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Genetic insights into the non-indigenous copepod *Pseudodiaptomus marinus* Sato, 1913 (Calanoida: Pseudodiaptomidae) in the Mediterranean Sea

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ABSTRACT

The calanoid copepod *Pseudodiaptomus marinus* Sato, 1913, native to the Indo-Pacific region, has rapidly expanded its range in European waters since its first detection in the Adriatic Sea in 2007, raising questions about its introduction pathways and population dynamics. In this study, we investigated the genetic diversity and phylogeography of *P. marinus* in the Mediterranean Sea, focusing on four Italian coastal areas, using a dual-marker approach based on the mitochondrial cytochrome oxidase subunit I (COI mtDNA) and the internal transcribed spacer of the ribosomal cistron (ITS) genes. Molecular data allowed the reconstruction of the phylogenetic relationships within the Pseudodiaptomidae family and the characterization of the species *P. marinus* across geographically distant areas. COI analyses revealed three main Pseudodiaptomidae clades corresponding to freshwater, brackish, and marine species, while all *P. marinus* sequences clustered together, showing no evidence of cryptic species. Haplotype networks indicate repeated human-mediated introductions, with Italian populations connected among themselves and to other introduced areas, though higher haplotype diversity was observed in putative native regions. ITS analyses confirmed the absence of clear biogeographic structuring among Italian populations, suggesting that some ITS sequences from Korean populations, within or near the presumed native range of *P. marinus*, may require further verification, although the observed divergence could also reflect native-range genetic diversity or distinct lineages within a broader species complex. Overall, our findings provide an overview of the genetic diversity of *P. marinus* across different Mediterranean areas, offering new insights into its characterization and suggesting possible introduction pathways in European waters. These findings underscore the value of integrated molecular approaches in supporting and enhancing monitoring programs for non-indigenous species, while also improving our ability to anticipate their potential spread under future environmental changes scenarios.

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INTRODUCTION

The Mediterranean Sea is a renowned hotspot of both endemic biodiversity and biological invasions (*Tsirintanis et al., 2022*). Several drivers have been hypothesized to be at play, including changes in sea surface temperature, increased propagule pressure, and augmented research efforts (as reviewed in *Zenetos et al., 2022*). Over an 11-year time window (2010–2021), the number of established non-indigenous species (NIS) has increased by 41%, scoring a total of ~1,000 validated introductions (*Zenetos et al., 2022*). Estimating the actual number of introduced species is made difficult by numerous factors, including the lack of taxonomic expertise and the presence of species complexes (*Bonanno & Orlando-Bonaca, 2019*; *Katsanevakis & Moustakas, 2018*). The impact of introduced species in the Mediterranean basin is still largely unknown (*Bonanno & Orlando-Bonaca, 2019*). The impacts of Mediterranean NIS are context-dependent, with both positive and negative effects reported according to the ecological target descriptor considered, namely biodiversity, ecosystem services, and human health (*Tsirintanis et al., 2022*).

In terms of Mediterranean subregions, as identified by the *European Environment Agency (2020)*, the Western Mediterranean Sea is the most invaded, with Italy, France and Spain scoring the highest records (*Tsiamis et al., 2019*). Given its particular position, Italian seas belong to three different subregions: the Ligurian and Tyrrhenian Sea to the Western Mediterranean Sea (MWE); the Ionian Sea, together with the southern and eastern coasts of Sicily, to the Ionian Sea and the Central Mediterranean Sea (MIC); and the Adriatic Sea to the homonymous subregion (MAD) (*European Environment Agency, 2020*). The trend in Italian introductions matches that of the Mediterranean one: a total of 111 species was censused by *Occhipinti-Ambrogi (2002)*, rising first to 165 (*Occhipinti-Ambrogi et al., 2011*) and then to 265 in less than two decades (*Servello et al., 2019*). This last figure is even more staggering considering that it equals nearly 25% of the introductions at the Mediterranean level (*Zenetos et al., 2022*). The Italian NIS are equally distributed among the MWE, MIC and MAD, and a large proportion of them are considered established (*Servello et al., 2019*). Crustaceans represent one of the most relevant groups among Italian marine NIS, with decapods being the dominant component, while planktonic copepods currently account for a smaller but increasing fraction of introduced taxa (*Di Capua & Boxshall, 2008*; *Di Capua & Mazzocchi, 2021*). Recent Mediterranean assessments further confirm Italy as one of the Mediterranean countries with the highest numbers of recorded marine NIS and highlight the continuing spread of crustacean invaders across different subregions (*Galanidi et al., 2023*).

Following its first record in the Adriatic Sea in 2007 (*De Olazabal & Tirelli, 2011*), the calanoid copepod *Pseudodiaptomus marinus* Sato, 1913, native to the Inland Sea of Japan (*Ohtsuka et al., 2018*), has increasingly spread its distribution in European seas and neighbouring waters (*Sabia et al., 2015*; *Uttieri et al., 2020*; *Uttieri et al., 2023*). *P. marinus* is

widely present in Italian waters, occurring in the coastal sectors of the Ligurian, Tyrrhenian, Ionian and Adriatic Sea (*Corona et al., 2026*; *Uttieri et al., 2023*). This species can inhabit a wide range of environments, from freshwater to hypersaline, due to specific biological and physiological traits (*e.g.*, *Sabia et al., 2017*; *Sabia et al., 2014*; *Svetlichny et al., 2019*).

Molecular tools are increasingly used not only to identify individuals at the species level, in the framework of the so-called "integrative taxonomy" (*Dayrat, 2005*; *Will, Mishler & Wheeler, 2005*), but also to gain insights into the range of expansion and the source of introduction of NIS (*e.g.*, *Bonanno & Orlando-Bonaca, 2019* and references therein). A multi-marker approach is often suggested as a proper approach to reduce the risk of species misidentifications (*e.g.*, *Varrella et al., 2025*; *Zhang et al., 2018*), and it can also provide information on the distribution and the differentiation of NIS (*Ghabooli et al., 2013*). Among the various alternative genes, the mitochondrial cytochrome oxidase subunit I (COI mtDNA) is one of the most commonly used for copepods, allowing reliable identification down to the species level (*e.g.*, *Bucklin, Steinke & Blanco-Bercial, 2011*; *Laakmann, Blanco-Bercial & Cornils, 2020*). As reviewed in *Bucklin, Steinke & Blanco-Bercial (2011)*, COI offers numerous advantages compared to other mitochondrial genes, and it has been successfully used as a diagnostic tool for cryptic species and for unveiling hidden diversity (*e.g.*, *Blanco-Bercial et al., 2014*; *Bucklin et al., 2010*; *Di Capua et al., 2022*; *Di Capua et al., 2023*). The internal transcribed spacer of the ribosomal cistron (ITS) is another gene that can be considered a suitable alternative to COI (*Bucklin, Steinke & Blanco-Bercial, 2011*). It is frequently used in phylogenetic and phylogeographic studies and is valued for its high variability and sequencing efficiency (*Rocha et al., 2019*). ITS has been successfully applied in copepods and has also demonstrated its utility in species from other orders (*e.g.*, *Chen & Hare, 2008*; *Di Capua et al., 2017*; *Jeon et al., 2018*; *Miller, Bradford-Grieve & Jillett, 1999*; *Sabia et al., 2017*). Additionally, analyses of ITS2 secondary structure and Compensatory Base Changes (CBCs) are used to refine species delimitation and assess the potential for interbreeding among genetically divergent populations.

By integrating mitochondrial and nuclear markers, our findings reveal patterns of genetic diversity among geographically distant populations of *P. marinus*, providing new insights into its spread, characterization, and possible introduction pathways in Mediterranean and European waters.

MATERIALS & METHODS

Study areas

The specimens of *P. marinus* were collected at four sites along the Italian coasts (Fig. 1): the Gulf of Trieste (GoT; Fig. 1A) and the Venice Lagoon (VL; Fig. 1B) in the Northern Adriatic Sea (MAD); Torre del Greco (TdG; Fig. 1C), a coastal site in the Gulf of Naples (GoN) in the Central Tyrrhenian Sea (MWE); and Lake Faro (LF; Fig. 1D), located at the northeastern tip of Sicily, at the boundary between the Central Tyrrhenian Sea (MWE) and the Ionian Sea (MIC).

The GoT, in the northernmost part of the Adriatic Sea, is a semi-enclosed coastal area of >600 km^2 and maximum depth of 26 m (*Olivotti, Faganelli & Malej, 1986*) (Fig. 1A).

