

A new species of *Lichtheimia* (Mucoromycotina, Mucorales) isolated from Brazilian soil

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Abstract During studies on Mucorales in semiarid and littoral dune areas in the northeast of Brazil, two cultures of an *Absidia*-like species were isolated from soil. They were characterized based on morphological, physiological and molecular data (5.8S and LSU rDNA sequences). The phylogenetic analyses of the isolates revealed that they belong to the Lichtheimiaceae and are closely related to species of *Lichtheimia*. The two isolates produced simple or branched, erect and circinate sporophores, occasionally with a septum under the sporangia, characteristics also common in *Lichtheimia* species. However, different from the described *Lichtheimia* species, the columellae of our isolates were mainly short hemispherical, never spatulate or elliptical and without projections. Sometimes, a long conical or bell shaped apophysis was found. Both isolates grew better at 30–35 °C, with no development at 42 °C, and giant cells were not observed. Based on the evidence of the analyzed datasets a new species of *Lichtheimia* is proposed.

Keywords Lichtheimiaceae · Phylogeny · Taxonomy · Temperature

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Introduction

The genus *Absidia* Tiegh. (Mucoromycotina, Mucorales) includes species commonly isolated from soil, characterized by the production of stolons, rhizoids and apophysate sporangia with deliquescent walls. One single septum is formed in the sporangiophore and the zygospores are formed from suspensor cells that contain several appendices (Benny 2010). Sporophores of *Absidia* are never produced opposite to the rhizoids like those of *Apophysomyces* P.C. Misra and *Rhizopus* Ehrenb. (Misra et al. 1979; Zheng et al. 2007). In general, taxa of *Absidia* present fast growth between 25 and 34 °C, although some representatives are capable of growing between 12 and 37 °C (Hoffmann et al. 2007).

Since *Absidia* was described (van Tieghem 1876), some species of this genus were transferred to other genera proposed later, such as *Tieghemella* Berl. & De Toni, *Mycocladus* Beauverie, *Proabsidia* Vuill., *Pseudo-Absidia* Bainier, *Lichtheimia* Vuill. and *Protoabsidia* Naumov. However, in time, with the exception of *Lichtheimia*, the other five genera were synonymized with *Absidia* (Hesseltine and Ellis 1964; Schipper 1990; Kirk et al. 2008).

Until 2007, the delimitation of *Absidia* species was mainly based on morphology criteria, such as size and form of vegetative structures and those of sexual and asexual reproduction. Beauverie (1900) proposed the genus *Mycocladus* Beauverie, containing only *M. verticillata*, which is morphologically identical to some species of *Absidia*. Mirza et al. (1979) also recognized the genus *Mycocladus* to group a few species of *Absidia* that grew well at 37 °C, never produced sporophores in whorls and had optimum temperature to form zygospores at 31 °C. Thus, *A. blakesleeana* Lendn., *A. corymbifera* (Cohn) Sacc. & Trotter, *A. hyalospora* (Saito) Lendn., *A. parvicida* Muskat and *A. ramosa* (Zopf) Lendn. were transferred to *Mycocladus*. However, these species (already within *Mycocladus*) were considered invalid, according

to Art. 36.1 of the International Code of Botany (ICBN) (Benny 2010). Therefore, in 2007, 27 species and nine varieties of *Absidia* were accepted (Benny 2010). Hoffmann et al. (2007), in a study that combined molecular techniques (with a phylogenetic approach) with physiology and micromorphology studies of *Absidia*, showed that known species presented differentiated growth under different temperatures, and divided them into three groups: thermotolerant—optimum growth temperatures above 37 °C (between 37 and 45 °C); mesophilic—optimum growth temperatures between 25 and 34 °C; mycoparasite—species potentially mycoparasitic on other Mucorales with optimum growth temperatures below 30 °C. Thus, Hoffmann et al. (2007) reintroduced the genus *Mycocladus* and proposed that those thermotolerant species of *Absidia* presenting zygospores with suspensor cells without appendices, and forming equator rings on the zygospores (*Absidia*-like species), were transferred again to this genus, as follows: *M. blakesleeana* (Lendn.) J.H. Mirza, *M. corymbifer* (Cohn) J.H. Mirza, *M. ramosus* (Zopf) J.H. Mirza and *M. hyalospora* (Saito) J.H. Mirza. The two mycoparasite species (*A. parricida* Renner & Muskat ex Hesseltine & J.J. Ellis and *A. zychnae* Hesseltine & J.J. Ellis) were transferred to the genus *Lentamyces* Kerst. Hoffm. & K. Voigt by Hoffmann and Voigt (2009), including *L. parricida* (Renner & Muskat ex Hesselt. & J.J. Ellis) Kerst. Hoffm. & K. Voigt and *L. zychnae* (Hesselt. & J.J. Ellis) Kerst. Hoffm. & K. Voigt.

