

## Research Article

## Canals and invasions: a review of the distribution of *Marenzelleria* (Annelida: Spionidae) in Eurasia, with a key to *Marenzelleria* species and insights on their relationships

Vasily I. Radashevsky<sup>1,\*</sup>, Victoria V. Pankova<sup>1</sup>, Tatyana V. Neretina<sup>2,3</sup> and Alexander B. Tzetlin<sup>2</sup>

<sup>1</sup>A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, 17 Palchevsky Street, Vladivostok 690041, Russia

<sup>2</sup>White Sea Biological Station, Faculty of Biology, Lomonosov Moscow State University, Moscow 119992, Russia

<sup>3</sup>Kharkevich Institute for Information Transmission Problems, Russian Academy of Sciences, 19/1 Bolshoy Karetny Pereulok, Moscow 127051, Russia

\*Corresponding author

E-mail: [radashevsky@gmail.com](mailto:radashevsky@gmail.com)

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### Abstract

Recent invasions of the North and Baltic Seas by three *Marenzelleria* species have extensively altered benthic communities in the region. Despite several studies on the morphology and biology of the worms, their morphological identifications are often challenging. Here we summarize and map the available records of *Marenzelleria* from Eurasia, distinguishing those based on morphology versus molecular data. Based upon the genetic similarity ( $p = 0.08\%$  for *COI*) between individuals from the Baltic Sea and individuals from the Barents and White Seas we propose, for the first time, a possible route for the invasion of the Baltic Sea by *M. arctica* from the White Sea through the White Sea–Baltic Sea Canal. At the same time, our analysis of the sequences of *COI* fragments showed a significant genetic distance ( $p = 4.28–4.29\%$ ) between individuals identified as *M. arctica* from the Baltic, Barents and White Seas and those from the Kara Sea. This genetic distance, as well as the isolated estuarine habitats of these Arctic worms, and the large geographic distance between the type locality of *M. arctica* in the Beaufort Sea (Alaska) and northern Europe, raise doubts about the conspecificity of North American, North European and Northwest Pacific populations. We report *M. neglecta* for the first time for the British Isles (River Thames). We also review the evidence for the role of the Baltic Sea–Volga Canal and the Volga–Don Canal in facilitating the dispersal of *M. neglecta* to the Caspian Sea and the Sea of Azov, respectively. We further provide new insight on the phylogeny of *Marenzelleria*, an updated diagnosis of the genus and a key for morphological identification of *Marenzelleria* adults greater than 1.2 mm wide.

**Key words:** Polychaete, *Marenzelleria arctica*, *Marenzelleria neglecta*, *Marenzelleria wireni*, molecular analysis, biological invasion, ballast water

### Introduction

*Marenzelleria* Mesnil, 1896 is a small group of spionid polychaetes that likely evolved in estuaries bordering the Arctic Ocean (Sikorski and Bick 2004; Bick 2005; Blank and Bastrop 2009). Subsequent dispersal, population isolation, and regional adaptation in isolated habitats on the Atlantic coast of North America apparently led to additional speciation in the Northwest

Atlantic Ocean. Two basal species, *M. wireni* Augener, 1913 and *M. arctia* (Chamberlin, 1920), have been considered indigenous in the Arctic region, and three derived species are regarded indigenous in the Northwest Atlantic: *M. viridis* (Verrill, 1873), *M. bastropi* Bick, 2005 and *M. neglecta* Sikorski and Bick, 2004 (Blank and Bastrop 2009; Radashevsky et al. 2021). The age of *Marenzelleria* has not yet been determined, but the high morphological similarity of larvae and adults of different species indicates a relatively recent divergence in this group.

In North European waters (North and Baltic Seas), alien *Marenzelleria* first appeared in the late 1970s–early 1980s (Atkins et al. 1987; Elliott and Kingston 1987; Essink and Kleef 1988, 1993; Bick and Burckhardt 1989). The rapid growth of the populations of these worms in the 1990s–2000s and their impacts on local benthic communities stimulated abundant studies on their distribution, morphology, reproductive biology, physiology, ecology, bioturbation and genetics (reviews by Bastrop et al. 1997; Blank et al. 2008; Blank and Bastrop 2009). Genetic analyses using either allozyme electrophoresis, PCR/sequencing (fragments of the mitochondrial *16S*, *COI* and *Cytb* genes) or combined PCR/RFLP analysis distinguished three species: *M. viridis*, *M. neglecta* and *M. arctia* (Bastrop et al. 1995, 1997; Röhner et al. 1996a, b; Bastrop and Blank 2006; Blank et al. 2008; Blank and Bastrop 2009). *Marenzelleria viridis* and *M. neglecta* are considered to have been introduced into European waters from the Atlantic coast of the United States. *Marenzelleria arctia* is only known in Northern Europe from the Baltic Sea, where its precise origin remains unclear.

The phylogenetic relationships of *Marenzelleria* species have been explored by the analyses of mitochondrial DNA by Blank and Bastrop (2009), Syomin et al. (2017), and Radashevsky et al. (2021). Worldwide records of *M. viridis* and records of *Marenzelleria* spp. from North America were recently summarized and mapped by Radashevsky et al. (2021). The purpose of the present study was to further explore the phylogeny of *Marenzelleria* based on an analysis of a larger set of data, including nuclear genes. We also review earlier reports, provide new records of *Marenzelleria* spp. (except *M. viridis*) from Eurasia, and, using molecular data, verify conspecificity of disjunct populations of *M. arctia*. In so doing, we update and refine our knowledge of the distribution of *Marenzelleria* species. Our additional purpose was also to hypothesize a possible route along which *M. arctia* could have been transported from the Arctic into the Baltic Sea, and to review the role of canals in facilitating the dispersal of *Marenzelleria* worms in Eurasia.

## Materials and methods

### *Material*

Collections were made in the intertidal in the Kandalaksha Gulf (White Sea, Russia). Sediments collected for this study were washed in the field on

a 500- $\mu$ m mesh sieve, and *Marenzelleria* worms retained in the residue were removed and examined alive under light microscopes in the laboratory. For molecular analysis, worm fragments were preserved in 95% ethanol. After morphological examination, worms were fixed in 10% formalin solution, rinsed in fresh water, transferred to 70% ethanol, and then deposited in the polychaete collections of the Museum of the A.V. Zhirmunsky National Scientific Center of Marine Biology (MIMB), Vladivostok, Russia, and the White Sea Branch of the Zoological Museum of the Lomonosov Moscow State University (ZMMU\_WS), the White Sea Biological Station, Poyakonda, Russia.

