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Development of the Parasite Nematode *Echinuria Uncinata* (Nematoda:Acuariidae) in the Intermediate Host in Uzbekistan

Maqsadjon Jumanovich Madumarov[1*]; Abdurakhim Ergashevich Kuchboev[2]; Hasanboy Kholiknazarovich Abdunazarov[3]; Amirov Oybek Olimlonovich[2]

[1] Department Zoology National University of Uzbekistan named after Mirzo Ulugbek, Uzbekistan.
[2]Laboratory Molecular zoology Institute of Zoology Uzbekistan Academy of Sciences, Uzbekistan.
[3]Department Biology Kokand State Pedagogical Institute named after Mukimi, Uzbekistan.
* maqsadjon_mj84@umail.uz

ABSTRACT
In this study, it was first established that larvae of the nematode *Echinuria uncinata* are found in the species *Daphnia magna* in the Fergana Valley, Uzbekistan. In the spring and autumn, 11.5% of the daphnia samples were infected larvae nematode. The morphology and morphometric parameters of nematode larvae are presented. To study the development of larvae in the organism of the intermediate host, *D. magna* infected nematode eggs with food and studied their development at different temperatures. The larvae reached the fourth invasive stage after 24-26 days at a low temperature of 1-15° C, 9-10 days at 20-22° C and 8-9 days at a relatively high temperature of 24-26° C. In this study was studied development of biology of the larvae of the nematode *Echinuria uncinata* in the organism of the intermediate hosts-daphnia in Uzbekistan.

Keywords:
nematode, invasive larva, intermediate host, *Daphnia magna.*

Introduction

The nematodes *Echinuria uncinata* from the family Acuaridae, which parasitize the wall of the glandular stomach of domestic and wild ducks, geese and many wild waterfowl. Echinuria registered in different regions of the world. Echinuria develops with the participation of definitive hosts (birds) and intermediate (*daphnia*). For many years, European and Russian researchers have conducted studies on the biology of the parasite nematode and its aquatic population. Furthermore, American scientists have also done a lot of work in this field. In one of these works, it was noted that juvenile forms of some nematode species live on *Daphnia pulex, D.magna, Simocephalus vetulus, Ceriodaphnia reticulata, C. acanthina, Moina macrocopa*, and other zooplankton (Clark, 1979). For the first time, Hamann (1891) found out the presence of nematode larvae in small crustaceans - *Daphnia pulex*, in the pond water in which ducks, infected with echinuria, were swimming. He managed to infect daphnia by means of the excrement of a duck. His observations were also confirmed by Wolfugel (1900). However, given that parasites cause significant damage to ducks, its prevention is important (PATRICIA et all., 2020; THIERRY et all., 2004). M.A. Sultanov noted that nematodes, found in birds living in water basins of Uzbekistan (Syrdarya and Chinoz districts), act as intermediate hosts in the species *D.pulex* and *D.magna* (SULTANOV, 1963).

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The aim of the research is to study the biology of daphnia as an intermediate host for the nematode *Echinuria uncinata* in the conditions of the Fergana Valley and to study the development of nematode *Echinuria uncinata* in the organism of Daphnia.

Material and methods

The research materials were collected from Lake Sariqamish in the Fergana Valley (ABDINAZAROV, 2017). (Figure 1, A). Specimens were collected in the spring (March-May) and autumn (late of August - early of November) of 2019-2020 (Figure 1, B). Zooplankton specimens were collected using hydrobiological methods from the lake shoreline and pond areas adjacent to the lake. Daphnia species were separated using Bogarov's camera and fixated in a mixture of 4% formalin and glycerin for morphological studies. A certain part was kept alive and brought to the laboratory in special containers in order to reproduce under laboratory conditions and infect them with nematode eggs. More than 2,000 specimens were collected from the 9 regions studied.