After a meticulous review of the original description of *M. verticillata*, Hoffmann et al. (2009) verified that this species is not thermotolerant, as it does not grow above 40 °C, having its optimum growth at 30 °C. Besides, the zygosporangia's suspensor cells of *M. verticillata* present appendices and the zygosporangial wall is ornamented with scales (or tubercles), sexual characteristic corresponding to *Lentamyces parricida* (Hoffmann and Voigt 2009). Therefore, Hoffmann et al. (2009), considering the elevated probability of the *M. verticillata* culture observed by Beauvérie to be a mesophilic co-culture of *Absidia* sp. with *Lentamyces* sp., transferred the thermotolerant species to the genus *Lichtheimia* Vuill. (Vuillemin 1903), of which the type species is *L. corymbifera* (Cohn) Vuill. (Vuillemin 1903). *Lichtheimia blakesleeana* (Lendn.) Kerst. Hoffm., Walther & K. Voigt, *L. corymbifera*, *L. hyalospora* (Saito) Kerst. Hoffm., Walther & K. Voigt and *L. ramosa* (Zopf) Vuill. have been validated and included in the new Lichteimiaceae K. Hoffm., G. Walther & K. Voigt family. Next, Alastruey-Izquierdo et al. (2010) transferred *A. ornata* A.K. Sarbhoy to *Lichtheimia* (*L. ornata* [A.K. Sarbhoy] A. Alastruey-Izquierdo & G. Walther), described *L. sphaerocystis* A. Alastruey-Izquierdo & G. Walther and placed *L. blakesleeana* in synonymy with *L. hyalospora*.

Although morphology and mating behaviour are important characteristics in traditional concepts of species recognition, delimitation of species within mucoralean fungi needs to be combined with molecular data to ensure a reliable delimitation.

Despite several proposed concepts for species recognition (Mayden 1997), the genealogical concordance phylogenetic species recognition (Taylor et al. 2000) seems to be the one to most likely recognize natural species (e.g., Alastruey-Izquierdo et al. 2010). One additional interesting finding is the correlation between sexual incompatibility and the occurrence of compensatory base changes (CBC) in the secondary structure of ITS2 sequences (Müller et al. 2007). CBCs are changes of paired nucleotides in the double-stranded regions of the secondary structure. With a confidence of 93 %, a single observed CBC seems to be meaningful in the detection of species boundaries (Coleman and Vacquier 2002; Müller et al. 2007).

During diversity studies of Mucoromycotina in soils from the semiarid region (Caatinga) and the Atlantic Forest in Brazil, we isolated two specimens of *Lichtheimia* presenting optimum growth temperatures below 37 °C. The isolates varied morphologically in relation to the other species within this genus. Based on the morphophysiology and molecular (LSU and 5.8S rDNA) analyses carried out, a new species of *Lichtheimia* is being proposed.

Materials and methods

Sampling sites

Soil samples were collected in the municipalities of Araripina (7°27'58"S, 40°24'53"W), Pernambuco State, and Mataraca (6°28'20"S, 34°57'10"W), Paraíba State, both located in the Northeast of Brazil. In Araripina, soils were collected in a preservation area of Caatinga belonging to the Instituto Agrônômico de Pernambuco, in September 2008. The area is covered by hyperxerophilic Caatinga vegetation with stretches of deciduous forest and the climate is tropical semiarid. The rainy season begins in November and ends in April. The average annual rainfall is 431.8 mm (MME 2005a). In Mataraca, soil sampling was carried out in July 2010, in sand dune areas of the Millennium Inorganic Chemicals Mining, a Cristal Company. The climate in this area is tropical rainy with dry summer. The rainy season extends from February to October. The average annual rainfall is 1634.2 mm. The vegetation is predominantly sub-evergreen forest, with sub-deciduous forest and Cerrado inclusions (MME 2005b).

Isolation of *Lichtheimia*

A volume of 5 mg of soil were added to Petri dishes containing culture medium wheat germ agar (Benny 2008) amended with chloramphenicol (NeoFenicol—Neo Química) (100 mg.L⁻¹). The dishes were left over the laboratory bench at room temperature (26±2 °C) for 7 days, in alternate periods of light and dark. Sporangiospores were transferred directly from the colonies by touching a single sporangium using a sterile needle

on a stereomicroscope (Leika EZ4), and placing the spores onto a fresh malt extract agar (MEA) plate (Benny 2008).

Experiments

Pure/axenic cultures from specimens were cultured in triplicate, in MEA and PDA, and incubated at 15, 20, 25, 30, 35, 37, 40 and 42 °C, during 15 days. Fragments of mycelia were removed from cultures, placed on microscope slides with KOH (3 %) and observed under a light microscope (Carl Zeiss Axioscope 40). The color designation of colonies was established according to Maerz and Paul (1950). Mating experiments were carried out on three SMA and PDA plates at 25, 33 and 35 °C, on which one 5 mm colony disk of each isolate was placed in opposite sides of the plates.

Molecular analyses

Fungal biomass was obtained from MEA cultures in test tubes kept at 28 °C for up to 6 days. All mycelia was removed from the test tube with a platinum loop and transferred to 2 mL screw capped microtubes containing 0.5 g of glass beads (1:1 [w:w], acid-washed, 150–212 µm and 425–600 µm; Sigma, U.S. sieve). The fungal material was macerated by vigorous agitation using a FastPrep homogenizer. Genomic DNA extraction was carried out with previously macerated material according to Góes-Neto et al. (2005), that includes a washing in chloroform/isoamyl alcohol (24:1) followed by homogenization of the material in 2 % CTAB buffer, isopropanol precipitation, 70 % ethanol washing and re-suspension in 50 µL of ultrapure water.

