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DIFFERENT ASPECTS OF STUDYING A DIATOM *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN ET LEWIN 1964 IN NATURAL AND LABORATORY CONDITIONS

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The article summarizes original and literary data on different aspects of studying *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin 1964 in two biotopes – phytoplankton and microphytobenthos – of the Black Sea, the Sea of Azov, and the Sea of Japan for the period from 1976 to 2016. The aim of the work is to present the results of the study mainly of own data on the morphology, systematics and ecology of *C. closterium* in different seas and under cultivation in the laboratory. Information on the history of the species origin and its nomenclature changes is given. *C. closterium* belongs to the phylum Bacillariophyta, class Bacillariophyceae, order Bacillariales Hendey 1937, family Bacillariaceae Ehrenb. 1831, genus *Cylindrotheca* Rabenhorst 1859 emend. Reim. et Lewin 1964. This benthoplanktonic species occurs in the plankton, in littoral and sublittoral zones of the seas. The species is marine and brackish-water; it is a cosmopolite common in different geographical zones of the World Ocean. The results of studying alga by various methods under natural and experimental conditions in light and transmission electron microscopes of C. Zeiss LIBRA-120 are presented. The quantitative data of *C. closterium* were determined by direct counting of the cells in the Goryaev' camera (V = 0.9 mm^3) in light microscopes BIOLAM L-212, C. Zeiss Axioskop 40 with the program AxioVision Rel. 4.6 at 10×40, 10×100, and Olympus BX41 (Tokyo, Japan) with lenses UPLanF140× and 100×1/30 oil immersion. Cultivation of *C. closterium* was carried out in the cumulative mode on the nutrient medium F, volume of 1 L under light intensity of 13.7 klx and temperature of +20…+21 °C. Morphology data of this species from different seas were obtained. The average cell sizes of *C. closterium* are: 25–260 µm length, 1.5–8 µm width; 12–25 fibulae in 10 µm. The results of cultivation in the laboratory conditions showed that the average cell sizes reached 148.17 µm (length) and 8 µm (width) at the temperature of +19…+20 °C and light intensity of 13 klx; length of cells reached 162.12 µm in the exponential phase of growth and 172.07 µm – in the stationary phase. *C. closterium* has an important practical significance as a source of fucoxanthin, since this alga is intensively cultivated for production of biologically active substances. Our experimental data showed that during laboratory cultivation the fucoxanthin concentration in a diatom biomass can reach 11 $mg \cdot g^{-1}$ of dry mass. The new data obtained are relevant and important; they can be used in different fields of science and medicine. The seasonal dynamics of population abundance of *C. closterium* in different ecotopes (epizoon of invertebrates and their food spectra, epiphyton of bottom vegetation, periphyton of the experimental and anthropogenic substrates of the different seas) is presented for the first time. The maximum abundance of the species population ($65.6 \cdot 10^3$ $cells \cdot cm^{-2}$) was registered in the epizoon of the mussel *Mytilus galloprovincialis* Lam. in March at the water temperature of +7.7 °C at a depth of 2.5 m in the Black Sea. The maximum abundance was registered in the epiphyton of green algae ($896 \cdot 10^3$ $cells \cdot cm^{-2}$) and in the periphyton of asbestos plates ($728 \cdot 10^3$ $cells \cdot cm^{-2}$) in August at the water temperature of +24.5 °C in the Sea of Japan.

The abundance dynamics of *C. closterium* natural populations in the local habitats changed depending on the season, the depth, and the type of substrate. The similarities and differences in the distribution of *C. closterium* in the sea microphytobenthos are discussed.

Keywords: diatom, *Cylindrotheca closterium*, taxonomy, morphology, phytogeography, ecology, ecotopes, abundance, laboratory cultivation, fucoxanthin

Cylindrotheca closterium is a species of great ecological and biotechnological importance. This alga is a rich source of proteins, polyunsaturated fatty acids, micro- and macroelements. It contains carotenoids, a pigment of fucoxanthin, which is found mainly in diatoms and brown algae [20]. As a part of the photosynthetic apparatus, fucoxanthin content reaches 78 % of the total content of carotenoids which makes up to 1.7 % of the dry mass. This species is also characterized by a high content of fat and ash [20]. Positive results of biomedical, pharmacological and clinical studies demonstrate the high efficiency of fucoxanthin in the treatment and prevention of obesity, diabetes, oncology, as well as diseases associated with disorders of the immune and cardiovascular system [8, 20]. It testifies the prospects of *C. closterium* study. The species is widely used in aquaculture for feeding aquatic organisms (larval shellfishes, mussels, oysters, etc.) and in medicine – as a source of fucoxanthin.

Working with the scientific papers related to different aspects of the taxa study it is important to identify them accurately, because sometimes definitions in the literary sources are incorrect. Therefore, for specialists not engaged in the taxonomy, the exact definition of the species in such papers is mandatory. Additional information for the species are ecological characteristics (relation to temperature, salinity, saprobity indicators, quantitative data on populations of the species in the different seasons of the year), as well as phytogeographic features (distribution of the species in local and main geographical areas of its habitat). Unfortunately, there are still very few papers on the synthesis of data on the study of individual species of diatoms, but they are necessary for having a complete picture. Long-term studies of *C. closterium* are of great interest not only for taxonomists and ecologists but also for those involved in laboratory cultivation. In general, the study of this species has not only theoretical but also practical significance in various fields of human activity.

The aim of this work is to generalize and analyze own and literary data on different aspects of *C. closterium* study in natural and laboratory conditions.

MATERIAL AND METHODS

The material for the analysis and generalization of the results of the study of *C. closterium* morphology, ecology and cultivation is used on the basis of original data from different natural geographical regions of the Black Sea, the Sea of Azov, and the Sea of Japan. Samples were collected in the microphytobenthos of the Black Sea in different years from 1987 to 2015, of the Sea of Japan – from 1976 to 2016. The quantitative data on populations of the species were determined by direct cell counting in the Goryaev's camera (V = 0.9 mm^3) in light microscopes BIOLAM L-212 at 10×40×2.5, 10×90×2.5 magnification, and C. Zeiss Axioskop 40 with the program AxioVision Rel. 4.6 at 10×40, 10×100. In addition, the morphology was examined using an Olympus BX41 (Tokyo, Japan) light microscope with lenses UPLanF140× and 100×1/30 oil immersion, and C. Zeiss (Germany) LIBRA-120 transmission electron microscope.

