Article

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Expansion in the distribution of *Blumenavia baturitensis* Melanda, M.P. Martín & Baseia (Phallales) in Southeastern, Brazil

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ABSTRACT – (Expansion in the distribution of *Blumenavia baturitensis* Melanda, M.P. Martín & Baseia (Phallales) in Southeastern, Brazil). *Blumenavia baturitensis* recently described for the Ceará State, Northeast Brazil, is reported for the first time from Espírito Santo State, Southeastern, Brazil. We present a description, photographs of microscopical and macroscopic structures, phylogenetic analyses, and distribution map of this species. Its conservation status, according to the most recent IUCN categories and criteria, should be tentatively as "Endangered".
Keywords: Basidiomycota, Clathraceae, Phallales, taxonomy

RESUMO – (Expansão na distribuição de *Blumenavia baturitensis* Melanda, M.P. Martín & Baseia (Phallales) no Sudeste do Brasil). *Blumenavia baturitensis* recentemente descrita para o Estado do Ceará, Nordeste do Brasil, é reportada pela primeira vez para o Estado do Espírito Santo, na região Sudeste. Nós apresentamos descrição, fotografias de estruturas microscópicas e macroscópicas, análises filogenéticas e mapa de distribuição da espécie. Seu estado de conservação, de acordo com as categorias e critérios mais recentes da IUCN, deve ser provisoriamente como "Em Perigo".
Palavras-chave: Basidiomycota, Clathraceae, Phallales, taxonomia

Introduction

Blumenavia Möller (Basidiomycota: Phallales), belong to the Clathraceae family known as cage fungi (Pegler and Gomez 1994) or lattice stinkhorns (Miller and Miller 1988). Until recently, only four species were described for the genus, based exclusively on morphological characters: *Blumenavia angolensis* (Welwitsch and Currey) Dring (with distribution for Angola and São Tomé), *B. toribiotalpaensis* Vargas-Rodríguez (Mexico: Jalisco), *B. usambarensis* Hennings (Tanzania: Usambara) and *B. rhacodes* Möller (Brazil: Rio Grande do Sul and Santa Catarina), the type species of *Blumenavia*.

However, in an important taxonomic revision for the genus, Melanda et al. (2020) based on morphological and phylogenetic evidence, recognize seven species as currently accepted, and of these, three new species: *B. baturitensis* Melanda, M.P. Martín & Baseia (Brazil: Ceará), *B. crucis-hellenicae* G. Coelho, Sulzbacher, Grebenc & Cortez (Brazil: Paraná and Rio Grande do Sul), *B. heroica* Melanda, Baseia & M.P. Martín (Mexico: Veracruz).

Recently a new sample of *B. baturitensis* was collected in the State of Espírito Santo, Southeastern Brazil. Here we present this new record, with the morphological description and molecular data from the specimen, preliminary extinction risk assessment and provide additional information about its distribution in the Atlantic Forest.

Material and Methods

Sample and Morphological Studies – The material was collected in Santa Teresa city, which belongs to the central

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montane region of Espírito Santo State. This region is mainly composed of Montane Ombrophylous Forest, which is conditioned by a climate with a dry period (< 100 mm/month) of up to 60 days in winter (which generally does not produce a pronounced water deficit), and annual rainfall totals of 1,300 to 1,800 mm (Saiter et al. 2015). An only specimen was found at the Instituto Nacional da Mata Atlântica (INMA) in July/2019, inside the Park of Museu de Biologia Professor Mello Leitão (MBML). The specimen was photographed, dehydrated and incorporated at the MBML Herbarium. The identification of the species was based on the taxonomic revision of *Blumenavia* (Melanda et al. 2020). The colors of the structures follow Küppers (2002).

