Heterotrophic flagellates and centrohelid heliozoa from littoral and supralittoral zones of the Black Sea (the Southern part of the Crimea)

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Summary

Species composition and morphology of heterotrophic flagellates and centrohelid heliozoa in littoral and supralittoral zones of the Black Sea in the Southern part of the Crimea were studied. Overall, 68 species of heterotrophic flagellates and 2 species of centrohels were encountered in this survey, of which 44 species are the first records for the Black Sea. Many species are widespread and known not only from marine but also from freshwater and soil habitats. Most observed species feed on bacteria, 14 species consume other protists (heterotrophic flagellates, algae, and naked amoebae). Micrographs and morphological descriptions of 20 species (Multicilia marina, Salpingoeca abyssalis, Salpingoeca infusionum, Stephanoeca diplocostata, Paraphysomonas cylicophora, Clathromonas butcheri, Protaspa tegere, Protaspa verrucosa, Helkesimastix marina, Heteronema ovale, Petalomonas labrum, Gweamonas unicus, Pendulomonas adriperis, Platychilomonas psammobia, Zoelucasa sablensis, Planomonas cephalopora, Planomonas melba, Amastigomonas muscula, Choanocystis aff. minima, Raineriophrys raineri) are given.

Key words: heterotrophic flagellates, centrohelid heliozoa, protists, species diversity, morphology, Black Sea, Crimea

Introduction

Heterotrophic flagellates and centrohelid heliozoa play an important role in the microbial food web, which transforms a significant part of carbon and other nutrients in water ecosystems (Pomeroy, 1974; Sherr et al., 1982; Porter et al., 1985; Stensdotter-Blomberg, 1998; Arndt et al., 2000). Precise identification of species is essential for ecological researches and their reliability. Most species of heterotrophic flagellates were described in late XIX and early XX centuries and need to be revised. Intense investigations of centrohelid heliozoans began only in the recent 30–40 years with the development of electron microscopy methods, and they are still in progress. These groups of protozoans remain the least studied components of aquatic ecosystems and precise morphological and ecological investigations using modern methods of light and electron microscopy are required.
In particular, very little is known about distribution of heterotrophic flagellates and heliozoans. Biogeography of protists is subject to lively debate but many questions are still unresolved. Current investigations show, that most morphospecies of free-living heterotrophic flagellates and centrohelids are cosmopolites (Azovsky et al., 2016; Leonov, 2012; Lee, 2015) and their distribution depends mostly on the type of habitats, rather than on geographic location (Finlay, 1998; Finlay et al., 1998; Lee and Patterson, 1998, 2000; Al-Qassab et al., 2002). But these results could be strongly affected by such factors as underestimation of cryptic diversity, misidentifications, under-sampling and under-exploration of some regions (Azovsky et al., 2016).

Particularly, the diversity of heterotrophic flagellates and centrohelid heliozoans of the Black Sea is poorly studied and reports on their observations are limited to a few papers (Ostroumov, 1917; Valkanov, 1940, 1970; Moiseev, 1980; Mikrjukov and Mylnikov, 1998; Mikrjukov, 1999; Leonov, 2010; Tikhonenkov, 2006). The aims of our study were to identify species composition and investigate details of the morphology of heterotrophic flagellates and centrohelids from the Black Sea (the Southern part of the Crimea) by methods of light and electron microscopy.

Material and methods

Benthic and periphytonic samples were collected from littoral and supralittoral zones of the Black Sea near Sevastopol (the Crimea peninsula) by Ilya S. Turbanov in May 8–17, 2017. The bays Kazachya (14 samples), Solenaya (2 samples), Kamyshtovaya (1 sample), Karantinnaya (3 samples), Kruglaya (3 samples) and Streletskaya (2 samples) were investigated. Water samples with bottom detritus or scrapings of periphyton were placed in 15-ml plastic tubes and transported to a laboratory at 4 °C. In the laboratory, samples were transferred into Petri dishes and enriched with a suspension of *Pseudomonas fluorescens* Migula bacteria for bacterivorous flagellate growth as well as by *Procryptobia sorokini* (Zhukov, 1975) Frolov et al., 2001 cell culture for heliozoan and predatory flagellate development. Organisms were cultured in Petri dishes at 22 °C in darkness and observed for 10 days to identify the cryptic species diversity. The light microscope AxioScope A1 (Carl Zeiss, Germany) with DIC and phase contrasts and water immersion objectives (total magnification 1120×) was used for observations of living cells. Videorecording was made by the analog AVT HORN MC1009/S video camera. Electron microscopy preparations for studying protists with external cell elements were carried out according to the described methods (Moestrup and Thomsen, 1980; Mikrjukov, 2002). Organisms were observed in transmission (JEM-1011) and scanning (Joel JSM-6510 LV) electron microscopes. Dendrogram of similarity of water bodies by species composition was drawn on the basis of the Bray-Curtis similarity index using the single linkage algorithm in the PAST software package.

Results

Descriptions of the sampling sites are listed in Table 1. Water temperature during the collection period varied within 16–20 °C, salinity ranged 16–18‰.

In total, 68 species and forms of heterotrophic flagellates and 2 species of centrohelids from four eukaryotic supergroups (Amoebozoa, Opisthokonta, SAR, and Excavata) were revealed. Eight species were identified only to genus level and eighteen species were of uncertain systematic position. Complete list of observed heterotrophic flagellates and centrohelids with descriptions of the morphology of 20 species is presented below. The current macrosystem of eukaryotes (Adl et al., 2012) was used; asterisks indicate ranks of taxa.

AMOEBOZOA Lühe, 1913 emend. Cavalier-Smith, 1998

*Multicilia* Cienkowsky, 1881

**Multicilia marina** Cienkowsky, 1881 [syn.: *Polymastix sol* Grüber, 1884; *Tetracilia paradoxa* Valkanov, 1970] (Fig. 1 a–d).

Found in the bays Kazachya, Solenaya, and Karantinnaya (samples 6–8, 12, 16, and 20).

Cell body is roundish, rarely oblong or irregular with slight swelling at the base of each flagellum. Diameter of the cell body is 3–15 µm. Unusually thick flagella are 1.5–3.5 times as long as the cell body, evenly distributed over the surface of the body and their number varies from 2 to 30. Flagella make very weak, slow, irregular movements without visible coordination. Small granules and large vacuoles are present in the cytoplasm. Round nucleus located medially. Several cell forms (stages of cell development) with different size and shape of the...
cell body, size of the nucleus, and number of flagella were observed in the samples.

Biflagellar cells (fig. 1a) – elongate oval, its size is 3–6×1.5–3.0 μm. Flagella insert from different “poles” of the cell and directed to opposite sides. Cells slowly gliding along the substrate toward the direction of one of the flagella. Direction of movement can abruptly change to the opposite without turning the cell, and the anterior end becomes the posterior one.

Cells with 5–7 flagella (Fig. 1 b) – roundish with pronounced swellings. Diameter of the cell body is 5–8 μm. Movement of the cell is slightly faster than that of the biflagellar form. Provisional anterior flagellum looks more direct compared with other flagella, and only its tip can slightly coil. Remaining flagella can slowly turn to different directions and smoothly squirm.

Multiflagellar cells (Fig. 1 c–d) – more roundish in comparison with other forms, 8–15 μm in diameter, and with about 30 long flagella. Sometimes cells bear also short flagella (2–4 μm long). Nucleus is well visible, 1.5–3.0 μm in diameter.

Also tetraflagellar, giant multi-flagellar (with up to 200 flagella), branching or horseshoe-shaped forms, developing in culture after sharp salinity decrease, have been described (Mikrjukov and Mylnikov, 1998). Cells feed on small naked amoebae

