
Short Note

On two endangered species (*Myriostoma coliforme* and *Battarrea* sp.) sharing the same microhabitat

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Received 20/11/2019; accepted 26/01/2020

Abstract

Specimens of *Myriostoma coliforme* and *Battarrea* sp. were found in the same habitat in a cypress-aligned alleyway in the town of Trevignano Romano in the province of Rome, Italy. Furthermore, other specimens of *M. coliforme* were found in the Villa Ada Urban Park in the city of Rome. Species attribution by morphological features is difficult in *Myriostoma* and still under investigation in *Battarrea* and was therefore also assessed by DNA-based analysis. Specifically, the *Battarrea* sample was found to not belong to the *phalloides/stevenii* complex. Although both genera are found widely spread, only small and scattered populations exist because their habitats are quite peculiar and in some terms coinciding. In fact, both species are presently relevant for assessment for the future European Red list of endangered macrofungi. The places of discovery could be useful for the study of the environmental and microclimatic aspects for the conservation of these two rare species.

Key words Red List; conservation; habitat; *Cupressus sempervirens*; microclimate; ITS

Introduction

The European Council for the Conservation of Fungi (ECCF) promotes the protection of fungi. Its activities comprise the redaction of a European Red List of endangered macrofungi. In the most recent evaluation of candidates (ECCF, 2019), two fungal species are present with the status of “relevant for assessment for the future European Red list”: *Myriostoma coliforme* (Dicks.) Corda (Agaricomycetes, Geastrales) and *Battarrea phalloides* (Dicks.) Pers. (Agaricomycetes, Agaricales). Both species are saprotrophic fungi with a wide global distribution but with small and scattered populations restricted to a few sites, mostly sandy soils. Although their final status could be “Least Concern” globally, at the local level both species are present in national Red Lists with a status ranging from “Vulnerable”, “Endangered”, “Critically Endangered” up to “Extinct” and are generally considered rare (Dahlberg and Croneborg, 2003; Amalfi, 2011; Fraiture and Otto, 2015).

Myriostoma Desv. was long considered a monotypic genus, however recently five species have been defined (Sousa et al., 2017; Sousa et al., 2019). We have found specimens of *M. coliforme* for the first time in 2016 in Rome, inside the Villa Ada Urban Park. They grew under olive trees nearby a secular cypress and were present in this same location every subsequent year. Furthermore, in spring/summer 2019 we found other specimens of *M. coliforme* in a cypress-aligned alleyway in Trevignano Romano, a village inside the Bracciano-Martignano Regional Natural Park.

The taxonomy of the genus *Battarrea* has undergone significant changes in time, especially upon molecular analyses (Ivančević et al., 2016). Most interestingly, by DNA barcoding the synonymy of *B. stevenii* with *B. phalloides* has been ascertained (Martín and Johannesson, 2000). At present, four distinct clades are included in the genus, one of which coincident with the type species *B. phalloides* (Martín et al., 2013).

Concomitantly with the finding of the *M. coliforme* specimens in Trevignano Romano, we noted specimens of *Battarrea* in the same cypress-aligned alleyway leading towards the Bracciano Lake.

These findings, together with the description of the two sites, represent an addition to the knowledge of the microambients of these two endangered species, useful for their preservation and in the specific case of the genus *Battarrea* also for the research of distinctive markers between the four clades recognised by DNA sequence data. With respect to the *Battarrea*, our intent is to study a major number of specimens, also in collaboration with interested researchers/mycologists, from a genetic and biochemical point of view in relation to their habitat because it does not seem a coincidence that other specimens found in Italy, also associated with cypresses in proximity of a body of water, belong to the same clade.

Materials and Methods

Collection of samples

Trevignano Romano is a small town, municipality of the Metropolitan City of Rome Capital and part of the Bracciano-Martignano Regional Natural Park. It is located about 35 km north-west of Rome on the north coast of the volcanic Bracciano lake and south at the toe of the slope of Monte Rocca Romana (612 m asl). The site of collection (Fig. 1, 42°09'36" N 12°13'44" E, 173 m amsl) is a 140 meters long cypress-aligned alleyway perpendicular to the lake's coast connecting the lake front with an interior road. The alleyway is private but freely accessible; it is a dirt road, covered by cypresses' debris. The collections examined are indicated in Table 1.

