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Species of the Genus *Eutreptiella* (Euglenophyceae) from Russian Waters of East/Japan Sea

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Abstract – The paper reviews data available on euglenophycean algae of the genus *Eutreptiella* from the Russian waters of East/Japan Sea. Five species (such as *E. braarudii, E. eupharyngea, E. gymnastica, E.* cf. *marina*, and *E. pascheri*) were identified in our study. *E.* cf. *marina* and *E. pascheri* were found for the first time in the far eastern seas of Russia (including East/Japan Sea, Bering and Okhotsk seas). Morphological descriptions of species are based on light and electron microscopical studies and supplied with information on ecology and distribution. Original micrographs of these species obtained by light and electron microscopy are presented. It was established that *E. eupharyngea, E. gymnastica,* and *E. pascheri* cause water "blooms" in spring and summer in eutrophic coastal areas of Peter the Great Bay (East/Japan Sea), where their cell concentrations at these periods exceed 1 x 10^6 cells l^{-1}.

Key words – Euglenophyceae, *Eutreptiella*, morphology

1. Introduction

The genus *Eutreptiella* da Cunha (Euglenophyceae) comprises nine marine and brackish-water species (Walne and Kivic 1990; Walne *et al.* 1986). The main results of taxonomic studies of the euglenophycean genera *Eutreptiella* and *Eutreptia* are presented in publications by Pringsheim (1953), Butcher (1961), Norris (1964), Throndsen (1969), and Walne *et al.* (1986). The type species of the genus *Eutreptiella, Eutreptiella marina* da Cunha, was only superficially described at its discovery (Da Cunha 1914). Later, Norris (1964) investigated materials from New Zealand and found specimens of the type species of the genus *Eutreptiella*. Algae of the genus inhabit mostly neritic zones of seas; some of them develop in a great mass (Throndsen 1993; Kato 1993).

Euglenophytes, like many other groups of unarmored flagellates in the far eastern seas of Russia (including East/Japan Sea, Bering and Okhotsk seas), are still poorly studied, which can be explained by technical difficulties of sampling and examination (Konovalova 2003). All our knowledge on representatives of the genus *Eutreptiella* found in East/Japan, Okhotsk and Bering seas is limited to findings of three species: *E. gymnastica, E. braarudii,* and *E. eupharyngea* (Konovalova 1999, 2000, 2003; Stonik and Selina 2001; Orlova *et al.* 2004). Of these species, a detailed morphological description based on light and electron microscopic examination is given only for *E. braarudii* (Stonik and Aizdaicher 2003).

The present study aims to examine composition and distribution of *Eutreptiella* species found in the Russian waters of the East/Japan Sea. The paper presents morphological descriptions and information on distribution of five species of the genus *Eutreptiella*, including morphological descriptions based on light microscopic examination of two species new for the far eastern seas of Russia (*E. pascheri* and *E.* cf. *marina*), as well as electron microscopic observations on the pattern of arrangement of flagellar hairs in *E. eupharyngea*.

2. Materials and Methods

The study used bathymetric samples collected in different seasons in 1993-2005 at ten stations in Peter the Great

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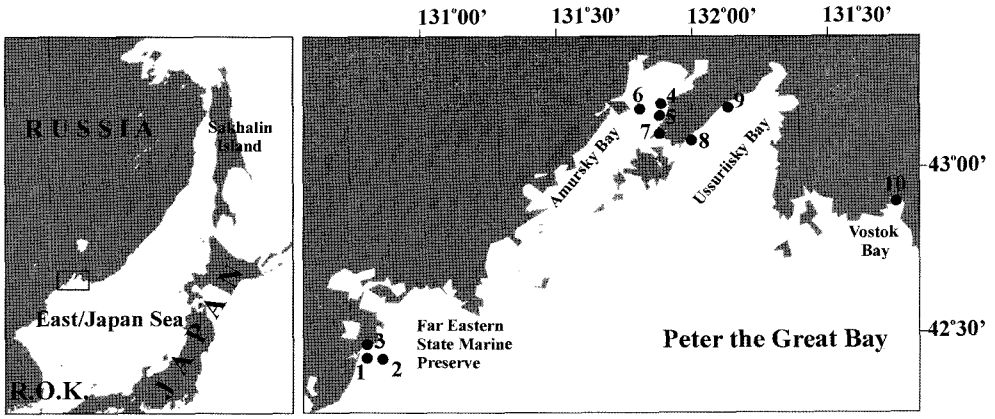

Fig. 1. Location of the sampling stations (1-10).

