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# A new host for *Hematodinium* infection among lithodid crabs from the Sea of Okhotsk

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ABSTRACT

A disease caused by parasitic dinoflagellates of the genus *Hematodinium* has been found in the soft crab *Hapalogaster grebnitzkii* from the Sea of Okhotsk, which is considered a new host for this agent among lithodid crabs. This report provides macro- and micromorphological descriptions (using light and transmission electron microscopy) of the disease, as well as molecular identification of *Hematodinium* sp. from soft crabs, based on 18S RNA sequence data.

## 1. Introduction

Parasitic dinoflagellates of the genus *Hematodinium* Chatton and Poisson (Syndinea: Syndiniophyceae: Syndiniales: Syndiniaceae) are recognized as the most significant parasites of crustaceans, causing serious damage to populations of commercial species in many regions of the World Ocean (Meyers et al., 1987, 1990, 1996; Wilhelm and Mialhe, 1996; Stentiford and Shields, 2005; Morado et al., 2012; Small, 2012; Stentiford et al., 2012). The disease is deadly to hosts, and its prevalence in populations can be very high (Messick, 1994; Meyers et al., 1990, 1996; Messick and Shields, 2000; Shields and Squyars, 2000; Stentiford and Shields, 2005). At the moment, the list of crustacean hosts comprises more than 40 species and is constantly being extended. There are reports on new geographic locations where this infection has been recorded, and the number of reports on *Hematodinium* sp. in crustacean populations is steadily increasing (Pestal et al., 2003; Stentiford and Shields, 2005; Morado et al., 2012; Small, 2012; Shields, 2012).

An external visual sign of *Hematodinium* dinoflagellate infection in most crustacean species is a change in the color of the shell, as well as in the color and consistency of hemolymph at late phases of the disease (Meyers et al., 1987; Shields, 1994; Field and Appleton, 1995; Stentiford and Shields, 2005). These signs make it possible in some cases to pre-diagnose the disease and monitor its distribution in natural populations (Field et al., 1992; Meyers et al., 1990, 1996; Stentiford et al., 2001, 2002; Pestal et al., 2003; Shields et al., 2005; Meyers et al., 1987, 1990, 1996). The presence of parasite stages in hemolymph and internal organs of crustaceans is the main diagnostic sign of *Hematodinium* infection (Field and Appleton, 1995; Messick, 1994; Meyers et al., 1987, 1990).

As molecular studies show, the same species of *Hematodinium* sp., not identified definitely in terms of taxonomy, probably parasitizes most of the boreal species from all over the world and is the only agent recorded from crustaceans in the North Pacific (Jensen et al., 2010; Small, 2012). In tanner crab (*Chionoecetes bairdi*), *Hematodinium* infection has been known since 1985 (Meyers et al., 1987). Currently, it is widespread in this species and in snow crabs (*Ch. opilio*) in southeastern Alaska, the eastern Bering and Chukchi seas (Meyers et al., 1987, 1990, 1996; Morado, 2011).

The first case of *Hematodinium* sp. infection in Russian waters was diagnosed by histological methods in a snow crab *Ch. opilio* caught from the Sea of Okhotsk off the western coast of Kamchatka in 2002 (Karmanova and Ryazanova, 2008). Four years later, the disease was found in red and blue king crabs from these regions (Ryazanova, 2008; Ryazanova et al., 2010). In 2012, in the northern Sea of Okhotsk, the infection was found by histological methods in a single individual of the golden king crab *Lithodes aequispinus* (Metelev and Ryazanova, 2013). In the period 2010–2012, *Hematodinium* infection was also diagnosed in tanner, snow, and blue king crabs from the western Bering Sea and in snow crab from the western Chukchi Sea (Ryazanova et al., 2016). The present work provides a report on the first case of *Hematodinium* sp. infection in the soft crab *Hapalogaster grebnitzkii*.

