Research Article

Alien species of the Romanian and Bulgarian Black Sea coast: state of knowledge, uncertainties, and needs for future research

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Abstract

In order to assist the implementation of the European policies on Invasive Alien Species (IAS Regulation) and mitigate the impact of alien species that threaten the marine ecosystem services and biodiversity, accurate lists of alien species per European Member State are required. Although inventories of marine alien species and relevant information about them have been reported for the Black Sea marine region of Romania and Bulgaria, a validated list of alien species for the two countries is still lacking. In this paper we (i) propose a validated list of the marine alien species occurring in the Romanian and Bulgarian Black Sea regions, and (ii) address errors, gaps and uncertainties associated with listing alien species from the marine waters of the two countries. The list of alien species includes 37 species for Romania and 26 for Bulgaria, with the highest number of alien species belonging to the phylum Arthopoda for both countries. The majority of alien species have been classified as successfully established in the marine waters of both countries. Thirteen alien species were classified as invasive in Romania whilst eight alien species were classed as invasive in Bulgaria. The historical overview indicates that the number of alien species has steadily increased over the last 50 years both in Romania and Bulgaria. The results of our study provide the baseline list and information for addressing marine alien species at a national level and a starting point for selecting the invasive alien species for risk assessment towards an effective implementation of the IAS Regulation. In conclusion, this study provides supporting information for implementing measures to tackle the introduction of alien species in the western region of the Black Sea. These measures are needed to build an early warning, prevention, and control of invasive alien species in the Black Sea, a unique and fragile ecosystem.

Key words: marine species, cryptogenic, crypto-expanding, species listing

Introduction

Alien species (i.e., species that certainly have been introduced through human activity) pose a serious threat to marine ecosystem services and biodiversity (Wallentinus and Nyberg 2007; Katsanevakis et al. 2014). A worldwide increase of the annual rate of first records of alien species has been reported for the last 200 years, 37% of first records referring to the period 1970 to 2014 (Seebens et al. 2017). Although the rate of introduction of new alien species in European Seas has slowed down over the last few years (Korpinen et al. 2019), the European Seas still hold the highest number of alien species worldwide (Tsiamis et al. 2018). To tackle the issue of alien species, a specific target, i.e., the Aichi Target 9, has been approved under the Strategic Plan for biodiversity (2011–2020), adopted by the conference of parties at the Convention on Biological Diversity (CBD) in 2010. The Aichi Target 9 aimed that by 2020 identification of invasive alien species and their introduction pathway(s) are prioritized. Moreover, the European Union (EU) adopted the Marine Strategy Framework Directive (MSFD) (Directive 2008/56/EC), which specifically aims to protect the marine ecosystem across Europe and to achieve Good Environmental Status (GES) by 2020, alien species being treated as a distinct Descriptor (i.e., D2) of GES. Furthermore, the EU Regulation 1143/2014 on Invasive Alien Species has also been adopted aiming at the prevention and management of the introduction and spread of invasive alien species. To assist the implementation of specific legal instruments on Invasive Alien Species (IAS) and mitigate the impact of alien species, accurate lists of alien species, per each European Member State and marine region as defined by MSFD, are required (Tsiamis et al. 2019). However, an evaluation of reported alien species prior to 2012 revealed geographical bias in sampling efforts associated with the reporting regions (Tsiamis et al. 2019) and errors in the listing of alien species (Zenetos et al. 2017). For example, updates of lists of alien species in the marine waters of Greece (Pancucci-Papadopoulou et al. 2005; Zenetos et al. 2009, 2011, 2018, 2020a) revealed that these lists suffer from a variety of errors. The errors included taxonomic issues (i.e., synonyms and species misidentification), erroneous reports, misclassification of native or natural range expansion species as alien species (Zenetos et al. 2018; Gómez 2019). Recurrent updates of inventories of alien species from the Mediterranean region (Zenetos et al. 2005, 2008, 2010, 2012, 2017; Zenetos and Galanidi 2020) have shown similar inaccuracies. Since these biases and errors might have serious consequences on the policy information and management of biological invasions (McGeoch et al. 2012), reporting updated and accurate lists of alien species is crucially needed. Shipping through the release of ballast water has been identified as the major and often the primary vector for introduction of alien species into the Black Sea. The ballast water management convention (BWMC, IMO 2004), which provides a set of management tools for ballast waters, entered into force in 2017. To adequately implement the BWMC and to reduce the number of ballast-transported alien species addressing the uncertainties associated with the identification, distribution, and pathway(s)/vector(s) of introduction of alien species is an important step.

The Black Sea is the largest anoxic body of water in the world (Bronfman 1995), with only 10% of its total water volume suitable for aquatic organisms (Andrussov 1918; Zenkevich 1963; Zaitsev and Mamaev 1997). It is an isolated sea connected to the Mediterranean Sea only through narrow Turkish Straits. Such particular conditions make the Black Sea a unique and fragile ecosystem, with low biodiversity, but with high biological productivity. It hosts nearly 800 phytoplankton species, 2000 invertebrates, and 200 fish species (Zaitsev and Alexandrov 1998). However, the Black Sea is a recipient of alien species from several donor regions (i.e., North Atlantic, East Atlantic-Mediterranean, West Pacific, South-East Asia, South-West Pacific, Indo-Pacific) (Zaitsev et al. 2004; Tsiamis et al. 2018). Studies on the invasion of alien species showed that the native biota of the Black Sea are severely threatened by alien species (Kideys 2002; Gomoiu et al. 2002; Seebens et al. 2017). Moreover, due to its geographical position, being a sea surrounded by land, the survival and establishment of marine alien species into the Black Sea might be amplified by climatic change. Increase of the mean annual water temperature might make the Black Sea environment more favorable or suitable to certain alien species, i.e., warmer waters better matching the thermal tolerance of alien species or certain alien species could invade based on climate change. The climatic challenge enhancing the survival and establishment of marine alien species has been already acknowledged for the Mediterranean Sea, another semi-enclosed sea, which is currently considered a true hotspot of marine bioinvasions (Mannino et al. 2017). The increase in the temperature of the Mediterranean waters' accounts for introduction of Erythrean species from the Suez Canal and the ingression of the Atlantic thermophilic species into the Mediterranean through the Gibraltar Strait (Mannino et al. 2017). It is also worth mentioning that since 2000, an intensification of penetration of species from the Mediterranean Sea into the Black Sea has been reported (Shalovenkov 2020). Thus, the warming of the Black Sea might be responsible for the increase of invasion of Mediterranean species into the Black Sea, through the Turkish Straits, the so-called process of "Mediterranization" of the Black Sea (Puzanov 1965).

