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OCCURRENCE OF SKRJABILLANID NEMATODES IN FISHES OF HUNGARY AND IN THE INTERMEDIATE HOST, *ARGULUS FOLIACEUS* L.

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Histozoic and coelozoic skrjabillanid-type nematodes belonging to the genera *Skrjabillanus, Molnaria, Sinoichthyonema, Esocinema, Daniconema* and *Lucionema* are recorded from the subcutaneous tissues, fins, swimbladder and abdominal cavity of different fish species living in natural waters and fish farms of Hungary. In addition to the nine taxonomically identified parasite species, one *Skrjabillanus* sp., two *Molnaria* spp. and three *Esocinema* spp. were identified to the genus level only. The histozoic larval stages of a *Molnaria, Daniconema* and *Lucionema* species each, living in a site different from that of the imagoes, were also detected. The presence of closely not identified first- to third-stage skrjabillanid larvae was demonstrated in 26.3% of the parasitic carp lice (*Argulus foliaceus* L.) collected from the eight fish species.

Key words: Nematoda, Skrjabillanidae, Lucionematidae, Daniconematidae, larval stages, fish hosts, *Argulus foliaceus*, intermediate host

Since the first skrjabillanid nematode, *Skrjabillanus tincae* was described by Shigin and Shigina in 1958, the number of known species belonging to that category has increased substantially. In his monograph, Moravec (1994) reported seven species of four genera (*Esocinema, Molnaria, Sinoichthyonema, Skrjabillanus*) of the Skrjabillanidae family from Europe. *Skrjabillanus cyprini* recently described from the common carp (Molnár and Moravec, 1997) is a further addition to this group. Although *Daniconema anguillae* Moravec et Køie, 1987 assigned to the Daniconematidae family and the most recently detected *Lucionema balatonense* Moravec, Molnár et Székely, 1998 classified into the Lucionematidae family cannot be considered skrjabillanid nematodes from the morphological and taxonomic point of view, they show many similarities to skrjabillanids in their development and site in the host and, therefore, can be described with the adjective 'skrjabillanid' in the broader sense. Relatively few data are available in the literature on the occurrence of these parasites. However, from the results re-

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ported by the few specialists dealing with the subject (Shigin and Shigina, 1958; Molnár, 1966; Mészáros, 1968; Molnár, 1970; Tikhomirova, 1970; Moravec, 1971; Garkavi, 1972; Wierzbicka and Wierzbicki, 1973; Tikhomirova and Rudometova, 1975; Moravec, 1977; Tikhomirova, 1980; Moravec and Køie, 1987; Molnár and Moravec, 1997; Moravec et al., 1998) it seems likely that these parasites are very common and often remain unnoticed only because of the inadequate examination techniques used.

The extrapiscine development of skrjabillanid nematodes was first studied by Rudometova (1974, 1975) and Tikhomirova (1975, 1980). These authors reported that the second- and third-stage larvae of *Skrjabillanus* and *Sinoichthyonema* species developed in the species *Argulus foliaceus* L. and *A. coregoni* Thorell (Branchiura) as intermediate hosts.

Since the first description of *Skrjabillanus scardinii* Molnár, 1966 and *Molnaria intestinalis* (Dogiel et Bychowsky, 1934) (Molnár, 1966), of the dracunculoids of extraintestinal development only the species *Skrjabillanus schigini, Sinoichthyonema amuri* and *Daniconema anguillae* have been detected in Hungary (Molnár, 1989; Molnár and Moravec, 1994; Molnár, 1997), apart from the occurrence of *Anguillicola crassus* and *Philometra* spp. having a different type of development. Recently, however, two new parasite species, *Skrjabillanus cyprini* Molnár et Moravec, 1997 and *Lucionema balatonense* Moravec et al., 1998 have also been recorded (Molnár and Moravec, 1997; Moravec et al., 1998).

