First finds of sessile ciliates (Ciliophora) in artificial and natural caverns on the Crimean coast of the Black Sea

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Abstract
The article presents sessile ciliates (Ciliophora) found during first studies of biological diversity of the underwater part of the artificial canal, created in the middle of the 20th century on the western shore of the Balaklava Bay (Sevastopol, the Black Sea), and the natural coastal Catherine Grotto (Cape Aya, the Black Sea). In total, 4 species of ciliates were found, one of which Cothurnia ovalis Kahl, 1933, is new not only for Balaklava Bay, but for the Black Sea. Description of the species, their systematic position, morphometric characteristics and information about their distribution are given.

Key words: biodiversity, biofouling, Black Sea, bottom sediments, peritrich ciliate, suctorian.

Introduction

To date, sea caves and grottoes of the Black Sea remain the least explored natural objects. There are practically no data of complex biological studies of sea caves. The first information about invertebrates in coastal caves of the Black Sea dates back to the middle of the 20th century.

There are practically no data of complex biological studies of sea caves. Moreover, most of the available biological studies on this topic are not systemic but situational. For this reason, there is very little information on the biodiversity of bottom communities of underwater in coastal caves (Vorobyova et al., 2012; Sergeeva et al., 2021).

There are a few works on the invertebrates and ciliates in extremal biotopes on the Black Sea (Sergeeva & Dovgal, 2014; Sergeeva & Mazlumyan, 2015), however, they are not concerning underwater caves.

In 2020 under the guidance of the first author of the article, began a comprehensive study of the coastal caves and grottoes of the Crimean Peninsula. The research was carried out in the underwater part of an artificial underground canal in Balaklava (the western coast of the Balaklava Bay) and Cape Aya, where the Catherine Grotto is located.

Previous studies of bottom sediments only in the entrance part of the canal date back to 1992, however, no living forms of macrozoobenthos were found there (Mironov et al., 1999).
The uniqueness of the Balaklava underground canal is determined by the fact that it is an analogue of the natural Black Sea cave, but of anthropogenic formation. A detailed description, physical, chemical and hydrodynamic characteristics of the underground canal, general description of biological diversity are given in the article (Sergeeva et al., 2021).

Catherine Grotto (Big Grotto) is located 8 km from Balaklava. It starts from a depth of 9 meters and deepens uphill by 50 meters. It is known that the unloading of underground fresh waters is concentrated in it.

This work is devoted to description of the sessile ciliates found in the bottom sediments and in walls fouling of the underground canal and the Catherine Grotto. The results obtained make it possible to expand information about the taxonomic diversity of ciliates living in underground canals and grottoes, about the biodiversity of the Balaklava Bay and the Catherine Grotto as a whole. Moreover, after analyzing the published lists of ciliates found in the Black Sea (Gassowski, 1960; Konstantinenko, 2014), and a number of review articles (for example, Dovgal et al., 2006; Dovgal, 2013; Sergeeva et al., 2020; Abibulaeva & Dovgal, 2021), we found that the species *Cothurnia ovalis* Kahl, 1933 is new not only for Balaklava Bay, but for the Black Sea.

**Material and methods**

The fauna of grotto and canal walls fouling and bottom sediments were studied. Sampling of bottom sediments and fouling of these grottoes for hydrobiological studies were carried out by scuba divers. Bottom sediments sampling for study of meiobenthos was carried out with a tubular sampler ($S = 18 \text{ cm}^2$) with three replications at each station. The fouling of grotto walls (macro- and meiobenthos) was excised using standard hydrobiological frames ($S=0.04 \text{ m}^2$) with a bag of mill gas sewn into them with a mesh size of 64 $\mu$m (two replications). Hydrobiological survey in the underground canal of Balaklava and Catherine grotto were carried out in March and July 2020, respectively.

During of material collecting the water temperature along the underground channel was 10 ºC, the salinity of water varied from 18.9 to 19.2‰. The acid-alkaline balance in the bottom sediments was 7.1–7.5, and the redox potential varied in range from -256.4 to -95.3.

Collected samples of bottom sediments and fouling were fixed in 75% alcohol. In the laboratory of the IBSS all samples were washed through two sieves, the upper one with a mesh size of 1 mm, the lower one with a mesh size of 63 $\mu$m, and stained with Rose Bengal solution targeting the “live” (i.e., stained) meiobenthic fauna before being sorted under a stereo-microscope using a Bogorov chamber. The morphological characteristics and dimensional parameters were studied under an Olympus CX41 microscope with magnifications of 100–1000x. Measurements were carried out using the program Toup View 3.7 for digital camera. Gelatin-glycerine preparats of ciliates deposited in the collection of the Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation.

