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Department of Zoology, University of Jodhpur, Jodhpur, India

A NEW SPECIES OF THE ANISAKID GENUS DUJARDINASCARIS FROM THE FISH CYBIUM GUTTATUM, WITH A KEY TO THE INDIAN SPECIES OF THE GENUS DUJARDINASCARIS (NEMATODA: HETEROCHEILIDAE)

SATYA NARAYAN ARYA and SYLVESTER JOHNSON

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Abstract: *Dujardinascaris cybii* sp. n. is described from the marine fish *Cybium guttatum*. It is compared with other species of the genus and is found to differ from these in being much smaller in size, and in having smaller oesophagus, ova and tail which terminates in a spine. The males, devoid of caudal alae, possess comparatively very small spicules and 13 pairs of sessile caudal papillae, 8 of which are precloacal and 5 postcloacal. This is the first record of the presence of the anisakid genus *Dujardinascaris* in a fish from Indian waters, and the fish *Cybium guttatum* is a new host for the members of this genus. A key to separate the Indian species of *Dujardinascaris* is provided.

In the latter half of 1975 the edible marine fish *Cybium guttatum* was subjected to periodical helminthological investigations. A number of different worms were thus collected. Among these are many forms belonging to the anisakid genus *Dujardinascaris* (Gedoelst, 1916) Baylis, 1947 which do not conform to any other species of the genus and, apparently, constitute a new species described hereunder.

Dujardinascaris cybii sp. n.

(Figs. 1—6)

(All measurements are in mm.)

Host: *Cybium guttatum* (Bl. & Schn.) Day
Location: Intestine
Locality: Fish Market, Jodhpur, India
Type specimens: Deposited in the Department of Zoology, University of Jodhpur, Jodhpur, India.

Worms thick, medium-sized, pale white, attenuated posteriorly. Cuticular striations well defined, 0.006—0.025 and 0.005—0.014 apart in female and male respectively. Oesophagus muscular throughout, somewhat dilated posteriorly. A well developed intestinal caecum present. Dimensions of the various structures are given in Table 1.

Female: Vulva inconspicuous and flush, at about middle of the body. Ovary didelphic and opisthodelphic. Eggs spherical, thin-shelled, 0.01—0.012 in diameter.

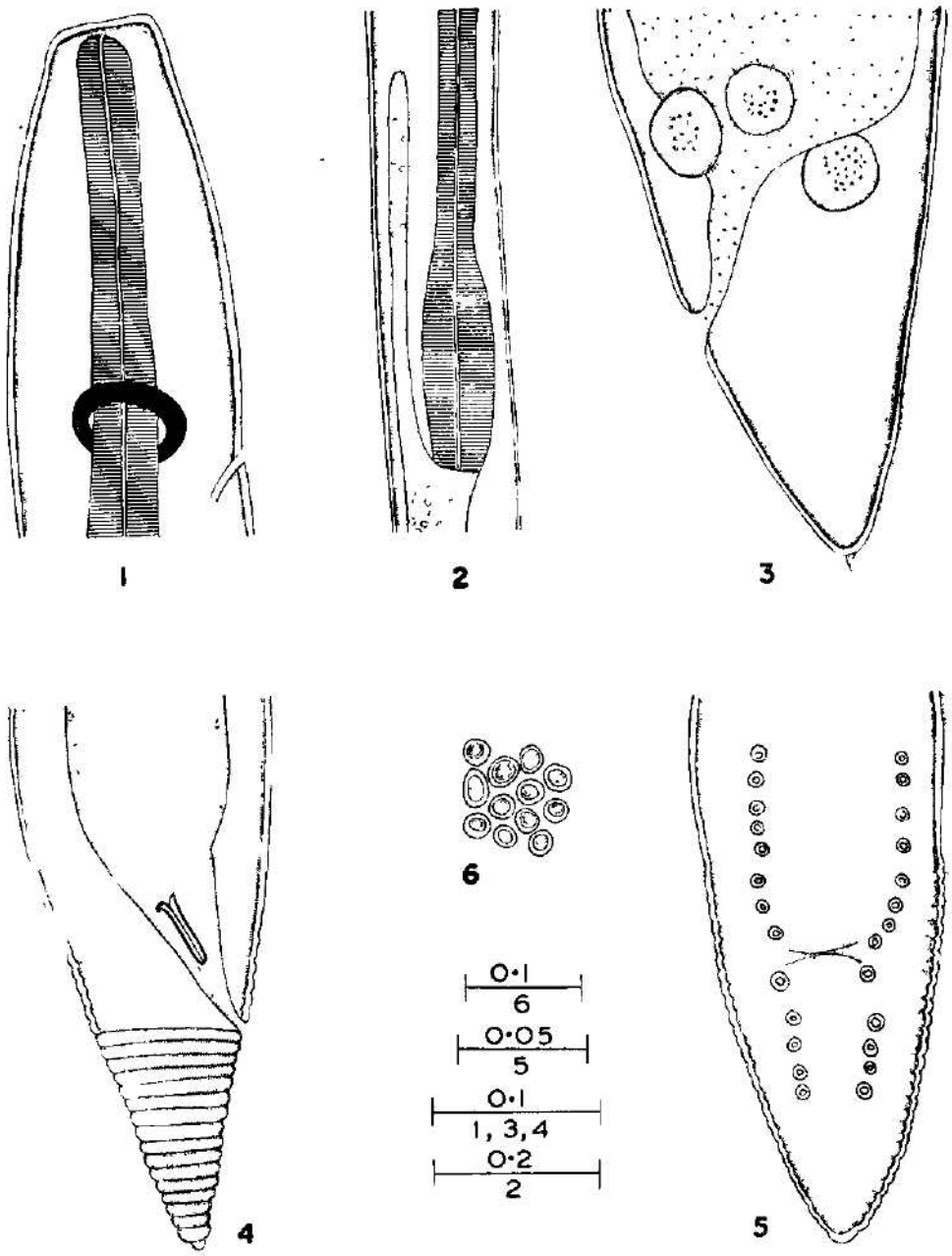
M a l e : Tail tapering, with prominent striations giving it a ring-like appearance. Spicules two, equal, similar, slender and small, 0.05—0.10 long. Gubernaculum absent. Caudal paillae sessile, 13 pairs, 8 precloacal and 5 postcloacal.

Table 1. Dimensions of various structures of *Dujardinascaris cybii* sp. n. (All measurement are in mm).

	Female	Male
Length	12.5—17.5	7.5 — 11.0
Maximum thickness	0.33—0.53	0.18 — 0.27
Tail	0.16—0.20	0.14 — 0.17
Stoma	0.02—0.04	0.021— 0.03
Oesophagus	1.07—1.60	1.06 — 1.18
Intestinal caecum	0.12—0.22	0.50 — 0.62
Nerve ring from anterior end	0.30—0.38	0.26 — 0.28
Excretory pore from anterior end	0.40—0.50	0.29 — 0.31

D i s c u s s i o n : Y a m a g u t i (1961) included 13 species in the genus *Dujardinascaris*. Of these two have been reported from fishes and 11 from reptiles. The fish parasites include *D. malapteruri* Baylis, 1923, reported from *Malapterurus electricus* from Sudan, and *D. cenotae* Pearse, 1936, described from *Rhamdia guatemalensis* from Yucatan. The species under discussion differs from both of these in its much smaller size, length of oesophagus, smaller ova, and shorter tail which terminates in a spine in the female. The males of these species differ markedly from each other. In the new species the caudal alae are absent and the spicules are very small measuring only 0.05—0.10 as against 1.3 in *D. malapteruri*. B a y l i s (1923) reported only 4 pairs of caudal papillae in *D. malapteruri*, although he hinted at the possibility of more of these existing though he could not make them out in the two specimens available to him. However, his postulate regarding the larger number of the caudal papillae was substantiated in the various species of the genus parasitizing reptiles. Thus, *D. helicina* (Molin. 1860) Baylis, 1947 has 9 pairs, 5 precloacal and 4 postcloacal; *D. woodlandi* (Baylis, 1923) Baylis, 1947 has 10 pairs, 5 precloacal, 1 adcloacal and 4 postcloacal; and *D. halicoris* (Owen, 1833) Baylis, 1947 has 8 pairs, 4 precloacal and 4 postcloacal. The species under discussion has 13 pairs of caudal papillae, 8 of which are precloacal and 5 postcloacal, and, thus, differs from all of these. Gubernaculum present in *D. malapteruri, D. helicina* and *D. woodlandi*, is absent in the new species as also in *D. halicoris*.

M a j u m d a r (1946) enumerated only three species of *Dujardinascaris* as occurring in India, viz., *D. helicina*, *D. woodlandi* and *D. halicoris*. All of these are parasites of reptiles. It would thus appear that the species under discussion is the first to be reported from a fish from Indian waters, and *Cybium guttatum* (Bl. & Schn.) Day is a new host for the members of this anisakid genus.



Fis. 1–6 *Dujardinascaris cybii* sp. n. 1 — male, anterior region, 2 — male, intestinal caecum, 3 — female, posterior region, 4 — male, posterior region, 5 — male, posterior region showing caudal papillae, 6 — eggs. All scales are in mm.

Majumdar (1964) has also presented a key to separate the three species of *Dujardinascaris* known from India till then. With the addition of the species under reference to the genus, the key is being modified as follows:

Key to the Indian species of *Dujardinascaris*

1.	Gubernaculum present	2
	Gubernaculum absent	3
2.	Gubernaculum simple at the tip	*D. woodlandi*
	Gubernaculum trifid at the tip	*D. helicina*
3.	Caudal papillae in male 8 pairs	*D. halicoris*
	Caudal papillae in male 13 pairs	*D. cybii*

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Authors' address: Dr. S. Johnson, Department of Zoology, University of Jodhpur. — 342 001, India

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Institute of Vertebrate Zoology, Czechoslovak Academy of Sciences, Brno
Institute of Zoology, Cuban Academy of Sciences, Havana

NEMATODES PARASITIZING CUBAN SNAKES (OPHIDIA)

VLASTIMIL BARUŠ and ALBERTO COY OTERO

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Abstract: Fifteen species of Cuban snakes were found to be parasitized with ten species (or subspecies) of nematodes. The following species are reported for the first time in Cuba: *Typhlopsia kratochvili* nov. gen. et nov. sp., *Kalicephalus costatus costatus*, *K. rectiphilus rectiphilus*, *Parapharyngodon cubensis* and *Stomachidae* gen. sp. — larvae. New data about the incidence and definitive hosts of the known species, *Oswaldocruzia lenteixeirai* (= syn. *O anolisi*), *Kalicephalus costatus alsophisi* nov. comb., *Hastospiculum cubaense*, *Abbreviata baracoa* and *Terranova caballeroi*, are presented. Morphological and metrical characters of most of the species are given and their zoogeographic distribution is evaluated.

The fauna of Cuban snakes is represented by 18 species belonging to eight genera and three families. Most of them are endemic species and the study of the helminth fauna of this vertebrate group is therefore of importance considering not only the taxonomy and systematics, but also the zoogeography.

The literary data dealing with this subject are not very numerous. The first report was published by Pérez Vigueras (1934) who described a new cestode species, *Ophiotaenia barbouri* from *Tretanorhinus variabilis* and later (Pérez Vigueras, 1942) the trematode *Ochetosoma adenodermis* from *Alsophis cantherigerus*. Baruš and Coy Otero (1966) described three new nematode species from the same host: *Terranova caballeroi*, *Abbreviata baracoa* and *Kalicephalus alsophisi*. In the host *Tretanorhinus variabilis* these authors found larvae of nematodes of the genus *Contracaecum*. A new species of filariae, *Hastospiculum cubaense*, from *Antillophis andreae* and *Tropidophis melanurus* was described by Baruš and Sonin (1971). Freze and Ryšavý (1975) in their paper devoted to cestodes of coldblooded vertebrates from Cuba reported the species *Ophiotaenia nattereri* from *Epicrates angulifer* and *O. viperis* from *T. variabilis*. They described also a new species, *Ophiotaenia habanensis* from *Tropidophis pardalis*.

During the years 1966—1976 we have collected further helminth specimens from this group of hosts. The results presented in this paper supply numerous new data on the helminth fauna of Cuban snakes, complementing the present knowledge of these parasites. Some problems of taxonomy and zoogeography are also being solved.

Table 1. Survey of examined snakes and the distribution of helminths in classes

Hosts	Total Exam.	Nematoda	Cestoidea	Trematoda	Acantho-cephala
Fam. Boidae					
+ *Epicrates angulifer*	11	—	1	—	—
+ *Tropidophis maculatus*	8	—	—	—	—
T. melanurus	15	10	5	5	—
+ *T. nigrivetris*	1	—	—	—	—
+ *T. pardalis*	6	4	1	—	—
+ *T. semicinctus*	1	1	1	—	—
+ *T. wrighti*	1	—	—	—	—
Fam. Colubridae					
Alsophis cantherigerus	32	26	11	14	7
+ *Antillophis andreae*	24	15	5	3	5
+ *Arrhyton dolicurum*	11	—	—	—	—
+ *A. taeniatum*	4	—	—	—	—
+ *A. vittatum*	2	1	—	—	—
Natrix fasciata	1	—	—	—	—
Tretanorhinus variabilis	12	7	7	—	—
Fam. Typhlopidae					
Typhlops lumbricalis	20	3	—	—	—

MATERIAL

A total of 149 Cuban snakes belonging to 15 species were examined at autopsy. The decreasing order of helminth incidence was: Nematoda in 67 hosts, Cestoidea in 30 hosts, Trematoda in 22 hosts and Acanthocephala in 12 hosts. A survey of the hosts examined and their systematic position is given in Table 1. (The endemic species of the Cuban fauna are marked by an asterisk.)

RESULTS

Strongylata

Family Trichostrongylidae

1. *Oswaldocruzia lenteixeirai* Pérez Vigueras, 1938

Hosts: ***Tropidophis pardalis*** (Boidae); ***Alsophis cantherigerus*** and ***Antillophis andreae*** (Colubridae).
Localization: intestine.
Locality: Guanahacabibes peninsula (province Pinar del Río); La Habana and Nueva Paz (province Havana).

One of the six *T. pardalis* examined was infected with two male and two female nematodes; one of the 32 *A. cantherigerus* was infected with one female nematode and one of the 24 *A. andreae* was infected with one male nematode.

The species *O. lenteixeirai* from *Hyla insulsa* (Amphibia: Hylidae) was described by Pérez Vigueras (1938) and later redescribed by Baruš and Moravec (1967). Other records from frogs of the genera *Bufo* and *Eleutherodactylus* were published by Baruš (1972, 1973). Outside the Cuban territory, this species was first reported by Schmidt and

Whittaker (1975) from *Eleutherodactylus portoricensis* from Puerto Rico Island.

Baruš and Coy Otero (1968) described a new species, *O. anolisi* from *Anolis equestris* (Iguanidae). Later Baruš and Coy Otero (1969b) and Coy Otero (1970) recorded this species from the hosts of the families Iguanidae and Teiidae and the differences between *O. lenteixeirai* and *O. anolisi* were established: the lateral alae are present in *O. anolisi* but absent in *O. teixeirai;* the cervical papillae differ in their shape and position and also some small metrical differences were observed.

Moravec and Vojtková (1975) have recently published a revision of the species of the genus *Oswaldocruzia* from Czechoslovakia. The conclusions drawn by these authors give evidence that the lateral alae, as well as the shape and position of cervical papillae (and other characters) are very variable in these species and cannot be therefore used for the differentiation of taxons. A considerable variability in the length of spicules and some other characters in relation to the definitive host has already been pointed out by Baruš (1972) for *O. lenteixeirai* and by Baruš and Coy Otero (1969) for *O. anolisi.* We have carried out a comparative analysis of our Cuban nematodes of the genus *Oswaldocruzia* from the hosts of the classes Amphibia and Reptilia. It may be stated that there are no significant differences which might serve for a reliable differentiation of individual host forms of these nematodes. Neither has a detailed study of the morphology revealed any characters separating the host forms of these two nematode species of the genus *Oswaldocruzia.* Although the specimens recovered from Iguanidae and Teiidae (and also Boidae and Colubridae) have conspicuous lateral alae, we agree with Moravec and Vojtková (1975) that this character is not suitable for species differentiation. Also the topography and shape of cervical papillae are more variable than we have supposed earlier. Consequently, we regard *O. anolisi* as a synonym of *O. lenteixeirai.*

O. lenteixeirai is a widely distributed parasite of hosts of the classes Amphibia and Reptilia in Cuba. We found this species for the first time in snakes, but these hosts seem to be only facultative. In view of the zoogeography, this species is evidently an element of the helminth fauna of the Antillean subregion.

2. *Typhlopsia kratochvili* nov. gen. et nov. sp. — (Fig. 1)

Host: *Typhlops lumbricalis* (Typhlopidae).
Localization: intestine.
Locality: Santiago de Cuba, Reparto Vista Alegre (province Oriente).
Only one of the 20 hosts examined was infected with two male nematodes.

Description of the holotype (measurements of the paratype are given in parentheses): Nematodes of whitish colour; cuticle with dense longitudinal striations and eight low longitudinal cuticular ridges. The mouth is terminal, rounded, measuring 0.014 mm in diameter. The mouth cavity is small and its walls are not sclerotized. The teeth are absent and the lips are not developed. The head vesicle is narrow, 0.066 (0.074) mm long and with fine transverse striations. Narrow lateral alae measure 0.014 mm in maximum width in cervical part of body.

The male body measures 6.65 (6.51) mm in length and 0.19 (0.21) mm in maximum width. The oesophagus is cylindrical, measuring 0.24

(0,29) mm in length and 0.051 (0.064) mm in maximum width. The nerve ring is situated 0.120 (0.080) mm and the cervical papillae 0.22 mm from the anterior end. The cervical papillae have the shape of a small spine. There are two spicules, equal and similar, sclerotized more on the ventral part than on the dorsal one. They measure 0.155 (0 167) mm in length and their distal ends are divided into two processes. The ventral process is somewhat shorter than the dorsal one. The gubernaculum is cymbiform

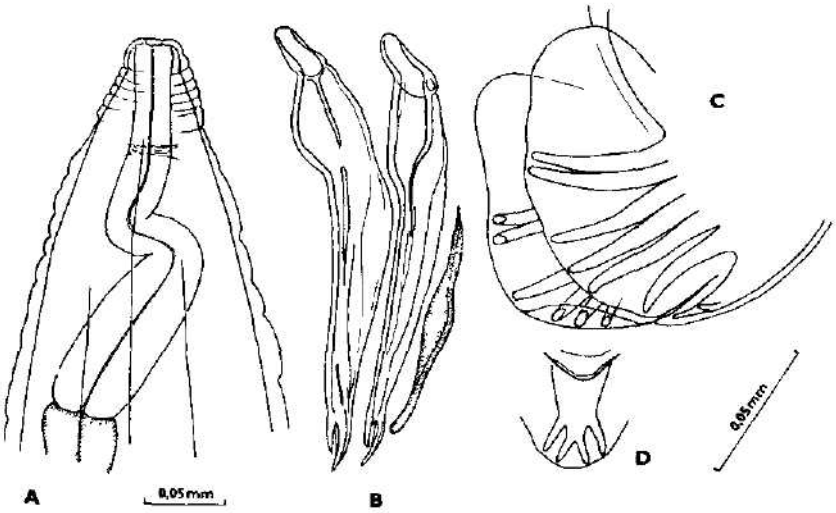

Fig. 1. *Typhlopsia kratochvili* nov. gen. et nov. sp. from *Typhlops lumbricalis*. A — anterior end of male body (ventral view); B — spicules and gubernaculum (lateral view); C — bursa copulatrix (lateral view); D — dorsal ray (detail). Original.

and 0.087 (0.095) mm long. The bursa copulatrix has a very small median lobe and wide lateral lobes. The medio-dorsal ray is bifurcated in its distal part. A small distance above the bifurcation runs off another branch on each side. The ventral and lateral rays are of almost the same thickness and length. The externo-dorsal rays are somewhat shorter. The ventral rays diverge only at their tips. The distance between the tips of lateral rays is slightly greater. All rays reach with their tips the margin of bursa copulatrix. The praebursal papillae were not found.

Discussion: The species of the family Trichostrongylidae Leiper, 1912 are only very rare parasites of coldblooded vertebrates. The nematodes of this family parasitizing Reptilia belong to five genera (Travassos, 1937; Skrjabin et al., 1954; Yamaguti, 1961): *Amphibiophilus* Skrjabin, 1916; *Oswaldocruzia* Travassos, 1917; *Herpetostrongylus* Baylis, 1931; *Trichoskrjabinia* Travassos, 1937 and *Schulzia* Travassos, 1937. The last two genera are monotypic. The genus *Oswaldocruzia* has a cosmopolitan distribution, whereas *Amphibiophilus* has been known from Africa and China, *Herpetostrongylus* from India and Australia, *Trichoskrjabinia* from Malaysia and *Schulzia* from Brazil

The new species described by us could not be included into any of the above-mentioned or hitherto known genera of the family Trichostrongylidae. The morphological characters in our specimens are so evident that we have established for them a new genus — *Typhlopsia* nov. gen belonging to the family Trichostrongylidae. Its diagnosis is as follows:

The head vesicle is narrow, but in the posterior part distinctly separated from the body. The mouth opening is rounded, without lips. The mouth cavity is very small and its walls are not sclerotized. The teeth are absent. The median lobe of the bursa copulatrix is very small, the lateral lobes are markedly longer and wider. The ventral and lateral rays are of almost the same length and the same thickness. The dorsal ray is bifurcated in the distal part and a small distance above the bifurcation runs off another process on each side. The spicules are massive and well sclerotized on the ventral side. The distal ends are divided into two branches. The gubernaculum is present. The members of this genus are parasites of the intestine of snakes in the Antillean zoogeographical subregion.

The genus *Typhlopsia* (with the type species *T. kratochvili* nov. sp.) erected by us differs from the genera *Amphibiophilus* and *Herpetostrongylus* in the form of the mouth apparatus. The members of the genus *Typhlopsia* have no tooth in their mouth cavity, whereas those of *Amphibiophilus* and *Herpetostrongylus* possess one or more teeth. There are also marked differences in the shape and topography of rays of bursa copulatrix and in the shape of spicules. *Typhlopsia* differs evidently from *Trichoskrjabinia* in the topography and shape of rays of bursa copulatrix. Particularly the characteristic shape of the dorsal ray should be noted in *Trichoskrjabinia malayana* (Baylis, 1933), which is a typical and hitherto single species of this genus. A characteristic feature of *Oswaldocruzia* is the absence of gubernaculum (which is present in *Typhlopsia*). These two genera (*Oswaldocruzia* and *Typhlopsia*) differ also in the shape of the distal end of spicules and topography of rays of the bursa copulatrix.

The genus *Typhlopsia* seems to be most closely related to *Schulzia*. They are distinctly separated from one another in the presence of the gubernaculum in *Typhlopsia* (absent in *Schulzia*) and the striations of the cuticle, which are longitudinal in *Typhlopsia* and transverse in *Schulzia*. The lateral alae are absent in *Schulzia* and present in *Typhlopsia*. Both genera differ also in the shape of the medio-dorsal ray. Besides the above-mentioned genera, the genus *Poekilostrongylus* Schmidt et Whittaker, 1975 with a single species *P. puertoricensis* parasitizing tree frog *(Eleutherodactylus coqui)* in Puerto Rico has been described recently. It differs from *Typhlopsia* in the absence of the gubernaculum, absence of longitudinal cuticular ridges and other characters. A key to the genus *Typhlopsia* nov. gen. is presented:

1. Mouth cavity without teeth, gubernaculum present or absent . 2
— Mouth cavity with one or more teeth, gubernaculum always present 3
2. Gubernaculum always absent 4
— Gubernaculum always present 5
3. Mouth cavity with one relatively large tooth and two small latero-ventral teeth . . . . . *Herpetostrongylus*
— Mouth cavity with one relatively large dorsal tooth, other teeth absent . . . . . *Amphibiophilus*
4. Distal ends of spicules divided into three and more branches *Oswaldocruzia*
— *Distal ends of spicules divided into two branches only* . . *Schulzia*
5. Medio-dorsal ray divided into three very short branches on distal end; antero-lateral ray markedly thicker and longer than medio- and postero-lateral . . . . . *Trichoskrjabinia*

— Medio-dorsal ray divided into two relatively large branches on distal end; antero-lateral ray of the same thickness and length as medio- and postero-lateral . . . . . *Typhlopsia*

The holotype is deposited in the collection of the Humboldt Museum in Berlin, the paratype in the collection of the Zoological Institute of the Cuban Academy of Sciences in Havana This new taxon is named in honour of Academician Josef Kratochvíl, the eminent Czechoslovak zoologist.

Family Diaphanocephalidae

1. *Kalicephalus costatus costatus* (Rudolphi, 1819) — (Fig. 2 A, B, C)

Host: *Antillophis andreae* (Colubridae).
Localization: intestine.
Locality: southern part of Isla de Pinos island.

This subspecies (1 and 2 specimens) was found in two of the 24 *A. andreae* examined. Two males and one female were recovered.

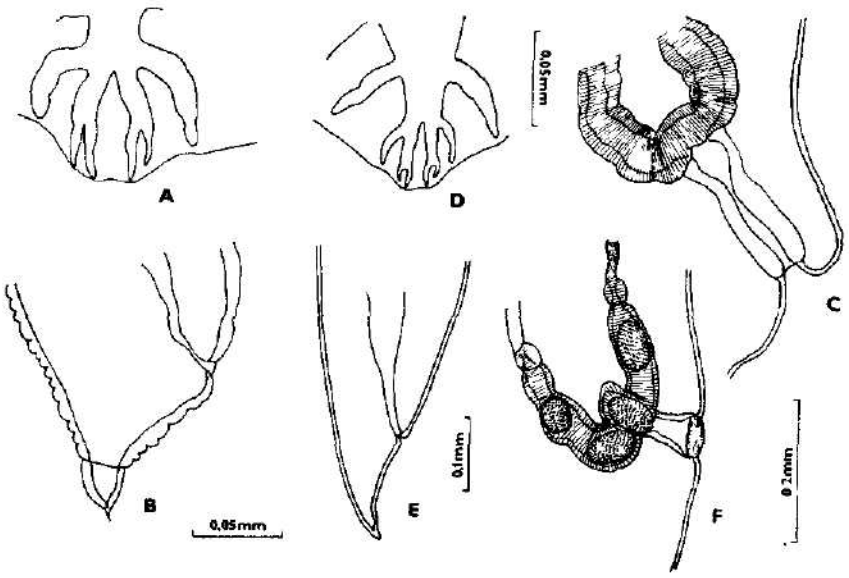

Fig. 2. *Kalicephalus costatus costatus* (Rudolphi, 1819) from *Antillophis andreae* (A, B, C) and *K. costatus alsophisi* (Baruš et Coy Otero, 1966) from *Alsophis cantherigerus* (D, E, F). A — dorsal ray; B — posterior end of female body (lateral view), C — vulva region (lateral view); D — dorsal ray; E — posterior end of female body (lateral view); F — vulva region (lateral view). Original.

Schad (1962) in his excellent monograph characterized this subspecies as follows: "*K. costatus* with breviconical female tail bearing terminal spike, vulva on ventral bulge of body, ovejector usually convergent". Our material fully corresponds to all morphological and metrical features of this subspecies characterizing and separating it from the remaining three subspecies of this species (*K. costatus*). In Table 2, the measurements of our specimens are compared with the data given by Schad (1962).

The subspecies *K. c. costatus* is distributed in South and Central America where it parasitizes more than 15 species of snakes of the families

Table 2. Comparative measurements of *Kalicephalus costatus costatus* (Rudolphi, 1819), after Schad (1962) and our material

Measurements	After Schad (1962) Male	After Schad (1962) Female	After our material Male	After our material Female
Length of the worm	4.88—9.00	5.90—12.45	4.80—4.96	5.11
Maximum width of the worm	0.28—0.52	0.29— 0.61	0.19—0.23	0.25
Dorsoventral diameter of head	0.19—0.31	0.20— 0.31	0.22—0.26	0.28
Deepth of buccal capsule	0.14—0.18	0.13— 0.23	0.17	0.23
Nerve ring to anterior tip	0.21—0.30	0.20— 0.34	0.26—0.29	0.35
Length of oesophagus	0.30—0.43	0.32— 0.48	0.35—0.36	0.40
Maximum width of oesophagus at bulb	0.18—0.27	0.18— 0.28	0.17—0.18	0.22
Vulvar ratio (+)	———	4.8 —11.7	———	7.3
Anus to posterior end of female	———	0.08— 0.22	———	0.12
Length of equal spicules	0.34—0.70	———	0.30—0.37	———

(+) Vulvar ratio = prevulvar body length divided by postvulvar body length.

Colubridae, Crotalidae and Viperidae. In the Antillean subregion, the occurence of this nematode depends on the hosts of the genus *Antillophis*, which are closely related to the genus *Dromicus* from South America. In the helminth fauna of Cuban snakes, *K. c. costatus* represents an element of South American region.

2. *Kalicephalus costatus alsophisi* (Baruš et Coy Otero, 1966) — (Fig. 2 D, E, F)

Host: *Alsophis cantherigerus* (Colubridae).
Localization: intestine.
Locality: Guanahacabibes peninsula (province Pinar del Rio).

This subspecies was found in one of the 32 *A. cantherigerus* examined (a total of 15 nematodes: 7 males and 8 females).

