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High genetic differentiation of *Daphnia pulex* s.lat. (Cladocera: Anomopoda) in northern Eurasia: evidence from an analysis of the mitochondrial 12S rRNA gene

Высокая генетическая дифференциация *Daphnia pulex* s.lat. (Cladocera: Anomopoda) в Северной Евразии: данные анализа фрагмента гена 12S митохондриальной ДНК

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KEY WORDS: 12S rRNA, *Daphnia pulex* sensu lato, phylogeny, genetic differentiation, polymorphism, evolutionary history, Northern Eurasia.

КЛЮЧЕВЫЕ СЛОВА: 12S рРНК, *Daphnia pulex* s.lat., филогения, генетическая изменчивость, полиморфизм, эволюционная история, Северная Евразия.

ABSTRACT. This work presents new data on population genetics structure of two species from *Daphnia pulex* sensu lato species group [*D. pulex* Leydig, 1860, and *D. middendorffiana* Fischer, 1851 (Anomopoda: Daphniidae)] from water bodies of Northern Eurasia. A fragment of the noncoding mitochondrial 12S rRNA gene was used to reconstruct phylogenetic relationships between the species, to identify genealogical links between haplotypes, and to evaluate genetic polymorphism of these species and their populations. A preliminary assessment of evolutionary history of these species is provided. We revealed an extremely high genetic diversity within the *D. pulex* s.lat. group in Northern Eurasia, primarily indicating a hidden species diversity. The magnitude of genetic polymorphism pointed to a mixture of different mitochondrial lineages and possibly species; this finding was most pronounced in the populations from Arctic regions. High genetic diversity of these closely related species in Northern Eurasia reflects paleogeographic events of the Late Pleistocene. Overall, there is an obvious need for a large-scale morphological revision combined with genetic analysis within this group to obtain a clear picture of their species diversity in this vast region in particular and in the Holarctic as a whole.

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РЕЗЮМЕ. В настоящей работе представлены новые данные о популяционно-генетической структуре видов группы *Daphnia pulex* sensu lato [*Daphnia pulex* Leydig, 1860 и *D. middendorffiana* Fischer, 1851 (Anomopoda: Daphniidae)] из водоемов Северной Евразии. Для реконструкции филогенетических отношений между видами, выявления генеалогических связей между гаплотипами, для оценки генетического полиморфизма видов и их популяций был использован фрагмент некодирующего митохондриального гена 12S рРНК. Дана предварительная оценка эволюционной истории видов. Полученные данные выявили чрезвычайно высокое генетическое разнообразие *D. pulex*, что в первую очередь свидетельствует о скрытом видовом разнообразии. Уровень генетического полиморфизма указывает на смешение различных митохондриальных линий, а, возможно, и видов, что

наиболее сильно проявляется в популяциях из арктических регионов. Высокое генетическое разнообразие этих близкородственных видов в Северной Евразии представляет собой отражение палеогеографических событий позднего плейстоцена. В целом, совершенно очевидна необходимость масштабной морфологической ревизии в пределах данной группы видов для четкого представления об их видовом, генетическом разнообразии и филогеографической структуре в этом обширном регионе и в целом в Голарктике.

Introduction

Northern Eurasia is a vast region that is important for biogeographic and phylogeographic studies. Comprehensive research on various taxa of cladocerans, as conducted at morphological and molecular-genetic levels [Bekker *et al*., 2016; 2018; Kotov, 2016; Zuykova *et al*., 2018, 2019; Kotov, Taylor, 2019], has confirmed that this region is a transitional zone between "eastern" and "western" faunas of the water fleas (Crustacea: Cladocera). To date, after the comparative analysis of the results of phylogeographic studies on different cladocerans, a sufficiently complete picture of the formation of their biodiversity in Northern Eurasia has been created [Korovchinsky *et al*., 2021]. Nevertheless, a final check of the internal logic of this model, it is necessary to evaluate phylogeographic structure of as many cladocerans taxa as possible. The practice of science in this field shows that geographically and taxonomically expanding research leads to the discovery of new mitochondrial lineages and phylogroups and possibly even new species [Zuykova *et al*., 2024a, b]. The latter statement is true for both relatively well-investigated Northern-Eurasian populations of *Daphnia magna* Straus, 1820, *Daphnia longispina* sensu lato (s.lat.), and *D. curvirostris* s.lat. [Bekker *et al*., 2018; Zuykova *et al*., 2018, 2019; Kotov *et al*., 2021] as well as for populations that have only just begun to be studied: *D. cristata* Sars, 1862, *D. longiremis* Sars, 1862 [Zuykova *et al*., 2024b], *D. middendorffiana* Fischer, 1851, and *D. pulex* Leydig, 1860 [Zuykova *et al*., 2022].

Of particular interest is the species group *D. pulex* s.lat., firstly, because their Northern Eurasian populations are at the initial phases of their studies (both in morphological and genetic aspects), and secondly, a comprehensive study on this species group will make a substantial contribution to the understanding of the cryptic diversity concept. Thirdly, taking into account the widespread obligate parthenogenesis within the group *D. pulex* s.lat., special attention should be given to the effects of different reproduction types (cyclic and obligate parthenogenesis) on both genetic diversity and on the formation of phylogeographic patterns within relatively short periods. Right now, information about phylogeographic and genetic structure of populations of the species group *D. pulex* s.lat. from Northern Eurasia is almost absent; research in this field is limited to only one publication so far [Zuykova *et al*., 2022]. The most complete data on the *D. pulex* s.lat. species group concerning molecular-genetic and morphological identification, phylogeographic patterns, types of reproduction, and invasion concern European and North-American populations [Colbourne *et al*., 1998; Weider *et al*., 1999; Mergeay *et al*., 2006; Jose, Dufresne, 2010; Dufresne *et al*., 2011; Jeffery *et al*., 2011; Crease *et al*., 2012; Duggan *et al*., 2012]. The necessity of a comparative analysis of existing information with the data on Northern-Eurasian populations is obvious because this is an important prerequisite for identifying general patterns in the formation of present-day cladocerans phylogeographic patterns throughout the Holarctic.

The aim of this work was to investigate phylogenetic relationships, genetic diversity, specific features of population genetics structure, to identify spatial localization of divergent mitochondrial lineages, and to assess evolutionary history of two closely related species — *D. pulex* and *D. middendorffiana* — in Northern Eurasia.

Materials and Methods

Sampling sites. Zooplankton samples containing individuals of *D. pulex* and *D. middendorffiana* were collected from water bodies located in different regions of Northern Eurasia: Kamchatka, Chukotka, Magadan Oblast, Republics of Sakha (Yakutia) and Karelia, the Baikal region, and Yamalo-Nenets Autonomous Okrug (YANAO; Suppl. Table 1, Fig. 1). Samples were fixed with 96% ethanol immediately after collection. Some samples (including those from Canadian water bodies) were kindly provided by A.A. Kotov (A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow).

DNA sequencing. Before genomic-DNA extraction, each individual was identified up to the species level if possible; in case of a good preservation of individuals, their photos were performed using a binocular microscope equipped with an Altami video camera (Altami, St. Petersburg, Russia) at 4× and 10× magnification. Total genomic DNA was extracted from each individual by a 5% solution of Chelex 100 resin (Bio-Rad, USA). Amplification of fragments of the noncoding 12S rRNA gene from mitochondrial DNA (mtDNA) was performed using the BioMaster HS-Taq PCR-Color (2×) reagent (Biolabmix, Novosibirsk, Russia, www.biolabmix.ru) in M111 thermocycler (BIS-N Ltd., Novosibirsk, Russia). Thermal profiles and primer sequences were identical to those described earlier [Zuykova *et al*., 2013]. The PCR products were sequenced with forward and reverse primers at the Syntol Company (Moscow, Russia, www.syntol.ru). The raw nucleotide sequences from species *D. pulex* and *D. middendorffiana* (143 sequences 568–573 bp long) were manually edited in BioEdit v.7 software [Hall, 1999] and were aligned for subsequent analyses using the MAFFT v.7 algorithm by means of a Web servicehttps://mafft.cbrc.jp/alignment/server/ [Katoh *et al*., 2019].

