

THE CAUCASUS ORIGIN OF DAPHNIA SPECIES BY MEANS OF PHYLOGENY AND FUNCTIONALITY

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Abstract. Species of the genus *Daphnia* Mueller, 1785, are ancient aquatic organisms with origins dating back to approximately 145 million years. Over time, geographical, ecological, and behavioral barriers have led to the diversification of these species. The mechanism of *Daphnia* survival and dispersal is predetermined by the capability of their ephippium-protected embryos to remain dormant for extended periods of time, thus facilitating their widespread dispersal by wind, water currents, and animal vectors. This paper presents the analysis of the *Daphnia* species diversity in the Caucasus region, specifically Georgia, conducted using mitochondrial 16S rDNA and cytochrome oxidase subunit I (COI) gene fragments. The phylogenetic analysis reveals distinct evolutionary lineages among *Daphnia* species, molecular clock estimates suggesting the early Miocene divergence pattern, followed by the glaciation events of the Pleistocene and the uplift of the Caucasus mountains. This research highlights the challenges that species-level identification represents, emphasizing the necessity for using multiple gene fragments for accurate identification. The findings provide unprecedented insights into the evolutionary history, dispersal mechanisms, and genetic identification of *Daphnia* species in the Caucasus region. These results contribute to the comprehensive understanding of the ecological role and adaptive strategies of *Daphnia*, with implications for biodiversity conservation and environmental monitoring in aquatic ecosystems.

INTRODUCTION

Species of the genus *Daphnia* Mueller, 1785 (Crustacea: Branchiopoda: Cladocera) have an ancient origin that dates back to approximately 145 million years (Chin and Cristescu 2021). It is believed that the two major genera, *Daphnia* and *Ctenodaphnia*, appeared during the breakup of the Pangea supercontinent (Kotov and Taylor 2011). Over time, these species have diversified in various ways, with the emergence of new lineages as a result of long-standing geographic, ecological, and behavioral barriers. It is important to note that speciation was triggered not by a single barrier, but rather by a combination of all the barriers that are successful (Chin and Cristescu 2021).

Daphniidae are known for their ability to colonize new habitats and maintain genetic continuity across large geographic ranges (Havel and Shurin 2004). Their embryos possess an ephippium that provides protection against harsh environmental conditions and temperature extremes (Geerts et al. 2015). These encapsulated embryos can remain dormant in undisturbed habitats for decades, and even centuries, providing an advantage for species survival (Fryer 1996; Orsini et al. 2013). Moreover, the ephippia enable dispersal across different locations and times via wind, birds, and water currents,

as well as through deposition in sediments and with a high potential for hatching (Geerts et al. 2015). This high probability of survival and dispersal, coupled with fast parthenogenetic reproduction, can lead to diversification of Daphniidae (Betini et al. 2020).

However, due to the frequent formation of hybrids and cryptic assemblages, species-level analysis of Daphniidae presents numerous challenges. Some *Daphnia* species complexes contain sibling species that make species identification by morphological features challenging or even impossible. The gene region that has been most frequently used for the species-level identification of *Daphnia* over the last decade (Hebert et al. 2003; Aarbakke et al. 2011; Bucklin et al. 2010) and is still most frequently used is a 650 bp fragment of the mitochondrial cytochrome oxidase subunit I gene, also known as the barcode region. However, other gene fragments such as 12S and 16S ribosomal DNA have also shown promising results in identifying Daphniidae. Currently, the suitability of different gene fragments for identifying water fleas is still a subject of discussion, with most studies using these three fragments to compare their effectiveness (Hamza et al. 2022).

The characterization of *Daphnia* species diversity is essential for a comprehensive understanding, assess-

ment, and prediction of the function and future of freshwater ecosystems worldwide (Lee et al. 2015). Planktonic organisms, particularly water fleas (*Daphnia*), have been extensively studied, and their ecological services, including nutrient cycling and bioindication, are well-known. Daphniidae, particularly *Daphnia* (*Ctenodaphnia*) *magna*, Straus, 1820 and *D.* (*Daphnia*) *pulex* Leydig, 1860, are believed to be highly sensitive indicators for screening the toxicity of common environmental chemicals and monitoring effluents and contaminated waters. Waterfowl and other wild birds are hosts to Influenza viruses, and their long migration routes facilitate dispersal of *Daphnia*'s ephippia in vast areas. As filter feeders, filtering 1 litre of water per day (Siciliano et al. 2015; Abbas et al. 2012), water fleas can accumulate Avian Influenza Virus (AIV) from the surrounding water systems.

It is well understood that community composition has a significant impact on ecosystem functioning, and the phenotypic variation within a single species has the potential to scale up and have an impact on the ecosystem (Fussmann et al. 2007; Palkovacs and Post 2009; Markova et al. 2013). The Caucasus region and the water systems wherefrom we sampled Daphniidae represent favorite and highly probable avian migration routes in Georgia existing since the Last Glacial Maximum (Waldenström et al. 2022). This study aimed to barcode the major groups of *Daphnia* species in different lakes in the Caucasus region for the first time and

to assess their potential dispersal pathways based on the time-calibrated phylogeny using the available data from other parts of the world.

MATERIALS AND METHODS

Samples, DNA Extraction, PCR & Sequencing

Figure 1 illustrates the geographic locations where from *Daphnia* samples were collected for genetic analysis. It should be noted that samples from Koruldi and Dali locations were damaged and excluded from the analysis. Two types of plankton nets were used to capture different *Daphnia* species through horizontal or vertical tows, as appropriate (a. mesh size 64 μm , mouth diameter 30 cm; b. mesh size 80 μm , mouth diameter 20 cm). Upon collection, samples were sorted out morphologically using an OMAX 3.5X-90X Digital Trinocular Stereo Microscope and stored immediately in 95% ethanol for subsequent DNA analysis. DNA was extracted from the whole organism using the Zymo Research Quick-DNA Tissue/Insect Micro prep kit in accordance with the manufacturer's instructions. The mitochondrial cytochrome *c* oxidase subunit I gene fragment (mtCOI) was amplified using the universal primer set LCO (5'-GGTCAACAAATCATAAA-GATATTGG-3') and ZplankF1-t1 (5'-TGTAACAC-GACGGCCAGTTCTASWAATCATAARGATATT-

