

Evolutionary history of the subgenus *Daphnia* (*Ctenodaphnia*) Dybowski et Grochowski, 1895 (Crustacea: Cladocera), inferred from a phylogenomic study of *D. (C.) studeri* (Rühe, 1914)

Эволюционная история подрода *Daphnia* (*Ctenodaphnia*) Dybowski et Grochowski, 1895 (Crustacea: Cladocera) в свете филогеномного исследования *D. (C.) studeri* (Rühe, 1914)

Dmitry D. Pereboev*, Alexey A. Kotov
Д.Д. Перебоев, А.А. Котов

A.N. Severtsov Institute of Ecology and Evolution, Leninsky Prospect 33, Moscow 119071 Russia.

Институт проблем экологии и эволюции им. А.Н. Северцова Российской академии наук, Ленинский просп., 33, Москва 119071 Россия.

*Corresponding author

Dmitry Pereboev: dm.pereboev@gmail.com; <https://orcid.org/0009-0004-8194-7585>

Alexey Kotov: alexey-a-kotov@yandex.ru; <https://orcid.org/0000-0002-8863-6438>

KEY WORDS: Branchiopoda, protective structures, phylogeography, phylogeny, Antarctic.

КЛЮЧЕВЫЕ СЛОВА: Branchiopoda, защитные структуры, филогеография, филогения, Антарктика.

ABSTRACT. Subgenus *Daphnia* (*Ctenodaphnia*) Dybowski et Grochowski, 1895 (Crustacea: Cladocera) is a diverse group inhabiting a wide range of biotopes spreading from the Arctic to the Antarctic. *D. (C.) studeri* (Rühe, 1914) is one of only two species of Cladocera present in continental Antarctica, it is also distributed in Subantarctic islands. Earlier research showed that this species is closely related to *D. atkinsoni* s.l., an example of the so-called “crowned” *D. (Ctenodaphnia)* having a “crown of thorns”, a protective structure induced by the presence of predators. Moreover, *D. (C.) studeri* was earlier placed in “*Daphniopsis* Sars, 1903” genus with another protective structure, the caudal needle, being highly reduced or absent. We performed phylogenomics and mitogenomic analyses to explore evolutionary history of *D. (C.) studeri*. In the mitogenomic analyses, the *D. (C.) atkinsoni* species group (*D. atkinsoni* s.l. and *D. studeri*) is monophyletic and *D. studeri* appears in the sister position to *D. atkinsoni* s.l. On the other hand, in the nuclear phylogeny *D. atkinsoni* s.l. is paraphyletic with respect to *D. studeri*. However, based on the morphological and mitogenomic evidence, and the results of the concordance factor analysis, we believe that the latter result is possibly an artefact.

According to fossil-calibrated dating based on mitogenomes, divergence of the *D. (C.) atkinsoni* species group and the closest outgroup, *D. (C.) tibetana*, happened ca. 80 MYA, and divergence of *D. (C.) studeri* and *D. (C.) atkinsoni* s.l. happened ca. 60 MYA. Such dates correspond well to the time of existence of the Oman–Kohistan–Ladakh Arc (~80–55 MYA), a hypothetical route between India and North Africa, possibly used by the earlier lineages for their dispersal. Moreover, an ancestor of *D. (C.) studeri* could have passed south via Kerguelen and Ninetyeast Ridge route, which had been open since

the Late Cretaceous. Tracing the evolution of protective structures, we can hypothesize that their loss was associated with colonisation of refugial habitats (extreme arid regions with saline waters, high mountains of Tibet and Antarctic-sub-Antarctic regions). Such habitats lack most predators, including small-sized invertebrates, against which protection is provided by the caudal needle and the “crown of thorns”.

How to cite this paper: Pereboev D.D., Kotov A.A. 2026. Evolutionary history of the subgenus *Daphnia* (*Ctenodaphnia*) Dybowski et Grochowski, 1895 (Crustacea: Cladocera), inferred from a phylogenomic study of *D. (C.) studeri* (Rühe, 1914) // Arthropoda Selecta. Vol.35. No.3. P.293–302, Suppl. Table 1. doi: 10.15298/arthscl.35.3.03

РЕЗЮМЕ. Подрод *Daphnia* (*Ctenodaphnia*) Dybowski et Grochowski, 1895 (Crustacea: Cladocera) — разнообразная группа, которая обитает в широком диапазоне биотопов от Арктики до Антарктики. В континентальной Антарктике *D. (C.) studeri* (Rühe, 1914) — это один из двух видов кладоцер, обитающих там. Предыдущие исследования показали, что этот вид близкородственен *D. (C.) atkinsoni* s.l., представителю так называемых «коронованных» ктенадафний, обладающих «терновым венцом», защитной структурой, индуцируемой в присутствии хищников. Более того, *D. studeri* ранее считалась принадлежащей к роду “*Daphniopsis* Sars, 1903”, у которого другая защитная структура, хвостовая игла, сильно редуцирована, если вообще присутствует. Мы провели фило- и митогеномные анализы чтобы исследовать эволюционную историю *D. (C.) studeri*. В митогеномных анализах, группа видов *D. (C.) atkinsoni* (*D. atkinsoni* s.l. и *D. studeri*) монофилетична, и *D. studeri*

оказывается в сестринской позиции к *D. atkinsoni* s.l. С другой стороны, в ядерной филогении *D. (C.) atkinsoni* s.l. парафилетична по отношению к *D. (C.) studei*. Однако, основываясь на морфологических и митогеномных доказательствах, а также результатах анализа факторов конкордности, мы считаем, что данная топология скорее всего является артефактом.

Согласно нашей митогеномной калиброванной датировке, расхождение группы видов *D. (C.) atkinsoni* и ближайшей внешней группы, *D. (C.) tibetana*, произошло около 80 MYA, и расхождение *D. (C.) studei* и *D. (C.) atkinsoni* s.l. — около 60 MYA. Такие временные рамки хорошо соответствуют времени существования Оман–Кохистан–Ладакской арки (~80–55 MYA), гипотетического пути между Индией и Северной Африкой, вероятно использованного ранними линиями для их распространения. Кроме того, предок *D. studei* мог уйти на юг через Кергеленский и Восточно-Индийский хребтовый путь, который был открыт начиная с позднего мела. Проследив эволюцию защитных структур, мы можем предположить, что их потеря была ассоциирована с колонизацией рефугиальных местообитаний (экстремально аридные регионы с солеными водами, высокогорье Тибета, Антарктико-Субантарктические регионы). В таких местообитаниях отсутствует большинство хищников, включая мелких беспозвоночных, от которых защищают хвостовая игла и «терновым венцом».

Introduction

Among the microscopic inhabitants of continental water bodies, the genus *Daphnia* O.F. Müller, 1776 (Crustacea: Cladocera: Anomopoda: Daphniidae) is probably the most famous taxon. The genus contains more than 100 known species assigned to three subgenera [Colbourne et al., 2006; Adamowicz et al., 2009]: *D. (Australodaphnia)* Colbourne, Wilson et Heber, 2006, with a single taxon endemic to Australia, and two species-rich subgenera: *Daphnia (Daphnia)* and *Daphnia (Ctenodaphnia)* Dybowski et Grochowski, 1895. The latter is a diverse group that inhabits a wide range of biotopes ranging from the Arctic to the Antarctic [Benzie, 2005]. In continental Antarctica *D. (C.) studei* is one of only two species of Cladocera present [Pugh et al., 2002; Kotov, 2007]. This taxon also on several sub-Antarctic islands: Kerguelen, Marion and Prince Edward, Crozet, Heard and Macquarie [Benzie, 2005].

