



## Detection and prevention of biological invasions in marinas and ports: Epibionts and associated fauna of *Mytilus galloprovincialis* revisited

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### ABSTRACT

Ports and marinas are gateways for introduction of non-indigenous species through 'shipping' worldwide. These anthropogenic environments concentrate a variety of artificial structures and substrates that are colonized by different types of organisms, being prone to biological invasions. One of the most outstanding taxa in European marinas and ports are the mussels of the genus *Mytilus*. Mussels live in patches forming a three-dimensional, multi-layered and permanent biostructure that favours the settlement of sessile and mobile fauna, creating a perfect environment for the establishment and development of different species. In this study we characterized the biodiversity associated to the mussel patches of *M. galloprovincialis* in the Marina of Gijón (northern Spain, Cantabrian Sea), according to their status (i.e., native, introduced or alien, invasive and cryptogenic). The samples were collected from different areas of the marina: the outer dock (with national and international traffic), the middle dock and the inner one (both with local and national transit). We identified a total of 102 species associated to *M. galloprovincialis*, from which 13 and 4 species were invasive and alien respectively. The exotic fanworm *Branchiomma luctuosum* is reported for the first time in the Bay of Biscay and the Cantabrian Sea. The inner dock was the area with the highest number of non-indigenous taxa. Most of the alien and invasive species belonged to the groups Ascidiacea and Bryozoa, all of them filter feeders with great plasticity and ecological tolerance. Furthermore, the elemental composition microanalysis of the invasive bryozoan *Watersipora subatra* showed high levels of aluminium (a common element in marinas, being present in the fuel and in ship paintings), which may imply a risk of aluminium bioaccumulation in the environment. From these results we can conclude that mussel patches may facilitate the settlement and development of non-indigenous species in marinas, highlighting the importance of monitoring these mussel aggregations as a management tool for the early control and prevention of marine bioinvasions.

### 1. Introduction

During the last decades, human activities, global warming, and habitat fragmentation have modified the distribution of species worldwide, dramatically increasing the spread of exotic, alien or non-indigenous species (NIS) (Vilá et al., 2008). Some of these introduced species can effectively colonize new ecosystems and eventually become invasive, provoking serious alterations in their new environment (Courchamp et al., 2017). Invasive species can imbalance the receiving ecosystems by competing against native species or preying on key organisms, disrupting the regulation of the trophic cascade and even causing ecosystem damage that can entail sanitary or economic costs for

humans (Arias and Anadón, 2012; Arias et al., 2013; Miralles et al., 2016). In the same way, neutral or facilitative effects of exotic species have been observed in some cases (Sellheim et al., 2010). The main challenge when managing biological invasions is the prevention of the arrival and settlement of exotic species, since their eradication after their settlement and expansion tend to be complex, sometimes even almost impossible. One of the factors that facilitate the establishment of alien species is the lack of biotic resistance, the colonization in degraded habitats being more feasible than in diverse ecosystems, due to the higher availability of niches for the settlement of new species (Miralles et al., 2016).

Ports and marinas are one of the most important gateways for the

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introduction of NIS through ‘shipping’ worldwide (Sellheim et al., 2010; Pejovic et al., 2016). Their introduction occurs mainly through two mechanisms: i) ballast water, in which planktonic organisms (such as larvae and juveniles of invertebrates, tunicates and fish) are easily transported (Seebens et al., 2013) and ii) biofouling, species that can adhere to the surface of the hull of boats. Other important introduction pathways are aquaculture activities, marine litter and transport for ornamental or aquarium purposes (Arias and Anadón, 2012; Habtemariam et al., 2015; Ibabe et al., 2020; Pergl et al., 2020). As anthropogenic environments, ports concentrate a variety of artificial structures and substrates that are colonized by different types of organisms, and their degradation in terms of biological diversity make them prone to biological invasions. The Cantabrian Sea is an ideal setting for the arrival and establishment of non-indigenous species, both due to its southern position (being able to attract subtropical fauna), and due to the increase in the temperature of its waters (Arias et al., 2012; Arias and Crocetta, 2016).

One of the most outstanding taxa in European marinas and ports are the mussels of the genus *Mytilus*, outstanding the Mediterranean mussel *M. galloprovincialis* Lamarck, 1819. This is a widespread species that constitutes an important marine resource, being harvested, and cultivated along the coasts of the Iberian Peninsula and France. *Mytilus galloprovincialis* lives in rocky marine substrates from the intertidal to shallow subtidal zones, being excluded from sedimentary or sandy areas (Tebble, 1976). This mussel adheres to the substrate through rows of byssus, which are protein fibers secreted by the byssus gland that also allow them to defend against predators or pathogens (Tebble, 1976). This species usually occurs in dense patches forming a three-dimensional and multi-layered net that favours the settlement of sessile and mobile taxa. These patches create a permanent, hard bio-substrate that is the perfect environment for the establishment and development of different species of marine fauna (Markert et al., 2009).

Mussel patches are the most abundant permanent, hard biosubstrate in European ports and marinas. These biostructures may function as ‘islands’ and are characterized on the basis of two assumptions as predicted by the model of MacArthur and Wilson (1967): i) the larger the size, the greater the abundance of individuals and ii) the more habitat heterogeneity, the greater the richness of species (Tsuchiya and Nishihira, 1985). Furthermore, both species diversity and richness increase with the age of the patch of mussels (Tsuchiya and Nishihira, 1985, 1986). As a consequence, these patches are suitable habitats for a large number of species due to facilitation relationships (Sellheim et al., 2010; Çinar et al., 2008). These patches provide a particularly suitable substrate for sessile animals like anthozoans, bryozoans, hydrozoans, barnacles, gastropods or bivalves, for which the substrate is a limiting resource (Markert et al., 2009; Çinar et al., 2020). In same way, mussel patches also offer benefits for mobile epifauna such as annelid polychaetes, nemertean worms, flatworms or crustaceans, since they can feed on sediment, biodepositions and other organisms that live/circulate in the patches. Furthermore, *Mytilus* mussels can also provide an ideal habitat for the settlement and development of exotic species, which may develop an invasive behaviour in the recipient ecosystems, as has been happened with the Pacific oyster *Magallana gigas* (Thunberg, 1793) in the Germanic bay of the Wadden Sea (Markert et al., 2009). Besides, some commensal species that live as mussels epibionts can damage them, either by deterioration of their valves or by preventing the circulation of water around them and subsequently impeding the water filtering for feeding (Perera et al., 1990).

The main goal of this work is to study the diversity of the associated fauna of *M. galloprovincialis* patches and to assess their status (native, alien, invasive or cryptogenic), taking the marina of Gijón (northern Spain) as a study case of a marina from a European temperate coast. With this purpose we aimed to evaluate the role of mussel patches in port ecosystems and their potential as facilitators of the settlement of alien species. We also provide useful information for the development of effective protocols and management tools to prevent and control the

spreading of invasive species in the Iberian Peninsula waters and other temperate areas of western Europe and the Mediterranean basin.

## 2. Material and methods

Mediterranean mussel samples and their associated fauna were collected from the Marina of Gijón, (43°32'46" N - 05°41'00" W), Cantabrian Sea, northern Iberian Peninsula, during the period comprised between October 2017 and April 2018. This marina is divided into three main areas (Fig. 1): the outer dock (with national and international traffic), the middle dock and the inner one (both with local and national transit). Samples were obtained from the floating docks, just below the waterline, at several points of the outer, middle and inner docks. Mussel patches from the selected sampling points were removed entirely by mechanical scraping. Samples were brought alive to the Zoology laboratory of the Department of Organisms and Systems Biology (University of Oviedo) and anaesthetised in a 7.5% MgCl<sub>2</sub> solution isotonic with seawater or relaxed with menthol crystals floated in seawater (in the case of cnidarian and ascidian specimens). Shortly thereafter, they were subjected to a rapid study to identify colour patterns and other external characteristics that can be lost with the preservation. Subsequently, they were fixed in 70% alcohol for their final conservation and storage in the Collection of the Department of Biology of Organisms and Systems (Zoology) of the University of Oviedo (BOS). Specimens were examined under both dissecting stereomicroscope and compound light microscope. Temporary glycerol slides of small animals or certain parts or structures of them (e.g., parapodia, chaetae or jaws in the case of polychaetes) were examined under a compound light microscope.

