Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest

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**ABSTRACT**

During a survey of Mucorales in soil from an upland forest area in Pernambuco, Brazil, two specimens were isolated and characterized based on their morphological, physiological, and molecular data (ITS and LSU rDNA). Phylogenetic analyses of the isolates revealed that the strains URM 8209 and URM 8210 are closely related to species of *Absidia*. URM 8209 forms conical, subglobose, and strawberry-shaped columellae and the sporangiospores are cylindrical and ellipsoid. URM 8210 produces hemispheric, subglobose, and strawberry-shaped columellae and the sporangiospores are globose, subglobose, ellipsoid, and short cylindrical. Based on evidence obtained through analysis of datasets (LSU and ITS rDNA regions), *A. saloaensis* sp. nov. (URM 8209) and *A. multispora* sp. nov. (URM 8210) are proposed here as novel species. A table with morphological characteristics of Neotropical *Absidia* spp. is provided.

**Keywords:** Cunninghamellaceae, Mucoromyceta, rDNA, soil, taxonomy

**Introduction**

The genus *Absidia* is composed of cosmopolitan fungal species commonly isolated from soil, herbivorous dung and decaying substrates (van Tieghem 1878). Species of this genus commonly produce sporangiophores in whorls, arising from stolons that bear apophysate and pyriform sporangia with a deliquescent wall. Rhizoids are never opposed to sporangiophores (Benny 2001), and columellae may be conical, subglobose, or aplanate, frequently showing an apical projection (van Tieghem 1878; Hoffmann et al. 2007). *Absidia* species reproduce asexually by the formation of sporangiospores and sexually through the formation of zygospores within zygosporangia supported by opposite suspensory cells that have appendages (Hoffmann et al. 2007; Hoffmann 2010).

Among mucoralean species of *Absidia* that have been studied for their industrial importance, *A. coerulea* specimens are capable of transforming saponins that show high yield and regioselectivity to 20 (S)-protopanaxatriol (Chen et al. 2007). *Absidia glauca* performs biotransformation of 3-OxOleandric acid resulting in hydroxylated metabolites and both species mentioned above are excellent chitosan producers used in food processing, antimicrobial production and biotransformation of steroid products (Abdel-Fattah et al. 1984; Smith et al. 1989; Muzzarelli et al. 1994; Brzezowska et al. 1996; Huszcz & Gladysz 2003 Rungsardthong et al. 2006; Dai et al. 2009). *Absidia griseola* has biotransformation
capacity, carrying out microbial hydroxylation of progesterone resulting in 14α-hydroxyprogesterone and 6β, 11α-dihydroxyprogesterone (Habibi et al. 2012). *Absidia fusca* and *A. cylindrospora* are used in bioremediation processes due to their ability to degrade polycyclic aromatic compounds, such as hydrocarbons (Guiraud et al. 2008). Specimens of *A. cylindrospora* have also been used for biosorption of Cadmiun, Copper and Lead metals under experimental conditions (Albert et al. 2018).

Hesseltine & Ellis (1964; 1966) and Ellis & Hesseltine (1965) monographed the genus *Absidia* and grouped its species based on the shape of sporangiospores. Later, in a molecular-physiology and micromorphology study of *Absidia*, Hoffmann et al. (2007) showed that the species of this genus basically consisted of three groups separated according to the growth temperatures: (1) mesophilic species, with ideal growth between 25 and 34 ºC (includes all currently accepted *Absidia s.s* species), (2) mycoparasite species of other mucoralean fungi, with optimal growth between 14 and 25 ºC (species after being transferred to *Lentamyces*), and (3) thermostolerant species, with optimal growth between 37 and 45 ºC (species after being transferred to *Lichtheimia* by Hoffmann et al. (2009)). In the last 10 years, six new *Absidia* species have been reported worldwide: *A. caatinguensis, A. jindoensis, A. koreana, A. panacisoli, A. stercorearia,* and *A. terrestris* (Ariyawansa et al. 2015; Li et al. 2016; Crows et al. 2018; Wanasinghe et al. 2018; Zhang et al. 2018).

