More than one fungus in the pepper pot: Integrative taxonomy unmasks hidden species within *Myriostoma coliforme* (Geastraceae, Basidiomycota)

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Abstract

Since the nineteenth century, *Myriostoma* has been regarded as a monotypic genus with a widespread distribution in north temperate and subtropical regions. However, on the basis of morphological characters and phylogenetic evidence of DNA sequences of the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (LSU), four species are now delimited: *M. areolatum* comb. & stat. nov., *M. calongei* sp. nov., *M. capillisporum* comb. & stat. nov., and *M. coliforme*. *Myriostoma coliforme* is typified by selecting a lectotype (iconotype) and a modern sequenced collection as an epitype. The four species can be discriminated by a combination of morphological characters, such as stomatal form, endoperidial surface texture, and basidiospore size and ornamentation.

Introduction

Correct species recognition is an essential requirement for the understanding of systematics, evolution and ecology. Furthermore, it is a prerequisite for population biological studies, reliable Red List assessments and effective conservation action. Recent molecular studies suggest that the magnitude of fungal taxonomic diversity is seriously underestimated [1–3]. Basidiomycete taxonomy has been revolutionized by the use of molecular techniques, which have been particularly valuable in revealing component cryptic or semi-cryptic taxa within species complexes or aggregates [4–6]. The drawbacks associated with the traditional morphology-only approach are succinctly expressed by Stielow et al. [7]: “The difficulties in defining characters and their states, and particularly the fact that distinct taxonomists assigned distinct
weights to morphological characters, have probably been the largest obstacles to the establishment of broadly acceptable classifications of numerous difficult groups of fungi.”

Historically, the gasteroid genera *Astraeus* Morgan and *Pisolithus* Alb. & Schwein. (Boletales) have been regarded as monotypic. However, recent molecular analyses, mainly of the universally adopted DNA barcode region for fungi [8], the internal transcribed spacer region of nuclear ribosomal DNA (ITS), have revealed the presence of previously hidden taxa. Several species have now been described within these genera and several more await formal naming and description [9–12].

The genus *Myriostoma* Desv., typified by *Myriostoma anglicum* Desv. (an illegitimate name for *M. coliforme*) is a very distinct and rare gasteroid genus of the family Geastraceae (Basidiomycota) which, until now, has been regarded as monotypic. Although *M. coliforme* (With.) Corda has been adopted as the correct name by some authors [13,14], the correct authorship for the name is *M. coliforme* (Dicks.) Corda. The single species is considered to have a worldwide distribution [13,15–22]. It is popularly known as pepper pot earthstar, and historically as cullender (colander) puffball, and is considered to be easily recognizable by its combination of unique characters, such as multiple endoperidial stomata (pores) and pedicels (stalks) and basidiospores with a wing-like reticulate ornamentation [21,23], characters that are absent in the earthstar genus *Geastrum* Pers. Two varieties have been described based on morphological characters: *M. coliforme* var. *capillisporum* V.J. Staněk from Cape Province, South Africa [24], and *M. coliforme* var. *areolatum* Calonge & M. Mata from Costa Rica [16].

Although *M. coliforme* is considered to be rare across the continent and red-listed in 20 European countries, evidence of large-scale population decline is lacking [22]. Moreover, contributors to the IUCN Global Fungal Red List Initiative (http://iucn.ekoo.se/iucn/species/122233/) indicated that populations are increasing in some European countries and likely to be currently underestimated globally. The main aim of the current study is to apply a combined molecular and morphological (integrative taxonomic) analysis to specimens identified as *M. coliforme* to investigate whether the name has been applied to a suite of hidden species as was previously shown to be the case in *Astraeus* and *Pisolithus*.

**Materials and methods**

**Morphological studies**

The morphological analyses were performed on specimens, including types, deposited in the Fungal Collection of the Federal University of Rio Grande do Norte (UFRN Herbarium), the collection of fungi of the Real Jardín Botánico of Madrid (MA-Fungi), the cryptogamy collection (PC) at the Herbarium of the Muséum national d’Histoire naturelle (MNHN—Paris), and the Fungarium of the Royal Botanic Gardens, Kew (K), (Table 1). Macromorphological studies were based on 26 exsiccates using a Nikon H600L stereomicroscope coupled with a Nikon DS-Ri camera for image capture. Colour descriptions followed Kornerup and Wanscher [25]. For micromorphological features, such as basidiospores, eucapillitium and exoperidial hyphae, a Nikon Eclipse Ni light microscope (LM) coupled with a Nikon DS-Ri camera was used. Basidiospore measurements were made at 1000× magnification following Sousa et al. [21] and include ornamentation. Scanning electron microscopy (SEM) was used to observe the patterns of ornamentation on basidiospores, eucapillitium and endoperidial surfaces.

