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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).

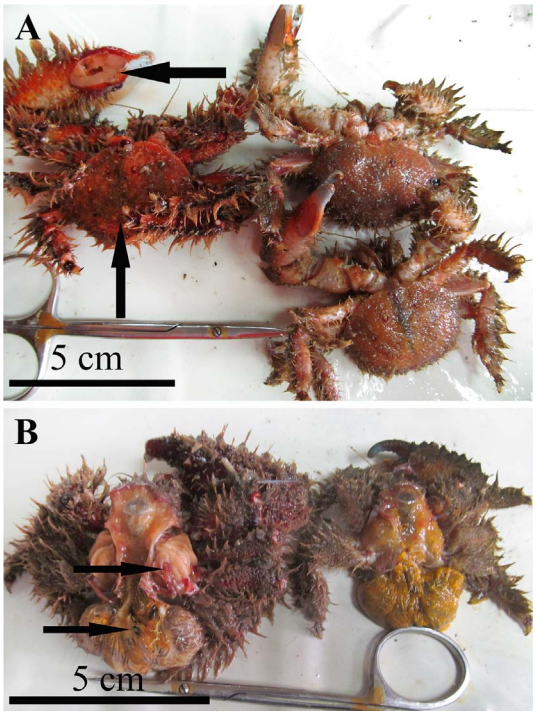

Fig. 1. Visual sings of *Hematodinium* sp. infection in the soft crab *Hapalogaster grebnitzkii*. (A) Carapace color in infected (left) and healthy (right) crabs. (B) Internal organs in an infected (left) crab are surrounded by creamy-yellow hemolymph in contrast to a healthy (right) individual (the 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).