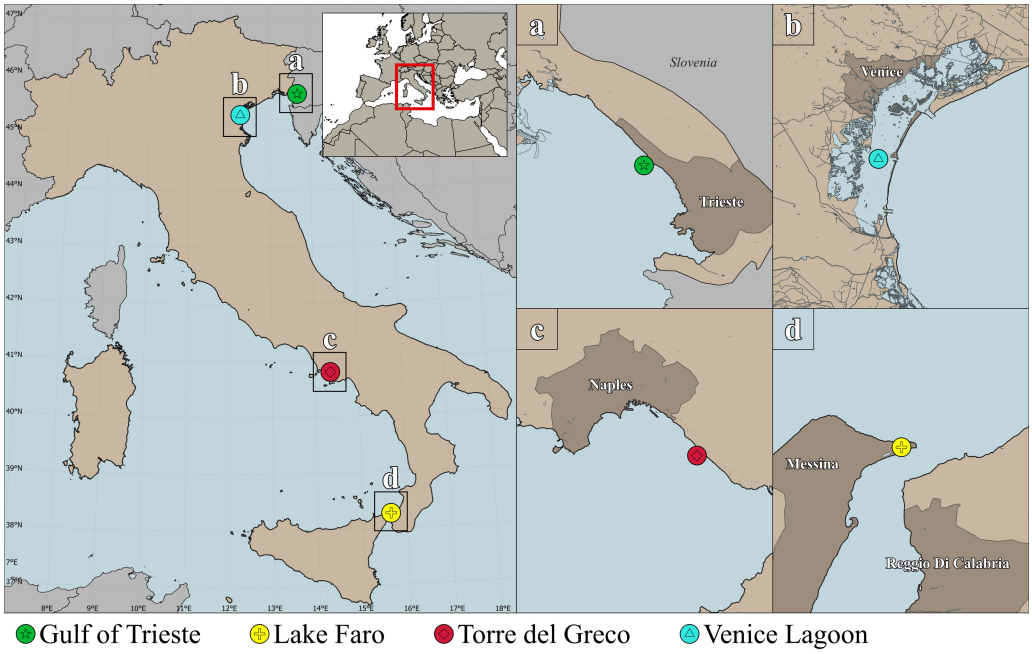


Figure 1 **Study area and sampling site along Italian coasts.** (A) Gulf of Trieste (GoT); (B) Venice Lagoon (VL); (C) Torre del Greco (TdG) in the Gulf of Naples (GoN) and (D) Lake Faro (LF).
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The individuals of *P. marinus* were recorded for the first time in this basin in an artificial canal near the port of Monfalcone (Italy) in May 2009 (*De Olazabal & Tirelli, 2011*). In the following years, observations of this NIS increased, at several sites in the central area of the GoT, in the ports of Trieste and Monfalcone, at the coastal Long-Term Ecological Research (LTER) Golfo di Trieste site and in the Marano and Grado Lagoon (*Uttieri et al., 2020*; *Uttieri et al., 2023*).

The VL is a large Mediterranean lagoon covering ~500 km^2 along the northwest coast of the Adriatic Sea (Fig. 1B). The depth of the lagoon is very shallow, averaging about 1.3 m, but it reaches over 10 m in the deepest channels (*Ghezzo et al., 2011*). The lagoon is surrounded by urban and industrial zones, and activities such as fishing, mariculture, and tourism are highly developed (*Solidoro et al., 2010*). The combination of simultaneous stressors determines high habitat heterogeneity (*Camatti et al., 2023*). The first record of *P. marinus* in the port of Venice dates to May 2014 (*Vidjak et al., 2019*), followed by additional records both in the lagoon and in the Gulf of Venice between 2014 and 2017 (*Uttieri et al., 2020*).

The GoN is a coastal sector of the Central Tyrrhenian Sea subject to intense anthropogenic pressure (*Cianelli et al., 2012*) (Fig. 1C). The first record of *P. marinus* dates back to July 2011, through eDNA barcoding (V4-18S rRNA; *Di Capua et al., 2021*). This NIS has been recurrently found as a rare species at the coastal LTER MareChiara site (https://deims.org/0b87459a-da3c-45af-a3e1-cb1508519411) (*Mazzocchi et al., 2023*; *Uttieri et al., 2020*; *Uttieri et al., 2023*), but occasionally also in offshore waters (*Sabia et al., 2015*), in the southeasternmost part of the GoN (TdG, Torre Annunziata and Sarno River

mouth; *Uttieri et al., 2023*), and in the Gulf of Pozzuoli (a sub-basin of the GoN; *Margiotta et al., 2020*).

The LF is part of the Laguna di Capo Peloro natural reserve (*Bertolino et al., 2022*; *Somma et al., 2024*) (Fig. 1D). It has a quasi-permanent anoxic layer below 10 m, owing to its unique bathymetric contour and meromictic regime (*Saccà, Guglielmo & Bruni, 2008*; *Somma et al., 2024*). Over the years, an increasing number of NIS has been recorded in the LF, including protozoans, sponges, polychaetes, and copepods (as reviewed in *Bertolino et al., 2022*). *P. marinus* has been recorded in the LF since October 2008 (*Sabia et al., 2014*), becoming the fourth copepod species in terms of abundance (*Pansera et al., 2014*).

Pseudodiaptomus marinus sampling and morphological identification

P. marinus specimens were sorted from mesozooplankton samples that were collected using standard vertical nets with a 200 μm mesh and preserved in 95% ethanol. In the Gulf of Trieste (GoT; LTER site Golfo di Trieste, https://deims.org/96969205-cfdf-41d8-979f-ff881ea8dc8b: 45.69911N, 13.70765E), samples were collected by vertical hauls of a WP2 net (57 cm mouth Ø) from near-bottom (18 m depth) to the surface. In the Venice Lagoon (VL; LTER Venice Lagoon site, https://deims.org/96969205-cfdf-41d8-979f-ff881ea8dc8b: 45.337N, 12.2747E), zooplankton was sampled using horizontal tows with an Apstein net equipped with a flowmeter to measure the filtered water volume, at a depth of 0.5 m. At TdG (40.7864N, 14.3517E) samples were collected by vertical hauls using a WP2 plankton net from 90 m depth to the surface, while in LF (38.269N, 15.637E), vertical tows with a WP2 net were conducted within the oxygenated layer (15 m to the surface).

P. marinus is currently the only representative of the genus reported in European waters, and its identification is supported by established taxonomic keys (*Zagami, Bonanzinga & Pansera, 2023*). Diagnostic features include the separation between the head and first thoracic segment, the presence of pointed posterolateral spines on the prosome, and the characteristic female P5 morphology.

Adult females were identified under stereomicroscopes at each sampling site (GoT: Olympus SZX16 and Leica M165C; VL: Zeiss Stemi 2000-C; TdG: Leica M165C; LF: Wild MDG-17). In addition, all female specimens were morphometrically examined, and high-definition images were acquired before DNA extraction.

Molecular analyses

The extraction, purification, and amplification of *P. marinus* DNA followed the modified cetyltrimethylammonium bromide (CTAB) protocol discussed in *Di Capua et al. (2022)*. COI was amplified using the *Folmer et al. (1994)* primers pairs (forward LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′; reverse HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), while ITS region was amplified using the forward ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers designed in *White et al. (1990)*.

BioEdit Sequence Alignment Editor 7.0.5.3 (*Hall, 1999*) was used to obtain consensus sequences from forward and reverse, while mothur v.1.44.3 (*Schloss et al., 2009*) collapsed

identical sequences. Molecular identifications for all generated sequences were confirmed by the similarity approach against the National Center for Biotechnology Information (NCBI) (*Altschul et al., 1997*) and (only for COI) also against the Barcode of Life Data Systems database (BOLD) (*Meiklejohn, Damaso & Robertson, 2019*; *Ratnasingham & Hebert, 2007*). For the COI dataset, we also used a reference sequence (NCBI accession number: MZ710190; *Di Capua et al., 2022*) obtained in the GoN (LTER site MareChiara, https://deims.org/0b87459a-da3c-45af-a3e1-cb1508519411, depth ca. 75 m). A phylogenetic tree was built using the ten COI sequences generated in this study and the 65 sequences downloaded from NCBI and BOLD databases (Table S1). *Pleuromamma xiphias* (Giesbrecht, 1889) (NCBI accession number: JN574428) was used as the outgroup. In detail, multialignment was generated using MAFFT v. 7 (*Katoh, Rozewicki & Yamada, 2019*; *Kuraku et al., 2013*) and manually checked using SeaView v.4.0 (*Gouy, Guindon & Gascuel, 2010*). A maximum-likelihood (ML) tree was inferred with IQ-TREE 2 software (*Minh et al., 2020*) using TIM+F+I+G4 substitution model, selected as best-fit model by ModelFinder within IQ-TREE (*Kalyaanamoorthy et al., 2017*), and 500 replicate bootstraps. The tree was visualized in iTOL4 (*Letunic & Bork, 2019*). Moreover, intraspecies population relationships were explored, producing phylogenetic networks generated both for COI and ITS markers using all the sequences available for *P. marinus*. Haplotype lists were generated with DnaSp v. 6 (*Rozas et al., 2017*), then Median-Joining (MJ) haplotype networks were inferred with Network 10 (*Bandelt, Forster & Röhl, 1999*) using default parameters.

