MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2017

January–March 2017—Volume 132, pp. 9–18

http://dx.doi.org/10.5248/132.9

Scutellospora tepuiensis sp. nov. from the highland tepuis of Venezuela

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Abstract—Examination of soil samples collected from the summit of Sororopán-tepui at La Gran Sabana, Venezuela, revealed an undescribed species of *Scutellospora* whose spores have an unusual and very complex ornamentation. The new species, named *Scutellospora tepuiensis*, is the fourth ornamented *Scutellospora* species described from La Gran Sabana and represents the first report of a glomeromycotan fungus for highland tepuis in the Venezuelan Guayana.

Key words—arbuscular mycorrhizal fungus, taxonomy, tropical species, *Gigasporaceae*, *Glomeromycetes*

Introduction

The Guayana shield occupies a vast area that extends approximately 1500 km in an east-to-west direction from the coast of Suriname to southwestern Venezuela and adjacent Colombia in South America. This region is mostly characterized by nutrient-poor soils and a flora of notable species richness, high endemism, and diversity of growth forms (Huber 1995). Vast expanses of hard rock (Precambrian quartzite and sandstone) that once covered this area as part of Gondwanaland (Schubert & Huber 1989) have been heavily weathered and fragmented by over a billion years of erosion cycles, leaving behind just a few strikingly isolated mountains (Huber 1995). These table mountains, with their sheer vertical walls and mostly flat summits, are the outstanding physiographic feature of the Venezuelan Guayana (Schubert & Huber 1989). The Pemón Amerindians of southeastern Venezuela call these mountains

"tepuis" (Huber 1995). A tepui is a sandstone mountain or tableland with a flat summit and vertical walls up to 600–3000 m tall. Rising abruptly over the forests and savannas of La Gran Sabana, Venezuela, these tepuis are quite isolated by their peculiar shape and harbor a high degree of endemic flora and fauna. Soils of tepuis are acid and extremely oligotrophic compared with their adjacent lowlands.

The often-steep tepui slopes are covered with dense mossy forests bathed in moisture from dense clouds that form along the cliffs. Many tepui summits have relatively little soil that is exposed to temperatures averaging 18–24 °C and heavy rainfall above 2000 mm. Dense clouds and prolonged mist are common and provide additional moisture at highland sites.

Arbuscular mycorrhizal fungi (AMF) are widespread in most terrestrial ecosystems where they form mutualistic associations with most plants and facilitate nutrient uptake from the soil via an extensive extraradical mycelium (Smith & Read 2008). This mycorrhizal association, which has a low degree of specificity between partners, involves a plant host and a fungus of the phylum *Glomeromycota* (Brundrett 2009, Smith et al. 2011). Although the fungi contribute many effects that may increase host plant survival and fitness, the presumed primary benefit to the plant is improved nutrition in exchange for carbohydrates to the fungal partner (Corrêa et al. 2014).

Previous works have reported an important role for glomeromycotan fungi in all lowland ecosystems of Venezuelan Guayana (Cuenca et al. 2003a,b), and three *Scutellospora* species have been described from the lowlands: *S. spinosissima* (Walker et al. 1998), *S. crenulata* (Herrera-Peraza et al. 2001), and *S. striata* (Cuenca & Herrera-Peraza 2008). However, the presence of this fungal group on tepui summits has remained unexplored.

During two brief trips to the Sororopán-tepui in Venezuelan Guayana, we found scutellosporoid spores with a very complex ornamentation representing an undescribed species. The fungus, described here as *Scutellospora tepuiensis*, constitutes the first report of an arbuscular mycorrhizal fungus for the highland tepuis.

