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Morphological and molecular evidences for a new species of *Datroniella* (Polyporales, Basidiomycota) from Brazil

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Abstract

Datroniella minuta sp. nov. from Northeast Brazil is described on the basis of morphological characteristics and ITS and nLSU sequence data analyses. This species is characterized by tiny and cupulate basidiomata and large cylindrical basidiospores. A key for species of *Datroniella*, drawings of the micro-characteristics, color photograph of the basidiomata and phylogenetic tree to show the placement of the new species are provided.

Key words: Basidiomycota, diversity, ITS, LSU, Neotropics, Polyporales s. l.

Introduction

Datroniella B.K. Cui, Hai J. Li & Y.C. Dai was recently segregated from *Datronia* Donk based in morphological and molecular evidences by Li *et al.* (2014) and accommodates the widespread *Datroniella scutellata* (Schwein.) Gilb. & Ryvarden (1985: 364) (type species) and four new East Asian species [*Datroniella melanocarpa* B.K. Cui, Hai J. Li & Y.C. Dai (2014: 173), *Datroniella subtropica* B.K. Cui, Hai J. Li & Y.C. Dai (2014: 175), *Datroniella tibetica* B.K. Cui, Hai J. Li & Y.C. Dai (2014: 175) and *Datroniella tropica* B.K. Cui, Hai J. Li & Y.C. Dai (2014: 176)]. The genus is characterized by annual pileate to effused-reflexed or rarely resupinate basidiomata. When pileate, the pileus is usually less than 3 cm long. The pileus surface is brown to black, glabrous, and the pore surface is white, cream to pale brown. The pores are large to small, round to angular and the context is pale brown, corky or brown. The hyphal system is dimitic, the generative hyphae have clamp connections and the skeletal hyphae are branched and dominate in the trama. The latter have no reaction in Melzer's reagent (IKI-), but are cyanophilous (CB+) in Cotton Blue and the tissues darkens in KOH. No dendrohyphidia and cystidia are present in hymenium. The basidiospores are cylindrical, hyaline, thin-walled, smooth, IKI- and CB- (Li *et al.* 2014).

Previously, the only representative species of the genus in Brazil was *D. scutellata* from the Atlantic Rain Forest (Gugliotta *et al.* 2015). *Datroniella minuta* Lira & Ryvarden is described on the basis of collections from the Brazilian semiarid. Morphological studies and molecular analyses and a key to species of the genus are presented below.

Material and methods

Area of study and morphological studies

The specimens were collected in April 2012, during a field trip in the Serra de Ibiapaba (03°52'47"S and 40°57'50"O), State of Ceará, in the Brazilian semi-arid. The material was identified based on macro- (measures, shape and color of the basidiomata) and micro-morphology (slide preparations with 5% KOH, stained with 1% aqueous phloxine, Melzer's reagent and Cotton Blue to analyze the hyphal system, dextrinoid, amyloid and/or cyanophilous reactions, presence/absence and measurements of at least 20 sterile structures and basidiospores, when possible (Ryvarden 1991). The designation of color followed Watling (1969). The material was deposited in the Herbarium Pe. Camille Torrend

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(URM), Department of Mycology, Universidade Federal de Pernambuco and in the Herbarium of the University of Oslo (O).

Genomic DNA extraction, Polymerase Chain Reaction (PCR) and sequencing

Fragments from the tubes and context of the basidioma (30–50mg) were removed and placed in tubes of 1.5 ml and stored at -20°C. The DNA was extracted according the method described in Góes-Neto *et al.* (2005). The reaction mix and parameters for PCR amplification of the full ITS regions was according to Smith & Sivasithamparam (2000) using the primers ITS4 and ITS5 (White *et al.* 1990). For LSU region, the amplification was performed following Goés-Neto *et al.* (2005), using the primers LR0R and LR7 (Moncalvo *et al.* 2000). Negative controls containing all components of the reaction mix, but exchanging DNA by water, were used in each procedure to detect possible contamination. The amplification products were purified with GenJET PCR Purification Kit (Thermo Scientific) and sequenced at the Plataform of Sequencing/LABCEN/CCB of the Universidade Federal de Pernambuco (UFPE, Brazil) in an ABI-310 Capillary Sequencer (PerkinElmer, Wellesley Massachusetts, USA). The cycle sequencing was carried with the same primers of amplification reactions (Moncalvo *et al.* 2000). All obtained sequences were deposited in the GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA).

