Journal of Applied Botany and Food Quality 95, 6 - 16 (2022), DOI:10.5073/JABFQ.2022.095.002

[1]Laboratorio de Biología y Genética Molecular, Universidad Nacional de San Martin, Morales, Peru
[2]Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Brazil
[3]Agroscope, Competence Division for Plants and Plant Products, Wädenswil, Switzerland

Acaulospora flavopapillosa, a new fungus in the Glomeromycetes from a coffee plantation in Peru, with an updated key for the identification of Acaulosporaceae species

Mike Anderson Corazon-Guivin[1*], Adela Vallejos-Tapullima[1], Ana Maria de la Sota-Ricaldi[1], Geomar Vallejos-Torres[1], María Emília Ruíz-Sánchez[1], Viviane Monique Santos[2], Gladstone Alves da Silva[2], Fritz Oehl[3*]

(Submitted: November 3, 2021; Accepted: January 6, 2022)

Summary

A new fungus of the arbuscular-mycorrhiza forming Glomeromycetes was found in a coffee plantation in Palestina, located in the Amazonian region of San Martín State in Peru. The fungus was propagated in bait cultures on *Brachiaria brizantha*, *Medicago sativa* and *Sorghum vulgare* as host plants. It forms typical acaulosporoid spores laterally on sporiferous saccule necks. The spores are brownish yellow to yellow brown, 125-160 µm in diam and are crowded with papillae on their surface. The papillae are approximately 1 µm wide as well as high. According to the color and surface structure of its spores, the fungus is here described under the epithet *Acaulospora flavopapillosa*. Phylogenetically, the new fungus clusters in a well-separated clade within a group that comprises *A. fragilissima, A. saccata, A. papillosa, A. morrowiae, A. delicata, A. rugosa, A. dilatata* and *A. longula*. Also *A. excavata* and *A. dilatata* were found by concomitant morphological and molecular phylogenetic analyses in San Martín State during this study: *A. excavata* in another coffee plantation, and *A. dilatata* in an inka nut plantation. An identification key for all species in the family Acaulosporaceae is updated in this study.

Key words: agroforestry, farming systems, Glomeromycota, Acaulosporaceae, soil biodiversity.

Introduction

In the family Acaulosporaceae, there are several major clades of *Acaulospora* species, which have either large spores or small spores. Major clades of *Acaulospora* species with rather large spores (> 130 µm) are for instance the species of the groups represented by *A. laevis*, or by *A. scrobiculata* and *A. spinosa* (Gerdemann and Trappe, 1974; Lin et al., 2019). In contrast, species of another *Acaulospora* group represented by *A. longula* form rather small spores (generally < 100 µm). A species group to which *A. cavernata* and *A. sieverdingii* belong and a species group arround *A. foveata*, form spores with different diameters varying from either large or small or intermediary sizes (Oehl et al., 2011; Błaszkowski et al., 2015; Corazon-Guivin et al., 2019a; Lin et al., 2019).

In the rhizosphere of coffee and the inka nut, several *Acaulospora* species have already been found, for instance *A. aspera* (Corazon-Guivin et al., 2019a) and *A. flava* (Corazon-Guivin et al., 2021). In our most recent survey from coffee and inka nut plantations in San Martín State of Peru, we found spores and obtained sequences of three other *Acaulospora* species. One of these species is new to the scientific community, while the other two species, *A. dilatata* and *A. excavata,* are already known from other habitats and continents (e.g. Morton, 1986; Ingleby et al., 1994; Pereira et al., 2015). The new fungal species is described hereafter under the epithet *A. flavopapillosa*. Additionally, we present spore illustrations and molecular phylogenies of the two other species, *A. dilatata* and *A. excavata*. Finally, the identification keys for the family Acaulosporaceae, published by our research group (Oehl et al. 2006; 2012; Lin et al. 2019), are improved and updated in the present study.

Material and methods

Study sites and soil sampling

Between July and December 2019, soil samples (0-30 cm depth) were repeatedly taken in Palestina (76°27'46.32"S 76°49'18.70"W, 745 m.a.s.l.) in a coffee plantation, in Bello Horizonte (6°31'39.54"S 76°17'57.85"W, 321 m a.s.l.) in a inka nut plantation, and in Nuevo Lamas (6°36'6.67"S 76°11'56.36"W, 973 m a.s.l.) in a coffee plantation. The first site is located in the province El Dorado, and the second and the third sites are located in the province San Martín. Both provinces belong to the Department (State) San Martín in the transition zone of Peruvian Amazonia lowlands and adjacent Andean low mountain ranges. These sites are traditionally cultivated under agroforestry systems, where coffee is grown with forest tree species and the inka nut is grown in mixed cultures together with banana as well as several other field crops such as *Manihot esculenta*. Both, inka nut and coffee grew without addition of chemical fertilizers and plant protection products. Soil pH (H_2O) was 6.5 in Palestina, 6.6 in Bello Horizonte and 4.6 in Nuevo Lamas, while available P ('Olsen-P', Olsen et al., 1954) was 10.9 mg P kg^{-1} in Palestina, 13.2 mg P kg^{-1} in Bello Horizonte, and 9.2 mg P kg^{-1} in Nuevo Lamas. In the province of El Dorado, the mean annual temperatures are about 28-32 °C, with variation between 22 and 36 °C throughout the year, mean annual precipitation is approximately 1200 mm. In the province of San Martín, the mean annual temperatures are about 23-33 °C, with variation between 19 and 37 °C throughout the year, mean annual precipitation is approximately 1380 mm (Senmhi, 2019).

AM fungal bait cultures

Bait cultures were established in the greenhouse of the Facultad de Ciencias Agrarias, Universidad Nacional de San Martín-Tarapoto, Peru, for 8 months under ambient temperature conditions, in cylindrical 3 L pots with 3 kg of substrate. Three bait cultures were established per field site with soil originating from the collection sites. The substrate consisted of a 2:1:1 mixture of field-collected soil samples, vermiculite and coarse river sand. The substrate mixtures were autoclaved at 121 °C for 60 min, three weeks before establishment of the bait cultures. The pots were first filled to 75% with the autoclaved substrate. Thereafter, layers of 200 g of non-sterilized rhizospheric soils were added to the substrate surface and five seeds either of *Sorghum vulgaris* L., *Medicago sativa* L. and *Brachiaria brizantha*

* Corresponding authors

(A. Rich.) Stapf were placed in order to establish the mycorrhizal association and reproduce spores of the new fungal species together with the complete native arbuscular mycorrhizal fungi (AMF) communities. The seeds were surface sterilized before seeding, using sodium hypochlorite (0.5%). Finally, the seeds were covered with the remaining 25% of the autoclaved substrate. The cultures were maintained in the greenhouse, with 21.4 °C ± 2.0 °C, 29.0 °C ± 3.0 °C and 36.0 °C ± 2.0 ºC as minimum, mean and maximum temperatures, respectively. The relative humidity was from 46 to 75% between January and August 2020. The pots were irrigated every other day and fertilized with a Long Ashton nutrient solution every two weeks, with reduced P contents (60% reduction; 20 µg P mL^{-1}; HEWITT, 1966).

Spore morphological analyses

Spores of the new fungus were found in the bait cultures from Palestina, while spores of two other *Acaulospora* species were found in Nuevo Lamas or Bello Horizonte, respectively. Single spores of each fungus were separated from their bait culture samples by a wet sieving process as described by SIEVERDING (1991). Spores were mounted on microscope slides in polyvinyl alcohol-lactic acid-glycerol (PVLG; KOSKE and TESSIER, 1983), Melzer's reagent, a mixture of PVLG and Melzer's reagent (BRUNDRETT et al., 1994), a mixture of lactic acid to water at 1:1, and in water (SPAIN, 1990). Morphological characteristics of the spores and their subcellular structures were observed in a high power light microscope at various magnifications. The terminology of the spore structures basically is that presented in BŁASZKOWSKI (2012) and OEHL et al. (2012) for species with acaulosporoid spore formation. Photographs were taken with a digital camera (Leika DFC 295) on a compound microscope (Leitz Laborlux S), using Leica Application Suite Version V 4.1 software. Specimens mounted in PVLG and a mixture of PVLG and Melzer's reagent (1:1) were deposited at Z+ZT (the joint herbarium of ETH Zurich and University of Zurich, Switzerland; https://www.herbarien.uzh.ch/en.html).

Molecular analyses

Intact, healthy spores were isolated from the bait culture samples, and cleaned by friction on fine filter paper (CORAZON-GUIVIN et al., 2019a, c). Spores were surface-sterilized (MOSSE, 1962) using a solution of chloramine T (2%), streptomycin (0.02%) and Tween 20 (2-5 drops in 25 mL final volume), for 20 min and rinsed five times in milli-Q water. For the new species from Palestina one independent group of sterile spores, containing 20-30 spores, was selected under a laminar flow hood and individually transferred into Eppendorf PCR tubes. Crude extract was obtained by crushing the spores with a sterile disposable fine-tipped pilon in 3 µL milli-Q water under the observation at 5× magnification using a stereoscope (Carl Zeiss, CORAZON-GUIVIN et al., 2019a, c). For the two other species, each 20-30 spores were also provided from their isolation sites and processed accordingly. Direct PCR of these crude extracts was performed in an automated thermal cycler (Eppendorf Mastercycler nexus, Germany) with a Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA) following manufacturer's instructions with 0.4 µM concentration of each primer. A two-step PCR was conducted to amplify the ribosomal fragment consisting of partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr, consecutively, according to KRÜGER et al. (2009). PCR products from the second round of amplifications (~1500 bp) were separated electrophoretically on 1.2% agarose gels, stained with Diamond™ Nucleic Acid Dye (Promega) and viewed by UV illumination. The band of the expected size was excised with a scalpel. The amplified DNA was isolated from the gel with the GFX™ PCR DNA and Gel Band Purification Kit (Sigma-Aldrich) following the manufacturer's protocol, cloned into the pCR2.1 TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA) and transformed into One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA). For the new species nine recombinant colonies were selected. For the two other species seven and five recombinant colonies, respectively, were selected by blue/white screening and the presence of inserts detected by PCR amplification with KOD DNA Polymerase (Sigma-Aldrich) using universal forward and reverse M13 vector primers. After isolation from transformed cells, plasmids were sequenced on both strands with M13F/M13R primers using the BigDye Terminator kit 3.1v (Applied Biosystems). The products were analyzed on an automated DNA sequencer (ABI 3730XL DNA analyzer-Macrogen Inc). All generated sequences were deposited at GenBank (OK360960-OK360968, OK356196-OK356202 and OK356203-OK356207).

