# Scutellospora crenulata, a new species of Glomales from La Gran Sabana, Venezuela

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# Scutellospora crenulata, a new species of Glomales from La Gran Sabana, Venezuela

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**Abstract**: A newly discovered glomalean fungus, *Scutellospora crenulata*, is described and illustrated. This species possesses a complex spore ornamentation composed of dome-like sub-polygonal papillae partly separated by pits. The presence of a "beaded" spore wall component is reported for the first time in a member of Gigasporaceae.

Key words: arbuscular mycorrhizal fungus, Gigasporaceae, tropical soil, sclerophyllous shrubland.

**Résumé**: Les auteurs décrivent et illustrent une espèce de Glomales récemment découverte, le *Scutellospora crenulata*. Cette espèce possède une ornementation complexe composée de papilles subpolygonales en forme de dôme, séparées par des cavités. C'est la première fois qu'on mentionne la présence de parois sporales perlées chez un membre des Gigasporaceae.

Mots clés: champignon mycorhizien arbusculaire, Gigasporaceae, sol tropical, formation arbustive sclérophylle.

[Traduit par la Rédaction]

#### Introduction

La Gran Sabana is a high, undulating plain situated in south-eastern Venezuela and is part of the Guayana Shield, one of the oldest substrates on earth. Its soils are derived from Precambrian rocks and are acid and poor in nutrients. Vegetation in La Gran Sabana is mostly open treeless savannahs but there are also sclerophyllous shrublands associated with rocky sandstone substrates, and upland meadows associated with organic soils (Huber 1995). Evergreen forests are also interspersed in the landscape dominated by savannahs. We have found various undescribed *Scutellospora* spp. in the sclerophyllous shrublands of La Gran Sabana. A new species, *Scutellospora crenulata*, in the Gigasporaceae is described from this locality. Spores of the species possess a complex ornamentation that distinguishes them from presently described *Scutellospora* spp.

#### Material and methods

Soil samples were taken in a sclerophyllous shrubland (5°36′N and 61°29′W) near the town of Liwo-riwo (1274 m a.s.l.) in La Gran Sabana. The sampling site is situated 7 km away from kilometre 32 of the road that connects the Troncal 10 highway to Kavanayén, the main Catholic mission of the area. *Scutellospora crenulata* was found together with at least three other species of *Scutellospora* and other glomalean fungi. Several attempts to ob-

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R.A. Herrera-Peraza. Instituto de Ecología y Sistemática,
CITMA, A.P. 8029, C. de La Habana 10800, Cuba.
G. Cuenca. Instituto Venezolano de Investigaciones
Científicas, IVIC, Centro de Ecología, Apdo. 21827, Caracas
1020 A, Venezuela.

C. Walker. School of Conservation Sciences, Bournemouth University, Talbot Campus, Fern Barrow, Poole, Dorset BH12 5BB, U.K.

<sup>1</sup>Corresponding author (e-mail: gcuenca@ivic.ve).

tain this species in pure culture were made but all failed. Trap cultures were attempted using *Vigna luteola* as a plant host, but the spores of *S. crenulata* recovered after 10 months of culture were apparently moribund.

Spores were extracted from the field by wet sieving and decanting followed by sucrose centrifugation (Sieverding 1991). They were then suspended in a dish of water and illuminated with light from a quartz–iodine fibre-optic source (Walker et al. 1993) to determine their colour by comparison with a fungal colour chart (Anonymous 1969). Specimens were mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier 1983) or in Melzer's reagent (1924) for examination under a compound microscope. Spore wall description and terminology follow those suggested by Walker (1983) and Walker and Vestberg (1998). Type material has been deposited in the Venezuelan National Herbarium (VEN) and in the Cuban National Herbarium (HAC).