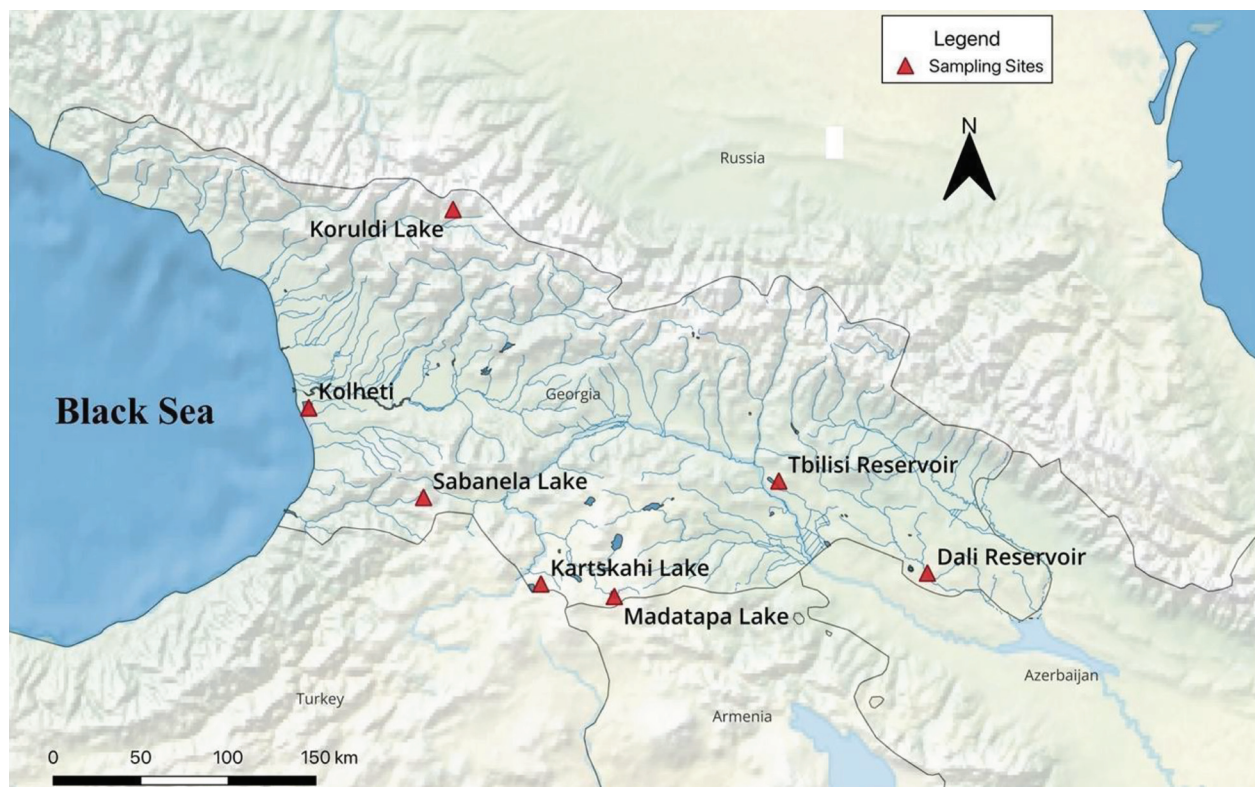


Figure 1. Sampling locations of *Daphnia* species in Georgia.

GG-3') (Prosser et al. 2013), while the small ribosomal subunit 16S rDNA gene fragment was amplified using the primer set (5'-TTTGTAATGGCCGCAGTA-3') (Zuykova et al. 2010). Amplification conditions for the mitochondrial fragments were identical. Twenty microliter PCR reactions contained 3 µl of template DNA, 1U Promega Taq polymerase, 1X Promega buffer, 2.0 mmol/L MgCl₂, 0.1 mmol/L of each dNTPs, and primer concentrations of 0.1 µmol/L. The thermal cycling protocol for polymerase chain reaction (PCR) was performed as described below: an initial denaturation step at 94° C for 2 minutes, followed by 35 cycles of denaturation at 94° C for 30 seconds, annealing at 50° C for 40 seconds, and extension at 72° C for 1.45 minutes. A final extension step was performed at 72° C for 2 minutes. Subsequently, 5 µl of PCR products were visualized on a 1.5% agarose gel to confirm amplification success. The PCR products were sent to MacroGen Europe B.V (Meibergdreef 31, Amsterdam 1105, AZ, Netherlands) for Sanger sequencing. The forward primer Plank_M13F – 5'-TGTAACGACGGCCAGT-3' was used for unidirectional sequencing of the COI amplicons. The unique sequences of the COI and 16S rRNA genes were deposited in the GenBank database under the following accession numbers: OQ428179, OQ435623, OQ435343, OQ435571, OQ434978, OQ434979, OQ435281, OQ459708 for COI and OQ407852-OQ407862 for 16S.

Sequence analysis

We aligned and corrected the raw sequences of the two mitochondrial genes, a 400–550 bp segment of the 16S rDNA and a 650-bp segment of COI, using BioEdit V.7.2 (Hall 1999). Then we constructed a Maximum Likelihood (ML) tree and estimated bootstrap support using MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura et al. 2021). The best-fit model was selected using the Bayesian Information Criterion (BIC) in MEGA 11. For the COI and 16S gene, we constructed an ML tree with the following parameters: Model: T92+G, Partial deletion with 1000 replicates. To visualize haplotypes of both COI and 16S gene, we used PopART software (Leigh and Briant 2015). A minimum spanning network was constructed and traits

were imported according to the standard guideline of the software. The mutations are shown by Hetch marks. To estimate divergence time and credible intervals, we used BEAST V.2.7.3. We applied a log-normal prior on the root describing the most recent common ancestor (MRCA). We calibrated the tree based on the following rationale: The first split of *D. pulex* and *D. magna* from the Jurassic-Cretaceous boundary indicates that Daphniidae originated over 145 million years ago (Kumar et al. 2022; www.timetree.org); Kotov and Taylor 2011; Cornetti et al. 2019). The split between closely related species of *Daphnia* (*D. galeata* (Ears, 1864) – *D. longispina* (O.F. Müller, 1776)) occurred 5–7 million years ago (Chin and Cristescu 2021). We used the same clock model (strict clock) for both COI and 16S, and the same substitution model (TN93), with a logistic tree prior and a million generations in BEAUti V.2.7.5. Tracer V.1.7.2 was used to examine the log files generated with BEAST. The maximum clade credibility time tree was generated using TreeAnnotator V.2.7.3 by excluding the first 20% of trees as burn-in. FigTree V.1.4.4 was used to visualize and edit the tree.

RESULTS

The sites were selected based on characteristics of the region, which encompasses a diverse array of ecosystems ranging from sea level to alpine lakes. For the first time, we have barcoded (i.e., short DNA sequences for species recognition and discovery) the major *Daphnia* species in Georgia. A total of five *Daphnia* species were identified (Table 1). The total number of downloaded sequences for both genes was 204 based on BLAST results. The complete list is presented in the supplementary Table S1. We filtered the sequences based on quality and length.

Phylogeny based on 16S and COI sequences

The ML analysis suggested the presence of well-supported major mitochondrial clades within the analyzed sequences for both genes (Figure 2 and Figure 3). The topology of the 16S tree suggests that: (a) *D. magna*, *D. pulex* and *D. obtusa* form a monophyletic clade.

Table 1. The DNA sampling locations details and *Daphnia* species identified in Georgia.

