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PROCEEDINGS OF THE
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VOLUME 43 JULY 1976 NUMBER 2

Body Wall Organization of the Acanthocephalan, *Macracanthorhynchus hirudinaceus*: A Reexamination of the Lacunar System[1]

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ABSTRACT: New components observed in the lacunar system of *Macracanthorhynchus hirudinaceus* (Acanthocephala) in relation to other body layers have indicated that the system is much more extensive than previously described. Using Procion dyes and India ink in inverted and injected living worms, the flow pattern from the dorsal or ventral longitudinal lacunar channel has been followed. A model of this system shows a major new pair of channels, the medial longitudinal lacunar channels, located adjacent to the pseudocoel and approximately equidistant from the dorsal and ventral longitudinal lacunar channels. Canals are also observed which interconnect the two major canals. In addition, an anastomosing canal system covers the medial surface of the longitudinal muscle layer. This canal system is separate from the lacunar system previously described and is much more fragile. The relationships between these new components of the body wall and those previously described indicate that the contents of the pseudocoel are readily accessible to the "circulatory" system of the worm.

The body wall and the associated musculature of acanthocephala has been of interest to biologists since the early studies of Goeze (1782) and Zeder (1800) who represented both circular and longitudinal muscle layers as a single homogeneous tissue. Later authors such as Schneider (1868) and Kaiser (1893) recognized and described a complex body wall consisting of different contractile elements, tubular channels, and fibrous epidermis. However, from the earliest studies authors have recognized the importance of the outer surface in the uptake of nutrients and regulation of osmotic balance and many of the more recent studies are directed to this end. The interest that has been generated in recent years in the construction of the body wall of Acanthocephala is the result of renewed efforts to understand its physiology. Much of the morphological work has been associated with an attempt to understand the ultrastructure of the outer most layers which in general terms consists of the tegument or epidermis (hypodermis of some authors). Butterworth (1969), Crompton and Lee (1963, 1965), and Hammond (1968) described the surface as consisting of a system of surface pores and canals which penetrate into shallow hypodermal cavities. Wright and Lumsden (1961, 1970) and Byram and Fisher (1973) further report that these peripheral canals are continuous with canalicular crypts. Work completed on the surface of *Moniliformis dubius* indicates that it is also covered by an extraneous coat which acts as a molecular sieve screening larger particles from the surface (Wright and Lumsden, 1968; Byram and Fisher, 1974). The cuticle itself is penetrated by numerous surface crypts which largely act as the absorptive surface of the worm. Pinocytosis and lysosomal activity associated with the surface of the crypts prepares the nutrients for immediate cellular use and for general distribution via the lacunar system. The smaller elements of the latter are in close prox-

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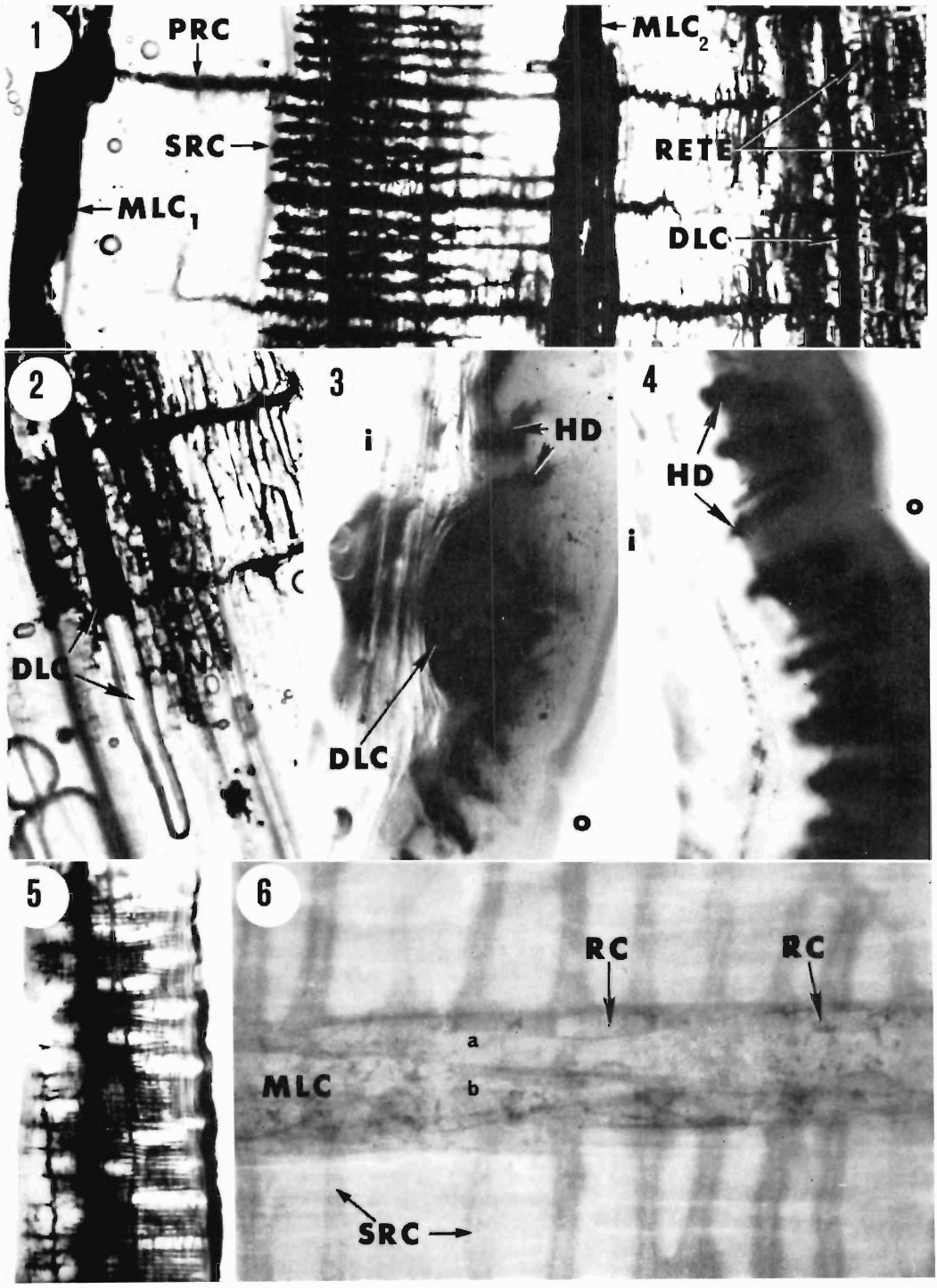

imity to the surface crypts and therefore readily available to remove the products of digestion or other types of metabolism. The generally accepted model is that all of these peripheral canals eventually lead into the main dorsal and/or ventral longitudinal lacunar channel also located in the hypodermis. However, there is no indication that this lacunar system ever extends beyond the hypodermis.

Lee (1966) indicated that about the only agreement between earlier authors and present day investigators seemed to be that the thin outermost layer should be called the cuticle and the innermost layers the circular and longitudinal muscles, the latter being adjacent to the pseudocoel. Crompton and Lee (1965) working on *Polymorphus minutus* use classic terminology, whereas Nicholas and Mercer (1965) described the ultrastructure of *Moniliformis dubius* body wall (called the tegument by them) using numbers for the different layers and not names. The term epidermis is suggestive of an organized cellular layer and cuticle connotes a non-living layer. Therefore, we have adopted the term, tegument. It is probable that the syncytial nature of the outer body wall has contributed to the many different terms used for the various layers. It would also appear that the use of fixed specimens has resulted in investigators overlooking some features.

It is the purpose of this article to present a model of the lacunar system in the body wall of *Macracanthorynchus hirudinaceus* (Pallas, 1781) and to put it into perspective with the muscles.

Materials and Methods

Acanthocephala, *M. hirudinaceus*, were collected at the Hunter Packing Company, East St. Louis, Illinois, and transported to the laboratory in a Dewar containing intestinal contents. Preparation of the worms for scanning electron microscopy has been described by Miller and Dunagan (1971). Preparation of the worms for light microscopy, including glycerol whole mounts, has been explained by Dunagan and Miller (1970) and the inversion technique by Hightower et al. (1975). Lacunar channels and canals were observed following injection with a variety of substances including India ink (Figs. 1–5), Procion Brilliant Red (H-3BNS) (Fig. 6), Azure Blue, Neutral Red, and Methylene Blue. Dye solutions were filtered immediately before use. Glycerol preparations enabled certain of the layers in the epidermis to be separated from the underlying dermis aiding photography as well as interpretation of structure.

To aid in the identification of the various channels and body parts a list of abbreviations is provided in Table 1.

Table 1.

Abbreviation	Term
CM	Circular muscle
DLC	Dorsal lacunar channel
HC	Hypodermal canals
HD	Hypodermal ducts
IRLT	Inner radial layer of tegument
LM	Longitudinal muscle
MLC	Medial longitudinal channel
N	Nucleus
PRC	Primary ring canal
RC	Radial canal
RN	Rete network
RNC	Rete network canal
SRC	Secondary ring canal
T	Tegument
VLC	Ventral lacunar channel

Results and Discussion

Hamann (1891) and Kaiser (1893) established that the Acanthocephala lack a direc-

←

Figures 1–6. Dye injected *M. hirudinaceus*. 1. India ink injection in inverted living worm. The medial longitudinal channels are designated MLC-1 and MLC-2 between which is the ventral lacunar channel where the dye was introduced. The dye has completely filled both MLC and both DLC and VLC. Notice the uniform number of SRC located between each PRC and the complicated morphology of the MLC. 2. Similar to Figure 1 except that an air bubble helps delineate the DLC. 3. A glycerinated worm sectioned with a razor blade into ¼" sections and viewed from a slight angle to the inside. Note the prominent position of the DLC with its medial covering of muscle. 4. Similar to Figure 3 but showing the HD apart from the DLC. 5. A portion of an inverted glycerinated worm injected with Azure Blue. 6. A Procion Red injection of the MLC of a living worm. Note the hole made by the entry of the RC. Note also the cellular complexity of the channel.

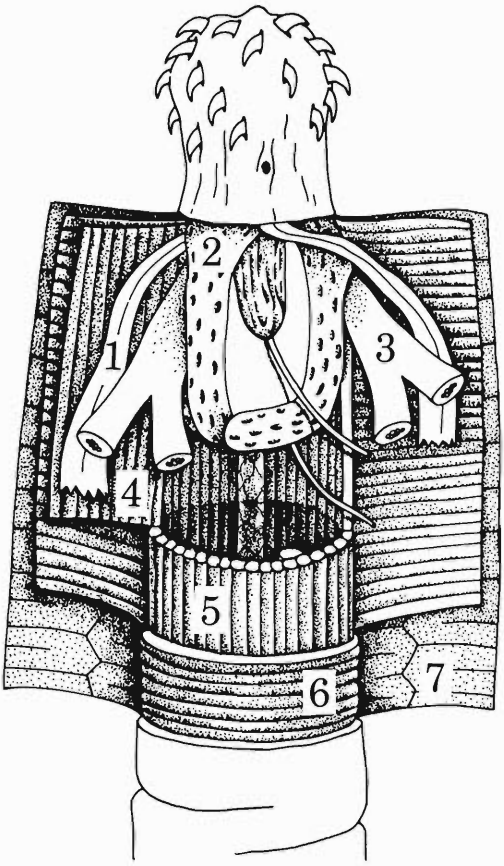

Figure 7. Schematic diagram of *M. hirudinaceus* showing the following major structures: 1) lemniscus, 2) proboscis protrusor muscle, 3) proboscis retractor muscle, 4–5) longitudinal muscle of body wall, 6) circular muscle of body wall, 7) tegument.

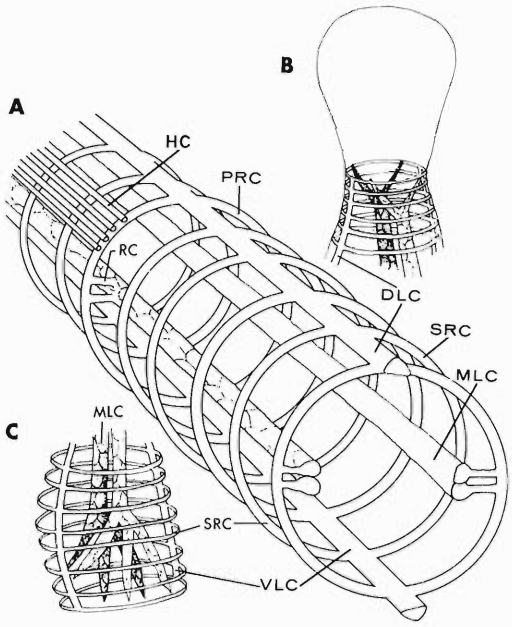

Figure 8. Diagrammatic illustration of the vascular system of *M. hirudinaceus*. Notice the anterior and posterior arbor-like termination of the MLC.

tional flow circulatory system but possess instead a loose network of tube-like cavities without linings or pumps. These cavities form channels known as the lacunar system which interconnect forming a definite pattern in some species but less so in others. Even in those genera such as *Centrorhynchus* where the lacunar system is described as being net-like and without a definite pattern (Hyman, 1951) there are usually a pair of main longitudinal channels that traverse the length of the body with numerous connections to the remainder of the system.

The general construction of the body of an acanthocephalan is depicted in Figure 7. In this view, the tegument is represented by the outer layer on top of which are located the circular and longitudinal muscles. The medial longitudinal lacunar channels are the only lacunar components represented.

According to Kaiser (1893), C. A. Rudolphi in 1809 was the first to observe the lacunar system (Gefässsystem) of Acanthocephala and C. H. A. Burow in 1836 was the first investigator to try to determine the distribution of tubes in this system. Kaiser (p. 24) indicates that Burows described two large lateral canals which extended to the area of the neck where they divided forming tree-like ramifications—an observation which we have confirmed (Fig. 8B, C). Schneider (1868) also studied the lacunar system of *M. hirudinaceus* and concluded that it consisted of two parts which were not connected with each other. The first part included the anterior proboscis to immediately posterior to the attachment of the lemnisci and thus included the lemnisci. The second part was distributed throughout the remainder of the body wall. Leuckart (1876) and Greeff (1864)

took a slightly different position and stated that both lacunar systems were "in limited connection" at the base of the neck. It is now generally accepted that the lacunar network of the trunk wall is distinct from that of the proboscis and praesoma, the latter presumably having no connection with the lacunar channels of the trunk body wall.

Workers that originally described the lacunar system as well as those that have written general descriptions (Rauther, 1930; Hyman, 1951; Baer, 1961) have all indicated that it is restricted to the inner radial layer of the tegument and does not open to the outside or communicate with any body structure medial to the muscles. However, it is clear from our studies that this is not the case with the lacunar system of *M. hirudinaceus*. Kaiser (1893) also indicated that several earlier investigators described additional components. Rauther (1930, fig. 497) illustrated the dorsal and ventral lacunar channels under the term "mediane hauptkanale." He also depicted the medial longitudinal lacunar channels (MLC) but did not label these structures in his illustration of a cross section of *M. hirudinaceus*. However, a pair of MLC are located adjacent to the circular muscles and are connected to circular lacunar channels (Figs. 1, 5, 6) which we call primary ring canals (PRC). The latter are connected to the MLC via regularly spaced transverse medio-lateral connections termed radial canals (RC) (Figs. 8A, 9). These small tubular connections pass between the circular and longitudinal muscles and are easily overlooked in fixed tissue. However, when the dorsal or ventral longitudinal lacunar channel of living worms is injected with dye or India ink (Figs. 1, 5), the substance moves progressively through the following sequence of channels (Fig. 9): dorsal or ventral longitudinal lacunar channel (DLC, VLC), primary ring canals (PRC), radial canals (RC), medial longitudinal lacunar channel (MLC). Meyer (1931, p. 124) included this channel in his text, Fig. 1, under the name: "linke (rechte) Muskelbeutel" but did not enlarge on the morphology of this structure and gave no information regarding its function or possible link with the "ventrales (dorsales) hypodermislängsgefäss."

Kilian (1932) studied the general morphology of the body wall of *Hamanniella microcephala*, a large acanthocephalan from South American opossums. In his discussion of the body wall and lacunar system (p. 252–257) he did not mention a MLC along the medial surface of the longitudinal muscles but did show (Fig. 1, p. 252) a structure referred to as a "Ringmuskelmarkbeutel" in the proper position for the MLC. Unfortunately, Kilian (Figs. 4 and 5, p. 255) schematically illustrated the lacunar system in such an abbreviated fashion that only the DLC and VCL (Hauptlacunenstamm) are apparent in his diagram of a cross section. His discussion also raises some interesting questions since he indicated (p. 271) that certain of these canals connect with the circular muscles—"Bei naherer Betrachtung sieht man jedoch (Abb. 15) dass diese "Gefässe" nur uber kurze Strecken frei verlaufen und dann an einer Ringsmuskelfaser ansetzen." Nevertheless, it is clear that this channel is a large and prominent feature of the inside surface of the pseudocoelomic cavity. It is observed as a pair of channels located approximately equidistant between the DLC and VLC in some specimens (Fig. 9) but more frequently closer to the DLC. With this description, the use of the term "main lacunar" channel which is a common expression in general works may need to be modified. At this stage of our knowledge it is not possible to judge which if either of these systems has a dominant role.

The two MLC are not always the same size and there is no known pattern in *M. hirudinaceus* as to which is generally larger. Their construction is unique. When viewed following injection with dyes, the channels are apparently formed by numerous interconnecting cells such as cells 'a' and 'b' in Figure 6. Notice that the cells have a complicated pattern of interconnection. An RC passes between the muscles of the body wall between the PRC and MLC. Each of the interconnecting cells is attached (Fig. 1, 5, 6) to the longitudinal muscles in a manner which surrounds the point where they pass between the muscle bundles via the RC to join the tegumental part of the lacunar system. The channel formed is somewhat irregular in its path as the connections between cells are not in a straight line. It should be noted that this type of periodic attachment permits free access for other systems to pass under the MLC channel.

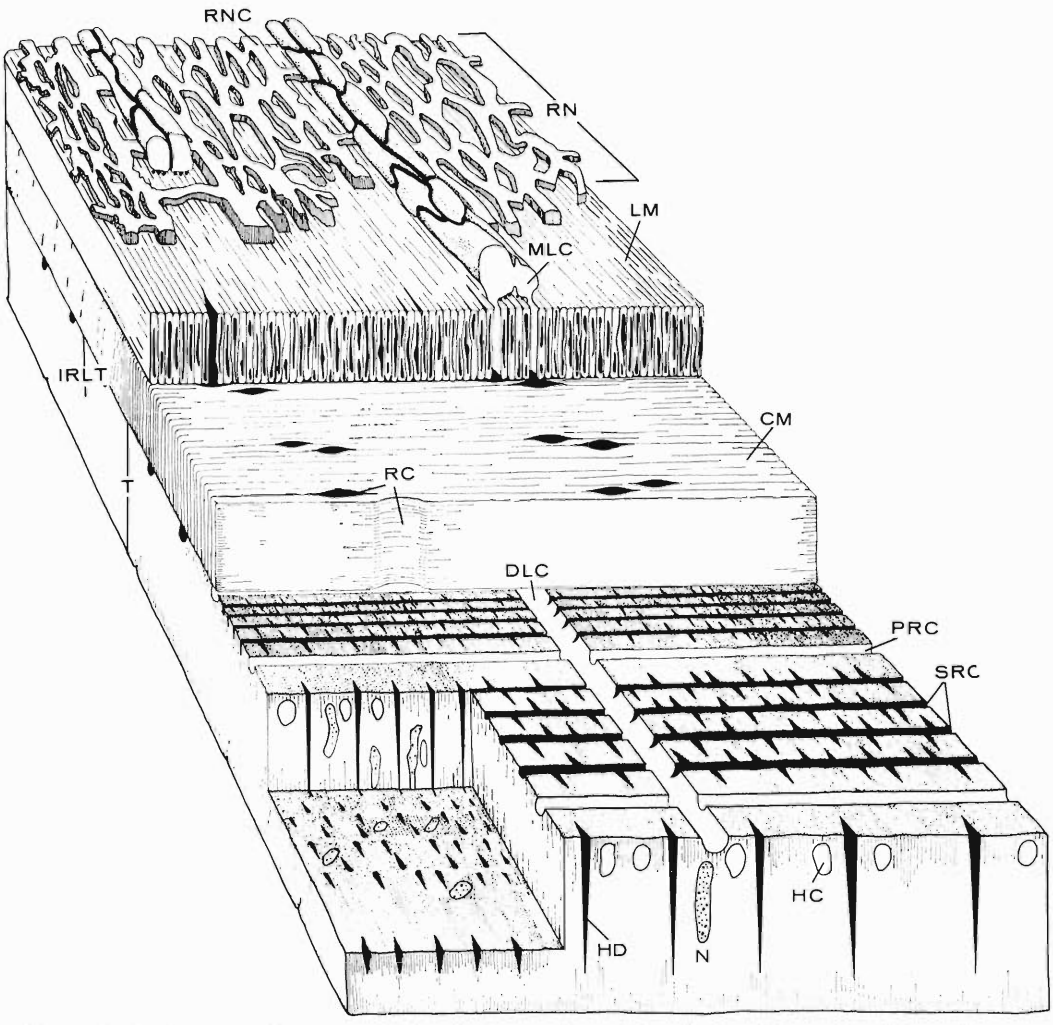

Figure 9. A diagrammatic representation of the acanthocephalan body wall, sectioned in various ways to show the relationship of the lacunar and previously undescribed rete system to the muscles and tegument. Note that the network of anastomosing channels making up this vascular system (RN) have longitudinal channels that parallel each side of the medial longitudinal channels (MLC). The lateral posterior nerves are located medial to the MLC. Note also that the radial canals (RC) have their long axis oriented in the direction of the respective muscle layer. The primary canals (PRC) do not possess hypodermal ducts (HD) as do the secondary ring canals (SRC).

Throughout the MLC large nuclei can be observed which seem to be held in position by fine protoplasmic strands. Kaiser (1893) stated that these large nuclei were first described by Zeder in 1800 as "large pores" and that Mueller's earlier (1777) description of "dark spots" were probably also nuclei. Greeff (1864) has been credited with the first clear recognization of these structures as nuclei with well-defined nucleoli. Rauther (1930) indicated that the nuclei observed in the lacunar system are held in position in the channels by means of fiber-like continuations.

Associated with the medial surface of the

longitudinal muscles is a network of thin-walled tubes. This rete network (RN; Fig. 9) covers the entire surface of this muscle layer throughout the length of the worm. In the area of the medial longitudinal canals of the lacunar system this network forms a pair of irregularly-shaped ducts (RNC; Fig. 9). These ducts parallel the MLC on each side and connect via smaller canals that pass between the MLC and longitudinal muscle. The impression one has when examining this system in living worms is that the main channels run parallel with the longitudinal muscle layers, with numerous rambling connection between parallel components. As this system is adjacent to the pseudocoel and very thin-walled, it seems evident that materials could easily move between the two compartments. However, there is no apparent connection between the lacunar system and this network of channels—at least none is evident in the trunk of the worm. Each system can be injected separately with no apparent movement of dye or ink between them.

The reason this extensive reticular network of channels may have been overlooked by previous investigators may be in the response of the thin-walled tubes to fixation and dehydration. The typical histological technique reduces these channels to a thin line of membranes erratically spaced on the medial surface of the longitudinal muscles. Occasionally they may appear as a "string" through the pseudocoel but in either case the typical histological section would not lend itself to the recognition of this system. In contrast, in *M. hirudinaceus* this reticular network of tubes can readily be seen in living worms or in glycerinated preparations. In these situations the tubes appear as an anastomosing network of translucent tubes. Of course, they are most easily viewed when injected (Fig. 1) with a dye.

To put the lacunar system in perspective an outline diagram is shown in Fig. 8A, B, C. It can be inferred from these results that the lacunar system can be a very effective circulatory network for the organism.

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Helminth Fauna of Saurians from Puerto Rico with Observations on the Life Cycle of *Lueheia inscripta* (Westrumb, 1821) and Description of *Allopharynx puertoricensis* sp. n.

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ABSTRACT: Two hundred and forty-six saurians comprising three species, *Anolis cristatellus* Dumeril and Bibron, *A. evermanni* Stejneger, and *Ameiva exsul* Cope, collected from 10 sites in Puerto Rico were examined for helminths. One hundred and eighty-five (75.2%) were infected. Nine species of parasites are recorded. *Pharyngodon anolis* (Chitwood, 1934) (Nematoda) was most prevalent. Several new host and locality records were established and polyinfections were encountered. The life cycle of *Lueheia inscripta* (Westrumb, 1821) Travassos, 1919 is discussed. The roach, *Periplaneta americana* is incriminated as the intermediate host and the lizard, *A. cristatellus*, as the paratenic host. The generic diagnosis of *Allopharynx* is emended and *Allopharynx puertoricensis* sp. n. described.

Although there are a few isolated reports on individual species of helminth parasites in lizards from Puerto Rico (Chitwood, 1934; Hoffman, 1935; Self and Garcia-Diaz, 1961; Cofresi-Sala, 1964; Garcia-Diaz, 1966), to my knowledge, no previous comprehensive helminthological survey of saurians had been conducted in this island. The present study was undertaken to fulfill the need for more knowledge on the helminth fauna of these hosts and was conducted between the springs of 1969 and 1973.

Materials and Methods

Two hundred and forty-six saurians representing three species (*Anolis cristatellus*, *A. evermanni*, and *Ameiva exsul*) were collected from 10 different localities in Puerto Rico most of which were in the western part (see map and Table 4), and examined for helminths. They were caught either by hitting them with a long stick or fly swatter. However, to capture them alive, the latter method was found more effective. On hitting them with the swatter, they

Table 1. Prevalence of helminths in Puerto Rican lizards.

Lizard species	No. examined	No. infected	Species of parasite	% of infection
1. *Anolis cristatellus*	234	175	*Mesocoelium danforthi* *Allopharynx puertoricensis* sp. n. *Lucheia inscripta* *Pharyngodon anolis* *Pharyngodon travassosi* *Centrorhynchus* sp. A. *Centrorhynchus* sp. B.	74.8
2. *Anolis evermanni*	4	3	*Pharyngodon anolis* *Pharyngodon travassosi*	75.0
3. *Ameiva exsul*	8	7	*Mesocoelium danforthi* *Oochoristica* sp. *Pharyngodon anolis* *Ozolaimus megatyphlon*	87.5
Total	246	185	9 species	75.2

fell from the trees stunned and were picked up while in this state from which they eventually recovered.

Animals captured alive were kept in small animal cages until autopsied. The animals which died during capture were autopsied on the same day or were refrigerated and examined later. The cestodes and trematodes were fixed in hot AFA (alcohol-formalin-acetic acid solution), stained in Mayer's HCl carmine and mounted in piccolyte. The acanthocephalans and nematodes were processed by the methods of Acholonu (1969) and Acholonu and Arny (1970), respectively. All measurements are in millimeters unless otherwise indicated; average measurements are in parentheses.

Results and Discussion

Of 246 saurians examined, 185 (75.2%) harbored one or more species of helminth parasites. One hundred and seventy-five (74.8%) of 234 *Anolis cristatellus*, 3 (75.0%) of 4 *A. evermanni*, and 7 (87.5%) of 8 *Ameiva exsul* were infected (see Table 1). Nine different species of helminths were recovered. The

Table 2. Species of helminths found.

Species of parasites	Locale in host	Hosts	Worm burden (range)	No. infected and %
TREMATODA		*Anolis cristatellus*	1–36	76 (32.5%)
1. *Mesocoelium danforthi*	small and large intestines	*Ameiva exsul*	1–5	2 (25%)
2. *Allopharynx puertoricensis* sp.n.†	body cavity	*A. cristatellus**	1	1 (0.43%)
CESTODA				
3. *Oochoristica* sp.†	small intestine	*A. exsul**	2–4	2 (25%)
ACANTHOCEPHALA				
4. *Lucheia inscripta*	intestine and body cavity	*A. cristatellus**	1–60	81 (34.6%)
5. *Centrorhynchus* sp. A†	body cavity	*A. cristatellus**	1	1 (0.43%)
6. *Centrorhynchus* sp. B†	body cavity	*A. cristatellus**	1	1 (0.43%)
NEMATODA				
7. *Pharyngodon anolis*	small and large intestines	*A. cristatellus* *A. evermanni** *A. exsul*	1–40 1 3–7	100 (42.7%) 1 (25.0%) 4 (37.5%)
8. *Pharyngodon travassosi*†	small intestine	*A. cristatellus** *A. evermanni**	1–2 1–2	4 (1.71%) 2 (50.0%)
9. *Ozolaimus megatyphlon*†	small intestine	*A. exsul**	1–16	2 (25.0%)

* = Host record.
† = Locality record.

Table 3. Prevalence of mono- and polyinfections in lizards.

1. *Anolis cristatellus*	Parasites	No. positive
I. Monoinfections:	*M. danforthi*	34
	A. puertoricensis sp. n.	1
	L. inscripta	23
	P. anolis	59
	P. travassosi	2
II. Polyinfections:		
2 worm species	*M. danforthi* + *L. inscripta*	11
	M. danforthi + *P. anolis*	23
	L. inscripta + *P. anolis*	33
	L. inscripta + *Centrorhynchus* sp. A	1
	L. inscripta + *Centrorhynchus* sp. B	1
	L. inscripta + *P. travassosi*	1
	P. anolis + *P. travassosi*	1
3 worn species	*M. danforthi* + *L. inscripta* + *P. anolis*	11
	L. inscripta + *P. anolis* + *P. travassosi*	1

2. *Anolis evermanni*	Parasites	No. positive
I. Monoinfections:	*P. anolis*	1
	P. travassosi	1
II. Polyinfections: 2 worm species	*P. anolis* + *P. travassosi*	1

3. *Ameiva exsul*	Parasites	No. positive
I. Monoinfections	*P. anolis*	1
	Oochoristica sp.	2
	O. megatyphlon	2
II. Polyinfections: 2 worm species	*M. danforthi* + *P. anolis*	2

nematode, *Pharyngodon anolis* (Chitwood, 1934), was the most frequently encountered parasite. It was recovered from 104 (42.3%) anolid lizards and iguanas (see Table 2).

Table 3 gives the prevalence of mono- and polyinfections in the respective species of saurians. One hundred and nineteen *A. cristatellus*, 2 *A. evermanni*, and 5 *A. exsul* harbored one worm species; 81, 1 and 2, respectively harbored 2 worm species and 12 *A. cristatellus* contained 3 species. Several host and locality records were established and these as well as the range of worm burdens are indicated in Table 2. A brief discussion of each parasite is presented below.

Trematoda

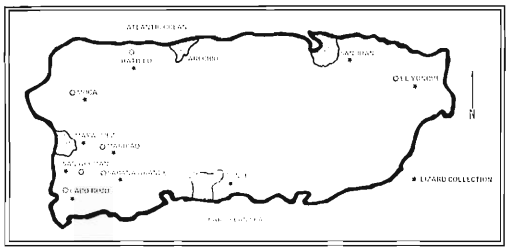
Genus *Mesocoelium* Odhner, 1911

1. *Mesocoelium danforthi.* Hoffman, 1935 (Brachycoeliidae)

This parasite which is considered to be a synonym of *M. monas* (Rudolphi, 1819) Freitas 1958 by Nasir and Diaz (1971) was recovered from the small and large intestines of 76 (32.5%) of 234 *A. cristatellus* and 2 (25%) of 8 *A. exsul*. The mature adult worms ranged from 1.24–2.76 in length (1.55) and 0.29–0.75 in width (0.43).

Hoffman (1935) described *M. danforthi* from the lizard, *Celestus pleii* Dumeril and Bibron, collected from El Yunque, Puerto Rico. Cofresi-Sala and Rodriquez de Vega (1963) reported it for the first time in the toad *Bufo marinus*. Cofresi-Sala (1964) reported it in 8 species of *Anolis* and *A. exsul* from 10 sites in Puerto Rico. In the present study, only 4 (El Yunque, Mayaguez, Ponce, and Maricao) of the 10 collection sites overlapped with those of Cofresi-Sala, and saurians at all sites harbored *M. danforthi*. Hence this study substantiates Cofresi-Sala's (loc. cit.) statement that *M. danforthi* seems to be well-distributed throughout the island.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 73287



Map of Puerto Rico showing collection sites.