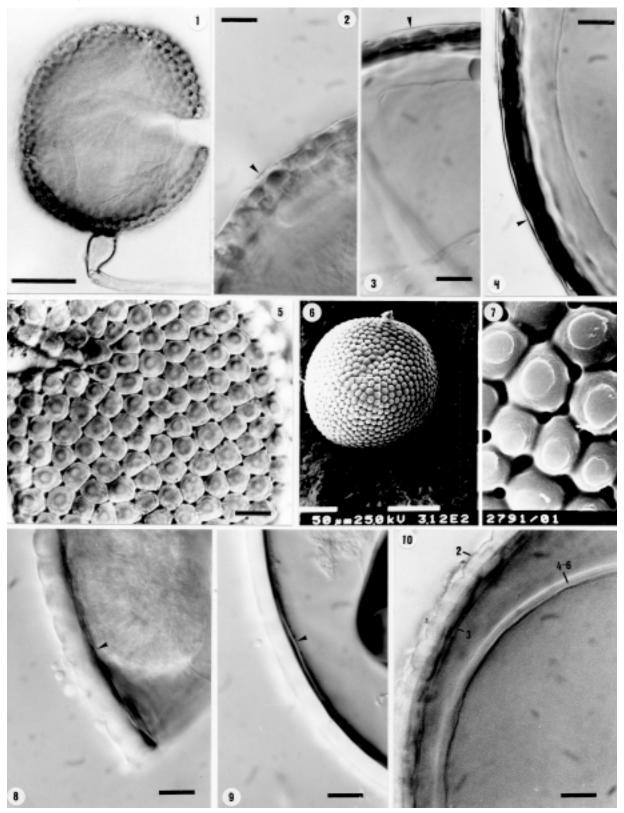
#### **Species description**

Scutellospora crenulata Herrera-Peraza, Cuenca & Walker sp.nov. Figs. 1–17

ETYMOLOGY: Latin, crenulata, referring to the crenulate appearance of spore surface.

Sporae in solo singillatim efformatae, terminaliter vel lateraliter sporophoro bulboso adhaerentes, globosae, subglobosae, vel late ellipsoides ochraceae, moribundae luteae. Sporae tunica stratis sex in turmis duabus tribusve constituta. Tunica prima exterior (1) raro observata, hyalina. Tunica secunda (2) laminata, 5.0-9-0 µm crassa, crenata, conspicue papillis cupuliformibus, subpolygonalibus, distinctis, constituta. Papillae basi plerumque haxagonae, interdum pentagonae vel tetragonae, a basi ad 2/3 partes 3.6-10.0 µm latae, apicaliter plerumque mammillis ellipticis 4.0–8.3 µm longis, 3.5–4.8 μm latis, minus 1 μm altis coronatae. Papillae ex lateribus polygonalibus, ponticulis medio tenuioribus, interconnexae, foraminibus ex rotundo ellipticis vel linearibus inter ponticulos praesentibus. Tunica tertia (3) stricte tunicae secundae adherens, minus 1 µm crassa, hyalina et flexibilis. Turma interior tunicis tribus (4-5-6) constituta. Tunica Herrera-Peraza et al. 675

**Figs. 1–10**. Photomicrographs of *Scutellospora crenulata* spores. Fig. 1. Slightly crushed spore showing the general appearance. Scale bar =  $50 \mu m$ . Figs. 2–4. External component 1 (arrows) and the laminate component 2 show a crenulate appearance. Scale bar =  $10 \mu m$ . Fig. 5. Surface view of the dome-like sub-polygonal papillae as observed with light microscopy. Scale bar =  $10 \mu m$ . Fig. 6. SEM image of the spore. Fig. 7. SEM image showing the flat warts occurring at the top of each papilla. Figs. 8 and 9. The internal component 3 (arrows). Scale bar =  $10 \mu m$ . Fig. 10. The spores wall structure (components 4–6) as observed in water. Scale bar =  $10 \mu m$ .



