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CONTRIBUTION TO THE KNOWLEDGE OF THE SPECIES GYRODACTYLUS APHYAE MALMBERG, 1957 AND G. PANNONICUS MOLNÁR, 1968 (MONOGENOIDEA: GYRODACTYLIDAE)

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Abstract: The extent of metrical and morphological variability of the hard parts of the opisthaptor has been determined for the species *G. aphyae* Malmberg, 1957 and *G. pannonicus* Molnár, 1968 parasitizing *Phoxinus phoxinus* (L.). The author claims that changes in the measurements of the individual hard parts of the opisthaptor occur directly in dependence on changes in the water temperature. For both *G. aphyae* and *G. pannonicus*, the bottom borderline of metrical variability is formed by individuals present in an environment with a higher temperature, the upper border by individuals present in an environment with a lower temperature.

INTRODUCTION

In 1957, Malmberg described a new subspecies of the genus *Gyrodactylus* Nordmann, 1832 from the skin and fins of *Phoxinus phoxinus* (L.) caught in brackish water of the Stockholm Archipelago at Nämdö on July 2, 1956. The author named this subspecies *G. wageneri aphyae*. In addition to drawings of the anchor complex, marginal hook, cirrus pouch and brief characteristics reading „. . . anchors rather narrow and posterior part of ventral bar membrane pointed . . .", the author gave data (Table 1, p. 50) on measurements of the body, oesophagus, cirrus pouch, receptaculum seminis, testis and hard parts of the opisthaptor concerning not only parasites from the type locality, but also those from brooks in the parishes Aneboda, Småland and Sunne (Jämtland) (fresh water). Later, on the basis of comparative studies on the marginal hooks and the anchor complex of the opisthaptor, the author (Malmberg, 1964) concluded that the worms represented an independent species, i.e., *G. aphyae*.

In 1970, Malmberg reopened the *G. aphyae*-problem. A re-examination of his *Gyrodactylus* material disclosed that the specimens used for the original description of *G. wageneri aphyae* represented, in fact, two species differing from one another in the shape of the hook proper of the marginal hooks, and in the overall length of the anchors. He stated in his redescription of *G. aphyae* that the only valid parts of his redescription of this species are the drawings of the cirrus, anchors, bars, marginal hook, and the measurements of the individual parasites from other than the type-locality, i.e., from Aneboda and Jämtland.

Table 1. Measurements (in μm) of the hard parts of the opisthaptor of *G. aphyae* Malmberg, 1957

	Malmberg		Ergens' results
	1957	1970	
Total length of anchors	52–64	56–64	51–69
Length of basal part	–	–	37–51
Length of point	23–32	28–32	25–34
Length of root	12–20	16–20	14–22
Length of ventral connecting bar	7–10	7–10	5– 9
Width of ventral connecting bar	23–31	24–31	22–32
Length of membranous extension	13–16	13–16	12–18
Length of dorsal connecting bar	1– 3	2– 3	1– 3
Width of dorsal connecting bar	18–28	20–24	16–24
Total length of marginal hooks	26–34	30–34	28–41
Length of hook proper	5– 6	5	5– 6

Although Malmberg disposed of 102 specimens of these parasites, he exactified, but did not extend, the knowledge of their diagnostic signs in his redescription. In spite of the fact that the characteristics of the species *G. aphyae* given in drawings of the anchor, the principal connecting bar and the marginal hook of the holotype, and also the metrical data obtained for individuals from other than the type locality, complied, formally, with the requirements of the International Code of Zoological Nomenclature, they did not comply with the requirements of the practice as regards the identification of the species. Under the present circumstances, the parasite may be mistaken for another, morphologically and metrically similar species, particularly for the species *G. pannonicus* Molnár, 1968. The author himself inferred that this species „ . . . ist am meisten der Art *G. wageneri aphyae* ähnlich, mit der es in Mischinfektionen vorkommt, doch unterscheidet es sich davon durch kleinere Körper- und Hakendimensionen". Hence, the species originally mistaken by Malmberg for *G. aphyae*, may, in fact, have been a *G. pannonicus*. This assumption may, or may not, be correct, but in view of the data available and, mainly, having regard to the considerable similarity of of *G. aphyae* to *G. pannonicus*, the elucidation of this problem requires additional knowledge of the variability of both species.

MATERIAL AND METHODS

In order to evaluate both morphologically and metrically the diagnostic signs of *G. aphyae* I used solely fixed material comprizing more than 400 specimens mounted in glycerine-jelly and ammonium picrate. I obtained the worms from various localities in Czechoslovakia (legit. author), Hungary (legit. Dr. K. Molnár), Yugoslavia (legit. author), the U.S.S.R. (from the collection of the Zoological Institute, Academy of Sciences, Leningrad) and from Mongolia (legit. author). Fixed material was used also for *G. pannonicus* (23 specimens) obtained from Hungary (including two syntypes — legit. Dr. K. Molnár), the U.S.S.R. (Kola-peninsula, legit. Dr. Mitenov), and Czechoslovakia (legit. author).

In addition, I studied metrical and morphological changes in the hard parts of the opisthaptor of these parasites in association with changes in the water temperature using for these studies other 120 specimens of *G. aphyae* and 90 specimens of *G. pannonicus*. The parasites were obtained from the skin and fins of equally large specimens of *Phoxinus phoxinus* (L.) (length 75 to 85 mm) caught in regular monthly intervals (from February till July) in one section of a small

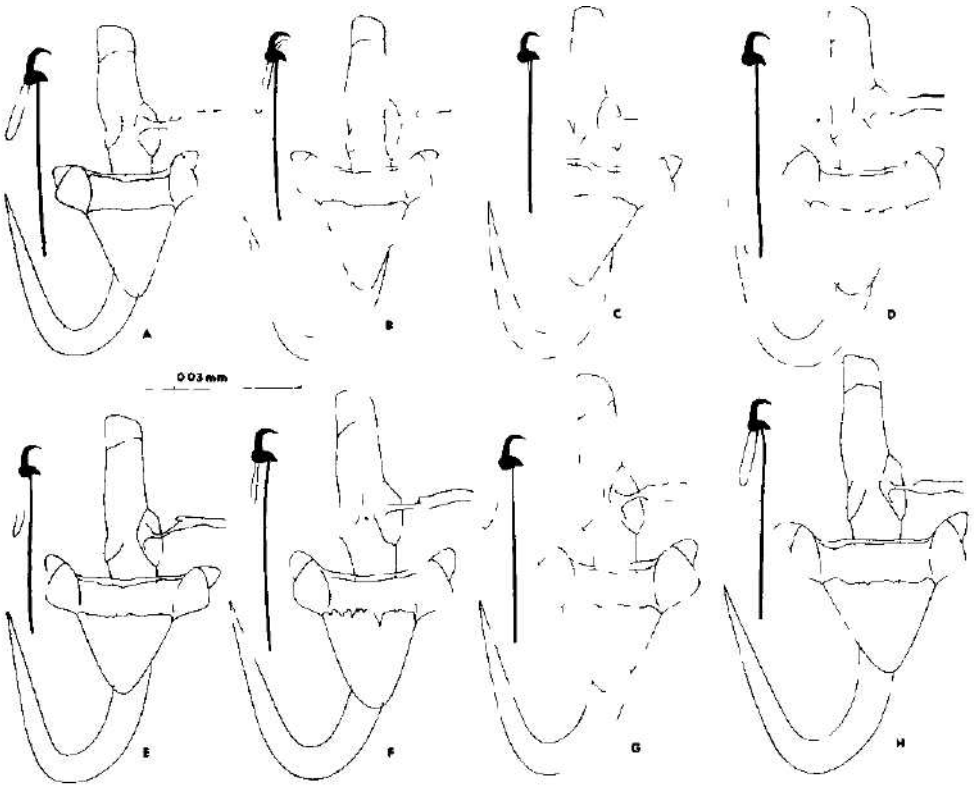


Fig. 1. Morphological variability of the hard parts of the opisthaptor of *Gyrodactylus aphyae* Malmberg, 1957 from the fins of *Phoxinus phoxinus* (L.). A — from the brook Rokytka (Czechoslovakia, July 17, 1973); B — from lake Ležiště (Czechoslovakia, September 19, 1960), C — from the Ponoj River (U.S.S.R., Kola Peninsula, July 13, 1969); D — from the brook Rokytka (Czechoslovakia, June 19, 1973), E — from the Osoblaha River (Czechoslovakia, May 16, 1961); F — from the Tul River (Mongolia, April 7, 1966); G — from the brook Rokytka (April 17, 1973); H — from lake Veliko Crno (Yugoslavia, November 7, 1969).

brook near Prague. The water temperature was taken at each sample collection. Apart from *P. phoxinus*, the only other fish species present in this locality was *Noemacheilus barbatulus* (L.).

The measurements (in μm) and drawings of the individual hard parts of the opisthaptor were made with a phase contrast microscope and a camera lucida.

RESULTS

Gyrodactylus aphyae Malmberg, 1957

According to Malmberg's redescription (1970), the extent of the originally determited metrical variability of most of the hard parts of the opisthaptor of *G. aphyae* was greatly reduced. E.g., that of the total anchor length by one third, of the point and anchor extension, and of the total length of the marginal hooks by one half, etc. (Table 1). With regard to the number of specimens measured (six parasites only, obtained from fishes caught in June and July in two localities — Malmberg, 1970, p. 214), the extent of variability need not be necessarily definitive. This assumption is supported by

the results of my study on a large material collected in various localities of the Palaearctic region (Table 1). Therefore, *G. aphyae* may be considered to be a species with a considerable metrical variability of its hard parts of the opisthaptor.

The results of studies on the morphological variability shown in Fig. 1 indicate that no marked morphological changes in the complex of anchors and marginal hooks occurr in association with the metrical variability. Certain deviations may occur if the individuals compared are at different morphogenetical stages (Fig. 2). Evidently, in fixed material, some of the

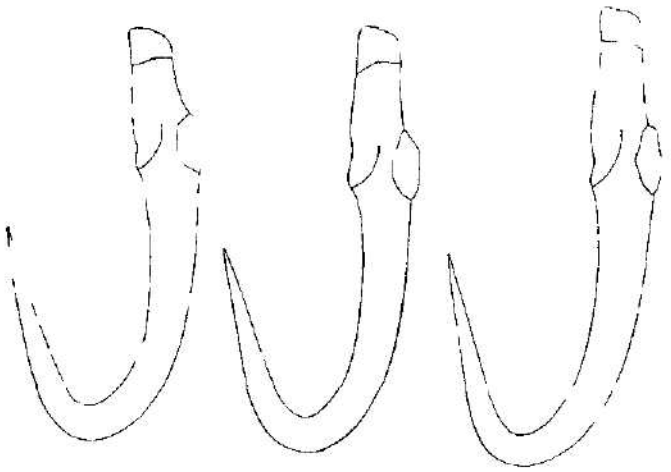


Fig 2 Morphological variability of the anchors of three specimens of *Gyrodactylus aphyae* Malmberg, 1957 obtained from a single specimen of *Phoxinus phoxinus* (L.) (Tul River near Ulan Bator, Mongolia, April 7, 1966)

changes observed may be due to the process of fixation such as a different angle between the point and the base of the anchors, the thickness of the bend of their extension, etc.

Table 2. Measurements (in μm) of the hard parts of the opisthaptor of *G pannonicus* Molnár, 1968

	Molnár, 1968	Ergens' results
Total length of anchors	45—47	45—54
Length of basal part	32—34	33—38
Length of point	24—26	23—27
Length of root	12—16	13—18
Length of ventral connecting bar	5—7	5—6
Width of ventral connecting bar	21—22	13—18
Length of membranous extension	8—10	9—13
Length of dorsal connecting bar	1—2	1—2
Width of dorsal connecting bar	19—20	13—20
Total length of marginal hooks	25—27	25—29
Length of hook proper	3—5	5—6

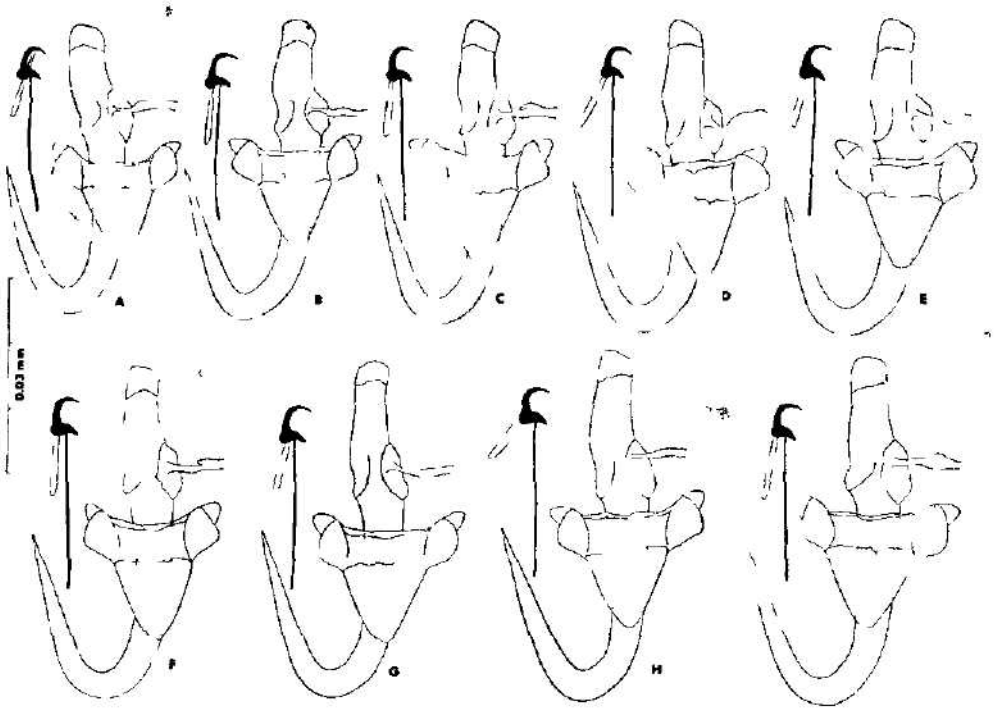


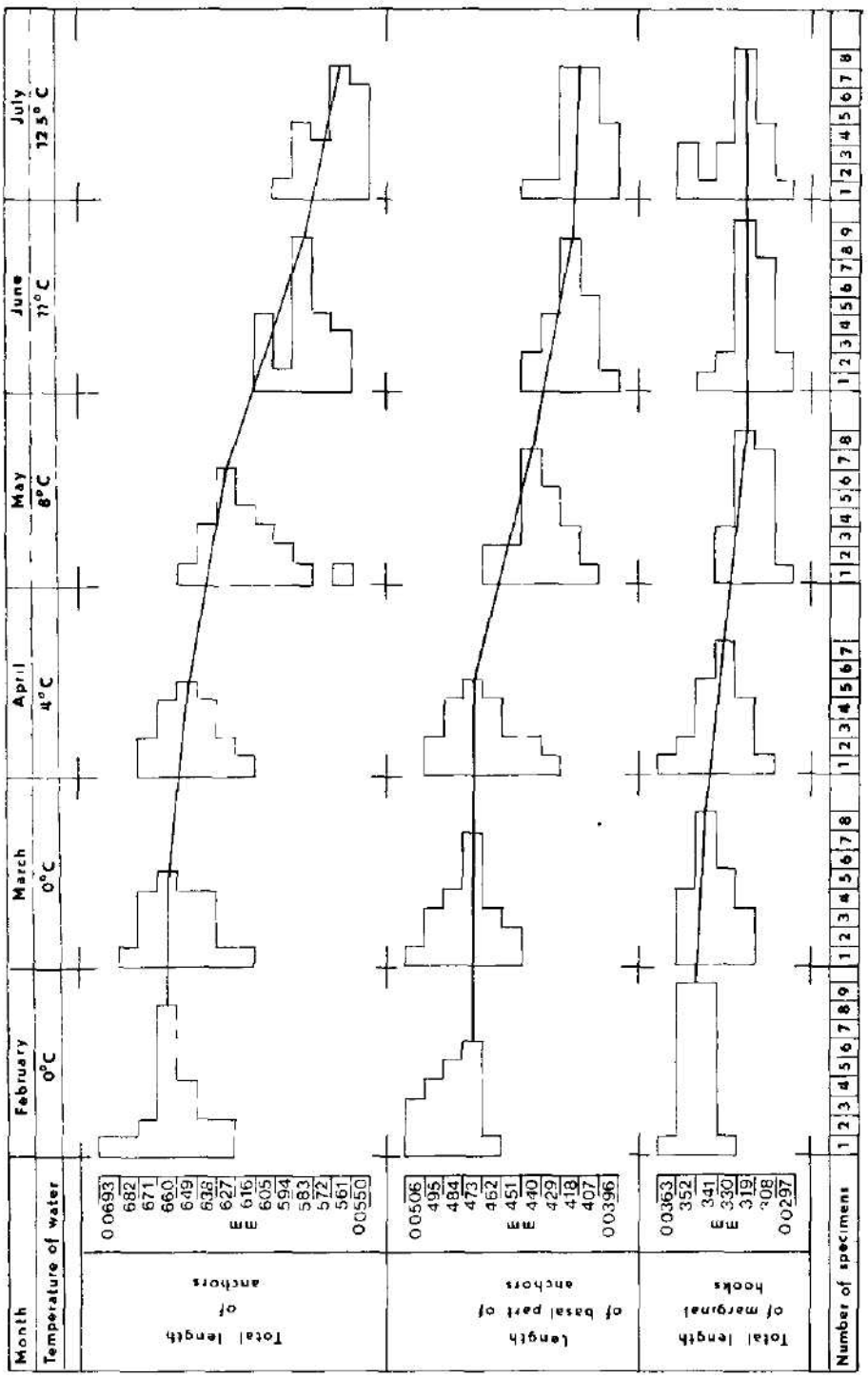
Fig. 3. Morphological variability of the hard parts of the opisthaptor of *Gyrodactylus pannonicus* Molnár, 1968 from the fins of *Phoxinus phoxinus* (L.) caught in the brook Rokytka A, B — July 17, 1973, C, D, April 19, 1973, E, F — January 1, 1973; G, H — March 12, 1973, I — January 15, 1973.

Gyrodactylus pannonicus Molnár, 1968

The original variability of the hard parts of the opisthaptor of *G. pannonicus* was determined on the basis of measurements obtained from 10 specimens. However, the results of my studies showed a considerable increase in the extent of this variability, particularly in the total length of the anchors, the length of the base and point, that of the membranous extension of the ventral connecting bar, the width of the dorsal connecting bar and the total length of the marginal hooks (Table 2).

The morphological variability of *G. pannonicus* (Fig. 3) can be evaluated in a way similar to that of *G. aphyae*. The shape and ratio of the individual hard parts of the opisthaptor remain unchanged regardless to an increase or decrease of their measurements.

The results indicate that *G. aphyae* and *G. pannonicus* are two very similar species. The maximum metrical values of all diagnostic signs of *G. pannonicus* are analogous to the minimum metrical values of diagnostic signs of *G. aphyae*. Differences in the shape of the anchors of both species are negligible, i.e., the extension of the anchors of *G. aphyae* is more or less straight, that of *G. pannonicus* shows a tendency to bend its distal portion in ventral direction. The shape of the ventral and dorsal connecting bars including the membranous extension is similar in both species. This fact causes frequent confusion (one species is mistaken for the other) particularly in imperfectly fixed


Month
February
March
April
May
June
July
Temperature of water
0°C
0°C
4°C
8°C
11°C
12.5°C
Total length of anchors
Length of basal part of anchors
Total length of marginal hooks
Number of specimens
0.0693
682
671
660
649
638
627
616
605
594
583
572
561
0.0550
0.0506
495
484
473
462
451
440
429
418
407
0.0396
0.0363
352
341
330
319
308
0.0297
mm
1 2 3 4 5 6 7 8 9

material (deformed by fixation etc.). The only reliable diagnostic sign enabling differentiation between the two species is the shape of the hook proper. In *G. aphyae*, its base is more massive and shorter than that of *G. pannonicus*, and the transition of its shaft to the point is not distinct in *G. pannonicus*, because both parts form an almost regular arch. In *G. aphyae*, the shaft is always straight and the transitional part from the shaft to the point is very distinct.

If we obtained both *G. aphyae* and *G. pannonicus* from either the same fish (*P. phoxinus*), or from several, simultaneously examined fish hosts from the same locality, it should be possible to distinguish them by the measurements of most of their diagnostic signs. The possibility of this differentiation is illustrated by the following results which are intended to serve only for the purpose of orientation, because they are based on the evaluation of material obtained only in order to test the method of sample collection at regular time intervals. I conclude from the measurements of 120 specimens of *G. aphyae* and 90 specimens of *G. pannonicus* collected from fishes of the same locality caught in monthly intervals within the course of 6 months that metrical changes in the hard parts of the opisthaptor occur in dependence on changes of the water temperature. Apart from the hook proper, all other hard parts of the opisthaptor decreased in accord with an increase in water temperature and vice versa. This fact is well evidenced by changes in the total length of the anchors, the length of their base and the total length of the marginal hooks (actually the handle of the marginal hook of *G. aphyae*). Each histogram (Fig. 4) shows one group of individuals (specimens) arranged in accord with the length of the pertinent hard part of the opisthaptor and the extent of its metrical variability. The curve obtained by joining the peaks of the individual histograms indicates the intensity and direction of the shifting of variability during an increase of the water temperature, in this case from zero to 12.5° C. Remarkable changes occur in the total length of the anchors, and in the length of their base: at 0—3°C, the minimum values of these hard parts are higher than the maximum values obtained at 12.5°C. This indicates that the bottom border of their metrical variability attained during the coolest period does not overlap the upper border of metrical variability attained during the warmest period. With regard to the fact that changes in the size of the individual hard parts of the opisthaptor of *G. pannonicus* follow a similar course, it may be inferred that the bottom border of total metrical variability is formed for both *G. aphyae* and *G. pannonicus* by individuals present in the "warm environment", and the upper border of this variability by individuals present in the "cool environment". The fact that the hard parts of the opisthaptor of *G. pannonicus* from the "cool environment" attain a similar size to those of *G. aphyae* from the "warm environment" is responsible for the afore-mentioned overlapping of the maximum values of measurements of *G. pannonicus* and the minimum values of measurements of *G. aphyae* only in the case of the comparison of their total metrical variability. If, however, the two parasite species are obtained from either the same sample, or from several samples collected in

Fig. 4. Graphical illustration of the parallelly occurring changes in the temperature of the water and the measurements of the anchors, the basal anchors part (shaft) and the total length of the marginal hooks in 120 specimens of *Gyrodactylus aphyae* Malmberg, 1957.

the same locality and at the same time, the measurements of their diagnostic signs are sufficiently different to enable a differentiation between the individual worm species.

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The Regional Museum in Teplice (Bohemia),
Department of Systematic Zoology, Charles University, Prague

ON THE OCCURRENCE OF THE FLOUNDER, PLEURONECTES FLESUS LINNAEUS, 1758 (OSTEICHTHYES, PLEURONECTIDAE) *IN BOHEMIA*

Ivo FLASAR and Ota OLIVA

Dedicated to 75th birthday of Professor S. A. Hrabě, DrSc.

Received on February 11, 1974

Abstract: The authors describe a single Bohemian specimen of the flounder, *Pleuronectes flesus* Linnaeus, 1758, which according to Michel (1929) was captured in 1912 in the mouth of the river Ploučnice that opens into the Labe at the town of Děčín. This specimen was obtained for the Agricultural Grammar School at Kadaň. The zoological collection of the museum in the town Teplice in Bohemia received this specimen in 1972. The old Bohemian documentary specimen of the flounder from the vicinity of Roudnice, coming From the river Labe (see Frič, 1908), was lost.

INTRODUCTION

The flounder is the single species of the family Pleuronectidae which has migrated upstream through the river Labe into Bohemia. From Bohemia this specimen was known already to Balbín (Frič, 1872, 1908). According to Schubert (1943) the flounder had ascended into the river Labe. The Emperor Ferdinand I, of the Habsburg dynasty (1503—1564), ordered to transport living flounders captured in the Labe near Ústí nad Labem to Prague. The specimens were given into wooden cage which was dragged behind the boat upstream to Prague. According to Schubert (l.c.) the flounder ascends regularly to Magdeburg and sometimes up to Meissen. In 1909 two flounders were captured by net near Ústí nad Labem. In 1914, another flounder was captured at Děčín in the castle's back-water of the river Ploučnice. Probably this specimen may be the same that is described hereunder.

Frič (1908) noted two flounders caught from the backwater of the river Ploučnice near Benešov nad Ploučnicí. Further, he described another specimen captured in the river Labe near Roudnice nad Labem, which was donated, to the National Museum in Prague. This specimen is 20 cm long, the eyer are on the left side of the body (in most specimens eyes are on the right side — see Berg, 1949). The photograph this documentary specimen was published in the book of Frič (1908), but the specimen was apparently lost in subsequent years, because during the revision of the whole collection

of Bohemian fishes, made by the second author (Oliva, 1963), this specimen was not found. In spring (1912) a small specimen of the flounder was caught in the mouth of the river Ploučnice into the Labe (Michel, 1929). It was stuffed (according to the manner of conservation by J. Michel or by E. Tschinkel in Děčín) for the Agricultural School in the town Kadaň. During the search for rare documents of northern Bohemian fauna in various school collections, this stuffed specimen was found by the senior author.

RESULTS

The description of this flounder is as follows: The total length is 250 mm, body length up to the base of the caudal fin rays is 210 mm. The smallest body depth is 8.6% of the body length, 7.2% of the total length, 20% of the largest depth. The largest body depth is 42% of its length. Maximum head length, measured from the foremost point of the lower jaw up to the end of the operculum, is 25% of the body length, or 21% of the total length. The length of the snout is 5.7% of the body length, 21% of the head length. Diameter of the eye is 3.8% of the body length, 14.6% of the head length. The length of the caudal peduncle is 12.4% of the body length. The P length is 10% of the body length.

The plastic characters are influenced by the mode of preservation, which are comparatively better. The fins, especially the paired ones, are damaged and their distal ends are broken up. Since the body surface was varnished it is not possible to ascertain the number of scales in the lateral line. There are about 80 scales. In dorsal fin and anal fin there are 56 and 39 rays respectively. The caudal peduncle is without bony tubercles, these are agglomerated only in the first portion of the body, above and below the lateral line.

DISCUSSION

For the description of our specimen the characters as used by Smitt (1892), Berg (1949) and Banarescu (1964) have been taken into account. Unfortunately, in preserved stuffed specimen the use of such characters as the number of gill rakers is not possible. This feature is important for the recognition of subspecies of the flounder The fact that our specimen was captured in the river Labe and no bony tubercles (knobs) can be found on the surface of the caudal peduncle shows that it belongs to the nominate form, *Pleuronectes flesus* Linnaeus, 1758. Concerning subspecies refer Berg (1949) and Banarescu (1964).

The presence of the flounder in the mouth of the river Ploučnice is interesting with regard to the older data of Frič (1908), who writes that "some years age I was informed by teacher Michel in Podmoklí, at Benešov (Bensen), that two adult specimens of flounder were captured in the Ploučnice, in the back-water, separated from the main river flow through the construction of the railway rampart. Here the fishermen sucked out the water and among other fishes also two flounders were captured and transported into the castel. Since I was un able to find anything about the rests of these fish, the thing itself remains uncertain . . ." (translated from Czech text).

It is evident that the specimen described here was captured in 1912 and is most probably the third documentary specimen from this locality, or even the fourth one, when we take into consideration the statement of Schubert

for 1914 (see Schubert, 1943). Frič's (1908) statement, that (translated from Czech) ". . . the specimen from Roudnice is the young one and grew up here apparently from eggs, laid here by the adult flounder, which returned then again into the sea . . .", is erroneous. The reproduction of the flounder takes place in the sea, as was known already to Hamilton (1943).