Phylogenetic analyses

The phylogeny was reconstructed by independent analyses of the ITS region and the partial SSU, 5.8S, and partial LSU rDNA data sequences. The AM fungal sequences obtained were aligned with other Acaulosporaceae sequences from GenBank (Supplementary Material 1) in ClustalX (LARKIN et al., 2007), generating two data sets (alignments). Two separate trees were constructed covering the ITS region of the rDNA (first data set) and the partial SSU, 5.8S and partial LSU rDNA (second data set). *Gigaspora margarita* W.N. Becker & I.R. Hall was included as outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (MILNE et al., 2004). Bayesian (two runs over 3×10^6 generations, with a sample frequency of 300 and a burnin value of 25%) and maximum likelihood (1,000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (RONQUIST and HUELSENBECK, 2003) and PhyML (GUINDON and GASCUEL, 2003), launched from Topali 2.5, using the GTR + G model.

Results

In the taxonomy section, the new species is firstly described morphologically, and its phylogenetic position shown. Secondly, its currently known distribution is shortly summarized. Finally, a key is updated for the morphological identification of the species attributed to the family Acaulosporaceae.

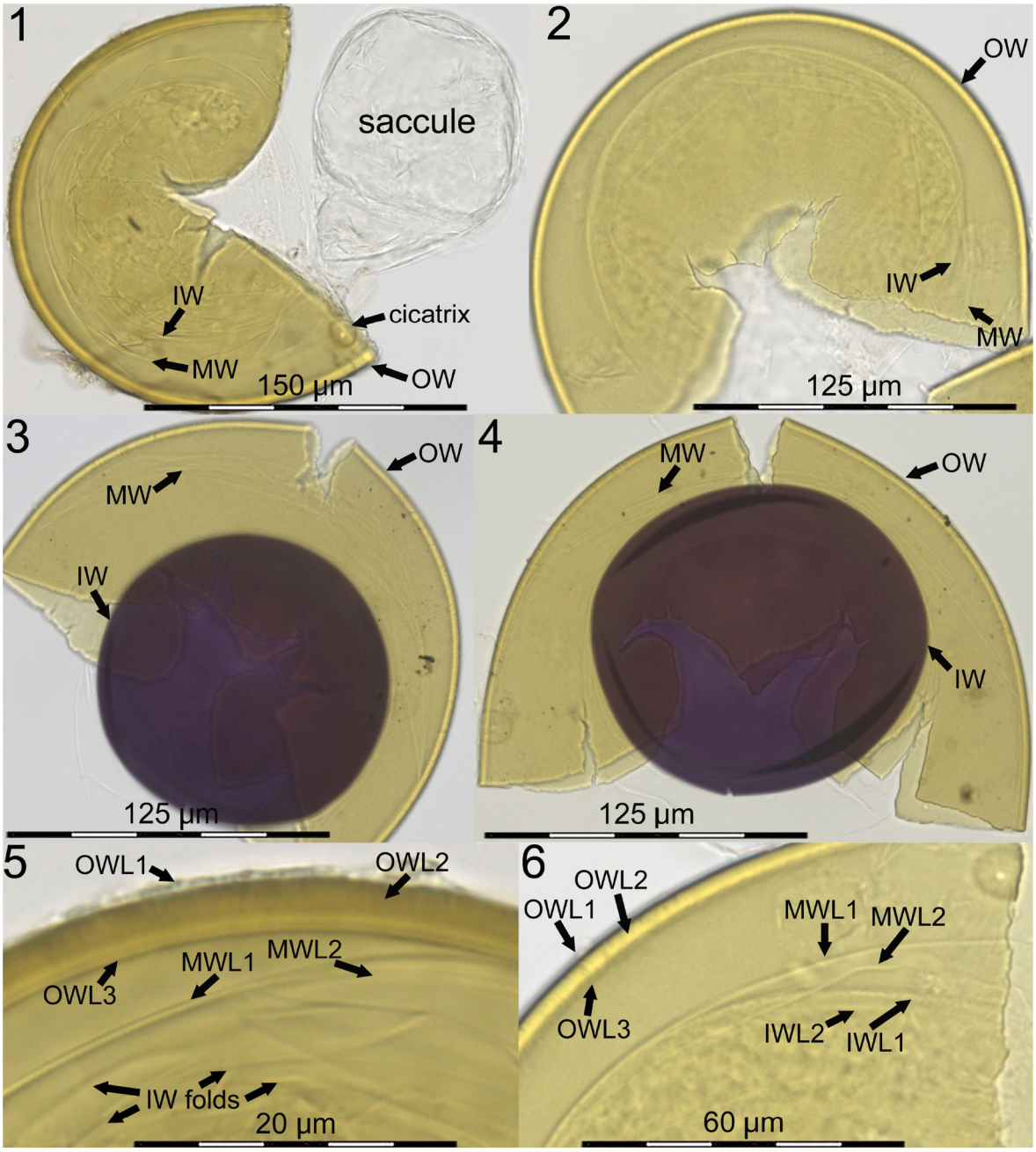
Taxonomy

Acaulospora flavopapillosa Corazon-Guivin, G.A. Silva & Oehl sp. nov. Figs. 1-6
MycoBank MB 841841

Diagnosis: Differing from *A. papillosa* in having larger and pigmented spores, which are brownish yellow to yellow-brown.

Etymology: Latin, *flavo-*, (= yellow to yellow brown) and *-papillosa* (papillate) referring to the spore color and the papillate spore surface structure of the new species.

Holotypus: Accession ZT Myc 66249, deposited at Z+ZT, specimen derived from a bait culture established on the host plants *Sorghum vulgare*, *Brachiaria brizantha* and *Medicago sativa* in the greenhouse of the Molecular Biology and Genetics Laboratory, Faculty of Agricultural Sciences, National University of San Martín-Tarapoto, Peru. Fungal inoculum for the culture originated from a coffee plantation in Palestina (Province El Dorado, San Martín State; 76°27'46.32"S 76°49'18.70"W, 745 m.a.s.l). Collector was Mike Anderson Corazon Guivin and collection date was 25.04.2019. Isotype (ZT Myc 66252) was also deposited at Z+ZT. Living cultures of the fungus are currently established at the Universidad Nacional de San Martín-Tarapoto.



Figs. 1-6: *Acaulospora flavopapillosa*. **1-2.** Crushed spores in PVLG with three walls (OW, MW & IW) formed laterally on the neck of sporiferous saccules, and forming a single permanent cicatrice on the outer wall. **3-4**. Crushed spores in PVLG + Melzer's. **5.** Papillate spore surface in cross view: Papillae are approximately 1 µm wide and high. OWL1 hyaline and evanescent to semi-persistent. OWL2 permanent, structural and brownish yellow to yellow-brown, OWL3 ususally closely adherent to OWL2. MWL1-2 hyaline and often not easily separated by pressure on the cover slide, or when separate, often each showing several wrinkling folds due to their high flexibility (e.g. Fig. 3). **6.** IWL1 generally only 0.8-1.1 µm thin, and thus, its beaded ornamentation is only observed, when clearly separating from IWL2. IWL2 stains purple to dark purple in Melzer's (Figs. 3-4). IWL3 difficult to observe as only 0.5-1.1 µm thick and usually closely adherent to IWL2.

Description: Sporiferous saccules are hyaline and singly formed at the end or intercalary of mycelial hyphae. The saccules are globose to subglobose, 95-140 × 90-135 µm, with 2-3 wall layers that are in total 2.3-4.0 µm thick. The saccule necks are 20-50 µm broad at the saccule termini, about 20-30 µm at the point of spore formation, and taper to 10-16 µm in 30-100 µm distance from the spore towards the mycelium. The saccule usually collapses after the spore wall has formed and usually is detached from mature spores.

Spores form laterally on the neck of sporiferous saccules in 40-100 µm distance from the sporiferous saccule. They are globose to subglobose, brownish yellow to yellow brown, 125-160 µm in diam and have three walls.

Outer wall consists of three layers (OWL1-OWL3). Outer layer (OWL1) is hyaline to subhyaline, 1.0-1.3 µm thick, crowded with fine papillae, which are approximately 1 µm wide as well as high,

and evanescent to semi-persistent. Second layer (OWL2) is brownish yellow to yellow brown, persistent, laminated, 3.0-5.0 µm thick. The inner layer of the outer wall (OWL3) is concolorous with OWL2, about 0.8-1.2 µm thick and regularly observed in crushed spores. None of the OW layers stains in Melzer's reagent.

Middle wall is hyaline, bi-layered and only 1.4-2.0 µm thick in total. Both layers (MWL1 and MWL2) are semi-flexible to flexible. They often appear as being only one wall layer, when tightly adherent to each other, but quite often they also show several wrinkling folds. None of the MW layers stains in Melzer's reagent.