Molecular phylogenetic studies – DNA extraction was performed using Qiagen DNeasy Plant Mini Kit (Germantown, MD, USA), with the modification made in Melanda et al. (2020). FIREPol® Master Mix – 5X (Solis Biodyne, Estonia) was used for the Polymerase Chain Reaction (PCR) to amplified three DNA regions: internal transcribed spacer (ITS nrDNA; fungi barcode), nuclear ribosomal large subunit (LSU nrDNA), and mitochondrial ATPase subunit 6 (*ATP*6), with the primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), and ATP6-3/ATP6-4 (Kretzer and Bruns 1999), respectively. The amplifications were purified with ExoSAP-IT™ PCR Product Cleanup Reagent (USB Corporation, OH, USA), following the fabricant instructions. Sequencing of the purified PCR products was carried out at Macrogen in Seoul, South Korea.

The visualization of the sequences after sequencing was made in Geneious R6.1 (Biomatters Ltd.) and the necessary cuts were made. The new sequences were deposited in GenBank® (http://www.ncbi.nlm.nih.gov/) under the numbers presented in table 1. Table 1 also presents the other sequences used in this work, all retrieved from Melanda et al. (2020). *Clathrus* species were selected as outgroup (*C. archeri* (Berk.) Dring [CA005] and *C. chrysomycelinus* Möller [PDD75096]) following Melanda et al. (2020). For the phylogenetic analyses the new sequences generated were aligned manually in Seaview v.4 (Gouy et al. 2010) with the alignment of Melanda et al. (2020).

The result to determine the position of the new specimen collected was based on the concatenated matrix: ITS nrDNA+LSU nrDNA+*ATP*6 submitted by Bayesian, and maximum likelihood (ML) phylogenetic analyses. The Bayesian analysis was performed in MrBayes 3.2.7a (Ronquist et al. 2012), considering five partitions (ITS1/5.8S/ITS2/LSU nr DNA/*ATP*6) which were set to use specific substitution models determined in jModelTest 2v.1.6 (Darriba et al. 2012) under Akaike Informational Criterion (AIC) for

Table 1. GenBank accession numbers for the three regions analyzed of *Blumenavia* specimens and outgroup (*Clathrus*). With asterisk type specimens. In bold new sequences generated in this study.

Taxa	Voucher	Country	ITS nrDNA	LSU nrDNA	ATP6
B. angolensis	JD772	Sao Tome and Principe	-	KC128653	-
B. baturitensis	MBML 55484	Brazil	**OP805930**	**OP805614**	**OP869946**
B. baturitensis	UFRN-Fungos 2868*	Brazil	MG817725	MG817733	MH061924
B. baturitensis	UFRN-Fungos 1943*	Brazil	MG817726	MG817734	MH061925
B. crucis-hellenicae	ICN 177268*	Brazil	-	MG817727	MH061920
B. crucis-hellenicae	ICN 177269*	Brazil	MG817717	MG817728	MH061921
B. crucis-hellenicae	ICN 200594*	Brazil	LN875254	-	-
B. crucis-hellenicae	SMDB 18116*	Brazil	LN875253	MK958819	MK975457
B. heroica	XAL S. Chacón 5257a*	Mexico	MG817720	MG817731	-
B. heroica	XAL E. Gandara 1455*	Mexico	MG817721	-	-
B. heroica	XAL D. Jarvio 778*	Mexico	MG817722	-	-
B. rhacodes	ICN 176968*	Brazil	MG817718	MG817729	MH061922
B. rhacodes	ICN 177266*	Brazil	MG817719	MG817730	MH061923
B. toribiotalpaensis	IBUG 456*	Mexico	MG817723	-	-
B. toribiotalpaensis	BPI 870955*	Mexico	MG817724	MG817732	-
Clathrus archeri	C A005	Poland, Marwice	KP688380	KP688386	-
C. chrysomycelinus	PDD 75096	New Zealand	-	DQ218626	DQ218915

