

## Mediterranean Marine Science

Vol 25, No 3 (2024)

Mediterranean Marine Science



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doi: [10.12681/mms.37998](https://doi.org/10.12681/mms.37998)

#### To cite this article:

TOSO, A., PUTIGNANO, M., FUMAROLA, L. M., BARICHE, M., GIANGRANDE, A., MUSCO, L., PIRAINO, S., & LANGENECK, J. (2024). A revised inventory of Annelida in the Lebanese coastal waters with ten new aliens for the Mediterranean Sea. *Mediterranean Marine Science*, 25(3), 715–731. <https://doi.org/10.12681/mms.37998>

## A revised inventory of Annelida in the Lebanese coastal waters with ten new aliens for the Mediterranean Sea

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Contributing Editor: Sarah FAULWETTER

Received: 03 June 2024; Accepted: 29 October 2024; Published online: 13 November 2024

### Abstract

Sampling activities conducted in the Tyre Coast Nature Reserve, southern Lebanon, to study the marine annelid fauna of the area, allowed for the revision of the diversity of this group in Lebanon. We particularly focused on non-indigenous species (NIS), which were characterised from morphological and molecular points of view. A total of 116 taxa were collected; 10 species are reported here for the first time in the Mediterranean Sea, while 6 species with presumable Indo-Pacific affinity are likely new to science. Seventy-three taxa were native, while 43 taxa were NIS; among the latter, 24 species are reported for the first time in Lebanon. Molecular data were obtained for 28 NIS, representing the first data from the Mediterranean Sea for 23 of them. Non-indigenous annelids occurring along the coast of Lebanon mainly have Indo-Pacific affinity. However, molecular data highlighted inconsistencies between the sampled material and the sequences available in public repositories, suggesting the widespread occurrence of species complexes in these taxa. These results suggest that further research on Indo-Pacific annelids is needed to understand their diversity patterns and invasion pathways. Additionally, the large number of new records of annelids in Lebanese waters indicate that further studies are needed to explore their diversity in comparison with neighbouring Mediterranean regions.

**Keywords:** Polychaetes; Non-indigenous species; Biological invasions; Climate change.

### Introduction

Marine annelids represent a key component of benthic communities in terms of both biomass and abundance, comprising 35-70% of the macroinvertebrates occurring in benthic environments (Tselepides *et al.*, 2000; Díaz-Castañeda & Reish, 2009; Kuk-Dzul *et al.*, 2012; Musco, 2012). Marine annelids play crucial roles in marine ecosystems (Hutchings, 1998), contributing to nutrient cycling (Davey & Watson, 1995) and habitat modification through bioconstruction and bioturbation (Palomo & Iribarne, 2000; Bertocci *et al.*, 2017). They represent important links in trophic webs, also being the main food source for several commercially relevant species (Winkler & Debus, 1996; Squires, 2003). Moreover, marine annelids can be employed as bioindicators of environ-

mental alterations, or as environmental bioremediators in polluted marine habitats, such as aquaculture facilities (Dean, 2008; Giangrande *et al.*, 2005; 2020). Additionally, several species have been used as models for studies in genetics, regeneration, reproductive biology, development, evolution, and neurobiology (Özpolat *et al.*, 2021). Lastly, some species hold significant economic value, being sold as ornamental species for aquariums or as bait for recreational and professional fishing (Gambi *et al.*, 1994; Arduini *et al.*, 2023). Annelids are one of most diverse groups of marine invertebrates distributed worldwide and exhibiting a wide range of sizes, shapes, and colours. This group includes more than 20,000 described species, with a comparable number of potentially undiscovered ones (Appeltans *et al.*, 2012; Capa & Hutchings, 2021). These organisms can thrive in almost all natural

and anthropogenic substrates and can be found in extreme environments, from shallow waters to the abyssal depths (Rouse *et al.*, 2022). Despite their abundance and importance in marine ecosystems, scientific knowledge on marine annelids is scarce compared to other taxa and uneven across different countries and geographical sectors. In many parts of the world, the diversity of this group remains largely unexplored, even though research focusing on the biodiversity of polychaetes is steadily increasing in several countries (Ribas & Hutchings, 2015; Pamungkas & Glasby, 2019; Al-Kandari *et al.*, 2019; Leon *et al.*, 2019; Ribeiro *et al.*, 2023; Shah *et al.*, 2023).

The Mediterranean Sea is an area of historical interest for the taxonomy of polychaetes. Numerous historical works detail the morphology and diversity of Mediterranean polychaetes (e.g., Grube, 1840; 1855; 1863; Claparède, 1864; 1868; Ehlers, 1864). These works have been extensively referenced by later authors. Overall, the diversity and biogeography of Mediterranean polychaetes are relatively well-known, especially when compared to other regions (Coll *et al.*, 2010). However, this knowledge remains incomplete, particularly regarding poorly explored habitats (Langeneck *et al.*, 2019a) or specific geographical areas. This knowledge gap is especially pronounced in the Levantine Sea, where recent, comprehensive checklists have been provided only for Türkiye (Çinar *et al.*, 2014; 2024) and Cyprus (Rousou *et al.*, 2023) and still lacking for most of the neighbouring countries, including Lebanon, where 143 species have been reported so far (Laubier, 1966; Zibrowius & Bitar, 1981; Bitar & Kouli-Bitar, 1998; 2001; Aguado & San Martin, 2007; Badreddine & Çinar *in* Fortič *et al.*, 2023).

The Levantine sub-basin, including the Mediterranean coasts of Egypt, Israel, Lebanon, Syria, Türkiye, and Cyprus, is highly oligotrophic and characterised by higher temperature and salinity compared to the rest of the Mediterranean Sea (D'Ortenzio & Ribera d'Alcala, 2009; Pastor *et al.*, 2020). Furthermore, the Levantine basin is directly connected to the Red Sea via the Suez Canal, resulting particularly susceptible to biological invasions. It is the first area of the Mediterranean Sea where Lessepsian species and other thermophilic non-indigenous species (NIS) are likely to settle (Zenetos *et al.*, 2012). In fact, benthic and nektonic assemblages in this region have been significantly altered by NIS, often resulting in cascading effects on fisheries and other human activities (Hooper *et al.*, 2005; Galil, 2007; Carpentieri *et al.*, 2009; Çinar, 2012). Additionally, the dominant surface circulation pushes water north-northeast (Fernández *et al.*, 2005), facilitating the spread of the species entering from the Suez Canal. Given these dynamics, studies conducted in the Levantine Sea are crucial for the early identification of new Lessepsian immigrants. These studies could help to understand the impact of NIS on native communities and inform future management strategies for the broader Mediterranean Sea.

A significant challenge in studying NIS, particularly in groups with complex and tangled taxonomy, is the scarcity and unreliability of molecular data, which are crucial for their rapid identification and detection, also in envi-

