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Биологические науки

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Testate Amoebae (Rhizopoda, Testacea) in Karelia Pine Biogeocenoses

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Аннотация: The aim of this work is to study the Testacea variety in pine biogeocenoses of Karelia. The species variety of soil testate amoebae (Rhizopoda, Testacea) was studied in the pine biogeocenoses of the PetrSU Botanical Garden (Oxalis Pine Forest) and the Kivach Reserve (Cowberry Pine Forest). Research results: during the research period (2019—2020), 28 species of testate amoebae were identified. The largest amount of species that were detected in the soils of the studied biotopes are Trinema lineare, Trinema complanatum, Nebela militaris, Cyclopyxis eurystoma and species of the genus Euglypha. The largest number of found species in the Pine Forest of the Kivach Reserve. There was a suggestion about the influence of anthropogenic load and small fertile soil layer on quantitative composition of protozoa in Botanical Garden. The number of Rhizopoda species changes during the whole survey period in both biotopes.
Ключевые слова: Protozoology; Testacea; Morphology; Seasonal Dynamics; Species; Microorganisms

Для цитирования: *Валдаева Е. В. Testate Amoebae (Rhizopoda, Testacea) in Karelia Pine Biogeocenoses // StudArctic Forum. 2021 № 3(23)*

The simplest microorganisms are species of Testacea. They have an important function in natural communities. Protozoa are widespread in forest ecosystems and are a constant component of soil microfauna.

These organisms mineralize organic matter and decompose cellulose and lignin, making it available to other animals and plants. As a matter of fact, they are involved in the transformation of organic materials into humus in the upper soil layers (Mazei, Tsyganov, 2006; Bulatova, 2010).

The number and variety of amoebae species depends on the structure of the stand in forests. The largest number of protozoa is located in the zone of the rhizosphere of trees, because there is a contact of microorganisms with roots of the plant in the soil.

For example, scientists discovered 9 species of Testacea (members of the *Cyclopyxis*, *Centropyxis* and *Nebela* genera) close to spruce and pine roots in the forest soil of West Siberia. There are all species in the undercrown zone of the pine. The maximum number of dominant species *Nebela collaris* were observed at a distance of 20-60 centimeters from the root system of both trees. This may be due to the fact that the trees can has an underdeveloped main root and only lateral roots absorb water and minerals (Kulyukina et al., 2016).

According to the scientific literature data, there is big variety of protozoa in pine forests, among eurybionts (kinds of the *Cyclopyxis* and *Trinema* genera). The species variety of testate amoebae is due to the great heterogeneity of the vegetation cover, where there are areas with coniferous litter and grass-shrub and moss-lichen layers. It was discovered that: 1) More Testacea of *Phryganella* and *Cyclopyxis* genera in the moss-lichen cover of pine forests. 2) More species of *Centropyxis* were detected in soil-lichen areas (Bulatova, 2010; Blinokhvatova et al., 2011).

There are several studies on the variety of Rhizopoda on the territory of Karelia, which were carried out in highly

swampy areas of pine and sedge-sphagnum forests (Mazei, Kabanov, 2008; Malysheva, Mazei, 2013). The authors noted 3 ecological groups of protozoan communities in the process of research in the sedge-sphagnum forest of the northern taiga: xerophiles, mesophiles, and hygrophils. Furthermore, it was suggested that in the distribution of testate amoebae over the area, other environmental factors, like humidity, are also important (Mazei, Kabanov, 2008).

There are few scientific works on protozoa in pine forests of Karelia, therefore the aim of this work is to study the Testacea variety in pine biogeocenoses of the Republic. The research sites are located on the territory of the Karelia Republic in the Northwestern Federal District of Russia.

