RESEARCH ARTICLE



# Non-indigenous macroalgal species in coralligenous habitats of the Marine Protected Area Isole Ciclopi (Sicily, Italy)

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#### Abstract

Biological invasions are considered one of the main threats for biodiversity. In the last decades, more than 60 macroalgae have been introduced in the Mediterranean Sea, causing serious problems in coastal areas. Nevertheless, the impacts of alien macroalgae in deep subtidal systems have been poorly studied, especially in the coralligenous habitats of the eastern coast of Sicily (Italy). Therefore, within the framework of the programme "Progetto Operativo di Monitoraggio (P.O.M.)" of the EU Marine Strategy Framework Directive (MSFD), the aim of the present study was to gain knowledge on the alien macroalgae present in coralligenous habitats of the Marine Protected Area (MPA) Isole Ciclopi, along the Ionian coast of Sicily. By Remotely Operated Vehicle (ROV) videos and destructive samples analysed in the laboratory, five alien species were identified: Caulerpa cylindracea, Antithamnion amphigeneum, Asparagopsis armata, Bonnemaisonia hamifera, and Lophocladia lallemandii. Since A. amphigeneum was previously reported only in the western Mediterranean and Adriatic Sea, the present report represents the first record of this species in the eastern Mediterranean. The ROV surveys showed that the alien species do not have a high coverage and do not appear to be invasive in the coralligenous area of the MPA. Since ocean temperatures are predicted to increase as climate change continues and alien species are favoured by warming of the Mediterranean Sea, the risk of biotic homogenisation caused by the spread of alien species is realistic. Therefore, further studies are needed to assess the incidence and invasiveness of alien species in phytobenthic assemblages of coralligenous in the MPA.

#### Keywords

Coralligenous, Macroalgae, Mediterranean Sea, Marine Strategy Framework Directive, Non-indigenous species

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# Introduction

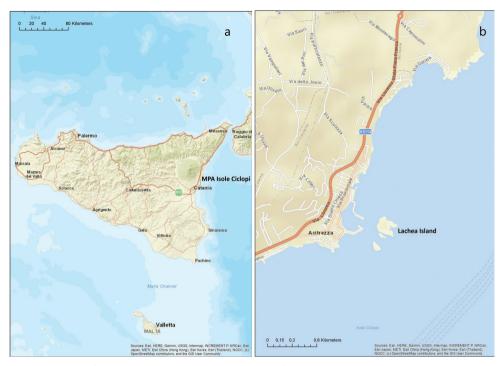
In the Mediterranean Sea, deep rocky bottoms are characterized by assemblages dominated by calcareous organisms, defined as coralligenous biocenosis (Pérès and Picard 1964), mainly built by Rhodophyta belonging to the orders Corallinales, Hapalidiales, and Peyssonneliales (Ballesteros 2006). In this system, the heterogeneity of the rocky bottom is increased by a complex microtopography related to the presence of a secondary substrate provided by building organisms, particularly calcareous encrusting algae (Piazzi et al. 2007). This high heterogeneity in the substrate favours the presence of a high number of species per unit area (Cocito 2004; Ballesteros 2006). However, coralligenous habitats are sensitive to several impacts, especially if caused by human activities (Hong 1983). Among these stressors, biological invasions caused by Non-Indigenous Species (NIS) have been recognized as an important threat in marine habitats (Walker and Kendrick 1998; Seebens et al. 2017). NIS are defined as organisms introduced intentionally or unintentionally outside their natural range by human activities (Olenin et al. 2010). Marine NIS are mainly introduced unintentionally by discharges of ballast waters and accumulated ballast sediments (David et al. 2012), attached to the hulls of vessels (Buschbaum et al. 2012), by the mariculture industry (Naylor et al. 2001), and by passage through canals (e.g., Suez Channel, Strait of Gibraltar, and Dardanelles Strait) (Galil 2012). In particular, port areas have been considered to be hotspots for the introduction of NIS (Tempesti et al. 2020). Introduction, spread, and establishment of NIS pose significant threats to biodiversity, at different scales and extent, and to economies worldwide (Streftaris and Zenetos 2006; Galil 2007; Saebi et al. 2020). For this reason, many environmental programmes, initiatives, policies and strategies have focused their attention on the protection against alien species. In particular, the Bern Convention on the Conservation of European Wildlife and Natural Habitats has developed the European Strategy on Invasive Alien Species, which offers specific advice to countries and international organisations on measures to limit this threat. The Convention on Biological Biodiversity (CBD) has highlighted the need to assemble and disseminate information on alien species that threaten ecosystems, habitats, and species in order to prevent any further introduction. Moreover, more studies on the impact of alien invasive species on biological diversity are strongly encouraged (CBD 2000). The EU Marine Strategy Framework Directive (MSFD 2008) includes measures to limit the spread of alien species in European Seas (Streftaris and Zenetos 2006). In fact, alien species are one of the eleven qualitative descriptors for the assessment of the environmental status of the water bodies, according to the MSFD (García et al. 2015). In particular, the descriptor D2 requires that the alien species remain at levels that do not adversely alter the ecosystem, in order to reach the Good Environmental Status (GES) of the marine environment. Therefore, data on the presence/absence, abundance, and impacts of the alien species on marine habitats have to be available (García et al. 2015).