We also examined museum samples of *Marenzelleria* spp. collected by recent expeditions of the Russian Academy of Sciences to the Arctic and Northwest Pacific. Ethanol-fixed specimens of *M. arctia* from the Kara Sea (Russia) were provided by Alexandra N. Stupnikova, and *M. neglecta* from Taganrog Bay (Sea of Azov, Russia) were provided by Vitaly Syomin. To map the distribution of *Marenzelleria* species, we considered reliable records made by earlier authors based on morphological characters, and records by Bastrop and Blank (2006), Blank et al. (2008), Blank and Bastrop (2009), and Syomin et al. (2017) based on genetic data. Complete information on newly collected material, museum samples examined during this study and by other authors, and records by other authors for which no museum deposits were noted, is provided in Supplementary material Tables S1–S3. Records by other authors are annotated in Tables in the *Comments*. A list of the museums and other collections (and their acronyms) holding the examined or reported specimens of *Marenzelleria* spp. is in Table S4.

When no coordinates were provided for sampling sites from other studies, they were estimated using Google Earth Pro according to the original descriptions of the locations. Sampling locations of *Marenzelleria* spp. are plotted on maps using QGIS 3.20.0 software and the geodata provided by the OpenStreetMap Project (<https://osmdata.openstreetmap.de>). Final maps and the plates were prepared using CorelDRAW®2019 software.

#### *DNA extraction, amplification and sequencing*

We used the ReliaPrep gDNA Tissue Miniprep System (Promega Corporation, Madison, WI, USA) for DNA extraction and purification with standard protocol for animal tissue. Polymerase chain reaction (PCR) amplification of nuclear *18S rDNA*, D1 region of *28S rDNA* and *Histone 3*, mitochondrial *16S rDNA* and *cytochrome C oxidase subunit 1 (COI)* gene fragments was accomplished with the primers and conditions described by Radashevsky et al. (2014, 2016, 2020). Purified PCR products were sequenced in both directions on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems) using the BrilliantDye Terminator v3.1 Cycle Sequencing Kit (NimaGen) and the same primers as for PCR. Sequence editing and contig

assembly were performed using SeqScape 2.5 (Applied Biosystems). GenBank accession numbers and brief information about sequences used in the present analysis are shown in Table S5. To link sequences with complete corresponding data, unique numbers from the first author's database (VIR) are given to samples in Tables S1–S3 and S5.

### Data analysis

We aligned DNA sequences using the ClustalW method implemented in the MEGA 5.1 software (Tamura et al. 2011). Ambiguous positions and gaps were excluded from subsequent analysis using GBlocks (Castresana 2000) with settings for a less stringent selection. Pairwise distances ( $p$ , see Nei and Kumar 2000) both within and between groups were calculated in MEGA 5.1 (Tamura et al. 2011). We concatenated DNA data partitions using SequenceMatrix (Vaidya et al. 2011) and specified substitution models for each partition individually. The best-fitting nucleotide substitution models for Bayesian analysis (TVM+G for *COI*, GTR+G for *16S*, SYM+I for *18S*, TVM for *28S*, and HKY+I+G for *Histone 3*) were selected in MrModeltest version 3.7 (Posada and Crandall 1998) using Akaike Information Criterion (AIC).

We used MrBayes 3.2.7 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) via the CIPRES web portal (Miller et al. 2010) for the Bayesian analyses of 10,000,000 generations, four parallel chains and sample frequencies set to 500, in two separate runs. Based on the convergence of likelihood scores, 25% of sampled trees were discarded as burn-in.

We performed two Bayesian analyses of sequences of *M. arctia* from the Kara and White Seas, including those obtained by Radashevsky et al. (2014, 2021), and sequences of *M. bastropi*, *M. neglecta*, *M. viridis* and *M. wireni* provided by Bastrop et al. (1998), Bastrop and Blank (2006), Blank et al. (2008), Blank and Bastrop (2009), and Syomin et al. (2017). A general analysis included available sequences of five genes: *COI*, *16S*, *18S*, *28S* and *Histone 3*. This analysis was done to formulate hypotheses about the phylogenetic relationships of *Marenzelleria* species for the first time using concatenated set of both mitochondrial and nuclear genes. The resulting tree was rooted using the sequences of *Spiophanes* cf. *bombyx* (Claparède, 1870) (provided by Radashevsky et al. 2020) according to a preliminary phylogenetic analysis of molecular data for spionid polychaetes, where *Spiophanes* Grube, 1860 appeared basal to *Marenzelleria* clade (Radashevsky et al. *unpubl. data*). We also performed an analysis of mitochondrial *COI* and *16S* genes of *Marenzelleria* species only. This analysis allowed to obtain more accurate  $p$ -distance estimates between *Marenzelleria* samples by reducing loss of data during exclusion of ambiguous positions and gaps after aligning *Marenzelleria* sequences with outgroup. It was rooted using sequences of *M. arctia* that in the general analysis appeared most basal among *Marenzelleria*.





*COI*, 302 bp (93.2%) for *16S* rDNA, 980 bp (96.3%) for *18S* rDNA (5'- and 3'-ends of the fragments; middle parts were excluded), 297 bp (96.4%) for *28S* rDNA, and 291 bp (100%) for *Histone 3*. The Bayesian analysis of the combined dataset resulted in a fully resolved consensus tree (Figure 1). The average *p*-distances for the individual gene fragments between groups of specimens are given in Table S6.

All ten *18S* sequences of *M. arctia* (five from the Kara Sea, three from the White Sea, one from the Baltic Sea, and one from the Barents Sea) were identical. Eight *28S* sequences (five from the Kara Sea and three from the White Sea) also were identical. *Histone 3* sequences of *M. arctia* from the Kara and White Seas differed by three substitutions (average *p* = 1.03%). No variability was found among the *Histone 3* sequences of conspecific individuals from the same location (Table S6).