Table 3. Comparative measurements between the susceptible *Kalicephalus costatus parvus* (Ortlepp, 1923) after Schad (1962) and *K. costatus alsophisi* (Barus et Coy Otero, 1966) after our material

Measurements	*Kalicephalus costatus parvus* After Schad (1962) Male	*Kalicephalus costatus parvus* After Schad (1962) Female	*Kalicephalus costatus alsophisi* After our material Male	*Kalicephalus costatus alsophisi* After our material Female
Length of the worm	3.39—4.91	4.38— 7.80	4.96—5.89	5.42—5.73
Maximum width of the worm	0.21—0.32	0.23— 0.35	0.32—0.34	0.37—0.41
Dorsoventral diameter of head	0.22—0.27	0.18— 0.24	0.24—0.27	0.36—0.37
Deepth of buccal capsule	0.11—0.14	0.12— 0.18	0.17—0.20	0.21—0.29
Nerve ring to anterior tip	0.18—0.24	0.21— 0.35	0.29—0.31	0.32—0.34
Length of oesophagus	0.26—0.30	0.27— 0.38	0.37—0.39	0.40—0.42
Maximum width of oesophagus at bulb	0.14—0.18	0.15— 0.23	0.20—0.25	0.26—0.36
Vulvar ratio	———	3.5 —10.6	———	4.8 —5.1
Anus to posterior tip of female	———	0.09— 0.15	———	0.13—0.15
Length of equal spicules	0.25—0.31	———	0.37—0.40	———

Baruš et Coy Otero (1966) described a new nematode species, *K. alsophisi*, from *A. cantherigerus* on the basis of four nematodes (1 ♂ and 3 ♀♀) from the locality Boca de Miel (province Oriente). On the basis of the new specimens from our collection we could define with more precision some morphological and metrical characters. In taxonomic evaluation of this material we consider the view of Schad (1962), who divided the species *K. costatus* (Rudolphi, 1819) into four subspecies: *K. costatus costatus (Rudolphi, 1819), characteristic for snakes of South*

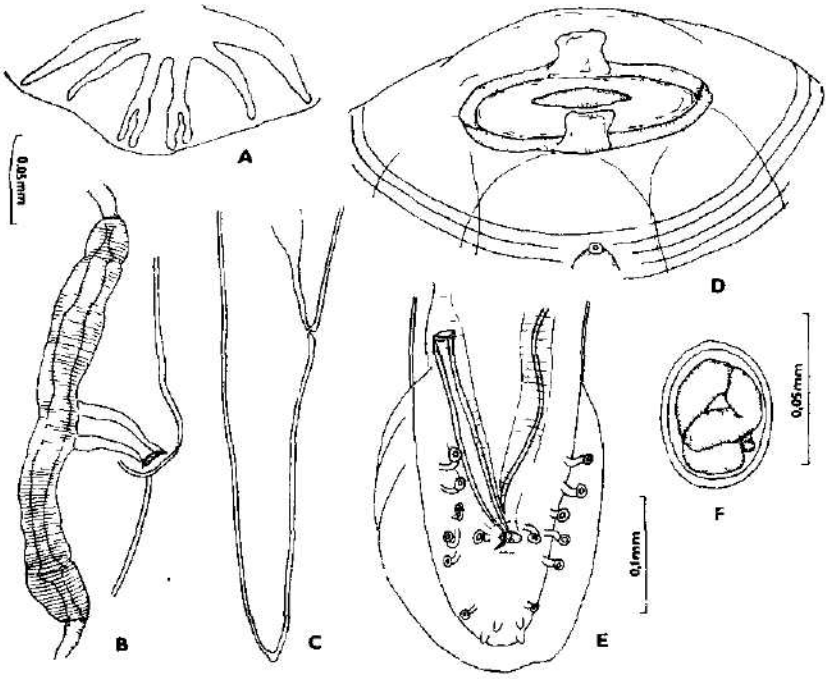

Fig. 3. *Kalicephalus rectiphilus rectiphilus* (Harwood, 1932) from *Antillophis andreae* (A, B, C) and *Hastospiculum cubaense* Baruš et Sonin, 1971 from *Tropidophis melanurus* (D, E, F). — A — dorsal ray; B — vulva region (lateral view); C — posterior end of female body (lateral view); D — anterior end of body (apical view); E — posterior end of male body (ventral view); F — egg. Original.

and Central America; *K. costatus parvus* (Ortlepp, 1923) from the Southern Nearctic region; *K. costatus micrurus* (Daubney, 1923) from the Ethiopian region and *K. costatus indicus* (Ortlepp, 1923) from the Oriental and Australian regions. Compared to the above-mentioned subspecies of *K. costatus* (Rudolphi, 1819), the morphological and metrical characters of *K. alsophisi* have not the value of species. but only of subspecies.

K. costatus alsophisi nov. comb. differs markedly from the nominal subspecies *K. costatus costatus* in the shape of female tail and also in the shape of vulva, which is not markedly salient in *K. c. alsophisi* (Fig. 2 E, F). *K. c. alsophisi* differs from the subspecies *K. c. parvus*, which is typical of snakes of the family Colubridae in the southern part of the

Table 4. Comparative measurements of *Kalicephalus rectiphilus rectiphilus* Harwood, 1932, after Schad (1962) and our material

Measurements	After Schad (1962)		Our material	
	Male	Female	Male	Female
Lenght of the worm	4.25—5.69	4.73—7.35	4.03—4.49	4.34—5.42
Maximum width of the worm	0.19—0.24	0.15—0.29	0.17—0.20	0.18—0.26
Dorsoventral diameter of head	0.16—0.20	0.15—0.24	0.17—0.19	0.19—0.24
Deepth of buccal capsule	0.11—0.14	0.11—0.17	0.13—0.18	0.14—0.18
Nerve ring to anterior tip	0.19—0.25	0.18—0.27	0.19—0.21	0.21—0.26
Length of oesophagus	0.18—0.24	0.20—0.29	0.22—0.23	0.22—0.27
Maximum width of oesophagus at bulb	0.08—0.11	0.09—0.13	0.08—0.10	0.09—0.14
Vulvar ratio	———	1.0 —1.5	———	1.0 —1.5
Anus to posterior tip of female	———	0.11—0.30	———	0.19—0.21
Length of equal spicules	0.24—0.28	———	0.25—0.29	———

Nearctic region, in larger size of body in both sexes and in the shape of vulva (Table 3). We consider the subspecies *K. c. alsophisi* to be a characteristic element of the helminth fauna of the Antillean subregion, probably specific for the hosts of the genus *Alsophis*.

3. *Kalicephalus rectiphilus rectiphilus* (Harwood, 1932) — (Fig. 3 A, B, C)

Hosts: *Antillophis andreae* and *Alsophis cantherigerus* (Colubridae).
Localization: large intestine.
Locality: La Habana (province Havana); Guanahacabibes peninsula (province Pinar del Río).

One specimen of the 24 *A. andreae* examined was infected with three nematodes (1 ♂ and 2 ♀♀) and one of the 32 *A. cantherigerus* examined was infected also with three nematodes 1 ♂ and 2 ♀♀).

According to Schad (1962), this nominal subspecies of the species *K. rectiphilus* Harwood, 1932 has the zoogeographical distribution from the southern Nearctic to norther Neotropical regions. The definitive hosts are snakes of the family Colubridae. We found this subspecies for the first time in the Antillean subregion in two new definitive hosts. On the basis of its morphological and metrical characters it can be easily distinguished from *K. rectiphilus neorectiphilus* Schad, 1962, which is distributed in South America. The morphology and measurements of our material fully conform to the diagnosis of the nominal subspecies (Fig. 3 A, B, C; Table 4). Its finding in Cuba suggests the north American origin.

Filariata

Family Diplotriaenidae

1. *Hastospiculum cubaense* Baruš et Sonin, 1971 — (Fig. 3 D, E, F)

Hosts: *Antillophis andreae* and *Alsophis cantherigerus* (Colubridae); *Tropidophis melanurus* (Boidae).
Localization: body cavity.
Locality: Viñales (province Pinar del Río), Ciénaga de Zapata (province Matanzas) and Isla de Pinos island.

Baruš and Sonin (1971) described this species from *A. andreae nebulatus* and *T. melanurus ericksoni* from Isla de Pinos. Their material was not very numerous (one male, one female and fragments of eight females).

The specimens in our new collections were obtained not only from the typical host in the typical locality (Isla de Pinos), but also from snakes of the central island of Cuba. The incidence of infection was the following: 2 of 24 *A. andreae* (1 and 4 nematodes), 3 of 15 *T. melanurus* (1, 6 and 7 nematodes) and 1 of 32 *A. cantherigerus* examined (1 nematode). Altogether we recovered 20 specimens of *H. cubaense* (9 males and 11 females). On the basis of the new material we are complementing some data which were not included in the original description (Fig. 3): Male body measures 29—32 mm in length and 0.13—0.19 mm in maximum width. The total length of the oesophagus is 6.98—8.06 mm. The spicules are 0.14—0.22 mm and 1.10—1.55 mm long. There is a pair of small chitinized tooth-like projections situated laterally on mouth, and measuring 0.015 mm in length and 0.015 mm in width. The sclerotized circumoral ring measures 0.079 × 0.030 mm.

Note: The long spicule was slightly longer than that in the original description (0.86 mm in the original description). The morphological characters of our material fully conform to the original diagnosis of *H. cubaense*.

Only two of the eight hitherto known species of the genus *Hastospiculum* Skrjabin, 1923 (see Sonin 1968) appertain to the Neotropic region. *H. digiticaudum* Freitas, 1955 has been reported from *Philodryas aestivus* (Colubridae) from Brazil and *H. onchocercum* Chitwood, 1932 from *Constrictor imperator* (Boidae) and *Crotalus terrificus* (Crotalidae) from Brazil and Panama. *H. cubaense* distinctly differs from *H. digiticaudum* in the form of male caudal alae and other characters. It differs from *H. onchocercum* in the form of the head end (Fig. 3 D), number and topography of caudal papillae (one pair of paracloacal papillae present in males of *H. cubaense*, but absent in *H. onchocercum*) and body measurements. Especially the length of spicules is different, measuring 1.84—2.75 mm and 0.24—0.30 mm in *H. onchocercum*, but only 0.86—1.55 mm and 0.14—0.22 mm in *H. cubaense*. We consider *H. cubaense* a typical element of the snake helminth fauna of the antillean subregion showing a zoogeographical relation to the fauna of filariae parasitizing snakes in South and Central America.

Family Physalopteridae

1. *Abbreviata (Didelphyoptera) baracoa* Baruš et Coy Otero, 1966 — (Fig. 4)

Host: *Alsophis cantherigerus* (Colubridae).
Localization: stomach.
Locality: Baracoa (province Oriente); La Esmeralda and Guaímaro (province Camagüey); La Habana and Santa Cruz del Norte (province Havana); Península de Guanahacabibes (province Pinar del Río); Cayo Cantiles islands; Santa Fe and La Vega (Isla de Pinos island).

This species was described by Baruš and Coy Otero (1966) from *Alsophis cantherigerus pepei* and *A. c. adspersus* from the locality Boca de Miel near Baracoa (province Oriente). The original description was based on the material consisting of three males, seven females and one larva. The new material enables us to complement the data about the distribution and variability of this species.

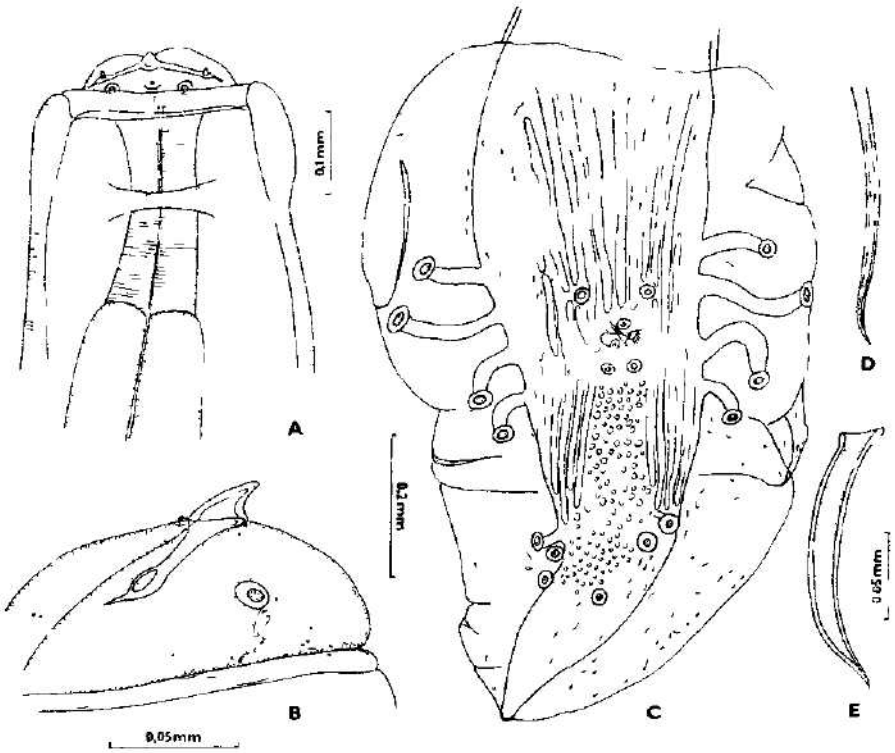

Fig. 4 *Abbreviata baracoa* Baruš et Coy Otero, 1966 from *Alsophis cantherigerus*. A — anterior end of body (lateral view), B — pseudolabium (ventral view); C — posterior end of male body (ventral view), D — distal end of long spicule; E — short spicule. Original.

Of the 32 *A. cantherigerus* examined, 13 specimens were positive (1—97 nematodes per host) Altogether we obtained 602 nematodes (203 males and 399 females). We are giving a redescription based on the material from the typical host (*A. cantherigerus*)

Description (based on 10 males and 10 females): Body whitish to rosy. Cuticle with transverse striations. Mouth terminal, with two lateral pseudolabia. Sclerotized thickening running near upper margin of pseudolabia and forming a conspicuous triangular tooth in its middle part. One or two small thorn-like teeth on its sides (more laterally). Inner part of pseudolabia divided by median longitudinal incision into two semispherical structures.

Male: Body length 11 62—14.57 mm and maximum width 0.38—0.53 mm. Total length of oesophagus 2.09—2.55 mm, anterior muscular part measuring 0.25—0.38 mm in length. Nerve ring 0.23—0.26 mm from anterior end of body. Posterior end of body surrounded by wide, relatively long caudal vesicle, supported by four pairs of long pedunculate papillae. Three sessile papillae on upper lip and four sessile papillae on lower lip of cloaca. Another three pairs of mostly sessile papillae on tail. Longitudinal thickened bands of cuticle on ventral side of caudal vesicle.

Small cuticular bosses distributed in median part behind cloaca and reaching the level of last pair of caudal papillae. Two spicules unequal and dissimilar, with pointed distal tips and measuring 1.00–1.24 mm and 0.15–0.17 mm. Cloaca 0.52–0.65 mm from tail end.

Female: Body length 14.88–18.83 mm, maximum width 0.46–0.56 mm. Total length of oesophagus 2.17–2.59 mm, of anterior muscular portion 0.26–0.34 mm. Nerve ring 0.22–0.31 mm, vulva 1.55–2.79 mm from anterior end of body. Vagina 0.54–0.56 mm, egg-chamber 0.51–0.54 mm and common trunk of oviduct 0.62–0.77 mm long. Two uterine branches present. Eggs 0.041–0.045 × 0.028–0.031 mm. Anus 0.40–0.42 mm from tail end.

This species is the most widespread and most frequent parasite of snakes in Cuba. According to hitherto available reports it is a specific parasite of *Alsophis cantherigerus*. Having regard to the zoogeographical distribution of the definitive host it is supposed that this parasite is an element of the helminth fauna of the Antillean subregion. Infective larvae of *A. baracoa* were found by Baruš (1973) in frogs *Bufo peltacephalus* and *B. taladai* which are evidently their reservoir hosts.

Ascaridata

Family Stomachidae

1. *Terranova caballeroi* Baruš et Coy Otero, 1966 — (Fig. 5)

Host: *Alsophis cantherigerus* (Colubridae).
Localization: stomach and intestine.
Locality: Pico Turquino and Baracoa (province Oriente); Guaímaro (province Camaguey); Ciénaga de Zapata (province Matanzas): La Habana and Santa Cruz del Norte (province Havana); Guanahacabibes peninsula (province Pinar del Río); Cayo Cantiles island.

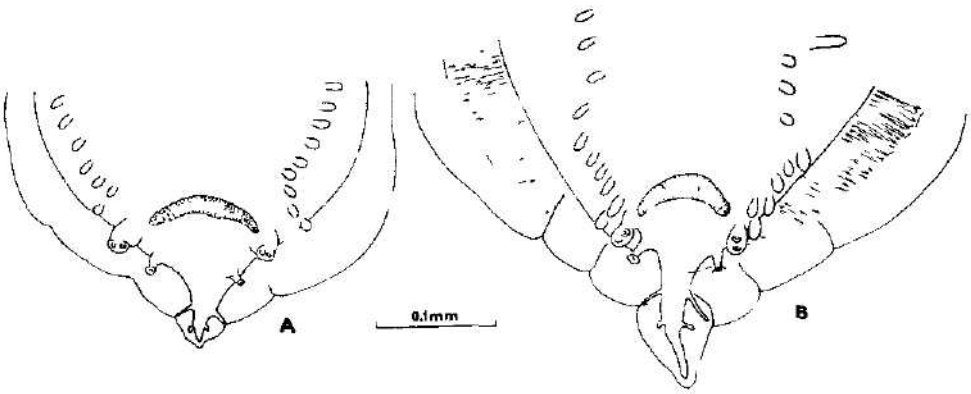

Fig. 5. *Terranova caballeroi* Baruš et Coy Otero, 1966 from *Alsophis cantherigerus*. A — posterior end of young male body (ventral view); B — posterior end of adult male body (ventral view) Original

Twenty-one specimens of the 32 *A. cantherigerus* examined were positive (1–55 nematodes per host). The material comprised 699 specimens (255 males and 444 females).

Table 5. Comparative measurements between the different forms of *Stomachidae* sp. larvae, found in snakes of the genus *Alsophis, Antillophis, Tropidophis* and *Arrhyton*

Measurements	Forms no. 1 +	Forms no. 2 +	Forms no. 3 +
Body length	10.38—14.06	5.73—16.50	10.85—14.70
Maximum width	0.38— 0.62	0.24— 0.54	0.25— 0.39
Oesophagus-length	0.81— 1.08	0.59— 1.19	0.93— 1.62
Oesophagus-maximum width	0.12— 0.15	0.09— 0.15	0.11— 0.17
Ventriculus-length	0.26— 0.32	0.17— 0.27	0.19— 0.29
Ventriculus width	0.12— 0.15	0.11— 0.22	0.10— 0.15
Intestinal caecum-length	0.25— 0.33	0.30— 0.44	0.39— 0.48
Intestinal caecum-width	0.06— 0.09	0.04— 0.08	0.05— 0.11
Anus to posterior tip	0.12— 0.15	0.09— 0.19	0.11— 0.22

+ Forms no. 1 are in the hosts: *Alsophis cantherigerus*
Forms no. 2 are in the hosts: *Antillophis andreae, Tropidophis melanurus T. pardalis* and *Arrhyton vittatum*
Forms no. 3 are in the hosts: *Antillophis andreae*

Baruš and Coy Otero (1966) described *T. caballeroi* from *Alsophis cantherigerus pepei* caught in the locality Boca de Miel near Baracoa (province Oriente). The original description was based on a rather rich material, 18 males and 64 females, and no substantial differences have been observed during the studies of our new material. In relation to the systematic position of this species, it should be noted that it possesses an important feature which does not conform of the diagnosis of the genus *Terranova* Leiper et Atkinson, 1914. It is the presence of two triangular teeth protruding from the mouth cavity. The teeth are well sclerotized and conspicuous, especially if the head end is observed from the ventral side. This feature might suggest some relation to the genus *Acanthocheilus* Molin, 1858, but all other characters correspond to the diagnosis of the genus *Terranova* (see Hartwich, 1957), i. e. "interlabia and cervical alae absent, stomach and caecum present, excretory pore lying between subventral lips, spicules equal and similar. Vulva situated at the boundary between anterior and middle third of body length."

The above survey of localities shows that *T. caballeroi* is a widely distributed species, specific for the host *A. cantherigerus*. According to the present knowledge it may be considered an endemic species of the Cuban helminth fauna. The morphology of this species is so caracteristic and different from other species of the family Ascaridoidea that no presumption on its zoogeographical relation can be expressed.

2. *Stomachidae* gen. sp. — larvae (Fig. 6)

Hosts: *Alsophis cantherigerus, Antillophis andreae* and *Arrhyton vittatum* (Colubridae); *Tropidophis pardalis* and *T. melanurus* (Boidae)
Localization: in cysts on walls of stomach and intestine
Locality: Guanahacabibes peninsula (province Pinar del Río); Ciénaga de Zapata (province Matanzas); Topes de Collantes (province Las Villas) and Isla de Pinos island.

The recovered larvae of the family Stomachide could be divided into three morphological groups:

1. The ratio between the length of stomach and the length of caecum is 1 : 0.9—1.0. The tail is conical, always with a short, fingerlike process The larvae were found

only in *Antillophis andreae*, in six of the 24 hosts examined (1–53 specimens per host). Our material comprised 109 larvae (Fig. 6 A, B).

2. The ratio between the length of stomach and the length of caecum is 1 : 1.6–1.7 mm. The tail is conical (Fig. 6 C), always with a short, fingerlike process (of the same shape as in group 1). The larvae were recovered from *A. andreae*, *T. melanurus*, *T. pardalis* and *A. vittatum*, the incidence of infection being as follows: 12 of 24 *A. andreae* (1–100 specimens per host; altogether 210 larvae); 4 of 15 *T. melanurus* (16–150 specimens per host; altogether 303 larvae); 1 if 6 *T. pardalis* (1 larva) and 1 of 2 *A. vittatum* examined (1 larva).

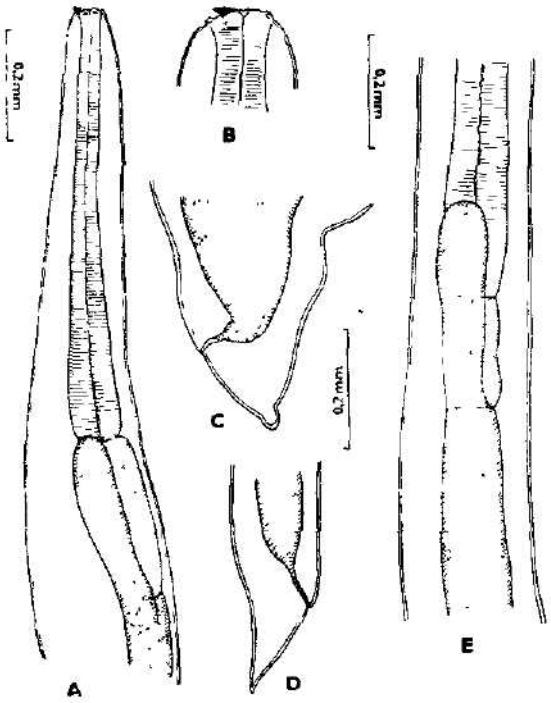

Fig. 6. Stomachidae gen. sp. — larvae from *Antillophis andreae* (A, B), *Tropidophis melanurus* (C) and *Alsophis cantherigerus* (D, E). A — anterior end of larval body (dorsal view); B — anterior end of body (detail, lateral view); C, D — posterior end of larval body (lateral view); E — region of oesophagus end and stomach (dorsal view). Original.

3. The ratio between the length of stomach and the length of caecum is 1 : 1.6–2.0. The tail is conical, always with pointed tip. The larvae were found only in *A. cantherigerus*, in two of the 32 specimens examined (1 and 3 larvae).

The taxonomic position of the larvae of groups 1 and 2 cannot be exactly determined. The larvae of group 3 markedly resemble adult specimens of *T. caballeroi* in the shape of tail and ratio of stomach length to caecum length. This ratio is 1 : 1.2–1.7 in males and 1 : 1.5–2.1 in females of *T. caballeroi*. The measurements of the larvae of all three types are given in Table 5.

Besides these larvae which we found for the first time in snakes in Cuba, Baruš and Coy Otero (1966) reported also nematode larvae of the genus *Contracaecum* from *Tretanorhinus variabilis* (Colubridae) from the locality Ciénaga de Zapata (province Matanzas).

Oxyurata

Family Oxyuridae

1. *Parapharyngodon cubensis* (Baruš et Coy Otero, 1969)

Hosts: *Tropidophis semicinctus* and *T. melanurus* (Boidae), *Alsophis cantherigerus* (Colubridae).

Localization: intestine.

Locality: Santiago de Cuba and Baracoa (province Oriente); La Habana – Marianao (province Havana).

Two nematodes were recovered from the single *T. semicinctus* examined 1 ♂ and 1 ♀); two of the 15 *T. melanurus* examined were infected with three nematodes (1 ♂ and 2 ♀♀); one of the 32 *A. cantherigerus* was infected with two female nematodes.

Baruš and Coy Otero (1969a) described this form as a new subspecies *P. senisfaciaecaudus cubensis* from the hosts of the families Iguanidae and Teiidae. Other records, also from Gekkonidae, were published by Baruš and Coy Otero (1969b), Coy Otero (1970) and Coy Otero and Baruš (1973). The characteristic morphology of these nematodes led Baruš (1973) to elevating this subspecies to the rank of species. *P. cubensis* is a frequent and widely distributed parasite of most species of the families Iguanidae (genera *Anolis* and *Leiocephalus*), Teiidae (genus *Ameiva*) and Gekkonidae (genera *Sphaerodactylus* and *Gonatodes*). This is the first record from snakes which seem to be accidental hosts of this species. Our finding may be also regarded as the case of the postcyclical parasitism (Bozhkov, 1969).

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Authors' address: Ing. Vlastimil Baruš, DrSc., člen korespondent ČSAV, Ústav pro výzkum obratlovců ČSAV, Května 8, 603 65 Brno, Czechoslovakia. Lic. Alberto Coy Otero, CSc., Instituto de Zoologia, Academia de Ciencias de Cuba, La Habana, Cuba.

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Institute of Parasitology, Czechoslovak Academy of Sciences, Prague
Laboratory of Aquaculture Biology, Faculty of Agriculture, University of Tokyo, Tokyo

REDESCRIPTION OF GYRODACTYLUS KOBAYASHII HUKUDA (MONOGENOIDEA)

R. ERGENS and K. OGAWA

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Abstract: *G. kobayashii* Hukuda, 1940 is redescribed on the basis of a detailed analysis of the original description and systematical evaluation of gyrodactylids from Japan goldfish. In agreement with the opinion of Yamaguti (1963), *G. elegans yamagutii* Yin et Sproston, 1948 (= *G. elegans sensu* Yamaguti, 1940), is considered to be a synonym of *G. kobayashii* and the possibility of another synonym, *G. elegans sinicus* Yin et Sproston, 1948 is also admitted.

In 1940, Hukuda described a new species of the genus *Gyrodactylus* Nordmann, 1832 — *G. kobayashii* from the fins and skin of the Korean goldfish, *Carassius auratus* (L.). The original description, complemented with figures of the whole mount (ventral view), complex of anchors and marginal hooks, included the following measurements: length and width of body (0.2—0.35 × 0.03—0.07 mm), diameter of pharynx (0.02 mm), length and width of cirrus pouch (0.016 × 0.012 mm), total length of anchors (0.085 mm) and marginal hooks (0.028 mm), width of dorsal connecting bar (0.015 mm), length and width of ventral connecting bar (0.006 × 0.022 mm) and length of its membranous extension (0.014 mm).

The type material of *G. kobayashii* was destroyed during the Second World War, as we were informed by Dr. S. Kamegai, Director of the Meguro Parasitological Museum in Tokyo.

We have tried to complement the measurements of *G. kobayashii* in respect of other parts of the complex of anchors (length of the shaft, root and point) and the hook proper of marginal hooks on the basis of Hukuda's (1940) illustrations (Plate I, Figs. 3a, 3b and 6). However, we have found some discrepancies which throw doubts of the applicability of the original description. The total length of anchors given by Hukuda in the text does not correspond to the length in figures. Supposing that the length of anchors is really 0.085 mm, as mentioned by the author, then the width of the dorsal connecting bar in the figure should be about 0.022 mm (and not 0.015 mm) and the ventral connecting bar should measure about 0.010 × 0.032 mm (not 0.006 × 0.022 mm as given in the paper). It is therefore assumed that, like in case of *G. macracanthus* Hukuda, 1940 and *G. micracanthus* Hukuda, 1940 (Ergens, 1975), the author considered the total length of anchors not the distance between the tip of root and the outer side of the arch formed by the shaft and point, but the length obtained by the addition of the lengths of the point

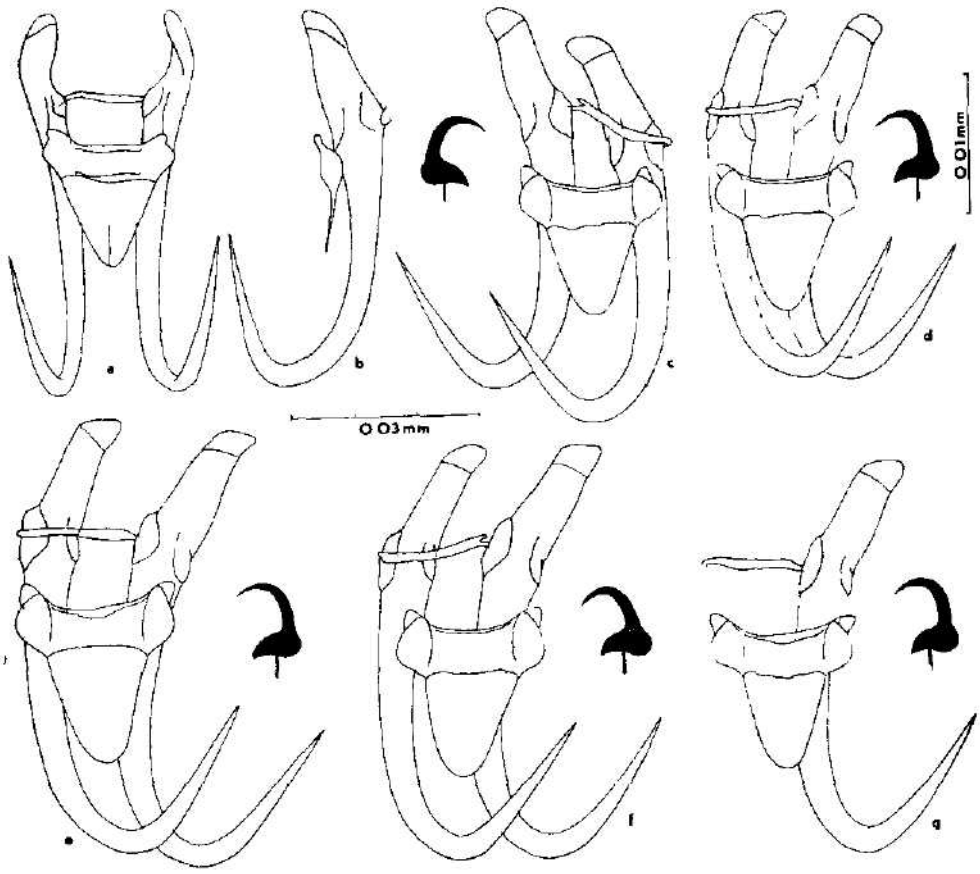

Fig. 1. *Gyrodactylus kobayashii* Hukuda, 1940 from skin and fins of *Carassius auratus* (L.), a, b — from Korea (after Hukuda, 1940); c — from Fisheries Experimental Station, Tokyo (December 22, 1975); d — from Sasaki Fish Farm, Tokyo (February 9, 1976); e, f — from Sasaki Fish Farm, Tokyo (December 15, 1975); g — from Fisheries Experimental Station, Tokyo (January 29, 1976)

and shaft with root. This presumption is confirmed by the comparison of the width of both connecting bars (which must have been measured without any error) with the total length of anchors (Hukuda, 1940, Fig. 3a). This includes almost three widths of the ventral connecting bar and approximately four widths of the dorsal connecting bar. That means that the total length of anchors in specimens measured by Hukuda was not 0.085 mm, but about 0.063 mm. Then the lengths of other parts must have been these: shaft 0.048 mm, point 0.028 mm, root 0.022 mm, dorsal connecting bar 0.001—0.002 mm and hook proper of marginal hooks 0.005—0.006 mm.

