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# Non-indigenous polychaetes along the Salento Peninsula: new records and first molecular data

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## Abstract

The Salento Peninsula represents the eastern-most edge of the Italian Peninsula, and one of the first areas to be invaded by thermophilic non-indigenous species. The diversity of non-indigenous polychaetes occurring along the Salento Peninsula is reviewed based on literature data and new samples. Overall, fifteen non-indigenous polychaetes were recorded; among them, *Syllis similisunzima* is reported for the first time in the Mediterranean Sea; *Lepidonotus tenuisetosus* is recorded for the first time in Italian waters; *Pseudonereis anomala*, until now known only for Sicily, is reported for the first time from the Italian Peninsula; *Dorvillea similis* is a first record for the Ionian and Adriatic Sea. 16S rDNA and COI sequences were obtained for eleven species, allowing us in some cases to confirm their identity and/or geographical origin, while in the case of some species they represent the first molecular data ever obtained.

**Keywords:** Annelida; alien species; Ionian Sea; Lessepsian species; DNA barcoding.

## Introduction

Non-indigenous species (NIS) can be regarded as one of the main elements contributing to the current biodiversity crisis (Olson *et al.*, 2002). In the Mediterranean Sea, NIS have been the focus of several studies detailing their distribution (Zenetos *et al.*, 2017; 2022), their impact (Tsiamis *et al.,* 2020; Tsirintanis *et al.,* 2022) and potential mitigation and management strategies (Galil *et al.,* 2018; Ojaveer *et al.,* 2018). However, in several cases the study of NIS is hindered by uncertainties surrounding their taxonomy or their native range, and a sizable part of the species reported as non-indigenous in the Mediterranean Sea are considered questionable (Zenetos *et al.*, 2005a; Tsiamis *et al.*, 2018). Uncertainties surrounding NIS typically refer to either the origin or the taxonomic identity of the organisms, or the reliability of the record (Marchini *et al.*, 2015; Langeneck *et al.*, 2020; Galanidi *et al.*, 2023). In the case of cryptogenic species, the taxonomic identity of the species is not in question, but the available data do not allow to precisely reconstruct their native and introduced range (Carlton, 1996). Cryptogenic species are particularly frequent among species associated with fouling communities, the introduction of which dates back to the 19th Century or before (Maltagliati *et al.*, 2016; Sun *et al.*, 2017); moreover, some recently described species with type locality in the Mediterranean Sea are potentially non-indigenous and would fall within this category (Langeneck *et al.*, 2020; Rousou *et al.*, 2023). Uncertainties about alleged areas of introduction led to consider some species questionable (Keppel *et al.*, 2019; Langeneck *et al.*, 2020; Lee *et al.*, 2020a); in this case, taxonomic revisions based on integrative taxonomy are often needed to disentangle issues surrounding their identity. In some cases, however, non-indigenous species are considered questionable because their record is based on species lists in community ecology papers, without any detailed description and/or deposited material allowing for further actual comparisons (Faulwetter *et al.*, 2017; Langeneck *et al.*, 2020). Regrettably, these last taxa are often reported in national and international checklists and even included in local/regional identification keys (e.g. D'Alessandro *et al.*, 2016a), increasing the risk of further misidentification and spread of errors.

Not all taxonomic groups are affected by the same degree of uncertainties about their introduction status. Some groups, such as fish, decapods and molluscs, have been addressed in a series of useful atlases (Zenetos *et* *al.*, 2005b; Galil *et al.*, 2008; Golani *et al.*, 2013) and, even though the taxonomy of some species within these groups has only recently been clarified (Malaquias & Reid, 2008; DiBattista *et al.,* 2012; Russell *et al.,* 2015), uncertainties are relatively rare in comparison with other groups, and the identification of a new NIS is usually rather solid. On the other hand, biogeographical and taxonomic uncertainties are rather widespread in some taxonomic groups (Marchini & Cardeccia, 2017; Gómez, 2019; Micael *et al.*, 2019), including polychaete annelids (Del Pasqua *et al.,* 2018; Langeneck *et al.*, 2020). In the case of polychaete annelids, uncertainties surrounding the identity and/or origin of newly recorded species are a particularly relevant issue. In the review conducted by Çinar (2013), approximately 17% of the polychaete species recorded as non-indigenous are in fact cryptogenic, while questionable non-indigenous species accounted for 23% of the non-indigenous polychaetes listed by Zenetos *et al.* (2005a) for the Mediterranean Sea as a whole, and for 47% of the non-indigenous polychaetes reported for the Italian coastline (Langeneck *et al.,* 2020). An accurate definition of the identity, origin and biology of non-indigenous species is necessary in order to understand pathways of introduction, to infer possible impacts on the native assemblages, to correctly predict invasion scenarios, and ultimately to plan mitigation and management strategies (Douglas *et al.,* 2009; Del Pasqua *et al.,* 2018; Bishop & Darbyshire, 2023; Golo *et al.*, 2023).

In this context, the increasing use of integrative taxonomy to unravel the diversity of marine organisms becomes a crucial tool in order to understand the identity and origin of alleged NIS, and to confirm or confute their non-indigenous status (Sun *et al.*, 2017; Simon *et al.*, 2019; Kupriyanova *et al.*, 2022; Radashevsky *et al.*, 2023). Moreover, in the last few decades, environmental DNA (eDNA) has been widely employed to indirectly reconstruct the diversity of natural assemblages (Yu *et al.,* 2012; Leray & Knowlton, 2015; Goldberg *et al.,* 2016) and it has also been successfully employed to trace the early stages of invasion of non-indigenous species (Brown *et al.,* 2016; Parrondo *et al.,* 2018; van den Heuvel-Greve *et al.,* 2021). However, metabarcoding studies based on eDNA are dependent on the presence of reliable barcoding libraries, which are still under development for most marine invertebrates occurring in European waters (Lobo *et al.,* 2016; Weigand *et al.,* 2019; Vieira *et al.,* 2021). The same situation concerns non-indigenous invertebrates, which are only partially covered by available barcoding libraries (Lavrador *et al.,* 2023). According to Lavrador *et al.* (2023), barcoding data are available for only 63 out of the 220 marine annelid species reported as non-indigenous for European waters, corresponding to 28.6%. Moreover, only a minor part of the barcodes shows univocal correspondence between Barcoding Index Numbers (BINs) and nominal species, while most records are characterised either by multiple BINs assigned to the same nominal species, highlighting the possible presence of cryptic species, or by various inconsistencies between BINs and morphologically identified taxa (Lavrador *et al.*, 2023). It is therefore clear that the data currently available hardly allow a straightforward application of metabarcoding techniques to the study of the spread of non-indigenous marine annelids; before this kind of monitoring can even start, reliable barcoding libraries should be built.

This contribution has the aim of detailing new records of non-indigenous annelids along the Salento coastline, which represents a biogeographical crossroads among the eastern and the western Mediterranean, and the Adriatic Sea (Musco & Giangrande, 2005), also providing barcoding data for the majority of the recorded species, especially those suffering from uncertainties in morphological identification. This paper also aims to discuss the state of molecular libraries referring to non-indigenous annelids in European waters.

## Material and Methods

The Salento Peninsula is a geographical region at the southern end of the administrative region of Apulia in southern Italy. This area represents the eastern-most edge of the Italian Peninsula, extending in direction NW – SE between approximately 40.5 and 39.5 °N, and 17.25 and 18.45° E, for approximately 100 km, with a linear extension of the coast around 260 km, and with an average width of 30-40 km. The western coast of Salento, facing the Gulf of Taranto (Northern Ionian Sea), extends from Taranto to Cape Santa Maria di Leuca, and includes the Marine Protected Area (MPA) of Porto Cesareo. The eastern coast of Salento extends from Brindisi to Cape Santa Maria di Leuca and is characterised by a more complex hydrography, as its northern part faces the Southern Adriatic Sea, its middle part faces the Strait of Otranto (considered as a transitional area between the Ionian and the Adriatic Sea), while its southern part faces the North-Eastern Ionian Sea (Bianchi *et al.,* 2012). The continental shelf is distinctly wider along the north-eastern part of the Salento Peninsula, which is mostly characterised by soft bottoms, while depth increase is steeper along the southern and western parts of the peninsula, with a prevalence of hard bottoms (Accogli *et al.*, 1993; Belmonte, 1993). Due to its geographical position, the Salento Peninsula often represents the first area where thermophilic non-indigenous and neonative species are observed in Italian waters (Guidetti *et al.,* 2012; Di Martino & Stancanelli, 2021; Toso & Musco, 2023) and might represent an early warning area for bioinvasions in the Ionian Sea and Adriatic Sea (Toso *et al.,* 2020; Toso *et al.,* 2022; Grech *et al.,* 2023). Additionally, the presence of important commercial and touristic ports, such as Brindisi and Taranto, an array of marinas, as well as aquaculture activities, make this area particularly susceptible to marine bioinvasions (Rubino *et al.,* 2011; Cecere *et al.*, 2016; Tempesti *et al.*, 2020a; Toso *et al.,* 2022).

Sampling activities were carried out along the coast of the Salento Peninsula between May 2022 and November 2023, focusing primarily on areas characterised by high likelihood of occurrence of non-indigenous species, such as ports and brackish-water environments. Whenever possible, samples were sorted while organisms were alive, and polychaetes were isolated, photographed 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, then fixed in either 70% or 96% molecular grade ethanol. Individuals of non-indigenous polychaetes were preserved in the polychaete collection of the Marine Biology Museum of the University of Salento, located in Porto Cesareo (Giangrande *et al.,* 2015).

A small tissue fragment (1 mm$^3$ or less) was removed from the individual, taking care not to damage or destroy any diagnostic features, and employed for DNA extraction. Total genomic DNA was extracted using the salting-out protocol (Aljanabi & Martínez, 1997) with the modifications detailed in Furfaro *et al.* (2022). The mitochondrial regions coding for 16S rRNA and cytochrome c oxidase subunit I (COI) were amplified. 16S rDNA amplification was obtained using the primer pair 16SL (5’-CGCCTGTTTAACAAAAACAT-3’) and H3080 (5’-CCGGTCTGAACTCAGATCACGT-3’) (Palumbi *et al.,* 1991), whereas for COI amplification the annelid-specific primers POLYLCO (5’-GAYTATWTTCAACAAATCATAAAGATATTGG-3’) and POLYHCO (5’-TAMACTTCWGGGTGACCAAARAATCA-3’) (Carr *et al.*, 2011) and the universal degenerate primers jGLCO1490 (5’-TITCIACIAAYCAYAARGAYATTGG-3’) and jGH2198 (5’-TAIACYTCIGGRTGICCRAARAAYCA-3’) (Geller *et al.*, 2013) were employed. Polymerase chain reaction (PCR) amplifications were carried out in 20 µL solutions using 4 µL of FIREPol® Master Mix (Solis BioDyne), 0.1 µM of each primer and 1 µL of template DNA. For 16S rDNA the PCR profile was set as follows: initial denaturing step at 94 °C for 4 min, 35 cycles of denaturing at 94 °C for 30 s, annealing at 50 °C for 30 s, and extending at 72 °C for 45 s, with a final extending step at 72 °C for 7 min. For COI, annealing temperature was set at 45 °C and the PCR profile included 40 cycles. A negative control was included in each reaction. PCR products were sent to Macrogen Europe for purification and sequencing. Molecular data were uploaded in BOLD (https://www.boldsystems.org) within the project MAPWORMS - Mimicking Adaptations and Plasticity in WORMS.