Benzie [1987], referring to data of Hebert [1978], pointed to contrasting distribution of the two extant subgenera: most species of *D. (Daphnia)* occur in the Northern Hemisphere, whereas those of *Daphnia (Ctenodaphnia)* occur in the Southern Hemisphere. He hypothesized that their differentiation was associated with the breakup of Gondwana and Laurasia. Subsequent studies have rejected this hypothesis because:

1) The non-calibrated molecular clock estimates indicated that the genus *Daphnia* had an Early Mesozoic origin (≥ 200 MYA, Triassic period) and that its subgenera originated at ca. 175 MYA (Jurassic), see Taylor et al.,

[1996]. This means that they had already formed in Pangaea, before the breakup of the continents.

2) Fossil ephippia belonging to two different subgenera were found at the Mongolian locality of Khotont, dated to the exact Jurassic/Cretaceous boundary (145 MYA), therefore, both subgenera were present in Laurasia at that time [Kotov, Taylor, 2011].

3) The basal clade of the genus *Daphnia*, namely *Daphnia (Australodaphnia)* is endemic to Australia; although most species of the subgenus *D. (Daphnia)* inhabit the Holarctic; the basalmost taxon of *D. (Ctenodaphnia)*, namely *D. (C.) ephemeralis* [Schwartz, Hebert, 1985], is endemic to the Great Lakes basin in North America [Adamowicz et al., 2009]. Therefore, the recent distribution ranges of earlier-derived clades strongly contradict Benzie's ideas. The very isolated position of earlier-derived clades possibly suggests their Pangaeian origin.

4) Finally, an accurate re-calculation of the species numbers of *D. (Daphnia)* and *D. (Ctenodaphnia)* in different latitudinal zones of the planet led to the conclusion that the hypothesis of Benzie [1987, 2005] was based on erroneous (outdated) estimates. In reality, “the distribution of *Daphnia* s.str. is dissymmetric between the hemispheres (antipolar), while that of *Ctenodaphnia* is sub-symmetric (bipolar)” [Popova, Kotov, 2013]. Note that both patterns are typical of “ejected relicts” and have a Cenozoic rather than Mesozoic origin [Korovchinsky, 2006; Kotov, Taylor, 2011].

Therefore, it is obvious that the genus *Daphnia* has a very long (from the Triassic through the entire Mesozoic and Cenozoic) and complex evolutionary history. We can conclude that the Quaternary history is relatively well-studied due to a large series of phylogeographic publications [Taylor et al., 1996, 1998; Petrusek et al., 2007; Mergeay et al., 2008; Zuykova et al., 2019]. However, the early evolutionary history of the main lineages within the genus *Daphnia* remains unclear to date. Adamowicz et al. [2009] discussed the differentiation of several species complexes within particular continents (e.g., Australia) and pointed to possible intercontinental transitions that occurred more recently than the breakup of Pangaea and the onset of Gondwana/Laurasia drift. Cornetti et al. [2019] performed a fossil-calibrated phylogenomic analysis of *Daphnia* and concluded that the tree based on nuclear genes gives “very different estimates of branching event times compared to those based on mtDNA”. At the same time, the major genome-based clades generally agree with the major clades earlier found in the mitogenomic trees [Adamowicz et al., 2009]; moreover, Beninde et al. [2026] reported “evidence of ancient introgression between species known to hybridize concurrently”. In general, information on the early evolutionary history of *Daphnia* is scarce due both to the absence of any palaeontological records older than 145 MYA and to a limited set of species included in full-genome studies.

Our recent research program on the subgenus *Daphnia (Ctenodaphnia)* combines analyses of full-genome phylogenies and traditional mitochondrial trees with morphology-based taxonomic revisions [Garibian et al., 2025; Pereboev et al., 2025]. The following conclusions have been drawn over the past two years:

1) There are four major clades within *D. (Ctenodaphnia)*, among which a single species, *D. (C.) ephemerialis*, represents an earlier-derived (=basal) clade.

2) In Major Clade 2, there are “crowned” *Ctenodaphnia* having a so-called “head plate” (in terms of Glagolev [1995]), sometimes provided with a marginal row of spines (=“crown of thorns” sensu Petrusek *et al.* [2009]). It is known that the “crown of thorns” is a protective structure induced by the presence of predators [Petrusek *et al.*, 2009]. We have found that the “crowned” *D. (Ctenodaphnia)* are non-monophyletic being represented by two lineages within the Major Clade 2: *D. triquetra* Sars, 1903 [Pereboev *et al.*, 2025] and the *D. atkinsoni* Baird, 1859 s.l. group, consisting of at least three taxa [Garibian *et al.*, 2025]. The same monophyletic clade contains several taxa completely lacking of the head plate: *D. mediterranea* Alonso, 1985, *D. tibetana* (Sars, 1903) and *D. studei* (Rühe, 1914); moreover, *D. hispanica* Glagolev *et al.*, 1990 is also close to this group [Pereboev *et al.*, 2025]. The genomes of most of the aforementioned taxa have been studied previously, while only a few COI and 16S sequences are available for *D. (C.) studei* to date.

To assess the hypothesis of ancient differentiation of the lineage leading to *Daphnia (C.) studei* and to reconstruct possible palaeobiogeographic implications of this species and related taxa, we set out to perform the most complete phylogenomic and mitogenomic analyses of the subgenus to date and to estimate divergence times for the Major Clades and some phylogenetic lineages within Major Clade 2.

Material and Methods

In this study, we obtained short-read whole-genome sequencing (WGS) data for a sample (an individual parthenogenetic female, fixed in 96% ethanol) of *Daphnia (C.) studei*. This animal was collected by V.L. Syomin in Lake LH-64 (−69.38661, 76.30254), Larsemann Hills, continental Antarctica on 11 February 2023.

DNA was isolated from the whole animal using the Qiamp Micro Kit (Qiagen) according to the manufacturer’s protocol into 50 µL of eluent solution. Library preparation and whole genome sequencing (ca. 6.7 Gbp of paired reads of 150 bp, the DNBSEQ Platform) were performed by the BGI Genomics Co., Ltd.

After filtering with fastp 0.23.4 [Chen, 2023], we assembled and annotated the mitochondrial genome as described earlier [Pereboev *et al.*, 2025] using GetOrganelle v1.7.7.1 [Jin *et al.*, 2020] and MitoZ v3.6 [Meng *et al.*, 2019]. Since we were not able to determine the exact repeat pattern, we extracted the largest edge using Bandage [Wick *et al.*, 2015]. Obtained data were combined with other mitogenomes of *Daphnia (C.)* publicly available (Supplementary Table 1), all protein-coding genes (PCGs) and rRNA genes were used for phylogenetic reconstruction. Individual genes were aligned using MAFFT v7.525 [Katoh *et al.*, 2019], with the L-INS-i algorithm, and gappy regions from the rRNA genes were removed by trimAl v1.4.rev15 [Capella-Gutierrez, Silla-Martinez *et al.*, 2009] with the -automated1 option. The data were partitioned in 4 partitions (rRNA genes, and 3 codon positions). We used IQ-TREE 3.0.1 [Wong *et al.*, 2025] with automatic model selection [Kalyaanamoorthy *et al.*, 2017] to reconstruct ML phylogenetic trees with 1000 ultrafast bootstrap replicates [Hoang *et al.*,

2018] and 1000 SH-aLRT replicates [Guindon *et al.*, 2010]. The final mitogenome dataset were composed of 20 taxa, 4 partitions with 13,472 total sites. The trees were rooted with *D. (Daphnia)* (represented by two different taxa) as an outgroup. Next, upon data used in previous publications [Adamowicz *et al.*, 2009; Pereboev *et al.*, 2025; Garibian *et al.*, 2026] for up to 3 mitochondrial loci (COI, 12S, 16S) and adding corresponding newly obtained sequences, a tree with wide taxonomic coverage was built as described above (see Supplementary Table 1 for the list of sequences).

