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# The influence of the temperature of the culture medium on the growth and development of naked Amoeba

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Abstract

We isolated 12 species of naked amoebae: *Saccamoeba stagnicola*, *Saccamoeba* sp., *Vexillifera bacillipedes*, *Vannella lata*, *Cochliopodium actinophorum*, *Mayorella cantabrigiensis*, *Mayorella vespertilioides*, *Thecamoeba quadrilineata*, *Thecamoeba striata*, *Stenamoeba stenopodia*, *Acanthamoeba* sp., *Vahlkampfia avara* from fresh water bodies. For these species, the generation time, cell density and speed of movement at different temperatures of the culture medium (+5 ºC, +10 ºC, +15 ºC, +20 ºC) were estimated. As the temperature of the medium increases, the density of cells and the speed of movement of naked amoebae increases, while their generation time decreases. The optimal temperature for the growth and development of the studied species of naked amoebae is +15 ºC and +20 ºC. As the temperature of the medium increases to +25 ºC, the speed of movement and density of naked amoebae decreases, the generation time increases. At this temperature of the medium, *Mayorella cantabrigiensis, Vannella lata, Stenamoeba stenopodia, Mayorella vespertilioides* in cultures are in the form of floating forms, and amoebae of the species *Saccamoeba stagnicola* and *Acanthamoeba* sp. enter the cyst stage. Eight species of naked amoebae showed optimal cell density at medium temperatures from +5 ºC to +20 ºC. The speed of movement of all studied amoeba species ranged from 12±3.5 to 148±8.5 µm/min$^{-1}$. The generation time of naked amoebae was from 3.98±0.5 at +20 ºC to 44.80±10.2 hours at +5 ºC.

Keywords

naked amoebae, cell density, speed of movement, generation time, floating forms.

# Influence de la température du milieu de culture sur la croissance et le développement des amibes nues

Résumé

Nous avons isolé 12 espèces d'amibes nues : *Saccamoeba stagnicola*, *Saccamoeba* sp., *Vexillifera bacillipedes*, *Vannella lata*, *Cochliopodium actinophorum*, *Mayorella cantabrigiensis*, *Mayorella vespertilioides*, *Thecamoeba quadrilineata*, *Thecamoeba striata*, *Stenamoeba stenopodia*, *Acanthamoeba* sp., *Vahlkampfia avara* de plans d'eau douce. Pour ces espèces, le temps de génération, la densité cellulaire et la vitesse de déplacement à différentes températures du milieu de culture (+5 ºC, +10 ºC, +15 ºC, +20 ºC) ont été estimés. À mesure que la température du milieu augmente, la densité des cellules et la vitesse de déplacement des amibes nues augmentent, tandis que leur temps de génération diminue. La température optimale pour la croissance et le développement des espèces d'amibes nues étudiées est de +15 ºC et +20 ºC. Lorsque la température du milieu augmente jusqu'à +25 ºC, la vitesse de déplacement et la densité des amibes nues diminuent, le temps de génération augmente. A cette température du milieu, *Mayorella cantabrigiensis*, *Vannella lata*, *Stenamoeba stenopodia*, *Mayorella vespertilioides* en culture se présentent sous forme de formes flottantes, et les amibes des espèces *Saccamoeba stagnicola* et *Acanthamoeba* sp. entrent dans le stade de kyste. Huit espèces d'amibes ont montré une densité cellulaire optimale à des températures moyennes de +5 ºC à +20 ºC. La vitesse de déplacement de toutes les espèces d'amibes étudiées variait de 12±3,5 à 148±8,5 µm/min$^{-1}$. Le temps de génération des amibes nues était de 3,98 ± 0,5 à + 20 ºC à 44,80 ± 10,2 heures à + 5 ºC.

Mots-clés

amibes nues, densité cellulaire, vitesse de déplacement, temps de génération, formes flottantes.

## Introduction

Naked amoebae are a common group of protists in natural biotopes, they are characterized by a quick reaction to changes in environmental conditions. Most representatives have large cells. Due to relatively simple cultivation in laboratory conditions, these protists are convenient objects for microbiological, cytological, biochemical, genetic, hydrobiological and pedological studies. The composition of species of naked amoebae and their abundance depend on adaptation to biotic and abiotic environments, which determine the distribution of these protists in the ecosystem. The distribution of species depends not only on the physical and chemical properties of the environment, but also on the physiological state of the organism itself, on its ability to survive in adverse conditions. It is known that naked amoebae form resting stages (cysts) to survive adverse environmental conditions. Species that do not form cysts, are small in size and cannot survive for a long time without food. Naked amoebae support the sustainability of ecosystems. By forming cysts, they store a supply of carbon and mineral nutrients. Considering their physiology, this reserve is necessary for the stability and productivity of plants and animals. There are species of naked amoebae that live in water bodies with a high content of organic substances, which contain high concentrations of bacteria, solid particles and often act as facultative aerobes (GEISEN *et al.,* 2017, 2018, 2019, 2020).

In order to assess the adequate role of naked amoebae in ecosystems, it is necessary to study their physiology, biology and ecology. We evaluated the growth and reproduction characteristics of these protists at different temperatures of the culture medium in laboratory conditions.

There are a few laboratory studies devoted to the study of the influence of environmental temperature on the development of naked amoebae. Such studies were carried out on *Chaos carolinense* Wilson, 1900 at a culture medium temperature of +25 °C (PACE & FROST, 1952); on *Acanthamoeba* sp. in the temperature range from +5 ºC to +25 ºC (HEAL, 1967); on *Amoeba proteus* Leidy, 1878 at temperatures of +20 ºC and +15 ºC, while *Tetrahymena pyriformis* was added to the medium as food (ROGERSON, 1980). The growth rates of such species of naked amoebae as *Polychaos fsciculatum* Penard, 1902, *Acanthamoeba polyphaga* Puschkarew, 1913, *Cochliopodium minus* Page, 1976, *Glaeseria mira* Glaser, 1912, *Saccamoeba limax* Dujardin, 1841, *Vannella* sp., *Vexillifera bacillipedes* Page, 1969 were described at different temperatures (BAKER, 1974; BALDOK & BAKER, 1980).

As for other protists, there are studies that describe the relationship between growth rate, cell size, food availability, and the prevalence of many types of ciliates (FENCHEL, 1968, 1974; FINLAY, 1970; LAYBOURN & STEWART, 1975; TAYLOR & BERGER, 1976a, 1976b; TAYLOR, 1978a, 1978b).