Cultivation of the Black Sea clone of *C. closterium* from the collection of the Kovalevsky Institute of Marine Biological Research of RAS (Sevastopol) was carried out in the cumulative mode in the nutrient medium, the volume of one liter (hereinafter L), under light intensity of 13.7 klx and temperature of +20…+21 °C. In the process of the clone growing, the culture medium was continuously bubbled with air through the compressor, using a sprayer to provide sufficient oxygen and carbon dioxide. The clone of *C. closterium* isolated from the Sea of Japan population is preserved in the collections of the Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of RAS (Vladivostok).

RESULTS AND DISCUSSION

The history of this species. *C. closterium* has a continuous history, with many details presented by the authors who described the species [25] and with a large number of names that were included in its extensive synonyms [9, 29, 33, 34]. Originally, the species was described by H. Ehrenberg in 1839 as *Ceratoneis closterium* Ehrenb. W. Smith first recorded species in May 1851 off the coast of Britain [39] and in 1853. W. Smith transferred the species to another genus and listed it as *Nitzschia closterium* (Ehrenb.) W. Smith 1853. However, Rabenhorst (1859) described a new genus *Cylindrotheca* with a single species *C. gerstenberger* Rabenh. 1859, which was later renamed to *C. gracilis* (Brèb. ex Kütz.) Grunow in Van Heurck 1882. In 1864, Rabenhorst described the form *Nitzschiella closterium*. A century later (1964), Reimann and Lewin, examining the range of *Nitzschia* forms in the laboratory with a light microscope and then with a scanning electron microscope, came to the conclusion that *Nitzschia closterium* by its morphological features belonged to the genus *Cylindrotheca*. Thus, the authors described the following taxa: *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin, *C. closterium* var. *californica* (Mereshck.) Reimann et Lewin, *C. fusiformis* Reimann et Lewin, and *C. signata* Reimann et Lewin. However, the authors emphasized that the species *C. closterium* and *C. fusiformis* possessed only two chloroplasts. Currently, the genus is named *Cylindrotheca* Rabenhorst emend. Reimann et Lewin 1964 and includes 10 taxa [9].

The taxonomic classification of the species *C. closterium* and its synonymy is as follows: the nomenclatural type of this species belongs to the phylum Bacillariophyta, class Bacillariophyceae, order Bacillariales Hendey 1937, family Bacillariaceae Ehrenb. 1831, genus *Cylindrotheca* Rabenhorst 1859 emend. Reim. et Lewin 1964 [9, 26].

Cylindrotheca closterium (Ehrenberg) Reimann et Lewin 1964 has many synonyms. Basionym: *Ceratoneis closterium* Ehrenb. 1839. Synonyms: *Nitzschia closterium* (Ehrenb.) W. Smith 1853; *N. reversa* W. Smith 1853; *N. closterium* var. *reversa* (W. Smith) Hauck; *Nitzschiella closterium* Rabenhorst 1864; *Nitzschia rostratum* Grunow 1880; *N. longissima* var. *closterium* (Ehrenb.) Van Heurck 1885; *N. curvirostris* var. *closterium* (Ehrenb.) De Toni 1892; *Nitzschiella longissima* var. *closterium* (Ehrenb.) Peragallo et Peragallo 1897; *Homoeocladia closterium* (Ehrenb.) Kuntze 1898; *Nitzschiella tenuirostris* Mereschk. 1901; *Nitzschia longissima* Gran 1930; *N. closterium* var. *recta* Gran 1931.

Comment. In the work of Crosby and Wood ([6], p. 38, pl. 1, Figs no. 16 and no. 17), Figure 16 shows the species *Nitzschia longissima* larger, and Figure 17 shows the *N. closterium* smaller with two chloroplasts. It should be noted that *N. longissima* is characterized by numerous granular chloroplasts, as noted by Proshkina-Lavrenko [22]. As detected by us in live samples from the Black Sea and the Sea of Japan, it is obvious that these authors specified the same species *Nitzschia closterium* (= *Cylindrotheca closterium*), which has typically only two chloroplasts. These data are confirmed by the authors [25].

The ecology. Ecological characteristics about *C. closterium* are given for a majority of the examined water areas of the Black Sea, the Sea of Azov, and the Sea of Japan on the basis of own and literary sources. This species is eurythermic, euryhaline, lives in marine and brackish waters. It is neritic, benthoplanktonic species that occurs in the plankton and in littoral and sublittoral zones of the seas. There are some limited data available on the phytoplankton of the Sea of Azov and estuaries, as well as on microphytobenthos [15, 21, 23, 34]. Thus, in the phytoplankton of summer of 1999 and 2003, *C. closterium* was observed at the temperature of +24…+25 °C with $59 \cdot 10^6$ cells·m^{-3} and the wet biomass of 41 mg·m^{-3} [14]. This species was identified in single individual cases in the microphytobenthos of the Crimean coastal waters of the Sea of Azov [2].

The study in natural conditions. Long-term observations and study of diatom from natural populations of microphytobenthos and phytoplankton from different regions and biotopes of the Black Sea and the Sea of Japan were carried out in different seasons and at different depths. *In vivo* cells of *C. closterium* from the Sea of Japan are shown on the Fig. 1.

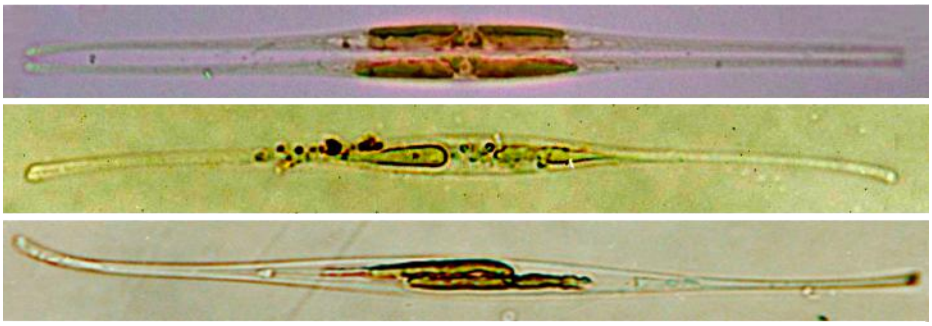

Fig. 1. Light microscopy. *In vivo* cells of *Cylindrotheca closterium* from the Sea of Japan with two chloroplasts (photo by A. Begun)

