diversity

Article

Resettlement of *Eurytemora velox* (Crustacea: Copepoda) in Europe, the Urals and Western Siberia

Natalia Sukhikh [1,2,*], Petr Garibian [2] and Elena Chertoprud [2]

[1] Zoological Institute RAS, 1 Universitetskaya Embankment, Saint-Petersburg 199034, Russia
[2] A.N. Severtsov Institute of Ecology and Evolution, Leninsky Prospect 33, Moscow 119071, Russia; petr.garibyan21@mail.ru (P.G.); horsax@yandex.ru (E.C.)
* Correspondence: natalia.sukhikh@zin.ru

Abstract: The recent distribution of the Ponto-Caspian calanoid copepod, *Eurytemora velox*, in Western and Eastern Europe has been well-documented; however, there are no studies on the genetic diversity of the recently discovered Western Siberian species population. To contribute towards filling this gap, genetic diversity and distribution were investigated for *E. velox* collected in the Urals and Western Siberia of the Russian Federation to compare with the European populations. In this study, 44 specimens were dissected for the morphological studies, and 22 specimens of *E. velox* from different geographical points were analyzed for mitochondrial cytochrome oxidase I (CO1), nuclear ITS1-ITS2 (nITS) and nuclear ribosomal RNA (18SrRNA) gene fragments. Analysis of the CO1 gene region showed that the studied populations from the Urals and Western Siberia differ significantly from European ones and represent a separate genetic line. However, the nuclear nITS and 18SrRNA genes, as well as the results of morphological analysis, did not show such isolation of the Trans-Ural populations from those in Europe. As in many studies on *E. velox*, we found atypical structural features of the fifth prosomal leg among females in the Ob Bay. Their share was 36% of the number of studied individuals. The CO1 shows that the divergence of genetic lines occurred approximately in the Middle Pleistocene, and the species itself is Paleogene–Neogene by origin. Based on these data, a different scenario of *E. velox* distribution or possible refuge survival is discussed.

Keywords: Copepoda; *Eurytemora velox*; morphometry; molecular–genetic methods; cytochrome oxidase I (COI); invasive species; Arctic region; Western Siberia; origin; distribution; refuge

check for updates

Citation: Sukhikh, N.; Garibian, P.; Chertoprud, E. Resettlement of *Eurytemora velox* (Crustacea: Copepoda) in Europe, the Urals and Western Siberia. *Diversity* **2024**, *16*, 47. https://doi.org/10.3390/d16010047

Academic Editor: Luc Legal

Received: 2 November 2023
Revised: 19 December 2023
Accepted: 30 December 2023
Published: 11 January 2024

1. Introduction

The significant range extension of Ponto-Caspian *Eurytemora velox* (Lilljeborg, 1853) in fresh and brackish European waters over the past decades was facilitated mainly due to the distribution of the species, it is supposed, through the big river basins interconnected by human-made canals, so-called migration corridors. The species was described for the first time in 1853 from the Baltic Scania [1] and then recorded in the lagoons of the Northern Caspian–Volga mouth in 1897 [2]. The range of this species covers the area from the Ponto-Caspian and Mediterranean region to Scandinavia, Iceland and the Volga River in Russia [3–9]. Such a wide range of an admittedly Ponto-Caspian [8] species is supposed to be a result of high species invasiveness.

In 2015–2019, *E. velox* was found in water bodies of Western Siberia for the first time [10–12], particularly in the Yamal Peninsula, as well as in the lower Ob River and its tributaries (below the city of Nizhnevartovsk) in the Ob and Gydan Bays. According to Gerasimova and coauthors [13], the species was also found much further south, in the Irtysh River of the Tyumen region. It is worth noting that intensive hydrobiological studies of the zooplankton in the lower Ob in 1979–2008 [14] resulted in a list of zooplankton species with more than 200 species, but *E. velox* was not observed at that time. As for Central Siberia, like the Novosibirsk region and further east, the species has not been seen so far [15,16].

In particular, *E. velox* is not found in the zooplankton of the Lena Delta [17–19], although the region has been quite well sampled and studied in recent years due to many ecological programs in the location of the Lena Delta Nature Reserve.

Records of *E. velox* in the region of the Urals are not documented in the literature sources. All the studies indicate the absence of any eurytemorid copepods in the zooplankton for this region [20–22]. The records of the species that are most geographically close to the Urals indicate the presence of *E. velox* populations in the lower tributaries of the Ural River back to 1923, as found by Muraveysky [23] and, in 1938, by Bening [24]. Repeatedly, in the 1960s, this species was also on the list of zooplankton taxa for that area [25]. In studies of zooplankton in the lower tributaries of the Ural River in 2016 [26], *E. velox* was not found; the authors noticed that the number of zooplankton taxa has decreased significantly compared to previous observations, and *E. velox* could not be caught simply with a net. Meanwhile, further south, Kazakh researchers noted the presence of *E. velox* in small brackish lakes of West Kazakhstan [27–29].

No genetic studies have been conducted for the recently found Siberian populations but, considering that *E. velox* was not there before 2015, it was supposed to be a European invasion [10–12].

Genetic studies of *E. velox* populations from Ukraine, Poland, Belarus and Central Russia (middle Volga River) show the close relationship between those populations [30,31]. Just a few nucleotide substitutions (0.8%) in the CO1 part of the gene differ between the Ukrainian (Black Sea drainage basin) and Central Russian (Caspian Sea drainage basin) populations. The Polish and Belarusian populations are genetically close to that of Central Russia. Here, we present the first study that combines genetic and morphological analysis of the Southern Ural and Ob Bay (Western Siberia) populations of *E. velox* in comparison with those from Ukraine, Belarus and the Volga reservoirs. Our data offers an interesting insight into the origin of the species and possible ways of spreading in the Urals and Western Siberia.

2. Material and Methods

2.1. Material and Methods Used for Morphological Analysis

Specimens of *E. velox* from the Rybinsk, Cheboksary and Kuybyshev Reservoirs (Russian Federation), the Feofania ponds in Kyiv (Ukraine) and the Mukhavets River (Belarus) were used for morphological studies (Figure 1, Table 1).

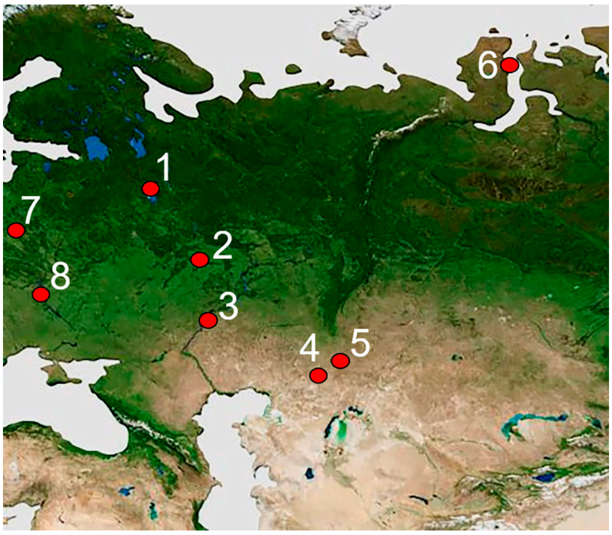

Figure 1. Map of studied populations of *Eurytemora velox*: 1—Rybinsk Reservoir; 2—Cheboksary Reservoir; 3—Kuybyshev Reservoir; 4—Zavodskoy Pond on the Ural River in Magnitogorsk (Chelyabinsk Region); 5—Smolino Lake in Chelyabinsk (Chelyabinsk Region); 6—Ob Bay (Yamal Region); 7—Mukhavets River (Brest region); 8—Feofania ponds (Kyiv).

Table 1. Studied material of *Eurytemora velox*.

Sampling Site	Drainage Basin	Number of Morphologically Studied Specimens	Number of Genetically Studied Specimens	Date of Sampling	Collector
Rybinsk Reservoir, Russia	Volga River, Caspian Sea basin	8 females		2017	Valentina Lazareva, IBIV RAS
Cheboksary Reservoir, Russia	Volga River, Caspian Sea basin	1 female	4 females	2020	Valentina Lazareva, IBIV RAS [31]
Kuybyshev Reservoir, Russia	Volga River, Caspian Sea basin	1 female		2018	Valentina Lazareva, IBIV RAS
Feofania ponds, Ukraine	Dnieper River, Black Sea basin	7 females	3 females	2020	Larysa Samchyshyna, IF NAAS [31]
Mukhavets River, Belarus	Western Bug, Baltic Sea basin	6 females		2019	Vasilii Vezhnovets, NAS of Belarus
Ob Bay, Russia	Kara Sea basin	11 females	2 females	2022	Petr Garibian, IEE RAS
Magnitogorsk Lake	Ob River, Kara Sea basin	14 females	2 females	2022	Alisa Neplyukhina, IEE RAS
Lake Smolino, Southern Urals region, Russia	Ob River, Kara Sea basin		7 females	2021	Maria Baturina, IB Komi SC UB RAS [32]
Dziwna Channel, Poland	Baltic Sea basin		4 specimens	2020	[33]

The specimens were identified by the morphological traits identified in [10,34,35].

