BioInvasions Records (2025) Volume 14, Issue 3: 673–686

Rapid Communication

First record of the non-indigenous *Myrianida pachycera* (Augener, 1913) (Annelida: Syllidae) in the Mediterranean Sea revealed by integrative taxonomy

Joachim Langeneck[1], Yann Toso[2,3], Alberto Castelli[4], Maria Giovanna Parisi[2,3], Stefano Piraino[1,3,5], Matteo Putignano[1,5], Jonathan Tempesti[4], Andrea Toso[1,3,5] and Luigi Musco[1,3,5]

[1]*Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), U.L.R. di Lecce, c/o Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (DiSTeBA), Palazzina A, Campus Ecotekne, Strada Provinciale Lecce-Monteroni, 73100 Lecce, Italy*

[2]*Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, Via Archirafi 22, 90123 Palermo, Italy*

[3]*National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133 Palermo, Italy*

[4]*Dipartimento di Biologia, Università di Pisa, via Derna 1, 56126 Pisa, Italy*

[5]*Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (DiSTeBA), Università del Salento, Palazzina A, Campus Ecotekne, Strada Provinciale Lecce-Monteroni, 73100 Lecce, Italy*

Corresponding author: Yann Toso (yann.toso@unipa.it)

Citation: Langeneck J, Toso Y, Castelli A, Parisi MG, Piraino S, Putignano M, Tempesti J, Toso A, Musco L (2025) First record of the non-indigenous *Myrianida pachycera* (Augener, 1913) (Annelida: Syllidae) in the Mediterranean Sea revealed by integrative taxonomy. *BioInvasions Records* 14(3): 673–686, https://doi.org/10.3391/bir.2025.14.3.16

Received: 2 October 2024
Accepted: 13 March 2025
Published: 11 August 2025

Handling editor: Tamar Guy-Haim
Thematic editor: April Blakeslee

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Abstract

The non-indigenous syllid polychaete *Myrianida pachycera* (Augener, 1913), originally described from Australia, is reported for the first time in the Mediterranean Sea, based on individuals sampled in port environments in the Tyrrhenian and Ionian Sea. The identity of the specimens was investigated through morphological identification and DNA barcoding of two individuals, resulting in COI sequences with almost 100% identity with an individual from California. *Myrianida pachycera* must be considered non-indigenous for the Mediterranean Sea. The distribution of this species seems to be relatively wide in the basin, but its detection has possibly been hindered by the loss of the majority of the diagnostic characters following fixation. Conversely, due to their striking live colour pattern, live individuals might be successfully tracked via citizen science.

Key words: Polychaeta, NIS, biological invasion, Sicily, Tyrrhenian Sea, Ionian Sea

Introduction

Non-Indigenous species (NIS) are known for their impacts on native communities and human activities, representing one of the major threats to marine biodiversity worldwide (Pimentel et al. 2001; Wittenberg and Cock 2001; Mooney 2005; Vilá et al. 2010). In recent years new records of NIS in the Mediterranean Sea have been published with high frequency (Golani et al. 2013; Marchini et al. 2015; Zenetos et al. 2017), resulting in a 40% increase of established species reports since 2010 (Zenetos et al. 2022). These periodical reports are essential to understand range shifts and invasion dynamics of NIS through space and time, and inform regional conservation planning and mitigation actions (Mačić et al. 2018; Giakoumi et al. 2019). However, most research on NIS traditionally focused on fishes, molluscs, and crustaceans, while several other taxa have been neglected or scarcely studied, resulting in unfilled gaps in NIS distribution knowledge.

Marine annelids are particularly affected by uncertainties regarding their taxonomy, origin and distribution. In 2010, 130 NIS annelid species were reported in the Mediterranean Sea (Zenetos et al. 2010), even though a sizable part of them was considered questionable (Zenetos et al. 2010; Çinar 2013; Langeneck et al. 2020). Moreover, a few records derived from misidentifications of other annelid species, either already described or still undescribed ones (Del Pasqua et al. 2018; Lezzi and Giangrande 2018; Çinar and Erdoğan-Dereli 2023). Often based on taxonomic revisions, additional marine NIS annelids were reported after 2010 (Langeneck et al. 2020; Dağli and Çinar 2022; Rousou et al. 2023; Sala-Mirete et al. 2023; Toso et al. 2024), increasing their total approximate number in the Mediterranean Sea up to around 160 species.

