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Data Paper

Unpublished Mediterranean and Black Sea records of marine alien, cryptogenic, and neonative species

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Abstract

To enrich spatio-temporal information on the distribution of alien, cryptogenic, and neonative species in the Mediterranean and the Black Sea, a collective effort by 173 marine scientists was made to provide unpublished records and make them open access to the scientific community. Through this effort, we collected and harmonized a dataset of 12,649 records. It includes 247 taxa, of which 217 are Animalia, 25 Plantae and 5 Chromista, from 23 countries surrounding the Mediterranean and the Black Sea. Chordata was the most abundant taxonomic group, followed by Arthropoda, Mollusca, and Annelida. In terms of species records, *Siganus luridus*, *Siganus rivulatus*, *Saurida lessepsianus*, *Pterois miles*, *Upeneus moluccensis*, *Charybdis* (*Archias*) *longicollis*, and *Caulerpa cylindracea* were the most numerous. The temporal distribution of the records ranges from 1973 to 2022, with 44% of the records in 2020–2021. *Lethrinus borbonicus* is reported for the first time in the Mediterranean Sea, while *Pomatoschistus quagga*, *Caulerpa cylindracea*, *Grateloupia turuturu*, and *Misophria pallida* are first records for the Black Sea; *Kapraunia schneideri* is recorded for the second time in the Mediterranean and for the first time in Israel; *Prionospio depauperata* and *Pseudonereis anomala* are reported for the first time from the Sea of Marmara. Many first country records are also included, namely: *Amathia verticillata* (Montenegro), *Ampithoe valida* (Italy), *Antithamnion amphigeneum* (Greece), *Clavelina oblonga* (Tunisia and Slovenia), *Dendostrea* cf. *folium* (Syria), *Epinephelus fasciatus* (Tunisia), *Ganonema farinosum* (Montenegro), *Macrorhynchia philippina* (Tunisia), *Marenzelleria neglecta* (Romania), *Paratapes textilis* (Tunisia), and *Botrylloides diegensis* (Tunisia).

Key words: non-native species, non-indigenous, distribution, invasive alien species, geo-referenced records, Mediterranean Sea, Black Sea

Introduction

Biological invasions have been a major focus of marine scientists and managers in the Mediterranean and Black Seas because of their severe impacts on marine biodiversity and ecosystem services (Micheli et al. 2013; Katsanevakis et al. 2014a, b; Azzurro et al. 2019; Tsirintanis et al. 2022). Furthermore, an increasing number of neonative species (i.e., range-expanding species that track human-induced environmental change; *sensu* Essl et al. 2019) have invaded the Mediterranean Sea through the Gibraltar Strait or the Black Sea through the Dardanelles Strait and the Sea of Marmara (e.g., Azzurro et al. 2022). Facilitated by climate change, alien and neonative species have contributed to great shifts in native ecosystems (Katsanevakis et al. 2018; Albano et al. 2021; Steger et al. 2021), the gradual tropicalization of the Mediterranean Sea (Bianchi and Morri 2003; Por 2009; Bianchi et al. 2018; Peleg et al. 2020), and mediterranization of the Black Sea (Kideys et al. 2000; Boltachev and Karpova 2014; Eyuboglu 2022). The latest reviews report a 40% increase in the established alien species in the Mediterranean Sea since 2010 and a steady increase in the Black Sea over the last five decades (Băncilă et al. 2022; Zenetos et al. 2022). Thus, the need arises for an improved theoretical and practical understanding of range shifts and biological invasion dynamics through space and time, the mechanisms of related impacts, and the functional role of alien and neonative species as drivers of change in the Mediterranean and Black Seas. This will contribute to effective regional conservation planning (Mačić et al. 2018) and prioritizing and implementing effective mitigation actions (Giakoumi et al. 2019), aiming to protect biodiversity and safeguard marine ecosystem services.

Although appropriate spatio-temporal data and information on the ecological characteristics of species are a prerequisite for effective management, they are often deficient in the Mediterranean and Black Seas (Levin et al. 2014). Hence, updated geo-referenced alien species records are valuable for assessing new species invasion progress and temporal dynamics (Katsanevakis et al. 2020a). Alien species records are often published when considered "first records" within a geographical region but rarely when a species is observed within its already documented invasion range. Consequently, spatio-temporal data of great value to researchers and managers remain unpublished and scattered in various repositories or personal files. Such information becomes even more valuable considering that the Mediterranean is warming at exceptionally high rates in comparison to the global ocean (Schroeder et al. 2016; Cramer et al. 2018; Pisano et al. 2020), whereas native species are becoming locally extinct (Rilov 2016; Albano et al. 2021) and may suffer high mortalities due to elevated temperatures and marine heatwaves (Garrabou et al. 2022). Consequently, even more suitable conditions are created for species of warm-water affinity to invade and replace native fauna (Bianchi et al. 2019).

A recent collective effort to compile such spatio-temporal information was made by Katsanevakis et al. (2020a). In that effort, 126 marine scientists from 16 countries contributed 5376 records of 239 alien and cryptogenic taxa from the Mediterranean, including one Mediterranean first record and nine first country records. Following that effort and expanding the geographical scope to cover the Black Sea, an invitation to submit unpublished alien, cryptogenic, and neonative species records was sent to marine scientists from all Mediterranean and Black Sea countries. This effort aimed to compile a second large open-access collective dataset, complementing existing information on alien and cryptogenic species in the region.

Dataset compilation

Overall, 173 scientists from 23 countries submitted their data, compiling a dataset of 12,649 records (see Supplementary material Table S1). Each Excel sheet line represents a specific record in space and time with relevant information. The required fields for each record were species name, species status, latitude, longitude, country, year, the observer of the record, the type of observation, and how it had been documented. "Status" refers to biogeographic status and was based on Zenetos et al. (2022) for Mediterranean records, Băncilă et al. (2022) for Black Sea records, and species-specific literature for debatable cases. "Status" took four possible values: "Alien" (*sensu* Essl et al. 2018), "Neonative" (*sensu* Essl et al. 2019), "Cryptogenic" (i.e., of uncertain biogeographic status; Carlton 1996; Essl et al. 2018) and "Data deficient" (for taxa for which an assessment of biogeographic status is unfeasible because of the lack of data; Essl et al. 2018). The "Observer" column has the name of the marine scientist(s) who identified the species. Records that a citizen-scientist had shared through an online platform like "Is it Alien to you? Share it!!!" (Giovos et al. 2019), personal communication, or a questionnaire are listed within brackets in the same column or within the "Comments" column. Only records adequately verified by scientists have been included; citizen science records that were not verified by marine experts were excluded.

"Type of observation" refers to the identification process for each record. Observations can be visual with or without photographic evidence (e.g., during SCUBA surveys or photo/video taken by a citizen), collected specimens by scientists, or even, for some fish species with no identification difficulties, answers to questionnaires by fishers.

Moreover, additional optional fields were available to include further information, exact or approximate date, depth of observation/collection, habitat, number of individuals observed or per cent substratum cover, and additional comments.

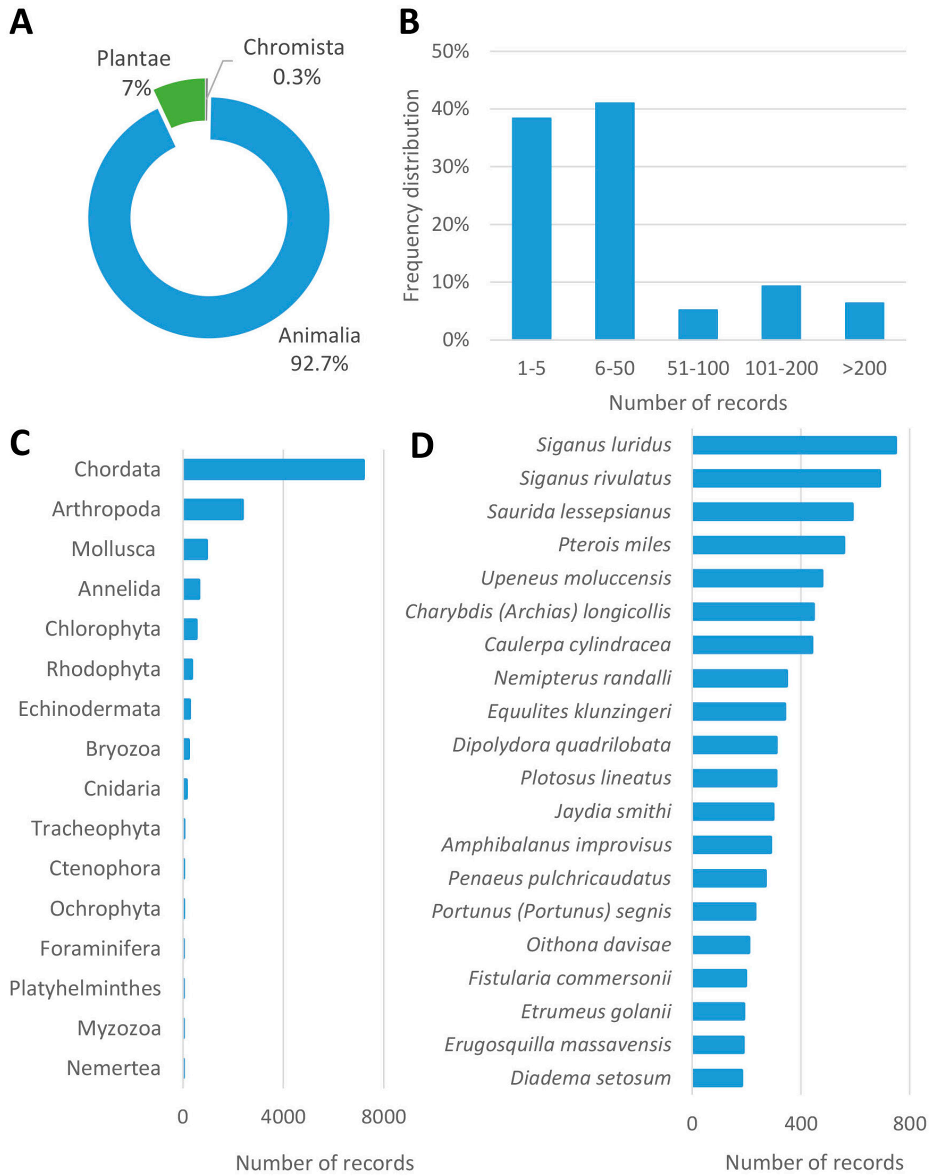

Figure 1. Taxonomic coverage of the dataset: (A) distribution pool by Kingdom; (B) frequency distribution of the number of records per species; (C) records by Phylum; (D) records by species (for the twenty most frequently observed species).