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Date</th>
<th>Characteristics of samples</th>
<th>Distance from waterfront, m</th>
<th>Depth, m</th>
<th>Number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bay Kazachya, 44°34'18.8&quot;N 33°24'40.2&quot;E</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>08.05</td>
<td>Littoral, scraping from a single stone</td>
<td>2</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>08.05</td>
<td>Littoral, sand</td>
<td>0.5</td>
<td>0.1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>08.05</td>
<td>Littoral, sand</td>
<td>1.5</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>08.05</td>
<td>Littoral, scraping from the rock</td>
<td>1</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>08.05</td>
<td>Supralittoral, storm emissions at the waterfront</td>
<td>–</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>08.05</td>
<td>Littoral, scraping from the base of the metal pier</td>
<td>3</td>
<td>1.5</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>11.05</td>
<td>Littoral, sand and sludge</td>
<td>0.5</td>
<td>0.1</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>11.05</td>
<td>Littoral, among thickets of <em>Phragmites australis</em></td>
<td>0.3</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>11.05</td>
<td>Littoral, sand and sludge</td>
<td>2</td>
<td>0.2</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>14.05</td>
<td>Littoral, scraping from the <em>Cystoseira barbata</em></td>
<td>2</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>14.05</td>
<td>Littoral, scraping from the <em>Ulva</em> sp.</td>
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<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>14.05</td>
<td>Littoral, sand</td>
<td>20</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>13</td>
<td>14.05</td>
<td>Supralittoral, Native Miocene clays (waterfront)</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>14.05</td>
<td>Littoral, scraping from stones</td>
<td>3</td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>15</td>
<td>10.05</td>
<td>Littoral, detritus among thickets of <em>Phragmites australis</em></td>
<td>0.5</td>
<td>0.1</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>10.05</td>
<td>Littoral, sludge</td>
<td>0.2</td>
<td>0.05</td>
<td>20</td>
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<tr>
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<td>10.05</td>
<td>Littoral, sand and sludge</td>
<td>0.1</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>Bay Karantinnaya, 44°36'44.3&quot;N 33°29'58.1&quot;E</td>
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<tr>
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<td>0.05</td>
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<tr>
<td>19</td>
<td>13.05</td>
<td>Littoral, scraping from the <em>Zostera marina</em></td>
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<td>6</td>
</tr>
<tr>
<td>20</td>
<td>13.05</td>
<td>Littoral, sand and sludge</td>
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<td>0.2</td>
<td>3</td>
</tr>
<tr>
<td>Bay Knigoyskaya, 44°36'13.0&quot;N 33°28'09.5&quot;E</td>
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<tr>
<td>21</td>
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<td>Littoral, sand and sludge</td>
<td>0.5</td>
<td>0.5</td>
<td>16</td>
</tr>
<tr>
<td>22</td>
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<td>Supralittoral, (waterfront)</td>
<td>–</td>
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<tr>
<td>23</td>
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<td>Littoral, sludge</td>
<td>5</td>
<td>0.3</td>
<td>10</td>
</tr>
<tr>
<td>Bay Streletskaya, 44°36'13.0&quot;N 33°28'09.5&quot;E</td>
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<td>24</td>
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<td>Littoral, sand</td>
<td>0.5</td>
<td>0.2</td>
<td>5</td>
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<tr>
<td>25</td>
<td>17.05</td>
<td>Supralittoral, (waterfront)</td>
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<td>–</td>
<td>2</td>
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</tbody>
</table>
and capture a prey by the ventral side of the body, remaining motionless for a short time, until food is absorbed (Nikolaev et al., 2006). Morphology of observed cells is in agreement with previous studies, but Mikrjukov and Mylnikov (1998) described larger cells (up to 40 µm in diameter in culture), while the largest cells observed in our investigation were 15 µm. This species is very easily distinguished from other flagellates because of its unusual morphology. It differs from other freshwater species of the genus (*M. lacustris* Lauterborn, 1901 and *M. palustris* Penard, 1903) by the ability to live in marine habitats.

Previously reported from marine waters of USA (Jones, 1974), White Sea (Cienkowski, 1881; Mikrjukov and Mylnikov, 1998; Tikhonenkov, 2006), Pechora Sea (Tikhonenkov, 2006), Baltic Sea (Mikrjukov and Mylnikov, 1998), Black Sea (Mikrjukov and Mylnikov, 1998; Tikhonenkov, 2006).

**OPISTHOKONTA** Cavalier-Smith, 1987 emend.

*HOLOZOA* Lang et al., 2002

**CRASPEDIDA** Cavalier-Smith, 1997 emend.

*Nitsche et al., 2011*

*Codosiga botrytis* (Ehrenberg, 1838) James-Clark, 1866 [bas.: *Epistyliis botrytis* Ehrenberg, 1838].

Found in the bay Kazachya (sample 8). See morphological descriptions by Vors (1992), Ekelund and Patterson (1997), and Shatilovich et al. (2010).

Previously reported from brackish waters of Siberia (Kopylov et al., 2002); White Sea, Pechora Sea, Black Sea, South China Sea (Tikhonenkov, 2006), Baltic Sea (Vors, 1992); from freshwaters of the European part of Russia (Tikhonenkov, 2006; Prokina and Mylnikov, 2017), Siberia (Shatilovich et al., 2010), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), Tailand (Charubhun and Charubhun, 2000), Japan (Takamura et al., 2000), Australia (Lee et al., 2005); from soils of China (Tikhonenkov et al., 2010), China (Tikhonenkov et al., 2012), Australia (Ekelund and Patterson, 1997).

*Salpingoeca abyssalis* Nitsche et al., 2007 (Fig. 1 e–g).

Found in the bays Kazachya and Solenaya (samples 12 and 16).

Cell body oval, without neck, completely fills the transparent lorica; its size is 3–4×2.0–2.5 µm. Large collar well developed, the same length as the lorica or slightly longer (Fig. 1 e). Flagellum is about 2 lengths of the lorica. Stalk is of the same length as the lorica, developed on attached cells (Fig. 1 f).

Floating forms, known for this species (Nitsche et al., 2007), were not observed.

Morphology of observed cells is in congruence with the first description from the Atlantic Ocean, from the depth of 5038 m (Nitsche et al., 2007). Species is similar to *S. infusionum* Kent, 1880. But lorica of *S. infusionum* narrows posteriorly and stalk is almost 1.2–2 times longer than the cell. *S. abyssalis*, unlike *S. infusionum*, occupies the whole space of the lorica. Also *S. abyssalis* is similar to *S. marina* James-Clark, 1867, but *S. marina* has narrowed anterior margin of the lorica, and the anterior end of the cell body has a neck. And conversely, expanded anterior margin of the lorica is typical for such species as *S. inquillata* Kent, 1880, *S. curvipes* Kent, 1880, *S. ringens* Kent, 1880, and *S. eurystoma* Stokes, 1886, which, possibly, are synonyms (Lee and Patterson, 2000).

*Salpingoeca amphoridium* James-Clark, 1868.

Found in the bay Kazachya (sample 7). See morphological descriptions by Vors (1992), Ekelund and Patterson (1997), and Prokina et al. (2017a).

Previously reported from marine waters of Australia (Lee, 2015); White Sea (Tikhonenkov, 2006), Baltic Sea (Vors, 1992), Red Sea (Tikhonenkov, 2009); from freshwaters of the European part of Russia (Tikhonenkov, 2006; Prokina and Mylnikov, 2017), Siberia (Shatilovich et al., 2010), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), Ethiopia (Prokina et al., 2017a); from soils of China (Tikhonenkov et al., 2012), Australia (Ekelund and Patterson, 1997).

*Salpingoeca infusionum* Kent, 1880 (Fig. 1 h–i).

Found in the bays Kazachya and Kruglaya (samples 7, 21, and 23).

Cell body is oval, 3–6 µm in length, occupies 2/3 of the lorica; the basal part of the lorica is empty. Protoplasmatic outgrows emerge at the posterior end of the cell body and attached to the inner side of the lorica (Fig. 1 h). Lorica is 4–9 µm in length, slightly tapers at the base, truncates anteriorly. Collar is equal to cell body length. Flagellum is 3 times longer than the lorica, stalk is 1.5–2 times longer than the lorica.

Morphology of observed cells corresponds to previous descriptions. However, some authors
described a shorter stalk (Lee and Patterson, 2000; Lee, 2002). Tong (1993) described very long flagellum (5–7 times longer than the cell body). On the contrary, Lee (2002) described very short flagellum, equal to cell body length. It is possible that a total length of flagella is difficult to estimate in living cells with light microscopy. Tong (1993) notes a spherical swelling at the base of the lorica, which we did not observe. Nevertheless, she mentioned that this morphological character was not observed in all cells and it was difficult to see in a light microscope. Some authors (Griessmann, 1913; Boucaud-Camou, 1967) consider *S. infusionum* as a synonym of *S. marina*, but we agree with Tong (1997a), that these organisms are different species; the main difference is a narrowing anterior margin of the lorica of *S. marina*, forming a short neck.

Previously reported from marine and brackish waters of the Northern Europe (Griessmann, 1913; Boucaud-Camou, 1967; Tong, 1993, 1997a), Korea (Lee, 2002), USA (Norris, 1965), Australia (Lee and Patterson, 2000; Lee et al., 2003), the Antarctic (Tong et al., 1997), White Sea, Black Sea (Tikhonenkov, 2006), Red Sea (Tikhonenkov, 2009).

**Salpingoeca megacheila** Ellis, 1929.

Found in the bay Kazachya (sample 1). See morphological descriptions by Tong et al. (1998) and Lee et al. (2003).

Previously reported from marine waters of Australia (Tong et al., 1998; Lee et al., 2003; Lee, 2015); White Sea (Tikhonenkov, 2006); from freshwaters of the European part of Russia (Tikhonenkov, 2006); from soils of China (Tikhonenkov et al., 2012).


**Stephanoeca diplocostata** Ellis, 1930 (Figs 1 j, 3 a).

Found in the bays Kazachya, Solenaya, and Karantinnaya (samples 6–9, 16, and 18).

**Stephanoeca** sp.

Found in the bays Kazachya, Solenaya, and Karantinnaya (sample 6–9, 16, and 18).

SAR

*Stramenopiles Patterson, 1989 emend. Adl et al., 2005


**Bicosoeca gracilipes** James-Clark, 1867.

Found in the bay Kazachya (sample 8). See morphological descriptions by Tong (1997b), Al-Quassab et al. (2002), Lee et al. (2003), and Lee (2007).

Previously reported from marine waters of U.K. (Tong, 1997b), Australia (Tong et al., 1998; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2007, 2015).
**Bicosoeca maris** Picken, 1841 [syn.: **Bicosoeca griessmanni** (Griessmann, 1914) Bourrelly, 1951].
Found in the bay Karantinnaya (sample 18). See morphological descriptions by Tong (1997b), Tong et al. (1998), and Lee et al. (2003).
Previously reported from marine waters of U.K. (Tong, 1997b) and Australia (Tong et al., 1998; Lee et al., 2003); from brackish waters of the European part of Russia (Tikhonenkov, 2006).