## Material and Methods

The samples were collected in fresh water bodies of the Zhytomyr region of Ukraine during 2019-2021. In total, more than 500 samples of water and bottom slime sediments were analysed during the research period. Samples were collected manually in glass vessels of up to 250 ml and delivered to the laboratory. The amoebae were isolated from samples that included the top layer of bottom soil and a small amount of bottom water. Then the protists were propagated in Petri dishes with a diameter of 100 mm on non-nutrient agar according to the method of Page (PAGE & SIEMENSMA, 1991) with the addition of rice grains as a food source. Cultures were maintained in laboratory conditions at different temperatures (+5 ◦C, +10 ◦C, +15 ◦C, +20 ◦C). All cultures were left for 48 hours to adapt protists to new environmental conditions. After two days, the examined samples were counted using a light microscope MBR-3, magnification ×40. Such calculations were performed for 7 days in order to obtain a sufficient amount of data to determine the growth rates and generation time of naked amoebae (COWIE, 2005; COWIE & HANNAH, 2006). Ten visual fields were counted every 24 hours. The density of naked amoeba cells was determined by the rate of increase in the number of individuals in each of 20 cultures, which were established for each species at temperatures of 5 ºC, +10 ºC, +15 ºC, +20 ºC. Subsequently, regressions were calculated based on all data for each species at each temperature.

The speed of movement was determined using a video camera attached to the microscope. The movement of amoebae was recorded by observing 15 amoebae that moved for 30 minutes (COWIE, 2005; COWIE & HANNAH, 2006).

The growth rate constant was calculated using the formula:

$$K= (log_{10}N_t – log_{10}N_0)/0.301×t,$$

where $N_t$ is the final number of cells, $N_0$ is initial number of cells, and $t$ is time in hours.

The generation time in hours was calculated according to the formula: $1/k$. All data were tested using one-way analysis of variance followed by Bonferroni pairwise comparison. Statistical data processing was carried out using Minitab version 19.1.1.0.

Photomicrographs were made using an Axio Imager MI light microscope (Center for Collective Use of Scientific Equipment “Animalia” of I.I. Schmalhausen Institute of Zoology) using differential interference contrast, depositing live cells in a drop of water on glass slides.

Genomic DNA was isolated using the guanidine isothiocyanate method (MANIATIS *et al*., 1982). The 18S rRNA gene was amplified using universal eukaryotic primers RibA 5'-ACCTGGTTGATCCTGCCAGT-3' and RibB 5'-TGATCCTTCTGCAGGTTCACCTAC-3' (MEDLIN *et al*., 1988). The same sequencing primers were used for each species. Comparison of the obtained DNA sequences with GenBank data was carried out using the BLAST program (NCBI)

(https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic analysis was performed using the MEGA 10.0 program (KUMAR *et al.*, 2018). In the analysis, I used the sequences obtained in the present study and the sequences for other species of naked amoebae that are available in the GenBank database.

## Results

The identification of amoebae was carried out in 2 stages. First, their morphotype was determined, then Page's taxonomic identifier was used (PAGE & SIEMENSMA, 1991). The identification of 7 species of naked amoebae is confirmed by molecular data: *Vannella lata* Page, 1988 (OL305063); *Cochliopodium actinophorum* Auerbach, 1856 (MZ079367); *Mayorella vespertilioides* Page, 1983 (OP739500); *Thecamoeba striata* Schaeffer, 1926 (OQ134482); *Stenamoeba stenopodia* Page, 1969 (OP375108); *Acanthamoeba* sp. (MZ079366); *Vahlkampfia avara* Page, 1967 (OP179657). In our study, we evaluated the cell density, movement speed, and generation time of 12 species of naked amoebae.

### Morphological characteristics and phylogenetic relationships of the identified species of naked amoebae

*Saccamoeba stagnicola* Page, 1974 (**Figure 1, *a***)

Amoeba of monotactic morphotype. The hyaline cap at the front end of the amoeba cell disappears during movement. Uroid is tuberous in shape. There are crystals in the cell cytoplasm. The length of the amoeba is from 40 to 70 µm, width 15-17 µm; the L/B ratio is 4.0. The diameter of the nucleus is 4.8-7.5 µm.

*Saccamoeba* sp. (**Figure 1, *b***)

Amoeba of monotactic morphotype. There are crystals in the cell cytoplasm. Uroid is tuberous in shape. Cell length 28-32 µm, width 9.5-10 µm; the L/B ratio is 1.3. The diameter of the nucleus is 1.6 µm.

*Vexillifera bacillipedes* Page, 1969 (**Figure 1, *c***)

Amoeba of the dactylopodial morphotype. The cell is triangular, with a wide front end. Uroid is absent. Subpseudopodia (from 2 to 6) are elongated, emerge from the front part of the cell. Cell length 10-18 µm (not including pseudopodia), width 7.5 µm; the L/B ratio is 1.0-2.2. The diameter of the nucleus is 1.6-3.8 µm.

*Vannella lata* Page, 1988 (**Figure 1, *d***)

Amoeba of the fan-shaped morphotype. Wide hyaloplasm occupies the front half of the cell. The rear end of the cell is rounded, uroid structures are absent. Cell width 35-42 µm; the L/B ratio is 0.8-1.0. Floating form with 5-10 long pseudopodia. The diameter of the nucleus is 2.1-2.2 µm. The sequence of the studied DNA sample is deposited in GenBank under the number OL305063.

*Cochliopodium actinophorum* Auerbach, 1856 (**Figure 1, *e***)

Amoeba of the lens-like morphotype, the tectum is present on the surface of the cell. Wide hyaloplasm sometimes forms thin, barely noticeable subpseudopodia. Cell length is from 48 to 68 µm, width 20-40 µm; the L/B ratio is 1.2-1.5. The diameter of the nucleus is 7.0-12.8 µm. The sequence of the studied DNA sample is deposited in GenBank under the number MZ079367.

*Mayorella cantabrigiensis* Page, 1983 (**Figure 1, *f***)

The species belongs to the mayorellian morphotype. Conical pseudopodia and subpseudopodia present. The floating form is rounded, with short subpseudopodia. The length of the amoeba is from 70 to 110 µm, the width is 50-52 µm, the L/B ratio is 4.5-6.8. The diameter of the nucleus is 9.0-10.2 µm.

*Mayorella vespertilioides* Page, 1983 (**Figure 1, *g***)

An amoeba of the mayorellian morphotype. Subpseudopodia are often absent. Floating form with radial subpseudopodia. There are crystalline inclusions in the cell cytoplasm. The length of the cell is from 50 to 75 µm, width 40-47 µm; the L/B ratio is 1.8-3.5. The diameter of the nucleus is 6.2-8.0 µm. The sequence of the studied DNA sample is in GenBank under the number OP739500.

