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First detection of *Hematodinium* sp. In spiny king crab *Paralithodes brevipes*, and new geographic areas for the parasite in tanner crab *Chionoecetes bairdi*, and red king crab Paralithodes camtschaticus

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Highlights

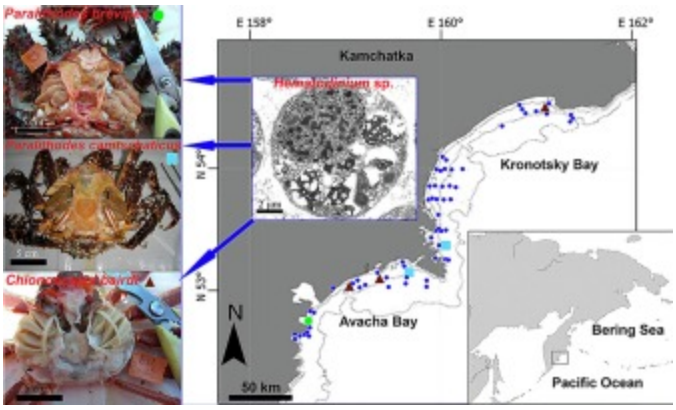
- Parasitic microalga Hematodinium sp. was found in three types of crabs.
- The Pacific coast of Kamchatka was a new area for such research.
- The spiny king crab Paralithodes brevipes has become the new host of this pathogen.
- Ultrastructure of Hematodinium sp. has been studied in three types of crabs.

- A high degree of identity was found between the parasites of the three crab species.

Abstract

A parasitic dinoflagellate of the genus *Hematodinium* was found off the Pacific coast of Kamchatka in three species of crabs: red king crab *Paralithodes camtschaticus*, tanner crab *Chionoecetes bairdi*, and spiny king crab *Paralithodes brevipes*. This is the first detection of *Hematodinium* in spiny king crab. The results of the genetic analysis showed that the pathogen found in *P. brevipes*, *P. camtschaticus*, and *C bairdi* from the Avacha and Kronotsky bays off the Pacific coast of Kamchatka was the same or very close to the *Hematodinium* sp., which infects many species of crustaceans in the Northern Hemisphere. The prevalence of infection was 0.2% for tanner crabs and 2.7% for red king crabs. Due to a limited sample size, we were unable to calculate the prevalence for spiny king crabs and female red king crabs. Both the macroscopic and microscopic signs of the pathology were similar in all diseased crabs. The differences in the micromorphology of the *Hematodinium* cells we found in the three crab species, including the presence or absence of trichocysts, the shape of the plasmodia, and the structure of pore complexes, are most likely related to the life cycle and the physiology of the parasite. The results of the genetic analysis showed that the pathogen found in *P. brevipes*, *P. camtschaticus*, and *C. bairdi* from the Avacha and Kronotsky bays of the Pacific coast of Kamchatka was the same or very close to the *Hematodinium* sp., which infects many species of crustaceans in the Northern Hemisphere.

Graphical abstract



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Introduction

The dinoflagellate parasite *Hematodinium* Chatton et Poisson (Syndinea: Syndiniophyceae: Syndiniales: Syndiniaceae) is one of the most dangerous crab parasites and is found in many areas of the World Ocean (Meyers et al., 1990, Meyers et al., 1996, Hudson and Shields, 1994, Messick, 1994, Stentiford and Shields, 2005, Shields, 2012, Small, 2012). With the exception of two reports in the literature, they have all been found in the Northern Hemisphere. New host crustaceans and geographic locations are increasingly being reported(Meyers et al., 1987, Meyers et al., 1990, Meyers et al., 1996, Wilhelm and Mialhe, 1996, Stentiford and Shields, 2005, Ryazanova, 2008, Xu et al., 2010, Morado, 2011, Morado et al., 2012, Small, 2012, Stentiford et al., 2012, Wang et al., 2017, Ryazanova et al., 2018).

Molecular studies show that the taxonomically unidentified *Hematodinium* sp. infects most boreal host crustaceans (Jensen et al., 2010, Small, 2012). In the North Pacific, the presence of *Hematodinium* sp. in tanner crabs *Chionoecetes bairdi* was first discovered in 1985 (Meyers et al., 1987). It has now been shown to be widespread in this species as well as in the snow crab *Chionoecetes opilio* of southeast Alaska, the eastern Bering and Chukchi seas (Meyers et al., 1987, Meyers et al., 1990, Meyers et al., 1996, Morado et al., 2012).