Non-indigenous species of annelid were categorised into the three categories employed by Langeneck *et al.* (2020). Accordingly, alien species were defined as species whose status of NIS can be confirmed based on the absence of discrepancies in morphology and/or genetics with the original description, and on the absence of doubts on their origin. Cryptogenic species were defined, in agreement with Carlton (1996), as species with reasonably clear taxonomy that are not demonstrably native nor introduced. Lastly, questionable species were defined, following Tsiamis *et al.* (2018), as species recorded as NIS in Mediterranean environments, but showing discrepancies in morphology and/or genetics towards topotypic individuals.

## Results

During the sampling period, individuals belonging to 15 non-indigenous species (Table 1) were sampled in 23 stations along the whole coast of the Salento Peninsula

**Table 1.** Non-indigenous annelid species recorded along the coast of Salento, with date of first record in the Mediterranean Sea and off Salento, introduction status in agreement with Langeneck *et al.* (2020) and molecular data available.

| Species | First record in the Mediterranean | First record off Salento | Introduction status | Molecular data |
|---|---|---|---|---|
| *Branchiomma boholense* (Grube, 1878) | 1927 (Israel) | 2004 | Alien | COI |
| *Branchiomma luctuosum* (Grube, 1870) | 1981 (Italy) | 1988 | Alien | COI |
| *Dorvillea similis* (Crossland, 1924) | 2005 (Türkiye) | 2022 | Alien | COI, 16S |
| *Hydroides dianthus* (Verrill, 1873) | Before 1865 (Türkiye) | 1964 | Questionable | - |
| *Hydroides dirampha* (Mörch, 1863) | 1866 (Italy) | 2013 | Alien | - |
| *Hydroides elegans* (Haswell, 1883) | Before 1844 (Italy) | 1964 | Alien | - |
| *Lepidonotus tenuisetosus* (Gravier, 1902) | 2018 (Tunisia) | 2023 | Alien | COI |
| *Lumbrineris perkinsi* Carrera-Parra, 2001 | 1973 (Spain) | 1985 | Alien | COI, 16S |
| *Naineris setosa* (Verrill, 1900) | 2003 (Italy) | 2003 | Alien | COI |
| *Neopseudocapitella brasiliensis* Rullier & Amoureux, 1979 | Before 1978 (Croatia) | 2019 | Questionable | - |
| *Nereis* aff. *agulhana* Day, 1963 | 1983 (Spain) | Before 2008 | Questionable | COI, 16S |
| *Palola valida* (Gravier, 1900) | 2005 (Türkiye) | 2018 | Alien | COI, 16S |
| *Prionospio* cf. *depauperata* Imajima, 1990 | 2005 (Türkiye) | 2018 | Questionable | COI, 16S |
| *Pseudonereis anomala* Gravier, 1899 | 1933 (Egypt) | 2022 | Alien | COI, 16S |
| *Syllis similisunzima* San Martín, Lucas & Hutchings, 2023 | 2023 (Italy) | 2023 | Alien | COI |

(Fig. 1). Amplification of at least one of the two mitochondrial genes was obtained for individuals of 11 species, while the amplification of mitochondrial genes was not possible for representatives of the families Capitellidae and Serpulidae, as already revealed by previous studies (Sun *et al.*, 2012; J. Langeneck, *pers. obs.*).

Family **Capitellidae** Grube, 1862

***Neopseudocapitella brasiliensis*** Rullier & Amoureux, 1979

***Material examined***. Cerano, Southern Adriatic Sea (40.57007°N; 18.0529°E), 11.5 m, coarse sand, 14 September 2022: 1 individual. Brindisi, Southern Adriatic Sea (40.66622°N; 17.95465°E), 9 m, fine sand, 15 September 2022: 2 individuals.

***Molecular data***. None.

***Remarks***. This species (the only one in the monotypic genus *Neopseudocapitella* Rullier & Amoureux, 1979) can be easily identified among all Mediterranean Capitellidae, with the exception of *Mastobranchus trinchesii* Eisig, 1887, for the co-occurrence of capillary chaetae and hooks in the anterior abdominal notopodia; it can be distinguished from *M. trinchesii* because of the absence of branchiae and because capillary chaetae are present at the notopodium to the 37th chaetiger and gradually decrease in number towards the pygidium, while in *M. trinchesii* branchiae are present, capillary chaetae are present at the notopodium to the 77th chaetiger, and the ratio between capillary chaetae and hooks does not change towards the pygidium. This species has been reported as non-indigenous from the whole Mediterranean Sea (Zenetos *et al.,* 2010). However, individuals from the Mediterranean Sea show clear differences in comparison with the original description, in particular referring to the number of abdominal chaetigers with capillary chaetae, which might suggest that they belong to a different, undescribed, and possibly indigenous species of *Neopseudocapitella* (Parapar *et al.,* 2015).

***Distribution in the study area***. Reported from the sediment under aquaculture cages off Taranto (Langeneck *et al.,* 2020). Along the Italian coastline this species has been reported mostly in the North Adriatic (Castelli *et al.,* 2008; Mikac, 2015) and in the Ionian Sea (Castelli *et al.,* 2008; Langeneck *et al.,* 2020). The present data extend its known distribution to the Southern Adriatic Sea.

Family **Dorvilleidae** Chamberlin, 1919

***Dorvillea similis*** (Crossland, 1924) (Fig. 2a)

***Material examined***. Marina di Pulsano, Ionian Sea (40.35069° N; 17.36656° E), intertidal, on natural hard bottom amongst coralline algae, 19 July 2022: 1 specimen. Baia d'Argento, Ionian Sea (40.36134° N; 17.34014° E), 7 m, on stone among sciaphilous algae, 5 October 2023: 1 specimen. Tricase, Strait of Otranto (39.93116°N; 18.3957°E), 1 m, on artificial hard substrate with sciaphilous bryozoans, 28 January 2023: 5 specimens. Brindisi, Southern Adriatic Sea (40.66103° N; 17.96413° E), 1 m, on artificial hard substrate with mussels, 01 February 2023: 5 specimens; same locality and environment, 09 November 2023: 13 specimens.

***Molecular data***. COI and 16S sequences were obtained for two individuals sampled in the port of Tricase (GenBank accession numbers: COI: OR795506-OR795507; 16S: OR795535-OR795536). COI gave a 98-99% identity in both GenBank and BOLD with an unidentified polychaete sampled in the Red Sea in association with sponges (GenBank accession numbers: KY262757, KY263450, Kandler *et al.,* 2019). Molecular data allow us therefore

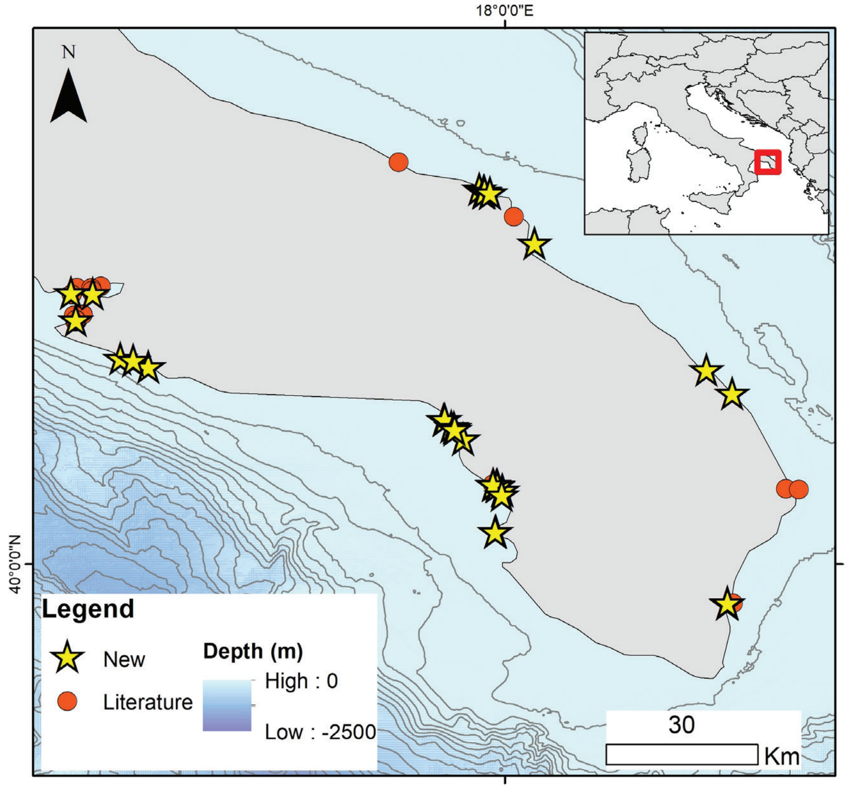

***Fig. 1:*** Map of the Salento Peninsula, showing historical (rounds) and new (stars) records of fifteen non-indigenous polychaetes.