ITS1, 5.8S, ITS2 nrDNA, and under Bayesian information criterion (BIC) for LSU rDNA and *ATP*6. The selection of the criterion was made testing the phylogeny for each one and choosing the one with higher support values. Bayesian inferences were performed with two independent runs, each one beginning from random trees with four simultaneous independent chains, using 20 million Markov chain Monte Carlo (MCMC) generations, with trees sampled every 1000 generations. The first 5×10^3 sampled trees were discarded as burn-in, whereas the remaining ones (all sampled after the average standard deviation of split frequencies reached < 0.01) were used to reconstruct a 50% majority-rule consensus tree and to calculate Bayesian posterior probabilities (PP) of the clades. ML analyses were performed using RAxML v.8 software (Stamatakis 2014), combined with the rapid bootstrapping algorithm with 1,000 replicates, with the model GTRCAT to obtain the maximum likelihood bootstrap (MLbs). The jModelTest 2v.1.6, RAxML v8.2.X, and MrBayes 3.2.7a were run from the CIPRES Science Gateway (Miller et al. 2010). Alignment is available in Figshare (https://doi.org/10.6084/m9.figshare.21592251). Trees were visualized and rooted in FigTree version 1.4.4, and the final images are done in GNU Image Manipulation Program (GIMP) v. 2.10.32.

Distance matrices were generated based on ITS sequences with one representative of each species and two vouchers of *Blumenavia baturitensis* in the R environment. The dist.dna function of the *ape* package (Paradis and Schliep 2019) was used with default values. The distances were plotted in a heatmap using heatmapSpp function of *spider* package (Brown et al. 2012).

Conservation status – The preliminary extinction assessment risk was based on known records of the species for the MBML and UFRN herbaria. IUCN criteria (IUCN 2012, 2022) were used to assess the risk of global extinction of the species, with extent of occurrence (EOO) and area of occupation (AOO) calculated using the Geospatial Conservation Assessment Tool (GeoCat) (Bachman et al. 2011). The Quantum-GIS 3.24.0 software was used to draw the distribution map.

Results and discussion

Blumenavia baturitensis Melanda, M.P. Martín & Baseia, PLoS One 15(5): e0232467: 12 (2020).
Figure 1, 2

Figure 1. *Blumenavia baturitensis* Melanda, M.P. Martín & Baseia. a-c. Mature basidiome. d. Volva. e. Receptacle apex. Bars a-c = 5 cm; c-d = 2 cm.