The amount of NIS annelid taxa reported across different areas of the Mediterranean Sea is highly variable. This is partially due to a pronounced "author effect", i.e. an uneven distribution of annelid specialists (Giangrande and Licciano 2004; Musco and Giangrande 2005), delivering much information in countries with strong taxonomic background on annelids, such as Italy and Türkiye (Castelli et al. 1987; Tempesti et al. 2020a), while creating apparent knowledge gaps in regions where annelids are less frequently studied. Nonetheless, there is also a clear biogeographical effect, with Eastern Mediterranean countries hosting a higher number of NIS and a higher proportion of thermophilic species with Indo-Pacific affinity due to their proximity to the Suez Canal (Rousou et al. 2023). However, in recent years several NIS established in the eastern part of the basin showed clues of expansion towards the central and western Mediterranean Sea (Langeneck et al. 2020; Palero et al. 2020; Bonifazi et al. 2023; Langeneck et al. 2024a). In this context, Italy represents a geographical crossroad among different Mediterranean subregions. Its geographic position and taxonomic background contributed to make Italy the Mediterranean country with the highest number of marine NIS annelid species reported. In fact, Langeneck et al. (2020) reported overall 68 annelid NIS, and another 10 species were recently added (Gravina et al. 2021; Bonifazi et al. 2023; Borghese et al. 2023; Langeneck et al. 2024a, b), even though questionable taxa represent more than half of the reported annelid NIS (42 out of 78) stressing the need for additional taxonomic studies on this group.

Syllidae, the most species-rich annelid family, is characterised by widespread occurrence of cryptic and pseudocryptic species (Álvarez-Campos et al. 2017a, b; Aguado et al. 2019; Del Olmo et al. 2024), leading to frequent uncertainties surrounding the identity and origin of NIS (Çinar 2013; Langeneck et al. 2020). Within this family, the subfamily Autolytinae is probably the most complex from a taxonomic point of view, showing a high degree of cryptic diversity, dramatic morphological divergence between vegetative (atoke) and reproductive (epitoke) forms, and few reliable taxonomic characters (Nygren and Sundberg 2003; Nygren 2004). In fact, while in the majority of Syllidae the shape of chaetae, antennae and dorsal cirri are often employed as diagnostic characters, they are less useful for the identification of Autolytinae. Species distinction is instead mostly based on live colour pattern, pharyngeal armature (trepan) and the presence and position of ciliary bands, which are generally difficult to examine in fixed material (Nygren and Gidholm 2001; Nygren 2004). As a consequence, the specific diversity of this subfamily is generally scarcely known, and the contribution of these species to biological invasions is generally overlooked. To date, no Autolytinae are reported as NIS in the Mediterranean Sea, and only two species, namely *Myrianida pachycera* (Augener, 1913) and *Proceraea* cf. *rubroproventriculata* Nygren and Gidholm, 2001, have been cited as possible invaders worldwide (Cohen et al. 2005; Carlton and Eldredge 2009; Keppel et al. 2019).

Another important gap of knowledge in the study of non-indigenous marine annelids is represented by the lack of molecular data, and in particular DNA barcoding sequences based on mitochondrial markers. So far, these are available only for approximately 30% of the annelid NIS reported in European waters (Lavrador et al. 2023). The lack of molecular data is worsening the taxonomic uncertainties surrounding several annelid species and has indirect negative consequences such as the impairment of environmental DNA monitoring (Maggio et al. 2023; Mugnai et al. 2023). At the same time, a significant part of the annelid sequences deposited in public repositories are identified only to the family, class or even phylum level (Langeneck et al. 2024; Toso et al. 2024), stressing the need for detailed taxonomic studies integrating morphological and molecular data. At present, DNA barcoding was carried out only on a few NIS annelids in the Mediterranean Sea (Del Pasqua et al. 2018; Grosse et al. 2021; Sala-Mirete et al. 2023; Langeneck et al. 2024a), therefore limiting our ability to trace NIS invasions in the area.