Most flounders have eyes on the right side of the body; the number of specimens having the eyes on the left side is smaller and changes considerably. At the coast of West Germany, the number of specimens with eyes on the left side is greater than at the coast of England. In the Baltic Sea, in the bay "Vismar Bucht", about 27% of the population possess eyes on the left side (Arndt and Nehls, 1964). Therefore, the specimen with the eyes on the left side, reported by Frič (1908, p. 36) from Bohemia, is very interesting.

As regards ascending of the flounder into various European rivers we have notes of Hamilton (1943), who mentioned the river Avon (in the area of 3 miles at Bath) and the river Thames to Toddington and Sunbury. Sélys-Longchamps (1842) mentioned the Neth at Waterloo, Liége, the Mosel up to Metz (the findings from 1818). Siebold (1863) quoted these findings and added also Trier, which lies naturally lower downstream the river Mosel, then the Rhine up to the town Mainz and — which is very curious — also the river Main up to Klinkenberg, which is more upstream than Aschaffenburg. Redeke (1941) also published findings from the river Rhine up to Mannheim and the river Mass up to Maastricht, where in the past the living flounders were offered in the fish-market. Also Bauch (1957) mentioned single specimens of flounder penetrating rivers — into the Rhine up to Mainz, into the Labe (Elbe) up to Magdeburg, through the river Saale up to Calbe; the flounders were captured rarely also in Berlin, and through the rivers Weser and Laine they penetrated up to Hannover. From England, Travis-Jenkins (1942) mentioned ascending of flounders in the river Severn up to Montgomeryshire, in the Dovey it has been recorded from Dinas Mawdwy, in the Concay above Trefriw and in the Wyre from Garstang. The anadromous migrations into the fresh water have nutritive character; after the decrease of water temperature flounders are descending into the sea again (Muus and Dahlstroem, 1968) Berg (1949) mentioned among rivers ascended by the flounder the river Neckar. Interesting is the recording of ascends through the Rhine up to Basel, a statement which could not be verified. From the facts quoted above it is evident that the findings from the mouth of the river Ploučnice are the evidence of the longest migrations of the flounders upstream into the continent.

SUMMARY

Only one documentary specimen of the flounder, *Pleuronectes flesus* Linnaeus, 1758, from the mouth of the river Ploučnice into the Labe (Elbe), captured in the spring 1912, was saved and it is now deposited in the collections of the Regional Museum in Teplice v Čechách. The total length is 250 mm, the body length 210 cm, the caudal peduncle without bony knobs in D56, in A39 rays. The specimen belongs to the Northern Sea nominate subsoecies, characterised among others also by the smooth surface of the caudal peduncle. This specimen can serve also as the example of the longest distance of penetration of the flounder into continental river.

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ENHANCEMENT OF PATHOGENICITY OF CONIDIOBOLUS CORONATUS FOR THE TERMITES COPTOTERMES FORMOSANUS AND RETICULITERMES LUCIFUGUS BY PRECULTIVATION ON AN INSECT HOST

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Abstract: The fungus *Conidiobolus coronatus* (Costantin) Srinivasan et Thirumalachar obtained by air contamination killed 30—95% of *Coptotermes formosanus* Shiraki and 10—75% of *Reticulitermes lucifugus* (Rossi). Out of its 15 reisolates examined only 7 showed higher pathogenicity for the workers of *Coptotermes formosanus* than the original strain. Six of them were precultivated on *C. formosanus*, one on the larvae of *Galleria mellonella* L. Of the 10 reisolates of *C. coronatus* examined only 4 were more pathogene for *R. lucifugus* than the original strain. Three of them were isolated from *C. formosanus*, one from *G. mellonella*. Reisolates from *R. lucifugus* showed no enhanced pathogenicity either for *C. formosanus* or for *R. lucifugus*.

INTRODUCTION

In most fungi pathogenic for insects an enhancement of pathogenicity was observed after precultivation on an insect host (in: Müller-Kögler, 1965). Also in our previous papers (Krejzová, 1971, 1972) we recorded that the strains of the species belonging to the genus *Entomophthora* and *Basidiobolus* freshly reisolated from the same host species, a relative one and in some cases also from a distant one, showed higher pathogenicity than the strains maintained in vitro for a long time.

The aim of our present experiments was to enhance the effectivity of *C. coronatus*, an insect-pathogenic fungus. The original species *Conidiobolus coronatus* (Costantin) Srinivasan et Thirumalachar (1964) used in our experiments was obtained as an air contamination in a new building (Krejzová, 1975). We were unable to ascertain its origin, whether it was a parasite or saprophyte. With regard to the fact that some strains of *C. coronatus* were isolated from termites and applied with success in their experimental infection (Kevorkian, 1937; Altson, 1947, Yendol and Paschke, 1965), this species seemed very suitable for our pathogenicity-enhancement experiments. Moreover this fungus occurs in the nature not only as a parasite but also as a saprophyte and thus would be capable of maintaining and growing in the nests of termites even without the direct contact with the host. On the basis of our previous experience we used the strains precultivated on the insects of the same, relative or distant, species.

MATERIAL AND METHODS

The reisolates were obtained in the following manner The insects which died of fungus infection were placed in sterile petri dishes with moistened filter paper on the bottom After 24 to 36 hours, when almost the whole surface of termites was covered with conidiophores with conidia (Figs 1, 2, 3), the insects were transferred to Sabouraud's glucose agar containing penicillin and streptomycin During the following 4—8 hours the fungus discharged a large number of conidia on the nutrient medium and the dead insect bodies were then removed In case of mass infection of animals, from which the reisolations were made, the fungus culture grew rapidly, the contamination with saprophytic fungi occurred only occasionally

We have studied the pathogenicity of 15 reisolates of *C coronatus* for the termites *Coptotermes formosanus* Shiraki and of 10 reisolates of the same fungus for *Reticulitermes lucifugus* (Rossi). The strains were precultivated on *C formosanus*, *R lucifugus* and *Galleria mellonella* L. We used the material first after the reinoculation of the isolated culture and then during the following 11 months

The cultures for infection with the original strain as well as reisolates were grown in small petri dishes containing coagulated egg yolk of Sabouraud's glucose agar Three-day old cultures grown on the same media in test tubes were used for inoculation After 3—5 days of cultivation of the fungus in petri dishes the termites were introduced into the lid of the dish under the culture and kept there for six hours The termites were then transferred to a larger petri dish with a piece of moistened cellulose cotton wool on the bottom We used the laboratory reared specimens of *C formosanus* or *R lucifugus*, 20 workers, or pseudergates and grown larvae of both species in each experiment The control specimens were kept under identical conditions without contamination of the fungus Every day the dead termites were collected and put on the bottom of a petri dish covered with moistened filter paper Examinations of the dead animals were made after 24 to 36 hours superficially under the microscope and by dissection Infected termites were covered with conidiophores with conidia within 24—36 hours and their body cavity was full of hyphal bodies.

The surviving termites from the controls of the experiments with the original strain, as well as from those of the following experiments with the reisolates, lived under the experimental conditions for even more than 90 days, after this period they were killed with ethyl alcohol

The experiments were repeated several times and the results were summarized The percentage of mortality caused by infection was corrected by the mortality of controls according to the following formula (Abbott, 1925)

$$\text{corrected mortality (\%)} = \frac{\text{test mortality (\%)} - \text{control mortality (\%)}}{100 - \text{control mortality (\%)}} \times 100$$

The probable error of the arithmetic mean was calculated after the formula Jakovlev, 1958

$$\rho_A = 0{,}6745 \sqrt{\frac{\Sigma\varepsilon^2}{n(n-1)}}$$

where

$\Sigma\varepsilon^2$ = sum of squared numbers of the deviations of the arithmetic mean

n = number of measurements

The results concerning the mortality caused by infection corrected by the mortality of controls and control mortality are given in graphs.

The values of effectivity of the original strain in comparison with individual reisolates were made precise by using distribution tables in the following way 1. percentage of mortality was divided into 5 intervals increasing gradually by 20%, 2. representation of individual intervals in each reisolate was evaluated in relation to the original strain

RESULTS

Seven of the 15 reisolates of *C. coronatus* produced higher mean mortality than the original strain. Of these six strains were precultivated on *C formosanus* (1—3, 9, 12, 13), one on *G. mellonella* (5). In other cases the mortality was lower than that of the original strain (Table 1).

When compared to the mortality rate caused by the original strain, the 7 reisolates mentioned produced the minimum mortality by 5—50% higher, maximum mortality was higher by 5% only with two reisolates (2, 3), while

Table 1. Pathogenicity of reisolates of *C. coronatus* for the termites *C. formosanus* in comparison with the original strain

No. of reisolate	Insect species used for precultivation	Pathogenicity for *C. formosanus**)
1	*C. formosanus*	+
2	*C. formosanus*	+
3	*C. formosanus*	+
4	*R. lucifugus*	−
5	*G. mellonella*	+
6	*R. lucifugus*	−
7	*R. lucifugus*	−
8	*G. mellonella*	−
9	*C. formosanus*	+
10	*C. formosanus*	−
11	*G. mellonella*	−
12	*C. formosanus*	+
13	*C. formosanus*	+
14	*C. formosanus*	−
15	*C. formosanus*	−

*) + enhancement of pathogenicity
− drop of pathogenicity

with the others it was either the same as with the original culture (1, 9, 13) or even lower (12) by 5%. The mean percentage of mortality of the 6 reisolates precultivated on *C. formosanus* increased by 12—37% in all cases. The reisolate from the larvae of *G. mellonella* (5) produced minimum mortality lower by 10%, maximum mortality was the same as with the original culture and the mean mortality was higher by 6% (Table 2, Graph 1).

As it follows from the distribution table (Table 7), in one third of experiments with the original strain of *C. coronatus* the percentage of mortality of *C. formosanus* corresponded to two lowest intervals. When using the reisolates precultivated on *C. formosanus* 1, 2, 9, 12 and 13, not a single result corresponded to these two intervals. In the experiments with the

Table 2. Mortality of the termites *C. formosanus* after treatment with the original strain and reisolates of *C. coronatus*

Original strain, reisolates*)	Percentage of mortality			Probable error of arithmetic mean	Number of experiments
	minimum	maximum	mean		
Original	30	95	56	4.86	15
1	45	95	79	5.27	8
2	75	100	93	2.09	8
3	35	100	68	4.71	13
5	20	95	62	7.10	8
9	65	95	80	2.57	6
12	80	90	85	4.16	2
13	65	95	81	6.62	4

*) Numbering of reisolates as in Table 1.

Table 3. Range of mortality period of the termites *C. formosanus* treated with *C. coronatus*

Original strain, reisolates*)	Range of mortality period in days		
	minimum	mean	maximum
Original	3	14	41
1	10	25	38
2	8	19	44
3	3	17	41
5	6	18	57
9	6	26	35
12	29	36	42
13	14	23	35

*) Numbering of reisolates as in Table 1.

reisolate No. 3 less than one third of results corresponded to two lowest intervals. Only with the reisolate No. 5 precultivated on *G. mellonella* more than one third of experiments gave the results corresponding to these two intervals.

When comparing the range of mortality period in individual strains after treatment of the termite *C. formosanus* with the reisolates from *C. formosanus*, the minimum range was 3—29 days, maximum 35—44 days and average 17—36 days. When a reisolate from *G. mellonella* was used for the termites *C. formosanus*, then the shortest period of mortality was 6 days, the longest 57 days and the average 18 days. After treatment with the original strain the shortest period of mortality was 3 days, the longest 41 days and the average 14 days (Table 3).

When using the reisolates from *C. formosanus*, we have succeeded in shortening the maximum range of mortality period in three cases, in the remaining four it was longer than that caused by the original strain. In all cases the mean range of period was higher than with the original strain. Minimum

Table 4. Pathogenicity of reisolates of *C. coronatus* for the termites *R. lucifugus* in comparison with the original strain

No. of reisolate	Insect species used for precultivation	Pathogenicity for *R. lucifugus*
1	*C. formosanus*	+
2	*C. formosanus*	+
3	*C. formosanus*	+
4	*R. lucifugus*	—
5	*G. mellonella*	+
6	*R. lucifugus*	—
7	*R. lucifugus*	—
8	*G. mellonella*	—
9	*C. formosanus*	—
10	*C. formosanus*	—

*) + enhancement of pathogenicity
— drop of pathogenicity

Table 5 Mortality of the termites *R. lucifugus* after treatment with the original strain and reisolates of *C. coronatus*

Original strain, reisolates*)	Percentage of mortality			Probable error of arithmetic mean	Number of experiments
	minimum	maximum	mean		
Original	10	75	40	8.14	7
1	15	95	40	8.82	6
2	30	85	55	4.88	7
3	25	85	54	5.25	9
5	25	65	43	8.53	3

*) Numbering of reisolates as in Table 1.

range of mortality period was also higher than with the original strain, except for one case. The course of infection with a reisolate from *G. mellonella* was longer than with the original strain, as regards the maximum, mean and minimum range of mortality period.

Out of 10 reisolates of *C. coronatus* only 4 were more effective for *R. lucifugus* than the original strain. Three of them were precultivated on *C. formosanus* (1, 2, 3), one on *G. mellonella* (5). On the other hand, no reisolate from *R. lucifugus* (4, 6, 7) showed higher pathogenicity for this termite species (Table 4).

Minimum mortality of *R. lucifugus* treated with reisolates of *C. coronatus* was in all cases higher than after treatment with the original culture (by 5 to 20%). Maximum mortality was increased when using the reisolates 1 (by 20%), 2 (by 10%) and 3 (by 10%). The average mortality was higher after treatment with the reisolates 2 and 3 (by 15 and 14% respectively), while the reisolate 1 produced the same mortality as the original strain. The reisolate 5 from *G. mellonella* produced higher minimum mortality (by 15%), lower maximum mortality (by 10%) and higher mean mortality (by 3%) than the original culture (Table 5, Graph 2).

As it follows from the distribution table (Table 8), in more than one third of experiments with the original strain *C. coronatus* the percentage of mortality of *R. lucifugus* corresponded to the lowest interval. When the reisolates precultivated on *C. formosanus* were used, one third of experiments with

Table 6. Range of mortality period of the termites *R. lucifugus* treated with *C. coronatus*

Original strain, reisolates*)	Range of mortality period in days		
	minimum	mean	maximum
Original	2	28	62
1	2	25	64
2	13	34	59
3	7	29	69
5	7	39	64

*) Numbering of reisolates as in Table 1.

Table 7. Distribution of mortality percentage of termites *C. formosanus* after treatment with the original strain and reisolates from *C. coronatus*

Interval	Original strain, reisolates							
%	P	1	2	3	5	9	12	13
0— 20	—	—	—	—	1	—	—	—
21— 40	5	—	—	3	2	—	—	—
41— 60	5	2	—	2	1	—	—	—
61— 80	—	1	1	4	2	4	1	2
81—100	5	5	7	4	2	2	1	2

P = original strain
1, 2, 3, 5, 9, 12, 13 = reisolates No. 1, 2, 3, 5, 9, 12, 13

reisolate No. 1 corresponded to the lowest interval, whereas the reisolates No. 2 and 3 gave no results corresponding to this interval. The same concerned also the reisolate No. 5 precultivated on *G. mellonella*.

When comparing the range of mortality period in individual strains after treatment of the termite *R. lucifugus* with the reisolates from *C. formosanus*, the minimum range was 2—13 days, maximum 59—69 days and average 25—34 days. When a reisolate from *G. mellonella* was used for infection, the shortest period of mortality was 7 days, the longest 64 days and the average 39 days (Table 6).

In comparison with the original strain, only one reisolate from *C. formosanus* showed lower range of mortality period, the maximum range of all others was higher. Also the mean range of period was lower only with one reisolate from *C. formosanus*. Minimum range of mortality period was higher after treatment with all but one reisolates from *C. formosanus*. The reisolate from *G. mellonella* showed longer period of mortality in all values.

Pathogenicity of all effective reisolates was not substantially changed after 11-month cultivation in vitro.

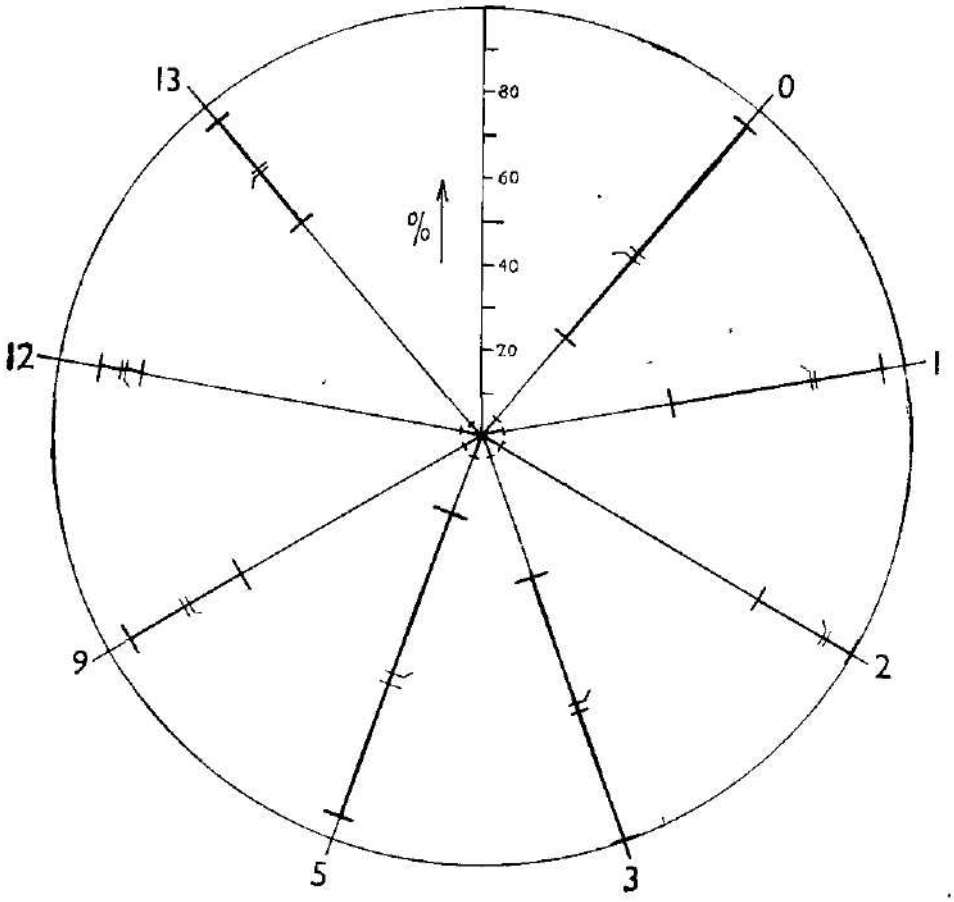
Other strains, whose pathogenicity was not changed by precultivation, are listed in Tables 1 and 4.

The maximum mortality in controls in all experiments was not higher than 5% (Graphs 1, 2).

Table 8. Distribution of mortality percentage of termites *R. lucifugus* after treatment with the original strain and reisolates from *C. coronatus*

Interval	*Original strain, reisolates*				
%	P	1	2	3	5
0— 20	3	2	—	—	—
21— 40	1	2	2	3	2
41— 60	1	1	3	3	—
61— 80	2	—	1	2	1
81—100	—	1	1	1	—

P = original strain
1, 2, 3, 5, = reisolates No. 1, 2, 3, 5

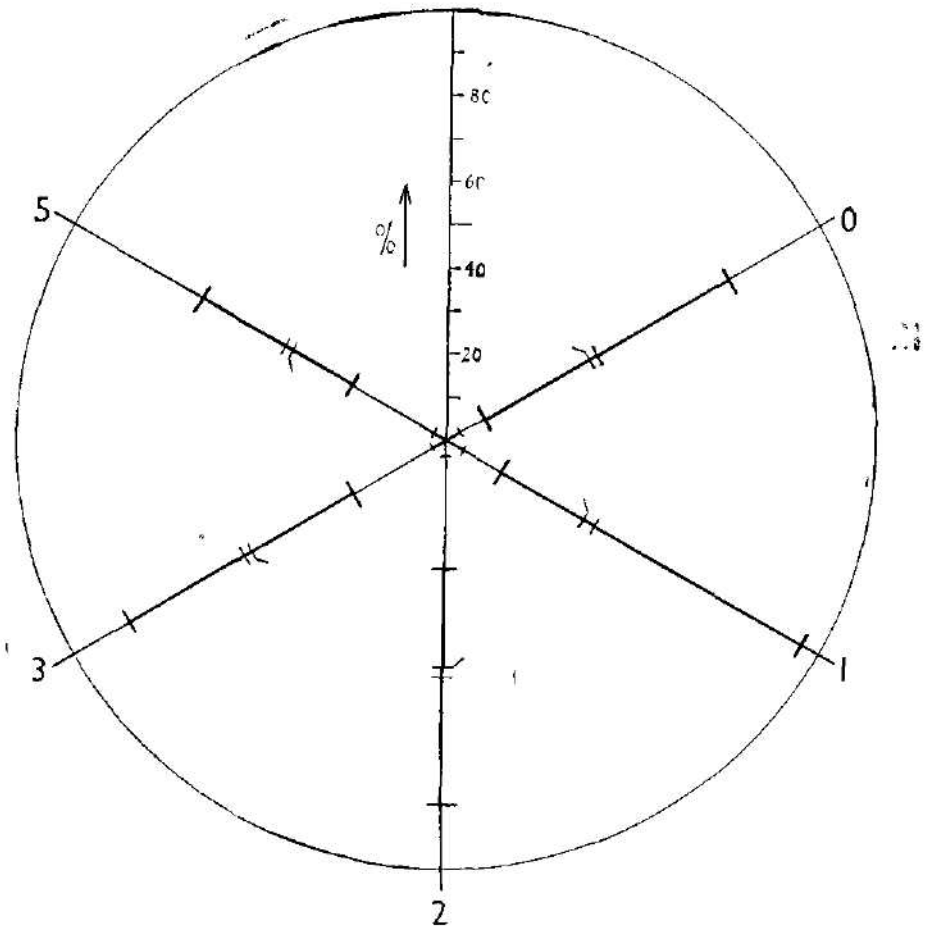


Graph 1. Mortality of the termites *C. formosanus* after treatment with the original strain and reisolates of *C. coronatus*. 0 — % mortality caused by the original strain, 1, 2, 3, 5, 9, 12, 13 — % mortality caused by the reisolates (see Tab. 1). On the obscissae running from the centre of the graphs the mortality of controls, minimum mortality of infected specimens, average mortality corrected by mortality of controls, average mortality and maximum mortality are given.

DISCUSSION AND CONCLUSION

Both strains of *C. coronatus* used in the experiments of Yendol and Paschke (1965) were isolated from insects *Therioaphis maculata* and *Reticulitermes flavipes* (Kollar), although they were kept in vitro for some time. The two strains of *C. coronatus* tried out by Kevorkian (Kevorkian, 1937), as well as the strain assayed by Altson (Altson, 1947), were also isolated from termites. In our investigations we used a strain of *C. coronatus* obtained and isolated as an air contamination. No information was available on the origin of this strain, similarly as with the strain of Costantin.

In comparison with the strains used by Yendol and Paschke and Altson, our original strain was less effective both on *C. formosanus* and *R. lucifugus* and the period of time for which the termites were killed was longer.



Graph 2. Mortality of the termites *R. lucifugus* after treatment with the original strain and reisolates of *C. coronatus*. 0 — % mortality caused by the original strain, 1, 2, 3, 5 — % mortality caused by the reisolates (see Tab. 1). On the obscissae running from the centre of the graphs the mortality of controls, minimum mortality of infected specimens, average mortality corrected by mortality of controls, average mortality and maximum mortality are given.

In our previous experiments (Krejzová, 1971) the termites were killed by *Entomophthora virulenta* Hall et Dunn, *Entomophthora thaxteriana* (Petch) Hall et Bell, and *Entomophthora destruens* Weiser et Batko during the first three days after treatment.

The long period of infection with the fungus *C. coronatus* suggests that the animals are not killed by the primary infection only, but that the fungus is transferred from the infected specimens or maintained on another suitable substrate. This provides the possibility that this strain of *C. coronatus* under suitable conditions might be maintained and disseminated in the termite nests both through the infected specimens and in the saprophytic way and cause thus a long-termed damage to them. With regard to the infectivity

of *C. coronatus* for higher mammals and man (Bridges, Romane, Emmons, 1962; Emmons, 1964; Emmons and Bridges, 1961; Williams, 1969), it is not possible to recommend the application of the fungus where it may come into contact with man.

In our experiments described in this paper the enhancement of pathogenicity for *C. formosanus* and *R. lucifugus* was achieved with almost one half of the reisolates.

Since we succeeded in increasing the pathogenicity of *C. coronatus* for the termites *R. lucifugus* not only by precultivation on the workers of *C. formosanus*, but also on the larvae of *G. mellonella*, which is distant from the termites, it may be concluded that the virulence of the fungal strain depends on the precultivation on some living organism.

However, with regard to higher percentage of mortality of both *C. formosanus* and *R. lucifugus* produced by a reisolate from *C. formosanus* in comparison with the reisolate from the larvae of *G. mellonella*, it may be assumed that the same or related organism is more suitable for precultivation than a distant one. The possible dependence of the virulence of *C. coronatus* on the host was also supposed by Prasertphon (Prasertphon, 1963).

The low percentage of mortality after treatment with a reisolate from *R. lucifugus*, which was observed not only with *C. formosanus* but also with *R. lucifugus*, gives evidence that there may exist an organism which is quite unsuitable as a substrate for cultivation of a certain species or strain of fungus.

Even after precultivation, however, there occurred reisolates whose pathogenicity for both termite species could not be enhanced in comparison with the original strain. Among them were those reisolated from the same host, the relative or a distant one. These negative results reveal that some properties of the fungus appearing as higher or lower pathogenicity are surely of genetic character and cannot be influenced by the host. Similarly also Donaubauer (1959), in contradistinction to other authors (in: Müller-Kögler, 1965), states that the virulence of *Beauveria bassiana* (Bals.) Vuill. does not depend on the host species used for precultivation.

The rate of pathogenicity might have been influenced also by the state of colony of termites from which the material was taken and the time at which it was done.

Besides, the phenomenon of the lower pathogenicity of the fungus for *R. lucifugus* than for *C. formosanus* seemed to be caused by the fact that the termites of *C. formosanus* which belong to a community with higher level of social organization are more sensitive in all because the experimental conditions cause greater stress to them than to the workers of *R. lucifugus* living in social organization of a lower level. We have already found lower pathogenicity of some strains of the genus *Entomophthora* and *Basidiobolus* for *R. lucifugus* than for *C. formosanus* in our previous experiments (Krejzová, 1971, 1972).

The prolongation of mortality period after treatment with the reisolates may be due to the fact that the reisolated strains have higher pathogenic effect even after a long-time saprophytic growth, which results from the total enhancement of their viability.

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The plate will be found at the end of this issue.

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THE DEVELOPMENT OF PROCAMALLANUS LAEVICONCHUS (WEDL, 1862) (NEMATODA: CAMALLANIDAE)

FRANTIŠEK MORAVEC

Received November 22, 1973

Abstract: A description is given of the life cycle of the nematode *Procamallanus laeviconchus* (Wedl, 1862), a common and widely distributed parasite of African freshwater fishes. As determined experimentally, the first intermediate host is *Mesocyclops leuckarti* (Claus) (Copepoda). The copepod feeds on free first-stage larvae of the parasite. These enter its haemocoel by way of the intestine; after two moults they attain the third, infective stage. At 23—24°C of the water, development in the intermediate host lasts 6 days. Experimental infection of the fish species *Gambusia affinis* disclosed that these may be utilized by the nematode as reservoir hosts. In addition to descriptions and drawings of the individual nematode stages in the intermediate and definitive hosts (*Clarias lazera*), several problems concerned with the development and morphology of *P. laeviconchus*, and the sources of infection with this parasite are discussed.