Inner wall is hyaline, with two to three layers (IWL1-IWL3). The IWL1 is about 0.8-1.1 µm thick with a 'beaded', i.e. granular structure, which usually is difficult to observe, since IWL1 is rather thin and generally adherent to IWL2. IWL2 is 1.1-2.1 µm thick and regularly stains pinkish purple to dark purple in Melzer's reagent. IWL3 is 0.5-1.1 µm and generally very difficult to detect since it is closely adherent to IWL2. As found for the two MW layers, also each of the IW layers is rather thin and may show several folds, due to their wrinkling nature.

Cicatrix remains after detachment of the connecting hypha, 8-13(-17) × 7-13 µm wide. The pore is closed by inner laminae of OWL2 and by OWL3.

Formation of *vesicular-arbuscular mycorrhizal structures* is not known.

Molecular analyses: The phylogenetic analyses of the ITS region and partial SSU, 5.8S, and partial LSU rDNA sequences placed *A. flavopapillosa* in a separated clade inside a group to which *A. fragilissima, A. saccata, A. papillosa, A. morrowiae, A. delicata, A. rugosa, A. dilatata* and *A. longula* belong (Figs. 7-8). The clade for the new species was full supported for Bayesian posterior probabilities in both analyses and was supported by 98% and 99% bootstrap values for ML analyses for the ITS and SSU-5.8S-LSU data set, respectively. In the BLASTn analyses, the sequences with closest match (96%) to the new fungus are from *A. morrowiae, A. dilatata* and *A. longula*. Four environmental sequences, related to *A. flavopapillosa* (97% of identity), were found in roots of tropical plants from a hydrocarbon-polluted soil in the amazon region of Ecuador (Garcés-Ruiz et al., 2019) and in roots from *Hedera rhombea* in Niigata, Japan (Ahulu et al., 2006).

Distribution of *A. flavopapillosa* and other *Acaulospora* species in San Martín State of Peru:
So far, the fungus was only found in a coffee plantation in Palestina (one agroforestry site, grown with forest species such as *Cordia alliodora* and *Calycophyllum spruceanum*) located in the Amazonia region, Province of El Dorado, Department of San Martín in Peru. In Corazon-Guivin et al. (2021), 14 *Acaulospora* species had already been reported in San Martín State either in coffee or inka nut plantations. Here, we add, besides *A. flavopapillosa*, also *A. dilatata* to this list (Figs. 7-8 and 9-11; ZT Myc 66250) and deliver for *A. excavata* the first phylogenetic data from Peru (Figs. 7-8 and 12-13; ZT Myc 66251, to compare with Pereira et al., 2015). *Acaulospora dilatata* was found in an inka nut plantation in Bello Horizonte and *A. excavata* in a coffee plantation in Nuevo Llamas, both located in the province San Martín. *Acaulospora flavopapillosa* is the third *Acaulospora* species, which is originally described from Peru, after *A. aspera* (Corazon-Guivin et al., 2019a) and *A. flava* (Corazon-Guivin et al., 2021).

Key for the morphological spore identification of *Acaulosporaceae* species
In order to establish an updated key for Acaulosporaceae, we followed Oehl et al. (2006; 2012; 2014) and Lin et al. (2019). As in Lin et al. (2019), a few species were not included in the key, since it was assumed that they do not belong to the genus *Acaulospora* (e.g. *A. soloidea*) or might be synonymous with previously described species (*A. walkeri* with *A. laevis*) or confused with species from other glomeromycotean orders ('*A. brasiliensis*' with *Ambispora brasiliensis*). Now, also *A. polonica* is excluded from the key following Błaszkowski et al. (2021). Six recently or newly described species are included here: *A. koreana* (Lee et al., 2018), *A. aspera* (Corazon-Guivin et al., 2019a), *A. flava* (Corazon-Guivin et al., 2021), *A. fanjing* (He et al., 2021), *A. jejuensis* (Park et al., 2021), and *A. flavopapillosa*. The references for the original and emended species descriptions for all Acaulosporaceae species included in the identification key are given in Supplementary Material 2:

1 Spores apparently not formed on stalk of sporiferous saccules . . . 2
1' Spores generally formed on/in stalk of sporiferous saccules 3

2 Spores white to pale ochraceous, 69-85 µm, IWL1 hyaline, beaded; IWL2 1.5-3.5 µm in PVLG, deep purple in Melzer's reagent . . . . . *A. saccata* D. Redecker et al.
2' Spores creamy white to pale ochraceous, 60-83 µm. IW not staining in Melzer's reagent except for IWL1, which may become light greenish yellow-ochre . . . . . *A. fragilissima* D. Redecker et al.

3 Spores with a single cicatrix at spore base 4
3' Spores with two persistent cicatrices at the spore, proximal and distal to the saccule 52

4 Spores without ornamentation on the outer spore wall 5
4' Spores with ornamentation on the outer spore wall 23

5 Spores generally ≤ 100 µm 6
5' Spores generally > 100 µm. 12

6 Spores hyaline to pale yellowish cream, 80-125 × 80-110 µm; OW turning slightly darker yellow, but IW staining orange-red in Melzer's . . . . . *A. delicata* C. Walker et al.
6' Spores yellow or ochreaous to light yellow brown to orange brown 7

7 Spores yellow or ochreaous to light yellow brown 8
7' Spores dark yellow brown to orange brown, (72-)95-105(-126) µm . . . . . *A. mellea* Spain & N.C. Schenck

8 Spores with a smooth surface. 9
8' Spores with a either papillae or rugose surface. 10

9 Spores without reaction in Melzer's reagent, spores pale yellow to yellow brown, (55-)65(-75) µm, a beaded wall hitherto not observed . . . . . *A. gedanensis* Błaszk.
9' Spores with reaction in Melzer's reagent 11

10 Spores with small papillae (0.5-1.1 µm wide, 0.5-1.2 µm high, and in 0.5-1.1 µm distance from each other), yellow white to light yellow to creamy, 65-100 µm; papillae often disappearing in lactic acid based mountants . . . . . *A. papillosa* C.M.R. Pereira & Oehl
10' Spores with a rugose surface, generally with ballooning OWL1 in lactic acid based mountants, subhyaline to straw colored, (49-)92 (-118) µm. . . . . *A. rugosa* J.B. Morton