The samples were sorted under stereomicroscopes (Micromed MC3 Zoom LED (Micromed, Saint Petersburg, Russia) and Olympus SZX10 (Olympus Corp., Tokyo, Japan) and measured with a ToupCam 14.0 MP camera (Hangzhou ToupTek Photonics Co., Hangzhou, China) with the software packages TopView and LevenhukLite (Public joint stock company Levenhuk, Saint Petersburg, Russia). The dissected appendages were placed on slides in pure glycerol, covered with Canadian balsam and a glass cover, and examined at up to 1000× resolution with immersion oil under compound microscopes Olympus CX 22 LED (Olympus Corp., Tokyo, Japan), Levenhuk D870T (Public joint stock company Levenhuk, Saint Petersburg, Russia) and Zeiss IMAGER (Zeiss, Oberkochen, Germany), equipped with the Nomarski system for differential interference contrast microscopy. Photographs were taken under a LOMO BLM-L microscope (LOMO, Saint Petersburg, Russia) equipped with a ToupCam 14.0 MP camera and TopView software version 9.12.226. The final photos were made by combining 8–12 images at different focal depths using the TopView software.

To compare *E. velox* populations, both sexes were analyzed. In total, 19 different measurements were made (Table 2) based on features proposed in [36]. They are the body size and width, caudal rami length and width, length of the abdominal segments and the number of spines on the exopod of the fifth pair of legs (P5) in the females. For the males, we measured body size, caudal rami length and width, the presence of spines on 10–11 segments of the right antennule and the length proportions of the segments of the P5.

Table 2. Measurements and morphometric indexes in studied populations of *Eurytemora velox*. Mean ± standard deviation (Min–Max), +/− is presence or absence.

Trait	**Gulf of Ob**	**Magnitogorsk**	**Kyiv**	**Belarus**	**Rybinsk Reservoir**	**Cheboksary Reservoir**	**Kuibyshev Reservoir**
Female							
Body length, mm mean +/− SD	1.85 +/− 0.11	1.61 +/− 0.06	1.77 +/− 0.02	1.51 +/− 0.02	1.43 +/− 0.06	1.48	1.52
Body length, mm range	1.63–1.97	1.54–1.72	1.68–1.86	1.42–1.58	1.68–1.87		
N of studied individuals	11	14	7	6	8	1	1
Body Length/Weight	2.89	3.16	3.9 +/− 0.06	3.5 +/− 0.08	3.6 +/− 0.09	3.32	3.31
Thoracic wings	+	+	+	+	+	+	+
Abdominal segments 1–3 length ratio	1.62/1/1.83	1.53/1/1.84	1.71/1/1.97	1.79/1/1.87	1.68/1/1.93	1.79/1/1.82	1.88/1/1.8
Furcal L/W	5.73 +/− 0.05	5.06 +/− 0.07	5.83 +/− 0.18	5.27 +/− 0.15	4.67 +/− 0.2		
Furcal L/W range	5.48–5.95	4.74–5.62	4.84–6.93	4.42–6.16	4.31–6.17		
Body L/Furcal L	7.91 +/− 0.14	8.3 +/− 0.20	5.91 +/− 0.09	6.28 +/− 0.16	6.47 +/− 0.08		
Body L/Furcal L range	6.79–8.48	6.91–9.53	5.37–6.58	5.57–7.77	6.16–6.91		
Furcal W/Body W	0.06 +/− 0.00	0.08 +/− 0.00	0.12 +/− 0.00	0.11 +/− 0.00	0.12 +/− 0.00		
Furcal W/Body W range	0.06–0.07	0.06–0.09	0.10–0.14	0.10–0.13	0.10–0.13		
Furcal L/Body W	0.37 +/− 0.01	0.39 +/− 0.01	0.69 +/− 0.15	0.58 +/− 0.02	0.58 +/− 0.15		
Furcal L/Body W range	0.34–0.4	0.35–0.46	0.58–0.78	0.46–0.64	0.52–0.65		
Furcal W/Body L	0.02 +/− 0.00	0.02 +/− 0.00	0.03 +/− 0.00	0.03 +/− 0.00	0.03 +/− 0.00		
Furcal W/Body L Range	0.021–0.025	0.020–0.028	0.025–0.036	0.028–0.034	0.026–0.034		
P5th leg. Exo. 1							
P5 right leg/left leg: one spine/one spine	+	+	+	+	+	+	+
P5 right leg/left leg: one spine/two spines	+	−	−	−	−	−	−
P5 right leg/left leg: two spines/one spine	+	−	−	+	−	−	−
P5 right leg/left leg: two spines/two spines	+	−	−	−	−	−	−
Male							
Body length mean +/− SD	1.69 +/− 0.11	1.35 +/− 0.1		1.09 +/− 0.17	1.14 +/− 0.01		
Body length range	1.54–1.86	1.23–1.51		1.06–1.13	1.12–1.15		
N of studied individuals	11	11		4	2		
Right 1st antennular spine 10	+	+		+/−	−		
Right 1st antennular spine 11	+	+		+/−	−		
Furcal L/W	7.24 +/− 0.07	5.92 +/− 0.13		5.86 +/− 0.16	5.43 +/− 0.46		
Furcal L/W range	6.88–7.65	4.85–6.43		5.24–9.46	5.17–6.04		
Body L/Furcal L	6.67 +/− 0.27	7.85 +/− 0.29		6.00 +/− 0.09	7.54 +/− 0.26		

Table 2. *Cont.*

Trait	Gulf of Ob	Magnitogorsk	Kyiv	Belarus	Rybinsk Reservoir	Cheboksary Reservoir	Kuibyshev Reservoir
Body L/Furcal L range	5.74–8.45	6.75–9.44		5.64–6.36	6.86–8.05		
Furcal W/Body L	0.021 +/− 0.001	0.022 +/− 0.001		0.029 +/− 0.001	0.025 +/− 0.002		
Furcal W/Body L range	0.017–0.024	0.018–0.026		0.026–0.032	0.021–0.028		
Left 5th leg length ratio Cox:Bas.:Exo1:Exo2	1/1.46/1.7/2.25	1/1.6/1.8/2.2		1/1.15/1.38/1.73	1/1.2/1.4/1.9		
Right 5th leg length ratio Cox:Bas:Exo1:Exo2:Exo3	1/2.3/2.4/2.16/1.55	1/2.3/2.8/2.3/1.5		1/2.1/2.3/2.0/1.83	1/2.0/2.3/1.9/1.7		

The principal component analysis (PCA) with an unconstrained ordination method was used to test the morphological differentiation between the studied populations in the PAST 4.03 software.

2.2. Genetic Studies

ExtractDNA Blood (Eurogen, RF) was used for lysis, DNA extraction and purification following the manufacturer's protocol. Species morphological identification was made following the taxonomical keys [8,36].

We obtained sequences of the mitochondrial cytochrome oxidase 1 gene (CO1) and nuclear genes ITS1-ITS2 (nITS) and 18SrRNA. The primers used for the amplifications were cited in the article by Sukhikh and coauthors [35]. The used conditions of the CO1 PCR are published in our previous work [35]. The PCR conditions for the used sets of primers started with DNA denaturing at 95 °C for 30 s, followed by 38 cycles of 30 s denaturing at 95 °C, 30 s, annealing at 50 °C for the nITS and 55 °C for the 18SrRNA, 70 s of extension at 72 °C and then a final extension at 72 °C for 7 min.

The amplification products were purified with a Cleanup Mini purification kit (Eurogen, RF), then sequenced using a 3500xL Genetic Analyzer with a BigDye Terminator v3.1 Cycle Sequencing Kit. Both DNA strands were sequenced to confirm the accuracy of each sequence.

The sequences were aligned using the algorithm CLUSTAL W [37] implemented in BIOEDIT v.7.2 [38], with manual editing of ambiguous sites.

