
AQUATIC FLORA
AND FAUNA

First Recorded Marine Species *Prorocentrum leve* Faust, Vandersea, Kibler, Tester & Litaker (Dinophyceae) in an Inland Waterbody

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Abstract—Data on the first recorded marine alga *Prorocentrum leve* Faust, Vandersea, Kibler, Tester & Litaker (Dinophyceae) in a Russian inland waterbody, the hypersaline Lake Manych-Gudilo, are given. As a result, in May, July, and October 2016 and February 2017 it was revealed that the species was found in small quantities, mainly in the northwestern part of the lake. Its morphology, studied with an electron scanning microscope, is largely consistent with that described in the literature. The presence of marine species *P. leve* can serve as indirect evidence of the fragmentation of marine algal areas as a result of paleobasin regression. The results expand information on the composition of the algal flora of inland water bodies in Russia, as well as on the environmental opportunities and general distribution of *P. leve*.

Keywords: *Prorocentrum leve*, dinophyte algae, benthic dinoflagellates, morphology, scanning electron microscopy, Lake Manych-Gudilo

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INTRODUCTION

Dinophyte algae (Dinophyceae) or dinoflagellates are one of the most diverse and widespread microalgae groups in the world's oceans. There are now 4000 known fossils and >2500 reference dinoflagellates, 90% of which live in seas and the rest in freshwater [17]. However, the study of continental hypersaline reservoirs is no less relevant, and data on the dinoflagellates of such reservoirs in southern European Russia and adjacent territories are extremely sporadic [2, 12, 24].

The greatest theoretical interest, especially in terms of florogenetic and palaeogeographic reconstructions, is the data on dinoflagellates of the continental reservoirs of the Ponto-Caspian Sea, which are historically connected with the epicontinental seas, which are the remnants of the ancient ocean of Eastern Paratetis. One such reservoir is the hypersaline Lake Manych-Gudilo located in the central part of the Kumo-Manych depression, the occurrence of which is historically connected with the existence of sea straits (the last one is the Khvalynsky strait) in the late Pleistocene. The final isolation of the reservoir from sea basins with a developed river network was established in the late Holocene [15]. Fauna and flora of water bodies of the Manych depression are extremely heterogeneous, which is connected not only with their subsequent fragmentation in the late Pleistocene and

Holocene, but also with modern human activity—the construction of a cascade of reservoirs and irrigation canals, as well as the introduction of commercial species of hydrobionts [7]. The existence of numerous fossil Mediterranean or Caspian molluscs, benthic, and planktonic foraminifera is evidence of the existence of at least four differently directed sea straits in the Manych depression [11, 15]. Unlike many benthic organisms, on the basis of which the paleontological chronicle of the Ponto-Caspian Sea is based, the presence of sea-based microalgae common to the Azov and Black Seas in the Manych depression reservoirs [5] can serve as indirect evidence of the fragmentation of their ranges as a result of paleobasin regression.

One such example is the detection of a seaweed of the genus *Prorocentrum* in Lake Manych-Gudilo, morphologically fully corresponding to the species *P. leve* Faust, Vandersea, Kibler, Tester & Litaker, recently described (as *P. levis* Faust, Vandersea, Kibler, Tester & Litaker) from the Caribbean Sea [22]. The genus *Prorocentrum* Ehrenberg was singled out in 1834 [21] and currently has ~80 species [26]; its representatives are mainly marine plankton or benthic inhabitants, except for two species found in fresh water [25]. Ten species of the genus are known to be potentially toxic [25, 28]. The species *P. leve*, collected and described from a floating detritus off the coast of Belize in the Caribbean Sea, has also been shown to be

capable of producing okadaic acid and dinophysistoxin-2 [22]. This species was later found in the northern part of the Aegean Sea, western and northwestern Adriatic Sea [18, 27, 29], and the southeastern part of the Bay of Biscay [19], but tests of these strains of *P. leve* did not confirm the presence of toxicity [18, 19, 29].