#### *COI-16S* analysis (Figure S1)

The combined aligned sequences of *Marenzelleria* spp., with gaps excluded, comprised in total 934 bp, including 612 bp (100% of original sequences) for *COI*, and 322 bp (99%) for *16S* rDNA. The combined concatenated dataset contained 254 (27.2%) variable sites, 243 (26%) of which were parsimony-informative. The frequency of variable sites in the aligned sequences of *COI* (34.2%) was greater than that for sequences of *16S* (14%).

The Bayesian analysis of the combined dataset of two mitochondrial markers resulted in a fully resolved consensus tree (Figure S1). It revealed two groups among specimens identified by morphology as *M. arctia*. One group included specimens from the Baltic, Barents and White Seas; the other included specimens from the Kara Sea. Three *COI* haplotypes and three *16S* haplotypes were identified in each group, but none of them was common to specimens from both groups. In the first group, specimens from all locations had one common *COI* haplotype and one *16S* haplotype. The maximum ingroup *p*-distances in each group were 0.33% for *COI* and 0.62% for *16S*. The *p*-distances between the two groups ranged from 4.09% to 4.58% (28 variable sites) for *COI* and 0.62% to 1.24% (6 variable sites) for *16S* (Table S7).

#### *Systematic Account*

##### ***Marenzelleria* Mesnil, 1896**

*Marenzelleria* Mesnil, 1896: 120. Augener 1913: 264–267. Fauchald 1977: 24. Maciolek 1984: 48. Sikorski and Buzhinskaya 1998: 1111–1112. Sikorski and Bick 2004: 255. Blank and Bastrop 2009: 311–318. Blake et al. 2020: 50.

*Synopsis.* Adults up to 140 mm long, 3 mm wide for 250 chaetigers in *M. viridis* (Verrill, 1873). Prostomium anteriorly wide, with entire, concave or weakly incised frontal margin, posteriorly narrowing and extending over first chaetiger as a low caruncle. Occipital antenna absent. Two pairs



**Figure 2.** Adult morphology of *Marenzelleria arctia* (live individuals from the Trosa Archipelago, Baltic Sea, Sweden). A, anterior end, dorsal view. B, general dorsal view. A – not fixed; B – SIO BIC A5893. Specimens likely about half a millimetre wide each. Photos by Fredrik Pleijel.

of small red eyes usually present. Nuchal organs U-shaped, over 1–5 anterior chaetigers. Each palp with frontal longitudinal ciliated groove only; basal sheath absent. Segment 1 well developed, with capillary chaetae and postchaetal lamellae in both rami. Posterior notopodia with hooded hooks in addition to capillary chaetae. Hooded hooks in neuropodia from chaetigers 10–51 onwards, alternating with thin capillary chaetae; hooks bi- or tridentate with outer hood only and slightly curved shaft. Inferior sabre chaetae in neuropodia usually from chaetiger 4 onwards. Branchiae from chaetiger 1 usually on anterior half of body, or throughout most of body, fused to notopodial postchaetal lamellae at least basally on anterior chaetigers, with surfaces oriented perpendicular to body axis; branchial blood vessels not interconnected by radial capillaries. Dorsal crests and lateral pouches absent. Pygidium with up to ten pairs of cirri. Digestive tract without gizzard-like structure. Main dorsal blood vessel without heart body. Nephridia from chaetiger 4 onwards, serving both excretory and gamete-releasing functions in fertile chaetigers.

Type species. *Marenzelleria wireni* Augener, 1913: 265.

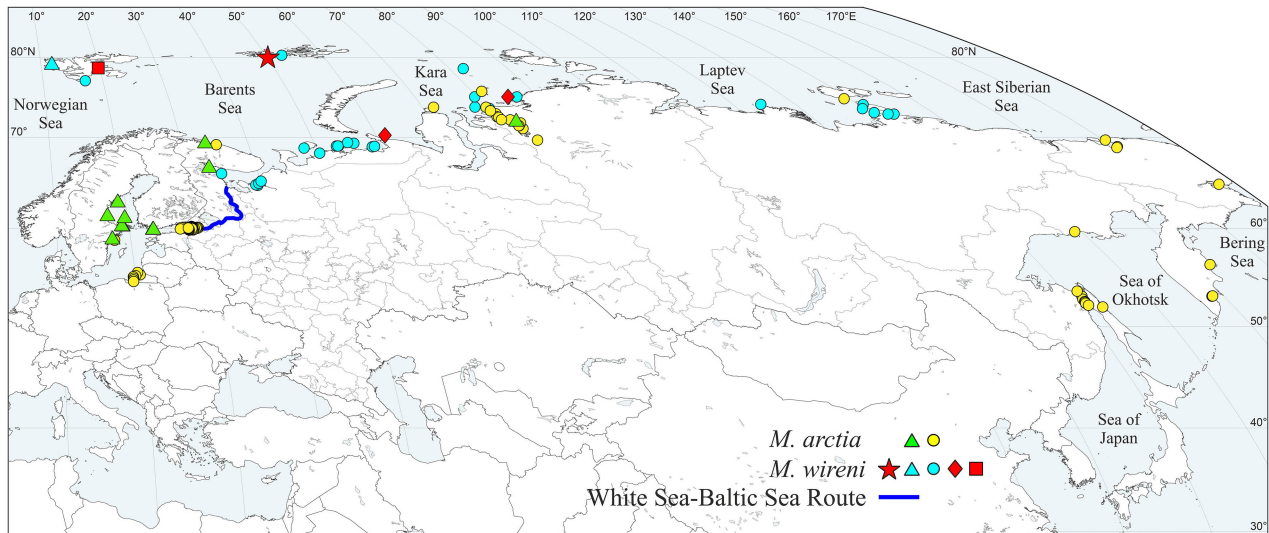
### ***Marenzelleria arctia* (Chamberlin, 1920)**

(Figures 2, 3)

*Scolecopides arctius* Chamberlin, 1920: 17–18, pl. III, figs. 5–7, pl. IV, fig. 1.

*Marenzelleria arctia*: Sikorski and Buzhinskaya 1998: 1115–1118, figs. 2, 3. Radashevsky et al. 2021: 359, figs. 1, 2 (References).

