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Microkamienskia gen. nov. and *Microkamienskia peruviana*, a new arbuscular mycorrhizal fungus from Western Amazonia

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With 10 figures

Abstract: A new arbuscular mycorrhizal (AM) fungus, *Microkamienskia peruviana*, was detected in bait cultures for arbuscular mycorrhizal fungi established with rhizospheric soil substrates of the inka nut (*Plukenetia volubilis*). The field soil derived from three agricultural plantations in the Amazonia lowlands of the province Lamas, San Martin State, in Peru. The fungus was subsequently propagated in single species cultures on *Sorghum* sp., *Brachiaria* sp., *Medicago sativa* and *P. volubilis* as host plants. The new species differentiates hyaline spores regularly in spore clusters, up to 500–800 × 400–600 µm. The spores are 16–31(–36) × 13–29(–35) µm in diam, formed on cylindrical or slightly funnel-shaped hyphae, without a septum at or close to the spore base. Phylogenetically, the new fungus belongs to a new genus, named *Microkamienskia*, which has as type species *M. perpusilla* comb. nov. and to which also *M. divaricata* comb. nov. belongs. Both are transferred from *Kamienskia* to *Microkamienskia* in the present study. The new fungus can be identified by the ballooning semi-persistent to evanescent outer spore wall layer in PVLG-based mountants that is not known for the other species of these two genera, nor for any other glomeromycotan species of similar small spore sizes. *Kamienskia* and *Microkamienskia* species can be distinguished by their position in the phylogenetic tree and by hyaline spores, open pores at the spore bases and

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in the subtending hyphae, and by their spore sizes that are for *Microkamienskia* among the smallest spore sizes so far detected for AM fungi (15–35 µm).

Key words: agroforestry; Glomeraceae; Glomerales; Glomeromycetes; mountain peanut; non-sporulating fungi; taxonomy

Introduction

Glomeraceae Piroz. & Dalpé (Pirozynski & Dalpé 1989) is the largest arbuscular mycorrhizal (AM) fungal family of the phylum Glomeromycota with approximately 120 AM fungal species known so far (Błaszkowski 2012, Jobim et al. 2019). It currently comprises 14 genera that is by far the highest genera number of all families within the Glomeromycota. Within the Glomeraceae, *Funneliformis* generally has the largest spore sizes ((100–)150–400 µm in diam), followed by *Septoglomus* (90–330 µm), *Funneliglomus* (90–150 µm) and the heterogenic *Rhizoglomus* sizes of (30–)80–250(–800 µm) reported (Oehl et al. 2011, 2019, Goto et al. 2013, Sieverding et al. 2014, Symanczik et al. 2014). Other genera have rather small spore sizes, such as *Dominikia* (20–70(–100 µm), *Kamienskia* (20–60 µm) and *Oehlia* (70–120 µm; Błaszkowski et al. 2015, 2018b, Oehl et al. 2015). Each of the Glomeraceae genera, besides having a distinct spore morphology, can also be distinguished by their phylogenetic clades (Oehl et al. 2011, Błaszkowski et al. 2015, 2018a, Jobim et al. 2019, Corazon-Guivin et al. 2019).

Species with small, hyaline spores (size < 50 µm) so far represent a rather small group in the Glomeraceae. Most belong to the recently described genera *Dominikia* or *Kamienskia* (Błaszkowski et al. 2015), or are still counted within the genus *Glomus*, because their phylogenetic position remains uncertain, such as *Glomus arborense* (Hall 1977) or *Glomus microcarpum* (Tulasne & Tulasne 1845). Also two *Rhizoglomus* species have small spores, *R. microaggregatum* and *R. proliferum* (Sieverding et al. 2014). *Dominikia* and *Kamienskia* spp. generally form so tiny and also rapidly degrading spores, which usually cannot be identified on the species level by classical morphological methods, and often they are not even detected from field samples (Schenck & Pérez 1990, Błaszkowski et al. 2018b). Their phylogenetic clades, obtained from so-called 'environmental sequences' derived from roots, rhizosphere or bulk soils, were often attributed to 'rarely sporulating' or 'non-sporulating' fungi (Schnoor et al. 2011, Avio et al. 2013, Lopez-Garcia et al. 2014, Oehl et al. 2017).