Taxonomic coverage

The compiled dataset included 247 taxa, of which 217 (88.38%) were Animalia, 25 (9.54%) Plantae, and 5 (2.08%) Chromista. Alien taxa were the majority (198); 31 species were classified as cryptogenic, 17 as neonative, and one as data deficient. Most records belonged to Animalia (92.7%), followed by Plantae (7%), and Chromista (0.3%) (Figure 1A). Most taxa (41%) were recorded between 6 and 50 times, while 37.3% less than six times (Figure 1B). In terms of Phyla, Chordata had the most records (7,180), followed by Arthropoda (2,359), Mollusca (925), and Annelida (621) (Figure 1C). Ninety per cent of the taxa were reported from 5 countries (Israel, Greece, Romania, Cyprus, and Italy). The five species with the highest number of records were all Osteichthyes, namely *Siganus luridus*

(Rüppell, 1829) (748), *Siganus rivulatus* Forsskål & Niebuhr, 1775 (689), *Saurida lessepsianus* Russell, Golani & Tikochinski, 2015 (589), *Pterois miles* (Bennett, 1828) (557), and *Upeneus moluccensis* (Bleeker, 1855) (477) (Figure 1D). Identification was based on collected specimens for 61% of the records in the dataset, only visually for 29.1% (either *in situ* or through photos/video), and through questionnaires to fishers for 9.8%.

Spatial and temporal coverage

The temporal coverage of observations extended from 1973 to June 2022. Records between 2014 and 2021 constituted 95.2% of the dataset, with most from 2020 (3,026), followed by 2021 (2,520) and 2014 (1,410) (Figure 2A). Regarding spatial coverage, records came from 23 countries, with 11,119 records from the Mediterranean and 1,530 from the Black Sea (the latter also includes the Sea of Marmara and the Istanbul Strait). The highest number of records were reported from Israel (5,304), Greece (2,900), Romania (1,365), Cyprus (744), and Italy (727) (Figure 2B). The species reported in most countries were *Callinectes sapidus* Rathbun, 1896 (11), *Caulerpa cylindracea* Sonder, 1845 (10), *Brachidontes pharaonis* (P. Fischer, 1870) (9), *Siganus luridus* (9), and *Pinctada radiata* (Leach, 1814) (9) (Figure 2C).

The records were unevenly distributed in the study area, as their spatial distribution is not only driven by the actual distribution patterns of the targeted taxa but is also influenced by the spatial variability of sampling effort and methodology and the uneven distribution of participating experts. A high concentration of records in the dataset is observed along the coast of Israel, in the South Aegean Sea and Cyprus, along the Romanian coast, but also, to a lesser extent, in Malta, Slovenia, and the Venice lagoon (Italy). Conversely, records were scarce along the Mediterranean coast of France, North Africa (except Tunisia), and the Black Sea coastlines of Turkey and Russia (Figure 3).

Remarkable new records of alien species

Among the records in the dataset, 20 are of particular importance. Most notably, the fish *Lethrinus borbonicus* Valenciennes, 1830 is reported for the first time in the Mediterranean (Tunisia), and the fish *Pomatoschistus quagga* (Heckel, 1837), the macroalgae *Caulerpa cylindracea* Sonder and *Grateloupia turuturu* Yamada, and the copepod *Misophria pallida* Boeck, 1865 are reported for the first time from the Black Sea (Crimea; Turkey; Romania). In addition, the red alga *Kapraunia schneideri* (Stuercke & Freshwater) Savoie & G.W. Saunders is reported for the first time in the Levant Sea (Israel; second time in the Mediterranean Sea). The polychaetes *Pseudonereis anomala* Gravier, 1899 and *Prionospio depauperata* Imajima, 1990 are reported for the first time in the Sea of Marmara (Turkey). Moreover, 12 first country records are included in the dataset: *Ampithoe valida* S.I.

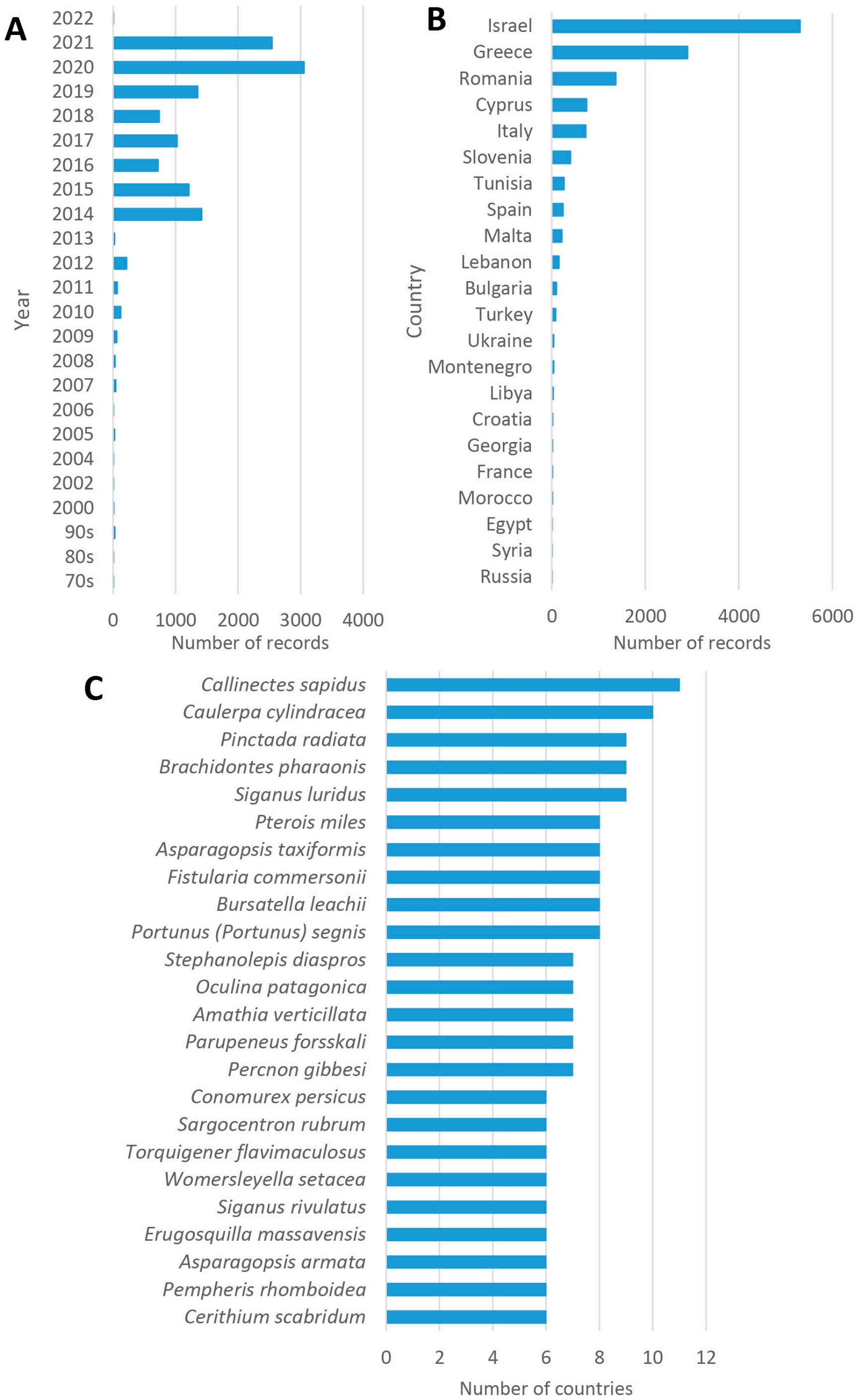

Figure 2. Spatio-temporal coverage of the dataset: (A) temporal distribution of records; (B) records per country; (C) country-coverage of species (for species recorded in more than 5 countries).

