

Minor harbours as dispersal hubs in the Mediterranean Sea: The case of non-indigenous species in an Italian port

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ABSTRACT

The Mediterranean Sea is one of the regions most severely affected by marine biological invasions, with maritime transport representing the principal pathway for the introduction and secondary spread of non-indigenous species (NIS). In this context, ports act as key nodes facilitating the establishment and dispersal of fouling-associated taxa. Nevertheless, small and medium-sized harbours remain poorly investigated. This study provides the first comprehensive assessment of NIS and cryptogenic invertebrates associated with the fouling communities of the port of Trapani (north-western Sicily, central Mediterranean Sea), a harbour directly connected to the Egadi Islands Marine Protected Area (MPA). The survey identified 27 taxa, with Peracarida and Polychaeta representing the most diverse components. To contextualise the findings, a Mediterranean-wide occurrence dataset was reconstructed through an extensive literature review. Species distributions were analysed across four Mediterranean biogeographical sectors, revealing strong spatial heterogeneity and marked geographical gaps in sampling effort, particularly along the North African coast. In parallel, maritime traffic data from the port of Trapani were analysed to evaluate potential pathways of dispersal. Results revealed intense liner and cruise vessel traffic connecting Trapani with several Mediterranean ports and transoceanic routes. The strategic position of Trapani, located between the Strait of Gibraltar and the Suez Canal, together with its proximity to the Egadi Islands MPA, highlights its importance as a potential stepping-stone hub for NIS dispersal in the central Mediterranean. Considering the ecological vulnerability of the neighbouring MPA, the high density of NIS recorded suggests a significant risk of spillover. Therefore, we recommend the implementation of early-detection protocols and a data-sharing system with the port authorities. Such management strategies are crucial to evaluate the progression of invasions from and towards this Mediterranean port and to mitigate the socio-economic and ecological impacts of NIS.

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1. Introduction

In the semi-enclosed Mediterranean Sea, maritime transport represents the primary pathway for the introduction of non-indigenous species (NIS), mainly via hull fouling and ballast water discharge (Katsanevakis et al., 2013; Seebens et al., 2013; Flagella and Abdulla, 2005). Once established, marine NIS can become invasive, out-competing native taxa, modifying habitats, altering trophic networks and ecosystem processes, posing risks to human health, and causing substantial economic losses (Katsanevakis et al., 2014; Tsirintanis et al., 2022). Consequently, identifying and assessing introduction and spread pathways are critical steps for developing effective prevention and management strategies aimed at mitigating biological invasions at their earliest stages (Katsanevakis et al., 2013; Nunes et al., 2014; Ulman et al., 2019a). The rate of introductions in the Mediterranean Sea associated with maritime transport has continued to rise markedly over recent decades, in parallel with the intensification of cruise and commercial shipping as well as international maritime trade (Antoniadou et al., 2011; Katsanevakis et al., 2013, 2014; Nunes et al., 2014; Ulman et al., 2019b). Ports serve as key nodes for the introduction and secondary spread of NIS, facilitating their subsequent dispersal to adjacent localities (Sghaier et al., 2019; Ulman et al., 2019b), including Marine Protected Areas (MPAs).

Harbour environments provide favourable conditions for the establishment of fouling communities, assemblages of marine organisms colonizing artificial or natural hard substrates, irrespective of their spatial or temporal persistence. These communities, often associated with ship hulls and submerged or floating structures, act as reservoirs for NIS, which can be translocated through maritime traffic (Ferrario et al., 2017; Ulman et al., 2017; Tempesti et al., 2020a; Rosso et al., 2025; Tempesti et al., 2025). The dominant component of fouling assemblages includes sessile and vagile fauna (Ferrario et al., 2017; Spagnolo et al., 2019; Tempesti et al., 2022a; López-Alonso et al., 2025), whose structural complexity enhances the three-dimensionality of substrates (Sedano et al., 2020) and thereby promotes the subsequent settlement of other sessile species and the persistence of vagile invertebrates.

Although a substantial portion of the literature addresses the distribution of non-indigenous invertebrates associated with biofouling communities in the Mediterranean port areas, numerous minor harbours remain completely unexplored (Sghaier et al., 2019; Tempesti et al., 2020b). This is the case of the Sicilian ports, although their strategic position in the basin suggests the high likelihood that several NIS have been established there (Langeneck et al., 2025). The port of Trapani exemplifies this knowledge gap, as available scientific records are scarce. To date, the only surveys conducted in the area have focused almost exclusively on the last two years, reflecting the fact that monitoring is still inadequate. Beyond the isolated records of the red alga *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon (Barone et al., 2003) and the polychaete *Myrianida pachycera* (Augener, 1913) (Langeneck et al., 2025), the available knowledge on non-indigenous species in the port of Trapani remains extremely limited. More recently, Lo Brutto et al. (2025) documented the occurrence of *Styela plicata* (Lesueur, 1823), *Microcosmus squamiger* Michaelsen, (1927), *Paranthura japonica* Richardson, (1909), *Caprella scaura* Templeton, 1836, *Elasmopus rapax* A. Costa, (1853), *Isognomon* sp., and *Pinctada radiata* (Leach, 1814). The occurrence of the latter two bivalve taxa had already been reported by Lo Brutto et al. (2024), whereas Badalucco et al. (2024) recorded *Mesanthura* cf. *romulea* Poore and Lew Ton, (1986) and *Brachidontes pharaonis* (P. Fischer, 1870). The port of Trapani has a strategic position in north-western Sicily, lying approximately equidistant from the Strait of Gibraltar and the Suez Canal. Moreover, its proximity to the Egadi Islands Marine Protected Area (MPA) underscores the ecological relevance of this study, given the potential risk of NIS spillover into protected environments.

The island is a crossroads of most shipping routes and acts as a "sentinel" for the early detection of NIS, as demonstrated by previous

studies (Servello et al., 2019; Iacifano et al., 2026). This strategic role is vital for monitoring strategies, serving as a frontline to detect new introductions before further spread into the Mediterranean Sea (Servello et al., 2019; Iacifano et al., 2026). Furthermore, its proximity to the Egadi Islands Marine Protected Area (MPA) underscores the ecological significance of this study, particularly regarding the risk of NIS spillover into susceptible protected environments.

In this context, the present study provides the first inventory of NIS and cryptogenic taxa associated with the fouling communities of the port of Trapani and computes the maritime traffic to assess the potential risk of NIS dispersal. This integrated approach aimed to address existing knowledge gaps regarding maritime traffic dynamics (Hilliam et al., 2024), mainly at the site where NIS were detected, and to evaluate the potential for secondary dispersal of the currently established NIS, including possible spread towards the nearby Marine Protected Area.

2. Materials and methods

2.1. Study area

Sicily is the largest island in the Mediterranean Sea with more than 1000 km of coastline, which is increased by several surrounding archipelagos. Sicily faces the Tyrrhenian Sea to the north, the Ionian Sea to the east, and the Strait of Sicily to the south side. The present study focused on the area of Trapani, a city whose characteristic sickle shape extends into a crucial geographical position between the Tyrrhenian Sea and the Strait of Sicily, where the Atlantic current, entering from the Strait of Gibraltar, bifurcates heading towards these two sub-basins. Additionally, Trapani is halfway between the Suez Canal and the Strait of Gibraltar, establishing itself as a crossroad of NIS dispersal pathways and international maritime traffic in Mediterranean Sea (Supplementary Material 1).

Five sampling stations within the harbour were selected: Molo Ronciglio (38.009722 N, 12.513083 E), Molo Columbus (38.014528 N, 12.500806 E), Lega Navale (38.013611 N, 12.498361 E), and Fishing port 1 and 2 (38.015306 N, 12.498889 E; 38.016444 N, 12.497750 E, respectively) (Fig. 1). Two samplings were carried out at Molo Ronciglio in December 2023 and May 2024; one at Molo Columbus on March 2024, two within the fishing port 1 and 2 on March and July 2024, and one at Lega Navale station on March 2025. The sampling stations were selected to ensure extensive coverage of the entire port area.

2.2. Samples collection

A qualitative-based sampling approach was employed. In each station, macroinvertebrates of the fouling communities were collected by scraping on mooring lines and port walls at the upper-infralittoral. On port walls, the sampling was carried out using a specific hand net consisting of a 0.2 mm mesh-size bag, attached to the mouth of a 20 × 20 cm stainless-steel frame. All the species were immediately placed in tanks with seawater for a preliminary 'live' sorting, and then transported to the laboratory, where they were observed. Subsequently, the species were fixed in absolute ethyl alcohol (96%) for future genetic analysis. Most of the organisms were identified at the lowest possible taxonomic level. Only NIS and cryptogenic species were selected for analysis.

2.3. Genetic data

DNA analyses were performed on collected species for which sequence data were unavailable in public databases. A few individuals of every species were processed molecularly, although not all of them worked due to the degraded conditions of some of them. For some amphipod species, it was possible to process them molecularly under the Biodiversity Genomic Europe (BGE) framework, in the project Mediterranean and Adjacent Seas Amphipod Reference Library (MEDAMP).



Fig. 1. (a) Geographical location of the city of Trapani on the western coast of Sicily, in the Mediterranean Sea. (b) Map of the harbour area of Trapani showing the five sampling stations: Fishing ports 1–2, Lega Navale, Molo Columbus, Molo Rongiglio. Satellite pictures by ©2026 Google, Landsat/Copernicus (a) and ©2026 Google, Airbus (b).

The specimens were dissected and only one pleopod or pereopod was taken for DNA isolation. The rest of the procedure, from DNA isolation to cytochrome oxidase subunit 1 (COI) amplicon sequencing, followed Pešić et al. (2024). All specimen metadata and sequences are available in the dataset DS-AMPTP (accessible at: <https://doi.org/10.5883/DS-AMPTP>) accessible in the public database Barcode of Life Data Systems (BOLD; Ratnasingham et al., 2024). Their identities were first confirmed through the Batch ID Engine tool and when their Barcode Index Numbers (BINs) were assigned in BOLD (Ratnasingham and Hebert, 2013).

