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Validated Inventories of Non-Indigenous Species (NIS) for the Mediterranean Sea as Tools for Regional Policy and Patterns of NIS Spread

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Abstract: This work presents refined, updated subregional and regional non-indigenous species (NIS) inventories for the Mediterranean Sea, validated by national and taxonomic experts, with species records observed until December 2020. These datasets will be used as the baselines for the implementation of the Integrated Monitoring and Assessment Programme for the Mediterranean (IMAP) and the Mediterranean Quality Status Report 2023. In total, 1006 non-indigenous species have been found in Mediterranean marine and brackish waters. The highest numbers of NIS were observed in Israel, Türkiye, Lebanon and Italy. Approximately 45 species were categorized as data deficient, either due to lack of consensus on their alien status or the validity of their identification. Polychaeta, Foraminifera and macroalgae were the groups with the highest numbers of controversial species. There was a general increase in the yearly rate of new NIS introductions after the late 1990s, which appears to be slowing down in the last decade, but this may be confounded by reporting lags and differential research efforts. Between 1970 and 2020 there has been a steep increase in the proportion of shared species present throughout all four Mediterranean subregions, which are predominantly transported via shipping and recreational boating. While Lessepsian species are gradually spreading westwards and northwards, there is still a considerable invasion debt accumulating in the eastern and central Mediterranean.

Keywords: non-indigenous species; Mediterranean Sea; validation; NIS baselines; IMAP

1. Introduction

Biological invasions are globally identified as one of the main direct drivers of biodiversity loss, with impacts ranging from loss of genetic diversity to native population decreases, species displacements, habitat modifications and even whole ecosystem shifts [1]. The Mediterranean Sea is one of the most invaded ecosystems in the world [2] with an accelerating rate of alien species spread and establishment [3–7]. Coupled with resource exploitation, sea use change, pollution and climate change [8], marine invasions in the Mediterranean have contributed to the homogenization of its native biota and the degradation of its distinctive communities and habitats [9–11]. As a consequence, alien species constitute one of the elements that are taken into consideration when assessing the health of the environment and formulating management strategies to achieve and maintain it.

With the adoption of the Ecosystem Approach (EcAp) towards the management of the Mediterranean Sea, the Contracting Parties (CPs) to the Barcelona Convention committed to developing and implementing a single integrated management framework that will function as a guiding principle to all policy implementation under the UNEP/Mediterranean Action Plan (MAP) [12] and agreed on a roadmap to its implementation [13]. Being a fundamental step in the EcAp process, the Integrated Monitoring and Assessment Programme of the Mediterranean Sea (IMAP) has as its main objective to assess the status of the Mediterranean Sea and coast with a specific set of criteria and targets [14] in line with global and regional policies. As an assessment framework, it allows the harmonization of the efforts of Mediterranean countries to assess Good Environmental Status (GES), using the Marine Strategy Framework Directive (MSFD) for the European Union [15,16] as a roadmap. IMAP is structured along 11 Ecological Objectives and 27 Common Indicators [14]. Specifically, for alien species, also called Non-Indigenous Species (NIS), Ecological Objective 2 (EO2) states that "Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem" and Common Indicator 6 (CI6) requires the monitoring of "Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species".

The national implementation and harmonization of IMAP across all Mediterranean countries requires the elaboration of a number of parameters, among which the establishment of a refined baseline of the NIS present at the national and regional level is fundamental as a starting point for any further evaluations. Furthermore, the dynamic nature of marine invasions and the continuous emergence of new information concerning species' identities and origins necessitate the regular production of refined inventories for accurate assessments and effective policy implementation [17,18].

One of the first concerted efforts to collate and present comprehensive data about introduced species in the Mediterranean was initiated by the 'Mediterranean Science Commission' (CIESM) with the publication of a series of atlases on exotic species in the early 2000s, addressing fishes [19,20], crustaceans [21,22], molluscs [23] and, later, macrophytes [24]. In the past 20 years, reviews and revisions of the Mediterranean NIS inventory have been periodically undertaken, e.g., [4–7,25–30]; similarly, full or partial national inventories of a number of Mediterranean countries have been sporadically published (Algeria [31], Cyprus [32], Croatia [33], Israel [34,35], Italy [36–38], Malta [39,40], Lebanon [41–43], Libya [44,45], Egypt [46], Montenegro [47], France [48], Greece [49–54], Slovenia [55], Spain [56,57], Syria [58,59], Tunisia [60,61] and Türkiye [62–64]). What most of these efforts have in common is that they were conducted by taxonomic experts, based on the literature, personal research and expert judgement, at irregular time intervals and without necessarily following uniform and agreed methodological standards and criteria, resulting in various discrepancies in, e.g., species status designations, or the inclusion/exclusion of species records and even whole taxonomic groups.

Following guidance for the implementation of the IMAP process [65], just as with NIS validation and assessment for policy implementation at the EU level [66–68], methodological standards have been developed both for monitoring and assessment, in accordance with the MSFD and with the cooperation and agreement of appointed experts from the Contracting Parties to the Barcelona Convention [14]. Furthermore, assessment criteria have been agreed upon and adopted concerning the collation of national and regional NIS inventories and their use in evaluating aspects of CI6 [69].

The present work stems from the IMAP requirement for updated NIS baselines [70]. It aimed to collect the available material on the presence of marine NIS in the Mediterranean countries in the form of existing national inventories, combine it with new and up-to-date information on new species records, the taxonomy and biogeography of the registered species and agreed methodological standards, in order to arrive at refined NIS baselines at national and regional levels. The final outcome is the result of a collaborative process between national and regional experts, involving detailed exchanges of information and the building of consensus on the final lists, as these will constitute a tool for the determination of thresholds for CI6 and will be used for the assessment of GES targets in the upcoming Mediterranean Quality Status Report, scheduled for 2023 [71,72].

2. Methods

The validated national inventories of marine and brackish NIS in EU Mediterranean countries, produced by the JRC (Joint Research Centre of the European Commission) in collaboration with EU national experts [67], updated to December 2020 in the framework of an EEA contract [68], formed the starting point for the revision process of the national lists of Croatia, Cyprus, France, Greece, Italy, Malta, Slovenia and Spain.

For the rest of the Mediterranean countries, national NIS inventories were provided by national experts designated by the Contracting Parties to the Barcelona Convention, for Albania, Algeria, Egypt, Israel, Lebanon, Libya, Morocco, Tunisia and Türkiye (only including data for the Aegean and Levantine Seas). For a small number of countries that had not submitted national inventories, initial national baselines were created with data retrieved from the Hellenic Centre for Marine Research (HCMR) offline database. Initial inventories were updated and revised according to the recently published literature and major national, regional and taxonomic reviews, as well as data from national monitoring campaigns and citizen science project observations until December 2020 (reported until May 2023). Nomenclature was revised where necessary following the World Register of Marine Species [73]. Revised inventories were validated through several rounds of communication with countries' national experts, invited consultations with taxonomic experts and two online workshops, whereby many discrepancies were resolved, and a number of controversial species were agreed upon. Subsequently, data were aggregated at the pan-Mediterranean and the subregional level, i.e., the Western Mediterranean Sea (WMED), the Adriatic Sea (ADRIA), the Ionian Sea and the Central Mediterranean Sea (CMED) and the Aegean and Levantine Sea (EMED).