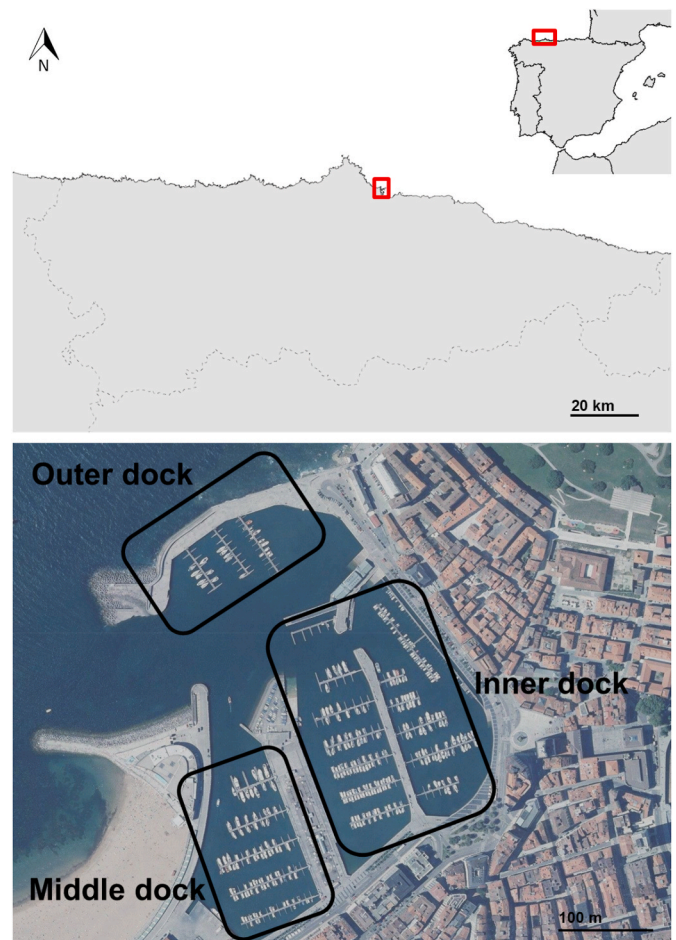


Fig. 1. Map of the Marina of Gijón, with the three sampling areas. Orthophotograph modified from puertodeportivogijon.es.

Selected specimens were stained with Methylene Blue solution in 30% ethanol to increase the contrast of some morphological structures. Samples were identified to the species level (except for four species that were only possible to the genus level due to their deterioration). Certain taxa were prepared for Scanning Electron Microscopy (SEM) for the study of their diagnostic features and general morphology. These specimens were dehydrated in an ascending series of ethanol, critical point dried using acetone as the transition liquid, mounted on aluminum stubs and sputter coated with gold. Samples were then imaged using a JEOL 6610 LV Scanning Electron Microscope. An elemental microanalysis of the bryozoan *Watersipora subatra* (Ortmann, 1890) was also performed with SEM for the following elements: carbon, nitrogen, oxygen, sulfur, calcium, gold, aluminium, magnesium and silicon.

The specimens were photographed with a Canon EOS 1200D Digital SLR Camera with Canon EF-S 18–55 mm f/3.5–5.6 III or Sigma 105 mm f/2.8 EX DG OS HSM Macro lens. Photomicrographs were taken with a Nikon Digital Sight DS-L1 camera mounted on a Nikon SMZ-U stereomicroscope. The species status was assigned following the guidelines of the International Union for the Conservation of Nature (IUCN) and the criteria of Zenetos et al. (2005, 2010, 2012, 2020) and Tsiamis et al. (2019), regarding their status (i.e., native, alien, invasive or cryptogenetic). Invasive species are a subset of alien species (Zenetos et al., 2020), so in the results of this study we will refer to alien species with a reported invasive behaviour as “invasive”, and we will use “alien” for those alien species with no reported invasive behaviour. Systematics and nomenclature follow the World Register of Marine Species (WoRMS Editorial Boards, 2021).

### 3. Results

#### 3.1. Species diversity

A total of 102 species/taxa were identified, belonging to 10 different phyla (Table 1). The most representative phyla were Annelida and Mollusca with 24 and 23 species, respectively, followed by Arthropoda and Cnidaria with 13 species each (Fig. 2). Regarding their status, 76.47% were native species, while NIS accounted for 16.67%, of which 12.75% were invasive and 3.92% were just alien species with no invasive behaviour reported (Figs. 3A and 4). Cryptogenic species represented 2.94% of the total of identified species, and the remaining 3.92% corresponded to unidentified at species-level specimens due to the deterioration and loss of diagnostic characteristics.

Most of the animals found as epibionts of mussels were suspension feeders (57.84%) or carnivorous species (27.45%), while only a few species were herbivorous (7.84%) or omnivorous (6.86%). Regarding the alien and invasive species, all of them were suspension feeders except for one omnivorous and one herbivorous species (see Table 1).

Among the invasive species, the most representative phylum was the Chordata with five species, which represented 35.71% of the total of invasive species found in this study (Fig. 3B). The eight remaining invasive species belonged to the phyla Bryozoa (three species), Arthropoda (two species), Mollusca (two species) and Annelida (one species). All these alien and invasive species were found in the outer dock, while in the middle and inner docks only seven and five of these species were found, respectively (Table 1).

Out of the 17 alien species found in the Gijón marina (Fig. 4), the columbellid gastropod *Columbella adansoni* Menke, 1853 and the sabellid polychaete *Branchioma luctuosum* (Grube, 1870) are found occupying their northernmost distribution to date. Furthermore, *B. luctuosum* (Fig. 5) represents a new record from the Bay of Biscay and the Cantabrian Sea. The dove snail *C. adansoni* (Fig. 6) is a medium-sized gastropod (2–2.5 cm), native from the Macaronesian and West African coasts. It has a biconical shell with 7–9 whorls, smooth surface with light unequal spiral ridges and covered with a thin periostracum. It has a thickened lip with 14–16 teeth and columella with 5–7 small teeth at the base. It presents variable colouration with yellowish or brownish spots

on white or cream background, forming bands or sinuous lines (Fig. 5A–C) (Gofas et al., 2011). The fanworm *B. luctuosum* (Fig. 5) was originally described from the Red Sea (Grube, 1870) and can be diagnosed as follows: large species, up to 120 mm in total length, up to 60 mm crown length; with 7–8 thoracic and 60–100 abdominal segments; about 30 pairs of spiralled radioles with 20–30 pairs of small digitiform stylodes each, not covering the small radiolar eyes (Fig. 5C). Body and crown brownish, reddish or dark velvet coloured; collar with a large dorsal gap (Fig. 5A), higher ventrally with well-developed ventral lap-pets (Fig. 5B); dorsal lips (Fig. 5A and B) one third smaller than the branchial crown; superior around a cluster of capillary chaetae; abdominal uncini avicular, 30 per torus.

#### 3.2. Microanalysis of the bryozoan *Watersipora subatra* (Ortmann, 1890)

The results of the microanalysis on a fragment of a colony of the bryozoan *W. subatra* (Fig. 4A) revealed high levels of gold (due to the metallization prior to observation at SEM), carbon and calcium, as expected, given the composition of the tegument of most bryozoans. However, the analysis also revealed high levels of aluminum, if compared to magnesium or calcium (Fig. 7, Table 2).

