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The Importance of Leeches in the Life Cycle of the Order Strigeidida (Trematoda)

J. VOJTEK, V. OPRAVILOVÁ, L. VOJTKOVÁ

Department of Comparative Physiology and General Zoology and Department of Zoology, University J. Ev. Purkyně, Brno

Abstract. The paper informs on four metacercaria species of the order Strigeidida, found in leeches in Czechoslovakia. Apart from *Apatemon gracilis* all findings are new to Czechoslovakia. The new species of larva, *Prohemistomulum sp.*, is described. The other three species of larvae are known to belong to flukes, which are most dangerous to waterfowl and game birds. The same may also be assumed of the species *Prohemistomulum sp.* In some localities the high extensity and intensity of invasion offer suitable conditions for massive invasions of fowl by these flukes.

The leeches in which the metacercariae of the order Strigeidida (La Rue, 1926) Sudarikov, 1959 develop, were collected in some helminthologically interesting localities. In Czechoslovakia practically no helminthological studies were made on leeches, although the fact that they may participate as supplementary hosts on the life cycle of some flukes causing dangerous helminthoses to waterfowl, has been known for some time.

MATERIAL AND METHOD

Most of the leeches were collected in two helminthologically very interesting areas: the surroundings of Komárno (southern Slovakia) and of Lednice (southern Moravia):

1. Kameničná 1: drainage canal near Kameničná
2. Kameničná 2: pool in the inundated area of the river Váh near Kameničná
3. Komárno 1: periodical or astatic pools on pasture in the inundated area of the river Váh near Komárno—Nová Osada
4. Komárno 2: drainage canal near Komárno—Nová Osada
5. Komárno 3: permanent pool at Komárno—Nová Osada
6. Lednice: inundation pool between Podivín and Lednice

Less numerous material was collected at:

7. Vranovice, periodical pool
8. Strachotín, old river arm "Saulochna"
9. Brook at Hostěradice near Moravský Krumlov
10. Rivulet near a duck propagating farm at Brno-Komín

The leeches belonged to 9 species. A review of the examined leeches, found in the individual localities, and of the larval load is given in Tab. 1. The total of metacercarial species numbered four.

The leeches were either dissected or smaller specimens were torn to pieces with needles, pressed between two slides and examined under the stereoscopical preparation microscope. The larval stages mostly capsulated, were isolated mechanically from the tissue and transferred to a saline solution (approx. 0.5 %). The metacercariae were isolated from the cysts in two ways. Less successful was the mechanical tearing or cutting of the cyst with a fine eye scalpel or fine needles, because, even with the greatest care, the metacercariae were often damaged. A more suitable way was the

Table 1. Review of leeches examined from the individual localities

	Kameničná 1.	Kameničná 2.	Komárno 1.	Komárno 2.	Komárno 3.	Lednice	Vranovice	Strachotín	Pohořelice	Hostěradice	Brno-Komín
Theromyzon tesselata	20/0										
Glossiphonia complanata	11/1	3/0		2/0	3/3	2/1	1/0				
Glossiphonia heteroclita				1/0							
Hemiclepsis marginata	1/0				1/1						
Piscicola geometra				4/0				6/0	15/0		
Hirudo medicinalis			4/3			1/1					
Haemopis sanguisiga	61/58		12/12			34/34		7/7		1/1	4/0
Erpobdella octoculata	67/16	6/2		48/44	13/6	1/0					2/0
Erpobdella testacea										25/22	

feeding of the cysts to the experimental animals (ducklings) or to freshly hatched chicks which had not yet taken any food. After 3—6 hours the animals were sacrified and recently liberated metacercariae were found in their intestine, which were not different from the larvae obtained from the mechanically opened cysts. The metacercariae were fixed in different manners. Most suitable was the fixation with a hot mixture of sublimate-acetate, preserving the natural shape of the body. Only a minor portion of the material was fixed under slight pressure of the cover slide. All measures of the larvae were taken from material fixed in the hot mixture. The measures of the cysts were taken from viable material, extracted mechanically from the leeches. The larvae, stained with Mayer's Carmalum were dehydrated in the alcoholic series and transferred to creosote, in which they were studied.

