

# *Absidia variicolumellata*, sp. nov., a new mucoralean fungus isolated from Atlantic Forest in Bahia state (Brazil)

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During a survey on the diversity of Mucoromycota in Atlantic Forest soils from northeastern Brazil, one strain belonging to *Absidia* that was morphologically and genetically [internal transcribed spacer (ITS) and large subunit (LSU) rDNA] distinct from other species was isolated. The new species produces simple or repeatedly branched (up to five times) sporangiophores that arise single or in whorls of up to eight from stolons, and hemispherical, globose, and fig-shaped columellae with no projections or with up to two projections on its surface. Sporangiospores are exclusively cylindrical and slightly constricted in the center. The specimen grew better at 25 and 30 °C, with no development at 40 °C. Based on the evidence of the analyzed datasets, a new species of *Absidia* is proposed. A table with the morphological characteristics of *Absidia* species from America (North, Central, and South) and species that are phylogenetically closest to the new species is provided.

Keywords: Mucorales, Cunninghamiellaceae, Monte Pascoal, taxonomy. – 1 new species.

*Absidia* Tiegh. species are saprobic and commonly isolated from soil and herbivore dung (Benny 2009). Morphologically, species of this genus are characterized by the production of erect, simple or branched sporangiophores that arise singly or in whorls from stolons. A septum below the apophysate pyriform sporangia is always present, and rhizoids are never arranged opposite the sporangiophores (Hesseltine & Ellis 1964). Columellae are commonly subglobose and hemispherical, without or with up to three projections on their surface (Lima et al. 2020). The sporangiospores are hyaline, smooth-walled, and cylindrical or globose to subglobose. Sexual reproduction, when observed, forms a zygospore in a zygosporangium, and the suspension cells that support the zygosporangium have appendages (Hoffmann et al. 2007).

*Absidia* species have traditionally been grouped according to the shape of the sporangiospores, being cylindrical, globose, and ovoid (Hesseltine & Ellis 1964, 1966; Ellis & Hesseltine 1965). This genus received special attention by Hoffmann et al. (2007), who reclassified the species of the genus

based on phylogenetic, morphological, and physiological studies, into thermotolerant species, later transferred to *Mycocladius* and then to *Lichtheimia* (Hoffmann et al. 2007, 2009); mesophilic species (currently *Absidia* spp.), and mycoparasites species, later transferred to *Lentamyces* Kerst. Hoffm. & K. Voigt (Hoffmann et al. 2009). Currently, *Absidia* comprises 44 species (www.speciesfungorum.org) of which 15 have been isolated in Brazil (Flora e Funga do Brasil 2022), including eight new species: *A. aguabelensis* J.D. Leitão, T.R.L. Cordeiro, H.B. Lee & A.L. Santiago (Leitão et al. 2021), *A. bonitoensis* C.L. Lima, D.X. Lima, Hyang B. Lee & A.L. Santiago (Lima et al. 2021), *A. caatingaensis* D.X. Lima & A.L. Santiago (as *A. caatinguensis*, Ariyawansa et al. 2015), *A. cornuta* D.X. Lima, C.A. de Souza, H.B. Lee & A.L. Santiago, *A. pernambucoensis* D.X. Lima, C.M. Souza-Motta & A.L. Santiago (Lima et al. 2020), *A. montepascoalis* L.W.S. Freitas, Hyang B. Lee, T.T.T. Nguyen, M.O. Cruz & A.L. Santiago (Crous et al. 2021), *A. multisporea* T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee & A.L. Santiago, and *A. saloaensis* T.R.L. Cordeiro, D.X.

Lima, Hyang B. Lee & A.L. Santiago (Cordeiro et al. 2020).

During a survey on Mucoralean diversity in soil from the Atlantic Rainforest in Northeastern Brazil, one specimen of *Absidia* that differs morphologically and genetically from others was identified. Based on morphological, physiological, and molecular (ITS and LSU rDNA regions) analyses, a new species of *Absidia* is proposed. We present a description and illustration of the new species, and aspects of its morphology are discussed. We also provide a table with morphological characteristics of *Absidia* species from America (North, Central, and South) and species that are phylogenetically closest to the new species.

## Materials and methods

### Collection site

Soil samples were collected in August 2019, at the biological Reserve Parque Nacional e Histórico do Monte Pascoal (PARNAH) (16° 55' 14" W, 39° 30' 20" S), a conservation unit of Atlantic Forest located in the State of Bahia, Brazil (Fig. 1). The climate is super-humid, with rainfall ranging between 1,400 and 1,800 mm/year. The average annual temperature of PARNAH is around 24 °C (ICMBio 2022).

### Isolation, purification, and identification

Five milligrams of soil were added to wheat germ agar (Wg) medium (Benny 2008) containing chloramphenicol (80 mg/l), in Petri dishes, in quadruplicate. Colony growth was monitored for 72 hours at room temperature (28 ± 2 °C), in alternating periods of light and dark. For the culture purification, fragments of the colonies were separately transferred to malt extract agar (MEA) (Benny 2008) containing chloramphenicol (80 mg/l) in Petri dishes and then transferred to test tubes containing potato dextrose agar (PDA) (Benny 2008). The specimen was identified by observing the macroscopic (color, appearance, and diameter of the colony) and microscopic (e.g., shape and size of sporangio-phores, sporangia, columellae, and sporangiospores) characteristics and confirmed by molecular analysis (ITS and LSU regions of rDNA). A slide corresponding to the holotype of the new species (URM 94648) is deposited in the Herbarium URM of the Universidade Federal de Pernambuco. Ex-type living cultures of the new species are deposited in the URM culture collection, Universidade Federal de Pernambuco (URM 8216), as well as in the culture collection (CNUFC) of the Environmental Microbiolo-

gy Laboratory Fungarium, Chonnam National University, Gwangju, Korea (CNUFC B190023).

