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DEVELOPMENT OF KHAWIA SINENSIS HSÜ, 1935 (CESTODA: CARYOPHYLLIDEA) IN THE FISH HOST

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Abstract. The development of the tapeworm *Khawia sinensis* has been observed up to the stage of sexually mature parasites releasing eggs in an experimentally infected definitive host (*Cyprinus carpio*) at 15—16 °C. Juvenile, maturing, adult and gravid tapeworms were found 2—12, 19—24, 36—62, and 78—91 days post infection, respectively. No apparent size difference between tapeworms from carp kept at 20—22 °C, examined 1—16 days post infection, and those from 15—16 °C was observed. In naturally infected and fed carp, kept at 21—22 °C, some tapeworms survived till the end of experiment (44 days) while in unfed fish they survived only 15 days from the beginning of experiment.

In past decades, the tapeworm *Khawia sinensis* Hsü, 1935 became a common and, from the veterinary point of view, an important parasite of carp in many European countries (see for example Kulakovskaya 1963, Bauer et al. 1981, and others). Most papers dealing with the biology of this tapeworm, especially with its developmental cycle, originated in the early 1960s (Kulakovskaya 1962a,b, 1963, Kulakovskaya et al. 1965). Still, a number of important aspects of the life cycle of this parasite remains unclear, including its course and length of the development in the definitive host. Available published data (Kulakovskaya 1963, Shcherban 1965, Sapozhnikov 1972) differ by as much as several months.

This paper presents results of the experimental study of the tapeworm *K. sinensis* development in the definitive host and its development and survival in naturally infected carp, kept in the laboratory under various food regimes (feeding, starvation) at 21—22 °C. These results continue our study of early development of the parasite (Scholz 1991) and observation of its biology under natural conditions (Scholz 1988, Scholz et al. 1990).

MATERIALS AND METHODS

For experimental infection of fish with the tapeworm *K. sinensis* both procercoids from experimentally infected intermediate hosts (see Scholz 1991) and larvae of the parasite found in tubificids *Tubifex tubifex* (Müll.) (Oligochaeta: Tubificidae) from the fish pond Dražský Skaličany (see Scholz et al. 1990) were used. Most fish used for these experiments were obtained from localities where the tapeworm *K. sinensis* does not occur. Moreover, all experimental fish were kept several weeks or months in the laboratory before infection, and before use were treated with medicated feed (Taenifugin carp).

The fish were infected in two ways: for a minority, tubificids containing procercoids were induced via thin Pasteur pipette directly into the anterior part of the digestive tube; for most fish, tubificids containing parasite larvae were added either to a large (500 ml) Erlenmayer flask or small aquaria with 1—2 litre of water where they remained until eaten. Fish were infected individually, labelled and transferred to 20-litre-aquaria. There they were kept in groups of 5—10, at water temperature 15—16 °C or 20—22 °C. Experimental fish were fed mainly pelletized feed and chironomid larvae. Only microscopically examined tubificids were fed. The fish were examined at definite intervals or at the time of death.

A total of 70 fish was used for experimental infection with the *K. sinensis* procercoids. Their survey together with results of experiments is given in Table 1. The number of procercoids used for

infection ranged from 2 to 5 (at maximum 10 larvae) in most of fish. However, a total number of all procercoids used could not be determined precisely, because in heavily infected tubificids used for infection of fish, the exact number of tapeworm larvae could not be identified without damage to the host. Moreover, it was not always possible to determine if procercoids from the body cavity of the tubificids *T. tubifex* from the fish pond Dražský Skaličany are already fully developed and infective.

In addition to the development of the parasite in experimentally infected fish, its survivability and sexual development in naturally infected carp (*Cyprinus carpio* L.) was observed. A total of 59 carp, length 10—21 cm, originated from an experimental fish pond of the Research Institute of Fish-Culture and Hydrobiology, Vodňany, where at veterinary control heavy infection of fish with this parasite was recorded (prevalence 90—100 %). Before the beginning of experiment 10 carp were dissected to detect the initial infection with tapeworms. The other carp were divided into two groups; fish in the first group were starved, while fish in the second group were fed *ad libitum*. All fish were put into 50-litre-containers with tap water (temperature 21—22 °C) which was regularly changed (in starving carp 1—2 times a day, in fed carp 2 times a day, later once a day). At regular intervals, or in case of death, fish were examined. All detected tapeworms were fixed in 4 % formalin, stained with Schuberg's carmine, dehydrated in an alcohol series and mounted in Canada balsam. Undamaged tapeworms were divided into four groups according to the degree of sexual maturity: group I — juvenile tapeworms (only genital primordium present), group II — — maturing tapeworms (genitalia developing), group III — mature tapeworms (genitalia fully developed, the uterus without eggs), group IV — gravid tapeworms (eggs in the uterus). The percentage of the above mentioned groups was calculated for every sample.