Genus *Allopharynx* Shtrom, 1928

2. *Allopharynx puertoricensis* sp. n. (Plagiorchiidae) (Fig. 1)

DESCRIPTION: Body elongate and broadly oval in cross section, 3.56 by 1.51 at level of anterior testis—the widest part. Devoid of cuticular spines. Oral sucker subterminal, 0.25 by 0.20. Acetabulum 0.18 by 0.19, about 0.89 from the anterior end of body. Prepharynx 0.21 long. Pharynx 0.22 by 0.18. Esophagus very short or nonexistent. Intestinal ceca very long, terminating near posterior extremity; right side, 0.36 and left, 0.20 from posterior tip. Genital pore prominent, slightly to the left of median line of body and just above anterior margin of acetabulum (0.03 from it). Cirrus pouch 0.30 by 0.12 at widest part, extends a little posterior to acetabulum passing dorsally and by the right side of it with partial overlap. Testes post-equatorial, obliquely placed, and fairly close (0.14 apart); each obliquely oriented. Anterior testis, slightly irregular, 0.41 by 0.23 and located to the left side; posterior one oval, 0.42 by 0.28. Ovary oval, obliquely oriented, 0.24 by 0.15, slightly toward left side and immediately pre-equatorial; closer to anterior testis (0.25 apart) than to acetabulum. Seminal receptacle small, 0.12 by 0.05, at posterodorsal side of ovary and partially overlapping it. Mehlis' gland present, Laurer's canal not discernible. Vitellaria lateral, extending on both sides from posterior margin of acetabulum to near ends of ceca; extracecal but a little intercecal in preovarian and post-testicular regions; right side 0.63 from posterior end; left one slightly longer, 0.48 from posterior end. Strands of vitelline ducts very conspicuous especially from dorsal aspect. Vitelline reservoir present. Excretory vesicle not visible, excretory pore terminal. Uterus highly convoluted, extending from posterior end to genital pore passing dorsally about middle of acetabulum. Uterine loops intercecal, more concentrated in preovarian and post-testicular regions. Eggs measured *in situ* 0.028–0.03 by 0.017–0.02.

HOST: *Anolis cristatellus*.

Table 4. No. of lizards collected from each site and infection record.

Collection sites	No. of lizards collected	No. infected	No. infected with trematodes	No. infected with acantho-cephalans	No. infected with nematodes	No. infected with cestodes
	Anolis cristatellus					
1. Cabo Rojo	46	30 (65.2%)	19	28	12	—
2. El Yunque	13	4 (30.8%)	1	1	3	—
3. Hatillo	31	17 (54.8%)	1	11	8	—
4. Maricao	9	9(100.0%)	6	—	7	—
5. Mayaguez	1	1(100.0%)	1	—	1	—
6. Moca	13	9 (69.2%)	4	7	4	—
7. Ponce	3	1 (33.3%)	1	—	—	—
8. Sabana Grande	59	48 (81.4%)	21	6	36	—
9. San German	50	48 (96.0%)	18	25	30	—
10. San Juan	9	8 (88.9%)	5	3	3	—
	234	175 (74.8%)	77	81	104	—
	A. evermanni					
1. El Yunque	4	3 (75.0%)	—	—	3	—
	Ameiva exsul					
1. San German	8	7 (87.5%)	2	—	5	2
	246	185 (75.2%)	79 (31.1%)	81 (32.9%)	112 (46.0%)	2 (0.81%)

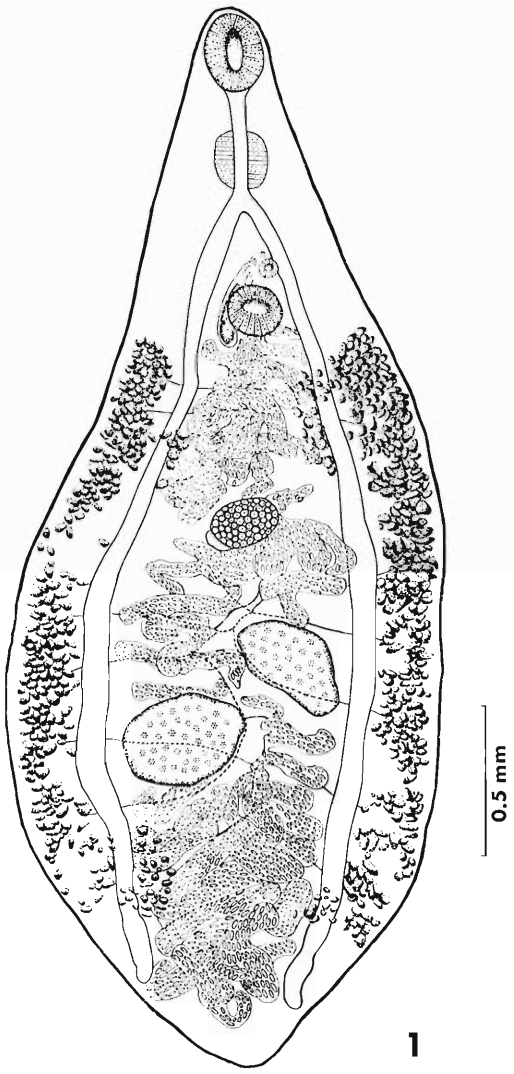


Figure 1. ***Allopharynx puertoricensis*** **sp. n. Whole Mount, holotype, ventral view.**

LOCATION: Body cavity.

LOCALITY: Hatillo, Puerto Rico.

HOLOTYPE: USHM Helm. Coll. No. 73286.

DISCUSSION: *Allopharynx puertoricensis* resembles *A. riopedrensis* Garcia-Diaz, 1966, but significantly differs from it by being smaller both in length and breadth, by possessing longer prepharynx and much shorter or nonexistent esophagus. The oral sucker is longer than wide as compared to that of *A. riopedrensis* which is spherical and slightly larger. The cirrus pouch is small and extends posterior to acetabulum whereas in *A. riopedrensis* it is larger and preacetabular. The vitelline follicles on both sides start from the posterior margin of the acetabulum and are extracecal and partly intercecal instead of starting on the right side at the level of intestinal bifurcation and on the left at the posterior margin of the acetabulum, and being entirely extracecal as in *A. riopedrensis*. The pharynx is longer than wide as compared to that of *A. riopedrensis* which is the opposite. The ovary is smaller and the eggs larger than in *A. riopedrensis*. The uterus passes dorsally about middle to acetabulum to genital pore instead of passing by the right side of acetabulum as in *A. riopedrensis*. It also resembles *A. tropidonoti* (MacCallum, 1919) Price, 1938 but differs from it by lacking cuticular spines; possessing longer prepharynx and shorter or nonexistent esophagus; being smaller in size; the arrangement and extent of the vitellaria; ovary not separated from anterior testis by a space about equal to the distance between the testes as in *A. tropidonoti*.

A. puertoricensis seems to be a rare trematode. Of 234 *A. cristatellus* examined, only one worm was found. Simha (1965) observed that his species, *A. leiperi*, was of rare occurrence causing only light infection. This appears to be the case with flukes of the genus *Allopharynx*. Descriptions of known species are based on one to a few worms.

Sharma and Gupta (1971) reviewed the genus *Glossimetra* Mehra, 1937 and synonymized *G. tamiansis* Dwivedi, 1967 and *G. narmadi* Dwivedi, 1967 with *G. orientalis* Mehra, 1937, the type species, thus leaving it as the only valid species in the genus. On the basis of phylogenetic relationships and similarities of characteristics which out number the differences, Gupta and Sharma (1973) proposed that the genera *Allopharynx* (Shtrom, 1928) and *Microderma* Mehra, 1931 be downgraded to subgeneric rank under the genus *Glossimetra* Mehra, 1937. But in accordance with the Law of Priority, Article 23c and e(1) of the International Code of Zoological Nomenclature ("The priority of the name of a taxon in the family-, genus- or species-group is not affected by elevation or reduction in rank within the group"; "A genus-group taxon

formed by the union of two or more genus-group taxa takes the oldest valid name among those of its components.") *Allopharynx* (Shtrom, 1928), the oldest name, should become the valid genus name. A review of the generic diagnosis of these genera (see Yamaguti, 1971) and the described species they contain, supports the views of Gupta and Sharma (loc. cit.) with the exception of their statement that "all the three genera are characterized by the absence of receptaculum seminis." Several described species of *Allopharynx* have it. In view of the several overlaps in the characteristics of the three genera, it is proposed that *Glossimetra* and *Microderma*, which contain only one and two species respectively, be further suppressed and synonymized with the genus *Allopharynx*. The diagnosis of this genus is emended.

Allopharynx (Shtrom, 1928)
Syn. *Megacustis* Bennett, 1935 (Nom. nud.)
Ophiorchis Mehra, 1937
Ptyasiorchis Mehra, 1937
Microderma Mehra, 1931
Glossimetra Mehra, 1937

GENERIC DIAGNOSIS (emend.): Plagiorchiidae, Astiotrematinae. Body variable, spatulate, elongate or slender with more or less pointed extremities; cuticle spinulate or non-spinulate. Oral sucker subterminal, small. Pharynx small to comparatively large. Esophagus from practically absent to moderate length, bifurcating some distance anterior to acetabulum; ceca terminating near posterior extremity. Acetabulum fairly close to oral sucker, slightly larger or smaller than oral sucker; in anterior third of body. Testes diagonal, anterior testis equatorial or postequatorial. Cirrus pouch elongate, reaching posterior acetabulum or turning back on itself in front of of acetabulum; enclosing winding seminal vesicle, short pars prostatica and short ejaculatory duct. Cirrus present or absent; when present with or without spines. Genital pore median or sub-median between acetabulum and intestinal bifurcation. Ovary median or sub-median, in midregion of body or pre-equatorial. Seminal receptacle present or absent. Laurer's canal present. Uterus intercecal, passing between two testes and reaching to posterior extremity; metraterm present, usually thick-walled. Vitelleria bunch-like, extending in lateral fields, leaving both extremities free. Excretory vesicle Y-shaped, with long median stem which empties at posterior tip of body through short muscular duct. Parasitic in intestine, gall bladder or body cavity of snakes, lizards, and tortoises.

Cestoda

Genus *Oochoristica* Lühe, 1898 *Oochoristica* sp. (Anoplocephalidae)

Tapeworms seem to be rare in the Puerto Rican saurians. Of the 246 examined, only 2 *Ameiva exsul* (0.81%) collected from San German were infected and the specimens were immature thus precluding specific identification. Two specimens were recovered from one host and 4 from the other and these occurred as monoinfections. To my knowledge this is the first time that cestodes are reported from this group in Puerto Rico.

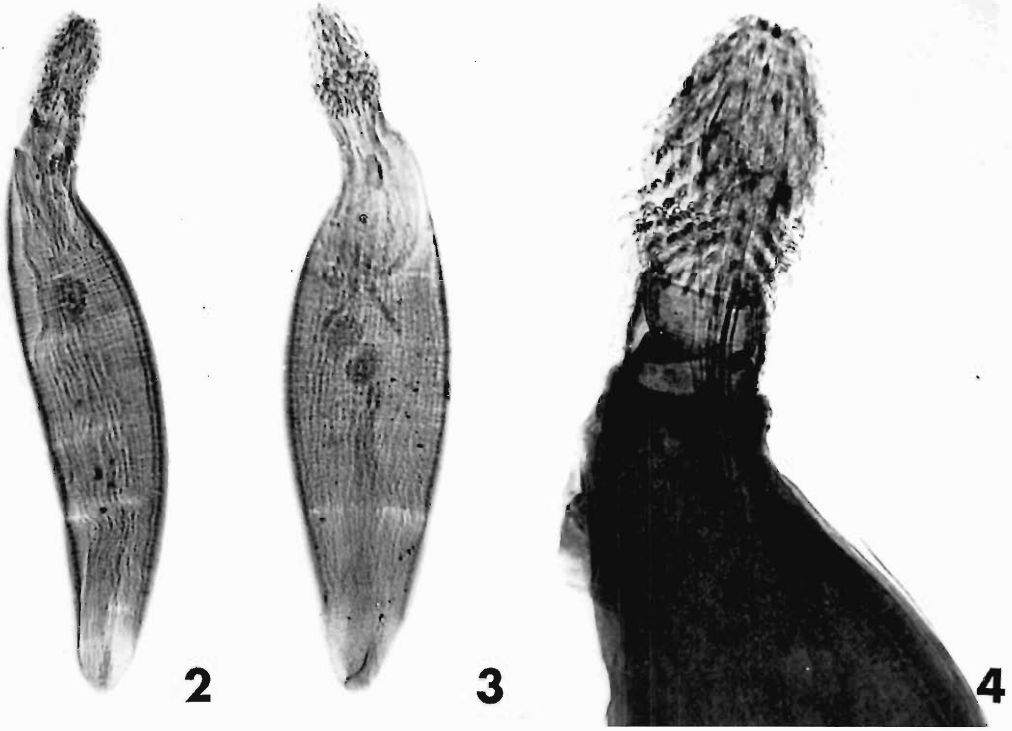
SPECIMEN DEPOSITED: USNM Helm. Coll. No. 73289.

Acanthocephala

1. Genus *Lueheia* Travassos, 1919 *Lueheia inscripta* (Westrumb, 1821) Travassos, 1919 (Plagiorhynchidae) (figs. 2–6)

This parasite characterized by its possession of subglobular proboscis and 4 tubular long lemnisci was recovered from the intestine and body cavity of 81 (34.6%) of 234 *A. cristatellus*. In general, the worms were all juvenile forms which, with their proboscis retracted, were fusiform or banana-shaped. The female specimens with their proboscis everted measured 2.13–3.07 (2.58) by 0.49–0.67 (0.56). The males were a little smaller in size; with their proboscis everted and bursa retracted, they measured 1.46–2.75 (2.11) by 0.30–0.60 (0.46). Lizards infected with this parasite were from all collection sites with the exception of Maricao, Mayaguez, and Ponce—areas where the fewest specimens were collected. Although acanthocephalans are known to inflict mechanical injuries on their hosts which become fatal (Webster, 1943; Boyd, 1951) there was no evidence that *L. inscripta* caused tissue or intestinal pathology or erratic behavior in lizards containing up to 60 parasites.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 73288.



Figures 2–4. Photomicrographs. 2. Juvenile *Lueheia inscripta* W.M., female, lateral view. ×40. 3. Same; W.M., male, lateral view. Note developing testes in the midregion. ×46. 4. Same; anterior end of female, lateral view. Higher magnification showing proboscis with spines. ×100.

Observations on the life cycle of *L. inscripta*

L. inscripta has been reported from Puerto Rican grackles (also called "Chango" or Mozambique de Puerto Rico), *Quiscalus niger brachypterus* Cassin; in this host females become ovigerous (Whittaker, et al., 1970). A borrowed mature but not fully grown female measured 5.28 by 1.09 (trunk region) and a male, 3.07 by 0.75. None of the total of 494 male and female worms recovered in the present study exceeded 3.07 in length (mature adult females measure up to 15 mm and males up to 9.23 mm (Yamaguti, 1963)), and their reproductive organs were never fully developed, implying that the lizard is a paratenic or transport host for this species. Grackles are known to feed on lizards in this area (V. Giaggi, University of Puerto Rico at Mayaguez, (pers. comm.); Acholonu, unpublished observations).

While one of the *A. cristatellus* collected from Cabo Rojo was being autopsied, a partially digested cockroach (*Periplaneta americana*) was found in its stomach. Probing of the cock-

→

Figures 5–8. 5. Juvenile *Lueheia inscripta*, posterior end of female, lateral view. Higher magnification showing developing genitalia. ×100. 6. Same; posterior end of male, lateral view. Higher magnification showing developing genitalia with the bursa everted. ×100. 7. *Centrorhynchus* sp. A. W.M., female, lateral view showing tripartite body with posterior trunk region resembling a tail; proboscis retractor muscle

5

6

7

8

prominent. ×42. 8. *Centrorhynchus* sp. B. W.M., male, lateral view showing cylindrical proboscis with swollen posterior part. Note the rounded or blunt distal end of worm and broad suspensory ligament. ×46.

roach's abdomen resulted in the emergence of about 60 larval acanthocephalans. From the work of Acholonu and Finn (1974) it was evident that the acanthellas were not those of *Moniliformis moniliformis*. After thorough examination, they were found to be identical with *L. inscripta* recovered from *A. cristatellus* but were smaller and less developed. This implied that the roach may serve as the intermediate host of this acanthocephalan. An attempt was made to verify the life cycle by feeding experiments conducted with the specimens obtained from both the roach and the lizards. About 10 worms were fed *per os* to each of 23-day-old chicks and to a wild caught grackle free from infection as attested by prior fecal sample examination. When the birds were examined 30 to 45 days post-feeding, no parasites were found.

The failure of the worms to establish themselves in the experimental birds may be attributed to the following: (1) the feeding method used did not simulate the natural mode of infection; the immature worms may have never survived passage through the gizzard with the feeding method used, (2) chicks may be unnatural hosts in which the parasite does not develop, (3) inadequate number of grackles used in the feeding experiments or unavailability of fledgling ones, (4) inadequate number of worms fed to each bird. Fledgling grackles or cattle egrets (*Bubulcus ibis*) which feed on lizards should be used in future life cycle studies of this parasite.

2. Genus *Centrorhynchus* Lühe, 1911
Centrorhynchus spp. (Centrorhynchiidae) (Figs. 7–8)

Single juvenile acanthocephalans identified as members of the genus *Centrorhynchus* were recovered from the body cavities of two *A. cristatellus* collected from Cabo Rojo. They had long cylindrical proboscis divided into two regions and beset with hooks arranged in longitudinal rows. One of these worms was a female measuring 3.34 by 0.48 at largest width. The everted proboscis measured 0.73 by 0.27 and had hooks measuring 0.04 at the anterior part and 0.03–0.035 at the posterior part. The worm was tripartite. It had a presoma, enlarged anterior, and attenuated, drawn-out posterior trunk that looked like a tail (Fig. 7). The other worm was a male which measured 1.30 by 0.36 at the largest width with everted proboscis measuring 0.43 by 0.24 at the widest part (the posterior swollen region). The hooks were smaller than in the female specimen. They measured 0.03 in the anterior part and 0.02 in the posterior part where they seemed to be arranged in diagonal rows. Unlike the female worm, it had undivided trunk with a blunt or rounded distal end and broad suspensory ligament (Fig. 8). Since these parasites were structurally dissimilar they are recorded as *Centrorhynchus* sp. A and B, respectively. They cohabited with *L. inscripta* with which they differ markedly (Figs. 2–6). Because of their peculiar morphology they may be new species but their immature conditions and insufficiency of materials preclude their description as such.

SPECIMENS DEPOSITED: USNM Helm. Coll. Nos. 73290, 73291.

Nematoda

Genus *Pharyngodon* Diesing, 1861
1. *Pharyngodon anolis* (Chitwood, 1934) (Oxyuridae)

This parasite was recovered from the intestine of 100 (42.7%) of 234 *A. cristatellus*, one (25.0%) of 4 *A. evermanni*, and 3 (37.5%) of 8 *Ameiva exsul* and was encountered at all collecting sites except Ponce. The females measured 2.30–4.15 (2.99) by 0.23–0.56 (0.39) and the males which were very rare measured 1.21–2.19 (1.69) by 0.36–0.49 (0.42).

Chitwood (1934) recovered and described this species from *A. cristatellus* collected from Viejo, Puerto Rico. My specimens were on the average, larger than those of Chitwood. Although he did not mention the number of spines on the tail of the female, his figure shows 7 or 8. In the present study the tail spines varied in number from 6 to 10 and seemed to occur in pairs or alternately.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 73292.

2. *Pharyngodon travassosi* Pereira, 1935

Five of 234 *A. cristatellus* and 2 of 4 *A. evermanni* collected from Cabo Rojo, Hatillo and El Yunque harbored female nematodes belonging to the genus *Pharyngodon* several of

which were distended with eggs. The parasites which measured 3.45–5.59 (4.48) by 0.48–0.73 (0.62) obviously differed from *P. anolis* with which they coexisted by possessing very short and conical tails devoid of spines. They showed most resemblance to *P. travassosi*. The measurements correspond approximately with those of this species. The size of the worms, the position of the vulva, the length of the esophagus, the shape and size of the tail, and the egg size fit closely the description of *P. travassosi* as given by Pereira. The Puerto Rican specimens are, therefore, assigned to this species.

Genus *Ozolaimus* Dujardin, 1845

3. *Ozolaimus megatyphlon* (Rudolphi, 1819) Dujardin, 1845. (Oxyuridae)

This parasite was recovered from the intestine of 2 (25%) of 8 *Ameiva exsul* collected from San German. The females measured 4.17–4.67 (4.42) by 0.34–0.36 (0.35); the males 3.65–4.06 (3.85) by 0.31–0.32 (0.31) with spicules 0.84–0.86 (0.85) long. The females were not gravid as they did not contain eggs.

Though *Ozolaimus* at one time contained two species viz., *O. megatyphlon* and *O. cirratus* (Linstow, 1906) both occurring in *Iguana tuberculata*, Thapar (1926) reduced them to synonymy leaving only *O. megatyphlon*. *Pereira* (1935) reported *O. megatyphlon* and *O. cirratus* as mixed infections in one specimen of *I. tuberculata*. He redescribed and refigured the two but neither brought out any significant taxonomic differences between the two to justify the resurrection of *O. cirratus* as a valid species nor expressed any objection to Thapar's synonymy. With the exception of a minutia of differences in size between the two species, his descriptions and figures of them show that they are strikingly similar and, therefore, truly conspecific. As such, Thapar's synonymy of *O. cirratus* remains valid. *O. megatyphlon* has been reported from *I. tuberculata* in Brazil, Venezuela, Mexico and Cuba (Yamaguti, 1961). It is here reported in Puerto Rico and from *A. exsul* for the first time.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 73293.

Acknowledgments

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The Trematode Genus *Glypthelmins* Stafford, 1905 (Plagiorchioidea: Macroderoididae) with a Redescription of *G. facioi* from Costa Rican Frogs[1, 2]

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ABSTRACT: The 28 described species of glypthelminth trematodes are divided into two groups on the basis of the form of the excretory bladder. Those possessing a Y-shaped bladder are referred to the Plagiorchiidae while those exhibiting an I-shaped bladder are placed in the Macroderoididae. The generic diagnosis of *Glypthelmins* is emended and the genus is included in the Macroderoididae. *Glypthelmins quieta* (Stafford, 1900), *G. rugocaudata* (Yoshida, 1916), *G. staffordi* Tubangui, 1928, *G. shastai* Ingles, 1936, and *G. facioi* Brenes Madrigal, Arroyo Sancho, Jimenez-Quiros, and Delgado Flores, 1959 are recognized as valid species. A redescription of *G. facioi* based on specimens collected from *Rana pipiens* in Costa Rica is presented. The presence of spines or tegumental scales, egg size, size of the testes, and length of the esophagus are used to distinguish these five species. *Reynoldstrema africana* (= *G. africana*) is transferred to the Astiotrematinae (family Plagiorchiidae), and *G. diana* is designated *incertae sedis*.

In June, 1969, 34 specimens of *Glypthelmins facioi* Brenes Madrigal et al., 1959, were recovered from the small intestines of 8 of 12 *Rana pipiens* Schreber. Worms were recovered from frogs collected at Turrialba, approximately 20 miles from the type locality, Coris, Cartago Province, Costa Rica. Study of whole mounted and serially sectioned worms provided additional data to those of Brenes Madrigal et al. (1959).

Specimens of *G. facioi* were compared to other glypthelminth trematodes (*sensu* Byrd and Maples, 1963) collected by the author in

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the United States, Costa Rica and Venezuela or borrowed from the U.S. National Parasite Collection and collections of other workers. These comparative studies focused attention on the taxonomic controversy surrounding this group of widely distributed plagiorchioid trematodes, parasitic in amphibians and reptiles. This controversy is evidenced by the inculsion of the group's 28 species in either one genus (e.g., Nasir, 1966), or, at one time or another, in as many as six genera (e.g., Cheng, 1959; Byrd and Maples, 1963). In view of the previously suggested relationships of these 28 species to the genus *Glypthelmins* Stafford, 1905, indicated by use of the neutral term glypthelminth, the status and familial assignment of the genus *Glypthelmins* is reexamined to provide a basis for assessing the relationships of these 28 species.

Trematodes were heat-killed in 0.7 per cent saline under slight coverslip pressure, fixed in alcohol-formalin-acetic acid solution (AFA), stained with Harris hematoxylin, and mounted in Canada balsam or Permount. Specimens of *G. facioi* sectioned at 8 μ had been previously whole mounted. Figures were drawn with the aid of a Wild drawing tube; unless otherwise indicated, all measurements are given in microns with the mean in parentheses. Individual measurements of the *G. facioi* and *G. quieta* referred to in this study are available elsewhere (Sullivan, 1972).

Literature citations in Tables 1–3 are listed as they appear in the Index Catalogue of Medical and Veterinary Zoology, U.S. Government Printing Office, Washington, D.C.

Glypthelmins Stafford, 1905, Char. Emend.

SYNONYM: *Margeana* Cort, 1919.

DIAGNOSIS: Macroderoididae. Body elongate, cylindrical to subcylindrical. Tegument spined or scaled. Oral sucker subterminal. Acetabulum medial, in anterior half of body. Pharynx well-developed. Esophagus present. Cecal bifurcation midway between pharynx and acetabulum. Ceca long, reaching to or into posterior quarter of body. Testes postacetabular, symmetrical or diagonal in position. Cirrus pouch elongate, usually overlapped by acetabulum. Ovary pretesticular, in close proximity to acetabulum. Seminal receptacle and Laurer's canal present. Uterus transversely coiled, intercecal, reaching to posterior extremity; uterine coils not developed in pretesticular region. Matraterm non-muscular, surrounded by numerous gland cells. Genital pore immediately preacetabular. Vitellaria follicular in lateral fields of body, overlapping ceca dorsally and ventrally, occasionally confluent dorsally; longitudinal extent highly variable, extending from level of oral sucker to posttesticular region. Excretory vesicle I-shaped. Parasitic in intestine of anurans.

TYPE SPECIES: *Glypthelmins quieta* (Stafford, 1900) Stafford, 1905.

Glypthelmins quieta (Stafford, 1900) Stafford, 1905

SYNONYMS: *Distomum quietum* Stafford, 1900; *Margeana californiensis* Cort, 1919; *Glypthelmins californiensis* (Cort, 1919) Miller, 1930; *Glypthelmins subtropica* Harwood, 1932.

DESCRIPTION: See Miller (1930).

HOSTS AND LOCALITIES: Table 1.

SPECIMENS: USNM Helm. Coll. No. 72268–72271. Other specimens in the author's collection.

Glypthelmins rugocaudata (Yoshida, 1916) Yahata, 1934

SYNONYM: *Enodiotrema rugocaudatum* Yoshida, 1916.

DESCRIPTION: See Yoshida (1916) and Yahata (1934).

HOSTS AND LOCALITIES: *Rana nigromaculata* Hollowell, Osaka, Japan (Yoshida, 1916) and P'yongyang (Heijo), North Korea (Ogata, 1937); *R. rugosa* Schlegel, Hiroshima (Yahata, 1934) and Kyoto, Japan (Yamaguti, 1936).

SPECIMENS: No specimens of this species were available for study.

Glypthelmins staffordi Tubangui, 1928

DESCRIPTION: See Fischthal and Kuntz (1967).

HOSTS AND LOCALITIES: Table 2.

SPECIMENS: Four specimens (USNM Helm. Coll. No. 61702) were studied. (Approximately 200 amphibia, including *Bufo melanostictus*, *Rana cancrivora*, *R. erythraea*, *R. limnocharis* and *R. macrodon*, from various localities in Malaysia as well as certain of Yuen's (1962) Singapore localities were examined for *G. staffordi* during the last six months of 1974. To

Table 1. *Glypthelmins quieta*: Hosts and localities. (Specimens from the collections of *E. E. Byrd and †F. A. Christian; **new locality records.)

Host	Locality	Author
Bufonidae		
Bufo americanus Holbrook	Presque Isle, Maine	Bouchard (1951)
Hylidae		
Acris crepitans Baird	Iowa	Ulmer (1970)
Hyla crucifer Weid	Canada	Stafford (1905)
(= *H. pickeringii* Kennicott)	Western Massachusetts	Rankin (1945)
	Athens, Clarke Co., Ga.	Byrd & Maples (1963)
Pseudacris nigrita (LeConte)	Athens, Clarke Co., Ga.	Byrd & Maples (1963)
P. triseriata Weid	Iowa	Ulmer (1970)
Ranidae		
Rana aurora (Baird & Girard)	San Francisco, Ca.	Cort (1919)
	San Diego Co., Ca.	Ingles (1936)
R. boylii (Baird)	Butte Co., Ca.	Ingles (1936)
	Marin & Sonoma Co., Ca.	Lehmann (1960)
R. catesbeiana Shaw	Canada	Stafford (1905)
	Urbana, Illinois	Miller (1930)
	Houston & Huntsville, Texas	Harwood (1932)
	Cleveland Co., Oklahoma	Trowbridge & Hefley (1934)
	Beaufort Co., N.C.	Brandt (1936)
	Florida	Manter (1938)
	Louisiana	Bennett (1938)
	Athens, Clarke Co., Ga.	Parker (1941)
	Gaspe Peninsula, Canada	Rankin (1944)
	Amherst, Massachusetts	Rankin (1944)
	North Carolina	Rankin (1944)
	Seattle, Washington	Rankin (1944)
	Havana, Cuba	Odening (1968)
	Iowa	Ulmer (1970)
	**Burke, Chatham & Taliaferro Co., Ga.	*Present study
	**Oconee & Screven Co., Ga.	Present study
	**Terrebonne & East Baton Parishes, La.	†Present study
	**Oktibbeha Co., Miss.	*Present study
R. clamitans Latreille	Western Massachusetts	Rankin (1945)
	Presque Isle, Maine	Bouchard (1951)
	**DeKalk & Oglethorpe Co., Ga.	Present study
	**Warren Co., New Jersey	Present study
R. montezumae Baird	Mexico, D.F.	Caballero y C. & Sokoloff (1934)
R. palustris LeConte	Presque Isle, Maine	Bouchard (1951)
R. pipiens Schreber	Canada	Stafford (1905)
(= *R. virescens* Garman)	Mexico, D.F.	Caballero y C. & Sokoloff (1934)
	**Franklin Co., Ohio	†Present study
	**Alamance Co., N.C.	*Present study
	**Franklin Co., Tenn.	Present study
R. septentrionalis Baird	Presque Isle, Maine	Bouchard (1951)
R. sphenocephala Cope	Cleveland Co., Oklahoma	Trowbridge & Hefley (1934)
	Houston & Huntsville, Texas	Harwood (1932)

Table 2. *Glypthelmins staffordi*: Hosts and localities.

Host	Locality	Author
Bufonidae		
Bufo melanostictus Schneider	Taihoku, Formosa	Yamaguti & Mitunaga (1943)
Ranidae		
Oocidozyga lima (Gravenhorst)	Hanoi Prov., North Vietnam	Odening (1968)
Rana cancrivora Gravenhorst	Republic of Singapore	Yuen (1962)
R. erythraea Schlegel	Republic of Singapore	Yuen (1962)
R. limnocharis vittigera Weigmann	Laguna Prov., Luzon, Philippine Is.	Tubangui (1928)
(= *R. vittigera* Weigmann)	Manila, Luzon, Philippine Is.	Fischthal & Kuntz (1967)
R. macrodon Dumeril & Bibron	Republic of Singapore	Yuen (1962)
R. tigrina rugulosa Weigmann	Amoy and Canton, China	Li (1937a)
(= *R. rugulosa* Weigmann)	Hanoi Prov., North Vietnam	Odening (1968)

Table 3. Comparison of body and esophageal lengths (in microns) for *Glypthelmins staffordi* and *G. rugocaudata*.

Glypthelmins staffordi			*Glypthelmins rugocaudata*		
Body	Esophagus	Author	Body	Esophagus	Author
2090–4150	40	Tubangui (1928)	Up to 3200	160–300	Yoshida (1916)
1940–4960	38–210	Yuen (1962)			
1454–1960	10–85	Fischthal & Kuntz (1967)			

date, the author has not found this species in Peninsular Malaysia or Singapore.)

Glypthelmins shastai Ingles, 1936

DESCRIPTION: See Ingles (1936). Certain measurements of the type specimen of *G. shastai* (USNM Helm. Coll. No. 8925) were not in accord with the size ranges presented by Ingles (1936). Since other aspects of the type's morphology agreed with the original description, only the measurements of the type, figured by Ingles (his pl. 16, fig. 1), are included here.

Body 3,200 long by 810 wide. Oral sucker 210 by 270. Acetabulum 160 by 160. Pharynx 110 by 140. Esophagus 300 long. Ovary 210 by 210. Anterior testis 320 by 270. Posterior testis 340 by 320. Cirrus pouch 570 by 120.

HOST AND LOCALITY: *Bufo boreas* Baird and Girard, Shasta County, California (Ingles, 1936).

SPECIMENS: Only the type specimen was available for study.