To amplify the ITS region, the *primers* ITS1 and ITS4 (White et al. 1990) were used and the LR1 (van Tuinen et al. 1998) and LSU2 (5'-GGTCCGTGTTTCAAGACGGGTCG-3') were employed to amplify the LSU rDNA. PCR reactions were carried out in a 50 µL volume containing 75 mM Tris-HCl pH 8.8, 200 mM (NH₄)₂SO₄, 0.01 % Tween 20, 2 mM MgCl₂, 200 µM each dNTP, 1 µM of each primer and 2 units of TaqTM DNA polymerase (Fermentas, Maryland, USA); cycling parameters were 5 min at 95 °C (1 cycle), 45 s at 94 °C, 1 min at 60 °C, 1 min at 72 °C (39 cycles), and a final elongation of 7 min at 72 °C followed the last cycle.

The final amplicons were purified with the PureLink PCR Purification Kit (Invitrogen), sequenced directly or cloned with a CloneJETTM PCR Cloning Kit (Fermentas; Carlsbad, USA), following the manufacturer's instructions, and sequenced. Sequencing was provided by the Human Genome Research Center (São Paulo, Brazil). Sequence data were compared to gene libraries (EMBL and GenBank) using BLASTn. The sequences derived from the new species were deposited in the NCBI database under the accession numbers KC740484–KC740489.

Phylogenetic analyses

The phylogeny was reconstructed using sequences of the 5.8S and partial sequences of the LSU rDNA gene. Fungal sequences were aligned in CLUSTALX (Larkin et al. 2007) and edited using BioEdit (Hall 1999). Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 1×10^6 generations with a burning value of 2500) and maximum likelihood (1000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and PhyML (Guindon and Gascuel 2003), launched from Topali 2.5, using the K2P+G model for the matrix generated for the 5.8S rDNA and GTR+G model for the matrix generated for LSU rDNA. Neighbor-joining (established with the models cited above) and maximum parsimony analyses were performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications. The sequence alignment files were deposited in TreeBASE and are available at <http://purl.org/phylo/treebase/phyloWS/study/TB2:S14094> and <http://purl.org/phylo/treebase/phyloWS/study/TB2:S14099>.

Sequence similarities based on alignments of the LSU, 5.8S rDNA or ITS1-5.8S rDNA-ITS2 sequences were calculated using BioEdit (Hall 1999) after shortening them to same length and exclusion of sequences containing indefinite bases.

Prediction of secondary structures and CBC estimations

Analyses for compensatory base changes (CBCs), as indicators for species boundaries, were performed after prediction of the putative secondary structures of the ITS2 sequences. Secondary structures for *L. brasiliensis* and *L. ramosa* were predicted using RNAfold (Vienna RNA website) (Gruber et al. 2008). Structures were used as templates for the other sequences of *Lichtheimia* using the 'model' option in the ITS2 Database (Schultz et al. 2006; Koetschan et al. 2012). CBCs were estimated using 4SALE (Seibel et al. 2006, 2008). Since no experimentally proven secondary structure for Lichtheimiaceae is known, the predicted structures used for CBC estimation remain highly putative. Applying the same parameters to different predictions may result in comparable structures and CBC values.

Results

Phylogenetic analyses

Phylogenetic analyses of the 5.8S and LSU rDNA sequences placed the two isolates in the family Lichtheimiaceae (bootstrap values >80 %). The specimens of *Lichtheimia* form a separate clade close to *Dichotomocladium* Benny & R.K. Benj. *Lichtheimia* and *Dichotomocladium* were supported with bootstrap values above 90 % in all analyses (Figs. 1 and 2).

