Heterotrophic flagellates from the saline lakes of the Crimean Peninsula

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The species composition of heterotrophic flagellates has been studied in saline inland water bodies of the Crimean Peninsula. Twenty-nine species and forms belonging to 20 genera and 5 supergroups have been found. All identified species are new to the studied lakes. *Rhynchomonas nasuta*, *Neobodo designis*, *Petalomonas poosilla*, *Percolomonas cosmopolitus* and *Cafeteria* sp. are the most common species. Euryhaline species form about a half of the species list. The number of the flagellate species was the lowest in the samples obtained in hypersaline ponds characterized by the salinity exceeding 100‰. There are no clear patterns observed in the cluster formation at the dendrogram of taxonomic similarity of the studied lakes.

Keywords: protists, Protozoa, flagellates, species composition, hypersaline habitats, inland waters.

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Introduction

Saline continental water bodies are among the most extreme water habitats of the Crimean Peninsula due to high water temperature and low oxygen content in addition to high water salinity (Shadrin et al., 2017); since such water bodies are often characterized by the shallow depths (1–3 m), they warm up to 35–40 °C in the summer. It is also noted that the alternation of dry and rainy periods preconditions the significant seasonal and interannual salinity variations (Balushkina et al., 2009). Saline lakes of the Crimean Peninsula were repeatedly studied by protistologists (Dagaeva, 1927; Goryacheva and Zhukov, 1976; Kulagin, 1888; Meyer, 1916; Nevrova and Shadrin, 2005, 2008; Pavlovskaya et al., 2009; Senicheva and et al., 2008; Zagorodnaya et al., 2008). However, the data on the species composition of heterotrophic flagellates are absent; and the species list is absent as well in the only known study of the flagellates of hyperhaline lakes of Crimea, namely, Sasyk-Sivash, Saki, and Moynaki lakes (Goryacheva and Zhukov, 1976).

Materials and Methods

Samples from the saline lakes of the Crimean Peninsula were taken by the author and A.P. Mylnikov in July 2017 and July 2018. All studied reservoirs were shallow, well warmed up, the water salinity ranged from 10 up to 204‰ during the sampling period. The studied water bodies could be divided into continental and marine by the origin. Lakes of continental origin were the Lake Marfovskoe, located in the central part of the Kerch Peninsula, as well as nameless saline lakes and puddles in its vicinity. Such reservoirs have a chloride-sulfate-carbonate ionic composition (Nevrova and Shadrin, 2005). Lakes of marine origin (estuary type) included Lake Chokrak and Lake Sivash (originated from the Sea of Azov), lakes Kuchuk-Adjigol' and Sasyk-Sivash (originated from the Black Sea). Such lakes were fed mainly by filtering the seawater through the dam, as well as by atmospheric precipitation, surface runoff, and seawater overflow during a storm. Lake Sivash was an exception, its western part was directly connected to the Sea of Azov through the Henichesk

Strait; the salinity of the lake increased significantly eastwards. One to three samples were taken at each study site; the data on the species composition were then pooled for the analysis. The detailed information on the sampling sites is presented in Table 1.

Water samples, partly with the bottom sediments, were placed into 15 mL plastic tubes and delivered alive at 4 °C in the dark to the laboratory. Under laboratory conditions, the samples were transferred into the Petri dishes, enriched with a bacterial suspension of *Pseudomonas fluorescens* Migula, 1895 (0.15 mL of bacterial suspension per 5 mL of sample) and reared in the incubator for 10 days in the dark at a temperature of 22 °C. Live flagellates were checked throughout the exposure time to reveal hidden species diversity according to the generally accepted methodology (Vørs, 1992). The species identification has been performed under AxioScope A1 light microscope (Carl Zeiss, Germany) at phase contrast, DIC, and water immersion lenses (total magnification $\times 1120$).

The dendrogram of the species similarity in the studied water bodies has been plotted in the PAST program using the single linkage Bray-Curtis similarity index (Hammer et al., 2001). The up-to-date eukaryotic system was applied for the taxonomy (Adl et al., 2019).

