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Established non-indigenous species increased by 40% in 11 years in the Mediterranean Sea

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Abstract

Using a 2010 review of non-indigenous species (NIS) reported in the Mediterranean Sea as a baseline, this study undertakes a paramount revision of the non-indigenous species list in the region up to December 2021, re-evaluating the established, casual and failed introduction events of over 1366 taxa. In the light of new data and expert judgement, 14 species have been removed from the "established list" of the Mediterranean Sea inventory. The total number of validated NIS is close to 1000-751 established taxa and 242 casual taxa –while 23 species are considered as failed introduction. The rest are tagged as cryptogenic (58 taxa), questionable (70 taxa) or excluded (223 taxa). Mollusca have the highest diversity among established and casual NIS (230 taxa), followed by Pisces and Crustacea with 173 and 170 NIS respectively. The changes in establishment status reveal an accelerated rate of establishment (13%) between January 2020 and December 2021 (>6% yearly), compared to an establishment rate of 27% in the period 2011-2021 (<3% yearly). This increased establishment success is more pronounced in Crustacea (47%) and Pisces (43%) than in Polychaeta (27%) and phytobenthos (30%). In the period 2011-2021, 42% of the newly reported species were established (149 out of 352). On a shorter timescale, out of 79 new species reported in the period 2020-2021, 17 NIS (21.5%) have already established, a figure well above the 10% prediction of invasion theory on establishment success for Mediterranean marine NIS.

Keywords: alien species; Mediterranean; established taxa.

Introduction

The invasion process of non-indigenous species (NIS) can be divided into a series of stages: transport to the new geographical area, introduction to the environment, establishment of self-sustaining populations and spread beyond the point of first introduction (Blackburn *et al*., 2011). Survival and reproduction in the new range may not suffice for establishment, which requires a positive long-term population growth. Scholars disagree on the number of introduced species which eventually manage to establish. A first hypothesis posited that 10% of introduced species eventually appear in the wild (as casual species), 10% of these manage to establish, and 10% of established species become invasive (the so-called "tens rule") (Williamson & Fitter, 1996). However, these figures may be too conservative, and more recent estimates suggest that 25% of introduced invertebrates and plants and 50% of vertebrates eventually manage to establish (Jeschke & Pyšek, 2018).

Detailed and accurate knowledge of the invasion status of NIS increases as the implementation of NIS-related policies progresses (Tsiamis *et al.*, 2019). Updated and validated NIS inventories constitute a milestone for the implementation of the European Marine Strategy Framework Directive (MSFD) Descriptor 2: "Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems" (Zenetos *et al.*, 2020; Tsiamis *et al.*, 2021). Similarly, at the Mediterranean level, the Integrated Monitoring and Assessment Programme (IMAP) of the United Nations Environment Programme evaluates NIS under Ecological Objective 2 (EO2) and requires refined, up-to-date inventories for the calculation of the relevant indicators (UNEP/MED,

2021). In more detail, the MSFD D2C2 criterion addresses the abundance and spatial distribution of established NIS, while monitoring for the abundance and spatial components of the IMAP Common Indicator 6 (CI6) is recommended for selected species, taking into consideration the establishment success and invasiveness potential (UNEP/MAP, 2021). Furthermore, the establishment success of NIS informs the selection process of species proposed for listing under the EU Regulation 1143/2014 for alien species (Roy *et al.*, 2015; Rabitsch *et al.*, 2020).

Catalogues of NIS in the Mediterranean Sea with notes on their invasion status have been addressed in a series of publications since 2005 (e.g., Zenetos *et al.*, 2005; 2010; 2012; 2017; Galil *et al.*, 2018). Zenetos & Galanidi (2020) recently published an updated inventory of established Mediterranean marine NIS taking into account records up to December 2019. Their work brought up to date the list of established NIS in the basin and includes new NIS reported between 2016-2019 (excluding Foraminifera). In the period 2020-2021, besides the new NIS that have appeared in the Mediterranean Sea, new information on the taxonomic identity, biogeographic origin, nomenclature and distribution of NIS has become available, resulting in change in the status of several species. But this information remains fragmented in the literature and may become a source of confusion when compiling updated species inventories for policy and management.

The present study aims to address this gap by reviewing the status of Mediterranean NIS since Zenetos *et al.* (2010). It includes new and previously unreported NIS up to December 2021. In addition, it provides a benchmark to test the predictions of invasion theory on establishment success for Mediterranean marine NIS.

Methods

We compiled a list of Mediterranean marine NIS and their known invasion status using as a starting point the extensive list of Mediterranean NIS compiled by Zenetos *et al.* (2010). As further main steps in our inventory we used the works of Zenetos *et al.* (2017), Zenetos & Galanidi (2020) and Stulpinaite *et al.* (2020). We further checked the recent literature and the HCMR (Hellenic Centre for Marine Research) offline database to add species and update their status (Annex 1). Nomenclature follows the World Register of Marine Species (WoRMS Editorial Board, 2021).

We then identified all the species that required an update of their invasion status by searching the relevant literature and by our assessment following the taxonomic expertise of the authors, specifically A. Zenetos (AZ) for Foraminifera, P.G. Albano (PGA) for Mollusca, N. Stern (NS) for Pisces, K. Tsiamis (KT) for macrophytes, E. Lopez (EL) for Polychaeta and M. Galanidi (MG) for Arthropoda and other taxa. For such identification, we first assessed the confidence level for identification accuracy (Table 1). We then assessed the new status according to the following criteria:

EXCLUDED: This includes native species wrongly reported as NIS, taxa proven or very likely to have been misidentified native species based on thorough examination of the provided image, drawing or description, taxa with confirmed mislabelled specimens (i.e., with wrong locality data), and species not reported in the wild (e.g., observed only on ship hulls). Records referring to non-living parts of organisms (i.e., mollusc shells, crustacean exuviae) were examined on a case-by-case basis, depending on the state of the finding, but for molluscs we usually excluded records based on empty shells found before 2010. With reference to Foraminifera in particular, species records from geological cores were excluded, whereas for extant species their absence from the fossil record gave a strong indication of introduction.

Additionally, we did not consider partially native species with clearly human-mediated introductions to different parts of the Mediterranean. Two characteristic examples are *Siphonaria pectinata* (Linnaeus, 1758), a native of the Alboran sea and coast of North Africa (Morrison, 1972) which was introduced to Saronikos in the 1970s and still thrives there, and *Steromphala albida* (Gmelin, 1791), a native of the Adriatic Sea which was introduced to the French lagoons and Ebro delta with oyster transfers (Gofas & Zenetos, 2003). Such cases of course may be considered at the subregional level but were excluded for the purposes of the current work at the pan-Mediterranean scale.

CRYPTOGENIC: This includes cryptogenic and crypto-expanding species. The second term refers to recently introduced species whose mode of introduction is uncertain (natural spread *vs* human-mediated). This is the case for species of the Tropical East Atlantic that may have entered the Mediterranean Sea through the Strait of Gibraltar, e.g., *Pagurus mbizi* (Forest, 1955), which is restricted to the Alboran Sea (Garcia-Raso *et al*., 2013), and *Calappa pelii* Herklots, 1851, which is also found in

Table 1. Confidence level of identification accuracy.

Confidence level	Identification accuracy
Low	The image, drawing or description provided in the report reveal a likely case of misidentification
Medium	No photograph, illustration or description is provided, or species identified provisionally (cf.) due to incomplete material, or species identified at the genus level
High	The image and/or description are in agreement with the description of the species and for newly described species

more distant locations in different parts of the basin (Pastore, 1995; Pancucci-Papadopoulou *et al.*, 2005) with no clear evidence of natural range expansion.

