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ORIGINAL ARTICLE

New records of one cryptogenic and two questionable alien species of polychaetes (Annelida) in Italian waters

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Abstract: We report new records for three potentially non-indigenous polychaetes in Italian waters. The Nereididae *Leonnates aylaoberi* Çinar & Dağli, 2013 is reported for the first time in the Tyrrhenian, Ionian and Adriatic Sea; this species has type locality in the Mediterranean Sea, but the genus *Leonnates* has Indo-Pacific affinity, and its distinctive characters suggest that it is a recent newcomer to this area. We here suggest that *L. aylaoberi* should be considered cryptogenic in the Mediterranean Sea. Moreover, we report the first records of *Paucibranchia* cf. *adenensis* Gravier, 1900 for the Italian coastline, in the Ionian and Tyrrhenian Sea, and the first records of *Isolda* cf. *pulchella* Müller, 1858 in the Tyrrhenian Sea. The meaning of the differences identified between the Mediterranean material and the available descriptions of these species is briefly discussed. Juvenile individuals of *L. aylaoberi* and *P.* cf. *adenensis* are also described for the first time.
Keywords: cryptogenic species; alien species; questionable species; polychaetes

Sažetak: NOVI NALAZI JEDNE KRIPTOGENE I DVIJE UPITNE STRANE VRSTE MNOGOČETINAŠA (ANNELIDA) U TALIJANSKIM VODAMA. Izvještavamo o novim zapisima za tri potencijalno neautohtone vrste mnogočetinaša u talijanskim vodama. Pripadnik porodice Nereididae *Leonnates aylaoberi* Çinar & Dağli, 2013 prvi je put zabilježen u Tirenskom, Jonskom i Jadranskom moru; iako je tipski lokalitet ove vrste u Sredozemnom moru, rod *Leonnates* ima indo-pacifički afinitet, a njegova karakteristična obilježja ukazuju da je nedavna pridošlica u ovo područje. Predlažemo da se vrsta *L. aylaoberi* smatra kriptogenom u Sredozemnom moru. Nadalje, izvještavamo o prvim nalazima vrste *Paucibranchia* cf. *adenensis* Gravier, 1900, za talijansku obalu, u Jonskom i Tirenskom moru te prvom nalazu vrste *Isolda* cf. *pulchella* Müller, 1858 u Tirenskom moru. Ukratko se raspravlja o naravi utvrđenih razlika između sredozemnog materijala i dostupnih opisa ovih vrsta. U radu se iznose i prvi opisi mladih jedinki vrsta *L. aylaoberi* i *P.* cf. *adenensis*.
Ključne riječi: kriptogene vrste; strane vrste; upitne vrste; mnogočetinaši

INTRODUCTION

The Mediterranean Sea experienced a steady increase in the number of non-indigenous species (NIS) in the last years (Zenetos and Galanidi, 2020) and they are generally believed to exert negative effects on native assemblages and ecosystems (Tsirintanis *et al.*, 2022). However, uncertainties surrounding the taxonomic identity and origin of several species might hinder a careful evaluation of their spread and potential impacts. Cryptogenic species have been defined as species with certain identity, but uncertain geographical origin which does not allow to conclusively assess whether they are native or introduced to a certain area (Carlton and Schwindt, 2024). In several cases, molecular data allowed to positively solve cases of cryptogenic species (Blakeslee *et al.*, 2008; Sun *et al.,* 2017; Conlan *et al.,* 2021) but, in other cases, genetic homogeneity or an ambiguous phylogenetic signal due to repeated introductions hinder a precise reconstruction of the origin and introduction pathways of cryptogenic species (Carmona *et al.,* 2014; Maltagliati *et al.,* 2016). Questionable non-indigenous species have been defined somewhat loosely as "NIS with insufficient information, or new entries not verified by experts, or NIS with unresolved taxonomic status" (Tsiamis *et al.*, 2018). Following Essl *et al.* (2018), and to equalise the terminology regarding NIS at the scale of the European Union, Tsiamis *et al.* (2019) suggested replacing "questionable" with "data deficient". However, the term "data deficient" was defined by Essl *et al.* (2018) as a term defining "taxa for which an assessment of biogeographic status is unfeasible because of the lack of data", making its meaning broader than the original use of "questionable" as defined by Tsiamis *et al.* (2018). Presumably for this reason, later works retained the use of "questionable" over "data deficient" (Langeneck *et al.*, 2020; Tsiamis *et al.*, 2021). In general, it can be stated that, while cryptogenic species are affected

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by doubts surrounding their origin, questionable non-indigenous species are affected by doubts surrounding their taxonomic identity, either because of the presence of discrepancies towards the original description, or because their ecological traits do not match those described for the species, or, lastly, because their records are based on species lists and have not been validated by experts of the group (Tsiamis *et al.,* 2018; Langeneck *et al.*, 2020, 2024).

While cryptogenic species are rather evenly spread across taxonomic groups, the occurrence of questionable species shows a large variation among different groups (Zenetos *et al.,* 2005), depending on the status of taxonomic research and the presence of updated literature. Marine annelids are particularly affected by this issue; in fact, almost half of the marine annelids reported as non-indigenous along the coasts of Italy are considered questionable (Langeneck *et al.*, 2020). While in some cases detailed taxonomic studies allowed to clarify the identity of alleged non-indigenous annelids, which turned out to represent new native species (e.g.: *Streptosyllis nunezi* Faulwetter, Vasileiadou, Papageorgiou & Arvanitidis, 2008: Faulwetter *et al.,* 2008; *Streblosoma pseudocomatum* Lezzi & Giangrande, 2018: Lezzi and Giangrande, 2018; *Pista labruneae* Lavesque, Daffe, Londoño-Mesa & Hutchings, 2021: Lavesque *et al.,* 2021), the identity of most questionable annelid NIS occurring in the Mediterranean remains unresolved. Moreover, in several cases the record of NIS refers to species lists not providing any description, iconography or even comments useful to define the status of the species (e.g., Cantone and Fassari, 1982; Cantone, 2001) and the reference material is not available anymore (Langeneck *et al.*, 2020); for these reasons, ascertaining the identity of several species recorded in the Mediterranean Sea as allegedly non-indigenous is often tricky.

This note has the purpose of detailing the occurrence in Italian waters of three marine annelid species with possible non-indigenous origin, characterised by uncertainties surrounding their native range, or their taxonomic identity. Two species (*Paucibranchia* cf. *adenensis* Gravier, 1900 and *Leonnates aylaoberi* Çinar & Dağli, 2013) represent the first records for Italy, while the third one (*Isolda* cf. *pulchella* Müller, 1858) was already known for Italy (Cantone, 2001) and Tunisia (Zaâbi *et al.*, 2012), but Mediterranean specimens have never been described, whilst the detailed description of Mediterranean specimens of cryptogenic species is always desirable to solve uncertainties.

MATERIAL AND METHODS

Macrozoobenthic samples were collected in the context of several monitoring programmes along the Italian coastline (Fig. 1); samples were obtained through a van Veen Grab and sifted with a 0.5 mm mesh, fixed in 4% buffered formalin and transferred to 70% ethanol after sorting. Polychaetes belonging to the genera *Isolda, Leonnates* and *Marphysa* were re-analysed carefully under a stereomicroscope and an optical microscope. In particular, microphotographs were obtained 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, permanent slides were obtained staining the dissected structures with Rose Bengal and mounting the slides with Faure's medium (Faure, 1910). Drawings of parapodia and 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).

