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# LOCUSTS (ORTHOPTERA: ACRIDIDAE) AS INTERMEDIATE HOSTS OF NEMATODES *APROCTA CYLINDRICA* AND *DIPLOTRIAENA ISABELLINA* (FILARIINA: APROCTIDAE, DIPLOTRIAENIDAE) IN UZBEKISTAN

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**ABSTRACT:** The main goal of the article is to study locusts as harmful insects considered to be dangerous intermediate hosts of nematodes. According to the authors, nematodes' harm lies in the fact that they are parasites causing some bad diseases in birds around the world, including Uzbekistan. In this article we determined scientifically the biological characteristics of the life cycle of nematode worms *Aprocta,* parasites of birds. These parasites use 3 species of locusts (Orthoptera) as their intermediate hosts.

***Keywords*:** Orthoptera, Locusts, Nematodes, Quelea queiea, Uzbekistan

## INTRODUCTION

Nematodes of the genus *Aprocta* Linstow, 1883 and *Diplotriaena* Henry et Ozoux, 1909 are common parasites of birds for a number of regions around the world (Sonin, 1966, 1968; Barus et al., 1978, Andersen, 2000; Saparov et al., 2013).

The genus *Aprocta* comprises nematodes parasitising eye sockets and nasal and other cavities in the bodies of birds from various ecological groups. Currently, 30 species are identified to comprise the genus.

Information on the biology of *Aprocta cylindrica* Linslow, 1883 is provided in the scientific research by Quentin et al (1976), who infected locusts *Locusta migratoria* (L) experimentally with nematode eggs taken from weaver birds Plocudae (*Quelea queiea*, *Ploceius capitalis*, *P. cucullatus* and *Euplectus oriz*) and established that the developing larvae lived in the locusts' hemocoel and that they became infective in 15 days, occupying the whole body of the insect. We believe that these are the only data on this species' biology.

The genus *Diplotiaena* (Henry, Ozoux, 1909) comprises about 80 species of the world's nematode fauna, whose representative's parasitise in the air sacs, lungs and body cavities of birds from various orders, in particular Passeriformes.

The life cycle of *Diplotriaena isabellina* Koroliowa, 1926 was studied by Anderson (1962: quoted from Sonin (1968)). Anderson established that the nematodes' main intermediate hosts are locusts (*Melanoplus bilituratus*, *Melanoplus fusciatus* and *Camnula pellucida*). Locust *Bryodema tubenculatum* was determined as the intermediate host of *Diplotriaena isabellina* in the foothills of Tashkent region, Uzbekistan (Kabilov, 1983). The rate of infection with the larvae of this Filariata is 3.9% (Kabilov, 1983).

The aim of our research is to study the life cycles of nematodes *A. cylindrica* and *D. isabellina*, parasites of birds in Uzbekistan.

### *Materials and methods*

The locusts (Acrididae) we collected between 2012 and 2016 in the places of birds' concentrations in Tashkent, Syrdarya, Jizzak, Kashkadarya and Bukhara provinces and in the Republic of Karakalpakstan were used as materials for this research. In various seasons (spring, summer and autumn) we collected and studied a total of 15,342

specimens of Acrididae in accordance with the common method suggested by Nelson (1960), Kabilov (1983), Bekuzin (1993), Lachininsky et al (2002) and Khamraev et al (2008).

We put the discovered nematode larvae in 1.5-2.0-per cent formalin. We placed most of the nematode larvae in drops of physiological solution on a slide and made them immobile by warming it slightly with the help of a spirit lamp, after which we covered them with a coverslip and studied under a microscope. We infected sterile birds with larvae taken from the bodies of the locusts. In the experiment we used 5 nestling individuals of the house sparrow *Passer domesticus*. Three bird individuals were infected with larvae of *Aprocta cylindrica* (34-37 individuals for each), and 2 nestlings served as reference samples. We infected 9 nestlings of the partridge, quail and mynah (3 from each species) with larvae of *Diplotriaena isabellina.* Each of the tested birds was infected with 33-45 larvae individuals. One (1) nestling of each species was used as a reference sample. The research was carried with the use of new up-to-date devices: inverted microscope SK-2TR (Olympus Japan), binocular ML-200 (Olympus, Japan).

***Results and discussion***

While collecting the biological materials within several regions and the Republic of Karakalpakstan we found nematode larvae in various phases of their development in the bodies of locusts (Acrididae). Among them we discovered some infective larvae from the 3rd phase. The larvae were found in the following species of locusts: *Calliptomus turanicus* (Tarbinskiy, 1930), *Dociostaurus kraussi* (Ingenizkij, 1897), *Locusta migratoriya* (Linnalus, 1758), with an infection intensity of 3-11 individuals (Table 1). The larvae occupied freely the fat bodies of locusts, their intermediate hosts. The infective larvae had cylindrical bodies, 0.462-0.508 mm long and 0.040-0.046 mm wide.

