Melzer's reagent. The innermost layer IWL3 is about 0.5 µm only, and, as generally tightly attached to IWL2, rarely visible.

Sporogenous cells are subglobose to oblong, 18–26 × 13–22 µm and generally persist on the spores. The two outermost layers of the spore wall continue on the sporogenous cell and the sporophore beneath. The sporophore is 25–90 µm long and tapers in diameter from 10–13 µm at the sporogeneous cell to 3.9–5.5 µm within these distances from the spore base and may bear 1–5 septae.

The germination shield is a circular to oval, mono-lobed, hyaline to subhyaline, germination orb formed on the outer surface of IW. It is 45–55 × 42–55 µm in size, with a central germ pore in its centre and one single peripheric germ tube initiation. Only in about 20 % of the spores investigated a germination orb was found.

Germination starts from the germ tube initiations at the lobe periphery of the germ orb. The initiations directly penetrate the middle and outer spore walls and branch already in a short distance from the spore (5–25 µm).

M o l e c u l a r a n a l y s e s . – The phylogenetic analyses and percentage of sequence identity place this new species in Gigasporales; however, outside of any other genera in this order (Fig. 10). Based on the partial sequences of the LSU rDNA, *S. calospora* (EU346867) and *S. dipurpurescens* (FJ461868) are most closely related to *B. minima*, with 91 % of maximum identity in the BLASTn analysis, while this percentage is slightly lower between *B. minima* and *O. pernambucana* (88 %). No environmental sequences were found for the partial LSU rDNA with close match to *B. minima*.

According to the morphological analysis and bayesian phylogeny, we have decided to place the new genus in the Scutellosporaceae. Nevertheless, there was no support to include the new genus *Bulbospora* in the family Scutellosporaceae with Maximum Parsimony and Maximum Likelihood analyses.