Using this amended and complemented original description and Hukuda's relatively exact illustration of the complex of anchors we managed to determine as *G. kobayashii* 36 specimens of *Gyrodactylus*

from fins and skin of *Carassius auratus* obtained from the Tokyo Fisheries Experimental Station and Sasaki Fish Farm, Tokyo. It is just this fact which enables us to submit the following redescription of *G. kobayashii*.

Gyrodactylus kobayashii Hukuda, 1940, Fig. 1

Host and location: *Carassius auratus* (L.), fins and skin.
Redescription: The totat length of relatively slender anchors varies within the range of 0.057—0.069 mm and their shaft (0.044—0.052 mm) with the root (0.017—0.023 mm) form a moderate, almost regular arch. The narrow and sharp point of anchors is 0.028—0.030 mm long. The ventral connecting bar with well developed lateral processes and with 0.014—0.016 mm long membranous extension measure 0.006—0.0007 × × 0.022—0.25 mm. The dorsal connecting bar is 0.001—0.002 mm long and 0.014—0.019 mm wide. The total length of marginal hooks is 0.025—0 028 mm, the hook proper with regularly arched point measures 0.005—0.006 mm.

DISCUSSION

Yamaguti (1940) reported *G. elegans* Nordmann, 1832 from Japan mentioning that "this species is common on the scales and fins of *Carassius carassius* and gold-fish kept in captivity". Besides a comprehensive description of the anatomy of these parasites the author gives also the measurements of the body and some hard parts of the opisthaptor: "Body 0.28—0.6 × 0.054—0.08 mm in whole mounts fixed in acetic sublimate; large central hooks 55—63 μ long from truncate anterior end to height of curve, 27—30 μ from tip to height of curve; ventral bar 20—22 μ long; dorsal bar 18 μ long; marginal hooklets in 8 pairs, 28 μ long".

There are obviously two errors in this description: the determinations both of the host (*C. carassius*) and the parasite. With regard to the fact that in the rivers emptying into the Pacific Ocean lives only one of the two species of the genus *Carassius* — *C. auratus* (Berg, 1949; Nikolski, 1971 and others) it may be supposed with certainty that the author obtained the parasites from this species and not from *C. carassius*. As to the determination of the parasites, it is sufficient to note that *G. elegans* occurs solely on gills of the members of the genus *Abramis* Cuvier.

The identification of Yamaguti's (1940) specimens is now difficult, since there are no exact drawings of their marginal hooks and anchors. We assume, however, that it is possible to agree with Yamaguti (1963) in considering *G. elegans* sensu Yamaguti, 1940, erroneously established by Yin and Sproston (1948) as a new subspecies of *G. elegans* — *G. elegans yamagutii*, to be conspecific with *G. kobayashii*. This is confirmed by the measurements of the hard parts of the opisthaptor which are identical in both species.

Yin and Sproston (1948) described *G. elegans sinicus* from the gills of the China goldfish, giving the following measurements: body length and width (0.22—0.55 × 0.058—0.087 mm), pharynx (0.025—0.027 mm), cirrus pouch (0.016—0.018 mm), total length of anchors (0.054—0.063 mm) and length of their point (0.020—0.032 mm), width of ventral connecting bar (0.019—0.021 mm), total length of marginal hooks (0.021—0.023 mm) and length of hook proper (0.0047—0 0054 mm). The

authors also mentioned: "This distinguishing feature of *G. elegans sinicus* subsp. n. particularly in relation to its closest relative, *G. e. yamagutii* n. n., are the shorter anchors with shorter point, and shorter characsteristically shaped ventral bar; while it differs from all other forms in the short marginal hooks and their sickle ends being only 4.7 to 5.4 μ. The pharynx is significantly larger than Yamaguti's form. yet it is smaller than any of the other forms described. The separation of the Chinese from the Japanese subspecies on goldfish is only provisional, for more detailed information on the latter may establish their identity; the relationship between the other subspecies is not nearly so close, and they are erected with fair confidence".

Similarly as Yamaguti (1940), also Yin and Sproston erroneously identified the host as *C. carassius* instead of *C. auratus* and the parasites regarded as *G. elegans*, though an independent subspecies. This error has also been mentioned by Malmberg (1970).

In spite of this fact, the comparison of the metrical values in Table I. columns 5* and 6 and in Figs. 2 and 3 (Yin and Sproston, 1940, pp. 60 and 62) gives evidence that *G. elegans sinicus* is almost identical with *G. elegans yamagutii* (= *G. kobayashii*). They differ only in the total length of marginal hooks (0.0215—0.0230 mm in *G. e. sinicus* and 0.025—0.028 mm in *G. kobayashii*) and in the location (*G. e. sinicus* on gills and *G. kobayashii* on fins adn skin). The conspecificity of these parasites, however, could be ascertained only on the basis of a further research, in case that *G. kobayashii* is found also on the gills of the goldfish and the total metrical variability of its marginal hooks is assessed.

Acknowledgement

We should like to express our gratitude to Dr. S. Kamegai, Director of the Meguro Parasitological Museum, Tokyo, Japan for his valuable information on the type material of *G. kobayashii*.

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Authors' address: Dr. R. Ergens, CSc., Parasitologický ústav ČSAV, Flemingovo nám. 2, 160 00 Praha 6, Dejvice, ČSSR.

Dr. K. Ogawa, Laboratory of Aquaculture Biology, Faculty of Agriculture, University of Tokyo, Tokyo, Japan.

* The total lenght of anchors is here erroneously 0.063 mm, but should be 0.063 mm (see Yamaguti, 1940).

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Institute of Parasitology, Czechoslovak Academy of Sciences, Praha
Instituto de Zoologia, Academia de Sciencias de Cuba, La Habana

OCCURRENCE OF INFIDUM SIMILIS (TREMATODA, DICROCOELIIDAE) IN CUBAN SNAKES

JINDŘICH GROSCHAFT & ALBERTO COY OTERO

Received October 14, 1976

Abstract: The tramtode *Infidum similis* Travassos, 1916 from three snake species is described and illustrated. The validity of some taxonomic characters used for differentiation of the known species of the genus *Infidum* is discussed.

During examination of reptiles in Cuba, trematodes of the genus *Infidum* Travassos, 1916 were recovered from the gall-bladder of three snake species captured in the vicinity of Havana. The description including metrical data is based on five specimens found in *Alsophis cantherigerus*. Figures of three trematodes originating from different hosts are attached. The trematodes from our collection conform in major taxonomic characters to the description of *I. similis* Travassos, 1916 published in the monograph by Travassos (1944). According to available literary sources, this is the first report of *I. similis* from Cuba, and the three snake species are new host records.

Infidum similis Travassos, 1916

Hosts. *Alsophis cantherigerus cantherigerus* (Bibron), *Antillophis* (= *Dromicus*) *andreae andreae* (Reinhardt et Lutken), *Tropidophis melanurus melanurus* (Schlegel).
Location: gall-bladder.
Locality: Havana (Cuba).

Description (all measurements in mm): Body length 3.63—4.30, width 1.93—2.31. Cuticle smooth, without spines. Oral sucker 0.364—0.392 × × 0.378—0.420. Acetabulum 0.467—0.560 × 0.490—0.630, situated 1.26 to 1.40 from anterior end of body. Pharynx 0.140—0.154 × 0.140—0.182. Oesophagus 0.280—0.322 long. Genital pore situated in region of intestinal bifurcation, somewhat on side of median line of body. Cirrus sac 0.700 to 0.770 × 0.088—0.126. Testes 0.364—0.574 × 0.350—0.504 and 0.476—0.518 × × 0.392—0.490. Ovary 0.196—0.294 × 0.280—0.364. Vitellaria of various extent; if there are few follicles, they are situated mostly in front of equatorial axis, if there are numerous fillicles, they are arranged symmetrically on both sides of equatorial axis of body.

Five trematode species have been included in the genus *Infidum* Travassos, 1916; four of them parasitize snakes of the Neotropical region: *I. infidum* (Faria, 1910) reported from Brazil and Bolivia; *I. intermedium* Ruiz et Leao, 1943 and *I. similis* Travassos, 1916 reported from Brazil; *I. luckeri* McIntosh,

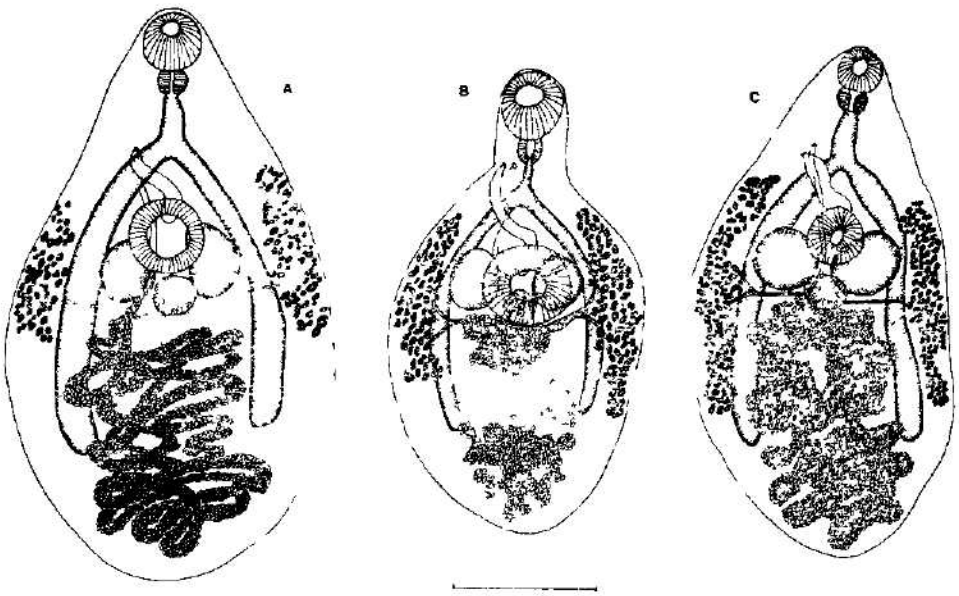

Fig 1 *Infidum similis* Travassos, 1916. A — specimen from *Alsophis c. cantherigerus;* B — specimen from *Tropidophis m melanurus*, C — specimen from *Antillophis a. andreae*. Drawings were made with the aid of camera lucida after whole mounts stained with borax-carmine Scale = 1 mm.

1939 reported from the Galapagos Islands. The fifth species, *I. nigerianum* Babero et Okpala, 1962 was found in *Agama colonarum* in Nigeria.

The key for the determination of neotropical species of the genus *Infidum* (in Skrjabin, 1952) is based only on the extent of vitellaria in relation to equatorial axis of body or length of intestinal branches The seminal receptacle is stated to be present in *I. luckeri*, but lacking in other members of this genus. It is very difficult to confirm its presence, because the area in which it is usually located is mostly covered with uterus filled with eggs. Travassos (1944) did not express his opinion on this question, he only adopted both the description and figure, but he considered *I. intermedium* Ruiz et Leao, 1943 to be identical with *I. similis* Travassos, 1916. He noted only the difference in the size of eggs.

We assume that the extent of vitellaria used as taxonomic character in the key (in Skrjabin, 1952) is not sufficient to separate individual species of the genus *Infidum*. It is valid only for *I. infidum* (Faria, 1910), in which the vitelline glands are extended along the whole length of intestinal branches. In other species, in which the vitellaria are situated practically only in the middle part of body in the region of acetabulum, their extent is variable. In the specimens from our collection, the vitellaria are situated either in front of or behind the equatorial axis or they are arranged symmetrically on its sides. As to other taxonomic characters, we have compared the sucker ratio and egg size of *I. similis* and *I. intermedium*. The sucker ratio in *I. similis* is 1 : 1.01—1.18 (after Travassos, 1944), in *I. intermedium* 1 : 1.02—1.45, i.e. 1 : 1.29 on the average (calculated by us from given

limit values) and in specimens from our collection 1 : 1.26—1.40. According to Travassos (1944) the eggs of *I. similis* measure 0.030—0.035 × 0.015 to 0.017 and those of *I. intermedium* 0.022—0.025 × 0.011—0.014. In our specimens the eggs measure 0.027—0.033 ×0.009—0.015. These results show that the measurements of specimens from our collection represent mean values between *I. similis* sensu Travassos (1916) and *I. intermedium* Ruiz et Leao, 1943. We assume that the extent of vitellaria in both species and the metrical differences are within the limits of both morphological and metrical variabilities. We therefore assigned our specimens to the species *Infidum similis* Travassos, 1916.

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Author's address RNDr. J. Groschaft, CSc , Parasitologický ústav ČSAV, Flemingovo nám. 2, 166 32 Praha 6, Czechoslovakia.

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Institute of Parasitology, Czechoslovak Academy of Sciences, Praha Department of Zoology, University of Agriculture, Brno

CHARAXICEPHALOIDES POLYORCHIS GEN. NOV., SP. NOV. (TREMATODA: CHARAXICEPHALINAE) FROM CHELONIA MYDAS MYDAS (TESTUDINATA) IN CUBA

JINDŘICH GROSCHAFT and FRANTIŠEK TENORA

Received January 12, 1977

Abstract: *Charaxicephaloides polyorchis* gen. nov, sp. nov. from turtles *Chelonia mydas mydas* (L.) from Cuba is described. Characteristics and differential diagnosis of *Charaxicephaloides* gen. nov are given. A key to trematodes of the subfamily Charaxicephalinae Price, 1931 (Pronocephalata Skrjabin, 1955) is attached.

During the studies of the material obtained at dissection of turtles caught on the west coast of Cuba, trematodes resembling the known species of the genus *Charaxicephalus* were recorved from *C. m. mydas*. A charasteristic feature of all members of the subfamily Charaxicephalinae is the large number of testes. The first representative of this subfamily, *Charaxicephalus robustus* Loos, 1901 was found in the same host near the Egyptian coast of the Mediterranean Sea. It was also the first species of the family Pronocephalidae differing from other members of this family in testes distributed in numerous oval formations arranged in two lateral rows. Stephens (1911) described another species with similar arrangement of testes which he placed in an independent genus *Desmogonius* with nominal species *D. desmogonius* Stephens, 1911. Johnston (1913 — after Skrjabin, 1955) recovered from *Chelone imbricata* (= *Erotmocheylus imbricata*) and *C. mydas* on the coast of Queensland (Australia) trematodes which he considered identical with *Monostomum pandum* Braun, 1901. He erected for them a new genus *Diaschistorchis* Johnston, 1913. Later also the genera *Wilderia* Pratt, 1914 and *Synorchis* Baker, 1922 were included in this genus as its synonyms (Mehra, 1932).

Members of the genus *Diaschistorchis* have also a larger number of testes, but these are situated in the posterior part of body under vitellaria and their lateral rows often unite in the posterior part.

According to Skrjabin (1955), the subfamily Charaxicephalinae includes three genera, namely *Charaxicephalus* with two species (*C. robustus* Looss, 1901 and *C. loossi* Mehra, 1939), *Desmogonius* with two species (*D. desmogonius* Stephens, 1911 and *D. loossi* Chattopadhyaya, 1972) and *Diaschistorchis* with seven species [*D. pandus* (Braun, 1901), *D. ellipticus* (Pratt, 1914), *D. gastricus* Mehra, 1932, *D. lateralis* Oguro, 1936, *D. takahashii* Fukui et Ogata, 1936, *D. multitesticularis* Rodhe, 1962 and *D. profullai* Chattopadhyaya, 1972].

An analysis of morphological characters of our specimens revealed that they differed substantially from all known members of Charaxicephalinae. We have therefore established a new genus of this subfamily, *Charaxicephaloides* gen. nov. including *Charaxicephaloides polyorchis* sp. nov.

We place in the subfamily Charaxicephalinae Price, 1931 also the genara *Charaxicephalus* Loos, 1901 and *Desmogonius* Stephens, 1911 and leave the genus *Diaschistorchis* Johnston, 1913 in the subfamily Diaschistorchinae Yamaguti, 1958. This problem will be discussed in a separate paper dealing with a detailed analysis of the system of Pronocephalidae.

MATERIAL AND METHODS

Four trematodes were recovered from one *Chelonia mydas mydas* (L.) caught on the north-west coast of Cuba. The material was kindly supplied by Dr. Coy of the Cuban Academy of Sciences, to whom our thanks are due. The whole mounts were stained with borax-carmine.

All measurements in the description of *Charaxicephaloides polyorchis* gen. nov. sp. nov. are given in millimetres. The drawings were made with the aid of camera lucida. The present paper offers further results obtained during the helminthological investigations carried out in cooperation by the Cuban Academy of Sciences and Czechoslovak Academy of Sciences.

Charaxicephaloides gen. nov.

Generic diagnosis: Charaxicephalinae: Body wide, with margins elevated to ventral side. Head collar developed. Two conical processes situated in the posterior part of body. Oral sucker well developed and subterminal. Oesophagus relatively short. Intestinal branches long, passing into conical processes with anal openings. Numerous diverticles present on both sides of intestinal branches along their whole length. Testes distributed into numerous spherical follicles arranged in two alternating lateral rows. Individual follicles not separated by uterine loops. Cirrus pouch in anterior part of body. Genital pore aside from longitudinal axis of body. Ovary on the opposite of body, in its posterior part. Vitellaria in posterior part of body, in a line with testes. Uterus intercaecal. Eggs with bundles of filaments at both poles. Parasite of turtles.

Type species: *Charaxicephaloides polyorchis* sp. nov.

Differential diagnosis: *Charaxicephaloides* gen. nov. differs from other known genera of the subfamily Charaxicephalinae primarily in the arrangement and number of male genital organs. It differs from *Charaxicephalus* Looss, 1901 in double rows of testicular follicles situated caecally. Individual follicles are not separated by uterine loops. The number of testicular follicles is up to three times as high. The vitellaria reach only to the lower margin of testes and lie in a line with them. *Charaxicephaloides* differs from *Desmogonius* Stephens, 1911 also in the number and arrangement of testicular follicles, in the presence of head collar and situation of genital openings which are nearer to the equatorial level of body in *Desmogonius*.

Charaxicephaloides polyorchis sp. n. (Fig. 1)

Host: *Chelonia mydas mydas* (L.)
Location: stomach (?)
Locality: Gulf of Guanahacabibes (Cuba)

Description of holotype (measurements of paratype in parentheses): Trematodes with developed head part provided with muscular head collar cut out on ventral side. Dorsal side of body convex, ventral side concave, with elevated margins. Body cymbiform. Cuticle smooth, without spines. Body length 5.87 (4.05—4.12), width 1.84 (1.41—1.51). Two lateral processes on posterior end of body. Oral sucker subterminal, 0.733 × 0.717 (0.436—0.717 × 0.499—0.748). Oesophagus reaching under lower margin of head collar and measuring 0.78 in length. Intestinal branches reaching up to terminal body processes where they open by anal

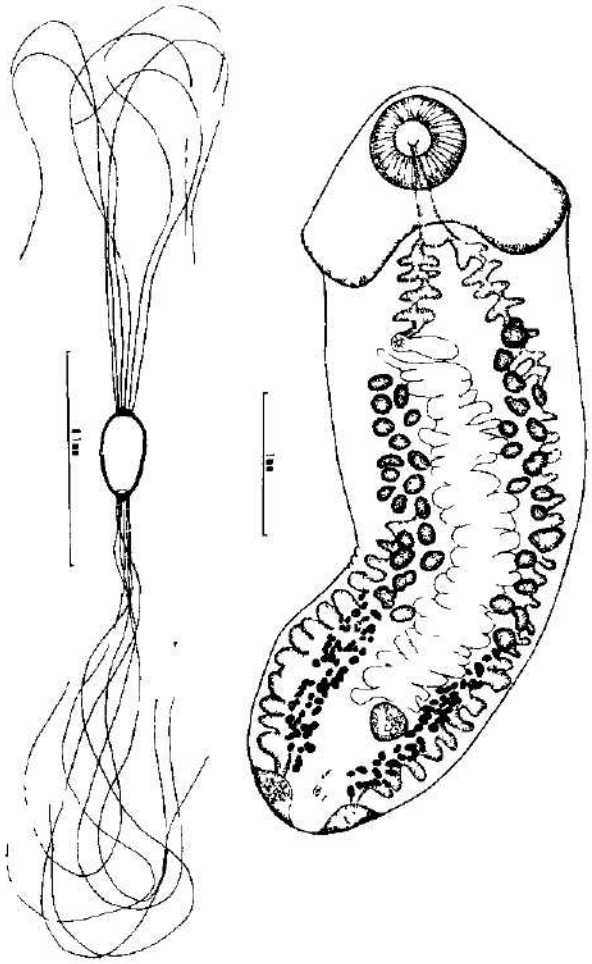

Fig. 1. *Charaxicephaloides polyorchis* gen. nov., sp. nov. — general view and egg.

pores. Both branches provided with numerous lateral diverticles on both sides along their whole length. Cirrus sac crossing longitudinal axis of body between first and second fourth of body. Opening of male and female genitalia aside longitudinal axis of body, but still in intercaecal

region. Cirrus pouch 0.46 × 0.18 (0.78 × 0.17). Testes distributed in numerous follicles arranged in two lateral double rows situated from upper margin of vitellaria to level of cirrus pouch. Individual follicles irregularly oval, measuring 0.15—0.21 in diameter, numbering 20—25 (18—25 on each side) and not separated by uterine loops. Number of testes on side of ovary always lower than on opposite side. Vitellaria consisting of minute follicles and situated between lower margin of testes and body end, lying in a line with testes. Ovary with smooth outline, irregularly oval. situated in lower part of body, somewhat aside longitudinal axis of body and measuring 0.234 × 0.343 (0.187 × 0.265). Mehlis' gland median subovarial. Uterus filled with eggs, situated in intercaecal region and opening above upper margin of testes, rather aside longitudinal axis of body. Eggs (0.034—0.038 × 0.015—0.018) with bundle of filaments on both poles; length of filaments 0.35, i. e., ten times more than length of eggs.

The holotype is deposited in the collection of the Institute of Parasitology, Czechoslovak Academy of Sciences in Prague (Coll. No. 581/1).

Key to the genera of the subfamily Charaxicephalinae Price, 1931 (Pronocephalata Skrjabin, 1955)

1. Head collar absent . . . . . *Desmogonius* Stephens, 1911
— head collar present 2
2. Testes arranged in simple rows, intercaecal, individual testes separated by uterine loops . . . . *Charaxicephalus* Looss, 1901
— testes arranged in irregular double rows, individual testes not separated by uterine loops . . . *Charaxicephaloides* gen. nov.

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Authors' addresses: Dr. J. Groschaft, CSc., Institute of Parasitology, Czechoslovak Academy of Sciences, Flemingovo nám. 2, Praha. 6. Doc. Dr. Tenora, CSc., Deparment of Zoology, University of Agricultur, Zemědělská 1, Brno.

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Department of Insect Pathology, Institute of Entomology, Czechoslovak Academy of Sciences, Praha

PLEISTOPHORA GROSSA SP. N. (PLEISTOPHORIDAE, MICROSPORIDIA) PARASITE OF CHRYSOMELID BEETLES IN YOUGOSLAVIA

ZDENĚK HOSTOUNSKÝ and JAROSLAV WEISER

Received January 17, 1977

Abstract: A new microsporidian from the midgut of *Chrysomella grossa* in Yougoslavia is described. No other tissues are infected. The microsporidian infects larvae of the Colorado potato beetle causing a chronical infection.

During a search for Microsporidia attacking different Chrysomelid beetles and possible infectious agents for the Colorado potato beetle series of different infections were collected in Czechoslovakia and these will be studied further. Most of them belong to the genus *Nosema* and they attack the gut wall, Malpighian tubules or the fat body of the hosts. In a group of adult beetles of *Chrysomella grossa* collected by jun. author in early autumn on plants at the road side in Radoviči, Boka Kotorska, Yougoslavia, a microsporidian infection was found which was included in the series of our investigations. The results are given in this paper.

MATERIAL AND METHODS

The original material included 11 adults and 10 larvae of *Chrysomella grossa* Fabr., collected on plants at the road side in Radoviči on the adriatic cost of the Boka Kotorska, Yougoslavia on Oct. 7, 1974. They were kept in the laboratory on *Nepeta mussini* till pupation. An egg batch was gained and 13 larvae were reared till Dec 17 when the rearings were finished due to lack of adequate food.

After the discovery of microsporidian spores in one dead adult, all subsequent dead animals were inspected in water-mounts Feces of living adults and larvae were inspected, collected in vials where they were kept for 4 hours. In dissections of living individuals the elytra were removed and the dorsal part cut open by scissors. The gut with Malpighian tubules, muscles and the fat body were ispected under cover slip in low magnification. Where spores were seen, the material was squashed in water and the suspension was smeared on the surface of a leaf which was fed to newly hatched first larvae. Feces of the infected larvae and all dead animals were stored in water, suspended and separated from remains of tissue by subsequent centrifugation at 100 g and 1300 g. Spores were washed several times and a clean suspension was stored over winter at 4 °C. The development of the infection in newly fed larvae was checqued 5 to 8 days after administration. The content of spores in feces was evaluated and larvae were dissected.

We used the suspension of spores after 5 months, in April 1975 for the infection of first larvae of *Leptinotarsa decemlineata*. The number of spores in feces of the infected animals indicated the progress of the disease. Infected larvae were fixed with Bouin's liquid, enbedded in paraffin and cut in sections 4 μm thick. Sections were stained with Heidenhain's iron hematoxylin. Dry smears were stained with Giemsa and the nuclei in spores with Giemsa after HCl hydrolysis (Weiser, 1976).

INFECTION IN THE ORIGIAL HOST

Only 3 of the 21 insects were infected with a microsporidian. In three other animals a larval *Tachina* caused the death of the larvae. The microsporidian infected the midgut, mainly its posterior half. Infected cells of the gut epithelial wall were slightly extended and protruding. In their central part are round centres with spores and developmental stages of the microsporidian. Sporoblasts and spores are deposed in the cytoplasm of the cells. During the maturation of the stages the group proceeds to the basal part of the cell under the membrane covering the epithelial layer from the gut. This membrane breaks soon and the spores leave single or in multisporous pansporoblasts the infected cell. During the development of the infection, the multinuclear plasmodia are rare, they break in single sporoblasts and these maturate in oval spores. Sporoblasts are uninuclear and so are spores when stained on smears after hydrolysis with HCl. The nucleus is spherical, located in the second third of the spore length. Spore size is 2.5 ± 0.5 μm by 1.7 ± 0.5 μm. The spore size and shape differ from known microsporidia in chrysomelid betles (see review in Hostounský and Weiser, 1973). We propose to name it *Pleistophora grossa* sp. n.

Spore material was used for the infection of *C. grossa* larvae. The rearing was difficult due to lack of suitable food during the winter. Eight of the 13 infected first larvae died 10 days after infection after minimum weight gain. They were fixed to the leaves with a sticky exudate from the anal opening. Feces contained microsporidian spores. Remaining 5 larvae were dissected after 5 weeks when they arrived to prepupae. They were not able to pupate. The spores of *P. grossa* were only in the gut wall, in groups of no more than 30 spores, in some areas the groups were confluent in common masses in adjacent cells. Malpighian tubules were infected, some parts were filled with spores, their cells hypertrophic and containing many spores. There was no infection in the fat body or in the muscles.

INFECTION IN THE COLORADO BEETLE

The suspension of spores was offered in a platinum loop to first larvae of *Leptinotarsa decemlineata* as soon as they fed on remains of egg shells. In their feces the first spores appeared after 7 days and the number of the spores there grew and they appeared in large spherical masses of 30 and more. At that time the larvae dispersed over the plant and they consummed more food. The usual moist feces changed into watery, black excretions which spread over the surface of the leaves. Black colour was the result of the blackening of the haemolymph which is a part of the feces. It enters the gut through ulcerations of the destroyed gut wall. The black spots contained many spores. During 14 days the larvae ceased feeding, the last fecal pellets were without spores. Dissections revealed only scarce spore groups in the gut. Nevertheless, the larvae died. The spore production in larval *Leptinotarsa* ranged from 17 to 40 million spores in total collected feces/animal.

The infection was localized in the posterior part of the midgut in the epithelial cells. Infected cells had vegetative stages of the microsporidian in their basal part. They grew in series of cells in the direction to the gut

lumen and there they produced sporonts which grew into spherical plasmodia with an irregular number of nuclei. They maturated in pansporoblasts with 10 to 30 spores, in many cases the pansporoblasts had their walls autolysed during the spore maturation and spores were free in the infected cells. The infected cells concentrated spores in the protruding inflated cell ends and these ruptured and released the spores in the gut. Some cells recovered, their nuclei were intact and the whole process represents a type of merocrine secretion. The most damaged parts were in the region of the constriction of the gut. In other parts the infected cells were scattered into the epithel. Bacteria invaded some ruptured cells before they closed their wall. In the cases studied, this was the reason of the mortality without a large mass of spores in the gut. The diffuse infection in host cells showed no tendency to form confluent focuses and there were no spores in Malphighian or silk glands in the histological material.