*Remarks on the identity of Marenzelleria arctia.* *Scolecopides arctius* was first described from a lagoon at Collinson Point (Camden Bay, Beaufort Sea, Alaska, USA) by Chamberlin (1920). The species was largely forgotten until Sikorski and Buzhinskaya (1998) redescribed it based on the type material (paratypes MCZ ANNb-2194, 2195) and transferred it to the genus



**Figure 3.** Map showing records of *Marenzelleria arctia* and *M. wireni* from Eurasia based on morphology (circles, rhombi, square and star) and molecular data (triangles). Sampling locations for specimens described by Wirén (1883) marked with rhombi, by Marenzeller (1892) with square, by Augener (1913) with star. Possible route of *M. arctia* invasion from the White Sea to the Baltic Sea through the White Sea–Baltic Sea Canal marked with a blue line. See Tables S1, S3, S5 for details.

*Marenzelleria*. Earlier reports of *M. arctia* from the Kandalaksha Gulf by Stolyarov (1994), Burkovsky et al. (1995), and Burkovsky and Stolyarov (1995) were based on the identifications by Andrey V. Sikorski.

It is noteworthy that various aspects of the biology of *M. arctia* have been studied in North European populations, whereas the American population remains unexplored. In the present study, we compared the sequences of gene fragments of individuals from the Baltic, Barents and White Seas and individuals from the Kara Sea, which were all morphologically identified as *M. arctia*. Genetic distances between European and the Kara Sea groups of specimens were significant ( $p = 4.28\text{--}4.29\%$ ) for *COI* fragments (in contrast to the distances between individuals from the same group  $p = 0.05\text{--}0.13\%$  for *COI*), while they were 0.84–0.88% for *16S*, 0.93% for *Histone 3*, and 0.0% for *18S* and *28S* (see Table S6). The high genetic distances between the *COI* fragments (which evolve faster), the low distances between the *16S* and *Histone 3* fragments, and the sequence identity of *18S* and *28S* can be interpreted as a result of the isolation of the Kara Sea population and ongoing speciation. At the same time, the high genetic distance between *COI* fragments, isolated estuarine habitats of these Arctic worms, and the large geographic distance between the type locality of *M. arctia* in the Beaufort Sea (Alaska, USA) and Northern Europe raise doubts about the conspecificity of the North American and North European populations. The phylogenetic relationships and the systematic position of these populations require careful further study.

*Remarks on Laonice annenkovae* Zachs, 1925. Zachs (1925) described *Laonice annenkovae* Zachs, 1925 from the Tuloma River estuary (Kola Bay, Barents Sea, Russia), and Uschakov (1939, 1950, 1953, 1955) reported this species from the White Sea and the Amur Liman (on the border between



the Okhotsk and the Sea of Japan). Sikorski and Buzhinskaya (1998) placed *L. annenkovae* into synonymy with *M. arctia* and for the first time reported this species from the Bering Sea in the Chukchi Peninsula and Kamchatka. Although Sikorski and Buzhinskaya (1998) noted that Uschakov's material was lost, they expanded the distribution of before-only-Arctic *M. arctia* along the Asian Pacific coast southward to the Amur Liman. New specimens of *Marenzelleria* from the Amur Liman and Sakhalinsky Gulf were collected by an Expedition of the Institute of Marine Biology, FEB RAS, in 2005 (MIMB 17905, 36659–36665). The worms from the Amur Liman are similar to those from Baffin Bay, Canada, but differ somewhat in the later start of sabre chaetae in neuropodia from chaetigers 10–12 instead of chaetiger 4. We also identified as *M. arctia* specimens from the Sea of Okhotsk collected in 1955 and 1997 (ZISP 13723 and 10/49457, respectively) that have not been previously reported in the literature. They are similar to *Marenzelleria* from the Amur Liman and Sakhalinsky Gulf. Note worthily, Sikorski and Bick (2004, p. 273) assumed that “In the Far East [Northwest Pacific], *M. arctia* and possibly *M. neglecta* occur.” In the Amur Liman, *Marenzelleria* is an important part of the prey of the Amur sturgeon *Acipenser schrenckii* and the Kaluga sturgeon *Huso dauricus* (Kolobov et al. 2013). The systematic position of *Marenzelleria* from the Northwest Pacific requires further study.

Sikorski and Buzhinskaya (1998) reported that designated a lectotype (ZISP 01/2210) and 23 paralectotypes (ZISP 02/2211 and 03/13765) from a type series of *Laonice annenkovae*. In reality, on the examination by one of us (VIR) on 12 Dec 2019, ZISP 01/2210 contained anterior fragments of 15 large worms. Therefore, one of these specimens, ca. 85-chaetiger anterior fragment, was designated by VIR as the lectotype (ZISP 01/2210) whereas the other 14 specimens were designated as paralectotypes and catalogued as ZISP 02a/50775 (12 specimens) and MIMB 42145 (2 specimens). Sample ZISP 03/13765 was not mentioned by Zachs (1925) in the original description of *L. annenkovae* and therefore cannot be considered as a part of the type series, as erroneously Sikorski and Buzhinskaya (1998) did.

*Invasion of the Baltic Sea by M. arctia.* In the Baltic Sea, *M. arctia* was first identified by genetic analysis of specimens collected in 2005 in two Swedish locations in the western part of the sea in Söderhamn and the Isle of Askö (Bastrop and Blank 2006: fig. 1). Soon after that, Blank et al. (2008: fig. 1) reported *M. arctia* from nine sites in the northern Baltic Sea (Sweden and Finland) and obtained new sequences of specimens from six sites in the Gulf of Bothnia, Åland Sea, and westernmost part of the Gulf of Finland (Figure 3). In 2009, many mature individuals of *M. arctia* were first found in the eastern part of the Gulf of Finland, after a series of hypoxic-anoxic events that led to the decline of native benthic communities (Maximov 2010, 2011, 2015, 2018). Currently, *M. arctia* dominates in the eastern deepwater (down to the depths of 70–80 m) part of the Baltic Sea

(Maximov et al. 2014, 2015; Golubkov et al. 2021; Kocheshkova and Ezhova 2018). Remarkably, the species has not been reported from the North Sea.