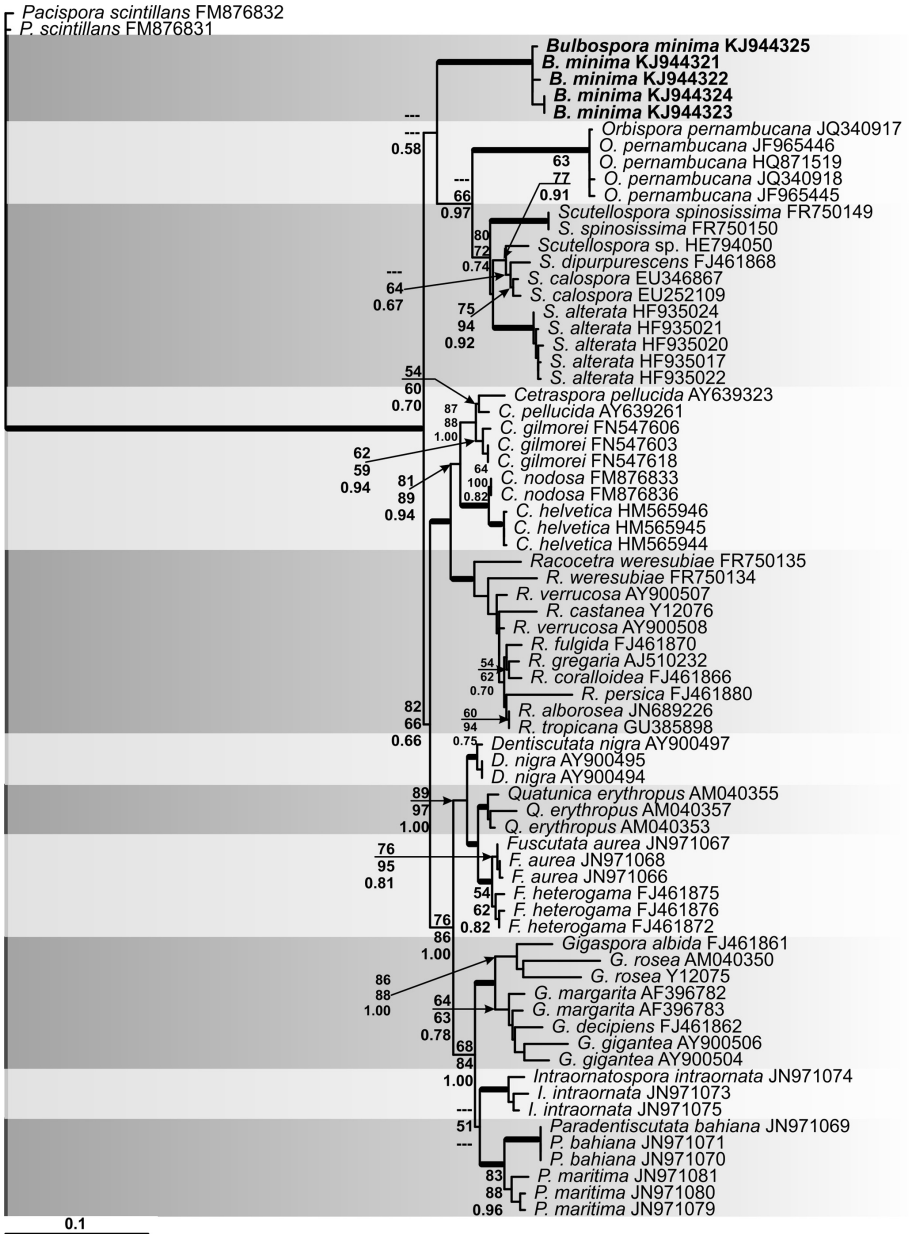


Fig. 10. Gigasporales. Partial LSU rDNA sequence-based phylogenetic tree rooted by *Pacispora scintillans*. Sequences are labeled with database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. *Bulbospora minima* sequences are in bold. Thick branches represent clades with more than 90 % of support in all analyses. Only bootstrap values of at least 50 % are shown. (Consistency Index = 0.52, Retention Index = 0.83).

D i s t r i b u t i o n . – Hitherto the fungus was only found in the valley of Catimbau, Pernambuco, and co-occurred with several other arbuscular mycorrhizal fungi such as *Acaulospora mellea, Gigaspora margarita, Glomus microcarpum, Paradentiscutata bahiana,* and *Scutellospora calospora*.

Discussion

Bulbospora minima can easily be distinguished from all other fungi in the Gigasporales just by pointing on the small spore and sporogenous cells sizes of the new species. There is no other fungal species of this order with spores < 100 µm in diam. or consistently smaller sporogenous cells sizes < 25 µm. These two morphological characteristics and the unique phylogenetic position justify the separation of the new genus *Bulbospora*.

Morphologically, *Orbispora pernambucana* is most similar to *B. minima* in terms of spore wall structure and color. However, this fungus has substantially larger spores, a thicker outer spore wall, and larger sporogenous cells. Moreover, the OW from *O. pernambucana* does not stain in Melzer's, while the white yellow to ochre OW of *B. minima* becomes dark yellow to yellow brown when exposed to this reagent. The other *Orbispora* species described so far, *O. projecturata* (Kramadibrata *et al.* 2000, Oehl *et al.* 2011 b), has projections on the spore surfaces, while *B. minima* and *O. pernambucana* have smooth surfaces (Silva *et al.* 2008, herein). *Scutellospora* species form violin-shaped to oval, bi-lobed germination shields, while *Bulbospora* and *Orbispora* species have the mono-lobed germination orbs of *Acaulospora* species. Thus, *Scutellospora* species can very easily be distinguished from both, *Bulbospora* and *Orbispora* spp. On genus level, *Bulbospora* and *Orbispora* spp. can currently be distinguished only by spore and sporogenous cell sizes, and by molecular phylogeny.

Bulbospora minima is the third Scutellosporaceae species, and the tenth Gigasporales species that was described during the last decade from the Brazilian Northeast. Beside these Scutellosporaceae species (Silva *et al.* 2008, Oehl *et al.* 2011 b, Pontes *et al.* 2013, and herein), also three Dentiscutataceae (Oehl *et al.* 2008, Goto *et al.* 2010 a, Mello *et al.* 2012), three Intraornatosporaceae (Goto *et al.* 2009, 2012), and one Racocetraceae (Goto *et al.* 2011) species were published during that time from this region, indicating an hitherto unrecognized high biodiversity of this glomeromycotan order in NE Brazil. This is particularly true for the semi-arid Caatinga, where twenty-two other gigasporalean species, that had previously been reported from other biomes, were also detected during the last two decades of intensified AM fungal diversity studies (Goto *et al.* 2010 b, Maia *et al.* 2010). Thirty-two species observed in Caatinga represent remarkable 60 % of all Gigasporales species described worldwide.