**DNA extraction, PCR amplification and sequencing**

Genomic DNA was extracted from approximately 10 mg of gleba of mature dry basidiomata. The DNeasy™Plant Mini Kit (Qiagen, Valencia, CA) was used to isolate DNA from UFRN and MA-Fungi specimens, following the manufacturer’s instructions with the following.
modifications: globular masses were macerated in 1.5 ml tubes with a micropestle before suspension in lysis buffer and again after overnight incubation at 55–60°C. Both ITS and the 5′–1450–base region of the LSU were analysed using the primer pairs ITS1F/ITS4 [26–27] and LR0R combined with LR7 or LR5 [28–29] respectively. DNA amplifications were carried out using illustra™ PureTaq™ Ready-To-Go™ PCR Beads (Healthcare, Buckinghamshire, UK), adding 1 μl [10 μM] of each primer and 23 μl of isolated DNA [1.5–5.0 ng/μl].

| Table 1. Specimens and sequences included in this study. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Species**     | **Country: Locality** | **Collection year** | **Fungarium number** | **GenBank accession number** |
| **Myriostoma areolatum** comb. & stat. nov. | Costa Rica: San José | 1991 | MA-Fungi 36165, paratype | KY096673 KY096690 |
| | Costa Rica: Guanacaste | 2005 | MA-Fungi 68596, isotype | - - |
| **Myriostoma calongei** sp. nov. | Argentina: Colón | 2012 | MA-Fungi 83759 (as M. coliforme), paratype | KF988467 KF988348 |
| | Brazil: Pernambuco | 2006 | UFRN-Fungos 386, paratype | KY096674 KY096691 |
| | Brazil: Pernambuco | 2007 | UFRN-Fungos 990, paratype | KY096675 KY096692 |
| | Brazil: Rio Grande do Norte | 2012 | UFRN-Fungos 2019, holotype | KY096676 KY096693 |
| | Brazil: Rio Grande do Norte | 2006 | UFRN-Fungos 2020, isotype | KY096677 KY096694 |
| **Myriostoma capillisporum** comb. & stat. nov. | South Africa: Grahamstown | 1930s | K(M)205482 (as M. coliforme) | KY096678 KY096695 |
| | South Africa: Groot River | 1930s | K(M)205483 (as M. coliforme) | KY096679 KY096696 |
| | South Africa: Cape of Good Hope | pre 1885 | K(M)205540 (as M. coliforme) | KY096680 KY096697 |
| **Myriostoma coliforme** | Channel Islands: Jersey | 1996 | K(M)37233 | EU784376 KY096698 |
| | Channel Islands: Jersey | 1999 | K(M)61641 | KY096681 KY096699 |
| | UK: England, East Suffolk | 2006 | K(M)138625, epitype | - KY096700 |
| | UK: England, East Suffolk | 2010 | K(M)166470 | KY096682 - |
| | UK: England, West Norfolk | 2014 | K(M)195584 | - KY096701 |
| | UK: England, West Norfolk | 1880 | K(M)81165 | KY096683 - |
| | France: Région de Nay | 1964 | PC 0723885 | KY096684 - |
| | Hungary: Felsolajos | 2003 | M. Jeppson 8714* | KC582020 KC582020 |
| | Portugal: Leiria | 1993 | MA-Fungi 31316 | KY096685 KY096702 |
| | Portugal: Madeira Island | 2007 | MA-Fungi 75818 | KY096686 - |
| | Russia: Rostov Region | 2004 | K(M)154620 | KY096687 KY096703 |
| | Spain: Menorca | 1998 | MA-Fungi 40288 | KY096688 - |
| | Spain: Jaén | 2004 | MA-Fungi 60898 | KY096689 KY096704 |
| | Spain: Madrid | - | JC. Zamora 496* | KF988337 KF988466 |
| | USA: Hawai‘i | - | TNS: TKG-GE-50801 | JN845203 JN845328 |
| **outgroup** | Geastrum saccatum | Sweden | - | TK950910 KS81968 KS81968 |
| | Sweden | 2000 | GH00909 | KS81969 KS81969 |

New sequences in bold.
*Personal Fungarium.

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conditions followed Martí and Winka [30]. PCR products were verified on 1% agarose gels (UltraPureTM Invitrogen), purified using ExoSAP-IT® (USB Corporation, OH, USA) and sequenced bidirectionally in Macrogen Inc. (Seoul, South Korea).

DNA from specimens K(M)138625 and K(M)61641 was extracted using an enzymatic digestion-glass fibre filtration protocol in 96-well plate format with a vacuum-manifold as described in Dentinger et al. [31]. PCR amplifications and sequencing were performed following Dentinger and Suz [32]. DNA from the rest of the specimens from the Kew Fungarium was extracted and ITS and LSU regions amplified using Extract-N-Amp (Sigma, Dorset, UK).