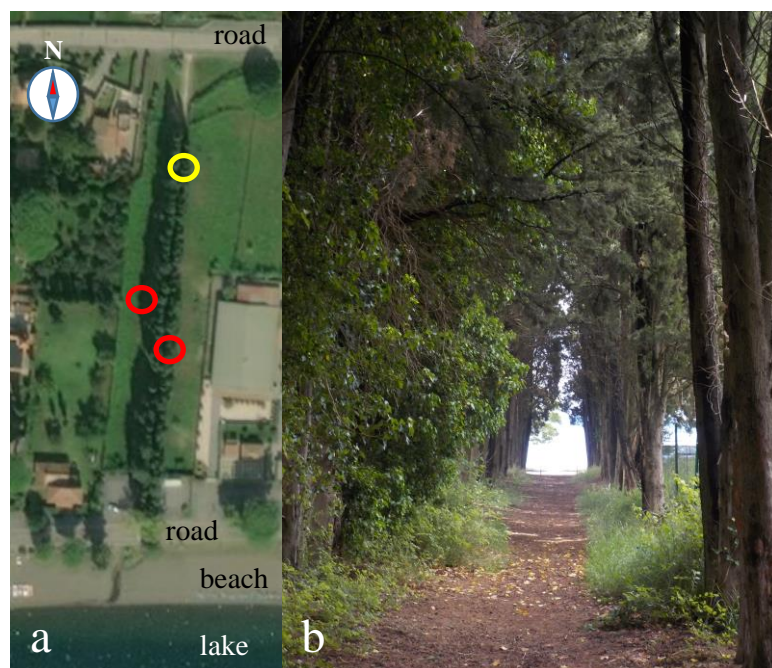


Fig. 1 – Satellite (a) and land (b) images from the site collection of the specimens in Trevignano Romano. The yellow circle (42°09'36" N 12°13'44" E) indicates the site of *Battarrea* sp., red circles of *M. coliforme*. (Source of the satellite image: Esri, i-cubed, USDA, USGS, AEX, GeoEye, Getmapping, Aerogrid, IGN, IGP, UPR-EGP and the GIS User Community)

Morphological analysis

Microscope images of the fungi were obtained with a digital camera AmScope (Irvine CA, USA) MU500 mounted on an AmScope B490 microscope, operating at $\times 800$ and $\times 2000$ magnifications. Samples were analysed dry mount.

Table 1 - The collected specimens

Species	Fungarium/ UNITE codes	Host	Date	Site
<i>Myriostoma coliforme</i>	TU114952/UDB0754090	Under <i>Olea</i> and <i>Cupressus</i>	2018/10/17	Rome
<i>M. coliforme</i>	TU114971/UDB0778014	Under <i>Cupressus</i>	2019/04/14	Trevignano Romano
<i>Battarrea</i> sp.	TU114972/UDB0778015	Under <i>Cupressus</i>	2019/05/12	Trevignano Romano

Molecular analysis

Ribosomal DNA-based analysis was performed on all three specimens of the fungi in the frame of the UNITE project (Kõljalg et al., 2013; <https://unite.ut.ee/>). DNA extraction, PCR amplification of SSU partial, ITS1, 5.8S, ITS2, LSU partial regions and sequencing were performed as in Voitk et al. (2018). Sequences were aligned with the MUSCLE v3.8.425 algorithm (Edgar, 2004) in Aliview 1.26 software (Larsson, 2014) and fine-tuned manually. The Basic Local Alignment Search Tool (BLAST 2.8.1+; Camacho et al., 2009) was used to assess sequence identity. For phylogenetic analyses, Bayesian inference was applied as before (Knijn and Ferretti, 2018) except that in this case GTR was used as the model for nucleotide evolution as suggested by MrModeltest 2.3 (Nylander, 2004). Phylogenetic trees were drawn using FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/>). The newly obtained sequences were submitted to UNITE (accession numbers in Table 1).