2.4. Mediterranean distribution of the identified NIS

A comprehensive dataset comprising all available Mediterranean records of the 27 species examined in this study was compiled from published sources. More than 250 scientific articles were reviewed (Supplementary Material 2, Table S2), and species occurrence records were extracted together with their associated geographical coordinates (latitude and longitude). All records were georeferenced and visualised using QGIS 3.40 Bratislava (QGIS.org, QGIS, 2025) on a Mediterranean basin map subdivided, according to the European Union (EU) Marine Strategy Framework Directive, into the Western Mediterranean Sea (WMDS), the Central Mediterranean Sea and Ionian Sea (CMDS), the Adriatic Sea (ADRIA), and the Eastern Mediterranean Sea (EMDS) (EEA,

2015). The corresponding dataset is provided in Supplementary Material 2, Table S2 and accessible at <https://doi.org/10.15468/x82kjj>. The creation of this extensive dataset was fundamental to contextualizing the results obtained in Trapani within the Mediterranean area. It allowed for the reconstruction of the spatial and temporal distribution of the 27 NIS found in the port, while also identifying Mediterranean NIS hotspots.

All publications used to construct the dataset were classified as records of single species, mono-taxa checklists or multi-taxa checklists, calculating their percentage frequency (Supplementary Material 3, Table S3).

2.5. Maritime traffic

To assess the extent of the maritime traffic of Trapani harbour over four years, 2022 – 2025, information on the number of liner and cruise ships arriving and departing from the port was retrieved from the official registers of the “Autorità di Sistema Portuale del Mare di Sicilia Occidentale” (Supplementary Material 1, Tables S1, A–D). The primary objective of this analysis was to quantify the activity of a minor port under the Mediterranean framework and to evaluate its connectivity with the neighbouring Egadi Islands MPA. Despite its strategic importance, the maritime traffic estimate is frequently overlooked in NIS inventories; therefore, providing these data was essential to characterize

the potential risk of primary introductions and subsequent secondary dispersal towards high-value natural areas.

The number of liner ships approaching the port was estimated per year, and it was constant throughout the four years examined. The liner ships followed fixed routes from and towards the Egadi Islands Marine Protected Area (MPA) and from and towards Pantelleria Island, which is a National Park. Regarding the cruises, the number of ships docking in the port of Trapani was recorded for each year.

Regarding commercial shipping, it was not possible to obtain a reliable estimate of the number of vessels and their trajectories. The official records for this sector are not accessible, and the available data were fragmented and incomplete. Such inconsistencies prevented a rigorous reconstruction for commercial ships, making these data unsuitable for the purposes of this study. Consequently, the potential NIS introduction risk associated with commercial traffic may be underestimated in our current analysis, representing a limitation that future studies should address.

The data were mapped using QGIS 3.40 Bratislava (QGIS.org, QGIS, 2025). QGIS Geographic Information System. QGIS Association. <http://www.qgis.org>.

2.6. Spatial and temporal analyses of NIS

Georeferenced occurrence records of NIS were compiled from the study dataset, which included country, year, locality, latitude, longitude, and species identity for each record. Temporal information was standardised by extracting the first available four-digit year from each entry, thereby harmonising heterogeneous date formats including year ranges, month-year combinations, and full calendar dates. Records lacking temporal information were retained for spatial analyses but excluded from temporal analyses.

All coordinates were converted into a spatial object using the WGS84 geographic coordinate system and subsequently projected to UTM zone 33 N to perform metric distance calculations. The harbour of Trapani (western Sicily, Italy) was represented as a fixed reference point located at 38.0140° N, 12.5070° E. Euclidean distances between each NIS record and Trapani harbour were calculated in kilometres.

To evaluate the potential hotspot role of the Trapani harbour, analyses were conducted at four spatial extents: (i) the entire Mediterranean dataset and (ii) three concentric buffers centred on Trapani harbour with radii of 300, 500, and 700 km. This hierarchical framework allowed the evaluation of whether harbour-related spatial effects remained detectable across progressively broader spatial scales.

Species richness was analysed using Generalized Linear Models (GLMs) with a negative binomial error distribution and logarithmic link function to account for overdispersion in count data. Prior to modelling, occurrence records were aggregated into 5-km grid cells by year. For each cell-year combination, the number of NIS species and the mean distance from Trapani harbour were calculated. The response variable was NIS richness per grid cell-year, whereas predictors included log-transformed distance from Trapani harbour and sampling year.

Spatial clustering around Trapani harbour was further assessed using Monte Carlo hotspot tests. For each spatial extent, the observed number of records occurring within increasing radii from the harbour (1–100 km) was compared against 999 random spatial simulations generated within the same spatial domain. Upper-tail probabilities were used to estimate the likelihood that the observed concentration of records around Trapani occurred by chance.

To visualise spatial patterns of NIS occurrence probability, kernel density estimation (KDE) was applied to the occurrence coordinates. KDE probability surfaces were calculated both at the Mediterranean scale and within the 300-km regional buffer around Trapani harbour. Probability values were constrained exclusively to marine areas by applying a land mask derived from Natural Earth coastlines; terrestrial cells were forced to zero probability prior to normalisation. Relative probability values therefore represented marine-only occurrence

likelihoods scaled between 0 and 1.

Temporal dynamics were analysed by examining the relationship between year of first detection and minimum distance from Trapani harbour for each species. Both Spearman rank correlations and ordinary least squares regressions were used to test whether newly detected species progressively occurred closer to the harbour through time.

3. Results

3.1. NIS detection

A total of 27 NIS and cryptogenic species were collected in the port of Trapani (Table 1; Fig. 2). Some of these had previously been documented in the area. Specifically, our data confirmed the presence of the polychaete *M. pachycera*, the tunicates *S. plicata* and *M. squamiger*, the peracarids *P. japonica*, *M. cf. romulea*, *C. scaura*, and *E. rapax*, and the bivalves *Isoptomon* sp., *P. radiata*, and *B. pharaonis* (Badalucco et al., 2024; Lo Brutto et al., 2024; Langeneck et al., 2025; Lo Brutto et al., 2025). All the remaining species (17) represented first records for the port of Trapani.

The recorded taxa covered eight high taxonomic groups: Porifera (1 species), Bivalvia (3 species), Polychaeta (5 species), Pycnogonida (1 species), Amphipoda (8 species), Isopoda (3 species), Bryozoa (3 species), and Tunicata (3 species). Overall, the fouling community comprised 23 families and 25 genera.

The NIS were predominantly represented by Amphipoda, with six species distributed across four families and five genera, followed by Polychaeta, with five species belonging to four families and four genera. Isopoda, Bivalvia, and Tunicata were each represented by three species, all belonging to distinct families and genera, while Bryozoa were represented by a single species.

Four taxa were classified as cryptogenic: two amphipod species (*Erichthonius brasiliensis* and *E. rapax*), belonging to two different families, and two bryozoan species (*Amathia verticillata* and *Bugula neritina*), similarly belonging to separate families. These species were assigned to the cryptogenic category and their native range is currently reported in the literature as uncertain (Table 1).

Regarding biogeographical origins, eight species were native to the Pacific Ocean, four to the Indo-Pacific region, three to the Indian Ocean, three to the Red Sea, and four to the Atlantic Ocean (Table 1).

3.2. Remarks on some species

Taking into account of historical misidentifications, weakly supported records, and/or taxonomic uncertainties arising from incomplete or ambiguous original descriptions, some species were identified with particular caution (Lorenti et al., 2009; Krapp-Schickel, 2015). Notably, the Pycnogonida *Anoplodactylus californicus*, the Amphipoda *Stenothoe gallensis*, and the Isopoda *Mesanthura romulea* present taxonomic issues that warrant further investigation and may benefit from targeted molecular analyses in future studies.

Anoplodactylus californicus was identified following Müller and Krapp (2009), Child (1992) and Lucena and Christoffersen (2018) (Fig. 2g). The collected *A. californicus* adult males showed the main diagnostic characters: trunk elongate, segments 3 and 4 fused. Ocular tubercle conical. Eyes oval, large, distinctly pigmented. Palp vestigial, palp buds present at base of anterior lateral processes. Legs long. Femur, tibia 1 and tibia 2 with a dorso-distal long seta. Genital spur on legs 3 and 4 relatively long. Cement gland tube situated dorso-medially on proximal half of femur, shaped like a short truncate cone. Propodus with 2 stout spines on heel. *Anoplodactylus californicus* was originally described by Hall in 1912. The type locality from the original description is Laguna Beach, California, located on the Eastern Pacific. The taxonomic history of the species is complex, as Hall (1912)'s initial description and illustration were considered insufficient, and the type material was thought to be lost until it was rediscovered and re-examined by Child (1987).

Table 1

List of NIS and cryptogenic species recorded in the port of Trapani. For each species, the native range or cryptogenic status and reference are indicated, as well as the reference of the first record in the Mediterranean Sea.