The resulting sequences were edited and the consensus sequence was obtained using Sequencher 5.2.4 (Gene Codes Corp., USA). Preliminary identifications were performed through megablast searches comparing the newly-generated sequences with those in GenBank [33]. Sequences were submitted to GenBank under the accession numbers indicated in Table 1.

Sequence alignments and phylogenetic analyses

Both ITS and LSU sequences were aligned separately using Se-Al v. 2.0a11 Carbon [34]. To infer phylogenetic relationships among Myriostoma specimens, homologous sequences retrieved from the EMBL/GenBank/DDBJ databases were included in the alignment [35]. Since Geastrum is the sister genus of Myriostoma [36], two sequences of Geastrum saccatum Fr. were included as outgroup. Where ambiguities in the alignment occurred, the alignment generating the fewest potentially informative characters was chosen [37]. Alignment gaps were marked “-”, unresolved nucleotides and unknown sequences were indicated with “N”. Three types of analyses were carried out for ITS and LSU individual alignments and the combined ITS/LSU alignment: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. The combined ITS/LSU alignment was submitted to the TreeBASE Number.

In the MP analyses, minimum length Fitch trees were constructed using heuristic searches with tree-bisection-reconnection branch swapping, collapsing branches if maximum length was zero, with the MulTrees option in PAUP* 4.0b10 [38], and a default setting to stop the analyses when reaching 100 trees. Gaps were treated as missing data. Nonparametric bootstrap (MPbs) support [39] for each clade, based on 10,000 replicates using the fast stepwise-addition, was tested [40]. The consistency index, CI [41], retention index, RI [42], and rescaled consistency index, RC [42], were obtained. The ML approach was carried out using RAxML [43] in the CIPRES portal (CIPRES Science Gateway v.3.3) assuming a GTR+I+G model as selected by PAUP* 4.0b10; MLbs support for each clade, based on 1,000 replicates was tested. The Bayesian analysis [44–45] was performed using MrBayes 3.2 [46], and assuming the general time reversible model [47], including estimation of invariant sites and assuming a discrete gamma distribution with six categories (GTR+I+G), as selected by PAUP* 4.0b10. Two independent and simultaneous analyses starting from different random trees were run for 2.000.000 generations with four parallel chains and trees and model scores saved every 100th generation. The default priors in MrBayes were used in the analysis. Every 1.000th generation tree from the two runs was sampled to measure the similarities between them and to determine the level of convergence of the two runs. The potential scale reduction factor (PSRF) was used as a convergence diagnostic and the first 25% of the trees were discarded as burn-in before stationary was reached. The 50% majority-rule consensus tree and the posterior probability (PP) of the nodes were calculated from the remaining trees with MrBayes. A combination of both bootstrap proportion and PP was used to assess the level of confidence for a specific node [2,48]. The phylogenetic trees were visualized using FigTree v. 1.3.1 (http://tree.
Moreover, Kimura-2-parameter (K2P) pairwise distances between ITS sequences were obtained using PAUP* Version 4.0b10, to delimit species following a barcoding approach [8].

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank, from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix at http://www.mycobank.org/MB. The online version of this work is archived and available from the following digital repositories: PubMed Central, LOCKSS and Digital-CSIC.

Results

This study generated 32 new Myriostoma sequences (Table 1). The ITS dataset included 24 sequences, 17 generated in this study and seven obtained from EMBL/GenBank/DDBJ databases. The alignment resulted in 613 unambiguously aligned nucleotide positions (496 constant, 40 parsimony-uninformative, and 77 parsimony-informative). The 100 most parsimonious trees gave a length of 129 steps, CI = 0.9535, HI = 0.0465, and RC = 0.9688. The ML tree and the 50% Bayesian majority rule combined consensus tree (not shown) showed essentially the same topology as the parsimony strict consensus tree (not shown). Myriostoma sequences were resolved as monophyletic in a highly supported clade (MPbs = 100%, MLbs = 100%, PP = 1.0). The specimen from Costa Rica (MA-Fungi 36165) was sister to the other Myriostoma specimens (MPbs = 100%, MLbs = 100%, PP = 1.0). The rest of the Myriotoma sequences clustered in two main groups: sequences from Europe and USA (Hawai’i) formed a highly supported clade (MPbs = 99%, MLbs = 100%, PP = 1.0), whereas sequences from Argentina, Brazil and South Africa grouped together in two subgroups, one including sequences of South Africa [K(M)205482, K(M)205483 and K(M)205540] and the other one with those of Argentina and Brazil.