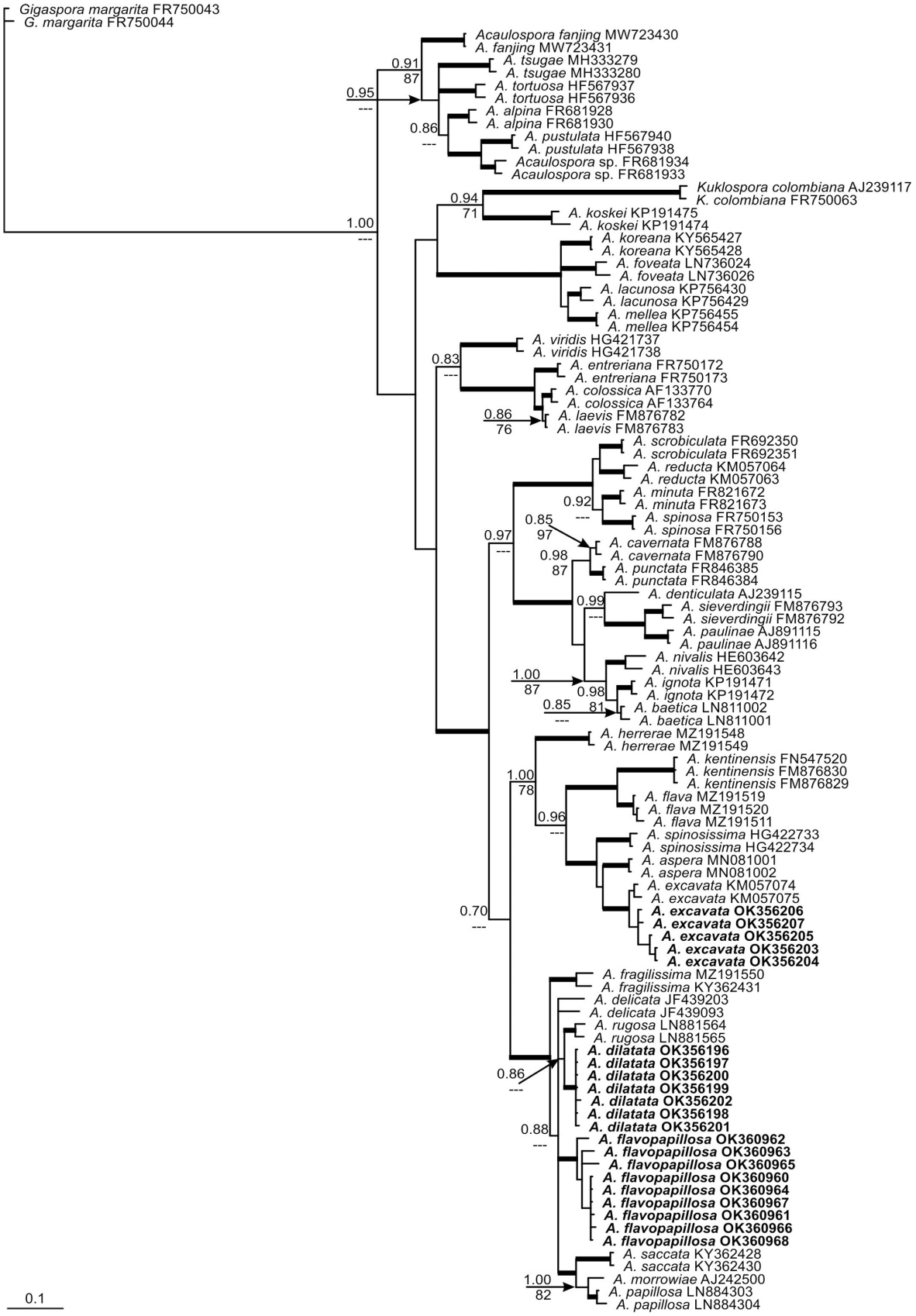
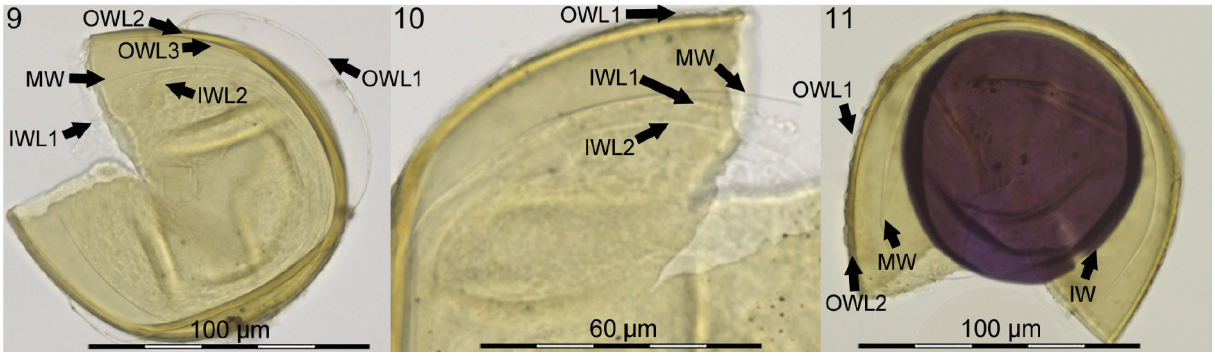
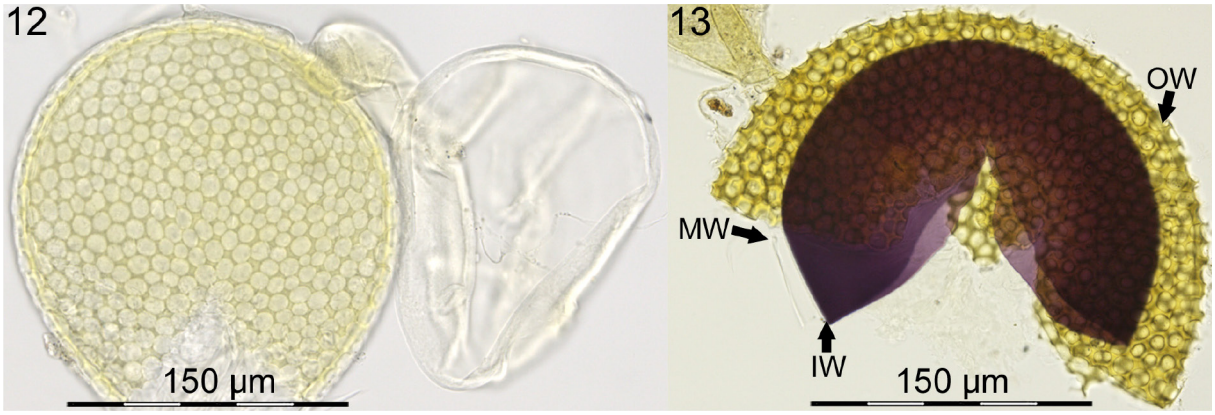


Fig. 7: Phylogenetic tree of the Acaulosporaceae obtained by analysis from sequences of the ITS region of the rDNA from different *Acaulospora* spp. sequences are labeled with their database accession numbers. Support values (from top) are from Bayesian inference – BI (performed with two runs over 3×10^6 generations, with a sample frequency of 300 and a burnin value of 25%) and maximum likelihood – ML (performed with 1,000 bootstrap), respectively. The GTR + G model was used in both analyses. Sequences obtained in this study are in boldface. Only support values of at least 70% are shown. Thick branches represent clades with more than 90% of support in all analyses. The tree was rooted by *Gigaspora margarita*.

Fig. 8: Phylogenetic tree of the Acaulosporaceae obtained by analysis of partial SSU, 5.8S, and partial LSU rDNA sequences from different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from Bayesian inference – BI (performed with two runs over 3×10^6 generations, with a sample frequency of 300 and a burnin value of 25%) and maximum likelihood – ML (performed with 1,000 bootstrap), respectively. The GTR + G model was used in both analyses. Sequences obtained in this study are in boldface. Only support values of at least 70% are shown. Thick branches represent clades with more than 90% of support in all analyses. The tree was rooted by *Gigaspora margarita*.



Figs. 9-11: *Acaulospora dilatata*. Crushed spores in PVLG or PVLG + Melzer's reagent with three walls (OW, MW & IW) and multiple wall layers (OWL1-3, bi-layered MW, IWL2). OWL1 initially smooth (Fig. 9), getting roughened in the course of time (Figs. 10-11), sometimes resembling as finely pitted. IWL2 staining purple to dark purple in the presence of Melzer's reagent.



Figs. 12-13: *Acaulospora excavata*. **12.** Young, crushed spore in PVLG formed laterally on the neck of a hyaline sporiferous saccule. The yellow spore wall layer is crowded by large pits on its outer surface. **13.** Crushed spore in PVLG + Melzer's reagent with three walls (OW, MW & IW). IW(L2) staining purple to dark purple in the presence of Melzer's reagent.

11 Spores with a mucilaginous wall, dull to pale yellow, 75-90(-100) µm . . . . . *A. longula* Spain & N.C. Schenck
11' Spores, without mucilaginous wall, bright yellow, sparkling in reflected light, 79-92(-120) µm . . . . . *A. morrowiae* Spain & N.C. Schenck

12 Spores hyaline to brilliant white, 145-317 µm . . . . . *A. splendida* Sieverd. et al.
12' Spores with pigmented spore wall. 13

13 Spores yellow to honey colored to yellow brown 14
13' Spores brown to dark brown, reddish orange, orange brown to red brown, or greenish brown 16

14 Spores generally < 150 µm, yellow to brownish yellow to yellow brown 15
14' Spores generally > 150 µm, (120-)150-300 × (120-)150-520 µm, honey colored, dull brown yellow to yellow brown to olive brown . . . . . *A. laevis* Gerd. & Trappe

15 Spores always smooth on surface, light yellow to bright yellow to yellow brown, (95-)105-160 × (95-)100-150 µm. . . . . *A. flava* Corazon-Guivin et al.
15' Spores with a smooth to roughened surface sometimes resembling a minute, pitted surface, (78-)106(-130) µm, deep yellow to brownish yellow. . . . . *A. dilatata* J.B. Morton

16 Spores without staining reaction on the outer wall in Melzer's reagent. 17
16' Spores with staining reaction on the outer wall in Melzer's reagent, spores reddish orange, (80-)183(-340) µm, IW purple to dark purple in Melzer's . . . . . *A. koskei* Błaszk.

17 Spores with a rather thin, evanescent to rarely semi-persistent outer hyaline spore wall layer. 18
17' Spores with a thick, semi-persistent to persistent outer hyaline spore wall layer, (150-)185(-240) µm, dark brown to black . . . . . *A. thomii* Błaszk.

18 Spores without or only pale pink staining reaction on the inner wall in Melzer's. 19
18' Spores with a purple to dark purple staining reaction on IW in Melzer's. 21

19 Spores (96-)127(-153) × (99-)132(-158) µm, light brown to dark brown; IWL2 staining light pink in Melzer's. . . . . *A. koreana* E.H. Lee et al.
19' Spores generally > 200 µm. 20

20 Spores brown, 260-330 µm. . . . . *A. entreriana* M.S. Velázquez & Cabello
20' Spores orange brown to dark red brown, 180-380 µm . . . . . *A. colossica* P.A. Schultz et al.

21 Spores greenish yellow brown to greenish brown, 140-205 × 140-193 µm. . . . . *A. viridis* Palenz. et al
21' Spores orange-red to capsicum-red, orange-brown to red-brown 22

22 Spores orange-red to capsicum-red, (170-)298-330 µm. . . . . *A. capsicula* Błaszk.
22' Spores orange-brown to red-brown, (109-)123(-138) µm. . . . . *A. fanjing* R.J. He et al.

23 Spores with papillae, spines, warts, pustules, or other regular to irregular projections 24
23' Spores with depressions (pits) or cerebriform folds 34

24 Spores with projections, but without reticulum. 25
24' Spores with projections and an additional reticulum 33

25 Spores with papillae, spines, tubercles, warts, pustules or other regular projections. 26
25' Spores with tortuous hyphae-like structures on the surface that are subhyaline to pale yellow to sometimes dark yellow. These structures are also highly irregular in length (2.6-10.5(-35) µm), width (2.5-7.5 µm, up to rarely 13 µm) and height (2.4-7.5 µm), and the distances between each other are also quite variable (0.0-6.5 µm); spores yellow orange to orange brown, 61-84(-94) × 61-80(-91) µm . . . . . *A. tortuosa* Palenz. et al.