**Caecitellus parvulus** (Griessmann, 1913) Patterson et al., 1993 [bas.: **Bodo parvulus** Griessmann, 1913]
Previously reported from marine waters of Denmark (Larsen and Patterson, 1990), U.K. (Larsen and Patterson, 1990; Tong, 1993, 1997b), Greenland (Vørs, 1993a), Belize (Vørs, 1993b), Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Patterson and Simpson, 1996; Tong, 1997c; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015), Antarctic (Tong et al., 1997); White Sea, Pechora Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Thomsen, 1975; Vørs, 1992), Aegean Sea (Aydin and Lee, 2012), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of Mongolia (Kopylov et al., 2006).

**Cafeteria marsupialis** Larsen et Patterson, 1990.
Previously reported from marine waters of U.K. (Tong, 1997b), Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Lee and Patterson, 2000; Lee et al., 2003; Lee, 2006); White Sea, Pechora Sea (Tikhonenkov, 2006), Red Sea (Tikhonenkov, 2009); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of Mongolia (Kopylov et al., 2006).

**Cafeteria roenbergensis** Fenchel et Patterson, 1988.
Found in all observed bays, except the bay Kruglaya (samples 1–6, 8, 10–12, 14–17, 19, and 24–25). See morphological descriptions by Larsen and Patterson (1990), Ekebom et al. (1995), Lee and Patterson (2000), Al-Quassab et al. (2002), Lee et al. (2003), and Aydin and Lee (2012).
Previously reported from marine waters of U.K. (Tong, 1997b), Greenland (Vørs, 1993a), Korea (Lee, 2002), Belize, Tenerife (Vørs, 1993b), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Patterson and Simpson, 1996; Tong, 1997c; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015), Antarctic (Tong et al., 1997); White Sea, Pechora Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Vørs, 1992), Aegean Sea (Aydin and Lee, 2012), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of Mongolia (Kopylov et al., 2006).

**Chrysophyceae Pascher, 1914**

**Paraphysomonas cylicophora** Leadbeater, 1972 (Fig. 3 b–d).
Found in the bay Karantinnaya (sample 19).
Cell body is covered by one type of siliceous scales. Scales have round plate base 0.7–0.9 µm in diameter, with radial, some times bifurcate ribs. Perforated cup with a dense marginal rim located on a small thick neck (0.12–0.15 µm in diameter) in the center of the base.

This species is easily identifiable by the peculiar form of siliceous scales, which can be visible only with electron microscope. Size and shape of scales agree well with previous descriptions (Tong et al., 1997; 1998).

Previously reported from marine waters of Norway (Leadbeater, 1972), Australia (Tong et al., 1998; Lee et al., 2003), Antarctica (Tong et al., 1997); Baltic Sea (Thomsen, 1975; Vørs, 1992).

**Clathromonas butcheri** (Pennick et Clarke, 1972). Scoble et Cavalier-Smith, 2014 [bas.: **Paraphysomonas butcheri** Pennick et Clarke, 1972; syn.: **Paraphysomonas inconspicula** Takahashi, 1976] (Fig. 3 e–h).
Found in the bays Kazachya, Karantinnaya, and Kruglaya (samples 3, 19, and 21).
Cell body is covered by two types of siliceous scales. The first type – round or oval plate mesh scales (Fig. 3 g) with 12–16 hollows at the first
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(external) row, 7–11 holes at the second (inner) row, and irregular holes at the center part of the scale. Size of plate mesh scales is 0.4–0.9 × 0.4–0.7 µm. The second type of scales – crown-scales (Fig. 3 h) with 5 columns and 5 oval holes between them. Anterior end of crown-scales is reticulate with small holes. Diameter of the second type of scales is 0.5–0.7 µm.

This species was originally described as Paraphysomonas butcheri, however, after phylogenetic studies, Scoble and Cavalier-Smith (2014) transferred it to a new genus Clathromonas. Number of holes and sizes of scales may vary at different cells (Bergesch et al., 2008). Size of scales of observed cells corresponds to previous descriptions (Tong et al., 1998; Scoble and Cavalier-Smith, 2014). However, Scoble and Cavalier-Smith (2014) pointed out that crown-scales can be observed with both five and six columns. Some species of the genus Clathromonas (C. caroni Scoble et Cavalier-Smith, 2014; C. cribosa (Lukas, 1968) Scoble et Cavalier-Smith, 2014; C. homolepis (Preisig et Hibberd, 1882) Scoble et Cavalier-Smith, 2014; C. tongi Scoble et Cavalier-Smith, 2014) have similar plate mesh scales. Therefore, identification of the species by observation of several scales is impossible. C. butcheri differs from C. cribosa and C. homolepis by shape of crown-scales. Studied organism differs from C. caroni and C. tongi by the size of scales and the number of hollows. C. tongi have fewer number of hollows in plate mesh scales (5–9 at the external row, 2–6 at the inner row), and is characterized by angular shape of hollows of crown-scales. C. caroni has larger size of scales of both type and shorter columns of crown-scales.

Previously reported from marine waters of U.K. (Tong, 1997b), Korea (Lee, 2002), Australia (Lee et al., 2003; Lee, 2015), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990), Antarctic (Tong et al., 1997); White Sea, Pechora Sea (Tikhonenkov, 2006), Northern Atlantic Ocean (Patterson et al., 1993); from freshwaters of the European part of Russia (Prokina and Mylnikov, 2017), Germany (Auer and Arndt, 2001), Japan (Takamura et al., 2000).


Previously reported from marine waters of Denmark, Baltic Sea (Patterson and Fenchel, 1985), U.K. (Tong, 1997b), Australia (Larsen and Patterson, 1990; Patterson and Simpson, 1996; Tong, 1997c; Tong et al., 1998; Lee, 2007, 2015), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990); White Sea, Black Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), equatorial Pacific Ocean (Vørs et al., 1995); freshwaters of Germany (Auer and Arndt, 2001), Brazil (Tikhonenkov, 2006), Mongolia (Kopylov et al., 2006).

*Alveolata Cavalier-Smith, 1991
**Incertae sedis Alveolata
Colponema sp.
Found in the bays Kazachya, Solenaya, and Kruglaya (samples 18 and 23).

*Rhizaria Cavalier-Smith, 2002
**Cercozoa Cavalier-Smith, 1998 emend. Adl et al., 2005
***Metromonadea Cavalier-Smith, 2007 emend. Cavalier-Smith, 2011
Metopion fluens Larsen et Patterson, 1990.
Found in the bays Kazachya, Kruglaya, and Streletskaya (samples 1, 21, and 23–24). See morphological descriptions by Larsen and Patterson (1990), Tong (1997b), Lee and Patterson (2000), Al-Quassab et al. (2002), and Lee et al. (2003).

Previously reported from marine waters of U.K. (Tong, 1997b), Korea (Lee, 2002), Tenerife (Vørs, 1993b), Fiji, Brazil (Larsen and Patterson, 1990), Australia (Patterson and Simpson, 1996; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015); White Sea,
Metromonas grandis Larsen et Patterson, 1990
Found in the bays Solenaya and Karantinnaya (samples 16 and 18). See morphological descriptions by Larsen and Patterson (1990), Lee and Patterson (2000), Al-Quassab et al. (2002), and Aydin and Lee (2012).

Previously reported from marine waters of Korea (Lee, 2002), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2006, 2015); White Sea, Pechora Sea (Tikhonenkov, 2006), Aegean Sea (Aydin and Lee, 2012), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993).

Metromonas simplex (Griessmann, 1913) Larsen et Patterson, 1990 [bas.: Phyllosomonas simplex Griessmann, 1913].

Previously reported from marine waters of U.K. (Larsen and Patterson, 1990; Tong, 1997b), Korea (Lee, 2002), Fiji, Hawaii, Brazil, Denmark (Larsen and Patterson, 1990), Canada, Greenland (Vørs, 1993a), Australia (Larsen and Patterson, 1990; Ekeborn et al., 1995; Patterson and Simpson, 1996; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015); Antarctic (Tong et al., 1997); White Sea, Pechora Sea, Black Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992), Mediterranean Sea (Hausmann et al., 2002), Red Sea (Tikhonenkov, 2009); from freshwaters of the European part of Russia (Tikhonenkov, 2006), Germany (Auer and Arndt, 2001); from soils of China (Tikhonenkov et al., 2012).

Micrometopion nutans Cavalier-Smith in Howe et al., 2011 (Fig. 1 k)
Found in the bay Kruglaya (samples 21 and 23). See the morphological description by Howe et al. (2011).

Previously reported from the White Sea (Howe et al., 2011).

Imbricatea Cavalier-Smith, 2011

Spongomonas sp.
Found in the bay Kazachya (samples 1 and 6).

Granifilosea Cavalier-Smith et Bass, 2009
Massisteria marina Larsen et Patterson, 1990.
Found in the bays Kazachya and Karantinnaya (samples 1—2, 4, 6, 12, and 18). See morphological descriptions by Larsen and Patterson (1990), Vørs (1992), Lee and Patterson (2000), Al-Quassab et al. (2002); Lee et al., 2003; Lee, 2006, 2015); White Sea, Pechora Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Vørs, 1992), Mediterranean Sea (Hausmann et al., 2002; Arndt et al., 2003), Northern Atlantic Ocean (Patterson et al., 1993), equatorial Pacific Ocean (Vørs et al., 1995); from brackish waters of Siberia (Kopylov et al., 2002).