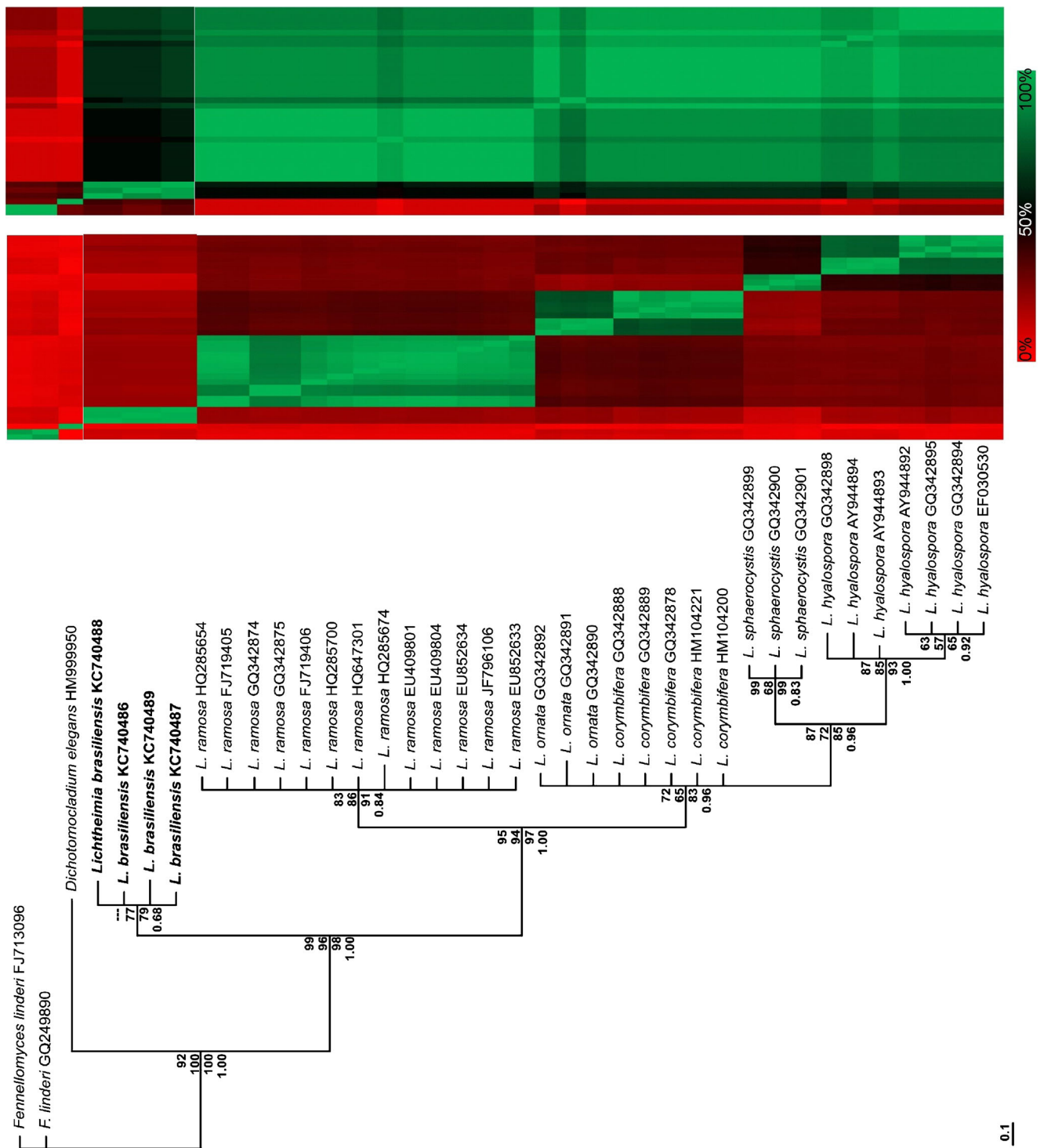


Fig. 1 Phylogenetic tree of the Lichtheimiaceae constructed using 5.8S rDNA sequences. *Fennellomyces linderi* was used as the outgroup. Sequences are followed by their respective access number. Bootstrap values are for neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis, respectively. Sequences

from this study are in bold letters. Consistency index=0.86; Retention index=0.96. Right site shows sequence similarity matrices for ITS1-5.8S rDNA-ITS2 (left matrix) or 5.8S rDNA (right matrix) alone. Similarities are summarized in Table 2

In the BLASTn analysis of the LSU rDNA sequences, the closest related species to the two isolates were *L. hyalospora* (CBS 102.36 and CBS 173.67), *Lichtheimia*

sp. AA-2009 (CBS 958.68) and *Lichtheimia* sp. AA-2009a (CBS 291.66) with 90 % identity. In relation to the 5.8S rDNA sequences, several isolates of *L.*