As previous studies [4, 7, 10, 16] have shown, Lake Manych-Gudilo is characterized by a change in the taxonomic composition of phytoplankton depending on the transformation of its hydrochemical regime. During the period of the greatest flooding, when the level of water salinity did not exceed 20 g/L, only one type of dinophyte algae *Gymnodinium* sp. has been noted [4]. With the subsequent increase in the salinity of the lake, the composition of dinophyte species has increased to seven species, which are typical mainly for marine reservoirs [5–7, 16].

In Lake Manych-Gudilo, *P. leve* was found for the first time in fixed samples in 2016 in the course of comprehensive monitoring studies of water bodies of the Lake Manych-Gudilo depression.

The goal of this work was to describe the morphology of *P. leve* in a hypersaline continental waterbody (using light and scanning electron microscopy) and its occurrence in this area.

MATERIALS AND METHODS

Physical and geographical characteristics and hydrochemical regime of the reservoir. Lake Manych-Gudilo is an inland shallow-water lake (average depth of 2.6 m), a hypersaline body of water located in the zone of unstable and insufficient humidification of the temperate climatic zone [7, 14]. The low rainfall, high summer temperatures, and prolonged hot dry winds in the region result in high evaporation rates, leading to an even higher salinity of the lake. Mineralization of this reservoir also depends on the volume of fresh water supplying it to the Bolshoy Egorlyk and Kalaus rivers [7, 9, 14]. At present, the total salinity of Lake Manych-Gudilo varies from 5.2 (at the river mouths) to ≥ 50 g/L. The lake waters are considered meso- and polyhalic and, in terms of the ratio of basic saline ions, they occupy an intermediate position between the carbonate–sulfate–calcium type of continental saline waters and the sodium chloride type of sea waters [9, 14].

Methods of investigation. In the course of the expedition studies at the reservoirs of the Manich system, the staffs of the Southern Scientific Center of the Russian Academy of Sciences (RAS) in 2016 took water samples in May at five stations, in July at three stations, and in October at 13 stations (from the surface horizon). In addition, in February 2017, subglacial water samples and aquatic vegetation residues deposited on the seabed were collected at two stations (Fig. 1).

To study the morphological features of organisms, we used light microscopy (microscope Mikmed-5), epiluminescence (fluorescent microscope Mikmed-

2-Var-11), and scanning electron microscopy (SEM EVO-40 XVP). Water samples were fixed with an acid solution of Lugol and 25% glutaric aldehyde with a final concentration in the sample of 1% [13].

For electron microscopy, algae cells were deposited on membrane nuclear filters with a pore diameter of 1 μm , washed twice with distilled water, dehydrated through a series of ascending concentration ethanol solutions (25, 40, 55, 70, and 96%), and dried in air at room temperature. The filters were then attached to the microscopic tables with a double-sided conductive carbon tape. Surface spraying of the samples was carried out with the help of Au/Pd-target in the Mini Sputter Coater SC7620 (Quorum Technologies) vacuum spraying unit.

The cells (24 examples) and their details were measured using electronic microphotographs using the Scandium software (v. 5.0), as well as the light microscope and Toupcam UCMOS 10 MP video eyepiece. The standard deviation is given as an indicator of the variation of characters. To identify the microalgae's calcareous plates, we used the terminology proposed by Hoppenrat et al. [25].

