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Phylogenetic placement of *Tritirachium* strains from the URM culture collection originally founded by Augusto Chaves Batista (1916–1967) in Brazil, and the description of *T. batistae* sp. nov.

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ABSTRACT

Twenty-seven *Tritirachium* strains were present in the URM culture collection originally founded in Brazil by Augusto Chaves Batista. Fifteen freshly-prepared cultures were obtained from these original strains preserved under mineral oil. DNA was extracted for analysing phylogenetic relationships using the sequence information available from *Tritirachium* type materials and reference strains. Phylogenetic analysis using the internal transcribed spacer (ITS) ribosomal DNA sequences revealed that eight of these strains belong to the same clade as of *T. oryzae* and that the four other strains belong to the same clade as of *T. candoliense*. The strain URM 38, which was previously identified as "*T. brumptii*", appeared to exist as a single lineage, related to *T. roseum* and *T. candoliense*. Based on morphological features and multi-locus phylogenetic analysis, including the analyses of ITS and LSU rDNA, and *rpb2* sequences, we propose that URM 38 belongs to the new species *T. batistae*. This novel species exhibited velutinous to cottony colonies of varying colour, septate hyphae without clamp connections, conidiophores reduced to conidiogenous cells, conidiogenous cells with a distinct sympodial rachis, and single-cell conidia that was globose to subglobose, obovoid, smooth, and hyaline. The morphological features of species accepted in *Tritirachium* are included in this study.

Keywords: Basidiomycota, Brazilian fungi, fungal preservation, phylogeny, taxonomy

Introduction

The genus *Tritirachium* was described by Limber in 1940 to accommodate the fungi with 'rachis-like or zigzag form of the fertile portions of the erect conidiophore' (Beguin *et al.* 2012). Upon observing the morphology of the asexual morph, these fungi were first included in the phylum Ascomycota (Kirk *et al.* 2008). Later on, several studies investigated the morphology of these fungi in more details (Limber 1940; Macleod 1954; Hoog 1972; 1973), and more recently, phylogenetic analyses based on DNA sequences were conducted, which placed these fungi in the phylum Basidiomycota (Schell *et al.* 2011; Beguin *et al.* 2012; Manohar *et al.* 2014).

In the phylum Basidiomycota (subphylum Pucciniomycotina), *Tritirachium* was placed in the class Tritirachiomycetes, order Tritirachiales and family Tritirachiaceae (Schell *et al.* 2011; Beguin *et al.* 2012; He *et*

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al. 2019). Four species originally placed in *Tritirachium* were revisited, and two other unidentified species were recognized and could be described as new *Tritirachium* species (Schell *et al.* 2011). Manohar *et al.* (2014) described the species *T. candoliense*, which was isolated from a coastal sediment sample of the Arabian Sea. A similar genus, *Paratritirachium*, was introduced in the family Tritirachiaceae, which currently accommodates two species, *P. cylindroconium* and *P. curvibasidium* (Beguin *et al.* 2012; Nguyen *et al.* 2014; He *et al.* 2019).

Tritirachium species have already been reported as saprophytes (Moraes *et al.* 2010; Schell *et al.* 2011; Naseri *et al.* 2013), biotrophic mycosymbionts (Beguin 2010), endophytes (Kaneko & Kaneko 2004; Moraes *et al.* 2010; Vieira *et al.* 2011; Bezerra *et al.* 2013; Qadri *et al.* 2014), and as opportunists (Rodrigues *et al.* 1975; Moraes *et al.* 2010; Beguin *et al.* 2012; Naseri *et al.* 2013; Martínez-Herrera *et al.* 2015; Vanam *et al.* 2018). *Tritirachium oryzae* is the most important species of this genus that causes human infections, and has occasionally been reported to cause corneal ulcers, otomycosis, onychomycosis, and dermatomycosis of the scalp (Rodrigues *et al.* 1975; Moraes *et al.* 2010; Beguin *et al.* 2012; Naseri *et al.* 2013; Martínez-Herrera *et al.* 2015; Vanam *et al.* 2018). However, other *Tritirachium* species were also reported to possess biotechnological potential, which includes producing enzymes, such as proteases, amylase, glucanase, xylanase, pectinases, lipase, and proteinase K (Ebeling *et al.* 1974; Wang *et al.* 2016).

The *Tritirachium* strains that were deposited by Augusto Chaves Batista and his collaborators in the University Recife Mycologia (Micoteca URM Profa. Maria Auxiliadora Cavalcanti, Universidade Federal de Pernambuco – UFPE) in Brazil were revisited in this study. Augusto Chaves Batista (Fig. 1) was an important Brazilian researcher, and he is respected as one of the most reputed mycologists in Brazil (Singer 1969; Carneiro 1968; Moura & Maia 2013; Bezerra *et al.* 2017a). Batista worked in collaboration with researchers from different fungal groups on projects in diverse areas such as agriculture, medical, microbiology, and taxonomy; however, his surveys were mostly related to phytopathology and soil microbiology (Moura & Maia 2013). It is estimated that his contribution to science includes around 700 published articles and five books (Moura & Maia 2013; Bezerra *et al.* 2017a). Among the publications of Batista and his collaborators, three of them are focussed on *T. dependens* (Batista *et al.* 1965; 1966; Luna & Neto 1971).