The secondary structure of the ITS2 region was predicted using mfold software (*Zuker, 2003*) and visualised in the PseudoViewer 3 Web Application (*Byun & Han, 2009*). The margins of each region were identified by secondary structure predictions using the ViennaRNA Web Service (*Gruber, Bernhart & Lorenz, 2015*). In particular, the ITS2 secondary structure prediction and CBC/Hemi-Compensatory Base Change (HCBC) search analyses were carried out (*Coleman, 2003*). The absence of CBCs in helix III, the most conserved ITS2 region, was verified to ensure that individuals belonged to the same biological species (*Coleman, 2000*). The *P. marinus* ITS sequences used in *Sabia et al. (2017)* were aligned with the sequences produced in the present study using MAFFT v. 7 (*Katoh & Standley, 2013*), and a ML tree was built following the same workflow described above for COI.

RESULTS

A total of ten COI and ten ITS reference sequences of *P. marinus* were successfully amplified and sequenced in this study (Table 1).

The COI phylogenetic tree consisted of a total of 75 sequences including all the references for Pseudodiaptomidae genera and *P. marinus* (Fig. 2 and Table S1). The tree included 21 different species grouped in three main clades following their adaptation to different habitats. Indeed, the first clade included ten species mainly inhabiting freshwater systems belonging to the genera *Skistodiaptomus* and *Acanthodiaptomus*, and the species *Pseudodiaptomus marshi* Wright S., 1936. The second clade grouped *Calanipedia aquaedulcis* Krichagin, 1873 and nine *Pseudodiaptomus* brackish water species. The third

Table 1 New COI and ITS consensus sequences obtained for the different sites investigated.

Sites	Individual code	COI (bp)	NCBI accession no	ITS (bp)	NCBI accession no
Gulf of Trieste (GoT)	Pmar_Trieste_Z1	576	PZ441184	no	no
	Pmar_Trieste_Z2	576	PZ441185	no	no
	Pmar_Trieste_Z13	no	no	667	PZ441404
Venice Lagoon (VL)	Pmar_Venezia_Z1	no	no	620	PZ441405
	Pmar_Venezia_Z2	576	PZ441183	no	no
	Pmar_Venezia_Z3	no	no	694	PZ441406
	Pmar_Venezia_Z4	576	PZ441186	418	PZ441408
	Pmar_Venezia_Z6	576	PZ441187	615	PZ441409
Gulf of Naples –Torre del Greco (TdG)	Pmar_Torre_N19	576	PZ441178	695	PZ441402
	Pmar_Torre_N20	576	PZ441179	687	PZ441400
Lake Faro (LF)	Pmar_Faro_Z7	576	PZ441180	651	PZ441401
	Pmar_Faro_Z8	576	PZ441181	566	PZ441403
	Pmar_Faro_Z10	576	PZ441182	696	PZ441407

clade comprised marine/brackish species belonging to the genus *Pseudodiaptomus*: *P. marinus* sequences from various geographic regions, including our newly obtained sequences, as well as *P. ishigakiensis* Nishida, 1985 and *P. nihonkaiensis* Hirakawa, 1983 (Fig. 2).

The haplotype networks generated using all the sequences available for *P. marinus* consisted of 28 and 14 sequences for COI and ITS regions, respectively (Fig. 3; Tables S2, S3). For both networks, the references downloaded included sequences generated from Korean areas that were assumed to represent the first genetic signature of the native populations of *P. marinus* (Northwest Pacific Ocean) (Tables S2, S3). Both networks highlighted the lack of a clear biogeographic pattern within *P. marinus* populations (Fig. 3). The COI network (Fig. 3) identified 13 haplotypes, eight of which included one sequence only (Tables S2, S3). Of the four references from Korea, two collapsed in a distinct haplotype (H_7-AY145436 and KR048958), while one was separate in the H_8 (KR048956). The fourth Korean sequence (KR048957) was shared with three individuals from LF (Faro_Z7_COI, Faro_Z8_COI and Faro_Z10_COI) in the H_2 haplotype (Fig. 3). This latter was part of a reticulate structure with other haplotypes from different geographic regions where *P. marinus* has been introduced over time (Mediterranean Sea, Eastern Atlantic Ocean, Eastern Pacific Ocean), without a clear phylogeographic pattern (Fig. 3; Tables S2, S3). Sequences from the GoT were grouped in the H_3 haplotype with sequences from California and the North Sea (Helgoland), while sequences from the VL and TdG were grouped in the H_1 haplotype with those from the North Sea (Helgoland). Finally, the H_5 haplotype included a previous sequence from the GoN (*Di Capua et al., 2022*) and the sequences from the Marmara Sea (Black Sea) and Bilbao (Iberian Coast).

ITS sequences produced in the present work were ca. 600 bp long and spanned the ITS1, the 5.8S and the ITS2 including a short portion of 18S and 28S at the 5′ and 3′ ends, respectively. The ITS sequence Faro_Z7_ITS resulted identical to KT808252, the first