Excrements of birds such as ducks (*Anos platyrhynchus*) and geese (*Anser anser*) were collected from the studied areas for experimental infestation of daphnia with invasive elements of nematodes. Two hundred specimens were obtained for the study. Helminthovoscopic examinations were performed on the excrement to examine the eggs of the nematode. Excrements that were positive were used for experimental work. Initially, collected excrements were soaked in a Petri bowl with warm water (20–22°C). Then they were mixed well and it transformed into a liquid state. Then, the presence of eggs was examined using a binocular microscope. The eggs that had sunk to the bottom of the Petri bowl were separated. In case the water temperature was different, the eggs were sprinkled along with the yeast as food for the daphnia.

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Figure 1: Map of the Fergana Valley (A). The areas where specimens were collected from Lake Sariqamish (B) area are shown in figures. The original map was taken from the Google Earth portal (https://earth.google.com/). It was processed in Macromedia Flash program.

Specimens of daphnia and nematode larvae obtained for morphological studies were placed on a microscopic glass slide. A mixture of glycerin and water was dripped on it, a cover glass was placed, and the edges were framed with attic varnish. In the study, the photographes were taken by a microscope camera of ToupCam company, using a MEIJI Microscope (Japan). The species composition of daphnia was determined using identifiers given in the specialized hydrobiological literature (TSALOLIKHIN, 1995.). Helminthological determinants and literature were used to identify eggs and larvae in nematodes (SKJABIN et all., 1965).

Results and discussion

Studies have shown that the *Daphnia magna* species, which belongs to the Daphnidae family, is common in Lake Sariqamish. The occurrence of these species as a dominant species has also been noted in our previous studies (MADUMAROV, 2020). However, during the study period, we also encountered small amounts of *D. pulex* and *D. curvirostris* (in ratio 1:12 with *Daphnia magna*).

When we helminthologically examined daphnia infestation with parasitic nematodes, it was found that 23 out of 200 specimens (11.5%) had eggs and larvae in the nematode. Intensity of invasion (II) of daphnia with nematode larvae was 2–6 copies.

Morphology: Its egg is in a oval form, 0.037-0.039 mm long, 0.021-0.023 mm wide (Figure 1, a). The larvae are whitish, brown, the head and tail parts are flattened, the head is blunt, the tip is bulging (Figure 1,b). The tail part is relatively sharp. The larvae are 0.950–1.689 mm long and 0.048–0.055 mm wide. At a distance of 0.04–0.05 mm from the anterior end of the body, the cuticle began to be covered with thorns, covering 0.03–0.04 mm from the posterior end. The length of the anterior end of the body is 0.124 mm, the length of the pharynx is 0.082-0.089 mm, cylindrical, the anterior end is funnel-shaped. The length of the anterior muscular part of the esophagus is 0.220-0.266 mm, the posterior glandular part is 0.706 mm. The nerve node is located 0.1 mm from the anterior end of the body.

Table 1: Morphological dimensions of nematode larvae *Echinuria uncinata*, n=10

Dimensions	(*Lim,M±m*)
The length of the body	0,950-1,689 (1,527±0,082)
The width of the body	0,048-0,055 (0,053±0,001)
The length of esophagus	0,920-0,966 (0,949±0,007)
The length of pharynx	0,082-0,089 (0,086±0,001)
The length of the tail	0,240-0,342 (0,306±0,013)

Note 1: *n*– the number of examined specimens, lim–variable limit of the characters, *M*–average arithmetic, *m*– average arithmetic error.