Phylogenetic analyses

The electropherograms were analyzed and edited using the 2.0 Staden Package software (Bonfield *et al*. 1995). One ITS and one partial LSU rDNA sequences of the new *Datroniella* and additional sequences for the ingroup based on studies of Li *et al.* (2014) were recovered from GenBank nucleotide database (Table 1). Sequences of *Pycnoporus sanguineus* (L.) Murrill (1904: 421) and *Trametes suaveolens* (L.) Fr. (1938: 491) were used as outgroup for phylogenetic analyses reconstruction (Li *et al.* 2014). The sequences were aligned and manually edited using MEGA6 (Tamura *et al.* 2013).

TABLE 1. Specimens presented in this study with GenBank accession numbers for the ITS and LSU sequences. The sequence in bold was generated in this study.

Species	Voucher	GenBank/NCBI accession number		Reference
		ITS	LSU	
Datronia mollis (Sommerf.) Donk (1966: 338)	Dai 11253	JX559258	JX559289	Li *et al.* (2014)
Datronia mollis	Dai 11456	JX559253	JX559292	Li *et al.* (2014)
Datronia mollis	RLG 6304	JN165002	JN164791	Justo & Hibbett (2011)
Datronia stereoides (Fr.) Ryvarden (1968: 42)	Holonen	KC415179	KC415196	Li *et al.* (2014)
Datronia stereoides	Niemelä 3020	KC415178	KC415195	Li *et al.* (2014)
Datroniella melanocarpa	Cui 10646	KC415186	KC415194	Li *et al.* (2014)
Datroniella minuta	**URM 87858**	**KX584447**	**KX619568**	**This study**
Datroniella scutellata	Cui 7265	JX559263	JX559300	Li *et al.* (2014)
Datroniella scutellata	RLG 9584	JN165004	JN164792	Li *et al.* (2014)
Datroniella subtropica	Dai 12883	KC415184	KC415191	Li *et al.* (2014)
Datroniella subtropica	Dai 12881	KC415183	KC415193	Li *et al.* (2014)
Datroniella tibetica	Cui 9486	JX559265	JX559299	Li *et al.* (2014)
Datroniella tropica	Dai 13147	KC415181	KC415189	Li *et al.* (2014)
Datroniella tropica	Dai 13152	KC415182	KC415190	Li *et al.* (2014)
Neodatronia gaoligongensis B.K. Cui, Hai J. Li & Y.C. Dai (2014: 177)	Cui 8055	JX559269	JX559286	Li *et al.* (2014)
Neodatronia gaoligongensis	Cui 8132	JX559270	JX559287	Li *et al.* (2014)
Neodatronia sinensis B.K. Cui, Hai J. Li & Y.C. Dai (2014: 178)	Cui 9434	JX559271	JX559282	Li *et al.* (2014)
Neodatronia sinensis	Dai 11921	JX559272	JX559283	Li *et al.* (2014)
Pycnoporus sanguineus	PRSC 95	JN164982	JN164795	Justo & Hibbett (2011)
Trametes suaveolens	FP 102529	JN164966	JN164807	Justo & Hibbett (2011)

The ITS and LSU regions were analyzed independently (data not shown), but there were no important topological differences between them and a combined analyses of the both regions into a single matrix was performed. Phylogenetic analyses and tree construction were performed using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Algorithm (BA). For MP analysis, gaps were treated as missing data. In MP and ML, the analyses were performed using PAUP* 4.0b10 (Swofford 2003) with 5000 bootstrap replications each. The models of evolution were indentified for each dataset and obtained from jModelTest (Posada 2008). To ML analysis was based on HKY + G for ITS sequences and TrN + G + I for LSU sequences. BA analyses were run in MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) with 3 000 000 generations. For ITS sequences, the BA analysis was based on HKY + G and for LSU sequences K80 + I + G. In the combined analysis (ITS + LSU), TVmef + G distance was used for the ML and SYM + G for BA, and all distances were obtained from MrModeltest 2.3 (Nylander 2004).