*Thecamoeba quadrilineata* Carter, 1856 (**Figure 1, *h***)

Amoeba belongs to the striate morphotype. The rear end of the cell is convex, the uroid is absent. On the dorsal surface, the hyaloplasm of the cell forms longitudinal folds. Cell length from 50 to 75 µm, width 23-35 µm; the L/B ratio is 1.6. The diameter of the nucleus is 9.2 µm in average.

*Thecamoeba striata* Penard, 1890 (**Figure 1, i**)

Amoeba belongs to the striate morphotype. The rear end of the cell is convex. Uroid structures are absent. Hyaline folds are present on the cell surface. Cell length from 50 to 65 µm, width 25-28 µm; the L/B ratio is 1.6. The diameter of the nucleus is 6.0-7.8 µm. The sequence of the studied DNA sample is in GenBank under the number OQ134482.

*Stenamoeba stenopodia* Page, 1969 (**Figure 1, *j***)

Amoeba belongs to the lingulate morphotype. The front part of the cell is occupied by hyaloplasm. The rear end of the cell is rounded, there are no uroid structures. The length of an amoeba is from 18 to 26 µm; width – 8-10 µm; the L/B ratio is 2.4. The diameter of the nucleus is 2.0 µm. The sequence of the studied DNA sample is in GenBank under the number OP375108.

*Acanthamoeba* sp. (**Figure 1, *k-l***)

Amoeba of the acanthopodial morphotype. Cyst with a diameter of 8 to 9 µm, two-layered. The round ectocyst is incompletely attached to the endocyst, which is star-shaped and has 5 to 6 arms. Triangular trophozoite with a wide anterior end. Hyaloplasm forms short conical subpseudopodia. Uroid structures

are absent. The diameter of the nucleus is from 1.5 to 1.6 µm. The sequence of the studied DNA sample is in GenBank under the number MZ079366.

*Vahlkampfia avara* Page, 1967 (**Figure 1, *m***)

Amoeba belongs to the eruptive morphotype. When moving slowly, the amoeba has a monotactic shape.

During rapid locomotion, short pseudopodia are formed, due to eruptive movements of the cytoplasm. Cell length from 55 to 60 µm; width 17-20 µm; the L/B ratio is 3.5. The diameter of the nucleus is 5.8 µm. The sequence of the studied DNA sample is in GenBank under the number OP179657.

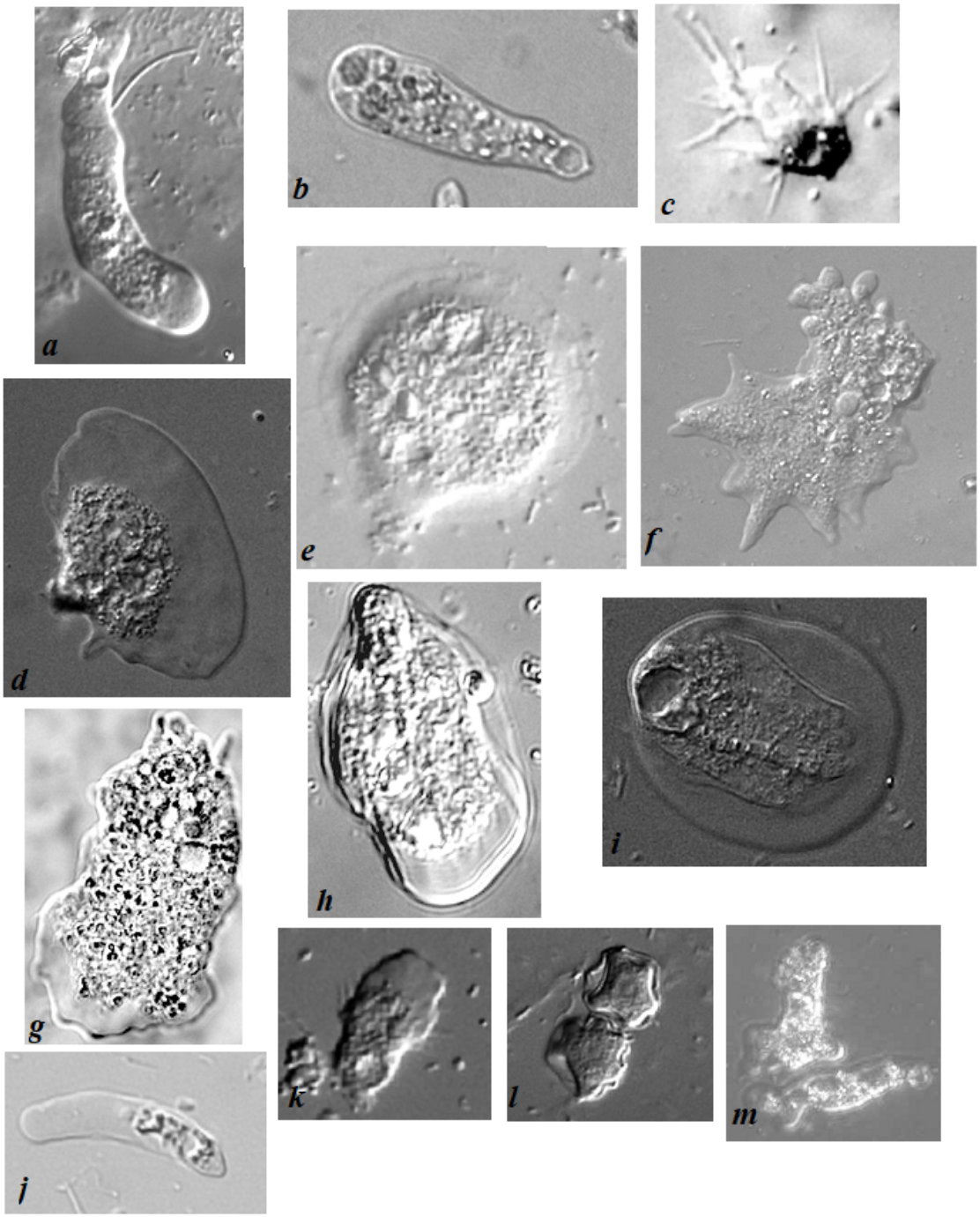


**Figure 1**. Fresh water species of naked amoebae (author's photos): *a – S. stagnicola*, *b – Saccamoeba* sp., *c – V. bacillipedes*, *d – V. lata*, *e – C. actinophorum*, *f – M. cantabrigiensis*, *g – M. vespertilioides*, *h – T. quadrilineata*, *i – T. striata*, *j – S. stenopodia*, *k – Acanthamoeba* sp., *l* – cysts of *Acanthamoeba* sp., *m – V. avara*.