Results, discussion and conclusion

Morphological characteristics

Both samples of *M. coliforme* basidioma display the typical morphological aspects of the genus (Fig. 2a). Basidiomata were globose/sub-globose with 7 to 9 exoperidial rays (or laciniae). The exoperidium was composed of an ochre or light brown mycelial layer, sometimes made up of irregular patches, a fibrous whitish-cream coloured layer and a light or dark yellow coloured pseudoparenchymatous layer. The endoperidial globose body (1.5 × 1.0 cm) was grey-light brown coloured, covered with evident minute warts. The number of stomata ranged from 9 to 17, and the ochre pedicels were laterally compressed. The basidiospores (Fig. 2b) were light brown and globose and measured 5.5 ± 0.4 µm (n = 18) in both specimens.

The samples of *Battarrea* sp. (Fig. 2c) were observed from spring to autumn. Their capitulum was initially covered by a white peridium surrounding the gleba, attached to the stem. In our specimens, the diameter of the capitulum measured about 3.5 cm. In mature samples the upper part of the veil was detached from the brown spore-bearing gleba. The basidiospores (Fig. 2d) were spherical or sub-spherical, brown and decorated, 4–6 µm in diameter. Elaters were sometimes evident at microscopic analyses (14–24 × 5 µm), cylindrical with spiral thickenings.

Mature stems protrude from the exoperidium, a volva-like veil of whitish colour, initially gelatinous then dry. The fibrillose and scaly stems changed colour from whitish to brownish due to the spores, were about 11 cm tall, buried in the terrain for about 4 cm together with the 2.5 cm long volva. The stem becomes hollow and woody with age; some specimens persisted in this dry state for several months.

Molecular data

Myriostoma coliforme is found mainly in Europe, but also in some sites in the United States; *M. capillisporum* (V.J. Staněk) Suz, A.M. Ainsw., Baseia & M.P. Martín is found in South Africa, *M. calongei* Baseia, J.O. Sousa & M.P. Martín in Brazil and Argentina, *M. areolatum* (Calonge & M. Mata) M.P. Martín, J.O. Sousa & Baseia in Costa Rica. These species differ from one another for morphological and microscopical characteristics as well as biogeography (Sousa et al., 2017).

Recently a new species has been described, *M. australianum* J.O. Sousa, Baseia & M.P. Martín, similar to *M. capillisporum* (Sousa et al., 2019).

Both *Myriostoma* specimens found by us in the two sites morphologically resembled *M. calongei*, but BLAST searches and phylogenetic analysis (Fig. 3) confirmed them to be *M. coliforme*.