Finally, the mitogenomic dataset partitioned as described were also used to estimate divergence times using the BEAST2 v2.7.6 package [Bouckaert *et al.*, 2019]. In accordance to Van Damme & Kotov [2016], we set priors in the Late Jurassic (143.1–161.5 MYA) for both subgenera and the root (the genus) assuming their uniform distribution and ensuring the monophyly of the taxa. Optimised Relaxed Clock Model and Calibrated Yule Model were used, and the HKY substitution model with 4 Gamma categories was applied for each partition. Two independent runs of 50,000,000 generations with sampling every 10000 generation were conducted, their convergence and ESS values (should be >200) were checked with Tracer v1.7.2 [Rambaut *et al.*, 2018]. First 10% were discarded as burn-in and the CCD0 tree was produced [Berling *et al.*, 2025].

Furthermore, raw reads were assembled by the MaSuRCA package [Zimin *et al.*, 2013]. Publicly available genomic data (Supplementary Table 1) were added to the dataset, and BUSCO 5.8.3 [Manni *et al.*, 2021] was run using lineage dataset crustacea_odb12 to extract single-copy BUSCO genes for subsequent analysis. Shared single-copy BUSCO genes (981 in total) were aligned by MAFFT and all gaps were trimmed with trimAl. The phylogeny was reconstructed by IQ-TREE 3.0.1 with 1000 ultrafast bootstrap replicates and automatic model selection for each gene in a separate partition [Chernomor *et al.*, 2016]. The final phylogenomic dataset were composed of 12 taxa and 981 partitions with 1,363,923 total sites. Moreover, site concordance factor analysis was performed, and to check violations of incomplete lineage sorting model we used a chi-square test on the counts of sites supporting the two discordant topologies (site discordance test). To investigate the strength of the phylogenetic signal, we performed site concordance factor (SCF) analysis [Mo *et al.*, 2023]. SCF corresponds to a fraction of decisive sites supporting given branch resolution against 2 alternatives [Lanfear, Hahn, 2024], and to check violations of incomplete lineage sorting model we used a chi-square test on the counts of sites supporting the two discordant topologies (onwards: site discordance test). The SCF values of about 33% is expected when each resolution is randomly assigned, and values more than 50% demonstrate the majority of sites support the given topology. A failed site discordance test could be explained by violations of the incomplete lineage sorting model (e.g. by reticulate evolution, etc.), or by some kind of systematic error (dis)favouring one of the topologies.

Results

All the Major Clades from Pereboev *et al.* [2025] were robustly recovered in our mitogenomic analyses (Figs 1A, 2), and the three-locus tree demonstrates a corresponding topology (Fig. 3). Since the taxa under discussion here belong to the Major Clade 2, all other major clades are not discussed below. The topology of the Major Clade 2 in the Bayesian tree is the same as the in the Maximum Likelihood (ML) tree, but shows maximal posterior probabilities for all nodes, with *D. hispanica* in the basal

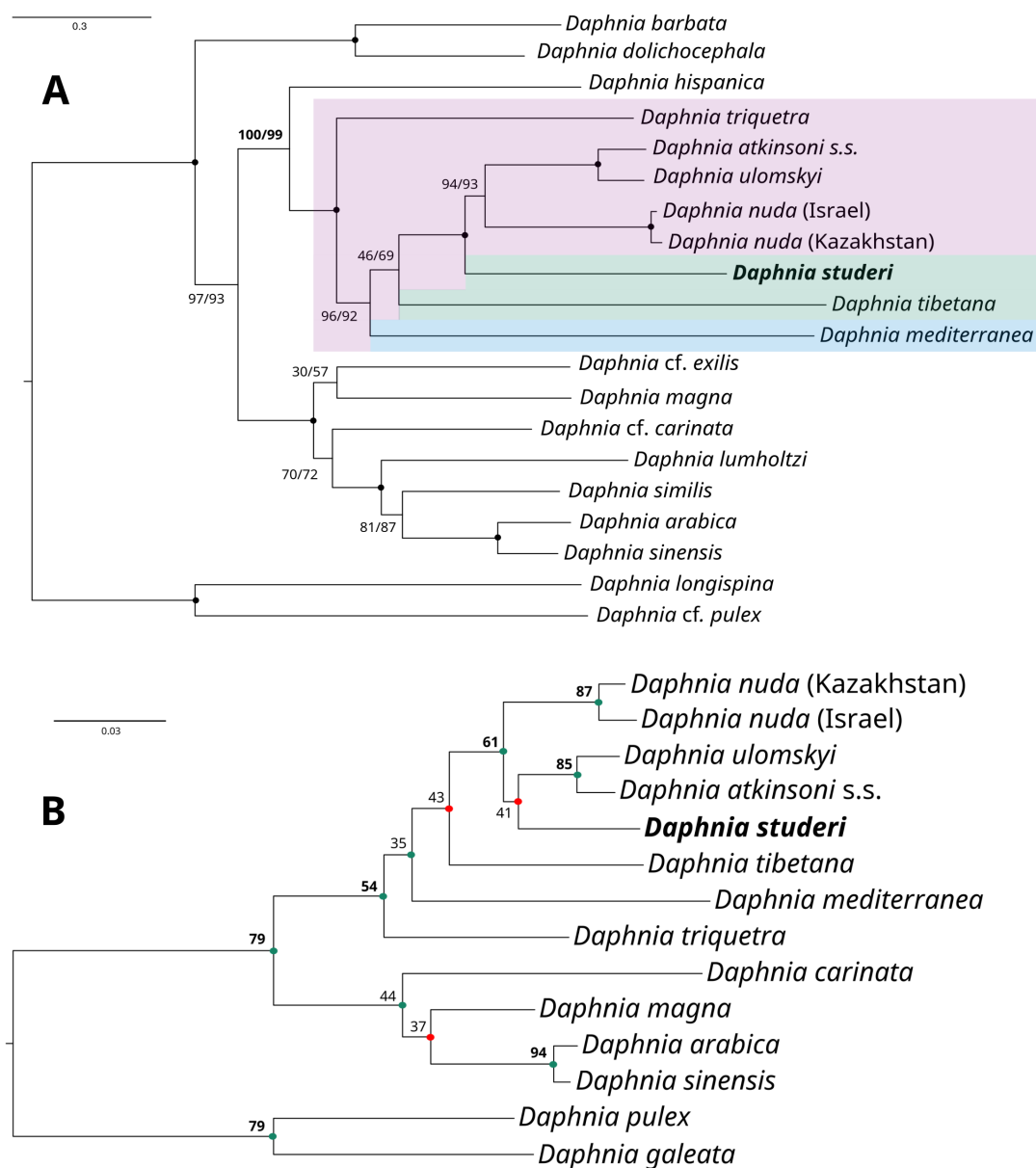


Fig. 1. Multigene phylogenetic reconstructions of the *Daphnia* (*Ctenodaphnia*) subgenus. Subgenus *D.* (*Daphnia*), represented by two different taxa, is used as an outgroup. **A** — maximum likelihood mitogenomic tree based on both ribosomal genes and full nucleotide sequences of all protein-coding genes partitioned by codon positions. SH-like approximate likelihood ratio test (SH-aLRT) / Ultrafast bootstrap (Ufboot) supports are given at the nodes. Supports of the nodes with SH-aLRT $\geq 80\%$ and Ufboot $\geq 95\%$ are highlighted by bold type. Nodes with maximal supports are marked with black dots. Purple box indicates the group of “crowned” *Ctenodaphnia*; blue box — secondary loss of the “crown” but retaining of the developed caudal needle; green box — former members of “*Daphniopsis*” with lost “crown” and the caudal needle in adult females. **B** — maximum likelihood phylogenomic tree based on 981 shared single-copy BUSCO genes. All Ultrafast bootstrap supports are maximal and not shown. Site concordance factor values are given at the nodes, values $> 50\%$ are highlighted in bold. Nodes (branches) passed the chi-square test on the assumptions of an ILS model are marked by green dots, otherwise with red dots.