Recently, a new AM fungal species, *Funneliglomus sanmartinensis*, was found in inka nut plantations in the Department San Martín, Peru (Corazon-Guivin et al. 2019). The host plant species is also called 'mountain peanut' or 'sacha inchi', and botanically is *Plukenetia volubilis* L. It belongs to the family Euphorbiaceae and is a perennial climbing plant, usually about 2–3 meters high, and native to the Peruvian Amazon. This plant has recently gained increasing agronomic interest also in other parts of the tropical world (Srichamnong et al. 2018, Wang et al. 2018). 'Sacha inchi' is known as a novel source of oil, snacks or tea, and its seeds are rich in unsaturated fatty acids (Wang et al. 2018).

In this study, we report another new AM fungal species from the rhizosphere of inka nut, belonging to a new genus and also originating from the Peruvian Amazon region. It was first detected in the greenhouse from rhizospheric substrates and roots of the inka nut and two bait culture plants, *Sorghum* and *Brachiaria* sp. that had been inoculated with soils from inka nut. The fungus forms tiny, fragile spore clusters, rather large in size, with up to 300–500 spores per cluster. Its spores are among the smallest so far described from AM fungi (< 35 µm) and would regularly pass the sieves, commonly used to separate AM spores from soils (Brundrett et al. 1994). The objectives of the present study were to describe the new fungus, based on both, morphological and phylogenetic analyses.

Material and Methods

Study sites, soil sampling: Soil samples (0–30 cm depth) were taken in agricultural field sites with inka nut at Pampamonte (06°21'08.6" S; 76°32'15.7" W; 500 m a.s.l.), Palmiche (06°20'02.40" S; 076°36'00.00" W; 858 m a.s.l.) and Morillo (06°23'09.6" S; 76°34'44.44" W; 520 m a.s.l.) in the Peruvian Amazonia lowlands and adjacent Andean low mountain ranges in the Department San Martín of the province Lamas. These areas are traditional agroforestry sites, in which the inka nut is grown in mixed cultures with maize, beans, and other field crops without addition of chemical fertilizers and pesticides. Mean annual temperatures are about 25–27 °C, with variation between 18 and 32 °C throughout the year. Mean annual precipitation is approximately 1300 mm.

AM fungal bait cultures: Spores of the new species were not detected in the field soils, but were extracted from pot trap cultures. Pot trap cultures were established in cylindrical 1 L pots with 1 kg of substrate. The substrate consisted of a 1:1 mixture of coarse river sand and collected rhizosphere soils with root fragments of inka nut and other smaller plants (Poaceae spp.), which likely hosted the fungus in the field. The culture and subsequent single-species cultures were grown under ambient temperature conditions in the greenhouse of the Facultad de Ciencias Agrarias, Universidad Nacional de San Martín-Tarapoto. The substrate for single-species cultures consisted of autoclaved 1:1 mixtures of collected field soil samples and coarse river sand. At inoculation and bait culture establishment, the pots were first filled to 75% with the autoclaved substrate. Thereafter spore clusters of 50–100 spores were added to the substrate and seeds of *Sorghum vulgaris* L., *Brachiaria* spp. and inka nut were seeded in order to establish the mycorrhizal association of the new fungal species and reproduce its spores without presence of any other AM fungal species. The seeds had been 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 for six months, with 21.4, 29 and 38.2 ºC as minimum, mean and maximum temperatures, respectively. The relative humidity was from 48 to 74%. The pots were irrigated every other day and fertilized with a Long Ashton nutrient solution (Hewitt 1966) with reduced P contents (60% reduction) every two weeks.

Morphological analyses: Spores of the new fungus were separated from the bait cultures and single species substrates by a wet sieving process as described by Sieverding (1991). The description of the morphological spore characteristics and their subcellular structures are based on observations of specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG; Koske & 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). The terminology of the spore structure basically is that presented in Błaszkowski (2012) and Oehl et al. (2015) for species with glomoid 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 (1:1) mixture of PVLG and Melzer's reagent were deposited at Z+ZT (ETH Zurich, Switzerland). Staining of the mycorrhizal root structures was carried out according to Vierheilig et al. (1998).