26 Spores with papillae, spines, tubercles, warts, or pustules. 27
26' Spores with inseparable polygonal segments (4-6 × 5-10 in diam and 4-6 µm thick) and circular to ellipsoidal projections (3-6 × 3-4 µm wide and up to 3.2 µm high); each projection with a central cavity; (112-)130-175 µm . . . . . *A. denticulata* Sieverd. & S. Toro

27 Spores with papillae, spines, tubercles, or warts 28
27' Spores crowded with pustules; pale brown when young, becoming yellow brown with age, 45-65(-72) × 44-62(-68) µm . . . . . *A. pustulata* Palenz. et al.

28 Spores with papillae, spines or tubercles 29
28' Spores with evenly distributed warts or flattened elevations on OWL2, up to 1 µm high on the upper surface, frequently deteriorating with age, and then gradually becoming invisible; spores yellowish white to orange-yellow, 65-80 µm . . . . . *A. ignota* Błaszk. et al.

29 Spores crowded with papillae, brownish yellow to yellow brown, 125-160 µm; papillae approximately 1 µm wide and high . . . . . *A. flavopapillosa* Corazon-Guivin et al.
29' Spores with spines or tubercles. 30

30 Spores with spines or tubercles formed on hyaline to subhyaline, evanescent to (semi-) persistent outer layer(s) of OW. 31
30' Spores crowded with fine spines formed below the evanescent OWL1 on the upper surface of the structural, laminated, pigmented layer; spines about 1.0-2.9 µm high, 0.9-1.4 µm at the base, pointed to 0.5 µm broad at the top, and <1 µm apart; spores yellow brown to brown 74-98(-107) × 73-98 µm . . . . . *A. spinulifera* Oehl et al.

31 Structural layer on OW generally < 2.5 µm thick; spores light yellow when young, becoming bright yellow to brownish-yellow, 120-187 × 116-180 µm; second evanescent layer (OWL2) subhyaline, densely crowded with short spiny projections that are 0.5-1.1 µm high and 0.4-0.8 µm wide at base . . . . . *A. spinosissima* Oehl et al.
31' OW generally > 2.5 µm thick 32

32 Spores with fine crowded, densely organized spines, 1-4 µm tall, 1 µm at base and tapering to 0.5 µm at the tip; spores yellow brown to brown to rarely dark brown, (110-)140-330 µm . . . . . *A. spinosa* C. Walker & Trappe
32' Spores with fine tubercles, 0.7-3.5 µm long and 1.5 µm broad at the base, tapering to 0.7-1.1 at the rounded tip, irregular distances (0.5-3 µm) between single tubercles; spores dark honey brown to reddish black, 250-340 µm. . . . . *A. tuberculata* Janos & Trappe

33 Reticulum three-layered enclosing polygonal projections ±1 × 1 µm; spores light brown to brown, generally 150-200 µm . . . . . *A. bireticulata* F.M. Rothwell & Trappe
33' Reticulum one-layered, overlaid over crowded, densely-organized spines ±2 µm high; spores yellow brown to dark brown, 140-330 µm. . . . . *A. elegans* Trappe & Gerd.

34 Spores with pits 35
34' Spores with cerebriform folds, light yellow to brown, (87-)112-168(-175) µm . . . . . *A. rehmii* Sieverd. & S. Toro

35 Spores in sporocarps, dark reddish brown to dark brown, 75-80 µm, ornamentation of 0.5-1 µm wide, 4-5 sided pits, 1.2 × 0.5-1.0 µm across, ridges form a mesh reticulum. . . *A. taiwania* H.T. Hu
35' Spores formed singly in soil, not in sporocarps. 36

36 Spores regularly < 100 µm. 37
36' Spores regularly > 100 µm. 43

37 Pits of irregular shape. 38
37' Pits of regular round shape 40

38 Spores with a reticulum forming ridges between the pits; spores yellow, becoming mostly yellowish brown when mature, (50-)70-95(-112) µm, or occasionally ellipsoidal or ovoid 79-126 × 50-92 µm; OWL2 yellowish brown, 2.1-3.5 µm thick, uniformly ornamented with rounded (0.5-1.4 µm) to elliptical pits, 1.3-1.9 µm long, 0.9-1.4 µm wide, and 0.6-2.3 µm deep; some pits vermiform or 'rugulate', 2.2-4.8 µm long and 0.5-1.0 µm wide. . . *A. herrerae* Furrazola et al.
38' Spores generally without a reticulum 39

39 Spores hyaline to subhyaline to rarely light yellow, 65-85 µm, irregular pits resembling small dots (0.8-1.8 µm) or lines (0.5-1.2 × 1.8-2.5 µm). . . . . *A. sieverdingii* Oehl et al.
39' Spores bright yellow to dark yellow, with irregular pits, 81-100 × 75-82 µm, 0.9-1.5 × (0.9-)1.5-3.5(-5.5) µm wide and 1.2-2.5 µm in depth. . . . . *A. nivalis* Oehl et al.

40 Spores hyaline, subhyaline, pale yellow to creamy. 41
40' Spores yellow to orange brown, or creamy brown to light brown 42

41 Spores hyaline to subhyaline, with concave round pits of widest diameter < 3.5 µm; (60-)72(-95) µm; pits 2.0-2.5 × 3.0-3.5 µm, when seen in a plan view, 0.8-1.0 µm deep . . . . . *A. paulinae* Błaszk.
41' Spores pale yellow to creamy, with concave, round and shallow pits of widest diameter (3.2-)3.5-7.8 µm; 70-95 × 60-70 µm. . . . . *A. verna* Błaszk.

42 Spores yellow to orange brown, 65-85 µm; truncated cone shape pits of widest diameter of 1.5-2.2 µm . . . . . *A. alpina* Oehl et al.
42' Spores creamy brown to light brown, often appearing with a grayish tint in water, 65-92 µm; pits about 0.8-1.6 × 0.7-1.4 µm wide, 0.6-1.3 µm deep and about (1.5-)2.2-5.1 µm apart . . . . . *A. baetica* Palenz. et al.

43 Pits of irregular shape. 44
43' Pits of regular round shape. 46

44 Spores whitish, subhayline to olive, greenish-yellow, turquoise or green 45
44' Spores reddish-yellow to yellow-brown, 100-180 µm, with irregular, saucer-shaped pits, 0.2-3 × 0.2-6 µm . . . . . *A. lacunosa* J.B. Morton

45 Spores subhyaline to light olive or brownish white, 100, 100-240 × 100-220 µm; circular to ellipsoid to y-shaped pits, 1.0-1.5 × 1.0-3.0 µm . . . . . *A. scrobiculata* Trappe
45' Spores green or turquoise, rarely yellowish-green, (142-)189(-244) × (146-)201(-246) µm; 'numerous pits with the form of crater', 'polygonal or irregular ellipsoid pits', 1.3-2.4 µm . . . . . *A. jejuensis* H. Park & A.H. Eom

46 Spores regularly 100-180 µm 47
46' Spores regularly 185-310(410) × 215-350(480) µm, yellowish brown to yellow brown to sometimes reddish brown, becoming brown to black brown with age, with concave round pits, 3.5-8.5(-12) × 3.5-12.5(-16) µm. . . . . *A. foveata* Trappe & Janos

47 Pits regularly < 2.0 µm 48
47' Pits regularly > 2.0 µm 50

48 Pits only 1.0-1.5(-1.8) µm apart, 0.4-0.7 µm in diam and up to 0.8 µm deep; spores yellow to yellow brown spores, (120-)135-195 × (120-)130-187 µm . . . . . *A. aspera* Corazon-Guivin et al.
48' Single pits generally separated by larger distances than 1.5 µm 49

49 Spores subhyaline to yellow-white, 105-129 µm, pits 1.1-2.0(-2.7) µm wide and at least as deep (1.4-3.5 µm) as wide; pits 2.0-3.2 µm apart . . . . . *A. punctata* Oehl et al.
49' Spores bright yellow orange to orange brown, 150-220 µm, crowded with minute pits, 0.5-1.2(-1.8) µm wide and 0.5-1.1 µm deep, and 1.5-2.5(-3.5) µm apart . . . . . *A. minuta* Oehl et al.

50 Spores without small pits and ridges within the large pits. 51
50' Spore with secondary small pits (ca. 0.5 µm broad and deep) and fine ridges within irregularly shaped, often edged to sometimes dumbbell-shaped pits (5.5-19 × 3.5-8.6 µm) large pits; spore whitish yellow, dark yellow to light brown, 135-205 µm . . . . . *A. reducta* Oehl et al.

51 Spores yellow brown, 115-170 µm, with concave round pits, 2.5-5.0 µm in diam and 1.7-2.5 µm in depth. . . *A. cavernata* Błaszk.
51' Spores ochre to brown, 100-180(-200) µm, with concave round pits, 4-20 µm in diam and 2-6 µm in depth . . . . . *A. excavata* Ingleby & C. Walker

52 Spores described to be formed either laterally on or within the neck of the sporiferous saccule, while saccule was never observed; spores hyaline or subhyaline to pale yellow, 75-140 µm; OWL1 covered with irregularly spaced, hemispherical, hyaline to subhyaline protrusions, 0.5-3 µm wide and up to 1 µm high . . . . . *A. colliculosa* Kaonongbua et al.
52' Spores exclusively formed intrahyphally within the neck of the sporiferous saccule 53

53 Spores with a strong proximal cicatrix, which continues from a few to several µm into the saccule neck 54
53' Spores with a fine proximal cicatrix, which resembles a ring and does not continue into the saccule neck 55

54 Spores with smooth surfaces, pale yellow to yellow brown, 64-74×84-99 µm. . . . . *A. tsugae* T.C. Lin & Oehl
54' Spores with pitted spore ornamentation, pale yellow to yellow brown, 85-140 × 95-210 µm; pits 1-3 µm in diam and 0.7-1.7 µm in depth, separated by ridges 2-6 µm wide. . . . . *A. kentinensis* (C.G. Wu & Y.S. Liu) Kaonongbua et al.