The objective of this paper is to demonstrate that skrjabillanids are much more prevalent in Hungarian fishes than is reflected by data of the literature, and this high prevalence manifests itself also in the intensive infection of the intermediate host *Argulus foliaceus*.

Materials and methods

The fish species included in the survey originated from different natural waters of Hungary, first of all from Lake Balaton, from the Kis-Balaton Water Reservoir and from backwaters of the River Körös. In the case of common carp (*Cyprinus carpio*) and grasscarp (*Ctenopharyngodon idella*), numerous fish obtained from fish farms were also dissected. Carp lice (*Argulus foliaceus*) were collected from fish originating from Lake Balaton, Kis-Balaton, and fish farms. After catching them, the fish were separated by species and transported to the laboratory for examination in water supplied with oxygen, in plastic bags. The investigations have been conducted continuously for several years, but the data reported in this paper include only the results obtained between July 1995 and November 1997, i.e. in a period when the technique suitable for the detection of skrjabillanids was consistently applied during the fish dissections.

In the 2.5-year period of study, a total of 656 specimens of 17 fish species were examined for the occurrence of skrjabillanid-type nematodes. The abdominal cavity and the swimbladders were examined for infection in all fish specimens, while the scales and the subcutaneous connective tissue were checked only occasionally.

After killing the fish, the fins, scales and gills were removed and placed into 0.6% physiological saline in a Petri dish. The abdominal cavity was opened, the inner organs were placed into saline, and the cavity was rinsed with the same solution. In the first step, the still moving nematodes washed out from the abdominal cavity were collected in a Petri dish. The parasites located on or under the abdominal serous membranes were released without hurting the gut, by carefully separating the abdominal organs. The serosa was peeled off the swimbladder. After inspecting the viscera, the skin was peeled off the fish, placed into saline in a tumbler, then after isolation for 1–2 h the nematodes released from the subcutaneous connective tissue were collected. To collect the fin-parasitic worms, the folds of the cut-off fins were torn into two halves in their entire length, starting from the base. From the scales, the skin-like layer covering the inner surface was removed. Attempts were made to complete the examinations within 3 h of killing the fish, as the otherwise agile movement of the worms in the tissues and in the solution gradually ceased over time, in which case the hair's-breadth thin, small-sized worms became unrecognisable even in intensive infection. The collected nematodes were either placed directly into lactophenol and fixed as slide preparations, or were conserved in 70% alcohol.

A systematic survey of the occurrence of skrjabillanid larvae in carp lice was carried out only in 1997, when 585 *Argulus* specimens collected from 8 fish species were examined for infection.

The *Argulus* specimens were collected from the fish before the fish were placed into flow-through water. The specimens that had fallen off the fish during transportation were also collected from the water of the plastic bags. The still living, transparent *A. foliaceus* specimens were examined under a coverslip at 100- to 200-fold magnifications of the microscope, and the larvae moving in them were counted and recorded. The larvae present in less transparent crustacean specimens containing eggs or spermatozoa were counted after partial destruction of the crustacean's body by strong pressure exerted on the coverslip. Only a certain proportion of the larvae released from *Argulus* were fixed. A proportion of the nematode larvae found in the carp lice were recorded on videotape, then the video images were transformed into digitalised pictures (Székely, 1997).

The species distribution and the number of fish and crustaceans examined for skrjabillanid infection and the results obtained are presented in Tables 1 and 2.