Species	Native range	Cryptogenic status	Reference of the native range or cryptogenic status	Reference of the first Mediterranean record
Porifera				
<i>Paraleucilla magna</i> Klautau, Monteiro and Borojevic, (2004)	Indo-Pacific	—	Klautau et al. (2004)	Longo et al. (2007)
Bivalvia				
<i>Brachidontes pharaonis</i> (P. Fischer, 1870) ^a	Red Sea	—	Battiata et al. (2024)	Fuchs (1878)
<i>Isognomon</i> sp. ^a	—	—	—	—
<i>Pinctada radiata</i> (Leach, 1814) ^a	NW Indian Ocean and Red Sea	—	Wada and Tëmkin (2008)	Monterosato (1878)
Polychaeta				
<i>Branchiommata bairdi</i> (McIntosh, 1885)	W Atlantic Ocean	—	McIntosh (1885)	Arias et al. (2013)
<i>Branchiommata luctuosum</i> (Grube, 1870)	Red Sea	—	Grube (1870)	Knight-Jones et al. (1991)
<i>Dorvillea similis</i> (Crossland, 1924)	Red Sea	—	Crossland (1924)	Toso et al. (2024)
<i>Lumbrineris perkinsi</i> Carrera-Parra, (2001)	Caribbean Sea	—	Carrera-Parra (2001)	Campoy (1982)
<i>Myrianida pachycera</i> (Augener, 1913) ^a	Indo-Pacific	—	Augener, (1913)	Langeneck et al. (2025)
Pycnogonida				
<i>Anoplodactylus californicus</i> Hall, (1912)	E Pacific Ocean	—	Hall (1912)	Stock (1958)
Amphipoda				
<i>Caprella scaura</i> Templeton, 1836 ^{ab}	W Indian Ocean	—	Krapp et al. (2006)	Mizzan (1999)
<i>Elasmopus rapax</i> A. Costa, (1853) ^a	—	Cryptogenic	Hughes and Lowry (2010)	Costa (1853)
<i>Erichthonius brasiliensis</i> (Dana, 1853) ^b	—	Cryptogenic	Dana (1853)–(1855)	Della Valle (1893)
<i>Jassa slatteryi</i> Conlan, (1990) ^b	N Pacific Ocean	—	Beermann et al. (2020)	Conlan (1990)
<i>Laticorophium baconi</i> (Shoemaker, 1934) ^b	NE Pacific Ocean	—	Shoemaker (1934)	Guerra-García et al. (2023)
<i>Paracaprella pusilla</i> Mayer, (1890)	W Atlantic Ocean	—	Mayer (1890)	Ros et al. (2013)
<i>Stenothoe gallensis</i> Walker, (1904)	Indian Ocean	—	Walker (1904)	Zakhama-Sraieb and Charfi-Cheikhrouha (2010)
<i>Stenothoe georgiana</i> Bynum and Fox, (1977)	W Atlantic Ocean	—	Bynum and Fox (1977)	Fernández-González and Sánchez-Jerez (2017)
Isopoda				
<i>Mesanthura</i> cf. <i>romulea</i> Poore and Lew Ton, (1986) ^a	Presumably W Pacific	—	Poore and Lew Ton (1986)	Lorenti et al. (2009)
<i>Paracerceis sculpta</i> (Holmes 1904)	N Pacific Ocean	—	Hewitt and Campbell (2001)	Rezic (1978)
<i>Paranthura japonica</i> Richardson, (1909) ^a	NW Pacific Ocean	—	Richardson (1909)	Marchini et al. (2014)
Bryozoa				
<i>Amathia verticillata</i> (delle Chiaje, 1822)	—	Cryptogenic? – Caribbean Sea	Galil and Gevili (2014)	delle Chiaje (1822)
<i>Bugula neritina</i> (Linnaeus, 1758)	—	Cryptogenic? – unknown American locality	Ryland et al. (2011)	Linnaeus (1758)
<i>Tricellaria inopinata</i> d'Hondt and Occhipinti Ambrogio, (1985)	Indo-Pacific	—	Dyrynda et al. (2000)	d'Hondt and Occhipinti Ambrogio (1985)
Tunicata				
<i>Distaplia bermudensis</i> Van Name, (1902)	W Atlantic Ocean	—	Van Name (1902)	Mastrototaro and Brunetti (2006)
<i>Microcosmus squamiger</i> Michaelsen, (1927) ^a	Indo-Pacific	—	Michaelsen (1927)	Monniot (1981)
<i>Styela plicata</i> (Lesueur, 1823) ^a	NW Pacific Ocean	—	de Barros et al. (2009)	Philippi (1843)

^a Indicates species recorded by Badalucco et al. (2024), Lo Brutto et al. (2024), Langeneck et al. (2025) and Lo Brutto et al. (2025) in the port of Trapani.

^b Indicates species identifications supported also with COI barcoding approach.

Due to this inadequate initial description and the resulting taxonomic confusion, the species has been misidentified under several names (Lucena and Christoffersen, 2018). According to Marcus (1940), *Anoplodactylus stictus* is very similar to *A. californicus*, but can be distinguished mainly by its larger size, the presence of a propodal cutting lamina and a small protuberance on the movable finger of the chela (Stock, 1992). Consequently, the two species cannot be misidentified.

The identification of the samples as *Stenothoe gallensis* was congruent with the original description by Walker (1904) and further observations by Krapp-Schickel (2015). The adult male of *S. gallensis* collected here (Fig. 3a) exhibited the main diagnostic characters; the first gnathopod merus was produced distally, with the tip extending beyond the end of the carpus (Fig. 3b–c). Regarding the second gnathopod, the merus margin was crenulated, with shallow incisions and a setae inserted in each notch; the propodus showed two distal palmar humps near the dactylus insertion; the palm was straight and densely covered with long setae; the dactylus was also densely covered in setae, approximately half the length of those on the palm, with sparse setae on the anterior margin

(Fig. 3d–e). The third uropod peduncle was spinose, and rami showed a geniculation and sculpture of the second article (Fig. 3f–g). It is noteworthy that our samples supported the presence of the species in the Mediterranean Sea.

Given the current state of knowledge, the collected specimens belonging to the genus *Mesanthura* were not identified as *M. romulea sensu stricto*, but as *Mesanthura* cf. *romulea*. The identification was based on the morphological description of *M. romulea sensu stricto* by Poore and Lew Ton (1986) and specifically on the pigmentation, the shape of the first pereopods, particularly the sixth article, and the shape of telson and uropodal exopods. Collected specimens showed an extensive dorsal pigmentation pattern and a uropodal exopod shape congruent with the description drawings (Fig. 4a–b–c). Regarding the first pereopod, the description showed the article 6 elongate, with palm oblique and concave, carrying a proximal toothed mesial projection; the dactyl posteriorly concave, characterized by a proximal prominent toothed area (Fig. 4d). The samples herein collected did not show such a shape (Fig. 4e). Furthermore, as already observed in the Mediterranean

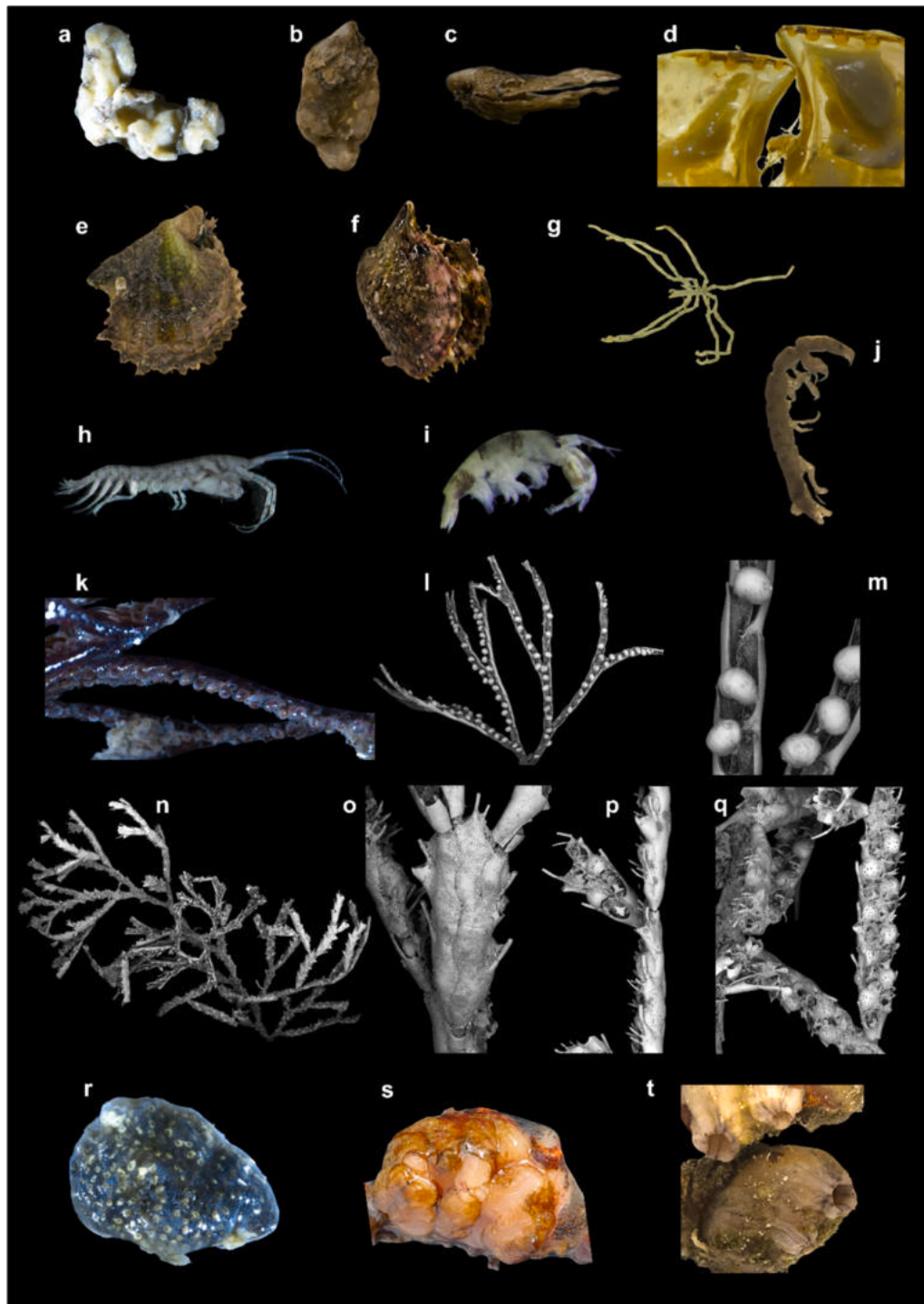


Fig. 2. Selected NIS and cryptogenic species collected in the port of Trapani: (a) Porifera: *Paraleucilla magna*; (b, c, d) Bivalvia: *Isognomon* sp.; (e, f) Bivalvia: *Pinctada radiata*; (g) Pycnogonida: *Anoplodactylus californicus*; (h) Amphipoda: *Erichthonius brasiliensis*; (i) Amphipoda: *Laticorophium baconi*; (j) Isopoda: *Paranthura japonica*; (k, l, m) Bryozoa: *Bugula neritina*; (n, o, p, q) Bryozoa: *Tricellaria inopinata*; (r) Tunicata: *Distaplia bermudensis*; (s, t) Tunicata: *Styela plicata*.

samples by Lorenti et al. (2009), the pleon showed a butterfly-shaped patch on the sixth free pleonite, which was not present in the original description (Fig. 4f). Finally, the original description reported a telson with lateral margins barely dilating proximally, apex broadly rounded, and 6 apical setae (see drawing by Poore and Lew Ton, 1986 in Fig. 4g), different from the moderately acute telson of the present samples (Fig. 4h).

