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Paraglomus occidentale, a new arbuscular mycorrhizal fungus from the sources of the Amazon river in Peru, with a key to the Paraglomeromycetes species

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A new arbuscular mycorrhizal fungus, *Paraglomus occidentale*, was found in an agricultural plantation of the inka nut (*Plukenetia volubilis*) in the Amazonia region of San Martín State in Peru. The inka nut was grown in mixed cultures together with *Zea mays* and *Phaseolus vulgaris*. The fungus was propagated in bait and single species cultures on *Sorghum vulgare*, *Brachiaria brizantha*, *Medicago sativa* and *P. volubilis* as host plants. The fungus differentiates hyaline spores terminally on cylindrical to slightly funnel-shaped hyphae, singly in soils or rarely in roots. The hyaline spores have a triple-layered outer wall and a bi- to triple-layered inner wall. They are (59)69–84(92) µm in diameter. The new fungus is distinguished from all other known *Paraglomus* spp. by spore wall structure including staining characteristics in Melzer's reagent, which is yellow-grayish to grayish on the outermost and generally dark yellow on the second spore wall layer. Phylogenetically, the new fungus is recognized in a well-separated clade, near to *P. laccatum* and *P. occultum*. Finally, an identification key to the Paraglomeromycetes species is included comprising all known species of the genera *Paraglomus*, *Innospora* and *Pervetustus*.

Keywords: agroforestry, farming systems, Glomerales, Glomeromycetes, mountain peanut. – 1 new species.

Since molecular phylogeny was introduced into the taxonomy of fungi, the number of Glomeromycota genera, comprising all species of Arbuscular Mycorrhizal Fungi (AMF), increased from six to currently fifty (Błaszkowski et al. 2015, 2018a, 2019; Baltruschat et al. 2019). Glomoid species, firstly attributed only to the genera *Glomus* or *Sclerocystis* (e.g. Gerdemann & Trappe 1974, Schenck & Pérez 1990, Błaszkowski 2012, Sieverding et al. 2014) were recently combined in more than twenty AMF genera belonging to the orders Glomerales, Diversisporales, Archaeosporales or Paraglomerales (e.g. Oehl et al. 2011a; Błaszkowski et al. 2012, 2018b, 2019; Symanczik et al. 2018; Turrini et al. 2018; Baltruschat et al. 2019). Only for the species of the order Gigasporales, glomoid spore formation is not known (Souza et al. 2018). Of all AMF orders, Paraglomerales might be the less diverse, as only about ten species have been described so far. However, only very recently, two new genera, *Innospora* and *Pervetustus*, were separated from *Paraglomus*, the type genus of this fungal order (Błaszkowski et al. 2017), while other species were attributed to the *Paraglomus* genus either due to concomitant morphological and molecular studies, or based on combined spore morphology and root colonization characteristics (Oehl et al. 2016), or solely on combined spore and germination morphology (Oehl et al. 2011b, c). Last but not least, environmental sequences deposited in the public databases suggest a much larger diversity of this fungal group than known so far (e.g. Mello et al. 2013).

More studies focusing on the diversity of AM fungi were recently conducted in the Western Amazonia lowlands in Peru (e.g. Ruíz et al. 2011; Rojas-Mego et al. 2014, Corazon-Guivin et al. 2019a, b).

During these studies, a new species was found, which resembled the small-spored species of the Paraglomerales. The new fungal species was found in a plantation of the inka nut (*Plukenetia volubilis* L., also called 'sacha inchi', 'inka peanut' or 'mountain peanut'), which is currently gaining increasing agronomic attention as new 'super-food' in many parts of the tropical world (e.g. Srichamnong et al. 2018, Wang et al. 2018). The objective of the present study was to describe the new fungus based on both morphological and phylogenetic analyses. A morphological identification key is included for a more accurate species determination of all Paraglomeromycetes species known so far.

Material and methods

Study sites, soil sampling

Between January 2016 and June 2018, soil samples (0–30 cm depth) were repeatedly taken in an agricultural field site planted with *P. volubilis* in Santa Cruz (6° 36' 41.4" S, 76° 44' 05.6" W, 436 m.a.s.l.) of the province EL Dorado in the Department San Martín in the transition zone of Peruvian Amazonia lowlands and adjacent Andean low mountain ranges. This site is a traditional agroforestry site, where the inka nut is grown in a mixed culture together with maize, beans, and other field crops without addition of chemical fertilizers and pesticides. 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.