Table 1

Occurrence of skrjabillanid nematodes in Hungary

Parasite species	Host	Period of examination	Locality	Site of parasite	No. of fish examined	No. of fish infected	Range of intensity (mean int.)
Molnaria intestinalis	*Scardinius erythrophthalmus*	1995–1997	LB, KB, FF	A. c.	30	24	1–8 (3)
Molnaria sp.	*Aspius aspius*	1995–1997	LB, KB	A. c., sb	20	12	1–21 (6)
Molnaria sp.	*Pelecus cultratus*	1996–1997	LB	A. c.	27	8	1–7 (4)
Molnaria sp. larva	*Pelecus cultratus*	1996–1997	LB	Fins	27	18	8–100 (47)
Sinoichthyonema amuri	*Ctenopharyngodon idella*	1995	FF	A. c.	10	10	6–9 (8)
Skrjabillanus scardinii	*Scardinius erythrophthalmus*	1995–1997	LB, KB, FF	Sb, skin	30	20	1–6
Skrjabillanus cyprini	*Cyprinus carpio*	1996–1997	LB, KB, FF	Scales	14	7	1–8 (3)
Skrjabillanus schigini	*Ctenopharyngodon idella*	1995	FF	Sb	10	10	1–7 (4)
Skrjabillanus tincae	*Tinca tinca*	1997	KB	Sb	2	2	1–3
Skrjabillanus sp.	*Abramis brama*	1996	LB	Scale	28	1	1
Lucionema balatonense[*]	*Stizostedion lucioperca*	1995–1997	LB	Sb	160	3	5–30 (23)
Lucionema balatonense larva[*]	*Stizostedion lucioperca,*	1995–1997	LB	Sb	160	5	1–32 (25)
	S. volgense	1996	LB	Sb	24	2	1–1
Daniconema anguillae[**]	*Anguilla anguilla*	1995–1997	LB	Fins, sb	82	6	1–2
Daniconema anguillae larva[**]	*Anguilla anguilla*	1995–1997	LB	Fins, sb	82	45	10–500
Esocinema bohemica	*Esox lucius*	1997	KB	Sb	7	2	1–2
Esocinema sp.	*Aspius aspius*	1996–1997	LB, KB	Sb, skin	20	13	1–9 (4)
Esocinema sp.	*Pelecus cultratus*	1996–1997	LB	Sb, skin	27	13	1–7 (3)
Esocinema sp.	*Abramis brama*	1997	LB	Skin	4	1	1

LB = Lake Balaton; KB = Kis-Balaton Water reservoir; FF = Fish Farm (Biatorbágy); A. c. = abdominal cavity; sb = swimbladder; [*]Classified into the Lucionematidae family; [**]Classified into the Daniconematidae family

Table 2

Infection of *Argulus foliaceus* specimens collected from different fish species with the larvae of skrjabillanid nematodes

Fish species	Collection		No. of carp lice		Prevalence of infection, %	Intensity of infection, ranges (mean)
	Place	Date	examined	infected		
Cyprinus carpio	Kis-Balaton	23.08.96	6	6	100	1–8 (4)
	Kis-Balaton	03.09	6	6	100	1–3 (2)
	Kis-Balaton	04.09	10	7	70	1–4 (2)
	Kis-Balaton	07.08.97	18	7	39	1–30 (7)
	Kis-Balaton	10.10	76	18	24	1–5 (3)
	Hortobágy	16.10	67	16	24	1–7 (4)
	Biatorbágy	21.10	23	4	17	1–3 (2)
	Biatorbágy	29.10	62	10	16	1–8 (3)
Aspius aspius	Balaton	26.09.96	1	1	–	5
	Kis-Balaton	07.08.97	22	4	18	1–2 (1.7)
	Kis-Balaton	10.10	12	4	17	1–7 (2.7)
	Biatorbágy	29.10	12	2	–	2–9
Aristichthys nobilis	Biatorbágy	29.10	9	2	22	2–2
Abramis brama	Balaton	02.10	1	0	–	
	Kis-Balaton	10.10	6	1	–	2
Ctenopharyngodon idella	Kis-Balaton	10.10	2	2	–	2–4
	Biatorbágy	29.10	14	5	36	1–3 (2)
Tinca tinca	Kis-Balaton	23.08.96	3	2	–	1–2
Stizostedion lucioperca	Kis-Balaton	21.07	8	3	–	1–2
	Kis-Balaton	03.09	3	1	–	3
	Kis-Balaton	26.09	1	1	–	2
	Kis-Balaton	07.08.97	30	4	13	1–2
	Balaton	02.10	3	0	–	
	Kis-Balaton	10.10	66	13	20	1–4 (1.7)
	Kis-Balaton	20.10	4	2	–	3–5
	Biatorbágy	21.10	12	4	33	1–2
	Biatorbágy	29.10	36	8	22	1–13 (2.6)
Esox lucius	Biatorbágy	21.10	3	1	–	1
	Biatorbágy	29.10	50	14	28	1–18 (4.6)
Mixed species	Kis-Balaton	07.08	19	6	32	1–10 (4.5)
Total	–	–	585	154	26.3	–