Here we report the occurrence of the Indo-Pacific annelid *Myrianida pachycera* in the Mediterranean Sea, backing the morphological identification with the mitochondrial cytochrome oxidase I gene (COI) barcoding data, and discuss its geographic distribution within the basin.

Materials and methods

Fouling assemblages were sampled by scraping artificial substrates in the harbours of Palermo, Trapani, Siracusa, Augusta and Livorno; individuals of *M. pachycera* were sorted alive, photographed and fixed in 4% paraformaldehyde in seawater or 96% ethanol for further morphological and genetic analyses. Macro photographs were obtained using a Sony ILCA-68camera equipped with a SubSee +10 magnifier lens. Microphotographs and measurements were performed in the BioForIU Laboratory of the Salento University using a stereomicroscope SMZ 25 equipped with DS-Ri2 video camera and a video-interactive image analysis system NIS–Elements BR 4.30.02 Nikon Instruments software. For the examination of smaller details and internal anatomy, permanent slides were obtained staining the dissected structures with Rose Bengal and mounting the slides with Faure's medium (Faure 1910). Drawings of chaetae were obtained with a Leica DM 2000 microscope equipped with a camera lucida and improved with Gimp 2.10 following the guidelines in Montesanto (2015). The examined specimens were deposited in the polychaete collections of the Museum of Marine Biology "Pietro Parenzan" of the University of Salento (PCZL) and of the Museum of Natural History of the University of Pisa (MSNP).

Genetic characterisation was carried out on two individuals of *M. pachycera*, as well as one individual of *Myrianida rubropunctata* (Grube, 1860) and one of *Amblyosyllis lineata* Grube, 1863 for comparison purposes. Total genomic DNA was extracted from a couple of posterior chaetigers using the salting-out protocol (Aljanabi and Martínez 1997) modified according to Furfaro et al. (2022). Folmer's fragment of the gene coding for the cytochrome *c* oxidase (COI) was amplified using the annelid-specific primers POLYLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and POLYHCO (5'-TAMACTTCWGGGTGACCAAARAATCA-3') (Carr et al. 2011), using the following PCR protocol: initial denaturing step at 94 °C for 5 min, 40 cycles of denaturing at 94 °C for 45 s, annealing at 45 °C for 45 s, and extending at 72 °C for 1 min, with a final extending step at 72 °C for 7 min. A negative control was included in each reaction. The PCR products were sent to Macrogen Europe for purification and sequencing.

Phylogenetic analyses were performed using the COI sequences of *M. pachycera* available on public repositories, as well as sequences of other Autolytinae, using *Amblyosyllis spectabilis* (Johnston *in* Baird, 1861) and *Amblyosyllis lineata* to root the tree. DNA sequences were aligned with ClustalX v. 2.1 (Larkin et al. 2007), and alignments were edited using the program BIOEDIT v. 7.2.5 (Hall 1999). The program jModelTest 2.1.6 (Guindon and Gascuel 2003; Darriba et al. 2012) was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike 1974). For all markers and genera, the most suitable substitution model was the generalised time reversible (GTR, Tavaré 1986) +I +G. A Bayesian consensus phylogenetic tree was constructed using MrBayes 3.2 (Ronquist et al. 2011). Four replicate runs were carried out with three Markov chains per run for 1×10^6 generations. The chain was sampled every 1,000 generations to obtain 2,000 sampled trees. The first 500 sampled trees (25%) were discarded as burn-in, with the remaining 1,500 trees used to estimate the Bayesian consensus, posterior probability (PP) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, which should reach a value < 0.01 at the end of the analysis (Ronquist et al. 2011).

Results

Myrianida pachycera (Augener, 1913)

?*Autolytus orientalis* Willey 1905: 270, pl. 4, figs. 81, 84.
?*Autolytus maculata* Potts 1911: 36–37.
Autolytus pachycerus Augener 1913: 257–260, pl. 2, figs. 11, 12, textfig. 40A–C; 1927: 157–158.
Myrianida pachycera Imajima 1966: 79–82, fig. 26A–L; 1967: 417; Nygren 2004: 143–146, fig. 71A–E; Keppel et al. 2019: 61.
Myrianida pachycerus Hartman 1966a: 362–363, fig. 1A–B.
Autolytus purpureimaculata Okada 1933: 332–338, figs. 6–11; Imajima and Hartman 1964: 100.
Myrianida crassicirrata Hartmann-Schröder 1965: 119–121, figs. 47–49; Hartman 1966b: 194–195.