The nematode *Procamallanus laeviconchus* (Wedl, 1862) is one of the most frequent and widespread parasites of African freshwater fishes. Paperna (1964) also recorded its incidence from Israel. From the African Continent, this species has been reported from Egypt (Wedl, 1862; Baylis, 1923; Törnquist, 1931; Imam, 1971; Moravec, 1973), the Sudan (Törnquist, 1931; Khalil, 1969), Zaire (Campana-Rouget, 1961), Senegal (Vassiliades, 1973), Ghana (Khalil, 1970) and Uganda (Khalil, Thurston 1973) from a number of fish hosts (mostly siluroid fishes, less frequently members of the families Mormyridae, Characidae, Tetraodontidae). Recently, I found this parasite in *Haplochromis desfontainesi* (fam. Cichlidae) from Egypt (unpublished). The nematodes are firmly attached to the stomach of the definitive host and feed, evidently, on blood as do most of the members of this family. In view of the fact that *P. laeviconchus* attacks mainly fishes of economic importance, studies on the biology of this parasite may disclose valuable information which may have practical implications and could be utilized particularly in African countries introducing artificial propagation of fish and developing fish culture.

Although approximately 25 nematode species of the genus *Procamallanus* are known to parasitize fishes of tropical and subtropical areas in Asia, Africa and America, the life history of members of this genus has not been studied as yet. A similar situation occurs with the closely related genus *Spirocamallanus* for which two papers only are available on these problems, one by Li (1935) describing larval development of *S. fulvidraconis* in cyclops from China, the other by Pereira et al. (1936) on the life history of the South American species *S. caerensis*. Of the remaining genera of the family Camalla-

nidae, the life histories have been studied only for the genera *Camallanus* (Mecznikow, 1866; Leuckart, 1876; Linstow, 1909; Leiper, 1910; Kuprjanova, 1954; Campana-Rouget, 1961b; Moravec, 1969, 1971a, 1971b; Stromberg Crites 1974) and *Zeylanema* (Moorthy, 1938). The present paper gives the results of studies on the life cycle of *P. laeviconchus*. The study has been made at the National Research Centre, Cairo, during the author's short stay in Egypt in the spring 1973.

MATERIAL AND METHODS

Adults of *P. laeviconchus* were obtained from the stomach of the karmote *Clarias lazera* bought at the fish market of Cairo (Gíza). This market was supplied mainly with fishes caught in the Nile, less frequently with those from the environment of irrigation canals and drains. Adult females of *P. laeviconchus* were placed in a petri dish filled with water, and from it we selected females with motile larvae in the uterus under the microscope. These were transferred to glass vesels (6—7 cm in diameter) with a small amount of filtered Nile water (2—3 nematodes per vessel). The nematode body was teased with mounted needles and the larvae released into the external environment to which water was added in order to obtain a water column of 7—8 cm in height. Copepods (20—30 specimens), sometimes also other plankton animals, a few fibrous algae and detritus were added to each vessel and these were kept at room temperature (23—24°C) in the laboratory; the cyclops were inspected at different intervals. For feeding experiments, one cyclops each was added to a small glass vessel with the fish to be infected in order to obtain evidence that the cyclops was swallowed by the fish. Less frequently the cyclops was forced into the fish or tadpole by means of a small pipette.

All larvae, i.e., those obtained from cyclops or fishes, and free larvae obtained from the uteri of female nematodes, were studied in the following manner. The larvae were placed in a drop of water on the slide and covered with a coverslip fixed to the slide with Noier lacquer. In order to stretch the larvae, the slide was carefully heated over the flame of a gas burner and a small drop of 2—4% formalin was added to the edge of the coverslip. The larvae were studied either without clearing or cleared in glycerine. All drawings were made with the camera lucida.

RESULTS

Experimental infection of cyclops

In order to clear the question of the intermediate hosts of *P. laeviconchus*, two cyclops species (*Mesocyclops leuckarti* and *Cyclops* sp.), one larger diaptomid species (Diaptomidae gen. sp.) and one species of the order Cladocera were exposed to experimental infection with the nematode larvae. Of these local plankton crustaceans infection was acquired only by the species *Mesocyclops leuckarti* (Claus) and this was used for study on larval development up to the third, infective, stage. This cyclops species is common in fresh waters of Egypt and, probably, utilized by *P. laeviconchus* as its intermediate host also in the natural environment.

P. laeviconchus is ovoviviparous, i.e., the mature female harbours first-stage larvae in its uterus. Larvae released into the water display considerable motility; they are mostly fixed to the substrate with their tail, continuously contracting and relaxing their body or swinging from one side to the other; these movements serve evidently for attracting the cyclops which swallow them eagerly. In the digestive tract of these hosts the larvae bore through the intestinal wall by means of their dorsal tooth and enter the haemocoel of the cyclops where larval development continues. At a water temperature of 23—24°C, the first larval moult was observed in as short a time as the end of the first and the beginning of the second day p.i. The second moult after which the larvae attain the third, infective, stage, occurred on day 5 and 6 p.i.

and afterwards larval development did not continue in the cyclops. Larvae found on day 16 p.i. in the cyclops were practically analogous in measurements and morphology to those found on day 5 and 6 p i. (Table 2). Third-stage infective larvae are located in the haemocoel of the cephalothorax of the cyclops, less frequently in its abdomen, and retain considerable motility. By contrast to infective larvae of other genera of the family Camallanidae (*Camallanus*, *Paracamallanus*), these larvae do not tend to coil in a circle or spiral. They are clearly visible even in living cyclops for their remarkable orange colouration.

In our experiments the incidence in *Mesocyclops leuckarti* was always 100%, the intensity of infection ranged from 1 to 5 larvae (average 3) per cyclops. The larvae are considerably pathogenic to their hosts; cyclops infected with 4 larvae became sluggish and had a tendency to remain at the bottom of the vessel; sometimes, they were incapable of active movement.

Experimental infection of fishes

Cyclops harbouring 2—5 infective larvae of *P. laeviconchus* (on day 16 p.i.) were fed individually to 7 specimens of the fish *Gambusia affinis* (body length 1—4 cm), and to 2 tadpoles (*Rana esculenta*). Inspection of the tadpoles (on day 1 and 5 p.i.) gave negative results. The fishes *G. affinis* were dissected on day 3, 5, 11 and 21 p.i. and infection (one larva per fish) was disclosed in 4 of them. The larvae were always found in the gut, firmly attached to the intestinal mucosa with their buccal capsule. The morphology and measurements of the larvae were analogous to those of infective larvae from cyclops (Table 2). This indicates that larval development does not continue in these fish hosts; they function evidently as reservoir hosts and may become a source of infection of the definitive hosts, mostly various predatory siluroid fishes.

Experimental studies on the development of *P. laeviconchus* in the definitive host could not be made in view of a shortage of time and the difficulty in obtaining suitable experimental fish hosts. However, we obtained fourth-stage larvae and adults at variously advanced stages of development from naturally infected fishes (*Clarias lazera*).

Description of the developmental stages of *P. laeviconchus*

a) First-stage larvae from the uterus of the female

Larval body slender, whitish to translucent. Body length 0.431—0.497 mm, maximum width 0.018—0.021 mm. Cuticle relatively thick, with either very fine transverse striation, or smooth. Anterior end of body armed with a clearly visible dorsal tooth utilized for piercing the intestinal wall of the intermediate host in order to enter its body cavity. Oral papillae present; these are minute and their exact number could not be determined. Mouth represented by a thin, feebly sclerotized tube (approximate length 0.003 mm). Oesophagus with very thin walls, with spacious cavity inside; length 0.074 mm, width 0.007 mm. It was difficult to distinguish from the intestine the oesophageal glands abutting the moderately muscular oesophagus end. Nerve ring at 0.056 mm from anterior end of body, excretory pore slightly above its level. Intestine wide, straight, with fine granulation, fairly light. Three

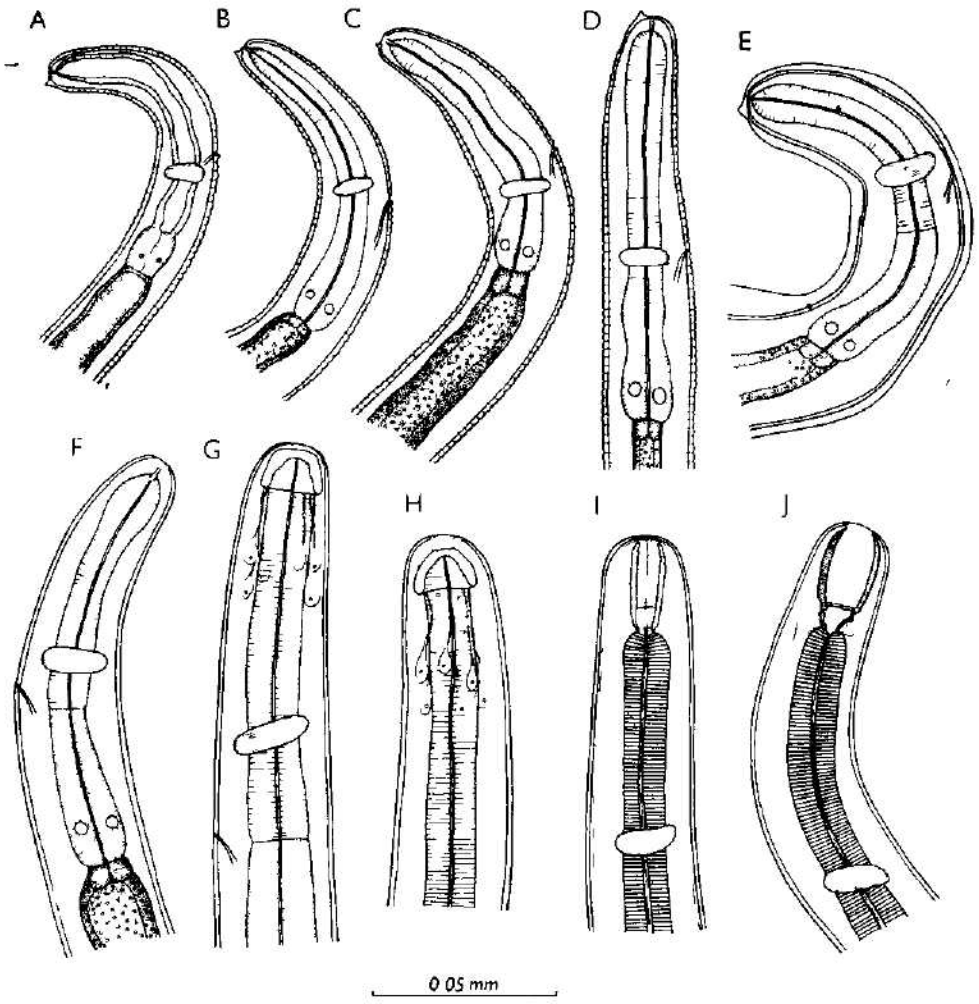


Fig 1 *Procamallanus laevi conchus* (Wedl, 1862) — development of the anterior part of larval body in cyclops. A — first-stage larva from the uterus of a female. B — first stage larva from cyclops on day 1 p i , C, D — first stage larva on day 2 p i ; E — larva during the first moult on day 2 p.i.; F — young second stage larva on day 2 p.i ; G, H — older second-stage larva starting the second moult (4 days p.i.), I — larva undergoing the second moult (6 days p.i.), J — young third-stage (infective) larva from cyclops (6 days p.i.)

distinct, oval, unicellular caudal glands present. Rectum a fine, narrow, straight tube. Tail elongate, slender, 0.147—0.161 mm long.

b) Development of first-stage larvae in the intermediate host

At first the larva entering the haemocoel of the cyclops does not change its morphology except for a moderate increase of its body measurements. Both the cuticle and walls of the oesophagus thicken and, consequently, the spacious oesophageal cavity disappears; the muscular region of the oesophagus

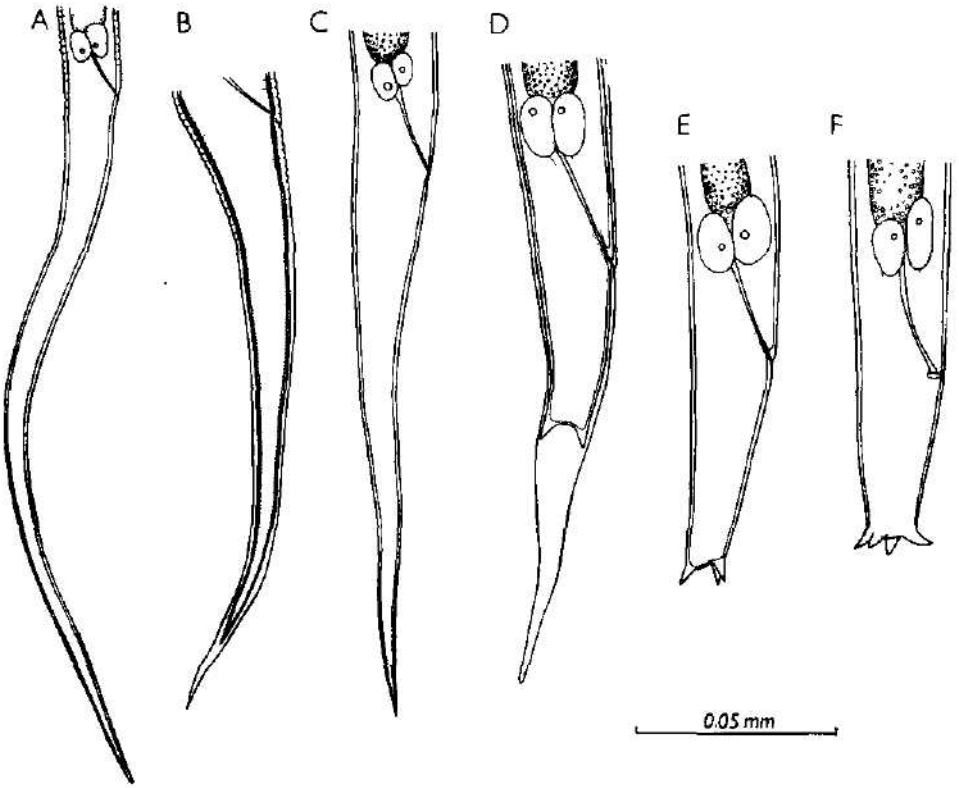


Fig. 2. *Procamallanus laeviconchus* (Wedl, 1862) — development of the tail in larvae from cyclops. A — first-stage larva from the uterus of a female; B — larva from cyclops undergoing the first moult (2 days p.i.); C — second-stage larva 3 days p.i.; D — larva undergoing the second moult (6 days p.i.); E — young third-stage larva (6 days p.i.); F — third-stage larva 16 days p.i. (sublateral view).

merges with the glandular region and forms a continuous oesophagus which remains glandular only in its posterior region; the division between these two regions is still indistinct. Cell nuclei of oesophageal glands increase considerably; three huge nuclei, each for the pertinent oesophageal sector, are present. Oesophagus opens into the intestine through the valvular apparatus. The colour of the intestine, although still light, becomes darker than that of free larvae in view of larval diet.

In as short a time as the end of the first day p.i., the larvae display readiness for their first moult. They are still slender, translucent, with a thick cuticle showing dense transverse striation. Dorsal cephalic tooth still distinct. Length of larval body 0.430—0.543 mm, maximum width 0.021—0.025 mm. Oral tube almost unchanged in length (approximately 0.0035 mm). Division of oesophagus into anterior muscular region (length 0.049—0.060 mm) and posterior, slightly wider glandular region (length 0.025—0.039 mm) observed in larvae ready for their first moult or in moulting larvae. Glandular oesophagus contains three huge cellular nuclei. Nerve ring close to posterior end of muscular oesophagus at 0.042 mm from anterior extremity; excretory pore

Table 1 Growth of first- and second-stage larvae of *P. laeviconchus* in cyclops

	First-stage larvae		Second-stage larvae		
	free larvae	1—2 days p.i.	2—3 days p.i.	4 days p.i.	6 days p.i.
Length of body	0.431—0.497	0.430—0.543	0.578—0.630	0.711—0.795	0.812
Width of body	0.018—0.021	0.021—0.025	0.028—0.035	0.032—0.039	0.032
Length of muse. oesophagus		0.049—0.060	0.063—0.067	0.116—0.123	0.151
Length of gland. oesophagus		0.025—0.039	0.035—0.046	0.091—0.112	0.123
Total length of oesophagus	0.074	0.074—0.098	0.105—0.112	0.207—0.235	0.274
Distance of nerve ring	0.056	0.042	0.042—0.049	0.088—0.098	0.088
Distance of excret. pore		0.049	0.074	0.102—0.130	0.088
Length of tail	0.147—0.161	0.140—0.179	0.147—0.189	0.116—0.140	0.123

slightly posterior to nerve ring level (0.049 mm from anterior extremity), occasionally anterior to it or level with it. Tail slender, conical, length 0.140—0.179 mm.

First larval moult occurred at the end of the first and the beginning of the second day p.i., but it was extremely difficult to recognize it. In moulting larvae this process was most distinct on the cephalic end and the tail (separation of the old cuticle).

c) Second-stage larvae

Young second-stage larvae are similar to larvae of the foregoing stage except for the absence of the dorsal tooth. The rate of larval growth increases after the moult. Length of second-stage larvae obtained from cyclops on day 2—3 after infection was 0.578—0.630 mm, maximum width 0.028 to 0.035 mm. Cuticle smooth, thin. Overall oesophagus length 0.105—0.112 mm. Division into anterior muscular and posterior glandular region still poor (length 0.063—0.067 mm and 0.035—0.046 mm respectively), the latter being slightly wider than the former. Mouth a narrow, feebly sclerotized tube, length approximately 0.003 mm. Anterior end of glandular oesophagus formed by two layers of large cells; these are glandular in nature and contain relatively large nuclei; similar cells surround the oral tube. Apparently these cells participate in the formation of the buccal capsule. Nerve ring at 0.042 to 0.049 mm from anterior extremity, excretory pore slightly posterior to it (at 0.074 mm from anterior extremity). Intestine straight, densely granulated, still without orange colouration. Genital primordium oval, situated moderately below mid-body. Tail slender, 0.147—0.189 mm long.

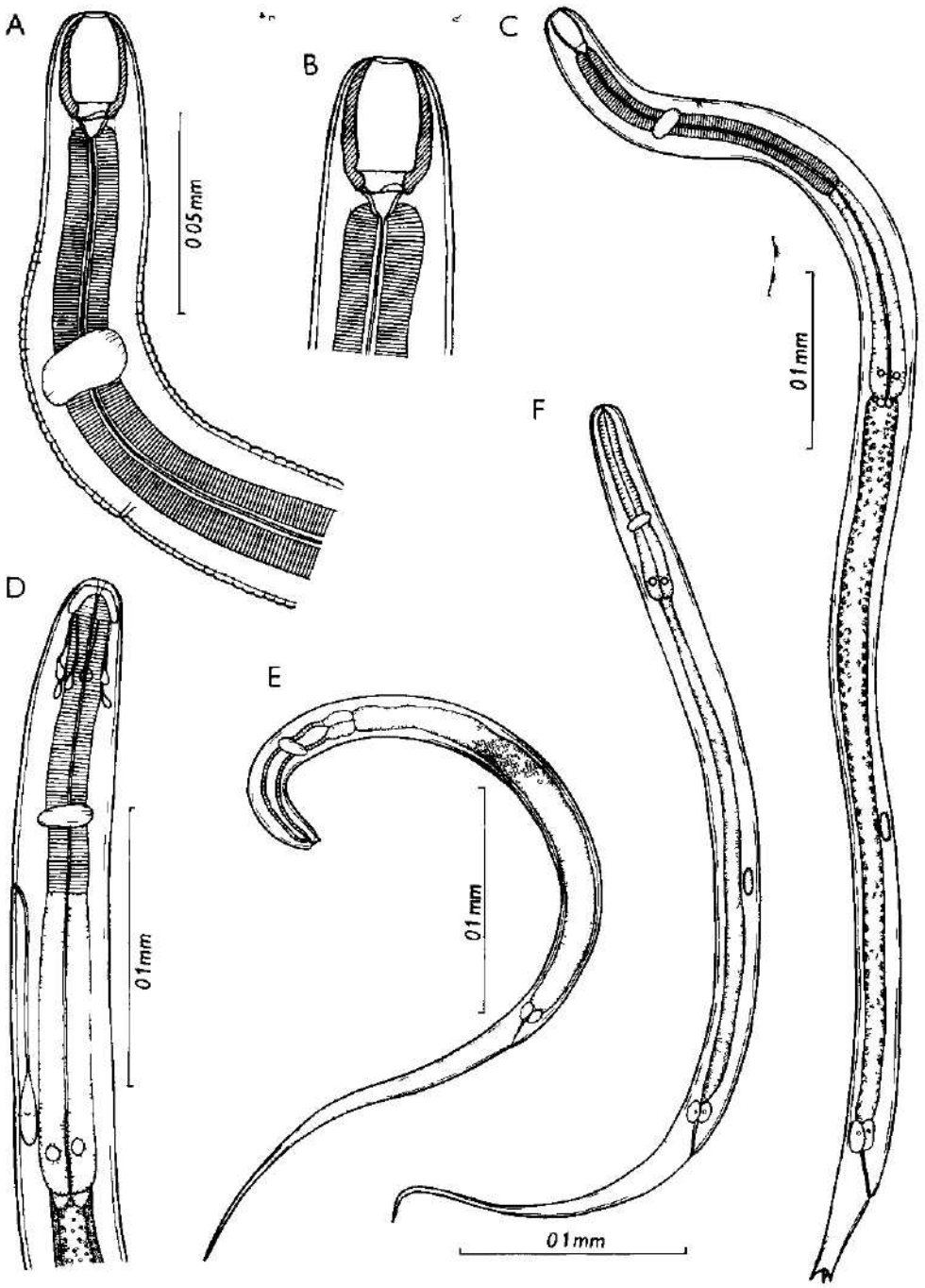
The second larval moult started in as short a time as 4 days p.i. and was clearly visible on the tail. At this time the larvae measured 0.711—0.795 mm, maximum width 0.032—0.039 mm. Formation of the buccal capsule at the cephalic end starts with the appearance of a hyaline, translucent bell-shaped, poorly sclerotized formation. This "bell" (length 0.011—0.014 mm, width 0.018 mm) abuts the anterior end of oesophagus. Approximately at the end of its first third, the glandular oesophagus is surrounded by six marked,

highly refractile, unicellular glands with long outlets leading into the base of the bell-shaped formation; these glands seem to be distributed regularly around the periphery of the oesophagus and participate apparently in the formation of the buccal capsule. Division of oesophagus into muscular and glandular region is distinct; length of muscular oesophagus 0.116—0.123 mm, of glandular oesophagus 0.091—0.112 mm. Nerve ring surrouds posterior part of muscular region at 0.088—0.098 mm for anterior extremity. Excretory cell slightly anterior to the end of glandular oesophagus, excretory pore approximately at the level of the junction of both oesophageal regions (at 0.102—0.130 mm from anterior extremity). Intestine becomes feebly orange in colour, very granulated, rectum and rectal glands colourless. Inside the old cuticle of the originally conical tail (length 0.116—0.140 mm), the new shorter tail (0.046—0.056 mm) is clearly visible. It terminates in four small conical processes. Genital primordium oval, consisting of several cells and situated at the ventral side of the body in the posterior half of the intestine, at 0.238—0.256 mm from posterior extremity.

Short to the second moult, the larva found on day 6 p.i. measured 0.812 mm (for other measurements see Table 1) and its general morphology resembles that of the infective larva; it was still in the exuviae of the old cuticle and its buccal capsule was very narrow, feebly sclerotized, uncoloured. The old cuticular oesophageal lining passed through the centre of this capsule; this is shed together with the old cuticle during the moult.

d) Third-stage larvae (infective larvae) from cyclops

These larvae first appeared in the cyclops on day 6 p.i.; their development was inspected for the following ten days. These are fairly large, body length 0.735—0.914 mm, maximum width 0.032—0.039 mm. Cuticle immediately after the second moult smooth, later with dense transverse striation, which is absent in the anterior extremity and on the tail; intervals between individual circlets in mid-body approximately 0.004 mm. Cephalic end with a big, continuous, highly sclerotized oral capsule, orange yellow to brownish in colour. General structure of buccal capsule similar to that in adult nematodes except for the absence of arched processes on its anterior margin (fourth-stage larvae bear 6 marked arched processes); posterior end of buccal capsule moderately attenuated, its inner wall thickened; it forms a kind of basal ring which is somewhat shorter than that in the adult nematode. The wall of the buccal capsule attenuates in direction towards the mouth opening which is widely circular. Length of buccal capsule generally 0.028 mm, less frequently 0.026 or 0.032 mm, maximum width 0.014—0.018 mm; length of "basal ring" 0.004—0.005 mm, width 0.006—0.011 mm. Posterior end of oral capsule abuts the sclerotized, colourless, basal (oesophageal) cup (0.004 × 0.004 mm). Oesophagus divided distinctly into anterior, almost cylindrical, muscular region with a strong cuticular lining and posterior glandular region bearing three huge cellular nuclei at its somewhat widened posterior end. Glandular oesophagus generally slightly longer than muscular oesophagus; this situation was reversed in the youngest larvae of this stage; length of muscular oesophagus 0.147—0.196 mm, of glandular oesophagus 0.123—0.207 mm. Nerve ring surrounds muscular oesophagus close to its mid-line, at 0.084—0.095 mm from anterior extremity. Excretory pore slightly posterior to nerve ring, at 0.095—0.126 mm from anterior extremity.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Oesophagus opens into intestine through a distinct three-lobate valvular apparatus. Intestine straight, wide, of orange colour, containing numerous granules. Three large, uncoloured, unicellular rectal glands present on its end. Rectum a thin, straight tube. Tail short (length 0.042—0.060 mm) attenuated in backward direction, with four conical processes (length 0.005 to 0.011 mm) at its tip. Two of these processes situated subdorsally, two subventrally. Viewed from the side, both subdorsal caudal processes overlap and their actual number can be assessed only by turning the larval body. Number of these processes is constant, they were present in all larvae examined. Genital primordium small in youngest third-stage larvae, oval, consisting of several cells, located in posterior part of body. In older infective larvae obtained from cyclops, primordium larger, changing gradually into the tubular anlage of the genital glands.

e) Third-stage larvae from the fish *Gambusia affinis*

Third-stage larvae of *P. laeviconchus* were obtained from fishes (*G. affinis*) with experimental infection on day 3, 5, 11 and 21 p.i. The morphology of these larvae was similar to that of infective larvae obtained from cyclops except for the slightly larger size of the body and that of several organs (Table 2). The structure of the oral capsule and its measurements were identical to those of larvae obtained from cyclops (without distinct arched processes at its anterior margin). Genital primordium oval, often more advanced in development, elongate, tubular. Cuticle considerably thick, with distinct transverse striation, but without signs of moulting (separation of the old cuticle). Tail end bears four small conical processes, similar to those of larvae from cyclops.

f) Fourth-stage larvae

Older fourth-stage female larvae were obtained from the stomach of the naturally infected *Clarias lazera*. These larvae were at an advanced stage of development, the largest of them undergoing their last moult. Colour of live larvae whitish, length 3.47—4.01 mm, maximum width 0.095 mm. Cuticle very thick, with dense transverse striation. Buccal capsule a dark orange, bearing six large, distinct arched processes on its anterior margin; these were practically absent in third-stage larvae. Posterior end of buccal capsule visibly thickened, forming a basal ring. Length of buccal capsule 0.048 mm, maximum width 0.039 mm, length of basal ring 0.009 mm. The capsule opens into muscular oesophagus through a feebly sclerotized oesophageal funnel. Muscular oesophagus with a thick, cuticular lining, length 0.381 to 0.540 mm, length of glandular oesophagus 0.510—0.546 mm. Three large cellular nuclei (one in each oesophageal sector) situated close to the posterior end of glandular oesophagus. Small oesophageal valves present. Nerve ring surrounds muscular oesophagus at 0.195—0.198 mm from anterior extremity; excretory pore at 0.210—0.339 mm. Intestine straight, narrow, of brownish

Fig. 3. *Procamallanus laeviconchus* (Wedl, 1862). A, B, C — third-stage larva from cyclops (A — anterior part of body, B — buccal capsule, C — total view) (A, B — 16 days p.i., C — 6 days p.i.); D — anterior part of body of older second-stage larva ready for the second moult (4 days p.i.); E — first-stage larva liberated from the uterus of a female; F — second-stage larva from cyclops (3 days p.i.).

