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Short communication

Are well-studied marine biodiversity hotspots still blackspots for animal barcoding?

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ABSTRACT

Marine biodiversity underpins ecosystem health and societal well-being. Preservation of biodiversity hotspots is a global challenge. Molecular tools, like DNA barcoding and metabarcoding, hold great potential for biodiversity monitoring, possibly outperforming more traditional taxonomic methods. However, metabarcoding-based biodiversity assessments are limited by the availability of sequences in barcoding reference databases; a lack thereof results in high percentages of unassigned sequences. In this study, we (i) present the current status of known vs. barcoded marine animal species at a global scale based on online taxonomic and genetic databases (NCBI and BOLD) and (ii) compare the current status with data from ten years ago. Then,

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we focused our attention on occurrence data of marine animal species from five Large Marine Ecosystems (LMEs) representing the most well studied biodiversity hotspots, to identify disparities in COI barcoding coverage between geographic regions and at phylum level. Barcoding coverage varied among LMEs (from 36.8% to 62.4% COI-barcoded species) and phyla (from 4.8% to 74.7% COI-barcoded species), with Porifera, Bryozoa and Platyhelminthes being highly underrepresented, compared to Chordata, Arthropoda and Mollusca. We demonstrate that barcoded marine species increased from 9.5% to 14.2% since the last assessment in 2011, due to new barcodes both on already described species and on newly described ones (about 15,000 new species were described from 2011 to 2021). The next ten years will thus be crucial to enroll concrete collaborative measures and long term initiatives (e.g., Horizon 2030, Ocean Decade) to boost animal barcoding libraries for the marine realm.

1. Introduction

Healthy and well-functioning ecosystems are crucial for providing essential goods and services (e.g., primary production, climate regulation, CO_2 sequestration). A critical ecohealth indicator (*sensu* Charron, 2012) is biodiversity (Myers et al., 2000), as its loss disrupts ecosystem processes and services. Unfortunately, global biodiversity is predicted to decline over the 21st century due to anthropogenic stressors such as commercial species overexploitation, habitat artificialization and destruction, eutrophication, pollution, and introduction of non-indigenous species (NIS) (Pereira et al., 2010). These pressures intensify the effects of climate change by accentuating associated alterations in oceanic biogeochemistry (Harley, 2011) and cause local and global biodiversity losses and spatial reshuffling of species (Blowes et al., 2019; Pecl et al., 2017). Marine biodiversity loss and redistribution are recognized as planetary challenges (Pimm et al., 2014; Worm et al., 2006), but the effective implementation of conservation initiatives depends on having an accurate knowledge of species diversity and their geographic distributions. Therefore, comprehensive biodiversity censuses, particularly of vulnerable marine ecosystems, are imperative to inform policy-making.

Biodiversity studies typically focus on charismatic or commercially-valuable species (Troudet et al., 2017) or well-known biodiversity hotspots (Jenkins and Van Houtan, 2016; Myers et al., 2000), where high species richness, endemism and human pressures coincide, leaving many oceanic regions largely unexplored (Snelgrove 2016). While there is a great need to characterize marine biodiversity more widely, few resources are dedicated to taxonomic research and training, which are fundamental for identifying and describing new species (Boero, 2010; de Carvalho et al., 2007), particularly of small and cryptic organisms. Furthermore, morphology based biodiversity assessments are time consuming and require highly specialized taxonomic knowledge, which is fast disappearing in the absence of funding. Although genetic characterization (barcoding) of species is highly effective in identifying known taxonomic entities (DeSalle and Goldstein, 2019), an entirely molecular approach applied to lesser known taxa or geographic regions goes little beyond identifying molecular operational taxonomic units (MOTUs). Thus, an integrative approach (combining barcoding and morphology) is needed for reliable species identification and high-quality biodiversity assessments.