During a survey on the diversity of mucoralean fungi in soils of upland forest fragments in the semiarid area of Brazil, two specimens of *Absidia* that varied morphologically and genetically in comparison with the other species of the same genus were isolated. Based on morphological, physiological, and molecular analyses (LSU and ITS rDNA regions), two new species of *Absidia* are being proposed here.

### Materials and methods

#### Sampling sites

Soil samples were collected from the Brejo Nature Reserve (09°00.418’ S 036°46.898’ W) located in Saloá municipality, Pernambuco State, Brazil. The average annual temperature in this region is 20 ºC, with the rainy season beginning in January/February and ending in September/October and precipitation ranging between 0 to 50 mm in the driest months and 50 to 100 mm in the wettest months. The vegetation is a predominant trait of the species of the Atlantic ombrophilous and semi-deciduous forests, being found in the herbaceous areas composed of litter, shrubby vegetation, grasses, and shrubs. The soil has a podzolic characteristic, with areas ranging from being clayey to containing granite blocks (Silva-Júnior et al. 2012).

### Isolation and purification of *Absidia* spp.

Five milligrams of soil were inoculated directly into Petri dishes containing wheat germ culture medium (Benny 2008) plus chloramphenicol (80 mg.L⁻¹), in triplicate. Growth was observed for seven days at room temperature (28 ºC) under alternating light and dark conditions. Fragments of mycelium were removed directly from the Petri dishes under a Leica EZ4 stereomicroscope and transferred to malt extract agar plates (MEA) (Benny 2008). Slides corresponding to the holotypes of *A. saloaensis* sp. nov. (URM 94180) and *A. multispora* sp. nov. (URM 94181) where deposited in the Herbarium URM of the Universidade Federal de Pernambuco. Ex-type living cultures of *A. saloaensis* sp. nov. (URM 8209) and *A. multispora* sp. nov. (URM 8210) where deposited in the culture collection Micoteca URM of the Universidade Federal de Pernambuco. Pure cultures were also deposited in the culture collection (CNUFC) of the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea (*A. saloaensis* sp. nov. CNUFC B190012 and *A. multispora* sp. nov. CNUFC B190013).

#### Growth experiments

Pure cultures were grown in triplicates in MEA and potato dextrose agar (PDA) and incubated at 15, 20, 25, 28, 30, and 35 ºC for 15 days. For morphological identification, fragments were removed from the cultures and observed under a stereomicroscope (Carl Zeiss Axioscope 40) and light microscope (Leica DMS500). The color designation of the colonies was performed according to previous literature (Maerz & Paul 1950).

#### Molecular analysis (DNA extraction, amplification, cloning and sequencing)

Genomic DNA was extracted from fresh fungal mycelia that were grown on cellophane at 25 ºC for four days using the SolgTM Genomic DNA Preparation Kit (Solgent Co. Ltd., Daejeon, Korea) according to the manufacturer’s instructions with a few modifications. The modifications included the DNA precipitated overnight at -20 ºC using an equal volume of ice-cold 100 % isopropanol and the DNA pellet was washed twice using 500 μL of ice-cold 70 % ethanol. The rDNA ITS region was amplified using the ITS1 and ITS4 primers (White et al. 1990), and LROR and LR3 were used to amplify the large subunit (LSU) rDNA (Vilgalys & Hester 1990; Rehner & Samuels 1995). The PCR products were purified using an Accuprep PCR Purification Kit (Bioneer Corp.). PCR products of the LSU region were used for direct sequencing on the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, California, USA) (Macrogen, Daejeon, Korea).
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Since direct sequencing of the ITS region from PCR products was unsuccessful, PCR products were cloned using the pGEM-T Easy Vector System (Promega) cloning kit, following the manufacturer’s instructions. These clones were sequenced using primers M13F forward (5'-GTAAAACGACGGCCAGT-3') and M13R-pUC reverse (5'-CAGGAAACAGCTATGAC-3') by ABI PRISM 3730XL Genetic Analyzer.