AM fungal bait cultures

Bait cultures were established in the greenhouse under ambient temperature conditions, in cylindrical 3 l pots with 3 kg of substrate. The substrate consisted of a 1:1 mixture of field-collected soil samples and coarse river sand. The substrate mixtures were autoclaved at 121 °C for 60 min, three weeks before establishment of the bait cultures. At inoculation and bait culture establishment, the pots were first filled to 75 % with the autoclaved substrate. Thereafter 10–20 spores were added to the substrate surface and five seeds either of *Sorghum vulgaris* L., *Medicago sativa* L., *Brachiaria brizantha* (A. Rich.) Stapf and the inka nut were seeded in order to establish the mycorrhizal association and reproduce spores of the new fungal species. 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 of the Facultad de Ciencias Agrarias, Universidad Nacional de San Martín-Tarapoto for eight months, with 21.4 °C±2.0, 29.0±3.0 °C and 36.0±2.0 °C as minimum, mean and maximum temperatures, respectively. The relative humidity was from 46 to 72 % between April and December 2018. 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).

Morphological analyses

Single spores of the new fungus were separated from the single-species culture samples by a wet sieving process as described by Sieverding (1991). The described 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. (2016) 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 mixture of PVLG and Melzer's reagent (1:1) 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

Intact, healthy spores were isolated from field soil and bait culture samples, and cleaned by friction on fine filter paper (Corazon-Guivin et al. 2019a). 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. The spores were 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 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. 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). Recombinant colonies (07) 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). *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker 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 1 x 10^6 generations, with a sample frequency of 100 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

Taxonomy

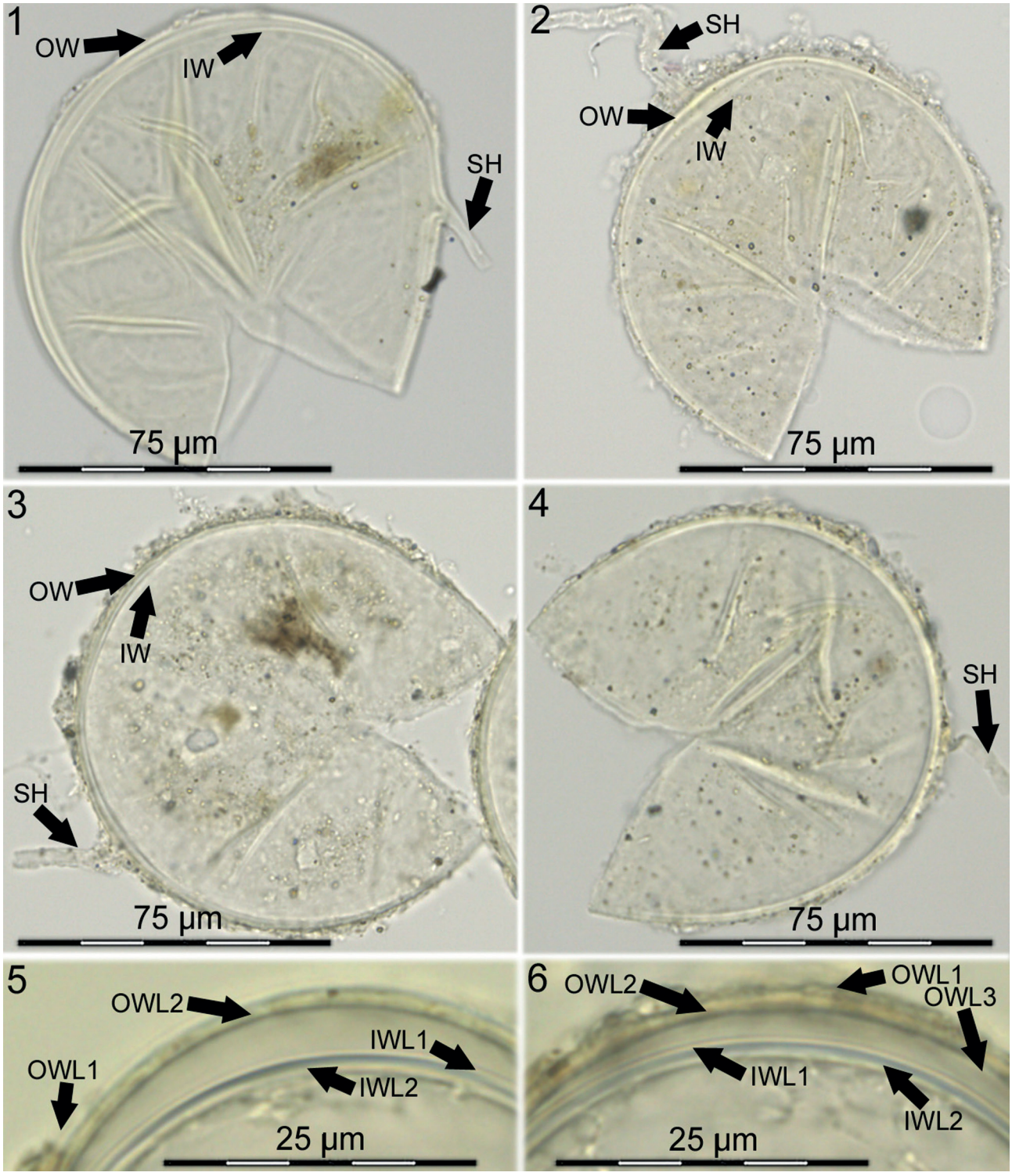
Paraglomus occidentale Corazon-Guivin, G.A. Silva & Oehl, **sp. nov.** – Figs. 1–6
MycoBank no.: MB 833756