In the last decades, more than 60 macroalgae have been introduced in the Mediterranean Sea (Verlague 1994; Galil 2000; Boudouresque and Verlague 2002) and in several coastal areas they have become dominant in the benthic assemblages (Verlague and Fritayre 1994; Piazzi and Cinelli 2003). The introduction of marine macroalgae represents a major threat for marine systems (Ribera and Boudouresque 1995; Scheibling and Gagnon 2006). Indeed, the spread of introduced benthic algae may lead to a complete cover of substrata, affecting native assemblages and reducing biodiversity (Viejo 1997; Britton-Simmons 2004; Casas et al. 2004; Buschbaum et al. 2006). The replacement of native benthic algae by alien macroalgae modifies environmental conditions and the functioning of ecosystems, causing an impoverishment of littoral systems (Rueness 1989; Staehr et al. 2000; Wikstrom and Kautsky 2004; Sanchez et al. 2005). In particular, the spread of species of the genus Caulerpa and turf-forming Rhodophyta have been considered among the most serious biological invasions in the Mediterranean (Meinesz et al. 2001; Boudouresque and Verlaque 2002). Nevertheless, the ecological impact of the majority of NIS in the Mediterranean is still unknown, since the few available studies were conducted at limited temporal and spatial scales, and the synergistic relations with other stressors affecting the marine environment are largely unknown (Raitsos et al. 2010; Galil et al. 2018). Furthermore, although all habitats are considered vulnerable to invasions (Lodge 1993), some are thought to be more sensitive than others (Wasson et al. 2005). In particular, the effects of introduced species in deep subtidal systems, such as coralligenous habitats, are still little-known (Piazzi et al. 2007). Therefore, the aim of the present study was to acquire knowledge on alien macroalgae in the coralligenous habitats of the Marine Protected Area (MPA) Isole Ciclopi, along the Ionian coast of Sicily (Italy) (Fig. 1A).

# Materials and methods

#### Study area

The MPA, established in 1998 and with a total extension of 6.23 km<sup>2</sup>, is located in the municipality of Aci Castello, along the central-eastern coast of Sicily (Italy). The MPA hosts three harbours, one of which, the largest in size, with a total extension of 0.028 km<sup>2</sup>. In this port, in summer, about 380 boats used for all authorized activities in the MPA (diving, professional fishing, recreational fishing, artisanal fishing, buoy field, recreational boat, touristic boat) are present. Indeed, especially in summer, daily input and output flows are more frequent. From a geological point of view, this area comprises a complex of subvolcanic rocks, mainly consisting of columnar basalts, and effusive submarine products forming extensive fields of pillow lavas (Cristofolini 1975; Corsaro and Cristofolini 1997). The underwater topography, from the coastline down to 25–40 m depth, is steeply sloping and consists largely of *in situ* basaltic bedrocks and large volcanic blocks (Sciuto et al. 2015).



**Figure 1.** Map of the study area **a** geographical location of the MPA Isole Ciclopi along the Ionian coast of Sicily **b** detail of the Lachea Island, the sampling site.