Smith, 1873 (Italy), *Amathia verticillata* (delle Chiaje, 1822) (Montenegro), *Antithamnion amphigeneum* A. Millar (Greece), *Clavelina oblonga* Herdman, 1880 (Slovenia and Tunisia), *Epinephelus fasciatus* (Forsskål, 1775) (Tunisia; third record in the Mediterranean Sea), *Dendostrea* cf. *folium* (Linnaeus, 1758) (Syria), *Ganonema farinosum* (J.V.Lamouroux) K.-C.Fan & Y.-C.Wang

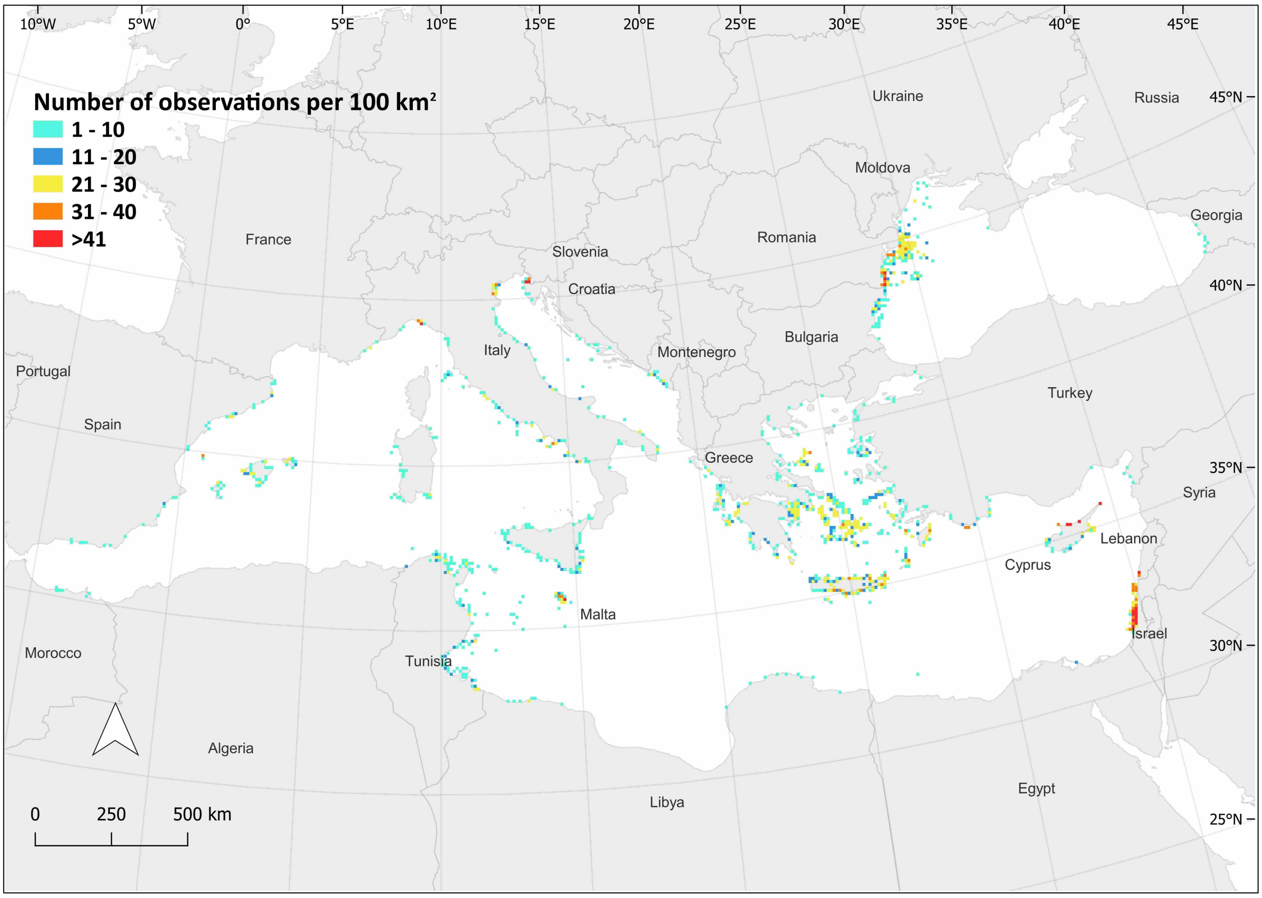

Figure 3. Number of alien, cryptogenic and neonative records per 10 km × 10 km grid cell, included in the dataset.

(Montenegro), *Marenzelleria neglecta* Sikorski & Bick, 2004 (Romania), *Macrorhynchia philippina* Kirchenpauer, 1872 (Tunisia), *Paratapes textilis* (Gmelin, 1791), and *Botrylloides diegensis* Ritter & Forsyth, 1917 (Tunisia).

The snubnose emperor *Lethrinus borbonicus* is the first representative of its genus in the Mediterranean Sea (Golani et al. 2021). It originates from the Western Indian Ocean, including the Red Sea and Arabian (Persian) Gulf to Reunion, and primarily occurs in sandy areas near reefs at depths of around 40 m (Carpenter and Allen 1989). The herein-reported specimen (Figure 4A) was captured on 20 March 2020 near the Marine Protected Area (MPA) of Zembra Island (eastern Tunisia) (37.11883°N; 10.7755°E) by a local fisher using gillnets at 75 m depth on a sandy bottom. It measured 203 mm in total length and weighed 122.6 g. The integrative taxonomic approach carried out on the sample confirmed the putative morphological identification. In particular, a 611 base pairs fragment of the *cytochrome c oxidase subunit I* (COX1) gene was amplified (GenBank accession number: OL441769), yelding a > 99% similarity with sequences of *L. borbonicus* from the Gulf of Suez (accession number: LC543919–LC543921), but also a 98–99% overlap with samples attributed to the congeneric species *L. lentjan* and *L. mahsena*. However, the inner surface of the pectoral fin base of the Zembra's specimen was covered with scales, thus excluding *L. lentjan*, and the specimen was characterized by 5½ longitudinal

Figure 4. Remarkable new records included in the dataset: (A) *Lethrinus borbonicus*, a first record in the Mediterranean Sea, observed in Tunisia, recorded by Jamila Ben Souissi; (B) first record of *Pomatoschistus quagga* in the Black Sea, recorded by Evgeniia Karpova and Elena Slynko.

scale rows between the lateral line and the base of middle dorsal spines, thus excluding *L. mahsena* (that has 4½) (Carpenter and Allen 1989). Shipping is a potential pathway of introduction of this species around the Zembra MPA.

The quagga goby *Pomatoschistus quagga* is a small, benthic goby, often associated with seagrasses in the coastal zone of the northern and western parts of the Mediterranean Sea, including the Adriatic and Aegean Seas (Kovačić 2003). Among individuals of the genus *Pomatoschistus* caught with a hand net in June 2018 in the bay of Sevastopol in the Black Sea (44.574698°N; 33.404630°E), and after fixing it in 96% ethanol, one quagga goby was identified through an integrative taxonomic approach (Figure 4B). In particular, a 524 base pairs fragment of the 16S ribosomal ribonucleic acid (16S rRNA) was amplified (GenBank accession number: MK457224), yelding > 97% similarity with three congeneric species, namely *P. bathi*, *P. minutus*, and *P. quagga*. However, its morphological features clearly pointed to *P. quagga*. In fact, it differred significantly from *P. minutus* in the number of scales of the lateral rows (32 *versus* more than 55) and it was characterized by the infraorbital row b that did not extend anteriorly below the infraorbital row a (*versus P. bathi* that is characterized by the infraorbital row b ending anteriorly under the infraorbital row a) (Kovačić 2008). This sighting constitutes the first record of *P. quagga* in the Black Sea. The preferred habitat of *Pomatoschistus* species, and specifically their epibenthic shallow-water lifestyle in open areas with soft substrates, implies that these fish rarely enter new water bodies. Like *Pomatoschistus bathi* Miller, 1982, the most probable vector for penetration of the quagga goby into the Black Sea is the natural introduction of planktonic larvae by the surface current, following the changing climate and the mediterranization of the Black Sea (Boltachev et al. 2016). Thus, the species should be considered as neonative in the basin.

Misophria pallida is a hyperbenthic neritic copepod species in the Order Misophroida (Family Misophriidae Brady, 1878), widely distributed in the NE Atlantic, Mediterranean, and Red Sea. Despite Misophrioids apparent wide occurrence, they are quite rare with Boxhall (1984) stating that "most copepod workers have probably never seen one". Misophroids present a peculiar combination of both podoplean body segmentation and gymnoplean-like characters, providing pieces of evidence for their ancestral evolution. Herein, we report the first record of *M. pallida* (Figure 5) in the Black Sea (44.75887°N; 30.11173°E) in six samples collected in June 2020 at depths of 50–55 m on mixed sediments (mud and shells). In total, 21 males, 12 females (five of which were ovigerous), and six juveniles were identified. This suggests that the species could develop large populations in Black Sea offshore deep habitats. We assume that the species entered the Black Sea only recently, possibly following an unusual climate-driven event that affected the subsurface circulation of the Mediterranean inflow into the Black Sea or through shipping. The recent first record of the hydrozoan *Podocorynoides minima* (Trinci, 1903) in the Black Sea, made in the summer of 2020, which could also have entered from the Mediterranean Sea (Muresan et al. 2021), supports our assumptions.