The LSU dataset included 21 sequences, 15 generated in this study and six obtained from sequence databases. The alignment resulted in 1391 unambiguously aligned nucleotide positions (1302 constant, 20 parsimony-uninformative, and 69 parsimony-informative). The 100 most parsimonious trees gave a length of 100 steps, CI = 0.9300, HI = 0.0700 and RC = 0.9533. The ML tree and the 50% Bayesian majority rule combined consensus tree (not shown) showed essentially the same topology as the parsimony strict consensus tree (not shown). In the three analyses, the sequences from South Africa [K(M)205482, K(M)205483 and K(M)205540] were sister to the other Myriostoma sequences, although this relationship was weakly supported (MPbs = 52%, MLbs = <50%, PP = 0.65). In the parsimony strict consensus tree, the sequence from Costa Rica appeared as the sister group to those from Argentina and Brazil, but this relationship had very low support (MPbs = 61%); moreover, in the ML and Bayesian analyses, the sequence from Costa Rica was the sister group to the clade formed by sequences from Europe and Hawai’i, a relationship with moderate support (MLbs = 54%, PP = 0.91).

In the ITS/LSU combined dataset there were 2004 unambiguously aligned nucleotide positions (1797 constant, 61 parsimony-uninformative, and 146 parsimony-informative). The 100
most parsimonious trees gave a length of 235 steps, CI = 0.9234, HI = 0.0766, and RC = 0.9474. The ML tree (not shown) and the 50% Bayesian majority rule combined consensus tree (Fig 1).
showed essentially the same topology as the parsimony strict consensus tree (not shown). Two main Myriostoma clades were produced: Clade I which comprised all sequences obtained from specimens from the Southern Hemisphere, in which the three sequences from South Africa grouped together (MPbs = 96%, MLbs = 54%, PP = 0.99) as did those from Argentina and Brazil (MPbs = 81%, MLbs = < 50%, PP = 0.99); and Clade II which comprised all sequences originating from material collected in the Northern Hemisphere, in which all sequences from Europe and the one from Hawai`i form a highly supported group (MPbs = 100%, MLbs = 85%, PP = 1.0) separated from the Costa Rica sequence.

A revision of the morphological characters present in the analysed material, such as basidiospore size and shape, together with endoperidial surface texture and stomatal morphology, supports the recognition of four distinct species: in Clade I, *M. capillisporum* comb. & stat. nov. from South Africa, with basidiospores 7.0–10.9 μm diam with long warts (2.9–6.6 μm high), and a wrinkled to slightly verrucose endoperidial surface; and *M. calongei* sp. nov. from Argentina and Brazil, with basidiospores 5.6–8.7 μm, verrucose endoperidium with prominent triangular processes (warts 0.13–0.28 mm high); and in Clade II, *M. coliforme*, with basidiospores 6.1–8.0 μm, with a wrinkled to slightly verrucose endoperidial surface (warts < 0.1 mm high), and *M. areolatum* comb. & stat. nov. from Costa Rica, with basidiospores 5.6–6.9 μm diam, with a similar endoperidial surface to that of *M. coliforme*, and *M. capillisporum*.

Furthermore, the K2P pairwise distance of *Myriostoma* ITS sequences included in Table 1 show high genetic variation between the four species considered (Table 2). There are clearly defined barcoding gaps within the ITS sequences of *Myriostoma* such that interspecific variation exceeds intraspecific variation [8]. Based on these results, a new species is described and two varieties are elevated to specific rank. No type material of *M. coliforme* is known [13,15] and as Persoon [49] referred to Dickson’s (not Withering’s) name [50], thereby sanctioning it, Dickson’s illustration is selected as lectotype and a recently sequenced collection from the same English region (East Anglia) is designated as epitype (see below).

### Taxonomic treatment

**Key to *Myriostoma* species**

1. Basidiomata with areolate and tubular stoma ....... *M. areolatum*
2. Basidiomata with non-areolate and non-tubular stoma ....... 2
3. Endoperidial surface strongly verrucose with warts > 0.1 mm high with (SEM) blunt triangular shape ............... *M. calongei*
4. Endoperidial surface wrinkled to slightly verrucose with warts < 0.1 mm having (SEM) rounded apices ............... 3

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**Table 2. Matrix of pairwise Kimura-2-parameter (K2P) distance between ITS sequences from the four species analysed in this paper.**

|        | 1  | 2         | 3         | 4         |
|--------|----|-----------|-----------|-----------|
| 1. *Myriostoma areolatum* | -* |           |           |           |
| 2. *M. capillisporum*     | 0.06918 | 0.00000   |           |           |
| 3. *M. calongei*          | 0.05623 | 0.01278   | 0.00227   |           |
| 4. *M. coliforme*         | 0.07711 | 0.03446   | 0.05219   | 0.00885   |

Maximum intraspecific distances in bold; the other values are the minimum interspecific distances.

* The intraspecific value for *M. areolatum* is not given in the table, since only one collection was sequenced.

