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Seyidova LM
Nakhchivan State University, Nakhchivan, Azerbaijan

Corresponding Author:
Seyidova LM
Nakhchivan State University, Nakhchivan, Azerbaijan

The sweet water amoeba species of the Nakhchivan Autonomous Republic with a shell (testacea)

Seyidova LM

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Abstract
This article examines the shelled amoebae of freshwater bodies. From 2017 to 2022, a total of 28 species, 2 classes, 2 orders, 7 families, and 28 genera of shelled amoebae have been identified within the Nakhchivan Autonomous Republic. As an ecological indicator, shelled amoebae are used to assess the environmental condition, particularly the level of pollution in soil and water.

Keywords: Testacea, species, bioindicator, protozoa, faunistic analysis, protist

Introduction
Free-living protozoa, that is, protists, are an essential component of aquatic ecosystems. They are morphologically and physiologically diverse and are widespread in almost all water and soil environments on the planet. These organisms, particularly ciliates, amoebae, and other protozoans, play important ecological roles and serve as bioindicators in assessing the condition of the environment.
The faunistic study of free-living protozoa provides valuable information not only about the composition, geographical distribution, and ecological characteristics of species but also about the ecological status of the region. Specifically, faunistic analyses of freshwater bodies reflect the dynamics of natural and anthropogenic processes occurring in these areas.
The main aim of the faunistic analysis of free-living protozoa is to identify the species composition of these organisms in aquatic habitats, map their distribution ranges, analyze their ecological and bioindicator characteristics, and determine endemic and new species to include in scientific literature.

Materials and Methods
The research was conducted from 2017 to 2022 in various freshwater bodies of the Nakhchivan Autonomous Republic. Samples were collected throughout the year. During the study period, more than 3,000 water samples were collected and analyzed. Samples were collected using polyethylene containers. The methods developed by Y. Tseeb and V. Gurvich were employed to determine the abundance of shelled amoebae. Existing monographs and extensive scientific articles were used as primary sources for species identification. Descriptions and micrographs of shelled amoebae were obtained from both permanent and temporary preparations through *in vivo* observations, using a GXCAM digital camera and a JEOL-6000 SEM device. Additionally, a simplified method for silver nitrate impregnation of shelled amoebae was applied for more precise processing.

Discussion and Results
Shelled amoebae (Latin: Testacea or Arcellinida) belong to the Amoebozoa suborder and are free-living or semi-parasitic protozoans with a cell structure. Despite being unicellular, they exhibit high adaptability to the environment, morphological diversity, and ecological importance. Their defining characteristic is the presence of a shell (test) that surrounds the cell and serves a protective function.
These organisms are mainly found in soil and freshwater ecosystems. In soil, they are present in the humus-rich upper layers, while in aquatic environments, they can be found in puddles, swamps, and stagnant water bodies. They feed on microorganisms, bacteria, and other protozoa, playing a crucial role in the nutrient cycle and regulating trophic relationships among organisms.

As ecological indicators, shelled amoebae are used to assess the state of the environment, especially the level of pollution in soil and water.

To date, 28 species of shelled amoebae belonging to 2 classes, 2 orders, 7 families, and 28 genera have been identified in the Nakhchivan Autonomous Republic.

Regnum: Protozoa
Phylum: Protozoa
Subphylum: Sarcodına
Superclassıs: Rhızopoda
1. Classıs: Lobosa
1. Ordo: Arcellınıda
Subordo: Arcellına

1.Famılıa: Arcellıdae
1. *Arcella arenaria* Greeff, 1866
2. *Arcell artocrea* Greeff, 1866
3. *Arcella costata* Ehrenberg, 1847
4. *Arcella discoides* Ehrenberg, 1832
5. *Arcella hemisphaerica* Perty, 1852
6. *Arcella patens* Claparede et Lachmann, 1859
7. *Arcella stellaris* Perty, 1849
8. *Arcella vulgaris* Ehrenberg, 1830