### Morphological study

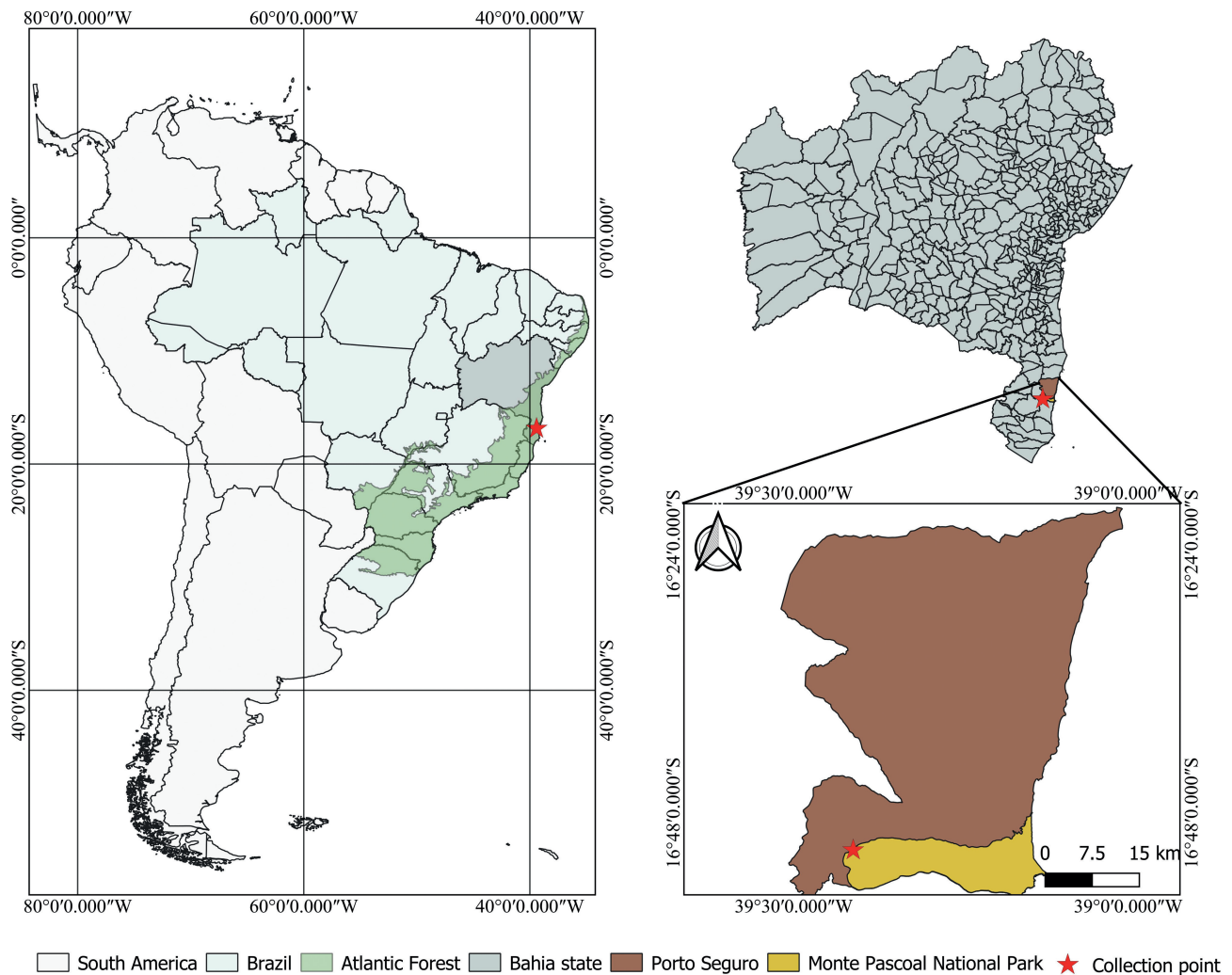
Pure cultures were grown in triplicates on MEA and PDA and incubated at 15, 20, 25, 28, 30, 35 and 40 °C for 15 days. For morphological identification, fragments of selected fertile areas of the colonies were removed from the plates to examine fungal structures. These were placed on microscope slides with a drop of KOH (3 %) or lactophenol blue and observed daily under a light microscope (Carl Zeiss Axioscope 40). Photomicrographs were taken using the Leica DM 500 microscope (Leica, Heerbrugg, Switzerland) combined with the Leica ICC50 camera and the Leica Application Suite software.

### Genomic DNA extraction

Fungal biomass was obtained from MEA cultures and kept in test tubes at 28 °C for a maximum of six days. The material was transferred to 2 ml microtubes with screw caps. We added 0.5 g of acid-washed glass beads with two different diameters (150–212 µm and 425–600 µm, 1:1; Sigma, USA) to each test tube. The material was crushed by stirring in a FastPrep homogenizer at high speed. The genomic DNA extraction procedure was conducted as described in Oliveira et al. (2014). Briefly, homogenized mycelia were transferred into 1.5 mL tube. CTAB lysis buffer (2 % cetyltrimethylammonium bromide, 20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl) was added and incubated at 65 °C. After incubation, chloroform:isoamyl alcohol (24:1) was added. Afterwards, the supernatant that contained the DNA from the hyphal residues was separated and mixed with an equal volume of isopropanol followed by DNA precipitation after incubation at –20 °C for 30 min. After centrifugation at 13,000 rpm for 15 min, the DNA pellet obtained was washed with 70 % ethanol and resuspended in 50 µl of ultrapure water.