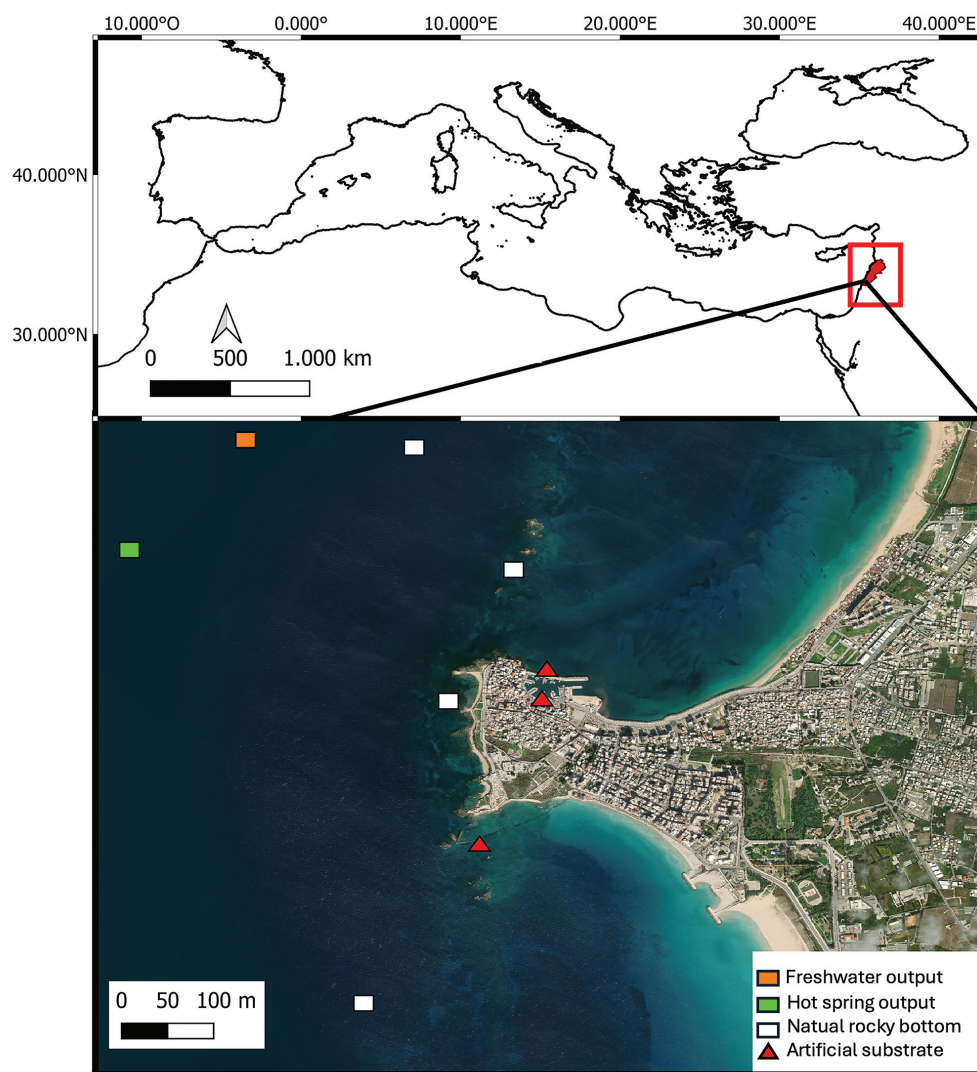
ronmental DNA samples (Simmons *et al.*, 2016; Uchii *et al.*, 2016; Holman *et al.*, 2019). However, barcoding libraries of marine invertebrates are currently inadequate for these tasks, especially for marine annelids. According to Lavrador *et al.* (2023), even when unreliable entries are considered, molecular data are available for only about one fourth of the non-indigenous marine annelids recorded in European waters. In the Mediterranean Sea, barcoding data are available for only a few species, primarily habitat-forming species of the families Sabellidae and Serpulidae (Del Pasqua *et al.*, 2018; Grosse *et al.*, 2021; Langeneck *et al.*, 2024a). Despite representing the majority of non-indigenous polychaetes reported in the Mediterranean Sea, most Errantia species remain unexplored from a molecular standpoint.

To address these gaps, we conducted a series of sampling activities in the Tyre Coast Nature Reserve, located on the southern coast of Lebanon. This work aims to enhance the understanding of annelid diversity in Lebanese waters by providing descriptions and molecular data for the most prevalent NIS found during the field activities of the project.

## Materials and Methods

Fresh material was collected during two expeditions in the Tyre Coast Nature Reserve, Lebanon (Fig. 1) in June and November 2022. Samples were obtained by scuba diving and snorkelling at depths ranging from 0 to 38 meters, collecting small rocks, different algae and sediments. Additionally, material from the port of Tyre was sampled by scraping the surface of docks and mooring lines. Overall, samples were collected from nine stations representing various habitats, including vermetid reefs, coralligenous formations, coralline algae, soft bottoms, non-calcareous algae, and freshwater and hot water springs (Table 1).

Samples were sorted alive using a stereomicroscope, and annelids were isolated and photographed (when-ever possible) using an Olympus Tough TG-9 camera. Specimens were then fixed in either 70% or 96% ethanol. Further identification, biometric measurements and photographs of the analysed material were obtained at the BioForIU Laboratory of the DiStEBA at 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. We were also able to examine additional material sampled in Lebanon between 2002 and 2003 and housed at the Senckenberg Natural History Museum in Frankfurt (Germany), which was included in the data obtained in the present work. Detailed descriptions and taxonomic remarks are provided in Supplementary Material 1.



**Fig. 1:** Map of the study area Tyre national reserve of Lebanon, with the sampling stations.

**Table 1.** Main characteristics of the sampling stations. The number of samplings refers to the number of sampling events in the locality at different period. NIS Non-Indigenous Species.

Environment	Coordinates	Depth	Habitat type	N° samplings	N° of NIS
Freshwater output	33.33295° N, 35.18800°E	35	Hard substrate with coralligenous formation and freshwater output	1	21
Hot spring water output	33.29111° N, 35.16858° E	38	Hard substrate with pre- coralligenous formation and hot water output	2	2
Artificial substrate	33.27395° N, 35.19532° E	0-1	Mooring lines in the port	2	7
	33.27538° N, 35.19548° E	0-5	Artificial wall covered by coralline algae and photophilous algae/ vermetid reef	4	22
	33.26500° N, 35.19059° E	0-4	Artificial hard substrate with photophilous algae	1	12
Natural rocky bottoms	33.28366° N, 35.19549°E	0-6	Hard substrate with coralline algae and photophilous algae/soft bottoms	3	16
	33.27453° N, 35.19079° E	0-3	Hard substrate with coralline algae and photophilous algae	2	4
	33.26061° N, 35.18264°E	20	Hard substrate with photophilous algae	2	4
	33.33019° N, 35.18475° E	15	Hard substrate with photophilous algae/ soft bottoms	1	6

A small tissue fragment (1 mm<sup>3</sup> or less) was detached from selected individuals of NIS, ensuring that no diagnostic features were damaged or removed, and used for DNA extraction. Total genomic DNA was extracted using the salting-out protocol (Aljanabi & Martínez, 1997) with modifications detailed in Furfaro *et al.* (2022). The Folmer fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using annelid-specific primers POLYLCO (5'-GAYTATWTCAACAAATCATAAAGATATTGG-3') and POLYHCO (5'-TAMACTTCWGGGTGACCAARAATCA-3') (Carr *et al.*, 2011), and universal degenerate primers jGLCO1490 (5'-TITCIACIAAYCAYAARGAYATTGG-3') and jGH2198 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3') (Geller *et al.*, 2013), while the mitochondrial gene coding for the large subunit of the ribosomal DNA (16S) was amplified using the universal primers 16Sar-L (5'-CGCCTGTTTAAACAAAACAT-3') and H3080 (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi *et al.*, 1991) and the annelid-specific primers 16SANNF (5'-GCGGTATCCTGACCGTRCWAAGGTA-3') and 16SANNR (5'-TCCTAAGCCAACATCGAGGTGCCAA-3') (Sjölin *et al.*, 2005). Polymerase chain reaction (PCR) amplifications were carried out in 20 µL solutions using 4 µL of FIREPol<sup>®</sup> Master Mix (Solis BioDyne), 0.1 µM of each primer and 1 µL of template DNA. The PCR profile for 16S rDNA was set as follows: initial denaturing step at 94 °C for 5 min, 35 cycles of denaturing at 94 °C for 30 s, annealing at 45-50 °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 amplification, the PCR profile was: initial denaturing step at 94 °C for 5 min, 40 cycles of denaturing at 94 °C for 30 s, annealing at 42-45 °C for 45 s, and extending at 72 °C for 1 min, with a final extending step at 72 °C for 7 min. A negative control was included in each reaction. PCR products were sent to Macrogen Europe for purification and sequencing. Sequences were compared with the ones deposited in GenBank and BOLD Systems through the Basic Local Alignment Search Tool (BLAST: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Identification Engine tool ([https://www.boldsystems.org/index.php/IDS\\_IdentificationRequest](https://www.boldsystems.org/index.php/IDS_IdentificationRequest)), respectively. Sequences were uploaded to BOLD and GenBank; GenBank accession numbers are included in Supplementary Material 1.

Comparative analyses were performed using sequences from both the Mediterranean/Eastern Atlantic and the Indo-Pacific region for the genera *Loimia*, *Palola*, *Subadyte* and *Trypanosyllis*. Gene sequences were aligned with ClustalX v. 2.1 (Larkin *et al.*, 2007), and alignments were edited using the program BIOEDIT v. 7.2.5 (Hall, 1999). The program jModelTest 2.1.6 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike, 1974). For all markers and genera, the most suitable substitution model was the generalised time reversible (GTR, Tavaré, 1986) +I +G. Sequences were concatenated using SequenceMatrix v. 1.8 (Vaidya *et al.*, 2010). Bayesian consensus phylogenetic trees based on the concatenated markers were

constructed using MrBayes 3.2 (Ronquist *et al.*, 2011). Four replicate runs were carried out with three Markov chains per run for 1 x 10<sup>6</sup> generations. The chain was sampled every 1,000 generations to obtain 2,000 sampled trees. The first 500 sampled trees (25%) were discarded as burn-in, with the remaining 1,500 trees used to estimate the Bayesian consensus, posterior probability (PP) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, which should reach a value < 0.01 at the end of the analysis (Ronquist *et al.*, 2011).