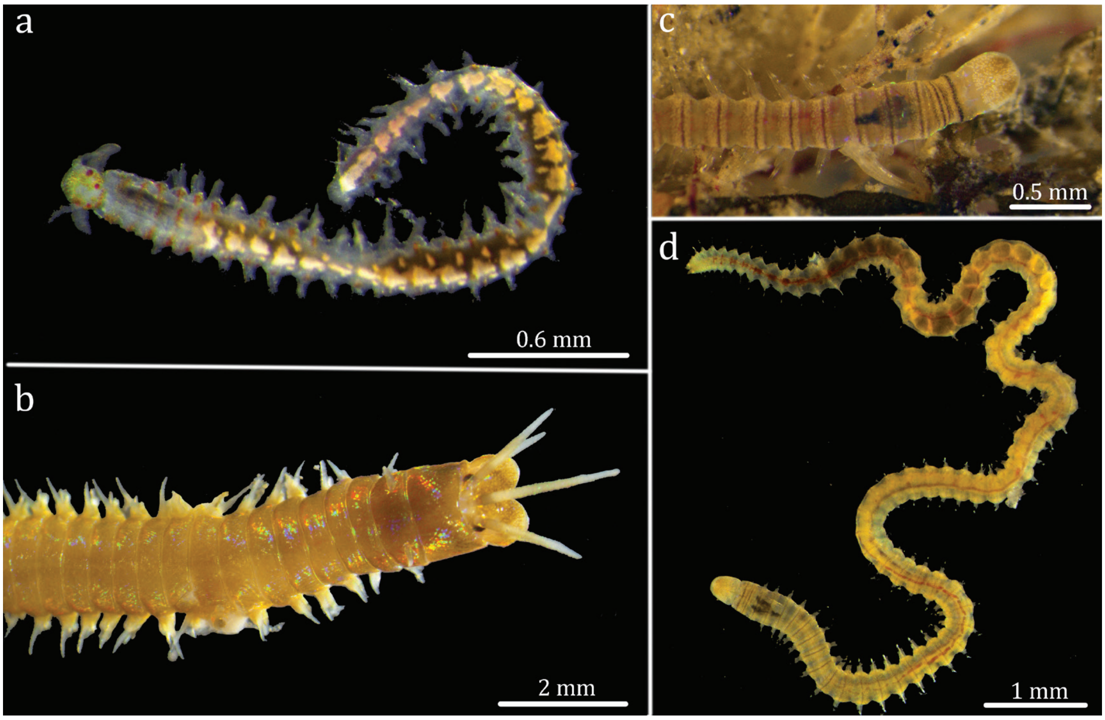

***Fig. 2:*** Live specimens of non-indigenous polychaetes: a) *Dorvillea similis*; b) *Palola valida*; c) and d) *Lumbrineris perkinsi*. All specimens have been sampled in the port of Tricase (north-eastern Ionian Sea).

to confirm the Lessepsian affinity of this species. The highest identity for 16S in GenBank was relatively low (83%) and was retrieved with *Dorvillea erucaeformis* (Malmgren, 1865) (accession numbers: AY838827, GQ478122). The identity with the two sequences of *D. similis* available on GenBank (COI: accession number DQ317857; 16S: accession number DQ317915), both belonging to a specimen with unknown origin used as outgroup by Schulze (2006), was very low (78% and 80%, respectively).

***Remarks.*** The examined individuals match well the available descriptions (Crossland, 1924; Çinar, 2009) and iconography (Corsini-Foka *et al.,* 2015; Dragičević *et al.*, 2019). In particular, the live colour, characterised by single, fragmented, orange transverse bands, particularly evident in the anterior part of the body (Fig. 2a), is clearly different from the characteristic colour of the widespread native *Dorvillea rubrovittata* (Grube, 1855). Also, the jaw apparatus, characterised by the absence of jaw pieces with an elongated, thread-like central tooth, is very different from that of *D. rubrovittata* and allows a ready identification of this species.

***Distribution in the study area.*** This is the first record of *D. similis* along the Salento coastline, as well as the first record in both the Ionian and the Adriatic Sea. This species occurs on both natural and artificial hard bottoms, confirming the ecological requirements reported in previous works (Çinar, 2009; Tempesti *et al.,* 2022). Elsewhere, *D. similis* is known from Türkiye (Çinar, 2009), Cyprus (Rousou *et al.*, 2023), Greece (Corsini-Foka *et al.*, 2015) and the Tyrrhenian Sea (Dragičević *et al.*, 2019; Langeneck *et al.,* 2020; Tempesti *et al.*, 2022). Moreover, an individual from Algiers, briefly described by Jumars (1974) as *D. rubrovittata,* is characterised by jaw pieces without the elongated, thread-like central tooth typical of this species, and seems closer to *D. similis*, suggesting that the occurrence of this species in the Mediterranean Sea might date back to the 1970s. However, Jumars (1974) did not fully describe this specimen, and only depicted a single jaw piece, therefore the evidence for the identity of the Algerian individual with *D. similis* is inconclusive.

Family **Eunicidae** Berthold, 1827

***Palola valida*** (Gravier, 1900) (Fig. 2b)

***Material examined***. Tricase, Strait of Otranto (39.93116°N; 18.3957°E), 1 m, on artificial hard substrate with sciaphilous bryozoans, 28 January 2023: 1 specimen.

***Molecular data***. COI and 16S sequences were obtained for the individual reported (GenBank accession numbers: COI: PP067668; 16S: OR795540). COI gave a 83% identity with *Palola* sp. from Lizard Island, Australia (accession number: KT124726) on Genbank, and a 84% identity with *Palola* sp. from Kosrae, Micronesia (Schulze, 2006; DQ317827) on BOLD). The identity with sequences assigned to *Palola siciliensis* (Grube, 1840) (GenBank accession numbers: MW277869, MW278709) was lower (around 80-81%); however, these sequences refer to specimens from the Hawaii, while Mediterranean COI sequences assigned to *P. siciliensis* seem to be altogether missing from all databases. The 16S sequence gave a 92% identity with a *Palola* sp. from Kosrae, Micronesia (Schulze, 2006; GenBank accession number: DQ317885), while the identity with *Palola siciliensis* (Grube, 1840) from the Mediterranean Sea (Zanol *et al.*, 2010: GenBank accession number: GQ478168) was much lower (83%).

***Remarks***. *Palola valida* is very similar to the native *P. siciliensis*; the main differences concern the jaw apparatus (with asymmetrical M2 and M3 in *P. valida*, symmetrical in *P. siciliensis*) and the live colour, overall whitish in *P. siciliensis* and brownish with honeycomb-like whitish pattern in *P. valida*, which is often preserved in fixed individuals. The individual found in the port of Tricase shows a good correspondence in size and general features with the description by Kurt Şahin & Çinar (2009) of individuals from the Levant Sea.

***Distribution in the study area***. Reported until now only for the Strait of Otranto (Gravina *et al.,* 2021; present data). The only other record refers to individuals sampled in a mesophotic assemblage dominated by *Neopycnodonte cochlear* (Poli, 1795) together with the native *P. siciliensis*. As coralligenous and mesophotic habitats are usually characterised by a low incidence of non-indigenous species, and considering the presence of the native congeneric species, we suspect that the previous record might represent a misidentification of *P. siciliensis*. Elsewhere in the Mediterranean Sea this species is currently known for the Levant Sea (Kurt Şahin & Çinar, 2009; Rousou *et al.,* 2023), the Aegean Sea (Çinar & Dağli, 2021) and the eastern Adriatic Sea (Spagnolo *et al.*, 2019).

Family **Lumbrineridae** Schmarda, 1861 (Fig. 2c-d)

***Lumbrineris perkinsi*** Carrera-Parra, 2001

***Material examined***. Taranto, Ionian Sea (40.47628° N; 17.2474° E), 1 m, on artificial hard bottom inside sponges, 17 May 2023: 1 specimen. Porto Cesareo, Ionian Sea (40.2565° N; 17.89197° E), intertidal, on artificial hard bottom amongst coralline algae, 01 June 2022: 1 specimen. Gallipoli, Ionian Sea (40.05781° N; 17.98556° E), 1 m, on artificial hard substrate amongst mussels, 26 April 2023: 4 specimens. Tricase, Strait of Otranto (39.93116°N; 18.3957°E), 1 m, on artificial hard substrate with sciaphilous bryozoans, 28 January 2023: 22 specimens.

***Molecular data***. COI and 16S sequences were obtained for two individuals sampled in the port of Tricase (GenBank accession numbers: COI: OR795510-OR795511; 16S: OR795537-OR795538). COI sequences showed a 99%-100% identity with *L. perkinsi* on both GenBank and BOLD (GenBank accession number: KP254185; BOLD sequences private). 16S sequences showed a 93% identity with *Lumbrineris inflata* Moore, 1911 (GenBank accession number: AY838832).

***Remarks***. The examined individuals match other Mediterranean individuals examined in previous studies (Langeneck *et al.,* 2020; Tempesti *et al.,* 2022) and the descriptions by Carrera-Parra (2001; 2006) and Çinar (2009). In addition, the live colour pattern of *L. perkinsi* depicted in Carrera-Parra (2009), characterised by the presence of two dark transverse stripes in anterior chaetigers, is identical to the one identified in the Mediterranean individuals (Fig. 2c; see also Campoy, 1982) and makes this species unmistakable among Mediterranean Lumbrineridae. Langeneck *et al.* (2020) suggested considering this species as questionable due to the extremely wide distribution of individuals morphologically consistent with this taxon, which casts doubts on the origin of this species. Nonetheless, while the origin of this taxon is still uncertain, its reports in the Mediterranean Sea are recent enough to confirm its status of non-indigenous species. In contrast with Langeneck *et al.* (2020), and in agreement with Zenetos *et al.* (2022), we regard this species as a confirmed NIS in the Mediterranean Sea.

***Distribution in the study area***. This species is widespread along the whole coastline of Salento. The first record (which was overlooked by Langeneck *et al.*, 2020) refers to artificial hard bottoms in Taranto (Gherardi *et al.*, 1985 – as *L. inflata*). The species was later reported for Brindisi (Langeneck *et al.*, 2020), even though precise georeferenced data are not available. In this study the distribution is extended to include the ports of Tricase and Gallipoli, as well as artificial hard bottoms in the bay of Porto Cesareo. The species occurs in the Mediterranean Sea since the 1970s (Campoy, 1982), even though the first records have been reported as *L. inflata*; the descriptions given by Giangrande *et al.* (1981) and Campoy (1982) correspond to *L. perkinsi*. Elsewhere, the species was reported for Israel (Langeneck *et al.*, 2020), Türkiye (Çinar, 2009), France (Tempesti *et al.,* 2022) and Spain (Campoy, 1982 – as *L. inflata*), while records for Greece cannot be confirmed (Faulwetter *et al.*, 2017). In Italian waters *L. perkinsi* was firstly recorded from Ischia (Giangrande *et al.,* 1981 – as *L. inflata*), and seems to be rather widespread in the Northern Tyrrhenian Sea (Langeneck *et al.,* 2020; Tempesti *et al.*, 2022).

Family **Nereididae** de Blainville, 1818

***Nereis* aff. *agulhana*** Day, 1963 (Fig. 3)

***Material examined***. Strea Inlet, Porto Cesareo, Ionian Sea (40.24474° N; 17.90515° E), 0.5 m, on fine sand and shell grit among *Cymodocea nodosa*, 06 October 2022: 5 specimens.

***Description***. All specimens very small. Complete individual with 52 chaetigers for 7.29 mm total length, 0.43 mm maximum width without parapodia (0.57-0.66 mm in incomplete specimens), 1.36 mm length at the 10th chaetiger (1.68-1.69 mm in incomplete specimens). Prostomium pear-shaped, slightly longer than wide, anteriorly truncated; antennae tapered, 2/3 of the prostomium length, palps shorter than antennae, with bulky, cylindrical palpophore and ogival, small palpostyle. Four sub-equal dark red eyes (black in preserved material) arranged in a rectangle. Four pairs of peristomial cirri; longest pair of peristomial cirri reaching the 5th chaetiger backwards, second-longest pair of peristomial cirri reaching the 2nd chaetiger backwards, remaining pairs reaching the 1st chaetiger. Peristomium achaetous, approximately half the length of the prostomium. Pharynx muscular, armed with corneous jaws bearing 10-11 well-developed teeth. Area I with 1 conical paragnath; areas II with 3 conical paragnaths; area III without paragnaths; area IV with 3-4 paragnaths; areas V and VI seemingly without paragnaths; areas VII-VIII with a continuous series of 3-5 rounded paragnaths.