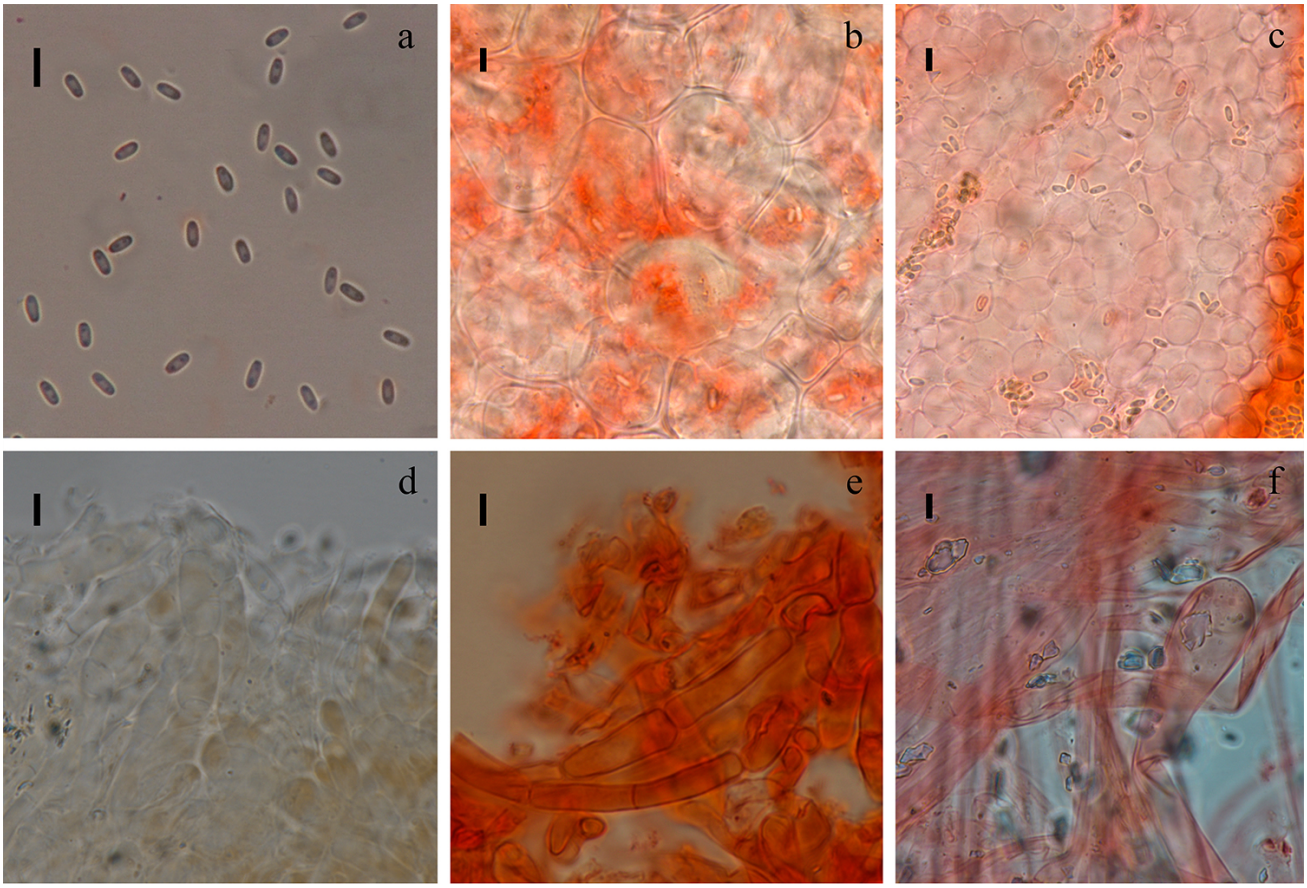

Figure 2. *Blumenavia baturitensis* Melanda, M.P. Martín & Baseia microscopical structures. a. Basidiospores in 5% KOH. b. Arms cells in 1% Congo red. c. Glebifer cells and basidiospores in 1% Congo red. d-e. Apical exoperidium hyphae; d. in 5% KOH, e. in 1% Congo red. f. Rhizomorphs hyphae in 1% Congo red. Bars = 5 µm.

Basidiome 10.9 cm high. Volva 3.3 cm high, brownish gray ($N_{60}Y_{00}M_{00}$–$N_{80}Y_{00}M_{00}$) in the upper half with whitish ($N_{00}C_{00}Y_{00}$–$N_{00}C_{00}Y_{10}$) base, composed of three layers, with single branched, white rhizomorph ($N_{00}M_{00}C_{00}$) attached. Receptacle with four robust arms that are free at the base and united at the apex, arms about 2 cm in diam. in the largest dimension, thinned from the middle to the top of the basidiomata, lack of a groove in the outer face of the arms, pale yellow color ($Y_{10}M_{00}C_{00}$), with a spongy texture and some larger pores and small perforations on the outer surface. Membranous glebifers adhered to the anterolateral angles of the arms, formed by triangular or irregular wrinkled, tentacled projections, regularly spaced in the upper half of the basidiomata, becoming smaller below. Glebifers covered with mucilaginous gleba, brown when fresh ($N_{90}C_{00}Y_{70}$). Basidiospores cylindrical, 3.1–4.2 × 1.4–1.9 [n = 30, $\overline{X}$ = 3.6 ± 0.2 × 1.7 ± 0.1, Q = 1.99–2.53, Qm = 2.19 ± 0.12] µm, smooth, with one inner guttule at each end of the length, hyaline in 5% KOH. Arms and glebifer exhibiting globose, subglobose to ellipsoid cells hyaline in 5% KOH with straight and regular walls: arms cells, 18–37.2 × 11.8–33.7 [n = 30, $\overline{X}$ = 26.8 ± 5.3 × 21.9 ± 4.6, Qm = 1.23 ± 0.16] µm, walls 0.4–1.1 [$\overline{X}$ = 0.7 ± 0.1] µm thick; glebifer cells, 7.8–14.3 × 5.4–10.9 [n = 30, $\overline{X}$ = 10 ± 1.7 × 8.2 ± 1.3, Qm = 1.23 ± 0.17] µm, walls 0.4–0.7 [$\overline{X}$ = 0.5 ± 0.1] µm thick. Apical exoperidium consisting of branched, septate filamentous hyphae, regularly spaced septa, without inflated tips and clamp connections, 3.1–7.5 [n = 30, $\overline{X}$ = 4.8 ± 1.1] µm broad, straight, and regular walls 0.2–0.6 [$\overline{X}$ = 0.3 ± 0.1] µm thick, hyaline in 5% KOH. Rhizomorphs consisting of branched, septate filamentous hyphae, irregularly spaced septa, with inflated tips, and without clamp connections, 2.8–10.1 [n = 30, $\overline{X}$ = 4.8 ± 1.9] µm broad, straight, and regular walls 0.2–0.8 [$\overline{X}$ = 0.4 ± 0.1] µm thick, hyaline in 5% KOH.