Thecofilosea Cavalier-Smith, 2003 emend. Cavalier-Smith, 2011

Cryomonadida Cavalier-Smith, 1993
Protaspa simplex (Vørs, 1992) Cavalier-Smith in Howe et al., 2011 [bas.: Protaspis simplex Vørs, 1992].

Previously reported from marine waters of U.K. (Tong, 1997b), Australia (Tong et al., 1998); White Sea, Pechora Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Vørs, 1992), Mediterranean Sea (Arndt et al., 2003); from freshwaters of the European part of Russia (Tikhonenkov, 2006); from freshwaters of China (Prokina et al., 2012), Australia (Lee et al., 2005), Ethiopia (Prokina et al., 2017a); from
soils of Siberia (Shatilovich et al., 2010), China (Tikhonenkov et al., 2012), Australia (Ekelund and Patterson, 1997).

*Protaspa tegere* (Larsen et Patterson, 1990) Cavalier-Smith in Howe et al., 2011 [bas.: *Protaspis tegere* Larsen et Patterson, 1990] (Fig. 1 k–m).

Found in the bays Kazachya, Solenaya, and Karantinnaya (samples 6, 16, and 18).

Cell body is oval, 10–13×6.5–9.0 µm, flattened in dorsoventral direction, and covered by small granules. Flagella emerge from subapical depression on the ventral side of the cell. Anterior flagellum is equal to cell body length; posterior flagellum is twice as long as cell body length. Pseudopodia produced from the longitudinal groove. Large round nucleus (4–6 µm in diameter) with nucleus caps (Fig. 1 m) located anteriorly. Cells gliding at the substrate, anterior flagellum makes a flapping movement.

This species is very similar to *P. glans* (Skuja, 1939) Cavalier-Smith in Howe et al., 2011. The main difference from *P. glans* is presence of nucleus caps. According to Aydin and Lee (2012), nucleus caps can be overlooked in a light microscope. Location of nucleus of these species is different, but Lee and Patterson (2000) notice that nucleus can change location with aging of the cell. Therefore, these features are unclear, and two species can be synonyms. Size of observed cells is smaller if compared to previous descriptions (Larsen and Patterson, 1990; Ekebom et al., 1995; Lee and Patterson, 2000; Aydin and Lee, 2002; Lee, 2015).

Previously reported from marine waters of Fiji, U.K. (Larsen and Patterson, 1990), Canada, Greenland (Vors, 1993a), Australia (Tong et al., 1998), White Sea, Pechora Sea (Tikhonenkov, 2006), Baltic Sea (Vors, 1992); from freshwaters of the European part of Russia (Tikhonenkov, 2006).

*Protaspa verrucosa* (Larsen et Patterson, 1990) Cavalier-Smith in Howe et al., 2011 [bas.: *Protaspis verrucosa* Larsen et Patterson, 1990] (Fig. 1 n–o).

Found in the bay Kazachya (sample 12).

Cell body is flattened in dorsoventral direction, oblong-oval, its size is 14–15×6–7 µm. Subapical depression located on the ventral side of the cell body. Thin flagella emerge from the subapical depression. Anterior flagellum is equal to cell body length; posterior flagellum is slightly longer. The deep longitudinal ventral groove runs along the entire body. Surface of the cell body is covered by well-marked granules. Large round nucleus (3.5–4 µm) without nucleus caps located anteriorly. Feeds on diatoms and green algae, as well as cysts of flagellates *Pseudopedinella* (Vors, 1992).

Observed cells almost completely correspond to morphological descriptions of other authors (Larsen and Patterson, 1990; Vors, 1992; Tong et al., 1998). However, Vors (1992) and Tong et al. (1998) described smaller cell body length (5–10 µm).

Previously reported from marine waters of Fiji, U.K. (Larsen and Patterson, 1990), Canada, Greenland (Vors, 1993a), Australia (Tong et al., 1998), White Sea, Pechora Sea (Tikhonenkov, 2006), Baltic Sea (Vors, 1992); from freshwaters of the European part of Russia (Tikhonenkov, 2006).

*Incertae sedis* Rhizaria

**Helkesimastix marina** Cavalier-Smith et al., 2009 (Fig. 1 p).

Found in the bay Kazachya (sample 1).

Cell body is oval, 6.0–6.5 µm long, rounded anteriorly and tapered posteriorly. Cell body is covered by numerous granules. Anterior flagellum is short and located under the cell; it can be overlooked, but is clearly visible when cell rotates. Posterior flagellum is long (twice as long as the cell body), pasted to the cell and bears an acroneme.

First record of this species was from marine habitats of Belize (Cavalier-Smith et al., 2009). Organism differs from the better-known freshwater *H. faecicola* Woodcock et Lapage, 1915 by several features. Anterior flagellum of *H. marina* is not visible in light microscope. There is no contractile vacuole in *H. marina*. Cavalier-Smith et al. (2009) adapted this species to low salinity and appearing contractile vacuole did not have a permanent localization (contractile vacuole of *H. faecicola* is located posteriorly); size of the vacuole was always greater than in *H. faecicola*.

EXCAVATA Cavalier-Smith, 2002 emend. Simpson, 2003

*Discoba Simpson in Hampl et al., 2009
*Discicristata Cavalier-Smith, 1998
****Euglenida Bütschli, 1884
*****Heteronematina Leedale, 1967
Anisonema acinus Dujardin, 1841.  
Found in the bay Kazachya (sample 12). See morphological descriptions by Larsen and Patterson (1990), Lee and Patterson (2000), Al-Quassab et al. (2002), Lee et al. (2005), and Al-Yamani and Saburova (2010).

Previously reported from marine waters of Kuwait (Al-Yamani and Saburova, 2010), Fiji (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003); Danish Wadden Sea (Larsen, 1987), Aegean Sea (Aydin and Lee, 2012); from freshwaters of the European part of Russia (Prokina and Mylnikov, 2017), Hungary (Kiss et al., 2008), Japan (Takamura et al., 2000), China (Tong et al., 1998), Australia (Schroeckh et al., 2003; Lee et al., 2005).


Previously reported from marine waters of Korea (Lee, 2002), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2008, 2015); White Sea (Tikhonenkov, 2006), Danish Wadden Sea (Larsen, 1987); from freshwaters of the European part of Russia (Prokina and Mylnikov, 2017), Australia (Schroeckh et al., 2003).

Dinema sp.  
Found in the bay Kazachya (sample 6).

Heteronema exaratum Larsen et Patterson, 1990.  
Found in the bays Kazachya, Solenaya, Kamyshevaya, and Kruglaya (samples 8, 15–17, and 22). See morphological descriptions by Larsen and Patterson (1990), Al-Quassab et al. (2002), Lee et al. (2003, 2005), Al-Yamani and Saburova (2010), and Aydin and Lee (2012).

Previously reported from marine waters of Korea (Lee, 2002), Kuwait (Al-Yamani and Saburova, 2010), Fiji (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Patterson and Simpson, 1996; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2008, 2015); White Sea, Pechora Sea (Tikhonenkov, 2006), Aegean Sea (Aydin and Lee, 2012); from freshwaters of the European part of Russia (Tikhonenkov, 2006), Australia (Schroeckh et al., 2003; Lee et al., 2005).

Heteronema ovale Kahl, 1928 (Fig. 1 q–t).  
Found in the bay Kazachya (sample 6).  
Cell body is oval, 12.0–20.0 × 8.5–11.0 µm, flattened, highly metabolic. Cell body with pellicular striations following a S-helix, with small irregular granules along striations (Fig. 1 r). Flagella emerge subapically from a flagellar pocket. Anterior flagellum slightly longer than the cell body, posterior flagellum about 1.5 times as long as the cell body, with knob at the base. Metabolical cells can strar-ching into an oval cylinder or, as opposite, can compress (Fig. 1 s–t). Nucleus located at the anterior part of the cell body. Ingestion organelle is well developed. Consumes diatoms (Lee and Patterson, 2000; Al-Quassab et al., 2002).

Morphology of observed cells agreed well with previous descriptions (Ekebom et al., 1995; Al-Quassab et al., 2002; Lee, 2002); however, some authors notice a larger size of the cell body – 20–33 µm (Lee, 2002; Aydin and Lee, 2012). This species is similar to H. exaratum by cell size. It differs by more vigorous squirming movements of a cell; lack of pointed posterior end of a cell body; as well as a characteristic feature of posterior flagellum of H. exaratum, which coils up at motionless cells. In addition, this species have the same pellicular striation on ventral and dorsal sides of cells (H. exaratum has more developed dorsal striation than ventral).

H. ovale is similar to H. larseni, which was originally defined and described as H. exaratum (Larsen, 1987), and then separated into a new species (Lee and Patterson, 2000). H. larseni differs by pointed posterior end of a cell body. This feature can also appear in H. exaratum, but only at metaboly (Al-Quassab et al., 2002).

Notosolenus tamanduensis Larsen et Patterson, 1990.  
Found in the bays Kruglaya and Streletskaya (samples 22 and 24). See morphological descriptions.
by Larsen and Patterson (1990), and Lee and Patterson (2000).

Previously reported from marine waters of Brazil (Larsen and Patterson, 1990), Australia (Lee and Patterson, 2000); from freshwaters of Australia (Schroeckh et al., 2003).

**Notosolenus urceolatus** Larsen et Patterson, 1990.

Found in the bays Kazachya and Karantinnaya (samples 1, 3–4, 6, 9, and 18). See morphological descriptions by Larsen and Patterson (1990), Lee and Patterson (2000), Al-Quassab et al. (2002), and Lee et al. (2003).