Despite the global increase of the number of alien species, the number of alien species reported from the Black Sea in recent assessments varies greatly. For instance, the assessment performed by Zaitsev et al. (2002) reported 41 alien species, the study carried out by Aleksandrov (2015) listed 261 alien species, whilst Shalovenkov (2019), in a recent publication on the alien species invasion in the Black Sea, reported 180 alien species. By contrast, a search in 2020 in the AquaNIS database (AquaNIS, Editorial Board 2015) for the Black Sea region returned 293 alien and cryptogenic species. These discrepancies make the information about the alien species in the Black Sea ambiguous and difficult to interpret. Although the reported number of alien species is not particularly high, due to the specific hydrological and environmental conditions of the Black Sea, some of them have developed mass populations, causing significant changes to native species communities and in the overall ecological properties of the sea (Kideys 2002). For example, the comb jelly *Mnemiopsis leidyi* A. Agassiz, 1865, a ctenophore originating from the eastern coast of the United States and introduced accidentally to the Black Sea reached enormous biomass levels (up to 4.6 kg m^{-2}) in the summer of 1989, devastating the food chain of the whole Black Sea basin (Shiganova et al. 2001). Their voracious feeding led to a significant decrease of the abundance of small pelagic fish species and to an order of magnitude lowering of zoopankton abundance. Moreover, geographical biases are evident in the Black Sea, such that more research has been conducted in the central and eastern part of the Black Sea, with important contribution from Russia (see Boltachev and Karpova 2014; Revkov 2016; Shalovenkov 2017, 2020), Turkey (see Serdar and Özcan 2018) and Georgia (see Vadachkoria et al. 2020), than in the western part of the Black Sea. Therefore, a reviewed list of alien species is essential prior to any risk assessment underpinning early warning, prevention and control methods of invasive alien species in the Black Sea.

The East and South European Network for Invasive Alien Species (ESENIAS), a regional network established in 2011 by the European Environment Agency, Ministry of Environment and Water of Bulgaria, Bulgarian Academy of Sciences, is an important initiative for assessing alien species in the Black Sea countries. One of the main activities of ESENIAS, supported throughout the ESENIAS-TOOLS and the largest project implemented by ESENIAS countries, was to compile a common list of alien species in the Balkan region (i.e., ESENIAS countries) (Trichkova et al. 2017). Although marine alien species in some taxonomic groups were reported as part of ESENIAS scientific outcomes (e.g., Karachle et al. 2017), an accurate inventory based on recent updates and scientific validation of the obtained data still needs to be done.

In this paper we aim to present a reviewed, updated, and validated list of alien species from the marine regions of Romania and Bulgaria, both Black Sea ESENIAS countries. The list will serve as a baseline towards the implementation of recent invasive species policies, both internationally and at the level of EU countries. Furthermore, we propose to address the uncertainties associated with the identification, distribution, and pathway(s)/vector(s) of introduction of alien species into Bulgarian and Romanian marine waters.

Materials and methods

To compile the list of alien species from Black Sea marine regions of Romania and Bulgaria we: (i) performed a comprehensive screening of the literature (i.e., peer reviewed and indexed and non-indexed papers, PhD theses, grey literature) published by December 2021 (in time interval 1912 to 2021) and (ii) accessed the data archived in the Hellenic Centre for Marine Research / European Environment Agency database and available on that date. For Bulgaria, additional data (not yet published nor included in relevant databases) was obtained from fieldwork surveys carried out in Varna Bay during the spring-summer-autumn season between 2015 and 2016 (ESENIAS project). As a result, our data include the first records, i.e., certain species reported for the first time during the sampling within the framework of ESENIAS project. The compiled list of species based on the bibliographic and field work surveys was checked against the AquaNIS database (AquaNIS, editorial Board 2015) and the Global Register of Introduced and Invasive Species (GRIIS) list. The additional species registered in the AquaNIS database and/or GRIIS list as alien or cryptogenic species, were added to the starting list. To avoid uncertainties associated with nomenclature for each species we checked its status according to the World Register of Marine Species (WoRMS Editorial Board 2020). When needed, species names were corrected taxonomically in agreement with WoRMS, and synonyms and alternate representations were provided (WoRMS status in Supplementary material Tables S1–S3).

Local experts were invited to check for possible errors, to validate the species on the list, and to suggest exclusions of species from the list. They were also asked to explain the reason for exclusion. For example, the species were excluded because they were extinct species (e.g., *Verruca spengleri* Darwin, 1854), misidentified species (e.g., *Oithona brevicornis* Giesbrecht, 1891 misidentification of *Oithona davisae* Ferrari F.D. & Orsi, 1984), native species (e.g., *Vaucheria dichotoma* f. *marina* Hauck, 1884), species expanding their range through natural means (e.g., *Bolinopsis vitrea* (L. Agassiz, 1860), and species that cannot survive in high salinity marine waters such as freshwater species or species that are occasionally found in slightly brackish estuarine habitats (e.g., *Umbra krameri* Walbaum, 1792). All species that fit these exclusion criteria were removed from the initial list.

For the species retained on the refined list we first gathered data on species status as follows: alien, cryptogenic and crypto-expanding. Cryptogenic is a term that includes species that cannot be assigned with certainty to either native or introduced species in their occurrence area (Carlton 1996). Crypto-expanding is a recent term (Zenetos et al. 2020b), first introduced in the ESENIAS-tools project (Trichkova et al. 2017) and adopted by Tsiamis et al. (2021). This term was used for species with an unclear arrival or introduction pathway(s)/vector(s). For example, a crypto-expanding species refers to a species that either arrived through natural range expansion from another area, as it is the case of some species from the Mediterranean Sea which arrived in the Black Sea via the Turkish Straits, or were introduced through human activity. Thus, in the context of the Black Sea invasions the term crypto-expanding serves our purpose better when dealing with uncertainties in alien species listing than the term cryptovectic (Carlton and Ruiz 2005). As defined by Carlton and Ruiz (2005) cryptovectic species are species for which the pathway(s)/vector(s) is unknown, which means that several vectors (polyvectism) move the species along the same or different multiple routes causing its occurrence in a new region. We also did not consider the widely accepted term secondary introduction. The term is intended for species which were initially introduced in a new region through human mediated translocation and from where they might continue to spread by natural or anthropogenic mechanisms (i.e., species introduced in the first instance to the Russian coast of the Black Sea that arrives to Romanian and Bulgarian shores). This is not the case for either cryptogenic or crypto-expanding species. Second, we gathered data on the species native distribution range, i.e., the distributional range where the species is considered native. For species validated as alien, we further classified their native distribution based on the global marine biogeographic realms proposed by Spalding et al. (2007). We assigned multiple realms to a species if the native distribution of that species covered more than one marine realm. For one alien species, i.e., *Polydora cornuta* Bosc, 1802, the native distribution range is unknown. Third, we gathered data on species first sighting or collection date (year or range of years); if the precise first sighting or collection date was not mentioned in the source article, we used the before symbol "<" and the year in which the source article was published. Finally, we gathered data on species establishment success. Following the terminology and classification of Blackburn et al. (2011), Zenetos et al. (2018, 2020a) species were assigned to one of the following four categories: (i) established (a species with at least one self-maintaining population currently occurring in the wild); (ii) invasive (an established species with rapid expansion of its distribution range and high probability to negatively affect the native biodiversity of the invaded ecosystem and its services; (iii) casual (a species reported based on one or very few individuals and with not yet proved ability to spread or reproduce in its occurrence area); (iv) unknown (a species with currently no available information on the establishment success, due to incomplete documentation of its abundance or distribution range, or unconfirmed occurrence in a particular location where it has been previously recorded or reported for the first time).