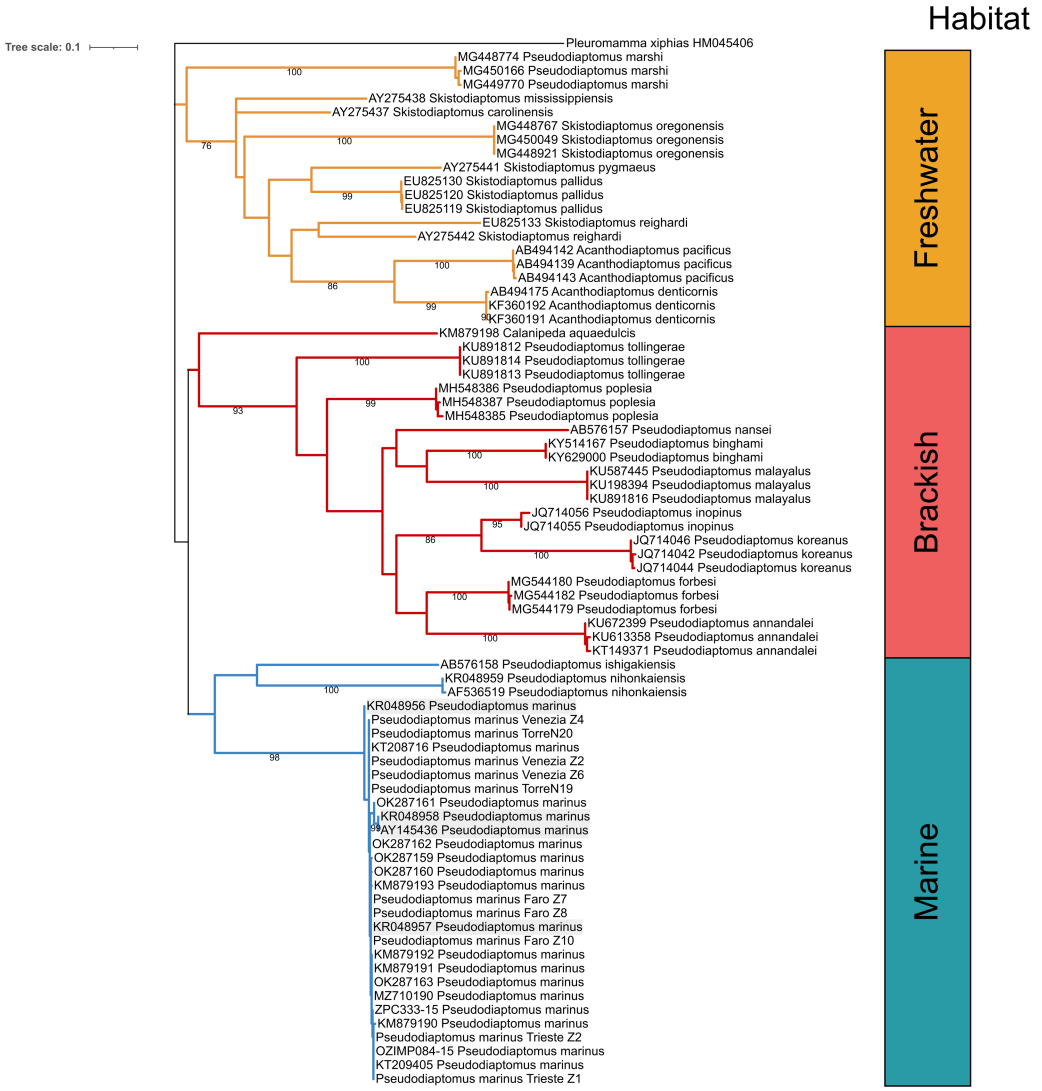


Figure 2 **Pseudodiaptomidae phylogeny.** Maximum Likelihood tree of 75 COI sequences including the ten sequences generated in this study and 65 sequences downloaded. *Pleuromamma xiphias* (JN574428) was used as outgroup. Bootstrap values > 70% are indicated in the tree. *Pseudododiaptomus marinus* sequences from Korea were highlighted with grey background in label.

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molecular record of *P. marinus* from the Mediterranean Sea (LF; *Sabia et al., 2017*). For this reason, only Faro_Z7_ITS was used in the further analyses. The ITS network identified seven haplotypes (Fig. 3). The only ITS sequence available from Korea formed a distinct haplotype (H_7- AY496261), which was clearly separate from the others. All sequences obtained in this study collapsed in the same haplotype (H_1), except for one sequence from LF (H_2) and one from VL (H_3) showing distinct haplotypes. Interestingly, the Black Sea sequences corresponded to three haplotypes (H_4-6), one of which (H_4) clearly separated from the other two. As for the COI, the ITS2 network also revealed no clear biogeographic pattern (Fig. 3).

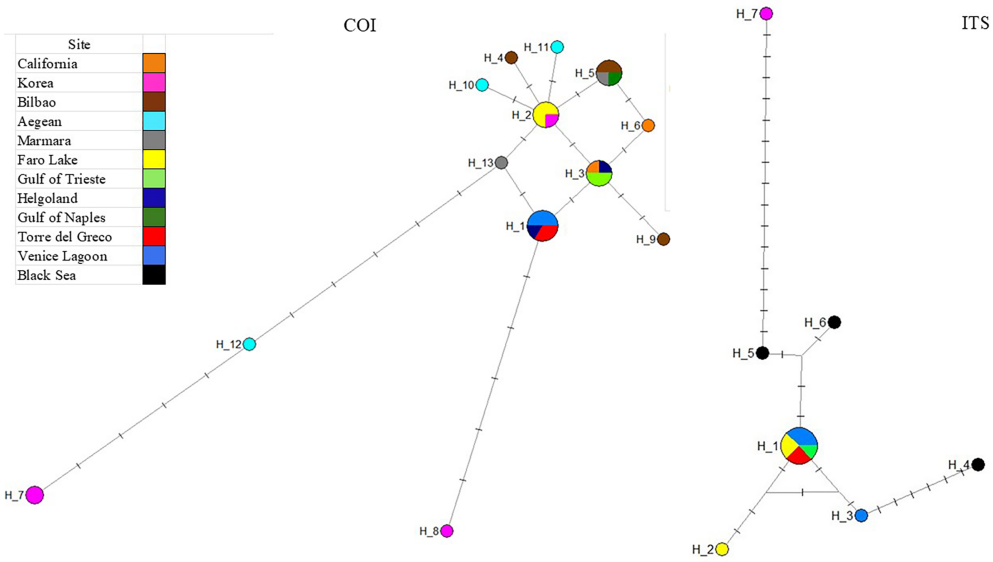


Figure 3 **Haplotype networks generated for COI (left) and ITS (right) molecular regions using the sequences of *Pseudododiaptomus marinus* from Italian waters (this study) and all the sequences available from other geographical areas.**

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To enhance the resolution of the genetic relationships among different populations, ITS2 secondary structure prediction and CBC search analyses were carried out. The ITS2 secondary structure predictions revealed a 185 bp long region (identified from the pairing of the 5.8S with the 28S), composed of the four canonical helices with the III being the longest (Fig. 4). The presence of the pyrimidine-pyrimidine mismatch in helix II validated the correctness of the folding. Using Faro_Z7_ITS2 as the reference sequence (Fig. 4), the analysis of the Italian and Black Sea sequences revealed no CBC in helix III, whereas three CBCs and two HCBCs were detected compared to the Korean reference. Helix I was the most variable among the analysed sequences, while the tip of helix IV presented subtle differences (Fig. S1).

DISCUSSION

Zooplankton invasions are reported almost worldwide (*Dexter & Bollens, 2020*). In marine ecosystems, copepods are particularly efficient colonizers, with widely documented intercontinental introductions (*e.g.*, *Cordell et al., 2008*; *Ruiz et al., 2011*; *Villate, Uriarte & Iriarte, 2018*; *Zagami et al., 2018*). Their success is largely attributed to the variety of anthropogenic vectors they can exploit (*Choi et al., 2005*; *Feis et al., 2019*; *Velasquez et al., 2021*; *Vidjak et al., 2019*). Among calanoid copepods, the genus *Pseudodiaptomus* includes several NIS of global concern, such as *Pseudodiaptomus forbesi* and *Pseudodiaptomus inopinus* (*Adams, Bollens & Bishop, 2015*; *Bollens et al., 2012*; *Bollens et al., 2014*; *Cordell & Morrison, 1996*; *Cordell, Rasmussen & Bollens, 2007*).

The dual-marker approach (COI and ITS) used in this study reinforces the taxonomic resolution for *P. marinus*, supporting its reliable molecular identification across the four

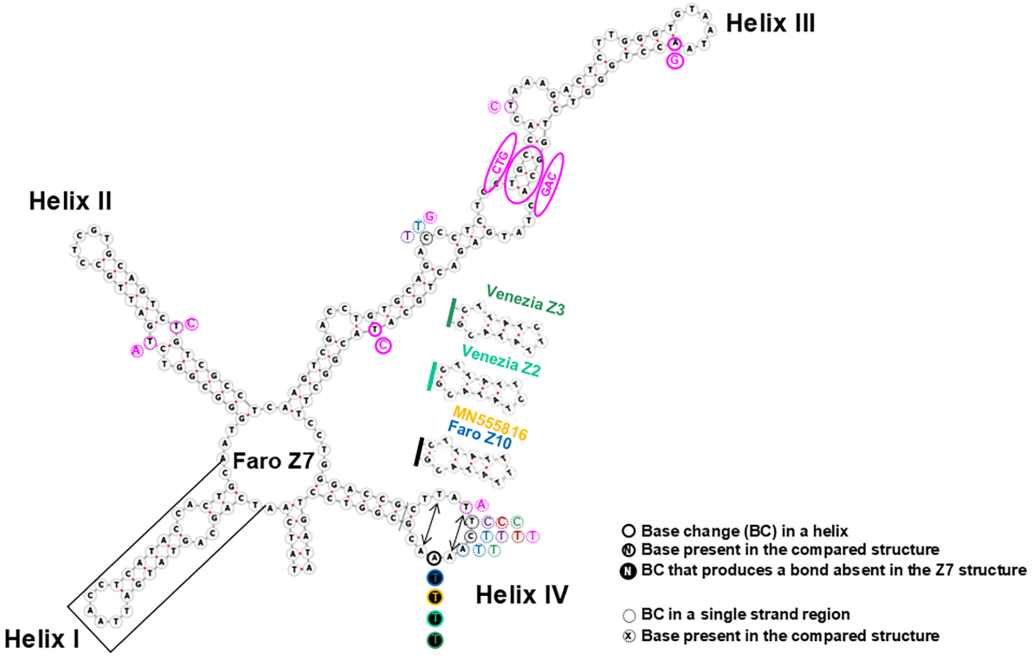


Figure 4 **The predicted secondary structures of the ITS2 helices of *P. marinus*.** The identified CBCs were shown on the helices.

