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Collaborative science narrows knowledge gaps in marine biological invasions to advance a national framework

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ABSTRACT

Large-scale data synthesis is a foundation pillar of modern macroecology, necessary for transitioning fragmented regional data into scalable biosecurity frameworks. This study presents the outcomes of a coordinated nationwide effort to analyze marine Non-Indigenous Species (NIS) records across mainland Portugal, the Azores and Madeira archipelagos using a dual-framework design. Our goals were to update the morphologically confirmed

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NIS inventory, identify primary introduction pathways, examine temporal and spatial trends, and evaluate genetic database coverage. We delivered an updated checklist comprising 231 morphologically confirmed marine NIS, including five new regional records. Spatial analysis reveals distinct biogeographical divergence: mainland NIS predominantly originate from the temperate North Pacific, probably due to shipping and aquaculture vectors, whereas archipelago NIS originate from the North Atlantic. This pronounced regional variability in pathway dynamics underscores the need for spatially specific management rather than uniform national policies. Also, a systematic genetic gap analysis showed that 71% of these species are cataloged in global repositories, whereas only 12.5% feature locally sourced sequences. To bridge this gap, the second framework introduces a precautionary molecular watchlist of 22 species detected exclusively via DNA-based methods awaiting morphological confirmation. Ultimately, this dual-framework structure demonstrates how integrating taxonomy with provisional molecular data provides a reliable, data-driven baseline, while moving beyond a localized dataset update to offer a scalable reference frame capable of informing regional and global marine biosecurity frameworks.

1. Introduction

Biological invasions are widely recognized as a major threat to marine biodiversity, ecosystem functioning and the sustainability of ecosystem services worldwide (Seebens et al., 2017; Gallardo et al., 2024). The introduction and establishment of non-indigenous species (NIS) can cause profound ecological changes, including competition with native species, habitat alteration, and disruption of trophic networks, often resulting in substantial socioeconomic costs (Molnar et al., 2008; Simberloff et al., 2013). Coastal marine ecosystems are particularly vulnerable, as they are already experiencing rapid environmental change and are exposed to multiple vectors that facilitate species introductions (Molnar et al., 2008; Chainho et al., 2015). These far-reaching consequences highlight the urgent need for collaborative monitoring and coordinated management strategies at local, regional, and global scales, supported by integrative and collaborative scientific efforts.

Collaborative science has become increasingly important in addressing complex ecological challenges that would otherwise be impossible to overcome or would take much longer to achieve (Nichols et al., 2019; Dusdal and Powell, 2021). This is particularly true for biological invasions since understanding and managing NIS requires coordinated efforts across scientific silos to integrate different areas of expertise, standardize methodologies, and share data across regions, countries, and even continents. International initiatives such as the European Alien Species Information Network (EASIN), the Information system on Aquatic Non-Indigenous and Cryptogenic Species (AquaNIS), the Smithsonian Environmental Research Center's National Estuarine and Marine Exotic Species Information System (NEMESIS) and the World Register of Introduced Marine Species (WRiMS), and various COST Actions (e.g., TD1209 - European Information System for Alien Species) have demonstrated how collaboration enhances monitoring capacity, facilitates the early detection of NIS and informs policy development. As the often-cited African proverb states, "*If you want to go fast, go alone; if you want to go far, go together.*", which highlights the collective strength needed to tackle global environmental issues.

Although invasion science has gained considerable momentum in the 21st century (Ojaveer et al., 2018), many fundamental questions and paradigms remain unsolved and continue to evolve as new technologies and observations emerge. For example, it is often unclear whether variations in the number of reported marine NIS reflect differences in propagule pressure, habitat or community susceptibility, and species-specific traits, or whether they primarily result from uneven sampling efforts, research investment over time (Carlton and Schwindt, 2024), and NIS backdating delays (Galanidi et al., 2025). Despite being considered relatively well-studied regions (Zenetos et al., 2022), European coastal waters remain severely undersampled for many taxonomic groups and overlooked species (e.g., Cnidaria, Tanaidacea) (Bailey et al., 2020; Stępień et al., 2023), as well as over extended temporal scales (Seebens and Kaplan, 2022). Additionally, the presence of cryptic species adds another layer of complexity to NIS detection, further challenging our understanding of invasion patterns and pathways (Morais and Reichard, 2018). Within the European context, Portugal stands out as a particularly relevant case study. With its continental coastline and the Atlantic Macaronesian archipelagos of the Azores and Madeira, the country encompasses a biogeographically complex marine region that still exhibits discrepancies in study coverage and sampling effort (Cardigos et al., 2006; Canning-Clode et al., 2013; Castro et al., 2022; Castro et al., 2024; Chainho et al., 2015). Its location along major global shipping routes, combined with active aquaculture facilities (Parretti et al., 2023), recreational boating, and maritime infrastructure development, provides multiple pathways for NIS introduction and spread (Chainho et al., 2015; Castro et al., 2020; Ribeiro et al., 2023). Over the first quarter of the 21st century, an increasing number of marine NIS have been recorded in Portuguese waters, including benthic macroalgae, invertebrates, and vertebrates (e.g., Chainho et al., 2015; Castro et al., 2023; Ribeiro et al., 2023). However, the extent of their ecological impacts remains poorly understood (ME, SRMP, SRAAC, 2025).

Traditional monitoring and identification of marine NIS have largely relied on morphological taxonomy, which is often hindered by the presence of cryptic species, morphological plasticity, and the difficulty in identifying different life stages (Morais and Reichard, 2018; Lavrador et al., 2023; Lavrador et al., 2024). Molecular tools, including DNA barcoding and metabarcoding of environmental DNA bulk samples, have reshaped marine bioinvasions research by enabling rapid species identification, detection of cryptic diversity, and early warning for new NIS introductions (Darling et al., 2017; Lavrador et al., 2024; Zaiko et al., 2015). However, the effectiveness of these molecular approaches depends critically on the completeness and quality of reference DNA barcode libraries (Pinna et al., 2021; Lavrador et al., 2023; Duarte et al., 2020). In Portuguese marine ecosystems, significant gaps persist in these databases, limiting the capacity for comprehensive molecular surveillance (Vieira et al., 2021).

Beyond species detection, understanding the biological traits and invasion pathways of NIS is critical for predicting establishment success, spread, and impacts (Ricciardi et al., 2013; Daly et al., 2023). This knowledge underpins the development of effective management and mitigation strategies. The European Union's regulatory framework, including Regulation (EU) No. 1143/2014 on invasive alien species, and the Marine Strategy Framework Directive (MSFD, 2008/56/EC), mandates the compilation of updated inventories and the implementation of monitoring programs to achieve good environmental status and protect marine biodiversity (Genovesi et al., 2015; Tsiamis et al., 2019). The first Portuguese NIS baseline inventory encompassing mainland Portugal and the archipelagos of the Azores and Madeira was compiled by Chainho et al. (2015). This database provided unique insights into abundance trends, temporal occurrence, spatial distribution, and introduction vectors of marine NIS along the Portuguese coast. Despite previous efforts (Cardigos et al., 2006; Canning-Clode et al., 2013; Chainho et al., 2015; Castro et al., 2022; Gabriel et al., 2023), there is an urgent need to update the NIS inventory by incorporating more recent studies, including molecular data, which enhance taxonomic resolution, improve the detection of cryptic and early-stage invasions and provide more robust assessments of species distributions (Darling and Mahon, 2011; Morais and Reichard, 2018). Additionally, a strategic framework is needed to address current knowledge gaps and improve monitoring and management efforts. Such an inventory would provide a harmonized baseline for monitoring, risk assessment, and management, while guiding future research priorities. Moreover, it would enhance regional and subregional cooperation with neighboring countries, that share the Bay of Biscay and the Iberian Coast, as well as the Macaronesia marine subregion, leading to improved coordinated responses to shared bioinvasion challenges (Oscoz et al., 2022).

Addressing such massive multi-regional pressures demands a shift from traditional, narrow hypothesis-testing investigations toward large-scale data harmonization, synthetic macro-ecology constitutes a foundational scientific pillar. Consolidating fragmented data silos allows transitioning regional compilations into scalable methodological frameworks capable of uncovering broad biogeographical insights that are invisible in isolated local studies. To demonstrate the scientific and methodological innovation of this macro-ecological approach, our study results from a collaborative effort among experts across Portugal to develop and update a comprehensive NIS database through two distinct frameworks. The first framework updates the list of NIS in Portugal comprised solely of species verified through morphological taxonomy and established reporting criteria. The second framework defines a precautionary national NIS watchlist to detect new NIS signaled exclusively by DNA-based surveillance. Emerging from these two frameworks, the goals of this study were: 1) update the morphological confirmed list of NIS in Portugal, 2) identify the main introduction pathways of these confirmed taxa, 3) examine the temporal and spatial trends of their introductions, and 4) evaluate gaps in genetic sequence availability to create a molecular NIS watchlist of species. Ultimately, our country-wide assessment will provide an updated, methodologically stratified baseline for national and European stakeholders. These findings will provide essential data for implementating science-based management programs and policies to prevent and mitigate the ecological, economic, and social impacts of biological invasions.

We tackled these goals by compiling and harmonising records curated by three public authorities responsible for evaluating the introduction and spread of non-native species at a national level, including Directorate-General for Natural Resources, Safety and Maritime Services (DGRM), the Portuguese Institute for Sea and Atmosphere, I.P. (IPMA, IP) and the Institute for Nature Conservation and Forests (ICNF), and by research centres associated to eleven national universities (in alphabetical order: Nova University of Lisbon, Polytechnic Institute of Setúbal, University of Algarve, University of Aveiro, University of Azores, University of Coimbra, University of Évora, University of Madeira, University of Minho, University of Lisbon, University of Porto) which operate across different coastal regions of mainland Portugal and the archipelagos.