Species	Sampling location	Type	Altitude (a. s. l)	DNA sequence no	GPS Coordinates (WGS84)
<i>D. obtusa</i>	Kolkheti	Pond	50 m	2	41.894207 41.775568
<i>D. longispina</i>	Goderdzi	Alpine lake	2458 m	2	41.667072 42.508072
<i>D. galeata</i>	Madatapa	Alpine lake	2208 m	2	41.171832 43.793319
<i>D. magna</i>	Kartsakhi	Lake	1800 m	3	41.227864 43.245719
<i>D. pulex</i>	Tbilisi	Reservoir	750 m	3	41.729550 44.879976

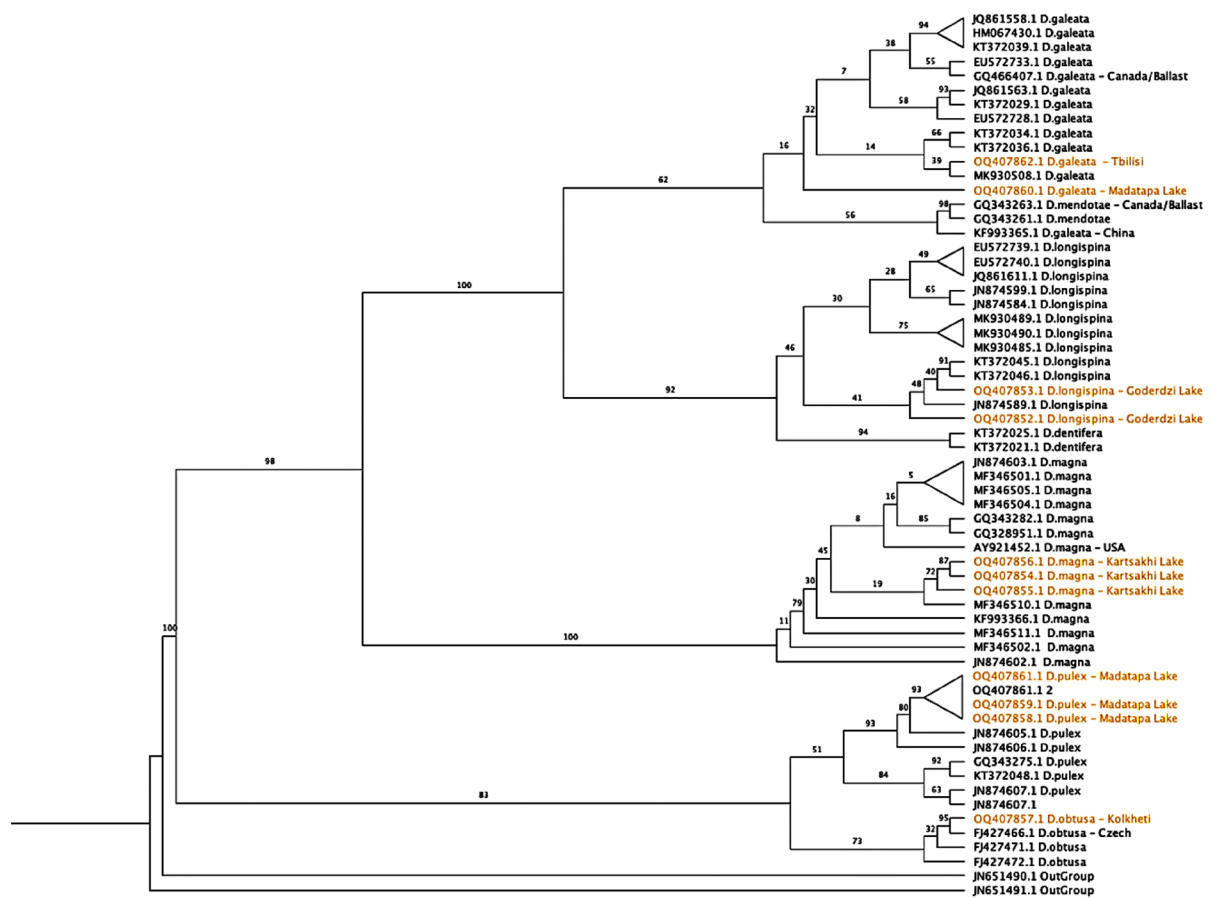


Figure 2. Maximum-likelihood phylogeny of the *Daphnia* species from North America, Asia, Russia (Siberia) and original sequences from Georgia based on 16S rDNA. Numbers above branches are bootstrap support values. Clades with bootstrap support below 30 are not annotated. The sequences highlighted in orange were obtained in this study.

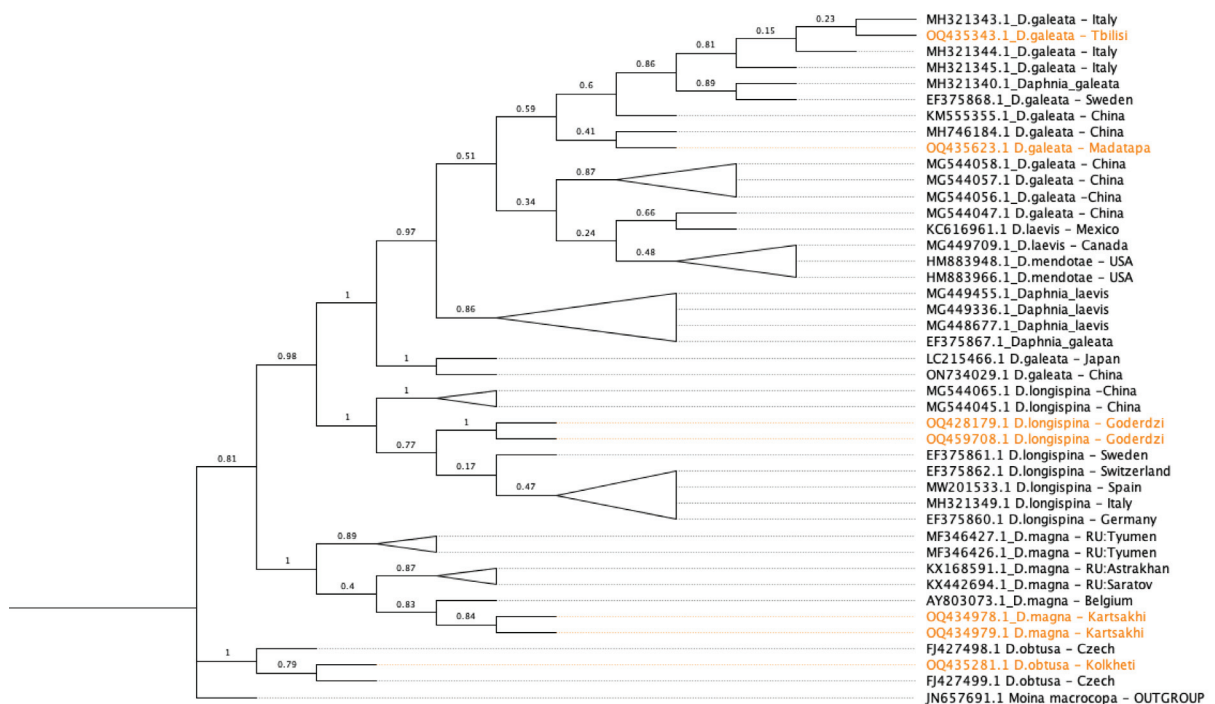


Figure 3. Maximum-likelihood phylogeny of the *Daphnia* species from North America, Asia, Russia (Siberia), Europe and original sequences from Georgia based on cytochrome oxidase subunit I gene (COI). Numbers above branches are bootstrap support values. Clades with bootstrap support below 30 are not annotated. The sequences highlighted in orange were obtained in this study.

