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Free-living heterotrophic flagellates from bays of Sevastopol (the Black Sea littoral)

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Summary

Species composition and morphology of heterotrophic flagellates in littoral and supralittoral zones of the Black Sea (southwestern part of the Crimea) were studied in September 2017. A total of 77 species and forms of heterotrophic flagellates were encountered in this survey, 35 of them are new records for the Black Sea, and one, *Oscillata sevastopoliensis* gen. et sp. n., was described for the first time. The most frequent were *Metromonas simplex* and *Neobodo designis* (observed in 14 samples), *Lentomonas corrugata* (13 samples), *Amastigomonas mutabilis* and *Petalomonas poosilla* (10 samples). Species diversity in the present study was higher in comparison with the data obtained in May 2017 in the same region. Micrographs and morphological descriptions of 20 species (*Ministeria vibrans, Salpingoeca oblonga, Savillea parva, Stephanoeca apheles, S. cupula, Volkanus costatus, Cafeteria ligulifera, Paraphysomonas lucasi, Pteridomonas danica, Colponema marisrubri, Ovaloplaca salina, Clautriavia cavus, Dinema platysomum, Lentomonas corrugata, Notosolenus stutulum, N. tamanduensis, Hemistasia amylophagus, H. phaeocysticola, Heterochromonas opaca*, and *Oscillata sevastopoliensis* gen. et sp. n.) are given.

Key words: heterotrophic flagellates, protists, species diversity, morphology, Black Sea, Crimea, *Oscillata sevastopoliensis* gen. et sp. n.

Introduction

Heterotrophic flagellates (HF) – a polyphyletic group of heterotrophic protists moving or feeding with flagella at least in one stage of their life cycle (Patterson and Larsen, 1991). Most of HF morphospecies have a cosmopolitan distribution (Azovsky et al., 2016; Lee, 2015). Free-living HF are an important component of the microbial food web since they feed on bacteria and small protists, also being food objects for ciliates and metazoa (Arndt et al., 2000; Pomeroy, 1974; Sherr et al., 1982). HF are essential for the process of self-purification of water bodies (Umorin, 1976; Zhukov and Mylnikov, 1983). Their diversity, morphology, and distribution have been actively studied in the recent decades (Al-Qassab et al., 2002; Lee, 2002b, 2006a, 2006b, 2008, 2015; Lee et al., 2005; Schroeckh et al., 2003; Shatilovich et al., 2010; Aydin and Lee, 2012, Duangjan et al., 2017, etc.). The spread of electron

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microscopy, as well as the appearance of more powerful light microscopes equipped with phase- and differential interference contrast, contributed to improving the quality of species identification.

The study of HF in the Black Sea has begun relatively recently (Valkanov, 1970; Moiseev, 1980; Murzov et al., 1999; Kopylov and Romanenko, 2004; Tikhonenkov, 2006; Prokina et al., 2017b). Only a few papers aimed at identifying the species composition were published (Valkanov, 1970; Tikhonenkov, 2006; Prokina et al., 2017b), and they do not reveal complete information on the species diversity of this region. The aim of our research was to supplement the database on the species composition, morphology, biological features and distribution of free-living HF in bottom sediments of littoral part of the Black Sea (Southwestern Crimea) using light and electron microscopy.

Material and methods

In this paper, we employ an extension of our previous study, which examined the species composition and morphology of HF on the same territory in May 2017 (Prokina et al., 2017b). Samples were collected by Ilya S. Turbanov on September 01, 2017 at 16 stations. HF in 5 bays of the Black Sea near Sevastopol (Southwestern Crimea) were investigated: Kazachya bay (44°34'18.8"N, 33°24'40.2"E), Solenaya bay (44°34'31.4"N, 33°24'13.8"E), Karantinnaya bay (44°36'44.3"N, 33°29'58.1"), Kruglaya bay (44°36'06.1"N, 33°26'41.4"E), and Streletskaya bay (44°36'13.0"N, 33°28'09.5"E). Detailed descriptions of sampling sites are given in Table 1 in Prokina et al. (2017b). The map of the sampling points is shown in Fig. 1. Water temperature during the collection period varied within 21–23 °C, salinity – 18–20‰.

Benthic and periphytonic samples 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 bacteria *Pseudomonas fluorescens* Migula. 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 contrast and water immersion objectives (total magnification ×1120) was used for observations of living cells. Video recording was made by the analog AVT HORN MC1009/S video camera. Electron microscopy preparations were carried out according to the described methods (Moestrup and Thomsen, 1980) and observed in transmission electron microscope JEM-1011 (Jeol, Japan).

Results

Lists of observed HF species are presented in table 1. Seventy-seven species and forms of HF were identified, 35 of them are new for the Black Sea. Morphological descriptions of 20 species are given below. Descriptions of the remaining species was presented earlier, including our works (Al-Qassab et al., 2002; Lee, 2002b, 2006a, 2006b, 2008, 2015; Lee et al., 2005; Schroeckh et al., 2003; Aydin and Lee, 2012, Prokina and Mylnikov, 2017; Prokina et al., 2017a, 2017b, 2017c, etc.). We do not consider these species here since they correspond to the already known diagnoses. Used abbreviations: CL – cell body length; LM – light microscope; TEM – transmission electron microscope.

OPISTHOKONTA Cavalier-Smith, 1987 emend. Adl et al., 2005.

*Holozoa Lang et al., 2002.

**Filasterea Shalchian-Tabrizi et al., 2008.

***Ministeria Patterson et al., 1993 emend. Tong, 1997.

Ministeria vibrans Tong, 1997 (Fig. 2 a-c).

Fourteen cells were observed in LM from samples 6, 9, 11, 14, and 15.

Spherical cell body is 2.5–3.5 µm in diameter, with 10–15 radial thin filopodia 4.0–5.5 µm in length. Cells attach to the substrate with a single flagellum and make fast pendulum movements with small amplitude (Fig. 2c). Sometimes cells stop moving for a while. Flagellates feed on bacteria, predominantly rod-like. Bacteria adhere to the surface of the cell body. Food pseudopodia envelop a bacterium and draw it into the cell (Tong, 1993). This species differs from another member of the genus (*M. marisola* Patterson et al., 1998) by a larger number of radial filopodia, a flagellum that is attached to a substrate, and pendulum movements.

Previously reported from marine waters of UK (Tong, 1993), Australia (Lee et al., 2003).

**Choanomonada Kent, 1880.

***Craspedida Cavalier-Smith, 1997 emend. Nitsche et al., 2011.

Salpingoeca oblonga Stein, 1878 (Fig. 2d).

Two cells were observed in LM from samples 8 and 20.

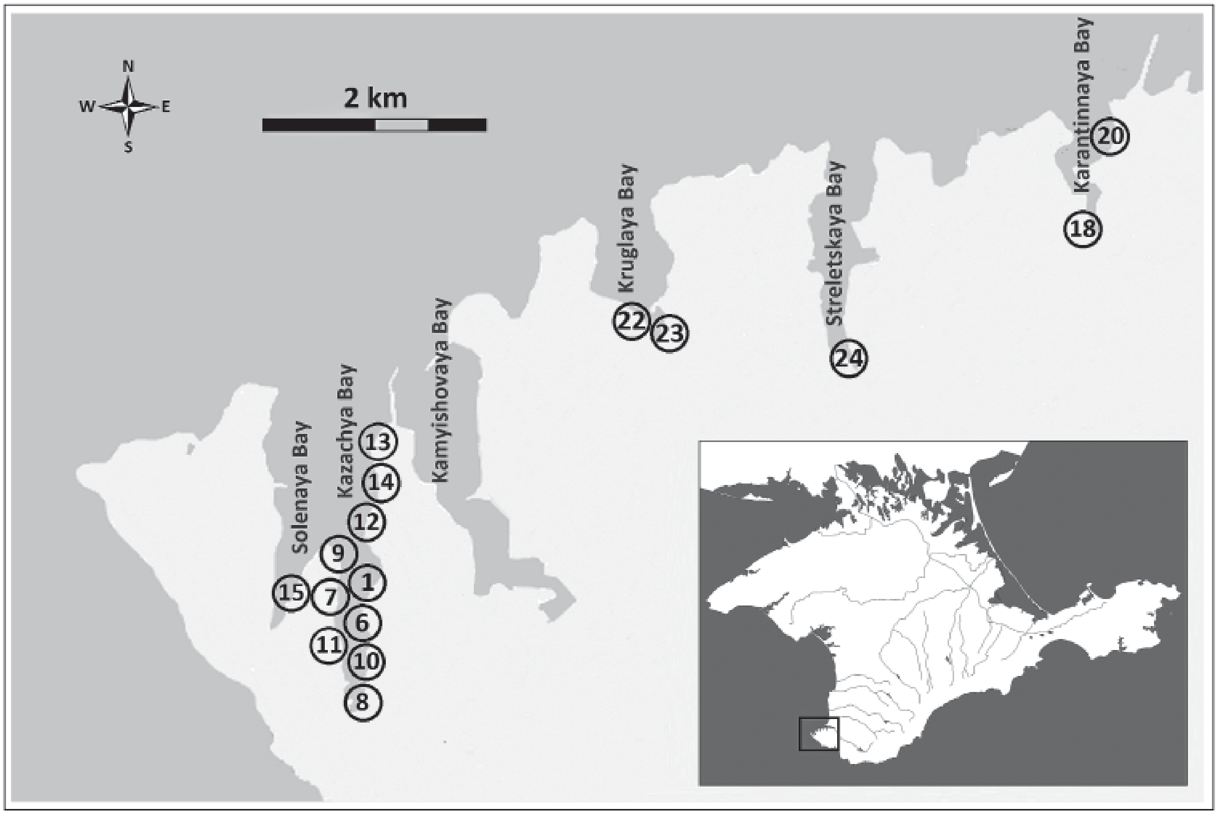

Fig. 1. Map of the observed sampling points; numbering in accordance with Prokina et al., 2017a (Table 1).

