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Rapid Communication

A new contribution to the alien macroalgal flora of the Ustica Island Marine Protected Area (Tyrrhenian Sea, Italy)

Anna Maria Mannino[1], Maria Cristina Gambi[2], Tiziana Dieli[3] and Paola Gianguzza[4,*]

[1]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Section of Botany and Plant Ecology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy*

[2]*Stazione Zoologica Anton Dohrn, Dept. of Integrative Marine Ecology, Villa Dohrn-Benthic Ecology Center, 80070 Ischia (Napoli), Italy*

[3]*AQUAPRO S.a.s., Via A. Di Giovanni 14, 90144 Palermo, Italy*

[4]*Department of Earth and Marine Science, University of Palermo, CoNISMa, Via Archirafi 18, 90123 Palermo, Italy*

Author e-mails: annamaria.mannino@unipa.it (AMM), gambimc@gmail.com (MCG), tdieli@hotmail.com (TD), paola.gianguzza@unipa.it (PG)

**Corresponding author*

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Abstract

A second record of the non-indigenous species *Botryocladia madagascariensis* G. Feldmann (Rhodophyta) along the coasts of the "Ustica Island" Marine Protected Area (Tyrrhenian Sea, Italy) is reported. With this additional record for the Sicilian coasts, we provide a description of collected specimens of *B. madagascariensis* and habitat details. Numerous fertile female gametophytes with cystocarps immersed in vesicles were also observed, for the second time in the Mediterranean Sea, and described.

Key words: non-indigenous species, *Botryocladia madagascariensis,* Mediterranean Sea

Introduction

The spread of non-indigenous species (NIS) is an ongoing phenomenon which is widely recognized as a major threat to biodiversity and natural ecosystem function (Wallentinus and Nyberg 2007). In the Mediterranean Sea, the annual number of recorded NIS has increased exponentially over the last 100 years (Occhipinti-Ambrogi et al. 2011a, b; Katsanevakis et al. 2013). Multiple human-related stressors (i.e. habitat fragmentation and destruction, pollution, maritime transport, tourism, aquaculture, exploitation of resources, global warming, and opening of the Suez Canal) have led to the introduction of ~ 1000 NIS, of which 134 are macrophytes (24 Chlorophyta, 79 Rhodophyta, 30 Ochrophyta, and 1 Tracheophyta) (Verlaque et al. 2015; Alós et al. 2016; Galil et al. 2017).

Identifying future NIS and taking effective steps to prevent their dispersal and establishment constitutes an enormous challenge to marine biologists. Sicily and its surrounding islands, located at the crossroads between the Western and Eastern Mediterranean, are particularly vulnerable to the introduction of NIS. In this region, the exposure to multiple transport pathways, including fisheries and recreational fleets, has promoted the introduction either accidentally or intentionally of several NIS, despite the high number of Marine Protected Areas (MPAs) already established. This area plays an important role as a receiver, transit and donor zone for NIS within the Mediterranean Sea, and monitoring and surveillance is required to reduce the risk of future NIS introductions. The creation of permanent habitat "alarm systems" might be an effective tool in the management of NIS introductions. In this regard, Sicilian MPAs can play an important role as "sentinel sites" where the effects of NIS invasion can be studied and management strategies can be developed to counter and contrast such negative effects. In order to develop effective managing plans for the prevention and control of NIS,