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**Figs. 11–17.** Photomicrographs of the innermost wall group components and germination shield of *Scutellospora crenulata*. Fig. 11. Structure in PVLG; the amorphous component (5) has expanded up to 8 μm. Scale bar = 50 μm. Fig. 12. The "beaded-like" component (4) shows its wrinkled appearance, and the innermost component (6) is observed adherent to the amorphous component (5) when mounted in PVLG. Scale bar = 10 μm. Fig. 13. The "beaded-like" component (4) as observed under its wrinkled appearance when broken; component 6, surrounding the spore content, is unbroken. Scale bar = 50 μm. Figs. 14–16. Melzer's reaction of components 5 and 6 (red-purple of Melzer's reaction, appears black in the photos). The "beaded-like" component (4) is shown (arrows) over the reacting components. Scale bar = 10 μm. Fig. 17. Germination shield. Scale bar = 10 μm. **Figs. 18 and 19.** Photomicrographs of *Acaulospora denticulata* (INVAM accession CL 139-1). Scale bar = 10 μm. Fig. 18. Component 6 has a "beaded" appearance in a spore of *A. denticulata*; the amorphous component (7) is also shown. Fig. 19. Internal components of an *A. denticulata* spore; component 6 appears wrinkled.

quarta (4) hyalina et 1  $\mu$ m crassa, sinuose flexibilis vel submoniliformis, stricte tunicae quintae adhaerens. Tunica quinta (5) hyalina, amorpha, flexibilis, in aqua singulis tunicis interioribus difficulter separatim observatis, e contra unicam membranam crassam simulantibus. Tunica sexta (6) tenuis, flexibilis. Sporophorus 18.6–26.5  $\mu$ m latus, 27.0–34.5  $\mu$ m altus; sporophori tunica proximaliter ad sporae basin 4–5  $\mu$ m crassa, distaliter vero sensim attenuata. Sporophori hypha plerumque ramosa. Scutellum germinale hyalinum, late lobatum, simplex. Cellulae auxiliares desiderantur.

Spores formed singly in the soil, terminally or laterally on a bulbous base (Fig. 1); globose (46% of spores, mean 133 × 136  $\mu$ m) to subglobose (44% of spores, mean 130.0 × 142.2  $\mu$ m) to broadly ellipsoidal (10% of spores, 103.0–130.0 (117.6) × 132.0–151.1 (144.0)  $\mu$ m); ochraceous (8G) to ochre (9H) in colour to luteous (51) when old; 103–165 (130.1) × 116–170 (139.6)  $\mu$ m (mean 130 × 140  $\mu$ m, SD = 12  $\mu$ m, n = 105). Bulbous base 22.0–46.0 (31.7) × 18.6–26.5 (23.0)  $\mu$ m generally with one or more hyphal projections.

Spore wall structure of six components in two or three groups. Component 1: outermost component, rarely observed unit, and hyaline (Figs. 2-4). Component 2: laminated, 5.0-9.0 µm thick, crenulate because of surface ornamentation of sharply separated dome-like sub-polygonal papillae (Fig. 5). Papillae hexagonal to pentagonal to tetragonal, rarely irregular or fused, 3-6 µm height overlaying the outermost lamina of component 2; basally 3.6-10.0 (-16) µm wide, tapering two thirds of their height outwards; from this height becoming circular to elliptical in section, and domeshaped or flattened (Figs. 5–7). Many spores show a flat wart occurring at the top of each papilla (Figs. 5–7). These warts are often elliptical, reaching 4.0-8.3 µm length × 3.5-4.8 µm width, and less than 1 µm height. Papillae laterally joined in part to produce rounded to elliptical to lineal depressions (pits) at each corner of the polygonal base of the papilla (Figs. 6 and 7). Component 3: hyaline, <1 µm thick, flexible, usually tightly adherent to component 2 (Figs. 8, 9, and 10).

Innermost wall consisted of a group of three components (4, 5, and 6) (Fig. 11). Component 4: hyaline, approx. 1 μm wide, smooth (Fig. 11) to wrinkled (Figs. 12 and 15), or with a granular "beaded" appearance (Fig. 16). Component 4 tightly adherent to component 5, separating and wrinkling (Fig. 13) under pressure applied to the cover glass when the innermost group is separated. Component 5: amorphous, flexible, <1 μm thick, hyaline, expanding to 5–19 μm thick when gently crushed in PVLG (Figs. 11 and 12). In water, the three inner components (4–6) are difficult to distinguish, appearing as a thick membrane (Fig. 10). Component 6: thin (≤1 μm thick), flexible, wrinkling considerably after crushing

in PVLG (Figs. 11 and 12) but not in water (Fig. 10) or when intact (Fig. 13). Component 5 and 6 becoming redpurple in Melzer's reagent (Figs. 14–17), component 2 becoming deep yellow; spore content yellowing. Colour reaction fading after 24 h, and after 3 to 4 days component 5 becoming pink to light purple and component 6 remaining red-purple. Other components not reacting. Bulbous base wall components contiguous with spore wall components 1 and 2, 4–5  $\mu$ m thick near the spore base and thinning distally. Germination shield: hyaline, broadly lobed and simple (Fig. 17) observed on the component 4. Auxiliary cells: unknown.