Each national inventory consists of the first record of each NIS in the country, accompanied by the year the species was reported and the associated reference, its alien status and the most likely pathway(s) of introduction, following the pathway categorization scheme of the Convention for Biological Diversity [74,75]. Regarding introduction status, range-expanding and partly native (i.e., native in one country/subregion but introduced in another) species in the Mediterranean were excluded at Pan-Mediterranean scale. However, species partly native in a subregion were included at the subregional scale. Cryptogenic and crypto-expanding species are included separately from the validated NIS lists. Due to the high degree of uncertainty regarding their origin and mode of introduction, the designation of species as cryptogenic was inconsistent among national experts in many cases; where differences in experts' opinions could not be resolved those species were flagged as data deficient at the regional/subregional level and are also reported separately from the validated NIS species. Additionally, the data deficient category includes species with unresolved taxonomic status, such as species belonging to species complexes and suspected undescribed native species (here referred to as debatable), species with questionable records, i.e., species records with insufficient information (e.g., no voucher or description provided), or with uncertain identification. Finally, regarding polychaetes, it should be noted that a number of species are present at the national or (sub)regional level, which were in the past listed as NIS but were considered debatable species by Langeneck et al. [76], in many cases without re-examination of the available material or comparison with type specimens. These species are termed here 'likely alien polychaeta', are annotated explicitly in the subregional inventories for future reference, but are presented separately from the final, refined baselines until further work clarifies their status and/or identity. Data deficient species and likely alien polychaeta were not included in any subsequent analyses and are, by general consensus, not to be taken into consideration for GES assessments [67,69].

Unicellular plankton species were in general not included in the inventories because the origin of most species is in doubt and subject to revisions [77]. However, in exceptional cases they were listed (case by case). Parasites on the other hand were included in accordance with the latest recommendations [67,69] and the recent literature (at the basin level see [78] and at the country level, e.g., Libya: [45]; Tunisia: [60]; Israel: [35]; Türkiye: [64]). Foraminifera were treated inconsistently, with most but not all countries including them in their inventories, and a strong divergence of opinion among national experts on the status of many species. As a result, particular attention was paid to this group, with the contribution of several invited taxonomic experts.

Records of species based on non-living animals (applies mostly to Mollusca with the observation of shells) and records of species reported solely from vectors (e.g., polychaeta and bryozoa, found only on ship hulls) were excluded from the final, refined lists.

Regarding the pathway of introduction, at the regional and subregional level, pathways were assigned according to the most likely means of primary introduction of the first record in the region/each subregion, respectively, based primarily on the published literature and, when the information was unavailable, on expert judgement. Pathway assignment follows the classification scheme of the Convention on Biological Diversity [74], as further refined by Pergl et al. [75]: (1) "Release in nature" (intentional introduction into the natural environment including fishery in the wild and game fishing, intentional release in the wild of species kept in domestic aquaria and other intentional release); (2) "Escape from confinement" (accidental escape of live organisms from confinement, including aquaculture/mariculture, botanical gardens/zoos/aquaria–excluding domestic aquaria, live food and live bait); (3) "Transport-contaminant" (species transported by host/vector, including contaminant on animals, parasites on animals, contaminant on plants); (4) "Transport-stowaway" (including Ship/boat ballast water, Ship/boat hull fouling, Hitchhikers on ship/boat, Angling/fishing equipment, Organic packing material and Other means of transport); (5) "Corridor" (interconnected waterways/basins/seas); (6) "Unaided" (natural dispersal across borders of invasive alien species that have been introduced through all other pathways). Species that were linked to more than one pathway were given a value of 1/k for each of the k associated pathways so that the overall contribution of each species to the total number of pathways equals one.

In our analysis of the trends in temporal occurrence and spatial distribution, we only considered the first new record of an NIS within each subregion. The number of species observed per 10-year cycle since 1970 were analyzed from these data sets to calculate rates in NIS introductions. Subsequently, the overlap in NIS between the four Mediterranean subregions at decadal time intervals was visualized with Venn diagrams, utilizing the package 'ggvenn' [79] in the R statistical environment, in order to examine the temporal evolution of the spread of NIS throughout the basin. For the purposes of this analysis, "unique" species are defined as those species that appear in one subregion only at a given time, while "shared" species are the species that are present and shared among all four subregions at that particular time. Finally, for the full dataset up to 2020, the percentage contribution of major taxa/taxa groups, as well as pathways, was calculated for the subregionally unique species and the shared species and juxtaposed with the respective values for the full subregional and regional species lists.

3. Results

Refined and validated national NIS inventories were produced for the Contracting Parties of the Barcelona Convention. The detailed lists will be uploaded to the MAMIAS Database (Marine Mediterranean Invasive Alien Species, https://dev.mamias.org/, accessed on 25 August 2023). The number of NIS together with the cryptogenic and data deficient species at the national level is displayed in Figure 1. The highest numbers of NIS were observed in Israel and Türkiye (457 and 437 NIS, respectively), followed by Italy (282 NIS), Lebanon (277 NIS), Egypt (266 NIS) and Greece (249 NIS), with values generally decreasing as we move towards the Adriatic and west Mediterranean countries.

During the validation process, 65 species emerged as data deficient; 35 characterized by divergence of opinion as to their alien or cryptogenic status and 23 as suspected questionable records (Table 1). The category of likely alien polychaeta includes seven species of different families, such as *Eurythoe complanata*, *Metasychis gotoi*, *Neopseudocapitella brasiliensis* and *Pista unibranchiata*, widely occurring across the Mediterranean Sea. They were regarded as alien species by Zenetos et al. [28,30] and Çinar et al. [62,64], but their occurrences were questioned on the coast of Italy by Langeneck et al. [76]. However, due to the widespread distribution of these species in the region and the fact that their morphological features are very similar to the original descriptions of the species, it was considered more consistent with a precautionary approach to keep them as likely alien species for the time being in the Mediterranean Sea.

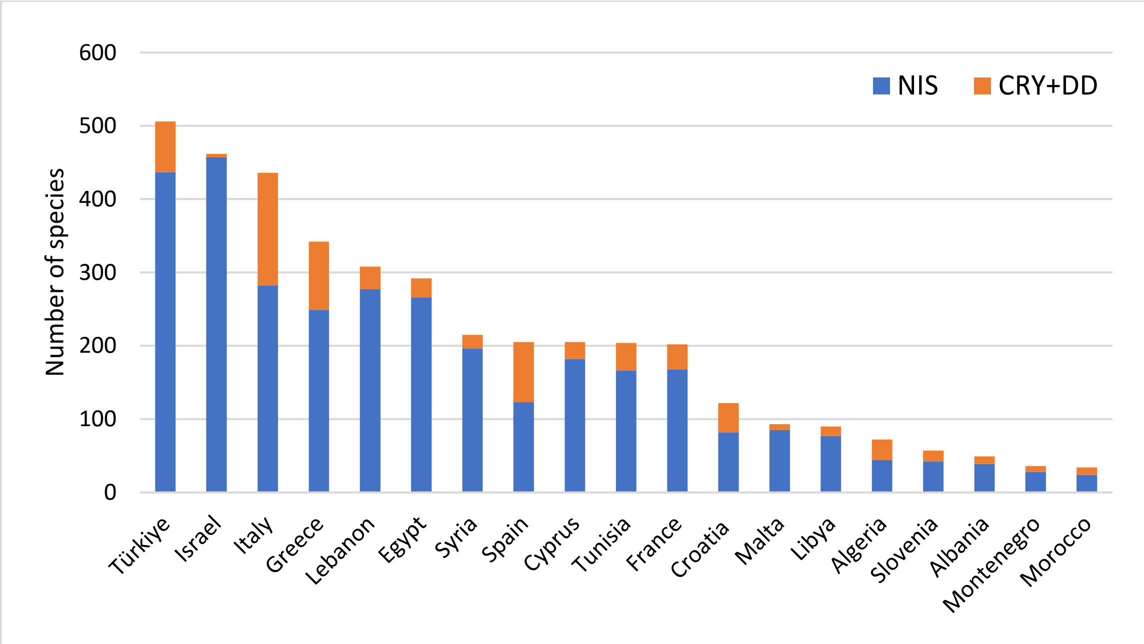

Figure 1. Number of NIS, cryptogenic (CRY) and data deficient (DD) species, detected in each Mediterranean country by December 2020.