55 Spores with smooth surfaces, pale yellow to light golden brown, (75-)100-115(-135) µm . . . . . *Kuklospora colombiana* (Spain & N.C. Schenck) Oehl & Sieverd.
55' Spores with spiny spore ornamentation, subhyaline to yellowish-white, 100-250 µm; OWL2 crowded with fine spines, 2-3 µm high and 0.5 µm in diam . . . . . *K. spinosa* B.P. Cai et al.

Discussion

Acaulospora flavopapillosa can be distinguished from all other species in the family Acaulosporaceae by the combination of its spore size (125-160 µm), brownish yellow to yellow brown spore color and the characteristics of the outer spore surface, which is crowded by papillae. The morphologically most similar species is *A. papillosa*, which forms smaller and less pigmented spores, 69-100(-110) × 65-93(-101) µm in diam and yellow white to light yellow or creamy (Pereira et al., 2016). Other similar species have also significantly smaller spores, such as *A. dilatata* (80-125 µm) and *A. rugosa* (49-118 µm), which has irregular folds on the spore surface instead of small papillae, and/or they have smooth spore surfaces, such as *A. delicata*, *A. longula* and *A. morrowiae* (all generally < 100 µm) or *A. flava* (130-190 µm; Corazon-Guivin et al., 2021). *Acaulospora dilata* was firstly described to have a pitted, roughened spore surface (Morton, 1986), but later on (http://fungi.invam.wvu.edu/the-fungi/classification/acaulosporaceae/acaulospora/dilatata.html), it was also presented with a smooth surface. Our observations confirm these diverging descriptions, depending on the degradation stage of the outermost spore wall layer (Figs. 9-11). We assume that also for *A. flavopapillosa* the presence of different degradation stages might complicate the morphological identification of spores especially, when isolated from field soil samples.

Also phylogenetically, *A. flavopapillosa* is closely related to *A. papillosa* (Pereira et al., 2016). Remarkably, *A. flavopapillosa* exclusively clusters together with *Acaulospora* species, which are characterized by smooth or roughenend to papillate spore surfaces, but rather small spores (generally < 100 µm: *A. longula*, *A. fragilissima*, *A. morrowiae*, *A. papillosa*, *A. rugosa*, *A. saccata*; approximately 100 µm: *A. dilatata*). *Acaulospora flavopapillosa* forms the largest spores of all the species within this group (Lin et al., 2019). With respect to spore surface ornamentation, this group still has only species with smooth or roughened spore surfaces, but not any species with permanent ornamentations, constituted by e.g. persistent projections or pits (Lin et al., 2019).

Interestingly, all these species discussed here above were firstly reported from tropical southern America (e.g. Schenck et al., 1984; Pereira, 2016), from the USA (Morton, 1986; Walker et al., 1986), or more recently also from tropical New Caledonia (Crossay et al., 2018). Except for the recently described *A. saccata*, a much wider distribution is already known for all other species, as they were all reported in several climatic zones and/or continents since their first records. Future research has to show also for *A. flavopapillosa*, if it has a wide distribution as most of the other fungi of the group, or if it is rather restricted to Western Amazonia, or even to traditional coffee plantations in San Martín State of Peru. The closest matches of ecological sequences (97% identity) reported from the West Amazonia region in Ecuador (Garcés-Ruiz et al., 2019) and in roots from *Hedera rhombea* in Niigata, Japan (Ahulu et al., 2006) suggest that the new fungus has a wider distribution. The matches,

however, were not close enough to confirm the identity. More applied research should elucidate, a) if the group of AMF species to which *A. flavopapillosa* belongs, and especially those found in the Neotropics, have positive effects on coffee, cacao or inka nut or any other crop growth, quality or yield in Peru, in southern and central American countries, or elsewhere in the Tropics, and b) if other Glomeromyota species, such as *Microkamienskia peruviana* of the Glomeraceae (Corazon-Guivin et al., 2019b) or *Paraglomus occidentale* of the Paraglomeraceae (Corazon-Guivin et al., 2020) have higher, lower or complementary benefits for agricultural production.

Acknowledgements
The authors thank all the members of the Laboratorio de Biología y Genética Molecular for collaborating in the publication of this article and to the farmers in Palestina (El Dorado), Nuevo Lamas and Bello Horizonte (San Martín) for providing us with the facilities for the collection of soil samples.

Funding information:
The study was financially supported by the Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica, (FONDECYT, Peru) for the financing granted within the frame work of the project with SUBVENTION AGREEMENT N° 163-2020-FONDECYT. Likewise, Instituto de Investigación y Desarrollo (IiyD) of the UNSM-T. Gladstone Alves da Silva thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Fellowship granted (Proc. 312227/2019-1).

Conflicts of interest
No potential conflict of interest was reported by the authors.

References

Ahulu, E.M., Gollotte, A., Gianinazzi-Pearson, V., Nonaka, M., 2006: Cooccurring plants forming distinct arbuscular mycorrhizal morphologies harbor similar AM fungal species. Mycorrhiza 17, 37-49. DOI: 10.1007/s00572-006-0079-0

Błaszkowski, J., 2012: Glomeromycota. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków. DOI: 10.5586/am.2013.015

Błaszkowski, J., Chwat, G., Góralska, A., 2015: *Acaulospora ignota* and *Claroideoglomus hanlinii*, two new species of arbuscular mycorrhizal fungi (Glomeromycota) from Brazil and Cuba. Mycol. Progess 14, 18. DOI: 10.1007/s11557-015-1042-2

Błaszkowski, J., Niezgoda, P., Meller, E., Milczarski, P., Zubek, S., Malicka, M., Usok, S., Casieri, L., Goto, B.T., Magurno, B.T., 2021: New taxa in Glomeromycota: Polonosporaceae fam. nov., *Polonospora* gen. nov., and *P. polonica* comb. nov. Mycol. Progress 20, 941-951. DOI: 10.1007/s11557-021-01726-4

Brundrett, M., Melville, L., Peterson, L., 1994: Practical Methods in Mycorrhizal Research. Mycologue Publications, University of Guelph, Guelph, Ontario, Canada.

Corazon-Guivin, M.A., Mendoza, A.C., Guerrero-Abad, J.C., Vallejos-Tapullima, A., Silva, G.A., Oehl, F., 2019a: *Acaulospora aspera*, a new fungal species in the Glomeromycetes from rhizosphere soils of the inka nut (*Plukenetia volubilis* L.) in Peru. J. Appl. Bot. Fruit Qual. 92, 250-257. DOI: 10.5073/JABFQ.2019.092.035

Corazon-Guivin, M.A., Cerna-Mendoza, A., Guerrero-Abad, J.C., Vallejos-Tapullima, A., Carballar-Hernández, S., Silva, G.A., Oehl, F., 2019b: *Microkamienskia* gen. nov. and *Microkamienskia peruviana*, a new arbuscular mycorrhizal fungus from Western Amazonia. Nova Hedwigia 109, 355-368. DOI: 10.1127/nova_hedwigia/2019/0551

Corazon-Guivin, M.A., Cerna-Mendoza, A., Guerrero-Abad, J.C., Vallejos-Tapullima, A., Carballar-Hernández, S., Silva, G.A., Oehl, F., 2019c: *Nanoglomus plukenetiae*, a new fungus from Peru, and a key to small-spored Glomeraceae species, including three new genera in the "*Dominikia* complex/clades". Mycol. Progress 18, 1395-1409. DOI: 10.1007/s11557-019-01522-1

Corazon-Guivin, M.A., Mendoza, A.C., Guerrero-Abad, J.C., Vallejos-Tapullima, A., Ríos-Ramírez, O., Vallesjos-Torres, G., de la Solta-Ricaldi, A.M., Santos, V.M., Silva, G.A., Oehl, F., 2020: *Paraglomus occidentale*, a new fungus from the sources of the Amazon river in Peru, with a key to the paraglomeromycetes species. Sydowia 72, 85-94. DOI: 10.12905/0380.sydowia72-2020-0085

Corazon-Guivin, A.M., Vallejos-Tapullima, A., de la Sota-Ricaldi, A.M., Cerna-Mendoza, A., Guerrero-Abad, J.C., Santos, V.M., Silva G.A., Oehl, F., 2021: *Acaulospora flava*, a new arbuscular mycorrhizal fungus from *Coffea arabica* and *Plukenetia volubilis* plantations at the sources of the Amazon river in Peru. J. Appl. Bot. Food Qual. 94, 116-123. DOI:10.5073/JABFQ.2021.094.014

Crossay, T., Cilia, A., Cavaloc, Y., Amir, H, Redecker, D., 2018: Four new species of arbuscular mycorrhizal fungi (Glomeromycota) associated with endemic plants from ultramafic soils of New Caledonia. Mycol. Progress 17, 729-744. DOI: 10.1007/s11557-018-1386-5

Garcés-Ruiz, M., Senés-Guerrero, C., Declerck, S., Cranenbrouck, S., 2019: Community composition of arbuscular mycorrhizal fungi associated with native plants growing in a petroleum-polluted soil of the Amazon region of Ecuador. MicrobiologyOpen 8:e703. DOI: 10.1002/mbo3.703

Gerdemann, J.W., Trappe, J.M., 1974: The Endogonaceae in the Pacific Nordwest. Mycol. Memoir 5, 1-76.

