

First Record of Nematode Larvae in the Amphipod *Ischyrocerus commensalis* Colonizing Red King Crabs in the Barents Sea

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Abstract: In this study, nematodes were first reported in the amphipods, *Ischyrocerus commensalis*, collected from the introduced and commercially important red king crabs, *Paralithodes camtschaticus*, in the coastal Barents Sea in July 2022. Commensal amphipods were registered on all red king crabs captured (n = 70, prevalence 100%). Further laboratory analysis revealed that 11 out of 467 amphipod individuals (prevalence 2.4%) harbored single third-stage larvae of *Hysterothylacium* sp. l. (Nematoda: Anisakidae). The nematode larvae ranged from 0.63 to 6.10 mm in body length. Due to the low prevalence of nematodes and lower vulnerability of the host amphipods to fish predators, negative effects on the Barents Sea ecosystem through the range expansion of crab-associated amphipods and their parasites are unlikely.

Keywords: nematode larvae; parasite; amphipod; *Ischyrocerus commensalis*; red king crab; *Paralithodes camtschaticus*; Barents Sea



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Biological invasions are recognized as some of the most dramatic examples of changes in species distribution [1]. In marine ecosystems, alien species may become invasive resulting in declines in biodiversity including displacing native species and the loss of native genotypes [2]. Invasive species may lead to disruption of ecological processes including changes in community structure, modified habitats, food-web properties, and ecosystem processes [3]. They impede the provision of ecosystem services and are responsible for detrimental impacts on human health and well-being and cause losses to multiple sectors of the economy [4]. Organisms that live in association with their hosts, both epibionts and parasites, are frequent partners in biological invasions because they are either introduced into new communities along with invading species or are left behind in the ancestral range of the host, affording the host ‘enemy release’ [5,6]. Moreover, parasites are frequently implicated in altering the outcome or impact of invasions, changing the strength of interactions between invasive and native species [7,8].

The vast majority of biological invasions are human-mediated and some of these are intentional [2]. An example of such introductions is the translocation of the red king crab, *Paralithodes camtschaticus*, from the Pacific Ocean to the Barents Sea conducted by Soviet scientists in the 1960s [9]. By the mid-1990s, this crab had formed a new self-sustaining population. In the subsequent decade, the crab abundance had increased to levels suitable for commercial and amateur fishing [9–13]. Positive effects from this introduction include the income from the fishery sector [12,14–18] and tourism while negative effects are associated with some alterations in the structure of benthic communities [19–22]. At the same time, red king crabs, like other marine crustaceans, are hosts for a variety of symbiotic and epibiotic organisms [23–29]. Previous research has shown that no symbiotic species were introduced into the Barents Sea from the Pacific Ocean together with the red king crab [30], but this species has become a suitable host for local organisms including various symbionts [23–25,28,29,31–35]. Moreover, the spread of red king crabs throughout the coastal Barents Sea has been assumed to promote range extension of their associated fauna.

For example, Norwegian authors reported that the red king crab can serve as a transport host for the fish leech *Johanssonia arctica*, thus acting as an indirect vector for *Trypanosoma murmanense* and promoting transmission of this blood parasite to the Barents Sea cod *Gadus morhua* [36].

Another local species whose life cycle is closely associated with red king crabs is the amphipod *Ischyrocerus commensalis* [34,37]. The advantages for this commensal amphipod include protection from predators and feeding on food remnants of the host [25]. In contrast to fish leeches, there are no data on the parasitic fauna of *Ischyrocerus commensalis*. For this reason, our purpose was to survey the prevalence of parasites infecting this crab-associated amphipod.

Red king crabs were collected by divers (depths 5–33 m, both soft- and hard-bottom locations, temperature 4–6 °C) according to standard protocols [27,33,38] in Dalnezelentskaya Bay in July 2022. The study area is a typical semi-closed small bay located on the eastern coast of the Kola Peninsula (for a more detailed description, see our previous papers [39–41]. In the coastal laboratory, each crab was visually examined for associated organisms. Amphipods were collected and fixed in 4% formaldehyde and then examined under a stereomicroscope MBS-10 for size and sex as described in Dvoretzky and Dvoretzky [37].

