BIOGEOGRAPHIA
The Journal of Integrative Biogeography

Biogeographia – The Journal of Integrative Biogeography 2026, 41 (1): a065

https://doi.org/10.21426/B6.63026

First record of the brine shrimp *Artemia* Leach, 1819 (Branchiopoda, Anostraca) in Armenia, with a synopsis of the anostracan fauna of the country

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Keywords: anostracans, *Artemia* parthenogenetic lineages, branchiopods, COI, South Caucasus.

SUMMARY

We report the first record of the brine shrimp genus *Artemia* (Branchiopoda, Anostraca, Artemiidae) from Armenia, based on specimens collected in two saline ponds (collectively referred to as “Lake Aghi”) in the Yerevan area. Individuals were identified through an integrative approach combining morphological examination and mitochondrial cytochrome c oxidase subunit I (COI) sequencing. All examined specimens were females and exhibited morphological traits compatible with parthenogenetic *Artemia*, although these characters alone are not sufficient for a definitive identification. Molecular analyses revealed a single COI haplotype attributable to a parthenogenetic lineage widely distributed across the Palaearctic region and closely related to the sexual species *A. amati*. No evidence of the occurrence of the invasive species *Artemia franciscana* was found. A synopsis of the anostracan fauna of Armenia based on available literature is also provided. This finding broadens current knowledge of anostracan diversity and distribution in the South Caucasus region and highlights the need for further surveys to better characterize the large branchiopod fauna of the country.

INTRODUCTION

The genus *Artemia* Leach, 1819 includes halophilic anostracans distributed in saline and hypersaline water bodies almost worldwide (Van Stappen 2002). This anostracan genus is characterized by an intricate taxonomy and includes at least nine sexual species and a wide array of parthenogenetic lineages exhibiting multiple ploidy levels (di-, tri-, tetra-, and pentaploid, see Sainz-Escudero et al. 2021; Asem et al. 2025, and references therein). Sexual *Artemia* species have usually a restricted distribution in comparison with the more widespread parthenogenetic lineages, with the only exception of *Artemia franciscana* Kellog, 1906. This last species has been anthropogenically introduced worldwide due to its wide use in aquaculture and aquarium hobby (e.g., Ruebhart et al. 2008; Emanifar et al. 2014; Sainz-Escudero et al. 2022).

The distinctive combination of life in extreme habitat, different reproductive modes, and polyploidy has long positioned *Artemia* as a model system for studying adaptation to extreme environments, mechanisms of evolution and differentiation, and biogeographic processes (e.g., Triantaphyllidis et al. 1998; Baxevanis et al. 2006; Maniatsi et al. 2011; Asem et al. 2016; Asem et al. 2020; Rode et al. 2022; Asem et al. 2023). Moreover, the widespread use of *Artemia* nauplii as live food in aquaculture and in the aquarium, hobby has driven the global trade of distinct evolutionary lineages. *Artemia* specimens have sometimes been deliberately or inadvertently released into natural environments, thereby potentially obscuring their original biogeographic distribution, and rendering the native versus introduced status of some populations uncertain.

Asia constitutes a biodiversity hotspot for *Artemia*, with several hundreds of reported localities and the occurrence of both sexual species and parthenogenetic lineages (Eimanifar et al. 2015; Asem et al. 2025, and references therein). This exceptional diversity, combined with the morphological plasticity of the genus, has produced a particularly complex taxonomic history in the region. As a result, Asian *Artemia* populations have often been subject to controversial nomenclature, synonymies, and recurrent misidentifications, further complicated by the coexistence of multiple reproductive lineages within the same habitats (e.g., Agh et al. 2007; Asem et al. 2021).