Material examined. Livorno, touristic port (43°32′54.74″N, 10°17′49.6″E), 1 m, fouling assemblages on concrete piers, 10 December 2024: 4 atoke individuals, one of them with developing stolon (MSNP: P/5224). Palermo, touristic marina (38°07′12.7″N, 13°22′01.8″E), 0.5 m, fouling assemblages on artificial substrate (concrete piers), 22 September 2023: 1 immature stolon (PCZL.SY.M.5.1). Palermo, touristic marina (38°07′10.0″N, 13°22′07.3″E), 1 m, same environment, 15 March 2024: 1 atoke individual with developing stolons (PCZL.SY.M.5.2). Palermo, touristic marina (38°07′10.0″N, 13°22′07.3″E), 1 m, same environment, 8 October 2024: 1 atoke individual with developing stolons (PCZL.SY.M.5.3), 1 detached epigamic stolon (PCZL.SY.M.5.4). Trapani, fishing port (38°00′55.4″N, 12°29′56.0″E), same environment, 11 October 2024: 1 atoke individual with developing stolons (PCZL.SY.M.5.5). Siracusa touristic marina (37°3′43.38″N, 15°17′21.37″E), 0.5 m, same environment, 29 October 2024: 1 atoke individual with developing stolons (PCZL.SY.M.5.6). Augusta, Cozzo Pisone saltern (37°14′48.52″N, 15°12′56.81″E), 0.5 m, fouling assemblages on artificial substrates, 07 November 2024: 3 atoke individuals with developing stolons (PCZL.SY.M.5.7; PCZL.SY.M.5.8; PCZL.SY.M.5.9).

Description

Body measurement and color. Largest complete individual 8.04 mm long, 1.13 mm maximum width, 50 chaetigers; longest individual 12.31 mm long, 0.51 mm maximum width, 118 chaetigers. Stock with 27–30 chaetigers, developing chain of stolons formed by up to 89 segments. Live individuals slightly flattened (Figure 1A) with two visible ciliary bands per segment, individuals after preservation in ethanol contracted, cilindrical (Figure 1B). Live colour yellowish, with a longitudinal orange stripe along each side and 5–8 transverse orange bands occurring every 2–4 chaetigers, varying between different specimens, rarely sequential over consecutive chaetigers; prostomium and peristomium dark blue, palps whitish, an unpaired dark blue triangular spot on the back of each segment starting from the 4th chaetiger. Colour pattern almost completely lost in ethanol-preserved individuals, with traces of blue pigmentation on the dorsal cirri and unpaired dark dorsal spots.

Head. Prostomium rectangular, wider than long. Two pairs of large, dark red to black eyes of similar size, inserted dorso-laterally, lateral pairs coalescent (Figure 1C). Palps short, fused, approximately as long as the