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3. Basidiospores (7.0)7.4–10.9 μm diam with prominent ornamentation (2.9–6.6 μm high) comprising (SEM) a reticulum of branching perforated ridges, crests and warts forming arcs and broken circles in face view. ......................... M. capillisporum

3. Basidiospores 6.1–8.0 μm diam with ornamentation (1.2–1.6 μm high) comprising (SEM) a relatively low simpler reticulum with less curvature in face view. ......................... M. coliforme

**Myriostoma areolatum** (Calonge & M. Mata) M.P. Martín, J.O. Sousa & Baseia, comb. & stat. nov.–Fig 2, Mycobank MB 818615

Etymology. Referring to the numerous areolate stomata in the endoperidium.

Basionym. *Myriostoma coliforme* var. *areolatum* Calonge & Mata, Bol. Soc. Micol. Madrid 30:116 (2006), MB 546689.

Holotype. COSTA RICA, Guanacaste, Parque Nacional Barra Honda, La Capilla, Caverna Pequeña, in soil, 1 Aug. 2005, leg. C. Aguilar 130–05 (USJ 82231!, under *Myriostoma coliforme* var. *areolatum* Calonge & M. Mata).

Diagnosis. *Myriostoma areolatum* can be distinguished from other known *Myriostoma* species by its tubular (up to 1 mm high) and areolate (up to 4 mm diam.) stomata. This species is very close to *M. coliforme*, but *M. areolatum* has smaller basidiospores (5.6–6.9 μm diam).

Description. Expanded basidiomata arched, 50–80 mm wide. Exoperidium splitting into 7–9 rays, revolute to horizontal, non-hygroscopic. Endoperidial body 35–45 mm wide, shiny,

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**Fig 2. Myriostoma areolatum** (MA-Fungi 68596, isotype). (a) Dried expanded basidioma ex situ, bar = 10 mm. (b) Stomata, bar = 5 mm. (c) Basidiospores under SEM, bar = 2 μm. (d) Endoperidial surface under SEM, bar = 50 μm.

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verrucose. Multiple circular stomata (up to 42) of about 4 mm diam, which have an areolate, tubular and fimbriate peristome (1 mm high, 1 mm diam). Endoperidial surface ornamentation comprised of small processes with rounded tips (sub SEM). Eucapillitium hyphae brownish, 2.0–5.0 μm diam, surface smooth or with rounded warts, lumen yellowish. Basidiospores globose to subglobose, 5.6–6.9 μm diam, with an ornament of winged ridges 0.8–1.5 μm high; under SEM, the ornamentation is reticulate, comprising warts and branching ridges with planar and curved apices.

Known distribution. Central America (Costa Rica).

Additional specimens examined. COSTA RICA, Guanacaste, Parque Nacional Barra Honda, La Capilla, Caverna Pequeña, in soil, 1 Aug. 2005, leg. C. Aguilar 130–05 (MA-Fungi 68596, under M. coliforme var. areolatum Calonge & M. Mata, isotype); San José, Ciudad Colón, Finca “El Rodeo”, 13 Jun. 1991, leg. M.P Núñez (MA-Fungi 36165, under M. coliforme var. areolatum Calonge & M. Mata, paratype).

Myriostoma calongei Baseia, J.O. Sousa, & M.P. Martín, sp. nov., Fig 3, Mycobank MB 818593.

Etymology. In honour of Prof. Francisco Diego Calonge, for his great contribution to the study of gasteroid fungi.

Holotype. BRAZIL, Rio Grande do Norte: Baía Formosa, RPPN Mata Estrela, 6°22′31.8″S 35°01′22.4″W, 61 msl, fruiting on leaf litter, under Ficus sp., 15 July 2012, leg. B.D.B. Silva & J.O. Sousa (UFRN-Fungos 2019!).

Diagnosis. Myriostoma calongei differs from other Myriostoma species mainly by the verrucose endoperidium, with prominent triangular processes (warts 0.13–0.28 mm high). It is closely related to M. capillusporum, but M. calongei has smaller basidiospores (5.9–8.7 μm diam) with less prominent ornamentation (1.0–2.3 μm high).

