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## Current status of the DNA barcode reference library of non-indigenous marine species occurring in European coastal regions

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Coastal habitats are among the most important, but also the most threatened ecosystems in the world, which may pose at risk many important services for human well-being (Solan et al. 2004). Along with global climate change, overexploitation, pollution and habitat destruction; the spread of invasive species is among the major threats to coastal ecosystems (Rilov 2009). Nonindigenous species (NIS), which can establish in areas outside their natural range and become invasive, can spread rapidly across the natural environment and displace and out-compete native species, driving to severe ecological changes that threaten ecosystem integrity (Rilov 2009). While morphologybased identification of taxa has largely contributed to appreciate the current status of NIS occurrence in coastal regions and marine environments (e.g. Chainho et al. 2015), this process is expertise-demanding, laborious and time consuming. Because of these limitations, most studies reporting bioinvasions and relying on morphotaxonomy are limited in time and space and only focus in the ecology of specific taxa or smaller groups at a time. Due to the abovementioned reasons, it becomes a priority to develop and optimize novel detection methods capable to overcome some of these challenges and that would allow an early detection and ease the monitoring of NIS in coastal ecosystems. Particularly, the combination of DNA barcoding with high-throughput sequencing (HTS) renders metabarcoding the capacity to bolster current biodiversity assessments techniques (Cristescu 2014). Within this approach, genetic information can be obtained through the use of standardized DNA barcode markers targeting a wide taxonomic range of organisms in mixed specimens or environmental samples (Cristescu 2014). DNA-based tools promise a number of potential benefits over traditional methods, including

increased sensitivity and specificity as well as greater throughput and cost effectiveness. However, the success of DNA-based methods is greatly dependent on the availability, taxonomic coverage and reliability of reference sequence databases, whose deficiencies can potentially compromise species identifications through HTS (Briski et al. 2016). In this study, we conducted a species-gap analysis on the availability of accessible DNA barcode sequences for marine NIS occurring in European coastal regions using the Barcode of Life Data System V4 (BOLD V4) (Ratnasingham and Hebert 2007). The checklist of NIS occurring in European seas was compiled by using two online databases: i) European Alien Species Information Network (EASIN) and ii) Information System on Aquatic Non-Indigenous and Cryptogenic Species (AquaNIS). The taxonomic classification of the NIS compiled in the checklist followed the World Register of Marine Species (WoRMS). All records without taxonomic assignments at the species level were removed from the list and only taxa with accepted scientific species names were maintained. The species list was submitted to BOLD v4 and the checklist progress report option implemented in BOLD was used to conduct our gap analysis (Ratnasingham and Hebert 2007). Barcoded species were considered to be those with sequences that meet the following requirements: i) minimum sequence length of 500 bp; ii) < 1% ambiguous bases; iii) presence of forward and reverse trace files; iv) minimum quality of trace files status and v) presence of a country specification, either with co-ordinates or country name. Barcode compliance flags were applied to the following markers if the sequences meet the above requirements: the mitochondrial cytochrome C oxidase gene (COI), for animals and the maturase K gene (matK) or the rubisco large subunit gene (rbcL), for plants. For Chromista, due to the high diversity within this kingdom, barcode compliance flags were applied to the following markers: COI for all Chromista groups, and, in addition to COI, matK and rbcL for Cryptophyta, Haptophyta and Ochrophyta. A list of 1,915 NIS occurring in European coastal regions was generated and uploaded to BOLD v4 (checklist: CL-NISEU). The species in the list were distributed by three kingdoms: i) Animalia (1,411 species), ii) Chromista (276 species) and iii) Plantae (228 species), comprehending 28 phyla. The most well represented phyla with NIS were Arthropoda (331 species), Mollusca (324 species) and Chordata (285 species) within Animalia; Ochrophyta (105 species), Myzozoa (79 species) and Foraminifera (70 species), within Chromista and Rhodophyta (171 species) and Chlorophyta (40 species), within Plantae. From the total species in the list, 1,109 were still missing a DNA barcode on BOLD (including private and public records), which corresponded to ca. 60% of the total NIS. In addition, within barcoded species ca. 13% were singletons (i.e. only one barcode sequence available). However, when only highimpact species were taken into account (146 species with the status of established or invasive), the gap was much lower (ca. 22% of the species).

Animalia NIS were the most well represented with DNA barcodes, but still missing for ca. 49% of the species. Only 5 out of the 16 represented phyla have a % of barcoded species higher than 50% (Acanthocephala, Arthropoda, Chordata, Echinodermata and Nemertea). For Chromista and Plantae the gap of missing species was higher than 80%. Among Chromista, only 2 out of 8 phyla (Myzozoa and Ochrophyta) were represented with DNA barcodes, but the % of covered species was lower than 30%. Among Plantae phyla, the % of barcoded species varied between 50 and 100%, but for Rodophyta NIS no DNA barcodes were yet deposited on BOLD. The current study allowed us to gauge the gaps of DNA barcodes for NIS occurring in European coastal ecosystems. The few marine NIS eradications attempts that proved successful in the past owe their success to an early detection, soon after introduction. DNA-based tools would allow the detection of early developmental stages or smaller organisms, reducing the time from introduction to detection and increasing the success of NIS control and/or eradication (Holman et al. 2018). However, the gaps found in reference libraries can have strong implications for an accurate species identification through DNA-based tools. Prioritization efforts should be conducted in order to fulfil these gaps, in particular for high impact species still missing DNA barcodes.

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