Acta Botanica Brasilica, 2023, 37: e20230061
doi: https://doi.org/10.1590/1677-941X-ABB-2023-0061

Original article

Discovery of *Backusella paraconstricta* sp. nov. (Mucorales, Mucoromycota) in an upland forest in northeastern Brazil with an identification key for *Backusella* from the Americas

Francisca Robervania Soares dos Santos[1], Thalline Rafhaella Leite Cordeiro[1], Catarina Letícia Ferreira de Lima[1], Maria Alice Barbosa dos Santos[1], Leslie Waren Silva de Freitas[1], Eleni Gentekaki[2, 3], Luciana Melo Sartori Gurgel[4], Cristina Maria de Souza-Motta[1], Hyang Burm Lee[5*] and André Luiz Cabral Monteiro de Azevedo Santiago[1*]

Received: March 16, 2023
Accepted: June 30, 2023

ABSTRACT

During a survey of mucoralean fungi in soil from an upland forest area located in Pernambuco, Brazil, a strain of *Backusella* (URM 8637) was isolated. Based on morphological, physiological, and molecular data [internal transcribed spacer (ITS) and large subunit (LSU) ribosomal DNA regions], it was recognized that this *Backusella* differed from all other species in the genus. Morphologically, the new species is characterized as forming varied-shaped columellae, including elongated, basally constricted, unisporate (rare) and multisporate sporangiola, and ellipsoidal sporangiospores. The maximum temperature growth of URM 8637 on malt extract agar and potato dextrose agar was 36 °C. In the phylogram, it was closely related to *B. constricta*. Based on the evidence from the analyzed datasets, a new species of *Backusella* is proposed. An updated identification key for *Backusella* from the Americas is provided.

Keywords: Backusellaceae, ITS and LSU rDNA, Soil, Taxonomy, New species.

Introduction

The genus *Backusella* was established by Ellis and Hesseltine in 1969 and typified with *B. circina* J.J. Ellis and Hesselt. Members of this genus can be found on various substrates, including excrement, invertebrates, leaf litter, soil, toads, and wood (Benny & Benjamin 1975; Walther *et al*. 2013; Lima *et al*. 2016; Nguyen & Lee 2018; Nguyen *et al*. 2021; Urquhart *et al*. 2021). *Backusella* belongs to Backusellaceae K. Voigt & P. M. Kirk, however it was previously associated with Mucoraceae Dumort.

1 Programa de Pós-Graduação em Biologia de Fungos, Departamento de Micologia, Universidade Federal de Pernambuco, 50670-420, Recife, Pernambuco, Brasil.
2 Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand.
3 School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand.
4 Instituto Agronômico de Pernambuco, 50761-000, Recife, Pernambuco, Brasil.
5 Environmental Microbiology Lab, Dept. of Agricultural Biological Chemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, Korea.

* Corresponding authors: andrelcabral@msn.com/hblee@chonnam.ac.kr

Species of this genus were later transferred to Thamnidiaceae Fitzp. due to morphological similarities with *Thamnidium* Link (Pidoplichko & Milko 1971; Wanasinghe *et al*. 2018). Species of *Backusella* are known to occur in Australia, Brazil, China, Japan, South Korea, the United States of America, and Thailand (Zheng *et al*. 2013; Lima *et al*. 2016; Nguyen *et al*. 2021; Urquhart *et al*. 2021; Hurdeal *et al*. 2022; de Lima *et al*. 2022).

Species of *Backusella* form sporophores that are transiently curved when young, and erect when mature. They may or not form laterally unispored and multispored pedicellate sporangiola, which have persistent walls. Short, simple, or sympodially branched sporangiophores proliferating only multispored and/or unispored sporangiola may form near the substrate (Benny 2005; Walther *et al*. 2013; de Souza *et al*. 2014). Based on the recognition of transiently curved sporophores as a distinctive feature of *Backusella*, Walther *et al*. (2013) transferred some species from *Mucor* to *Backusella*. As of January 2023, 34 species have been accepted in *Backusella* (Wijayawardene *et al*. 2022; Hurdeal *et al*. 2022; de Lima *et al*. 2022; Cordeiro *et al*. 2023).

During a study on the diversity of mucoralean fungi in an upland forest area in Pernambuco, northeastern Brazil, a strain of *Backusella* was found. Its identity was confirmed using morphological and molecular data, which included internal transcribed spacer (ITS) and large subunit (LSU) of ribosomal DNA (rDNA). In this work we describe and illustrate this new species. This is not the first new species of *Backusella* discovered in Brazil; *Backusella azygospora*, *B. brasiliensis*, *B. constricta*, *B. obliqua*, and *B. pernambucensis* were previously isolated and described for the first time in this country. This indicates that Brazil is a tropical hotspot for discovering new mucoralean fungi (de Lima *et al*. 2022). In this study, we updated the identification key of *Backusella* from the Americas provided by de Lima *et al*. (2022) with two additional species.