Parapodia biramous, with two stocky, dark aciculae, dorsal cirri tapered, short. Pattern of ligulae and lobes showing a limited variation along the body. Dorsal ligule conical, well-developed throughout the body, notopodial pre-chaetal lobe well-developed, bluntly conical, medi-

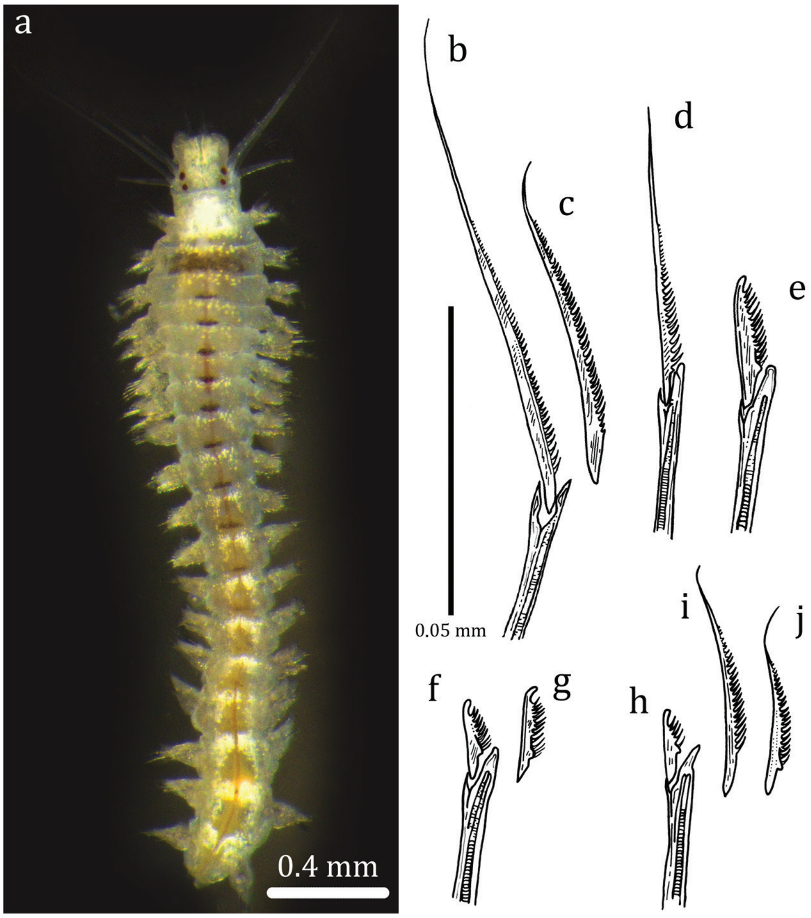

***Fig. 3:*** *Nereis* cf. *agulhana* from the Strea Inlet. a) live specimen, anterior part of the body; b) notopodial homogomph spiniger from anterior parapodia; c) blade of neuropodial homogomph spiniger from anterior parapodia; d) neuropodial heterogomph spiniger from anterior parapodia; e) neuropodial heterogomph falciger from anterior parapodia; f, g) neuropodial heterogomph falcigers from midbody parapodia; h) neuropodial heterogomph falciger from posterior parapodia; i) blade of notopodial homogomph spiniger from posterior parapodia; j) blade of neuropodial homogomph spiniger from posterior parapodia.

an ligule as large as dorsal ligule. Neuroacicular ligule conical, short, ventral ligule conical, shorter than dorsal and median ligule, ventral cirri tapered, short. Anterior parapodia with 4 homogomph spinigers at the notopodium (Fig. 3b), 2-3 homogomph spinigers (Fig. 3c), 1-2 heterogomph spinigers (Fig. 3d), and 4-5 heterogomph falcigers (Fig. 3e) with rather elongated blades at the neuropodium. Midbody parapodia with 3-4 homogomph falcigers at the notopodium, 2-4 homogomph spinigers and 5-6 heterogomph falcigers at the neuropodium (Fig. 3f, g). Posterior parapodia with 3-4 homogomph spinigers at the notopodium (Fig. 3i), 2-3 homogomph spinigers, 1 heterogomph spiniger (Fig. 3j) and 3-4 heterogomph falcigers at the neuropodium (Fig. 3h). Pygidium with two thread-like, tapering cirri, as long as the last 5-6 chaetigers.

Live colour pale grey with white peristomium and 1st chaetiger, 2nd chaetiger completely brown, and a single, unpaired dark spot along the posterior margin of chaetigers 3-12, gradually fading (Fig. 3a). Traces of the live colour often remain in preserved individuals.

***Molecular data***. COI and 16S sequences were obtained for only one out of the two specimens assayed (GenBank accession numbers: COI: OR795513; 16S: OR795539). The COI sequence gave a 81% identity with *Hediste diversicolor* (O. F. Müller, 1776) on GenBank (accession number: OP038883) and a 84% identity with *Perinereis* sp. on BOLD (GenBank accession number: KX525497), while the 16S sequence gave a 85% identity with *Alitta virens* (M. Sars, 1835) (GenBank accession number OW028587).

***Remarks***. *Nereis agulhana*, with type locality in South Africa, was described within the genus *Nereis* and later moved to *Neanthes* on the basis of the alleged absence of notopodial homogomph falcigers. López & Richter (2017) stressed relevant differences between the individuals described from the Mediterranean Sea and the original description, suggesting that Mediterranean material might represent a different species. Later on, Villalobos-Guerrero *et al.* (2022) redescribed *N. agulhana* on the basis of the type material, and found homogomph falcigers in the posterior-most notopodia of the largest individuals. This character does not match the current definition of *Neanthes* and led these authors to re-establish the original combination. The individuals sampled in the Strea inlet were very small; therefore, inconsistency with the redescription of the type material, such as absence of homogomph falcigers, might be due to the size of the examined material. However, some characters of the pharynx are puzzling and might suggest a distinction at the species level. In particular, areas III and VI were devoid of paragnaths in the individuals from the Strea Inlet, while paragnaths occur in these areas in all individuals examined by Villalobos-Guerrero *et al.* (2022); however, areas I and VII-VIII match those of the type material, suggesting that these characters show a relatively limited size-related variation. The presence of a single row of paragnaths in areas VII-VIII is relatively infrequent in Mediterranean Nereididae, and is currently known only for *N. agulhana*, *Nereis rava* Ehlers, 1868 and *Nereis usticensis* Cantone, Catalano & Badalamenti, 2001. The two latter species are characterised by the presence of typical homogomph falcigers, also in individuals of comparable size to those examined, and do not share the peculiar live colour pattern, which is closer to that observed in the type material of *N. agulhana*. However, considering the discrepancies observed between the Mediterranean material and the redescription and the inconclusive result of the molecular analyses, we are here regarding *Nereis agulhana* as a questionable non-indigenous species in the Mediterranean Sea.

***Distribution in the study area***. The species is known for the Ionian Sea, most likely along the Salento coastline, at least since 2008 (Giangrande *in* Zenetos *et al.,* 2008). Unfortunately, more precise data about this early occurrence are not available. During the present study, *N.* cf. *agulhana* was found only in Porto Cesareo Bay, with a relatively abundant population. Elsewhere along the Italian coastline this species is currently known for the Sea of Sardinia (Langeneck *et al.,* 2020), Tyrrhenian Sea (Bedini *et al.,* 2015; Langeneck *et al.,* 2020), Strait of Sicily (Musco *et al.,* 2013) and Adriatic Sea (Spagnolo *et al.,* 2019).

***Pseudonereis anomala*** Gravier, 1899 (Fig. 4a-c)

***Material examined***. Mar Piccolo di Taranto, Ionian Sea (40.48210° N; 17.22921° E), 1 m, on artificial hard bottom amongst sponges, 5 October 2023: 2 specimens. Marina di Pulsano, Ionian Sea (40.35069° N; 17.36656° E), intertidal, on natural hard bottom amongst coralline algae, 19 July 2022: 2 specimens. Porto Pirrone, Ionian Sea (40.36520° N; 17.31723° E), intertidal, on natural hard bottom amongst coralline algae, 5 October 2023: 12 specimens. Tricase, Strait of Otranto (39.92989°N; 17.39522°E), intertidal, on natural hard substrate among coralline algae, 04 July 2022: 1 specimen. Same locality and environment, 13 June 2023: 1 specimen.

***Molecular data***. COI sequences were obtained for two specimens from Tricase and Marina di Pulsano, respectively (GenBank accession numbers: OR795517-OR795518) and yielded a >99% identity with *Pseudonereis* sp. from Greece (GenBank accession numbers: OP347391, OP347537) in both GenBank and BOLD. The identity with four sequences deposited as *P. anomala* (GenBank accession numbers: JX420268, JX420270, JX420271, MZ560609) was distinctly lower (82-84%); however, it should be noted that these sequences come from Australia and Papua-New Guinea, quite distant from the type locality, and might belong to a different species. The 16S sequence was obtained from the specimen from Tricase only (GenBank accession number: OR795544) and yielded a >99% identity with *Pseudonereis* sp. from Greece (GenBank accession numbers: OP347584, OP347660).