## Results

A total of 116 taxa belonging to 27 families were sampled and identified to the lowest possible taxonomic level (Table 2). This work adds 73 species and 4 new families to the annelid fauna of Lebanon: Capitellidae, Golfingidae, Iphionidae, and Sigalionidae. The most diverse families were Syllidae with 35 species, Nereididae with 11 species, Eunicidae with 7 species, and Hesionidae with 6 species.

Native species represented 62% (73 taxa) of the species collected, while the remaining 38% (43 taxa) were NIS (including potential alien species, questionable and cryptic species). 24 non indigenous species are herein reported for the first time for Lebanon: among them, 14 are NIS already known in the other areas of the Mediterranean Sea, whilst 10 species (8.5%) are new records for the Mediterranean waters, namely *Loimia* cf. *ingens* (Grube, 1878), *Ceratonereis tentaculata* Kinberg, 1865, *Lumbrineris annulata* Hartmann-Schröder, 1960, *Neanthes* cf. *unifasciata* (Willey, 1905), *Subadyte* cf. *albanyensis* Hanley & Burke, 1990, *Syllis* cf. *crassicirrata* (Treadwell, 1925), *Syllis* cf. *picta* (Kinberg, 1866), *Hololepidella nigropunctata* (Horst, 1915), *Trypanosyllis* cf. *cristoboi* Álvarez-Campos, Taboada, San Martín, Leiva & Riesgo, 2018, and *Trypanosyllis luzonensis* (Pillai, 1965) (Fig. 2). Through the re-examination of samples obtained in 2002-2003 and deposited in the Senckenberg Natural History Museum, we were able to backdate the first occurrence of 10 species for Lebanon (*Ceratonereis tentaculata*, *Neanthes* cf. *unifasciata*, *Syllis* cf. *crassicirrata*, *Trypanosyllis luzonensis*, *Dorvillea similis*, *Oenone fulgida*, *Nereis persica*, *Branchiosyllis exilis*, *Branchiomma bairdi* and *Branchiomma boholense*). In addition, 6 taxa (*Dorvillea* sp. 1, *Lysidice* sp. 1, *Nereis* sp. 1, *Pseudobranchiomma* sp. 1, *Sabellomma* sp. 1, and *Syllis* sp. 1) were identified as undescribed species with potential Indo-Pacific affinity. In particular, *Lysidice* sp. 1 morphologically corresponds to individuals from the Red Sea misidentified by Ben-Eliahu (1976) as *Lysidice collaris* Grube, 1869, the identity of which was recently clarified by Kurt-Şahin & Çınar (2009). Conversely, morphological and distributional data suggest an Indo-Pacific affinity for the remaining new species. *Pseudobranchiomma* sp. 1 and *Sabellomma* sp. 1 belong to genera until now unknown in the Mediterranean Sea (Putignano *et al.*, 2023), but well known in tropical waters of the Austral

**Table 2.** List of the annelids collected in the project Blue-Tyre (2022); \* represent the species for which the DNA amplification was unsuccessful. In bold the new NIS for Lebanon, and in bold with \* the new NIS for the Mediterranean Sea. The term NIS encompasses the concept of alien, potential alien, questionable and cryptogenic species.

Family	Species	Introduction year	Status	Marker (COI/16S)
<b>Amphinomidae</b>	<b><i>Eurythoe complanata</i> (Pallas, 1766)</b>	2022	<b>Alien</b>	<b>COI</b>
	<i>Hermodice carunculata</i> (Pallas, 1766)		<b>Native</b>	
	<i>Linopherus canariensis</i> Langerhans, 1881	1965	<b>Alien</b>	<b>COI</b>
<b>Aphroditidae</b>	<i>Pontogenia chrysocoma</i> (Baird, 1865)		<b>Native</b>	
<b>Aspidosiphonidae</b>	<i>Aspidosiphon muelleri</i> Diesing, 1851		<b>Native</b>	
<b>Capitellidae</b>	<i>Dasybranchus gajolae</i> Eisig, 1887		<b>Native</b>	
	<b><i>Leiochrides australis</i> Augener, 1914</b>	2022	<b>Alien</b>	*
	<b><i>Notomastus aberans</i> Day, 1957</b>	2022	<b>Alien</b>	*
<b>Chrysopetalidae</b>	<i>Chrysopetalum debile</i> (Grube, 1855)		<b>Native</b>	
<b>Cirratulidae</b>	<i>Aphelochaeta</i> sp. 1		<b>Native</b>	
	<i>Cauleriella</i> sp. 1		<b>Native</b>	
	<i>Protocirrinieris</i> sp. 1		<b>Native</b>	
	<b><i>Timarete cf. caribous</i> (Grube, 1859)</b>	2022	<b>Alien</b>	*
	<i>Timarete punctata</i> (Grube, 1859)	1965	<b>Alien</b>	*
	<i>Timarete</i> sp. 1		<b>Native</b>	
<b>Dorvilleidae</b>	<b><i>Dorvillea similis</i> (Crossland, 1924)</b>	2002	<b>Alien</b>	<b>COI + 16S</b>
	<i>Dorvillea</i> sp. 1	2022	<b>Potential alien</b>	<b>COI + 16S</b>
<b>Eunicidae</b>	<i>Eunice vittata</i> (Delle Chiaje, 1828)		<b>Native</b>	
	<i>Leodice antennata</i> Savigny in Lamarck, 1818	2002	<b>Alien</b>	<b>COI</b>
	<i>Lysidice margaritacea</i> Claparède, 1868		<b>Native</b>	
	<i>Lysidice</i> sp. 1	2022	<b>Potential alien</b>	*
	<i>Lysidice unicornis</i> (Grube, 1840)		<b>Native</b>	
	<i>Palola siciliensis</i> (Grube, 1840)		<b>Native</b>	
	<b><i>Palola valida</i> (Gravier, 1900)</b>	2022	<b>Alien</b>	<b>COI</b>
<b>Golfingiidae</b>	<i>Golfingia vulgaris</i> (de Blainville, 1827)		<b>Native</b>	
	<i>Nephasoma rimicola</i> (Gibbs, 1973)		<b>Native</b>	
	<i>Phascolion convestitum</i> Sluiter, 1902		<b>Native</b>	
<b>Hesionidae</b>	<i>Hesione pantherina</i> Risso, 1826		<b>Native</b>	
	<i>Hesiospina aurantiaca</i> (M. Sars, 1862)		<b>Native</b>	
	<i>Leocrates claparedii</i> (Costa in Claparède, 1868)		<b>Native</b>	
	<i>Oxydromus flexuosus</i> (Delle Chiaje, 1827)		<b>Native</b>	
	<i>Oxydromus pallidus</i> Claparède, 1864		<b>Native</b>	
	<b><i>Podarkeopsis capensis</i> (Day, 1963)</b>	2022	<b>Questionable</b>	
<b>Iphionidae</b>	<b><i>Iphione muricata</i> (Lamarck, 1818)</b>	2022	<b>Alien</b>	<b>COI</b>
<b>Lumbrineridae</b>	<b>*<i>Lumbrineris annulata</i> Hartmann-Schröder, 1960</b>	2022	<b>Alien</b>	<b>COI</b>
	<b><i>Lumbrineris perkinsi</i> Carrera-Parra, 2001</b>	2022	<b>Alien</b>	<b>COI</b>
<b>Nereididae</b>	<b>*<i>Ceratonereis tentaculata</i> Kinberg, 1865</b>	2002	<b>Alien</b>	<b>COI</b>
	<i>Composetia costae</i> (Grube, 1840)		<b>Native</b>	