Materials & methods

The 2050 m high Sororopán-tepui is part of the Ptari massif, a small mountain system lying north of La Gran Sabana at 5°40–50′N 61°40–50′W, where annual precipitation exceeds 2000 mm (Huber 1995). During two short trips made by helicopter to the summit of Sororopán-tepui, composite soil samples (15 subsamples taken 0–15 cm below ground level) were collected in two different tepui vegetation zones. Zone 1 was characterized by a low shrubland growing on acidic (pH 5.7), relatively deep soils rich (20%) in organic matter; the dominant vegetation (≤6 m tall) comprised woody species in the *Clusiaceae* and *Melastomataceae*. Zone 2 was characterized by pioneer vegetation growing between fissures and depressions of sandstone on shallow soil with

dominant species representing *Poaceae*, *Cyperaceae*, *Melastomataceae*, and *Orchidaceae;* zone 2 soils are also acidic (pH 5.9) but with less (16.5%) organic matter than in zone 1. We found our new *Scutellospora* species in both vegetation types accompanied by *Scutellospora spinosissima* C. Walker & Cuenca, *Acaulospora morrowiae* Spain & N.C. Schenck, several *Glomus* species, and another undescribed *Scutellospora*.

Open pot trap cultures started from the soil samples were maintained in a glasshouse for three months and then dried for one week. Two pre-germinated *Vigna luteola* seeds (the host plant) were placed in 1-liter pot with undiluted field soil; the pot contents were stored at room temperature (≈20°C) for almost one year to break spore latency (Morton et al. 1993), after which the spores were isolated and used to start pure cultures. Because these last pure cultures were unsuccessful, the description of the species presented below is based only on spores isolated from trap cultures (IVIC-38) and field soils.

Spores were isolated from the trap pots or field soils by wet sieving, decanting, and sucrose centrifugation (Sieverding 1991). The isolated spores were suspended in water and illuminated with light from a quartz-iodine fibre-optic source. Their color was determined by comparison with a British color chart for fungi (Anon 1969). The specimens were mounted in polyvinyl alcohol lacto-glycerol (PVLG) or in PVLG mixed with Melzer's reagent (1:1, v/v). Wall description and terminology follow Walker (1983) and Walker & Vestberg (1998). Type material was deposited in the Venezuelan National Herbarium, Universidad Central de Venezuela, Caracas, Venezuela (VEN) and the Cuban National Herbarium, IES-CITMA, La Habana, Cuba (HAC).

Spore wall ornamentation and structure was also examined with scanning electron microscopy (SEM). After removing or crushing the outermost spore wall layer with fine tweezers under a dissecting microscope, we rinsed the spores in a phosphate buffer solution prior to fixing in 1% osmium tetroxide at 4 °C for 1 h. Fixed samples were dehydrated for 5 min in each dilution of an acetone series (20, 40, 70, 80, and 100%), dried at critical point with liquid CO_2, placed on aluminum metal holders, coated with 200 Å gold–palladium, and then carefully examined by SEM.

Taxonomy

Scutellospora tepuiensis Furrazola & Cuenca, **sp. nov**. Figs 1–3

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Differs from all other *Scutellospora* spp. by its spores formed singly in soil and by the complex spore ornamentation comprising numerous annulate pores, each surrounded by a lip composed of either gemmae or columellae.

Type: Venezuela, La Gran Sabana Region, Sororopán tepui; isolated from the rhizospheric soil of sclerophyllous shrubland, and pioneer vegetation growing between fissures and depressions of sandstones on a shallow soil under *Poaceae* and *Cyperaceae,* isolation date December 1996 by Z. De Andrade (**Holotype:** VEN-438658 [De Andrade 114-slide 2]; **isotype:** HAC-G-VE01 [De Andrade 114-slide 5).

Etymology: Latin *tepuiensis*, referring to the "tepui" tableland where the species was found.

Spores formed singly in soil terminally (rarely laterally) on a bulbous base (sporophore); saffron (49) to orange (48) when young, becoming sienna (11)

to fulvous (12) when mature; generally globose or subglobose, 162–216 × 159–219 µm (mean 191 × 190 µm, n = 28), and containing oily globular drops 8–35 µm diam.

Spore wall arrangement A(EL$_o$M)B(MMM) or A(EL$_o$)B(M)C(MMM) (following Walker 1983), here referred to A(12$_o$3)B(456) or A(12$_o$)B(3)C(456), with layer 2$_o$ comprising two sub-layers: 2$_o$A and 2$_o$B.

Outermost layer (1) rarely observed; when present, hyaline, smooth, ≤1 µm thick, readily visible in younger spores but frequently detaching in older ones; considered evanescent as not commonly found in mature spores.