### PCR amplification, cloning and sequencing

Two genomic regions were amplified, the internal transcribed spacer (ITS) region using primers ITS1 and ITS4 (White et al. 1990) and the large subunit rDNA region using primers LR0R and LR5 (Vilgalys & Hester 1990, Vilgalys & Sun 1994). The PCR reactions and conditions for PCR were performed as previously described (Nguyen et al. 2021). Amplified fragments were purified using an Accu-prep PCR Purification Kit (Bioneer Corp., Daejeon,



**Fig. 1.** Map of sample collection sites.

Korea). The amplicons of the LSU were sequenced with the same PCR primer using an ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at Macrogen (Daejeon, Korea). Since direct sequencing of the ITS region from PCR products failed, PCR products were cloned using the pGEM-T Easy Vector (Promega, Madison, WI, USA), following the manufacturer's instructions. These clones were sequenced using primers M13F forward (5'-GTAAAACGACGGCCAGT-3') and M13R-pUC reverse (5'-CAGGAAACAGCTATGAC-3').

#### Phylogenetic analyses

Each sequence was checked for ambiguous bases and were assembled using SeqMan v.7 (DNASTAR Inc., Madison, WI, USA). All sequences data of

closely related *Absidia* spp. used in this study were obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (Tab. 1). Sequences of each locus were aligned using MAFFT v.7 with the algorithm L-INS-I (<http://mafft.cbrc.jp/alignment/server>) (Katoh et al. 2019) and the resulting alignment was trimmed using trimAl (Capella-Gutiérrez et al. 2009). The aligned sequences of multiple loci were concatenated using MEGA 7 (Kumar et al. 2016). The data were converted from a FASTA format to nexus and phylip formats using the online tool "ALTER" (<https://sing.ei.uvigo.es/ALTER/>). The most suitable substitution model was determined using jModelTest v.2.1.10 software (Guindon & Gascuel 2003, Darrriba et al. 2012). The best-fit model GTR+G+I was selected for the ML and BI analyses. Phylogenetic trees were construct-

ed using maximum likelihood analyses (ML) in PhyML 3.0 (Guindon et al. 2010) with 1,000 bootstrap replicates. Bayesian inference (BI) analysis was performed in MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). Four Markov chain Monte Carlo (MCMC) chains were run from a random starting tree for five million generations and trees were sampled every 100th generation. The first 25 % of

trees were removed as burn-in and the remaining trees were used to calculate the posterior probabilities. Bayesian posterior probabilities (BPP)  $\geq 0.70$  and bootstrap (BS)  $\geq 70$  % values are indicated above and under the nodes. The newly obtained sequences were deposited in the GenBank database as MZ331545 (ITS), MZ331546 (ITS clone), and MZ331547 (LSU) (Tab. 1).

**Tab. 1.** Culture collection accession numbers and voucher numbers of sequences used for the phylogenetic analysis.

Species name	Collection No.	GenBank accession No.	
		ITS	LSU
<i>Absidia anomala</i>	CBS 125.68 <sup>T</sup>	JN205815	JN206593
<i>Absidia aguabelensis</i>	URM 8213 <sup>T</sup>	MW763074	MW762874
<b><i>Absidia variicolumellata</i></b>	<b>URM 8216<sup>T</sup></b>	<b>MZ331545</b>	<b>MZ331547</b>
<i>Absidia bonitoensis</i>	URM 7889 <sup>T</sup>	MN977786	MN977805
<i>Absidia caatingaensis</i>	URM 7156 <sup>T</sup>	KT308168	KT308170
<i>Absidia californica</i>	CBS 126.68	AY944872.1	–
<i>Absidia californica</i>	CBS 314.78	JN205816	JN206582
<i>Absidia coerulea</i>	CBS 102.28	JN205821	JN206584
<i>Absidia coerulea</i>	CBS 104.08	JN205811	HM849703
<i>Absidia cornuta</i>	URM 6100 <sup>T</sup>	NR_172976.1	MN625255.1
<i>Absidia cuneospora</i>	CBS 101.59 <sup>T</sup>	EF030524	JN206580
<i>Absidia cuneospora</i>	CBS 102.59	JN205819	JN206579
<i>Absidia cylindrospora</i> var. <i>cylindrospora</i>	CBS 100.08	JN205822	JN206588
<i>Absidia cylindrospora</i> var. <i>nigra</i>	CBS 127.68 <sup>T</sup>	–	JN206589
<i>Absidia cylindrospora</i> var. <i>rhizomorpha</i>	CBS 153.63 <sup>T</sup>	–	JN206594
<i>Absidia edaphica</i>	MFLUCC 20-0088 <sup>T</sup>	NR_172305.1	NG_075367.1
<i>Absidia fusca</i>	CBS 346.97	JN205817	–
<i>Absidia fusca</i>	CBS 102.35 <sup>T</sup>	JN205814	HM849707
<i>Absidia glauca</i>	CBS 101.08 <sup>T</sup>	JN205810	JN206581
<i>Absidia glauca</i>	CBS 100.48	JN205820	HM849705
<i>Absidia globospora</i>	CGMCC 3.16031 <sup>T</sup>	MW671537.1	MW671544.1
<i>Absidia healeyae</i>	U0MAU1 <sup>T</sup>	MT436027.1	MT436028.1
<i>Absidia heterospora</i>	CBS 101.29 <sup>T</sup>	–	JN206595
<i>Absidia jindoensis</i>	CNUFC-PTII-1 <sup>T</sup>	MF926622	MF926616
<i>Absidia koreana</i>	EML-IFS45-1 <sup>T</sup>	KR030062	KR030056
<i>Absidia macrospora</i>	CBS 697.68 <sup>T</sup>	AY944883	HM849704
<i>Absidia medulla</i>	CGMCC 3.16034 <sup>T</sup>	MW671542.1	MW671549.1
<i>Absidia montepascoalii</i>	URM 8218 <sup>T</sup>	NR_172995.1	MW561560.1
<i>Absidia multispora</i>	URM 8210 <sup>T</sup>	MN953780	MN953782
<i>Absidia ovalispora</i>	CGMCC 3.16018 <sup>T</sup>	MW264071.1	MW264130.1
<i>Absidia panacisoli</i>	CBS 140959 <sup>T</sup>	NR_159563.1	NG_063948.1
<i>Absidia pararepens</i>	CCF 6352	MT193669.1	MT192308.1
<i>Absidia pernambucoensis</i>	URM 7219 <sup>T</sup>	MN635568	MN635569
<i>Absidia pseudocylindrospora</i>	CBS 100.62 <sup>T</sup>	NR_145276	JN206591
<i>Absidia psychrophilia</i>	CBS128.68 <sup>T</sup>	AY944874.1	–
<i>Absidia repens</i>	CBS 115583 <sup>TT</sup>	JN205813	HM849706
<i>Absidia saloensis</i>	URM 8209 <sup>T</sup>	MN953781	MN953783