Continued

Table 2 continued

Family	Species	Introduction year	Status	Marker (COI/16S)
	<i>Neanthes acuminata</i> (Ehlers, 1868)		Native	
	<i>Neanthes rubicunda</i> (Ehlers, 1868)		Native	
	<b>*<i>Neanthes cf. unifasciata</i> (Willey, 1905)</b>	2002	Alien	16S
	<i>Nereis</i> sp. 1	2022	Potential alien	16S
	<i>Nereis perivisceralis</i> Claparède, 1868		Native	
	<b><i>Nereis persica</i> Fauvel, 1911</b>	2002	Alien	*
	<i>Nereis rava</i> Ehlers, 1868		Native	
	<i>Platynereis dumerilii</i> (Audouin & Milne Edwards, 1833)		Native	
	<i>Pseudonereis anomala</i> Gravier, 1899	1965	Alien	*
<b>Oeonidae</b>	<i>Arabella geniculata</i> (Claparède, 1868)		Native	
	<b><i>Oenone fulgida</i> (Lamarck, 1818)</b>	2003	Alien	COI
<b>Onuphidae</b>	<i>Aponuphis bilineata</i> (Baird, 1870)		Native	
<b>Opheliidae</b>	<i>Arandia polyophthalma</i> Kükenthal, 1887		Native	
	<i>Polyophthalmus pictus</i> (Dujardin, 1839)		Native	
<b>Orbiniidae</b>	<i>Protoaricia oerstedii</i> (Claparède, 1864)		Native	
<b>Paraonidae</b>	<i>Cirrophorus nikebianchii</i> Langeneck, Barbieri, Maltagliati & Castelli, 2017		Native	
	<i>Paradoneis cf. perdidoensis</i> (McLelland & Gaston, 1994)		Native	
<b>Phascolosomatidae</b>	<i>Phascolosoma stephensoni</i> (Stephen, 1942)		Native	
<b>Phyllodocidae</b>	<i>Nereiphylla cf. lugens</i> (Ehlers, 1864)		Native	
	<i>Nereiphylla rubiginosa</i> (de Saint-Joseph, 1888)		Native	
	<i>Phyllodoce madeirensis</i> Langerhans, 1880		Native	
	<i>Pterocirrus macroceros</i> (Grube, 1860)		Native	
<b>Polynoidae</b>	<i>Harmothoe impar</i> (Johnston, 1839)		Native	
	<b>*<i>Hololepidella nigropunctata</i> (Horst, 1915)</b>	2022	Alien	*
	<i>Lepidonotus clava</i> (Montagu, 1808)		Native	
	<b>*<i>Subadyte cf. albanyensis</i> Hanley &amp; Burke, 1990</b>	2022	Alien	COI + 16S
	<i>Subadyte pellucida</i> (Ehlers, 1864)		Native	
<b>Sabellidae</b>	<i>Pseudobranchiomma</i> sp. 1	2002	Potential alien	COI
	<i>Branchiomma boholense</i> (Grube, 1878)	1965	Alien	COI
	<i>Branchiomma bairdi</i> (McIntosh, 1885)	2002	Alien	COI
	<i>Sabellomma</i> sp. 1	2022	Potential alien	16S
<b>Serpulidae</b>	<i>Hydroides dianthus</i> (Verrill, 1873)	1998	Cryptogenic	*
	<i>Serpula concharum</i> Langerhans, 1880		Native	
	<i>Spirobranchus</i> aff. <i>tetraceros</i> (Schmarda, 1861)	1965	Alien	*
	<i>Vermiliopsis infundibulum</i> (Philippi, 1844)		Native	
<b>Sigalionidae</b>	<i>Fimbriosthenelais minor</i> (Pruvot & Racovitza, 1895)		Native	
	<i>Pholoe inornata</i> Johnston, 1839		Native	
	<i>Pisione guanache</i> San Martín, López & Núñez, 1999	2022	Cryptogenic	COI
<b>Spionidae</b>	<i>Prionospio ergeni</i> Dagli & Çınar, 2009		Native	
	<i>Pseudopolydora pulchra</i> (Carazzi, 1893)		Native	

Continued

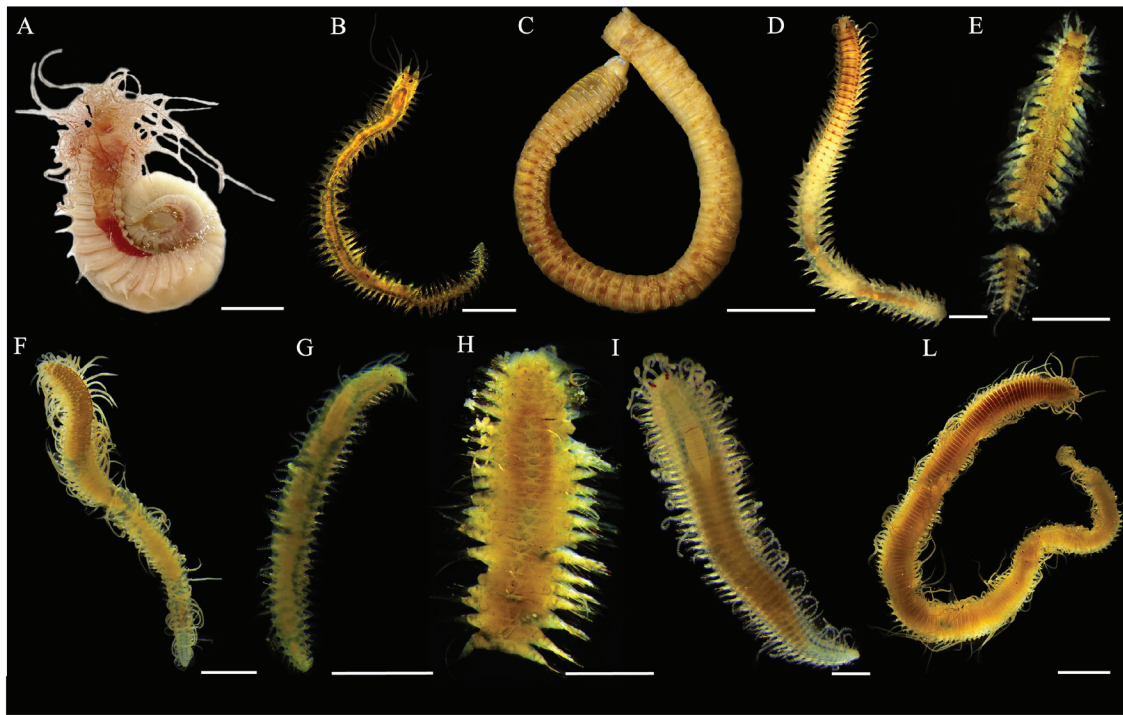
Table 2 continued

Family	Species	Introduction year	Status	Marker (COI/16S)
	<i>Spio filicornis</i> (Müller, 1776)		Native	
Syllidae	<i>Amblyosyllis madeirensis</i> Langerhans, 1879		Native	
	<i>Branchiosyllis exilis</i> (Gravier, 1900)	1999	Questionable	COI
	<i>Eusyllis kupfferi</i> Langerhans, 1879	1999	Alien	*
	<i>Eusyllis lamelligera</i> Marion & Bobretzky, 1875		Native	
	<b><i>Exogone africana</i> Hartmann-Schröder, 1974</b>	2022	Alien	*
	<i>Exogone brevi antennata</i> Hartmann-Schröder, 1959	1999	Alien	*
	<i>Exogone verugera</i> (Claparède, 1868)		Native	
	<i>Haplosyllis granulosa</i> (Lattig, San Martín & Martin, 2007)		Native	
	<i>Myrianida convoluta</i> (Cognetti, 1953)		Native	
	<i>Myrianida prolifera</i> (O.F. Müller, 1788)		Native	
	<i>Odontosyllis fulgurans</i> (Audouin & Milne Edwards, 1833)		Native	
	<i>Opisthosyllis brunnea</i> Langerhans, 1879		Native	
	<i>Paraehlersia dionisi</i> (Núñez & San Martín, 1991)		Native	
	<i>Proceraea aurantiaca</i> Claparède, 1868		Native	
	<i>Pseudosyllis brevipennis</i> Grube, 1863		Native	
	<i>Salvatoria neapolitana</i> (Goodrich, 1930)		Native	
	<i>Sphaerosyllis pirifera</i> Claparède, 1868		Native	
	<i>Sphaerosyllis</i> sp. 1		Native	
	<i>Syllis armillaris</i> (O.F. Müller, 1776)		Native	
	<i>Syllis variegata</i> Grube, 1860		Native	
	<i>Syllis</i> sp. 1	2022	Potential alien	COI
	<i>Syllis columbretensis</i> (Campoy, 1982)		Native	
	<i>Syllis corallicola</i> Verrill, 1900		Native	
	<b>*<i>Syllis</i> cf. <i>crassicirrata</i> (Treadwell, 1925)</b>	2002	Alien	COI
	<i>Syllis truncata cryptica</i> (Ben-Eliahu, 1977)		Native	
	<i>Syllis gerundensis</i> (Alós & Campoy, 1981)		Native	
	<i>Syllis prolifera</i> Krohn, 1852		Native	
	<b>*<i>Syllis</i> cf. <i>picta</i> (Kinberg, 1866)</b>	2022	Alien	COI
	<i>Syllis pulvinata</i> (Langerhans, 1881)		Native	
	<b><i>Syllis schulzi</i> (Hartmann-Schröder, 1960)</b>	2022	Cryptogenic	*
	<i>Synmerosyllis lamelligera</i> (Saint-Joseph, 1887)		Native	
	<b>*<i>Trypanosyllis luzonensis</i> (Pillai, 1965)</b>	2002	Alien	16S
	<b>*<i>Trypanosyllis</i> cf. <i>crisoboi</i> Álvarez-Campos, Taboada, San Martín, Leiva &amp; Riesgo, 2018</b>	2022	Alien	COI + 16S
	<i>Trypanosyllis zebra</i> (Grube, 1860)		Questionable	COI
Terebellidae	<b>*<i>Loimia</i> cf. <i>ingens</i> (Grube, 1878)</b>	2022	Alien	COI
	<i>Nicolea venustula</i> (Montagu, 1819)		Native	
	<b><i>Streblosoma pseudocomatum</i> Lezzi &amp; Giangrande, 2019</b>	2022	Cryptogenic	COI