*Figure 1. Espèces d'eau douce d'amibes nues (photos personnelles) :* a – S. stagnicola, b – Saccamoeba sp., c – V. bacillipedes, d – V. lata, e – C. actinophorum, f – M. cantabrigiensis, g – M. vespertilioides, h – T. quadrilineata, i – T. striata, j – S. stenopodia, k – Acanthamoeba sp., l – kystes d'Acanthamoeba sp., m – V. avara.

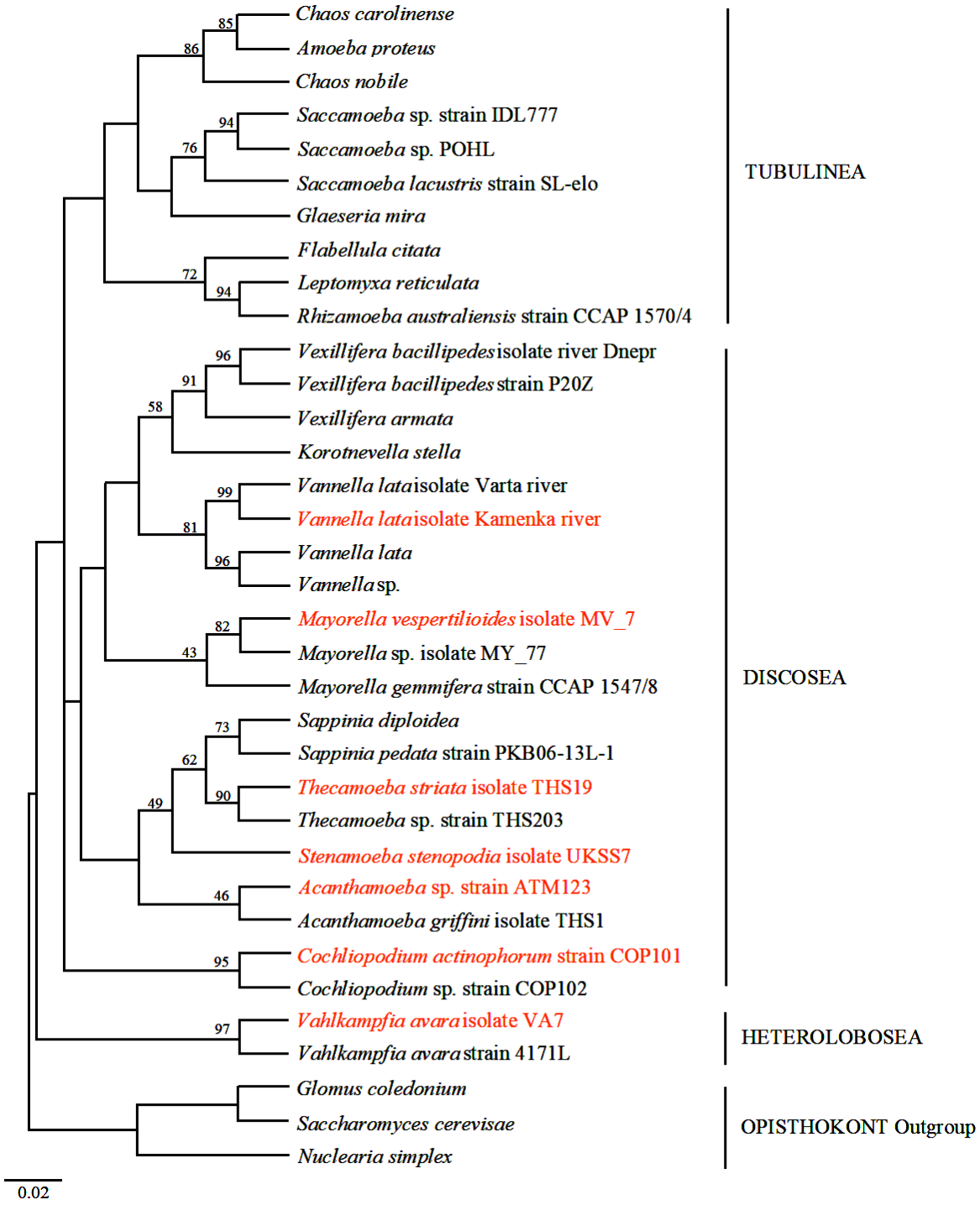


**Figure 2.** Fragment of a phylogenetic tree of naked amoebae. The original sequences are highlighted in red. Opisthokont was selected as outgroup.

*Figure 2. Fragment d'un arbre phylogénétique d'amibes nues. Les séquences originales sont surlignées en rouge. Opisthokont a été sélectionné comme groupe externe.*

We have constructed a phylogenetic tree of naked amoebae based on the 18S rRNA gene. The largest number of sequences of representatives of the Discosea group were selected for phylogenetic analysis and several representatives of the groups Tubulinea and Heterolobosea. The overall tree topology and placement of groups within Tubulinea and Discosea are not different from those presented in other studies of naked amoebae phylogeny (CAVALIER-SMITH *et al.*, 2016; LAHR *et al.*, 2013). The Tubulinea group includes species of naked amoebae from the families Amoebidae (*Amoeba*+*Chaos*), Hartmannellidae (*Saccamoeba* + *Glaeseria*) and Leptomyxidae (*Leptomyxa*+*Rhizamoeba*). Representatives of these families belong to the polytactic, monopodial and branched morphotypes.

The Discosea group has the largest number of naked amoebae. *Vannella lata* (*Vannella lata* isolate Kamenka river) according to the results of morphogenetic analysis was included in the group of *Vannella*-like amoebae, which belong to the family Vannellidae. The Mayorellidae family includes naked amoebae of the genus *Mayorella*, including *Mayorella*

*vespertilioides*, which we identified during our research. Naked amoebae of the above groups belong to the fan-shaped and mayorellian morphotypes. *Stenamoeba stenopodia* (lingulate morphotype) is sister to the *Thecamoeba*+*Sappinia* group (striate morphotype). Sister to this group of naked amoebae is the group of acanthopodial amoebae (*Acanthamoeba* sp.+*Acanthamoeba griffini*). *Cochliopodum actinphorum* strain COP101 is grouped with *Cochliopodium* sp. strain COP102, which belong to the lens-like morphotype and are included in the Discosea molecular cluster.

Representatives of the genus *Vahlkampfia* form a separate group of heterolobose amoebae.

## Growth and development of naked amoebae at different temperatures of the culture medium

As a result of the study, the dependence of cell density, movement speed and generation time of naked amoebae on the temperature of the environment was demonstrated. As the temperature of the medium increases, the density and speed of movement of naked amoebae increases, while the generation time of these protists decreases.