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investigated Italian sites and contributing to a broader set of validated reference sequences for the Mediterranean Sea (*Di Capua et al., 2022*; *Kourkoutmani et al., 2026*; *Sabia et al., 2017*; *Tiralongo et al., 2022*; *Tirelli et al., 2024*). Furthermore, the COI and ITS genes, incorporating ITS2 secondary structure and CBCs as additional features, were applied for species delimitation also within Harpacticoida and provided the first molecular data for a harpacticoid species from Türkiye (*Bakmaz, Sönmez & Korkmaz, 2025*). These findings reinforce the results of the present study and further highlight the need for a comprehensive taxonomic revision of the family, integrating both morphological and molecular approaches to clarify species boundaries and phylogenetic relationships.

The COI phylogenetic tree distinguishes three main Pseudodiaptomidae clades, corresponding to freshwater, brackish, and marine species, in agreement with previous ecological groupings based on ITS2 (*Sabia et al., 2017*). Overall, our results do not support the existence of cryptic species within *P. marinus* populations sampled across different biogeographic regions: all European and extra-European sequences cluster together, and both COI and ITS haplotype networks show no clear geographic structuring. While some haplotypes are shared across regions, this pattern suggests repeated human-mediated introductions rather than ongoing gene flow.

P. marinus COI data support the occurrence of high genetic diversity within the species, particularly in Korean populations, as previously reported by *Ohtsuka et al. (2018)*. However, the limited geographic coverage and incomplete metadata associated with several sequences deposited in public databases currently hamper a robust reconstruction of

introduction routes and source populations. The reticulate structure of the COI haplotype network, showing shared or closely related haplotypes among Mediterranean, European Atlantic, Southern North Sea, and Eastern Pacific populations, is consistent with repeated introduction events followed by secondary spread across invaded regions (*Uttieri et al., 2020*; *Uttieri et al., 2023*). Such a pattern likely reflects the role of maritime traffic and port connectivity in facilitating dispersal.

Analyses based on the ITS marker, particularly the ITS2 region, confirm the absence of clear biogeographic structuring among Italian *P. marinus* haplotypes, while highlighting higher differentiation in Black Sea populations, consistent with previous findings from Sevastopol Bay (*Gubanova et al., 2020*). The lack of CBCs in helix III between Italian and Black Sea specimens supports their conspecific status and suggests potential gene flow between these populations. In contrast, the presence of CBCs and HCBCs in sequences attributed to Korean populations (AY496261; *Gubanova et al., 2020*) suggests a possible misidentifications, emphasizing the importance of an integrated molecular-assisted α-taxonomy approach, correct identifications and the need for robust, validated sequence databases (*Di Capua et al., 2022*; *Di Capua et al., 2023*; *Peters et al., 2025*), or indicating a markedly higher divergence among different population. Differences observed in helix I of the ITS2 secondary structure remain difficult to interpret due to limited comparative data for *Pseudodiaptomus*. Further integrative analyses combining multiple species concepts, together with broader sampling across the native distribution range of *P. marinus*, are needed to clarify the taxonomic significance of these patterns and the phylogenetic position of the Korean lineage. Nonetheless, although ITS sequences of *P. marinus* are still relatively scarce in public repositories, our findings confirm ITS as a powerful gene for species delimitation and detection of cryptic diversity (*Li et al., 2024*; *Liu et al., 2024*; *Van Wormhoudt et al., 2019*). Future studies integrating ITS with other molecular markers will be essential to further elucidate the bioinvasion dynamics and evolutionary trajectories of planktonic species (*e.g.*, *Kochanova et al., 2021*; *Kourkoutmani et al., 2026*; *Mouth et al., 2024*).

CONCLUSIONS

P. marinus forms a well-supported genetic clade, with Italian populations showing genetic connections not only among themselves but also with those from other introduced areas, without evident geographic patterns. At the same time, higher haplotype diversity is observed in the presumed native area of origin, supporting the hypothesis of multiple introduction events followed by secondary dispersal in invaded areas. Overall, the present study provides additional insight into the genetic diversity and phylogeography of *P. marinus*, contributing to a better understanding of its invasion pathways. Given the ongoing environmental changes and the increasing spread of NIS, investigating the phylogeography of *P. marinus* and other marine invaders remains essential for understanding the evolutionary and ecological processes that facilitate adaptation, establishment, and persistence in newly colonized environments.

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Competing Interests

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Author Contributions

- Iole Di Capua conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

- Roberta Piredda analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Alberto Amato conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Elisa Camatti performed the experiments, prepared figures and/or tables, and approved the final draft.
- Ylenia Carotenuto performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Stefano Corona analyzed the data, prepared figures and/or tables, and approved the final draft.
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- Andrea Marcucci analyzed the data, prepared figures and/or tables, and approved the final draft.
- Alenka Goruppi performed the experiments, prepared figures and/or tables, and approved the final draft.
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- Giacomo Zagami performed the experiments, prepared figures and/or tables, and approved the final draft.
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DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The raw sequence data are available at NCBI. The complete list of accessions is available in the Supplemental Files.

Data Availability

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REFERENCES

Adams JB, Bollens SM, Bishop JG. 2015. Predation on the invasive copepod, Pseudodiaptomus forbesi, and native zooplankton in the lower Columbia River: an experimental approach to quantify differences in prey-specific feeding rates. *PLOS ONE* **10**:e0144095 DOI 10.1371/journal.pone.0144095.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**:3389–3402 DOI 10.1093/nar/25.17.3389.

Bakmaz D, Sönmez S, Korkmaz EM. 2025. Evaluating COI and ITS2 dual barcoding for molecular delimitation and taxonomic insights in *Arenosetella* Wilson, 1932 (Harpacticoida: Ectinosomatidae) along Turkish coasts. *PeerJ* **13**:e19870 DOI 10.7717/peerj.19870.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**:37–48 DOI 10.1093/oxfordjournals.molbev.a026036.

Bertolino M, Costa G, Ruocco N, Esposito R, De Matteo S, Zagami G, Costantini M. 2022. First certain record of Demospongiae class (Porifera) alien species from the Mediterranean Sea. *Marine Genomics* **63**:100951 DOI 10.1016/j.margen.2022.100951.

Blanco-Bercial L, Cornils A, Copley N, Bucklin A. 2014. DNA barcoding of marine copepods: assessment of analytical approaches to species identification. *PLOS Currents: Tree of Life* **6**.

Bollens SM, Breckenridge JK, Cordell JR, Rollwagen-Bollens G, Kalata O. 2012. Invasive copepods in the Lower Columbia River Estuary: seasonal abundance, co-occurrence and potential competition with native copepods. *Aquatic Invasions* **7**:101–109 DOI 10.3391/ai.2012.7.1.011.

Bollens SM, Breckenridge JK, Cordell JR, Simenstad CA, Kalata O. 2014. Zooplankton of tidal marsh channels in relation to enrivonmental variables in the upper San Francisco Estuary. *Aquatic Biology* **21**:205–219 DOI 10.3354/ab00589.

Bonanno G, Orlando-Bonaca M. 2019. Non-indigenous marine species in the Mediterranean Sea—myth and reality. *Environmental Science & Policy* **96**:123–131 DOI 10.1016/j.envsci.2019.03.014.

Bucklin A, Ortman BD, Jennings RM, Nigro LM, Sweetman CJ, Copley NJ, Sutton T, Wiebe PH. 2010. A Rosetta Stone for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean). *Deep Sea Research Part II: Topical Studies in Oceanography* **57**:2234–2247 DOI 10.1016/j.dsr2.2010.09.025.

Bucklin A, Steinke D, Blanco-Bercial L. 2011. DNA barcoding of marine metazoa. *Annual Review of Marine Science* **3**:471–508 DOI 10.1146/annurev-marine-120308-080950.

Byun Y, Han K. 2009. PseudoViewer3: generating planar drawings of large-scale RNA structures with pseudoknots. *Bioinformatics* **25**:1435–1437 DOI 10.1093/bioinformatics/btp252.

Camatti E, Acri F, De Lazzari A, Nurra N, Pansera M, Schroeder A, Bergamasco A. 2023. Natural or anthropogenic variability? A long-term pattern of the zooplankton communities in an ever-changing transitional ecosystem. *Frontiers in Marine Science* **10**:1176829 DOI 10.3389/fmars.2023.1176829.

Chen G, Hare MP. 2008. Cryptic ecological diversification of a planktonic estuarine copepod, Acartia tonsa. *Molecular Ecology* **17**:1451–1468 DOI 10.1111/j.1365-294X.2007.03657.x.