For determining the individual larval species we tried to obtain adult trematodes from artificially infected ducklings, freshly hatched chicks or other hosts.

RESULTS

Apatemon gracilis (Rudolphi, 1819) Fig. 4

A review of the supplementary hosts and their invasion with metacercariae of *A. gracilis* in the individual localities is given in Tab. 2.

The metacercariae are in thick-walled ovoid cysts 0.386—0.461 mm long and 0.318—0.386 mm wide. The walls of the cysts measure 0.040—0.083 mm at the poles and only 0.026—0.042 mm at the sides. The cysts are situated in the parenchyma and the musculature of the leeches. The structure of the metacercarial body is characteristic for the type *Tetracotyle*. It measures 0.246—0.391 by 0.166 to 0.224 mm (0.316 by 0.211 mm in diameter). The body is distinctly differentiated

Table 2. *Apatemon gracilis* — review of the supplementary hosts and their worm invasion in the individual localities

Supplementary hosts \ Locality		Kameničná 1.	Komárno 1.	Komárno 2.	Lednice	Strachotín	Hostěradice
Hirudo medicinalis	a	—	4	1	—	—	—
	b	—	—	1	—	—	—
	c	—	—	—	—	—	—
	d	—	—	2	—	—	—
	e	—	—	—	—	—	—
Haemopis sanguisuga	a	61	12	—	34	7	1
	b	3	12	—	34	7	1
	c	4.9%	100%	—	100%	100%	—
	d	8—364	38—3735	—	1—2143	2—1032	15
	e	137	825	—	245	345	—
Erpobdella octoculata	a	67	—	48	1	—	—
	b	3	—	44	—	—	—
	c	4.5%	—	91.5%	—	—	—
	d	1—10	—	1—100	—	—	—
	e	6.5	—	27.9	—	—	—
Erpobdella testacea	a	—	—	—	—	—	25
	b	—	—	—	—	—	22
	c	—	—	—	—	—	88%
	d	—	—	—	—	—	1—52
	e	—	—	—	—	—	8.3

a = total number of leeches examined
b = number of attacked leeches
c = extensity of invasion
d = minimum and maximum intensity of invasion
e = average intensity of invasion

into a larger, cup-shaped anterior part and a very small posterior part. The anterior part is 0.176—0.302 (0.237) mm long. The two lobes of the Brandes organ protrude from the bottom of the cavity of the anterior part, extending sometimes even above its anterior margin. On the internal side of the dorsal wall of the anterior part lies the subterminal oral sucker—length 0.042—0.062 (0.056) mm, width 0.053—0.070 (0.064) mm. This is followed by a muscular pharynx, measuring 0.017—0.028 (0.022) by 0.015—0.022 (0.018) mm. The distinct oesophagus continues in two intestinal caeca, extending almost to the termination of the posterior extremity. At both sides at approximately the pharynx level are situated the oval pseudosuckers with an irregularly longitudinal slit. The acetabulum, situated at the bottom of the cup, can sometimes be extruded quite far in forward direction. It measures 0.053—0.067 (0.059) by 0.062—0.078 (0.072) mm. At the base of the lobes of the Brandes organ lies close to the line dividing the fore- from the hindbody a distinct gland. It measures 0.064—0.109 (0.084) by 0.067—0.092 (0.083) mm. Our description of the metacercariae are generally in agreement with the data by various authors, found in the literature.

The definitive hosts of these flukes are birds, belonging mostly to the order Anseriformes. The diseases caused by them can be fatal to the domestic ducks. The first intermediate hosts are various snails, mostly Pulmonata (see Opravilová, Vojtek 1965). In our findings the most frequently occurring parasite belonged to the subspecies *Apatemon gracilis minor* (Yamaguti, 1933). Only the larvae found at Hostěradice belonged to the nominate form *A. gracilis gracilis* (Rudolphi 1819).