Table 1. Data deficient (DD) species at regional/subregional level, reported in the baselines but not included in further calculations or used for GES assessments. QR = Questionable Record, WMED = Western Mediterranean, ADRIA = Adriatic Sea, EMED = Eastern Mediterranean, FR = France. Taxonomic experts: MEC = Melih Ertan Çınar, MB = Murat Bilecenoglu.

Phylum	Species	Notes
Annelida	*Armandia intermedia* Fauvel, 1902	QR in Zenetos et al. [6]/MEC expert judgement
Annelida	*Eunice cf tubifex* Crossland, 1904	QR in Zenetos et al. [6]
Annelida	*Eurythoe complanata* (Pallas, 1766)	Likely alien polychaeta
Annelida	*Eurythoe laevisetis* Fauvel, 1914	Likely alien polychaeta
Annelida	*Lumbrinerides neogesae* Miura, 1981	Likely alien polychaeta
Annelida	*Metasychis gotoi* (Izuka, 1902)	Likely alien polychaeta
Annelida	*Neopseudocapitella brasiliensis* Rullier & Amoureux, 1979	Likely alien polychaeta
Annelida	*Novafabricia infratorquata* (Fitzhugh, 1973)	Inconsistent status assignement
Annelida	*Oenone* cf. *fulgida* (Lamarck, 1818)	Likely alien polychaeta
Annelida	*Pista unibranchia* Day, 1963	Likely alien polychaeta
Annelida	*Polydora colonia* Moore, 1907	Inconsistent status assignement
Arthropoda	*Calappa pelii* Herklots, 1851	Inconsistent status assignement
Arthropoda	*Latigammaropsis togoensis* (Schellenberg, 1925)	Inconsistent status assignement
Arthropoda	*Percnon gibbesi* (H. Milne Edwards, 1853)	Inconsistent status assignement
Arthropoda	*Amphibalanus improvisus* (Darwin, 1854)	Inconsistent status assignement
Bryozoa	*Amathia verticillata* (delle Chiaje 1822)	Inconsistent status assignement
Bryozoa	*Microporella harmeri* Hayward, 1988	Inconsistent status assignement

Table 1. *Cont.*

Phylum	Species	Notes
Chlorophyta	*Bryopsis pennata* J.V.Lamouroux 1809	Inconsistent status assignement—excluded in Verlaque et al. [24]
Chlorophyta	*Caulerpa denticulata* Decaisne, 1841	QR in Zenetos et al. [6]
Chordata/Ascidiacea	*Ascidiella aspersa* (Müller, 1776)	Inconsistent status assignement
Chordata/Ascidiacea	*Diplosoma listerianum* (Milne Edwards, 1841)	Inconsistent status assignement
Chordata/Teleostei	*Abudefduf* spp.	WMED, ADRIA, EMED—Specimens of the genus Abudefduf visually classified may belong to different cryptic species of genus Abudefduf [80,81]. Molecularly identified specimens of the introduced species are retained as valid records
Chordata/Teleostei	*Aphanius dispar* (Rüppell, 1829)	QR in Zenetos et al. [6]
Chordata/Teleostei	*Cephalopholis taeniops* (Valenciennes, 1828)	Inconsistent status assignement
Chordata/Teleostei	*Enchelycore anatina* (Lowe, 1838)	Inconsistent status assignement
Chordata/Teleostei	*Ophioblennius atlanticus* (Valenciennes, 1836)	Inconsistent status assignement
Chordata/Teleostei	*Pseudotolithus senegallus* (Cuvier, 1830)	QR in Zenetos et al. [6]/MB expert judgement
Chordata/Teleostei	*Siganus javus* (Linnaeus, 1766)	QR in Zenetos et al. [6]
Chordata/Teleostei	*Trachurus indicus* Nekrasov, 1966	QR in Zenetos et al. [6]
Cnidaria	*Filellum serratum* (Clarke, 1879)	Inconsistent status assignement
Echinodermata	*Ophiactis savignyi* (Müller & Troschel, 1842)	Inconsistent status assignement
Foraminifera	*Amphisorus hemprichii* Ehrenberg, 1840	Inconsistent status assignement
Foraminifera	*Articulina alticostata* Cushman, 1944	QR this work
Foraminifera	*Articulina mayori* Cushman, 1922	QR this work
Foraminifera	*Astacolus insolitus* (Schwager, 1866)	QR this work
Foraminifera	*Bulimina biserialis* Millett, 1900	QR this work
Foraminifera	*Cymbaloporetta plana* (Cushman, 1915)	Inconsistent status assignement
Foraminifera	*Cymbaloporetta squammosa* (d'Orbigny, 1839)	Inconsistent status assignement
Foraminifera	*Dentalina albatrossi* (Cushman, 1923)	QR this work
Foraminifera	*Euthymonacha polita* (Chapman, 1904)	Inconsistent status assignement
Foraminifera	*Heterocyclina tuberculata* (Möbius, 1880)	QR this work
Foraminifera	*Peneroplis pertusus* (Forsskål in Niebuhr, 1775)	Inconsistent status assignement
Foraminifera	*Polymorphina fistulosa* Williamson, 1858	Inconsistent status assignement
Foraminifera	*Pseudonodosaria brevis* (d'Orbigny, 1846)	QR this work
Foraminifera	*Pyramidulina perversa* (Schwager, 1866)	QR this work
Foraminifera	*Quinqueloculina* sp. C	QR this work
Foraminifera	*Sigmoihauerina bradyi* (Cushman, 1917)	QR this work
Foraminifera	*Triloculina* sp. A	QR this work
Foraminifera	*Triloculinella asymmetrica* (Said, 1949)	QR this work
Foraminifera	*Vaginulinopsis sublegumen* Parr, 1950	QR this work
Mollusca	*Spondylus nicobaricus* Schreibers, 1793	QR in Zenetos et al. [6]
Mollusca	*Bursatella leachii* Blainville, 1817	Inconsistent status assignement
Mollusca	*Thecacera pennigera* (Montagu, 1813)	Inconsistent status assignement
Myzozoa	*Alexandrium taylorii* Balech	Inconsistent status assignement

Table 1. *Cont.*

Phylum	Species	Notes
Ochrophyta	*Ectocarpus siliculosus* var. *hiemalis* (P.L.Crouan & H.M.Crouan) Gallardo, 1992	Inconsistent status assignement
Ochrophyta	*Pylaiella littoralis* (Linnaeus) Kjellman, 1872	Inconsistent status assignement
Porifera	*Niphates toxifera* Vacelet, Bitar, Carteron, Zibrowius & Pérez, 2007	Inconsistent status assignement
Rhodophyta	*Pyropia koreana* (M.S.Hwang & I.K.Lee) M.S.Hwang, H.G.Choi Y.S.Oh & I.K.Lee, 2011	Inconsistent status assignement
Rhodophyta	*Acanthophora nayadiformis* (Delile) Papenfuss, 1968	Inconsistent status assignement
Rhodophyta	*Anotrichium okamurae* Baldock, 1976	Inconsistent status assignement (*A. furcellatum* in FR)
Rhodophyta	*Ganonema farinosum* (Lamouroux) Fan & Wang, 1974	Inconsistent status assignement
Rhodophyta	*Laurencia minuta* Vandermeulen, Garbary & Guiry 1990	Inconsistent status assignement
Rhodophyta	*Palisada maris-rubri* (K.W.Nam & Saito) K.W.Nam, 2007	Inconsistent status assignement
Rhodophyta	*Spyridia aculeata* (Bory de Saint-Vincent) Papenfuss, 1968	Inconsistent status assignement
Rhodophyta	*Vertebrata fucoides* (Hudson) Kuntze, 1891	Inconsistent status assignement

At the pan-Mediterranean level, a total of 1006 validated, non-indigenous species have been found throughout the basin until the end of 2020 (Supplementary Material), of which 222 are Mollusca, 188 Arthropoda, 171 Fishes, 143 Macrophytes, 83 Annelida, 47 Foraminifera, 42 Cnidaria, 32 Bryozoa, 29 Ascidiacea and 49 taxa belong to other taxonomic groups. The different taxonomic groups are variably represented in the four subregions (Table 2). Macrophytes are the most dominant group in the ADRIA and WMED, primarily as contaminants in shellfish consignments towards the major shellfish culture areas of the northern Adriatic and the French coast. Mollusca, Arthropoda and fishes are the main taxa groups in EMED, with more than 140 species each. Many of these are species of Indo-Pacific origin and warm water affinity. Annelida almost invariably constitutes approximately 10% of the NIS found, regardless of subregion, while Osteichthyes (fishes) is the most common taxon reported in the CMED. Foraminifera are represented by 44 species in the EMED (or 5%) but much lower species numbers (between 6 and 8 or 2–3%) in the other subregions.