Molecular analyses: Healthy and intact spore clusters were isolated from the single species cultures and superficially cleaned of soil particles by friction on cellulose filter paper (WHATMAN, Grade 50; Corazon-Guivin et al. 2019). 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. Two independent groups of sterile spore clusters, containing each 20–30 spores, connected by a common hypha were selected under a laminar flow hood and individually transferred into Eppendorf PCR tubes. Crude extract was obtained by crushing the individual spore clusters with a sterile disposable micropestle in 23 µL milli-Q water, as described by Palenzuela et al. (2013). 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 and 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 vector (Invitrogen, Carlsbad, CA, USA) and transformed into One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA). Recombinant colonies (10) 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).

Phylogenetic analyses: The AM fungal sequences (partial SSU, ITS region, and partial LSU rDNA) obtained were aligned with other related glomeromycotan sequences from

GenBank in ClustalX (Larkin et al. 2007). *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler 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 5×10^6 generations, with a sample frequency of 500 and a burnin value of 25%) and maximum likelihood (1,000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5, using the GTR + G model.

Results

Molecular analyses: The phylogenetic analyses from the partial SSU, ITS region, and partial LSU rDNA sequences placed the new fungus in a distinct clade near to *Kamienskia perpusilla* and *K. divaricata* (Fig. 1). The support values for the clade of the new species were 100% in all analyses. In the BLASTn analysis, the rDNA sequences with closest match (94%) to the new fungus are from *K. perpusilla*. In the phylogenetic tree, the genus *Kamienskia* is divided in two main subclades. The first subclade comprises *K. bistrata* (KA), while *K. perpusilla*, *K. divaricata* and our new species constitute the second cluster (KB). In the BLASTn analysis, the rDNA sequences of the species of subclade KA demonstrated only 85% of maximum identity (MI) with sequences of the species of subclade KB. The clear separation of the subclades and the low identity of the sequences for both clades indicate that a new genus (including *K. perpusilla*, *K. divaricata* and our new species) should be described.

Taxonomy

Microkamienskia Corazon-Guivin, G.A. Silva & Oehl gen.nov.

MycoBank MB 830814

Diagnosis: Hyaline spores generally < 40 µm, formed singly or in small clusters in soil or roots, without bridging septum at or close to the spore base.

Etymology: *Micro-* and *-kamienskia*, referring to the smaller spore size, but similar spore morphology as described for *Kamienskia* (Błaszkowski et al. 2015).

Typus generis. – *Microkamienskia perpusilla* (Błaszk. & Kovács) Corazon-Guivin, G.A. Silva & Oehl

Description: *Microkamienskia* species differentiate generally hyaline, small spores (< 40 µm diam.), singly or in spore clusters, in soils or in roots, terminally or intercalary on cylindrical to slightly funnel-shaped or slightly constricted hyphae. The spore pore is regularly not closed by a septum at or close to the spore base. So far, they can be clearly differentiated from *Kamienskia* spp. by the smaller spore size and by molecular phylogeny on the partial SSU, ITS region, and partial LSU rDNA.