The Kivach reserve is a protected state zone with a special nature, where scientific research works are carried out annually. Most of the reserve (84%) is covered with forest, 41% of which is pine forests. Cowberry and blueberry pine forests are formed on the slopes of the hills, where the soil cover is an Ferric Podzols (Bakhmet, 2017). These soils contain the greatest amount of humus in mineral horizons, the value of which decreases with depth. It provides high density of colonization by the simplest organisms in the upper soil layers. The pH indicates a highly acidic reaction of podzols especially in the upper horizons (Fedorets et al., 2006).

The second research site is the Botanical garden. That place was formed on the slopes of a relict volcano. There are sandy loam burozems in the Oxalis Pine Forest. These soils differ from the others by having a humus horizon, where substances are humified. Soils are highly acidic too especially in litter (Krasilnikov, Platonova, 2001).

Material and method

The species variety of soil testate amoebae (Rhizopoda, Testacea) was studied in the pine biogeocenoses of the PetrSU Botanical Garden (Oxalis Pine Forest) and the Kivach Reserve (Cowberry Pine Forest) in the period from 2019 to 2020.

Soil samples were taken annually from June to October in the Botanical Garden (61°84′ N 34°38′ W) and from June to September in the Kivach Reserve (62°29′ N 33°91' W) in four areas: two samples from each studied biotope (Fig. 1).

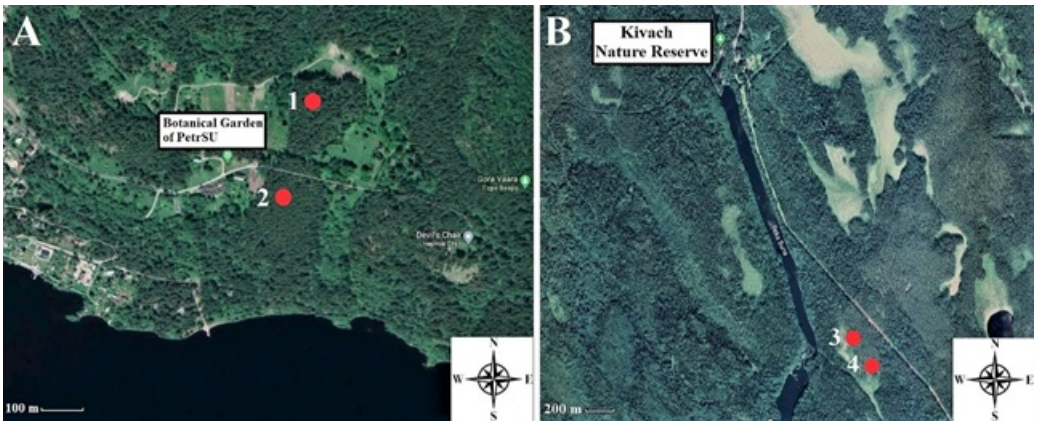

Fig. 1. Sampling sites (studied sampling sites marked): A — Oxalis Pine Forest of the Botanical Garden (PetrSU) (1, 2); B — Cowberry Pine Forest of the Kivach nature reserve (3, 4)

The depth of sampling of the soil samples was less than 20 cm, which allowed us to study the species and quantitative composition of microorganisms in the upper mineral horizons.

To identify seasonal dynamics, soils from one site have been studied monthly from June to October for a two-year period.

We used the method of A.A. Rakhleeva, G.A. Korganova (2005) for species identification of soil testate amoebae. Exact 5 grams of the substrate were placed in a flask, poured with water (150—200 ml) and left for several hours to soak the soil particles. Then the suspension was shaking during 10 minutes and filtered through the sieve with 0.8 mm holes into large beakers. The suspension was settled for 2 hours, the upper liquid was drained, the filtrate residue was transferred to a graduated container and allowed to settle again. Excess liquid was drained and 10 ml left. The suspension was colored with carmine and fixed with formalin for 24 hours. Living cells of protozoa and cysts were coloured in pink, which allowed us to identify active organisms and empty shells. More than 10 preparations were reviewed from each sample.

