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Records of sessile ciliates (Ciliophora, Peritrichia) on the green filamentous algae *Cladophora sivashensis* in the Sivash Bay (the Sea of Azov)

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Summary

Three species of sessile ciliates, i.e. *Cothurnia trophoniae, C. angusta* and *Cothurniopsis valvata*, were found in the Sivash Bay (the Sea of Azov) on the floating mats of the green filamentous algae *Cladophora sivashensis* at salinities from 68 to 84‰. All three species of ciliates are new for the Azov-Black Sea basin as well as for hypersaline waters in general. The systematic position and short descriptions of the discovered species are presented.

Key words: *Cothurnia, Cothurniopsis*, hypersaline waters, peritrich ciliates, Sea of Azov, Sivash Bay

Introduction

Hypersaline waters are among the extreme habitats for aquatic organisms and, therefore, they are characterized by a lower species richness (Por, 1980; Williams et al., 1990; Anufriieva, 2015; Dovgal and Sergeeva, 2016).

The Sivash, also known as the Rotten Sea, is a bay in the western part of the Sea of Azov. This is the world's largest hypersaline lagoon, with an area of about 2700 km^2 (Vorobyev, 1940; Shadrin et al., 2018; Sergeeva et al., 2019). Before construction of the North-Crimean canal to provide fresh water from the River Dnieper into the Crimea in the 1960–1970s, it was a hypersaline shallow lagoon with the average salinity of 140‰. After the canal construction, fresh water source was used for agriculture, and then wastewaters started to enter the Sivash Bay. Salinity gradually decreased down to the average of 22‰, and the bay ecosystem underwent an ecological change. However, in 2014 water salinity in the Sivash started to increase due the North-Crimean canal closure and, in turn, the fauna composition changed (Shadrin et al., 2018; Sergeeva et al., 2019). Currently, the salinity gradient stretches from 30‰ in the northern part of the bay up to more than 100‰ in the southern part.

Nowadays, the intensive development of the green filamentous algae *Cladophora sivashensis* Meyer, 1922 occurs in the bay, forming the floating

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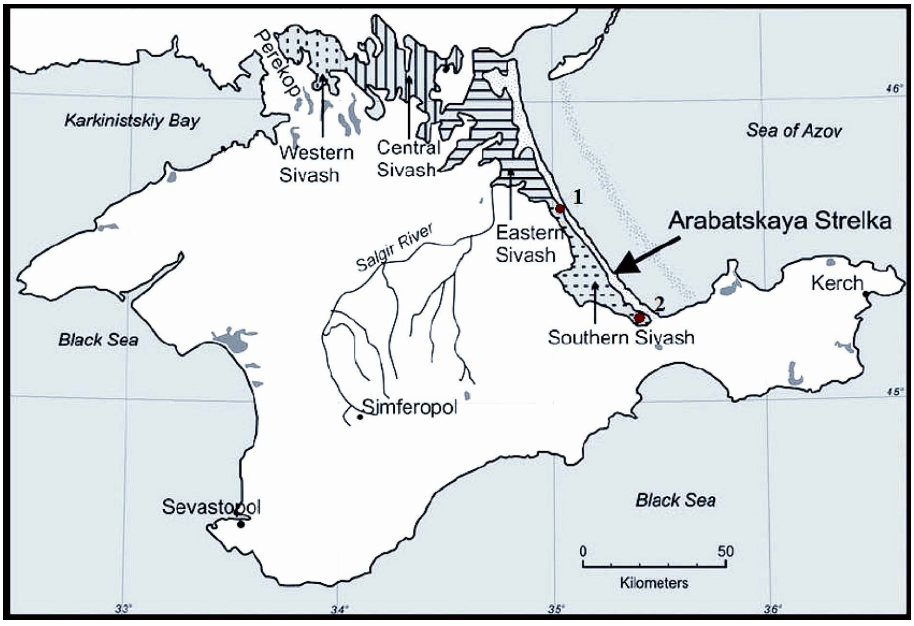

Fig. 1. Map of sampling area (June 2019). Station 1 – (45°37′9.0″N, 35°04′40.0″E); station 2 – (45°17′14.3″N, 35°28′01.2″E).

mats with biomass of up to 2–2.5 kg/m^2 over a large area (Shadrin et al., 2018; Sergeeva et al., 2019; Anufriieva and Shadrin, 2020).