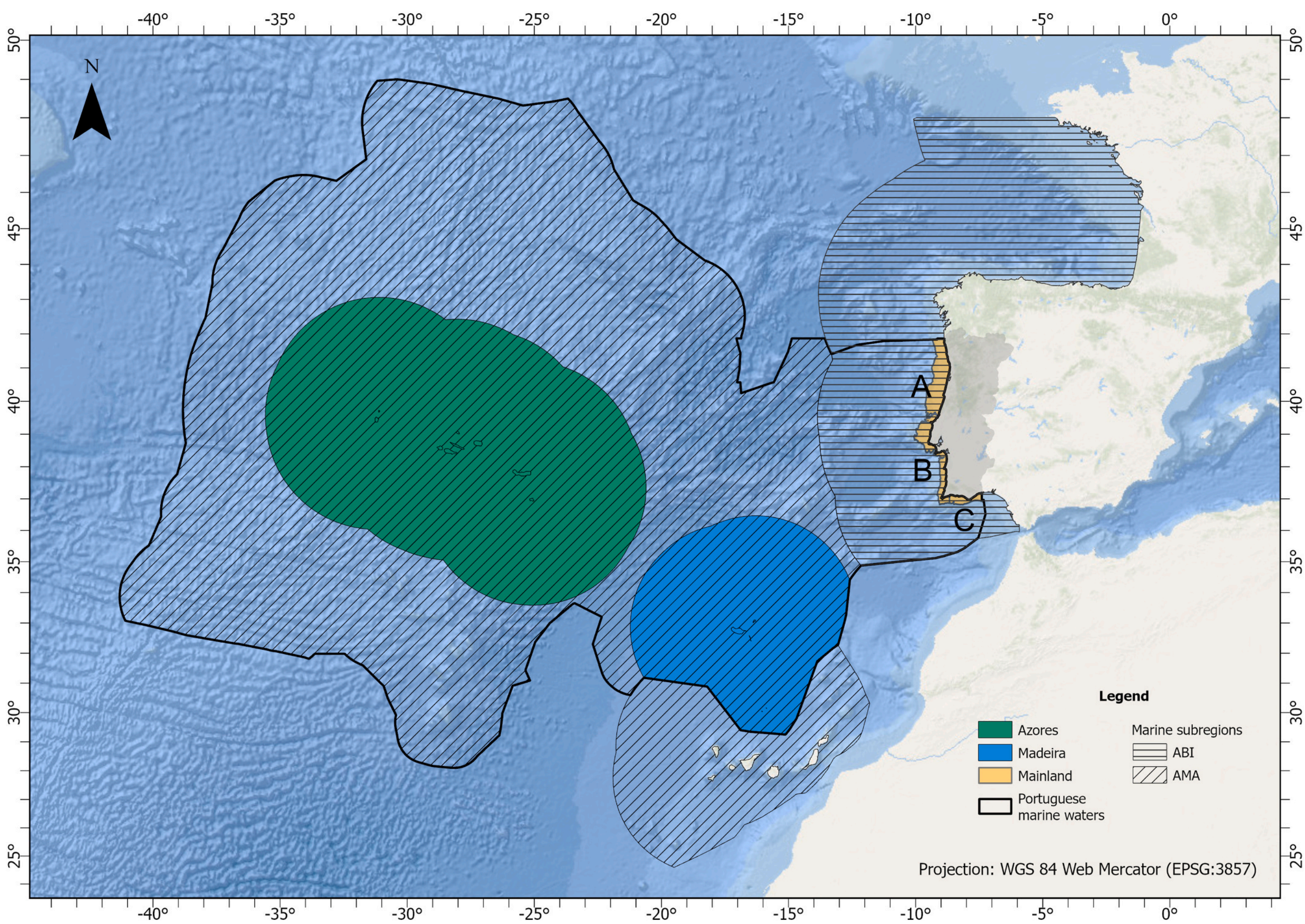

Fig. 1. Study area, including mainland Portugal (Orange) divided into three Marine Reporting Units (A, B, C), as well as the Azores (Green) and Madeira (Blue) archipelagos. AMA - Macaronesia; ABI - Bay of Biscay and the Iberian Coast; MRU D2 - Marine Reporting Units used in Descriptor 2 of the Marine Strategy Framework Directive (MSFD). Source: ESRI; EEA (2022) - MSFD regions and subregions; EMEPC - Portuguese Continental Shelf Extension; DGT - Mainland Portugal; IH - Portugal EEZ. Cartography: E. Dias (2025).

2. Materials and methods

2.1. NIS inventory

In 2011, a pioneering collaborative informal working group was created in Portugal (NIS-Pt), comprising researchers and policymakers, with the aim of addressing the implementation of policies for the introduction of marine species. NIS-Pt currently comprises 56 people, most of whom work in academic research centers (professors, researchers, and postgraduate students) distributed across mainland Portugal and the archipelagos of Madeira and the Azores (Fig. 1).

NIS-Pt also includes individuals from public bodies with expertise in implementing, coordinating and advising on decisions in the framework of national and international policies related to the introduction and spread of non-native species, such as DGRM, and IPMA. The members of this group have updated and revised the national list of NIS for mainland Portugal and the archipelagos of Madeira and the Azores, developed collaborative projects focused on biological invasions. They supported the preparation of reports to inform the implementation of national and international policies on this topic. These results were published by Chainho et al. (2015), updated within the scope of the MSFD implementation and the WGBIOINV-ICES reports, and also used as the baseline for the present update.

We adapted the guidance of Carlton and Schwindt (2024) to assess and update the diversity and history of marine bioinvasions, as well as the occurrence of cryptogenic species (defined below) in Portugal. First, a systematic literature search was conducted across multiple scientific databases to identify records of non-indigenous species (NIS), cryptogenic species (CRY), and natural range-expanding species (NRE), in Portuguese marine and estuarine environments. The databases used were Scopus and Web of Science, due to their broad coverage of ecological, environmental, and biological sciences studies. To complement these sources, Google Scholar was searched to identify additional references. A combination of keywords with Boolean operators was used, including "non-indigenous species", "invasive species", "alien species", "cryptogenic species", "Portugal", "Madeira", "Azores", "marine", and "estuarine". The selected works were published in English, Portuguese and Spanish up to 2024 and were organized by year, language, and subject area to ensure relevance. The bibliographic search was further extended to grey literature (e.g., academic theses, governmental and scientific project reports, institutional databases) and records of natural history museums.

We also retrieved data from global and regional biodiversity and invasion databases, including the WRiMS (Costello et al., 2025), AlgaeBase (Guiry and Guiry, 2025), Global Biodiversity Information Facility (GBIF, 2025), the Invasive Species Specialist Group (ISSG) of the International Union for Conservation of Nature (ISSG, 2025), the European Alien Species Information Network (EASIN, 2025) and AquaNIS (2025). In addition, unpublished data from the team's field surveys and monitoring campaigns were incorporated to ensure the dataset's completeness.

The species nomenclature followed the World Register of Marine Species (WoRMS, 2025), plus Guiry (2024) for algae.

2.2. Species status classification

Species were classified into three categories based on criteria adapted from Chapman and Carlton (1991), Carlton (1996) and Carlton and Schwindt (2024) as follows: NIS) species introduced through human activities, either intentional or unintentional, and established beyond their natural biogeographical barriers (Essl et al., 2018; Richardson et al., 2010; CRY) species of uncertain or unknown biogeographic origin, where it remains unclear whether they are native or introduced (Carlton, 1996); NRE) species that have spread naturally to new areas adjacent to their historical native ranges, typically through natural dispersal or indirectly influenced by human-driven environmental changes (Simberloff et al., 2013).

Species status was independently assessed for each of the three subdivisions (mainland Portugal, Madeira, and the Azores), as a given species may hold different statuses across geographic-subdivisions. Species were excluded from the final database to ensure data quality and accuracy, including taxa detected only once without recent confirmation; taxa with multiple records but no confirmed presence over the past 30 years; and taxa with ambiguous or doubtful identifications requiring genetic confirmation. Additionally, species that were previously considered NIS but lacking sufficient evidence to support their classification were re-evaluated as cryptogenic.

Finally, we reassessed the native distribution of species with unclear historical records to confirm their biogeographical status (native or non-indigenous). Only records with clear geographic and temporal information were retained. Portuguese marine waters belong to the North-East Atlantic Ocean regional geographic level, which encompasses the Bay of Biscay and the Iberian Coast, as well as the Macaronesia subregions. The inclusion criteria required that the species had been reported within the marine or estuarine ecosystems in at least one of the Portuguese subdivisions: mainland Portugal, the Azores, or Madeira. When multiple records existed for a species, the earliest confirmed introduction date was prioritized. Oligohaline species were included if recorded in estuarine or coastal areas within the marine subregions by following the criteria defined by Png-Gonzalez et al. (2023).

2.3. Native distribution and pathways of introduction

The native distribution range of NIS was classified according to the Marine Ecoregions of the World (MEOW) framework (Spalding et al., 2007), which provides a standardized global system for classifying coastal and shelf areas into biogeographic realms. Species with uncertain or ambiguous native ranges were designated as CRY and excluded from native origin analyses.

Widely distributed species spanning multiple ecoregions were assigned to all relevant areas with equal weighting, to prevent overrepresentation in any single region. Species with uncertain native ecoregions, but whose potential native ranges lay entirely outside the ecoregions encompassing Portugal, were classified as having an uncertain native distribution range.

The most probable pathways of NIS introduction for each region were determined using the Convention on Biological Diversity (CBD) classification framework (Hulme et al., 2008), following the guidance provided by Harrower et al. (2018). Six main categories were used: release, escape, transport-contaminant, transport-stowaway, corridor and unaided.

Pathway attribution was based on an extensive review of literature, species traits, and expert knowledge. To ensure contextual relevance and regional accuracy, experts from each subdivision independently reviewed the data for their respective areas, accounting for differences in geographic isolation, maritime traffic, ecological conditions, and human activities that may influence species introduction dynamics. When multiple plausible pathways existed, more than one category was assigned. In cases in which evidence was insufficient or inconclusive to infer a likely pathway reliably, it was classified as "uncertain" to avoid overestimation.

2.4. Spatial and temporal trends

The NIS dataset includes species observed in mainland Portugal, the Azores and the Madeira archipelagos (Fig. 1). For spatial analyses, the Portuguese mainland coast was divided into three Marine Reporting Units (MRU-A, MRU-B, MRU-C) defined under the MSFD (Descriptor 2). These reflect ecological and environmental heterogeneity along the coast.

NIS records were categorized into three MRUs to analyze geographical trends and identify region-specific introduction patterns along the Portuguese mainland coast. For the Azores and Madeira archipelagos, analyses were performed treating each archipelago as a single unit, while still recording the specific island for each species detection.

An overlap analysis was performed between subdivisions and within MRUs to assess species overlap across different geographic levels. UpSet plots were generated using R to visualize shared and unique species occurrences among these spatial units.

The temporal scope of the inventory spans all recorded first introductions, from the earliest documented occurrences before 1700 up to December 2024. The year of the first record for each species was determined using associated references and cross-verified information whenever possible. Museum specimens and historical records were also consulted to identify the earliest possible detection date (Carlton and Schwindt, 2024). When records lacked an exact year and confirmation was not possible, the publication year was assigned as the introduction year. Similarly, for records reporting a range of years (e.g., duration of sampling campaign), the initial year of the interval was used.

The temporal dynamics of non-indigenous species (NIS) introductions in Portugal were analysed using an approach that distinguishes between descriptive and inferential components. First, cumulative NIS detections were used to visualize long-term accumulation patterns across regions and time. Second, the number of newly detected NIS per historical period (<1900, 1901–1920, 1921–1940, 1941–1960, 1961–1980, 1981–2000, 2001–2020, and > 2020) was used for statistical modelling. To account for uneven sampling effort and differences in the duration of historical periods, period-specific counts of new introductions were standardized by including the log-transformed length of each period (in years) as an offset term. Temporal trends in the rate of NIS introductions were then evaluated using a generalized linear model (GLM) with a negative binomial error distribution and a log link function to account for overdispersion. The response variable was the number of newly detected NIS per period, and time was included as a continuous predictor representing the sequential order of periods.

To assess potential differences in temporal dynamics among geographic regions (Mainland, Madeira and Azores), an extended model including Region and its interaction with Time (Time × Region) was also fitted. Data were analysed to compare the temporal dynamics between the year of species detection and the reporting year for each geographical-subdivision. These three datasets were then subsequently merged, and the region of origin of each record was added as a new categorical variable. The distribution of the time differences across regions was visually explored using violin plots, boxplots, and jittered individual points to illustrate variability and sample sizes. To statistically assess differences between detection and reporting years, and whether these differences varied among subdivisions, non-parametric Kruskal-Wallis tests were applied, after testing normality and homoscedasticity of variances using the Shapiro–Wilk and Levene's tests, respectively. When a significant overall effect was detected, pairwise comparisons were conducted using Wilcoxon rank-sum tests with the Bonferroni correction. Mean and standard deviation of detection and publication years were also computed per subdivision to identify periods of higher propagule pressure and sampling effort.

In addition, analyses were conducted to assess both regional differences and shared trends in the taxonomic composition of marine NIS across Portugal.

All statistical analyses and visualizations were performed in R (R Core Team, 2024), primarily using the packages tidyverse (Wickham et al., 2019) and dplyr for data manipulation and cleaning (Wickham et al., 2023), ggplot2 (Wickham, 2016) for data visualization, ggpubr (Kassambara, n.d.) for statistical annotations, and patchwork for figure composition (Pedersen, 2020).