Table 1. Sampling stations in Peter the Great Bay

Area	No stations	Sampling date	No samples	Latitude N	Longitude E
Far Eastern State Marine Preserve	1	July – August 2003	4	42°26′	130°50′
	2	July – August 1996	2	42°26′	130°52′
	3	July – August 1996	4	42°28′	130°48′
Amursky Bay	4	January 1997 – May 1998 January – December 2005	24 21	43°11′	131°54′
	5	January 2003 – September 2004	28	43°09′	131°54′
	6	January 1993 – January 1994	26	43°11′	131°50′
Golden Horn Bay	7	January 1993 – January 1994 May 2001 – May 2002	21 11	43°06′	131°52′
Ussuriisky Bay	8	May – November 1999	7	43°05′	131°58′
	9	May 2001 – May 2002	19	43°11′	132°06′
Vostok Bay	10	July 2001 – June 2004	23	42°54′	132°46′

Bay, East/Japan Sea (including Amursky, Ussuriisky, Vostok and Golden Horn bays) (Fig. 1, Table 1). Stations 5 and 7 were located at a distance of about 600 and 500 m from industrial and domestic water discharges (Stonik and Orlova 2002; Zvyagintsev and Kondrateva 2002). Station 5 was located in a semi-closed man-made eutrophic sea basin within the city of Vladivostok. This sea basin of an area of about 70 thousand sq. m is separated from Amursky Bay with a rock-fill coffer-dam and has a free water exchange with Amursky Bay through a shallow canal (Begun *et al.* 2004).

Samples for quantitative analysis were taken from the surface horizon (0–0.5 m) with the use of a 4-liter Molchanov bathometer. One-liter sample was fixed with Utermöhl's solution and concentrated through sedimentation (Utermöhl 1958) to a volume of 10–15 ml. Algal cells were counted in a 0.05–1 ml Nojott chamber. During water "Bloom", a direct cell counting was done in non-concentrated water samples.

Live cells as well as fixed material were examined, photographed and measured using a light microscope (LM) Olympus BX41. Preparations for transmission electron microscopy (TEM) were prepared by the method of "shadow-cast microscopy" proposed by Moestrup and Thomsen (1980); the method involves contrasting of preparations for studying details of the cell fine structure in unarmored flagellates. The material was sprayed with chrome and examined with a TEM JEM-100 B (JEOL, Tokyo, Japan).

The detected species were classified according to the system adopted by Chretiennot-Dinnet *et al.* (1993).

3. Results

Five species of the genus *Eutreptiella*, namely *E. braarudii* Throndsen, *E. eupharyngea* Moestrup and Norris, *E.*

gymnastica Throndsen, *E.* cf. *marina* Da Cunha, and *E. pascheri* (Schiller) Pascher were found in phytoplankton samples from Peter the Great Bay of the East/Japan Sea.

Class Euglenophyceae Schoenichen, 1925
Order Eutreptiales Leedale, 1967
Family Eutreptiaceae Hollande, 1942
Genus *Eutreptiella* da Cunha, 1914

Eutreptiella braarudii Throndsen
(Figures 2a-d)

References: Throndsen (1969), p.181, fig. 17a, b, fig. 29a-d

Description: Cells 46-140 μm long and 17.5-32 μm wide. The cells are fusiform, capable of metabolic movements (stretching and pumping of the anterior end of the cell). The anterior end of the cell is blunt, the posterior end elongated and very acutely pointed (Figs. 2a-d). The pellicle has spiral striation, 10-12 striae per 10 μm. The reservoir system is middle-sized. Chloroplasts are arranged in two stellate clusters, anterior and posterior to the nucleus (Fig. 2b). Pyrenoids are numerous, arranged in two clusters and surrounded by large paramylon grains. Two or two duplicate flagella are unequal in length. Cells usually swim in a straight line, rotating around the longitudinal axis.