RESULTS

Morphology of *Prorocentrum leve*. These are cells of highly oval shape, symmetrical, and concave/double concave from the side of the belt (Figs. 2a–2g). Cell length is 43.74–50.37 μm ($47.99 \pm 1.52 \mu\text{m}$), width is 39.73–45.31 μm ($42.85 \pm 1.38 \mu\text{m}$), and the length to width ratio is 1.05–1.18 (1.12 ± 0.03). The cells contain chloroplasts, a large, clearly visible pyrenoid in the centre, and an oval nucleus in the lower part of the cell (Figs. 2b, 2c). The cell is covered with two large plates, which are joined by the sagittal suture (Fig. 2i). At the apical end of the left plate there is a small indentation (Fig. 2e), and at the right end there is a narrow V-shaped depression with a width of 5.56 μm and depth of 4.01 μm (Figs. 2d–2g). This is the periflagellar zone, where the flagellum and apical pores are located, as well as a number of small platelets (Figs. 2j–2l). The large thecal plates are smooth, with small depressions of 0.45–0.93 μm ($0.64 \pm 0.12 \mu\text{m}$, $n = 48$) scattered over them with two types of pores: the predominant large ones of 0.12–0.21 μm (0.16 ± 0.03 , $n = 59$) and small ones of 0.06–0.11 μm (0.09 ± 0.02 , $n = 13$) in diameter (Fig. 2h). Depressions with pores are scattered almost all over the surface of large plates, are more densely located on the margins, and in most cases are absent in their central part. The number of pores on the plate is 203–310 (267 ± 34.9) and, at the marginal of zone, 134–164 (151 ± 9.2 , $n = 7$). The Intercalary band is smooth (Fig. 2i). In the upper part of the periflagellar zone there are four small platelets; the second one is the smallest (Figs. 2j–2l).

***Prorocentrum leve* algae occurrence in Lake Manych-Gudilo.** In a study of phytoplankton in May

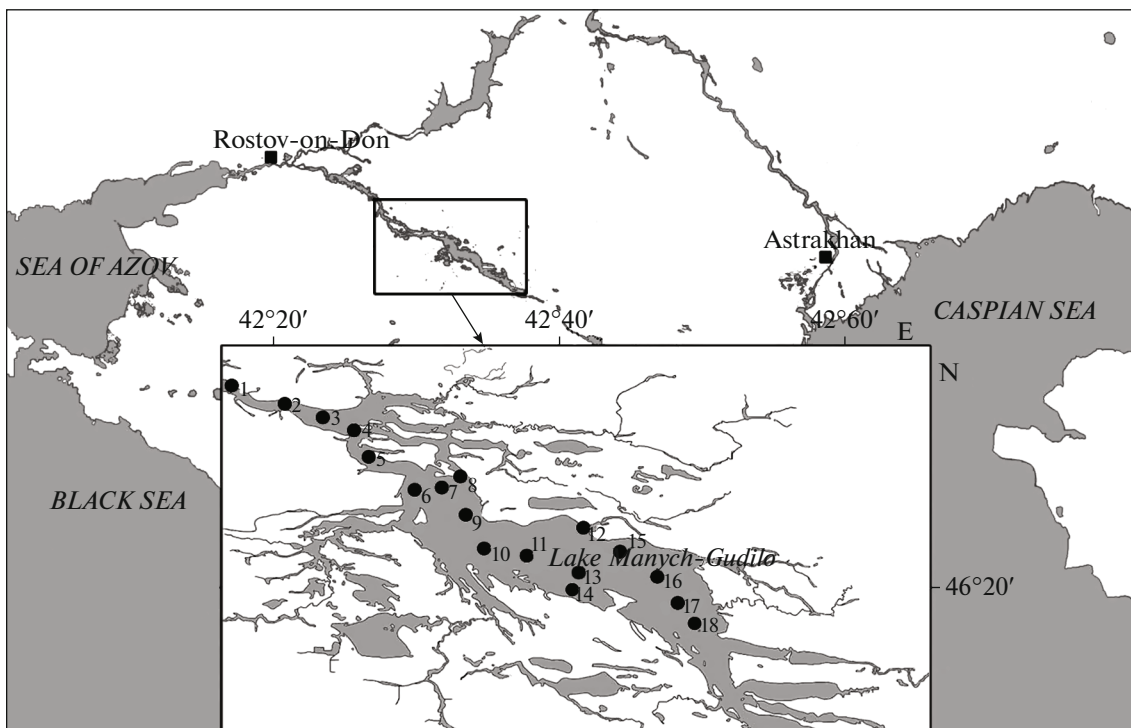


Fig. 1. Map-scheme of Kumo-Manych depression reservoirs. In the footnote there are locations of water sampling stations on the Lake Manych-Gudilo: in 2016 May (stations nos. 1–5); July (nos. 6–8); October (nos. 6–18); and, in 2017, February (nos. 8 and 12).