Table 2 Measurements of third-stage larvae of *P. laeviconchus*

	from cyclops			from *Gambusia affinis*			
	6 days p.i.	13 days p.i.	16 days p.i.	3 days p.i.	5 days p.i.	11 days p.i.	21 days p.i.
Length of body	0.805	0.837–0.914	0.735–0.886	1.050	1.030	1.150	1.138
Width of body	0.032	0.035–0.039	0.035	0.049	0.046	0.053	0.049
Length of buccal capsule	0.028	0.028	0.026–0.032	0.028	0.028	0.032	0.028
Width of buccal capsule	0.014	0.018	0.014–0.018	0.018	0.018	0.018	0.021
Length of musc. oesophagus	0.158	0.161–0.196	0.147–0.161	0.179	0.175	0.182	0.179
Length of gland. oesophagus	0.123	0.179–0.207	0.147–0.175	0.235	0.186	0.210	0.238
Distance of nerve ring	0.088	0.091–0.095	0.084–0.091	0.098	0.098	0.098	0.088
Distance of excret. pore	0.105	0.105–0.109	0.095–0.126	0.158		0.123	
Length of tail	0.060	0.042–0.053	0.049–0.053	0.053	0.056	0.046	0.053

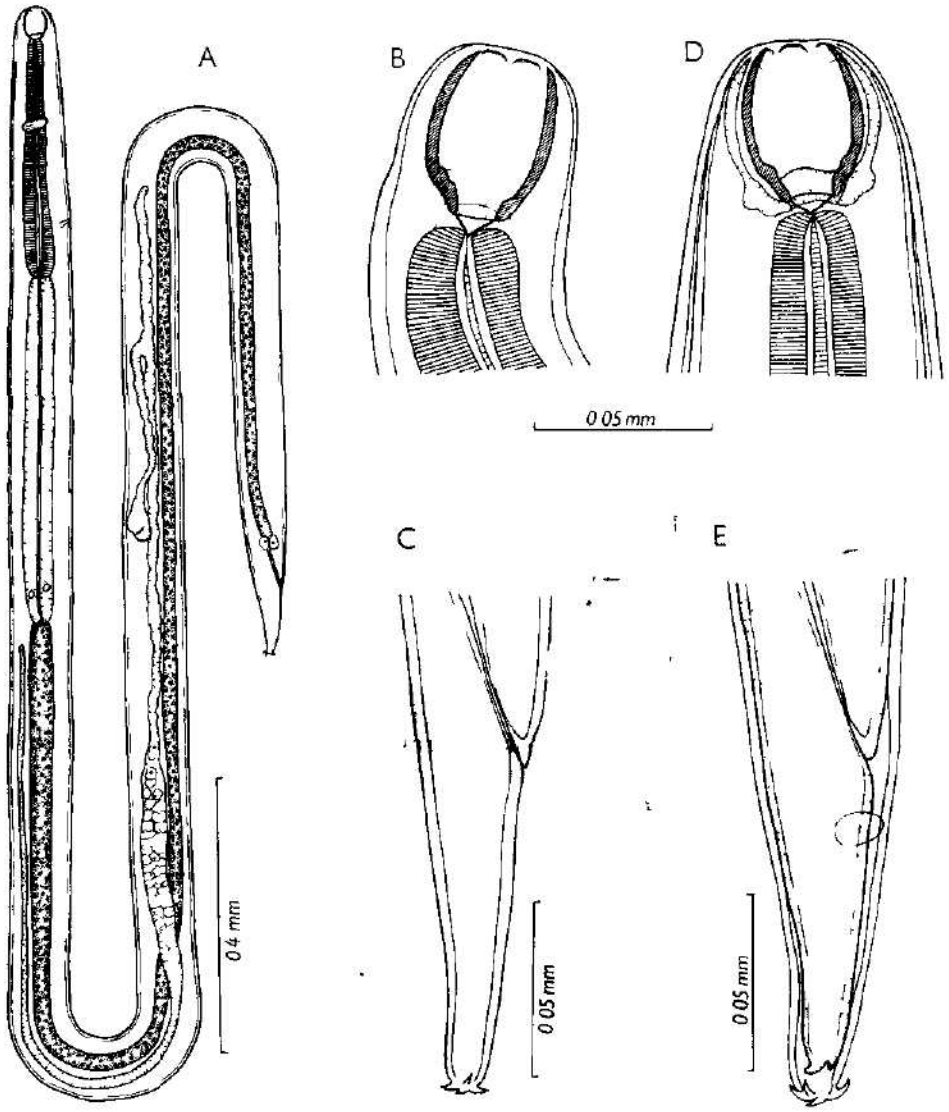

Fig 4 *Procamallanus laeviconchus* (Wedl, 1862) — fourth stage larva from *Clarias lazera*. A — general view, B — buccal capsule, C — tail, D — head end close to the fourth moult, E — tail close to the last (fourth) moult

colour. Three small rectal glands present; rectum a straight, narrow, colourless tube. Ovary distinctly developed, its anterior narrow end reaches almost the end of oesophagus; posterior ovary absent, uterus terminates in a blind process in direction of posterior extremity. Uterus tubular, thin, fairly long. Vagina narrow, length 0.26 mm; it extends in backward direction; vulva still covered with the cuticle starts to form at 1.36 to 1.41 mm from posterior

extremity. Tail conical, 0.105—0.111 mm long; it bears four small sharply pointed processes (length 0.009 mm) at its posterior end.

During the last larval moult (body length 4 mm) the separation of the cuticle is best visible at the body ends (Fig. 4d, e). Apart from the intensively coloured buccal capsule typical of fourth-stage larvae, the new buccal capsule becomes visible; this is wider than the former, with a more distinctly separated ring, but still very light in view of its faint sclerotization. The new tail is present in the exuviae of the old cuticle; it is similar in shape to that of fourth-stage larvae, but bears only three conical processes, i.e., one large dorsal and two smaller ventral processes, while fourth-stage larvae have four processes (this being still visible on the old cuticle).

g) Adults

Adult nematodes were obtained from the stomach of naturally infected specimens of *Clarias lazera*. Body colour of most adults reddish, buccal capsule a distinct yellowish brown colour. Males slightly smaller than females, their tail bent ventrally. Cuticle very thick, with dense transverse striation. Oral opening circular, with a narrow, colourless, cuticular rim. Four oral papillae and two lateral amphids present. Yellowish brown buccal capsule continuous, its anterior margin formed by six large, arched processes. Posterior end of capsule wall visibly thickened, forming a basal ring. It opens into the oesophagus through a small, colourless, feebly sclerotized, oesophageal funnel. Muscular oesophagus lined with a thick cuticular lining. Glandular oesophagus distinctly longer than muscular oesophagus; three large cellular nuclei present close to the posterior end of glandular oesophagus; oesophageal valves present. Intestine straight, narrow, of brownish colour. Nerve ring surrounds muscular oesophagus at its anterior half, excretory pore anterior to the end of muscular oesophagus. Small, simple cervical papillae present in the region of the nerve ring.

Males (6 specimens): Body length 3.03—4.08 mm, maximum width 0.109—0.163 mm. Length of buccal capsule 0.054—0.057 mm, width 0.048 mm, length of basal ring 0.009—0.012 mm. Muscular oesophagus 0.330—0.432 mm, glandular oesophagus 0.504—0.876 mm. Nerve ring at 0.159—0.216 mm from anterior extremity, excretory pore at 0.258—0.399 mm, cervical papillae at 0.165—0.276 mm. Narrow tail alae present. Eight pairs of pedunculate, subventral, preanal papillae present; a similar pair of papillae situated laterally at the level of the cloaca. Postanal papillae — 3 pairs subventral, 3 pairs of very small lateral papillae. Three additional pairs of small papillae surround the cloaca. Number of papillae unstable, the lateral pair, situated in the cloaca region, was absent in one male, in another male there were only two pairs of cloacal papillae. Spicules simple, slender, with sharp distal ends. Larger spicule 0.129—0.165 mm, smaller spicule 0.087—0.114 mm; the latter is less sclerotized than the former and, therefore, sometimes not distinct. A gubernaculum-like formation present, but without clear sclerotization. Tail short, 0.045—0.072 mm, with obtuse tip.

Females (8 specimens): The specimens in our material were mostly younger nematodes; in addition to eggs, larvae were present in the uterus of the largest of these females. Body length 3.74—7.37 mm, maximum width 0.136—0.204 mm. Length of buccal capsule 0.069 mm, width 0.057 to

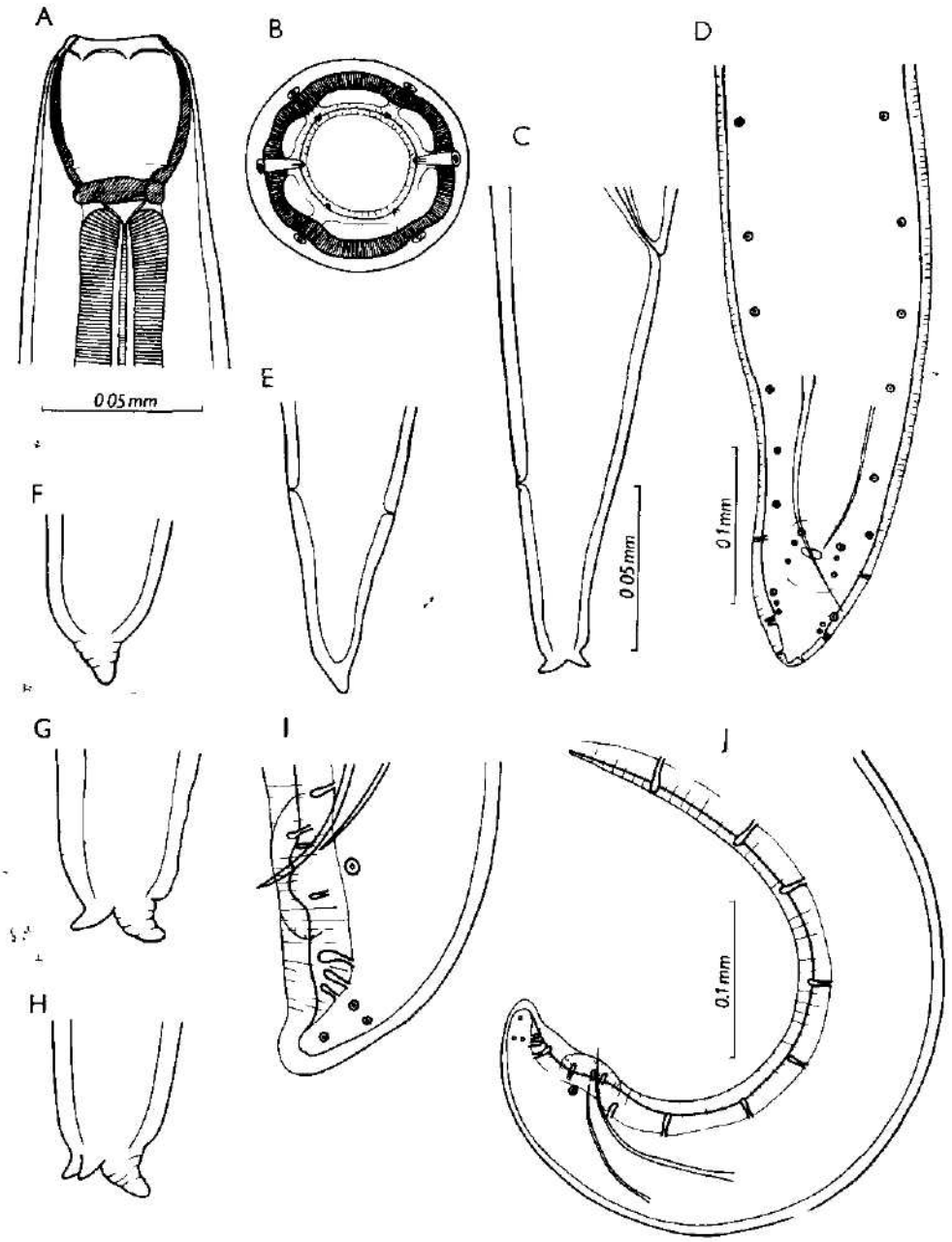

Fig. 5. *Procamallanus laeviconchus* (Wedl, 1862) — adults. A, B — buccal capsule (lateral and apical view); C — female tail; D — posterior extremity of male; E — posterior part of female tail (dorsal view); F, G, H — tip of female tail (dorsal, lateral and sublateral view); I — tail of male; J — posterior extremity of male.

0.063 mm, length of basal ring 0.012 mm. Length of muscular oesophagus 0.360—0.516 mm, of glandular oesophagus 0.666—0.990 mm. Nerve ring at 0.189—0.207 mm from anterior extremity, excretory pore at 0.240 to 0.384 mm, cervical papillae at 0.162—0.252 mm. Intestine straight, narrow, of brownish colour. Ovary fairly large, its anterior process reaches almost the end of oesophagus. Uterus of older females occupies a considerable portion of body cavity; posteriorly it forms a blind process which terminates at a short distance anterior to the anus. Uterus with spherical eggs (approximate diameter 0.066 mm) at various stages of development, or motile first-stage larvae. Vagina narrow, directed backwards. Vulva situated in posterior half of body at 1.78—2.99 mm from posterior extremity; vulval lips clearly elevated. Tail conical ending in three processes, i.e., a larger dorsal process, length 0.007 mm, and two smaller ventral processes (0.005 mm).

DISCUSSION

According to our results the development of *P. laeviconchus* proceeds as follows: Liberated first-stage larvae survive for a certain period in the water. Their persistent movements attract cyclops which swallow them readily. Development proceeds inside the body of these intermediate hosts. Soon after ingestion by the cyclops, the first-stage larvae pierce the intestine with their cephalic dorsal tooth and penetrate into the haemocoel of the intermediate host. At a water temperature of 23—24°C the first moult occurs within 24—48 hrs p.i. Second-stage larvae, in which the dorsal tooth is absent, grow at a considerable rate in the host's body; the second moult starts on day 5—6 p.i. and the larva attains the infective third stage. This is accompanied by considerable morphological changes (particularly the development of the large orange yellow buccal capsule and the conical processes on the tip of the tail). Development in the cyclops is arrested in larvae which have attained the infective stage. The definitive host (siluroid fish) acquires infection by either ingestion of an infected cyclops, or a reservoir host (apparently various small fishes). The infective larvae attach themselves with the buccal capsule to the wall of the stomach; after two more moults they attain the adult stage.

In general, the course of development of *P. laeviconchus* is similar to that of other members of the family Camallanidae. Although in our experiments we succeeded to confirm only one intermediate host of this nematode, i.e., *Mesocyclops leuckarti*, it is probable that it develops in a number of members of Cyclopoidea. This appears to depend solely on the bodily measurements of the pertinent cyclops species and on its capability of ingesting first-stage larvae. Floating copepods (e.g. Diaptomidae) seem unlikely to acquire infection, because the nematode larvae keep to the bottom and, hence, are not readily available for these crustaceans. This has been suggested by our unsuccessful tests with artificial infection of diaptomes with larvae of *P. laeviconchus*, similar to experiments with *Camallanus lacustris* (see Moravec, 1969). Pereira et al. (1936) determined *Diaptomus* sp. as the intermediate host of *Spirocamallanus caerensis*, but both the incidence and intensity of infection were extremely low in these copepods both under natural and experimental conditions.

As regards the morphology of the individual larval stages, we found first- and second-stage larvae to be similar to those larval stages of the genera

Spirocamallanus, Camallanus and *Zeylanema*. Differences, however, occur in third-stage larvae, i.e., in the structure of the buccal capsule, typical of this stage which, by contrast to the latter two genera, was continuous. Li (1935) and Pereira et al. (1936) considered infective larvae of *Spirocamallanus fulvidraconis* and *S. caerensis* from copepods to be second-stage larvae· it is evident that these authors failed to observe the first larval moult which is often difficult to observe and that their second-stage larvae were, in fact, third-stage larvae. A comparison of infective larvae of *S. fulvidraconis* and *S. caerensis* with infective larvae of *P. laeviconchus* did not disclose generic differences. Although Pereira et al. (1936) referred the buccal capsule of infective *S. caerensis* larvae to be supported by spiral ribs similarly to that of the adults, Li (1935) observed a smooth buccal capsule without spiral supports in *S. fulvidraconis* larvae which were at the same developmental stage. The number of conical processes (4) at the end of the tail of third-stage larvae is the same in both *S. caerensis* and *P. laeviconchus*; Li (1935), however, found three processes only on the tail end of infective *S. fulvidraconis* larvae; this may be due to the fact that he failed to differentiate the two dorsal processes which, upon lateral view, appear to be one. In this case the number of processes would be similar to that recorded for *P. laeviconchus*. Pande et al. (1963) described larvae from India, which they identified as third-stage larvae of *Procamallanus mathurai;* the morphology of these larvae, however, was different from that of members of the family Camallanidae suggesting that they were members of the genus *Spiroxys*.

In our experiments we observed a considerable rate of development of *P. laeviconchus* larvae in the cyclops, in which the larvae attained the infective stage at as early a time as on day 5—6 p.i. This speedy development is evidently due to the temperature under which our experiments were conducted. It has been indicated in earlier papers (Moorthy, 1938; Kuprjanova, 1954; Moravec, 1969 and others) that temperature is one of the most important factors influencing the rate of development of members of this family, and, evidently, an important limiting factor in the distribution and occurrence of the individual species. During the hot summer months, the period required for the development of *P. laeviconchus* shortens and the wealth of plankton animals available at this time provide optimal living conditions for this nematode. This was reflected in a considerable increase of the incidence of *P. laeviconchus* in the definitive hosts at the end of the spring and in the summer as indicated by Imam (1971) and confirmed by our observervations.

As evident from our experiments, fishes of the species *Gambusia affinis* may be utilized by infective larvae of *P. laeviconchus* as reservoir hosts. Under natural conditions, the definitive hosts, mainly predatory siluroids, acquire infection with *P. laeviconchus* apparently by feeding mainly on various small fish species (reservoir hosts), but the direct mode of infection by swallowing infected cyclops is also possible. Pereira et al. (1936) reported a similar case of reservoir parasitism for *Spirocamallanus caerensis* in the fish *Curimatus elegans*. Reservoir parasitism appears to be frequent with members of the family Camallanidae; it has also been recorded for the European species *Camallanus lacustris* and *C. truncatus* (Kuprjanova, 1954; Moravec, 1971a), the North American species *C. oxycephalus* (Stromberg, Crites 1974) and for the Indian species *Zeylanema sweeti* (see Moorthy. 1938).

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The plate will be found at the end of this issue.

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ON THE REPRODUCTION OF PHOXINUS PHOXINUS (LINNAEUS, 1758) (PISCES : CYPRINIDAE) WITH NOTES ON OTHER ASPECTS OF ITS LIFE HISTORY

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Abstract: 551 specimens of *Phoxinus phoxinus* (Linnaeus, 1758) from various districts of Romania with regard to several aspects, namely size structure of populations, histology of sexual glands, fecundity and growth have been studied by the authors.

The European Minnow *Phoxinus phoxinus* (Linnaeus, 1758) is a small fish living in all mountain and hill rivers and brooks of Romania, with the exception of the Dobroudja and of the rivers from southern Banat, between the Nera and the Cerna rivers. It is the only species of Cyprinidae occurring in the upper, trout zone of the rivers. One meets it also in dam lakes and in some small mountain lakes and ponds, but never in the glacial lakes of high mountains. It occurs even in some running waters at a rather low altitude here, mainly in shaded areas of the rivers, in their lateral arms with moderately running water and in rather slowly running brooks whose ground is a mixture of gravel, sand and silt (Antipa, 1909; Cărăusu, 1952; Antonescu, 1957; Vasiliu, 1959; Bănărescu, 1964).

Like other species of small-sized Cyprinidae (*Leucaspius delineatus*, *Alburnus alburnus*), it is an active and rapid swimmer, living in numerous and large groups which consist of specimens of the same size and age. Small groups occur only in places which do not represent, the normal habitat of the species, such as low altitude rivers and places where the minnow have been carried down by the stream. Yet even in such places minnows do not live isolated, but in small groups, which search for places with comparatively rapid water and shadow (under roots, dead treese, etc.). It is interesting to notice that when the groups are small the fish has a less active, more hidden mode of life, and inversely.

In summer, the minnow lives, in the upper reaches of rivers and in brooks with gravel grounds, where it finds adequate spawning conditions and abundant food. In autumn, when the water becomes cooler, the groups usually desagregate. Some specimens hide themselves under roots, in holes, or burrow into the silt, adopting thus a less active life; others retire to deeper water, where they often become the prey of trouts, burbots or other criophilous fishes.

Table 1 — The composition correlated with sizes and body weights

The absolute and relative number and the average weight of fishes								
		The length (without C) mm						
The date and the fishing place		15 — 20	— 25	— 30	— 35	— 40	— 45	— 5
1. The Roşu lake	n. abs.	—	—	—	—	—	—	1
1956, Sept., 2—3	n % %	—	—	—	—	—	—	0.8
	weight g	—	—	—	—	—	—	2.43
2. The Prahova river	n abs.	2	4	22	15	3	2	1
Buşteni 1958, oct. 13	n % %	4.0	8.0	44.0	30.0	6.0	4.0	2.0
	weight g	0.08	0.22	0.37	0.58	0.71	1.38	1.80
3. The Prejmer brook	n. abs.	—	—	—	—	—	—	—
(Braşov) 1961, Nov. 10	n % %	—	—	—	—	—	—	—
	weight g	—	—	—	—	—	—	—
4. The Doftana river	n. abs.	—	—	2	7	33	46	39
(the lower area)	n % %	—	—	1.3	4.7	22.0	30.7	26.0
1965, Nov. 21	weight g	—	—	0.70	0.75	0.83	1.30	1.77
5. The Căpuş brook	n. abs.	—	—	—	—	1	—	6
infer Someşul Mic	n % %	—	—	—	—	1	—	6
super Căpuş 1959	weight g	—	—	—	—	0.98	—	1.95

Like other criophilous fishes, for example the trout and the sculpin, the minnow requires a high amount of dissolved oxygen. Normally it needs 7—11 cm^3 O_2/l; at a concentration of 5 cm^3/l it shows evident symptoms of anxiety. (Wunder, 1936, cited by Nikolskij, 1944.)

Contrary to other small-sized fishes, of no or only reduced and strictly local economical importance, the ecology and life history of the minnow have been investigated by a number of authors, including recent contributions (Tack, 1940; Frost, 1943; Nikolskij & colab., 1947; Popescu-Gorj, 1950; Dottrens, 1952; Starmach, 1961, 1963; Bănărescu, 1964; Balon, 1965; Žukov, 1965).

During the investigations whose results are summarized in the present paper, the authors tried to give a more comprehensive characterization of this fish in order to complete and elaborate some incompletely studied and therefore little known aspects of the reproductive biology and pattern of growth of this widely ranging and common species.

MATERIAL AND METHODS

In order to investigate the main features of the reproductive biology (sexual maturity, fecundity, type of the gametogenesis and of the spawning), the pattern of growth as well as some aspects of the population ecology (relations between length-classes and sexes) of the minnow, a number of five lots of specimens were analyzed, captured from several provinces of Romania (Moldavia, Wallachia, Banat). (The samples from the rivers Căpuş, Minis and Bega have been collected by Dr. Bănărescu, to whom the authors are thankful.) In total there were 551 specimens (413 adults and juveniles, 38 quite young ones); all preserved in alcohol (Table 1).

0 — 55	55 — 60	60 — 65	65 — 70	70 — 75	75 — 80	n total absolute and in %	The body length mm The weight (g) the total average	the individual variation
3	31	44	43	7	1	130		
2.3	23.9	33.8	33.1	5.3	0.8	100	62.60 mm	49.00—80.00
2.56	3.65	4.57	5.61	7.62	8.40	—	4. 66g	2.40— 8.40
1	—	—	—	—	—	50		
2.0	—	—	—	—	—	100	28.00 mm	18.60—52.00
2.20	—	—	—	—	—	—	0.81 g	0.07— 2.20
8	28	24	8	4	—	72		
11.1	39.0	33.3	11.1	5.5	—	100	62.10 mm	53.50—71.00
3.10	3.68	4.28	4.95	6.80	—	—	4.01 g	2.80— 6.80
21	2	—	—	—	—	150		
14.0	1.3	—	—	—	—	100	42.70 mm	27.00—56.00
2.03	2.80	—	—	—	—	—	1.41 g	0.40— 3.40
5	9	36	25	17	1	100		
5	9	36	25	17	1	100	62.70 mm	34.90—77.00
2.42	3.65	4.20	5.73	6.45	7.45	—	4.95 g	0.98— 7.92

All specimens were measured and weighed; many of them (more than 200) were dissected in order to determine the sex, the degree of maturation of the gonads, the gono-somatic relation, the absolute and relative fecundity, etc. The degree of maturation of the gonads was established after the scale of Kulaev and Meien for Cyprinidae and Percidae and after the gono-somatic relation (weight of the gonads in percent of the whole body weight). The absolute fecundity which is an expression of the reproductive potential, was established by a numerical differential analysis of the ovocytes and eggs in ripe females (stage IV to V) collected at the beginning, the spawning season (13.—14. V. 1962) in Bega River. The relative fecundity (number of eggs for one unity of the body weight) was calculated for 1 g of the fish body. The pattern of growth in length was established after the body length and the fish age, this being determined, rather with difficulty, according to the scales of juveniles and, especially of mature specimens collected at the beginning of the spawning season (9.—14. V.) or during it, and of young specimens collected at the end of the spawning season (21. VIII.). The ponderal growth was established by the method of logarithmic correlation between body length and weight. The degree of fleshiness (coefficient of condition) was estimated after the well known formula of Fulton: $K = \frac{W \cdot 100}{l^3}$, W being the weight, l the body length.

RESULTS AND DISCUSSION

The statistical data obtained through the biometrical study and the macro- and microscopical analysis of the gonads of the 551 specimens of *Phoxinus* collected in different rivers and different seasons are presented in the tables 1—8.

The structure or composition of the populations of *Phoxinus* from Lacul Roşu (the eastern Carpathians), the Prahova R, (the southern Carpathians), the Doftana R., the Prejmer brook (near Braşov, Transylvania) and the Căpus R (near Cluj, Transylvania) is shown in Table 1.

