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## Barcodes of marine invertebrates from north Iberian ports: Native diversity and resistance to biological invasions

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## ABSTRACT

Ports are gateways for many marine organisms transported by ships worldwide, especially non-indigenous species (NIS). In this study carried out in North Iberian ports (Cantabrian Sea, Bay of Biscay) we have observed 38% of exotic macroinvertebrates. Four species, namely the barnacle *Austrominius modestus*, the tubeworm *Ficopomatus enigmaticus*, the Pacific oyster *Crassostrea gigas* and the pygmy mussel *Xenostrobus securis*, exhibited clear signs of invasiveness. A total of 671 barcode (cytochrome oxidase subunit I or 18S rRNA) genes were obtained and confirmed the species status of some cryptic NIS. Negative and significant correlation between diversity estimators of native biota and proportion of NIS suggests biotic resistance in ports. This could be applied to management of port biota for contributing to prevent the settlement of biopollutants in these areas which are very sensitive to biological invasions.

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### 1. Introduction

Biological pollution is a main challenge in marine settings (Molnar et al., 2008). In marine biological invasions, as in other fields related to environmental risks and biosecurity, there is a general consensus: prevention is more efficient than treatment. Once established in a new location, eradicating invasive populations is extremely challenging, costly and in many cases not feasible (Bax et al., 2003; Thresher and Kuris, 2004; Williams and Schroeder, 2004). Actions for controlling biological invasions are the most efficient at the early stage of incursion (Myers et al., 2000; Hopkins et al., 2011; Miralles et al., 2016). Thus early detection of invasive species is a crucial step for successful post-introduction management (e.g. Pochon et al., 2015; Devloo-Delva et al., 2016). For the successful prevention of new invasions it is especially important to identify the main features of recipient communities that may make them resistant to invasions. Both habitat conditions and biological properties of the recipient ecosystem determine the success of aquatic invasions (e.g. Zaiko et al., 2007, 2011; Valiente et al., 2010a, 2010b), being generally facilitated in degraded environments (e.g. MacDougall and Turkington, 2005; Linde et al., 2008).

One of the factors that may confer resistance to invasions is native biodiversity of the recipient community, a phenomenon called biotic resistance. More diverse communities are expected to be more resistant to invasions than impoverished communities (e.g. Stachowicz et al., 1999; Byers and Noonburg, 2003). This fact has been explained from the presence of fewer niches available for invaders in rich communities (Stachowicz et al., 1999), although the effect is not clear and there are discrepancies between studies (Fridley et al., 2007).

Ports are gates for marine invasions (Molnar et al., 2008; Ardura et al., 2015; Pejovic et al., 2016). Their generally degraded environment adds to the constant entrance of biota carried by ships (e.g. Seebens et al., 2013). If biotic resistance occurs also in degraded ecosystems, for similar environment and number of ship arrivals, ports with rich local communities should be less prone to biological invasions than ports with fewer native species. Here we tested this hypothesis in the central part of the Iberian Bay of Biscay, coast of Asturias region in Northern Spain. We have inventoried fouling animal communities from ports of different size, quantified native biodiversity and determined its correlation with the abundance of non-native species. Samples of fouling communities attached to artificial port structures were sampled from eight ports. DNA barcoding was carried out for accurate species identification, including cryptic species, and biodiversity estimated.

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## 2. Material and methods

### 2.1. Study region

The considered ports are located in the region of Asturias (43°20'N 6°00'W) in the Cantabrian Sea coast (Bay of Biscay) in the northern Iberian Peninsula. Eight ports were studied, from West to East: Figueras, Luarca, Cudillero, Aviles, Gijon, Villaviciosa, Ribadesella and Llanes (Fig. 1). Aviles and Gijon are commercial ports under national Spanish authority that receive large international cargo vessels, and also have adjacent fishing ports and marinas. The other six locations are fishing ports and associated marinas under Asturias regional authority, serving for local maritime traffic, arrival of fishing catch (from national and international waters) and recreational boating.

In this specific region introduction of exotics from shellfish aquaculture (Pacific oyster *Crassostrea gigas*, Japanese carpet shell *Ruditapes philippinarum*, hard clam *Mercenaria mercenaria*) has been reported previously (e.g. Arias and Anadón, 2012; Habtemariam et al., 2015; Semeraro et al., 2015). Currently aquaculture production is scarce and is majorly represented by Pacific oyster farms located exclusively in the Eo estuary near Figueras port (Semeraro et al., 2015). Pacific oysters were also grown in Ribadesella in the past and are still present in the wild (Fabioux et al., 2002). Other marginal shellfish production is carpet shell and razor clam that are harvested in Villaviciosa (artisanal harvesting). They are supported with stocking practices. In the rest of the region

there is no marine aquaculture, so it does not contribute much as a potential pathway for NIS introductions.

