Acaulospora herrerae, a new pitted species in the Glomeromycetes from Cuba and Brazil

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With 9 figures and 1 table

Abstract: A new *Acaulospora* species was isolated from the rhizosphere of *Panicum maximum*, *Sporobolus indicus* and *Byrsonima crassifolia* of a calcareous soil in Bayamo (Cuba). It was successfully propagated in single species cultures obtained after inoculation with multiple spores on *Bracharia decumbens* and *Sorghum vulgare*, respectively. It was also detected in several natural and agricultural sites in Northeastern Brazil. The spores are brown-yellow to yellowish brown, (50–)70–95(–112) μm in diam., and have a diagnostic pitted ornamentation on the outer surface of the structural wall layer. The pits are rounded, elliptical to elongated (vermiform or rugulate), and uniformly distributed leaving narrow bridges that regularly give the impression of a raised reticulum. Partial sequences of the LSU rRNA gene confirm the new fungus in a well separated, monophyletic clade within the Acaulosporaceae. The new fungus is here presented under the epithet *Acaulospora herrerae* in honor to Dr. Ricardo Herrera-Peraza, a highly appreciated former mycorrhizologist from La Habana, Cuba.

Key words: Caribbean, South America, Tropics, taxonomy, Glomeromycota, Diversisporales, arbuscular mycorrhizal fungi.

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Introduction

The combined consideration of morphological and molecular analyses has outstandingly improved the knowledge about the taxonomy of arbuscular mycorrhizal fungi – AMF (Gamper et al. 2009, Oehl et al. 2011b), especially for those forming spores on or in the necks of sporiferous saccules (Morton & Redecker 2001, Spain et al. 2006, Walker et al. 2007, Palenzuela et al. 2010). Recently some new *Acaulospora* species were described using both morphological and molecular phylogenetic analysis with LSU rRNA sequences, ITS region, or both (e.g. Oehl et al. 2011a, c, d, 2012). Differences in spore morphologies could be confirmed by clear phylogenetic differences between multiple species (e.g. Oehl et al. 2012), and surface ornamentation of spores was a valuable character of comparison between species.

A new ornamented *Acaulospora* species was discovered during a survey of AM fungi in a calcareous soil from Bayamo, Granma province, in Eastern Cuba. The fungus produced abundantly spores in single species cultures on *Brachiaria decumbens* and *Sorghum vulgare*. The cultures have been successfully maintained for several culture generations in La Habana, Cuba. In recent years, a series of independent AMF diversity studies were performed in Northeastern Brazil, in which this fungus was recovered from several natural and agricultural sites. Thus, the objective of this study is to describe the new species thoroughly, combining morphological and molecular phylogenetic analyses of the partial LSU rRNA sequences, and to refer to the biogeographic distribution of the fungus, as far this has been already possible.

Materials and methods

STUDY SITES AND SOIL SAMPLING: In 2000, soil samples were taken in Bayamo (Granma province, Eastern Cuba) in a Calcaric Leptosol from the rhizospheres of Guinea grass (*Panicum maximum* Jacq.), smut grass (*Sporobolus indicus* (L.) R.Br.), an acerola species (*Byrsonima crassifolia* (L.) Kunth), and a neighbored sugar cane plantation. At the site, climate is humid tropical with about 26°C mean annual temperatures and 1200 mm mean annual precipitation. In tropical NE Brazil, the new fungus was detected during several independent AMF diversity surveys in the semi-arid Caatinga and in the semi-humid coastal rainforest biomes (mean annual temperatures 25–29°C; mean annual rainfall 400–1190mm) between 2006 and 2011. In Taperoá (Paraíba State), soil samples were taken in maize, cowpea and cotton intercropping systems. In Pernambuco State, the new fungus was found in rhizospheric soils of passion fruit (*Passiflora cincinnata* Mast.) cultivated at the Experimental Station of Embrapa Semi-Árido in Petrolina, while it was also detected under natural Caatinga vegetation within the Experimental Station of the Instituto Agronômico de Pernambuco (IPA) in Sertânia. In Santa Maria da Boa Vista and Jacobina (Pernambuco and Bahia State, respectively), the fungus was also found in the rhizospheric soil of the Barbados Nut (*Jatropha curcas* L.). Recently, the new fungus was also detected in sand dunes of the Parque das Dunas de Natal in Rio Grande do Norte State, and in a higher altitude semi-deciduous tropical rainforest in the middle of the natural Caatinga (dry forest) in the municipality Crato, Ceará State. The isolation sites of the new fungus are summarized in Table 1 presenting their geographical data and selected soil parameters of the sites.