At those times water was changed, sediment at the bottom was examined and detected tapeworms or their remnants were collected. Permanent slides were not prepared from these specimens because of the high decomposition of most of them. The degree of their sexual maturity was determined after compression between glasses under a dissection microscope.

Table 1. Survey of experimentally infected fish and results of experiments

Fish species	Length of fish (cm)	Number of fish		Number of tapeworms found	Fish examination (days p.i.)
		used	infected		
Ctenopharyngodon idella	5.7— 5.8	3	3	19	4—35
Cyprinus carpio	5.5—11.5	55	19	53	1—91
Tinca tinca	7.0—18.5	8	1	3	5—20
Aspius aspius	10.0	1	0	—	45
Gobio gobio	14.0	1	0	—	35
Carassius auratus	7.0— 8.0	2	1	8	6—23
		70	24	83	1—91

RESULTS

Development in carp

(Table 2, Figs. 1—3)

From 2 to 91 days post infection, a total of 45 tapeworms was found in 15 out of 45 experimentally infected carp, kept at 15—16 °C (intensity of infection 1—10 specimens). Juvenile tapeworms (group I) were found from 2 to 12 days p.i., maturing ones (group II) from 19 to 24 days p.i., mature ones (group III) from 36 to 62 days p.i. and gravid (group IV) 78 and 91 days p.i. (Table 2). Live spermatozoons were observed in the seminal receptacle in tapeworms 36 and 55 days old. In the 36-day-old specimen they filled only the distal part of the receptacle, while in the older tapeworm they filled the whole receptacle. In parasites found 55 and 60 days p.i., clusters of vitelline cells were observed in postovarial (proximal) parts of the uterus. All tapeworms from carp examined 78 days p.i. spontaneously released a small number of eggs into water. The number of eggs released by the 91-day-old specimen was apparently higher.