For the species classified as alien we also gathered data on: (i) the most plausible primary pathway(s)/vector(s) of introduction, in accordance with CBD classification (CBD 2014), and (ii) the certainty of introduction pathway(s)/vector(s) assignment. Since the terms pathway and vector are used frequently in the text, for more clarity we use herein both interchangeably. Pathway would be an adequate term and the ideas surrounding the term vector are included in our use of the term pathway and vice-versa. According to CBD classification the pathways of alien species introduction in European Seas are: (i) corridor (COR) (e.g., movement of alien species via canals); (ii) escape from confinement (EC) (e.g., aquaculture); (iii) transport stowaway (TS) (e.g., moving of live alien species by maritime transport via ballast water and sediments, bio-fouling of water vessels and dredging, angling or fishing equipment); (iv) release in nature (REL) (e.g., intentional introduction of live alien species for fishing); (v) transport contaminant (TC) (unintentional movement of live pests, through international trade); (vi) unaided (UNA) (e.g., natural dispersal of invasive alien species that have been introduced by different pathways). We defined four certainty levels (Katsanevakis et al. 2013) as follows: (i) level "1" which corresponds to a very high certainty (i.e., the pathway was documented), (ii) level "2" refers to a medium certainty (i.e., the pathway is very likely), (iii) level "3" attributed to low certainty (i.e., the pathway is likely), and (iv) level "4" assigned to unknown pathway.

To show how the number of new records reported since 1970 for the alien, crypto-expanding, and cryptogenic species varied in time (MSFD descriptor D2, criterion D2C1) we grouped the number of new reports per six-year time intervals and calculated the cumulative number of species over time. The chi-square (χ^2) test was used to test for significant differences between countries and among categories (i.e., alien, crypto-expanding and cryptogenic species) of the taxonomic affiliation, establishment success, origin, variation in new records reported over time, and introduction pathway(s). All the graphics in the manuscript were generated using the package ggplot2 in R, version 3.5.1. (R Core Team 2022).

Results

The literature search generated 218 references from the year 1912 to 2021 (see Table S4). The initial list included 194 species, out of which 43 species were suggested by the local experts to be excluded from the list (see Table S1 for detailed information about them including reason for exclusion), 38 species were validated as alien (Table 1 and Table S2) and a total of 113 species were classified as crypto-expanding, cryptogenic, and data deficient (Table S3). There was a nonsignificant difference in the overall number of alien, cryptogenic and crypto-expanding species between the two countries (χ^2 = 4.6709, df = 2, P = 0.096). When each country was considered separately, the validated list of alien species included 37 species for Romania and 26 for Bulgaria, respectively (Table 1 and Table S2 for detailed information). Eleven alien species were exclusive to Romania, while from all 26 alien species present in Bulgaria only one was not shared with Romania. Furthermore, 29 cryptogenic and 72 crypto-expanding species were documented for Romania while for Bulgaria we found 22 cryptogenic, 28 crypto-expanding, and 6 data deficient species. Eleven cryptogenic and 46 crypto-expanding species were exclusive to Romania and four cryptogenic,

Table 1. The validated list of alien species recorded in the Black Sea marine region of Bulgaria (BG) and Romania (RO) (see Table S2 for detailed information about them) and their establishment success following the terminology and classification of Blackburn et al. (2011), and Zenetos et al. (2018, 2020a): casual (cas), established (est), invasive (inv), and unknown (unk).

Phylum	Taxon	Species	Country	
			BG	RO
Annelida	Polychaeta	*Alitta succinea* (Leuckart, 1847)	est	est
	Polychaeta	*Amphiglena mediterranea* (Leydig, 1851)		cas
	Polychaeta	*Dipolydora quadrilobata* (Jacobi, 1883)	inv	inv
	Polychaeta	*Ficopomatus enigmaticus* (Fauvel, 1923)	inv	inv
	Polychaeta	*Laonome xeprovala* Bick & Bastrop, in Bick et al. (2018)		est
	Polychaeta	*Polydora cornuta* Bosc, 1802	inv	inv
	Polychaeta	*Streblospio gynobranchiata* Rice & Levin, 1998		inv
Ciliophora	Oligotrichea	*Eutintinnus lusus-undae* (Entz, 1885)		est
Chordata	Ascidiacea	*Molgula manhattensis* (De Kay, 1843)		est
	Ascidiacea	*Styela clava* Herdmann, 1881		est
Ctenophora	Nuda	*Beroe ovata* Bruguière, 1789	inv	inv
	Tentaculata	*Mnemiopsis leidyi* A. Agassiz, 1865	inv	inv
Cnidaria	Hydrozoa	*Calyptospadix cerulea* Clarke, 1882	unk	est
	Anthozoa	*Diadumene lineata* (Verrill, 1869)		inv
Arthropoda	Cirripedia	*Amphibalanus amphitrite* (Darwin, 1854)		cas
	Cirripedia	*Amphibalanus eburneus* (Gould, 1841)	est	est
	Cirripedia	*Chthamalus stellatus* (Poli, 1791)	cas	est
	Copepoda	*Acartia tonsa* Dana, 1849	est	est
	Copepoda	*Oithona davisae* Ferrari F.D. & Orsi, 1984	est	est
	Decapoda	*Alpheus dentipes* Guérin, 1832	est	
	Decapoda	*Callinectes sapidus* Rathbun, 1896	est	est
	Decapoda	*Eriocheir sinensis* H. Milne Edwards, 1853		est
	Decapoda	*Eurypanopeus depressus* (Smith, 1869)	est	est
	Decapoda	*Hemigrapsus sanguineus* (De Haan, 1835)		cas
	Decapoda	*Palaemon macrodactylus* Rathbun, 1902	cas	est
	Decapoda	*Rhithropanopeus harrisii* (Gould, 1841)	est	inv
	Isopoda	*Sphaeroma walkeri* Stebbing, 1905		est
Mollusca	Bivalvia	*Anadara kagoshimensis* (Tokunaga, 1906)	inv	inv
	Bivalvia	*Arcuatula senhousia* (Benson, 1842)	cas	cas
	Bivalvia	*Magallana gigas* (Thunberg, 1793)	est	est
	Bivalvia	*Crassostrea virginica* (Gmelin, 1791)		est
	Bivalvia	*Mya arenaria* Linnaeus, 1758	inv	inv
	Gastropoda	*Corambe obscura* (A.E. Verrill, 1870)	est	inv
	Gastropoda	*Rapana venosa* (Valenciennes, 1846)	inv	inv
Ochrophyta	Phaeophyceae	*Desmarestia viridis* (O.F. Müller) J.V. Lamouroux, 1813	unk	est
	Bacillariophyceae	*Lauderia pumila* Castracane, 1886	est	est
	Bacillariophyceae	*Thalassiosira nordenskioeldii* Cleve, 1873	unk	est
Chordata	Pisces	*Planiliza haematocheila* (Temminck & Schlegel, 1845)	est	inv