2. Famılıa: Centropyxıdae
9. *Centropyxis aculeata* (Ehrenberg, 1838)
10. *Centropyxis* laevigata Penard, 1890

3. Famılıa: Dıfflugıdae
11. *Difflugia acuminata* Ehrenberg, 1832
12. *Difflugia assulina seminulum* Ehrenberg, 1871
13. *Difflugia corona* Wallich, 1864
14. *Difflugia globulosa* Dujardin, 1837
15. *Difflugia lanceolata* Penard, 1890
16. *Difflugia lemani* Blanc, 1892
17. *Difflugia limnetica* Levander, 1900
18. *Difflugia lobostoma* Leidy, 1879
19. *Difflugia mamilaris* Penard, 1893
20. *Difflugia pyriformis* Party, 1852
21. *Pontigulasia compressa* (Carter, 1864)

4. Famılıa: Nebelıdae
22. *Nebela caudata* Leidy, 1879
23. *Nebela galeata* Penard, 1902

5. Famılıa: Paraquadrulıdae
24. *Quadrulella symmetrica* Wallich, 1863

6. Famılıa: Phryganellıdae
25. *Phryganella paradoxa* Penard, 1902

1. Classıs: Fılosıa
2. Ordo: Aconchulınıda
Subordo: Gromına

7. Famılıa: Euglyphıdae
26. *Euglypha mucronata* Leidy, 1878
27. *Euglypha alveolata* Dujardin, 1841
28. *Euglypha ciliata* Ehrenberg, 1848

The most widely distributed families include *Difflugiidae*, represented by 11 species (40.74%), and *Arcellidae.* The main criteria used for the taxonomic identification of these organisms include the shape of the shell (oval, conical, flattened, etc.), the position of the aperture, the texture of the shell, and the type of pseudopodia.

Arcellidae is one of the most important and historically earliest described families in the systematics of shelled amoebae (Testacea Lobosia). This family was first described in 1830 by the German microbiologist Christian Gottfried Ehrenberg. Members of the *Arcellidae* family are predominantly found in freshwater bodies, swamps, and moist soil layers, preferring environments rich in organic matter.

A key characteristic of organisms belonging to *Arcellidae* is their asymmetric or flattened shell structure. These shells are mostly round or oval and composed of organic material, exhibiting a homogeneous structure. In some species, the shell surface is smooth, while in others it may be wrinkled or covered with fine granules. The aperture is located on the ventral side of the shell, which is an important taxonomic feature distinguishing them from other families.

The pseudopodia (Lobopodia) of the amoeba located inside the shell usually extend through the aperture and are responsible for locomotion and feeding. One or more vacuoles, as well as a nucleus, are visible within the cell.

Representatives of the *Arcellidae* family are mainly saprophytic, feeding on bacteria, decomposed organic particles, and other microscopic organisms. They are primarily distributed in freshwater bodies, ponds, swamps, upper soil layers, and algal mats.

Species belonging to this family are widely used as bioindicator organisms. Their abundance, distribution, and species diversity are of great significance for assessing trophic conditions and ecological stability in aquatic ecosystems. Polluted or oxygen-poor waters are typically associated with a decline or disappearance of *Arcellidae* species.

Arcellidae belongs to the order Arcellinida and the phylum Amoebozoa. The genus Arcella includes about eight species. Members of this genus usually possess brownish, yellowish, or transparent shells and range from 30 to 150 µm in size.

Representatives of the Arcellidae family play a role in nutrient cycling, microbial population regulation, and mineralization in soil and water ecosystems. Their fossil remains are also used in paleoecological studies. In addition, these species serve as effective bioindicators for ecological monitoring of water bodies.

This family belongs to the phylum A*moebozoa* and the order Arcellinida (shelled amoebae). The cell is surrounded by a shell (test), usually chitinous or covered with mineral particles. The shell is often flattened or egg-shaped, with a single aperture (ostium) located at the upper part. The aperture is generally situated anteriorly, from which the pseudopodia extend. Pseudopodia are mostly lobose in type.

Members of this family are found in freshwater ecosystems (lakes, rivers, ponds) and sometimes in soil. Many species are benthic, living at the bottom of water bodies within mud or sediments.