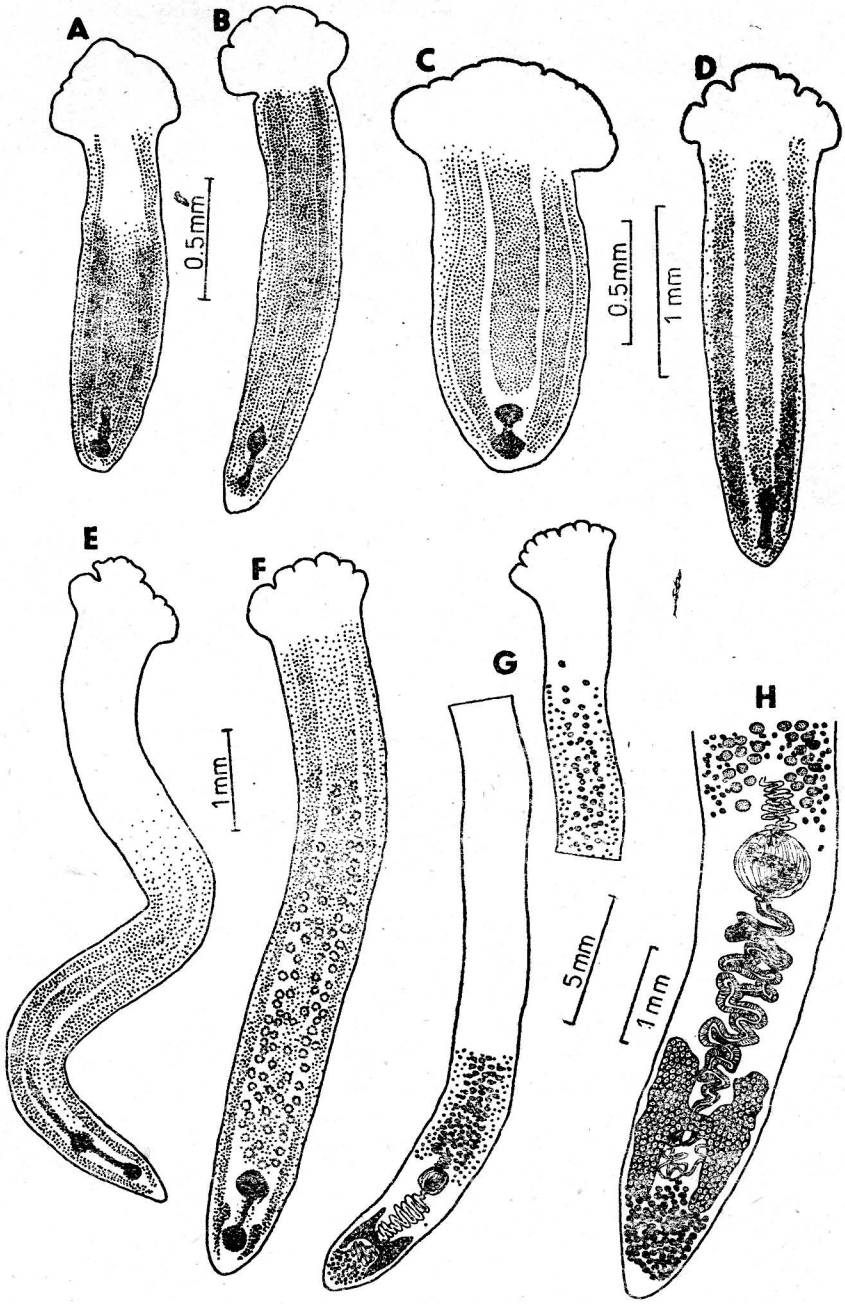

Fig. 1. *K. sinensis* tapeworms from experimentally infected carp (*Cyprinus carpio*). (A — 2 days p.i., B — 7 days p.i., C — 10 days p.i., D — 12 days p.i., E — 16 days p.i., F — 19 days p.i., G — 51 days p.i., H — 55 days p.i., A—D, F—H — temperature 15—16 °C, E — 20—22 °C).

Embryonal development of these eggs at water temperature 20—22 °C was similar to that in eggs from naturally infected carp (Scholz 1991) — the oncospheres were formed after 19 days.

At water temperature 20—22 °C, development of the tapeworm *K. sinensis* was observed in 10 experimentally infected carp. A total of 8 tapeworms were found in 4 of these (intensity of infection 1—3). The tapeworms from fish examined 1, 8 and 9 days p.i. were juveniles (group I), while parasites found 16 days p.i. were maturing (group II) — Table 2.

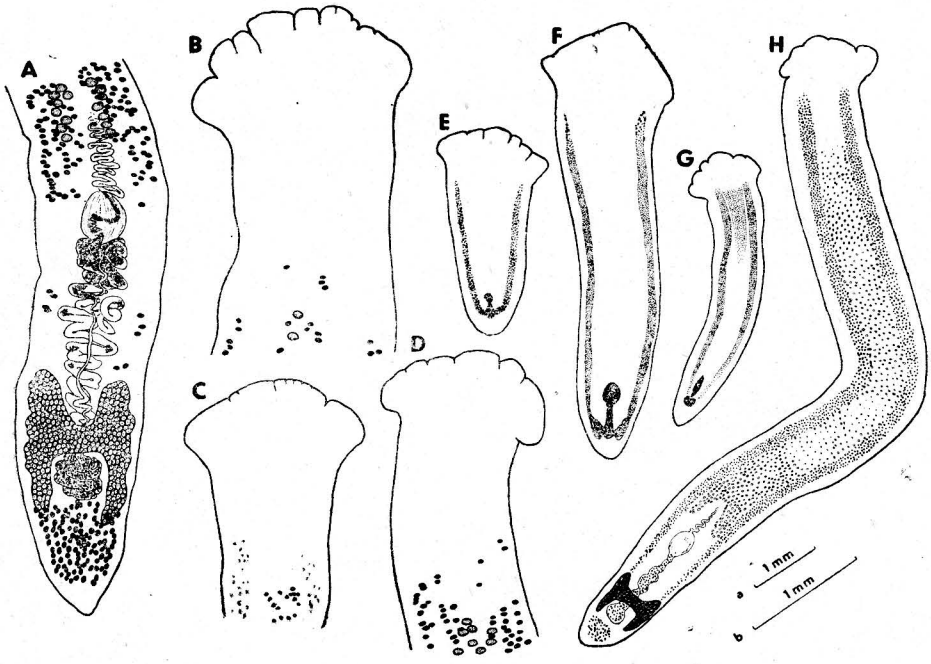

Fig. 2. *K. sinensis* tapeworms from experimentally infected carp (*Cyprinus carpio*) (A—D), gold fish (*Carassius auratus*) (E—F) and grass carp (*Ctenopharyngodon idella*) (G—H). (A, D—78 days p.i., B — 91 days p.i., C — 60 days p.i., E—F 6 days p.i., H — 35 days p.i.; all temperature 15—16 °C). Scales: a — A—D, H; b — E—G.