### 4. Discussion

Ports and marinas are excellent contact pathways between species from all over the world, and mussel patches micro-environments are extremely rich in biodiversity, due to their three-dimensional multilayer structure (Tsuchiya and Nishihira, 1985). We found 102 different taxa associated to *M. galloprovincialis* patches, which were predominantly annelids and molluscs in juvenile phases. Many bivalves and polychaetes are suspension or filter feeders that benefit from the currents rich in nutrients generated by mussels during their filter feeding (Saier, 2002). Moreover, herbivorous animals (like gastropods) shelter in mussel patches, which also attracts carnivorous species like some annelids, turning mussel patches into highly rich small communities (Tsuchiya and Nishihira, 1985).

The currents generated by mussels can attract invertebrate larvae from the surrounding water, which may contain non-indigenous animals released from ships. This mechanism favours the settlement of native, but also alien species in the patch (Saier, 2002). Many of these attracted larvae need hard substrates to settle and develop, so they attach to mussels' valves, where they benefit from shelter and nutrients. As a consequence, mussel patches are probably an ideal environment for the settlement and development of some non-indigenous species, as they provide them with substrate, shelter and food (Tsuchiya and Nishihira, 1985). Our results support this hypothesis, as 16.67% of the species found as mussels epibionts were alien (3.92%) and/or invasive (12.75%) that were predominantly suspension feeders, benefiting in the same way explained above.

A common characteristic of all the invasive species found in this study is their great plasticity, as many of them are able to tolerate large ranges of temperature and salinity. This allows them to easily colonize many habitats and optimize the use of resources, competing against native species and even displacing them (Sakai et al., 2001). Another common feature is that almost all of them have been introduced through two main pathways: i) attached to ship hulls (biofouling) either in adult or egg/juvenile phases, and ii) contained in ballast waters as juvenile phases, which are collected at the ship's origin and released at the destination (Gouletquer, 2016).

A good example is the invasive bryozoan *W. subatra* which exhibits a high capacity to grow on artificial substrates with anthropogenic disturbance and thus is increasingly common on ports and marinas of European and North American coasts, from which it is progressively spreading to the nearby natural habitats (Viola et al., 2018; Reverter-Gil and Souto, 2019). *Watersipora subatra*, like many bryozoans, have a

Table 1

List of species found associated to *Mytilus galloprovincialis* patches, as well as data on substrate, mobility, feeding, habitat, status and port area where they have been located during the samplings carried out in the Marina of Gijón (alien species highlighted in bold italics; Heterog: heterogeneous; s: sessile; m: mobile; NA: Not Assessed).

Species	Phylum	Class	Family	Substratum	Feeding strategy	Habitat	Status	Outer Dock	Middle Dock	Inner Dock
<i>Clathrina primordialis</i>	Porifera	Calcarea	Clathrinidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Sycon ciliatum</i>	Porifera	Calcarea	Syctetidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	x
<i>Pachymatisma jonhstonia</i>	Porifera	Demospongiae	Geodiidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Halichondria panicea</i>	Porifera	Demospongiae	Halichondriidae	Hard, s.	Suspensivore	Inter/sub	Native	x		
<i>Anemonia viridis</i>	Cnidaria	Anthozoa	Actiniidae	Hard, s.	Carnivorous	Intertidal	Native	x	x	
<i>Bougainvillia muscus</i>	Cnidaria	Hydrozoa	Bougainvilliidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Obelia geniculata</i>	Cnidaria	Hydrozoa	Campanulariidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Clytia hemisphaerica</i>	Cnidaria	Hydrozoa	Campanulariidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Obelia bidentata</i>	Cnidaria	Hydrozoa	Campanulariidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Laomedea neglecta</i>	Cnidaria	Hydrozoa	Campanulariidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Laomedea flexuosa</i>	Cnidaria	Hydrozoa	Campanulariidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	x
<i>Halophteris catharina</i>	Cnidaria	Hydrozoa	Halopterididae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Kirchenpaueria pinnata</i>	Cnidaria	Hydrozoa	Kirchenpaueriidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Plumularia setacea</i>	Cnidaria	Hydrozoa	Plumulariidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	x
<i>Sertularella gayi</i>	Cnidaria	Hydrozoa	Sertularellidae	Hard, s.	Suspensivore	Inter/sub	Native	x		
<i>Sertularella polyzonias</i>	Cnidaria	Hydrozoa	Sertularellidae	Hard, s.	Suspensivore	Inter/sub	Native	x		
<i>Sertularella gaudichaudi</i>	Cnidaria	Hydrozoa	Sertularellidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Emplectonema gracile</i>	Nemertea	Hoplonemertea	Emplectonematidae	Heterog, m.	Carnivorous	Intertidal	Native	x		
<i>Cephalothrix rufifrons</i>	Nemertea	Palaeonemertea	Cephalothrichidae	Hard, m.	Carnivorous	Intertidal	Native	x		
<i>Lineus longissimus</i>	Nemertea	Piliidophora	Lineidae	Heterog, m.	Carnivorous	Intertidal	Native	x	x	
<i>Bugulina stolonifera</i>	Bryozoa	Gymnolaemata	Bugulidae	Hard, s.	Suspensivore	Intertidal	Cryptogenic	x		
<i>Bugula neritina</i>	Bryozoa	Gymnolaemata	Bugulidae	Hard, s.	Suspensivore	Inter/sub	Cryptogenic	x	x	
<i>Crisularia plumosa</i>	Bryozoa	Gymnolaemata	Bugulidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Penetrantia concharum</i>	Bryozoa	Gymnolaemata	Penetrantiidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Tricellaria inopinata</i>	Bryozoa	Gymnolaemata	Candidae	Hard, s.	Suspensivore	Inter/sub	Alien/Invasive	x		
<i>Schizoporella cf. japonica</i>	Bryozoa	Gymnolaemata	Schizoporellidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x		
<i>Watersipora subatra</i>	Bryozoa	Gymnolaemata	Watersiporidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x	x	x
<i>Crisidia cornuta</i>	Bryozoa	Stenolaemata	Crisiidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Filicrisia geniculata</i>	Bryozoa	Stenolaemata	Crisiidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Phascolosoma stephensoni</i>	Sipuncula	Phascolosomatidea	Phascolosomatidae	Heterog, m.	Suspensivore	Inter/sub	Native	x		
<i>Limaria hians</i>	Mollusca	Bivalvia	Limidae	Hard, m.	Suspensivore	Inter/sub	Native	x		
<i>Musculus costulatus</i>	Mollusca	Bivalvia	Mytilidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Musculus subpictus</i>	Mollusca	Bivalvia	Mytilidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	x
<i>Modiolus barbatus</i>	Mollusca	Bivalvia	Mytilidae	Hard, s.	Suspensivore	Inter/sub	Native	x		
<i>Modiolula phaseolina</i>	Mollusca	Bivalvia	Mytilidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Mytilus galloprovincialis</i>	Mollusca	Bivalvia	Mytilidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	x
<i>Mytilaster minimus</i>	Mollusca	Bivalvia	Mytilidae	Hard, s.	Suspensivore	Intertidal	Cryptogenic	x		
<i>Magallana gigas</i>	Mollusca	Bivalvia	Ostreidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x	x	x
<i>Irus irus</i>	Mollusca	Bivalvia	Veneridae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Crepidula fornicata</i>	Mollusca	Gastropoda	Calyptraeidae	Hard, m.	Suspensivore	Intertidal	Alien/Invasive	x		
<i>Crepidatella dilatata</i>	Mollusca	Gastropoda	Calyptraeidae	Hard, m.	Suspensivore	Intertidal	Alien	x	x	
<i>Haliotis tuberculata</i>	Mollusca	Gastropoda	Haliotidae	Hard, m.	Suspensivore	Intertidal	Native	x	x	
<i>Columbella adansoni</i>	Mollusca	Gastropoda	Columbellidae	Hard, m.	Herbivorous	Intertidal	Alien	x		
<i>Tritia incrassata</i>	Mollusca	Gastropoda	Nassariidae	Hard, m.	Carnivorous	Intertidal	Native	x		
<i>Patella depressa</i>	Mollusca	Gastropoda	Patellidae	Hard, m.	Herbivorous	Intertidal	Native	x		
<i>Patella vulgata</i>	Mollusca	Gastropoda	Patellidae	Hard, m.	Herbivorous	Intertidal	Native	x	x	
<i>Patella rustica</i>	Mollusca	Gastropoda	Patellidae	Hard, m.	Herbivorous	Intertidal	Native	x	x	
<i>Patella ulyssiponensis</i>	Mollusca	Gastropoda	Patellidae	Hard, m.	Herbivorous	Intertidal	Native	x	x	
<i>Cingula trifasciata</i>	Mollusca	Gastropoda	Rissoidae	Hard, m.	Herbivorous	Intertidal	Native	x	x	x
<i>Trivia monacha</i>	Mollusca	Gastropoda	Triviidae	Hard, m.	Carnivorous	Intertidal	Native	x		
<i>Ocenebra erinaceus</i>	Mollusca	Gastropoda	Muricidae	Hard, m.	Herbivorous	Inter/sub	Native	x		
<i>Aeolidiella glauca</i>	Mollusca	Gastropoda	Aeolidiidae	Hard, m.	Carnivorous	Intertidal	Native	x		