Let us consider how the density of naked amoebae changed with an increase in the temperature of the medium. *Saccamoeba* sp., *V. lata, M. cantabrigiensis, M. vespertilioides, C. actinophorum, T. striata, Acanthamoeba* sp., *V. avara* showed growth rates at temperatures from +5 ºC to +20 ºC (Figures 4, 6-9, 11, 13, 14; Table 1). At environmental temperatures of +5 ºC and +10 ºC, the number of cells of these amoeba species in cultures was almost at the same level, with an increase in the temperature of the environment to +15 °C and +20 °C, cell density increased.

Density of naked amoeba species *S. stagnicola*, *T. quadrilineata*, *V. bacillipedes*, *S. stenopodia* was low at the culture medium temperature of +5 ºC. With an increase in the temperature of the environment to +10 ºC, their number in the cultures increased with the increase in the temperature of the medium (Figures 3, 5, 10, 12; Table 1).

**Table 1.** Density of naked amoebae at different temperatures of the medium
*Tableau 1. Densité des amibes nues à différentes températures du milieu*

| № | Naked amoebae | Temperature, °C | Hours, h | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 98 h |
| 1. | *Saccamoeba stagnicola* | 5 °C | 66±9,4 | 68±8,8 | 83±10,2 | 85±9,9 |
| | | 10 °C | 135±28 | 158±32 | 193±28 | 201±44 |
| | | 15 °C | 284±37 | 314±50 | 338±54 | 352±47 |
| | | 20 °C | 320±56 | 336±63 | 368±58 | 385±43 |
| 2. | *Saccamoeba* sp. | 5 °C | 354±38 | 372±41 | 485±28 | 530±40 |
| | | 10 °C | 372±34 | 388±28 | 491±37 | 584±38 |
| | | 15 °C | 495±42 | 593±40 | 614±28 | 780±42 |
| | | 20 °C | 568±74 | 684±73 | 735±69 | 820±84 |
| 3. | *Vexillifera bacillipedes* | 5 °C | 48±3,4 | 63±4,2 | 80±7,7 | 82±7,6 |
| | | 10 °C | 268±29 | 274±37 | 316±36 | 342±38 |
| | | 15 °C | 428±40 | 430±46 | 475±43 | 490±40 |
| | | 20 °C | 485±40 | 502±42 | 534±42 | 560±48 |
| 4. | *Cochliopodium actinophorum* | 5 °C | 54±10 | 68±12 | 89±26 | 107±27 |
| | | 10 °C | 63±30 | 84±32 | 92±37 | 121±40 |
| | | 15 °C | 106±42 | 134±40 | 138±37 | 152±35 |
| | | 20 °C | 112±28 | 136±26 | 148±30 | 163±30 |
| 5. | *Thecamoeba striata* | 5 °C | 113±25 | 128±28 | 149±32 | 184±48 |
| | | 10 °C | 125±28 | 184±30 | 212±37 | 253±36 |
| | | 15 °C | 185±31 | 223±36 | 284±40 | 300±39 |
| | | 20 °C | 220±26 | 235±32 | 312±40 | 348±42 |
| 6. | *Thecamoeba quadrilineata* | 5 °C | 54±9,2 | 62±9,5 | 74±8,8 | 85±8,3 |
| | | 10 °C | 203±38 | 220±42 | 245±40 | 248±41 |
| | | 15 °C | 331±51 | 343±54 | 374±48 | 402±53 |
| | | 20 °C | 404±48 | 428±62 | 485±68 | 520±64 |
| 7. | *Stenamoeba stenopodia* | 5 °C | 51±5,2 | 53±5,1 | 72±6,8 | 80±6,5 |
| | | 10 °C | 167±25 | 173±28 | 184±30 | 195±26 |
| | | 15 °C | 203±31 | 218±32 | 235±38 | 250±35 |
| | | 20 °C | 254±38 | 268±40 | 293±42 | 304±52 |
| 8. | *Vannella lata* | 5 °C | 168±24 | 184±33 | 198±32 | 220±41 |
| | | 10 °C | 174±45 | 198±48 | 220±52 | 245±55 |
| | | 15 °C | 243±60 | 264±62 | 269±84 | 280±80 |
| | | 20 °C | 266±52 | 278±84 | 300±80 | 312±82 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9. | *Mayorella cantabrigiensis* | 5 °C | 142±23 | 160±28 | 168±30 | 174±32 |
| | | 10 °C | 152±32 | 168±34 | 163±28 | 188±27 |
| | | 15 °C | 212±34 | 234±40 | 248±37 | 252±50 |
| | | 20 °C | 254±47 | 268±52 | 284±50 | 288±54 |
| 10. | *Mayorella vespertilioides* | 5 °C | 103±43 | 124±47 | 127±30 | 145±28 |
| | | 10 °C | 153±50 | 168±53 | 185±41 | 204±60 |
| | | 15 °C | 223±57 | 243±52 | 267±48 | 280±50 |
| | | 20 °C | 295±64 | 312±82 | 337±73 | 384±85 |
| 11. | *Acanthamoeba* sp. | 5 °C | 248±35 | 284±30 | 363±36 | 420±41 |
| | | 10 °C | 315±43 | 381±29 | 495±40 | 603±48 |
| | | 15 °C | 682±50 | 735±56 | 820±67 | 951±68 |
| | | 20 °C | 730±70 | 798±71 | 910±69 | 1023±78 |
| 12. | *Vahlkampfia avara* | 5 °C | 266±51 | 285±58 | 320±62 | 398±40 |
| | | 10 °C | 317±66 | 364±59 | 416±68 | 495±68 |
| | | 15 °C | 580±82 | 677±80 | 785±87 | 981±95 |
| | | 20 °C | 734±73 | 825±94 | 981±112 | 1002±113 |

The generation time of naked amoeba cells differed at different temperatures of the medium ($p<0.01$). Mean generation (doubling) time for *Saccamoeba* sp. varied from 113 at +15 ºC to 140 h at +20 ºC; for *T. quadrilineata* – from 113 at +5 ºC to 264 h at +15 ºC; for *S. stenopodia* – from 114 at +5 ºC to 331 h at +10 ºC; for *V. lata* – from 150 at +10 ºC to 362 h at +15 ºC; for *M. cantabrigiensis* – from 241 at +10 ºC to 408 h at +20 ºC; for *M. vespertilioides* – from 150 at +5 ºC to 225 h at +15 ºC; for *S. stagnicola* – from 129 at +10 ºC to 277 h at +20 ºC; for *V. bacillipedes* – from 96 at +5 ºC to 379 h at +15 ºC; for *C. actinophorum* – from 75 at +5 ºC to 142 h at +15 ºC; for *T. striata* – from 73 at +10 ºC to 112 h at +20 ºC; for *Acanthamoeba* sp. – from 79 at +10 ºC to 154 h at +15 ºC; for *V. avara* – from 98 at +15 ºC to 165 h at +20 ºC.