Remarks: The species is morphologically very similar to *E. eupharyngea* Moestrup and Norris in cell form, in the number and arrangement of chloroplasts, but differs from that by having a larger cell size and a smaller reservoir system.

Distribution: Neritic, cold-water species. It was found in North Atlantic and Arctic (Throndsen 1969, 1993). *E. braarudii* is annually found in Peter the Great Bay at stations from 4 to 8 from January until May. Concentration maximum (about 3×10^5 cells l^{-1}) was recorded in Amursky Bay at Station 4 in January at a water temperature of -1.8°C and salinity of 33.5‰.

Eutreptiella eupharyngea Moestrup and Norris
(Figures 2e-k)

References: Walne *et al.* (1986), p.110, figs 1-32; Kato (1993), p. 50, figs 4, 11-13.

Description: Cells 26-48 μm long and 6-12 μm wide. The cells are fusiform, capable of metabolic movement. The anterior end is blunt and slightly narrower than the mid-region (Fig. 2e). A characteristic hump (or s-shaped crick) is seen in the mid-region of the cell (Figs. 2f, g). The colorless posterior end pointed, although not as acutely as in *E. braarudii*. Pellicle has numerous striae arranged helically, 19-24 striae per 10 μm. The reservoir system is extensive. Bottom of the reservoir extends nearly to the anterior portion of the centrally located nucleus. Chloroplasts arranged in two stellate clusters, anterior and posterior to the nucleus (Fig. 2f). Two clusters of pyrenoids are surrounded by large paramylon granules. Some cells have disc-shaped chloroplasts distributed throughout cytoplasm (Fig. 2e). Two flagella are unequal in length, heterodynamic. Cells swim in a fairly straight line, rotating around the longitudinal axis. Examination of shadow-cast preparations revealed the presence of flagellar hairs (Fig. 2i, j). Some flagellar hairs are arranged in numerous unilateral acuminate hair bundles or tufts. Both flagella contain the bundles of flagellar hairs. The bundles consist of seven or more hairs (Fig. 2k) that are often interwined in a ropelike fashion, splaying out at the tips in some instances. Flagellar hairs of other types, constituting a felt or hairy mat, are located external to the flagellar membrane (Fig. 2k).

Remarks: A similar pattern of the arrangement of flagellar hairs was reported for *E. braarudii* Throndsen (Stonik and Aizdaicher 2003). The species is morphologically similar to *E. braarudii* Throndsen, but differs from it by having a smaller size, a much larger reservoir system and a greater number of pellicular striae (Walne *et al.* 1986).

Distribution: Neritic. The species was found in the coastal waters of Denmark, USA and Japan (Throndsen 1993). The species is common in the phytoplankton of Peter the Great Bay. *E. eupharyngea* occurs at all stations throughout the year. The relatively high concentrations (up to 1.3×10^6 cells l^{-1}) were registered in Golden Horn Bay from April to June. Intensive blooms of *E. eupharyngea* were observed in the semi-closed man-made sea basin within the city of Vladivostok (station 5) from May to June. Maximum concentration (5.6×10^7 cells l^{-1}) was recorded at station 5 in June at a water temperature of 23°C and a salinity of 27.5‰.

Eutreptiella gymnastica Throndsen
(Figures 3a-e)

References: Throndsen (1969), p. 181, figs 18-24; Kato (1993), p. 48, figs 3, 8-10.

Description: Cells 16-40 μm long and 4.8-12 μm wide.