Experimental infection of other fish species

Tapeworms were found in all three infected grass carp (*Ctenopharyngodon idella* (Valenc.)) — Table 2. Specimens found 4 and 6 days p.i. were juveniles (group I) while specimens found 35 days p.i. were maturing (Fig. 2). The first two grass carp were examined after death, which was probably caused by the presence of tapeworms. The intestines of the fish were completely blocked by parasites and after experimental infection the fish became languid and soon died.

Eight juvenile *K. sinensis* were found in the intestine of one out of 2 experimentally infected gold fish (*Carassius auratus*) 6 days p.i. (Fig. 2). They were firmly attached to the intestinal mucosa of the host. The gold fish was fed only one tubificid *Limnodrilus udekemianus* with 10 larvae of the parasite measuring 1.5—2.5 mm. Live tapeworms found in the gold fish measured 1.7—3.3 × 0.53—0.59 mm (after fixation 1.7—4.2 × 0.52—0.86 mm) and their scolex reached width 0.68—0.80 mm

Table 2. Survey of *K. sinensis* tapeworms from experimentally infected definitive hosts

	Length of fish (cm)	Dissection (days p.i.)	Procercoids used for challenge		Tapeworms recovered from fish		Length (mm) of living tapeworms	Size of fixed tapeworms (mm) average (minimum — maximum)		
			Age (Days	Number	Number	Degree of maturation		Body length	Body width	Scolex width
Cyprinus carpio	Temperature 15—16 °C									
	7	2	65	5	5	I[1]	0.8—2.9	1.64(0.78—2.19)	0.43(0.23—0.58)	0.61(0.29—0.86)
	7	5	B[2]	1—2	1	I	0.95	2.00	0.40	0.61
	6.5	7	62	6—8	6	I	2.0—3.0	1.77(1.41—2.07)	0.31(0.20—0.35)	0.39(0.23—0.52)
	10.5	10	51—53	?	10	I	—	1.44(1.12—1.93)	0.33(0.43—0.55)	0.65(0.55—0.89)
	7	12	B	1	1	I	—	2.45	0.46	0.58
	8	19	76	?	4	II	5.0—7.0	6.8(6.3—7.5)	0.93(0.84—1.01)	1.27(1.09—1.38)
	7	24	62	5—8	3	II	—	5.1(4.1—6.8)	0.86(0.84—0.89)	1.11(0.92—1.27)
	7.5	36	68	2—3	1	III	17	33.3	2.13	3.02
	7.5	42	B	1	1	II—III	15	15.1	1.04	1.90
	8	51	76	1—2	1	III	25	22.9	1.61	2.56
	6	55	B	1	1	III	20	33.0	1.87	3.54
	8.5	60	74	10	4	III	max. 20	19.2(18.7—20.1)	2.08(1.62—2.40)	2.47(2.27—2.62)
	7.5	62	62	?	2	III	12	18.1	1.30	1.87
	9.5	78	74	10	4	IV	50—70	29.8(26.2—33.5)	1.98(1.68—2.19)	2.34(2.07—2.94)
	9	91	74	16	1	IV	32	36.5	2.64	2.80
	Temperature 20—21 °C									
	7.5	1	70	3	2	I	3.0—4.0	4.38(3.46—5.30)	0.52(0.51—0.52)	0.57(0.55—0.59)
	7	8	B	1	1	I	11.5	1.97	0.69	0.75
	6	9	B	2	2	I	1.5—1.8	2.4(2.3—2.4)	0.60(0.59—0.60)	0.68(0.67—0.69)
	11	16	51—53	?	3	II	7.0—8.0	7.7(7.1—8.6)	0.68(0.66—0.69)	0.95(0.89—1.07)
Ctenopharyngodon idella	5.8	4	74	6	4	I	2—3	5.8(5.3—6.3)	0.77(0.69—0.85)	0.87(0.79—0.91)
	5.7	6	74	7	6	I	2.5—3	2.2(1.5—2.6)	0.39(0.32—0.46)	0.50(0.34—0.62)
	5.7	35	74	7	9	II	6—9	6.1(4.1—9.5)	1.12(0.81—1.36)	1.09(0.77—1.58)