Roughly half of the non-indigenous species present in the Mediterranean have Corridor as their primary pathway of introduction, i.e., have most likely entered through the Suez Canal (Figure 2). This number reaches 57% in the EMED, but this pathway is not applicable as we move westwards and northwards to the other subregions, where Lessepsian species (i.e., species that have been introduced freely moving through the Suez Canal) spread to a large extent by natural dispersal (pathway Unaided). CMED has the largest proportion of Unaided species, as it accepts naturally dispersing NIS propagules from all other subregions. Noteworthy also is the higher percentage of contaminant species in ADRIA (21%) and the WMED (22%), which are inadvertently transported with aquaculture activities, while escapees have their largest representation in ADRIA, with 6% of the species assumed to have escaped from mariculture or from non-domestic aquaria. Intentional releases from domestic aquaria represent only 1–2% of all introductions, with the highest number of species appearing in the WMED and the EMED. Transport—Stowaway, including the two main shipping vectors (i.e., Ballast water and Hull fouling), constitutes the primary pathway for almost one third of the NIS entering the Mediterranean but as high as 49% of the NIS present in the ADRIA.

Table 2. Representation of the major taxa groups among the validated NIS present in the different Mediterranean subregions.

	MED	WMED	CMED	ADRIA	EMED
Macrophytes	143	90	52	55	72
Annelida	83	34	29	21	74
Mollusca	222	40	46	30	197
Arthropoda	188	65	46	38	149
Fishes	171	42	65	26	139
Ascidiacea	29	18	20	6	17
Cnidaria	42	21	10	13	31
Bryozoa	32	7	10	5	30
Foraminifera	47	6	8	6	44
Miscellanea	49	15	17	11	34
Total	1006	337	303	211	787

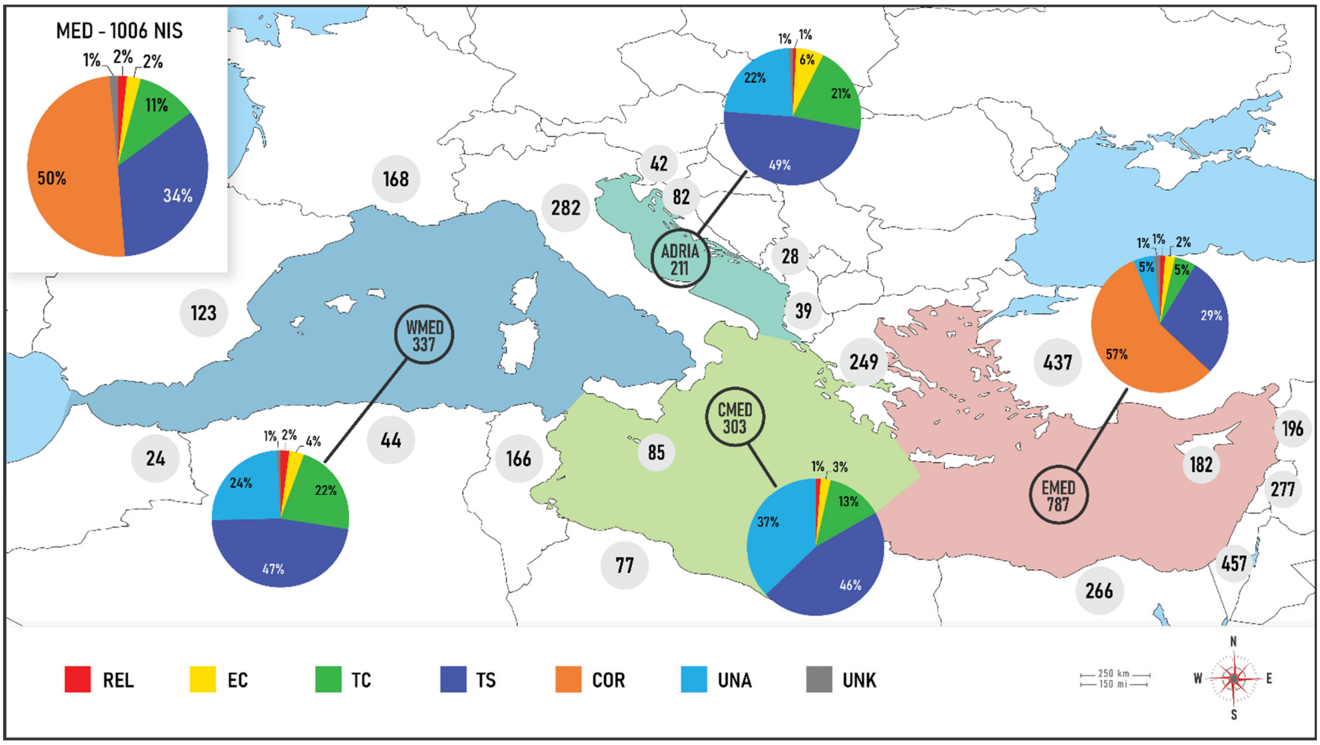

Figure 2. Primary pathways of introduction of marine NIS per Mediterranean subregion and regionally. REL = Release in nature, EC = Escape from confinement, TC = Transport-contaminant on animals (including parasites, species transported by host/vector), TS = Transport-stowaway (including Ship/boat ballast water, Ship/boat hull fouling and Other means of transport), COR = Corridor: interconnected waterways/basins/seas, UNA = Unaided: natural dispersal across borders of invasive alien species that have been introduced through all other pathways, UNK = Unknown. Numbers in circles represent total number of validated NIS per country.

Temporal trends in the Mediterranean Sea are displayed for the 807 NIS taxa observed for the first time since 1970 (Figure 3a). The rate of new NIS introductions at the decadal scale demonstrates a generally upwards trend with a notable increase in the slope after the 1990s in all Mediterranean subregions, which is more noticeable in the EMED and the CMED. Indeed, the annual introduction rates more than doubled between 1990 and 2020 at all geographic levels, except for the WMED, where the rate increased by 1.6 times. In the last decade this trend appears to be levelling off in most subregions and the basin as a whole, the only exception being the CMED, where new NIS continue to be detected at an unabatedly increasing rate even after 2010. At a finer scale (Figure 3b), the increase observed in the period 2000–2010 was more pronounced between 2000 and 2005.