Oval cell body with rounded posterior and truncated anterior end, without neck, 2.5–3.5×5.0–9.5 µm. Collar is well developed, 1.5×CL, flagellum is 2–3×CL. Nucleus is located medially, food vacuole in the basal part of the cell body. Cell occupies about half length of the lorica and is located in its anterior part. Oval lorica with slightly expanded middle part and slightly narrowed basal part, 10–20 µm in length.

Previously reported from fresh waters of the European part of Russia (Kosolapova, 2005), Germany (Auer and Arndt, 2001), Japan (Takamura et al., 2000).

***Acanthoecida Norris, 1965 emend. Cavalier-Smith, 1997 emend. Nitsche et al., 2011.

Savillea parva (Ellis, 1929) Loeblich, 1967 [bas.: *Diaphanoeca parva* Ellis, 1929; syn.: *Ellisiella parva* Norris, 1965] (Figs 2e, 3a–b).

One living cell in LM and one lorica in TEM were observed from sample 6.

Round cell body is 4 µm in diameter and located in posterior part of lorica. Pear-shaped lorica with an extended basal part and narrowed anterior part with truncated margin. Lorica is 12 µm in length, 2.5–7.5 µm in diameter. Lorica is formed by almost transverse costae strips, twisted into a spiral, and longitudinal costae strips. Inner side of lorica is enveloped by a thin network (Fig. 3b).

Previously reported from marine waters of USA (Norris, 1965), Antarctica (Marchant and Perrin, 1990), Denmark (Leadbeater, 1972), Baltic Sea (Vørs, 1992).

Stephanoeca apheles Thomsen et al., 1991 (Fig. 3c).

Loricae of 2 cells were observed in TEM from sample 13.

Lorica consists of two chambers, separated by a slight narrowing. Posterior chamber is 4.5–5.0×5.0 µm, with numerous irregularly located longitudinal and transverse costae. Anterior chamber is 10–12×8.5–10 µm, consist of 13–14 longitudinal costae, slightly spirally twisted. Each longitudinal costa contains 3 rod-like strips, connected with each other due to a little overlap. Two transverse costae. The first is located at the margin of the lorica, contains 6 rod-like strips, slightly overlapping. Each strip joins with 4 longitudinal costae. The second transverse costa lies just below the widest part of

Table 1. Distribution of heterotrophic flagellates at the collection points* (map of sampling points see in Fig. 1; detailed descriptions of sampling sites are given in Table 1 in Prokina et al., 2017a).

	1	6	7	8	9	10	11	12	13	14	15	18	20	22	23	24
AMOEBOZOA Lühe, 1913, emend. Cavalier–Smith 1998																
**Multicilia* Cienkowsky, 1881																
Multicilia marina Cienkowsky 1881	–	+	–	+	–	–	+	+	+	+	+	–	+	–	–	–
OPISTHOKONTA Cavalier–Smith, 1987, emend. Adl et al., 2005																
*Holozoa Lang et al., 2002																
**Filasterea Shalchian–Tabrizi et al., 2008																
***Ministeria Patterson et al., 1993, emend. Tong, 1997																
Ministeria vibrans Tong, 1997	–	+	–	–	+	–	+	–	–	+	+	–	–	–	–	–
**Choanomonada Kent, 1880																
***Craspedida Cavalier–Smith, 1997, emend. Nitsche et al., 2011																
Codosiga botrytis (Ehrenberg, 1838) James–Clark, 1866	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Salpingoeca balatonis Lemmermann, 1910	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
S. infusionum Kent, 1880	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
S. gracilis James–Clark, 1867	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
S. marina James–Clark, 1867	–	–	–	–	+	+	–	–	–	–	–	–	–	–	+	+
S. napiformis Kent, 1880	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. oblonga Stein, 1878	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–
S. ringens Kent, 1880	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
S. schilleri (Schiller, 1953) Starmach, 1968	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. vaginicola Stein, 1878	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	+
Salpingoeca sp.	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
***Acanthoecida Norris, 1965, emend. Cavalier–Smith, 1997, emend. Nitsche et al., 2011																
Savillea parva (Ellis, 1929) Loeblich, 1967	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stephanoeca apheles Thomsen et al., 1991	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
S. cupula (Leadbeater, 1972) Thomsen, 1988	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+
S. diplocostata Ellis, 1930	–	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–
Volcanus costatus (Valkanov, 1970) Özdikimen, 2009	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
SAR																
*Stramenopiles Patterson, 1989, emend. Adl et al., 2005																
**Bicosoecida Grasse, 1926, emend. Karpov, 1998																
Bicosoeca exilis Penard, 1921	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
B. lacustris (James–Clark, 1867) Skuja, 1948	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caecitellus parvulus (Griessmann, 1913) Patterson et al., 1993	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
Cafeteria ligulifera Larsen et Patterson, 1990	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. minuta (Ruinen, 1938) Larsen et Patterson, 1990	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. roenbergensis Fenchel et Patterson, 1988	–	–	+	–	+	+	–	–	+	+	+	–	–	–	–	–
Cafeteria sp. 1	–	+	–	–	–	+	–	+	+	–	+	–	–	+	–	–
Cafeteria sp. 2	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–

Table 1. (Continuation).

	1	6	7	8	9	10	11	12	13	14	15	18	20	22	23	24
Pseudobodo tremulans Griessmann, 1913	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	+
**Chrysophyceae Pascher, 1914																
Paraphysomonas cylicophora Leadbeater, 1972	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
P. lucasi Scoble et Cavalier–Smith, 2014	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
**Dictyochophyceae Silva, 1980																
***Pedinellales Zimmermann et al., 1984																
Actinomonas mirabilis Kent, 1880	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	+
Ciliophrys infusionum Cienkowski, 1876	+	–	+	+	+	+	–	–	+	+	+	–	+	–	–	–
Pteridomonas danica Patterson et Fenchel, 1985	+	–	–	+	–	–	+	–	–	–	–	–	+	–	–	–
*Alveolata Cavalier–Smith, 1991																
***Incertae sedis* Alveolata																
Colponema marisrubri Mylnikov et Tikhonenkov, 2009	+	–	–	–	+	+	–	–	–	+	–	+	+	–	–	–
*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	+	–	–	–	–	+	+	+	–	+	+	–	+	–	+	–
Metromonas grandis Larsen et Patterson, 1990	+	+	–	–	+	–	–	–	–	+	–	–	+	–	–	–
M. simplex (Griessmann, 1913) Larsen et Patterson, 1990	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+
***Imbricatea Cavalier–Smith, 2011																
****Silicofilosea Adl et al., 2005, emend. Adl et al., 2012																
*****Thaumatomonadida Shirkina, 1987																
******Thaumatomonadidae Hollande, 1952																
Ovaloplaca salina (Birch–Andersen, 1973) Scoble et Cavalier–Smith, 2014	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+
*** Granofilosea Cavalier–Smith et Bass, 2009																
Massisteria marina Larsen et Pattersen, 1990	+	–	–	+	+	–	–	–	+	+	–	–	+	–	–	–
**** Incertae sedis Imbricatea																
Clautriavia cavus Lee et Patterson, 2000	+	+	–	–	–	+	–	+	+	–	–	–	–	+	–	–
***Thecofilosea Cavalier–Smith, 2003, emend. Cavalier–Smith, 2011																
****Cryomonadida Cavalier–Smith, 1993																
Protaspa gemmifera (Larsen et Patterson, 1990) Cavalier–Smith in Howe et al., 2011	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
P. simplex (Vørs, 1992) Cavalier–Smith in Howe et al., 2011	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
P. tegere (Larsen et Patterson, 1990) Cavalier–Smith in Howe et al., 2011	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–
EXCAVATA Cavalier–Smith, 2002, emend. Simpson, 2003																
*Discoba Simpson in Hampl et al., 2009																
**Discicristata Cavalier–Smith, 1998																
***Euglenozoa Cavalier–Smith, 1981, emend. Simpson, 1997																
****Euglenida Bütschli, 1884																
*****Heteronematina Leedale, 1967																

Table 1. (Continuation).