Legend:

[1] group I — juvenile, II — maturing, III — mature tapeworms, IV — gravid tapeworms

[2] B = procercoids from natural infections of *Tubifex tubifex* by the tapeworm *K. sinensis* in the fish pond Dražský Skaličany, near Blatná (Scholz et al. 1990)

(0.72—1.21 mm after fixation) — Fig. 3. In a tench, 14 cm long, three tapeworms were found 2 days (53 hours) p.i. One specimen, localized in the first third of the intestine, was firmly attached; two others were not attached and were found in the second (posterior) half of the intestine. Attempted experimental infections of other fish species were not successful (Table 1).

Survival and sexual development of *K. sinensis* in naturally infected carp at 21—22 °C

Results of these experiments are summarized in Table 3. Sexual maturity was apparently proceeding in the course of the experiment. This demonstrates gradual maturation of the parasite in fish kept in the laboratory. The finding of a mature tapeworm without eggs, even 44 days from the beginning of this experiment, was interesting and noteworthy. The results show evident differences of parasite burden in fed and starving fish (Table 3).

An additional 28 tapeworms or their remnants were found in the course of the experiment, at the bottom of the fish containers. These tapeworms were found from 3 to 10 days in fed carp (a total of 24 parasites) or after 4 days in the experiment with starving carp (a total of 4 specimens). Except for one gravid tapeworm recorded 3 days p.i., only maturing and mature specimens of *K. sinensis* were found (groups II and III).

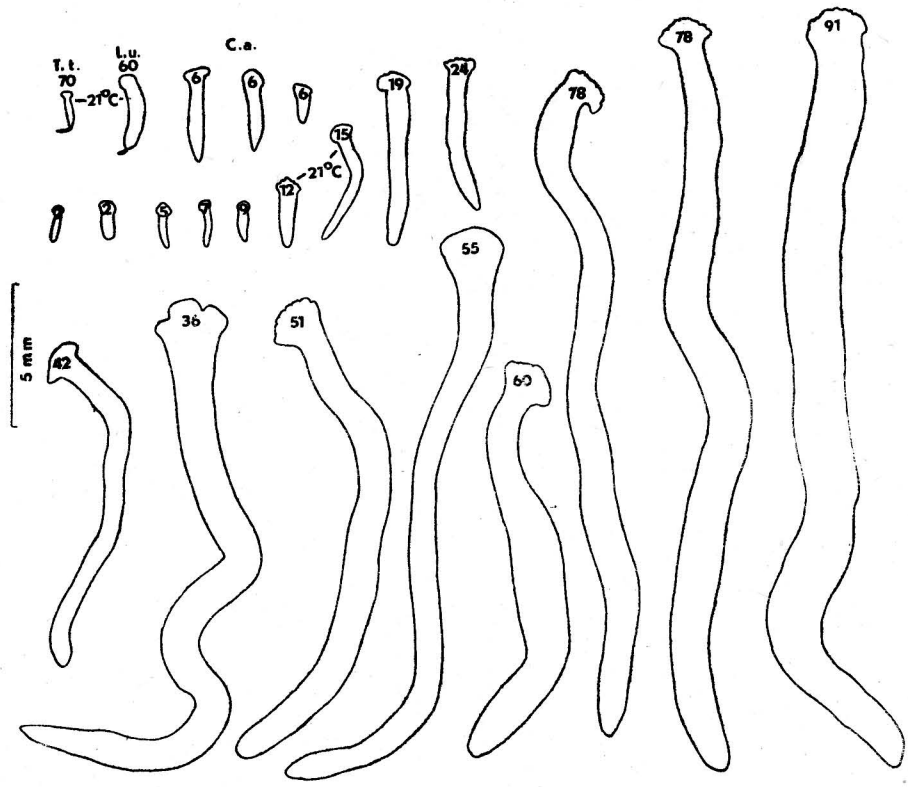

Fig. 3. Comparison of *K. sinensis* tapeworms from experimentally infected fish and infective procercoids from the intermediate hosts (tubificids). Figures represent number of days post infection, T.T. = *Tubifex tubifex*, L.u. = *Limnodrilus udekemianus*, C.a. = *Carassius auratus*, 21 °C — temperature of water, other cestodes are from *Cyprinus carpio* kept at temperature 15—16 °C.

