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Development of the parasite nematode *Echinuria uncinata* (Nematoda: Acuariidae) in the intermediate host *Daphnia magna*, in Uzbekistan

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ABSTRACT

The current study is the first to record that larvae of the nematode *Echinuria uncinata* were found in *Daphnia magna* species in the Fergana Valley, Uzbekistan. Zooplankton specimens of *Daphina magna* were collected using hydrobiological methods from the Sariqamish Lake shoreline and the adjacent pond areas (9 spots) in spring and autumn, 2019-2020. It was noticed that 11.5% of the samples were infected by nematode larvae. To detect the impact of infection on the intermediate host, samples were subjected to morphological and morphometric parameters. In addition, the development of larvae was addressed in the intermediate host, *D. Magna*. The infected nematode eggs were supplied with food and their development was studied at different temperatures. After 24-26 days, at low temperatures (1-15°C), the fourth invasive stage was reached. While, on days 9-10 of the experimental period, and at 20-22°C, the invasive stage was recorded. Meanwhile, on days 8-9 days at a relatively high temperature of 24-26° C, the same stage was noted.

INTRODUCTION

The nematodes *Echinuria uncinata* from the family Acuaridae live as parasites on the wall of the glandular stomach of domestic and wild ducks, geese and many wild waterfowls. *Echinuria*, recorded in different regions of the world, develops with the participation of definitive hosts (birds) and intermediate *Daphnia*. European and Russian studies have been conducted on the biology of the parasite nematode and its aquatic population. Furthermore, American scientists have also done a lot of work in this field. It was reported that juvenile of some nematode species live on *Daphnia pulex, D. magna*, *Simocephalus vetulus, Ceriodaphnia reticulata, C. acanthina, Moina macrocopa*, and other zooplanktons **(Clark, 1979)**. Nematodes, found in birds living in water basins of Uzbekistan (Syrdarya and Chinoz districts), act as intermediate hosts in the species of *D. pulex* and *D.magna* **(Sultanov, 1963)**. Moreover, **Hamann (1891)** detected the presence of nematode larvae in small crustaceans of *Daphnia pulex* in the pond water in which ducks, infected with *Echinuria*, are found. The pevious author managed to infect *Daphnia* by means of the excrement of a duck. His observations were confirmed by **Wolfugel (1900)**. However, given that parasites cause significant damages to ducks, its prevention is highly recommended **(Patricia *et al.*, 2020; Thierry *et al.*, 2004)**.

The present research was organized to study the biology of *Daphnia* as an intermediate host for the nematode *Echinuria uncinata* under the conditions of the Fergana Valley and assess the development of nematode *Echinuria uncinata* in *Daphnia*.

MATERIALS AND METHODS

The research materials were collected from Lake Sariqamish in the Fergana Valley (Fig. 1A). Specimens were collected in the spring (March-May) and autumn (late of August - early of November) of 2019-2020 (Fig. 1B). Zooplankton specimens were collected using hydrobiological methods from the lake shoreline and its adjacent pond areas. *Daphina* species were separated and identified 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 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 study 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 transformed into a liquid state. Then, the presence of eggs was examined using a binocular microscope. The eggs that sank at 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*.

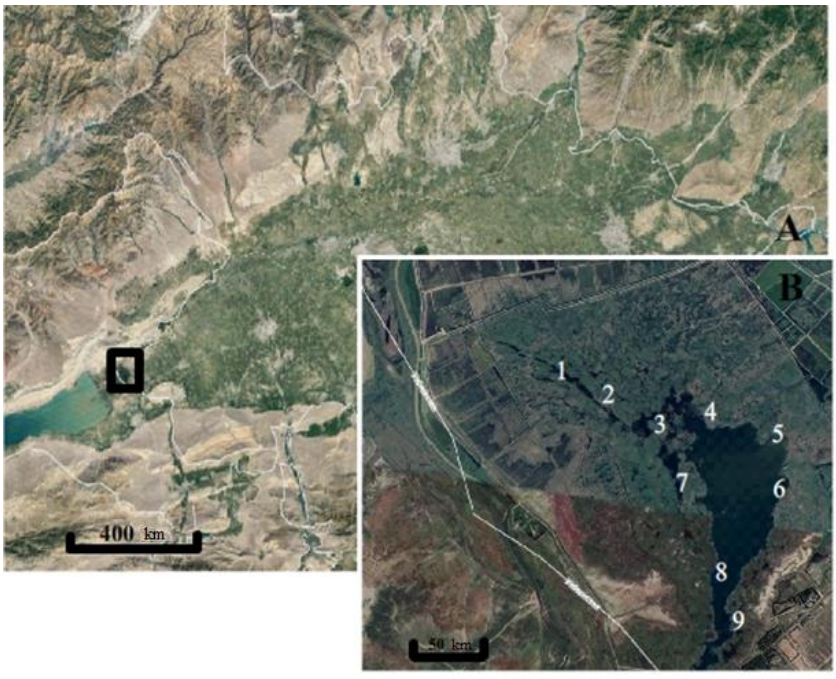

Fig. 1. A map of the Fergana Valley
(A). The areas where specimens were collected from Lake Sariqamish (B) are shown in figures.
*The original map was taken from the Google Earth portal (https://earth.google.com/ . It was processed in Macromedia Flash program.

Specimens of *Daphnia* and nematode larvae were subjected to morphological studies using a microscopic glass slide. A mixture of glycerin and water was dripped on the slide under study; a cover glass was placed, and the edges were framed with attic varnish. In the study, the photographs were taken by a microscope camera of ToupCam company, using a MEIJI Microscope (Japan). The species composition of *Daphnia* was determined following descriptions of **Tsalolikhin (1995)**. Helminthological determinants and literature were used to identify eggs and larvae in nematodes **(Skrjabin *et al.*, 1965)**.