QUESTIONABLE: The term "questionable," as used in this work, reflects uncertainties in the taxonomic validity of the species records.

FAILED INTRODUCTIONS: This includes species reported before 1970 but not reported later on. These are species that have been referred to in the NIS literature as "extinct" (e.g., Gravili *et al.*, 2015). We have not used the term "extinct" as it often points to evolutionary processes or processes at the population level that we cannot presume happened. For single records in particular, the term "failed introduction" reflects better the low likelihood that self-sustaining populations developed in the first place.

CASUAL: This includes non-established populations (not even locally) and/or accidental findings, i.e., species with one or two records of live specimens reported in the period 1970-2021.

ESTABLISHED: This includes species with more than two records distributed in space and time, indicating self-sustaining populations and locally established species. Established species correspond to Categories D1 and D2 of Blackburn *et al.* (2011), viz.: D1: self-sustaining population in the wild, with individuals surviving at a significant distance from the original point of introduction; D2: self-sustaining population in the wild, with individuals surviving and reproducing at a significant distance from the original point of introduction.

For each these categories we also assessed the level of confidence in the invasion status following the criteria in Table 2. Our literature search was updated on December 2021.

For molluscs, several species recently reported by Albano *et al.* (2021) were left in open nomenclature due to the very limited knowledge about the taxonomy of several micromollusc families in most tropical seas. These taxonomic entities are clearly species that do not belong to the Mediterranean native fauna as they often belong to genera without native representatives or because of their striking morphological affinities with tropical taxa. Marchini *et al.* (2015) understandably warned against including in NIS inventories species recorded with identification qualifiers such as cf., aff., etc.. However, in our opinion, when such species are well illustrated and thus unambiguously recognised, they should be fully considered for NIS inventories. The taxonomic impediment in tropical species, especially small-sized species (e.g., Albano *et al.*, 2011), is so large that the exclusion of these NIS would imply a significant underestimation of invasion processes.

Another special situation involves species detected based only on skeletal parts (e.g., molluscan shells, fish otoliths, foraminiferan tests). In this case, we used the available text and illustrations to assess if the skeletal part was in pristine taphonomic condition (e.g., preservation of shine or colour for shells (Powell & Davis, 1990; Kowalewski *et al.*, 1994) and completeness or translucency for otoliths (Agiadi *et al.*, 2021)). Such pristine skeletons testify to recently produced skeletons and, thus, very recent (in ecological time scale) populations, and were consequently treated as the records of living individuals. Skeletons with some degree of taphonomic damage were considered older occurrences, and the assessment of status or confidence was adjusted accordingly.

Table 2. Assessment criteria for the establishment status.

Status		**Confidence of the final Assessment**		
	ID confidence	**High**	**Medium**	**Low**
Established	High	>2 confirmed records (both in space and time) Locally established		
Casual	High/medium	1 or 2 records of live specimens between 1970-2000 and 1 or more pathways with low propagule pressure (e.g., aquarium related escapees or releases)	1 or 2 records of live specimens after 2000 with high likelihood of re-introduction (corridor – Mollusca)	1 or 2 records after 2010 and 1 or more pathways which make re-introduction likely (e.g., Corridor, Shipping)
Excluded	High/medium/low	Proven or very likely misidentification, mislabeling of specimens Non-living parts of organisms recorded before 2010 Specimens not in the wild Native and range expanding species		
Failed introductions	High	Records before 1970 never reported again		
Cryptogenic	High/medium/low	Unknown origin, Possible native, Crypto-expanding		
Questionable	High/medium/low	Uncertain identification Sibling species, species complexes Possibly undescribed species Possible mislabeled specimens		

Results and Discussion

Over 1366 taxa were examined, 666 of which were reported as established by Zenetos & Galanidi (2020). Seven hundred and fifty-one (751) taxa were assessed as established, over 242 as casual, over 23 as failed introductions, over 70 as questionable, and over 57 as cryptogenic, with over 223 taxa excluded (Annex 1, Fig. 1). Ninety-four species are considered as established in this work based on recent literature (Albano *et al.*, 2021; Castelló *et al.*, 2020; Galil *et al.*, 2020; Langeneck *et al.*, 2020; Stulpinaite *et al.*, 2020; Çinar *et al.*, 2021; Kovačić *et al.*, 2021). Finally, 14 species have been removed from the established NIS list and redstributed among casual, cryptogenic and excluded.

For molluscs we added 97 recent records to the 208 species classified as established, casual or questionable in Zenetos *et al.* (2010) to get a total of 305 species. Of these, 39 were reported as established in previous papers (literature 2010–2020), while 21 more species are evaluated as established in this work (Table 3). Out of 126 re-evaluated species (Annex 2), 57 are here assessed as casual. These are mostly newly recorded species, such as the 30 new non-indigenous species recently reported in Israel (Steger *et al.*, 2018; Albano *et al.*, 2021). Most of the 61 excluded species belong to one of two categories: species recorded from only empty shells likely related to the souvenir trade (e.g., the cowrie *Monetaria annulus*) or misidentifications (e.g., *Centrocardita akabana*). In addition to the three reported cryptogenic species (*Murchisonella columna*/*mediterranea*, *Discodoris lilacina* (Gould, 1852*)* and *Aplysia dactylomela* Rang, 1828), the four species here considered cryptogenic are: *Perna perna (*Linnaeus, 1758), *Anteaeolidiella lurana* (Ev. Marcus & Er. Marcus, 1967), *Turbonilla flaianoi* Mazziotti, Agamennone, Micali & Tisselli, 2006) and *Leucotina* cf. *eva* Thiele, 1935. Some species with early records based on empty shells are here confirmed as established because of continuous records of empty shells which suggest low detectability of living populations. A prime example is *Conus fumigatus*, which was first recorded in Libya in the mid-1970s (Röckel, 1986) but had no subsequent records until recent ones from Syria (Ammar, 2019) and Israel (Albano *et al.*, 2021). Some species recorded only very recently and in a single country are here considered established because they were found in multiple locations and with several living individuals. This is chiefly the case with *Musculus coenobitus*, *Musculus* aff. *Viridulus*, *Joculator problematicus*, *Elachisina* sp., *Parthenina cossmanni* and *Parthenina typica*, which were all found along the Israeli coastline in 2018. Ambiguous is the status of two species among the earlier invaders. The status of *Bursatella leachii* Blainville, 1817 and *Brachidontes pharaonis* (Fischer, 1870) is here retained as NIS despite some divergent opinions among experts. Both are widely known as Lessepsian immigrants but recent molecular studies suggest that they may be cryptogenic (Bazzicalupo *et al.*, 2020) and native (Belmaker *et al.*, 2021), respectively. Regarding *B. pharaonis*, the hypothesis of a native, previously overlooked population seems very implausible considering the long history of malacological research in the region and the focus on the Suez Canal area. Furthermore, the hypothesis is weakly supported by the limited geographical coverage of the sequenced samples which did not allow for a wide comparison of Indo-Pacific and Mediterranean populations and the detection of the origin of different *B. pharaonis* populations in the Mediterranean. As far as *B. leachii* is concerned, the colonisation pattern from the Levantine westwards, as well as the morphological differences between the West African populations and the Moroccan and Western Mediterranean populations (S. Gofas, pers. Commun), makes a Lessepsian invasion more tenable.