This family includes protozoans belonging to the Testate Amoebae group. Their cells are covered with a shell (test), typically composed of mineral particles (sand grains and other fragments) or organic materials. The shell is usually round, pear-shaped, or elongated. Through the aperture (ostium), one or several lobose pseudopodia emerge.

Locomotion and feeding are carried out by these pseudopodia.

They mainly inhabit freshwater ecosystems, soils, and algal mats. In freshwater ecosystems, they act as microscopic predators, feeding on bacteria, algae, and other microorganisms. Due to the preservation of their shells in sediments, they are important in paleoecological and bioindication studies. Typical species include Difflugia acuminata, *Difflugia oblonga*, *Difflugia corona*, and others.

Nebelidae is a family of testate amoebae with distinctive characteristics. Their cells are enclosed in an organic shell. These shells are typically flexible, with wavy and uneven edges. The shell is often dark-colored and weakly transparent. The aperture may appear irregular in shape. Pseudopodia extend from this opening to enable movement and feeding.

Members of this family occur mainly in bottom sediments of freshwater bodies, among algae and sphagnum mosses, as well as in soil. They play an important role as bioindicators in moist ecosystems. Representatives include *Nebela collaris*, *Nebela tincta*, *Nebela penardiana*, and others. Due to the preservation of their shells in peat and sediments, they are widely used in paleoecological and bioindication research. They participate in nutrient cycling by feeding on bacteria and algae.

Family: Paraquadrulidae Deflandre, 1958

Paraquadrulidae is one of the less studied and relatively rare families of Testate Amoebae. Their cells contain a single nucleus. The external skeleton (shell) is typically chitinous and reinforced with mineral particles. The shell often appears quadrangular or polygonal (a feature reflected in the name derived from the genus *Paraquadrula*). Their pseudopodia are mostly lobose.

They occur in bottom sediments of freshwater bodies, among algae, and in soil. The family is mainly represented by the genus *Paraquadrula*. Only a few species have been described, and they are rarely encountered. They take part in the decomposition of microscopic organisms and exhibit both saprophytic and predatory feeding characteristics. They may serve as bioindicators for assessing the ecological condition of certain freshwater bodies.

This family of testate amoebae is found predominantly in soil and freshwater ecosystems. Their cells contain a single nucleus. The shell (test) is usually small, thin, and reinforced with mineral particles. The shape of the shell is spherical or oval, with a small and simple aperture. Pseudopodia are lobose.

The main genus is *Phryganella.* Species are microscopic (15-40 µm). They are found in bottom sediments of freshwater bodies, soil layers, and among algae. They participate in microbial nutrient cycles, feeding on bacteria and organic particles. They can serve as bioindicators of ecological quality in soil and aquatic environments.

Euglyphidae is one of the most studied and widely distributed families of testate amoebae. Their cells are uninucleate. The shell (Testa) consists of silica plates arranged in a mosaic pattern. The shell is elongated or oval and symmetrical. The aperture is usually circular or oval, with a distinctive arrangement of plates around it. Pseudopodia are thread-like (filopodia), which clearly distinguishes them from lobose amoebae.

The genus *Euglypha* is the most widespread and species-rich. *Trinema*, *Assulina*, and others are sometimes regarded as separate families but are often included within *Euglyphidae*. These organisms are common in freshwater bodies, among algae, in soil, and in sphagnum mosses. They feed on bacteria and small organic particles. They serve as important bioindicators of soil and water ecosystems, particularly reflecting soil acidity and moisture regimes.

This study covers the species diversity and distribution characteristics of testate amoebae in various freshwater bodies of the administrative regions of the Nakhchivan Autonomous Republic. As a result of the research, 28 species and subspecies belonging to 7 families of testate amoebae were recorded. Among these, members of the families *Difflugiidae* (11 species), *Arcellidae* (8 species), and *Centropyxidae* (2 species) were dominant.

The taxonomic identification of the recorded species was performed based on modern systematic approaches. Regarding species diversity across regions, the highest number of species was recorded in the Nehram reservoir (22 species), followed by the Araz reservoir (20 species), H. Aliyev reservoir (18 species), Dəstəgöl reservoir (18 species), Arpaçay River (15 species), Uzunoba reservoir (13 species), and the lowest species number was recorded in Nakhchivanchay (12 species) and Gümüşlü Lake (15 species).