Two macroalgae reported from the Sea of Marmara, *Caulerpa cylindracea* and *Grateloupia turuturu*, are here first recorded from the Black Sea. Both species are included in the proposed inventory of alien marine species with reported moderate to high impacts on biodiversity and ecosystem services in the Mediterranean Sea (Tsirintanis et al. 2022). The green alga *C. cylindracea* ranks first among the ten worst invasive species in terms of reported negative impacts on biodiversity. It was first reported in the Mediterranean Sea from Tunisia in 1985 (Hamza et al. 1995). It is now widespread throughout the Mediterranean Sea, becoming one of the most invasive species of the basin (Verlaque et al. 2015; Katsanevakis et al. 2016; Zenetos et al. 2017; Morri et al. 2019), although its population dynamics are not yet fully understood (Piazzi et al. 2016). Çinar et al. (2021) have monitored the distribution of *C. cylindracea* along the Aegean coasts of Turkey since 1993 and noticed its considerable range expansion towards the northern Aegean Sea (Güreşen et al. 2015). The species was detected on 28 February 2020 in the Dardanelles Strait, Turkey (40.0464°N; 26.3463°E) on rocky substratum (Figure 6A). The rhodophyte *Grateloupia turuturu* was first detected in the Mediterranean Sea in southern France in 1982 (Riouall et al. 1985) and has spread across the Mediterranean basin (Verlaque et al. 2015). It reached

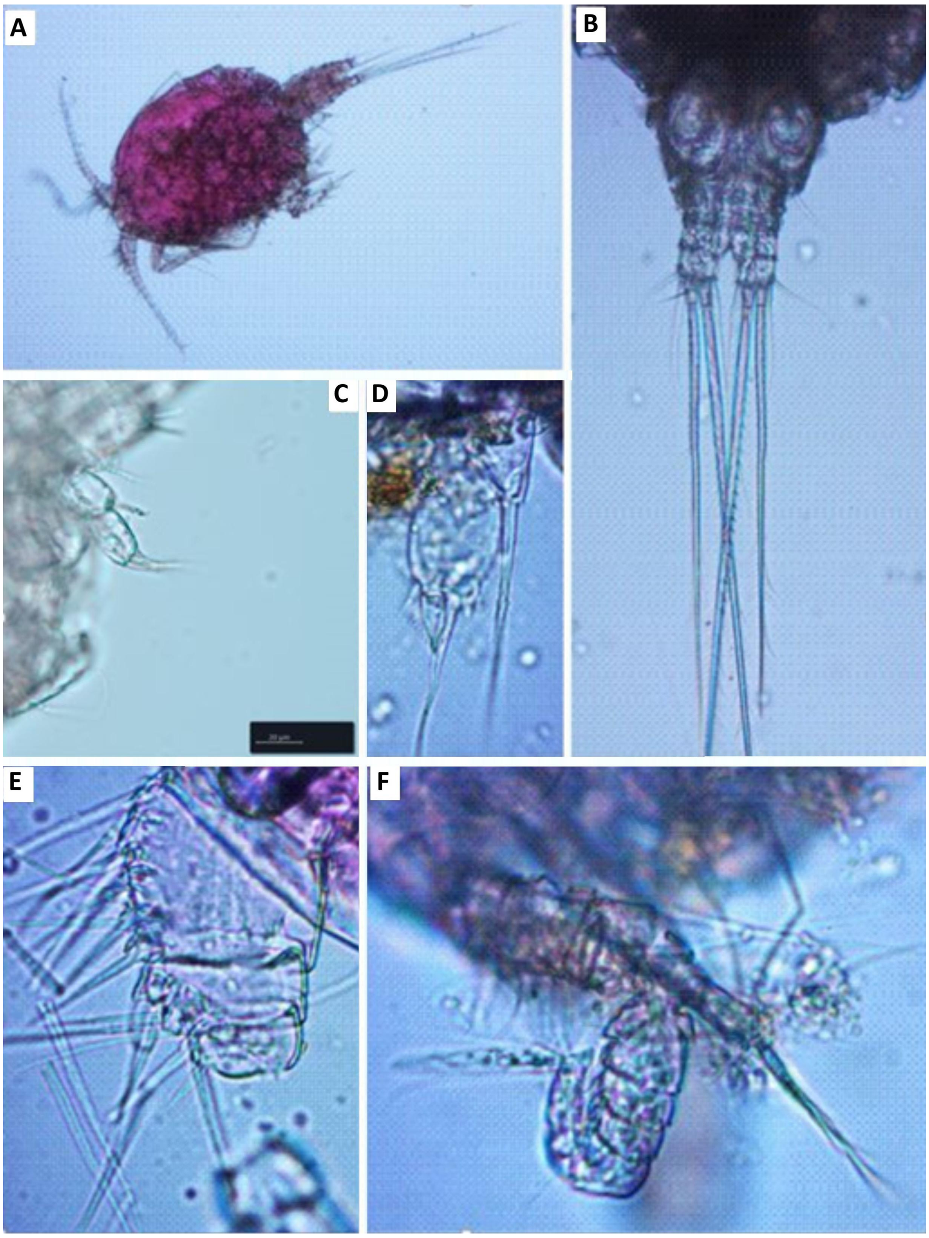

Figure 5. First record of *Misophria pallida* in the Black Sea, recorded by Muresan Mihaela: (A) *M. pallida* male; (B) male abdomen and furca; (C) male P5 and P6; (D) detail P5 (protopodal segment with outer basal seta and 2 setae on inner distal margin); (E) geniculate part of A1 male; (F) details of 13 segmented A1 male.

the Turkish coasts of the Aegean Sea in 2015 (Çinar et al. 2021). This red alga was often misidentified as *Grateloupia doryphora* (Montagne) M.Howe (Verlaque et al. 2015). *Grateloupia turuturu* was detected on 26 June 2021 in the Sea of Marmara (40.4008°N; 27.9145°E) on rocky substrata where it was abundant at 0–1 m depth (Figure 6B).

Kapraunia schneideri (previously *Polysiphonia schneideri*) (see Díaz-Tapia et al. 2013), is a marine red alga that has a mainly central-western Atlantic and Caribbean distribution (Guiry and Guiry 2019). Since its erection, this species was collected from the northwestern Atlantic in Connecticut and the central-eastern Atlantic in southwestern Spain (Stuercke and Freshwater 2010; Díaz-Tapia et al. 2013). The first reported introduction of this Atlantic

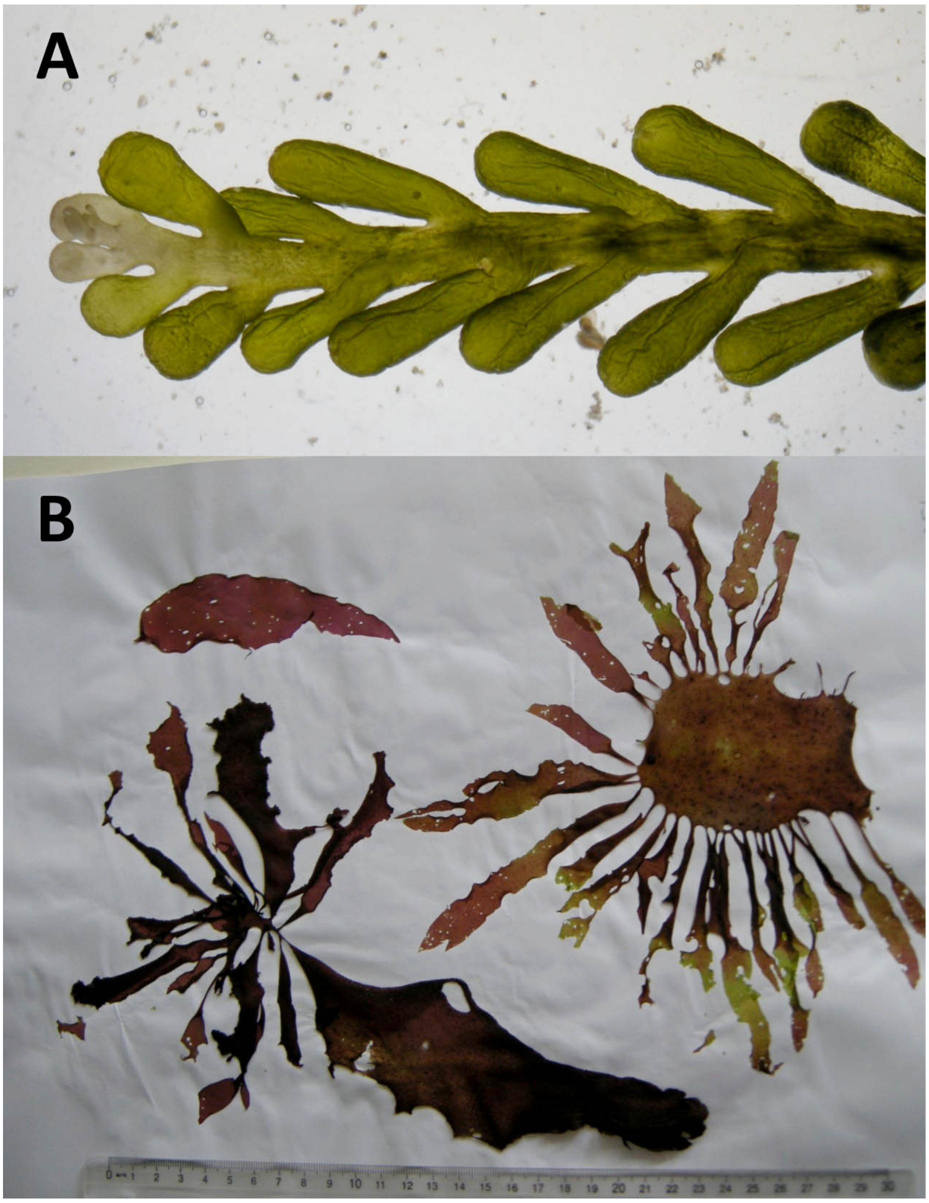

Figure 6. Remarkable new records included in the dataset: (A) *Caulerpa cylindracea*, a first record for the Black Sea reported by Ergün Taşkın; (B) *Grateloupia turuturu*, also a first record for the Black Sea reported by Ergün Taşkın.

native species from the Mediterranean was from the north Adriatic Sea, specifically the Venice Lagoon, in 2016 (Wolf et al. 2018). However, in February 2014, specimens were collected from wave breaker rocks inside the marina of the city of Ashkelon, located on the southern Levantine Mediterranean shore of Israel (31.68080°N; 34.55400°E). These sterile specimens were initially identified as *Polysiphonia denudata* (Dillwyn) Greville ex Harvey based on cross-sections of the thallus showing six pericentral cells surrounding each central siphon. Further study of the reproductive and vegetative morphology of some newly collected specimens from the same site indicated that the tetrasporangia of this species are not spirally arranged and that the basal portion of the axes is ecorticated. Other vegetative features that agree with the species description of *K. schneideri*