3.3. Mediterranean NIS occurrence

A dataset of the Mediterranean occurrences for the 27 species considered in this study was compiled from the literature (Supplementary Material 2, Table S2), comprising 2079 occurrence records and 1447 georeferenced coordinates (Fig. 5a). Among the analysed taxa, *P. radiata* exhibited the highest number of records across the Mediterranean basin, whereas *D. bermudensis*, *M. pachycera*, and *S. gallensis* were represented by the fewest records (Fig. 5b).

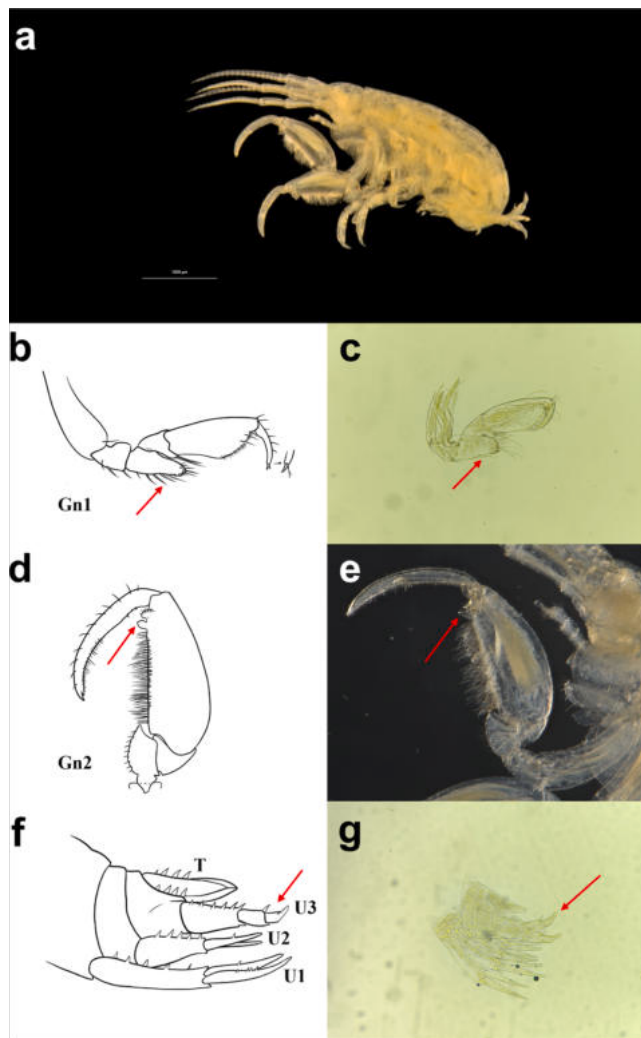


Fig. 3. (a) Lateral view of an adult male specimen of *Stenothoe gallensis* sampled in Trapani; (b) illustration of gnathopod 1 (Gn1) of the *S. gallensis* original description; (c) gnathopod 1 of *S. gallensis* sampled in Trapani. The red arrows indicate the merus, with the tip surpassing the end of the carpus; (d) illustration of gnathopod 2 (Gn2) of the *S. gallensis* original description; (e) gnathopod 2 of *S. gallensis* sampled in Trapani. The red arrows indicate the two palmar distal humps near dactylus insertion in the propodus of gnathopod 2; the palm is straight and beset with long and dense setae. (f) Illustration of the telson and uropods of the *S. gallensis* original description (Krapp-Schickel, 2015); (g) photograph of the telson and uropods of *S. gallensis* sampled in Trapani. The red arrows indicate the third uropod peduncle which is spinose and the distal article of the ramus which is geniculate.

The highest number of reports was obtained in the western Mediterranean and Adriatic sectors (Fig. 5c). Twenty-two species were found in ADRIA, 24 in EMDS, 26 in CMDS, and 27 in WMDS. The records originated from 19 countries. The country with the highest number of records was Italy (791 records), followed by Slovenia (238) and Spain (223). Similarly, Italy had the highest number of the species considered (27), followed by Spain (22), France (20), and Türkiye (20). Syria, Libya, Montenegro, and Albania showed the lowest numbers of records and species.

3.4. Maritime routes

A high level of maritime traffic was detected. The liner vessel movements, exclusively serving the routes connecting Trapani with the Egadi Islands MPA and Pantelleria Island, were calculated to be 6708

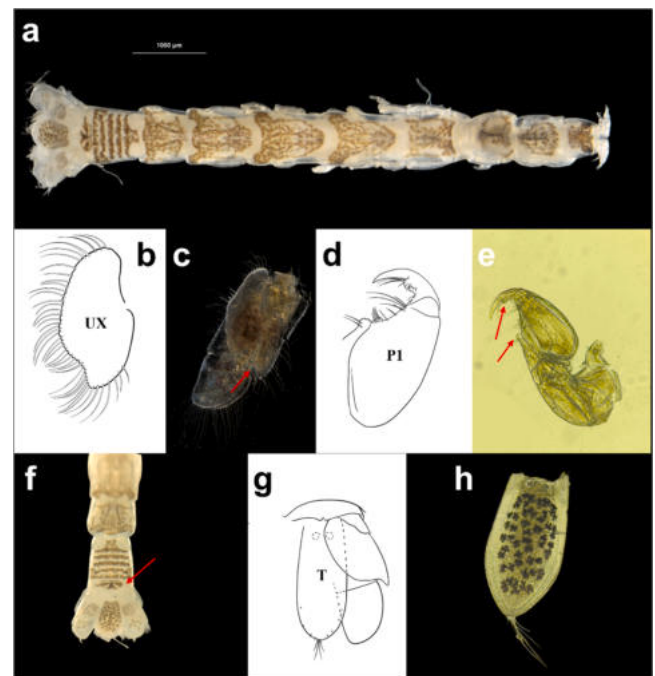


Fig. 4. (a) Dorsal view of an adult female specimen of *Mesanthura cf. romulea* sampled in the port of Trapani (Italy); (b) illustration of uropodal exopod (UX) of the *M. romulea* original description (Poore and Lew Ton, 1986), congruent with (c) uropodal exopod of *M. cf. romulea* sampled in Trapani; (d) illustration of pereopod 1 propodus (P1) of the *M. romulea* original description congruent with (e) pereopod 1 of *Mesanthura cf. romulea* from Trapani. The toothed structures of *Mesanthura romulea* in the propodus palm and dactylus are present in both (indicated with red arrows) but appear less pronounced in the Trapani specimens. (f) Dorsal view of the pleon, telson and uropods of an adult female specimen of *Mesanthura cf. romulea* sampled in Trapani. Note the detail of the butterfly-shaped pigmentation on the posterior margin of the pleon (indicated with red arrow), which is absent in the original description. (g) Illustration of the urotelson of the *M. romulea* original description; (h) telson of *Mesanthura cf. romulea* sampled in Trapani, with a shape triangular towards the apex.

arrivals/departures per year. Regarding the cruise traffic, the first three years (2022–2024) were characterised by a constant level of activity, with an average of 41 port calls, whereas a significant increase to 64 cruise ship calls was detected in 2025, representing a growth of 57.4%. This surge is primarily linked to new regional tourism development policies and recent infrastructural enlargement of the docks, which have increased the harbour's capacity for larger vessels. A substantial proportion of these cruises operated within the western Mediterranean basin (Supplementary Material 1, Tables S1, A–D).

The longest itinerary in 2025 was undertaken by a cruise vessel departing from Fort Lauderdale (Florida, USA) and concluding its voyage in Barcelona (Spain), after calling at 74 ports across 25 countries (Supplementary Material 1, Tables S1, E–F). The port of Trapani was the 68th port of call along this route, after traversing the Atlantic Ocean, the Pacific Ocean, the Antarctic, and the Mediterranean Sea (Fig. 6).

3.5. Trapani harbour as a regional hotspot for NIS occurrence

Spatial probability surfaces revealed a marked concentration of NIS occurrence around the western Sicilian sector centered on Trapani harbour (Fig. 7). At the Mediterranean scale, elevated occurrence probabilities were distributed along several coastal sectors, although the highest regional concentration was consistently detected in the Trapani area. Within the 300 –km regional buffer, the KDE analysis showed a pronounced spatial aggregation immediately surrounding Trapani harbour and adjacent coastal waters (Tunisia and Malta), supporting the hypothesis that the harbour acts as a regional hotspot for NIS occurrence