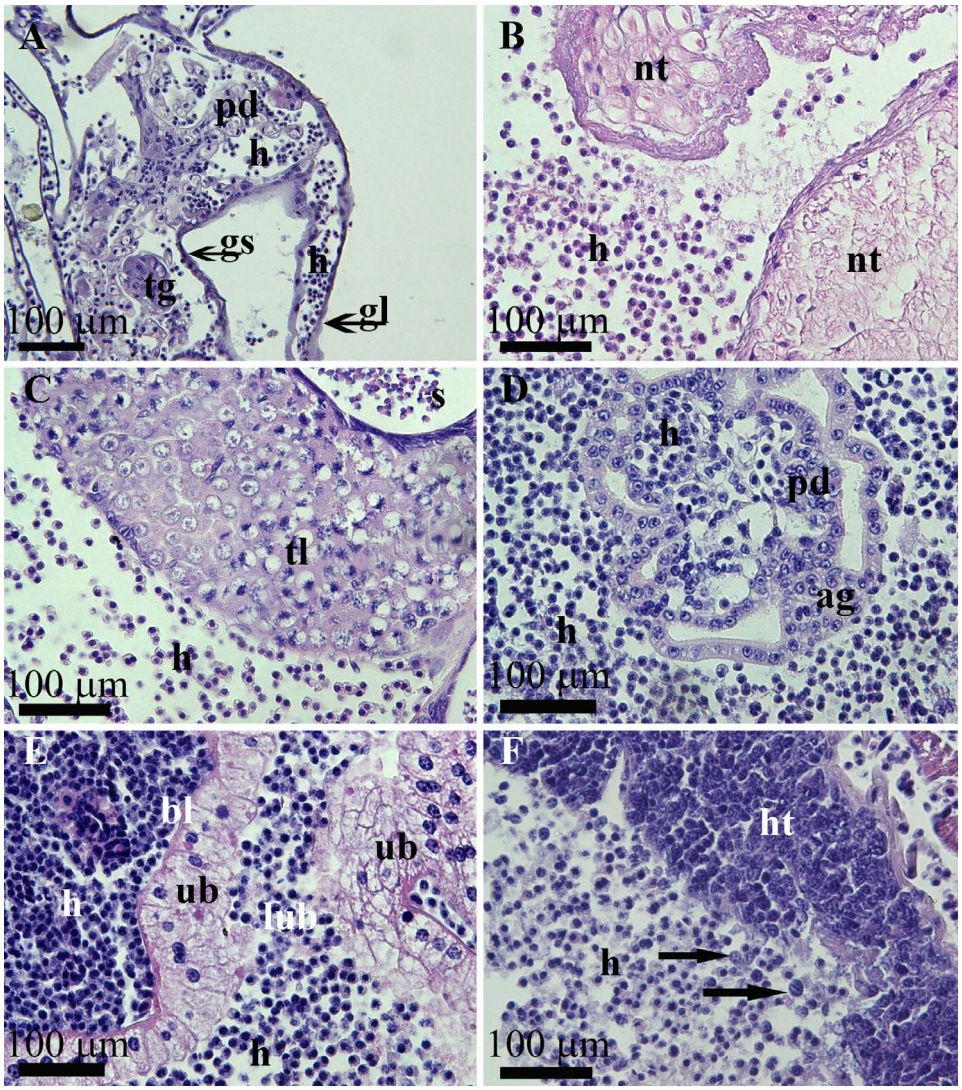

Fig. 2. Light micrographs of tissues of a soft crab infected by *Hematodinium* sp. Vegetative *Hematodinium* cells found in hemal spaces of gill stem and gills lamella (A), in interstitial connective tissue near thoracic ganglion (B), in connective tissue surrounding testicular lobe (C), in interstitial connective tissue of antennal glang and inside of the labyrinth (D), in interstitial connective tissue of urinary bladder and inside of the lumen (E), and in interstitial connective tissue of hemopoietic tissue. Arrow points at a young hemocyte (F). Legend: h, *Hematodinium* cells; gl, gill lamella; gs, gill stem; tg, tegmental gland; pd, podocytes; nt, nerve tissue; tl, testicular lobe; s, spermatids; ag, epitelium of antennal gland labyrinth; ub, urinary bladder; bl, basal laminae; lub, lumen of urinary bladder; ht, hemopoietic tissue. Young hemocytes are indicated by arrows.

The connective tissue under the epidermis, around the tubules of the hepatopancreas, and the connective tissue component of the walls of the gastrointestinal tract sections were completely filled with parasite cells. Nevertheless, no changes were found in cells of the epidermis, epithelium of the gastrointestinal tract, and tegmental glands (Fig. 3B and C). In some areas, invasion of *Hematodinium* into visceral and skeletal muscles was observed. Sarcolemma was absent here; a degeneration of muscle fibers and penetration of the trophonts between myofibrils occurred (Fig. 3D). In hepatopancreas, epithelium in most tubules looked vacuolized and, in some of the tubules its lysis was observed. Walls of most hemal vessels present here were enlarged and swollen, and the fixed hemocytes, connected to endothelium, were enlarged and contained a melanized matter. Granuloma formations in connective tissue and around blood vessels was also noted (Fig. 4A and B). A large number of granulomas of various sizes were found in subepidermal connective tissue and in connective tissue of the stomach wall. The central part of the granulomas consisted of a homogeneous pigmented substance and degranulated cells. Hemocytes of elongated shape were located distally down the spiral around the center (Fig. 4C and D).

Among the parasite stages, mainly mononuclear rounded trophonts were present. Chromatin in their nuclei was dense, sometimes in the form of variously sized beads or V-shaped. Plasmodia of a rounded and oval shape, containing 2 or more nuclei, were recorded less frequently. In addition, filamentous plasmodia with up to 8 nuclei were found. Most filamentous plasmodia were attached to the outersurfaces of the endothelium of blood vessels in the hepatopancreas. As a rule, they were tightly arranged, forming syncytia. Only single filamentous plasmodia were attached to the basal membrane of hepatopancre tubules and to the sarcolemma of the cardiac musculature (Fig. 5).

With TEM, only the vegetative stages of the parasite were found. Trophonts filled the lumen of hemal vessels in hepatopancreas (Fig. 6A). Along with *Hematodinium* cells, single hemocytes were observed in vessel lumens (Fig. 6B). They could contact with the surface of trophonts, however, retaining a rounded shape; any signs of the phagocytosis reaction were absent. At the same time, in the dense connective tissue there were isolated, possibly encapsulated, trophonts surrounded with an envelope formed by cellular processes and a loose fibrillar matrix (Fig. 6C). Cells of *Hematodinium* were, as a rule, mononuclear or, less frequently, bi- and trinuclear (Fig. 6C–E). Multinuclear plasmodia divided by cytotomy, finally forming mononuclear trophonts (Fig. 6F). The size of mononuclear trophonts was 9.915 ± 2.819 µm (n = 30); bi- and trinuclear ones, about 18.05 ± 1.5 µm (n = 10). Trophonts had nuclei of the mesokaryotic type with a diameter of 5.056 ± 0.923 µm (n = 30): nuclear membrane was not expressed; nuclei were filled with diffuse chromatin; chromosomes were condensed and electron-dense. Numerous trichocysts were located in perinuclear cytoplasm of trophonts (Fig. 7A). These were elongated organelles containing crystalline core bounded by the closely adjoining membrane. In cross sections, the crystalline