#### Collection examined

TYPE: Consisting of spores on microscope slides, and spores in 0.025% sodium azide solution from soil from Liwo-riwo shrubland. Holotype: VEN, Venezuelan National Herbarium, Fundación Instituto Botánico, Caracas, Venezuela, Cuenca 604-7, 29 April 1998, Liwo-riwo shrubland. Isotypes: HAC, Cuban National Herbarium, Instituto de Ecología y Sistemática, CITMA, La Habana, Cuba, Cuenca 604-12, 29 April 1998, Liwo-riwo shrubland. Other specimens examined: IVIC, Centro de Ecología, Cuenca 279-1, March 1995, Liwo-riwo shrubland; Cuenca 321, June 1995, trap culture (M-28) with an unidentified member of the Cyperaceae as "host" plant, from Liwo-riwo shrubland; Cuenca 322, November 1995, Liwo-riwo shrubland; Cuenca 588 (1-6), January 1998, Liwo-riwo shrubland.

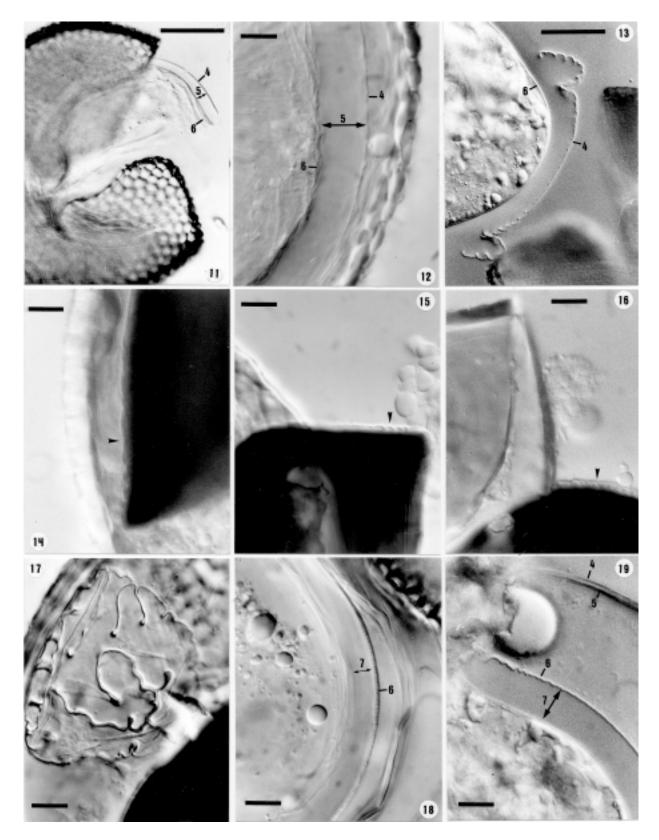
#### Distribution and habitat

Known only from the sclerophyllous shrubland at Liworiwo in La Gran Sabana, Venezuela. The vegetation in this locality is composed by dense shrub communities 1–3 m tall, distributed in patches on a quartz sand substrate embedded in a matrix of exposed sandstone rocks (Huber 1995). Most roots of these shrubs grow inside rock fissures. *Gongylolepis benthamiana* (Asteraceae), *Clusia pusilla* (Clusiaceae), *Calliandra* sp. (Mimosaceae), and *Euphronia guianensis* (Euphroniaceae), dominate this shrubland, among other species. Soils are acid (pH 5.4) and rich in organic matter (11.7%).