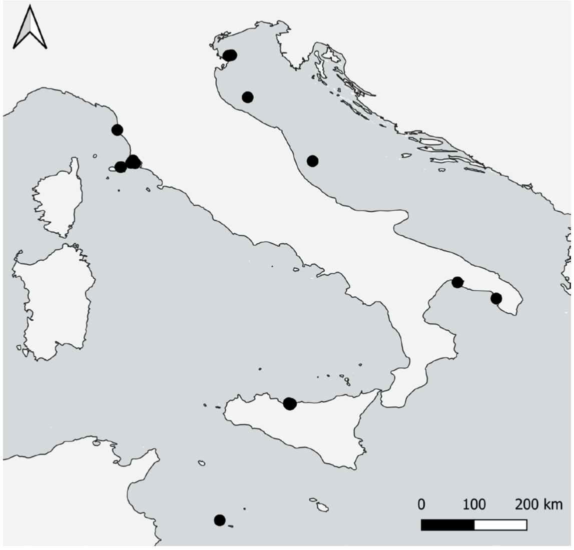

Fig. 1. Sampling localities along the Italian coastline where the three species were found.

The analysed specimens are deposited in the following repositories:

MSNP = Museum of Natural History of the University of Pisa (Calci, Italy)

PCZL = Museum of Marine Biology "Pietro Parenzan" of the University of Salento (Porto Cesareo, Italy)

ISPRA = Laboratory of Benthos Ecology collection, ISPRA (Rome, Italy)

ARPA = Reference collection of ARPA Puglia (Bari, Italy).

RESULTS

Leonnates aylaoberi Çinar & Dağli, 2013 (Figs. 2-4)

Leonnates aylaoberi Çinar & Dağli, 2013: 933-941, Figs. 6-10.

Material examined. Gulf of Follonica, Northern Tyrrhenian Sea: station PB-A (42.9415° N, 10.5922° E), 10 m, sand patch within a *Posidonia oceanica* meadow, 27 April 2022: 1 specimen (MSNP: P/5207); station

PB-B (42.9482° N, 10.5946° E), 10 m, clean fine sand, 27 April 2022: 1 specimen (MSNP: P/5208); station ST0 (42.8929° N, 10.6341° E), 20-35 m depth, silt and clay, 23 October 2018: 1 specimen (ISPRA.65); station ST2 (42.8920° N, 10.6349° E), 20-35 m depth, silt and clay, 23 October 2018: 2 specimens (ISPRA.65); station ST3 (42.8939° N, 10.6333° E), 20-35 m depth, silt and clay, 23 October 2018: 1 specimen (ISPRA.65); station ST4 (42.8961° N, 10.6320° N), 20-35 m depth, silt and clay, 23 October 2018: 2 specimens (ISPRA.65). Gulf of Portoferraio, Northern Tyrrhenian Sea: station PF-A (42.8132° N, 10.3365° E), 25 m, muddy sand, 28 April 2022: 5 specimens (MSNP: P/5209); station PF-B (42.8149° N, 10.3506° E), 30 m, muddy sand, 28 April 2022: 1 specimen (MSNP: P/5210). Termini Imerese, Southern Tyrrhenian Sea: station FT1-L (38.0027° N, 13.7610° E), 26.6 m depth, 20 January 2022: 4 specimens (MSNP: P/5211); station FT2-I (37.9996° N, 13.7697° E), 24 m depth, 20 January 2022: 1 specimen (MSNP: P/5212); station FT2-L (38.0038° N, 13.7665° E), 30.1 m depth, 20 January 2022: 1 specimen (MSNP: P/5213); station FT2-N (38.0120° N, 13.7599° N), 47 m depth, 20 January 2022: 1 specimen (MSNP: P/5214); station FT-G (37.9934° N, 13.7908° E), 18 m depth, 26 January 2022: 1 specimen (MSNP: P/5215); station FT-I (38.0001° N, 13.7979° E), 25 m depth, 26 January 2022: 2 specimens (MSNP: P/5216); station FT-L (38.0020° N, 13.8030° E), 26.5 m depth, 26 January 2022: 1 specimen (MSNP: P/5217). Santa Caterina di Nardò, Ionian Sea (40.1408° N, 17.9796° E), 17 m depth, *Halophila stipulacea* meadow, 28 July 2022: 4 specimens (PCZL. NR.L.1.1 and PCZL.NR.L.1.2). San Benedetto del Tronto, Central Adriatic Sea: station A6 (42.9349° N, 14.2436° E), 80 m depth, muddy silt, 01 July 2014: 1 specimen (ISPRA.67); station A7 (42.9344° N, 14.2439° E), 80 m depth, muddy silt, 06 February 2008: 1 specimen (ISPRA.68). Rimini, Northern Adriatic Sea: Station AS09 (44.2325° N, 12.9255° E), 63 m depth, sandy mud, 21 June 2019: 5 specimens (ISPRA.66). Porto Viro, North Adriatic Sea: station TERM (45.0892° N, 12.5915° E), 29-33 m depth, muddy sand, 01 May 2014: 13 specimens (ISPRA.64); station C027 (45.0767° N, 12.5317° E), 27 m depth, sand, 25 October 2008: 1 specimen (ISPRA.69).

Description. All adult specimens incomplete, with 17-69 chaetigers, 3.64-26.50 mm total length, 1.54-4.04 mm length at the 10th chaetiger, 0.46-1.03 mm maximum width without parapodia (Fig. 2). Body elongated, slightly flattened, slightly wider in the anterior part (Fig. 3A). Prostomium triangular, flattened longer than wide, in the majority of the examined individuals with four very small eyes, easy to overlook in preserved individuals (Fig. 3B). Seven individuals from station TERM and two individuals from station AS09 with four relatively large, sub-equal dark brown eyes, in rectangular arrangement (Fig. 3D). Two individuals from station TERM with large eyes with unripe oocytes (diameter: 0.123 ± 0.019 mm) in the coelome after chaetiger 28-34; gametes seemingly absent in the coelome of the remaining specimens with large eyes. Two antennae, slightly shorter than prostomium, thick and tapering. Two palps, longer than antennae, articulated, with palpophore long, cylindrical, palpostyle oval, very short. Peristomium achaetous, shorter than prostomium. Tentacular cirri elongated, tapered; anterior dorsal tentacular cirri reaching chaetiger 5-7 backwards, posterior dorsal tentacular cirri easily broken at cirrophore, but when entire reaching chaetiger 10 backwards, ventral tentacular cirri much shorter (Fig 3B).

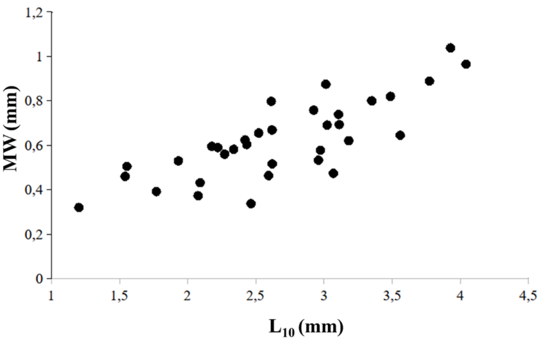

Fig. 2. *Leonnates aylaoberi* Çinar & Dağli, 2013, ratio between length at the 10th chaetiger (L_{10}) and maximum width (MW) in the examined specimens.

Protrusible pharynx with two muscular rings bearing papillae with corneous, light brown tips. One pair of corneous, brown jaws, each with 8-9 teeth. Maxillary ring: area I with 2 papillae in longitudinal series; area II with 8-10 papillae in 3-4 transverse rows; area III with 20-25 small papillae on 2-3 irregular rows; area IV with 10-14 papillae in three arcuate, oblique rows. Oral ring: area V without paragnaths; area VI with a single, large papilla; areas VII and VIII with approximately 50 papillae in three transverse rows; anterior row relatively distant from the remaining two, posterior row with distinctly smaller papillae (Fig. 3B, 3C).