Рис. 1. Световая микроскопия. Прижизненные клетки *Cylindrotheca closterium* из Японского моря с двумя хлоропластами (фото А. Бегуна)

To study the morphology of *C. closterium* we conducted analyses of samples from the Black Sea and the Sea of Japan (Fig. 2) clones made with the transmission electron microscope. The analysis of original and literary data on the morphological parameters of the valves of *C. closterium* from different seas showed a wide size range. The valve sizes are: 25–180 µm length, 1.5–8.0 µm width, 12–25 fibulae in 10 µm [25]; 30–260 µm length, 2.0–6.0 µm width for the Black Sea population of the species; 45–65 µm length, 3–4 µm width for the Black Sea [10]; 50–160 µm length, 3–5 µm width, 14–16 fibulae in 10 µm for the Sea of Japan population [19]; 47.5–118.4 µm length, 2.9–5.0 µm width, 12–16 fibulae in 10 µm [22, 23, 24]; 12–14 fibulae in 10 µm [27, 33]. In the morphological parameters study of the valves in the Black Sea populations of *C. closterium*, the annual dynamics of development on the experimental glass-plates periphyton was characterized by a wide size range. In 2011–2012, the cells length varied from 24.9 to 97.2 µm, the width varied from 1.5 to 5.0 µm [1].

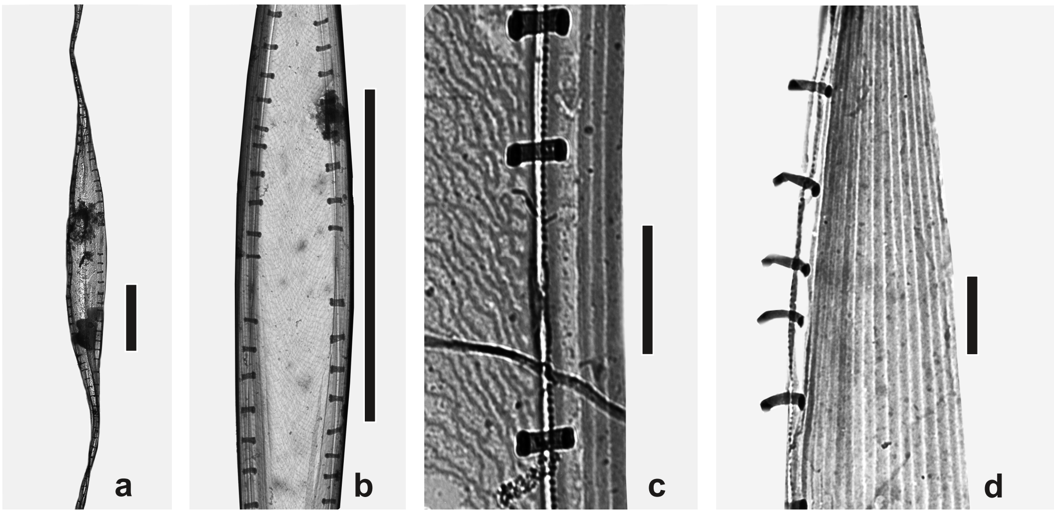

Fig. 2. Transmission electron microscopy. The structure of *Cylindrotheca closterium* valves from the Black Sea (a, b) and the Sea of Japan (c, d). Scale bar: 10 µm (a, b); 1.0 µm (c, d) (photo by I. Stonik)

Рис. 2. Трансмиссионная электронная микроскопия. Структура фрагментов створок *Cylindrotheca closterium* из Чёрного (a, b) и Японского (c, d) моря. Шкала измерения: 10 мкм (a, b); 1,0 мкм (c, d) (фото И. Стоник)

The occurrence and abundance of this species are registered in different ecotopes of the Black Sea [28, 30]. In the studies on “Ectocarpus” model substrates imitating macroalgae, the abundance of *C. closterium* ranged from $5.7 \cdot 10^3$ to $21.0 \cdot 10^3$ cells·cm^{-2} during the exposure period near the Karantinnaya Bay (station of observations) from 1988.05.26 to 1988.06.07 [35]. The abundant growth of *C. closterium* was recorded in different seasons, on substrates and at depths in the water area at Sevastopol (Table 1).

Table 1. The occurrence of the diatom *Cylindrotheca closterium* on different substrates of the Black Sea (near Sevastopol)

Таблица 1. Встречаемость диатомовой водоросли *Cylindrotheca closterium* на разных субстратах Чёрного моря (возле г. Севастополя)

Sampling date	Sampling area	Depth, m	Substrate	Species
1987.04.28	Karantinnaya Bay	0.1	stone with detrite	in a mucous capsule
1987.08.27	Fiolent Cape	0.5	stone	normal cells
1995.09.27	Kazach'ya Bay	0.5	*Mytilus galoprovincialis*	normal cells
1995.10.25	Kazach'ya Bay	0.5	*Mytilus galoprovincialis*	high abundance

The species abundance of the phytoplankton in different regions of the Black Sea was noted in a number of works [16, 22, 24]. The maximum abundance ($16 \cdot 10^6$ cells·L^{-1}) was observed in phytoplankton of the eutrophic north-western part of the Black Sea [18].

The seasonal dynamics of abundance of *C. closterium* populations was investigated from May 1995 to May 1996 in the Kazach'ya Bay, simultaneously in phytoplankton and in microphytobenthos, at the depth of 0.5–4.5 m [30]. The phytoplankton maximum abundance $38 \cdot 10^3$ cells·L^{-1} was recorded in March; it decreased to the minimum of $5.3 \cdot 10^3$ cells·L^{-1} in summer. In the microphytobenthos of this bay the species occurred year-round, but the greatest abundance was recorded in spring in the range of water temperature of +6.8…+7.7 °C (Fig. 3).