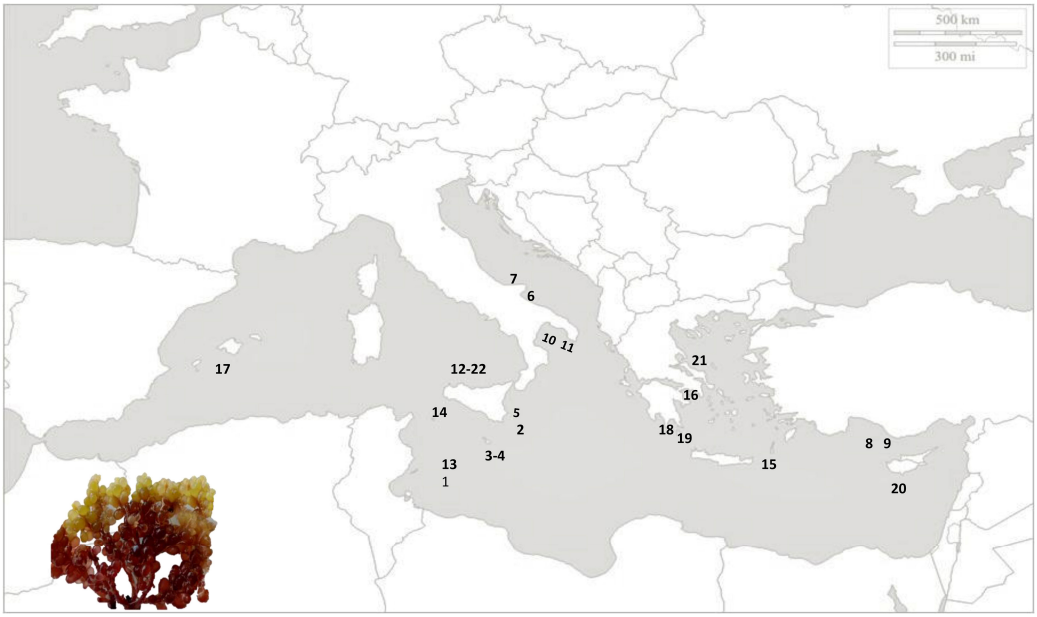

Figure 1. Map showing the localities in the Mediterranean Sea where *Botryocladia madagascariensis* has been recorded. The records are chronologically arranged in the following order: 1 and 2 (Lampedusa Island and Castelluccio (Syracuse), respectively; Cormaci et al. 1992), 3 (Malta; Cormaci et al. 1997), 4 (Comino; Cormaci et al. 1997), 5 (Riserva Ciclopi; Pizzuto 1999), 6 (Gargano promontory; Cecere et al. 2000), 7 (Tremiti Islands; Cormaci et al. 2000), 8 (Seki Beach, Gulf of Antalya; Turna et al. 2000), 9 (Side region, Gulf of Antalya; Turna et al. 2000), 10 (Gulf of Taranto; Cecere and Petrocelli 2004), 11 (Porto Cesareo; Cecere et al. 2005), 12 (Ustica Island; Catra et al. 2005), 13 (Linosa Island; Serio et al. 2006), 14 (Favignana Island; Catra et al. 2006), 15 (Karpathos Island; Catra and Giardina 2009), 16 (Porto Germeno, Korinthiakos Gulf; Tsiamis and Verlaque 2011), 17 (Baleari Islands; Joher et al. 2012), 18 (Messenian Gulf; Nicolaidou et al. 2012), 19 (Lakonic Gulf; Nicolaidou et al. 2012), 20 (Cyprus; Taşkın et al. 2013), 21 (Limni, Evia Island; Tsiamis and Panayotidis 2015), 22 (Ustica Island; present study).

Table 1. Comparison of taxonomic characters of *Botryocladia botryoides* and *B. madagascariensis* specimens described in Wilkes et al. (2006) and examined in the present study.

Species	Vesicle morphology			Secretory cell		
	Shape	Cortex	Cell layers	Shape	No. secretory cells/group	Position within vesicle
Botryocladia botryoides (Wulfen) Feldmann 1941	pyriform	continuous	2-3	~ spherical	1/2	borne directly on inner medullary cells
Botryocladia madagascariensis G. Feldmann 1944	ovoid	continuous	6?	ovoid/subspherical	2–4 (rarely 1)	borne on support cells and directly on medullary cells
Botryocladia madagascariensis (present study)	obovate/ovoid	continuous	3-4	ovoid/spherical	1 (rarely 2)	borne on support cells and directly on medullary cells

data on the distribution and spread dynamics of current NIS are essential.

Botryocladia madagascariensis G. Feldmann, a Rhodophyta macroalgal, is widely distributed along the Indian Ocean coast of South Africa (e.g., along the coasts of Madagascar, Natal and the Canary Islands) and has already been reported within the Mediterranean Sea (for details on localities and chronology see Figure 1). *B. madagascariensis* may be easily confused with the native species *Botryocladia botryoides* (Wulfen) Feldmann, but they differ in morphology at the cellular level (Table 1). This species was first recorded in the Mediterranean Sea at Lampedusa Island and Castelluccio (Syracuse) (Sicily; Cormaci et al. 1992), and later at Malta (170 and 210 kilometres, respectively, from the first recorded site). Afterwards, it was reported from southern Italy, several Sicilian localities, Turkey, Greece, Balearic Islands, and Cyprus (Figure 1). Both shipping or Suez Canal have been speculated as pathways of introduction for *B. madagascariensis* into the Mediterranean Sea (Cormaci et al. 2004; Verlaque et al. 2015). The goal

of this study was to provide a new record of *B. madagascariensis* along the coasts of the Ustica Island MPA, a description of recorded specimens, and habitat details.