## 2. Materials and methods

The studies were carried out during a bottom trawl survey on the

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western Kamchatka shelf aboard R/V "TINRO". Work was conducted in July 2016 between the latitudes 51°10′ N and 57°40′ N, within a depth range of 15–200 m. A total of 238 stations were performed. Trawl hauls were carried out at a speed of 3 knots; the duration was 30 min, and a bottom trawl 27.1/33.7 m was used.

The main points of focus in the study were crabs of commercial species inhabiting these waters: red king crab *Paralithodes camtschaticus*, blue king crab *P. platypus*, spiny king crab *P. brevipes*, snow crab *Ch. opilio*, tanner crab *Ch. bairdi*, and horsehair crab *Erimacrus isenbeckii*. Attention was also paid to crustaceans of other species, including non-commercial species, such as the helmet crab *Telmessus cheiragonus*, lyre crab *Hyas coarctatus*, and soft crab *H. grebnitzkii*.

At each station, all crabs from the catch were sorted out by species, sex, size, and molting stage as described in the "Manual for a description of Far Eastern Decapods" (Rodin et al., 1979). Catch structure was determined. The individuals with visual signs of various diseases, including the parasitic infection caused by the dinoflagellate *Hematodinium* sp., were selected.

The crabs with macroscopic signs of diseases were examined and dissected. Pieces of the heart, skeletal muscle, gills, epidermis, gonads, gastrointestinal tract, hepatopancreas, whole antennal gland, and the thoracic nerve ganglia were collected for histological study and fixed for 24–48 h in Davidson's fluid (Bell and Lightner, 1988), prepared from seawater. The material was further treated using standard histological techniques. Sections were stained with Meyer's hematoxylin-eosin (H&E). The obtained slides were examined under an Olympus Al-2 light microscope with a digital photographic camera DP21 (Olympus, Japan). The specimens for transmission electron microscopy were fixed with 2.5% glutaraldehyde in sterile seawater, post-fixed with 1% $OsO_4$ in seawater, dehydrated in an acetone series and embedded in Epon-Araldite. Ultrathin sections were cut on a Leica EM UC6 (Leica Microsystems, Germany) ultramicrotome. Semithin sections were stained with methylene blue and examined using a Leica DM 4500 (Leica Microsystems, Germany) microscope. The ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Zeiss Libra 200 FE (Carl Zeiss, Germany) transmission electron microscope. Measurements were done using the ITEM A4899600 (Olympus) software installed on the Libra 200 FE. Mean values and standard deviation from the mean were calculated in MS Excel 2016.

Pieces of hepatopancreas and interstitial connective tissue were preserved in 96% ethanol for the molecular study. Total genomic DNA was extracted from the hepatopancreas and interstitial connective tissue of infected soft crabs *H. grebnitzkii* using a Qiagen DNeasy Blood and Tissue Kit in compliance with the manufacturer's protocol for Animal Tissues and eluted in 70 μL total volume. Approximately 1700 base pairs of the 18S rRNA were amplified from the diluted genomic DNA through polymerase chain reaction (PCR) in a total volume of 10 μL with 0.1 μL Dream Taq DNA Polymerase (Thermo Scientific), 1 μL Polymerase buffer, 0.25 μL dNTP Mix, 0.4 μL each primer (100 ng/μL), 7.7 μL nuclease-free water, and 0.5 μL total DNA. The PCR thermal regime consisted of one cycle of 1 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 54 °C, and 2 min at 72 °C; and a final cycle of 5 min at 72 °C. We used the primers Univ-F-15 (5′-CTCCCAGTAGTCATATGC-3′) and Hemat-R-1654 (5′-GGCTGCCGTCCGAATTATTCAC-3′) according to Gruebl et al. (2002). The PCR reaction was repeated in 8 tubes with similar thermal conditions and reagents ratio. Reaction products were tested for size and purity on 1.5% agarose gels. The PCR products were bidirectionally sequenced in 8 reactions (4 reactions for each primer) using a BigDye Terminator v 3.1 cycle kit and run on an ABI 3500 DNA analyzer (Applied Biosystems). All the obtained sequences were aligned and manually edited in MEGA 7 (Kumar et al., 2016). Four assembled sequences were identical, and the consensus sequence of *Hematodinium* sp. has been deposited in GenBank (KY853757).