	1	6	7	8	9	10	11	12	13	14	15	18	20	22	23	24
Anisonema acinus Dujardin, 1841	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dinema platysomum (Skuja, 1939) Lee et Patterson, 2000	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–
Heteronema exaratum Larsen et Patterson, 1990	+	–	–	–	+	–	+	–	–	+	–	–	+	–	–	+
Lentomonas corrugata (Larsen et Patterson, 1990) Cavalier–Smith, 2016	+	+	–	+	+	+	+	+	+	+	+	–	+	+	–	+
Notosolenus alatellus Larsen et Patterson, 1990	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Notosolenus apocamptus Stokes, 1884	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
N. scutulum Larsen et Patterson, 1990	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
N. tamanduensis Larsen et Patterson, 1990	–	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–
N. urceolatus Larsen et Patterson, 1990	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Petalomonas abscissa (Dujardin, 1841) Stein, 1859	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. labrum Lee et Patterson, 2000	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–
P. minor Larsen and Patterson, 1990	+	+	+	–	–	+	+	+	–	+	–	+	–	+	–	–
P. poosilla (Skuja, 1948) Larsen et Patterson, 1990	+	+	–	–	+	+	+	–	–	+	+	+	–	+	–	+
Ploeotia pseudanisonema Larsen et Patterson, 1990	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
****Kinetoplastea Honigberg, 1963																
*****Metakinetoplastina Vickerman in Moreira et al., 2004																
******Neobodonida Vickerman in Moreirae et al., 2004																
Neobodo designis (Skuja, 1948) Moreira et al., 2004	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+
N. saliens (Larsen et Patterson, 1990) Moreira et al., 2004	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
Rhynchomonas nasuta (Stokes, 1888) Klebs, 1893	–	–	–	–	+	–	–	–	+	+	–	–	+	–	–	–
*****Incertae sedis Kinetoplastea																
Bordnamonas tropicana Larsen et Patterson, 1990	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–
Hemistasia amylophagus (Klebs, 1893) Lee, 2002	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
H. phaeocysticola (Scherffel, 1900) Elbrächter et al., 1996	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Incertae sedis EUKARYOTA																
Developayella elegans Tong, 1995	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Glissandra innuerende Patterson et Simpson, 1996	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Heterochromonas opaca Skuja, 1948	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Platychilomonas psammobia Larsen et Patterson, 1990	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Zoelucasa sablensis Nicholls, 2012	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
*Ancyromonadida Cavalier–Smith, 1998																
Ancyromonas sigmoides Kent, 1880	–	–	–	–	+	–	–	–	+	+	–	–	–	+	–	–
Ancyromonas sp.	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	+
Planomonas cephalopora (Larsen et Patterson, 1990) Cavalier–Smith in Cavalier–Smith et al., 2008	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
*Apusomonadidae Karpov et Mylnikov, 1989																

Table 1. (Continuation).

	1	6	7	8	9	10	11	12	13	14	15	18	20	22	23	24
Amastigomonas caudata Zhukov, 1975	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Amastigomonas griebensis Mylnikov, 1999	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Amastigomonas muscula Mylnikov, 1999	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Amastigomonas mutabilis (Griessmann, 1913) Molina et Nerad, 1991	+	+	–	+	+	–	–	+	+	+	–	–	+	–	+	+
*Cryptophyceae Pascher, 1913, emend. Schoenichen, 1925, emend. Adl et al., 2012																
**Goniomonas Stein, 1878 [Goniomonadales Novarino et Lucas, 1993]																
Goniomonas pacifica Larsen et Patterson, 1990	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
G. truncata (Fresenius, 1858) Stein, 1878	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
Oscillata sevastopoliensis gen. et sp. n.	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes. * Collection points: Kazachya bay (1 – littoral, scraping from a single stone; 6 – littoral, scraping from the base of the metal pier; 7 – littoral, sand and sludge; 8 – littoral, among thickets of *Phragmites australis*; 9 – littoral, sand and sludge; 10 – littoral, scraping from the *Cystoseira barbata*; 11 – littoral, scraping from *Ulva* sp.; 12 – littoral, sand; 13 – supralittoral, Native Miocene clays, waterfront; 14 – littoral, scraping from stones), Solenaya bay (15 – littoral, detritus among thickets of *Phragmites australis)*, Karantinnaya bay (18 – littoral, sand and sludge; 20 – littoral, sand and sludge), Kruglaya bay (22 – supralittoral, waterfront; 23 – littoral, sludge), Streletskaya bay (24 – littoral, sand).

the anterior chamber. It consists of 11–12 strips, located with significant overlapping of each other. Several transverse costae surround the narrowing which divides lorica into two chambers. Tong et al. (1998) described a greater number of longitudinal costae – 15–16.

Previously reported from brackish waters of Germany (Auer and Arndt, 2001), marine waters of USA (Thomsen et al., 1991), Australia (Tong et al., 1998), Baltic Sea (Vørs, 1992).

Stephanoeca cupula (Leadbeater, 1972) Thomsen, 1988 [bas.: *Pleurasiga cupula* Leadbeater, 1972] (Fig. 3d–e).

Loricae of three cells were observed in TEM from sample 13, 14, and 24.

Lorica consists of two chambers, separated by a small narrowing. Anterior chamber is broad, 6–7×7–9 µm, consists of 10 longitudinal and 3 transverse costae. Each longitudinal costa contains 3 strips, which are overlapping at the point of intersection with the transverse costae. Transverse costae contain numerous strips, which significantly overlap each other. They intersect with longitudinal costae approximately in the middle of their length. Posterior chamber is 2.5–5.0×5.0 µm, with a large number of longitudinal and transverse strips, darkened by protoplast.

Previously reported from marine waters of Denmark (Leadbeater, 1972), Antarctica (Marchant and Perrin, 1990), Australia (Tong et al., 1998).

Volkanus costatus (Valkanov, 1970) Özdikimen, 2009 [bas.: *Diplotheca costata* Valkanov, 1970] (Fig. 3f).

Lorica of one cell in TEM was observed from sample 13.

Lorica contains 2 chambers. Collar and flagellum located in anterior chamber, cell body – in posterior (basal) chamber. Posterior chamber is 5×5 µm, consists of evenly spaced expanded flattened perforated longitudinal strips. Anterior chamber is 6.5×10.0 µm, slightly expanded in basal and distal parts. It contains 21 longitudinal and 2 transverse costae. Longitudinal costae include rod-like strips with slightly expanded distal tips, where they are joined to the transverse strips. Basal part of longitudinal strips more expanded and flattened, similar to longitudinal strips of the posterior chamber. The most distal transverse costa consists of short (1.5–2.0 µm) extended flattened strips. The second transverse costa from the distal margin located closer to the junction of chambers and consists of rod-shaped strips. This species differs from other members of the genus (*V. elongata* (Nitsche et Arndt, 2008) Özdikimen, 2009 and *V. tricyclica* (Bergesch et al., 2008) Özdikimen, 2009) by lack of the stalk and smaller lorica (Bergesch et al., 2008). The anterior chamber of lorica of *V. tricyclica* is most widened in the middle part near the second transverse costae; posterior chamber more elongated and conically tapered towards the posterior end (Bergesch et al., 2008). *V. costatus*