Results

I. Surveys on fish

Skrjabillanid-type nematodes were detected from 11 fish species (Table 1). Of the fish species included in the survey with at least 10 specimens examined, 252 specimens of 10 species proved to be free of skrjabillanid infection: *Alburnus alburnus* (30), *Barbus barbus* (17), *Blicca bjoerkna* (30), *Carassius auratus gibelio* (62), *Rutilus rutilus* (11), *Aristichthys nobilis* (14), *Hypophthalmichthys molitrix* (14), *Silurus glanis* (16), *Perca fluviatilis* (16), *Gymnocephalus cernuus* (42).

As regards the skrjabillanid species detected, the results presented in the table must be complemented with the following data:

(1) *Skrjabillanus cyprini* Molnár et Moravec, 1997. This parasite occurred in common carp (*Cyprinus carpio*) in natural waters and fish farms alike. Its typical site is the subsquamal space between the cartilaginous plate of the scales and the thin epithelial layer covering them on the inside. Less commonly the parasite was found also under the scales of the mirror carp, a species having only a few scales.

(2) Numerous breams (*Abramis brama*) were examined, but only a single *Skrjabillanus* female was found on one occasion. That specimen was found subsquamally, in the same location as *S. cyprini*, and resembled the latter also in shape and size; however, its fixation was unsuccessful. In the abdominal cavity and swimbladder of the breams examined there was 100% prevalence of infection with another dracunculoid nematode, *Philometra ovata* (Zeder, 1803). Mostly specimens of arrested development were found, and mature nematodes were only rarely detected.

(3) In several years old specimens of the rudd (*Scardinius erythrophthalmus*), *Molnaria intestinalis* (Dogiel et Bychowsky, 1934) and *Skrjabillanus scardinii* Molnár, 1966 occurred almost always as concurrent infection. At the same time, in 2–3 years old fish usually the specimens of only one of these parasite species could be detected.

(4) In the grasscarp (*Ctenopharyngodon idella*), the species most closely related to the rudd, the occurrence of also two nematode species, *Skrjabillanus schigini* Tikhomirova et Rudometova, 1975 and *Sinoichthyonema amuri* (Garkavi, 1972) was commonly recorded.

(5) The tench (*Tinca tinca*) was found to be parasitised by *S. tincae* Schigin et Schigina, 1958, the type species of the *Skrjabillanus* genus. It has been detected in Hungary for the first time in this survey.

(6) In asp (*Aspius aspius*) specimens exceeding 30 cm in size, the occurrence of three dracunculoids was commonly recorded. Specimens of *Philometra*

kotlani (Molnár, 1969) and a hitherto not closely identified *Molnaria* sp. were regularly found in the abdominal cavity. In the swimbladder serosa and subcutaneous connective tissue of the same fish species specimens of an *Esocinema* sp. occurred. Occasionally all three parasite species were simultaneously present; however, in the majority of cases specimens of the *Molnaria* sp. could not be detected in asps infected by *Philometra kotlani.*

(7) In the chekhon (*Pelecus cultratus*) a *Molnaria* and an *Esocinema* species could be detected in a similar site as in the asp.