(continued on next page)

Table 1 (continued)

Species	Phylum	Class	Family	Substratum	Feeding strategy	Habitat	Status	Outer Dock	Middle Dock	Inner Dock
<i>Acanthochitona crinita</i>	Mollusca	Polyplocophora	Acanthochitonidae	Hard, m.	Herbivorous	Intertidal	Native	x		
<i>Lysidice ninetta</i>	Annelida	Polychaeta	Eunicidae	Hard, m.	Carnivorous	Inter/sub	Native	x	x	
<i>Leodice torquata</i>	Annelida	Polychaeta	Eunicidae	Hard, m.	Carnivorous	Inter/sub	Native	x	x	
<i>Leodice harassii</i>	Annelida	Polychaeta	Eunicidae	Hard, m.	Carnivorous	Inter/sub	Native	x	x	x
<i>Lumbrineris</i> sp.	Annelida	Polychaeta	Lumbrineridae	Hard, m.	Carnivorous	Inter/sub	NA	x		
<i>Neanthes</i> sp.	Annelida	Polychaeta	Nereididae	Heterog, m.	Omnivorous	Inter/sub	NA	x	x	x
<i>Platynereis dumerilii</i>	Annelida	Polychaeta	Nereididae	Hard, m.	Carnivorous	Intertidal	Native	x	x	
<i>Perinereis cultrifera</i>	Annelida	Polychaeta	Nereididae	Heterog, m.	Carnivorous	Intertidal	Native	x	x	
<i>Nereididae</i> sp.	Annelida	Polychaeta	Nereididae	Heterog, m.	Carnivorous	Intertidal	NA	x	x	
<i>Eulalia clavigera</i>	Annelida	Polychaeta	Phyllococidae	Hard, m.	Carnivorous	Intertidal	Native	x		
<i>Harmothoe impar</i>	Annelida	Polychaeta	Polynoidae	Hard, m.	Carnivorous	Inter/sub	Native	x		
<i>Lepidonotus squamatus</i>	Annelida	Polychaeta	Polynoidae	Hard, m.	Carnivorous	Intertidal	Native	x		
<i>Alentia gelatinosa</i>	Annelida	Polychaeta	Polynoidae	Heterog, m.	Carnivorous	Inter/sub	Native	x		
<i>Lepidonotus clava</i>	Annelida	Polychaeta	Polynoidae	Hard, m.	Carnivorous	Intertidal	Native	x	x	
<i>Sabella spallanzanii</i>	Annelida	Polychaeta	Sabellidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Branchiomma bombyx</i>	Annelida	Polychaeta	Sabellidae	Hard, s.	Suspensivore	Inter/sub	Native	x	x	
<i>Branchiomma luctuosum</i>	Annelida	Polychaeta	Sabellidae	Hard, s.	Suspensivore	Inter/sub	Alien	x	x	
<i>Ficopomatus enigmaticus</i>	Annelida	Polychaeta	Serpulidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x	x	
<i>Spirobranchus triquetus</i>	Annelida	Polychaeta	Serpulidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Vermiliopsis striaticeps</i>	Annelida	Polychaeta	Serpulidae	Hard, s.	Suspensivore	Intertidal	Native	x		
<i>Spirorbis spirorbis</i>	Annelida	Polychaeta	Serpulidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	
<i>Polydora</i> sp.	Annelida	Polychaeta	Spionidae	Hard, m.	Carnivorous	Intertidal	NA	x	x	x
<i>Laonice cirrata</i>	Annelida	Polychaeta	Spionidae	Hard, m.	Carnivorous	Intertidal	Native	x	x	
<i>Trypanosyllis zebra</i>	Annelida	Polychaeta	Syllidae	Hard, m.	Carnivorous	Intertidal	Native	x		
<i>Eupolyornia nebulosa</i>	Annelida	Polychaeta	Terebellidae	Heterog, m.	Suspensivore	Subtidal	Native	x		
<i>Grandierella japonica</i>	Arthropoda	Malacostraca	Aoridae	Heterog, m.	Omnivorous	Inter/sub	Alien/Invasive	x		
<i>Pseudoprotella phasma</i>	Arthropoda	Malacostraca	Caprellidae	Hard, m.	Carnivorous	Inter/sub	Native	x	x	
<i>Caprella linearis</i>	Arthropoda	Malacostraca	Caprellidae	Hard, m.	Omnivorous	Inter/sub	Native	x		
<i>Caprella equilibra</i>	Arthropoda	Malacostraca	Caprellidae	Hard, m.	Omnivorous	Subtidal	Native	x		
<i>Diogenes pugilator</i>	Arthropoda	Malacostraca	Diogenidae	Soft, m.	Carnivorous	Subtidal	Native	x		
<i>Galathea strigosa</i>	Arthropoda	Malacostraca	Galatheididae	Hard, m.	Carnivorous	Subtidal	Native	x		
<i>Pachygrapsus marmoratus</i>	Arthropoda	Malacostraca	Grapsidae	Hard, m.	Omnivorous	Intertidal	Native	x	x	
<i>Macropodia rostrata</i>	Arthropoda	Malacostraca	Inachidae	Heterog, m.	Carnivorous	Inter/sub	Native	x		
<i>Pilumnus hirtellus</i>	Arthropoda	Malacostraca	Pilumnidae	Heterog, m.	Carnivorous	Inter/sub	Native	x	x	
<i>Maera grossimana</i>	Arthropoda	Malacostraca	Maeridae	Hard, m.	Omnivorous	Intertidal	Native	x		
<i>Perforatus perforatus</i>	Arthropoda	Thecostraca	Balanidae	Hard, s.	Suspensivore	Intertidal	Native	x	x	x
<i>Balanus trigonus</i>	Arthropoda	Thecostraca	Balanidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x		
<i>Austrominius modestus</i>	Arthropoda	Thecostraca	Elminidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x		
<i>Paracentrotus lividus</i>	Echinodermata	Echinoidea	Parechinidae	Hard, m.	Omnivorous	Inter/sub	Native	x	x	
<i>Pawsonia saxicola</i>	Echinodermata	Holothuroidea	Cucumariidae	Hard, m.	Suspensivore	Inter/sub	Native	x		
<i>Holothuria tubulosa</i>	Echinodermata	Holothuroidea	Holothuriidae	Hard, m.	Suspensivore	Inter/sub	Native	x		
<i>Amphipholis squamata</i>	Echinodermata	Ophiuroidea	Amphiuroidae	Hard, m.	Suspensivore	Inter/sub	Native	x		
<i>Ophiothrix fragilis</i>	Echinodermata	Ophiuroidea	Ophiotrichidae	Hard, m.	Suspensivore	Intertidal	Native	x	x	
<i>Ophiocten affinis</i>	Echinodermata	Ophiuroidea	Ophiuridae	Hard, m.	Suspensivore	Inter/sub	Native	x	x	
<i>Phallusia mammillata</i>	Chordata	Ascidacea	Ascidiidae	Hard,s.	Suspensivore	Intertidal	Native	x		
<i>Didemnum vexillum</i>	Chordata	Ascidacea	Didemniidae	Hard, s.	Suspensivore	Intertidal	Alien/Invasive	x		
<i>Corella eumyota</i>	Chordata	Ascidacea	Corellidae	Hard, s.	Suspensivore	Inter/sub	Alien/Invasive	x	x	x
<i>Styela plicata</i>	Chordata	Ascidacea	Styelidae	Hard,s.	Suspensivore	Intertidal	Alien/Invasive	x	x	x
<i>Styela clava</i>	Chordata	Ascidacea	Styelidae	Hard,s.	Suspensivore	Inter/sub	Alien/Invasive	x	x	x
<i>Botrylloides violaceus</i>	Chordata	Ascidacea	Styelidae	Hard,s.	Suspensivore	Intertidal	Alien/Invasive	x	x	x