Layer 2$_o$ 7–10 µm thick, laminated, composed of two sub-layers (2$_o$A, 2$_o$B). Sub-layer 2$_o$A dark yellow, 5–7 µm thick, perforated on its upper surface with 1–5 µm rounded to irregular pores, further inward apparently annulated and completely filled by round to elliptical or irregular 1.5–7 µm wide annuli separated by 1–7 µm with the in-between surface slightly depressed; each annulus with ~1-µm thick lip surrounded by 10–20 rounded gemmae or elliptical to clavate columellae, 1–3 µm long and ~1 µm thick, extending radially around the annulus pore as though hanging down into the lips (pores and annuli best seen in surface view); inward from each gemma or columella tip arise yeast-like projections, seemingly composed of chains or clusters of tiny (much smaller than 0.5 µm) wall material granules, the chains and clusters brush-like to diffuse and lacking in structure; the granules sometimes fusing together closer to 2$_o$A or 2$_o$B and then enlarging to ~1 µm; annuli with suspended gemma/columellae and yeast-like granule chains resembling a shower. [The information above based on crushed spores, with sub-layers somehow separating and granules disaggregating; we presume that the granules stay more closely together in nature]. Sub-layer 2$_o$B concolorous with 2$_o$A, but seeming darker when superimposed over sub-laminae; 2–3 µm thick, uniformly covered with hyaline to yellow bacula, 1–1.5 µm long, ≤0.5 µm wide; in unbroken spores, the bacula of this lower sub-layer intermingling with the yeast-like granule chains from the upper sub-layer, generally taller when just under the annulus pore.

Layer 3 tightly adhering to 2$_o$B, rarely separating from it and forming a separate group (B); <1 µm thick, hyaline, membranous.

Innermost wall group comprising layers 4, 5 and 6.

Layer 4 hyaline, ca. 1µm thick; in crushed spores smooth, slightly wavy or with a granular surface resembling a "beaded" membrane; commonly tightly adhering to layer 5, but separating or shrinking to form waves under applied pressure when the innermost group (endospore) is separated.

Layer 5 hyaline; 1 µm thick, expanding to 9-30 µm (in PVLG) or to 2–5 µm (in PVLG-Melzer's reagent), thus revealing its amorphous nature; when