Two *Marenzelleria* specimens were collected from Trosa Archipelago (Baltic Sea, Sweden) and photographed by Fredrick Pleijel in June 2008 (Figure 2D, E). One of these specimens was preserved (SIO BIC A5893), and, although not examined in this study, according to the features shown on the picture (i.e., nuchal organ length, arrangement of branchiae), we refer it to *M. arctia*.

Blank et al. (2008) showed that Baltic *M. arctia* shared haplotypes with specimens from the Tuloma River (Kola Bay, Barents Sea, Russia) and suggested an introduction by ship ballast water from the European Arctic to harbours in the central or northern parts of the Baltic Sea. The exact route of that introduction remained unknown, however, because of most likely delayed record of the first appearance of the species in the Baltic Sea due to difficulties in identification of *Marenzelleria* specimens based on morphological characters, and possible inadequate genetic characteristics of populations within the range of distribution of *M. arctia*.

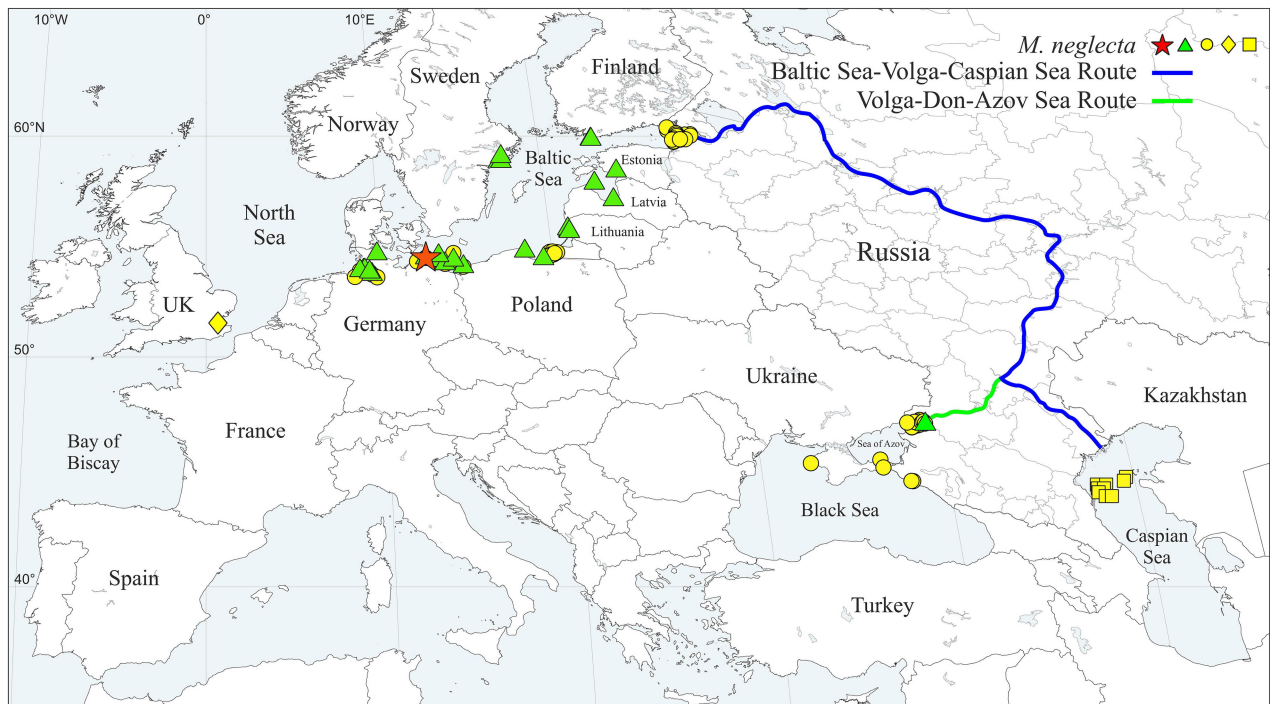
Here, for the first time, we propose a possible route for the invasion of the Baltic Sea by *M. arctia* from the White Sea through the White Sea–Baltic Sea Canal. The Canal was opened in 1933 and connects the White Sea with Lake Onega, and then with Lake Ladoga, which further connects with the Gulf of Finland (Figure 3). The most likely vector of this invasion is the transportation of larvae with ship ballast. The proposed route seems to contradict the first reports of this species from the western part of the Baltic Sea, but not from the Gulf of Finland. However, this may be due to the delayed record of the first appearance of the species in the area, as well as due to the locations of ballast water discharge by ships from the White Sea and further movement of larvae by local currents and subsequent successful settling of larvae, phenomena about which we have no data at this time.

### ***Marenzelleria neglecta* Sikorski and Bick, 2004**

(Figure 4)

*Marenzelleria neglecta* Sikorski and Bick, 2004: 264–268, figs. 2B, 3C, 5A–I, 6. Blank et al. 2008: 134 (molecular key). Blank and Bastrop 2009: 316–318, fig. 1 (phylogeny). Maximov 2015: 301–309. Kocheshkova and Ezhova 2018: 221–223. Kauppi et al. 2017: 195; 2018: 48–55. Syomin et al. 2017: 977–979, figs. 2–5. Wasmund et al. 2018: 78. Boltachova and Lisitskaya 2019: 137, fig. 2b, c. Boltachova et al. 2021: 17–18. Radashevsky et al. 2021: 360–361, figs. 1, 2. *Marenzelleria viridis*: Lyakhin et al. 1997: 431–434. Maximov and Panov 2003: 192. Not Verrill 1873. *Marenzelleria* sp. 1: Syomin et al. 2016: 112–113, fig. 2. *Marenzelleria* sp. 2: Syomin et al. 2016: 113–115, fig. 3. *Marenzelleria* sp. (nectochaeta): Syomin et al. 2016: 115, fig. 4.