Parapodia biramous, notopodium and neuropodium supported by one dark acicula. Anterior and midbody notopodia with dorsal cirri tapering, shorter than both notopodial ligules; dorsal and median ligule of similar size, conical, pointed, tapered distally, median ligule conical, slightly shorter than dorsal ligule; 4-6 homogomph spinigers and 1-2 homogomph falcigers (disappearing after chaetiger 8). Anterior neuropodia with neuroacicular ligule conical, shorter than notopodial ligules but well-developed, ventral ligule conical, slightly longer than neuroacicular ligule, ventral cirri tapering, as long as ventral ligule; supra-acicular fascicle with up to 5 homogomph spinigers; sub-acicular fascicle with up to 6 homogomph spinigers and 6-8 homogomph falcigers, with elongated blade, rounded tip and a strong serration along the ventral margin (Fig. 3E, 3F). Posterior parapodia (after the 35th chaetiger) larger with respect to body width, with stouter dorsal cirri and ventral ligule

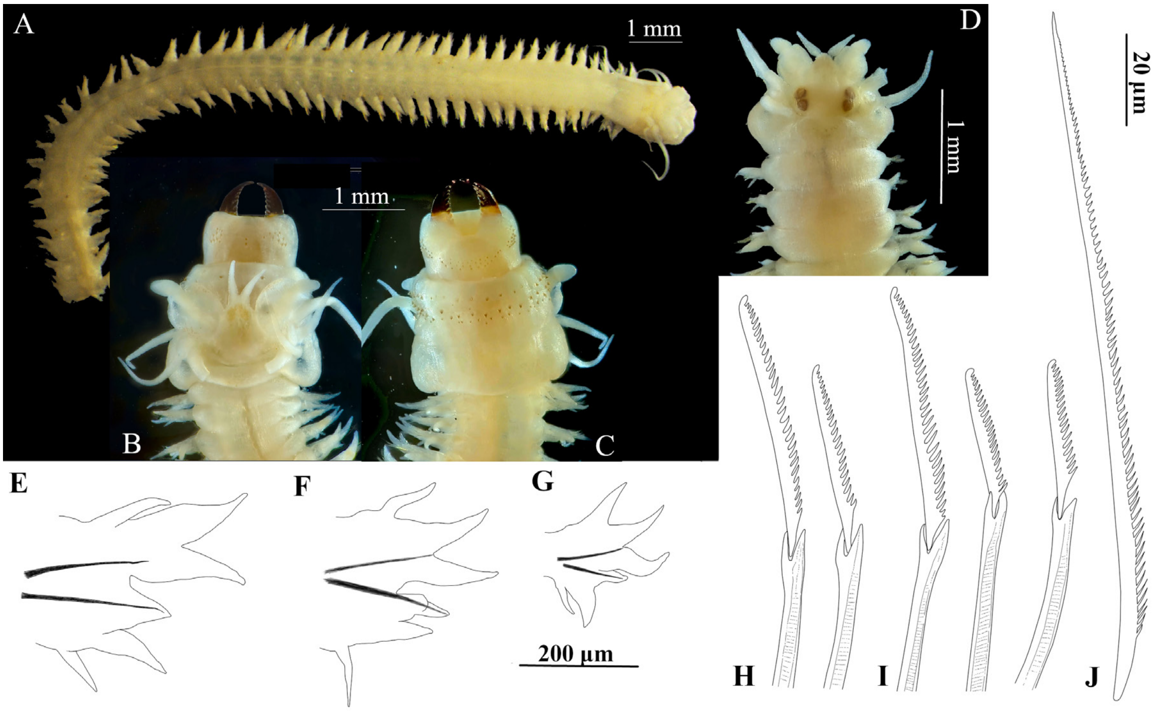

Fig. 3. *Leonnates aylaoberi* Çinar & Dağli, 2013, adult specimens: anterior fragment, Gulf of Portoferraio (Tyrrhenian Sea), dorsal view (**A**); everted pharynx, dorsal view, Porto Viro (Adriatic Sea) (**B**); everted pharynx, ventral view, Porto Viro (Adriatic Sea) (**C**); female epitoke specimen, anterior end, Porto Viro (Adriatic Sea) (**D**); anterior parapodium, lateral view (**E**); midbody parapodium, lateral view (**F**); posterior parapodium, lateral view (**G**); notopodial falcigers (**H**); neuropodial falcigers (**I**); spiniger, blade (**J**).

(Fig. 3G), number of homogomph falcigers decreasing towards the posterior part of the body. Homogomph spinigers with very long, unidentate blade, strongly serrated for the whole length (Fig. 3H, 3I). Homogomph falcigers with a strong dorso-ventral gradation, typically with very long, unidentate blade, with rounded tip and coarsely serrated ventral edge (Fig. 3J).

Juveniles complete, approximately 1.5 mm long for 12 chaetigers, 0.25 mm maximum width without parapodia (Fig. 4A). Prostomium with four dark eyes; anterior pair of eyes elliptic, much larger than posterior pair of eyes. Anterior dorsal peristomial cirri only slightly longer than prostomium; posterior dorsal peristomial cirri reaching backwards chaetiger 2 (Fig. 4A). Serrated jaws with 7 teeth (Fig. 4B). Parapodial ligules less developed than in the adult, dorsal and ventral cirri proportionally longer with respect to the parapodium. Notopodia with 3-4 homogomph spinigers (Fig. 4C) and 2-3 homogomph falcigers (Fig. 4D) (chaetigers 11 and 12 with spinigers only); neuropodia with 3-4 homogomph spinigers and 4-5 homogomph falcigers (Fig. 4E). Chaetae of the juvenile almost identical to those of the adult, only shorter. Pygidium rounded, with anus dorso-terminal and two tapering anal cirri, as long as the last 2-3 chaetigers.

Distribution. Known until now for the Levantine and Aegean Seas and for the Sea of Marmara (Çinar and Dağli, 2013; Çinar *et al.*, 2015; 2022; Rousou *et al.*, 2023); the current records considerably extend the distribution of this species to the Western Mediterranean Sea (Tyrrhenian Sea), the Ionian Sea and the Adriatic Sea.

Ecology. On soft sediments (sand to mud) between 10 and 80 m depth (Çinar *et al.*, 2022; present data), sometimes in association with vegetation (*Posidonia oceanica*, *Caulerpa* spp., *Halophila stipulacea*) (Rousou *et al.*, 2023; present data).

Remarks. The examined individuals correspond very well to the original description in all features (Çinar & Dağli, 2013). While the individuals from the Tyrrhenian Sea and the majority of those from the Adriatic Sea are consistent with the type material in having very small eyes that easily go unnoticed, a part of the individuals from the Adriatic Sea is characterised by larger, darker eyes, more similar to those of the other *Leonnates* species reported for the Mediterranean Sea. All other characters are not significantly different from those of the forms with small eyes; these differences might depend on a polymorphism known in other groups of Phyllodocida (e.g., *Erinaceusyllis barbarae* Langeneck, Musco & Castelli, 2018, and *Parexogone wolfi* (San Martín, 1991): Langeneck *et al.*, 2018; *Erinaceusyllis serratosetosa* (Hartmann-Schröder, 1982): Langeneck *et al.*, 2020) or on an early stage of epitoke modifications, even though parapodial lobes and ligulae are not

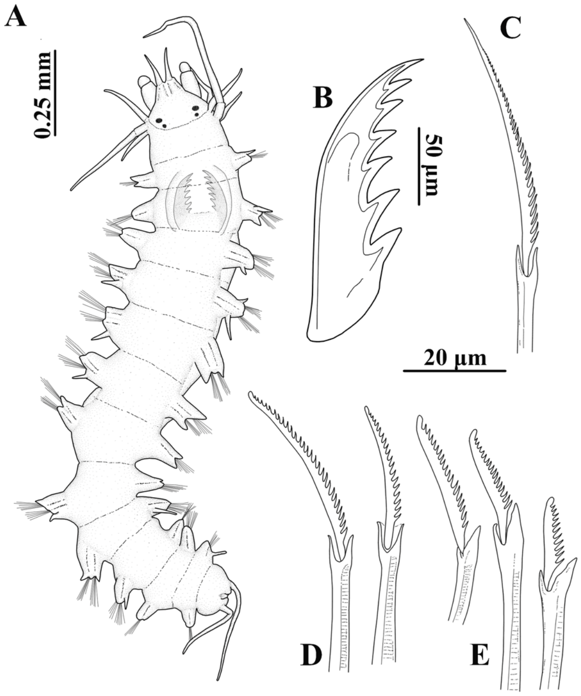

Fig. 4. *Leonnates aylaoberi* Çinar & Dağli, 2013, juvenile specimen from Santa Caterina di Nardò (Ionian Sea): entire specimen, dorsal view (**A**); left jaw (**B**); notopodial spiniger (**C**); notopodial falcigers (**D**); neuropodial falcigers (**E**).

significantly larger than in the other specimens, there is no trace of epitoke chaetae and only two specimens out of nine had noticeable gametes in the coelome. Epitoke forms have not been described for *L. aylaoberi* but are known in other species of the genus *Leonnates* (Gravier and Dantan, 1934; Wu *et al.*, 1985). It is possible that eyes, usually very small and inconspicuous in this species, are developed during the reproductive phase, leading to a morphology convergent with that of the other species of *Leonnates*.