Choi KH, Kimmerer W, Smith G, Ruiz GM, Lion K. 2005. Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. *Journal of Plankton Research* **27**:707–714 DOI 10.1093/plankt/fbi044.

Cianelli D, Uttieri M, Buonocore B, Falco P, Zambardino G, Zambianchi E. 2012. Dynamics of a very special Mediterranean coastal area: the Gulf of Naples. In: Williams GS, ed. *Mediterranean ecosystems: dynamics, management and conservation*. New York: Nova Science Publishers Inc, 129–150.

Coleman AW. 2000. The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. *Protist* **151**:1–9 DOI 10.1078/1434-4610-00002.

Coleman AW. 2003. ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics* **19**:370–375 DOI 10.1016/S0168-9525(03)00118-5.

Cordell JR, Bollens SM, Draheim R, Sytsma M. 2008. Asian copepods on the move: recent invasions in the Columbia-Snake River system, USA. *ICES Journal of Marine Science* **65**:753–758 DOI 10.1093/icesjms/fsm195.

Cordell JR, Morrison SM. 1996. The invasive Asian copepod *Pseudodiaptomus inopinus* in Oregon, Washington, and British Columbia Estuaries. *Estuaries* **19**:628–638 DOI 10.2307/1352523.

Cordell JR, Rasmussen M, Bollens SM. 2007. Biology of the introduced copepod *Pseudodiaptomus inopinus* in a northeastern Pacific estuary. *Marine Ecology Progress Series* **333**:213–227 DOI 10.3354/meps333213.

Corona S, Becker ÉC, Carotenuto Y, de Olazabal A, Di Capua I, Goruppi A, Tirelli V, Uttieri M. 2026. Biogeographic spread of the non-indigenous copepod *Pseudodiaptomus marinus* in Italian waters –insights by the marine strategy framework directive surveys. *Diversity and Distributions* **32**:e70226 DOI 10.1111/ddi.70226.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**:407–415 DOI 10.1111/j.1095-8312.2005.00503.x.

De Olazabal A, Tirelli V. 2011. First record of the egg-carrying calanoid copepod *Pseudodiaptomus marinus* in the Adriatic Sea. *Marine Biodiversity Records* **4**:e85 DOI 10.1017/S1755267211000935.

Dexter E, Bollens SM. 2020. Zooplankton invasions in the early 21st century: a global survey of recent studies and recommendations for future research. *Hydrobiologia* **847**:309–319 DOI 10.1007/s10750-019-04096-x.

Di Capua I, Boxshall GA. 2008. Records of Oncaeidae (Copepoda) from the Gulf of Naples, with new records of three species of *Triconia*. *Journal of the Marine Biological Association of the United Kingdom* **88**:1407–1415 DOI 10.1017/S002531540800194X.

Di Capua I, Maffucci F, Pannone R, Mazzocchi MG, Biffali E, Amato A. 2017. Molecular phylogeny of Oncaeidae (Copepoda) using nuclear ribosomal internal transcribed spacer (ITS rDNA). *PLOS ONE* **12**:e0175662 DOI 10.1371/journal.pone.0175662.

Di Capua I, Mazzocchi MG. 2021. Crustacea Copepoda Calanoida, Cyclopoida, Harpacticoida, Mormonilloida, Siphonostomatoida (marine planktonic). In: Bologna MA, Zapparoli M, Oliverio M, Minelli A, Bonato L, F. C, Stoch F, eds. *Checklist of the Italian Fauna, Version 1.0*. Last update: 2021-2005-2031.

Di Capua I, Piredda R, Mazzocchi MG, Zingone A. 2021. Metazoan diversity and seasonality through eDNA metabarcoding at a Mediterranean long-term ecological research site. *ICES Journal of Marine Science* **78**:3303–3316 DOI 10.1093/icesjms/fsab059.

Di Capua I, D'Angiolo R, Piredda R, Minucci C, Boero F, Uttieri M, Carotenuto Y. 2022. From phenotypes to genotypes and back: toward an integrated evaluation of biodiversity in calanoid copepods. *Frontiers in Marine Science* **9**:833089 DOI 10.3389/fmars.2022.833089.

Di Capua I, Piredda R, D'Angiolo R, Minucci C, Montalbano A, Boero F, Carotenuto Y, Uttieri M. 2023. Is integrated taxonomy useful to study diversity and ecology? An example from crustacean zooplankton at the long-term ecological research site MareChiara (LTER-MC). *Marine Ecology* **44**:e12752 DOI 10.1111/maec.12752.

European Environment Agency. 2020. Marine regions and subregions. *Available at https://www.eea.europa.eu/data-and-maps/figures/marine-regions-and-subregions* (accessed on 19 April 2023).

Feis ME, Goedknegt MA, Arzul I, Chenuil A, den Boon O, Gottschalck L, Kondo Y, Ohtsuka S, Shama LNS, Thieltges DW, Wegner KM, Luttikhuizen PC. 2019. Global invasion genetics of two parasitic copepods infecting marine bivalves. *Scientific Reports* **9**:12730 DOI 10.1038/s41598-019-48928-1.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**:294–299.

Galanidi M, Aissi M, Ali M, Bakalem A, Bariche M, Bartolo AG, Bazairi H, Beqiraj S, Bilecenoglu M, Bitar G, Bugeja M, Carbonell A, Castriota L, Chalabi A, Çinar ME, Dragičević B, Dulčić J, El-Haweet AEA, Farrag MMS, Evans J, Galil B, Guerin L, Hyams-Kaphzan O, Kapedani R, Kamberi E, Livi S, Mačić V, Masse C, Mavrič B, Orlando-Bonaca M, Ouerghi A, Petović S, Png-Gonzalez L, Schembri PJ, Shenkar N, Sghaier YR, Shakman E, Yahyaoui A, Yokeş MB, Zenetos A. 2023. Validated inventories of non-indigenous species (NIS) for the Mediterranean Sea as tools for regional policy and patterns of NIS spread. *Diversity* **15**:962 DOI 10.3390/d15090962.

Ghabooli S, Shiganova TA, Briski E, Piraino S, Fuentes V, Thibault-Botha D, Angel DL, Cristescu ME, MacIsaac HJ. 2013. Invasion pathway of the ctenophore *Mnemiopsis leidyi* in the Mediterranean Sea. *PLOS ONE* **8**:e81067 DOI 10.1371/journal.pone.0081067.

Ghezzo M, Sarretta A, Sigovini M, Guerzoni S, Tagliapietra D, Umgiesser G. 2011. Modeling the inter-annual variability of salinity in the lagoon of Venice in relation to the water framework directive typologies. *Ocean & Coastal Management* **54**:706–719 DOI 10.1016/j.ocecoaman.2011.06.007.

Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**:221–224 DOI 10.1093/molbev/msp259.

Gruber AR, Bernhart SH, Lorenz R. 2015. The ViennaRNA web services. In: Picardi E, ed. *RNA bioinformatics*. New York: Springer New York, 307–326.

Gubanova A, Drapun I, Garbazey O, Krivenko O, Vodiasova E. 2020. Pseudodiaptomus marinus Sato, 1913 in the Black Sea: morphology, genetic analysis, and variability in seasonal and interannual abundance. *PeerJ* **8**:e10153 DOI 10.7717/peerj.10153.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* **41**:95–98.

Jeon D, Lim D, Lee W, Soh HY. 2018. First use of molecular evidence to match sexes in the Monstrilloida (Crustacea: Copepoda), and taxonomic implications of the newly recognized and described, partly *Maemonstrilla*-like females of *Monstrillopsis longilobata* Lee, Kim & Chang, 2016. *PeerJ* **6**:e4938 DOI 10.7717/peerj.4938.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**:587–589 DOI 10.1038/nmeth.4285.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**:1160–1166 DOI 10.1093/bib/bbx108.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**:772–780 DOI 10.1093/molbev/mst010.

Katsanevakis S, Moustakas A. 2018. Uncertainty in marine invasion science. *Frontiers in Marine Science* **5**:38 DOI 10.3389/fmars.2018.00038.

Kochanova E, Nair A, Sukhikh N, Väinölä R, Husby A. 2021. Patterns of cryptic diversity and phylogeography in four freshwater copepod crustaceans in European lakes. *Diversity* **13**:448 DOI 10.3390/d13090448.