(b) *D. longispina* and *D. galeata* are subdivided into two monophyletic clades and are nested with Russian specimens, mostly of the Siberian region. These clades are geographically distinct, and barriers between them are significant. The topology of the COI tree suggests that: (a) *D. magna* forms a monophyletic clade and is closer to European haplotypes and samples from the eastern part of Russia. (b) *D. galeata* – Tbilisi is nested with the European clade and *D. galeata* – Madatapa forms an monophyletic sub-clade with the Asian clade. The latter was found in the catchment of Madatapa lake, which is located in the southern part of Georgia, and the former one was found in samples from the Tbilisi reservoir, which is located in the eastern part of Georgia. (c) *D. longispina* forms a monophyletic clade and is very distinct from Asian and European Clades at the same time. These samples are found in the Alpine Pond from the southern part of Georgia. (d) *D. obtusa* is nested with the European haplotype.

Minimum spanning network

Eleven and ten haplotypes were identified among the 16S sequences of *D. galeata* and *D. longispina*, respectively. Eight, nine and thirteen haplotypes were revealed among the COI sequences of *D. longispina*, *D. magna*

and *D. galeata*, respectively. Three haplogroups were identified in *D. magna* samples from the Russian, European and Armenian regions and two haplogroups of *D. galeata* from the North American, European and Georgian regions. Nucleotide diversity of the COI gene was: *D. longispina* = 0.0208, *D. galeata* = 0.058, *D. magna* = 0.0061. Nucleotide diversity of the 16S gene was determined as follows: for *D. longispina* = 0.0100 and for *D. galeata* = 0.0074 (Figure 4 and Figure 5).

Evolutionary time tree

If the time of split between *D. magna* and *D. pulex* (150 Mya) is set based on fossil records, the divergence between species of this clade is dated as shown in Figure 6. The split between European and Asian clades of *D. longispina* occurred 17.8 Mya; Original samples from Goderdzi form a clade with Asian species and a split occurred 13.1 mya; a split between European and Asian clades of *D. galeata* occurred 14.1 mya. *D. galeata* from the Tbilisi reservoir split from the European *D. galeata* (MH321344.1) in 0.8 Mya. However, about 8.8 million years ago a split occurred between *D. galeata* from Madatapa lake and Asian haplotypes of this species. The earliest split between Georgian haplotypes from those of other parts of the world occurred 14.1 Mya.

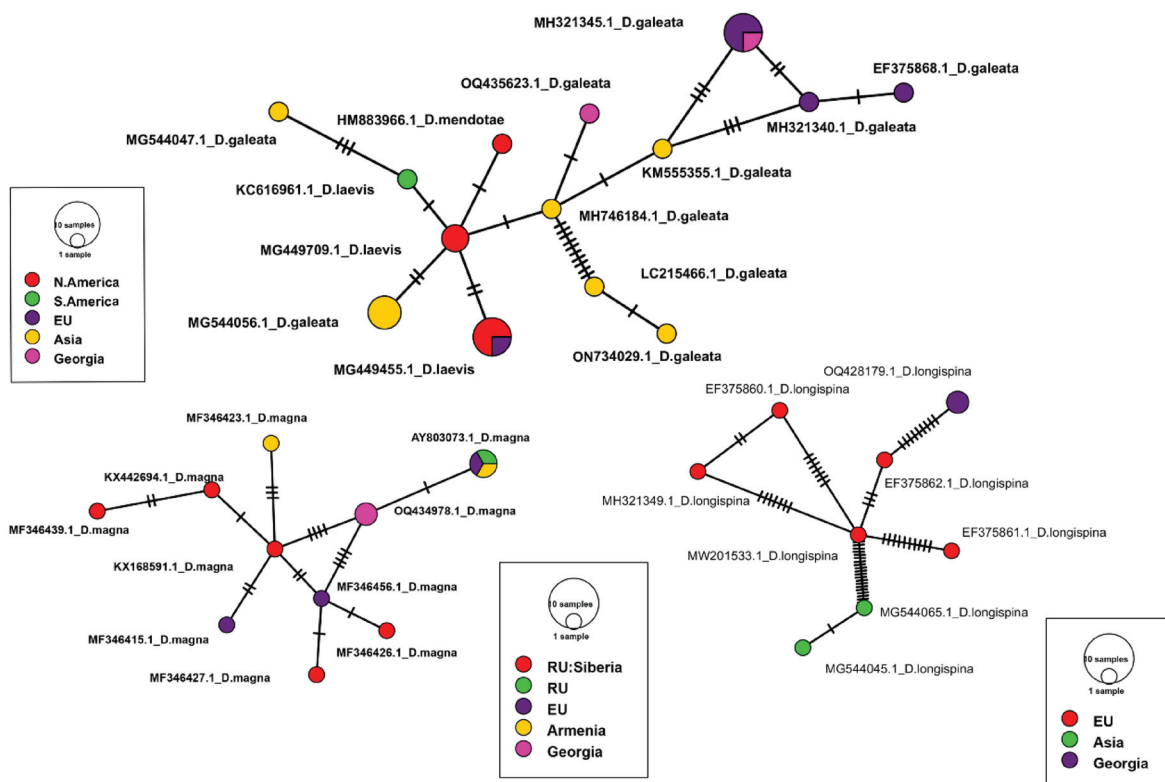


Figure 4. Median-joining network showing COI haplotypes of *D. galeata* (Up), *D. longispina* (Right) and *D. magna* (Left). Circle size is proportional to the number of individuals with a specific haplotype. Hatch marks indicate the number of substitutions. The colors show the regions of different parts of the world. (Left) – N. America, S. America, Europe, Asia and Georgia (Right) Europe, Asia and Georgia; (Down) RU: Siberia, RU: Eastern part, Europe, Armenia and Georgia.