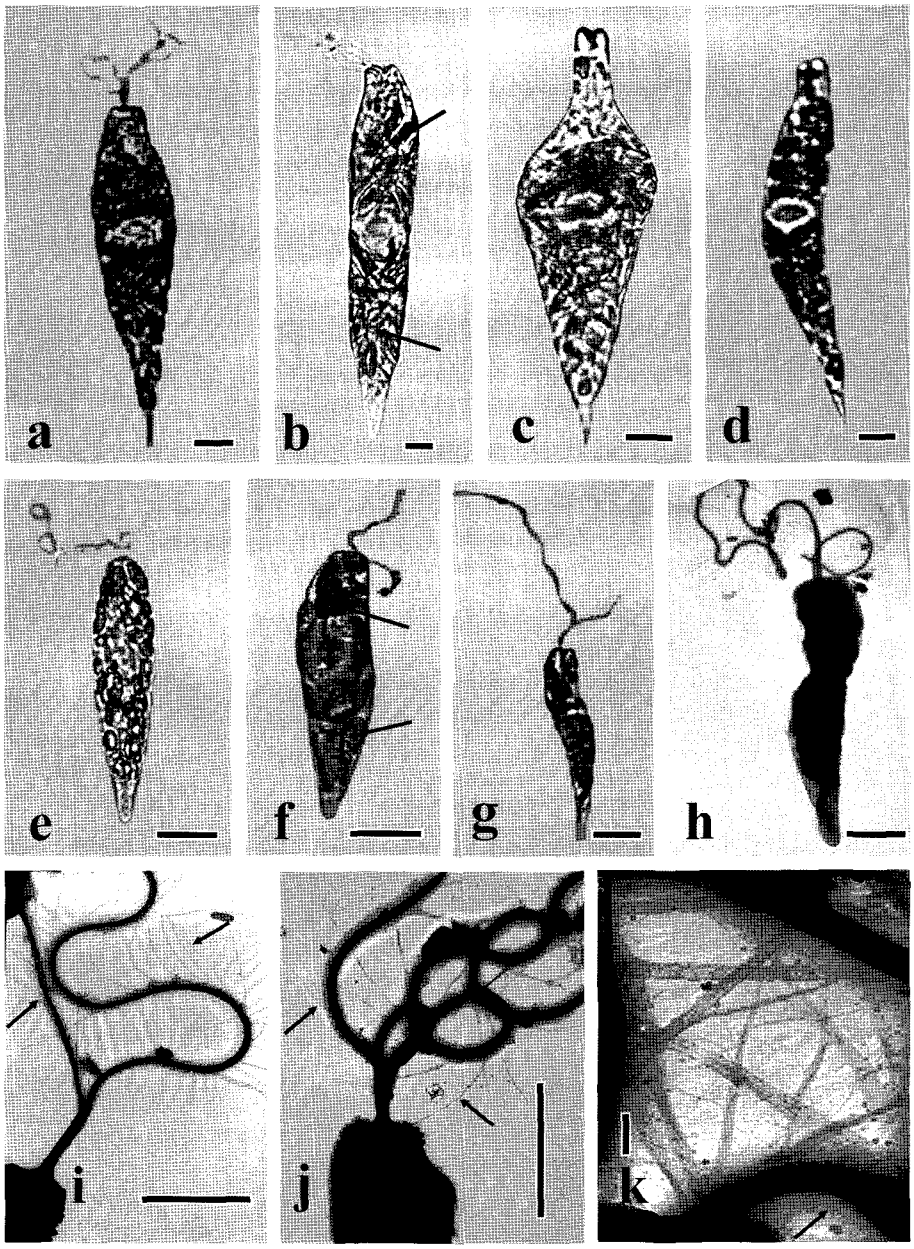

Fig. 2. (a)-(d) *Eutreptiella braarudii*, (a) a fusiform cell with duplicate flagella, (b) a cell shows chloroplasts in two stellate clusters (arrows), (c, d) two live cells in metabolic movement; (e)-(k) *Eutreptiella eupharyngea*, (e) a cell with separate discoid chloroplasts distributed throughout cytoplasm, (f) a cell shows chloroplasts in two stellate clusters and two pyrenoid centers (arrows) that are composed of many small pyrenoids, (g) a cell with a characteristic hump approximately halfway along its longitudinal axis, (h) longitudinal overview of the cell shows pellicular striations, (i, j) anterior ends of two cells reveal the characteristic arrangement of acuminate hair bundles (arrows) on both flagella, (k) flagellar hairs arranged in bundles and some in disarray (arrow). (a)-(g) LM, (h)-(k) TEM, shadow-cast preparation. Scale bar is equal to 10 μm (a)-(j) and 1 μm (k).

The cells show intensive metabolic movements, they are elliptical or cone-shaped, the anterior end slightly rounded (Figs. 3a-e). The reservoir system is extensive, extending nearly to the anterior portion of the centrally located nucleus. Stigma is large. Chloroplasts are disc- or irregular-shaped, varying in number (Figs. 3b, c). The pyrenoid is single, parietal, covered by one or two opposed cup-shaped paramylon shields (Figs. 3c, d). Paramylon grains are numerous and scattered in the cytoplasm. Two flagella are unequal in length.

Distribution: Neritic, worldwide (Throndsen 1993) and common species in the phytoplankton of Peter the