Results and Discussion

In the studied saline lakes of the Republic of Crimea, 29 species of heterotrophic flagellates from 20 genera and 5 supergroups were identified, two species belong to eukaryotes of an uncertain systematic position (Table 2). *Rhynchomonas nasuta*, *Neobodo designis*, *Petalomonas poosilla*, *Percolomonas cosmopolites*, and *Cafeteria* sp. have been noted the most often (in 30% or more of the studied lakes). Seventeen species (58.6%) were rare, i.e. noted for a single sample only.

The number of the flagellate species per sample averaged 5.4, which was 2–3 times lower than in the samples obtained in the Black Sea (Prokina et al., 2017, 2018). Our data are consistent with the studies of other protist groups, when a significantly lower species richness of diatoms has been noted in saline lakes compared with coastal marine areas (Nevrova and Shadrin, 2005). A total of six species identified in saline lakes within the present study were not previously found in the marine area of the Crimean Peninsula; these species were *Salpingoeca rosetta*, *Halocafeteria seosinensis*, “Protist 4”, *Percolomonas cosmopolitus*, *Phyllomitus undulatus*, and *Pleurostomum flabellatum* (Prokina et al., 2017, 2018). Most of the flagellate species have been found

Table 1. Characteristics of the studied saline lakes of the Crimean Peninsula.

No.	Water body, sediment type	Date, dd.mm.yyyy	Coordinates	Salinity, ‰	T, °C
1	Kerch Peninsula, Marfovka village, Lake Marfovskoe, clay	09.07.2018	N 45°11'49.2" E 36°06'10.7"	135	24
2	Ibid., a stream from a nearby brackish lake, clay	09.07.2018	N 45°11'49.2" E 36°06'10.7"	84	22
3	Ibid., nameless lake near the Lake Marfovskoe, silt	02.07.2018	N 45°11'33.4" E 36°04'56.4"	204	23
4	Kerch Peninsula, Marjevka village, salt puddle, clay	09.07.2018	N 45°06'31.6" E 36°14'06.5"	41	27
5	Kerch Peninsula, Lake Chokrak, black clay	10.07.2018	N 45°28'13.5" E 36°18'43.6"	135	29
6	Beregovoe village in Feodosia District, Lake Kuchuk-Adjiröl', silt	02.07.2018	N 45°06'11.7" E 35°27'15.4"	27	32
7	Lake Sivash, south-eastern part, silt	03.07.2018	N 45°17'53.3" E 35°27'42.6"	99	28
8	Ibid., dried puddle near Lake Sivash, silt	03.07.2018	N 45°18'04.8" E 35°27'51.0"	210	30
9	Evpatoria city, Lake Sasyk-Sivash, northern part, stone scraping	03.07.2018	N 45°12'17.2" E 33°24'23.0"	10	24
10	Sevastopol city, Lyubimovka microdistrict, nameless lake, sand	17.06.2017	N 44°39'40.2" E 33°32'45.7"	19	23

Table 2. Distribution of heterotrophic flagellates in the studied saline lakes of the Crimean Peninsula. * – hierarchical ranks of taxa are given from higher rank to lower one. The numbering of reservoirs is given in accordance with Table 1.