Previously reported from marine waters of Korea (Lee, 2002), Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2008, 2015); Pechora Sea (Tikhonenkov, 2006); from freshwaters of the European part of Russia (Tikhonenkov, 2006), Australia (Schroeckh et al., 2003); from soils of China (Tikhonenkov et al., 2012).

**Petalomonas labrum** Lee et Patterson, 2000 (Fig. 2a, b).

Found in the bays Kazachya, Solenaya, and Kruglaya (samples 6, 16, and 21).

Cell body is oval with elongated posterior end, slightly curved to the right. Size of the cell body is 12–15×6.5–9 µm. Ventral side is slightly concave. Anterior end is round, with wide opening of the flagellar pocket, which has a dense marginal rim (Fig. 2b). Single flagellum approximately equal to cell body length or slightly longer. Nucleus on the right-hand side. Cells gliding, while the posterior end of the body raised above the substrate.

Morphology of observed cells almost completely corresponds to the original description (Lee and Patterson, 2000). This species similar to *P. poosilla*; however, *P. labrum* differs by larger body size, narrowed posterior end of the body, and a wide dense marginal rim of an opening of flagellar pocket.

Previously reported from marine waters of Australia (Lee and Patterson, 2000; Schroeckh et al., 2003) and White Sea (Tikhonenkov, 2006).

**Petalomonas minor** Larsen and Patterson, 1990.


Previously reported from marine waters of Korea (Lee, 2002), Kuwait (Al-Yamani and Saburova, 2010), Fiji (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Lee and Patterson, 2000; Al-Quassab et al., 2002; White Sea, Pechora Sea, Black Sea (Tikhonenkov, 2006), Red Sea (Tikhonenkov, 2009); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina et al., 2017b), China (Tikhonenkov et al., 2012), Australia (Schroeckh et al., 2003); from soils of China (Tikhonenkov et al., 2012).

**Petalomonas minuta** Hollande, 1942 [syn.: *Petalomonas minuta* Christen, 1962].

Found in the bay Kazachya (samples 4 and 9). See morphological descriptions by Larsen and Patterson (1990), Vørs (1992), Lee and Patterson (2000), and Lee et al. (2003, 2005).

Previously reported from marine waters of U.K. (Tong, 1997b), Korea (Lee, 2002), Fiji, Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Patterson and Simpson, 1996; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2015); White Sea, Pechora, Black Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992), Danish Wadden Sea (Larsen, 1987), Aegean Sea (Aydin and Lee, 2012), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993); from freshwaters of the European part of Russia (Prokina and Mylnikov, 2017), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), China (Tikhonenkov et al., 2012), Japan (Takamura et al., 2000), Australia (Schroeckh et al., 2003; Lee et al., 2005).

**Petalomonas poosilla** (Skuja, 1948) Larsen et Patterson, 1990.


Previously reported from marine waters of U.K. (Tong, 1997b), Korea (Lee, 2002), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990), Belize, Tenerife (Vørs, 1993b), Australia (Larsen and Patterson, 1990; Patterson and Simpson, 1996; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab
Fig. 2. Light microscopy of heterotrophic flagellates (DIC): a, b – Petalomonas labrum; c–e – Gweamonas unicus (d – turning of the cell, e – pellicular striation); f–g – Pendulomonas adriperis; h–j – Platychilomonas psammobia; k–m – Zoelucasa sablensis; n–o – Planomonas cephalopora; p–q – P. melba; r–t – Anastigomonas muscula (s – turning of the cell). Scale bars: a,b, h–j – 10 µm; c–g, k–t – 5 µm.
et al., 2002; Lee et al., 2003; Lee, 2008, 2015); White Sea, Pechora Sea, Black Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992), Danish Wadden Sea (Larsen, 1987), Aegean Sea (Aydin and Lee, 2012), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina and Mylnikov, 2017; Prokina et al., 2017b), Siberia (Romanov, 2005), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), China (Tikhonenkov et al., 2012), Japan (Takamura et al., 2000), Australia (Schroeckh et al., 2003; Lee et al., 2005), Ethiopia (Prokina et al., 2017a); from soils of Australia (Ekelund and Patterson, 1997).

**Ploeotia oblonga** Larsen et Patterson, 1990.

Found in the bay Kazachya (sample 8). See morphological descriptions by Larsen and Patterson (1990), Lee and Patterson (2000), and Al-Yamani and Saburova (2010).

Previously reported from marine waters of Kuwait (Al-Yamani and Saburova, 2010), Fiji (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Patterson and Simpson, 1996; Lee and Patterson, 2000; Lee, 2008, 2015); from freshwaters of the European part of Russia and China (Tikhonenkov, 2006), Hungary (Kiss et al., 2008), Australia (Schroeckh et al., 2003).

**Urceolus cristatus** Larsen et Patterson, 1990.

Found in the bay Kazachya (sample 4). See the morphological description by Larsen and Patterson (1990).

Previously reported from marine waters of Fiji (Larsen and Patterson, 1990).

**Urceolus pasheri** Skvortzow, 1924.


Previously reported from White Sea (Tikhonenkov, 2006), Danish Wadden Sea (Larsen, 1987); from freshwaters of Siberia (Romanov, 2005), Ukraine (Vetrova, 1980), Japan (Takamura et al., 2000).

****Kinetoplastea Honigberg, 1963

*****Metakinetoplastina Vickerman in Moreira et al., 2004

******Neobodonida Vickerman in Moreira et al., 2004

**Neobodo curvifilus** (Griessmann, 1914) Moreira et al., 2004 [bas.: *Bodo curvifilus* Griessmann, 1913].

Found in the bay Solenaya (sample 15). See morphological descriptions by Tong et al. (1997), Lee et al. (2003), and Shatilovich et al. (2010).

Previously reported from marine waters of U.K. (Tong, 1997b), Greenland (Vørs, 1993a), Australia (Lee and Patterson, 2000; Lee et al., 2003; Lee, 2015), Antarctic (Tong et al., 1997); White Sea, Pechora Sea, Black Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina, Mylnikov, 2017), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), China (Tikhonenkov et al., 2012), Antarctic (Butler et al., 2000); from soils of Siberia (Shatilovich et al., 2010).

**Neobodo designis** (Skuja, 1948) Moreira et al., 2004 [bas.: *Bodo designis* Skuja, 1948].

Found in the bays Kazachya, Kamyshovaya, Karantinnaya, and Kruglaya (samples 1–2, 4, 6, 8–12, 17–18, and 23). See morphological descriptions by Larsen and Patterson (1990), Vørs (1992), Lee and Patterson (2000), Al-Quassab et al. (2002), and Lee et al. (2003).

Previously reported from marine waters of U.K. (Larsen and Patterson, 1990; Tong, 1997b), Greenland (Vørs, 1993a), Korea (Lee, 2002), Belize, Tenerife (Vørs, 1993b), Fiji, Hawaii, Panama, Brazil, Danish Wadden Sea (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Patterson and Simpson, 1996; Lee and Patterson, 2000; Lee, 2008, 2015); from freshwaters of the European part of Russia and China (Tikhonenkov, 2006), Hungary (Kiss et al., 2008), Australia (Schroeckh et al., 2003).
Rhynchomonas nasuta (Stokes, 1888) Klebs, 1893 [bas.: Heteromita nasuta Stokes, 1888].

Found in all observed bays (samples 2–8, 13–19, 22, and 24). See morphological descriptions by Larsen and Patterson (1990), Lee and Patterson (2000), Al-Quassab et al. (2002), and Lee et al. (2005).

Previously reported from marine waters of U.K. (Tong, 1997b), Canada (Vørs, 1993a), Korea (Lee, 2002), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Patterson and Simpson, 1996; Tong et al., 1997; Lee et al., 2000; Al-Quassab et al., 2002, 2015), Antarctic (Tong et al., 1997); White Sea, Bering Sea, Black Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Vørs, 1992; Ikävalko, 1998), Mediterranean Sea (Hausmann et al., 2002), Northern Atlantic Ocean (Patterson et al., 1993), equatorial Pacific Ocean (Vørs et al., 1995); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina and Mylnikov, 2017; Prokina et al., 2017b), Hungary (Kiss et al., 2008), Germany (Auer and Arndt, 2001), Mongolia (Kopylov et al., 2006), China (Tikhonenkov et al., 2012, Tikhonenkov, 2006, Tong et al., 1998), Japan (Takamura et al., 2000); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina and Mylnikov, 2017; Prokina et al., 2017b), Hungary (Kiss et al., 2008), Germany (Auer and Arndt, 2001), U.K. (Tong, 1997b), Mongolia (Kopylov et al., 2006), China (Tikhonenkov et al., 2012, Tikhonenkov, 2006), Japan (Takamura et al., 2000), Thailand (Charubhun and Charubhun, 2000), Ethiopia (Prokina et al., 2017a), Australia (Lee et al., 2005), Antarctic (Butler et al., 2000).

Parabodo nitrophilus Skuja, 1948.

Found in the bay Kazachya (samples 2 and 4). See the morphological description by Mylnikov et al. (2002).

Previously reported from White Sea (Tikhonenkov, 2006); from freshwaters of the European part of Russia (Tikhonenkov, 2006), China (Tikhonenkov et al., 2012); from soils of China (Tikhonenkov et al., 2012).

Parabodo caudatus (Dujardin, 1841) Moreira et al., 2004 [bas.: Amphimonas caudata Dujardin, 1841; syn.: Diplomastix caudata Kent, 1880; Bodo caudatus (Dujardin, 1841) Stein, 1878].