Description. Unexpanded basidiomata semi-hypogeous, globose to depressed globose, 22–30 mm × 18–37 mm, surface brown (6E4), papery, with longitudinal cracks, not encrusted to slightly encrusted with debris. Expanded basidiomata arched to saccate, 15–59 × 23–120 mm. Exoperidium splitting into 4–8 rays, arched to involute, rolling up under the endoperidial body, semi-hygroscopic to non-hygroscopic. Mycelial layer brown (6E4), dark brown (6F4) to greyish brown (6F3), papery, slightly encrusted to not encrusted with debris, peeling off in longitudinal cracks or in irregular patches. Fibrous layer brownish orange (5C3), white (5A1), orange white (5A2), greyish orange (5B3), coriaceous. Pseudoparenchymatous layer dark brown (6F4; 7F4), brown (6E4; 6E5), persistent or peeling off in irregular patches. Endoperidial body greyish brown (6D3), light brown (6D4) to orange grey (6B2), brownish grey (6D2), depressed globose to globose, 9–22 × 15–55 mm, surface slightly metallic and shiny, verrucose, warts 0.13–0.28 mm high. Multiple pedicels (5–13), 1.6–3.6 mm high, concolorous with the endoperidium, laterally compressed. Multiple stomata (3–11), fibrillose, scattered across the surface of the endoperidial body, slightly delimited, non-depressed on the endoperidium, lacerate with age, 2.5–3.8 mm diam. Gleba brown (6E5) to dark brown (6F3; 6F5).

Endoperidial surface with prominent triangular warts, 0.13–0.28 mm high under SEM. Mycelial layer composed of hyaline to brownish hyphae 2.5–5.1 μm diam, thin-walled (0.6–1.1 μm), non-incrusted, lumen not seen. Fibrous layer composed of hyaline sinuous hyphae 3.6–6.5 μm diam, thin-walled (0.4–1.0 μm), lumen not seen. Pseudoparenchymatous layer composed of hyaline to yellowish, thin to thick-walled hyphal cells, pyriform, subglobose to elongated, 20.4–41.1 × 10.5–32.8 μm. Eucapillitium of brownish hyphae 1.6–4.7 μm diam, thick-walled (0.3–0.9 μm), sinuous, incrusted, lumen seen. Basidiospores yellowish, subglobose, 5.9–8.7 × 5.6–7.6 μm [x = 6.9 ± 0.6 × 6.6 ± 0.5, Qm = 1.06, n = 120], warts prominent (1.0–2.3 μm high) under LM; under SEM, the ornamentation is reticulate formed by confluent warts and ridges which are planar or curved when seen in face view.
Known distribution. South America (Brazil and Argentina).

Additional specimens examined. ARGENTINA, Colón, Ubajay, El Palmar, S. Suaza, next to *Allophyllus edulis* and *Ligustrum lucidum*, 31 May 2012, leg. J. Maller & J.C. Zamora (MA-Fungi 83759, paratype). BRAZIL, Rio Grande do Norte, Baía Formosa, RPPN Mata Estrela, 6°22’32.1”S 35°01’21.6”W, 12 Jun. 2006, leg. B.D.B. Silva, J.O. Sousa & A.G. Leite (UFRN-Fungos 2020, isotype); Pernambuco, Buíque, Parque Estadual Vale do Catimbau, 8°30’22”S 37°...
19°23′W, fruiting on humid ground, 4 Aug. 2006, leg. J.F. Silva (UFRN-Fungos 386, paratype);
Morro do Cachorro, 8°34′01″S 37°14′19″W, fruiting on ground under Ziziphus sp., 16 Apr.
2007, leg. T. Ottoni, (UFRN-Fungos 990, paratype).

Remarks. Specimens of this new species were identified in Sousa et al. [21] as M. coliforme.
In Brazil, this species occurs in two vegetation types with quite different characteristics: Atlantic
Rain Forest and "Caatinga". The Atlantic Rain Forest is a “hotspot” of biodiversity comprising
tropical forest formations, which extend along the east coast of the South American
continent, while "Caatinga" is a vegetation type endemic to Brazil found in semiarid regions
and specialized for life in a dry climate [51,52,53,54]. According to the specimen label, in
Argentina this species was found next to endemic (Allophyllus edulis) and introduced (Ligustrum
lucidum) plants.

Myriostoma capillisporum (V.J. Staněk) L.M. Suz, A.M. Ainsw., Baseia & M.P. Martín,
comb. & stat. nov., Fig 4, Mycobank MB 818616.
Etymology. Derived from Latin *capillus*, meaning hair and referring to the prominent hair-like spore ornamentation.

Basionym. *Myriostoma coliforme* var. *capillisporum* V.J. Staněk, in Pilát (ed.) Flora ČSR B1–Gasteromycetes: 402 (1958), MB 347330.

Type. **SOUTH AFRICA**, Cape Province, Belvidere, A.V. Duthie No. 31355 (Herb?).

Diagnosis. The basidiospore size, (7.0–)7.4–10.9μm, and ornamentation comprising prominent warts under LM (2.9–6.6μm high), which under SEM are formed by warts and ridges with confluent tips forming arcs and circles in face view, clearly distinguish this species from the other *Myriostoma* spp.

Etymology. Derived from Latin *capillus*, meaning hair and referring to the prominent hair-like spore ornamentation.