hemisphere (Grube, 1878; Wehe & Fiege, 2002; Nogueira *et al.*, 2010; Capa & Murray, 2016). Specimens morphologically matching *Pseudobranchiomma* sp. 1 and

*Sabellomma* sp. 1 were found in samples collected in the Red Sea (M. Putignano, *pers. obs.*) and molecular data reveals *Pseudobranchiomma* sp. 1 is genetically close to





**Fig. 2:** Pictures of the 10 species herein reported for the first time in the Mediterranean Sea. A. *Loimia* cf. *ingens*, scale-bar: 10 mm; B. *Ceratonereis tentaculata*, scale-bar: 2 mm; C. *Lumbrineris annulata*, scale-bar: 1 mm; D. *Neanthes* cf. *unifasciata*, scale-bar: 4 mm; E. *Subadyte* cf. *albanyensis*, scale-bar: 1 mm; F. *Syllis* cf. *crassicirrata*, scale-bar: 2 mm; G. *Syllis* cf. *picta*, scale-bar: 2 mm; H. *Hololepidella nigropunctata*, scale-bar: 1 mm; I. *Trypanosyllis* cf. *cristoboi*, scale-bar: 0.5 mm; L. *Trypanosyllis luzonensis*, scale-bar: 3 mm.

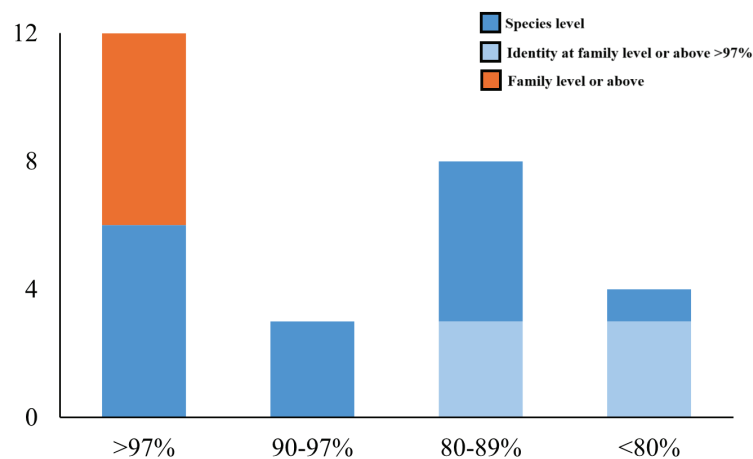
an undescribed species from Australia (Capa & Murray, 2016). *Syllis* sp. 1 is identical to an undescribed *Syllis* species reported by Abd-Elnaby & San Martín (2011) for the Egyptian Mediterranean Sea and it is also close to the Australian *Syllis boggemanni* San Martín, Álvarez-Campos & Hutchings, 2017 (San Martín *et al.*, 2017); *Nereis* sp. 1 shows similarities with the Red Sea species *Nereis ethiopiae* Day, 1965 in the number and distribution of paragnaths, but the colour pattern and the shape of parapodia do not match (Day, 1965; Ben-Eliahu, 1975); lastly, *Dorvillea* sp. 1 is morphologically close to *D. similis*, but shows a distinctive live colour, different jaw apparatus and is clearly different from the molecular point of view. These species and their potential allochthonous origin for the Mediterranean Sea will be the object of a dedicated taxonomic paper.

For 28 of the alien species found in this study, including 3 of the potentially undescribed species (*Dorvillea* sp. 1, *Pseudobranchiomma* sp. 1, *Syllis* sp. 1), we were able to obtain DNA barcoding data based on the mitochondrial marker COI, and for 8 species, including 3 of the potentially undescribed species (*Dorvillea* sp. 1, *Nereis* sp. 1, *Sabellomma* sp. 1), we obtained 16S sequences (Table 2; Supplementary Material 1). The comparison of the COI sequences obtained with the online repositories highlighted a rather variable situation; for 6 species we were able to highlight a >97% identity with sequences assigned to the species level, while for 3 the identity is between 90 and 97%, for another 8 between 80 and 89%, and for 4, below 80% (Fig. 2). It is noteworthy that, including sequences identified at the family level or below, the number of species with a match above 97% of sequence iden-

tity increases to 12; all such cases were retrieved in the categories below 90% sequence identity (Fig. 3). 16S sequences were overall much less abundant, and a sequence identity above 98% was retrieved only in the cases of *Syllis* cf. *crassicirrata* (again, with an unidentified Syllidae), and *Trypanosyllis* cf. *krohnii*. Detailed descriptions and molecular characterisation of the non-indigenous species are reported in Supplementary Material 1, together with comments and remarks on their specific identity and on taxonomic issues surrounding their identity.

## Discussion

In the present study we investigated the diversity of the annelids of the Lebanon waters, adding 73 new species for Lebanon. Among these, 10 are new NIS for the Mediterranean Sea, and 14 are NIS already known in other areas of the basin. Some previous NIS records are now considered misidentifications of species later reported from the same area; for instance, the old records of *Branchiomma cingulatum* (Grube, 1870) and *Cirriformia semicineta* (Ehlers, 1905) most likely correspond to the species currently identified as *B. bohoolense* and *T. punctata*, respectively (Knight-Jones *et al.*, 1991; Çinar, 2007). The updated checklist for Lebanon now includes 215 species from 37 families (Supplementary Material 2), with 27% (N=58) non-indigenous taxa. Nevertheless, the number of annelid NIS reported from Lebanon is close to that reported from neighbouring countries (Türkiye: 72; Cyprus: 51) and confirms the trend of a higher diversity of annelid NIS in the eastern Mediterranean Sea (Rousou



**Fig. 3:** Percentual identity of the COI sequences obtained in this study. Blue: highest identity with sequences identified at the species level; pale blue: sequences for which a higher identity was retrieved with sequences identified at the family level or above; orange: highest identity with sequences identified at the family level or above.

*et al.*, 2023; Çinar *et al.*, 2024). The majority of the annelid NIS reported from Lebanon have Indo-Pacific affinity and have presumably entered the Mediterranean Sea through the Suez Canal (Supplementary material 1). This accounts for most of the species reported for the first time in the Mediterranean Sea, except for *L. annulata*, which is currently known only from the southeastern Pacific Ocean only (Carrera-Parra, 2006). Some species, such as *P. guanche* and *L. canariensis*, have warm Atlantic affinity, and their introduction is probably due to shipping or a natural expansion through Gibraltar (Moreira *et al.*, 2010). A recent work excluded *B. bairdi* from the Mediterranean area (Del Pasqua *et al.*, 2018). However, reports of this species in the Mediterranean Sea suggest its introduction at a later time (Cepeda & Rodríguez-Flores, 2018; Abd-Elnaby, 2020; Langeneck *et al.*, 2020; Fortić *et al.*, 2023). Individuals of *B. bairdi* found in this study show minor morphological differences with the redescription by Del Pasqua *et al.* (2018), which might be assigned to ontogenetic variation, and a 3% distance at the level of COI sequences. Even though a 3% distance was generally identified as the threshold value between intraspecific and interspecific distances (Hebert *et al.*, 2003), several studies on annelids highlighted the absence of a general barcoding gap (Kvist, 2016), and distances as high as 6-8% on the COI gene often correspond to incompletely sorted lineages on more conserved mitochondrial and nuclear genes (Elgetany *et al.*, 2020; Langeneck *et al.*, 2022; Teixeira *et al.*, 2022). The divergence retrieved between Lebanese *B. bairdi* and the other sequences available for this species might represent a clue of geographical structuring, and thus of a different origin of the Lebanese population, or of incipient speciation, but additional samples from a wider geographical range are needed to test these hypotheses.