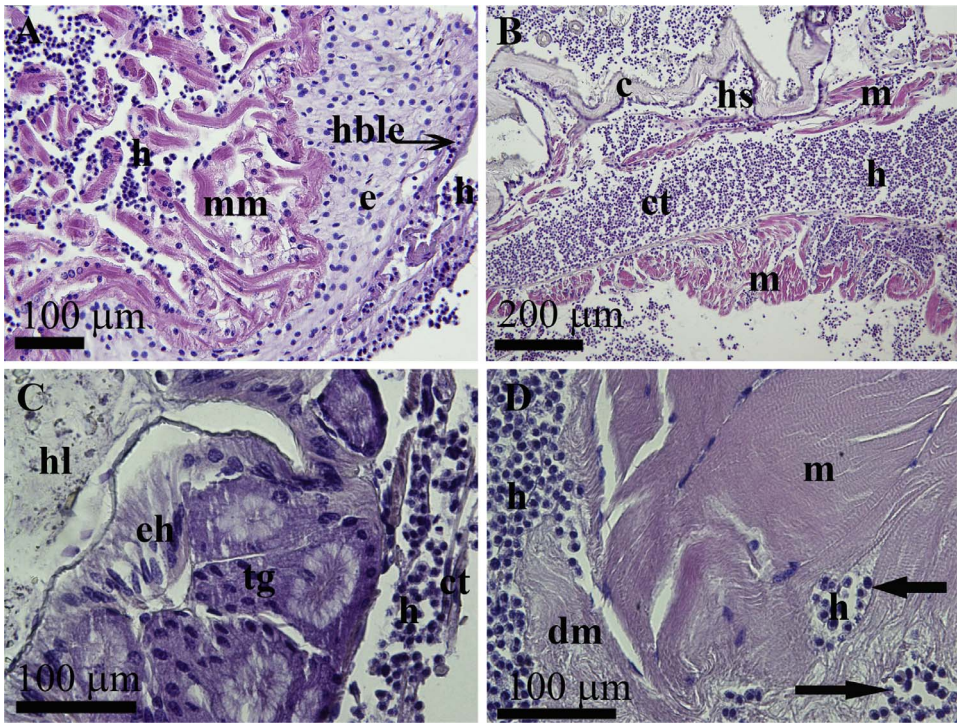

Fig. 3. Light micrographs of tissues of a soft crab infected by *Hematodinium* sp. Vegetative *Hematodinium* cells in heart miocadium and epicardium. Arrow indicates the *Hematodinium* cells that penetrated under the basal lamina of epicardium (A). Connective tissue and hemal sinuses of stomach completely occupied by parasite cells (B). Vegetative cells of the parasite in connective tissue of the hindgut wall (C). Vegetative *Hematodinium* cells in the interstitial connective tissue of skeletal muscle. A section of degraded muscle is visible. Arrow indicates trophonts of the parasite that penetrated between muscle fibrils (D). Legend: h, *Hematodinium* cells; hble, *Hematodinium* cells under basal lamina of epicardium; e, epicardium; mm, miocardial muscle; ct, connective tissue; hs, hemal sinus; c, cuticle; m, muscle; tg, tegmental gland; eh, hindgut epithelium; el, hindgut lumen.