Guindon, S., Gascuel, O., 2003: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. System Biol. 52, 696-704. DOI: 10.1080/10635150390235520

He, R.-J., Long, C.-L., Yao, L.-M., Jiang, L., 2021: *Acaulospora fanjing* (Glomeromycota), a new species of arbuscular mycorrhizal fungi from Fanjingshan in China. Phytotaxa 524, 191-198. DOI: 10.11646/phytotaxa.524.3.3

Hewitt, E.J., 1966: Sand and water culture methods used in the study of plant nutrition. Farnhan Royal, Commonwealth Agricultural Bureau, Farnham, England.

Ingleby, K., Walker, C., Mason, P.A. 1994: *Acaulospora excavata* sp. nov. – an endomycorrhizal fungus from Cote d'Ivoire. Mycotaxon 50, 99-105.

Koske, R.E., Tessier, B., 1983: A convenient, permanent slide mounting medium. Mycol. Soc. Am. Newsletter 34, 59.

Krüger, M., Stockinger, H., Krüger, C., Schüssler, A., 2009: DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol. 183, 212-223. DOI: 10.1111/j.1469-8137.2009.02835.x

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A. et al., 2007: Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947-2948. DOI: 10.1093/bioinformatics/btm404

Lee, E.H., Park, S.-H., Eo, J.-K., Ka, K.-H., Eom, A.-H., 2018: *Acaulosproa koreana*, a new species of arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of woody plants in Korea. Mycobiology 46, 341-348. DOI: 10.1080/12298093.2018.1548805

Lin, T.C., Silva, G.A., Oehl, F., 2019: *Acaulospora tsugae*, a new species in the *Glomeromycetes* from Taiwan, and a key to species in Acaulosporaceae. Nova Hedwigia 108, 475-488. DOI: 10.1127/nova_hedwigia/2018/0513

Milne, I., Wright, F., Rowe, G., Marshal, D.F., Husmeier, D., et al., 2004: TOPALi: Software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20, 1806-1807. DOI: 10.1093/bioinformatics/bth155

Morton, J.B., 1986: Three new species of *Acaulospora* (Endogonaceae) from high aluminum, low pH soils in West Virginia. Mycologia 78, 641-648.

Mosse, B., 1962: Establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J. Genetic Microbiol. 27, 509-520.

Oehl, F., Palenzuela, J., Sánchez-Castro, I., Kuss, P., Sieverding, E., Silva, G.A., 2012: *Acaulospora nivalis*, a new fungus in the Glomero-

mycetes, characteristic for high alpine and nival altitudes of the Swiss Alps. Nova Hedwigia 95, 105-122. DOI: 10.1127/0029-5035/2012/0038

Oehl, F., Sýkorová, Z., Błaszkowski, J., Sánchez-Castro, I., Coyne, D., Tchabi, A., Lawouin, L., Hountondji, F.C.C., Silva, G.A., 2011: *Acaulospora sieverdingii*, an ecologically diverse new fungus in the Glomeromycota, described from lowland temperate Europe and tropical West Africa. J. Appl. Bot. Food Qual. 84, 47-53.

Oehl, F., Sýkorová, Z., Redecker, D., Wiemken, A., Sieverding, E., 2006: *Acaulospora alpina*, a new species in the Glomeromycetes characteristic for high mountainous and alpine regions of the Swiss Alps. Mycologia 98, 286-294.

Oehl, F., Tchabi, A., Silva, G.A., Sánchez-Castro, I., Palenzuela, J., Monte Júnior, I.P., Lawouin, L., Coyne, D., Hountondji, F., 2014: *Acaulospora spinosissima*, a new arbuscular mycorrhizal fungus from the Southern Guinea Savanna in Benin. Sydowia 66, 29-42. DOI: 10.12905/0380.sydowia66(1)2014-0029

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954: Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular, Vol 939. US Department of Agriculture, Washington, DC. p. 19.

Park, H., Ka, K.-H., Eom, A.-H., 2021: *Acaulospora jejuensis*, a new species of Glomeromycota from Korea. Korean J. Mycol. 49, 425-431. DOI: 10.4489/KJM.20210041

Pereira, C.M., Goto, B.T., Silva, D.K.A., de Ferreira, A.A., Souza, F.A., Silva, G.A., Maia L.C., Oehl, F., 2015: *Acaulospora reducta* sp. nov. and *A. excavata* – two glomeromycotan fungi with pitted spores from Brazil. Mycotaxon 130, 983-995. DOI: 10.5248/130.983

Pereira, C.M., Maia, L.C., Sanchez-Castro, I., Palenzuela, J., Silva, D.K.A., Sudova, R., Kolaříková Z, Rydlová J, Čtvrtlíková M, Goto BT, Silva GA, Goto, B.T., Silva G.A., Oehl, F., 2016: *Acaulospora papillosa*, a new mycorrhizal fungus from NE Brazil, and *Acaulospora rugosa* from Norway. Phytotaxa 260, 14-24. DOI: 10.11646/phytotaxa.260.1.2

Ronquist, F., Huelsenbeck, J.P., 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574. DOI: 10.1093/bioinformatics/btg180

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.

Senamhi, 2019: Datos Meteorológicos, San Martín. https://www.gob.pe/senamhi

Sieverding, E., 1991: Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit Nr. 224. Hartmut Bremer Verlag, Friedland, Germany.

Spain, J.L., 1990: Arguments for diagnoses based on unaltered wall structures. Mycotaxon 38, 71-76.

Walker, C., Pfeiffer, C.M., Bloss, H.E. 1986: *Acaulospora delicata* sp. nov. – an endomycorrhizal fungus from Arizona. Mycotaxon 25, 621-628.

ORCID:
Mike Anderson Corazon-Guivin https://orcid.org/0000-0001-6027-4255
Adela Vallejos-Tapullima https://orcid.org/0000-0002-4183-3741
Ana Maria de la Sota Ricaldi https://orcid.org/0000-0003-4048-4049
Geomar Vallejos-Torres https://orcid.org/0000-0001-7084-977X
María Emília Ruíz-Sánchez https://orcid.org/000-0002-9933-9017
Viviane Monique Santos https://orcid.org/0000-0002-2846-7513
Gladstone Silva https://orcid.org/0000-0002-5652-7800
Fritz Oehl https://orcid.org/0000-0003-1492-7985

Addresses of the corresponding authors:
Mike Anderson Corazon-Guivin, Laboratorio de Biología y Genética Molecular, Universidad Nacional de San Martín, Jr. Amorarca N° 315, Morales, Peru
E-mail: macorazong@unsm.edu.pe
Fritz Oehl, Agroscope, Competence Division for Plants and Plant Products, Plant Protection Products – Impact and Assessment, Müller-Thurgau-Str. 29, CH-8820 Wädenswil ZH, Switzerland
E-mail: fritz.oehl@agroscope.admin.ch

Supplementary material 2:

References for all *Acaulospora* species included in the identification key:

Błaszkowski, J., 1988a: Four new species of the Endogonaceae (Zygomycotina) from Poland. Karstenia 27, 37-42.

Błaszkowski, J., 1988b: Three new vesicular-arbuscular mycorrhizal fungi (Endogonaceae) from Poland. Bulletin Polish Acad. Biol. Sci. 36, 271-275.

Błaszkowski, J., 1989: *Acaulospora cavernata* (Endogonales) – a new species from Poland with pitted spores. Crypt. Bot. 1, 204-207.

Błaszkowski, J., 1990: Polish Endogonaceae. IV. *Acaulospora capsicula* sp. nov. Mycologia 82, 794-798.

Błaszkowski ,J., 1995: *Acaulospora koskei*, a new species in Glomales from Poland. Mycol. Res. 99, 237-240.

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

Błaszkowski, J., Chwat, G., Góralska, A., 2015: *Acaulospora ignota* and *Claroideoglomus hanlinii*, two new species of arbuscular mycorrhizal fungi (Glomeromycota). Mycol. Progress 14, 18 (1-11).
DOI: 10.1007/s11557-015-1042-2

Cai, B.P., Guo, L.D., Chen, J.Y., Zhang, Q.X., 2013: *Glomus mume* and *Kuklospora spinosa*: two new species of Glomeromycota from China. Mycotaxon 124, 263-268. DOI: 10.5248/124.253

Corazon-Guivin, M.A., Mendoza, A.C., Guerrero-Abad, J.C., Vallejos-Tapullima, A., Silva, G.A., Oehl, F., 2019a: *Acaulospora aspera*, a new fungal species in the Glomeromycetes from rhizosphere soils of the inka nut (*Plukenetia volubilis* L.) in Peru. J. Appl. Bot. Fruit Qual. 92, 250-257. DOI: 10.5073/JABFQ.2019.092.035

Corazon-Guivin, M.A., Vallejos-Tapullima, A., de la Sota-Ricaldi, A.M., Cerna-Mendoza, A., Guerrero-Abad, J.C., Santos, V.M., Silva, G.A., Oehl, F., 2021: *Acaulospora flava*, a new arbuscular mycorrhizal fungus from *Coffea arabica* and *Plukenetia volubilis* plantations at the sources of the Amazon river in Peru. J. Appl. Bot. Fruit Qual. 94, 116-123. DOI: 10.5073/JABFQ.2021.094.00X

Corazon-Guivin, M.A., Vallejos-Tapullima, A., de la Sota-Ricaldi, A.M., Vallejos-Torres, G., Ruíz-Sánchez, M.E., Santos, V.M., Silva, G.A., Oehl, F., 2022: *Acaulospora flavopapillosa*, a new fungus in the Glomeromycetes from a coffee plantation in Peru. J. Appl. Bot. Food Qual. 95, 6-16.