For Pisces, 243 species were examined (Annex 1). By removing the species reported established by 2020, range expanding and the cryptogenic records, the status and validity of 106 species were re-evaluated in this study. Out of these, 76 species (72%) are retained as "casual" species based on a single observation; seven species have been excluded from the NIS list, based on taxonomic revisions, misidentifications, poor descriptions or old non-substantiated records. Of the 97 established species, 30 were

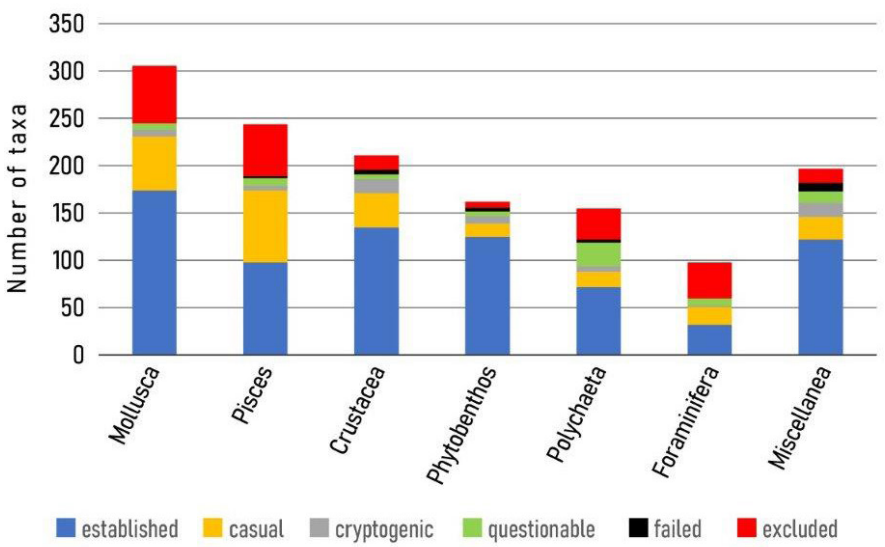

Fig. 1: Status of non-indigenous species in the Mediterranean Sea according to their taxa and introduction stages.

Table 3. List of species newly evaluated as established. Abbreviation of groups: MOL= Mollusca, PISC=Pisces, CRU=Crustacea BRY=Bryozoa, PHY=phytobenthos, POL=Polychaeta, FOR=Foraminifera, CNI=Cnidaria, ECH=Echinodermata, NEM=Nematoda POR=Porifera, TUN=Tunicata. "Additional records" in the text of column 3 refers to records reported after the first casual findings as documented either in Zenetos *et al.,* 2010 or in subsequent individual publications.

Group	species	Reasoning for establishment
MOL	*Lioberus ligneus* (Reeve, 1858)	Multiple records from Israel and Cyprus by Albano *et al.* (2021).
MOL	*Musculus* aff. *viridulus* (H. Adams, 1871*)*	Living individuals reported from multiple locations in Israel (Albano *et al.*, 2021).
MOL	*Musculus coenobitus* (Vaillant, 1865)	Living individuals reported from multiple locations in Israel (Albano *et al.*, 2021).
MOL	*Isognomon* aff. *australicus* (Reeve, 1858)	Reported also from Cyprus by Albano *et al.* (2021).
MOL	*Pinctada fucata* (A. Gould, 1850)	This species was recognized as a distinct non-indigenous species in the Mediterranean from *Pinctada radiata* by Scuderi *et al.* (2019). It was reported from Spain by Cunningham Aparicio & Mulero Mendez 2021. Still, it should be noted that the identity of populations around the Mediterranean would benefit from confirmation with molecular methods.
MOL	*Mimachlamys sanguinea* (Linnaeus, 1758)	Numerous reports from southern Israel.
MOL	*Rugalucina angela* (Melvill, 1899)	First reported with a single living individual; a second living individual reported by Albano *et al.* (2021).
MOL	*Nudiscintilla* cf. *glabra* Lützen & C. Nielsen, 2005	Living individuals reported from Israel by Albano *et al.*, (2021).
MOL	*Gari pallida* (Deshayes, 1855)	Additional records from Israel reported by Albano *et al.* (2021).
MOL	*Dikoleps micali* Agamennone, Sbrana, Nardi, Siragusa & Germanà, 2020	Living individuals reported also from Israel by Albano *et al.* (2021).
MOL	*Eunaticina papilla* (Gmelin, 1791)	An additional living individual reported from Israel by Albano *et al.* (2021).
MOL	*Epitonium vaillanti* (Jousseaume, 1912)	Multiple records between 2015-2019.
MOL	*Joculator problematicus* Albano & Steger, 2021	Several living individuals from multiple locations in Israel (Albano *et al.*, 2021). Native range unknown, but most likely Indo-Pacific.
MOL	*Elachisina* sp.	Several living individuals from multiple locations in Israel (Albano *et al.*, 2021). Native range unknown, but most likely Indo-Pacific.
MOL	*Circulus novemcarinatus* (Melvill, 1906)	Multiple records from the Levantine Basin (B. Öztürk pers. comm.).
MOL	*Conus fumigatus* Hwass, 1792	Extremely rare, but recently reported also from Syria (Ammar, 2019) and Israel (Albano *et al.*, 2021).
MOL	*Phidiana militaris* (Alder & Hancock, 1864)	Recorded again from Israel by Steger *et al.* (2018) after its first record in 2016 by Rothman *et al.* (2017).
MOL	*Atys angustatus* E. A. Smith, 1872	Multiple findings.
MOL	*Parthenina cossmanni* (Hornung & Mermod, 1924)	Multiple records in Israel (Albano *et al.*, 2021).
MOL	*Parthenina typica* (Laseron, 1959)	Multiple records in Israel (Albano *et al.*, 2021).
MOL	*Crepipatella dilatata* (Lamarck, 1822)	Multiple records in Spain (J. Lopez Sorriano, pers. com).
PISC	*Ablennes hians* (Valenciennes, 1846)	Additional recent records from Malta (Deidun *et al.*, 2021) and Syria (Alshawy *et al.*, 2019).
PISC	*Acanthopagrus bifasciatus* (Forsskål, 1775)	Further records from Egypt (Al Mabruk *et al.*, 2021a) and Turkey (Şensurat-Genç *et al.*, 2020).
PISC	*Ambassis dussumieri* Cuvier, 1828	Documentation of a school of hundrend individuals (Stern *et al.*, 2022).
PISC	*Cryptocentrus steinhardti* (Goren and Stern, 2021)	Several individuals from Israel and possible observations from Turkey (Stern, pers. observation).
PISC	*Epinephelus areolatus* (Forsskål, 1775)	Further records from Lebanon (Bariche & Edde in Bariche *et al.*, 2020) and Syria (Al Mabruk *et al.*, 2021b).
PISC	*Himantura leoparda* Manjaji-Matsumoto & Last, 2008	Locally established in Israel (Barash, pers. com.).