Рис. 1. Мультигенные филогенетические реконструкции для подрода *Daphnia* (*Ctenodaphnia*). Подрод *D.* (*Daphnia*), представленный двумя различными таксонами, использован в качестве внешней группы. **A** — митогеномное дерево, построенное методом максимального правдоподобия на основе обоих рибосомальных и полных нуклеотидных последовательностей всех протеин-кодирующих генов, разбитых по позициям в кодоне. Поддержки теста приближенного отношения правдоподобия с непараметрической коррекцией на базе алгоритма Шимодайры-Хасегавы (SH-aLRT) / ультрабыстрого бутстрэпа (Ufboot) даны возле узлов. Поддержки узлов с SH-aLRT $\geq 80\%$ и Ufboot $\geq 95\%$ выделены жирным. Узлы с максимальными поддержками отмечены черными точками. Лиловым выделением обозначена группа “коронованных” ктенодафний; синим — вторичная потеря “короны” с сохранением развитой хвостовой иглы; зеленым — бывшие члены “*Daphniopsis*”, потерявшие “корону” и хвостовую иглу у взрослых самок. **B** — митогеномное дерево, построенное методом максимального правдоподобия на основе 981 общих однокопийных генов-ортологов BUSCO. Все поддержки ультрабыстрого бутстрэпа максимальны и не показаны. Значения сайтовых факторов конкордности даны возле узлов, значения $> 50\%$ выделены жирным. Узлы (ветви) прошедшие хи-квадрат тест на предположения модели неполной сортировки линий отмечены зелеными точками, остальные — красными.

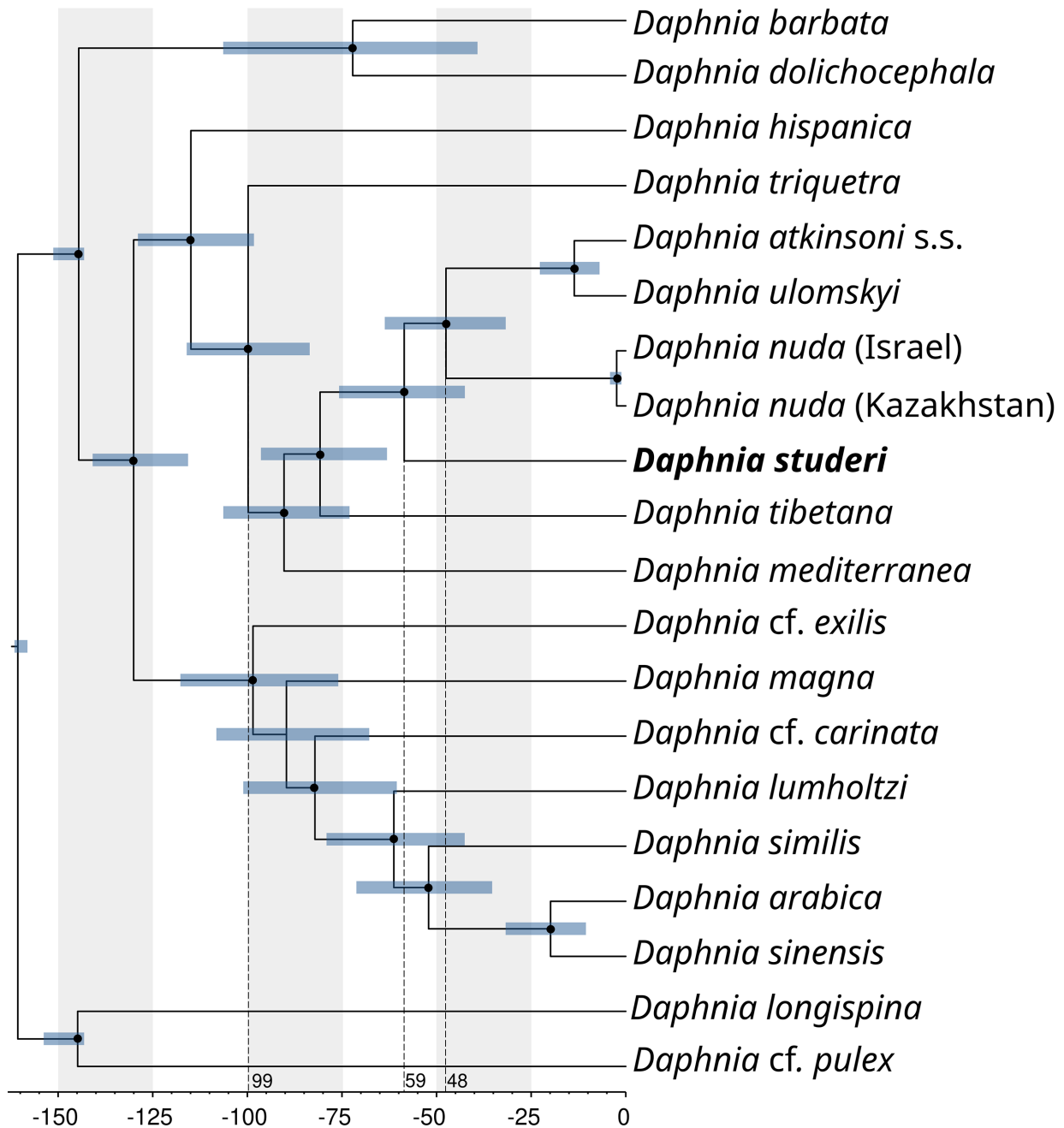


Fig. 2. Fossil-calibrated Bayesian inference mitogenomic tree of *Daphnia* (*Ctenodaphnia*) based on both ribosomal genes and full nucleotide sequences of all protein-coding genes partitioned by codon positions. Nodes with maximal posterior probabilities (PP) are marked by black dots, nodes with PP less than 95% are not labelled. Bars represent 95% Highest Posterior Density. Subgenus *D. (Daphnia)*, represented by two different taxa, is used as an outgroup.

Рис. 2. Байесово калиброванное митогеномное дерево подрода *Daphnia* (*Ctenodaphnia*), построенное на основе обоих рибосомальных и полных нуклеотидных последовательностей всех протеин-кодирующих генов, разбитых по позициям в кодоне. Узлы с максимальной апостериорной вероятностью (AB) отмечены черными точками, узлы с AB меньше 95% не подписаны. Полосы показывают область 95%-й наивысшей апостериорной плотности. Подрод *D. (Daphnia)*, представленный двумя различными таксонами, использован в качестве внешней группы.

position and the *D. triquetra* branch next after it. The congruent topology of the trees allows us to focus on the results of the ML analysis (Fig. 1A), without referring to the BI tree.