**Sequence alignment and phylogenetic analyses**

Raw sequences were assembled and edited (edges trimmed) using BioEdit (Hall 1999). All sequence data used in this study were obtained from GenBank (https://www.ncbi.nlm.nih.gov). Sequences were aligned using MAFFT 7 (https://mafft.cbrc.jp/alignment/server) (Katoh et al. 2019) and then manually refined in MEGA7 (Kumar et al. 2016). Bayesian inference (two runs over 3 × 10⁶ generations with a burn-in of 2500) and maximum likelihood (with support estimated by bootstrap analysis with 1000 replicates) analyses were performed with Mrbayes 3.2.2 (Ronquist et al. 2012) and PhyML 3.0 (Guindon et al. 2010), respectively. The best-fit model of nucleotide substitution for each data set was obtained using jModelTest v.2.1.10 software (Guindon & Gascuel 2003; Darriba et al. 2012).

Sequence data were compared with those of similar sequences available in the National Center for Biotechnology Information GenBank database using BLASTn. The newly obtained sequences were deposited in the GenBank database: *A. saloaensis* sp. nov.: ITS (MN953781), LSU (MN953783), and *A. multispora* sp. nov: ITS (MN953780), LSU (MN953782) (Tab. 1).

**Results**

**Phylogenetic analyses**

The phylogenetic relationship of two novel species and related species was determined by analysis of concatenated sequences datasets of two loci (ITS and LSU) (Fig.1). The concatenated alignment consisted of 1516 characters (including alignment gaps) with 816 and 700 characters used in the ITS and LSU, respectively. TIM2+I+G was found to be the most suitable model for the analysis of the concatenated ITS-LSU sequences. *Absidia multispora* URM 8210 was closely related to *A. anomala* CBS 125.68. The BLASTn search revealed that the ITS and LSU sequences of URM 8210 strain were 92.7% and 97.7% identical with *A. anomala* (GenBank accession numbers: EF030523 and JN982937), respectively. *Absidia saloaensis* URM 8209 was clustered together with *A. koreana* EML-IFS45-1 in the concatenated ITS-LSU tree. In addition, the BLASTn search showed that ITS and LSU sequences of URM 8209 were 83.14% and 93.9% homologous with *A. koreana* (GenBank accession numbers: KR030062 and KR030056), respectively.

**Taxonomy**

*Absidia multispora* T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee & A.L. Santiago sp. nov. (Fig. 2A–I).

**Etymology:** multispora. Reference to variable-shaped sporangiospores that are produced.

**Diagnosis:** Differs from other species of *Absidia* by the combination of the following characters: globose, subglobose, ellipsoid, cylindrical, short-cylindrical, and irregular sporangiospores. Sporangia subglobose and pyriform and columellae which are hemispheric, subglobose and strawberry-shaped, some of them with a projection on their surface.

**Type:** Brazil, Pernambuco: Saloá, Fazenda Brejo Nature Reserve (09°00.418’ S 036°46.898’ W) isolated from soil samples, 10 Nov 2018, T.R.L. Cordeiro (Holotype: URM 94181, Herbarium URM; Ex-type: URM 8210, Micoteca URM). Index Fungorum number: IF557224. GenBank accession numbers: MN953780 and MN953782 (ITS and LSU, respectively).

**Description:** Colony brownish gray turning dark gray (MP21 A1, gray-drab), zoned, colonizing the entire Petri dish (9 cm in diam) within five days at 28 °C; light gray reverse (MP20 A1, minera-grey). Odor absent. Rhizoids present, short or long, branched or unbranched. Stolons light gray, with slightly encrusted wall. Sporangio phyloes slightly brownish-gray, arising from stolons, usually unbranched, single or in whorls of 2 (4), occasionally with aging, up to 270 μm in length and 5 μm in width, some with one swelling, thick–walled; successive branches may originate from abortive sporangia; one septum was observed near the apophysis, two septa were rarely present. Sporangia brownish-gray, apophysate, subglobose, and pyriform, up to 30 μm in diam, multisporated, and smooth-walled. Columellae hyaline, hemispheric, subglobose, and strawberry-shaped, (7–) 10–16 × (–7) 10–15 (~20) μm, smooth-walled. Projection on columellae present or absent; when present, mostly conical, short or thin, elongated, needle-like, up to 5 × 2.5 μm. Collar usually evident. Sporangiospores brownish-gray, globose, subglobose (2.5–) 5–7.5 (~9), ellipsoid, short cylindrical, broadly-ellipsoidal, irregular, 5–9.5 (~12) × (3.5) 5–7.5 (~9) μm, smooth, and thick-walled. Chlamydospores absent. Zygo spores not observed.