Taxa	Water bodies* (water salinity, ‰)									
	1 (135)	2 (84)	3 (204)	4 (41)	5 (135)	6 (27)	7 (99)	8 (210)	9 (10)	10 (19)
Domain AMORPHEA Adl et al., 2012										
Supergroup Obazoa Brown et al., 2013										
*Apusomonadida Karpov et Mylnikov, 1989										
<i>Amastigomonas caudata</i> Zhukov, 1975	–	–	–	–	–	–	–	–	–	+
<i>A. mutabilis</i> (Griessmann, 1913) Molina et Nerad, 1991	–	–	–	–	–	–	–	–	–	+
*Opisthokonta Cavalier-Smith, 1987										
**Holozoa Lang et al., 2002										
***Filasterea Shalchian-Tabrizi et al., 2008										
<i>Ministeria vibrans</i> Tong, 1997	–	–	–	–	–	–	+	–	–	–
***Choanoflagellata Kent, 1880–1882										
****Craspedida Cavalier-Smith, 1997										
*****Salpingoecidae Kent, 1880–1882 sensu Nitsche et al., 2011										
<i>Salpingoeca infusionum</i> Kent, 1880–1882	–	–	–	–	–	+	–	–	+	–
<i>S. rosetta</i> Dayel et al., 2011	–	–	–	–	–	–	+	+	–	–
****Acanthoecida Cavalier-Smith, 1997										
*****Acanthoecidae Norris, 1965 sensu Nitsche et al., 2011										
<i>Savillea parva</i> (Ellis, 1929) Loeblich, 1967	–	–	–	–	–	–	–	–	+	–
Domain DIAPHORETICKES Adl et al., 2012										
Supergroup SAR Burki et al., 2008										
*Stramenopiles Patterson, 1989										
**Bigyra Cavalier-Smith, 1998										
***Opalozoa Cavalier-Smith, 1991										
****Bicosoecida Grasse, 1926										
<i>Bicosoeca maris</i> Picken, 1841	–	–	–	–	–	–	+	–	–	–
<i>Caecitellus parvulus</i> (Griessmann, 1913) Patterson et al., 1998	–	+	–	–	–	–	+	–	–	–
<i>Cafeteria ligulifera</i> Larsen et Patterson, 1990	–	–	–	–	–	–	+	–	–	–
<i>C. roenbergensis</i> Fenchel et Patterson, 1988	–	–	–	+	–	–	–	–	–	+
<i>Cafeteria</i> sp.	–	+	+	+	–	+	–	–	–	–
<i>Halocafeteria seosinensis</i> Park et al., 2006	–	+	–	–	–	–	–	–	–	–
<i>Pseudobodo tremulans</i> Griessmann, 1913	–	–	–	–	–	–	+	–	–	–

Taxa	Water bodies* (water salinity, ‰)									
	1 (135)	2 (84)	3 (204)	4 (41)	5 (135)	6 (27)	7 (99)	8 (210)	9 (10)	10 (19)
**Gyrsta Cavalier-Smith, 1998										
***Ochrophyta Cavalier-Smith, 1986										
****Diatomista Derelle et al., 2016										
*****Dictyochophyceae Silva, 1980										
*****Pedinellales Zimmermann et al., 1984										
<i>Ciliophrys infusionum</i> Cienkowski, 1876	–	–	–	–	–	–	+	–	–	–
Supergroup Cryptista Adl et al., 2018										
*Cryptophyceae Pascher, 1913										
**Cyathomonadacea Pringsheim, 1944										
<i>Goniomonas pacifica</i> Larsen et Patterson, 1990	–	–	–	–	–	–	–	–	–	+
<i>G. truncata</i> (Fresenius, 1858) Stein, 1878	–	–	–	–	–	–	–	–	–	+
Incertae sedis EUKARYA										
Formal group Excavates [ex-supergroup Excavata Cavalier-Smith, 2002]										
*Discoba Simpson, 2009										
**Euglenozoa Cavalier-Smith, 1981										
***Kinetoplastea Honigberg, 1963										
<i>Incertae sedis</i> Kinetoplastea										
<i>Bordnamonas tropicana</i> Larsen et Patterson, 1990	–	–	–	–	–	–	+	–	–	+
****Metakinetoplastina Vickerman, 2004										
*****Neobodonida Vickerman, 2004										
<i>Neobodo curvifilus</i> (Griessmann, 1913) Moreira et al., 2004	–	–	–	–	–	–	–	–	–	+
<i>N. designis</i> (Skuja, 1948) Moreira et al., 2004	+	–	–	–	–	–	+	–	+	+
<i>Rhynchomonas nasuta</i> (Stokes, 1888) Klebs, 1893	+	+	+	–	–	–	+	–	+	+
*****Eubodonida Vickerman, 2004										
<i>Bodo saltans</i> Ehrenberg, 1838	–	–	–	–	–	–	+	–	–	–
***Euglenida Butschli, 1884										
****Heteronematina Leedale, 1967										
<i>Notosolenus apocamptus</i> Stokes, 1884	–	–	–	–	–	–	–	–	+	–
<i>N. urceolatus</i> Larsen et Patterson, 1990	–	–	–	–	–	–	+	–	–	–
<i>Petalomonas poosilla</i> (Skuja, 1948) Larsen et Patterson, 1990	–	–	+	–	–	–	+	–	+	–