Morphological characteristics of the species were determined and described based on biometric measurements. Microscopic images obtained using a scanning electron microscope and a GXCAM-3 digital camera were employed in the morphological analyses.

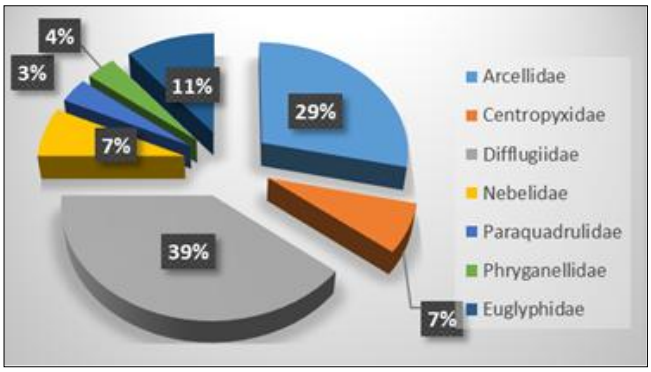

Diagram 1: Distribution of Testate Amoebae by Families

The conducted research shows that a total of 28 species of testate amoebae belonging to 8 genera were recorded in the freshwater bodies of the Autonomous Republic. Based on their distribution across genera, the genus *Difflugia*

dominates with 11 species (15.9%). The genera *Arcella* and *Centropyxis* include 8 species (11.6%) and 2 species (13%), respectively. The remaining 16 genera together account for 23% of the total species composition (Figure 1).

Analysis by regions shows the following distribution patterns:

Arpaçay River: Arcellidae - 6 (75%); Centropyxidae - 1 (50%); Difflugiidae - 5 (45.45%); Nebelidae - 1 (50%); Euglyphidae - 2 (66.7%)

Nakhchivanchay: Arcellidae - 4 (50%); Centropyxidae - 1 (50%); Difflugiidae - 7 (63.63%); Nebelidae - 1 (50%)

H. Aliyev Reservoir: Arcellidae - 7 (87.5%); Centropyxidae - 2 (100%); Difflugiidae - 7 (63.63%)

Uzunoba Reservoir: Arcellidae - 5 (62.5%); Centropyxidae - 2 (100%); Difflugiidae - 8 (72.72%); Nebelidae - 1 (50%); Euglyphidae - 1 (33.33%)

Nehram Reservoir: Arcellidae - 6 (75%); Centropyxidae - 1 (50%); Difflugiidae - 9 (9.90%); Nebelidae - 1 (50%); Paraquadrulidae - 1 (100%); Euglyphidae - 3 (100%)

Dəstədöl Reservoir: Arcellidae - 5 (62.5%); Centropyxidae - 2 (100%); Difflugiidae - 6 (54.54%); Paraquadrulidae - 1 (100%); Phryganellidae - 1 (100%); Euglyphidae - 3 (100%)

Araz Reservoir: Arcellidae - 8 (100%); Centropyxidae - 2 (100%); Difflugiidae - 7 (87.5%); Nebelidae - 2 (100%); Euglyphidae - 1 (33.33%)

Gümüşlü Lake: Arcellidae - 5 (62.5%); Difflugiidae - 7 (63.63%)

These results clearly demonstrate that the genus *Difflugia* dominates across all regions and constitutes the main component of the taxonomic structure of testate amoebae. The genus *Arcella* ranks second in abundance. The distribution of other genera varies only slightly between regions and is largely influenced by local ecological conditions (Diagram 2).

As shown in the diagram, 8 species (34%) belong to the genus *Arcella*, while 10 species (42%) belong to the genus *Difflugia*. All remaining genera together represent 24% of the total species composition.