Continued

Table 3 continued

Group	species	Reasoning for establishment
PISC	*Istiblennius meleagris* (Valenciennes, 1836)	Broadly distributed along the Israeli coast (Rothman *et al*., 2020).
PISC	*Paranthias furcifer* (Valeciennes, 1828)	Four records within 2020-21, the last ones from Spain: Tiralongo and Azzurro in Orfanidis *et al.* (2021) and Turkey (Çinar *et al.*, 2021).
PISC	*Priacanthus hamrur* (Forsskål, 1775)	One record from Tunisia (1980) and one individual from Turkey (Erguden *et al.*, 2018).
PISC	*Encrasicholina gloria* Hata & Motomura, 2016	Large population in the Israeli pelagic stock (Stern, unpublished. data).
PISC	*Synchiropus sechellensis* Regan, 1908	Multiple records from Greece, Cyprus and Egypt.
CRU	*Ampithoe bizseli* Özaydinli & Coleman, 2012	Established in Turkey (Çinar *et al.*, 2021).
CRU	*Labidocera orsinii* Giesbrecht, 1889	First record in Lebanon in 1972, later established in Syria (Durgham, 2002).
CRU	*Laticorophium baconi* (Shoemaker, 1934)	Locally established based on Gouillieux & Sauriau (2019).
CRU	*Actaeodes tomentosus* (H. Milne Edwards, 1834)	Additional specimens in Tunisia (non-ovigerous females) (Ounifi-Ben Amor *et al.*, 2016).
CRU	*Alpheus* cf. *lobidens* De Haan, 1849 [in De Haan, 1833-1850]	Additional records in Syria and Egypt (Hamdy & Dorgham, 2019).
CRU	*Ashtoret lunaris* (Forskål, 1775)	New records in Syria (2 specimens in 2017) and Turkey (4 females, Iskenderun Bay, in 2015).
CRU	*Sphaerozius nitidus* Stimpson, 1858	Repeated findings up to 1971 in Egypt - another record from the Alboran Sea (MEDITS surveys). One more record in Israel from a buoy from Port Said (Ivkić *et al.*, 2019).
CRU	*Notopus dorsipes* (Linnaeus, 1758)	Two more records in Egypt (2000 and 2016), both reported in Abdelsalam & Ramadan (2016).
CRU	*Hatschekia siganicola* El-Rashidy & Boxshall, 2011	It was first reported off Alexandria, Egypt in large numbers on 3 *Siganus luridus*. Reported again in Libya in 2016 (Abdelnor *et al*., 2019).
CRU	*Euchaeta concinna* Dana, 1849	Two more records found in Greece (off western Peloponnese) in OBIS, collected in 1987 - verified by Greek experts. Another record in Libya (Halim, 1990), cited in Zakaria et al., (2016) and Shakman *et al.* (2019).
CRU	*Leptochela aculeocaudata* Paulson, 1875	See Ammar (2019) updated Syrian NIS.
CRU	*Cosmocalanus darwinii darwinii* (Lubbock, 1860)	Widespread Indo-Pacific and south-eastern Pacific, sporadic records in the Carribean, present in the Red Sea (according to Razouls *et al.*, 2005-2021). First reported in the Mediterranean by Nowaczyk *et al.* (2011)
CRU	*Zeuxo coralensis* Sieg, 1980	Additional records in Spain (Sánchez-Moyano & García-Gómez, 1998) and Algeria (Bensari *et al.*, 2020).
PHY	*Acanthosiphonia echinata* (Harvey) A.M.Savoie & G.W.Saunders	Well established in the Venice lagoon (M.Wolf pers. com.).
PHY	*Aglaothamnion halliae* (Collins) Aponte, D.L.Ballantine & J.N.Norris	Well established in the Venice lagoon (M.Wolf pers. com.).
PHY	*Batophora occidentalis* var. *largoensis* (J.S. Prince & S. Baker) S. Berger & Kaever ex M. J.Wynne	Established locally in Spain (Ballesteros, 2020).
PHY	*Calliblepharis rammediorum* Hoffman, Wynne & Saunders	Established in Israel (Galil *et al.*, 2020).
PHY	*Derbesia boergesenii* (M.O.P.Iyengar & Ramanathan) Mayhoub	Established in Israel (Hoffman pers. com.).
PHY	*Dictyota acutiloba* J.Agardh	Established in Israel (Katsanevakis *et al.*, 2020).
PHY	*Ectocarpus siliculosus* var. *hiemalis* (P.L.Crouan & H.M.Crouan) Gallardo	Found at least in three locations in the Mediterranean Sea (Verlaque *et al.*, 2015).
PHY	*Grateloupia gibbesii* Harvey	Established in Egypt (Rodríguez-Prieto *et al.*, 2021).
PHY	*Hypnea corona* Huisman & Petrocelli	Established in Italy, previously records assigned as *Hypnea cornuta* (Huisman *et al.*, 2021).
PHY	*Lobophora lessepsiana* C.W.Vieira, 2019	Established in Israel (Galil *et al.*, 2020).

Continued

Table 3 continued

Group	species	Reasoning for establishment
PHY	*Pachymeniopsis gargiuli* S.Y.Kim, Manghisi, Morabito & S.M.Boo	Established in Italy (Kim *et al.*, 2014).
PHY	*Palisada maris-rubri* (K.W. Nam & Saito) K.W. Nam	Several records across the Mediterranean (Tsiamis *et al.*, 2015).
PHY	*Ulva tepida* Masakiyo & S.Shimada	Established in Israel (Krupnik *et al.*, 2018).
PHY	*Ulva chaugulii* M.G.Kavale & M.A.Kazi 2016	Established in Israel (Krupnik *et al.*, 2018).
POL	*Acromegalomma claparedei* (Gravier, 1906)	Several reports from Italy (Giangrande & Licciano, 2008). At least an established population in Lake Faro, Messina, Italy (Rizzo *et al.*, 2015).
POL	*Aricidea bulbosa* Hartley, 1984	Established in Turkey (Çinar *et al.*, 2021).
POL	*Branchiomma boholense* (Grube, 1878)	Many records, usually as *B. bairdi* (Del Pasqua *et al.*, 2018), all over the Mediterranean Sea since an early report from Levantine coasts.
POL	*Dodecaceria capensis* Day, 1961	Description not provided, but identification checked by a specialist (see Faulwetter *et al.*, 2017).
POL	*Novafabricia infratorquata* (Fitzhugh, 1983)	Several reports from the Mediterranean coast of Spain (Bick, 2005; Cepeda & Lattig, 2016).
POL	*Lumbrineris perkinsi* Carrera-Parra, 2001	Despite the argumements raised by Langeneck *et al.* (2020), the material described by Çinar (2009) shows no important difference with the description of *L. perkinsi*.
POL	*Polydora colonia* Moore, 1907	Considered as established in Spain (López & Richter, 2017).
POL	*Protodorvillea biarticulata* Day, 1963	Established: Langeneck *et al.* (2020).
POL	*Semiodera cinari* Salazar-Vallejo, 2012	Established in Turkey (Çinar *et al.*, 2021).
POL	*Syllis ergeni* Çinar, 2005	Established in Turkey (Çinar *et al.*, 2021).
FOR	*Ammodiscus gullmarensis* Höglund, 1948	multiple records
FOR	*Amphistegina lessoni* d'Orbingy in Guérin-Méneville, 1932	Established: Zenetos *et al.* (2010).
FOR	*Amphistegina lobifera* Larsen, 1976	Established: Zenetos *et al.* (2010).
FOR	*Amphistegina* cf. *papillosa* Said, 1949	Established: Stulpinaite *et al.* (2020).
FOR	*Borelis schlumbergeri* (Reichel, 1937)	Established: Zenetos *et al.* (2010).
FOR	*Clavulina* cf. *multicamerata* Chapman, 1907	Established: Zenetos *et al.* (2010).
FOR	*Cornuspiroides striolata* (Brady)	Established in Turkey (Çinar *et al.*, 2021).
FOR	*Cyclorbiculina compressa* (d'Orbigny, 1839)	Established: Zenetos *et al.* (2010).
FOR	*Elphidium striatopunctatum* (Fichtel & Moll, 1798)	Established: Zenetos *et al.* (2010).
FOR	*Epistomaroides punctulata* (d'Orbigny, 1826)	Established: Stulpinaite *et al.* (2020).
FOR	*Haddonia* sp.	Established: Zenetos *et al.* (2010).
FOR	*Heterostegina depressa* d'Orbigny, 1826	Established: Zenetos *et al.* (2010).
FOR	*Loxostomina* cf. *africana* (Smitter, 1955)	Established: Stulpinaite *et al.* (2020).
FOR	*Nodophthalmidium antillarum* (Cushman, 1922)	Established: Zenetos *et al.* (2010).
FOR	*Nodobacullariella cristobalensis* McCulloch, 1977	Established in Turkey (Çinar *et al.*, 2021).
FOR	*Operculina ammonoides* (Gronovius, 1781)	Established: Stulpinaite *et al.* (2020).
FOR	*Parasorites orbitolitoides* (Hofker, 1930)	Established in Spain: Alvira Romero (2019).
FOR	*Planispirinella exigua* (Brady, 1879)	Established: Stulpinaite *et al.* (2020).
FOR	*Planogypsina acervalis* (Brady, 1884)	Established: Stulpinaite *et al.* (2020).