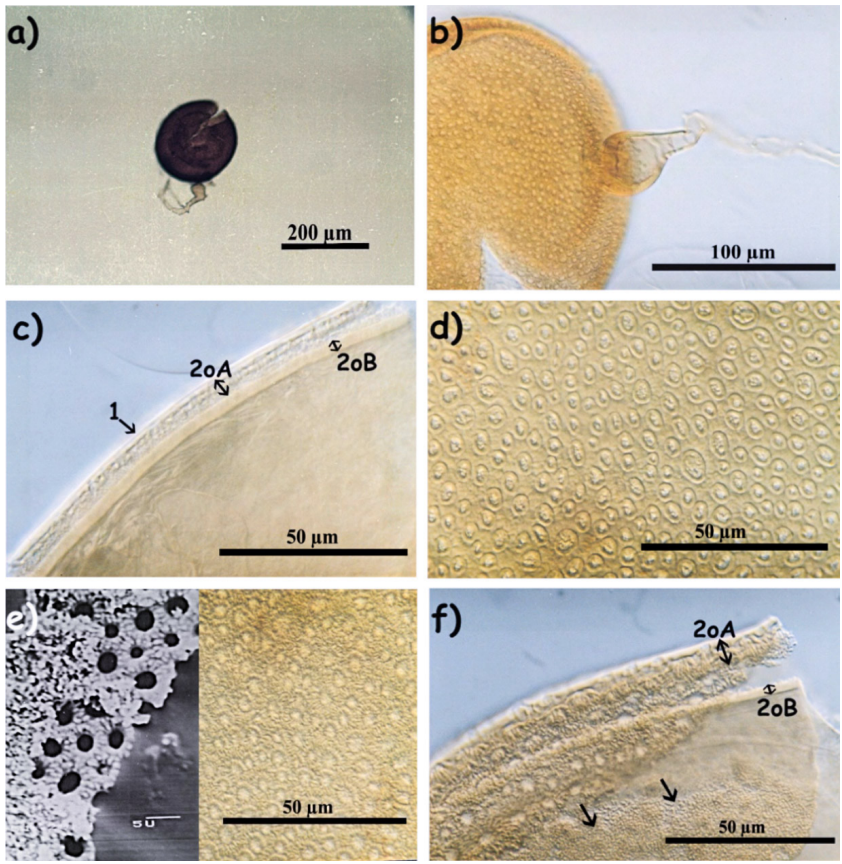

Fig. 1. *Scutellospora tepuiensis*. a) Whole spore mounted in water. b) Young spore crushed and mounted in PVLG. c) Crushed spore showing the external wall layer 1 and the two 2_0 layers. d) Surface view of layer 2 showing the ring-shaped ornamentation or annuli. e) Left: SEM image of the underside of layer 2_0A, showing gemmae radiating from the pores to form the "showers". Right: Underside (inner view) of the layer 2_0 surface, showing the rounded gemmae radiating from the annulus pores. f) Crushed spore showing the two sub-layers A and B that comprise layer 2_0. See the bacula of 2_0 B (arrowed) at lower right.

mounted in water, flexible and membranous and difficult to observe in the endospore wall.

Layer 6 (innermost) a thin (<1 µm thick) flexible membrane that wrinkles considerably after crushing when mounted in PVLG but not in water or when intact.

In Melzer's layers 5 and 6 becoming red-purple while layer 2_0 turning deep yellow with the spore content partly yellow; after 24 hours the color reaction

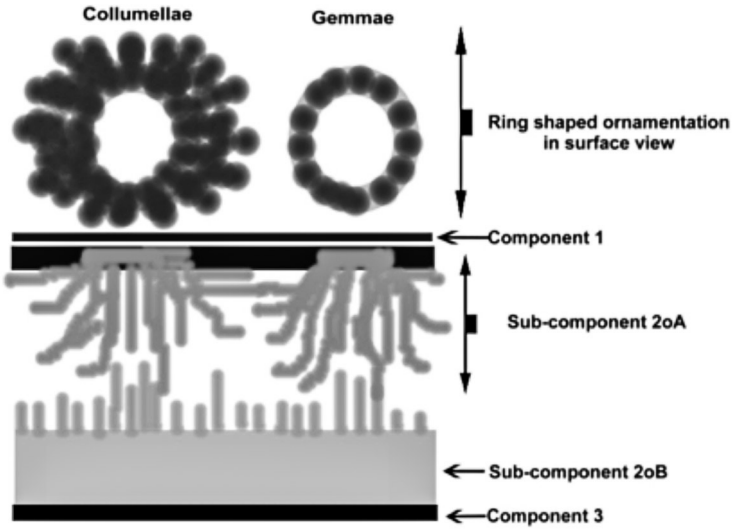

Fig. 2. Schematic representation of outermost wall layers of *Scutellospora tepuiensis*. Annular lips are defined by either columellae (at left) or gemmae (at right) radiating from a pore.

beginning to fade and after 3–4 days layer 5 turning pink to light purple and layer 6 remaining red-purple. Other layers do not react.

Sporophore bulbous, 27–43 µm diam., 40–57 µm tall (from spore base to the subtending hypha septum); wall ornamented or smooth, contiguous with spore layers 1 and 2, thickness reaching 4–6 µm (≤9 µm near spore base), with one stout (10–35 µm) projection connected to a slender hypha.

Germination shield (on layer 4) broadly lobed, simple, hyaline, 87–110 × 30–65 µm, thin (<1 µm thick) walled.

Auxiliary cells: unknown.

Distribution & ecology—Known only from the summit of Sororopán-tepui, La Gran Sabana, Venezuela. Spores have been collected only from the low high-tepui shrubland and from the saxicolous community growing on sandstone at the summit. Soils are acidic and very low in exchangeable P (0.78–1.8 ppm).

Mycorrhizal associations unknown. Attempts to form mycorrhizas in pure culture failed, although the species sporulated abundantly in a multispecies pot culture with *Vigna luteola* as host.