Table 2 — The composition correlated with the ages and the sexes, and the variation of the average body weight in proportion to the sizes of both the *Phoxinus phoxinus* (Linnaeus) females and males, from the Doftana river (1965), Nov. 10)

Sex	The absolute and the relative number and the average weight of the specimens	The length (without C) mm 25 — 30 — 35 — 40 — 45 — 50 — 55 — 60							n totale absolute and relative	The body length mm the weight g. average	variation
♂♂	absolute nr.	—	1	8	7	5	5	1	27		
	n % %	—	3.7	29.6	26.0	18.5	18.5	3.7	100	42.8 mm	3.10—5.60
	weight g	—	0.60	0.86	1.16	1.70	2.15	2.20	—	1.37 g	0.60—2.35
♀♀	absolute nr.	2	6	25	39	34	16	1	123		
	n % %	1.6	4.8	20.3	31.7	27.6	13.0	0.8	100	42.7 mm	2.70—5.60
	weight g	0.70	0.78	0.82	1.32	1.78	2.02	3.40	—	1.42 g	0.40—3.40

Table 4 — The variation of body and ovaries weights, the gono-somatic report correlated with the *Phoxinus phoxinus* (Linnaeus) females sizes, collected in the Bega river at the beginning of their spawning season (1962, May, 13—14)

The lenght without C (mm)	The body weight (g) average	variation	The ovaries weight (g) average	variation	The gono-somatic report I average	variation	II average	variation	n
47.5—57.4	2.91	1.76—4.06	0.40	0.05—0.70	15.6	8.1—21.1	20.0	8.8—30.0	6
57.5—67.4	5.10	4.16—6.44	0.98	0.69—1.28	18.9	14.7—24.7	23.3	17.3—32.9	5
67.5—77.0	7.99	7.04—9.60	1.43	1.30—1.60	18.1	15.0—19.8	22.1	17.7—24.8	4
47.5—77.0	4.99	1.76—7.60	0.86	0.05—1.60	17.4	8.1—24.7	21.8	8.8—32.9	15

Table 3 — The composition correlated with ages, body sizes and the state of maturation of the gonades concerning the *Phoxinus phoxinus* (Linnaeus) populations (females and males) fished in both the Miniș and the Bega rivers (1962, May 9, 13—14)

Age years	The length (without C) mm average	variation	The body weight (g) average	variation	State of maturation of the gonades	n abs.	%
			The Miniș river — females				
I	25.0	—	0.22	—	II	1	9.0
II	53.1	49.0—57.0	2.70	1.93—2.97	IV—V	8	73.0
III	59.9	58.7—61.0	3.72	3.55—3.90	IV—V	2	18.0
			males				
II	47.6	41.3—54.0	1.67	1.00—2.56	IV	3	100
			The Bega river — females				
I	27.4	—	0.29	—	II	1	6.2
II	52.9	47.5—57.5	2.93	1.76—4.16	IV—V	6	37.4
III	62.1	56.4—68.7	5.21	4.06—7.04	IV—V	5	31.5
IV	70.8	66.0—77.0	7.84	6.44—9.60	IV—V	4	24.9
			males				
III	60.0	57.0—63.0	3.91	3.51—4.32	IV—V	2	100

As seen in Table 1, the standard length of juvenile and mature specimens of *Phoxinus phoxinus* collected varies between 27.0 and 80.0 mm and their weight between 0.4 and 8.4 g. The samples collected in Lacul Rosu (the 2-nd and 3-rd Sept. 1956), the Prejmer brook (the 10. XI. 1961) and the Căpus R. (the 26. Nov. 1959) consist practically only of mature specimens; among them specimens dominated whose length ranged between 55.0 and 65.0 to 70.0 mm and the weight between 3.5 and 5.5—6.0 g. The 150 specimens from the lower reach of the Doftana R., and from the Prahova R. at Busteni (near Sinaia) were juveniles and adults, having 27.0—56.0 mm and 0.4—3.4 g (in the Prahova R.) and 18.0—48.0 mm (the Doftana R.). Both populations were dominated by young, immature specimens, whose length ranged between 25.0—35.0 mm (the Prahova R.) or 35.0—50.0 mm and the averange weight between 0.83 and 1.8 g.

In Romania, the standard length of mature specimens of *Phoxinus* ranges usually between 50.0 and 70.0 mm, rarely up to 80.0 mm, and the weight between 2.0 and 7.0 g., rarely 8—9 g. These values are similar to those found by other authors in other river and lake drainages of Europe: Tack, 1940; Frost, 1943; Dottrens, 1952; Starmach, 1961, 1963; Žukov, 1965; Balon, 1966. Popescu-Gorj (1950) mentions much larger specimens, in Lacul Rosu, having 90—127 mm st. length and 10—29 g (in 16 specimens examined). It seems that these values are the highest reached by the species.

The sex ratio is rather variable during the year. According to Dottrens (1952) the males are always more numerous during the spawning season than the females, while in the other seasons the females are more numerous, the sex ratio being 2/3 females for 1/3 males or even more in older age-classes.

We noticed the same phenomenon in the Doftana R. specimens collected on Nov. 10, 1965, i.e. several months after the spawning season. Among the 150 dissected specimens there were 123 females (82%). Frost (1943) suggested the possibility of a sexual inversion occurring after the spawning season, when the females might become males.

The minnow belongs to the group of early maturating fishes, like the bitterling, the bleak, the pumpkinseed sunfish and the roach (Papadopol, 1960—1968). In these species the great majority of the specimens belonging to both sexes become mature at the age of two years and in very favourable conditions some specimens even at one year.

The data included in Table 3 show the age and size composition and the degree of maturity of the male and female minnows collected in the Bega and Minis rivers, Banat, at the beginning of the spawning season.

These data confirm that all specimens having two or more years and those collected between the 9th and the 14th May were sexually mature, their gonads being in the stages IV and IV to V. Both series were dominated by spawning specimens having two (the Minis R.) or two and three years (the Bega R.). The minimum standard length of mature males was 41.3 mm (corresponding to 1.0 g), that of mature females 47.5 mm (1.76 g).

The data obtained correspond to those recorded by other authors· Nikolskij & colab. (1947) mention that the minnow becomes mature, in the Petchora R., at the size of 35—40 mm; Berg (1949) that it spawns at the age of two, sometimes even one year; according to Dottrens (1952) the males become mature at two years, the females at two or three years; according to Žukov (1965) the maturity is attained at the age of two years and a size of 5 mm; Cărăusu, (1952) and Bănărescu (1964) report the data mentioned by Berg. Only Sterba (1959) asserts that the maturity occurs at the age of 3 or 4 years, but these data refer to specimens reared in aquaria.

Table 4 presents our data concerning the variation of both the body and ovaries weight and the gono-somatic relation, all obtained from mature females collected at the beginning of the spawning season (13—14/V/1962) in the Miniș R., South Banat. The gono-somatic relation was calculated both in percent of the total body weight (I) and of the body weight without gonads (II). The last value was rarely recorded in the bibliography, but is more corect.

The weights of body and of the ovaries increase, normally, in proportion to the body length, while the gono-somatic relation (I and II) has lower value in small-sized females comparatively to larger ones, the average values being slightly higher than 17% (I) or 21% (II) of the total body weight or of the body weight without ovaries. The amplitude of the individual variations is rather high within the limits of each length class for all three values and especially for the gono-somatic relation which can reach to 32.9% of the body weight without gonads (Table 4).

The absolute fecundity (total number of ovocytes and eggs) and the relative one (number of ovocytes and eggs for 1 g body) were established, as mentioned, by counting the eggs in 100 mg ovaries samples from each mature female (stage IV to V) collected in the Bega R., on May 13 and 14, 1962.

Table 5 — The variation of the values of the absolute fecundity of the *Phoxinus phoxinus* (Linnaeus) females from the Bega river, in proportion to their ages and sizes

Body length without C (mm)	The total number of eggs ovules and ovocytes		n	Body weirght g	The total number of eggs ovules and ovocytes		n	Age years	The total number of eggs ovules and ovocytes		n
	average	variation			average	variation			average	variation	
47.5—57.4	1988	1359—3256	6	1.76—3.75	1836	1359—3256	5	II	1912	1359—3256	6
57.5—67.4	3377	2288—4536	5	3.76—5.75	3019	2288—3782	5	III	3386	2748—4121	5
67.5—77.0	4894	4121—5515	4	5.76—7.75	4370	4121—4536	3	IV	4998	4484—5515	4
—	—	—	—	7.76—9.75	5485	5456—5515	2	—	—	—	—
47.5—77.0	3226	1359—5515	15	1.76—9.75	3226	1359—5515	15	II—IV	3226	1359—5515	15

Table 6 — The variation of the relative fecundity of the females of *Phoxinus phoxinus* (Linnaeus) in the Bega river, in relation to their sizes and ages

The length (without C) mm	The number of eggs for 1 g of body		n	The body weight	The number of eggs for 1 g of body		n	Age years	The number of eggs for 1 g of body		n
	average	variation			average	variation			average	variation	
47.5—57.4	683	503—930	6	1.76—3.75	685	503—930	5	II	662	503—930	6
57.5—67.4	655	550—731	5	3.76—5.75	649	550—731	5	III	656	585—731	5
67.5—77.0	613	574—677	4	5.76—7.75	635	585—704	3	IV	643	574—704	4
—	—	—	—	7.76—9.75	626	574—677	2	—	—	—	—
47.5—77.0	655	503—930	15	1.76—9.75	655	503—930	15	II—IV	655	503—930	15

Table 5 shows the variation of the values of the absolute fecundity in the Bega R. specimens, in correlation with the body size and age.

As shown in Table 5, the absolute fecundity in 2—3 years old minnow females, having 47—77 mm standard length and 1.76—9.60 g, ranged between 1359 and 5515 eggs, this variation depending on the size and age. As in other fishes, the mean value is higher in larger and older specimens.

Table 6 shows the values of the relative fecundity in the Bega R. specimens.

The analyses of the values included in Table 6 shows that the range of variation of the relative fecundity is strong (503 to 930 eggs), the average value decreasing with the age and the size, as in the bream, the bleak and pumpkinseed sunfish (*Lepomis gibbosus*) (Papadopol, 1962, 1967, 1968). These data also demonstrate that a female minnow of normal size can lay between 500 and 930 eggs for each g of its body. We point out also that two years old females, which spawn for the first time, are comparatively more prolific than three years old ones.

Our data, summarized in Table 5 and 6, as well as those recently obtained by Žukov (1956) for the minnow from the Western Dwina R., Bjelorussia (absolute fecundity of 52—74 mm long females ranging between 1746 and 3620 eggs), and by Balon (1966) for the minnow from the Orava R. in Slovakia (absolute fecundity 500—2000 eggs), enable us to assert that the absolute and relative reproductive potential of this species is much higher than as reported earlier: (Nikolskij & colab., 1947, record 200—600 eggs for 50—65 mm long females from the Petchora R.; Berg, 1949 and Suchoverkov, 1953 between 700 and 1000 eggs; Căraușu, 1952, Vasiliu, 1959, Spillmann, 1961 and Bănărescu, 1964 up to 1000 eggs). Hence the minnow, although a rheophillous fish, is, like many small-sized limnophilous species — bleak, crucian-carp, pumpkinseed sunfish — a prolific and early maturating species. These ecological characters are adaptive, allowing an increasing and rapid restoration of the populations. The relatively low absolute fecundity of this species in some northern rivers, as for example the Petchora (Nikolskij & colab., 1947), could be explained by the synchronous ovogenesis and spawning of the populations inhabiting these rivers. The same phenomenon was recorded in other species of Cyprinidae, for example the bream (Papadopol, 1962).

Another important feature of the reproductive biology of this fish, which we tried to establish for the Romanian populations, is the type of gametogenesis and, in correlation with it, the spawning-type. For establishing these features, we counted separately (after size and colour) the ovocytes and eggs from ovary at the beginning of the spawning season and made also a general histological examination of the ovary after the spawning. We made also some direct field observations upon the environmental conditions of the reproduction during the spawning season (the first half of June, 1967, on the Pingărați brook, near Bicaz, in July and the second half of August, 1964, on the Șipa brook, near Sinaia, and on the Dimbovița river, near Cetățeni, 1969.

According to the statistical data, correlated with both the numerical — structural and dimensional composition of the ovaries elements at the beginning of the spawning season (1962, May, 13—14) in the Bega R. (Table 7) and the histological examination of the ovaries after spawning (November, 10), we can conclude that:

Table 7 — The numerical and qualitative-dimensional composition of the ovules and the ovocytes extant in the *Phoxinus phoxinus* (Linnaeus) ovaries, in proportion to their sizes in the Bega river (1962, May, 13—14)

The length without C mm	The absolute number of eggs within ovaries					The relative number (%) of eggs out of their total		n
	ovules		ovocytes		ovules and ovocytes	ovules	ovocytes	
	average	variation	average	variation				
47.5—52.4	464	354— 550	995	900—1080	1459	31.8	68.2	3
52.5—57.4	591	550— 632	1927	1000—2264	2518	23.4	76.6	3
57.5—62.4	686	675— 697	2018	1613—2424	2704	25.3	74.7	2
62.5—67.4	874	774— 966	2951	2384—3654	3835	22.8	77.2	3
67.5—72.4	1216	936—1616	3473	3185—3840	4689	25.9	74.1	3
72.5—77.0	1195	—	4320	—	5515	21.6	78.4	1
47.5—77.0	800	354—1616	2426	900—4320	3226	24.4	75.6	15

1) The development and maturation of the ovocytes in *Phoxinus* is asynchronous, as in the already mentioned limnophilous Cyprinidae (*Rhodeus*, *Alburnus*, *Carassius*, etc.) (Papadopol, 1960—1969);

2) The ovocytes and eggs in different stages of their development are uniformly distributed within the ovary. In each small fragment of the ovary one can thus distinguish four categories of elements: a) almost ripe or (in May and June) ripe yellow eggs, containing much yolk, whose diameter ranges between 0.9 and 1.2 mm; they represent 25% (average value) of the total amount; b) large, white yellowish ovocytes with a vacuolar cytoplasma, having a diameter of 0.4—0.7 mm; c) semi-transparent small-sized ovocytes, with one or two rows of vacuoles in their peripheral cytoplasma, with a diameter of 0.1—0.3 mm; d) minute (less than 0.1 mm diameter) ovocytes in early stages of development, with an evidently basophilic cytoplasma (b, c and d in Fig. 1). The large and small-sized ovocytes (b and c) represent 75% of the total number of the sexual elements; the minute ovocytes are not included in this number (which represents also the absolute fecundity), since they would be laid next year only (Table 7);

3) The eggs, whose number ranged, in the 2—4 years old and 47—77 mm long females from the Bega R., between 354 and 1616, representing 21.6 to 31.8% of the absolute fecundity; they are deposited in the first portion. The ovocytes of the categories b and c represent 68.2—78.4% of the absolute fecundity; they are laid as soon as they maturate and achieve their development, in three or four succesive portions, with an interval of 15 days between them. According to these data, the Bega river females were able to lay between 900 and 4320 eggs during one summer (Table 7).

It is rather difficult to distinguish between these categories of sexual elements by counting them, since the differences of size and colour are not well marked; yet the differences are very evident in histological sections (*Fig. 1*).

There are quite few references and statistical data in the literature about the spawning-type of this common European fish. Starmach (1961) makes some remarks that the ovaries of females captured during the spawning season are in different stages of maturation; Žukov (1965) shows that the

Table 8 — The linear sizes and the weights of the *Phoxinus phoxinus* (Linnaeus) alevines proceeded from different eggs, in the Sipa brook (Sinaia) (1964, August, 21)

The number	The length mm without C	total	The weight mg	The number	The length mm without C	total	The weight mg
1	10.0	12.0	6.0	20	13.0	14.5	18.5
2	10.0	11.5	7.0	21	13.0	15.0	19.0
3	10.0	11.5	7.5	22	13.0	15.0	22.0
4	10.3	12.3	7.5	23	13.2	15.0	19.0
5	11.0	12.7	8.0	24	13.5	15.0	17.7
6	11.0	12.0	10.0	25	13.5	15.5	19 0
7	11.0	12.5	10.5	26	13.6	15.6	17.5
8	11.1	12.9	10.0	27	14.0	16.0	14.5
9	11.2	13.0	11.5	28	14.0	15.5	16 0
10	11.3	13.0	9.0	29	14.0	16.0	16.5
11	11.3	13.0	9.5	30	14.0	16.0	17.0
12	11.5	12.9	10.0	31	14.0	16.0	21.5
13	11.8	13.5	11.5	32	14.0	15.8	23.0
14	12.0	14.0	13.0	33	14.8	17.0	24.0
15	12.0	13.5	14.0	34	15.0	16.0	28.0
16	12.0	13.8	14.5	35	16.4	18.3	30 5
17	12.0	13.2	16.0	36	16.5	18.5	34.2
18	13.0	15.0	15.0	37	16 5	18.6	40.5
19	13.0	15.0	18.5	38	21.0	25.0	90.5

The length without C mm		The weight mg		n
average	variation	average	variation	
13.4	10.0—21.0	18.6	6.0—90.5	38

spawning occurs in successive portions; Lebedev (1969) remarks the same. All other general books on ichthyology or on fish faunas, as well as many papers dealing with the minnows from different river drainages, mention only the spawning season (Antipa, 1909; Berg, 1949; Cărăusu, 1952; Dottrens, 1952; Suchoverchov, 1953; Nikolskij, 1954; Antonescu, 1957; Vasiliu, 1959; Sterba, 1959; Spillmann, 1961; Bănărescu, 1964; Balon, 1966).

The examination of the gonads of mature specimens from the Minis and Bega rivers (collected on May 9 and 13—14, 1962), as well as the field observations carried on in June 1967, in July and August 1964 and 1969 on the Pingărați and Sipa brooks and on the upper Dimbovita R. convinced us that the spawning of the minnow (*Phoxinus phoxinus*) usually begins, in the lower altitude rivers, during the second half of May and lasts till the end of July; at higher altitudes, the spawning begins during the first decade of June and lasts till the middle of August. The populations from low altitudes undergo short upstream migrations, searching more favourable reproductive conditions (stony ground, a higher amount of oxygen, etc: Dottrens, 1952).

Before spawning the sexual dimorphism becomes more evident, a nuptial colouration develops. The dorsal side of male becomes dark blue-blackish, the ventral side slight red; small pointed white breeding tubercles occur on

Table 9. The rate of growth both in length and in weight of the *Phoxinus phoxinus* (Linnaeus) specimens, from different waters

Agein years	The Minis				The Bega				The Mszanka				The Windemere		The Barthay		The Niemec
	The body length mm		Body weight g	Coef. of condition	The body length mm		Body weight g	Coef. of condition	The body length mm		Body weight g	Coef. of condition	The body length mm		The body length mm		The body lenght mm
	without C	absolute			without C	absolute			without C	absolute			♂♂	♀♀	♂♂	♀♀	♂ + ♀
I	25.0	30.4	0.22	0.71	27.4	33.3	0.29	0.78	46.0	—	—	—	35.3	33.2	38.3	38.3	34.3
II	53.1	63.8	2.70	1.04	52.9	63.5	2.93	1.14	62.0	71.6	3.4	0.92	55.7	56.9	54.0	54.7	53.1
III	59.9	72.5	3.73	0.98	61.4	73.7	4.52	1.13	70.0	82.8	5.3	0.93	63.9	70.2	62.5	65.6	69.4
IV	—	—	—	—	70.8	84.2	7.84	1.27	80.0	97.4	7.75	0.84	—	—	—	—	—

the upper side of head and of body. In females the ventral side becomes intensively red.

The females lay the eggs in shallow water (a few cm deep), on sony ground, close to the shore of the river or brook. The spawning occurs during warm days when the water temperature can reach to 17—20° C.

The level of the water in the spawning places is sometimes so low that the back of the fishes come out of the water and the fishes can easily be captured by hand (Dottrens, 1952; Papadopol made the same observation on the the Pingărați brook, in the first decade of June, 1967).

At a temperature of 18—21° C the hatching takes place after 6—7 days. The larval period lasts 35 days and the whole development period (embryonal and larval) 45 days. When hatching, the larvae have 12—13 mm total length (Starmach, 1961). The growth is rather slow during the first summer. On the 21 August, 1964, the standard length of young specimens from the Șipa brook near Sinaia, resulting from different spawning portions, ranged between 10 and 21 mm, the total length between 11.5 and 25 mm and the weight between 6 and 90.5 mg (n = 38 specimens) (Table 8).

According to the observations carried out by Starmach (1961) in experimental conditions, the above-mentioned body sizes correspond to the following ages (in days): 11.5 mm, to 7—9 days (i.e. immediately after the hatching), 14—15 mm to 17 days, 16—17 mm to 28 days, 24—25 mm total length to 42 days.

The further growth of *Ph. phoxinus* is rather slow, except during the first two years, before the specimens reach sexual maturity and spawn for the first time. The same phenomenon occurs in other Cyprinidae from the temperate zone (Table 9). At the age of two years the minnow reaches a body length of 53—62 mm, at three years 60—70 mm, at four years only 71 to 80 mm. The pattern of ponderal growth is also rather slow, nevertheless it remains strong enough even after the occurrence of the sexual maturity; this results from the data in Table 9.

The value of the coefficient of condition (after Fulton) shows a positive variation in relation with the age, which is more evident at the beginning of the spawning season (May, 13—14, the Bega R.).

The pattern of growth, especially of length growth, is practically the same in the Minis and Bega rivers populations, being lower than in the populations from the Mszanka (Poland), the Barthay, and Windermere lake (England) and similar to that of the specimens from the Niemec brook. The values of the ponderal growth and especially those of the coefficient of condition are evidently higher in sexually mature, 3—4 years old specimens from Romania.

CONCLUSIONS

1. The analysis of the size structure of *Phoxinus phoxinus* populations from some Romanian waters (The Roșu lake, the Prejmer, Doftana and Căpus rivers) and of the sex ratio led to the following conclusions:

a) Sexually mature specimens, 50—70 mm long and 2—7 g heavy dominated in the samples examined (Table 1).

b) Except during the spawning season, females are by far more numerous than males (82% in the Doftana r.) (Table 2).

2. The qualitative and quantitative study of the gonads and their component elements in mature specimens, at the beginning of the spawning season and somewhat later showed that:

a) Males and females reach the sexual maturity at the age of two years, at a minimal size of 41 mm and 1 g (males) and 47 mm and 1,76 g (females) (Table 1). The minnow, like other freshwater fishes (bitterling, bleak, roach, crucian carp, sunfish, etc), belongs to the group of polycyclic early maturating species (Papadopol, 1960—1969).

b) The absolute fecundity of the females from the Bega R, having 47 to 77 mm standard length, 1.76—9.6 g and 2—4 years, ranges between 1359 and 5515 eggs (Table 5); the relative fecundity between 503—930 eggs/g (Table 6); the variation depends on age and size. The relative fecundity is higher in two years old females, which spawn for the first time.

c) Our data, summarized in tables 5 and 6, as well as those recently published by Žukov (1965) and Balon (1966), demonstrate that the absolute and relative reproductive potentiality of the minnow is higher than it has been reported by older authors: Berg (1949); Nikolskij (1954), Cărăușu (1952), Vasiliu (1959), Spilmann (1961), and Bănărescu (1964) (up to 1000 eggs).

d) The gametogenesis is asynchronous; eggs and ovocytes in different stages of development (b, c, d) are distributed rather uniformly in the ovary mass (Fig. 1). The spawning occurs in 4—5 successive portions during a prolonged spawning season which lasts in Romania from the second half of May or the beginning of June till the second half of July or the middle of August. The eggs laid in the first portion represent about 24.4% of the total amount of eggs and ovocytes; in 47—77 mm long females from the Bega R., this value corresponds to 354—1616 eggs. The remaining ovocytes (representing 75.6% of the total amount) are laid in 3—4 successive portions, at intervals of 15 days (Table 7).

3) The study of the pattern of growth in very young, juvenile and adult specimens showed that:

a) The young specimens reached 10—11 mm (without C) and 6 mg at the age of 7—8 days, 20—21 mm and more than 90 mg after 40—45 days (specimens from the Sipa brook, near Sinaia) (Table 8).

b) The pattern of linear growth of both juveniles and adults is rather slow, this growth being, however, somewhat stronger during the first two years, before the reaching of sexual maturity. Yet the pattern of ponderal growth remains comparatively strong during the whole life, as in other species of Cyprinidae. The pattern of linear growth is practically the same in the populations from the Minis and Bega rivers, being similar to that recorded in the population from the Niemec brook (Poland), but somewhat lower than in the rivers Mszanka (Poland), Barthay, and in Widermere lake (England). The pattern of ponderal growth and especially the coefficient of condition are evidently higher in Romanian specimens, especially in mature ones (Table 9).

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The plate will be found at the end of this issue.

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WACHSTUMSVARIABILITÄT DER KÖRPERLÄNGE VERSCHIEDENER FISCHARTEN

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Abstrakt: Bei Anwendung des χ^2 Testes wurden sowohl die eigenen Ergebnisse als auch die anderer Autoren bezüglich des Längenwachstums verschiedener Fischarten kontrolliert. Dabei ergaben sich in allen Fällen nicht signifikante Unterschiede. Die signifikant unterschiedliche Mindestabweichung ist in Prozent des erwarteten Wachstumswertes angegeben. Die Mindestabweichungen betrugen bei grossen Fischarten (Hecht) 5,4%, bei mittelgrossen (Plötze, Döbel) 11,2 und 9,4%, bei ausgesprochen kleinen Fischarten (Kaulbarsch) erreichten sie dagegen 16,6%.

EINLEITUNG

Trotz langjähriger Wachstumsstudien widmete man der Kontrolle der erzielten Ergebnisse nicht genügend Aufmerksamkeit. Das Schuppenmaterial von Plötzen aus dem Klíčava Stausee gewährleistet zwei Kontrollmöglichkeiten der Wachstumsergebnisse: a) durch Rückrechnung werden die Wachstumswerte bei den einzelnen Altergruppen im Jahresablauf festgestellt und mittels χ^2 Testes bewertet, b) man testet Wachstumswerte verschiedener Altersgruppen in aufeinander folgenden Jahren; diese Kontrolle kann auch zur Prüfung von Ergebnissen anderer Autoren verwendet werden.

MATERIAL UND METHODIK

In den Jahren 1967—68 wurde das Schuppenmaterial der Plötzen im Laufe des ganzen Jahres gesammelt. Die Proben aus den Jahren 1970—72 enthalten dagegen nur Fische aus den Frühjahrs- und Herbstmonaten. In allen Fällen fanden Netze von 30 m Länge, 3 m Höhe, mit einer Maschenweite von 3 × 3 cm Verwendung. Es wurden folgende Fachausdrücke (Abkürzungen) und Formeln benutzt:

l^t_{ijk} — die Körperlänge der i-ten Altersgruppe in j-tem Zeitabschnitt der k-ten Lokalität und in der Zeit „t"

l^t_{jk} — durchshnittliche Körperlänge der i-ten Altersgruppe aus mehreren bzw. allen Zeitabschnitten in der k-ten Lokalität und in der Zeit „t"

l^t_k — durchschnittliche Körperlänge aus mehreren bzw. allen Altersgruppen und Zeitabschnitten in der k-ten Lokalität und in der Zeit „t"

l^t — durchschnittliche Körperlänge aus mehreren bzw. allen Altersgruppen, Zeitabschnitten und Lokalitäten in der Zeit „t"

$$l^t_{ik} = \frac{\sum_{j=1}^{m} l^t_{ijk}}{j}, \qquad l^t_k = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m} l^t_{ijk}}{ij} \qquad l^t = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\sum_{k=1}^{p} l^t_{ijk}}{ijk}$$

Ab_{ik} — durchschnittliche Abweichung

Ab_{ik} in % l^t_{ik} — durchschnittliche Abweichung in Prozent der durchschnittlichen Körperlänge (l^t_{ik})

$$Ab_{ik} = \frac{\sum_{j=1}^{m} (l^t_{ijk} - l^t_{ik})}{j}, \qquad Ab_{ik} \text{ in } \% \; l^t_{ik} = \frac{Ab_{ik}}{l^t_{ik}} \cdot 100$$

i = 1, 2, 3, . . . n — 1, n Altersgruppe
k = 1, 2, 3, . . . p — 1, p Lokalität
j = 1, 2, 3, . . . m — 1, m Zeitabschnitt der k-ten Lokalität
t = 1, 2, 3, . . . u — 1, u Lebensjahre

min. Ab in % l_{ik} — die Mindestabweichung in Prozent der durchschnittlichen Körperlänge (l_{ik})

Zur Prüfung der Signifikans des Unterschiedes der Wachstumswerte diente der χ^2 Test,

$\chi^2_{ik} = \sum_{t=1}^{u} \left[\frac{(l^t_{ijk} - l^t_{ik})^2}{l^t_{ik}} \right]$, wobei l^t_{ijk} den festgestellten Wert und l^t_{ik} den erwarteten (theoretischen) Wert darstellt.