**Remarks.** Bastrop et al. (1995) and Röhner et al. (1996a) examined *Marenzelleria* populations from northern Europe using allozyme electrophoresis and suggested the presence of two different species in the region: one in the North Sea and the other in the Baltic Sea. In an attempt



**Figure 4.** Map showing type locality (red star) and records of *Marenzelleria neglecta* from Eurasia based on morphology (yellow circles, squares and rhomb) and molecular data (green triangles); records from the Caspian Sea by Mikhailova et al. (2021, as *M. arctica*) marked with squares; our new record from the UK (River Thames) marked with a rhomb. Possible route of *Marenzelleria* invasion from the Baltic Sea to the Caspian Sea through the Baltic Sea–Volga Canal and the Volga River marked with a blue line. Possible routes of *Marenzelleria* invasion from the Baltic Sea to the Sea of Azov through the Baltic Sea–Volga Canal, the Volga River, the Volga–Don Canal and the Don River marked with blue and green lines. See Tables S2, S5 for details.

to determine the origin of these worms, Röhner et al. (1996b) compared their allozymes to *Marenzelleria* from the Atlantic coast of North America. Three species were distinguished and referred to as *Marenzelleria* Types I, II and III. The North Sea population was found similar to *Marenzelleria* Type I from the US coastal waters between Barnstable Harbour (Massachusetts) and Cape Henlopen (Delaware). The Baltic Sea population was found similar to *Marenzelleria* Type II from the US coastal waters between Chesapeake Bay (Trippe Bay) and Ogeechee River (Georgia). Therefore, it was suggested that the North and Baltic Seas were colonized by two *Marenzelleria* species from the North American Atlantic coast. Bastrop et al. (1997, 1998) confirmed this assumption when analyzing 16S rDNA fragments from the same populations. Sikorski and Bick (2004) revised the genus *Marenzelleria* and described *Marenzelleria* Type II as a new species *M. neglecta*. Darss-Zingst-Boddendchain (Germany) was chosen at the type locality of the species (Figure 4). Based on new material from Currituck Sound (North Carolina, USA), Bick (2005) described *Marenzelleria* Type III as the new species *M. bastropi*. The distribution of *M. neglecta* and *M. bastropi* in North America was reviewed and mapped by Radashevsky et al. (2021).

In the first half of the 1990s, *M. neglecta* began to spread in the Gulf of Finland in Estonia and Finland (Norkko et al. 1993; Stigzelius et al. 1997; Kotta and Kotta 1998). In 1996, rare *Marenzelleria* (initially identified as

*M. viridis*) were first discovered in the eastern part of the Gulf in Russia (Lyakhin et al. 1997), but the next year the worms spread over large areas and soon became a common component of both shallow and deepwater benthic communities (Maximov and Panov 2003; Maximov 2011, 2015). Dramatic changes in the zoobenthos of the eastern part of the Gulf of Finland took place in 2009, shortly after the devastating hypoxic-anoxic events in 2003 and 2006. Alien *M. arctia* quickly occupied most of the deepwater zone and became the dominant species in the communities (Maximov 2015). A similar distribution of the two *Marenzelleria* species was found in the southeastern part of the Baltic Sea in 2001–2014: *M. arctia* was mainly found in relatively deep (down to the 70–80 m depth), mesotrophic areas with salinity above 5‰, whereas *M. neglecta* inhabited shallow, eutrophic and hypertrophic, brackish waters of the Vistula and Curonian lagoons (Kocheshkova and Ezhova 2018).

In February–March 2014, the adults and larvae of *Marenzelleria* sp. were first found in the Don River and the Taganrog Bay of the Sea of Azov (Syomin et al. 2016). Their morphological characters varied greatly and corresponded to both *M. neglecta* and *M. arctia*. However, genetic analysis (mainly *COI* sequences, but also *16S*, *28S*, *cytb*, and *Histone 3*) showed that only *M. neglecta* was present in the region (Syomin et al. 2017). Soon after the first find in Taganrog Bay, *M. neglecta* became dominant in the region and also appeared in the centre of the Sea of Azov, in the Strait of Kerch, and on the Caucasian coast of the Black Sea (Taman Peninsula; Syomin et al. 2017). Syomin et al. (2016) suggested that, most likely, the worms could have entered the Don River and Taganrog Bay with the ballast waters of ships coming from the Baltic Sea through the Baltic Sea–Volga Canal, the Volga River, the Volga–Don Canal, and then the Don River (Figure 4). Syomin et al. (2017) warned about the further spread of *M. neglecta* in the Black Sea, as well as about the possible invasion of the Caspian Sea by this species. Indeed, in April 2016, *M. neglecta* was first collected off the western coast of Crimea (Boltachova and Lisitskaya 2019; Boltachova et al. 2021), and in October 2018, *Marenzelleria* was first found in the northern part of the Caspian Sea (Mikhailova et al. 2021). Mikhailova et al. (2021) noted that Caspian worms were similar to *M. arctia*, but since *M. neglecta* was already identified by molecular methods in the neighbouring Sea of Azov, they referred them to as *Marenzelleria* sp., pending reliable identification by molecular analysis. Because Syomin et al. (2016) also identified by morphology some worms from the Don River and Taganrog Bay as *M. arctia*, but later, based on molecular data, re-identified them as *M. neglecta*, here we tentatively refer the Caspian worms to *M. neglecta*. The worms could have entered the Caspian Sea with ballast waters of ships sailing from the Baltic Sea through the Baltic Sea–Volga Canal, and then through the Volga River (Figure 4).

Here we report for the first time *M. neglecta* in the British Isles (River Thames; MIMB 36644), recording the further spread of this species in the North Sea. Complete information on *M. neglecta* records is given in Table S2 (mapped in Figure 4).

### ***Marenzelleria wireni* Augener, 1913**

(Figure 3)

*Marenzelleria wireni* Augener, 1913 (*Part.*): 264–267, figs. 1, 2. Annenkova 1952: 126. Maciolek 1984 (*Part.*): 49–51, fig. 1a–g. Sikorski et al. 1988 (*Part.*): 835–837, fig. 4a–k. Sikorski and Buzhinskaya 1998: 1112–1115, fig. 1. Sikorski and Bick 2004: 255–261, figs. 1A–F, 2A, 3A. Bick 2005: 269–270, fig. 3 (references).

*Marenzelleria* sp.: Radashevsky et al. 2021: 363–364, figs. 2, 4–6.

*Microspio wireni*: Söderström 1920 (*Part.*): 249–250.

*Paraspio wireni*: Hartman 1959: 382.

*Nerine vulgaris*: Wirén 1883 (*Part.*): 408–409. *Fide* Augener 1913: 264.