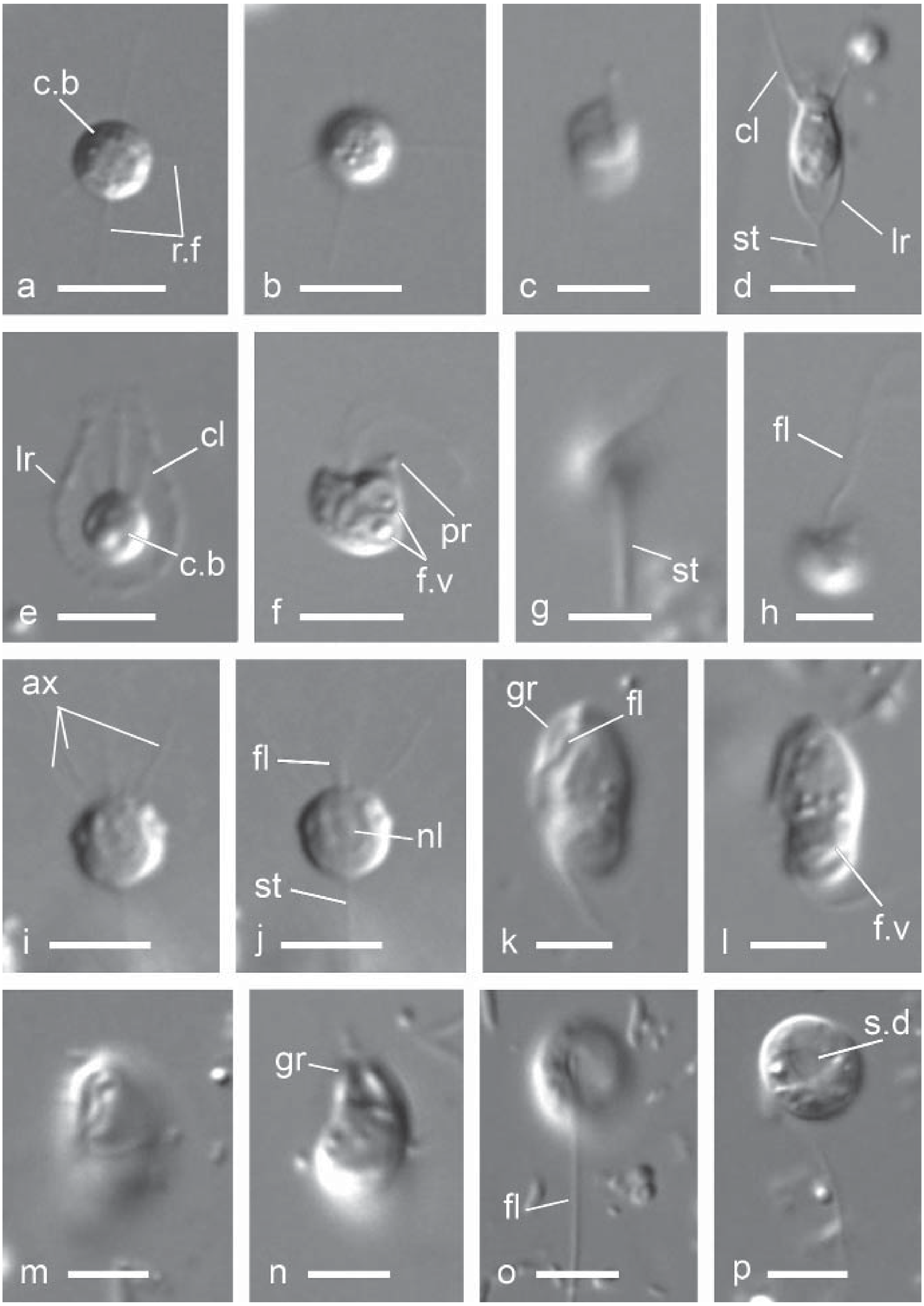

Fig. 2. Morphology of heterotrophic flagellates (LM): a–c – *Ministeria vibrans*; d – *Salpingoeca oblonga*; e – *Savillea parva*; f–h – *Cafeteria ligulifera*; i–j – *Pteridomonas danica*; k–n – *Colponema marisrubri*; o–p – *Clautriavia cavus*. *Abbreviations*: ax – axopodia, c.b – cell body, cl – collar, fl – flagellum, f.v – food vacuole, gr – groove, lr – lorica, nl – nucleus, pr – protrusion, r.f – radial filopodia, s.d – subapical depression, st – stalk. Scale bars: 5 μm.

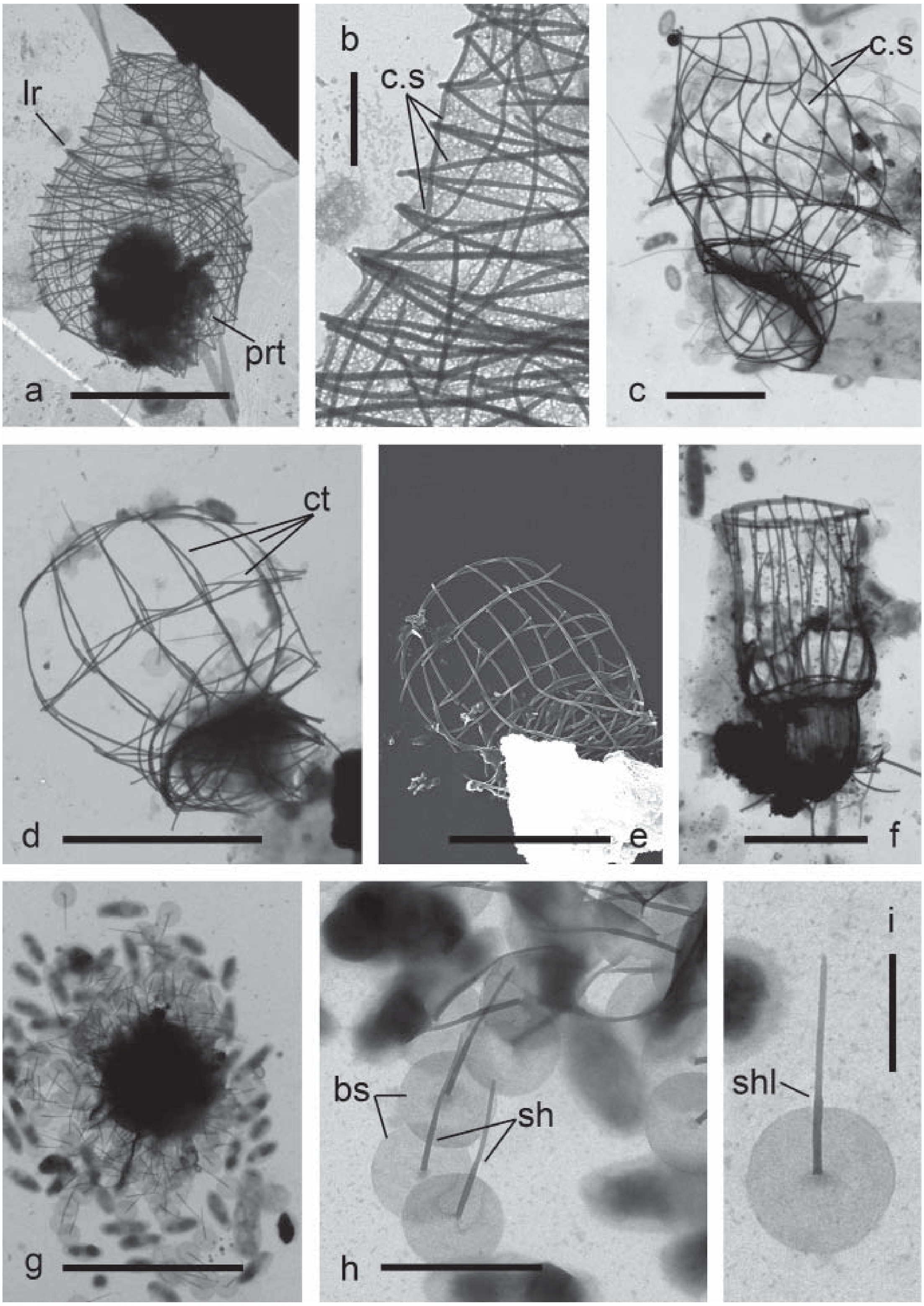

Fig. 3. Morphology of heterotrophic flagellates (a–d, f–I – TEM, e – SEM): a–b – lorica of *Savillea parva*; c – lorica of *Stephanoeca apheles*; d–e – lorica of *Stephanoeca cupula* (e – micrographs by D.G. Zagumyonnyi); f – lorica of *Volkanus costatus*; g–i – scales of *Paraphysomonas lucasi* (g – dryed cell with scales; h–i – scales). *Abbreviations*: prt – protoplast, c.s – costae strips, ct – costae, bs – base, sh – shaft, shl – sholders, the remaining notations as in Fig. 1. Scale bars: a, c–g – 5 μm; b, h – 1; i – 0.5 μm.

similar to *Stephanoeca elegans* (Norris, 1965) Throndsen, 1974, but differs by the presence of expanded flattened perforated strips in the posterior chamber.

Previously reported from marine waters of Denmark (Leadbeater, 1972), Croatia (Leadbeater, 1973), Australia (Tong, 1997; Lee et al., 2003), Black Sea (Valkanov, 1970).

SAR

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

**Bicosoecida Grasse, 1926 emend. Karpov, 1998.

Cafeteria ligulifera Larsen et Patterson, 1990 (Fig. 2f–h).

Two cells were observed in LM from sample 1.

Round cell body is 3.0–4.5 µm in diameter, with depression on the ventral side. Equal flagella are 2.5×CL. They emerge subapically from a ventral depression. Anterior flagellum slightly curved in an arc and oscillating along the entire length (Fig. 2f). Posterior flagellum directed backwards and attached to a substrate (Fig. 2e). A small sharp protrusion located on the ventral side of the body (Fig. 2g). Nucleus located anteriorly. This species is easy to distinguish from other members of the genus *Cafeteria* by the presence of a small anterior-lateral beak-like protrusion, as well as an equal length of flagella. It differs from *Pseudobodo tremulans* Griessmann, 1913 by the absence of a small collar in the base of the flagella.

Previously reported from marine waters of Hawaii (Larsen and Patterson, 1990), Australia (Patterson and Simpson, 1996), White Sea (Tikhonenkov et al., 2006), Pechora Sea (Mazei and Tikhonenkov, 2006), fresh waters of China (Tikhonenkov and Mazei, 2008).

**Chrysophyceae Pascher, 1914

Paraphysomonas lucasi Scoble et Cavalier-Smith, 2014 (Fig. 3g–i).

Scales of one cell were observed in TEM from sample 7.

Cell covered by one type of hobnail-like scales. Shaft of scales is 0.7–1.0 µm in length. Shaft slightly tapers approximately in the middle of length, forming small shoulders (Fig. 3i). Distal part of shaft conically tapers to a rounded apex. Round base of scales is 0.6–0.7 µm in diameter, without thickened margin and medial ring. This species differs from *P. imperforata* Lukas, 1967 by smaller scales, presence of small shoulders in the middle of a shaft length, and absence of a medial ring of the base.

Previously reported from marine waters of USA (Scoble and Cavalier-Smith, 2014).

**Dictyochophyceae Silva, 1980

***Pedinellales Zimmermann et al., 1984

Pteridomonas danica Patterson et Fenchel, 1985 (Fig. 2i–j).

Seventeen cells were observed in LM from samples 1, 8, 11, and 20.

Round to square in outline cell body is 3.5–6.0 µm in diameter, with swellings of food vacuoles on a surface of the cell body. One flagellum is 2.0–2.5×CL. Cell is attached to a substrate by a long (15–25 µm) thin contractile stalk. One ring of axopodia, 4.5–8.0 µm in length, located around the flagellum. Flagellate feed on bacteria and small protists. When victim sticks to the axopodia, they bend back to the medial part of the cell body, where food is absorbed (Tong, 1993). Several predators are able to unite around a common victim (Patterson and Fenchel, 1985; Mikrjukov, 2001). This species is very similar to *A. mirabilis* Kent, 1880 by its external morphology, but differs by the absence of the second ring of axopodia around flagellum and additional irregular axopodia in the middle and posterior parts of the cell body (Larsen and Patterson, 1990). *P. salina* Bourrelly 1953 differs by its ability to form tree-like colonies (Larsen and Patterson, 1990). *P. pulex* Penard 1890 differs by a preference to freshwater habitats and arrangement of axopodia – they depart from the anterolateral part of the cell body; *P. pulex* also has a larger cell body size – up to 12 µm (Patterson and Fenchel, 1985).