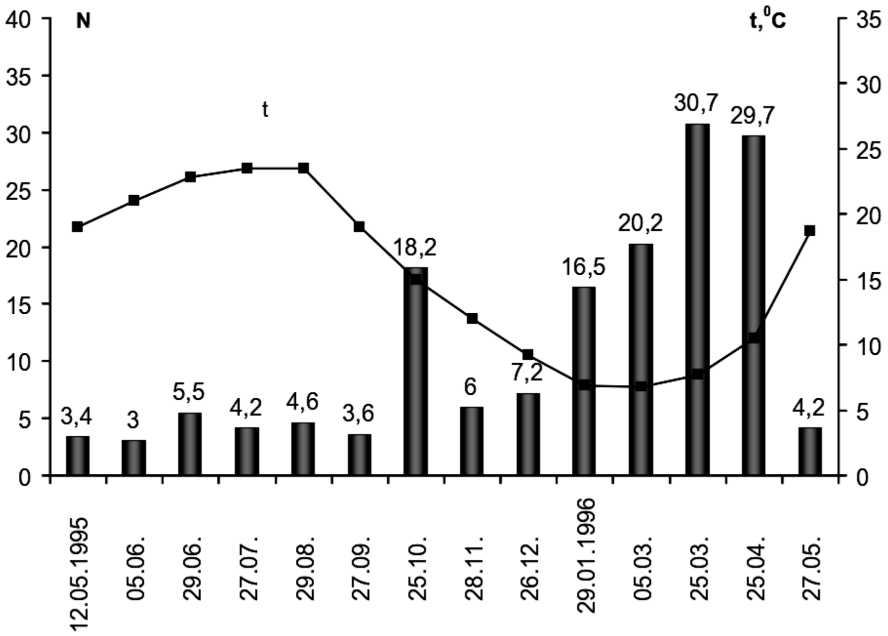

Fig. 3. The seasonal dynamics of abundance (N, 10^3 cells·cm^{-2}) of *Cylindrotheca closterium* in the epizoon of mussel *Mytilus galloprovincialis* Lam. and water temperature (*t*) in the Kazach'ya Bay of the Black Sea (1995–1996) [28]

Рис. 3. Сезонная динамика численности (N, 10^3 кл·см$^{-2}$) *Cylindrotheca closterium* в эпизооне мидии *Mytilus galloprovincialis* Lam. и температура воды (*t*) в бухте Казачья Чёрного моря (1995–1996) [28]

The abundance of *C. closterium* on the surface of shells of live mussels *Mytilus galloprovincialis* Lam. was investigated for the first time depending on the age in the range from 0.5 to 10 years. The abundance of species ranged from $3.0 \cdot 10^3$ to $65.6 \cdot 10^3$ cells·cm^{-2} (June and March, respectively).

In October 1995 in the same bay the average abundance of *C. closterium* population was from $20 \cdot 10^3$ to $28 \cdot 10^3$ cells·cm^{-2} at a depth of 0.5 m at the water temperature of +15 °C. In July 1998 the population abundance was $70.88 \cdot 10^3$ cells·cm^{-2}, or 48 % of diatom community of periphyton polyethylene film (mesocosm) [30]. In the experiments on the exposure of the glass-plates in the sea during 2007–2008, the species was found year-round, and the greatest abundance ($152.9{\cdot}10^3$ cells·cm^{-2}) was observed at the water temperature of +10.5 °C [30] in December. During the study of experimental glass-plates in the sea at monthly exposure, maximum abundance ($868{\cdot}10^3$ cells·cm^{-2}, or 71 % of the diatom community of the phytoperiphyton) was registered in January at the water temperature of +8 °C (Fig. 4) [1].

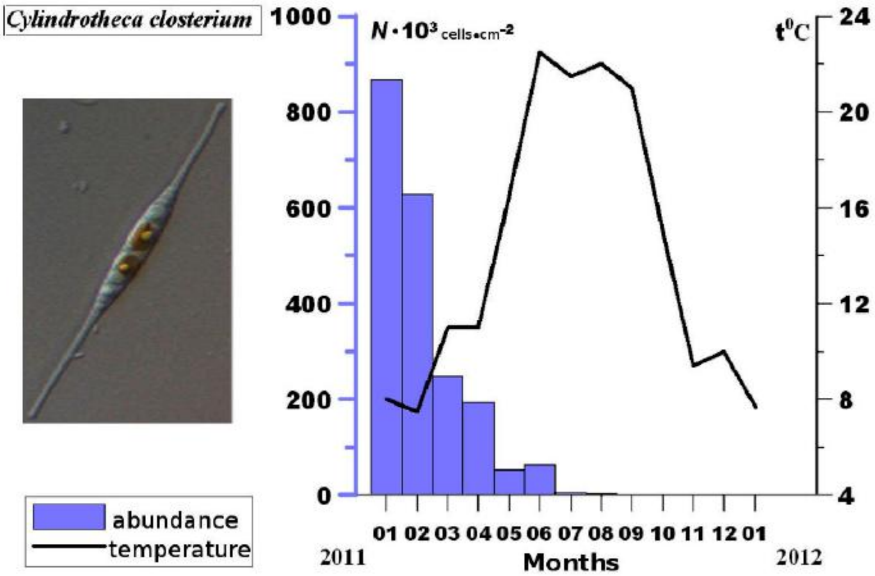

Fig. 4. The abundance of *Cylindrotheca closterium* on the glass-plates periphyton in the Karantinnaya Bay of the Black Sea (2011–2012) [1]

Рис. 4. Численность *Cylindrotheca closterium* перифитона стеклянных пластин в Карантинной бухте Чёрного моря (2011–2012) [1]

The degree of its dominance by the Berger – Parker index decreased to 57 %. After the peak of development, the abundance decreased to $0.3{\cdot}10^3$ cells·cm^{-2} by June. In the following months the alga cells of the species were only sparse. The abundance values of *C. closterium* cells changed depending on the cumulative exposure on the glass-plates (Table 2). In July 2015, at the water temperature of +21.4 °C the species dominated on the shells mussel in the Karantinnaya Bay, reaching $21{\cdot}10^3$ cells·cm^{-2} at depth of 6 m.