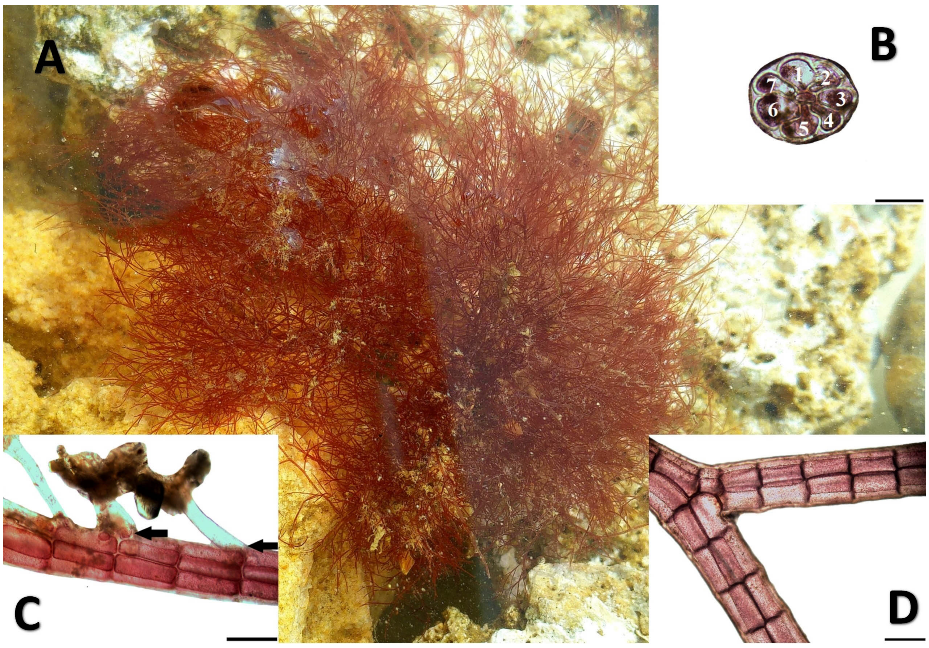

Figure 7. *Kapraunia schneideri*: (A) *in situ*; (B) Seven pericentral cells per segment of thallus; (C) Indicating rhizoids cut off from pericentral cells (arrows); (D) Segmented thallus; Sterile plant, scale bars 100 µm; a first record in the Levantine Sea, Israel and second in the Mediterranean Sea, recorded by Razy Hoffman.

(Stuercke and Freshwater 2010; Díaz-Tapia et al. 2013) are: plants are up to 8 cm long, attached to rocks by rhizoids (Figure 7A) that grow from short, decumbent basal parts; rhizoids are cut off from pericentral cells (Figure 7C), and segments (Figure 7D) have six or seven (Figure 7B) pericentral cells. Molecular identification of specimens collected from the marina of Ashkelon in February 2020 (GenBank accession number: OP797406) confirmed the morphological identification. The fact that *K. schneideri* was found in harbours and marinas in the Venice Lagoon, in Barbate (Spain), and Ashkelon (Israel) (present work) points to vessels and recreational boats as the vectors of introduction of this species in the Mediterranean Sea.

Pseudonereis anomala is a nereidid species of Indo-Pacific origin, also found in the Red Sea. In the Mediterranean, it was first reported from the coast of Alexandria, Egypt, by Fauvel (1937) and has since expanded widely to become the most successful alien nereidid species in the Mediterranean Sea (Kurt et al. 2021). Ten *P. anomala* individuals were extracted from an assemblage dominated by *Mytilus galloprovincialis* Lamarck, 1819 and sampled just below the water surface on 21 December 2018, near the South entrance of the Istanbul Strait (41.02870°N; 28.98830°E). The morphological features of the specimens examined agreed with the original and subsequent descriptions of *P. anomala* (Figure 8A). This species is mainly characterized by homogomph falcigers in the notopodia, dorsal cirrus subterminally, a dorsal ligule foliose (twice longer than wide) and markedly longer than median ligule in posterior chaetigers, areas VI with cones only, and few paragnaths on areas VII–VIII (up to 20 paragnaths

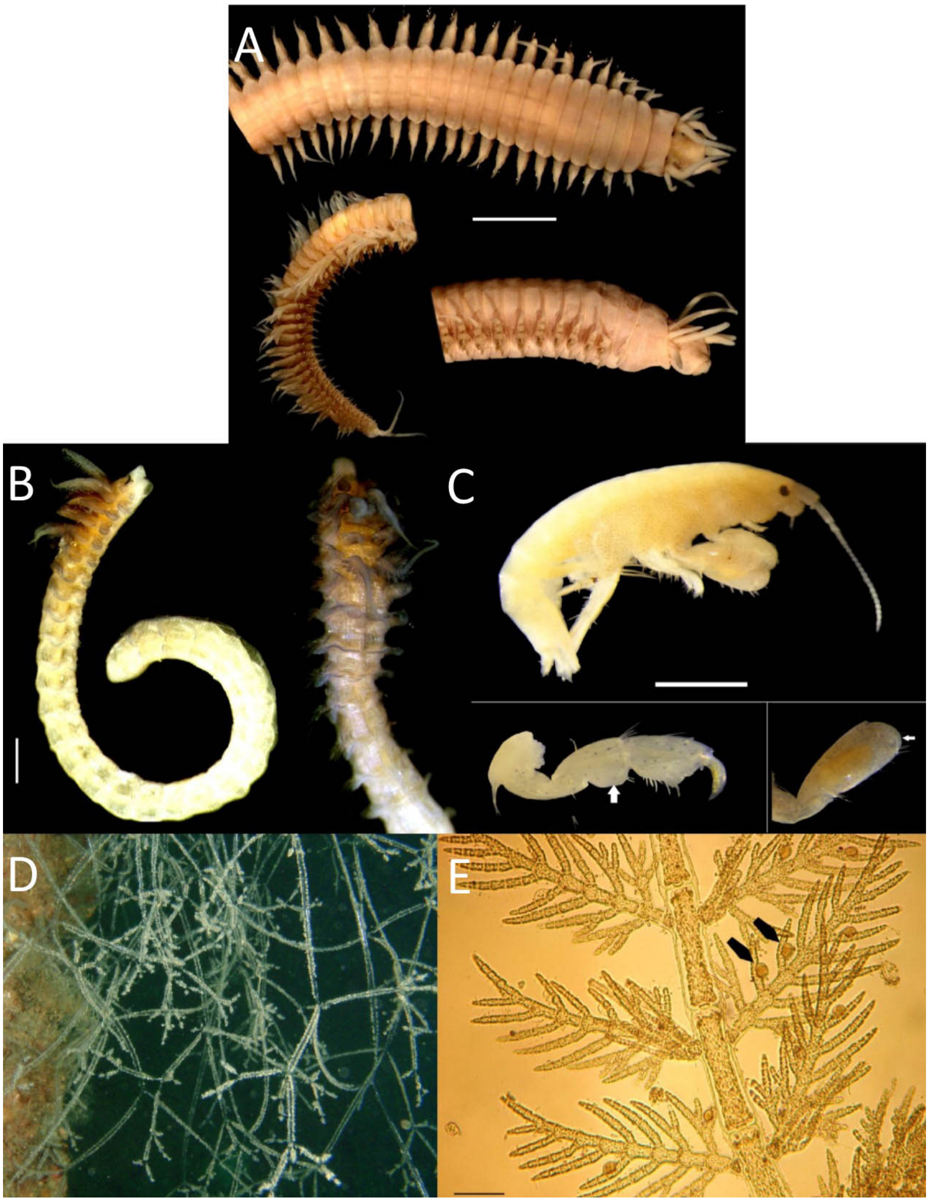

Figure 8. Remarkable new records included in the dataset: (A) *Pseudonereis anomala* and (B) *Prionospio depauperata*, first records in the Sea of Marmara by Ertan Dağlı; (C) *Ampithoe valida* from the Venice Lagoon, top: male specimen (scale bar: 1 cm), low left: gnathopod 1 (the arrow indicates carpal lobe), low right: gnathopod 2 (the arrow indicates the process stemming from the central part of the propodus palm), first record from Italy by Agnese Marchini and Renato Sconfietti; (D) *Amathia verticillata*, a first record for Montenegro by Slavica Petović; (E) *Antithamnion amphigeneum*, a first record from Greece by Konstantinos Tsiamis, whorl-branches (pinnae) with gland cells born adaxially (black arrows). Scale bar = 40 µm.

in a single band). *Pseudonereis anomala* is a new species to the marine fauna of Marmara Sea and the Straits System (Bosphorus).

Prionospio depauperata was described from the coast of Japan by Imajima (1990). It was reported for the first time in the Mediterranean Sea in Izmir Bay (Dagli and Çinar 2009) and later found along the Levantine coast of Turkey (Çınar et al. 2014). On 13 December 2019, eight further specimens were collected near the south entrance of the Istanbul Strait (41.02130°N; 28.97630°E) at 15 meters depth, fixed and identified morphologically. The morphological features of the specimens examined agree with the original and subsequent descriptions of *P. depauperata* (Figure 8B). This species is mainly characterized by pinnate branchiae on chaetigers 2 and 5 and apinnate branchiae on chaetigers 3 and 4, large posterior eyes, dorsal crests extending from chaetigers 7 to 16, and no dorsolateral skin folds. It is a new species to the marine fauna of the Marmara Sea and the Bosporus Straits System.