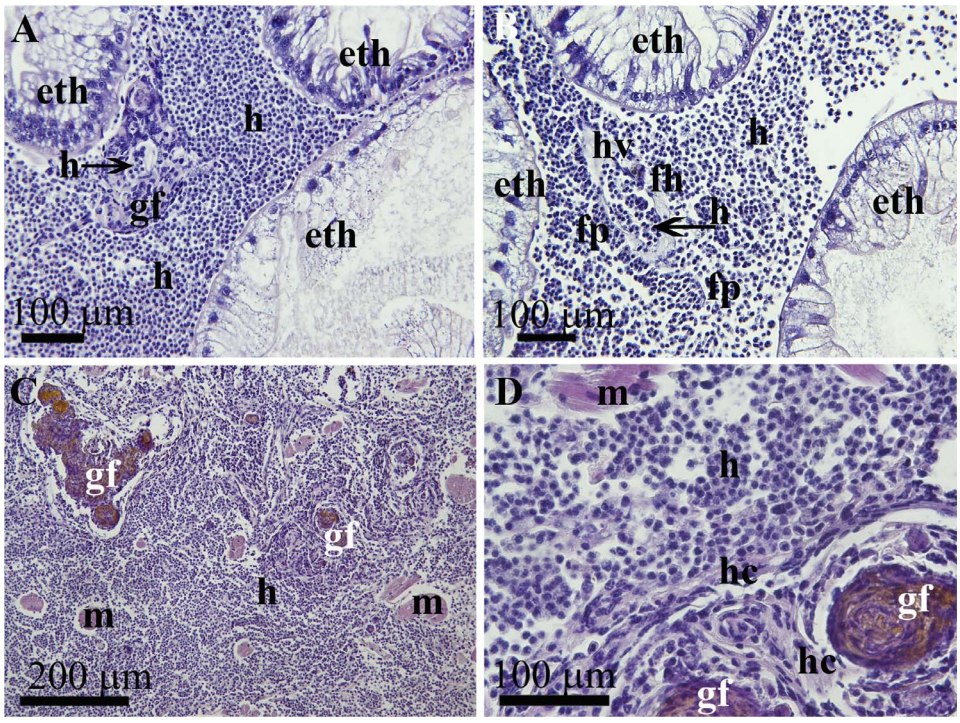

Fig. 4. Light micrographs of granuloma formation in tissues of a soft crab infected by *Hematodinium* sp. Transverse section of hepatopancreatic tubules showing replacement of interstitial connective tissue by parasite cells. Note the lysis of epithelial cells of tubule (at the right) and formation of granuloma around one hemal vessel. Arrow points at vegetative cells of parasite inside a hemal vessel (A). Hemal vessel with swollen walls in infected connective tissue of interstitial connective tissue of hepatopancreas. Note a filamentous plasmodium at the hemal vessel wall. Arrow points at one fixed hemocyte containing the pigmented matter (B). Granulomas in subepidermal connective tissue infected by *Hematodinium* cells (C). Enlarged micrographs of granuloma in subepidermal connective tissue infected by *Hematodinium* cells (D). Legend: h, *Hematodinium* cells; eth, epithelium of hepatopancreatic tubules; gf, granuloma formation; fp, filamentous plasmodium; hv, hemal vessel; fh, fixed hemocyte; m, muscle; hm, hemocytes.

core had the shape of a square with a side of 222 ± 15.8 nm (n = 30). Sometimes the reticulate structure of crystalline core was revealed in cross sections; the width of square cells forming the grid was 10 ± 1.5 nm, n = 10 (Fig. 7A, insert). The apical part of trichocyst represented a narrowed, sometimes curved, process, devoid of crystalline core (Fig. 7B). Inside the apical process, there was a fibrillary band of medium electron density, which connected the crystalline core and the membrane at the end of the apical process. Transverse striation was visible on the surface of the apical process throughout its length. Developing trichocysts, formed within vesicles with fibrillar content (primordial vesicles), were present in cytoplasm of trophonts along with fully formed (mature) trichocysts. At the initial stages, primordial vesicles had an electron-dense granule, and then the crystalline core with a layered or reticulate structure was formed (Fig. 7C and D).

In cytoplasm of *Hematodinium* cells, there were mitochondria with dense matrix and tubular cristae (Fig. 7D). In addition, trophont cytoplasm included vacuoles containing lipids (type 1), vacuoles with electron-lucent content (type 2), and also small electron-dense granules, which were located both immediately in cytoplasm and in the type 2 vacuoles (Fig. 7D). In the latter vacuoles, heterogeneous bodies were also present. They were formed through invagination of the membrane surrounding vacuole and represented a membrane-bounded vesicle