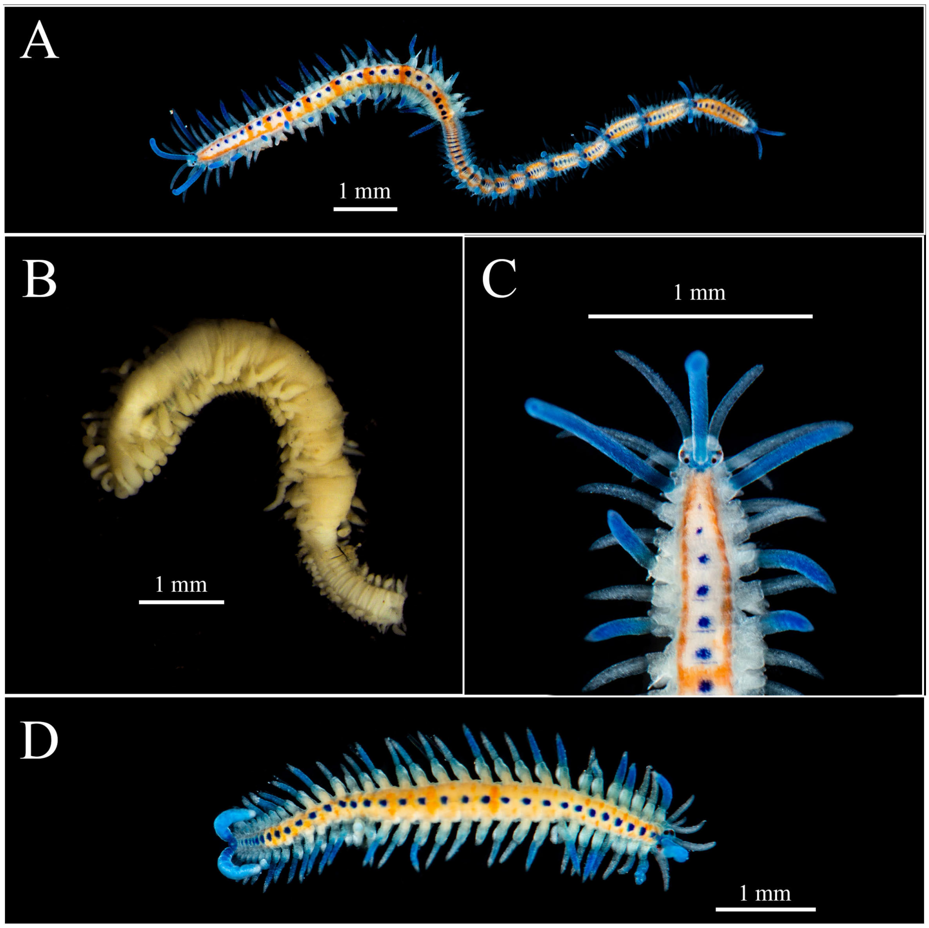

Figure 1. *Myrianida pachycera* (Augener 1913): A) Atoke individual with stolon chain from Augusta, Sicily, live; B) Atoke specimen preserved in ethanol; C) Close-up on the anterior segments; D) Detached epigamic stolon from the port of Palermo. Photographs A, C, D by Yann Toso, photograph B by Joachim Langeneck.

prostomium. Nuchal epaulettes as grooves, reaching the 4th chaetiger. Antennae, dorsal cirri and tentacular cirri club-shaped and slightly flattened, smooth but with irregular constrictions along their length. Median antenna reaching chaetiger 5–6 backwards, lateral antennae slightly thinner, approximately 3/4 the length of the median antenna. Dorsal tentacular cirri as long as median antenna, ventral tentacular cirri approximately 2/5 of dorsal tentacular cirri. Pharynx over 6 segments with one sinuation, trepan with 36 denticles, irregularly alternating between large and small ones (one larger denticle every 2–5 small ones) (Figure 2A), proventricle over 3 segments with 27 muscle cell rows.

Parapodia. All chaetigers uniramous and similar throughout the body. Parapodia with dorsal cirri around 2/3 of the length of the median antenna, approximately maintaining the same length throughout the whole body length. Dorsal cirri showing a slight alternation in size and colour, with thicker, slightly longer, blue cirrostyles in chaetigers 3, 5, 8, 10 and following even chaetigers, thinner, slightly shorter, whitish cirrostyles in chaetigers 1, 2, 4, 6, 7, 9 and following odd chaetigers. Cirrophores approximately as long