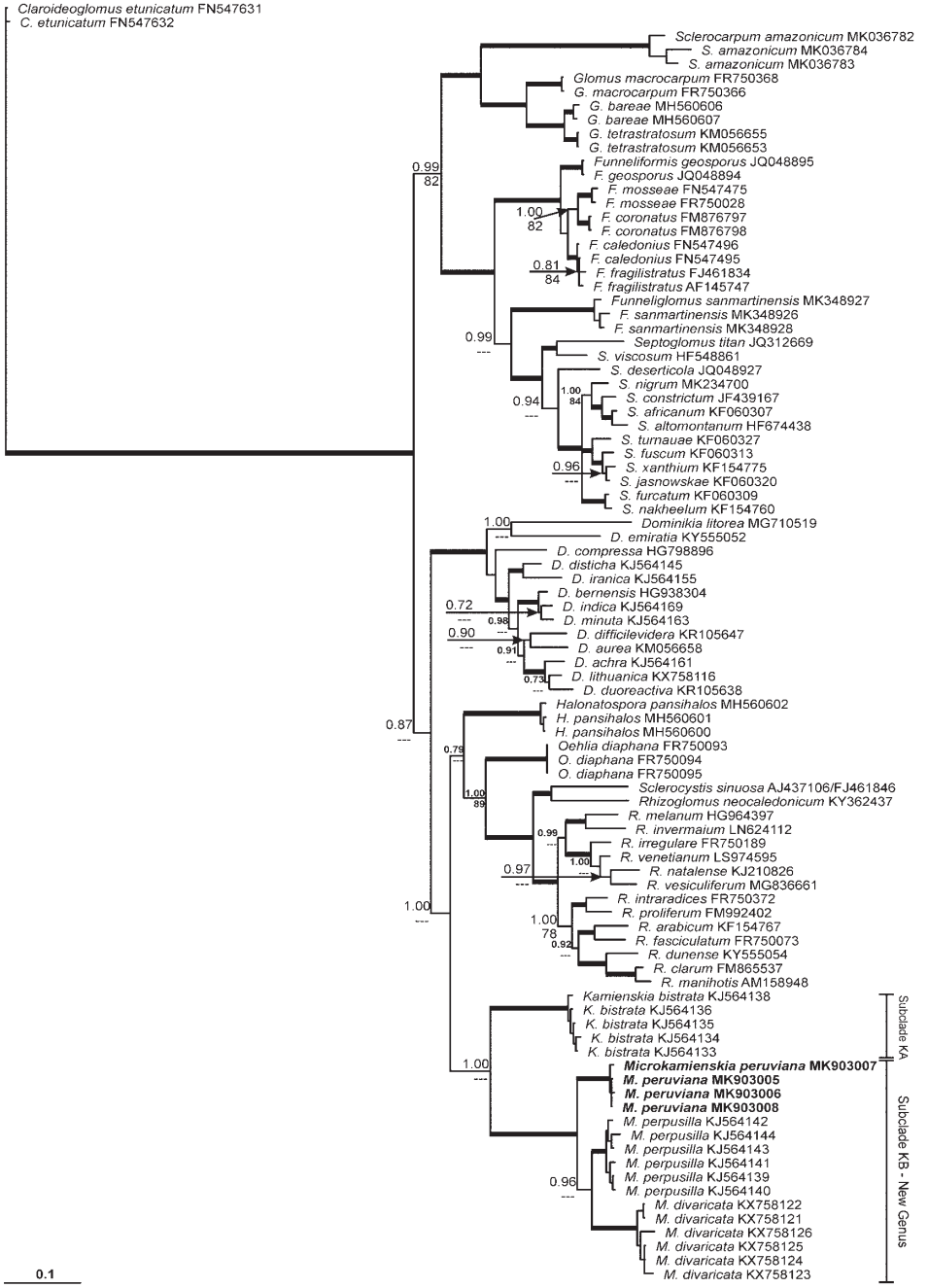

Claroideoglomus etunicatum FN547631
C. etunicatum FN547632
Sclerocarpum amazonicum MK036782
S. amazonicum MK036784
S. amazonicum MK036783
Glomus macrocarpum FR750368
G. macrocarpum FR750366
G. bareae MH560606
G. bareae MH560607
G. tetrastratosum KM056655
G. tetrastratosum KM056653
Funneliformis geosporus JQ048895
F. geosporus JQ048894
F. mosseae FN547475
F. mosseae FR750028
F. coronatus FM876797
F. coronatus FM876798
F. caledonius FN547496
F. caledonius FN547495
F. fragilistratus FJ461834
F. fragilistratus AF145747
Funneliglomus sanmartinensis MK348927
F. sanmartinensis MK348926
F. sanmartinensis MK348928
Septoglomus titan JQ312669
S. viscosum HF548861
S. deserticola JQ048927
S. nigrum MK234700
S. constrictum JF439167
S. africanum KF060307
S. altomontanum HF674438
S. turnauae KF060327
S. fuscum KF060313
S. xanthium KF154775
S. jasnowskae KF060320
S. furcatum KF060309
S. nakheelum KF154760
Dominikia litorea MG710519
D. emiratia KY555052
D. compressa HG798896
D. disticha KJ564145
D. iranica KJ564155
D. bernensis HG938304
D. indica KJ564169
D. minuta KJ564163
D. difficilevidera KR105647
D. aurea KM056658
D. achra KJ564161
D. lithuanica KX758116
D. duoreactiva KR105638
Halonatospora pansihalos MH560602
H. pansihalos MH560601
H. pansihalos MH560600
Oehlia diaphana FR750093
O. diaphana FR750094
O. diaphana FR750095
Sclerocystis sinuosa AJ437106/FJ461846
Rhizoglomus neocaledonicum KY362437
R. melanum HG964397
R. invermaium LN624112
R. irregulare FR750189
R. venetianum LS974595
R. natalense KJ210826
R. vesiculiferum MG836661
R. intraradices FR750372
R. proliferum FM992402