Phylogenetic reconstructions were performed by maximum likelihood (ML) and a general time–reversible model with gamma rate distribution (G), and without invariable sites (I), with the MEGA XI software package [39]. The best-fitting model under the ML criterion was selected from the "Bayesian Information Criterion" (BIC) and "Akaike Information Criterion" (AIC) output of jMODELTEST v0.1.1 [40]. The level of nucleotide differences between the species was calculated with the Tamura–Nei 93 model, as commonly used for *Eurytemora* species [31,32,34,35,41], in the MEGA XI software package [39].

Paleontological information and points based on molecular phylogenetic data were used for the estimation of possible divergence age for different clades. The maximum likelihood test in MEGA XI was applied to test the fit of our data to molecular clock models [39,42]. Nucleotide substitution parameters (using a maximum likelihood substitution model statistical method) were also made in MEGA XI, based on the lowest BIC (Bayesian information criterion) scores. The null hypothesis of equal evolutionary rate throughout the tree was not rejected at a 5% significance level.

The divergence times of lineages were estimated using BEAST2 (ver. 2.6) [43] with Bayesian inference, using the calibrated Yule model for the tree prior and the strict clock model. BEAST2 used a random tree with 6×10^6 generations and a sample frequency of 5×10^3 generations. We chose the rate of substitutions of 2.8% per MYR, as no properly calibrated rate estimates are available for copepods, in particular, and for other crustaceans, estimates of COI rates in the range of 1.4% to 4% divergence per MYR have been obtained [44–48]. The chosen value yields results that are congruent with the paleontological data.

As calibration points of the species and genetic lineages (with 15% standard deviations), the fossil-based minimum age was applied for the split: *Lepidurus*/*Triops* 122 MYA [49,50], plus, additional calibration points based on molecular phylogenetic data were used within the outgroups: *Acartia*–*Eurytemora*—170 MYA [51], *Acanthocyclops*–*Mesocyclops* group—24 MYA [51] and inner group *Eurytemora* aff. *affinis*–*Eurytemora carolleeae*—11.5 MYA [41].

3. Results

3.1. Genetic Studies

Sequences of the mitochondrial cytochrome C oxidase 1 gene (CO1) and nuclear genes ITS1–ITS2 (nITS) and 18SrRNA from *E. velox* specimens collected in the Ob Bay and a lake in Magnitogorsk were obtained (Table 3). All the other sequences for the rest of the populations were used from published data [31,32,52] for comparison (Table 3).

Table 3. Sequences with accession numbers of GenBank used in the study.

Sampling Place	CO1	nITS	18SrRNA	Source
Cheboksary Reservoir, Russia	2 sequences MZ373321-MZ373322	3 sequences MZ400502-MZ400504	1 sequence MZ373302	[31]
Feofania ponds in Kyiv, Ukraine	3 sequences MZ373318-Z373320	3 sequences MZ400499-MZ400501	OR921659	[31]
Gulf of Ob, RF	1 sequence OR578622	1 sequence OR583034	2 sequences OR578728-OR578729	This paper
Magnitogorsk Lake, Russia		2 sequences OR583032-OR583033	2 sequences OR921657-OR921658	This paper
Lake Smolino, Southern Urals region, Russia	3 sequences OR578619-OR578621	7 sequences OR583025-OR583031	3 sequences OR578725-OR578727	[32]
Dziwna Channel, Poland	2 sequences MT146446-MT146445	3 sequences MT787212-MT787214		[33]

The results of the CO1 (520 bp length) gene region analysis showed that the specimen from the Ob Bay is identical to the *E. velox* population from Chelyabinsk and belongs to one shared haplotype (Figure 2). These populations in the CO1 gene differ from the previously studied *E. velox* from Europe [36] by 4%.

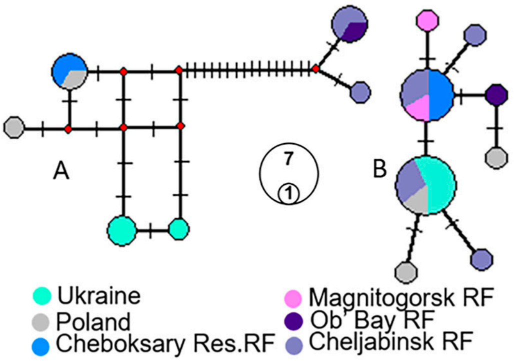

Figure 2. Median-joining network of haplotypes of *Eurytemora velox* built on 11 and 19 nucleotide sequences of the CO1 (**A**) and nITS (**B**) part of genes. The lines indicate the number of mutations between haplotypes. Circles are proportional to the haplotype frequency.

The results of the nITS (803 bp length) gene region analysis showed that three specimens from the Ob Bay and Magnitogorsk are represented by three different haplotypes, one of which is shared with one of the Chelyabinsk haplotypes (Figure 2). These haplotypes are closer to the Volga population [31], which differs from the Kyiv one by 0.1% of nucleotide substitutions.

Analysis of the DNA sequences of the conservative 18SrRNA (333 bp length) gene showed no differences among the studied populations of *E. velox* from the Ob Bay, Chelyabinsk, Kyiv and the Volga, as expected.

Our molecular clocks revealed a Paleogene–Early Neogene *E. velox* origin (13.3–41.1 MYA). The European and the Ural–Siberian lineages of *E. velox* were differentiated possibly in the mid-Pleistocene 0.133–0.928 MYA according to our molecular clock calibration (Figure 3).

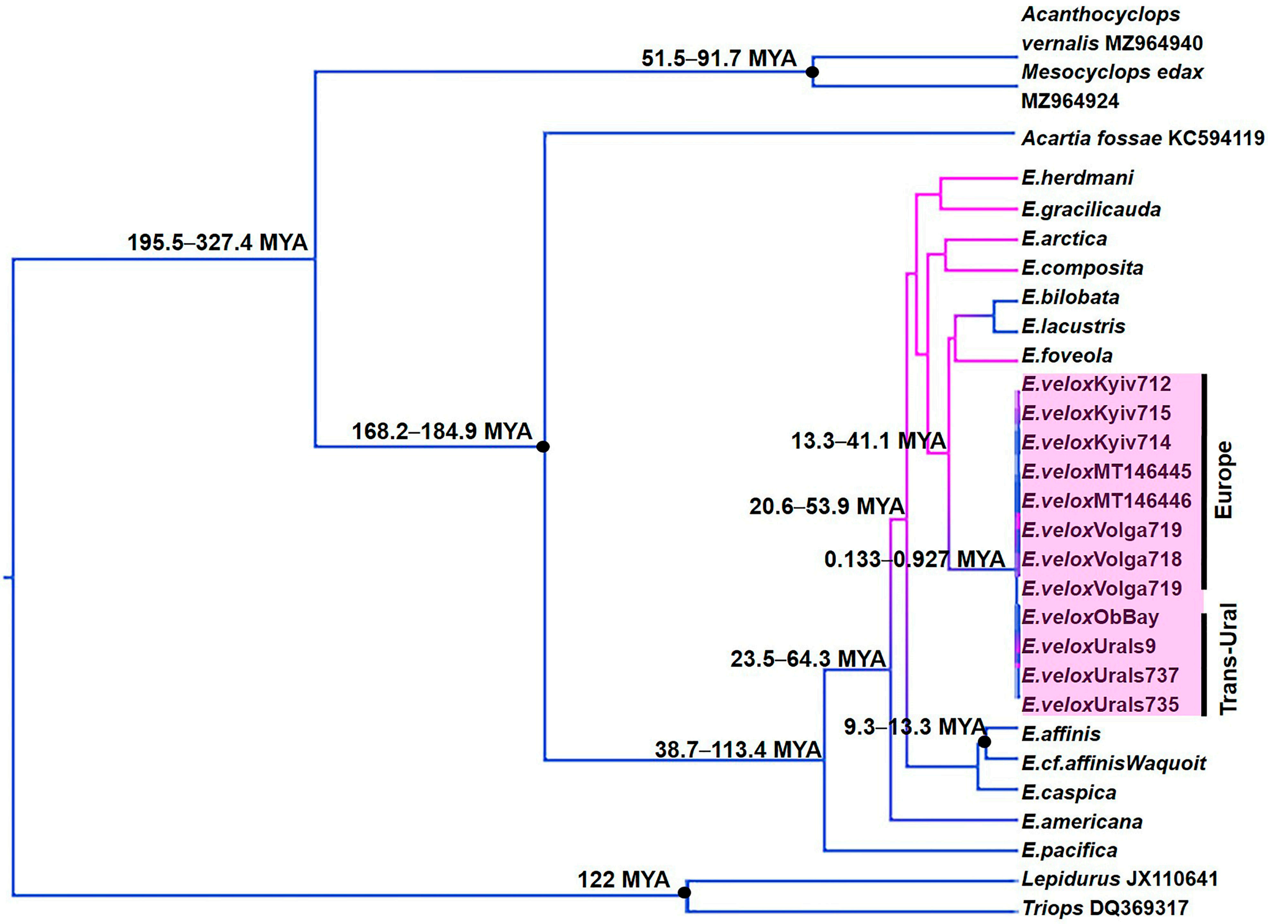

Figure 3. Phylogenetic ultrametric tree constructed in BEAST2 v.2.6 for *Eurytemora velox*. Phylogenetic analysis of the mitochondrial data set (CO1) with strict clock estimates based on fossil calibration point (*Lepidurus*/*Triops* 122 MYA). Statistic support (posterior probabilities (BI)) of branches is coded by the color gradient from pink (low) to blue (high). Probable time of divergence between clades (in MYA) based on the rate of substitutions of 2.8% per MYA given in nodes.