The *D. atkinsoni* species group (*D. atkinsoni* s.l. and *D. studeri*) is monophyletic with maximum support; non-

monophyly of the crowned *Daphnia* (*D. atkinsoni* s.l. and *D. triquetra*) is confirmed once more in the present study. *D. studeri* appears in the sister position to *D. atkinsoni* s.l. (*D. atkinsoni*, *D. nuda*, and *D. ulomskyi*), but the support (94 / 93) is rather modest. Unfortunately, the three-locus tree (Fig. 3) provided is rather uninformative, supporting

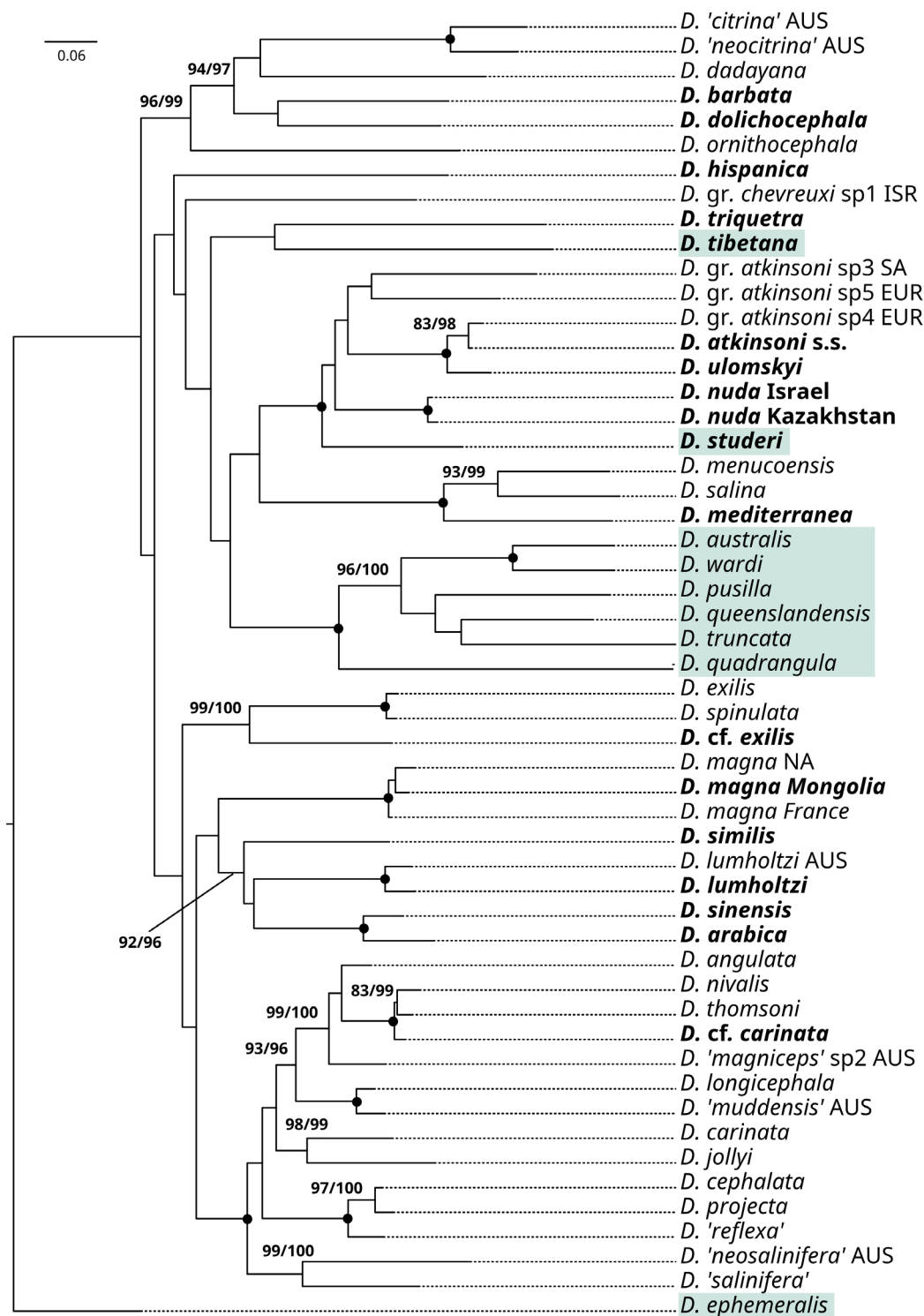


Fig. 3. Maximum likelihood tree of *Daphnia* (*Ctenodaphnia*) based on up-to three mitochondrial genes (COI, 12S, 16S). Taxa used in the mitogenomic analyses are marked by bold type. SH-like approximate likelihood ratio test (SH-aLRT) / Ultrafast bootstrap (Ufboot) supports are given at the nodes. Only nodes with SH-aLRT $\geq 80\%$ and Ufboot $\geq 95\%$ are shown. Nodes with maximal supports are marked with black dots. Green boxes indicate former members of "Daphniopsis". *D. (C.) ephemeris* is used as an outgroup.

Рис. 3. Митогеномное дерево подрода *Daphnia* (*Ctenodaphnia*), построенное методом максимального правдоподобия с использованием до трех митохондриальных генов (COI, 12S, 16S). Таксоны, использованные в митогеномных анализах, выделены жирным. Поддержки теста приближенного отношения правдоподобия с непараметрической коррекцией на базе алгоритма Шимодайры-Хасагавы (SH-aLRT) / ультрабыстрого бутстрэпа (Ufboot) даны возле узлов. Указаны только поддержки узлов с SH-aLRT $\geq 80\%$ и Ufboot $\geq 95\%$. Узлы с максимальными поддержками отмечены черными точками. Зеленым выделением обозначены бывшие члены "Daphniopsis". *D. (C.) ephemeris* использован в качестве внешней группы.

the basal position of *D. studei* but with insufficient support for any accurate conclusions.

Finally, fossil-calibrated molecular dating based on mitogenomic data (Fig. 2) estimated the mean age of the MRCA of the crowned *Daphnia* at ca. 99 MYA, that of the *D. atkinsoni* species group at ca. 59 MYA, and *D. atkinsoni* s.l. at ca. 48 MYA.

In the phylogenomic tree based on single-copy BUSCO genes (Fig. 1B) all nodes were maximally supported. The topology is mostly consistent with the mitogenomic one; however, the structure of the *D. atkinsoni* species group is different. *D. studei* appears in sister position to the *D. atkinsoni* + *D. ulomskyi* clade, effectively making *D. atkinsoni* s.l. paraphyletic. Although all other branches inside the *D. atkinsoni* species group show a rather strong phylogenetic signal, the position of *D. studei* is weakly supported by the SCF analysis. Moreover, this branch failed the site discordance test, what further undermines credibility of the inference.

Discussion

Earlier molecular studies [Adamowicz *et al.*, 2009] already demonstrated that the closest relative of the Antarctic *D. (C.) studei* is *D. (C.) atkinsoni* s.l., a species complex native to Western Eurasia and Northern Africa [Garibian *et al.*, 2026]; although some sequence data from South America grouped with them, they do not appear to be unequivocally the closest lineage to the Antarctic species. Such situation is quite intriguing and may be explained either by ancient differentiation of the species groups in *Daphnia* (Ctenodaphnia) or by later dispersal. Since Cladocera appears to be a very old, relict group [Korovchinsky, 2006] and the earliest fossils of *D. (Ctenodaphnia)* date back to the Late Jurassic, the former explanation seems plausible to us.