Even though the type locality of this species is in the Mediterranean Sea (Çinar and Dağli, 2013), the genus *Leonnates* mostly includes species with Indo-Pacific affinity, and the very distinctive features of *L. aylaoberi* (in particular, the small eyes of the atoke form, the pharynx with partially sclerotized papillae instead of paragnaths, and the presence of homogomph falcigers at the neuropodium) suggest that this is a relatively recent newcomer in the Mediterranean Sea (Rousou *et al.*, 2023). This hypothesis is supported by these recent findings of *L. aylaoberi* in Italian waters; in particular, the northern Tyrrhenian and Adriatic areas where the species was found have been extensively studied for several decades, and none of the previous monitoring studies found this very characteristic species (Albertelli *et al.*, 1983; Bianchi *et al.*, 1993; Crema *et al.*, 1993; Occhipinti-Ambrogi *et al.*, 2002; Mikac *et al.*, 2011), suggesting that a westward expansion from the eastern Mediterranean is currently ongoing. Based on the present data, *L. aylaoberi* is present in the Adriatic Sea at least since 2008, and in the northern Tyrrhenian Sea at least since 2018. A non-indigenous origin was already suggested for other species with type locality in the eastern Mediterranean Sea (Galil *et al.*, 2010; Çinar *et al.*, 2017; Mutlu *et al.*, 2020), and in our opinion this is the most likely explanation for the presence of *L. aylaoberi* in the Mediterranean Sea. Nonetheless, this species is currently known only from the Mediterranean Sea; for this reason, in agreement with Rousou *et al.* (2023) we consider it as cryptogenic in the Mediterranean Sea.

Paucibranchia cf. ***adenensis*** **(**Gravier, 1900) (Figs. 5, 6)

Marphysa adenensis Gravier, 1900: 270-272, Figs. 140-143, pl. XIV, Figs. 91-92; Katsiaras *et al.*, 2014: 203-208, Figs. 1-5.

Paucibranchia adenensis Molina-Acevedo, 2018: 9-15, Figs. 3-6.

Material examined. Port of Livorno, Northern Tyrrhenian Sea: station DE09bis (43.5686° N, 10.2736° E), 11 m depth, dead *Posidonia* root mat with coarse sand and organogenic detritus, 07 September 2023: 2 specimens (ISPRA.63). Gulf of Follonica, Northern Tyrrhenian Sea: station PB-E (42.8975° N, 10.5540° E), 33 m depth, dead *Posidonia* root mat with sand, 04 May 2022: 2 specimens (MSNP: P/0297/V). Off Lampione island, Ionian Sea: station IS25 (35.6294° N, 12.3563° E), 158 m depth, sandy silt with organogenic detritus, 13 June 2020: 1 specimen (ISPRA.70). Taranto, Ionian Sea: M3_TA02 (C1) (40.46816° N, 17.18965° E), 9 m depth, fine sand with organic matter, 2023: 1 specimen (ARPA).

Description. All adult specimens incomplete, largest individual 12.28 mm long for 48 chaetigers, 1.31 mm maximum width without parapodia, 3.43 mm length at the 10th chaetiger (Fig. 5A). Prostomium oval, 1½ times longer than wide, with two small dark red eyes, three antennae and two prostomial palps, all smooth. Individual from Lampione island with unpigmented eyes. Median antenna longer, approximately 1½ times the prostomium, lateral antennae slightly longer than prostomium, palps shorter than prostomium. Peristomium without dorsal cirri, formed by two achaetous rings, anterior ring 1½ times the posterior ring (Fig. 5B). Parapodia with chaetigers uniramous, with ventral cirri conical, dorsal cirri elongated, cirriform. Branchiae from chaetiger 11-12 to chaetiger 20-23, pectinate, with up to 8 filaments (Fig. 5A). Dorsal cirri as long as branchiae in the branchial region, remaining of the same length but distinctly thinner, thread-like in the post-branchial region. Anterior parapodia with up to 10 limbate chaetae with finely serrated edge in the dorsal part of the parapodium, and up to 20 compound heterogomph chaetae with strongly bidentate blade surrounded by a finely serrated