Testate amoebae were counted in aqueous suspensions. A drop of the solution (~ 45μl) was placed on a glass slide using a Pasteur pipette. Microscopy of the preparations was performed using Axio Scope A1 and Motic

microscope magnifications of 45x and 60x. We studied more than 3000 preparations in total.

The discussion of the results

The Pearson index ($\chi 2$) was used to assess the credibility differences in species abundance, confinement to biotope. Significant differences were considered under the following conditions: if p

Table. Occurrence of the soil testate amoebae (Testacea) in research biotopes. The Pearson index values obtained ($\chi 2$) and the significance level values obtained (p) when the number of degrees of freedom df = 1.

Species	Cowberry Pine Forest of the Kivach Reserve	Oxalis Pine Forest of the Botanical Garden (PetrSU)	$\chi 2$	p
Corythion dubium (Taranek, 1871)	200	8	177,2	< 0,001
Cyclopyxis eurystoma (Deflandre, 1929)	174	159	0,7	0,41
Euglypha laevis (Ehrenberg, 1845)	126	66	18,8	< 0,001
Euglypha tuberculata (Dujardin, 1841)	102	57	12,7	0,01
Nebela militaris (Penard, 1890)	227	100	49,3	< 0,001
Nebela tincta (Leidy, 1879)	126	91	5,6	0,02
Trinema complanatum (Penard, 1890)	155	54	48,8	< 0,001
Trinema lineare (Penard, 1890)	285	213	10,4	0,01
Total	1395	748		

In the studied pine forests, 28 species belonging to 14 genera were observed.

The most numerous species that were detected in the soils of the studied biotopes are*Trinema lineare*, *Trinema complanatum*, *Nebela militaris*, *Cyclopyxis eurystoma* and species of the genus *Euglypha*.

The greatest variety of spotted species is in the Pine Forest of the Kivach Reserve. There are such dominants as *Trinema lineare*, *Corithion dubium*, *Nebela militaris*, *Euglypha strigosa glabra* (Fig. 2A) in this biogeocenosis.

The *Trinema lineare* includes little elongated shell. We can distinguish these species of amoebae by the round mouth is 10 μm in diameter. Its shell is small in size, 16-35 μm in length, 7-17 μm in width.

The shell of *Nebela militaris* is pear-shaped and has medium size (50-80 μm in length, 25-50 μm in width), which tapers towards the mouth, but the mouth a little expanded. *Nebela militaris* is covered with round, elliptical rod-shaped or irregularly shaped idiosomes. The mouth is elliptical, 16-20 μm in width (Fig. 2B).

Corithion dubium, which was located in every Cowberry Pine Forest soil sample, has an ovoid shell consisting of very small oval plates that overlap. Its sizes are average: 33-43 μm in length; 25 μm in width.

Trinema lineare and *Nebela militaris* were detected in the biotope samples of the Botanical Garden, as well as in the Forest of the Kivach Nature Reserve. In addition, *Cyclopyxis eurystoma* became the dominant species in the Oxalis Pine Forest.

The *Cyclopyxis eurystoma* includes a middle half-round shell in a hemispherical profile, 30-80 μm in diameter and 30-50 μm in height. Its mouth is large (22-34 μm in diameter). Mineral components of shell are big and visible well enough.

The shell of *Nebela tincta,* which has been identified in large numbers as well, is big, pear-shaped or ovoid. The color range is different: from light yellow to colorless. The shell is 75–95 μm in length, 55–64 μm in width, the mouth is up to 30 μm in width (Fig. 2C).

An interesting specie is *Trigonopyxis arcula*. It is located in the soil of the Cowberry Pine Forest, the mouth of which is of an irregular shape, resembling a triangle. The shell is medium, hemispherical. Coating of flat grains of sand or detritus. Size: 65 μm in height, 64 μm in width (Fig. 2D).