*Absidia saloaensis* T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee & A.L. Santiago sp. nov. (Fig. 3A–I).

**Etymology:** saloaensis. Reference to the city (Saloá) from where the species was first isolated.
Table 1. Accession numbers in collection cultures and voucher numbers of sequences used for the phylogenetic analysis

<table>
<thead>
<tr>
<th>Species name</th>
<th>Collection No.</th>
<th>GenBank accession No.</th>
<th>ITS</th>
<th>LSU</th>
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<td><em>Absidia anomal a</em> Hesselt. &amp; J.J. Ellis</td>
<td>¹CBS 125.68²FSU5798</td>
<td>JN205815, EF030523</td>
<td>JN206593</td>
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<td><em>Absidia caatinguensis</em> D.X. Lima &amp; A.L. Santiago</td>
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<td>KT308170</td>
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<td><em>Absidia californica</em> J.J. Ellis &amp; Hesselt.</td>
<td>CBS 314.78</td>
<td>JN205816</td>
<td>JN206582</td>
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<td><em>Absidia coerulea</em> Bainier</td>
<td>CBS 102.28, CBS 104.08</td>
<td>JN205821, JN205811</td>
<td>JN206584, HM849703</td>
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</tr>
<tr>
<td><em>Absidia cuneospora</em> G.F. Orr &amp; Plunkett</td>
<td>CBS 101.59, CBS 102.59</td>
<td>-</td>
<td>JN206580, JN206579</td>
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<tr>
<td><em>Absidia cylindrospora</em> var. cylindrospora Hagem</td>
<td>CBS 100.08</td>
<td>JN205822</td>
<td>JN206588</td>
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<tr>
<td><em>Absidia cylindrospora</em> var. nigra Hesselt. &amp; J.J. Ellis</td>
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<td>-</td>
<td>JN206589</td>
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<td><em>Absidia cylindrospora</em> var. rhizomorpha* Hesselt. &amp; J.J. Ellis</td>
<td>CBS 153.63²</td>
<td>-</td>
<td>JN206594</td>
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<td><em>Absidia fusca</em> Linnem.</td>
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<td>JN205810, JN205820</td>
<td>HM849705, JN206581</td>
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<td>-</td>
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<td><em>Absidia jindoensis</em> Hyang B. Lee &amp; T.T.T. Nguyen</td>
<td>CNUFC-PTT1-1</td>
<td>MF926622</td>
<td>MF926616</td>
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<tr>
<td><em>Absidia macrospora</em> Vaňová</td>
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<td>AY944882</td>
<td>EU736305, HM849704</td>
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<td><em>Absidia multispora</em> sp. nov. T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee &amp; A.L. Santiago</td>
<td>URM 8210²</td>
<td>MN953780</td>
<td>MN953782</td>
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<td><em>Absidia saloensis</em> sp. nov. T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee &amp; A.L. Santiago</td>
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<td>MN953781</td>
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<td>LT795003</td>
<td>LT95005</td>
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<td><em>Chlamydoabsidia</em> padenii Hesselt. &amp; J.J. Ellis</td>
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<td>JN206586</td>
<td></td>
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<tr>
<td><em>Cunninghamella phaeospora</em> Boedijn</td>
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<td>JN205864</td>
<td>HM849697</td>
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<td><em>Cunninghamella vesiculosa</em> P.C. Misra</td>
<td>CBS 989.96⁴</td>
<td>JN205897</td>
<td>HM849693</td>
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<td><em>Halteromyces radiatus</em> Shipton &amp; Schipper</td>
<td>CBS 162.75⁴</td>
<td>-</td>
<td>JN206596</td>
<td></td>
</tr>
</tbody>
</table>