*Scolecopsis* sp.: Marenzeller 1892: 427–429, fig. 5. *Fide* Augener 1913: 264.

*Spio gorbunovi* Averintsev, 1990: 165–166, fig. 13. *Fide* Sikorski and Buzhinskaya 1998: 1112. Sikorski and Bick 2004: 255.

**Remarks.** The taxonomic history of *M. wireni* dates back to the material collected by the Swedish expedition on R/V *Vega* (1878–1880; the first Arctic expedition that passed the Northeast Passage and the first voyage around Eurasia) in the Kara Sea in 1878 and reported by Wirén (1883) as likely *Nerine vulgaris* Johnston, 1838. Marenzeller (1892) received one incomplete specimen (41 mm long, 2.75 mm wide for 148 chaetigers) collected by a German expedition (sponsored by Bremer Geographischen Gesellschaft) from Whalespointbucht (eastern Spitsbergen, northwest Barents Sea) in 1889. Marenzeller (1892) noted that his specimen looked similar to Wirén’s *N. vulgaris* and possibly represented a new species. However, due to the lack of good material for a complete description, he reported that it was *Scolecopsis* sp.

In revising the Spionidae family, Mesnil (1896) used Wirén’s (1883) and Marenzeller’s (1892) descriptions to create a new genus, *Marenzelleria*, although he did not give a species name for the corresponding material. Following the descriptions, Mesnil (1896: p. 117) mistakenly diagnosed the new genus as having branchiae beginning from chaetiger 2.

Augener (1913) received three specimens collected by the Scottish Jackson-Harmsworth Expedition of Professor W.S. Bruce near the Cape Flora (Franz Joseph Land, northeastern Barents Sea) in 1896. Augener (1913: p. 265) re-examined Wirén’s (1883) and Marenzeller’s (1892) materials, found them similar to his material from the Franz Joseph Land, and named it after its first discoverer as *Marenzelleria wireni*.

Revising the genera *Scolecopsis* Malmgren, 1867, *Marenzelleria* Mesnil, 1896, and *Scolecopides* Ehlers, 1907, Maciolek (1984: p. 49) noted the problem: “According to the current rules of the ICZN (not in effect in 1896), Mesnil’s generic name *Marenzelleria* would be a *nomen nudum*” (that is, Mesnil erected *Marenzelleria* without specifying the type species for the



new genus, VIR). To save the genus and solve the problem, Maciolek (1984: p. 49) suggested that “Augener (1913) effectively emended Mesnil’s (1896) diagnosis of *Marenzelleria* when he assigned his new species, *M. wireni*, to that genus.” Maciolek (1984) transferred *Scolecoplepis viridis* Verrill, 1873 to *Marenzelleria*, and described a new species *M. jonesi* (later synonymized with *M. viridis* by Rodi and Dauer 1996) based on material from Cape Henlopen (Delaware, USA). Maciolek (1984) overlooked the *Scolecoplepides arctius* (= *M. arctia*) described by Chamberlin (1920) from the Beaufort Sea (Alaska, USA), so her “material might contain specimens of both *M. wireni* and *M. arctia*” (Sikorski and Bick 2004: p. 261).

Bick (2005) collected and described *M. wireni* from Kongsfjorden (eastern Greenland Sea, western Spitsbergen). Blank and Bastrop (2009) sequenced 16S, COI and *Cytb* gene fragments, while Syomin et al. (2017) sequenced 28S and *Histone 3* from this material; these are the only sequences of the species available until present.

*Marenzelleria wireni*/*M. cf. wireni*/*Microspio wireni* were reported from the North Sea (Wohlenberg 1937; Elliott and Kingston 1987; Schiedek 1999; Essink and Dekker 2002; Wolff 2005), but these reports were misidentifications. Until now, there is no genetically confirmed record of *M. wireni* from the North and Baltic Seas, as well as from North America. Sikorski and Buzhinskaya (1998) and Sikorski and Bick (2004) provided new records of *M. wireni* for the Barents, White, Pechora, Kara, Laptev, East Siberian and Chukchi Seas (all in the Arctic Russia). Complete information on *M. wireni* records is given in Table S3 (mapped in Figure 3).

## Discussion

### *Phylogeny of Marenzelleria*

Earlier analyses of phylogenetic relationships among *Marenzelleria* species were based on sequences of the mitochondrial DNA only. Blank and Bastrop (2009) and Syomin et al. (2017) used 16S, COI and *cytochrome b* sequence data, whereas Radashevsky et al. (2021) used only 16S and COI data. All three analyses suggested a basal position for two Arctic species, *M. arctia* and *M. wireni*, and, thus, an Arctic origin of *Marenzelleria*. However, they resulted in slightly different hypotheses about relationships among boreal Northwest Atlantic species. Blank and Bastrop (2009: fig. 1) proposed sister relationships of *M. bastropi* with *M. viridis: neglecta* (*bastropi+viridis*), while Syomin et al. (2017: fig. 6) proposed sister relationships of *M. bastropi* with *M. neglecta: viridis* (*bastropi+neglecta*). The analysis by Radashevsky et al. (2021: fig. 1) suggested sister relationships of *M. neglecta* with *M. viridis: bastropi* (*neglecta+viridis*). The latter hypothesis was supported by the present analysis of five genes and seems more plausible given that the nodes in the phylogenetic tree received higher support.

Tree topology (Figure 1) and the distribution of *Marenzelleria* along the Atlantic coast of North America (see Radashevsky et al. 2021: fig. 2) allow us to assume that the boreal Northwest Atlantic species had evolved from a common ancestor that was originally widely distributed along the Atlantic coast of North America. Population isolation and adaptation in isolated habitats apparently led to the divergence of the ancestral population and the origin of three species: *M. bastropi*, *M. neglecta* and *M. viridis*.

### *Identification of Marenzelleria species*

Although *Marenzelleria* includes only five described species, identifying worms by morphological characteristics is often challenging. Diagnostic characters such as the length of the nuchal organs, the distribution of branchiae, hooded hooks and sabre chaetae are age-dependent and individually variable (Sikorski and Bick 2004: table 1, fig. 6; Syomin et al. 2016: table), resulting in complex correlations between body size (width, length) and morphological features (see Radashevsky et al. 2021: figs. 5, 6). The usual presence of only anterior fragments in samples further complicates their identification. Hence, molecular data is an important complementary diagnostic tool (Röhner et al. 1996a; Bastrop and Blank 2006; Blank et al. 2008).