two crypto-expanding and three data deficient species to Bulgaria, respectively. Forty-four species, i.e., 18 cryptogenic, 26 crypto-expanding and three data deficient species, were shared by both countries (Table S3).

We found a significant difference in the number of species among the taxonomic groups for both countries (Romania: $\chi^2 = 82.905$, df = 24, $P < 0.001$; Bulgaria: $\chi^2 = 56.388$, df = 20, $P < 0.001$). The highest number of alien species belonged to the phylum Arthopoda, whilst the phylum Myzozoa was the best represented for both cryptogenic and crypto-expanding species in both counties (Figure 1). The chi-square test also revealed significant differences in the establishment success among alien, cryptogenic and crypto-expanding species in both counties (Romania: $\chi^2 = 25.495$, df = 4, $P < 0.001$; Bulgaria: $\chi^2 = 42.023$, df = 6, $P < 0.001$). Most of the alien and

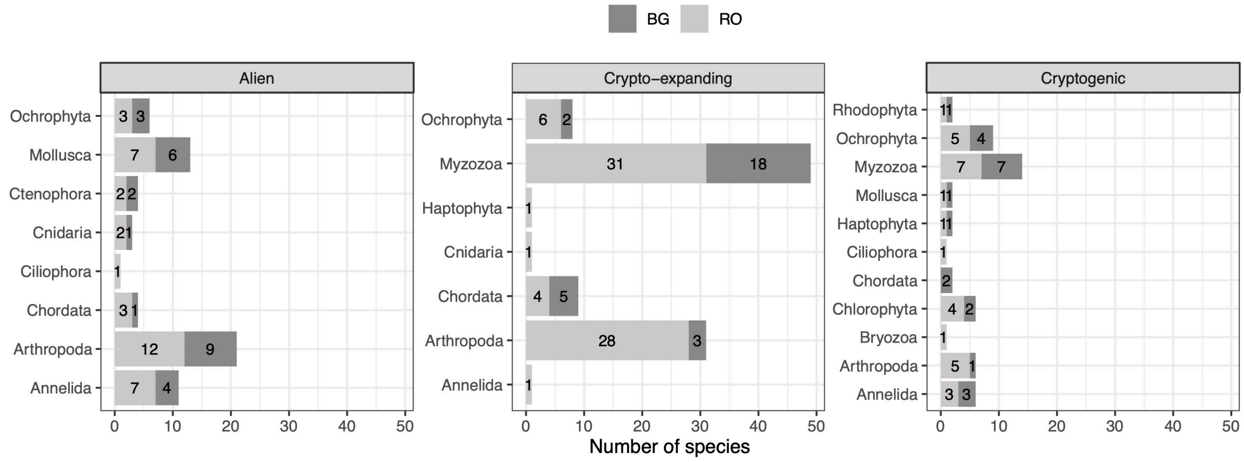

Figure 1. Number of alien, crypto-expanding, and cryptogenic species per taxonomic group from the Black Sea marine region of Bulgaria (BG) and Romania (RO), respectively.

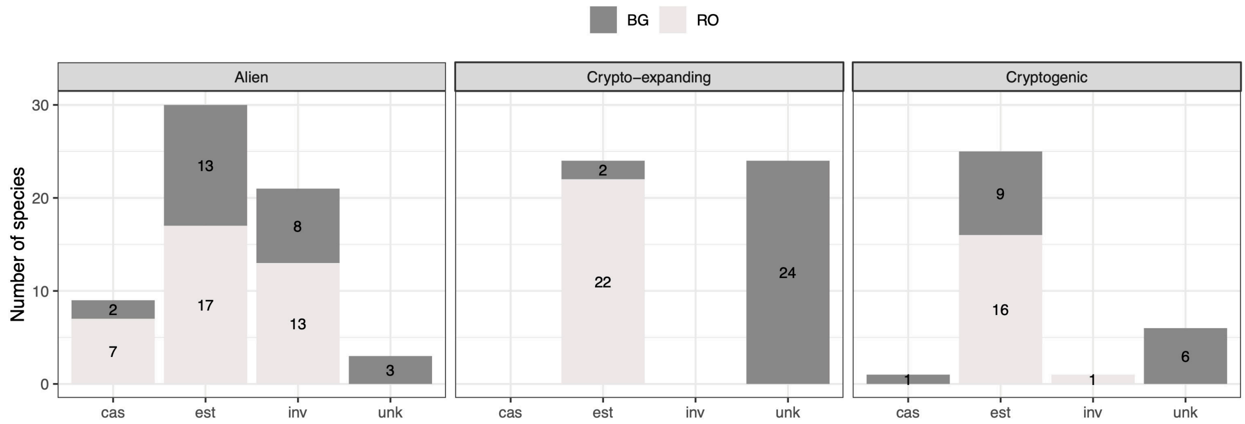

Figure 2. The establishment success (following the terminology and classification of Blackburn et al. (2011), Zenetos et al. (2018, 2020a): casual (cas), established (est), invasive (inv), and unknown (unk), of the alien, crypto-expanding and cryptogenic species from the Black Sea marine region of Bulgaria (BG) and Romania (RO), respectively.

cryptogenic species were classified as established according to the establishment success in both countries (Figure 2). Thirteen alien species classified as invasive occurred in Romania whereas only eight in Bulgaria, respectively (Figure 2). The majority of crypto-expanding species had self-sustaining established populations in Romania and on the contrary in Bulgaria where an unknown establishment success was reported for all of them (Figure 2).