Materials and methods

Collection site

Soil samples were collected in April 2022 in the district of Jenipapo, municipality of Sanharó (8°17'08.6" S 36°30'53.9" W), located in the state of Pernambuco, Brazil. The local vegetation comprises of subdeciduous and deciduous forests. The climate is tropical and rainy with dry summers. The rainy season starts in January/February and ends in September, but it can continue until October. The average annual temperature is 31 °C, with an average annual rainfall of 496 mm (MME 2005). Using sterilized spatulas, soil samples were collected at a depth of 5 cm, packed in sterile plastic bags and stored in styrofoam boxes with ice for transport to the Laboratory of Fungos Zigospóricos at the Universidade Federal de Pernambuco (UFPE).

Isolation, purification and morphological description

Five milligrams of soil was added to wheat germ agar culture medium (Benny 2008), supplemented with chloramphenicol (80 mg L^{-1}), contained in Petri plates. Colony growth was monitored for 72 h at room temperature (26 ± 2 °C). Mycelial fragments were removed directly from the Petri dishes under a Leica EZ4 stereomicroscope (Leica Microsystems, Wetzlar, Germany) and transferred to malt extract agar (MEA) plates (Benny 2008). At least 50 measurements were made for each fungal structure from plates incubated at 25 °C for seven days on MEA in the dark. Mycelial fragments from the specimens were transferred to slides with 2% KOH or lactophenol blue and observed using a light microscope (Leica DM500). A slide corresponding to the holotype of the new species (URM 8367) was deposited in the Herbarium URM, and the ex-type living culture of the new species (URM 8367) was deposited in the URM Culture Collection of the Universidade Federal de Pernambuco.

Growth experiments and macro and microscopy

URM 8637 was grown in triplicate on both MEA and potato dextrose agar (PDA; HiMedia, Vadhani, India) and incubated at 15, 20, 25, 30, 35, and 40 °C in the dark for morphological analysis. Colony growth was measured every 24 h and monitored for 10 d. The maximum growth temperature was determined by growing the strains on MEA at one degree increments. For morphological observation, culture slides (with fragments of the fungal mycelia) were prepared, stained with 2% KOH or lactophenol blue, and observed using a light microscope (Leica DM500). Colony color was determined according to Kornerup and Wanscher (1978).

DNA extraction, amplification, purification, and sequencing

Fungal biomass was obtained from MEA slant cultures incubated at 28 °C for up to five days and was transferred to 2-mL microtubes with screw caps. To each tube, 0.5 g of acid-washed glass beads (Sigma-Aldrich, Darmstadt, Germany) of two different diameters (150–212 µm and 425–600 µm, 1:1) were added and the fungal biomass was crushed by stirring at high speed in a FastPrep homogenizer (FastPrep-24, MP Biomedicals, California, USA). Genomic DNA was extracted as described by de Oliveira *et al*. (2016), whereby the mycelium was homogenized in CTAB lysis buffer [2% cetyltrimethylammonium bromide, 20 mM EDTA, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl (Doyle & Doyle 1987; 1990)], and washed with chloroform: isoamyl alcohol (24:1). The DNA-containing supernatant was then separated from the hyphal residues. The supernatant was 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 resulting DNA pellet was washed with 70% ethanol and resuspended in 50 µL ultrapure water.

For the amplification of ITS and LSU rDNA, the primer pairs ITS1/ITS4 and LR1/LSU2 (White *et al*. 1990; van Tuinen *et al*. 1998; Santiago *et al*. 2014) were used, respectively. The final amplicons were purified with the NucleoSAP enzymatic mix (Molecular Biotecnologia, Belo Horizonte, Brazil) and sequenced at Plataforma de Tecnologia Genômica e Expressão Gênica of the Centro de Ciências Biológicas - UFPE (Pernambuco, Brazil).

Sequence alignment and phylogenetic analysis

The sequences of the URM 8637 strain were used as queries to perform BLASTn in the GenBank database to identify the closest matching sequences. Raw reads were edited to remove ambiguous bases at both ends. Two separate datasets, one for ITS and one for LSU, were assembled using sequences of all available described species in the database. The datasets were aligned using MAFFT v.7 (https://mafft.cbrc.jp/alignment/server) (Katoh & Standley 2013) for each molecular marker. The sequences were manually edited using MEGA version 7 (Kumar *et al*. 2016). The ITS and LSU rDNA region alignments were concatenated before the phylogenetic analyses (Supplementary Material 01). Bayesian inference (BI) and maximum likelihood (ML) analyses were performed with MrBayes v.3.2.2 (Ronquist *et al*. 2012) on XSEDE and RAxML-HPC BlackBox v.8.2.8 (Stamatakis *et al*. 2008; Stamatakis 2014), respectively, using the CIPRES Science Gateway (http://www.phylo.org/) (Miller *et al*. 2010). The ML analysis was performed using the GTR+I+G standard nucleotide substitution model, and BI was performed using the best nucleotide model selected by AIC in MrModeltest 2.3 (Nylander 2004). Bayesian inference analysis was conducted using 1×10^6 generations with a tree burn-in value of 25%. Phylogenetic trees were viewed and arranged using the Interactive Tree of Life (iTOL) v4 (https://itol.embl.de/) (Letunic & Bork 2019). Values less than 0.95 BI posterior probability and 70% ML bootstrap were not considered. The newly obtained sequences were deposited in the GenBank database. GenBank accession numbers are listed in Tab. 1.