Continued

Table 3 continued

Group	species	Reasoning for establishment
FOR	*Pseudohauerina diversa* (Cushman, 1946)	Established: Zenetos *et al.* (2010).
FOR	*Pseudomassilina reticulata* (Heron-Allen & Earland, 1915)	Established: Zenetos *et al.* (2010).
FOR	*Pyrgo denticulata* (Brady, 1884)	Established: Zenetos *et al.* (2010).
FOR	*Quinqueloculina* cf. *mosharrafai* Said, 1949	Established: Zenetos *et al.* (2010).
FOR	*Schlumbergerina alveoliniformis* (Brady, 1879)	Established: Zenetos *et al.* (2010).
FOR	*Sigmamiliolinella australis* (Parr, 1932)	Established: Stulpinaite *et al.* (2020).
FOR	*Siphonaperta distorqueata* (Cushman, 1954)	Established: Stulpinaite *et al.* (2020).
FOR	*Sorites variabilis* Lacroix, 1940	Established: Zenetos *et al.* (2010).
FOR	*Spiroloculina* aff.*communis*	Established: Stulpinaite *et al.* (2020).
FOR	*Spiroloculina angulata* Cushman, 1917	Established: Stulpinaite *et al.* (2020).
FOR	*Spiroloculina antillarum* d'Orbigny, 1839	Established: Zenetos *et al.* (2010).
FOR	*Triloculina ex Miliolinella fichteliana* d'Orbigny, 1839	Established: Zenetos *et al.* (2010).
FUN	*Nosema ceratomyxae* Diamant & Paperna, 1985	Missing from previous reviews.
BRY	*Celleporaria vermiformis* (Waters, 1909)	Five new records in Greece and Cyprus (Ulman *et al*., 2017), on harbour walls and marinas.
BRY	*Microporella coronata* (Audouin, 1826)	Re-described in Harmelin *et al.* (2011) with new neotype from Lebanon - clearly established in Lebanon. Ferrario *et al.* (2018) consider all records acceptable, except for the Zabala (1986) one from Corsica.
TUN	*Botrylloides giganteus* (Pérès, 1949)	Initially identified as *B. pizoni*, it was reassigned to *B. giganteus* based on a molecular study (Rocha *et al.*, 2019).
CNI	*Arctapodema australis* (Vanhöffen, 1912)	Established: Gravili *et al.* (2013).
CNI	*Cordylophora caspia* (Pallas, 1771)	Established: Galanidi (2019); Çinar *et al.* (2021).
CNI	*Aequorea globosa* Eschscholtz, 1829	In Turkey it was observed all year round for a year (Turan *et al.*, 2011). One more record of a single specimen in Syria in 2012 (Mamish *et al.*, 2012).
CNI	*Eucheilota ventricularis* McCrady, 1859	Established: Gravili *et al.* (2013).
CNI	*Moerisia carine* Bouillon, 1978	Established: Gravili *et al.* (2013).
CNI	*Tetrorchis erythrogaster* Bigelow, 1909	Established: Gravili *et al.* (2013).
ECH	*Holothuria (Theelothuria) hamata Pearson, 1913*	Additional specimens found in Turkey (ME Çinar pers. com.; Çinar *et al.*, 2021).

Note: Foraminifera include the 17 species recorded as established in Zenetos *et al.* (2010) but missing from Zenetos & Galanidi (2020).

reported to have established viable populations or even become invasive (e.g., *Pterois miles* (Bennett, 1828): Dimitriadis *et al.,* 2020; *Parupeneus forsskali* (Fourmanoir & Guézé, 1976): Evagelopoulos *et al.,* 2020) between 2010 and 2019 while 11 more species were tagged as established in their invaded territories based on multiple observations or locally established populations in the last 24 months (Table 3). Overall, 55 species have been excluded, eight species are marked "questionable" due to poor descriptions of their corresponding documentations or merely due to a single non-substantiated old record, five species are tagged as cryptogenic and two species have been deemed as failed introductions.

In relation to crustaceans, 63 species were added to the 147 already reported as established, casual or questionable by Zenetos *et al.* (2010), making a total of 210 species. Of these, 121 species were already recognized as established by December 2019 (Zenetos & Galanidi, 2020). Thirteen species are here confirmed as established (Table 3), primarily decapods which have established populations along the coasts of the East and Central Mediterranean. Among these, *Ampithoe bizseli* emerged as established in Turkey (Çinar *et al.*, 2021). Screening of the list (Annex 2) resulted in 36 species registered as casual. Among them, questions have been raised about the validity of the *R. whitei* record in Egypt (Horton *et al.*, 2021 in WoRMS). Five crustacean species with single records before 1970 are now considered failed introductions

since no further records have been reported since. Fifteen species have been assigned to cryptogenics; indicatively, *Delavalia minuta* [Por's (1964) *Stenhelia* aff. *minuta* from the coast of Israel] was recently reassigned to the new species *Willenstenhelia urania* Karanovic & Kim 2014 following a major revision of the genus *Delavalia* (Karanovic & Kim 2014). We here consider it cryptogenic in the Mediterranean.

The decapods *Pagurus mbizi*, *Calappa pelii*, *Cryptosoma cristatum* and *Processa macrodactyla* have now been moved to the cryptogenic category on the basis of their Atlantic East/Tropical East origin, which makes them candidates for crypto-expansion and casts doubt on their alien status. Two species are considered questionable on the basis of dubious records: one is the decapod *Actumnus globulus* Heller, 1860, whose Italian record is suspected to be a mislabelled specimen originating in the Red Sea (Innocenti & Crocetta, 2020), and the other is the isopod *Apanthura sandalensis* Stebbing, 1900, whose Libyan and Israeli records lack sufficient description (Marchini *et al.*, 2015). Finally, 15 species have been excluded from the list of Mediterranean NIS (Annex 2). *Scottolana longipes* (Thompson I.C. & Scott A., 1903) represents a species group and is associated with nomenclatural problems (Huys, 2009). Three more excluded copepods are *Robertsonia salsa* Gurney, 1927, *Enhydrosoma vicinum* Por, 1967 and *Canuellina insignis* Gurney, 1927, which were only reported from the hypersaline Bardawil (Sirbonian) lagoon and were considered by Por (1978) to be members of the native Isthmus fauna, whose migrational movements might have preceded the opening of the Suez Canal. The calanoids *Calanopia biloba, Calanopia minor* and *Parvocalanus latus*, all single records from Turkish waters, were recently excluded from the Turkish marine alien fauna due to lack of proper description and subsequent confirmation of their presence in the region (Çinar *et al.*, 2021).