Phylogenetic reconstructions. A phylogenetic tree was reconstructed based on a fragment of the 12S rRNA gene from mtDNA. The following sequences from the GenBank (NCBI) database served as an outgroup in the analyses: *D. obtusa* Kurz, 1875 (Acc. No. AY626364 and AY626366), *D. ambigua* Scourfield, 1947 (AF064175, AF523723, AF523716, AF523728, AF523732, and AF523733), and *D. mitsukuri* Ishikava, 1986 (MH632069). Additionally, 12S rRNA sequences of *D. pulex*, *D. pulicaria* Forbes, 1893, and *D. middendorffiana* from the GenBank database were included in the analysis (see accession numbers in Suppl. Table 1). The models that best described the evolution of the sequences under study were selected in the

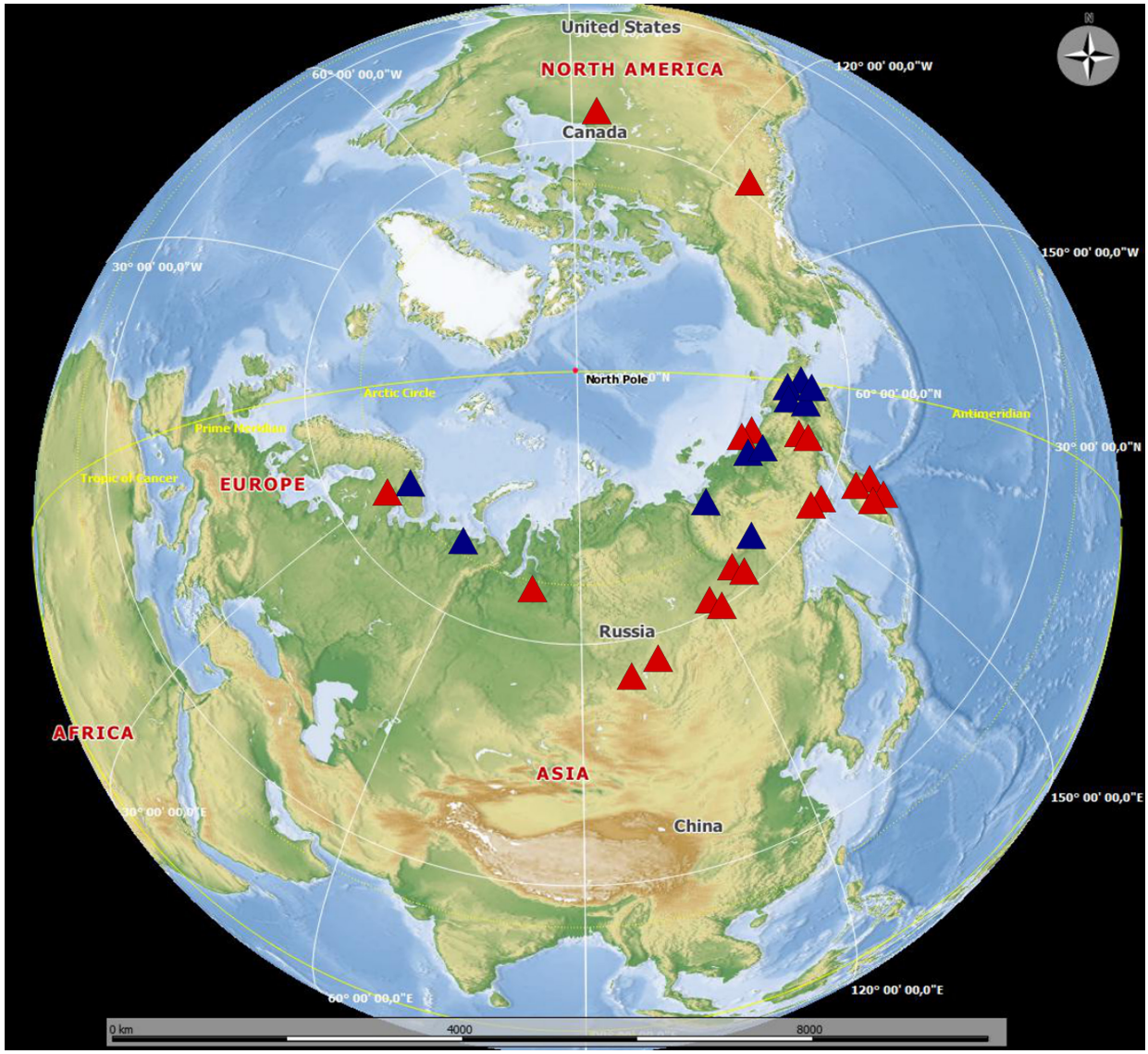

Fig. 1. Sampling sites of *Daphnia pulex* (red triangles) and *D. middendorffiana* (blue triangles) in Northern Eurasia. The base map is available at https://marble.kde.org/

Рис. 1. Точки сбора образцов *Daphnia pulex* (красные треугольники) и *D. middendorffiana* (синие треугольники). Карта доступна на https://marble.kde.org/

jModelTest v.2.1.7 software on the basis of likelihood parameters of 88 different models, the Akaike information criterion (AIC), and the Bayesian information criterion (BIC) [Guindon, Gascuel, 2003; Darriba *et al*., 2012]. According to aforementioned analysis, the best models were selected: Tamura's three-parameter model with a gamma distribution (TN92+G, parameter $\alpha = 0.619$) [Tamura, 1992] and the general time reversible model with a gamma distribution (GTR+G, parameter $\alpha = 0.538$) [Tavaré, 1986]. The former model was used to reconstruct a 12S rRNA phylogeny by the maximum likelihood method in MEGA v.7.0 software; branch support was assessed by bootstrappingwith 1000 replications [Saitou, Nei, 1987; Kumar *et al*., 2016]. The latter model was employed to implement Bayesian analysis in MrBayes v.3.2 software [Ronquist, Huelsenbeck, 2003]. Two synchronous runs with four Markov chains each were executedfor 10^6 generations with sampling every 500 generations. Stationarity of runs was confirmed by of the posterior probability and log-likelihood metrics. To test the convergence of Markov chains and of Markov chain Monte Carlo trace plots, parameters of effective sample size (ESS > 300) were evaluated in Tracer v.1.6 software [Rambaut *et al*., 2018]. Bayesian inference (BI) phylogeny was visualized in FigTree v.1.4.4 software (http://tree.bio.ed.ac.uk/).

MtDNA polymorphism, haplotype networks, and evolutionary history. Evolutionary divergence between populations and species based on the 12S rRNA sequences was estimated using uncorrected *p*-distances in MEGA v.7.0 software. The analysis was performed on groups formed by populations from different geographic regions, including sequences from the GenBank database. Polymorphism of the mitochondrial 12S rRNA gene fragment was assessed by means of only our original nucleotide sequences for each species *D. pulex* and *D. middendorffiana* overall and for groups of geographic populations. Standard parameters were calculated — the number of polymorphic (segregating) sites (S), the number of haplotypes (h), and haplotype (H_d) and nucleotide diversity (π) — using

DnaSP v.5.10 software [Librado, Rozas, 2009]. Reconstruction of the genealogical relationships between 12S rRNA haplotypes of species *D. pulex* and *D. middendorffiana* (both our original data and data retrieved from GenBank) was performed by the median-joining algorithm (MJ) and was implemented in PopART v.1.7 software [Bandelt *et al*., 1999; Leigh, Bryant, 2015]. A preliminary assessment of the evolutionary history of investigated species was performed based on the ratio of haplotype diversity (H_d) to nucleotide diversity (π) [Grant, Bowen, 1998; Avise, 2000], results of neutrality tests (Tajima's *D* and Fu's F_S) [Fu, 1997; Tajima, 1989], and the structure of the haplotype networks.

Results

Mitochondrial phylogeny. Reconstruction of phylogenetic relationships showed that most nucleotide sequences of the mitochondrial 12S rRNA gene fragment got separated into two main clades, corresponding to species *D. pulex* and *D. middendorffiana* (Figs 2 and 3). Haplotypes of *D. obtusa*, *D. ambigua*, and *D. mitsukuri* formed clades that were external to the species of the *D. pulex* s.lat. group.

Major clade I of *D. pulex* is represented by haplotypes of a "pan-Arctic" mitochondrial lineage, which includes several subclades that have small geographic ranges: Magadan Oblast (subclade Ia), Yakutia (subclade Ib), and Chukotka (subclade Ic). Aside from the pan-Arctic phylogroup, three other clades of *D. pulex* can be distinguished, their positions in the general phylogenetic scheme of the group were are unstable. Similarly, haplotypes of the major clade I of *D. middendorffiana* are separated into several subclades confined to different geographic regions. At the same time, subdivision of *D. middendorffiana* haplotypes into distant subclades is noted in Magadan Oblast (subclade Ia), Chukotka (subclades Ib and Ic), and Yakutia (subclades Id and Ie). Some haplotypes of *D. middendorffiana* from Yakutia populations constitute the clade II, and clade IV was formed by haplotypes from YANAO (Figs 2 and 3).

Topology of BI and ML phylogenetic trees is different in unclear positions of the subclades II, III, and IV of *D. pulex* and of the subclade III of *D. middendorffiana*. In the BI tree, subclades II and IV of *D. pulex* occupy a basal position relative to the subclade I, whereas the *D. pulex* JN903685 haplotype of Lake Chany from subclade III constitutes a basal branch to all haplotypes of *D. middendorffiana*.