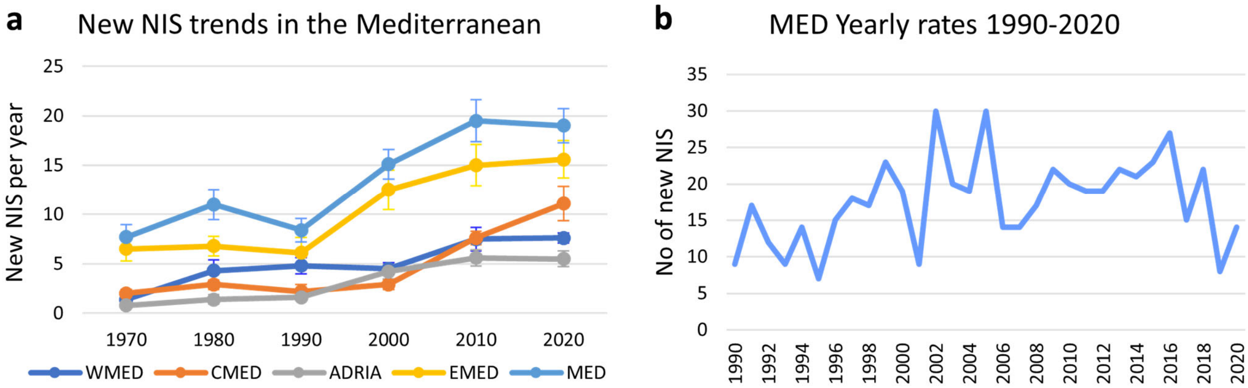

Figure 3. (**a**) Annual rate of NIS introductions (10-year mean and vertical Standard Error bars) at the regional and subregional scale. (**b**) Yearly introduction rates for the period 1990–2020 (to illustrate the large fluctuations masked by the decadal scale).

Moreover, the CMED is also distinctive among Mediterranean subregions in that the percentage of unique NIS in this area has been steadily increasing since 1970, whereas in all other subregions the percentage of unique species has been decreasing overall (Figure 4). The EMED still contains the highest number of unique species, even though the percentage has declined from 68% to 50% since 1970. In the WMED the percentage of unique species peaked at 14% by 1990 but has almost halved since, while in the ADRIA, with the smallest proportion of unique species, the decline has been steady as well. Meanwhile, the total number of species shared among all subregions has risen from six in 1970 to 86 in 2020 (2.2% to 8.5%, respectively). Among the shared species subset by 2020, polychaetes and macrophytes constitute a higher proportion compared to their percentage contribution to the total NIS diversity (Figure 5); similarly, species introduced by means of ballast water, hull fouling and aquaculture are proportionately higher in the shared subset than in the complete set of Mediterranean NIS. In contrast, Lessepsian molluscs, arthropods and fish are under-represented among the shared species of the whole basin. It is worth mentioning that the sharp increase in new NIS introductions observed in the EMED between 1990 and 2000 is also reflected in the temporal pattern of shared species between the EMED and the CMED, which was at its lowest rate (5.6%) by 2000 but started increasing again thereafter, peaking at 7.1% by the end of 2020 (Figure 4). At that time, only eight of the 72 shared EMED-CMED species were first reported in the CMED, but, even so, Corridor is implicated as a pathway of primary introduction for all eight of them (namely: *Ablennes hians*, *Atactodea striata*, *Iphione muricata*, *Isognomon legumen*, *Morula aspera*, *Padina boryana*, *Priacanthus hamrur*, *Syphonota geographica*).

Among the unique species, the pathway Corridor is over-represented in the EMED (70% compared to 62% for all EMED species), where also molluscs, particularly small gastropods, display a higher percentage compared to all EMED species (Figure 6). On the other hand, arthropods, fishes and fish parasites (within the Miscellanea phyla) are disproportionately high among species unique to the CMED (22%, 30%, respectively, in contrast with 15% and 22% among all CMED species), and the same applies to stowaway and contaminant species (60% and 20% for unique species −46% and 13%, respectively, for the total CMED dataset). In contrast, non-indigenous macrophytes are the highest contributing group among unique species both in the WMED and the ADRIA (39% and 33%, compared with 27% and 26% in the respective full subregional datasets), however, the respective over-represented pathways are different in the two subregions, namely Transport-Contaminant in the former and Transport-Stowaway in the latter. Notably, 50% of the shared species in the whole basin were introduced in the Mediterranean before 1970, while 29% were introduced between 1970 and 1990 and only 21% are recent introductions after 1990.

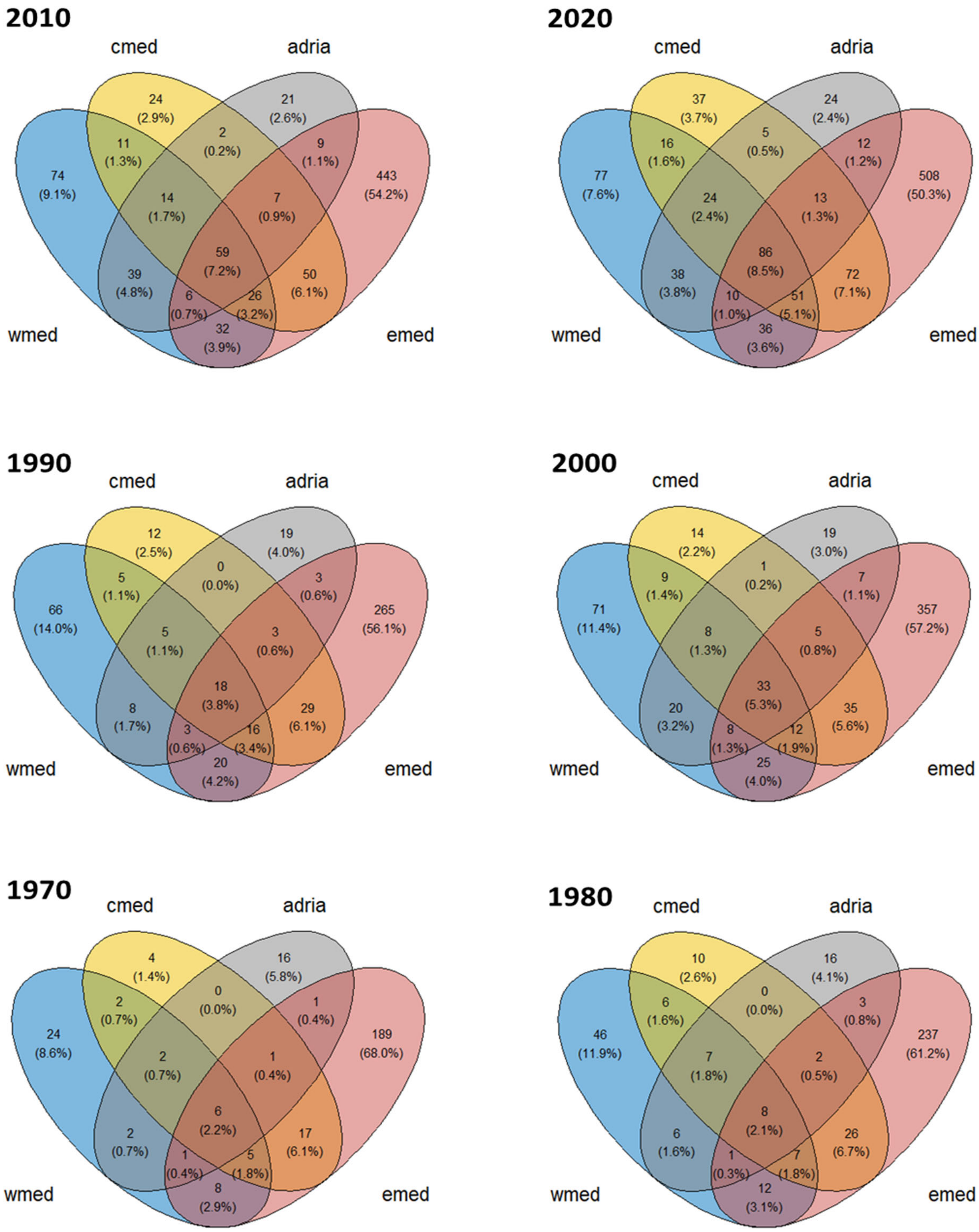

Figure 4. Cumulative number of species that are unique to or shared between the four Mediterranean subregions at a decadal scale between 1970 and 2020. Each intersection in the Venn diagrams represents the number (percentages in parentheses) of species contained only within the specific combination of geographic areas and nowhere else. wmed = western Mediterranean, cmed = Central Mediterranean and the Ionian Sea, adria = Adriatic Sea, emed = eastern Mediterranean.