Our results cannot unambiguously resolve the phylogenetic position of *D. (C.) studei* within the Major Clade 2 due to the apparent mito-nuclear discordance. Indeed, the nuclear phylogeny supports an unexpected resolution in which the highly morphologically similar [Garibian *et al.*, 2026] *D. (C.) atkinsoni* species complex proves to be paraphyletic. Although researchers usually tend to explain mito-nuclear discordance by biological means, e.g. sex-biased dispersal, adaptive introgression, etc. [Toews, Brelsford, 2012], we believe that this resolution is rather an artefact. Our concordance factor analysis has revealed that nuclear phylogenetic signal is weak, and the failed chi-square test is another reason to doubt this phylogeny. Thus, integrating both morphological and mitogenomic data, we assume the sister position of *D. (C.) studei* to *D. (C.) atkinsoni* s.l.

D. (C.) studei was initially [Rühe, 1914] placed to the genus *Daphniopsis* Sars, 1903, described as “somewhat intermediate between *Daphnia* and *Simocephalus*” [Sars, 1903] and most characteristically lacking a dorsal carina and a caudal needle (= caudal spine, = tail spine) in adult females [Schwartz, Hebert, 1985]. This genus has now been shown to be incorporated into *D. (Ctenodaphnia)* into four distinctive species complexes, and all of them

except *D. ephemeralis* appear to be somewhat related [Adamowicz *et al.*, 2009]. Although we do not have genomic data on the *D. pusilla* complex, we think that these Australian endemics is a part of the second major ctenodaphnian clade sensu Pereboev *et al.* [2025], and our three-locus tree (Fig. 3) is congruent with this assumption. Moreover, another former member of *Daphniopsis*, *Daphnia (C.) tibetana* is placed basal to *Daphnia (C.) studei* in both our nuclear and mitogenomic phylogenies. This is remarkable because *D. studei* (inhabiting Antarctica and nearby islands) proves to be situated in the trees between species occurring, on the one hand, in Tibet and Mongolia (*D. tibetana*), and, on the other hand, in Europe, West Asia and North Africa (*D. atkinsoni* s.l.). Therefore, the question arises as to how such a biogeographical pattern could be explained, is arising. As Cladocera is a very ancient group [Van Damme, Kotov, 2016], such a pattern could be explained by extinction of all intermediate lineages, whereas only a few have managed to survive to this day. On the other hand, this implies an ancient origin of the lineages in question.

According to our fossil-calibrated dating, the divergence of the *D. (C.) atkinsoni* species group and *D. (C.) tibetana* occurred ca. 80 MYA, and the divergence of *D. (C.) studei* and *D. (C.) atkinsoni* s.l. occurred ca. 60 MYA. Such dates correspond well to the time of existence of the Oman–Kohistan–Ladakh Arc (~80–55 MYA), hypothetical route between India and North Africa (Fig. 4) [Chatterjee, Scotese, 2010; Gobbo, Bertini, 2026]. We can assume that the ancestor of Major Clade 2 has a Gondwanan origin. The common ancestor of *D. (C.) tibetana* and *D. (C.) studei* inhabited Africa and at the moment of the opening of the Arc migrated into India, where lineages of *D. tibetana*, *D. studei*, and *D. atkinsoni* became separated. After that, the ancestor of *D. atkinsoni* s.l. migrated back, and the ancestor of *D. studei* went south via Kerguelen and Ninetyeast Ridge route, which had been open since the Late Cretaceous [Chatterjee, Scotese, 2010; Gobbo, Bertini, 2026]. Interestingly, *D. studei* was originally described from the Kerguelen Islands [Rühe, 1914]. Another possible scenario is the separation of the *D. (C.) studei* lineage group in Africa and its subsequent colonisation of Antarctica through the aforementioned routes. In any case, these routes are not unique for our reconstructions; for example, they were proposed to explain global dispersal patterns in non-marine Ostracoda [Gobbo, Bertini, 2026].

Therefore, we propose two plausible palaeobiogeographic scenarios of dispersal of *Daphnia (C.) studei* and related taxa. Although these scenarios fit our data well, some precautions should be taken. The molecular clock, especially over such long time spans, could be quite inaccurate, and palaeogeographic reconstructions are not immune to errors. Nonetheless, this scenario seems to be the most realistic one. Further research is needed to elucidate the phylogeography of *D. studei*; currently, molecular data are available only for some nearby populations from continental Antarctica. It will be very intriguing to obtain sequences from other more distant populations, first of all, from the type locality, the Kerguelen Islands.

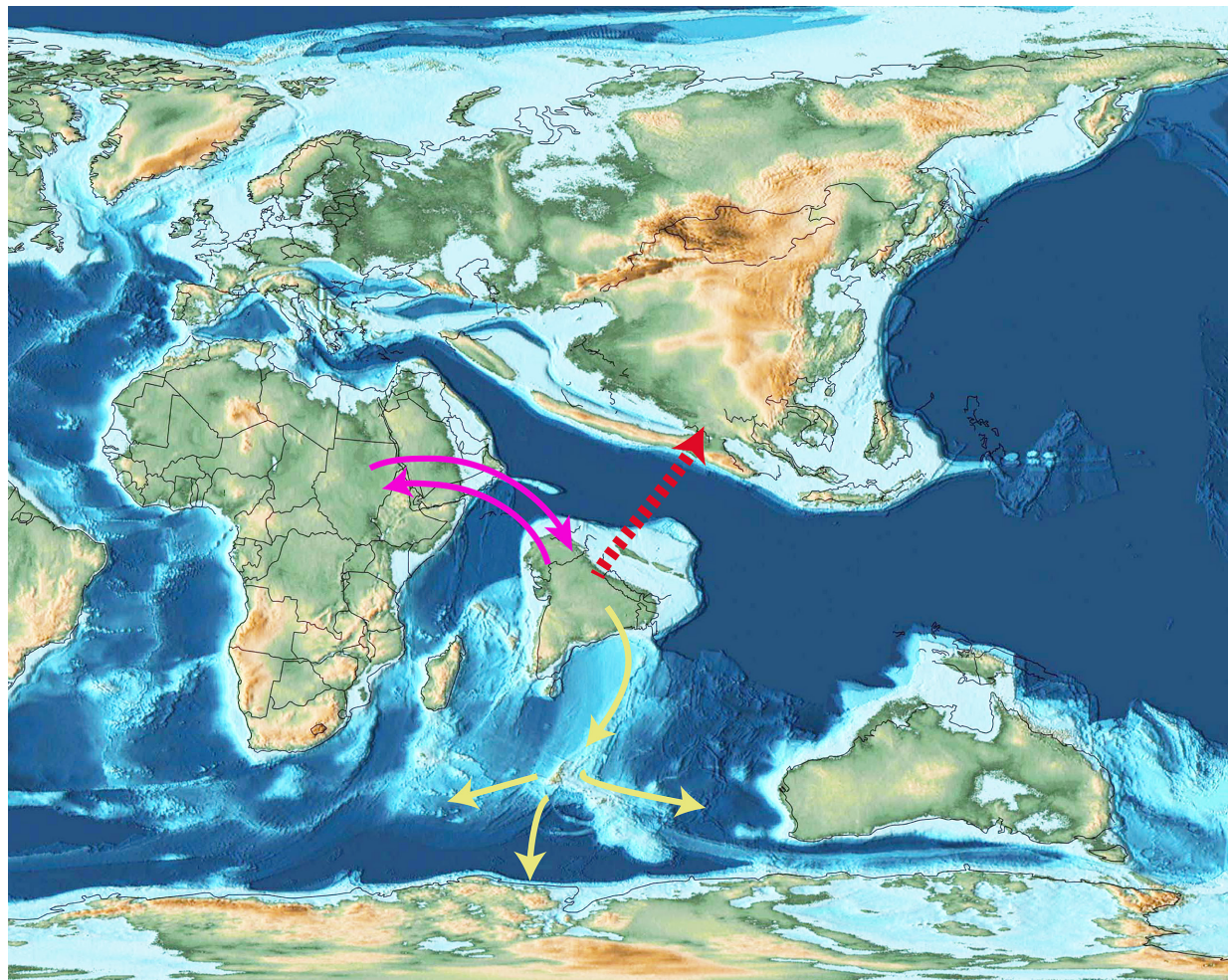


Fig. 4. Hypothetical ancient intercontinental routes at the Mesozoic/Cenozoic boundary. Purple arrows indicate migrations through the Oman–Kohistan–Ladakh Arc; yellow arrows indicate migrations through the Kerguelen and Ninetyeast Ridge route; dashed red arrow indicates the movement of India towards Proto-Asia. Base map of K/T Boundary (66 MYA) from Scotese [2016] licensed under CC BY 4.0.

