First finding of epibiont peritrich and suctorian ciliates (Ciliophora) on oligochaetes and harpacticoid copepods from the deep-water hypoxic/anoxic conditions of the Black Sea

NELLI SERGEEVA¹ and IGOR DOVGAL²

¹Institute of Biology of the Southern Seas, 2, Nakhimov av., 299011, Sevastopol, Russia. E-mail: nserg05@mail.ru
²Schmalhausen Institute of Zoology, 15, B. Khmelnitsky str., 01601, Kiev, Ukraine. E-mail: dovgal@izan.kiev.ua

Abstract
Three species of commensal ciliates (Cothurnia maritima Ehrenberg, 1838 on oligochaete Tubificoides sp.; Paracineta livadiana (Mereschkowsky, 1881) and Corynophrya lyngbyi (Ehrenberg, 1834) on harpacticoid copepods Amphiascella subdebilis (Willey, 1935), Haloschizopera pontarchis Por, 1959, Cletodes tenuipes Scott, 1896 and Enhydrosoma longifurcatum Sars, 1909) were found in the Black Sea deep-water under hypoxic/anoxic conditions for the first time. Corynophrya lyngbyi is reported for the first time in the Black Sea.

Key words: ciliates, hypoxic/anoxic conditions, the Black Sea, commensal.

Introduction
The Black Sea is known to be permanently anoxic below a water depth of about 120 m in the open sea, and of about 180 m at its shelves, down to its deepest parts at 2200 m depth.

Some authors (Zaitsev et al. 2007, Zaitsev and Polikarvov 2008) assume an absence of eukaryotic life in anoxic Black Sea waters and in hydrogen-sulfide sediments considered then as “toxic” environment.

Ciliates are present in aerobic Black Sea habitats, both in pelagic and benthic environments. The total number of species (29) found in pelagic aerobic waters (Zaika et al. 1976) is low compared to the benthic species diversity (476) (Azovsky and Mazei 2003a, b, 2005; Polikarpov et al. 2003).

As a result of investigations of free-living psammophilic ciliate species composition on the Northeastern (Caucasian) coast of the Black Sea at a depth from 0 to 432 m, the highest diversity of ciliates was found in heterogeneous sands at a depth of 3-10 m, the lowest one - in unstable sands of the tidal zone and in silt deeper than 25 m. At the same time no ciliates were found in the zone enriched with H₂S (deeper than 100 m) (Azovsky and Mazei 2005).

Earlier studies of the oxic/anoxic chemocline of the Black Sea water column revealed only two ciliate species: one of them was being a representative of the Mesodiniidae, and the other one was a small unidentified ciliate with symbiotic bacteria on its surface (Sazhin et al. 1991). However, it is possible that previous deep water sampling methods were inadequate for microorganisms, thus abundance and diversity of ciliates below 100 m water depth were underestimated.

Later additional data concerning taxonomic richness and abundance of deep-water Protozoa and Metazoa in hypoxic and anoxic zones were obtained (Sergeeva et al. 2008, 2011a, b, 2013). Several ciliate
and foraminifera taxa have been known to occur in anoxic sediments of the Black Sea and could live in the Black Sea (e.g. Sergeeva et al. 2011a). The bathymetric distribution of the benthic ciliates at depths from 120 to 2075 m near the Dnieper Canyon and the Sorokin Trough (eastern part of the Black Sea) was described from samples of near bottom water, sediment surface detritus, and the upper layer (0–1 cm) of sediment (Zaika & Sergeeva 2009). The ciliates found in mentioned samples were the representatives of genera Chilodonella Strand, 1928, Trachelocerca Ehrenberg, 1834, Tracheloraphis Dragesco, 1960 and Loxophyllum Dujardin, 1841. At the same time more than 30 morphological species were recognized among mentioned materials (Sergeeva and Zaika 2008). The peaks of ciliate abundances were registered at depths of 120, 160–190, and 240 m in the same region (Sergeeva et al. 2008).

It should be noted that all ciliate species identified in the Black Sea under hypoxic/anoxic conditions were free-swimming forms.

Thus the present article represents the first investigation of commensal ciliates on hosts from the mentioned peculiar biotope.

