Short communication

Strengthening *Myriostoma* (Geastraceae, Basidiomycota) diversity: *Myriostoma australianum* sp. nov.

Julieth O. Sousa^a, Iuri G. Baseia^b, María P. Martín^{c*}

^a Programa de Pós-Graduação em Sistemática e Evolução, Universidade Federal do Rio Grande do Norte, Campus Universitário Natal 59072-970, Brazil

^b Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Campus Universitário Natal 59072-970, Brazil

^c Departamento de Micología, Real Jardín Botánico-CSIC, Plaza de Murillo 2, Madrid, Spain

*Corresponding author

María P. Martín

Tel: +34 914203017

Fax: +34 914200157

E-mail: maripaz@rjb.csic.es

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ABSTRACT

A new species in the genus *Myriostoma* (Geastraceae, Basidiomycota) is described from Australia. Phylogenetic analyses of the internal transcribed spacer (ITS) and large subunit (LSU) of nuclear ribosomal DNA, as well as morphological data are evidence that the new species, *Myriostoma australianum*, is closely related to *M. capillisporum* from South Africa. Additional collections under *M. coliforme* from Brazil and USA (New Mexico) were analyzed and confirmed as belonging to *M. calongei*.

Keywords

Distribution; Earthstar; Gasteroid fungi; Systematics; Taxonomy

The genus *Myriostoma* Desv. is a rarely-occurring, star-shaped gasteroid fungus. It is very similar in macro-morphology to other star-shaped gasteroid genera, such as *Geastrum* Pers. and *Astraeus* Morgan, but *Myriostoma* is clearly distinct based on the presence of an endoperidium with several stomata and pedicels, and reticulate basidiospores (Phosri et al. 2014; Sousa et al. 2014, 2017).

Until Sousa et al. (2017) the genus was considered monotypic. Based on morphological features, as well as molecular data (ITS and LSU nrDNA), these authors recognized four species. Sousa et al. (2017) proposed the epitype *Myriostoma coliforme* (With.: Pers.) Corda, type species of the genus, and the species *M. areolatum* (Calonge & M. Mata) M.P. Martín, J.O. Sousa and Baseia from Costa Rica, *M. calongei* Baseia, J.O. Sousa, and M.P. Martín from Argentina and Brazil, and *M. capillissporum* (V.J. Staněk) L.M. Suz, A.M. Ainsw., Baseia and M.P. Martín from South Africa. Moreover, according to their data, *M. coliforme* is restricted to the Northern Hemisphere. However, Rees et al. (2005) and Moore and O’Sullivan (2014) cited this species from Australia.

Therefore, following Sousa et al. (2017), this work aimed to examine more specimens of *Myriostoma* worldwide, and to investigate whether specimens of *M. coliforme* from Australia belong to this species or, as we hypothesize, correspond to a new species. Phylogenetic analyses of ITS and LSU nrDNA are presented, along with morphological description, illustrations, and discussion of the new species in relation to the other species of the genus. This work raises the known number of *Myriostoma* species to five.

The new specimens analyzed were located in four fungus collections: Naturalis (L, Netherlands, Leiden); Universidade Federal do Rio Grande do Sul (ICN, Porto Alegre, Rio Grande do Sul, Brazil); Universidade Federal de Pernambuco (URM, Recife, Pernambuco, Brazil), and National Herbarium of Victoria (MEL, Melbourne, Victoria, Australia) (Table 1). Macro- and micro-morphological studies followed Sousa et al. (2017); basidiospore measurements were made at 1000×, and include ornamentation. Color descriptions were based on Küppers (2002). Analyses of Scanning Electron Microscopy (SEM) of basidiospores and capillitium were performed with a Hitachi S-3000N microscope.

Genomic DNA was extracted from approximately 10 mg of peridium or gleba from dry basidiomata. The Speedtools Tissue DNA Extraction Kit (Biotools B&M Labs.S.A) was used to isolate DNA based on the manufacturer's instructions with the following modifications: fungal material was macerated in 1.5 mL tubes with glass balls utilizing TissueLyser (Qiagen), and incubated 24–48 h at 56 °C. PCR amplifications, purifications, sequencing, and