Table 2. Seasonal dynamics of the abundance (N) of *Cylindrotheca closterium* cells on the glass-plates of the periphyton of cumulative exposure in the Black Sea (2007–2008)

Таблица 2. Сезонная динамика численности (N) клеток *Cylindrotheca closterium* в перифитоне стеклянных пластин при накопительной экспозиции в Чёрном море (2007–2008)

Date of substrate exposure	Temperature, °C	N, cells·cm^{-2}
2007.01.17 – 2007.02.21	+8.5	single
2007.01.17 – 2007.03.20	+9.0	$15.7{\cdot}10^3$
2007.01.17 – 2007.04.08	+10.0	$33.3{\cdot}10^3$
2007.01.17 – 2007.05.21	+18.0	$279.1{\cdot}10^3$
2007.01.17 – 2007.06.21	+22.0	$36.1{\cdot}10^3$
2007.01.17 – 2007.07.19	+25.0	$46.9{\cdot}10^3$
2007.01.17 – 2007.08.21	+28.0	$17.5{\cdot}10^3$
2007.01.17 – 2007.09.19	+21.0	single
2007.01.17 – 2007.10.22	+18.0	single
2007.01.17 – 2007.11.20	+11.0	single
2007.01.17 – 2007.12.20	+10.5	$206.8{\cdot}10^3$
2007.01.17 – 2008.01.22	+9.0	single
2007.01.17 – 2008.02.21	+6.0	single

Laboratory cultivation. Along with the data obtained in the study of the species under natural conditions, we present some complementary results of cultivation in the laboratory. Traditionally, *C. closterium* is cultivated in the laboratory on the nutrient medium F, specially formulated for the diatoms (Fig. 5).

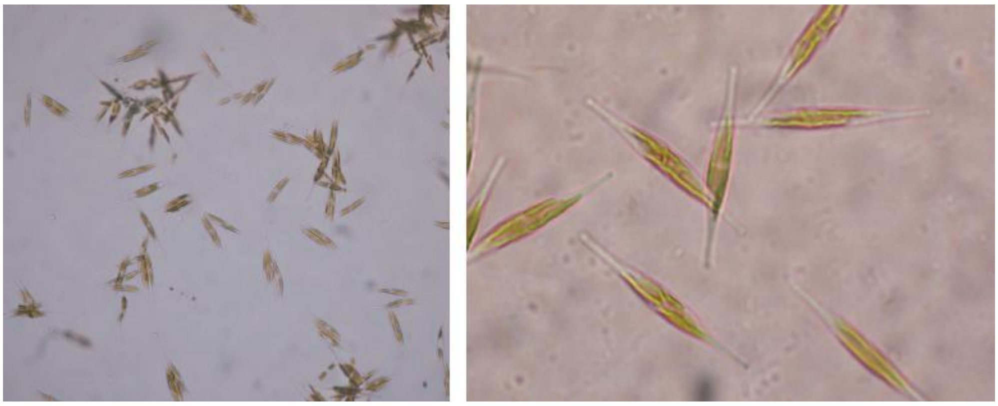

Fig. 5. Light microscopy. The cells of *Cylindrotheca closterium* (from the Black Sea) in the medium F (photo by S. Zheleznova)

Рис. 5. Световая микроскопия. Клетки *Cylindrotheca closterium* (из Чёрного моря) в среде F (фото С. Железновой)

In this medium, the ratio of nitrogen to phosphorus is close to optimal and is 12. The results of determining the cell size in the culture of *C. closterium* at various growth stages in the experimental conditions on the nutrient medium F for 24 hours with light intensity of 13.7 klx, temperature of (19 ± 1) °C and pH of 8–9 are presented. The length of *C. closterium* cells varied from 120 to 220 µm, and the width of the cells – from 6 to 9 µm for 3 days. Our experimental data showed that under these conditions the concentration of fucoxanthin in the diatom biomass can reach 11 $mg \cdot g^{-1}$ of dry mass. The optimum temperature for the growth of this species is +16 °C. At the temperature of +26…+27 °C the culture stops growing. The growth of diatom in the culture is described by an S-shaped curve. In the stationary phase of growth, the culture reaches its maximum density of 0.1 $g \cdot L^{-1}$ of dry biomass on the 8th day. At this stage, larger cells dominate; numerous cells appear mainly due to an increase in lipid content. This shows that cells are able to accumulate valuable biologically active substances.

In the composition of the photosynthetic apparatus of *C. closterium*, the content of fucoxanthin under certain conditions of cultivation can reach 1.5–1.7 % of the dry weight of the algae [36]. An increase of the nitrogen concentration in the nutrient medium F leads to a noticeable increase in the productivity of the cells and the accumulation of fucoxanthin in the culture. At a sodium nitrate concentration of 225–300 $mg \cdot L^{-1}$, the amount of fucoxanthin in the dry biomass of *C. closterium* reaches 15 $mg \cdot g^{-1}$. Despite the fact that fucoxanthin has a high antitumor activity and is able to inhibit the growth of human leukemia cells, prostate cancer and breast cancer [37], its use in the medical and food industries has not been wide enough yet.

The Sea of Japan. For comparison with the Black Sea and the Sea of Azov, we present similar data for the Sea of Japan. *C. closterium* is widely found in the phytoplankton of the Peter the Great Bight [12, 13], as well as in macrophytes epiphyton of the Amursky Gulf [37], among epilithon and epiphyton of the Posyet Bay [19]. The species is regularly observed on different substrates (sand, rock, in the stomachs of invertebrates) at depths of 0.5–10.0 m in different seasons of the year in the Vostok Bay [27, 31, 33]. In addition, *C. closterium* was found in periphyton of anthropogenic substrates of different types and in epiphyton of macrophytes, including for the first time sea flax *Phyllospadix iwatensis* Makino in the Lazurnaya Bay, as well as in the gulfs of Amursky, Uglovoy, Ussuriisky, Vostok, Nakhodka and Slavyanka, in the Tavrichansky estuary, and in the Golden Horn Bay in the artificial lagoon near Vladivostok [33]. The size of the population cells of *C. closterium* from the Sea of Japan varied: there were valves of 50–160 µm length, 3–5 µm width, and 14–16 fibulae in 10 µm [19]; of 47.5–118.4 µm length, 2.9–5.0 µm width, and 12–14 fibulae in 10 µm [28]. Seasonal changes of *C. closterium* cell sizes on different substrates in the Sea of Japan are shown (Table 3).