Found in the bay Kazachya (samples 5 and 11). See morphological descriptions by Al-Quassab et al. (2002) and Lee et al. (2005).

Previously reported from marine waters of Australia (Al-Quassab et al., 2002); from freshwaters of the European part of Russia (Prokina and Mylnikov, 2017; Prokina et al., 2017b), China (Tikhonenkov et al., 2012), Japan (Takamura et al., 2000), Australia (Lee et al., 2005); from soils of China (Tikhonenkov et al., 2012).

Parabodonida Vickerman in Moreira et al., 2004

Parabodo nitrophilus Skuja, 1948.

Found in the bay Kazachya (samples 2 and 4). See the morphological description by Mylnikov et al. (2002).

Previously reported from White Sea (Tikhonenkov, 2006); from freshwaters of the European part of Russia (Tikhonenkov, 2006), China (Tikhonenkov et al., 2012); from soils of China (Tikhonenkov et al., 2012).
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Patterson, 1990), Australia (Larsen and Patterson, 1990; Ekebom et al., 1995; Patterson and Simpson, 1996; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015); White Sea, Pechora Sea, Black Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992), Aegean Sea (Aydin and Lee, 2012), Mediterranean Sea (Tikhonenkov, 2006), Northern Atlantic Ocean (Patterson et al., 1993), equatorial Pacific Ocean (Vørs et al., 1995); from freshwaters of the European part of Russia (Prokina, Mylnikov, 2017).

**Incertae sedis EUKARYOTA**

**Devopaela elegans** Tong, 1995.

Found in the bay Kazachya (samples 1 and 8). See morphological descriptions by Tong (1997b), Lee and Patterson (2000), Al-Quassab et al. (2002), and Aydin and Lee (2012).


**Discocelis punctata** Larsen et Patterson, 1990.

Found in the bays Kazachya, Solenaya, and Karantinnaya (samples 1, 9, 16, 18). See morphological descriptions by Larsen and Patterson (1990), Tong et al. (1998), Lee and Patterson (2000), and Lee (2002).

Previously reported from marine waters of Korea (Lee, 2002), Fiji, Brazil (Larsen and Patterson, 1990), Australia (Tong et al., 1998; Lee and Patterson, 2000).

**Gweamonas unicus** Lee, 2002 (Fig. 2 c–e).

Found in the bays Kazachya and Karantinnaya (samples 6, 9, and 18).

Cell body is oval, 5–6×3–3.5 µm. Single flagellum is half as long as cell body length, directed posteriorly and coils up when the cell stops moving. Flagellum lies in a longitudinal groove, which is located on the ventral side of the cell body. Surface of the cell body with pellicular striations (Fig. 2 e). Cells slides slowly and often change direction of movement. When a cell turns, flagellum can leave the longitudinal groove (Fig. 2 d).

This species was originally described from marine waters of Australia (Lee, 2002). All features (size, cell morphology and behavior) correspond to the original description.

**Kiitoksia ystava** Vørs, 1992

Found in the bays Kazachya, Solenaya, and Karantinnaya (samples 6, 16, and 18). See morphological descriptions by Vørs (1992), Tong (1997b), Lee et al. (2003), and Aydin and Lee (2012).

Previously reported from marine waters of U.K. (Tong, 1997b), Canada (Vørs, 1993a), Australia (Tong et al., 1998; Lee et al., 2003; Lee, 2006, 2015); White Sea, Black Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992), Aegean Sea (Aydin and Lee, 2012), Mediterranean Sea (Hausmann et al., 2002), Red Sea (Tikhonenkov, 2009); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of Hungary (Kiss et al., 2008).

**Pendulomonas adriperis** Tong, 1997 (Fig. 2 f–g).

Found in the bay Kazachya (samples 4 and 8).

Cell body is oval, 4–5×3–4 µm, with small subapical depression on the ventral side. Flagella emerge from small prominence, located in the upper third part of the ventral side of cell body (Fig. 2 g). Cell attached to the substrate by the posterior flagellum, which periodically cowers. Anterior flagellum directed anteriorly and slightly vibrates. Length of flagella is approximately equal to the length of the cell body. Large nucleus located near flagella emergence. Cells can swim (Tong, 1997b, 1997c; Al-Quassab et al., 2002), but only attached cells were observed.

These species were originally described from marine waters of U.K. and Australia (Tong, 1997b). Morphology of observed cells almost completely corresponds to the first description, except for a narrowed posterior end of a cell body. Many authors described both narrowed and rounded posterior ends of a body (Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2002; Lee et al., 2003). Lee et al. (2003) described larger cell body sizes (7–10 µm). *P. adriperis* similar to species from the genus *Cafeteria* Fenchel et Patterson, 1988. However, flagella of *Cafeteria* emerge apically, while flagella of *Pendulomonas* emerge subapically. Moriya et al. (2000) described a very similar species *Wobblia lunata* Moriya et al., 2000 without references to *P. adriperis*. According to most researchers, *W. lunata* is a junior synonym of *P. adriperis* (Karpov et al., 2001; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006).

Previously reported from marine waters of U.K. (Tong, 1997b), Japan (as *Wobblia lunata* Moriya et al., 2000), Australia (Tong, 1997b; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2002; Lee et al., 2003); Red Sea (Tikhonenkov, 2009).
**Platychilomonas psammobia** Larsen et Patterson, 1990 (Fig. 2 h–j).

Found in the bay Kazachya (sample 10).

Cell body is flattened in dorsoventral direction, 11–14 × 7–10 µm, oval with slightly narrowed anterior and posterior ends. Cells attached to the substrate by posterior flagellum, distal part of which lies entirely on the substrate and folds into several turns of a spiral (Fig. 2 j). Anterior flagellum makes rhythmic beats, straightens out and collapses, slightly twisting the front end of the body (Fig. 2 h–i). Cells slightly swaying. Flagella emerge from the right-hand side of the cell and lie at the longitudinal groove. Row of extrusomes lies along the groove. Nucleus situated in the posterior part of the cell body.

Morphology of observed cells corresponds to known descriptions (Larsen and Patterson, 1990; Lee and Patterson, 2000; Al-Yamani and Saburova, 2010). However, the size of observed cells was slightly smaller. Consumers of algae (Lee and Patterson, 2000).

Previously reported from marine waters of U.K. (Tong, 1993, 1997b), Greenland, Canada (Tong et al., 1997), Tenerife (Vørs, 1993b), China (Tikhonenkov et al., 2012), Korea (Lee, 2002), Australia (Ekebom et al., 1995; Patterson and Simpson, 1996; Tong, 1997c; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015), Antarctic (Tong et al., 1997); White Sea, Pechora Sea, Black Sea, South China Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Vørs, 1992), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993), equatorial Pacific Ocean (Vørs et al., 1995); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina and Mylnikov, 2017; Prokina et al., 2017b), Siberia (Tikhonenkov, 2006), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), Ethiopia (Prokina et al., 2017a), Australia (Lee et al., 2005), Antarctic (Butler et al., 2000); from soils of China (Tikhonenkov et al., 2012), Australia (Ekelund and Patterson, 1997).

**Zoelucasa sablensis** Nicholls, 2012 (Fig. 2 k–m).

Found in the bay Kazachya (samples 12 and 14).

Cell body is ovoid, 8–10 × 5–7 µm, with elongated posterior and slightly narrowed anterior end. Cell body fills a lorica and is covered by large round siliceous scales with diameter 3–4 µm. Large flagellar pocket situated at the anterior part of the cell body. Flagella emerge from a small depression at the anterior end of the cell body and positioned in parallel (Fig. 2 l). Anterior flagellum is short (2–3 µm), directed anteriorly and makes weak flapping movements. Posterior flagellum is slightly longer than the cell body (Fig. 2 m). Cells gliding slowly. Empty lorica of dead cells were also observed in samples.

This species was originally described from Atlantic Ocean (Nicholls, 2012) and apparently is very rare. Morphology of observed cells corresponds to the original description.

*Ancyromonadida* Cavalier-Smith, 1998

**Ancyromonas sigmoides** Kent, 1880.

Found in all observed bays, except the bay Kamyshovaya (samples 1, 3–4, 6, 8–10, 12, 14–16, 18, 22, and 24). See morphological descriptions by Vørs (1992), Tong (1997b), Al-Quassab et al. (2002), and Lee et al. (2003, 2005).

Previously reported from marine waters of U.K. (Tong, 1993, 1997b), Greenland, Canada (Tong et al., 1997), Tenerife (Vørs, 1993b), China (Tikhonenkov et al., 2012), Korea (Lee, 2002), Australia (Ekebom et al., 1995; Patterson and Simpson, 1996; Tong, 1997c; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006, 2015), Antarctic (Tong et al., 1997); White Sea, Pechora Sea, Black Sea, South China Sea (Tikhonenkov, 2006), Kara Sea (Tikhonenkov et al., 2015), Baltic Sea (Vørs, 1992), Mediterranean Sea (Arndt et al., 2003), Red Sea (Tikhonenkov, 2009), Northern Atlantic Ocean (Patterson et al., 1993), equatorial Pacific Ocean (Vørs et al., 1995); from brackish waters of Siberia (Kopylov et al., 2002); from freshwaters of the European part of Russia (Tikhonenkov, 2006, Prokina and Mylnikov, 2017; Prokina et al., 2017b), Siberia (Tikhonenkov, 2006), Hungary (Kiss et al., 2008), Mongolia (Kopylov et al., 2006), Ethiopia (Prokina et al., 2017a), Australia (Lee et al., 2005), Antarctic (Butler et al., 2000); from soils of China (Tikhonenkov et al., 2012), Australia (Ekelund and Patterson, 1997).