Glypthelmins facioi

Brenes Madrigal, Arroyo Sancho, Jimencz-Quiros, and Delgado Flores, 1959 (Figs. 1–4)

DESCRIPTION (measurements based on 29 specimens): Body elongate, 1,820–3,440 (2,800) long by 390–900 (640) wide. Tegument entirely scaled, with scales diminishing in number posteriorly. Oral sucker subterminal, 170–240 (200) by 170–250 (220). Acetabulum medial, in middle third of body, 90–140 (120) by 80–150 (120). Ratio of oral sucker to acetabulum 1:0.58 ± 0.03. Prepharynx short. Pharynx muscular, 70–110 (90) by 110–160 (130). Esophagus 110–280 long. Ceca terminating near posterior extremity. Testes smooth, diagonal, in mid-region of body; anterior testis dextral, 130–240 (200) by 100–230 (170), in immediate postovarian zone; posterior testis 130–290 (240) by 110–250 (190), with its anterior border extending into the zone of the anterior testis. Cirrus pouch 150–340 (270) by 50–100 (70), extending from preacetabular region to ovarian level, containing saccular seminal vesicle; cirrus eversible, unarmed. Ovary sinistral, pretesticular, spherical, overlapped by acetabulum or not, 100–240 (170) by 90–210 (160). Laurer's canal and seminal receptacle present. Uterus intercecal, with numerous transverse coils reaching to posterior extremity of body; pretesticular coiling absent. Metraterm as long as or slightly longer than cirrus pouch. Metraterm and cirrus pouch opening separately into a small genital atrium; genital pore median, immediately preacetabular. Vitellaria follicular, commencing at level of cecal bifurcation and terminating at a level behind posterior testis; follicles distributed laterally, overlapping ceca dorsally and ventrally, and often confluent dorsally at varying levels throughout their extent. Eggs operculate, 27–32 (29) by 12–20 (15). Excretory bladder I-shaped, reaching to level slightly behind posterior testis. Excretory system mesostomate; common collecting tubules receiving anterior and posterior main collecting tubules in immediate postovarian region.

HOST: *Rana pipiens* Schreber.

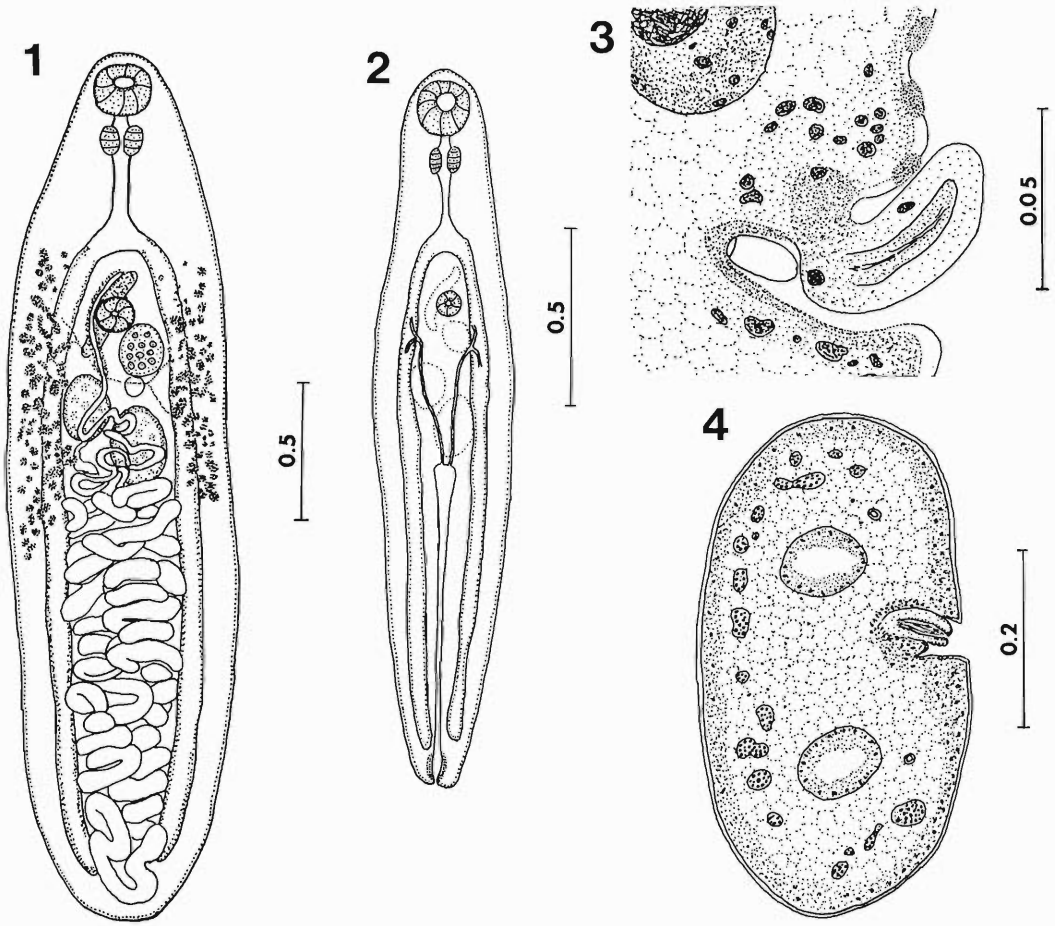
SITE OF INFECTION: Small intestine.

LOCALITIES: Coris and Turrialba, Cartago Province, Costa Rica.

SPECIMENS: USNM Helm. Coll. No. 72275. Other specimens in the author's collection.

Discussion

Of the 28 described species of glypthelminths, the form of the adult excretory vesicle is unrecorded for *Glypthelmins facioi*, *G.*



Figures 1–4. ***Glypthelmins facioi*** **from Turrialba, Costa Rica (scales in millimeters). 1. Ventral view; note uterine configuration. 2. Ventral view showing form of the excretory bladder. 3. Section through the genital atrium; note unarmed cirrus and three adjacent tegumental scales. 4. Section showing dorsally confluent vitellaria.**

palmipedis, *G. parva*, *G. proximus*, *G. pseudium*, *G. shastai*, *G. simulans*, *G. vitellinophilum*, and *Choledocystus intermedius*. Life histories are known only for *G. quieta* (Rankin, 1944; Leigh, 1946), *G. hyloreus* (Martin, 1969) and *C. pennsylvaniensis* (Sullivan and Byrd, 1970), all three of which are characterized by I-shaped bladders in both cercarial and adult stages. Y-shaped bladders are recorded for *G. repandum* and *G. linguatula* (Travassos, 1924), *G. subtropica* (Harwood, 1932), *G. africana* (Beverly-Burton, 1963), *G. incurvatum* and *G. ramitesticularis* (Nasir, 1966), and *Rauschiella tineri* (Babero, 1951). The excretory vesicle of *G. staffordi* is variously described as Y-shaped (Tubangui, 1928; Yuen, 1962), as a central canal with two large collecting trunks (Li, 1937), or as I-shaped (Odening, 1968). The bladder of *G. rugocaudata* is described as extending as far forward as the testes and dividing into two short lateral branches (Yoshida, 1916), as Y-shaped (Yahata, 1934), or as club-shaped (Yamaguti, 1936). Similarly, Travassos (1926) described the blad-

der of *G. elegans*, later designated the senior synonym of both *Choledocystus eucharis* and *C. vesicalis* by Ruiz (1949), as Y-shaped. The bladders of *C. eucharis* and *vesicalis* were described, respectively, as tubular (Pereira and Cuocolo, 1941) and as Y-shaped with short arms and a long trunk (Ruiz and Leao, 1942). However, Ruiz (1949) considered the bladder in *elegans* as transitional between the I- and Y-shaped forms. Finally, Cordero's (1944) descriptions of *G. sera* and *G. festina*, while not stating the bladder forms, suggested that they are Y-shaped.

Comparison of the shape of the excretory bladder in the glypthelminth trematodes (*sensu lato*) indicates that they are divisible into two groups: 1) those possessing Y-shaped, and 2) those possessing I-shaped bladders. South American forms, represented by 15 "species" (two of which were also reported from Central America and one only from Mexico), can be included in the first group. North American forms, as well as one Central American form, and those reported from Southeast Asia, Japan and Korea, can be included in the second group. Although exhibiting a Y-shaped bladder, the sole representative of the group described from Africa, *Reynoldstrema africana* Cheng, 1959 (= *Glypthelmins a.* Dollfus, 1950), demonstrated certain characters which suggest its removal from consideration with this group (see below).

While placement of the glypthelminths in the superfamily Plagiorchioidea Dollfus, 1930, is accepted (Schell, 1962; Byrd and Maples, 1963; Odening, 1964; Sullivan and Byrd, 1970), various members of the group, or the group as a whole, have been included in the Macroderoididae McMullen, 1937 (Schell, 1962; Odening, 1964; Sullivan and Byrd, 1970) or in the Plagiorchiidae Luhe, 1901 (Fischthal and Kuntz, 1967; Martin, 1969; Ulmer, 1970). However, in establishing the Macroderoididae, McMullen (1937) separated it from the Plagiorchiidae primarily on the basis of the I-shaped excretory vesicle of the former as opposed to the Y-shaped vesicle of the latter. Since the glypthelminths are divisible by the I- or Y-shape of the bladder and exhibit other morphological characters of the Plagiorchiidae and the Macroderoididae, as emended by Odening (1964) and Sullivan and Byrd (1970), respectively, it is proposed that those glypthelminths with a Y-shaped bladder be restricted to the Plagiorchiidae, whereas those with an I-shaped bladder be referred to the Macroderoididae. The taxonomy of those glypthelminths possessing a Y-shaped bladder as well as that of *Choledocystus pennsylvaniensis* Byrd and Maples, 1963 (= *Glypthelmins p.* Cheng, 1961) and *Glypthelmins hyloreus* Martin, 1969, will be considered elsewhere.

Citing Manter's (1969) statement, "To distinguish between a 'tubular excretory vesicle' and a Y-shaped vesicle is not realistic. All excretory vesicles are tubular, whether I-shaped, Y-shaped, or V-shaped," Nasir and Diaz (1970) emended the diagnosis of the Macroderoididae to include forms possessing a tubular bladder rather than only an I-shaped bladder as originally proposed by McMullen (1937). Considering the morphological similarities of the adult worms assigned to the two families, such an all inclusive treatment of excretory vesicles could render the family Macroderoididae a synonym of the Plagiorchiidae, if the intramolluscan stages were not known (Yamaguti, 1971). Although Manter's statement regarding separation of tubular and Y-shaped bladders is well founded, the basic I-, Y-, or V-shape of the bladder is, as Manter suggests, independent of its tubular nature.

The author accepts the conclusions of Miller (1930) in considering *Margeana* Cort, 1919, a synonym of *Glypthelmins*, and also agrees with Byrd and Maples (1963) in suppressing the reinstatement of *Margeana* by Cheng (1959).

Although Miller (1930) differentiated *G. californiensis* from *G. quieta* by the anterior extent of the vitellaria in *G. californiensis*, Caballero y C. and Sokoloff (1934) noted that the vitellaria in their *G. californiensis* did not conform with Cort's (1919) account and attributed this difference to the effects of flattening. The variability in the position of the vitellaria in species of *Glypthelmins*, which was emphasized by Miller (1930), as well as the findings of Caballero y C. and Sokoloff (1934) with regard to *G. californiensis*, leads to the conclusion that vitelline position is insufficient for the separation of *G. californiensis* from *G. quieta*. Nasir and Diaz (1970) considered these two species conspecific, a conclusion which is supported by the author.

In separating *G. subtropica* from *G. quieta*, Harwood (1932) maintained that *G. subtropica*

> . . . most closely resembles *G. quieta*, but it may be . . . distinguished from this form by the transverse band of vitellaria, and the location of the testes behind the transverse vitelline duct, and the tendency of the uterus to pass ventral to the testes rather than between them.

Olsen (1937), in his key to the species of *Glypthelmins*, further distinguished *G. subtropica* from *G. quieta* by indicating that the former possessed a pharynx larger than the ventral sucker.

Concurring with Miller's (1930) findings regarding morphological variability in *G. quieta*, Rankin (1944) also figured nine specimens (his figs. 11–19) of *G. quieta* from a single natural infection and called attention to the vitelline distribution; three specimens (his figs. 14, 17, 19) showed marked transverse bands of vitellaria, obviating one of Harwood's (1932) distinctions between *G. subtropica* and *G. quieta*. Further, Harwood's use of the relative position of the testes and transverse vitelline ducts is considered highly questionable in view of 1) Stafford's (1900) inability to accurately demonstrate the ducts themselves or their position, and 2) Rankin's (1944) findings which indicate that the relative positions of the ducts and testes depend to some degree on the oblique or symmetrical position of the testes. Ventral overlap of the testes by the ascending uterus is constant although the degree of overlap is highly variable. No taxonomic significance, therefore, is attached to a "tendency" of the uterus to pass ventral to the testes rather than between them. Preliminary study of size allometry, based on measurements of 74 *G. quieta*, indicates that although pharyngeal size is greater than that of the acetabulum in smaller specimens, this size relationship is reversed in larger specimens (Sullivan, 1972). Therefore, the ratio of pharyngeal size to acetabular size cannot be considered a valid taxonomic character as proposed by Olsen (1937) for the separation of *G. subtropica* and *G. quieta*. Since the characters used by Harwood (1932) and Olsen (1937) are insufficient for the separation of these two species and no characters could be found to distinguish them from each other, it is suggested that *G. subtropica* be considered a synonym of *G. quieta* as proposed by Manter (1938).

Tubangui (1928) distinguished *G. staffordi* from *G. quieta* by vitelline extent, arrangement of testes and ovary, and egg size. Yahata (1934), in transferring *Enodiotrema rugocaudatum* to *Glypthelmins*, separated this species from *G. quieta* by the relative positions of the ovary and ventral sucker, as well as the position of the genital pore and that of the testes. The author's inability to obtain sufficient specimens of *G. staffordi* or *G. rugocaudata* precludes any assessment of morphological characters which could be used to differentiate these two forms from each other or from *G. quieta*, *G. shastai* or *G. facioi*. It should be noted that the literature concerning the glypthelminths contains no statements which purport to distinguish *G. staffordi* from *G. rugocaudata*. Examination of pertinent figures and descriptions of *G. rugocaudata* (Yoshida, 1916; Yahata, 1934) and *G. staffordi* (Tubangui, 1928; Yuen, 1962; Fischthal and Kuntz, 1967; Odening, 1968) and examination of four specimens of *G. staffordi* (USNM Helm. Coll. No. 61702) indicated that the only discernible difference between the two species was the relative length of the esophagus (Table 3). Although not giving measurements, Yahata (1934) figured specimens of *G. rugocaudata* which show a relatively longer esophagus than that of *G. staffordi*. In view of the lack of supportive evidence to the contrary and the wide geographic separation of these two species from the North and Central American species, both *G. staffordi* and *G. rugocaudata* are tentatively accepted as valid species. Of Parenthetic interest in this regard is the report of a *Glypthelmins* sp. from *Rana temporaria* in eastern Siberia (Milogradova and Spasskii, 1957) which extends the northern range of the genus in Asia. Further study of this northern form could provide data for evaluating both the systematics of the Asian species of *Glypthelmins* and their relation to the North and Central American forms.

Nasir and Diaz (1970) considered *G. shastai* a synonym of *G. linguatula*, a species transferred to *Choledocystus* by Byrd and Maples (1963) and the position with which the author

is in accord. It is difficult to determine on what basis the synonymy was advanced since Nasir and Diaz' key was constructed around those forms with a known cercaria and those for which the cercaria was unknown. Study of the holotype of *G. shastai* indicates that the species was described from immature forms, judging by the development of the uterus. However, the presence of *G. shastai* in a bufonid host as well as the large testes suggests that this species may be more closely allied to *Choledocystus* than is presently suspected. Since the form of the excretory bladder is unknown, *G. shastai* is retained in *Glypthelmins* pending further study. As such, it can be distinguished from *G. facioi* by the presence of tegumental spines rather than scales and by the relatively greater size of the testes, a character together with egg size which distinguishes it from *G. quieta.*

Glypthelmins facioi can be distinguished from *G. quieta* by the presence of scales (Fig. 3) as well as by egg size. It should be noted, however, that although Brenes Madrigal et al. (1959) reported that *G. facioi* possessed eggs measuring 33–47 by 20–21, eggs of specimens collected at Turrialba measured 27–32 by 12–20.

Cheng's (1959) establishment of *Reynoldstrema* for *Glypthelmins africana* has much to support it; however, its relationship to the other glypthelminths is questionable. He maintained that "The posteriorly located uterus and the posteriorly situated testes are sufficient criteria to justify the erection of . . . *Reynoldstrema.*" Cheng regarded this genus more closely allied to *Brachycoelium* (Dujardin, 1845), *Glypthelmins* and *Margeana* than to *Astiotrema* Looss, 1900, since *Reynoldstrema* lacked a prepharynx, long Laurer's canal, and an excretory bladder reaching the seminal receptacle, characteristics of *Astiotrema* as outlined by Olsen (1937). However, the prime generic characters of *Reynoldstrema* strongly suggest a closer relationship to *Astiotrema* than that advanced by Cheng (1959).

While Beverly-Burton (1963) noted that Dollfus (1950) included *Glypthelmins* in the Brachycoeliidae Johnston, 1912, on the basis of the I-shaped excretory bladder, she agreed with Yamaguti (1958) and Skrjabin and Antipin (1958) in assigning *Glypthelmins* to the Plagiorchiidae, after she demonstrated a Y-shaped bladder in *G. africana*, recovered from *Rana adspersa* (Tschudi) and *Mabuya striata* (Peters) in Southern Rhodesia. She further maintained that *Plagiorchis himalayi* (Jordan, 1930), *P. momplei* Dollfus, 1932, and *P. ramlianus* (Looss, 1896) were more similar to *G. africana* "than . . . the accepted species of *Glypthelmins* . . . ," in that the uterus filled the area posterior to the ceca and vitellaria. Consequently, she suggested that a separate genus might be erected for some of these forms.

In reviewing *Reynoldstrema*, Fischthal and Thomas (1968), after considering the suggestions of Beverly-Burton (1963), indicated that Cheng (1959) had previously established a new genus for *G. africana.* These authors disagreed with the placement of *G. africana* in *Plagiorchis* Luhe, 1899, by Vercammen-Grandjean (1960) or *Haplometra* Looss, 1899, by Manter and Pritchard (1964). Fischthal and Thomas maintained the validity of *Reynoldstrema* to which they transferred *Plagiorchis laurenti* Vercammen-Grandjean, 1960, and *P. berghei* Vercammen-Grandjean, 1960.

In view of the generic revision of *Plagiorchis* by Odening (1959) and the findings of Beverly-Burton (1963) and Fischthal and Thomas (1968), the author does not accept the synonymy of *Reynoldstrema* with *Plagiorchis* proposed by Yamaguti (1971). The Y-shaped excretory vesicle exhibited by species of *Reynoldstrema* indicates placement in the Plagiorchiidae rather than the Macroderoididae as proposed by Odening (1964). Additionally, the morphological characters of *Reynoldstrema* are sufficient to allocate this genus to the subfamily Astiotrematinae Baer, 1924.

Skrjabin and Antipin (1958) recorded a V-shaped excretory bladder for *Glypthelmins diana* Belouss, 1959. The presence of a V-shaped bladder in this species precludes its placement in either the Plagiorchiidae or the Macroderoididae. Accordingly, *G. diana* is removed from *Glypthelmins*, which is characterized by an I-shaped bladder. However, the V-shaped bladder of this species appears to indicate a lecithodendriid relationship, but since the species does not conform to any of the presently known lecithodendriid genera, it seems advisable to designate the species *incertae sedis* pending further investigation.

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Eimeria tenella: Comparative Oocyst Production in Primary Cultures of Chicken Kidney Cells Maintained in Various Media Systems

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ABSTRACT: *Eimeria tenella* sporozoites were inoculated into primary cultures of chicken kidney cells maintained in 15 media systems. Oocyst production in 14 of these systems was compared with that in a system designated by Doran (1971) as A–B, in which cultures were maintained before inoculation in Medium A and after inoculation in Medium B. Medium A was composed of 80% Hanks' balanced salt solution (HBSS), 10% lactalbumin hydrolysate (LAH, 2.5% solution in HBSS), and 10% fetal calf serum (fcs); Medium B was composed of 90% HBSS, 5% LAH, and 5% fcs.

Oocyst yields were greater than yields in system A–B when cells were maintained before inoculation in Medium A and after inoculation in a medium composed of (1) 90% of either Basal Medium of Eagle, Minimal Essential Medium of Eagle, or Medium 199 with HBSS, (2) 5% LAH, and (3) 5% fcs.

Doran (1971) compared the effect of four media systems on infection of primary chicken kidney cells and subsequent oocyst production by *Eimeria tenella*. He found most oocysts in systems designated as A–B and B–B where cells were either maintained before inoculation in Medium A and after inoculation in Medium B or were maintained before and after inoculation in Medium B. Medium A was composed of 80% Hanks' balanced salt solution (HBSS), 10% lactalbumin hydrolysate (LAH, 2.5% solution in HBSS), and 10% fetal calf serum (fcs); Medium B was composed of 90% HBSS, 5% LAH, and 5% fcs. He found very few or no oocysts in system C–C (Basal Medium of Eagle and 10% fcs) and system D–D (Basal Medium of Eagle and 5% fcs). In later experiments, Doran (1971) also found that the oocyst yield in media system B–B could be increased by using 100,000 sporozoites free of debris (oocyst and sporocyst hulls) and 10 ml rather than 2 ml of media for maintenance after inoculation. We have tested many media systems in attempts to increase the yield in culture even more. In the present paper, system A–B was used as a baseline standard for comparison of oocyst production in primary chicken kidney cells maintained in 14 other media systems.

Materials and Methods

Sporozoites: Sporozoites of the Beltsville strain of *E. tenella* were cleaned of debris (oocyst and sporocyst hulls) as previously described (Doran and Augustine, 1973). They were suspended in Medium B of Doran (1971) and then frozen and stored as previously described (Doran, 1969). All sporozoites were from the same batch of oocysts, 4–7 weeks old when excysted and frozen, and 2–3 years old when thawed.

Cell cultures: Kidneys were obtained from 2 to 3-week-old chickens. The procedures for trypsinizing minced kidney tissue, treating cells after trypsinization, and establishing cultures in Leighton tubes (10.5 × 35 mm coverslip) were the same as those previously described (Doran, 1971).

Media systems: The media systems tested are shown in Table 1. All media contained streptomycin sulfate (100 μg/ml), penicillin G potassium (100 units/ml), and phenol red indicator (100 μg/ml). They were adjusted to pH 7.0–7.2 with 1N NaOH or HCl.

When the experiments comparing four media systems in the previous study (Doran, 1971) were completed, system B–B rather than A–B was arbitrarily chosen for use in the experiments that followed. We have compared oocyst yields in A–B and B–B many times (unpublished) under experimental conditions that Doran (1971) found best. However, there was less than a 7% average difference between oocyst yields in the two systems. The choice of system A–B for comparison in the present study was again arbitrary.

Table 1. Media systems used for maintaining chicken kidney cells.

System	Before inoculation	After inoculation
A–B	Medium A*	Medium B*
C–D	Medium C*	Medium D*
E	90% Minimal Essential Medium of Eagle with HBSS[1] (HMEM) + 10% fcs[2]	95% HMEM + 5% fcs
F	90% Medium 199 with HBSS (H199) + 10% fcs	95% H199 + 5% fcs
G	Medium A*	Medium D*
H	Medium A*	95% HMEM + 5% fcs
I	Medium A*	95% H199 + 5% fcs
J	Medium C*	Medium B*
K	90% HMEM + 10% fcs	Medium B*
L	90% H199 + 10% fcs	Medium B*
M	Medium C*	90% HBME, 5% LAH,[3] + 5% fcs
N	90% HMEM + 10% fcs	90% HMEM, 5% LAH, + 5% fcs
O	90% H199 + 10% fcs	90% H199, 5% LAH, + 5% fcs
P	Medium A*	90% HBME, 5% LAH, + 5% fcs
Q	Medium A*	90% HMEM, 5% LAH, + 5% fcs
R	Medium A*	90% H199, 5% LAH, + 5% fcs

* Doran (1971).
[1] Hanks' balanced salt solution.
[2] Fetal calf serum.
[3] Lactalbumin hydrolysate (2.5% solution in HBSS).

INOCULATION AND MAINTENANCE OF CELL CULTURES: Cell cultures were inoculated when they were 48 hr old. Sporozoites were quickly thawed, placed in 50 ml of Medium B (Doran, 1971) without antibiotics, and counted with a counting chamber. The concentration was adjusted so that 1 ml contained approximately 100,000 sporozoites. Media in the culture tubes were then decanted and replaced with 1 ml of the sporozoite suspension. After 3½ hr, the inoculation medium was replaced with 10 ml of new medium. Cultures were kept at 41.5 C.

EVALUATION OF CULTURES: From 5 to 9 of the media systems were tested at one time. Eight cultures were prepared for each system, including system A–B, which was used as a comparison. At 4 hr after inoculation with sporozoites, coverslips were removed from three of the cultures maintained in each medium. At 4 days, at least two of the remaining cultures in each medium were examined using an inverted microscope to determine whether clusters or islets of epithelial-like cells were present. At 8 days, coverslips were removed from the remaining five cultures in each medium. Cells on the 8 coverslips were fixed and stained as previously described (Doran and Vetterling, 1967). The medium from which coverslips had been removed at 8 days was pooled for each medium and centrifuged at 500× g for 5 min. All but 1 ml of the supernatant was removed, and triplicate counts with a counting chamber were made of the number of oocysts present.

Under 625× magnification, 240 microscopic fields on each stained coverslip were examined as previously described (Doran, 1971). Counts were made of the number of sporozoites at 4 hr and oocysts at 8 days. The following equation

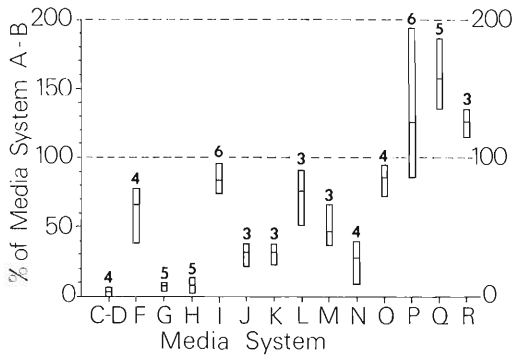

Figure 1. Oocyst production in various media systems as compared with production in system A–B. Number above bar indicates number of replicates. Horizontal line within bar indicates average. 3–5 coverslips/replicate.

was used to calculate the oocyst index (OI; number of oocysts/intracellular sporozoite):

$$OI = \frac{\text{number of oocysts in 240 fields} \times 25.9 + \dfrac{\text{number of oocysts in pooled medium}}{\text{number of cultures}}}{\text{number of sporozoites in 240 fields} \times 25.9}$$

where 25.9 is a multiplication factor representing the area of Leighton tube window and coverslip not covered by the 240 microscopic fields (Leighton tube window, 440 mm^2; 240 fields, 17 mm^2).

Results and Discussion

No oocysts were found in medium system E. Oocyst production in the other systems as compared with that in system A–B is shown in Figure 1. Fewer oocysts were produced in systems C–D and F through O than in system A–B. However, more oocysts were produced in systems P, Q, and R than in system A–B by an averages of 26, 57, and 26 per cent, respectively. In system P, the average OI in one replicate was nearly twice as great as that in system A–B.

Islets or elevated areas of epithelial-like cells have been thought necessary for gametogony (Bedrnik, 1967; Strout and Ouellette, 1968, 1970; Doran, 1970, 1971). In the present study, these areas were abundant in all systems that involved H199 (F, I, L, O, R) and in the systems in which cells were maintained in Medium A before inoculation and in media containing either HBME or HMEM and LAH (P, Q) after inoculation. These were the seven systems in which oocyst yield was greatest. Islets were absent or few in number in all systems involving HBME and HMEM except in systems P and Q.

Data indicate that LAH influenced oocyst production, especially in the systems involving HBME and HMEM. In the systems involving either HBME, HMEM, or H199, oocyst yield was lowest when LAH was absent from media both before and after inoculation. The yield increased when LAH was present in media after inoculation, and increased to levels higher than in system A–B when present in media both before and after inoculation. LAH may have produced a better physical environment by causing a greater retention of the islets of epithelial-like cells. Because the yields in systems P, Q, and R were greater than in system A–B, LAH possibly contains one or more growth factors that in combination with those in HBME, HMEM, and H199, provided a more satisfactory nutritional environment than the environment in system A–B.

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New Monogenetic Trematodes from Freshwater Fishes of Western Colombia with the Proposal of *Anacanthoroides* gen. n. (Dactylogyridae)[1]

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ABSTRACT: *Anacanthoroides* gen. n. is proposed and the following new species are described from the gills of freshwater fishes of Colombia: *Urocleidoides lebedevi* sp. n. from *Pimelodus grosskopfi* Steindachner, Pimelodidae; *U. mamaevi* sp. n. from *Cephalosilurus zungaro* (Humboldt), Pimelodidae; and *Anacanthoroides mizellei* sp. n. from *Prochilodus reticulatus* Steindachner, Characidae. *U. lebedevi* is the only species of *Urocleidoides* reported with blind intestinal crura. *U. mamaevi* differs from all other members of the genus by having overlapping gonads. *Anacanthoroides* is similar to *Anacanthorus* Mizelle and Price, 1964, from which it differs principally by having members with disc-shaped haptors armed with 12 marginal and four central hooks.

Three new monogenetic trematodes (Dactylogyridae), one a member of the new genus *Anacanthoroides*, were found on freshwater fishes from western Colombia. Fish hosts were identified with the aid of the monograph by Miles (1943). Methods of collection, preservation, and preparation of helminths for study were those described by Kritsky and Thatcher (1974). Terminology is that of Mizelle and Kritsky (1967) and Mizelle et al. (1968). All measurements are in microns and were made according to the recommendations of Mizelle and Klucka (1953). Types are in the US National Museum and the Museum of the Instituto Oswaldo Cruz (IOC) as indicated below.

Urocleidoides lebedevi sp. n. (Figs. 1–12)

HOST AND LOCALITIES: *Pimelodus grosskopfi* Steindachner, Pimelodidae; Rio Cauca, Juanchito, Valle, Cali (type), and Rio Frio near Tulua, Valle.

LOCATION ON HOST: Gills.

SPECIMENS STUDIED: 18.

TYPES: USNM Helm. Coll. 73278 (holotype), 73279, 73280; IOC 31222.

Description

Body fusiform, 943 (680–1,069) long; greatest width 192 (119–313) in posterior half or near midlength. Tegument thin, smooth. Two terminal and two lateral cephalic lobes incipient; four pairs of head organs; cephalic glands inconspicuous, near posterolateral margin of pharynx. Four eyes; members of posterior pair larger, closer together, with small lens; accessory granules few in cephalic region. Mouth subterminal; pharynx subovate, 111 (98–123) × 86 (78–94); esophagus extends to level of cirral pore; intestinal crura blind. Peduncle broad; haptor subhexagonal, posterior border concave; haptor length 104 (75–122), width 185 (158–216). Anchors similar, each with large base, short shaft, evenly curved point; ventral anchor 61 (58–65) long, base 36 (32–41) wide; dorsal anchor 49 (44–54) long, base 27 (23–34) wide. Anchor filament well developed. Bars V shaped; ventral bar 92 (78–112) long, dorsal bar 64 (53–79) long. Hook distribution ancyrocephaline (Mizelle, 1936); hooks 22 (20–26) long; prs. 1, 3, 4, 7 with recurved point, depressed thumb, heavy shank; pr. 2 occasionally absent, with proximal bean-shaped termination of shank; prs. 5, 6 with delicate shank; FH loop 0.7 shank length. Gonads tandem; testis ovate, 199 (162–257) × 112 (87–129); vas deferens loops left intestinal crus; seminal vesicle coiled, a dilation of vas deferens; cirrus with two to three rings, largest ring diameter 72 (56–83); accessory piece a sclerotized portion of wall of cirral duct, 49 (44–54) long; cirral pore midventral. Ovary C shaped (lateral view) with

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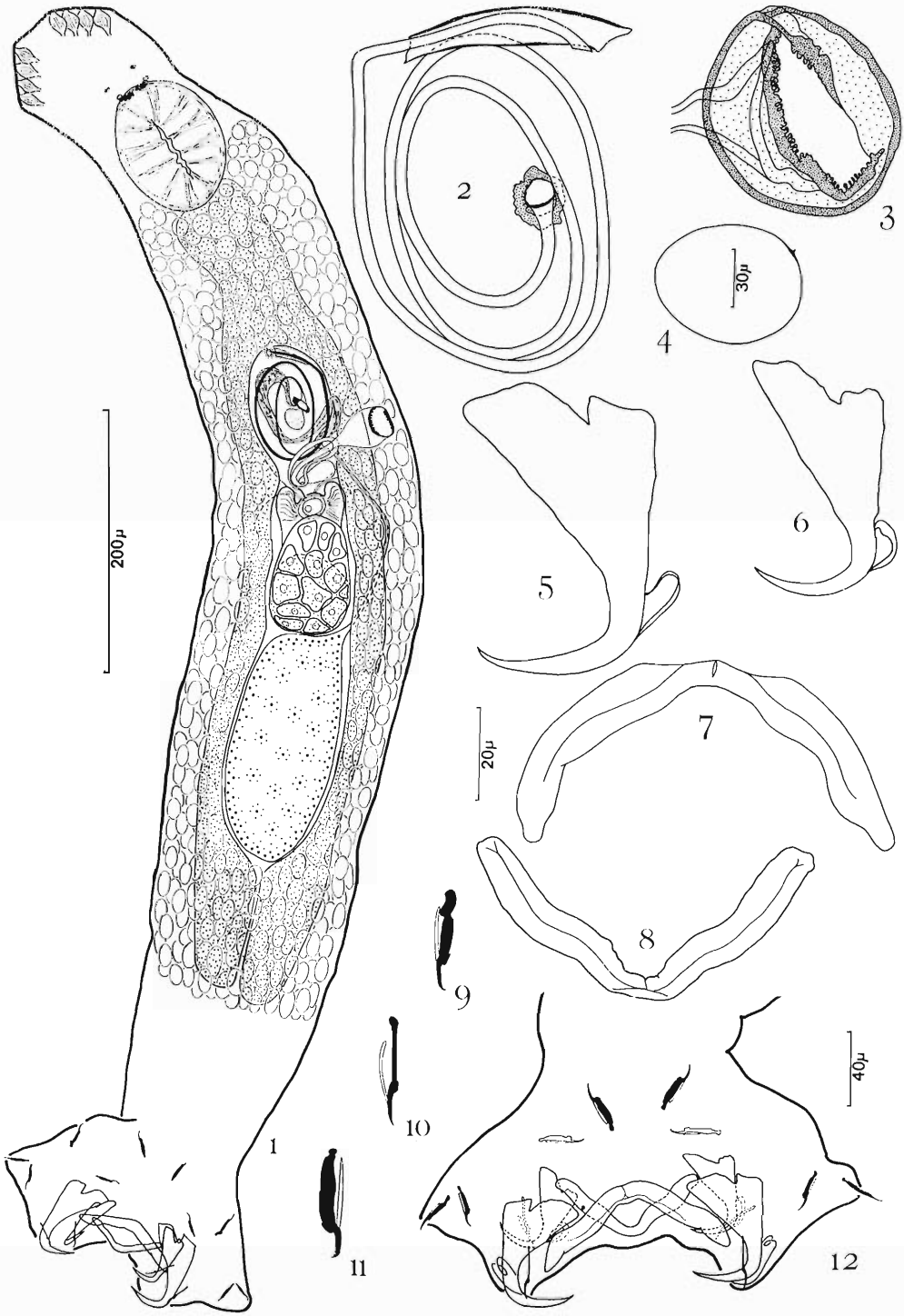

open portion dorsal, greatest width 89 (70–98); ootype conspicuous; vagina sinistroventral, with sclerotized papillated orifice and coiled tube; seminal receptacle subovate; vitellaria random in trunk except absent in regions of other reproductive organs; egg ovate, 81 × 67, with short filament.