In the recent years, a number of works have been devoted to the study of the bay and different taxa therein (Shadrin et al., 2018; Sergeeva et al., 2019; Anufriieva and Shadrin, 2020), but the ciliates have been hardly investigated.

Twenty-four species of Ciliophora have been found to date in the hypersaline waters of the Crimea (Dovgal et al., 2006; Pavlovskya et al, 2009), including the sessile species *Acinetides infundibuliformis* (Wang et Nie, 1933) found in the Sivash (Class Suctorea Claparéde et Lachmann, 1859) on the alga *Cladophora* sp. by Dovgal et al. (2006); *Cothurnia maritima* Ehrenberg, 1838 (Subclass Peritrichia) on the harpacticoids (Dovgal et al., 2006; Dovgal, 2013; Dovgal and Sergeeva, 2016); and *Conidophrys fucatum* (Averinzeff, 1916) (Order Pilisuctorida Jankowski, 1966) found on the amphipods *Gammarus subtipicus* Stock, 1966 and *G. aequicauda* (Martynov, 1931; Dovgal and Mayén-Estrada (2015).

Epibiont organisms can achieve great abundances and play an important role in the functioning of various aquatic ecosystems (Shadrin, 1990; Weissman et al., 1993; Shadrin et al., 2002; Gilbert and Schröder, 2003). The sessile ciliates are an essential component of the epibiont communities; among those, the sessile peritrich ciliates attached to aquatic plants and submerged abiotic substrates are studied best of all (Lu et al., 2020).

This article provides the descriptions of three species of peritrich ciliates found in the Sivash Bay on the thalli of the algae *C. sivashensis.*

Material and methods

Mats formed by macroalgae, mainly *Cladophora* sp., are locally distributed in the water mass and on the bottom surface of the Sivash Bay.

The samples were collected at two stations (Fig. 1) under different salinity conditions in June 2019. At the first station located in the north-west of the bay (45°37′9.0″N, 35°04′40.0″E), water salinity was 68‰ and temperature 26 °C; at the second station in the southern part of the bay (45°17′14.3″N, 35°28′01.2″E), salinity reached 84‰ and temperature 29 °C.

Samples of algae were preserved in 4% formaldehyde. The mats were carefully washed with water through a nylon sieve with the mesh size of 63 μm. Algae were weighed after washing the samples; the mats and the resulting deposits were placed in a small volume of distilled water, stained with Bengal Rose, and analyzed with an optical microscope CX41 at the magnifications of 600–1000×. All observed organisms were counted (Shadrin et al., 2018), with the subsequent identification to the species level.

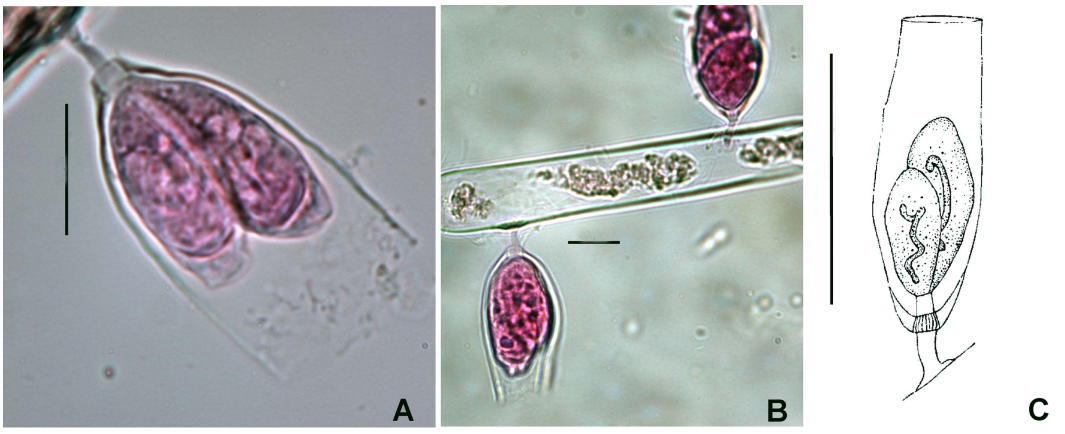

Fig. 2. *Cothurnia trophoniae* Dons, 1946. A, B – On the green filamentous algae *Cladophora sivashensis* C. Meyer, 1922 (original); C – modified from Warren and Paynter, 1991. Scale bars: A, B – 20 μm, C – 100 μm.