DISCUSSION

Pleistophora grossa sp. n. produces rather untypical pansporoblasts, where the spores are free, without pansporoblastic membrane and only uninuclear spores differenciate them from spores of the genus *Nosema*. This phenomen is common among the species of *Pleistophora* in the intestine, such as *Pleistophora schubergi* or *P. fidelis*. (Hostounský and Weiser, 1975). The proposed new species differs from *P. fidelis* especially by spore dimensions and spore shape. They are broad oval to almost spherical (Fig. 4). They differ also in their infectivity fort the Colorado beetle and in their original host. The tissues of the Colorado beetle seems not to be an optimal environment for their development and they are eliminated during late molts and before pupation. Pathogenity is very low, the disease is long lasting, the effect is more or less a stress which may be combined with other factors. On the other side, infected feces bring the spores into contact with other host larvae during the subsequent heavier defoliation of the nutrient planst. The production of spores in the original as well as in the secondary host is relatively low and does not offer enough material for field application.

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

Authors' address: Ing. Z. Hostounský, CSc., RNDr. J. Weiser, Dr. Sc., Entomologický ústav ČSAV, Flemingovo nám. 2, 160 00 Praha 6, Dejvice, Czechoslovakia.

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Czechoslovak Academy of Sciences, Hydrobiological Laboratory of the Botanical Institute

THE GROWTH RATE OF DAPHNIA PULEX AND DAPHNIA PULICARIA (CRUSTACEA: CLADOCERA) AT DIFFERENT FOOD LEVELS

MARTA HRBÁČKOVÁ and JAROSLAV HRBÁČEK

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Abstract: The species *Daphnia pulex* L. and *Daphnia pulicaria* Forbes were reared in various concentrations of natural food obtained by centrifugation. As a first approximation Monod's equation was used for the description of the relation between the length of the postembryonic development and the concentration of food. The species from more eutrophic conditions *(Daphnia pulex)* have only somewhat higher maximal rate of the development but considerably higher value of the half velocity coefficient.

INTRODUCTION

The length of the postembryonic development is one of the factors that effects the population dynamics of Cladocera; it is influenced by the amount and quality of food. The influence of the quality of the food on the growth of Cladocera interested many authors, for instance Kerb (1910), Grogh (1930), Woltereck (1928), Ryther (1954) and others. Ingle, Wood and Banta (1937) and later on Green (1935) found that the number of instars and the length of development increased if food declined. Hrbáčková (1963) recorded that the food concentration in Slapy reservoir for *Daphnia* were markedly below the optimum of food found during the cultivation of Cladocera under Laboratory conditions (Hrbáčková, 1962). The aim of the present study is to gain more comprehensive data on the relationship between the length of development and the amount of food in the species *Daphnia pulex* and *Dophnia pulicaria*. According to earlier investigations (Hrbáčková, 1963) these species had different development (rate) under comparable food condition. Blažka (1966) found in Slapy and some others reservoirs that the ratio of protein to the sum of carbohydrates and lipids in the seston is relatively constant. In the present study, therefore the amount of Kjeldahl N in the seston obtained by centrifugation was used as the measure of food.

MATERIAL AND METHOD

The species *Daphnia pulicaria* Forbes, sampled from the pond near Čim and *Daphnia pulex* L. from the village pond in Křeničná (both localities are in the region of Slapy reservoir) were used. The two species were reared for three weeks under laboratory conditions before the experiments are initiated. In one series of them the speciments of *Daphnia pulicaria* were cultivated in 100-ml flasks, which were fastened on a rotating drum (rev. rate 1× per min.) and placed in a water bath heated up to 20 °C (stirred medium). In other series, the specimens were reared in 100-ml flasks with unstirred medium placed in a box at 20 °C under daylight

conditions. In xperiments in which both species were investigated one specimen of both were cultivated in the same flask. The experiments were made during July and August 1972, 1974 and 1975. The cultivation medium and the food obtained from Slapy reservoir were changed daily. Water samples were obtained by pumping from a depth of 0.4 m, filtered through silk bolting cloth No. 8. and cenrtrifugated by a continuous centrifuge (20 000 g). The centrifugate was diluted by the centrifuged water in a ratio of 0.5, 1, 2, 4, 8, and 16 times the concentration of algae and the other particles in the water sampled from the reservoir. From a parallel sample organic N was determined by the Kejdahl method. The values of organic N according to Blažka (1966) were converted to calories and further on to Joules. In all the experiments, we followed the length of the postembryonic development. Under the length of the postembryonic development we understand the time-period that has elapsed from the release of the young from the brood chamber of the mother until the appearence of the first eggs in their own brood chambers. Because the normal distribution could not be considered as guaranted the modus was used as an average figure. It was calculated from the following formula:

$$D = x_1 + \frac{n_0 - n_{-1}}{2n_0 - n_{-1} - n_{+1}} d$$

where:
D = Modus
x_1 = *the value of the lower range of the most frequent class.*
n_0 = the number of the individuals in the most frequent class.
n_{-1} and n_{+1} = the number of the individuals in the both neighbouring classis
d = the width of the class.
The obtained values of modus were plotted in Tables and Figures. The average concentrations in the Tables do not correspond precisely to the multiples of the concentrations because of the day to day variation of the food concentration values varied. As the difference is not too great, it is evident that the use of the concentration average for the studied period is adequate.

Before the experiments were started, the females were observated frequently and care taken that the young used in experiments are only several hours old. Specimens, before reaching the adult stage, were observed twice a day.

For the evaluation of the dependence of the length of the postembryonic development of the food concentration the mathematical apparatus developed for the study of the dependence of the growht of bacteria on the concentrion of the substrat was used (Williamson and McCarty, 1975) The parameter of the Monod equation (maximal growth rate and concentration of the food at the half of food concentration) were calculated from the expected linear relation between inverse value of the food concentration and the length of the postembryonic development

The Kjeldahl nitrogen of the samples from individual days has been estimated after the end of the experiments. The highest concentration used in these experiments was not adequate for the maximal development rate of *Daphnia pulex* observed in the earlier experiments. Therefore we overcalculated the data from the experiments in the year 1961 in which the calorific content of the added algae (1 million of cells to 1 ml of water) was estimated by the bichromate method. (Hrbáčková, 1974).

RESULTS

A. The cultivation in the flasks with stirred medium

Experiment in 1972.
The concentration of seston was equivalent to about 10 J/1.

Daphnia pulicaria Forbes

From 22 neonates cultivated at the food level 12 J/1 4% finished the postembryonic development on the 8th day, 29% on the 9th day, 48% on the 10 day and 19% on the 11 day. M = 9.6. The average egg number of the primiparae was 2.5 per female.

From 22 neonates reared at the food level of 98 J/1 25% maturated on the 5th day, 15% on the 6th day, 50% on the 7th day and 10% on the 8th day. M = 5.9. The average egg number of the primiparae was 5 per female.
The data are summarised in Table 2. and in Fig. 2.

B. Cultivation in the flasks with unstirred medium

I. Experiment in 1974

The concentration of seston was equivalent to about 9 J/1.

Daphnia pulicaria Forbes.

None of the neonates cultivated at the food level of 6 J 1 completed the postembryonic development within a fortnight.

From 20 neonates kept at the food level of 12 J 1 50% reached the maturity on the 9th day, 30% on the 12th day and 20% on the 13th day. M = 9.8. The average egg number of the primiparae was 2.5 per female.

From 20 neonates cultivated at the food level of 25 J/1 60% finished the postembryonic development on the 7th day. 10% on the 8th day, 10% on the 9th day and 20% on the 10th day. M = 7.7. The average egg number of the primiparae was 2 per female.

From 20 neonates reared at the food level of 40 J/1 60% of individuals reached the matutiry on the 6th day, 10% on the 7th day, 10% on the 8th day and 20% on the 9th day. M = 6.7. The average egg number of the primiparae was 2.3 per female.

20 neonates cultivated at the food level of 90 J/1 reached the stage of primipara on the 6th day.
The data are summarised in Table 2. and in Figs. 1. and 2.

I. Experiments in 1975

Experiment initiated on 7th July.

The concentration of seston was equivalent to about 17 J/1.

In all the concentrations all individuals of the species *Daphnia pulex* died within 3 to 4 days. Only 3 individuals out of 20 of the species *Daphnia pulicaria* completed the postembryonic development in a eight fold concentration within 16 to 18 days. Both species were found in a very bad condition having broken antennae and spines and grown over with mould. The same was observed during approximately the same period in other species of *Daphnia* in 1973.

Experiment initiated on 17th July.

The concentration of seston was equivalent to about 13 J/1.

Daphnia pulex L.

None of 15 neonates cultivated at the food level of 13 J 1 and of 15 neonates at the food level of 24 J/1 completed the postembryonic development. They died within 3–7 days.

From 15 neonates kept at the food level of 53 J 1 20% finished the postembryonic development on the 9th day, 33.3% on the 10th day and

Table 1. The constituens of the biomas of the fytoplancton in the Slapy reservoir in μg/l fresh weight in 1975

	18.7.	22 7.	25.7.	29.7.	2.8.	12.8.	15.8.	18.8.	21.8.
Blue greens: *Anabaena circinalis Rabenh. Aphanizomenon flos aquae* Ralfs	100	290	1390	1550	1280	1160	275	1920	380
Diatomes: *Melosira italica* (Ehrenb.) Kutz *Fragilaria crotonensis* Kitton	300	170	159	250	850	4190	3730	7600	1165
Flagalates: *Cryptomonas curvat* Ehrenb. *Cryptomonas reflexa* Skuja	650	470	1480	740	1590	1570	1200	1660	280
Green algae: *Oocystis sp. Coelastrum* Chlorococcales	190	800	66	90	55	23	55	80	38

The table is based on the unpublished results of Dr.B. Desortová.

46.7% on the 11th day. M = 10.4. The average egg number of the primiparae was 1 per female.

From 15 neonates reared at the food level of 101 J 1 33.3% maturated on the 9th day, 53.4% on the 10th day and 13.3% on the 11th day. M = 9.2. The average egg number of the primiparae was 2 per female.

From 15 neonates cultivated at the food level of 183 J/1 40% maturated on the 7th day, 46% on the 8th day, 6.6% on the 9th day and 6.5% on the 10th day. M = 7.6. The average egg number was 2.3 per primiparae.

Daphnia pulicaria Forbes

From 15 neonates cultivated at the food level of 13 J/1 40% finished the postembryonic development on the 9th day, 33.3% on the 10th day, 6.7% on the 11th day and 20% on the 12th day. M = 10. The average egg number of primiparae was 2.2 per female.

From 15 neonates reared at the food level of 28 J/1 20% maturated on the 7th day, 20% on the 8th day, 46.7% on the 9th day and 13.3% on the 10th day. M = 8.5. The average egg number of primiparae was 2.2 per female.

From 15 neonates kept at the food level of 53 J/1 13.3% of individuals completed the postembryonic development on the 6th day, 26.7% on the 7th day, 53.3% on the 8th day and 6.7% on the 9th day. M = 7.5. The average egg number of the primiparae was 2.5 per female.

15 neonates cultivated at the food level of 101 J/1 reached maturity on the 6th day.

Table 2. The dependence of the length of the postembryonic development and number of eggs of the food level in *Daphnia pulicaria* and *Daphnia pulex*

Species	Start	Food concentration in J/l	Length of the postembr. development Modus	Range	The average egg number per female
D. pulicaria	11.8.	12	9.6	8—11	2.5
	1972	98	5.9	5—8	5.0
D. pulicaria	8.8.	6	postembr. d. was not finished		
	1974	12	9.8	9—13	2.5
		25	7.7	7—10	2.0
		46	6.7	6—9	2.3
		90	6.0	—	4.0
D. pulex	17.7.	12	all indiv. died within 3—7 days		
	1975	24	all indiv. died within 7 days		
		53	10.4	9—11	1.0
		001	9.2	8—10	2.1
		183	7.6	7—9	2.3
D. pulicaria	17.7.	13	10.0	9—12	2.0
	1975	28	8.5	7—10	2.3
		53	7.5	6—9	2.5
		101	6.0	—	2.3
D. pulex	26.7.	10	all indiv died within 2—10 days		
	1975	20	all indiv. died within 10 days		
		51	9.7	8—11	2.3
		98	7.9	6—9	2.0
		200	6.5	5—8	2.4
D. pulicaria	26.7.	10	10.9	9—12	2.0
	1975	20	9.2	7—10	2.3
		52	7.4	6—8	2.2
		106	6.0	—	2.7
D. pulex	11.8.	13	all indiv. died within 4—9 days		
	1975	34	all indiv. died within 9 days		
		74	9.3	8—10	2.3
		140	6.5	5—8	2.5
		298	6.0	5—7	3.0
D. pulicaria	11.8.	17	9.2	9—11	3.0
	1975	34	8.2	7—10	2.5
		69	7.1	7—9	2.1
		140	5.9	5—6	1.3
		289	5.5	5—6	2.8
D. pulex	July 1961	500	4.7	4.5—5	6.8
D. pulicaria	July	500	5.0	4.7—5.3	9.4

The data are summarised in Table 2. and in Figs. 1. and 2.
Experiment initiated on 26th July.
The concentration of seston was equivalent to about 14 J/l.

Daphnia pulex L.

15 neonates cultivated at the food level of 10 J/1 and 15 neonates at the food level of 20 J/1 died within 2–10 days.

From 15 neonates reared at the food level of 51 J 1 13.3% maturated on the 8th day, 26.6% on the 9th day, 40% on the 10th day and 20% on the 11th day. M = 9.7. The average egg number of the primiparae was 2.3 per female.

From 15 neonates cultivated at the food level of 98 J/1 6.7% completed the postembryonic development on the 6th day, 20% on the 7th day, 60% on the 8th day and 13.3% on the 9th day. M = 7.9. The average egg number of primiparae was 2.1 per female.

From 15 neonates kept at the food level of 200 J/1 6.7% maturated on the 5th day, 40% on the 6th day, 46.6% on the 7th day and 6.7% on the 8th day. M = 6.5. The average egg number of primiparae was 2.4 per female.

Daphnia pulicaria Forbes

From 15 neonates cultivated at the food level of 10 J/1 6.7% reached maturity on the 9th day, 40% on the 10th day, 33.3% on the 11th day and 20% on the 12th day. M = 10.9. The average egg number of the primiparae was 2 per female.

From 15 neonates reared at the food level of 20 J 1 20% maturated on the 7th day, 6.7% on the 8th day, 33.3% on the 9th day and 40% on the 10th day. M = 9.2. The average egg number of the primiparae was 2.3 per female.

From 15 neonates kept at the food level of 52 J/1 13.3% finished the postembryonic development on the 6th day, 46.6% on the 7th day, 40% on the 8th day. M = 7.4. The average egg number of the primiparae was 2.2 per female.

15 investigated neonates cultivated at the food level of 106 J/1 reached the stage of the primipara on the 6th day. M = 6.0. The average egg number of primiparae was 2.7 per female. The data are sumarised in Table 2. and in Figs. 1. and 2.

Experiment iniated on the 11th July.

The concentration of seston was equivalent to about 17 J/1.

Daphnia pulex L.

None of 12 neonates cultivated at the food level of 13 J/1 and of 12 neonates cultivated at the food level of 34 J/1 finished the postembryonic development. All individuals died within 4–9 days.

From 12 neonates reared at the food level of 74 J/1 16.7% maturated on the 8th day, 50% on the 9th day and 33.3% on the 10th day. M = 9.3. The average egg number of the primiparae was 2.3 per female.

From 12 neonates kept at the food level of 140 J/1 16.7% finished the postembryonic development on the 5th day, 25% on the 6th day, 41.6% on the 7th day and 16.6% on the 8th day. M = 6.5. The average egg number of the primiparae was 2.5 per female.

From 12 neonates cultivated at the food level of 298 J/1 16.6% maturated on the 5th day, 58.3% on the 6th day and 25% on the 7th day. M = 6.0. The average egg number of the primiparae was 3.0 per female.

Daphnia pulicaria Forbes

From 12 neonates kept at the food level of 17 J/1 25% reached the stage of the primiparae on the 9th day, 58.3% on the 10th day and 16.7% on the 10th day. M = 9.2. The average egg number of primiparae was 3 per female.

From 12 neonates reared at the food level of 34 J/1 8.3% reached maturity on the 7th day, 25% on the 8th day, 41.7% on the 9th day and 16.7% on the 10th day. M = 8.2. The average egg number of the primiparae was 2.5 per female.

From 12 neonates cultivated at the food level of 69 J/1 50% completed the postembryonic development on the 7th day, 33.3% on the 8th day and 16.6% on the 9th day. M = 7.1. The average egg number of the primiparae was 3.1 per female.

From 12 neonates kept at the food level of 140 J/1 8.3% maturated on the 5th day and 91.7% on the 6th day. M = 5.9. The average egg number of the primiparae was 1.3 per female.

From 12 neonates reared at the food level of 289 J/1 59% finished the postembryonic development on the 5th day, 41% on the 6th day. M = 5.5. The average egg number of the primiparae was 2.0 per female.
The data are summarised in Table 2. and in Figs. 1. and 2.

Experiment in 1961. (A part of these results was elaborated in Hrbáčková, 1963.)

Daphnia pulex L.

From 22 neonates cultivated at the food level of 500 J/1 54.5% finished the postembryonic development on the 4.5th day, 27.3% on the 4.6th day and 18.2% on the 5th day. M = 4.7. The average egg number of the primiparae was 6.0 per female.

Daphnia pulicaria Forbes

From 29 neonates reared at the food level of 500 J/1 10.3% maturated on the 5.3th day, 68.9% on the 5th day and 20.7% on the 4.7th day. M = 5.0. The average egg number of the primiparae was 9.4 per female. The data are summarised in Table 2. and in Figs. 1. and 2.

The dependence of the length of the postembryonic development on the concentration of the food

Monod (1942) used the equation $\mu = \mu m \frac{S}{K_s + S}$

μ, μm = actual and maximum development rate, respectively (time^{-1})
S = rate-limiting food concentration (mass volume)
K_s = half-velocity coefficient (mass/volume)

to describe the relation of the developmental rate of bacteria on the concentration of the substrate. We found it exciting whether the same typ

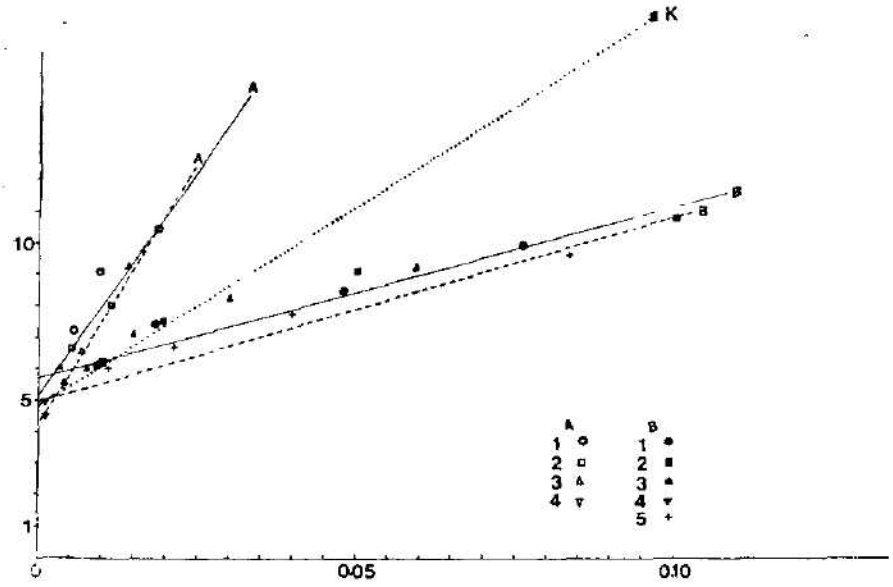

Fig. 1 — The ralation between the inversed values of the food concentration in J/l and the length of the postembryonic development (in days). Lineweaver-Burk plot of the regression. Full lines A *(D. pulex)* and B. *(D. pulicaria)* are sample regression lines. Dashed lines are based only on the points of lowest and highest food concentration. Dotted line in *D. pulicaria* is based on the points at the highest and lowest food cencentration corrected for the smaller size of the primiparae at this concentration. 1. Experiment in July 1975. 2. Experiment in July 1975. 3. Experiment in August 1975. 4. Experiment in July and August 1961. 5. Experiment in August 1974. K = value korrected for smaller size of females (see text).

of equation can be used in *Daphnia* for the description of the dependence of the length of the postembryonic development on the concentration of food. Fig. 1. shows the Lineweaver-Burk plot of the length of the postembryonic development on the inverted value of the concentration of food. In *Daphnia pulex* this relation is nearly linear whereas in *Daphnia pulicaria* slightly curvilinear. The coefficient of the correlation is in *Daphnia pulex* 0.92 and in *Daphnia pulicaria* 0.96. The shortest length of the postembryonic development actually measured is shorter than the length of the postembryonic development derived from regression lines (4.7 in *Daphnia pulex* against 5.0, in *Daphnia pulicaria* 5.0 against 5.8), because the points from the highest food level are below the regression line. The line which passes the points of the postembryonic development at highest and lowest food level gives much more adequate figures for the shortest length of the postembryonic development (in *Daphnia pulex* 4.7 days, in *Daphnia pulicaria* 5.0 days) but all other experimental points are above these lines. The same relation can be seen from the more usual plot of the development of food. (Fig. 2). The value of the food concentration at the half of the maximal development rate (half velocity coefficient) derived from the Lineweaver-Burk plot is considerably higher when the line passing the points at maximal and minimal concentration of food is used than when it is based on population regression line (97.7 against 64.8 in *Daphnia pulex* and 12.3 against 9.6 in *Daphnia pulicaria*).

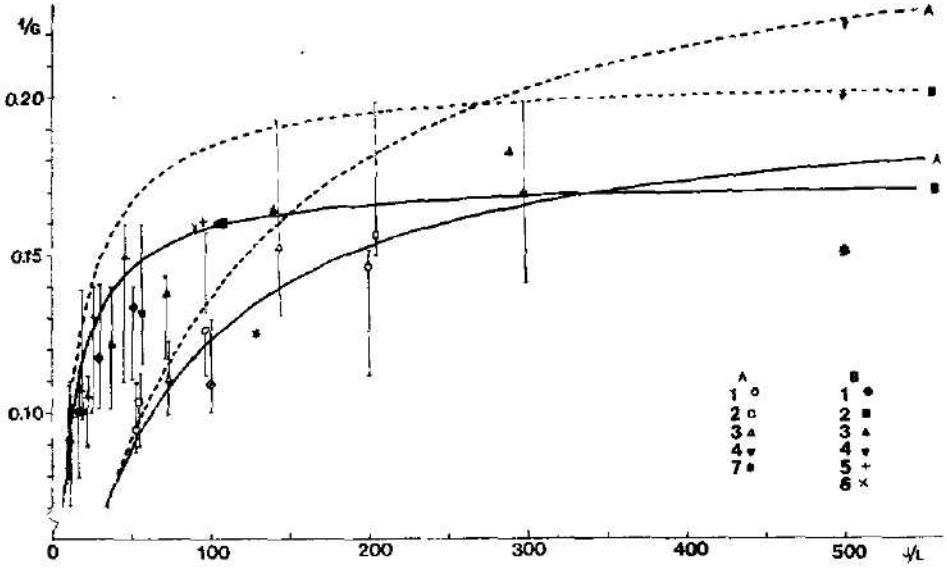

Fig. 2 — The relation between the concentration of food J/1 and the inversed values of the length development (in days). Curves are based on the Monod equation with parametrs derived from the linear regression in Fig. 1. Full lines A *(D. pulex)* and B *(D. pulicaria)* are sample regression lines. Dashed lines are based only on the points of lowest and highest food concentration. 1. Experiment in July 1975. 2. Experiment in July 1975. 3. Experiment in August 1975. 4. Experiment in 1961. 5. Experiment in August 1974. 6. Experiment in August 1973 (stirred medium). 7. Data of Richman's experiments.

DISCUSSION

The relationship between the food concentration and the length of the postembryonic development was studied by Richman (1958), who reared *Daphnia pulex* under laboratory conditions fed on sterile algae of *Chlamydomonas* Rh. The lowest concentration of algae by Richman used was 25,000 cells/ml (1.308×10^{-6} cal/cell); this corresponds to about 130 J/1. Richman concentration is therefore much above the values found in the water of Slapy reservoir getting near to our 8 to 16 fold concentrations. In our experiments with the food concentration approaching the lowest values given by Richman, *Daphnia pulicaria* reached maturity on the 5th to 6th day, *Daphnia pulex* on the 7th day, whereas the specimens of *Daphnia pulex* in Richmans' experiments at four different concentrations (according to our conversion from 130 to 540 J/1 for the fifth to the sixth instar on the 8th day. Richman did not report the effect of different food concentration on the length of the postembryonic development, but rather on the size of the primiparae and the egg number. As can be seen from the Fig. 1 and Fig. 2 the overcalculated Richman's data fit rather well to our data of *Daphnia pulex*.

For our observation of the development of *Daphnia* in the course of various years using a various species composition of algae, and the low dispersion of the points in Figs. 1. and 2. we may conclude that the

assimilation of various species of algae is not very different. One may object that this result is somewhat affected by centrifugation, which causes a damage to algae with a resulting reproduction of bacteria, so that a great part of the food for Cladocera during 24 hours before the medium is changed is based on a secondary bacterial reproduction. As the lowest concentration used by Richman fits well to the curve for *Daphnia pulex* based on the natural food, it does not seem that the effect of this secondary bacterial development would be substantial as it always results in a decrease in the caloric values of the original biomas. On the other hand, the importance of the bacterial development may be supported by the fact that there was no difference in the length of the postembryonic development in the cultures with a stirred an unstirred medium. The influence of various food concentration on the length of the postembryonic development was followed by Weglenska (1971) for the species *Daphnia cucullata* and *Daphnia longispina*. It is difficult to compare exactly her results with ours, baceuse one cannot convert precisely the volume of algae given by Weglenska in mg/l of fresh weight to the caloric value which is the basis of our comparison. The volume of algae for Slapy reservoir (Table 1.) is approximatelly half to three fold the volume of Weglenska for lake Mikolajky. Weglenska found that *Daphnia longispina* attained the shortest postembryonic development of 6 and a half day at a food level of 2.5 mg/l (this is when converted about 10 J 1); a further increase of the concentration does no more shorten the length of the postembryonic development. *Daphnia cucullata* has the shortest postembryonic development, i. e. seven days at the food concentration of 3.7 mg l (which is in the order 15 J 1). A decline in the rate of the postembryonic development set in at a raised concentration. Weglenska made her experiments at 17—18 °C. As at 20 °C, the species *Daphnia longispina* and *D. cucullata* may reach under favourable feeding conditions the stages of primiparae after three molts, on 4th day (Hrbáček, Hrbáčková, 1960), one may assume that the concentration was below the optimum in the experiments conducted by Weglenska. It is interesting to note that the food increase from the concentration of 2.5 to 3.7 mg l had not positive influence on the rate of development in *Daphnia longispina*, whereas the development of *Daphnia cucullata* was slightly shortened. Noteworthy is that the postembryonic development in *Daphnia cucullata* lasted longer than in *Daphnia longispina* whereas Hrbáček and Hrbáčková (1960), who studied the postembryonic development in the above mentioned species, give evidence that under favourable food conditions the opposite occured. The comparison of the length of the postembryonic development at various temperatures under various food concentrations is not univocal. Blažka (1966) shows that the dependence of metabolism in Cladocera on temperature is largely influenced by the nutrimental condition. That is why one cannot tell whether the shortest time of the postembryonic development found by Weglenska, when feeding *Daphnia* on natural food, is comparable with the results obtained by Hrbáček and Hrbáčková (1960) by experiments under optimum feeding conditions. Weglenska gives as the cause of a small effect of the increase of food cencentration above the level of natural food, which she carried through her experiments, the

clogging of the filtering apparatus; we have never noted this in our experiments

From our results, it is clear that the difference between the utilization of food in *Daphnia pulex* and *Daphnia pulicaria* is very substantial. The lowest values in *Daphnia pulicaria* at which the postembryonic development was terminated was 10 J/1, in *Daphnia pulex* more than five times as much, namely 51 L/1. However, in both species the length of the postembryonic development at the lowest tolerable concentrations was approximately the same, about 10 days; this is roughly a twice longer postembryonic development as compared with the shortest length observed. The difference between both species was maintained during the food concentrations under study till highest food concentration when the postembryonic development in *Daphnia pulex* is a little shorter that that of *Daphnia pulicaria*. One of the possible explantations of the cause of this difference between the above mentioned species may be a different ability of filtration. According to Johnson (1952) the distance of the filtration bristles in the genus *Daphnia* is approximately alike. This would indicate that this difference may be conditioned by the difference in the size of the filtering area or in the frequency of the filtering movements, or in the combination of both. Egloff (1971) ascertained the difference in the relative size filtering area between the species *Daphnia magna* and *Daphnia rosea*, which are morphologicaly more distant than *Daphnia pulex* and *Daphnia pulicaria*, but also here the difference in the relative size of the filtering area is much smaller than the difference we found in the ability of the above mentioned species to use various food concentrations. That is why the main difference must be sought in the frequency of the action of the filtering apparatus and thus also in the metabolism. From the occurrence in nature, one can assume that *Daphnia pulex* is a species which is adapted to a less effective, but quick utilization of food (periodical pools), whereas *Daphnia pulicaria* utilises food in a very effective manner, but slowly (high mountain lakes and large ponds). Influence of food concentration on the number of eggs was not recorded. In laboratory cultures, *Daphnia pulex* and *Daphnia pulicaria* fed on a surplus of chlorococcal algae, in which the concentration of food in terms of Joules was subtantially higher than under natural conditions, we found more eggs in the first batch at the identical number of preadult stages.

It is noteworthy that the ranges are different on different parts of the curve in Fig. 2. In *Daphnia pulicaria* it is highest at a value around 0.15, in *Daphnia pulex* at a value around 0.16. At lower values there exists a uniform strategy how to maintain a species, i. e. to form an egg at the physiologically smallest possible size of the mother. On the other hand, the length of the postembryonic development at highest food concentration cannot be shortened and therefore the increase of food concentration is utilized for the production of eggs. A greater number of eggs at higher food concentrations, was observed e. g. by Richman (1958). In the values between these extremes, there is evidently no uniform strategy how to use more abundant food whether for growth and in connection with this for a prolonged length of the postembryonic development or for forming eggs and in connection with this a shortened length of the post-

embryonic development and therefore a considerable fluctuation of the length of the postembryonic development is found.