Table 3. Seasonal changes of *Cylindrotheca closterium* cell sizes on different substrates of the Sea of Japan

Таблица 3. Сезонные изменения размера клеток *Cylindrotheca closterium* на разных субстратах Японского моря

Date	Sampling area	Depth, m	Substrate	Cell size
1976.06.01	Vostok Bay	0.5	stone	68–78 µm length, 3.4 µm width
1979.07.09	Vostok Bay	0.5–10.0	stone	23.8–85 µm length, 3.4 µm width
1979.07.11	Vostok Bay	5.0	sand	76 µm length, 3.5 µm width
1979.09.30	Vostok Bay	0.5	coral	110 µm length, 1.5–5 fibulae in 10 µm
1984.05.08	Vityaz Bay of the Posyet Gulf	0.5–1.0	*Zostera marina*	in a mucous capsule with *Parlibellus delognei* diatom

The abundance of *C. closterium* in phytoplankton of the Sea of Japan changed in different years from $4.87 \cdot 10^6$ cells·L^{-1} at the temperature of +22 °C (August 1999, the Amursky Bay) to $122.5 \cdot 10^6$ cells·L^{-1} at the temperature of +24.5 °C and salinity of 27 ‰ (August 2002, an artificial lagoon near the city of Vladivostok). The values of species abundance on different substrates of microphytobenthos and in the periphyton of experimental plates of plexiglas were $40 \cdot 10^3$ cells·cm^{-2}; on wood substrate – $120 \cdot 10^3$; on high-alloy steel – $160 \cdot 10^3$; on asbestos – $728 \cdot 10^3$ cells·cm^{-2}; in the epizoon of barnacles *Amphibalanus improvisus* (Darwin), on valves of the mussels *Mytilus trossulus* Gould, on the ascidians *Aplidium tenuicaudum* (Beniaminson) and *Styela clava* (Herdman) – $100 \cdot 10^3$ cells·cm^{-2}; in epiphyton of green algae – $896 \cdot 10^3$; on brown algae – $463 \cdot 10^3$; on red algae – $292 \cdot 10^3$; on sea grasses – $15 \cdot 10^3$ cells·g^{-1} of their wet biomass [33].

Indicator role. *C. closterium* is the causative algal bloom in the sea. Sometimes it is found in the area of “red tides” [28]. Being alkaliphilic and β-mesosaprobic, the species is an organic indicator of moderately polluted waters [10].

Phytogeography. *C. closterium* is cosmopolitan, it is found in all geographical zones of the World Ocean. The species is known in the Amursky Liman [11], in the ice of the Laptev Sea, in Arctic, Barents, White, Bering, Caribbean, Mediterranean, Adriatic, Aral, Caspian, Norwegian, Kara, Chukchi, Baltic, Black seas, in the Sea of Azov. There are numerous literary data on the occurrence of this species in the East China Sea, off the coast of Greenland, Northern California, Spitsbergen, Brazil, Mexico, Finland, Sweden, Spain, Croatia, Romania, Germany, Denmark, Turkey, Kuwait, West India, Japan, Australia, New Zealand, Singapore, Sakhalin and Primorye, the Hawaiian and Canary Islands [6, 9, 12, 13, 27, 30, 31, 33, 38], as well as in the microphytobenthos of the Antarctic [32]. The abundant growth of the species in the phytoplankton of the Urias Estuary (Mexico) was recorded in the winter of 1980; it gave $0.5 \cdot 10^6$ cells·L^{-1}, decreased in January and increased again in February [5]. The species was found in the columns of sea ice and on the ice surface in the Weddell Sea of Antarctic [4], as well as in sand of the Florida Bay [7]. *C. closterium* was found in the northern Adriatic Sea in August at the water temperature of +24.0…+25.5 °C [17], as well as on the northwest coast of Brazil in the periphyton of experimental glass-plates year-round at the water temperature of +16…+26 °C [3].

Conclusion. The paper presents a retrospective analysis and synthesis of the results of original studies of *C. closterium* on the systematics, morphology, ecology and phytogeography. Comparative data on the study of morphology and ecology of *C. closterium* from different seas showed that this marine and brackish-water, cosmopolitan species occurs throughout the World Ocean, so the optimum of its development in different seas and in laboratory cultivation have a wide range of environmental factors (temperature, salinity, and illumination of water). This species is found in marine phytoplankton and microphytobenthos, including different ecotopes: epizoon of invertebrates and their food spectra, epiphyton of bottom vegetation, periphyton of the experimental and anthropogenic substrates.

The average cell sizes of *C. closterium* range for different seas: 25–260 µm length, 1.5–8 µm width, 12–25 fibulae in 10 µm; in the culture at the temperature of +19…+20 °C and light intensity of 13 klx the cell sizes reached 148.17 µm length and 8.0 µm width in the culture inoculum, 162.12 µm length – in the exponential growth phase, 172.07 µm length – in the stationary phase.

The abundance dynamics of *C. closterium* natural populations in the local habitats changed depending on the season, the depth of habitat and the type of substrate. The maximum abundance ($65.6 \cdot 10^3$ cells·cm^{-2}) was registered in the epizoon of the mussel in March at the water temperature of +7.7 °C at a depth of 2.5 m in the Black Sea. The maximum abundance was in the epiphyton of green algae ($896 \cdot 10^3$ cells·cm^{-2}) and in the periphyton of asbestos plates ($728 \cdot 10^3$ cells·cm^{-2}) in August at the water temperature of +24.5 °C in the Sea of Japan.

C. closterium has an important practical significance, since this alga is intensively cultivated for production of biologically active substances, for example of fucoxanthin. *C. closterium* contains a unique set of components necessary for humans and is a promising fucoxanthin manufacturer and a biotechnology object. In this regard, further thorough study of this species production and of biochemical characteristics of the obtained substances is necessary for the widespread introduction of scientific developments into practice.

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REFERENCES / СПИСОК ЛИТЕРАТУРЫ