Table 1. Specimens used in the phylogenetic analyses with their GenBank accession numbers.

Species	Strain number	GenBank accession No.		References
		ITS	LSU	
Backusella australiensis	UoMAU34^T	MK959062	MK958800	Urquhart *et al*. (2021)
Backusella australiensis	UoMAU90	MK959064	MK958797	Urquhart *et al*. (2021)
Backusella azygospora	URM 8065^T	MK625216	MK625222	Crous *et al*. (2019)
Backusella brasiliensis	URM 8395^T	OM458082	OM458083	de Lima *et al*. (2022)
Backusella circina	CBS 128.70^T	JN206258	JN206529	Ellis and Hesseltine (1969)
Backusella circina	CBS 129.70	JN206257	MH871299	Walther *et al*. (2013)
Backusella chlamydospora	CNUFC PS1^T	MZ171385	MZ148709	Nguyen *et al*. (2021)
Backusella chlamydospora	CNUFC HL7	MZ171386	MZ148710	Nguyen *et al*. (2021)
Backusella constricta	URM 7322 RV05	KT937157	-	Lima *et al*. (2016)
Backusella constricta	URM 7322 RV06	KT937158	-	Lima *et al*. (2016)
Backusella constricta	URM 7322 RV07	KT937159	-	Lima *et al*. (2016)
Backusella constricta	**URM 8701**	**OQ354764**	**-**	**This study**
Backusella paraconstricta	**URM 8637^T**	**OQ625517**	**OQ625516**	**This study**
Backusella dispersa	CBS 107.09^T	JN206269	MH866118	Urquhart *et al*. (2021)
Backusella dispersa	CBS 195.28	JN206271	JN206530	Urquhart *et al*. (2021)
Backusella gigacellularis	CCIBt 3866^T	KF742415	-	de Souza *et al*. (2014)
Backusella gigaspora	CBS 538.80^T	HM999964	HM849692	Cordeiro *et al*. (2023)
Backusella 'group X'	UoMAU121	MK959103	MK958792	Urquhart *et al*. (2021)
Backusella 'group X'	UoMAU152	MK959102	MK958791	Urquhart *et al*. (2021)
Backusella indica	CBS 786.70	JN206255	MH871743	Walther *et al*. (2013)
Backusella koreana	CNUFC CM05^T	MZ171387	MZ148711	Nguyen *et al*. (2021)
Backusella koreana	CNUFC CM06	MZ171388	MZ148712	Nguyen *et al*. (2021)

Table 1. Cont.

Species	Strain number	GenBank accession No.		References
		ITS	LSU	
Backusella lamprospora	CBS 118.08 T	NR_145291	NG_058650	Benny and Benjamin (1975)
Backusella liffmaniae	UoMAU58 T	MK959065	MK958734	Urquhart *et al.* (2021)
Backusella liffmaniae	UoMAU128	-	MK958735	Urquhart *et al.* (2021)
Backusella locustae	EML-SFB2 T	KY449291	KY449292	Wanasinghe *et al.* (2018)
Backusella locustae	EML-SFB4	KY449293	KY449290	Wanasinghe *et al.* (2018)
Backusella luteola	UoMAU6 T	MK959058	MK958795	Urquhart *et al.* (2021)
Backusella luteola	UoMAU36	-	MK958794	Urquhart *et al.* (2021)
Backusella macrospora	UoMAU7 T	MK959107	MK958628	Urquhart *et al.* (2021)
Backusella macrospora	UoMAU54	-	MK958629	Urquhart *et al.* (2021)
Backusella mclennaniae	UoMAU11	MK959077	MK958776	Urquhart *et al.* (2021)
Backusella mclennaniae	UoMAU12 T	MK959087	MK958777	Urquhart *et al.* (2021)
Backusella morwellensis	UoMAU14	-	MK958806	Urquhart *et al.* (2021)
Backusella morwellensis	UoMAU16 T	MK959059	MK958808	Urquhart *et al.* (2021)
Backusella obliqua	URM 8427 T	ON858475	ON858467	de Lima *et al.* (2022)
Backusella oblongielliptica	CBS 568.70 LT	JN206278	JN206533	Walther *et al.* (2013)
Backusella oblongielliptica	CNUFC IL02	MZ171391	MZ148715	Nguyen *et al.* (2021)
Backusella oblongispora	CBS 569.70 T	JN206251	JN206407	Walther *et al.* (2013)
Backusella oblongispora	CNUFC TKB11	MZ420786	MZ148717	Nguyen *et al.* (2021)
Backusella parvicylindrica	UoMAU35 T	MK959109	MK958727	Urquhart *et al.* (2021)
Backusella parvicylindrica	UoMAU39	-	MK958728	Urquhart *et al.* (2021)
Backusella pernambucensis	URM 7647 T	OP339860	OP339863	Cordeiro *et al.* (2023)
Backusella pernambucensis	URM 7648	OP339861	OP339864	Cordeiro *et al.* (2023)
Backusella psycrophilia	UoMAU26	-	MK958748	Urquhart *et al.* (2021)
Backusella psycrophilia	UoMAU55 T	MK959093	MK958749	Urquhart *et al.* (2021)
Backusella recurva	CBS 196.71	JN206265	JN206523	Walther *et al.* (2013)
Backusella recurva	CBS 317.52	JN206262	MH868593	Walther *et al.* (2013)
Backusella recurva	CBS 318.52 ET	JN206261	JN206522	Walther *et al.* (2013)
Backusella solicola	MFLUCC 22-0067 T	ON899832	ON892503	Hurdeal *et al.* (2022)
Backusella tarrabulga	UoMAU5 T	MK959060	MK958804	Urquhart *et al.* (2021)
Backusella tarrabulga	UoMAU187	-	MK958805	Urquhart *et al.* (2021)
Backusella thermophila	CNUFC CS02 T	MZ171389	MZ148713	Nguyen *et al.* (2021)
Backusella thermophila	CNUFC CS03	MZ171390	MZ148714	Nguyen *et al.* (2021)
Backusella tuberculispora	CBS 562.66 LT	JN206267	JN206525	Walther *et al.* (2013)
Backusella tuberculispora	CBS 570.70	JN206266	MH871631	Walther *et al.* (2013)
Backusella variabilis	CBS 186.87 T of *B. grandis*	JN206252	JN206527	Walther *et al.* (2013)
Backusella variabilis	CBS 564.66 LT	JN206254	JN206528	Walther *et al.* (2013)
Backusella westeae	UoMAU4 T	MK959061	MK958796	Urquhart *et al.* (2021)
Mucor indicus	CBS 226.29 ET	NR_077173	NG_057878	Walther *et al.* (2013)