**Material and methods**

The sediment sampling was performed in the Bosporus Strait’s outlet area of the Black Sea during the RV ‘Maria S. Merian’ (Germany) cruise.

For benthos studies we got virtually undisturbed bottom sediments at the stations in the range of depths 80-300 m by TV-multi-corer Ø=9.5cm or gravity corer Ø=7cm. Benthic stations were chosen along this transect, ranging from the oxic to the anoxic zone.

Sediment cores, obtained by the multiple-corer, were sectioned at 0–1, 1–2, 2–3, 3–4 and 4–5 cm intervals and all samples immediately preserved in 75% ethanol. The sediments were washed by distilled water through sieves with a mesh size of 1 mm and 63 µm. The organisms recovered in the sieves fractions were stained with vital Rose Bengal and investigated in the Bogorov’s chamber using a stereomicroscope.

The isolated organisms were first identified up to higher taxa and then were examined and identified to the lowest possible taxa using the Olympus CX41 microscope. Photomicrographs were obtained by using an Olympus E–410 digital camera. Measurements of ciliates were made using the computer program Scope Photo v. 2.0 for processing of digital images.

For slide preparation the material was mounted in Canada balsam. Permanent slides of oligochaetes and harpacticoids were deposited in the collections of the Department of Ecology of Benthos of the Institute of Biology of the Southern Seas, Sevastopol.

The systematic positions of ciliate species were determined according to review works on peritrichs (Warren and Paynter 1991) and suctorians (Curds 1987, Dovgal 2002, 2013).

**Table 1. Water depths and stations position where ciliates were found (Istanbul Strait’s outlet of the Black Sea).**

| Station       | Date       | Position         | Depth (m) | Gear   |
|---------------|------------|------------------|-----------|--------|
| MSM15/224-1   | 14.04.2010 | 41° 29.59’N      | 199.9     | TV-MUC |
| MSM15/263-1   | 16.04.2010 | 41° 29.92’N      | 247.7     | TV-MUC |

**Results**

The macrobenthic oligochaetes were found in bottom sediments almost at all studied depths (80-253 m). We found three ciliate species from the body surface of oligochaetes and harpacticoids recovered from two stations (Table 1) at the depths of 200 and 248 m.

The oligochaetes were identified belonging to the genus Tubificoides Lastěkin, 1937. The 58 oligochaete individuals were analyzed and 13 (22.5%) of them were infected with peritrich ciliates Cothurnia maritima Ehrenberg, 1838.

Four species of harpacticoid copepods were found in both of sampling stations: Amphiascella subdebilis (Willey, 1935), Haloschizopera pontarchis Por, 1959, Cletodes tenuipes Scott, 1896 and Enhydrosoma longifurcatum Sars, 1909. Among 12 analyzed individuals of harpacticoid copepods included
in the above listed species, 9 (75%) were infected with suctorians *Paracineta livadiana* (Mereschkowsky, 1881) and *Corynophrya lyngbyi* (Ehrenberg, 1834).

The systematic positions and descriptions of found ciliate species are given below.

**Phylum Ciliophora** Doflein, 1901

**Subphylum Intramacronucleata** Lynn, 1996

**Class Oligohymenophorea** Puytorac et al., 1974

**Subclass Peritrichia** Stein, 1859

**Order Sessilida** Kahl, 1933

**Family Vaginicolidae** Fromentel, 1874

**Genus Cothurnia** Ehrenberg, 1831

*Cothurnia maritima* Ehrenberg, 1838
(Fig. 1-2)

**Diagnosis.** Loricate, stalked peritrich ciliate. Lorica much longer than wide, occasionally with irregular ridges and furrows. The external stalk is relatively long; the short and broad endostyle is also present, mesostyle absent. Macronucleus straight. Marine species, attached to different substrates.

It should be mentioned that in our materials the individuals with furrows of lorica neither were observed.

**Measurements** (in µm): lorida height 39-48 (35-56 after Warren and Paynter 1991), width 29-42 (28-33 after Warren and Paynter 1991); external stalk length 8-19 (10-15 after Warren and Paynter 1991); endostyle length 4-11, width 6-11.

