

## Article

# The First Record of *Marenzelleria neglecta* and the Spread of *Laonome xeprovala* in the Danube Delta–Black Sea Ecosystem

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**Abstract:** Biological invasions can have major impacts on freshwater and marine ecosystems. Therefore, it is vital that non-indigenous species are accurately identified and reported when potential or confirmed invasions occur. The present study reports the first occurrence of *Marenzelleria neglecta* (Annelida, Spionidae) and the spread of *Laonome xeprovala* (Annelida, Sabellidae) in the Danube Delta–Black Sea ecosystem. Spionidae is one of the most diverse families of annelid worms and is a dominant group in terms of the number of species that have been introduced to non-native areas, while the members of Sabellidae are among the most visible polychaetes commonly found in fouling communities and are colonizing new geographic areas. Based on 20 samples collected in 2021, we provide an overview of the distribution of the investigated species and possible arrival pathways for *Marenzelleria neglecta*. Specimens were identified based on morphological descriptions. Both species have invasive behaviour, colonizing large areas in relatively short time periods and reaching relatively high densities (*M. neglecta*—1400 ind.m<sup>−2</sup>; *L. xeprovala*—40 ind.m<sup>−2</sup>). Due to their distribution and high abundances, the biology and ecology of these species in the Danube River–Danube Delta–Black Sea system need to be investigated further in order to assess their impact on ecosystem structure and functioning.

**Keywords:** *Marenzelleria neglecta*; *Laonome xeprovala*; polychaeta; biological invasion; Danube Delta; Black Sea



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## 1. Introduction

European continental coastal waters are natural corridors of dispersal with continuously changing species communities, ecosystems [1], and native species [2–4]. Over recent centuries, the frequency at which human actions have facilitated the movement of species into habitats outside their natural range has markedly increased [5]. This has made the introduction of non-indigenous species (NIS) a major driver of biodiversity changes [6]. Some NIS may have economic consequences on fisheries and the tourism industry, causing irreversible damage to vessels, water pipes, piers, and other port and canalization infrastructure as fouling organisms [4], and a few may affect human health [7]. Therefore, monitoring for the presence of impacting species, assessment of the current phase of an invasion process, evaluation of the effects on native biota [1], and follow-up on the evolution of already established NIS and newly recorded ones to detect eventual sudden outbursts [8] are required for environmental management. Early interception by controlling potential pathways is probably the most efficient method for preventing future impacts on native ecosystems [9], and this largely depends on the correct identification, if possible, of species [10], which is sometimes problematic; misidentifications with native species or other NIS frequently occur [8].

Due to the threats posed by NIS, they are the subject of several legislative instruments, e.g., international agreements (i.e., the Convention on Biological Diversity, and the United

Nations Sustainable Development Goals), regulations (i.e., EU Regulation 1143/2014 [11]), European directives (MSFD, EC, 2008 [12]), and conservation strategies (EU Biodiversity Strategy to 2020 [13]) that aim to tackle biodiversity loss by preventing the spread of invasive alien species, prioritizing monitoring, early eradication, and long-term control. However, the principal piece of EU legislation governing the management of freshwater and coastal environments, the European Water Framework Directive (WFD), does not specifically mention NIS [14–17].

The Danube Delta–Black Sea system has always been a region of introduction of NIS into Romania and Ukraine, and their further distribution through the Southern Invasion Corridor, which links the basins of the Black and the North Seas. Over the last two decades, dozens of invertebrate species have been introduced and have naturalized over time [10,18,19].