While searching in the URM database, 27 *Tritirachium* strains appeared to be preserved in the collection, and most of them were deposited when Batista was still working as the director of the former Instituto de Micologia da Universidade do Recife (IMUR) (Bezerra *et al.* 2017a; b). The main preservation method for these cultures used back then was mineral oil, as proposed by Sherf (1943), which was also introduced by Augusto Chaves Batista, but later, other fungal preservation methods were also included in the routine of the URM collection, such as using distilled water (Castellani 1967), lyophilisation (Raper & Alexander 1945), and freezing at –80 °C (Kitamoto *et al.* 2002; Nakasone *et al.* 2004; Souza-Motta *et al.* 2015). In this study, we aimed to revisit the *Tritirachium* strains that were previously deposited at the URM culture collection, to determine their phylogenetic placement using the ITS rDNA sequences of these available strains, to report species isolated from new geographical and substrate/host records, and to provide detailed description of a new species based on the multi-gene phylogenetic analyses (ITS, LSU, and *rpb2*), and its morphological features.

Materials and methods

Fungal strains and morphological studies

The *Tritirachium* strains used in this study were obtained from the URM culture collection (WCDM 604) hosted at the Departmento de Micologia of the Universidade Federal de Pernambuco, Recife, Brazil. These strains were first preserved using mineral oil, and fresh cultures were obtained using these original materials. In Tab. 1, the information

Figure 1. Augusto Chaves Batista (Brazil, 1916-1967) (Photo: Departamento de Micologia, UFPE, Recife, Brazil).

Table 1. *Tritirachium* strains deposited in the URM culture collection at the Universidade Federal de Pernambuco, Recife, Brazil.

Strain number	Original name	Substrate/host	Country	Year[a]
URM 38	*T. brumptii*	Atmospheric air	Brazil	1955
URM 498	*T. roseum*	-	England	1957
URM 499	*T. album*	-	England	1955
URM 500	*T. dependens*	-	England	1955
URM 690	*T. dependens*	-	-	1956
URM 1653	*T. album*	-	Peru	1960
URM 1689	*T. dependens*	Atmospheric air	Brazil	1960
URM 1806	*T. dependens*	Pulmonary secretion	Brazil	1961
URM 2379	*T. album*	-	Brazil	1974
URM 2886	*T. oryzae*	Atmospheric air	Brazil	1986
URM 3561	*T. oryzae*	Oat	Brazil	1994
URM 3775	*T. album*	River water	Brazil	1997
URM 4088	*T. album*	River water	Brazil	1998
URM 4289	*T. album*	Knee biopsy	Brazil	-
URM 5436	*T. oryzae*	Water from water park	Brazil	2007
URM 5442	*T. oryzae*	Pool filter soil	Brazil	2007
URM 5452	*T. oryzae*	Water from water park	Brazil	2007
URM 6015	*T. oryzae*	Endophytic fungus (*Gossypium hirsutum*)	Brazil	2009
URM 6336	*T. oryzae*	Endophytic fungus (*Hancornea speciosa*)	Brazil	2011
URM 6517	*T. oryzae*	Herbal medicine (artichoke)	Brazil	2011
URM 6518	*T. oryzae*	Herbal medicine (Indian chestnut)	Brazil	2011
URM 6519	*T. oryzae*	Herbal medicine (artichoke)	Brazil	2011
URM 6705	*T. dependens*	Endophytic fungus (*Cereus jamacaru*)	Brazil	2012
URM 6821	*T. dependens*	Endophytic fungus (*Cereus jamacaru*)	Brazil	2012
URM 6822	*T. dependens*	Endophytic fungus (*Cereus jamacaru*)	Brazil	2012
URM 6856	*T. oryzae*	Endophytic fungus (*Plantago major*)	Brazil	2012
URM 8181	*T. oryzae*	Cake	Brazil	2019

[a] Refers to the year that the strain was deposited in the URM culture collection.

about the strains obtained from the URM database is summarized. The strain URM 38 is also deposited in the personal collection of Jadson D.P. Bezerra (Fungal Culture Collection of the Universidade Federal de Goiás – FCCUFG), which is maintained at the Instituto de Patologia Tropical e Saúde Pública (IPTSP) of the Universidade Federal de Goiás (UFG), Brazil.

A fraction of the *Tritirachium* strains that were preserved under mineral oil were transferred to glucose broth medium and incubated for seven days at 25 °C under a natural day-night cycle. After growth of these strains, they were transferred to Petri dishes with Malt Extract Agar (MEA), and were incubated using the conditions described above. For the morphological study of the strain URM 38 = FCCUFG 01, it was cultured on MEA, Potato Dextrose Agar (PDA), Czapek Yeast extract Agar (CYA), Czapek yeast autolysate agar with 5 % NaCl (CYAS), Yeast Extract Sucrose agar (YES), Dichloran-glycerol (DG18), Oatmeal Agar (OA), Malt agar with 20 % Sucrose (M20S), and Czapek Yeast with 20 % Sucrose agar (CYA20S), and incubated in dark at 25 °C. The morphological features of the newly appearing colonies on different culture medium were visualized after two weeks, and the reproductive structures were observed on PDA after four weeks. Slide were prepared and mounted in clear lactic acid, and culture colours were evaluated using the chart of Rayner (1970).

DNA extraction, DNA sequences, and phylogenetic analyses

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA), according to the manufacturer's instructions. The primer sets LR0R/LR5 (Vilgalys & Hester 1990), and ITS5/ITS4 (White *et al*. 1990) were used to amplify a part of the nuclear ribosomal large subunit (LSU) of the rDNA and the ITS region (first and second internal transcribed spacer regions and intervening 5.8 S nrDNA), respectively. The primer set 5f2/7cr (O'Donnell *et al*. 2010) was used to amplify the second largest subunit of the RNA polymerase II (*rpb2*) gene. PCR amplification, sequencing, and consensus sequences were performed and obtained as described by Bezerra *et al*. (2017c) and Silva *et al*. (2019).