(8) In the pikeperch (*Stizostedion lucioperca*), the specimens of *Lucionema balatonense* could be detected in only three cases. These nematodes, which are similar to skrjabillanids, have been described and assigned to the independent Lucionematidae family belonging to the Dracunculoidea superfamily by Moravec, Molnár and Székely (1998). In the three infected pikeperch specimens, however, more than 30 nematodes (all females with only a single male present) were found freely in the swimbladder.

(9) *Esocinema* spp. were detected in four fish species (*Esox lucius, Pelecus cultratus, Aspius aspius, Abramis brama*). Of them, the nematodes found in the pike were identified with the species *Esocinema bohemicum* Moravec, 1977, while those found in cyprinids were determined to the genus level only. In asp and chekhon the worms were detected also in the swimbladder, while in pike and bream they were isolated only from the subcutaneous connective tissue. Such nematodes were not found, however, under the skin of *Blicca bjoerkna, Rutilus rutilus, Leuciscus idus, Cyprinus carpio, Carassius carassius* and *Silurus glanis* specimens examined by a similar technique.

(10) *Daniconema anguillae* Moravec et Køie, 1987 was detected in eels in both abdominal and fin site. Infection of the fins was found to be more common.

II. Infection of fish with skrjabillanid larvae

Infection with skrjabillanid larvae occurred in four fish species, i.e. in the eel, pikeperch, Volga pikeperch, and chekhon.

(1) Infection of the fins of eel with third-stage *Daniconema anguillae* larvae has been reported in detail by Molnár and Moravec (1994). That infection proved to be common also in the present survey, affecting about 40% of the eels examined.

(2) The 360–400 μm long first-stage larvae of *Lucionema balatonense* were present under the serosa of the swimbladder in 5 out of the 160 pikeperch and in 2 out of the 24 Volga pikeperch specimens examined.

(3) Coiled-up nematode larvae were detected in the caudal, anal and dorsal fins of 18 out of the 27 chekhons examined. They were located in the skin fold between the fin rays and less often in the fin rays themselves (Figs 1 and 2).

By their characteristic buccal capsule they could be identified with the third-stage larvae of stagnating development of the *Molnaria* sp. parasitic in the abdominal cavity of chekhon. The prevalence of infection was 66% and its intensity varied between 1 and 450 (mean: 47).

III. Survey of Argulus foliaceus *for infection with nematode larvae (Table 2)*

In Hungary, the infection of *A. foliaceus* with nematode larvae was first observed in 1995, when infection of the swimbladder of pikeperch with *Lucionema balatonense* larvae was first detected. At that time, a young larva with tapered caudal end (Fig. 3) was detected in an *Argulus* specimen found on an infected fish. In that year, 7 out of the 9 *Argulus* specimens collected from pikeperch were found to be infected with larvae. In the subsequent year (1996), 33 out of the 44 *A. foliaceus* specimens collected from pikeperch, common carp, asp and tench at random proved to be infected with skrjabillanid-type larvae.

In the present survey conducted in 1997, infection with nematode larvae was detected in 26.3% of the *Argulus* specimens examined. The highest prevalence of infection was found in carp louse specimens collected from grasscarp. In the majority of cases the intensity of infection was restricted to one or two larvae; however, there were *Argulus* specimens infected with as many as 18 and 30 larvae, respectively.

The larvae occurred freely in the body cavity of the carp louse and were constantly changing their location. Young larvae preferred staying in the suckers but commonly occurred also in the crustacean's legs (Fig. 3), uropods (Fig. 4), and other sites of the body (Fig. 5). In their size and tapered caudal ends (Fig. 3) the youngest larvae resembled the first-stage larvae developing in the body of imago *Skrjabillanus* and *Molnaria* females; however, after the first moulting they lost their tapered tail (Fig. 6 and inset of Fig. 6) and both their head and tail ends became rounded. Larger specimens were detected around the stylet or in its cavity (Figs 7 and 8). By their tapering, slightly bent tail and the primordium of the buccal capsule, some of these advanced larvae resembled the larvae isolated from the fins of chekhon (Fig. 2) and displayed a typical *Skrjabillanus* character.