In our material, the larvae of *A. gracilis* were found in most localities investigated. Also the extensity and intensity of invasion was extremely high. From our material we could not draw any conclusions as to the specifity of the larvae to their supplementary hosts, although we presume that the most important supplementary hosts of *A. gracilis* are leeches of the genus *Erpobdella* and the species *Haemopis sanguisuga*. Especially in the latter we observed heavy invasions which may be attributed to the big size of this leech species. The much smaller leeches of the genus *Erpobdella* would certainly succumb to similar invasions.

***Cotylurus* sp.** Figs. 1, 5

A review of the invasion of supplementary hosts on the various localities is given in Tab. 3.

Tetracotyle are in relatively thin-walled pear-shaped cysts. The wall of the cyst (thickness 0.014—0.028 mm) lies close to the larval body. The cysts are flattened dorsoventrally and measure alive 0.379—0.469 mm in length and 0.297—0.345 in width. The metacercariae, when liberated from the cysts, are pear-shaped, the widened forebody is distinctly differentiated from the attenuated hindbody. The entire length of the metacercaria is 0.290—0.400 mm. The anterior end is spherical, in the shape of a widely opened cup, with its anterior wall shorter than its posterior wall. It attains a length of 0.207—0.290 mm and a width of 0.193—0.235 mm.

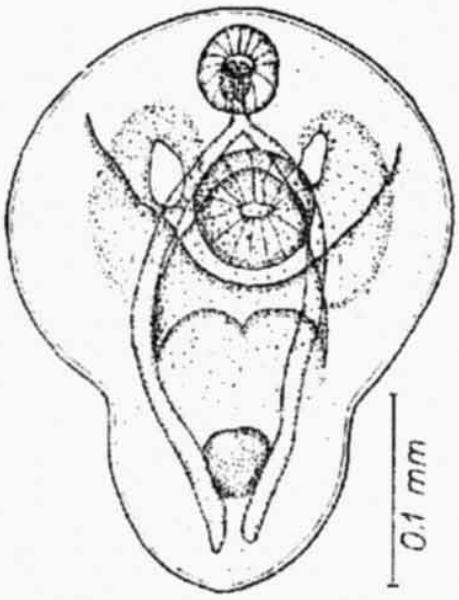

Fig. 1. *Cotylurus sp.*

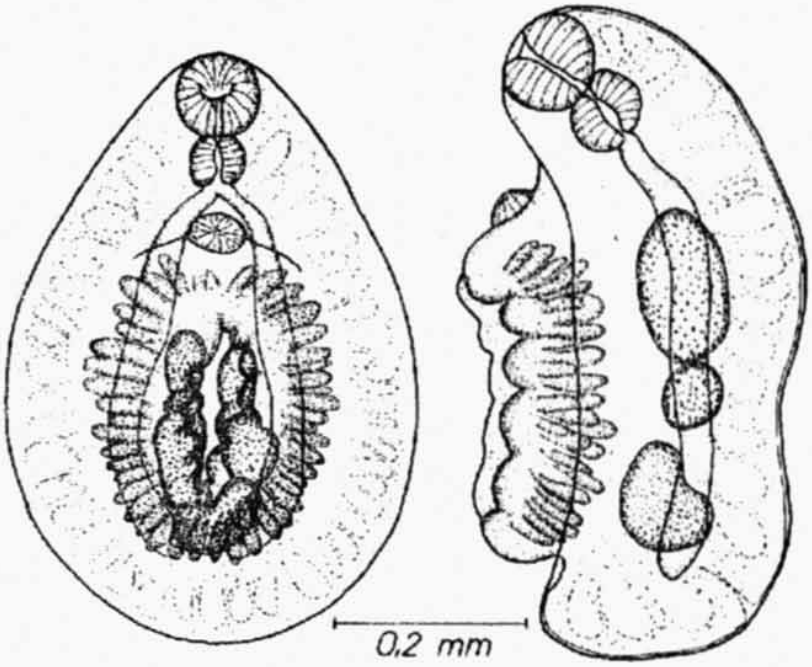

Fig. 2. *Prohemistomulum opacum*

Table 3. *Cotylurus* sp.—Review of supplementary hosts and their worm invasion in the individual localities