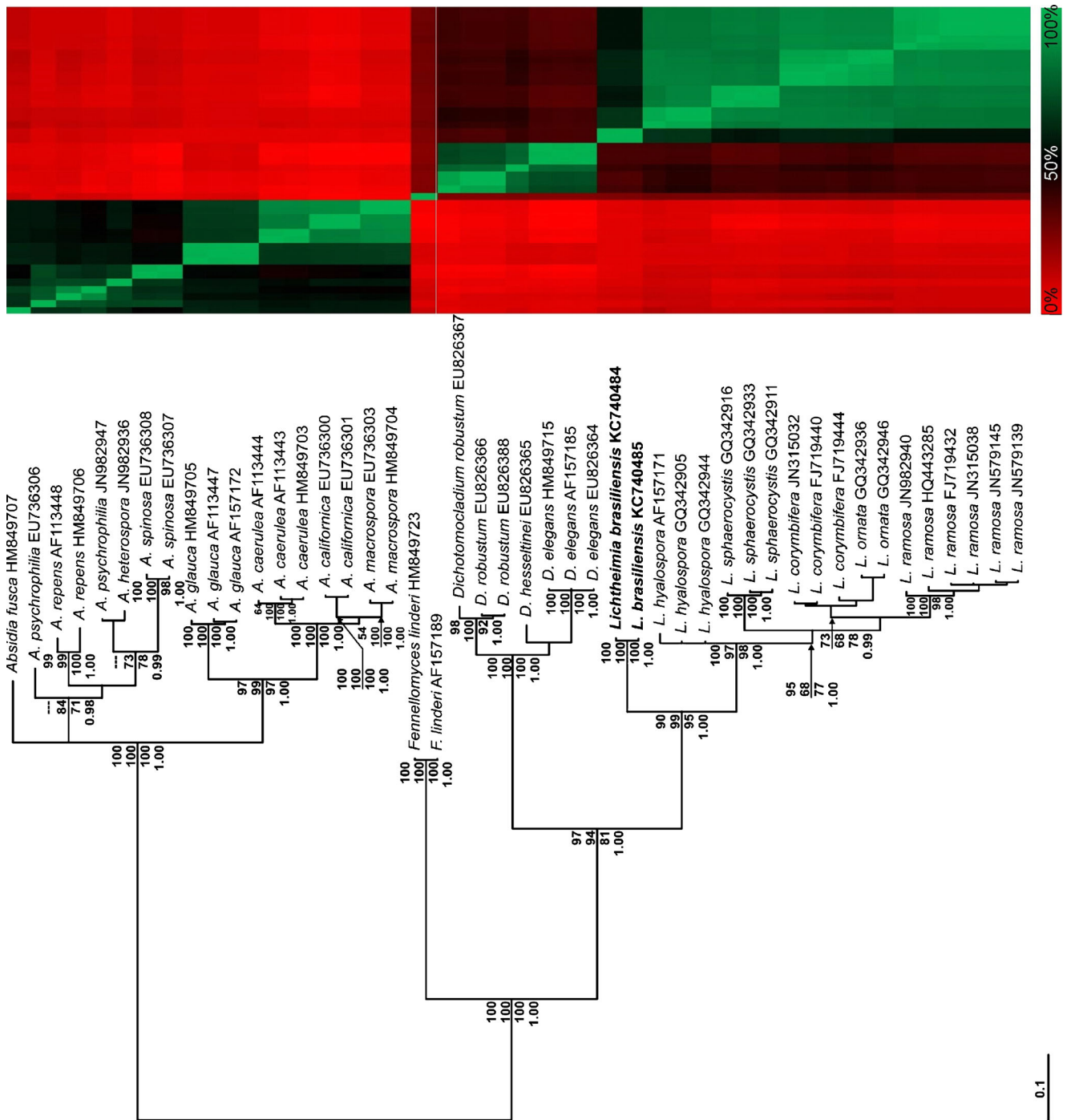


Fig. 2 Phylogenetic tree of the Lichtheimiaceae and related taxa constructed using LSU rDNA sequences. Representatives of the genus *Absidia* were used as the outgroup. Sequences are followed by their respective access number. Bootstrap values are for neighbor-joining

(NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis, respectively. Sequences from this study are in bold letters. Consistency index=0.5r; Retention index=0.91. Right: Sequence similarity matrix for LSU sequences. Similarities are summarized in Table 1

corymbifera and *L. hyalospora* showed 96 % identity with the new species. The 5.8S rDNA is a highly conserved region and can be used to separate genus and above, but does not give good resolution at the species level. The ITS 1 and ITS 2 regions were not used in the analyses due to the high level of variation between

species and genera, resulting in difficulties to properly align the sequences. Based on LSU sequences, similarities between the different species of *Lichtheimia* and other genera include ranges overall from 60 % to 77 % and 81–100 % within *Lichtheimia* itself (Table 1). Sequence similarities of full-length ITS sequences (including 5.8S

Table 1 LSU-sequence similarities (%) of the source sequences for the phylogenetic analyses shown in Fig. 2. Similarities were summarized for the single clades

Mucorales	<i>A</i>	<i>F</i>	<i>D</i>	<i>Lb</i>	<i>Lh</i>	<i>Ls</i>	<i>Lo & Lc</i>	<i>Lr</i>
<i>Absidia</i>	78–100	59–63	59–63	61–64	62–65	60–64	60–65	60–64
<i>Fennellomyces</i>	59–63	100	69	69	70	70	70–71	71
<i>Dichotomocladium</i>	59–63	69	87–100	73–75	74–77	72–75	74–76	73–75
<i>Lichtheimia brasiliensis</i>	61–64	69	73–75	100	85	82	83–84	81–82
<i>L. hyalospora</i>	62–65	70	74–77	85	99–100	93–95	93–94	92–93
<i>L. sphaerocystis</i>	60–64	70	72–75	82	93–95	99–100	93–94	93–94
<i>L. ornata & L. corymbifera</i>	60–65	70–71	74–76	83–84	93–94	93–94	98–100	94–95
<i>L. ramosa</i>	60–64	71	73–75	81–82	92–93	93–94	94–95	94–100

rDNA) range from 38 % to 45 % identity between the investigated genera *Fennellomyces*, *Dichotomocladium* and *Lichtheimia* and 44–65 % between species within the genus *Lichtheimia* itself (Table 2). *Lichtheimia brasiliensis* shows similar, but slightly lower interspecies similarities (44–52 %) when compared to the other *Lichtheimia* species (50–65 %). Yet, the highly conserved 5.8S rDNA shows 87–90 % intergeneric and 93–100 % interspecies similarities (Table 2). Comparing the secondary structures of ITS2 and analyzing them for CBCs, the species appears to be well delimited (Table 3). The number of observed CBCs for *L. brasiliensis* is comparable to other CBCs observed in this genus, supporting its proposed unique status as new species. The observed CBCs should be considered carefully, since different parameters used for the prediction of the secondary structures will lead to different structures and therefore to different values of CBCs, although in similar comparable relations (data not shown). Nevertheless, estimation of CBCs could be considered as supportive information in full accordance with all observed morphological features, including the phylogenetic analyses and the so far unsuccessful crossings.