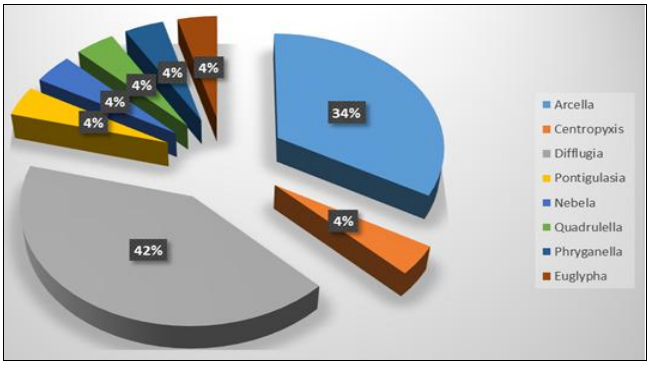

Diagram 2: Distribution of Testate Amoebae by Genera

Based on the collected material from the freshwater bodies, permanent and temporary preparations were made, and the morphology of characteristic testate amoebae species was studied using *in vivo* observations, impregnation, and glycerin-gelatin methods. The morphological structure and characteristic features of the species were described based on high-resolution images obtained with both a light microscope (CX-41RF-Olympus) and a JEOL-6000 scanning electron microscope. Biometric measurements were taken under at least 40× magnification during microscopic observations. This approach has created an essential scientific basis for refining the systematic composition of testate amoebae and evaluating their ecological characteristics.

Table 1 presents the species composition and distribution of testate amoebae observed in the studied water bodies. A total of 12 species were recorded in the Nakhchivanchay, 18 species in the H. Aliyev Reservoir, 13 species in the Uzunoba Reservoir, 22 species in the Nehram Reservoir, 18 species in the Dəstədöl Reservoir, 20 species in the Araz Reservoir, 15 species in the Arpaçay River, and 12 species in Gümüşlü Lake.

Table 1: Species Composition of Testate Amoebae in the Freshwater Bodies of Nakhchivan Autonomous Republic

S. No	Növlər	Sututarlar							
	Regnum: Protozoa **Phylum:** Protozoa **Subphylum:** Sarcodına **Superclassıs:** Rhızopoda **1. Classıs:** Lobosa **1. Ordo:** Arcellınıda **Subordo:** Arcellına **1. Famılıa:** Arcellıdae	1	2	3	4	5	6	7	8
1.	*Arcella arenaria* Greeff, 1866	+	+	+	+	-	+	+	+
2.	*Arcell artocrea* Greeff, 1866 (8; 29,62%)	-	+	-	+	+	+	+	-
3.	*Arcella costata* Ehrenberg, 1847	+	+	+	+	+	+	+	-
4.	*Arcella discoides* Ehrenberg, 1832	-	+	+	+	-	+	+	+
5.	*Arcella hemisphaerica* Perty, 1852	-	-	-	-	+	+	-	+
6.	*Arcella patens* Claparede et Lachmann, 1859	+	+	+	+	+	+	+	+
7.	*Arcella stellaris* Perty, 1849	-	+	-	+	+	+	+	+
8.	*Arcella vulgaris* Ehrenberg, 1830	+	+	+	-	-	+	-	-
2. Famılıa: Centropyxıdae									
1.	*Centropyxis aculeata* (Ehrenberg, 1838)	-	+	+	+	+	+	+	-
2.	*Centropyxis laevigata* Penard, 1890	+	+	-	-	+	+	-	-
3. Famılıa: Dıfflugıdae									
1.	*Difflugia acuminata* Ehrenberg, 1832	+	+	+	+	+	+	-	+
2.	*Difflugia assulina seminulum* Ehrenberg, 1871	+	+	+	+	+	+	+	+
3.	*Difflugia corona* Wallich, 1864	+	+	+	+	+	+	+	-