		Kamenična 1.	Komárno 3.	Lednice	Strachotín
Glossiphonia complanata	a	11	3	2	–
	b	1	3	1	–
	c	9.1%	100%		–
	d	18	19 - 26	2	–
	e	18	22	2	–
Hemiclepsis marginata	a		1	–	–
	b	–	1	–	–
	c	–		–	–
	d	–	6	–	–
	e	–	6	–	–
Haemopis sanguisuga	a	61	–	34	7
	b	50	–	5	2
	c	82%	–	14.7%	28.6%
	d	1 - 200	–	1 – 28	95 – 592
	e	29.4	–	12.2	345
Erpobdella octoculata	a	67	13	1	–
	b	11	5	–	–
	c	16.4%	38.5%	–	–
	d	1 – 16	1 – 15	–	–
	e	5.45	5	–	–

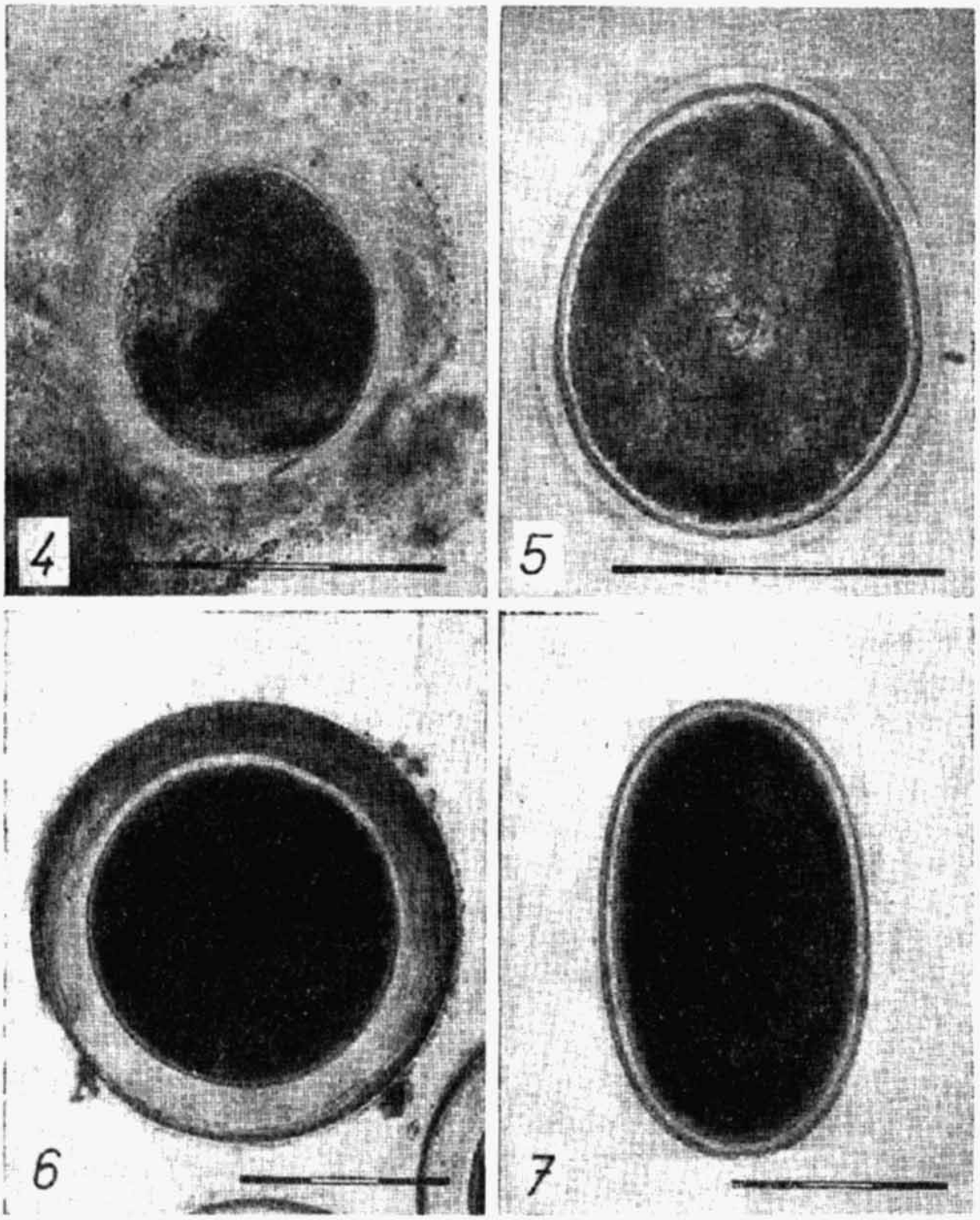

Fig. 4.—7. Cysts with metacercariae (measures = 0.3 mm). 4 — *Apatemon gracilis;* 5 — *Cotylurus sp.;* 6 — *Prohemistomulum opacum;* 7 — *Prohemistomulum sp.*

The posterior part measures 0.062—0.110 by 0.103—0.131 mm. The oral sucker is placed on the anterior margin of the dorsal wall of the forebody cup and measures 0.051—0.063 by 0.045—0.060 mm. The pharynx (0.018—0.030 mm in diameter) is followed by a 0.018—0.033 mm long oesophagus. The intestinal caeca extend along the margins of the Brandes organ, which covers them in some parts, terminating at the hindbody in a distance of 0.018—0.045 mm from the end of the body. On the

bottom of the cup lies the acetabulum, which measures 0.51—0.072 mm in length and 0.060—0.069 mm in width. It is covered by the lobes of the Brandes organ. The pseudosuckers are placed at the sides of the body between the oral sucker and the acetabulum, measuring 0.075—0.112 mm in length and 0.042 to 0.060 mm in width. In the hindbody between the caeca is situated the genital primordium (0.039—0.048 mm long and 0.036—0.045 mm wide).

Metacercariae of this type, designated *Tetracotyle typica* de Fillipi have been known for a long time in the literature. The data concerning their supplementary hosts mention both leeches and mollusks. Some authors consider metacercariae from mollusks and leeches to belong to the same larval species—*Cotylurus cornutus* (Rudolphi, 1808) Szidat, 1928, other authors consider them to belong to two species.