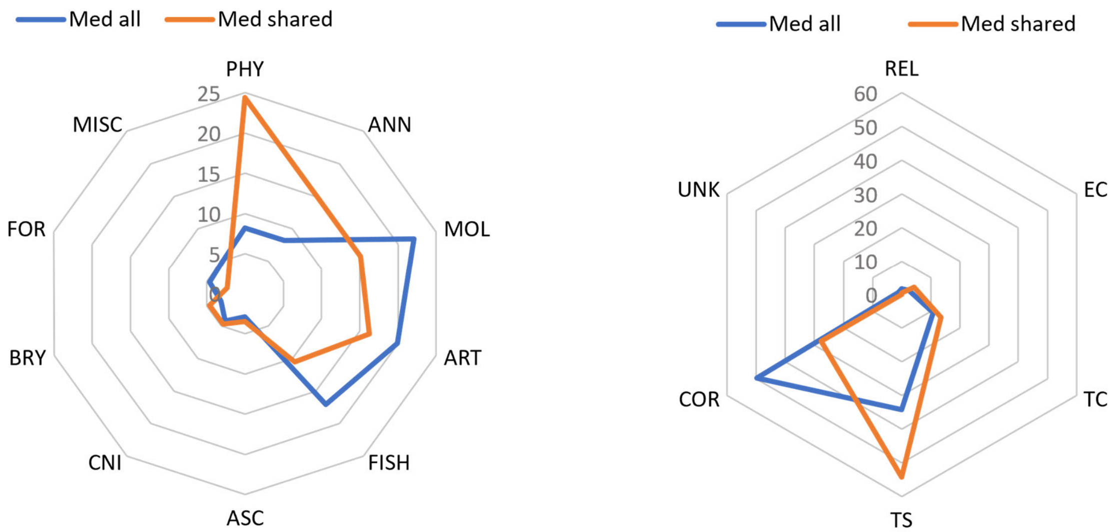

Figure 5. Representation of broad taxa groups (**left**) and primary pathways of introduction (**right**) among all Mediterranean NIS (blue line) and among the subset of 86 NIS shared between the four subregions (brown line) by 2020. Pathway abbreviations as in Figure 2, taxa group abbreviations as follows: PHY = Macrophytes, ANN = Annelida, MOL = Mollusca, ART = Arthropoda, FISH = Fishes, ASC = Ascidiacea, CNI = Cnidaria, BRY = Bryozoa, FOR = Foraminifera, MISC = Miscellanea. Y-axis expressed as %.

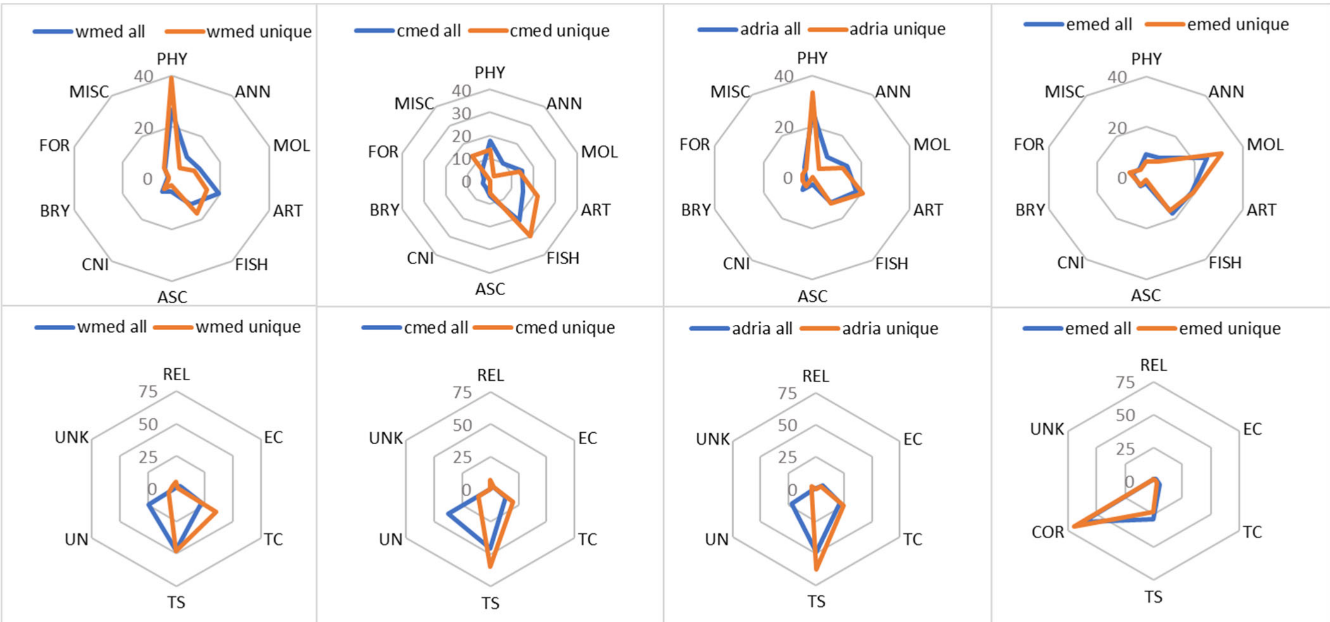

Figure 6. Representation of broad taxa groups (**top**) and primary pathways of introduction (**bottom**) among all NIS for each Mediterranean subregion (blue line) and among the subset of unique NIS for each subregion (brown line) by 2020. Y-axis expressed as %. Abbreviations as in Figures 4 and 5.

4. Discussion

Mandated by requirements of the IMAP implementation for the Mediterranean coordinated by the Regional Activity Centre for Specially Protected Areas (SPA/RAC), the current work presents refined and updated baseline NIS inventories at the regional and subregional level for the Mediterranean Sea, including species detected up to 2020. Refined inventories, starting from the national level, have been agreed upon and endorsed by countries' national experts, an essential step for the coherent assessment within and across the region and its subregions of the CI6 NIS indicator in the upcoming Mediterranean Quality Status

Report (QSR) later this year, within the IMAP context. During the validation process, a number of issues emerged with regards to species identification, alien status assignment and criteria employed when compiling national NIS inventories, which are discussed in the following sections. Trends in new NIS introductions were briefly addressed, as they have been the subject of recent studies at different geographical scales (national: Greece: [54]; Italy: [37]; Israel: [35]; Türkiye: [64]; regional–subregional [26,68,82]); however, here we aim to highlight factors that affect their interpretation. Finally, we used the new baseline dataset with the associated pathway information to illustrate the homogenization process of the Mediterranean xenodiversity, broach the invasion debt developing in the different subregions and indicate further steps in tackling non-indigenous species in the region.

4.1. Species Validation and Uncertainties

In common with what was observed in an equivalent study of EU marine waters [68], it was ascertained also at the pan-Mediterranean level that the NIS data gathering process is not standardized (there is no consistent methodology) among Contracting Parties. A number of basic ground criteria are generally followed for the validation of NIS records, namely (a) at least one record has been sufficiently documented (with detailed descriptions and/or illustrations); (b) and/or voucher specimen(s) are provided for inspection, which are deposited in a museum collection; (c) and/or genetic data have confirmed the record where ambiguities from morphological identification have arisen (e.g., Greece—[53]; Israel—[35]; Slovenia—[83]). All three criteria, however, are not applicable for all NIS national inventories, some of which had not been updated for several years, while in a few cases NIS baselines had never been established (e.g., Morocco).