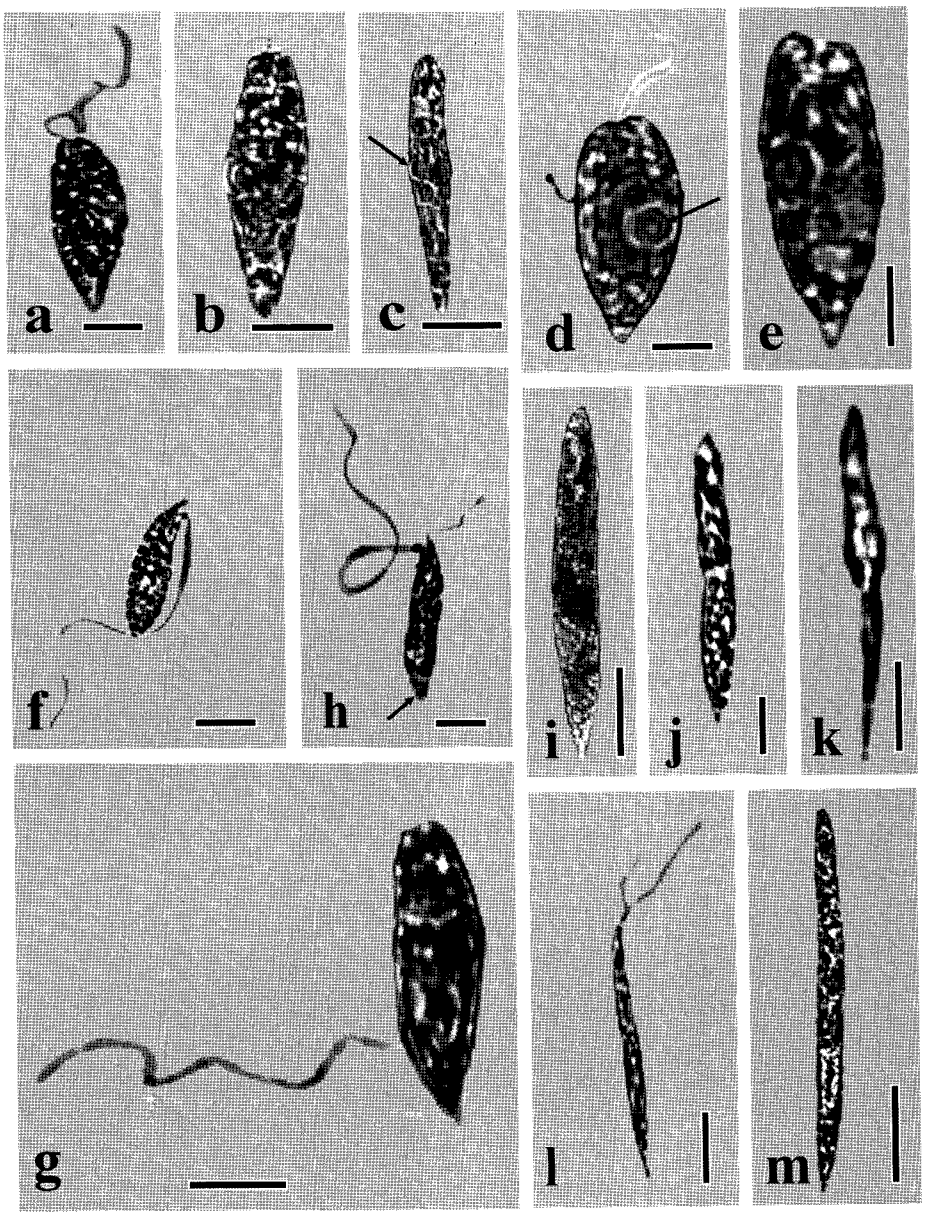

Fig. 3 (a)-(e) *Eutreptiella gymnastica*, (a) a cell with two unequal heterodynamic flagella, (b) a cell shows numerous disc- or irregular-shaped chloroplasts, (c, d) two cells show pyrenoid covered by two cup-shaped paramylon shields (arrows), (e) longitudinal overview of a fusiform cell; (f)-(h) *Eutreptiella* cf. *marina*, (f, g) two cells with longer flagellum trails behind, (h) a fusiform cell shows numerous paramylon grains and disc-shaped chloroplasts, note a duct-like apparatus ended in the posterior end (arrow); (i)-(m) *Eutreptiella pascheri*, (i) a fusiform cell shows pellicular striations, (j) a cell with numerous disc-shaped chloroplasts, (k) metabolic cell, (l) a cell with two flagella unequal in length, (m) a fusiform cell. (a-m) LM. Scale bar is equal 10 μm.

Great Bay. *E. gymnastica* annually occurs at stations from 4 to 8 throughout the year. The species might often cause water blooms in Amursky Bay (station 5) and in Golden Horn Bay (station 7) from March to June. Maximum concentration (1×10^7 cells l^{-1}) was registered in March in Golden Horn Bay at a water temperature of 3°C.

Eutreptiella* cf. *marina da Cunha

(Figures 3f-h)

References: Da Cunha (1914), p. 172, Taf. 24, fig.6; Norris (1964), p. 271, fig. 8.

Description: Cells 24-32 μm in length, 6-7 μm in width. The cells are fusiform. The anterior end slightly narrower than the mid-region (Figs. 3f-h). Stigma is small. Disc-