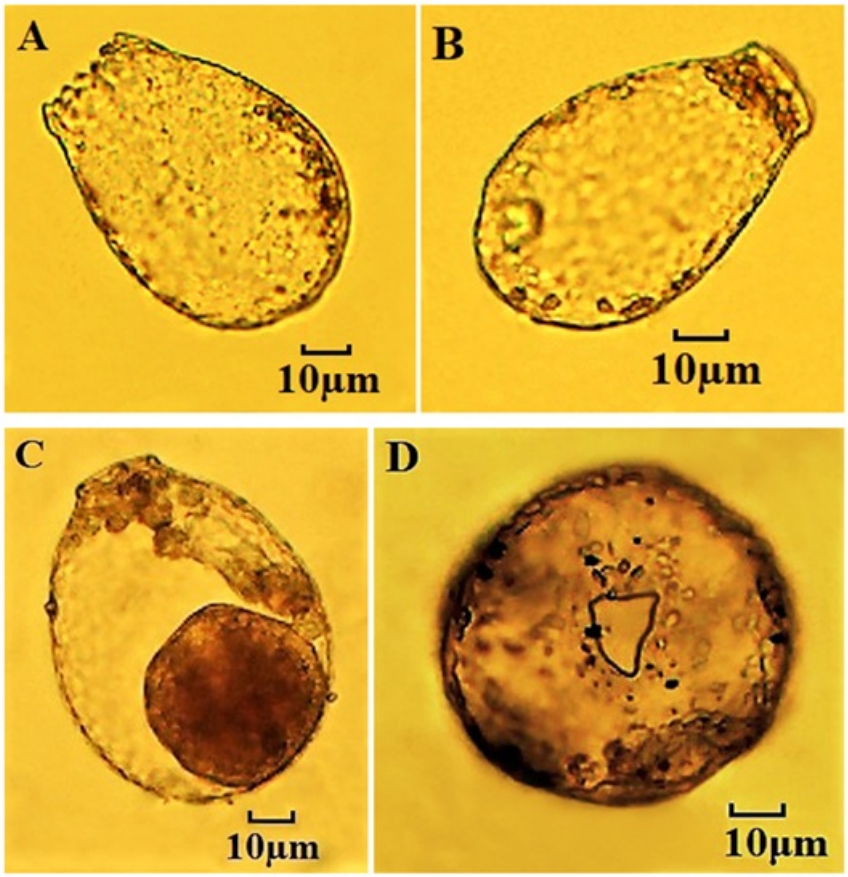

Fig. 2. Species of testate amoebae:

A — *Euglypha strigosa glabra* (Wailes, 1898); B — *Nebela militaris* (Penard, 1890); C — *Nebela tincta* (Leidy, 1879) with amoeba cyst inside; D — *Trigonopyxis arcula* (Penard, 1912)

The total amount of Testacea located in the biogeocenosis of the Botanical Garden is 788 and 4006 in the Kivach Reserve. It is established, that out of 9 species identified in the Oxalis Pine Forest, only 1 was not detected in the soils of the Cowberry Pine Forest.

In the comparison between smooth forms and forms with spines from the genus*Euglypha* shows that the first are more common in drier habitats. The species of *Euglypha strigosa* are present in the Oxalis Pine Forest soil samples, and it is absent in the Cowberry Pine Forest. It informs us that the environment in the first biotope is wetter than in the second. Therefore, it cannot be denied that in the process of life (when moving and growing), living organisms can lose the shell. This may affect the result of determining species in the future (Mazei, Tsyganov, 2006).

Availability of xerophilous representatives in pine ecosystem of the Kivach Reserve such as*Assulina muscorum*, *Corithion dubium* and *Centropyxis orbicularis* can confirm the assumption about the difference in the humidity of the environment of both forests. As a matter of fact, it is well-known that *Assulina muscorum*, *Corithion dubium* also were determined in dry conditions of Botanical Garden in Neuchatel, Switzerland (Koenig, I. et al., 2018). The difference in humidity is demonstrated by larger number and variety of Testacea forms with a round shell in the soils of the Kivach Reserve (which have been detected during this research). The spherical shape provides protection against the water evaporation in the soil. It is believed that this particular feature of some part of the individuals is the adaptation to low moisture content in habitat (Mazei, Tsyganov, 2006).