The species representation of the *Tetracotyle* from mollusks has been confirmed repeatedly in experiments by ERCOLANI 1881, LUTZ 1921, MATHIAS 1925, TIMON—DAVID 1943, ZAJÍČEK and VALENTA 1964, ŽDÁRSKÁ 1964. SZIDAT (1929) first succeeded to rear adult flukes from leeches. The metacercariae found by him in the gonads of *Erpobdella octoculata* and *Haemopis sanguisuga*, developed 5 days after feeding them to ducklings into adult flukes, which he determined as *Cotylurus cornutus*. *Tetracotyle* from leeches are considered the larvae of the species *C. cornutus* also by TIMON—DAVID (1943), DOBROWOLSKI (1958) and WISNIEWSKI (1958). SUDARIKOV, KARMANOVA and BAKHMETEVA (1962) assumed that *Tetracotyle* from the lacunary system of leeches *Erpobdella octoculata*, *Glossiphonia complanata* and *Helobdella stagnalis* in the Volga delta, belong probably to another species than *C. cornutus*, designating them therefore *Tetracotyle sp.* Their assumption was based on the results of experimental invasions with cysts (*Anas platyrhyncha* L., the young of *Larus ridibundus* L., of *Sterna hirundo* L., of *Tringa glareola* L. and of *Columba livia f. domestica* L.). The results of all these experiments were negative.

In our experiments we obtained adults from cysts fed to ducklings (*Anas platyrhyncha f. dom.*), to freshly hatched chicks (*Gallus gallus f. dom.*) and to doves (*Columba livia f. dom.*). However, the percentage of adult flukes developing from the cysts was quite negligeable, apart from some experiments with the same hosts which were also negative. In our opinion the *Tetracotyle* of the genus *Cotylurus* from leeches belong to a different, although closely related, species than the *Tetracotyle* from mollusks. Evidence supporting this fact was found in experiments with cercarial invasions of the supplementary hosts. The cercariae developing in *Planorbarius corneus* penetrated the leeches of the species *Haemopis sanguisuga*, *Erpobdella octoculata*, *Glossiphonia complanata*, *G. heteroclita*, *Hemiclepsis marginata* and *Protoclepsis tesselata*. In these leeches the *Tetracotyle* became fully developed. Contrary to that, experiments with invasions of mollusks (*Lymnaea stagnalis*, *Planorbarius corneus*) with the same cercariae remained always negative. A definitive decision to which species the metacercariae from leeches belong, will be possible only after more profound studies of both life cycles and a comparison of all important developmental stages.