2016 (stations nos. 1–5) (Fig. 1) *P. leve* cells were not detected. In July, samples of plankton were taken in the northwestern part of the lake (stations nos. 6–8) at a heavy wave level; *P. leve* was present at these stations at a number of 1300–3826 cells/L (2–4% of the total phytoplankton number). Water temperature was 25.3–26.8°C and salinity was 44 g/L. Small green algae were dominated in the plankton (83–94% of total phytoplankton abundance). Dinoflagellates and diatoms accounted for 11 and 6%, respectively. In addition to *P. leve*, other marine species of dinoflagellates were found, such as *Heterocapsa rotundata* (Lohmann) Hansen and *Akashiwo sanguinea* (Hirasaka) Hansen et Moestrup.

In October, almost the entire water area of the lake was studied (stations nos. 6–18). However, *P. leve*, as in July, was recorded only on stations nos. 6–8 in the northwestern part of the lake. Numbers of the species reached 1111–1342 cells/L and were dominated by *A. sanguinea*. During this period, the water surface temperature reached 7–10°C and salinity was 38–44 g/L.

In February 2017, at 0.2–0.3°C and salinity 6–38 g/L, *P. leve* was absent in plankton and only a few fixed cells with compressed content were found on dead filamentous algae.

DISCUSSION

The morphology of *P. leve* from Lake Manych-Gudilo coincides with the first description of the species in terms of cell shape, absence of thecal ornamentation, and structure of the periplagellar zone [22]. According to M. Faust et al. [22], these features distinguish *P. leve* from other species of the genus *Prorocentrum*. Cells from the lake are larger than cells from the Caribbean and Aegean Seas, but fall within the size range specified for *P. leve* from the Bay of Biscay (Table 1). In contrast to cells from the sea coast, specimens from the lake had more pores on their large plates and in their marginal zone (Table 1). As in the cultures of *P. leve* from the Bay of Biscay, two types of pores were noted in the studied specimens on large plates: large pores and small ones. The diameter of the large pores coincided with that in the first description of the species (Table 1). In addition, the diameter of the depressions in which the pores were located was noticeably larger than that of the cells from the Bay of Biscay (Table 1). The morphological differences may be due to the process of the organism adapting to an unusual environment, which is characterized by a high degree of equally chloride and sulfate salinity.

Until now, *P. leve* has been observed exclusively in the zones of tropical [22], subtropical (northern Mediterranean Sea [18, 27, 29]), and temperate (Atlantic, off the coast of the Iberian Peninsula in the Bay of Bis-