Additional collections examined: **VENEZUELA, La Gran Sabana Region, Sororopán-tepui,** sclerophyllous shrubland, Dec. 1996, Z. De Andrade, De Andrade-110

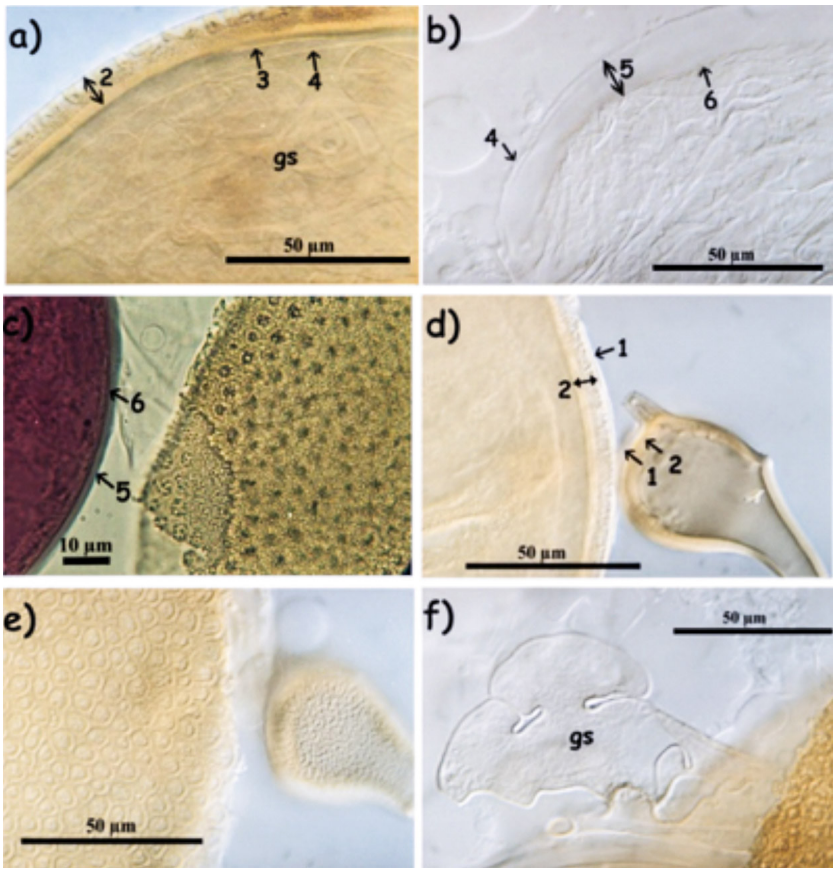

Fig. 3. *Scutellospora tepuiensis*. a) Crushed spore showing germination shield (gs) in lateral view. b) Crushed spore showing the inner group (4–6) of membranous layers and illustrating the amorphous nature of layer 5. c) Crushed spore showing the Melzer's reaction in layers 5 and 6. d) Bulbous suspensor showing its two layers contiguous with the spore layers. e) Ornamented bulbous suspensor. The ornamentation is not present in all specimens. f) Partial view of the germination shield.

(De Andrade personal herbarium); undiluted soil, trap culture (IVIC-38) with *Vigna luteola* as host plant, Dec. 1996, Z. De Andrade, De Andrade-118 (De Andrade personal herbarium); pioneer vegetation, Dec. 1996, De Andrade-123-(1/4) (De Andrade personal herbarium).

Discussion

The taxonomy and systematics of AMF species forming a bulbous suspensor and differentiating germinal walls (scutellosporoid spores) have changed dramatically over recent years. Oehl et al. (2008), who revised *Scutellospora*

based on morphological and genetic characters, split the genus into five genera and three families by weighting germination shield attributes as a diagnostic character. However, there are overlaps in germination shield morphology among these segregate genera. Analyses using different molecular tools and cladistics of 23 morphological characters by Morton & Msiska (2010) did not support most genera and families of Oehl et al. (2008) but indicated instead that species producing spores with a bulbous suspensor belong in a single family, *Gigasporaceae*, with only three genera: *Scutellospora*, *Gigaspora*, and *Racocetra*. More recently Redecker et al. (2013), who revised the classification of arbuscular mycorrhizal fungi—particularly the new taxa proposed by Oehl et al (2008)—accepted *Dentiscutata* and provisionally accepted *Cetraspora* (pending further study) but rejected *Quatunica, Intraornatospora*, and *Paradentiscutata* and retained all taxa of uncertain affinity in *Scutellospora.*