**Class Suctorea** Claparède & Lachmann, 1858

**Subclass Exogenia** Collin, 1912

**Order Metacinetida** Jankowski, 1978

**Family Paracinetidae** Jankowski, 1975

**Genus Paracineta** Collin, 1911

*Paracineta livadiana* (Mereschkowsky, 1881)
(Fig. 3)

**Diagnosis.** Loricate suctorian ciliate. Lorica of stylotheca-type covering the whole of body, the stylotheca mouth slightly dips into lorida. The tentacles are capitate, arranged in apical bundle. The stalk-like stylotheca protuberance long, thin, and does not extend up to bottom of the lorida. Macronucleus ellipsoidal, central. There is one laterally positioned contractile vacuole. Marine species, attached to different substrates.

**Measurements** (in µm): lorida height 115, width 50; body height 50 (26-65 after Dovgal 2013), width 50 (20-40 after Dovgal 2013); length of the tentacles 40-50; macronucleus dimensions 11×7 (15-20×20-25 after Dovgal 2013).

**Order Vermigemmida** Jankowski, 1973

**Family Corynophryidae** Jankowski, 1981

**Genus Corynophrya** Kahl, 1934
Figures 1-4. 1 Distribution of *Cothurnia maritima* Ehrenberg, 1838 on *Tubificoides* body. 2 A microphotography of *Cothurnia maritima* Ehrenberg, 1838, from *Tubificoides* sp. 3 *Paracineta livadiana* (Mereschkowsky, 1881), from harpacticoid copepod. 4 *Corynophrya lyngbyi* (Ehrenberg, 1834), from harpacticoid copepod.

*Corynophrya lyngbyi* (Ehrenberg, 1834)
(Fig. 4)

**Diagnosis.** Aloricate suctorian ciliate with cell body irregular-shaped oval, slightly wider anteriorly. The retractile capitate tentacles located on the anterior body surface. The length of stalk is at least four times the body length. Macronucleus is horseshoe-shaped.

It should be mentioned that length of the stalk in observed individual of the species was not exceeding the height of the body.
Marine species, attached to various substrates such as hydroids and algae. This is the first record of the species in the Black Sea.

**Measurements** (in μm): body height 42 (40-80 after Curds 1987), width 44; stalk length 32 (120-400 after Curds 1987); length of the contracted tentacles 7-13.

The distribution of ciliates on the body surface of both oligochaete and harpacticoid hosts had no a specific distributional pattern at certain areas, and were distributed from the head to the end of the body. In the case of the harpacticoid hosts the suctorian ciliates were attached on the antennae, furcas, carapaces and egg sacs.

**Acknowledgements**
Part of this work was supported from EC 7th FP project HYPOX 226213 and National Programs of NAS Ukraine. We wish to thank the Dr. Sci. N.M. Shurova from Odessa Branch of the Institute of the Southern Seas, NAS of Ukraine, for consultations under identifying the deep-sea oligochaetes and Dr. E.A. Kolesnikova from the Institute of the Southern Seas (Sevastopol) for identification of Harpacticoida. We are grateful to Professor Antje Boetius for providing an opportunity to participate in RV ‘M.S. Merian’ cruise 15/1, and Dr. S.A. Mazlumyan for collaboration in sample collection of bottom sediments.

**References**

Azovsky, A. I. & Mazei, Yu. A. (2003a) Ciliates of coarse ground on the Northeastern Black Sea coast. *Zoologichesky Zhurnal*, 82 (2), 899–912.

Azovsky, A. I. & Mazei, Yu. A. (2003b) A conspectus of the Black Sea fauna of benthic ciliates. *Protistology*, 3 (2), 72–91.

Azovsky, A.I. & Mazei, Yu. A. (2005) Distribution and community structure of benthic ciliates in the North-eastern part of the Black Sea. *Protistology*, 4 (2), 83–90.

Curds, C. R. (1987) A revision of the Suctoria (Ciliophora, Kinetofragminophora) 5. The *Paracineta* and *Corynophrya* problem. *Bulletin of the British Museum (Natural History). Zoology*, 52 (2), 71–106.

Dovgal, I.V. (2002) Evolution, phylogeny and classification of Suctorea (Ciliophora). *Protistology*, 2 (4), 194–270.

Dovgal, I.V. (2013) Fauna of Ukraine: in 40 vol. Vol. 36: Ciliates – Ciliophora. Issue 1: Class Suctorea, Naukova dumka, Kiev, 267 pp.