RESULTS AND DISCUSSION

Studies have shown that the *Daphnia magna* species, which belongs to the Daphniidae 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* (with ratio 1:12 for *Daphnia magna*).

Helminthological examination of *Daphnia* infestation with parasitic nematodes revealed that, 23 out of 200 specimens (11.5%) had eggs and larvae of the nematode. Intensity of invasion (II) of *Daphnia* with nematode larvae was 2–6 copies.

The egg of *Echinuria uncinata* is in an oval form, 0.037-0.039 mm long, 0.021-0.023 mm wide (Fig. 1a). The larvae are whitish brown, the head and tail are flattened, the head is blunt, the tip is bulging (Fig. 1b). The tail 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 begins 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

Dimension	(*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.

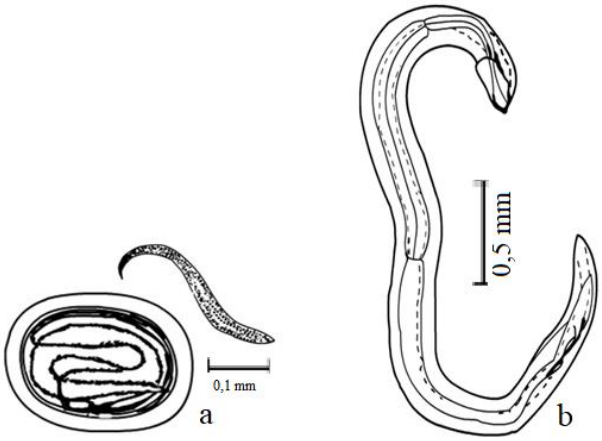

Fig. 2. *Echinuria uncinata*: a) eggs and larvae; b) invasive larvae.

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 is Echinuriinae (Raillet, Henry & Sisoff, 1912),of the family Acuariidae (Railliet, Henry & Sisoff, 1912), order is Spirurida (Chitwood, 1933).

For the experimental infection of *Daphnia* with invasive larvae of nematode *Echinuria uncinata*, daphnia was given the excrements of ducks and geese infested with nematodes. Eggs were sprinkled with yeast as feed for *Daphnia* in case of variation in water temperature (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

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

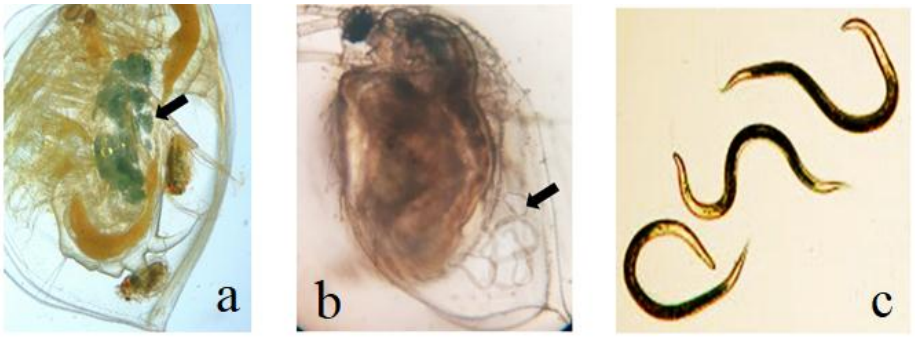

Fig. 3. Development of *Echinuria uncinata* in *Daphnia* a) eggs in the intestine (20^x); b) larvae in the body cavity (20^x); c) appearance of isolated larvae (40^x)

In experiment №1, 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 experiment №2 (20–22°C), parasites developed over 4–5 days, and larvae hatched from nematode eggs in the *Daphnia* gut and entered the body cavity. On days 6–7, the second stage was 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 infect the main hosts – ducks and geese.

In experiment №3, conducted under the temperature 26–28°C, *E. uncinata* developed rapidly, reaching the first stage in 3–4 days, and on the 6th–7th day, they reached 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 are in consistent with those of **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 those eggs along with tiny organic matters, micronutrients and bacteria. Initially, the eggs fall into the *Daphnia'*s gut, where they develop and undergo the first stage. It then, passes from the intestinal wall into the body cavity. There, it develops until a mature invasive larva is formed. Ducks and geese that consume the infected *Daphnia* along with the water infected with the *E. uncinata* larva. Thus, *E.uncinata* passes the lifespan. It has not been studied yet how long the eggs can survive **(Work *et al.*, 2004; Ponomarenko, 2014)**.

Daphnia specimens collected from the study areas were infested with an average of 15% *E.uncinata* larvae. They are mainly found on the shores of lakes, in areas where waterfowls are dense. Damage is especially high in small pools and lake ditches. These data suggest that along with wild waterfowls, there is also a transmission to farm birds. Among the *Daphnia*, *D.magna* is 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 are identified in the studies of **Austin (1970)** and **Ponomarenko (2014)**. In comparison, the current observations under laboratory conditions differed slightly from the results of the afore- mentioned studies because larvae developed as invasive on days 9–10 at 20–22°C.

However, the present 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 developmental period of larvae was prolonged, extending from 11-16 days to even 20-24 days.

In Uzbekistan, *E. uncinata* was found with adult representatives of the species in the glandular stomach of a grey duck by **Sultanov (1963)**. In Tajikistan, it has been studied by **Borgarenko (1990)** in ducks and geese as the main host. However, no studies have been conducted on the intermediate host of this nematode - the *Daphnia* species.

CONCLUSION

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 investigated.

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