The class Polychaeta is the most prominent taxonomic group within Annelida in terms of species richness, with about 100 families and over 12,000 valid species [20]. They are a major component of benthos, and their study is crucial to understand ecosystem structure and functioning [10,21–27]. Although members of some species are sensitive to pollution [28], many are opportunistic, withstanding high levels of perturbation [29], and have biological traits that allow NIS to overcome negative genetic effects of small founder populations, therefore promoting successful establishment and spread [30]. Members of NIS have a variety of attributes such as wide environmental tolerance, high genetic variability, short generation time, early sexual maturity, high reproductive capacity, and a broad diet [31]. Some opportunistic species of polychaetes have such traits and represent a dominant group among NIS in Europe. Of the more than 12,000 species of polychaetes [20,32] described in the world, 292 are NIS [33]. Out of them, nine occur on the Romanian Black Sea coast and adjacent areas [10].

The aim of this paper is to contribute to the growing knowledge of NIS in the Danube Delta–Black Sea ecosystem by (1) reporting on the presence of a new NIS, the polychaete *Marenzelleria neglecta* Sikorski and Bick, 2004, based on material collected in 2021; and (2) presenting an overview of the expansion of invasive *Laonome xeprovala* Bick, Bastrop, Kotta, Meißner, Meyer, and Syomin, 2018 (Sabellidae Latreille, 1825) along the Danube Delta from the first record of it in 2018 until 2021.

## 2. Materials and Methods

### 2.1. Area Description

Our research was conducted in the Danube Delta–Black Sea system, part of the Danube Delta Biosphere Reserve (DDBR). The Danube Delta is the largest wetland of the complex system pertaining to the Danube River and it forms a complex network of river channels, bays, lakes, sandy banks, and numerous marshes that contribute to improving the quality of Black Sea water, acting as a buffer zone for pollutants in the Danube [34]. The Danube is the second longest river in Europe, extending over 2860 km, and crossing or bordering the territories of ten Central and Eastern European countries before reaching the Black Sea [35]. The Danube's major branches are Sf. Gheorghe to the south (70 km), Sulina in the middle (64 km), and Chilia to the north (120 km), representing the border between Romania and Ukraine. The Musura Spit and Bay, at the Chilia distributary mouth, and Sakhalin Island, at the Sf. Gheorghe mouth, have rapidly developed in front of the large river discharge mouth [36] and exhibited a gradual transition from marine to a semi-enclosed brackish lagoons and later freshwater lakes [37].

The sediments of the Danube branches are composed mostly of sand, while artificial canals of meanders are composed of fine sand and clay [38,39].

### 2.2. Data Collection and Analysis

In June 2021, 20 stations were used from on board R/V “Istros” along the Danube branches and coastal lagoons of the Romanian Black Sea coast (Musura and Sakhalin). Field activities were conducted within the National CORE Program—project PN19200401. A

single 0.1 m<sup>2</sup> van Veen grab sample was taken at each station (Figure 1). Samples were washed with water from the sampling site using a 0.5 mm mesh sieve, and the retained organisms were fixed in 4% buffered water formalin. Density and biomass (as wet weight) were referred to one square meter (indv·m<sup>-2</sup>, g·m<sup>-2</sup>).



**Figure 1.** Sampling stations in the branches of the Danube and coastal lagoons of the Romanian Black Sea coast (Musura and Sakhalin) (green polygons ROSCI: MPA's).

The fauna, including polychaetes, were subsequently preserved in 70% ethanol for morphological and biometric analyses, which were carried out using a Zeiss SteREO Discovery.V8 stereomicroscope, an AxioLab.A1 transmitted light microscope, and an AxioVert.A1 inverted microscope with DIC technology. The identification of *Marenzelleria neglecta* specimens was carried out according to the keys published by Sikorski and Bick [40,41], and for *Laonome xeprovala* Bick et al. [42]. Unstained specimens were used for full-body and diagnostic character micrographs, taken with a Zeiss Axiocam 305 colour digital camera. Measurements were taken with Zeiss Image software (ZEN 2.5—blue edition) in the licensed measurement module. Plates were prepared using CorelDRAW 2017 software. The best-preserved and complete specimens (representative of the variability among individuals) were measured and analysed. All the material was deposited at the National Research Development Institute for Marine Geology and Geoecology—GeoEcoMar.