Material and methods

Study area

The study was carried out at Ustica Island, which is the protruding part of an extinct volcano located in the southern Tyrrhenian Sea (Western Mediterranean; 38.7ºN; 13.18333ºE), 60 km north of the Sicilian coast. Its base lies at about 2000 m depth and is composed mainly of alkaline basalts and sedimentary rocks. The Marine Protected Area (MPA), created in 1986, encompasses a total area of 16.000 ha and contains three zones with different degrees of protection. The no-take zone (zone A) covers 65 ha along the western part of the Island, while the general reserve (zone B) and the take zone (zone C) share the remaining area equally (Figure 2). According to the institutive decree of the MPA (D.I. 12/11/1986), only research activities are authorized in the no-take zone, whereas recreational activities, such as SCUBA diving, boat anchoring, swimming and angling, are strictly prohibited. There are no restrictions on recreational activities in either the B or C zone (Gianguzza et al. 2006).

During surveys carried out on August 17 and October 12, 2017 at Colombara Bank off Ustica Island (38.7256ºN; 13.1883ºE), specimens of *B. madagascariensis* were observed and collected. Colombara Bank is a large basaltic rocky bank located approx. 1 km offshore the northern coast of Ustica Island in the B zone of the MPA. The summit of the bank, with a surface of approx. 500 square meters, is shallow (3–8 m depth) and almost horizontally orientated. The bank summit is almost entirely covered by a dense and rich photophilous macroalgal assemblage, mainly composed of brown algae including habitat-formers *Cystoseira* spp. (Giaccone et al. 1985). The perimeter of the summit abruptly breaks off in steep cliffs with a columnar basaltic structure, which descends almost vertically down to 40–45 m depth.

Sampling and processing

Specimens of *B. madagascariensis* were sampled by SCUBA diving and hand collection since the thalli were easily detachable from the substrate. Since *B. madagascariensis* is similar in habit and morphology to the native *B. botryoides*, visual identification in the field can be challenging. In the laboratory, fresh, whole specimens were photographed, manually sectioned with a razor blade, and observed under a light microscope for the analyses of diagnostic characters. Photos were taken with a Stereo Microscope (Stemi SV 11 Apo Zeiss) and photomicrographs with an Inverted Microscope (Axio Observer Zeiss). The main distinctive character between the native and invasive species is the disposition of the gland cells, which in *B. botryoides* are usually solitary and only occur on the inner layer of medullary cells (Table 1).

Figure 2. Map showing the zonation of the Ustica Island MPA. The site of Colombara Bank is indicated by the black arrow.

Results

Habitat description

In August some specimens (N = 30) were found at 7 m depth on the summit of Colombara Bank, a few meters from the margin of the break off the southern oriented cliff. In October all the observed thalli (N = 35) were collected at 7.5 m depth on the top of the vertical cliff of the same relief, exposed to the south-west/west. The collected thalli (Figure 1) clumped together and were attached directly to the rocky substrate via holdfasts. They settled in spots among or below other algal species, such as *Halopteris scoparia* (Linnaeus) Sauvageau and *Cystoseira* spp., but were not overgrown by them and visible by a naked eye. In October, the specimens were smaller and settled relatively close to specimens of the Mediterranean endemic scleractinia *Astroides calycularis* (Pallas, 1766).

Specimen description

All of the analyzed specimens (N = 35), 2.5–3.5 cm high, presented erect and cartilaginous red thalli that

were pseudodichotomously branched with numerous elliptical/obovate hollow and mucilage-filled vesicles (up to 5 mm long and 4 mm wide) all around the axis (Figure 3a). Vesicles showed a continuous layer of small cortical cells (4–5 µm in diameter) and 3–4 layers of medullary cells gradually increasing in size inward (up to 80 µm long and 60 µm wide) (Figure 3b, c). Secretory cells, single or rarely in pairs, were produced both on innermost medullary cells and special bearing cells that protruded into the vesicular cavity (Figure 3d, e). Female gametophytes with immersed cystocarps scattered irregularly over the vesicles (4–5 per vesicle) were present (Figure 3a, f). Cystocarps reached up to 950 µm in diameter and 550 µm in height (including the pericarp). The mature gonimoblast was sub-spherical and composed of carposporangia (14 to 23 µm in diameter).