Species name	Collection No.	GenBank accession No.	
		ITS	LSU
<i>Absidia spinosa</i> var. <i>spinosa</i>	CBS 106.08	JN205809	JN206590
<i>Absidia soli</i>	MFLU 20-0413 <sup>T</sup>	MT394046.1	MT393985.1
<i>Absidia stercoraria</i>	EML-DG8-1 <sup>T</sup>	KU168828	KT921998
<i>Absidia terrestris</i>	FMR:14989 <sup>T</sup>	LT795003	LT795005
<i>Absidia turgida</i>	CGMCC 3. 16032 <sup>T</sup>	MW671540.1	MW671547.1
<i>Absidia zonata</i>	CGMCC 3. 16034 <sup>T</sup>	MW671542.1	MW671549.1
<i>Chlamydoabsidia padenii</i>	CBS 172.67 <sup>T</sup>	–	JN206586
<i>Cunninghamella phaeospora</i>	CBS 692.68 <sup>NT</sup>	JN205864	HM849697
<i>Cunninghamella vesiculosa</i>	CBS 989.96 <sup>T</sup>	JN205897	HM849693
<i>Halteromyces radiatus</i>	CBS 162.75 <sup>T</sup>	–	JN206596

CBS, Culture Collection of the Westerdijk Fungal Biodiversity Institute, The Netherlands; URM, Culture Collection, Universidade Federal de Pernambuco, Recife, Brazil; MFLU, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; CGMCC, China General Microbiological Culture Collection Center, Beijing, China; U0MAU, National Herbarium of Victoria, Australia; CNUFC, Chonnam National University Fungal Collection, Gwangju, South Korea; EML, Environmental Microbiology Laboratory (Fungarium, Chonnam National University, Gwangju, South Korea); CCF, Culture Collection of Fungi, Charles University, Prague, Czech Republic; FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain.

## Results

### Phylogenetic analyses

Topologies of the best phylogenetic tree of strain URM 8216 and related species were based on maximum likelihood (ML) analysis of combined ITS and LSU rDNA. The concatenated alignment consisted of 1,244 characters including gaps (544 for ITS; 700 for LSU). The substitution model GTR+I+G was selected for the ML and BI analyses. The ML tree with bootstrap support values for both the ML and BI analyses is presented in Fig. 2. The phylogenetic analysis showed that URM 8216 is related to *A. healeyae* (U0MAU1, ex-type strain), *A. cylindrospora* var. *cylindrospora* (CBS 100.08), and *A. terrestris* (FMR:14989, ex-type strain).

### Taxonomy

***Absidia variicolumellata*** L.W.S. Freitas, M.O. Cruz, Hyang B. Lee & A.L. Santiago, **sp. nov.** – Fig 3.  
MycoBank no.: MB 844363

Holotypus. – URM 94648.

**Description.** – Colony initially white, becoming light brown after seven days at 25 °C on PDA; reverse light gray with a wave edge. Stolons brown, 4–12 µm in diam., irregularly septate after 9 days, some with two septa near the place of origin of the sporangiophore. Rhizoids brown, poorly branched, filiform, some with lobed ends (12)60–140 × 8–25(35) µm. Sporangiophores brown, short or

long, emerging along the stolon, single or in whorls of 2–6(8), simple, monopodially or sympodially branched up to four times (12)25–700 × 2.5–5 µm, with slightly incrustated wall, rarely with a swelling; one septum below the sporangium is always present. – **Sporangia** brown, pyriform, 25–65 × 12.5–35 µm, with a funnel shaped apophysis, 5–12 × 5.5–17 µm, smooth-walled. Columellae brownish, hemispherical, 4.5–7.5 × 7–9 µm, subglobose, 7–15 × 9.5–17 µm and fig-shaped, 5–19.5 × 7.5–25 µm, smooth-walled, with up to two filiform (majority), some bulbous at distal end, or conical projections, 2.5–6 × 1.2–3.5 µm. Projections not always present. Collar sometimes evident. – **Sporangiospores** brown, cylindrical and slightly constricted in the center, 3.5–7.2 × 2.5–4.5 µm, smooth-walled. – **Chlamydospores** and **zygospores** not observed.