Dubilier, N., Giere, O. & Grieshaber, M. K. (1994) Concomitant effects of sulfide and hypoxia on the aerobic metabolism of the marine oligochaeta *Tubificoides benedii*. *Journal of Experimental Zoology*, 269, 287–297.

Polikarpov, I.G., Saburova, M.A., Manzhos, L.A., Pavlovskaya, T.V. & Gavriloiva, N.A. (2003) Biologic diversity of the microplankton in near shore Black Sea waters (Sevastopol area, 2001 -2003). In: Eremeev, V. N. & Gavetskaya, A. V. (Eds.), *Modern state of biodiversity in Crimea nearshore waters*, Ecosi-Hydrophysika, Sevastopol, pp. 16–42.

Sazhin, A.F., Subkov, M.V. & Drabkova, V.G. (1991) Heterotrophic microplankton of lower part of oxygenated waters during spring 1988. In: Vinogradov, M. E (Ed.), *Variability of the Black Sea ecosystem: natural and anthropogenic factors*, Nauka, Moscow, pp. 204–210.

Sergeeva, N.G., Gooday, A.J., Mazlumyan, S.A., Kolesnikova, E.A, Lichtschlag, A., Kosheleva, T.N. & Anikeeva, O.V. (2011a) Meiobenthos of the oxic/anoxic interface in the Southwestern region of the Black Sea: abundance and taxonomic composition. In: Altenbach, A.V., Bernhard, J.M., Seckbach, J. (Eds.), *Anoxia: paleontological strategies and evidence for eukaryotes Survival*, Springer, pp. 369–401.

Sergeeva, N.G., Lichtschlag, A. & Mazlumyan, S.A. (2011b) Protozoa and Metazoa living under hypoxia/anoxia conditions in the Black Sea: Discovery of actively moving animals in situ. Abstract 3-rd Bi-annual BS Scientific Conference and UP-GRADE BS-SCENE Project Joint Conference, 188–189.
Sergeeva, N.G., Mazlumyan, S. A., Cagatay, N & Lichtschlag, A. (2013) Hypoxic meio-benthic communities of the Istanbul strait’s (Bosporus) outlet area of the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences, 13*, 33–41.

Sergeeva, N.G. & Zaika, V.E. (2008) Ciliophora in hydrogen sulfide zone of the Black Sea. *Marine Ecological Journal, 7* (1), 80–85.

Sergeeva, N.G., Zaika, V.E. & Lichtschlag, A. (2008) Preliminary data on the presence of diverse benthic ciliate species in deep anoxic Black Sea. *Environmental Micropaleontology, Microbiology and Meiobenthology. 5th international conference*, 279–282.

Warren, A. & Paynter, J. (1991) A revision of Cothurnia (Ciliophora, Peritrichia) and its morphological relatives. *Bulletin of the British Museum (Natural History). Zoology, 57* (1), 17–59.

Zaika, V.E., Moraykova, C. K., Ostrovskaya, N. A & Tsalkina, A. C. (1976) Distribution of marine microzooplankton. *Naukova dumka, Kiev*, 92 pp.

Zaika, V.E. & Sergeeva, N.G. (2009) The vertical distribution of the deep-water ciliates in the Black Sea. *Marine Ecological Journal, 8* (1), 32–36.

Zaitsev, Yu.P., Polikarpov, G.G., Egorov, V.N., Alexandrov, B.G., Garkusha, O.P., Kopytina, N.I., Kirilov, A.V., Nesterova, D.A., Nidzvetskaya, L.M., Nikonova, S.E., Polikarpov, I.G., Popovichev, V.N., Rusnak, E.M., Stokozov, N.A., Teplinskaya, N.G. & Tereńko, L.M. (2007) Accumulation of the remnants of oxybiotic organisms and a bank of living spores of higher fungi and diatoms in bottom sediments of the hydrogen-sulphide bathyal zone of the Black Sea. *Reports of the National Academy of Sciences of Ukraine*, 7, 159–164.

Zaitsev, Yu.P. & Polikarpov, G.G. (2008) Recently discovered new biospheric pelicocontour function in the Black Sea reductive bathyal zone. *Journal of the Black Sea / Mediterranean Environment, 14* (3), 151–165.