Discussion

This finding represents the second record of *Botryocladia madagascariensis* from the "Ustica Island" MPA (the first one reported in Catra et al. 2005). Since the species has been recorded at least twice and spread over time and space (see Zenetos et al. 2005), we can consider it as established at Ustica Island MPA. While Catra et al. (2005), reported the species in a list with no description or images, this study provides the first description of specimens collected in this MPA with detailed observations on location and habitat. Our finding is also the first record of female gametophytes with cystocarps from Sicily, and the second one for the Mediterranean Sea (Cormaci et al. 1997). In general, reproductive structures were rarely reported in the Mediterranean Sea (but see sporophytes by Cormaci et al. 1992 and female gametophytes by Cormaci et al. 1997). Reproductive structures have only been observed on *B. madagascariensis* in the Western basin of the Mediterranean Sea (i.e., Lampedusa and/or Castelluccio, Ustica Island and Maltese Islands). It is possible that *B. madagascariensis,* a warm-temperate species, spreads mainly vegetatively in the Mediterranean, and sexual reproduction occurs only in areas where environmental conditions are suitable (Norris 1989).

Examining chronological records (Figure 1), the alga was reported first from the eastern coast (Lampedusa Island and Castelluccio, Syracuse), then from the western (Egadi Islands) and northern (Ustica Island MPA) coasts (see Figure 1), confirming the warm-temperate affinity hypothesis (Norris 1989). Giaccone et al. (1985) highlighted, through an analysis of the marine flora of Ustica, that this Island is subtropical. The subtropical character has also been highlighted by all the other NIS species occurring in the Island, namely the macroalgae *Acrothamnion preissii* (Sonder) E. M. Wollaston, *Asparagopsis armata* Harvey, *Caulerpa cylindracea* Sonder and *Womersleyella setacea* (Hollenberg) R.E. Norris (Catra et al. 2007; Di Trapani et al. 2016; Gianguzza P. personal observation), the heterobranch mollusc *Aplysia dactylomela* (Rang, 1828) (Gambi et al. 2017) and the decapod crustacean *Percnon gibbesi* (H. Milne Edwards, 1853) (Noè et al. 2018).

Few previously published reports of *B. madagascariensis* in the Mediterranean provide descriptions or images of the species. Since confusion with *B. botryoides* is possible without the aid of microscopes, it is natural to wonder how many of these records are really records of *B. madagascariensis* and how many are the result of misidentification. Therefore, we currently do not know the exact distribution of *B. madagascariensis* in the Mediterranean Sea. In addition, *B. madagascariensis* could not be a recently introduced species in the Mediterranean Sea (Ribera and Boudouresque 1995). Seagrief (1984) highlighted a misidentification between *B. madagascariensis* and *B. botryoides* in South Africa in the 1980s; therefore, it is possible that the distribution areas of the two species also overlap in the Mediterranean Sea (Turna et al. 2000). This study confirms that each new NIS record must be accompanied by either illustrations or descriptions of diagnostic traits that are difficult to check in the field, and which are often necessary for taxonomic certainty. Moreover, when morphological diagnostic characters of a NIS are not easy to check, as for the genus *Botryocladia* within which the species delineation remains complex (see Afonso-Carrillo and Sobrino 2003; Wilkes et al. 2006), additional molecular techniques should be used. Therefore, further molecular research is needed to verify all of the records of *B. madagascariensis* reported for the Mediterranean Sea.

The presence of NIS in "Ustica Island" MPA, but also in other MPAs (Cecere et al. 2005; Mannino and Balistreri 2017; Mannino et al. 2014, 2017), confirms that MPAs, despite their recognized role as a management tool for the protection of local biodiversity, are not immune from NIS invasions. However, NIS have been largely disregarded in marine conservation plans (Giakoumi et al. 2016). For this reason, it is crucial to establish a long-term monitoring programme to limit the introduction of NIS and control the spread of existing ones. The creation of permanent observatories involving all Sicilian MPAs will enable scientists and managers to detect new introductions and follow the spread of species already present to help manage NIS in the MPAs and in neighboring areas.