2.5. Genetic data gap analysis and watchlist

To identify gaps in the availability of genetic sequences in public repositories for marine NIS, CRY, and NRE occurring in Portugal, a species-level gap analysis was conducted in July 2025 using public DNA barcode reference libraries. Searches were performed, in the Barcode of Life Data System (BOLD; https://boldsystems.org/) using the "include public records" option for each species to assess the availability of genetic data across different marine regions of Portugal and among different taxonomic and invasion-status groups. BOLD was selected for its stringent data curation standards and for the recommended inclusion of metadata such as voucher specimen details, georeferenced collection data, and trace files. Unlike other sections, this particular stage of the study required a comprehensive assessment of all recorded species, regardless of their evaluated status (NIS, CRY, and NRE). The inclusion of molecular analyses was critical, as they provide essential resolution in cases where morphological or distributional data alone are insufficient to determine species origin or status.

To complement this effort, a comprehensive watchlist for marine NIS detected exclusively through DNA-based approaches was compiled. The term 'watchlist' hereinafter used reflects the provisional nature of these species until further verification is made, since a confirmed physical specimen, properly georeferenced, is required for a new registered record of a NIS.

3. Results

3.1. NIS inventory and species status classification

The bibliographic review yielded 297 validated documents for NIS records in Portugal, including 129 for mainland Portugal, 91 for Madeira, and 78 for the Azores. Some references were relevant to more than one subdivision, which may account for overlaps in these numbers. In total, more than 800 documents were examined, from which 294 were validated as confirming or, to the best of our knowledge, indicating the first records of each species in each region.

As a result, a total of 383 NIS, CRY, and NRE species were cataloged in Portuguese marine waters until December 2024 (Supplementary Table S1). Of these, 231 were classified as NIS, with 159 occurring in mainland Portugal, 81 in the Azores, and 62 in Madeira (Fig. 6). Alongside the identified NIS, mainland Portugal, Madeira, and the Azores hosted 37, 77, and 18 CRY, and 25, 26, and 5 NRE, respectively. A total of five species previously recorded as present in Portugal and described as NIS were excluded from the final database to ensure data quality and accuracy. *Jasus lalandii* (H. M. Edwards, 1937), detected in 1980 in Cascais (Tuaty-Guerra and Gaudêncio, 1982) was excluded because it was not detected again. For instance, *Schizoporella japonica* Ortmann, 1890, was removed because no specimens are available for confirmation by a bryozoan specialist or for DNA sequencing. Since its initial identification (Ribeiro et al., 2023), no further detections have been reported. Given the potential for misidentification with other members of the genus *Schizoporella*, this species was removed from the list until further records allow confirmation. *Polydora cornuta* Bosc, 1802, was also excluded as NIS, as it may be native to mainland Portugal, given the insufficient data on its evolutionary and biogeographic origins.

In addition to reassessing the status of previously reported taxa, we also inventoried new records of NIS for mainland Portugal, namely the amphipods *Cerapopsis alexandri* Munari, 2014 and *Ericthonius pugnax* (Dana, 1852), the tanaid *Hexapleomera bultidactyla* Esquete & Fernandez-Gonzalez, 2016, the isopod *Ianiropsis serricaudis* Gurjanova, 1936 and the ascidian *Botrylloides niger* Herdman, 1886. All these species were recorded along the southwest coast (MRU-B). *C. alexandri* was first reported in 2019 (CIEMAR, 2012) and continued to be found in samples collected in 2012, 2015, 2018, 2021, and 2024 (CIEMAR, 2015, 2018, 2021b, 2024, 2026 *in press*) in the aim of a long-term monitoring programme funded by the Administration of the Ports of Sines and the Algarve. *Ericthonius pugnax* was found in high abundance in subtidal environments of the Sines area (CIEMAR, 2021, 2025). The tanaid

H. bultidactyla was first detected in 2019 and has since been found in high densities (CIEMAR, 2021). Only one individual of *I. serricaudis* was recorded in 2022 on PVC plates using Autonomous Reef Monitoring Structures (ARMS) at the recreational marina of Sines (CIEMAR, 2025), and another in 2024 in sediment samples collected in a terminal of the Port of Sines (CIEMAR, 2026 in press). *Botrylloides niger* was also occasionally detected in the Sines area (CIEMAR, 2021).

3.2. Native distribution and pathways of introduction

The analyses of the biogeographic realms of origin of the marine NIS occurring in Portuguese waters revealed different patterns between the mainland coast and the archipelagos of Madeira and Azores. Most NIS registered along mainland Portugal were native to the Temperate Northern Pacific (39%), followed by other provinces in the Temperate Northern Atlantic (24%; Fig. 2). In contrast, the Portuguese archipelagos showed different trends. Most NIS found in Madeira originated from other provinces in the Temperate Northern Atlantic (27%), with a notable contribution from the Tropical Atlantic (24%). In the Azores, NIS were predominantly from the Temperate Northern Atlantic (37%), followed by the Temperate Northern Pacific (19%), mirroring the mainland pattern.

When examining Portugal as a whole, the composition of NIS is dominated by taxa originating from other provinces in the Temperate Northern Atlantic region, with a substantial proportion of species that are native to both the temperate West Atlantic and the Mediterranean Sea (31%).

In all three geographical-subdivisions, transport-stowaway related vectors were the predominant pathway for the introduction of marine NIS (Fig. 4). Although this pathway accounted for most introductions overall, its relative contribution was approximately 12% lower in mainland Portugal than in the archipelagos.

On the other hand, aquaculture-associated pathways, including escape from confinement (10%) and contaminants on imported animals (13%), played a more prominent role in shaping the mainland introduction patterns. These contributions were higher than those observed in the archipelagos, where aquaculture-related pathways contributed minimally, around 2% in each subdivision, to overall NIS introductions (Fig. 3).

A proportion of introduction events ranging from 0 to 9% across the three subdivisions was categorized as *Uncertain*. These cases reflect situations in which evidence was insufficient or inconclusive to infer a likely pathway, often due to the limited historical information, geographic context at the time of first detection, or incomplete metadata associated with early records.

3.3. Spatial and temporal trends

A comparative analysis of NIS detected across the three studied subdivisions revealed different degrees of species overlap. Several shared *taxa* exhibited differing statuses across subdivisions, with some species being considered CRY, NRE, or even native in subdivision.

A total of 25 common NIS were found across all three subdivisions; however, four of these were considered CRY in at least one of the subdivisions (e.g., *Botryllus schlosseri* (Pallas, 1766)), and one species, *Clavelina lepadiformis* (Müller, 1776), is regarded as native to mainland Portugal but NIS in the Azores archipelago. Mainland Portugal and the Azores shared the highest number of NIS, with 15 species recorded in

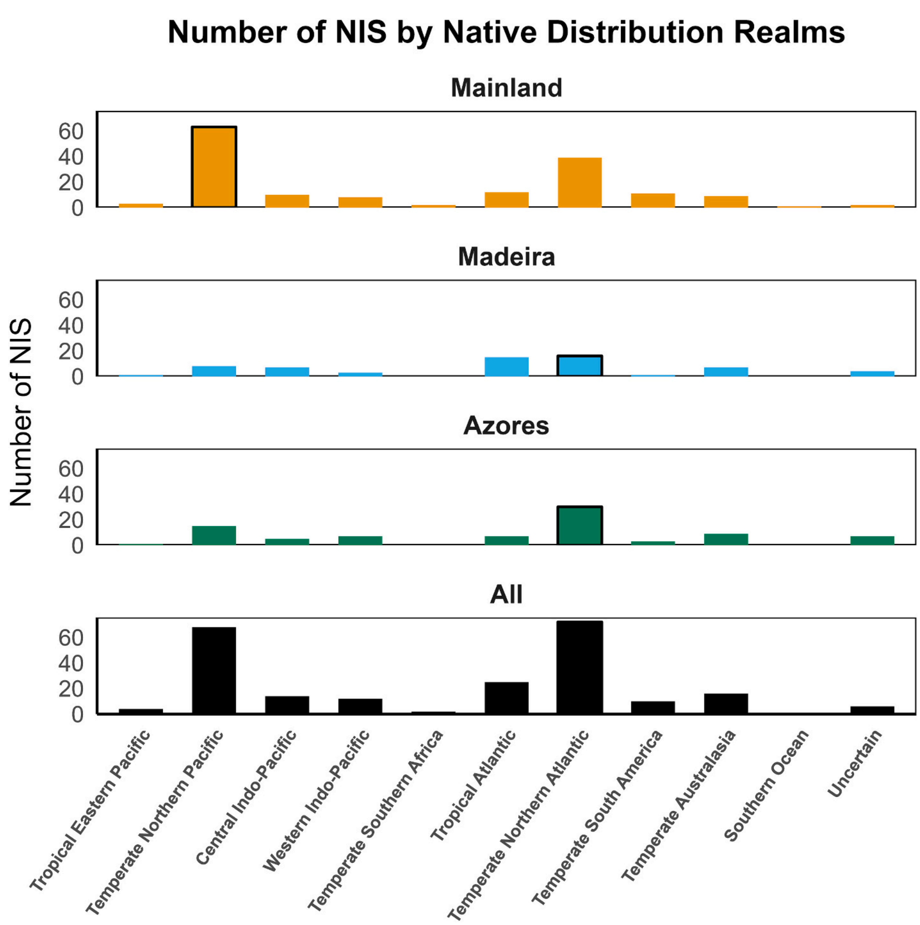

Fig. 2. Marine non-indigenous species (NIS) recorded in Portugal (Mainland, Madeira and the Azores), categorized by native biogeographic realm following Spalding et al. (2007). Bar charts outlined in bold indicate the realms from which the highest number of NIS are native.