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References

Andrade K. V. S., Rodal M. J. M., Lucena M. F., Gomes A. P. S. (2004) Composição florística de um trecho do parque nacional do Catimbau. *Hoehnea* **31**: 337–348.

Błaszkowski J. (2012) *Glomeromycota*. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Conceição A. A., Pirani J. R., Meirelles S. T. (2007) Floristics, structure and soil of insular vegetation in four quartzite-sandstone outcrops of "Chapada Diamantina", Northeast Brazil. *Revista Brasileira de Botânica* **30**: 641–656.

Gerdemann J. W., Trappe J. M. (1974) The *Endogonaceae* in the Pacific Northwest. *Mycologia Memoir* **5**.

Goto B. T., Maia L. C., Silva G. A., Oehl F. (2009) *Racocetra intraornata*, a new species in the *Glomeromycetes* with a unique spore wall structure. *Mycotaxon* **109**: 483–491.

Goto B. T., Silva G. A., Maia L. C., Oehl F. (2010 a) *Dentiscutata colliculosa*, a new species in the *Glomeromycetes* from Northeastern Brazil with colliculate spore ornamentation. *Nova Hedwigia* **90**: 383–393.

Goto B. T., Silva G. A., Yano-Melo A. M., Maia L. C. (2010 b) Checklist of the arbuscular mycorrhizal fungi (*Glomeromycota*) in the Brazilian semiarid. *Mycotaxon* **113**: 251–254.

Goto B. T., Silva G. A., Maia L. C., Souza R. G., Coyne D., Tchabi A., Lawouin L., Hountondji F., Oehl F. (2011) *Racocetra tropicana*, a new species in the *Glomeromycetes* from tropical areas. *Nova Hedwigia* **92**: 69–82.

Goto B. T., Silva G. A., Assis D. M. A., Silva D. K. A., Souza R. G., Ferreira A. C. A., Jobim K., Mello C. M. A., Vieira H. E. E., Maia L. C., Oehl F. (2012) *Intraornatosporaceae* (*Gigasporales*), a new family with two new genera and two new species. *Mycotaxon* **119**: 117–132.

Guindon S., Gascuel O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.

Hall T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleid Acids Symposium Series* **41**: 95–98.

Janos D. P., Trappe J. M. (1982) Two new *Acaulospora* species from Tropical America. *Mycotaxon* **15**: 515–522.

Kramadibrata K., Walker C., Schwarzott D., Schüßler A. (2000) A new species of *Scutellospora* with a coiled germination shield. *Annals of Botany* **86**: 21–27.

Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H., Valentin F., Wallace I. M., Wilm A., Lopez R., Thompson J. D., Gibson T. J., Higgins D. G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947–2948.

Lemos J. R., Rodal M. J. N. (2001) Fitossociologia do componente lenhoso de um trecho da vegetaçãoo de caatinga no parque nacional Serra da Capivara, Piauí, Brasil. *Acta Botanica Brasilica* **16**: 223–242.

Maia L. C., Silva G. A., Yano-Melo A. M., Goto B. T. (2010) *Fungos micorrízicos arbusculares no bioma Caatinga*. In: Micorrizas: 30 anos de pesquisas no Brasil. (Org. Siqueira J. O., de Souza F. A., Cardoso E. J. B. N., Tsai S. M.) 1st edn. Lavras, Editora UFLA: 311–339.

Mello C. M. A., Silva G. A., Vieira H. E. E., Silva I. R., Maia L. C., Oehl F. (2012) *Fuscutata aurea*, a new species in the *Glomeromycetes* from cassava and maize fields in the Atlantic rainforest zone of Northeastern Brazil. *Nova Hedwigia* **95**: 267–273.

Mello C. M. A., Silva G. A., Assis D. M. A., Pontes J. S., Ferreira A. C. A., Leão M. P. C., Vieira H. E. E., Maia L. C., Oehl F. (2013) *Paraglomus pernambucanum* sp. nov. and *Paraglomus bolivianum* com. nov., and biogeographic distribution of *Paraglomus* and *Pacispora*. *Journal of Applied Botany and Food Quality* **86**: 113–125.