Previously reported from fresh waters of the European part of Russia (Kosolapova and Kosolapov, 2009), Germany (Auer and Arndt, 2001), Brazil (Tikhonenkov, 2006), Mongolia (Kopylov et al., 2006), marine waters of Denmark (Patterson and Fenchel, 1985), UK (Tong, 1993), Australia (Larsen and Patterson, 1990; Lee, 2015; Patterson and Simpson, 1996; Tong, 1997; Tong et al., 1998), Fiji, Hawaii, Brazil (Larsen and Patterson, 1990); White Sea (Mikrjukov, 2001; Tikhonenkov et al., 2006), Black Sea (Tikhonenkov, 2006; Prokina et al, 2017b), Baltic Sea (Patterson and Fenchel, 1985), North Atlantic Ocean (Patterson and Fenchel, 1985; Patterson et al., 1993), equatorial part of the Pacific Ocean (Vørs et al., 1995).

*Alveolata Cavalier-Smith, 1991

**Incertae sedis Alveolata

Colponema marisrubri Mylnikov et Tikhonenkov, 2009 (ig. 2k–n).

Five cells were observed in LM from samples 1, 9, 10, 14, 18, and 20.

Oval cell body is 7–10×3.5–5.5 μm, not flattened, with round posterior ends and small narrowing in the middle. Starving cells with a narrowed posterior end. Posterior half of the cell body is occupied by one large food vacuole (Fig. 2k–l). Well-marked wide groove on ventral side of an anterior part of cell body (Fig. 2k, 2m–n). Nucleus located anteriorly. Thin anterior flagellum is equal to cell body length and makes flapping movement. Thick posterior flagellum is 2×CL. Proximal part of posterior flagellum lies in a longitudinal groove and undulates (Fig. 2k, 2m). Flagellate quickly swims along a zigzag path. The flagellate is a predator, it feeds on small bodonids (Mylnikov and Tikhonenkov, 2009). Feeding behavior is the same as in all species of *Colponema* – whole cells absorption through a longitudinal groove. This species differs from other *Colponema* species by the absence of a contractile vacuole, smaller length of a longitudinal groove relative to cell body length, and smaller size of cells. General body plan and behavior of the observed organism resemble to a great degree the cell of recently described Ancoracysta twista (Janouškovec et al., 2017).

Previously reported from the Red Sea (Mylnikov and Tikhonenkov, 2009; Tikhonenkov, 2009).

*Rhizaria Cavalier-Smith, 2002.

** Cercozoa Cavalier-Smith, 1998 emend. Adl et al., 2005.

*** Imbricatea Cavalier-Smith, 2011.

****Silicofilosea Adl et al., 2005 emend. Adl et al., 2012.

*****Thaumatomonadida Shirkina, 1987.

******Thaumatomonadidae Hollande, 1952.

Ovaloplaca salina (Birch-Andersen, 1973) Scoble et Cavalier-Smith, 2014 [bas.: *Chrysosphaerella salina* Birch-Andersen, 1973; syn.: *Thaumatomastix salina* (Birch-Andersen, 1973) Beech et Moestrup, 1986] (Fig. 4a–b).

Five scales were observed in TEM from samples 13 and 24.

Two types of somatic scales – inner oval plate scales and external radial spicules (Tong, 1993). We observed only the first type of somatic scales. Plate scales consist of 2 plates 0.8–1.0×0.5–0.6 μm, pressed against each other (Fig. 4b). Lower plate without texture. Upper plate with a wide flat margin and a middle part, which divides across into 2 unequal parts. In each part along the margin, there passes a row of 5–7 openings, and 3–5 irregularly arranged openings located in a center (Fig. 4a).

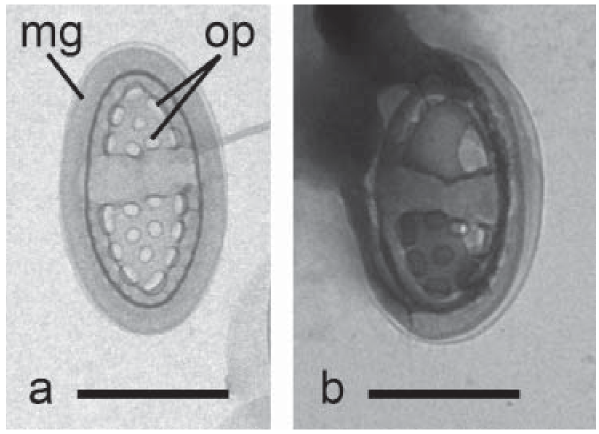

Fig. 4. Morphology of heterotrophic flagellates (TEM): a–b – scales of *Ovaloplaca salina*. *Abbreviations*: mg – margin, op - openings. Scale bar: 0.5 μm.

Previously reported from marine waters of UK (Tong, 1993), Norway (Ota et al., 2012), Baltic Sea (Vørs, 1992).

**** Incertae sedis Imbricatea

Clautriavia cavus Lee et Patterson, 2000 (Fig. 2o–p)

Seven cells were observed in LM from samples 1, 6, 10, 12, 13, and 22.

Round or oval rigid and flattened cell body is 5.5–7.0×4–7 μm. Ventral side slightly concave, with a subapical depression. Single flagellum is 2.5×CL. It emerges from subapical depression and is directed posteriorly. Nucleus located anteriorly, several food vacuoles – posteriorly. Flagellate is moving fast and directly. This species is distinguished from other members of *Clautriavia* by the smaller size. *C. mobilis* Massart, 1900 also differs by a nucleus located in the posterior part of the cell, and its flagellum is shorter than the cell body length (Lee and Patterson, 2000; Al-Quassab et al., 2002). *C. biflagellata* Chantangsi et Leander, 2010 differs by the presence of the second short motionless and slightly noticeable flagellum (Chantangsi and Leander, 2010).

Previously reported from marine waters of Korea (Lee, 2002b), Australia (Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2006b); White Sea (Tikhonenkov et al., 2006).

EXCAVATA Cavalier-Smith, 2002 emend. Simpson, 2003.

*Discoba Simpson in Hampl et al., 2009.

**Discicristata Cavalier-Smith, 1998.

***Euglenozoa Cavalier-Smith, 1981 emend. Simpson, 1997.

****Euglenida Bьtschli, 1884.

*****Heteronematina Leedale, 1967.

Dinema platysomum (Skuja, 1939) Lee et Patterson, 2000 [bas.: *Anisonema platysomum* Skuja, 1939; syn.: *Dinema inaequalis* Larsen et Patterson, 1990; *Dinematomonas inaequalis* Larsen et Patterson, 1990] (Fig. 5a–d).

Three cells were observed in LM from samples 1, 8, and 15.

Oval flattened cell body is 15–22×6–12 µm, with high metaboly (Fig. 5b–c) and well-marked longitudinal pellicular striation. Flagellar pocket and curved ingestion organelle are located on the left (Fig. 5b–c), nucleus located on the right. Thin anterior flagellum is equal to the cell body length when body is stretched, flapping from side to side. Trailing posterior flagellum is 2×CL. Several food vacuoles located in the posterior part of the cell (Fig. 5d). Flagellates feed on diatoms (Lee and Patterson, 2000). Size of the observed cells was smaller than described earlier – 20–32 µm (Larsen and Patterson, 1990; Ekebom et al., 1995; Lee and Patterson, 2000; Lee, 2006a; Aydin and Lee 2012; Duangjan et al., 2017). Differs from other species of the genus *Dinema* by smaller size of cells.

Previously reported from marine waters of Turkey (Aydin and Lee, 2012), Fiji ([as *D. inaequalis*] Larsen and Patterson, 1990), Australia (Lee and Patterson, 2000; [as *D. inaequalis*] Ekebom et al., 1995; [as *D. inaequalis*] Larsen and Patterson, 1990; Lee, 2006a); from fresh waters of Thailand (Duangjan et al., 2017), Australia ([as *D. inaequalis* and *D. platysomum*] Schroeckh et al., 2003).

Lentomonas corrugata (Larsen et Patterson, 1990) Cavalier-Smith, 2016 [bas.: *Ploeotia corrugata* Larsen et Patterson, 1990; syn.: *Lentomonas applanatum* Farmer et Triemer, 1994 non *Entosiphon applanatum* Preisig, 1979] (Fig. 5e–h).

Thirteen cells were observed in LM from samples 1, 6, 8, 9, 10, 11, 12, 13, 14, 15, 20, 22, and 24.

Oval cell body is 7.5–11.5×6–7 µm, with small concave in the anterior and posterior poles. Ingestion organelle equal to the cell body length, with 2 clearly visible rods. It inclines relative to the longitudinal axis of the body (Fig. 5e–f). Anterior flagellum is equal to the cell body length, oscillates along the entire length. Posterior flagellum is 2×CL. Several large food vacuoles located in posterior part of the body (Fig. 5h). Pellicula with 7 rounded longitudinal ribs on the dorsal side of the cell body (Fig. 5g–h), and 2 of them located laterally (Fig. 5f). Flagellar pocket located in the left side, nucleus – in the right.