The amphipods were found on all red king crabs captured ($n = 70$; carapace length, range 68.8–184 mm, mean \pm SE 144.8 ± 2.4 mm), thus demonstrating a 100% prevalence of infestation. The intensity of *Ischyrocerus commensalis* varied from 4 to 340 averaging 102.5 ± 6.4 ind. per crab. Larger amphipods were found predominantly on mouthparts and limbs (Figure 1).

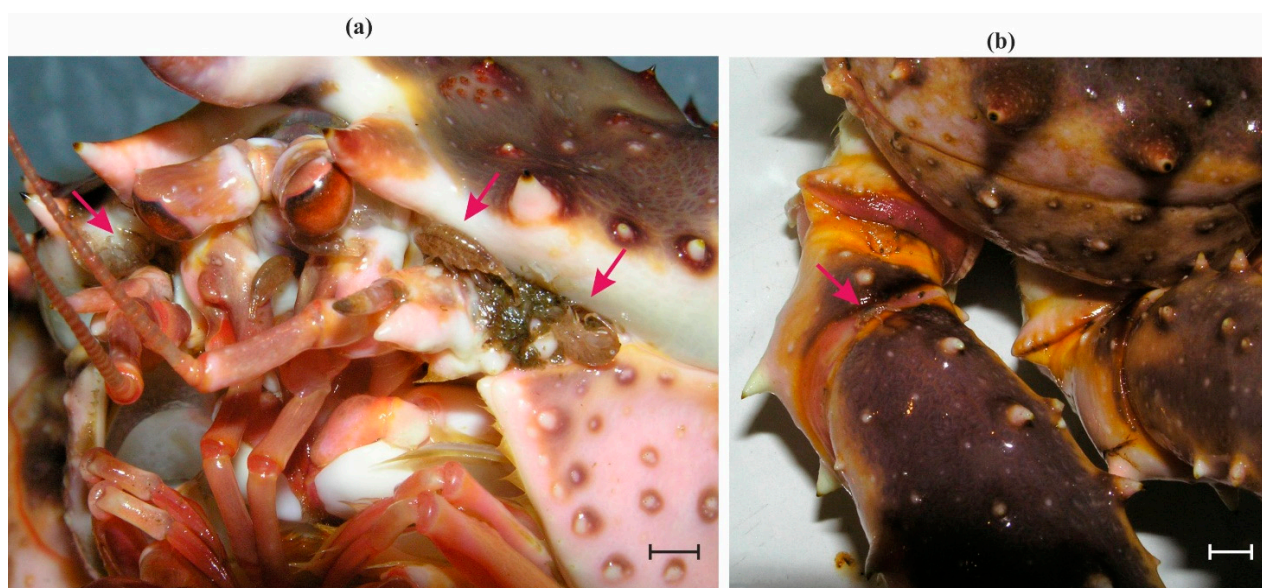


Figure 1. *Ischyrocerus commensalis* amphipods (arrows) on the mouthparts (a) and limbs (b) of red king crabs from Dalnezelentskaya Bay, July 2022. Scale bar 5 mm.

A total of 467 amphipods ranging from 3.8 to 12.1 mm in body length were dissected and examined for parasites under a MBS-10 stereomicroscope.

For the first time, third-stage nematode larvae belonging to the genus *Hysterothylacium* were found in *Ischyrocerus commensalis* collected on red king crabs (Figure 2a). The worm specimens were fixed with a mixture of glycerin and lactic acid and then examined and photographed using a ZEISS Imager D2 equipped with an AxioCamMRc5 photo camera (Figure 2b–d).