During the process of investigation, numerous attached and free-living ciliates were found. A careful study of these organisms revealed several ciliate species that are unknown for the Sivash Bay and the Sea of Azov. Pictures were made with a stereomicroscope Nikon E200.

Measurements were carried out using the program Toup View 3.7 for digital camera. For identification of the ciliate species, we used the works of Kahl (1935) and Warren and Paynter (1991). Classification of peritrich ciliates follows Lynn (2008).

Results and discussion

Three species of peritrich ciliates: *Cothurnia trophoniae* Dons, 1946, *C. pedunculata* Dons, 1918 and *Cothurniopsis valvata* Stokes, 1893 were observed on thalli of the green filamentous algae *C. sivashensis*, collected in the Sivash Bay. There were from 10 to 16 ciliates registered per 1 mm of the thalli.

These species were not mentioned in the review articles by Gassowski (1960) and Konstantynenko (2014) that contained the earlier data on ciliates of the Azov-Black Sea basin. Thus, the species listed above are new for both the Sivash Bay and the Azov-Black Sea basin as a whole. In addition, these species were not previously recorded in the hypersaline waters.

The brief diagnoses, the systematic position and illustrations of the found species are given below.

Phylum: Ciliophora Doflein, 1901
Subphylum: Intramacronucleata Lynn, 1996
Class: Oligohymenophorea de Puytorac et al., 1974
Subclass: Peritrichia Stein, 1859
Order: Sessilida Kahl, 1933
Family: Vaginicolidae Fromentel, 1874
Genus: *Cothurnia* Ehrenberg, 1831

Cothurnia trophoniae Dons, 1946 (Fig. 2)

Description (after Warren and Paynter, 1991 with modifications). Lorica roughly cylindrical, 53.4 μm long × 31.5 μm wide (125–140 × 40–50 μm after Warren and Paynter, 1991). Lorica aperture oval when viewed from above, 30 μm long. External stalk 8 μm long (65–135 μm long after Warren and Paynter, 1991), provided with extension (basal disc), which is used for attachment; mesostyle short and broad with conspicuous longitudinal striae, 3.5 μm long × 5.5 μm wide; endostyle absent. Lorica contains two zooids attached to its base, contracted zooids are 36 μm long × 15 μm wide (uncontracted are 70 μm long × 35 μm wide after Warren and Paynter, 1991). Macronucleus vermiform and coiled irregularly. Pellicular striations inconspicuous.

Habitat. Initially the species was found in the Kiel Bight (the North Sea) as an epibiont of the alga *Polysiphonia violacea* Sprengel 1827, the polychaetes *Trophonia plumosa* (Müller 1771) and *Stylarioides plumosa* (Müller 1776), the cnidarian *Laomedea loveni* Allman, 1859 and the pantopod *Pallene brevirostris* Johnston, 1837 (Precht, 1935). The species was previously described as an inhabitant of marine water bodies (Warren and Paynter, 1991), in part on alga *Enteromorpha flexuosa* (Wulfen, 1803) in Temiahua Lagoon, Veracruz, Mexico (Alardo-Lubel et

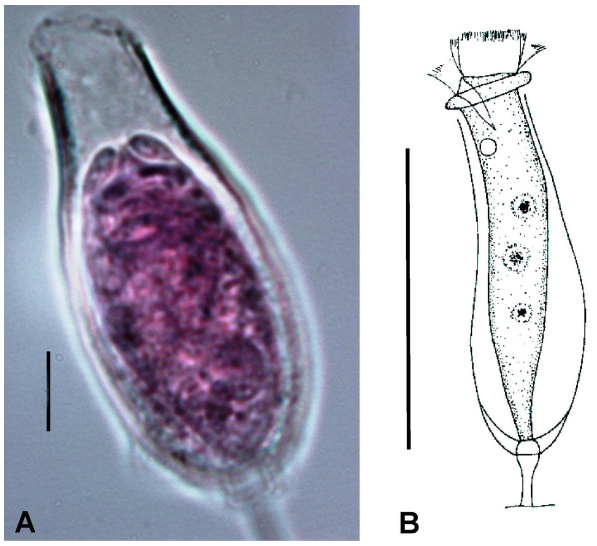

Fig. 3. *Cothurnia angusta* Kahl, 1933. A – Original; B – modified from Warren and Paynter, 1991. Scale bars: A – 20 μm, B – 50 μm.