***Remarks***. The examined individuals are relatively small (around 0.4-1 mm maximum width), but match very well the original description and subsequent redescriptions

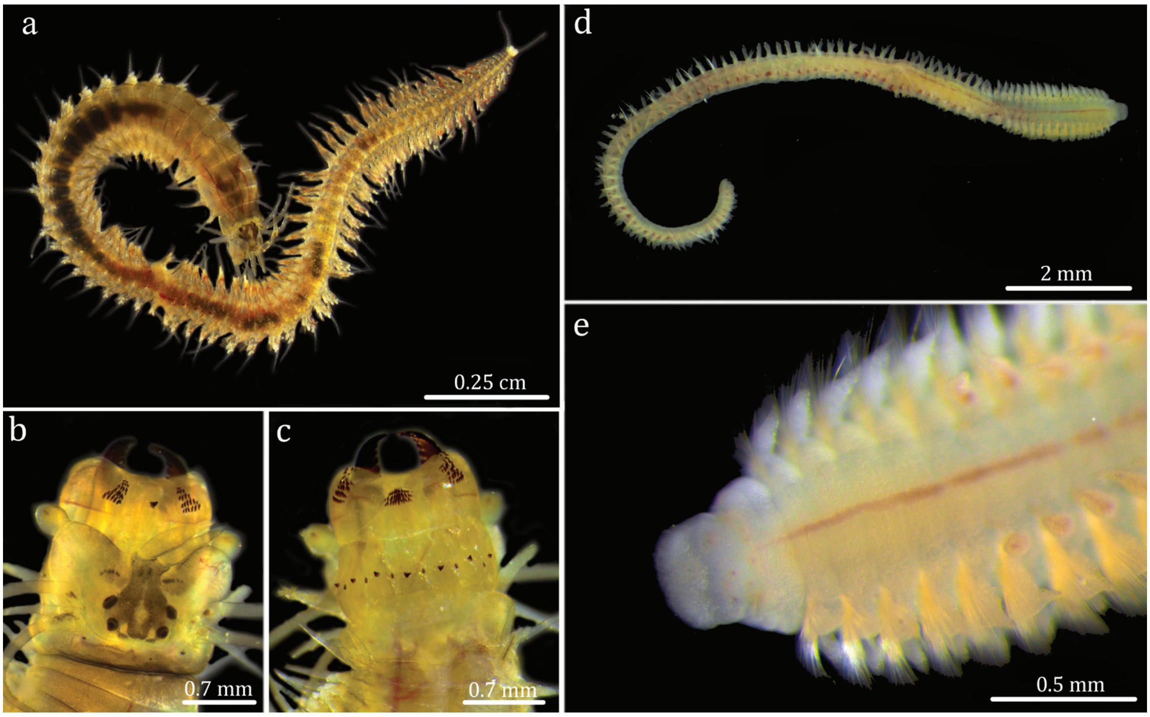

***Fig. 4:*** Live specimens of non-indigenous polychaetes: a) *Pseudonereis anomala* from natural hard bottoms off Porto Pirrone, complete specimen; b) *P. anomala*, everted pharynx, dorsal view; c) *P. anomala*, everted pharynx, ventral view; d) *Naineris setosa* from the Mar Piccolo of Taranto, complete sub-adult specimen; e) *N. setosa*, detail of the anterior end.

(Gravier, 1899; Çinar & Ergen, 2005; D'Alessandro *et al.,* 2016b). Dorsal cirri, which are very long and flattened in large adults, are short and thin in small individuals, which might be easily misidentified as the native *Perinereis cultrifera* (Grube, 1840) if the pharynx is not protracted.

***Distribution in the study area***. Found on intertidal coralline algae in the Gulf of Taranto and off Tricase, and on sponges in the Mar Piccolo of Taranto. Interestingly, the species seems to be more widespread in pristine natural hard substrates than in port environments. The current records represent the first report of *P. anomala* for the Italian Peninsula. Elsewhere, along the Italian coastline this species is only known for Sicily (D'Alessandro *et al.,* 2016b). Due to its invasive potential, and the possibility of competitive interactions with native Nereididae (Çinar & Altun, 2007), the spread of *P. anomala* along the Salento Peninsula deserves a close monitoring.

Family **Orbiniidae** Hartman, 1942

***Naineris setosa*** (Verrill, 1900) (Fig. 4d)

***Material examined***. Mar Piccolo di Taranto, Ionian Sea (40.48075° N; 17.26817° E), 6 m, on hypoxic fine sand under rhodolith bed, 07 February 2023: 4 specimens. Brindisi, Southern Adriatic Sea (40.66103° N; 17.96413° E), 1 m, on artificial hard substrate with mussels, 09 November 2023: 2 specimens.

***Molecular data***. A COI sequence was obtained for only one out of the two specimens assayed. This sequence (GenBank accession number: OR795512) gave a 97% identity with an unidentified polychaete sampled in a lagoon in Florida (GenBank accession numbers: KP254343, KP254455, KP254461: Leray & Knowlton, 2015) on GenBank, while on BOLD it yielded a >99% identity with private sequences assigned to *Naineris setosa*, confirming both the identification and the west-Atlantic origin of this species.

***Remarks.*** Among Mediterranean Orbiniidae, *N. setosa* can be easily identified due to the combination of roundish to square prostomium (typical of the genera *Naineris* and *Protoaricia*) and absence of subuluncini in thoracic chaetigers (Blake & Giangrande, 2011). The morphology of this species shows some ontogenetic variation: juveniles usually show two distinct peristomial rings, a round prostomium, and the presence of several small eyespots, whereas during growth the peristomial rings become fused into a single ring, the prostomium becomes square, and eyespots tend to disappear (Solis-Weiss & Fauchald, 1989; Blake & Giangrande, 2011).

***Distribution in the study area***. Originally reported for artificial canals in an aquaculture facility near Brindisi, southern Adriatic Sea (Blake & Giangrande, 2011) and later found in the soft sediment under aquaculture cages in the Mar Grande of Taranto (Langeneck *et al.,* 2020). The present record confirms the occurrence of this species in the Taranto area, extending its range to the inner basin of Mar Piccolo. Elsewhere along the Italian coastline the species is known for the Sea of Sardinia (Atzori *et al.,* 2016) and for the northern Tyrrhenian Sea (Tempesti *et al.,* 2020b). In the Mediterranean Sea, *N. setosa* was reported for Tunisia (Khedhri *et al.,* 2014) and Cyprus (Rousou *et al.,* 2023). In all cases, occurrences of this species are associated with organically enriched environments, as ports, coastal ponds, and aquaculture facilities.

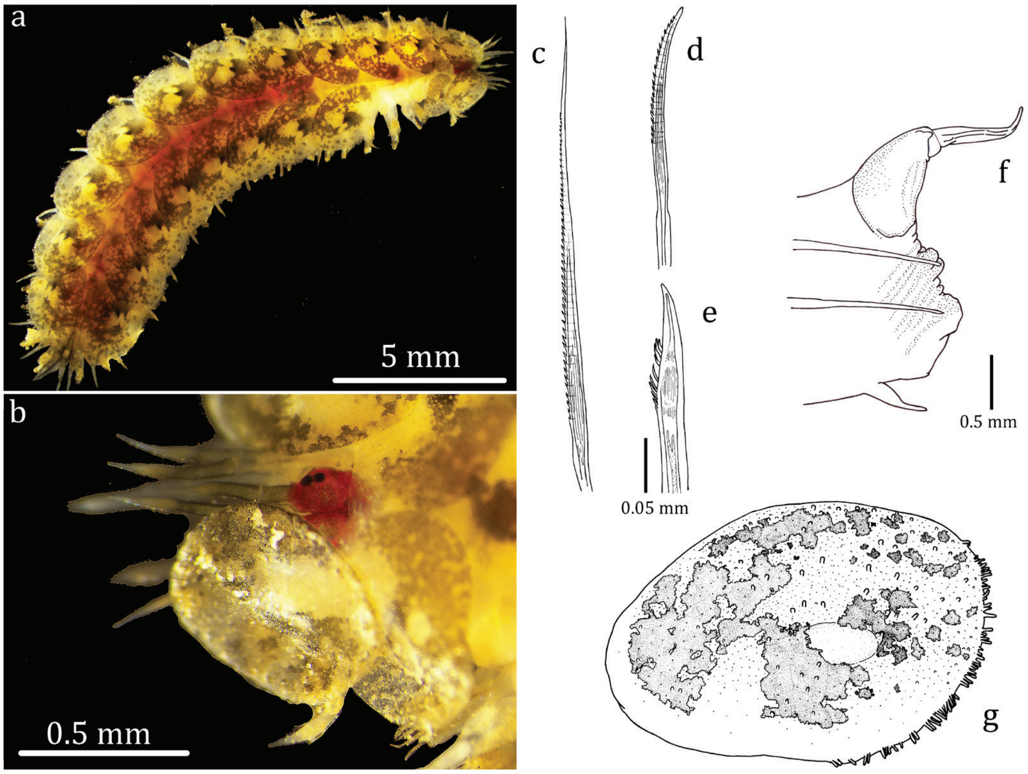

***Fig. 5:*** *Lepidonotus tenuisetosus* from the port of Brindisi. a) complete specimen; b) detail of the prostomium; c) slender notochaeta; d) curved notochaeta; e) neurochaeta; f) left parapodium in anterior view; g) elytron.

Family **Polynoidae** Kinberg, 1856

***Lepidonotus tenuisetosus*** (Gravier, 1902) (Fig. 5)

***Material examined***. Brindisi, Adriatic Sea (40.34544° N; 18.35873° E), 1 m, on artificial hard substrate, 01 February 2023: 2 specimens.

***Description***. Largest individual complete, 29 chaetigers for 15.72 mm total length, 7.72 mm length at the 10th chaetiger, 4.22 mm maximum width. Body slightly flattened, prostomium without cephalic peaks; median antenna with ceratophore in anterior notch, style smooth, 2½ times the prostomium length, slightly inflated subdistally. Lateral antennae inserted terminally, with ceratophores fused to prostomium, styles smooth, 2 times the prostomium length, slightly inflated subdistally. Palps smooth, three times the prostomium, flattened, with a very short tapered tip. Two pairs of sub-equal, rounded black eyes; anterior pair of eyes dorso-lateral, just below the insertion of lateral antenna; posterior eye dorsal, very close to the anterior eye (Fig. 5b). Tentaculophores inserted laterally to prostomium, with few chaetae, and a pair of dorsal and ventral tentacular cirri with smooth styles, slightly inflated subdistally. Second segment with first pair of elytra, and sub-biramous parapodia.

Twelve pairs of large, oval elytra. External elytral margin fringed by a series of filiform papillae (approximately 1/3 of the perimeter). A few scattered macrotubercles, oval to cylindrical with rounded tip, more numerous and smaller along the external edge of the elytron; microtubercles very small and tightly packed over the whole elytral surface (Fig. 5g). Styles of dorsal cirri smooth, weakly inflated subdistally in the anterior part, with tapering tip, in the anterior part of the body and in the median part; cirri evenly tapered in the posterior part of the body. Styles of ventral cirri smooth, tapering, shorter than neuropodia (Fig. 5f). Pygidium elongated, with dorsal anus and truncated tip, two elongated anal cirri, slightly longer than dorsal cirri, evenly tapering.

Parapodia sub-biramous, notopodia and neuropodia with elongate acicular lobe; tips of both aciculae penetrating the epidermis (Fig. 5f). Up to 16 notochaetae and 31 neurochaetae. Notochaetae slender, mostly rather straight and with tapering tip (Fig. 5c), some notochaetae shorter, slightly curved (Fig. 5d); all notochaetae with serrated ventral edge and unidentate tip. Neurochaetae much stouter than notochaetae, with slightly falcate, unidentate tip, and an evident subdistal spinulation along the ventral edge (Fig. 5e).

Live colour well preserved in the fixed specimens. Body yellowish, with indistinct greenish transverse bands on segments, prostomium bright red, antennae and tentacular cirri greenish-grey with whitish tip, dorsal cirri pale grey with a subdistal brownish ring. Elytra yellowish with a greenish-brown marbled pattern (Fig. 5a).