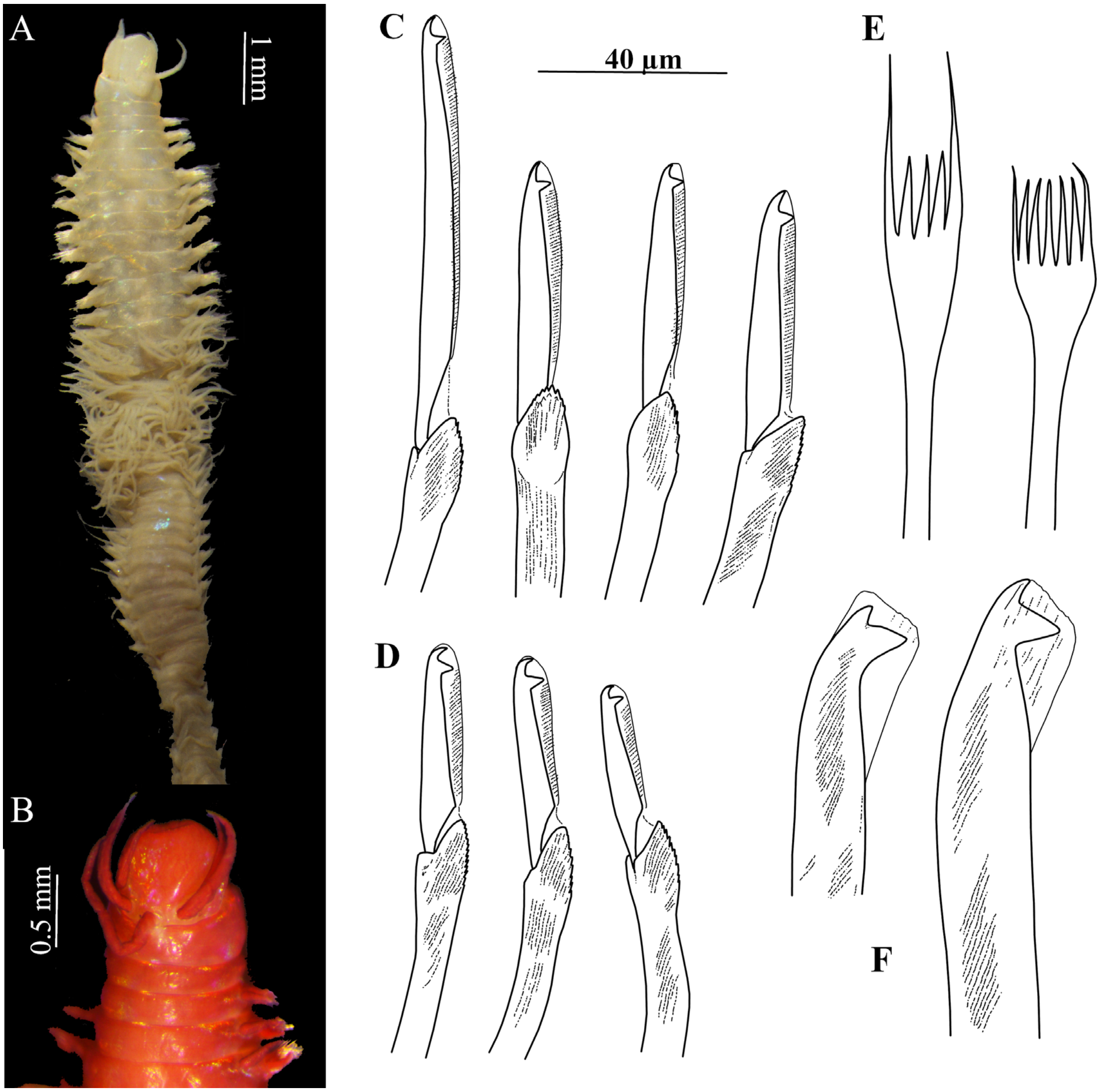

Fig. 5. *Marphysa* cf. *adenensis* Gravier, 1900, adult specimen from Taranto (Ionian Sea): anterior part of the body (**A**); close-up of prostomium and peristomium, highlighted with Shirlastain A (**B**); compound chaetae from the anterior part of the body (**C**); compound chaetae from the midbody (**D**); pectinate chaetae from the midbody (**E**); sub-acicular hooks (**F**).

hyaline hood; up to 4 chaetae with distinctly longer blade, the remaining ones almost without dorso-ventral gradation in size (Fig. 5C). Chaetae decreasing in number after the branchial region, reducing to 4 limbate chaetae and up to 6 compound heterogomph chaetae, stouter and with a less pronounced dorso-ventral gradation in length of articles (Fig. 5D). Pectinate chaetae isodont, large, with 5-7 teeth, external ones with tapering tip (Fig. 5E), not easy to find in the majority of parapodia. Starting from chaetiger 22-26, one sub-acicular hook, stout, bidentate, with subdistal tooth much larger than the distal one, with which it forms a 90° angle, partially shrouded by a hyaline, sub-rectangular sheath (Fig. 5F). Preserved individual uniformly light tan, with iridescent glare, without contrasted colour markings.

Juveniles all incomplete, up to 4.84 mm long, 0.39 mm maximum width, for 26 chaetigers. Prostomium oval, with two dark red eyes, not very small, three antennae as long or just slightly longer than prostomium. Peristomium without dorsal cirri, formed by two subequal rings (Fig. 6A). Parapodia with chaetigers uniramous, with ventral cirri conical, dorsal cirri elongated, cirriform. Branchiae from chaetiger 7 to 11-12, bifid to trifid, last pair consisting of a single filament (Fig. 6A). Dorsal cirri as long as the branchiae in the branchial region, remaining of the same length but distinctly thinner, thread-like in the post-branchial region. Anterior parapodia with up to 5 limbate chaetae in the dorsal part of the parapodium, and up to 6 compound heterogomph chaetae with blade surrounded by a hyaline hood; a strong dorso-ventral gradation in length of the articles (Fig. 6B). Chaetae decreasing in number after the branchial region, reducing to 2 limbate chaetae and 4 compound heterogomph chaetae, stouter and with a

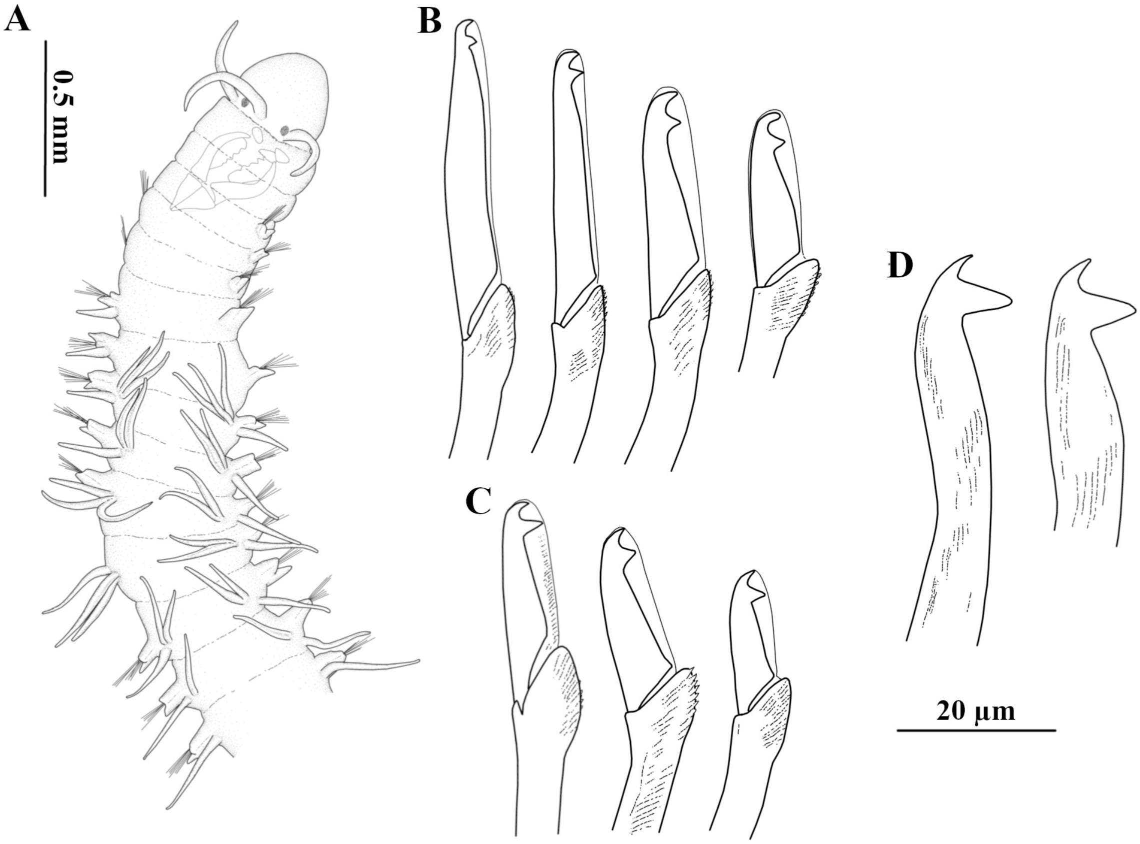

Fig. 6. *Marphysa* cf. *adenensis* Gravier, 1900, juvenile specimen, Gulf of Portoferraio (Tyrrhenian Sea): anterior part of the body (**A**); compound chaetae from the anterior part of the body (**B**); compound chaetae from the midbody (**C**); sub-acicular hooks (**D**).

less pronounced dorso-ventral gradation in the length of articles (Fig. 6C). Starting from the chaetiger 11, one sub-acicular hook, stout, bidentate, with subdistal tooth much larger than the distal one, with which it forms a 90° angle (Fig. 6D). Pectinate chaetae absent. Dorso-ventral gradation in size of blades less pronounced in juveniles with respect to adults.

Distribution. *Paucibranchia adenensis* was originally described from the Gulf of Aden, Red Sea (Gravier, 1900) and later reported for several localities in the Indian and Pacific Ocean (Day, 1967; Mak, 1980; Aungtonya *et al.*, 2002; Bigot *et al.*, 2008). It was reported as a non-indigenous species for the Aegean and southern Ionian Sea (Katsiaras *et al.*, 2014) and for the Levantine Sea (Rousou *et al.*, 2023); the present records extend its distribution to the northern Ionian Sea and the Tyrrhenian Sea.

Ecology. Usually occurring in shallow soft sediments (4-42 m depth) (Katsiaras *et al.*, 2014; Rousou *et al.*, 2023), with just two records for circalittoral muds at 140-160 m depth (Rousou *et al.*, 2023; present data).