In the ML tree, the haplotypes of subclades II and III of *D. pulex* occupy a basal position relative to *D. middendorffiana* (Fig. 3). It is likely that the haplotypes of subclades II–IV of *D. pulex*, as well as the haplotypes of subclades II and III of *D. middendorffiana*, belong to completely different species. Moreover, the maximum likelihood method does not give a significant branch support to major clades and subclades and does not uncover monophyletic relationships between the major clades of *D. pulex* and *D. middendorffiana*, whereas the Bayesian analysis indicates a clear-cut monophyly (100%). Separately, it should be mentioned that some individuals from Canada were incorrectly identified as *D. middendorffiana* (AAK-0893 and FJ427429); their haplotypes are clustered with haplotypes of the pan-Arctic lineage of *D. pulex*.

Median networks of 12S rRNA haplotypes. The median-joining method, which was used to identify genealogical connections between 12S rRNA haplotypes, more clear defined their subdivision into major clades and subclades. The *D. pulex* haplotypes form a complicated median network which was characterized by a star-shaped structure with the central haplotype H_1 having a large geographic range in entire Holarctic (Fig. 4). Three distant mitochondrial clades are associated with the central structure (clade I). The first of these clades of *D. pulex* (clade II) is composed of 12S rRNA haplotypes of *D. pulex* from YANAO; the second one (clade III) consists of haplotypes from Lake Chany and of *D. pulicaria* from China (Acc. No. MH632078); and the third one (clade IV) contains haplotypes from YANAO and Western Europe (Finland, Czech Republic, and Italy). These groups of haplotypes are related to the central group via several hypothetical haplotypes and 18–23 mutation steps. Most haplotypes in these clades are unique, are weakly related to each other, and are engaged in cyclic connections. Clade IV is related — through 64 mutational steps — to a haplotype of *D. pulex* from China (H_58, Acc. No. KT003819) which apparently belongs to another species. Peripheral haplotypes from Magadan Oblast and a haplotype from Canada form subclade Ia, haplotypes from Yakutia give rise to subclade Ib, and subclade Ic consists of haplotypes from Chukotka (H_35–H_37) and Canada (H_5–H_7 and H_44). Thus, some haplotypes of *D. pulex* from Magadan Oblast and Yakutia are located more distantly from the central star-shaped structure (consisting of haplotypes of the pan-Arctic phylogroup), than some haplotypes of North America, Japan, and New Zealand. Overall, the median network of 12S rRNA haplotypes of *D. pulex* indicates a strong genetic differentiation between haplotypes even within a single region, thereby most likely presumes cryptic species diversity.

In the median network of 12S rRNA haplotypes of *D. middendorffiana*, no well-defined star-shaped structure is found (Fig. 5). A common haplotype, H_11, is detected in populations of *D. middendorffiana* from water bodies of Chukotka, Yakutia, and Murmansk Oblast. A small number of haplotypes from Chukotka, Yakutia, and Komi Republic are most closely related (2–6 mutation steps) to the central structure.

Some haplotypes of *D. middendorffiana* from Chukotka, Magadan Oblast, and Yakutia are unique, they are related to each other weakly, and form separate subclades (Ia–Ic). Groups of haplotypes from YANAO and Yakutia are the most distant ones from the clade I, occupy a peripheral position, and form distant clades II and III, characterized by small geographic ranges (regional). Haplotype H_42 from Yakutia is only weakly related to other haplotype groups from same region. As in the case of *D. pulex*, the most divergent mitochondrial lineage is identified in YANAO.

MtDNA polymorphism and neutrality tests. Between *D. pulex* and *D. middendorffiana*, differences in

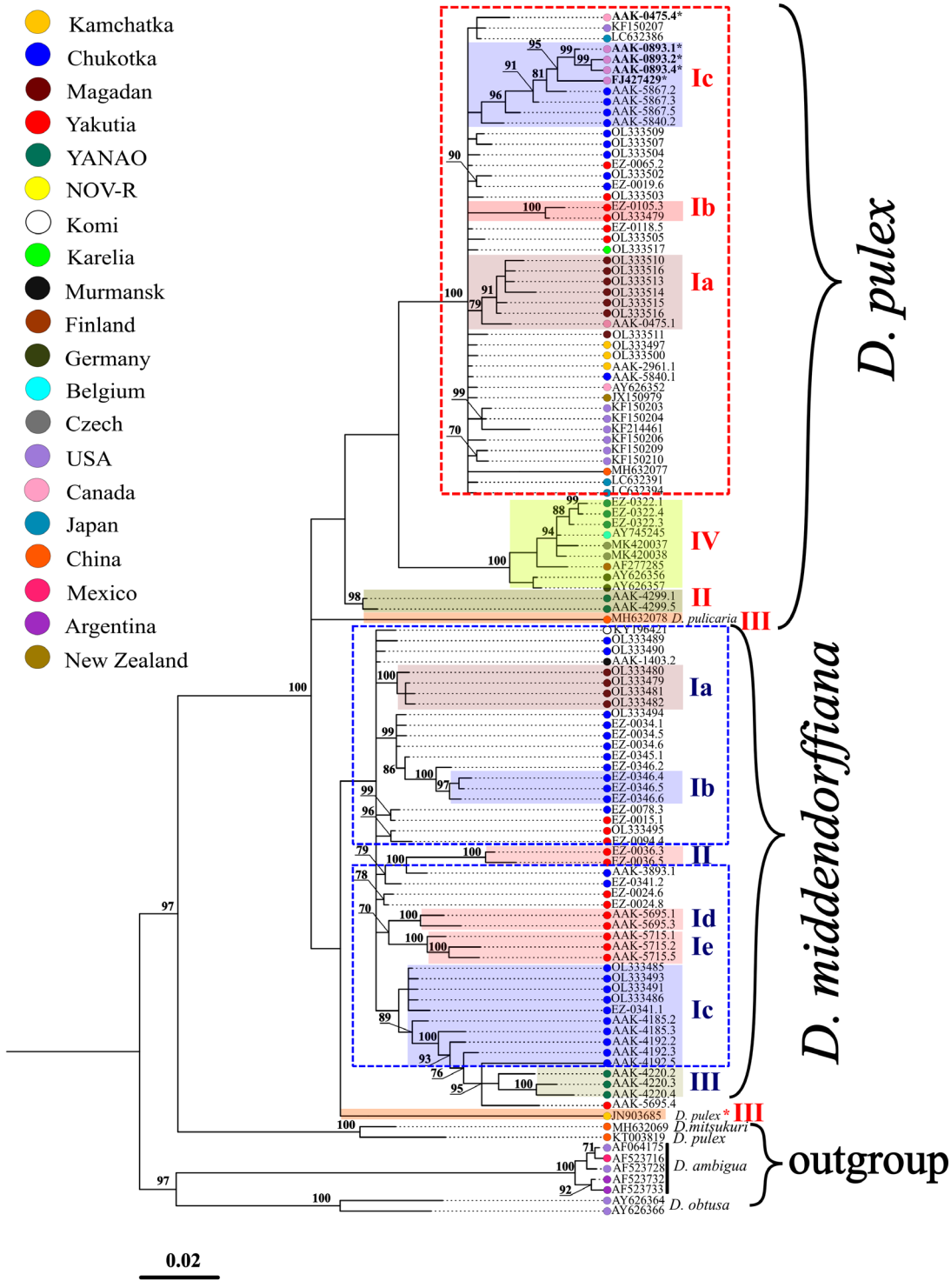

Fig. 2. The BI phylogenetic tree for *D. pulex* and *D. middendorffiana* based on the fragment of the 12S rRNA gene of the mtDNA (according to haplotypes). Posterior probabilities (BI) above 70% are shown. The scale represents expected substitutions per site. I–IV (red) — *D. pulex* major clade and subclades, I–III (blue) — *D. middendorffiana* major clade and subclades; **D. pulex* (JN903685) from Lake Chany; bold faced with* — misidentification of the specimens in course of their morphological identification. YANAO — Yamalo-Nenets Autonomous Okrug, NOV-R — Chany Lake (Novosibirsk Oblast).

Рис. 2. Байесовское филогенетическое дерево для *Daphnia pulex* и *D. middendorffiana* на основе фрагмента гена 12S митохондриальной ДНК (согласно выявленным гаплотипам). Показаны апостериорные вероятности выше 70%. Масштаб — число ожидаемых замен на сайт. I–IV (красный цвет) — главная клада и субклады *D. pulex*, I–III (синий цвет) — главная клада и субклады *D. middendorffiana*; **D. pulex* (JN903685) из оз. Чаны; жирный шрифт с звездочкой (*) — неверная видовая идентификация особей по морфологическим признакам. YANAO — Ямало-Ненецкий АО, NOV-R — оз. Чаны (Новосибирская обл.).