Discussion

The results of the survey indicate that skrjabillanid nematodes assigned to the order Dracunculoidea are extremely common parasites of Hungarian fishes and can consistently be detected in some fish species if a suitable examination technique is used.

Skrjabillanus cyprini seems to be specific for the common carp, as it could not be found in the closely related gibel carp (*Carassius auratus gibelio*). The detection of a closely not identified specimen from the same site in the bream, however, queries that explicit statement.

According to data obtained over a period of several years, *Skrjabillanus scardinii* and *Molnaria intestinalis* infection can be considered almost universal in the rudd (Molnár, 1989). It is striking, therefore, that no skrjabillanids could be found in *Rutilus rutilus*, another cyprinid belonging to the Leuciscinae subfamily, although the species *Molnaria leucisci* had been described from another member of that subfamily, *Leuciscus idus* (Agapova, 1963).

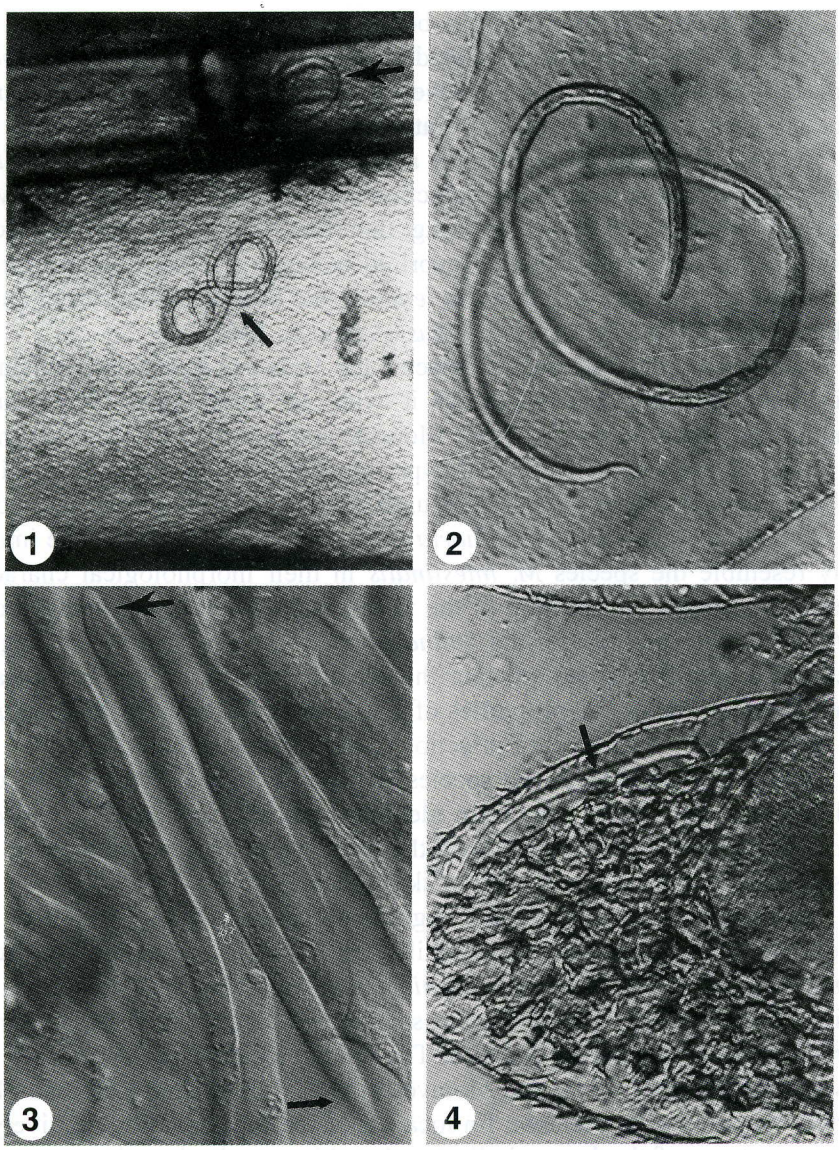
Mészáros (1968) had already detected the *Molnaria* species parasitising the asp in Lake Balaton, and identified it with *M. intestinalis*. Other authors (Tikhomirova, 1980; Boni et al., 1990; Moravec and Scholz, 1991) also claimed to have found *M. intestinalis* in several cyprinids. Despite this fact, the specimens found are regarded only as *Molnaria* sp. until more detailed studies are carried out.