SINGLE SPECIES CULTURES: In 2001, several single species cultures of the new fungus were initiated at the Instituto de Ecología e Sistemática in La Habana, Cuba, after initial inoculation with 20 spores per pot to *Bracharia decumbens* Steud. in 500 mL pots. Before inoculation, the pots had been filled with a 1:1 autoclaved substrate mixture (pH 5.4) of sand and native soil, which derived from the type location of the fungus in Bayamo. Since the first successful establishment, the cultures have been maintained for several cycles (each 6–10 months), either on *Brachiaria decumbens* or *Sorghum*

Table 1. Isolation sites of *Acaulospora herrerae* with geographic data, soil pH, organic carbon and available phosphorus.

Municipality (Locality)	Province/ State	Country	Plant species community	Geographic position	Altitude m asl	Mean annual temperature	Mean annual precipitation	pH (H$_2$O)	Organic C (g kg^{-1})	Available P (mg kg^{-1})	Year of sampling
Bayamo	Granma province	Cuba	Guinea grass, smuth grass, acerola sp. Byrsonima crassifolia (sugar cane)	20°17'00"N; 76°41'00"W	115	26	1200	7.3	17.6	10.0	2000
Taperoá (Agroecological Station of Vila Maria Rita)	Paraíba State	Brazil	maize, cowpea and cotton intercropping systems (field plot experiment)	7°12'23"S; 36°49'25"W	520	26	560	7.1–7.9	12.7–14.9	145.6–180.3	2006 & 2007
Petrolina (Experimental Station of Embrapa Semi-Árido)	Pernambuco State	Brazil	passion fruit	9°23'33"S; 40°30'7"W	380	26	550	6.7	13.3	28.0	2007
Sertânia (Exp. Station of the Instituto Agronômico de Pernambuco – IPA)	Pernambuco State	Brazil	natural Caatinga vegetation	08°04'25"S; 37°15'52"W	560	25	630	6.0	10.0	8.0	2007
Santa Maria da Boa Vista	Pernambuco State	Brazil	barbados nut (*Jatropha curcas*)	8°47'17"S; 39°49'22"W	350	27	520	7.4	12.9	106.0	2007
Jacobina	Bahia State	Brazil	barbados nut (*Jatropha curcas*)	11°11'07"S; 40°32'11"W	500	29	400	6.0	26.8	7.5	2007
Natal (Parque das Dunas)	Rio Grande do Norte State	Brazil	Coastal sand dunes	5°46'S; 35°12'W	20	26	1190	5.8	16.6	3.0	2010
Crato	Ceará State	Brazil	semi-deciduous Atlantic Rainforest	07°17'S; 39°33'W	930	25	1090	4.4	19.1	6.0	2011

vulgare Pers. as host plants. Plants were fertilized every other week using a Hoagland solution with reduced P contents.

MORPHOLOGICAL ANALYSES: Spores were separated from the field soil samples and the single species cultures by wet-sieving and decanting followed by gradient centrifugation (Sieverding 1991). Subsequently, the spores were placed in Petri dishes with water and separated under a dissecting microscope with up to 100 fold magnification. For taxonomic analysis, spores were mounted on microscope slides in water (Spain 1990), in poly-vinyl-lacto-acid-glycerin (PVLG) or in a 1:1 mixture of PVLG and Melzer's reagent (Brundrett et al. 1994). Spore terminology follows Palenzuela et al. (2008, 2010, 2011) for species with spore formation on sporiferous saccules, while Glomeromycota classification follows Oehl et al. (2011b). The original species descriptions of all *Acaulospora* species with pitted ornamentation on the spore surfaces, type material of many of those deposited in mycological herbaria, and an identification key recently published for all known *Acaulospora* species (Oehl et al. 2012) were considered for taxonomic comparisons. Spore dimensions reported here are measured averages of 135 spores. As additional character, pit density 500 μm^{-2} has also been analyzed for the spores of the new species as well as for *Kuklospora kentinensis* (C.G.Wu & Y.S.Liu) Oehl & Sieverd. Type material has been deposited in the Herbarium of the Academy of Science (HAC) at the Institute of Ecology and Systematic (IES), Ministry of Science, Technology and Environment (CITMA) in La Habana (Cuba) and other recognized herbaria: FH (USA), MUCL (Belgium), URM (Brazil), UFRN (Brazil), and Z+ZT (Switzerland).