Diagnosis. – Spores hyaline, (59)69–84(92) µm in diameter, with a triple-layered outer wall and a bi-to-triple layered inner wall, differing from spores of *P. occultum* and *P. laccatum* by a more intensive staining reaction in Melzer's reagent on the two outermost spore wall layers: yellow-grayish to grayish on OWL1 and dark yellow on OWL2.

Etymology. – Latin *occidentale*, referring to the neotropic isolation site from one of the source areas of the Amazonia river in Peru, from which the fungus was reported first.

Holotype. – Deposited at Z+ZT (accession ZT Myc 60652), derived from a single species culture established on the host plant inka nut 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 originated from an inka nut plantation in Santa Cruz (6° 36' 41.4" S, 76° 44' 05.6" W, 436 m.a.s.l), where the trees were cultured in agroforestry systems together with *Z. mays* and *P. vulgaris*. *Leg*. Mike Anderson Corazon Guivin, 22.01.2018. Isotype slides (ZT Myc 60653) from the same culture grown on *S. vulgare* and *B. brizantha* also deposited at Z+ZT. Living cultures of the fungus currently maintained at the Universidad Nacional de San Martín-Tarapoto.

Description. – The fungus differentiates globose to subglobose, hyaline, bi-walled spores, (59)69–84(92) µm in diameter, terminally or intercalary on cylindrical to slightly funnel-shaped subtending hyphae, singly in soils or rarely in roots. – Outer wall (OW) triple-layered, hyaline. Outer layer OWL1 is mucilaginous, evanescent, 0.4–0.7 µm thick, adhering a large quantity of debris in degradation stages. OWL2 is persistent, 1.7–3.1 µm thick. OWL3 flexible to semi-flexible, 0.5–1.0 µm thick, generally tightly adherent to OWL2. OWL1 staining yellow-grayish to grayish, while OWL2 generally stains dark yellow in Melzer's. – Inner wall (IW) 1.5–3.0 µm thick, divided in two to three tightly adherent layers (IWL1–3), but often only IWL2, unite to finely laminate and 1.0–2.2 µm thick, is clearly observable, especially in uncrushed spores. – Subtending hyphae (SH) of the spores sometimes straight to often recurved, i.e. S-shaped, generally cylindrical to slightly funnel-shaped, to rarely flared or inflated, (12)25–75 µm long, and 3.5–5.5(7.2) µm broad at the spore base, usually tapering to 3.0–5.0(5.5) µm within a short distance to the spore base towards the mycelia hyphae. The outer spore wall continues in the subtending hyphae, while the inner wall seems to form *de novo* within the spore and closing the pore at the spore base. SH wall 1.4–3.0 µm thick at the spore base tapering to 1.0–2.3 µm towards the mycelia. Pore approximate-



Figs. 1–6. *Paraglomus occidentale*. **1–4.** Crushed spores in PVLG with two walls (OW & IW), often with a rather large quantity of debris on the outer spore surface. Subtending hyphae (SH) straight to usually recurved formed by the continuation of OW towards the mycelia hyphae. Spore pore at the spore base closed by IW. **5–6.** Spore segments in PVLG+Melzer's reagent. OW triple-layered and IW bi-layered to triple-layered (OWL1-3, here IWL1-2 only). OWL1 mucilaginous, degrading, staining grayish. OWL2 unit to laminate, staining dark yellow. IW not reacting in Melzer's. IWL1 thin, flexible and tightly adherent to IWL2, which is ca. 2.0 µm thick. IWL3 not visible, generally very thin, flexible and also tightly adherent to IWL2.

ly 2.5–5.2 µm wide at spore base, separated from the spore by IW and rarely closed by an additional septum arising from the outer wall.