Remarks. This species was first reported for the Mediterranean Sea by Katsiaras *et al.* (2014), who considered it a possible Lessepsian immigrant. Later on, Molina-Acevedo (2018) stressed some differences between the Mediterranean specimens and the type material; in particular, Mediterranean specimens are characterised by compound falcigers with a strong dorso-ventral gradation in the length of blades, and the longest blades measuring twice the length of the shortest ones, while the type material had shorter, more uniform blades. The individuals from Italian waters have somewhat intermediate features between the type material redescribed by Molina-Acevedo (2018) and the Mediterranean specimens described and depicted by Katsiaras *et al.* (2014), as in adult specimens the longest blades in the anterior chaetigers are around 1½ times the shortest ones, while they are almost of the same length in midbody segments (as depicted in Molina-Acevedo, 2018). However, the substantial size difference between the specimens depicted in Katsiaras *et al.* (2014) and those examined in this study, coupled with the ontogenetic changes in this character, suggests a size-dependent relationship between the proportions of long- and short-bladed chaetae. The length of blades of the specimens described by Katsiaras *et al.* (2014) is similar to that observed in another Indo-Pacific species, *Paucibranchia gemmata* (Mohammad, 1973), which also shares the presence of longer dorsal cirri in the post-branchial region; however, this species is characterised by the absence of pectinate chaetae in the posterior part of the body (Katsiaras *et al.*, 2014; Molina-Acevedo, 2018), while the Mediterranean specimens described by Katsiaras *et al.* (2014) and the present material have pectinate chaetae in posterior parapodia. It is likely that the Mediterranean individuals identified as *P. adenensis* actually belong to a different, probably undescribed species. The Mediterranean *Paucibranchia* cf. *adenensis* might represent a non-indigenous species, as suggested by Katsiaras *et al.* (2014); in fact, the most similar species within the genus *Paucibranchia* have Indo-Pacific affinity (Molina-Acevedo, 2018), and it seems to be a relatively recent newcomer to the western Mediterranean Sea. Nonetheless, if one does not take into consideration the complete absence of spinigerous compound chaetae (present in all other species of *Paucibranchia* reported in the Mediterranean Sea), this species appears quite similar to the widespread *Paucibranchia bellii* (Audouin & Milne-Edwards, 1833), and it is possible that it went unnoticed in the majority of the environmental monitoring programmes due to this similarity.

Lastly, this species, together with all "*Marphysa bellii*-like species" (*sensu* Zanol *et al.*, 2010 – corresponding to the subgroup I of *Marphysa* as defined by Fauchald, 1970), deserves a brief comment on systematics and nomenclature. Molina-Acevedo (2018) identified the "*Marphysa bellii*-like species" as a coherent group and described it as a new genus, *Paucibranchia* Molina-Acevedo, 2018. The genus is currently accepted by WoRMS (2024), but it was apparently not considered valid by some authors; for instance, Lavesque *et al.* (2022) used the genus *Marphysa* Quatrefages, 1866 for three species, one of which (*Marphysa papuensis* Lavesque, Daffe, Glasby, Hourdez & Hutchings, 2022) clearly corresponds to the diagnosis of *Paucibranchia*. Zanol *et al.* (2010; 2014) consistently retrieved with all analyses the "*Marphysa sanguinea*-like species" as a clade, but only a part of the analyses retrieved the monophyly of the group including "*Marphysa bellii*-like species" and, on the other hand, the genus *Marphysa* as a whole is a highly supported clade. At the same time, while stressing some common morphological characters between the species of the genus *Paucibranchia*, Molina-Acevedo (2018) did not test the phylogenetic informativeness of these characters through a cladistic analysis. In case further analyses would confirm the monophyly of the "*Marphysa bellii*-like species", it should also be noted that the genus *Paucibranchia* would have a senior synonym, corresponding to the genus *Lysibranchia* Cantone, 1983. This genus was created for *Lysibranchia paucibranchiata* Cantone, 1983, a taxon that, upon further examination, was found to correspond to juvenile individuals of *P. bellii* (Cantone, 1983; Çinar, 2005) and was put into synonymy with this latter taxon. As *P. bellii* is the type species of the genus *Paucibranchia*, and the type species of *Lysibranchia* is synonymous with *P. bellii*, the two genera should be treated as synonyms, with *Lysibranchia* having priority.

Isolda cf. ***pulchella*** Müller, 1858 (Fig. 7)

Isolda pulchella Müller, 1858: 218-219, pl. VII, Figs. 26-27; Day, 1967: 690-691, Figs. 35.1k-n; Díaz-Díaz & Liñero-Arana, 2012: 175, Fig. 2E-G.

Material examined. Termini Imerese, Southern Tyrrhenian Sea, Station FT1-E (37.9819° N, 13.7830° E), 9.5 m depth, 21 January 2022: 2 specimens (MSNP: P/5218); Station FT2-M (38.0080° N, 13.7631° E), 38.5 m depth, 20 January 2022: 1 specimen (MSNP: P/5219).

Description. Largest specimen complete, 22.75 mm long, 1.66 mm wide for 57 chaetigers. Body elongated, maximum width reached in post-branchial region, tapering posteriorly (Fig. 7A). Seventeen thoracic chaetigers and up to 40 abdominal chaetigers. Prostomium rounded, without eyespots and glandular ridges. First two segments achaetous, dorsally covered by branchiae, ventrally forming the lower margin of mouth. Notopodial chaetae from the V segment (chaetiger 3); dorso-lateral longitudinal ridges connecting the chaetigers 1-4. Two groups of four branchiae in dorsal region of the 1st chaetiger; two inner pairs of branchiae pinnate, with two rows of elongated, flattened and numerous lateral lamellae. A pair of large post-branchial hooks inserted on dorsum of the IV segment (2nd chaetiger) (Fig. 7B). Hooks flattened, elongated, with briskly crooked tip (Fig. 6f). Dorsum of the 4th chaetiger covered by a wide membranous fold, with smooth margin. Notopodial limbate capillary chaetae (Fig. 7C) occurring in chaetigers 3-17; fine acicular neurochaetae in chaetigers 1-3; chaetiger 4 without neurochaetae. Neuropodium of the following thoracic chaetigers with distinct uncinigerous tori, bearing uncini arranged in a single row; thoracic uncini with one single row of 5-6 teeth above the rostral tooth, a projecting basal prow below the rostral tooth, posterior margin of the hook with a slight concavity defining a small handle (Fig. 7D). Abdominal chaetigers without rudimentary notopodia, neuropodia uncinigerous, with uncini arranged in a single row, decreasing in size ventrally; abdominal uncini with one single row of 4-6 teeth above the rostral tooth, a projecting basal prow; rostral tooth missing in the smallest, ventral-most uncini, posterior handle absent in all abdominal uncini (Fig. 7E). Live colour unknown, preserved specimens whitish, without a definite colour pattern, aside from a slight banding on the branchiae.

Distribution. Originally described from shallow environments off Brazil (Müller, 1858), subsequently recorded from South Africa (Day, 1963; 1967), the Caribbean region (Díaz-Díaz & Liñero-Arana, 2012), the Portuguese shelf (Ravara and Moreira, 2013), the Mediterranean Sea (Cantone, 2001; Zaâbi *et al.*, 2012) and, with some uncertainty, the Red Sea (Wehe and Fiege, 2002). After the synonymisation of *Isolda warnbroensis* Augener, 1914 (originally described from Australia: Augener, 1914), *Isolda whydahensis* Augener, 1918