As the temperature of the medium increased, the speed of movement of naked amoebae changed. In *S. stagnicola*, it ranged from 12±3.5 to 72±8.9 μm/min.$^{-1}$; in *Saccamoeba* sp. – from 58±3.8 to 148±8.5 μm/min.$^{-1}$; in *V. bacillipedes* – from 20±1.5 to 81±3.9 μm/min.$^{-1}$; in *C. actinophorum* – from 22±2.7 to 82±4.0 μm/min.$^{-1}$; in *T. striata* – from 37±4.2 to 89±6.8 μm/min.$^{-1}$; in *T. quadrilineata* – from 24±3.4 to 92±5.6 μm/min.$^{-1}$; in *S. stenopodia* – from 20±3.6 to 113±6.3 μm/min.$^{-1}$; in *V. lata* – from 45±6.2 to 123±10.2 μm/min.$^{-1}$; in *M. cantabrigiensis* – from 39±2.8 to 119±9.3 μm/min.$^{-1}$; in *M. vespertilioides* – from 40±4.1 to 125±8.8 μm/min.$^{-1}$; in *Acanthamoeba* sp. – from 34±2.4 to 89±8.4 μm/min.$^{-1}$; in *V. avara* – from 42±6.0 to 104±6.8 μm/min$^{-1}$ (**Table 2**).

**Table 2.** The speed of movement of naked amoebae at different temperatures of the culture medium
*Tableau 2. Vitesse de déplacement des amibes nues à différentes températures du milieu de culture*

| Amoeba species | 5 ◦C | 10 ◦C | 15 ◦C | 20 ◦C |
|---|---|---|---|---|
| *Saccamoeba stagnicola* | 12±3.5 | 26±2.8 | 68±6.4 | 72±8.9 |
| *Saccamoeba* sp. | 58±3.8 | 69.5±3.2 | 134±7.7 | 148±8.5 |
| *Vexillifera bacillipedes* | 20±1.5 | 33±2.8 | 66±2.6 | 81±3.9 |
| *Cochliopodium actinophorum* | 22±2.7 | 28±2.6 | 67±4.2 | 82±4.0 |
| *Thecamoeba striata* | 37±4.2 | 37±3.8 | 80±6.3 | 89±6.8 |
| *Thecamoeba quadrilineata* | 24±3.4 | 40±3.5 | 85±6.2 | 92±5.6 |
| *Stenamoeba stenopodia* | 20±3.6 | 46±3.1 | 98±6.3 | 113±6.3 |
| *Vannella lata* | 45±6.2 | 54±4.8 | 115±4.8 | 123±10.2 |
| *Mayorella cantabrigiensis* | 39±2.8 | 41.5±4.9 | 98±5.1 | 119±9.3 |
| *Mayorella vespertilioides* | 40±4.1 | 44±3.7 | 120±7.8 | 125±8.8 |
| *Acanthamoeba* sp. | 34±2.4 | 48±2.8 | 81±5.6 | 89±8.4 |
| *Vahlkampfia avara* | 42±6.0 | 48±4.7 | 91±7.2 | 104±6.8 |

We did not observe any morphological changes in the cells of all studied species of naked amoebae at different temperatures of culture medium. At a temperature of +25 ºC, the density of naked amoeba cells and the speed of movement of these protists decrease, however, the generation time of naked amoebae increases. Naked amoebae of the species *M. cantabrigiensis, V. lata, S. stenopodia, M. vespetilioides*, were in the form of floating forms in the cultures (mostly spherical, without cytoplasmic outgrowths), while *S. stagnicola* and *Acanthamoeba* sp. amoebae entered the cyst stage.

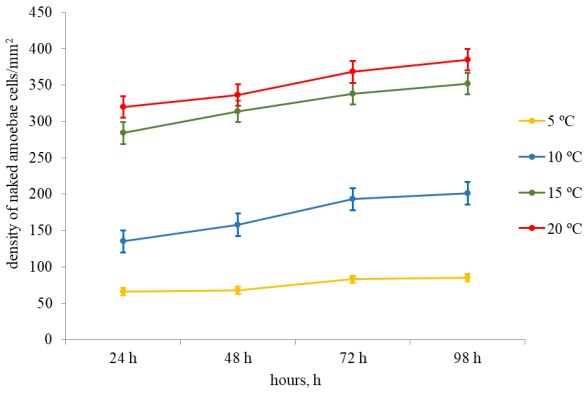


**Figure 3.** Density of *S. stagnicola* at different temperatures of the medium.
*Figure 3. Densité de* S. stagnicola *à différentes températures du milieu.*

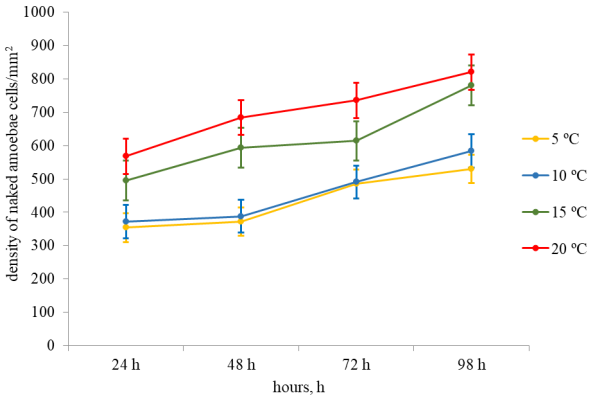


**Figure 4.** Density of *Saccamoeba* sp. at different temperatures of the medium.
*Figure 4. Densité de* Saccamoeba *sp. à différentes températures du milieu.*

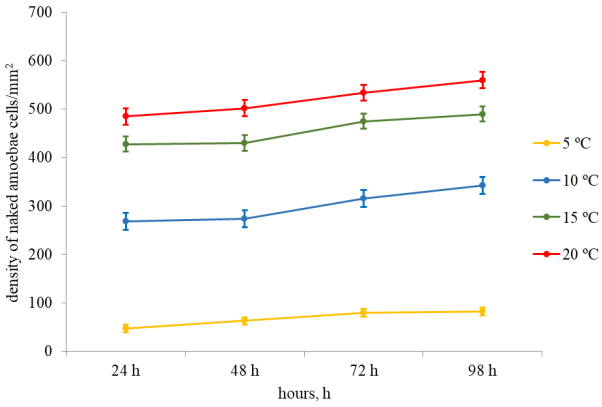


**Figure 5.** Density of *V. bacillipedes* at different temperatures of the medium.
*Figure 5. Densité de* V. bacillipedes *à différentes températures du milieu.*