Expanded basidiomata arched 31–37 × 33–68 mm. Exoperidium splitting into 6–7 rays, arched to revolute, rolling up under the basidioma, non-hygroscopic. Pseudoparenchymatous layer ephemeral, absent in some basidiomata. Endoperidial body depressed globose, 11–12 × 26–35 mm (excluding pedicels), slightly verrucose, stalked. Multiple pedicels (6–7), 2.3–2.9 mm high, paler than or concolorous with the endoperidium. Multiple stomata (up to 4), fibrillose, scattered across the surface of the endoperidial body, lacerate with age. Endoperidial surface with irregular processes under SEM. Basidiospores light yellowish, subglobose, (7.0–)7.4–10.9μm [x = 8.5 ± 0.8 n = 27], warts prominent (2.9–6.6μm high) under LM; the ornamentation is reticulate under SEM, formed by warts and ridges with confluent tips, forming arcs and circles in face view.

Known distribution. South Africa.

Additional material studied. **SOUTH AFRICA**, Cape of Good Hope, pre 1885, Mac Owan (ex herb. M.C. Cooke), (K(M)205540, Kew Fungarium); Grahamstown, 1930s, leg. N.J.G. Smith, Smith 334, (K(M)205482, Kew Fungarium); Groot River, 1930s, leg. N.J.G. Smith, Smith 320, (K(M)205483, Kew Fungarium).

*Myriostoma coliforme* (Dicks.) Corda 1842 [55], Fig 5, Mycobank MB 122233

Etymology. The specific epithet *coliforme* means colander- or strainer-like, because “*colum*” means strainer in Latin, referring to the multistomatous endoperidium.

Lectotype (designated here). Dickson’s illustration [as *Lycoperdon coliforme*], in Dickson (1785) Fasc. pl. crypt. brit. (London) 1:24 (Tab. III: Fig 4), reproduced as Fig 6, IF 552745.

Epitype (designated here). **UNITED KINGDOM**: England. East Suffolk (vice county 25), north of Ipswich, Nat. Grid Ref. TM15, fruiting in sandy soil, 19 February 2006, leg. C. Povey (K(M)138625!), IF 552746.

Supported lectotypification. See above.

Basionym. *Lycoperdon coliforme* Dicks., Fasciculus plantarum cryptogamicarum Britanniae 1:24 (1785), MB 228521.

Diagnosis. *Myriostoma coliforme* is closely related to *M. areolatum*, but clearly distinguished by the presence of flattened stomata, whereas in *M. areolatum* the stomata are areolate and tubular.

Description. Expanded basidioma arched 38–135 mm wide. Exoperidium splitting into 6–11 rays arched, involute to horizontal, non-hygroscopic. Mycelial layer greyish yellow (3D4), papery, encrusted with debris, squamous or slightly peeling in longitudinal cracks in some basidiomata. Fibrous layer pastel yellow (1A4) to pale yellow (2A2), papery. Pseudoparenchymatous layer greyish yellow (3C4; 4B4) to light brown (5C6), persistent or peeling in some basidiomata. Endoperidial body pale yellow (2A3), blond (4C4) to yellowish brown (5D4), depressed globose to globose, 18–40 × 24–50 mm (excluding pedicels), surface wrinkled to slightly verrucose (warts < 0.1 mm high with rounded tips), stalked. Multiple pedicels (3–9), 3–10 mm high, concolorous with the endoperidial body. Multiple stomata (6–24), >30
visible in some Swedish specimens illustrated in Sunhede (1989), fibrillose, flattened, non-delimited, lacerate with age, 1.5–5 mm diam. Gleba light brown (5E5) to brown (6E7).

Endoperidial surface comprised of small processes under SEM. Eucapillitial hyphae brownish 3.3–4.0 μm diam, thick-walled (0.9–1.5 μm), sinuous, non-encrusted, lumen seen. Basidiospores yellowish, subglobose 6.1–8.0 μm [x = 7.0±0.4 n = 60], warts 1.2–1.6 μm high under
LM; under SEM, the ornamentation is reticulate, formed by warts and ridges with planar or slightly curved tips in surface view.

Known distribution. Europe, North America and Oceania (Hawai‘i).