The results showed that the origin of alien species based on the classification of global marine biogeographic realms proposed by Spalding et al. (2007) did not differ significantly between Romania and Bulgaria (χ^2 = 13.856, df = 20, P = 0.838). For both countries the majority of alien species originated from the Atlantic Ocean followed by Pacific and Indo-Pacific biogeographic realms (Figure 3). Alien species with native ranges in the Arctic Ocean and Australia were less represented (Figure 3). Most crypto-expanding and cryptogenic species listed from Romania have had an obscure origin as they are nowadays regarded as cosmopolitan while most of the crypto-expanding and cryptogenic species listed from Bulgaria originated from the Atlantic Ocean (Table S3).

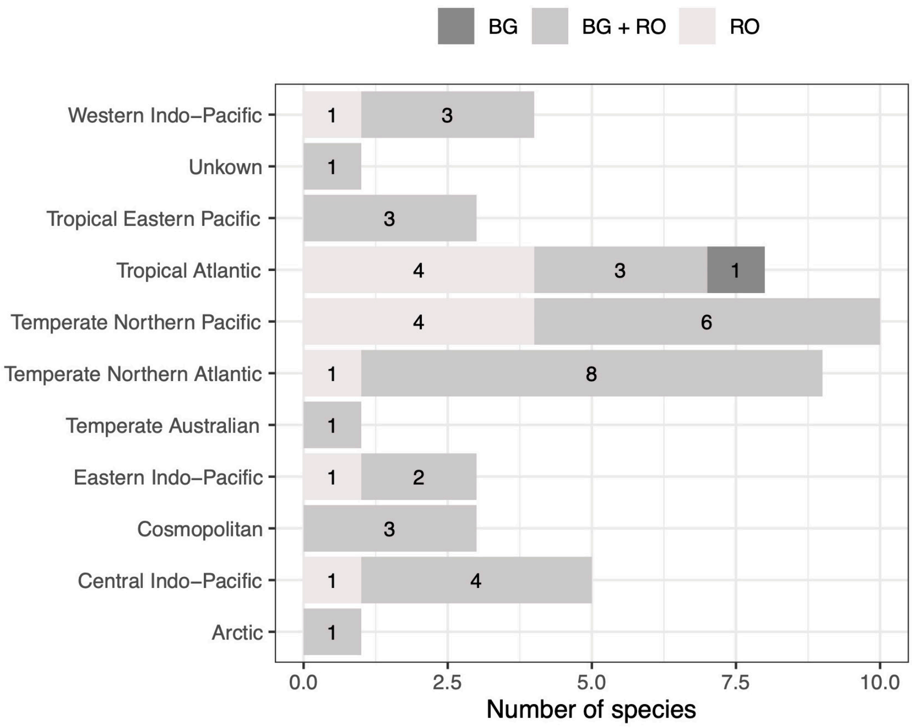

Figure 3. Native distribution ranges of the alien species depicted per marine biogeographic realms following Spalding et al. (2007), from the Black Sea marine region of Bulgaria (BG) and Romania (RO), and from both countries' (BG + RO) region, respectively.

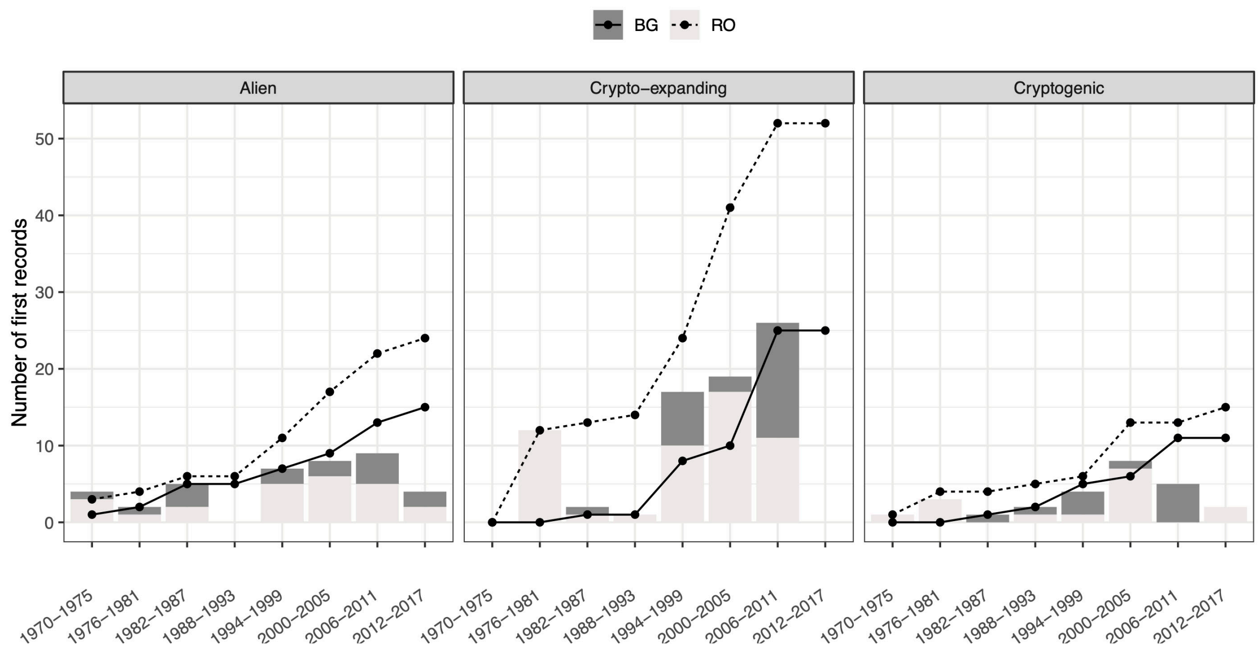

Figure 4. The number of new reported records since 1970 at six-year time intervals (bars) and the cumulative number of species (lines) for alien, crypto-expanding, and cryptogenic species from the Black Sea marine region of Bulgaria (BG) and Romania (RO), respectively.