3.2. Morphological Studies

Among all of the studied populations, the population from Ob Bay turned out to be the most variable in the morphology of the P5 in females and the biggest in body size for both males and females. In this population, Exp I of the P5 in females was found to be morphologically highly variable (Figure 4, Table 2).

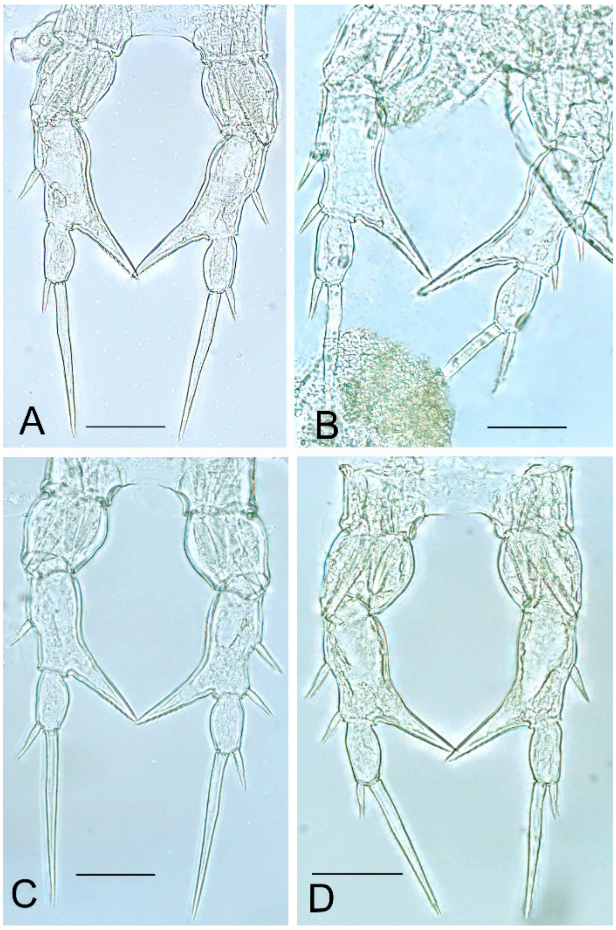

Figure 4. Females of *Eurytemora velox* from the Ob Bay (Yamal Region): (**A**–**D**)—different types of leg 5. Scale 50 µm—(**A**–**D**).

The proportion of females with a P5 structure deviating from the normal with one spine on exopod 1 was very high (four out of eleven individuals)—36% in the Ob Bay population. In the Belarusian specimens, one of the six studied females had two spines on one of the exopods on the P5. Among the other studied populations, we did not observe deviations from the normal morphology, and all the P5s of females had a standard structure with one spine on exopod 1. All males of the population from Magnitogorsk and Ob Bay had spines on the 10th and 11th segments of the geniculate antennae (Figure 5). In the Belarusian population, among the four studied males, we observed either the absence or presence of these spines. No spines at all on the 10–11 segments of the geniculate antennae of males were observed on specimens from the Rybinsk Reservoir.

To assess the morphological variability between the studied populations of females, the following indices were calculated: L/W Body, Furcal L/W, Furcal W/Body W, Furcal W/Body L and abdominal segment length ratio indices 1–3 (Table 2). The indices used among females showed that, as in the genetic data, the Belarusian and Volga populations do not differ significantly from each other. The populations of Belarus and Ukraine differ in one index (L/W Body). At the same time, the populations from Magnitogorsk and Ob Bay differ significantly from each other, both in body size and in the proportions of furcal branches and abdominal segments, as well as in the degree of variability of the P5. Females from the Ob Bay population are larger in size, have thinner and longer furcal rami, and the first segment of the abdomen is longer than in individuals from the population of reservoirs near Magnitogorsk (Table 2, Figures 5A and 6A).

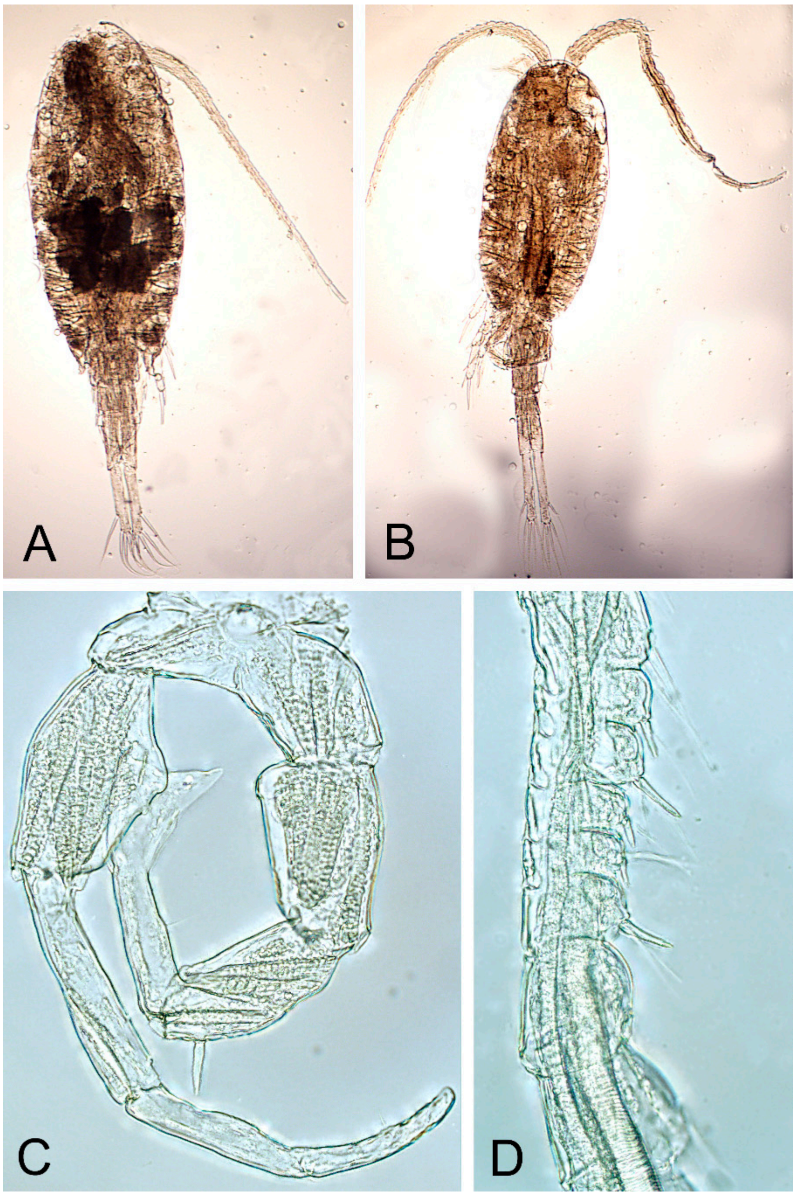

Figure 5. *Eurytemora velox* from the Ob Bay (Yamal Region): (**A**)—female: (**B**)—male; (**C**)—leg 5 of male; (**D**)—7–14 segments of male antennule (ventral). Scale 500 µm—(**A**,**B**); scale 50 µm—(**C**); scale 30 µm—(**D**).