Kourkoutmani P, Iliadou E, Minoudi S, Kontse A, Rados C, Vlachodimou T, Castelli M, Carotenuto Y, Di Capua I, Uttieri M, Michaloudi E. 2026. The non-indigenous copepod *Pseudodiaptomus marinus* Sato, 1913 in an Eastern Mediterranean coastal area: day-night pattern and genetic characterization. *Scientific Reports* DOI 10.1038/s41598-026-69749-z.

Kuraku S, Zmasek CM, Nishimura O, Katoh K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research* **41**:W22–W28 DOI 10.1093/nar/gkt389.

Laakmann S, Blanco-Bercial L, Cornils A. 2020. The crossover from microscopy to genes in marine diversity: from species to assemblages in marine pelagic copepods. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**:20190446 DOI 10.1098/rstb.2019.0446.

Letunic I, Bork P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research* **47**:W256–W259 DOI 10.1093/nar/gkz239.

Li T, Zhang T, Liu M, Zhang Z, Zhang J, Niu J, Chen X, Al-Farraj SA, Song W. 2024. Findings on three endocommensal scuticociliates (Protista, Ciliophora) from freshwater mollusks, including their morphology and molecular phylogeny with descriptions of two new species. *Marine Life Science & Technology* **6**:212–235 DOI 10.1007/s42995-024-00230-4.

Liu M, Jiang L, Zhang Z, Wei F, Ma H, Chen Z, Al-Rasheid KAS, Hines HN, Wang C. 2024. Linking multi-gene and morphological data in the subclass Scuticociliatia (Protista, Ciliophora) with establishment of the new family Homalogastridae fam. nov. *Marine Life Science & Technology* **7**:1–22 DOI 10.1007/s42995-024-00264-8.

Margiotta F, Balestra C, Buondonno A, Casotti R, D'Ambra I, Di Capua I, Gallia R, Mazzocchi MG, Merquiol L, Pepi M, Percopo I, Saggiomo M, Sarno D, Zingone A. 2020. Do plankton reflect the environmental quality status? The case of a post-industrial Mediterranean Bay. *Marine Environmental Research* **160**:104980 DOI 10.1016/j.marenvres.2020.104980.

Mazzocchi MG, Di Capua I, Kokoszka F, Margiotta F, Ribera d'Alcalà M, Sarno D, Zingone A, Licandro P. 2023. Coastal mesozooplankton respond to decadal environmental changes *via* community restructuring. *Marine Ecology* **44**:e12746 DOI 10.1111/maec.12746.

Meiklejohn KA, Damaso N, Robertson JM. 2019. Assessment of BOLD and GenBank – their accuracy and reliability for the identification of biological materials. *PLOS ONE* **14**:e0217084 DOI 10.1371/journal.pone.0217084.

Miller KJ, Bradford-Grieve JM, Jillett JB. 1999. Genetic relationship between winter deep-dwelling and spring surface-dwelling female *Neocalanus tonsus* in the Southern Ocean. *Marine Biology* **134**:99–106 DOI 10.1007/s002270050528.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**:1530–1534 DOI 10.1093/molbev/msaa015.

Mouth C, Ferreira F, Sukhikh N, Bou E, Bernard A, Tackx M, Azémar F, Meire P, Maris T, Legal L. 2024. Exploring the genetic structure and phylogeographic patterns of the copepod genus *Eurytemora* in Europe. *Diversity* **16**:483 DOI 10.3390/d16080483.

Occhipinti-Ambrogi A. 2002. Current status of aquatic introductions in Italy. In: Leppäkoski E, Gollasch S, Olenin S, eds. *Invasive aquatic species of europe distribution, impacts and management*. Dordrecht: Springer Netherlands, 311–324.

Occhipinti-Ambrogi A, Marchini A, Cantone G, Castelli A, Chimenz C, Cormaci M, Froglia C, Furnari G, Gambi MC, Giaccone G, Giangrande A, Gravili C, Mastrototaro F, Mazziotti C, Orsi-Relini L, Piraino S. 2011. Alien species along the Italian coasts: an overview. *Biological Invasions* **13**:215–237 DOI 10.1007/s10530-010-9803-y.

Ohtsuka S, Shimono T, Hanyuda T, Shang X, Huang C, Soh HY, Kimmerer W, Kawai H, Itoh H, Ishimaru T, Tomikawa K. 2018. Possible origins of planktonic copepods, Pseudodiaptomus marinus (Crustacea: Copepoda; Calanoida), introduced from East

Asia to the San Francisco Estuary based on a molecular analysis. *Aquatic Invasions* **13**:221–230 DOI 10.3391/ai.2018.13.2.04.

Olivotti R, Faganelli J, Malej A. 1986. Impact of 'organic' pollutants on coastal waters. *Gulf of Trieste Water Science & Technology* **18**:57–68 DOI 10.2166/wst.1986.0078.

Pansera M, Granata A, Guglielmo L, Minutoli R, Zagami G, Brugnano C. 2014. How mesh-size selection reshape the description of zooplankton community structure in coastal lakes? *Estuarine, Coastal and Shelf Science* **151**:221–235 DOI 10.1016/j.ecss.2014.10.015.

Peters J, Albaina A, Blanco-Bercial L, Bucklin A, Di Capua I, Dos Santos A, Falkenhaug T, Fernandez de Puelles M, Fileman E, Garić R, Holst S, Hosia A, Laakmann S, Majaneva S, Mańko MK, Mazzocchi MG, O'Brien TD, Peijnenburg KTCA, Renz J, Rossel S, Weydmann-Zwolicka A, Winkler G, Yebra L, Cornils A. 2025. Taxonomic uncertainty in North Atlantic and Mediterranean zooplankton limits species-level monitoring accuracy. *ICES Journal of Marine Science* **82**:fsaf077 DOI 10.1093/icesjms/fsaf077.

Ratnasingham S, Hebert PDN. 2007. BOLD: the barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes* **7**:355–364 DOI 10.1111/j.1471-8286.2007.01678.x.

Rocha GM, Martinelli AB, Utz LRP, Loureiro Fernandes LF. 2019. Using internal transcribed spacers for Calanoida (Crustacea, Copepoda) species identification. *Marine Biodiversity* **49**:1565–1571 DOI 10.1007/s12526-018-0902-9.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* **34**:3299–3302 DOI 10.1093/molbev/msx248.

Ruiz G, Fofonoff P, Steves B, Dahlstrom A. 2011. Marine crustacean invasions in North America: a synthesis of historical records and documented impacts. In: Galil B, Clark PF, Carlton JT, eds. *In the wrong place - alien marine crustaceans: distribution, biology and impacts*. Dordrecht: Springer, 215–250.

Sabia L, Di Capua I, Percopo I, Uttieri M, Amato A. 2017. ITS2 in calanoid copepods: reconstructing phylogenetic relationships and identifying a newly introduced species in the Mediterranean. *The European Zoological Journal* **84**:104–115 DOI 10.1080/11250003.2016.1276639.

Sabia L, Uttieri M, Schmitt FG, Zagami G, Zambianchi E, Souissi S. 2014. Pseudodiaptomus marinus Sato, 1913, a new invasive copepod in Lake Faro (Sicily): observations on the swimming behaviour and the sex-dependent responses to food. *Zoological Studies* **53**:49 DOI 10.1186/s40555-014-0049-8.

Sabia L, Zagami G, Mazzocchi MG, Zambianchi E, Uttieri M. 2015. Spreading factors of a globally invading coastal copepod. *Mediterranean Marine Science* **16**:460–471 DOI 10.12681/mms.1154.

Saccà A, Guglielmo L, Bruni V. 2008. Vertical and temporal microbial community patterns in a meromictic coastal lake influenced by the Straits of Messina upwelling system. *Hydrobiologia* **600**:89–104 DOI 10.1007/s10750-007-9179-x.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**:7537–7541 DOI 10.1128/AEM.01541-09.

Servello G, Andaloro F, Azzurro E, Castriota L, Catra M, Chiarore A, Crocetta F, D'Alessandro M, Denitto F, Froglia C, Gravili C, Langer MR, Lo Brutto S, Mastrototaro F, Petrocelli A, Pipitone C, Piraino S, Relini G, Serio D, Xentidis NJ, Zenetos A. 2019. Marine alien species in Italy: a contribution to the implementation of descriptor D2 of the marine strategy framework directive. *Mediterranean Marine Science* **20**:1–48 DOI 10.12681/mms.18711.