The spatial data distribution was projected using Ocean Data View, version 5.4.0 software [43] and QGIS v 3.24.1 (QGIS Development Team (2022). QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>, accessed on 31 March 2022). Species nomenclature was checked according to the World Register of Marine Species [44].

### 2.3. Morphological Examination

Taking into account that there are few external morphologic characters which are suitable for discrimination of *Marenzelleria* species, many authors recommend the use of genetic analysis to complement morphological analysis [Radase, Syomin]. We followed the methodology of Sikorski and Bick [40] regarding characters and measured body width (without parapodia) at chaetiger 10. The morphological characters of *Marenzelleria* species

are, in general, size-dependent [40]. Even if complete specimens were rarely found in samples, width was used as a size group parameter.

The following morphological characters were used for the species identification: VHH—first chaetiger with neuropodial hooded hooks; DHH—first chaetiger with notopodial hooded hooks; Br—last chaetiger with branchiae; NO—last chaetiger with nuchal organs. Furthermore, the arithmetic differences between above-mentioned morphometric variables (Br-VHH, Br-DHH, DHH-VHH), proposed as additional characteristics of the species, were used in the present study.

### 3. Results

#### 3.1. Benthic Communities

The sediments in Musura Bay and Sakhalin Lagoon were characterised by muddy (silty clay). On the Danube arms, the sediments were sand, except at stations P12 and P13, where mixed sediments with large amounts of shell debris dominated.

Representatives of 45 benthic species belonging to 11 taxonomic groups were found, with the Amphipoda being dominant in number of species and abundance, followed by Oligochaeta, Polychaeta, and Bivalvia, with a low representation of Porifera, Gastropoda, Cumacea, Mysida, Isopoda, Decapoda, and Insecta. Out of 45 species, 37 were recorded on the Danube branches and 12 in the Musura and Sakhalin coastal lagoons. In the coastal lagoons, most of the recorded species were marine, while in the Danube branches, freshwater and Ponto–Caspian forms were present. The latter declined in the study area [45], as a result of changing environmental conditions largely due to anthropogenic impact and climate changes.

Fifty-eight specimens of *Marenzelleria neglecta* and six of *Laonome xeprovala* were collected from branches of the Danube and coastal lagoons of the Romanian Black Sea coast.