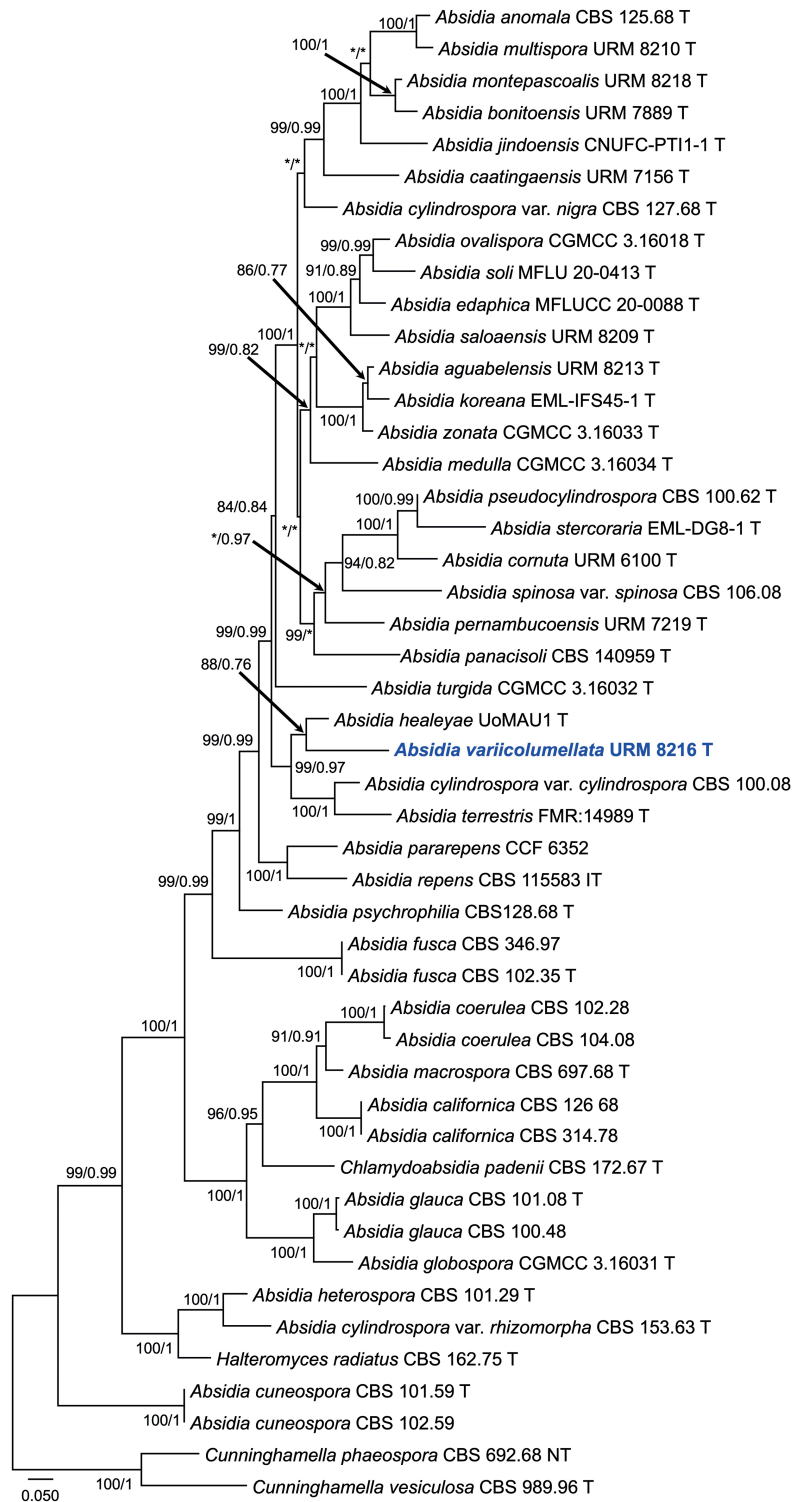
**Etymology.** – *variicolumellata*. Referring to the varied-shaped columellae formed.

**Habitat.** – Soil.

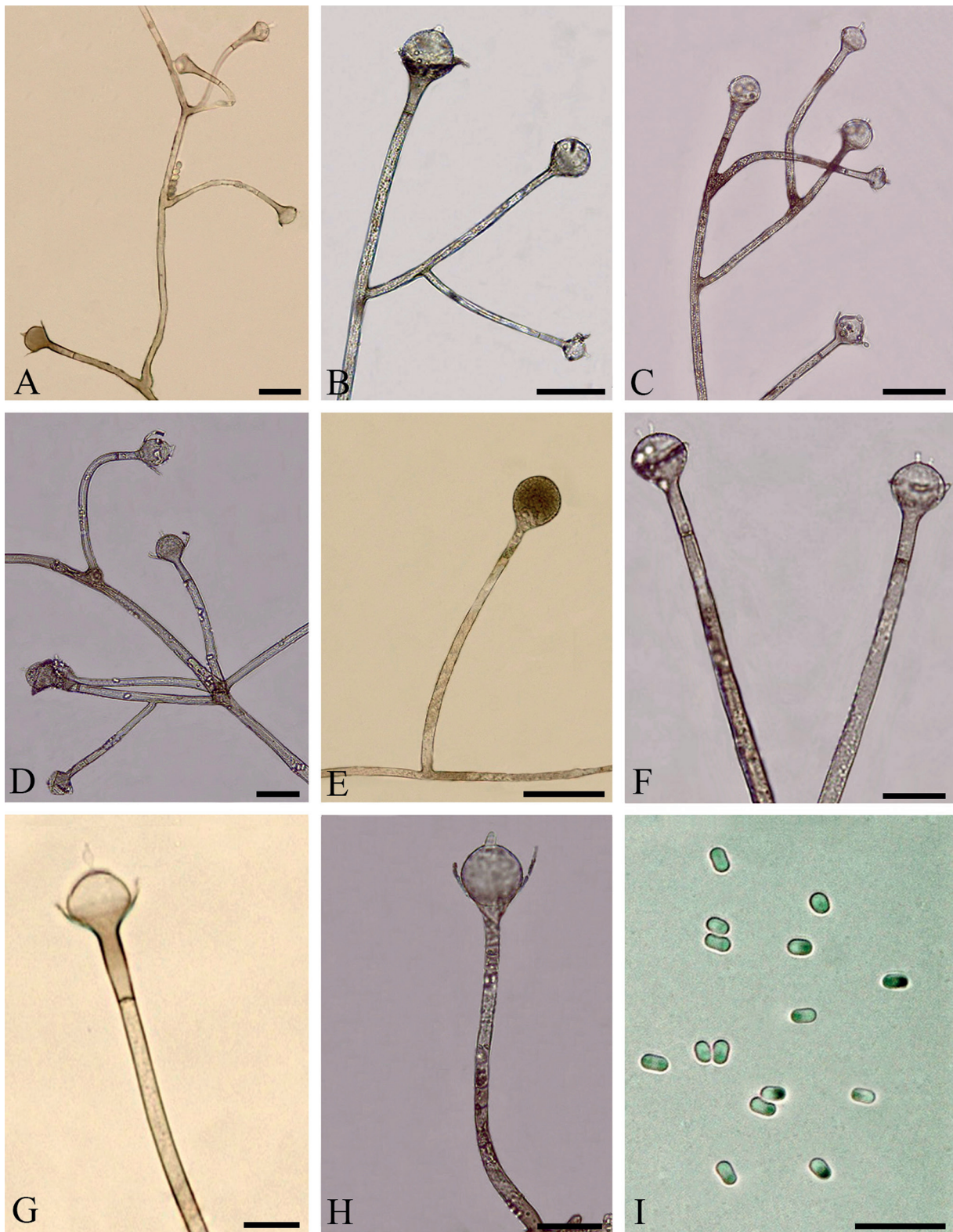
**Distribution.** – Atlantic Forest area, Porto Seguro, Bahia, Brazil.