The current study allowed us to obtain molecular data for 28 of the 43 annelid NIS found in Lebanon. For 5 species, these are the first data ever obtained, while for 23 species these are the first molecular data from the Mediterranean Sea. In most cases, the identity between the sequences obtained in this work and those deposited

in GenBank and BOLD for the same taxon was rather low (75-87%; Supplementary material 1). The only exceptions are *P. guanche*, showing a 100% identity with sequences from the type locality (Gonzalez *et al.*, 2017), and *E. complanata*, which corresponds to a lineage of this species complex that is widespread in the Indo-Pacific region (Barroso *et al.*, 2010). In some cases (*C. tentaculata*, *L. antennata*, *S. cf. albanyensis*, *S. cf. crassicirrata*), a high sequence identity was found with polychaetes from the Red Sea that had not been identified to the species level. This paradoxically allows us to confirm the Lessepsian origin but not to back the species identification, as already observed for this group (Langeneck *et al.*, 2024a).

The low identity of the remaining species sequenced compared to the sequences deposited in GenBank and BOLD is likely due to the limited number of sequences from the Red Sea, representing the probable origin of these organisms, and suggests the widespread occurrence of species complexes in these Indo-Pacific taxa. Based on the comparison between the new COI data and those available in literature, despite morphological homogeneity, *L. ingens* includes 3 divergent lineages with an average identity around 87%, *P. valida* includes at least 2 lineages in the Mediterranean Sea, with 93% identity, *D. similis* has a 95% identity with specimens from Italy and the Red Sea, and *O. fulgida* has a 85% identity with allegedly conspecific specimens from Hawaii (see Supplementary Material 1). Despite having been reported from the Red Sea (Wehe & Fiege, 2002; Wehe, 2006), several Indo-Pacific taxa identified in this work have type locality in the Philippines (e.g., *L. ingens*, *T. luzonensis*, *T. cristoboi*), Indonesia (e.g., *H. nigropunctata*), the Hawaii Islands (e.g., *C. tentaculata*, *S. cf. crassicirrata*) or Australia (e.g., *S. albanyensis*, *S. aff. tetraceros*). Even though the examined material shows a good correspondence from the morphological point of view, molecular data together with the relatively distant type locality suggest that the individuals from Lebanon do not correspond to the species *sensu stricto* and might rather represent western Indian vicariants, which are probably still undescribed. In particular, the Red Sea is considered an endemism hotspot

for several animal groups (DiBattista *et al.*, 2016; Bogorodsky & Randall, 2019) and its fauna is overall scarcely known from the molecular point of view. It is then possible that the species sequenced from Lebanon, the barcoding of which does not perfectly match Indo-Pacific sequences, might be either endemic to the Red Sea or to the western Indian Ocean. In this case, expansion into the Mediterranean Sea through the Suez Canal is the most likely explanation for their occurrence. This hypothesis is strongly supported by the finding of *Pseudobranchiomma* sp. 1 and *Sabellomma* sp. 1 in material collected from the Red Sea, since these genera were unknown for the Mediterranean Sea (Putignano *et al.*, 2023). The same inference, albeit with more caution, could be made for four of the probably undescribed species (*Dorvillea* sp. 1, *Lysidice* sp. 1, *Nereis* sp. 1, *Syllis* sp. 1): while it is true that the Mediterranean annelid fauna is still partially undescribed, and new species are described rather frequently (e.g., Lezzi & Giangrande, 2018; Enrichetti *et al.*, 2022; Çinar *et al.*, 2022; Çinar & Erdoğan-Dereli, 2023), all these genera are overall well-known in the area, and these species show clear discrepancies with Mediterranean congeneric ones, while being closer to Indo-Pacific species. Similarly, the examined individual of *T. krohnii* did not correspond to the native lineages of the species complex but rather to a widespread lineage known until now from Australia and Brazil (Álvarez-Campos *et al.*, 2017), suggesting that cryptic non-indigenous lineages might be detected in allegedly native species as well.

Genetic and morphological discrepancies between Indo-Pacific taxa and material reported from the eastern Mediterranean Sea are not limited to annelids (Langeneck *et al.*, 2024a; present data), but have been recorded also in other comparatively better-known taxa, such as molluscs (Albano *et al.*, 2021; 2024), fishes (Azzurro *et al.*, 2015; Deidun *et al.*, 2016), macroalgae (Golo *et al.*, 2023), and decapod crustaceans (Galil, 2001; Rifi *et al.*, 2023). Although current alien species checklists tend to only include species with allegedly certain identification, the lack of detailed studies on the diversity and biology of species with Indo-Pacific affinity both in the eastern Mediterranean and in the Red Sea reflects into a high degree of taxonomic uncertainties, which are generally not addressed by non-indigenous species checklists (Albano *et al.*, 2024). Uncertain identifications presented as certain can potentially have an impact on the evaluation of spread capabilities and environmental risks posed by non-indigenous species (Golo *et al.*, 2023). At the same time, the option to lump all species affected by some taxonomic uncertainty under the ‘wastebasket’ category of ‘questionable’ species increases the risk of underestimating the relevance of these species for native assemblages (Albano *et al.*, 2024; Langeneck *et al.*, 2024b). For this reason, recent works decided to stress taxonomic uncertainties surrounding non-indigenous species while still considering them as non-indigenous for monitoring and management purposes (Albano *et al.*, 2021; 2024; Langeneck *et al.*, 2024b). This approach is in our opinion particularly suited for groups which have not been the object of detailed taxonomic revision in the western In-

do-Pacific, leading to a general uncertainty surrounding their identity; as observed by Langeneck *et al.* (2024a) and confirmed by the present observations, in several polychaete groups the lack of taxonomic expertise and updated revision based on integrative taxonomy has as a consequence the fact that reconstructing the origin of a non-indigenous annelid is currently easier than assigning it to a described or undescribed taxon. While assigning species surrounded by taxonomic doubts to the alien species category is often considered controversial, in our opinion it is the only suitable way to address the current biodiversity crisis affecting the eastern Mediterranean Sea, at least for groups that are affected by the widespread occurrence of species complexes and limited taxonomic expertise, such as annelids (Nygren, 2014; Hutchings & Kupriyanova, 2018; Capa & Hutchings, 2021).

Aside from the new records and data on annelid NIS, this study shows that the native marine biota of Lebanon is largely unknown, at least concerning the annelid fauna. While molluscs and fish are overall well studied and have been the subject of comprehensive checklists (Crocetta *et al.*, 2013; Bariche & Fricke, 2020), data about most of the invertebrate groups along the Lebanese coast are scanty. During this study, 40 native annelid species, corresponding to 25% of all native species recorded, were found for the first time in Lebanon. Moreover, the study highlighted the occurrence of taxonomic uncertainties in some of the native species identified; this is the case of the phyllodocid *Nereiphylla* cf. *lugens*, and of the paraonid *Paradoneis* cf. *perdidensis*. *Nereiphylla lugens* was described for the Adriatic Sea by Ehlers (1864) and put into synonymy with *Nereiphylla paretii* Blainville, 1828 by Fauvel (1923), despite being a much smaller species, comparable in size to *N. rubiginosa*, from which it can be separated based on the shape of the cirri and the live colour pattern; however, the identification of Lebanese specimens as *N. lugens* should be backed by the study of topotypic material. *Paradoneis perdidensis* is a pedomorphic paraonid species originally described from the Caribbean region (McLelland & Gaston, 1994) and subsequently reported from the Canary Islands (Monterroso *et al.*, 2012) and the Mediterranean Sea (Langeneck *et al.*, 2019b); considering the extremely simple anatomy and the few characters available for this species, it is likely that eastern Atlantic and Mediterranean records of *P.* cf. *perdidensis* should in fact be referred to different, native species, but also in this case further investigations are needed. The total number of species currently known from this area is approximately one fourth of those reported from other Mediterranean countries (Castelli *et al.*, 2008; Bakalem *et al.*, 2020; Rousou *et al.*, 2023; Çinar *et al.*, 2024). Considering the limited sampling effort and the relatively homogeneous environments explored, this number is likely an underestimate of the actual annelid diversity in Lebanon. This is confirmed by the re-examination of samples obtained in 2002-2003 and deposited in the Senckenberg Natural History Museum, which allowed us to backdate the introduction of 10 of the species newly reported for Lebanon. Four of these species (*C. tentaculata*, *N.* cf. *unifasciata*, *S. crassicirrata*, *T. luzon-*

*ensis*) are here reported for the Mediterranean Sea for the first time. Yet, according to the museum samples examined, their first occurrence in the Mediterranean Sea dates back to more than twenty years ago. It is evident that the lack of monitoring and taxonomic studies in the eastern Mediterranean Sea is affecting the identification of NIS and the reconstruction of their distribution, invasion pathways and spread.