R. arabicum KF154767
R. fasciculatum FR750073
R. dunense KY555054
R. clarum FM865537
R. manihotis AM158948
Kamienskia bistrata KJ564138
K. bistrata KJ564136
K. bistrata KJ564135
K. bistrata KJ564134
K. bistrata KJ564133
Microkamienskia peruviana MK903007
M. peruviana MK903005
M. peruviana MK903006
M. peruviana MK903008
M. perpusilla KJ564142
M. perpusilla KJ564144
M. perpusilla KJ564143
M. perpusilla KJ564141
M. perpusilla KJ564139
M. perpusilla KJ564140
M. divaricata KX758122
M. divaricata KX758121
M. divaricata KX758126
M. divaricata KX758125
M. divaricata KX758124
M. divaricata KX758123
Subclade KA
Subclade KB - New Genus
0.1

Fig 1. Phylogenetic tree of the Glomeraceae obtained by analysis from partial SSU, ITS region, and partial LSU rDNA sequences of different Glomeraceae spp. Sequences are labeled with their database accession numbers. Support values (from top) are from Bayesian inference (BI) and maximum likelihood (ML), respectively. 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 *Claroideoglomus etunicatum*.

New combinations in *Microkamienskia*

Microkamienskia perpusilla (Błaszk. & Kovács) Corazon-Guivin, G.A. Silva & Oehl, comb.nov.

MycoBank MB 830815

Basionym: *Glomus perpusillum* Blaszk. & Kovács, Mycologia 101 (2): 249 (2009) [MB#512346]

Synoymym: *Kamienskia perpusilla* (Błaszk. & Kovács) Błaszk., Chwat & Kovács. Nova Hedwigia 100 (1–2): 231 (2015) [MB#808264]

Microkamienskia divaricata (Błaszk., Chwat & Góralska) Corazon-Guivin, G.A. Silva & Oehl, comb.nov.

MycoBank MB 830816

Basionym: *Kamienskia divaricata* Błaszk., Chwat & Góralska. Botany 94 (12): 1078 (2016) [MB#818100]