Remarks

Urocleidoides lebedevi sp. n. is apparently closest to the type species, *U. reticulatus* Mizelle and Price, 1964 from the gills of *Lebistes reticulatus* in Trinidad. They differ as follows: (1) *U. lebedevi* possesses blind intestinal crura (confluent in *U. reticulatus*); (2) the haptoral hooks of *U. lebedevi* occur as three morphologic types (one type in *U. reticulatus*); (3) a vaginal sclerite is absent in *U. lebedevi* (present in *U. reticulatus*); (4) the distal portion of the cirrus shaft of *U. lebedevi* has an angular bend (straight in *U. reticulatus*); and (5) host. These differences are deemed sufficient for designating *U. lebedevi* as a new species. It is named in honor of Dr. Boris Lebedev, Institute of Biology and Pedology, USSR Academy of Sciences, Vladivostok, in recognition of his many contributions in parasitology.

Urocleidoides mamaevi sp. n. (Figs. 13–20)

HOST AND LOCALITY: *Cephalosilurus zungaro* (Humboldt), Pimelodidae; Rio Palo near Puerto Tejada, Cauca.

LOCATION ON HOST: Gills.

SPECIMENS STUDIED: 5.

TYPES: USNM Helm. Coll. 73281 (holotype), 73282; IOC 31223.

Description

Body fusiform; length 634 (540–778); greatest width 93 (73–141) at level of gonads. Tegument thin, smooth. Cephalic lobes, head organs, cephalic glands indistinct. Eyes four, poorly developed, variable in size and position; accessory granules usually in cephalic region. Mouth subterminal; pharynx muscular, subspherical, 27 (23–29) in diameter; esophagus extends to level of cirrus; intestinal crura confluent posteriorly. Peduncle broad; haptor usually bilobed, 86 (75–98) long, 116 (98–132) wide. Anchor pairs widely separated, dorsal pair anterior; anchors similar, with two angular bends of shaft and point; ventral anchor 27–28 long, base 11–12 wide; dorsal anchor 30 (29–31) long, base 12 (11–13) wide; anchor filament conspicuous. Bars broadly U or V shaped; ventral bar with posteromedial process, bar 43 (41–47) long; dorsal bar 37 (34–43) long. Hook distribution ancyrocephaline (Mizelle, 1936), except prs. 2, 3, 4, 6, 7 being situated anterior to ventral bar and hook pr. 1; hooks similar, 23–24 long, with dilated shank, short FH loop. Gonads in midregion; testis dorsal to ovary, saccate, 120–121 long, 28–29 wide; vas deferens enlarged, loops left intestinal crus; seminal vesicle large, spindle shaped, dorsal in body, a dilation of vas deferens, 68 (61–73) long; prostatic reservoir C shaped; prostates indistinct. Cirrus with 2 to 3 rings, largest ring diameter 24 (22–26); accessory piece articulated to cirrus base, distally forming sclerotized wall of cirral duct. Ovary rod shaped, 204 long, 20–21 wide; oviduct short; uterus, vitelline ducts, and associated organs not observed; vagina sinistral, wrinkled, opening into large spindle-shaped seminal receptacle; seminal receptacle 68 (64–70) long; vitellaria as two dorsal bilateral bands confluent anterior to cirrus and posterior to ovary.

Remarks

Urocleidoides mamaevi sp. n. is the only species in the genus reported to have overlapping gonads. In all other species, in which the position of the gonads is known, the ovary is pretesticular. *U. mamaevi* is most similar in the morphology of the

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Figures 1–12. *Urocleidoides lebedevi* sp. n. 1. Composite illustration of whole mount (Ventral). 2. Copulatory complex (dorsal). 3. Vagina. 4. Egg. 5. Ventral anchor. 6. Dorsal anchor. 7. Ventral bar. 8. Dorsal bar. 9. Hook (pr. 2). 10. Hook (pr. 6). 11. Hook (pr. 7). 12. Enlargement of haptor lacking hook-pair 2 (hooks situated ventral in haptor are black). All figures are to the same scale (20 μ) except Figures 1, 4, 12 (200 μ, 30 μ, 40 μ, respectively).

haptoral armament and the copulatory complex to *U. variabilis* Mizelle and Kritsky, 1969. These species are easily differentiated by the ventral bar: a posteromedial process like that on the ventral bar of *U. mamaevi* is absent in *U. variabilis*. The new species is named for Dr. Yu. Mamaev, Institute of Biology and Pedology, USSR Academy of Sciences, Vladivostok, who has contributed significantly to the knowledge of the Monogenea.

Anacanthorinae Price, 1967

EMENDED DIAGNOSIS: Dactylogyridae: Haptor lacking anchors and bars. Haptoral hooks 16 or 18; one or two pairs reduced, without thumb. Eyes present. Intestinal crura confluent. Gonads intercecal, tandem (testis posterior) or overlapping. Vagina sinistral or sinistroventral. Terminal portion of uterus with metraterm. Vas deferens looping left intestinal crus.

Anacanthoroides gen. n.

DIAGNOSIS: Dactylogyridae: Anacanthorinae: Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Cephalic area rounded, lacking lobes. Head organs, cephalic glands present. Four eyes. Pharynx muscular, glandular; esophagus short; intestinal crura (two) without diverticulae, confluent posteriorly. Gonads near midlength, testis dorsal to ovary; seminal vesicle a dilation of vas deferens; prostatic reservoir present; cirrus base small, shaft a loosely coiled attenuated tube; accessory piece variable. Ovary ventral; oviduct short; vagina sinistral; seminal receptacle preovarian; terminal portion of uterus heavily muscular forming well-developed metraterm; uterine pore sinistroventral, anterior to cirrus. Vitellaria well developed in trunk and peduncle. Haptor disc shaped with 12 (6 pairs) hooks situated along margin and 2 pairs (one reduced) situated near center; anchor and bars absent. Parasitic on gills of freshwater fishes.

TYPE AND ONLY SPECIES: *A. mizellei* sp. n.

TYPE HOST: *Prochilodus reticulatus* Steindachner.

Remarks

Haptoral anchors are also lacking in the following genera of Dactylogyridae: *Acolpenteron* Fischthal and Allison, 1940; *Anacanthorus* Mizelle and Price, 1965; *Anonchohaptor* Mueller, 1938; *Icelanonchohaptor* Leiby, Kritsky, and Pederson, 1972; and *Pseudacolpenteron* Bychowsky and Gussev, 1955. *Anacanthoroides* differs from them by possessing a disc-shaped haptor armed with six pairs of marginal and two pairs of central hooks.

Comparison of the internal anatomy of members of each of these genera indicates that *Anacanthoroides* most closely resembles *Anacanthorus* (*A. colombianus* Kritsky and Thatcher, 1974, examined) in that both possess (1) a metraterm, (2) confluent intestinal crura, (3) an intercecal ovary, and (4) at least one pair of reduced haptoral hooks. In addition to the haptor, *Anacanthoroides* differs from *Anacanthorus* by lacking cephalic lobes and by having overlapping gonads (tandem in *Anacanthorus*) and a loosely coiled cirrus (rod-shaped in *Anacanthorus*).

Our assignment of *Anacanthoroides* to the Anacanthorinae is provisional. The criteria used to originally establish the subfamily, "Anchors lacking. Hooks 18." (See Price, 1966 (1967)), alone would not appear to justify the taxon. However, the modification of the distal portion of the uterus into a metraterm in *Anacanthoroides* and *Anacanthorus* (poorly developed in latter) may indicate a natural grouping, since this structure does not occur in other Dactylogyridae.

Anacanthoroides mizellei sp. n. (Figs. 21–23)

HOST AND LOCALITIES: *Prochilodus reticulatus* Steindachner, Characidae; Rio Palo near Puerto Tejada, Cauca (type), Rio Frio near

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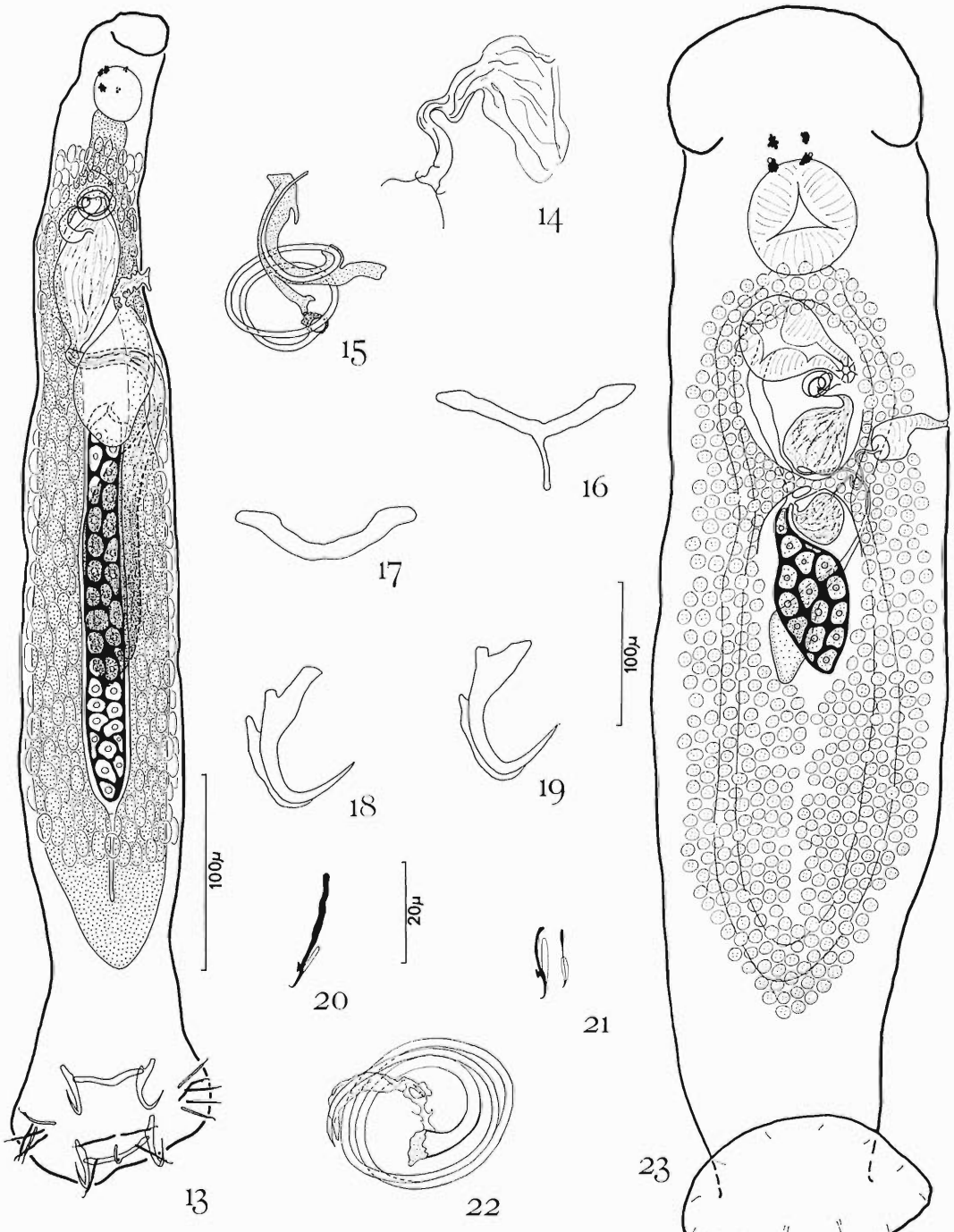
Figures 13–20. ***Urocleidoides mamaevi*** **sp. n. 13. Ventral view of holotype. 14. Vagina and distal seminal receptacle. 15. Copulatory complex. 16. Ventral bar. 17. Dorsal bar. 18. Dorsal anchor. 19. Ventral anchor. 20. Hook. Figures 21–23.** ***Anacanthoroides mizellei*** **gen. et sp. n. 21. Hooks. 22. Copulatory complex. 23. Holotype (ventral). The 20 μ scale refers to Figures 14–22.**



Tulua, Valle; Rio Guachinte and Rio Pance, Valle.

LOCATION ON HOST: Gills.

SPECIMENS STUDIED: 8.

TYPES: USNM Helm. Coll. 73283 (holotype), 73284, 73285; IOC 31221.

Description

Body robust; length 917 (567–1,177), greatest width 204 (140–281) near midlength. Tegument thin, smooth. Head organs poorly developed in lateral cephalic area; pre- and post-pharyngeal cephalic glands in dorsal cephalic region. Eyes equidistant; members of posterior pair slightly larger with small anterolateral lens; accessory granules absent. Mouth subterminal, pharynx subovate, 81 (62–90) wide. Peduncle broad. Haptor disc shaped; diameter 146 (108–170). Hooks (7 pairs) similar, subequal, 14 (13–15) long, with recurved point, depressed thumb, proximally expanded shank; FH loop 0.7 shank length. One pair of central hooks reduced, 8 (7–9) long. Testis saccate, 79 (67–90) long, 42 (30–48) wide; seminal vesicle pyriform; prostatic reservoir variable; cirrus with two to three loose rings, largest ring diameter 35 (33–37); accessory piece variable, delicate, articulated to cirrus base; cirral pore ventral. Ovary pyriform, greatest width 79 (72–92); oviduct large; vagina sinistral, variable, with expanded sclerotized wall distally and delicate tube proximally; seminal receptacle usually conspicuous, filled with sperm; vitellaria coextensive with gut.

Remarks

This species is named for Dr. J. D. Mizelle (Washington State University), Roche Harbor, Washington, in respect and in recognition of his many works on the monogenetic trematodes of the Nearctic and Neotropical Regions.

Acknowledgment

We wish to thank Dr. A. Gussev, USSR Academy of Sciences, Leningrad, for his useful comments on our new genus and the subfamily Anacanthorinae.

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Comparative Anthelmintic Efficacy in Dogs Treated with Vincofos, Ticarbodine, or Mebendazole[1, 2]

D. K. Hass and J. A. Collins
Shell Development Company, Modesto, California 95352

ABSTRACT: Three broad-spectrum anthelmintics have been tested in dogs for the purpose of comparing their overall parasitical effect. Vincofos was highly effective for ascarids, hookworms, whipworms, and taeniid tapeworms, while *Dipylidium caninum* was slightly less susceptible to single dosages of 18 mg/kg. Ticarbodine was highly effective for ascarids, hookworms, and all tapeworms but ineffective for the dog whipworm at the recommended dosage of 100 mg/kg. Mebendazole was highly effective for ascarids, hookworms and whipworms but lacked efficiency against tapeworms following a treatment regimen of 100 mg given twice daily for five consecutive days.

KEY WORDS: anthelmintics, efficacy, dogs, comparative evaluation.

During the past three years (1972–1974), several new candidate anthelmintic materials have been reported as effective broad-spectrum canine anthelmintics (Boisvenue et al., 1972; Vanparijs and Thienpont, 1973; and Hass and Collins, 1974). These materials represent a wide range of chemistry and are truly significant in that they claim anthelmintic efficacy for both nematode and cestode parasites. As would be expected, however, the diversity of chemical type would cause somewhat different responses with regard to the many species of helminths which can be found in parasitized dogs. Therefore, a study was undertaken to compare the anthelmintic efficiency of three candidate materials, vincofos, ticarbodine, and mebendazole, which were representative (Fig. 1) of an organophosphate, a piperidinecarbothioamide, and benzimidazolecarbamate, respectively. This report presents the collected data from a series of critical tests which were conducted for the purpose of making a comparative evaluation of each material's individual anthelmintic properties.

Materials and Methods

Specific testing for the combined nematocidal and cesticidal anthelmintic efficacy of vincofos, ticarbodine, and mebendazole was accomplished with 60 dogs of mixed breeding and 13 Beagle puppies. Older dogs were used for their hookworm, whipworm and/or tapeworm infections, while the Beagle puppies were primarily utilized for their natural ascarid and artificially induced (each was given 500 infective larvae) *Uncinaria stenocephala* infections. All dogs obtained from commercial sources were held for a four-week period and received standard immunization for rabies, canine distemper, infectious canine hepatitis, and leptospirosis. Each dog was placed in an individual indoor pen with continuous availability to dry dog chow and water at all times. Fecal egg counts (sodium dichromate floatation) were made to identify the parasite spectrum present in each dog. In addition, several daily inspections were made for tapeworm segments in the feces so as to allow for uniform distribution of parasite populations for each material being investigated.

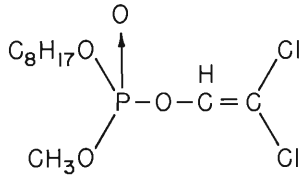
The vincofos was prepared as an oil solution in soft gelatin capsules and administered orally as a single dose of 18 mg/kg. The ticarbodine[3] was triturated with lactose, and individual dosages of 100 mg/kg were prepared in hard gelatin capsules for oral administration. The mebendazole[4] was triturated with lactose and prepared in hard gelatin capsules containing 100 mg of active ingredient per capsule. One 100 mg capsule of mebendazole was given orally twice daily (b.i.d.) for a total of 5 consecutive days. All of the dogs were on full free

[1] Presented in part at the 3rd International Congress of Parasitology, Munich, Germany, 25–31 August, 1974.

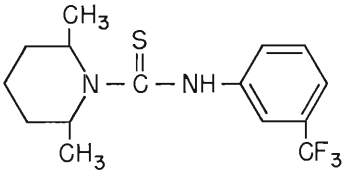
[2] Due to unforeseen toxicological problems in the field, Vincofos (Shell) has been withdrawn from the market and is no longer commercially available. Ticarbodine (Eli Lilly) has been cleared by the U.S. Food and Drug Administration but remains to be introduced to the anthelmintic market. Mebendazole is sold in various countries outside the United States as Telmin® Dog Wormer (Ethnor).

[3] Obtained from Eli Lilly and Company, Greenfield, Indiana.

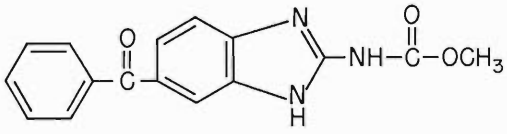
[4] Obtained from Janssen Pharmaceutica, Beerse, Belgium.



VINCOFOS



TICARBODINE



MEBENDAZOLE

Figure 1. Chemical structures for the anthelmintics: vincofos, ticarbodine, and mebendazole.

choice feeding and none were fasted as a condition of treatment.

With the vincofos and ticarbodine treated dogs, each test animal was treated on a Monday morning and subjected to a routine parasitological necropsy on the following Friday morning. During the ensuing 4 days, 24-hr fecal collections were washed on 60 mesh screens and the washings retained in 10% formalin for future recovery of nematodes and cestodes and subsequent identification and tabulation of helminths expelled following therapy. With the mebendazole-treated dogs, the test animals were held and fecal washings made for an additional 5 days because of the delay in passage of worms associated with this drug. On the day prior to necropsy, the dogs were taken off feed so as to facilitate the procedures and reduce food present in the gastrointestinal tracts. At necropsy, all of the intestinal contents and mucosal scrapings were washed onto a 60 mesh screen and the washings retained in 10% formalin for further recovery of any parasitic worms that may be remaining after treatment. Comparative critical anthelmintic evaluations were made on the basis of the tabulated data following identification and counting of the parasites recovered from the feces versus those found at necropsy.

Results and Discussion

The results for this comparative study are summarized in Table 1. As will be noted, there were considerable numbers of worms present in these dogs, and each of the seven most common parasites was well represented. With the vincofos-treated dogs, all or nearly all of the ascarids, hookworms, whipworms, and the tapeworm, *Taenia pisiformis*, were expelled following therapy. The tapeworm, *Dipylidium caninum*, was also effectively removed by the vincofos treatment, but it appeared that a slightly greater dosage would be required to obtain maximum anthelmintic effect.

The ticarbodine treatment was also very effective for the ascarids, hookworms and both types of tapeworms. Ticarbodine is not effective for the dog whipworm, *Trichuris vulpis*. Of specific interest is that six of the dogs were confirmed to be infected with tapeworms on the basis of fecal egg counts and observations of segments passed in the feces. Yet, no intact worms or scolices were removed from the fecal or necropsy washings. These observations support the theory that ticarbodine causes dissolution of the tapeworms before they are passed in the feces or softens the tapeworm so that it is washed through the screens.

The multiple dose mebendazole treatment effectively expelled all of the ascarids, hookworms and whipworms from the dogs. However, the cesticidal properties of mebendazole demonstrated in this study were somewhat less than had been reported previously (Vanparijs and Thienpont, loc. cit.). Only 40% of the *taenia* and 6% of the *Dipylidium* tapeworms were expelled following mebendazole treatment.

In recapitulation, vincofos was effective for all of the nematode and most of the tapeworm

Table 1. Anthelmintic response in dogs treated with vincofos, ticarbodine and mebendazole.*

Parasite	Vincofos—40 dogs			Ticarbodine—14 dogs			Mebendazole—19 dogs		
	No. of worms		Per cent efficiency	No. of worms		Per cent efficiency	No. of worms		Per cent efficiency
	Passed	Necropsy		Passed	Necropsy		Passed	Necropsy	
Toxocara canis	146	0	100	70	0	100	82	0	100
Toxascaris leonina	212	4	98	408	3	99	129	0	100
Ancylostoma caninum	1516	9	99	262	0	100	609	0	100
Uncinaria stenocephala	1541	18	99	353	47	88**	356	0	100
Trichuris vulpis	277	3	99	45	855	5	354	0	100
Taenia pisiformis	35	3	92	†	0	100	46	69	40
Dipylidium caninum	269	56	83	†	0	100	10	165	6

* Treatment dosages: Vincofos = 18 mg/kg; ticarbodine = 100 mg/kg; mebendazole = 100 mg b.i.d. × 5 days.
** All 47 *U. stenocephala* remaining at necropsy were from 4 puppies artificially exposed to infective larvae.
† Six dogs were positive for fecal egg counts and tapeworm segments in the feces, but no tapeworms or scolices were passed or recovered at necropsy.

parasites of dogs. Ticarbodine was effective for the ascarids, hookworms, and tapeworms but not the whipworm of dogs. Mebendazole with its multiple dose treatment was highly effective for all of the nematodes but lacked significant efficacy for the tapeworm parasites.

Acknowledgment

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Vanparijs, O., and D. Thienpont. 1973. Anthelminthische Wirkung des Mebendazols gegen Nematoden und Zestoden Bei Hunden. Deutsche Tierärztliche Wochenschrift 80: 320–322.

The 500th meeting of the Helminthological Society of Washington featured the presentation of three invited papers on the subject: "Control of Parasitic Infections." The authors, T. C. Cheng, E. J. L. Soulsby and W. C. Campbell, discussed the respective roles of the parasite, the host and of drugs. Publication of these papers is planned for the January 1977 issue of the *Proceedings*—Ed.

A Diagnostic Compendium of the Genus *Meloidogyne* (Nematoda: Heteroderidae)

R. P. Esser,[1] V. G. Perry[2] and A. L. Taylor[1]

ABSTRACT: Tabular morphometric and morphological data supported by illustrations are presented to facilitate identification of 35 species of *Meloidogyne*. *M. acrita* and *M. bauruensis*, formerly subspecies, are established at a specific level, and *Hypsoperine megriensis* becomes *Meloidogyne megriensis* (Pogosyan, 1971) n. comb.

Diagnostic compendia are engendered by necessity. Addition of new species in an already large genus tends to render existing taxonomic keys obsolete. Species determination also consumes excessive amounts of time in the search for, and comparison of, diverse species descriptions.

The diagnostic compendium differs from dichotomous keys in that keys go directly to a single species using gross and finite characteristics. The compendium eliminates most species from consideration and provides one or more specific possibilities that are confirmed by the original description or descriptions using finite characteristics. Keys become obsolete the moment a species (that is not contained in the key) is described. New species can be included in the compendium table as they occur, thus preventing early obsolescence.

The principal objective of this work is to facilitate the identification of *Meloidogyne* species.

The first Meloidogyne compendium was constructed in 1966 on 5 × 7 cards. Each card included male, female, larva, and egg characteristics. New cards were prepared as new species were described. After 2 years of use, the cards were mimeographed (6), an explanation added, and the compilation sent to a number of nematologists for comment.

Whitehead (1968) published a monograph of Meloidogyne, which included an excellent literature review and contributed invaluable data to many species in the genus. In his work *M. poghossianae* described by Kiryanova (1963) was placed in *species inquirenda*.

The senior author assisted and was taught identification of *Meloidogyne* spp. by B. G. Chitwood in the years 1956–57. Chitwood's system of identification was to first diagnose the female posterior cuticular pattern characteristics, and then corroborate his diagnosis with larval, male, female, and egg characteristics. Corroboration of stages was employed in the 1966 and subsequent compendia. Whitehead came to a similar conclusion regarding stage corroboration in his analysis of the genus in the 1968 monograph.

The compendium tables presented here comprise selected data considered essential for identification of the developmental stage represented in each table. Identification criteria selection was based on the following: The structure must be readily observable (*en face* views not utilized); the structure must be easily measurable (esophageal gland measurements rejected); the measurement must be well represented by all species (anal body diameter rejected); and the measurement must be sufficiently discrete to use comparatively (male body length rejected). The criteria selected and rejected in the establishment of the tables are presented below.

Female (Table 1)

Posterior protuberance: The first consideration in female differentiation was given to the presence of a posterior protuberance on the mature female body (Fig. 1A). In most species having this character, the protuberance is pronounced and unmistakable. Posterior cuticular patterns on a protuberance are not subject to maximum cuticle stretching, therefore lateral incisures are apt to be deeper and more pronounced (Fig. 5O, N). Female tail tips also are likely to be more definitive (Fig. 5O). In *M. spartinae* (Rau and Fassuliotis, 1965; Whitehead, 1968), a well-defined tail

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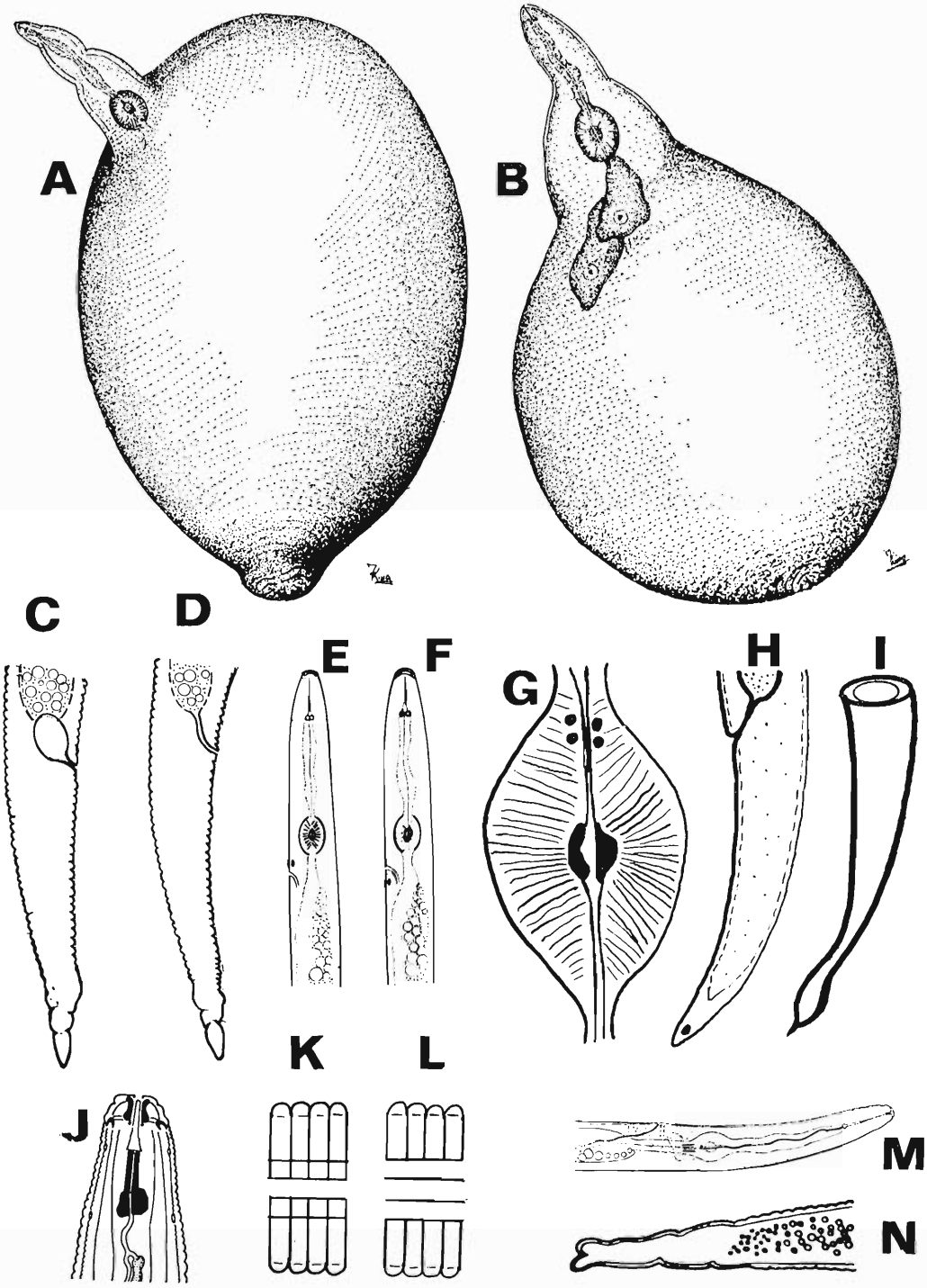
Florida Agricultural Journal Series No. 6083.

Table 1. Diagnostic characters of mature females.