Taxonomy

Lichtheimia brasiliensis A.L. Santiago, Lima & Oliveira, sp. nov. Fig. 3

*Mycobank*803795

White colonies at first then turning light grey (MP 11A1), higher in the central portion, covering the entire Petri plate and touching the plate lid in the central region and in some points in the periphery of the colony after 15 days in MEA at 25 °C. Reverse yellow (MP 11I2). Coenocytic or irregularly septate stolons, with few septa, mainly in proximity to the place of sporophore origin. Rhizoids branched and with bulbous dilatation in some points. Sporophores simple, few branched, erect, bent or circinate, raising from aerial hyphae or from stolons (the latter, in several points and terminally), solitary or in pairs, never in whorls, occasionally with one septum below the sporangium, colorless and becoming grey near the columella (2.6)3.6–7.2(12) µm diameter (diam.). Yellowish sporangia, turning brown, multispored, sphaerical or subpyriform, apophysate, sometimes with long conical or bell shaped apophysis, 19–55 µm diam. with a smooth,

Table 2 Sequence similarities (%) of the source sequences for the phylogenetic analyses shown in Fig. 1. Similarities are displayed for ITS1-5.8S rDNA-ITS2 (lower triangle) and 5.8S rDNA alone (upper triangle, grey). Similarities were summarized for the single clades

ITS \ 5.8S	<i>F</i>	<i>D</i>	<i>Lb</i>	<i>Lr</i>	<i>Lo & Lc</i>	<i>Ls</i>	<i>Lh</i>
<i>Fennellomyces</i>	100	90	90-91	87-89	88-89	89	88-90
<i>Dichotomocladium</i>	100	100	91-92	87-88	86-88	88	87-88
<i>Lichtheimia brasiliensis</i>	38	100	99	93-94	93-95	95	94-96
<i>L. ramosa</i>	44-45	40	99	99-100	97-99	98-99	97-99
<i>L. ornata & L. corymbifera</i>	41-42	42-43	50-51	89-100	99-100	99-100	98-99
<i>L. sphaerocystis</i>	43-45	40	49-52	57-60	81-100	99-100	98-99
<i>L. hyalospora</i>	40-41	41	44-45	54-55	50-53	100	99-100
	41-43	39-41	49-52	55-56	54-57	93-100	99-100
						63-65	85-100

Table 3 Compensatory base changes (CBCs) summarized for ITS2 sequences used in this study

Species	Lb	Lr	Lo	Lc	Ls	Lh
<i>L. brasiliensis</i>	0	3–4	2	2	1	3–4
<i>L. ramosa</i>	3–4	0–1	1–2	2–5	1–2	3–4
<i>L. ornata</i>	2	1–2	0	0	1–2	1–2
<i>L. corymbifera</i>	2	2–5	0	0	2–3	1–3
<i>L. sphaerocystis</i>	1	1–2	1–2	2–3	0	1–2
<i>L. hyalospora</i>	3–4	3–4	1–2	1–3	1–2	0–1

transparent wall. Columellae short hemispherical, sometimes almost non-existing, subglobose, never spatulate nor conical, smooth and without projections, $7.2\text{--}26.5 \times 2.5\text{--}26.5$ μm . Collar present or absent. Sporangiospores hyaline with smooth wall, ellipsoid, subglobose, $2.8\text{--}5.0 \times 2.4\text{--}3.4$. Giant cells absent. Zygosporangia not observed.

Holotype URM 6910.

Media, temperature tests and mating experiments: On MEA. At 15 °C—slow growth (4.2 cm in 120 h). Few sporophores formed; poor sporulation. At 20 °C—slow growth (5.3 cm in 96 h); medium sporulation. At 25 °C—faster growth than at 20 °C (9 cm in 96 h); good sporulation. At 30 °C—better growth than at 25 °C (9 cm in 72 h); excellent sporulation. At 35 °C—similar growth to 30 °C (9 cm in 72 h); excellent sporulation. At 37 °C—slower growth than at 35 °C (9 cm in 96 h); good sporulation. At 40 °C—limited growth (6.5 cm in 168 h); rare sporophores produced and sporulation poor; colonies with irregular borders and many peripheral colonies. At 42 °C—lack of growth and sporulation. The growth of *L. brasiliensis* on PDA was similar to that on MEA for the temperatures of 15, 20, 25, 30, 37 and 40 °C. At 35 °C the growth was faster in PDA than in MEA (9 cm in 48 h). The sporangiospores of URM 6911 are mostly subglobose or subglobose to ellipsoid, differing from URM 6910 that are mostly ellipsoid. Colonies in the former are gray to deep grey, differing from the latter that is light grey. Plates from mating experiments were analyzed at 5, 7, 14, 21, 28 and 35 d and zygosporangia were not observed.

Influence of light: not detected.

Specimen examined: BRAZIL, Pernambuco, Araripina, Instituto Agronômico de Pernambuco (IPA), $7^{\circ}27'58''\text{S}$ and $40^{\circ}24'53''\text{W}$. Soil, 09. 2009, R.J. Oliveira. Holotype (URM 6910).

Other examined specimen. BRAZIL, João Pessoa, Mataraca, dune areas of the Millennium Inorganic Chemicals Mining, a Cristal Company, $6^{\circ}30'00''\text{S}$ and $34^{\circ}57'10''\text{W}$, Soil, 03. 2010, D.X. Lima, (URM 6911).

Etymology: Brasiliensis. Referring to country where the species was first isolated.