***Molecular data***. COI sequences were obtained from both specimens (GenBank accession numbers: OR795508-OR795509). The two sequences had a 99% identity; a comparison with sequences deposited in BOLD and GenBank gave as best results an 84% identity with *Harmothoe quadrituberculata* Augener, 1922 (sequence ID: YCHAE253-08.COI-5P), and an 82% identity with *Harmothoe fuligineum* (Baird, 1865) (accession numbers: KX865415, KX865418, KX867420), respectively.

***Remarks***. This species was first recorded from the Mediterranean Sea off Israel by Barnich & Fiege (2003), and later reported from Türkiye (Çinar, 2009), Croatia (Mikac, 2015) and Greece (Chatzigeorgiou *et al.*, 2016). Chaibi et al. (2023) reported *L. tenuisetosus* from Tunisia and re-examined the previous reports, concluding that the species reported by Barnich & Fiege (2003) has both unidentate and bidentate neurochaetae, and is therefore not *L. tenuisetosus* but rather *Lepidonotus carinulatus* (Grube, 1869). Based on the same reasons, the specimen reported by Mikac (2015) is certainly not *L. tenuisetosus*, while the material from Greece was re-examined and turned out to belong to the native *Lepidonotus clava* (Montagu, 1808). Although the chaetal features match well with *L. tenuisetosus*, Chaibi *et al.* (2023) suggested that the material examined by Çinar (2009) does not belong to this species either, due to differences in the microtubercles and the position of the eyes on the prostomium. The morphological characters of the two specimens examined match well the redescription by Chaibi *et al.* (2023), allowing us to extend the distribution of this species to the Italian coast.

***Distribution in the study area***. Currently known only for the port of Brindisi. This record represents the first report for Italian waters.

Family **Sabellidae** Latreille, 1825

***Branchiomma boholense*** (Grube, 1878)

***Material examined***. Marina di Pulsano, Ionian Sea (40.35047° N; 17.36661° E), intertidal, on natural hard bottom amongst coralline algae, 12 October 2022: 2 specimens. Baia d'Argento, Ionian Sea (40.36216° N; 17.34028° E), 0.5 m, under stones, 05 October 2023: 25 specimens. Porto Cesareo, Ionian Sea (40.25713° N; 17.89345 ° E), 0.5 m, on natural, barren hard bottom, 01 September 2022: 2 specimens. Porto Cesareo, Ionian Sea (40.25607° N; 17.8917° E), 0.2 m, on scarcely vegetated natural hard bottom, 01 September 2022: 4 specimens. Sant'Isidoro, Ionian Sea (40.22203° N; 17.92603° E), 2 m, on natural hard bottom inside a submarine freshwater spring, 02 February 2023: 15 specimens. Santa Caterina di Nardò, Ionian Sea (40.14145° N; 17.97998° E), 1.5 m, natural hard substrate, 01 July 2022: 5 specimens. Santa Caterina di Nardò, Ionian Sea (40.14059° N; 17.97981° E), 0.2 m, on floating plastic buoy, 01 July 2022: 2 specimens. Santa Caterina di Nardò, Ionian Sea (40.14025° N; 17.98698° E), 0.5 m, on natural hard substrate, 01 July 2022: 4 specimens. Santa Caterina di Nardò, Ionian Sea (40.13967° N; 17.98571° E), 0.5 m, on natural hard substrate, 01 July 2022: 5 specimens. Santa Caterina di Nardò, Ionian Sea (40.13965° N; 17.98564° E), 0.5 m, on natural hard substrate with photophilous algae, 09 September 2022: 10 specimens. Santa Maria al Bagno, Ionian Sea (40.13153° N; 17.9962° E), 1 m, on barren natural hard substrate, 01 June 2022: 4 specimens. Santa Maria al Bagno, Ionian Sea (40.13095° N; 17.99549° E), 1 m, on artificial hard substrate, 01 June 2022: 5 specimens. Tricase, Strait of Otranto (39.93116°N; 18.3957°E), 1 m, on artificial hard substrate with sciaphilous bryozoans, 28

January 2023: 6 specimens; 13 June 2023: 1 specimen.

***Molecular data***. A COI sequence was obtained for only one out of two specimens from Marina di Pulsano (GenBank accession number: OR795504). The sequence had a 99.8% identity with *B. boholense* sequences deposited on GenBank (accession numbers: MG457324-MG457371), confirming the morphological identification.

***Remarks.*** Apart from *Branchiomma bairdi* (McIntosh, 1885), this species can be distinguished from the other *Branchiomma* species reported in the Mediterranean due to the presence of macro- and micro-stylodes. It can be distinguished from *B. bairdi*, a species that seems less widespread in the Mediterranean Sea, for its larger size and smaller eyes (Del Pasqua *et al.*, 2018).

***Distribution in the study area***. Known hitherto for Taranto (Giangrande *et al.,* 2021) and Brindisi (Arias *et al.,* 2013); precise georeferenced data are available in literature only for the Taranto area, where the species is largely widespread on artificial substrates. Present data allow us to extend the distribution of this species to several localities along the Ionian coastline; along the eastern coast, *B. boholense* was found only in the port of Tricase, in the Strait of Otranto, and it seems to be less widespread. In addition, while most historical records (between 2004 and 2018) referred to artificial hard substrates, the majority of the new records have been obtained by samples collected on natural substrates, often not even in proximity of ports and marinas, highlighting that this species was able to establish itself along the whole Ionian coastline successfully, forming dense aggregate in the immediate sublittoral (M. Putignano *pers. obs*.).

***Branchiomma luctuosum*** (Grube, 1870)

***Material examined***. Brindisi, Adriatic Sea (40.66224° N; 17.96311° E), 0.5 m, artificial hard substrates, 10 April 2022: 10 specimens. Brindisi, Adriatic Sea (40.66103° N; 17.96413° E), 1 m, artificial hard substrates, 01 February 2023: 3 specimens. Brindisi, Adriatic Sea (40.6603° N; 17.96218° E), 0.5-3 m, barren natural hard substrate, 10 April 2022: 15 specimens. Brindisi, Adriatic Sea (40.65943° N; 17.97364° E), 0.5 m, plastic buoy heavily colonised by mussels, 23 February 2023: 2 specimens.

***Molecular data***. A COI sequence was obtained for only one of the two specimens assayed (GenBank accession number: OR795505). The sequence had a 99-100% identity with two short *B. luctuosum* sequences deposited in GenBank (accession number: ON911309-ON911311), and a 99.8% identity with a private sequence identified as *Branchiomma* sp. on BOLD, confirming the morphological identification.

***Remarks.*** This species is unmistakable among Mediterranean species of the genus *Branchiomma* due to its large size, stocky body with relatively long branchial crown, and live colour (black-spotted greenish body with orange crown and purple pinnulae) (Licciano & Giangrande, 2008).

***Distribution in the study area***. The first records for the Salento Peninsula date back to 1988 (Licciano & Giangrande, 2008) but, unlike *B. boholense*, the species does not seem to have been able to spread along the whole coastline. Literature data mostly refer to the port areas of Brindisi (Cavallo *et al.,* 2007) and Taranto (Licciano & Giangrande, 2008), with a single record for natural hard substrates in Santa Caterina di Nardò (Ionian Sea) (Toso *et al.,* 2022). During this study the species was found only in the port of Brindisi, where it was already known.

Family **Serpulidae** Rafinesque, 1815

***Hydroides dianthus*** (Verrill, 1873)

***Material examined***. San Foca, Adriatic Sea (40.30357° N; 18.40467° E), 0.5 m, on artificial hard substrate with *Cladophora* spp., 12 January 2023: 2 specimens.

***Molecular data***. None.

***Remarks***. This species can be easily identified based on the operculum, which is characterised by a verticil with approximately ten stocky, sub-equal spines with bent tip, usually facing ventrally (Bianchi, 1981). Sun *et al.* (2017) identified *H. dianthus* as a species complex comprising at least two morphologically indistinguishable lineages. Lineage A, tentatively identified as *Hydroides dianthus s.s.*, was considered native in the Mediterranean Sea, while Lineage B likely originated in the Caribbean, and had been introduced in the Black Sea. Since all Mediterranean samples hitherto analysed belonged to *Hydroides dianthus s.s.* and displayed a remarkably higher diversity in comparison with extra-Mediterranean ones, Langeneck *et al.* (2020) regarded this species as native in the Mediterranean Sea and suggested removing it from NIS lists. This approach was followed in subsequent lists of NIS occurring in the Mediterranean Sea (Zenetos *et al.*, 2022). However, Grosse *et al.* (2021) stressed the occurrence of both lineages in port environments of Mallorca. Pending further investigations on Mediterranean populations of *H. dianthus*, we suggest considering this species as a questionable NIS.

***Distribution in the study area***. The species was reported only for the area of Taranto, where it is known at least since 1964 (Zibrowius, 1970; Gherardi *et al.,* 2011; Lezzi & Giangrande, 2018). Present data allow us to extend its distribution to the eastern coast of the Salento Peninsula, even though this occurrence is not surprising, provided that *H. dianthus* is widespread in port environments and brackish-water habitats of the Western Adriatic coastline (Mikac, 2015; Spagnolo *et al.,* 2019).

***Hydroides dirampha*** (Mörch, 1863)

***Material examined***. Brindisi, Adriatic Sea (40.66103° N; 17.96413° E), 1 m, artificial hard substrates, 01 February 2023: 4 specimens.

***Molecular data***. None.

***Remarks.*** This species is unmistakable among Mediterranean Serpulidae due to the operculum with verticil comprised of about ten flattened spines with widened, rounded tip (Bianchi, 1981).

***Distribution in the study area***. Possibly originating from the Caribbean region (Sun *et al.*, 2015; Bastida-Zavala *et al.*, 2017), this species was reported for Taranto (Lezzi *et al.*, 2018) and Santa Caterina di Nardò (Toso *et al.,* 2022); both localities belong to the Ionian Sea. The present record is the first along the Eastern coast of the Salento

Peninsula, even though the species is already known in the Southern Adriatic Sea (Spagnolo *et al.,* 2019). Elsewhere along the Italian coastline *H. dirampha* is known for the Tyrrhenian Sea (Claparède, 1870; Tempesti *et al.,* 2020b; Tempesti *et al.,* 2022) and for the Strait of Sicily (Mangano *et al.,* 2019). This species, initially restricted to the southern part of the Mediterranean Sea (Bianchi, 1981), shows a tendency to northward expansion, and its spread along the Adriatic coastline deserves a careful monitoring.