Results

A combined dataset which includes four sequences of *Datronia*, nine of *Datroniella* (one of which generated in this study, in bold in Table 1), four of *Neodatronia*, and *Trametes suaveolens* and *Pycnoporus sanguineus* as outgroups. The combined alignment (ITS + LSU) had 1880 characters of which 1514 were parsimony informative, 366 were variable and parsimony uninformative, and 296 were parsimony informative. The MP analysis resulted in trees equally parsimonious (tree length = 637, CI = 0.7221, RI = 0.8285, RC = 0.598), one of which is shown in Fig. 1.

The results of the phylogenetic analyses generated from ML, MP and BA showed small differences in statistical support values and quite similar topologies. Thus, the ML tree was used as a base of this work (Fig. 1).

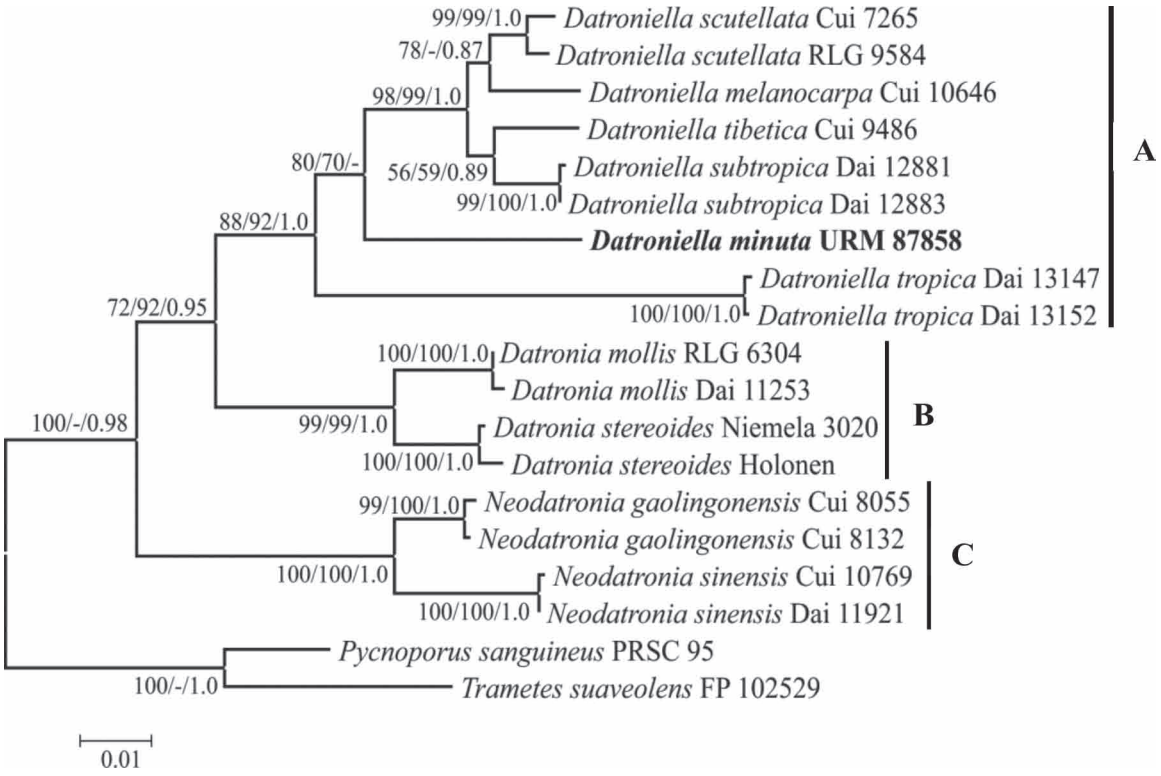

FIGURE 1. Phylogenetic reconstruction of *Datronia*, *Datroniella* and *Neodatronia* inferred from a combined dataset of ITS and nLSU. Parsimony bootstrap generated by ML, MP (higher than 50%) and BA posterior probabilities (higher than 0.70) are showed along the branches, respectively.