L. applanatum Farmer et Triemer, 1994 is synonyms of *L. corrugata*, since both species have the same dimensions and morphology. *L. applanatum* Preisig, 1979 was originally described as *Entosiphon applanatum* due to presence of a protruding ingestion organelle (Larsen and Patterson, 1990). However, when Farmer and Triemer (1994) studied the ultrastructure of this species and separated it into a new genus, they did not point to the protruding ingestion organelle. So, they probably studied a different species (Ekebom et al., 1995; Cavalier-Smith, 2016). *L. azurina* (Patterson et Simpson, 1996) Cavalier-Smith, 2016 differs by a less elongated body shape, flattened cell body, the absence of depressions at the anterior and posterior poles, and shorter flagella (Al-Quassab et al., 2002).

Previously reported from marine waters of Turkey (Aydin and Lee, 2012), Australia (Larsen and Patterson, 1990; Ekebom 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, 2006a, 2008, 2015); White Sea (Tikhonenkov et al., 2006), Black Sea (Tikhonenkov, 2006), Red Sea (Tikhonenkov, 2009), Northern Atlantic (Patterson et al., 1993); fresh waters of the European part of Russia (Tikhonenkov and Mazei, 2007), Abkhazia, China (Tikhonenkov, 2006), Australia (Schroeckh et al., 2003).

Notosolenus scutulum Larsen et Patterson, 1990 (Fig. 5i–j).

Three cells were observed in LM from samples 15, 18, and 20.

Rhomboid flattened cell body is 7–11×4.5–6.0 µm, with narrowed anterior and posterior ends of the body and widened middle part. A slightly expanded aperture of the flagellar pocket at the anterior end of the body. Anterior flagellum is 1.5–2.0×CL. Posterior flagellum is equal to the length of the cell body (Fig. 5i). Medial longitudinal grooves are located on ventral and dorsal sides. A ventral groove is thinner, a dorsal groove expanding toward the posterior end of the cell body (Fig. 5j). Flagellar pocket located on the right side, nucleus – on the left.

Size of the observed cells was slightly smaller than of those previously described (9–15 µm), and length of posterior flagella was smaller – 0.3–0.5 ×CL (Larsen and Patterson, 1990; Lee and Patterson, 2000; Al-Quassab et al., 2002). This species is distinguished from others by the expansion of

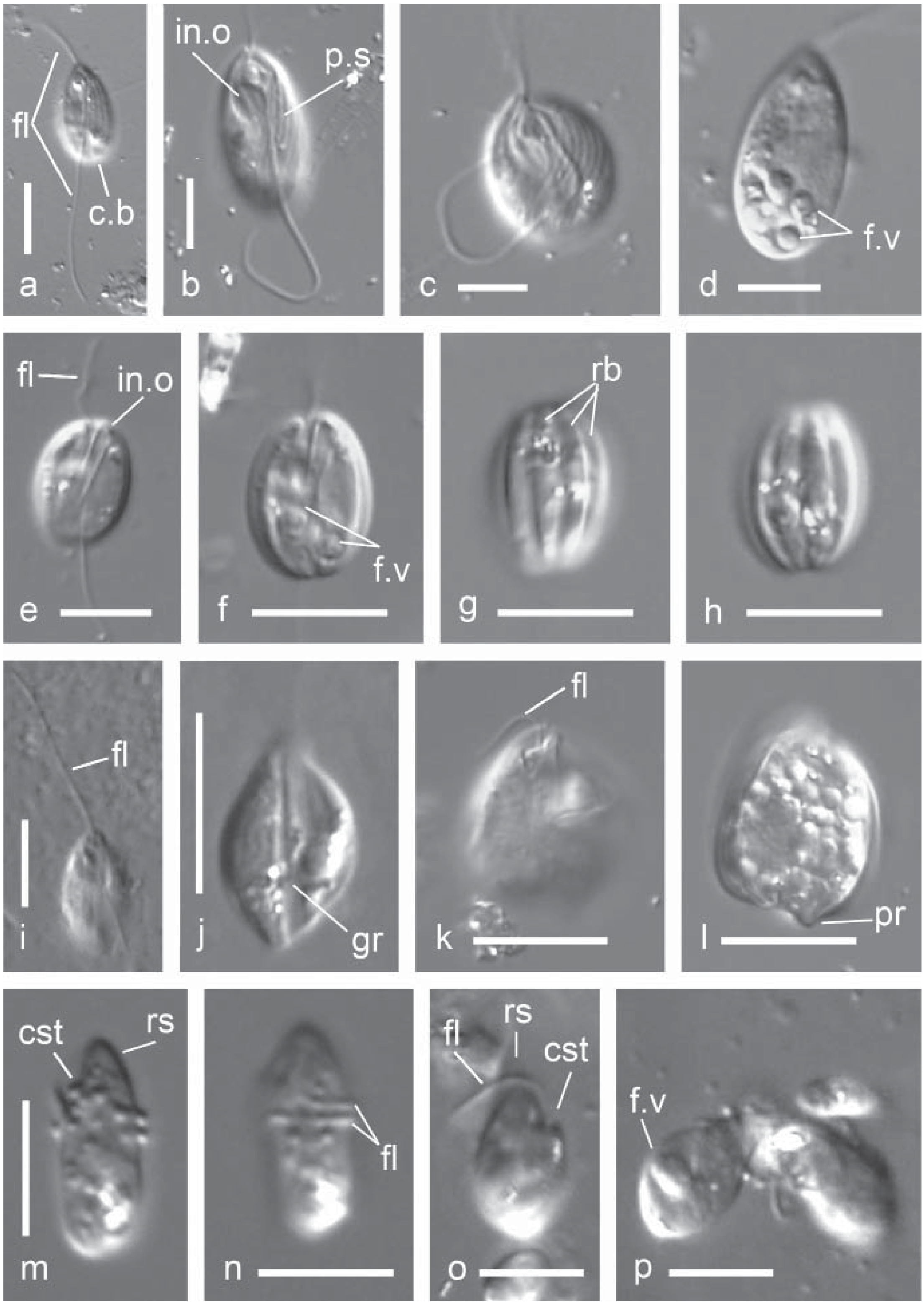

Fig. 5. Morphology of heterotrophic flagellates (LM): a–d – *Dinema platysomum*; e–h – *Lentomonas corrugata*; i–j – *Notosolenus scutulum*; k–l – *Notosolenus tamanduensis*; m–n – *Hemistasia amylophagus*; o–p – *Hemistasia phaeocysticola*. *Abbreviations*: in.o – ingestive organelle, rb – ribs, cst – cytostome, rs – rostrum, the remaining notations as in Figs 1 and 2. Scale bar: 10 µm.

a dorsal longitudinal groove to the posterior end of the body. Similar species (*N. mediocanellatus* (Stein, 1878) Schroeckh et al., 2003, *N. canellatus* Skuja, 1948, *N. lashue* Lee et Patterson, 2000, and *N. pyriforme* Lee et Patterson, 2000) differ by their shape and dimensions of cells (Schroeckh et al., 2003; Lee et al., 2005; Duangjan et al., 2017).

Previously reported from fresh waters of the European part of Russia (Tikhonenkov and Mazei, 2006); marine waters of Australia (Lee and Patterson, 2000; Al-Quassab et al., 2002); Brazil (Larsen and Patterson, 1990); Red Sea (Tikhonenkov, 2009).

Notosolenus tamanduensis Larsen et Patterson, 1990 (Fig. 5k–l).

Three cells were observed in LM from samples 8, 10, and 12.

Rigid oval cell body with wedge-shaped protrusion at the posterior end and two lateral protrusions (Fig. 5l). Size of cell body is 13.0–16.5×10–12 µm. Expanded opening of flagellar pocket at anterior end of cell body (Fig. 5k). Anterior flagellum is 0.5–1.0×CL, posterior flagellum half shorter than the anterior one. This species is similar to *Petalomonas platyrhyncha* Skuja 1948, *P. praegnans* Skuja 1948 and *P. tricarinata* Skuja 1939, but differs from those by presence of the second flagellum.

Previously reported from marine waters of Australia (Larsen and Patterson, 1990); Black Sea (Prokina et al., 2017b).

****Kinetoplastea Honigberg, 1963

*****Incertae sedis Kinetoplastea

Hemistasia amylophagus (Klebs, 1893) Lee, 2002a [bas.: *Phyllomitus amylophagus* Klebs, 1893] (Fig. 5m–n).

One cell was observed in LM from sample 22.

Elongate-oval, not flattened cell body with a weak metaboly, 12–15×4.5–7.0 µm. Rostrum located on the anterior end of the cell body, a well-marked cytostom located at its base (Fig. 5m). Flagella depart subapically from the lateral flagellar pocket and twist around the cell body (Fig. 5n). Large nucleus is located medially. Flagellate is a predator, quickly (in a few seconds) engulfs the prey entirely, while the cytostom greatly expands (Mylnikov, 1998).

This species is similar to *Pseudophyllomitus apiculatus* and *Rhynchobodo armata*, but differs by the absence of a contractile vacuole, and by the ultrastructural features (Mylnikov, 1998). Differs from *H. phaeocysticola* by a more elongated and cylindrical body shape, and the process of food absorbing (see below).

Previously reported from fresh waters of the European part of Russia (Tikhonenkov, 2007/2008), Germany (Auer et al., 2003), Japan (Takamura et al., 2000); Black Sea (Mylnikov, 1998; Tikhonenkov, 2006).