Melo J. I. M. (2012) Flora do parque nacional do Catimbau, Pernambuco, Brasil: Boraginaceae *sensu lato*. *Biotemas* **25**: 109–120.

Milne I., Wright F., Rowe G., Marshal D. F., Husmeier D., McGuire G. (2004) TOPALi: Software for automatic identification of recombinant sequences within dna multiple alignments. *Bioinformatics* **20**: 1806–1807.

Morton J. B., Benny G. L. (1990) Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporinae, and two families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon* **37**: 471–491.

Nelson W. L., Mehlich A., Winters E. (1953) The development, evaluation and use of soil tests for phosphorus availability. In: *Soil and fertilizers phosphorus in crop nutrition*. (eds. Pierre W. H., Norman A. F.) Aponany Monographs. Academic Press, New York: 153–188.

Nicolson T. H., Gerdemann J. W. (1968) Mycorrhizal *Endogone* species. *Mycologia* **60**: 313–325.

Nicolson T. H., Schenck N. C. (1979) Endogonaceous mycorrhizal endophytes from Florida. *Mycologia* **71**: 178–198.

Oehl F., de Souza F. A., Sieverding E. (2008) Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming Glomeromycetes. *Mycotaxon* **106**: 311–360.

Oehl F., Sieverding E., Palenzuela J., Ineichen K., Silva G. A. (2011 a) Advances in Glomeromycota taxonomy and classification. *IMA Fungus* **2**: 191–199.

Oehl F., Silva D. K. A., Maia L. C., Sousa N. M. F, Silva G. A. (2011 b) *Orbispora* gen. nov., ancestral in the Scutellosporaceae of the Glomeromycetes. *Mycotaxon* **116**: 161–169.

Oehl F., Silva G. A., Goto B. T., Maia L. C., Sieverding E. (2011c) *Glomeromycota*: two new classes and a new order. *Mycotaxon* **116**: 365–379.

Pontes J. S., Sánchez-Castro I., Palenzuela J., Maia L. C., Silva G. A., Oehl F. (2013) *Scutellospora alterata*, a new gigasporalean species from the semi-arid Caatinga biome in NE Brazil. *Mycotaxon* **125**: 169–181.

Ronquist F., Huelsenbeck J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Schenck N. C., Pérez Y. (1990) Manual for the identification of VA mycorrhizal fungi, 3rd edn. Synergistic Publications, Gainesville, Florida.

Schenck N. C., Spain J. L., Sieverding E., Howeler R. H. (1984) Several new and unreported vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Colombia. *Mycologia* **76**: 685–699.

Sieverding E. (1991) *Vesicular–arbuscular mycorrhiza management in tropical agrosystems*. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Germany.

Silva D. K. A., Freitas N. O., Cuenca G., Maia L. C., Oehl F. (2008) *Scutellospora pernambucana*, a new fungal species in the Glomeromycetes with a characteristic germination orb. *Mycotaxon* **106**: 361–370.

Silva G. A., Lumini E., Maia L. C., Bonfante P., Bianciotto V. (2006) Phylogenetic analysis of Glomeromycota by partial LSU rDNA sequences. *Mycorrhiza* **16**: 183–189.

Silva G. A., Maia L. C., Oehl F. (2012) Phylogenetic systematics of the *Gigasporales*. *Mycotaxon* **122**: 207–220.

Spain J. L., Sieverding E., Oehl F. (2006) *Appendicispora*: a new genus in the arbuscular mycorrhiza-forming Glomeromycetes, with discussion of *Archaeospora*. *Mycotaxon* **97**: 163–182.

Souza F. A., Declerck S., Smit E., Kowalchuk G. A. (2005) Morphological, ontogenetic and molecular characterization of *Scutellospora reticulata* (Glomeromycota). *Mycological Research* **109**: 697–706.

Swofford D. L. (2003) PAUP*. phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

Van Tuinen D., Zhao B., Gianinazzi-Pearson V. (1998) PCR in studies of AM fungi: from primers to application. In: *Mycorrhizal manual* (ed.Varma A.K.). Springer, Berlin, Heidelberg, New York: 387–399.

White T. J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*. (eds. Innis M. A., Gelfand D. H., Sninsky J. J., White T. J.). Academic Press, San Diego, California: 315–322.

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