¹ CBS culture collection of the Westerdijk Fungal Biodiversity Institute, The Netherlands; ² CNUFC: Chonnam National University Fungal Collection, Gwangju, South Korea; ³ EML: Environmental Microbiology Laboratory (Fungarium, Chonnam National University), Gwangju, South Korea; ⁴ FMR: Universitat Rovira i Virgili (URV), Tarragona, Spain; ⁵ FSU: Friedrich Schiller University, Jena, Germany; ⁶ URM Micoteca and URM herbarium: Universidade Federal de Pernambuco, Recife, Brazil; ⁷ and ⁸⁴ = ex-type and ex-neotype strains.
Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest

Figure 1. Phylogenetic tree of *Absidia multispora* URM 8210 and *Absidia saloaensis* URM 8209 and related species based on maximum likelihood (ML) analysis of a combined DNA data set of ITS and LSU sequences. Bootstrap values for Bayesian posterior probabilities (BYPP) over 0.95 and maximum likelihood greater than 70% are placed above the branches. Bootstrap values lower than 0.95 and 70% are marked with “*”, and absent are marked with “-”. The bar indicates the number of substitutions per position. *Cunninghamella phaeospora* CBS 692.68 and *Cunninghamella vesiculosa* CBS 989.96 were used as outgroups. The new species are in blue and the type species are indicated with “^T” (ex-type) or “^NT” (neotype).
Figure 2. *Absidia multispora* (URM 8210). A. Surface of colony on PDA at 28°C; B, C. Branched sporangiophore with fertile sporangia and an abortive sporangium; D. Branched sporangiophore with fertile sporangia; E. Unbranched sporangiophore with sporangium; F, G. Unbranched sporangiophore with a columella with one projection on its surface; H. Rhizoids; I. Sporangiospores. Bars: B, C, D, E, F, G, I = 10 μm; H = 20 μm.
Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest

**Figure 3.** *Absidia saloaensis* (URM 8209). **A.** Surface of colony on PDA at 28°C; **B.** Two sporangiophores in a whorl with sporangia and rhizoids; **C, D, E, F, G.** Unbranched sporangiophore with a columella with one projection on its surface; **H.** Rhizoids; **I.** Sporangiospores. Bars: B = 50 μm; C, D, E, F, G, I = 10 μm; H = 100 μm.
**Diagnosis:** Differs from other species of *Absidia* by the combination of the following characters: strawberry-shaped columellae, and cylindrical and elliptical sporangiospores.

**Type:** Brazil, Pernambuco: Saloa, Fazenda Brejo Nature Reserve, 09°00.418’ S 036°46.898’ W, isolated from soil samples, 10 Nov 2018, T.R.L. Cordeiro (Holotype: URM 94180, Herbarium URM; Ex-type: URM 8209, Micoteca URM). Index Fungorum number: IF557227. GenBank accession: MN953781 and MN953783 (ITS and LSU, respectively).

**Description:** Colony grayish-brown (MP22 C1, dusty-gr.) colonizing the entire Petri dish (9 cm in diam) within five days at 28 °C; reverse grayish-white zoned (MP21 B2, olive-gray). Odor absent. Rhizoids present, weakly branched. Stolons hyaline, smooth-walled. Sporangiohyphes hyaline, long and short, growing along the stolons and terminally, up to 280 μm in length and 6 μm in width, erect, slightly encrusted walled, with one septum near the apophysis, and occasionally with one swelling; solitary or more often in whorls of 5 (6); sporangia hyaline, pyriform (15–) 20–35 μm in diam, multisporate, deliquescent, smooth-walled, apophysate. When the sporangium wall liquefies, some sporangiospores may remain attached to the columellae. Columellae hyaline, conical to subglobose and strawberry-shaped, (4.5–) 7–22 × (5–) 8.5–20 (–25) μm, smooth-walled; collar visible. Projection on the columella generally elliptical, conical, or needle-shaped, up to 5 × 3.5 μm, occasionally short, almost inconnspicuous. Sporangiospores hyaline, mostly cylindrical and elliptical, (3.5–) 5–7 (9.5) × 2.5–3.5 (–5) μm, some slightly constricted in the center, smooth-walled. Chlamydospores absent. Zygosporangiospores not observed.

**Discussion**

Morphologically, *A. saloaensis* sp. nov. and *A. multispora* sp. nov. present characteristics of the *Absidia* sensu stricto group, such as apophysate sporangiohyphes arising from stolons, rhizoids never opposed to sporangiophores, and pyriform sporangia (Benny 2001). For the Neotropical region, only nine species had been reported (Tab. 2).