The rapid generation of high volumes of data resulting from the recent advances in “Omics” technologies and high-throughput sequencing methods, poses great opportunities for metabarcoding, metagenomics and metatranscriptomics studies (Holman et al., 2019; Raupach et al., 2016; Wangensteen et al., 2018). Yet, substantial gaps remain in public DNA barcoding databases, leading to a high proportion of unassigned sequences in metabarcoding-based studies (e.g., Martin et al., 2021), and substantial uncertainty in the estimated number of species living in a surveyed area (Valentini et al., 2016). Furthermore, many national and regional initiatives aimed at expanding DNA barcode databases are not providing complete species documentation, including morphology, ecology, and biology. Moreover, databases are usually not fully curated and harmonized by the time funds are exhausted after project completions (Collins et al., 2020). Consequently, some databases may have limited accessibility or are taken offline only a few years after their publication, and the obtained sequences are not always uploaded on public barcoding databases (see Supplementary material S1, based on Radulovici et al., 2010 and Trivedi et al., 2016). In addition, most DNA barcoding initiatives were, and still are, focusing on terrestrial or freshwater environments (see Supplementary material S1) or on marine organisms that can serve as bioindicators of environmental impacts (Weigand et al., 2019). However, more substantial contributions are needed to improve the genetic knowledge for underrepresented marine taxa at a global scale.

A previous attempt to quantifying the proportion of all barcoded species across the 192,702 described marine animal species worldwide found that only 9.5% of them had a molecular cytochrome *c* oxidase subunit I (COI) barcode (Bucklin et al., 2011). Moreover, evidence of COI sequence gaps characterizing some taxa rather than others at different taxonomic resolutions (Bergsten et al., 2012; Vargas et al., 2012; Ratnasingham and Hebert, 2013; Aylagas et al., 2018; Weigand et al., 2019; Leite et al., 2020), as well as disparities in barcoding coverage when comparing different molecular markers (Duarte et al., 2020; Hestetun et al., 2020), have been reported during the last decade. In addition, progresses in molecular barcoding knowledge led to the identification of dedicated and taxa-specific barcodes (Bahia et al., 2017; Nyman et al., 2021; Van Steenkiste et al., 2015), albeit COI molecular marker still remains the most employed one (Andújar et al., 2018).

2. Actual status of marine biodiversity knowledge

The number of discovered marine species has significantly progressed on a global scale in the recent years (Appeltans et al., 2012; Costello et al., 2012; Mora et al., 2011). Currently, around 238,000 marine species have been described (WoRMS Editorial Board,

2021), with about 2500 new species added each year (www.lifewatch.be/en/worms-stats), most of them within Animalia (i.e., about 204,000). Here, we provide an updated worldwide estimate of the actual knowledge of COI barcodes related to marine animal species. Moreover, we selected five Large Marine Ecosystems (LMEs, www.lmehub.net) considered as biodiversity hotspots (Mediterranean Sea, Caribbean Sea, North Sea, Indonesian Sea, and Red Sea) (*sensu* Myers et al., 2000, Spalding et al., 2007), to detect possible disparities in COI barcoding coverage of marine animals at regional scale and to underline where more efforts should be enrolled.

Our data were gathered from the World Register of Marine Species (WoRMS Editorial Board, 2021), while their presence within the five LMEs was retrieved from the Ocean Biodiversity Information System (OBIS, Intergovernmental Oceanographic Commission of UNESCO; www.iobis.org accessed on May 2021). The availability of COI barcode records at species level were retrieved from the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov, accessed on May 2021) and Barcode of Life Data System (BOLD, www.boldsystems.org, accessed on May 2021) repositories using custom Perl scripts (www.osf.io/qsn5e/).