It is important to note, the number of protozoa species and its variety in the Pine Forest of the Botanical Garden is much less than in the biotope of the Kivach Reserve. This may be due to location of the first natural complex on the slope in a mountainous area where the fertile soil layer is less compared to the second biogeocenosis. It is possible that the anthropogenic load also affects the composition of testate amoebae and prevails in the Oxalis Pine Forest of the Botanical Garden.

Seasonal dynamics

Important changes were identified in many species of Rhizopoda over the research period in samples taken from the territory of the Botanical Garden forest changes. There is a tendency for most species to decrease in numbers

during the autumn.

The largest number of *Cyclopyxis eurystoma* were detected in July, *Nebela militaris* also prevailed in summer. Most species of *Euglypha* were identified evenly during the observation period.

There was a certain decrease in the numbers of *Nebela tincta* from June to October (Fig. 3). It was noted that these species reach its maximum values in the summer. The decrease in soil temperature may cause the death of testate amoebae (Heal, 1964). The species of the genus *Nebela* identified by us are spotted in the soils of pine forests in Western and Eastern Siberia (Marfina, 2011; Kulyukina et al., 2016).

Cyclopyxis eurystoma and *Trinema lineare* are dominant in the community; their high number is noted in pine forests and swamps of the Southern Taiga (Bulatova, 2010; Tsyganov et al., 2020). During the entire growing season, these species remain prevalent in the litter of the Yukseevskoe forestry (The northern border of the Krasnoyarsk forest-steppe) (Smolyaninova, Grenaderova, 2018).

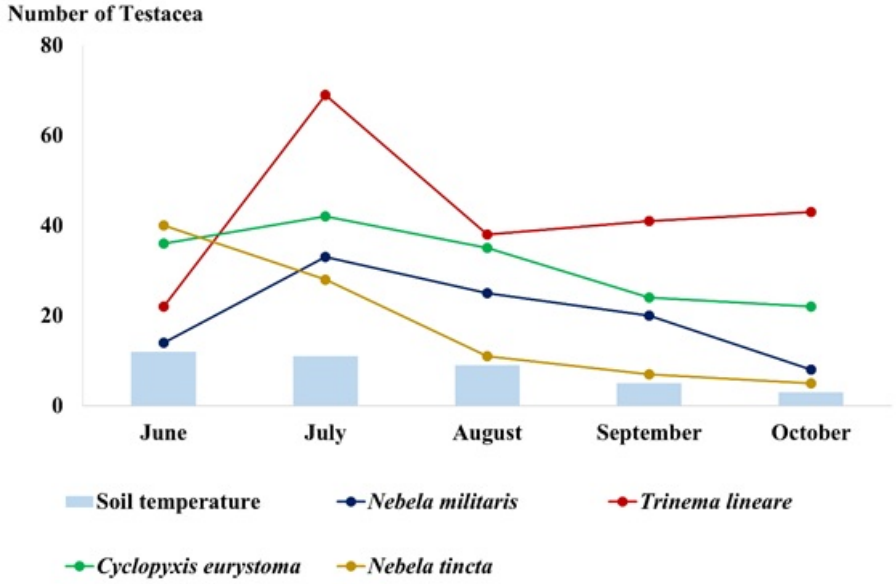

Fig. 3. Seasonal population dynamics of the soil testate amoebae species (The Botanical Garden Oxalis Pine Forest)

Xerophilous Testacea of the genus *Centropyxis*, *Schoenbornia humicola* and *Assulina muscorum* prevail in the summer driest conditions in the Cowberry Pine Forest of the Kivach Reserve.

On the other hand, the multi-peak of *Arcella arenaria* (Fig. 4) and *Trigonopyxis arcula* was in September. It occurs when a large amount of precipitation falls on the territory of Karelia, and therefore this confirms the hydrophilicity of these species.