Table 1. Rate of infection with larvae *Aprocta cylindrical* in locusts in the natural conditions of Uzbekistan

| Locusts species | Number of examined locust specimens | Infected | | |
|---|---|---|---|---|
| | | Number of specimens | Prevalence, % | Infection intensity |
| Calliptamus turanicus | 1050 | 26 | 2.2 | 3-7 |
| C. sephalotes | 656 | - | - | - |
| Dociostaurus kraussi | 1610 | 65 | 3.8 | 5-11 |
| Locusta migratoria | 725 | 16 | 2.2 | 6-9 |
| | 4041 | 117 | 2.6 | 3-11 |

The body of the larvae narrows towards the end. The nerve ring is situated at a distance of 0.052 mm, and the excretory aperture – 0.072-0.078 mm from the head. The oesophagus is 0.410-0.520 mm long. The cuticle has crosswise cuts. The body of the larva, particularly the intestine, is heavily filled with granular pieces. (Fig. 1).

The morphology of the studied larvae is very much like that of the larvae of *Aprocta cylindrica* from locusts (*Locusta migratoria*) described by Quentin et al (1976).

The experimental nestlings of the house sparrows *Passer domesticus* (Linnaeus, 1758) were fed with infective larvae from each locust species. The experimental infection of the nestlings was carried out in July 2015.

The nestlings were dissected in 25, 35 and 45 days after being infected with the purpose of studying the development of the infective larvae up to the mature nematode forms in the organs of the definitive host. (Table 2)

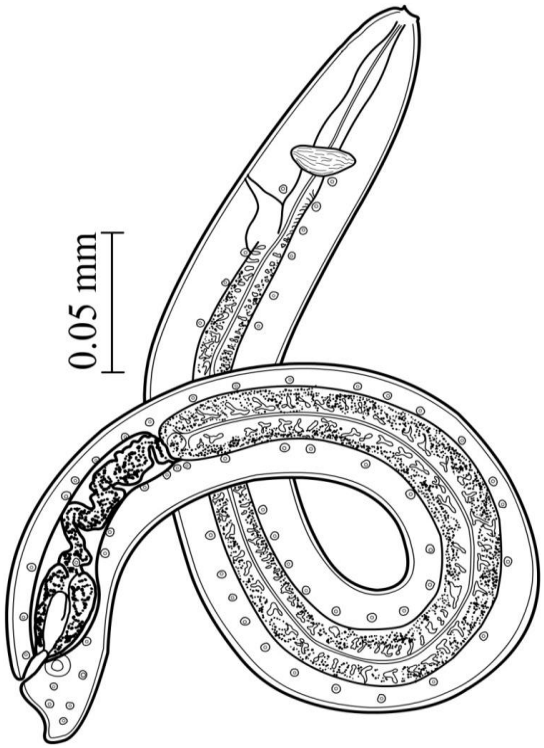


Figure 1. *Aprocta cylindrica* Linstow, 1883. Larvae from locusts *Calliptomus turanicus* Tarb: infective larva in the 3rd phase.

The results of the research showed that in 25-35 days after the infection developing juvenile forms of nematodes, both males and females with distinct sexual organs, were recorded in the nasal cavities of nestling Nos. 1 and 2. On the 45th day mature nematodes were discovered in the nasal cavity of nestling No. 3: 5 males and 11 females. The reference birds (Nos. 4 and 5) did not contain any nematodes.

Table 2. Experimental infection of birds with infective larvae of nematodes *Aprocta cylindrica* Linstow, 1883, taken from the locusts of Uzbekistan

| Nestlings | Calliptamus turanicus | Dociostaurus kraussii | Locusta migratoria | Research duration (Number of days after the infection) |
|---|---|---|---|---|
| | Number of larvae | | | |
| Passer dom.: | | | | |
| No. 1 | 34 | - | - | 25 |
| No. 2 | - | 36 | - | 35 |
| No. 3 | - | - | 37 | 45 |
| Nos. 4 and 5 | Reference samples | | | 45 |

The morphological parameters of the males and females completely corresponded with the characteristics of mature worms *Aprocta cylindrica* we had discovered in a number of sparrow species and with data from scientific literature (Sonin, 1966; Borgarenko, 1990). In addition, we also observed a considerable number of eggs with thick shells. The size of the eggs ranged between 0.042 mm and 0.056 mm in length and 0.28 mm and 0.036 mm in width.

Thus, the development of larvae in the organs of a vertebrate host is characterised by further increase of the body's length and width, with the growth speed in this phase different in males and female. The reproductive systems in both sexes develop actively at this stage. On the 45th day after the infection practically all the organs in the studied Filariata proved to be fully developed. Probably, this is the time when they begin coupling. This is confirmed by the eggs found within the nasal cavity and surrounding tissues in the infected birds.