Globally, from the 207,821 known marine animal species, only 14.5% (NCBI) and 13.8% (BOLD) possessed COI barcodes, representing increases of 4.3% and 5%, respectively (see Supplementary material S2) since the estimates by Bucklin and colleagues (2011). This relative increase could be explained both by (i) progresses in barcoding of species already described until 2011 and (ii) identification of new species with consequent barcoding. The five LMEs encompass a total of 28,219 nominal marine animal species, with 7775 (27.6%, NCBI) and 8200 (29.1%, BOLD) having at least one COI sequence. Evidently, the proportion of barcoded species in these hotspots doubles that at global level. Nevertheless, the current LMEs barcoding data are still not sufficient for comprehensive biodiversity assessments. Furthermore, this barcoding coverage and species richness varies across LMEs (Fig. 1). Variable research intensity and disparity in sampling efforts and access to marine biological collections may explain the differences in number and relative occurrences (i.e., frequency) of detected species, as well as the variable barcoding coverage among the LMEs (Collins et al., 2020) (Fig. 2A). We can clearly notice this in the North Sea, which is the most intensively studied LME, with species occurrence records starting in 1753 vs. as late as the 1970 s in the other four LMEs. Moreover, the magnitude of occurrence records does not follow that of species richness. The North Sea has a lower marine animal species richness compared to Caribbean, Mediterranean and Indonesian Seas. However, it has several millions more species occurrences (9.57 millions) than the other four LMEs (Fig. 2B).

Regarding taxon-specific statistics, Chordata, Arthropoda and Mollusca showed the highest numbers of nominal and COI-sequenced species in the five LMEs, followed by Annelida and Cnidaria (Fig. 3) (see Supplementary material S3 for LME-specific data).

COI barcoding coverage for Porifera, Bryozoa, and Platyhelminthes was very low, which is likely due to (i) high crypticity (Fehlauer-Ale et al., 2014; Trontelj and Fišer, 2009), (ii) variability in the annealing position of traditional COI primers leading to failure of PCR amplification, (iii) co-amplification of epibionts and endosymbionts (Vargas et al., 2012), (iv) lack of discovered (and barcoded) over predicted species (Appeltans et al., 2012). Moreover, COI alone is not sufficient for species identification for certain phyla such as anthozoans due to slow mitochondrial genome evolution (Huang et al., 2008). Another example are barcoding studies on