alignments followed Sousa et al. (2017). Preliminary identifications were performed through megablast searches (Altschul et al. 1997) comparing the newly-generated sequences with those in GenBank. The new ITS and LSU *Myriostoma* sequences were compared with homologous sequences from GenBank, mainly published in Sousa et al. (2017) (Table 1), but only from collections with both sequences. The two alignments were optimized visually in MEGA v. 5.2. As in Sousa et al. (2017) three types of analyses were carried out for the combined ITS/LSU alignment, including *Geastrum saccatum* as outgroup: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. The combined ITS/LSU alignment was submitted to TreeBASE; the only modification was related to the ML analyses that were performed using PAUP* v.4.0b10 (Swofford 2003). The ML and the Bayesian analyses were performed assuming the general time reversible model (Rodriguez et al. 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six categories (GTR+I+G), as selected by PAUP* v.4.0b10. A combination of both bootstrap proportions and PP was used to assess the level of confidence for a specific node (Lutzoni et al. 2004; Wilson et al. 2011). The phylogenetic trees were visualized using FigTree v. 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>) and edited with Adobe Illustrator CS3 v. 11.0.2 (Adobe Systems).

Thirty-eight new sequences of *Myriostoma* are provided (Table 1, in bold). In the MP analysis of 1,590 positions, 1,390 positions were constant, 51 parsimony-uninformative and 138 parsimony-informative; gaps are treated as a "missing" character. Parsimony tree scores were identical for all the 100 trees retained: length = 220, consistency index (CI) = 0.9136, retention index (RI) = 0.9637 and homoplasy index = 0.1131. The 100 MP consensus trees (not shown) and the three ML consensus trees (not shown) have topology identical to the 50% Bayesian majority rule combined consensus tree (Fig.1); bootstrap percentages (MPbs and MLbs), as well as the posterior probability (PP) values are indicated in Fig. 1.

The collections ICN 175617, ICN 177080, L 3961249 and URM 31433, previously identified as *M. coliforme*, generated sequences which grouped into the clade of *M. calongei* (Fig. 1). The morphology of these exsiccates was revised, and the prominent warts on the endoperidium surface, as well as the basidiospore size and ornamentation, confirmed them as belonging to *M. calongei*. Exsiccates from Leiden, except L 3961249, were confirmed as indicated in the table, as belonging to *M. coliforme*. However, the sequences obtained from collections from Australia (MEL 2060796, MEL 2091620, MEL 2095275, and MEL 2305388), previously identified as *M. coliforme*, are grouped in their own well-supported clade (MPbs = 93%, MLbs = 94%, PP= 0.99), forming a sister group of *M. capillisporum*

specimens. Based on molecular analyses and morphological data, we propose the new species *Myriostoma australianum* described here.

Taxonomy

Myriostoma australianum J.O. Sousa, Baseia, & M.P. Martín, sp. nov.

Figs. 2, 3

Mycobank no.: MB 823778.

Diagnosis: *Myriostoma australianum* is closely related to *M. capillisporum*, but *M. australianum* has smaller basidiospores 6.7–8.3 μm ($x = 7.9$) and shorter warts (1.3–3 μm high) than *M. capillisporum*, which has basidiopores with 7.4–10.9 μm ($x = 8.5$) diam. and warts with 2.9–6.6 μm high.

Type: AUSTRALIA, New South Wales, Central Coast, National Park Mort Bay, Balmain, 33°51'15" S 151° 11'01" E, 1 May 2007, leg. Wilson, K.L. 10443 (holotype MEL 2305388, ITS and LSU sequence GenBank, MG675901 and MG675882).

Etymology: In reference to the type locality.

Expanded basidiomata arched, 27–55 $\times$ 24–51 mm. Exoperidium splitting into 6–9 rays, mostly arched, rarely involute, rolling up under the endoperidial body, non-hygroscopic. Mycelial layer brown (N80Y50M40), slightly encrusted to not encrusted with debris, peeling off in irregular patches. Pseudoparenchymatous layer brown (N70Y60M40) to dark brown (N80Y70M40), peeling off, rimose or absent. Endoperidial body greyish brown (N30Y20M10 to N60Y20M10), depressed globose to globose, 19–54 mm wide, surface slightly metallic and shiny, verrucose. Multiple pedicels (5–8), 2.8–6.1 mm high, concolorous with the endoperidium, laterally compressed. Multiple stomata (3–5), fibrillose, scattered across the surface of the endoperidial body, non-depressed on the endoperidium, slightly conic, lacerate with age, 1.1–3.4 mm diam. Gleba pulverulent, brown (N50Y50M40).

Mycelial layer composed of yellowish to brownish, thick-walled hyphae (0.5–0.9 μm), 2.7–6.8 μm diam., some sinuous, non-incrusted, lumen not seen. Fibrous layer composed of hyaline sinuous, thick-walled hyphae (0.5–1.0 μm), 3.5–6.3 μm diam., lumen conspicuous. Pseudoparenchymatous layer composed of hyaline to brownish, thick-walled hyphal cells, pyriform, subglobose to oval, 19.1–35.1 $\times$ 16.5–29.0 μm . Basidiospores yellowish, subglobose, (6.5) 7.1–8 $\times$ (6.3) 6.7–8.3 μm [$x = 7.9 - 0.5 \times 7.5 - 0.5$, $Q_m = 1.052$, $n = 30$], warts prominent (1.32–3 μm high) under LM; under SEM, the ornamentation is reticulate formed by warts and ridges with confluent tips, forming arcs and circles in face view.