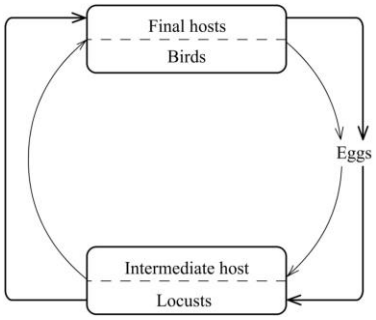


Figure 2. Life cycle of *Aprocta cylindrica* Linstow, 1883

At a temperature of 25-32°C the larvae of *Aprocta cylinrica* in the organs of the intermediate host *Locusta migratoria* become infective on the 13th-15th day after the swallowing of the egg. Nematodes in the experimental birds became sexually mature on the 45th day after the birds had been fed with their larvae. (Fig. 2).

While studying the locusts of Uzbekistan we discovered the larvae of *D. isabellina* in various phases of development in the following species: *Bryodema tuberculatum* (Fabricius, 1775), *Aiolopus oxianus* (Uvarov, 1926) *Melanoplus frigidus* (Boheman, 1846) and *Calliptamus italicus* (Lunnaeus, 1958). The recorded invasion intensity ranged from 1 to 7 specimens in different locust species (Table 3). Infective larvae were found in the fat bodies enveloped in thin, transparent substance (Fig. 3)

Table 3. Natural rate of infection with larvae *Diplotriaena isabellina* in locusts

| Locust Species | No. of examined specimens | Infected | | |
|---|---|---|---|---|
| | | Number of specimens | Prevalence, % | Infection intensity |
| *Aiolopus oxianus* | 1430 | 35 | 2.3 | 2-5 |
| *Bryodema tuberculatum* | 1437 | 45 | 3.1 | 3-7 |
| *Calliptamus italicus* | 1410 | 30 | 2.1 | 1-5 |
| *C. turanicus* | 1355 | - | - | - |
| *C. barbarous* | 1360 | - | - | - |
| *Locusta migratoria* | 1500 | - | - | - |
| *Dociostaurus maroccanus* | 1470 | - | - | - |
| *Melanoplus frigidis* | 1380 | 56 | 4.0 | 3-7 |

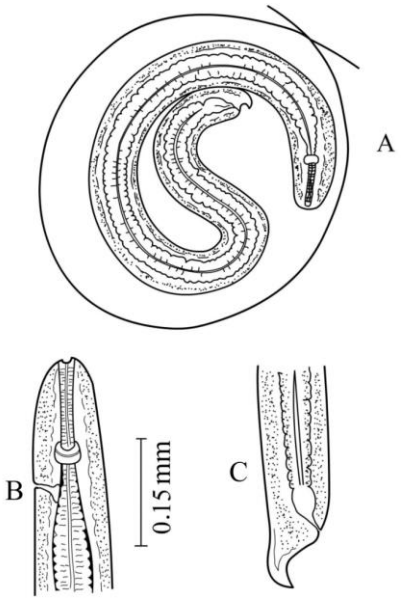


Fig. 3.*Diplotriaena isabellina* Korolova, 1962. Larvae from *Calliptamus italicus* (L.), infective larva in the 3rd phase: A - General view of the larva in a cyst. B - head end. C - tail end.

The body of the infective larva is cylindrical. The length is 0.528-0.641 mm, with a maximum width of 0.042-0.048 mm. The body narrows abruptly towards the head and tail. The distance from the head to the nerve ring is 0.057-0.060 mm, to the excretory hole – 0.076-0.080 mm. The oesophagus consists of two sections. The muscular section of the oesophagus is 0.074-0.076 mm long, the glandular part is 0.460-0.464 mm. The tip of the tail is situated 0.038-0.040 mm from the anus.

The cuticle is crossed with thin cuts. The body of the larva is filled with granular pieces (Fig. 3). Morphometrically the studied larvae are very similar to larvae of *D. isabellina*, recorded in *B. tuberculatum* (Kabilov, 1983).

After studying carefully the infective larvae we carried out a series of experiments to establish the infection rate in the nestlings of the quail, partridge and mynah (Table 4).