***Hydroides elegans*** (Haswell, 1883)

***Material examined***. Brindisi, Adriatic Sea (40.34544° N; 18.35873° E), 1 m, on artificial hard substrate, 01 February 2023: 36 specimens. Le Cesine Beach, Adriatic Sea (40.66103° N; 17.96413° E), beached on a drifting plastic buoy, 12 January 2023: 23 specimens.

***Molecular data***. None.

***Remarks.*** This species can be distinguished from all Mediterranean *Hydroides* species based on its operculum, with a verticil comprised of sub-equal spines with lateral branches. A similar operculum also occurs in the native *Hydroides norvegica* Gunnerus, 1768 and *Hydroides stoichadon* Zibrowius, 1970; however, in *H. norvegica* the tip of each spine is much longer than the lateral branches, and the specialised chaetae of the first chaetiger with two large sub-distal teeth, while in *H. elegans* the tip of each spine is not much longer than the lateral branches, and the specialised chaetae have several small denticles. *H. stoichadon* clearly differs from both species due to the single pair of lateral branches on each spine of the verticil, while both *H. elegans* and *H. norvegica* have 2-4 pairs of lateral branches on each spine (Bianchi, 1981).

***Distribution in the study area***. Widespread along the whole Salento Peninsula, usually in port environments (Lezzi & Giangrande, 2018), with few records referring to natural environments (Giangrande *et al.,* 2003; Musco *et al.,* 2021). The first documented record dates back to 1964 (Zibrowius, 1970), even though this species is known in the Mediterranean Sea since the 19th Century (Langeneck *et al.*, 2020) and this is possibly true for the Salento Peninsula as well. The record by Micaroni *et al.* (2022) for coralligenous outcrops at around 60 m depth is most likely a misidentification of the native *H. norvegica*, a species widespread on deep bioconstructions along the whole Salento coastline.

Family **Spionidae** Grube, 1850

***Prionospio*** cf. ***depauperata*** Imajima, 1990

***Material examined***. Santa Caterina di Nardò, Ionian Sea (40.14123° N; 17.97915° E), 20 m, on coarse sand with slight organic enrichment, 14 November 2022: 2 specimens. Santa Caterina di Nardò, Ionian Sea (40.13879° N; 17.98693° E), 5 m, on gravel, 03 November 2022: 1 specimen. Santa Maria al Bagno, Ionian Sea (40.12281° N; 17.9949° E), 8 m, on coarse sand, 30 April 2023: 1 specimen.

***Molecular data***. COI sequences were obtained from two out of three specimens (GenBank accession numbers: OR795515-OR795516). The two sequences had a 98.5% identity and gave a 81% maximum identity with *Prionospio membranacea* Imajima, 1990 (accession number: MW054861) and *P. depauperata* from Korea (accession number: MW054865) on both GenBank and BOLD. 16S sequences were obtained from all three specimens (GenBank accession numbers: OR795541-OR795543) and gave a 84% identity with *P. membranacea* (accession number: MW077198).

***Remarks***. The specimens examined match very well the redescription based on Mediterranean material (Dağli & Çinar, 2009), even though they show some variability in size and shape of the posterior pair of eyes. As pointed out by Dağli & Çinar (2009), some differences with regards to the original description are noticeable, especially regarding the presence of dorsal crests across 10 chaetigers starting from chaetiger 7 (dorsal crests across 7 segments in the type material), and the relative length of branchiae. Molecular data show that the closest match is indeed *P. depauperata* (together with *P. membranacea*), but the identity is very low (81%), and the Mediterranean specimens here examined clearly do not belong to the same species of the deposited sequence from Korea (Lee *et al.*, 2020b). This outcome, together with the morphological differences observed between the Mediterranean and the Japanese material, raises the possibility that *P. depauperata* might represent a species complex. Pending further investigations, based on the morphological and molecular discrepancies between Mediterranean and Pacific material, we suggest to consider *P. depauperata* as a questionable NIS in the Mediterranean Sea, even though the spatio-temporal distribution of records suggests a westward expansion from the eastern Mediterranean Sea, which is compatible with a NIS with Indo-Pacific affinity.

***Distribution in the study area***. Reported for Taranto in organically enriched environments (Borghese *et al.,* 2023). The present data allow us to extend the distribution of this species to the area of Santa Caterina di Nardò, in far less impacted environments. The species was previously known for the Eastern Mediterranean Sea (Dağli & Çinar, 2009), reaching westwards to Malta (Katsanevakis *et al.,* 2020). The present data together with those reported by Borghese *et al.* (2023) suggests that it is expanding towards the western Mediterranean Sea.

Family **Syllidae** Grube, 1850

***Syllis similisunzima*** San Martín, Lucas & Hutchings, 2023 (Fig. 6)

***Material examined***. Brindisi, Adriatic Sea (40.34544° N; 18.35873° E), 0.5 m, on plastic buoys colonised by *Mytilus galloprovincialis* Lamarck, 1819, 23 February 2023: 11 specimens; same locality and environment, 08 May 2023: 12 specimens.

***Description***. Largest complete individual 10.6 mm long for 66 chaetigers, 0.68 mm maximum width, 1.81 mm length at the 10th chaetiger. Prostomium oval, approximately 1½ times wider than long, with four bright red eyes. Anterior pair of eyes oval, slightly larger than the posterior one, posterior pair rounded (Fig. 6b), eye-spots absent. Palps basally fused, approximately 1½ longer than the prostomium. Antennae, dorsal cirri and tentacu-

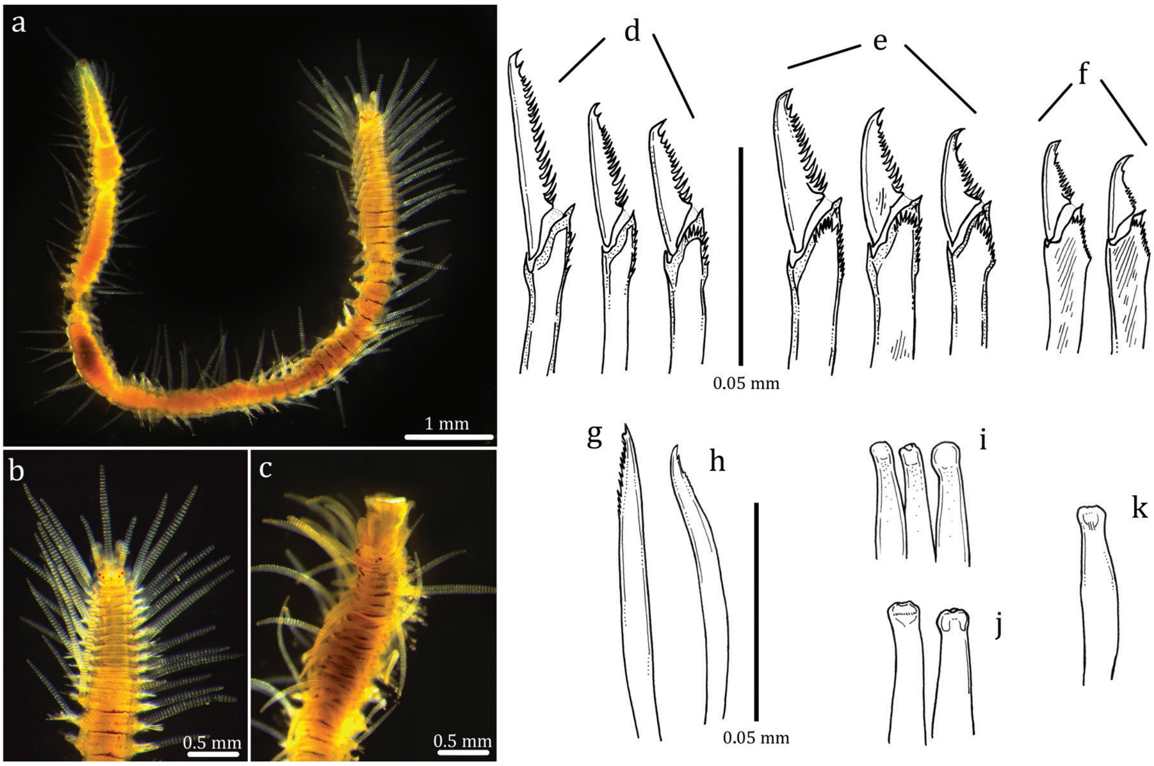

***Fig. 6:*** *Syllis similisunzima* from the port of Brindisi; a) complete specimen; b) detail of the anterior part; c) individual with partially everted pharynx; d) compound chaetae, anterior chaetigers; e) compound chaetae, midbody chaetigers; f) compound chaetae, posterior chaetigers; g) dorsal simple chaeta; h) ventral simple chaeta; i) aciculae, anterior chaetigers; j) aciculae, midbody chaetigers; k) acicula, posterior chaetiger.

lar cirri moniliform, with short, wide, rectangular articles with paired glandular inclusions heavily stained by Rose Bengal; the number of the articles close to the basis of the cirrus is difficult to estimate because the articles are very short and very close. Median antenna with 27-36 articles, lateral antennae with 18-23 articles, approximately 2/3 the length of the median one. Dorsal tentacular cirri with 25-36 articles, ventral tentacular cirri with 17-22 articles. Dorsal cirri alternating between longer and thicker, and shorter and slender; 1st, 3rd, 4th, 5th, 8th, 9th, 11th, 12th dorsal cirri longer, 2nd, 6th, 7th, 10th dorsal cirri shorter, after the 12th chaetiger there is a regular alternation between longer and shorter cirri. Longer dorsal cirri with up to 45 articles, shorter dorsal cirri with up to 25 articles. Dorsal cirri in the first 8-12 chaetigers distinctly thicker than the remaining ones, slightly flattened. Ventral cirri slightly longer than the parapodium, not articulated.

Anterior parapodia supported by 3 sub-equal aciculae with rounded tip (Fig. 6i), with up to 6 compound chaetae (Fig. 6d). Midbody parapodia supported by 2 aciculae of different thickness with rounded tip (Fig. 6j), with 4-5 compound chaetae (Fig. 6e). Posterior parapodia supported by one single acicula with rounded tip (Fig. 6k), with 4-5 compound chaetae (Fig. 6f) and, in the posterior-most chaetigers, one dorsal (Fig. 6g) and one ventral chaeta (Fig. 6h). Compound chaetae heterogomph falcigers, showing a pronounced dorso-ventral gradation in size of blades; blades bidentate, with secondary tooth smaller than the primary tooth, much smaller in posterior parapodia, with a fine, regular serration along the ventral edge, almost unnoticeable in ventral-most, posterior parapodia (Fig. 6d-f). Dorsal and ventral simple chaetae of similar thickness; dorsal simple chaeta strongly bidentate, with concave edge subdistally serrated, ventral simple chaeta indistinctly bidentate, with smooth concave edge (Fig. 6g, h).