Bold letters indicate the strains obtained in this study. CBS culture collection of the Westerdijk Fungal Biodiversity Institute, The Netherlands; CNUFC Chonnam National University Fungal Collection, Gwangju, South Korea; UoMAU National Herbarium of Victoria, Australia; MFLUCC Mae Fah Luang University Culture Collection. Ex-type, ex-epitype, and ex-lectotype strains are marked with T, ET, and LT, respectively.

Results

Phylogenetic analyses

Phylogenetic relationships within *Backusella* were estimated using BI and ML approaches. The alignment of ITS and LSU consisted of 60 sequences and 1699 characters with 1058 and 641 characters used in the ITS and LSU, respectively. Phylogenetic analysis identified the isolate as a new species that was sister to *B. constricta* with statistical support (94%ML/1.00PP). The topology of the concatenated tree, as well as the ML bootstrap values and BI posterior probabilities (>70% and >0.95, respectively) are shown in Fig. 1.

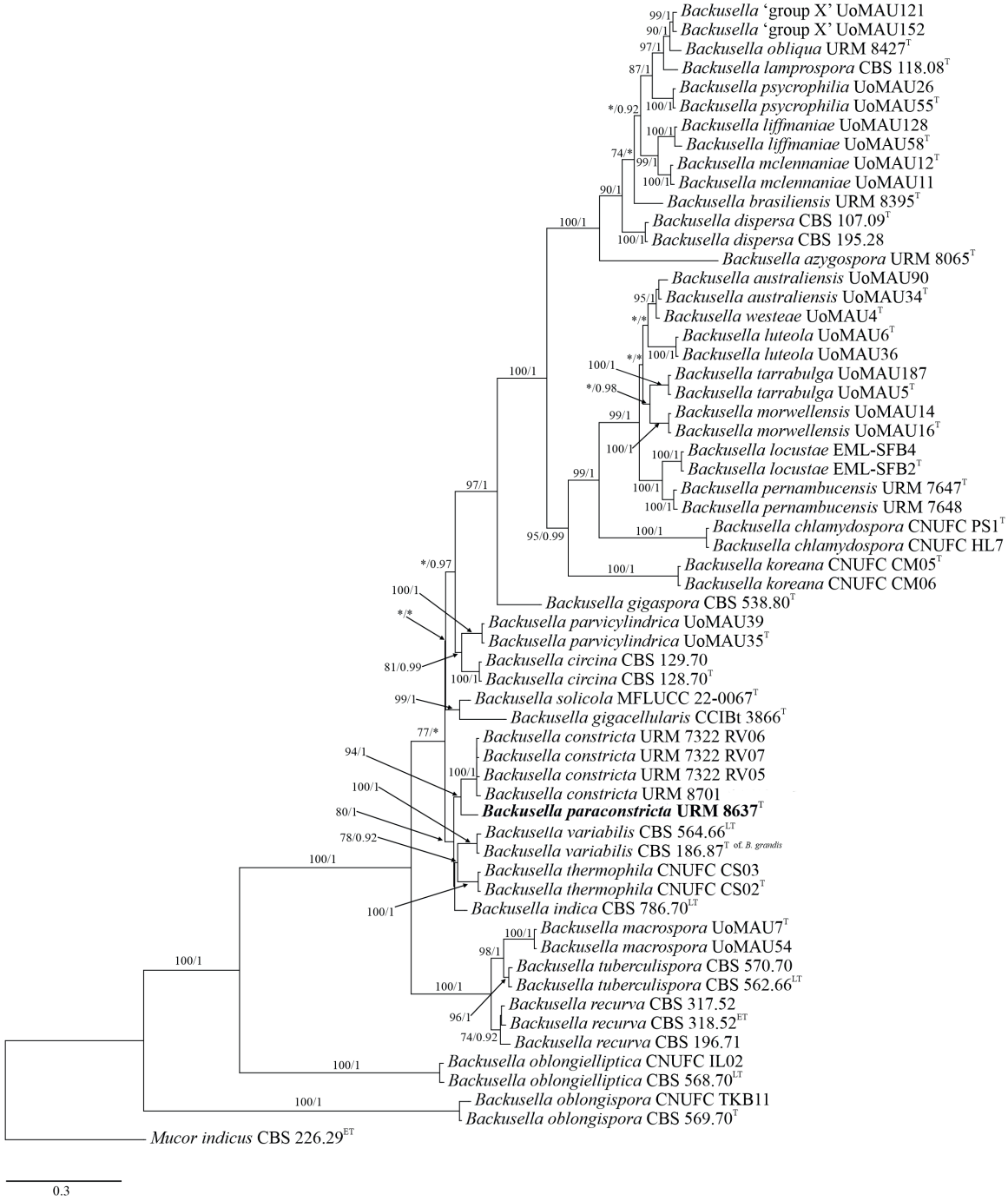

Figure 1. Phylogenetic tree of *Backusella* inferred from the combined internal transcribed spacer (ITS) and large subunit (LSU) ribosomal DNA (rDNA) sequences. Support values on the branches represent maximum likelihood bootstrap support and Bayesian inference posterior probabilities in this order. Bootstrap values lower than 70% or 0.95 are marked with "*". New taxa are in bold font. *Mucor indicus* CBS 226.29 was used as outgroup. Ex-type, ex-epitype, and ex-lectotype strains are marked with T, ET, and LT, respectively.

Taxonomy

Backusella paraconstricta F.R.S. Santos, T.R.L. Cordeiro, Hyang B. Lee & A.L. Santiago **sp. nov.** – Fig. 2

MycoBank number: 847766

Colonies rapidly growing, initially white, becoming pale to gray (1–1 B), reaching the entire Petri dish (9 cm diameter and 1.5 cm high) after 4 days at 25 °C on MEA; reverse yellow (2–8A). Rhizoids well branched. Long sporophores hyaline, curved when young and erect at maturity, frequently simple, infrequently sympodially branched up to three times (rarely four times), with rarely recurved branches, up to 12 