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PRELIMINARY DATA ON THE PRESENCE OF DIVERSE BENTHIC CILIATE SPECIES IN DEEP ANOXIC BLACK SEA

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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. Ciliates were reported to be present in aerobic Black Sea habitats, both in pelagic as well as in the benthic environments. The total number of species (29) found in pelagic aerobic waters is low compared to the number of benthic species (476) (Azovsky, Mazei, 2005; Polikarpov *et al.*, 2003). Earlier studies of the oxic/anoxic chemocline of the Black Sea water column revealed only two ciliate species: one of them being a representative of the Mesodiniidae, the other one a small-unidentified ciliate with symbiotic bacteria on its surface (Sazhin *et al.*, 1991).

Many authors have assumed an absence of eukaryotic life in anoxic Black Sea waters and sediments, and have stressed the impossibility of eukaryotic life in this hydrogen-sulfide rich and thus "toxic" environment. However, it is possible that previous deep water sampling methods were inadequate for microorganisms, so that the abundance and diversity of ciliates below 100 m water depth was underestimated (Polikarpov *et al.*, 2003; Sazhin *et al.*, 1991). Here we conducted a study of the meio- and microbenthos in anoxic environments as well as in cold seeps, where methane is actively emitted into the water column (Sergeeva, 2000, 2004; Sergeeva, Gulin, 2007; Sergeeva, Zaika, 1999, 2000; Zaika *at al.*, 1999).

METODOLOGY

Samples were collected in February and March 2007 during the M 72/2 MICROHAB cruise with the German RV Meteor in the NE and NW part of the Black Sea. The undisturbed sediment samples were obtained using a multi-corer (modified by Barnett *et al.*, 1984).

Sampling stations were chosen along a transect from the oxic into the anoxic zone, crossing the shelf and the NW Crimea slope. Recent publications showed that in this area the appearance of hydrogen sulfide - indicating the upper boundary of the anoxic zone – is located at 150 - 170 m water depth (Luth *et al.*, 1998). Samples for micro- and meiobenthos were collected at 10 m intervals ranging from 120 m to 230 m water depth. In addition, samples were taken at 832 m depth in a hydrate-rich area, and at the actively methane seeping Dvurechenskii mud volcano NE of the Crimean peninsula, at 1807 m and 2075 m water depth.

The sediment cores (28.3cm^2 and 63.6cm^2) obtained by the multiple corer and push-corer (44.15 cm^2) were sectioned in 0-1, 1-2, 2-3, 3-4 cm intervals. In addition, near bottom water and detritus were studied separately. All samples were fixed in alcohol (75%). In the Sevastopol laboratories, samples were carefully wet sieved (upper sieve mesh size: 1 mm, lower sieve mesh size: 0.064 mm) using sterile filtered seawater. The retained fraction was stained with a rose Bengal solution and carefully examined under the microscope.

In addition, oxygen and sulfide concentrations were obtained from the water overlying the multi-corer sediment samoles taken on the transect on the shelf and the NW Crimea slope. Oxygen concentration was determined by Winkler titration; samples for sulfide were fixed in 5% ZnAc and subsequently measured by the diamine complexation method described by Cline (1969).

RESULTS

Ciliates were found in almost all samples from 120, 130, 140, 150, 160, 170, 180, 190, 210, 230 m, 832m, 1807 and 2075 m water depth. They were present in the near-bottom water overlying the sediment cores, in the detritus as well as in the upper layer (0-1cm) of the sediments. A total about 30 species (or morphospecies) could be distinguished. Examples of the retrieved Ciliates are shown in Figure 1.