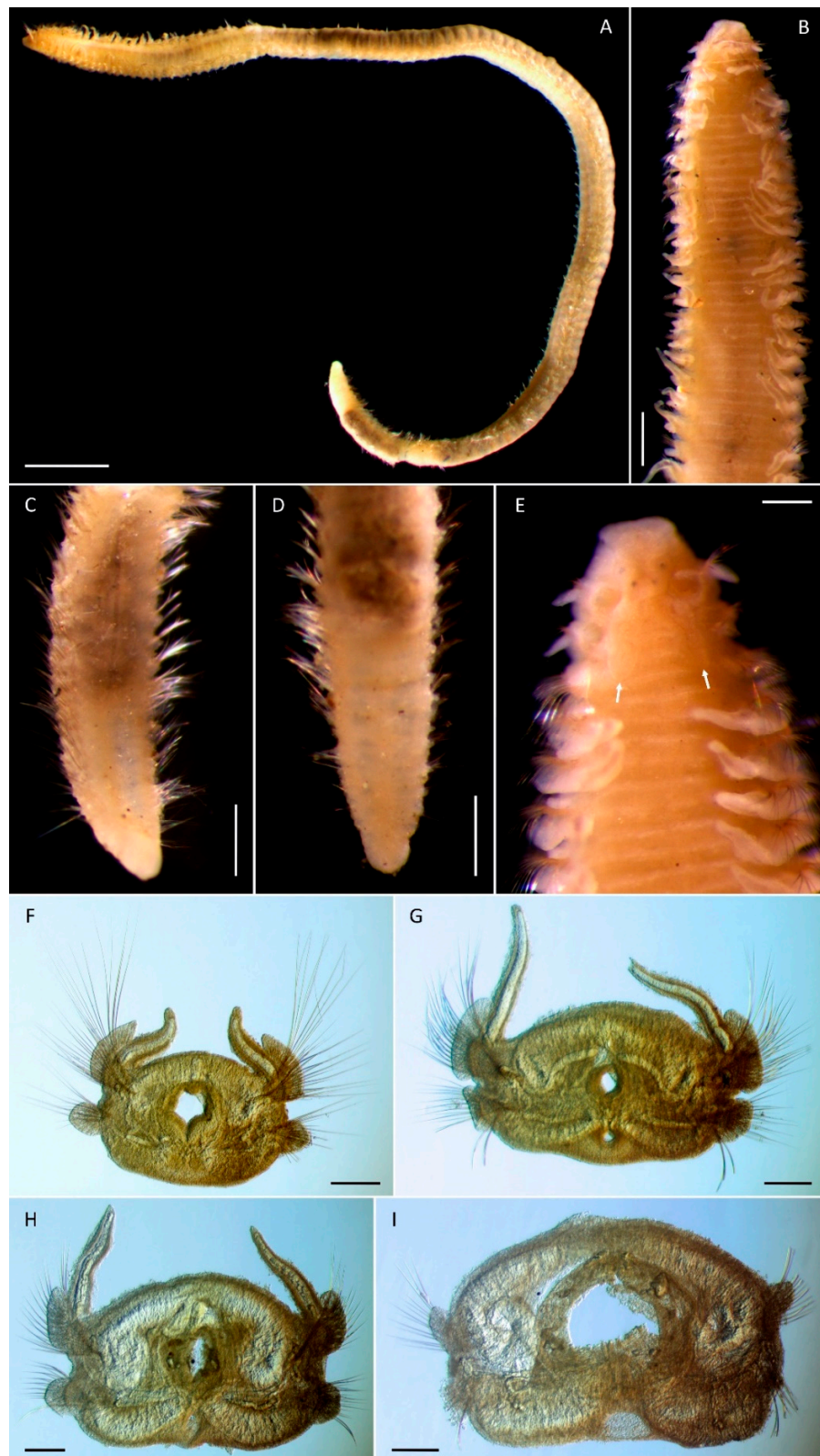
#### 3.2. *Marenzelleria neglecta*

**Material examined.** Romania, Sakhalin Lagoon, June 2021, S6a-2021 (07.06.2021; 44.809503° N, 29.522897° E; 1.5 m; mud substrate)—56 ind.; S5-2021 (07.06.2021; 44.827739° N, 29.565136° E; 1.5 m; mud substrate)—2 ind. (Figure 2).

**Description.** Up to 1.0 mm in width and 27 mm in length, with up to 133 chaetigers. Prostomium bell-shaped, broadly rounded anteriorly, often incised medially. Occipital papilla was absent. Two pairs of eyespots arranged in line or in trapeziform with posterior pair were closer together. No specimens bearing it were encountered. Nuchal organs, such as narrow grooves bounded with cilia, usually reach the mid ciliary band of chaetiger 3. The number of branchiae ranged from 27 to 38 pairs (depending on size; Table 1). Branchia usually end between setigers 30–38, with the last pairs sharply decreasing in length. Neuropodial hooded hooks ranged from chaetiger 27 to 34, with two to eight per fascicle in middle to posterior half of body. Notopodial hooded hooks ranged from chaetiger 33 to 42. Hooded hooks were bidentate, sometimes tridentate posteriorly (more in larger specimens), with two apical teeth in tandem above the main fang. Ventral inferior fascicles of neuropodium with sabre chaetae ranged from chaetigers 1 to 5. Anal cirri was not observed. The measurements confirmed the species identity according to the original description offered by Sikorski and Bick (Table 1).