Blank et al. (2008) developed a PCR/RFLP protocol and provided a molecular identification key for three *Marenzelleria* species from the Baltic Sea. The protocol used the restriction fragment length polymorphism (RFLP) method, when the polymerase chain reaction (PCR) products of two mitochondrial DNA gene segments (*16S*, *COI*) were cut using restriction enzymes. Sikorski and Bick (2004) provided the first key for the morphological identification of five *Marenzelleria* species. The key was mainly based on species specific arithmetic differences of three numerical characters: the first chaetiger with neuropodial (ventral) hooded hooks (VHH), the first chaetiger with notopodial (dorsal) hooded hooks (DHH), and the last chaetiger with branchiae (Br). Because all these characters, as well as the nuchal organs length (the fourth important diagnostic character), were found to be size/age-dependent, Sikorski and Bick (2004) suggested that the key be used only for specimens more than 1.0 mm wide. Bick (2005) modified and updated the first morphological key, but noted that the revised version was best for specimens larger than 1.2 mm wide. Below is a further update to the key, which includes morphological data recently published by various authors.

### *Key to species of Marenzelleria*

*(complete adults with body width greater than 1.2 mm)*

1. Nuchal organs extending beyond chaetiger 3 ..... 2
- . Nuchal organs not extending beyond middle of chaetiger 3..... 3

2. Nuchal organs to middle of chaetiger 5. Branchiae throughout most of body. Up to 180 chaetigers in total ..... *M. wireni*
- . Nuchal organs to middle of chaetiger 4. Branchiae on less than one third of body (up to 69 branchiate chaetigers). Up to 250 chaetigers in total ..... *M. neglecta*
3. Nuchal organs to middle of chaetiger 3. More than 20 chaetigers between start of hooded hooks in neuro- and notopodia. More than 60 branchiate chaetigers ..... *M. bastropi*
- . Nuchal organs to middle of chaetiger 2. Less than 20 chaetigers between start of hooded hooks in neuro- and notopodia. Branchiate chaetigers more or less than 60 ..... 4
4. Up to 40 branchiate chaetigers. Dorsal hooded hooks start in postbranchiate chaetigers (Br-DHH < 0). Up to 120 chaetigers in total ...  
..... *M. arctia*
- . Up to 130 branchiate chaetigers. Dorsal hooded hooks start in branchiate chaetigers (Br-DHH > 0). Up to 250 chaetigers in total.....  
..... *M. viridis*

## Conclusions

Polychaetous annelids, and especially spionids, are among the lists of alien species in the various regions discussed in the present work. Ballast water and hull fouling have been major vectors for the introduction of polychaetes, including spionids, worldwide (Çinar 2013). Because polychaetes are mainly marine or estuarine, transoceanic or long-distance coastal movements have been considered as main transportation routes. Ships transporting ballast via canals have rarely been reported and may be underestimated. The invasion of the Sea of Azov by *M. neglecta* thus likely occurred via transport of worms (larvae or adults or both) from the Baltic Sea via the Baltic Sea–Volga–Don Canal (Syomin et al. 2016, 2017). Similarly, *M. arctia* may have invaded the Baltic Sea by transport from the White Sea via the White Sea–Baltic Sea Canal.

The Arctic *M. arctia* has been extensively examined based on the North European populations located far from the type locality of the species in the Beaufort Sea (Alaska). However, molecular analysis of specimens, initially identified morphologically as *M. arctia*, found significant genetic distances between the Baltic, Barents and White Seas and those worms from the Kara Sea. This genetic distance, as well as the isolated estuarine habitats of these Arctic worms, and the large geographic distance between the type locality of *M. arctia* in the Beaufort Sea (Alaska) and northern Europe, raises doubts about the conspecificity of North American, North European and Northwest Pacific populations. Sequence data from the North American population are urgently needed to characterize the molecular identity of the species and to verify the conspecificity of the disjunct

populations. A similar situation may exist for *M. wireni*, which has been reported from isolated estuaries along much of the Arctic coast. Molecular data are only available for the Spitsbergen population of *M. wireni* from the Greenland Sea, which is approximately 800 kilometers from the proposed type locality of the species in Franz Josef Land (Kara Sea) and thousands of kilometers from the Eurasian mainland. Further molecular studies will be required to ensure a systematic revision of *Marenzelleria* and further development of the hypothesis of the origin and evolution of these spionid polychaetes.

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### Authors' contribution

VIR conceptualized and designed the research, collected and analyzed the data, prepared figures, and tables, wrote the manuscript and approved the final version. VVP sequenced material, analyzed and interpreted genetic data, prepared tables, reviewed drafts and approved the final version. TVN sequenced material, organized funding provision and approved the final version. ABT collected material, interpreted the data and approved the final version.

### Ethics and permits

Marine worms of the annelid family Spionidae are not listed as species at risk of extinction. The authors have complied with all policies relative to the collection and handling of marine species, and no ethics approval was required.

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Sampling location data and museum registration numbers of *Marenzelleria arctica*.

**Table S2.** Sampling location data and museum registration numbers of *Marenzelleria neglecta*.

**Table S3.** Sampling location data and museum registration numbers of *Marenzelleria wireni*.

**Table S4.** List of the museums and collections (and their acronyms) holding the examined or reported specimens of *Marenzelleria* spp.

**Table S5.** Taxa, sampling location data, references, and GenBank accession numbers of sequences used in the present study.

**Table S6.** Uncorrected pairwise average genetic distances ( $p$ , in %) between *Marenzelleria* spp. calculated in the analysis of five genes.

**Table S7.** Uncorrected pairwise average genetic distances ( $p$ , in %) between *Marenzelleria* spp. calculated in the analysis of two mitochondrial genes.

**Figure S1.** Majority rule consensus tree of the Bayesian inference analysis of the combined *COI* (612 bp) and *I6S* (322 bp) sequences (934 bp in total) of *Marenzelleria* spp. rooted with sequences of *Marenzelleria arctica*.

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