A major unresolved issue in *Artemia* systematics concerns the taxonomical and nomenclatural distinction between sexual species and parthenogenetic lineages. Parthenogenetic forms do not correspond to a biological species in the classical sense; instead, they represent a paraphyletic assemblage closely related to the Eurasian sexual taxa *A. urmiana* Günther, 1899, *A. amati* Asem, Eimanifar, Hontoria, Rogers and Gajardo 2023, *A. tibetiana* Abatzopoulos et al. 1998, and *A. sinica* Cai 1989 (Muñoz et al. 2010; Eimanifar et al. 2014; Asem et al. 2021, 2025).

In recent years, the integration of molecular, morphological, and morphometric analyses has improved our understanding of Asian *Artemia* diversity. These approaches have led both to the characterization of additional sexual species (Asem et al. 2023) and to a deeper, though still incomplete, understanding of the evolution and biogeography of *Artemia* parthenogenetic lineages (e.g., Sainz-Escudero et al. 2021; Rode et al. 2022; Asem et al. 2025).

To date, the anostracan fauna of the Transcaucasian region remains comparatively underexplored, and Armenia, located at the intersection of Anatolia, the Caucasus, and the endorheic basins of western Asia, represents a biogeographically strategic area for investigating the crustacean diversity and the faunal relationships across the Palaearctic region. Here, we document the first record of the occurrence of the brine shrimp *Artemia* in Armenia, and frame this finding within the currently available knowledge about the anostracan fauna of the country.

MATERIALS AND METHODS

Brine shrimps were collected in Armenia from two saline ponds, locally known as “Lake Aghi” (Աղի լիճ): the “Big Lake Aghi” and the “Small Lake Aghi”. They are located at about 1008 m a.s.l. in Yerevan City (WGS84 geographical coordinate 40.156773° N, 44.533203° E, and 40.15723° N, 44.532904° E, respectively; Fig. 1).

Detailed historical and origin-specific information about this limno-system is relatively scarce. Lake Aghi was formed in the late 1970s as a result of the operations of a nearby gypsum factory (A. Khanjyan, pers. comm.). The lake’s high salinity is most likely driven by groundwater inputs, combined with the absence of a significant outflow