**Material examined.** – *Absidia variicolumellata* L.W.S. Freitas, M.O. Cruz, Hyang B. Lee, and A.L. Santiago: BRAZIL. Bahia, Porto Seguro (16° 51' 20.2" S, 39° 24' 40.6 W), soil., 7 August 2018, *leg.* L.W.S. Freitas (Holotype: URM 94648. Ex-type: URM 8216).

**Media and temperature test.** – On MEA: at 10 °C lack of growth. 15 °C slow growth (0.3 cm in 96 h), poor sporulation; at 20 °C good growth, good sporulation (7.8 cm in 96 h); at 25 °C good growth, excellent sporulation (8.7 cm in 120 h);



**Fig. 2.** Phylogenetic tree of *Absidia variicolumellata* URM 8216 and related species based on maximum likelihood (ML) analysis of combined ITS and LSU rDNA using sequence data. Bayesian posterior probabilities (BPP)  $\geq 0.70$  (right) and bootstrap (BS)  $\geq 70\%$  (left) values are placed above and under the branches. Bootstrap values lower than 0.70 and 70% are marked with “\*”. The bar indicates the number of substitutions per position. Ex-type, ex-isotype, and ex-neotype strains are marked with “T”, “IT”, and “NT”, respectively. Strain in the current study is shown in bold blue. *Cunninghamella phaeospora* CBS 692.68 and *Cunninghamella vesiculosa* CBS 989.96 were used as outgroups.



**Fig. 3.** *Absidia variicolumellata* URM 8216, ex-type. **A–D.** Branched sporangiophore with columellae; **E.** Unbranched sporangiophore with sporangium; **F.** Two sporangiophores with columellae showing two projections each; **G, H.** Sporangiophore with columella showing one projection; **I.** Sporangiospores. Bars: 25  $\mu$ m.



at 30 °C better growth, excellent sporulation (9 cm in 120 h); at 35 °C slow growth (2.3 cm in 120 h), good sporulation; at 40 °C lack of growth. On PDA: at 10 °C lack of growth; at 15 °C slow growth (0.3 cm in 96 h), poor sporulation; at 20 °C good growth (5.9 cm in 96 h), good sporulation; at 25 °C good growth, excellent sporulation (8.2 cm in 120 h); at 30 °C better growth (8.9 cm in 120 h), excellent sporulation; at 35 °C slow growth (2 cm in 120 h), good sporulation; at 40 °C lack of growth.

### Discussion

The concatenated ITS/LSU trees showed that *Absidia variicolumellata* is phylogenetically closest to *A. healeyae* and close to *A. cylindrospora* var. *cylindrospora* and *A. terrestris* (Fig. 2). The main morphological features of species of *Absidia* from America and species genetically closer to *A. variicolumellata* are shown in Tab. 2. Morphological similarities between *A. variicolumellata* and *A. healeyae* are minimal, with both forming sporangiophores with short branches. However, *A. healeyae* colonies are initially white with visible purple pigmentation, and it does not grow at temperatures above 30 °C. In contrast, colonies from the new species are initially white, turning light brown, and grow at 35 °C. Sporangia of *A. healeyae* are 25.7–30.4 × 32–39.1 µm, smaller than those of the new species. Finally, sporangiospores of *A. healeyae* are spherical (Urquhart & Idnurm 2021), differing from the ones of *A. variicolumellata* that are cylindrical and slightly constricted in the center.

*Absidia variicolumellata* can be easily differentiated from *A. cylindrospora* var. *cylindrospora* as the latter forms sporangiophores 36–300 µm long, arising singly or up to 4(–5) in a whorl. Columellae are hemispherical with one projection each (Hesseltine & Ellis 1964), whereas *A. variicolumellata* forms longer sporangiophores, arising singly or in whorls of 2–6 (8). Columellae are hemispherical, globose, and fig-shaped, often with one or two projections.

*Absidia terrestris* initially forms white colonies that turn greyish brown. The sporangiophores measure 25–215 × 2.5–5 µm, and columellae are globose with one projection (Crous et al. 2018). In contrast, *A. variicolumellata* initially forms white colonies, turning light brown, with (12)25–700 × 2.5–5 µm sporangiophores, and hemispherical, globose, fig-shaped columellae with one or two projections.