Habitat: Soil

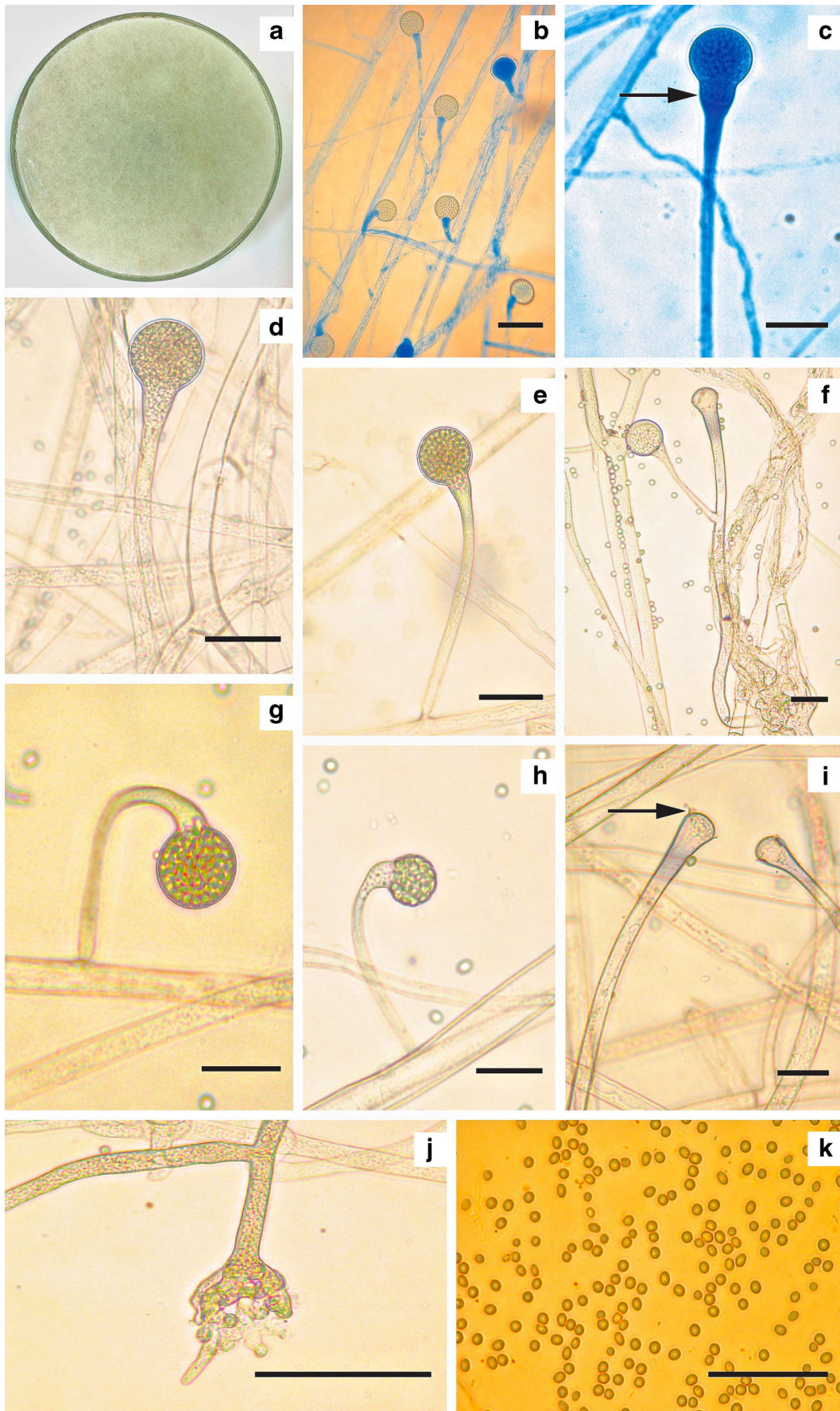
Distribution: Araripina (Pernambuco, Brazil) and Mataraca (Paraíba, Brazil).

Discussion

Several studies have reported the variability of nucleotide sequences of the ITS region (Iwen et al. 2002; Schwarz et al. 2006; Hoffmann et al. 2009) and LSU rDNA (Álvarez et al. 2011; de Souza et al. 2012) as reliable indication of taxon differentiation in Mucorales. According to our phylogenetic and morphophysiological analysis, *L. brasiliensis* is genetically distinct from other described species within the genus. Evidences from the analyzed datasets support the delimitation of a new species. The analysis of either the 5.8S region or the LSU rDNA showed the two sequenced specimens grouping strongly in the family Lichtheimiaceae, in the genus *Lichtheimia* and close to *Dichotomocladium*. Topology and statistical support of the phylogenetic trees, as well as genetic differences (maximum identity) between the studied specimens and representatives in Lichtheimiaceae confirm the proposal of a new species for this family.

Morphologically, *L. brasiliensis* resembles the described species of *Lichtheimia* as it presents erect or circinate sporophores emerging from aerial hyphae or from stolons, occasionally with a septum below the sporangium, besides producing apophyses sporangia that are common characteristics of the genus *Lichtheimia* (Hoffmann et al. 2007, 2009). However, the presence of short and very short hemispheric columellae that are never spatulate and lack projections, besides the absence of giant cells in the mycelium also differentiate *L. brasiliensis* from the described *Lichtheimia* species. Some bell shaped apophyses, sometimes very dilatated can eventually be observed in *L. brasiliensis*.