Mycorrhiza formation. – The fungus forms arbuscular mycorrhizal symbiosis with *Sorghum* sp., *Brachiaria* sp. and *Plukenetia volubilis* as host plants in pot cultures. AM structures do not or only faintly stain, when exposed to conventional staining procedures such as Trypan blue or ink (Vierheilig et al. 1998).

Molecular analyses. – The phylogenetic analyses from the partial SSU, ITS region, and partial LSU rDNA sequences placed the new fungus in a separated clade near to *Paraglomus laccatum* (Fig. 7). The support values for the clade of the new species were 100 % in all analyses. In the BLASTn analysis, the rDNA species sequences with closest match (89 %) to the new fungus are from *P. laccatum*. No environmental sequences deposited in the GenBank correspond to the new fungus in the BLASTn analysis.

Distribution. – So far, the fungus was found in one agroforestry site (inka nut plantation of Santa Cruz) in the Province of EL Dorado, Department of San Martín, (Amazonia lowlands and adjacent low mountain ranges up to 436 m a.s.l.). Soil texture was sandy-clayey loam. Soil pH at the site was 8.1, and available P was 15.2 mg P kg^{-1}).

Key to species of the Paraglomeromycetes including *Paraglomus*, *Innospora* and *Pervetustus* species

An identification key is presented for all species currently attributed to the fungal class Paraglomeromycetes. The species comprised are nine *Paraglomus*, one *Innospora* and one *Pervetustus* species. *Paraglomus*, *Innospora,* and *Pervetustus* species generally form hyaline to subhyaline spores with the exception of *Paraglomus bolivianum*, which forms yellow brown to brown spores (Mello et al. 2013). Mycelia hyphal and intraradical root colonization structures are generally tiny and do not or only faintly stain in Trypan blue, ink or comparable staining reagents (Renker et al. 2007; Oehl et al. 2011a, 2016), as also known for the *Archaeosporaceae* family (Oehl et al. 2019). The spores are usually formed terminally on subtending hyphae, singly in soils or rarely also in roots (Oehl et al. 2011 a, b). *Paraglomus* species are usually known to germinate from the innermost wall, penetrating directly the spore wall, which is generally known for AM fungal genera forming an inner germinal wall (Oehl et al. 2011a). For *Innospora* and *Pervetustus* such germinal procedures have not yet been described. Thus, germination features cannot be used as an identification tool for single Paraglomerales species or genera. In the key, diameter sizes are given for globose to subglobose spores, and for the width of the subtending hyphae (SH) and the pore channel in the SH at the spore base. The color, thickness, and the persistence degree (evanescent, semi-persistent, persistent/permanent) are presented generally for each single spore wall layer (OWL1-3; IWL1-3) and for the SH wall (and, if possible, for single SH wall layers).