#### Mycorrhizal associations

Mycorrhizal associations are unknown. Attempts to establish the fungus as a mycorrhizal symbiont in single-species pot culture have failed. Reproduction of this species in trap culture also failed, but healthy spores were recovered from field soil that was left in closed plastic bags in the laboratory  $(20-25^{\circ}C)$  for 13 months.

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### **Discussion**

Populations of *S. crenulata* are low in its natural environment, with a maximum of 152 spores/dm<sup>3</sup> soil. Sporulation seems to occur between November and March, during the driest season in La Gran Sabana. *Scutellospora crenulata* 

may be classified as a small Scutellospora sp., belonging to the group of Scutellospora minuta with small (less than 200- $\mu$ m diam.) ornamented spores.

The complex surface ornamentation of *S. crenulata* is strikingly different from that of most other species of the genus. The arrangement of papillae superficially resembles the

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ocular phases of an insect eye or the surface of some infrutescens, e.g., *Rubus* spp., *Annona squamosa*. Depending upon the observation plane in the light microscope, transverse views of papillae vary considerably. Therefore, in transverse view, papillae might seem to be laterally fused (Figs. 3 and 4) or they are not at all observed (Fig. 9). Papillae are laterally joined by bridges that may be observed under the compound microscope. However, when scanning electron microscopy (SEM) is used the bridges are observed near the papillae bases. These bridges are thinner towards the central portion between the polygonal sides of papillae giving rise to rounded to elliptical, rarely linear, pits (Fig. 6 and 7) which are easily observed with either the compound microscope or SEM.

The spores of *S. crenulata* tend to loose their bulbous base during sieving from the soil and could then be confused with spores of *Acaulospora denticulata* because the ornamentation of both species is very similar. The papillae only differ in that those of *A. denticulata* end in a depression and not in warts as in the convex tip papillae of *S. crenulata*. Also, spores of *A. denticulata* lack the pits in the corner of the base of papillae, which is characteristic of *S. crenulata*.

Component 1 of the spore wall is presumed to be detached from the spore surface very early during spore ontogeny. Its detachment might occur also during spore storage or as a result of soil microorganisms. Although we are convinced of the existence of this wall component, more detailed study will be necessary once the fungus can be cultured to establish if it really exists.

The nature of component 4 (beaded-like) of *S. crenulata* is uncertain. According to Morton (1988), the "beaded" component is an elastic membrane ornamented with granular excrescences. Indeed, this seems to be the appearance of component 4 of *S. crenulata* in Fig. 16. But this component in this species also can be seen as an elastic flexible component which is wrinkled (Figs. 13–15) or smooth (Fig. 11). A similar behaviour of this type of component has been observed in *Acaulospora* spp. and *Entrophospora* spp. Therefore, it seems probable that component 4 of *S. crenulata* could be somehow comparable with the "beaded" component of Acaulosporaceae (compare Fig. 16 with Fig. 18; and Figs. 13 and 15 with Fig. 19). This requires more research.

A component somehow comparable to a "beaded" one has not previously been reported from spores of Gigasporaceae. Spore walls showing a combination of a "beaded" component followed by an amorphous layer have been reported for Acaulospora dilatata, Acaulospora lacunosa, and Acaulospora rugosa (Morton 1986). Among Entrophospora spp. this combination has been found in Entrophospora colombiana (Morton and Benny 1990) and Entrophospora kentinensis and detected in other unreported species of Entrophospora. However, among Scutellospora spp. showing amorphous components in the innermost wall group, e.g., Scutellospora calospora, (Koske and Walker 1986), Scutellospora dipurpurascens (Morton and Benny 1990), and Scutellospora pellucida (Koske and Walker 1986), the outer group's component is not "beaded." The discovery of a "beaded-like" wall component in a Scutellospora sp. may suggest a close evolutionary link between this genus and some members of the Acaulosporaceae.

To date, only *Scutellospora spinosissima* (Walker et al. 1998) and *S. crenulata* (present study) have been reported from La Gran Sabana, but there are several other undescribed *Scutellospora* spp., some also with an apparently "beaded" component, awaiting description from the same locality. Future investigation of La Gran Sabana should yield interesting discoveries of great importance in the elucidation of the phylogeny of the Glomales.

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