MOLECULAR ANALYSES: Spores isolated from the single species cultures were firstly washed in ultrapure water and sonicated three to four times. Crude extracts were obtained from three individual spores, isolated from the Cuban single species cultures maintained at the Instituto de Ecología e Sistemática in La Habana (Cuba). The spores were singly placed on a slide in a drop (5–10 μl) of ultrapure water, and crushed with a sterile needle. PCR reactions were performed according Goto et al. (2012) with some modifications. Crude DNA extract was used as template for a semi-nested PCR using the primers ITS3 (White et al. 1990) – LSUAC (5'-CCATTACGTCAGCATCCTTAGCG-3') and LR1 (van Tuinen et al. 1998) – LSUAC consecutively. 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, 1μM of each primer and 2 units of Taq™ DNA polymerase (Fermentas, Maryland, USA); cycling parameters were 5 min at 95°C (1 cycle), 45s at 94°C, 1 min at 55°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 (~700bp) were purified with a PureLink PCR Purification Kit (Invitrogen), sequenced directly (product from spores 1 and 2) or cloned (product from spore 3) with a CloneJET™ PCR Cloning kit (Fermentas; Carlsbad, USA) following the manufacturer's instructions and sequenced. Sequencing was provided by the Human Genome Research Center (São Paulo, Brazil). Through a BLASTn query of the National Center for Biotechnology Information databases, we verified that the sequences obtained were affiliated with Acaulosporaceae. The new sequences were deposited in the NCBI database under the accession numbers JX135569-JX135573.

PHYLOGENETIC ANALYSES: The AM fungal sequences (partial LSU rRNA) obtained were aligned with other glomeromycotan sequences from GenBank in ClustalX (Larkin et al. 2007) and edited with the BioEdit program (Hall 1999) without any manipulation on the sequences. *Claroideoglomus etunicatum* W.N.Becker & Gerd. was included as an outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 1×10^6 generations with a burn in value of 4000) and maximum likelihood (1,000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon &Gascuel2003), launched from Topali 2.5, using the GTR + G model. Neighbor-joining (established with the model cited above) and maximum parsimony analyses were performed using PAUP*4b10 (Swofford 2003) with 1,000 bootstrap replications.

Results

TAXONOMY: The morphological description of the spores were based on the spores isolated from the rhizosphere of Guinea grass, smut grass and the acerola species as

well as from the single species cultures, maintained in Cuba on *Bracharia decumbens* and *Sorghum vulgare*. The spores isolated from the Brazilian field soil samples had identical spore morphology for all characters investigated (spore size and color, spore ornamentation, numbers, thickness and staining of spore walls; and size and thickness of saccules and their walls, which was measured when the saccules were still attached to the spores in the field soil samples).

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Figs 1–8

Sporae singulae in solo efformatae, flavae vel flavo-fuscae, (50–)70–95(–112) μm. Sporae tunicibus tribus; stratum structurale 2.1–3.5 μm crassum, laminatum, depressionibus 0.5–1.4 μm foveoreticulatae; mycorrhizam vesiculari-arbuscularem formans. Holotypus: CUBMO 1a (HAC, Cuba).

HOLOTYPE: In Herbario de la Academia de Ciencias (HAC) CUBMO 1a, permanent slide in PVLG/PVLG-Melzer, obtained from single species culture MFO-IES-51/177, from which about 500 g of air-dried substrate containing spores have been conserved. Isotypes: FH (USA; CUBMO 1d), HAC-CUBMO 1b in MUCL (Belgium; CUBMO 1c), at URM (Brazil), UFRN (Brazil) and at Z+ZT (Switzerland; ZT Myc 24211).