For *S. tepuiensis*, we were unable to see the germination shield in plane view as required by Oehl et al. (2008) for generic identification, although what we did see indicates that it is broadly lobed, a trait shared by at least two genera, *Cetraspora* and *Racocetra*. In addition, *S. tepuiensis* spores comprise six layers (or components) arranged in two or three groups, as found in most new genera erected by Oehl et al. (2008). Only *Racocetra* spores have one inner group of layers called a germinal wall (Morton & Msiska 2010). In the absence of molecular support for our new species and in view of its relatively simple germination shield, we prefer to adopt a conservative approach by describing this species in *Scutellospora*.

Scutellospora tepuiensis can be distinguished from other scutellosporoid species by its extremely complex ornamentation. Only *Dentiscutata reticulata* (Koske et al.) Sieverd. et al. has an ornamentation of such complexity. In addition, mature spores of both species are similar in color. However, S. *tepuiensis* spores have an annulate ornamentation, while *D. reticulata* spores are covered with a reticulum overlaying spines. *Dentiscutata nigra* has rounded holes in the outer wall, but its spores are much larger (≈500 µm) and darker-colored (dark brown to black) than those of *S. tepuiensis*. Other scutellosporoid species with a remarkable ornamentation are *S. crenulata* R.A. Herrera et al. (with spores covered with dome-like papillae) and *S. striata* Cuenca & R.A. Herrera (with spore ornamentation resembling a fingerprint). The other ornamented species retained in *Scutellospora* after the papers by Morton and Msiska (2010) and Redecker et al. (2013) are *S. nodosa* Błaszk. (with the outermost layer forming a knobbed spore surface) and *S. spinosissima* (with the laminate spore wall layer 2 ornamented with spines).

Three inner layers similar to those of *S. tepuiensis* have also been noted in other *Scutellospora* species, e.g., *S. spinosissima* (Walker et al. 1998),

S. projecturata Kramad. & C. Walker (Kramadibrata et al. 2000), *S. striata* (Cuenca & Herrera-Peraza 2008), and *S. crenulata* (Herrera-Peraza et al. 2001). According to Walker et al. (1998), the morphological features of the innermost wall layers of this fungal group probably differ from germinal wall 2 of other scutellosporoid species. In addition the layers forming this inner group frequently tightly adhere to each other and thereby this wall may resemble a structure consisting of one coriaceous layer (Walker et al. 1998).

Layer 4 of the inner wall group B or C of *S. tepuiensis* spores occasionally resembles a beaded layer commonly occurring in the innermost spore wall in species of *Acaulosporaceae*. A similar layer was also identified in spores of *S. crenulata* described from specimens found in La Gran Sabana (Herrera-Peraza et al. 2001). Molecular and cladistic morphological analyses support *Gigasporales* as more closely related to *Acaulosporaceae* than to *Glomerales* (Morton & Benny 1990). The presence of a beaded layer in *S. crenulata* and *S. tepuiensis* additionally supports this finding.

Future sampling of *S. tepuiensis* at the summit of Venezuelan tepuis will enable us to conduct the necessary molecular analysis that should indicate its phylogenetic position within *Scutellospora*. Nevertheless, its unique ornamentation when compared to all other described *Scutellospora* species distinguishes *S. tepuiensis* within *Gigasporaceae*. The La Gran Sabana ecosystem has become an important one for discovering new AMF species.

Acknowledgements

We are in debt to our late fellow Zita De Andrade, who discovered *S. tepuiensis* during a short trip to the Sororopán-tepui, and to late Dr. Ricardo Herrera-Peraza and Roberto Ferrer from the Institute of Ecology and Systematics of La Habana, Cuba, who prepared the first draft of species description. Milagros Lovera gave invaluable comments to the manuscript. We thank Drs. J. Błaszkowski and S. Stürmer for reviewing the manuscript. We are also grateful to Mycotaxon, Ltd. for underwriting the cost of this publication.

Literature cited

Anon. 1969. Flora of the British Fungi. Colour identification chart. Royal Botanic Garden Edinburgh, Her Majesty´s Stationery Office, Glasgow.