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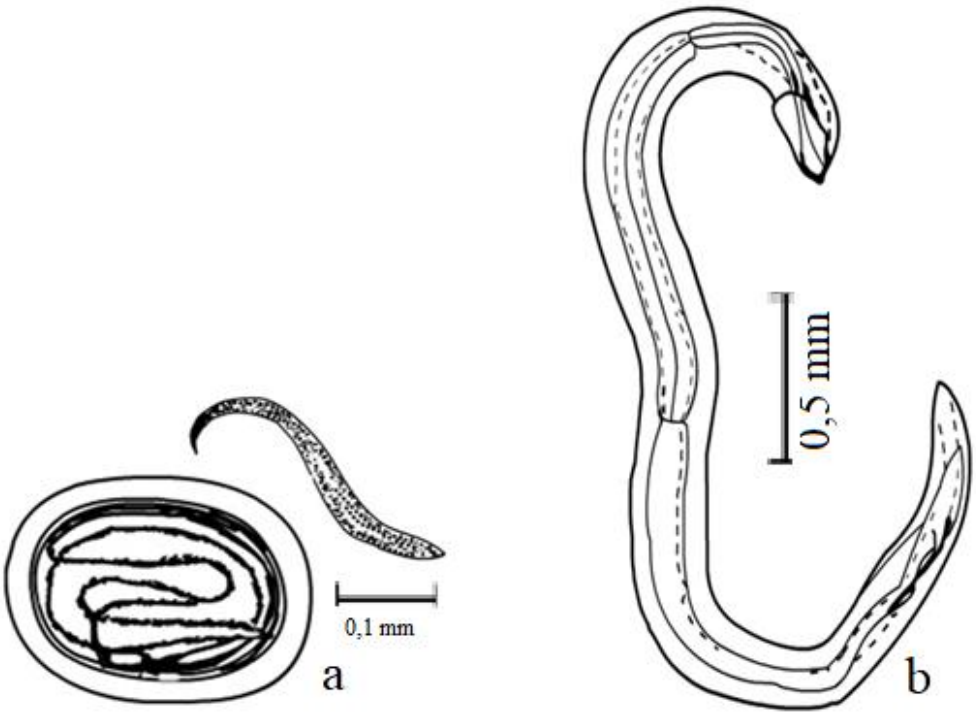

Figure 2: Larvae of *Echinuria uncinata* nematode: a) eggs and larvae; b) invasive larvae.

Based on the results of morphological and morphometric analysis and the literature review, it was determined that this is a species of *Echinuria uncinata* (Rudolphi, 1819), which belongs to the genus *Echinuria* Soloviev, 1912, the subfamily Echinuriinae Raillet, Henry & Sisoff, 1912.of the family Acuariidae Railliet, Henry & Sisoff, 1912, order Spirurida Chitwood, 1933.

The next phase of the study consisted of experimental infection of daphnia with invasive elements of nematodes in the laboratory. In order to do this, daphnia was given the excrements of ducks and geese infested with nematodes. Eggs were sprinkled with yeast as feed for daphnia in case the water temperature was different (Table 2).

Table 2: Infecting process of daphnia in different aquatic environments of *Echinuria uncinata*
nematode, number of eggs n = 50

Experiment number	Water temperature	Total number of daphnia	EI (a unit, %)	II (a unit)	Reaching the invasive stage (days)
№1	+13 -15 °C	30	3 (10)	1-4	24-26
№2	+20 -22 °C	30	7 (23,3)	2-6	9-10
№3	+26 -28 °C	30	8 (26,6)	2-6	8-9

30 minutes after eggs were sprinkled, E.uncinata parasitic eggs were observed in the gut of daphnia. The nematode eggs in the affected daphnia were then examined binocularly every 3 hours to check for egg development and larval phase transition (Figure 2).

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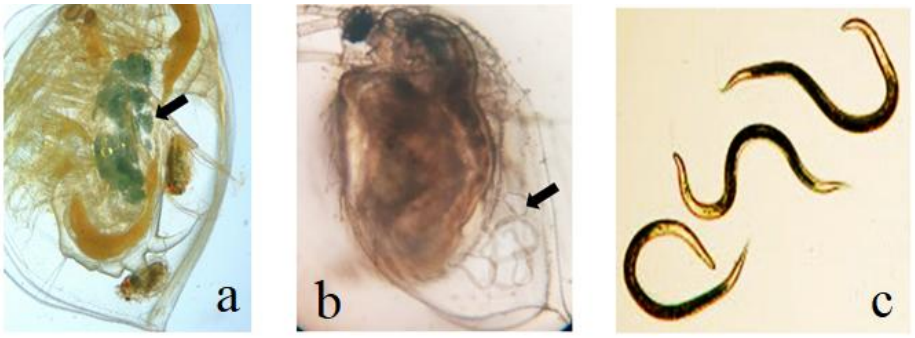

Figure 3: Development of *Echinuria uncinata* in the organism of daphnia. a) eggs in the intestine (20^x); b) larvae in the body cavity (20^x); c) appearance of isolated larvae (40^x).