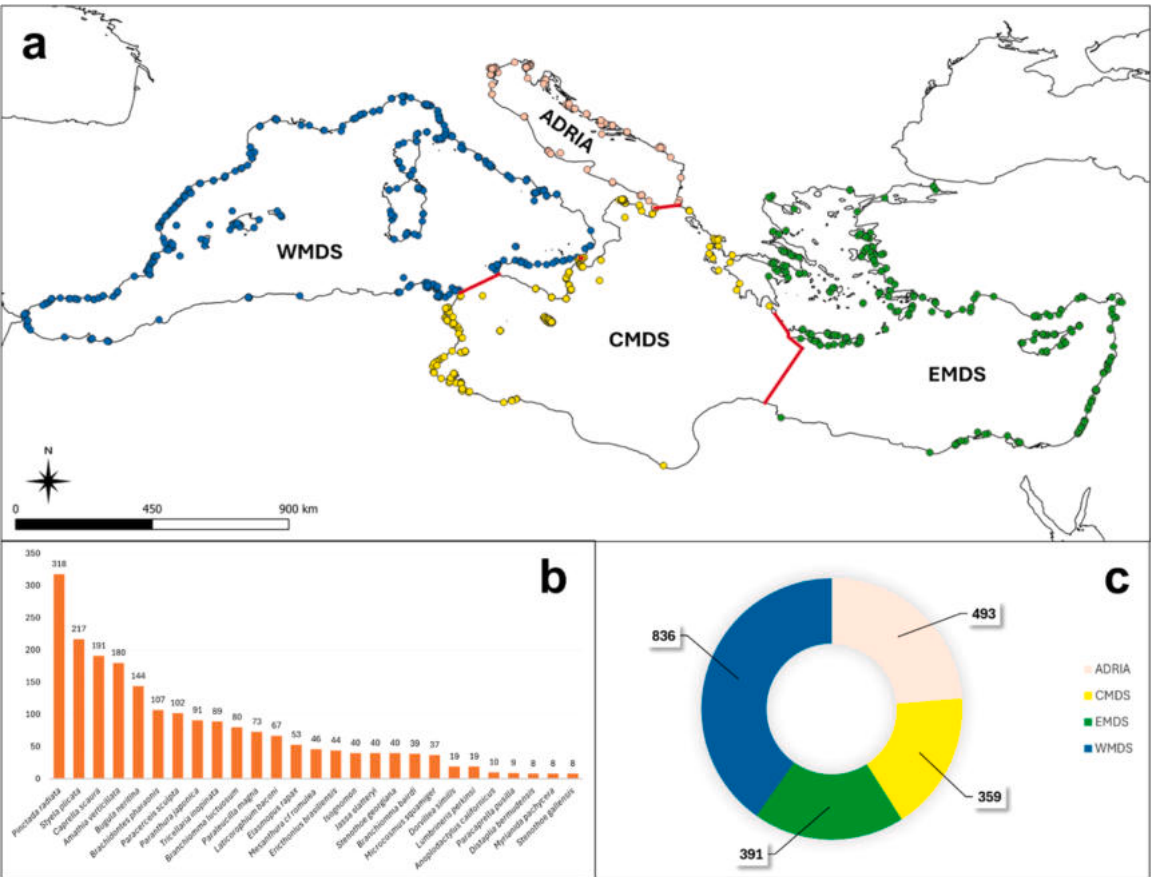


Fig. 5. Distribution of the 27 studied species in the Mediterranean Sea. (a) Map of the 2079 records for a total of 1447 geographic coordinates, (b) number of records per species (c) number of records based on the four biogeographical areas. WMDS: Western Mediterranean Sea, CMDS: Central Mediterranean and Ionian Sea, ADRIA: Adriatic Sea, EMDS: Eastern Mediterranean Sea. The dataset is available in [Supplementary Material 2, Table S2](#).

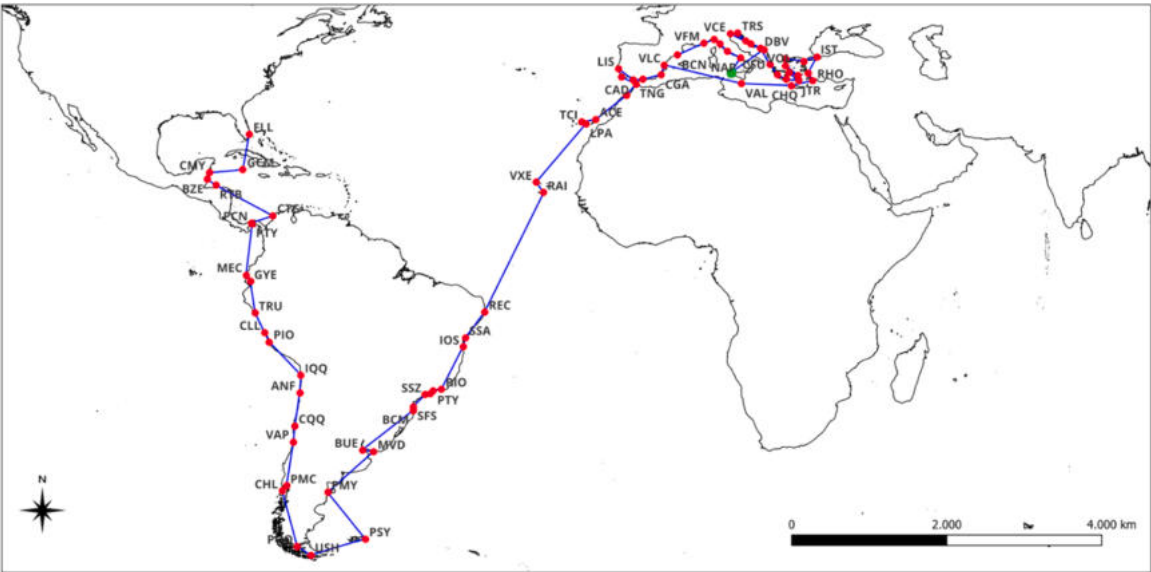


Fig. 6. Reconstruction of the case of an international cruise ship that docked at the port of Trapani in 2025. Acronyms indicate port localities, as reported in [Supplementary Material 1, Tables S1, E–F](#). The green point indicates the harbour of Trapani.

and potential secondary spread.

Monte Carlo hotspot analyses strongly supported non-random spatial aggregation around Trapani harbour across all spatial extents. In all tested radii and buffers, observed record counts near the harbour were

significantly higher than expected under random spatial distributions (all upper-tail p – values = 0.001).

Negative binomial GLMs consistently identified a significant negative relationship between NIS richness and distance from Trapani

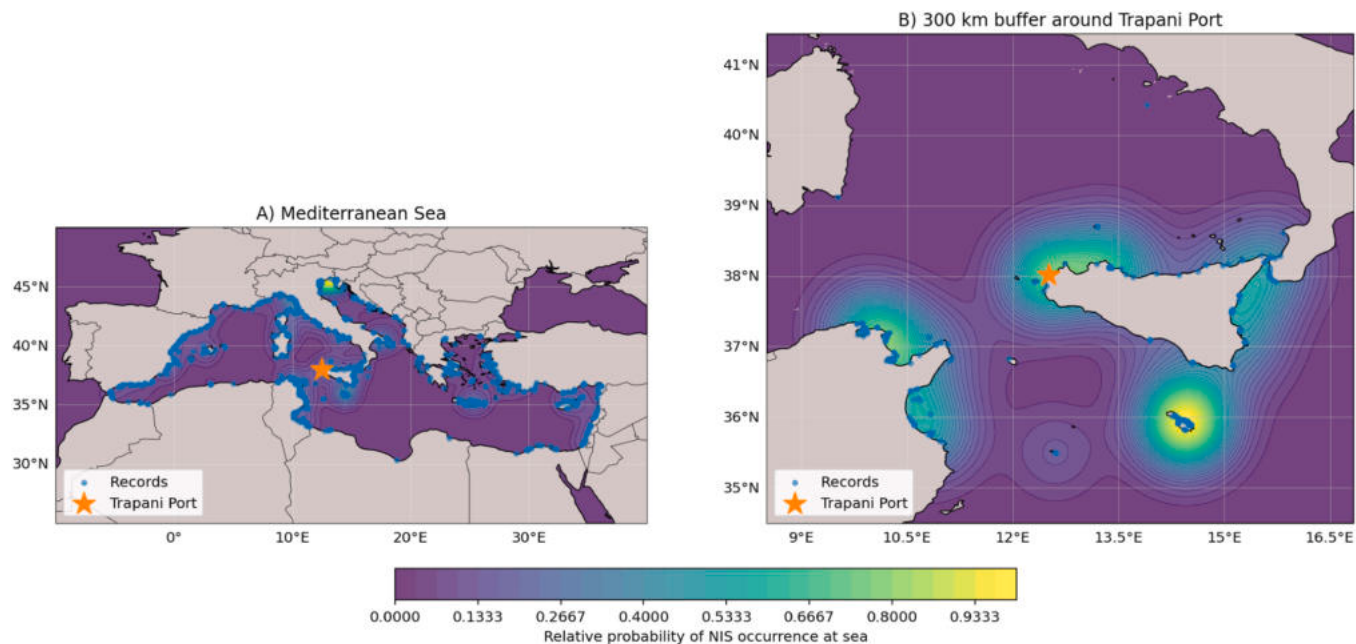


Fig. 7. Kernel density estimation (KDE) probability maps of non-indigenous marine species occurrence. (A) Mediterranean-wide relative probability surface showing regional hotspots of occurrence probability across the basin. (B) Relative probability surface within the 300 –km buffer centered on Trapani harbour, highlighting the marked spatial aggregation of records surrounding the harbour area. Probability values were constrained exclusively to marine environments by assigning zero probability to terrestrial cells prior to normalisation.

harbour across all spatial extents (Table 2). The effect size was strongest within the 300 –km buffer and progressively weakened at broader scales, although remaining statistically significant at the Mediterranean level. Specifically, the coefficient associated with log–transformed distance declined from -0.2898 in the 300 –km buffer to -0.1503 at the Mediterranean scale. These results indicate that the richness of the shared NIS decreases with increasing distance between the Trapani harbour and all around Mediterranean georeferenced records and that the harbour effect as “dispersal hub” is stronger at regional scale.

A positive effect of year was detected at the Mediterranean scale and within the 700 –km buffer, suggesting a gradual increase in NIS richness through time across broader spatial extents. In contrast, temporal effects were not significant within the 300 –km and 500 –km buffers.

Temporal analyses based on first species detections showed a significant negative relationship between year of first occurrence and distance from Trapani harbour only within the 300-km buffer (Spearman $\rho = -0.522$, $p = 0.005$; linear regression slope $= -1.98 \text{ km year}^{-1}$, $p = 0.045$). No significant temporal trends were detected at broader spatial extents, including the Mediterranean–wide dataset.

4. Discussion

This study aimed to assess the occurrence and diversity of NIS within an unexplored minor harbour of the Mediterranean Sea and to evaluate

the potential connectivity of the port of Trapani with other harbours, both within and beyond the Mediterranean basin. A total of 27 species, including non–indigenous and cryptogenic taxa (hereafter collectively referred to as NIS), were identified (Table 1), the majority of which are widely distributed across the Mediterranean Sea. Among the recorded taxa, Peracarida and Polychaeta were the most represented groups.

4.1. Peracarida

Peracarida was the most representative taxon in the fouling of the port of Trapani, specifically eight amphipod and three isopod species were detected.