For phytobenthos (macroalgae), the vast majority (77% = 124 taxa) of the 161 total taxa are established. Fourteen species were assessed as established in the last 24 months (Table 3). For example, *Acanthosiphonia echinata* and *Aglaothamnion halliae* are well established in the Venice lagoon (M. Wolf pers. com.), while *Ectocarpus siliculosus* var. *hiemalis* has been found in more than two locations across the Mediterranean Sea (Verlaque *et al.*, 2015). Four species initially considered as established by Zenetos *et al.* (2010) were later treated as casual by Verlaque *et al.* (2015), but eventually as established by Zenetos *et al.* (2017) (see Annex 1).

Four macroalgae should be considered today as failed introductions in the Mediterranean Sea, namely *Hypnea flagelliformis* Greville ex J. Agardh, *Acrochaetium robustum* Børgesen, *Acrochaetium subseriatum* Børgesen and *Rhodymenia erythraea* Zanardini, all casual species never reported in the Mediterranean Sea in the last 50 years (Annex 1). Our results suggest that 14 of the re-evaluated taxa should be retained as casuals (Annex 2). Moreover, six macroalgae should be excluded from the list of alien species (Annex 1, 2), considered mostly as native to the Mediterranean Sea (e.g., *Microspongium stilophorae*; Verlaque pers. com.). Finally, five species have been tagged as questionable [e.g., *Acrochaetium spathoglossi* Børgesen*, Padina antillarum* (Kützing) Piccone] and eight species as cryptogenic.

Out of the 154 polychaete species recorded in our list, 61 species were already reported as established (Zenetos *et al.*, 2010; Zenetos & Galanidi 2020), while another 10 have been added to the list of established taxa in this work (Table 3). Among them *Semiodera cinari* and *Syllis ergeni* deserve further comment. *Semiodera cinari* was first reported in Iskenderun Bay in Turkey as the alien species *Pherusa parmata* by Çinar (2009). Subsequently, Salazar-Vallejo (2012) re-examined some of the reported specimens and used them to describe a new species, which is only known from the type locality and might be considered as native. However, some of the characters reported are subjective and size-dependent, and the genus *Semiodera* shows a clear tropical affinity (Çinar pers. com.). This and the appearance of the species in high numbers in a heavily anthropized locality where it was previously absent make its non-native nature quite likely (Çinar *et al.*, 2021). *Syllis ergeni* was first considered as native (Çinar, 2005), but its sudden occurrence in a regularly monitored Mediterranean location and its presence in the Red Sea led to its re-categorisation as an established alien species (Çinar *et al.*, 2021). Seven species initially included among the alien established ones but assigned to Data Defficient by Tsiamis *et al.* (2019) have been re-examined here, including *Metasychis gotoi* (Izuka, 1902) and *Neopseudocapitella brasiliensis* Rullier & Amoureux, 1979. Overall, 33 species have been excluded, 25 have been classified as questionable, three as failed introductions and 16 species as casuals.

For Foraminifera, 97 species were scrutinized, including 17 species that were reported as established in Zenetos *et al*. (2010). Following the extensive review of alien Foraminifera in 2020 by Stulpinaite *et al*. (2020), 14 more species have been tagged as established in this work (Table 3), with 38 species excluded and 8 species evaluated as questionable because of their high taxonomic uncertainty (e.g., *Cushmanina striatopunctata* (Parker & Jones, 1865), *Polymorphina fistulosa* Williamson, 1858 as taxon inquirendum (WoRMS Editorial Board, 2021). The presence of 19 taxa is known from casual records.

The Miscellaneous group covers species belonging to the phyla Bryozoa, Sipuncula, Echinodermata, Platyelminthes, Cnidaria and Tunicata. In total, the group contains 196 taxa, among which 56 were already identified as established by Zenetos *et al.* (2010), making 110 established taxa by the end of 2019 (Zenetos & Galanidi, 2020). Eleven additional species are newly tagged as established in this work (Table 3). Overall, 24 species have been assessed as casuals and 15 as cryptogenics; 15 species did not qualify as aliens and have been consequently excluded. Among the species considered established in this work, the bryozoan *Celleporaria vermiformis* was recently recorded in a number of harbours and marinas in the Eastern Mediterranean (Ulman *et al.*, 2017). On the other hand, its congeneric *Celleporaria pilaefera* was only recorded once in Malta on oyster baskets and cages

and cannot be considered part of the Mediterranean NIS fauna (Marchini *et al.*, 2015).

The phylum Cnidaria includes seven established species, six of which were inconsistently classified in the past (Annex 1, see comments under the following section); only *Aequorea globosa*, which was recorded once in Syria (Mamish *et al.*, 2012), is a new species established locally in Turkey (Çinar *et al.*, 2021).

An interesting case is the pycnogonid *Endeis biseriata* Stock, 1968, which was only recently reported for the first time in the Mediterranean in preserved material (Colasanto & Galli, 2021), even though the collection of the samples was made in 1979 from the cooling water discharge area of a power plant. The population appeared to have been established at the time but no further records were found in the literature; we therefore consider it a casual species.

The majority of the failed introductions are Red Sea Porifera, which were reported by Tsurnamal (1969) in Israel with no follow-up and previously assigned to questionable (Annex 1, 2).

Fifteen species with inconsistent classification of their invasion status in previous works are here assessed as established. Among macroalgae, four species initially considered as established by Zenetos *et al.* (2010) –namely *Botrytella parva* (Takamatsu) H.S. Kim; *Cladophora patentiramea* (Montagne) Kützing; *Derbesia rhizophora* Yamada and *Neomeris annulata* Dickie– were later treated as casual by Verlaque *et al.* (2015), but eventually as established by Zenetos *et al.* (2017) (see Annex 1).

For Crustacea, *Triconia umerus* (Böttger-Schnack & Boxshall, 1990), *Triconia rufa* (Boxshall & Böttger, 1987) and *Triconia hawii* (Böttger-Schnack & Boxshall, 1990) were already considered established by Zenetos *et al.* (2010). They were not included in the revision by Zenetos *et al.* (2017), and only two of them (*T. umerus* and *T. rufa*) were listed by Zenetos & Galanidi (2020) but with incomplete distribution information. *Triconia umerus* is established both in the East and West Mediterranean (Böttger-Schnack, 1997; Böttger-Schnack & Schnack, 2009; Di Capua & Boxshall, 2008). *Triconia rufa* has been reported in Egypt (Abd El-Rahman, 2005) and Italy (Di Capua & Boxshall, 2008), while *Triconia hawii* is now considered as locally established in the Western Mediterranean, according to Di Capua and Boxshall (2008). *Leptochela aculeocaudata* Paulson, 1875, with one casual record in Egyptian waters in 1933 (Balss, 1936), was erroneously registered as established by Zenetos *et al.* (2010), and was later mistakenly omitted from the inventory of established Mediterranean NIS (Zenetos *et al.*, 2017). Recent records in Syria (Ammar, 2019) indicate that the species is locally established in the region.