## Methods

The present study was conducted in October 2018 along the border of zone A of the MPA Isole Ciclopi. A visual census of the coralligenous habitats was carried out by a ROV in three transects (each with an extension of ca. 200 m and at depth of 32–39 m) located along the northeastern side of the island of Lachea (37°33'40.51"N, 15°09'05.43"E), the largest outcrop of the Isole Ciclopi (Fig. 1B). The type of ROV used for this study was a Marine Scope, FO II model, equipped with a high-definition video camera (GoPro 5), a digital camera with depth sensor and an integrated compass, two laser beams placed 10 cm apart and used as a metric scale for the images and the visual field, and two led strobes of 13,000 lumen. Subsequently, an average of 20 frames for each transect were extrapolated from the ROV videos to allow the identification of the main macroalgal taxa.

Moreover, samplings in scuba diving were performed in the same area as the ROV surveys in two different seasons (spring and autumn) at a depth of 36 m. Two samples, one for each season, were collected by removing with a hatchet all sessile organisms from  $20 \times 20$  cm quadrats. We preferred to use the hatchet rather than the traditional hammer and chisel since it was easier to use on organogenic substrates. The samples collected in scuba diving were stored in a solution of seawater and 90% ethyl alcohol and carried to the Laboratory of Algology of the University of Catania for the identification of the flora.

# Results

Through the observation of frames extrapolated by the ROV videos and the analysis of the samples in the laboratory, a total of 92 taxa were found (Costanzo et al. 2020). Among them, five NIS were observed, including one Chlorophyta (*Caulerpa cylindra-cea* Sonder) and four Rhodophyta [*Antithamnion amphigeneum* A.Millar, *Asparagopsis armata* Harvey, *Bonnemaisonia hamifera* Hariot and *Lophocladia lallemandii* (Montagne) F.Schmitz].Comments on morphology, distribution, and likely way of introduction for each species are reported below.

Ceramiales Ceramiaceae

## Antithamnion amphigeneum A.Millar

**Description.** The collected specimens match with the descriptions of Verlaque and Seridi (1991), Cormaci et al. (2004), Rodríguez-Prieto et al. (2013) and Verlaque et al. (2015). The thalli are pink-reddish, filamentous and uniseriate, consisting of creeping and erect axes, bearing distichous opposite whorl branches. The whorl branches have an isodiametric basal cell and bear opposite distichous simple or branched branchlets with only abaxial branchlets near the distal portion. As reported by Secilla et al. (1997), we noticed in our samples that the apical cells are blunt and sometimes provided with a single hyaline hair (Fig. 2A). The lateral indeterminate branchlet graph arise replacing a branchlet and with the suppression of the opposite branchlet (Fig. 2B). Moreover, in the collected specimens there were abundant gland cells located on the adaxial side of both normal and special branchlets, touching 2–3 cells. We found only sterile specimens.

## Chorology. Indo-Pacific.

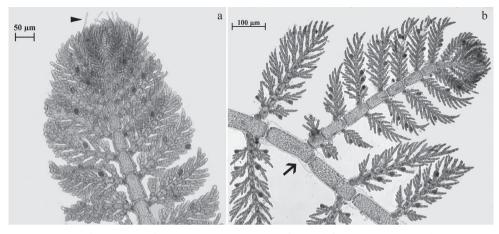
Presumed introduction vector. Fouling.

**Remarks.** Hitherto in the Mediterranean, this species has only been reported in the western basin (Verlaque et al. 2015) and in the Adriatic Sea (Mačić and Ballesteros 2016). Therefore, the present report represents the first record of *A. amphigeneum* in the eastern Mediterranean.

# Bonnemaisoniales Bonnemaisoniaceae

## Asparagopsis armata Harvey

**Description.** In our samples, only the tetrasporophytic phase (known as *Falkenbergia rufolanosa*) was found. The tetrasporophytes are filamentous and usually form small tufts. The tetrasporophytic thalli consist of an axis with apical growth, with 3 periaxial cells around each axial cell. In the collected specimens the gland cells, which are cut off



**Figure 2.** *Antithamnion amphigeneum* **a** apical portion of an indefinite axes: the arrowhead shows a terminal hyaline hair **b** detail of lateral indeterminate branches, which arise by replacing a branchlet and with the suppression of the opposite branchlet (arrow).

by each periaxial cell, were well-identifiable. Previously, in the MPA Isole Ciclopi the gamethophytes were also reported (Giaccone and Pizzuto 2001). Gametophytic thalli are pale pink, with a pyramidal outline and fixed to the substrate by cylindrical stolons. The frond consists of a cylindrical main axis, irregularly and radially branched, bearing plumose branches on all sides. Furthermore, the branches are scattered and with a harpoon shape for the presence of several retroverted spines.