Also in our material, the larvae of *Cotylurus sp.* were well-distributed. In some

localities (the canal at Kameničná, Strachotín) the extensity and intensity of invasions was very high. On the grounds of our experiments with the cercariae, on findings in nature and according to the literature, the range of supplementary hosts seems to be wider than that of the species A. gracilis. Also here, the leech *H. sanguisuga* is probably playing the most important role. The flukes of the genus *Cotylurus* can cause serious diseases to waterfowl.

Prohemistomulum opacum Wisniewski, 1934 Figs. 2, 6

A review of the hosts and their worm load in the individual localities is given in Tab. 4.

The metacercariae are enclosed in thick-walled spherical, or almost spherical cysts situated in the musculature, the parenchyma or the sexual organs of the leeches. They are opaque, measuring 0.461—0.704 mm in diameter, which makes them macroscopic. Sometimes they shine through the skin of the leeches like opaque grains. The walls of the cysts are very thick with a distinct structure of concentric layers. Two layers are observable in the wall: the external layer (thickness 0.063 to 0.069 mm) with a more evident stratification, the thinner internal layer (0.013 to 0.019 mm) which is almost homogeneous. A thin refractive hyaline membrane (0.003—0.004 mm) covers the cavity of the cyst. Inside the cyst the metacercariae are coiled. After liberation their structure is characteristic for the larvae of the

Table 4. *Prohemistomulum opacum* — review of supplementary hosts and their worm invasion in the individual localities

		Kameničná 1.	Kameničná 2.	Komárno 1.	Komárno 2.	Lednice	Strachotín
Hirudo medicinalis	a	–	–	4	–	1	–
	b	–	–	1	–	1	–
	c	–	–	25%	–		–
	d	–	–	1	–	2	–
	e	–	–	1	–	2	–
Haemopis sanguisuga	a	61	–	12	–	34	7
	b	31	–	11	–	7	1
	c	52%	–	91.5%	–	20.6%	14.3%
	d	1–105	–	2–132	–	1–11	2
	e	39	–	40.4	–	5.1	2
Erpobdella octoculata	a	67	6	–	48	1	–
	b	2	2	–	7		–
	c	3%	33%	–	14.6%	–	–
	d	1	1–2	–	1–4	–	–
	e	1	1.5	–	1.9	–	–

family Cyanthocotylidae, designated complexly *Prohemistomulum*. A review of the larval measures and a comparison with the data by WISNIEWSKI (1934) and by Soviet writers (SUDARIKOV, KARMANOVA, BAKHMETEVA 1962) is given in Tab. 5.

The body is widely oval, the forebody often more attenuated, its maximum width at approximately the end of the second third of its length. On the ventral side there is a slight cavity, from which the enormous Brandes organ protrudes. The body surface is covered with fine spines, visible especially in the anterior half of the body. The oral sucker is slightly subterminal, moderately muscular and relatively large. On the dorsal side it is followed by a muscular pharynx approximately by one third smaller than the oral sucker. The caeca, bifurcating closely behind the pharynx extend almost to the posterior end of the body. The acetabulum is widely oval, approximately half the size of the oral sucker. It is situated on the anterior margin of the Brandes organ, mostly slightly elevated above the ventral field. The Brandes organ, shaped as a sucker with irregular lobate margins covers most of the ventral area and is highly elevated above it. Its median hollow is either oval or irregularly lobate. Below the Brandes organ, the fairly large but not distinctly differentiated genital primordium is situated close to the caeca. In living larvae a dense network of canals of the secondary excretory system is visible.