Table 2. Morphometric characteristics of *Eutreptiella* species

Species	Cell length, μm	Cell width, μm	Length of flagella, μm or ratio of flagella length/cell length		Pellicular striae number/10 μm	References
			long	short		
E. braarudii	46-140	17.5-32	60-110	20	10-12	Our data
	64-115	-	-	-	8-11	Throndsen (1969)
E. eupharyngea	26-48	6-12	40-60	16-20	19-24	Our data
	35-70	7.5-12	60-80	20-24	18-25	Walne *et al.* (1986)
E. gymnastica	16-40	4.8-12	16-46	8-17	-	Our data
	17-38	-	20-32	8-13	-	Throndsen (1969)
E. cf. marina	24-32	6-7	1.5-2 cell length	0.4-0.6 cell length	-	Our data
	40-50	8-10	1.5-2 cell length	0.25 cell length	-	Butcher (1961)
	33	-	-	-	-	Norris (1964)
E. pascheri	28-44	4-8	0.5-0.6 cell length	0.25 cell length	10-12	Our data
	30-40	5-7	0.5 cell length	-	-	Schiller (1925)

-: data not available

shaped chloroplasts are numerous, scattered throughout the cell periphery. Paramylon grains circular to oblong in outline are numerous and scattered in the cytoplasm (Figs. 3f, h). Two flagella are unequal in length. The longer flagellum often trails behind the cell, and its length is considerably longer than the cell length, while the shorter flagellum projects in front of the cell. The longer flagellum probably can be moved backwards and forwards around its point of attachment within an angle of 180°.