4.	*Difflugia globulosa* Dujardin, 1837	-	-	-	+	-	-	-	+
5.	*Difflugia lanceolata* Penard, 1890	-	+	-	+	-	+	-	+
6.	*Difflugia lemani* Blanc, 1892	-	-	-	+	-	-	-	+
7.	*Difflugia limnetica* Levander, 1900	+	+	+	+	-	+	+	-
8.	*Difflugia lobostoma* Leidy, 1879	-	-	-	-	+	-	-	-
9.	*Difflugia mamilaris* Penard, 1893	-	-	+	+	-	-	-	-
10.	*Difflugia pyriformis* Party, 1852	+	+	+	+	+	+	+	+
11.	*Pontigulasia compressoidea* Jung, 1942	+	+	+	+	+	+	+	+
4. Famılıa: Nebelıdae									
1.	*Nebela caudata* Leidy, 1879	+	+	-	+	-	+	+	_
2	*Nebela galeata* Penard, 1902	-	-	-	-	-	+	-	-
5. Famılıa: Paraquadrulıdae									
1.	*Quadrulella symmetrica* Wallich, 1863	-	-	-	+	+	-	-	-
6. Famılıa: Phryganellıdae									
1.	*Phryganella paradoxa* Penard, 1902	-	-	-	-	+	-	-	-
2. Classıs: Fılosıa 2. Ordo: Aconchulınıda Subordo: Gromına 7. Famılıa: Euglyphıdae									
1.	*Euglypha mucronata* Leidy, 1878	-	-	-	+	+	-	-	-
2.	*Euglypha alveolata* Dujardin, 1841		+	-	+	+	+	+	-
3.	*Euglypha ciliata* Ehrenberg, 1848	-	-	-	+	+	-	+	-
4.		12	18	13	22	18	20	15	12

1. Nakhchivanchay, 2. H. Aliyev Reservoir, 3. Uzunoba Reservoi, 4. Nehram Reservoir, 5. Dəstədöl, 6. Araz Reservoir, 7. Arpaçay River,8. Gümüşlü Lake

The distribution of species in the studied water bodies was determined at different proportions. For instance, in the

Arpaçay River: Arcellidae (6) 75%, Centropyxidae (1) 50%, Difflugiidae (5) 45.45%, Nebelidae (1) 50%, Euglyphidae (2) 66.7%.

In Nakhchivanchay: Arcellidae (4) 50%, Centropyxidae (1) 50%, Difflugiidae (7) 63.63%,
Nebelidae (1) 50%.

In the H. Aliyev Reservoir: Arcellidae (7) 87.5%, Centropyxidae (2) 100%, Difflugiidae (7) 63.63%.

In the Uzunoba Reservoir: Arcellidae (5) 62.5%, Centropyxidae (2) 100%, Difflugiidae (8) 72.72%, Nebelidae (1) 50%, Euglyphidae (1) 33.33%.
In Nehram Reservoir: Arcellidae (6) 75%, Centropyxidae (1) 50%, Difflugiidae (9) 90%, Nebelidae (1) 50%, Paraquadrulidae (1) 100%, Euglyphidae (3) 100%.

In Dəstədöl: Arcellidae (5) 62.5%, Centropyxidae (2) 100%, Difflugiidae (6) 54.54%, Paraquadrulidae (1) 100%, Hryganellidae (1) 100%,
Euglyphidae (3) 100%.

In Araz Reservoir: Arcellidae (8) 100%, Centropyxidae (2) 100%, Difflugiidae (7) 87.5%, Nebelidae (2) 100%, Euglyphidae (1) 33.33%.
In Gümüşlü Lake: Arcellidae (5) 62.5%, Difflugiidae (7) 63.63%.

Overall, the faunistic analysis showed that in all the studied water bodies, the *Difflugia* genus has maintained a dominant position, significantly surpassing other genera in species diversity. The *Arcella* genus, on the other hand, is recorded as a subdominant group. These findings suggest that while the faunistic composition of testate amoebae in various freshwater bodies of the Nakhchivan Autonomous Republic shares common structural elements, the relative distribution of species exhibits significant differences depending on ecological conditions and habitat characteristics.

Results

1. A total of 28 species of testate amoebae were identified in the water bodies and rivers of the Nakhchivan Autonomous Republic, representing 2 classes, 2 orders, 7 families, and 8 genera.
2. The study of water bodies indicated that species from the families *Arcellidae* (13%), Centropyxidae (14%), and *Difflugiidae* (55%) were dominant.
3. The periphytic forms of testate amoebae are widely distributed across various aquatic ecosystems of the autonomous republic and play a crucial role as indicators of both biodiversity and ecosystem health. Information on their morphological and ecological characteristics, as well as their distribution, serves as a valuable source for hydro-ecological monitoring and bioindication activities.

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