Taxa	Water bodies* (water salinity, ‰)									
	1 (135)	2 (84)	3 (204)	4 (41)	5 (135)	6 (27)	7 (99)	8 (210)	9 (10)	10 (19)
**Heterolobosea Page et Blanton, 1985										
***Tetramitia Cavalier-Smith, 1993										
****Eutetramitia Hanousková et al., 2018										
*****Percolomonadidae Cavalier-Smith, 2008										
<i>Percolomonas cosmopolitus</i> (Ruinen, 1938) Fenchel et Patterson, 1986	-	-	+	-	+	-	+	-	-	-
*****Tulamoebidae Kirby et al., 2015										
<i>Pleurostomum flabellatum</i> Ruinen, 1938	-	-	-	-	-	-	-	+	-	-
Clade Ancyromonadida Cavalier-Smith, 1998										
<i>Ancyromonas sigmoides</i> (Kent, 1880–1882) Heiss et al., 2010	-	-	-	-	-	-	+	-	-	+
<i>Incertae sedis</i> EUKARYOTA										
<i>Phyllomitus undulatus</i> Stein, 1878	-	-	-	-	+	-	-	+	-	-
“Protist–4”	-	-	-	-	-	-	-	+	-	-
Total species number	2	4	4	2	2	2	15	4	6	10

by the author both in saline continental water bodies and in marine biotopes of the Crimean Peninsula.

When comparing the saline lakes of other regions of Russia and of the world with the studied saline water bodies of the Republic of Crimea, the latter were characterized by a low species diversity of heterotrophic flagellates: 74 species were recorded in the saline lakes of the Orenburg Region (Russia), including 41 species found in hyperhaline lakes with salinity of 92–291‰ (Plotnikov et al., 2011); 46 species, in the hyperhaline habitats of Western Australia (60–150‰) (Patterson and Simpson, 1996). The resulting low species diversity of the flagellates in the study area comparing with other regions may be explained by the incompleteness of our studies and the insufficient number of samples taken.

The hyperhaline Lake Sivash (15 species) and the nameless saline lake in the Lyubimovka microdistrict, the city of Sevastopol (10 species) turned out to have the richest species composition, from 2 to 6 species have been found in the other studied lakes. Here, no decrease in the species richness of the flagellates of saline habitats has been found in regard to the water salinity increase, as it was shown earlier (Patterson and Simpson, 1996; Plotnikov et al., 2011; Post et al., 1983).

Two flagellate species (*Halocafeteria seosinensis* and *Pleurostomum flabellatum*), found within the study, are extremely halophilic according to the

known distribution (Park et al., 2006, 2007; Plotnikov et al., 2011); our data testify for their confinement to the hyperhaline biotopes. About a half of the heterotrophic flagellates identified down to the species level (13 species) were previously noted not only in saline continental and marine biotopes, but also in fresh waters, so they can be considered euryhaline. About the same number of species (14 species) were previously found in the sea waters only.

According the Venice system (Romanenko, 2004), the studied lakes can be divided into brackish (mesogaline (5–18‰) and polyhaline (18–30‰)) and hyperhaline (exceeding 40‰) by their degree of mineralization. Moreover, hyperhaline lakes with salinity up to 100‰ had a higher flagellate species diversity and the greatest number of the species found there exclusively comparing to all other lakes. Meantime, hyperhaline lakes with salinity exceeding 100‰ were characterized by the lowest species number per sample (Table 3). Among brackish lakes, a greater number of species were noted in polyhaline lakes.