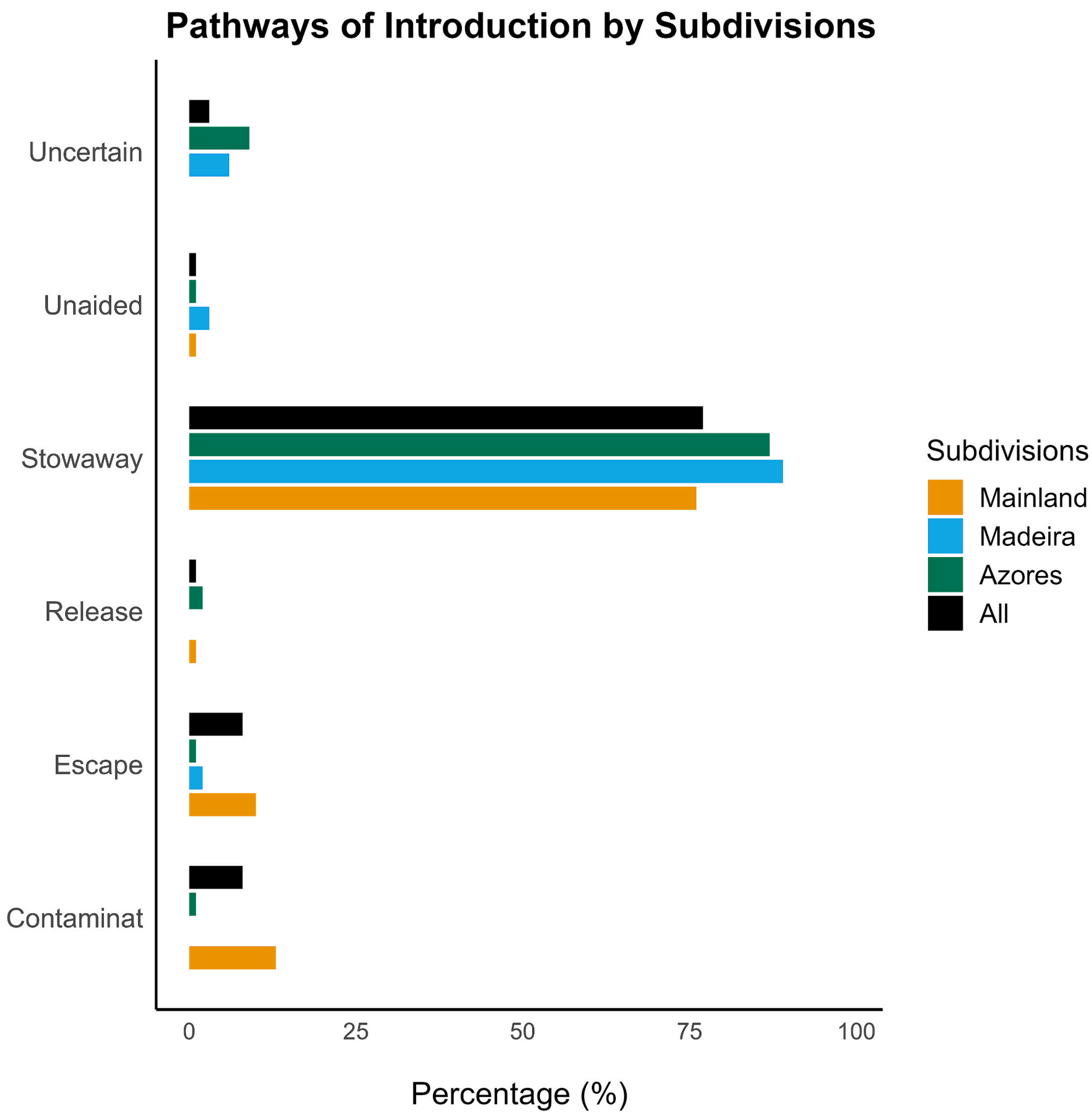

Fig. 3. Most probable pathways of introduction of all the marine non-indigenous species reported for the three subdivisions of Portugal (Mainland, Madeira and the Azores) following the classification framework of the Convention on Biological Diversity. Pathways represent the primary introduction routes for each subdivision, based on the first recorded introduction event. Secondary dispersal pathways between regions are not included.

both areas (Fig. 4A), whereas 11 species were shared between the Azores and Madeira, and 10 between mainland Portugal and Madeira. The highest number of exclusive species was found in mainland Portugal (119), while only 38 and 24 species were found exclusively in the Azores and Madeira archipelagos (Fig. 4A).

Regarding mainland Portugal, NIS detections also varied among the three MRUs. MRU-B recorded the highest number (120 NIS), followed by MRU-C (89 NIS) and MRU-A (83 NIS). MRU-B also had the largest number of exclusive NIS (37 species), with MRU-C and MRU-A having twenty-one and thirteen exclusive species, respectively. Despite these differences, 30% of NIS were present across all three MRUs, indicating substantial overlap in species distribution (Fig. 4B).

The taxonomic composition of NIS was different across the three Portuguese subdivisions. The phylum Arthropoda represented the largest proportion of NIS detections (20%), followed closely by the phyla Mollusca, Chordata, and Rhodophyta, each contributing around 16% (Fig. 5). In Madeira, Chordata was the most prominent group (29%), with Bryozoa and Arthropoda representing approximately 19% and 14%, respectively. In the Azores, Rhodophyta dominated (25%), followed by Chordata (19%) and Arthropoda and Mollusca (12% each). In mainland Portugal, Arthropoda again accounted for the majority of introductions (25%), with Mollusca and Rhodophyta each accounting for

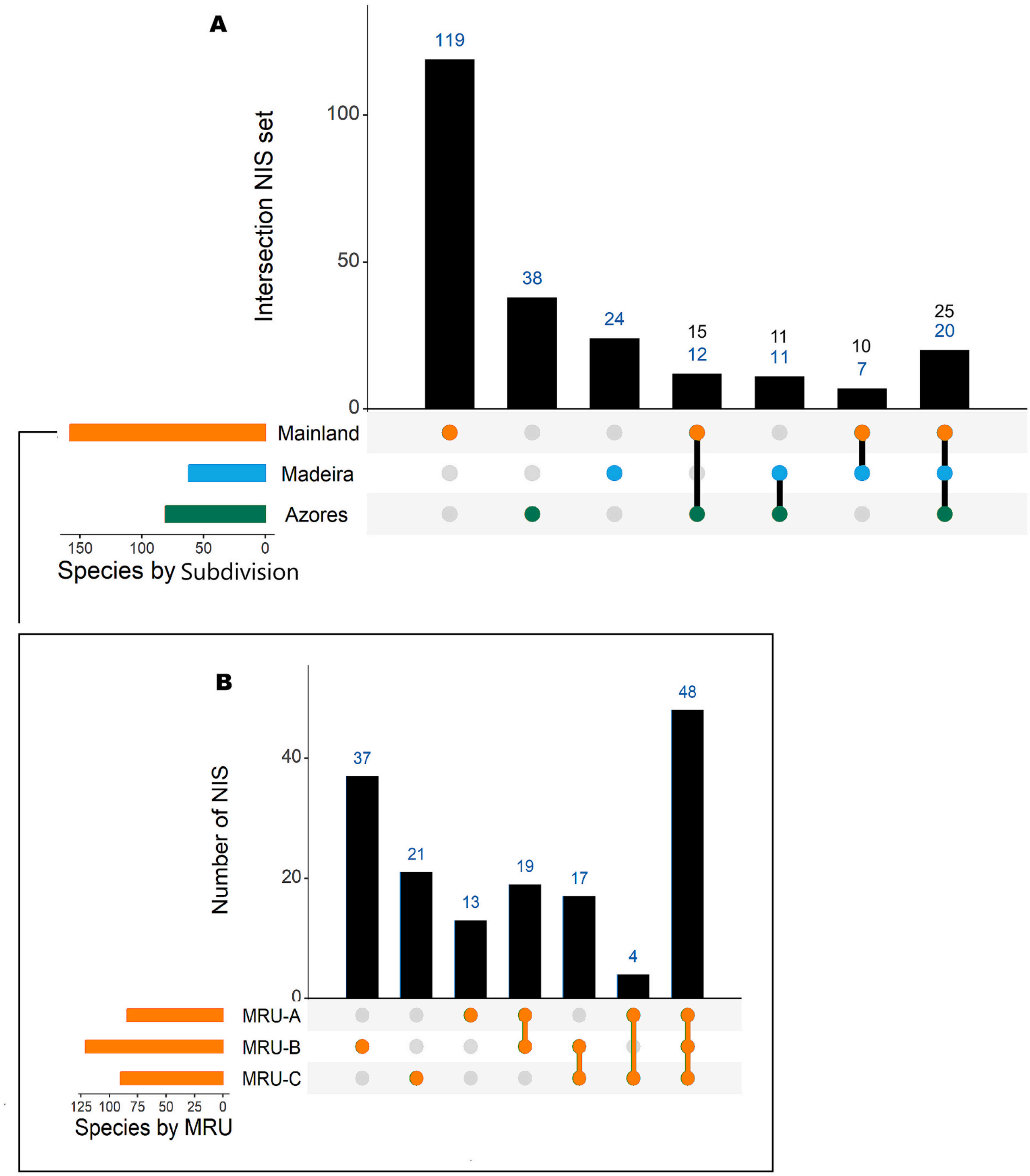

Fig. 4. UpSet plots representing the overlap of marine non-indigenous species between Portuguese subdivisions: Mainland, Madeira and the Azores **(A)** and among marine reporting units **(B)**. The regions shown are illustrated in Fig. 1. Blue values represent species classified as NIS across all areas, whereas black values include species whose status varies between subdivisions, encompassing those highlighted in blue.

about 15%. These results highlight both regional differences and shared trends in the taxonomic groups most representative of marine NIS across Portugal, while acknowledging that differences may be influenced by sampling effort, propagule pressure, and the availability of local taxonomic expertise on specific groups.

The rate of NIS introductions increased markedly over time, reaching a peak in the 2001–2020 period (5.7 introductions per year). However, a decrease was observed in the most recent period (>2020; 2.75 introductions per year). Overall, these results indicate a strong long-term increase in NIS introduction rates over the study period, despite recent short-term variability. This pattern was supported by the negative binomial GLM, which revealed a significant positive temporal trend in the rate of NIS introductions (Time effect, $p < 0.001$), after accounting for unequal period lengths through an offset term. No significant interaction between Time and Region (Mainland, Madeira, and Azores) was detected, indicating similar temporal trajectories across regions.

Differences between the year of first detection and the year of publication were observed within all regions, with varying levels of statistical significance (Mainland: $p = 4.4 \times 10^{-6}$; Azores: $p = 0.0215$; Madeira: $p = 0.0044$). However, no significant differences were found among regions (Kruskal–Wallis, $p > 0.05$) (Fig. 7). In mainland Portugal and Madeira, the average year of first record and publication were

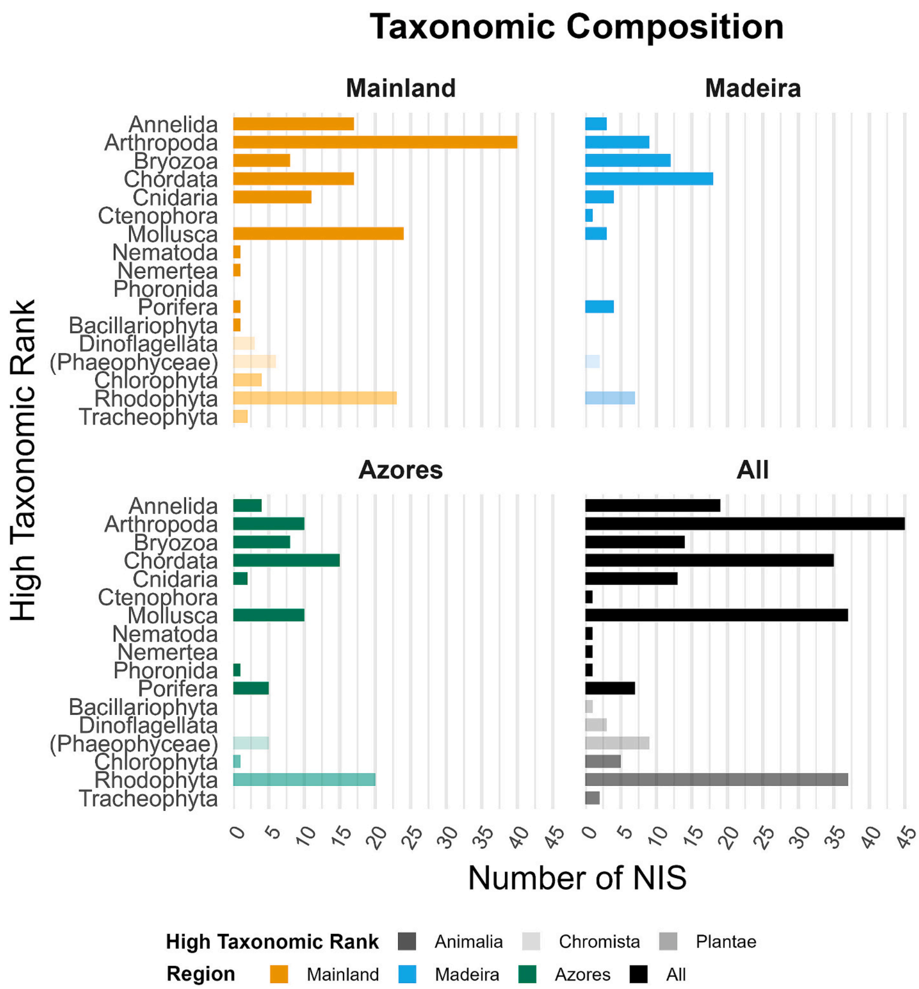

Fig. 5. Taxonomical composition of marine NIS detected in the three subdivisions of Portugal (Mainland, Madeira and the Azores) at high taxonomic rank following Guiry (2024) and WoRMS Editorial Board (2025) recommendations within Animalia, Chromista and Plantae.

similar, indicating a broadly concurrent timeline between NIS arrival and formal recognition (Fig. 7). The lag time differed slightly among regions, with mainland Portugal showing an average delay of approximately eight years, Madeira around six and the Azores, about seven years, between detection and publication. Notably, the Azores began detecting and reporting new species nearly a decade earlier than the other two regions. Temporal gap analysis indicated that most first detections of NIS in the Azores occurred earlier in the overall timeline but were reported within a similar delay period to those in mainland Portugal and Madeira, despite their earlier onset.