These populations also differ in the following indices: L/W Body, Furcal L/W, Furcal W/Body W, Furcal W/Body L and L abdominal segment 1/L abdominal segment 2. As for the general appearance of females from all the studied populations—the ones from Kyiv have the slimmest shape and the highest L/W Body index (3.9 +/− 0.06). They are also the largest of all: the body length is 1.85 +/− 0.11 mm. The stockiest females came from the Ob Bay; their L/W Body ratio is 2.89. The population from the Rybinsk Reservoir had the smallest size of all the females studied—1.43 +/− 0.06 mm. The longest furcae in comparison with their width were found in females from Kyiv—almost seven times longer than the width. The average for the population from Kyiv was 5.83 +/− 0.18. Slightly shorter furcae were observed in females from the Ob Bay—5.73 +/− 0.05. The next population in terms of the length/width ratio of furcae is the Belarusian one—5.27 +/− 0.15. In the Volga population from the Rybinsk Reservoir and the population of females from the Urals; the ratio was 4.67 +/− 0.2 and 5.06 +/− 0.07, correspondingly. The Ob Bay and Ural populations of females have the shortest furcae in relation to the length of the entire body; the index fluctuates in the range of 6.79–9.53. In European populations, this index is approximately the same on average and does not exceed 7.77.

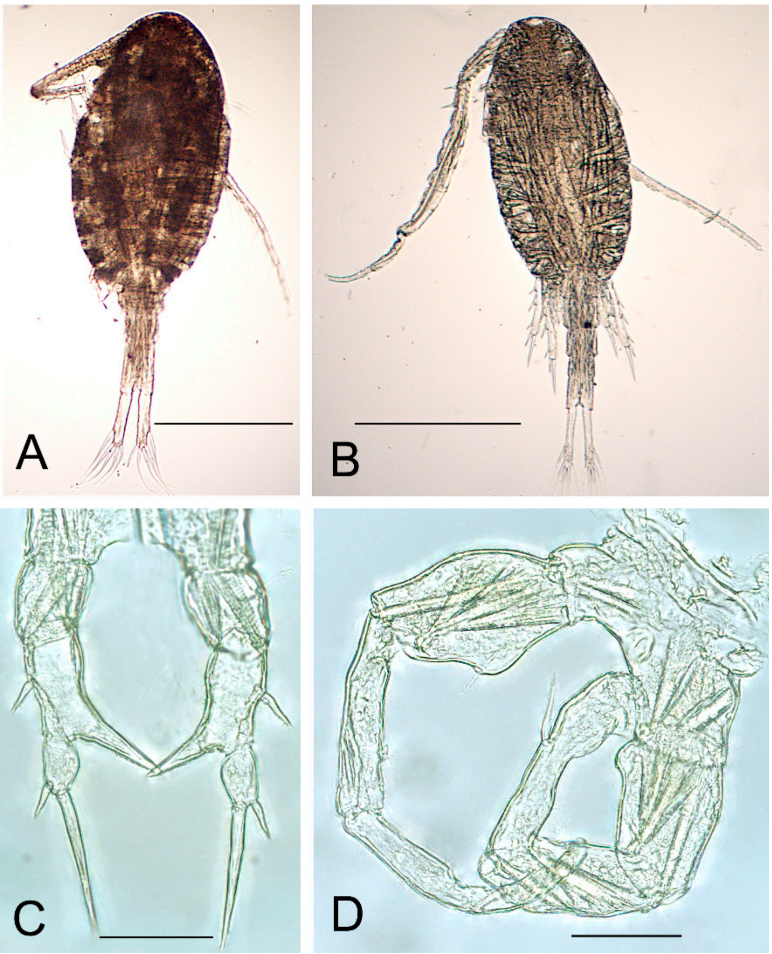

Figure 6. *Eurytemora velox* from the Zavodskoy Pond in Magnitogorsk (Chelyabinsk Region): (**A**)—female; (**B**)—male; (**C**)—P5 of female; (**D**)—P5 of male. Scale 500 µm—(**A**,**B**); scale 50 µm—(**C**,**D**).

On a PCA plot built using the indices, females look more or less uniform. The PCA showed the maximum weight of furcal index and body length/width ratio (Figure 7A).

To assess the morphological variability between the studied populations of males, the ratio indices of the segments of the left and right parts of the P5, as well as Furcal L/W, Body L/Furcal L, Furcal W/Body W, Furcal W/Body L, were measured (Table 2). Among the males, the Belarusian and Volga populations also do not significantly differ from each other in any of the selected indices. At the same time, genetically identical populations from Magnitogorsk and Ob Bay differ significantly from each other in body size, proportions of furcal rami and P5 segment lengths. Specimens from the Ob Bay population have larger sizes, thinner and longer furcal rami, as well as shorter bases of the left P5 and the first exopod segment of the right leg P5 relative to the coxa than in individuals from the population of reservoirs near Magnitogorsk. (Figures 5B and 6B). Populations from the Ob Bay and Magnitogorsk have significant differences in the following indices: Furcal L/W, Body L/Furcal L, Furcal W/Body W, Left P5 Bas/Cox and Right P5 Exo1/Cox (Table 2). The Ob Bay and Magnitogorsk populations are significantly different from the Volga and Belarusian populations according to all the selected Left P5 indices. The Magnitogorsk and Belarusian populations also differ in the ratio of the lengths of exopods 1–3 to the length of the Right P5 coxa (Exo/Cox). The Belarusian population differs from the Ob

Bay population in the ratio of the lengths of the basipod, exopod 3 and coxa (Bas/Cox, Exo3/Cox). The largest males were found in the Ob Bay (1.69 +/− 0.11 mm) and the smallest in Belarus (1.09 +/− 0.17 mm). The longest furcae in comparison with the width were observed in males from the Ob Bay; the length/width ratio is 7.24 +/− 0.07, and the shortest are in males from the Rybinsk Reservoir (Volga region); the index does not exceed six.

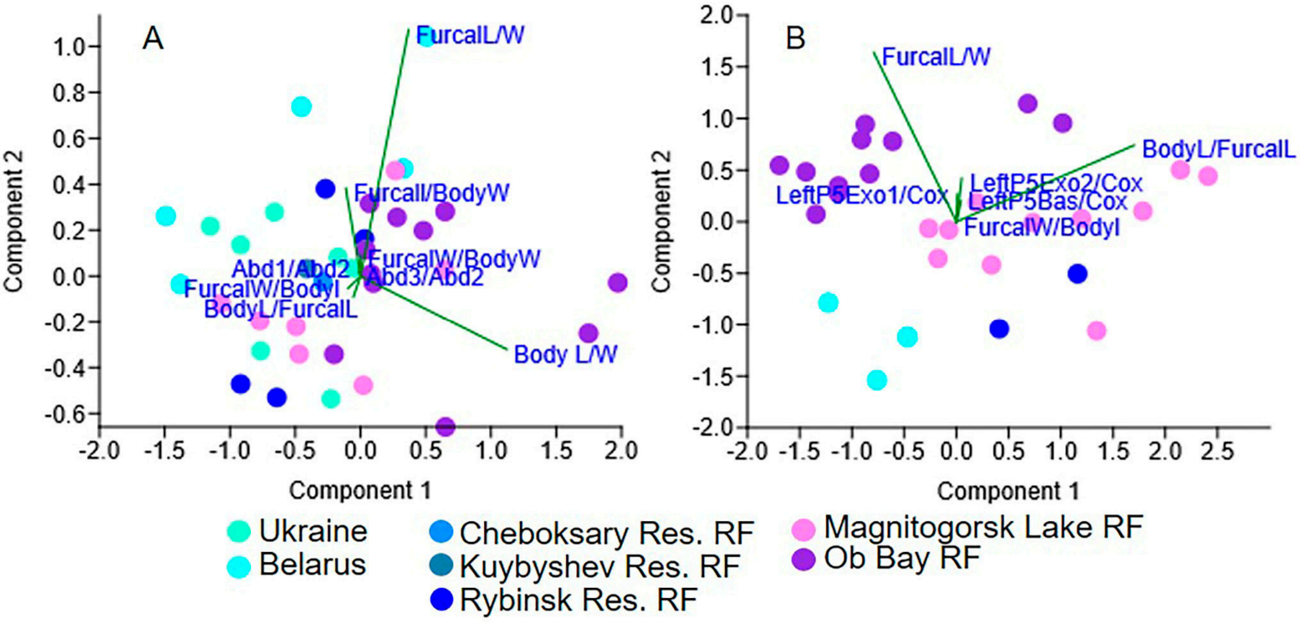

Figure 7. Two-dimensional PCA ordination of *Eurytemora velox* female (**A**) and male (**B**) populations on the two first axes. Morphological indices are indicated as vectors.

On the PCA plot, built using the indices, Ob Bay males appear the most isolated. The PCA showed the maximum weight of furcal index and the ratio of furcal length to whole body length (Figure 7B).