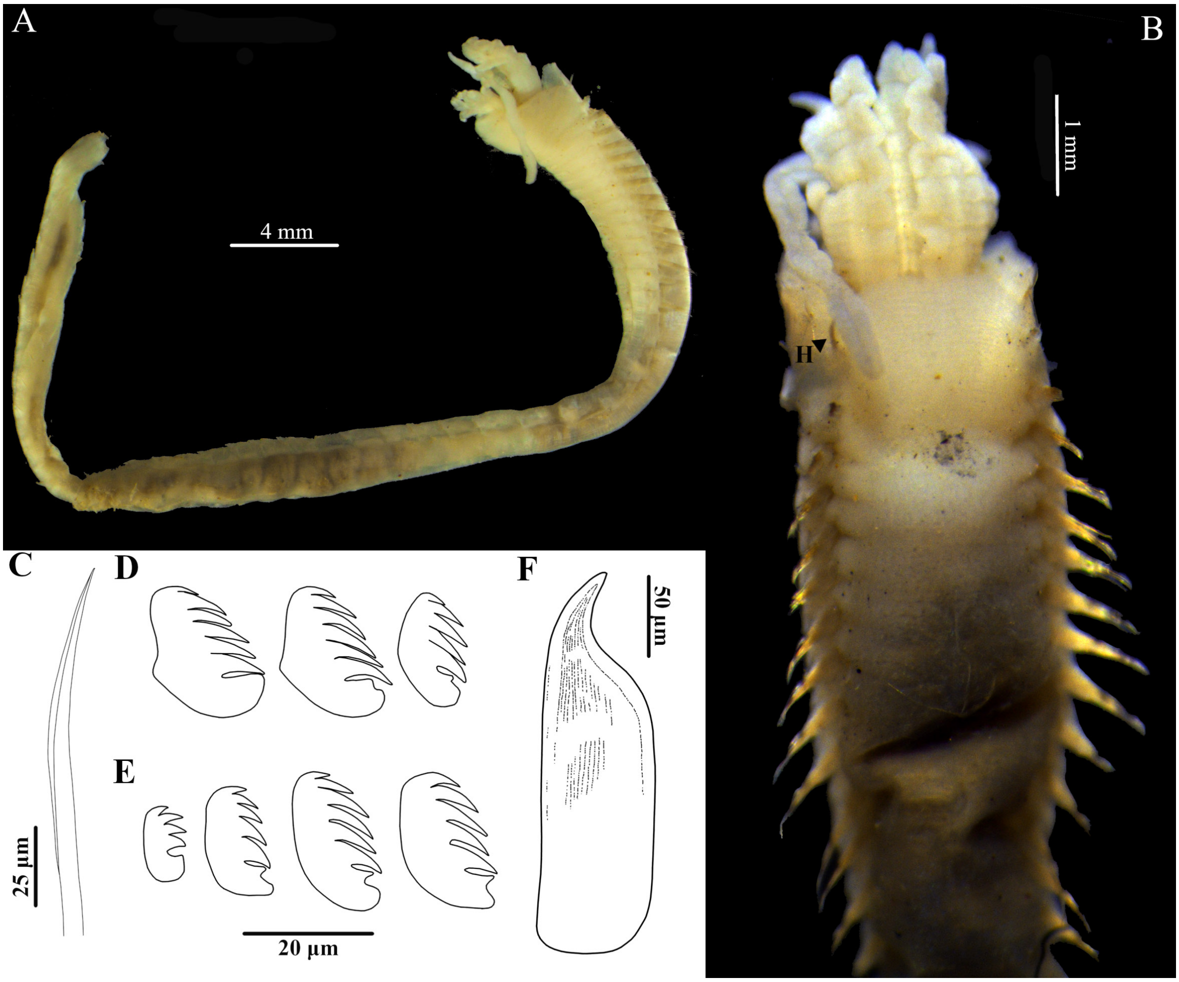

Fig. 7. *Isolda* cf. *pulchella* Müller, 1858, adult specimen from Termini Imerese (Tyrrhenian Sea): entire individual (**A**); anterior part of the body, showing the flattened, pinnate pairs of branchiae, the dorsal ridge at the 4th chaetiger, and the position of the dorsal hooks of the 4th chaetiger (**B**); Thoracic limbate notopodial chaeta (**C**); thoracic uncini (**D**); abdominal uncini (**E**); dorsal hook from the 4th chaetiger (**F**).

(originally described from Benin, also reported from Congo and Angola: Augener, 1918) and *Isolda sibogae* Caullery, 1944 (originally described from Indonesia: Caullery, 1944), this species was also considered occurring off West Africa and in the Indian Ocean. The species was considered non-indigenous in the Mediterranean Sea and in the northeastern Atlantic by Zenetos *et al.* (2010) and López and Richter (2017) but is missing in more recent revisions of alien species in the Mediterranean (Zenetos *et al.*, 2017; Galanidi *et al.*, 2023).

Ecology. Although Müller (1858) did not explicitly state the environment where he found this species, the fact that he was able to examine the specimens alive strongly suggests that they were sampled in shallow environments. Further records from the tropical western Atlantic also refer to soft bottoms in very shallow, often estuarine environments (Díaz-Díaz and Liñero-Arana, 2012; Passos Ribeiro *et al.*, 2018). Conversely, records from South Africa and the European waters consistently refer to environments below 60 m depth (Day, 1963; Cantone, 2001; Zaâbi *et al.*, 2012; Ravara and Moreira, 2013). The present records, while shallower, confirm the occurrence of this species in fully marine Mediterranean environments.

Remarks. Langeneck *et al.* (2020) suggested to treat *I. pulchella* as a questionable NIS based mostly on the absence of descriptions and/or iconography of Mediterranean specimens, and on the discrepancies between the ecology of the West-Atlantic *I. pulchella* (a very shallow, often estuarine species) and the East-Atlantic *I. pulchella* (a species occurring in deeper, fully marine environments). The current examination of the available literature confirms the ecological discrepancies and suggests the occurrence of some morphological differences as well. In particular, East-Atlantic individuals seem to be larger than West-Atlantic ones, and the uncini are slightly different; West-Atlantic individuals are apparently characterised by a shorter, higher profile, and up to 7 teeth above the rostral tooth, which is almost absent (Augener, 1918; Díaz-Díaz and Liñero-Arana, 2012), while the South African individuals described by Day (1967) and the Mediterranean ones here examined are characterised by a stockier shape and 5-6 teeth above a well-developed rostral tooth. The uncini of the East-Atlantic *I. pulchella* are in fact more similar to those of the Indopacific *Isolda albula* Mohammad, 1971, which however differs from the examined specimens by the presence of 16 thoracic chaetigers (Mohammad, 1971). The importance of this character has been the subject of some discussion: Müller (1858) did not state the number of thoracic chaetigers, but Augener (1918) and Day (1967), based on the examination of material identified by F. Müller himself, consistently reported the presence of 17 thoracic chaetigers. Later sources reported the possible variation between 16 and 17 thoracic chaetigers (Uebelacker, 1984; Passos Ribeiro *et al.*, 2018), even if Díaz-Díaz and Liñero-Arana (2012) suggested that this might be a clue of overlooked diversity within the genus *Isolda*. As the number of thoracic chaetigers shows a certain stability in the Terebelliformia and is usually considered as a sound diagnostic character (Lavesque *et al.*, 2021), forms with 16 thoracic chaetigers likely represent different species from those with 17 thoracic chaetigers. The two forms of *I. pulchella* reported in literature share the same number of thoracic chaetigers, but they also show consistent differences between the western Atlantic and the eastern Atlantic, suggesting that the species occurring in the eastern Atlantic is not the "true" *I. pulchella*. Similarly, the Indopacific taxa listed among the synonymies of *I. pulchella* (*I. warnbroensis* and *I. sibogae*) might also represent valid species. However, the majority of the species of the genus *Isolda* are known only from the original description, therefore in order to disentangle the diversity of this group a critical revision would be needed.

Isolda cf. *pulchella* was firstly reported in the Mediterranean Sea by Cantone (2001), who found the species in the Gulf of Noto (Ionian Sea). It was later found in Tunisia (Zaâbi *et al*., 2012) and off the Portuguese coast (Ravara and Moreira, 2013). The current records confirm the occurrence of the species in the Mediterranean Sea and extend its occurrence to the southern Tyrrhenian Sea. The species is rather distinctive, and the absence of records before the early 2000s suggests that it arrived in European waters only recently. It was accordingly considered as a possible introduction in European waters (Cantone, 2001; Zenetos *et al.*, 2005), even though the introduction means are unclear. The majority of the members of the clade Terebelliformia are characterised by either direct development, or a short lecitotrophic planktonic larval phase (Giangrande, 1997), which would hinder an introduction through ballast waters. A possible explanation for the presence of *I.* cf. *pulchella* in European waters might entail a natural expansion from the tropical eastern Atlantic, possibly fostered by the current trend of temperature increase. Nonetheless, the family Melinnidae has never been the object of a thorough revision in Mediterranean waters, and the possibility that this species historically occurred in the Mediterranean Sea cannot be completely ruled out.