According to Santos et al. (2003) and Hoffmann et al. (2007, 2009), production of giant cells is considered an important morphological characteristic in *Lichtheimia*. However, giant cells are not produced by all isolates of this genus. Alastruey-Izquierdo et al. (2010) cited that on MEA, at 24 °C, many samples (not all) of all known species of *Lichtheimia* developed giant cells. These structures were also common on PDA, with higher degree of differentiation at 24 °C and lower degree of differentiation at 33 and at 37 °C. Hoffmann et al. (2007) reported the presence of giant cells as an important characteristic together with thermotolerance to include *L. hyalospora* in the family Mycocladiaceae K. Hoffmann, S. Discher & K. Voigt (= Lichtheimiaceae), as the zygosporangia in this species were never observed. These structures were cited by Ellis and Hesselstine (1966) to *L. ramosa* (cited as *A. ramosa* [Lindt] Lendn) and to *L. corymbifera* (cited as *A. corymbifera* [Cohn] Sacc. & Trotter). In the former species, the giant cells were common on PDA and absent on Synthetic Mucor Agar (SMA) and, to the latter, giant cells were common on SMA and PDA. Hesselstine and Ellis (1966) cited the presence of giant cells in *L. hyalospora* (cited as *A. hyalospora* [Saito] Lendn. and as *A. blakesleeana* Lendn.). In this latter species, giant cells were observed in cultures grown on SMA and MEA. Alastruey-



◀ **Fig. 3** *Lichtheimia brasiliensis*. **a** Colony surface; **b** Sporophores and sporangia; **c** Simple sporangiophore with a bell-shaped apophysis (*arrow*) under the sporangium; **d**, **e** Simple sporangiophore with a sporangium; **f** Branched sporangiophore with sporangium and columella; **g**, **h** Circinate sporangiophore; **i** Two sporangiophores with short hemispherical columellae and collars (*arrow*); **j** Rhizoids; **k** Sporangiospores. Bars: B, C, D, E, K=50 μm ; F, G, H, I=25 μm ; J=100 μm

Izquierdo et al. (2010) observed the presence of giant cells in *L. sphaerocystis* and in *L. ornata* on MEA and yeast extract agar (YEA), respectively, and used the globose form of these structures, associated with molecular information, to separate *L. sphaerocystis* from the other species within the genus. Thus, as some isolates of *Lichtheimia* do not produce giant cells, the total absence of these structures in *L. brasiliensis*, either on MEA or PDA, at the tested temperatures, cannot be seen as an indication to exclude it from *Lichtheimia*.

The presence of projections in the columellae has been cited to all known species of *Lichtheimia*. Hesseltine and Ellis (1966) reported projection up to 3.5 μm diam. in columellae of *L. hyalospora*, while Ellis and Hesseltine (1966) observed that columellae of *L. ramosa* and *L. corymbifera* presented, frequently, one to several projections of 2.5 and 4.5 μm length, respectively. Alastruey-Izquierdo et al. (2010) cited occasional production of projections of 8.5–29 μm length in *L. sphaerocystis*. As these structures were not observed in *L. brasiliensis*, the absence can be considered as distinctive characteristic between our isolate and the other *Lichtheimia* species. Likewise, globose, elliptical, elongated and spatulate columellae, besides the hemispheric, were described for *Lichtheimia* species. In *L. brasiliensis* only hemispheric (short and very short) columellae and few subglobose (never spatulate) were observed. Moreover, some columellae are so small that they seem inconspicuous, differently from the observed in *Lichtheimia* species so far.

Besides the presence of giant cells, growth temperature has been an important physiological characteristic to separate *Lichtheimia* species from other “*Absidia*-like” species. According to Hoffmann et al. (2007), growth capacity at 37 °C, or at temperatures above this, distinguish species included in the group of thermolerant (*Lichtheimia*) from the mesophilic (*Absidia*) group, being permissive to the former and suppressive to the latter group. These authors showed that *L. hyalospora* (as *A. hyalospora* and *A. blakesleeana*), and *L. corymbifera* (as *A. corymbifera*) presented optimum growth temperatures between 37 and 45 °C, and were capable of growing at 42, 50 and 53 °C, respectively. However, Alastruey-Izquierdo et al. (2010) observed that specimens of *L. sphaerocystis* presented optimum growth temperatures on MEA at 33 °C and, among the three studied specimens, two presented maximum growth temperatures at 37 °C and only one was capable of growing at 40 °C. Regarding *L. brasiliensis*, the two specimens studied in this work were able to grow at 37 °C, with optimum growth temperature at 30–

35 °C, and very limited growth at 40 °C. The growth temperatures observed to *L. sphaerocystis* and *L. brasiliensis* indicate that these species of *Lichtheimia* do not exhibit strict thermotolerance.

In conclusion, none of the described species of *Lichtheimia* have all the characteristics as found in *L. brasiliensis*, therefore it is described as new. Although both specimens of *L. brasiliensis* have been grouped in a subclade slightly distant from the other species of the genus, when considering the two phylogenetic trees shown (5.8S region and the LSU rDNA), we did not find morphological characteristics sufficiently strong to propose a new genus.

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