3.4. Genetic data gap analysis and watchlist

The results of the genetic gap analysis of the cataloged species revealed that approximately 71% have at least one genetic sequence available in BOLD, totaling 26,671 sequences. However, only 48 species (12.5%) have sequences derived from specimens collected in Portuguese waters.

The most widely represented genetic marker is the mitochondrial cytochrome *c* oxidase I (COI), with 24,351 sequences (91%), underscoring its status as the standard barcode for animals and its broad adoption in taxonomic and ecological studies. Other markers are less commonly represented, with 18S rRNA accounting for 558 sequences, and the chloroplast gene *rbc*L, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase-oxygenase (frequently used for plants and algae), contributing to 499 sequences.

Certain taxonomic groups exhibited full representation in BOLD (Fig. 8). These include the phyla Ctenophora, Echinodermata, Nematoda, Nemertea, and Phoronida. Other well-represented groups include the Phyla Chlorophyta (92%) and Chordata (88%), as well as the Class Phaeophyceae (91%).

In contrast, several groups are poorly represented or entirely lacking barcodes. Notably, Entoprocta had 0% representation (1 species only inventoried), while Porifera and Dinoflagellata showed only 18% and 29% coverage, respectively.

Regarding species status (Fig. 8), NRE species are the most represented, with 88% having at least one sequence in BOLD. They are followed by NIS (76%), while CRY are the least represented (56%).

Focusing specifically on species with NIS status, the analysis revealed notable differences in barcode coverage among taxonomic groups. The best-represented groups, each with 100% coverage of the listed NIS, include Ctenophora, Nematoda, Nemertea, Phoronida, Bacillariophyta, and Chlorophyta (Fig. 9). Other well-covered groups include Mollusca (92%), Phaeophyceae (89%), and Chordata (89%). Conversely, some taxonomic groups were poorly representated. Dinoflagellata had no sequences available in BOLD for any of the NIS-listed species, indicating a data gap for this group. Similarly, Porifera accounted for only 29% of NIS barcodes, and Annelida for 53%.

In terms of geographic representation (Fig. 9), the highest proportion of NIS with at least one DNA barcode available in BOLD was recorded for mainland Portugal (79%), followed closely by Madeira (78%) and the Azores (75%). Within mainland Portugal, representation varied slightly among the MRUs. The most well-represented was MRU-A (84%), followed by MRU-C (83%) and MRU-B (79%).

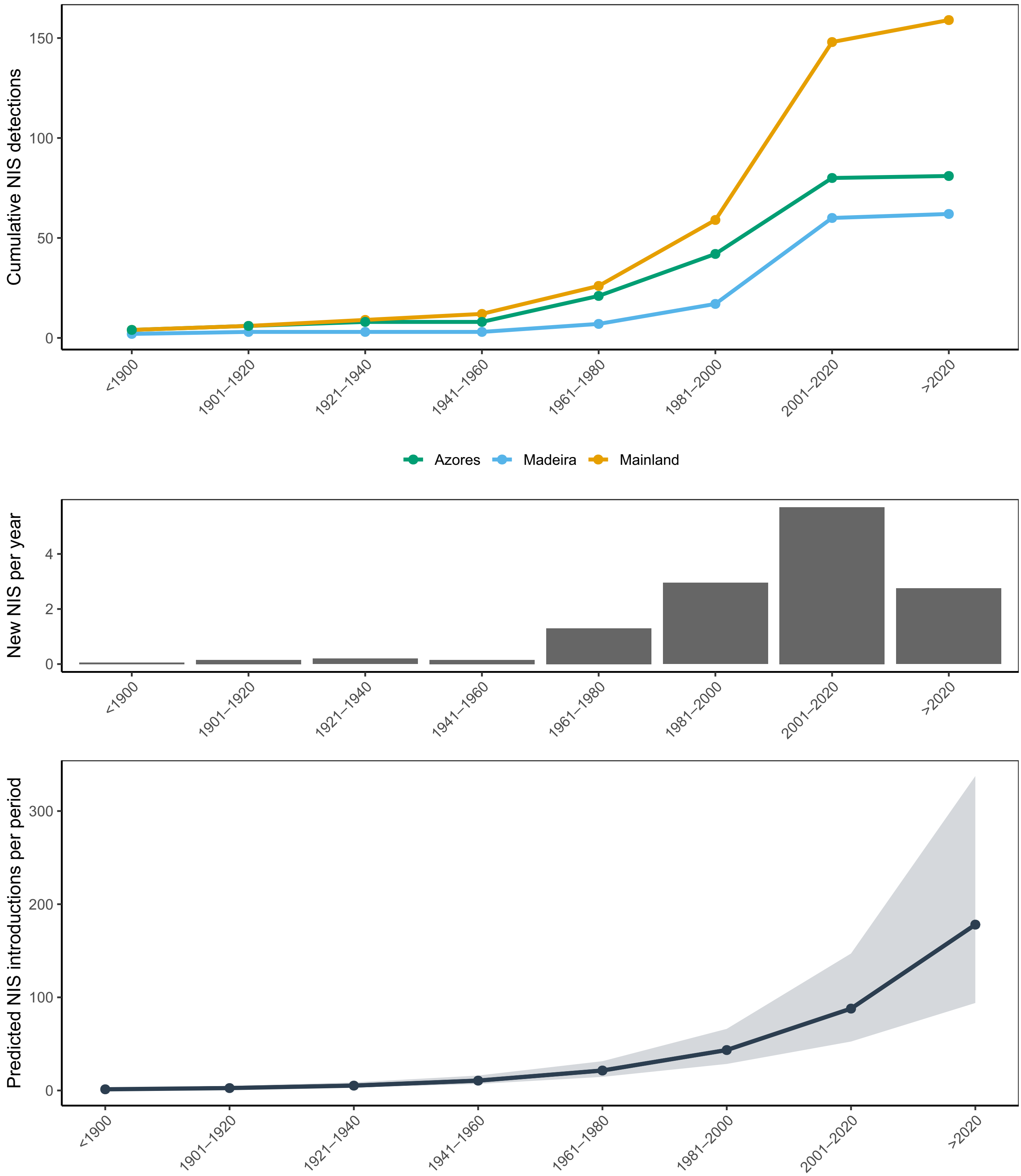

Fig. 6. Temporal dynamics of non-indigenous species (NIS) introductions in Portugal across Mainland, Madeira, and the Azores. (A) Cumulative number of NIS detections over time by region. (B) Rate of new NIS introductions per period, standardized by period length (years). (C) Predicted NIS introduction rates from a negative binomial GLM with a log link, including time as a predictor and an offset for period length; shaded areas represent 95% confidence intervals.

A watchlist of 22 NIS was compiled, considering records detected only through DNA-based methods (DNA metabarcoding of eDNA or bulk samples) in Portuguese marine waters (Table 1) (four publications). Among these, twenty invertebrates (three Annelida, eight Arthropoda, one Bryozoa, one Chordata, two Cnidaria, three Mollusca and one Porifera) and three algae (one Heterokontophyta (Phaeophyceae) and two Rhodophyta) were detected.

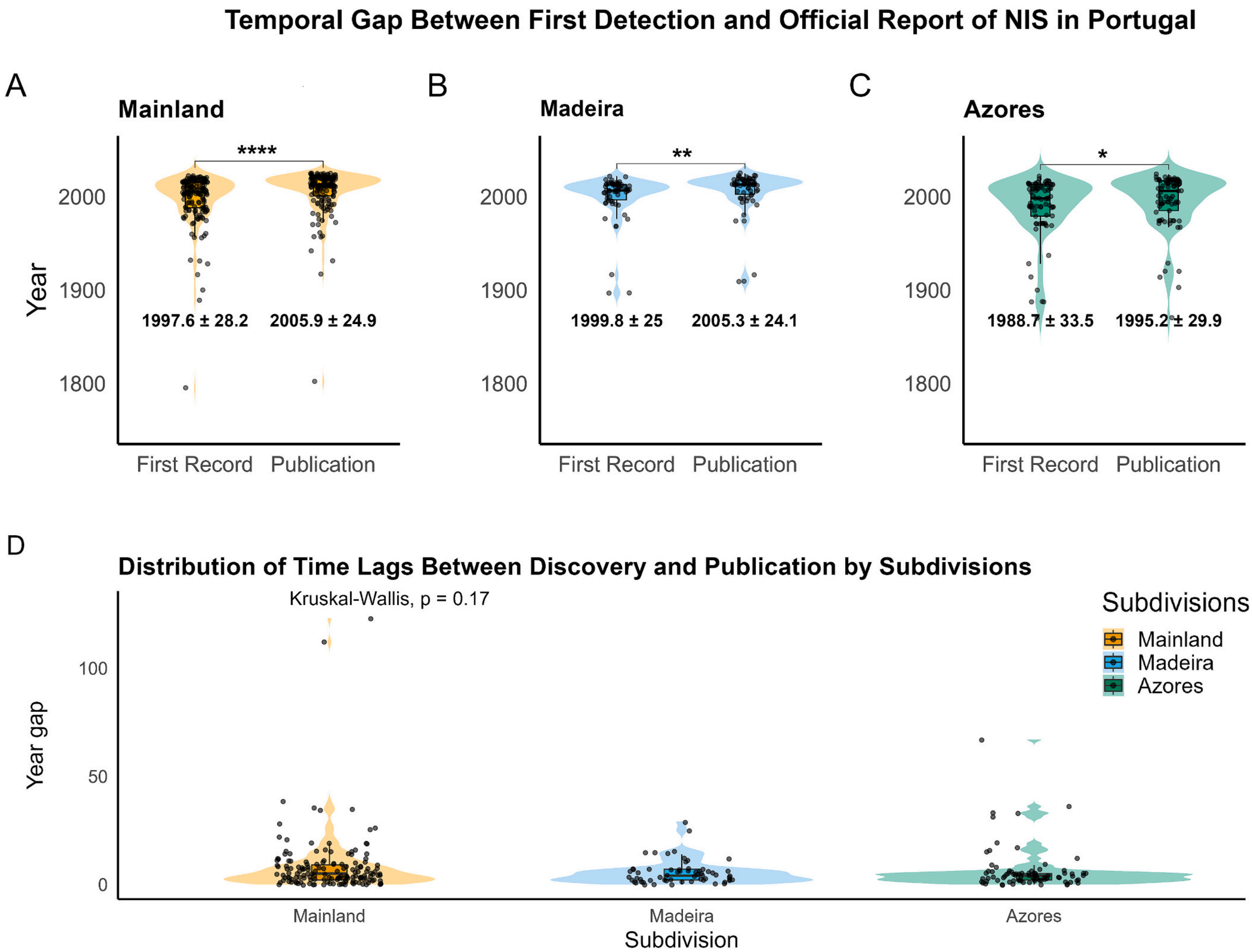

Fig. 7. Temporal gap between first detection and official report date (publication) of marine non-indigenous species in Portugal across the 3 subdivisions analysed: Mainland, Madeira, and the Azores. Asterisks indicate significant differences based on Wilcoxon tests: **** $p \leq 0.0001$; ** $0.001 < p \leq 0.01$; *$0.01 < p \leq 0.05$. Numerical values below each violin plot indicate the group's mean and standard deviation.