In research, carried out in the Pine Forests of the Middle Volga region, the species of *Nebela militaris* quantitatively prevail in summer, and *Euglypha laevis* prevails in autumn (Trulova et al., 2013). Probably, the conditions in the studied biotope of the Kivach Reserve and Nikonovsky Pine Forest in the summer-autumn period are related and favorable for these species.

During the entire summer-autumn period, *Trinema complanatum*, *Corythion delamarei*, *Corythion dubium* and *Trigonopyxis minuta* have low dynamics.

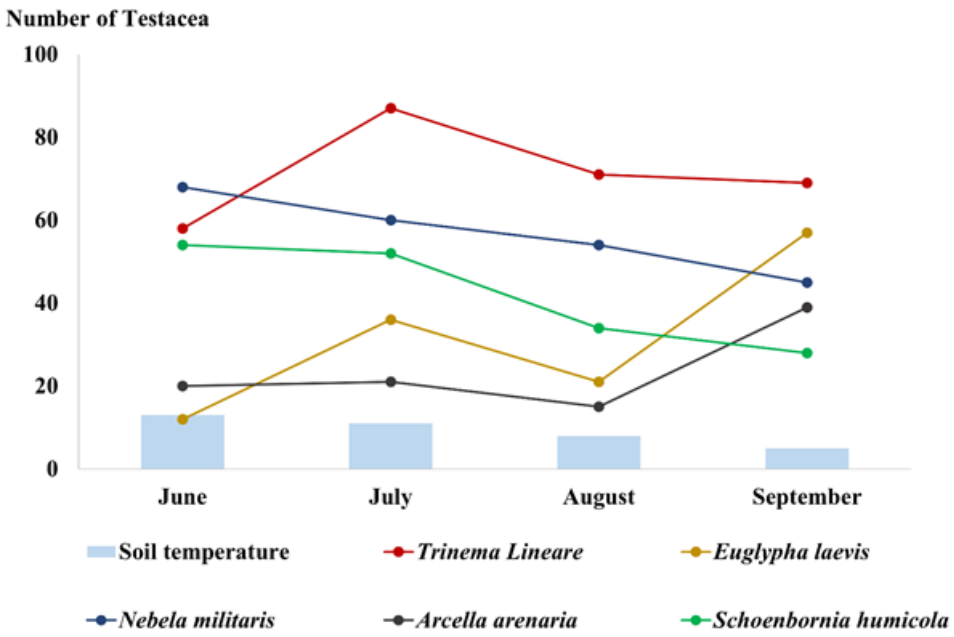

Fig. 4. Seasonal population dynamics of the soil testate amoebae species (Kivach Reserve Cowberry Pine Forest)

Microorganisms are sensitive indicators that react sharply to various changes in the environment. Consequently, in many of the experiments carried out, we may see highly dynamic of the microbiological indicators.

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Biological sciences

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Testate Amoebae (Rhizopoda, Testacea) in Karelia Pine Biogeocenoses

Scientific adviser:
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Abstract: The aim of this work is to study the Testacea variety in pine biogeocenoses of Karelia. The species variety of soil testate amoebae (Rhizopoda, Testacea) was studied in the pine biogeocenoses of the PetrSU Botanical Garden (Oxalis Pine Forest) and the Kivach Reserve (Cowberry Pine Forest). Research results: during the research period (2019—2020), 28 species of testate amoebae were identified. The largest amount of species that were detected in the soils of the studied biotopes are Trinema lineare, Trinema complanatum, Nebela militaris, Cyclopyxis eurystoma and species of the genus Euglypha. The largest number of found species in the Pine Forest of the Kivach Reserve. There was a suggestion about the influence of anthropogenic load and small fertile soil layer on quantitative composition of protozoa in Botanical Garden. The number of Rhizopoda species changes during the whole survey period in both biotopes.
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