Table 3. Survey of burden of naturally infected carp (both fed and unfed), kept at 21–22 °C

Group		Fed fish								Unfed fish				
Days post infection	0	2[1]	3[1]	8	15	25	35	44	Total	2[1]	8	15	25	Total
Number of examined fish	10	3	5	5	5	5	2	3	28	1	5	5	10	21
Number of infected fish	9	2	5	5	4	2	1	2	21	1	4	2	0	7
Number of found tapeworms	155	73	35	88(10[2])	26	16	7	9	254	18	30(9)	7(6)	—	55
Medium intensity of infection	17	37	7	18	7	8	—	5	12	—	8	4	—	8
Intensity (min. –max.)	1–48	19–54	1–17	6–39	1–12	2–14	7	2–7	1–54	18	1–21	1–6	—	1–21
Sexual maturity (number of specimens)														
group I	3[3]	3	—	—	—	—	—	—	3	—	—	—	—	—
group II	61	22	2	21	2	2	—	—	49	+11	—	3	—	14
group III	73	37	18	44	10	2	—	1	112	6	1	4	—	11
group IV	12	6	10	12	14	12	7	7	68	—	12	—	—	12

Legend:

[1] fish examined because of death
[2] number of dead, decomposed tapeworms from total number in sample given in brackets
[3] in some samples, due to damage of part of tapeworms, total number of evaluated specimens is lower than total of all found parasites

DISCUSSION

Development of the tapeworms of the order Caryophyllidea in the experimentally infected definitive host has been studied only by a few authors up to now. Attention was concentrated mainly on North American members of the genera *Archigetes*, *Biacetabulum* and *Glaridacris* (Mackiewicz 1972). However, all authors reviewed by Mackiewicz (1972) observed the development only a few days, to a maximum of 16 days in the tapeworm *Caryophyllaeus laticeps* (Pallas, 1781) by Kennedy and Walker (1969).

Up to now, little has been known about the development of the species *K. sinensis* in the definitive host. Kulakovskaya (1962a, 1963) has reported a 4—6 months' maturation period in this parasite. However, it is not apparent if this is based on results of experiments or deduced from the results of occurrence and maturation observation of the tapeworm in natural conditions during the year. Begoyan (1977) made similar observations in the tapeworm *Khawia armeniaca* from *Varicorhinus capoeta sevangi:* on the basis of tapeworm morphology and occurrence during individual months, he described stages of their development in the definitive host. According to Begoyan (1977), it is possible to estimate the length of the *K. armeniaca* development in the definitive host to be at least 5 months. This is in agreement with the period given by Kulakovskaya (1962a, 1963). Shcherban (1965), reporting 1.5—2 months' period of the tapeworm *K. sinensis* development in carp, also has not indicated how this determination was made; it is probable that they are also not based on the results of experiments.

Completely different data are given by Sapozhnikov (1972), who experimentally infected 24 carp with tubificids containing either young larvae of the parasite or its invasive procercoids. After infection experimental fish were put into cages and immersed in a pond. Average daily water temperature varied from 16.8 to 25.2 °C. As early as 15 days later, tapeworms 5.6—7.8 cm long with eggs in the uterus were found in the intestine of fish. Twenty days later, parasites reached a body length of 7.1—10 cm and released eggs into water. After 25 days, the tapeworms measured 10—11.3 cm. After 30 days no parasites were detected in fish, either in carp infected with young larvae or in control fish. According to Sapozhnikov (1972), the parasite had developed completely within 2—3 weeks in the definitive host, with an average daily growth of the body of 3.5—5 mm.

In our experiments, development of the tapeworm *K. sinensis* in the definitive host was apparently slower. At 15—16 °C, gravid specimens were first noted after 11 weeks (78 days) of development. It should be stressed that Sapozhnikov (1972) carried out his experiments at higher water temperature than we did, but our results at 20—22 °C in the initial phases did not differ substantially from results at 15—16 °C. Results of our observation of sexual development of parasite in naturally infected carp at 21—22 °C (see Table 3) also correspond with the results of our experimental study of *K. sinensis* in carp.