Species	Posterior protuberance	Lateral incisures	Stylet length	Vulva lip striae	Perinium striae	Zone 1 striae	Zone 2 striae	Zone 3 striae	Zone 4 striae	Fig. 5	Excretory pore to stylet	Ex. pore* level in stylet lengths
mali	slight	yes	13–17	no	no	no	SBf	SBa	SBf	Z	posterior	2
*naasi***	slight	no	11–15	no	1	few	SU	SU-SBm	SBf	CC	anterior	¾
graminis	yes	yes (deep)	10(12–13)16	no	no	few	SBf	SBf	SBf	O	posterior	1
africana	yes	yes	15	yes	no	few	SBa	SBm	SBa	C	posterior	3
ardenensis	yes	weak	15–19	no	few	few	SBa	SBa	SBa	E	anterior	½
ottersoni	yes	no	10–12	no	no	no	SBa	SBm	SBm	EE	posterior	1
acronea†	yes	no	10(11–13)14	no	few	few	SBm	SBa	SBm	B	posterior	3
spartinae	yes	no	11–17	no	few	few	SBf	SBa	SU	HH	posterior	1½
megriensis	yes	no	13–18	no	few or no	few	SBa	SBa	SBa	BB	posterior	2
decalineata	yes or no	no	12–17	no	few	few	SBa	SBa	SBa	J	posterior	1½
megadora	yes or no	obscure	13–17	no	many	many	SBa	SBa	SBa	AA	anterior	½
lucknowica	yes & no	yes	15–21	no	many	many	SBf	SBf	SBf	Y	posterior	2
hapla	no	no	10(12–14)	no	no	no or few	SU or SBf	SBf	SBf	P	posterior	1½
graminicola	no	no	11	no or few	few	few	SBf	SBf	SBf	N	posterior	2½
exiqua	no	no	(11)14	no	few	few	SBa	WBa	SBf	M	posterior	2
ethiopica‡	no	no	11–15	no	few	few	SBm	SBa	SBf	L	posterior	2
artiellia	no	no	12–16	no	no	no	0	SU	SBa	F	posterior	1¾
oteifae	no	no	13–14	yes	yes	many	SBm	SBa	SBa	DD	posterior	1
incognita	no	no	15–16	no	no	no or few	WBf	WBf	WBf	Q	posterior	1
arenaria	no	no	14–16	no	few or no	few	SBf	SBf	SWBm	D	posterior	2
inornata	no	no	15–17	no	few	few	SBm	SBa	SBm	S	posterior	2½
coffeicola	?	no	15–18	no	SUa	SU	SU	SU	SU	I	posterior	1½
acrita	no	no	16	no	no	few	SBf	SBf	SBf	A	?	?
deconincki	no	no	16–20	no	few	few	SBa	SBm	SBf	K	anterior	½
ovalis	no	no	17–24	no	no	few	SU	SU	SU	FF	posterior	1½
brevicauda	no	no	17(22)25	no	SUm	many	SU	SBm	SBf	H	anterior	½
indica	no	sometimes	12–16	no	many	many	SBm	SBm	SU	R	posterior	1
lordelloi	no	yes	12–15	no	SBa	many	SBm	SBm	SU	X	posterior	4
kirjanovae	no	yes	13–15	no	many	many	SBf	SBf	SU	V	?	?
kikuyensis‖	no	yes	14–16	no	few	many	SBa	SBf	SBf	U	posterior	2
javanica	no	yes	14(16)18	no	1 or 0	few	SWf	SWf	SBf	T	posterior	2½
bauruensis	no	yes	14(15–17)18	no	no	few	SBm	SWf	SWBf	G	anterior	½
litoralis	no	yes	14–18	yes	few	few	SBm	SBf	SBf	W	anterior	½
tadshikistanica	no	yes	15	yes	many	many	WU	SU	SU	II	?	?
thamesi	no	yes	15–18	no	no	few	SBf	SBf	SBf	JJ	?	?

CODE: *S* mooth; *W* avy; *U* nbroken; *B* reaks; *f* ew; *m* oderate; *a* bundant.
* Stylet lengths measured from apex of head.
** Phasmids conspicuous.
† Posterior cuticular pattern very obscure.
‡ Posterior cuticular pattern analysis based on photos in Whitehead (1968).
‖ Lateral vulva cheeks.



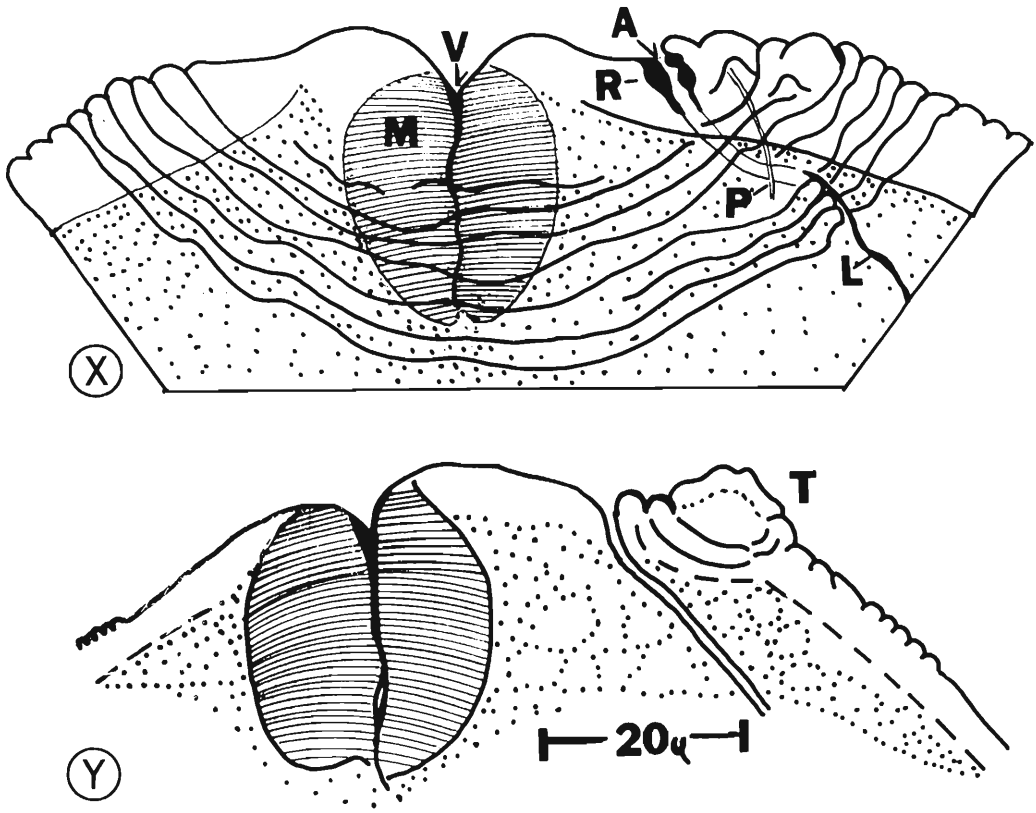


Figure 2. *M. arenaria* (Neal, 1889) (Chitwood, 1949) (X), nonprotuberant posterior region of a mature female: A. Anus; L. Lateral incisure; M. Muscular area of vagina; P. Phasmid lumen; R. Rectum; and *M. spartinae* (Y), protuberant posterior region of a mature female. T. Tail.

tip was seen in a lateral view (Fig. 2Y). In some posterior cuticular patterns of this species the tail appeared as a small round balloon. The set-off tail resembled that of *M. poghossianae* (Fig. 5GG). Other specimens had less defined but definite protuberant tail remnants.

LATERAL INCISURES: The presence or absence of lateral incisures usually is a strong differentiating character. Occasionally some weak incisures will be seen in a posterior cuticular pattern of a species where such lines normally do not occur. One must guard

←

Figure 1. Selected diagnostic morphological characteristics: A. Mature *M. graminis* with vulva on a protuberance. B. *Meloidogyne* sp. without vulva on a protuberance. C. Larval tail with dilated rectum. D. Larval tail with undilated rectum. E. Larvae with hemizonid anterior to excretory pore. F. Larvae with hemizonid posterior to excretory pore. G. Vesicles in metacorpus of *M. naasi* larva. H. *Subterminal spot on tail tip of *M. africana* (Whitehead, 1959). I. Inflated tail tip of *M. spartinae*. J. *M. megadora* Whitehead, 1968, male with indented telorhabdions. K. Lateral fields areolated. L. Lateral fields not areolated. M. Minute telorhabdions of *M. hapla* (Chitwood, 1949) larva. N. Bifid tail tip of *M. thamesi* (Chitwood in Chitwood, Specht and Havis, 1952).

* Thought to be a phasmid in the original description.

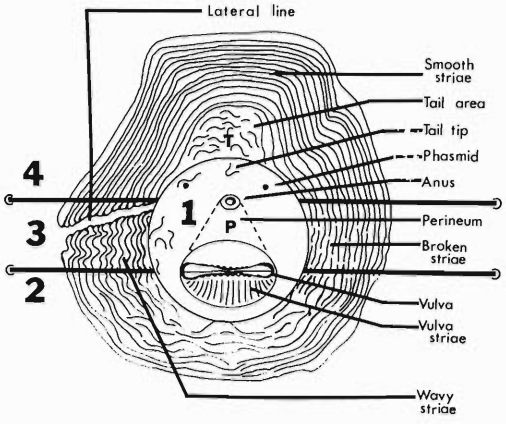


Figure 3. Schematic posterior cuticular pattern, P = perineum, T = tail area. 1. Zone 1: area in pattern center containing perineum and adjacent area usually free of continuous striae; 2. Zone 2: striated area just below (anterior to) vulva; Zone 3: striated area lateral to but bounded by perineum; Zone 4: striated area above (posterior to) anus and tail area.

against the interpretation of folds in the posterior cuticular pattern as lateral incisures.

STYLET LENGTH: Stylet length ranges are rather narrow, and 25 of 36 species fall within a single range. They have, however, some use as a supporting character. In Table 1, stylet lengths are presented in increasing order of magnitude within each equiponderant lateral incisure group.

VULVA LIP STRIAE: Five species have this feature (Table 1). A few lip striae were observed on two specimens of *M. graminicola* (Golden and Birchfield, 1965). Several other specimens of the same species did not have vulva lip striae.

POSTERIOR CUTICULAR PATTERN: Original descriptions contain detailed information concerning striae development and modifications, each peculiar to itself. No attempt is made in this work to include such data due to interpretive diversity. In the final diagnostic step the posterior cuticular pattern under study should be compared with the pattern in the original description. Whenever possible, each species in this work is represented by a posterior cuticular pattern illustration from the original description. A single pattern illustration can rarely represent the variation that occurs in a single population but, it can serve as a guide in the analysis. An unsuccessful attempt was made to utilize morphometric procedures in pattern interpretation. Anus vulva distance, vulva width, and anus vulva width times distance from the anus to the apogee of the pattern were considered. Intraspecific variability in these measurements and anal obscurity in some species negated the attempt. Using any pattern perimeter side as a point of reference is questionable since such boundaries under oil immersion are not clearly defined.

To utilize the features of the posterior cuticular pattern more effectively it was divided into 4 definitive zones (Fig. 3). The first area is the perineum within Zone 1 which is defined in this work as the triangle formed by the anus and the vulva slit (Fig. 3P). Zone 1 (Fig. 3) is a roughly circular area in the center of the pattern usually free of continuous striae. Striae of Zone 1 are usually few, broken, and scattered. Zone 2 (Fig. 3) is the area under (anterior to) the vulva, and specifically refers to the mass or band of striae directly below (anterior to) the perineum. Zone 3 encompasses the group of striae lateral to the perineum (Fig. 3). Zone 4 is that group of striae above (posterior to) the anus. The tail area (Fig. 3T) is a roughly circular area just above the anus characterized by the tail and short broken striae. Tail area is not considered in pattern striae analysis. When lateral incisures are present they should be considered as discrete structures and not broken striae in the analysis.

Analysis of patterns revealed that certain characteristics of each zone could be of value in differentiating patterns. Absence of striae in the perineum (Fig. 5C, O, Z); presence of a single striae (Fig. 5CC); presence of many Zone 1 striae (Fig. 5H, R, AA, DD); unbroken Zone 2 striae (Fig. 5H, I, FF); wavy Zone 3 striae (Fig. 5G), and abundant breaks in Zone 4 striae (Fig. 5AA, CC) are all useful in the analysis of patterns. Some patterns have individual characteristics, such as the pronounced phasmids in *M. naasi* (Franklin, 1965) (Fig. 5CC), obscurity of the *M. acronea* Coetzee, 1956, pattern, sparseness of inner striae in *M. artiellia* Franklin, 1961 (Fig. 4), and the pronounced lateral cheeks in *M. kikuyensis* (DeGrisse, 1960) (Fig. 5U).

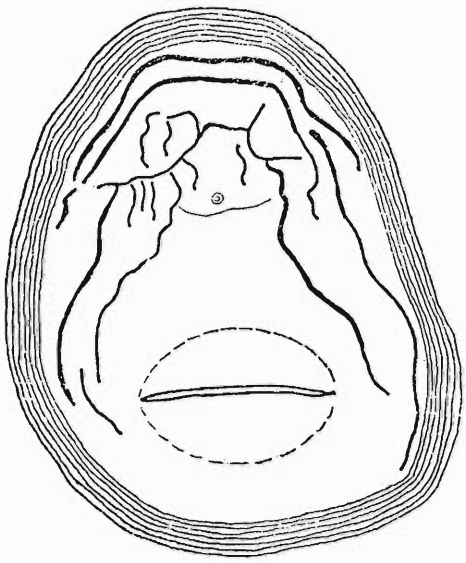


Figure 4. Posterior cuticular pattern of *M. artiellia* showing coarse inner striae, fine outer striae, and an absence of inner striae in Zone 2.

An analysis of pattern zones is presented in Table 1, which complements the basic female morphometric data. Two types of striae are considered. First is the prominent, usually coarse striae that comprises nearly all of the posterior cuticular pattern (inner striae) (Fig. 4). Second is the perimeter of fine, usually unbroken striae (outer striae) (Fig. 4) that surrounds most inner striae. In *M. artiellia* (Fig. 4) outer striae predominate. Inner striae in this species are represented by a few coarse lines in the anterior two-thirds of the pattern; Zone 3 area is almost all outer striae. The circular sclerotized pre-anal part of the rectum (Fig. 2R, 3P, 4A) is the best point of reference for the perineum, since the actual anus appears as a thin ill-defined slit often not apparent in a posterior cuticular pattern.

EXCRETORY PORE:[1] The excretory pore of the female lies anterior to the telorhabdions in seven species (Table 1). Position of the excretory pore should serve as a good corroborating character in the analysis. It is best seen in freshly prepared specimens. In Table 1 an excretory pore value of ½ means the pore is at the level of ½ the stylet length from the head apex. A value of 1 indicates the pore opens just behind the telorhabdions and a value of 3 means the pore lies 3 stylet lengths from the head apex.

REJECTED CRITERIA: Body length and alpha measurements were omitted due to excessive variation. Beta was excluded from all tables due to the difficulty and unreliability of esophageal gland measurements. Dorsal gland orifice (DGO) distance from the telorhabdion base was rejected due to overlap in a restricted range. Twenty-nine species had a DGO of 4 μ within their range.

Male (Table 2)

Stylets are presented in increasing magnitude of lower range length.

Dorsal gland orifice, spicule length, and head annules are useful to corroborate the analysis. The head annule number is subject to morphological clarification, intraspecific variation (Whitehead, 1968) and differences in observer interpretation so its utilization as a differentiating character is rejected. Lateral lines and areolation (Fig. 1K, L) are definitive and easily seen and can therefore be used with some degree of reliability. The hemizonid of *M. spartinae* only was located posterior to the excretory pore.

REJECTED CRITERIA: Length, alpha, and gamma of males were omitted due to the extreme range length. Males of 33 species had an average variation between minimum and maximum length of 662 μ. The maximum variation between minimum and maximum length was 1,947 mm in *M. kirjanovae* (Terenteva, 1965). Such extremes expand alpha and gamma ranges to impractical limits.

Larvae (Table 3)

BODY LENGTH: Larval length was selected as the starting point in the table and larvae were placed in increasing magnitude of lower range length.

RECTUM DILATION: This character (Fig. 1C) is readily seen with an oil immersion objective in live specimens, and is considered a strong point in the diagnosis. Fixation procedures tend to obscure this character.

[1] Suggested usage as a differentiating character by A. M. Golden.

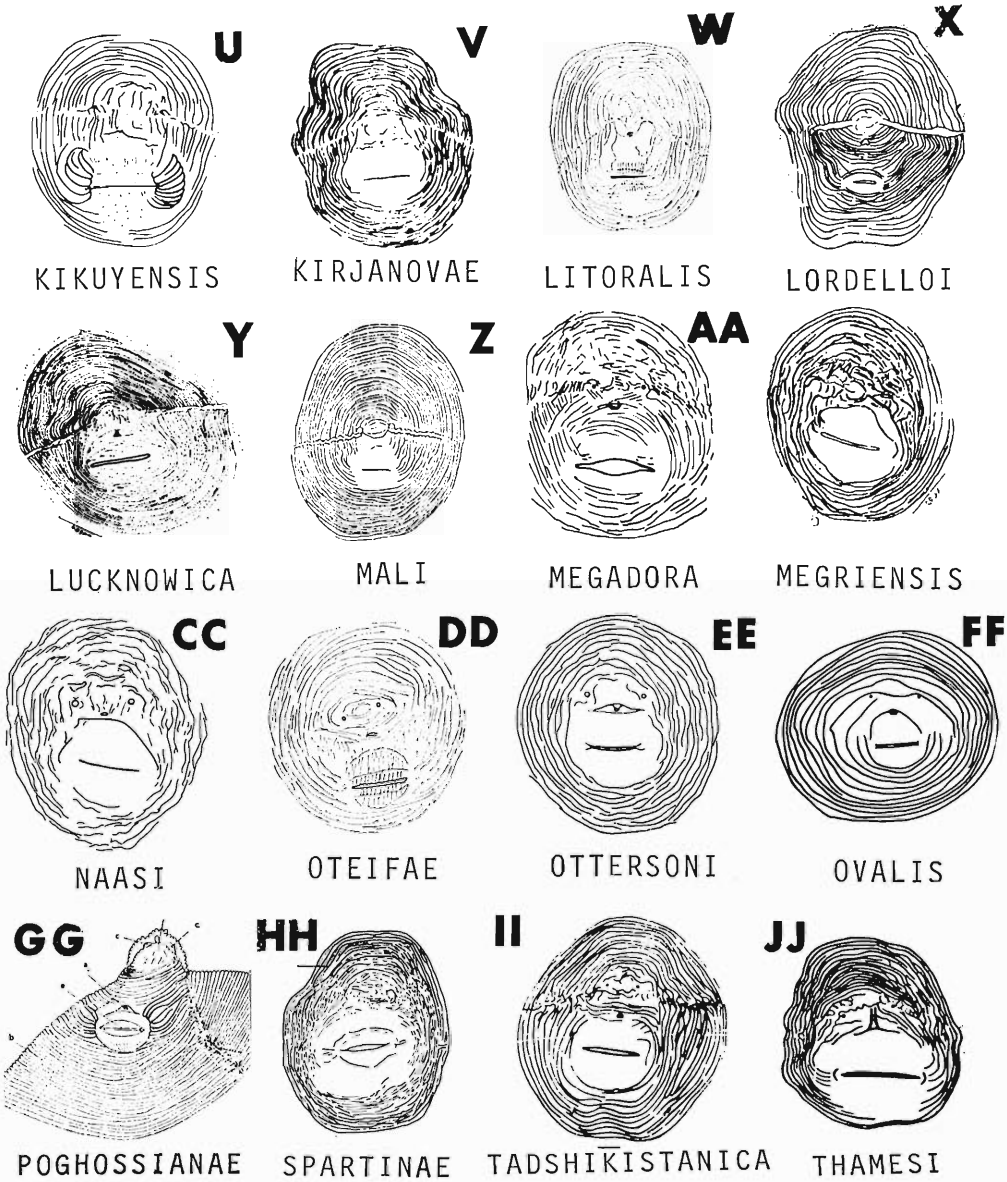


Figure 5. A–JJ Posterior cuticular patterns of the genus *Meloidogyne*. Illustration credit B,J,L,N,P,R, and AA from Whitehead, 1968; C, Whitehead, 1960; D, Santos, 1967; E,Q,T, and JJ, Chitwood, 1949; G, Lordello, 1956 (redrawn); H, Loos, 1953; I, Lordello and Zamith, 1961; K,W,DD, Elmilgy, 1968; M, Lordello and Zamith, 1958; S, Lordello, 1956; U, Grisse, De, 1960; V, Terenteva, 1965; X, Ponte, 1969; Y, Singh, 1969; Z, Itoh, Ohshima, and Ichinohe, 1969; DD, Pogosyan, 1961; CC, Franklin, 1965; EE, Thorne, 1969; FF, Riffle, 1963; GG, Kirjanova, 1963; HH, Rau and Fassuliotis, 1965; II, Kirjanova, and Ivanova, 1965.

Table 2. Diagnostic characteristics of *Meloidogyne* males.

Species	Stylet	Dorsal gland orifice	Spicule length	Lateral Incisures		Head annules
				no.	areolated	
megriensis	13–18	3–4	23–30	4–6	no	2
ottersoni	14–16	?	19–23	4–5	yes	2
ethiopica	14–24	2	29–36	4–5	yes	2
lucknowica	15–24	?	?	6	no	1
graminicola	16–17	3–4	27–29	4–8	yes	2
naasi	16–19	2–4	25–30	4	yes	3
acronea	(16–18)20	2–7	24(32–34)36	4	yes & no	1
kikuyensis	17–20	5–6	31–35	4	yes	1
spartinae	17–21	4–7	25–40	4	no	?
hapla	(17–18)23	3(4–6)	22(29–31)	4	no	2
ardensis	17–24	3–4	28–38	4–5	yes	4
artiellia	17–27	5–7	25–30	4–5	no	1
indica	18	?	?	4	yes	2
*exigua**	18–20	3	(20–26)27	4	yes	1
graminis	(18–19)21	(2–3)5	21(28–29)30	4	no	1
mali	18–22	6–13	28–35	4	(tail only)	1
*megadora***	18–22	4–8	25–36	4–6	yes	1
ovalis†	(18–23)25	3–5	31–38	4	no	2
decalineata	19–20	4	33–37	10	no	2
africana	19–22	4–6	26–35	5	yes	?
oteifa	19–23	3–4	29–37	4–5	yes	2
litoralis	19–24	4–5	29–33	5	yes	3–4
javanica	20–21	3	30–31	4	no	3
bauruensis	20–23	3–4	28–33	4	yes	2
arenaria	20–24	4–7	31–24	4	yes	2
brevicauda	(20–21)24	5–8	30(34–43)	4	yes	1
acrita	20–24	2–4	29–34	4	?	3
kirjanovae	20–24	2–3	28–36	4	no	1
inornata	20–25	4–5	27–33	4–5	yes	2
thamesi†	21–28	3	22–28	4–6	yes	2
tadshikistanica	22–25	5	27–37	4	no	4
deconincki	22–28	5–7	29–37	5	yes	1–2
coffeicola	23–26	4–5	20–29	4–5	yes	1
incognita	(23–26)33	1(2–4)	29(34–36)40	4	yes & no	3
lordelloi	Males unknown					

* Body untwisted.
** Telorhabdions indented (Fig. 1J).
† Telorhabdions asymmetric.

HEMIZONID: Hemizonid position (anterior or posterior) to the excretory pore is considered a strong larval characteristic.

Alpha, gamma, and stylet length are considered corroborating characters, but stylet length might be untrustworthy since one rarely knows at what point anterior to the telorhabdion base the original measurements were made. A suggestion is made that future describers of species in this genus measure from the telorhabdion base to the top of the head as a stylet measurement and state the procedure in their methodology.

Lateral line number and areolation were omitted due to the difficulty in seeing these structures in larvae. Measurements of the dorsal gland orifice distance posterior to the stylet base were omitted since little change occurred in this measurement among 35 species.

Diagnosis

SPECIMEN REPRESENTATION: Prior to analysis it should be ascertained that sufficient larvae and females are available to make an adequate diagnosis (at least 10 each). Males confirm the diagnosis but are not essential to all identifications.

Table 3. Diagnostic characteristics of *Meloidogyne* larvae.

Species	Length	Rectum	Hemizonid to excretory pore	Alpha	Gamma	Spear
exigua	289(334–359)370	undilated	?	(22–26)29	7–8	9(11)
kikuyensis	290–360	undilated	anterior	17–23	10–12	12–15
artiellia	301(334–370)	undilated	anterior	20(22–26)	(13–16)21	10(14–16)
ovalis	302(350–430)	undilated	anterior	19(21–24)	8–9	9–12
hapla	312(395–466)*	undilated	anterior	20(28–35)	7(8)10	8(10)11
oteifa	320–400	undilated	anterior	22–29	8–9	11–13
litoralis	330–450	undilated	?	19–30	10–12	11–15
coffeicola	337–424	undilated	?	22–25	10–14	9–11
lordelloi	340–380	?	?	25–28	?(Anus obscure)	10–11
deconincki	340–400	undilated	anterior	27–33	7–10	10–11
javanica	340–400	d or u	anterior	24–26	6–7	10
bauruensis	345–352	undilated (obscure)	?	23–29	7–11	11–12
acrita	345–396	undilated	anterior	22–28	7–8	10–11
tadshikistanica	350–435	undilated	?	32	9	12–15
acronea	354(440–460)	undilated	anterior	22(32)35	8(9)11	10(12)
megriensis	358–467	?	?	19–26	6–8	13–15
kirjanovae	359–433	?	?	20–30	6–8	11
incognita	360–393	d	anterior	29–33	8–9	10
ardenensis	372–453	undilated	posterior	22–32	9–12	9–14
*inornata**	375–420	obscure	?	28–36	12–13	10–13
africana	380–470	undilated	?	22–28	7–14	12–18
indica	381–448	undilated	?	?	21–31	10–14
ethiopica	383–432	dilated	anterior	29–35	8–10	9–11
mali	390–450	undilated	?	27–31	12–15	12–15
graminis	409(420–510)	d or u	posterior	24(29–34)	(6–7)8	10(12–13)
*thamesi***	410–476	dilated	posterior	30–38	8–9	10–13
lucknowica	410–575	dilated?	?	21–37	9–14	11–18
megadora	413–548	undilated	anterior	23–33	8–11	11–13
graminicola	415–484	undilated	anterior	22–27	6–7	11–12
naasi†	418–465	undilated	anterior	25–32	6	13–15
ottersoni	430–500	undilated	anterior	23–30	?	13–15
arenaria	450–490	d or u	?	26–32	6–8	10
brevicauda	460–590	undilated	anterior	23–33	21–29	(14)16
decalineata	471–573	undilated	anterior	33–40	10–12	11–14
spartinae‡	612–912	u(obscure)	posterior	43–65	7–9	14–17

* Telorhabdions minute (Fig. 1M).
** Bifid or trifid tail (Fig. 1N).
† Vesicles in metacorpus (Fig. 1G).
‡ Swollen spiked tail tip (Fig. 1I).

SPECIMEN CONDITION: Diagnostic characters and measurements of males and larvae should be taken from living nematodes or specimens shortly after a gentle death. Immobility in a natural state can be achieved in two ways. Live specimens in water under a coverslip sealed with Zut attain quiescence in about 10 min (Esser, 1973). Specimens mounted in 2% formaldehyde cease movement in a few minutes. In either case all data should be taken within an hour. Within a few hours deterioration characters such as the inflated rectum, hemizonid position, and dorsal gland orifice become obscure. Twenty-four hours after either treatment, striae will be more definitive, and lateral lines and areolation interpretation will be facilitated.

Female criteria can be taken from live or fixed specimens. Galled roots cut up and blended in 100 cc of water for 10–20 sec (succulent tissue), or 30 sec to 1 min (woody tissue) yield cleaned female cuticles with excellent posterior cuticular patterns. When females are abundant 10 to 20 females, either entire specimens or cuticles, may be placed in a small drop of water and an 18 mm coverslip dropped thereon. Three to six excellent fresh posterior cuticular patterns are often obtained in this manner, and female stylet and excretory pore measurements also can be made. When feasible, it is advisable to take posterior cuticular pattern, spear, and excretory pore data from the same specimen. Two or more species of root-knot nematodes may appear on a single root. *M. graminis* (Sledge and Golden, 1964; Whitehead, 1968) and a different species of root-knot have been found side-by-side on roots of St. Augustine grass. *M. graminis* egg masses were yellow and located inside the junction of a branch root. The other species produced a white egg mass at an unbranched root site. In cases where posterior cuticular patterns or larval characteristics show wide variation, mixed populations should be considered.

IDENTIFICATION PROCEDURE: It is suggested that data be taken in the sequence presented in Tables 1, 2, and 3 for each specific stage. For example in Table 3 (larva) length, rectum dilation, hemizonid, alpha, gamma, and stylet length should be recorded in that order. Larva characteristics are the starting point for identification. The diagnostic data of the larval species under analysis is checked with Table 3. This comparison should eliminate all but a few species for the final specific diagnosis. Original descriptions of the species delineated by analysis with Table 3 should be consulted in conjunction with mounted posterior cuticular patterns of the species under diagnosis. Final analysis will be facilitated by utilizing the data in Table 1. If males are present, characteristics of this stage should be used in confirming the diagnosis.

Writing the proper measurements and data horizontally on a paper slip to match the tabular presentation is the easiest method to utilize the compendium tables.

TAXONOMIC CONSIDERATIONS: Whitehead (1968) considered *M. incognita acrita* Chitwood, 1949, a synonym of *M. incognita incognita* Chitwood, 1949. The present authors believe Chitwood erred when he established *M. incognita* var. *acrita* as a variety rather than a species, thereby engendering research and conjecture regarding its validity as a discrete taxon. Terenteva (1967) utilizing variational statistics showed that the two subspecies were discrete. Separation was based on anal vulva plate, stylet head shape, and head height in males; 5.75–8 μ in *M. incognita incognita* and 5.2–6 μ in *M. incognita acrita*. Dr. Chitwood (pers. comm.) considered the undilated rectum of *M. incognita acrita* larva the principal diagnostic character that distinguished it from *M. incognita incognita*. He also separated posterior cuticular patterns of the two species on the basis of striae coarseness. *M. incognita acrita* usually has relatively coarse striae (Fig. 5) and *M. incognita incognita* has fine (close together) usually wavy striae. The difference is well illustrated by Sasser (1954). *M. incognita acrita* also has a smaller alpha and gamma (Table 1) and the male has a smaller spicule (Table 3). Based on these criteria all of which are contained in the original description, *M. incognita* is considered a discrete species (Article 50b, c of the International Code (31)). Chitwood's *M. incognita acrita* is herein recognized and elevated to full specific rank as *M. acrita*. According to Dr. A. M. Golden (pers. comm.) the hololectotype female of *M. acrita* is contained in the USDA nematode collection at Beltsville, Md. (Slide T–268t) in addition to 12 paralectotype slides.

Whitehead (1968) also synonymized *M. javanica bauruensis* (Lordello, 1956) with *M. javanica* (Treub, 1885; Chitwood, 1949). In this work *M. bauruensis* is considered a discrete species based on areolation in the lateral fields of the male, two male head annules, a larger larval gamma, and a difference in appearance of the gross posterior cuticular pattern (Fig. 5G, T).

Following Whitehead's (1968) synonymy of *Hypsoperine* to *Meloidogyne*, *Hypsoperine megriensis* (Pogosyan, 1971) is hereby placed in the genus Meloidogyne as follows: *M. megriensis* (Pogosyan, 1971) n. comb. Syn. *H. megriensis* (Pogosyan, 1971).

M. carolinensis (Fox, 1967) has not yet been properly published according to articles 7, 8, and 9 of the International Code (Stoll et al., 1964).

Acknowledgment

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In Memoriam

Gerard Dikmans

December 8, 1885–December 7, 1975
Member since 1927
President 1937–1938
Elected to Life Membership 1953

Satyu Yamaguti

March 11, 1976
Member since 1954

A Revision of the Genus *Cephaluris* Akhtar, 1947 (Nematoda, Oxyuridae) with Redescriptions of the North American Species

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ABSTRACT: The genus *Cephaluris* Akhtar, 1947 is reorganized, based on specimens collected in Canada (Alberta and Yukon Territory), USA (Colorado), USSR (Kazakhstan), and Japan. Both North American pikas, *Ochotona princeps* and *O. collaris*, are hosts to *C. coloradensis* Olsen, 1949, and *C. alaskensis* Akhtar, 1958. The latter species is a new host record for *O. princeps*. The North American species are redescribed, and differentiated on the basis of male morphology, the females being indistinguishable to the author. The genus is reduced from 8 to 6 species. *Cephalurus collaris* Akhtar, 1958 and *C. vakhanica* Erhardova-Kotrla and Daniel, 1970, are considered synonyms of *C. coloradensis* and *C. ochotonae* Akhtar, 1947, respectively. *Cephaluris andrejevi* Shul'ts, 1948 from *O. rutila* of Kazakhstan and *O. hyperborea yesoensis* of Japan (a new host record), is morphologically close to *C. alaskensis*, as is *C. ochotonae* from *O. rutila* (a new host record) to *C. coloradensis*. Evidence is presented to suggest that species of *Ochotona* are each infected with two species of *Cephaluris*, one from each of two species-groups.

The pinworm genus *Cephaluris* Akhtar, 1947, of pikas (*Ochotona* spp.), is in a state of confusion. Much of the confusion has arisen due to the literature lag, as seven of the eight species were described within a 12 year period. Three species have been found previously in the two species of North American pikas: *C. coloradensis* Olsen, 1949 in *O. princeps*, and *C. alaskensis* Akhtar, 1958, and *C. collaris* Akhtar, 1958, in *O. collaris*. This paper revises host listings and redescribes the North American species, compares these with species from Asian hosts, and reduces the genus to six species.