#### Chorology. Cosmopolitan.

#### Presumed introduction vector. Fouling.

**Remarks.** This species has been widely reported in almost all the Mediterranean. Hitherto, in the coralligenous of the MPA only the tetrasporophytes have been found (Furnari and Scammacca 1970; Furnari et al. 1977). In fact, usually, the gametophytes occur in the upper infralittoral (Cormaci et al. 2020).

#### Bonnemaisonia hamifera Hariot

**Description.** In the collected samples, only the tetrasporophytic stage (known as *Trailliella intricata*) was observed. The specimens found match with the descriptions of Cormaci et al. (2014), Verlaque et al. (2015) and Cormaci et al. (2020). The tetrasporophytic thalli are pinkish-red or pinkish-brown and consist of uniseriate and irregularly branched filaments. One or rarely two small refractive gland cells are formed at the upper end of each cell. The gamethophytic thalli are dark red and consist of a main axis, which bears opposite branches, lacking in lower portions. Primary axes are arranged on several levels forming a 3/8 spiral. Few branches are modified to form reflexed crozier-shaped hooks, which are present particularly in the middle-upper parts of the thallus and serve for anchoring and vegetative propagation.

#### Chorology. Circumboreal.

**Presumed introduction vector.** Fouling and/or spontaneously through the Strait of Gibraltar.

**Remarks.** Hitherto, in the Mediterranean Sea, *B. hamifera* gametophytes have only been reported in Spain: in the Strait of Gibraltar and along the coasts of Catalonia (Cormaci et al. 2020). Previously, in the MPA the tetrasporophytes were only found in 1978 at a depth of 45 m (Cormaci and Furnari 1979).

## Bryopsidales Caulerpaceae

## Caulerpa cylindracea Sonder

**Description.** The found specimens clearly show the features described by Rodríguez-Prieto et al. (2013), Cormaci et al. (2014), and Verlaque et al. (2015). The thalli of this species consist of stolons attached by numerous short slender rhizoids and bearing erect photosynthetic cylindrical axes. The erect fronds, with a simple axis, are rarely branched and slightly swollen at the base, bearing loose to moderately dense ramules, distichous or radial, inclined towards the apex. The ramules are sub-cylindrical to claviform, rounded at the apex and slightly attenuated downwards, without constraint at the point of attachment to the axis.

Chorology. Pantropical.

#### Presumed introduction vector. Ballast waters.

**Remarks.** In the Mediterranean, *C. cylindracea* is widely distributed from the lower eulittoral to the upper circalittoral (Cormaci et al. 2014). Most of the previous reports of this species were referred to as *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque. In 2014, molecular studies assessed the identity of Mediterranean specimens as *C. cylindracea* (Belton et al. 2014). In the MPA this species has been previously reported as *C. racemosa* by Giaccone and Pizzuto (2001).

# Ceramiales Rhodomelaceae

#### Lophocladia lallemandii (Montagne) F.Schmitz

**Description.** The collected specimens correspond to the description of Cormaci et al. (2004), Rodríguez-Prieto et al. (2013) and Verlaque et al. (2015). The thalli of this species are dark red, filamentous and are attached to the substrate by rhizoids with multicellular discs. The axes are cylindrical and dichotomously branched, with a monopodial structure. Each axial cell develops four pericentral cells. Trichoblasts are pigmented and are formed radially, one per segment, in a regular spiral. Branches are mainly exogenous and replace a trichoblast. In our samples, only gamethophytes were found.

Chorology. Indo-Pacific.

Presumed introduction vector. Spontaneously through the Suez Canal.

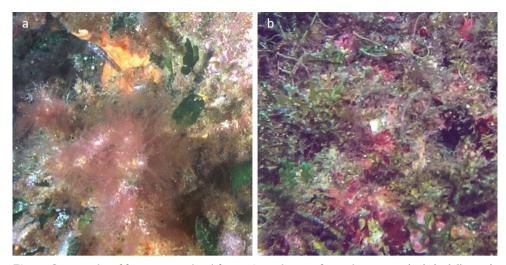
**Remarks.** This species has spread throughout the Mediterranean Sea, colonising subtidal communities from shallow waters to deep environments (Verlaque et al. 2015). According to Bedini et al. (2011), the percentage cover of *L. lallemandii* increase with depth. In the coralligenous habitats of the MPA, this species has been recorded since the 1970s (Furnari and Scammacca 1970; Furnari et al. 1977).