Fig. 4. Гипотетические древние межконтинентальные коридоры около границы мезозоя и кайнозоя. Лиловые стрелки обозначают миграции через Оман–Кохи́стан–Лада́хскую арку; желтые стрелки — Кергеленский и Восточно-Индийский хребтовый путь; пунктирная красная стрелка — движение Индии в сторону Прото-Азии. Карта для границы мела и палеогена (66 млн.л.н.) для основы взята из Scotese [2016].

We can trace not only the biogeographic pattern in our trees, but also the appearance/disappearance of two protective morphological structures in the evolutionary history of the subgenus, namely the caudal needle and the head plate (Fig. 1A). Although adult females of *D. (C.) ephemeralis* (the basalmost member of *Ctenodaphnia*) have a rudimentary caudal needle, the needles of males and juvenile are more pronounced, therefore more or less developed caudal needle could be regarded as a plesiomorphic state for the subgenus. In the evolution of Major Clade 2, a normally developed caudal needle disappeared independently in several taxa (e.g. *D. studeri* and *D. tibetana*, and possibly in the *D. pusilla* species group if it belongs to Major Clade 2). Also, the head plate appeared early within Major Clade 2, but then it was lost in several taxa (e.g. *D. studeri*, *D. tibetana* and *D. mediterranea*). We can hypothesize that such loss of protective structures was associated with colonisation of refugial habitats (extreme arid regions with saline waters, high mountains of

Tibet and Antarctic–sub-Antarctic regions). Such habitats lack most predators, including small-sized invertebrates protection against which is provided by the caudal spine and the “crown of thorns”. For example, notostracans are very rare or absent in sub-Antarctic and saline waters [Brendonk *et al.*, 2008], and also in the Himalayas [Rogers, Padhye, 2015] which are close to Tibet, with its poorly studied notostracan fauna. Note that the induction of the “crown of thorns” was initially demonstrated just for notostracans [Petrusek *et al.*, 2009].

Therefore, the formation of biogeographic pattern in *D. (Ctenodaphnia)* happened in parallel with ecological evolution, namely the penetration into new types of habitats and loss of protective structures useless in species-poor refugial ecosystems. The appearance of fundamentally new water bodies of different refugial types was happened due to global climatic cooling and aridisation in the second half of the Cenozoic which led to a mass extinction of cladocerans and their recent relict status [Korovchinsky, 2006].

Data availability

We uploaded the newly sequenced raw reads, and the de novo assembled and annotated partial mitogenome to NCBI SRA (BioProject 1523874 acc. no. SRX35112952) and NCBI GenBank (acc. no. PZ769985), respectively.

Compliance with ethical standards

Conflict of interests: The authors declare that they have no conflict of interest.

Ethical approval: No ethical issues were raised during our research.

Supplementary data.

The following spreadsheet table is available online.

Supplementary Table 1. List of public data used in molecular phylogenetic analyses.

Acknowledgements. The study was supported by the Russian Science Foundation (grant no. 23-14-00128-II). Many thanks to G.D. Kolbasova for the sample with *D. studei* and to I.A. Dadykin for his assistance in preparation of the figures.