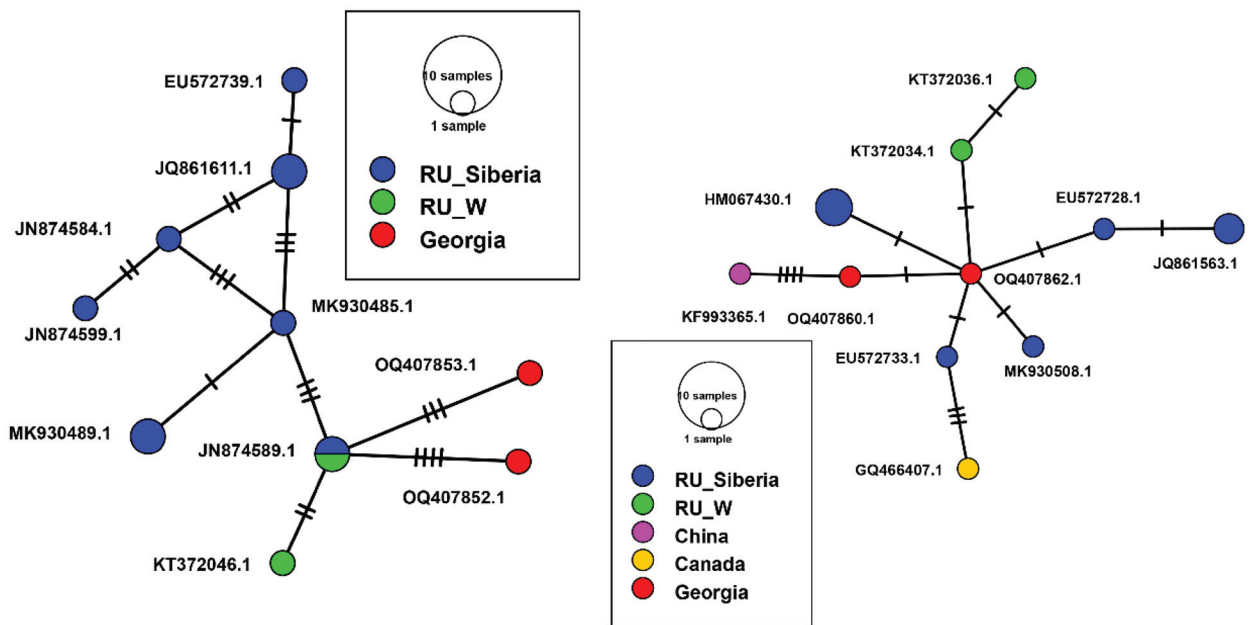


Figure 5. Median-joining network showing the 16S rDNA haplotypes of *D. longispina* (Left) and *D. galeata* (right). Circle size is proportional to the number of individuals with a specific haplotype. Hatch marks indicate the number of substitutions. The colors show the regions of different parts of the world. (Left) – RU: Siberia, RU: Western part, Georgia. (Right) RU: Siberia, RU: Western part, China, Canada and Georgia.

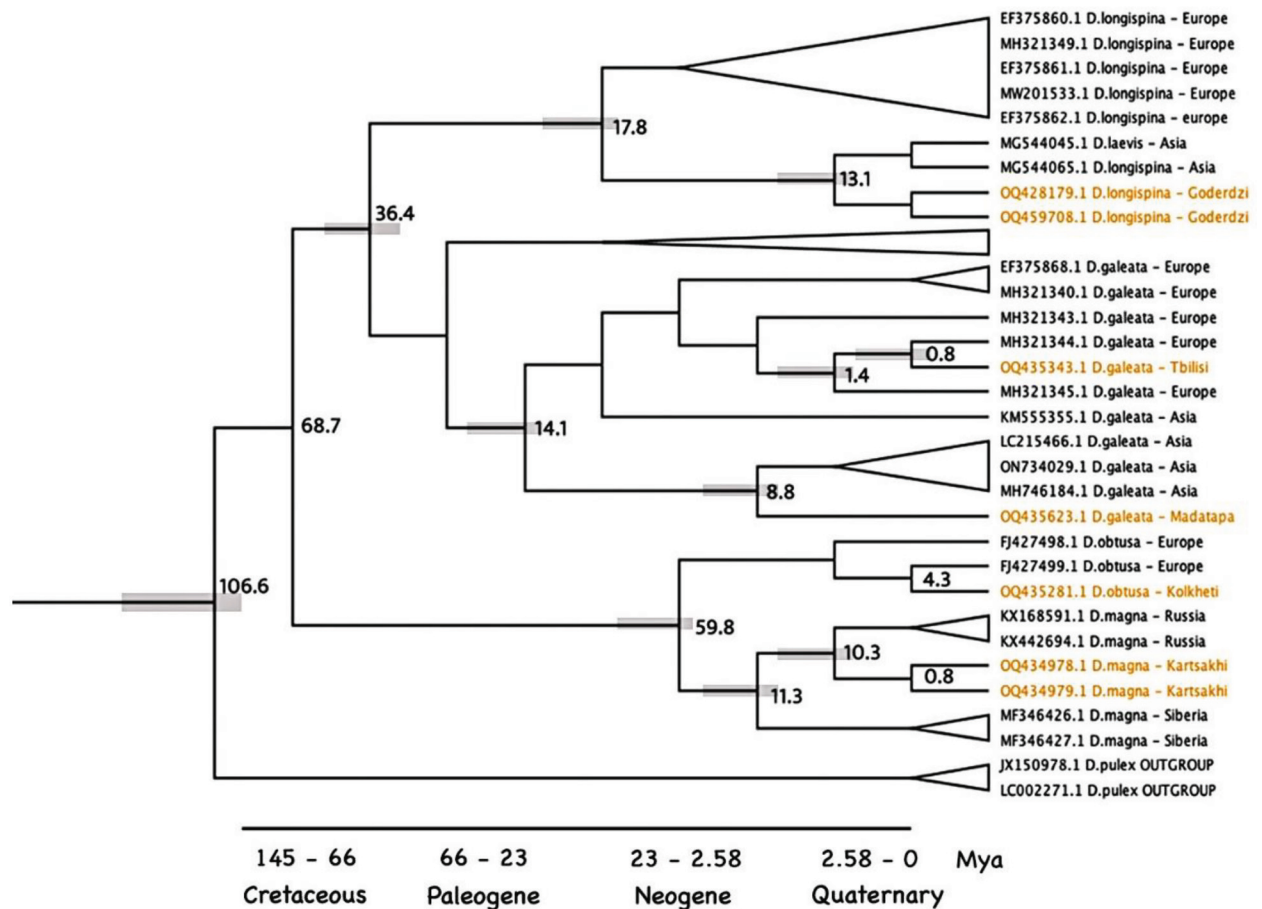


Figure 6. Timescale of divergence of focal clades based on the COI marker. The numbers at the nodes are estimated divergence times (millions of years, Mya). Gray horizontal bars are 95% HPD intervals of clade age.

DISCUSSION

The obtained sequences of *Daphnia* from Georgia form a monophyletic evolutionary sub-lineage that is distinct yet closely related to Siberian haplotypes. The earliest divergence of Georgian *Daphnia* species took place around 14.1 million years ago (Mya), marking the onset of the Late Miocene epoch. This coincides with the retreat of the Eastern Paratethys Sea, the emergence of the Caucasus mountains, and the persistence of the Black and Caspian Seas as predominantly freshwater systems during that period (Neubauer et al. 2015; Esin et al. 2018). During the Pliocene epoch, there was minimal interaction observed between the Caspian and the Black Seas. The hypotheses proposed by various authors suggest that the differentiation of *Daphnia* species began at the beginning of the Pleistocene epoch, as indicated by references detailing distinct evolutionary scenarios. The previous findings provide support for these differentiation events and establish a connection to the effects of the Pleistocene glaciations. Glacial events in Southern and Eastern Siberia commenced in the Early Pleistocene and reached their zenith in the Late Pleistocene (Zuykova et al. 2021). As the phylogeography of *Daphnia* species holds great interest, it is plausible to mention that it was in the Pleistocene that the diversification of *Daphnia* species first occurred. Glacial events during the Pleistocene are widely recognized as significant drivers of speciation processes that have shaped the present distribution patterns of *Daphnia*. This theoretical framework also holds true for their populations in North America (Gelas and Meester 2005). In the case of *D. obtusa* in North America, the speciation event took place less than 1 Mya. The analysis of original samples revealed that the time of divergence between *D. galeata* and *D. magna* was less than 0.8 Mya. This relatively short divergence time can be attributed to the influence of glacial events in Georgia, as this region hosted several refugia, primarily located in the southern and eastern parts of the country (Gavashelishvili and Tarkhnishvili 2016). However, the limited availability of original sequences from these locations provides only preliminary insights into the speciation of *Daphnia* species within Georgia. Despite this limitation, these findings offer a starting point for identifying promising haplotypes that warrant further investigation.