New species in *Microkamienskia*

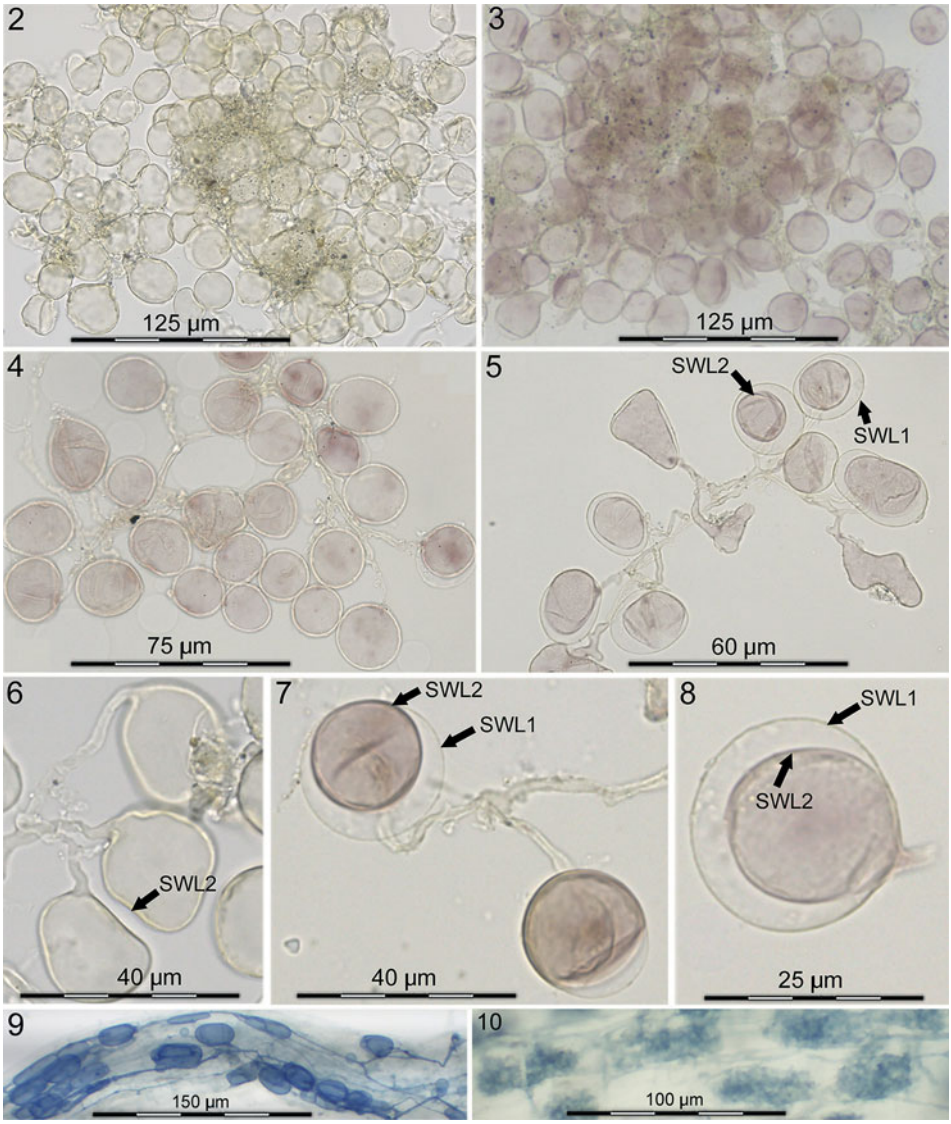
Microkamienskia peruviana Corazon-Guivin, G.A. Silva & Oehl, sp. nov. – Figs. 2–8

MycoBank MB 830817

Diagnosis: Differing from *Microkamienskia perpusilla* and *M. divaricata* by the semi-persistent to evanescent outer spore wall layer, generally ballooning in PVLG-based mountants.

Etymology: *peruviana*, referring to the country Peru, in which this species was found for the first time.

Holotype: deposited at Z+ZT (accession ZT Myc 60102), derived from a single species culture established on the host plant inka nut (*Plukenetia volubilis*) in the greenhouse of the Molecular Biology and Genetics Laboratory, Faculty of Agricultural Sciences, National University of San Martin-Tarapoto, Peru. Fungal inoculum for the culture derived from a bait culture, inoculated with field soils originating from an inka nut plantation in Palmiche (06°20'02.40" S, 076°36'00.00" W; 462 m a.s.l.), where inka nut is cultured in



Figs. 2–10. *Microkamienskia peruviana.* **2.** Hyaline spores clusters mounted in PVLG. **3–5.** Spore clusters and spore cluster segments mounted in PVLG+Melzer's. Spores bi-layered (SWL1-2). SWL2 staining pale purple in Melzer's. **6–8.** Spores formed terminally on hyphae with open pore channels at or close to the spore bases. SWL1, if present, ballooning in PVLG-based mountants. **9–10.** Intraradical hyphae, vesicle and arbuscule structures obtained from roots of *Sorghum vulgare*.

agroforestry systems together with *Zea mays* and *Phaseolus vulgaris*. Collector was Mike Anderson Corazon Guivin and collection date was 25.03.2016. Isotypes (ZT Myc 60103) and Paratypes (ZT Myc 60104) from other cultures grown on *Sorghum* and *Brachiaria* sp., and from other collection dates (15.03.2018) or other sites from Pampamonte (06°21'08.6" S; 76°32'15.7" W; 500 m a.s.l.) and Morillo (06°23'09.6" S; 76°34'44.44" W; 520 m a.s.l.), in the Peruvian Amazonian lowlands were also deposited at Z+ZT. Living cultures of the fungus are currently maintained at the Universidad Nacional de San Martín-Tarapoto.