Table 4. The results of the experimental infection of birds with infective larvae of *Diplotriaena isabellina*

| Bird species[1] | | Number of larvae specimens | Number of days after the infection |
|---|---|---|---|
| *Coturnix coturnix* | №1 | 33 | 35 |
| | №2 | 36 | 40 |
| | №3 | 45 | 50 |
| | №4 | Reference samples | 55 |
| *Alectoris chucar* | №1 | 35 | 40 |
| | №2 | 45 | 55 |
| | №3 | 50 | 65 |
| | №4 | Reference samples | 65 |
| *Acridotheres tristis* | №1 | 30 | 45 |
| | №2 | 33 | 55 |
| | №3 | 35 | 65 |
| | №4 | Reference samples | 65 |

When we dissected quail No. 1, partridge No. 1 and mynah No. 1 35 days, 40 days and 40 days after the infection, respectively, we found juvenile forms of Filariata males and females. The nematodes were observed in the lumina of the lungs and air sacs. In 45-55 days the nematodes were also discovered in the other experimental birds, in the lumina of the air sacs. The male and female reproductive organs were clearly seen, but not quite mature. Among the nematodes there were single specimens, both males and females, the size of which corresponded with that of mature parasites. The length of the males was 19-20 mm, the females – 28-35 mm. Most of the parasites in the dissected experimental birds (partridge №3 and mynah №3) were mature. The air sacs of the mynah contained 8♂ and 13♀, and that of the partridge – 7♂ и 11♀.

Thus, we observed a complete development of males and females of *D. isabellina* in the bodies of the partridge, quail and mynah. The morphology of mature nematodes *D. isabellina* found in the artificially infected birds conformed with earlier data (Sonin, 1968; Bargorenko, 1990.).

Overall, the biological life cycle of the nematode under study corresponds to earlier data (Anderson, 1962), with the only difference that we recorded three locust species, *Aiolopus oxianus*, *Calliptamus italicus* and *Melanoplus frigidis* as *D. isabellina*'s intermediate hosts. In our experiments the parasites developed in their definitive hosts much faster than in the experiments by Anderson (1962). Probably, this is because of the experimental conditions and different susceptibility in different bird species. Probably, the other species of the genus that concentrate in the organs connected with the external environment (air sacs, eye sockets and nasal cavities) will have a similar biological cycle scheme. This viewpoint is confirmed by data from a number of other researchers (Bain, Voucher, 1973; Cawthorn, Anderson, 1980), who registered locusts (*Camnula pellucida, Melanoplus bilituratus, M. fusciatus, M. sanguinipes, Locusta migratoria, Schistocerca gregaria*) as the intermediate hosts of some nematode species from the genus *Diplotiaena* – *D. agelaeus* (Walton, 1927), *D. tricuspis* (Fedtshenko, 1874), *D.tridens* (Molim, 1858) and *D. bargusinica* (Skryabin, 1927).

Generalising the results of our research on the life cycles of *Aprocta cylindrica* and *Diplotriaena isabellina* and literary data (Anderson, 1962, 2000; Bain, Vausher, 1973; Quentin and others, 1976; Cauthorn, Anderson, 1980; Kabilov 1983) we would like to note that these nematodes live in the air sacs and nasal cavities in a number of bird species from various ecological groups, mainly in sparrows. Females lay eggs containing larvae. The eggs get into the bird's digestive tract via the respiratory tube and then go out into the external environment with excrement. The intermediate hosts (locusts) swallow the eggs together with food. The larvae that emerge from the eggs migrate and

penetrate into the hemocoel and fat body. There they develop across two ecdysis phases into the infective stage. Birds become infected through eating locusts containing infective larvae.

As we were collecting locusts from natural biotopes in July and August 2015-2016, we noted unusual behavior in some individuals of the locust species *Calliptamus turanicus, C. barbarous; Dociostaurus morocanus* inhabiting the areas in the lower course of the Amudarya (Kungrad and Muynak districts of the Republic of Karakalpakstan). Some individuals looked inactive on the stems and branches of plants or on the ground. Their inactivity was accompanied by slow reaction to threat (they did not escape) – they could be easily caught by hand. In the meanwhile, most of the insects were quite active and we could hardly catch them with the help of a butterfly net. Further research revealed that the less active individuals were infected with nematode larvae.

The infected locusts usually became easy preys of birds and other predators. This phenomenon requires additional research. Here it should only be noted that the intensively infected locust individuals have subdued or no reaction to threat, although other reactions, such as the reaction to touch, remain unchanged.

In some cases, alongside a debilitated organism, an infected animal demonstrates specific changes in behaviour (Ginetsinskaya, Dobrovolsky, 1978; Alexeyev, 1993; Kulmamatov et al., 1994). The deviation from the normal behaviour places the organism apart from the rest of the population and makes it an easier prey for predators. This situation increases the possibility of taking the infected Orthoptera by their definitive host.

The described locust species belong to common plant pests and inhabit all the studied regions of Uzbekistan and the Republic of Karakalpakstan (Khamraevet al.,2008), on the one hand, and act as the intermediate hosts of nematodes – parasites of wild and game birds, on the other hand.

Thus, we conclude that Orthoptera, their intermediate hosts, play an important role in the life cycle of the studied nematode species, as they inhabit grass-covered areas and are eaten by the nematodes' definitive hosts – birds from various ecological groups.

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