We found a significant difference in the number of new records reported since 1970 among the defined six-year time intervals for Romania (χ^2 = 25.57, df = 14, P = 0.029) but not for Bulgaria (χ^2 = 19.133, df = 14, P = 0.159). Figure 4, that shows the introduction history of the alien, crypto-expanding,

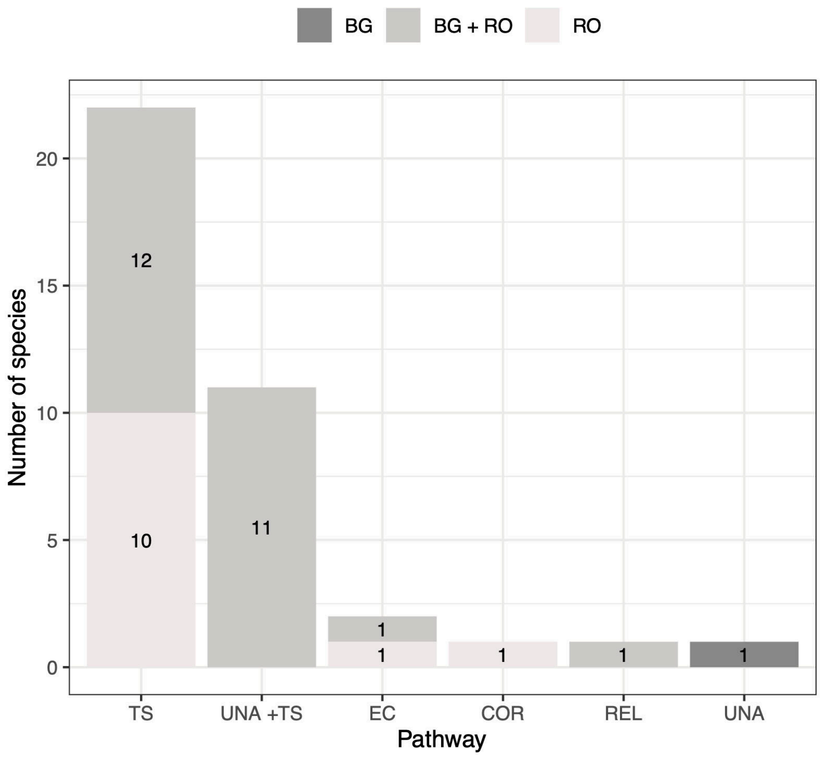

Figure 5. The introduction pathway(s) of alien species from the Black Sea marine region of Bulgaria (BG) and Romania (RO), and from both countries' (BG + RO) region, respectively. The pathway classification follows the Convention on Biological Diversity (2014): corridor (COR), escape from confinement (EC), transport-stowaway (TS), release in nature (REL), and unaided (UNA).

and cryptogenic species reported since 1970 per six-year intervals, indicated that: (i) the number of alien species has steadily increased over the last 50 years for both Romania and Bulgaria; (ii) there is a steep increase of reported crypto-expanding species in the time interval 1994–2011 for Romania, and (iii) there is an important increase of reported crypto-expanding species in the time interval 2006–2011 for Bulgaria.

The chi-square test indicated that the introduction pathway(s) of alien species differ significantly between the two countries ($\chi^2 = 48.093$, df = 10, $P < 0.01$). Transport-stowaway was the most important pathway of arrival for species listed as alien in both countries (Figure 5). A significant number of alien species were introduced by two pathways. For them, the Transport-stowaway was the main introduction pathway and natural dispersal, i.e., unaided introduction, was less important (Figure 5). Concerning the certainty of introduction pathway(s) the majority of alien species were clearly associated to a specific pathway at the time of introduction or the most likely pathway could be inferred. Consequently, certainty levels of "1" and "2", respectively, were assigned to introduction pathway(s) of more than 89% of the species (Table S2).

Discussion

We propose a validated list of alien, cryptogenic and crypto-expanding in the western Black Sea region, i.e., Bulgarian and Romanian Black Sea region.

The list represents the baseline information for addressing marine alien species on a national level and a coherent and representative list for future updating. Moreover, the list serves as a starting point towards an effective implementation of MSFD (D2C1: trends in introduction) as well as for selecting the invasive alien species for risk assessment and inclusion on the Union list (EU IAS Regulation). Concerning the validation of previously reported alien species the present study revealed: (i) errors in listing of alien species, (ii) changes in the alien status and/or establishment success of alien, cryptogenic and crypto-expanding species, and (iii) gaps in the scientific literature associated with reporting of alien species. This suggests that both the opinion of local experts (Crocetta et al. 2017) and filling gaps in the knowledge of alien species are essential to define future research priorities and focus conservation efforts.

As highlighted in previous works (Zenetos et al. 2017), the main reasons for excluding species from the initial list, species that failed to be classified as alien, cryptogenic or crypto-expanding included: (i) synonymizing of existing taxa (i.e., *Mugil soiuy* Basilewsky, 1855, which is synonymous with *Liza haematocheila* (Temminck & Schlegel, 1845) and for which the new accepted name is *Planiliza haematocheila* (Temminck & Schlegel, 1845); *Peridinium quinquecorne* T.H.Abé, 1927 which is synonymous with *Protoperidinium quinquecorne* (Abé, 1927) Balech, 1974 and for which the new accepted name is *Blixaea quinquecornis* (Abé) Gottschling, 2017; *Distephanus speculum* var. *octonarius* (Ehrenberg) Jørgensen, 1899 synonym of *Octactis octonaria* (Ehrenberg) Hovasse, 1946; *Oxyphysis oxytoxoides* Kofoid, 1926 synonym of *Phalacroma oxytoxoides* (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011; *Rhizosolenia calcar-avis* Schultze, 1858 synonym of *Pseudosolenia calcar-avis* (Schultze) B.G.Sundström, 1986; *Glenodinium gymnodinium* Penard, 1891 synonym of *Naiadinium polonicum* (Woloszynska) Carty, 2014; *Fogedia finmarchica* (Cleve & Grunow) A.Witkowski, D.Metzeltin & Lange-Bertalot, 1997 synonym of *Navicula finmarchica* (Cleve & Grunow) Cleve, 1895), (ii) erroneous reports (i.e., *Acrochaetium leptonema* (Rosenvinge) Børgesen, 1915, *Kylinia rosulata* Rosenvinge, 1909, *Asterionellopsis glacialis* (Castracane) Round, 1990, *Pteria hirundo* (Linnaeus, 1758), *Bacteriastrum hyalinum* Lauder, 1864, *Lepas* sp., *Neptunea arthritica* (Valenciennes, 1858), which never occurred in any of the Romanian or Bulgarian Black Sea regions), (iii) species misidentification (i.e., *O. brevicornis*, a misidentification of *O. davisae*; *Alexandrium affine* (H. Inoue & Y. Fukuyo) Balech, 1995, for which description does not correspond to that of individuals collected from its native range; *Ruditapes philippinarum* (A. Adams & Reeve, 1850), most likely a misidentification of *Polititapes aureus* (Gmelin, 1791), (iv) misclassification of native species as alien (i.e., *Blackfordia virginica* Mayer, 1910, *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, 1976, *V. dichotoma* f. *marina*, *Pseudosolenia calcar-avis* (Schultze) B.G. Sundström, 1986, *Ulva lactuca* Linnaeus, 1753, *Acrochaetium mahumetanum* G. Hamel, 1927, and *Prionace glauca* (Linnaeus, 1758) – all were previously reported as alien but currently are regarded as native species), and (v) inclusion of freshwater species in marine species lists (i.e., *Lepomis gibbosus* (Linnaeus, 1758), *Pseudorasbora parva* (Temminck & Schlegel, 1846), *U. krameri*, *Lithognathus mormyrus* (Linnaeus, 1758), *Naiadinium polonicum* (Woloszynska) Carty, 2014, *Urnatella gracilis* Leidy, 1851 and *Podonevadne trigona ovum* (Zernov, 1901)). Several species were excluded from the list because they involved extinct species (i.e., *V. spengleri*), natural range expansion species (i.e., *B. vitrea*, *Sphyrna zygaena* (Linnaeus, 1758) and *Chelon labrosus* (Risso, 1827)) and species not validated by local experts (i.e., *Erythrocladia irregularis* Rosenvinge, 1909, *Hildenbrandia crouaniorum* J. Agardh, 1851, and *Hillea fusiformis* (J. Schiller) J. Schiller, 1925, which proved to be AquaNIS miss-reports). Also, data deficient species could not be assigned to any of the previous categories due to lack of data, i.e., unsubstantiated records with no description or photos of species and taxa of unresolved taxonomic status.