Ampithoe valida Smith, 1873 (Figure 8C) is known from the Atlantic coast of North America (Pilgrim and Darling 2010). Faasse (2015) has reported this species in the western Mediterranean, from specimens collected in 2000 in Balaruc-les-Bains (Bassin de Thau), France. Here we report its presence in the northern Venice Lagoon (45.50550°N; 12.39050°E), accounting for the first record from Italy, based on two male individuals collected in June 2017 from the subtidal fouling community of a wooden pole. The European distribution of *A. valida* reflects the routes of introduction of Pacific oysters. The Ria de Aveiro (Cunha et al. 1999), Arcachon Bay (Gouillieux 2017), Berre Lagoon (Faasse 2015), as well as the Venice Lagoon (present work), are all brackish sites where Japanese oysters have been introduced for aquaculture, and which share several alien species of NW-Pacific origin, probably introduced along with imported shellfish stocks. The individuals collected present the diagnostic characters well described in Conlan and Bousfield (1982) and Gouilleux (2017), and especially the large posterior carpal lobe in gnathopod 1 and the transverse palm of male gnathopod 2, bearing a central process (Figure 8C). Previous records of the morphologically similar congener *Ampithoe ferox* (Chevreux, 1901) in Mediterranean aquaculture sites (e.g., Marchini et al. 2007) may be due to misidentifications of introduced populations of *A. valida*, and the first introduction event of this American amphipod could be backdated by several years.

Amathia verticillata is a bryozoan probably native to the Caribbean ecoregion (Galil and Gevili 2014) that uses its ability to produce vegetative fragments and shipping as its main pathway of introduction (as fouling) (Nascimento et al. 2021). First described from Italian waters (delle Chiaje, 1822) and long considered native of the Mediterranean Sea, *A. verticillata* is known from several localities in the basin (e.g., Galil and Gevili 2014). While surveying the Boka Kotorska Bay, Montenegro (42.431767°N; 18.691783°E) on the 1st of August 2016, extensive colonies of this cryptogenic bryozoan were observed and collected for identification (Figure 8D). This is the first record of *A. verticillata* in Montenegro.

Antithamnion amphigeneum is a minuscule filamentous red alga which is an alien in the Mediterranean Sea, originating from the Indo-Pacific region. The species was first reported for the Mediterranean Sea from Algeria in 1989, possibly introduced through shipping (Verlaque et al. 2015). Since then, it has also been recorded from Spain, Morocco, Monaco, France, Italy, and most recently from Montenegro (Mačić and Ballesteros 2016, and references therein). The species was found as an epiphyte on the alien alga *Dictyota cyanoloma* Tronholm, De Clerck, A.Gómez-Garreta & Rull Lluch (Figure 8E), located in the upper sublittoral zone at a semi-exposed shore in March 2013, near the port of Argostoli (Kephallonia Island, Ionian Sea). This finding represents the first record of the species in Greece and the Ionian Sea and its easternmost distribution record in the Mediterranean Sea.

Clavelina oblonga is an ascidian originally described from Bermuda, whose native range is considered the tropical western Atlantic Ocean (Rocha et al. 2012). The species has a long invasion history, at least since 1929 in the Mediterranean, which has led to confusion regarding its biogeographical status (Carlton 2009). Here we report for the first time on the occurrence of *C. oblonga* in Slovenia (45.48778°N; 13.58532°E) and Tunisia (34.30650°N; 10.15590°E) (Figure 9A, B). The observations occurred on 3 July 2018 and 31 July 2019, respectively. In Slovenia, most specimens were found within a mussel farm site, while in Tunisia many colonies were observed on artificial structures within Skhira port. The observers reported colonial ascidians united by stolons, forming closed clusters; zooids had thorax and abdomen regions up to 2–3 cm long. The tunic was transparent without the white bands—like *Clavelina lepadiformis* (Müller, 1776)—around oral and atrial siphons, dorsal and ventral parts, and base of thorax, and only showed fine white dots. Branchial sac had 15–18 rows of stigmata. *Clavelina oblonga* is associated with bivalve mariculture, which is believed to be the original pathway of introduction into the Mediterranean Sea, later expanding its distribution naturally (Ordóñez et al. 2016).

The blacktip grouper *Epinephelus fasciatus*, one of the most common and widespread species of the genus in the tropical Indo-West Pacific (Heemstra and Randall 1993), was first recorded in the Mediterranean Sea in Syria in 2002 (Foulquie and Dupuy de la Grandrive 2003) and then once more in 2011 off the coast of Lebanon (Bariche and Heemstra 2012). Here we report the third occurrence of *E. fasciatus* in the Mediterranean Sea (Figure 9C), which constitutes the first record of this species from Tunisia. On 31 May 2020, a single individual of the blacktip grouper was caught along the Kelibia coast, northern Tunisia (36.8289°N; 11.1357°E). It was entangled in gillnets at a depth of 35 m over a sandy bottom covered by seagrasses. The specimen measured 201 mm in total length and weighed 102 g. The fresh colouration of the body was pale yellowish-red with orange-red bars, the margin of inter-spinous dorsal fin membranes black, and the dark reddish-brown dorsal part of the head and nape are characteristic features of the species. The present record in the vicinity of a port suggests that the introduction of the species in Tunisia was via maritime shipping.

Dendostrea cf. *folium* is a Lessepsian species belonging to the Ostreidae family, found on hard substrata in the infralittoral zone (Zenetos et al. 2011). This species was reported for the first time in the Mediterranean from Greece in 2010 as *Dendrostrea frons*, and is expanding its range

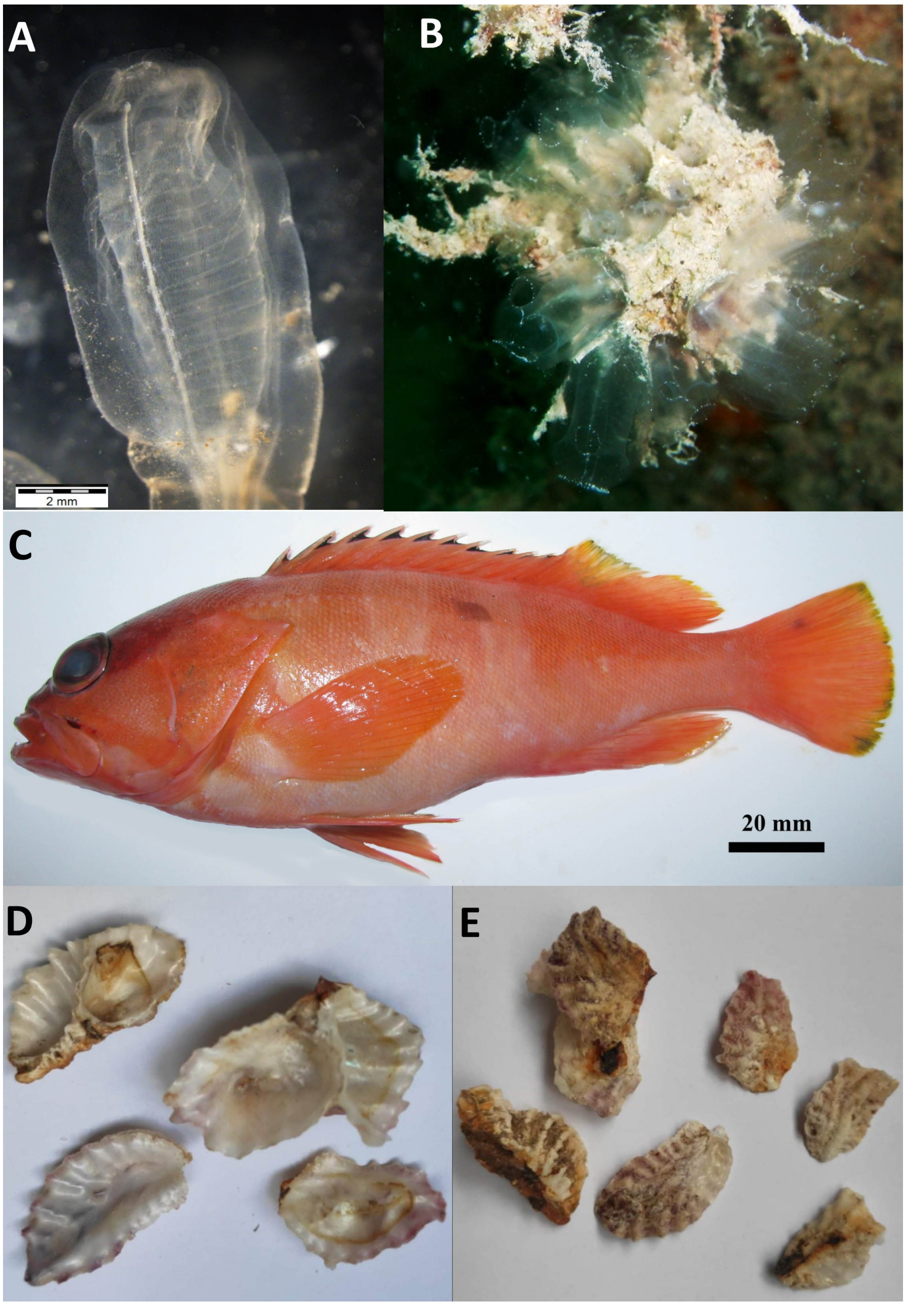

Figure 9. Remarkable new records included in the dataset: (A) *Clavelina oblonga*, a first record from Slovenia reported by Domen Trkov and Ana Fortič; (B) *Clavelina oblonga*, a first record from Tunisia reported by Alfonso Ramos; (C) *Epinephelus fasciatus*, a first record from Tunisia reported by Raouia Ghanem; (D and E) upper and lower valvae of *Dendostrea* cf. *folium* from Syria, a first record reported by Izdihar Ammar and Alaa Alo.

(Zenetos et al. 2011; Crocetta et al. 2013; Karachle et al. 2016; Ivkić et al. 2019). Based on molecular analysis and although it is a species that exhibits high morphological variability, it seems to be the only representative of its genus in the Mediterranean (Crocetta et al. 2015). Here we report the first record from Syria. Many samples were collected at 0.5 m depth, North of the Latakia port (35.567553°N; 35.739105°E) in April 2019 and near Al-bassit marina (35.865836°N; 35.866614°E) in August 2021, where they were attached to rock and fishing gear (Figure 9D, E). All samples are currently in the High Institute of Marine Research collection (Latakia).