4. Discussion

4.1. Morphological Studies

According to a study by Gaviria and Forro [36], almost all the populations they studied from Austria, Hungary and Romania, belonging to the Black Sea basin, had variability in the structure of the P5 of females and had spines on the 10–11 segments of the male geniculate antennae. Variation in the structure of the P5 of females was also noted for other European populations (Poland, France and Hungary) [52–54]. It is supposed that such variability is connected with environmental stress as a result of human activity (pollution, hydraulic work, etc.) [36]. There is also a hypothesis that this is a result of species hybridization between *E. velox* and *E. affinis* [32,55], although these species are never found cohabiting. In our studies, among populations of *E. velox* from the basins of four seas, the Caspian, Black, Baltic and Kara Seas, populations from the Ob Bay and Belarus show the highest variability in terms of the mentioned above morphological characteristics, while other populations (Volga region, Kyiv) have stable structural features typical of *E. velox*. But in general, we did not find significant morphological differences among all the studied populations.

4.2. Genetic Studies

The results of the CO1 and nITS gene region analysis showed that the specimens from the Ob Bay are close to the *E. velox* population from Chelyabinsk, which was studied earlier [32]. The Southern Ural populations (Chelyabinsk and Magnitogorsk) differ in the CO1 gene from the previously studied *E. velox* from Europe [31] by 4% (Figure 2A), which corresponds to the subspecies level among Copepoda [35,56,57]. Interestingly, the nITS genes did not show such homogeneity of the populations from the Southern Urals, and eight sequences are represented by five haplotypes that differ from each other by 1–2 replacements (Figure 2B). These haplotypes are identical to the Kyiv and Volga populations [31], which differ by 0.1% of nucleotide substitutions in the nITS genes.

Analysis of the DNA sequences of the conservative 18SrRNA gene shows no differences between the studied populations from the Ob Bay, Chelyabinsk (Southern Urals), Kyiv, and the Volga of *E. velox*, as expected. A similar picture, when we observe high intraspecies and species divergence in mitochondrial genes and very low one among nuclear genes, was observed among different groups of Copepoda [35,56,57]. It was shown that the level of interspecific differences in the CO1 gene among *Eurytemora* is 12.0–30.2%. The nuclear 18SrRNA gene is more useful in a wide phylogenetic analysis, and the level of pairwise divergence among the *Eurytemora* species is 0.4–12.9%. The level of differences among *Eurytemora* species in nITS genes is 4.9–34.5%.

Data from molecular clock calculations based on a region of the mitochondrial CO1 gene showed that *E. velox* appeared approximately at the end of the Paleogene–early Neogene (13.3–41.1 MYA). The divergence within the species into European (Ponto-Caspian) and Ural-Siberian lines occurred no later than 0.928 MYA, which is approximately in the Middle Pleistocene, probably during one of the interglacial phases. Later, the Ponto-Caspian *E. velox* split into the Volga and Black Sea genetic lines. We assume that the common ancestor of all present populations survived glaciation in a refuge in the Ponto-Caspian region. From there, judging by the network of CO1 haplotypes, one lineage spread towards Europe and later split up into two other lineages (probably the Black Sea and Caspian), while the other line spread along the eastern part of the Ural Mountains and gave source to the Ural–Siberian populations. The common origin of all the lines is confirmed by the structure of the haplotype network, built on sections of nuclear genes, nITS, in which we see that some of the Ural haplotypes are grouped with the Ukrainian and some with the Cheboksary haplotypes.

4.3. Urals and Western Siberian Populations

Considering the significant number of previous studies on the zooplankton species composition in the reservoirs of Chelyabinsk and Magnitogorsk [20–22], the recent appearance of *E. velox* obviously indicates an invasive nature. Until recently, long-term studies of the lower reaches of the Ob Bay (1979–2008) also did not reveal the presence of this species [14], although there is no data on this species for the Ob Bay. Taking into account the proximity of the studied reservoirs to the lower Ural River, where the population of *E. velox* has long been known [23,24], it is possible that this population is a donor for the Chelyabinsk region and the Arctic coast of the Western Siberia. At the same time, despite the short distance between Magnitogorsk and Chelyabinsk, which is only three hundred kilometers, Magnitogorsk stands on the Ural River, which flows into the Caspian Sea, and the reservoirs of Chelyabinsk already belong to the Kara Sea basin, as does the Ob Bay. Considering the migration routes of birds, a bird-mediated dispersal ability by diapausing eggs [56,57] may explain the existence of these two populations divided by a geographical barrier, although, for species of the genus *Eurytemora*, there is no confirmed evidence of this fact in the literature. The most common means of the genus invasion is in the ballast water of ships. Hovewer this seems unlikely since the drainage basins of the Caspian and Kara Seas are not connected. We can assume that the species spread in Siberia naturally along water corridors from the lower Ural River to the Arctic coast. Nevertheless, given the huge distance covered and the geographical barrier—a watershed between the basins of the Caspian and Kara Seas, this hypothesis needs more confirmations. The origin of the Trans-Ural population of *E. velox* from the Caspian Sea and spread in the Ural River seems to us the most likely. Unfortunately, we do not have for study genetic material from the lower Ural River, the basin of the Caspian Sea. Furthermore, the data from another population inhabiting the northern tributary of the Caspian Sea—the Volga River and its drainage basin—do not confirm this hypothesis, since the Volga populations show genetic similarity to the European ones, but not to the Trans-Ural populations [31]. It is obviously that the increase in sampling efforts in the Caspian region and Siberia is needed and future genetic studies will help to identify pathway of distribution. In general, a similar pattern of different genetic lineage distribution within one drainage basin and within small areas is also known for other species of the genus *Eurytemora* [35].

Due to the lack of studies on the Ob Bay before the recent data, it should not be excluded that the bay mouths in Western Siberia (Ob Bay, Gydan Bay) are the natural habitats for this species. Those areas might be the initial spot from where *E. velox* began to spread toward the south and explains *E. velox* records in the lower Ob River up to Nizhnevartovsk only in recent years [11] and our new records of species in Chelyabinsk and Magnitogorsk. In this case, the idea of a reverse direction in the Siberian invasion of *E. velox* from the north to the southern Ponto-Caspian reservoirs may explain such a pattern in genetic results. In addition, it cannot be excluded that we are observing in Siberia not an invasive species, but a species having a disrupted range as a result of the last glaciation.

Another possible scenario for the appearance of *E. velox* populations under study in the Urals and Western Siberia is the introduction of populations from Europe that we have not yet studied, at least genetically. For example, in the article by Gaviria and Forro [36], several populations from the Black Sea drainage basin (Danube River from Austria to Romania) are morphologically described, and, according to the available data, these several studied populations themselves look quite heterogeneous in a small area. Nevertheless, we do not see any significant differences between our specimens and those described in this article. In general, according to the morphological indices and appearance of females from Regelsbrunn (Austria), cited in the article by Gaviria and Forro [36], this Austrian population looks most similar to the Volga eurytemoras. Also, the population from the Baltic region has not been genetically studied, except for the findings about the invaders in Belarus and Poland, genetically identical to the Volga population [30,31]. The morphological works of Kiefer [58] and Lucks [52] describe *Eurytemora* from water bodies of Germany and Poland (again the Baltic region) showing variability in relation to the P5 in females, although spines on the 10–11 segments of the geniculate antennae were invariably present.

4.4. Baltic Population

In addition to populations from Belarus, Poland and Germany, fresh and brackish water populations from the Neva River, the Gulfs of Finland and Bothnia are also known from the Baltic region [2]. Probably, unlike the Belarusian and Polish populations of invaders, these populations are native. Unfortunately, we were unable to find any of these populations, despite annual sampling in the Neva River and in the Gulf of Finland. Material from the Gulf of Bothnia was also available to the author, but it did not contain the species sought. It was assumed that, in addition to the Ponto-Caspian refuge, the species could survive glaciation in a certain Baltic refuge (Littorina Sea), from which the Baltic population originated [59]. It seems that today, if these ancestral populations exist, they inhabit very limited habitats.

It should be noted that the original description of the species *E. velox* was made specifically of those from the Baltic Sea—the coast of the Gulf of Bothnia in Sweden [1]. In the picture of the male in the original description, we see extremely narrow and long furcal rami, the length/width ratio of which is 12, and the ratio of body length to furcal length is 5.52. We did not observe such values for any of the populations we studied or previously described. At the same time, the female furcal index of 5.3–5.5 is quite consistent with our data, although the ratio of abdominal segments differs: 1.38/1/1.83. It is also worth noting that the original description does not provide information about the variability of the P5 structure in females. In general, this population could be special and different from the populations we studied in Europe and could be the possible progenitor of the Trans-Ural *E. velox*.