The most controversial group among Mediterranean NIS was Foraminifera, which is often lacking from Mediterranean NIS checklists, e.g., [18]. It has been argued that Foraminifera is not a reliable group of NIS because geological records are lacking in many areas and, therefore, recent revisions point to possible misidentifications or to doubts about the alien status of certain species. Indeed, there was a large number of differences regarding Foraminifera species in the national lists provided by the countries, the most pronounced discrepancies being observed in the list provided by Çinar et al. [64] with the status designation reported by Stulpinaite et al. [84]. Following a series of correspondence with Mediterranean Foraminiferan experts it was agreed to include Foraminifera through validation by expert taxonomists case by case. By examining the Foraminifera species by species, it was confirmed that many species reported from Türkiye are not alien or cryptogenic forms, but were previously reported from Mediterranean Pleistocene and Holocene assemblages and, as such, were excluded. Moreover, thirteen species were classified as data deficient because (a) there are no pictures to document their presence, or (b) the specimens illustrated differ from the original ones. Such is the case of *Heterocyclina tuberculata* (Möbius, 1880) (no picture) and *Pyramidulina perversa* (Schwager, 1866) (The specimen illustrated by Meriç et al., 2014 [85] is very different from the original of Schwager, 1866). Finally, the species *Nodophthalmidium antillarum* was removed from the NIS list since it was found in core material dating to the pre-Lessepsian period [86].

Another taxonomic group where a strong divergence of opinions among experts was observed is that of polychaeta, the primary reason being that the polychaete diversity of the Mediterranean Sea is still underestimated, while taxonomic revisions for several genera are lacking. In recent years, detailed studies on some genera (e.g., *Levinsenia*, *Amphiglena*) revealed several new species, even in a limited geographical area [87,88]. Some of these species are likely to be alien species, as they suddenly appear in monitoring locations and increase their numbers over time [89]. It is not surprising that the new species identified in the highly invaded areas (the Levantine Basin) were shown to be alien species (e.g., *Syllis ergeni*, [90,91]). This is mainly because of the fact that there is relatively little information on polychaetes in the Northern Red Sea and Suez Canal. In addition, incomplete original descriptions without figures have caused the confusion of anatomically similar species. For example, Kinberg's *Eurythoe* species (*E. syriaca* and *E. hedenborgi*) described in the Levantine

Sea have not been found/re-described by subsequent researchers in the region, thus were considered as a synonym of *E. complanata* or *nomen dubium* [92,93]. The real taxonomic positions of some alien polychaete species (e.g., *Pista unibranchiata*) should be re-evaluated with integrative taxonomic approaches, including examining the type specimens. However, they should be kept on the alien species list with a special annotation until new evidence for their taxonomical entities is postulated.

Out of 65 data deficient species 12 were macroalgae, whose alien status is debated among experts due to complexities in taxonomic identification, the existence of species complexes (e.g., the *Laurencia* complex [94,95]) or the possibility of Tethyan relicts (e.g., *Acanthophora nayadiformis* and *Spyridia aculeata* [96]). On the other hand, one characteristic example of a macroalgal species which was revised in the current inventory is *Halophila decipiens*. The seagrass *H. decipiens* was reported with a single population in Salamina, Greece in 2018 [97] but its identification was based only on vegetative morphological characters; a subsequent study employing DNA barcoding demonstrated that the Greek population actually corresponds to a morphologically variant *H. stipulacea* [98], hence *H. decipiens* was removed from the Greek national inventory and consequently from the Mediterranean baseline. Despite ongoing progress in elucidating the identity and origin of many macroalgal cryptic species [99], there is a clear need for more molecular and phylogeographic studies of this species group.

Taxonomic ambiguities and uncertainties are often exacerbated by the lack of sufficiently experienced experts for certain taxonomic groups, as is the case for example in Croatia in relation to polychaetes; consequently, evaluation of the NIS list for this particular taxonomic group was not as reliable, in contrast to other taxonomic groups such as fishes, decapod crustaceans, and some other well studied groups including planktonic ones. Although the mentioned lack of expertise represents an obstacle in portraying NIS status uniformly across taxa, efforts such as the current one and similar, large-scale validation works of regional NIS inventories [6,66–68] have resulted in more pronounced collaboration among national and taxonomic experts who can provide additional quality assurance when local expertise is lacking. In the current work, for example, critical re-evaluation of published records led to the characterization of a number of records as questionable, e.g., records of *Armandia intermedia* Fauvel, 1902 and *Pseudotolithus senegallus* (Cuvier, 1830). With respect to recent updates to the Mediterranean NIS inventory, it is worth clarifying the differences between the current work and the work of Zenetos et al. [6,7]. In both Zenetos et al. [6,7] and in this work, we include species detected until the end of 2020. However, in the 1.5 years separating the two studies (December 2021 for updates of the previous dataset and May 2023 for this one) there have been new publications addressing (1) newly reported species, still detected until the end of 2020 but published after Zenetos et al. [6,7]; (2) species revisions (e.g., with regards to taxonomy, origin, nomenclature, etc.); (3) reporting/discovery of previously missed records of existing species, backdating the date of the first record; and (4) validation of records (also resulting from consensus building during the current work). This new information is taken into account herein and is responsible for the small differences between the two NIS updates, rendering the current work the most up-to-date Mediterranean NIS inventory.

Regarding uncertainties, another relevant aspect is the one that considers older records which may have not been subjected to such scientific rigor in the past as is needed in order to achieve certainty in species identification. Therefore, in addition to list curation through addition of novel NIS records, future work should also be aimed at re-examination of older records in order to reduce ambiguity. This becomes particularly important when it comes to small-sized, poorly studied and taxonomically challenging groups [17], but applies equally to larger, more conspicuous and easily identifiable taxa [6,7].

Bearing all the above in mind, it would be advisable to include all records of cryptogenic and debatable species (appropriately annotated) besides the NIS in national lists, in order to facilitate the regular updating of inventories as required when new information comes to light.

4.2. Trends

The rate of new introductions in the Mediterranean Sea appears to fluctuate with time and the span of the assessing period. Zenetos et al. [78] reported 94 new alien species for the period of January 2006 to April 2008 alone, which suggests one introduction every 9 days. A subsequent assessment by Galil [4] for the period 2000–2007 estimated 10 new alien species per year. On the coasts of Türkiye, while one new alien species was introduced every 22 weeks from 1951 to 1970, the time span decreased to four weeks between 1991 and 2010 [63]. However, Zenetos [100] reported the rate of introductions to be one NIS every 1.5 weeks for the period 2008–2009, a rate that slowed down in the following period (2011–2012) to one new introduction every 2 weeks [29].

In agreement with Zenetos et al. [29], our results, calculated at the decadal scale, showed a generally upwards trend after the 1990s in all Mediterranean subregions. At a finer scale the increase observed in the period 2000–2010 was more pronounced between 2000 and 2005, reaching 30 species in 2002 and 2005, which corresponds to one new NIS every 1.8 weeks, a number close to that calculated by Zenetos [100].

The most recent assessment of trends in NIS introductions [68] revealed that the number of newly introduced NIS has increased in the Mediterranean since the late 1990s, reaching 14 species per year in the period 2012–2017. However, this figure is not comparable to our results, as (a) it was calculated at six-year intervals and (b) it is based on NIS in Mediterranean EU countries only. As expected, the pattern is markedly different, especially for the eastern Mediterranean, precisely because of the inclusion of data from southern Mediterranean countries in our dataset. Due to the importance of this subregion as a gateway for Lessepsian species and the further use of the Baseline for future policy implementation, it is essential that trends are assessed at the pan-Mediterranean level.

In the last decade the increasing trend appears to be levelling off, although values for 2010–2020 may still change, given the lag between introduction, observation and reporting of new NIS sightings [26,101,102] and the rather low NIS numbers reported thus far for the years after 2018 (Figure 3b). The decadal scale appears to ameliorate to some extent the reporting lag's effect on the 2010–2020 yearly average, but it is evident that yearly introduction rates can fluctuate considerably, which accounts to a large extent for the differences reported in different publications.