**Ecology.** *Marenzelleria neglecta* was found at 1.5 m depth in the Sakhalin Lagoon on muddy bottoms. Only adult specimens were found. The density ranged from 50 ind.m<sup>-2</sup> to 1400 ind.m<sup>-2</sup> and the biomass ranged from 0.5 to 9.5 g.m<sup>-2</sup>, respectively. *Marenzelleria neglecta* accounted for 36% of the total density of macrobenthos. It was found together with three other marine species of polychaetes (*Alitta succinea* (Leuckart, 1847)—740 ind.m<sup>-2</sup>, *Hediste diversicolor* (O.F. Müller, 1776)—50 ind.m<sup>-2</sup>, and *Prionospio maciolekae* (Dagli and Çinar, 2011)—25 ind.m<sup>-2</sup>), which were dominant prior to the appearance of *M. neglecta*. Among amphipods, *Corophium orientale* (Schellenberg, 1928) (508 ind.m<sup>-2</sup>) and *Ampelisca sarsi* (Chevreux, 1888) (17 ind.m<sup>-2</sup>) were found.





**Figure 2.** *Marenzelleria neglecta* collected in the Sakhalin Lagoon, Black Sea. (A)—Complete specimen; (B)—anterior end with no palps; (C,D)—posterior end with pygidium, ventral, and dorsal view; (E)—anterior end, dorsal view showing the ends of nuchal organs (arrows) (first three pairs of branchiae were removed); (F–I)—transverse section showing anterior views of parapodia from chaetigers 3, 10, 25, and 40, respectively. Scale bars: (A)—2 mm; (B–D)—500  $\mu$ m; (E–I)—200  $\mu$ m.

**Table 1.** Variability of morphometric characters for different sizes of *M. neglecta* (L—length, W—width, Chae—number of chaetigers, \* incomplete specimen).

No. Crt.	L [mm]	W [mm]	Chae	VHH	DHH	Br	NO	Br-VHH	BR-DHH	DHH-VHH
1	8.07	0.69	41 *	28	33	30	m3	2	−3	5
2	15.56	0.70	67 *	28	33	30	b3	2	−3	5
3	8.01	0.72	38 *	30	38	32	b3	2	−6	8
4	5.96	0.72	32 *	29		31	b2	2		
5	12.97	0.73	61 *	27	33	27	b3	0	−6	6
6	8.83	0.73	40 *	27	36	34	b3	7	−2	9
7	4.81	0.74	24 *				m3			
8	21.85	0.75	99 *	29	40	31	m3	2	−9	11
9	27.75	0.75	133	34	42	38	m3	4	−4	8
10	7.94	0.75	36 *	28		32	m3	4		
11	6.48	0.75	28 *				m3			
12	6.72	0.76	35 *	33		35	b3	2		
13	9.23	0.80	42 *	31	37	34	m3	3	−3	6
14	6.60	0.80	29 *				b3			
15	8.7	0.8	38 *	29		31	m3	2		
16	6.86	0.81	29 *				b3			
17	5.37	0.81	30 *	28			m3			
18	9.47	0.82	44 *	30	36	32	m3	2	−4	6
19	8.21	0.82	37 *	33		36	m3	3		
20	11.19	0.83	49 *	30	36	35	e3	5	−1	6
21	14.72	0.85	57 *	32	41	37	m3	5	−4	9
22	8.72	0.85	36 *	31		34	m3	3		
23	22.83	0.86	97 *	31	35	32	m3	1	−3	4
24	8.30	0.86	39 *	33		34	m3	1		
25	14.93	0.87	62 *	32	40	34	m3	2	−6	8
26	9.90	0.87	38 *	31		33	m3	2		
27	10.73	0.89	47 *	32	38	33	e3	1	−5	6
28	13.09	0.92	56 *	32	39	32	m3	0	−7	7
29	9.48	0.93	41 *	29	38	35	m3	6	−3	9
30	9.16	0.94	40 *	29	38	30	m3	1	−8	9

### 3.3. *Laonome xeprovala*

**Material examined.** Romania, Sulina branch, June 2021, P12-2021 (06.06.2021; 45.181569° N, 29.346622° E; 4.7 m; mixed substrate)–4 ind; P13-2021 (06.06.2021; 45.176483° N, 29.474628° E; 5 m; mixed substrate)–2 ind.