It is important to note that although our data cannot conclusively establish glaciation events as the predominant drivers of speciation, they do indicate that *Daphnia* species from the studied locations may represent some of the earliest haplotypes, originating just before the divergence of lineages after the closure of the Black and Caspian Seas. The inferred phylogenetic trees provide additional clarity regarding the sequence of events, revealing an initial split between European and

Siberian species, which was followed by the emergence of Siberian-Georgian species.

Besides geological events, there may have been other contributing factors in *Daphnia* speciation. Tarkhnishvili (2013) proposed that morphological and genetic speciation could be associated with habitat suitability rather than genetic divergence time. In the context of *Daphnia*, habitat suitability could be a key determinant of its diversification success, even when considering *Daphnia*'s remarkable adaptability to environmental conditions.

Regrettably, the available original samples and analyses do not provide substantial support for the possibility of dispersal between Siberian and Georgian regions. However, waterfowl migrations could serve as a significant dispersal mechanism between these two notably distinct geographical regions, particularly in instances where isolation is sufficiently pronounced. This discovery suggests that bird migration routes may have contributed to the dispersal of *Daphnia* species within Georgia. Furthermore, the Caucasus region, encompassing Georgia, boasts a diverse and distinctive array of freshwater ecosystems that offer favorable habitats for various *Daphnia* species. During the Last Glacial Maximum, the western part of Georgia, known as Colchis, acted as a notable refugium for numerous species, leading to a bottleneck effect (Dagtekin et al. 2020). It is postulated that after the glacial period species dispersed from the Caucasus or from other global refugial regions. The insights yielded from bird migration surveillance indicate that Madatapa Lake is influenced by the western and Central Asian flyways (Waldenström et al. 2022), suggesting a pivotal role for these flyways in potential dispersal of *Daphnia* species. Thus, it is reasonable to hypothesize that wind and waterfowl represent the primary means of *Daphnia* species dispersal, given the challenging accessibility of these alpine lakes for navigation or waterborne movement.

Regarding the efficacy of using the 16S and COI genes for species identification, the 16S gene exhibits considerable variations. However, the limited information available in GenBank restricts its applicability. Consequently, we advocate the concurrent use of both COI and supplementary gene fragments in order to enhance the accuracy of species identification. The COI gene is widely recognized as a universal marker for animal taxa in DNA barcoding and holds particular value when assessing zooplankton diversity in bulk samples (Bucklin et al. 2010). Nevertheless, when dealing with closely related species, it is imperative to include additional gene fragments alongside COI to ensure precise identification. Thus, we recommend the incorporation of multiple gene fragments to enhance the reliability of species identification.

CONCLUSION

The current study into the diversity of *Daphnia* species within the Caucasus region, elucidates their ancient origins, evolutionary processes, and intricate dispersal mechanisms. It also sheds light on the Early Miocene divergence patterns, formed by the glaciation events of the Pleistocene and the unique geographic landscape of the region. The insights gained from the genetic analyses, using mitochondrial 16S rDNA and COI gene fragments, reveal distinct evolutionary lineages and challenges associated with species-level identification. Moreover, this study underscores the broader significance of *Daphnia* species in the context of freshwater ecosystem dynamics, bioindication, and environmental monitoring. With its contributions to understanding evolutionary history, dispersal mechanisms, and genetic identification, this study marks a first step in advancing knowledge about *Daphnia* species diversity within Georgia.

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Supplementary Table 1. List of sequences obtained from the GenBank database.

##	Species	Country	Acc. No	Gene
1	<i>Daphnia magna</i>	EU: Belgium	AY803073.1	COI
2	<i>Daphnia mendotae</i>	USA	AY921412.1	COI
3	<i>Daphnia magna</i>	Israel	DQ166849.1	COI
4	<i>Daphnia longispina</i>	EU: Germany	EF375860.1	COI
5	<i>Daphnia longispina</i>	EU: Sweden	EF375861.1	COI
6	<i>Daphnia longispina</i>	EU: Switzerland	EF375862.1	COI
7	<i>Daphnia galeata</i>	EU: Netherlands	EF375867.1	COI
8	<i>Daphnia galeata</i>	EU: Sweden	EF375868.1	COI
9	<i>Daphnia magna</i>	Canada	EU702133.1	COI
10	<i>Daphnia magna</i>	Mexico	EU702138.1	COI
11	<i>Daphnia obtusa</i>	EU: Czech	FJ427498.1	COI
12	<i>Daphnia obtusa</i>	EU: Czech	FJ427499.1	COI
13	<i>Daphnia mendotae</i>	Canada: Ballast	GQ475272.1	COI
14	<i>Daphnia mendotae</i>	USA	HM883947.1	COI
15	<i>Daphnia mendotae</i>	USA	HM883948.1	COI
16	<i>Daphnia mendotae</i>	USA	HM883964.1	COI
17	<i>Daphnia mendotae</i>	USA	HM883966.1	COI
18	<i>Daphnia galeata</i>	Turkey	JF821192.1	COI
19	<i>Daphnia magna</i>	Turkey	JF821194.1	COI
20	<i>Moina macrocopa</i>	Outgroup	JN657690.1	COI
21	<i>Moina macrocopa</i>	Outgroup	JN657691.1	COI
22	<i>Daphnia laevis</i>	Mexico	KC616937.1	COI
23	<i>Daphnia laevis</i>	Mexico	KC616956.1	COI
24	<i>Daphnia laevis</i>	Mexico	KC616957.1	COI
25	<i>Daphnia laevis</i>	Mexico	KC616958.1	COI
26	<i>Daphnia laevis</i>	Mexico	KC616959.1	COI
27	<i>Daphnia laevis</i>	Mexico	KC616961.1	COI
28	<i>Daphnia laevis</i>	Mexico	KC616964.1	COI
29	<i>Daphnia laevis</i>	Mexico	KC616966.1	COI
30	<i>Daphnia laevis</i>	Mexico	KC616968.1	COI
31	<i>Daphnia galeata</i>	China	KM555355.1	COI
32	<i>Daphnia galeata</i>	China	KM555359.1	COI
33	<i>Daphnia galeata</i>	China	KM590523.1	COI
34	<i>Daphnia magna</i>	RU: Astrakhan	KX168590.1	COI
35	<i>Daphnia magna</i>	RU: Astrakhan	KX168591.1	COI
36	<i>Daphnia magna</i>	RU: Saratov	KX442694.1	COI
37	<i>Daphnia magna</i>	RU: Astrakhan	KX442699.1	COI
38	<i>Daphnia galeata</i>	Australia	KY700828.1	COI
39	<i>Daphnia galeata</i>	Japan	LC215466.1	COI
40	<i>Daphnia magna</i>	EU: Germany	MF346415.1	COI
41	<i>Daphnia magna</i>	RU: Vladimir area	MF346416.1	COI
42	<i>Daphnia magna</i>	RU: Vladimir area	MF346417.1	COI
43	<i>Daphnia magna</i>	RU: Vladimir area	MF346418.1	COI
44	<i>Daphnia magna</i>	RU: Ryazan Area	MF346420.1	COI
45	<i>Daphnia magna</i>	RU: Ryazan Area	MF346421.1	COI
46	<i>Daphnia magna</i>	RU: Volgograd Area	MF346422.1	COI
47	<i>Daphnia magna</i>	Armenia: Sevan Lake	MF346423.1	COI
48	<i>Daphnia magna</i>	RU: Tyumen	MF346426.1	COI
49	<i>Daphnia magna</i>	RU: Tyumen	MF346427.1	COI
50	<i>Daphnia magna</i>	RU: Tyumen	MF346428.1	COI
51	<i>Daphnia magna</i>	RU: Kalmyk	MF346437.1	COI
52	<i>Daphnia magna</i>	RU: Kalmyk	MF346438.1	COI
53	<i>Daphnia magna</i>	RU: Novosibirsk	MF346439.1	COI