1 Spores with one spore wall consisting of 1–3 layers (SWL1-3).....2
1 Spores with two walls (OW, IW) and 4–6 layers (OWL1-3 & IWL1-3) 3
2 Spores hyaline, bi-layered, (17)51(67) µm, rarely ovoid, 52–58 × 58–63 µm. .....
Pervetustus simplex Błaszk., Chwat, Kozłowska, Crossay, Symanczik & Al-Yahya'ei
SWL1 evanescent, (1.0–)1.5(–2.3) µm, short-lived, usually highly deteriorated or completely sloughed. SWL2 laminate, smooth, (3.8–)5.1(–7.3) µm, consisting of very thin laminae, each <0.5 µm, tightly adherent to each other. SH hyaline; straight or recurved, cylindrical to slightly funnel-shaped, sometimes slightly constricted at the spore base; (3.0–)4.2(–5.8) µm at the spore base. SW layers not staining in Melzer's reagent. SH wall (1.0–) 1.3(–1.8) µm at the spore base, continuous with SWL1-2. Pore (0.8–)1.8(–2.8) µm, open or occluded by a straight or curved septum connecting the inner surfaces of SWL2.
2 Spores hyaline, triple-layered, (35)63(78) µm, sometimes egg-shaped; 50–70 × 65–90 µm. .....
Innospora majewskii Błaszk. & Kovács sp. nov.
SWL1 evanescent, usually slightly roughened on its outer surface, (0.5)0.7(1.0) µm, often deteriorated or completely sloughed in mature spores. SWL2 laminate, smooth, (2.5)3.5(4.4) µm, frequently stratifying into groups of laminae (sublayers) in vigorously crushed spores. SWL3 flexible, ca. 0.5 µm, usually tightly adherent to SWL2 in uncrushed spores, but sometimes separating in vigorously crushed spores. None of the spore wall layers stain in Melzer's. SH hyaline; straight or curved, cylindrical to flared, (3.2)4.6(5.9) µm at the spore base. SH wall (0.7)1.4(2.0) µm at the spore base; composed of two layers continuous with SWL1-2. Pore (1.2)1.9(2.5) µm, open.
3 Spore hyaline to subhyaline 4
3 Spores yellow brown to brown, 70–95 µm, or 71–105 × 57–80 µm, when ellipsoid or irregular.....

Paraglomus bolivianum (Sieverd. & Oehl) Oehl & G.A. Silva

OWL1 smooth, sometimes finely laminated, 1.3–3.0 µm, evanescent. OWL2 yellow brown to brown, laminated, 2.5–4.5 µm, densely covered with shallow, usually pentagonal pits on the outer surface, 3.5–5.5 µm wide and 0.5–1.1 µm deep with ridges 0.5–1.6 µm wide between the pits. OWL3 subhyaline to light brown, ca. 0.5 µm, often difficult to observe as tightly adherent to OWL2. IW hyaline. IWL1 about 0.5 µm. IWL2 is 1.2–2.0 µm. IWL3 ca. 0.5 µm, flexible, often showing several folds in crushed spores. None of the layers staining in Melzer's. SH usually straight and cylindrical, 5.0–10.0 µm at the spore base. SW wall continuous with OW. Pore often closed by 1–2 bridging septa arising from OWL2 at the spore base or in a short distance from the base.

4 Spore regularly < 150 µm 5

4 Spore regularly ≥ 150 µm ... ***Paraglomus lacteum*** (S.L. Rose & Trappe) Oehl, F.A. Souza, G.A. Silva & Sieverd.

Spores 150–220 µm, opaque, milky white., with two spore walls, each double to possibly triple layered, 3–7(9) µm in total. Germination from IW as known for several more recently described *Paraglomus* spp. SH, as typical for many *Paraglomus* spp., cylindrical to slightly funnel-shaped, often recurved, S-shaped. For this species, an emended description would be needed, but this was not possible here due to the lack of newly collected spores (see Rose & Trappe 1980).

5 Spore regularly < 50 µm ..... ***Paraglomus turpe*** Oehl, V.M. Santos & Palenz.

Spores hyaline in water, often becoming slightly creamy in lactic acid based mountants, and creamy to yellow in Melzer's reagent, 32–49 × 29–48 µm. OW triple-layered and entirely evanescent. OWL1 hyaline, 0.5–1.0 µm, and, as rapidly degrading, rarely visible. OWL2 is 0.7–1.3 µm in young spores, rapidly degrading, and expanding to 2.0–6.0 µm when aging. OWL3 is 0.6–1.2 µm. OWL2 may stain slightly yellow, and OWL3 stains slightly yellow, yellow to pinkish-yellow in Melzer's reagent. IW hyaline, (bi-) to triple-layered: IWL1 is 0.6–1.1 µm. IWL2 1.1–2.6(3.5) µm. IWL3 is 0.5–1.0 µm. IWL1 & IWL3 are difficult to observe, as they are generally tightly adherent to IWL2. IWL1 may stain yellow to pinkish-yellow in Melzer's. SH extremely rarely found on spores isolated from the field or bait and single species cultures. SH hyaline, cylindrical, 2.4–5.1 µm, and formed by OWL1-2. SH wall 1.1–2.2 µm, continuous with OWL1 and OWL2.