Hemistasia phaeocysticola (Scherffel, 1900) Elbrachter et al., 1996 [bas.: *Oxyrrhys phaeocysticola* Scherffel, 1900; syn.: *Pronoctiluca phaeocysticola* (Scherffel, 1900) Pavillard, 1922; *Hemistasia klebsi* Griessmann, 1913] (Fig. 5o–p).

Three cells were observed in LM from sample 1.

Oval or ovoid, not flattened cell body with metaboly, 7–15×3.5–5.5 µm. Well-fed cells have a rounded posterior half of the body with one or more large food vacuoles. The anterior end is narrowed to a small rostrum (Fig. 5o). Flagella are 1.5×CL, emerge in parallel from the antero-lateral flagellar pocket. Cytostome is located at the base of the rostrum (Fig. 5o). Cell surface is smooth. Cells can rotate around the longitudinal axis of the body during stops and feeding. Flagella twist around the cell body. Flagellate swims quickly, straight or spirally, and can quickly stop moving and change direction.

Feeds on diatoms, dinoflagellates, copepods, dead ciliates (Elbrachter et al., 1996; Lee and Patterson, 2000), cannibalism is also present (Elbrachter et al., 1996). Elbrachter et al. (1996) described the process of digestion: small portions of the cytoplasm of decaying cells are consumed through the cytostome. Many authors noted larger cell body sizes – 16–33 µm (Elbrachter et al., 1996; Al-Quassab et al., 2002; Lee, 2006b, 2015). From *H. amylophagus* differs by the shape of cell body and way of food absorbing (see above).

Previously reported from marine waters of Germany (Elbrachter et al., 1996), Korea (Lee, 2002b), Australia (Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee, 2006b); Baltic Sea (Vørs, 1992).

EUKARYOTA incertae sedis

Heterochromonas opaca Skuja, 1948 (Fig. 6a–d).

Two cells were observed in LM from sample 9.

Round cell body is 7.0–10.5 µm in diameter, rigid and flattened, with a small protrusion on anterior end of the cell body. Flagella emerge from a subapical depression on ventral side of the cell body. Anterior flagellum approximately equal or slightly longer than the cell body, directed forward and flapping. Posterior flagellum is 2.5–3.0×CL. Surface of cells is rough, with hillocks. Sometimes the cells stop moving, and flagella are twisted into a spiral (Fig. 6d). This species differs from other flagellates by the presence of an apical anterior protrusion. Lee and Patterson described larger size of cells (18 µm), shorter posterior flagellum (1.0–1.5 times longer than the cell body) (Lee and Patterson, 2000; Lee, 2002b).

Previously reported from marine waters of Korea (Lee, 2002b), Australia (Lee and Patterson, 2000).

Oscillata sevastopoliensis gen. et sp. n. (Fig. 6e–j).

Two cells were observed in LM from sample 6.

Free-living heterotrophic biflagellate protists of unknown affinity. Spherical cell body is about 2 µm in diameter. Two unequal flagella depart from the posterior part of the cell body and are directed to the opposite sides. Flagella are very long relative to the size of cell body. Short flagellum is about 15 µm, long flagellum is about 25 µm. Both flagella are elevated above the substrate, forming an arc, attached to the substrate only by tips (Fig. 6j). Cell body rapidly oscillates from side to side with small amplitude – 2–4 µm (Fig. 6h–i). Swimming forms were not observed.

Discussion

A total of 73 species of HF and 4 forms, not defined to the species level, were encountered in this survey (Table 1). The greatest number of species refer to SAR (24 species) and Excavata (20 species). Stramenopiles (14 species) and Rhizaria (9 species) prevail among SAR. Fifteen species are of the uncertain systematic position. The most frequent were *Metromonas simplex* and *Neobodo designis* (observed in 14 samples), *Lentomonas corrugata* (13 samples), *Amastigomonas mutabilis* and *Petalomonas poosilla* (10 samples). Thirty-two species were rare and found only in one sample: *Amastigomonas caudata, A. griebensis, Anisonema acinus, Bicosoeca exilis, B. lacustris, Cafeteria ligulifera, C. minuta, Cafeteria* sp. 2, *Codosiga botrytis, Hemistasia amylophagus, H. phaeocysticola, Heterochromonas opaca, Notosolenus alatellus, N. urceolatus, Paraphysomonas cylicophora, P. lucasi, Petalomonas abscissa, Platychilomonas psammobia, Protaspa gemmifera, P. simplex, Salpingoeca balatonis, S. infusionum, S. gracilis, S. napiformis, S. ringens, S. schilleri, Salpingoeca sp., Savillea parva, Stephanoeca apheles, Volcanus costatus, Zoelucasa sablensis*, and *Oscillata sevastopoliensis* gen. et sp. n. Only 5 of them were also rare in May (Prokina et al., 2017b).

Most of the observed species (65) were previously reported from marine habitats (Norris, 1965; Larsen and Patterson, 1990; Vørs, 1993; Ekebom et al., 1995; Patterson and Simpson, 1996; Tong, 1997; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003; Lee, 2006b; Mazei and Tikhonenkov, 2006; Tikhonenkov et al., 2006; Tikhonenkov, 2006, 2009; Prokina et al., 2017b, etc.). Many of them were also registered in brackish (20 species) and saline inland waters (21 species): *Actinomonas mirabilis, Amastigomonas griebensis, A. muscula, Ancyromonas sigmoides, Bicosoeca lacustris, Bordnamonas tropicana, Caecitellus parvulus, Cafeteria minuta, C. roenbergensis, Ciliophrys infusionum, Codosiga botrytis, Goniomonas pacifica, G. truncata, Massisteria marina, Metromonas simplex, Neobodo designis, N. saliens, Petalomonas minor, P. poosilla, Protaspa gemmifera, P. simplex, Pseudobodo tremulans*, *Pteridomonas danica, Rhynchomonas nasuta, Salpingoeca infusionum, S. marina, Stephanoeca apheles*, and *S. diplocostata* (Mylnikov, 1999; Auer and Arndt, 2001; Kopylov et al., 2002; Plotnikov et al., 2011). Four species were recorded in saline habitats for the first time: *Bicosoeca exilis, Salpingoeca gracilis, Salpingoeca napiformis, Salpingoeca schilleri*; previously they were found only in freshwaters (Kopylov et al., 2006; Tikhonenkov, 2006; Tikhonenkov et al., 2012).

Forty-one species were previously reported not only from saline but also from freshwater habitats: *Actinomonas mirabilis, Amastigomonas caudata, A. mutabilis, Ancyromonas sigmoides, Anisonema acinus, Bicosoeca exilis, B. lacustris, Bordnamonas tropicana, Cafeteria ligulifera, C. minuta, C. roenbergensis, Ciliophrys infusionum, Codosiga botrytis, Dinema platysomum, Goniomonas truncata, Hemistasia amylophagus, Heteronema exaratum, Lentomonas corrugata, Metromonas simplex, Neobodo designis, N. saliens, Notosolenus alatellus, N. apocamptus, N. scutulum, N. urceolatus, Petalomonas abscissa, P. minor, P. poosilla, Ploeotia pseudanisonema, Protaspa gemmifera, P. simplex, Pseudobodo tremulans, Pteridomonas danica, Rhynchomonas nasuta, Salpingoeca balatonis, S. gracilis, S. napiformis, S. oblonga, S. ringens, S. schilleri, S. vaginicola* (Takamura et al., 2000; Schroeckh et al., 2003; Lee et al., 2005; Kopylov et al., 2006; Tikhonenkov, 2006; Tikhonenkov and Mazei, 2006; Kiss et al., 2008; Tikhonenkov et al., 2012; Prokina and Mylnikov,