Further studies and additional monitoring programs are needed to unravel the diversity of annelids in Lebanese waters and to ascertain the distribution of NIS, considering its proximity to the Suez Canal. The abundance of NIS in coastal benthic and nektonic assemblages is typical of the eastern Mediterranean Sea (Stergiou, 1988; Cardecia *et al.*, 2018). However, there is increasing evidence of a westward expansion of Indo-Pacific thermophilic species and a gradual replacement of native species by NIS, even in scarcely anthropized environments (Azzurro & D'Amen, 2022; Langeneck *et al.*, 2024a). In this context, eastern Mediterranean countries represent important study areas to understand future scenarios for the rest of the Mediterranean Sea. Therefore, international programs aimed at monitoring and raising awareness on environmental problems in the region are crucial. These programs will help obtain new data on poorly studied organisms and their interactions with the native assemblages, and to plan and implement mitigation activities.

## Acknowledgements

The project *Blue Tyre. Local Partnership For Sustainable Marine And Coastal Development* (AID 012314/01/6) is developed by the Municipality of Tricase (Italy) and the Municipality of Tyre (Lebanon). This project is funded by Italian Agency for Development Cooperation (Promotion of Territorial Partnerships and implementation of 2030 Agenda). Project partners and collaborators: Municipality of Tricase, Municipality of Tyre, Cooperation in World Territories (CTM), Department of Biological and Environmental Sciences and Technologies (Di.S.Te.B.A.) - University of Salento, Naturalia (Civic Museum of Natural History of Salento), Tyre Coast Nature Reserve (TCNR), Magna Grecia Mare Association, Tyros Lag, Lebanon Diving Center. This project was partially funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union-NextGenerationEU. Project code CN\_00000033, Concession Decree No. 1034. Of 14 June 2022 adopted by the Italian Ministry of University and research, CUP D33C22000960007, Project title "National Biodiversity Future Center - NBFC". Moreover, we thank Ekin Tilic (Senckenberg Natural History Museum), who allowed us to examine material sampled in Lebanon in the years 2002-2003, and Giulia Furfaro (University of Salento) for her support in the setting of the molecular characterisation protocol.

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## Supplementary Material

The following supplementary material is available for this article:

**Supplementary Material 1.** Non-indigenous annelid species sampled in Lebanon, with material examined, updated descriptions of the Mediterranean specimens, molecular data and taxonomic remarks.

**Table S1.** Comparison between different descriptions of *Subadyte albanyensis* and *S. pellucida* from the Mediterranean Sea.

**Fig. S1:** *Leodice antennata* (Savigny, 1818). A. Almost complete live specimen; B. Close up to prostomium and peristomium; C. Maxillary apparatus everted, frontal view; D-J. Chaetae, line drawings (D, E. Adult compound chaetae; F. Juvenile bidentate compound chaetae; G. Juvenile tridentate compound chaetae; H. Pectinate chaeta; I. Aciculae; J. Acicular chaeta); K-M. Parapodia, line drawings (K. Parapodium of the 1<sup>st</sup> chaetiger; L. Mid-body parapodium; M. Posterior parapodium).

**Fig. S2:** *Palola valida* (Gravier, 1899). A. Maxillae 1 and 2; B. Mandible; C. Maxillae 3 and 4, line drawings; D, E. Compound chaetae, line drawings; F-H. Parapodia, line drawings (F. Parapodium of the 1<sup>st</sup> chaetiger; G. Mid-body parapodium; H. Posterior parapodium).

**Fig. S3:** Bayesian phylogenetic tree of Mediterranean and Indo-Pacific individuals belonging to the genus *Palola* based on concatenated 16S rDNA and COI sequences (accession numbers: [16S/COI]). *Lumbrineris perkinsi* is used as outgroup. Node values represent Bayesian posterior probabilities, specimens in bold were sequenced in the present study.

**Fig. S4:** *Lumbrineris annulata* Hartmann-Schröder, 1960. A. Incomplete specimen; B. Maxillae 2, 3 and 4; C. Maxillary apparatus, dissected; D-G. Chaetae, line drawings (D, E. Compound multidentate hooded hooks; F. Long-hooded simple multidentate hook; G. Short-hooded simple multidentate hook); H-J. Parapodia, line drawings (H. Anterior parapodium; I. Mid-body parapodium; J. Posterior parapodium).

**Fig. S5:** *Lumbrineris perkinsi* Carrera-Parra, 2001. A. Almost complete specimen; B. Maxillary apparatus, line drawings; C. Mandible, line drawings; D-G. Chaetae, line drawings (D, E. Limbate chaetae; F. Compound multidentate hooded hook; G. Simple multidentate hooded hook); H-J. Parapodia, line drawings (H. Anterior parapodium; I. Mid-body parapodium; J. Posterior parapodium).

**Fig. S6:** *Oenone fulgida* (Lamarck, 1818). A. Anterior end (prostomium and peristomium); B. Prostomial antennae after partial dissection; C. Maxillary apparatus; D. Close up of maxillary apparatus; E. Mandible; F, G. Sub-acicular hooks, line drawings; H, I. Parapodia, line drawings (H. Anterior parapodium; I. Mid-body parapodium).

**Fig. S7:** *Hololepidella nigropunctata* (Horst, 1915). A. Specimen incomplete; B. Close up of prostomium and peristomium; C. Parapodium, line drawing; D. Elytra, line drawing; E-I. Chaetae, line drawings: E-G. Neurochaetae (E. ventral neurochaeta; F. intermediate neurochaeta; G. dorsal neurochaetae); H, I. Notochaetae.

**Fig. S8:** *Subadyte albanyensis* Hanley & Burke, 1990. A. Full specimen; B. Close up to prostomium and peristomium; C. Pygidi-



um; D. Parapodium, line drawing; E. Elytra, line drawing; F-J. Chaetae, line drawings: F-H. Neuropodial chaetae; I, J. Notopodial chaetae.

**Fig. S9:** *Subadyte pellucida* (Ehlers, 1864) from coralligenous assemblages. A-C. Live colour patterns (A. Checkered/striated pattern; B. Absence of coloration pattern; C. Striped pattern); D-H. Chaetae, line drawing; D-F. Neuropodial chaetae; G, H. Unidentate notopodia chaetae.

**Fig. S10:** *Subadyte pellucida* (Ehlers, 1864) from shallow water biocenosis. A-D. Live colorations (A. Longitudinally striped pattern; B. Close-up to prostomium and peristomium; C. Checkered pattern; D. Close-up to prostomium and peristomium); E. Elytra, line drawing; F-K. Chaetae, line drawing; F-H. Neuropodial chaetae; I-K. Bidentate notopodial chaetae.

**Fig. S11:** Bayesian phylogenetic tree of Mediterranean individuals belonging to the genus *Subadyte* based on concatenated 16S rDNA and COI sequences (accession numbers: [16S/COI]). *Iphione muricata* is used as outgroup. Node values represent Bayesian posterior probabilities, all sequences were obtained in the present study.

**Fig. S12:** *Pisione guanche* San Martín, López & Núñez, 1999. A. Full specimen; B. Close-up to prostomium and peristomium, dorsal view; C. Close-up to prostomium and peristomium, ventral view; D. Parapodium, line drawing; E, F. Chaetae, line drawing; E. Compound chaetae; F. Simple chaetae.