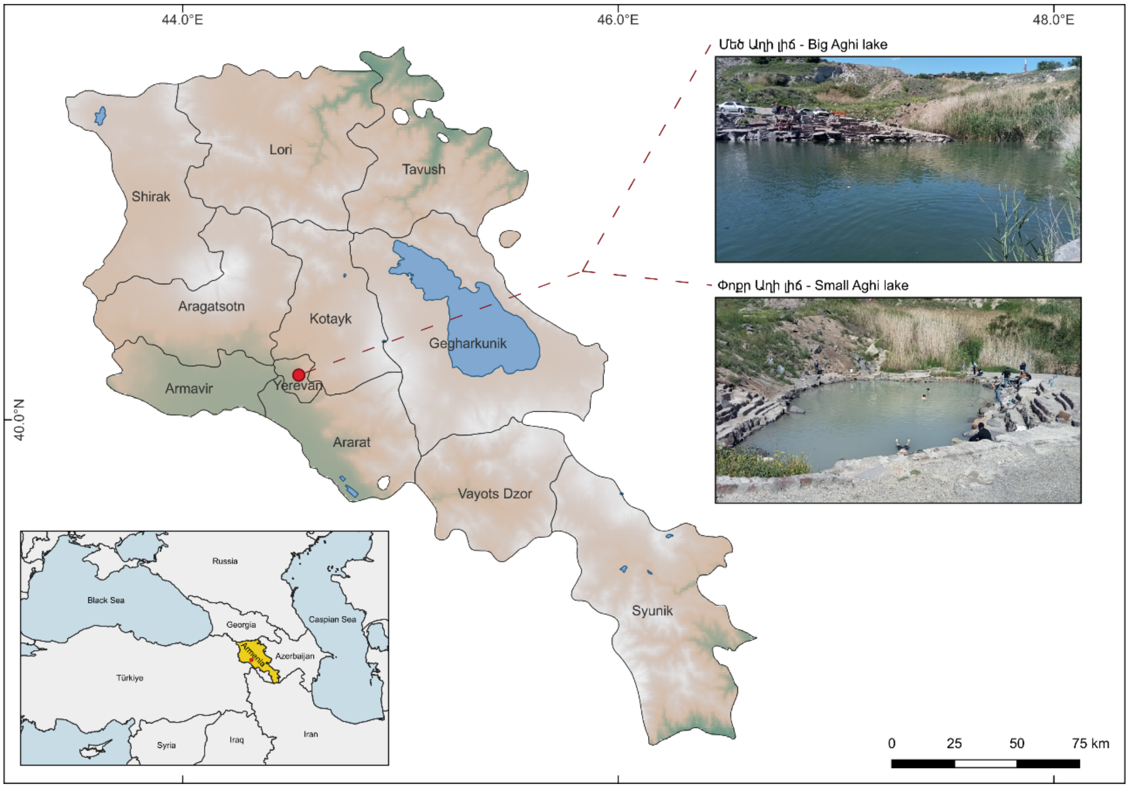

Figure 1. Location of the "Lake Aghi" (Աղի լիճ), and the aspect of the two water bodies on 16th May 2025.

from the basin and intense evaporation due to high air temperatures in the area. The lake consists of two basins: "Big Lake Aghi", with a surface area of 3000 m^2, and "Small Lake Aghi", with a surface area of only 500 m^2. The basins, with a maximum depth of no more than 4 m, are currently used as recreational pools by the local community.

Hydrobiological sampling was carried out on 16 May 2025 using a 200 µm mesh hand net from the northern and southern shores of each site, with sampling performed both in open water and near the bottom of the basins. During the sampling, water environmental parameters were also determined. Temperature (T), pH, dissolved oxygen (DO), electrical conductivity (EC), and salinity (Sal) were recorded using a multiparameter sonde (ProDSS, YSI, Yellow Springs, OH, USA), while chlorophyll-a (Chl-a) concentration and turbidity (Turb) were measured using a fluorescence sensor (AlgaeTorch, bbe Moldaenke, Göttingen, Germany). The biological collected samples were fixed *in situ* using 95% ethanol; crustaceans were sorted out in laboratory under a Leica M205 C stereomicroscope and identified at the genus level based on Brtek and Mura (2000) and Rogers et al. (2019). The studied samples are currently stored in FM's branchiopod collection at the University of Palermo, Italy. Moreover, ten individuals are now stored in the Crustacea Collection of the "Museo di Storia Naturale - La Specola" of the University of Florence (Italy) with the catalogue numbers MZUF 731 and MZUF 732, and ten more in the biological collection of the Scientific Center of Zoology and Hydroecology, National Academy of Sciences of Republic of Armenia, Yerevan (Armenia).

Two *Artemia* individuals from each water body were carefully cleaned of any impurities and soaked in clear water for 5 minutes to remove any residual ethanol. Subsequently, a thoracic leg was excised and processed for DNA extraction from each specimen. The selective amplification of a fragment of the gene encoding the cytochrome oxidase subunit 1 (COI), was carried out by PCR using the Folmer primers pair (Folmer et al. 1994). Composition of the PCR mix and thermal cycles were performed according to Vecchioni al. (2017). Purification and sequencing were performed by Macrogen Europe (Milan, Italy) using a capillary electrophoresis sequencer with fluorescent detection (ABI 3130xL, Applied Biosystems, Waltham, MA, USA). The forward and reverse chromatograms were carefully inspected using Chromas (ver. 2.6.2, Technelysium

Pty Ltd, South Brisbane, Australia) and aligned with MEGA12 (Kumar et al. 2024), thus obtaining the consensus sequence. Novel COI sequences were translated into amino acids to check for any possible presence of frameshift or stop codons, eventually showing the presence of sequencing errors or pseudogenes. Furthermore, to compare our novel Armenian *Artemia* COI sequences with those publicly available, we retrieved 850 *Artemia* spp. COI sequences from GenBank, based on the dataset produced by Rode et al. (2022; see also their dataset available online at: https://datadryad.org/downloads/file_stream/1346182).