The same applies to the *Molnaria* sp. found in the chekhon. The imagoes greatly resemble the species *M. intestinalis* in their morphological characters; however, the unique fin location of the third-stage larvae, found neither for *M. intestinalis* of the rudd nor for the *Molnaria* sp. of the asp, makes the diagnosis difficult. The question arises whether the specific location of the larvae can be explained by the biological properties of a species different from *M. intestinalis* or by the especially high level of infection found in the chekhon.

As regards the *Esocinema* species found in cyprinids it has to be elucidated whether the species found are identical with the species *Esocinema bohemicum* described from the type host, the pike. If yes, this would mean a loose host specificity which is less typical of skrjabillanids.

Two species belonging to the Anguillicolidae and Daniconematidae families of the Dracunculoidea superfamily are common parasites of the eel (*Anguilla anguilla*). Of them, only *Daniconema anguillae* is characterised by a location similar to that of *Skrjabillanus* species and by the intermittent excretion of larvae from the viviparous females. The unique location of the parasite's larvae was described by Molnár and Moravec (1994). Subsequently, Molnár (1997) reported the previously unknown fin location of the imagoes. The relatively frequent occurrence of the larvae indicates that this parasite is rather common in the eel despite the fact that the imagoes are rarely detectable. The swimbladder and the fins are likely to represent only one of the possible site, and worms colonising the intermuscular connective tissue or other sites of the organism remain hidden from the examiner.

The survey clearly proves that skrjabillanids develop via the carp louse as intermediate host, as has been demonstrated by Tikhomirova (1970, 1975) and Rudometova (1974, 1975) for *Skrjabillanus scardinii, Molnaria intestinalis* and



Figs 1–2. Video-recorded images of the fin of chekhon infected with *Molnaria* larvae
Fig. 1. Molnaria larva in the fin ray (a) and skin fold between the fin rays (b) of the chekhon. × 60
Fig. 2. Third-stage *Molnaria* larva released from the fin ray of a chekhon. × 200
Figs 3–8. Video-recorded images of carp lice infected with skrjabillanid larvae;
Fig. 3. Young skrjabillanid (probably *Lucionema*) larva from the leg of a carp louse (*Argulus foliaceus*) collected from pikeperch. Note the blunt cephalic end (large arrow) and tapered caudal end (small arrow) of the larva. × 800
Fig. 4. Skrjabillanid larva (arrow) in the uropod of an *Argulus foliaceus* collected from common carp. × 60