Three species of polychaetes have re-enterered the established list. The alien status of *Novafabricia infratorquata* (Fitzhugh, 1983) and *Lumbrineris perkinsi* Carrera-Parra, 2001 had been questioned, leading to their classification as cryptogenics (Zenetos *et al.*, 2017). Licciano & Giangrande (2006) described some differences with *N. infratorquata* with type material, which caused Langeneck *et al.* (2020) to consider it a questionable record. Langeneck *et al.* (2020) also questioned the identification of *L. perkinsi*, stating that this material could refer to *L. albifrons*, although they mentioned stable populations of four-teethed *Lumbrineris* in the Levantine Sea (Çinar, 2009) as well as in the Red Sea, which supported the alien status of the taxon irrespective of its specific identity. However, in both species, the differences in type specimens fall within the known variability of the species in the native range and, thus, we have reinstated them as aliens. After the work by Tovar-Hernández *et al.* (2009), *Branchiomma boholense* (Grube, 1878), a species long reported in the Mediterranean, was considered absent from the area, and records referred to the similar *B. bairdi* (McIntosh, 1885). However, Del Pasqua *et al.* (2018) revised material from several locations around the world both morphologically and from a molecular point of view and concluded that most reports from the Mediterranean referred to *B. boholense*.

Due to the complex life cycle of the hydrozoans –e.g., *Moerisia carine* Bouillon, 1978, *Tetrorchis erythrogaster* Bigelow, 1909 and *Arctapodema australis* (Vanhöffen, 1912)– as well as the paucity of information on the biology and worldwide distribution of many of them, there is high uncertainty about their origin and possible alien status in the Mediterranean (P. Schuchert, pers. com.), hence their inconsistent classification in previous publications. In this work, we have followed taxonomic experts from the Mediterranean and their previous assessments of cnidarian records (e.g., Gravili *et al.*, 2013; Gravili & Rossi 2021) while conceding that hydrozoan records should be treated with caution.

Cordylophora caspia (Pallas, 1771), on the other hand, is an entirely different case, being a well-known and well-studied hydrozoan species. As an oligohaline non-indigenous species of Ponto-Caspian origin, it is generally not treated consistently across the Mediterranean and European countries, as in some countries it occurs exclusively in their inland systems, but in others (i.e., Baltic Sea countries) it is found in coastal lagoons and estuaries and is thus included in marine NIS inventories (Tsiamis *et al.*, 2019). In this work we have followed the definition adopted by the European Commission, which considers the species as partly native to the EU but introduced to the Mediterranean countries (Galanidi, 2019; see also Çinar *et al.*, 2021 for Turkey).

Species to be removed from the "established NIS" list

In the light of new data and personal experience, 14 species (Table 4) have been removed from the "established list" by Zenetos & Galanidi (2020). It appears they were inaccurately reported as established. They are here classified as casuals (9 species), cryptogenics (2 species) or excluded (3 species).

The status of four mollusca was revised: *Iolaea neofelixoides* and *Lienardia mighelsi*, represented only by single records, and *Gregariella* cf. *ehrenbergi*, recorded in Israel on a stranded buoy originating in Port Said (Steger *et al.*, 2018), are here considered casual species, where-

Table 4. Species to be removed from the Zenetos & Galanidi (2020) "established NIS" list.

Group	Species	New assessment (this work)
MOL	*Iolaea neofelixoides* (Nomura, 1936)	Casual
MOL	*Lienardia mighelsi* Iredale & Tomlin, 1917	Casual
MOL	*Gregariella* cf. *ehrenbergi* (Issel, 1869)	Casual
MOL	*Planaxis savignyi* (Brocchi, 1821)	Excluded
PISC	*Rhabdosargus haffara* (Forsskål, 1775)	Casual
CRU	*Percnon gibbesi* (H. Milne Edwards, 1853)	Cryptogenic (crypto-expanding)
CRU	*Delavalia inopinata* Scott A., 1902	Excluded
PHY	*Rhodophysema georgei* Batters	Casual
PHY	*Sarconema scinaioides* Børgesen	Casual
POL	*Spiophanes algidus* Meißner, 2005	Casual
POL	*Laonice norgensis* Sikorski, 2003	Casual
POL	*Eurythoe laevisetis* Fauvel, 1914	Cryptogenic (crypto-expanding)
CIRR	*Tetraclita rufotincta* Pilsbry, 1916	Casual
MISC	*Monilicaecum ventricosum* Yamaguti, 1942	Excluded

as *Planaxis savignyi*, whose occurrence in Egypt (Tillier & Bavay 1905) was later disputed by Moazzo (1939), is here excluded.

Among pisces, *Rhabdosargus haffara* has been removed as a rare record.

Among Crustacea previously reported as established is the nibble crab *Percnon gibbesi*, whose pathway of introduction has been questioned (possibly unaided via Gibraltar), hence it is re-classified as a crypto-expanding species, while *Delavalia inopinata* has only been found in the hypersaline Bardawil lagoon in Egypt (Por, 1972). The barnacle *Tetraclita rufotincta* is another case of a single record (Zaouali *et al.*, 2007) mistakenly reported as established by Zenetos *et al* (2017).

Two macroalgal species *Rhodophysema georgei* and *Sarconema scinaioides* are here classified as casuals. *Sarconema scinaioides was* initially considered as established, but its last specimens were found cast ashore in Salamina (Greece) in 2016, which indicates that the Agios Kosmas (Saronikos Gulf, Greece) population is possibly extinct and, hence, casual. *Rhodophysema georgei*, on the other hand, is known from a single record (Verlaque *et al.*, 2015).

The status of two polychaetes, namely *Spiophanes algidus* and *Laonice norgensis*, which were erroneously reported as established by Çinar *et al.* (2021), has been confirmed as casual (M.E. Çinar pers. com.). *Eurythoe laevisetis*, reported in Spain and Malta, is re-assessed here as cryptogenic (crypto-expanding) due to the proximity to its native range (Cape Verde and Canary Islands).

Monilicaecum ventricosum is considered taxon inquirendum (WoRMS Editorial Board, 2021), as it is thought to represent several different species of trematode larvae (Overstreet & Hochberg, 1975), and is thus excluded.

Following the progress in new invasions and change in establishment status since 2010, we report a 40% increase in the number of established species in the Mediterranean Sea (Fig. 2, 3). It is worth noting that of the 298 NIS established in the last 11 years (including the

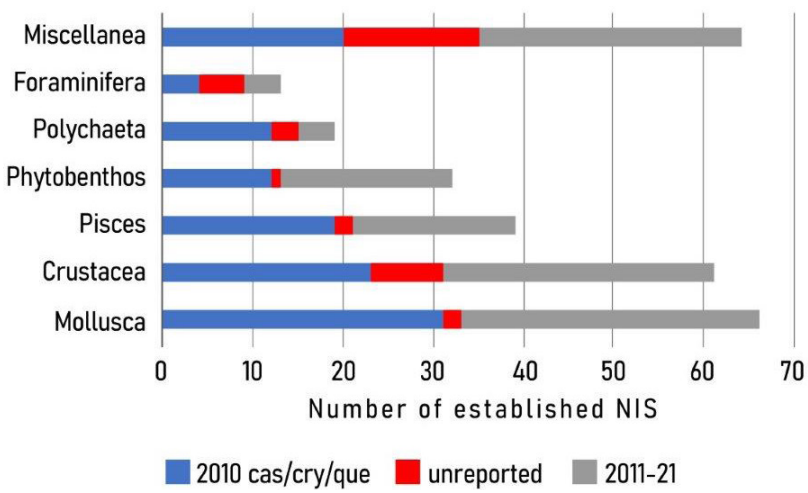

Fig. 2: Change in establishment status of NIS since 2010 (blue bars) and newly reported (2011-21) NIS (grey bars) that are established. Red bars indicate NIS that were already established but unreported in 2010.