1. Balycheva D. S. *Species composition, structure and function characteristics of microalgae of antropogenic substrates periphyton in the Crimean coast of the Black Sea*. PhD thesis. Sevastopol, 2014, 24 p. (in Russ.)
2. Bondarenko A. V., Ryabushko L. I. Diatoms of the benthos of the Crimean coast of the Sea of Azov. In: *Modern Problems of Algology* : proceedings of the International Science Conference and the VII School for Marine Biology, Rostov-on-Don, 9–13 June, 2008. Rostov-on-Don: SSC RAS, 2008, pp. 61–63. (in Russ.)
3. Brandini F. P., de Silva E. T., Pellizari F. M., Fonseca A. L., Fernandes L. F. Production and biomass accumulation of periphytic diatoms growing on glass slides during a 1-year cycle in a subtropical estuarine environment (Bay of Paranagua, southern Brazil). *Marine Biology*, 2001, vol. 138, iss. 1, pp. 163–171. https://doi.org/10.1007/s002270000427
4. Burckle L. H. Diatom distribution in the Weddell gyre region during late winter. *Micropaleontology,* 1987, vol. 33, no. 2, pp. 177–184. https://doi.org/10.2307/1485492
5. Cortés A. R., Miranda N. P. Composición, abundancia y distribución del fitoplancton del Estero Urias, Sin. México. IV periodo de invierno (1980). *Revista Latinoamericana de Microbiología*, 1985, vol. 27, no. 2, pp. 123–133.
6. Crosby L. N., Wood E. J. F. Studies on Australian and New Zealand diatoms. II. *Transactions and Proceedings of the Royal Society of New Zealand*, 1959, vol. 86, no. 1–2, pp. 1–58.
7. De Felice D. R., Lynts G. W. Benthic Marine Diatom associations. Upper Florida Bay (Florida) and associated sounds. *Journal of Phycology*, 1978, vol. 14, iss. 1, pp. 21–24. https://doi.org/10.1111/j.1529-8817.1978.tb00627.x
8. Gammone M. A., D'Orazio N. Anti-Obesity Activity of the Marine Carotenoid Fucoxanthin. *Marine Drugs,* 2015, vol. 13, iss. 4, pp. 2196–2214. https://doi.org/10.3390/md13042196
9. Guiry M. D., Guiry G. M. *AlgaeBase.* Galway: National University of Ireland, 2018. URL: http://www.algaebase.org [accessed 2019.02.20].
10. Guslyakov N. E., Zakordonec O. A., Gerasimuk V. P. *Atlas diatomovykh vodoroslei bentosa severo-zapadnoi chasti Chernogo morya i prilegayushchikh vodoemov*. Kiev: Naukova dumka, 1992, 112 p. (in Russ.)
11. Kiselev I. A. Sostav i rasprostranenie fitoplanktona v ust'e reki Amur. *Issledovaniya morei SSSR.* Leningrad: Gidrologicheskii institut, 1931, vol. 14, 116 p. (in Russ.)
12. Kiselev I. A. Sostav i periodichnost' fitoplanktona v zalive Patrokl Yaponskogo morya. *Issledovaniya*

morei SSSR. Leningrad: Gidrologicheskii institut, 1935, vol. 22, pp. 82–118. (in Russ.)

13. Konovalova G. V., Orlova T. Yu., Pautova L. A. *Atlas fitoplanktona Yaponskogo morya*. Vladivostok: Nauka, 1984, 160 p. (in Russ.)
14. Maltsev V. N., Klyuchnikov A. V. O massovoi gibeli ryb u krymskogo poberezh'ya Azovskogo morya. *Veterinarnaya meditsina*, 2004, vol. 84, pp. 457–463. (in Russ.)
15. Mereschkowsky C. S. A list of California diatoms. *Annals and Magazine of Natural History including Zoology, Botany and Geology*, 1901, vol. 7, no. 39–42, pp. 292–300; 474–480; 505–520.
16. Morozova-Vodyanitskaya N. V. Fitoplankton Chernogo morya. Ch. 1. Fitoplankton v raione Sevastopolya i obshchii obzor fitoplanktona Chernogo morya. *Trudy Sevastopol'skoi biologicheskoi stantsii AN SSSR*, 1948, vol. 6, pp. 39–172. (in Russ.)
17. Munda I. M. Seasonal fouling by diatoms on artificial substrata at different depths near Piran (Gulf of Trieste, Northern Adriatic). *Acta Adriatica*, 2005, vol. 46, no. 2, pp. 137–157.
18. Nesterova D. A. Water bloom in the north-western part of the Black Sea (Review). *Al'gologiya*, 2001, vol. 11, no. 4, pp. 502–513. (in Russ.)
19. Nikolaev V. A. *Diatomovye vodorosli bentosa zaliva Pos'et Yaponskogo morya*. [dissertation]. Leningrad, 1970, 227 p. (in Russ.)
20. Peng J., Yuan J.-P., Wu C., Wan G. J. Fucoxanthin, a Marine Carotenoid Present in Brown Seaweeds and Diatoms: Metabolism and Bioactivities Relevant to Human Health. *Marine Drugs*, 2011, vol. 9, no. 10, pp. 1806–1828. https://doi.org/10.3390/md9101806
21. Pitsyk G. K. O kachestvennom sostave fitoplanktona Azovskogo morya. *Trudy Sevastopol'skoi biologicheskoi stantsii SSSR*, 1963, vol. 14, pp. 71–89. (in Russ.)
22. Proshkina-Lavrenko A. I. *Diatomovye vodorosli planktona Chernogo morya*. Moscow ; Leningrad: Akademiya nauk SSSR, 1955, 222 p. (in Russ.)
23. Proshkina-Lavrenko A. I. *Diatomovye vodorosli planktona Azovskogo morya*. Moscow ; Leningrad: Akademiya nauk SSSR, 1963, 190 p. (in Russ.)
24. Proshkina-Lavrenko A. I. *Diatomovye vodorosli bentosa Chernogo morya*. Moscow ; Leningrad: Nauka, 1963, 244 p. (in Russ.)
25. Reimann B. E. F., Lewin J. C. The diatom genus *Cylindrotheca* Rabenhorst (with a reconsideration of *Nitzschia closterium*). *Journal of the Royal Microscopical Society*, 1964, vol. 83, iss. 3, pp. 283–296. https://doi.org/10.1111/j.1365-2818.1964.tb00542.x
26. Round F. E., Crawford R. M., Mann D. G. *The diatoms. Biology and morphology of the genera*. Cambridge: Cambridge University, 1990, 747 p.
27. Ryabushko L. I. *Diatoms of the upper sublittoral North-Western part of the Japan Sea*. [dissertation]. Sevastopol, 1986, 244 p. (in Russ.).
28. Ryabushko L. I. *Potentially harmful microalgae of the Azov and Black sea basin* / V. I. Ryabushko (Ed.). Sevastopol: EKOSI-Gidrofizika, 2003, 288 p. (in Russ.)
29. Ryabushko L. I. *Microalgae of the Black Sea benthos (check-list, synonyms, comment)* / A. V. Gaevskaya (Ed.). Sevastopol: EKOSI-Gidrofizika, 2006, 143 p. (in Russ.)
30. Ryabushko L. I. *Microphytobenthos of the Black Sea* / A. V. Gaevskaya (Ed.). Sevastopol: EKOSI-Gidrofizika, 2013, 416 p. (in Russ.)
31. Ryabushko L. I. Diatoms (Bacillariophyta) of Vostok Bay, the Sea of Japan. *Biota and environment of nature reserves in the Far East*, 2014, vol. 2, pp. 4–17. (in Russ.)
32. Ryabushko L. I. The state of knowledge microphytobenthos Argentine islands of Antarctica. *Proceedings of the Belarusian State University*, 2016, vol. 11 (1), pp. 337–350. (in Russ.)
33. Ryabushko L. I., Begun A. A. *Diatoms of the microphytobenthos of the Sea of Japan*. In 2 vols. Sevastopol: PK "KIA", 2016, vol. 2, 324 p. (in Russ.)
34. Ryabushko L. I., Bondarenko A. V. *Microalgae of the plankton and benthos of the Sea of Azov (check-list, synonyms, comment)* / A. V. Gaevskaya (Ed.). Sevastopol: EKOSI-Gidrofizika, 2011, 211 p. (in Russ.)
35. Ryabushko L. I., Zavalko S. E. Microphytocovering of artificial and natural substrates in the Black Sea. *Botanicheskii zhurnal*, 1992, vol. 77, no. 5, pp. 33–39. (in Russ.)
36. Ryabushko V. I., Zheleznova S. N., Nekhoroshev M. V. Effect of nitrogen on the accumulation of fucoxanthin by diatom *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin. *International Journal on Algae*, 2017, vol. 19, no. 1, pp. 79–84. https://doi.org/10.1615/InterJAlgae.v19.i1.70
37. Satomi Y. Antitumor and Cancer-preventative Function of Fucoxanthin: A Marine Carotenoid. *Anticancer Research*, 2017, vol. 37, no. 4, pp. 1557–1562.
38. Skvortzow B. W. Marine diatoms from the Kanazawa oyster experimental station of Japan.