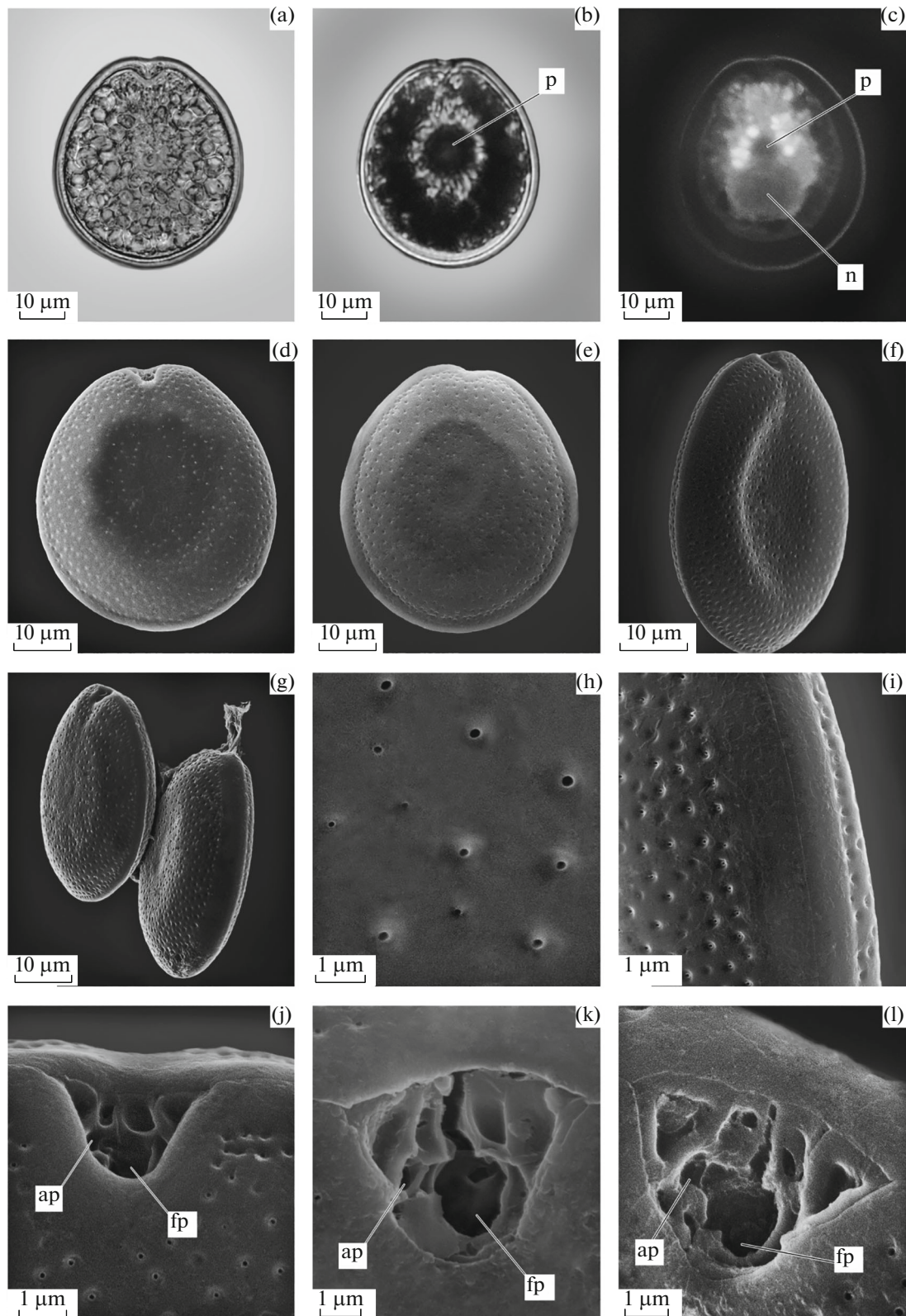


Fig. 2. Light (a–c) and electronic (d–l) microphotographs *Prorocentrum leve*. (a, b) view of different cells on the right side – (a) fixation with glutare and (b) fixation with Lugol solution; (c) view of the cell on the left side (fluorescent microscope); (d) view on the right side; (e) view on the left side; (f, g) lateral view of the cells; (h) pores; (i) view of the insertion belt; (j–l) periflagellar zone. (ap) is the apical pore, (fp) is the flagellar pore, (p) is the pirenoid, and (n) are the nucleus.

Table 1. Comparative morphological characteristics of *Prorocentrum leve*

Features	Caribbean Sea, Belizean coast [22]	North Aegean Sea [18]	Biscay Bay [19]	Lake Manych-Gudilo (authors' data)
Cell shape	Oval	Oval	Oval	Highly oval
Cell length, μm	40–44	38–49	37–51 (45 ± 4)	44–50 (48 ± 2)
Cell width, μm	37–40	33–37	30–42 (36 ± 3)	40–45 (43 ± 1)
Length/width ratio	–	1.1–1.3	1.3*	1.1–1.2
Number of pores:				
on plate	221–238	–	211–222	203–310
on edge	99–130	–	105	134–164
Diameter of depression, μm	–	–	0.27–0.59	0.45–0.93
Pora diameter, μm :				
large pora	0.13–0.19	–	0.12–0.19	0.12–0.21
small pora	–	–	0.05–0.10	0.06–0.11
Biotop	Floating detritus	Macrophytes, water column, precipitation	Macrophytes	Water column, macrophytes