The amphipod *Caprella scaura*, native to the western Indian Ocean, is a species dispersed in all seas and oceans around the world (Krapp et al., 2006). Its first record in Italy occurred in 1994 in the northern Adriatic basin (Mizzan, 1999), and it is considered to be widespread along the entire Italian coastline (Dailianis et al., 2016; Tempesti et al., 2020b; Badalucco et al., 2026).

The amphipod *Elasmopus rapax* was originally described in Italy (Costa, 1853). Subsequently, Hughes and Lowry (2010) analysed material from the Mediterranean and Australian areas and argued for its cryptogenic status. The same status can be attributed to *Erichthonius brasiliensis*, which was described from Brazil (Dana, 1853–1855) and shows a wide distribution.

Table 2
Negative binomial generalized linear models testing the effects of distance from Trapani harbour and year on non-indigenous species richness across spatial extents. Significant negative coefficients for log–transformed distance indicate decreasing species richness with increasing distance from the harbour.

Spatial extent	Number of observations	Predictor	Estimate	SE	z-value	p-value
Mediterranean	954	log distance to port	-0.1503	0.048	-3.161	0.002
Mediterranean	954	year	0.0069	0.003	2.426	0.015
300 km buffer	182	log distance to port	-0.2898	0.096	-3.007	0.003
300 km buffer	182	year	0.0124	0.009	1.448	0.148
500 km buffer	337	log distance to port	-0.1991	0.086	-2.305	0.021
500 km buffer	337	year	0.0099	0.005	1.844	0.065
700 km buffer	438	log distance to port	-0.1608	0.079	-2.047	0.041
700 km buffer	438	year	0.0122	0.005	2.313	0.021

Jassa slatteryi is an invasive amphipod that has long been considered cryptogenic (Fernández-González and Sánchez-Jerez, 2017; Conlan et al., 2021). The first documented Italian record was in the central Tyrrhenian Sea, although it had already been present in the northern Adriatic (Badalucco et al., 2026), where it was originally described as a new species (Bonifazi et al., 2018). A recent study has clarified its North Pacific origin, and suggested that, since its historical invasion events began centuries ago, *J. slatteryi* can be classified as a neocosmopolitan species (Beermann et al., 2020).

Laticorophium baconi is a small invasive and opportunistic amphipod native to the Pacific coast of North and Central America. It was recorded for the first time in the Mediterranean Sea in 2018 in association with fouling assemblages on mooring buoys along the Spanish coast (Gouillieux and Sauriau, 2019). It was reported along the Italian coast only in 2023, but likely already occurred for longer, albeit unrecorded due to misidentification with the strikingly similar species *Apocorophium acutum* (Guerra-García et al., 2023).

The amphipod *Paracaprilla pusilla* is native to the western Atlantic (Cabezas et al., 2019). After its first record in the European waters in 2010 (Ros and Guerra-García, 2012) and the subsequent arrival in the western Mediterranean (Ros et al., 2013), the species moved eastward to Israel (Lo Brutto et al., 2019). Our findings in Trapani document the first record of the species and the genus *Paracaprilla* for Italy.

Stenothoe gallensis was originally described from the Indian Ocean, Sri Lanka. Several species were misidentified with it (Krapp-Schickel, 2015), leading to its designation as a cosmopolitan amphipod species. Such inaccuracies in taxonomic identification created confusion. This paper includes the first record of *S. gallensis* for the Italian seas and the third record for the Mediterranean Sea, after the ones from Tunisia (Zakhama-Sraieb and Charfi-Cheikhrouha, 2010) and Türkiye (Çinar et al., 2017). *Stenothoe gallensis* seems to be a rare NIS in the Mediterranean Sea; however, it cannot be excluded that it has been overlooked and misidentified with the Atlanto-Mediterranean *Stenothoe cattai*, as argued by Krapp-Schickel (2013, 2015). Furthermore, the records in the Mediterranean Sea of the invasive congeneric NIS *S. georgiana* (Martínez-Laiz et al., 2020) might have diverted attention from *S. gallensis*.

Stenothoe georgiana is a species described from the Western Atlantic Ocean, North Carolina (USA), that in recent years has successfully invaded both the Eastern Atlantic and Pacific Oceans, alongside the Mediterranean Sea (Martínez-Laiz et al., 2020). This small amphipod quickly spread throughout the Mediterranean and is now present in many Italian ports (Fernández-González and Sánchez-Jerez, 2017; Ulman et al., 2017; Servello et al., 2019; Martínez-Laiz et al., 2020; Calabrese et al., 2025; Badalucco et al., 2026). *Stenothoe georgiana* shows a high invasion potential because of a high affinity for artificial environments and plasticity in settling on different biogenic substrata (Martínez-Laiz et al., 2020).

The Mediterranean *Mesanthura* cf. *romulea* has a taxonomic status that currently remains unresolved (see the Remarks section and Lorenti et al., 2009). It is presumed that the isopod collected in the port of Trapani is the one widespread in the Mediterranean. *Mesanthura romulea sensu stricto* is native to the Western Pacific; it was first described in Australia (South Wales coast) by Poore and Lew Ton (1986). The earliest record of the genus *Mesanthura* in the Mediterranean Sea was reported in a brackish lagoon of the northern coast of Egypt (Samaan et al., 1989). The first records of *M. cf. romulea* occurred in Italy, in the southern Adriatic Sea (Lorenti et al., 2009). Successive records were from the Tyrrhenian basin (island of Ischia, Kroeker et al., 2011; Liguria region, Ferrario et al., 2017; Stamouli et al., 2017; Civitavecchia, Livorno, Tempesti et al., 2020a; many touristic marinas in the Tuscan Archipelago and Corsica, Tempesti et al., 2025), Adriatic Sea (Ragkousis et al., 2023), and Ionian Sea (Calabrese et al., 2025), confirming a progressive distribution expansion.

Our observations support the idea of Lorenti et al. (2009), namely that Mediterranean records should be interpreted with caution, as the

original description was performed on juvenile specimens. In the present study, we provided a detailed photographic documentation of adult male specimens, thereby attempting to ensure the reliability of species identification. Although some characters in adult males can be singled out without uncertainty, the recognition in juvenile specimens remains often problematic, as several diagnostic morphological characters in peracarids are strongly influenced by sex and growth stage (Lo Brutto et al., 2022; Iaciovano et al., 2026). This limitation frequently hampers the accuracy and completeness of species delimitation. The present study highlights taxonomic ambiguities and emphasises the need for genetic confirmation. Although detailed morphological diagnosis remains central to the detection of NIS (Battiata et al., 2024; Iaciovano et al., 2026), its effectiveness is increasingly constrained by the growing recognition of species complexes. Notable examples include the amphipod *Talitrus saltator*, considered widely distributed for a long time, which was only recently subdivided into three valid species (Lowry and Myers, 2019), or the amphipod *Leucothoe spinicarpa*, also shown to represent a complex of cryptic species (Gouillieux and Droual, 2020). These cases underscore the need for caution when relying exclusively on morphology and suggest that *M. romulea* may likewise represent a cryptic species complex requiring integrative taxonomic approaches. The specimens herein collected showed minute morphological differences, though constant among other Italian samples (Authors' personal observations).

Paracerceis sculpta is an isopod native to the Northeast Pacific Ocean. The first documented occurrence of the species in Italian waters was in 1981, in the northern Adriatic (Forniz and Sconfietti, 1983). To date, the species has been observed to be widespread throughout the Italian seas (Dailianis et al., 2016; Calabrese et al., 2025).

The isopod *Paranthura japonica* is native to the Western Pacific, Sea of Japan (Marchini et al., 2014). It was reported in Italy for the first time in the Northern Adriatic (Marchini et al., 2014), subsequently in the Tyrrhenian Sea (Tempesti et al., 2020a) and later in the Ionian Sea (Calabrese et al., 2025).

4.2. Polychaeta

The second representative taxon was represented by the polychaetes, with five species.

Branchiomma bairdi, described from the Caribbean and subsequently reported from localities in the Atlantic and Pacific Ocean (Del Pasqua et al., 2018), has been the object of a long lasting, and only partially solved, taxonomic confusion with the Indo-Pacific *Branchiomma boholense*. While adult specimens of the two species can be easily distinguished (Del Pasqua et al., 2018), the identification of juveniles is challenging. Through an integrative approach, Del Pasqua et al. (2018) recognized only *B. boholense* in the Mediterranean Sea, leading to retrospectively reassigning all Mediterranean records of *B. bairdi* to this species. However, the situation appears to be more complex, and some records of *B. bairdi* from the Mediterranean Sea do indeed refer to this species. In particular, records for Malta by Arias et al. (2013) should probably be referred to this taxon based on the presence of asexual reproduction by architomy, known with some certainty for *B. bairdi* and not described for *B. boholense*. Additionally, Langeneck et al. (2020) identified as *B. bairdi* specimens from a touristic marina from north-western Sicily (Villa Ignea marina). Lastly, based on molecular analyses, Toso et al. (2024) confirmed the occurrence of both *B. bairdi* and *B. boholense* off Lebanon. An integrative revision of Mediterranean material, including a critical revision of previous literature, is currently ongoing, with the aim of better defining the morphological characters allowing for the distinction of the two species, and reconstructing their actual distribution across the Mediterranean Sea.

Branchiomma luctuosum is a species originally described from the Red Sea, currently widespread in the Indian Ocean (Capa et al., 2013), in the Mediterranean and adjacent Atlantic (Fernández-Romero et al., 2021; Langeneck et al., 2022), and in the south-western Atlantic Ocean

(Oricchio et al., 2019), where it is considered invasive. It was first reported in the Mediterranean (southern Tyrrhenian Sea) in 1979 (Knight-Jones et al., 1991) and subsequently observed across the whole basin (Langeneck et al., 2022). The first record from Sicily refers to specimens sampled in 2010 in Faro Lake, Strait of Messina (Giangrande et al., 2012).