µm in diameter. Lateral pedicellate sporangia not formed on sporophores. Sporangia brownish-yellow, globose, smooth-walled with vitreous aspect, 30–70 µm in diameter. Columellae of sporangia light grey, conical, subglobose, infrequently applanate and very rarely elongate to ellipsoid or cylindrical, 20–35 × 15–35 µm, smooth-walled. Some columellae may rarely show a slight constriction at the base. Short sporophores, unbranched, or less commonly sympodially branched (up to three times), bearing only multispored (frequent) and/or unispored sporangiola (rare) formed near the substrate. Multispored sporangiola brownish, globose, 15–40 µm in diameter, containing 3–15 sporangiospores each, persistent and spinulose-walled. Sporangiola unisporate globose, up to 20 µm in diameter, minutely spinulose. Columellae of sporangiola conical to flattened, subglobose, elongated, rarely globose, hyaline or grayish, 15–20 × 12–40 µm, smooth-walled. Collar evident. Sporangiospores hyaline, with greenish contents, ellipsoid (mostly), subglobose, some globose and irregular, 7–16 (–20) × 4.5–9.5 (–12) µm, smooth-walled. No chlamydospores or zygosporangia were observed.

Etymology: referring to the phylogenetic proximity to *Backusella constricta*.

Material examined: Brazil, Pernambuco; the district of Jenipapo, municipality of Sanharó (8°17'08.6" S 36°30'53.9" W), from soil, 12 Apr. 2022, F.R.S. Santos (Holotype URM 95258; ex-holotype URM 8637). GenBank accessions: OQ625517 (ITS) and OQ625516 (LSU).

Habitat: Soil.

Distribution: Pernambuco state (Brazil).

Media and temperature test: On MEA, at 10 °C – no growth; at 15 °C – slow growth (6 cm in diameter after 168 h); at 20 °C – good growth (8 cm in diameter after 120 h); at 25 °C – excellent growth (9 cm in diameter after 96 h); at 30 °C – good growth (9 cm in diameter after 144 h); at 35 °C – slow growth (9 cm in diameter after 192 h); at 40 °C – no growth. *Backusella paraconstricta* exhibited similar growth and development of reproductive structures on MEA and PDA culture media. Maximum temperature growth on both MEA and PDA was 36 °C.