Crossay, T, Cilia, A., Cavaloc, Y., Amir, H, Redecker, D., 2018: Four new species of arbuscular mycorrhizal fungi (Glomeromycota) associated with endemic plants from ultramafic soils of New Caledonia. Mycol. Progress 17, 729-744. DOI: 10.1007/s11557-018-1386-5

Furrazola, E., Goto, B.T., Silva, G.A., Torres-Arias, Y., Morais, T., Lima, C.E.P, Ferreira, A.C.A., Maia, L.C., Sieverding, E., Oehl, F., 2013: *Acaulospora herrerae*, a new pitted species in the Glomeromycetes from Cuba and Brazil. Nova Hedwigia 97, 401-413.
DOI: 10.1127/0029-5035/2013/0128

Gerdemann, J.W., Trappe, J.M., 1974: The Endogonaceae in the Pacific Northwest. Mycol. Memoirs 5, 1-76.

Hu, H.T., 1988: Study on the Endomycorrhizae of Chinese Fir (Cunninghamia lanceolata Hooker) and Taiwania (Taiwania cryptomerioides Hay.). Quarterly Journal of Chinese Forestry 21, 45-72.

Ingleby, K., Walker, C., Mason, P.A., 1994: *Acaulospora excavata* sp. nov. – an endomycorrhizal fungus from Cote d'Ivoire. Mycotaxon 50, 99-105.

He, R.-J., Long, C.-L., Yao, L.-M., Jiang, L., 2021: *Acaulospora fanjing* (Glomeromycota), a new species of arbuscular mycorrhizal fungi from Fanjingshan in China. Phytotaxa 524, 191-198.
DOI: 10.11646/phytotaxa.524.3.3

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

Kaonongbua, W., Morton, J.B., Bever, J.D., 2010: Taxonomic revision transferring species in *Kuklospora* to *Acaulospora* (Glomeromycota) and a description of *Acaulospora colliculosa* sp. nov. from field collected spores Mycologia 102, 1497-1509. DOI: 10.3852/10-011

Lee, E.H., Park, S.-H., Eo, J.-K., Ka, K.-H., Eom, A.-H., 2018: *Acaulosproa koreana*, a new species of arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of woody plants in Korea. Mycobiology 46, 341-348. DOI: 10.1080/12298093.2018.1548805

Lin, T.C., Silva, G.A., Oehl, F., 2019: *Acaulospora tsugae*, a new species in the *Glomeromycetes* from Taiwan, and a key to species in Acaulosporaceae. Nova Hedwigia 108, 475-488.
DOI: 10.1127/nova_hedwigia/2018/0513

Morton, J.B., 1986: Three new species of *Acaulospora* (Endogonaceae) from high aluminium, low pH soils in West Virginia. Mycologia 78, 641-648.

Oehl, F., Palenzuela, J., Sánchez-Castro, I., Hountondji, F., Tchabi, A., Lawouin, L., Barea, J.M., Coyne, D., Silva, G.A., 2011a: *Acaulospora minuta*, a new arbuscular mycorrhizal fungal species from sub-Saharan savannas of West Africa. J. Appl. Bot. Food Qual. 84, 213-218.

Oehl, F., Palenzuela, J., Sánchez-Castro, I., Kuss, P., Sieverding, E., Silva G.A., 2012: *Acaulospora nivalis*, a new fungus in the Glomeromycetes, characteristic for high alpine and nival altitudes of the Swiss Alps. Nova Hedwigia 95, 105-122.
DOI: 10.1127/0029-5035/2012/0038

Oehl, F., Silva, G.A., Palenzuela, J., Sanchez-Castro, I., Castillo, C., Sieverding, E. 2011b: *Acaulospora punctata,* a new fungal species in the *Glomeromycetes* from mountainous altitudes of the Swiss Alps and Chilean Andes. Nova Hedwigia 93, 353-362.
DOI: 10.1127/0029-5035/2011/0093-0353

Oehl F., Sýkorová Z., Błaszkowski J., Sánchez-Castro I., Coyne D., Tchabi A., Lawouin L., Hountondji F.C.C., Silva G.A., 2011c: *Acaulospora sieverdingii*, an ecologically diverse new fungus in the Glomeromycota, described from lowland temperate Europe and tropical West Africa. J. Appl. Bot. Fruit Qual. 84, 47-53.

Oehl F., Sýkorová Z., Redecker D., Wiemken A., Sieverding E., 2006: *Acaulospora alpina*, a new arbuscular mycorrhizal fungal species characteristic for high mountainous and alpine regions of the Swiss Alps. Mycologia 98, 286-294.

Oehl, F., Tchabi, A., Silva, G.A., Sánchez-Castro, I., Palenzuela, J., Monte Júnior, I.P., Lawouin, L., Coyne, D., Hountondji, F., 2014: *Acaulospora spinosissima*, a new arbuscular mycorrhizal fungus from the Southern Guinea Savanna in Benin. Sydowia 66, 29-42.
DOI: 10.12905/0380.sydowia66(1)2014-0029

Palenzuela, J., Azcón-Aguilar, C., Barea, J.M., Silva, G.A., Oehl, F., 2013: *Acaulospora pustulata* and *Acaulospora tortuosa*, two new species in the Glomeromycota associated with endangered plants in Sierra Nevada (Spain) and the Swiss Alps. Nova Hedwigia 97, 305-319.
DOI: 10.1127/0029-5035/2013/0129

Palenzuela, J., Azcón-Aguilar, C., Barea, J.M., Silva, G.A., Oehl, F., 2014: *Acaulospora viridis,* a new species in the Glomeromycetes from two mountain ranges in Andalucía (Spain). Nova Hedwigia 99, 71-82.
DOI: 10.1127/0029-5035/2014/0180

Palenzuela, J., Azcón-Aguilar, C., Barea, J.M., Silva, G.A., Oehl, F., 2015: *Acaulospora baetica*, a new arbuscular mycorrhizal fungal species from two mountain ranges in Andalucía (Spain). Nova Hedwigia 101, 463-474. DOI: 10.1127/nova_hedwigia/2015/0285

Park, H., Ka, K.-H., Eom, A.-H., 2021: *Acaulospora jejuensis*, a new species of Glomeromycota from Korea. Korean J. Mycol. 49, 425-431.
DOI: 0.4489/KJM.20210041

Pereira, C.M., Goto, B.T., Silva, D.K.A., de Ferreira, A.A., Souza, F.A., Silva, G.A., Maia L.C., Oehl, F., 2015: *Acaulospora reducta* sp. nov. and *A. excavata* – two glomeromycotan fungi with pitted spores from Brazil. Mycotaxon 130, 983-995. DOI: 10.5248/130.983

Pereira, C.M., Maia, L.C., Sanchez-Castro, I., Palenzuela, J., Silva, D.K.A., Sudova, R., Kolaříková, Z., Rydlová, J., Čtvrtlíková, M., Goto, B.T., Silva, G.A., Goto, B.T., Silva, G.A., Oehl, F., 2016: *Acaulospora papillosa*, a new mycorrhizal fungus from NE Brazil, and *Acaulospora rugosa* from Norway. Phytotaxa 260, 14-24.
DOI: 10.11646/phytotaxa.260.1.2

PONTES, J.S., SANTOS, V.M., PEREIRA, C.D., MAIA, L.C., OEHL, F., 2017: *Acaulospora spinulifera*, a new arbuscular mycorrhizal fungal species from the Brazilian Cerrado and Atlantic Rain forest. Nova Hedwigia 105, 219-229. DOI: 10.1127/nova_hedwigia/2017/0407

ROTHWELL, F.M., TRAPPE, J.M., 1979: *Acaulospora bireticulata* sp. nov. Mycotaxon 8, 471-475.

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., TORO, S.T., 1987: *Acaulospora denticulata* sp. nov. and *Acaulospora rehmii* sp. nov. (Endogonaceae) with ornamented spore walls. Angewandte Botanik 61, 217-223.

SIEVERDING, E., CHAVERRI, A., TORO, S.T., 1988: *Acaulospora splendida*, a new species in the Endogonaceae from Costa Rica. Mycotaxon 33, 251-256.

SCHULTZ, P.A., BEVER, J.D., MORTON, J.B., 1999: *Acaulospora colossica*, sp. nov. from an old field in North Carolina and morphological comparisons with similar species, *A. laevis* and *A. koskei*. Mycologia 91, 676-683.

TRAPPE, J.M., 1977: Three new Endogonaceae: *Glomus constrictus*, *Sclerocystis clavispora* and *Acaulospora scrobiculata*. Mycotaxon 6, 359-366.

TREJO, D., GUZMAN, G., LARA, L., ZULUETA, R., PALENZUELA, J., SANCHEZ-CASTRO, I., SILVA, G.A., SIEVERDING, E., OEHL, F., 2015: Morphology and phylogeny of *Acaulospora foveata* (*Glomeromycetes*) from Mexico. Sydowia 67, 119-126. DOI: 10.12905/0380.sydowia67-2015-0119

VELÁSQUEZ, M.S., CABELLO, M., IRRAZABAL, G, GODEAS, A., 2008: Acaulosporaceae from El Palmar National Park, Entre Ríos, Argentina. Mycotaxon 103, 171-187.

WALKER, C., PFEIFFER, C.M., BLOSS, H.E., 1986: *Acaulospora delicata* sp. nov. – an endomycorrhizal fungus from Arizona. Mycotaxon 25, 621-628.

WALKER, C., TRAPPE, J.M., 1981: *Acaulospora spinosa* sp. nov. with a key of *Acaulospora*. Mycotaxon 12, 515-521.

WU, C.-G., LIU, Y.-S., 1995: Glomales of Taiwan: V. *Glomus chimonobambusae* and *Entrophospora kentinensis*. Mycotaxon 53, 283-294.