Morphologically, *A. variicolumellata* is relatively similar to *A. aguabelensis* D. Leitão, T.R.L. Cordeiro, H.B. Lee & A.L. Santiago (Leitão et al. 2021), *A. cornuta* D.X. Lima, C.A. de Souza, H.B. Lee & A.L. Santiago (Lima et al. 2020), and *A. montepascoalis* L.W.S. Freitas, Hyang B. Lee, T.T.T. Nguyen, M.O. Cruz & A.L. Santiago (Crous et al. 2021). All these species form single or branched sporangiophores, with at least seven in a whorl; the columellae vary in shape, some are fig-shaped (described as conical in *A. cornuta*) with up to two (rarely three in *A. cornuta*) projections on its surface, and the sporangiospores are cylindrical (Leitão et al. 2021, Lima et al. 2020, Crous et al. 2021). However, *A. variicolumellata* can be morphologically differentiated from *A. aguabelensis* as the latter forms short or long branched or unbranched rhizoids, while rhizoids of the former are elongated, some terminally bulbous, and slightly branched. Sporangia of *A. aguabelensis* are 13–26.5 × 9.5–30 µm, smaller than the ones of *A. variicolumellata*. The sporangiospores of *A. aguabelensis* are globose, subglobose, and cylindrical, differing from the ones of the new species that are cylindrical and slightly constricted in the center. Lastly, chlamydospores are well evidenced in *A. aguabelensis* on PDA at 25 °C but absent in the new species using the same medium and temperature (Leitão et al. 2021). The main differences between *A. cornuta* and *A. variicolumellata* are that both sporangiophores [(65)70–250(300) × 2.5–5 µm] and sporangia (20–35 µm diam.) of the former (Lima et al. 2020) are smaller than those of the new species. *Absidia montepascoalis* may be differentiated from *A. variicolumellata* by producing sporangiophores up to 400 µm long, solitary or in whorls of up to seven. In contrast, sporangiophores of *A. variicolumellata* are bigger, solitary, or in whorls up to eight. Sporangiospores of *A. variicolumellata* are bigger than those of *A. montepascoalis* which are 3–5 × 1.5–3 µm (Crous et al. 2021). Chlamydospores are common in the latter species on PDA at 25 °C, but not observed in the new species using the same medium and temperature.

Even with those abovementioned morphological differences, in our opinion, differentiation among *A. variicolumellata*, *A. aguabelensis*, *A. cornuta*, and *A. montepascoalis*, may be challenging, especially if done by untrained taxonomists. Therefore, the sequencing of at least the LSU and ITS rDNA regions is highly recommended as both are efficient for the differentiation of *Absidia* species (Walther et al. 2013, Lima et al. 2020, Leitão et al. 2021).



**Tab. 2.** Morphological characteristics of *Absidia* species from America (North, Central, and South) and species phylogenetically closest to *A. variicolumellata*. Adapted from Cordeiro et al. (2020).

Species	Colony color	Whorls	Sporangiophores	Sporangia	Columellae	Projection	Sporangiospores
<i>Absidia anomala</i> <sup>1</sup>	Gray to light violet	Up to 2	40–105 × 3–9 µm	Pyriiform, 12–26 µm diam.	Hemispherical, 6.5–20 µm diam.	One (shape not known); up to 2 µm long	Cylindrical to somewhat constricted in the center to short oval, 3–4 × 2.2 µm
<i>Absidia aguabelensis</i> <sup>2</sup>	White to brownish grey	Up to 8	Up to 440 × 2.5–7.5 µm	Subglobose, pyriform, 13–26.5 × 9.5–30 µm	Fig-shaped, 5–15.5 × 5–17 µm, hemispherical, 2.5–15.5 × 7–17 µm diam.	One, rarely 2; conical, up to 4.5 µm long, or needle-shaped with or without a bulbous tip, up to 7.2 µm long, some almost inconspicuous	Globose, 2.5–5 µm diam., subglobose, 4.5–6 × 3.6–5 µm, cylindrical, 3.5–9.5 × 2–6 µm
<i>Absidia variicolumellata</i>	White to light brown	Up to 6	25–700 × 2.5(–5) µm	Pyriiform, 25–65 × 12.5–35 µm	Hemispherical, 4.5–7.5 × 7–9 µm, subglobose, 7–15 × 9.5–17 µm and fig-shaped, 5–19.5 × 7.5–25 µm	Up to 2, (rarely 3) conical, filiform or filiform bulbous at distal end, 2.5–6 × 1.2–3.5 µm	Cylindrical and slightly constricted in the center, 3.5–7.2 × 2.5–4.5 µm
<i>Absidia bonitoensis</i> <sup>3</sup>	White to brown–gray	Up to 5(6)	60–330(670) × 2.4–5 µm	Pyriiform, 16.5–33.5 µm diam.	Hemispherical, subglobose, 9.5–21.5 µm diam.	Rarely one (shape not known); less than 1 µm	Globose and subglobose, 2.4–5 (7) µm diam., rarely short cylindrical 4–7 × 2.5–5 µm
<i>Absidia caatingaensis</i> <sup>4</sup>	Brownish gray	Up to 6(7)	40–150 × 2.5–5 µm	Pyriiform, 17.5–27.5 µm diam.	Mostly hemispherical, some subglobose, 10–20 µm diam.	One; bulbous at distal end, up to 5.75 × 2.5 µm	Cylindrical, slightly constricted at the central portion, 5–7.5 × 2.5–3.7 µm
<i>Absidia californica</i> <sup>5</sup>	Light gray to grayish olive	Not formed	150–250 × 7–11 µm	Globose to oval, 10–30 µm in diam.	Hemispherical, some hemispherical, 14.5–26.5 µm in diam.	One (shape not known); up to 4.5 × 5.5 µm	Globose, 3.5–4 µm in diam.
<i>Absidia comua</i> <sup>6</sup>	White to brownish gray	Up to 6(8)	(65) 0–250 (300) × 2.5–5 µm	Pyriiform to subglobose, 15–35 µm diam.	Subglobose, 9.5–25 × 7.2–21.5 µm, conical 7–17 × 6–15 µm	One; up to 7.5 × 2.5 µm, and 2 (rarely 3) up to 4.5 × 2 µm	Cylindrical, (3)4.5–6(7.5) × 1.5–2(3.5) µm
<i>Absidia cuneospora</i> <sup>7</sup>	Light gray to pearl gray	Up to 3	30–150 × 2–4 µm	Globose, 13–32 µm in diam.	Hemispherical to nearly globose, 9–19.5 µm in diam.	One; swollen at apex, 6–7 × 2.5–3 µm	Lacrimoid to wedge-shaped, 4.5–6.5 µm long
<i>A. cylindrospora</i> <sup>1</sup>	Pale olive–buff	Up to 4(5)	36–300 × 2–7 µm	Pyriiform, 10–35 µm diam.	Hemispherical, 8.5–26 µm diam.	One; straight or slightly swollen at the apex	Cylindrical to slightly constricted in center, some slightly broader at one end, 3.3–5.5 × 2.2–3.5 µm
<i>Absidia fusca</i> <sup>1</sup>	White to pale olive–gray	Up to 6	(35)78–234(490) × 3–8.6 µm	Pyriiform, 15–36 µm diam.	Hemispherical, 8.6–21.5 µm diam.	One; bulbous and rounded terminally, up to 6.5 µm long	Cylindrical, 3.3–4.5 × 1.8–3.5 µm
<i>Absidia glauca</i> <sup>7</sup>	White to olive	Up to 4	(135)300–750(1000) × 8–12 µm	Pyriiform, 28.5–65 µm diam.	Hemispherical, 27.5–50 µm diam.	One; narrow pointed	Globose, 2.5–5 µm in diam.