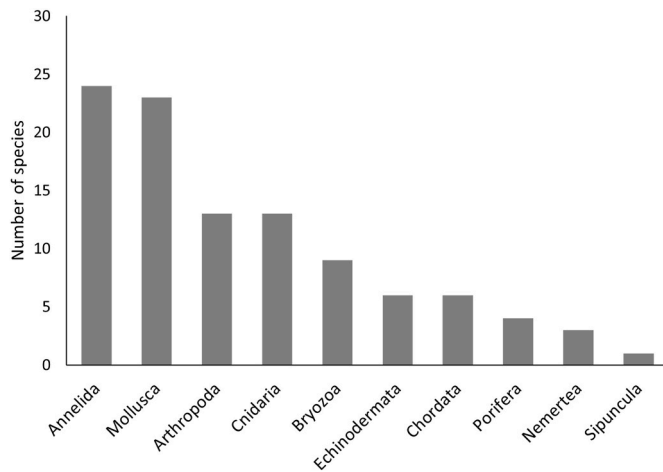


Fig. 2. Number of species from each phylum found as epibionts on *Mytilus galloprovincialis*.

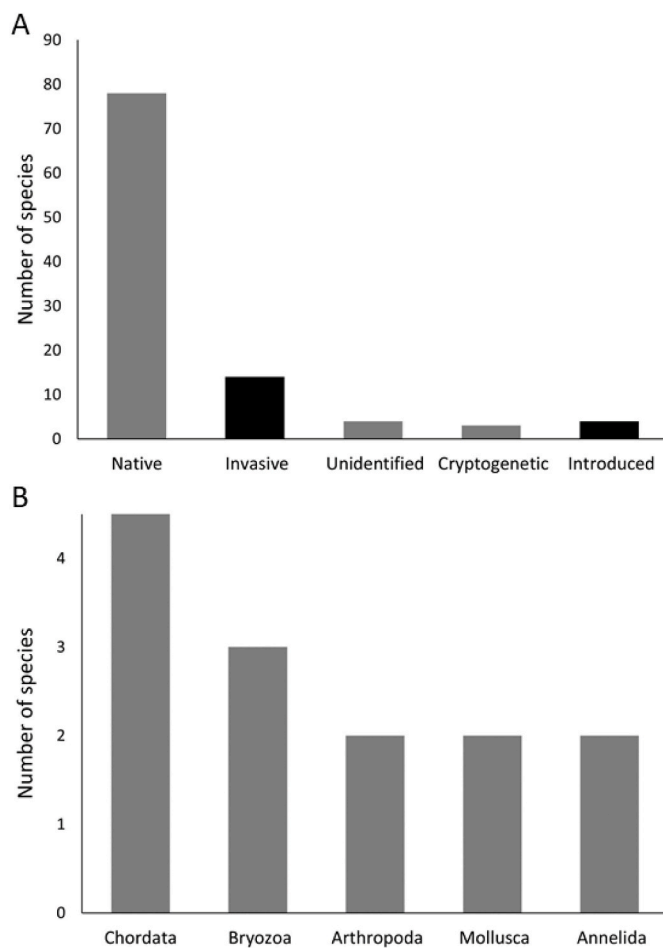


Fig. 3. A, Number of identified species from each status (introduced and invasive species are highlighted in black colour); B, Distribution of the identified invasive species among different phyla.

short larval stage of maximum 24 h and larvae can only spread 4.5 km from their origin due to limitations morphological limitations (Page et al., 2019). Therefore, the only explanation for their great dispersal is shipping transport (Page et al., 2019), acting as a ‘stepping-stone’ model on which they settle and generate adult individuals that produce new larvae, starting the cycle again and making it possible to colonize new

ecosystems. *Watersipora subatra* has a great capacity to compete for space with other sessile species, displacing them and potentially reducing the biodiversity of the host environment. In addition, this species is usually associated with the oyster *M. gigas* (Gouletquer, 2016), also present and widespread in the Marina of Gijón. Besides, the elemental microanalysis revealed high levels of aluminum in relation to other elements, such as calcium, which is part of most bryozoan covers (Tebble, 1976). No information was found in the literature to explain the high levels of aluminium in this bryozoan, but the aluminium is common in port waters due to the paintings of boats and fossil fuels. The results of this analysis suggest that there may be a bioaccumulation of this element in *W. subatra*, likely posing a risk of accumulation in the food chain. Many bryozoans are preyed on by commercial animals like crustaceans or sea urchins (Gouletquer, 2016), being able to pass into our tissues through their consumption of them (Walton et al., 2010).

The true identity of the specimens identified as *Schizoporella* cf. *japonica* Ortmann, 1890 is open to question due to the great variability of the species and its resemblance with *S. unicornis* (Johnston in Wood, 1844) (Tompsett et al., 2009; Ryland et al., 2014). However, the usual habitats of the two species are different: *S. japonica* is a typical fouling species, only known from harbours and marinas (within its European distribution range), while *S. unicornis* tends to occur in non-fouling situations, on stones, rocks, shells and kelp holdfasts from the lower shore to the sublittoral (Ryland et al., 2014). The ability of *S. japonica* and allied species to foul a variety of substrata, e.g. plastic, wood, pontoons, buoys, ropes and recreational and commercial boat hulls, may facilitate its spreading among ports and marinas around the world (Tompsett et al., 2009).

Juvenile and small-sized Pacific oyster *M. gigas* are here reported as abundant epibionts of Mediterranean mussels. This species reached northern Iberian coasts both through ‘biofouling’ and through an intense aquaculture activity (Gofas et al., 2011; Gouletquer, 2016). It is an engineering species that modifies the substrate and the sedimentary processes due to its intense filtration, competes for space with other sessile species and can introduce other species associated to it. Besides, it has long larval stages (2–4 weeks) and fast growth rates that allow a rapid colonization of distant areas (Gofas et al., 2011; Gouletquer, 2016). Although mussel patches may facilitate the development of juvenile *M. gigas*, as reported in this study, the ability of the Pacific oyster to displace other sessile organisms leads to the complete eradication of mussel patches in some areas, as it happened in the Wadden Sea (North Sea) with *Mytilus edulis* Linnaeus, 1758 (Kochmann et al., 2008).