Philippine Journal of Science, 1932, vol. 47, no. 1, pp. 119–126.
39. Smith W. F. *A synopsis of the British Diatomaceae*: with remarks on their structure, functions and distribution; and instructions for collecting and preserving specimens. In 2 vols. Vol. 1. London: Printed for Smith and Beck, Pub. by J. Van Voorst, 1853, 89 p. https://doi.org/10.5962/bhl.title.10706

РАЗЛИЧНЫЕ АСПЕКТЫ ИЗУЧЕНИЯ ДИАТОМОВОЙ ВОДОРОСЛИ *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN ET LEWIN 1964 В ПРИРОДНЫХ И ЛАБОРАТОРНЫХ УСЛОВИЯХ

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Обобщены собственные и литературные данные по разным аспектам изучения диатомовой водоросли *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin 1964 в двух биотопах — фитопланктоне и микрофитобентосе — Чёрного, Азовского и Японского морей за период с 1976 по 2016 г. Цель работы — представление результатов изучения в основном собственных данных по морфологии, систематике и экологии *C. closterium* в разных морях и при культивировании в лабораторных условиях. Приведена информация об истории происхождения вида и о номенклатурных изменениях. *C. closterium* принадлежит к отделу Bacillariophyta, классу Bacillariophyceae, порядку Bacillariales Hendey 1937, семейству Bacillariaceae Ehrenb. 1831, роду *Cylindrotheca* Rabenhorst 1859 emend. Reim. et Lewin 1964. Этот бентопланктонный вид встречается в планктоне, в литоральной и сублиторальной зонах морей. Вид является морским и солоноватоводным. Это космополит, распространённый в различных географических зонах Мирового океана. Приведены результаты изучения водоросли разными методами в естественных и экспериментальных условиях в световых и трансмиссионном электронном микроскопах C. Zeiss LIBRA-120. Численность *C. closterium* определяли путём прямого подсчёта клеток в камере Горяева (объём — 0,9 мм^3) в световых микроскопах BIOLAM L-212, C. Zeiss Axioskop 40 с программой AxioVision Rel. 4.6 при 10×40, 10×100, Olympus BX41 (Токио, Япония) с иммерсионным объективами UPLanF140× и 100×1/30. Культивирование *C. closterium* осуществляли в кумулятивном режиме в питательной среде F объёмом 1 л при освещённости среды 13,7 клк и температуре +20…+21 °C. Получены данные по морфологии этого вида из разных морей. Средние размеры клеток *C. closterium* составляли: 25–260 мкм длина, 1,5–8 мкм ширина; количество фибул в 10 мкм — 12–25. По результатам культивирования в лабораторных условиях, средние размеры клеток — 148,17 мкм (длина) и 8 мкм (ширина) при температуре +19…+20 °C и освещённости 13 клк; длина клеток в экспоненциальной фазе роста достигала 162,12 мкм, в стационарной — 172,07 мкм. Как источник фукоксантина, *C. closterium* имеет важное практическое значение; водоросль интенсивно культивируют для получения биологически активных веществ. Результаты получения биомассы фукоксантина из клеток диатомовой водоросли при лабораторном культивировании — 11 мг·г$^{-1}$ сухой массы. Новые данные актуальны и важны, они могут быть использованы в различных областях науки и медицины. Впервые представлена сезонная динамика численности популяций *C. closterium* в разных экотопах: эпизооне беспозвоночных и их пищевых спектрах, эпифитоне донной растительности, перифитоне экспериментальных и антропогенных субстратов различных морей. Максимальная численность популяции вида (65,6·10^3 кл.·см$^{-2}$) зарегистрирована в эпизооне мидии *Mytilus galloprovincialis* Lam. в марте при температуре воды +7,7 °C на глубине 2,5 м в Чёрном море. Максимальная численность отмечена в эпифитоне зелёных водорослей-макрофитов (896·10^3 кл.·см$^{-2}$) и перифитоне асбестовых пластин (728·10^3 кл.·см$^{-2}$) в августе при +24,5 °C в Японском море. Динамика численности природных популяций *C. closterium* в локальных местообитаниях изменялась в зависимости от сезона, глубины, типа субстрата. Рассмотрены сходства и различия в распределении *C. closterium* в микрофитобентосе нескольких морей.

Ключевые слова: диатомовая водоросль, *Cylindrotheca closterium*, таксономия, морфология, фитогеография, экология, экотопы, численность, лабораторное культивирование, фукоксантин