4. Discussion

This study provides a scalable synthetic framework that uncovers four critical macro-ecological insights, rather than serving merely as a passive data update. First, it reveals a clear biogeographic divergence in invasion sources, showing that most species introduced to the archipelagos originate from the temperate Northern Atlantic. At the same time, the North Pacific dominated introduction pathways for the mainland. Second, it quantifies regional variability in pathway dynamics, demonstrating that while transport-stowaway dominates nationwide, aquaculture plays a major role exclusively on the continent. Third, by tracking the historical time lags between initial discovery and formal reporting, the dataset revealed critical temporal patterns in propagule pressure. Fourth, the study introduces an active early-warning innovation by establishing a precautionary molecular-based monitoring of 22 NIS, providing a forward-looking tool to capture new introductions before they can fully establish.

4.1. NIS inventory

This work provides a comprehensive revision and synthesis of previously reported first-detection dates and includes five new records. We emphasize that this primary national inventory comprises 231 taxa grounded entirely on morphologically confirmed records, ensuring that the historical baseline remains free from unverified molecular signals. A comparative analysis between the detection years reported by Chainho et al. (2015) and those compiled here, revealed a consistent temporal difference between the date of first record with an estimated average gap of six years. The most extreme case was observed for *Chaetopleura angulata* (Spengler, 1797), for which the first recorded detection date was revised by 121 years, from 1916 (Hidalgo, 1917) to 1795 (Tilesius, 1802). Such discrepancies have also been reported for other regions (Galanidi et al., 2025) and may be attributed to factors such as misidentification, overlooked historical records, or limited monitoring efforts at the time of introduction (Morais and Reichard, 2018). Collectively, these results emphasize the importance of retrospective data validation, the use of historical and museum records (Castro et al., 2021), citizen science as an approach to detect NIS (Morais and Teodósio, 2016; Encarnação et al., 2020) and the continuous updating of NIS databases (Carlton and Schwindt, 2024).

The five new NIS records presented in this study were detected through a long-term monitoring programme, funded by the Administration of the Ports of Sines and the Algarve, conducted along the southwest coast (MRU-B) since 1997. Although the programme was initially designed to assess coastal ecological conditions, the detection and tracking of NIS has become a key focus since 2018 (CIEMAR, 2021; 2025). One of the detected NIS was *Cerapopsis alexandri* Munari, 2014, an amphipod differing from the Photidae-related species previously

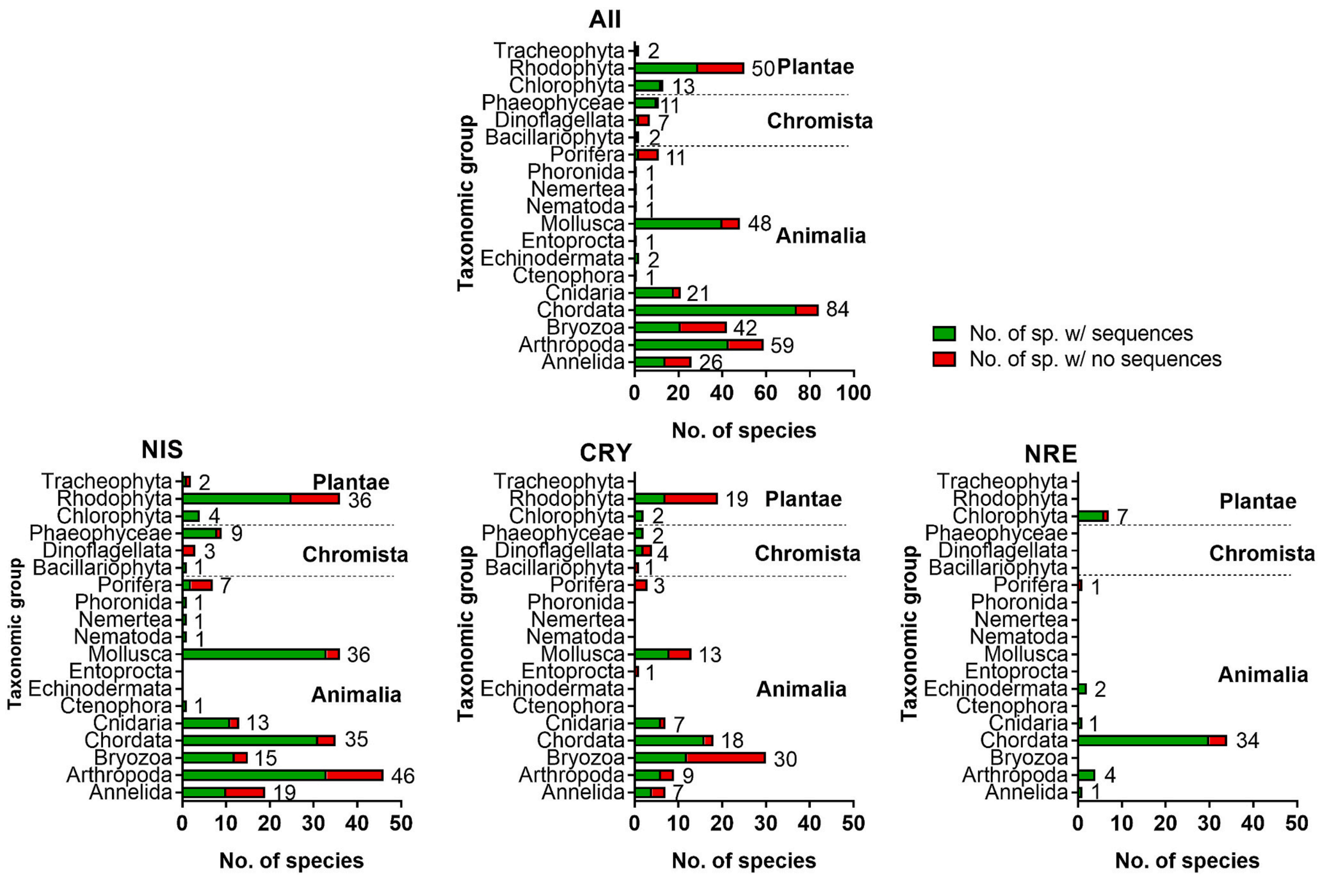

Fig. 8. Gap analysis of the 383 species catalogued in Portuguese marine waters, including non-indigenous species (NIS), cryptogenic species (CRY), and natural range expanding species (NRE). Barcode coverage (in green) and barcode gap (in red) are shown at high taxonomic rank within Animalia, Chromista, and Plantae.

reported for the Portuguese coast. It was first detected in sediment samples in 2009 (CIEMAR, 2012); however, the species' original description was published only in 2014, based on specimens collected in the Strait of Messina in the central Mediterranean Sea (Munari, 2024). This species appears to be well established in the Sines area, having been recorded by the monitoring programme intermittently since 2012 (CIEMAR, 2015, 2018, 2021b, 2024, 2026 *in press*).

Another amphipod species, *Ericthonius pugnax* (Dana, 1852), was found in high abundance in subtidal environments of the Sines area (CIEMAR, 2021; CIEMAR, 2025). This species has a broad Indo-West Pacific distribution (Ariyama, 2009). Similarly, the tanaid *Hexapleomera bultidactyla* Esquete & Fernandez-Gonzalez, 2016, originally described from fish-farm cages in the western Mediterranean, was detected in high densities (CIEMAR, 2021).

The isopod *Ianiropsis serricaudis* Gurjanova, 1936, originally described from the NW Pacific Ocean (from the Sea of Okhotsk to the Sea of Japan), has recently been detected in MRU-B. This species has been spreading through northern Europe, possibly introduced to France via oyster culture or ship hull fouling (Raoux et al., 2020), and to Italy via shellfish trade (Marchini et al., 2016a, 2016b). Only two individuals have been recorded to date, in separate years, suggesting an early stage of species establishment.

The ascidian *Botrylloides niger* Herdman, 1886, an invasive marine filter-feeding invertebrate that is believed to be native to the western Atlantic, was also occasionally detected (CIEMAR, 2021a). Although the species has been recorded at several locations along the coast of Israel and near the Suez Canal, it has not yet been detected along the coasts of the Northeastern Mediterranean Sea, supporting the hypothesis of an ongoing Lessepsian migration following its initial introduction to these regions (Temiz et al., 2023). This spatial pattern suggests that the species may still be in an early phase of regional spread, with its current distribution shaped by ship-mediated transport rather than continuous natural dispersal. One plausible hypothesis for the introduction of *B. niger* is its transfer from Funchal, Madeira. The species has been established in this archipelago since at least 2013 (Gestoso et al., 2017; Ramalhosa et al., 2019, 2021), and there is heavy maritime traffic between the port of Funchal and several mainland Portuguese ports, including the Port of Sines (Castro et al., 2020). However, a direct introduction from Israel is also plausible, as Israel ranks among the top 20 countries of origin for vessels arriving at the Port of Sines (Rosa et al., 2026). Sustained maritime connectivity between these regions strongly supports ship-mediated transport, particularly via ballast water discharge and biofouling on vessel hulls, as the most likely vectors facilitating the spread of the species.

The Port of Sines, is the largest and westernmost deep-water port in mainland Europe, functioning as a major hub for international maritime traffic and allowing access for large ocean-going vessels and cruise ships. Its location at the intersection of east-west routes (linking Asia, the Mediterranean, and the United States) and north-south routes (connecting Africa to Europe) makes it a strategic node for global shipping. In addition, it hosts fishing and leisure ports, serving as a common stopover for pleasure boats traveling along national (Castro et al., 2020) and international routes (Batista et al., 2025; Rosa et al., 2026).

These findings reinforce the importance of long-term, standardized monitoring programmes in high-risk areas, such as the port of Sines, to improve early detection, track invasion pathways and support timely management responses in Portuguese coastal waters.