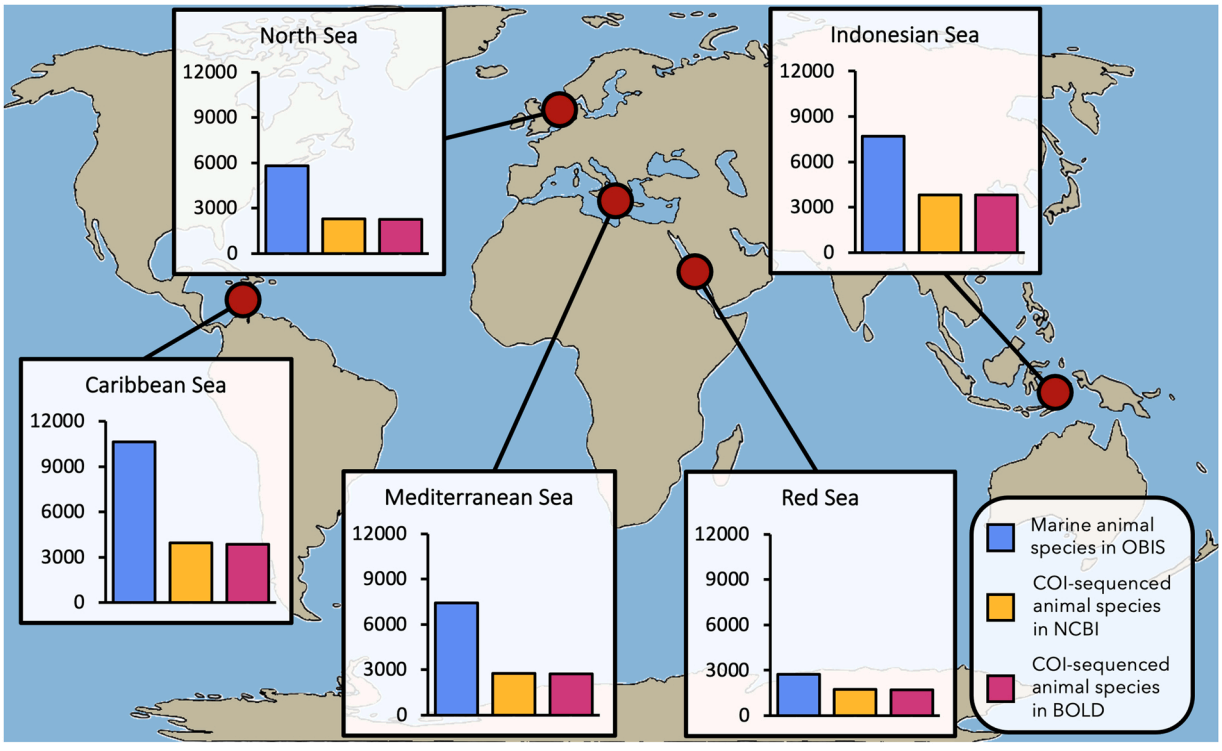


Fig. 1. Number of nominal marine (retrieved from OBIS database) and COI-sequenced (retrieved from NCBI and BOLD databases) animal species for the five target Large Marine Ecosystems (data accessed on 20 May 2021).

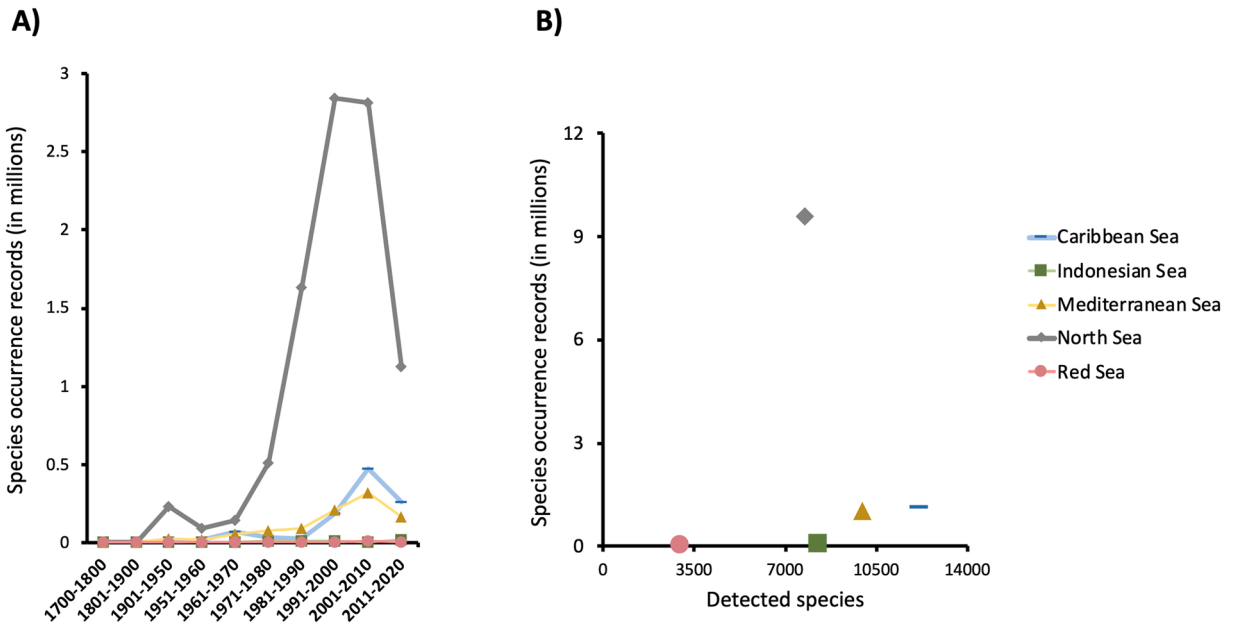


Fig. 2. A) Species occurrences over time for each target Large Marine Ecosystem and B) total species occurrences relative to total number of described species per each LME (data retrieved from OBIS database and accessed on May 20th, 2021).