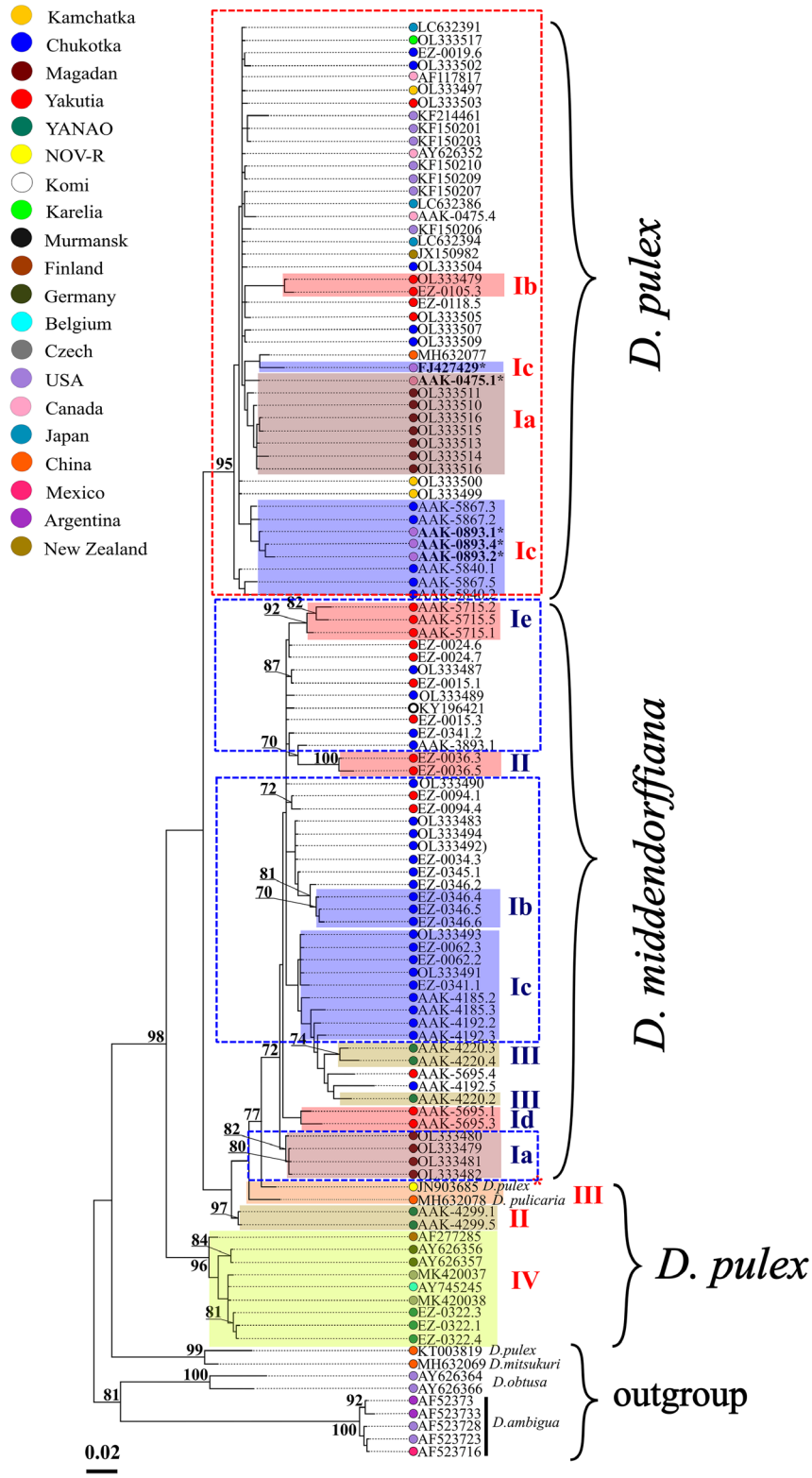

Fig. 3. The ML phylogenetic tree for *D. pulex* and *D. middendorffiana* based on the 12S rRNA gene of the mtDNA (according to haplotypes). Bootstrap values for branch support above 70% are presented. The scale is given in expected substitutions per site. For meanings of the Roman numbers, see Fig. 2.

Рис. 3. ML-филогенетическое дерево для *Daphnia pulex* и *D. middendorffiana* на основе фрагмента гена 12S митохондриальной ДНК (согласно выявленным гаплотипам). Показаны бутстрэп значения поддержки ветвей выше 70%. Масштаб — число ожидаемых замен на сайт. Обозначения см. рис. 2.

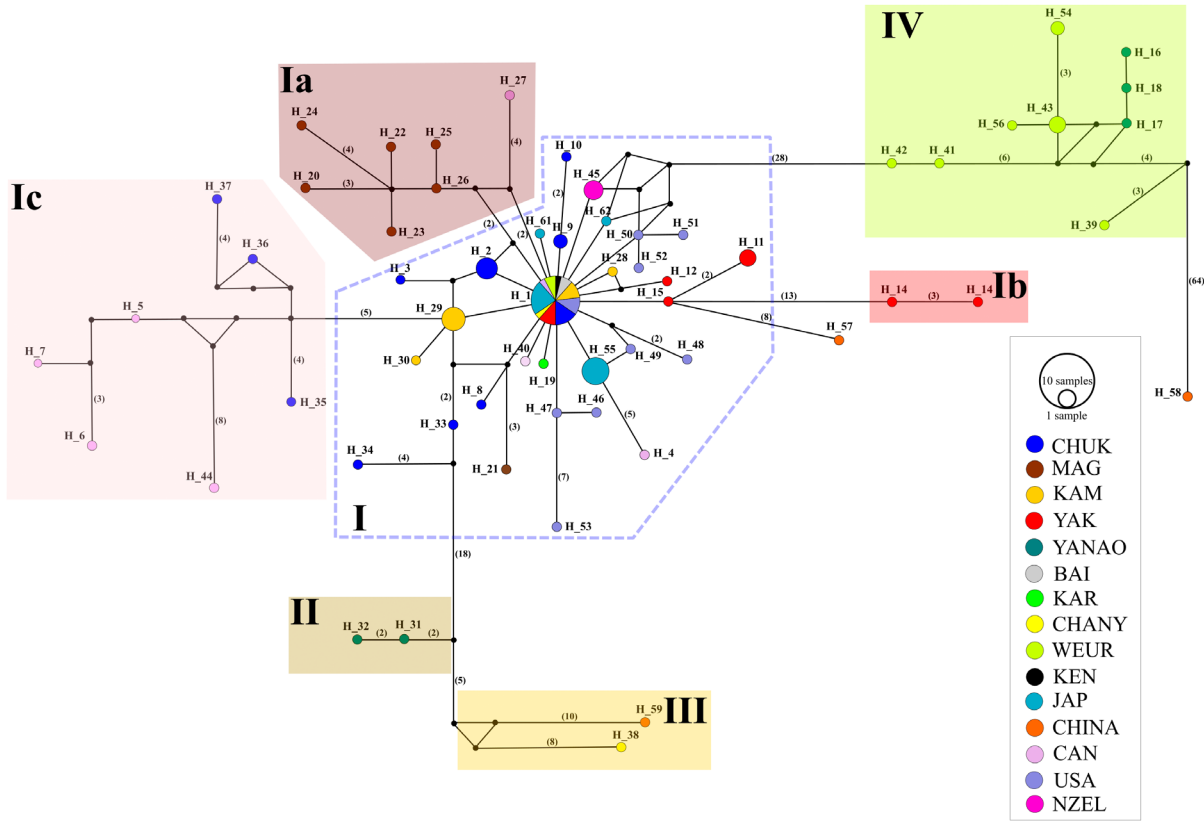

Fig. 4. The median-joining (MJ) network for the *D. pulex* 12S rRNA haplotypes. Each circle of the MJ network is proportional to relative haplotype frequencies (the scale is shown in the upper right corner). The numbers of mutations are indicated for each branch in parentheses (if not 1.0); black small circles are median vectors; I–IV — clades and subclades. CHUK — Chukotka, MAG — Magadan Oblast, KAM — Kamchatka, YAK — Yakutia, YANAO — Yamalo-Nenets Autonomous Okrug, BAI — the Baikal Lake basin, KAR — Karelia, CHANY — Chany Lake (Novosibirsk Oblast), WEUR — Western Europe, KEN: Kenya, JAP — Japan, CAN — Canada, NZEL — New Zealand.

Рис. 4. Медианная сеть (MJ) 12S гаплотипов *Daphnia pulex*. Размер кружков соответствует относительной частоте гаплотипов; цифры на ветвях в скобках — число мутаций, если оно не равно 1; черные маленькие кружки — медианные векторы; I–IV — клады и субклады. CHUK — Чукотка, MAG — Магаданская обл., KAM — Камчатка, YAK — Якутия, YANAO — Ямало-Ненецкий АО, BAI — бассейн оз. Байкал, KAR — Карелия, CHANY — оз. Чаны (Новосибирская обл.), WEUR — Западная Европа, KEN — Кения, JAP — Япония, CAN — Канада, NZEL — Новая Зеландия.

genetic polymorphism parameters are insignificant (Table 1). A slightly greater number of haplotypes is found in *D. middendorffiana* (45), but it should be pointed out that the number of analyzed nucleotide sequences is also greater (82) as compared to *D. pulex* (63). The number (*S*) of polymorphic (segregating) sites is slightly greater in the sample of *D. middendorffiana*. High values of the haplotype diversity (H_d) is noted for both species along with high values of nucleotide diversity (**π**). Neutrality tests yield negative significant values (Tajima's *D* and Fu's F_S) for both species (Table 1).