##	Species	Country	Acc. No	Gene
54	<i>Daphnia magna</i>	RU: Volgograd Area	MF346440.1	COI
55	<i>Daphnia magna</i>	RU: Volgograd Area	MF346441.1	COI
56	<i>Daphnia magna</i>	RU: Volgograd Area	MF346446.1	COI
57	<i>Daphnia magna</i>	RU: Saratov	MF346448.1	COI
58	<i>Daphnia magna</i>	RU: Saratov	MF346449.1	COI
59	<i>Daphnia magna</i>	RU: Volgograd Area	MF346451.1	COI
60	<i>Daphnia magna</i>	EU: Czech	MF346456.1	COI
61	<i>Daphnia magna</i>	RU: Rostov	MF346457.1	COI
62	<i>Daphnia magna</i>	RU: Razan Area	MF346459.1	COI
63	<i>Daphnia magna</i>	RU: Samara Area	MF346474.1	COI
64	<i>Daphnia magna</i>	RU: Krasnodar	MF346477.1	COI
65	<i>Daphnia magna</i>	Armenia: Sevan Lake	MF346478.1	COI
66	<i>Daphnia laevis</i>	Canada	MG448677.1	COI
67	<i>Daphnia laevis</i>	Canada	MG449108.1	COI
68	<i>Daphnia laevis</i>	Canada	MG449336.1	COI
69	<i>Daphnia laevis</i>	Canada	MG449455.1	COI
70	<i>Daphnia laevis</i>	Canada	MG449540.1	COI
71	<i>Daphnia laevis</i>	Canada	MG449709.1	COI
72	<i>Daphnia laevis</i>	Canada	MG450239.1	COI
73	<i>Daphnia laevis</i>	Canada	MG544043.1	COI
74	<i>Daphnia laevis</i>	Canada	MG544044.1	COI
75	<i>Daphnia longispina</i>	China	MG544045.1	COI
76	<i>Daphnia galeata</i>	China	MG544047.1	COI
77	<i>Daphnia galeata</i>	China	MG544048.1	COI
78	<i>Daphnia galeata</i>	China	MG544049.1	COI
79	<i>Daphnia galeata</i>	China	MG544056.1	COI
80	<i>Daphnia galeata</i>	China	MG544057.1	COI
81	<i>Daphnia galeata</i>	China	MG544058.1	COI
82	<i>Daphnia longispina</i>	China	MG544065.1	COI
83	<i>Daphnia galeata</i>	China	MG544066.1	COI
84	<i>Daphnia galeata</i>	China	MG544067.1	COI
85	<i>Daphnia galeata</i>	China	MG544068.1	COI
86	<i>Daphnia galeata</i>	China	MG544069.1	COI
87	<i>Daphnia galeata</i>	China	MG544070.1	COI
88	<i>Daphnia longispina</i>	China	MG544080.1	COI
89	<i>Daphnia longispina</i>	China	MG544081.1	COI
90	<i>Daphnia longispina</i>	China	MG544082.1	COI
91	<i>Daphnia galeata</i>	EU: Italy	MH321339.1	COI
92	<i>Daphnia galeata</i>	EU: Italy	MH321340.1	COI
93	<i>Daphnia galeata</i>	EU: Italy	MH321342.1	COI
94	<i>Daphnia galeata</i>	EU: Italy	MH321343.1	COI
95	<i>Daphnia galeata</i>	EU: Italy	MH321344.1	COI
96	<i>Daphnia galeata</i>	EU: Italy	MH321345.1	COI
97	<i>Daphnia galeata</i>	EU: Italy	MH321347.1	COI
98	<i>Daphnia galeata</i>	EU: Italy	MH321348.1	COI
99	<i>Daphnia longispina</i>	EU: Italy	MH321349.1	COI
100	<i>Daphnia galeata</i>	China	MH746123.1	COI
101	<i>Daphnia galeata</i>	China	MH746124.1	COI
102	<i>Daphnia galeata</i>	China	MH746125.1	COI
103	<i>Daphnia galeata</i>	China	MH746126.1	COI
104	<i>Daphnia galeata</i>	China	MH746132.1	COI
105	<i>Daphnia galeata</i>	China	MH746133.1	COI
106	<i>Daphnia galeata</i>	China	MH746134.1	COI
107	<i>Daphnia galeata</i>	China	MH746135.1	COI

##	Species	Country	Acc. No	Gene
108	<i>Daphnia galeata</i>	China	MH746136.1	COI
109	<i>Daphnia galeata</i>	China	MH746137.1	COI
110	<i>Daphnia galeata</i>	China	MH746138.1	COI
111	<i>Daphnia galeata</i>	China	MH746139.1	COI
112	<i>Daphnia galeata</i>	China	MH746140.1	COI
113	<i>Daphnia galeata</i>	China	MH746141.1	COI
114	<i>Daphnia galeata</i>	China	MH746144.1	COI
115	<i>Daphnia galeata</i>	China	MH746146.1	COI
116	<i>Daphnia galeata</i>	China	MH746148.1	COI
117	<i>Daphnia galeata</i>	China	MH746149.1	COI
118	<i>Daphnia galeata</i>	China	MH746150.1	COI
119	<i>Daphnia galeata</i>	China	MH746151.1	COI
120	<i>Daphnia galeata</i>	China	MH746156.1	COI
121	<i>Daphnia galeata</i>	China	MH746157.1	COI
122	<i>Daphnia galeata</i>	China	MH746159.1	COI
123	<i>Daphnia galeata</i>	China	MH746160.1	COI
124	<i>Daphnia galeata</i>	China	MH746161.1	COI
125	<i>Daphnia galeata</i>	China	MH746162.1	COI
126	<i>Daphnia galeata</i>	China	MH746163.1	COI
127	<i>Daphnia galeata</i>	China	MH746164.1	COI
128	<i>Daphnia galeata</i>	China	MH746167.1	COI
129	<i>Daphnia galeata</i>	China	MH746169.1	COI
130	<i>Daphnia galeata</i>	China	MH746170.1	COI
131	<i>Daphnia galeata</i>	China	MH746171.1	COI
132	<i>Daphnia galeata</i>	China	MH746174.1	COI
133	<i>Daphnia galeata</i>	China	MH746175.1	COI
134	<i>Daphnia galeata</i>	China	MH746178.1	COI
135	<i>Daphnia galeata</i>	China	MH746184.1	COI
136	<i>Daphnia galeata</i>	China	MH746186.1	COI
137	<i>Daphnia magna</i>	USA	MN164019.1	COI
138	<i>Daphnia longispina</i>	EU: Spain	MW201533.1	COI
139	<i>Daphnia galeata</i>	China	ON734022.1	COI
140	<i>Daphnia galeata</i>	China	ON734023.1	COI
141	<i>Daphnia galeata</i>	China	ON734027.1	COI
142	<i>Daphnia galeata</i>	China	ON734029.1	COI
143	<i>Daphnia galeata</i>	China	ON734033.1	COI
144	<i>Daphnia galeata</i>	China	ON734035.1	COI
145	<i>Daphnia galeata</i>	China	ON734036.1	COI
146	<i>Daphnia galeata</i>	China	ON734039.1	COI
147	<i>Daphnia galeata</i>	China	ON734040.1	COI
148	<i>Daphnia galeata</i>	China	ON734041.1	COI
149	<i>D. longispina</i>	Georgia: Adjara	OQ428179.1	COI
150	<i>D. longispina</i>	Georgia: Adjara	OQ459708.1	COI
151	<i>D. galeata</i>	Georgia: Tbilisi	OQ435343.1	COI
152	<i>D. galeata</i>	Georgia: Javakheti	OQ435623.1	COI
153	<i>D. magna</i>	Georgia: Javakheti	OQ434978.1	COI
154	<i>D. magna</i>	Georgia: Javakheti	OQ434979.1	COI
155	<i>D. obtusa</i>	Georgia: Kolkheti	OQ435281.1	COI
156	<i>Daphnia magna</i>	US: Nebraska	AY921452.1	16S
157	<i>Daphnia galeata</i>	RU: S. Siberia: Tuva	EU572728.1	16S
158	<i>Daphnia galeata</i>	RU: S. Siberia: Tuva	EU572733.1	16S
159	<i>Daphnia longispina</i>	RU: S. Siberia: Altai Republic	EU572739.1	16S
160	<i>Daphnia longispina</i>	RU: S. Siberia: Altai Republic	EU572740.1	16S
161	<i>Daphnia obtusa</i>	CZ: puddle near Blatna	FJ427466.1	16S