The use of the Monod equation for the examined relation assumes that the number of the cells and their size in the female which has first eggs in its brood chamber does not change with the change of the food concentration and that only the rate of the division of the cells is changed. This assumption is not fullfillet in our experiments as the females reared at higher food level are definitely larger than the females reared at the lower food level. This is the reason why the curvefitting in Fig. 2. is not very satisfactory. Wimberg (1971) has described the relation between the fresh weight and the length of the females of *Daphnia pulex* by the equation $W = 0.87 \times 1^{2.57}$

where

W = fresh weight
1 = length of the female

The average length of the well fed primiparae in the 1961 experiments was 2.31 mm and of the low fed primiparae 1.95 mm. The corresponding weight was by 1.48 larger in well fed than in the low fed individuals. When the development of the individuals on the low food condition is prolonged by the same ratio we get the corrected value of the development rate of a female having the same weight under low food condition as under high food condition. This corrected value is plotted in Fig. 1. It is evident that the points corresponding to higher food condition are very close to the line connecting this corrected value with the value obtained at the highest food conditions.

Irrespective of all shortcomings mentioned in the preceedings paragraphs the Monod equation can be used as a description of the dependence of developmental rate of various *Daphnia* species on the food concentration.

Acknowledgements

It is a pleasant duty to express our thanks to J. Kratochvíl for the analysis of seston, to Dr. P. Blažka for the methodological approach how to evaluate the energy content of seston and last not least to Dr M. Legner for the help in the evaluation of the results in the terms of Monod equation.

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Authors' address: Dr. Marta Hrbáčková, CSc., Doc Dr. J. Hrbáček, CSc., Hydrobiologická laboratoř Botanického ústavu ČSAV, Vltavská 17, 150 00 Praha 5, ČSSR.

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Department of General Zoology and Animal Physiology, University of J. E. Purkyně, Brno

EFFECTS OF COOLING ON OSMOTIC, PRESSURE, CATION CONTENTS, AND PROTEIN SYNTHESIS IN THE BLOOD OF GALLERIA MELLONELLA PREPUPAE AND PUPAE (LEPIDOPTERA)

MILAN MAREK

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Abstract: The influence of cooling at 4 °C on the formation of "cooling protein", on osmotic pressure, the ionic contents of K^+, Na^+, Mg^{2+}, and Ca^{2+} cations and the number of hemocytes in the hemolymph of prepupae and pupae of *Galleria mellonella* was investigated. Possible influence of ionic concentration on protein synthesis is discussed.

INTRODUCTION

It is known that at low temperatures many insects fall into diapause, during which their neurohormonal and metabolic processes decrease to a minimum (Gersch, 1964; Wigglesworth, 1965; Bursell, 1970; Lozina-Lozinsky, 1972). Nevertheless we succeded in determining synthesis of esterases in the hemolymph of *Galleria mellonella* pupae under the influence of cooling, which eventually led to the synthesis of "cooling protein", a gamma globulin, in hemocytes (Marek, 1970a).

The effect of ions on the binding of histones with DNA in the cell nucleus has been thoroughly investigated (Katchalski, 1964). Since DNA, whose binding to nucleoproteins has been loosened by ions, can act as a template for m-RNA (Egelhaaf, 1966; Kroeger and Lezzi, 1966; Lezzi and Gilbert, 1970; Robert, 1971; Lezzi and Robert, 1972), which should lead to the synthesis of new proteins, and since and since cooling influences the size of cell membrane pores considerably (Kleinzeller, 1963), the question of the role of ions present in the hemolymph arose, to wit, which -if any- change their concentration under the influence of cooling, and whether these changes are reflected in the total osmotic pressure.

The experiments were designed to answer the following questions:

- In what way do the ionic concentration and the blood osmolarity changes during normal development?
- What is the influence of cooling on the number of hemocytes, ionic concentration and osmolarity in the hemolymph?
- In what way does an altered food composition influence the osmolarity of the hemolymph?

MATERIALS AND METHODS

Rearing: Larvae, prepupae and pupae of *Galleria mellonella* were reared in the dark at 30 °C and 60% relative humidity. Larvae of one part of the breed received an artificial diet no. 1. With individuals of this group the major part of experiments was carried out. Another part of the breed was fed on artificial diet no. 2. With individuals reared on this diet only the osmotic pressure in the hemolymph was measured.

The age of spinning larvae prepupae was determined by the pigmentation of the eyes (eyeclasses, medofied after Kühn and Piepho, 1936), the age of pupae by the pigmentation of the base of the developing wings (Marcus, 1962).

Collection of hemolymph samples: Hemolymph was collected after narcosis (animals were submerged in water for 10 min) by incising the legs of larvae and prepupae. Pupae were placed in a glass test tube filled with water and centrifuged at 1000 rev/min for 10 min. After piercing the pupae at the site of the accumulated hemolymph with a glass needle, a sufficient amount could be obtained.

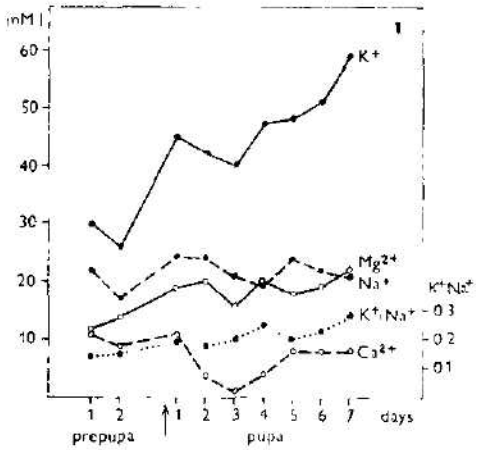
Measurement of osmotic presure: The osmotic pressure in the hemolymph was described by freezing point depression with a Knauer-semi-micro-osmometer, Value of Δ t °C was calculated relative to doubly distilled water. The results were expressed in mOsm ($= \frac{\Delta t\,°C}{1{,}86}$) $\pm$ standard error.

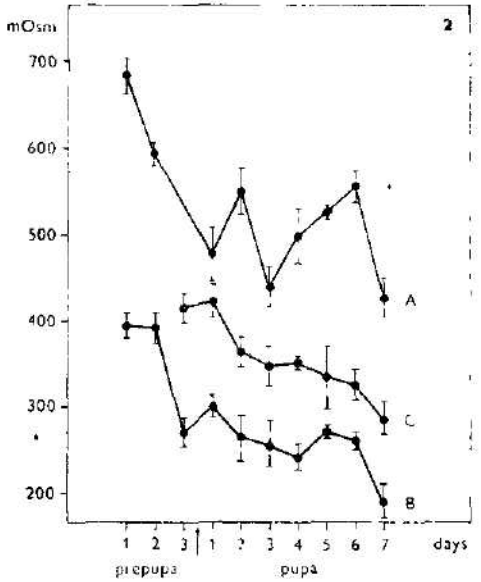
Measurement of ionic concentration: The ionic content of Na^+, K^+, and Ca^{2+} in the hemolymph was analysed with a flame photometer (Eppendorf), Mg^{2+} concentration by a Helium Glow Photometer (Aminco). The standard solution consisted of 50 mM NaCl (KCl) for Na^+ (K^+) or 20 mM $CaCl_2$ + 20mM NaCl for Ca^{2+} in Cesiumchloridealuminiumnitrate-buffer-solution (1 : 50) acc. to Schuhknecht and Schinkel (Merck). For the determination of Mg^{2+} it consisted of 20 (or 100) mM $CaCl_2$ + 50 mM KCl + 30 mM NaCl in distilled water. Hemolymph samples were diluted in Schuhknecht-Schinkel-solution (1 : 50) at a ratio of 20 μl hemolymph to 3000 μl buffer. The results are expressed in mM/1 $\pm$ standard error.

Counting of hemocytes: The number of hemocytes in the pupal hemolymph was determined according to Stephens (1963) in a Bürker cell with an area of 1/25 mm^2. The collected hemolymph was diluted in a melangeur (marked up to 11). The hemolymph was taken up to the 0,25 mark, diluted with 2% acetic and gently stained with methylene blue, up to the 11 mark. After shaking for 3 min, the fifth drop was used the Bürker cell. The number of hemocytes was calculated in the usual way (Bykow, 1969) for the volume of 1 mm^3.

Electrophoresis on starch gel: To determine the appearance of "cooling protein", the hemolymph proteins were separated on horizontal starch gel electrophoresis, modified acc. to Smithies (1955, 1959). For the determination of esterases, the starch strips with separated hemolymph proteins were submerged for 30 min in 100 ml phosphate buffer (pH 6.7), to which 2 ml of 1% acetone solution of

Composition of diets	no. 1	no. 2
maize meal	220g	—
wheat meal	110g	—
wheat flour	110g	—
milk powder	110g	130g
dried yeast	55g	—
yeast	—	235g
bee's wax	175g	10g
honey	110g	260g
glycerol	110g	130g
oat flakes	—	235g
4-Hydroxy-benzoicacid-methylester (Nipagin M)	—	1,5g





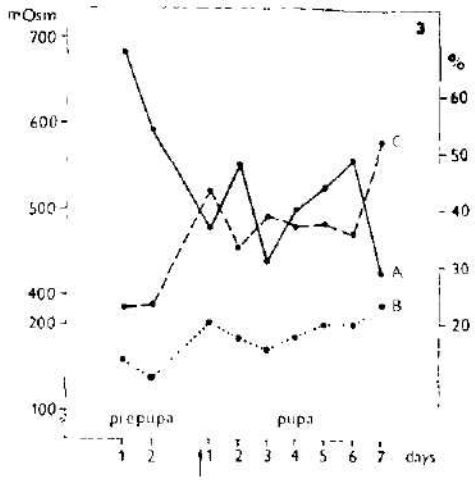


Fig. 1. Changes of ionic concentration of K^+, Na^+, Mg^{2+}, Ca^{2+}, an of the K^+/Na^+ ratio in the hemolymph during prepupal and pupal development of *Galleria mellonella*.

Fig. 2. Change of osmotic pressure in the hemolymph during the development of *Galleria mellonemla*.

A: Controls, diet no. 1

B: Controls, diet no. 2

C: After cooling at 4 °C for 17 days, diet no. 2.

Fig. 3. Percental proportion of the osmotic pressure of K^+, Na^+, Mg^{2+} and Ca^{2+} in the total osmotic pressure in the hemolymph of *Galleria melonella*.

A: Total osmotic pressure, in mOsm (diet no. 1)

B: Total osmotic pressure of K^+, Na^+, Mg^{2+}, Ca^{2+}, calculated from their concentrations.

C: Osmotic pressure of abovementioned ions in % of total osmotic pressure.

1-naphthyl butyrate with 250 mg diazonic salt fast blue BB (Laufer, 1960) were added.

A total of 1250 individuals of prepupae and pupae of *Galleria mellonella* were used in the experiments.

RESULTS

1. Changes of the ionic content in the hemolymph during development:

Fig. 1 shows the changes of concentration of K^+, Na^+, Mg^{2+}, and Ca^{2+} ions (in mM/l) in the hemolymph of prepupae and pupae of *Galleria mellonella*. Of these ions, K^+ shows the highest values and is the only one to increase markedly during development. Its concentration declines in the period of spinning to 25.5 mM, and rises at the pupal ecdysis to 45.3 mM. The third day after ecdysis is distinguished by a slight decrease of K^+ to 40.2 mM; afterwards the content of K^+ increase reaching a maximum (58.9 mM) on the seventh day.

Compared with above values, the content of Na^+ is lower. On the whole, the concentration of Na^+ fluctuates reaching maxima of 23—24 mM in early prepupae and in pupae 1—2 days and 5 days after ecdysis (Fig. 1). The content of Ca^{2+} is mostly 8—10 mM except the 2nd, 3rd and 4th days after pupal ecdysis when it deaps to 0.7 mM.

Values of Mg^{2+} were measured with a different type of spectrophotometer. Nevertheless, the results obtained are similar to those of Ca^{2+}, especially on the third day after pupal ecdysis, where the curves of both Ca^{2+} and Mg^{2+} show a depression (Fig. 1).

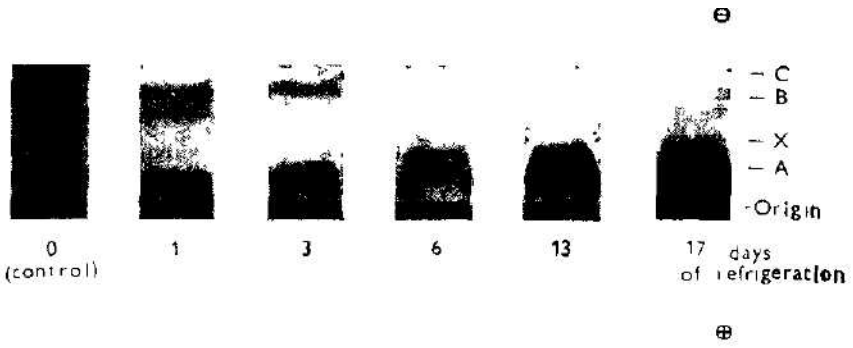
The value of the K^+ (Na^+ ratio is higher than 0.1 and in the course of development the trend of a slow increase can be observed, mainly due to the rising K^+ (Fig. 1).

2. Changes of osmotic pressure in the hemolymph during development:

2.a. Animals fed on diet no. 1: The osmotic pressure of hemolymph during the development is higly variable (Fig. 2-A). In the period of prepupae it declines from 682.6 to 479 mOsm. In the pupae it reaches two peaks, one on the second day (553.2 mOsm) and one on the sixth day (555.6 mOsm) after ecdysis. On the last day the osmotic pressure decreases to 328.8 mOsm.

2.b. Animals fed on diet no. 2: On the whole, lower values of osmotic pressure (Fig. 2-B) than those described in the foregoing paragraph were observed. Here, too, peaks of osmotic pressure were found on the first and one on the fifth day of pupal instar. Osmotic pressure is higher in prepupae than in pupae. The lowest value was found on the last day of pupal instar. Except for the overall lover values, the results are fairly similar to those given in paragraph 2.a.

3. Contribution of ions to the osmotic pressure of the hemolymph: A comparison between the total hemolymph osmotic pressure (Fig. 3-A) and the share of the investigated cations in the osmotic pressure was made in animals fed on died no. 1. The cumulated osmotic pressure of these ions was calculated from their respective concentrations (Fig. 3-B). Their percental share is given in curve 3-C, the



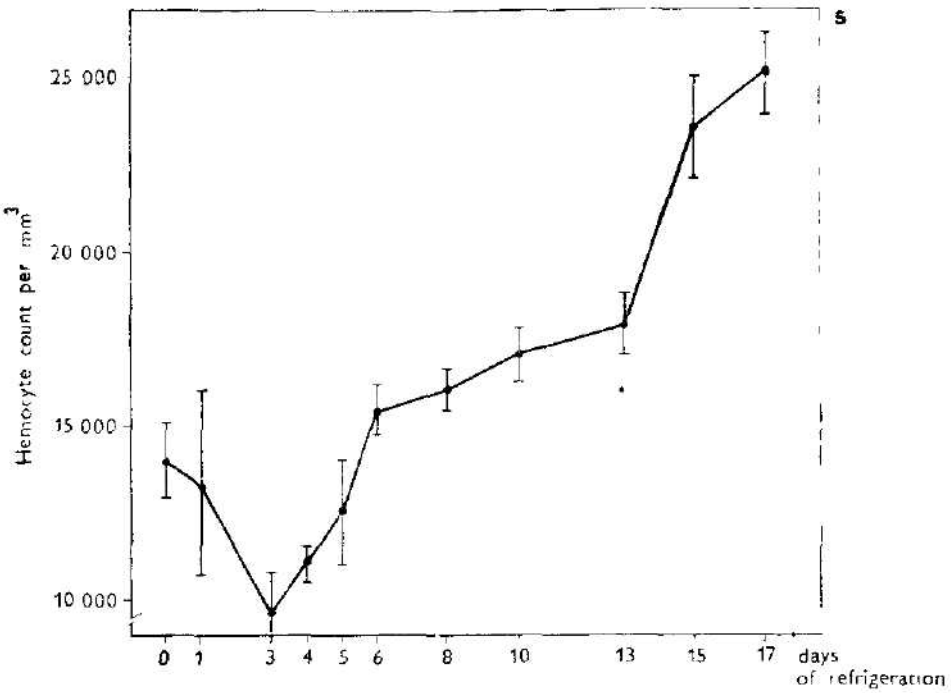


Fig. 4. Influence of cooling at 4 °C on the appearance of "cooling protein" in the hemolymph of *Galleria mellonella* pupae A, B, C, X isoenzymes of esterases; X: "*Cooling protein*". *Untreated pupae (0 — control); Time of cooling: 1 day (1), 3 days (3),* 6 days (6), 13 days (13), 17 days (17)
Fig. 5. Influence of cooling at 4 °C on the number of hemocytes per mm³ in the hemolymph of *Galleria mellonella* pupae.

latter showing, that the changes in total osmotic pressure are not exclusively due to the changes in total Na^+, K^+, Mg^{2+}, and Ca^{2+} contents.

4. Synthesis of "cooling protein" and changes in the number of hemocytes: Pupae kept at 4 °C contained in their hemolymph a new "cooling protein". Fig. 4 confirms that the protein is synthesized only after three days of cooling (see also Marek, 1970, a).

The effect of cooling is also evident in the increase of the number of hemocytes in the hemolymph (Fig. 5). A distinct minimum (9600 ± 1170) is reached on the third day of cooling, after which the number of hemocytes rises sharply up to 25100 ± 1230 per mm^3 on day 17.

5. Effect of cooling on the ionic content of hemolymph: For a clearer demonstration of the cooling effect we calculated the percentage of ionic concentration gain or loss in relation to the values found in controls, kept at 30 °C. During all incubation periods at 4 °C the values of K^+ never fall below 100% and eventually reach about 165% of the control value (Fig. 6). The most striking effect of cooling is the increase of the K^+ concentration after 14 to 23 hours. In this relatively short time the concentration increases by 18%, which is about 16 times faster than during the following days.

The opposite was observed for Na^+ at the beginning of the incubation period. Its concentration decreased by 6% during the first 24 hours, but between 24 and 48 hours, simultaneously with a slight decrease in K^+ concentration, the Na^+ content increased by 10.9%. Otherwise, the Na^+ concentration remains on nearly the same level, showing that cooling exerts no substantial influence on it during longer incubation periods.

The graph of the percental change of the K^+/Na^+ ratio (Fig. 6) reflects the trend of the K^+ concentration, i. e. rapid increase on the first day (by 35%) and slower increase between day 6 and 17—18. The rising Na^+ concentration on the second day affects a decrease of the ration by 11%.

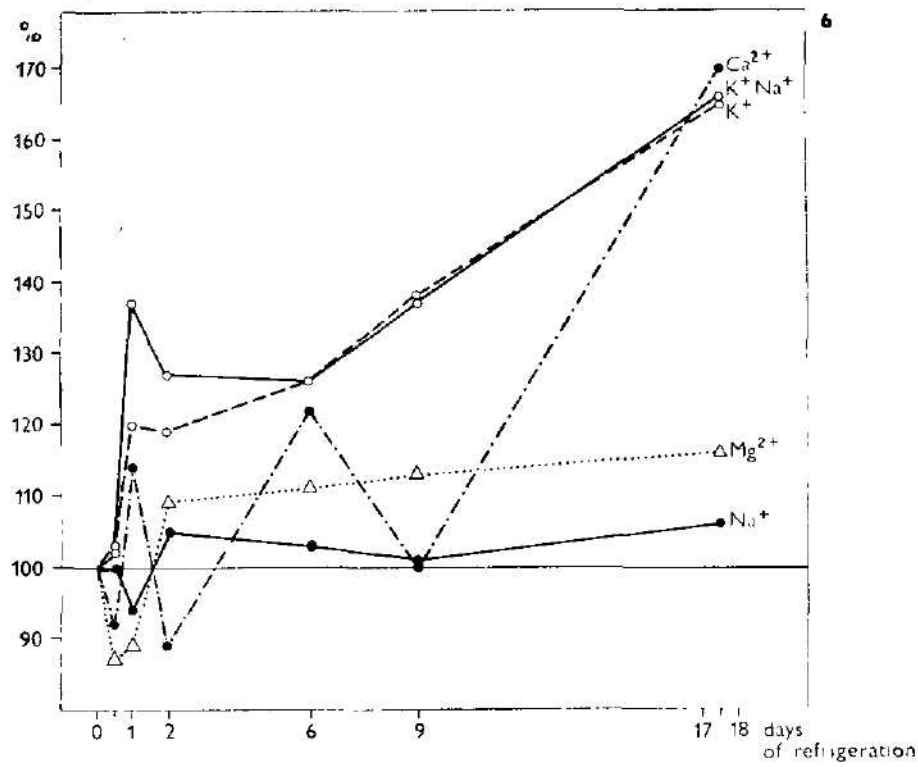
Similar to Na^+, the concentration of Mg^{2+} is lowered on the first day (– 10.5%) and increase sharply on the second day 9%, to climb slowly to the level of 16.3% between days 2 and 17—18 (Fig. 6).

The concentration of Ca^{2+} changes differently from those of the other ions (Fig. 6): it declines in 14 hours by 7.5% and increases in the following 19 hours by 21.5%. During the next two days it decreases again (by 25%), and after six days the increase amounts to 33%. Then again, after another 3 days (on the 9th day), the value decreases by 22%, to rise finally to 69% on day 17—18. Thus, it is evident that the Ca^{2+} concentration varies highly under the effect of cooling and contributes most diversely to the changing osmotic pressure.

6. Effect of cooling on the osmotic pressure of hemolymph:

6.a Animals fed on diet no. 1: In general we found that cooling at 4 °C increases hemolymph osmotic pressure in prepupae and pupae. Fig. 7 shows the percental increase of osmotic pressure during 17 days of cooling compared to values found in controls reared at 30 °C. The most striking increase (30%) occurs during the first 6 days, the fastest increase is found between the first and second day. The maximum is reached on day 17—18 (58%).

6.b. Animals fed on diet no. 2: Here, too, the osmotic pressure of the hemolymph was raised by prolonged cooling. Test measure-



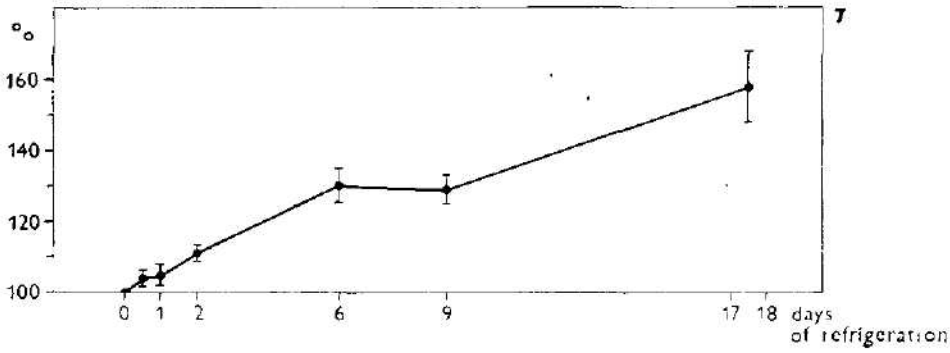


Fig. 6. Influence of cooling at 4 °C on the ionic concentrations of K^+, Na^+, Mg^{2+} Ca^{2+}, and the K^+/Na^+ ratio in the hemolymph of Galleria mellonella prepupae and pupae. Controls = 100%.
Fig. 7. Influence of cooling at 4 °C on the osmotic pressure in the hemolymph of *Galleria mellonella* prepupae and pupae. Controls = 100%.

ments with prepupae of the third day of development, carried out on the 2nd, 4th, and 9th day of cooling are quite similar to those obtained with diet no. 1. Comparing curve C on Fig. 2, which shows the changes of

osmotic pressure of prepupae and pupae kept in the cold for 17 days, with curve B on Fig. 2, which gives the values of osmotic pressure without cooling influence, we notice a striking linearity between them. This shows that cooling affects all stages of prepupal and pupal development to nearly the same degree, though some deviations can be observed of days 3—5.

DISCUSSION

The problem of the stress of cooling on insect is connected with a number of unsolved questions. In the present study, we tried to shed light on some of them.

It has been known that a "cooling protein" is synthesized in pupae of *Galleria mellonella*, kept at 4 °C (Marek, 1970a, c). As this protein is synthesized only by hemocytes (Marek, 1970b) we have investigated in the present study some factors acting in the liquid milieu of hemolymph, namely its total osmotic pressure and its ionic composition. We found that the inorganic ions are responsible for 20—50% of the total osmotic pressure (Fig. 3-C).

In control animals kept under normal temperature of 30 °C and reared on two different diets the hemolymph osmotic pressure is much higher in feeding larvae (Rouchal, 1940; Laviolette and Mestres, 1967; Mestres and Laviolette, 1968; Czaja-Topińska and Klekowski, 1970) than in spinning larvae, prepupae, and pupae.

Remarkable were the relations between the total osmotic pressure and the osmotic pressure resulting from the concentration of K^+, Na^+, Ca^{2+} and Mg^{2+} cations. We found that the cations are increasing their share in the total osmotic pressure when its value is declining and vice versa.

Our experiment involving 2 diets revealed great influence of food on the blood osmotic pressure (Fig. 2). The most significant difference between the two diets was the content of wax, whose weight in diet 1 was 20-times higher than diet 2. It should be mentioned that wax contains about 10- times more K^+ and Ca^{2+} ions than the hemolymph (Rockstein, 1964).

As to the influence of ions on the osmotic pressure at cooling, and perhaps also their influence on the synthesis of the "cooling protein" in hemocytes, potassium plays an unmistakable part. Its content is almost steadily increasing for 17—18 days during the cooling. This observation is interesting in regard to the results of Molinar and Hultin (1965) who established that an increased content of K^+ -ions has a stimulating effect on the protein synthesis in the eggs of sea urchin. Importance of K^+ concentration for the synthesis of the "cooling protein" was indicated by previous experiments (Marek, 1970c).

The synthesis of the "cooling protein" did not begin until the 3 rd day of cooling when the magnesium content in hemolymph decreassed. However, the content of Mg^{2+} gradually increased with further keeping at 4 °C. Considerable fluctuations occured in the Ca^{2+} content in the hemolymph. This fluctuations may be significant because Price (1967) reported that Ca^{2+} -ions influence the permeability of cell membrane and that they can exert an indirect influence on protein synthesis; this role the author ascribes also to the Mg^{2+} -ions.

Changes in the number of hemocytes can also provide clue for understanding stimulation of the "cooling protein" synthesis. Pichon (1970) demonstrated that an increase of the concentration of K^+ -ions exerts an influence on the increase of the number of hemocytes. Meeker, 1970 reported that an optimal K^+ -ion concentration is necessary for the cell division and in case of its lack the synthesis of protein necessary for the constriction of mitotic apparatus does not take place. Findings of Lubin, 1967; Molinaro and Hultin, 1965; and Terskikh and Malenkov, 1973 are in agreement with the results of the two authors just mentioned.

Our results show that the maximum synthesis the "cooling protein" coincides with the maximum content of K^+ -ions in hemolymph. The influence of K^+ on the synthesis of this protein was shown in experiments with the injection of KCl into the cooled pupae of *Galleria mellonella* (Marek, 1970c). The synthesis was increased after the injection and was inhibited both by actionmycin D and by cycloheximide.

Marek and Kroeger, 1974; 1976; determined variations in proteosynthesis of several isoenzymes in insect tissues kept in vitro. These experiments indicated that both cooling and incubation media directly affect the ion patterns in caryoplasma.

The achieved results and data in the literature indicate that the stress from cooling could affect the semipermeability of the cell membrane (of the hemocytes) and by this also their homeostasis. This could result in the transcription of new loci of the genom.

Indeed Kroeger (unpublished) and Dörr, 1972 found changes in puffs in the nucleus of salivary glands by *Chironomus thummi* owing to cooling.

SUMMARY

1. The osmotic pressure of hemolymph in prepupae of *Galleria mellonella* dropped from 628 mOsm to 479 mOsm. During the development of the pupae it was reduced from 479 mOsm to 428 mOsm. The breed was fed on diet no. 1.

2. When rearing our animals on diet no. 2, we observed a decrease in osmotic pressure during the prepupal and pupal development from 396 mOsm to 190.9 mOsm. After cooling for seventeen days, the osmotic pressure in the hemolymph of prepupae rose to 416 mOsm and reached 286 mOsm at the end of the development of the coldtreated pupae.

3. The cooling of pupae prepupae reared at 4 °C on diet no. 1 brings about a 58% elevation of the osmotic pressure of hemolymph in 17 days.

4. During the development of prepupae and pupae the hemolymph content of K^+ -cations increases from 30.4 mM to 58.9 mM/l. Within the 17 days of cooling its amount increased by 65.2%.

5. The content of Na^+ -ions in prepupal and pupal hemolymph ranged from 17.2 mM to 23.9 mM/l. During the cold treatment their amount was lower by 6.15% at the beginning of the cooling period, while it was higher by 6.22% at the end of cooling, when compared with the normal level.

6. During the prepupal and pupal development, the content of Ca^{2+} -ions varied between 11.4 mM and 0.7 mM/l. As a result of cooling the Ca^{2+}

-ions content fluctuated, as often as three times within 48 hours, viz. by 26%. *In 17 days of cooling their amount increased by 69.6%.*

7. The content of Mg^{2+} -ions rose in hemolymph during the development of the prepupae and pupae from 12 mM/l to 22 mM l. During the period *of cooling the amount of these cations increased by 16.3%.*

8. The K^+ -ions could be essential to the hemocyte mitoses and to the "cooling protein" synthesis. The Ca^{2+} -ions could also carry out an im*portand function in the hemolymph.*

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Authors' addres: Dr. Milan Marek, Oddělení obecné zoologie a fysiologie živočichů, Universita J. E. Purkyně, 611 37 Brno, Kotlářská 2, ČSSR.

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Department of Zoology, University of Calcutta, India

EFFECTS OF PROSTAGLANDIN $PGF_{2\alpha}$ ON THE CEREBRAL NEUROSECRETORY CELLS OF PERIPLANETA AMERICANA (BLATTODEA)

D. K. NANDA and M. S. GHOSAL

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Abstract: $PGF_{2\alpha}$ causes alterations in the neurosecretory profile of the brain of *Periplaneta americana* (L.). Neurosecretory cells in general exhibit drastic depletion of NSM with the appearance of vacuoles in the perikarya and eventual enhancement in the distribution of NSM throughout the intracerebral course of the protocerebrum. Release of NSM from the cell in question has been considered as due to the $PGF_{2\alpha}$ which is known to furnish strong pharmacodynamic effects and may thus facilitate the "stimulation-secretion coupling" mechanism.