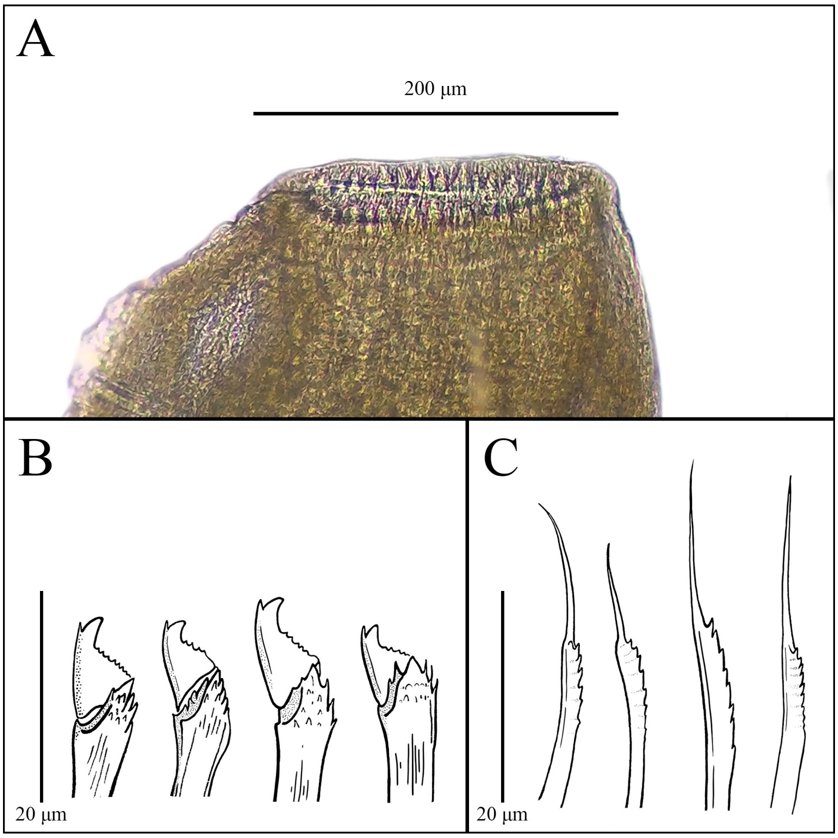

Figure 2. *Myrianida pachycera* (Augener 1913): A) Dissected trepan; B) Compound chaetae respectively (left to right) from the 5th, 5th, 22nd chaetiger from the stock, with the last one from the 2nd stolon; C) Bayonet chaetae respectively (left to right) from the 20th, 22nd chaetiger, with the last two from the 2nd stolon. Photograph by Yann Toso, drawing by Matteo Putignano.

as the parapodium, of the same length between cirri with long and short cirrostyle, slightly thinner in the latter. Ventral cirri pillow-shaped, massive, fused to the parapodium.

Chetae. Parapodia supported by up to 3 aciculae, number of chaetae increasing towards the midbody, anteriorly with around 20 compound chaetae without dorso-ventral gradation in size of blades, in the midbody with up to 34 compound chaetae. Compound chaetae heterogomph, with short, strongly bidentate blade, secondary tooth distinctly larger than the primary one, with serrated ventral edge (Figure 2B). Bayonet chaetae (Figure 2C) present from chaetiger 11 in some specimens, starting at the end of the stock or even already in the stolon in other individuals.

Stolons and pygidium. Stolon chain with 7–33 developing stolons. Unripe stolons with smaller chaetigers, devoid of chaetae, gradually increasing in size and number of chaetae towards the pygidium. Ripe stolon with 33 chaetigers, flattened, with eyes larger than those of the atoke form, with parapodia provided with a small number of compound chaetae, one bayonet chaeta, and several natatory chaetae. Posterior chaetigers very short and numerous, pygidium with two long, thick anal cirri.

Molecular data. COI sequences were obtained for two individuals from Palermo (PCZL SY.M.5.1 and SY.M.5.2, GenBank accession numbers: PQ135964–PQ135965), both sequences showing a 99.8% identity with a