Die Nullhypothese setzt eine Übereinstimmung der festgestellten und erwarteten Werte voraus. Mit Hilfe der Testformel kann die signifikant unterschiedliche Mindestabweichung in Prozent der erwarteten Wachstumswerte (min Ab_{ik} in % l^t_{ik}) errechnet werden. Von diesem Wert an sind alle Unterschiede zwischen den festgestellten (l^t_{ijk}) und erwarteten (l^t_{ik}) Körperlängen, in Prozent der erwarteten Werte signifikant unterschiedlich. Die Formel zur Berechnung lautet wie folgt:

$$\chi^2_{ik} = \sum_{t=1}^{u} \left[\frac{\left(\frac{l^t_{ik} x}{100} \right)^2}{l^t_{ik}} \right].$$

für P (5 %). Eine Teillösung ergibt: $\sum_{t=1}^{u} 0{,}0000\, l^t_{ik}\, x^2 = \chi^2$, *wobei* x = *min.* Ab_{ik} *in* % l^t_{ik}, P (5 %) die 5% Wahrscheinlichkeit der Nullhypothese bei u — 1 Freiheitsgraden darstellt. Die mathematischen Ausdrücke Ab_{ik}, Ab_{ik} in % l^t_{ik} und χ^2_{ik} wurden in Hinsicht zu den Werten l^t_{ijk} und l^t_{ik} errechnet. Dasselbe gilt für l^t_k und l^t.

Beispiel: Für die dritte Altersgruppe wurden im Jahre 1968 folgende erwartete Körperlängen festgestellt — 57, 144 und 180 mm (Tab. 1). Nach der angegebenen Formel beträgt die Mindestabweichung $0{,}0057\, x^2 + 0{,}0144\, x^2 + 0{,}0180\, x^2 = 6$, x = 12,6 % (P (5%) = 6).

ERGEBNISSE UND DISKUSSION

Die häufigsten Fehler bei der Rückablesung und Rückrechnung der Länge der Fische sind der Unzulänglichkeit des Materials, der Ungleichartigkeit der Population bzw. der schwierigen Rückablesung der Annulen zuzuschreiben. Wenn wir die schlechte Detektion der Annulen als Fehlerquelle ausscheiden, so bewirken die beiden anderen Faktoren eine falsche Rückberechnung der Längen oft dort, wo wir theoretisch nicht signifikant unterschiedliche Ergebnisse erhalten sollten. Als nicht homogen betrachte ich solches Material, das nicht von den gleichen Elementarpopulationen stammt (Lebedev, 1969). Dies bewirkt, dass Ergebnisse der Wachstumsforschung gleicher Altersgruppen, die aber aus verschiedenen Jahren stammen, sich erheblich voneinander unterscheiden können.

Als Beispiel für die Variabilität der Wachstumswerte bei einzelnen Altersgruppen der Plötze im Laufe eines Jahres sei das Jahr 1968 angeführt. Nur in einem Fall, bei der 6. Altersgruppe (das Material vom Monat Mai) ergab sich ein signifikanter Wert des χ^2 Testes. In allen anderen Fällen waren die Testwerte bei den in Frage kommenden Altersgruppen (3, 4, 5, 6) für den Zeitraum April—Oktober nicht signifikant. Weiterhin wurde errechnet, dass die

Tabelle 1. Das Wachstum (in mm) verschiedener Altersgruppen der Plotze im Jahre 1968. Stausee von Klíčava

Monat	Fischanzahl	l_{ijk}^{t-2}	l_{ijk}^{t-1}	l_{ijk}^{t}	X_{ik}^{2}	P (in %)	Fischanzahl	l_{ijk}^{t-5}	l_{ijk}^{t-4}	l_{ijk}^{t-3}	l_{ijk}^{t-2}	l_{ijk}^{t-1}	l_{ijk}^{t}	X_{ik}^{2}	P (in %)
3 Altersgruppe (i = 3)							**6 Altersgruppe (i = 6)**								
IV	-						5	64	106	128	155	171	189	10,235	8
V	1	59	149	190	0,8	70	15	60	132	167	195	214	226	12,118	3
VI	2	54	143	175	0,368	80	5	55	121	160	180	200	210	1,878	90
VII	1	58	149	193	1,133	50	3	56	115	163	181	204	215	3 313	70
VIII	4	54	144	174	0,358	80	4	50	115	146	169	182	197	0,998	96
IX	—						2	51	109	134	155	171	188	7 911	20
X	3	61	136	166	1,813	40	1	54	121	142	170	194	213	0.852	98
l_{ik}^{t} (theor.)		57	144	180	4,472	80		56	118	149	172	191	205	37,305	15
Ab in % l_{ik}^{t}		4,6	2,8	5,3				5,4	5,7	15,4	6,6	12,2	5,9		
4 Altersgruppe (i = 4)							**5 Altersgruppe (i = 5)**								
IV	2	54	123	164	187	0,426	94	46	59	119	152	169	187	0,339	99
V	29	61	127	161	182	0,607	85	98	57	118	153	169	185	0,143	99
VI	6	56	120	155	177	0,262	97	27	57	116	151	169	187	0,099	99
VII	14	57	125	163	184	0,203	98	62	56	119	152	169	187	0,177	99
VIII	23	54	118	153	177	0,587	90	60	55	114	146	163	183	0,261	99
IX	27	57	119	154	177	0,277	96	40	55	110	145	163	181	0,641	96
X	27	56	130	161	183	0,445	94	39	55	116	148	164	181	0,149	99
l_{ik}^{t} (theor.)		56	123	159	181	2,807	99		56	116	150	167	184	1,810	99
Ab in % l_{ik}^{t}		2,8	2,9	2,5	2 0				2,8	2,0	1,8	1,6	1 3		

Mindestabweichungen in % l_k für die 3., 4., 5. und 6. Altersgruppe 12,6 %, 12,2 %, 11,8 % und 11,3 % erreichen müssten, um signifikant unterschiedlich zu sein, Tabelle 1. Solche hohen Abweichungen lagen auch nicht im Jahr 1967 vor, so dass auch ohne Berechnung des entsprechenden Testes nicht signifikante Unterschiede bestätigt werden können.

Die erwähnte Methode gehört zu denen, die zur Kontrolle der eigenen Ergebnisse der Wachstumsforschung dienen können. Eine andere Möglichkeit, die Genauigkeit der Wachstumsstudien zu prüfen, bietet die Arbeit mit markierten Fischen (Oliva, 1955), oder mit Fischen bekannten Alters (Živkov, 1967). Die beiden hier zitierten Autoren verwendeten Wachstumswerte zu anderen Zwecken. Schliesslich wurde bei uns auch noch nicht die Methode der zwei- oder mehrmaligen Annulenablesung derselben Schuppen durch mehrere Personen angewandt, die sonst Verwendung findet (Regier, 1968; Mina, 1973).

Das Wachstumsstudium der Plötzen im Klíčava Stausee ermöglicht noch eine weitere Art der Kontrolle. Man vergleicht hierbei das Wachstum einer bestimmten Altersgruppe über mehrere aufeinander folgenden Jahre (Tab. 2a). Aus der Tabelle ist gut ersichtlich, dass die Länge der Fische gleicher Altersgruppen nach und nach mit zunehmendem Alter abnimmt. Dies erklärt sich auch durch das bekannte Phänomen nach R. Lee (Ricker, 1969). Weiter kann nach den Angaben dieser Tabelle durch die Berechnung der Mindestababweichung auch die Kontrolle eigener Wachstumswerte durchgeführt werden. Ab in % l_k sind unbedeutend; χ^2 Wert fehlt.

Eine derartige Kontrolle der Wachstumswerte kann natürlich auch an den Angaben anderer Autoren vorgenomen werden. Als Beispiel seien hier die Wachstumswerte aus der Arbeit von Cabejšek, Frank (1968) angeführt, die sich auf Plötzen aus dem Stausee Lipno (1960—1962) beziehen (Tab. 2d). Die Berechnung des χ^2 Testes wurde nur für die 5. Altersgruppe vorgenomen. Bis auf eine Ausnahme wurden nur die durchschnittlichen Abweichungen in Prozent der durchschnittlichen Körperlänge berechnet und nicht signifikante Unterschiede festgestellt. Dieser Fall wurde auch noch auf Grund des Körpergewichtes berechnet. Hier fällt der hochgradig signifikante Wert des Testes auf, was aber selbstverständlich erscheint, da das Verhältnis zwischen Länge und Gewicht durch die Formel $w = aL^3$ gegeben ist. Berechnungen auf Grund des Gewichtes ergeben weit ausdrucksvollere Differenzen. Das gleiche Bild ergeben auch die durchschnittlichen Abweichungen, welche 20% und mehr Differenz aufweisen. Als weiteres Beispiel dienten die Angaben über den Katzenwels von Hensel (1966). Der Wert des χ^2 Testes erreicht hier in einem Falle fast 5% der Wahrscheinlichkeit der Nullhypothese, Tabelle 2b. In diesem Zusammenhang muss noch erwähnt werden, dass die Ergebnisse der beiden angeführten Beispiele stark durch die geringe Stückzahl des Materials beeinträchtigt sind. Weiterhin wurden auch noch die Wachstumswerte des Barsches aus dem Klíčava Stausee von Holčík (1969) und Lohnisky (1967) für zwei nachfolgende Jahre (1962 und 1963) herangezogen. Wie die Tabelle 2c zeigt, ergaben sich auch in diesem Falle keine signifikanten Unterschiede. Zusammenfassend kann gesagt werden, dass trotz anscheinend bedeutender Differenzen der gegebenen Wachstumswerte in keinem Fall signifikante Unterschiede erzielt wurden.

Berücksichtigen wir stets, dass die Testwerte in der Biologie nur eine Hilfsrolle spielen. Die Ergebnisse sollten immer exakt in biologischen Fach-

Tabelle 2

a) Das Wachstum der Plotzen vom Ablaich im Jahre 1964. Stausee von Kličava

Altersgruppe	Jahr	Fischanzahl	l_{ijk}^{t-7}	l_{ijk}^{t-6}	l_{ijk}^{t-5}	l_{ijk}^{t-4}	l_{ijk}^{t-3}	l_{ijk}^{t-2}	l_{ijk}^{t-1}	l_{ijk}^{t}	X^2	P in %
3	1967	69						54	131	171		
4	1968	127					57	123	159	180		
5	1969	12				59	121	156	176	186		
6	1970	128			56	116	148	167	183	194		
7	1971	55		54	115	149	170	186	199	207		
8	1972	48	57	111	141	159	176	191	204	217		
l_k^t			56	120	154	170	183	193	206	217		
Ab in % l_k^t			2.7	4.6	4.0	3.5	1.8	1 6	0.7	—	—	—

b) Das Wachstum der Katzenwelse vom Ablaich im Jahre 1954. (Hensel, 1966)

5+	1959	1				92	119	146	176	200		
6+	1960	5			109	152	186	212	232	254		
l_k^t					100	136	166	194	216	254		
Ab in % l_k^t					8.0	12.1	12.6	9.3	7.4	—	8.020	9

c) Das Wachstum der Flussbarsche vom Ablaich im Jahre 1959. (Lohnisky, 1967; Holčík, 1969)

3	1962	11						76	104	128		
4	1963	104					79	128	144	155		
l_k^t							78	116	136	155		
Ab in % l_k^t							2.6	10.3	5.9	—	1.741	40

d) Das Wachstum der Plotzen vom Ablaich im Jahre 1955. (Cabejšek, Frank, 1968)

5	1960	3				51	92	126	177	203		
6	1961	9			38	73	101	140	176	205		
7	1962	2		45	75	118	154	196	238	258		
l_k^t				45	80	115	157	192	221	258		
Ab in % l_k^t				11.1	10.0	7.8	8.3	5.2	7.3	—	5.051	28

ausdrücken wiedergegeben werden. Ihre biologische und mathematische Bedeutung können nicht auf die gleiche Stufe gestellt werden (Pivničková, 1973). Die Testwerte stellen eigentlich nur Anhaltspunkte dar, von denen man bei der Bewertung des Wachstums der Fische ausgehen kann. Dennoch ist empfehlenswerter, die Ergebnisse nach diesen Werten abzufassen, als das Wachstum in der Form wie „verschieden, gleich" oder „ähnlich"zu kennzeichnen.

Noch zu einigen weiteren Angaben: Brožová (1972) errechnete auf Grund veröffentlichter Angaben die durchschnittlichen Körperlängen (l^t) der Plötzen aus der ČSSR (aus den Lokalitäten — Stauseen Slapy, Pastviny, Orava,

Lipno, einem Tümpel bei Čelákovice, der Donau und der Thaya) für neun Lebensjahre wie folgt: 48, 78, 106, 130, 144, 170, 192 und 195 mm. Mit Ausnahme des Stausees Slapy stellte sie in allen Lokalitäten ein signifikant unterschiedliches Wachstum der Plötzen fest. Die Wahrscheinlichkeit der Nullhypothese erreicht hier Werte, die kleiner als 1% sind. Wenn wir von den hier angegebenen durchschnittlichen Körperlängen ausgehen, müsste die Mindestabweichung in % l^t für die 9 Lebensjahre (t = 9) 11,2 % betragen. Jede Plötzenpopulation, deren Körperlängen sich durchschnittlich von diesem Wachstumsmittelwerte wenigstens um 11,2 % unterscheiden, könnte als signifikant verschieden gelten. Auf ähnliche Weise lassen sich auch die durchschnittlichen Körperlängen des Döbels in der ČSSR auf Grund der Tabelle von Pecl (1969) berechnen. Es handelt sich um 9 Lebensjahre aus verschiedenen Lokalitäten (den Stauseen von Orava, Slapy und Klíčava und den Flüssen Svratka, Moravice, Thaya und Lučina), für die der Autor Körperlängen von 61, 105, 143, 175, 203, 229, 253, 279 und 299 angibt. Die Mindestabweichung müsste 9,4 % erreichen, um signifikant unterschiedlich zu sein. In unserem Gebiet können Plötzen und Döbel als mittelgrosse Fische gelten. Als Beispiel für eine grosse, schnell wachsende Art dient der Hecht. Die durchschnittlichen Körperlängen dieser Art können für die 9 Lebensjahre mit 196, 313, 415, 533, 618, 713, 780, 814 und 970 mm angegeben werden. Es handelt sich hier um die Lokalitäten: Stausee Lipno. Durchschnitt der Jahre 1959—1967 (Vostradovský, 1974), Klíčava Stausee (Holčík, 1968), Tümpel bei Čelákovice (Oliva, 1956) Stausee Slapy (Oliva, Frank, 1959). Die Mindestabweichung müsste in diesem Falle 5,4 % erreichen. Als Beispiel für eine kleine Art sei der Kaulbarsch angeführt. Seine durchschnittliche Körperlänge in den Stauseen von Slapy und Pastvina beträgt nach Oliva, Vostradovský (1960): 51, 69, 87, 96, 104 und 106 mm. Die Mindestabweichung müsste hier den Wert von 14,6 % erreichen. Zusammenfassend kann gesagt werden, dass die Mindestabweichungen bei kleinen Fischen (Kaulbarsch) etwa 15 %, bei mittelgrossen (Plötze, Döbel) etwa 10 % und bei grossen Arten (Hecht) ca. 5 % ausmachen. Ich halte es nicht für notwendig für weitere Arten Mindestabweichungen anzuführen. Die Berechnung ist einfach und zur flüchtigen Orientierung können die angeführten Grenzen dienen.

Zum Schluss möchte ich dem Herrn RNDr. S. Frank CSc. für seine wertvollen Ratschläge und Bemerkungen zu meiner Arbeit herzlichst danken.

ZUSAMMENFASSUNG

1. Mit Hilfe des χ^2 Testes wurden bei Plötzen der 3., 4., 5. und 6. Altersgruppe aus dem Klíčava Stausee im Laufe des Jahres 1968 nicht signifikante Unterschiede der Wachstumswerte festgestellt.

2. Nicht signifikante Unterschiede ergaben sich auch dann, wenn die Wachstumswerte einer bestimmten Altersgruppe über mehrere Jahre hinweg verglichen wurden.

3. Es wurde eine Methode zur Berechnung der Mindestabweichung entworfen. Sie ermöglicht es, Wachstumswerte einer bestimmten Art von den erwarteten (durchschnittlichen) zu testen, damit diese Werte (min. **Ab**) gleichzeitig als signifikant unterschiedlich bezeichnet werden können.

4. Bei Plötzen aus dem Klíčava Stausee wurden für die 3., 4., 5. und 6. Altersgruppe Mindestabweichungen in Prozent der durchschnittlichen Körperlängen (l'_{ik}) von 12,6, 12,2, 11,8 und 11,3 % festgestellt.

5. Aus Arbeiten anderer Autoren wurden durchschnittliche Körperlängen (l^t) für 9 Lebensjahre der Plötze und des Döbels (mittelgrosse Art), des Hechtes (grosse Art) und für 6 Lebensjahre des Kaulbarsches (kleine Art) übernommen und Mindestabweichungen von 11,2, 9,4, 5,4 und 14,6 % errechnet. Für die meisten, bei uns vorkommenden Fische gelten demnach Mindestabweichungen von 5—15 %.

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CONTRIBUTION TO THE KNOWLEDGE OF HELMINTH FAUNA OF SMALL MAMMALS (RODENTIA AND INSECTIVORA) IN SPAIN

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Abstract: The helminth fauna of small mammals occurring in Spain has been studied. The parasites were recovered from 241 specimens of rodents of the family Microtidae, 11 specimens of rodents of the family Muridae and 26 specimens of insectivores. The rodents examined were found to harbour 10 species of helminths. Among them were the larval stages of cestodes of the following 5 species: *H. taeniaeformis*, *T. crassiceps*, *T. martis*, *T. polyacantha* and *T. tenuicollis*. In addition to them adult cestodes of the species *A. macrocephala*, *P. brevis* and *H. horrida* and nematodes of the species *H. costellatum* and *C. hepatica* were identified. Insectivores harboured only 1 larval stage of the cestode *C. globifera*. The trematodes and acanthocephalans were not found in the material examined.

The helminth fauna of small mammals in Spain has not yet been studied in detail (Bernard, 1961; Tenora and Mészáros, 1972 Quentin, 1973). We welcomed therefore the possibility to study the material of helminths from some species of small mammals collected by Dr. H. Winking during the scientific expedition of the Institute of Zoology, University of Bonn, headed by Prof. Niethammer, from 15th March to 13th May 1972. We are grateful to both these scientists for kindly making specimens available for this study.

MATERIAL AND METHODS

A total of 198 *Pitymys mariae*, 5 *P. pyrenaicus*, 21 *P. duodecimcostatus*, 14 *Microtus arvalis asturianus*, 2 *M. aurestis*, 1 *Clethrionomys glareolus*, 11 *Apodemus sylvaticus* and 26 specimens of undetermined species of the genera *Talpa*, *Sorex*, *Neomys* and *Crocidura* were dissected and examined for helminths. The rodents harboured 5 species of larval stages of cestodes, 3 species of adult cestodes and 2 species of nematodes, while in insectivores (*Crocidu a russula*) only 1 larval stage of cestode was found.

HELMINTH SPECIES RECOVERED

A. Cestoidea

1. *Hydarigera taeniaeformis* (Batsch, 1786) (larvae)

Intermediate host: *Pitymys mariae*.
Location in intermediate host: liver.
Locality: Riano, La Robla.

This species is a common parasite of felids. It has a cosmopolitan distribution (Abuladze, 1964; Verster, 1969). As intermediate hosts serve

different species of rodents. An unusual finding was reported by Ryšavý (1973), who recovered the larval stage of this cestode from the liver of the pheasant *Phasianus colchicus*. In Spain, this larval stage was found in *P. savii* (Tenora and Mészáros, 1972). The species *P. mariae* has been reported for the first time as intermediate host of this cestode.

2. *Taenia crassiceps* (Zeder, 1800) (larvae)

Intermediate host *Microtus arvalis asturianus*.
Location in intermediate host: body cavity.
Locality: Saldana.

The adult cestodes of this species parasitize the intestine of fox or other carnivores in the Holoarctic Region (Abuladze, 1964; Verster, 1969). The larval stage develops under the skin and in abdominal cavity of rodents. This parasite has recently been reported from many species of rodents of the genera *Microtus*, *Pitymys*, *Clethrionomys*, *Arvicola*, *Ondatra*, *Rattus*, *Apodemus*, and *Marmota* (Erhardová, 1958; Tenora, 1967; Wandeler and Hörning, 1969—1971; Prokopič, 1972; Pfaller and Tenora, 1972; Murai and Tenora, 1973a; Prokopič and Genov, 1974 and others). The larval stages of *T. crassiceps* have often been reported also from rodents occurring on the territory of the U.S.S.R. (Abuladze, 1964; more recently Prokopič and Madzaberidze, 1972)

In Spain, the larval stage of this parasite has been known to parasitize *P. mariae* (Tenora and Mészáros, 1972) *Microtus arvalis asturianus* is a new intermediate host registered.

3. *Taenia tenuicollis* Rudolphi, 1819 (larvae)

Intermediate hosts: *Putymys mariae*, *P pyrenaicus*, *Microtus arvalis asturianus*, *Apodemus sylvaticus*.
Location in intermediate host: liver.
Locality: Santo Tirso, Villalba, Mayorga, Riano, Tazones, Espanilla, San Vicente, Saldano, Burguete.

The adult cestodes of this species parasitize the intestine of the representatives of the family Mustelidae. It is distributed in the Holoarctic Region (Abuladze, 1964; Verster, 1969). Adult specimens of this parasite show a large morphological variability of hooks, as it was mentioned for example by Freeman (1956). A considerable morphological variability of hooks was observed also in larval stages (according to Andrejko, 1963 and Prokopič, 1965). This cestode employs as intermediate hosts numerous species of rodents; of insectivores, the species *Talpa europea* was recorded (Abuladze, 1964). The larval stages have often been reported in the literature under the name *T. mustelae* (Verster, 1969) or *Cysticercus talpae* (Abuladze, 1964). Critical remarks to the nomenclature of larval stages of *T. tenuicollis* were published by Tenora and Vaněk (1969). Besides the intermediate hosts mentioned in the compendium by Abuladze (1964) also *P. subterraneus* and *P. tatricus* (Erhardová, 1958) as well as *Citellus citellus* (Agapova, 1953) were reported.

The rodents *Pitymys mariae*, *P. pyrenaicus* and *Microtus arvalis asturianus* are new intermediate hosts of this cestode.

4. *Taenia martis* (Zeder, 1803) (larvae)

Intermediate host: *Clethrionomys glareolus.*
Location in intermediate host: thoracic cavity.
Locality: Burguete.

The hosts, as well as the geographic distribution, are the same as with the species *T. tenuicollis. T. martis* has also a great metrical and morphological variability. The nomenclatoric problems are dealt with in the paper by Murai and Tenora (1973a). In the compendia by Abuladze (1964) and Verster (1969) the species *Clethrionomys glareolus, C. gapperi* and *Microtus agrestis* are mentioned as intermediate hosts of this cestode. Recently also *Sciurus altaicus* (Prokopič and Macaberidze, 1972) and *Apodemus flavicollis* (Murai and Tenora, 1973a) have been reported to serve as intermediate hosts.

5. *Taenia polyacantha* (Leucart, 1856) (larvae)

Intermediate host: *Pitymys mariae.*
Location in intermediate host: body cavity.
Locality: Remosa.

The adult specimens of this cestode parasitize the representatives of the family Canidae in the Holoarctic Region (Abuladze, 1964; Verster, 1969). Besides many other hosts reported by Abuladze (1964) also *Pitymys tatricus* and *Microtus agrestis* were recorded (Tenora, 1967). *Pitymys mariae* is a new intermediate host of this cestode.

6. *Cladotaenia globifera* (Batsch 1786) (larvae)

Intermediate host: Crocidura russula.
Location in intermediate host: liver.
Locality: Tazones.

This species parasitizes birds of the order Falconiformes in the Holoarctic Region. The larval stages are known to infect numerous species of rodents and insectivores (Abuladze, 1964). Besides the intermediate hosts mentioned by Abuladze, many other species were reported, namely *Crocidura leucodon* (Dimitrova et al., 1962), *Sorex minutus, Neomys anomalus* and *Apodemus sylvaticus* (Prokopič, 1972; Prokopič and Genov, 1974), *Apodemus flavicollis* and *Clethrionomys glareolus* (Murai and Tenora, 1973a). *Crocidura russula* is a new intermediate host of this cestode.

7. *Aprostatandrya macrocephala* (Douthitt, 1915)

Hosts: *Pitymys mariae, Microtus arvalis asturianus, M. agrestis.*
Location in host: small intestine.
Locality: Malveira, Santa Maria, Caldas de Reyes, Villalba Cabones, Reinosa, Sorria, Espinilla, Astorga, Mayorga, La Robla.

This cestode species was reported to parasitize the rodents of the Holoarctic Region (Douthitt, 1915; Rausch and Schiller, 1949; Spassky, 1951 and others). In recent years it has been found in Czechoslovakia (Prokopič, 1970, 1972), Poland (Kisielewska and Zubczewska, 1973), Hungary (Tenora, Murai, Mészáros, 1973) and Bulgaria (Prokopič and Genov, 1974). In the U.S.S.R., it was recorded by Nadtochiy (1970) in the

Far East Region. Erhardová-Kotrlá and Daniel (1971) recovered this species from a new host, *Alticola argentata*, in Pakistan, Tenora (1972), similarly as Rausch (1957), points out that the findings reported under the name *A. macrocephala* concern the specimens of great morphological and metrical variability and that the material described under this name should be revised.

Only a single report on the cestodes of the family Anoplocephalidae, without species determination, has been available from Spain (Tenora and Mészáros, 1972). The species *Pitymys mariae* and *Microtus arvalis asturianus* are new hosts of *A. macrocephala*.

8. *Paranoplocephala brevis* Kirschenblat, 1938

Host: *Pitymys mariae*.
Location in host: caecum.
Locality: Santa Maria, Villalba, La Robla, Reinosa.

This species is a parasite of the rodents in the Palaearctic Region and its nomenclature has often been discussed (compare the compiled data in the paper by Tenora, Pfaller and Murai, 1971). In Europe, it was reported mostly from the rodents of the family Microtidae, namely from the representative of the genera *Pitymys*, *Microtus* and *Clethrionomys*, occasionally in rodents of the genus *Apodemus* (see the results of the author's own studies, as well as literary data, mentioned in the papers by Prokopič, 1970, 1972; Prokopič and Genov, 1974). The species *P. mariae* is a new host of this cestode in Spain.

9. *Hymenolepis horrida* (Linstow, 1901)

Hosts: *Pitymys mariae, P. pyrenaicus, Microtus arvalis asturianus*.
Location in host: small intestine.
Locality: Guimaraes, Santa Maria, Caldos de Reyes, Cabanes, Burguete, Saldana.

This species of cestodes parasitizes rodents of the Holoarctic Region. Murai and Tenora (1973), on the basis of their own studies and on literary data, arrived at the conclusion that this species occurs mostly in the representatives of the family Microtidae (subfamily Microtinae) and not in the rodents of the family Muridae (Murinae), from which it was originally described as a parasite of *Rattus norvegicus*.