Thus, we observed a new CO1 genetic lineage of *E. velox* from the Trans-Ural region, which does not differ in the nITS and 18SrRNA part of genes from known ones from Europe. The origin of the *E. velox* species in the Urals and Western Siberia remains not entirely clear. It is obvious that the species is currently actively spreading beyond the Urals and Western Siberia. We can say with certainty that the source, in this case, is not the populations we studied from Europe since, genetically, the European populations are significantly different and diverged from the Trans-Ural populations about the Middle Pleistocene according to our date.

Author Contributions: N.S. and E.C.: conceptualization of the paper; N.S. and E.C. wrote the main manuscript text and prepared the figures; validation, P.G. All the authors discussed the results and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The studies were supported by the Russian Science Foundation, 22-14-00258.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author. Accession numbers for sequences provided in Table 3 can be found in GenBank (https://www.ncbi.nlm.nih.gov accessed on 12 December 2023).

Acknowledgments: We are very grateful to Larysa Samchyshyna IF NAAS for the valuable comments and great im-proving of the paper. We are thankful to Valentina Lazareva, IBIV RAS, Vasilii Vezhnovets, NAS of Belarus, Alisa Neplyukhina, IEE RAS and Maria Baturina IB, Komi SC UB RAS for their help in sampling. We also thank the anonymous reviewers and editor-in-chief for their careful reading of our manuscript and their many insightful comments and suggestions. The genetic part of the work was performed in the Core Facilities Centre "Taxon", ZIN RAS, and sequencing was performed in the Evrogen Joint Stock Company (Moscow).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lilljeborg, W. *De Crustaceis ex Ordinibus Tribus: Cladocera, Ostracoda et Copepoda, in Scania Occurrentibus*; Berlingska Boktryckeriet: Lund, Sweden, 1853; pp. 1–222. Available online: https://www.biodiversitylibrary.org/page/39045453 (accessed on 12 December 2023).
2. Sars, G.O. Temorella lacinulata (Fischer). In *Yearbook of the Zoological Museum of the Imperial Academy of Sciences*; Printing House of the Imperial Academy of Sciences: St. Petersburg, Russia, 1897; p. 66.
3. Pandourski, I.; Stoichev, S. Sur la faune de l'eau interstitielle littorale de la bande sableuse entre le système lacustre de "Chabla-Ezeretz" et la Mer Noire. *Hist. Nat. Bulg.* **1999**, *10*, 125–131.
4. Pandourski, I.; Evtimova, V. First record of *Eurytemora velox* (Lilljeborg, 1853) (Crustacea, Copepoda, Calanoida) in Iceland with morphological notes. *Hist. Nat. Bulg.* **2006**, *17*, 35–38.
5. Vranovský, M. *Eurytemora velox* (Lilljeborg, 1853) (Crustacea, Copepoda), a new immigrant in the middle Danube. *Biol. Bratisl.* **1994**, *49*, 167–172.
6. Samchyshyna, L.; Gromova, Y.; Zorina-Sakharova, K. Recent distribution of Eurytemora velox (Lilljeborg, 1853) (Copepoda, Calanoida) in brackish and fresh waters of Ukraine. *Crustaceana* **2020**, *93*, 275. [CrossRef]
7. Quintana, X.; Antón-Pardo, M.; Bas-Silvestre, M.; Boix, D.; Casamitjana, X.; Compte, J.; Cunillera-Montcusí, D.; Gascón, S.; Menció, A.; Obrador, B.; et al. Identifying critical transitions in seasonal shifts of zooplankton composition in a confined coastal salt marsh. *Aquat. Sci.* **2021**, *83*, 69. [CrossRef]
8. Borutsky, E.S.; Stepanova, L.A.; Kos, M.S. *Key to Calanoida Fresh Waters of the USSR*; Nauka: Leningrad, Russia, 1991; p. 504. (In Russian)
9. Sabaneev, P.L. *Eurytemora velox* (Lill.) in the flat part of Dnieper. *Zb. Pr. Dnipr. Biol. Stn.* **1930**, *5*, 201–203.
10. Ermolaeva, N.I. Zooplankton of different types of water bodies of the Yamal Peninsula in 2015. *Sci. Bull. Yamalo-Nenets Auton. Okrug* **2016**, *2*, 56–62. (In Russian)
11. Ermolaeva, N.I. Species composition and spatial distribution of zooplankton in the Gulf of Ob and Gydan Bay. In Proceedings of the III All-Russian Scientific Conference with International Participation, Yekaterinburg, Russia, 5–10 October 2020; Institute of Water and Environmental Problems SB RAS: Novosibirsk, Russia, 2017; pp. 91–99. (In Russian).
12. Abdullina, G.K.; Bondar, M.S. Zooplankton of water bodies of the Arctic tundra of the Yamal Peninsula. In Proceedings of the XXI International Scientific and Practical Conference I, Moscow, Russia, 20 May 2019; pp. 297–304. (In Russian).

13. Gerasimova, A.; Sharapova, T.; Gerasimov, A.; Babushkin, E.; Gerasimova, A. Alien Species of Hydrobionts in the Tyumen Region. Version 1.4. Institute of the Problems of Northern Development is a Subdivision of Tyumen Scientific Centre SB RAS. Occurrence Dataset. 2022. Available online: https://www.gbif.org/dataset/93d951ea-c37d-4062-9007-dbe966f7f9d9 (accessed on 29 August 2023).
14. Semenova, L.A.; Aleksyuk, V.A. Zooplankton of the Lower Ob. *Bull. Ecol. For. Landsc. Sci.* **2010**, 156–169. (In Russian)
15. Ermolaeva, N.I. The fauna of brackish-water and freshwater invertebrates (Rotifera, Cladocera, Copepoda) of lakes in the south of Western Siberia. In Proceedings of the Materials of the International Scientific Internet Conference «Biodiversity of Terrestrial and Aquatic Animals and Bioresources», Kazan, Russia, 12 February 2013. (In Russian).
16. Yermolaeva, N. Some results concerning zooplankton species composition in the lakes of the south of Ob-Irtysh interfluve. *World Sci. Cult. Educ.* **2015**, *50*, 390–398.
17. Abramova, E.; Vishnyakova, I.; Boike, J.; Abramova, A.; Solovyev, G.; Martynov, F. Structure of freshwater zooplankton communities from tundra waterbodies in the Lena River Delta, Russian Arctic, with a discussion on new records of glacial relict copepods. *Polar Biol.* **2017**, *40*, 1629–1643. [CrossRef]
18. Chertoprud, E.S.; Novichkova, A.A. Crustaceans in the meiobenthos and plankton of the thermokarst lakes and polygonal ponds in the Lena River Delta (Northern Yakutia, Russia): Species composition and factors regulating assemblage structures. *Water* **2021**, *13*, 1936. [CrossRef]
19. Chertoprud, E.S.; Novichkova, A.A.; Tsyganov, A.N.; Vorobjeva, L.V.; Esaulov, A.S.; Krylenko, S.V.; Mazei, Y.A. Species diversity and driving factors of benthic and zooplanktonic assemblages at different stages of thermokarst lake development: A case study in the Lena River delta (Middle Siberia). *Diversity* **2023**, *15*, 511. [CrossRef]
20. Rechkalov, V.V.; Kuznetsova, A. Study of zooplankton in the Magnitogorsk Reservoir. *Bull. Chelyabinsk St. Univ.* **2008**, *17*, 91–97. (In Russian)
21. Rechkalov, V.V.; Permyakova, T.A. Composition and seasonal dynamics of zooplankton in Lake Second (Chelyabinsk region). *Bull. Chelyabinsk St. Univ.* **2010**, *4*, 86–93. (In Russian)
22. Golubok, O.V.; Rechkalov, V.V. Identification of signs of anthropogenic eutrophication of dimictic lakes based on indicators of zooplankton development. *Bull. Orenbg. State Univ.* **2013**, *10*, 61–68. (In Russian)
23. Muraveysky, S.D. Observations on the spring plankton of the river. The Urals and its oxbow lakes. *Russ. Hydrobiol. J.* **1923**, *11*, 14–23. (In Russian)
24. Bening, A.L. Materials on the Hydrobiology of the River Ural. *Proc. KazFAN USSR* **1938**, *11*, 153–157. (In Russian)
25. Saduakasova, P.E. Zooplankton in the lower reaches of the Ural River. Fish resources of water bodies of Kazakhstan and their use. *Alma-Ata Sci.* **1966**, *5*, 346–351. (In Russian)
26. Demesinova, G.T.; Kurashov, E.A. Current state of zooplankton in the lower reaches of the Ural River. *Water Chem. Ecol.* **2016**, *12*, 42–53.
27. Troshina, T.T.; Idayatov, P.V.; Gorelov, O.P. Zooplankton of Lake Chelkar (June, 2005). Biodiversity of the animal world of Kazakhstan, problems of conservation and use. In *Book of Abstracts of Conference: 75-th Anniversary of the Institute of Zoology "Biodiversity of the Fauna of Kazakhstan, Problems of Conservation and Use." 17–20 October*; Adebiet: Almaty, Kazakhstan, 2007; pp. 81–83. (In Russian)
28. Troshina, T.T.; Idayatov, P.V.; Gorelov, O.P. Structural and functional characteristics of zooplankton in the Bitik reservoir and lake. In *Book of Abstracts of Conference: «Biodiversity of the Animal World of Kazakhstan, Problems of Conservation and Use". 17–20 October*; Adebiet: Almaty, Kazakhstan, 2007; pp. 84–86. (In Russian)
29. Krupa, E.G.; Dobrokhotova, O.V.; Stuge, T.S. *Fauna of Calanoida (Crustacea: Copepoda) of Kazakhstan and Adjacent Territories*; Etalon Print: Almaty, Kazakhstan, 2016; p. 208. (In Russian)
30. Vezhnovets, V.; Litvinova, A. Representatives of the genus *Eurytemora* Giesbrecht, 1881 (Calanoida, Temoridae) in aquatic ecosystems of Belarus. *Crustaceana* **2020**, *93*, 283–298. [CrossRef]
31. Sukhikh, N.M.; Lazareva, V.I. First Results of a Molecular Genetic Analysis of the European Invader *Eurytemora velox* (Crustacea, Calanoida). *Inland Water Biol.* **2022**, *15*, 201–203. [CrossRef]
32. Sukhikh, N.M.; Fefilova, E.B. The first record of *Eurytemora velox* (Lilljeborg, 1853) (Crustacea, Calanoida) outside of Europe, genetic identification with surprise. *Russ. J. Biol. Invasions* **2023**, *4*, 139–140. [CrossRef]
33. Rymaszewska, A.; Kirczuk, L. Eurytemora carolleeae (Alekseev & Souissi, 2011) as a Non-Native Copepod Invader into Central European Large River. Available online: https://www.ncbi.nlm.nih.gov/nuccore/?term=Eurytemora+velox (accessed on 12 December 2023).
34. Sukhikh, N.M.; Lazareva, V.I.; Alekseev, V.R. Copepod *Eurytemora caspica* Sukhikh et Alekseev, 2013 (Crustacea, Calanoida) in Volga and Kama River Reservoirs. *Inland Water Biol.* **2020**, *13*, 198–205. [CrossRef]
35. Sukhikh, N.M.; Souissi, A.; Souissi, S.; Winkler, G.; Castric, V.; Holl, A.-C.; Alekseev, V. Genetic and morphological heterogeneity among populations of *Eurytemora affinis* (Crustacea: Copepoda: Temoridae) in European waters. *Comptes Rendus Biol.* **2016**, *339*, 197–206. [CrossRef] [PubMed]
36. Gaviria Melo, S.; Forro, L. Morphological characterization of new populations of the copepod *Eurytemora velox* (Lilljeborg, 1853) (Calanoida, Temoridae) found in Austria and Hungary. *Hydrobiologia* **2000**, *438*, 205–216. [CrossRef]