The red alga *Ganonema farinosum* has a heteromorphic life history with alternation of erect gametophyte and filamentous tetrasporophytes. The gametophyte is erect and bushy, light purple to reddish brown, axes cylindrical, subdichotomously branched, moderately calcified, and up to 25 cm high (Lin et al. 2014; Verlaque et al. 2015). The species was described from the Red Sea (Suez) by Lamouroux (1816, as *Liagora farinosa*), and it is widespread from the Indo-Pacific through western Africa and the eastern Atlantic Ocean. It was recorded for the first time in the Mediterranean Sea from Alexandria (Egypt) in 1808 (Hamel, 1931, as *Liagora farinosa*) and later mainly in the eastern Mediterranean: Greece (1931), Israel (1964), Syria (1976), Lebanon (2000), Cyprus (2000), Turkey (1978), but also in south Italy (1969), south Spain (1987), Albania (2011), and Malta (Verlaque et al. 2015; Crocetta et al. 2021). Verlaque et al. (2015) suggested the introduction or the co-occurrence of introduced and native populations in the Mediterranean, given that the oldest Mediterranean record dates before the opening of the Suez Canal. Contrary to this, Cormaci et al. (2004) considered this species a Tethyan relict. Hence, the species is treated as cryptogenic due to its uncertain biogeographic status. *Ganonema farinosum* has not been previously reported from the Adriatic Sea except for the Strait of Otranto (Katsanevakis et al. 2011). We may presume that this species could be transported in the form of floating spores by water currents and ballast waters or as fouling of ship hulls, but the northward expansion of this thermophilic species could also be a consequence of climate change. In some tropical waters, *G. farinosum* is utilized for human consumption (Trono 2001), but in the Mediterranean, although well established, it has no importance to humans (Verlaque et al. 2015). Here we report on the first record of *G. farinosum* (Figure 10A) from Montenegro (42.09259°N; 19.07768°E), found in October 2018 in the port of Bar area, on an artificial hard substratum, at 7 m depth. This new record is the northernmost record of the species in the Adriatic.

The red gilled mud worm *Marenzelleria neglecta* is an oligohaline polychaete whose invasion history in the Baltic Sea received much attention at the turn of the last millennium (Leppäkoski and Olenin 2000; Zettler et al. 2002). First recorded (as *Marenzelleria viridis*) in the North Sea in 1983 (Essink and Kleef 1988), the species appeared in the Baltic Sea in 1985 (Bick and Burckhardt 1989). After only a dozen years, the red gilled mud worm expanded its distribution into almost the whole Baltic Sea. Though the native region of *M. neglecta* is not known with certainty, genetic data suggest that it most likely originates from the Atlantic coast of North America (Bastrop et al. 1998). In the Ponto-Caspian region, the species was reported for the first time in 2014 in the Don River delta and Taganrog Bay of the Sea of Azov and spread rapidly (Syomin et al. 2017; Mikhailova et al. 2021). The first specimens from Romanian waters that we report here (Figure 10B) were collected on 27 May 2021 from black detritic mud at 0.5 m

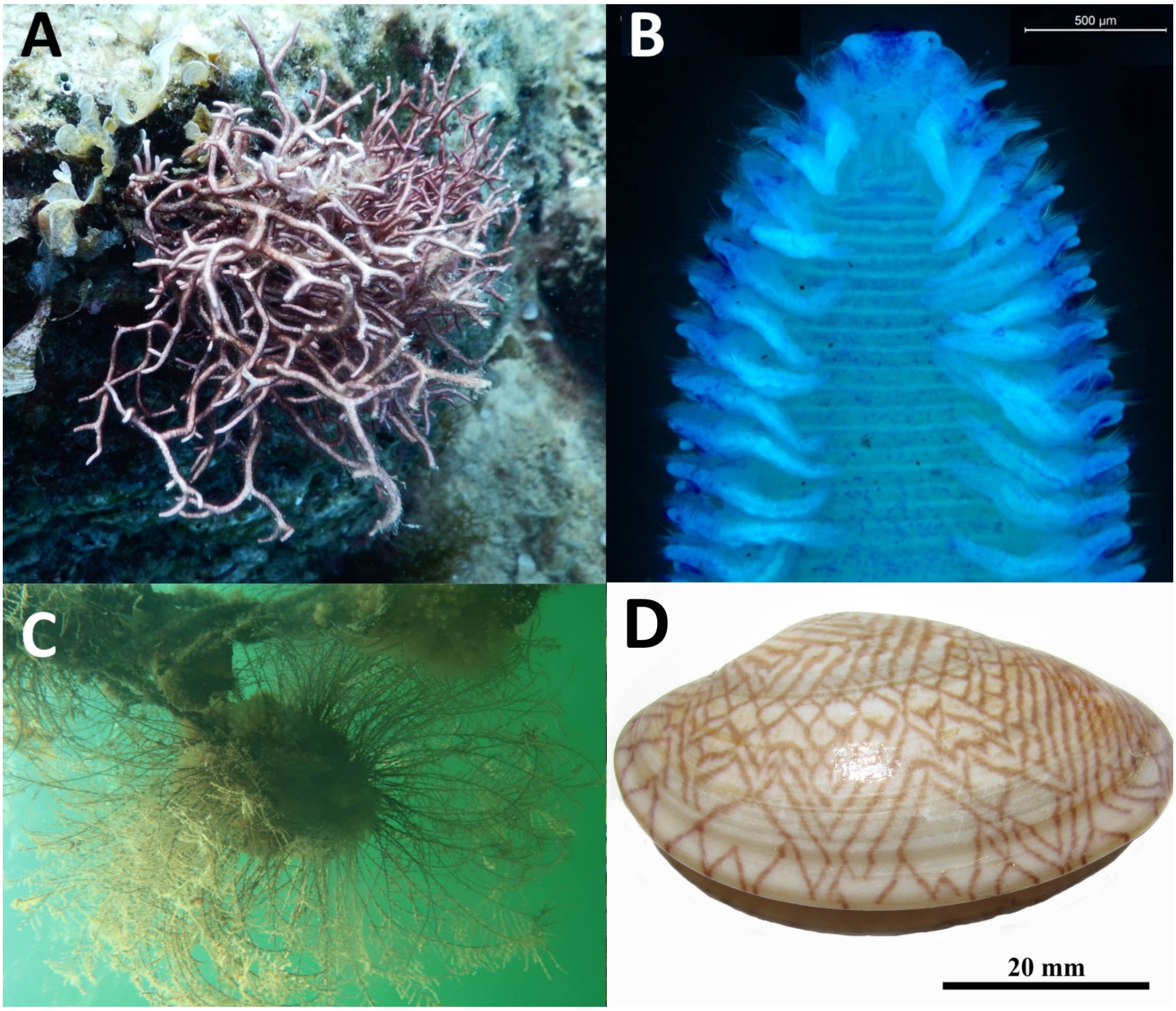

Figure 10. Remarkable new records included in the dataset: (A) *Ganonema farinosum*, a first record for Montenegro reported by Vesna Mačić; (B) *Marenzelleria neglecta*, a first record from Romania reported by Victor Surugiu; (C) *Macrorhynchia philippina*, a first record for Tunisia reported by Raouia Ghanem; (D) *Paratapes textilis*, a first country record from Tunisia reported by Wafa Rjiba.

depth in the upper reaches of the Mangalia Gulf (43.81180°N; 28.51840°E), at a salinity of 5.9 PSU. In the Sea of Azov, *M. neglecta* is already well established and locally has become a major component of the benthic fauna, reaching high densities (6823 ind. m^{-2}) and large biomass (31.2 g m^{-2}) (Syomin et al. 2017). The most probable vector of its introduction into the Sea of Azov and the Caspian Sea is by ship ballast water through the Volga-Baltic and Volga-Don canals (Syomin et al. 2017; Mikhailova et al. 2021). The secondary spread along the Black Sea shores is probably due to larval dispersal by the quasi-permanent anti-clockwise Main Rim Current. Therefore, the species is expected to occur soon on the Bulgarian Black Sea coast. On 26 May 2022, and while this work was ongoing, Teaca et al. (2022) published their study on "The First record of *M. neglecta* and the Spread of *Laonome xeprovala* in the Danube Delta–Black Sea Ecosystem". The samples containing *M. neglecta* that we report on were collected less than two weeks prior to the records described in their work, and though far in terms of distance, our results completely agree. Teaca et al. (2022) found *M. neglecta* in the northern part of the Romanian Black Sea coast, where it accounted for 36% of the total density of macrobenthos (50 to 1400 ind m^{-2}). The fact that the species was found in high densities in the northern part of the Romanian coast (Teaca et al. 2021, 2022), while only one specimen was found in the south, indicates that the northern part offers much more suitable habitat conditions for *M. neglecta*.

The feathery stinging hydroid *Macrorhynchia philippina* is distributed in tropical and subtropical regions (Rees and Vervoort 1987) and is common in the Red Sea (Vervoort 1993). The species has been observed in the Mediterranean along the coast of Lebanon at 0–40 m depth (Bitar and Bitar-Kouli 1995; Zibrowius and Bitar 2003; Morri et al. 2009) and has since expanded northwards to the Turkish coast (Çinar et al. 2006). About twenty colonies were observed and photographed, for the first time, on southern Tunisian coasts at Bibane lagoon (33.260283°N; 11.236798°E) on a wreck at 2 m depth (Figure 10C). This species has probably been introduced via shipping.

