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Species gap analysis in DNA barcode reference libraries of marine non-indigenous species in the Azores archipelago

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Coastal regions are widely recognised for their ecologic and socio-economic importance. The spread of invasive species, along with global climate change, overexploitation, pollution and habitat destruction, is a major threat to coastal ecosystems (Solan et al. 2004). Some of these species, in particular macroalgae and invertebrates, can spread quickly and become invasive, causing severe ecologic and economic impacts. When prevention fails, the early detection and surveillance of NIS could be essential for coordinating a timely and effective response to invasions. Marine ecosystems on oceanic islands, such as Azores, can be especially prone to invasions by non-indigenous species since they are characterized by a small number of native species and by a high availability of empty niches, and thus, the list of NIS is relatively long (Micael et al. 2014). There are no doubts that traditional morphology-based methods have highly contributed to the current knowledge about NIS communities inhabiting Portuguese coastal waters, including the Portuguese oceanic islands (Micael et al. 2014, Chainho et al. 2015). However, the hurdles associated with morphological methods may compromise the early detection and monitoring of the most risky species. On the other hand, DNA-based tools promise advantages over traditional ones, particularly the identification of individuals and life stages that cannot be recognized by using morphological approaches (Darling and Mahon 2011). Plus, most surveys typically target specific species or taxonomic groups, which may neither accurately represent overall ecosystems' condition nor allow an early detection of NIS. Due to the above-mentioned 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 of DNA barcode sequences available for marine NIS occurring in the Azores archipelago in the Barcode of Life Data System V4 (BOLD V4) (Ratnasingham and Hebert 2007) and in the GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). The list of NIS occurring in Azores was compiled from a wide variety of sources, mainly from literature searches through scientific papers and reports, including recent field and taxonomic studies, and supplemented with existing databases and this is the list that was submitted to the 2019 interim ICES WGITMO report (International Council for the Exploration of the Sea; Working Group on Introductions and Transfers of Marine Organisms). The taxonomic classification of the compiled NIS followed the AlgaeBase and World Register of Marine Species (WoRMS) databases. A checklist of 80 marine NIS occurring in Azores archipelago was compiled and the species in the list were checked for the presence of public sequences on both BOLD v4 and GenBank databases. The species in the list were distributed by three kingdoms: i) Animalia (48 species), ii) Plantae (30 species) and iii) Chromista (2 species), comprehending 10 phyla. The most well represented phyla with NIS were Chordata (15 species), Mollusca and Bryozoa (both with 8 species) within Animalia, and Rhodophyta within Plantae (26 species); while the only represented phyla within Chromista was Ochrophyta with 2 species. In total, 3,884 sequences belonging to 25 different markers were found for the 80 species, published in BOLD and GenBank. Among these published sequences, the COI-5P is the most well represented loci, with 3,224 records belonging to 44 species. However, from the total species in the list, 25 were still missing a DNA sequence, which corresponded to ca. 31% of the total NIS. In addition, within sequenced species ca. 14% were singletons (i.e. only one sequence available). Animalia NIS were the most well represented with sequences, missing only for ca. 27% of the species, while for Chromista and Plantae the gap of missing species was higher than 35%. The current study

allowed us to characterize the gaps in available sequences in public repositories for marine NIS occurring in the Azores archipelago. Actions developing DNA-based tools should be a priority for detection and effective management of biological invasions. DNA-based tools would allow the detection of early developmental stages or smaller organisms, reducing the time from introduction to discovery 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.

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