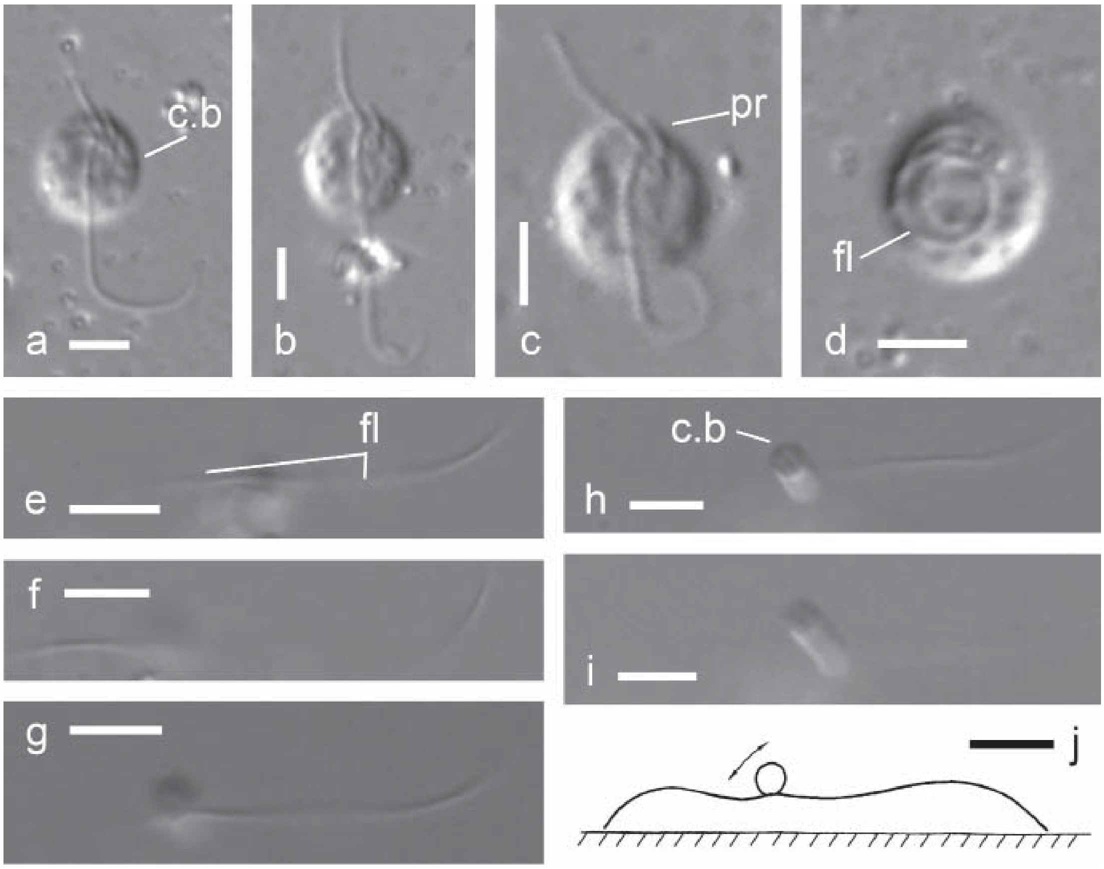

Fig. 6. Morphology of heterotrophic flagellates (a–i – LM; j – drawing): a–d – *Heterochromonas opaca*; e–j – *Oscillata sevastopoliensis* gen. et sp. n. *Abbreviations* as in Figs 1, 2 and 3. Scale bar: 5 µm.

2017). And 12 species were known from soil habitats: *Ancyromonas sigmoides, Codosiga botrytis, Goniomonas truncata, Massisteria marina, Metromonas simplex, Neobodo designis, Notosolenus apocamptus, Petalomonas minor, P. poosilla, Protaspa gemmifera, P. simplex, Rhynchomonas nasuta* (Ekelund and Patterson, 1997; Shatilovich et al., 2010; Tikhonenkov, 2010; Tikhonenkov et al., 2012).

The biogeography of protists is actively discussed. It is considered that most of the currently known morphospecies of HF have a cosmopolitan distribution (Lee and Patterson, 1998; Azovsky et al., 2016). Accordingly, most species observed here were previously reported from different geographical zones of many continents. Meanwhile, some species (*Salpingoeca napiformis, S. schilleri, Paraphysomonas lucasi, Colponema marisrubri, Hemistasia amylophagus, Heterichromonas opaca, Zoelucasa sablensis, Amastigomonas muscula*) are extremely rare and poorly studied (Mylnikov, 1999; Lee and Patterson, 2000; Takamura et al., 2000; Tikhonenkov, 2006, 2009; Nicholls, 2012; Scoble and Cavalier-Smith, 2014; Prokina et al., 2017b). However, the insufficient investigation of many regions of the planet prevents the formulation of certain conclusions since data for some regions are lacking; while other regions have been studied quite well. For example, more than a half of the observed species (51) were previously found in Australia (Larsen and Patterson, 1990; Patterson and Simpson, 1996; Ekelund and Patterson, 1997; Tong, 1997; Tong et al., 1998; Lee and Patterson, 2000; Al-Quassab et al., 2002; Lee et al., 2003, 2005; Schroeckh et al., 2003; Lee, 2006b, 2008; Lee, 2015).

Thirty-five species are new for the Black Sea: *Amastigomonas griebensis, A. mutabilis, Bicosoeca exilis, B. lacustris, Cafeteria ligulifera, C. minuta, Ciliophrys infusionum, Clautriavia cavus, Colponema marisrubri, Dinema platysomum, Glissandra innuerende, Hemistasia phaeocysticola, Heterochromonas opaca, Lentomonas corrugata, Ministeria vibrans, Notosolenus alatellus, N. apocamptus, N. scu-*

tulum, Oscillata sevastopoliensis gen et sp. nov., *Ovaloplaca salina, Paraphysomonas lucasi, Petalomonas abscissa, Ploeotia pseudanisonema, Protaspa gemmifera, Salpingoeca balatonis, S. gra-cilis, S. marina, S. napiformis, S. oblonga, S. ringens, S. schilleri, S. vaginicola, Savillea parva, Stephanoeca apheles, S. cupula.*

Among the studied sites of the Black Sea, the greatest species richness was observed in the samples collected by scraping from stones in the Kazachya bay (in sample 1 – 30 species, and in sample 14 – 24 species). Among all observed bays, the greatest species richness was found in the Kazachya bay (67 species), which, however, may be caused by the larger number of samples taken in this bay in comparison with the others. Species diversity in the present study was higher in comparison with the data obtained in May 2017 on the same territory: the average number of species in the sample in September was 15.7, while in May – 9.4. Lesser than a half (30 species) among all observed species were detected in the same territory in May, while the majority (43 species) previously were not observed (Prokina et al., 2017b). This indicates a high under-investigation of the bays in the vicinity of Sevastopol and the Black Sea as a whole. There is a high probability that further studies in this region will yield more new species.

A protist with unique morphology and cell behavior was observed in this study, wherefore we described the new genus and species *Oscillata sevastopoliensis* gen. et sp. n. This organism is characterized by very small spherical cell body and very long oppositely directed flagella. Both flagella are elevated above the substrate forming an arc. The cell rapidly oscillates in the upper part of this arc and does not touch substrate, while flagella ends are not moving, being constantly attached to the substrate.

Small spherical cell body, attachment to the substrate by flagellum, and vibrating movements also correspond to *Ministeria vibrans* Tong, 1997, but the latter species differs by a single flagellum and presence of numerous thin radial filopodia departing from the entire surface of the cell body. Also, a few species with trembling movements and a flagellum attached to the substrate are known: *Pendulomonas adriperis* Tong, 1997; *Wobblia lunata* Moriya, Nakayama et Inouye, 2000 (most likely, a synonym of *P. adriperis*); *Metromonas simplex* (Griessmann, 1913) Larsen et Patterson, 1990 and *M. grandis* Larsen et Patterson, 1990; *Symbiomonas scintillans* Guillou et Chretiennot-Oinet in Guillou et al., 1999.

However, only this newly observed organism is attached to the substrate with both flagella and keeps them in the arc position, while the cell, lo-cated medially, quickly oscillates from side to side. Both known species of the genus *Metromonas* (*M. simplex* and *M. grandis*), as well as *Metromonas* sp. described by Tikhonenkov (2007/2008) are attached to the substrate only by one flagellum and their cells make pendulum movements, while the second flagellum is reduced and sometimes not visible (Larsen and Patterson, 1990). Cells of *Pendulomonas adriperis* are with one flagellum attached to the substrate and periodically cowers, while the other flagellum is free and directs anteriorly (Tong, 1997). *Symbiomonas scintillans* is similar to the observed new species by the presence of very small and sometimes spherical cell body with vibrating movement but differs by possessing a free flagellum directed anteriorly (Tikhonenkov, unpubl.). Two known *Tremula* species (*T. longifila* Howe et Cavalier-Smith in Howe et al., 2011 and *T. vibrans* (Sandon, 1927) Cavalier-Smith in Howe et al., 2011) are also characterized by trembling movement, but their cells are gliding with both flagella stretched along the substrate (Howe et al., 2011).

The taxonomical position of the observed organism is currently unknown. It could be related to the described above stramenopiles or rhizarians with vibrating movements. Unfortunately, an effort to isolate this organism as a cell culture failed; but it will be undertaken again in the future. Therefore, we cannot exclude the possibility that the studied organism may represent a stage of the life cycle of some other protist, but it is unlikely. Also, the studied protist does not resemble any of the known dispersal flagellate stages of fungi, algae, plants, or Metazoa, which are actively swimming and are predominantly uniflagellar or heterokont. Molecular and ultrastructural data for this organism are necessary for clarification of its exact taxonomic position.

Taxonomic summary

EUKARYOTA incertae sedis

Oscillata n. gen.

Diagnosis: free-living heterotrophic biflagellate protist with spherical cell body. Two unequal flagella depart from the posterior part of the cell body and going in two different directions, attached to the substrate by tips. Cells elevated above the substrate, forming an arc and rapidly oscillating from side to side.

Etymology: The genus name comes from the oscillatory movement of the cell.

Zoobank Registration: urn:lsid:zoobank.org:act: 1B4A8B6F-B4CC-4F24-9844-0CBCDAA8AA8F.

Type species: *Oscillata sevastopoliensis*.

Oscillata sevastopoliensis n. sp.

Diagnosis: spherical cell body is about 2 µm in diameter and oscillates from side to side with small amplitude (2–4 µm). Flagella are very long relative to the size of cell body, short flagellum is about 15 µm, long flagellum is about 25 µm. Swimming forms were not observed. Type figure: figure 6e illustrates a live oscillating cell of the species.

Type locality: scraping of the base of the metal pier in Kazachya bay, Black Sea, Sevastopol.

Etymology: the species name means "Sevastopol-dwelling".

Zoobank Registration: urn:lsid:zoobank.org:act: 986F65F0-6263-4614-BBB1-F148E3EBC908.

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