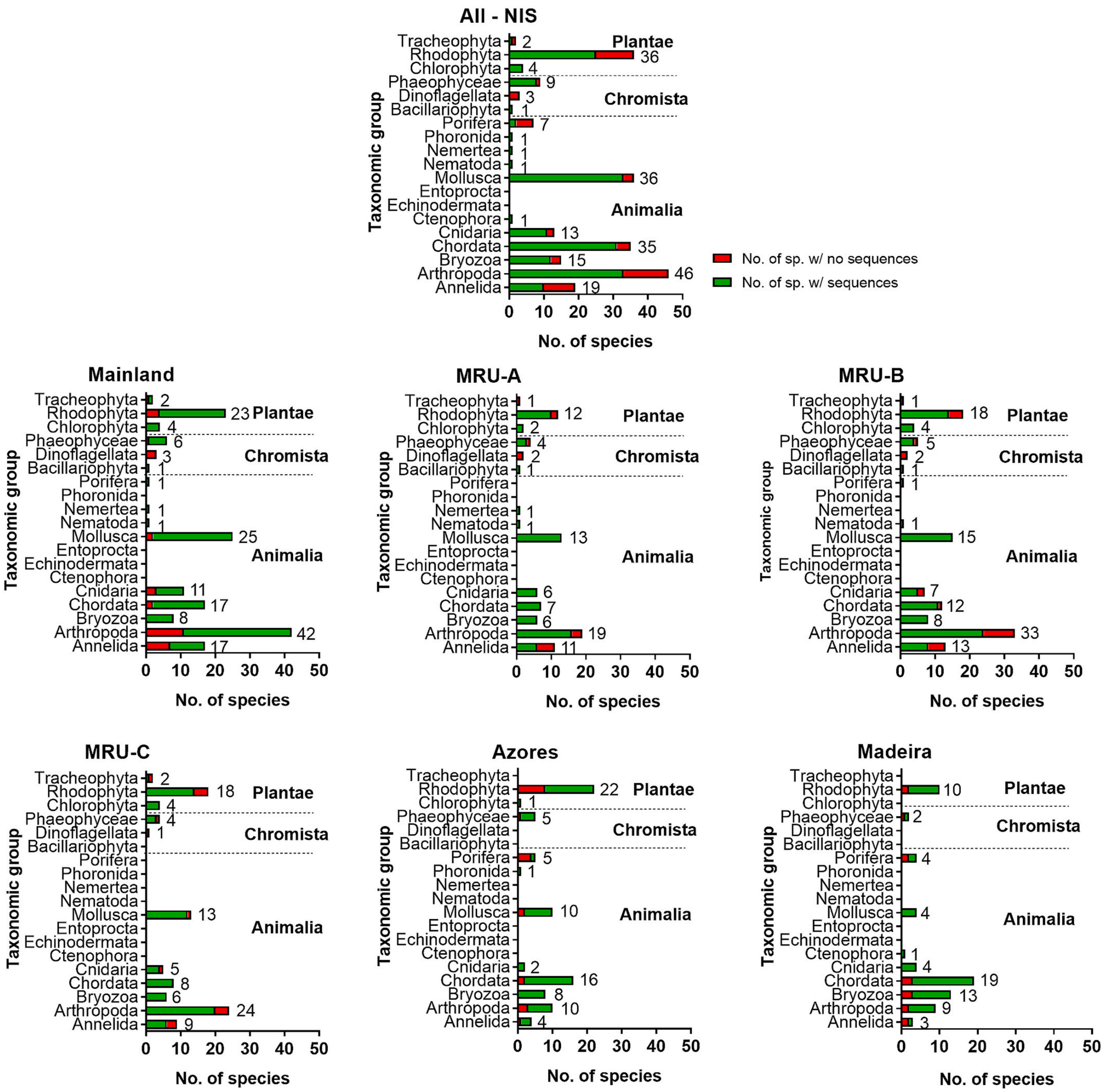

Fig. 9. Gap analysis of species with NIS status recorded in Portuguese marine waters. Barcode coverage (in green) and barcode gap (in red) are shown at high taxonomic rank within Animalia, Chromista, and Plantae, and across the three Portuguese subdivisions (Mainland, Madeira, and Azores). Within mainland Portugal, coverage by each Marine Reporting Unit (MRU) was also assessed.

4.2. Native distribution and pathways of introduction

Regional differences in the biogeographic realms of origin of the marine NIS are likely due to both ecological and anthropogenic factors. The higher prevalence of Tropical Atlantic species in Madeira is probably influenced by its proximity to West Africa and its location within subtropical Atlantic currents, which facilitate the dispersal and establishment of tropical species (Albano et al., 2024; Ribeiro et al., 2019; Ramalhosa et al., 2025).

The apparent paradox of Portugal receiving numerous Pacific-origin marine NIS despite limited direct traffic is explained by indirect pathways. Many Pacific marine species likely arrived via secondary spread through European ports, particularly from major maritime hubs such as those in the Netherlands, the United Kingdom, and France, which maintain strong connections with the North Pacific (Galil et al., 2014). Some macroalgae and invertebrates are also associated with oyster and shellfish aquaculture, historically involving translocation from Asia to Europe, including Portugal (Williams and Smith, 2007; Ribeiro et al., 2023). Thus, even without direct transpacific shipping, Portugal remains vulnerable to Pacific-origin introductions probably via complex, multiple, co-occurring anthropogenic vectors.

Interestingly, Temperate Southern Africa appears to contribute relatively little as a source region for NIS recorded in Portugal. This is somewhat unexpected given that (1) both regions fall within the same

Table 1
List of marine non-indigenous species detected exclusively through DNA metabarcoding of environmental or bulk samples. The Portuguese subdivision(s) where each species was detected (MP- Mainland Portugal, MA- Madeira, AZ-Azores), status (NIS - non-indigenous, CRY - cryptogenic, NRE - natural range expansion species), year of first record, and references are shown. References: [A] Azevedo et al., 2020; [B] Amaral, 2022; [C] Lavrador et al., 2024; [D] Lavrador et al., 2026.

Species	Subdivision	Status	Year of first record	References
Rhodophyta				
Carradoriella denudata (Dillwyn) Savoie & G.W. Saunders, 2019	MP	CRY	2016–2017	[A]
Polysiphonia morrowii Harvey, 1857	MP	NIS	2016–2017	[A]
Phaeophyceae				
Halothrix lumbricalis (Kützing) Reinke, 1888	MP	NIS	2016–2017	[A]
Porifera				
Callyspongia (*Callyspongia*) *siphonella* (Lévi, 1965)	MP	NIS	2016–2017	[A]
Mollusca				
Musculus lateralis (Say, 1822)	MP, AZ	NIS	2020–2021	[C] [D-Azores]
Ostrea angasi G. B. Sowerby II, 1871	MP	NIS	2020–2021	
Spisula solidissima (Dillwyn, 1817)	MP	NIS	2020–2021	[B]
Cnidaria				
Cordylophora caspia (Pallas, 1771)	AZ	NIS	2020	[D]
Haliclystus tenuis Kishinouye, 1910	MP	NIS	2016–2017	[A]
Chordata				
Phallusia nigra Savigny, 1816	MP	NIS	2020–2021	[B]
Bryozoa				
Tricellaria occidentalis (Trask, 1857)	MP	NIS	2020–2021	[B]
Arthropoda				
Austrominius modestus (Darwin, 1854)	AZ	NIS	2020	[D]
Calocalanus plumulosus (Claus, 1863)	MP	NRE	2020–2021	[B]
Caprella mutica Schurin, 1935	MP	NIS	2020–2021	[C]
Jassa slatteryi Conlan, 1990	AZ	NIS	2020	[D]
Laticorophium baconi (Shoemaker, 1934)	AZ, MA	NIS	2020	[D]
Mytilicola intestinalis Steuer, 1902	MP	NIS	2020–2021	[C]
Oithona davisae Ferrari F.D. & Orsi, 1984	MP, AZ	NIS	2020–2021	[B] [D-Azores]
Pseudodiaptomus marinus Sato, 1913	AZ	NIS	2020	[D]
Annelida				
Ctenodrilus serratus (Schmidt, 1857)	MP	NIS	2016–2017	[A]
Polydora onagawaensis Teramoto, Sato-Okoshi, Abe, Nishitani & Endo, 2013	MP	NIS	2020–2021	[C]
Pseudopolydora paucibranchiata (Okuda, 1937)	AZ	NIS	2020	[D]

broad ecological zone, (2) they are influenced by similar oceanographic features, namely the Benguela and Iberian upwelling systems, and (3) there are historically strong maritime links between Portugal and its former overseas territories in Southern Africa. One possible explanation for this apparent underrepresentation is that many species native to the Temperate Southern African region may have naturally expanded their ranges into Iberian waters (e.g., *Acanthurus monroviae* Steindachner, 1876; *Lysmata uncicornis* Holthuis & Maurin, 1952) and thus are considered NRE. Additionally, the lack of a clear biogeographical discontinuity between source and recipient regions may obscure the detection of introductions, especially when historical distribution data are limited or absent.

Regarding the pathways of introduction, transport-stowaway is the dominant pathway for marine NIS in Portugal (76%), with other pathways, such as corridors, being almost negligible according to previous assessments (Chainho et al., 2015). This contrasts with the broader European context, where corridors accounted for approximately 14% of NIS introductions, while transport-stowaway related vectors accounted for nearly 50% (European Environment Agency EEA, 2025). Similar patterns were observed in neighboring Spain, where transport-stowaway related vectors accounted for 68% of NIS records in the ABI (Bay of Biscay and the Iberian Coast) subregion (Png-Gonzalez et al., 2023). The trend is even more pronounced in the Macaronesian subregion: Spain reported 85% of introductions through transport-stowaway pathways for the Canary Islands (Png-Gonzalez et al., 2023), while Portugal reported 89% and 87% for Madeira and the Azores, respectively. These figures clearly highlight the dominant role of maritime transport, particularly ballast water and hull fouling, in shaping NIS introductions in the Iberian Peninsula and associated islands. The Portuguese government has developed the action plan, Priority Pathways of Unintentional Introduction of Invasive Alien Species since 2023 (Portuguese Council of Ministers Resolution n. ° 45/2023); however, its implementation has not yet begun. This action plan excludes ballast water since this vector is addressed by the International Convention for the Control and Management of Ships' Ballast Water and Sediments. Similarly, implementing and enforcing this Convention remain challenging despite a clear legal framework. We highlight that this is not exclusive to Portugal, numerous other countries worldwide face the same problem (Bailey et al., 2025; Outinen et al., 2024), requiring specific regulation and enhanced monitoring of shipping activities (Tsiamis et al., 2019).

At the national level, a clear geographical variation in dominance regarding the introduction pathways was demonstrated. Aquaculture played a pivotal role in marine NIS introductions on the continent compared to the insular systems, likely due to the more intensive and widely established aquaculture operations in mainland Portugal (Rocha, Cabral, Marques and Gonçalves, 2022). Existing records from archipelagos primarily document fish escapes from aquaculture operations (Parretti et al., 2023). These findings also underscore the importance of region-specific pathways management, even within the same country (Lapointe et al., 2016), as the predominance of certain pathways varies considerably depending on local economic activities, artificial infrastructure availability, and historical patterns of human-mediated marine species movement across boundaries (Pinochet et al., 2025).

Variations may influence differences in the proportion of uncertain records across subdivisions in expert-judgment profiles, as pathway assignments were based on regional expert knowledge. Some of these pathway-related uncertainties, particularly those observed in the archipelagos, may also result from the prominence of marine debris as a vector for secondary dispersal of NIS (Miralles et al., 2018). Although this vector is not yet formally recognized among those listed by the CBD, it appears to be crucial to include it in the near future. On oceanic islands, especially in the Madeira archipelago, marine debris has been shown to play a significant role in NIS dispersal, with a proportion of NIS associated with this vector reaching levels comparable to those found in marinas and ports (Ramalhosa et al., 2025).

In mainland Portugal, uncertainty regarding pathways of introduction could, in some cases, be addressed by examining analogous introduction events in neighboring countries, such as Spain and France (Png-Gonzalez et al., 2023), and bearing in mind the specific characteristics of the detection site. In contrast, such resolution is more difficult to attain in remote islands, where introduction dynamics differ markedly due to their geography and distinct socio-economic and ecological contexts. These regional differences in data availability between islands and the mainland, as well as among islands, together with differences in contextual knowledge, likely contributed to varying levels of confidence in attributing of the most probable pathways across subdivisions.