5 Spore regularly ≥ 50 µm, but < 150 µm 6

6 Spores with ornamentation on OW 7

6 Spores without ornamentation on OW.....8

7 OWL1 with regular, round but shallow pits that are 0.5–1.1 µm wide, 0.5–1.0 µm deep and 1.3–2.4 µm apart. Spores hyaline to brilliant white in young mature spores, to yellowish white in older spores, 66–95 × 62–75 µm.....***Paraglomus pernambucanum*** Oehl, C.M. Mello, Magna & G.A. Silva

Spores globose to subglobose to ovoid. OWL1 0.5–0.9 µm. OWL2 is 2.0–2.6 µm, stains yellow in Melzer's. OWL3 hyaline, 0.4–0.7 µm, separable under pressure, but usually adherent to OWL2 and then extremely difficult to observe. IW is hyaline and triple-layered. IWL1 0.5–0.8 µm. In crushed spores, it sometimes separates under light pressure from IWL2. IWL2 1.0–2.4 µm, finely laminate. IWL3 0.4–0.7 µm, flexible, sometimes showing several folds in crushed spores, but usually adherent to IWL2 and thus also very difficult to observe. None of the layers stain in Melzer's. SH is straight or recurved, 4.0–5.5 µm at the spore base tapering to 3.5–4.5 µm within 6–15 µm distance from the base, generally slightly funnel-shaped to cylindrical, or rarely slightly constricted. SH wall continuous with OWL1 and OWL2, and of the same thickness, tapering to 0.5–1.1 µm within 25–90 µm distance. SH length persistently remaining at the spore is often only 10–15 µm from the spore base. Pore usually open at the spore base, but IW generally works as pore closure at spore base.

7 OWL3 with characteristic labyrinthiforme ornamentation on outer surface. Spores hyaline, (47.5)60–80(113 µm) ..... ***Paraglomus brasilianum*** (Spain & J. Miranda) J.B. Morton & D. Redecker

Spores formed in soils or occasionally in senescent roots. OWL1 mucilagenous, 0.5–0.9 µm. OWL2 brittle, ca. 0.9–1.1 µm. OWL3 is 1.0–2.0 µm, expanding in acid lactic based mountants. IWL1 flexible, ca. 0.5 µm. IWL2 laminate, flexible, 1.0–3.0 µm, slightly expanding in acid lactic based mountants. IWL3 flexible, membranous, 0.5–0.9 µm. Spores not staining in Melzer's reagent. SH straight to often recurved, 5.0–6.0 µm, continuous with OWL1-3 of the spore wall.