References

- Adamowicz S.J., Petrusek A., Colbourne J.K., Hebert P.D.N., Witt J.D.S. 2009. The scale of divergence: a phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus // *Molecular Phylogenetics and Evolution*. Vol.50. P.423–436.
- Beninde J., Olave M., Möst M., Mergeay J., Dennis S.R., Spaak P. 2026. Whole-genome sequencing reveals cryptic diversity and ancient hybridization in key aquatic herbivore *Daphnia* // *Hydrobiologia*. P.1–25 (accepted). <https://doi.org/10.1007/s10750-026-06154-7>
- Benzie J.A.H. 2005. The genus *Daphnia* (including *Daphniopsis*) (Anomopoda: Daphniidae) // *Guides to the identification of the microinvertebrates of the continental waters of the world* 21. Leiden: Kenobi Productions, Ghent & Backhuys Publishers. 376 p.
- Berling L., Klawitter J., Bouckaert R., Xie, D., Gavryushkin, A., Drummond A.J. 2025. Accurate Bayesian phylogenetic point estimation using a tree distribution parameterized by clade probabilities // *PLoS Computational Biology*. Vol.21. No.2. Art.e1012789.
- Bouckaert R., Vaughan T.G., Barido-Sottani J., Duchene S., Fourment M., Gavryushkina A., Heled J., Jones G., Kuhnert D., Maio N., Matschiner M., Mendes F.K., Muller N.F., Ogilvie H.A., Du Plessis L., Poppinga A., Rambaut A., Rasmussen D., Siveroni I., Suchard M.A., Wu C.-H., Xie D., Zhang C., Stadler T., Drummond A.J. 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis // *PLoS Computational Biology*. Vol.15. Art.e1006650.
- Brendonck L., Rogers D.C., Olesen J., Weeks S., Hoeh W.R. 2008. Global diversity of large branchiopods (Crustacea: Branchiopoda) in freshwater // *Hydrobiologia*. Vol.595. P.167–176.
- Capella-Gutierrez S., Silla-Martinez J., Gabaldón T. 2009. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses // *Bioinformatics*. Vol.25. P.1972–1973.
- Chatterjee S., Scotese C. 2010. The Wandering Indian Plate and Its Changing Biogeography During the Late Cretaceous-Early Tertiary Period // S. Bandyopadhyay (ed.). *New Aspects of Mesozoic Biodiversity*. Berlin, Heidelberg: Springer, P.105–126.
- Chen S. 2023. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp // *iMeta*. Vol.2. Art.e107.
- Chernomor O., von Haeseler A., Minh B.Q. 2016. Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices // *Systematic Biology*. Vol.65. No.6. P.997–1008.
- Colbourne J.K., Wilson C.C., Hebert P.D.N. 2006. The systematics of Australian *Daphnia* and *Daphniopsis* (Crustacea: Cladocera): a shared phylogenetic history transformed by habitat-specific rates of evolution // *Biological Journal of the Linnean Society*. Vol.89. P.469–488.
- Cornetti L., Fields P.D., Van Damme K., Ebert D. 2019. A fossil calibrated phylogenomic analysis of *Daphnia* and the Daphniidae // *Molecular Phylogenetics and Evolution*. Vol.137. P.250–262.
- Garibian P.G., Pereboev D.D., Karabanov D.P., Galimov Y.R., Petrusek A., Kotov A.A. 2025. A revision of “crowned” *Daphnia* (*Ctenodaphnia*) *atkinsoni* species group (Cladocera: Daphniidae) combining morphological and genomic evidence // *Zootaxa*. Vol.5792. No.3. P.401–456.
- Glagolev S.M. 1995. [*Daphnia*] // S.J. Tsalolikhin (ed.). *Opredelitel' presnovodnykh bespozvonochnykh Rossi*. St Petersburg: ZIN RAN. Vol.2. Rakoobraznye. P.48–58 [in Russian].
- Gobbo S.R., Bertini R.J. 2026. Global dispersal patterns of non-marine ostracods in the Late Cretaceous (Campanian–Maastrichtian) // *Cretaceous Research*. Vol.179. Art.106242.
- Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0 // *Systematic Biology*. Vol.59. No.3. P.307–321.
- Hebert P.D.N. 1978. The population biology of *Daphnia* // *Biological Reviews*. Vol.53. P.387–426.
- Hebert P.D.N., Witt J.D.S., Adamowicz S.J. 2003. Phylogeographical patterning in *Daphnia* *ambigua*: Regional divergence and intercontinental cohesion // *Limnology and Oceanography*. Vol.48. No.1. P.261–268.
- Hoang D.T., Chernomor O., Von Haeseler A., Minh B.Q., Vinh L.S. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation // *Molecular Biology and Evolution*. Vol.35. No.2. P.518–522.
- Jin J.J., Yu W.B., Yang J.B., Song Y., Pamphilis C.W., Yi T.S., Li D.Z. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes // *Genome Biology*. Vol.21. Art.241.
- Kalyaanamoorthy S., Minh B.Q., Wong T.K., Von Haeseler A., Jermini L.S. 2017. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates // *Nature Methods*. Vol.14. No.6. P.587–589.
- Korovchinsky N.M. 2006. The Cladocera (Crustacea: Branchiopoda) as a relict group // *Zoological Journal of the Linnean Society*. Vol.147. No.1. P.109–124.
- Kotov A.A. 2007. Revision of the hirsuticornis-like species of *Macrothrix* Baird, 1843 (Cladocera: Anomopoda: Macrothricidae) from Subantarctic and temperate regions of the southern hemisphere // *Journal of Natural History*. Vol.41. No.41–44. P.2569–2620.
- Kotov A.A., Korovchinsky N.M. 2006. First record of fossil Mesozoic Ctenopoda (Crustacea, Cladocera) // *Zoological Journal of the Linnean Society*. Vol.146. No.2. P.269–274.
- Kotov A.A., Taylor D.J. 2011. Mesozoic fossils (>145 Mya) suggest the antiquity of the subgenera of *Daphnia* and their coevolution with chaoborid predators // *BMC Evolutionary Biology*. Vol.11. Art.e129.
- Kotov A.A., Taylor D.J. 2019. Contrasting endemism in pond-dwelling cyclic parthenogens: the *Daphnia curvirostris* species group (Crustacea: Cladocera) // *Scientific Reports*. Vol.9. Art.e6812.
- Lanfear R., Hahn M.W. 2024. The Meaning and Measure of Concordance Factors in Phylogenomics // *Molecular Biology and Evolution*. Vol.41. No.11. Art.msae214.
- Meng G., Li Y., Yang C., Liu S. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization // *Nucleic Acids Research*. Vol.47. Art.e63.
- Mergeay J., Aguilera X., Declercq S., Petrusek A., Huysse T., De Meester L. 2008. The genetic legacy of polyploid Bolivian *Daphnia*: the tropical Andes as a source for the North and South American *D. pulicaria* complex // *Molecular Ecology*. Vol.17. No.7. P.1789–1800.
- Mo Y.K., Lanfear R., Hahn M.W., Minh B.Q. 2023. Updated site concordance factors minimize effects of homoplasy and taxon sampling // *Bioinformatics*. Vol.39. No.1. Art.btac741.
- Pereboev D.D., Garibian P.G., Karabanov D.P., Efeikin B.D., Galimov Y.R., Petrusek A., Kotov A.A. 2025. A non-monophyly of “crowned” *Daphnia* (*Ctenodaphnia*) Dybowski et Grochowski, 1895 (Cladocera: Daphniidae): from genomes to morphology // *Zoologica Scripta*. Vol.54. No.1. P.69–90.
- Petrusek A., Cerny M., Mergeay J., Schwenk K. 2007. *Daphnia* in the Tatra Mountain lakes: multiple colonisation and hidden species diversity revealed by molecular markers // *Fundamental and Applied Limnology*. Vol.169. No.4. P.279–291.
- Petrusek A., Tollrian R., Schwenk K., Haas A., Laforsch C. 2009. A “crown of thorns” is an inducible defense that protects *Daphnia* against an ancient predator // *Proceedings of the National Academy of Sciences USA*. Vol.106. P.2248–2252.

- Pugh P.J.A., Dartnall H.J.G., McInnes S.J. 2002. The non-marine Crustacea of Antarctica and the Islands of the Southern Ocean: biodiversity and biogeography // *Journal of Natural History*. Vol.36. No.9. P.1047–1103.
- Rambaut A., Drummond A.J., Xie D., Baele G., Suchard M.A. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7 // *Systematic Biology*. Vol.67. No.5. P.901–904.
- Rogers D.C., Padhye S. 2015. Review of the large branchiopod crustacean fauna of the Indian Subcontinent (Anostraca, Notostraca, Laevicaudata, Spinicaudata, Cyclestherida) // *Journal of Crustacean Biology*. Vol.35. No.3. P.392–406.
- Rühe F.E. 1914. Die Süßwassercrustaceen der Deutschen Südpolar-Expedition 1901–1903 mit Ausschluss der Ostrakoden // *Deutsche Südpolar-Expedition, 1901–1903. Ser.8. Zoologie. Bd.16. S.5–66*.
- Schwartz S.S., Hebert P.D.N. 1985. *Daphniopsis ephemeralis* sp. n. (Cladocera, Daphniidae): a new genus for North America // *Canadian Journal of Zoology*. Vol.63. No.11. P.2689–2693.
- Scotese C.R. 2016. PALEOMAP PaleoAtlas for GPlates and the Paleo-Data Plotter Program, PALEOMAP Project. <http://www.earthbyte.org/paleomap-paleoatlas-for-gplates/>
- Taylor D.J., Finston T.L., Hebert P.D.N. 1998. Biogeography of a widespread freshwater crustacean: Pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex // *Evolution*. Vol.52. No.6. P.1648–1670.
- Taylor D.J., Hebert P.D.N., Colbourne J.K. 1996. Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation // *Molecular Phylogenetics and Evolution*. Vol.5. No.3. P.495–510.
- Toews D.P.L., Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals // *Molecular Ecology*. Vol.21. No.16. P.3907–3930.
- Van Damme K., Kotov A.A. 2016. The fossil record of the Cladocera (Crustacea: Branchiopoda): Evidence and hypotheses // *Earth-Science Reviews*. Vol.163. P.162–189.
- Wick R.R., Schultz M.B., Zobel J., Holt K.E. 2015. Bandage: interactive visualization of de novo genome assemblies // *Bioinformatics*. Vol.31. No.20. P.3350–3352.
- Wong T.K., Ly-Trong N., Ren H., Demotte P., Baños H., Roger A.J., Susko E., Bielow C., De Maio N., Goldman N., Hahn M.W. 2026. IQ-TREE 3: phylogenomic inference software using complex evolutionary models // *Molecular Biology and Evolution*. Vol.43. No.5. Art.msag117.
- Zimin A.V., Marçais G., Puiu D., Roberts M., Salzberg S.L., Yorke J.A. 2013. The MaSuRCA genome assembler // *Bioinformatics*. Vol.29. No.21. P.2669–2677.
- Zuykova E.I., Bochkarev N.A., Taylor D.J., Kotov A.A. 2019. Unexpected endemism in the *Daphnia longispina* complex (Crustacea: Cladocera) in Southern Siberia // *PloS ONE*. Vol.14. No.9. Art. e0221527.

Responsible editor K.G. Mikhailov