Sequence analysis using the BLASTn tool of the GenBank database at NCBI was performed to verify the relationship between ITS, LSU rDNA, and *rpb2* sequences from different strains that were obtained in this study. Using the sequences obtained from GenBank, the online MAFFT interface (Katoh & Standley 2013) was used to perform further sequence alignment, and MEGA v. 7.0 (Kumar *et al*. 2016) was used for sequence adjustments. An initial phylogenetic analysis was performed using the ITS sequences of all the strains based on Maximum Likelihood (ML) and Bayesian Inference (BI)

methods. For the one-gene (ITS) phylogeny analysis, ML and BI methods were used in PhyML (Guindon *et al*. 2010) and MrBayes (Ronquist & Huelsenbeck 2003), respectively, which were launched from Topali 2.5 (Milne *et al*. 2004). The nucleotide substitution model was also applied by using Topali 2.5. The BI analysis was conducted using two subsequent runs over 1×10^6 generations with a burning value of 25 %, while the ML analysis was performed using 1000 bootstrap replicates.

A second multi-gene (ITS, LSU, and *rpb2*) sequence alignment representing the fungal strains placed in the family Tritirachiaceae, was performed to infer the phylogenetic position of the strain URM 38 = FCCUFG 01, which was identified as a new species based on the ITS phylogeny analysis. The three-gene phylogenetic analyses based on ML and BI methods were conducted in the RAxML-HPC BlackBox v.8.2.8 (Stamatakis 2008), and MrBayes on XSEDE, respectively, using the CIPRES Science Gateway (http://www.phylo.org/). For the combined analysis, the MrModel Test v.2.3 (Nylander 2004) was used to estimate the best nucleotide model for the sequence alignment. In both of these analyses, values less than 0.96 BI posterior probability (BPP), and 70 % ML bootstrap (ML-BS) were not considered. The new sequences are deposited in the GenBank database (Tab. 2), and the alignments are deposited in TreeBASE (study ID S24520).

Results

Phylogenetic analyses

Out of the 27 *Tritirachium* strains that were preserved under mineral oil in the URM culture collection, only 15 strains (55.5 %) were still viable and thus were available for

Table 2. Species used to perform the phylogenetic relationship of *Tritirachium* strains deposited at the URM culture collection at the Universidade Federal de Pernambuco, Recife, Brazil. GenBank numbers in bold were generated in this study.

Taxa	Strain number	ITS	LSU	*rpb2*
Paratritirachium curvibasidium	DAOM 242438^T	KF258724	KF258728	-
Paratritirachium curvibasidium	DAOM 242437	KF258723	KF258727	-
Paratritirachium curvibasidium	DAOM 242439	KF258725	KF258729	-
Paratritirachium cylindroconium	CBS 838.71^T	KF258726	KF258730	-
Sakaguchia dacryoidea	CBS 6353^T	AF444597	AF189972	DQ832208
Symmetrospora coprosmae	CBS 7899^T	NR_073317	KY109807	-
Tritirachium sp.	CBS 473.93	JF779664	JF779649	JF779651
Tritirachium sp.	CBS 265.96	JF779668	JF779652	JF779654
***T. batistae* sp. nov.**	URM 38^T	**MN496401**	**MN496416**	**MN505211**
T. candoliense	CBS 123151^T	KC879157	KC879158	KC879159
T. candoliense	URM 3561	**MN496404**	-	-
T. candoliense	URM 5442	**MN496405**	-	-
T. candoliense	URM 6518	**MN496409**	-	-
T. candoliense	URM 6519	**MN496410**	-	-
T. cinnamomeum	CBS 182.42^T	JF779667	-	-
T. dependens	IHEM 3497	JF779666	-	-
T. dependens	BPI 414589	JF779665	-	-
T. oryzae	URM 1689	**MN496402**	-	-
T. oryzae	URM 1806	**MN496403**	-	-
T. oryzae	URM 6821	**MN496411**	-	-
T. oryzae	URM 5452	**MN496406**	-	-
T. oryzae	URM 6856	**MN496412**	-	-
T. oryzae	URM 6015	**MN496407**	-	-
T. oryzae	URM 6336	**MN496408**	-	-
T. oryzae	URM 8181	**MN496413**	-	-
T. oryzae	NRRL 2018	JF779663	-	-
T. oryzae	CBS 164.67	GQ329853	GQ329853	JF779648
T. oryzae	NRRL 1210	JF779662	-	-
T. oryzae	CBS 896.70	JF779660	-	-
T. oryzae	CBS 388.39	JF779661	-	-
T. oryzae	CBS 442.70	JF779659	-	-
T. oryzae	CBS 837.71	JF779658	-	-
T. oryzae	CBS 116300	JF779657	-	-
T. roseum	CBS 183.42^T	JF779669	KF258731	JF779656

Strains URM 498, URM 500, URM 690, URM 1653, URM 2379, URM 2886, URM 3775, URM 4088, URM 5436, URM 6517, URM 6705, and URM 6822 were not viable to perform the study.

morphological and/or phylogenetic studies. The ITS rDNA was amplified and used to perform phylogenetic analysis of all the available strains to verify their relationship with *Tritirachium* species (Fig. 2). The phylogenetic analysis was performed using JC+I as the best substitution model for ML and BI analyses. These analyses revealed that eight strains grouped in the same clade as of *T. oryzae* with 80 % support according to ML analysis. The other four strains were placed in the same clade of *T. candoliense* (BPP = one and ML-BS = 100 %), which is a recently described species. The *Tritirachium* strain, URM 38 = FCCUFG 01, which was previously designated as "*T. brumpti*", was now identified as a single lineage related to *T. roseum* and *T. candoliense*. Other two strains including URM 499 and URM 4289, which were previously identified as "*T. album*", were excluded from this phylogenetic analysis because they were reclassified as *Parengyodontium album* (Cordycipitaceae, Ascomycota) (GenBank accession numbers for ITS URM 499 = MN496414 and URM 4289 = MN496415) via BLASTn analysis.