**Fig. S13:** *Ceratonereis tentaculata* Kinberg, 1865. A. Full specimen; B. Pharynx and paragnaths disposition, dorsal view; C. Pharynx and paragnaths disposition, ventral view; D-F. Parapodia, line drawing (D. Anterior parapodium; E. Mid-body parapodium; F. Posterior parapodia); G-P. Chaetae, line drawing; G. Anterior parapodia homogomph compound spiniger notochaetae; H. Anterior parapodia homogomph compound spiniger neurochaetae I. Anterior parapodia heterogomph compound spiniger neurochaetae; J. Mid-posterior parapodia homogomph compound spiniger notochaetae; K. Mid-posterior parapodia homogomph compound spiniger neurochaetae; L. Mid-posterior parapodia heterogomph compound spiniger neurochaetae; M. Anterior parapodia heterogomph supra-acicular falciger neurochaetae; N. Anterior parapodia heterogomph sub-acicular falciger neurochaetae; O. Mid-posterior parapodia homogomph falciger notochaetae; P. Mid-posterior parapodia heterogomph falciger neurochaetae.

**Fig. S14:** *Neanthes cf. unifasciata* (Willey, 1905). A. Specimen incomplete; B. Close-up to prostomium and peristomium; C. Close-up anterior portion of the body; D-E. Parapodia, line drawing (D. Anterior parapodium; E. Mid-body parapodium); F-I. Chaetae, line drawing; F. Anterior parapodia homogomph compound spiniger notochaetae; G. Anterior parapodia hemigomph compound spiniger neurochaetae; H. Anterior parapodia heterogomph falciger neurochaetae; N. Mid-body parapodia heterogomph falciger neurochaetae.

**Fig. S15:** *Nereis persica* Fauvel, 1911. A. Full specimen; B. Pharynx and paragnaths disposition, ventral view; C. Pharynx and paragnaths disposition, frontal view; D-F. Parapodia, line drawing (D. Anterior parapodium; E. Mid-body parapodium; F. Posterior parapodia); G-P. Chaetae, line drawing; G. Anterior parapodia homogomph compound supra-acicular spiniger neurochaetae; H. Anterior parapodia heterogomph compound sub-acicular spiniger neurochaetae; I. Posterior parapodia heterogomph compound spiniger chaetae; J. Posterior parapodia homogomph compound spiniger chaetae; K. Anterior parapodia heterogomph supra-acicular falciger neurochaetae; L. Anterior parapodia heterogomph sub-acicular falciger neurochaetae; M. Mid-body parapodia heterogomph falciger chaetae; N. Mid-body parapodia homogomph falciger chaetae; O. Posterior parapodia heterogomph falciger chaetae; P. Posterior parapodia homogomph falciger chaetae.

**Fig. S16:** *Branchiosyllis exilis* (Gravier, 1900). A. Complete specimen; B. Close-up of prostomium and peristomium; C. Close-up of posterior end with developing stolon; D-I. Chaetae, line drawing (D. Compound chaetae of anterior parapodia; E. Compound chaetae of mid-body parapodia; F. Compound chaetae and unguiae (Ug) of posterior parapodia; G. Anterior parapodia aciculae; H. Mid-body parapodia aciculae; I. Posterior parapodia aciculae).

**Fig. S17:** *Syllis cf. crassicirrata* (Treadwell, 1925). A. Complete specimen; B. Close-up to anterior end; C-K. Chaetae, line drawing (C. Anterior parapodia compound chaetae; D. Mid-body parapodia compound chaetae; E. Posterior parapodia compound chaetae; F. Anterior parapodia aciculae; G. Mid-body parapodia aciculae; H, I. Posterior parapodia aciculae; J. Posterior parapodia ventral simple chaetae; K. Posterior parapodia dorsal simple chaetae).

**Fig. S18:** *Syllis cf. picta* (Kinberg, 1866). A. Full specimen, fixed material; B. Close-up to anterior end, alive specimen; C-I. Chaetae, line drawing; C. Anterior parapodia compound chaetae; D. Mid-body parapodia ypsiloid pseudo-compound (yps) and compound chaetae; E. Posterior parapodia compound chaetae; F. anterior parapodia aciculae; G. Mid-body parapodia aciculae; H. Posterior parapodia aciculae; I. Posterior parapodia ventral simple chaetae.

**Fig. S19:** *Trypanosyllis luzonensis* (Pillai, 1965). A. Full specimen; B. Close-up to anterior end; C-H. Chaetae, line drawing; C. Anterior parapodia compound chaetae; D. Mid-body parapodia compound chaetae; E. Posterior parapodia compound chaetae; F. Anterior parapodia aciculae; G. Mid-body parapodia aciculae; H. Posterior parapodia aciculae.

**Fig. S20:** *Trypanosyllis cf. cristoboi* Álvarez-Campos, Taboada, San Martín, Leiva & Riesgo, 2018. A. Complete specimen; B. Close-up to anterior end; C-H. Chaetae, line drawing; C. Anterior parapodia compound chaetae; D. Mid-body parapodia compound chaetae; E. Posterior parapodia compound chaetae; F. Anterior parapodia aciculae; G. Mid-body parapodia aciculae; H. Posterior parapodia aciculae.

**Fig. S21:** Bayesian phylogenetic tree of individuals belonging to the genus *Trypanosyllis* based on concatenated 16S rDNA and COI sequences (accession numbers: [16S/COI]). *Syllis prolifera* Krohn, 1852 is used as outgroup. Node values represent Bayesian posterior probabilities, specimens in bold were sequenced in the present study.

**Fig. S22:** *Loimia cf. ingens* (Grube, 1878). A. Full specimen, alive; B. Close-up to anterior end, alive specimen; C. Close-up to prostomium and peristomium, fixed material (arrows: branchial lobes); D. Pygidium, fixed material; E-H. Chaetae, line drawing; E. Notopodial limbate chaetae; F, G. Thoracic neuropodial uncini; H. Abdominal neuropodial uncini.

**Fig. S23:** Bayesian phylogenetic tree of individuals belonging to the genus *Loimia* from the Indopacific and European waters based

on COI sequences. *Streblosoma pseudocomatum* is used as outgroup. Node values represent Bayesian posterior probabilities, specimens in bold were sequenced in the present study.

**Fig. S24:** *Streblosoma pseudocomatum* Lezzi & Giangrande, 2019. A. Live specimen; B-E. Chaetae, line drawing: B. Notopodial limbate chaetae; C. Neuropodial uncini, lateral view; H. Neuropodial uncini, upper view.

**Fig. S25:** Comparison of radiolar tip and stylodes among *Branchiommma* K lliker, 1858 material analysed in this study. A-C. *Branchiommma boholense* (Grube, 1850) from Lebanon coasts (A. Anterior end and crown, fixed material; B Radiolar tip, fixed material; C. Close-up to macro- and micro-stylodes, fixed material); D-F. *Branchiommma boholense* (Grube, 1850) from Italian coasts (D. Medium size alive specimen, with macro- and micro-stylodes distinct; E. Small size alive specimen with stylodes in development; F. Close-up to macro-stylodes, frontal view); G-I. *Branchiommma bairdi* (McIntosh, 1885) from Lebanon coasts (G. Anterior end and crown, fixed material; B Radiolar tip, fixed material; C. Close-up to stylodes, fixed material). Ms. macro-stylodes; ms. micro-stylodes; st. stylodes.

**Fig. S26:** Comparison between radioles and uncini among *Branchiommma* K lliker, 1858 material analysed in this study. A-G. *Branchiommma boholense* (Grube, 1850) from Lebanon coasts: A. Radiole; B-G. Uncini, line drawings (B. Thoracic neuropodial uncini, lateral view; C. Thoracic neuropodia uncini, latero-frontal view; D. Anterior to middle abdomen notopodial uncini, lateral view; E. Anterior to middle abdomen notopodial uncini, frontal view; F. Posterior abdomen notopodial uncini, lateral view; G. Posterior abdomen notopodial uncini, frontal view); H-N. *Branchiommma bairdi* (McIntosh, 1885) from Lebanon coasts: H-M. Uncini, line drawings (H. Thoracic neuropodial uncini, lateral view; I. Thoracic neuropodia uncini, latero-frontal view; J. Anterior to middle abdomen notopodial uncini, lateral view; K. Anterior to middle abdomen notopodial uncini, frontal view; L. Posterior abdomen notopodial uncini, lateral view; M. Posterior abdomen notopodial uncini, frontal view); N. Radiole.

**Supplementary Material 2.** Complete checklist of marine annelids recorded off Lebanon, including reference for the first record, status as native or alien species, and year of the first record for alien species.