So far, no information has been available on the life cycle of these larvae and there are only various views to which genus the adult fluke belongs. By feeding duckling of the species *Anas platyrhyncha f. dom.* with metacercariae we recovered adult flukes belonging to the genus *Cyathocotyle* MUHLING 1896. Their more precise systematic position will depend on more detailed studies of their life cycle. The intermediate host of these flukes is *Bithynia tentaculata*.

Also the flukes of the genus *Cyathocotyle* can be dangerous to waterfowl if the invasion is heavy (SHEVTSOV 1958).

In our material the finding of the species *Prohemistomulum opacum* in 5 localities indicates its wide distribution. Three of these localities are in the Komárno region, where the intensity and extensity of invasions were found highest. On the two Moravian localities their occurrence was rather sporadic. In the investigated localities the most important supplementary hosts of the developmental stages of this species are leeches of the species *Haemopis sanguisuga*.

***Prohemistomulum* sp.** Figs. 3, 7

A review of the hosts and their worm load on the individual localities is given in Tab. 6.

These larvae were found only in two localities in the Komárno region where they accompanied the foregoing species. Very often, we found both species in one host. The intensity of invasion was slightly lower than in *Prohemistomulum opacum*.

The location of the cysts is similar to that of *P. opacum*. The cysts are large, elongated and, during the dissection of the leeches, can be differentiated macroscopically as opaque formations. The walls are less thick than in the previous species

Table 5. Measurements of the metacercariae of the family Cyathocotylidae from leeches

		Prohemistomulum opacum				Prohemistomulum sp.
		own material	Wisniewski 1934 living flukes	Wisniewski 1934 fixed flukes	Sudarikov et al. 1962	own material
the cyst	external measurements	0.461—0.704 0.580	0.39—0.54		0.45—0.60	0.610—0.835 0.727 × 0.388—0.538 0.461
	measurements of cyst cavity	0.371—0.512 0.478				0.590—0.785 0.668 × 0.315—0.490 0.414
	thickness of walls	0.060—0.080	0.040—0.050	up to 0.070	up to 0.070 0	0.018—0.050
length of body		0.563—0.704 0.617	0.40—0.60		0.50—0.64	0.627—0.806 0.728
width of body		0.333—0.435 0.390	0.46—0.29			0.474—0.640 0.565
oral sucker	l	0.087—0.112 0.099	0.057 × 0.070 to	0.060 × 0.085 to	0.049—0.064 ×	0.112—0.154 0.125
	w	0.104—0.123 0.111	0.079 × 0.066	0.085 × 0.080	0.086	0.134—0.168 0.145
pharynx	l	0.053—0.081 0.064	0.066 × 0.044	0.06—0.07 ×	0.042 × 0.060	0.078—0.101 0.089
	w	0.064—0.081 0.073		0.06		0.084—0.112 0.094

Table 5. (Continued)

		Prohemistomulum opacum				Prohemistomulum sp.
		own material	Wisniewski 1934 viable flukes	Wisniewski 1934 fixed flukes	Sudarikov et al. 1962	own material
acetabulum	l	0.039 – 0.056 0.047	0.037 × 0.050 to 0.055 × 0.055	0.040 × 0.040 to 0.050 × 0.050	distinctly smaller than oral sucker	absent
	w	0.048 – 0.070 0.062				
Brandes organ	l	0.358 – 0.397 0.386	1/2 – 2/3 of the body		0.224 – 0.259	0.371 – 0.486 0.442
	w	0.256 – 0.294 0.277				0.346 – 0.474 0.415