To further verify the phylogenetic relationship of the strain URM 38 = FCCUFG 01, a combined dataset of ITS, LSU, and *rpb2* sequences was constructed, and the results of ML and BI analyses revealed that URM 38 = FCCUFG 01 belongs to a single lineage, which is related

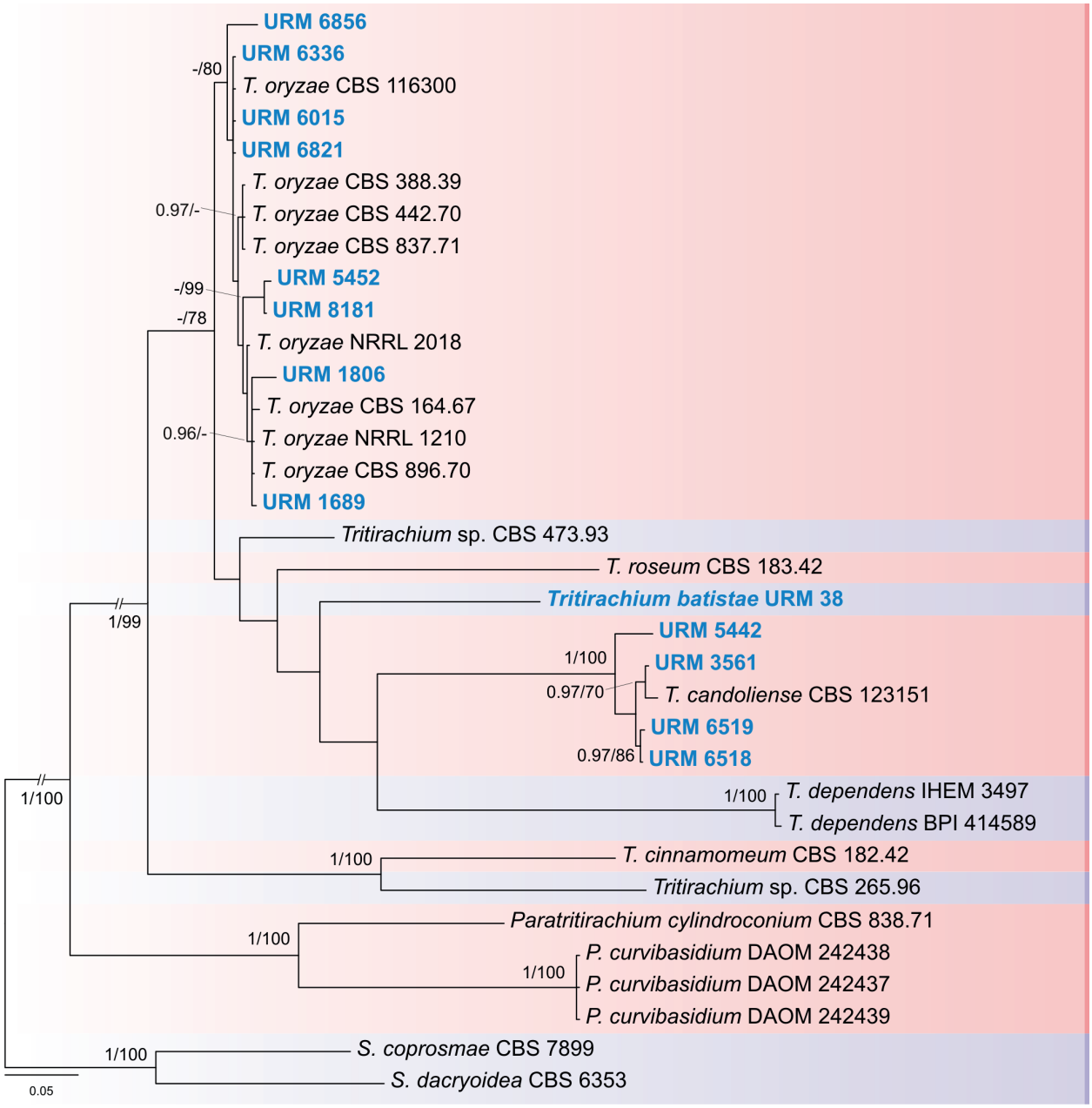

Figure 2. Bayesian inference (BI) tree obtained using ITS rDNA sequences from species representing Tritirachium and *Paratritirachium* (Tritirachiaceae). The new species and the strains studied are in bold and blue. BPP and ML-BS above 0.95 and 70 %, respectively, are shown near nodes. *Sakaguchia dacryoidea* (CBS 6353) and *Symmetrospora coprosmae* (CBS 7899) were used as outgroup.

to *T. roseum* and *T. candoliense* (Fig. 3). To perform the BI analysis, the nucleotide models GTR+I+G, GTR+G, and SYM+G, were used for ITS, LSU, and *rpb2*, respectively. The nucleotide model GTR+I+G was defined as the best model in the ML analysis. Based on the morphological features and the results of phylogenetic analyses, a new species is introduced in this study to accommodate the strain URM 38 = FCCUFG 01.