Three clades were formed with strong statistical support and represented the monophyletic and distinct genera *Datroniella* (A), *Datronia* (B) and *Neodatronia* (C). The clade A grouped, with high support (88/92/1.0; Fig. 1), five species already described in *Datroniella* from China and United States, besides the new species *Datroniella minuta* described here. The clade B included *Datronia mollis* and *Datronia stereoides*. The clade C grouped *Neodatronia gaolingonensis* and *N. sinensis*. The results of the present study are similar of those in Li *et al.* (2014).

Taxonomy

Datroniella minuta Lira & Ryvarden, *sp. nov*., Fig. 2
Mycobank: MB816069

Diagnosis:—*Datroniella minuta* is characterized by very tiny and cupulate basidiomata and its large cylindrical basidiospores.

Holotype:—BRAZIL. Ceará: Tianguá, Serra de Ibiapaba, C.R.S. Lira 787, April 19, 2012 (URM87858, Isotypus O).

Etymology:—*Minuta*: referring to the small basidiomata.

Basidiomata annual, pileate, cupulate, very small, up to 1 cm wide and 2 mm thick at the base. Abhimenial surface azonate to slightly sulcate, glabrous, smooth, corky to dark reddish brown (52 buff, 19 bay to 16 cigar brown). Sterile margin cream to cork (6 F to 52 buff), up to 1 mm wide, slightly involute when dry, sometimes longitudinally sulcate. Pore surface cork to light brown (52 buff to 32 clay buff), pores round, 2–3 per mm, dissepiments thin and entire. Context pale brown (31 vinaceous buff to 52 buff), up to 1.5 mm thick. Tubes concolorous with the pore surface, up to 0.5 mm long. Hyphal system dimitic, generative hyphae hyaline, 2.5–3 µm wide, bearing clamp connections; skeletal hyphae IKI-, CB-, pale yellowish brown, straight or with arboriform branches, 3.5–4 µm wide, thick-walled. Dendrohyphidia, cystidia and cystidiole absent. Basidia cylindrical, clavate, with four sterigmata and a basal clamp connection, 12.5–15 × 4–5 µm. Basidiospores 9–10(11) × 3–4 µm, cylindrical, apiculate, hyaline, thin-walled, smooth, with few guttules, IKI-, CB-.

Substrate:—On dead wood.

Distribution:—Until now, known only from the type locality in Brazilian semi-arid.

Remarks:—*Datroniella minuta* is macroscopically characterized by its small, reddish to dark brown basidiomata, margin lighter than the abhymenial surface and becoming slightly involute when dry. The basidiosporos are similar to those of *Datroniella melanocarpa*, but this Asian species has applanate, black basidiomata and cystidioles (Table 2) (Li *et al*. 2014).

Contrarily to what described for the genus, the microstructures of *D. minuta* are acyanophilous and thus this character should be revaluated in the genus delimitation. Besides, the cyanophilous reaction turns blue the walls and ornamentations of the microstructures and not the cytoplasm and precaution is necessary when using this character.

FIGURE 2. Macro- and micro-morphologic aspects of *Datroniella minuta*. A. Basidiomata; B. Basidiospores; C. Arboriform skeletal hyphae. Scale: A = 1 cm; B and C = 10 µm.

TABLE 2. Main morphological characteristics of *Datroniella* species (updated from Li *et al.* 2014).