**Description.** Body length, without branchial crown, varied from 13 to 23 mm, and width from 0.6 to 1 mm, with 8 thoracic (in all individuals) and 38–46 abdominal (depending on size) segments. Branchial crown was 2.1–3.2 mm long with a total of 14–16 branchial radioles.

**Ecology.** *Laonome xeprovala* was found in mixed sediments dominated by mollusk shells in two stations on Sulina branch, 24.86 km (St. P13) and 35.1 km (St. P12), respectively, away from the discharge mouth. Abundance of *L. xeprovala* was 20–40 ind.m<sup>−2</sup>

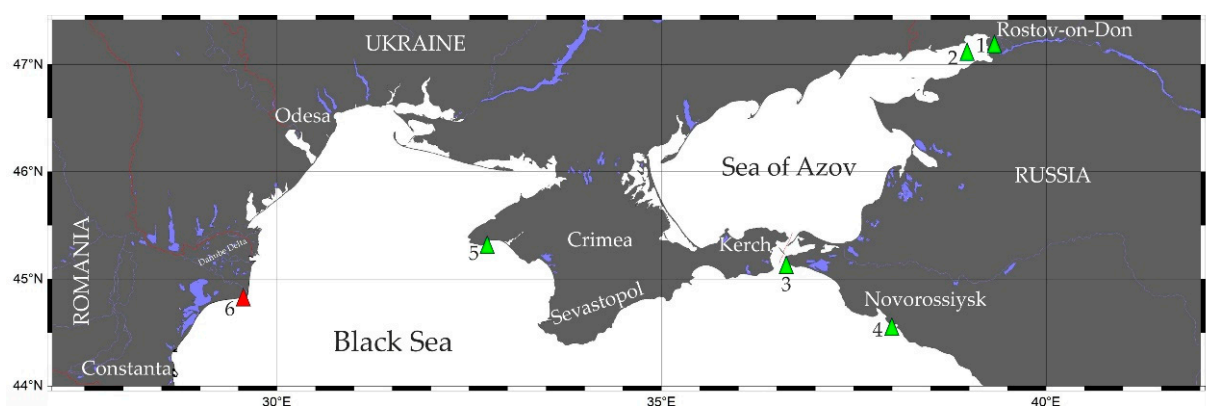
and biomass was  $0.19\text{--}0.2\text{ g.m}^{-2}$ , respectively. It was noted in association with Ponto-Caspian polychaetes (*Hypania invalida* (Grube, 1860) and *Hypaniola kowalewskii* (Grimm, 1877)), which recorded high densities ( $1600\text{--}2000\text{ ind.m}^{-2}$ ), together with the amphipods *Chelicorophium sowinskyi* (Martynov, 1924) ( $9800\text{ ind.m}^{-2}$ ), *C. curvispinum* (G.O. Sars, 1895) ( $650\text{ ind.m}^{-2}$ ), and *C. robustum* (G.O. Sars, 1895) ( $420\text{ ind.m}^{-2}$ ).

#### 4. Discussion

##### 4.1. *Marenzelleria neglecta* Invasion

*Marenzelleria* is a small group of spionid polychaetes currently comprising five valid species: *M. wireni* (Augener, 1913); *M. arctia* (Chamberlin, 1920); *M. viridis* (Verrill, 1873); *M. bastropi* (Bick, 2005); and *M. neglecta* (Sikorski and Bick, 2004) [40,41,46]. It is a species group native to meso- and oligohaline estuaries on the northeastern coast of North America [47]. They first appeared in the Baltic Sea in 1985, and quickly invaded large areas in the South Baltic Sea, occupying a dominant position in some benthic habitats [48–57]. The polychaetes that had invaded the Baltic Sea were initially identified as *M. viridis*, but following a revision of the genus, they were treated as a separate species, *M. neglecta* [40].