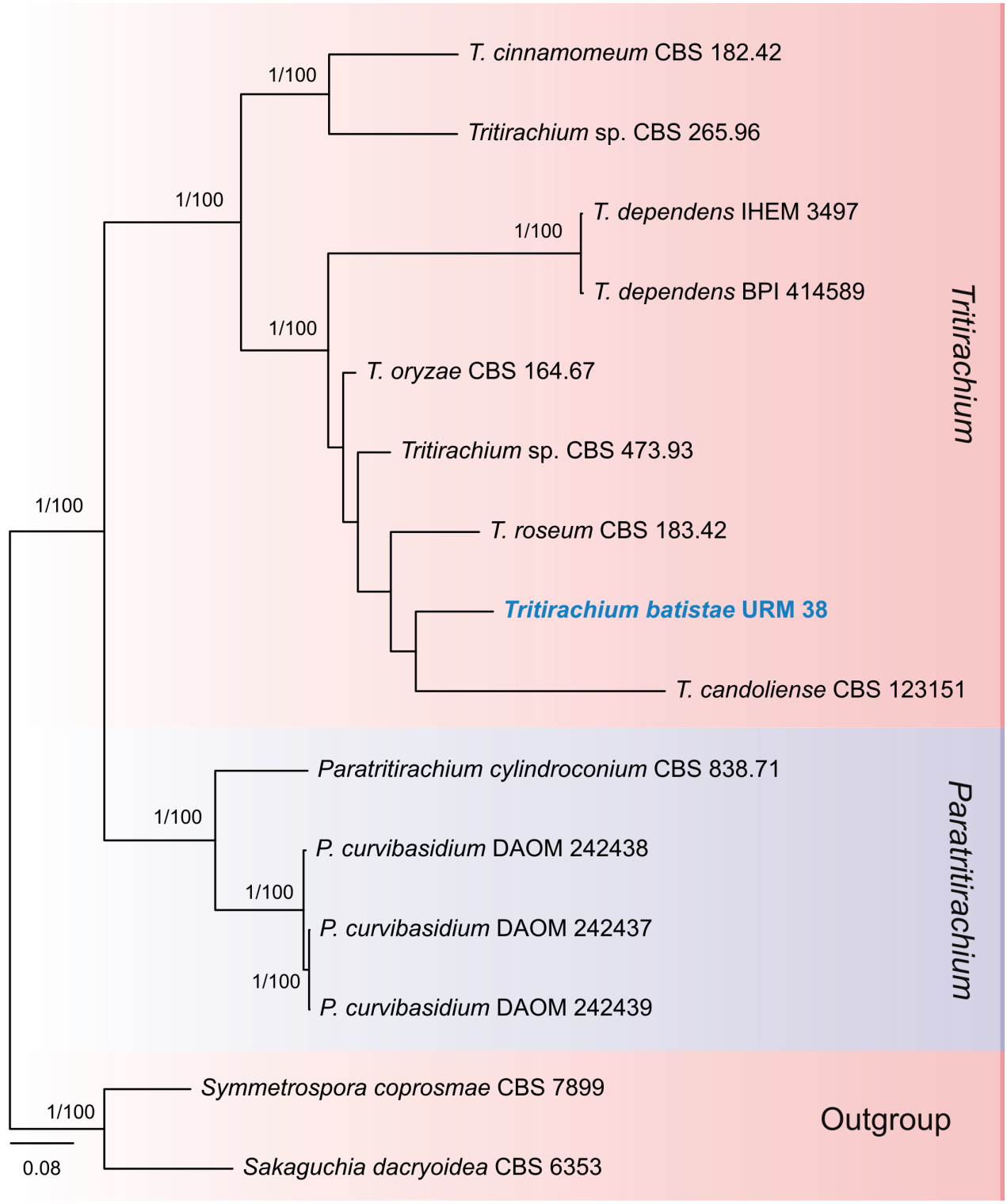

Figure 3. Bayesian inference (BI) tree obtained using ITS and LSU rDNA and *RPB2* sequences from *Tritirachium* and *Paratritirachium* species (Tritirachiaceae). The new species is in bold and blue. BPP and ML-BS above 0.95 and 70 %, respectively, are shown near nodes. *Sakaguchia dacryoidea* (CBS 6353) and *Symmetrospora coprosmae* (CBS 7899) were used as outgroup.

Taxonomy

Tritirachium batistae J.D.P. Bezerra & C.M. Souza-Motta **sp. nov.** (Fig. 4)

MycoBank MB833847

Etymology. Named in the honour of Augusto Chaves Batista (1916-1967), a brilliant Brazilian mycologist who founded the former Instituto de Micologia da Universidadede Recife (IMUR), Brazil.

Holotype. URM 93562

Description. Colonies after seven days at 25 °C: on PDA, were smooth, velutinous, white to light grey in colour, reverse luteous, and exhibited lower growth up to 1.2 cm. On MEA, the colonies were predominantly white similar to that on PDA, reverse luteous, and were growing up to 1.5 cm. On CYA, colonies with a cottony appearance similar to MEA, greyish to green in colour, and growing up to 1.9 cm. On CYAS, colonies had similar appearance as on MEA and CYA, were amber in colour, reverse pale luteous to luteous, and reaching up to 0.8 cm. On YES, colonies were cottony, honey-like, reverse umber to brown in colour, reaching up to 1.5 cm, and there was a reddish pigment diffused in the medium near the colonies. On AO, colonies were apiculate with a cottony appearance as observed on MEA, CYA, and CYAS, predominantly saffron coloured, and formed lightly aerial hyaline mycelium, reverse saffron to umber, and were growing up to 1.7 cm. On DG18, colonies were cerebriform-like, with a velvety aspect, pale olivaceous grey in colour, and had a diameter reaching up to 1.1 cm. On M20S, colonies were cottony, amber to honey in colour, and had a diameter reaching up to 1 cm. On CYA 20S, colonies were cottony, honey-like, and had a diameter reaching up to 0.7 cm. Hyphae on PDA after two weeks of growth in the dark at 25 °C, were straight to somewhat undulating, septate, changing from hyaline to light brownish in colour with age, without clamp connections, and 1–2 µm wide. Conidiophores were erect, ascendent, smooth, lightly tapered, zero(–one)-septate, reduced to conidiogenous cells, changing from hyaline to light brownish in colour with age, and were 9–23.5(–52) × 1–2 µm. Conidiogenous cells were slightly tapered towards the apex, with a distinct sympodial rachis on which conidia are formed and were 18.5–24.5 × 1–2 µm. Conidia were single-celled, globose to subglobose, obovoid, smooth, hyaline, and were 2.5–3.5(–4.5) × 2–2.5(–3) µm. Sexual morph was not observed.