Species	Colony color	Whorls	Sporangio-phores	Sporangia	Columellae	Projection	Sporangiospores
<i>Absidia montepascoalis</i> <sup>7</sup>	White to pale grey	Up to 6(7)	(45)120–325(400) × 4–8 µm	Pyriiform, 25–40 × 21.5–35 µm diam.	Subglobose, hemispherical, fig-shaped, 9.5–25 × 10–30 µm diam.	Up to 2 (rarely 3); cylindrical, conical, bulbous at distal end. Some may show a constriction on its apical portion, 2.5–5.5 × 1.5–5 µm	Cylindrical, slightly constricted in the center, 3–5 × 1.5–3 µm
<i>Absidia multispore</i> <sup>8</sup>	Brownish gray, turning dark gray	Up to 2(4)	Up to 270 × 5 µm	Pyriiform, subglobose, up to 30 µm in diam.	Hemispherical, subglobose, and strawberry-shaped, (7)10–16 × (7)10–15(20) µm	One; mostly conical, or elongated, needle-like, up to 5 × 2.5 µm	Globose, subglobose, (2.5)5–7.5(9), ellipsoidal, short cylindrical, broadly-ellipsoidal, irregular, 5–9.5(12) × (3.5)5–7.5(9) µm
<i>Absidia pernambucoensis</i> <sup>8</sup>	White to dark gray	Up to 5(8)	(65)85–220(240) × 2.5–5 µm	Pyriiform to subglobose, 20–35 µm diam.	Subglobose, hemispherical 10–25 µm	One (3.5–6.5 × 1.2–2.5 µm) or 2 (up to 5 × 2 µm.) (shape not known)	Cylindrical, cuneiform, 3.5–5 × 1.5–2.5 µm
<i>A. pseudocylindropora</i> <sup>1</sup>	Pale to dark olive-gray	Up to 5–7(11)	45–172 × 3–6 µm	Pyriiform, 15–35 µm in diam.	Globose to nearly hemispherical, 9–26 µm in diam.	One; globose to hemispherical, up to 6 µm long	Cylindrical or nearly so, up to 3.5–5 × 2.4 µm
<i>Absidia repens</i> <sup>9</sup>	Light grayish olive to olive gray	Not formed	(50)140–250(450) × 2.5–6 µm, short sporangio-phores, 12–78 × 2.2–4.5 µm	Oval to elliptical, 15–36 × 7–15 µm, turning globose, 19–26.5 µm in diam.	Hemispherical, 5–25 µm in diam.	One; with a bulbous swelling at end, up to 9 µm long	Short oval to irregular oval, 2.8–5.5 × 2–3 µm, few globose up to 6.5 µm in diam.
<i>Absidia sabaensis</i> <sup>8</sup>	Grayish brown	Up to 5(6)	Up to 280 × 6 µm	Pyriiform, 20–35 µm in diam.	Conical to subglobose and strawberry-shaped, 7–22 × 8.5–20 µm	One; elliptical, conical, or needle-shaped, up to 5 × 3.5 µm	Cylindrical and elliptical, some slightly constricted in the center, 5–7 × 2.5–3.5 µm
<i>A. spinosa</i> <sup>1</sup>	Smoke gray to drab	Up to 4(8)	100–250 × 5–10.5 µm	Pyriiform, 12–30 µm in diam.	Hemispherical, 8–21 µm in diam.	One; short spine to pointed or rounded cylindrical, 1.5–4.5 × 0.5–1 µm	Short cylindrical with rounded ends, 5 × 5.5 µm
<i>A. terrestris</i> <sup>10</sup>	Grayish brown	Not formed	25–215 × 2.5–5 µm	Pyriiform, 17.5–27.5 × 17.5–22.5 µm	Globose, 5–7.5 µm diam.	One (shape not known); 5–7.5 µm	Cylindrical, 4–5 × 2–4 µm

<sup>1</sup> Hesselatine & Ellis (1964); <sup>2</sup> Leitão et al. (2021); <sup>3</sup> Lima et al. (2021); <sup>4</sup> Ariyawansa et al. (2015); <sup>5</sup> Ellis & Hesselatine (1965); <sup>6</sup> Lima et al. (2020); <sup>7</sup> Crous et al. (2021); <sup>8</sup> Cordeiro et al. (2020); <sup>9</sup> Hesselatine & Ellis (1966); <sup>10</sup> Crous et al. (2018).

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