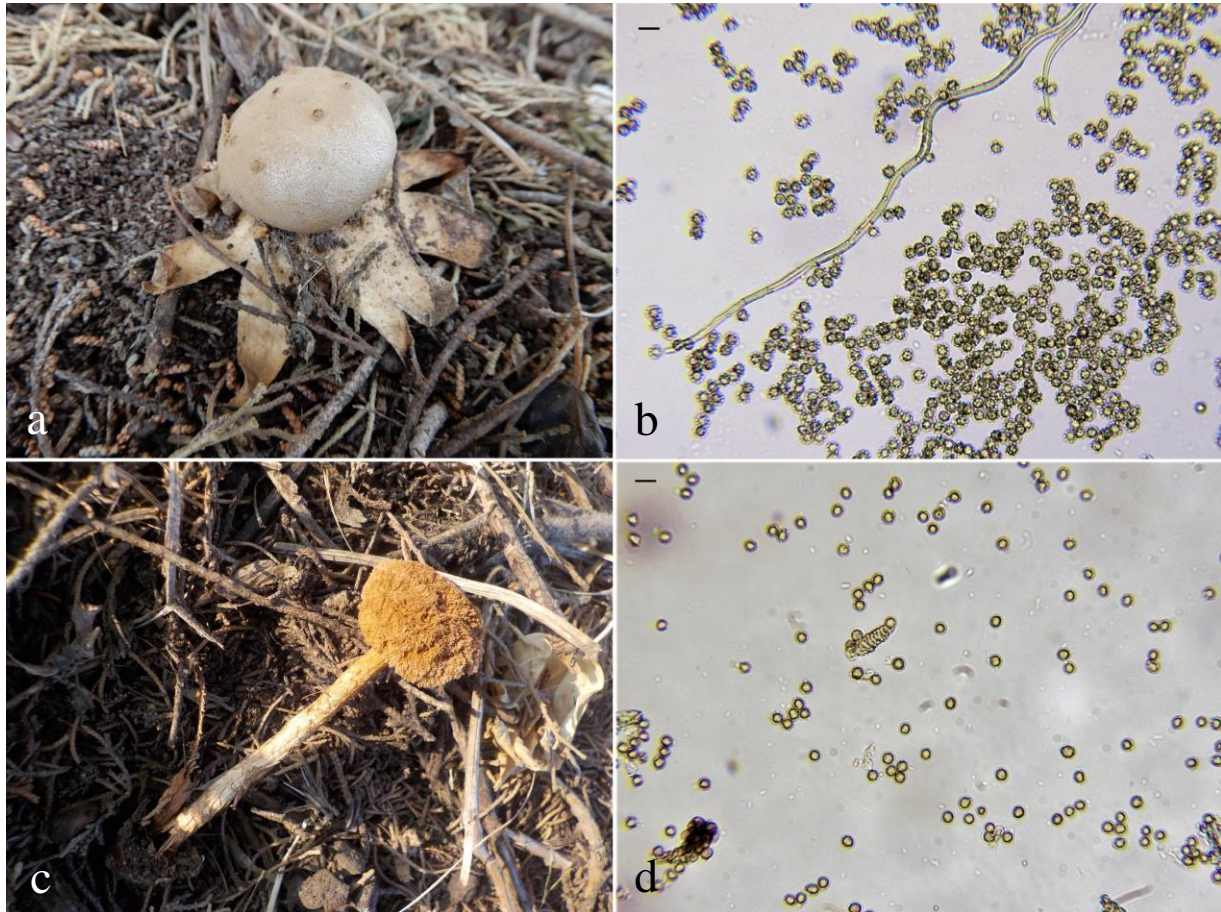


Fig. 2 - Basidioma of *M. coliforme* collected in Trevignano Romano under *Cupressus sempervirens* (a); basidiospores and capillitium of *M. coliforme* (b); basidioma of *Battarrea* sp. collected in Trevignano Romano under *Cupressus sempervirens* (c); basidiospores and an elater of *Battarrea* sp. (d). Bar = 10 μ m

The sample found in Rome (accession number UDB0754090) together with other three specimens is somewhat distant from the central clade of *M. coliforme* sequences due to only one single nucleotide polymorphism in the ITS2 region about 10 nucleotides away from the LSU region (a Cytosine transformed in Adenine in these four samples).

A relevant aspect of the *Battarrea* sample is that phylogenetic analysis (Fig. 4) proved that the specimen did not belong to the *phalloides/stevenii* complex (clade B2 in the phylogenetic tree) but fitted well into what has been defined clade A (Martín et al., 2013), which has not yet been assigned a species name. No evident morphological or geographical differences, specific and reproducible, have been found between the specimens belonging to the four clades (Garrido-Benavent, 2014), indicating that the genetic variations could be due to cryptic characteristics. It would be an interesting study to assert if the differences at the genetic level are correlated with a particular habitat or growth substrate and thus to a relative different enzymatic content.