Table 3. Morphological features of *Tritirachium* species.

Species	Type	Country	Host/ substrate	Colony colour	Conidiophores (µm)	Conidiogenous cells (µm)	Conidia (µm)	References
T. batistae J.D.P. Bezerra & Souza-Motta	URM 38	Brazil	From atmospheric air	On PDA, white to light grey	Reduced to conidiogenous cells, 9–23.5(–52) × 1–2	Slightly tapered towards apex, 18.5–24.5 × 1–2	Globose to subglobose, obovoid, smooth, hyaline, 2.5–3.5(–4.5) × 2–2.5(–3)	This paper
T. candoliense C.S. Manohar, Boekhout & Stoeck	CBS 123151	India	From anoxic marine sediment	On MEA, vinaceous	Often terminate in whorls of conidiogenous cells	Strongly tapered, flask-shaped, 15–50 × 0.8–2.0	Ellipsoidal to clavate-ellipsoidal, pale brownish, 2.5–3.5 × 1.6–2.5	Manohar *et al.* 2014
T. cinnamomeum J.F.H. Beyma	CBS 182.42	Netherlands	On flies from a chalk-pit	On PDA, light yellow-brown to tan	Terminate in whorls of conidiogenous cells, up to 100 × 1.3–1.5	60–80 long	Ellipsoid, smooth, hyaline, brownish in mass, 3.3–4.7 × 2.7–3.3	Beyma 1942 Schell *et al.* 2011
T. dependens Limber	BPI, Limber s. n.	Cuba	From branch of *Yucca treculeana*	Pale yellowish to brownish yellow	Often terminate in whorls of conidiogenous cells, up to 1000 long and 3–4.5 wide	Elongate, slightly swollen below the middle, tapering towards the tip, geniculate, 13–35 × 2–3.5	Globose to ellipsoidal, slightly yellowish, (2–)2.5–3.5(–4) × (2–)2–3(–3.5)	Limber 1940 Hoog 1973 Manohar *et al.* 2014
T. oryzae (Vincens) Hoog	?	Vietnam	On *Oryza*	At first rosy vinaceous to rosy buff, when older lilac or cinnamon	Ascendent, often terminate in whorls of conidiogenous cells, 200–1500 long, 1.5–3 wide	Elongate basal part, slightly swollen below the middle, tapering towards the tip, (7–)9–20(–25) × 1.5–3	Globose to ellipsoidal, rarely with a slightly apiculate base, hyaline (1.5–)2–3(–3.5) × 1.5–3	Hoog 1972 Schell *et al.* 2011
T. roseum J.F.H. Beyma	CBS 183.42	Germany	On litter from a cowshed	Initially purple or brown, or one mix of both colours; the purple colour appears with age against the brown back	Erect, rarely branched	Strongly tapered, 30–50 long, 1.7–2 wide	Subglobose, hyaline, 2.3–2.7 × 2	Beyma 1942 Schell *et al.* 2011