*Absidia multispora* sp. nov. is phylogenetically closely related to *A. anomala* (Fig. 1). However, morphologically, the former differs from *A. anomala* in the size and shape of sporangiospores and columellae. *Absidia anomala* produces cylindrical sporangiospores with 3–4 × 2.2 μm, differing from *A. multispora* sp. nov. that produces sporangiospores globose, subglobose (2.5–) 5–7.5 (–9) μm, ellipsoidal, short cylindrical, broadly-ellipsoidal, and irregular with 5–9.5 (–12) × (3.5) 5–7.5 (–9) μm. Additionally, the columellae of *A. multispora* are hemispherical, subglobose, and strawberry-shaped, unlike the ones of *A. anomala* that are hemispherical only (Hesseltine & Ellis 1964).

*Absidia saloaensis* sp. nov. is phylogenetically related to *A. koreana* (Fig. 1), but morphologically both species are different. *A. saloaensis* produces bigger columellae and sporangiospores than those observed in *A. koreana*. Furthermore, *A. saloaensis* produces strawberry-shaped columellae, while *A. koreana* has globose columellae. The sporangiospores of *A. koreana* are cylindrical with 2.07–4.28 × 1.73–1.98 μm (Ariyawansa et al. 2015), unlike *A. saloaensis* sp. nov. sporangiospores that are cylindrical and elliptical with (3.5–) 5–7 (9.5) × 2.5–3.5 (–5) μm.

In conclusion, our molecular analyses (ITS and LSU rDNA) show that *A. multispora* sp. nov. and *A. saloaensis* sp. nov. are genetically different from other *Absidia* species. Additionally, both novel isolates exhibit a combination of morphological traits that are not yet described for other *Absidia* species. In addition to their bioremediation capacity, *Absidia* species present great potential as chitosan producers and biotransformers of saponins, organic acids, and steroids. Therefore, future experiments to elucidate whether *A. saloaensis* and *A. multispora* exhibit industrial potential should be highly encouraged.