Molecular identification of the examined specimens and inference of their phylogenetic relationships were carried out by constructing a COI haplotype network including both newly generated and previously published *Artemia* spp. sequences, using PopART (v. 1.7; Leigh et al. 2015; https://popart.maths.otago.ac.nz/, accessed 13 February 2026). The haplotype network was estimated under the Median-joining algorithm (Bandelt et al. 1999).

A database of the Anostraca reported for Armenia was compiled based on an extensive bibliographical review based on the datasets of the authors, coupled with a literature search through the databases of SCOPUS (https://www.scopus.com, last accessed on 10 February 2026) and Google Scholar (https://scholar.google.it/, last accessed on 10 February 2026).

RESULTS

Environmental conditions in both basins on the sampling date are shown in Table 1. A small inflow of freshwater from uphill fed the northernmost sector of the lower lake, where a reed belt (*Phragmites* sp.) and a small population of mosquitofish (*Gambusia* sp.) were present; both species are absent from the remaining parts of both ponds. In contrast, brine shrimps were abundant in both basins and could be easily spotted from the shoreline.

In the collected zooplankton samples, *Artemia* was the only crustacean detected, represented by individuals at all developmental stages. All observed and collected adults were females, and the adults exhibited a characteristic non-reduced pattern of cercopods (cf. Asem et al. 2024a). The only other invertebrates occurring in both lakes were abundant Ephydridae larvae (Diptera).

Overall, four novel COI *Artemia* sequences were produced and included in the analyses. The newly obtained sequences were deposited in GenBank (Accession Numbers: PZ015493-PZ015496). The readable COI PCR products ranged from 674 to 720 bp; after trimming low-quality ends, a final alignment of 540 bp was obtained. The haplotype network (Fig. 2) showed the main genetic clusters corresponding to sexual species and distinct asexual lineages, which mirrors the haplotype's structure reported by Rode et al. (2022; their Fig. 5). All sequenced individuals from the present study collapsed into a single haplotype and this haplotype is identical to the "APD05" haplotype previously reported by Rode et al. (2022; see their Fig. 5 and associated Supplementary Materials). APD05 falls within a parthenogenetic *Artemia* lineage positioned close to the sexual species *Artemia amati* and includes both diploid and triploid individuals, corresponding to the "mt-2nk" and "mt-3n" in Rode et al. (2022), which are reported in Fig. 2 as "2na" and "3na".

To the best of our knowledge, only four papers provide primary occurrence data for Anostraca in Armenia: Smirnov (1936), Ter-Poghossian (1942), Meshkova (1968), and Hakobyan and Kalashian (2019). An additional work mentioning the occurrence of *Branchipus schaefferi* Fischer, 1834 (sub "*B. stagnalis*") in Armenia, actually refers to Bingol Dagh, a region nowadays located in Turkey (Cavalier, 1901). Overall, four anostracan species belonging to the families Branchipodidae, Chirocephalidae, and Streptocephalidae are mentioned to date for the country (Table 2).

Table 1. Environmental conditions in the Lake Aghi basins on 16th May 2025. Temp: Water temperature; DO: Dissolved oxygen; EC: electrical conductivity; Sal: Salinity; Chl-a: Chlorophyll-a concentration; Turb: Turbidity.

	Temp (ºC)	pH	DO (mg/L)	EC (mS/cm)	Sal (g/L)	Chl-a (μg/L)	Turb (FTU)
Big Aghi Lake	27.9	7.59	8.12	95.40	68.85	1.5	24.1
Small Aghi Lake	27.8	7.14	1.09	217.01	195.90	0.0	24.0