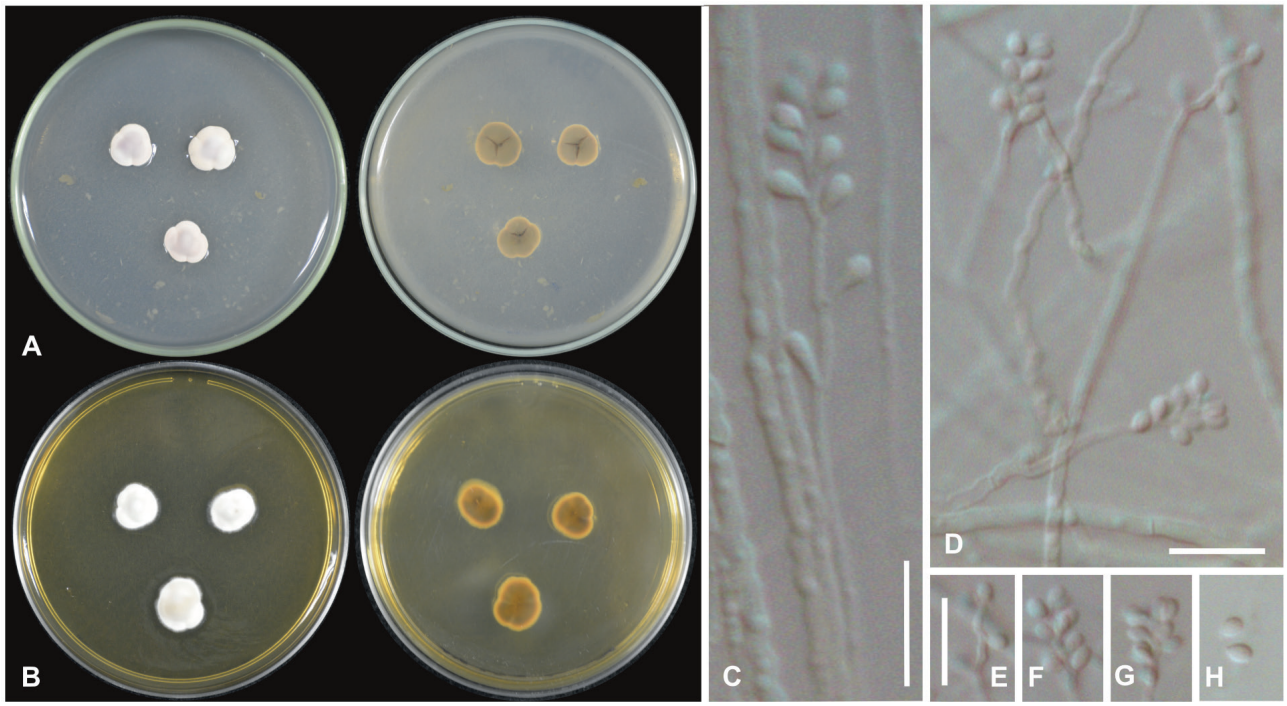

Figure 4. *Tritirachium batistae* (URM 38 = FCCUFG 01, ex-type-living culture). **A**. Culture on PDA after seven days. **B**. Culture on MEA after seven days. **C**, **D**. Conidiophores and conidia. **E**-**G**. Details of sympodial rachis and conidia. **H**. Conidia. Scale bars: 10 µm.

Material examined. BRAZIL, Pernambuco: Recife, isolated from atmospheric air, 1955, A.C. Batista (URM 93562, holotype), ex-type-living culture URM 38 = FCCUFG 01.

Notes. *Tritirachium batistae* was described to accommodate the strain URM 38 = FCCUFG 01, which was previously designated as "*T. brumptii*". Later, "*T. brumptii*" was synonymized under *Beauveria brumptii* (Langeron 1947). Our BLASTn search showed that the ITS rDNA sequences of this strain are 80-90 % identical to *Tritirachium* species (e.g. MH865598.1, CBS 130019), and had no identity to *Beauveria* species. Similar results were obtained using the analysis of LSU and *rpb2* sequences. Based on these results and the multi-gene phylogenetic analysis (Fig. 3), we conclude that the strain URM 38 = FCCUFG 01 can be recognized as a single lineage related to *T. roseum* and *T. candoliense*. *Tritirachium batistae* differs from *T. candoliense* and *T. roseum* in regard to texture and colour of the colonies, size, format, and colour of conidiophores, conidiogenous cells, and conidia (Tab. 3).

Tritirachium candoliense C.S. Manohar, T. Boekhout, W.H. Muller & T. Stoeck, Fungal Biology 118(2): 143 (2014)

Description and illustration. Manohar *et al*. (2014).

Material examined. BRAZIL, Pernambuco: isolated from oats, February 1997, M. Cavalcanti, living culture URM 3561. BRAZIL, Pernambuco: Recife, water park, isolated from soil from pool filter, February 2007, M.A. Sousa, living culture URM 5442. BRAZIL, Pernambuco: Recife, isolated from Indian chestnut (*Aesculus hippocastanum*) capsule (phytotherapeutic medicine), December 2011, A.C. Regis, living culture URM 6518. BRAZIL, Pernambuco: Recife, isolated from artichoke (*Cynara scolymus*) capsule (phytotherapeutic medicine), December 2011, A.C. Regis, living culture URM 6519.

Notes. *Tritirachium candoliense* was described by Manohar *et al*. (2014) based on a strain that was isolated from the marine sediments growing under anoxic conditions on the Goa coast, in the Arabian Sea. In this study, one of the strains was isolated from oats (URM 3561), another from soil of a pool filter from a water park (URM 5442), and two other strains (URM 6518 and URM 6519) were isolated from phytotherapeutic medicine. To our knowledge, this is the first time that this species has been reported after its original description, and thus it highlights the importance of studying the lifecycle and geographical distribution of many more *Tritirachium* species.

Tritirachium oryzae (Vincens) G.S. de Hoog, Studies in Mycology 1: 22 (1972).

≡*Beauveria oryzae* Vincens, Revue Path. vég. Ent. agric. Fr.: 122 (1910).

=*Beauveria brumptii* Langeron & Lichaa, Bull. Acad. Méd. Paris: 133 (1934).

=*Tritirachium purpureum* Saito ex J.F.H. Beyma, Antonie van Leeuwenhoek 8: 118 (1942).

=*Tritirachium violaceum* Tatarenko, Notulae Syst. Sect. Crypt. Inst. Bot. Acad. Sci. U.S.S.R.: 90 (1952).

For more synonyms see Index Fungorum and/or MycoBank.

Description and illustration. Hoog (1972).

Material examined. BRAZIL, Pernambuco: Recife, isolated from atmospheric air of Igreja das Graças, September 1960, A.C. Batista, living culture URM 1689. BRAZIL, Ceará: isolated from pulmonary secretion, July 1961, unknown collector and/or depositor, living culture URM 1806. BRAZIL, Paraíba: Santa Teresina, Tamanduá farm, isolated as endophyte from *Cereus jamacaru* (Cactaceae), August 2012, J.D.P. Bezerra, living culture URM 6705. BRAZIL, Paraíba: Santa Teresina, Tamanduá farm, isolated as endophyte from *C. jamacaru*, December 2012, J.D.P. Bezerra, living culture URM 6821. BRAZIL, Paraíba: Santa Teresina, Tamanduá farm, isolated as endophyte from *C. jamacaru*, December 2012, J.D.P. Bezerra, living culture URM 6822. BRAZIL, Pernambuco: Recife, water park, isolated from water, February 2007, M.A. Sousa, living culture URM 5452. BRAZIL, Pernambuco: Jaboatão dos Guararapes, isolated as endophyte from leaves of *Plantago major*, December 2012, P. Vieira, living culture URM 6856. BRAZIL, Pernambuco: Recife, isolated as endophyte from leaves of *Gossypium hirsutum*, September 2008, P. Vieira, living culture URM 6015. BRAZIL, Pernambuco: Recife, isolated as an endophyte from *Hancornia speciosa*, March 2011, M.B.O. Chagas, living culture URM 6336. BRAZIL, Pernambuco: Jaboatão dos Guararapes, isolated from cake, 2017, G.M.R. Albuquerque, living culture URM 8181.