Remarks: Cell lengths of specimens found by us were shorter than in the Butchers' description of the species (Table 2), but similar to those reported by Norris (1964) for cells from Wellington Harbour, New Zealand. However, we have not detected two clusters of paramylon grains, anterior and posterior to the nucleus, as Norris describes (Norris 1964). In some specimens, we observed a duct-like apparatus similar to that described by Norris (1964); the duct ended in the posterior acute end of the cell with dense protoplasm (Fig. 3h). The specimens found by us resembled *E. eupharyngea* in cell sizes, flagellum lengths and the presence of a number of disc-shaped chloroplasts characteristic of *E. eupharyngea* cells grown in adverse conditions (Walne *et al.* 1986). But the flagellum length and the location of the long flagellum behind the cell - that is characteristic of the species *Eutreptiella marina* (Norris 1964) - allowed us to identify our specimens as *Eutreptiella* cf. *marina*.

Distribution: Neritic. The species was found in the coastal waters of Atlantic Ocean (Throndsen 1993) and New Zealand (Norris 1964). It is rare, not numerous species in Peter the Great Bay. The species occurs at stations 4 and 5 from June to July. The species was found for the first time in the far eastern seas of Russia.

Eutreptiella pascheri (Schiller) Pascher
(Figures 3i-m)

References: Pascher (1927), p. 597.

Synonym: *Gymnastica pascheri* Schiller (1925), p. 96, Taf. 4, fig. 21.

Description: Cells 28-44 μm in length, 4-8 μm in width. The cells show metabolic movements, they are elongated, and fusiform. The anterior end slightly narrower than the mid-region, the posterior end pointed (Figs. 3i-m). Pellicle with numerous striae arranged helically (Fig. 3i), 10-12 striae per 10 μm. The reservoir system is not perceptible. There are many small disc-shaped chloroplasts (Figs. 3, j, m). Two flagella, unequal in length, are projected in front of the cell.

Distribution: Findings of the species are reported for the Adriatic Sea and in the coastal waters of England (Schiller 1925; Butcher 1961). The species occurs in Peter the Great Bay from January to August at stations 4-8. Intensive water blooms caused by *E. pascheri* were registered in the semi-closed man-made sea basin within the city of Vladivostok at station 5 from July to August. Maximum concentration (1.7×10^7 cells l^{-1}) was registered at station 5 in July at a water temperature of 23°C and a salinity of 27‰. The species was found for the first time in the far eastern seas of Russia.

4. Discussion

Algae of the genus *Eutreptiella* are a common component of the phytoplankton in Peter the Great Bay. The species *E. braarudii* dominated in Amursky Bay in winter, its density being 3×10^5 cells l^{-1} or 30-46% of the total density of the phytoplankton population. The species *E. eupharyngea*, *E. gymnastica,* and *E. pascheri* (their cell concentrations of over 1×10^6 cells l^{-1} or 56-98% of the total phytoplankton population) were responsible for water "Bloom" in spring and summer in the eutrophic coastal areas of Amursky and Golden Horn bays.

Some species of euglenoids found by us (*E. eupharyngea*, *E. gymnastica*) caused water "Bloom" in eutrophic coastal waters of Japan (Kato 1993). However, regular surveys of phytoplankton conducted in Peter the Great Bay since the beginning of 70s have only recently detected these algal species in the area. It can be explained, first of all, by technical difficulties in studying these algae that loose their cell form at the use of generally accepted fixation methods and their collapse at a long deposition of samples. Identification of euglenoids requires special methods of fixation. We can expect that the use of proper methods of examination coupled with cultivation of microalgae will significantly extend our knowledge on species composition, morphological characters and ecology of this important group of microalgae that contribute significantly to phytoplankton biomass in coastal sea areas.

Acknowledgements

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