ETYMOLOGY: *herrerae*, in honor to Dr. Ricardo Herrera-Peraza (1950–2006), a worldwide highly appreciated and knowledgeable mycorrhizologist from La Habana, Cuba (e.g Ferrer & Herrera 1980, Herrera-Peraza et al. 2001).

Sporiferous saccules are 65–105 μm in diameter (Figs 1–2) often detaching from mature spores. The saccule wall is composed by three thin layers, with the intermediate structural one being the thickest (up to 2.0 μm thick) and the outermost mucilaginous. The innermost saccule layer is very thin and difficult to observe. Often a short persistent pedicel connects the neck of the sporiferous saccule with the spore wall. It is cylindrical, up to 11 μm long and 6.4–9.0 μm wide. The wall of the pedicel consists also of three layers that are continuous between the outer spore wall and the wall of the saccule terminus. The pedicel usually breaks close to the base of the spore, revealing the cicatrix, which is 5.4–10.8 μm wide.

Spores formed singly in soils, are globose to subglobose, (50–)70–95(–112) μm (mean 83 μm) diameter (Figs 1–2), or occasionally ellipsoidal or ovoid 79–126 × 50–92 μm. When young, spores are yellow, becoming mostly yellowish brown when mature. The spore wall consists of three walls: outer, middle and inner wall (OW, MW, and IW).

Outer wall is triple-layered (Figs 3–4). OWL1 is hyaline, smooth, up to 1 μm thick, evanescent, commonly absent in mature spores (Fig. 8). OWL2 is yellowish brown, 2.1–3.5 μm thick, uniformly ornamented with rounded (0.5–1.4 μm) to elliptical (1.3–1.9 μm long and 0.9–1.4 μm wide) pits that are 0.6–2.3 μm deep (Figs 5–8). Some pits are vermiform or rugulate (2.2–4.8 long and 0.5–1.0 μm wide). At spore surface, 187–212 (mean = 199) pits can be observed 500 μm^{-2}. Surfaces are densely crowded with pits, leaving narrow ridges of 1.0–1.5 μm width in between each other, and giving the appearance of a raised reticulum (Fig. 7). OWL3 is hyaline, 0.5–1.1 μm thick, and often adherent to OWL2 (Figs 3–4), although a slight separation can occur in some crushed spores.
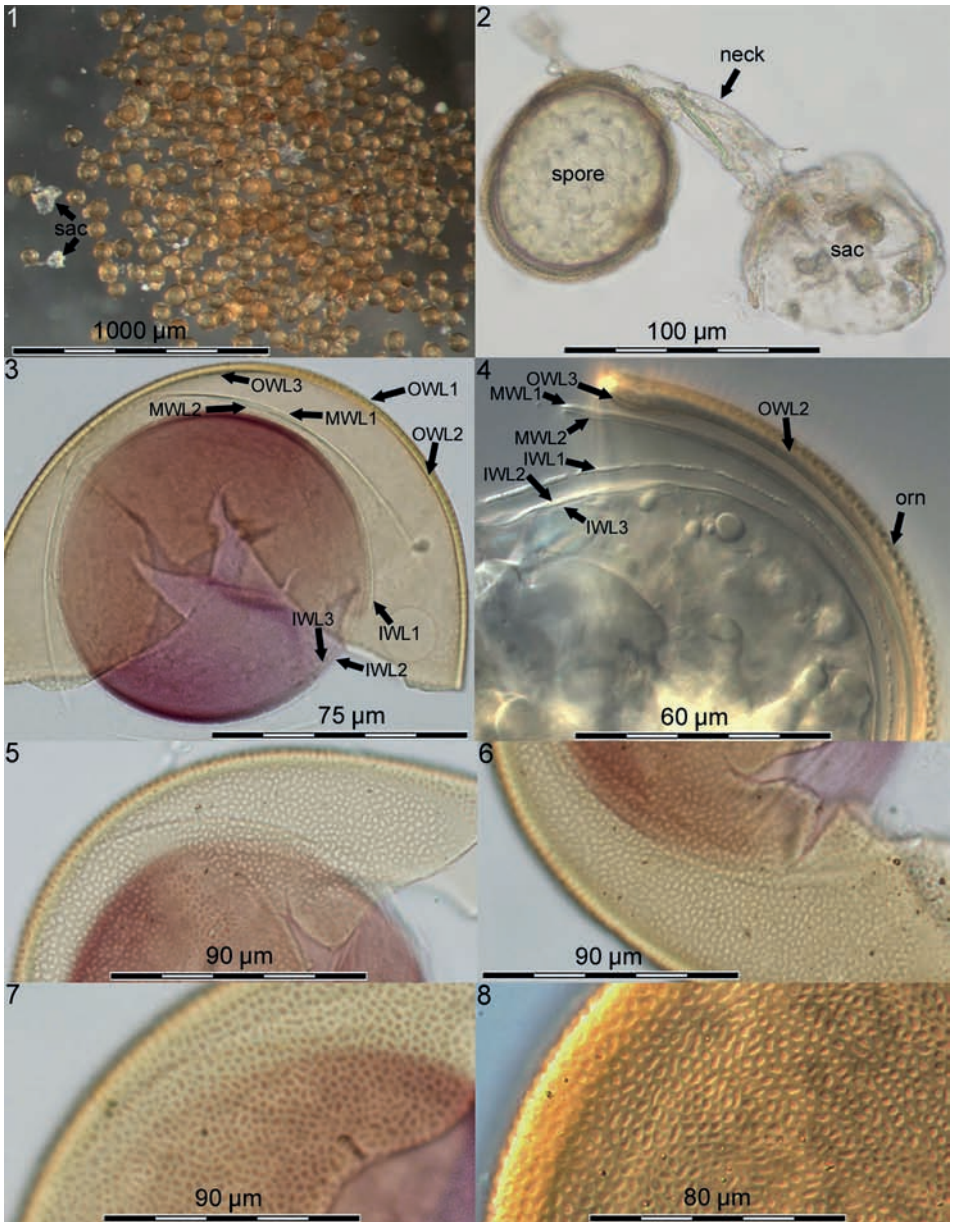
Figs 1–8. *Acaulospora herrerae*. Figs 1–2. Spores with or without sporiferous saccules (sac) attached; spores form laterally on the saccule neck. Figs 3–4. Crushed spores in cross view with three walls: triple-layered outer wall (OWL1–OWL3), bi-layered middle wall (MWL1–MWL2), and triple layered inner wall (IWL1–IWL3). Outer wall with pitted ornamentation on OWL2 (orn); IWL1 with granular (‚beaded') appearance, as typical in the family Acaulosporaceae; IWL2 & IWL3 staining purple to dark purple in Melzer's reagent. Figs 5–8. Diagnostic pitted spore surface ornamentation in planar view, showing high pit density per μm^2, and the raised reticulate appearance (Fig. 7).