## Discussion

Through the observation of ROV frames, we saw that in the investigated area the encrusting layer is well developed and formed by a high coverage of calcareous Rhodophyta. In both samples and frames analysis, it was observed that alien species do not show a high coverage or an invasive attitude in the coralligenous of the MPA. Indeed, in ROV frames only few thalli of C. cylindracea and L. lallemandii (corresponding to a coverage of 5–25% of the Braun-Blanquet's Scale), were observed (Fig. 3A, B). Instead, the other alien species, due to their microscopic sizes, were observed only by analysis of the destructive samples in the laboratory. In particular, we found specimens of A. amphigeneum, previously reported only in the western Mediterranean (Verlque et al. 2015) and more recently in the Adriatic Sea (Mačić and Ballesteros 2016). Therefore, the present record constitutes a further eastward step of this species in the Mediterranean Sea. Moreover, in the present study only the tetrasporophytic stages of A. armata and B. hamifera have been observed. This could depend on the daylength and temperature requirements of these species. In fact, in culture experiments, it has been observed that the tetrasporophytes of both species reproduce under short-day conditions [less than 9 h of light for Falkenbergia (Guiry and Dawes 1992) and less than 12 h of light for Trailliella (Breeman et al. 1988)] and within a narrow temperature range of about 15 °C (Breeman et al. 1988; Oza 1989). Conditions of low irradiance and relatively constant temperature are typical of coralligenous habitats (Garrabou and Ballesteros 2000) and, thus, this might explain the presence of the only tetrasporophytes in the coralligenous habitat of the study area.

Almost all NIS found in this study, except *B. hamifera*, are warm-water species, whose origin is from Australasia or from the Red Sea. Generally, the presence of warm-water alien species has been related to warming of the Mediterranean Sea. In fact, this warming not only stresses the native species, but also facilitates the arrival of other NIS, adding extra pressure on the ecosystem (Harris and Tyrell 2001). The survival, reproduction, and establishment of warm alien species in a new environment depends on the thermal regime, which has to match the thermal physiological requirement of the species (Raitsos et al. 2010). The concurrent increase in seawater temperature and abundance of warm alien species is a phenomenon called 'tropicalization' of the Mediterranean Sea, which has especially affected the south-eastern sectors of the basin (Bianchi et al. 2018).

Most marine introductions take place by dispersal operated by cargo ships; thus, ports are considered one of the principal dispersal hotspots for alien species (Hulme



**Figure 3.** Examples of frames extrapolated from ROV videos **a** a frame showing *Lophocladia lallemandii* **b** a frame showing *Caulerpa cylindracea*.

2009). Moreover, ports also provide several artificial structures that create favourable habitats for NIS (Mineur et al. 2012), particularly sessile benthic invertebrates (Cangussu et al. 2010), and macroalgae (Petrocelli et al. 2019). Due to the presence of three harbours within the MPA, it is likely that the main way of access of the NIS in this area is related to shipping. In fact, particularly during summer, there is a considerable flux of authorized vessels in the MPA. Moreover, most of the found NIS could have easily been transported by fouling. In fact, the recent ban of Tributyltin based antifouling paints, applied to limit metal pollution (Campbell and Hewitt 2011), and the long periods spent by ships in port (Floerl and Coutts 2009) are factors which further enhance hull fouling transport. Therefore, surveillance, eradication, and monitoring programmes should be suggested and established (Rohde et al. 2017).

In conclusion, in the present study, we observed that the occurrence of alien species contributes to an increase of the percentage incidence of Rhodophyta in the flora of the coralligenous habitats of the MPA. Nevertheless, the presence of NIS does not currently compromise this Mediterranean biodiversity hotspot. Since ocean temperatures are predicted to increase as climate change continues (IPCC 2007) and alien species are favoured by warming of Mediterranean waters, the risk of biotic homogenisation is possible. Therefore, further studies are needed to fully assess the incidence and invasiveness of alien species in the coralligenous phytobenthic assemblages of this MPA.

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