A slightly different picture is observed at the level of geographic groups (populations) (Table 2). Similarly, differences in the number of haplotypes may be due explained by different sample sizes to some extent. Very large numbers of polymorphic sites are found in populations of *D. pulex* from YANAO (45), Chukotka (24), Yakutia (21), and Magadan Oblast (16). For *D. middendorffiana*, high values of this parameter are registered in Chukotka (64), Yakutia (64), and YANAO (20). Significant differences in the ratio of major parameters of genetic polymorphism (H_d and π) are detected among different populations of each species (Table 2). For instance, high values of both parameters are noted in all populations of *D. pulex*, except for populations from the Lake Baikal basin and Karelia, where the sample size does not allow us to evaluate these parameters. In the *D. middendorffiana* populations, the pattern is similar. High values of haplotype diversity with low values of nucleotide diversity are registered in populations of *D. pulex* from Kamchatka water bodies and in populations of *D. middendorffiana* from Magadan Oblast.

Tajima's *D* and Fu's F_S neutrality indices have negative values in the majority of populations. Nonetheless, a significant negative value of Tajima's *D* is recorded only in *D. middendorffiana* populations from Yakutia, whereas a positive significant value of Fu's F_S are found on in the group of *D. pulex* populations from Magadan Oblast (Table 2). Positive insignificant values of Tajima's *D* are noted in populations of *D. pulex* from YANAO and Canada, whereas positive insignificant values of Fu's F_S in populations from Yakutia, YANAO, and Lake Chany. In

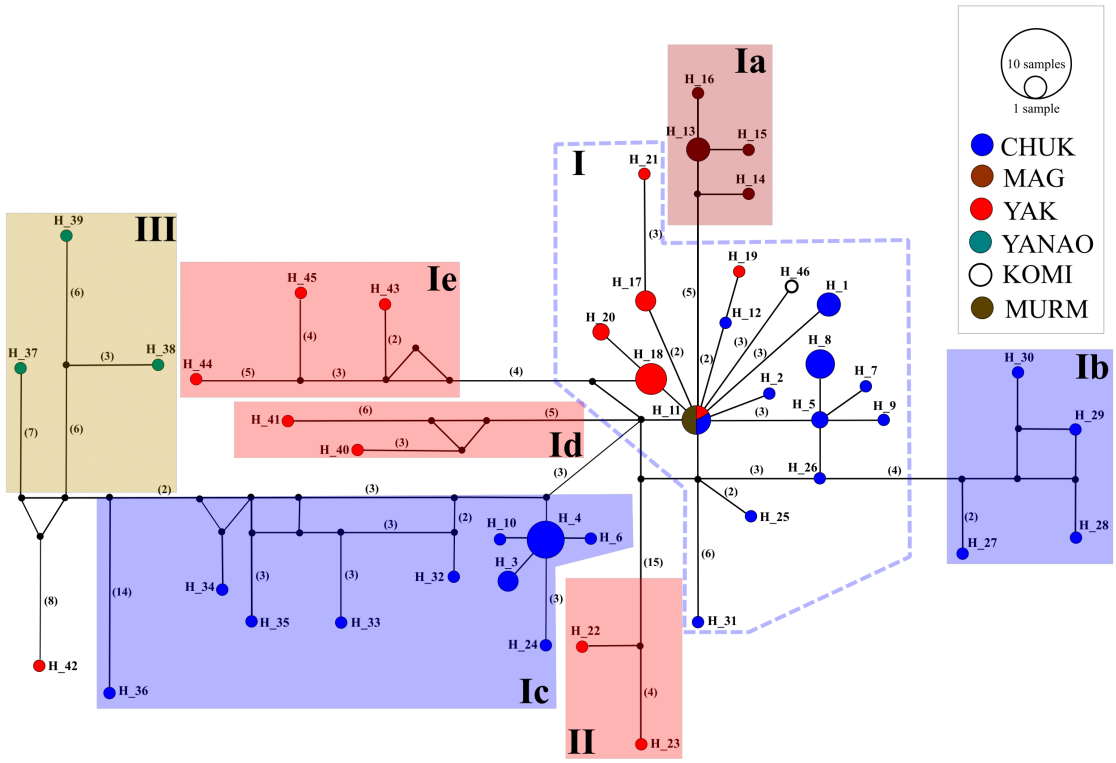

Fig. 5. The MJ network for *D. middendorffiana* 12S rRNA haplotypes. Designations are the same as in Fig. 4. I–III — *D. middendorffiana* clades and subclades. CHUK — Chukotka, MAG — Magadan Oblast, YAK — Yakutia, YANAO — Yamalo-Nenets Autonomous Okrug, KOMI — Komi Republic, MURM — Murmansk Oblast.

Рис. 5. Медианная сеть (MJ) 12S гаплотипов для *Daphnia middendorffiana*. Обозначения см. рис. 4. CHUK — Чукотка, MAG — Магаданская обл., YAK — Якутия, YANAO — Ямало-Ненецкий АО, KOMI — Республика Коми, MURM — Мурманская обл.

Table 1. Polymorphism of the fragment of the mtDNA 12S rRNA gene and neutrality tests in species *Daphnia pulex* and *D. middendorffiana*.

Таблица 1. Значения индексов генетического полиморфизма и тестов на нейтральность эволюции для видов *Daphnia pulex* и *D. middendorffiana* на основе фрагмента гена 12S митохондриальной ДНК.

Species	*n*	*h*	*S*	H_d ± st.d.	π ± st.d.	Tajima's *D*	Fu's F_S
D. pulex	63	38	116	0.944±0.019	0.0205±0.0019	–1.817*	–11.362***
D. middendorffiana	82	45	112	0.965±0.009	0.0194±0.0018	–1.739**	–14.623***

NOTE. *n* — the number of analyzed nucleotide sequences; *S* — the number of polymorphic (segregating) sites; *h* — the number of haplotypes; H_d — haplotype diversity; π — nucleotide diversity; st.d. — standard deviation; Tajima's *D* and Fu's F_S — neutrality tests; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.02$.

ПРИМЕЧАНИЕ: *n* — число анализируемых нуклеотидных последовательностей; *S* — число полиморфных (сегрегирующих) сайтов; *h* — число гаплотипов; H_d — гаплотипическое разнообразие; π — нуклеотидное разнообразие; st.d. — стандартное отклонение; Tajima's *D* и Fu's F_S — тесты на нейтральность эволюции; $^*P < 0{,}05$; $^{**}P < 0{,}01$; $^{***}P < 0{,}02$.

populations of *D. middendorffiana*, positive insignificant values of Fu's F_S are registered in Yakutia and YANAO.

Evolutionary divergence and differentiation of populations. Judging by the fragment of the mitochondrial 12S rRNA gene, the greatest evolutionary divergence (uncorrected *p*-distances) is noted within populations of *D. pulex* in China, the Lake Chany basin, Western Europe, and YANAO: 12.1, 6.7, 3.0, and 5.0%, respectively (Table 3). Besides, rather high values of this parameter are foundin Canada (1.9%), Yakutia (1.3%), Chukotka (0.9%), Magadan Oblast (0.8%), and the USA (0.7%). Judging by the fragment of the 12S rRNA gene, the lowest *p*-distances are recorded within populations from Kamchatka (0.2%) and Japan (0.1%); in populations from the Lake Baikal basin, evolutionary divergence is not detectable (Table 3). Likewise, for *D. middendorffiana*, the strongest evolutionary divergence is observed within populations from YANAO and Yakutia: 2.3 and 2.0%, respectively (Table 4). In Chukotka, intrapopulation divergence is slightly lower: 1.6%, and the lowest value is registered in the group of populations from Magadan Oblast (0.2%).

Very high *p*-distances, exceeding 1.5–2.0% and in some cases >4.9–9.0%, are noted between most popu-

Table 2. Polymorphism of the fragment of the mtDNA 12S rRNA gene and neutrality tests ingeographical groups of *Daphnia pulex* and *D. middendorffiana* populations.
Таблица 2. Значения индексов генетического полиморфизма и тестов на нейтральность эволюции для географических групп популяций *Daphnia pulex* и *D. middendorffiana* на основе фрагмента гена 12S митохондриальной ДНК.