Because the number of *K. sinensis* tapeworms in individual groups varied in fed carp, and mainly because a mature tapeworm without eggs was found even 44 days after the challenge, we believe that maturation of some parasites occurred only after 7—8 weeks at water temperatures of 21—22 °C.

Experimental conditions certainly influenced parasite development in the definitive host, but neither this fact nor the fact that Sapozhnikov (1972) used larger fish (weight 16.6—65.5 g, length approximately 9—15 cm) in his experiments can fully explain the differences between our results and his (and also results of Kulakovskaya 1962a, 1963, and Shcherban 1965). To explain them, it would be

helpful to repeat the experiment of Sapozhnikov (1972) in analogous conditions.

Successful experimental infection of one goldfish (*Carassius auratus*) and one tench with apparent enlargement of the tapeworms found in the former fish 6 days post-infection have proved the possibility of short-term survival and growth of the parasite in these atypical hosts. However, another experiment employing larger numbers of fish is needed to clarify the possibility that sexual development of the tapeworm *K. sinensis*, up to now found only in carp, wild carp and grass carp (*Cyprinus carpio, C.c. haematopterus* and *Ctenopharyngodon idella*), can also occur in crucian carp (*Carassius* spp.).Host specificity of the tapeworms of the genus *Khawia* in the definitive host plays an important role in evaluation of validity of some doubtful taxons of this genus, mainly three "species" — *Bothrioscolex* (= *Khawia*) *rossittensis*, *B. prussicus* and *B. dubius* from *Carassius carassius* from East-Prussia, described by Szidat (1937).

Experiment with naturally infected carp, bred in the laboratory at 21—22 °C, showed that starvation of this host is very important for survival of the parasite. The burden in fed fish was conspicuously higher that in carp without feed. While in fed carp parasites were found even at the end of experiment (44 days), in unfed carp tapeworms were found only for 15 days after the beginning of experiment. Moreover, most specimens from unfed fish, including tapeworms found after 8 days, were dead. An apparently higher number of tapeworms and their remnants was recorded in the sediment of fed fish than in sediments of unfed carp. This was probably due to their quicker decomposition in starving hosts — making it more difficult to detect them in the sediment. This idea is supported by representation of dead parasites from examined hosts. While in unfed carp these specimens represented 27 % of found tapeworms (15 out of 55), in fed fish they represented only 4 % (10 out of 254). According to the findings of tapeworms' remnants in the sediment of both experimental groups, it can be supposed that the greatest number of tapeworms was expelled from fish between day 3 and 10 of the experiment. Loss of parasite is probably not related to the spontaneous shedding of sexually mature tapeworms with eggs from hosts at the end of spring and at the beginning of summer, as observed in the tapeworm *K. sinensis* in naturally infected carp from the pond Dražský Skaličany (Scholz et al. 1990), because only specimens without eggs were found in the sediment.

Kennedy (1971) has studied the effect of temperature on survival of the tapeworm *Caryophyllaeus laticeps* in experimentally infected orfe (*Leuciscus idus* v. *orphus*). The author recorded only a small loss of parasites at 4 and 12 °C and some specimens survived in these fish more than a month. In contrast, at 18 °C tapeworms were dead and expelled from the host even 3 days after the beginning of the experiment. According to Kennedy (1971), there is a relation between the water temperature and "establishment" of tapeworms and their survival in hosts. The critical value is about 13 °C in the given case. However, according to the author, death and loss of the parasites are not caused solely by the temperature of water. On the basis of his experiments, Kennedy (1971) excludes the influence of starvation on survival of the tapeworm *C. laticeps* in the definitive host. This is in contrast with our findings. The negative effect of starvation of the definitive host on its intestinal parasites, including tapeworms, is known in a wide range of helminths (see, for example, Dogel et al. 1958).

Differences between our results and the data of Kennedy (1971) are apparent. It should be emphasized that in the initial phase of our experiment with naturally infected carp exit of the tapeworms *K. sinensis* from fish also occurred. Because the host burden was high, this was demonstrated only by a mild reduction of number of infected carp and number of tapeworms detected (Table 3). Kennedy (1971)

used only one tubificid for the experimental infection of orfe. Therefore, the loss of even a few specimens of *C. laticeps* could lead to complete disappearance of the parasite from experimental fish over a period of several days. The number of unfed experimental fish and the burden of these fish with the tapeworm *C. laticeps* were also apparently lower in Kennedy's (1971) experiment than during our observation.

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