Middle wall is bi-layered (MWL1-2) and smooth. The layers are tightly adherent, hyaline to pale yellow, of almost equal thickness, and together 1.1–1.7 μm thick (Figs 3–4).

Inner wall is triple layered (IWL1–3). The layers are commonly tightly adherent (Figs 3–4). IWL1 is 0.5–1.5 μm thick, moniliform and may desegregate as granular excrescences or "beads" dislodging outward with applied pressure. These beads also tend to disappear after heating at 60°C in lactic acid based mountants. IWL2 is 1.4–2.8 μm thick, expands sometimes up to 4.3 μm in PVLG (Fig. 7). IWL3 is a very thin (< 0.5 μm thick) and highly flexible layers that commonly wrinkles showing several folds on the inner IW surface (Fig. 8). IWL3 is not observed in all spores, but frequently. IWL2 and IWL3 stain purple to dark purple in Melzer's reagent just after staining. However, hours to days later staining of IWL3 might become pale or disappear completely.

Forming vesicular-arbuscular mycorrhizae as proven for *Bracharia decumbens* and *Sorghum vulgare* in multiple single species cultures.

PHYLOGENETIC ANALYSES: Partial sequences of the LSU rRNA gene obtained from three single spores confirm the new fungus in a well separated monophyletic clade next to *Kuklospora kentinensis* and several other ornamented species in the Acaulosporaceae such as *Acaulospora spinosa*, *A. tuberculata*, *A. scrobiculata* and *A. minuta* (Fig. 9). The variation between the LSU rRNA sequences of intra- and inter-individuals of the new species was around 1–2%. The closest species related to *A. herrerae* was *K. kentinensis* with 93% of identity in the BLASTn analysis.