The hosts *Pitymys mariae*, *P. pyrenaicus* and *Microtus arvalis asturianus* represent new host records of this cestode species.

B. Nematoda

1. *Heligmosomum costellatum* (Dujardin, 1845)

Host: *Pitymys mariae*.
Location in host: small intestine.
Locality: Villalba, Astorga, Riano, Reinosa.

This species of nematodes parasitizes rodents of the Palaearctic Region (see compiled data in the paper by Durette-Desset, 1968; Tenora and Mészáros, 1971). According to Durette-Desset (1968) and Prokopič and Genov (1974), *H. costellatum* parasitizes mostly the representatives of

the family Microtidae. The latter authors pointed out that in the northern parts of its distribution area *H. costellatum* was found in the hosts of the family Microtidae in all regions, while in the southern part its occurrence is limited to mountainous region. A similar phenomenon was observed by Rosický (1966) during the study of distribution of some flea species. In Spain, Tenora and Mészáros (1972) reported *H. costellatum* as being parasitic in *Pitymys duodecimcostatus* in the Pyrenees. The species *P. mariae* is a new host of this nematode.

2. *Hepaticola hepatica* (Bancroft, 1893)

Host: *Apodemus sylvaticus*.
Location in host: liver.
Locality: Burguete.

This species of nematodes has often been placed in the genus *Capillaia*. It is parasitic in the liver of rodents of the Holoarctic Region and has also been found in man (Šlais, 1970). A wide variety of hosts have been reported to harbour this parasite in the Palaearctic Region: *A. flavicollis*, *Cl. glareolus* (Erhardová, 1958), *Arvicola terrestris* (Mozgovoj et al., 1966), *Ellobius talpinus* and *Cricetus cricetus* (Agapova, 1953), *Rattus rattus* (Merkuševa, 1963).

DISCUSSION

A majority of the rodents examined belong to the family Microtidae (241 specimens). They were found to harbour the larval stages of 5 species of cestodes, adult specimens of 3 species of sectodes and 1 species of nematodes. The most frequently encountered parasite was the cestode *Aprostatandrya macrocephala* showing the highest incidence of infection in *P. mariae*. According to Prokopič (1972), the incidence of infection with *A. macrocephala* in the same hosts is different in different localities. This author found *A. macrocephala* in 25% of *Microtus arvalis* examined in a locality (I) and in 9.6% of the same host from other locality (II) on the territory of Czechoslovakia. In *Clethrionomys glareolus* the ratio of incidence of infection with the same cestode was reverse. In the locality I the incidence of infection was 2%, whereas in the locality II it was 16.7%. A detailed study of this phenomenon from ecological view is comparised in the paper by Prokopič and Genov (1974).

Also the larvae of the cestode *Taenia tenuicollis* were frequently encountered in the material examined, namely in the liver of *P. mariae*. These findings give evidence of the large occurrence of the definitive hosts — Mustelidae and their infection with adult cestodes in the localities investigated. Among other species also *Paranoplocephala* brevis and *Hymenolepis horrida* were found in larger numbers.

Although the material obtained from Spain was not large, its study revealed some aspects which should be dealt with in the future. One of them is the fact that although primarily the species of the genus *Pitymys* were examined, which do not live, e.g., in Central and Eastern Europe, they were found to harbour only helminths parasitizing also other rodent species, mainly vole-like, from the Central and Eastern Europe. No helminth species has hitherto been found to be typical only of the representatives of different species belonging to the genus *Pitymys* in Spain. Another aspect follows from

Table 1. Survey of hosts and their infection with parasitic species of helminths

Hosts	1	2	3	4	5	6	7	8
H. taeniaeformis	2 ✓	0	0	0	0	0	0	0
T. crassiceps	0	0	0	2 ×	0	0	0	0
T. martis	0	0	0	0	0	1 ×	0	0
T. tenuicollis	13 ✓	1 ×	0	1 ×	0	0	1 ×	0
T. polyacantha	2 ×	0	0	0	0	0	0	0
C. globifera	0	0	0	0	0	0	0	1 ×
A. macrocephala	16 ✓	0	0	1 ×	1 ×	0	0	0
P. brevis	10 ×	0	0	0	0	0	0	0
H. horrida	7 ×	1 ×	0	0	1 ×	0	0	0
H. costellatum	3 ×	0	0	0	0	0	0	0
C. hepatica	0	0	0	0	0	0	1 ×	0

1. *P. mariae*, 2. *P. pyrenaicus*, 3. *P. duodecimcostatus*, 4. *M. arvalis asturianus*, 5. *M. agrestis*, 6. *C. glareolus*, 7. *A. sylvaticus*, 8. *C. russula*.

the comparison of the data on helminth fauna of small mammals available from Spain with those from other parts of the Palaearctic Region (see the above-cited authors as well as the following ones: Guex, 1934; Kiršenblat, 1938; Andrejko, 1960; Schmidt, 1961; Chiriac and Hamar, 1966; Wahl, 1967; Babajev, 1968; Dorosz, 1968; Prokopič and Mahnert, 1970; Prokopič et al., 1973). In the material from Spain many known cestode species are lacking, as for example *Hymenolepis straminea*, *H. asymmetrica*, *Catenotaenia pusilla*, *Skrjabinotaenia lobata* etc. Under other ecological conditions, this species have been found in many rodent species, even in vole-like rodents. On the other hand, it should be stressed that in the material under investigation the larval stages of cestodes markedly prevailed in comparison with other groups of helminths (larval stages of 6 cestode species, adult specimens of 3 cestode species and only 2 species of nematodes). Of nematodes only the species *Heligmosomum costellatum* and *Capillaria hepatica* were recorded. The latter species is of epidemiological importance, since it may parasitize also in liver of man. The trematodes and acanthocephalans were not found in the material examined.

CONCLUSIONS

1) The helminth parasites dealt with in this paper were recovered from the following hosts: 198 specimens of *Pitymys mariae*, 5 specimens of *P. pyrenaicus*, 21 specimens of *P. duodecimcostatus*, 14 specimens of *Microtus arvalis asturianus*, 2 specimens of *M. agrestis*, 1 specimen of *Clethrionomys glareolus*, 11 specimens of *Apodemus sylvaticus* and 26 specimens of undetermined species of the genera *Talpa*, *Sorex*, *Neomys* and *Crocidura*.

2) The 252 examined specimens of rodents of the families Muridae and Microtidae were found to harbour 10 species of helminths; from the 26 specimens of insectivores 1 helminth species was recovered at dissection.

3) In the total number of parasites the cestodes prevailed (9 species), among them their larval stages (6 species). The highest incidence and intensity of infection was observed with the species *A. macrocephala*. Of the 2 ne-

matode species recovered, *Hepaticola hepatica* is of epidemiological importance. The trematodes and acanthocephalans were not found.

4) Most part of the material from Spain consisted of rodents of the genus *Pitymys* (224 specimens). Up to the present time, no records have been available on a species whose helminth fauna would differ from the common helminth fauna of *P. subterraneus* and *P. tatricus* or other species of the family Microtidae from the Palaearctic Region.

5) Small terrestrial mammals of the species *P. mariae*, *P. pyrenaicus*, *M. arvalis asturianus* and *C. russula* are recorded as new hosts or intermediate hosts of some identified species of cestodes and nematodes.

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The plate will be found at the end of this issue.

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BOTHRIOCEPHALUS AEGYPTIACUS SP. N. (CESTODA : PSEUDOPHYLLIDEA) FROM BARBUS BYNNI, AND ITS LIFE CYCLE

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Abstract: In *Barbus bynni*, a Nile fish, bought at the fish market in Old Cairo (in March and May 1973) we found numerous cestodes of the genus *Bothriocephalus*, order Pseudophyllidea. These are described in the text as the new species, *Bothriocephalus aegyptiacus* sp. n. The large number of individuals and mature eggs enabled studies on the exogenous phase of the life cycle of this cestode and its development in the intermediate host.

Bothriocephalus aegyptiacus sp. n. (Fig. 1)

Host: *Barbus bynni* (Forsk.)
Location: intestine
Locality: River Nile near Cairo

Description:

Holotype: Body length 598 mm, width 4 mm. Scolex conical, 1.1 mm in diameter. An indistinct apical disk was seen on the scolex of the live worm which disappeared during fixation and further treatment. Two oval bothria with prominent margins imitating suckers situated at each side of scolex, shape triangular in pressed, fixed and stained material. Scolex abuts strobilum which shows typical "secondary fragmentation" (a term suggested by van Cleave and Mueller, 1934). Strobilar segments 7—8 times wider than long, last gravid segments 3—4 times wider than long. The first anlages of the sexual organs appeared in segments 40—60.

Sexually mature segments harboured approximately 140—200 testes situated in the lateral fields along both sides of the segment, in the medullar parenchyma; shape oval, measurements 0.026—0.048 × 0.040—0.056 mm; cirrus sac (0.130—0.156 mm in diameter) opened on median line in mid-segment. Diameter of cirrus 0.016 mm. The coils of the tubular vas deferens descended along the left side of the cirrus sac. Vagina opened close to cirrus sac, tubular uterus extended in parallel direction to the upper margin of the segment, then turned to the posterior margin and opened in an uterin pore. Ovary transversely elongate, moderately lobate, situated in the centre of posterior segment margin; transverse diameter 0.480—0.540 mm. Vitellaria dispersed in cortical parenchyma, formed two wide lateral fields which were wider than the lateral fields formed by the testes. Their number exceeded that of testes which they overlapped. Measurements of individual follicles

0.006—0.020 by 0.004—0.010 mm. Eggs oval with a plug, size 0.034—0.046 by 0 066 mm. Several segments contained as many as 8% of teratological forms of eggs producing couples of tightly joined eggs.

Paratypes: These differed from the holotype mainly in outer measurements. Length 502—611 mm, width 3.8—4.3 mm. Scolex diameter 0.980—1. 3 mm. Remaining measurements consistent with those of holotype. The holotype

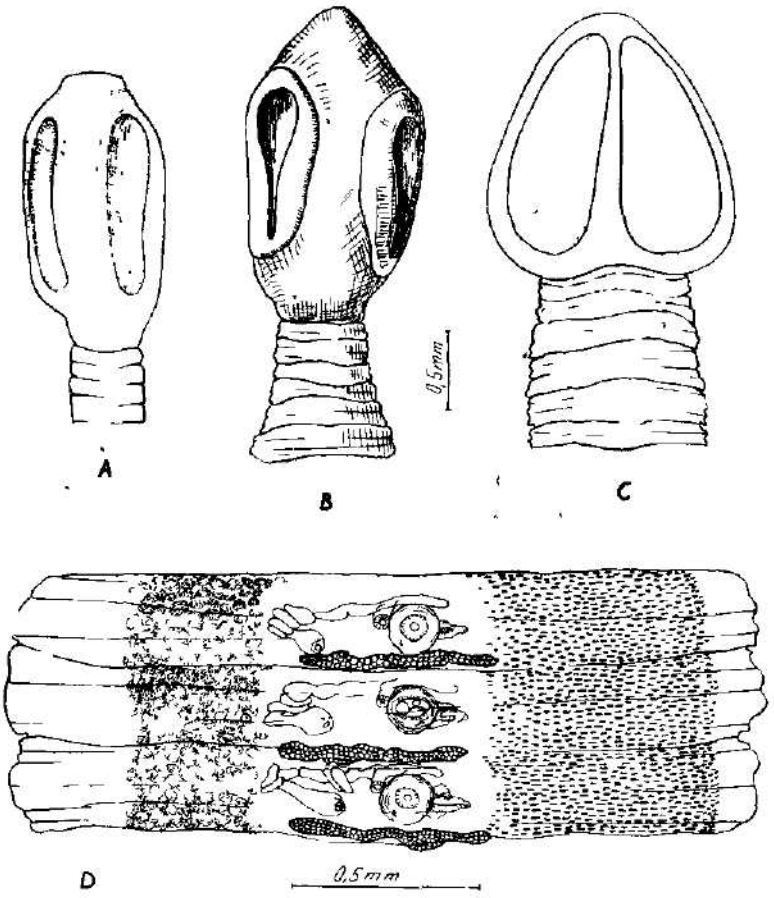

Fig 1 — *Bothriocephalus aegyptiacus* sp. n.: A, B — scoleces (live material), C — pressed and fixed scolex, D — sexually mature segments.

is deposited in the collection of the Institute of Parasitology, Prague, several paratypes are in the helminthological collection of the Humboldt Museum (Department Zoology), Berlin (German Democratic Republic).

Discussion

Bothriocephalus aegyptiacus sp. n. resembles *B. claviceps* (Goeze, 1782) particularly in the position of the uterus. It differs from it in the larger number of testes (50—60 for *B. claviceps*, 140—200 for our species), and in general bodily measurements which are bigger than those of *B. claviceps*.

In the latter, the apical disk on the scolex is clearly visible by contrast to our species, where it is feebly visible in live specimens only.

The scolex of our species is similar to that of *B. opsalichthydis* Yamaguti, 1934, our species differs from it in the smaller number of testes (60—100 as compared with 140—200) and mainly in general measurements of the strobila (100 × 1.25 mm as compared with 502—611 × 3.8—4.3 mm). Other different features are the shape and location of the ovaries.

The anatomy of the reproductive organs and the shape of the scolex of our species are similar to those of *B. phoxini* Molnár, 1968. Different are mainly the bigger measurements of the strobila (45 × 1.4 mm for *B. phoxini*; 502—611 × 3.8—4.3 mm for our species), the larger number of testes (60—70 as compared with 140—200) and the smaller diameter of the cirrus sac (0.08 mm and 0.130—0.156 mm). Our species differs from the remaining species of the genus *Bothriocephalus* in the different type of the uterus, in the number of testes and in the different shape and position of the uterus. The ovary of our species is similar to that of *B. cuspidatus* Cooper, 1917 (after Wardle et McLeod, 1952), the number of testes, however, is much lower (50), the shape and position of the uterus is completely different. Also the shape of the scolex is different.

Development of *Bothriocephalus aegyptiacus* sp. n. (figs. 2) in the intermediate host

Information on the life cycle of cestodes of the genus *Bothriocephalus* is scarce. It was reported by Essex (1928) that the cestode *B. cuspidatus* Cooper, 1917 utilizes cepepods as intermediate hosts. Markowski (1935) studied the life cycle of *B. scorpii* (Müller, 1776) and suggested that cestodes of the genus *Bothriocephalus* require two hosts, i.e. copepods as the first intermediate host, fishes as the second. Jarecka (1959, 1963, 1964) who studied the life cycle of *B. claviceps* (Goeze, 1782) found that this species utilizes the copepod *Macrocyclops albidus* Jur. as its intermediate host. In 1964, this author confirmed that one intermediate host only, and that a copepode, is involved in the life cycle of this species. Molnár (1971) described the procercoid of *B. phoxini* Molnár, 1968 which develops in copepods (the copepod species has not been determined exactly).

The results of our experimentàl studies on the development of *B. aegyptiacus* sp. n. in *Mesocyclops leuckarti* Jur. are these:

We obtained mature eggs of *B. aegyptiacus* sp. n. from the uterus of gravid segments. They were kept in glass beakers (volume 50 ml). in filtered Nile water which was changed daily. Average temperature of the water was 22—24° C. Part of the eggs was added to glass vessels (volume 1 l) containing the plankton crustacean *Mesocyclops leuckarti**) collected in a blind arm of the Nile near the village of Warak El Arab, North of Cairo, and transferred to the laboratory for reproduction. Each day we inspected 10 crustaceans in order to study the development of the cestodes in these hosts.

The brown or brownish yellow content of mature eggs started to cleave soon after transfer into the water. Division of the content into 16—32 cells lasted 6 hr, the formation of the embryo 12—20 hr, the coracidium completed

*) The intermediate hosts were identified by RNDr. V. Kořínek, Department of parasitology and hydrobiology, Faculty of Natural Sciences, Charles University, Prague, to whom our thanks are due.

its development in 48 hr and was released from the egg after an additional 12—24 hr. At 24° C the egg completed its development in 72 hr, at 18—20° C in 144 hr, i.e., in 6 days. Coracidium circular, 0.056 0.74 mm in diameter, covered with ciliae (length 0.014—0.016 mm). Diameter of outer membrane 0.045—0.056, of the larva proper (oncosphere) 0.026—0.032 mm. Soon after the release of the coracidium from the egg, swim bladders are formed in the space between the outer and inner membrane, which facilitate the movements

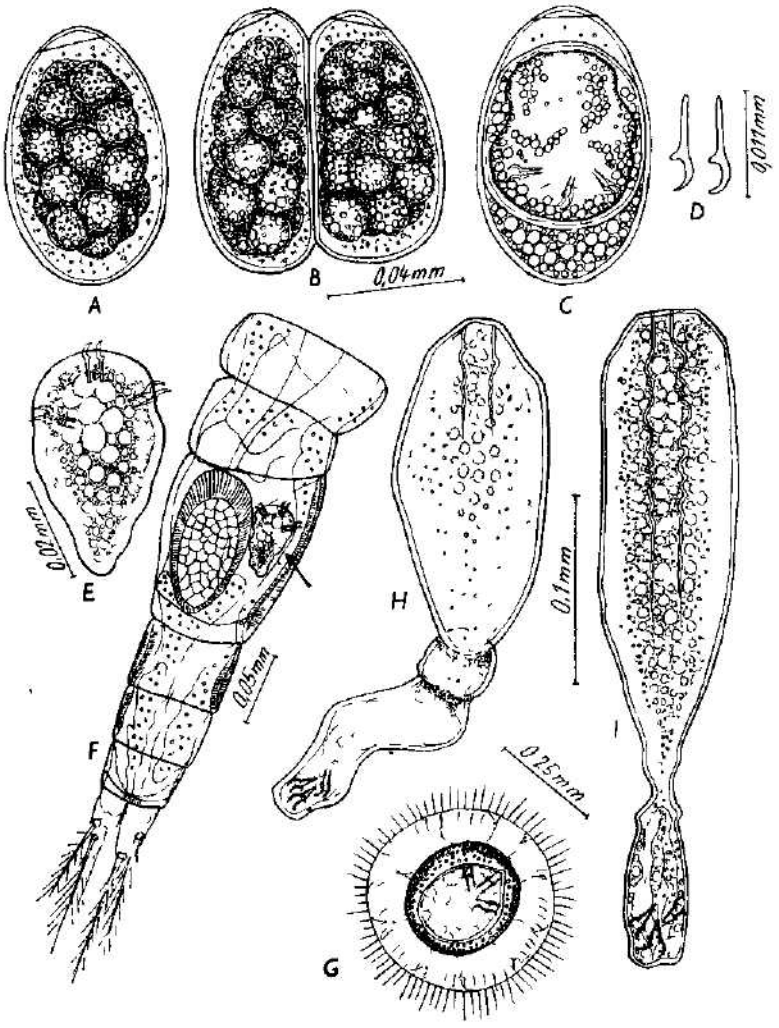

Fig 2 — *Bothriocephalus aegyptiacus* sp. n.: A — cleaved egg removed from the uterus after 6 hr, B — teratological form of double eggs, C — egg with developed coracidium, after 48 hr of development, D — embryonic hooks of the coracidium, E — oncosphere pressed out of the body cavity of *Mesocyclops leuckarti*, F — posterior extremity of *Mesocyclops leuckarti* containing a 24 hr old oncosphere, G — free coracidium H — procercoid from the body cavity of *M. leuckarti* on day 7 of development, I — completely formed procercoid on day 8 of development.

of the coracidium. The oncosphere is armed with 3 pairs of embryonic hooks (length 0.011—0.012 mm).

The coracidium is ingested by the intermediate host, *Mesocyclops leuckarti*, the oncospheres are released in the host's gut and enter the body cavity through the wall of the gut. This process is completed in 2—4 hr (after ingestion of the coracidium). At this stage, the oncospheres concentrate in the posterior part of the body, mainly in the tail cavity of the crustaceans. Their measurements are 0.048—0.058 × 0.030—0.038 mm, the length of their embryonic hooks remains unchanged. Their movements, very active 48 hr after the ingestion of the coracidium, become slower, the larvae grow to about twice their original size (0.95—0.103 × 0.024—0.030 mm). During this phase they move to the anterior part of the crustacean body. They still bear paired embryonic hooks on the anterior extremity. At 24 hr after ingestion of the coracidium, the larvae measure 0.240—0.064 × 0.080 mm, their body is filled with dark nutritive granules, the embryonic hooks have shifted to the posterior extremity, but remain arranged in pairs. At 144 hr after ingestion of the coracidium, the size of the larvae is 0.230—0.286 by 0.118—0.136 mm. A spherical or oval cercomere, size 0.046—0.064 by 0.032—0.042 mm, containing paired embryonic hooks is formed by the constriction of the posterior body end. At this phase the larva is a typical procercoid which completes its development on day 8—10 after ingestion of the coracidium by the crustacean, i.e., in 192—240 hrs. The length of a fully developed procercoid is 0.340—0.390 mm, (length of cercomere 0.120—0.134 mm), the width of the procercoid is 0.074—0.088 mm (that of the cercomere 0.042—0.048 mm). The embryonic hooks arranged in pairs in the posterior part of the cercomere, measure 0.012—0.014 mm. Two longitudinal excretory canals starting in the anterior part of the procercoid body, extend along both sides to the posterior third of its body length. Studies on the development of the procercoid in its definitive host had to be discontinued because we had no facilities for rearing these hosts in the laboratory.

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A POPULATION OF GAMMARUS FOSSARUM KOCH (AMPHIPODA) IN A KARSTIC STREAM

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Abstract The population dynamics of *Gammarus fossarum* Koch has been investigated at two stations on a karstic stream with a stony bottom during a period of 15 months The annual average abundance of the population of *Gammarus* in the Křtiny river was 991 ind sqm, the annual average biomass being 8.65 g/sqm In the investigated locality the sex of 5 mm long and longer Gammarus could be distinguished The mean size was found to be 7.4—7.6 mm for males, 7.2—7.6 mm for females and 3.5 mm for juvenils.

Gammarus fossarum represents an important component of the fauna of trout streams. In the case of mass occurrence Amphipoda may become an important part of fish food (Illies, 1961 Straškraba, 1966) *Gammarus* as a consumer of leaf fall (Bick, 1959) contributes also to self-purification processes in streams

Nowadays great attention is paid to problems of production in small streams (Kubíček et al , 1971). When studying the annual cycle of rheobenthos in the Křtiny river — Moravian Karst (Sukop, 1973) we paid attention also to the composition of the *Gammarus fossarum* population As only some data about the composition of individual populations of Czechoslovak streams are available (Straškraba, 1966, Obrdlík. 1972; Helan et al 1973) this paper enables to compare our results with those obtained in earlier studies on Amphipoda populations in Czechoslovakia.

BIOTOPE AND SAMPLING METHODS

Studies on annual changes in numbers of *Gammarus* were carried out at two stations on the rocky bottom of the Křtiny river in the Moravian Karst The stream rises at an altitude of 455 metres, it is 14 kilometres long, and its catchment area covers 31 square kilometres Below Křtiny village the brook reaches the limestone bedrock and goes underground, emerging approximately 2 8 kilometres away and continuing in the direction of a small town called Adamov where it empties into the left bank of the Svitava

Sampling was carried out at two stations the upper one (No 1) was situated approximately 400 metres below the place of reappearance of the underground stream, near the rock called Býcí skála (Bull's Rock). The lower station (No. 2) was situated approximately 500 metres upstream from Adamov

There was a predominantly stony bottom at both stations investigated. Stones were often covered with diatoms and filamentous algae For further details about individual stations see Tab 1.

Quantitative samples were taken by means of a water net from stony bottom The area of the sampled substrate was determined according to the method described by Schrader (1932) and it was greater than 2000 square centimetres in every sampling. Samples were fixed immediately

Table 1. Basic data of both stations

Station	No. 1	No. 2
Height above sea level	315.00m	265.00 m
Width	2.50 m	4.50 m
Depth	0.15 m	0.25 m
Stream speed (min. – max.)	0.6 – 1.2 m/sec	0.5 – 1.2 m/sec
Discharge (min. – max.)	0.1 – 0.5 m³/sec	0.2 – 0.9 m³/sec
pH (min. – max.)	6.7 – 7.5	7.3 – 7.5
Water temperature	3 – 9°C	2 – 11°C

under field conditions with 4 per cent formaldehyde, weighing was carried out after three months. Prior to weighing the material was dried according to the method described by Albrecht (1959) and Kubíček (1969).

RESULTS

Abundance and biomass

As far as the frequency is concerned *Gammarus* belongs to a very important part of fauna of the Křtiny river. As shown in Tabs 2 and 3 this species represented in many cases approx. 50 per cent of the total biomass in both localities during the year and its share in the total values of abundance was also high. In locality No. 1 its abundance was high during summer (July to August), a second peak was observed in autumn (October—November). Amphipoda were frequent also in early spring. In this locality the annual average abundance was 1,007 ind/sqm, the annual average biomass was 9.68 g/sqm. In locality No. 2 a high abundance was observed from August to March, during this period the values of biomass were also high. The highest quantitative values were found in October. In this locality the annual average abundance was 976 ind/sqm, the annual average biomass was 7.62 g/sqm.

Sexual distribution of the population

In this study 6,260 specimens were analyzed. Sex might be distinguished in individuals of more than 5 mm. The population was divided into four sexual categories: males, females without embryos, females with embryos and juvenile specimens. In locality No. 1 the sexual composition of the population was as follows: males, females without embryos, females with embryos and juveniles represented 36.1, 29.7, 12.3 (females together 42.0) and 21.9 per cent, respectively. In locality No. 2 males formed 36.0% of the total population of *Gammarus*. Females formed 40.2% (females without embryos 27.1%, females with embryos 13.1%) and juvenile specimens formed 23.8%. Females with embryos occurred all the year round, their highest occurrence was found in spring and summer. The ratio between mature and juvenile forms was approx. 3 : 1 in both localities.