Materials and Methods

Pikas were collected by shooting: *O. princeps* from the Rocky Mountains, east of the Kananaskis River in Alberta (51°N 115°W), and *O. collaris* from the Ogilvie Mountains of the Yukon Territory (65°N 138°W). They were necropsied within 6 hr of death. Nematodes were killed in dilute saline, fixed in glycerine-ethanol, and cleared in lactophenol-creosote. Measurements were made with the aid of an ocular micrometer, or camera lucida and measuring wheel. *En face* mounts in glycerine jelly were made of several other specimens from these two collections, after the method of Anderson (1958). Descriptions were based on these collections.

Other specimens of *O. princeps* were collected by shooting, in Colorado (39°N 107°W), and Sheep River Alberta within 15 km of the Kananaskis River collection. Specimens from these two collections were made available through the courtesy of Drs. D. G. Cameron and J. S. Millar, respectively. An incision was made in the abdomen and carcasses were placed in AFA. Nematodes from these pikas were later transferred to glycerine-ethanol, and cleared in lactophenol-creosote for measurement.

Specimens of *Cephaluris* from freshly killed *Ochotona hyperborea yesoensis* collected in the Daisetzusan National Park Japan (43°N 142°E) by Dr. F. C. Zwickel, were treated as described above.

Specimens from one *O. rutila* collected in the Altai Mountains of Kazakhstan were sent by Dr. E. V. Gvozdev to Dr. R. L. Rausch, who loaned them to the author for examination. The collecting procedures used for these worms were not known to the author, and they had been stored in an unknown preservative.

Analyses of covariance (Steel and Torrie, 1960) were used to test differences between collection groups, using the body length of the worm as the independent variable. For the significance of regression, the 95% level was used, and for comparisons of means, the 99% level. In cases where there was not a significant regression, analyses of variance were applied using the 99% level of significance.

Table 1. Diagnosis of the two North American species of *Cephaluris* by male characteristics. Measurements are based on specimens from Kananaskis Alberta, and Ogilvie Mountains Yukon, for both species, and are given in micrometers.

	Cephaluris alaskensis	*Cephaluris coloradensis*
Buccal cavity	Cuticular ridges reach to about 5 behind anterior end of tooth (Fig. 5)	Cuticular ridges reach anterior end of tooth (Fig. 10)
Precloacal ventral ridge	Blunt rugged teeth often bunching into groups (Fig. 2)	Fine thin teeth, never bunching into groups (Fig. 7)
Distance of anterior end of ventral ridge from cloaca	942 (720–1310)*	1,372 (960–1660)
Tail tip (from caudal alae)	200 (155–244)	293 (224–348)
Caudal alae	5 to 8 subcuticular longitudinal bands present (Fig. 3)	Longitudinal bands absent (Fig. 8)
Width of transverse striations	10 (7–14)	11.5 (9–16)
Cervical alae	Continuous with, but clearly delimited from lateral alae (Fig. 1)	Posterior end not clearly delimited from lateral alae (Fig. 6)
Cloacal papillae	12 paired and 2 single (Fig. 3) One pair on anterior lips Bilobed papilla immediately posterior to cloaca, with a tiny pair on posterior lobe Second lateral papilla simple Cushion-like papilla greatly expanded	10 paired and 2 single (Fig. 8) None on anterior lips Papilla immediately posterior to cloaca not bilobed, with narrow pedunculate laterals Second lateral papilla with large medially projecting lobe Cushion-like papilla not greatly expanded

* Mean (range).

Logarithmic transforms did not increase the significance of regression so were not used.

Measurements are in micrometers unless otherwise indicated.

Results

Two species, *C. alaskensis* and *C. coloradensis*, were present in all North American collections studied. Although the males of these species were easily distinguishable (Table 1), the author was unable to differentiate two kinds of females.

Cephaluris alaskensis Akhtar, 1958

Redescription

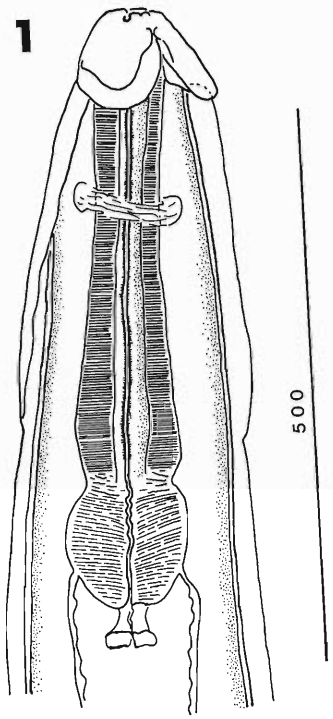
Medium-sized worms, with the cuticle striated transversely. Anterior end with a pair of posteriorly directed cuticular shields (Figs. 1, 12). Mouth surrounded by 3 bilobed lips, one dorsal, and 2 latero-ventral, 2 pairs of papillae, and a pair of lateral amphids (Figs. 5, 12). A pair of cervical alae begins just posterior to the shields, and extends posteriad, becoming continuous with the lateral alae, which extend almost the length of the body. The buccal cavity is 2-storied, and heavily lined with cuticular structures (Fig. 4). Esophagus club-shaped, with a muscular bulb at the posterior end, and an esophago-intestinal valvular apparatus, which extends from the bulb into the lumen of the intestine (Fig. 1). The triradiate lumen of the esophagus anterior to the bulb is lined with fine transverse striations, approximately 2 apart (Fig. 4).

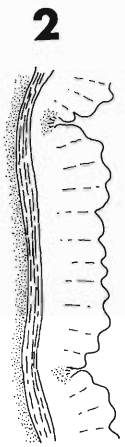
Females

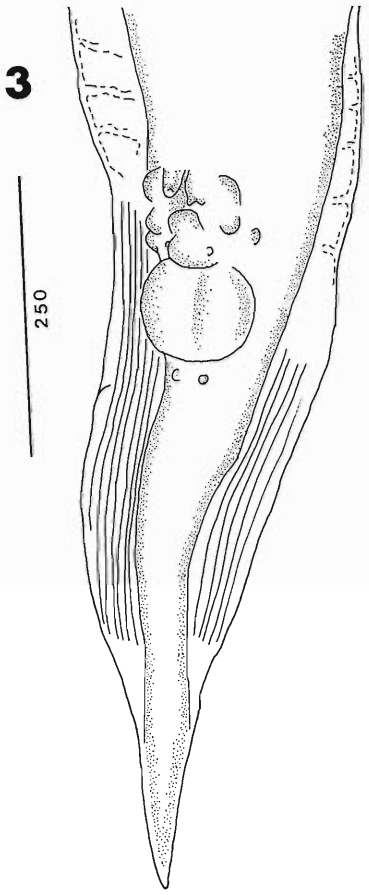
Measurements are shown in Table 2. Vulva anterior to mid-body, without flap (Fig. 11). A muscular ovejector runs anteriad from the vulva, then curves posteriad. At the curve is a ring-shaped glandular structure surrounding the ovejector. Posterior to the level of the vulva, the ovejector widens into a thin-walled

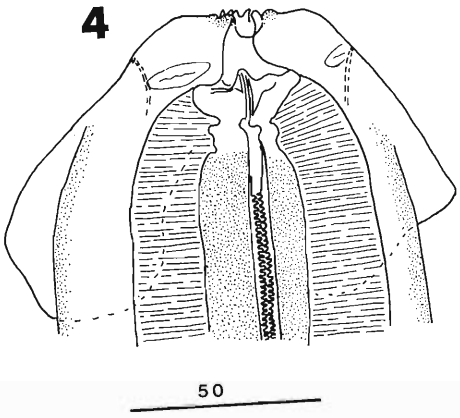
→

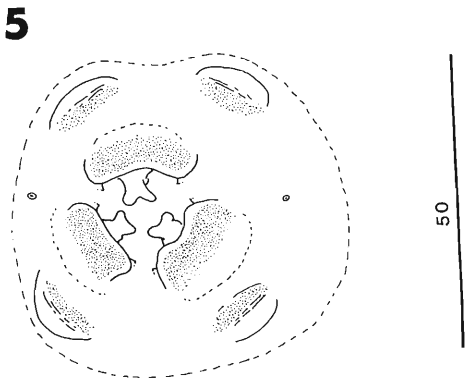
Figures 1–5. Males of *Cephaluris alaskensis* Akhtar, 1958. 1. Dorsal view of anterior end. 2. Detail of precloacal ventral ridge. 3. Ventral view of posterior end. 4. Ventral view of anterior end. 5. *En face* aspect.

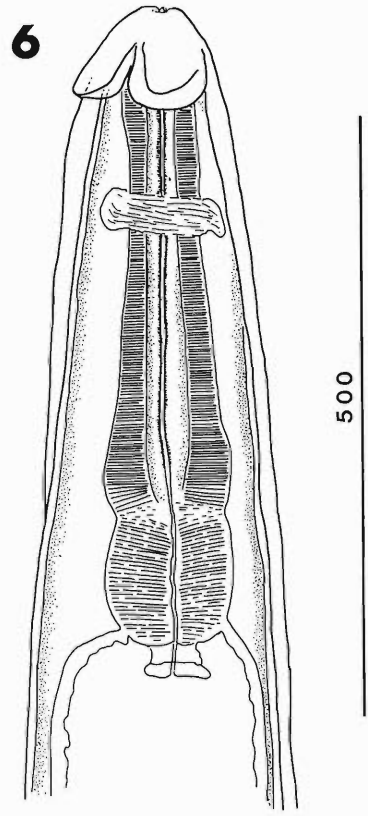


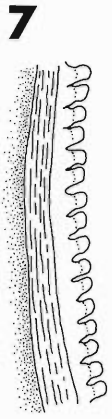


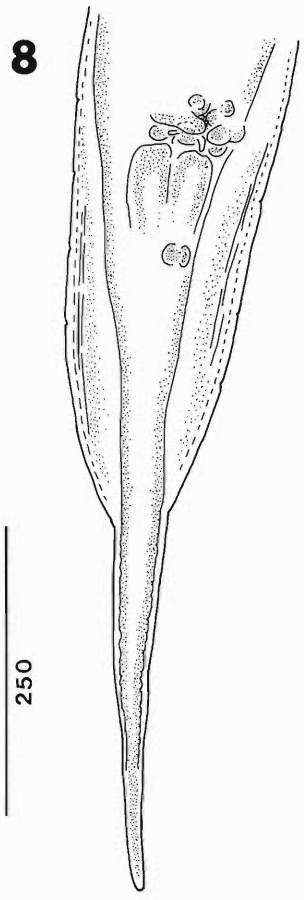


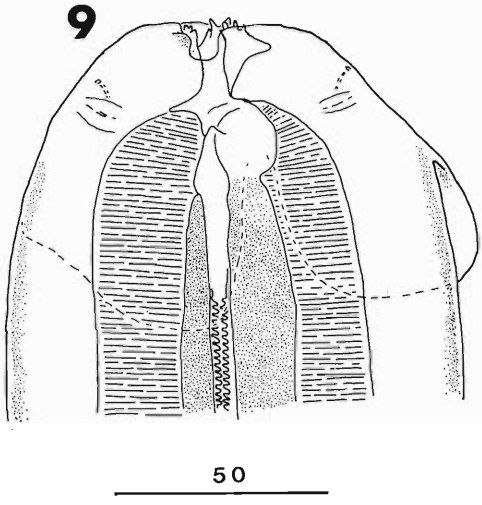












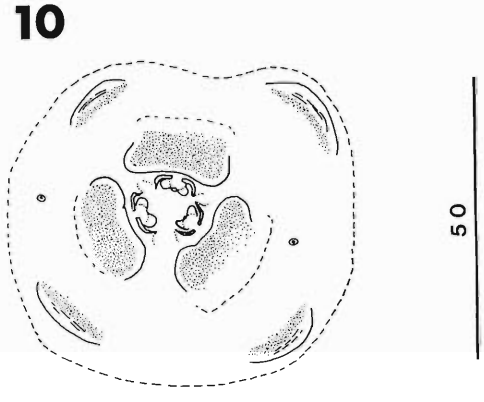


Table 2. Means (and ranges) of measurements of female *Cephaluris* spp. Measurements are in micrometers unless otherwise stated.

Worm species*	*Cephaluris andrejevi*				*C. ochotonae*		*C. alaskensis* and *C. coloradensis*	
Host species	*Ochotona rutila*		*O. hyperborea yesoensis*		*O. rutila*		*O. princeps* and *O. collaris*	
Number of worms	7		6		8		43	
Length (mm)	14.1	(11.5–14.9)	12.6	(11.3–13.1)	14.9	(14.3–15.4)	11.3	(9.1–13.7)
Width	767	(640–820)	663	(550–790)	785	(740–840)	629	(540–760)
Striations	18.5	(16.8–20.9)	13	(10.4–17.4)	15.5	(13.3–17.4)	13.3	(10–19)
Lateral alae width	111	(93–123)	110	(93–123)	111	(102–123)	86	(58–137)
Esophagus length	951	(770–1020)	822	(790–840)	979	(950–1040)	774	(640–960)
Esophagus bulb width	196	(155–216)	180	(172–184)	199	(184–216)	159	(134–204)
Buccal cavity depth	58	(53–61)	51	(44–58)	58	(53–61)	45	(41–53)
Shield length	116	(96–137)	108	(99–117)	123	(102–134)	105	(88–128)
Nerve ring	256	(230–290)	236	(219–260)	275	(250–290)	217	(180–250)
Tail length (mm)	2.28	(1.97–2.53)	2.07	(1.89–2.21)	2.36	(2.15–2.50)	2.02	(1.65–2.42)
Distance of vulva from anterior end (mm)	5.83	(4.7–6.5)	4.93	(4.5–5.5)	6.05	(5.5–6.6)	4.63	(3.7–5.6)
Ring gland to vulva (along ovejector)	983	(910–1130)	1070	(940–1190)	1070	(910–1170)	931	(730–1270)
Egg length	107	(99–104)	105	(93–111)	107	(99–104)	113	(96–123)
Egg width	51	(50–53)	55	(53–58)	53	(50–55)	55	(47–64)

* Determined only on the basis of presence (*C. ochotonae*) or absence of vulval flap for Asian worms.

uterus which extends to at least mid-way between the anus and the tail tip (Fig. 13). In this region, the uterus divides; both arms run anteriad to about the level of the ovejector. A narrow oviduct runs back along each uterus a short distance, and then recurves and runs anteriad to the ovary (Fig. 11). The ovary continues anteriad, gradually tapering, and recurves near its termination usually posterior to the esophagus. Each lip of the mouth bears on its medial surface, a blunt double-ended tooth, which has a sharp cuticular ridge, approximately 2 wide on each side (Fig. 12). The ridges extend to a depth of about 20 into the buccal cavity. Rectum with a cuticular lining (Fig. 13). Eggs asymmetrical, with a rugose shell, and a plug near one end (Fig. 14).

Males

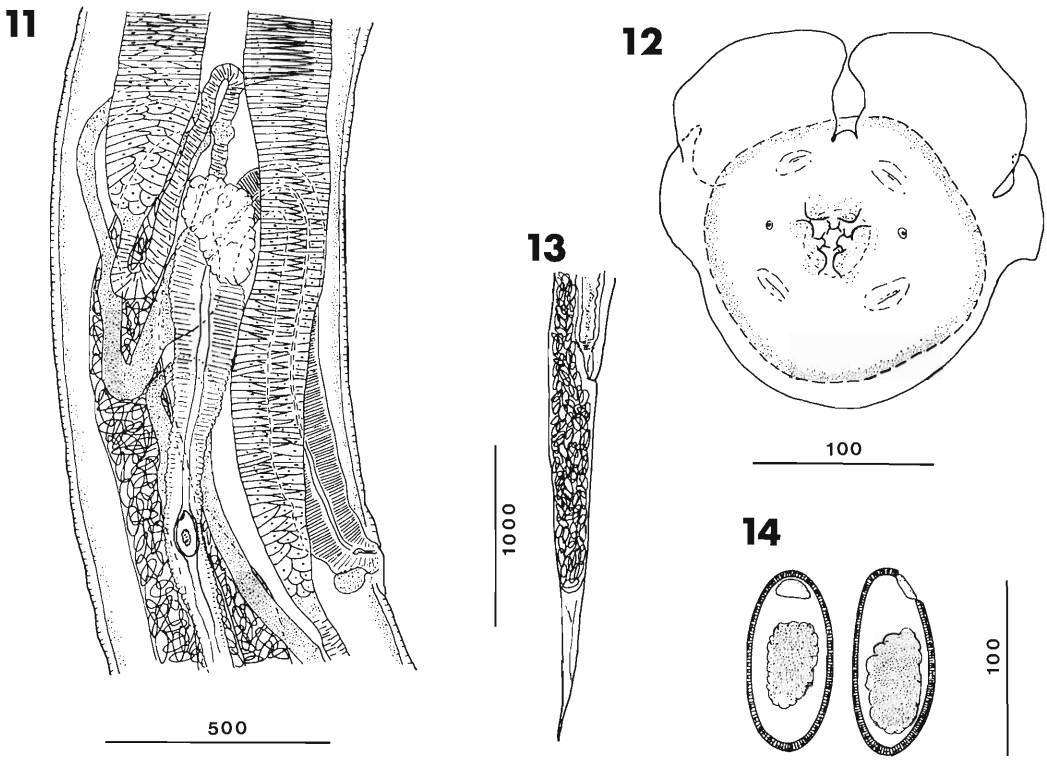
Measurements are shown in Table 3. Cervical alae continuous posteriorly with lateral alae, but clearly delimited from them (Fig. 1). An antero-medially directed tooth on the medial surface of each of the 3 lips (Figs. 4, 5). Teeth are double-ended and blunt, and not ornamented at their anterior margin. A pair of sharp cuticular ridges approximately 1 wide, on each side of the tooth, begins about 5 behind the anterior extremity of the tooth, and extends about 5 deeper (Fig. 5). Caudal alae continuous anteriorly with lateral alae. Five to 8 subcuticular longitudinal bands inside each caudal alae (Fig. 3). Twelve paired and 2 unpaired cloacal papillae; 3 pairs lateral to cloaca; 1 pair on anterior lips of cloaca; an unpaired posterior papilla, divided into anterior and posterior lobes, the posterior one with a pair of tiny lateral papillae; a large cushion-like papilla posterior to those; and a small pair posterior to it (Fig. 3). Spicules and gubernaculum absent. Rectum with cuticular lining. A long ventral precloacal cuticular ridge with rounded serrations, often bunching into groups, present (Fig. 2).

HOSTS: *Ochotona collaris, O. princeps.*

DISTRIBUTION: Ogilvie Mountains, Yukon; Talkeetna Mountains, Alaska; Rocky Mountains, southwest Alberta; Gunnison County, Colorado.

←

Figures 6–10. Males of *Cephaluris coloradensis* Olsen, 1949. 6. Dorsal view of anterior end. 7. Detail of precloacal ventral ridge. 8. Ventral view of posterior end. 9. Ventral view of anterior end. 10. *En face* aspect.



Figures 11–14. Females of *Cephaluris alaskensis* or *C. coloradensis*. Since these species are distinguishable on the basis of male characteristics only, separate figures for females were not possible. 11. Details of the reproductive system. 12. *En face* aspect. 13. Lateral view of posterior end. 14. Eggs. Scales in all figures are in micrometers.

Table 3. Means (and ranges) of measurements of male *Cephaluris* spp. For the North American species, measurements are given only for worms from the type host species. Measurements are in micrometers unless otherwise stated.

Worm species	*C. alaskensis*	*C. andrejevi*		*C. coloradensis*	*C. ochotonae*
Host species	*Ochotona collaris*	*O. rutila*	*O. hyperborea yesoensis*	*O. princeps*	*O. rutila*
Number of worms	22	10	4	20	1
Length (mm)	7.5 (6.0–9.1)	8.8 (7.6–9.6)	7.1 (6.1–8.5)	6.8 (5.1–7.5)	8.7
Width	346 (270–440)	363 (300–400)	338 (250–430)	319 (260–350)	440
Striations	10 (7–13)	12 (8–14)	8 (7.5–8.7)	12 (10–14)	14.5
Lateral alae width	57 (41–76)	72 (67–76)	74 (61–88)	50 (38–70)	96
Esophagus length	554 (450–700)	673 (550–730)	580 (520–660)	508 (410–550)	730
Esophagus bulb width	116 (96–157)	140 (111–152)	127 (108–163)	110 (93–131)	169
Buccal cavity depth	33 (29–35)	43 (38–47)	38 (35–41)	34 (29–40)	47
Shield length	755 (640–880)	82 (70–96)	75 (67–82)	72 (58–88)	85
Nerve ring	168 (140–200)	212 (190–230)	197 (175–219)	171 (150–180)	210
Distance from cloaca to tip of tail	544 (410–760)	816 (540–930)	643 (520–790)	595 (530–670)	680
Tail tip (from caudal alae)	186 (155–235)	296 (204–360)	170 (151–209)	285 (250–348)	250
Distance from anterior of ventral ridge to cloaca	940 (750–1310)	924 (670–1200)	1040 (820–1410)	1364 (990–1620)	1950

LOCATION IN HOST: Caecum and colon.
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 73536.

Cephaluris coloradensis Olsen, 1949

Since only males of this species were found to differ from *C. alaskensis*, the general and female descriptions will not be repeated.

Males

Measurements are shown in Table 3. Cervical alae continuous posteriorly with lateral alae; the posterior delineation of the cervical alae inconspicuous (Fig. 6). An anteromedially directed double-ended tooth present on the medial surface of each of the 3 lips (Figs. 9, 10). Two sharp cuticular ridges about 2 wide, begin at the anterior extremity between the tips of each tooth and the lip, and extend about 10 into the buccal cavity, curving to the lateral sides of the tooth (Fig. 10). Caudal alae are continuous anteriorly with lateral alae. Longitudinal bands in caudal alae absent. Spicule and gubernaculum absent. Ten paired and 2 unpaired cloacal papillae (Fig. 8); none on anterior lips of cloaca; 3 pairs of lateral papillae, the second of which is enlarged, and has a medially-directed lobe; papilla immediately posterior to cloaca consists of one lobe with a pair of narrow pedunculate lateral papillae; large cushion-like papilla posterior to this, not greatly expanded, and is indistinctly divided medially; posterior pair of papillae very close together. Rectum with cuticular lining. Long cuticular ventral ridge with fine teeth, never bunching into groups (Fig. 7).

LECTOTYPE: USNM Helm. Coll. No. 37085.
HOSTS: *Ochotona princeps*, *O. collaris*.
DISTRIBUTION: Colorado; Rocky Mountains, Alberta; Ogilvie Mountains, Yukon.
LOCATION IN HOST: Caecum and colon.
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 73535.

Comparisons of measurements of males by analyses of covariance (Fig. 15), show that while there are a large number of differences among worm species within a collection group in North America, very few differences exist among collection groups for each worm species. However, measurements of a relatively large number of structures (4 of 8) in

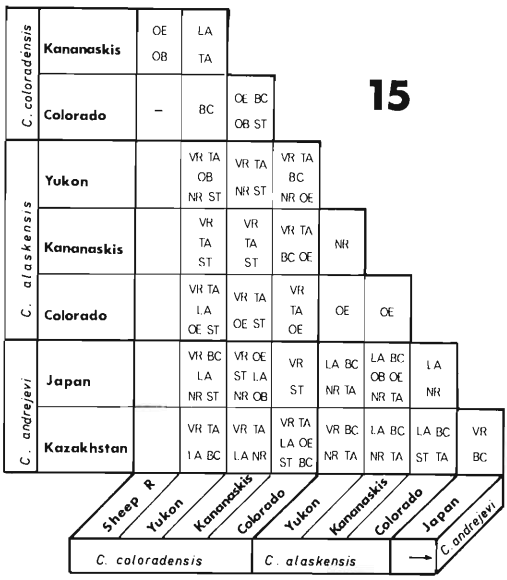


Figure 15. Comparisons of lengths or positions of structures for male *Cephaluris* spp. by analysis of covariance. Symbols in the boxes represent measurements which are significantly different ($P < 0.01$) for each pair. Boxes marked with (–) represent no significant differences. Where there were no analyses between members of a pair, the box is empty. The 8 measurements compared in the analyses are: width of transverse striations (ST), width of lateral alae (LA), depth of buccal cavity (BC), total length of esophagus (OE), width of esophageal bulb (OB), distance of nerve ring from anterior end (NR), distance of cloaca from posterior end (TA), and distance of anterior end of precloacal ventral ridge from cloaca (VR).

the Colorado sample of male *C. coloradensis* were found to differ significantly from the Kananaskis sample. Since these worms had been preserved differently, and it has been shown that procedural differences can affect measurements (Lamberti and Sher, 1969), the effect of differences in procedures was tested by comparing males from Sheep River with those from the Kananaskis and Colorado samples. The Sheep River worms were treated in the same manner as the Colorado group. No differences were found between the samples from Sheep River and Colorado (Fig. 15), suggesting that the differences between the samples from Kananaskis and Colorado were

due to different methods, rather than true differences in the worms.

Lists of measurements for worms from Asian hosts are included in Tables 2 and 3. All but one of the male worms were similar in appearance to *C. alaskensis*, but the author considers them to belong to a separate species because of size differences indicated by the analyses of covariance (Fig. 15). Worms from Kazakhstan were labelled as *C. andrejevi* Shul'ts, 1948 by Dr. Gvozdev, and although they are larger than the published descriptions, the author agrees with his determination, with the following exception. One male from Kazakhstan is definitely not *C. andrejevi*, but is probably *C. ochotonae* Akhtar, 1947, a new host record for *O. rutila*. Two kinds of females, distinguishable by the presence or absence of a vulvar flap, were found in the same collection. Akhtar (1947) described a flap for *C. ochotonae*; neither Shul'ts (1948) nor Spassky and Ryzhikov (1951) did so for *C. andrejevi*.

Only four males were found in *O. hyperborea yesoensis*. These are similar in appearance to both *C. alaskensis* and *C. andrejevi*, but are placed in the latter species because of the greater similarities of measurements (Fig. 15). The females, all without a vulvar flap, appear to be more similar to *C. andrejevi* from *O. rutila*, than to the pooled North American species (Table 2), although they are smaller than the former.

Discussion

Both *C. alaskensis* and *C. coloradensis* were universally present in the North American pika populations of this study. *Cephaluris alaskensis* in *O. princeps* is a new host record.

Akhtar (1958) described *C. collaris* from *O. collaris* collected in the Talkeetna Mountains of Alaska. He differentiated it from *C. coloradensis* by the following six characteristics: shallower buccal cavity, shorter esophagus, shorter tail, shorter ovejector, smaller eggs, and the presence of a doubly-papillate cushion-like papilla in the males. Inspection by the present author of syntype specimens of *C. coloradensis* established that Olsen (1949) had based his description of this species on specimens of both *C. coloradensis* and *C. alaskensis*. It is therefore necessary to review the differences between *C. coloradensis* and *C. collaris*.

Akhtar's (1958) measurements of esophagus length and eggs for *C. collaris* are similar to those of *C. coloradensis* in this study, as is the appearance of the large cushion-like papilla in the males. The tail length of females also agrees with *C. coloradensis* in this study. His shorter tail length in the males may be due to a sample size of eight, as the present author has found this character to be quite variable. It is difficult to compare ovejector lengths, as those in the present study were measured along the ovejector to the ring gland, while Akhtar measured the straight line distance from the vulva to the ring gland. The latter method can be misleading and results in lower values, due to differing states of curvature of the ovejector. Akhtar's measurements of the buccal cavity agree with those in this study for females, but are smaller for males. However, he measured this structure in only one male, the smallest specimen.

The above-listed lack of differences between Akhtar's *C. collaris*, and *C. coloradensis* as redescribed in this study, the same host species, and the close proximity of the collecting site for this study to the type locality for *C. collaris* (500 km), indicate that they are one and the same species. Therefore, *C. collaris* is a synonym for *C. coloradensis*.

In his key to the helminth species of North American pikas, Seesee (1973) uses the presence of a spicule to differentiate *C. coloradensis* from both *C. alaskensis* and *C. collaris*. Akhtar (1956b), Inglis (1959), and Spassky and Ryzhikov (1951) all pointed out that the structure referred to as the spicule in this genus (Olsen, 1949), is actually the cuticular lining of the rectum. Apparently Seesee (loc. cit.) ignored their arguments on this point, as no mention of them appears in his paper, although all three were cited. Seesee (loc. cit.), following Akhtar (1958), further differentiated *C. alaskensis* from *C. collaris* on the basis of presence in the former species of six long glands surrounding the anterior part of the esophagus. This has been found by the present author to be of no value in differentiating *C. alaskensis* from *C. coloradensis*. Some specimens of both species have the glands, while others appear not to have them.

Erhardova-Kotrla and Daniel (1970), apparently unaware of descriptions of *C. alaskensis*, *C. collaris*, *C. chabaudi* Inglis, 1959, and *C. andrejevi* males (first described by Spassky

and Ryzhikov, 1951), described *C. vakhanica* from *O. roylei* of Afghanistan. The only major characteristic they used to distinguish this species from *C. ochotonae* was absence of a spicule. As mentioned above, none of the species possess a spicule; Akhtar (1956b) revised his description of *C. ochotonae*. Therefore *C. vakhanica* is a synonym of *C. ochotonae*.

The genus *Cephaluris* now contains 6 species, 2 in North America, and 4 in Asia. These can be divided into 2 groups by morphology of males.

Key to Males of *Cephaluris* Akhtar, 1947

1A. Blunt, rugged teeth on ventral precloacal ridge; subcuticular longitudinal bands or bosses in caudal alae; greatly expanded cushion-like posterior papilla .. Group 1 . . .(2)

1B. Fine, thin teeth on ventral ridge; longitudinal bands or bosses absent; relatively deflated cushion-like posterior papilla ..... Group 2 . . .(4)

2A. Discontinuous bosses in caudal alae ..... *C. hashmi* Akhtar, 1956

2B. Continuous longitudinal bands in caudal alae ..... (3)

3A. Cloaca closer than 700 from tail tip; nerve ring closer than 195 from anterior end; buccal cavity shallower than 35; Nearctic distribution ..... *C. alaskensis* Akhtar, 1958

3B. Cloaca further than 700 from tail tip; nerve ring further than 195 from anterior end; buccal cavity deeper than 35; Palearctic distribution ..... *C. andrejevi* Shul'ts, 1948

4A. Cloacal papillae asymmetrical ..... *C. chabaudi* Inglis, 1959

4B. Cloacal papillae symmetrical ..... (5)

5A. Body shorter than 8 mm, and narrower than 400 mm; Nearctic distribution .. *C. coloradensis* Olsen, 1949

5B. Body longer than 8 mm, and wider than 400 mm; Palearctic distribution ..... *C. ochotonae* Akhtar, 1947

The differences between *C. andrejevi* and *C. alaskensis* in Group 1 were so slight that these may not represent separate species. It was imposible to distinguish between females of these two species, since there was no certainty as to the identity of the North American females, as stated above. Although differences in the males (Fig. 15) could be host-induced, or even an artifact caused by procedural differences, the author considers it safer at the present time to regard the two as separate species until more is known. It should be kept in mind that they are very closely related.

A similar situation exists with *C. ochotonae* and *C. coloradensis* in Group 2. Males of the latter species differ only in body length and width from the published description of *C. ochotonae*. The females of *C. ochotonae* possess a vulval flap, whereas no North American specimens of this genus had one.

With the exception of *O. hyperborea yesoensis* in Japan, from which only 4 male *Cephaluris* were recovered, the host population in each geographic location surveyed, harbored two species of *Cephaluris*, one from each group. This is a similar situation to that found in chimaeroid fishes, four species of which are host to two species of *Gyrocotyle*, one from each of two morphological groups (Land and Templeman, 1968). The situation in pikas differs however, in that both species are often present in the same individual. There also appears to be no competitive exclusion between *C. alaskensis* and *C. coloradensis* in either *O. collaris* or *O. princeps* (unpublished data). In addition, there is no obvious habitat segregation of these species within the caecum and colon of *O. collaris* (unpublished). That these species pairs should coexist in this fashion, and that the situation seems widespread in pikas, poses an evolutionary puzzle which requires further investigation.

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A Key to Larval Cestodes of Shallow-Water, Benthic Mollusks of the Northern Gulf of Mexico

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ABSTRACT: Eleven distinct species of larval cestodes recovered from shallow-water, benthic mollusks of the northern Gulf of Mexico are differentiated in an illustrated taxonomic key. They represent nine recognized genera in seven families of four orders and include the trypanorhynchs, *Eutetrarhynchus* sp. and *Parachristianella* sp., the tetraphyllideans, *Anthobothrium* sp., *Dioecotaenia cancellata* (Linton, 1890), *Rhinebothrium* sp., *Acanthobothrium* sp. (of Regan, 1963), *Acanthobothrium* sp. (of Harry, 1969), and "*Scolex pleuronectis quadrilocularis*"; the lecanicephalideans, *Polypocephalus* sp. and *Tylocephalum* sp.; and the diphyllidaen, *Echinobothrium* sp. Infected mollusks are widely distributed along the Gulf of Mexico coastline. Benthic marine mollusks serve as intermediate or paratenic hosts for these larvae while elasmobranch fishes serve as final hosts.