Species	Pores/mm	Basidiomata	Basidiospores (µm)	Cystidioles
D. minuta	2–3	Pileate	9–10(11) × 3(4)	-
D. melanocarpa	2–3	Pileate	8.8–11 × 3–4	+
D. subtropica	6–8	Effused-reflexed/Pileate	6.8–8 × 2–2.7	+
D. tibetica	4–6	Effused-reflexed/Pileate	8–10.2 × 2.5–3	-
D. tropica	5–7	Effused-reflexed	8–9.8 × 2.5–3.5	+
D. scutellata	3.5–5	Effused-reflexed/Pileate	7.8–9.2 × 3–3.6	-

Key to *Datroniella* species (updated from Li *et al.* 2014)

1. Pores 2–3/mm 2
- Pores 3–8/mm 3
2. Basidiomata cupulate, basidiospores 9–10(11) × 3–4 µm, cystidioles absent ..... *D. minuta*
- Basidiomata applanate, basidiospores (8.7)8.8–11 × (2.9) 3–4 µm, cystidioles present ..... *D. melanocarpa*
3. Basidiospores 6.8–8 µm long ..... *D. subtropica*
- Basidiospores > 8 µm long 3
4. Cystidioles absent ..... *D. tibetica*
- Cystidioles present 4
5. Pores 3.5–5/mm ..... *D. scutellata*
- Pores 5–7/mm ..... *D. tropica*