Size distribution of population

The size of individuals of the *Gammarus* population of the Křtiny river ranged between 2 and 14 mm. The total length of *Gammarus* was measured from rostrum to telson tip. The most frequent were individuals of the size

Table 2. Abundance, biomass and percentage of *Gammarus fossarum* Koch in total quantitative values (Station No. 1)

Day 1970–71	Number of individuals ind/sqm	Biomass g/sqm	Share in the total abundance (%)	Share in the total biomass (%)
3 May	408	3.88	19.7	28.2
31 May	293	2.54	13.9	13.1
27 Jun.	710	8.26	16.7	28 4
19 Jul.	1,282	10.92	21.6	37.0
8 Aug.	1,565	9.68	41.2	36.4
13 Sep.	838	6.12	36.3	55.0
18 Oct.	1,371	11.01	55.2	69.8
7 Nov.	1,571	12.64	44.0	64.5
13 Dec.	719	6.08	32.6	55 0
9 Jan	670	6.06	23.7	47.8
13 Feb.	1.766	15.90	41.6	60.6
20 Mar.	1,354	19.73	24.4	49.0
18 Apr.	830	12.22	31.2	42.0
15 May	722	10.48	20.4	32.4
$\bar{x}$	1,007	9.68		

of 5 to 10 mm, those larger than 10 mm were less frequent. The average size of males was 7.6 and 7.4 mm in localities No. 1 and No. 2, resp. The size of females ranged between 5 and 12 mm. Individuals of the size of 7 to 8 mm were the most frequently. Within the category of females without embryos individuals of the size of 6 to 7 mm, were the most frequent within the cate-

Table 3. Abundance, biomass and percentage of *Gammarus fossarum* Koch in total quantitative values (Station No. 2)

Day 1970–71	Number of individuals ind/sqm	Biomass g/sqm	Share in the total abundance (%)	Share in the total biomass (%)
10 Mar.	770	6.06	18.8	37.4
3 May	92	1.29	3.2	7.3
31 May	151	0.96	7.5	5.4
27 Jun.	394	2.41	7.4	5.6
19 Jul.	623	6.10	13.8	16.9
8 Aug.	1,808	10.63	22.7	36.9
13 Sep.	1,851	10.49	30.2	41 2
18 Oct.	2,991	13.91	65.2	70.7
7 Nov.	1,257	12.80	26.6	57.2
13 Dec.	1,089	12 21	35.8	56.3
9 Jan.	1,032	10.04	29.3	53 4
13 Feb.	375	2.65	12.5	13 3
20 Mar.	1,062	12.36	29.3	39.0
18 Apr.	965	10.53	20.6	27.9
15 May	187	1.91	2.3	4.3
$\bar{x}$	976	7.62		

Table 4. Sexual distribution of the population of *Gammarus fossarum* Koch on Station No. 1 and Station No. 2
(M = males, Fa = females without embryos, Fe — females with embryos, J = juvenil, F = females total)

Day 1970—71	Station No. 1						Station No. 2					
	M	Fa	Fe	M+F	J	M+F+J	M	Fa	Fe	M+F	J	M+F+J
10 Mar.							88	71	23	182	46	228
3 May	42	22	14	78	21	99	7	4	7	18	1	19
31 May	49	28	17	94	9	103	12	8	4	24	4	28
27 Jun.	61	38	31	130	1	131	30	17	11	58	3	61
19 Jul.	103	52	48	203	34	237	44	22	41	107	7	114
8 Aug.	79	41	28	148	116	264	93	48	74	215	109	324
13 Sep.	37	25	38	100	59	159	113	56	64	233	120	353
18 Oct.	124	106	17	247	114	361	185	179	10	374	303	677
7 Nov.	121	163	1	285	99	384	137	111	1	249	41	290
13 Dec.	69	95	0	164	39	203	102	89	9	200	20	220
9 Jan.	59	63	0	122	28	150	87	83	24	194	36	230
13 Feb	110	126	34	270	124	394	28	21	4	53	18	71
20 Mar.	113	86	50	249	26	275	96	70	62	228	18	246
18 Apr.	88	61	71	220	17	237	77	48	65	190	7	197
15 May	86	31	40	157	3	160	19	13	8	40	5	45
Total	1,141	937	389	2,467	690	3,157	1,118	840	407	2,365	738	3,103
%	36.1	29.7	12.3		21.9	100.0	36.0	27.1	13.1		23.8	100.0

gory of females with embryos those of the size of 8 to 9 mm. The average size of females was 7.6 and 7.2 mm in localities No. 1 and No. 2, resp.

DISCUSSION

Although *Gammarus* represents an important part of the fauna of trout streams, in Czechoslovakia relatively low attention has been paid to the population composition of this species. The research was carried out in several Moravian streams only. Straškraba (1966) studied populations of the Lucina and Morávka rivers. Hower, sampling was carried out only in June and July.

Obrdlík (1972) investigated the rocky and sandy substrate of a tributary of the Ponávka river. This tributary was without fish. It was found out that the rocky substrate was more productive than sand. The average size of individual sex categories was greater on the rocky bed, too. The mean abundance on stones was 1,080 ind/sqm, on sand 509 ind/sqm; the biomass on stones was 6.8 g/sqm, on sand 1.6 g/sqm.

Helan et al. (1973) studied populations in the Lušová and Brodská rivers in the Beskydy Mountains. In the Lušová river the annual average abundance was 201 ind/sqm, the mean biomass 2,527 g/sqm. In the Brodská river the population was investigated in the stretch with normal fishstock, in the stretch with excessive fishstock, and in the stretch without fish. The mean quantitative values see Tab. 6.

The Křtiny river is a karstic stream with fishstock (mainly trout and bullhead). The annual average abundance of a population of *Gammarus* in the Křtiny river was 991 ind/sqm, the annual average biomass being

Table 5. Distribution of size of the population of *Gammarus fossarum* Koch in a karstic stream of the period 1970/71

Station No 1												
size in mm	5	6	7	8	9	10	11	12	13	14	T	$\bar{X}$
					males							
number	284	198	146	119	124	117	95	47	8	3	1,141	7.6
%	24.9	17.4	12 8	10.4	10 9	10 3	8.3	4.1	0.7	0.2	100.0	
					females with embryos							
number	139	205	245	176	118	39	11	4	0	0	937	
					females with embryos							
number	0	1	38	144	133	59	12	2	0	0	389	
					females total							
number	139	206	283	320	251	98	23	6	0	0	1,326	7.6
%	10.5	15.5	21.3	24.2	18.9	7.4	1.7	0.5	0	0	100.0	
size in mm	2	3	4	T	$\bar{X}$							
			juvenil									
number	57	212	421	690	3 5							
%	8.3	30 7	61.0	100 0								

Station No 2												
size in mm	5	6	7	8	9	10	11	12	13		T	$\bar{X}$
					males							
number	250	185	159	165	198	102	47	10	2		1,118	7.4
%	22.4	16 5	14.2	14 8	17 7	9.1	4.2	0.9	0.2		100.0	
					females without embryos							
number	149	238	227	140	71	13	2	0	0		840	
					females with embryos							
number	0	13	95	156	112	27	4	0	0		407	
					females total							
number	149	251	322	296	183	40	6	0	0		1,247	7.2
%	11.9	20.1	25.8	23.8	14.7	3.2	0.5	0	0		100.0	
size in mm	2	3	4	T	$\bar{X}$							
			juvenil									
number	59	268	411	738	3.5							
%	8.0	36.3	55.7	100 0								

T – total

8.65 g/sqm. On the rocky be of the tributary of the Ponávka river females with embryos represented 1.6 per cent of population and occurred predominantly in the late summer and early autumn. In the Křtiny river the percentage of females with embryos occurred practically all the year round with maxima in July—September and March—April. In the Ponávka river

Table 6. Populations of *Gammarus fossarum* Koch in different streams

stream	formation of oostegits in mm	mean size of the body in mm		mean abundance ind/sqm	mean biomasa g/sqm	fishstock
		males	females			
Lucina (Straškraba, 1966)	—	10.8—11.5	7.6—9.2	—	—	present
Morávka (Straškraba, 1966)	—	11.5	9.1	—	—	present
Breitenbach (Lehmann, 1967)	5.5	7.7—8.6	6.0—7.2	1,944	—	absent
Tributary of Ponávka (stone) (Obrdlík, 1972)	4.0	5.2	5.3	1,080	6.8	absent
Lušová (Helan et al., 1973)	5.5	8.2	7.7	201	2.527	present
Brodská (Helan et al., 1973)	5.5	8.2	7.7			
stretch with normal fishstock				313	2.726	present
stretch with excessive fishstock				318	2.657	present
stretch without fish				415	3.775	absent
Křtiny stream	5.0	7.4—7.6	7.2—7.6	991	8.65	present

the most frequent were females with embryos of the size of 6 to 7 mm, in the Křtiny river those of the size of 8 to 9 mm.

The reproduction season of *Gammarus* was influenced by the water temperature. In the Breitenbach river the reproduction season started in January (Lehmann, 1967). The first peak in the number of females with embryos occurred in March, the second one in July—August, reproduction ceased completely in November and December. In the Křtiny river the *Gammarus* population showed two peak numbers of females with embryos: the first one in March—April, the second one in July—September. In the locality No. 1 the reproduction nearly ceased in November—January. In the locality No. 2 females with embryos occurred during the whole period of investigations, the minimum occurrence was registered in November.

When following the annual cycle of *Gammarus* populations in individual streams a considerable fluctuation of abundance may be observed both in individual years and in individual months. Obrdlík (1972) for example found very different abundances in the same locality in Februaries of two different years: 96 *ind/sqm* and 5,720 *ind/sqm*. The fluctuation of abundance in individual localities in the Křtiny river was also found within one research period.

In the Bobrava river (Sukop, 1968) a great quantity of Amphipoda was registrated on a muddy bed with a thick sediment of leaf fall in October, this value exceeding many times the average annual values. It is possible that Amphipoda migrated to places where a greater amount of leaf fall sedimented which represented a source of nutrition for *Gammarus*. *Gammarus* belongs to very active migrants in the stream (Meijering, 1972).

Although the changes in the population density depend on many biotic and abiotic environmental factors (fish predation, thermal and hydrological conditions) a high density of *Gammarus* occurred in the streams investigated most frequently in the period of October—February (in the Beskydy Mountains also in July). The lowest population density occurred in May—June.

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The publication of the Soviet national commitee of International Biological Program contains practically only tables on the abundance and biomass of components of zooplankton (determined mostly to species level) under 1 m^2 of surface in one reservoir and six lakes of very different position in geographical and trophic range of lakes. The periods covered by observation on individual lakes are to my knowledge the longest in the biological literature: Lake Mjastro (P. G. Petrovič, Minsk) 1955–1970, Lake Naroch (P. G. Petrovič, Minsk) 1955–1970, Lake Batorino (P. G. Petrovič, Minsk) 1955–1969, Lake Sevan (T. M. Meshkova, Jerevan) 1937–1969, Lake Bajkal (M. M. Kozhov and G. J. Pomazkova, Irkutsk) 1964–1971, Lake Dalnee on Kamchatka (E. M. Krokhin, B. P. Kozhevnikov) 1938–1970 and Irkutsk Reservoir (G. L. Vasiljeva, Irkutsk) 1957–1971. Only the data on Lake Dalnee are restricted to yearly averages on prevailing forms whereas in all other lakes the data on numbers and biomas presented according individual sampling dates. It is fully understandable that the frequency of sampling from such a large number of lakes and such a long periods are not adhered on a strictly comparable procedure. In some lakes (e.g. Belorussian) in summer in some years several samples per month are reported whereas samples especially from some winter months are lacking. In others (e.g. Bajkal or Sevan) there is almost a complete set of data from each month of the whole period.

Most of the observations are summarized with regard to yearly averages. In the observations of lake Baikal such a summarisation is lacking. It is a pitty that an elementary statistic of the data has not been worked out to enable the testing of the simplest hypotheses. At first look the diffeence between typical eutrophic and oligotrophic lakes is not great, the year variability also does not seem to be drastically different. In lake Sevan there is a definite trend for increase (presumably due to changes in hydrological regime), in lake Dalnee is such tendency lacking.

In summary an extremely valueable set of data which enables to examine long term changes in results of biological processes of Lakes of different geographical position and typology.

J. Hrbáček

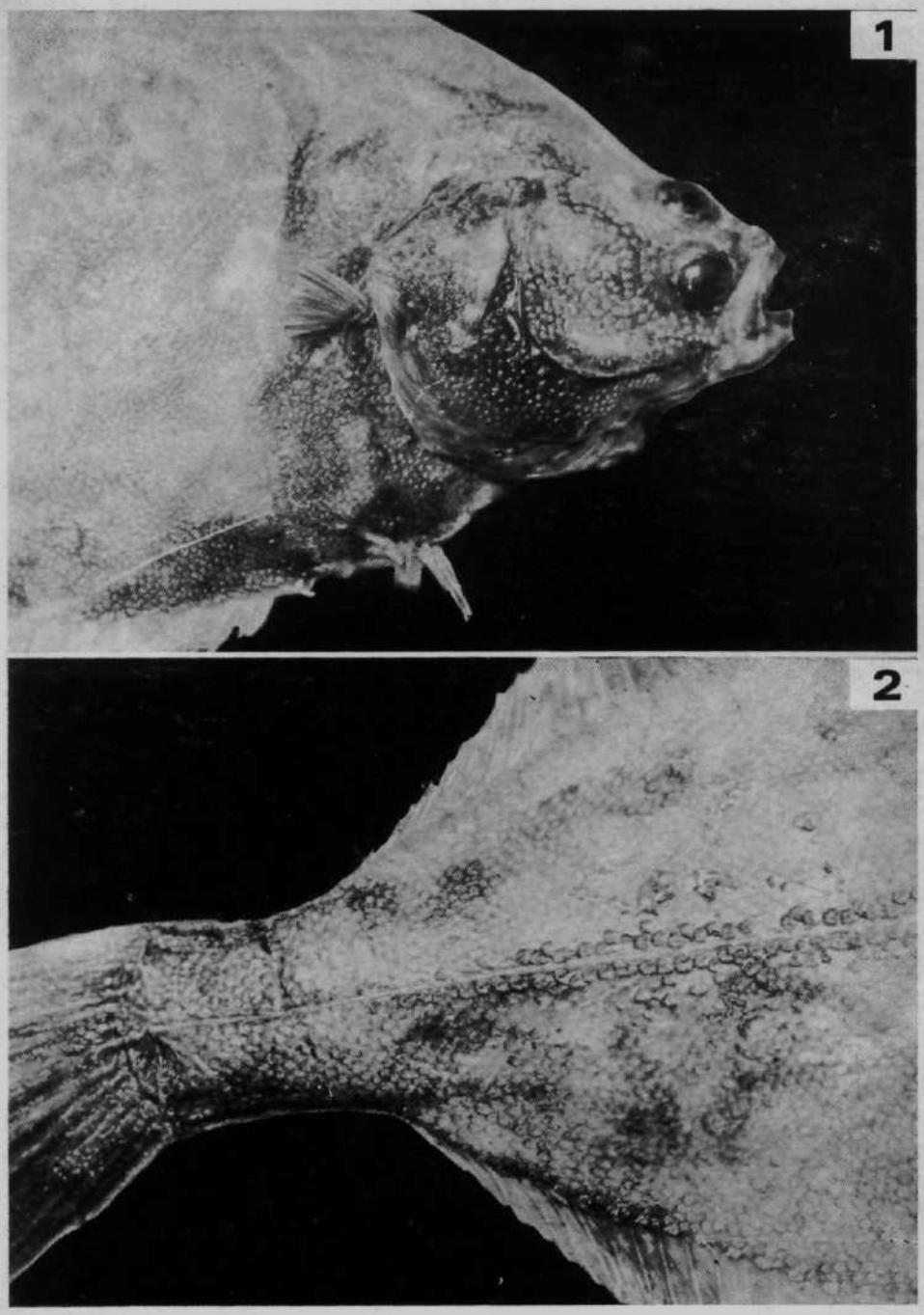
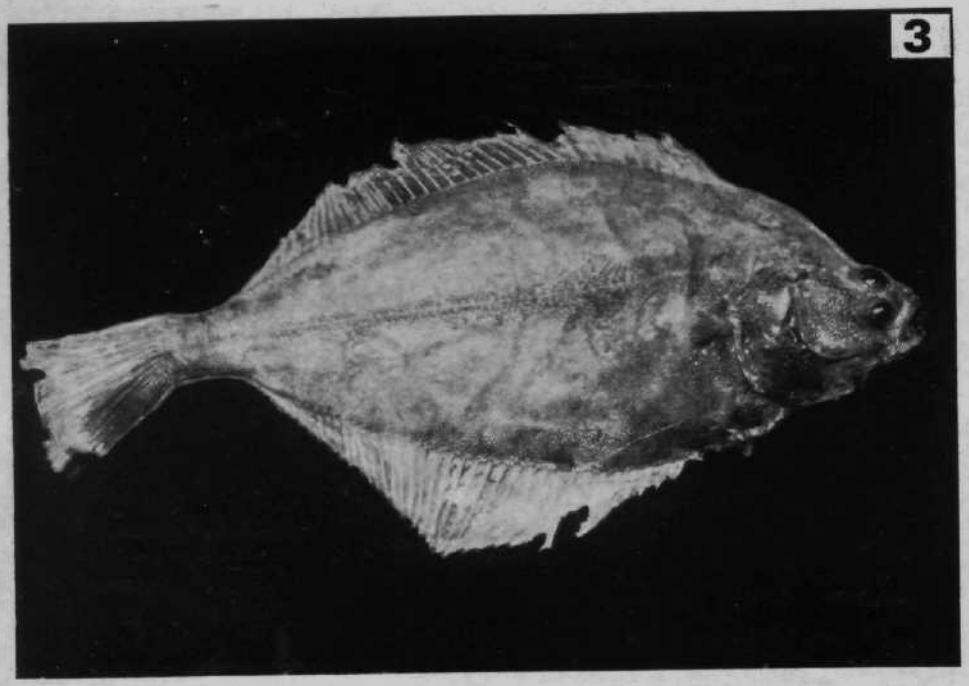


Table 1: 1. — *Pleuronectes flesus* Linnaeus, 1758. Total length 250 mm, body length 200 mm. The mouth of the river Ploučnice into the Labe near Děčín, 1912. The accumulation of bony knobs in the anterior part of body above and below the lateral line. 2. — The same specimen, the posterior part of the body. One can clearly see the diminishing of bony knobs, which lack on the caudal peduncle and only the course of the lateral line as visible.



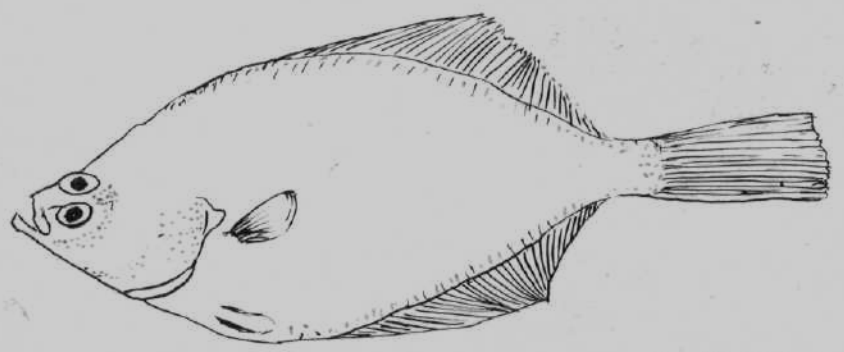
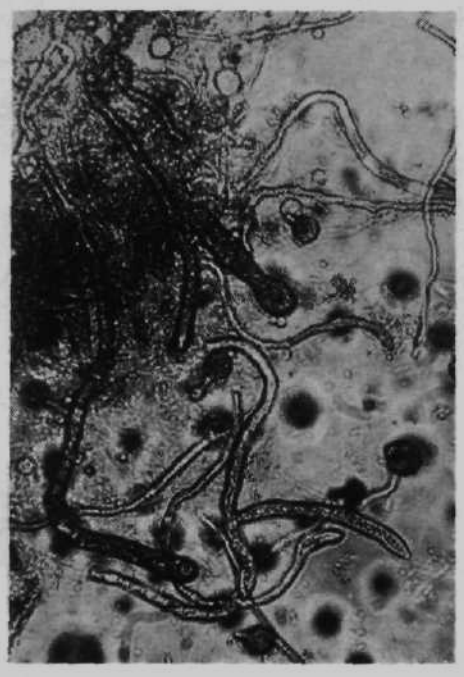


Table 2: 3. — The total view on the specimen from the river Ploučnice. 4. — The total view on the Frič (1908) specimen from the river Labe at Roudnice, which in contrast to our specimen, has eyes on the left side. Redrawn from the photograph in Frič (1908).

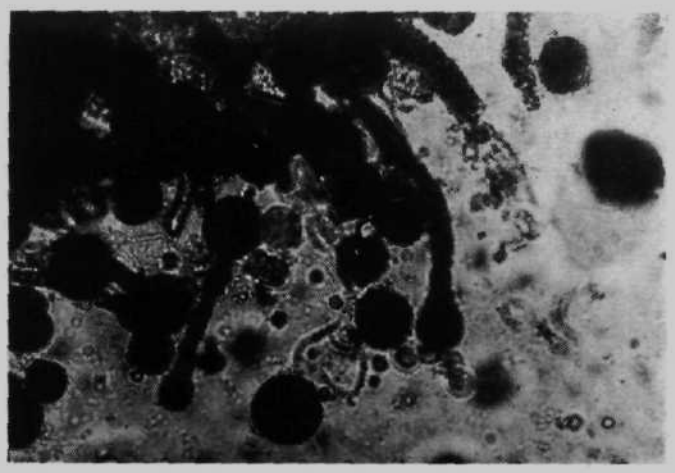


100μm

1

200μm

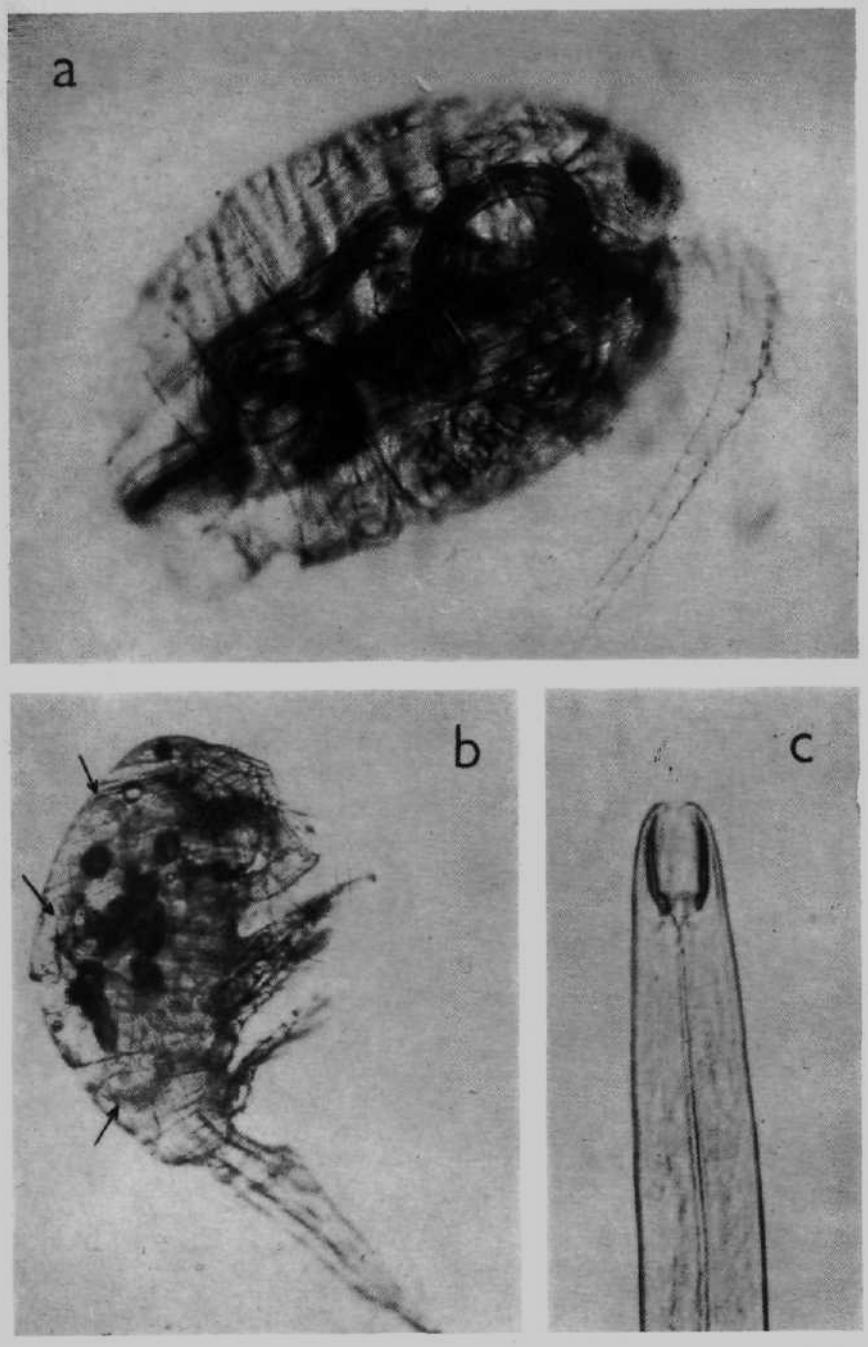
2



100μm

3

Fig. 1. 24 hours after death of mycosis: the conidiophores are growing through the body surface of *C. formosanus*, the conidia of *C. coronatus* start to form.
Figs. 2, 3. 36 hours after death of mycosis: conidiophores with mature conidia of *C. coronatus* form a dense cover of the body, some of the mature conidia have already been discharged. Material embedded in Colley's medium.



a — invasive larvae of *Procamallanus laeviconchus* in the haemocoel of a cyclops. Photo Z. Mauer.
b — cyclops harbouring invasive larvae of *P. laeviconchus*. Photo B. Ryšavý.
c — anterior extremity of *P. laeviconchus* invasive larva obtained from the cyclops. Photo B. Ryšavý.

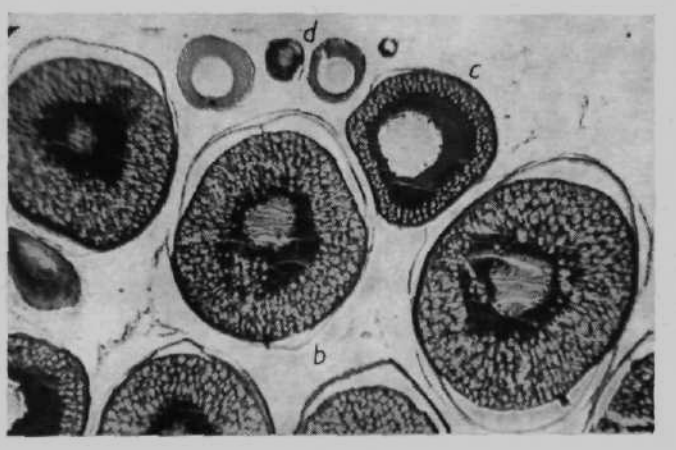


Fig. 1 — Histological section through the *Phoxinus phoxinus* (L.) ovary in the third stage, fished in the Prejmer river on Nov. 10, 1961 (the length without C 72 mm, the body weight 6.1 g): b, c, d — ovocytes in different stages of development (ob. 6×, oc. 6×).

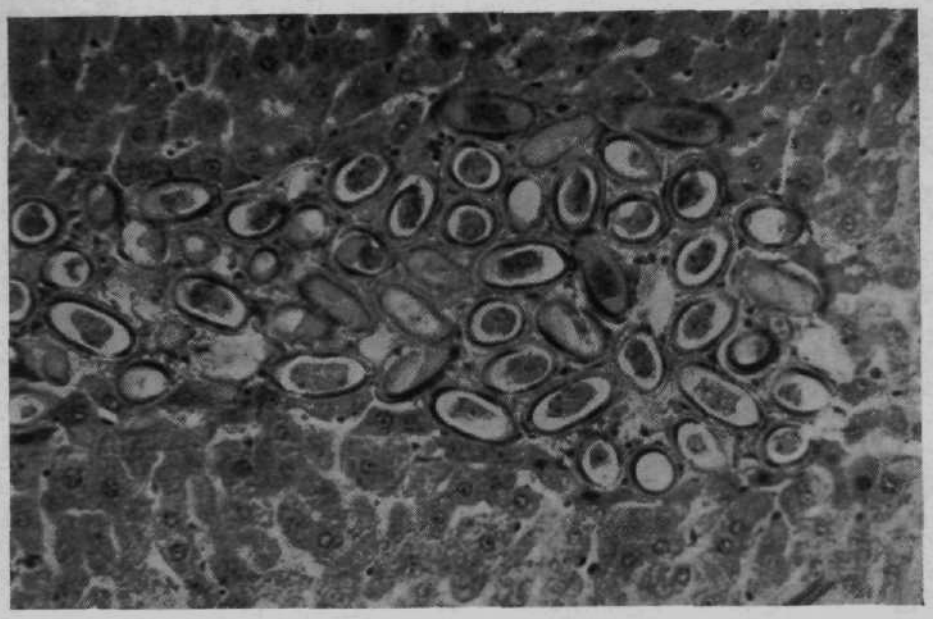


Fig. 1: Section through liver tissue infected with eggs of *C. hepatica*. Host *A. sylvaticus*.