8 Spores with maximum 2–4 fine laminae on structural layer OW2 9

8 Spores regularly with > 4 laminae on structural layer OW2. Spores hyaline, glistening, (50)87(130)

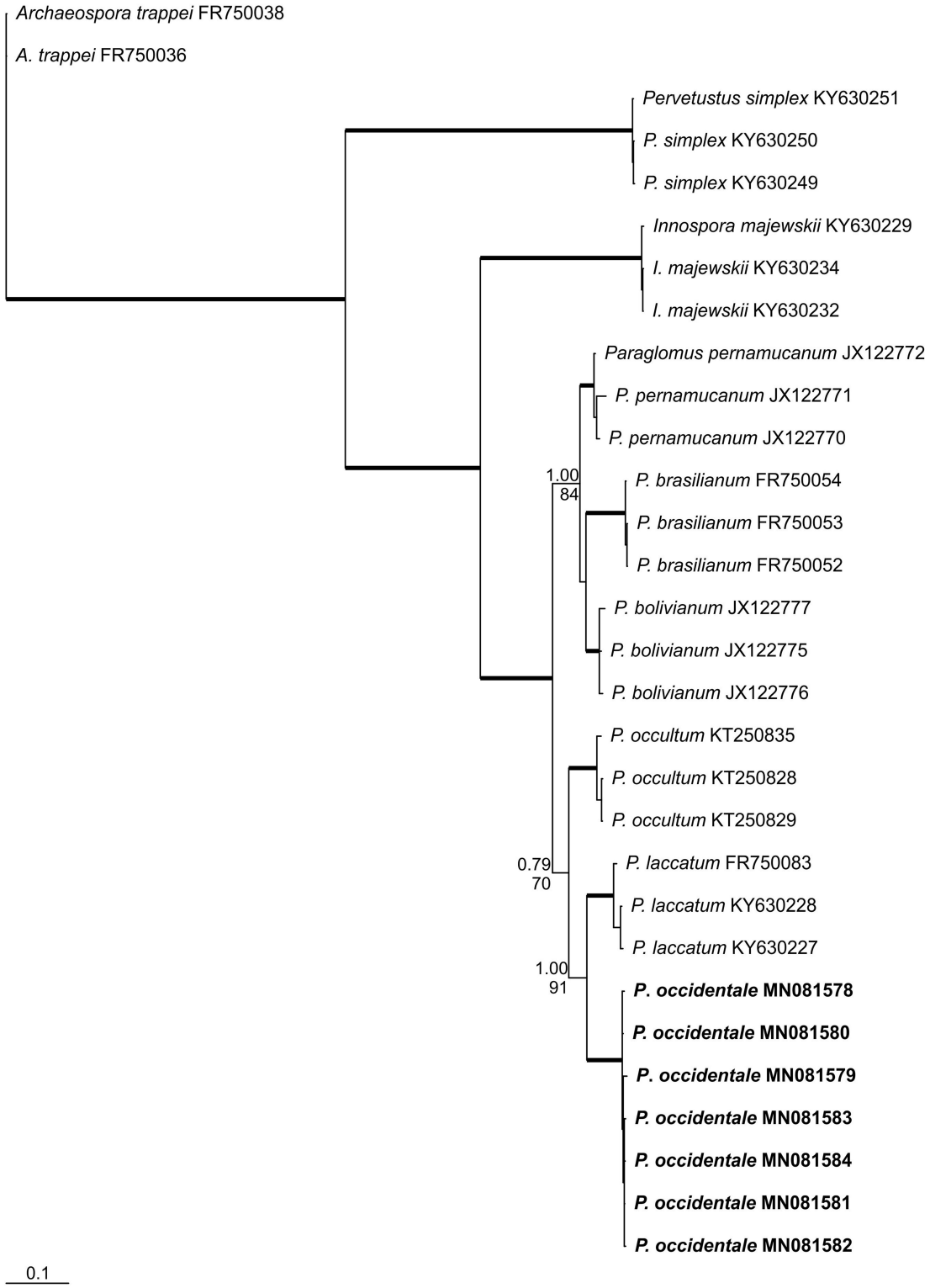


Fig. 7. Phylogenetic tree of the Paraglomeromycetes obtained by analysis from partial SSU, ITS region, and partial LSU rDNA sequences of different Paraglomerales species. Sequences are labeled with their database accession numbers. Support values (from top) are from Bayesian inference (BI) and maximum 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 95 % of support in all analyses. The tree was rooted by *Archaeospora trappei*.

µm, sometimes ovoid; 120–130 × 80–85 µm..... ***Paraglomus laccatum*** (Blaszk.) Renker, Blaszk. & Buscot

Spores consisting of 4–6 layers, which are considered to represent two walls. OWL1 evanescent, hyaline, 0.5–0.8 µm, tightly adherent to OWL2, usually completely sloughed in mature spores. OWL2 laminate, hyaline, smooth, (5.9)7.2(7.9) µm, composed of up to 15 easily separating laminae, each (0.5)1.2(2.2) µm. OWL3 ≤ 0.5 µm, rarely observed, as often enclosed between OWL2 and IW. IW1-2 in total 0.7–1.3 µm. A thin IWL3 is hardly observed as usually tightly adherent to IWL2. None of the wall layers stain in Melzer's. SH hyaline, straight or curved, cylindrical to slightly flared; (7.4)9.7(12.9) µm at the spore base. SH wall (3.0)3.6(3.9) µm at the spore base continuous with OWL1-2. Pore open in young spores, gradually narrowing with age because of thickening of the inner layer of subtending hypha. Germination by a germination tube arising from IW and penetrating directly OW.

9 Young spores hyaline, at maturity white, becoming yellowish with age, 85–145(198) × 85–140(168) µm .. ***Paraglomus albidum*** (C. Walker & L.H. Rhodes) Oehl, F.A. Souza, G.A. Silva & Sieverd.

OWL1 about 0.5 µm. OWL2 is 0.7–1.5 µm, finely laminated, possibly expanding temporarily in lactic acid based mountants, staining yellow in Melzer's. IWL1 < 0.5 µm, rarely to observe as tightly adherent to IWL2. IWL2 0.5–2.0 µm. An IWL3 is rarely observed as very thin and adherent to IWL2. SH straight to recurved, cylindrical to slightly funnel-shaped or slightly constricted, 5–15 µm at the spore base, continuous with OW. Germination by a germination tube arising from IW and penetrating directly OW.