Larval cestode parasites of shallow-water, benthic mollusks were collected during a three-year period from the eastern Gulf of Mexico (Florida Keys to the Mississippi Sound). During that study, 2,470 mollusks representing 36 gastropod species, 55 pelecypod species, and one octopus from 30 sampling localities were examined for larval cestodes. Eleven distinct species of larvae representing nine or ten recognized genera in seven families and

Table 1. Larval cestode parasites of shallow-water, benthic mollusks of the northern Gulf of Mexico.

Cestode larvae	Gastropod hosts	Pelecypod hosts
Order Trypanorhyncha		
Family Eutetrarhynchidae		
Eutetrarhynchus sp.	*Busycon spiratum pyruloides* (Say) *Crepidula fornicata* (Linné) *Fasciolaria lilium hunteria* (Perry) *Fasciolaria tulipa* (Linné) *Pleuroploca gigantea* (Kiener)* *Thais haemastoma canaliculata* (Gray)	*Argopecten irradians concentricus* (Say) *Atrina rigida* (Lightfoot) *Atrina seminuda* (Lamarck) *Dosinia discus* (Reeve)
Parachristianella sp.	*Cantharus cancellarius* (Conrad) *Crepidula fornicata* (Linné) *Fasciolaria lilium hunteria* (Perry) *Fasciolaria tulipa* (Linné) *Polinices duplicatus* (Say)	*Anadara transversa* (Say) *Argopecten irradians concentricus* (Say)* *Atrina rigida* (Lightfoot) *Atrina seminuda* (Lamarck)* *Chione cancellata* (Linné) *Donax variabilis* (Say) *Macrocallista maculata* (Linné) *Macrocallista nimbosa* (Lightfoot)* *Noetia ponderosa* (Say) *Raeta plicatella* (Lamarck) *Spisula solidissima similis* (Say)*
Order Lecanicephalidea		
Family Lecanicephalidae		
Polypocephalus sp.		*Argopecten irradians concentricus* (Say)*
Family Cephalobothriidae		
Tylocephalum sp.	*Busycon contrarium* (Conrad)* *Busycon spiratum pyruloides* (Say)* *Cantharus cancellarius* (Conrad)* *Crepidula fornicata* (Linné) *Crepidula maculosa* Conrad *Crepidula plana* Say *Fasciolaria lilium hunteria* (Perry)* *Fasciolaria tulipa* (Linné)* *Melongena corona* (Gmelin) *Murex florifer dilectus* (A. Adams)* *Murex fulvescens* Sowerby *Murex pomum* (Gmelin)* *Oliva sayana* Ravenel* *Pleuroploca gigantea* (Kiener)* *Polinices duplicatus* (Say) *Thais haemastoma canaliculata* (Gray)*	*Anadara floridana* (Conrad) *Anadara transversa* (Say)* *Anomia simplex* Orbigny *Arca zebra* (Swainson) *Argopecten irradians concentricus* (Say)* *Artrina rigida* (Lightfoot)* *Atrina seminuda* (Lamarck)* *Chama macerophylla* (Gmelin) *Chione cancellata* (Linné) *Chlamys sentis* (Reeve)* *Crassostrea virginica* (Gmelin)* *Cyrtopleura costata* (Linné)* *Dinocardium robustum* (Lightfoot)* *Donax variabilis* (Say) *Dosinia elegans* Conrad* *Ensis minor* Dall* *Laevicardium mortoni* (Conrad) *Macrocallista maculata* (Linné) *Macrocallista nimbosa* (Lightfoot)* *Mactra fragilis* Gmelin *Mercenaria campechiensis* (Gmelin)* *Mercenaria mercenaria texana* (Dall)* *Modiolus modiolus squamosus* Beauperthuy *Noetia ponderosa* (Say)* *Periglypta listeri* (Gray)* *Pinctada imbricata* Röding *Pinna carnea* Gmelin *Pseudochama radians* (Lamarck) *Pteria colymbus* (Röding) *Raeta plicatella* (Lamarck) *Spisula solidissima similis* (Say) *Spondylus americanus* Herman *Trachycardium egmontianum* (Shuttleworth)
Order Tetraphyllidea		
Family Dioecotaeniidae		
Dioecotaenia cancellata (Linton)	*Melongena corona* (Gmelin)	[*Anadara ovalis* (Bruguière)]† *Chione cancellata* (Linné)
Family Phyllobothriidae		
Anthobothrium sp.		*Anadara transversa* (Say)* *Argopecten irradians concentricus* (Say) [*Donax variabilis* (Say)]† *Macrocallista nimbosa* (Lightfoot) *Spisula solidissima similis* (Say) *Tellina versicolor* DeKay
Rhinebothrium sp.	*Busycon contrarium* (Conrad) *Busycon spiratum pyruloides* (Say)* *Cerithium atratum* (Born) *Cantharus cancellarius* (Conrad) *Crepidula fornicata* (Linné)† *Crepidula maculosa* Conrad *Crepidula plana* Say† *Fasciolaria lilium hunteria* (Perry)	*Amygdalum papyrium* (Conrad) *Anadara transversa* (Say)* *Argopecten irradians concentricus* (Say)* *Atrina rigida* (Lightfoot) [*Atrina seminuda* (Lamarck)]† *Donax variabilis* (Say) *Dosinia discus* (Reeve)*† *Ensis minor* Dall

Table 1. ***Continued.***

Cestode larvae	Gastropod hosts	Pelecypod hosts
Rhinebothrium sp. (continued)	*Fasciolaria tulipa* (Linné)*	*Mactra fragilis* Gmelin
	Melongena corona (Gmelin)	*Noetia ponderosa* (Say)*†
	Nassarius vibex (Say)	[*Periploma inequale* (C. B. Adams)]†
	Oliva sayana Ravenel	*Raeta plicatella* (Lamarck)†
	Pleuroploca gigantea (Kiener)	*Spisula solidissima similis* (Say)*
	Polinices duplicatus (Say)	*Tagelus divisus* Spengler
		Tagelus plebeius (Solander)
		Trachycardium egmontianum (Shuttleworth)
Family Onchobothriidae		
Acanthobothrium sp. (of Regan, 1963)	*Busycon spiratum pyruloides* (Say)*	*Noetia ponderosa* (Say)
	Cantharus cancellarius (Conrad)	
	Fasciolaria lilium hunteria (Perry)*	
	Fasciolaria tulipa (Linné)*	
	Melongena corona (Gmelin)	
	Murex pomum Gmelin	
	Oliva sayana Ravenel	
	Pleuroploca gigantea (Kiener)*†	
	Polinices duplicatus (Say)	
	[*Thais haemastoma canaliculata* (Gray)]†	
Acanthobothrium sp. (of Harry, 1969)	*Polinices duplicatus* (Say)	*Argopecten irradians concentricus* (Say)
		Ensis minor Dall*
		[*Macoma constricta* (Bruguière)]†
		Pseudomiltha floridana (Conrad)
		Raeta plicatella (Lamarck)*
		Tagelus divisus Spengler*
		Tagelus plebeius (Solander)*†
"Scolex pleuronectis quadriloculoris"	(OCTOPOD) *Octopus joubini Robson*	
Unidentified Tetraphyllideans *"Scolex pleuronectis"*	*Busycon spiratum pyruloides* (Say)	*Anomalocardia auberiana* (Orbigny)
	Cantharus cancellarius (Conrad)	*Argopecten irradians concentricus* (Say)
	Crepidula fornicata (Linné)	*Chione cancellata* (Linné)
	Crepidula plana Say	*Cyrtopleura costata* (Linné)*
	Fasciolaria lilium hunteria (Perry)	*Dosinia discus* (Reeve)*
	Fasciolaria tulipa (Linné)	*Dosinia elegans* Conrad
	Melongena corona (Gmelin)	*Ensis minor* Dall
		Modiolus modiolus squamosus Beauperthuy
		Noetia ponderosa (Say)
Order Diphyllidea Family Echinobothriidae		
Echinobothrium sp.	*Cantharus cancellarius* (Conrad)	
	Nassarius vibex (Say)	

* Major intermediate or paratenic hosts.
† Source: W. J. Wardle, Moody College of Marine Science, Texas A & M University, Galveston, Tex., pers. comm., 1975. Hosts enclosed in brackets were identified by Wardle only.

four orders were conditionally identified. Because those larvae lacked taxonomically important characteristics, most could be identified only to generic level. Recently developed artificial culture techniques facilitated generic identification of several tetraphyllidean larvae (*vide* Read et al., 1960; Hamilton and Byram, 1974). Improved culture methods and experimental infection studies may permit specific identifications of many of these larvae in the future.

I have reviewed all known reports of cestodes from benthic mollusks in the Gulf of Mexico and adjacent waters and a synoptic review is in press. The present taxonomic key includes all larval cestodes presently known to infect benthic mollusks of the northern Gulf of Mexico. William J. Wardle (Moody College of Marine Science, Texas A & M University, Galveston, Tex., pers. comm., 1975) provided larval cestode infection data from Galveston Bay and adjacent coastal mollusks, thereby extending the geographic range of this key. A list of the molluscan hosts of each larval cestode included in this key is presented in Table 1.

This key should be used judiciously and with the realization that it includes only those cestodes recovered from benthic mollusks to date. Additional species of larval cestodes infect squid, but those pelagic mollusks were not examined during the study from which this key was derived. Bibliographic references are provided within the key to facilitate specific

identifications should advanced specimens be encountered or eventually cultured by others. The cestode nomenclature follows that of Schmidt (1970) with the exception of *Anthobothrium* Beneden, which he splits, and *Rhinebothrium* Linton, which he does not recognize. The molluscan nomenclature follows that of Abbott (1974).

For the sake of convenience and clarity all cestodes covered by this key are considered metacestodes (a term for any larval form between the egg and adult) or larvae in the general or collective sense. I have utilized Freeman's (1973) system of metacestode classification in an attempt to avoid the present confusion in larval terminology. Freeman's system utilizes descriptive prefixes (e.g., acanthobothrio-, bothridio-, tentaculo-, uniacetabulo-, etc.) in conjunction with standard metacestode terms (e.g., procercoid, plerocercoid, cysticercoid, postplerocercoid, etc.) to characterize larvae.

Pelecypods appear to serve as primary intermediate hosts while molluscivorous gastropods appear to serve as secondary intermediate or paratenic hosts for these larval cestodes. Other marine organisms including crustaceans and fish may also be involved as intermediate hosts in the life cycle of some of these elasmobranch cestodes. Many of the molluscan hosts are confirmed prey of demersal elasmobranch fishes of the Gulf of Mexico (Bigelow and Schroeder, 1953). Pelecypods become infected by ingesting reproductive products released by definitive hosts (e.g., eggs and gravid proglottids) or free-swimming coracidia. Molluscivorous gastropods become infected by ingesting infected pelecypods or gastropods. The first mode of infection is based on circumstantial feeding and infection data (Cheng, 1966) and the second mode was demonstrated during this investigation (Cake, in press). For the parasitologist who is interested in surveying the cestode fauna of any coastal marine habitat, molluscivorous gastropods would serve as ideal "indicator" organisms (or cestode collectors).

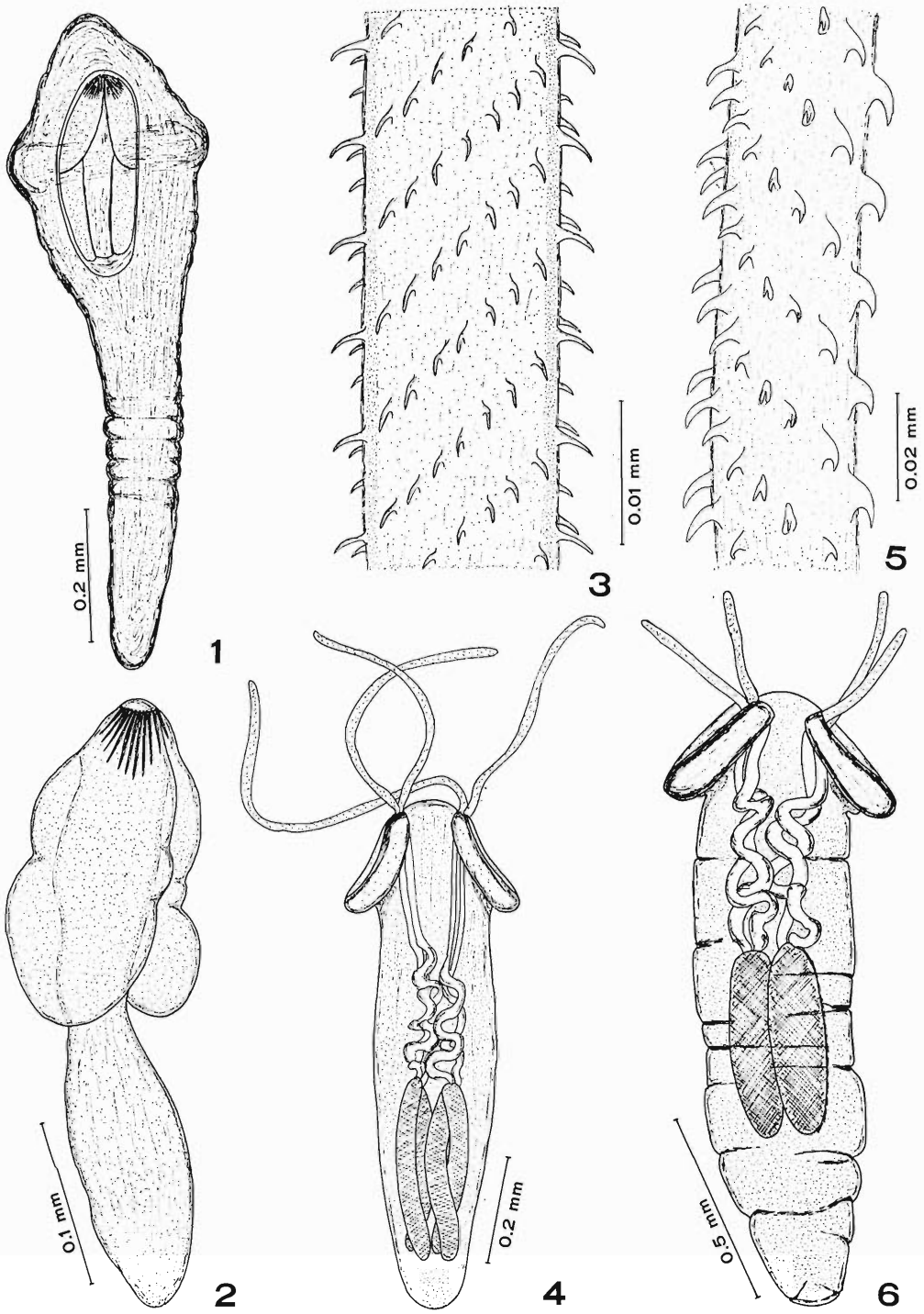
Materials and Methods

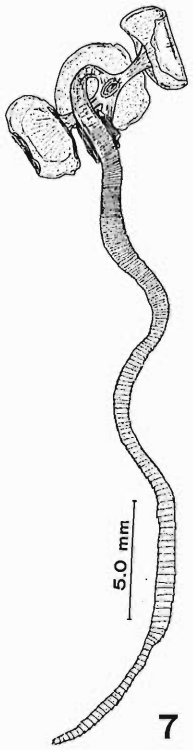
Benthic marine mollusks were collected at thirty Gulf coast localities between Dry Tortugas, Florida, and Bay St. Louis, Mississippi, from shallow, subtidal, estuarine and marine environments (e.g., sand-, mud- and grassflats and coral and oyster reefs, etc.) via selective sampling methods (SCUBA, snorkling, wading, shoveling, etc.). Based on preliminary parasite studies (Cake, 1972), large pelecypods (e.g., clams, oysters, pen shells, scallops, etc.) and molluscivorous gastropods (e.g., conchs, tulips shells, whelks, etc.) were collected, maintained alive in large Styrofoam containers and transported to various coastal laboratories for dissection and examination. Again, based on preliminary studies, the following tissues and locations were examined for cestode larvae: (in pelecypods) gills, labial palps, stomach and stomach walls, intestine, intestine walls, and intestinal pouches (if present), digestive gland and diverticula, and foot musculature; (in gastropods) valve of Leiblein (if present), esophagus and esophageal pouches (if present), stomach and stomach wall, digestive gland and diverticula; and

→

Figures 1–6. Larval cestodes of Gulf of Mexico Mollusca. 1. Longicaudate, invaginated acanthorostellobothridio-cysticercoid of *Echinobothrium* sp. from *Nassarius vibex*. (Cut-away view showing invaginated scolex.) 2. Scolex of *Echinobothrium* sp. from *N. vibex*. 3. Metabasal armature of internal surface of tentacle of *Eutetrarhynchus* sp. tentaculo-neoplerocercoid from *Pleuroploca gigantea*. 4. Tentaculo-neoplerocercoid of *Eutetrarhynchus* sp. from *P. gigantea*. 5. Side view of metabasal armature of tentacle of *Parachristianella* sp. tentaculo-neoplerocercoid from *Macrocallista nimbosa*. 6. Tentaculo-neoplerocercoid of *Parachristianella* sp. from *M. nimbosa*.

Figures 7–12. Larval cestodes of Gulf of Mexico Mollusca. 7. Bothridio-postplerocercoid of *Anthobothrium* sp. from *Anadara transversa*. 8. Claviform capsule containing bothridio-postplerocercoid of *Anthobothrium* sp. from *A. transversa*. (Cut-away view showing coiled postplerocercoid.) 9. Bothridio-plerocercoid of *Acanthobothrium* sp. (of Regan, 1963) from *Oliva sayana*. 10. Bothridio-plerocercoid of *Acanthobothrium* sp. (of Harry, 1969) from *Ensis minor*. 11. Bothridio-plerocercoid of "*Scolex pleuronectis quadrilocularis*" from *Octopus joubini*. 12. Bothridio-plerocercoid of *Rhinebothrium* sp. from *Argopecten irradians concentricus*.





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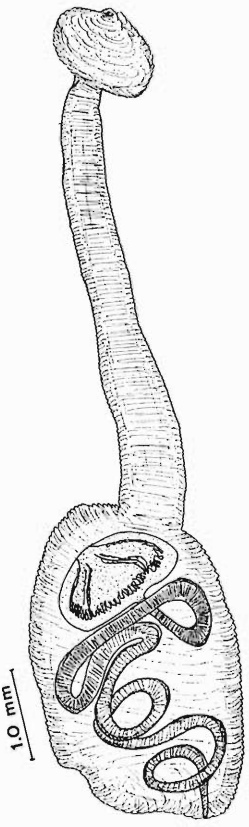

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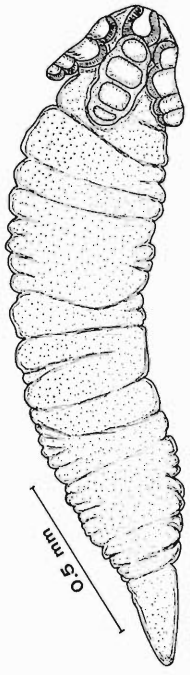

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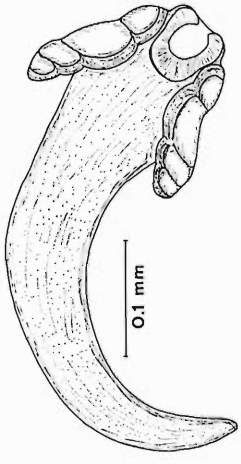

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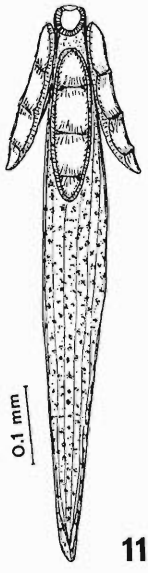

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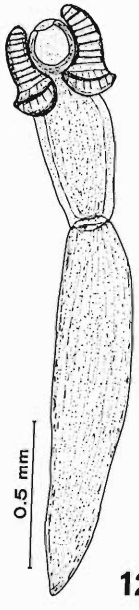

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(in octopods) crop, stomach, intestine, digestive gland, and connective tissues in and around the visceral mass.

All examinations were made using a stereoscopic, dissecting microscope. When located, the larvae were removed or excised (if encysted), identified (usually to family or "type"), counted and either fixed and preserved or placed in dishes of filtered seawater (at 30‰ salinity and ambient temperature) for further observations. Selected larvae of *Acanthobothrium* spp., *Rhinebothrium* sp. and *Polypocephalus* sp. were incubated for up to 150 hr in a glucose-enriched, artificial elasmobranch saline medium (*vide* Read et al., 1960; Hamilton and Byram, 1974). During incubation some larval features (e.g., apical suckers) were lost or modified (quadriloculate to triloculate bothridial condition with terminal suckers or pads), and some rudimentary adult features developed (e.g., bothridial hooks).

The larvae were killed in an expanded or relaxed condition with tepid tap water or hot AFA (ca. 50 C), fixed in AFA, and preserved in 70% ethanol and 5% glycerine. Large postplerocercoids of *Anthobothrium* sp. were killed in an expanded and relaxed condition (all four bothridia attached to bottom of dish) with liquid nitrogen and fixed and preserved as above. The larvae were stained with either Van Cleave's combination hematoxylin or Ehrlich's acid hematoxylin and mounted on slides via standard helminthological techniques. Illustrations of larvae were made with the aid of a micro-projector.

A Key to Larval Cestodes of Shallow-Water, Benthic Mollusks of the Northern Gulf of Mexico

1a. Scolex with two bothridia 2
1b. Scolex with four bothridia 4
1c. Scolex without bothridia 8
2a. Bothridia leaflike and spoon-shaped; rostellum (myzorhynchus) armed with a crown of apical hooks (Diphyllidea); longicaudate, invaginated acanthorostellobothridio-cysticercoid (Figs. 1 and 2); cysticercoids embedded in digestive gland of small, omnivorous gastropods (*Cantharus cancellarius* and *Nassarius vibex*) ..... *Echinobothrium* sp.
(Possibly *E. musteli* Pintner, 1889.)
[Key to adult species: Rees, 1961.]
2b. Bothridia patelliform, each with posterior notch and two-armed protrusile tentacles (Trypanorhyncha) 3
3a. Tentacle armature heteroacanthous and homeomorphous, consisting entirely of small, falciform hooks arranged in continuous, spiraling rows (Fig. 3); tentacle sheaths sinuous; tentaculo-neoplerocercoids (Fig. 4) encysted singly or in small groups (up to five per cyst) in muscular folds of valve of Leiblein of *Pleuroploca gigantea* and other molluscivorous gastropods ..... *Eutetrarhynchus* sp.
[Keys to adult species: Dollfus, 1942; Wardle and McLeod, 1952.]
3b. Tentacle armature heteroacanthous and heteromorphous (internal surface hooks large like rose thorns, others spiniform), arranged in spiral, half-turn rows (Fig. 5); tentaculoneoplerocercoids (Fig. 6) commonly encysted along intestine walls of numerous pelecypods (Table 1) ..... *Parachristianella* sp.
[Description of Gulf of Mexico species: Kruse, 1959.]
4a. Bothridia simple, variable in shape and without accessory suckers (Fig. 7); apical sucker absent; bothridiopostplerocercoids encapsulated singly in muscular, thin-walled, claviform capsules (Fig. 8); small end of capsule embedded in visceral mass of infected pelecypods (Table 1); large, bulbous end containing coiled postplerocercoid hangs free in host's mantle cavity ..... *Anthobothrium* sp.
[Key to adult species: Wardle and McLeod, 1952.]
4b. Bothridia multiloculate, apical sucker present 5
5a. Bothridia quadriloculate 6
5b. Bothridia not quadriloculate 7
6a. Bothridio-plerocercoids 3.4 to 6.2 mm long (Fig. 9); free in digestive tract of numerous molluscivorous gastropods (Table 1) ..... *Acanthobothrium* sp. (of Regan, 1963)
(Possibly *A. paulum* Linton, 1890.)

6b. Bothridio-plerocercoids 0.2 to 0.9 mm long (Fig. 10); encapsulated singly or in groups (up to 150 per per capsule) in gut wall pouches or distended digestive diverticula immediately adjacent to intestine of small, burrowing pelecypods (Table 1) ..... *Acanthobothrium* sp. (of Harry, 1969) (Possibly *A. brevissime* Linton, 1908.) [Key to adult species: Goldstein, 1967.]

6c. Bothridio-plerocercoids 0.4 to 1.5 mm long (Fig. 11); free or encapsulated (in mucoid sheath) in digestive tract of *Octopus joubini* ..... *"Scolex pleuronectis quadrilocularis"* (Probably species of *Acanthobothrium*.)

7a. Bothridia spoon-shaped, divided into narrow anterior region with eight transverse loculi and cuplike posterior region with eight radially arranged loculi (Fig. 12) or leaflike with up to 33 transverse loculi (Fig. 13); small bothridio-plerocercoids, 0.3 to 4.6 mm long, "confined" in digestive diverticula of numerous pelecypods (Fig. 12 and Table 1); larger (0.4 to 15.1 mm) and more advanced plerocercoids free in digestive tract of numerous, molluscivorous gastropods (Figs. 13 and 14, Table 1) ..... *Rhinebothrium* sp. [Review of genus: Campbell, 1970.]

7b. Bothridia oval, slightly cupped; each bothridium divided into 21 loculi either as three rows of seven each or as five central loculi surrounded by 16 marginal loculi (Fig. 15); large bothridio-plerocercoids (ca. 8 mm) rare in stomach of *Melongena corona* and digestive diverticula of *Chione cancellata* ..... *Dioecotaenia cancellata* (Linton, 1890) [Species description: Schmidt, 1969.]

8a. Small (0.6 to 1.6 mm long) unencysted, uniacetabulo-plerocercoids (Figs. 16 and 17); scolex occasionally with four bothridial precursors immediately posterior to apical sucker; osmoregulatory canals distinct; (early tetraphyllidean plerocercoids) 9

8b. Small (0.1 to 0.4 mm long) encysted metacestodes (Figs. 18 and 19); apical region of scolex protrusile, forming compact cushion or sucker, voluminous glandular mass, or tentacles; posterior scolex region with or without four simple suckers; (lecanicephalidean metacestodes) 10

9a. Uniacetabulo-plerocercoids (Fig. 16) free in digestive tract of molluscivorous gastropods (Table 1) concurrently infected with advanced bothridio-plerocercoids of *Acanthobothrium* sp.; probably early plerocercoids of ..... *Acanthobothrium* sp. (of Regan, 1963)

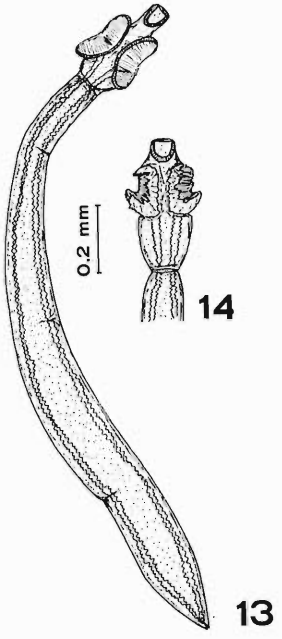
9b. Uniacetabulo-plerocercoids (Fig. 17) frequently confined in digestive diverticula (tubules) of pelecypods (Table 1) concurrently infected with advanced bothridio-plerocercoids of *Rhinebothrium* sp.; probably early plerocercoids of ..... *Rhinebothrium* sp.

10a. Scolex with muscular, cuplike apical sucker, with protrusile, domelike protuberance originating from sucker floor; acaudate glando-procercoids small, 0.14 to 0.34 mm long, globose (Fig. 18); encysted singly in thick-walled cysts throughout visceral mass of numerous pelecypods and in digestive gland of numerous gastropods (Table 1) ..... *Tylocephalum* sp. [No key to adult species available; species list: Yamaguti, 1959.]

10b. Scolex with 16, simple, unarmed, protrusile, apical tentacles and four simple suckers (Fig. 19); tentaculo-plerocercoids small, 0.26 to 0.41 mm long, thin and pyriform; encysted singly in clumps of up to eight individuals in thin, transparent capsules in connective tissues of digestive gland in *Argopecten irradians concentricus* ..... *Polypocephalus* sp. [No key to adult species available; species list: Yamaguti, 1959.]

Discussion

I have restricted this key to larval cestodes of benthic mollusks because more definitive data are available on molluscan cestodes in the Gulf of Mexico than on other invertebrate



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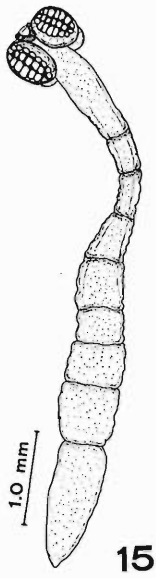

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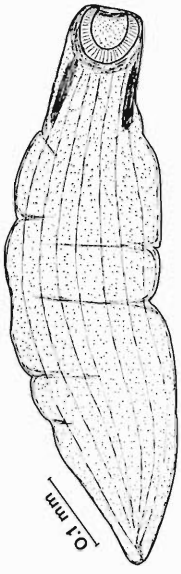

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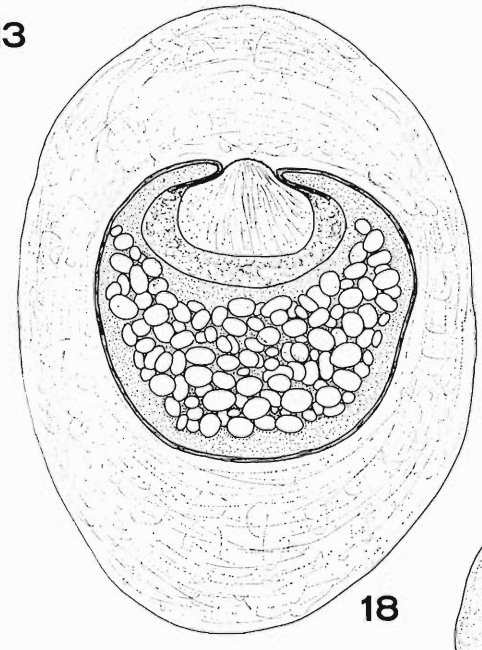

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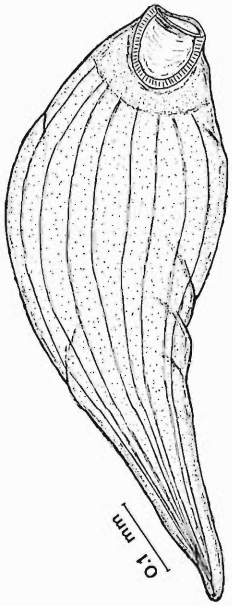

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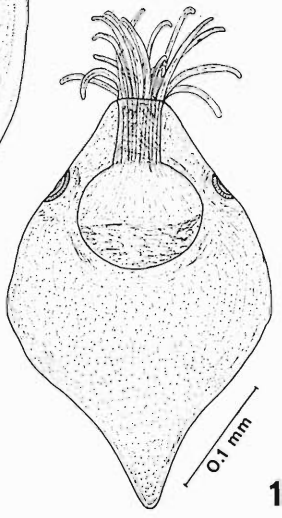

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hosts, and because the collection and maintenance of pelagic mollusks such as squid was beyond the scope of the investigation from which this key was derived. That investigation was designed to determine the cestode parasites of resident mollusks rather than transient mollusks. Because most molluscan cestodes exhibit no host specificity (see Table 1), and because many molluscivorous gastropods are capable of concentrating cestode larvae from their prey, one may acquire definitive information on the cestodes of a particular coastal area by studying molluscan hosts. That is not to say that other hosts (e.g., elasmobranchs) should not be examined. In most instances the adult cestode fauna of most coastal areas in the Gulf of Mexico is well-known, but relatively little is known about their life cycles and intermediate hosts. This key was designed specifically to assist those who wish to investigate the complete identities and life cycles of these molluscan cestodes.

In the body of this key I have suggested specific identities for several larval forms which closely resemble adult cestodes from the same area. I am particularly indebted to Mr. Tom Mattis (Gulf Coast Research Laboratory, Parasitology Section, pers. comm., 1973–1975) for providing information on the elasmobranch cestodes of the northeastern Gulf of Mexico. The suggested identity of *Echinobothrium* sp. cysticercoids was based on scolex morphology which matches that of *E. musteli* Pintner, 1889, the only adult species reported from the northeastern Gulf. The identities suggested for the two *Acanthobothrium* forms were based on meristic comparisons of incubated plerocercoids and adults (as reported in the literature).