Distribution: Australia, Sydney.

Habitat: Specimens from the collection MEL 2305388 were found in a garden bed amongst leaf litter and woodchip mulch, next to young eucalypts. Specimens from collection MEL 2091620 were found on the ground among leaf litter.

Additional specimens/cultures examined: AUSTRALIA. New South Wales, Central Coast, Royal Botanic Gardens, Sydney, 33°52' S 151°13' E, 22 May 1979, Coveny, R. GAC X27 (paratype MEL 2060796, ITS and LSU sequence GenBank, MG675902 and MG675883); Royal Botanic Gardens, Sydney, 33°52' S 151°13' E, 03 Jun 1978, Coveny, R. F172 (paratype MEL 2091620, ITS and LSU sequence GenBank, MG675903 and MG675884); Royal Botanic Gardens, Sydney, 33°52'52" S 151°13' E, May 1978, Coveny, R. s.n. (paratype MEL 2095275, ITS and LSU sequence GenBank, MG675904 and MG675885).

Recently, based on integrative taxonomy, we demonstrated the hidden high diversity in the genus *Myriostoma* (Sousa et al. 2017); however, no specimens from Oceania were included. From this continent, there are records only from Australia, and specimens were identified as *M. coliforme* (Rees et al. 2005; Moore & O'Sullivan 2014). In our study, after molecular and morphological analyses, specimens analyzed from Australia represent a distinct and new species of this genus, *Myriostoma australianum*.

The new species is very closely related to *M. capillisporum*, and the current geographic distribution of these two species (Australia and South Africa) could suggest the following hypothesis: common origin in the Gondwanan age, then by allopatric speciation, new species were established; although, more collections and loci should be studied to confirm the origin and distribution of *Myriostoma* species. These species are distinct, not only by ITS and LSU sequences, but also by the pedicel height, higher in *M. australianum* (2.8–6.1 mm high) than in *M. capillisporum* (2.3–2.9 mm high); and also by the size of basidiospores, which have different medians: *M. australianum* $7.9 \pm 0.5 \mu\text{m}$, and *M. capillisporum* $8.5 \pm 0.8 \mu\text{m}$ (Fig. 4; Table 2).

Another species close to *M. australianum* is *M. calongei*, but the latter species has distinct basidiophore ornamentation formed by confluent warts and ridges, which are planar or curved when seen in face view, and also slightly delimited stomata. *Myriostoma areolatum* is easily differentiated from *M. australianum* by the morphology of the stomata (tubular and delimited by an areola); whereas *M. coliforme* differs by its slightly verrucose endoperidium surface, and the greater number of stomata (6–24).

This study confirms that the genus *Myriostoma* is even more diverse than recently published, and that *M. coliforme* is restricted to the Northern Hemisphere.

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Figure legends

Fig.1 –Phylogenetic tree, 50% Bayesian majority rule combined consensus tree of ITS and LSU nrDNA. Numbers over branches are parsimony bootstrap (MPbs), maximum likelihood bootstrap (MLbs) values, and posterior probabilities (PP). Voucher numbers, locality and GenBank codes are indicated in Table 1.

Fig. 2 – *Myriostoma australianum* (holotype MEL 2305388). A: Expanded dried basidiomata B: Stoma in detail. C: Basidiospore (LM). D–E: Basidiospores (SEM). *Bars*: A 20 mm; B 5 mm; C–E 5 μ m.

Fig. 3 – *Myriostoma australianum* (holotype MEL 2305388), Exoperidium layers under LM. A: Mycelial layer under LM. B: Fibrous layer. C: Pseudoparenchimatous layer. *Bars*: A–C 10 μ m.

Fig. 4 – Line drawing of basidiospores. A: *Myriostoma australianum* (holotype MEL 2305388). B: *M. capillisporum* (K(M)205483). *Bars*: A, B 10 μ m.

A

B

Table 1. Specimens and sequences used to reconstruct the phylogenetic trees. New ITS and LSU sequences in bold.