Material examined: Brazil. Espírito Santo: Santa Teresa. Instituto Nacional da Mata Atlântica, Parque do Museu de Biologia Professor Mello Leitão, 02 July 2019, *S. S. Recla 01* (MBML 55484, ITS nrDNA, LSU nrDNA and *ATP6* GenBank sequences: OP805930, OP805614, OP869946, respectively).

Distribution and habitat: In Brazil *B. baturitensis* has a known distribution for Northeast Brazil (Ceará State). This new record, found for the first time for the Southeast of Brazil, comprises 1753 km in a straight line from the record to the Ceará State (figure 3). This is the first record in a montane region in Southeastern Brazil (altitude of 650 m), the specimens from Northeast Brazil were also found at high altitudes, above 844m (Melanda et al. 2020).

Comments: Morphologically, *B. baturitensis* is similar to *B. heroica*, *B. rhacodes* and *B. toribiotalpaensis* by the whitish yellow arm color. The main differences between *B. baturitensis* and the other species is the lack of a groove in the outer face of the arms (vs. present in the others); differs also from *B. rhacodes* by the arms thinned from the middle to the top of the basidiomata (vs. arms with even thickness throughout its length in *B. rhacodes*) and the apical exoperidium composed only by filamentous hyphae (vs. filamentous and elongate hyphae in *B. rhacodes*).

Molecular phylogeny: The final aligned matrix has 2224 characters (604 for ITS nrDNA, 919 for LSU nrDNA and 701 for *ATP6*) to 17 specimens, with corresponds 14 ITS nrDNA, 13 LSU nrDNA and 9 *ATP*6 sequences (table 1).

Figure 3. Distribution of *Blumenavia baturitensis* Melanda, M.P. Martín & Baseia in Brazil. Black spots indicate known distribution. White dot indicates the new record for Santa Teresa, Espírito Santo State.

The evolutionary models selected for the final dataset were as follows: ITS1: TPM3;5.8S: TrNef; ITS2: TPM2uf+I; LSU nrDNA: TrN; *ATP*6: F81.

The concatenated tree is shown in Figure 4. Despite *Blumenavia rhacodes* are on a branch shared with *B. baturitensis* (UFRN-1943), the support of this branch is low (0.61 PP, 51 MLbs), which leads us to not confirm the exact location of this specimen of *B. baturitensis*. The other *B. baturitensis* specimen (UFRN-F 2868) as well as the collection of our study (MBML 55484) are placed in a major clade sharing ancestor with *B. rhacodes* and *B. heroica*. The ITS sequence of the specimen analyzed (MBML 55484) did not present genetic distance (0.00%) with the specimen *B. baturitensis* (UFRN-Fungos 1943) (Figure 5), revealing their similarity. The undefined position of *B. baturitensis* in phylogeny can be inferred to the lack of other markers (e.g. RPB2 and TEF-1α), as well as the small number of the specimens available. It was not possible to amplify these markers with the specimen MBML 55484 despite many attempts. This fact highlights the importance of continuous study with this genus to improve the knowledge about it.