**Ancyromonas** sp.

Found in the bay Kazachya (samples 1 and 4).

**Planomonas cephalopora** (Larsen et Patterson, 1990) Cavalier-Smith in Cavalier-Smith et al., 2008 [bas.: *Bodo cephalopora* Larsen et Patterson, 1990; syn.: *Ancyromonas cephalopora* (Larsen et Patterson, 1990) Heiss et al., 2010] (Fig. 2 n–o).

Found in the bays Kazachya, Karantinnaya, and Kruglaya (samples 12, 16, and 21).

Cell body is roundish with flattened ventral side and truncated anterior end. Size of the cell body is 4–6 × 3.5–5 µm. Two rows of extrusomes located in the wide rostrum (Fig. 2 n). Anterior flagellum is equal to cell body length, thin and weakly visible, and makes flapping movements. Posterior flagellum twice as long as the cell body, and bears acroneme. There are several large vacuoles in the posterior part of the cell.

This species differs from other species of the genera *Planomonas* and *Ancyromonas* by a larger size of a cell body, wide rostrum with two rows of extrusomes and large vacuoles in a posterior part of a cell. But sometimes the second row of extrusomes can be very difficult to see. Organism
was originally described as *Bodo cephaloporus* (Larsen and Patterson, 1990). However, the authors made a notice that placement of a new species in Kinetoplastida Honigberg, 1963 is questionable due to lack of electron microscopic studies, and assumed the change of a systematic position of this species in future.

Previously reported from marine waters of U.K., Fiji, Hawaii, Panama, Australia, Northern Atlantic (Larsen and Patterson, 1990).

*Planomonas melba* (Patterson et Simpson, 1996). Cavalier-Smith in Cavalier-Smith et al., 2008 [bas.: *Ancyromonas melba* Patterson et Simpson, 1996] (Fig. 2 p–q).

Found in the bay Kruglaya (sample 21).

Cell body is oval, dorsoventrally flattened, with slightly concave ventral side. Size of the cell body is 5–6 × 3.5–4.5 µm. Ventral groove is located at the anterior lateral part of the body and turns into a longitudinal depression along the ventral side of the body (Fig. 2 q). Rostrum exits into the ventral groove and carries extrusomes. Anterior flagellum emerges from the base of the rostrum; it is equal in length to the cell body, and in thickness corresponds to the posterior flagellum (Fig. 2 p). Posterior flagellum is acronematic, 1.5–2 times of cell body length, emerges from the ventral groove.

This species resembles the widespread *A. sigmoides* (Kent, 1880) Heiss et al., 2010. It can be distinguished from *A. sigmoides* (as well as from other species of *Ancyromonas* and *Planomonas*) by thick anterior flagellum, which is equal in thickness to posterior one. Also, *P. melba* is longer than *A. sigmoides* and their ventral side of a body concaved stronger. Some authors pointed out a complete reduction of an acronema of posterior flagellum (Tong et al., 1997), but it was observed in our investigation. Tong et al. (1998) mistakenly used the name *A. magna*, when described *A. melba*. However, *A. magna* Zhang et al., 1993 is a completely different species.

Previously reported from hypersaline waters of Australia (60–150‰) (Patterson and Simpson, 1996; Al-Quassab et al., 2002); from marine waters of Antarctica (Patterson and Simpson, 1996; Tong et al., 1997), Australia (as *A. magna* Tong et al., 1998).

*Planomonas micra* Cavalier-Smith in Cavalier-Smith et al., 2008 [syn.: *Ancyromonas micra* (Cavalier-Smith in Cavalier-Smith et al., 2008) Heiss et al., 2010].

Found in the bay Kazachya (samples 2 and 3). See the morphological description by Aydin and Lee (2012).

Previously reported from the Aegean Sea (Aydin and Lee, 2012).

*Amastigomonas caudata* Zhukov, 1975

Found in the bays Kazachya, Karantinnaya, and Kruglaya (samples 4, 9–10, 12, 18–19, and 22). See the morphological description by Prokina and Mylnikov (2017).

Previously reported from the White Sea, Black Sea (Tikhonenkov, 2006); from freshwaters of the European part of Russia (Tikhonenkov, 2006; Prokina and Mylnikov, 2017; Prokina et al., 2017b), China (Tikhonenkov et al., 2012).

*Amastigomonas muscula* Mylnikov, 1999 (Fig. 2 r–t).

Found in the bay Kazachya (sample 4).

Cell body is oval, 4–5 µm long. Anterior end of the cell body is tapered and slightly curved, forming a small proboscis. Posterior end of the cell body is rounded. Anterior flagellum is short, poorly visible. Posterior flagellum extends beyond the cell, 6.5–7 µm in length. Anterior one third of the body separated from posterior two thirds by the constriction, which is well visible during cell rotation (Fig. 2 s). There are many small granules in the cytoplasm. Flagellates move fast and directly.

This species was originally described from the Baltic Sea with salinity 8–9‰ (Mylnikov, 1999). Morphology of observed cells almost completely corresponds to the original description, but the author specified cell sizes almost 2 times larger. Nevertheless, we consider this fact as intraspecific variability.

*Cryptophyceae Pascher, 1913 emend. Schoenichen, 1925 emend. Adl et al., 2012

**Goniomonas Stein, 1878 [Goniomonadales Novarino et Lucas, 1993]**

*Goniomonas amphinema* Larsen et Patterson, 1990.

Found in the bay Kazachya (samples 4 and 6). See morphological descriptions by Larsen and Patterson (1990), Vørs (1992), Lee and Patterson (2000), and Al-Quassab et al. (2002).

Previously reported from marine waters of Korea (Lee, 2002), Belize, Tenerife (Vørs, 1993b), Fiji, Panama, U.K. (Larsen and Patterson, 1990),
Goniomonas truncata (Fresenius, 1858) Stein, 1878 [bas.: Monas truncata Fresenius, 1858].

Found in the bays Kazachya and Solenaya (samples 1, 6, 8, 12, and 16). See morphological descriptions by Vørs (1992), Ekelund and Patterson (1997), Lee et al. (2005), and Prokina et al. (2017).

Previously reported from marine waters of Belize (Vørs, 1993b); Baltic Sea (Vørs, 1992); from freshwaters of the European part of Russia (Tikhonenkov, 2006; Prokina and Mylnikov, 2017; Prokina et al., 2017b), Siberia (Romanov, 2005; Tikhonenkov, 2006), Hungary (Kiss et al., 2008), Germany (Auer and Arndt, 2001), Mongolia (Kopylov et al., 2006), China (Tikhonenkov, 2006; Tikhonenkov et al., 2012), Ethiopia (Prokina et al., 2017b), Australia (Lee et al., 2005); from soils of Siberia (Shatilovich et al., 2010), China (Tikhonenkov et al., 2012), Australia (Ekelund and Patterson, 1997).

**Kathablepharidae Vørs, 1992**


Previously reported from marine waters of U.K. (Tong, 1997b), Greenland, Canada (Vørs, 1993a), Australia (Lee and Patterson, 2000; Lee, 2006); White Sea (Tikhonenkov, 2006), Baltic Sea (Vørs, 1992; Ikävalko, 1998), Aegean Sea (Aydin and Lee, 2012), equatorial Pacific Ocean (Vørs et al., 1995).

**Kathablepharis sp.**

Found in the bays Kazachya and Karantinnaya (samples 9 and 18).

*Centroplasthelida Febvre-Chevalier et Febvre, 1984

**Pterocystida Cavalier-Smith, 2012

***Choanocystidae Cavalier-Smith et von der Heyden, 2007

Chaoanocystis aff. minima Zlatogursky, 2010 (Fig. 3 i–l).

Found in the bays Kazachya, Karantinnaya, and Kruglaya (samples 12, 20, and 21).

Cell body is covered by two types of siliceous scales. Radial scales with hollow shaft and heart-shaped flattened base. Slightly curved shaft is 3.5–6.0 μm in length and 0.05–0.07 μm in diameter, departs from the excavation of base. Base is 0.8–1.0×0.5–0.7 μm, with a dense marginal rim and 25–30 radial, sometimes branching ribs (Fig. 3 l). Ovoid plate scales are 3.5–4.5×1.5–2.0 μm, with wide longitudinal depression.

The species C. minima was originally described from freshwater lake in the European part of Russia (Zlatogursky, 2010). Morphology of observed cells is similar to the original description of C. minima: size of scales is completely consistent. However, we observed some differences in morphology: base of a shaft of C. minima with thin margin, without dense marginal rim, and without radial ribs; plate scales flattened, without longitudinal depression. Radial ribs on a base of shaft are known for C. ebelii Wujek et Elsner, 1992, but these species are different in many other aspects (size of radial scales of C. ebelii is larger; plate scales larger and with more elongated shape, possess subparallel ribs) (Wujek and Elsner, 1992; Mikrjukov, 2002). Morphology of C. lepidula Penard, 1904 is similar to observed cells, but size of scales in C. lepidula is larger, and its plate scales ornamented with granules (Siemensma and Roijackers, 1988; Mikrjukov, 2002). C. rossica (Mikrjukov, 1995) Mikrjukov, 2002 has larger size of scales, which are characterized by different morphology: shaft of radial scales expands to a base; base of radial scales with straight thin margin; plate scales flattened, with medial constriction (Mikrjukov, 2002).