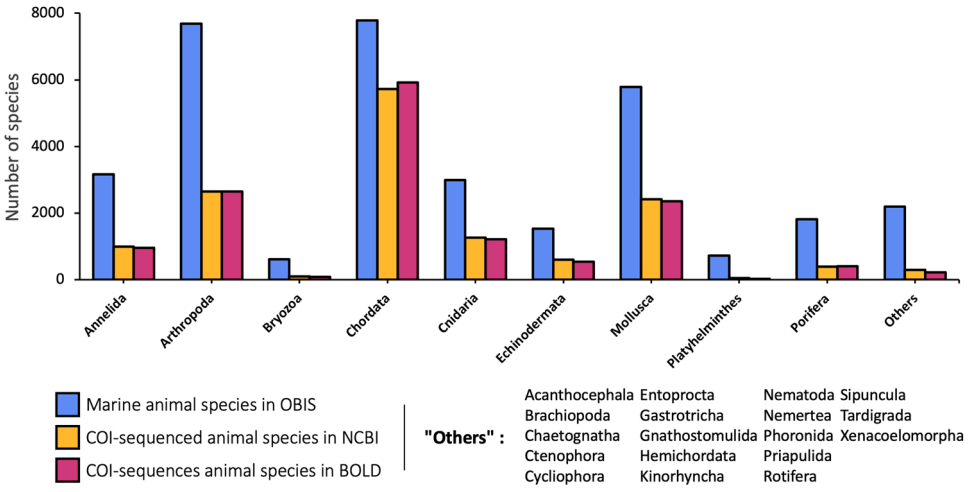


Fig. 3. Cumulative number of nominal marine animals (retrieved from OBIS database) and COI-sequenced species (retrieved from NCBI and BOLD databases) per phylum in the five target Large Marine Ecosystems (data accessed on 20 May 2021).

Platyhelminthes (specifically polyclads) which mainly focus on 28 S (Bahia et al., 2017; Ip et al., 2019), and appropriate COI primers were only developed and tested more recently (Van Steenkiste et al., 2015). Even then, there is often no overlapping region shared by the panoply of COI primers available (Nyman et al., 2021). For these groups, the percentage of COI barcoding coverage may only indicate an initial status in barcoding efforts, instead of being related to how well the group has been studied in terms of barcoding by now.

Chordata clearly shows the highest number of nominal species and barcoding coverage (68.6% NCBI, 71.5% BOLD), particularly among Vertebrata (70.1% NCBI, 73.1% BOLD). Conversely, non-vertebrate Chordata (ascidians) are highly underrepresented (36.3% NCBI, 35.0% BOLD) (see Supplementary material S4).

Taxa being underrepresented in terms of barcoding could be understudied and/or undersequenced. For example, looking at Table 2 in Appeltans et al. (2012), over the 8700–11100 estimated bryozoan species only 5900 are known/described (thus, 53.1–67.8% of bryozoan species are currently known). Compared to well-studied groups such as mammals (94–99% of current knowledge), bryozoans could be considered understudied. However, the 1863 COI bryozoan sequence records in BOLD belong to 245 species that have been sequenced. This represents only (i) 4.1% of the known/described species and (ii) 2.2–2.8% of the estimated species, leading to

consider bryozoans also heavily undersequenced.

3. Final remarks

Our results evidence a clear lack of COI sequences available worldwide in respect to the total described species. This poor COI barcode availability is still maintained when focusing on marine animals, particularly invertebrates. We also clearly observe that the North, Caribbean, and Mediterranean Seas are highly underrepresented in barcode databases, thus requiring further efforts. In addition, giving the higher species richness characterizing the LMEs hotspots, we noticed clear morpho-genetic gaps that could possibly be less evident in non-hotspot geographic areas. The high frequency of non-barcoded species could be also related to overwhelming barcode presences of the most common, widespread or problematic (e.g. invasive) species, as well as data unavailability in certain geographic areas (Supplementary S5). From our results, 3374 species with at least one barcode are shared between at least two LMEs, with Chordata, Artrhopoda and Mollusca leading this tendency (1211, 626, 571 shared species respectively) (Supplementary S5).