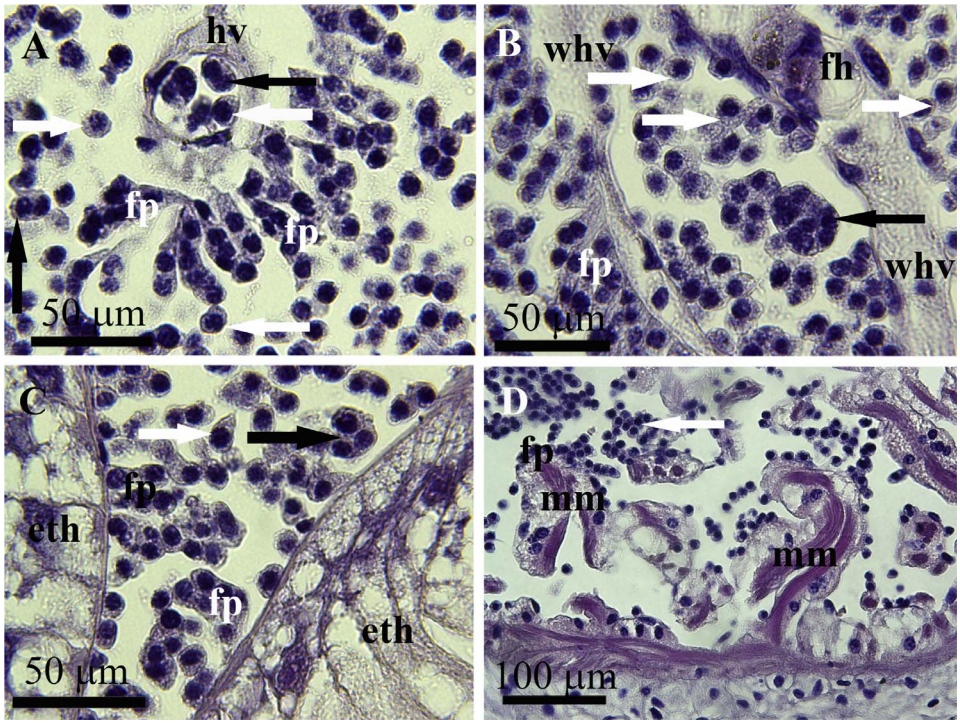

Fig. 5. Light micrographs of *Hematodinium* sp. stages in tissues of a soft crab. Stages of parasite around and inside a hemal vessel of interstitial connective tissue of hepatopancreas. Note a filamentous plasmodium attached to the wall of hemal vessel (A, B). Filamentous plasmodium attached to basal lamina of epithelium of hepatopancreatic tubule (C). Filamentous plasmodium attached to sarcolemma of miocardial muscle (D). Legend: hv, hemal vessel; p, plasmodium; t, trophont; fp, filamentous plasmodium; fh, fixed hemocyte; eth, epithelium of hepatopancreatic tubules; whv, wall of hemal vessel; mm, miocardial muscle. Trophonts are indicated by white arrows; plasmodia, by black arrows.

with the heterogeneous internal part. The outer membrane of trophonts formed invaginations, which sometimes contacted with the type 2 vacuoles and ended as pores in the alveolar membrane. Together, these structures formed pore complexes. In the pore complexes, as well as in bulging of the alveolar membrane on the cell surface, there were membrane-bounded vesicles of different sizes with a heterogeneous content (Fig. 7E). Similar formations were lying freely among *Hematodinium* cells (Fig. 7F).

The final alignment of the 18S rRNA fragment yielded 1638 bp. The obtained sequence of *Hematodinium* sp. from *H. grebnitzkii* was identical to the comparable gene fragments of *Hematodinium* sp. found in *Lithodes couesi* (Genbank Accession Number FJ844413), *Hyas coarctatus* (FJ844412), *Chionoecetes bairdi* (FJ844414-FJ844417), *Ch. opilio* (FJ844418-FJ844422), *Ch. tanneri* (FJ844423-FJ844425), *Ch. angulatus* (FJ844426), *Nephrops norvegicus* (FJ844427-FJ844429), and *Paralithodes camtschaticus* (EU856716). Based on this comparison, the parasite of soft crab was identified as *Hematodinium* sp. (see Jensen et al., 2010; Small et al., 2012; Small, 2012).

4. Discussion

The identity of 18S (small subunit) rRNA sequence of *Hematodinium* sp. from the soft crab shows that it is the same species or closely related to *Hematodinium* sp. isolated from crabs of many genera and also from lobsters. According to Small et al. (2012), this pathogenic species is widely distributed in the North Pacific and North Atlantic. The closest geographic localization of the crabs infected by *Hematodinium* sp. to the point of collection of the infected *H. grebnitzkii*, was *Paralithodes camtschaticus* found in the same area of the Sea of Okhotsk (Ryazanova et al., 2010), *Hyas coarctatus*, *Ch. bairdi*, and *Ch. opilio* from the Bering Sea, and *Lithodes couesi* from Vancouver Island (Jensen et al., 2010). The high similarity of the sequence to the comparable 18S rRNA gene fragments among *Hematodinium* sp. from these crabs, including the new host, *H. grebnitzkii*, confirms that the infection is widespread among many decapods in the North Pacific (Jensen et al., 2010)

The structure of the parasite cells found in the crab *H. grebnitzkii* was typical for *Hematodinium*: large nuclei of the mesokaryotic type with chromatin in the form of electron-dense lumps or ribbons, the amphiesmal membrane, the presence of “empty” vacuoles and those containing lipid-like material, and the presence of pore complexes (Hudson and Shields, 1994; Meyers et al., 1987; Field et al., 1992; Stentiford and Shields, 2005; Small et al., 2012). Vacuoles with a granular content, which we observed inside the alveoli of the cell membrane and lying freely between cells, apparently represent the exudate excreted by the parasite. Meyers and co-authors reported on a significant amount of exudate in the form of small droplets on the surface of the vegetative stages of *Hematodinium* (Meyers et al., 1987).

In the cytoplasm of the trophonts, there are trichocysts, or extrusomes, performing the function of protection and dispersal, the presence of which is characteristic for most species of dinoflagellates (Dodge and Greuet, 1987). The appearance of trichocysts defines the initiation of a morphological transition in the parasites life cycle from a vegetative stage to the transmission stage (Gaudet et al., 2014). Trichocysts at different stages of development—mature trichocysts and young ones, formed in primordial vesicles—are found in trophonts, which indicates asynchronous development of trichocysts in a parasite cell. The structure of mature trichocysts in *Hematodinium* spp. from tissues of *H. grebnitzkii*, in particular the presence of a crystalline core with an elaborate grid pattern surrounded by the closely adjoining outer membrane, the structure of the apical region, as well as their linear dimensions, are similar to those described for *Hematodinium* spp. from the Atlantic snow crab *Chionoecetes opilio* (Gaudet et al., 2014), which is an additional argument for the identity of the pathogens.