In 2014, Syomin et al. [58] reported the presence of *M. neglecta* in the Don Delta and Taganrog Bay in the Sea of Azov (Figure 3), where they probably entered the ballast waters of ships transiting the Volga-Don Canal. Later, the species was found in the Strait of Kerch and the Taman peninsula [59] and the western coast of Crimea on the Black Sea [60,61]. In 2018–2019, *M. arctia* was reported in the Caspian Sea [62], and the authors do not rule out that, after genetic analysis, the species may be confirmed as *M. neglecta* as a result of its presence in the Sea of Azov [59], closer to the Caspian basin and with a possible pathway trough in the Volga-Don channel.



**Figure 3.** Known records of *M. neglecta* in the Azov and Black Seas. 1–2—Syomin et al., 2016 [58]; 3–4—Syomin et al., 2017 [59]; 5—Boltacheva and Lisitskaya (2019) [60]; Boltacheva et al. (2021) [61]; 6—current study.

So far, *M. neglecta* has not been reported in the Romanian waters of the Black Sea. Studies conducted [63] in Romanian coastal lagoons during 2018 did not report the presence of the species. Thus, our findings represent the first report in a Danube estuarine environment (Figure 3). For the time being, the distribution of *M. neglecta* is limited to shallow areas with brackish water. However, this does not exclude the possibility of it spreading to the shallow water of the Black Sea under the Danube’s water influence, coastal lakes, and harbour areas. At this moment, the pathways of its arrival in the area are uncertain. However, larval ecology and life-history traits suggest a secondary spreading through shipping.

##### 4.2. *Laonome xeprovala* Invasion

After its first occurrence reported in 2018 in estuarine habitats [10], where it reached the highest abundance ( $100.8\text{ ind.m}^{-2}$ ) in the Sakhalin Lagoon, we report now the spread of the species upstream into the Danube mouth on the Sulina branch, where we recorded

an abundance of up to 40 ind.m<sup>-2</sup>. We specify that the previous results [63,64] regarding the analysis of the benthos from the Danube–Danube Delta ecosystem did not mention the presence of the species. It seems that among the ten known species of *Laonome* Malmgren, 1866 worldwide, *L. xeprovala* has one of the highest potentials for invasion, being recently identified in several European water bodies [65–69]. The rapid spreading in different regions seems to confirm the hypothesis of accidental introduction with ballast waters due to intense maritime and fluvial traffic. The fresh and brackish waters of canals, rivers, and estuaries, therefore, face the greatest amount of risk since the species prefers these types of habitats. Lately, the association of *L. xeprovala* and different *Marenzelleria* species in the same habitat has been reported [70], which brings up the idea of an “invasion outburst” driven by increasing anthropogenic and climate pressure factors that favour the spread of opportunistic species [53,61,67]. Sabelliidae, and likewise Spionidae, possess the biological and ecological features necessary to thrive and even to disrupt the local communities once they settle within a habitat. According to Rouse and Fitzhugh [71] and Kotta et al. [67], the reproductive strategy of sabellid polychaets as well as its active suspension filter feeding and burrowing behaviour make *L. xeprovala* a potential allogenic engineer species of ecosystems.

## 5. Conclusions

Our paper brings new evidence on the further spreading of *L. xeprovala* after its first reporting in 2018 in the Danube mouth area of the Romanian Black Sea. As its presence was reconfirmed in 2021 upstream of the original place (Sulina branch of the Danube), we deem that the species has already established a new habitat in the Romanian brackish and freshwaters.

The incoming of *M. neglecta*, in the summer of 2021 point out the vulnerability of the Danube estuarine habitats to aliens, especially those undergoing anthropogenic stress. We predict that, once settled in the new benthic habitats, the species has the capacity to change the current state and equilibrium of the ecosystem.

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