Another invasive species detected in the Marina of Gijón was the Japanese amphipod *Grandidierella japonica* Stephensen, 1938, which is also associated with the aquaculture of *M. gigas* and competes for resources with other autochthonous amphipods (Lavesque et al., 2014; Gouletquer, 2016). This is the second record for this species in the Cantabrian Sea (the first one was from the Bidasoa estuary (Foulquier et al., 2018)), which may indicate that *G. japonica* is in an expansion process across this area. The invasive colonial ascidians *Botrylloides violaceus* Oka, 1927 and *Didemnum vexillum* Kott 2002 were also reported here as mussel epibionts. These colonial sea squirts spread rapidly, covering large areas that prevent the settlement of other sessile individuals due to their gelatinous cover (Cordell et al., 2013) and produce chemical substances that make them resistant to potential predators, such as echinoderms or gastropods (Dijkstra and Harris, 2009; Gouletquer, 2016). There is no apparent competition for food against mussels because, despite being all of them filter feeders, the size of the particles ingested by these sea squirts is much smaller than for mussels (in *M. edulis* and *M. galloprovincialis* they range from 2 to 16 µm, while in these ascidians it ranges from 2 to 3 µm). However, they can completely cover the mussels preventing their feeding by filtration (Arens et al., 2011). Another invasive sea squirt found in this study was *Styela clava* Herdman, 1881, whose spread reduces the space available for other species, reducing the local biodiversity (Gouletquer, 2016). The invasion capacity of all these species is deeply fostered by the lack of

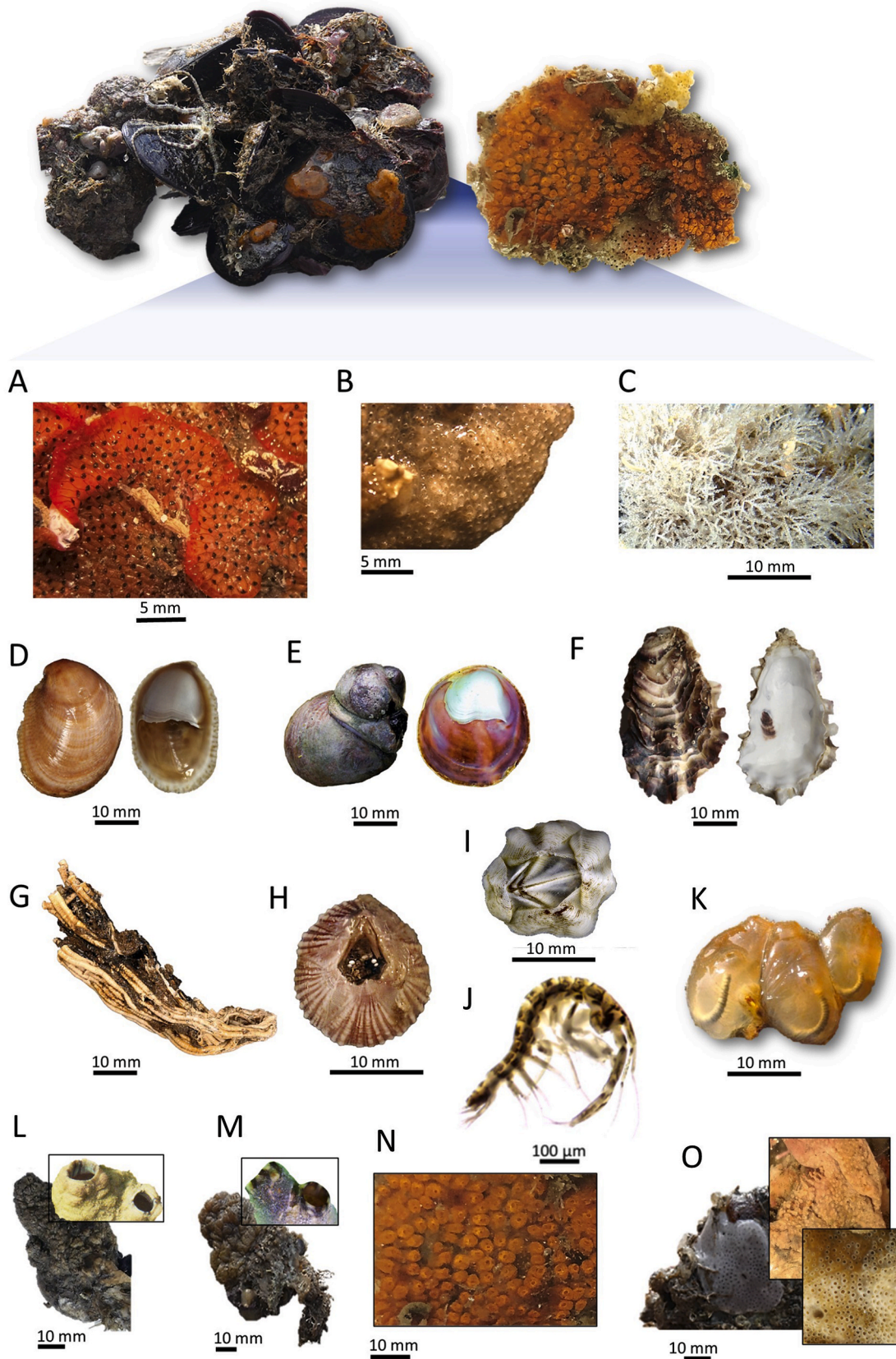


Fig. 4. Photographs of the associated alien fauna of *Mytilus galloprovincialis* from the study area (top, overall view of a *M. galloprovincialis* patch). A, *Watersipora subatra*; B, *Schizoporella* cf. *japonica*; C, *Tricellaria inopinata*; D, *Crepidula fornicata*; E, *Crepidatella dilatata*; F, *Magallana gigas*; G, *Ficopomatus enigmaticus*; H, *Balanus trigonus*; I, *Austrominius modestus*; J, *Grandidierella japonica*; K, *Corella eumyota*; L, *Styela plicata*; M, *Styela clava*; N, *Botrylloides violaceus*; O, *Didemnum vexillum*.