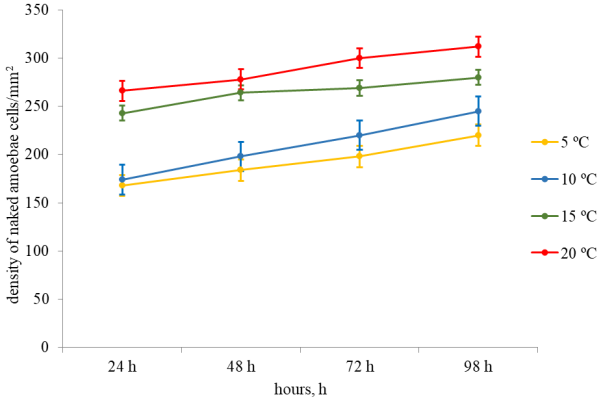


**Figure 6.** Density of *V. lata* at different temperatures of the medium.
*Figure 6. Densité de* V. lata *à différentes températures du milieu.*

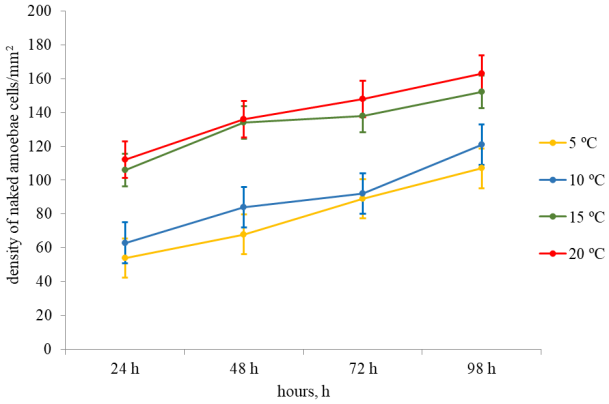


**Figure 7.** Density of *C. actinophorum* at different temperatures of the medium.
*Figure 7. Densité de* C. actinophorum *à différentes températures du milieu.*

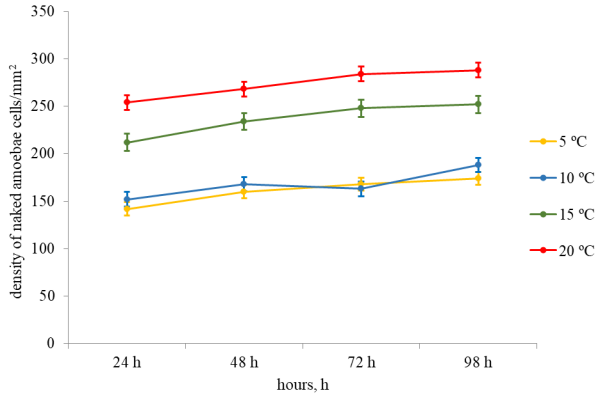


**Figure 8.** Density of *M. cantabrigiensis* at different temperatures of the medium.
*Figure 8. Densité de* M. cantabrigiensis *à différentes températures du milieu.*

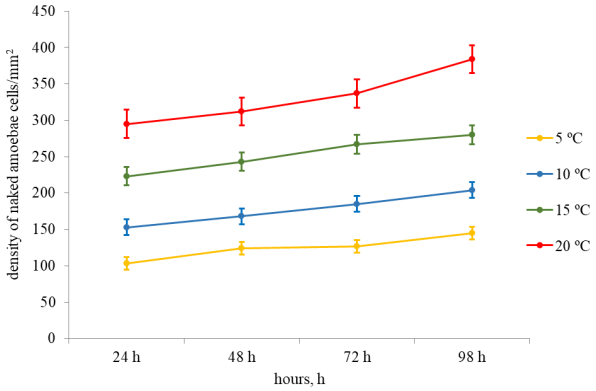


**Figure 9.** Density of *M. vespertilioides* at different temperatures of the medium.
*Figure 9. Densité de* M. vespertilioides *à différentes températures du milieu.*

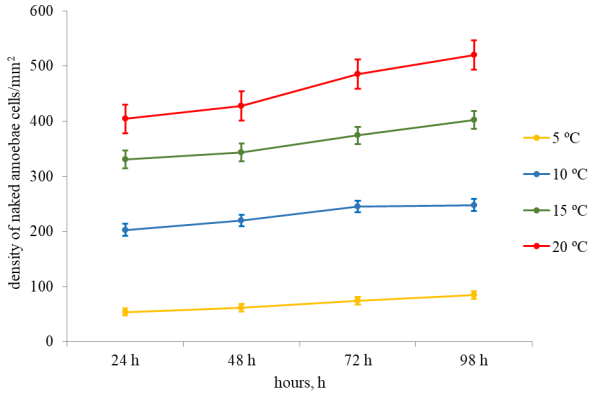


**Figure 10.** Density of *T. quadrilineata* at different temperatures of the medium.
*Figure 10. Densité de* T. quadrilineata *à différentes températures du milieu.*

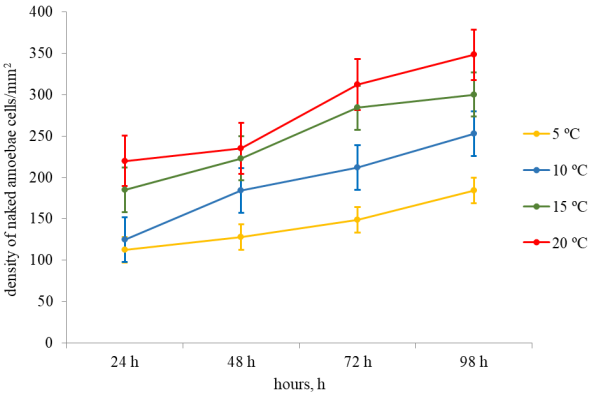


**Figure 11.** Density of *T. striata* at different temperatures of the medium.
*Figure 11. Densité de* T. striata *à différentes températures du milieu.*

We analyzed the final cell concentration after 98 hours compared to the initial one (after 24 hours), i.e., the production yield. The following features should be noted.

Such species of naked amoebae as *T. striata*, *C. actinophorum*, *Acanthamoeba* sp. are able to double their initial concentrations when cultivated at temperatures of +5 °C and +10 °C, however, with a doubling time of 72 hours.