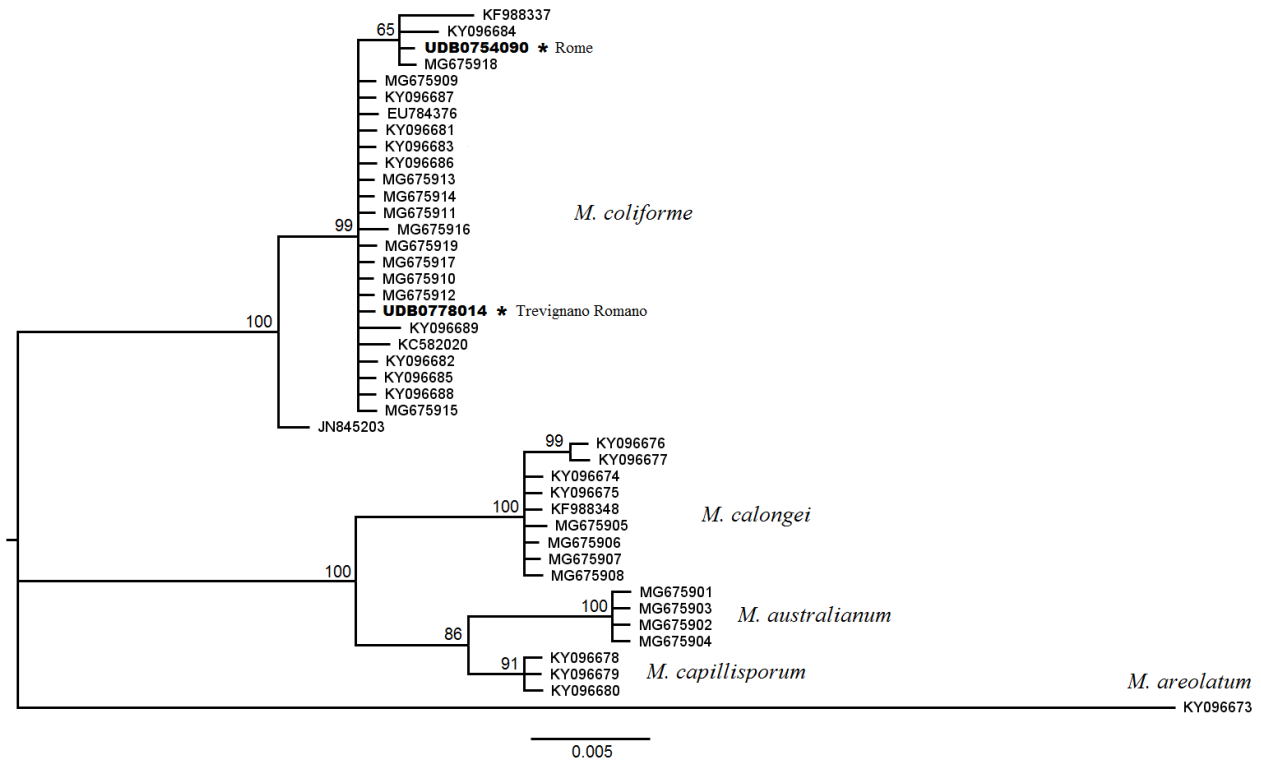


Fig. 3 - Phylogenetic tree with posterior probability values obtained through Bayesian inference based upon alignment of the nuclear ribosomal ITS sequences of *Myriostoma* species, identified by their UNITE/GenBank accession number and associated species. The sequence from *M. areolatum* was used to root the phylogenetic tree. The new samples described in the article are indicated in bold. Bayesian posterior probabilities are reported on each branch. The final mean standard deviation of the separated frequencies was 0.0076.