However, albeit relying on WoRMS for taxonomy, OBIS database does not include all described marine species, particularly when extracting data from specific geographic areas. A clear example is the Red Sea, which hosts more marine animal species (Dibattista et al., 2016) than those we found in OBIS (2734). This incompleteness is not due to scarce OBIS database curation, but rather to insufficient efforts in transferring new and archived biogeographic data coming from publications devoted to biological monitoring campaigns. The gaps are most marked in those regions (e.g. the Red Sea and the Indonesian Sea) where initiatives and regulations aimed at sharing data on public repositories are probably not yet well implemented.

There is also a need to consider the accuracy of the barcodes deposited at NCBI and, even, at BOLD. Misidentified sequences hinder the broader utility of DNA barcoding, probably more so than the use of open nomenclature and unspecific identification. NCBI and BOLD DNA barcodes derived from unintentionally misidentified organisms have to be tracked down and reduced to zero, preventing the whole barcoding workflow to be meaningless and not reflecting progress(es) with respect to biodiversity assessments. While low barcoding error rate presences at genus level have only been suggested (Leray et al., 2019), instances of species-level misidentification have been effectively detected (Meiklejohn et al., 2019) and are still ongoing (Radulovici et al., 2021).

The ongoing EU's key funding programme for research and innovation "Horizon 2030″ (2021–2027) allocates 8953 Bi€ for Pillar II, Cluster 6 "Food, Bioeconomy, Natural Resources, Agriculture & Environment", offering co-funded initiatives such as "Rescuing Biodiversity to Safeguard Life on Earth" (www.ec.europa.eu/info/horizon-europe_en) that could be pivotal for increasing the biodiversity knowledge at European level, including the marine realm.

To bridge the gaps in biodiversity assessment and monitoring worldwide, national and international collaborations among multidisciplinary teams (including taxonomists, molecular biologists, bioinformaticians, evolutionary biologists, ecologists, and environmental scientists) must be fostered. Such an integrated approach would enable physical specimens (accessioned in permanent natural history museum collections) to be linked to molecular, morphological and ecological data, creating meaningful and long-lived reference data repositories. In parallel, an interactive virtual platform able to interface with different database sources and showing up-to-date species-specific ecological, morphological and molecular data must be created (such as the work in progress within LifeWatch Species Information Backbone, available at www.LifeWatch.eu). Configuring these public repositories following a top-down layout linking other subset databases based on FAIR (Findable, Accessible, Interoperable and Reusable, www.wilkinsonlab.info/node/FAIR, Wilkinson et al., 2016) principles is mandatory to allow efficient data sharing. This will be particularly useful to increase the knowledge in all biodiversity hotspots, particularly in developing countries that are experiencing a lot of economy, pollution and climate change associated issues, allowing to preserve more efficiently and soundly their local marine environments in a growing economy framework.

Other European initiatives, such as DNAquanet (www.dnaqua.net), MBON (www.marinebon.org), SEAMoBB (www.seamobb.osupytheas.fr), are working to reduce morpho-genetic gaps and to expand our knowledge on marine biodiversity. Such initiatives should be further promoted worldwide. The BIOSCAN initiative from the iBOL Consortium (2019–2027, www.ibol.org/programs/bioscan/) aiming at "Revealing species, their interactions, and dynamics" is expected to promote new species descriptions and molecular characterization of terrestrial, freshwater, and marine species. Last but not least, in the present era of unprecedented marine biodiversity loss, society as a whole must be enthused about marine life in view to support measures that preserve marine biodiversity at a global scale. To attain this goal, long-term endeavors driven by scientists leading to involve citizens in educational initiatives (like www.trainingcatalogue.lifewatch.eu/; www.lifewatch.eu/sounds) and filed activities (e.g., marine BioBlitz - www.abol.ac.at/en/abol-bioblitz-2020/) will be pivotal, as emphasized by the UN Decade of Ocean Science for Sustainable Development (www.oceandecade.org).

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01909.

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