Species/populations	*n*	*h*	*S*	H_d± st.d.	π ± st.d.	Tajima's *D*	Fu's F_s
D. pulex							
KAM	11	4	3	0.673±0.123	0.0015±0.0004	–0.506	–1.026
CHUK	20	11	24	0.900±0.044	0.0088±0.0019	–1.001	–1.711
MAG	7	7	16	1.000±0.076	0.0087±0.0018	–1.341	–3.033*
YAK	10	6	21	0.867±0.085	0.0124±0.0040	–0.212	1.233
BAI	2	1	0	n/a	0	n/a	n/a
YANAO	5	5	45	1.000±0.126	0.0462±0.0129	1.674	0.840
KAR	1	1	0	n/a	n/a	n/a	n/a
CHANY	2	2	35	1.000±0.500	0.0612±0.0306	0	3.355
CAN	5	5	23	1.000±0.126	0.0199±0.1260	0.245	–0.029
D. middendorffiana							
CHUK	46	25	64	0.931±0.024	0.0160±0.0018	–1.314	–4.958
MAG	7	4	4	0.714±0.181	0.0020±0.0007	–1.434	–1.217
YAK	23	14	64	0.901±0.051	0.0195±0.0041	–1.424**	0.041
YANAO	3	3	20	1.000±0.272	0.0234±0.0071	0	1.493
MURM	3	1	0	0	0	0	n/a

NOTE. Indexes are the same as in Table 1; CAN — Canada; KAM — Kamchatka; CHUK — Chukotka; YAK — Yakutia; YANAO — Yamalo-Nenets Autonomous Okrug; BAI — Baikal Lake basin; CHANY — Chany Lake (Novosibirsk Oblast); MAG — Magadan Oblast; KAR — Karelia; MURM — Murmansk Oblast; n/a — estimation is impossible.

ПРИМЕЧАНИЕ: показатели см. табл. 1; CAN — Канада; KAM — Камчатка; CHUK — Чукотка; YAK — Якутия; YANAO — Ямало-Ненецкий АО; BAI — бассейн оз. Байкал; CHANY — оз. Чаны (Новосибирская обл.); MAG — Магаданская область; KAR — Карелия; MURM — Мурманская область; n/a — оценка невозможна; *$P < 0.02$; **$P < 0.05$.

Table 3. Evolutionary divergence (uncorrected *p*-distances, %) between pairs of nucleotide sequences within and between geographical groups of *Daphnia pulex* populations based on the fragment of the mtDNA 12S rRNA gene. The analysis included 112 nucleotide sequences.
Таблица 3. Эволюционная дивергенция (нескорректированные *p*-дистанции, %) между парами нуклеотидных последовательностей в пределах и между географическими группами популяций *D. pulex* на основе фрагмента гена 12SмтДНК. В анализ включено 112 нуклеотидных последовательностей.

##	Within pop.	KAM	CHUK	MAG	YAK	YA-NAO	BAI	CHA-NY	KAR	W_EUR	KEN	JAP	CHI-NA	CAN	USA
KAM	0.2±0.1														
CHUK	0.9±0.2	0.6													
MAG	0.8±0.2	1.2	1.5												
YAK	1.3±0.3	0.9	1.2	1.8											
YANAO	5.0±0.8	6.5	6.8	7.3	7.2										
BAI	0	0.1	0.5	1.0	0.8	6.5									
CHANY	6.7±1.1	3.4	3.7	3.5	3.9	6.3	3.3								
KAR	n/c	0.3	0.7	1.2	1.0	6.8	1.0	3.5							
W_EUR	3.0±0.5	5.8	6.1	6.6	6.4	4.2	5.8	6.7	6.0						
KEN	n/c	0.1	0.5	1.0	0.8	6.5	0	3.3	0.2	5.8					
JAP	0.1±0.1	0.3	0.6	1.1	0.9	6.6	0.1	3.3	0.3	5.9	0.1				
CHINA	12.1±1.3	7.9	8.2	8.4	8.5	8.8	7.9	8.2	8.1	9.0	7.9	7.9			
CAN	1.9±0.4	1.3	1.6	2.1	2.0	7.3	1.2	4.4	1.4	6.5	1.2	1.3	8.7		
USA	0.7±0.2	0.5	0.9	1.4	1.2	6.9	0.4	3.7	0.6	6.1	0.4	0.5	8.3	0.9	
N_ZEL	n/c	0.3	0.7	1.2	1.0	1.0	0.2	3.3	0.4	5.6	0.2	0.3	8.0	0.7	0.6

NOTE. KAM: Kamchatka; CHUK — Chukotka; MAG — Magadan Oblast; YAK — Yakutia; YANAO — Yamalo-Nenets Autonomous Okrug; BAI — Baikal Lake basin; CHANY — Chany Lake (Novosibirsk Oblast); KAR — Karelia; W_EUR — Western Europe; KEN — Kenya; JAP — Japan; CHINA — China; CAN — Canada; USA — USA; N_ZEL — New Zealand; n/c — estimation of evolutionary distances is impossible.

ПРИМЕЧАНИЕ: KAM — Камчатка; CHUK — Чукотка; MAG — Магаданская обл.; YAK — Якутия; YANAO — Ямало-Ненецкий АО; BAI — бассейн оз. Байкал; CHANY — бассейн оз. Чаны (Новосибирская обл.); KAR — Карелия; W_EUR — Западная Европа; KEN — Кения; JAP — Япония; CHINA — Китай; CAN — Канада; USA — США; N_ZEL — Новая Зеландия; n/c — оценка эволюционных дистанций невозможна.

Table 4. Evolutionary divergence (uncorrected *p*-distances, %) between pairs of nucleotide sequences within and between geographical groups of *Daphnia middendorffiana* populations based on the fragment of the mtDNA 12S rRNA gene. The analysis included 83 nucleotide sequences.

Таблица 4. Эволюционная дивергенция (нескорректированные *p*-дистанции, %) между парами нуклеотидных последовательностей в пределах и между популяциями *Daphnia middendorffiana* на основе фрагмента гена 12SмтДНК. В анализ включено 83 нуклеотидных последовательности.

##	Within population	CHUK	MAG	YAK	YANAO	KOMI
CHUK	1.6±0.3					
MAG	0.2±0.1	2.2				
YAK	2.0±0.3	2.0	2.3			
YANAO	2.3±0.5	3.8	4.9	4.2		
KOMI	n/c	1.6	1.7	1.7	4.2	
MURM	n/c	1.0	1.1	1.1	3.7	0.5

NOTE. CHUK — Chukotka; MAG — Magadan Oblast; YAK — Yakutia; YANAO — Yamalo-Nenets Autonomous Okrug; KOMI — Komi Republic; MURM — Murmansk Oblast.

ПРИМЕЧАНИЕ: CHUK — Чукотка; MAG — Магаданская обл.; YAK — Якутия; YANAO — Ямало-Ненецкий АО; KOMI — Республика Коми; MURM — Мурманская обл.

lations of *D. pulex* and between most populations of *D. middendorffiana* from different geographic regions (Tables 3 and 4). High divergence is observed mainly in pairwise comparisons of North-Asian populations with their European and/or North-American counterparts. Nevertheless, high *p*-distances are also revealed in pairwise comparisons of North-Asian populations, for example, from YANAO with all the other populations (Tables 3 and 4). The greatest divergence is noted in pairwise comparisons of *D. pulex* populations from China with all others (8.2–9.7%); this finding casts doubt on the correctness of the species identification of the individuals under study.

In pairwise comparisons of *D. pulex* populations from water bodies of YANAO and Canada with other populations, significant high values of the fixation index (F_{ST} up to 1.0) indicates a genetic differentiation and a limited gene flow. High differentiation is found between populations from Kamchatka, Chukotka, Yakutia, and Magadan Oblast as well as between populations from Magadan Oblast and populations from the Baikal and Chany lake basins (Fig. 6A). A limited gene flow is detected between populations of *D. middendorffiana* from Magadan Oblast, Murmansk Oblast, and YANAO (Fig. 6B). Low F_{ST} values (0.1) were found in pairwise comparisons of populations