Identification key for ***Backusella*** species in the Americas

1. Sporangiola formed 2
1. Sporangiola not formed ..... *B. oblongielliptica*
2. Unispored sporangiola abundant ..... *B. circina*
2. Unispored sporangiola rare or not formed 3
3. Giant cells formed 4
3. Giant cells not formed 6
4. Columellae of sporangia mostly hemispherical, some applanate, or subglobose; some rhizoids arising from sporophores and surrounding sporangium entangled ..... *B. pernambucencis*
4. Columellae of sporangia not hemispherical; rhizoids never surrounding the sporangium entangled 5
5. Columellae of sporangia ellipsoidal, cylindrical, rarely pyriform; chlamydospores absent ..... *B. gigacellularis*
5. Columellae of sporangia conical (majority), but ellipsoidal with a truncate base, globose to subglobose, subglobose to conical, or rarely conical or cylindrical with slight constriction at the center; chlamydospores abundant ..... *B. brasiliensis*
6. Azygospores formed ..... *B. azygospora*
6. Azygospores not formed 7
7. Sporangiospores elipsoidal or mostly ellipsoidal 8
7. Sporangiospores not ellipsoidal 10
8. Sporangia never extending 70 µm in diameter ..... ***B. paraconstricta***
8. Sporangia commonly extending 70 µm in diameter 9

9. Sporangia up to 150 (–200) µm diam.; sporangiospores 20–26 × 10–12 µm ..... *B. recurva*

9. Sporangia up to 100–125 µm in diameter; sporangiospores 11–15 × 7–9 µm ..... *B. variabilis*

10. Sporangiospores with irregular polyhedral shape, with protrusions ..... *B. tuberculispora*

10. Sporangiospores with neither an irregular polyhedral shape nor protrusions 11

11. Sporophores forming a terminal sporangium and few lateral pedicellate sporangiola ..... *B. lamprospora*

11. Sporophores forming a terminal sporangium with no lateral pedicellate sporangiola 12

12. Columellae of sporangia with varied shapes, some arranged obliquely on sporangiophores, some with one side more swollen than the other; sporangiospores globose to sub-globose ..... *B. obliqua*

12. Columellae of sporangia conical and cylindrical, sometimes constricted at the center, never arranged obliquely on the sporangiophores or with one side more swollen than the other; sporangiospores subglobose to broadly ellipsoidal, some slightly irregular ..... *B. constricta*

Discussion

In this study, we describe the novel species *Backusella paraconstricta* URM 8637. Our ML and BI phylogenetic analyses demonstrated that this species is genetically distinct from all other species of *Backusella* and sister to *B. constricta* in the tree inferred using a concatenated ITS/LSU dataset. The new species belongs to a major clade containing also *B. variabilis*, *B. thermophila* and *B. indica*. Cordeiro *et al*. (2023) proposed that the maximum growth temperature represents a valuable taxonomic feature in *Backusella*, and that temperature plays an important role in the evolution of this genus. They identified seven *Backusella* spp. capable of growing at temperatures ≥36 °C, four of which (*B. constricta*, *B. variabilis*, *B. thermophila* and *B. indica*.) were placed in the same clade in the inferred phylogenies. Herein, we present a new species that grows at 36 °C and also belongs to this clade (Fig. 1). This further confirms that maximum growth temperature is a taxonomically relevant character in *Backusella*.

Morphologically, *B. paraconstricta* differs from *B. constricta* by forming sporangia up to 70 µm in diameter, whereas those of the latter reach 100 µm in diameter. *Backusella constricta* forms columellae that are conical (majority) or cylindrical, and slightly or strongly constricted in the center (Lima *et al*. 2016). *Backusella paraconstricta,* instead, forms columellae that are mostly conical, subglobose, infrequently applanate, and very rarely elongate to ellipsoid, cylindrical and with a slight constriction at the base. The new species predominantly forms ellipsoidal sporangiospores, although some are subglobose, globose, and irregular, whereas *B. constricta* only forms sporangiospores that are subglobose to ellipsoidal and slightly irregular. Cordeiro *et al*. (2023) observed that most species of *Backusella* form subglobose to broadly ellipsoidal sporangiospores, and that ellipsoidal sporangiospores occur in *Backusella* species that are in the deeper branches of the ITS and *RPB1* phylogenetic trees, namely *B. indica, B. oblongielliptica, B. oblongispora, B. parvicylindrica, B. recurva, B. thermophila,* and *B. variabilis*. This was also observed in our ITS/LSU phylogeny, which includes *B. paraconstricta*. Finally, *B. paraconstricta* can grow at temperatures up to 36 °C, whereas *B. constricta* can grow up to 39 °C.

In conclusion, our results demonstrate that *B. paraconstricta* is morphologically and genetically different from the other *Backusella* species described to date. Therefore, it was described as new. This study contributes to our knowledge of the distribution of mucoralen fungi.

Acknowledgements

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for scholarships awarded to Francisca dos Santos and Thalline Cordeiro, and the National Council for Scientific and Technological Development (CNPq) for the research grant provided to André Santiago. This research was supported by the 'Mucoromycotin Diversity in the different ecosystems of the Atlantic Forest of Pernambuco' project (FACEPE - APQ - 0842-2.12/14). It was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2022R1I1A3068645).

References

Benny GL. 2005. Zygomycetes. http://zygomycetes.org. 09 Jan. 2023.