Widespread occurrence of prostaglandins in a number of animal tissues coupled with their mode of actions on a varied range of physiological systems has lent support to the proposition that these agents may have a role as mediators or modulators of cellular responses to a variety of stimuli (Bergström, 1967). No standard term has yet been attributed to fit their role as "hormones", "local hormones" or "counter hormones". They are also implicated in the process of transmission across the adrenergic synapses and thus be considered as a new class of trasmitter neurohormones (Holmes and Horton, 1967). Prostaglandins are the natural constituents of many tissues including the brain and spinal cord where $PGF_{2\alpha}$ remains universal but PGs of other categories are variable in rabbit, bovine, cat and other mammals (Kirschner and Vogt, 1961: Ambache et al., 1963; Samuelsson, 1964; Čoceani and Wolf, 1965; Ambache et al., 1966; Horton and Main, 1967). Up to now there has been no conclusive evidence for the occurrence of PGs in the central nervous system of invertebrates in general and insects in particular. Neither is there any record for the involvement of PGs in the neurosecretory control of releasing factors (R. F.) in the metabolism of insects.

The present communication deals with the impact of one of the prostaglandins, $PGF_{2\alpha}$, a naturally occurring constituent of mammalian brain, on the cephalic neurohormogenic cells of dictyopteran insect, *Periplaneta americana*. Our aim is to elucidate the pharmacodynamic effects of the said agent to substantiate "supplementary" control for neuroendocrine integration with reference to cytoarchitectural alterations in the neurosecretory cells, their secretory dynamics and transport of NSM in the intracerebral course of the brain.

MATERIAL AND METHODS

Female roaches, *Periplaneta americana* (average body weight 1.7 grams) were used for this study. They were acclimated in the laboratory for more than a week. The animals were divided into six groups — each group consisting of five individuals — and were administered $PGF_{2\alpha}$ at 1 25 μg/roach. Controls were similarly treated with PG-free vehicle. The insects were killed by decapitation 1, 3, 6, 12, 24 and 48 hours after injection. Brains were quickly removed and processed for demonstration of neurosecretory material (NSM) (Cameron and Steele, 1959 and Bargmann, 1949).

OBSERVATIONS

Control: Neurosecretory cells of the pars intercerebralis comprising large, medium and small types exhibit secretory cycle, i. e. they are in various states of secretion. Larger cell groups (A- and B-types) contain granular inclusions in general (Fig. 1B); but the smaller (C- and D-type) posses deeply stained coarse particles (Fig. 1A). Some of the members of these cells are found to transport secretory material along the axonal processes which could be traced by their staining intensities.

Experimental:

Group 1 (one hour after injection) — Large and medium types of cells show acute depletion and the cytoplasm has been found to contain vacuoles particularly at the peripheral part of the cell. The cell membrane sometimes attains wavy appearance (Fig. 2). Cells of smaller dimension also show similar types of cytomorphic alterations along with the reduction in their number.

Group 2 (3 hours after injection) — In larger cells dirth of secretory particles still persists. Axonal processes in most cases are not conceivable. Some of the small cells may contain meagre concentration of coarse particles in their perikarya.

Group 3 (6 hours after injection) — Cells of larger dimension in general contain very few secretory inclusions dispersed in the cytoplasm. The smaller cells, on the other hand, show an indication for the repletion of NSM but discrete nature of the inclusions has not been restored.

Group 4 (12 hours after injection) — Most of the large cells are in the same state of depletion as found in the previous experiment. A few of them may contain traces of secretory particles trailing along the axon. Smaller cell groups, however, are found to bear detectable secretory inclusions in their scanty cytoplasmic perikarya but their abundance and tinctorial intensities have not yet been fully revived.

Group 5 (24 hours after injection) — Depletion of neurosecretory material is maintained in the large cells. Vacuoles of different size are still conspicuous in their perikarya. The axonal tract becomes silent with regard to secretory inclusions. Medium types of cells, however, seem to contain low concentration of NSM in their perikarya and occurrence of vacuoles is not very rare (Fig. 3). The discharged secretory material from the neurosecretory cells remains accumulated in the neuropile in course of their intracerebral dispatch (Figs. 4 a, 4 b). Cells of smaller dimension contain moderate to rich amount of secretory inclusions in their cytoplasm. Texture of the secretory inclusions does not deviate much from that of the control.

Group 6 (48 hours after injection) — Larger cells contain but marginal quantity of secretory inclusions, yet a tendency of a return to normal

condition is indicated both by the concentration and texture of cytoplasmic inclusions and by the virtual absence of vacuoles in the cytoplasm. The smaller cells restored more or less the normal condition in regard to the number, tinctorial richness, discrete appearance and axonal transport of their secretory inclusions (Fig. 5).

DISCUSSION

Endocrine dependent prostaglandin production has been conceived (Hawkins and Labrun, 1961; Pickles, 1967) more than a decade ago in human beings. But the neuropharmacological effects of prostaglandins on the neurohormogenic cells has not yet been recorded precisely, especially in invertebrates. As far as the effects of prostaglandins on the nervous system (Horton and Main, 1965) are concerned, it has been ascertained that they affect specific physiological functions which depend upon the difference in the chemical nature of PGs. We found that the $PGF_{2\alpha}$ causes the folowing cytomorphic alterations in the protocerebral neurosecretory cells of *Periplaneta americana:* appearance of vacuoles, undulation of the cell membrane and drop in the cytoplasmic inclusions (NSM). The changes are possibly due to biochemical interactions which principally affect the cell membrane (Douglas and Poisner, 1964) and subsequently involve intricate phenomenon like inhibition of cyclic AMP. The change in the secretory behaviour of the neurosecretory cells from the secretion of large to the production of small granules is indeed interesting, but overall initial evacuation (depletion) of NSM from the neurosecretory perikarya tends to indicate participation of prostaglandin in the release of neurohormones (Harms et al., 1973) and is dependent upon the relative sensitivity of a number of different receptors (Tobias, 1964). Effect of such activity eventually prompted accumulation of NSM in the intracerebral course of protocerebrum. Besides these, biological actions of prostaglandins on secreting connective tissue cells as recorded by Cabut et al., 1967 should be considered in this context. Fluctuation in the extent of repletion of neurosecretion in the course of experimental period further indicates that the $PGF_{2\alpha}$ caused a specific pharmacodynamic effect and is involved in "stimulation-secretion coupling" mechanism (Douglas and Poisner, 1964; Biswas and Ghosh, 1976).

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The figures 1–5 will be found at the end of this issue.

Authors' address: D. K. Nanda, M. S. Ghosal, Neuroendocrinology Laboratory, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Calcutta – 700019, India.

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Abteilung fur Forstschutz, Forschungsanstalt für Forstwirtschaft, Zvolen

ZUR PUPPENMORPHOLOGIE UND -TAXONOMIE DER UNTERFAMILIE ENNOMINAE, INSBESONDERE DER TRIBUS BISTONINI (LEPIDOPTERA, GEOMETRIDAE)

JAN PATOČKA

Eingegangen am 14. Februar 1977

Abstract: A modern classification of the subfamily Ennominae (Lepidoptera, Geometridae) is examined from the viewpoint of pupal morphology. A key for determining the pupae from the economic important tribe Bistonini is given and an explanation of the phylogenetic relationships within the subfamily Ennominae is suggested on the base of pupal morphology.

Die Morphologie der Puppen der Schmetterlinge überhaupt ist wenig durchgearbeitet und taxonomisch ausgenützt. Als Hauptquellen sind die Arbeiten von Chot'ko (1971), Koehler (1937), Mosher (1916), Patočka (1955, 1960), Nordström & Wahlgren (1941) zu bezeichnen. In der vorliegenden Arbeit wird das neue System der Familie Geometridae nach Herbulot (vgl. Bradley et al., 1972, Urbahn, 1967) vom Gesichtspunkte der Puppentaxonomie untersucht und es wird zur Diagnostik der Puppen aus der Tribus Bistonini beitgetragen.

MATERIAL UND METHODIK

Durch lange Jahre wurden die Puppen und Puppenexuvien gesammelt und trocken aufbewahrt, sodass dem Verfasser bei den meisten erwähnten Arten grössere Serien zur Untersuchung vorlagen. Sie wurden mit Hilfe eines Stereomikroskopes beschrieben, gemessen und (teilweise mit dem Abbe schen Zeichenapparat) gezeichnet. Als geeignete Unterlage für die Untersuchungen von Trockenpräparaten der Puppen hat sich Verbandzeugwatte (in einer Schale gelegt) bewährt, in welcher das Objekt die gewünschte Lage behält.

DIE WICHTIGSTEN MERKMALE UND IHRE VARIABILITÄT

Auser den bei den Puppen allgemein untersuchten Merkmalen wie die Grösse, Körperform, Färbung, der Glanz, die Skulptur, Form, An oder Abwesenheit der Sklerite, Mundteile und Gliedmassen, Beschaffenheit der letzten Hinterleibsringe und insbesondere des Kremasters usw. wurde noch zwei Merkmalen grössere Aufmerksamkeit gewidmet, weil sie sich als besonders geeignet zur Unterscheidung vieler sonst sehr ähnlichen Puppen der Unterfamilie Ennominae gezeigt haben: einem Höcker kaudal von dem (nicht immer sichtbaren) Brustluftloch, dem Brustluftlochhöcker (Brlh.). Fig. 22, 26, 28. 42—36. sowie einem Leistengebilde, welches sich bei vielen Puppen dieser Subfamilie lateral an der Basis des 5. Hinterleibsringes befindet (Fig. 20, 24, 25, 32—34). Dieses wird an der Puppe oft

durch den Kaudalrand des 4. Ringes verdeckt, welchen man manchmal beim Trockenpräparat mit einer Nadel wegpräparieren muss. Bei der Puppenexuvie ist es meistens frei und gut sichtbar.

Die Puppen varieren stark in der Grösse, was durch Ernährung der Raupe, das Geschlecht und zuweilen auch die Generation bedingt wird. Eine abweichende Form besitzen die Weibchen, insbesondere der in diesem Geschlecht brachypteren Arten. Dort ist die weibliche Puppe viel gedrungener, ihre Flügel sind Kürzer, Metanotum schmäler usw. Variabilität in der Färbung ist bei den Vertretern der Subfam. Ennominae nicht gross. Ebenfalls der Glanz und die Skulptur varieren sehr wenig, ähnlich wie die Brlh. und Seitenleisten am 5. Hinterleibsring. Gebilde and den zwei letzten Hinterleibsringen sind auch rel. wenig veränderlich. Der Kremaster ist in Detailen, nicht aber im Grundbau, ziemlich variabel. So unterscheiden sich in Detailen die Runzeln, Länge und Dicke des Endstieles, die Seitendornen, die auch vorkommen oder ganz fehlen können. Die Endgabel kann einfach oder verzweigt sein. Die Form der Sklerite und die Gliedmassen varieren mässig.

CHARAKTERISTIK DER PUPPEN DER FAMILIE GEOMETRIDAE UND SUBFAMILIE ENNOMINAE

Mumienpuppen (pupae obtectae), mittelgross, rel. stark sklerotisiert, vorne abgerundet, nach hinten kegel-, zuweilen keilförmig. 1—8. Hinterleibsring fast immer, Brustrücken selten mit Punktgrübchen (Fig. 20). Unterlippentaster fehlen. Unterlippe nur als ein 3—5 eckiges Plättchen vorhanden (Fig. 2, 6) oder fehlend (Fig. 11, 36). Vorderflügel berühren sich miteinander nicht. Kremaster immer entwickelt, entweder mit höchstens 4 Paaren von Häkchen (Fig. 1, 9, 13), oder mit einer, zuweilen gezweigten Doppelspitze am Ende (fig. 19, 21, 29, 35).

In dieser Weise kann man die Vertreter der Familie Geometridae fast eindeutig charakterisieren. Eine Verwechslung — von den mitteluropäischen Familien — wäre wohl nur mit den Vertretern der Familie Thyatiridae möglich; sie unterscheiden sich aber durch die rel. Grösse der Mandibel. Ferner ist die Grenze des Rüssels mit den Backen rel. kürzer, die der Vorderbeine rel. länger als bei Geometridae. Die ebenfalls ähnlichen Puppen der Familie Drepanidae unterscheiden sich durch weisse Bereifung, abweichende Form der Vorderflügel und gleichzeiting am Ende des Kremasters zusammengedrängte Häkchen. — Die Ähnlichkeit der Puppen dieser drei Familien entspricht deren Folge in der Oberfamilie Geometroidea in den modernen Systemen der Lepidopteren (vgl. Bradley et al., 1972).

Die einzelnen Unterfamilien der *Geometridae* lassen sich nicht immer so eindeutig charakterisieren.

Vertreter der Subfamilie Ennominae sind als Puppen meist über 9 mm lang, braungelb bis schwarz, nicht z. B. grün gefärbt, sie sind fast immer zeichnunglos und nicht bereift. Am Kopf gibt es selten Erhabenheiten (Fig. 14). Kremaster besitzt entweder 2—4 Paare von Häkchen (Fig. 1, 8, 9, 13), oder eine Doppelspitze am Ende (Fig. 5, 10, 19, 35), welche ausnahmsweise fehlen kann (Fig. 39); in diesem Falle gibt es am Kremaster keine Häkchen mehr. Die meisten Puppen ruhen am oder im Boden.

Durch diesen Komplex von Merkmalen können fast alle Vertreter dieser Unterfamilie charakterisiert werden. Schwierigkeiten bietet haptsächlich die Unterscheidung mancher Vertreter der Subfamilie Larentiinae.

Herbulot hat die Gattungen der Unterfamilie Ennominae in mehrere Tribus eingeteilt:

1. Tribus Abraxini — ist vom Gesichstpunkte der Puppenmorphologie uneinheitlich und zerfällt in zwei Gruppen: die eine — Gattung *Abraxas* Leach, 1815, z. B. *Abraxas grossulariata* (Linnaeus, 1758), steht durch den Bau des Kremasters (Fig. 1) und andere Merkmale der Tribus Ennomini nahe. Andere (*Stegania* Duponchel, 1846 — Fig. 7, *Lomaspilis* Hübner, 1825 — Fig. 5) weisen durch Anwesenheit der Unterlippe (Fig. 2, 6) und durch die Form des Kremasters Beziehungen zu der Tribus Semiothisini, sowie Boarmiini auf.

2. Tribus Semiothisini — ist im Puppenbau einheitlich: Unterlippe bezw. Vorderschenkel vorhanden. Kremaster basal dreieckig und gefurcht, kaudal glatt, stielartig, mit einer (manchmal verzweigten) Dorngabel am Ende (Fig. 10, 12). Diese Kremasterform ist denen bei Bistononi und Boarmiini ähnlich. Von der ersteren eindeutig durch Vorhandensein der Unterlippe bezw. Vorderschenkel unterscheidbar. Mit der letzteren jedoch in der Puppenmorphologie weitaus übereinstimmend und nicht eindeutig unterscheidbar. Meistens sind aber die Puppen der Tribus Semiothisini rel. rauher skulpturiert mit auffallend grossen Punktgrubchen, die der Boarmiini glatter, glänzender, die Punktgrübchen am Hinterleib (und zuweilen auch am Thoraxrücken rel. feiner.

Die Einreihung der Vertreter der Gattung *Itame* Hübner, 1826 in die Nähe — oder in die Gattung *Semiothisa* Hübner, 1818 selbst — scheint vom Gesichstpunkte der Puppenmorphologie gerechtigt zu sein (Fig. 10, 12).

3. Tribus Ennomini — wird meist durch rel. kurzen und mehr oder weniger gerunzelten Kremaster mit 3—4 Häkchenpaaren ausgezeichnet (Fig. 3). Diese Charakteristik bezieht sich aber auch auf die Gattung *Abraxas* aus der Tribus Abraxini und an die Vertreter der Tribus Angeronini, Caberini und Campaeini, welche überhaupt einen recht ähnlichen Puppenbau aufweisen (Fig. 1, 13, 47, 48), mit keiner Andeutung der Berechtigung der drei letztgenannten Tribus auf Grund der Puppenmarkmale. Bei den Puppen, die oberirdisch in einem leichten Kokon ruhen (Gattung *Ennomos* Treitschke, 1825 — Fig. 4) verlängert sich der Kremaster stark und die Häkchen verschieben sich zu seinem Kaudalende. Die Puppen der Arten *Plagodis dolabraria* (Linnaeus, 1758) und *Anagoga pulveraria* (Linnaeus, 1758) sind einander recht ähnlich und bestätigen die Auffassung, beide Arten in die gemeinsame Gattung *Plagodis* Hübner, 1823 zu vereinigen.

4. Tribus Ourapterygini — diese kleine Tribus, in Mitteleuropa nur mit einer Gattung, *Ourapteryx* Leach, 1814, weist im Puppenbau manche Besonderheiten auf, die ihre Existenz auch von der Hinsicht der Puppenmerkmale einigermassen berechtigen; keilartige Form, Höckerskulptur am Kopf, Mangel an Punktgrübchen am Hinterleib und umgekerhte, winkelige Stellung der Endhäkchen am Kremaster (Fig. 8). Es existiert hier aber auch Ähnlichkeit mit der vorgehenden Tribus Ennomini, insbesondere mit der Gattung *Ennomos* selbst. Ähnlich wie diese zeichnet sich auch hier die Puppe — dem oberirdischen Vorkommen entsprechend — durch

braungelbe Färbung und stark verlängerten Kremaster, mit den Häkchen im Endteil konzentriert (Fig. 4 und 8).

5. T r i b u s Colotoini — bei dieser Tribus — in Mitteleuropa auch nur mit einer Gattung, *Colotois* Hübner, 1823, vertreten — ist der Kremaster dagegen kurz und ungerunzelt, mit nur 2 Paar Häkchen (Fig. 9). Obzwar dadurch die Tribus eindeutig charakterisiert ist, besteht im Grundbau des Kremasters und in anderen Merkmalen eine deutliche Ähnlichkeit mit der Tribus Ennomini und den schon erwähnten anderen.

6. T r i b u s Angeronini — in Mittelueuropa auch nur durch eine sinzige Gattung *Angerona* Duponchel, 1829 vertreten — ist auf Grund der Puppenmerkmale der Tribus Ennomini sehr ähnlich und die Trennung von dieser Hinsicht kaum berechtigt (Fig. 13).

7. T r i b u s Bistonini — ist einheitlich und durch die Kremasterform (dreieckig, dorsal gerunzelt, am Ende ein glatter Stiel mit einer Doppelspitze — Fig. 15, 27, 29, 31) und zugleich durch den Mangel der Unterlippe (Fig. 30, 36) und der Vorderschenkel charakterisiert. Kremasterform der von Boarmiini (Fig. 41) und Semiothiosini (Fig. 10, 12) sehr ähnlich.

Die Vertreter der Tribus Bistonini stellen neben *Bupalus piniarius* (Linnaeus, 1758) — Tribus Bupalini — im Rahmen der Unterfamilie Ennominae die wichtigsten Schädlinge der Wald- und Obsthölzer vor. Sie werden auf Grund der Puppenuntersuchung oft kontrolliert und deshalb ist ihre richtige Bestimmung schon im Puppenstadium auch von besonderer praktischer Bedeutung. Ich führe hier deshalb eine Bestimmungstabelle der dendrophilen Arten der Bistonini Mitteleuropas nach Puppenmerkmalen an:

1 10. Hinterleibsring (und ausserdem noch der Kremaster im Mittelteil) mit einem Seitenzahn (Fig. 15, 17, 23) 2
— 10. Hinterleibsring ohne-, Kremaster im Mittelteil aber oft mit einem Seitenzahn (Fig. 18, 21, 27, 29) 4
2 (1) Brlh. vorhanden, niedrig (Fig. 16). Am 8. Hinterleibsring dorsal 2 Höcker. Seitenleiste am 5. Hinterleibsring einfach, nicht länger als dieser Ring selbst (vgl. Fig. 24), die Vertiefung darunter rugulos. Puppe 20—26 × 6—8 mm, rot- bis schwarzbraun, grob skulpturiert . . . . . *Lycia hirtaria* (Clerck, 1759).
— Brlh. fehlend. A 8. Hinterleibsring fehlen die Dorsalhöcker. Puppe meist kleiner (15—20 × 4.5—6 mm), dunkel rotbraun, grob skulpturiert 3
3 (2) Seitenleiste am 5. Hinterleibsring länger als dieser Ring selbts, gerade, Vertiefung davor rugulos (vgl. Fig. 25). Seiten- Höcker am 10. Hinterleibsring gross, dornartig (Fig. 15). Kremaster dorsal rel. grob gerunzelt (Raupe an Laubhölzern) . . . . . *Lycia pomonaria* (Hübner, 1790).
— Seitenleiste am 5. Hinterleibsring nicht länger als dieser Ring selbst (Vgl. Fig. 24). Seitenhöcker am 10. Hinterleibsring rel. kleiner, Kremaster dorsal meist rel. feiner gerunzelt (Fig. 17) (Raupe an Lärchen) . . . . . *Lycia isabellae* (Heslop & Harrison, 1914).
4 (1) Puppe 17—26 × 5—8 mm 5
— Puppe 11—14 × 3,5—4,5 mm 8
5 (4) Vor dem 10. Hinterleibsring dorsal eine tiefe, kaudalwärts gewellte Querfurche (Fig. 21, 27). Seiten dieses Ringes mit tiefen Einschnitten. Brlh. gross, ohrartig (Fig. 22, 26, 28). Seitenleiste am 5. Hinterleibsring einfach oder doppelt, die Vertiefung davor frontalwärts geöffnet, rugulos (Fig. 20, 24, 25) 6
— Vor dem 10. Hinterleibsring dorsal keine tiefere Querrinne (Fig. 18). Ohne Seiteneinschnitte am 10. Hinterleibsring. Brlh. reduziert, nicht ohrartig. Seitenleiste am 5. Hinterleibsring kürzer als dieser Ring selbst. Die Vertiefung davor glatt, geschlossen, frontalwärts durch eine weitere, gezähnte Leiste umgeben (vgl. Fig. 37). Puppe 17—21 × 4,8—6,5 mm, rotbraun, rel. glänzend, Hinterleib grob skulpturiert . . . . *Phigalia pilosaria* (Denis et Skiffermüller, 1775).

6 (5) Seitenleiste basal am 5. Hinterleibsring doppelt, viel kurzer als dieser Ring selbst (Fig. 20). Beim Brlh. der vordere Absturz steil, der hintere seicht (Fig. 22). Puppe so gross und gefärbt wie die vorige, Hinterleib feiner skulpturiert. . . . . *Apocheima hispidaria* (Denis et Schiffermuller, 1775)

— Seitenleiste basal am 5. Hinterleibsring einfach, kaum kürzer bis länger als dieser selbst (Fig. 24, 25) Der vordere Absturz des Brlh. etwa glech steil oder seichter als der hintere (Fig. 26, 28). Puppe 20—26 × 6—8 mm 7

7 (6) Punktgrübchen am Hinterleib rel. gröber (Fig 24). Frontal- und Kaudalabsturz des Brlh. rel. gleich gross und -steil (Fig. 26). Seitenleiste basal am 5 Hinterleibsring etwa so lang wie dieser Ring selbst. Sie reicht rd. gleichweit dorsal- und ventralwärts von dem Luftloch (Fig. 24). Puppe rel. rauher, dunkel rotbraun. Querrinne dorsal vor dem 10. Hinterleibsring mit wenigen, aber starken Auslaufern kaudalwarts (Fig. 21). Kremaster meist mit Seitenzahnen (Fig. 21) . . . . . *Biston strataria* (Hufnagel, 1767).

— Punktgrübchen am Hinterleib rel. feiner (Fig. 25). Frontalabsturz des Brlh. viel grösser und seichter als der Kaudalabsturz (Fig. 28). Seitenleiste basal am 5. Hinterleibsring meistens länger als dieser Ring selbst und reicht viel weiter dorsal- als ventralwarts vom Luftloch (Fig. 25). Puppe rel. glatter und oft auch dunkler als die vorige. Querrinne dorsal vor dem 10. Hinterleibsring mit zahlreicheren und kleineren Ausläufern kaudalwärts (Fig. 27). Kremaster schlank, ohne (Fig. 27), oder mit Seitenzähnen . . . *Biston betularia* (Linnaeus, 1758).

8 (4) Brlh. rel. gross, tomentos. Seine Lange grösser als 1/3 der Entfernung zwischen seinem Rande und der Rückenmittellinie (Fig. 42). Dorsal, vor dem 10. Hinterleibsring eine tiefe, kaudalwärts gewellt begrenzte Querfurche (Fig. 29). Lateral an diesem Ringe je ein tiefer Einschnitt. Seitenleiste basal am 5. Hinterleibsring einfach, gerade, länger als ihre doppelte Entfernung vom Luftloch (Fig 32). Die Vertiefung frontalwärts davon glatt. Kremaster oft mit Seitenzähnen, Endgabel oft gezweigt (Fig. 29). Puppe 12—14 × 3,8—4,5 mm, rotbraun, rel. grob skulpturiert . . . . . *Erannis defoliaria* (Clerk, 1759)

— Brlh. rel. kleiner, tomentiert, rugulos, gerunzelt oder glatt. Seine Länge meist höchstens 1/3 des Abstandes zwischen seinem Rand und der Rückenmittellinie Fig. 43—46). Dorsal vor dem 10. Hinterleibsring keine tiefe, nach hinten gewellt begrenzte Querrinne. Es fehlen auch Seiteneinschnitte am 10 Hinterleibsring. Seitenleiste basal am 5. Hinterleibsring doppelt (iFg. 34, 37, 38) oder viel kurzer (Fig. 33) 9

9 (8) Seinteleiste basal am 5 Hinterleibsring doppelt, parallel, nicht geschlossen (Fig. 34). Brlh. tomentiert (Fig. 45). Kremaster schlank, nicht selten mit Seitenzähnen. Puppe 10—13 × 3,5—4,2 mm, rotbraun, rel. rauh skulpturiert . . . . . *Agriopis bajaria* (Denis et Schiffermuller, 1775).

— Seitenleiste basal am 5. Hinterleibsring einfach, kurz, Vertiefung frontal davon glatt (Fig. 33). Brlh. kahl, rel. gross, keilförmig (Fig. 44). Sonst der vorigen Art ähnlich . . . . *Agriopis leucophaearia* (Denis et Schiffermüller, 1775).

— Seitenleiste basal am 5. Hinterleibsring doppelt, geschlossen; die frontale der beiden Leisten gezähnt, die kaudale glatt, Innenraum glatt (Fig. 37, 38). Brlh. klein, oval oder langlich, nicht tomentiert 10

10 (9) Seitenleisten am 5. Hinterleibsring länger als ihr doppelter Abstand von Luftloch (Fig. 37). Frontale Leiste meist mit mehr als 5 Zähnen. Brlh. niedrig, länglich, rugulos (Fig. 46). Oberlippe meist mit weniger schrägen Seiten (Fig. 30). Kremaster oft ohne Seitenzähne und mit kurzer, scharfwinkliger Endgabel. Sonst den vorigen ähnlich . . . *Agriopis aurantiaria* (Hübner, 1796 — 99)

Seitenleisten am 5. Hinterleibsring kurzer als der doppelte Abstand zwischen Ihnen und dem Luftloch (Fig. 38). Frontalleiste meist mit höchstens 5 Zähnen Brlh. erhaben, oval, oben gerunzelt (Fig 43). Oberlippe meist mehr trapezförmig (Fig. 36). Sonst den vorigen ähnlich . . *Agriopis marginaria* (Fabricius, 1777).

(Die Puppe von *Agriopis ankeraria* (Staudinger, 1861) stand mir nicht zur Verfügung, ist in der Tabelle also nicht erwähnt).

BEMERKUNGEN ZU DEN PUPPEN DER EINZELNEN ARTEN DER TRIBUS BISTONINI

Die Tribus Bistonini stellt vom Gesichstpunkte der Puppenmorphologie eine geschlossene, gut begrenzte Gruppe vor. Die Puppenmerkmale wider-

sprechen der Auffassung, die Arten *pilosaria* und *hispidaria* in eine gemeinsame Gattung *Apocheima* Hübner, 1825 (vgl. Bradley et al., 1972) zu vereinigen. Die Puppe von *A. hispidaria* ist mehr denen der Gattung *Biston* Leach, 1815, die von *P. pilosaria* (bis auf die Grösse) denen der Gattung *Agriopis* Hübner, 1825, insbes. *A. aurantiaria*, ähnlich.

Die Puppenmerkmale unterstützen das Zusammenziehen der früheren Gattungen *Lycia* und *Poecilopsis* Harrison, 1910 in eine einzige Gattung *Lycia* Hübner, 1825. Es gibt jedoch deutlichere (vielleicht subgenerische) Unterschiede zwischen der Puppe von *L. hirtaria* und den Vertretern der früheren Gattung *Poecilopsis (L. pomonaria, L. isabellae)*, welche einander besonders nahe stehen.

Die Puppenmerkmale (ähnlich wie die der Raupen) zeugen für eine nahe Verwandschaft der Arten *Biston strataria* und *B. betularia* nicht. Im Komplex mit den Mermalen der Imago würden sie vielleicht ebenfalls für eine subgenerische Einteilung der beiden Arten zeugen.

Auf Grund der Puppenmerkmale kann die Einteilung der früheren Gattung *Erannis* in zwei: *Erannis* Hübner, 1825 für die Art *defoliaria* und *Agriopis* Hübner, 1825 für die übrigen mitteleuropäischen Arten als ziemlich berechtigt angesehen werden. Im Rahmen der Gattung *Agriopis* sind die Arten *aurantiaria* und *marginaria* (auch auf Grund der Raupenmerkmale) einander besonders nahe, die übrigen unterscheiden sich (auch bei den Raupen) deutlich voneinander.

8. Tribus Boarmiini — weist mit Semiothisini und Bistonini einen gemeinsamen Kremasterbautyp auf. Von Bistonini unterscheidet sich durch Anwesenheit der Vorderschenkel und meist auch der Unterlippe. Wenn beide ausnahmsweise fehlen bezw. sehr klein sind, kommen die Punktgrübchen auch am Brustrücken vor.