Preliminary conservation status: Tentatively, the species may be considered Endangered (B2a, B2b(ii;iii), and D) according to the IUCN red list criteria (IUCN 2012, 2022), due to the very small area of occupancy (<12 km²) and extent of occurrence (1,317.400 km²), with just two places of occurrence and two populations. Although *B. baturitensis* has an occurrence in a Conservation Unit in the Ceará State (APA of the Massif de Baturité), in the Espírito Santo State, the only individual so far confirmed, occurred in the MBML Park, and since it receives an approximate average of 80.000 annual visitors, the species may suffer some threat, such as

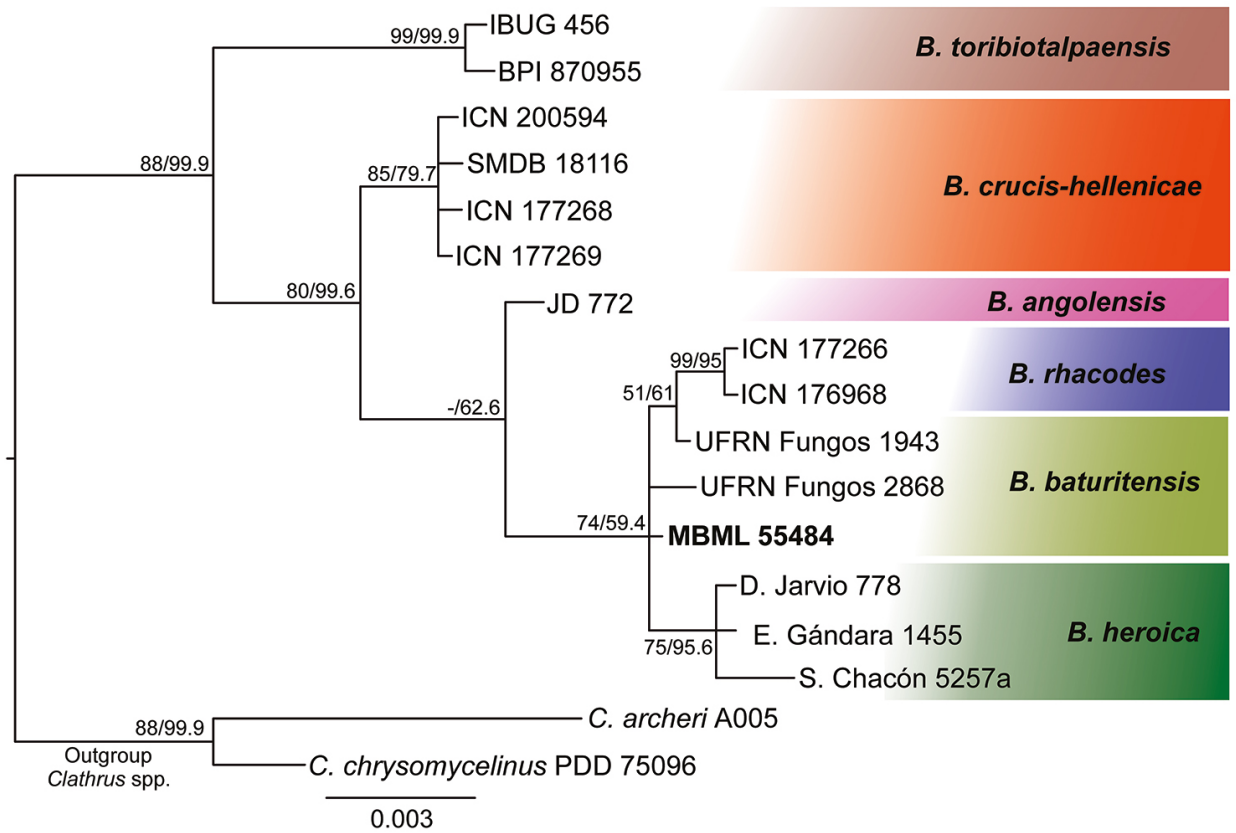

Figure 4. The 50% majority rule Bayesian tree inferred from combined dataset (ITS nrDNA/LSU nrDNA/*ATP*6) of *Blumenavia* spp. Bold name represents the species of this study. Scale bars indicate expected changes per site. Bayesian posterior probabilities (PP) and maximum likelihood bootstrap (MLbs) are indicated on the branches respectively.