4.3. Spatial and temporal trends

The occurrence of NIS in Portugal shows a prevalence on the mainland, where 69% of the species recorded for the national territory occur, while the islands accounted for 35% and 27% of the total NIS, in the Azores and Madeira archipelagos, respectively. Although the numbers published in 2015 (Chainho et al., 2015) indicated greater similarity NIS occurrence between the mainland and the islands, the present work shows that those numbers were underestimated due to the omission of overlooked species. Updated cumulative numbers show that the occurrence of more NIS on the mainland than in the archipelagos has always been pronounced. Therefore, although the high number of NIS on the islands corroborates the classic hypothesis of greater susceptibility to invasion due to lower natural diversity and lower biotic resistance (Moser et al., 2018), propagule pressure appears to play a predominant role in NIS colonization, as previously concluded by Castro et al. (2022), when they compared the number of NIS among different archipelagos of Macaronesia. Beyond the numerical differences, the present study also identified differences in the taxonomic composition of NIS across subdivisions and between mainland MRUs. However, the differences between subdivisions are much more pronounced, with the dominance of species from the Phyla Arthropoda, Rhodophyta and Chordata, respectively in the mainland, Azores and Madeira. These differences are reinforced by the fact that the number of species that occur exclusively in each of the subdivisions (i.e., 119 in Mainland; 38 in Azores; 24 in Madeira) is considerably higher than the number of NIS common between the three subdivisions (25). Biogeographical segregation of NIS has been observed in other studies conducted across different regions, such as the French and Spanish marine regions (Western Mediterranean Sea, Bay of Biscay, Northern Seas, and Macaronesia), which are associated with the environmental preferences of the species (Massé et al., 2023; Png-Gonzalez et al., 2023).

On the other hand, our results indicate that at least 88 NIS are already dispersed in two or more MRUs of mainland Portugal, some even across three (48), suggesting that over 66% of the recorded NIS in mainland Portugal are colonizing and establishing across multiple ecosystems, thus highlighting the urgency of prioritizing the risk management of these NIS (Ojaveer et al., 2014). Furthermore, by translating previously fragmented datasets into an integrated macro-ecological baseline, this study represents a key methodological advancement. It establishes the rigorous platform and reference framework required by the international community to reliably formulate and test future quantitative hypotheses regarding biogeographical shifts and global environmental change.

Interpretation of the recent decrease in the annual rate of newly detected NIS (2020–2024) requires caution, as there is always a time lag between the actual detection of new NIS and the subsequent publication (Zenetos et al., 2019; Galanidi et al., 2025). Moreover, the observed increase over the past 20 years is likely to have been influenced, not only by a real rise in introductions, but also by a greater investment in this field of research (Castro et al., 2021; Outinen et al., 2026) and monitoring, major advancements in molecular detection techniques, improved species differentiation within cryptic groups, and intensified sampling efforts (Lavrador et al., 2024; Katsanevakis et al., 2023). In Portugal, the expansion of marine research funded by the Fundação para a Ciência e a Tecnologia (FCT) since the early 2000s, together with increasing requirements for systematic monitoring under the MSFD and WFD, substantially increased sampling effort and taxonomic expertise, accelerating the detection and publication of marine NIS. Consequently, the apparent stabilization or decline in recent first records should be interpreted cautiously, as it may reflect the interaction between expected discovery-reporting delays and the increasing completeness of baseline inventories, rather than direct evidence of a genuine reduction in propagule pressure or introduction rates." The manuscript has been revised accordingly.

Another relevant point is that the most recent period of data analysis covers only four years. This limited timeframe may mask the actual pattern of species introductions in the current era when compared to earlier analysis periods. The shorter duration could therefore lead to an underrepresentation of recent invasion dynamics.

Our findings clearly highlight the challenges in real-time data availability and underscore the importance of accounting for the time gap from discovery to publication in official assessments. In the latest MSFD assessment cycle, the inclusion of records was limited to those available up to the end of 2021, corresponding to the six-years for the assessment due for reporting in 2024 (European Commission, 2022). This approach was also adopted also to mitigate the impact of reporting delays and ensure consistency in the data considered by member states, in line with suggestions by the European Commission (e.g., Magliozzi et al., 2025).

4.4. Genetic data gap analysis and watchlist

Uneven taxonomic representation in molecular databases underscores the need for targeted barcoding efforts in poorly represented groups (Weigand et al., 2019). Addressing these gaps is vital to the effective use of DNA and eDNA-based tools in biodiversity monitoring and the early detection of marine NIS in Portuguese waters (Duarte, Parente, Costa and Costa, 2019; Leite et al., 2020; Vieira et al., 2021). For CRY species, genetic molecular tools can help clarify whether these taxa are truly native, introduced, or the result of historical uncertainty about their native geographic extent, by revealing phylogeographic patterns and population structure that may indicate source regions or long-term establishment (Blakeslee et al., 2008). Similarly, in the case of NRE species, haplotype diversity and population connectivity are fundamental sources of information allowing for discerning whether the observed distributional shifts reflect a continuous, natural expansion, or are the result of human-mediated translocations (Rius et al., 2012).

A gap analysis highlighted an uneven representation of Portuguese marine species in the BOLD genetic reference database, with significant taxonomic and geographic biases. Large datasets that provides adequate coverage of some taxonomic groups, such as Chlorophyta, likely reflect a combination of factors, including global research interest, ecological or economic importance, and the relative ease of sampling and taxonomic identification. While overall coverage is relatively high, several ecologically important groups remain underrepresented, notably Porifera, Myzozoa, Entoprocta, and Annelida. These gaps may stem from taxonomic challenges, limited global focus, or technical difficulties in DNA extraction and amplification. This limits the effectiveness of DNA-based monitoring tools and hinders early detection of marine NIS, making accredited inventories and morphological identification still essential tools. To bridge this infrastructural gap without losing valuable early-detection signals, we segregated unverified releases from the core checklist into a distinct national watchlist. The term watchlist explicitly highlights the provisional nature a of these occurrences, since these species are not integrated into the formal Portuguese baseline until a physical voucher specimen is obtained, geolocated and morphologically validated by a taxonomic specialist.

Overall, marine species occurring in Portuguese waters are relatively well covered in public reference libraries, regardless of region. Some minor differences likely reflect uneven historical sampling effort, changes in research priorities and variations in species composition across regions. Since only 12.5% of sequences originate from Portuguese-collected specimens, there is an urgent need for local sampling efforts. Expanding region-specific reference libraries would improve taxonomic resolution and reduce misidentifications in metabarcoding studies.

Additionally, the detection of 22 NIS solely through molecular methods underscored the growing importance of eDNA-based surveillance as an early-warning system. In the Azores, eight NIS represent the first molecular detections for Vila Franca do Campo and Ponta Delgada marinas in June 2020, retrieved from three sample types using metabarcoding: zooplankton, water eDNA and bulk samples collected from hard substrates in marinas (e.g., pontoons and buoys) (see Lavrador et al., 2026). These NIS were detected targeting COI or 18S sequences, except for *Oithona davisae* Ferrari F.D. & Orsi, 1984, *Pseudodiaptomus marinus* Sato, 1913 and *Pseudopolydora paucibranchiata* (Okuda, 1937), which were identified by both markers.

Regarding mainland Portugal, five NIS were detected using COI markers in a DNA-metabarcoding biofouling assessment on stainless-steel plates in the Port of Leixões (northern mainland Portugal) (Azevedo et al., 2020). Additionally, six NIS were detected in 2020–2021 at Porto Atlantic marina (also located in northern mainland Portugal) using metabarcoding of zooplankton, water eDNA, and hard substrate samples (Amaral, 2022). Finally, four NIS were detected on different sampled substrates in the marinas of Porto Atlântico and Viana do Castelo, located in northern mainland Portugal. These species were identified from different sample types, including artificial sponges and polystyrene plates, water eDNA and zooplankton. Molecular identification was performed targeting COI and 18S sequences (Lavrador et al., 2024). However, several key actions are required to maximize the effectiveness of molecular identification: i) to focus barcoding efforts on poorly represented taxonomic groups; ii) to adopt standardized sampling designs and laboratory workflows; iii) to implement harmonized bioinformatic pipelines and data curation procedures across regions to ensure comparability and reproducibility; and iv) to integrate molecular monitoring into existing biodiversity and biosecurity programmes.

Addressing these gaps will strengthen Portugal's capacity for early detection, refine and improve NIS management strategies, thereby contributing to global referenced libraries of high-quality molecular data and supporting coordinated international efforts for marine biosecurity and biodiversity conservation.

4.5. Future research directions

Beyond providing an updated inventory of marine non-indigenous species in Portuguese waters, this harmonized dataset establishes a robust and standardized baseline for future hypothesis-driven research. By integrating this dataset with complementary information, such as shipping connectivity, propagule pressure proxies, environmental similarity between donor and recipient regions, climate variability, and anthropogenic pressures, future studies can investigate the processes and drivers shaping marine NIS distributions and accumulation patterns. This framework will enable the evaluation of hypotheses related to the influence of biogeographical origin, introduction pathways, environmental suitability, and human-mediated pressures on the occurrence and spread of marine NIS across Portuguese waters. Addressing these mechanistic questions will require the integration of additional environmental and anthropogenic datasets and dedicated analytical approaches. Still, the standardized baseline established here provides an essential foundation for such future investigations.

5. Conclusion

This study demonstrates the power of synthetic macroecology by transitioning a fragmented dataset into an innovative, methodologically stratified national framework. This cross-regional collaborative approach 1) delivered a robust checklist of 231 morphologically confirmed marine NIS, including few new regional records, 2) documented a nation-wide dominance of shipping vectors while uncovering distinct biogeographical difference in origin between the main (Pacific-dominated) and the archipelagos (Atlantic-dominated), 3) traced long-term invasion rates and reporting lags, highlighting the critical need for continuous, synchronized inventory updates, 4) identified significant gaps in the availability of genetic sequence data for many NIS occurring in Portuguese waters and 5) proposed a molecular NIS watchlist as a forward-looking early-warning tool to flag new NIS before they can fully establish. The case of Portugal illustrates how national efforts, when grounded in comprehensive data and supported by national and international collaboration, can meaningfully contribute to regional and global frameworks for marine NIS management. Ultimately, our dual framework connects taxonomy with modern molecular tracking, and provides stakeholders with a reliable, scalable approach to test macroecological hypotheses and build proactive biosecurity policies.

CRediT authorship contribution statement

Romeu S. Ribeiro: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cristina Espírito Santo:** Writing – review & editing, Resources, Data curation. **Susana Celestino:** Writing – review & editing, Resources, Data curation. **Marta Mamede:** Writing – review & editing, Resources, Data curation. **João J. Castro:** Writing – review & editing, Resources, Data curation. **Teresa Cruz:** Writing – review & editing, Resources, Data curation. **Teresa Silva:** Writing – review & editing, Resources, Data curation. **David Jacinto:** Writing – review & editing, Resources, Data curation. **Cátia Bartilotti:** Writing – review & editing, Resources, Data curation. **Jorge Lobo-Arteaga:** Writing – review & editing, Resources, Data curation. **Miriam Tuaty-Guerra:** Writing – review & editing, Data curation. **João Canning-Clode:** Writing – review & editing, Resources, Data curation. **Sofia Duarte:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Ana S. Lavrador:** Writing – review & editing, Formal analysis, Data curation. **Ana Cristina Costa:** Writing – review & editing, Data curation. **Manuela Isabel Parente:** Writing – review & editing, Data curation. **Paola Parretti:** Writing – review & editing, Data curation. **Patrício Ramalhosa:** Writing – review & editing, Data curation. **Nuno Castro:** Writing – review & editing, Data curation. **Pedro Morais:** Writing – review & editing, Data curation, Conceptualization, Methodology, Writing – original draft. **Maria Alexandra Teodósio:** Writing – review & editing, Data curation. **João Encarnação:** Writing – review & editing, Data curation. **Inês Afonso:** Writing – review & editing, Data curation. **Sara Cabral:** Writing – review & editing, Data curation. **Frederico Carvalho:** Writing – review & editing, Data curation. **Maria Ana Dionísio:** Writing – review & editing, Data curation. **Ricardo Melo:** Writing – review & editing, Data curation. **Ana Amorim:** Writing – review & editing, Data curation. **António Sousa:** Writing – review & editing, Data curation. **Tiago Cidade:** Writing – review & editing, Data curation. **Paula Chainho:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Data curation.

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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. Supplementary data

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Data availability

Data will be made available on request.

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