We observed the envelope, consisting of a loose fibrillar extracellular matrix and a cellular process, around some *Hematodinium* cells. A similar structure, or “halo”, was previously reported in the case of a *Hematodinium* infection combined with yeast-like organisms isolated from the crabs *Cancer pagurus* and *Necora puber* (Stentiford et al., 2003). Some yeast-like cells were surrounded by an electron-lucent “halo” that separated them from the surrounding host’s material. The author suggested that this “halo” can be either a consequence of fixation, or a result of emission of some material by the cell wall of the yeast-like organisms. It is difficult to identify the mechanism that could cause this “halo” (or zone, or envelope) in the soft crab. We may assume this to be a result of some interaction of the exudate produced by parasite cells with the components of the host tissues.

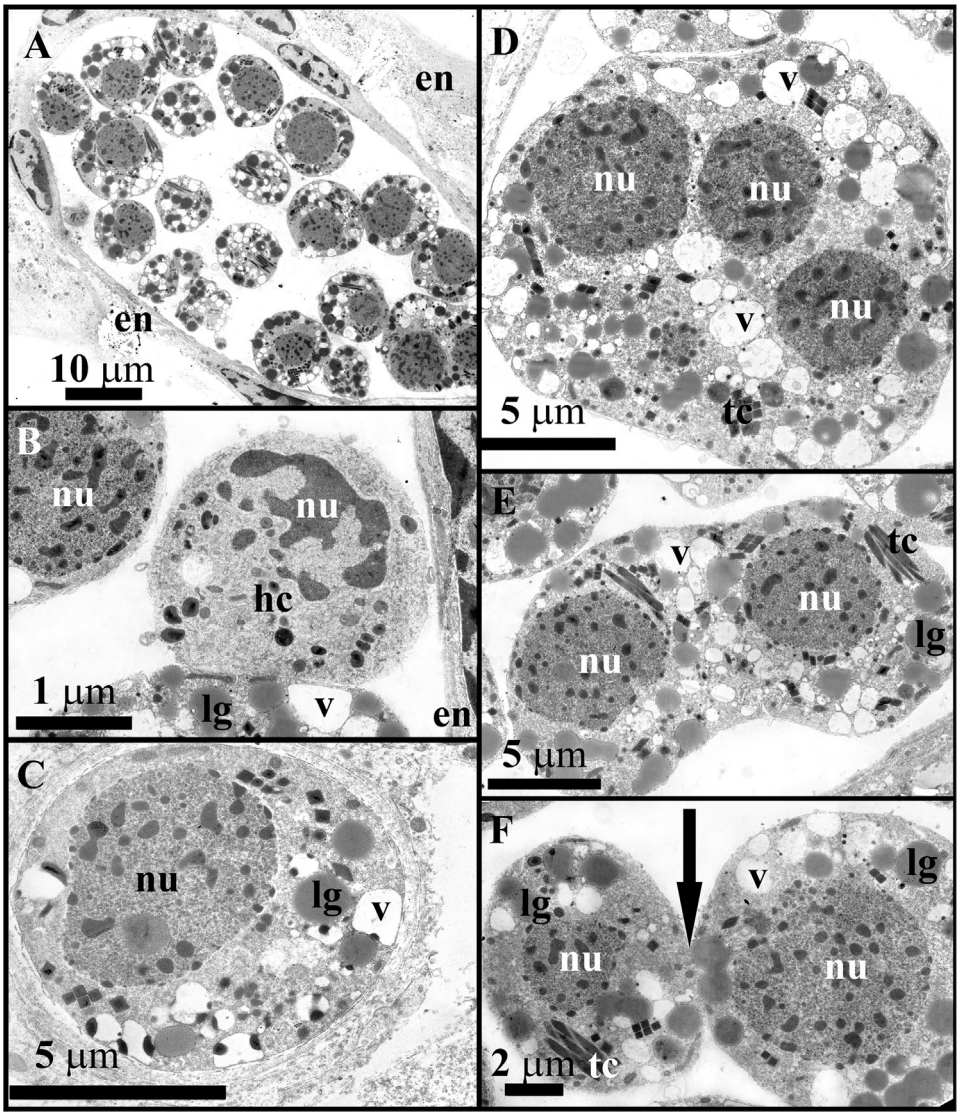

Fig. 6. Transmission electron micrographs of trophonts in hemal vessels of hepatopancreas of *Hapalogaster grebnitzkii*. (A) Hemal vessel filled with trophonts. (B) Hemocyte contacting a trophont in the lumen of hemal vessel. (C) Trophont surrounded by the envelope formed by cellular processes and loose fibrillar matrix in connective tissue. (D) Trinuclear plasmodium. (E) Binuclear plasmodium in the beginning of cytotomy. (F) The final stage of cytotomy; only a small isthmus (arrow) remains between daughter cells. Legend: en, envelope; hc, hemocyte; lg, lipid granule; nu, nucleus; tc, trichocysts; v, vacuoles with electron-lucent content.