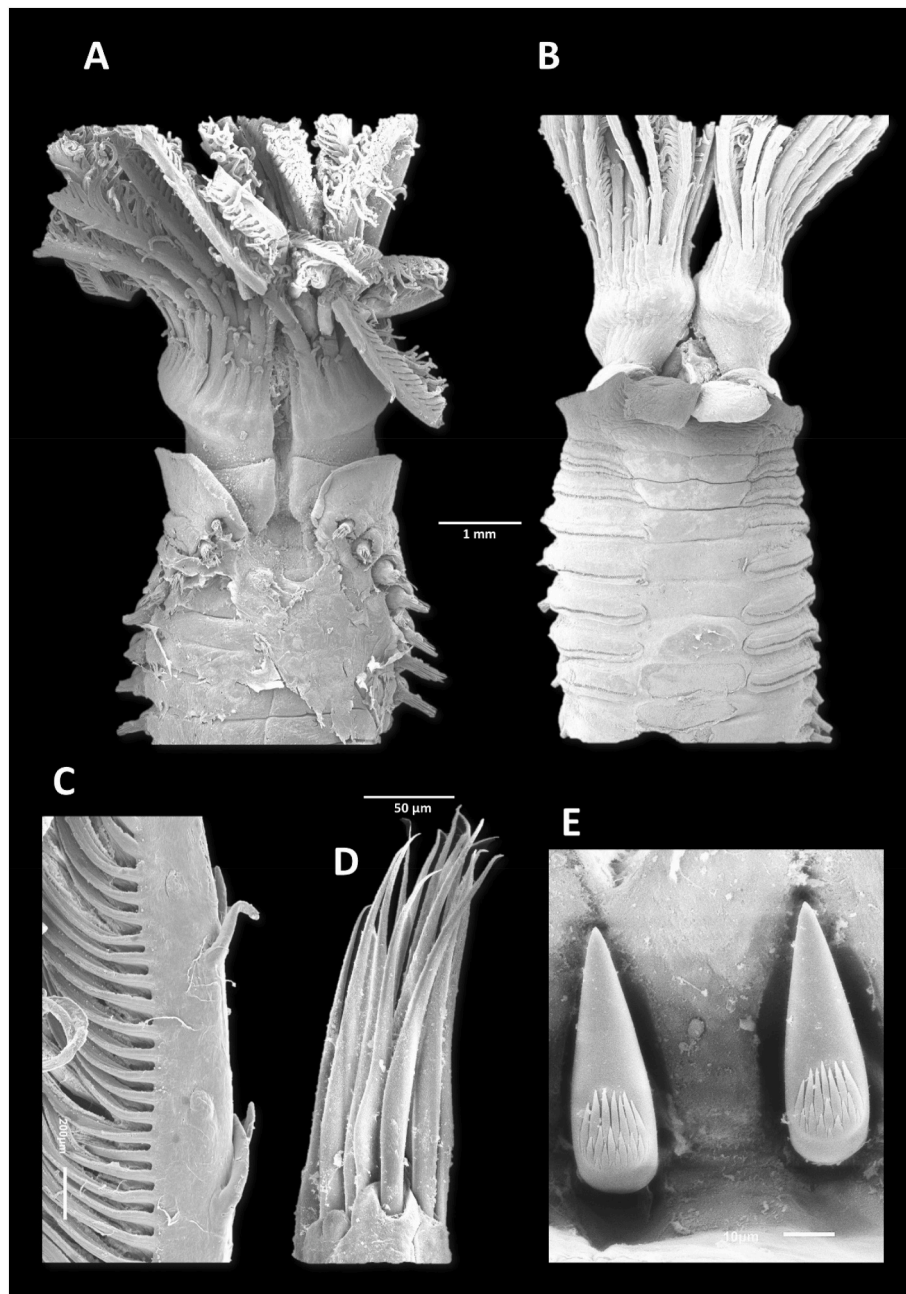


Fig. 5. *Branchiommma luctuosum* scanning electron micrographs. A, anterior end, dorsal view; B, anterior end, ventral view; C, detail of radiole, showing stylodes and radiolar eyes; D, thoracic inferior notochaetae; E, thoracic uncini.

autochthonous analogues (Lützen, 1999).

The invasive barnacles *Balanus trigonus* Darwin, 1854 and *Austrominius modestus* (Darwin, 1854), found as epibionts of *M. galloprovincialis* in this study, may represent an important threat for mussels growth, as suggested by several experiments performed with the similar species *Balanus crenatus* Bruguière, 1789 (Buschbaum and Saier, 2001). These barnacles modify the mussel morphology, which alters the micro-currents and compromises their feeding (Buschbaum and Saier, 2001). Barnacles also produce a “cascade of epibionts”, facilitating the recruitment of other epibiontic organisms and thus potentially covering the mussels and difficulting their survival (Gutiérrez and Palomo, 2016; Page et al., 2019).

*Columbella adansoni*, a gastropod belonging to the family Columbellidae, is native along the Macaronesian and West African coasts, from Senegal to Angola, where it is abundant in shallow infralittoral waters

(Moolenbeek and Hoenselaar, 1991; Gofas et al., 2011). This species is similar to *C. rustica* (Linnaeus, 1758), but they differ in the type of larval development and, consequently, in the type of protoconch presented. *Columbella adansoni* has a planktotrophic development and multispiral protoconch (Fig. 6D and E), while *C. rustica* has a direct development and paucispiral protoconch (Moolenbeek and Hoenselaar, 1991; Gofas et al., 2011). To date, this species was only known to be present on the beach of l'Arbeyal, very close to the Marina of Gijón, where its presence was reported in 2010 (Arias, 2012; Arias et al., 2012). This species likely arrived as a stowaway on artificial floating rafts dragged by sea currents from the Macaronesian coasts. *Columbella adansoni* has still not developed an invasive behaviour in the area, since it does not seem to have expanded its local distribution area and increased the number of individuals. However, it would be necessary evaluate the evolution of this species in the Asturian coast, since it is an herbivorous species that may



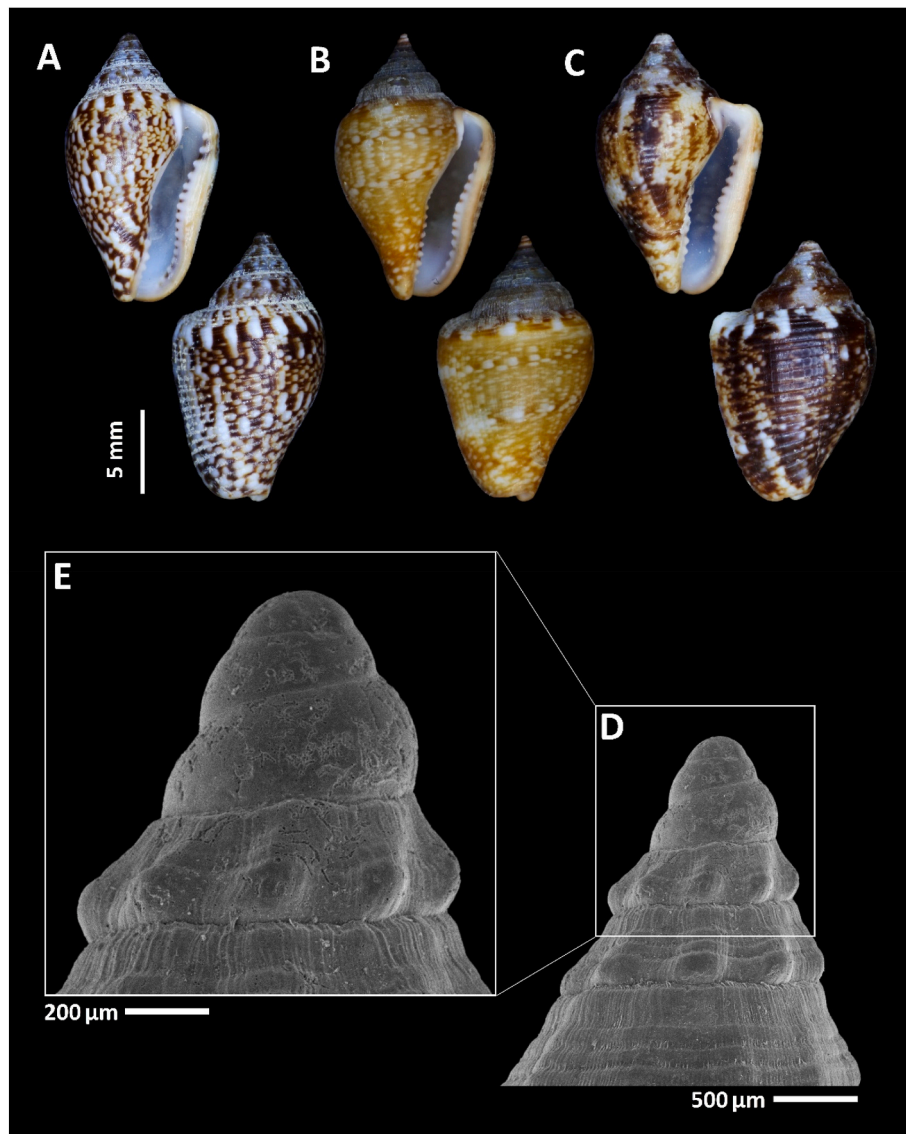


Fig. 6. *Columbella adansoni*. A-C, different adult morphotypes; D, protoconch, scanning electron micrograph; E, detail of the protoconch, scanning electron micrograph.