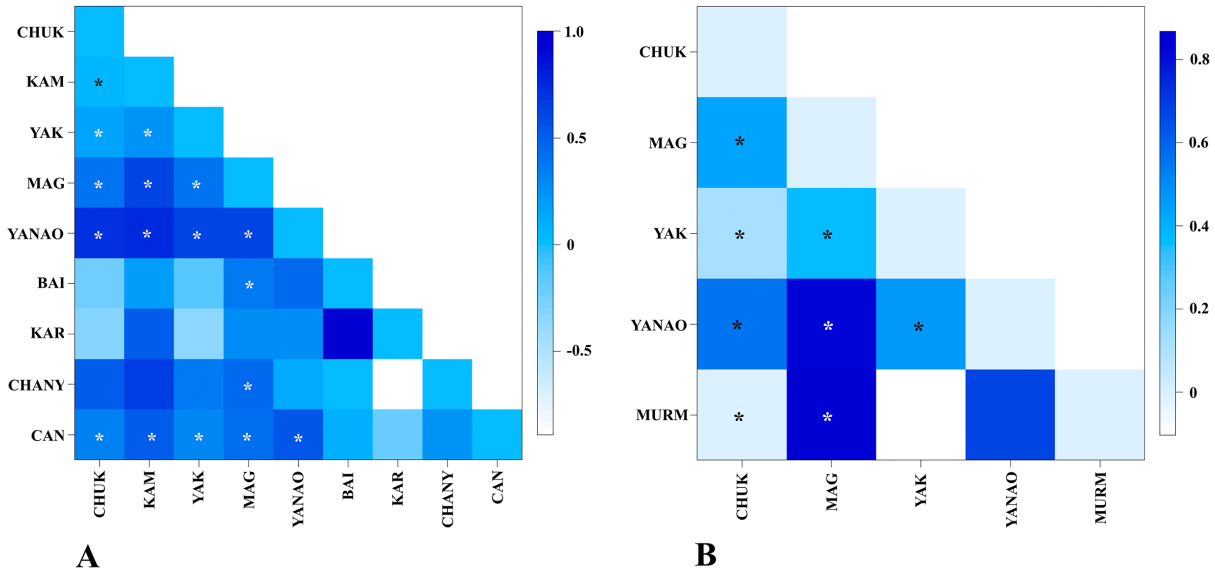

Fig. 6. The graph of pairwise F_{ST} distance matrices between geographical populations of either *D. pulex* (A) or *D. middendorffiana* (B). Abbreviations are the same as in Table 2; *$P < 0.05$. CHUK — Chukotka, MAG — Magadan Oblast, KAM — Kamchatka, YAK — Yakutia, YANAO — Yamalo-Nenets Autonomous Okrug, BAI — the Baikal Lake basin, KAR — Karelia, CHANY — Chany Lake (Novosibirsk Oblast), MURM — Murmansk Oblast, CAN — Canada.

Рис. 6. Графики матриц парных F_{ST} дистанций между географическими популяциями видов *D. pulex* (А) и *D. middendorffiana* (Б). Аббревиатуры см. табл. 2; *$P < 0{,}05$. CHUK — Чукотка, MAG — Магаданская обл., KAM — Камчатка, YAK — Якутия, YANAO — Ямало-Ненецкий АО, BAI — бассейн оз. Байкал, KAR — Карелия, CHANY — оз. Чаны (Новосибирская обл.), MURM — Мурманская обл., CAN — Канада.

from Chukotka, Yakutia, and Murmansk Oblast. Overall, however, the highest genetic differentiation was revealed when geographically distant populations of each species (*D. pulex* or *D. middendorffiana*) were compared.

Discussion

Phylogenetic relationships and phylogeography. The results of our study clearly indicate a deep genetic differentiation within each of the two closely related species, *D. pulex* and *D. middendorffiana*; the level of which for the former species are so high that it implies a cryptic species diversity. Previously, we have identified three distant mitochondrial lineages (subclades) of *D. pulex* in Northeastern Eurasia [Zuykova *et al*., 2022], one of which is widespread throughout the Holarctic and belongs to the so-called "pan-Arctic" lineage of this species; this lineage has been previously identified by an analysis of protein-coding mitochondrial genes COX1 and ND5 [Crease *et al*., 2012]. As already mentioned, the pan-Arctic lineage of *D. pulex* should be regarded as a mitochondrial phylogroup widespread in the Holarctic rather than an independent species. The prevalent 12S rRNA haplotype of this phylogroup forms a central star-shaped structure and could be regarded as an ancient ancestral haplotype. It is also possible that the multiple cyclic connections found in the network of *D. pulex* 12S rRNA haplotypes point to exchange of genetic material between different species and/or mitochondrial lineages (processes of hybridization and incomplete sorting) and therefore to a reticulate evolution [Iersel *et al*., 2010; Solís-Lemus, Ané, 2016; Elworth *et al*., 2018].

The other mitochondrial lineages of *D. pulex* found in Northern Eurasia are composed of unique haplotypes and mostly have restricted (regional) geographic ranges: Chukotka, Magadan Oblast, Yakutia, or YANAO. In turn, the haplotypes of *D. pulex* found in YANAO form two separate subclades. One of them is closely related to *D. pulicaria* from China (H_59, GenBank Acc. No. MH632078) and to a haplotype (H_38) from the Lake Chany basin (Western Siberia, Novosibirsk Oblast). It is possible that these haplotypes belong to different mitochondrial lineages of another species close to *D. pulex*, *D. pulicaria*. Nonetheless, at this stage of studies, this notion cannot be stated with certainty, because in some cases, instances of erroneous identification of individuals are too obvious, e.g. in the GenBank database [Pereboev *et al*., 2025]. Other haplotypes of *D. pulex* from YANAO share with *D. pulex* haplotypes from Western Europe (GenBank Acc. No. AY745245, AF277285, MK420038, and KY196422) and apparently belong to the "European" lineage of this species [Marková *et al*., 2013]. Considering the magnitude of divergence between subclades of *pulex*-like specimens from water bodies of YANAO, they should be regarded as different species according to recent ideas on a genetic differentiation within Cladocera [Hebert *et al*., 2003].

The median network of *D. middendorffiana* 12S rRNA haplotypes does not contain such a clear-cut star-shaped structure; however, haplotypes of *D. middendorffiana* are also clustered into several clades. Only one common haplotype, H_11 (Chukotka, Yakutia, and Murmansk Oblast) is detected and also appears to be an ancient haplotype. This haplotype is closely related to the haplotypes from Chukotka, Yakutia, and Magadan Oblast (through 4-7 mutational steps); they form several internal subclades. Distant mitochondrial clades of *D. middendorffiana* contain haplotypes from water bodies in Yakutia and YANAO; however, a single haplotype from Yakutia (H_42) is adjacent to the clade of YANAO. The magnitude of genetic differentiation of intraspecific clades from the main gene pool, just as in the case of *D. pulex*, implies the presence of cryptic species diversity. All distant groups of *D. middendorffiana* haplotypes, just as in *D. pulex*, have a regional geographic range, are unique, and are only weakly related to each other through multiple hypothetical haplotypes and mutational steps.

The distribution of unique haplotypes of *D. pulex* and *D. middendorffiana* in Northern Eurasia resembles the distribution pattern of mitochondrial haplotypes of other *Daphnia* taxa: *D. galeata*, *D. longispina* s.str., *D. dentifera*, and *D. cristata* [Zuykova *et al*., 2018b, 2021, 2024a, b]. There are many peripheral unique haplotypes that possess a regional geographic range. It is believed that multiple unique haplotypes represent remains of ancient fauna that had stayed isolated for a long time in refugia free from the Pleistocene glaciations [Avise, 2000; Hewitt, 2004]. At the present stage of these studies, such a geographic distribution of haplotypes should be considered a natural phenomenon common among *Daphnia* members' modern phylogeographic patterns that formed in Northern Eurasia during the Late Pleistocene to Early Holocene. Regarding haplotypes of *D. pulex* from North America (Canada and USA), they are affiliated with two distant clades (one of which obviously belongs to the pan-Arctic lineage). Our data agree well with those on phylogeographic analyses in North-American and European populations of *D. pulex* s.lat. based on other mitochondrial markers [Colbourne *et al*., 1989; Dufresne *et al*., 2011; Crease *et al*., 2012; Marková *et al*., 2013].

Hypothetical evolutionary history. According to our data on the polymorphism of the mitochondrial 12S rRNA gene fragment, values of Tajima's *D* and Fu's F_S, and to the structure of median haplotype networks, a preliminary assessment of evolutionary history is performed for *D. pulex* s.lat. in Northern Eurasia. At the species level, high values of haplotype diversity (H_d) and nucleotide diversity (π) are noted, suggesting that the analyzed sets of individuals consist of genetically highly differentiated divergent mitochondrial lineages/populations or even cryptic species [Grant, Bowen, 1998; Avise, 2000]. This statement is also supported by the large evolutionary divergence (*p*-distance) and genetic differentiation (F_{ST}) identified within geographic groups (populations) and between the populations based on sequences of the fragment of the mitochondrial 12S rRNA gene. Previously, a similar ratio of parameters H_d and π has been documented for the "Siberian" clade of *D. longispina* s.str. and for species *D. cristata* and *D. longiremis* from water bodies of Northern Eurasia [Zuykova *et al*., 2024a, b]. Negative significant

values of Tajima's *D* and Fu's F_S for both studied species and star-shaped structures of central mitochondrial clades in the median networks of 12S rRNA haplotypes suggest a relatively recent spatial expansion [Tajima, 1989; Fu, 1997; Garrigan *et al*., 2010; Holsinger, 2015].