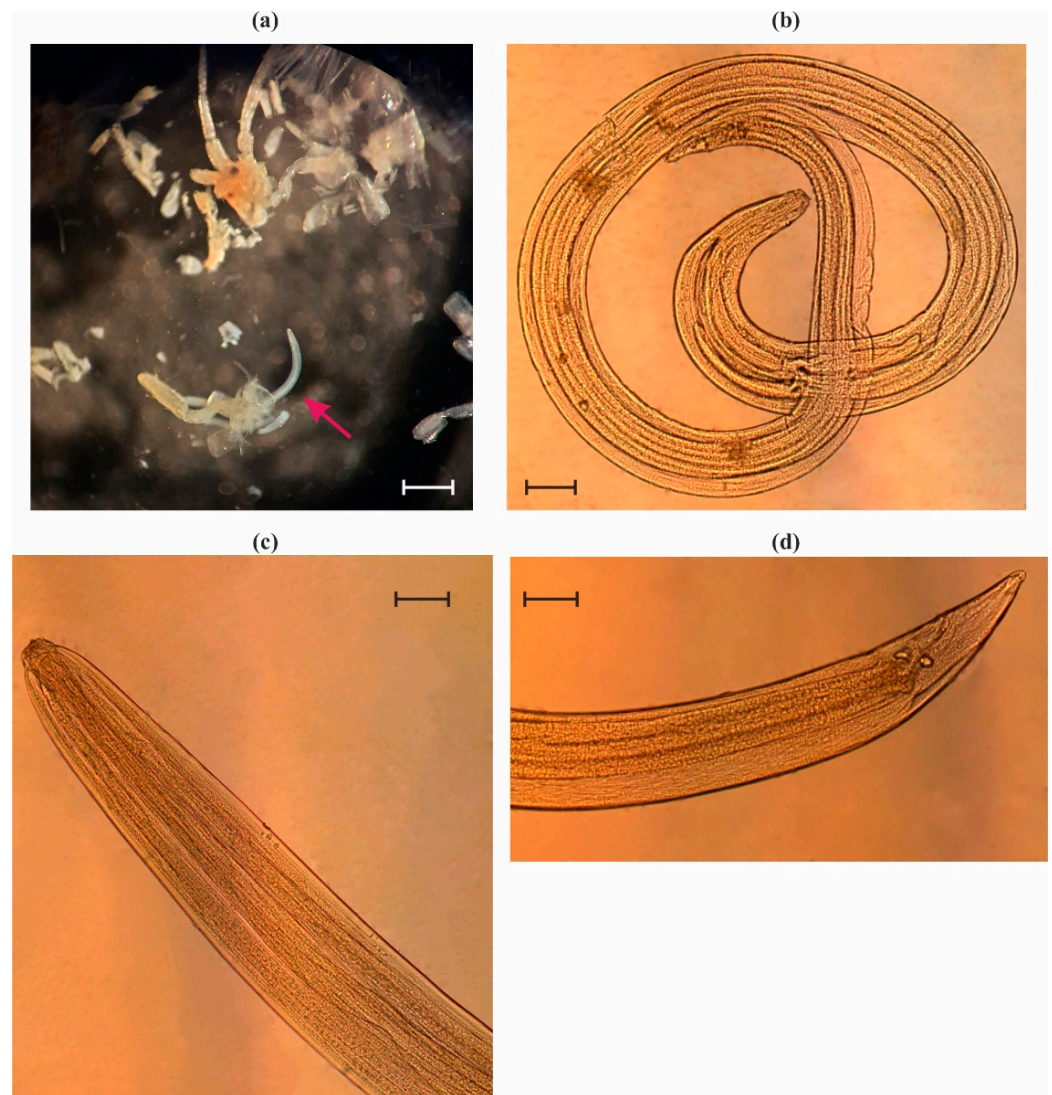


Figure 2. Nematode larva in *Ischyrocerus commensalis*. (a)—dissected amphipod with nematode larva (arrow), scale bar 1 mm; (b)—light microphotograph of nematode larva, overall view, scale bar 0.1 mm; (c)—anterior end, scale bar 0.1 mm; (d)—posterior end, scale bar 0.1 mm.

The total body length of the larvae varied from 0.63 to 6.10 mm. Maximum body width, labial region width, nerve ring diameter, oesophagus region width, and anal body width were 0.25–0.41, 0.10, 0.08, 0.15, and 0.09 mm, respectively. The larvae had striated zones in their cuticles. A nerve ring was located 0.281 mm from the anterior end. The excretory pore was located at the level of the nerve ring, 0.413 mm from the anterior end. The muscular oesophagus, ventricular appendix, and intestinal caecum were 0.938, 0.64–0.84, and 0.46–0.67 mm long, respectively. Each larvae had well-developed labia with a noticeable tooth and a conical tail with a single prominent spine.