DISTRIBUTION: *Acaulospora herrerae* was firstly detected in Eastern Cuba. It was originally isolated from a Calcaric Leptosol in Bayamo (Granma Province), with soil pH 7.3 and organic carbon content of 17.6 g kg⁻¹(Table 1). Then, the new fungal species was frequently detected in the semi-arid to semi-humid NE Brazil (e.g. in Petrolina, in Sertânia and Santa Maria da Boa Vista (Pernambuco State) and in Crato (Ceará State), in Taperoá (Paraíba State), in Jacobina (Bahia State), and in coastal dunes of Natal (Rio Grande do Norte State). The findings suggest a wide distribution of *A. herrerae* over tropical Central and South America in sandy to loamy soils of a wide range of soil pH (4–8), in semi-arid to semi-humid environments (Table 1).

Discussion

Acaulospora herrerae represents a distinct AM fungus of the Acaulosporaceae that might have been difficult to identify in the past through the similarities to *A. scrobiculata* and the slow progresses made in the morphological characterization of spore ornamentations since this first ornamented *Acaulospora* species with pitted

Fig. 4 & Fig. 8 were taken with DIC (differential interference contrast, Normansky's optic) on a Carl Zeiss Axioskop compound microscope, the others without DIC on several dissecting or compound microscopes in various laboratories in Cuba, Brazil and Switzerland, but always on the same type material derived from the single species culture.

Fig. 9. Phylogenetic tree of the Acaulosporaceae based on nuclear LSU rRNA gene sequence analysis. Sequences are labeled with database accession numbers. Support values (from up to down) are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Sequences obtained in this work are in bold. NA = not available. (Consistency Index = 0.54; Retention Index = 0.86).

spore surfaces was described (Trappe 1977). Due to the concomitant advances in morphological and molecular phylogenetic AMF species identification of the recent

years (e.g. Oehl et al. 2006, 2012), this new fungus can easily be identified, firstly through its diagnostic reticulate spore surface, and secondly by the LSU rRNA gene sequence phylogenetic analysis.

There are several other species in the Acaulosporaceae with similar spore sizes, colors and pitted surface ornamentations, e.g. *A. alpina*, *A. cavernata*, *A. excavata*, *A. lacunosa*, *A. minuta*, *A. nivalis*, *A. paulinae*, *A. punctata*, *A. sieverdingii* and *Kuklospora kentinensis* (basionym *Entrophospora kentinensis*) (Morton 1986, Błaszkowski 1988, 1989, Ingleby et al. 1994, Wu et al. 1995, Oehl et al. 2006; 2011a, c, d; 2012, Sieverding & Oehl 2006). Nevertheless, *A. herrerae* can be distinguished from all of them through its conspicuously raised and dense reticulum and the shape of its rounded, elliptical to elongated (vermiform or rugulate) pits. While spore size and color might be highly variable characters intraspecifically, especially when species occur in different environments, spore ornamentation is a much more stable character (e.g. Oehl et al. 2011d, 2012). Recently, an identification key was presented that facilitate the identification of all Acaulosporaceae species, especially for those species with ornamented spore surfaces (Oehl et al. 2012). In this key, the known variability of ornamentations is considered for each *Acaulospora* species.