On the other hand, values of H_d, π, Tajima's *D*, and Fu's F_S in population groups of both species from different regions of Northern Eurasia and Canada suggest that genetic structure of these groups has been shaped by different evolutionary processes, in some cases different from those that are applicable at the species level. For instance, a different ratio of genetic-polymorphism indices — a high H_d value with a low π value — was noted here for *D. pulex* populations from Kamchatka and for *D. middendorffiana* from Magadan Oblast. This observation confirms a rapid population growth from an ancient population having a small effective size [Grant, Bowen, 1998; Avise, 2000]. Here it is appropriate to draw an analogy with species *D. cristata* and *D. longiremis* because a similar ratio of H_d to π has been noted in populations of these species from the same or nearby regions of northeastern Eurasia [Zuykova *et al*., 2024b].

For most groups of *D. pulex* and *D. middendorffiana* populations, Tajima's *D* and Fu's F_S show negative but insignificant values. Exceptions represent the populations of *D. pulex* from YANAO (where both parameters were positive), from Yakutia (where a positive value was noted only for Fu's F_S), and from Canada (a positive value of Tajima's *D)*. A significant negative value of Fu's F_S is recorded for populations of *D. pulex* from Magadan Oblast. For groups of *D. middendorffiana* populations from Yakutia and YANAO, a positive value was noted for the Fu's test, while a significant negative value is registered in groups of populations from Yakutia. Positive values of Fu's F_S provide evidence of a sharp reduction in genetic diversity owing to a critical decrease in abundance; that is, we see a result of populations' passing through a "bottleneck" event. In this case, the positive value of Tajima's *D* (if not significant) for *D. pulex* from YANAO confirms the impact of this phenomenon.

As for populations of *D. pulex* from the Baikal region and of *D. middendorffiana* from Murmansk Oblast, to date, only a single haplotype (just a widespread one) has been detected in them (with a low sample size: two and three specimens, respectively); consequently, it is impossible to estimate the magnitude of their genetic polymorphism. It is likely that large-scale research projects will reveal a higher genetic diversity of *D. pulex* and *D. middendorffiana* in these regions. Thus, at this stage of studies, it is difficult to characterize an evolutionary history of populations of *Daphnia* species in the Baikal region and Murmansk Oblast with sufficient confidence. It is possible that, similarly to European populations of *D. cristata* [Zuykova *et al*., 2024b], *D. middendorffiana* from Murmansk Oblast demonstrates well-pronounced consequences of a spatial expansion of the prevalent haplotype between the Late Pleistocene and the Early Holocene: during the melting of the most powerful glacier at the Late Pleistocene. For *D. pulex* from YANAO, the problem of assessment of evolutionary history cannot be resolved unambiguously and requires additional morphological and genetic studies.

The origin and evolutionary history of North-American and European members of the *D. pulex* s.lat. group are covered in detail in numerous articles [Colbourne *et al*., 1998; Paland *et al*., 2005; Dufresne *et al*., 2011; Crease *et al*., 2012; Marková *et al*., 2013]. According to results of those studies, most phylogenetic lineages of *D. pulex* s.lat. species arose during the Pleistocene, and a decisive influence on the evolution of these species and on their modern phylogeographic structure was exerted by paleoclimatic events of the Late Pleistocene.

Papers about population genetic structure of different species of the genus *Daphnia* in Northern Eurasia (albeit by means of a single mitochondrial marker) also confirm the decisive influence — on their evolutionary history — of Pleistocene climatic oscillations (an increase and decrease of ice sheets) [Zuykova *et al*., 2018, 2019, 2024a, b]. A role of Pleistocene refugia in the formation of modern phylogeographic structure is convincingly demonstrated in the current study. During the Last Glacial Maximum (approximately 30,000–20,000 years ago), most of northwestern Europe and the Barents Sea shelf were covered by an ice sheet, while the eastern Arctic was free of glaciation. In this period, there was a well-pronounced transition from a marine climate in western Eurasia to a sharply continental one in the eastern part of Eurasia [Astakhov, 2020]. During periods of a cold and arid climate in northeastern Eurasia, only few populations of *Daphnia* (probably, as dormant stages) could persist either in temporary water bodies (periodically emerging along glacier margins) or in a large lake under a dammed reservoirs being refugia for freshwater fauna [Grosswald, Kotlyakov, 1989; Astakhov, 2020; Mangerud *et al*., 2024]. Such water bodies, just as the crustacean populations inhabiting them, had been isolated from each other for long time periods, thereby contributing to intraspecific genetic divergence and to the formation of a high genetic diversity [Hewitt, 2004; Paland *et al*., 2005; Chin, Cristescu, 2021]. Evidently, the abundance of unique haplotypes of *D. pulex* and *D. middendorffiana* in Yakutia and Chukotka — territories free from powerful glaciation — is a result of an effect of Pleistocene paleoclimatic events on the populations.

During the last glaciation, the territory of North America was almost completely covered by a huge continuous glacier; however, proglacial lakes existed along edges of the ice sheet [Dyke *et al*., 2002; Lambeck *et al*., 2017]. These lakes, just as in Northern Eurasia, were represented refugia for the cladocerans. According to the paleoclimatic conditions on the North-American continent in the Pleistocene, it has been theorized that the place of origin of the *D. pulex* "pan-Arctic" lineage is North America, The start of the expansion of the "pan-Arctic" phylogroup has been assigned to a period at the end of the Last Glacial Maximum, approximately 22,000–8,800 years ago, and currently, haplotypes of this phylogroup are found on different continents [Colbourne *et al*., 1998; Mergeay *et al*., 2006; Marková *et al*., 2013]. In particular, the presence of haplotypes of this phylogroup in Northern

Eurasia is explained by those authors as introduction of dormant stages of *Daphnia* species by waterfowl migrating from Alaska to the Eurasian regions and other continents (Africa, New Zealand, South America, and Southeast Asia). It is believed that the method of reproduction, obligate parthenogenesis (most common in *D. pulex* s.lat. populations from high latitudes), and capacity for interspecific hybridization play a substantial role in the ability of the "pan-Arctic" phylogroup to occupy new habitats [Mergeay *et al*., 2008; Dufresne *et al*., 2011], moreover, some asexual clades intensively invade new territories during last decade due to a human transportation [Kotov *et al*., 2022].

Despite the seemingly convincing evidence of the North-American origin of the *D. pulex* "pan-Arctic" phylogroup, we think that this conclusion is not accurately justified. Firstly, *D. pulex* s.lat. from the Russian part of Northern Eurasia were almost not investigated, with few exceptions [Colbourne *et al*., 1998; Weider *et al*., 1999; Crease *et al*., 2012]. Meanwhile, such a vast region with numerous freshwater bodies clearly deserves close attention regarding an accurate evaluation of evolutionary history of *D. pulex* s.lat. species and identification of routes of spatial expansion of large mitochondrial phylogroups. Secondly, in our study, haplotypes of the "pan-Arctic" lineage of *D. pulex* were not found in Magadan Oblast. If we speculate that haplotypes of the "pan-Arctic" lineage of *D. pulex* penetrated the Eurasian continent through Alaska, it is likely that they would colonize water bodies of Magadan region, which is located in close proximity to the North-American continent and is adjacent to Yakutia, where these haplotypes occur. Undoubtedly, for a complete understanding of evolutionary mechanisms and routes of dispersion and for solving taxonomic problems in the *D. pulex* s.lat. group, it is necessary to carry out a larger-scale comparative phylogeographic analysis. To date, we can be certain only about similar directions of evolutionary processes in the group *D. pulex* s.lat. on different continents of the Holarctic.

Conclusion

Our study confirms existence of the gaps in the taxonomy of *D. pulex* s.lat. We revealed a high genetic differentiation within populations in Northern Eurasia, thereby indicating a hidden species diversity. A solution to this problem requires additional morphological and genetic studies. Genetic-polymorphism indices, neutrality tests, and the structure of haplotype networks point to a rapid spatial expansion of the "pan-Arctic" mitochondrial phylogroup of *D. pulex*. In *D. middendorffiana*, a widespread haplotype also occurs in populations from different regions of Northern Eurasia, but its rapid spatial expansion is not so obvious. Within both species, regional mitochondrial subclades and clades with increased haplotype diversity were identified: it is a consequence of paleogeographic events in the Late Pleistocene to the Early Holocene. Our results expand knowledge about genetic diversity of *D. pulex* s.lat. in Northern Eurasia. It is demonstrated above that the data — obtained with the help of this marker — on genetic diversity, evolutionary history, phylogenetic relationships, and the phylogeography of *D. pulex*s. lat. in Northern Eurasia are all consistent with previously obtained results on Western-European and North-American populations. At the same time, the new findings suggest that the question on the place of origin of the *D. pulex* "pan-Arctic" phylogroup remains to be opened.

Compliance with ethical standards

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest.

Ethical approval: No ethical issues arose during our research.

Supplementary Issues

Supplementary Table 1. The list of the mtDNA 12S rRNA gene sequences from *Daphnia pulex* and *D. middendorffiana* specimens, their IDs, haplotypes, GenBank accession numbers, and the list of species from the GenBank database (GB) used in this study

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