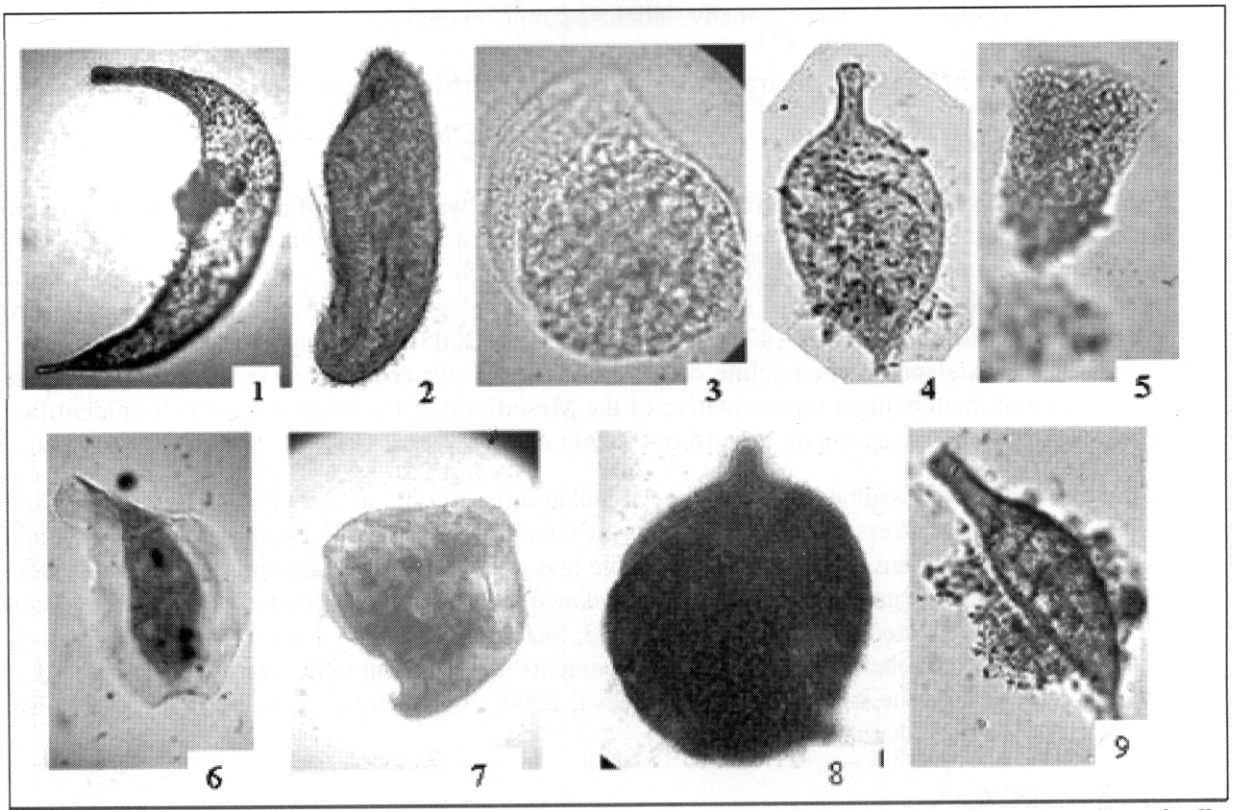

Figure 1. Typical representatives of the Ciliophora from the deep-water zone of the Black Sea. Size of cells: 1 – 185 mkm, 2 – 205 mkm, 3 – 107.5 mkm, 4 – 225 mkm, 5 – 164 mkm, 6 –112.5 mkm, 7– 165 mkm, 8 – 125 mkm, 9 – 262.5 mkm.

The sizes of the individuals ranged from 80 - 100 μm to 230 - 800 μm. Cell numbers of Ciliophora found per sample varied between from 1 – 223 cells /63.6cm^2. An exceptionally high abundance of cells (156 – 223 cells/63.6cm^2) was present at a water depth of 160 - 170 m. In contrast, in a water depth of 832 m, 21 cells/28.26cm^2 were recovered, 5 cells/28,26cm^2 were found in samples from 1807m, and only 1cell /28,26cm^2 was obtained from 2075 m water depth. On-board measurements of oxygen in the samples from the depth transect on the Crimean shelf showed the increasing depletion of oxygen with depth. Sulfide concentration started to increase with water depth below 157 m.