For identification the ciliates, we used the works of Kahl (1935), Warren and Paynter (1991) and Dovgal (2013). The taxonomic position of peritrich ciliate taxa is given according to Lynn (2008) and suctorian taxon according to Dovgal (2013).

The canal is located in coordinates (44°30'1.8"N, 33°35'48.48"E).

Station №1 is located at a distance of 50 m from the canal exit. The collected sample is bottom sediment (silt) with strong smell of hydrogen sulfide. The walls of the canal are covered with biofouling from *Mytilaster lineatus* (Bivalvia), Bryozoa, Porifera and Ascidiae.

Station №2 is located 150–200 meters away from the canal exit.

Station №3 of Catherine Grotto is located in coordinates (44°26'26"N, 33°38'37"E). The walls of the grotto are covered with biofouling mostly from Bryozoa.

Specimens are kept in the collection of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (Sevastopol, Russian Federation).

**Results and discussion**

The following sessile ciliates were found: in the bottom sediments of the canal – *Cothurnia ovalis* Kahl, 1933 attached to Oligochaeta (station № 1); and two species of *Zoothamnium* were found in the canal walls
fouling (station № 2 and № 1). *Praethecacineta halacari* Schultz, 1933 were found in large numbers attached to Halacarida in grotto walls fouling sampled from Catherine Grotto (station № 3).

Descriptions, taxonomy and illustrations of the collected 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

1. *Cothurnia ovalis* (Wailes, 1928) Kahl, 1933 (fig. 1, table 1).

= *Cothurnia innata* Wailes, 1928

**Description.** The species was originally described from Departure Bay, Vancouver (Wailes, 1928). Marine loricate peritrichs, with one zooid per lorica. Diameter of aperture slightly smaller than that of lorica. Aperture oval when viewed from above, aperture border without clefts. External stalk short with bulbous thickening at its point of attachment to lorica. Endostyle and mesostyle absent. Pellicular striations inconspicuous. After Warren and Paynter (1991) macronucleus short, curved and lies longitudinally in centre of zooid.

**Our material.** Gelatin-glycerine prepare ibss.Cil.2.v, deposited in the collection IBSS RAS (Sevastopol).

**Locality.** In present find this species is reported as an epibiont attached to Oligochaeta collected from underwater canal of the Balaklava Bay (the Black Sea) (station №1). Samples were collected at a depth of 3,8 m.

**Measurements.** Morphometric measurements of *Cothurnia ovalis* observed in the present study (based on 8 individuals, in μm): Lorica length 73–86, lorica width 28–47 (40 μm long × 22 μm wide after Warren and Paynter, 1991), zooid when contracted length 29–51, width 19–29 (50–58 μm long × 12–18 μm wide after Warren and Paynter, 1991), external stalk length 16–38 (according Warren and Paynter, 1991 up to 27 μm), aperture 25–45 in diameter (27 μm × 14 μm after Warren and Paynter, 1991).

Table 1 gives detail measurements of various parts found in each of 8 individuals.

| Sample number | Lorica (length), μm | Lorica (width), μm | Lorica aperture (length), μm | Zooid when contracted (length), μm | Zooid when contracted (width), μm | External stalk, μm |
|---------------|---------------------|-------------------|-----------------------------|----------------------------------|---------------------------------|-------------------|
| 1             | 73                  | 32                | 30                          | 51                               | 24                              | 17                |
| 2             | 77                  | 40                | 25                          | 30                               | 27                              | 16                |
| 3             | 80                  | 40                | 29                          | 31                               | 25                              | 38                |
| 4             | 80                  | 28                | 23                          | 48                               | 21                              | 17                |
| 5             | 86                  | 47                | 45                          | 34                               | 27                              | 20                |
| 6             | 79                  | 43                | 41                          | 49                               | 29                              | 25                |
| 7             | 79                  | 43                | 31                          | 31                               | 23                              | 24                |
| 8             | 73                  | 34                | 27                          | 29                               | 19                              | 16                |
Family Zoothamniidae Sommer, 1951
Genus: *Zoothamnium* Bory de St. Vincent, 1826

1. *Zoothamnium* sp. 1 (fig. 2)

**Description.** The colony is alternately branched stalk up to 150 μm high with up to 40 zooids in present collection. The diameter of the main branch of the stalk is about 5 μm. Zooids elongate, measuring about 5–16 μm in diameter when contracted.

**Our material.** Prepare ibss.Cil.3.v deposited in the collection IBSS RAS (Sevastopol).

**Locality.** In present find this species is reported in fouling of the canal walls from underwater part of the canal (Balaklava Bay, Black Sea) at station № 2 (central least illuminated part). Samples were collected at a depth of 5,5 m.