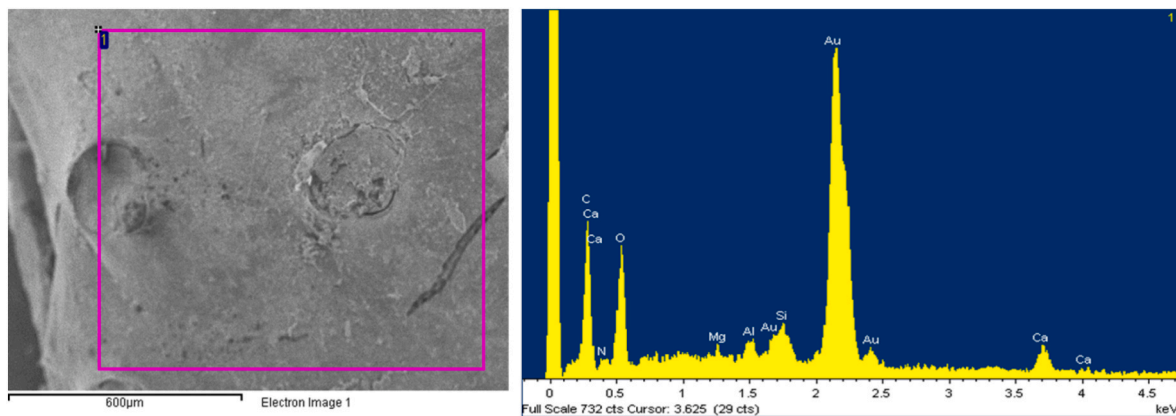


Fig. 7. Microanalysis of *Watersipora subatra*. The X-ray spectrum of dispersive energy represents the counts of x-ray photons emitted by each element of the sample.

compete with local species for food and shelter.

*Branchiommma luctuosum* is a tube-building, filter-feeding polychaete that forms aggregates of three to four individuals in shallow water. It is

usually associated with corals or sponges from sheltered areas of coral reefs (Grube, 1870). This species is native from the Red Sea and was reported as introduced in the Mediterranean Sea (Phyllis et al., 1991)

**Table 2**  
Percentage of content of each of the elements measured in the microanalysis on the exotic bryozoan *Watersipora subatra*.

Element	Percentage
Carbon	38.48
Nitrogen	11.01
Oxygen	37.96
Magnesium	0.43
Aluminium	1.60
Silicon	2.72
Calcium	7.80

and in the South Atlantic coasts of the Iberian Peninsula (Fernández-Romero et al., 2021). *Branchiommata luctuosum* has been reported as one of the 100 worst invasive alien marine species in the Mediterranean (Streftaris and Zenetos, 2006) due to i) its high densities in artificial and natural habitats, ii) ability to growth in nutrient-enriched water, iii) efficient anti-predation strategies, iv) reproduction by simultaneous hermaphroditism and v) very short larval pelagic phase (Sordino and Gambi, 1992; Giangrande and Gambi, 1998; Licciano et al., 2002; Mastrotoaro et al., 2015). The large and discontinuous distribution of *B. luctuosum* suggests that the main introduction and spread vector was likely the transport of larval stages with ships' ballast waters, in which the species can even survive during long interoceanic transits (El Hadad et al., 2008).

Calyptraeid gastropods have been commonly introduced in bays, estuaries and ports worldwide, and several species are highly invasive, such as *Crepidula fornicata* (Linnaeus, 1758) (Collin et al., 2009). *Crepidula fornicata* has caused considerable damage to French shellfisheries and has become the dominant benthic species in the Solent (UK), reaching densities that surpass 1000 individuals/m<sup>2</sup> (Blanchard, 1997; Collins et al. 2009). To date, for West Europe, only two alien calyptreids have been reported: *Cr. fornicata*, widespread across the European Atlantic and *Crepidipatella dilatata* (Lamarck, 1822) restricted to Vigo and Pontevedra estuaries in the Atlantic Galicia and the central Cantabrian Sea (NW Spain) (Rolan and Horro, 2005; Collins et al. 2009; Richter et al., 2012). *Crepidipatella dilatata* has been previously found in the vicinities of the Gijón Musel Port and the Gijón Marina in an intertidal rocky community as epibiont on *M. galloprovincialis* and directly attached to rubble, rocks and the artificial port breakwater (A. Arias, unpublished data). *Crepidipatella dilatata* can be differentiated from other South American members of the genus by its non-planktotrophic development with nurse eggs that never cleave (Collins et al. 2009). The most probable vector of introduction of *C. dilatata* to the Marina of Gijón is the 'shipping', due to its proximity to an important merchant port. The Musel Port has had and still has regular routes to Vigo and Pontevedra. So, it is most likely that the population of Gijón was a result of the subsequent spread of specimens from Galicia as fouling species. However, further research involving genetic studies would be required to corroborate this hypothesis. *Crepidipatella dilatata* certainly has the potential to become invasive. Its preference for living as epibiont on other marine molluscs, may turn this species into a plague on mussel cultures and aquaculture facilities (Collins et al. 2009), competing with bivalves for food and generating economic losses in aquaculture production, as has occurred with *Cr. fornicata* (Blanchard, 1997).

This work represents a baseline study of the diversity and status of the associated fauna to *M. galloprovincialis* from a northern Spain marina. This analysis allows to identify the starting points for a forthcoming program or project to assess the biodiversity of native and alien species within the tridimensional, biological structures in European ports and marinas. Further research comparing these results with "naked" patches (not colonized by mussels or other structuring organisms), but also with patches of *Bugula neritina* (Linnaeus, 1758) (an arborescent bryozoan that creates tridimensional structures that can provide shelter and nutrients to other epibionts) would be of great

interest to understand the mechanisms of facilitation of the settlement of alien species.

## 5. Conclusions

Mussel patches may facilitate the settlement and development of non-indigenous species in marinas, especially in juvenile and larval stages, which may mask the presence of alien species in ports and delay its detection until they spread and reach adulthood. In this context, in addition to preventive measures like the use of "anti-fouling" paintings and biocides in ships and in aquaculture, we highlight the importance of monitoring these mussel aggregations as a management tool for the early control and prevention of marine bioinvasions. The conducted elemental microanalysis of the widely distributed exotic bryozoan *W. subatra* revealed high levels of aluminium in relation to other elements, representing a potential bio-risk in receiving environments and thus requiring a more in-depth study. The newly recorded fanworm *B. luctuosum* has developed an invasive behaviour across the Mediterranean and Atlantic coasts of Iberia and is now spreading into similar habitats. Therefore, a monitoring program of its occurrence and distribution along the Cantabrian Sea coastline and the Bay of Biscay, is highly recommended.

## CRedit authorship contribution statement

**Irene Fernández-Rodríguez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ricardo López-Alonso:** Writing – review & editing, Investigation, Formal analysis. **Omar Sánchez:** Writing – review & editing, Investigation, Formal analysis. **Isaac Suárez-Turienzo:** Writing – original draft, Methodology, Investigation, Formal analysis. **Rocío Gutiérrez-Martínez:** Writing – original draft, Methodology, Investigation, Formal analysis. **Andrés Arias:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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