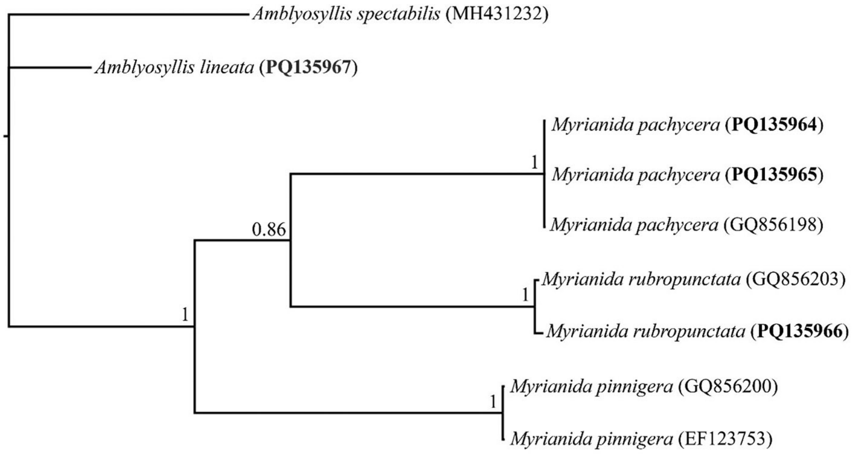

Figure 3. Bayesian phylogenetic tree of selected species of the genus *Myrianida* based on COI sequences. *Amblyosyllis lineata* and *A. spectabilis* are used as outgroup. Node values represent Bayesian posterior probabilities, sequences in bold were obtained in the present study.

deposited sequence of *M. pachycera* from California (GenBank accession number: GQ856198; Nygren et al. 2010) as well as private sequences of the same species on BOLD (Figure 3).

Remarks. The examined individuals match well the redescription by Nygren (2004), who stressed the distinctive colour pattern of this species as one of the diagnostic characters for *M. pachycera*. The only differences we identified between our material and Nygren's (2004) redescription are represented by the number of compound chaetae (up to 34 *vs* up to 25) and the starting point of bayonet chaetae (from chaetiger 11 *vs* from chaetiger 24), suggesting that these characters are subject to a higher degree of variation. All other characters, including the pharyngeal and proventricular traits, show instead a very good correspondence. Augener (1913) described *Autolytus pachycerus* from Western Australia; Imajima (1966) transferred the species to the genus *Myrianida*, proposing *Autolytus* purpureimaculata Okada, 1933 (described from Japan) as a junior synonym. Later on, Nygren (2004) confirmed the synonymy with this last taxon and suggested a further synonymy with *Myrianida crassicirrata* Hartmann-Schröder, 1965 (described from the Hawaii Islands), suggesting that *M. pachycera* has a wide distribution in the tropical and sub-tropical Pacific Ocean. Molecular and morphological data confirm that Mediterranean specimens assayed in this study belong to *M. pachycera*, as shown by the close match to a specimen from California (Nygren et al. 2010).

Discussion

Myrianida pachycera is a tropical and sub-tropical species, currently known with some certainty mainly from the Indo-Pacific region, where it was reported from Australia (Augener 1913), Japan (Imajima 1966), south China Sea (Huang 2001), the Seychelles (Böggemann et al. 2003), the Hawaii Islands (Nygren 2004), California (Nygren 2004; Cohen et al. 2005), Pacific Mexico (Tovar-Hernández et al. 2014), and the Galapagos Islands (Keppel et al. 2019). Nygren (2004) suggested that *Autolytus orientalis*, described by Willey (1905) for Ceylon and *Autolytus maculata* described by Potts (1911) from an unknown locality might be synonymous with *M. pachycera*, but the descriptions are too scanty to confirm this hypothesis. Outside the Indian and Pacific Oceans, *M. pachycera* is currently known from Florida only (Nygren 2004). Nygren (2004) suggested that the wide distribution of *M. pachycera* might be due to anthropogenic introduction in a sizable part of its range. The absence of historical records, as well as the high sequence identity with the individual of *M. pachycera* from California, confirm that this species is a recent introduction in the Mediterranean Sea.

Interestingly, the distribution of *M. pachycera* in Italian waters seems to be rather fragmented, as the species was not detected in previous, detailed surveys of port environments focusing on non-indigenous species both in the northern Tyrrhenian Sea and along the Apulia coastline (Tamburini et al. 2021; Tempesti et al. 2022; Langeneck et al. 2024a). Recently, we were informed that a single specimen of *M. pachycera* was found in the Venice lagoon in 2016 (E. Keppel *unpublished obs.*). Still, no published data is available from 2016 to date, which suggests that a reproductive population of *M. pachycera* was never established in the Northern Adriatic after that unpublished record. In the case of the Italian harbours investigated, the subsequent finding of reproductive stages suggests the occurrence of stable, although probably small populations in the northern and southern Tyrrhenian Sea, as well as in the western Ionian Sea. While Sicilian harbours are comparatively poorly studied, and the species could have been present and undetected for several years, the port of Livorno has been continuously monitored from 2019 onwards (Tempesti et al. 2020b).