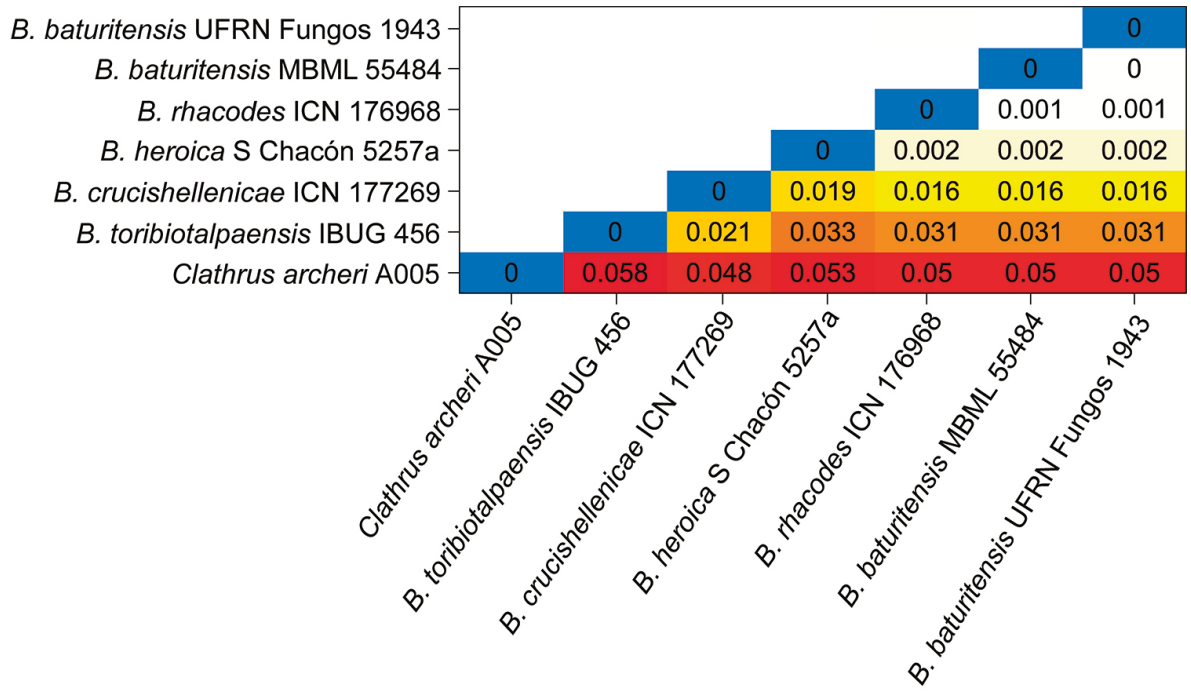

Figure 5. Heat map representing the distance matrix between the species studied in this study based on pairwise distances from ITS sequences.

trampling, at the edges of the internal tracks. After 2019, new samples were not found, so new efforts are needed to determine the actual distribution and conservation of this species.

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Author Contributions

Sâmela da Silva Recla: data collection and analysis, manuscript preparation.

Juliana Paulo da Silva: data collection and analysis, manuscript preparation.

Lorena Tonini: data collection and analysis, manuscript preparation.

Cristina Jaques da Cunha: data collection and analysis, manuscript preparation.

Francieli Loss Pugnal: data collection and analysis, manuscript preparation.

Gislaine Cristina de Souza Melanda: analysis and identification of the species, manuscript, figures and table preparation, phylogenetic analysis.

Jefferson Santos de Gois: manuscript preparation, phylogenetic analysis.

Joelcio Freitas: data collection and analysis, manuscript and figures preparation.

Conflicts of interest

There is no conflict of interest.

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