Solidoro C, Bandelj V, Aubry Bernardi F, Camatti E, Ciavatta S, Cossarini G, Facca C, Franzoi P, Libralato S, Canu DM, Pastres R, Pranovi F, Raicevich S, Socal G, Sfriso A, Sigovini M, Tagliapietra D, Torricelli P. 2010. Response of the Venice lagoon ecosystem to natural and anthropogenic pressures over the last 50 years. In: Kennish MJ, Paerl HW, eds. *Coastal Lagoons critical habitats of environmental change*. Boca Raton: CRC Press, 483–511.

Somma R, Giuffrè E, Amonullozoda S, Spoto SE, Giacobbe A, Giacobbe S. 2024. Geological and ecological insights on the Lake Faro Global Geosite within the Messina Strait framework (Italy). *Geosciences* **14**:319 DOI 10.3390/geosciences14120319.

Svetlichny L, Hubareva E, Khanaychenko A, Uttieri M. 2019. Response to salinity and temperature changes in the alien Asian copepod *Pseudodiaptomus marinus* introduced in the Black Sea. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* **331**:416–426 DOI 10.1002/jez.2309.

Tiralongo F, Akyol O, Al Mabruk SAA, Battaglia P, Beton D, Bitls B, Borg JA, Bouchoucha M, Çinar ME, Crocetta F, Dragičević B, Dulčić J, Evangelopoulos A, Evans J, Fortič A, Gauff RPM, Georgiadis CG, Gökoğlu M, Grech D, Guy-Haim T, Huseyinoglu MF, Lombardo A, Marletta G, Mastrototaro F, Montesanto F, Nunes F, Özgül A, Öztürk B, Rammou D-L, Scuderi D, Terbiyik Kurt T, Trainito E, Trkov D, Ulman A, Ünal V, Velasquez X. 2022. New alien Mediterranean biodiversity records (2022). *Mediterranean Marine Science* **23**:725–747 DOI 10.12681/mms.31228.

Tirelli V, Di Capua I, Putelli E, Carotenuto Y, Uttieri M. 2024. First insight on molecular identification and nycthemeral vertical distribution of Pseudodiaptomus marinus (Copepoda: Calanoida) in Italian waters. *Rapports de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée* **43**:222.

Tsiamis K, Palialexis A, Stefanova K, Gladan ŽN, Skejić S, Despalatović M, Cvitković I, Dragičević B, Dulčić J, Vidjak O, Bojanić N, Žuljević A, Aplikioti M, Argyrou M, Josephides M, Michailidis N, Jakobsen HH, Staehr PA, Ojaveer H, Lehtiniemi M, Massé C, Zenetos A, Castriota L, Livi S, Mazziotti C, Schembri PJ, Evans J, Bartolo AG, Kabuta SH, Smolders S, Knegtering E, Gittenberger A, Gruszka P, Kraśniewski W, Bartilotti C, Tuaty-Guerra M, Canning-Clode J, Costa AC,

Parente MI, Botelho AZ, Micael J, Miodonski JV, Carreira GP, Lopes V, Chainho P, Barberá C, Naddafi R, Florin A-B, Barry P, Stebbing PD, Cardoso AC. 2019. Non-indigenous species refined national baseline inventories: a synthesis in the context of the European Union's Marine Strategy Framework Directive. *Marine Pollution Bulletin* **145**:429–435 DOI 10.1016/j.marpolbul.2019.06.012.

Tsirintanis K, Azzurro E, Crocetta F, Dimiza M, Froglia C, Gerovasileiou V, Langeneck J, Mancinelli G, Rosso A, Stern N, Triantaphyllou M, Tsiamis K, Turon X, Verlaque M, Zenetos A, Katsanevakis S. 2022. Bioinvasion impacts on biodiversity, ecosystem services, and human health in the Mediterranean Sea. *Aquatic Invasions* **17**:308–352 DOI 10.3391/ai.2022.17.3.01.

Uttieri M, Aguzzi L, Aiese Cigliano R, Amato A, Bojanić N, Brunetta M, Camatti E, Carotenuto Y, Damjanović T, Delpy F, de Olazabal A, Di Capua I, Falcão J, Fernandez de Puelles ML, Foti G, Garbazey O, Goruppi A, Gubanova A, Hubareva E, Iriarte A, Khanaychenko A, Lučić D, Marques SC, Mazzocchi MG, Mikuš J, Minutoli R, Pagano M, Pansera M, Percopo I, Primo AL, Svetlichny L, Rožić S, Tirelli V, Uriarte I, Vidjak O, Villate F, Wootton M, Zagami G, Zervoudaki S. 2020. WGEUROBUS –working group towards a European OBservatory of the non-indigenous calanoid copepod *Pseudodiaptomus marinus*. *Biological Invasions* **22**:885–906 DOI 10.1007/s10530-019-02174-8.

Uttieri M, Anadoli O, Banchi E, Battuello M, Beşiktepe Ş, Carotenuto Y, Cotrim Marques S, de Olazabal A, Di Capua I, Engell-Sørensen K, Goruppi A, Guy-Haim T, Hure M, Kourkoutmani P, Lučić D, Mazzocchi MG, Michaloudi E, Morov AR, Terbıyık Kurt T, Tirelli V, Vannini J, Velasquez X, Vidjak O, Wootton M. 2023. The distribution of *Pseudodiaptomus marinus* in European and neighbouring waters - a rolling review. *Journal of Marine Science and Engineering* **11**:1238 DOI 10.3390/jmse11061238.

Van Wormhoudt A, Adjeroud M, Rouzé H, Leray M. 2019. Recent and old duplications in crustaceans internal transcribed spacer 1: structural and phylogenetic implications. *Molecular Biology Reports* **46**:5185–5195 DOI 10.1007/s11033-019-04976-4.

Varrella S, Livi S, Corinaldesi C, Castriota L, Maggio T, Vivona P, Pindo M, Fava S, Danovaro R, Dell'Anno A. 2025. A comprehensive assessment of non-indigenous species requires the combination of multi-marker eDNA metabarcoding with classical taxonomic identification. *Environment International* **199**:109489 DOI 10.1016/j.envint.2025.109489.

Velasquez X, Morov AR, Terbıyık Kurt T, Meron D, Guy-Haim T. 2021. Two-way bioinvasion: tracking the neritic non-native cyclopoid copepods *Dioithona oculata* and *Oithona davisae* (Oithonidae) in the Eastern Mediterranean Sea. *Mediterranean Marine Science* **22**:586–602 DOI 10.12681/mms.26036.

Vidjak O, Bojanić N, de Olazabal A, Benzi M, Brautović I, Camatti E, Hure M, Lipej L, Lučić D, Pansera M, Pećarević M, Pestorić B, Pigozzi S, Tirelli V. 2019. Zooplankton in Adriatic port environments: indigenous communities and non-indigenous species. *Marine Pollution Bulletin* **147**:133–149 DOI 10.1016/j.marpolbul.2018.06.055.

Villate F, Uriarte I, Iriarte A. 2018. Impact of the invasive species *Acartia tonsa* on the distribution of autochthonous Acartiidae species in estuaries of the Bay of Biscay. In: Uttieri M, ed. *Trends in copepod studies - distribution, biology and ecology*. New York: 83–117.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. New York: Academic Press Inc, 315–322.

Will KW, Mishler BD, Wheeler QD. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* **54**:844–851 DOI 10.1080/10635150500354878.

Zagami G, Bonanzinga V, Pansera M. 2023. Pseudodiaptomidae G.O. Sars. 1902. ICES Identification Leaflets for Plankton No. 201. International Council for the Exploration of the Sea, Copenhagen, Denmark, 19 pp. DOI 10.17895/ices.pub.23963307.

Zagami G, Brugnano C, Granata A, Guglielmo L, Minutoli R, Aloise A. 2018. Biogeographical distribution and ecology of the planktonic copepod *Oithona davisae*: rapid invasion in Lakes Faro and Ganzirri (Central Mediterranean Sea). In: Uttieri M, ed. *Trends in copepod studies - distribution, biology and ecology*. New York: Nova Science Publishers Inc, 59–82.

Zenetos A, Albano PG, López Garcia E, Stern NIR, Tsiamis K, Galanidi M. 2022. Established non-indigenous species increased by 40% in 11 years in the Mediterranean Sea. *Mediterranean Marine Science* **23**:196–212 DOI 10.12681/mms.29106.

Zhang GK, Chain FJJ, Abbott CL, Cristescu ME. 2018. Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. *Evolutionary Applications* **11**:1901–1914 DOI 10.1111/eva.12694.

Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* **31**:3406–3415 DOI 10.1093/nar/gkg595.