Description: Spore cluster formation prevailing, in soils and in roots on mycelia hyphae. Several spore clusters, each 80–300 × 60–200 µm, might be separated from one mycelia hypha in a short distance from each other to form larger interwoven spore cluster complexes, or loose sporocarps of up to 500–900 × 400–600 µm.

Spores hyaline, globose to subglobose, 16–31(–36) × 13–29(–35) µm in diam, sometimes egg-shaped, oblong or irregular, formed terminally or intercalary, without septa at or close to the spore base.

Spore wall (SW) bi-layered, hyaline. Outer layer SWL1 is semipersistent to evanescent, 0.5–1.1 µm thick, and partly or completely separating from SWL2, often ballooning in PVLG-based mountants. SWL2 is persistent, unit to finely laminated, 0.7–1.5 µm thick. SWL2 stains pale purple to reddish purple, when exposed to Melzer's reagent.

Subtending hyphae (SH) of spores 10–50 µm long straight or recurved, to rarely flared, generally cylindrical to funnel-shaped or rarely constricted. SH 4–8 µm broad at spore base, usually tapering within 10–20 µm distance to the spore base. Both spore wall layers continue in the subtending hyphae. SHL1 usually thin and evanescent to semi-persistent, or sometimes tightly adherent and, thus, difficult to observe. Pore approximately 3.0–7.0 µm in diam at the spore base, regularly not closed by a septum. Mycelia hyphae 2–5 µm in diam, irregularly branching in the soils.

Mycorrhiza formation: forming vesicular-arbuscular mycorrhiza with *Sorghum* sp., *Brachiaria* sp. and inka nut as plant hosts in pot cultures. Intraradical vesicles similar in size as the spores, and thus, even for experts difficult to distinguish from intraradically formed spores. Intraradical hyphae 2–6 µm in diam, irregularly branching within the roots (Figs. 9–10).

Distribution of *Microkamiensikia peruviana*, *Microkamienskia* and *Kamienskia* species

Microkamienskia peruviana has so far been found only in trap cultures inoculated with rhizosphere soils and root fragments of inka nut, and in single spore pot cultures with *Sorghum vulgare* and *Brachiaria* spp. The trap pot culture soil inocula derived from the three agroforestry field sites in Palmiche, Pampamonte and Morillo (Province of Lamas, Department of San Martín), belonging to the Peruvian Amazonian lowlands and the adja-

cent low mountain areas that range up to 858 m a.s.l. Soil pH at the sites was 7.0–8.1 and available P was rather low (12.1–19.0 mg P kg^{-1}). Hitherto, spores of this fungus have not been detected in the rhizospheric soils of any of the three collection sites.

BLASTn analysis of the entire DNA fragment sequenced from *M. peruviana* revealed that an environmental sequence obtained from roots of a semi-mangrove plant communities (KJ484704) from China (Wang et al. 2015) had 97% of MI with our new species, and thus most probably belongs to *M. peruviana*.

ITS environmental sequences related to *M. divaricata* and *M. perpusilla* have not been found so far. In relation to *M. perpusilla*, LSU rDNA environmental sequences with 98% MI were found in rhizosphere soil from agroecosystems in China (JX683742), rhizosphere soil of *Miscanthus sinensis* and *Leymus mollis* in Japan (AB640733 and AB640741, respectively) and soil from an abandoned field in Czech Republic (KP265018; NCBI). LSU rDNA environmental sequences related to *M. divaricata* with 97–98% MI were obtained from soil fungal communities in a grassland ecosystem (KC410895, KC410909, KC410914, KC410944, KC410989, KC411038, KC411043, KC411061, KC411078, KC411095, KC411134, KC411237, KC411263, KC411270) in the USA-Texas (Procter et al. 2014).