***Pterocystidae Cavalier-Smith et von der Heyden, 2007


Found in the bays Kazachya and Kruglaya (samples 12 and 21).

Cell body is covered by two types of siliceous scales. Radial scales with cylindrical hollow shaft and rounded flattened base. Shaft of radial scales is 3.8–4.5 μm in length, 0.05–0.07 μm in diameter. Apex of shaft slightly tapers, with two teeth. Proximal part of the shaft strongly curved, connected to the base with lateral membranes, and forms deepening. Base of the shaft is 0.5–0.7 μm in diameter. Plate scales
Fig. 3. Electron microscopy of heterotrophic flagellates and centrohelid heliozoans (a–h, k–p – TEM; i–j – SEM). a – *Stephanoeca diplocostata*; b–d – *Paraphysomonas cyllicophora*; e–h – *Clathromonas butcheri* (g – plate mesh scales; h – crown-scale); i–l – *Choanocystis aff. minima* (j – radial scale; l – base of radial scale); m–p – *Raineriophrys raineri* (o – plate scales; p – radial scale). Scale bars: a, e–f, i, k, m–o – 5 µm; b–c – 3 µm; d, g–h, j, p – 1 µm; l – 0.5 µm.
Discussion

Among observed organisms, only 18 species have been indicated previously in the Black Sea (Mikrjukov and Mylnikov, 1998; Tikhonenkov, 2006). Forty-four species were found here for the first time. Most species are common and widespread, known not only from marine, but also from fresh–water habitats (35 species): Actinomonas mirabilis, Amastigomonas muscula, Anisonema acinus, Bicosoeca maris, Bodo saltans, Bordhamonas tropicana, Cafeteria marsupialis, C. roenbergensis, Clathromonas butcheri, Codosiga botrytis, Goniomonas amphiemena, G. truncata, Heteronema exaratum, H. ovale, Kiitoksia ystava, Metromonas simplex, Neobodo curvifilus, N. designis, N. urceolatus, Parabodo caudatus, P. nitrophila, Petalomonas minor, P. minutia, P. poosilla, Plocotia oblonga, Protaspa simplex, P. verrucosa, Pteridomonas danica, Raineriophrys raineri, Rhynchomonas nasuta, Salpingoeca amphoridium, S. megacheila, Urceolus pasheri. Fourteen species are also known from soils: Ancyromonas sigmoides, Codosiga botrytis, Goniomonas truncata, Metromonas simplex, Neobodo curvifilus, N. designis, Parabodo caudatus, P. nitrophila, Petalomonas minor, P. poosilla, Protaspa simplex, Rhyynchomonas nasuta, Salpingoeca amphoridium, S. megacheila. The data obtained here confirm the euryhalinity and ubiquity of the most observed flagellate and centrohelid morphospecies. But some species are known only from marine habitats, 11 of which (Amastigomonas muscula, Bicosoeca maris, Gweamonas unicus, Helkesimastix marina, Multicilia marina, Pendulomonas adriperis, Planomonas cephalopora, P. micra, Platychilomonas psammobia, Salpingoeca abyssalis, Zoelucasa sabelensis) are rare and probably poorly studied.

Most of observed species are common in different geographical zones from all continents. More than a half of studied species (50 species) were previously found in Australia. This is an indicator of a good study of the region, rather than greater species diversity in comparison with other continents. Thus, for a better understanding of the biogeography of protists, studies of species composition of protozoa throughout the world are necessary. The distribution of some species has not yet been studied, since they have recently been described, they are very rare or difficult to identify (Gweamonas unicus, Helkesimastix marina, Micrometopion nutans, Salpingoeca abyssalis, Zoelucasa sabelensis).

Most common species (found in more than a half of observed samples) were Cafeteria roenbergensis (17 samples), Rhynchomonas nasuta (16 samples) and Ancyromonas sigmoides (15 samples). Twenty two species were rare and found only in one sample (Amastigomonas muscula, Anisonema acinus, Bicosoeca gracilipes, B. maris, Bodo saltans, Codosiga botrytis, Dinema sp., Helkesimastix marina, Heteronema ovale, Neobodo curvifilus, Notosolenus sp., Metromonas simplex, Paraphysomonas cylindrophora, Planomonas melba, Platychilomonas psammobia, Plocotia oblonga, Protaspa simplex, P. verrucosa, Salpingoeca amphoridium, S. megacheila, Urceolus cristatus, U. pasheri).

Most of observed species are bacterivorous, but 14 species consume eukaryotes. Heterotrophic and phototrophic flagellates are consumed by 11 species: Actinomonas mirabilis; Choanoecystis aff. minima; Colponema sp.; Goniomonas amphiemena; G. truncata; Kathablepharis remigera; Kiahtablepharis sp.; Paraphysomonas cylindrophora; Platychilomonas psammobia; Pteridomonas danica; Raineriophrys raineri (Vors, 1992; Lee and Patterson, 2000). Nacked amoebae are consumed by Multicilia marina (Nikolaev et al., 2006). Diatoms are consumed by Heteronema exaratum and H. ovale (Lee and Patterson, 2000; Al-Quassab et al., 2006).

Morphology of observed cells of 18 species of heterotrophic flagellates and 2 species of heliozoans is consistent with previous descriptions; however, some variations in size of cell bodies, lorica, flagella, and stalks were observed. Cell bodies of Multicilia marina, Protaspa tegere, Pendulomonas adriperis,
Amastigomonas muscula and Heteronema ovale were smaller than described earlier (Larsen and Patterson, 1990; Ekebom et al., 1995; Mikrjukov and Mylnikov, 1998; Mylnikov, 1999; Lee and Patterson, 2000; Lee, 2002; Lee et al., 2003; Aydin and Lee, 2012). While observed cells of Protaspa verrucosa are characterized by larger size than previously reported (Vors, 1992; Tong et al., 1998).

Some morphological features (size and shape of body cells, siliceous scales and costae of lorica) can vary in different specimens due to intraspecific variability, influence of environmental parameters, or even variations within the same organism in different periods of ontogenesis (Leadate, 1979; Mikrjukov and Mylnikov, 1998; Lee and Patterson, 2000; Bergesch et al., 2008; Cavalier-Smith et al., 2009; Scoble and Cavalier-Smith, 2014). Changing of the water salinity can affect shape and size of the cell body of Multicilia marina (Mikrjukov and Mylnikov, 1998), as well as formation of contractile vacuoles and movement change in Helkesinastix marina (Cavalier-Smith et al., 2009). Studies of the life cycle in clonal cultures have shown changes of some morphological features in ontogenesis. E.g., number of ribs, size and shape of the cell body of Stephanoeca diplocostata, size of siliceous scales and numbers of hollows in scales of Clathromonas butcheri, localization of the nucleus in Protaspa tegere (Leadate, 1979; Lee and Patterson, 2000; Bergesch et al., 2008; Scoble and Cavalier-Smith, 2014).

Different authors use different approaches for systematics of heterotrophic flagellates and species identification and also interpret differently a morphological variability in cells, presence or absence of some features. For example, Salpingoeoca marina differs from Salpingoeoca infusionum by such a doubtful feature like narrowed anterior end of the lorica. Difference of S. inquillata, S. curvipes, S. ringens and S. eurystoma from S. infusionum is the widened anterior end of the lorica. As the result, different authors have different opinions on whether these organisms should be considered as different species or they need to be reduced to synonyms. For a better understanding of intraspecific variability, molecular phylogenetic and morphological studies in clonal cultures are needed.

Among the studied sites of the Black Sea, the greatest species richness was observed in scrapings from the base of the metal pier in the bay Kazachya (sample 6 – 22 species) and in sludge from the bay Solenaya (sample 16 – 20 species). Among all six observed bays, most species were found in the bay Kazachya (60 species), which may be caused by the larger number of samples taken in this bay in comparison with other bays. Only one species (Rhynchosomas nasuta) was found in all the observed bays.

Analysis of the similarity of flagellate communities by species composition in different types of habitats reveals four groups of microbiotopes with more than 50% similarity (Fig. 4). However, the most noticeable is the cluster of 5 samples with the similarity of more than 65%: (1) storm surges at the waterfront (the bay Kazachya, sample 5); (2) scrapings with Ulva sp. (the bay Kazachya, sample 11); (3) scrapings from stones (the bay Kazachya, sample 14); (4) detritus among thickets of Phragmites australis (the bay Solenaya, sample 15); (5) littoral sand (the bay Streletskaia, sample 24). There is no relationship between these microbiotopes (type of bottom sediment, distance from a shore, or a depth). Probably, there is not enough data for such analysis of species distribution within the microbiotopes and further researches are needed.

A total of 68 species of heterotrophic flagellates and 2 species of centrohelid heliozoans were encountered in this survey, of which 44 species are the first records for the Black Sea. Many species are known not only from marine but also from freshwater and soil habitats; many of them have a cosmopolitan distribution. Most observed species feed on bacteria, 14 species consume other protists (heterotrophic flagellates, algae, and naked amoebae). Morphology of observed cells almost completely corresponds with previous descriptions. New details of the structure and behavior of cells obtained during this study have complemented our knowledge of intraspecific variability of these organisms.

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Fig. 4. Dendrogram showing the Bray–Curtis similarity (%) between observed sites (numbering in accordance with Table 1).


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