Benny GL. 2008. The methods used by Dr. R. K. Benjamin, and other mycologists to isolate zygomycetes. Aliso 26: 37–61. doi: 10.5642/aliso.20082601.08

Benny GL, Benjamin RK. 1975. Observations on Thamnidiaceae (Mucorales). New taxa, new combinations, and notes on selected species. Aliso 8: 301–351. doi: 10.5642/aliso.19750803.10

Cordeiro TRL, Walther G, Souza CAF *et al*. 2023. A polyphasic approach to the taxonomy of *Backusella* reveals two new species. Mycological Progress 22: 16 doi: 10.1007/s11557-023-01864-x

Crous P, Carnegie AJ, Wingfield MJ, Sharma R, Mughini G, Noordeloos ME *et al*. 2019. Fungal Planet description sheets: 868–950. Persoonia 42: 291–473. doi: 10.3767/persoonia.2019.42.11

De Lima CLF, Lundgren JDAL, Nguyen TTT *et al*. 2022. Two new species of *Backusella* (Mucorales, Mucoromycota) from Soil in an Upland Forest

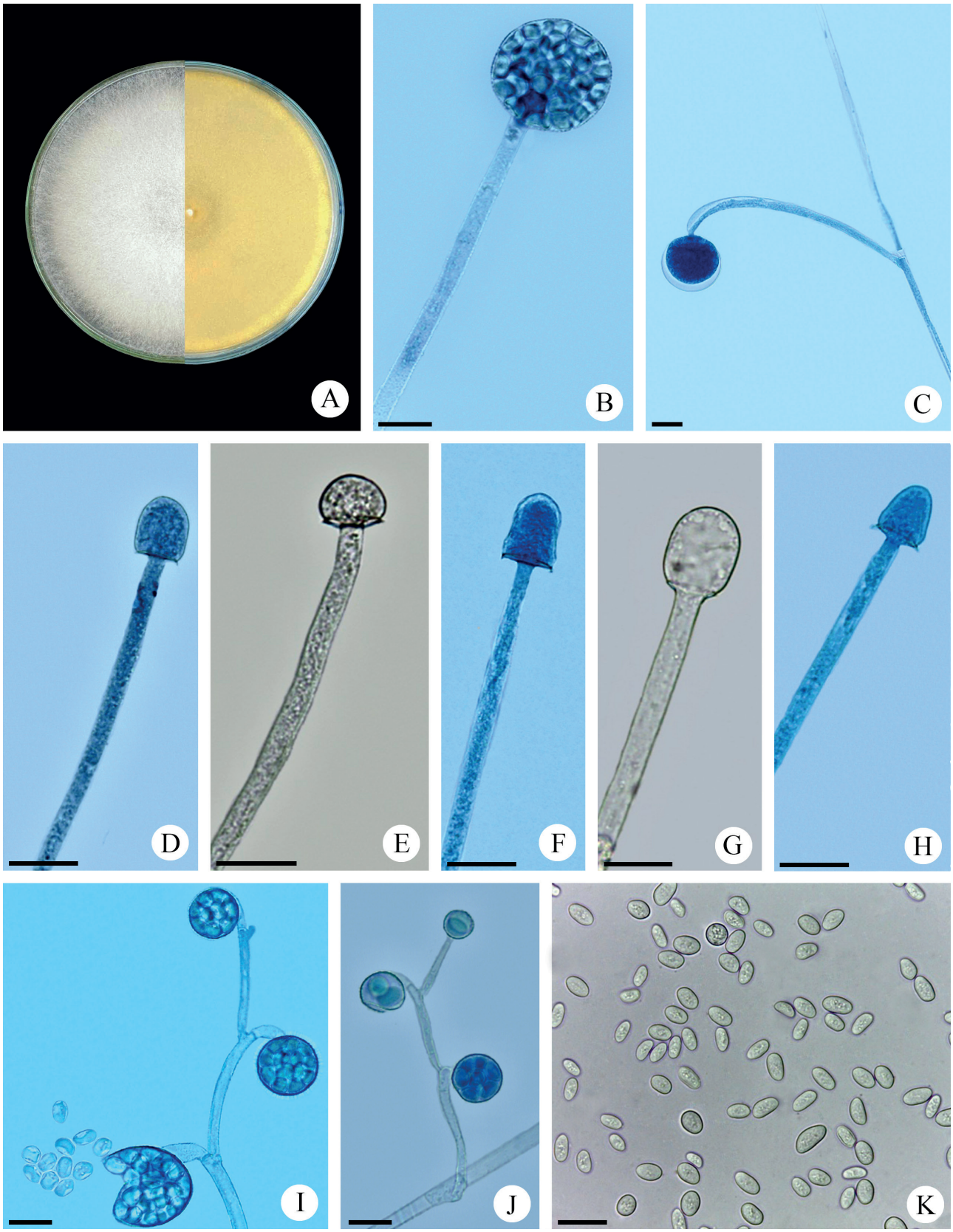

Figure 2. *Backusella paraconstricta* sp. nov. (URM 8637) **A.** Colony surface (left) and reverse (right) on malt extract agar (MEA) at 25 °C, **B.** sporophore with sporangium**, C.** sporophore branch with sporangium, **D–H.** sporophore with columella **I, J.** short sporophore with sporangiola, **K**. sporangiospores**.** Scale bars = 20 µm.

in Northeastern Brazil with an Identification Key of *Backusella* from the Americas. Journal of Fungi 8: 1038. doi: 10.3390/jof8101038