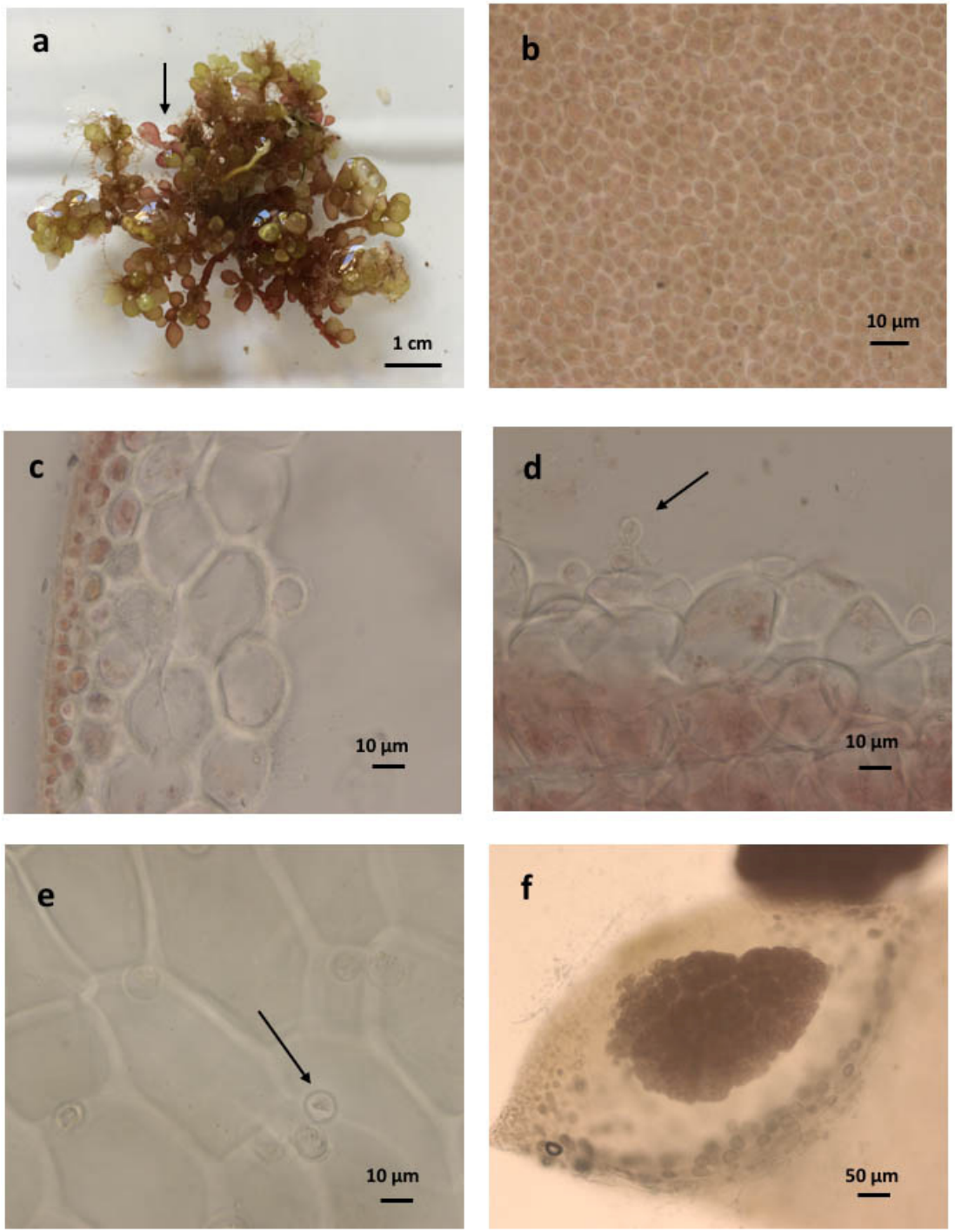

Figure 3. *Botryocladia madagascariensis* collected from the "Ustica Island" Marine Protected Area (Tyrrhenian Sea, Italy). Female gametophytes showing cystocarps (arrow) on vesicles (a), surface view of cortical cells of vesicles (b), transverse section of vesicle wall showing cortical and medullary cell layers (c), a secretory cell (arrow) on a special bearing cell (d), inner surface of the vesicle wall showing a secretory cell (arrow) on a special bearing cell (e), transverse section of a cystocarp showing the pericarp of smaller cells and carposporangia (f). Photographs by A.M.

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