Von Semiothisini nicht eindeutig zu unterscheiden. Meist sind die Puppen von Boarmiini glatter und glänzender, am Abdomen feiner skulpturiert, die Punktgrübchen manchmal auch dorsal am Thorax anwesend. Oft (zum Unterschied von Semiothisini — Fig. 10, 12 — aber gemeinsam mit vielen Bistonini — Fig. 21, 29) gibt es eine tiefe, kaudalwärts gewellt begrenzte Querrinne dorsal vor dem 10 Hinterleibsring und je ein tiefer Einschnitt lateral an diesem Ring.

9. Tribus Bupalini — durch den Kremaster des vorgehenden Typus, aber ohne Endgabel (Fig. 39), charakterisiert. Anwesenheit der Unterlippe und Vorderschenkel weist auf die Beziehungen zu Boarmiini bezw. Semiothisini auf. In Mitteleuropa nur eine Gattung.

10. Tribus Theriini — nur durch eine mitteleuropäische Gattung *Theria* Hübner 1825 vertreten. Von allen übrigen Tribus der Subfamilie Ennominae stark abweichend und den Gattungen *Alsophila* Hübner, 1825 aus der Unterfamilie Alsophilinae (= Oenochrominae p. p.) sowie *Operophthera* Hübner, 1825 aus der Unterfamalie Larentiinae in Puppenmerkmalen sehr ähnlich. Die Puppe ist gedrungen mit rel. dünner Chitinhaut, Skulptur, insbes. Punktgrübchen fein, Unterlippe, Vorderschenkel und Brlh. nicht anwesend. Kremaster klein, häkchenlos mit zwei stumpfwinklig oder gegeneinander stehenden Spitzen (Fig. 19, 40, 49). Die Gattung *Theria* zeichnet sich z. B. durch vergrösserte und gewölbt hervortretende Mandibel aus (Fig. 11).

11. Tribus Gnophini — ich besitze allzu wenig Material, um diese Tribus beurteilen zu können. Die Puppe von *Siona lineata* (Scopoli, 1763) unterscheidet sich von anderen Vertretern der Subfamilie stark: An der Basis der Fühler gibt es je ein Höcker (Fig. 14), was sonst häufig bei den Vertretern der Subfamilie Geometrinae vorkommt. Kremaster trägt nur zwei ziemlich parallele Dorne am Ende (Fig. 35). Färbung der Puppe rel. bunt, Adern der Vorderflügel stark hervortretend. Mikroskulptur aus sehr dichten, seichten Grübchen. Auch die Art der Verpuppung und das Kokon sind merkwürdig, einigermassen an die der vertreter von Zygaenidae erinnernd.

12. Tribus Caberini und 13. Campaeini entsprechen im Kremasterbau (Fig. 47, 48) und in anderen Merkmalen ganz der Tribus Ennomini und ich konnte im Puppenstadium keine brauchbaren Unterscheidungsmerkmale finden. Bessere Unterscheidungsmöglichkeit gibt es bei den Raupen.

DISKUSSION

Die taxonomische Ausnützung der Puppenmerkmale erschweren zahlreiche Adaptations- und Konvergenzerscheinungen. Der Kremaster als einer der taxonomisch wichtigsten Puppenmerkmale wird infolge Adaptationen besonders stark umgeändert, diese begehen jedoch bei den verwandten Gruppen meist ähnliche, bei den unverwandten unähnliche wege. Diese Adaptationen entsprechen den Funktionen des Kremasters als Haftorgans im Kokon und Stützorgans beim Glättern der Innenwände des Puppengehäuses durch kreisende Bewegungen der Puppe, sowie beim Schlüpfen der Imago (vgl. Giljarov, 1953) Bei den Puppen der Subfamilie Ennominae findet man die schon erwähnten zwei Grundbauformen des Kremasters: eine mit 2—4 Häkchenpaaren und eine mit einer Gabelspitze am Ende. Die erstere besitzt wahrscheinlich vor allem die Haftfunktion im Kokon, meistens in einem Erdkokon in der Waldstreu, aber ursprünglich möglicherweise in einem oberirdischen Kokon, wie bei der Gattung *Abraxas,* wo alle Häkchen gleichgross und stark zerstreut sind. Diese Bauform des Kremasters (Fig. 1) scheint die primitivste zu sein. Von dieser ist die häufigste Bauform bei der Tribus Ennomini (und ebenfalls Angeronini, Caberini und Campaeini — Fig. 3, 13, 47, 48) abzuleiten, die dem Vorkommen der Puppe am Boden in einem Erdkokon entspricht. Der Kremaster bleibt hier kurz, Die Häkchen verschieben sich mehr zu seinem Endteil, die Endhäkchen vergrössern sich. Als spezialisiertere Bauform kann man den Kremaster der Tribus Colotoini bezeichnen: er ist noch stärker verkürzt mit reduzierter Häkchenzahl (Fig. 9). Als Modifikationen, für das oberirdische Vorkommen adaptiert, sind wohl die Kremasterformen der Gattungen *Ennomos* und *Ourapteryx* abzuleiten: Der Kremaster verlängert sich, die Häkchen sind noch mehr im Spitzenteil konzentriert (Fig. 4, 8). Bei *Ourapteryx* betritt diese Adaptation einen abweichenden Weg (umgekehrte Lage der Endhäkchen, Puppenform, Skulptur), was auch von der Hinsicht der Puppenmorphologie die Aufstellung einer gesonderten Tribus (Ourapterygini) für diese Gattung berechtigt.

Den bisher besprochenen Bautyp des Kremasters kann man als einen primitiveren bezeichnen, von dem der andere Hauptbautyp — mit einer Endgabel am Ende (Fig. 12, 21, 41) — sich wohl als Adaptation auf das

Vorkommen der Puppe im Boden — zum Glättern des Puppengehäuses durch Drehbewegungen der Puppe — entwickelt hat. Das geschah in der Weise, dass die Seitenhäkchen verschwanden, die Endhäkchen sich vergössert haben und bis auf die Enden zusammengewachsen sind. Man kann es mit bestimmten Übergangsformen beweisen (Fig. 5, 7); bei der Gattung *Lomaspilis* sind die zwei verwachsenden Endhäkchen noch deutlich sichtbar. Die Unterlippe und Vorderschenkel erscheinen an der Puppenhülle oft parallel. Ihr Vorhandensein kann man als primitiveres, ihr Verschwinden als spezialisiertes Merkmal bezeichnen. Besonders bei der Tribus Boarmiini findet man alle Übergänge des Prozesses der Verschwindung dieser Sklerite der Puppenhülle. In dieser Weise kann man die Tribus Semiothisini als die primitivste und Bistonini als die spezialisierteste von dieser Gruppe bezeichnen. Durch Reduktion der Endgabel als weitere Adaption für das Vorkommen im Boden (eine analoge findet man z. B. am Kremaster der Sphingidae) hat sich, wahrscheinlich von Boarmiini (Vorhandensein der Unterlippe) die Tribus Bupalini entwickelt.

Den Kremasterbautyp der Tribus Theriini kann man ebenfalls als Adaptation auf den Aufenthalt der Puppe im Boden erklären, welche aber einen anderen Weg betritt. Dieser Weg war von dem bei Bistonini stark abweichend und dem der Gattungen *Alsophila* und *Operophthera* sehr ähnlich. Bei allen Arten der erwähnten Gruppen gibt es Brachypterie der Weibchen und ähnliche Grundrisse der Bionomie und Ökologie. Vom Gesichstpunkte des obenerwähnten Prinzipes der Adaptationen des Kremasters kann man bei den Beziehungen zwischen Theriini und Bistonini von reiner Konvergenz, bei denen zwischen Theriini und der Gattung *Alsophila* bezw. *Operophthera* wohl eher von Verwandtschaft sprechen.

Bei der Gattung *Siona* findet man einen extremen Fall der Adaptation auf das oberirdische Vorkommen der Puppe, welche aber einen abweichenden Weg beging, wie bei der Unterfamilie Geometrinae, Rhodometrinae (Gattung *Cyclophora* Hübner, 1882) usw. In dieser Weise sind die Höcker an der Fühlerbasis der Puppe (Fig. 14) als Konvergenzerscheinung (mit den ähnlichen bei der Unterfamilie Geometrinae) zu deuten.

Vom Gesichstpunkte der Puppenmorphologie kann man also die Gattung *Abraxas*, im Rahmen der Unterfamilie Ennominae als die primitivste bezeichnen, von der die Tribus Ennomini (zusammen mit den vom Gesichstpunkte der Puppenmorphologie ganz ähnlichen Angeronini, Caberini und Campaeini) abzuleiten sind. Spezialisierte Modifikationen dieser Gruppen stellen die Tribus Colotoini und Ourapterygini vor. Durch Adaptation auf das Vorkommen im Boden haben sich über Übergangsformen (Gattungen *Lomaspilis*, *Stegania*) wohl Semiothisini, Boarmiini und, als die spezialisiersten, Bistonini entwickelt. Als eine spezialisiertere Abzweigung (wohl von Boarmiini) sind auch Bupalini zu bezeichnen. Anderen Weg betritt die Adaptation zum Vorkommen im Boden bei der Tribus Theriini (mit verwandtschftlichen Beziehungen zu den Gattungen *Alsophila* und *Operophthera*), sowie zum oberirdischen Vorkommen — bei der Gattung *Siona*.

ZUSAMMENFASSUNG

Die vorliegende Arbeit untersucht vom Gesichstpunkte der Puppenmorphologie das System der Unterfamilie Ennominae (Lepidoptera, Geometridae) in der Auffassung von Herbulot (vgl. Urbahn, 1967), bringt

Bestimmungstabellen für die Puppen der wirtschaftlich wichtigen Tribus Bistonini und versucht auf Grund der Puppenmorphologie die verwadtschaftlichen Beziehungen im Rahmen der genannten Unterfamilie zu erklären.

Vom Gesichstpunkte der Puppenmorphologie zeigen sich die Tribus Ennomini, Caberini, Campaeini u. a. als die primitiveren, Semiothisini, Boarmiini und Bistonini einerseits und Theriini anderseits als die spezialisierteren. Die Tribus Abraxini ist uneinheitlich. Zwischen Ennomini, Angeronini, Caberini und Campaeini gibt es im Puppenstadium keine brauchbaren Unterscheidungsmerkmale.

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

Author's address: doc. RNDr. Ján Patočka CSc., Výskumný ústav lesného hospodárstva, Strakonická 2175, 960 92 Zvolen, ČSSR.

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Bezirkmuseum in Tachov

DIE FRUCHTBARKEIT VON MODERLIESCHEN (LEUCASPIUS DELINEATUS) IN SÜDWESTBÖHMEN (PISCES, CYPRINIDAE)

PAVEL ŘEPA

Eingegangen am 27. Dezember 1976

Abstract: Author gives data about the absolute and relative fecundity and about the diameter of eggs in dependence on age and length of the body of sunbleak *(Leucaspius delineatus)* Heckel, 1843) from the Elbe-bassin in southwest Bohemia.

Im folgenden werden die Daten über die Fruchtbarkeit von Moderlieschen bearbeitet, die am ziemlich umfangreichen Material des Bezirksmuseums in Tachov festgestellt wurden. Das Material selbst habe ich im Verlauf einiger Jahre in der Umgebung von Tachov angesammelt. Ich bin bemüht mit dieser Arbeit zur Durchforschung allgemeiner Gesetzmässigkeiten der Fischenfruchtbarkeit, sowie mit Angaben über Moderlieschen aus einem noch nicht bearbeiteten Gebiet zur Klärung der Variabilität biologischer Eingeschaften dieser Art beizutragen.

Es ist mir eine angenehme Pflicht Herrn Dr. K. Pivnička CSc. von der Naturwissenschaftlichen Fakultät der Prager Karls-Universität für das Durchlesen des Manuskripts und wertvolle Hinweise zu danken. Für die Übersetzung ins Deutsch bin ich dem Herrn K. Hofman aus Plzeň zu Dank verpflichtet.

MATERIAL

Ich hatte zur Verfügung 15 Weibchen von Moderlieschen, die am 4. 4. 1969 in einem Nebenarm des Flusses Mže (Mies), bei der Ortschaft Světce, cca 2 km gegen den Strom von Tachov entfernt, erbeutet wurden, ferner 27 Weibchen, die in einem kleinen Teich bei der Ortschaft Mýto, ebenfalls 2 km von Tachov, am 25. 4. 1969 gefangen worden sind, 27 Weibchen aus derselben Lokalität vom 20. 5. 1968 und schliesslich 57 Weibchen aus einem namenlosen Teich bei der Ortschaft Březí, 3 km von Tachov, welche am 19. 5. 1969 erbeutet wurden.

Nähere Angaben über die Grösse der Fische findet man in der Tab. 1. Das gesamte Material befindet sich, wie schon gesagt, im zoologischen Depositarium des Bezirksmuseums in Tachov.

METHODIK

Die gefangenen Fische wurden in 4%iger Formalinlösung fixiert und nach einigen Monaten in 75%igen Alkohol übertragen. Die Körperlänge wurde mittels Schublehre mit Genauigkeit auf 0,1 mm, das Gewicht mit Genauigkeit aut 0,1 g festgestellt. Die Rogenzahl wurde durch direkte Abzählung aller Eier im Eierstock ermittelt. Da es sich um einen Fisch mit portionalem Laichen handelt, wurden beide Portionen (die mit blossem Auge oder unter Lupe leicht voneinander zu unterscheiden sind) getrennt abgezählt; die absolute Fruchtbarkeit ergibt sich dann aus der Summe beider Portionen. Unter dem Mikroskop ist nachher noch eine weitere, und zwar die kleinste Rogenkategorie festgestellt worden, die mit blossem Auge nicht zu erkennen war. Es handelt sich offensichtlich um Reserveoozyten (Drjagin, 1952), die in die absolute Fruchtbarkeit nicht einbezogen wurden. Der Rogendurchmesser

Tab. 1. Durchschnittliche Reifekoeffizienten bei den Moderlieschenweibchen aus verschiedenen Lokalitäten in einzelnen Grössen- und Alterskategorien

Alter Körperlänge in mm	2. Lebensjahr 30–35	35–40	40–45	45–50	50–55	Zusammen	3. Lebensjahr 40–45	45–50	50–55	55–60	60–65	Zusammen	4. Lebensjahr 60–65	65–70	70–75	Zusammen
Světce, Mže 4. 4. 1969	2	3	4	—	—	9	3	3	—	—	—	6	—	—	—	—
	10,0	9,6	6,0	—	—	8,1	11,7	13,0	—	—	—	12,3	—	—	—	—
Mýto, namenloser	—	1	19	2	—	22	—	3	2	—	—	5	—	—	—	—
Teich, 25. 4. 1969	—	8,0	10,2	8,0	—	9,9	—	10,8	10,0	—	—	10,4	—	—	—	—
Aprilfänge Zusammen	2	4	23	2	—	31	3	6	2	—	—	11	—	—	—	—
	10,0	9,1	9,4	8,0	—	9,0	11,7	11,9	10,0	—	—	11,4	—	—	—	—
Mýto, namenloser	—	—	—	4	5	9	—	—	2	7	7	16	—	2	—	2
Teich, 20. 5. 1968	—	—	—	14,2	19,0	16,9	—	—	27,0	20,4	18,8	20,5	—	17,5	—	17,5
Březí, namenloser	—	—	6	12	6	24	—	—	7	15	1	23	5	3	2	10
Teich, 19. 5. 1969	—	—	12,2	14,2	17,7	14,5	—	—	26,3	22,1	13,0	22,9	21,2	15,0	15,0	18,1
Maifänge Zusamen	—	—	6	16	11	33	—	—	9	22	8	39	5	5	2	12
	—	—	12,2	14,2	18,7	14,9	—	—	26,4	21,6	18,1	21,9	21,2	16,0	15,0	18,0

Bemerkung: Die obere Zahl bedeutet Individuenzahl, die untere Reifekoeffizient.

Tab. 2. Abhangigkeit der Rogengrosse der 1. und 2. Portion vom Reifekoeffizienten

Reife-koeffizient	Zahl der Ex.	Eierdurchmesser 1. Portion min.	max.	durchschn.	2. Portion min.	max	durchschn.
0—3	—	—	—	—	—	—	—
3—6	6	0,606	1,114	0,779	0,241	0,461	0,318
6—9	21	0,615	1,110	0,867	0,252	0,680	0,403
9—12	13	0,703	1,465	0,910	0,286	0,745	0,388
12—15	21	0,780	1,370	1,032	0,208	0,682	0,452
15—18	17	0,758	1,390	1,081	0,292	0,685	0,499
18—21	15	0,850	1,560	1,222	0,300	0,685	0,551
21—24	3	1,030	1,600	1,333	0,234	0,680	0,508
24—27	7	0,920	1,510	1,320	0,399	0,790	0,631
27—30	6	1,070	1,490	1,310	0,425	0,785	0,504
30—33	2	0,947	1,530	1,239	0,486	0,682	0,583
33—36	1			1,140			0,670
36—39	—	—	—	—	—	—	—
39—42	—	—	—		—	—	—
42—45	1			1,170			0,685

wurde durch direkte Messung unter Mikroskop mit einem Ocularmicrometer ermittelt. Von jedem Weibchen wurden 50—70 Rogen aus jeder Portion gemessen und dann der Durchschnitt berechnet. Für jeden Fisch wurde Reifekoeffizient nach Nikolskij (1975) festgesetzt. Die relative Fruchtbarkeit wurde durch Dividieren der Eiergesamtzahl durch Gewicht in g der Fische ohne Eingeweide (ohne Nieren) festgestellt (Skorikov, 1911). Koeffizient der Portionalitat zeigt, welchen Prozentsatz die Eierzahl der ersten Portion aus der Gesamtzahl bildet

Tab. 3. Abhangigkeit der absoluten Fruchtbarkeit vom Reifekoeffizienten bei Moderlieschen der gleichen Grossen- und Alterskategorien

Reife-koeffizient	Absolute Fruchtbarkeit 2. Lebensjahr, Korperlange 40—50 mm n	min	max.	ø	3. Lebensjahr, Korperlange 50—60 mm n	min.	max.	ø
0—3	1			210	—	—	—	—
3—6	5	210	678	470	—	—	—	—
6—9	16	220	1202	632	3	1624	1288	
9—12	9	322	1205	769	1			1202
12—15	8	451	1433	893	3	1491	2440	1830
17—18	3	354	1870	812	2	1350	2200	1775
18—21	—	—	—	—	7	1270	2087	1598
21—24	—	—	—	—	—	—	—	—
24—27	—	—	—	—	8	1150	2163	1702
27—30	—	—	—	—	2	1435	1910	1672
30—33	—	—	—	—	1			1692
33—36	—	—	—	—	1			1643
36—39	—	—	—	—	—	—	—	—
39—42	—	—	—	—	—	—	—	—
42—45	—	—	—	—	1			1372

Tab. 4. Abhängigkeit der durchschnittlichen absoluten und relativen Fruchtbarkeit von der Körperlange der Moderlieschen mit Reifekoeffizienten grösser als 1%

Korperlange in mm	Zahl. der Ex.	Absolute Fruchtbarkeit von-bis	Durchschn.	relative Fruchbarkeit von-bis	Durchschn.
40—45	3	555—1140	676	1150—1280	1423
45—50	10	706—1870	1035	360—1030	675
50—55	19	1150—2163	1596	595—1160	849
55—60	22	1270—2200	1614	450—1100	669
60—65	12	1274—2717	2015	400—925	652
55—70	6	1120—3498	2081	330 —720	487
70—75	2	1472—2956	2214	228— 515	372

EIGENE ERGEBNISSE

In der Tab. 1 findet man durchschnittliche Reifekoeffizienten in einzelnen Proben des Materials, die nach Alter und Körpergrösse der Fische eingeteilt wurden. Es ist ersichtlich, dass in den Aprilfängen wesentlich

Tab. 5: Abhängigkeit der absoluten und relativen Fruchtbarkeit von Alter bei den Moderlieschen mit Reifekoeffizienten von 13% und mehr

Lebensjahr	Zahl. der Ex.	Absolute Fruchtbarkeit von-bis	Durchschn.	Relative Fruchtbarkeit von-bis	Durchschn.
2.	23	555—2045	1219	360—1280	813
3.	39	1150—2717	1755	430—1100	712
4.	12	1349—3498	2133	228— 850	524

niedrigere Reifekoeffizienten vorkommen als in den Fängen von Ende Mai. In April findet man auch keine wesentlichen Unterschiede der Reifekoeffizienten in einzelnen Grössenkategorien gleichaltriger Fische und zwischen

Tab. 6. Abhängigkeit der absoluten und relativen Fruchtbarkeit vom Körpergewicht der Moderlieschen mit Reifekoeffizienten über 13%

Gewicht in g	Zahl der Ex.	Absolute Fruchtbarkeit von-bis	Durschchn.	Relative Fruchtbarkeit von bis	Durchschn.
0—0,5	2	555 602	578	1150—1280	1215
0,5—1	1		1140		1209
1—1,5	5	842 —1433	1139	682—1150	834
1,5—2	18	706 —1880	1222	360—1452	856
2—2,5	13	1309— 2163	1703	588—1100	784
2,5—3	16	1274—2717	1789	430— 925	657
3—3,5	7	1350—2625	1819	460— 850	564
3,5—4	3	1585—2717	2177	490— 720	603
4—4,5	1		1877		460
4,5— 5	4	1642—3490	2415	364— 720	514
5—5,5	1		1701		330

den 2- und 3jährigen Fischen. Dagegen in Maifängen unterscheidet sich der jüngste Jahrgang bereits sehr deutlich von älteren Fischen.

In der Tab. 2 wird die Abhängigkeit der durchschnitlichen Rogengrösse vom Reifekoeffizienten festgehalten. Wir sehen, dass vom Reifekoeffizienten von 12% an die Grösse der Rogen aus der ersten Portion nur noch langsam anwächts, und von 18% an praktisch unverändert bleibt. Wenn wir die Abhängigkeit der absoluten Fruchtbarkeit vom Reifekoeffi-

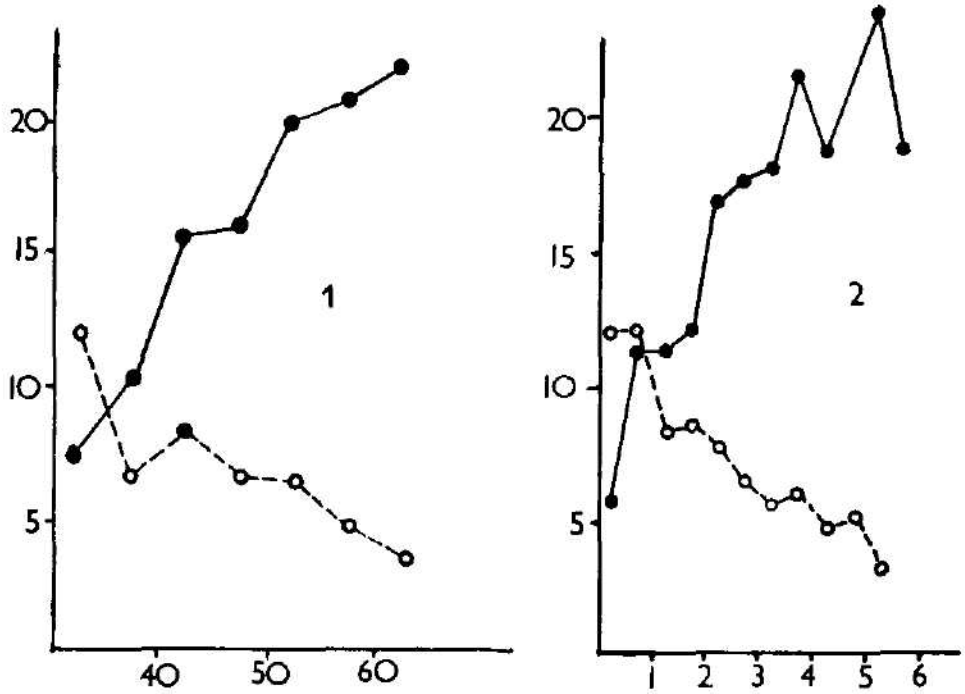

Abb. 1: Die Abhängigkeit relativer und absoluter Fruchbarkeit von der Körperlänge bei moderlieschen.
Vertikalachse: Absolute und relative Fruchtbarkeit in Hunderten.
Horizontalachse: Körperlänge des Fisches in Milimetern.
Leere Kreise-unterbrochene Linie: Relative Fruchtbarkeit.
Volle Kreise-volle Linie: Absolute Fruchtbarkeit.

Abb. 2: Die Abhängigkeit relativer und absoluter Fruchtbarkeit vom Körpergewicht bei Moderlieschen.
Horizontalachse: Grammgewicht.
Die übrigen Erläuterungen siehe Abb. 1.

zienten verfolgen (Tab. 3), sehen wir, dass die Rogenzahl bis zum Reifekoeffizienten von 12% anwächst und nachher ziemlich konstant wird. Daraus ergibt sich, dass bei den Fischen mit einem Reifekoeffizienten von 12% die endgültige Zahl der Rogen erreicht wird.

Bei der Feststellung der relativen und absoluten Fruchtbarkeit und ihrer Abhängigkeit von Alter, Köpergrösse und Gewicht der Fische, zog ich in Erwägung nur Weibchen mit dem Reifekoeffizienten von 13% und mehr. Von diesem Wert höher ist die Rogenzahl vom Reifekoeffizienten

Tab. 7. Abhängigkeit des Portionalitätskoeffizienten vom Reifekoeffizienten bei Moderlieschen. Weibchen aus Maifangen

Reifekoefizient	Zahl der Ex.	Portionalitätskoeffizient von-bis	Durchschnitt
6— 9	7	59—77	65,8
9—12	5	44—75	58,8
12—15	16	48—68	58,9
15—18	19	46—65	52,5
18—21	10	52—68	60,0
21—24	3	60—70	66,9
24—27	8	43—60	56,9
27—30	5	58—69	62,0
30—33	2	58—66	62,0
33—36	1		48,0

nicht mehr abhängig. In den Tab. 4, 5 und 6, finden wir Angaben über die Änderungen der relativen und absoluten Fruchtbarkeit im Zusammenhang mit Alter und Grösse der Fische. Bei Moderlieschen im zweiten Lebensjahr, mit der Körperlänge von 30,9 mm bis 54,0 mm, war die

Tab. 8 Abhängigkeit des Portionalitätskoeffizienten von der absolutenFruchtbarkeit der Moderlieschen

Absolute Fruchtbarkeit	Zahl der Ex.	Portionalitätskoeffizient Von-bis	Durchschnitt
0— 600	18	45—72	60,7
600—12000	31	46—69	59,3
1200—1800	42	44—77	59,7
1800—2400	12	46—66	58,9
2400—3000	5	53—61	56,4

Fruchtbarkeit 555 bis 2045 Eier, durchschnittlich 1219. Bei Fischen im dritten Lebensjahr (Körperlänge 41,2—61,0 mm) gab es 1150 bis 2717 Eier (durchschn. 1755). Bei 12 Fischen im vierten Lebensjahr, deren Köperlänge zwischen 60,0 und 74,3 mm schwankte, war die absolute Frucht-

Tab. 9:Abhängigkeit der Rogengrösse beider Portionen von der Körperlänge der Moderlieschen-Weibchen mit Reifekoeffizienten über 18%

Körpelänge lange in mm	Zahl. der Ex.	Rogendurchmesser 1. Portion von-bis	Durchschn.	2 Portion von-bis	Durchschn.
45—50	2	0,800—1,410	1,100	0,300—0,890	0,538
50—55	15	0,910—1,490	1,245	0,234—0,890	0,541
55—60	19	0,945—1,530	1,218	0,462—0,790	0,757
60—65	4	1,145—1,372	1,270	0,535—0,0665	0,593
65—70	2	1,160—1,610	1,385	0,535—0,602	0,593

barkeit 1349—3498 Eier (durchschn. 2133). Man sieht, das in allen Fällen die absolute Fruchtbarkeit anwächst und die relative Fruchtbarkeit zurückgeht. Die Abhängigkeit von Grösse und Gewicht des Fisches ist aber keineswegs linear (Abb. 1 und 2); von einer bestimmten Grössenkategorie an wird die Eierzahlzunahme deutlich langsamer. Bei Fischen über 65 mm beobachtet man mit anwachsender Körpergrösse ständige Verminderung der Eierzunahme, aber ein völliger Stillstand tritt in dieser Beziehung bei der verfolgten Grössenspanne (bis 75 mm) nich ein. Dagegen bei den Fischen, die das Gewicht von 2,5 g erreicht haben, vermindert sich die Eierzahlzunahme so stark, dass die absolute Fruchtbarkeit über diese Grenze praktisch unverändert bleibt.

Ferner verfolgte ich den Anteil beider Portionen an der Gesamtzahl der Rogen. Der Koeffizient der Portionalität wies im Zusammenhang mit Alter und Grösse keine Änderungen auf (wegen Platzmangel führe ich diese Angaben nicht an). Interessant ist, dass beim Anwachsen des Reifekoeffizienten keine Änderungen des Portionalkoeffizienten zu verzeichnen waren (Tab. 7). Bis zu einem gewissen Grad zeigt sich der Portionalkoeffizient von der absoluten Fruchtbarkeit abhängig (Tab. 8). Mit anwachsender Eierzahl kann man mässiges Sinken des Anteils der ersten Portion beobachten. Interessant ist auch der Vergleich der von mir festgestellten Portionalitätskoeffizienten mit den Angaben von Abdurachmanov (1962) über *Leucaspius delineatus caucasicus*. Während bei Moderlieschen aus Südwestböhmen die erste Portion ein wenig grösser ist als die zweite, ist bei Moderlieschen aus Kaukasus die zweite Portion deutlich grösser als die erste.

Ich verfolgte ferner die Abhängigkeit der Rogengrösse von Alter und Köpergrösse der Fische. Mit wachsender Körpergrösse steigt der Durchmesser der Rogen aus der ersten Portion mässig an. Betracht wurden allerdings nur Fische mit Reifekoeffizienten von 18% und mehr gezogen, bei denen die Rogengrösse nicht mehr vom Reifekoeffizienten beeinflusst wird. Wegen Platzmangel führe ich nur die zahlenmässigen Angaben über die Abhängigkeit des Rogendurchmessers von der Körperlänge der Fische an.