According to many studies, a dinospore, after penetrating into the host organism, first develops into a mononuclear trophont, and then passes through a series of multinuclear stages of various shapes and sizes. Then the vegetative stages of the parasite are replaced by the prespore stages, and sporulation occurs shortly before the host's death (Meyers et al., 1987; Appleton and Vickerman, 1998; Shields and Squyars, 2000; Stentiford and Shields, 2005; Wheeler et al., 2007). In the analyzed specimens of the infected crab *H. grebnitzkii*, a major part of parasite cells were represented by mononuclear trophonts, and a smaller part were plasmodia with a small number of nuclei. Already at this stage, the massive occupation of the circulatory system and connective tissue of all organs by the parasite occurred and changes appeared in the cells of the epithelium of hepatopancreas tubuli. Pathological changes, similar to those found in *H. grebnitzkii*, are described from various species of crustaceans parasitized by *Hematodinium* (Field and Appleton, 1995; Meyers et al., 1987; Stentiford et al., 2002; Sheppard et al., 2003; Stentiford and Shields, 2005; Wheeler et al., 2007).

A great number of studies confirm that the rapid proliferation of *Hematodinium* stages in tissues of crustaceans is accompanied by hemocytopenia (Field and Appleton, 1995; Meyers et al., 1987; Shields, 1994; Shields and Squyars, 2000; Stentiford and Shields, 2005; Wheeler et al., 2007). We observed hemocytopenia in *H. grebnitzkii* as well, but a significant number of granulomas (haemocytic nodules, granuloma-like foci) were registered also in connective tissue of the hepatopancreas, connective tissue under epidermis and intestinal tract. This is one of the signs of a normal protective response of crustacean hemocytes to damage, invasion of pathogenic organisms, etc. But formation of melanized hemocytic inclusions (hemocytic nodules) in crustaceans infected by dinoflagellates is rarely recorded. In some cases, the cause of formation of these inclusions (in *N. norvegicus* and *C. pagurus*) remained unclear (Field and Appleton, 1995; Stentiford et al., 2002); in others, it was associated with concomitant secondary infections caused by bacteria, ciliated protozoa (*Ch. bairdi*), or yeast-like fungi (*C. pagurus* and *N. puber*) (Meyers et al., 1987; Stentiford et al., 2003). It is believed that the encapsulation response was aimed either at removing the cellular debris that appear during infection, or at the secondary pathogen, but not at *Hematodinium* cells (Meyers, 1987; Messick, 1994; Stentiford et al., 2002, 2003). We did not identify bacteria or any other secondary pathogenic organisms in soft crab. Since *H. grebnitzkii* is not a commercial species, studies usually pay very little attention to it, as well as to other non-commercial species. However, the role of these crustacean