Average values and their errors are given in brackets. An asterisk (*) indicates the value calculated from the average value.

cay [19]) zons. This is the first record of *P. leve* in a continental waterbody, which is also characterized by high salinity. In addition to this species, representatives of other genera of marine dinoflagellates are registered in the lake, such as *Akashiwo* Hansen & Moestrup, 2000, *Heterocapsa* Stein, 1883, and *Gyrodinium* Kofoid & Swezy, 1921. Types of these genera were also found in the lake earlier under waterbody salinity ≤ 30 g/L [7, 16]. Under current conditions, at salt content ≥ 50 g/L, marine species *Glenodinium pilula* (Ostenfeld) Schiller, *Scrippsiella acuminata* (Ehrenberg) Kretschmann et al., *Prorocentrum scutellum* Schröder, *Heterocapsa rotundata* (Lohmann) Hansen were found among dinoflagellates [5, 6].

Dinoflagellate *P. leve* has not been previously found in Russian reservoirs. However, as early as in the 1930s, dinoflagellates of *Exuviaella caspica* Kisselew were described by I.A. Kiselev [1] from the Northeastern Caspian bays with water salinity of 31–53‰. Later this species was found in the eastern part of the Sivash Bay of the Sea of Azov, water bodies of Ukraine, and lakes of flat Georgia [8]. In the mid-1970s, the genus *Exuviaella* Cienkowski, 1881, was abolished and the species was reduced to synonyms with *Prorocentrum lima* (Ehrenberg) Stein [20]. A.F. Krahnalny [3] considered this species to be an independent taxon and proposed a new nomenclature combination—*Prorocentrum caspica* (Kisselew) Krahnalny, but *P. caspica* was not included in the list of modern free-living dinoflagellates [23]. Actually, the shape and size of *Exuviaella caspica* coincide with those of *P. leve*. However, the wide oval shape and pyrenoid in the center of the cell are present not only in *P. leve*, but also in several

types of benthic dinoflagellates, including *P. lima*, characterized by wide variability [25]. The description of the ornamentation of the *E. caspica* cell as “smooth or punctured” [1, p. 112] suggests that two morphologically similar species of benthic *Prorocentrum* inhabit the Caspian Sea. It is necessary to verify the species from the typical location described as *E. caspica* using modern research methods.

It's hard to tell how and when *P. leve* entered Lake Manych-Gudilo. It is possible that this is an autochthonous species with a wide tolerance to salinity fluctuations, which the species successfully experienced in the form of cysts. In previous studies of plankton, *P. leve* was not noted [4–7, 10, 16], probably because it leads a predominantly epiphytic lifestyle [18, 22], and no studies of microalgae in the growths have been conducted here. The species was detected by us for the first time during a strong wave in the lake, as a result of which epiphyte organisms could be carried out in the water column. It remains unclear which biotope in this pond *P. leve* prefers, its seasonal dynamics and distribution in the lake, and whether the local population is able to produce a toxin.

CONCLUSIONS

Marine dinoflagellate *P. leve* was registered for the first time in an inland waterbody in Russia in hypersaline Lake Manych-Gudilo. Morphological characteristics of the species in general correspond to the first description, but there are a number of structural features. *P. leve* mass development in lake Manych-Gudilo was not achieved and was recorded only in the

northwestern part of the reservoir in a vegetative state in July and October in plankton and in transition to cysts in February on algae mats. The finding of this species in Lake Manych-Gudilo may serve as indirect evidence of the fragmentation of seaweed habitats as a result of paleobasin regression and significantly expands knowledge about the composition of the algal flora of inland water bodies in Russia, as well as about the ecology and distribution of this marine species.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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