Brundrett M. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320(1): 37–77. http://dx.doi.org/10.1007/s11104-008-9877-9

Corrêa A, Cruz C, Pérez-Tienda J, Ferrol N. 2014. Shedding light onto nutrient responses of arbuscular mycorrhizal plants: Nutrient interactions may lead to unpredicted outcomes of the symbiosis. Plant Science 221–222: 29–41. http://dx.doi.org/10.1016/j.plantsci.2014.01.009

Cuenca G, Herrera-Peraza RA. 2008. *Scutellospora striata* sp. nov., a newly described glomeromycotan fungus from La Gran Sabana, Venezuela. Mycotaxon 105: 79–87

Cuenca G, De Andrade Z, Lovera M, Fajardo L, Meneses E. 2003a. Mycorrhizal response of *Clusia pusilla* growing in two different soils in the field. Trees 17: 200–206.

Cuenca G, De Andrade Z, Lovera M, Fajardo L, Meneses E, Márquez M, Machuca R. 2003b. Pre-selección de plantas nativas y producción de inóculos de hongos micorrízicos arbusculares (HMA) de relevancia en la rehabilitación de áreas degradadas de la Gran Sabana, Estado Bolívar, Venezuela. Ecotrópicos 16(1): 27–40.

Goto BT, Silva GA, De Assis DMA, Silva DKA, Souza RG, Ferreira ACA, Jobim K, Mello CMA, Vieira HEE, Maia LC, Oehl F. 2012. *Intraornatosporaceae (Gigasporales),* a new family with two new genera and two new species. Mycotaxon 119: 117–132. http://dx.doi.org/10.5248/119.117

Huber O. 1995. Geographical and physical features. 1–61, in: JA Steyermark et al. (eds). Flora of the Venezuelan Guayana, vol. 1. Timber Press, Oregon.

Herrera-Peraza RA, Cuenca G, Walker C. 2001 *Scutellospora crenulata*, a new species of *Glomales* from La Gran Sabana, Venezuela. Canadian Journal of Botany 79: 674–678. http://dx.doi.org/10.1139/b01-042

Kramadibrata K, Walker C, Schwarzott D, Schüβler A. 2000. A new species of *Scutellospora* with a coiled germination shield. Annals of Botany 86(1): 21–28. http://dx.doi.org/10.1006/anbo.2000.1146

Morton JB, Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, *Glomales,* two new suborders, *Glomineae* and *Gigasporineae*, and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of *Glomaceae*. Mycotaxon 37: 471–492.

Morton JB, Msiska Z. 2010. Phylogenies from genetic and morphological characters do not support a revision of *Gigasporaceae* (*Glomeromycota*) into four families and five genera. Mycorrhiza 20: 483–496. http://dx.doi.org/10.1007/s00572-010-0303-9

Morton JB, Bentivenga SP, Wheeler WW. 1993. Germ plasm in the International Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development documentation and storage. Mycotaxon 48: 491–528.

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

Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (*Glomeromycota*). Mycorrhiza 23: 515–531. http://dx.doi.org/10.1007/s00572-013-0486-y

Schubert C, Huber O. 1989. La Gran Sabana, panorámica de una región. Lagoven booklets. Refolit Venezuela.

Sieverding E. 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. GTZ Eschborn Germany.

Smith SE, Read D. 2008 Mycorrhizal symbiosis 3rd ed. Academic Press

Smith SE, Jakobsen I, Grønlund M, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology 156(3): 1050–1057. http://dx.doi.org/10.1104/pp.111.174581

Walker C. 1983. Taxonomic concepts in the *Endogonaceae:* spore wall characteristics in species descriptions. Mycotaxon 18: 443–455.

Walker C, Vestberg M. 1998. Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum*, *G. maculosum, G. multisubstensum* and *G. fistulosum*. Annals of Botany 82: 601–624. http://dx.doi.org/10.1006/anbo.1998.0714

Walker C, Cuenca G, Sánchez F. 1998. *Scutellospora spinosissima* sp. nov., a newly described glomalean fungus from acidic, low nutrient plant communities in Venezuela. Annals of Botany 82: 721–725. http://dx.doi.org/10.1006/anbo.1998.0728