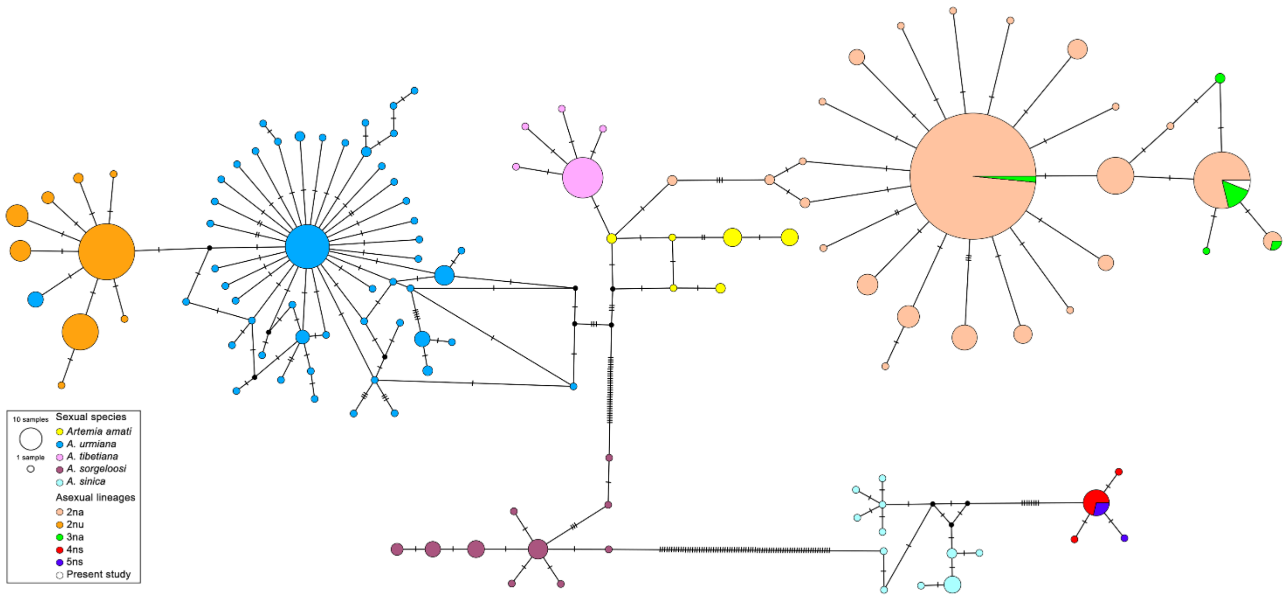

Figure 2. Median-joining haplotype network of novel and selected published *Artemia* COI sequences. The haplotype network was reconstructed using the 850 COI sequences analysed by Rode et al. (2022; see their Figure 5) and our four newly generated COI sequences. Circle size is proportional to the number of individuals sharing each haplotype. Connections between haplotypes represent mutational steps, while black dots represent inferred intermediate haplotypes. Asexual lineages, 2na: parthenogenetic diploid lineage close to *A. amati*; 2nu: parthenogenetic diploid lineage close to *A. urmiana*; 3na: parthenogenetic triploid lineage close to *A. amati*; 4ns: parthenogenetic tetraploid lineage close to *A. sinica*; 5ns: parthenogenetic pentaploid lineage close to *A. sinica*.

Table 2. Checklist of the Anostraca of Armenia. 1: Smirnov (1936); 2: Ter-Poghossian (1942); 3: Meshkova (1968); 4: Hakobyan and Kalashian (2019); 5: Present work. See the text for details.

Taxa	References
Artemiidae	
Artemia parthenogenetic lineage	5
Branchipodidae	
Branchipodopsis terpogossiani Smirnov 1936	1, 2, 3, 4
Branchipus blanchardi Daday 1908	3
Chirocephalidae	
Chirocephalus skorikowi Daday 1913	1, 3
Streptocephalidae	
Streptocephalus torvicornis (Waga 1842)	1, 2, 3