In *S. stagnicola* no significant difference was found in the increase in cell concentration at temperatures of +5 °C, +10 °C and +20 °C; in *V.*

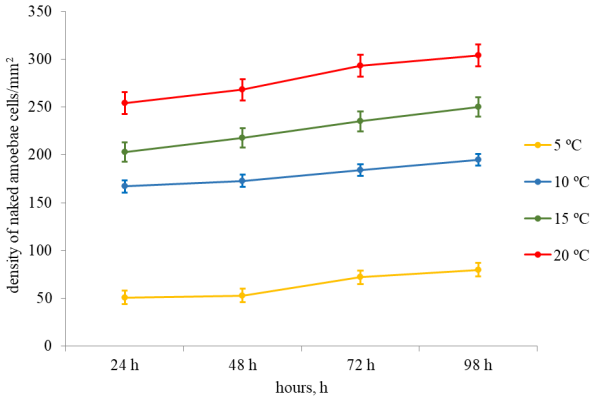


**Figure 12.** Density of *S. stenopodia* at different temperatures of the medium.
*Figure 12. Densité de* S. stenopodia *à différentes températures du milieu.*

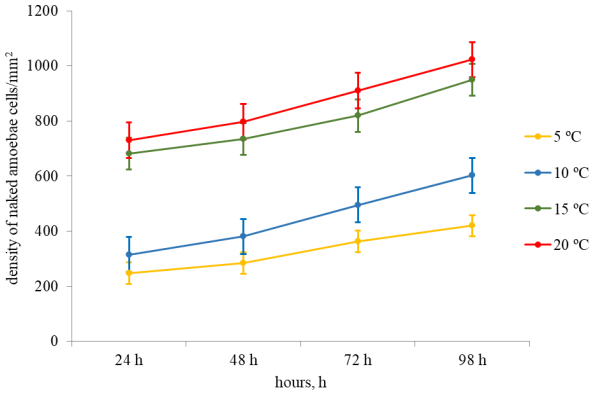


**Figure 13.** Density of *Acanthamoeba* sp. at different temperatures of the medium.
*Figure 13. Densité d'*Acanthamoeba *sp. à différentes températures du milieu.*

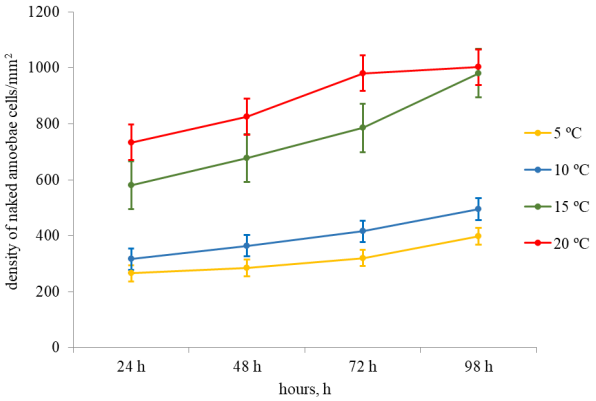


**Figure 14.** Density of *V. avara* at different temperatures of the medium.
*Figure 14. Densité de* V. avara *à différentes températures du milieu.*

*bacillipedes*, *T. quadrilineata*, *S. stenopodia* – +10 °C, +15 °C and +20 °C; in *C. actinophorum* – +15 °C and +20 °C; in *T. striata* – +5 °C, +15 °C and +20 °C; *V. lata* and *M. cantabrigiensis* – +5 °C, +10 °C, +15 °C and +20 °C; *Acanthamoeba* sp. – +15 °C and +20 °C, *V. avara* – +5 °C, +10 °C and +15 °C.

The generation (doubling) time varied from 73 hours for *T. striata* at an environmental temperature of +10 °C to 408 hours for *M. cantabrigiensis*. This feature may be associated with the species of naked amoebae, temperature and environmental characteristics (naked amoebae were propagated on non-nutritive agar-agar, which was prepared on PJ salt solution (PAGE, 1988), and rice grains were added to the culture as a food source). Generation time can be reduced by adding sufficient food to the culture medium, such as bacteria or *Tetrahymena pyriformis*, etc.

## Discussion

There is virtually no information in the literature regarding laboratory data on the variability of cell number and generation time at any temperature with which the data presented in our study could be compared.

For *Acanthamoeba* sp. the generation time has been determined from 7 to 300 hours at temperatures of culture medium ranging from +25 º to +5 ºC (HEAL, 1967). Amoebae were propagated in cultures with the addition of yeast as a food source. At a temperature of +30 °C for *Acanthamoeba* sp. the generation time has been observed from 6.5–3.8 h (BYERS *et al*., 1969), and at a temperature of +28 °C, the doubling time of *Acanthamoeba* sp. has been determined a number of days (HOLST-SORENSEN, 1971). These studies were carried out in pure cultures without added food. The dependence between the speed of movement and the temperature of the medium was demonstrated by a number of scientists for ciliates (FENCHEL, 1968; MITCHELL, 1929; PHELPS, 1946; THORMAR, 1962) and amoeba (BALDOCK & BAKER, 1980). It was established that *V. bacillipedes*, which was isolated from the Tandol source at a water temperature of +9.8 ºC, cannot grow in a medium at a temperature of +10 ºC (PINDER, 1974).

Our data show that the optimal temperature for the growth and reproduction of the studied naked amoeba species is a temperature of +15 ºC and +20 ºC. With an increase in the temperature of the medium to +25 ºC, the density and speed of movement of naked amoeba species significantly decreases, while the generation time increases, compared to a culture medium temperature of +20 ºC. *Saccamoeba* sp., *V. lata, M. cantabrigiensis, M. vespertilioides, C. actinophorum, T. striata, Acanthamoeba* sp., *V. avara* showed optimal growth rates at culture medium temperatures from +5 °C to +20 °C. A significant increase in the number of naked amoebae cells was observed when the culture medium temperature increased to +15 °C in comparison with the values observed at +5 °C and +10 °C. In natural conditions, these species of naked amoebae belong to the eurythermic ecological group and can withstand significant fluctuations in the temperature of the aquatic environment (PATSYUK, 2016, 2018). At a low temperature of the environment, the movement of naked amoebae and the density of cells decreases, which is related to the processes that take place in the cells: cytoplasmic fluidity and protein metabolism decreases, the water content in the cell decreases, etc. Some species of naked amoebae can enter the cyst stage.

Note that in natural conditions, living organisms can withstand different environmental temperatures, which are significantly different from those studied in laboratory conditions. Extrapolation of laboratory data to natural conditions should be done with caution, because in natural conditions there is interspecific competition and food is a limiting factor. It is necessary to take into account the age of the cultures themselves. The density of naked amoeba species, the speed of movement and the generation time of these protists should be evaluated on fresh cultures that are at the same stage of growth.

## Conclusions

From fresh water bodies, we isolated 12 species of naked amoebae which react differently to different temperatures of the culture medium. At temperatures of +15 °C and +20 °C in cultures, density and speed of movement of these protists increase, while their generation time decreases. At the culture medium temperature above +20 °C, the abundance of species in cultures decreases, some species are in the form of floating forms or enter the cyst stage. *T. striata*, *C. actinophorum*, *Acanthamoeba* sp. are able to double their initial concentrations when cultivated at temperatures of +5 °C and +10 °C.

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