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**References**


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<table>
<thead>
<tr>
<th>Species</th>
<th>Colony color</th>
<th>Whorls</th>
<th>Sporangiophores</th>
<th>Sporangia</th>
<th>Columellae</th>
<th>Projection</th>
<th>Sporangiospores</th>
</tr>
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<tbody>
<tr>
<td><em>A. anomala</em>¹</td>
<td>Gray to light violet.</td>
<td>Up to 2</td>
<td>40–105 × 3–9 μm</td>
<td>Pyriform, 12–26 μm in diam.</td>
<td>Hemispherical, 6.5–20 μm in diam.</td>
<td>Up to 2 μm in length.</td>
<td>Cylindrical to somewhat constricted in the center to short oval, 3–4 × 2.2 μm.</td>
</tr>
<tr>
<td><em>A. californica</em>¹</td>
<td>Light gray to grayish olive.</td>
<td>Not formed</td>
<td>150–250 × 7–11 μm</td>
<td>Globose to oval, 10–30 μm in diam.</td>
<td>Hemispherical, some hemielliptical, 14.5–26.5 μm in diam.</td>
<td>Up to 4.5 × 5.5 μm.</td>
<td>Globose, 3.5–4 μm in diam.</td>
</tr>
<tr>
<td><em>A. cuneospora</em>¹</td>
<td>Light gray to pearl gray.</td>
<td>Up to 3</td>
<td>30–150 × 2–4 μm</td>
<td>Globose, 13–32 μm in diam.</td>
<td>Hemispherical to nearly globose, 9–19.5 μm in diam.</td>
<td>Swollen at apex, 6–7 × 2.5–3 μm.</td>
<td>Lacrimoid to wedge-shaped, 4.5–6.5 μm in length.</td>
</tr>
<tr>
<td><em>A. cylindrospora</em>¹</td>
<td>Pale olive-buff.</td>
<td>Up to 4</td>
<td>36–300 × 2–7 μm</td>
<td>Pyriform, 10–35 μm diam.</td>
<td>Hemispherical, 8.5–26 μm diam.</td>
<td>Rounded and often bulbous, up to 4.5 μm in length.</td>
<td>Cylindrical to slightly broader at one end, 3.3–5.5 × 2.2–3.5 μm.</td>
</tr>
<tr>
<td><em>A. caatinguensis</em>⁶</td>
<td>Brownish gray.</td>
<td>Up to 6 (7)</td>
<td>40–150 × 2.5–5 μm</td>
<td>Pyriform 17.5–27.5 μm diam.</td>
<td>Mostly hemispherical, some subglobose, 10–20 μm diam.</td>
<td>Bulbous at distal end up to 5.75 × 2.5 μm.</td>
<td>Cylindrical, slightly constricted at the central portion, 5–7.5 × 2.5–3.7 μm.</td>
</tr>
<tr>
<td><em>A. multispora</em> sp. nov.⁸</td>
<td>Brownish gray, turning dark gray.</td>
<td>Up to 2 (4)</td>
<td>Up to 270 × 5 μm</td>
<td>Pyriform and subglobose, up to 30 μm in diam.</td>
<td>Hemispherical, subglobose, or strawberry-shaped, 10–16 × 10–15 μm in diam.</td>
<td>Mostly conical, or elongated, needle-like, up to 5 × 2.5 μm.</td>
<td>Globose, subglobose 5–7.5 diam, ellipsoid, short cylindrical, broadly-ellipsoidal, irregular, 5–9.5 × 5–7.5 μm.</td>
</tr>
<tr>
<td><em>A. pseudocylindrospora</em>²,³</td>
<td>Pale to dark olive-gray.</td>
<td>Up to 5–7 (11)</td>
<td>45–172 × 3–6 μm</td>
<td>Pyriform, 15–35 μm in diam.</td>
<td>Globose to nearly hemispherical, 9–26 μm in diam.</td>
<td>Globose to hemispherical, up to 6 μm in length.</td>
<td>Cylindrical or nearly so, up to 3.5–5 × 2.4 μm.</td>
</tr>
<tr>
<td><em>A. repens</em>²</td>
<td>Light grayish olive to olive gray.</td>
<td>Not formed</td>
<td>140–250 × 2.5–6 μm; short sporangiophores 12–78 × 2.2–4.5 μm.</td>
<td>Oval to elliptical, 15–36 × 7–15 μm, turning globose, 19–26.5 μm in diam.</td>
<td>Hemispherical, 5–25 μm in diam.</td>
<td>With a bulbous swelling at end, up to 9 μm in length.</td>
<td>Short oval to irregular oval, 2.8–5.5 × 2–3 μm, few globose up to 6.5 μm in diam,</td>
</tr>
<tr>
<td><em>A. saloaensis</em> sp. nov.⁸</td>
<td>Grayish brown.</td>
<td>Up to 5 (6)</td>
<td>Up to 280 × 6 μm</td>
<td>Pyriform, 20–35 μm in diam.</td>
<td>Conical to subglobose and strawberry-shaped, 7–22 × 8.5–20 μm.</td>
<td>Elliptical, conical, or needle-shaped, up to 5 × 3.5 μm.</td>
<td>Cylindrical and elliptical, 5–7 × 2.5–3.5 μm, some slightly constricted in the center.</td>
</tr>
<tr>
<td><em>A. spinosa</em>²</td>
<td>Smoke gray to drab.</td>
<td>Up to 1–4 (8)</td>
<td>100–250 × 5–10.5 μm</td>
<td>Pyriform, up to 12–30 μm in diam.</td>
<td>Hemispherical, 8–21 μm in diam.</td>
<td>Cylindrical to rounded, up to 1.5–4.5 × 0.5–1 μm.</td>
<td>Short cylindrical with rounded ends, up to 5 × 5.5 μm.</td>
</tr>
</tbody>
</table>

¹ Orr & Plunkett (1959); ² Hesseltine & Ellis (1961); ³ Hesseltine & Ellis (1964); ⁴ Ellis & Hesseltine (1965); ⁵ Hesseltine & Ellis (1966); ⁶ Ariyawansa et al. (2015); ⁷ Crows et al. (2018); ⁸ This article.