Species	Locality	Year	Collection number	GenBank accession number	
				ITS	LSU
<i>Myriostoma areolatum</i>	Costa Rica: San José	1991	MA-Fungi 36165, paratype	KY096673	KY096690
<i>Myriostoma australianum</i> sp. nov.	Australia: Sydney	1979	MEL 2060796 (as <i>M. coliforme</i>),	MG675902	MG675883
	Australia: Sydney	1978	MEL 2091620 (as <i>M. coliforme</i>)	MG675903	MG675884
	Australia: Sydney	1978	MEL 2095275 (as <i>M. coliforme</i>),	MG675904	MG675885
	Australia: Sydney	2007	MEL 2305388 (as <i>M. coliforme</i>), holotype	MG675901	MG675882
<i>Myriostoma calongei</i>	Argentina: Colón	2012	MA-Fungi 83759 (as <i>M. coliforme</i>), paratype	KF988467	KF988348
	Brazil	-	L 3961249 (as <i>M. coliforme</i>)	MG675905	MG675886
	Brazil: Pernambuco	2006	UFRN-Fungos 386, paratype	KY096674	KY096691
	Brazil: Pernambuco	2007	UFRN-Fungos 990, paratype	KY096675	KY096692
	Brazil: Río Grande do Norte	2012	UFRN-Fungos 2019, holotype	KY096676	KY096693
	Brazil: Río Grande do Norte	2006	UFRN-Fungos 2020, isotype	KY096677	KY096694
	Brazil: Rio Grande do Sul	2011	ICN 175617 (as <i>M. coliforme</i>)	MG675906	MG675887
	Brazil: Rio Grande do Sul	2011	ICN 177080 (as <i>M. coliforme</i>)	MG675907	MG675888
	USA: New Mexico	1963	URM 31433 (as <i>M. coliforme</i>)	MG675908	MG675889
<i>Myriostoma capillisporum</i>	South Africa: Grahamstown	1930s	K(M)205482 (as <i>M. coliforme</i>)	KY096678	KY096695

<i>Myriostoma coliforme</i>	South Africa: Groot River	1930	K(M)205483 (as <i>M. coliforme</i>)	KY096679	KY096696
	South Africa: Cape of Good Hope	pre 1885	K(M)205540 (as <i>M. coliforme</i>)	KY096680	KY096697
	Austria: Niederösterreich	1985	L 3961241	MG675909	MG675890
	Austria: Niederösterreich	1985	L 3961244	MG675910	MG675891
	Austria:	1986	L 3961247	MG675911	MG675892
	Bulgaria:	1977	L 3961237	MG675912	MG675893
	Channel Islands: Jersey	1996	K(M)37233	EU784376	KY096698
	Channel Islands: Jersey	1999	K(M)61641	KY096681	KY096699
	United Kingdom: Isle of Jersey	2003	L 3961239	MG675913	MG675894
	France: Languedoc-Roussillon	1983	L 3961240	MG675914	MG675895
	Germany:	-	L 3961242	MG675915	MG675896
	Hungary: Bács-Kiskun	-	L 3961243	MG675916	MG675897
	Hungary: Felsőajos	2003	M. Jeppson 8714	KC582020	KC582020
	Netherlands: Noord-Holland	1980	L 3961251	MG675917	MG675898
	Portugal: Leiria	1993	MA-Fungi 31316	KY096685	KY096702
	Russia: Rostov Region	2004	K(M)154620	KY096687	KY096703
	Slovakia:	1981	L 3961238	MG675918	MG675899
	Spain: Jaén	2004	MA-Fungi 60898	KY096689	KY096704
	Spain: Madrid	-	JC. Zamora 496*	KF988337	KF988466
outgroup <i>Geastrum saccatum</i>	USA: Hawai'i	-	TNS: TKG-GE-50801	JN845203	JN845328
	USA: Ohio	1911	L 3961250	MG675919	MG675900
	Sweden	-	TK950910	KC581968	KC581968
	Sweden	2000	GH000909	KC581969	KC581969

Table 2. Diagnostic characters to *Myriostoma asutralianum* and *M. capilliporum*

	<i>M. australianum</i>	<i>M. capilliporum</i>
Pedicel height	2.8–6.1 mm high	2.3–2.9 mm high
Spores size	6.7 –8.3 μm diam.	7.4–10.9 μm diam.
Spores media	7.9 μm diam.	8.5 μm diam.
Spores warts	1.32–3 μm high	2.9–6.6 μm high

Julieth O. Souza, Iuri G. Baseia and María P. Martín confirm that the manuscript **“Strengthening *Myriostoma* (Geastraceae, Basidiomycota) diversity: *Myriostoma australianum* sp. nov.”** has not been previously published, and the information is original. All author agree to publish this paper. This version has been modified according to the comments, both reviewers and editors.