DISKUSSION

Unsere Ergebnisse (Tab. 1) zeigen, dass das Moderlieschen seine Geschlechtsreife im 2. Lebensjahr erreicht, was mit den Angaben von Balon, 1968; Lebeděv et all., 1969; Holčík, Hensel, 1971 und Žukov, 1965 übereinstimmt. Was die absolute Fruchtbarkeit betrifft, so stimmen meine Angaben (550—3500 Rogen mit denen von Holčík, Hensel, 1971 auffälig überein; eine Ähnlichkeit zeigt sich auch im vergleich mit den Daten von Žukov (1965) — nur die maximale Grösse der absoluten Fruchtbarkeit des Moderlieschens wird bei diesem Autor Autor mit grösserer Rogenzahl (4000) angegeben. Wesentliche Differenzen ergeben sich beim Vergleich meiner Daten mit denen von Abdurachmanov (1962): die Fruchtbarkeit der Moderlieschen aus Kaukasus überstieg in beiden Portionen die Rogenzahl von 600 niemals; sie ist also wesentlich niedriger als bei den Moderlieschen aus Südwestböhmen. Abdurachmanov (1962) verfolgte allerdings nur kleinere Fische;

alle seine Individuen befanden sich erst im 2. Lebensjahr. Trotzdem ist eine wesentlich kleinere Fruchtbarkeit der Moderlieschen aus Kaukasus, im Vergleich mit denen aus Südwestböhmen, kaun zu bezweifeln.

ZUSAMMENFASSUNG

Es wurden Rogenzahl, Anteil beider Portionen der Eier und Grösse derselben bei 126 Weibchen von Moderlieschen verfolgt, welche in April–Mai 1968–1969 in der Umgebung von Tachov in Südwestböhmen erbeutet wurden.

Es wurde festgestellt:

1. Moderlieschen erreicht seine Geschlechtsreife im 2. Lebensjahr.
2. Vom Reifekoeffizienten von 13% an ist bei ihm die Rogenzahl als definitiv anzusehen; weitere Zunahme ist unbedeutend.
3. Die absolute Fruchtbarkeit schwankt zwischen 500–3500 Rogen in beiden Portionen; relative Fruchtbarkeit beträgt 228–1300 Rogen auf 1 g des Fischgewichts.
4. Mit anwachsender Grösse und höheren Alter der Fische sinkt die relative Fruchtbarkeit, während die absolute höher wird.
5. Anteil der 1. und 2. Eierportion weist im Zusammenhang mit Grösse und Alter der Fische keine Änderungen auf; geringe Differenzen zeigen sich in Abhängigkeit von der absoluten Fruchtbarkeit.
6. Grösse der Rogen aus der ersten Portion schwankt zwischen 0,8–1,5 mm; mit anwachsender Körpergrösse und höherem Alter des Fisches kann man eine geringe Zunahme des Rogendurchmessers beobachten.

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Department of Zoology, University of Agriculture, Brno
Institute of Zoology, University of Oslo

SOME NOTES ON THE MORPHOLOGY OF THE NEMATODE HELIGMOSOMOIDES POLYGYRUS

FRANTIŠEK TENORA, RICHARD WIGER, VLASTIMIL BARUŠ

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Abstract: New data on the morphology of *Heligmosomoides polygyrus* (Dujardin, 1845) sensu Durette-Desset, 1968 are presented. The ultrastructure of body surface and the morphology of bursa copulatrix have been studied by scanning electron microscopy. The importance of the observed morphological characters in systematics of *H. polygyrus* is discussed.

The metod of scanning electron microscopy (SEM) has not been used in other studies concerning morphological and anatomical characters of *H. polygyrus*. We have applied this metod in our studies.

MATERIAL AND METHODS

Our material consisted of *H. polygyrus* (males, 15 specimens) from *Apodemus sylvaticus* (loc. Oslo, Norway). Scanning electron microscopic studies were carried out at the University of Oslo (the method see Ogden (1971).

RESULTS

Heligmosomoides polygyrus (Dujardin 1845)

Syn.: *Heligmosomum skrjabini* (Schulz, 1926) Skrjabin et Schikhobalova, 1952 nec *Heligmosomum polygyrum* (Duj., 1845) sensu Travassos, 1937; Skrjabin, Schikhobalova, Schulz 1954. Other syn. Tenora, 1966, Durette-Desset, 1971.

Attention was paid to the morphology and anatomy of bursa copulatrix and body cuticle. It was found that bursa copulatrix is supported by a solid cuticular border, which forms an independent structure (Figs. 1, 2). This border is of irregular shape and forms a part of the genital apparatus. Most probably it directs also the movement of spicules. The spicules are joined in their lower part and form a chained in the median longitudinal line to lead spermiae (Fig. 3). Longitudinal cuticular ridges are regulary arranged on the surface of body (Fig. 4).

DISCUSSION

Our results show the importance of the study of the structure of bursa copulatrix in the systematics of species belonging to *Heligmosomoides*. The presence of one or two cuticular borders in bursa copulatrix was the reason of an earlier discussion about the synonymy of *H. azerbaidjani* Schachnazarova, 1949 and *H. polygyrus* (Duj., 1845). In *H. azerbaidjani*, two cuticular

borders in bursa copulatrix were observed in whole mounts (Schachnazarova, 1949; Tenora, 1958), while in *H. polygyrus*, there was only one cuticular border (Baylis, 1926; Tenora, 1958). In spite of this difference, Tenora (1966) and Durette-Desset (1971) consider *H. azerbaidjani* a synonym of *H. polygyrus*.

Further S.E.M. studies of this character, using the material from both geographical regions, will probably elucidate the problem of the validity of *H. azerbaidjani*. The scanning electron micrographs of the body cuticle of *H. polygyrus* showed that both the number and arrangement of cuticular ridges are characteristic of this species and fully conform to the diagnosis of this genus published by Durette-Desset (1971).

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

Authors' addresses Doc. Dr. F. Tenora, CSc., Department of Zoology, University of Agriculture, Zemědělská 1, 662 65 Brno, ČSSR.

Dr. R. Wiger, Institute of Zoology, University of Oslo, Oslo 3, Blindern, Norway.

Ing. V. Baruš, DrSc., coresp. member of ČSAV, Institute of Vertebrate Zoology ČSAV, 603 65 BRNO, Květná 8.

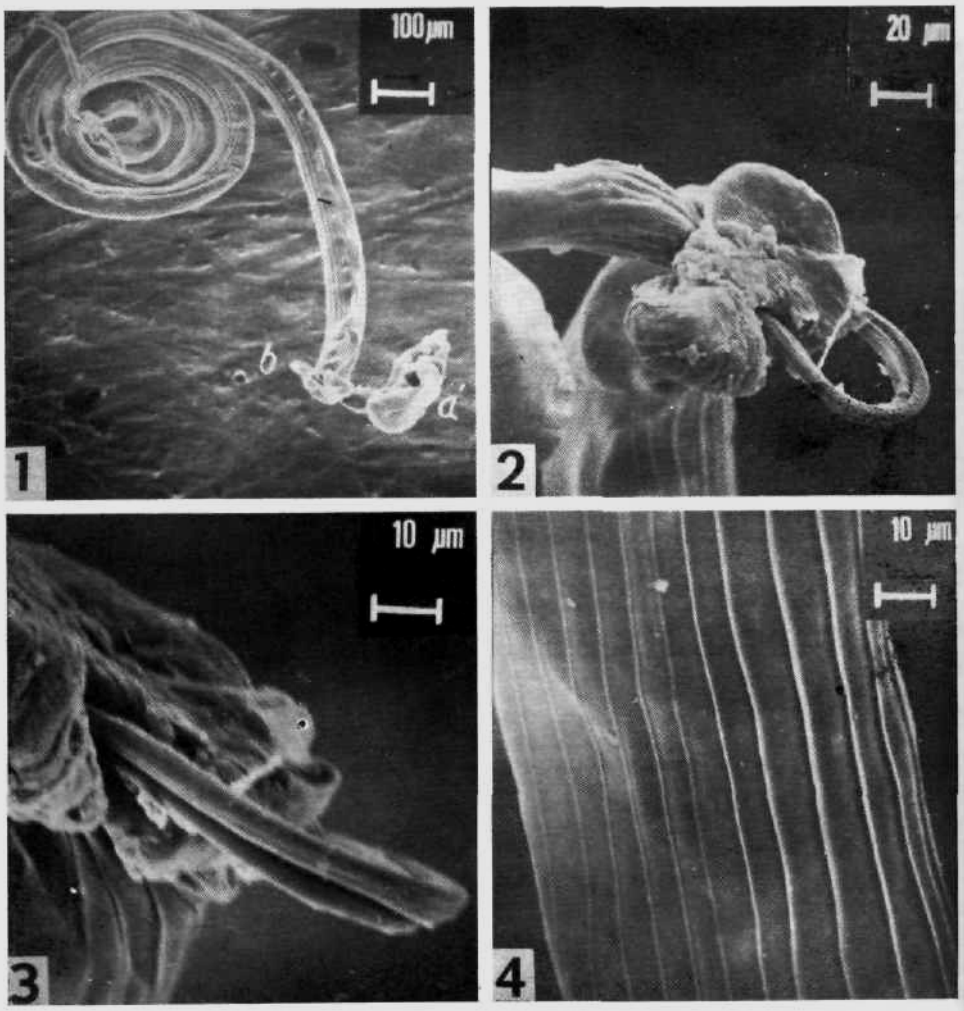

Fig. 1: *H. polygyrus* — male — from *Apodemus sylvaticus*
a) bursa copulatrix, b) solid cuticular border
Fig. 2: *H. polygyrus*. Solid cuticular border — detail
Fig. 3: *H. polygyrus*. The part of the genital apparatus with spicules forming a channel
Fig. 4: *H. polygyrus*. Longitudinal cuticular ridges arranged on the surgace of body

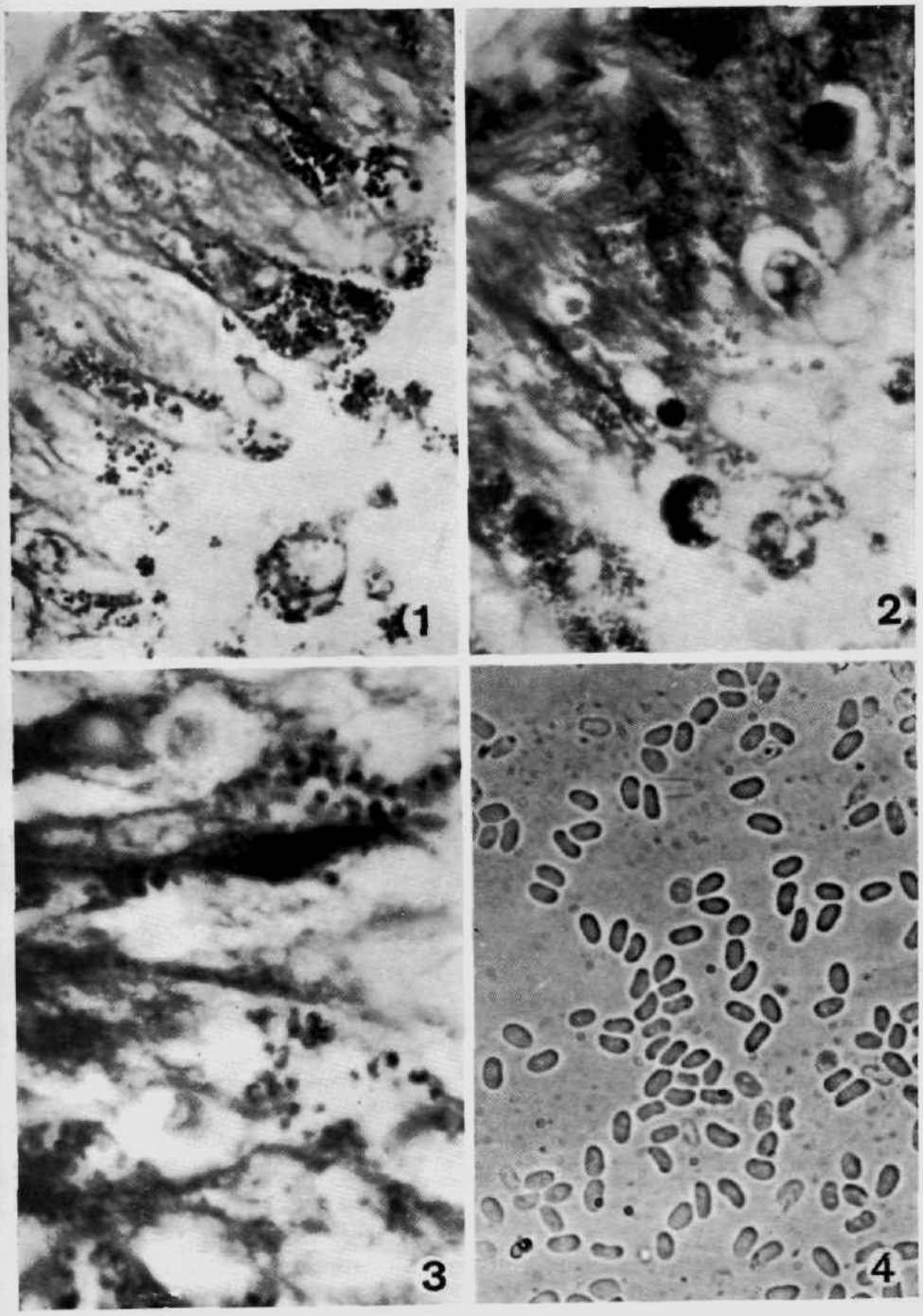

Fig. 1. *Pleistophora grossa* in the midgut of *Chrysomella grossa,* Infected cells are scattered in the epithel. Magn. = 400×.
Fig. 2. *Pleistophora grossa* is liberated from infected cells in round groups. Magn. 330×.
Fig. 3. Vacuolated epithelial cells of *Leptinotarsa decemlineata* at the time of rupture of the cells. Magn. 1000×.
Fig. 4. Groups of *Pleistophora grossa* spores in wet mount. Magn. 1500×.

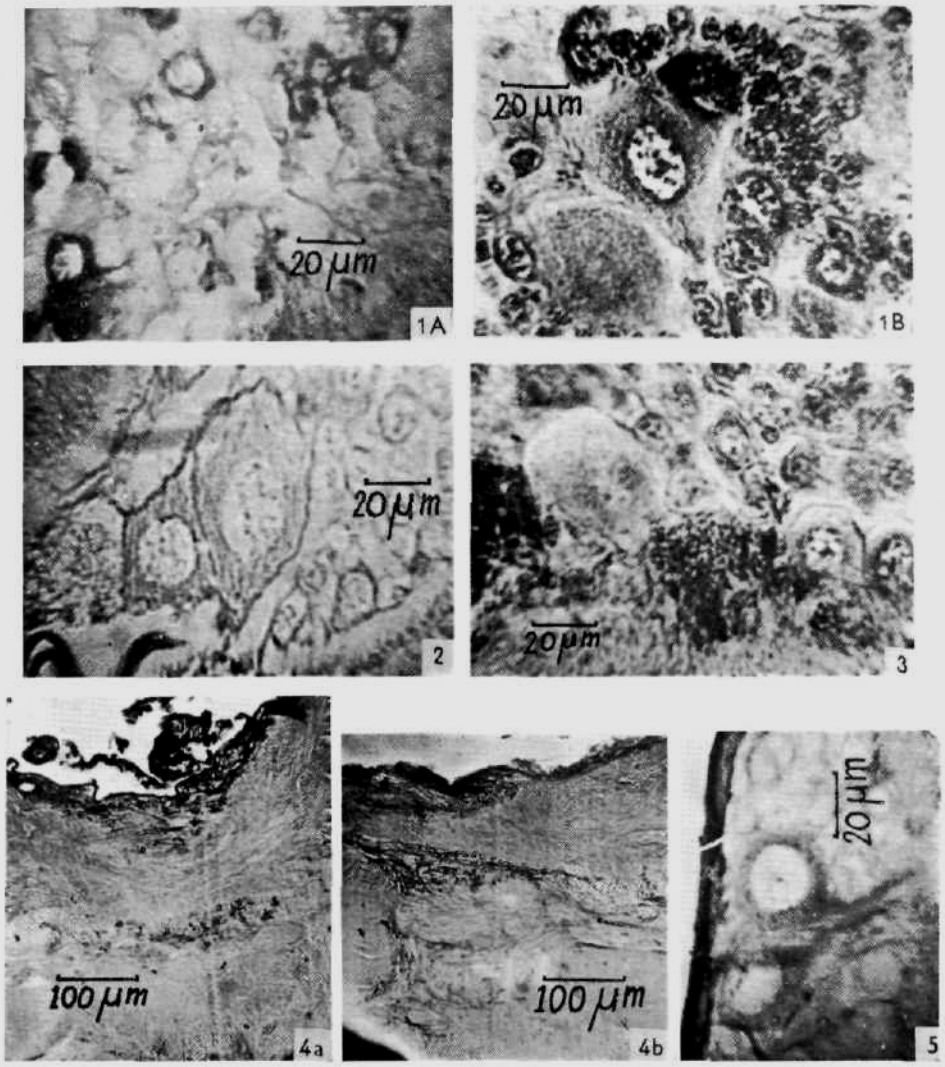

Fig. 1. Control sections showing pars intercerebralis neurosecretory cells in the brain of *P. americana*
A) AF (aldehyde fuchsin) — reaction, small cells
B) CAHP (chrome alumhaematoxylin-phloxin of Gomori) — reaction
Fig. 2. Section showing the effect of $PGF_{2\alpha}$ on the neurosecretory neurocytes, one hour after injection. Note general depletion of AF-positive material, appearance of cytoplasmic vacuolations and undulation of the cell membrane in the large and medium types of cells.
Fig. 3. Section showing the conditions of the large and medium cells, 24 hours after $PGF_{2\alpha}$ injection. Note persistent depletion of CAH-positive NSM and peripheral vacuolation in the cytoplasm.
Fig. 4. Section showing the neuropile area of the protocerebrum.
a) Control. (Note poor concentration of CAHP-positive material.)
b) Experimental. (Note abundance of CAHP-positive material 24 hrs after $PGF_{2\alpha}$ administration.)
Fig. 5. Section showing the condition of the small cells, 48 hrs after $PGF_{2\alpha}$ injection. Note distinct AF-positive material invading the perikarya and the axonal tracts.

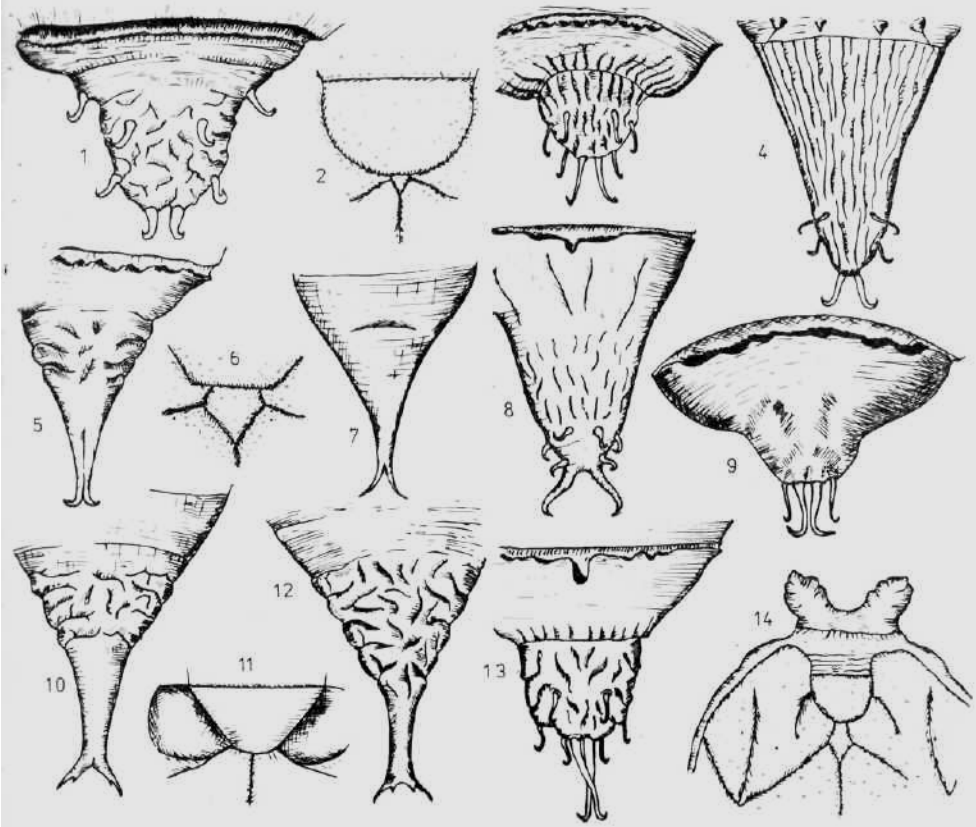

Fig. 1. Kremaster der Puppe von *Abraxas grossulariata* (Linnaeus, 1758), von oben. — Fig. 2. Oberlippe und Unterlippe der Puppe von ders. Art. — Fig. 3. Kremaster der Puppe von *Selenia dentaria* (Fabricius, 1775), von oben. — Fig. 4. Kremaster der Puppe von *Ennomos quercinaria* (Hufnagel, 1767), von oben. — Fig. 5. Kremaster der Puppe von *Lomaspilis marginata* (Linnaeus, 1758), von oben. — Fig. 6. Ober- und Unterlippe der Puppe von *Stegania dilectaria* (Hübner, 1790). — Fig. 7. Kremaster der Puppe ders. Art, von oben. — Fig. 8. Kremaster der Puppe von *Ourapteryx sambucaria* (Linnaeus, 1758), von oben. — Fig. 9. Kremaster der Puppe von *Colotois pennaria* (Linneaeus, 1761), von oben. — Fig. 10. Kremaster der Puppe von *Semiothisa wauaria* (Linnaeus, 1758), von oben. — Fig. 11. Oberlippe und Mandibel der Puppe von *Theria rupicapraria* (Denis et Schiffermüller, 1775). — Fig. 12. Kremaster der Puppe von *Semiothisa liturata* (Clerk, 1759), von oben. — Fig. 13. Kremaster der Puppe von *Angerona prunaria* (Linnaeus, 1758), von oben. — Fig. 14. Vorderteil des Kopfes der Puppe von *Siona lineata* (Scopoli, 1763), von unten.

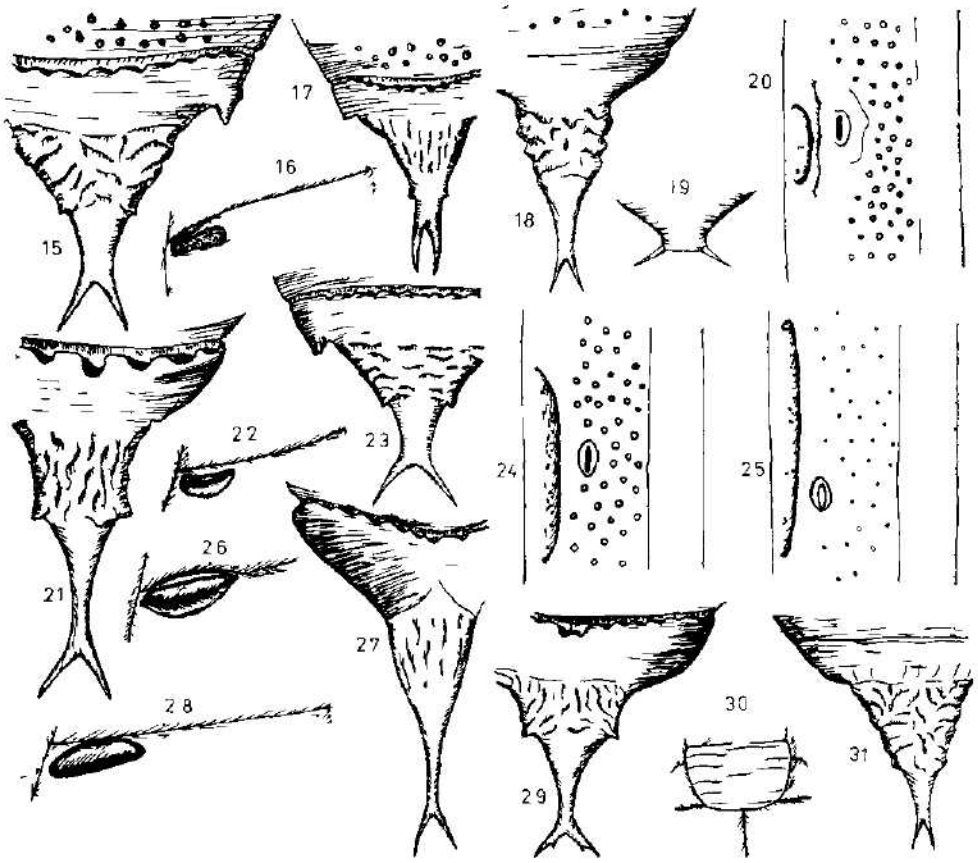

Fig. 15 Kremaster der Puppe von *Lycia pomonaria* (Hubner 1790), von oben — Fig 16 Brlh der Puppe von *Lycia hirtaria* (Clerk, 1759) — Fig 17 Kremaster der Puppe von *Lycia isabellae* (Heslop — Harrison, 1914), von oben — Fig 18 Kremaster der Puppe von *Phigalia pilosaria* (Denis et Schiffermuller, 1775), von oben — Fig 19 Kremaster der Puppe von *Theria rupicapraria* (Denis et Schiffermuller, 1775), von oben — Fig 20 Seitenleiste am 5 Hinterleibsring der Puppe von *Apocheima hispidaria* (Denis et Schiffermuller, 1775) — Fig 21 Kremaster der Puppe von *Biston strataria* (Hufnagel, 1767), von oben — Fig 22 Brlh der Puppe von *Apocheima hispidaria* — Fig 23 Kremaster der Puppe von *Lycia hirtaria*, von oben — Fig 24 Seitenleiste am 5 Hinterleibsring der Puppe von *Biston strataria* — Fig 25 Dasselbe bei *B betularia* (Linnaeus, 1758) — Fig 26 Brlh der Puppe von *Biston strataria* — Fig 27 Kremaster der Puppe von *B betularia*, von oben — Fig 28 Brlh der Puppe ders Art — Fig 29 Kremaster der Puppe von *Erannis defoliaria* (Clerk, 1759), von oben — Fig 30 Oberlippe der Puppe von *Agriopis aurantiaria* (Hubner, 1796–99) — Fig 31 Kremaster der Puppe von ders Art

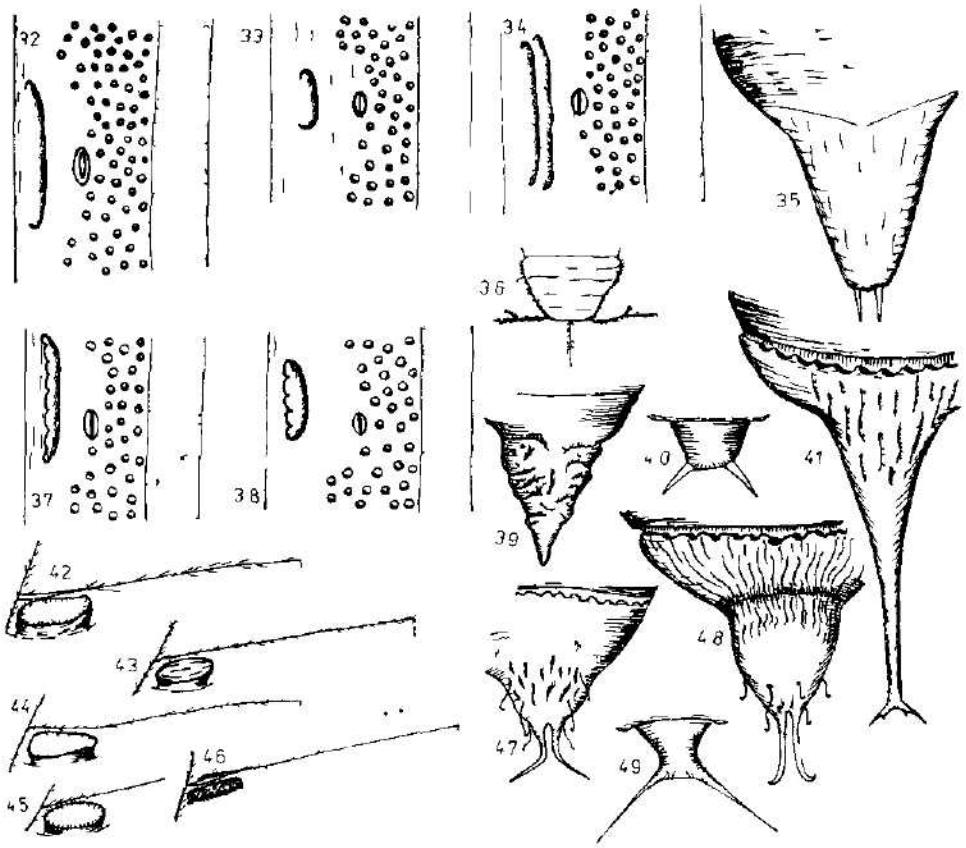

Fig 32 Seitenleiste am 5 Hinterleibsring der Puppe von *Erannis defoliaria* — Fig 33 Dasselbe der Puppe von *Agriopis leucophaearia* (Denis et Schiffermuller 1775) — Fig 34 Dasselbe der Puppe von *A bajaria* (Denis et Schiffermuller 1775) — Fig 35 Kremaster der Puppe von *Siona lineata*, von oben — Fig 36 Oberlippe der Puppe von *Agriopis marginaria* (Fabricius 1777) — Fig 37 Seitenleiste am 5 Hinterleibsring der Puppe von *A aurantiaria* — Fig 38 Dasselbe der Puppe von *A marginaria* — Fig 39 Kremaster der Puppe von *Bupalus piniarius* (Linnaeus, 1758) von oben — Fig 40 Kremaster der Puppe von *Alsophila aescularia* (Denis et Schiffermuller, 1775) von oben — Fig 41 Kremaster der Puppe von *Ematurga atomaria* (Linnaeus 1758) von oben — Fig 42 Bilh der Puppe von *Erannis defoliaria* — Fig 43 Dasselbe der Puppe von *Agriopis marginaria* — Fig 44 Dasselbe der Puppe von *A leucophaearia* — Fig 45 Dasselbe der Puppe von *A bajaria* — Fig 46 Dasselbe der Puppe von *A aurantiaria* — Fig 47 Kremaster der Puppe von *Lomographa bimaculata* (Fabricius, 1775) von oben — Fig 48 Dasselbe von der Puppe von *Campaea honoraria* (Denis et Schiffermuller 1775) — Fig 49 Dasselbe von der Puppe von *Operophthera fagata* (Scharfenberg, 1805)