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**Figure 1.** *Cothurnia ovalis* Kahl, 1933. A, B – Original; C – General view of *Cothurnia ovalis* attached to Oligochaeta; D – After Kahl, 1933, scale bar: 50 μm.
Unfortunately, due to fixation of the material, it is not possible to identify these specimens to the species level. But it can also be argued that these are different species of Zoothamnium, which is due to different types of branching, which is one of the key aspects in determining this genus to species.

Figure 2. Zoothamnium sp. 1. A, C – General view of Zoothamnium sp.; B – Type of branching of Zoothamnium sp.; D – Zooid of Zoothamnium sp.

2. Zoothamnium sp. 2 (fig.3)

Description. The colony is asymmetrical alternately branched, up to 200 μm high with up to 30 zooids in present collection. The diameter of the main branch of the stalk is about 4 μm. Basal part of primary stalk comprises up to 50% of colony height. Zooids elongate, measuring about 5–17 μm in diameter when contracted. Peristomial lip with a medial circumferential infolding.

Our material. Preparate ibss.Cil.4.v deposited in the collection IBSS RAS (Sevastopol).

Locality. In present find this species is reported attached to Halacarida in fouling of the canal walls from underwater part of the canal (Balaklava Bay, Black Sea) at station № 1 (exit of the canal).
Class Suctorea Claparède et Lachmann, 1859  
Subclass Exogenia Collin, 1912  
Order Metacinetida Jankowski, 1978  
Family Praethecacinetidae Dovgal, 1996  
Genus Praethecacineta Matthes, 1956

3. **Praethecacineta halacari** Schultz, 1933 (fig.4, table 2)

**Description.** Marine loricate suctorian attached to marine Halacarida. Lorica surface smooth, without transverse folds. The body expands somewhat towards the point of attachment to the lorica. The body occupy the entire lorica size, partly protruding beyond its aperture. Tentacles are club-shaped, collected in one bundle on the upper surface of the body. After Dovgal (2013) spherical macronucleus located in the lower body, single contractile vacuole.
**Measurements.** Morphometric measurements of specimens of *Praethecacineta halacari* observed in the present study (based on 5 individuals, in μm): Lorica length 36–47, lorica width 25–27, lorica aperture diameter 22–27, body length 35–47, width 18–22, external stalk length 7–36, tentacles length 4–49.

Table 2 gives detail measurements of various parts found in each of 5 individuals.

**Habitat and host specificity.** This species is widespread. Originally described from Halacarida collected from Tromsø, Norway (type locality) (Dovgal, 2013). Subsequently, it was repeatedly noted in the North, Black and Caspian Seas, off the Atlantic coast of Brazil (Dovgal et al., 2008) and Canada, in the Pacific Ocean off the island of Taiwan, and also in the Indian Ocean off the coast of Tanzania (Dovgal et al., 2009). In the Crimean water area of the Black Sea, it was first found on *Copidognathus brachystomus* Viets, 1940 off the coast of the Karadag Nature Reserve (Boshko & Dovgal, 2004). Later found on the halacarid mite from the coast near the Cape Martyan nature reserve near the village of Nikita (the Black Sea) (Gelmboldt & Dovgal, 2005). Hosts – Halacarida genera *Copidognathus* Trouessart, 1888 and *Caspialacarus* Viets, 1928 (Dovgal, 2013).

List of recorded *P. halacari* with geographical distributions and their host species is presented in the article (Durucan & Boyaci, 2019).
Table 2. Measurements of Praethecacineta halacari Schultz, 1933.

| Sample number | Lorica (length), µm | Lorica (width), µm | Lorica aperture (diameter), µm | Body (length), µm | Body (width), µm | External stalk, µm | Tentacle (length), µm |
|---------------|---------------------|--------------------|-------------------------------|------------------|--------------------|-----------------|---------------------|
| 1             | 46                  | 27                 | 26                            | 35               | 18                 | 11              | 6                   |
| 2             | 47                  | 27                 | 27                            | 42               | 19                 | 12              | 9                   |
| 3             | 36                  | 25                 | 24                            | 47               | 18                 | 7               | 4                   |
| 4             | 40                  | 25                 | 22                            | 47               | 21                 | 36              | 5                   |
| 5             | 36                  | 26                 | 27                            | 36               | 22                 | 21              | 4                   |

Our material. Prepare ibss.Cil.1.v deposited in the collection IBSS RAS (Sevastopol).

Locality. In our samples this species is reported as numerous epibionts of Halacarida attached on all areas of its body (more 25 ind. on specimen mite) found from Black Sea Catherine Grotto (station № 3). Samples were collected at a depth of 4 m.

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