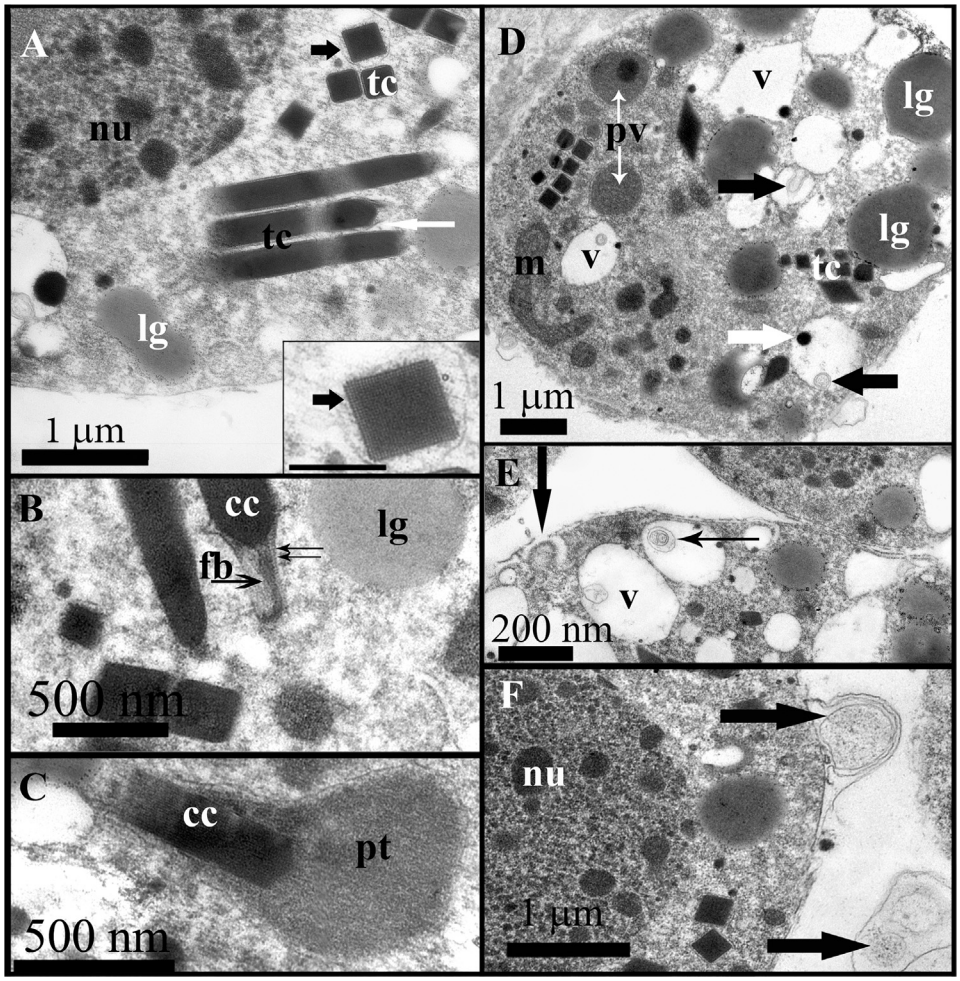

Fig. 7. The structure of cytoplasm of *Hematodinium* sp. trophonts. Transmission electron micrographs. (A) Longitudinal (white arrow) and cross sections (dark arrows) of trichocysts containing electron-dense crystalline core and separated from the cytoplasm by a membrane layer. Insert: cross section of a crystalline core with the grid consisting of regular squares with a side of 10 ± nm; scale bar, 250 nm. (B) Apical region of mature *Hematodinium* trichocysts; note the outer membrane of trichocysts, the fibrillar band between the crystalline core and the tip of apical structure (big dark arrow), and the transverse striation on the surface of membrane (small dark arrows). (C) Formation of crystalline core in a primordial trichocyst. (D) Cytoplasmic inclusions are as follows: vacuoles containing lipids, vacuoles with electron-lucent content, small electron-dense granules (white arrow), heterogeneous bodies (dark arrows), and primordial vesicles. (E) Pore complexes on the surface of trophont (dark arrow). Heterogeneous bodies inside a vacuole (small dark arrow). (F) Drops of exudate on the surface and between cells of *Hematodinium* sp. (dark arrows). Legend: en, envelope; lg, lipid granule; m, mitochondria; nu, nucleus; pv, primordial vesicles, pt, primordial trichocyst, tc, trichocysts; v, vacuoles with electron-lucent content.

species as a possible reservoir host and a vector of disease transmission can be very important, especially for such host generalists with a broad geographic range as *Hematodinium* (Small and Pagenkopp, 2011; Pagenkopp et al., 2012). We noted the infected soft crab individual among five soft crabs and 16 red king crabs in the catch by the changes of its shell color. It should be mentioned that no changes in the exoskeleton color have ever been observed in lithodid (red and blue king) crabs infected by *Hematodinium* (Ryazanova, 2008; Ryazanova et al., 2010). In the Sea of Okhotsk, the *Hematodinium* infection has been known for lithodid (red and blue king) crabs since 2004. It was also diagnosed by histological methods in one golden king crab, *Lithodes aequispinus*, from the northern Sea of Okhotsk. Since this was first recorded, the prevalence of infection in red and blue king crabs has remained at a very low level (Ryazanova et al., 2010). It is possible to assume that the abiotic conditions on the shelf of western Kamchatka are quite unfavorable for *Hematodinium*. This area is characterized by a very shallow indentation of the coastline, a mostly sandy and sandy/silty bottom, and large currents along the coast (Shuntov, 2001).

Unfortunately, we can only speculate on the prevalence of the infection *H. grebnitzkii* on the western Kamchatka shelf, a this species is very rare found during trawl survey. *H. grebnitzkii* mainly inhabits the littoral zone, although it is known occur at depths of up to 90 m (Slizkin, 2010). If transmission of the infection to deep-sea crabs such as blue and, especially, golden king crabs directly from *H. grebnitzkii* seems impossible, a transmission to red king crabs may occur in summer, when individuals of this species migrate to shallow waters. In the spring and summer seasons, aggregations of red king crabs are located at depths of 5–60 m, where they breed and molt (Levin, 2001).

Thus, *H. grebnitzkii* is currently known as another crustacean from which *Hematodinium* infection has been recorded and is the fifth species of host crabs from the superfamily Lithodoidea. The issue of the significance of soft crab in the maintenance and distribution of the *Hematodinium* infection remains open and requires special attention. It should be noted that this boreal Pacific species is widespread and occurs along the coasts from southern Primorsky Krai to the Bering Strait and from the La Perouse Strait to California.

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Competing interests

The authors declare no competing interests.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2018.02.002.

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