l = length, w = width

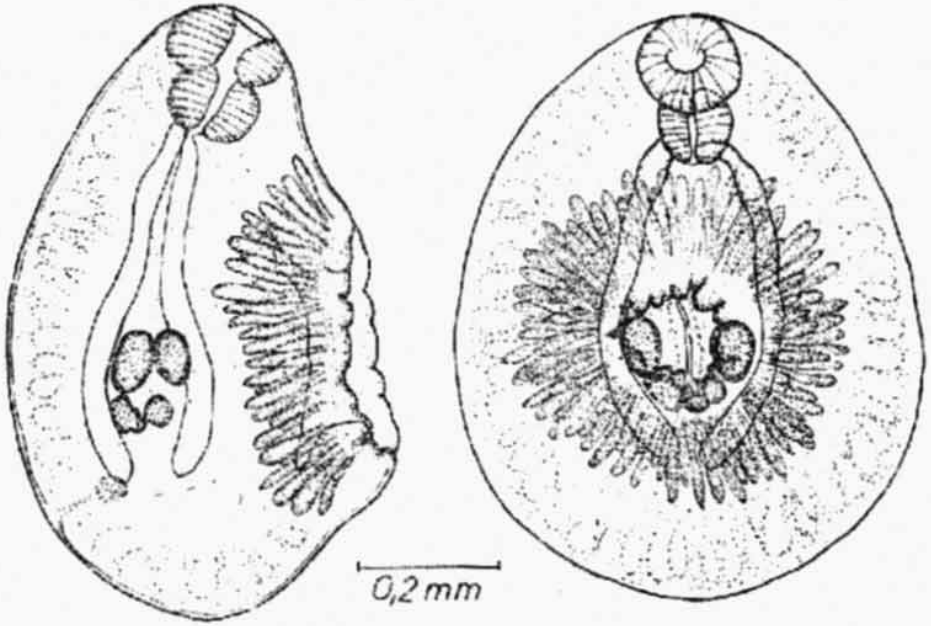

Fig. 3. *Prohemistomulum sp.*

Table 6. *Prohemistomulum* sp.—review of supplementary hosts and their worm invasion in the individual localities

		Kameničná 1	Komárno 1
Hirudo medicinalis	a	–	4
	b	–	2
	c	–	50%
	d	–	1 – 26
	e	–	13.5
Haemopis sanguisuga	a	61	12
	b	28	9
	c	46%	75%
	d	1 – 79	2 – 52
	e	25.4	19.3

and also the mechanical liberation of the larvae is easier. All measurements of the cyst and the metacercariae are given in Tab. 5 together with the data of *P. opacum*.

The metacercariae isolated from the cysts and fixed with the hot fixation are of a widely oval shape. The body is moderately flattened. The dorsal side is vaulted, the enormous Brandes organ protrudes from the flat ventral field. The very muscular oral sucker is subterminal, followed by the pharynx, which is approximately by one third smaller than the oral sucker. The oesophagus is missing, the caeca terminate close in front of the posterior border of the Brandes organ. The acetabulum is not developed. The Brandes organ covers more than half the length and about two thirds of the width of the ventral field of the larval body. It has a relatively large deep central hollow surrounded by irregularly lobate borders. The anlage of the gonads lies under the Brandes organ and is arranged similarly as in the foregoing species. In relation to the size of the body it is slightly smaller.

The larvae were fed to young ducklings coot (*Fulica atra* L.), crows (*Corvus corone cornix*, L.) and freshly hatched chicks, however no adult worms were recovered. This fact together with the observation of a less developed gonad anlage, the absence of the acetabulum and, finally, the finding of isolated cysts of a distinctly wider and shorter shape lead to the assumption that the larvae concerned are not fully developed larvae of *Prohemistomulum opacum*. We therefore started to rear for three months in the laboratory leeches from the canal near Kolárovo, where the intensity and extensity of invasion was found rather high. In part of the leeches dissected immediately after being brought to the laboratory, no liberated larvae were found. After the three months the shape of the oval cysts was still the same and practically no changes were observed in their intensity. The feeding experiment with freshly hatched chicks showed that the larvae from these oval cysts can live in the digestive tract of the definitive host. Six hours after the feeding of the cysts we found the larvae strongly attached to the mucous membrane of the blind intestinal branches of the chick. The borders of the Brandes organ were strongly attached to the mucous membrane and the place of attachment was distinctly hyperemic.

Having found no information in the literature on similar larvae we consider them a new species, most probably belonging to the genus *Cyathocotyle* Mühling, 1896, probably to one of its species which have either no acetabulum or with only a poorly developed acetabulum. Only careful studies of the life cycle or at least the recovering of the adult fluke will definitely solve the problem which of the species these larvae belong to.

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J. V., Zoologický ústav
přírodověd. fak.,
Kotlářská 2, Brno, ČSSR