DISCUSSION

The taxonomy and nomenclature of the genus *Artemia* are complex and remain partially unresolved, limiting a comprehensive understanding of its diversity. Moreover, since morphological traits in *Artemia* are poorly understood, highly plastic and influenced by environmental salinity, the molecular approaches provide a robust and reliable tool for species delimitation and accurate sample assignment and are now widely regarded as essential. Although based on a relatively limited number of individuals and lacking data on ploidy, the congruence between reproductive mode, morphology, and mitochondrial data supports the assignment of the *Artemia* population inhabiting two basins of Lake Aghi to a yet unnamed parthenogenetic lineage closely related to Central Asian *Artemia* species. Despite the anthropogenic origin of the sampled sites and their intense human recreational use, the detected *Artemia* population belongs to a parthenogenetic lineage which can be considered native in the area. No evidence for the presence of the non-native sexual species *Artemia franciscana*, known to occur in several sites in Iran (e.g., Eimanifar et al. 2014), was detected.

This represents the first documented record of the whole genus *Artemia* in Armenia (cf. Triantaphyllidis et al. 1998; Emanifar et al. 2015). Although the genus is widespread in neighboring Turkey and Iran (e.g., Eskandari and Saygi 2019; Asem et al. 2024b), information on its occurrence in Georgia and Azerbaijan is scarce or absent (cf. Smirnov 1936; Mura and Nagorskaya 2005; Emanifar et al. 2015), highlighting the need for further surveys in the region.

This finding expands the known anostracan fauna of Armenia by adding one new species and one new family. Previously, the Armenian anostracan fauna was known to comprise only representatives of the families Branchipodidae, Chirocephalidae, and Streptocephalidae (Table 2). Moreover, the taxonomic identity of some previously reported species remains uncertain and requires further verification. The endemic branchipodid *Branchipodopsis terpogossiani*, spelled *Branchipodopsis ter-pogossiani* in the original description (Smirnov, 1936) and *Branchipodopsis ter-poghossiani* in Ter-Poghossian (1942), was considered a junior synonym of the widespread Asian species *Branchipodopsis affinis* Sars 1901 by Hartland-Rowe (1968). His opinion was followed by some authors (e.g., Rogers 2013; Kangarloei and Roohi, 2021) but not by others (e.g., Vekhoff 1992). As stressed by Brtek et al. (1984) and Belk and Brtek (1995), a revision of specimens from the type locality of the species is needed to check for the actual taxonomical status of this interesting species. The occurrence of *Branchipus blanchardi* Daday 1908 (reported sub *B. alpinus* Colosi 1922 by Meshkova 1968) on the Lori plateau is rather unexpected, since this species is currently considered an endemic of the western European Alps (Alonso 1989; Belk and Brtek 1995). The identity of this branchipodid species needs to be checked as well.

Several anostracan species reported for neighboring countries (e.g., Vekhoff 1992b, 1993; Atashbar et al. 2014) have not to date been reported in Armenia, suggesting that the currently available picture is unlikely to be representative of the actual diversity of the country, and that further targeted surveys are required. The present study thus expands the currently available knowledge of the anostracan fauna of Armenia and emphasizes the need for comprehensive, integrative faunistic and molecular surveys to more accurately assess biodiversity patterns in inland water bodies of the South Caucasus region.

ACKNOWLEDGEMENTS

The study was supported by the Higher Education and Science Committee of the Ministry of Education, Science, Culture, and Sports (MESCS) of the Republic of Armenia under Research Project No.24WS-1F009. N.O. Rode (CBGP, INRAE, France) is warmly acknowledged for kindly providing information regarding the Dryad data associated with Rode et al. (2022). We sincerely thank D.C. Rogers (GRDA Scenic Rivers and Watershed Research Laboratory, USA) for his valuable assistance in locating hard-to-access literature. We are grateful to Mr. Artyush Khanjyan for providing historical information on the investigated limno-system. Two anonymous reviewers are gratefully acknowledged for their constructive criticisms of an earlier version of the manuscript.

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Submitted: 18 February 2026

First decision: 28 March 2026

Accepted: 30 March 2026

Published online: 1 April 2026

Edited by Loris Galli