##	Species	Country	Acc. No	Gene
162	<i>Daphnia obtusa</i>	Argentina: Laguna Quichaura	FJ427471.1	16S
163	<i>Daphnia obtusa</i>	Argentina: Laguna Quichaura	FJ427472.1	16S
164	<i>Daphnia magan</i>	Unknown	GQ328951.1	16S
165	<i>D. mendotae</i>	Canada: Ballast waters	GQ343261.1	16S
166	<i>D. mendotae</i>	Canada: Ballast waters	GQ343263.1	16S
167	<i>Daphnia pulex</i>	Canada: Ballast waters	GQ343275.1	16S
168	<i>Daphnia magna</i>	Canada: Ballast waters	GQ343282.1	16S
169	<i>Daphnia galeata</i>	Canada: Ballast waters	GQ466407.1	16S
170	<i>Daphnia galeata</i>	RU: S. Siberia: Novosibirsk Reservoir	HM067430.1	16S
171	<i>Moina Brachiata</i>	Outgroup	JN651490.1	16S
172	<i>Moina Brachiata</i>	Outgroup	JN651491.1	16S
173	<i>Daphnia longispina</i>	RU: S. Siberia: Novosibirsk	JN874584.1	16S
174	<i>Daphnia longispina</i>	RU: S. Siberia: Novosibirsk	JN874589.1	16S
175	<i>Daphnia longispina</i>	RU: S. Siberia: Novosibirsk	JN874599.1	16S
176	<i>Daphnia magna</i>	RU: S. Siberia: Novosibirsk	JN874602.1	16S
177	<i>Daphnia magna</i>	RU: S. Siberia: Novosibirsk	JN874603.1	16S
178	<i>Daphnia pulex</i>	RU: S. Siberia: Novosibirsk	JN874605.1	16S
179	<i>Daphnia pulex</i>	RU: S. Siberia: Novosibirsk	JN874606.1	16S
180	<i>Daphnia pulex</i>	RU: S. Siberia: Novosibirsk	JN874607.1	16S
181	<i>Daphnia galeata</i>	RU: S. Siberia: Tuva	JQ861558.1	16S
182	<i>Daphnia galeata</i>	RU: S. Siberia: Tuva	JQ861563.1	16S
183	<i>Daphnia longispina</i>	RU: S. Siberia: Altai Republic	JQ861611.1	16S
184	<i>Daphnia galeata</i>	China : Unpublished	KF993365.1	16S
185	<i>Daphnia magna</i>	China : Unpublished	KF993366.1	16S
186	<i>Daphnia dentifera</i>	RU: Sverdlovsk Oblast	KT372021.1	16S
187	<i>Daphnia dentifera</i>	RU: Sverdlovsk Oblast	KT372025.1	16S
188	<i>Daphnia galeata</i>	RU: S. Siberia: Irkutsk	KT372029.1	16S
189	<i>Daphnia galeata</i>	RU: W. Moscow	KT372034.1	16S
190	<i>Daphnia galeata</i>	RU: W. Moscow	KT372036.1	16S
191	<i>Daphnia galeata</i>	RU: S. Siberia: Irkutsk	KT372039.1	16S
192	<i>Daphnia longispina</i>	RU: W. Moscow	KT372045.1	16S
193	<i>Daphnia longispina</i>	RU: W. Moscow	KT372046.1	16S
194	<i>Daphnia pulex</i>	Mongolia: Lake Zhaakhan	KT372048.1	16S
195	<i>Daphnia magna</i>	RU: Volgograd	MF346501.1	16S
196	<i>Daphnia magna</i>	RU: Volgograd	MF346502.1	16S
197	<i>Daphnia magna</i>	RU: Saratov	MF346504.1	16S
198	<i>Daphnia magna</i>	RU: Volgograd	MF346505.1	16S
199	<i>Daphnia magna</i>	RU: Krasnodar	MF346510.1	16S
200	<i>Daphnia magna</i>	RU: S. Siberia: Novosibirsk	MF346511.1	16S
201	<i>Daphnia longispina</i>	RU: S. Siberia: Altai Republic	MK930485.1	16S
202	<i>Daphnia longispina</i>	RU: S. Siberia: Altai Republic	MK930489.1	16S
203	<i>Daphnia longispina</i>	RU: S. Siberia: Altai Republic	MK930490.1	16S
204	<i>Daphnia galeata</i>	RU: S. Siberia: Tuva	MK930508.1	16S
205	<i>D. longispina</i>	Georgia: Adjara	OQ407852.1	16S
206	<i>D. longispina</i>	Georgia: Adjara	OQ407853.1	16S
207	<i>D. galeata</i>	Georgia: Tbilisi	OQ407862.1	16S
208	<i>D. galeata</i>	Georgia: Javakheti	OQ407860.1	16S
209	<i>D. pulex</i>	Georgia: Javakheti	OQ407858.1	16S
210	<i>D. pulex</i>	Georgia: Javakheti	OQ407859.1	16S
211	<i>D. pulex</i>	Georgia: Javakheti	OQ407861.1	16S
212	<i>D. magna</i>	Georgia: Javakheti	OQ407854.1	16S
213	<i>D. magna</i>	Georgia: Javakheti	OQ407855.1	16S
214	<i>D. magna</i>	Georgia: Javakheti	OQ407856.1	16S
215	<i>D. obtusa</i>	Georgia: Kolkheti	OQ407857.1	16S