### 2.2. Sampling methodology

Sampling protocol followed Pejovic et al. (2016), was inspired by the Rapid Assessment Survey (RAS) approach (Minchin, 2007) and adapted for assemblages living on artificial port structures. For this study, eight ports were selected across Asturias coastline, in the northern Iberian Peninsula (Fig. 1). Three sites were sampled inside each port: one near the port mouth, one in the inner section, one half way between these two. The sites were similarly uncovered by algae and sheltered (inside the port) to avoid environmental sampling biases. To standardize the sampling effort, the surface sampled from each site within each port was approximately 200 m<sup>2</sup>. Roughly 1% of the animals visually detected attached on that surface were collected at random. For a representative sampling, and preventing biased collection of species with patchy distribution, a visual inspection prior to sampling was made to determine the phenotypically different organisms (presumably different species) present in the sampling site. The number of individuals picked of each morphotype was approximately proportional to the abundance of such morphotype. All individuals were instantly transported in ambient water (contained in 10 L buckets) to the Genetic Laboratory of Natural Resources (University of Oviedo) for visual species identification and follow-up molecular analyses. In the laboratory, individuals were

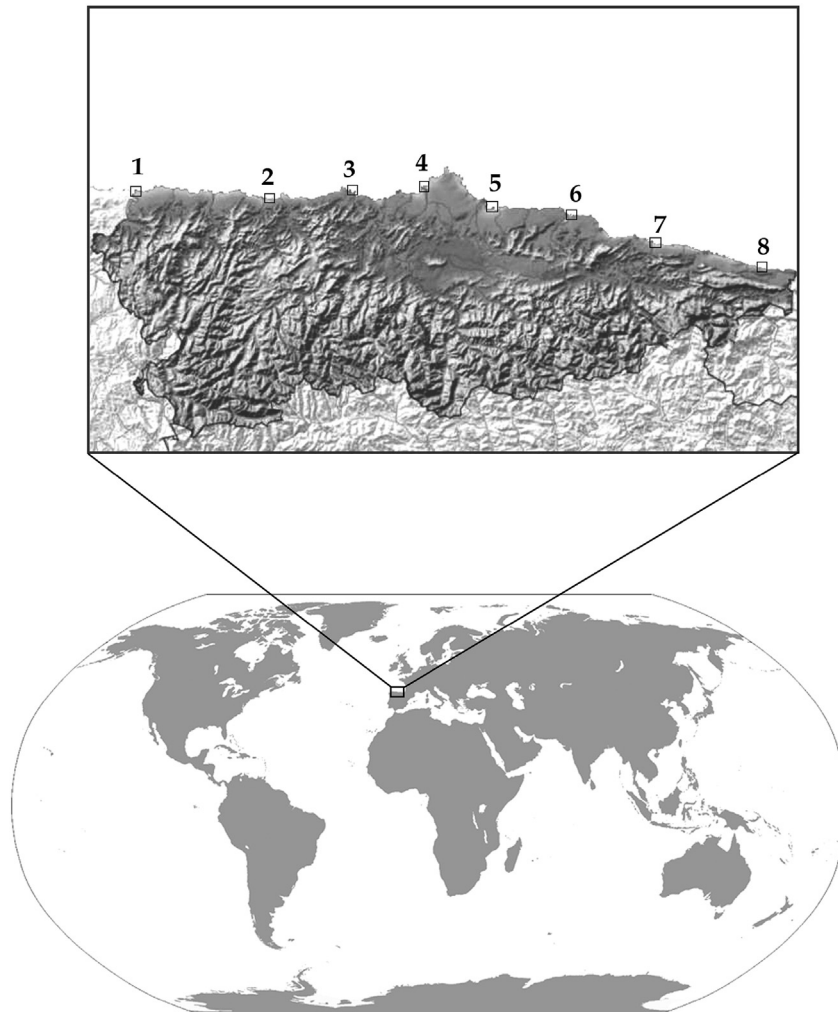


Fig. 1. Map of the Asturias coast showing the ports considered: from 1 to 8 are Figueras, Luarca, Cudillero, Aviles, Gijon, Villaviciosa, Ribadesella and Luarca.

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