37. Thompson, J.D.; Higgins, D.J.; Gibson, T.J.; Clustal, W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef]
38. Hall, T.A. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Res.* **1999**, *41*, 95–98.
39. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
40. Posada, D. jModelTest: Phylogenetic model averaging. *Mol. Biol. Evol.* **2008**, *25*, 1253–1256. [CrossRef]
41. Winkler, G.; Souissi, S.; Poux, C.; Castric, V. Genetic heterogeneity among *Eurytemora affinis* populations in Western Europe. *Mar. Biol.* **2011**, *158*, 1841–1856. [CrossRef]
42. Takezaki, N.; Rzhetsky, A.; Nei, M. Phylogenetic test of the molecular clock and linearized trees. *Mol. Biol. Evol.* **1995**, *12*, 823–833. [CrossRef]
43. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchêne, S.; Fourment, M.; Gavryushkina, A.; Heled, J.; Jones, G.; Kühnert, D.; De Maio, N.; et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [CrossRef]
44. Knowlton, N.; Weigt, L.A. New dates and new rates for divergence across the Isthmus of Panama. *Proceeding R. Soc. B Biol. Sci.* **1998**, *265*, 2257–2263. [CrossRef]
45. Zofkova, M.; Timms, B.V. A conflict of morphological and genetic patterns in the australian anostracan *Branchinella longirostris*. *Hydrobiologia* **2009**, *635*, 67–80. [CrossRef]
46. Marino, I.A.M.; Pujolar, J.M.; Zane, L. Reconciling deep calibration and demographic history: Bayesian inference of post glacial colonization patterns in *Carcinus aestuarii* (Nardo, 1847) and *C. maenas* (Linnaeus, 1758). *PLoS ONE* **2011**, *6*, e28567. [CrossRef] [PubMed]
47. Ketmaier, V.; Marrone, F.; Alfonso, G.; Paulus, K.; Wiemann, A.; Tiedemann, R.; Mura, G. Mitochondrial DNA regionalism and historical demography in the extant populations of *Chirocephalus kerkyrensis* (Branchiopoda: Anostraca). *PLoS ONE* **2012**, *7*, e30082. [CrossRef] [PubMed]
48. Sworobowicz, L.; Mamos, T.; Grabowski, M.; Wysocka, A. Lasting through the ice age: The role of the proglacial refugia in the maintenance of genetic diversity, population growth, and high dispersal rate in a widespread freshwater crustacean. *Freshw. Biol.* **2020**, *65*, 1028–1046. [CrossRef]
49. Kotov, A.A.; Taylor, D.J. Mesozoic fossils (>145 Mya) suggest the antiquity of the subgenera of *Daphnia* and their coevolution with chaoborid predators. *BMC Evol. Biol.* **2011**, *11*, 129. [CrossRef]
50. Wolfe, J.M.; Daley, A.C.; Legg, D.A.; Edgecombe, G. Fossil calibrations for the arthropod Tree of Life. *Earth-Sci. Rev.* **2016**, *160*, 43–110. [CrossRef]
51. Eyun, S. Phylogenomic analysis of Copepoda (Arthropoda, Crustacea) reveals unexpected similarities with earlier proposed morphological phylogenies. *BMC Evol. Biol.* **2017**, *17*, 23. [CrossRef]
52. Lucks, R. Die crustaceen und rotatorien des messinasees. *Ber. Westpreuss Bol-Zool.* **1937**, *59*, 59–101.
53. Petit, G.; Schachter, D. Contributions a l'titude biologique de la Camargue. Sur la presence du genre Eurytemora en Camargue. *Bull. Mus. Hist. Nat. Marseille* **1947**, *7*, 103–114.
54. Forro, L.; Gulyzis, P. *Eurytemora velox* (Li11jeborg. 1853) (Copepoda. Calanoida). *Misc. Zool. Hung.* **1992**, *7*, 53–58.
55. Smirnov, C.C. On the so-called hybrids in the genus *Eurytemora* Giesbr. (Copepoda). *Rep. Acad. Sci. USSR* **1940**, *26*, 615–618. (In Russian)
56. Bucklin, A.; LaJeunesse, T.C. Molecular genetic variation of *Calanus pacificus* (Copepoda: Calanoida): Preliminary evaluation of genetic structure and subspecific differentiation based on mt-DNA sequences. *Calif. Coop. Ocean. Fish. Investig. Rep.* **1994**, *35*, 45–51.
57. Kochanova, E.; Nair, A.; Sukhikh, N.; Väinölä, R.; Husby, A. Patterns of cryptic diversity and phylogeography in four freshwater copepod crustaceans in European lakes. *Diversity* **2021**, *13*, 448. [CrossRef]
58. Kiefer, F. *Freilebende Copepoda. Das Zooplankton der Binnengewässer*; Teil 2, E. Schweizerbartsche Verlag: Stuttgart, Germany, 1978; p. 380.
59. Ekmann, S. Uber das crustacea plankton des Ekoln (Mälaren) und überver schiedene Kategorien von marinen Relikten in schwedischen Binnenseen. *Zool. Stud. Tillägn. T. Tullberg. Upsala Almqvist Wik-Sells* **1907**, 42–65.