The major morphological differences between the new fungus and the fungi mentioned above are summarized hereafter: *A. herrerae* produces smaller spores and more irregular pits than *A. cavernata* and *A. punctata*, respectively (Błaszkowski 1989, Oehl et al. 2011c), and the later two species rarely show the appearance of a raised reticule. *Acaulospora excavata* (Ingleby et al. 1994) produces larger spores and much larger pits than *A. herrerae*, and *A. lacunosa* (Morton 1986) forms spores larger than those of *A. herrerae* (98–186 μm vs 50–112 μm). Also, *A. lacunosa* shows a reddish-yellow color, and its ornamentation differs from the one described for *A. herrerae* as spores of *A. lacunosa* are ornamented with highly irregular saucer-shaped pits $0.2–3.0 \times 0.2–6.0$ μm broad, 0.2–2.0 μm deep and highly variable in number, showing also cone-shaped raised edges (Morton 1986). The spore sizes of *A. paulinae* (Błaszkowski 1988) and *A. nivalis* (Oehl et al. 2012) overlap with those of *A. herrerae* as well as their pit diameters. However, *A. paulinae* and *A. nivalis* pits were not described to show a raised reticule, respectively. Spores of *A. scrobiculata* (Trappe 1977) are much bigger (up to 240 μm) than those of *A. herrerae*. Also, *A. scrobiculata* spores are evenly pitted with circular, elliptical or linear to y-shaped depressions $1.0–1.5 \times 1.0–3.0$ μm, and they also do, like minutely pitted *A. minuta*, not have a raised reticule (Trappe 1977, Maia et al. 1994). Spores of *A. sieverdingii* are much lighter in color, and also lack a reticulum. Finally, spores of *Kuklospora kentinensis* are formed within the neck of sporiferous saccules, while spores of *A. herrerae* are always formed laterally on the neck of their saccules. Spores of *K. kentinensis* do also never form a reticulum. Moreover, pit density of *A. herrerae* is high (187–212 pits 500 μm^{-2}; mean = 199), while the pits of *K. kentinensis* isolate SM-71 from Taiwan conserved in sodium azide solution (courtesy of Dr. C.G.Wu) are less numerous (30–43 pits 500 μm^{-2}; mean = 36), and a similar Cuban isolate of *K. kentinensis* (CCHMA accession IES-56) had 44–68 pits/500 μm^{-2} (mean = 58). Pit density analyses, so far rarely performed on AMF species, might indeed be useful in the future for the identification of ornamented glomeromycotan species but this character will need to be investigated more in detail for all of these species.

Remarkably, *K. kentinensis* phylogenetically is the species most closely related to *A. herrerae* of all known *Acaulosporaceae*, despite of the fact that the spore formation mode of both species is different. Nevertheless, of all Acaulosporaceae species, formation of > 5–10 µm long, pigmented pedicels is regularly expressed only in exactly these two species (e.g. Wu et al. 1995, Sieverding & Oehl 2006), while it has been rarely if ever observed in other Acaulosporaceae spp. Recently, it was stated that Acaulosporaceae species might have more than one genus, or even have several genera (e.g. Schüßler & Walker 2010), an opinion which is shared by us. Morphological characters separating the species on the genus level could be the germination and germination shield structures that might have one to multiple lobes (see Spain 1992), or also other specific characters like the absence/presence of pigmented pedicels formed on the spore base. Such novel characters, however, have to be studied in more detail in the future. In all our phylogenetic analyses performed in the past, *Kuklospora colombiana* (the type species of the genus) was basal in the Acaulosporaceae (e.g. Oehl et al. 2012), and this is confirmed in the current study. This justified maintaining *Kuklospora* as a valid genus in the classification of Oehl et al. (2011b). Thus, we reject the transfer of all *Kuklospora* spp. to *Acaulospora* by Kaonongbua et al. (2010). Much more investigation appears to be necessary to disprove the existence of this genus. Our present findings place *Kuklospora kentinensis* together with *A. herrerae, A. spinosa, A. tuberculata, A. scrobiculata* and *A. minuta* into a major sub-clade within the Acaulosporaceae, which might suggest that they belong to a new genus within this family. Molecular analyses on multiple marker genes, however, will be needed for a greater resolution of the relationships between species and in particular between the major phylogenetic groups within the Acaulosporaceae. Just a single molecular marker was common in new *Acaulospora* species descriptions in the past. One exception was *A. nivalis* (Oehl et al. 2012), where the authors used the ITS region and the LSU rRNA sequences to construct the phylogenetic trees.

The new fungus was found in a calcareous soil in Eastern Cuba, and in NE Brazil it was often found in soils with pH > 6.0. However, recently it was also found in two more acidic tropical soils (pH 4.4–5.8), and it had been easily propagated in single species cultures with soil pH of 5.4 during the last decade. Many *Acaulospora* species had often been reported to prefer such more acidic soil pH conditions for their occurrence. We assume that *A. herrerae* has a major distribution in tropical Central and South America, and that this fungus has a better adaption to higher pH soils in tropical areas than several other *Acaulospora* species.

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