9 Spores hyaline to white, sometimes becoming creamish white with age.....10

10 OWL1 staining grayish and OWL2 dark yellow in Melzer's reagent. Spores hyaline, (59)69–84(92) µm..... ***Paraglomus occidentale*** Corazon-Guivin, G.A. Silva & Oehl

See detailed description above.

10 OWL2 and IWL2 staining light yellow in Melzer's reagent. Spores hyaline to white, 60–120 µm ... ***Paraglomus occultum*** (C. Walker) J.B. Morton & D. Redecker

Spores consisting of two double to triple-layered walls. OWL1 mucilagenous, 0.5–1.4 µm often degrading to form a granular layer, attracting debris in degrading stages. Not staining in Melzer's. OWL2 is 0.5–1.2 µm. OWL3 < 0.5 µm thin and thus, usually difficult to observe. IWL1 ca. 0.5 µm, closely adherent to IWL2. IWL2 is 0.5–1.2 µm, usually 1.4–1.7 µm at the spore base. IWL3 only ca. 0.5 µm and thus, regularly not observed. SH cylindrical to slightly flared, straight to often recurved, 3.0–10 µm. SH wall layers continuous with OW layers of the spore wall. Pore closed by IW. Germination by a germination tube arising from IW and penetrating directly OW.

Discussion

Paraglomus occidentale can be distinguished from all other species in the Paraglomerales by the spore wall structure including the staining features in Melzer's reagent, as the most similar species, *P. occultum* and *P. laccatum* do not stain in Melzer's on the outermost spore wall layer, while the yellow staining of the second wall layer is less intensive for *P. occultum* (Morton & Redecker 2001) and the lamination of this layer is usually much more pronounced in *P. laccatum* than in any other *Paraglomus* sp. described so far (Błaszkowski et al. 1988, Renker et al. 2007). All other *Paraglomus* species might have at least one ornamented spore wall layer (e.g. *P. brasilianum* and *P. pernambucanum*; Spain & Miranda 1996, Mello et al. 2013), bigger spores (e.g. *P. albidum*, *P. lacteum*, Rose & Trappe, 1980, Walker & Rhodes 1981, Oehl et al. 2011a), or, a rare case for Paraglomerales spp., even a pigmented outer spore wall (*P. bolivianum*, e.g. Oehl & Sieverding 2004, Mello et al. 2013). Also phylogenetically, *P. occidentale* is most closely related to *P. occultum* and *P. laccatum*.

In the past, *Glomus diaphanum* and *Glomus viscosum* were cited to be morphologically similar to *P. laccatum* and *P. occultum* (e.g. Renker et al. 2007). However, the staining reactions of the extraradical mycelia and intraradical mycorrhizal structures for Paraglomerales species are only faint to absent in Trypan blue, ink or comparable staining reagents (Mello et al. 2013), while they are blue to dark blue in *G. diaphanum* and *G. viscosum*, as typical for all species of the Glomerales. Furthermore, these two species do not have characteristically recurved, i.e. S-shaped, subtending hyphae, as often found for *Paraglomus* species, and they have only one spore wall (Walker et al. 1995, Oehl et al. 2011b, Błaszkowski et al. 2018b) instead of an additional inner, germinal wall, as observed in *Paraglomus* species. Thus, all *Paraglomus* species are supposed to germinate directly through the spore wall, and not through the subtending hyphae, as known for the Glomerales. Recently, *G. diaphanum* and *G. vis-*

cosum were transferred into two new genera within the Glomerales, *Oehlia* and *Viscospora*, respectively, due to their phylogenetic position (Oehl et al. 2011b, Błaszkowski et al. 2018a). After recent sequence correction for *Glomus viscosum*, newest phylogenetic findings now are suggesting that both these genera form monophyletic clades within the Glomeraceae family, where *Oehlia diaphana* clusters next to the *Rhizoglomus* clade (Corazon-Guivin et al. 2019c) and *Viscospora viscosa* closest to *Septoglomus* clade (Chimal-Sánchez et al. 2019; presented there as the late synonym *S. viscosum* of *G. viscosum*).

Paraglomerales species have a worldwide distribution from coldest and moist regions (e.g. Oehl & Körner 2014) up to the hottest and (semi-)arid regions around the globe (e.g. Al-Yahya'ei et al. 2017, Mello et al. 2013). The new species is so far known only from San Martin State in Peru, isolated from the rhizosphere of the inka nut. Future investigations will be needed, to show if the fungus might be endemic to Western Amazonia or, more likely, has either neotropical, pantropic, or even ubiquitous biogeography.

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