Conclusion

In №1 experiment conducted under the low-temperature (13–15°C), the full development of E. uncinata nematodes in the daphnia body was somewhat delayed. The larvae reached the stage of invasion within 24–26 days.

In №2 experiment (20–22°C), parasites developed over 4–5 days, and larvae hatched from nematode eggs in the daphnia gut and entered the body cavity. At 6–7 days, the second stage developed, the length of the larva reached 0.4–0.9 mm. On days 9–10 of the experiment, the third-stage larvae were 1.0–1.7 mm in body length and 0.048–0.055 mm in width. At this stage, the larvae were fully developed and reached an invasive state, that is, the level of being able to infecti the main hosts – ducks and geese.

In №3 experiment conducted under the temperature 26–28°C, E. uncinata developed rapidly, reaching the first stage in 3–4 days, and on 6–7 days, reaching the second stage, with a body length of 1–1.2 mm. On days 8–9 of the experiment, the third-stage-larvae was formed, whose body length was 1.5–1.7 mm. II was in 2–6 copies, and was the same as in the experiment №2. These data were consistent with some literature data (AUSTIN, 1970).

According to the literature, nematodes develop and lay eggs in the digestive system of infected wild ducks and geese. Eggs enter the water along with the digestive products through the digestive tract (AUSTIN; WELCH, 1972; Silveira; AMATO, 2006]. Excrements falling into the water are broken down into smaller pieces. Daphnia swallows these eggs along with tiny organic matter, micronutrients, bacteria. Initially, the eggs fall into the daphnia gut, where they develop and undergo the first stage. It then passes from the intestinal wall into the body cavity. There it develops until there is a mature invasive larva. Ducks and geese that consume the infected daphnia along with the water infect the *E. uncinata* larva. Thus *E.uncinata* passes the lifespan. It has not yet been studied how long the eggs can survive (PONOMARENKO, 2014; WORK et all., 2004).

Daphnia specimens collected from the study areas were infested with an average of 15% *E.uncinata* larvae. Mainly found on the shores of lakes, in areas where waterfowl are dense. Damage is especially high in small pools and lake ditches. These data suggest that along with wild waterfowl, there is also transmission to farm birds. Among the daphnia, *D.magna* led as the intermediate host of *E.uncinata*. As a result of studies conducted under natural conditions, the peak of invasion occurred in May and September.

While nematodes reached an invasive state at moderate temperatures for up to 15 days, changes in temperature were shown to have an effect on larval development along with daphnia vital activity. These factors have been identified by a number of scientists, such as F. Austin and V.Y. Ponomarenko. Our observations under laboratory conditions differed slightly from their results and developed as invasive larvae at 9–10 days at 20–22°C.

However, our research suggests that daphnia with a body length greater than 1.4 mm may be affected by *E. uncinata*. Because such a size can correspond to the size of the digestive system. As a result of observations, in the first half of spring and the second half of autumn, the development period of larvae was prolonged, extending from 11-16 days to even 20-24 days.

Resumo

In Uzbekistan, E. uncinata was found with adult representatives of the species in the glandular stomach of a gray duck by M.A. Sultanov (1963). In Tajikistan, it has been studied by L.F. Borgarenko (1990) in ducks and geese as the main host (BORGARENKO, 1990). However, no studies have been conducted on the intermediate host of this nematode - the daphnia species.
In conclusion, for the first time in Uzbekistan it was noted that the intermediate host of *E. uncinata* nematode is *D. magna*. At the same time the developmental biology of larvae in the body of the intermediate host of this nematode – daphnia was studied.

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