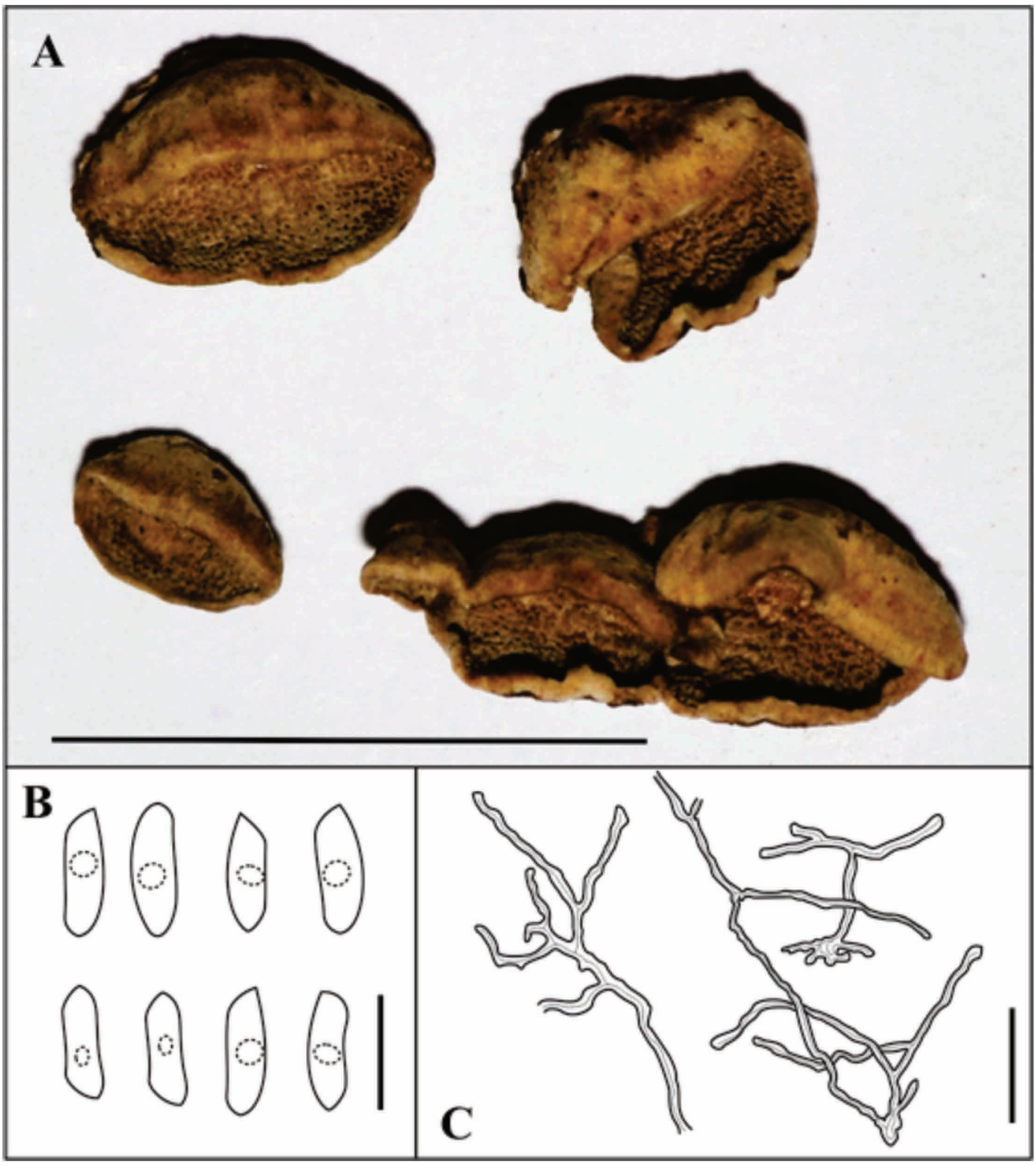

FIGURE 2. Macro- and micromorphologic aspects of *Datroniella minuta* Lira & Ryvarden. A. Basidiomata; B. Basidiospores; C. Arboriform skeletal hyphae. Scale: A = 1 cm; B and C = 10 µm.

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References

Bonfield, J.K., Smith, K.F. & Staden, R. (1995) A new DNA sequence assembly program. *Nucleic Acids Research* 23: 4992–4999. http://dx.doi.org/10.1093/nar/23.24.4992

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772. http://dx.doi.org/10.1038/nmeth.2109

Donk, M.A. (1966) Notes on European polypores - I. *Persoonia* 4 (3): 337–343.

Fries, E. (1838) Epicrisis Systematis Mycologici. *Typographia Academica*, *Uppsala* 1838: 1–610.

Gilbertson, R.L. & Ryvarden, L. (1985) Some new combinations in Polyporaceae. *Mycotaxon* 22 (2): 363–365.

Goés-Neto, A., Loguercio-Leite, C. & Guerrero, R.T. (2005) DNA extraction from frozem field-colected and dehydrated herbarium fungal basidiomata: performance of SDS and CTAB-based methods. *Biotemas* 18 (2): 19–32.

Gugliotta, A.M., Gibertoni, T.B., Drechsler-Santos, E.R., Silveira, R.M.B., Chikowski, R.S., Pires, R.M., Montoya, C.A.S., Souza, J.F., Palacio, M. & Rezende, D.H.C. (2015) *Polyporales* in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available from: http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB92517 (accessed 1 October 2016)

Justo, A. & Hibbett, D.S. (2011) Phylogenetic classification of *Trametes* (Basidiomycota, Polyporales) based on a five-marker dataset. *Taxon* 60: 1567–1583.

Li, H.J., Cui, B.K. & Dai, Y.C. (2014) Taxonomy and multi-gene phylogeny of *Datronia* (*Polyporales*, *Basidiomycota*). *Persoonia* 32: 170-182. http://dx.doi.org/10.3767/003158514X681828

Moncalvo, J.M., Lutzoni, F.M., Rehner, S.A., Johnson, J. & Vilgalys, R. (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* 49: 278–305. http://dx.doi.org/10.1093/sysbio/49.2.278

Murrill, W.A. (1904) The Polyporaceae of North America: VIII. *Hapalopilus*, *Pycnoporus* and new monotypic genera. *Bulletin of the Torrey Botanical Club* 31 (8): 415–428. http://dx.doi.org/10.2307/2478892

Nylander, J.A.A. (2004) *MrModeltest v2.* Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Ryvarden, L. (1991) Genera of polypores – Nomenclature and taxonomy – *Synopsis Fungorum* 5: 1–363.

Swofford, D.L. (2002) *PAUP*: Phylogenetic analysis using parsimony (*and other methods).* Sunderland, pp. 1–142. Available from: http://www.sinauer.com/paup-phylogenetic-analysis-using-parsimony-and-other-methods-4-0-beta.html (accessed 1 October 2016)

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. http://dx.doi.org/10.1093/molbev/mst197

Watling, R. (1969) *Colour identification chart.* Her Majesty's Stationary Office, Edinburgh (Scotland).

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications* 8: 315–322. Available from http://nature.berkeley.edu/brunslab/papers/white1990.pdf (accessed 1 October 2016)