Environmental sequences (from the LSU rDNA) related to *K. bistrata* with 97–98% of MI were obtained from roots of *Pancratium maritimum* (MH702390, MH702391, MH702395, MH702399, MH702423, MH702436, MH702438) in Italy. Procter et al. (2014) have also found a sequence (KC411235) similar to *K. bistrata* with 96% of MI from soil fungal communities in a grassland ecosystem in Texas, USA. Environmental sequences for the ITS rDNA related to *K. bistrata* with 96–99% of MI were found in roots of *Pancratium maritimum* (MH702391, MH702399, MH702423, MH702436, MH702438) in Italy.

Discussion

Microkamienskia peruviana can be distinguished from the other species in the genus by the ballooning nature of SWL1, which is not known for *M. perpusilla* or *M. divaricata*. Ballooning outer spore wall layers are described rarely for Glomeromycota spores, for instance for *Acaulospora rugosa* (Pereira et al. 2016). The new species can easily be distinguished from small-sized species of other genera, e.g. from *R. microaggregatum*, which forms similarly small, but pigmented, brownish yellow spores and subtending hyphae (Koske et al. 1986), or *Dominikia* spp., which regularly form pore closures (septa) at or close to the spore base (Błaszkowski et al. 2015). Morphological differentiation from small-sized glomoid spores of the bimorphic *Archaeospora* spp. seems to be possible mainly by the staining reaction in Melzer's reagent, which is pink, reddish to purple in *Microkamienskia* species, but yellowish to not-existing in *Archaeospora* spp.

Kamienskia and *Microkamienskia* species can be recognized by their small, hyaline spores and open pore channels in the subtending hyphae at and close to the spore bases. They can easily be distinguished by their position in the phylogenetic tree (the sequences

display a 85% MI between the genera), and by their spore sizes. *Microkamienskia* spores are among the smallest spores so far detected for AM fungi (14–35 µm; Błaszkowski et al. 2009a, 2016), while *Kamienskia* spores are slightly, but significantly larger (55–65 × 15–45 µm of *K. bistrata*; Błaszkowski et al. 2009b).

Kamienskia and *Microkamienskia* are not the only genera in the Glomeromycetes having usually an open spore pore at or close to the spore base. This feature is also known for *Rhizoglomus* (Sieverding et al. 2014). For most of the other Glomeraceae genera, pore closure/occlusions are commonly described. *Dominikia*, *Funneliformis*, *Funneliglomus* and *Septoglomus* spp. have rather wide spore pore channels in the subtending hyphae, which are generally closed by well visible, strong and broad septa at or close to the spore base (e.g. Oehl et al. 2011, Corazon-Guivin et al. 2019). The spore pores in *Glomus*, *Sclerocarpum* and *Sclerocystis* spp. (e.g. Oehl et al. 2011, Błaszkowski et al. 2015, Jobim et al. 2019) are usually closed by a combination of introverted wall thickening of their subtending hyphae and fine, straight to recurved bridging septa arising from the inner SW layers.

Microkamienskia species have hyaline spores with small sizes (approximately 12–35 µm), with thin wall layers, which might degrade rather rapidly, especially in soils of the humid Tropics, and thus can hardly be detected in field soil samples by classical spore extraction and morphological identification. In the past, sieve mesh sizes of > 40 µm were commonly used and certainly one major reason, why such spores could not be detected. Another reason could have been the uncertainty, if such small spores were not young, immature spores of larger-spored species/genera, or were glomoid spores of the bi-morphic *Archaeospora* genus. For the identification of such small-sized spores, morphological methods might be at their limits and only molecular methods can clearly identify them.

Kamienskia and *Microkamienskia* species were so far identified from single species cultures originating from maritime sand dunes of Mediterranean Greece (*K. bistrata*; Błaszkowski et al. 2009b) and Italy (*M. perpusilla*; Błaszkowski et al. 2009a), or from maritime dunes of South Africa (*M. divaricata*). Our BLASTn analyses suggest that at least *Microkamienskia* species might have a global distribution from cold to hot climates, from natural to man-made ecosystems, and from humid to semi-arid climates.

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