The refined list of alien species recorded in Romanian Black Sea region proposed here comprised 37 species. This number is lower in comparison with the number of species (i.e., 49 species) listed in the last inventory of Skolka and Preda (2010). Since then, five new species – *Hemigrapsus sanguineus* (De Haan, 1835 [in De Haan, 1833–1850]), *Eurypanopeus depressus* (Smith, 1869), *O. davisae*, *Amphiglena mediterranea* (Leydig, 1851), *Alitta succinea* (Leuckart, 1847) and *Laonome xeprovala* Bick & Bastrop, in Bick et al., 2018 – have entered the list (Micu et al. 2010a, b; Zinevici et al. 2011; Timofte and Tabarcea 2012; Petrescu et al. 2016; Surugiu and Capa 2020; Teacă et al. 2021), whereas many have been excluded following the aforementioned criteria. We refer to species that changed their status from alien to cryptogenic (i.e., *Teredo navalis* Linnaeus, 1758, *Amphibalanus improvisus* (Darwin, 1854), *Einhornia crustulenta* (Pallas, 1766), *Thalassiothrix mediterranea* var. *pacifica* Cupp, 1943) or to crypto-expanding (i.e., *Pontella mediterranea* (Claus, 1863), *Mecynocera clausi* Thompson I. C., 1888, *Neocalanus gracilis* (Dana, 1852), *Euterpina acutifrons* (Dana, 1847), *Triconia dentipes* (Giesbrecht, 1891), *T. minuta* (Giesbrecht, 1893), *Oncaea mediterranea* (Claus, 1863), *Calocalanus tenuis* Farran, 1926, *C. pavoninus* Farran, 1936, *Agetus flaccus* (Giesbrecht, 1891), *A. typicus* Krøyer, 1849, *Corycaeus clausi* Dahl F., 1894, *Urocorycaeus furcifer* (Claus, 1863), *Microsetella rosea* (Dana, 1847), *Ctenocalanus vanus* Giesbrecht, 1888, C. *pavo* (Dana, 1852), *C. plumulosus* (Claus, 1863), *Clausocalanus arcuicornis* (Dana, 1849), *Paracalanus nanus* Sars G.O., 1925, *P. aculeatus* Giesbrecht, 1888, *Mesocalanus tenuicornis* (Dana, 1849), and *Neocalanus gracilis* (Dana, 1852)).

Surprisingly, there are no studies that provide an inventory of all marine alien species for the Bulgarian Black Sea region. The refined list of 26 alien species we came up with here, is the first comprehensive list (i.e., including all taxonomic groups) of marine alien species for this region. Separate taxonomic group inventories for Bulgarian Black Sea region have been published, i.e., inventories of invertebrate marine species (Hubenov 2014, 2015) and fish species (Yankova 2016). Hubenov (2014) reported a total of 31 alien invertebrate marine species and Yankova (2016) reported six alien fish species. Several species of invertebrates and fish which have been classified as alien by Hubenov (2014) and Yankova (2016), are currently regarded as cryptogenic (i.e., *A. improvisus*, *Hesionides arenaria* Friedrich, 1937, *Streblospio shrubsolii* (Buchanan, 1890), *Streptosyllis varians* Webster & Benedict, 1887, *T. navalis*, *Pomatoschistus marmoratus* (Risso, 1810) and *P. bathi* Miller, 1982) or crypto-expanding (i.e., *T. minuta*, *Sardinella aurita* Valenciennes, 1847, *Sphyraena sphyraena* (Linnaeus, 1758)). However, *P. marmoratus*, which was mentioned from the Black Sea as *P. microps leopardinus* (Nordmann, 1840) (Bănărescu 1964), is a subspecies of *P. microps* (Krøyer, 1838). Bănărescu (1964) reported the subspecies as native to the Black Sea and mentioned it as naturally occurring at the Romanian Black Sea coast.

We found that most of the alien species were either established or invasive in the Romanian Black Sea region. Since 2010 (Skolka and Preda 2010), there was a substantial change compared to the present days in the establishment success of alien species from casual to established (i.e., *Calyptospadix cerulea* Clarke, 1882, *Crassostrea virginica* (Gmelin, 1791), *Magallana gigas* (Thunberg, 1793), *Sphaeroma walkeri* Stebbing, 1905, *Palaemon macrodactylus* Rathbun, 1902, *Callinectes sapidus* Rathbun, 1896 and *Styela clava* Herdman, 1881) and from established to invasive (i.e., *M. leidyi*, *Beroe ovata* Bruguière, 1789, *Polydora cornuta* Bosc, 1802, *Corambe obscura* (A. E. Verrill, 1870), *Planiliza haematocheila* (Temminck & Schlegel, 1845), *Rhithropanopaeus harrisii* (Gould, 1841)). We also report four new invasive alien species in Black Sea region of Romania (i.e., *Dipolydora quadrilobata* (Jacobi, 1883), *Diadumene lineata* (Verrill, 1869), *Streblospio gynobranchiata* Rice & Levin, 1998, and *L. xeprovala*), and changes in the establishment success of two cryptogenic species, i.e., *Einhornia crustulenta* (Pallas, 1766) from casual (Skolka and Preda 2010) to established. The observed increase in the success of establishment and invasion of alien and of species that might be alien, is most likely the result of the increase in habitat degradation (Spear et al. 2013) and international trade (Westphal et al. 2008). Also, since the second half of the 20th century marine invasions are being amplified by the increase in use of non-biodegradable plastics. Billions of tons of plastics are deposed at the land-sea interface creating rafts that last for decades and permit more species to be transported as passengers far longer and further (Carlton et al. 2017). After World War II global shipping expanded dramatically and many thousands of species are transported around the world as stowaways in ballast water (Carlton and Geller 1993) and contaminants of transported goods to regions that are increasingly favorable to new invasions due to climate warming.