De Oliveira RJV, Bezerra JL, Lima TEF, Silva GA, Cavalcanti MAQ. 2016. *Phaeosphaeria nodulispora*, a new endophytic coelomycete isolated from tropical palm (*Cocos nucifera*) in Brazil. Nova Hedwigia 103: 185–192. doi: 10.1127/nova_hedwigia/2016/0343

de Souza JI, Marano AV, Pires-Zotarelli CLA, Chambergo FS, Harakava R. 2014. A new species of *Backusella* (Mucorales) from a Cerrado reserve in Southeast Brazil. Mycological Progress 13: 975–980. doi: 10.1007/s11557-014-0981-3

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Ellis JJ, Hesseltine CW. 1969. Two new members of the Mucorales. Mycologia 61: 863–872.

Hurdeal VG, Jones EG, Santiago ALCMA, Hyde KD, Gentekaki E. 2022. Expanding the diversity of mucoralean fungi from northern Thailand: A novel soil *Backusella* species. Phytotaxa 559: 275–284.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. doi: 10.1093/molbev/mst010

Kornerup A, Wanscher JH. 1978. Methuen handbook of colours. 3rd. edn. London, Eyre Methuen.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. doi: 10.1093/molbev/msw054%20

Letunic I, Bork P. 2019. Interactive Tree Of Life (iTOL) v4: Recent updates and new developments. Nucleic Acids Research 47: W256–W259. doi: 10.1093/nar/gkz239

Lima DX, Voigt K, de Souza CAF, Oliveira RJV, Souza-Motta CM, Santiago ALCMA. 2016. Description of *Backusella constricta* sp. nov. (Mucorales, ex Zygomycota) from the Brazilian Atlantic Rainforest, including a key to species of *Backusella*. Phytotaxa 289: 59–68. doi: 10.11646/phytotaxa.289.1.4

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans. p. 1–8. doi: 10.1109/GCE.2010.5676129

MME - Ministério das Minas e Energia. 2005. Projeto cadastro de fontes de abastecimento por água subterrânea. Diagnóstico do Município de Sanharó, Pernambuco. Companhia de Pesquisa de Recursos Minerais/Programa de Desenvolvimento Energético dos Estados e Municípios. Recife, CPRM/PRODEEM. https://rigeo.cprm.gov.br/jspui/bitstream/doc/16670/1/Rel_Sanhar%C3%B3.pdf. 24 Dec. 2021.

Nguyen TTT, Lee HB. 2018. Isolation and characterization of three zygomycetous fungi in Korea: *Backusella circina*, *Circinella muscae*, and *Mucor ramosissimus*. Mycobiology 46: 317–327 doi: 10.1080/12298093.2018.1538071

Nguyen TTT, Voigt K, Santiago ALCMA, Kirk PM, Lee HB. 2021. Discovery of novel *Backusella* (Backusellaceae, Mucorales) isolated from invertebrates and toads in Cheongyang, Korea. Journal of Fungi 7: 513. doi: 10.3390/jof7070513

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pidoplichko NM, Milko AA. 1971. Atlas of mucoralean fungi. Ukranian, Academic Science of the Ukranian SSR.

Ronquist F, Teslenko M, van der Mark P. *et al*. 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi: 10.1093/sysbio/sys029

Santiago ALCMA, Hoffmann K, Lima DX *et al*. 2014. A new species of *Lichtheimia* (Mucoromycotina, Mucorales) isolated from Brazilian soil. Mycological Progress 13: 343–352. doi: 10.1007/s11557- 013-0920-8

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. doi: 10.1093/bioinformatics/btu033

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for RAxML web-servers. Systematic Biology 57: 758–771. doi: 10.1080/10635150802429642

Urquhart AS, Douch JK, Heafield TA, Buddie A, Idnurm A. 2021. Diversity of *Backusella* (Mucoromycotina) in south-eastern Australia revealed through polyphasic taxonomy. Persoonia 46: 1–25. doi: 10.3767/persoonia.2021.46.01

van Tuinen D, Zhao B, Gianinazzi-Pearson V. 1998. PCR in studies of AM fungi: From primers to application. In: Varma AK (ed.) Mycorrhizal manual. Berlin, Springer. p. 387–399.

Walther G, Pawłowska J, Alastruey-Izquierdo A *et al*. 2013. DNA barcoding in Mucorales: an inventory of biodiversity. Persoonia-Molecular Phylogeny and Evolution of Fungi 30: 11–47.

Wanasinghe DN, Phukhamsakda C, Hyde KD *et al*. 2018. Fungal diversity notes 709–839: Taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Diversity 89: 1–236.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA 640 genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.) PCR protocols: A guide 641 to methods and applications. San Diego, Academic Press. p. 315–322.

Wijayawardene NN, Hyde K D, Dai DQ *et al*. 2022. Outline of fungi and fungus-like taxa –2021. Mycosphere 13: 53–453. doi: 10.5953/mycosphere/13/1/2

Zheng RY, Liu XY, Wang YN. 2013. Two taxa of the new record genus *Backusella* from China. Mycosystema 32: 330–341.