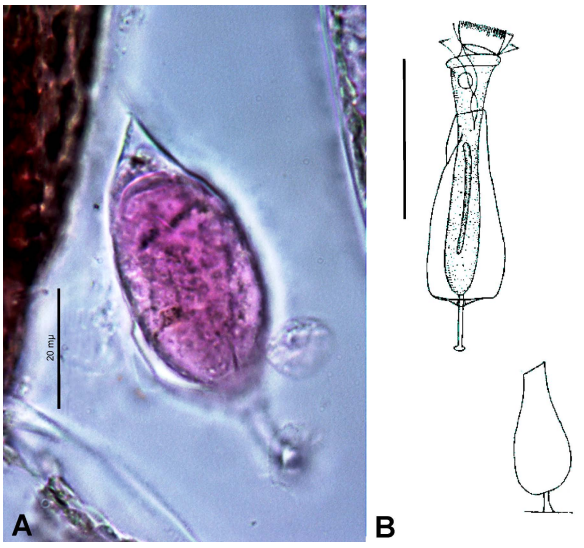

Fig. 4. *Cothurniopsis valvata* Stokes, 1893. A – Original (aperture closed); B – modified from Warren and Paynter, 1991. Scale bars: A – 20 μm, B – 50 μm.

al., 2006). In addition, the species was registered in the Baltic Sea (Mironova et al., 2014).

Cothurnia angusta Kahl, 1933 (Fig. 3)

Description (after Warren and Paynter, 1991 with modifications). Lorica elongated, 64.4 μm long × 26.2 μm wide (60 μm × 15–23 μm after Warren and Paynter, 1991), rounded posteriorly. Aperture 12.8 μm (10 μm after Warren and Paynter, 1991) in diameter. External stalk about 11.5 μm long (10 μm after Warren and Paynter, 1991), mesostyle short, endostyle absent. Zooid 43.5 μm long × 20 μm wide (70 μm × 12 μm after Warren and Paynter, 1991) and does not extend beyond aperture. Pellicular striations inconspicuous.

Habitat. Initially the species was found attached to ostracods of the family Cypridae from the Kiel Bight, the North Sea (Kahl, 1933; Precht, 1935); also found on *Ulva intestinalis* Sommer, 1951 (Warren and Paynter, 1991). Earlier the species was considered to inhabit both brackish and fresh waters (Warren and Paynter, 1991).

Genus: *Cothurniopsis* Stokes, 1893

Cothurniopsis valvata Stokes, 1893 (Fig. 4)

Description (after Warren and Paynter, 1991 with modifications). Lorica 53 μm long ×25.5 μm wide (50–70 μm × 25–35 μm after Warren and Paynter, 1991) and rounded posteriorly. Lorica aperture closes when zooid is contracting. External stalk 11 μm long (10–18 μm after Warren and Paynter, 1991). Zooid 39.5 μm long × 22.1 μm wide (75–80 μm × 10–15 μm after Warren and Paynter, 1991), and does not extend beyond aperture. Macronucleus straight, lies longitudinally in posterior half of body.

Habitat. Originally found attached to filamentous algae from the Coney Island, New York, USA (Stokes, 1893); also isolated from moss in Switzerland (Penard, 1914).

Is considered to inhabit brackish or fresh waters (Warren and Paynter, 1991).

Conclusions

Peritrich ciliates are quite common in natural water bodies and play an important role in maintaining the ecological equilibrium and the processes of water self-purification therein. They are also indicator organisms of the sanitary and hygienic state of water basins. Consequently, these organisms deserve further in-depth investigations.

In the present study, we obtained new data about ciliates inhabiting the floating mats of the green filamentous algae in the conditions of hypersalinity. Three species of sessile ciliates, i.e. *Cothurnia trophoniae, C. angusta* and *Cothurniopsis valvata*, were found in the Sivash Bay (the Sea of

Azov) on *Cladophora sivashensis* at salinities from 68 to 84‰. All three species of ciliates are new for the Azov-Black Sea basin as well as for hypersaline waters in general.

The floating mats are widespread in the hypersaline water bodies of the Crimea (Prazukin et al., 2018; Shadrin et al., 2018) and are a convenient substrate for the epibiont ciliates. The fact that all species found in the mats turned out to be new to the studied region indicates that the current knowledge of this group in the region is largely insufficient.

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