Considering that *M. pachycera* has only been found inside ports and marinas in the Mediterranean Sea, its dispersal is probably strictly navigation-mediated within the basin, and the establishment of reproductive populations and their spread northwards is probably fostered by the recent increase in surface temperatures. However, it is likely that the distribution of *M. pachycera* in the Mediterranean Sea is wider than the current data would suggest. In fact, a recent video focusing on marine worms from Antalya, Türkiye uploaded on YouTube for dissemination purposes, (https://www.youtube.com/watch?v=7Z-dIFZ9dCA) shows several good-quality frames of a live specimen having the characteristic live colour pattern of *M. pachycera*. The species might also occur in the Aegean and the Levantine Sea; however, surveys of living specimens in port environments of this areas coupled with molecular analyses would be desirable to ascertain its presence.

Analysing living specimens is often crucial for the correct identification of many annelid species, but this kind of observations are seldom carried out. The spread of *M. pachycera* in the Mediterranean Sea may be underrated due to the limited number of studies focusing on living specimens. In fact, this species is as easily recognisable when alive, as poorly characterizable when fixed. Based on our observations, direct fixation in ethanol causes a general shrinkage of the organism, and a deformation of the appendages, as well as the complete loss of the colour pattern, which ultimately leads to the impossibility to use the key provided by Nygren (2004) which is mostly based on live colour. Conversely, the unique contrasted and colourful pattern of this species makes it a popular subject for underwater photographers (http://www.flickriver.com/photos/top_down/9429696118/; http://www.nikonsmallworld.com/galleries/entry/2003-photomicrography-competition/2; Fofonoff et al. 2018) suggesting that the distribution and spread of this species could be reconstructed and monitored through citizen science, either through underwater photography contests, or through the organisation of events involving citizen scientists, such as BioBlitzs (Matassa and Hitchcock 2021; Innocenti et al. 2022). This approach was successfully used for cryptobenthic marine vertebrates (Colombo and Langeneck 2013; Ragkousis et al. 2021) and invertebrates (Trainito and Doneddu 2014; Rothman et al. 2017; Langeneck et al. 2022) and, albeit seldom employed for the study of marine annelids (but see Righi et al. 2020; Langeneck et al. 2022; Fourreau et al. 2024), may help to depict the actual distribution of *M. pachycera* in the Mediterranean Sea.

Authors' contribution

Research conceptualization JL, YT, LM; sample design and methodology JL, YT, LM, MGP, SP; investigation and data collection JL, YT, AC, JT, AT; data analysis and interpretation JL, YT, LM, MP; funding provision LM, MGP, SP; writing – original draft JL, YT; writing – review and editing LM, MGP, SP, AC, MP, JT, AT.

Authors' ORCIDs

JL: 0000-0003-3665-8683; YT: 0000-0001-6289-0676; AC: 0000-0002-0762-1403; MGP: 0000-0001-5462-0596; SP: 0000-0002-8752-9390; MP: 0000-0002-0575-224X; JT: 0000-0002-7027-3480; AT: 0000-0001-6252-924X; LM: 0000-0003-4750-4129.

Acknowledgements

We are grateful to Erica Keppel for sharing with us her unpublished data about *M. pachycera* in the Venice lagoon and to two anonymous reviewers for their comments that contributed to ameliorate the manuscript.

Funding declaration

This study has been funded in part by the project "National Biodiversity Future Center – NBFC" funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 – Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP D33C22000960007 (YT, MGP, SP, LM), and in part by the Horizon-EIC Pathfinder project MAPWORMS (Mimicking Adaptation and Plasticity in WORMS), grant agreement number 101046846 (JL, LM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability

Species georeferenced records are available at the European Alien Species Information Network: https://easin.jrc.ec.europa.eu/easin/RJD/Download/c0d4ae33-fbc7-4895-8cafa4af986b5fe7.

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