Paratapes textilis was first recorded as *Tapes undulatus* in the Mediterranean in 1939 from Egypt (Moazzo 1939) and then from Palestine Authority in 1935 (Haas 1948). Subsequently, it was reported from Israel (1948), southern Turkey (Niederhöfer et al. 1991), Syria (Kucheruk and Basin 1999), and Cyprus (Zenetos et al. 2009). The first specimen from Tunisia (Figure 10D) was caught by trawling off Kelibia coasts in Tunisia (36.82341°N; 11.13739°E) at 50 m depth. The specimen was characterized by the absence of lateral teeth in the hinge, colour externally beige and pale yellow with a characteristic zig-zag pattern in brown, and internally white.

The colonial ascidian *Botrylloides diegensis* is native to the North Pacific but has already colonized several European areas, namely the English Channel, where it was well established in marinas of the United Kingdom, the Atlantic coast of France, and the Italian Adriatic (Viard et al. 2019). In October 2019, colonial ascidians of the genus *Botrylloides* Milne Edwards, 1841 were observed for the first time in the shallow waters of Bahiret el Bibane lagoon (33.22909°N; 11.172793°E) on a rocky substratum (Figure 11A). Subsequently, during surveys carried out at the same location in May 2020, similar colonies were observed on *Pinna nobilis* Linnaeus, 1758 shells and collected for identification. As morphological identification of botryllid taxa could be deceiving, and several alien species are now spreading in the Mediterranean basin (Rocha et al. 2019; Viard et al. 2019; Della Sala et al. 2022; Virgili et al. 2022), the specimen was identified through DNA barcoding. A 631 base pairs fragment of the *cytochrome c oxidase subunit I* (COX1) gene was amplified (GenBank accession number: OP802711), yielding a > 98% similarity with sequences of both *B. diegensis* and *B. leachii*. However, the latter species was excluded based on the recent work of Viard et al. (2019), thus the reported ascidians are the first records of *B. diegensis* found in Tunisia.

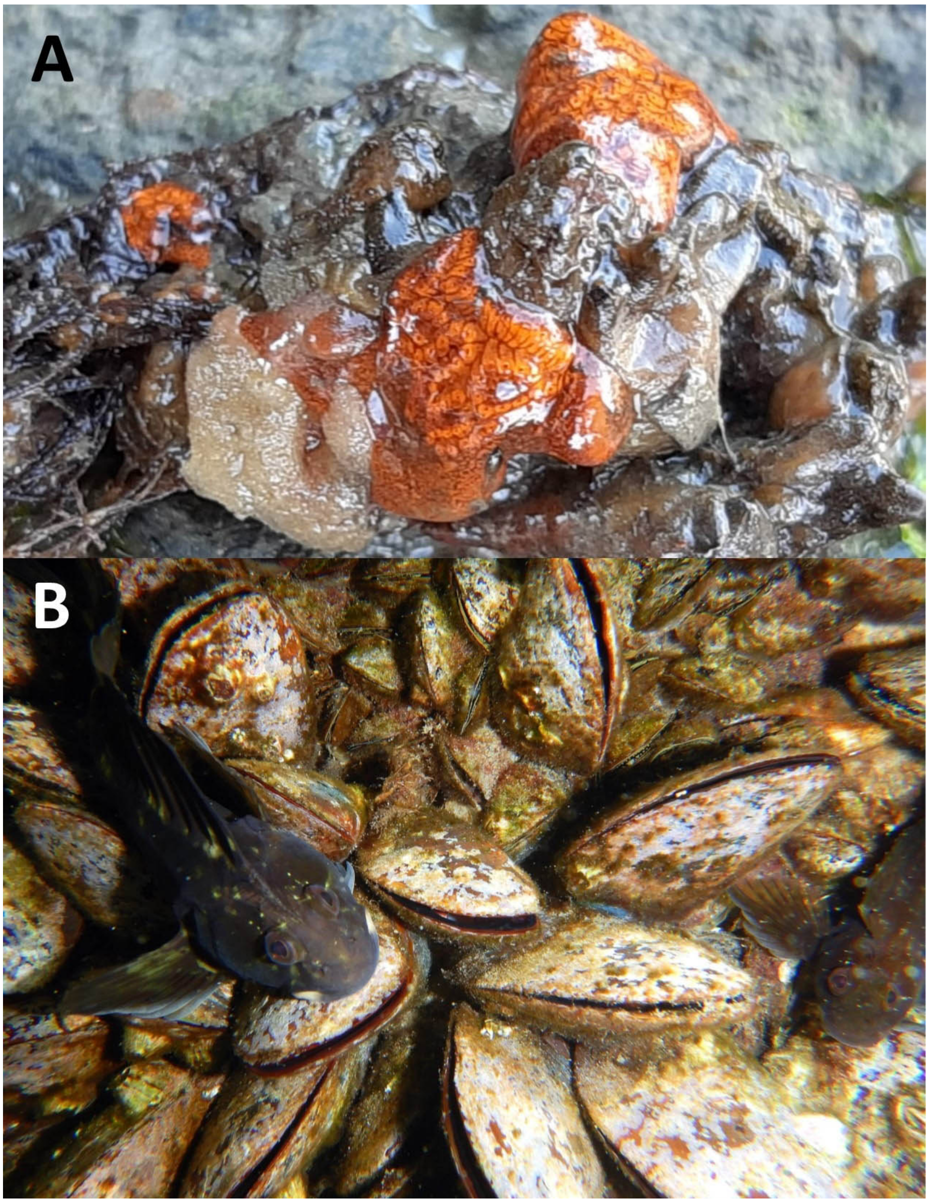

Figure 11. Remarkable new records included in the dataset: (A) *Botrylloides diegensis*, a first record for Tunisia reported by Raouia Ghanem; (B) *Perna perna* as ecosystem engineer providing shelter and a new habitat for the local fish *Scartella cristata* and barnacle species, and free surface for settlement, establishment and prosperity of the encrusting coralline red alga *Hydrolithon* sp.; this bivalve's aggregate was observed in 8.10.2020 at 1 m depth in Haifa. The fish is ca. 7 cm in length. Photo by Moti Mendelson.

One last very interesting observation included in the dataset, that does not constitute a first record, belongs to the widely invasive bivalve *Perna perna* (Linnaeus, 1758), initially observed and collected by a citizen scientist during the summer of 2020 in Haifa, located in north Israel (Douek et al. 2021), and spread all along the Levantine Mediterranean shore of Israel like wildfire. At the end of 2020, less than six months since its first record from Israel, this species of western Indian Ocean origin (Gardner et al. 2016; Fofonoff et al. 2018) reached the southernmost beach of Israel in Zikim (31.60630°N; 34.49940°E), by the border with Gaza Strip (Figure 11B). Although it is not the first introduction of this invasive mytilid mussel on the Israeli shoreline, the previous introduction (Barash and Danin 1992) was regarded as an ephemeral occurrence, and the species remained cryptic until recently (Douek et al. 2021). Moreover, seven years of steady ongoing seasonal "Bioblitz" surveys of the marine fauna and flora of the shallow and deep subtidal in Haifa, conducted by the Israeli Nature and Parks Authority, proved that the species is indeed a new invader because it was not observed or collected until 2020. The extensive and dense beds observed near Haifa port may point to vessels as the vector of introduction (Douek et al. 2021). In Haifa, Zikim and Tel Aviv, this invasive species is an ecosystem engineer, providing a habitat for marine fauna and epiflora, as depicted in Figure 11B. Moreover, although a recently published study reported that a marine heatwave-induced mass mortality event "laid waste to the entire mussel population" in July 2021 and suggests that *P. perna* outbreaks in Israel might be short-lived (Galil et al. 2022), further observation from April 2022 testify that, at least in part, the population seems to be slowly recovering in Zikim.

Discussion

Just two years after Katsanevakis et al. (2020a) paper came out, we managed to collect more than double the records of alien, cryptogenic and neonative species, complementing existing data with an additional 12,649 open-access records. This effort was motivated by the need to demonstrate that a huge amount of valuable information exists, and new data are continuously accumulating that need to be retrieved, harmonized, and openly shared. Despite the requirements of the Barcelona Convention (e.g., UNEP/MAP 2017) and the EU (e.g., for the implementation of the Marine Strategy Framework Directive 2008/56/EC), a state-level monitoring network is still largely missing from the Mediterranean and Black Seas (Tsiamis et al. 2021). Regional collaboration and networking among scientists can be valuable in partially filling this gap.

This extensive and large-scale cooperation, aiming to collect data from a large geographic area, facilitates networking among colleagues in the biological invasions field, promoting future cooperation. Scientists have been very positive in sharing their data and working together despite regional geopolitical issues for the benefit of science and society. By continuing this effort regularly, we believe we will further promote invasion science in the region and increase opportunities for further research and analyses that will improve our understanding of ecosystem change and the impacts of biological invasions in the Mediterranean and the Black Sea.

As expected, our dataset is not unbiased in the spatial and taxonomic representation of biological invasions in the Mediterranean and the Black Sea. Species size and habitat distribution, the feasibility of visual identification, the location of participating scientists, varying sampling effort and methodology among countries, the uneven distribution of citizen science initiatives, socioeconomic differences, and field methods used to obtain data may have introduced biases in our dataset. Still, the emerged patterns can highlight the invasive potential of certain species (e.g., *Caulerpa cylindracea*, *Charybdis (Archias) longicollis*, *Upeneus moluccensis*, *Pterois miles*, *Saurida lessepsianus*, *Siganus rivulatus*, *Siganus luridus*) that appear abundant and widespread. Moreover, the ever-growing use of advanced machine learning and artificial intelligence technology for big-data analysis can help overcome biases and produce novel applications to spatially and temporally map alien species advances in our seas using large datasets such as the one collated in the present study.

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Disclaimer

The responsibility for correct identification and reporting rests with the observer of each record, as stated in the Supplementary material Table S1.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Dataset of unpublished Mediterranean and Black Sea Records of alien, cryptogenic, and neonative species.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Ragkousis_etal_SupplementaryMaterial.xlsx