Dorvillea similis is a small hard bottom polychaete originally described based on material from the Red Sea, even though the inclusion of material from Cape Verde and some morphological variation in the original description (Crossland, 1924) suggest that the type material might include more than one species. It was subsequently widely reported from the Indo-Pacific region, and more recently the Mediterranean Sea (Çinar, 2009), where the first individuals were sampled in Lebanon in 2002 (Toso et al., 2024). The species is currently widespread across the Mediterranean Sea (Çinar, 2009; Toso et al., 2024; Tempesti et al., 2025). In Italy, *D. similis* is known from the northern Tyrrhenian Sea (Langeneck et al., 2020) and the coast of Apulia, in both the Ionian and the Adriatic Seas (Langeneck et al., 2024).

Lumbrineris perkinsi is a species originally described from the Caribbean region, but its distribution also includes the Pacific Ocean (Carrera-Parra, 2006), the south-western Atlantic Ocean (Berlandi et al., 2012), and the Mediterranean Sea, where the species has been known since the 1970s. The first records (from Spain, Italy, and Algeria) were reported as *Lumbrineris inflata*, but Carrera-Parra (2001) clarified the differences between the two species, showing that all records for the Mediterranean Sea should be assigned to *L. perkinsi*. Despite the lack of records between the late 1980s and 2005, this species is widespread across the Mediterranean Sea and has probably been overlooked by the majority of historical studies (Giangrande et al., 1981; Campoy, 1982; Çinar, 2009; Toso et al., 2024). Records from Greece could not be confirmed (Faulwetter et al., 2017). Along the Italian coast it occurs in the Tyrrhenian Sea (Giangrande et al., 1981; Tempesti et al., 2025) and the Apulian coast, with the majority of records in the Ionian Sea (Langeneck et al., 2024). However, the occurrence of populations of *L. perkinsi* even in very small and isolated marinas suggests that this apparently fragmented distribution is most likely an artefact due to the uneven research effort along the Italian coastline.

Myrianida pachycera is a tropical and subtropical syllid polychaete primarily distributed throughout the Indo-Pacific region (Tovar-Hernández et al., 2014; Keppel et al., 2019). Outside this region, it has been recorded in the Caribbean from Florida (Nygren, 2004) and Colombia (Palacio-Miranda et al., 2025), off Brazil (Álvarez et al., 2024), and in some Italian harbours with a fragmented distribution (Langeneck et al., 2025).

4.3. A Mediterranean view

The Mediterranean distribution of the species examined in this study was reconstructed through an extensive review of the available literature. Occurrence records were compiled and analysed based on four biogeographical sectors: Western Mediterranean Sea (WMDS), Central Mediterranean and Ionian Sea (CMDS), Adriatic Sea (ADRIA), and Eastern Mediterranean Sea (EMDS) (Fig. 6).

Species occurrence frequency across the Mediterranean basin exhibited a pronounced heterogeneity, both among taxa and across biogeographical sectors. *Pinctada radiata* accounted for the highest number of records, whereas several taxa were represented by only a limited number of occurrences (e.g. *S. gallensis*, with eight records). Overall, the number of records revealed a marked concentration in the Western Mediterranean, while the central and eastern sectors were characterised by substantially fewer records.

This uneven distribution of data reflects persistent gaps in knowledge of the spatial distribution of NIS in the Mediterranean Sea. Such gaps largely stem from the predominance of studies focused on individual species reports (e.g. Fernández-González and Sánchez-Jerez, 2014), rather than broader surveys encompassing multiple taxa (e.g. Çinar

et al., 2006; Ragkousis et al., 2023; Spagnolo et al., 2019; Ulman et al., 2017). Consistent with this pattern, our literature-derived dataset indicates that nearly half of the reviewed publications (46.1%) reported records of single species, compared with the mono-taxa checklists (29.9%) and the multi-taxa checklists (24%) (Supplementary material 3, Table S3).

These limitations may be partially addressed through coordinated and collaborative surveys targeting biodiversity hotspot regions and integrating both large commercial ports and smaller harbour systems, as demonstrated by the present study.

The present survey further highlights pronounced geographical gaps in research effort across the Mediterranean basin. Data from the North African coastline remains scarce (except for Tunisia), with an almost complete absence of information for Libya. This lack of data is largely attributable to the limited accessibility of grey literature and non-indexed sources (Ragkousis et al., 2023) and reflects geopolitical and linguistic barriers rather than a genuine absence of biological invasions (Tempesti et al., 2020b; Ragkousis et al., 2023). Recent investigations conducted in Tunisia (Bay of Monastir) and Algeria (Port of Arzew) documented the presence of 19 and 18 non-indigenous and cryptogenic species, respectively (Chebaane et al., 2019; Bensari et al., 2025); however, these studies remain isolated examples within a region still characterised by substantial knowledge gaps.

Within this context of fragmented information, Sicily emerges as a key “sentinel” area for the early detection of NIS (e.g. Iacofano et al., 2026), owing to its proximity to the North African coast and its central position within the Mediterranean basin. The island represents a natural crossroads for the introduction and secondary spread of invasive species between the western and eastern Mediterranean. In this framework, the port of Trapani warrants particular attention despite its relatively small size. Its strategic location renders it a potentially important hub within regional maritime routes (Fig. 7; Supplementary material 1, Tables S1, A–D). Although several Italian ports, such as Venice, Civitavecchia, Livorno, and Taranto, are substantially larger and more intensively studied, the relatively high number of invertebrate species recorded in Trapani (27 taxa) is noteworthy, especially considering that the occurrence of NIS in Sicilian ports is likely significantly underestimated (Langeneck et al., 2025).

Minor ports such as Trapani may therefore play a disproportionate role in the dispersal dynamics of NIS, acting as intermediate nodes within a “stepping-stone” dispersal model. The data generated for the port of Trapani, an area previously almost unexplored, enable a critical comparison with the extensive literature on introduction pathways and dispersal routes across the Mediterranean (Fig. 7). Moreover, the proximity of the port to the Egadi Islands Marine Protected Area (MPA) substantially increases the ecological and management relevance of this study, as it highlights the potential risk of NIS spillover into protected ecosystems. Consequently, this study represents an important contribution to biological risk assessment and to the development of targeted monitoring strategies in a region that has, until now, been largely overlooked in the scientific literature.

4.4. Impact of Trapani maritime traffic on NIS dispersal

In the context of the increasing bioinvasion risk, the port of Trapani emerges as an emblematic case of an overlooked harbour despite being a strategic hub for maritime connections. The port had not been investigated yet and was missing in the literature on the potential NIS dispersal routes.

The present study aimed to demonstrate that the port of Trapani represents a significant node within Mediterranean maritime traffic networks and, consequently, a potential hotspot for the dispersal of NIS. A high intensity of maritime traffic was documented in the port, with liner vessel movements, particularly those serving routes connecting Trapani with the Egadi Islands MPA, reaching large numbers of arrivals and departures per day. The cruise traffic linked Trapani to ports across

both the eastern and western Mediterranean basins and exhibited a marked increase of 57.4% in 2025. Moreover, international cruises included long-distance itineraries encompassing transoceanic routes, therefore enhancing the port's connectivity at a global scale.

The spatial analyses performed in the present study further support the hypothesis that Trapani harbour operates as an important regional hub for the secondary spread of NIS across the Mediterranean Sea. In particular, the significant negative relationship detected between the NIS richness and the distance from Trapani harbour across all analysed spatial extents indicates that NIS richness progressively declines with increasing distance from the port, suggesting a central role of this harbour in structuring regional invasion patterns. This relationship was strongest within the 300-km buffer, where both the negative binomial GLM and temporal analyses identified significant distance-decay patterns, implying that the area surrounding Trapani may represent an active source region for ongoing dispersal processes. Conversely, the progressive weakening of the relationship at broader spatial scales likely reflects the increasing influence of additional Mediterranean introduction hubs and secondary dispersal pathways.

In this scenario, the port of Trapani and, consequently, Sicily Island are a natural laboratory and a strategic crossroads for monitoring marine bioinvasion processes.

Kernel density estimations revealed a pronounced concentration of occurrence probabilities in the western Sicilian sector surrounding Trapani harbour, consistent with the intense maritime connectivity documented for this port. These results align with the observed predominance of western Mediterranean occurrence records for the analysed taxa and reinforce the interpretation of maritime traffic as the primary vector driving regional spread dynamics. The absence of significant temporal trends at broader Mediterranean scales further suggests that local harbour-mediated dispersal processes may be partially obscured by the complexity of basin-wide invasion histories and by the coexistence of multiple introduction sources throughout the Mediterranean Sea.

The co-occurrence of species of different biogeographical origins demonstrates the complexity of the pathways of entry and the synergistic role of anthropogenic and climatic factors in shaping the fouling coastal communities of the Mediterranean Sea. In this context, the integration of spatial probability modelling, hotspot analyses, and temporal trends provides quantitative support for the ecological role of Trapani harbour as both a local reservoir and a potential stepping-stone node for NIS expansion, highlighting the importance of continuous monitoring programmes in strategically connected minor Mediterranean ports. Although literature indicates that marinas exhibiting high NIS richness are often located in proximity to other major introduction vectors, such as aquaculture facilities (Ulman et al., 2019a, 2019b), in the specific case of Trapani port, the influence of shipping traffic appears to be predominant. This inference is supported by the near absence of aquaculture activities in the surrounding area, which suggests that maritime transport represents the principal pathway for both the introduction and secondary dispersal of NIS.

4.5. The role of basibionts

Under the above considerations, we must also consider the spread of some NIS through basibionts. Basibionts are sessile organisms capable of colonising bare surfaces, giving rise to a secondary substrate (Sedano et al., 2020). The main taxonomic groups that structure this secondary substrate are bryozoans, bivalve molluscs, barnacles, and serpulid polychaetes, together with various macroalgal taxa (Ferrario et al., 2017; Spagnolo et al., 2019; Tempesti et al., 2022b). These organisms increase the three-dimensional complexity of bare substrates (Cunha et al., 2017; Sedano et al., 2020), allowing colonisation by an associated community composed of sessile and vagile epibionts. Sessile epibionts are mainly represented by colonial ascidians, encrusting colonies of bryozoans, filamentous algae, and sponges (Antoniadou et al., 2013). On

the other hand, vagile para-epibionts are mainly represented by peracarid crustaceans, wandering polychaetes, gastropods, echinoderms, and pycnogonids (Ferrario et al., 2017; Spagnolo et al., 2019; Tempesti et al., 2020a, 2022a).