Additional material studied: CHANNEL ISLANDS, Jersey, Les Vaux Cuisin, fruiting on sandy soil, 23 May 1999, leg. B.M. Spooner (K(M)61641, Kew Fungarium); St Ouen’s, fruiting on old sand dunes, Feb. 1996, leg. N. Armstrong (K(M)37233, Kew Fungarium). FRANCE, 64 Pyrénées-Atlantiques, Région de Nay (Basses Pyrénées), 1964, leg. J. Beller (PC 0723885). HUNGARY, Bacs-Kiskun, Ladánybene, Felsolajos, 8 Sep. 2003, leg. L. Nagy & M. Jeppson (personal herbarium MJ8714; ITS and LSU nrDNA Acc. Number KC582020). PORTUGAL, Leiria, Mato das Acacias, 2 Apr. 1983, leg. L. Freire & M. Castro (MA-Fungi 31316); Maderia, Jardines de la Universidad, under Cupressus sp., 8 Aug. 2007, leg. M. Sequeira (MA-Fungi 75818). RUSSIA, Rostov region, Sholokhovsky District, Schebunyevsky village, fruiting on pasture soil, 20 July 2004, leg. Y. Rebriev 1090 (K(M)154620, Kew Fungarium). SPAIN, Jaén, Andújar, Las Viñas, under Eucalyptus sp. 18 Nov. 2004, leg. F. Jiménez (MA-Fungi 60898); Balearic Islands, Menorca, Maó, Sant Antoni, under Quercus ilex, 27 Nov. 1998, leg. B. Mateo (MA-Fungi 40288). UNITED KINGDOM: England, East Suffolk, near Harleston, fruiting on sandy soil, 30 July 2010, leg. N. Mahler (K(M)166470, Kew Fungarium); idem, 18 Nov. 2014, leg. N. Mahler (K(M)195584, Kew Fungarium); England, West Norfolk, Hillington, fruiting on soil, Oct. 1880, leg. P. Hebgin (via Lady Ffolkes) (K(M)81165, Kew Fungarium).

Sequences retrieved from GenBank (specimens not studied morphologically). USA, Hawai‘i, TKG-GE-50801 (TNS Herbarium; ITS sequence Acc. Number JN845203; LSU sequence Acc. Number JN845328).

Remarks. Although we have not analysed DNA from specimens from the USA, other than those from Hawai‘i, this species is widespread in North America. Descriptions of North...
American material provided in Coker and Couch [56] and Bates [15] accord with the lectotypification proposed here. Specimens were recorded under desert hackberry (*Celtis pallida*), juniper (*Juniperus* spp.), mesquite (*Prosopis* spp.) or cactus species [15]. The presence of *M. coliforme* on Hawai‘i Island was also recorded in Smith and Ponce de León [57] and Gilbertson et al. [58], under *Sophora chrysophylla*, an endemic Fabaceae; moreover, Hemmes and Desjardin [18] collected numerous specimens in Manuka Wayside Park (Hawai‘i Island) under several introduced and endemic plants.

Tejera et al. [59] provided descriptions of specimens identified as *M. coliforme* from the Canary Islands that are also in accordance with the lectotypified concept. Esqueda-Valle et al. [19,60] recorded *M. coliforme* in Mexico (Sonora Desert) under *Prosopis* sp.; however, there are no descriptions to confirm that these authors are referring to the lectotypified concept. In South America, there are several records of *M. coliforme*, mostly from southeast Brazil [61–62] and from areas of “Caatinga” vegetation in northeast Brazil: under *Spondias tuberosa* [63] and under *Ficus* sp. [21, 64]. With the exception of the specimens in Sousa et al. [21], the South American collections were not subjected to DNA sequence analysis, but based on morphological characters alone they should be assigned to *M. calongei*.

**Discussion**

Since the nineteenth century, the genus *Myriostoma* has been regarded as monotypic. Pegler et al. [14] indicated that *M. coliforme* is widespread in north temperate and subtropical regions. However, our study reveals that the name *M. coliforme* has been applied to at least four members of a species complex each of which is well characterized by a combination of

![Fig 7. Distribution map of the Myriostoma specimens included in the phylogenetic analyses of this study (geometric figures in colour).](https://doi.org/10.1371/journal.pone.0177873.g007)
morphological characters, of which the stomata, endoperidial surface and spore size and ornamentation are the most important. Consequently, the distribution of M. coliforme in the original sense has been overestimated (IUCN webpage: http://iucn.ekok.se/iucn/species_view/122233/; [22]; Fig 7). Although further worldwide sampling is clearly required, current DNA-based evidence supports a European and North American range for M. coliforme.

A lack of knowledge about dispersal mechanisms coupled with insufficient molecular data on Neotropical fungi have resulted in speculative interpretations of their biogeographic distribution, especially for saprotrophic taxa such as Myriostoma [20,65]. Recent studies demonstrate that fungal species with a cosmopolitan distribution are the exception [66,67]. In general, most names applied to species with an apparent worldwide distribution represent species complexes rather than good species [68]. Based on the conclusion of Kasuya et al. [20] regarding the earthstar Geastrum triplex Jungh., which has a similar, bellows-like, spore dispersal mechanism, Myriostoma dispersal capacity is not expected to be very effective over long distances.

This work opens new perspectives on this striking genus through the application of integrative taxonomy using a combined molecular and morphological approach. Indeed, the production of revised distribution maps of Myriostoma species is an essential prerequisite for ecological studies and robust and reliable IUCN-compliant conservation assessments.

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