Time lags of NIS reporting, in particular, need to be taken into account for more accurate assessments of introduction patterns of marine NIS and related management measures [103]. Zenetos et al. [102] documented that the time lags of NIS reporting at country level are at least partly dependent on the available taxonomic expertise of the human resources related to marine NIS. Moreover, there is a noticeable difference in the time lag of reporting NIS in association with the taxonomic group of the species, ranging from 4.3 years for fishes to 9.7 years for Annelida [102]. However, such factors were not considered in our analysis because of the different spatial and temporal scale of our calculations. Although the time lag in NIS reporting in the Mediterranean Sea has considerably decreased during the last decades due to networks such as INVASIVESNET [104], there have been many cases of backdating the first detection year due to the discovery of specimens in museum collections, re-examination of specimens misidentified as native, and the contribution of citizen scientists [105].

Additional biases may arise from the lack and/or heterogeneity of dedicated monitoring programs among Mediterranean countries [68]. Even though, within the MSFD and IMAP frameworks, most if not all Mediterranean countries have now elaborated MSFD/IMAP-aligned national monitoring programs (e.g., see the outputs of the GEF Adriatic Project [106,107] and the EcAp-MED II Project—https://www.unep.org/unepmap/what-we-do/projects/ECAP-MED-II, accessed on 24 August 2023), this does not alleviate the existing data limitations. Combined with a notable increase in NIS-related research efforts and the well-established positive relationship between the number of surveys/publications and the number of NIS reported [54,108], it is difficult to confidently evaluate the significance of these trends without considering a measure of "effort". Modeling approaches may help to shed some light on this issue, e.g., a study by Samaha et al. [109] using the model by Solow & Costello [110], showed that the rate at which new demersal NIS fish species are recorded has been steadily increasing, but particularly so since 2000, and this trend is due to changes in the true introduction rate rather than detection.

4.3. Spatial Patterns of the Mediterranean Xenodiversity and Invasion Debt

Our results illustrate that between 1970 and 2020 there has been a steep increase in the proportion of shared species throughout all four Mediterranean subregions, which are predominantly transported via shipping and recreational boating. This is not surprising given the predominance of the Transport-Stowaway pathway in WMED, CMED and ADRIA, the number of studied ports, the intensity of shipping traffic [108,111] and the increasingly evinced role of marinas as hotspots of NIS introduction and spread [112–114]. Almost 25% of the shared species in the basin are macrophytes, some of which, such as *Asparagopsis taxiformis*, *Caulerpa cylindracea*, *Codium fragile* subsp. *fragile*, *Lophocladia lallemandii* and *Womersleyella setacea* are highly invasive, with severe impacts demonstrated on native biodiversity, community and habitat structure, and the ecosystem functioning of sensitive habitats [11,115,116].

Lessepsian species are also expanding their distribution towards the rest of the Mediterranean [5] and by 2020 about 28% of the shared species had entered the basin through the Suez Canal. In France, for example, fish species probably dispersed unaided, such as *Siganus rivulatus* and *S. luridus*, observed first in Israel in 1924 and 1955, respectively, appeared in French Mediterranean waters in 2008 and 2010 [117,118]. However, this phenomenon is also observed for sedentary/attached species with, for example, the red algae *Lophocladia lallemandii* recorded for the first time in the Mediterranean in 1908 in Greece, but observed in 2011 in France in Corsica (unpublished data, during the CARTHAM Program). This phenomenon does not appear likely to abate, as shown by prediction models used with fish species [119].

Nevertheless, Lessepsian molluscs, crustaceans and fish are still largely restricted to the eastern and central Mediterranean. A similar pattern was demonstrated by Galil et al. [35], who showed a positive relationship between the time since first recorded and the westward spread of Lessepsian species, corroborated by the findings of the current study, indicating a considerable invasion debt from the eastern Mediterranean towards the rest of the basin. This is further supported by the fluctuations in shared species between EMED and CMED, being at its lowest rate in 2000 (Figure 4), following the large influx of new NIS in the EMED in the late 1990s, which has been linked to the abrupt shift in Sea Surface Temperatures (SSTs) in the Mediterranean between the mid-1990s and the mid-2000s [120,121]. Sea surface temperature in the last decades has continued to rise at a rate in the range between +0.29 and +0.44 °C per decade but has been spatially heterogenous, with stronger trends in the eastern basin (Adriatic, Aegean, Levantine and North-East Ionian Seas) [122]. This is undoubtedly one of the reasons for the increased establishment success of NIS observed in the Mediterranean over the past two decades [6] and particularly the increased penetration of warm water NIS into the cooler regions of the basin. As the proliferation of Lessepsian species continued, species such as *Sepioteuthis lessoniana* Férussac [in Lesson], 1831, *Biuve fulvipunctata* (Baba, 1938), *Lamprohaminoea ovalis* (Pease, 1868), *Conomurex persicus* (Swainson, 1821) had already entered the Adriatic, exhibiting their northernmost records between 2016 and 2019 [5]. The lionfish *Pterois miles* (Bennett, 1828) was first reported in the southern Adriatic in 2019 [123] but was recently recorded as far north as Croatia in the middle Adriatic [124] and as far west as the Alboran Sea in 2022 [125]. Winter isotherms of 14 °C and 15 °C, considered important physiological barriers for Mediterranean biogeography [126], have already shifted northwards and westwards and will continue to do so in the future [127], as the overall sea warming trend is expected to continue throughout the 21st century [122]. Thus, elucidating patterns of secondary NIS spread and establishment at the subregional and national level can provide powerful tools for prediction and prioritization with respect to early detection and pathway management. For example, scrutinizing the list of unique species per subregion, potentially combined with exact location information, can offer insights into the predominant gateways of introduction, where control measures would need to be adopted in order to minimize new introductions and prevent further spread. Vector risk analyses can incorporate these spatial patterns alongside pathway information to help compile future invader watch lists in association with particular vectors [128]. Subregionally unique species constitute strong candidate "door knockers" [129] for other subregions depending on their environmental requirements and limitations. Thus, horizon scanning approaches focused on assessing risk of arrival both by anthropogenic vectors and natural dispersal, combined with species distribution models, can inform the development of early detection plans [48] and even augment the fine-tuning of molecular tools both for targeted surveillance and passive monitoring [130–132].

In the context of the Barcelona Convention, the dedicated instrument for NIS management in the Mediterranean Sea is the Regional Action Plan concerning species introductions and invasive species in the Mediterranean Sea (NIS Action Plan), first adopted in 2005 [133] and further updated in 2017 [134]. A new update of the NIS Action Plan, currently under consultation and due in 2023 [135], takes into consideration the post-2020 international and regional policy framework focusing on more concrete actions for the management of pathways and the drastic reduction in invasive alien species populations and their impacts (e.g., [136,137]) to promote actions such as systematic risk analysis, prioritization and risk assessment studies, both for species and for vectors/pathways and concrete priority species action plans. The refined baselines produced through this work comprise one of the primary data sources to be used for the prioritized actions and an essential dataset for the elaboration of future steps in tackling marine invasions in the region. The network of national and taxonomic experts played an active role in the validation and update of the NIS inventories, further fostering CPs' cooperation and coordination across and within marine subregions, which will strengthen and enhance the planned work for GES achievement within the context of the Barcelona Convention.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15090962/s1, Excel spreadsheet containing the four subregional and the regional refined baselines, including validated NIS, cryptogenic and data deficient species, as well as questionable records.

Author Contributions: M.G. and A.Z.: conceptualization, review, and data collation. All authors: providing and validating national data, contribution with taxonomic expertise, interpretation of data. M.G. and A.Z.: data analysis, writing the first draft of the manuscript. All authors wrote, revised, and contributed to the editing of the final manuscript. All authors have read and agreed to the published version of the manuscript.

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