The basibionts identified at the port of Trapani include two species of bivalves and three species of bryozoans.

The pearl oyster *P. radiata* commonly colonises artificial substrates, although its spread has been significantly increased by deliberate introduction for mariculture purposes (Lodola et al., 2013). In addition to natural dispersal, the species spreads through secondary vectors such as maritime transport (Png-Gonzalez et al., 2021) and marine debris. Historically known for its economic value linked to pearl production, this species hosts a rich associated community, mainly composed of polychaetes and barnacles (Abdelsalam and Elebiary, 2023). In the specific context of the port of Trapani, the associated species found include *D. similis*, *L. perkinsi*, *B. bairdi*, *B. pharaonis*, *P. sculpta*, *M. cf. romulea*, *E. brasiliensis*, *B. neritina*, and *S. plicata* (Abdelsalam and Elebiary, 2023).

Brachidontes pharaonis, a Lessepsian invader, is one of the pioneer species introduced from the Red Sea into the Mediterranean via the Suez Canal (Battiata et al., 2024). Its expansion has caused a sharp decline, sometimes to the point of total exclusion, in populations of the native mussel *Mytilaster minimus*. Although the faunal communities associated with this invader have not yet been analysed in detail in the Mediterranean basin, the available literature reports the presence of polychaetes, crustaceans, sipunculids, and molluscs in association with *B. pharaonis* (Çinar et al., 2017). In line with this evidence, sampling carried out in Trapani confirms the association of *L. perkinsi*, *D. similis*, and *S. gallensis* with this species (Çinar et al., 2017).

Amathia verticillata is a bryozoan which colonises hulls and keels rapidly, acting as a primary and secondary vector of spread (Minchin, 2012; Martínez-Laiz et al., 2018). It is one of the most frequently recorded species in Mediterranean enclosed habitats, such as coastal lagoons, harbours, and marinas (Minchin, 2012; Tempesti et al., 2022b), and its robust, broad stolons enable it to attach to a variety of substrates, including smooth surfaces, allowing it to thrive in highly variable hydrodynamic conditions, also facilitated by its unmineralized flexible colonies. All these characters make this species highly adaptable in terms of hull fouling compared to natural and other anthropogenic substrates (Minchin, 2012; Marchini et al., 2015). Consequently, this basibiont has contributed to the dispersal of several NIS, particularly peracarids (Dailianis et al., 2016; Furfaro et al., 2025). Some species found in Trapani Harbour (e.g. *L. baconi*, *P. sculpta*, *C. scaura* and *P. japonica*) are closely associated with *A. verticillata* colonies (Dailianis et al., 2016; Guerra-García et al., 2023, 2024), and this bryozoan has probably contributed to their establishment and spreading in neighbouring coastal area. Small recreational boats and yachts primarily spread *A. verticillata* and its associated fauna, and many studies have shown that *A. verticillata* fragment propagation is directly related to the number and characteristics of recreational boats in a given port and the intensity of vessel traffic in a specific area (Lacoursière-Roussel et al., 2016; Castro et al., 2020, 2022).

Bugula neritina is one of the main fouling organisms in numerous Mediterranean ports (Spagnolo et al., 2019). This species effectively colonises oyster shells, ballast tank walls and floating materials inside them (Ryland et al., 2011). Thanks to its wide distribution in marinas around the world and its complex tree-like structure that hosts a highly diverse epifaunal community (Guerra-García et al., 2025), *B. neritina* is an ideal model for monitoring and comparing associated macrofauna, particularly caprellids (Guerra-García et al., 2015). In particular, the caprellid *C. scaura*, despite being present in different habitats, shows a marked affinity for *B. neritina* (Ros et al., 2013), probably favoured by a cryptic adaptation due to the morphological and chromatic similarity between the two species (Lolas et al., 2021). A similar association has been documented between bryozoans and *P. pusilla* (Ros et al., 2013). The use of *B. neritina* as a substrate has proven essential for monitoring

the presence of NIS peracarids such as *J. slatteryi*, *S. georgiana*, *C. scaura*, *P. sculpta*, and *P. japonica* (Martínez-Laiz et al., 2018; Guerra-García et al., 2025). This evidence is directly confirmed by the data collected during the present study, which confirms the presence of these species associated with the bryozoan in the port of Trapani.

The bryozoan *T. inopinata* is among the fouling species typically found in Mediterranean ports and marinas (Gavira-O'Neill et al., 2018). Thanks to its generalist nature, this species shows a high tolerance to different environmental conditions, a factor that favours its invasive character, especially in stressed ecosystems such as ports (Occhipinti Ambrogi, 2000). The complex structural conformation of *T. inopinata* allows it to host a rich variety of small benthic organisms. Investigations conducted in the port of Trapani confirm the presence of crustaceans such as *C. scaura*, *L. baconi*, *J. slatteryi*, *E. rapax*, *S. georgiana*, *P. japonica*, and *P. sculpta* in association with the bryozoan, as already known from literature (Gavira-O'Neill et al., 2018; Guerra-García et al., 2025).

Many of these basibionts, particularly *A. verticillata*, are especially abundant in the port of Trapani, mainly on the keels of pleasure boats, ropes and floating objects, but further data on pleasure boat traffic is needed to define their contribution to the spread of NIS in the coastal area of Trapani.

5. Conclusions

The spatial analyses performed in the present study supported the hypothesis that Trapani harbour acts as an important regional hub for the secondary spread of non-indigenous species (NIS) across the Mediterranean Sea. In particular, the significant negative relationship detected between NIS richness and distance from Trapani harbour across all analysed spatial extents indicates that NIS richness progressively declines with increasing distance from the port, suggesting a central role of this harbour in structuring regional invasion patterns. On the contrary, this relationship was stronger within the 300-km buffer, implying that the area surrounding Trapani may represent an active source region for ongoing dispersal processes.

In conclusion, the results of this study demonstrate that the port of Trapani plays a strategic role within the maritime network of the Mediterranean Sea, acting as a key dispersal hub for NIS. The high richness of NIS recorded, combined with the increasing intensity of tourist and cruise activities, renders the area particularly vulnerable to biological invasions, with a concrete risk of spillover toward the nearby Egadi Islands MPA. To address these challenges, it is essential to transition from opportunistic surveys to coordinated and continuous monitoring programs. Specifically, we recommend the establishment of a permanent regional cooperation mechanism, such as a technical table involving Port Authorities and MPA management departments, to align conservation goals with port activities. A crucial step in this direction would be the implementation of a shared digital platform for real-time data exchange regarding vessel arrivals and early detection alerts, allowing for rapid response interventions. Furthermore, NIS risk assessments should be formally integrated into the planning of future port expansions and dredging activities to mitigate the creation of new niches for invasive species. By fostering such integrated management strategies and regional data-sharing protocols, it will be possible to safeguard the biodiversity of both the harbour system and the adjacent protected marine environments.

Author contributions

Antonina Badalucco and Sabrina Lo Brutto contributed to the study conception and design. Material preparation, data collection and analysis were performed by Antonina Badalucco and Yann Toso. The first draft of the manuscript was written by Antonina Badalucco and all authors commented on previous versions of the manuscript. Taxonomic identifications were carried out by Fabio Crocetta (molluscs); Cengiz Koçak (pycnogonids); Joachim Langeneck, Luigi Musco and Yann Toso

(polychaetes); Emanuele Mancini, Antonina Badalucco, Caterina Martino and Sabrina Lo Brutto (peracarids); Francesco Mastrototaro (tunicates) and Antonietta Rosso (bryozoans). Andrea Desiderato and Serena Mucciolo were responsible for genetic analysis. Fabio Bozzeda carried out the statistical analysis. Sabrina Lo Brutto contributed to conceptualisation, supervision and revision. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Yann Toso: Writing – review & editing, Formal analysis. **Joachim Langeneck:** Writing – review & editing, Formal analysis. **Emanuele Mancini:** Writing – review & editing, Formal analysis. **Sabrina Lo Brutto:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Andrea Desiderato:** Writing – review & editing, Formal analysis. **Fabio Bozzeda:** Formal analysis, Writing – review & editing. **Cengiz Koçak:** Writing – review & editing, Formal analysis. **Serena Mucciolo:** Writing – review & editing, Formal analysis. **Luigi Musco:** Writing – review & editing, Formal analysis. **Caterina Martino:** Writing – original draft, Formal analysis. **Francesco Mastrototaro:** Writing – review & editing, Formal analysis. **Fabio Crocetta:** Writing – review & editing, Formal analysis. **Maria Giovanna Parisi:** Writing – review & editing, Formal analysis. **Antonietta Rosso:** Writing – review & editing, Formal analysis. **Antonina Badalucco:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

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Supplementary Information

[Supplementary material](#) is attached.

Supplementary material

The data compiled for the study are available in the form of Excel spreadsheets in the [Supplementary Material](#). The file “Supplementary_Material_1_Maritime routes.xlsx” contains data on the cruise ships that docked in the port of Trapani between 2022 and 2025, retrieved from the official registers of the “Autorità di Sistema Portuale del Mare di Sicilia Occidentale”. The dataset is structured as follows: [Tables S1 A–D](#) describe the routes of origin and destination; [Table S1 E](#) lists the acronyms and geographical coordinates of the ports used to create the [Fig. 5](#); [Table S1 F](#) presents the reconstruction of an international cruise ship case study docked in Trapani in 2025 (shown in [Fig. 5](#)). The file “Supplementary_Material_2_Dataset.xlsx” lists the Mediterranean records collected from literature of the 27 non-indigenous species collected at the port of Trapani. The file “Supplementary_Material_3_Table S3.xlsx” contains the list of the references used to build the map in [Fig. 6](#) and

subdivided into the three groups: 1. references that included multi-taxa checklists; 2. references that included mono-taxon checklists; 3. references reporting records of single species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.rsma.2026.105169](https://doi.org/10.1016/j.rsma.2026.105169).

Data availability

The data will be published in a free-to-use repository after publication.

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