Regard must be paid to the species found within the present study in hyperhaline lakes only; these species are also characteristic of the hyperhaline habitats of the Orenburg Region (Russia), as well as Australia and Korea, namely, these are *Halocafeteria seosinensis*, *Percolomonas cosmopolitus*, and *Pleurostomum flabellatum* (Park et al., 2006, 2007, 2007; Patterson and Simpson, 1996; Plotnikov et al., 2011; Post et al.,

1983). Significantly, these organisms were not found in the marine habitats along the Crimean coast during earlier studies (Prokina et al., 2017, 2018). Enigmatic and morphologically unique organism ("Protist 4") has been found only in hyperhaline biotopes, this one has been also registered in the marine area of Curaçao (unpublished original data); we suppose it is probably a new genus and species not yet described. All flagellate species found in hyperhaline lakes were marine, less commonly euryhaline species, while in meso- and polyhaline waters, the species typical for the fresh water (*Goniomonas truncata* and *Neobodo curvifilis*) have been registered.

Comparison of the species richness of heterotrophic flagellates of different types of bottom sediments and fouling communities (Table 4) evidences on the maximum species diversity, the highest average species number and the number of unique species per sample in the samples obtained at the sites characterized by silty and sandy sediments, while the minimum values of these parameters have been noted in the samples obtained at clay sediments and in periphyton samples.

Several small clusters are visible on the dendrogram of taxonomic similarities (Fig. 1) when analyzing the species composition of the flagellates in saline continental reservoirs of the Crimean Peninsula. One cluster includes the lakes located in Marfovka village (two sites within Lake Marfovskoe

and the unnamed lake located nearby); another cluster comprises the reservoirs with similar salinity (Lake Kuchuk-Adjigol' and a puddle in the village of Marjevka). However, there is no general relationship between the formation of the flagellate species composition and the lake salinity, which is probably due to a significant change in the salinity of these lakes during the year.

Conclusions

In the saline lakes of the Republic of Crimea, 29 species of heterotrophic flagellates have been identified, including two flagellates unidentified down to the species level. The species diversity of the studied saline lakes is significantly lower than that of the Black Sea and saline water bodies of the Orenburg Region (Russia), Australia, and Korea, according to the literature data. There is no direct pattern trend of a decrease in the species richness of flagellates in regard to the water salinity increase, which may be due to a low number of studied reservoirs and samples analyzed. Nevertheless, there is a sharp decrease in the number of flagellate species in the water bodies characterized by the water salinity over 100‰, which is consistent with previously published studies of the flagellate composition in hyperhaline biotopes. There are no clear patterns in the formation of the species composition of the flagellate communities in the water bodies with different salinity.

Табл. 3. Species number of heterotrophic flagellates in the studied saline lakes of different types.

Type of lake, water salinity	Total species number	Species number in the sample	Species found exclusively for the lake type
Mesohaline, 5–18‰	6	6.0	2 (33.3%)
Polyhaline, 18–30‰	12	6.0	5 (41.7%)
Hyperhaline, 40–100‰	18	7.0	9 (50.0%)
Hyperhaline, >100‰	9	3.3	3 (33.3%)

Табл. 4. Species number of heterotrophic flagellates in different types of bottom sediments and periphyton.

Sediment/sample type	Total species number	Species number in the sample	Species found exclusively for the lake type
Silt	19	6.3	9 (47.4%)
Sand	10	10.0	5 (50%)
Clay	8	2.7	1 (12.5%)
Periphyton	6	6.0	2 (33.3%)

Fig. 1. Dendrogram of the species similarity of heterotrophic flagellates (%) of the studied saline lakes on the Crimean Peninsula. The numbering of reservoirs is given in accordance with Table 1.

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