A total of 11 nematode larvae were found in the amphipods (Table 1).

The prevalence of infection for the whole sample was 2.4% (95% confidence interval, 1.2–4.2%) and the mean intensity was 1 ind. per amphipod. No significant differences were found for the prevalence of Nematoda with regard to size and sex of the host amphipods (Chi-squared tests, $p > 0.05$).

Table 1. Infestation indices of *Hysterothylacium* nematode larvae in crab-associated *Ischyrocerus commensalis* amphipods from Dalnezelentskaya Bay, Barents Sea, in July 2022.

Size Class, mm	Male					Female				
	N	NI	PI	95-% C.I.	II	N	NI	PI	95-% C.I.	II
<6.1	80	2	2.5	0.3–8.7	1	75	0	0.0	–	–
6.1–8.0	63	2	3.2	0.4–11.0	1	96	6	6.3	2.3–13.1	1
8.1–10.0	30	0	0.0	–	–	83	1	1.2	0.0–6.5	1
>10.0	14	0	0.0	–	–	26	0	0.0	–	–
Total	187	4	2,1	0.6–5.4	1	280	7	2.5	1.0–5.1	1

Note: N—sample size, NI—number of infected amphipods, PI—prevalence (%), 95-% C.I.—95% confidence interval for prevalence (%), II—intensity of infection, (ind. per amphipod).

Previous studies conducted in the coastal Barents Sea have shown that the caprellid amphipod *Caprella septentrionais* is a host for the nematode *Anisakis* sp. with as low prevalence as 0.12% [42] and amphipods belonging to the family Gammaridae (*Gammarus oceanicus*, *G. duebeni*, and *Marinogammarus obtusatus*) are hosts for nematodes *Spirurida* sp. (prevalence, 0.6–37.6%) and *Ascarophis* sp. (0.2–0.4%) [43,44]. There is only one record on parasitic fauna of ischyrocerid amphipods in the Barents Sea: the amphipod *Ischyrocerus anquipes* was reported to be a host for metacercaria of the trematode *Podocotyle atomon* (prevalence, 4.0%) [42]. Thus, our findings update a list of amphipod parasites in the Russian part of the coastal Barents Sea.

Nematodes belonging to the genus *Hysterothylacium* (family Raphidascauridae) infect various species of marine fish in both the larval and adult stages [45–47]. Their larvae also are known to occur in marine invertebrates [48–50]. Laboratory studies have shown that the calanoid copepod *Acartia tonsa* and harpacticoid copepods became infected by ingesting eggs containing infective third-stage larvae. Mysids, corophiid and gammarid amphipods, and isopods easily became infected when exposed to a large number of eggs [51]. Under natural conditions, the prevalence of *Hysterothylacium* was found to be 7.7% in the gammaridean amphipod *Proboloides holmesi*, 0.06% in the caprellid amphipod *Caprella linearis* from eastern Canada [50], and 1.95% in hyperiid amphipods from the North Sea [52].

Amphipods are known to be significant prey for fish and invertebrates and are considered a major food web link between pelagic production and upper trophic levels [53,54]. Although parasites can alter food web dynamics, in the case of nematodes infecting *Ischyrocerus commensalis*, this process seems to be negligible because of the low prevalence of the nematode larvae and low vulnerability of the host amphipods to potential fish predators such as cod, haddock, and saithe which are mostly infected when consuming small planktivorous pelagic fishes [55]. Our assumption is supported by the fact that the red king crab introduction and its growing population did not affect the production of major fish stocks and traditional fisheries in the Barents Sea [56].

Finally, our findings seem to have no major practical implications for public health because a laboratory study on cultivation of *Hysterothylacium* from third-stage larvae to egg-laying adults showed that the highest survival rates and percentage of molting to fourth-stage larvae occurred at 13 °C whereas at 37 °C nematodes survived only for a few hours [57], thus indicating that *Hysterothylacium* are non-pathogenic to organisms with high body temperatures including humans [58].

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