DISCUSSION

The last comprehensive review of the diversity of non-indigenous marine annelids in Italian waters reported 25 confirmed alien species, three cryptogenic species, and 40 questionable species (Langeneck *et al.*, 2020). Gravina *et al.* (2021) added to this list *Palola valida* (Gravier, 1900), Bonifazi *et al*. (2023) reported for the first time *Laonome triangularis* Hutchings & Murray, 1984 for Italian waters, and Borghese *et al*. (2023) reported four additional non-indigenous polychaetes (*Caulleriella cabbsi* Pocklington & Coates, 2010, *Prionospio depauperata* Imajima, 1990, *Prionospio pulchra* Imajima, 1990, and *Stylarioides grubei* Salazar-Vallejo, 2011). Lastly, Langeneck *et al.* (2024) confirmed the occurrence of *P. valida* and *P. depauperata* and recorded *Lepidonotus tenuisetosus* (Gravier, 1902) and *Syllis similisunzima* San Martín, Lucas & Hutchings, 2023; morphological and genetic differences retrieved by Langeneck *et al.* (2024) between Mediterranean and Pacific individuals of *P. depauperata* led to the evaluation of this species as a questionable NIS in the Mediterranean Sea, while the status of NIS of *P. valida* was confirmed. With the addition of the present data, the non-indigenous annelids reported for Italian waters currently include 32 confirmed alien species, four cryptogenic species, and 42 questionable species.

As shown by the prevalence of questionable species, doubts and uncertainties about the identity of polychaete species reported as non-indigenous are a widespread issue. In the review by Langeneck *et al.* (2020), the term "questionable species" was used in a somewhat polysemic way; in fact, this category included both species listed in general ecology papers, the presence of which could not be confirmed nor rejected based on the absence of deposited material, and species for which the presence in Italian waters could be confirmed, but were surrounded by doubts on their identity. It is noteworthy that the result of a critical re-examination of questionable species based on new material and/or molecular data was not obvious: some species, such as *Pista unibranchia* Day, 1961 and *Erinaceusyllis belizensis* (Russell, 1989), were found to be misidentifications of native, undescribed species (Lavesque *et al.*, 2021; Çinar and Erdoğan-Dereli, 2023), while the identity of *Lumbrineris perkinsi* Carrera-Parra, 2001 and its alien status in the Mediterranean Sea were confirmed by molecular data (Langeneck *et al.*, 2024). Nonetheless, this kind of evidence is still lacking for a sizable part of the polychaete species identified as questionable NIS in the Mediterranean Sea. Despite the examination of further material, we were not able to ascertain the identity of the individuals reported as *P. adenensis* and *I. pulchella* in the Mediterranean Sea; this is due to the limited number of studies focusing on these genera in the Mediterranean

Sea, but especially outside of the Mediterranean area. Molecular data are often considered a crucial tool to unravel doubts surrounding the identity of an allegedly non-indigenous species (Sun *et al.*, 2017; Golo *et al.*, 2023); however, barcoding libraries contain data for a minor part of the non-indigenous annelids reported in Europe (Lavrador *et al.*, 2023), and several nominal taxa correspond to multiple MOTUs, suggesting the widespread occurrence of cryptic species in non-indigenous taxa as well, and further complicating the situation (Lavrador *et al.*, 2023). In fact, the widespread occurrence of species complexes in marine annelids, combined with the scarcity of molecular data from the type localities and/or the low taxonomic resolution of a sizable part of the available DNA barcodes, often hinders the resolution of questionable species issues even in the presence of molecular data (Langeneck *et al.*, 2024). In the case of the three species herein reported, DNA sequences are not available for *L. aylaoberi* and *P. adenensis*, while there are a few sequences of *I. pulchella*, but they originate from areas far from its type locality. Molecular data from the Mediterranean Sea are therefore not enough to solve this kind of issue, and further studies in the areas of alleged origin are needed to further advance our knowledge about these species.

Even though it is still considered controversial by several scholars working on biological invasions, the use of open nomenclature arose in recent years as a possible way to highlight taxonomic issues surrounding questionable NIS, especially when taking into consideration large reviews. Open nomenclature was sporadically employed to stress the complex situation surrounding some alleged NIS (e.g., Lorenti *et al.*, 2009; Langeneck *et al.*, 2019), but its use was supported mostly by Albano *et al.* (2021; 2024) in their revisions of non-indigenous molluscs and ostracods with alleged Lessepsian origin. In particular, Albano *et al.* (2024) suggested that the use of open nomenclature, when dealing with species with likely non-indigenous origin but complex taxonomy, might help the scientific community to avoid overconfidence on their distribution, origin and diversity. In this regard, Albano *et al.* (2024) also recommend to provide not just species lists, but also detailed descriptions and readable iconography, to allow for further comparisons with new material and other literature sources. In this framework, using open nomenclature for questionable species is not equivalent to consider them less important or interesting than confirmed alien species; on the contrary, it stresses the need for additional studies clarifying the taxonomy and origin of these taxa (Carlton and Schwindt, 2024).

A further support for the alien origin of species with unclear taxonomy comes from the criteria suggested by Chapman and Carlton (1991). These criteria were originally developed to test hypotheses about the alien origin of peracarids, and would probably require some revision to be applied to a different group, especially one with such a wide ecological and biological diversity as polychaetes. However, some of the criteria listed by Chapman and Carlton (1991) can be successfully applied to a wide range of macrobenthic organisms. In particular, criteria 1 (“appearance in local regions where not found previously”), 2 (“initial expansion of local range subsequent to introduction”), 6 (“relatively restricted distribution […] compared to distributions of native species”) and 10 (“exotic evolutionary origin”, i.e., the closest species from a phylogenetic point of view are native to an area far away from the alleged introduction range) can be applied to the three species reported in this manuscript, considering that, despite the long tradition of studies dealing with polychaete taxonomy in the Mediterranean Sea and the mandatory monitoring required by European regulations (Marine Strategy Framework Directive and Water Framework Directive), the first records of *I.* cf. *pulchella* date back to 2001, those of *L. aylaoberi* to 2005, and those of *P.* cf. *adenensis* to 2009, and all these species are clearly different from their Mediterranean relatives, and close to organisms described from areas outside of the biogeographical provinces contributing to Mediterranean biodiversity. The diversity of Mediterranean polychaetes is still partially unknown, and the description of new species is rather frequent (see for instance Çinar *et al.*, 2011; 2022; Langeneck *et al.*, 2018); thus, in principle, the hypothesis that these three species are in fact native cannot be completely excluded. Nonetheless, applying the criteria defined by Chapman and Carlton (1991), the relatively recent record of all species in Italian waters, and the apparent trend of expansion from south-east to north-west, strongly support the hypothesis of a non-indigenous origin, or at least of a range expansion related to water warming.

Despite the absence of molecular data, the data herein presented represent a baseline to track the spread of these species in the western and northern Mediterranean Sea, and to support other researchers in the identification of these species, and possibly in the revision of the diversity of these groups. In fact, current data suggest that the biodiversity of the Mediterranean fauna is still largely unknown even in shallow and well-monitored environments, and that continuous exchanges of information, expertise and material between parataxonomists and taxonomists is fundamental for the development of updated identification keys and taxonomic revisions (Boero, 2015). In this context, building and developing collaborations between environmental monitoring agencies and research units (Cirino *et al.*, 2016) is fundamental to advance the state of knowledge about benthic macrofauna.

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