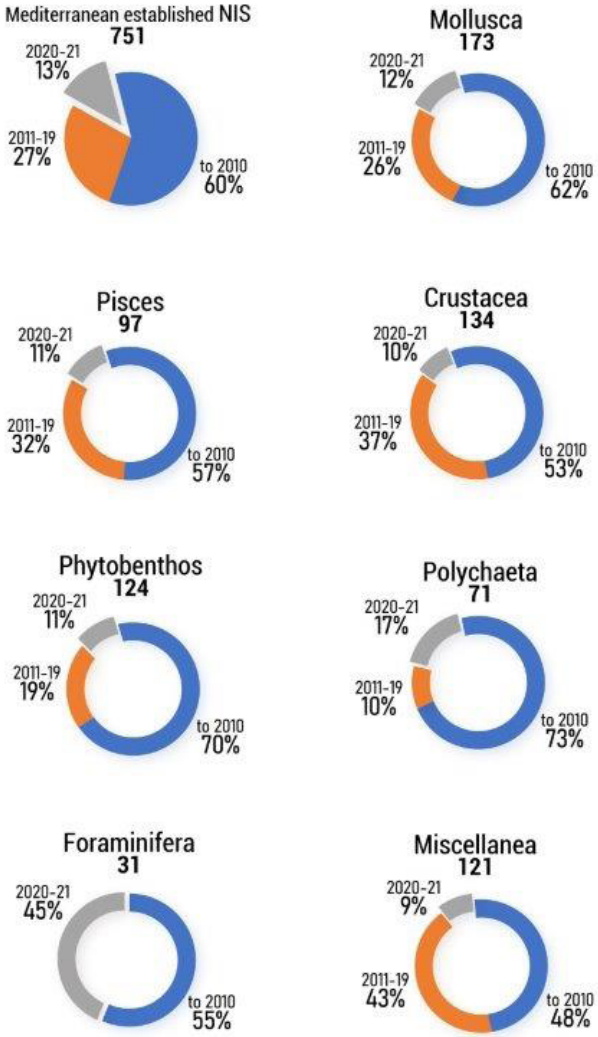

Fig. 3: Overall and per taxonomic group rate of establishment success.

38 unreported taxa, i.e., NIS that escaped our attention or were not included in previous reviews), 121 species were already reported as present (casual/cryptogenic/questionable) by 2010 while 139 taxa were first reported in the period 2011-2021. Figure 2 depicts the number of taxa per group already known by 2010 that were classified as established in the period 2011-2021 and those newly reported that are already established.

In the period 2011-2019, the establishment success was 27% (Fig. 3). The high rate reported in the last 24 months (13%) is more pronounced in polychaetes (17%) and molluscs (12%), whereas it is approximately 10% in most other groups (Fig. 3). The excessive 45% establishment success rate in Foraminifera is due to the fact that there was no account of their status in 2020.

While the number of established species increases, the number of casual NIS declines (Fig. 4). Despite the large number of new publications on new NIS based on a few specimens (casual findings), the number of casual NIS has decreased by 18.8% in the last 11 years. This is clear in molluscs, crustaceans, polychaetes and the miscellaneous taxa where many of the 2010 "casuals" have become established (see Fig. 2).

By December 2021, the total number of established NIS reported in the Mediterranean had reached 751 taxa. Two hundred and forty-two more taxa have been assessed in this study as validated casual NIS, leading to a total of more than 993 NIS. As dynamic as the biological invasion in the Mediterranean is, the inventory of established NIS is very flexible due to changes induced by the fact that: a) some of the casual species will inevitably establish; b) molecular analysis of cryptogenic species may reveal non-indigenous strains; c) some new alien invasive species will make an entrance and establish rapidly; and d) better collaboration and consensus on the status of questionable Foraminifera NIS may lead to numerous established NIS.

The share of established NIS (13%) in the last 24 months is noteworthy. Out of 79 new NIS reported in the period 2020-2021, 17 taxa (21.5%) have already established, a figure well above the 10% prediction of the "tens rule" on establishment success of introduced species (Williamson & Brown, 1986) and much closer to more recent estimates of 25% for introduced invertebrates and plants and 50% for vertebrates, as calculated by Jeschke & Pyšek (2018). On a longer scale (2011-2021), this figure rises even higher –149 NIS have established (42%) out of the 352 taxa reported in the same period. Taking into account the validated species records throughout the whole study period (230 years), the figure climbs to 76%

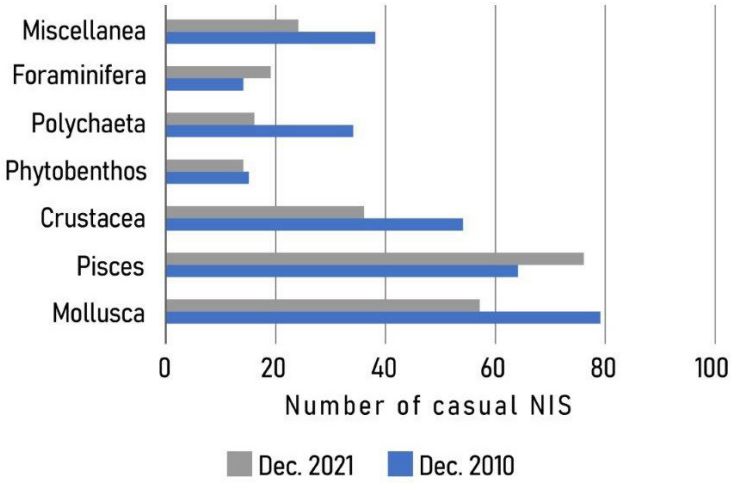

Fig. 4: Comparison of casual NIS numbers registered in 2010 (*Zenetos et al*., 2010) and currently current casual NIS numbers for selected groups.

on average (751 established vs. 242 casual NIS), being higher for phytobenthos and invertebrates (i.e., Polychaeta) and lower for vertebrates.

The seemingly increased rates of NIS establishment in the last two decades may have been triggered by an abrupt regime shift in Sea Surface Temperatures (SST) in the Mediterranean between the mid-1990s and the mid-2000s, which was particularly pronounced in the Eastern Mediterranean and the Adriatic (Nykjaer, 2009), and was indeed identified as a potential cause of an increased rate of introduction of Lessepsian immigrants by Raitsos et al. (2010). The potential contribution of increased propagule pressure due to the enlargement of the Suez Canal (Galil *et al.*, 2015) was refuted by Zenetos (2017), who demonstrated that, in fact, the rate of new NIS introductions slightly decreased in the period 2015-2017. Nevertheless, another important factor is undoubtedly the increase in dedicated research efforts as well as Citizen Science initiatives (Zenetos, 2019; Bailey *et al.*, 2020; Galanidi & Zenetos, 2022) in response to policy requirements and management concerns. This is particularly true for the Southern Mediterranean, which is only starting to be systematically surveyed in recent years. At the same time, the lack of taxonomic expertise in certain taxonomic groups and the concurrent tightening of criteria for accepting species records as valid (e.g., see Marchini *et al*., 2015) may have resulted in the exclusion of a number of casual records, thereby inflating the proportion of established species. These possible artifacts notwithstanding, the continuously rising share of established NIS suggests that Mediterranean communities would be increasingly invaded in the future, marking the unrevertability of biological invasions in the basin.

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Supplementary Data

The following supplementary information is available online for the article:

Annex 1 (xls): List of examined species with their assessed status in the literature and this work.

Annex 2 (xls): Casual or questionable NIS records that were re-examined in this study and their re-assigned status.