Notes. *Tritirachium oryzae* was described by de Hoog (1972) based on *Beauveria oryzae* that was isolated from *Oryza* sp. (Vincens 1923; Hoog 1972). This species has been reported as an opportunist, causing mycoses in humans (Silva *et al*. 1999; Moraes *et al*. 2010; Martínez-Herrera *et al*. 2015), and is also reported from several substrates and habitats, such as decaying organic matter and plants. It has also been reported as a symbiont, saprotroph, and an endophyte. This fungal species was also isolated from the wood and the ceiling tiles, found associated with leaves, stems, and twigs of *Pinus wallichiana*, and was also isolated from the branches of cotton, nails, cornea, and scalp, causing infections in humans (Rodrigues *et al*. 1975; Moraes *et al*. 2010; Naseri *et al*. 2013; Qadri *et al*. 2014; Vanam *et al*. 2018). In this study, we further expanded the information on geographical distribution of this species and reported it from several new substrates/hosts.

Discussion

Tritirachium species are found to be present in various environments and substrates/hosts, but their ecological relationships need to be further investigated in order to better understand their lifecycle. For example, *T. oryzae* was isolated from the branches of cotton in Brazil (Vieira *et al*. 2011), which was later found to be associated with roots, stems, and leaves of *P. wallichiana* in the Himalayas (Qadri *et al*. 2014), and was also isolated from the wood and the ceiling tiles (Moraes *et al*. 2010). *Tritirachium* sp. was isolated from the leaves of Japanese beech trees in Japan (Kaneko & Kaneko 2004), and *T. dependens* [here re-evaluated and classified as *T. oryzae*] was isolated from cladodes of the cactus *Cereus jamacaru* in Brazil (Bezerra *et al*. 2013). Some members of this genus were isolated from soil and organic matter, and they are considered as saprotrophic (Moraes *et al*. 2010). *Tritirachium candoliense* was isolated from the coastal sediments of the Arabian Sea (Manohar *et al*. 2014; Wang *et al*. 2016), and another *Tritirachium* sp. was isolated from a sponge present in the Adriatic Sea (Wang *et al*. 2016). Another *Tritirachium* strain was isolated as a symbiont associated with *Penicillium rugulosum* (Beguin 2010).

The clinical importance of *Tritirachium* was reported for the first time by Wang and colleagues in 2016. They described about the representatives of this genus that are responsible for human infections and also reported that the species of *Tritirachium* could be potential airborne contaminants. Clinical reports are also linked to *T. oryzae* and *T. roseum*, which have occasionally been isolated from the patients with corneal ulcers, otomycosis, onychomycosis, and dermatomycosis of the scalp (Rodrigues *et al*. 1975; Moraes *et al*. 2010; Beguin *et al*. 2012; Naseri *et al*. 2013; Martínez-Herrera *et al*. 2015; Vanam *et al*. 2018). *Tritirachium* species have been demonstrated to possess biotechnological importance. For example, "*Tritirachium album*" was reported as a producer of protease K (Ebeling *et al*. 1974), and *Tritirachium* sp. was reported as a producer of protease, amylase, glucanase, xylanase, pectinases, and lipase (Wang *et al*. 2016).

In this study, a new species was proposed, which was designated as *Tritirachium batistae*, and it is based on the strain URM 38 = FCCUFG 01. The strain URM 38 = FCCUFG 01 was isolated from the atmospheric air in Brazil (1955) and was deposited in the URM culture collection. Phylogenetic analyses using the ITS sequences of representative strains of *Tritirachium* species that are maintained in the URM culture collection, along with reference sequences acquired from the GenBank database, confirmed that the strain URM 38 = FCCUFG 01 could be introduced as a new species, which is identified to be related to *T. candoliense* and *T. roseum*. These two species mainly differ from *T. batistae* in colony colour and texture, their conidiogenous cells often terminate in verticils, and the size and colour of their conidia is also different (Tab. 3) (Schell *et al*. 2011; Manohar *et al*. 2014). Another important aspect of this study was the re-classification of some strains that were previously identified as *T. oryzae*, *T. dependens*, "*T. brumptii*", "*T. album*" (= *Parengyodontium album*), and *T. candoliense*, which further helped to expand the information about their geographical distribution, lifecycle, environment, and substrate/host association of these species.

We resolved the phylogenetic placement of the old *Tritirachium* cultures deposited in the URM culture collection since 1955, and introduced *T. batistae* as a new species in the genus. In addition, in this study, we also reported

species occurring in different regions and/or substrates, which can further add to the knowledge of global fungal diversity. The URM culture collection is the most important fungal collection in Brazil, representing an abundant and diversified strain collection, which contains about 8,000 fungal names and have preserved 98,000 fungal cultures belonging to the most diverse groups. Among these cultures, there are strains deposited by Augusto Chaves Batista and his collaborators during their work in the former Instituto de Micologia da Universidade do Recife (IMUR), highlighting this collection as a reservoir of fungal diversity, which is recently being reviewed based on the modern taxonomic analyses (morphological and molecular), and is also being biotechnologically characterized in relation to the production of enzymes, and other secondary metabolites (Maciel *et al*. 2013; 2014; Moreira *et al*. 2014; Sousa *et al*. 2015; Bezerra *et al*. 2017a; b; Cruz *et al*. 2017; Lisboa *et al*. 2017; Barbosa *et al*. 2018; Bezerra *et al*. 2019).

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