Hematodinium sp. was first recorded in Russia in the Sea of Okhotsk in 2002 in snow crab *C. opilio* (Karmanova and Ryazanova, 2008) and was later found in four more species of commercial crabs: red king *Paralithodes camtschaticus*, blue king *P. platypus,* golden king *Litodes aequispinus* and tanner *C. bairdi* (Ryazanova et al., 2010, Metelev and Ryazanova, 2013, Ryazanova et al., 2016). More recently, the parasite has been reported in the non-commercial soft crab *Hapalogaster grebnitzkii* (Ryazanova et al., 2018). *Hematodinium* sp. are found in an area which covers the northern and eastern parts of the Sea of Okhotsk and the western part of the Bering and Chukchi seas. This article reports the detection of *Hematodinium* sp. off the Pacific coast of Kamchatka in three species of crabs: red king crab *P. camtschaticus*, tanner crab *C. bairdi*, and spiny king crab *Paralithodes brevipes*. This is the first detection of *Hematodinium* sp. in spiny king crab. This report presents macroscopic and microscopic descriptions (light and transmission electron microscopy) of the disease and molecular identification of *Hematodinium* sp. in crabs.

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Materials and methods

In May 2019, during a standard trap survey off the Pacific coast of Kamchatka (Avacha and Kronotsky bays, see Fig. 1), we investigated diseases in the captured crustaceans. At depths of between 15 and 94m, the crew set nine standard Japanese conical traps for each of the 67 stations in the study area using frozen herring as bait. Depending on weather conditions, the traps were deployed for between 18 and 24h.

The commercial crab species inhabiting the area: tanner crab *C. bairdi*, snow crab *C.*…

Results

We examined a total of 2344 specimens visually and pathological analysis was carried out on 1104 crabs (Table 2).

We found *Hematodinium* sp. in three species of crabs at six separate stations: spiny king crabs (2 males), red king crabs (3 males/1 female) and 2 male tanner crabs (Fig. 1). The prevalence of the infection was 0.2% for tanner crabs and 2.7% for red king crabs. We did not find *Hematodinium* sp. in snow crabs. Due to a limited sample size, we were unable to calculate the prevalence for…

Visual and histological signs of infection

The condition of the integument of all diseased crustaceans corresponded to stage 3 of the molting cycle. The colour of the carapace of the infected tanner crabs was pink on the dorsal and chalk on the ventral side. We didn't observe any changes in the colour of the shell of the spiny and red king crabs. Upon necropsy, the same visual signs of the disease appeared in all three species. Their hemolymph was opaque and the colour was creamy or light yellow, unlike healthy hemolymph, which is…

Ultrastructure of Hematodinium sp

In the spiny king crab, the polymorphic amoeboid trophonts had a nucleus with small condensed chromosomes surrounded by a thin nuclear envelope, not always detectable by electron microscopy. The nucleus was divided with the formation of a constriction without destruction of the nuclear membrane, which is characteristic of dinomitosis (Fig. 3A).

The cytotomy process was not synchronized with karyokinesis, causing formation of binucleate and multinucleate cells. The size of the uninucleate…

Genetic analysis

We used all the primer combinations recommended by Jensen (Jensen et al, 2010) to obtain 18S rRNA - ITS1 *Hematodinium* fragments from three crab species. All combinations of the forward primer with the Hsp2R primer (Hudson and Adlard,1996) lead to either unreadable sequence or to a fragment of the ribosomal gene of the host DNA. In addition, in parasites from red king and spiny king crabs, the results were unsatisfactory when sequencing the ITS1 region of various amplified fragments…

Discussion

The results of genetic analysis showed that the pathogen we found in the spiny king crab, as well as in the red king and tanner crab from Avacha and Kronotsky bays of the Pacific coast of Kamchatka is *Hematodinium* sp. and it is the same or close to that which was previously isolated in the red king and blue crabs, as well as the soft crab from the Sea of Okhotsk (Ryazanova et al., 2010, Ryazanova et al., 2018), as well as in crabs of the genus *Chionoecetes* and the genus *Lithodes* (Jensen et…

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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We would like to recognize our colleagues for their collaboration on this project and offer our sincere gratitude to the crew of the research vessel *Engineer Martynov* for their dedication and courage shown in performing their duties on this voyage. We are deeply grateful to the reviewers for their valuable comments, which made it possible to

significantly improve the original version of this article. The material for ultrastructural investigation was processed and analyzed at the Far Eastern…

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Milky hemolymph syndrome (MHS) associated with a virus in tanner crab Chionoecetes bairdi off the Pacific coast of Kamchatka

2023, Journal of Invertebrate Pathology

> *Citation Excerpt :*

> …Off the coast of Kamchatka, this infection was registered in 7 species of crustaceans, including tanner and snow crabs (Ryazanova et al., 2010; Ryazanova et al., 2016; Ryazanova et al., 2018). In 2019, we found both MHS (this article) and Hematodinium sp. infection in tanner crabs (Ryazanova et al., 2021). Thus, the initial diagnosis based on visual signs needs to be confirmed in each case by microscopic or molecular methods.…

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