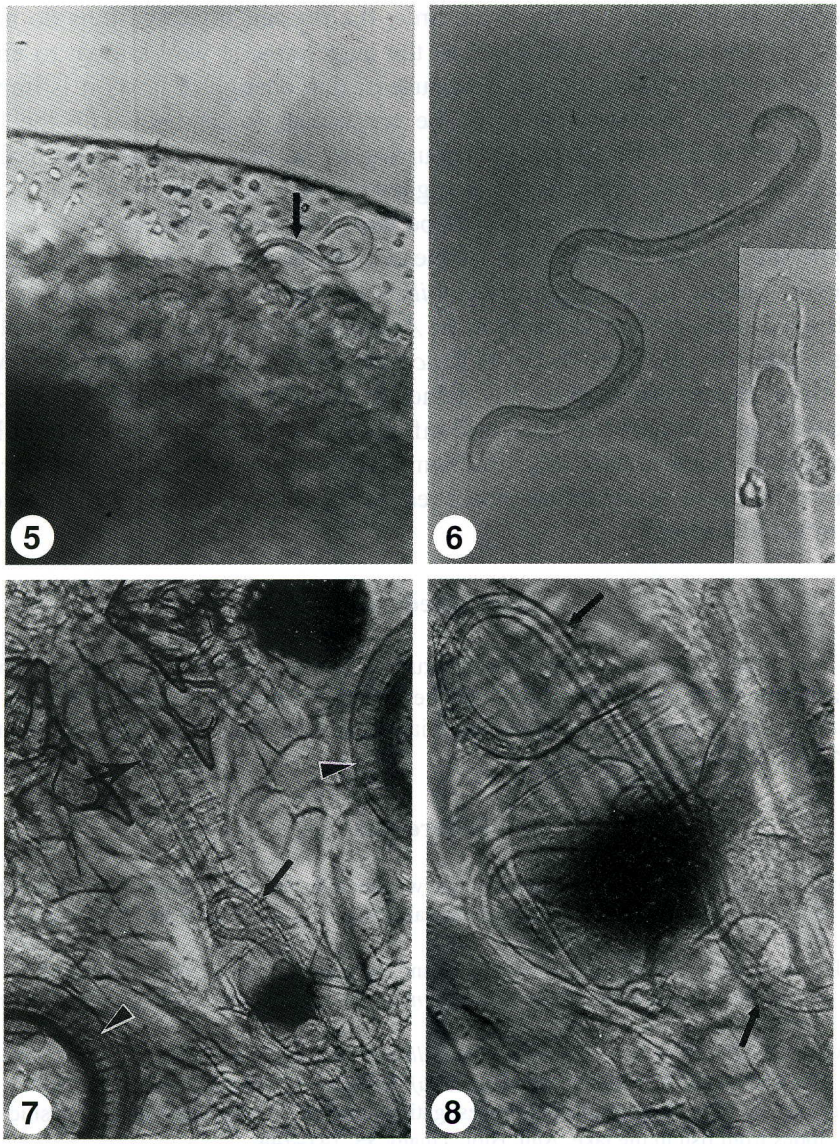


Fig. 5. Skrjabillanid larva (arrow) in the lateral part of the body of a carp louse collected from common carp. × 40

Fig. 6. Young skrjabillanid larva released from a carp louse. × 150. Inset: Larva released from a carp louse bearing a moulted cuticle at its cephalic end. × 300

Fig. 7. Skrjabillanid larva of advanced development (small arrow) in the stylet of a carp louse. The suckers of the carp louse are marked with an arrowhead. × 40

Fig. 8. Skrjabillanid larva of advanced development, wriggling in the basal part of the stylet of a carp louse (arrows). × 120

Sinoichthyonema amuri. At the same time, the present investigations do not allow us to draw further conclusions, as the carp louse is well known to change hosts several times during its life. Because of that change of hosts it is impossible to prove that the larva found in the crustacean is identical with the larval form of the skrjabillanid sp. or spp. occurring in the fish host, although infection of the carp louse by young stages is suggestive of a very likely infection of the host. Larvae of extremely variable shape and size were obtained from the carp lice. Obviously only a proportion of these corresponded to the larval stages of species belonging to the genera *Skrjabillanus, Molnaria* and *Sinoichthyonema,* which have been proved to develop in *Argulus* intermediate host. These larval stages assumedly included those of *Esocinema* and *Daniconema* species. At the same time, the present survey has demonstrated that the majority of carp lice collected from fish harbours a natural infection and, therefore, are unsuitable for use in reliable experiments. This problem could only be solved by the laboratory culture of *Argulus* intermediate hosts reared on parasite-free fish that have not been infected earlier.

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