Pharynx on 9-10 chaetigers, not slender, with a very large sub-terminal tooth, and a series of around 10 thick papillae around the opening (Fig. 6c). Proventriculum slightly shorter than the pharynx, on 4-6 chaetigers, with 50 muscle cell rows. Pygidium rounded, with dorsal anus, two anal cirri with around 30 articles. Live colour pale yellowish to pale tan on the anterior part, more translucent with the bright yellow gut content clearly visible in the median and posterior parts. Large individuals with 3 thin transverse brown stripes across each chaetiger; after the 12th chaetiger, the posterior-most stripe becomes darker and much more marked, while the other two stripes tend to disappear; stripes completely disappear after the 20th-25th chaetiger (Fig. 6a). Small individuals with shorter, thinner dorsal cirri, and only two transverse stripes, disappearing after the proventriculum.

***Molecular data***. COI sequences were obtained for two specimens (GenBank accession numbers: OR795519-OR795520). The sequences had a 91% identity with *Syllis* sp. from Korea on GenBank (accession number: ON312495), and a 99-100% identity with *Typosyllis* sp. sequences from California deposited in BOLD (sequence IDs: DISA454-18.COI-5P, DISA455-18.COI-5P, DISA456-18.COI-5P, DISA480-18.COI-5P). The latter taxon is clearly conspecific with the material from Brindisi, and the pictures on BOLD show a good correspondence of the colour pattern. Even though these individuals were not identified at the species level, these data confirm the Indo-Pacific affinity of this species.

***Remarks***. The individuals from the port of Brindisi show a good correspondence with the recently described *S. similisunzima* from Australia in all morphological characters, with the exception of eye-spots, which are present in the type material from Australia (San Martín *et al.*, 2023) and absent in all the examined individuals from Brindisi. Molecular data are not available from the type locality of this species, but the correspondence with sequences of an unidentified *Typosyllis* from California confirms the Indo-Pacific affinity of the population of Brindisi. The available evidence indicates that the Brindisi specimens as well as the specimens from California belong to *S. similisunzima*. However, the molecular characterisation of specimens from the type locality in Australia is necessary. Among Mediterranean species, this species can be easily identified by the combination of the distinctive colour pattern (which is often noticeable in ethanol-fixed material), the very large, sub-terminal pharyngeal tooth, the thicker anterior cirri and the aciculae with rounded tip throughout the body. The most similar Mediterranean species are *Syllis prolifera* Krohn, 1852 and *Syllis vivipara* Krohn, 1869, as both share with *S. similisunzima* the aciculae with rounded tip and a large, sub-terminal pharyngeal tooth; however, both species lack thicker anterior cirri and the distinctive colour pattern, even if sometimes live *S. prolifera* can show thin dark stripes along the posterior edge of parapodia (J. Langeneck, *pers. obs.*); moreover, *S. vivipara* has unidentate chaetae, while in *S. prolifera* the secondary tooth is almost as large as the primary one and becomes more distinct in posterior parapodia. The position of the pharyngeal tooth is shared with *Syllis pectinans* Haswell, 1920, which however has a different pigmentation pattern, only unidentate falcigers, and posterior aciculae with bent tip; the thicker anterior cirri are shared with *Syllis krohnii* (Ehlers, 1864), another heavily pigmented species, but in this case too chaetal features and aciculae are quite different.

***Distribution in the study area***. Only known for the port of Brindisi, on artificial substrates (plastic buoys densely colonised by mussels). In this environment, *S. similisunzima* is the dominant species, and was found with an abundant population in both winter and late spring. This is the first record of the species in the Mediterranean Sea.

## Discussion

The present data about non-indigenous polychaetes along the Salento Peninsula confirm the important role of this area for the study of early stages of biological invasions, already highlighted for other taxa (Di Martino & Stancanelli, 2021; Toso & Musco, 2023). Overall, fifteen NIS were found along the Salento Peninsula during one year of sampling; out of these species, *Syllis similisunzima* is here reported for the first time in the Mediterranean Sea, *Lepidonotus tenuisetosus* is reported for the first time in Italian waters, *Pseudonereis anomala* is reported for the first time for the Italian Peninsula, and *Dorvillea similis* is reported for the first time in the Ionian Sea. All the other species were already known for the study area, even though some of them, such as *Palola valida* and *Prionospio* cf. *depauperata*, have been recorded only recently (Gravina *et al.,* 2021; Borghese *et al.,* 2023) and are not known outside of the Ionian Sea, which suggests that their westward expansion is relatively recent and still ongoing. Conversely, some species, such as *Lumbrineris perkinsi* and *Branchiomma luctuosum* have been known along the Salento Peninsula since the 1980s (Gherardi *et al.,* 1985; Licciano & Giangrande, 2008), and are established in several of the sampled localities. Previous data on non-indigenous species identified the Taranto area as a bioinvasion hotspot, due to the co-occurrence of commercial port, touristic marinas, and aquaculture (Mastrototaro *et al.,* 2004; Tempesti *et al.,* 2020a). The present study highlighted a rather more complex situation, with the possible presence of additional invasion routes along the Salento Peninsula. While several species were found off Taranto in this study, or were reported by other studies (Giangrande *et al.*, 2008; Borghese *et al.,* 2023), some species (*L. tenuisetosus*, *P. valida*, *S. similisunzima*) are seemingly restricted to the eastern coast of Salento. Interestingly, all these species have Indo-Pacific affinity and are probably Lessepsian invaders. Their occurrence along the eastern coast is possibly due to maritime traffic between the port of Brindisi and the coasts of Greece and Albania, and by secondary traffic with the adjacent touristic marinas. As typical for non-indigenous species, most records refer to heavily anthropised environments and artificial substrates; only a few species showed the capability to colonise less impacted environments. In particular, all records of *Prionospio* cf. *depauperata* in the study area refer to coarse sand or gravel bottoms, characterised by very limited human impact; however, the species is also known from sediments under fish cages in the Gulf of Taranto (Borghese *et al.,* 2023). Another interesting case regards the invasive *B. boholense*, reported from both artificial substrates in port environments, where the species had already been widely reported, and natural substrates in slightly impacted environments. Unlike in other areas (Langeneck *et al.,* 2020; Fernández-Romero *et al.,* 2021), the congeneric species *B. luctuosum* did not show the same ability to colonise environments outside of ports. As already observed in the Ligurian Sea (Dragičević *et al.,* 2019; Ragazzola *et al.,* 2021), *D. similis* showed a higher abundance in port environments, but it was also found on natural rocky bottoms, among coralline algae; *P. anomala* was instead found mostly on natural substrates outside of port and marinas.

Out of the fifteen species reported, two (*Neopseudocapitella brasiliensis* and *Nereis* aff. *agulhana*) are still considered questionable, in agreement with Langeneck *et al.* (2020), despite the examination of additional material. In particular, the examined individuals of *N.* aff. *agulhana* show some differences towards the redescription based on type material (Villalobos-Guerrero *et al.,* 2022), but this might be due to the very small size of the sampled specimens, and molecular data, in the absence of sequences assigned to this species in public databases, did not allow us to solve the issue. The absence of molecular data did not allow us to identify with certainty the specimens identified as *Hydroides dianthus*. This species was regarded as native in the Mediterranean Sea by Sun *et al.* (2017) and subsequently excluded from non-indigenous polychaetes by Langeneck *et al.* (2020). However, Grosse *et al.* (2021) reported the co-occurrence in a Mediterranean port of both the allegedly native lineage and a lineage with Caribbean affinity identified by Sun *et al.* (2017); while more complete data on the distribution of these lineages are still missing, we think that *H. dianthus* should be precautionarily considered cryptogenic in the Mediterranean Sea. All other species are here regarded as confirmed non-indigenous species, even if in some cases molecular data suggest that more detailed studies comparing Mediterranean specimens with specimens from the type locality might be needed to ascertain their identity.

The present work allowed us to obtain molecular data (COI and 16S) for eleven out of fifteen species; in the case of all species, except *B. boholense*, *B. luctuosum* and *P. anomala*, these are the first molecular data from the Mediterranean Sea. Molecular data confirm the identity of *B. boholense*, *B. luctuosum*, *L. perkinsi*, and *N. setosa*. In some cases, the situation is more complicated. The sequences of *D. similis* obtained in this work do not match the available sequences assigned to this species, but the correspondence with an unidentified polychaete from the Red Sea confirms their Lessepsian origin. A similar case regards *S. similisunzima*, which perfectly corresponds to an unidentified Indo-Pacific species of *Syllis*. In the case of *P. anomala*, we identified a good correspondence with sequences of *Pseudonereis* sp. from Crete, but the identity with the other sequences identified with this taxon, originating from Australia, is low. In all these cases, molecular data from the type localities are needed to ascertain or disprove the identity of these specimens. Lastly, the sequences of *L. tenuisetosus*, *N.* aff. *agulhana*, *P. valida* and *Prionospio* cf. *depauperata* show a rather low identity with all polychaete sequences available on public databases. The first three taxa are completely missing from both BOLD and GenBank, while the only available COI sequence of *P. depauperata* is the closest match for the sequences from Salento, but the identity is around 81%, and it clearly belongs to a different species. The use of molecular data and genetic databases for the identification of organisms is affected by the lack of sequences assigned to several taxa, the limited geographical coverage and the presence of errors of attribution. While attribution errors and low quality of the data are mostly a matter of quality of the process of genetic characterisation, and can be addressed, to a certain extent, after the upload of the sequences (Goudey *et al.*, 2022), the absence of some species in public databases and the limited geographical coverage depend on limits and unevenness in the research effort and can further affect the interpretation of molecular data. This is particularly true for studies dealing with non-indigenous polychaetes (Lavrador *et al.,* 2023), and more complete data, as well as critical revisions based on integrative taxonomy techniques, are needed to solve several uncertainties pertaining to the identity and origin of these taxa. In fact, in the present study molecular data allowed us to confirm the identity of a minor part of the annelids assayed, seemingly leaving more questions than answers; this should however be seen as an effect of the incompleteness of the available libraries, rather than as a shortcoming of the approach followed. In fact, the molecular data provided here might represent a useful contribution to further taxonomic studies, as well as a useful tool for comparing results of future metabarcoding studies focusing on non-indigenous species.

The current data for the Salento Peninsula show that non-indigenous polychaetes, and in particular Lessepsian ones, are currently spreading westwards and northwards, experiencing a hitherto unknown success in Italian waters, probably fostered by the current trend of water warming, and by milder winter conditions. Some species (as *D. similis* and *L. perkinsi*) are already widespread along the whole Italian coastline, and their absence from some areas might be an artifact of a limited sampling effort; however, other species, such as *L. tenuisetosus*, *P. valida*, *P. anomala* and *Prionospio* cf. *depauperata*, are probably in an initial phase of their spread in Italian waters. In any case, a careful monitoring of these species, and detailed data on their spread along the Italian Peninsula, are crucial to investigate their impact on native assemblages and identify and implement mitigation measures.

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