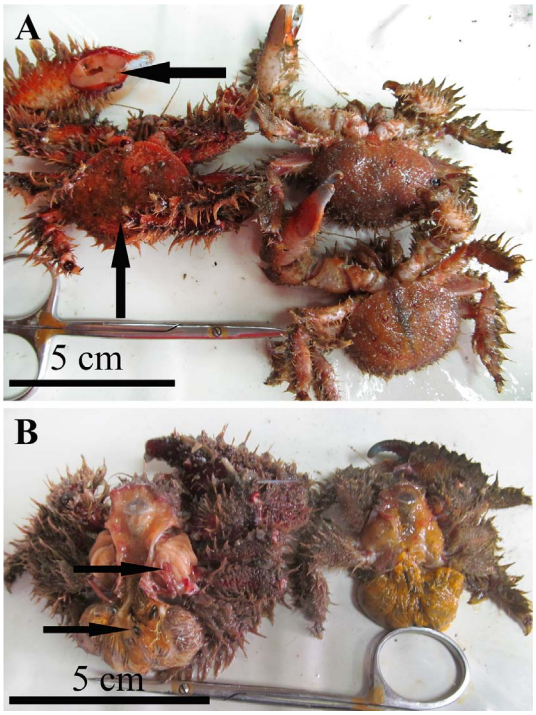


**Fig. 1.** Visual sings of *Hematodinium* sp. infection in the soft crab *Hapalogaster grebnitzkii*. (A) Carapace color in infected (left) and healthy (right) crabs. (B) Internal organs in an infected (left) crab are surrounded by creamy-yellow hemolymph in contrast to a healthy (right) individual (the carapace and integument of abdomen are removed). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

## 3. Results

At one of the stations at a latitude of 56°0′ and a depth of 16 m, the catch consisted of 16 male red king crabs (with a carapace width of 135–202 mm) and five individuals of soft crab *H. grebnitzkii*. The color of shell in one of the soft crabs was distinguished by a more saturated red hue than in the other individuals of this species; its hemolymph was creamy in color, opaque, and more viscous than normal (Fig. 1A and B). These macroscopic signs suggested that the crab was infected by parasitic dinoflagellate *Hematodinium* sp. All five individuals of soft crab *H. grebnitzkii*, both with signs of infection and without them, were examined histologically. The *Hematodinium* sp. infection was detected only in those with visual signs.

A histological study of specimens of internal organs and tissues of soft crab showed cells of a parasite with the structural characteristics of *Hematodinium*. Hemal spaces and vessels, as well as connective tissue of all internal organs, were occupied predominantly by mononuclear trophonts of the parasite. Hemocytes of the host were rare in all areas. Numerous protozoans were recorded from the hemal spaces of stems and lamellae of the gills, connective tissue surrounding the nerve ganglia, gonads, antennal glands and urinary bladder, and hematopoietic tissue. At the same time, no changes in the structure of nerve tissue proper were observed; hemopoietic tissue remained active, and young hemocytes were present here. In gonads, sex cells of different types (including spermatozoa) had a normal structure. In the antennal gland and bladder, the parasite cells were found not only in the surrounding connective tissue, but also inside the labyrinth among podocytes, as well as in the bladder lumen. The structure of podocytes, epithelium of labyrinth of antenatal glands, and bladder remained normal (Fig. 2A–F). A large number of *Hematodinium* stages were located in the heart: freely in the trabecular channels of myocardium and attached to sarcolemma of cardiac muscles. An invasion of the trophonts under the basal lamina of epicardium was also noted (Fig. 3A).