Concerning the Bulgarian Black Sea region, although Hubenov (2014, 2015) reported nine alien invasive species of invertebrates (i.e., *M. leidyi*, *B. ovata*, *Ficopomatus enigmaticus* (Fauvel, 1923), *P. cornuta*, *D. quadrilobata*, *A. improvisus*, *Rapana venosa* (Valenciennes, 1846), *Anadara kagoshimensis* (Tokunaga, 1906), and *Mya arenaria* Linnaeus, 1758), and Yankova (2016) five alien invasive species of fish (i.e., *Sparus aurata* Linnaeus, 1758, *S. aurita*, *P. marmoratus*, *Sarpa salpa* (Linnaeus, 1758), and *P. bathi*), in our study local experts classified eight invertebrate species (i.e., *M. leidyi*, *B. ovata*, *F. enigmaticus*, *P. cornuta*, *D. quadrilobata*, *R. venosa*, *A. kagoshimensis* and *M. arenaria*) as alien invasive species, while all alien fish species were classified either as cryptogenic (i.e., *P. marmoratus* and *P. bathi*) or crypto-expanding (*S. aurata*, *S. aurita*, and *S. salpa*), having established populations or an unknown establishment success. *Amphibalanus improvisus*, reported by Hubenov (2014, 2015) as an alien invasive species in the Black Sea region of Bulgaria, was reassigned by the local experts in this study as an established cryptogenic species.

We found that the vast majority of alien, cryptogenic and crypto-expanding species have their native distribution in the Atlantic Ocean and a significant proportion in the Pacific and Indo-Pacific regions both for Romanian and Bulgarian Black Sea marine regions. The result is in line with other studies which have addressed the native distribution of European alien species. The Atlantic, Pacific and Indo-Pacific regions represented important realms of alien species recorded in the main European Seas (Tsiamis et al. 2018). On the other hand, the connection of the Black Sea with the Mediterranean Sea, where many alien Indo-Pacific species have entered the Mediterranean Sea via Suez Canal (Zenetos and Galanidi 2020), is worth mentioning. Very few alien, cryptogenic and crypto-expanding species were native to the Arctic or Sea of Azov, most likely due to climatic differences.

Trends in the introduction of alien species clearly showed that there was a steady increase in the number of introduced alien species in marine regions of both Romania and Bulgaria over the last 50 years. Previous studies evaluating the dynamics of introduction of alien species to Europe indicated that the number of newly reported alien species over the past century is generally increasing in the marine environment (Richardson and Pyšek 2008). This increase partly reflects new introductions and is partly due to the progress in conducting inventories of alien species for many taxonomic groups. There was also strong evidence that the number of crypto-expanding and cryptogenic species recorded in the recent decades in the marine regions of Romania and Bulgaria, respectively, sharply increased until 2011. In the time interval 2012–2017 the number of introduced species clearly decreased with only one report in the last four years, i.e., *L. xeprovala* from the Romanian marine region (Teacă et al. 2021). The most recent record of a non-native species in the Black Sea was the amphipod *Melita nitida* S.I. Smith in Verrill, 1873, found in 2019 along the Black Sea coast of Georgia (Copilaș-Ciocianu et al. 2020). This suggests that, although overall the amount of data increased, the knowledge on the distribution and the natural dispersal mechanisms is far from being complete, which leads to confusion regarding the status of many species. However, we cannot rule out that the reason of a decreasing trend in the introduction of marine species over the past few years might be the reduced sampling effort for investigating the communities of marine species in the Black Sea region. For example, *R. venosa*, ranked as one of the 100 worst alien species in Europe, and *C. sapidus*, which is among the 100 worst invasive species in the Mediterranean, are considered for inclusion on the Union list under EU Regulation on invasive alien species. Recent studies have shown that *C. sapidus* is spreading on the southern coast of the Black Sea (Ceylan 2020). Moreover, observations from citizen scientists of *C. sapidus* are verified by experts and is promoted as legitimate. Yet, there are few recent publications on *R. venosa* (Janssen et al. 2014) and *C. sapidus* (Uzunova 2016) for the Bulgarian Black Sea and no recent publications for Romania. Publications stemming from inventory and monitoring activities in hot spots of invasive alien species (i.e., ports, lagoons and aquaculture farms) identified per regional sea and MSFD area are not available in both countries. Moreover, the lack of studies documenting the world distribution and vectors for alien species is emphasized by the fact that the true status of many species (i.e., classified as crypto-expanding and cryptogenic) is still unclear. This adds to the necessity of taxonomic verification which is dependent on the availability of taxonomic expertise. Thus, our study highlights the need for further studies, including molecular studies, that are able to elucidate the true status of introduced species in the western Black Sea region, as well as the mechanisms of introduction (pathways) and the actual spread of alien species. Without appropriate, harmonized and integrated data on alien species, future efforts toward implementation of MSFD instruments may render ineffective. Nonetheless, the data collected so far allows us to identify key vectors presumably responsible for the introduction of alien species in the western Black Sea region.

Conclusions

We present an updated and validated list of alien species for the Romanian Black Sea region and a first comprehensive list of alien species for the Bulgarian Black Sea region. This information is essential for future risk assessment and implementation of biodiversity conservation and management policies in the western part of Black Sea. The data provided here are particularly useful for the development of priority lists of invasive alien species present in marine waters of Romania and Bulgaria. The list supplies key information to identify species to be included on the Bulgarian and

Romanian national concern lists or/and on the Union list. Also, the results of this study open new research directions including future focus on poorly studied alien species and species reported based on the information available in AquaNIS with no recent scientifically documented record. Moreover, our results reveal some disparity in the number of alien species, species status and establishment success between the Romanian and Bulgarian Black Sea regions, which might result from uneven monitoring of alien species in the two countries. This emphasises the need for study of the overall distribution of alien species in the Black Sea but also could be useful information to direct the management efforts of alien species where priority is needed and tailor them per Black Sea sector. In conclusion, this study provides supporting information for implementing measures to tackle the introduction of alien species in the western region of the Black Sea towards an early warning, prevention and control of invasive alien species and application of the MSFD directive in the Black Sea, a unique fragile ecosystem.

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Authors' contribution

RIB and AZ conceived the idea of the study. AZ, MS, PI, VS, KS, and VT contributed to data collecting. RIB and AZ wrote the manuscript with input from VS.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Species to be excluded.
Table S2. List of alien species.
Table S3. List of cryptogenic, crypto-expanding, and data deficient species.
Table S4. References for Tables S1–S3.

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