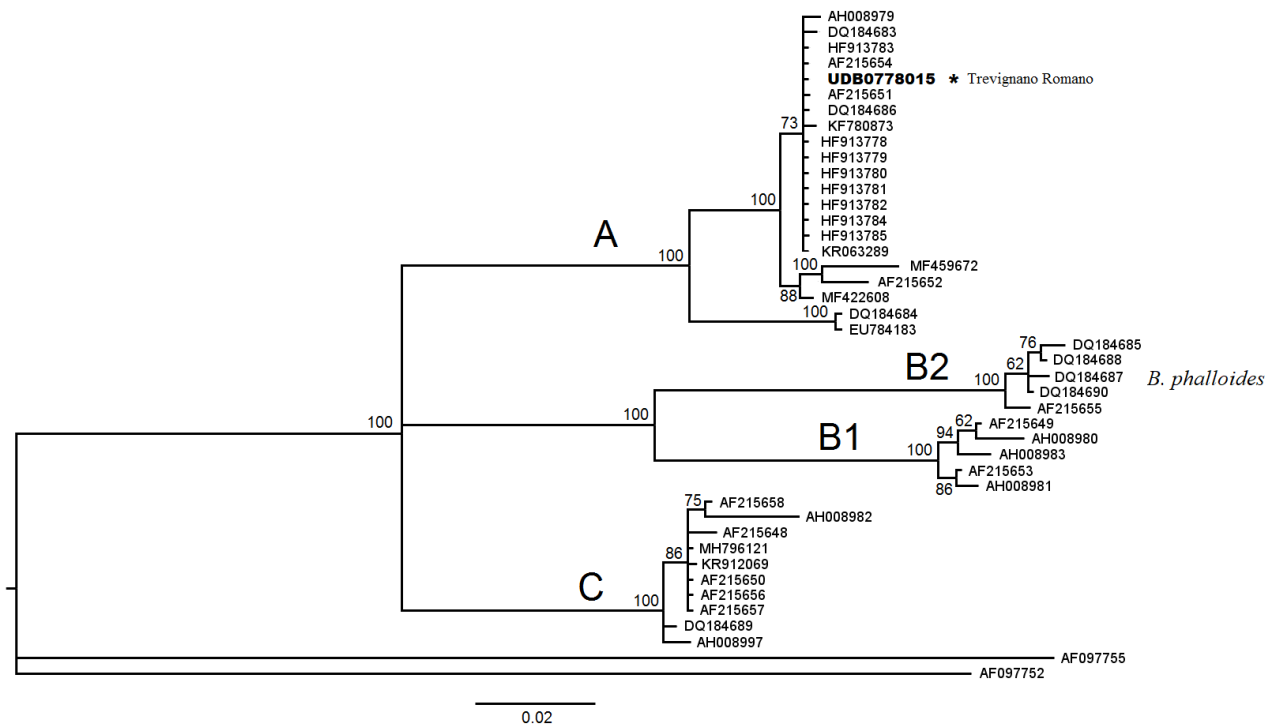


Fig. 4 - Phylogenetic tree with posterior probability values obtained through Bayesian inference based upon alignment of the nuclear ribosomal ITS sequences of *Battarreia* specimens, identified by their UNITE/GenBank accession number. Sequences from *Chlamydopus meyenianus* (AF097755) and *Tulostoma beccarianum* (AF097752) were chosen as outgroups to root the tree. The new sample described in the article is indicated in bold. Clades are indicated as A, B1, B2 and C according to Martín et al. (2013). Bayesian posterior probabilities are reported on each branch. The final mean standard deviation of the separated frequencies was 0.0053.

Conservation

An interesting result of this study is that specimens of *M. coliforme* and *Battarrea* sp. clade A were growing contemporarily in a cypress-aligned alleyway in Trevignano Romano, probably because they share a particular type of habitat. In Italy, both are frequently associated with cypress trees but certainly other ambient factors like soil composition are influent since especially central Italy is very rich in cypress groves. However *Myriostoma* and *Battarrea* specimens are not equally frequently found. The same concomitance of these two species has previously been reported in Italy for the Polvese island in the Trasimeno lake (Pecoraro et al., 2014; Angelini et al., 2016). Similarly, in this case the *Battarrea* specimen belonged to clade A (KF780873 in Fig. 4). The Polvese island was declared scientific and didactic park as well as a territorial laboratory for sustainable development inside the Regional Park of the lake in 1995. Furthermore, in 2012 the island was included in the European ecological network “Natura 2000” as a site of community interest in the form of special conservation zones (SCZ) (Angelini et al., 2016). Thus, in the latter case the habitat is well protected and could serve as a virtuous example of conservation.

The situation in Trevignano Romano results more complicated, despite the area making part of a Regional Natural Park. In fact, as a private property with public access, the habitat could be the object of various threats. A European Red List of endangered macrofungi or at least an Italian Red List would be important to convince local authorities to act for the preservation of such a particular environment. An Italian Red List has not yet been redacted, although efforts in this direction are being made based upon the Checklist of Italian fungi (Onofri et al., 2005) for instance by the Italian Institute for Environmental Protection and Research (ISPRA; Siniscalco et al., 2019) or at a Regional level in Tuscany (Antonini et al., 2006) and in Umbria (Angelini et al., 2017).

The main point is that, especially in the light of the recent division in species/clades of both genera which makes each individual species even more rare, there is a strong need to maintain and protect current populations. A comparison between the two places of discovery could prove valuable for the study of the particularities of their habitat in terms of environment and microclimate.

Acknowledgements

Irja Saar was supported by the Estonian Research Council (IUT20-30), the European Regional Development Fund (Centre of Excellence EcolChange).

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