New ciliate molecular identification methods (Chen *et al.*, 2003; Fried *et al.*, 2002) have proven that even well distinguishable phylotypes can have morphological similar phenotypes. Species adapted to life in extreme conditions, like hydrogen sulfide rich water, can have physiological, biochemical and genetic differences at the species level. The taxonomic identification of the inhabitants of the Black Sea anoxic waters will thus be a future goal.

This study, which proves the presence of ciliates in the Black Sea hydrogen sulfide zone, is supported by data obtained during the past few decades-. It is known from previous studies that specific groups of protozoa, fungi and lower metazoa can be found in the sediments of coastal "sulfidic systems". They disappear with sediment oxidation, which reflects the adaptation of these specific communities to the sulfidic environment. In addition sulfur bacteria, colorless euglenids

and ciliates can be adapted to anoxic habitats in silt sediments. Often life is even more diverse in these spots (Lackey, 1961).

An example of the presence of ciliates in those environments is *Geleia nigriceps,* which is known for its ability to inhabit coastal anoxic sediment (Hartwig, Wieser, 1977). Species of *Plagiopyla* and *Metopus* genera are also found in such habitats (Embley *et al.*, 1992; Massana & Pedros-Alio, 1994). Real anaerobes among the free-living ciliates are found as well, for example *Trimyema* sp. (Dando, 1993)

Anaerobic conditions can also prevail near deep sea vents. These waters often contain hydrogen sulfide, which can be used as and electron donor by sulfide oxidizing prokaryotes. The occurrence of symbiotic ciliates in the presence of hydrogen sulfide can be expected (Polikarpov *et al.*, 2003).

Furthermore, ciliates were found in constantly anoxic waters of the Framvaren fjord (Norway) (Behnke *et al*,, 2005; Behnke *et al.*, 2006). At the bottom of this fjord the hydrogen sulfide concentrations are even 25 times higher than in the Black Sea. Phylogenetic analyses of these samples were carried out with the help of 18S rRNA gene sequencing (Behnke *et al.*, 2006). The results have shown that the highest number of phylotypes (147) occurred near the upper boundary of the H_2S appearance (at 23 m); at 18 m, 64 phylotypes, and at 36 m, 27 phylotypes could be detected. Representatives of Plagiopylidae, Strombidiidae, Nyctotheridae, Cycliidae and Prorodontidae ciliate groups have been described in this fjord system. Also in the Cariaco basin (Caribbean Sea), 18-S rRNA sequences of ciliates related to Spirotrichea, Litostomatea, Heterotrichea, Karyorelictea and other groups have been found (Stoek *et al.*, 2003).

Our finding of a diverse Ciliophoran fauna in the waters of the deep, hydrogen sulfide enriched Black Sea is supported by new data on the presence of Ciliophora in the permanently anoxic waters of Framvaren fjord and Cariaco basin, and even of meiobenthic metazoans at the bottom of the deeper part of the Black Sea (Sergeeva, 2004; Sergeeva, Gulin, 2007). The specific physiological – biochemical processes allowing the survival of eukaryotes in such "extreme" environments are an important question for future studies.

CONCLUSIONS

Ciliates are present in the near-bottom water, in the detritus and in the upper layer (0 –1cm) of Black Sea sediments from various sites and water depths.

We were able to distinguish between about 30 species (morphospecies), and many different phenotypes were present, including moving and attached ciliates. Sizes of individual ciliates ranged from 80 – 100 μm to 230 – 800 μm. Cell numbers of *Ciliophora* generally varied between 1 – 223 cells/63.6 cm^2. The sample with the highest number of 156 – 223 cells/63,6cm^2 was found at a water depth of 160 – 170 m, close to the oxic/anoxic interface. In the permanently anoxic waters at 832 m, 21 cells/28,26cm^2 were present and 5 cells/28.26cm^2 were present at 1807 m water depth, but only 1 cell/28.26cm^2 was found in samples at 2075 m water depth.

Several previous studies found an absence of eukaryotes in permanently anoxic and sulfidic environment of the deeper part of the Black Sea. The new data on the presence of Ciliophora in permanently anoxic waters presented here support previous findings from the Framvaren fjord and the Cariaco basin, where Ciliates are also present under anoxic and sulfide rich conditions.

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