The two trypanorhynch larval forms included in this key appear to be undescribed species, and will be formally described in a subsequent publication. Only one species of *Eutetrarhynchus*, *E. lineatus* (Linton), and two species of *Parachristianella*, *P. monomegacantha* Kruse and *P. dimegacantha* Kruse, have been reported from the eastern Gulf of Mexico to date (Linton, 1908; and Kruse, 1959, respectively). The descriptions of those three eutetrarhynchids do not match those of the larval forms recovered from marine mollusks. There is a slight resemblance, however, between the *Parachristianella* larvae recovered from penaeid shrimps (Kruse, 1959) and pelecypod mollusks in the vicinity of Alligator Harbor, Florida (Cake, 1972).

Regan (1963) and Harry (1969) recovered and described unidentified tetraphyllidean larvae from crown conchs, *Melongena corona*, and channeled duck clams, *Raeta plicatella*, in Florida and Texas, respectively. I obtained living specimens from the same gastropod host and locality reported by Regan (1963), and from the same pelecypod host, but from a different locality than that reported by Harry (1969). I also obtained specimens of Harry's larvae that he had given to James Byram (Brigham Hospital, Pathology Dept., Boston, Mass.). The plerocercoids that I collected are the same as those described in the original publications. Subsequent incubation experiments by Hamilton and Byram (1974) and this investigator revealed that those plerocercoids were two distinct species, that they were in the family Onchobothriidae, and that they belonged in the genus *Acanthobothrium* Beneden. The onchobothriid plerocercoids recovered from Joubini's octopus, *O. joubini*, probably belong in the same genus, but because none were incubated (*in vitro*) in artificial elasmobranch saline, that generic identity cannot be confirmed. That octopus does consume small pelecypods such as the hosts of the plerocercoid reported by Harry (1969) (pers. observ.), and the two larval forms [*Acanthobothrium* sp. (of Harry, 1969) and "*Scolex*

←

Figures 13–19. Larval cestodes of Gulf of Mexico Mollusca. 13. Bothridio-plerocercoid of *Rhinebothrium* sp. from *Busycon spiratum pyruloides*. 14. Contracted scolex (bothridia) of *Rhinebothrium* sp. from *B. s. pyruloides*. 15. Bothridio-plerocercoid of *Dioecotaenia cancellata* (Linton, 1890) from *Chione cancellata*. 16. Uniacetabulo-plerocercoid of *Acanthobothrium* sp. (of Regan, 1963) from *Fasciolaria tulipa*. 17. Uniacetabulo-plerocercoid of *Rhinebothrium* sp. from *C. cancellata*. 18. Encysted, acaudate glando-procercoid of *Tylocephalum* sp. from *Argopecten irradians concentricus*. 19. Tentaculo-plerocercoid of *Polypocephalus* sp. from *A. i. concentricus*.

pleuronectis quadrilocularis"] do share meristic and morphologic characteristics.

The fact that the phyllobothriid plerocercoids which infect numerous pelecypods and gastropods are the same species of *Rhinebothrium* was confirmed by artificial culture and infection studies. The bothridial morphology of small plerocercoids from pelecypods matched that of advanced forms from molluscivorous gastropods following incubation. The transfer of *Rhinebothrium* sp. plerocercoids from pelecypod to gastropod hosts was demonstrated with artificial infection experiments using ponderous arks, *Noetia ponderosa*, and banded tulips, *Fasciolaria lilium hunteria*. Those experiments will be detailed in a subsequent publication.

Infection data obtained from hosts of two tetraphyllideans, *Acanthobothrium* sp. (of Regan, 1963) and *Rhinebothrium* sp., strongly suggest that the larvae which possess only an apical sucker and concurrently infect hosts with advanced bothridio-plerocercoids are in fact early plerocercoids of those respective species. The larvae fall within the size ranges of the advanced plerocercoids; they occur simultaneously in the same hosts; and they possess the distinctive osmoregulatory canal systems of their respective advanced forms. Some of the larvae possess bothridial precursors that range from undeveloped, darkly staining areas immediately posterior to the apical sucker to poorly developed, but definite bothridia.

Formal descriptions and analyses of infection and occurrence data for all larval cestodes covered by this key will be published at a later date.

Acknowledgments

I should like to express my appreciation to Dr. R. W. Menzel (Florida State University Oceanography Department, Tallahassee, Fla.) and Dr. J. E. Byram (Peter Bent Brigham Hospital Pathology Department, Boston, Mass., formerly with the F.S.U. Biological Sciences Department) for financial, logistical, and technical assistance. Dr. W. J. Wardle (Moody College of Marine Science, Texas A & M University, Galveston, Tex.) and Mr. Tom Mattis (Gulf Coast Research Laboratory Parasitology Section, Ocean Springs, Miss.) kindly shared their extensive knowledge of Gulf coast cestodes. Thanks are also extended to Dr. Robin Overstreet and Mr. Richard Heard (G.C.R.L. Parasitology Section) for critically reviewing this manuscript.

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Small Dung Beetles as Biological Control Agents: Laboratory Studies of Beetle Action on Trichostrongylid Eggs in Sheep and Cattle Feces[1]

R. C. Bergstrom[2], L. R. Maki[2] and B. A. Werner[3]

ABSTRACT: Four species of the genus *Aphodius* and *Canthon practicola* beetles were placed on sheep and cattle feces containing trichostrongylid eggs. Ten to 20 beetles were allowed to feed 1–5 days on 5–100 g of feces containing 12–3,000 trichostrongylid eggs per g. Numbers of trichostrongylid eggs in cultures with beetles decreased 24–90% more than in those without beetles within five days.

Researchers of Coleoptera have shown that coprophagous, scarabaeoid beetles are strongly attracted to fresh feces of various animals (Fincher et al., 1970). Fincher et al. (1971) studied flight activities of several species of beetles during various hours of the day and found a consistent periodicity of activity which was affected by temperature change.

Geographically nearer the present study are the observations of McDaniel and Balsbaugh (1968) who showed that bovine manure is used as an overwintering medium for Coleoptera in South Dakota; in the same state Kessler and Balsbaugh (1972) noted a regular succession of adult Coleoptera in bovine manure during the growing season. Sanders and Dobson (1966) reported on insects associated with bovine manure in Indiana. More recently, Waterhouse (1974) reported on the biological control of dung by beetles and Fincher (1973) showed dung burying beetles could serve as biological control agents of *Ostertagia ostertagia*, a trichostrongylid nematode parasite of cattle. Dung beetle mouth parts are efficient in macerating food and could be effective in breaking nematode eggs in feces (Miller, 1961).

The objective of the present study was to determine whether or not relatively small

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Table 1. Small dung beetles acting on *Trichostrongylus colubriformis* eggs in sheep feces.

Trial no.	Hours of beetle action	Fecal subsample size (g)	Initial no. eggs/g	No. eggs recovered		% decrease in egg no. due to beetle action
				no beetles control	with beetles principal	
1	48	10	3000	2200	1500	32
2	28	10	3000	2200	1400	36
3	40	10	3000	2400	600	75
4	30	10	3000	2100	900	57
5	22	10	3000	2100	1300	38
6	40	10	3000	2100	200	90
7	49	6	3000	1300	600	54
8	24	5	2500	1700	900	47
9	25	10	2200	2200	1200	45
10	29	5	1700	1400	600	57
11	23	4	3000	2900	1200	59
Ave.	32.5	8	2764	2055	945	54

beetles such as the *Aphodius* spp. and *Canthon practicola* might affect trichostrongylid egg numbers in feces of sheep and cattle.

Materials and Methods

Ovine fecal material used in the trials was collected with diapers on lambs which had been orally infected via drench with third-stage larvae of *Trichostrongylus colubriformis* (Giles, 1892). About one month after exposure, *T. colubriformis* egg numbers in ovine feces ranged from about 1,700–3,000 eggs per gram (e.p.g.) with the higher number being found in many of the samples used in these trials.

Feces taken from the diapers were placed in plastic sacks and weighed. Feces not used immediately were stored 1–3 weeks at 0–4 C. From the large fecal samples, equal 5–30 g subsamples were used as control (no beetles) and principal (with beetles) samples for each trial. Eleven trials were carried out at laboratory temperatures of ± 20 C. All trials were conducted with feces on filter paper over moist, sterile soil in capped polyethylene jars with 40 1 mm air holes.

Four to 10 *Aphodius vittatus* Say, 6–10 *A. coloradensis* Horn and 2–4 *A. granarius* (L.) were placed on or near feces containing *T. colubriformis* eggs whereas 1–4 *Canthon practicola* LeConte, 3–5 *A. coloradensis* and 3–5 *A. fimetarius* (L.) were used in 6 trials with mixed species of trichostrongylid eggs.

The beetles were allowed to act on the feces and trichostrongylid eggs for 22–49 hrs. After the period of exposure, smaller (4–10 g) subsamples were withdrawn from the control and principal samples and checked by use of the McMaster technique for the number of *T. colubriformis* eggs. Per cent decreases in numbers of eggs in the principal samples were recorded. Attempts were made to find trichostrongylid eggs in the intestinal tract and/or on the external surfaces of the beetles.

Fecal samples containing the mixed trichostrongylid ova were gathered from various, naturally infected sheep and cattle in Wyoming. Trichostrongylid (*Nematodirus* spp., *Ostertagia* spp., and *Marshallagia marshalli*, Orloff, 1933) egg numbers in such fecal samples were much lower (12–184 e.p.g.) than in those of the artificially infected lambs. Eggs were concentrated by a centrifugation-flotation technique using a sucrose solution (specific gravity 1.18) in a modified Lane technique. Subsample size in the latter trials was larger (100 g).

Data were analyzed by use of Student's *t* test in order to detect possible significant differences in egg numbers between control and principal samples after beetle exposure. Statistical analyses were made with a Sigma 7 (Xerox Data Systems) computer.

Results

Data presented in Table 1 show the per cent decrease in the numbers of *Trichostrongylus colubriformis* eggs in sheep feces when *Aphodius* beetles were allowed to feed on relatively small amounts of feces (4–10 g) for relatively brief periods of time (1–2 days).

Table 2. Small dung beetles acting on various trichostrongylid eggs* in cattle and sheep feces.

Trial no.	Hours of beetle action	Fecal subsample size (g)	Initial no. eggs/g	No. eggs recovered		% decrease in egg no. due to beetle action
				no beetles control	with beetles principal	
1	48	100	16	16	8	50
2	48	100	184	156	118	24
3	48	100	36	36	18	50
4	48	100	40	28	12	57
5	120	100	18	18	10	44
6	120	100	12	12	6	50
Ave.	72	100	51	44	29	46

* Eggs of *Nematodirus* sp., *Ostertagia* sp., and *Marshallagia marshalli*.

Recovery of eggs in control samples was good (mean = 74%) and decrease in egg numbers due to beetle action was moderate to large, (32–90 with a mean of 54%). The effect of the beetle action was significant at the 1% level.

Data in Table 2 indicate the results of 6 trials where the research procedures were similar to those of the *Aphodius* spp.–*T. colubriformis* trials but with mixed trichostrongylid (*Nematodirus* sp., *Ostertagia* sp. and *Marshallagia marshalli*), nematode eggs in sheep and cattle feces.

No trichostrongylid eggs were found in the intestinal tracts or on the external parts of the beetles after they had been exposed to the feces.

Discussion

Much of the previous research with coprophagous beetles, pertinent to this discussion, has been done with a larger, burying beetles (e.g., *Phanaeus* spp.) and results show that pasture contamination is decreased (Waterhouse, 1974) or that livestock infection by trichostrongylid larvae on vegetation is decreased (Fincher, 1973). Smaller, coprophagous beetles such as species of *Aphodius* and *Canthon* may also be effective biological control agents. Smaller beetles do not bury feces directly but do ingest a portion of the feces and spread much of the remaining fecal material over surrounding areas of soil, grass, etc. Nematode eggs are then exposed to detrimental micrometeorological factors.

We have not attempted to separate beetles by species but have collected and maintained them by complexes of species found together in feces in the field at various times of the year.

With longer periods of interaction (trials 5 and 6, Table 2), decreases in numbers of trichostrongylid eggs due to beetle action was relatively low as compared to the shorter term trials. In trials 5 and 6, beetles were sluggish and only 1 or 2 beetles had penetrated the fecal mass within the 5 day exposure.

When the numbers of beetles dropped to 1–3, control and principal fecal samples contained about the same number of eggs post-beetle exposure.

More research is needed as to the effect of monospecific beetle populations in interaction with parasite eggs in feces.

Acknowledgments

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Some Digenetic Trematodes from the Atlantic Hawksbill Turtle, *Eretmochelys imbricata imbricata* (L.), from Puerto Rico[1]

JACOB H. FISCHTHAL AND ALEXANDER D. ACHOLONU[2]

ABSTRACT: Ten families, 20 genera, and 28 species of digenetic trematodes are reported from 14 Atlantic hawksbill turtles, *Eretmochelys imbricata imbricata* (L.), from Cabo Rojo, Puerto Rico. Seven new species are described: Spirorchiidae, *Amphiorchis caborojoensis*; Pronocephalidae, *Epibathra stenobursata*, *Glyphicephalus latus*, *Pleurogonius laterouterus*, *P. puertoricensis*; Pachypsolidae, *Pachypsolus puertoricensis*; Calycodidae, *Calycodes caborojoensis*. Previously known species are: Spirorchiidae, *Learedius orientalis*, *Hapalotrema synorchis* (described), *Amphiorchis amphiorchis*; Angiodictyidae, *Microscaphidium reticulare*, *Octangium sagitta*, *O. travassosi*; Paramphistomidae, *Schizamphistomum scleroporum*; Gorgoderidae, *Plesiochorus cymbiformis*; Pronocephalidae, *Cricocephalus albus*, *C. megastomus*, *Pyelosomum posterorchis*, *Diaschistorchis pandus*, *Metacetabulum invaginatum*, *Glyphicephalus lobatus*, *Pleurogonius linearis*, *P. trigonocephalus*; Pachypsolidae, *Pachypsolus ovalis*; Plagiorchiidae, *Enodiotrema reductum*, *Styphlotrema solitarium*; Telorchiidae, *Orchidasma amphiorchis*; Rhytidodidae, *Rhytidodes gelatinosus*; all represent new geographical distribution records, while 12 are new host records.

The trematodes of this report were collected by one of us (ADA) between Fall 1969 and Spring 1971 while associated with the Inter American University of Puerto Rico. Fourteen Atlantic hawksbill turtles, *Eretmochelys imbricata imbricata* (L.), from the Caribbean Sea at Cabo Rojo (southwesternmost point of the island) were examined; all had trematodes. The worms were killed in hot AFA fixative without coverglass pressure, stained in Mayer's acid carmine, Mayer's carmalum or Harris' hematoxylin, and mounted in xylo-damar or permount. All represent new geographical distribution records. An asterisk (*) preceding the name of previously known species indicates a new host record. Specimens have been deposited in the U.S. National Museum Helminthological Collection as noted. All measurements are in microns.

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Amphiorchis caborojoensis sp. n. (Figs. 1, 2)

HOST: *Eretmochelys i. imbricata.*

HABITAT: Blood vessels of lungs.

LOCALITY: Cabo Rojo, Puerto Rico.

DATE: 11 October 1969.

SPECIMEN DEPOSITED: No. 73311 (holotype); No. 73312 (paratypes).

DESCRIPTION (based on 11 worms from one host; seven measured): Spirorchiidae. Body elongate, constricted at acetabular level, extremities rounded, entirely spined, 3,140–4,915 long by 510–915 wide at anterior testis level. Forebody 1,265–2,010 long; hindbody 1,760–2,720 long; forebody–hindbody length ratio 1:1.20–1.54. Eyespot pigment granules scattered on each side of esophagus at about its midlength. Oral sucker ventroterminal, round to slightly longitudinally elongate, 120–225 by 105–200. Acetabulum fungiform and large when relaxed, outline occasionally quadrate or irregular when retracted and size smaller, 115–345 by 140–355. Sucker length ratio 1:0.76–

1.72, width ratio 1:1.00–1.82. Esophagus very long, terminating in muscular bulb, 905–1,400 (including bulb) by 80–150, surrounded by gland cells, latter in thicker mass posteriorly than anteriorly. Cecal bifurcation 120–360 preacetabular. Ceca narrow, ascending sides of esophageal bulb before looping posteriorly, terminating near posterior extremity, just posttesticular or slightly more anteriorly. Excretory vesicle Y-shaped, posttesticular.

Testes two, tandem, 405–685 apart, usually S-shaped, smooth to slightly lobed, usually overlapping ceca laterally; anterior testis usually transversely elongate, 420–605 by 430–690, lying 400–795 postacetabular and 1,780–2,850 from anterior extremity, latter distances 56.7–62.5 per cent of body length; posterior testis usually longitudinally elongate, 420–640 by 325–590, close to posterior extremity; posttesticular space 80–170 long. External seminal vesicle large, diagonal, elongate, 215–290 by 160–195, intercecal, lying between anterior testis and ovary. Cirrus sac thick-walled, muscular, arcuate, 245–390 (longitudinal extent) by 50–82, commencing ventrally posterosinistral to external seminal vesicle, passing dorsally before extending posteromedianly, passing dorsal to ovary to genital pore, containing internal seminal vesicle (46–56 in longitudinal extent by 41–60), prostatic vesicle (32–51 by 12–32), few prostate cells, and long cirrus (256–320 by 8–12). Genital pore sinistromedian, opening ventrally at posterior part of ovary, gland cells radiating from pore region. Ovary lobed, transversely elongate, occasionally overlapping ceca, 165–300 by 285–435, lying 230–350 posterior to anterior testis and 15–95 anterior to posterior testis. Oviduct emerging from dextroposterior ovarian lobe. Seminal receptacle dextral, between ovary and posterior testis, 36–61 by 44–80. Mehlis' gland large, postovarian. Vitellaria follicular, extending from cecal bifurcation to posterior extremity; ceca more or less separating follicles on each side of body into extracecal and intercecal rows with few follicles overlapping ceca; extracecal rows uninterrupted; intercecal rows interrupted at levels of acetabulum, anterior testis, ovary, and posterior testis; fields confluent posttesticular in four of 11 worms; collecting duct between extra- and intercecal fields, ventral to ceca, one anterior and one posterior duct uniting on each side of body forming transverse duct; vitelline reservoir large, median, between ovary and posterior testis. Uterus very short, between ovary and posterior testis; eight worms with one egg each and three with none. Eggs all collapsed, large, with single filament at each pole, best egg 230 (including filaments) by 40, next best about 215 by 48.

DISCUSSION: Our new species is closest to *A. lateralis* Oguro, 1938, from *Eretmochelys squamosa* (Girard) from Palao Islands. The latter differs from ours in being aspinose, in lacking a muscular esophageal bulb and an internal seminal vesicle, and in having esophageal gland cells only at the distal end, the testes slightly lobed, a much longer posttesticular space, a bipartite seminal vesicle, the genital pore anterosinistral to the ovary, and the vitelline fields completely interrupted between the genital pore and the vitelline reservoir.

Epibathra stenobursata sp. n. (Figs. 3, 4)

HOST: *Eretmochelys i. imbricata.*

HABITAT: Large intestine.

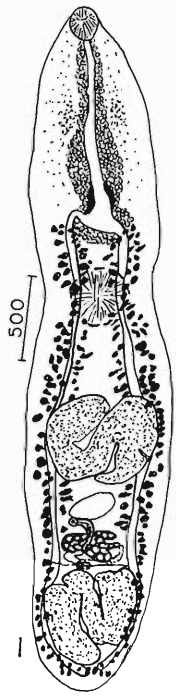
LOCALITY: Cabo Rojo, Puerto Rico.

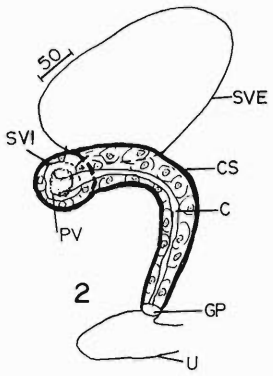
DATE: 11 October 1969.

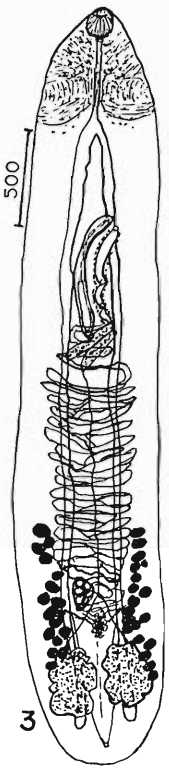
SPECIMEN DEPOSITED: No. 73313 (holotype).

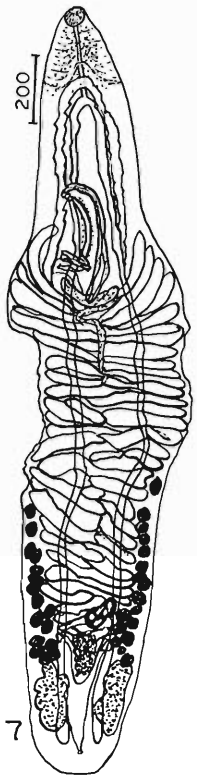
DESCRIPTION (based on one young adult): Pronocephalidae. Body elongate, aspinose, lateral margins nearly parallel and not folded ventrally, extremities rounded, 3,900 long by 755 wide at level of posterior end of cirrus sac. Head collar 560 by 595. Eyespot pigment granules scattered from oral sucker to cecal bifurcation. Oral sucker small, truncated posteriorly, diameter 130. Esophagus 490 long, distance 12.6 per cent of body length; ceca narrow, walls sinuous, passing dorsal to testes; postcecal space 144 long. Excretory vesicle Y-shaped, stem bifurcating just posterior to Mehlis' gland, arms extending to side of esophagus; pore dorsal, 120 from posterior extremity, surrounded by gland cells.

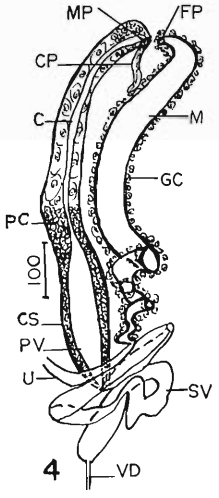
Testes two, lobed, symmetrical, separated by stem of excretory vesicle; right testis 350 by 280, left 350 by 265; posttesticular space 225 long. Vas efferens emerging from anterodorsal part of each testis, uniting medianly at ovarian level. Vas deferens sinuous, enlarging as tubular, coiled external seminal vesicle lying in

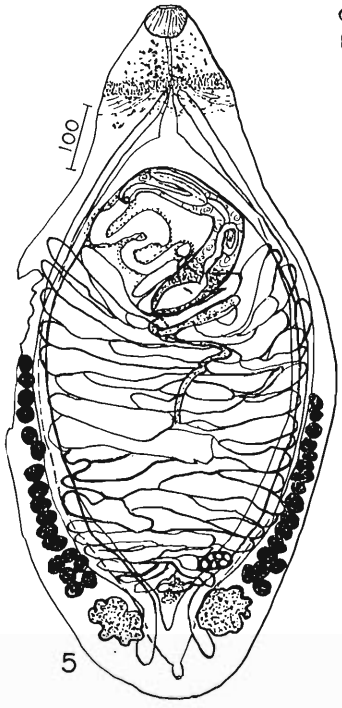


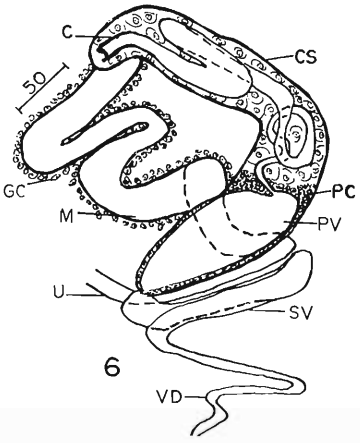


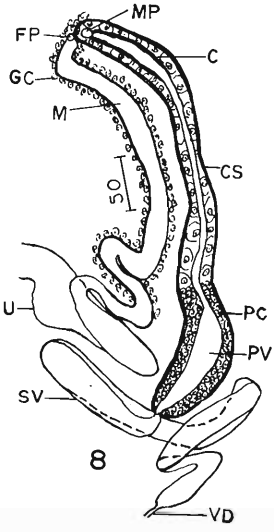












transverse plane just posterior to cirrus sac. Latter elongate, narrow, comma-shaped in dorsal view, 720 by 90, containing saccular prostatic vesicle (265 by 53), and long, muscular, protrusible cirrus. Male pore ventral to left cecum, lying 460 postbifurcal. Ovary somewhat triangular, dextral, 155 by 105, lying 180 anterior to right testis. Oviduct emerging from mesial side of ovary. Mehlis' gland large, posteromedian to ovary, just reaching testicular level, 245 by 115. Vitelline follicles large, 18 in each lateral field, mainly extracecal but some overlapping ceca, in posterior third of body, follicles overlapping right testis 105 and left 200. Vitelline reservoir small. Uterine coils transverse between testes and posterior part of cirrus sac. Metraterm muscular, sinuous, slightly shorter in longitudinal extent than and lying sinistral to cirrus sac. Female pore just extracecal, sinistral to male pore. Eggs few, with several small filaments on opercular end and one stout filament on other end, 10 measuring 32–39 (35.7) (excluding filaments) by 18–20 (18.9).

Discussion: The genus contains only the type species *E. crassa* (Looss, 1901) Looss, 1902. The latter species differs from ours in having a relatively much larger oral sucker which is rounded posteriorly, a shorter esophagus, a relatively shorter and much broader cirrus sac, the vitellaria entirely pretesticular, and longer eggs (42–52 long). Although Looss' (1902) worms are 4–6 mm long the oral sucker is more than twice as large as in our worm. The esophagus of *E. crassa* in Looss' figure 83 is about 6.5 per cent of the body length and in Ruiz' (1946) specimens about 3.4–4.6 per cent compared to 12.6 per cent in our worm. Although Ruiz' worms are 8,370–8,930 long the esophagus is only 300–430 long, whereas in our worm which is less than half that length it is 490 long. Looss noted that the cirrus sac is a short, club-shaped structure with a thin neck and a strongly swollen posterior part; Ruiz illustrated an even greater contrast between the neck and posterior part. The name *stenobursata* refers to the presence of a narrow cirrus sac in our new species.

Glyphicephalus latus sp. n. (Figs. 5, 6)

Host: *Eretmochelys i. imbricata.*

Habitat: Small intestine, occasionally stomach and large intestine.

Locality: Cabo Rojo, Puerto Rico.

Dates: 7 October 1969; 11 February, 9 May 1970; 26 February 1971.

Specimens deposited: No. 73314 (holotype); No. 73315 (paratypes).

Description (based on 38 adult worms; eight measured): Pronocephalidae. Body elongate, bottle-shaped, anterior part triangular, 985–1,710 long by 450–780 wide. Head collar 182–290 by 206–315, ventral lappets with shallow posterior incision, connected dorsally by prominent ridge. Eyespot pigment granules scattered in collar region. Oral sucker ventroterminal, transversely elongate, 42–65 by 49–85. Esophagus 153–210 long, narrow along most of length, dilated just before bifurcation; ceca without diverticula, extending to posterior extremity. Excretory vesicle Y-shaped, stem very short, arms long and extending to sides of esophagus, not uniting; pore dorsal, 38–90 from posterior extremity.

Testes two, symmetrical, lobed, mostly extracecal, overlapping ceca dorsally; right testis 80–180 by 82–185, left 80–175 by 82–170; posttesticular space 42–125 long. Vasa efferentia uniting in midline at ovarian level. Vas

←

Abbreviations in figures. C, cirrus; CP, protruded cirrus; CS, cirrus sac; E, egg; FP, female genital pore; GA, genital atrium; GC, gland cells; GP, genital pore; M, metraterm; MP, male genital pore; PC, prostate cells; PV, prostatic vesicle; SV, seminal vesicle; SVE, external seminal vesicle; SVI, internal seminal vesicle; U, uterus; VD, vas deferens.

Figures 1–8. ***Amphiorchis caborojoensis*** **sp. n. 1. Whole mount, holotype, dorsal view. 2. Terminal genitalia, holotype.** ***Epibathra stenobursata*** **sp. n. 3. Whole mount, holotype, ventral view. 4. Terminal genitalia, holotype.** ***Glyphicephalus latus*** **sp. n. 5. Whole mount, holotype, dorsal view. 6. Terminal genitalia, holotype.** ***Pleurogonius laterouterus*** **sp. n. 7. Whole mount, holotype, dorsal view. 8. Terminal genitalia, holotype.**

deferens long, sinuous, enlarging just posterior to cirrus sac forming tubular, transversely coiled external seminal vesicle. Cirrus sac 305–462 by 62–90, C-shaped in ventral view, thick-walled, muscular; proximal end median, curving to right cecum, following curvature of latter and cecal bifurcation to left cecum. Prostatic vesicle saccular, occupying proximal two-fifths to one-half of cirrus sac. Cirrus long, winding, protrusible. Male genital pore just postbifurcal, next to or overlapping left cecum, lying 260–425 from anterior extremity. Ovary small, margins smooth to slightly irregular, dextral, transversely to obliquely elongate, 35–60 by 52–82. Mehlis' gland postovarian, median. Vitelline follicles in single row in lateral extracecal fields except posteriorly where two rows for one or two levels of follicles occurring, pretesticular, anteriormost extent of fields 495–785 from anterior extremity, distances 44–52 per cent of body length. Uterus with transverse coils between Mehlis' gland and approximate midlength of cirrus sac, coils overlapping ceca, anterolateralmost coils looping anterolaterally. Metraterm thick-walled, muscular, very undulating in ascent to female genital pore, commencing anterior to posteriormost part of cirrus sac. Female pore just posterior to male pore. Eggs numerous, light yellow, operculate, with single long filament at each end, 24 measuring 38–45 (41.0) (excluding filaments) by 15–21 (17.6).

DISCUSSION: Our collection contains one, one, 12, and 24 adult worms from four hosts. The genus contains two species: *G. solidus* Looss, 1901; *G. lobatus* Looss, 1901. They differ from our new species in being considerably longer, and in having the sides of the body parallel or nearly so, the cirrus sac and genital pores lying more posteriorly from the cecal bifurcation, and shorter eggs. The species designation *latus* refers to the broad body so characteristic of all our specimens.

Pleurogonius laterouterus sp. n. (Figs. 7, 8)

HOST: *Eretmochelys i. imbricata.*

HABITAT: Large intestine.

LOCALITY: Cabo Rojo, Puerto Rico.

DATE: 9 May 1970.

SPECIMENS DEPOSITED: No. 73316 (holotype); No. 73317 (paratypes).

DESCRIPTION (based on eight worms from one host; four measured): Pronocephalidae. Body elongate, preuterine part slender and with smooth sides, then widening considerably with lateral margins sinuous, starting at anteriormost part of vitellaria tapering posteriorly with smooth sides to slender posterior extremity, 1,825–2,420 long by 410–640 wide. Head collar with slight ventral incision, dorsal ridge absent, 212–285 by 194–245. Eyespot pigment granules scattered in collar region. Oral sucker ventroterminal, longitudinally elongate, 56–68 by 47–59. Esophagus 175–215 long; ceca without diverticula, sinuous, extending posttesticular to posterior extremity. Excretory vesicle V-shaped, arms extending to esophageal level, uniting dorsally; pore dorsal, 29–60 from posterior extremity.

Testes two, symmetrical, with large lobes, longitudinally elongate; right testis 162–219 by 80–133, left 157–213 by 80–152; posttesticular space 75–95 long. External seminal vesicle winding intercecally, occasionally with extracecal loop. Cirrus sac 336–414 by 36–53, crescent-shaped, sinuous, thick-walled, muscular, commencing medianly or sinistrally, containing saccular prostatic vesicle (110–168 by 25–45), prostate cells, and long, winding cirrus. Male genital pore just mesial or ventral to left cecum. Ovary dextral, lightly lobed, 70–97 by 68–110. Mehlis' gland median, postovarian, compact, 94–104 by 41–65. Vitelline follicles mostly in single row in lateral extracecal fields, two rows for short distance from posteriormost level, overlapping anterior part of testes dorsally, anteriormost extent 1,200–1,625 from anterior extremity, latter distances 63–78 per cent of body length. Uterus in transverse coils from Mehlis' gland to approximate midlength of cirrus sac, coils extending laterally to vitelline fields and to body margins anterior to latter, anterolateral coils looping anterolaterally. Female genital pore lateral to male pore, ventral to left cecum. Eggs numerous, light yellow, operculate, with single long filament on each end, 15 measuring 36–41 (38.4) (excluding filaments) by 18–20 (18.8).

DISCUSSION: The new species designation *laterouterus* refers to the extension of the uterine coils to the lateral body margins, and it differs from all known species in the genus in this characteristic. It is closest to *P. linearis* Looss, 1901, of Ruiz (1946) and Caballero and

Zerecero (1950). In the latter the uterus extends extracecally but does not reach the body margins. *P. linearis* differs further in body shape (a relatively broader anterior part, and nearly parallel sides extending to the posterior extremity without tapering posteriorly), and in having smooth lateral body margins, and shorter eggs.

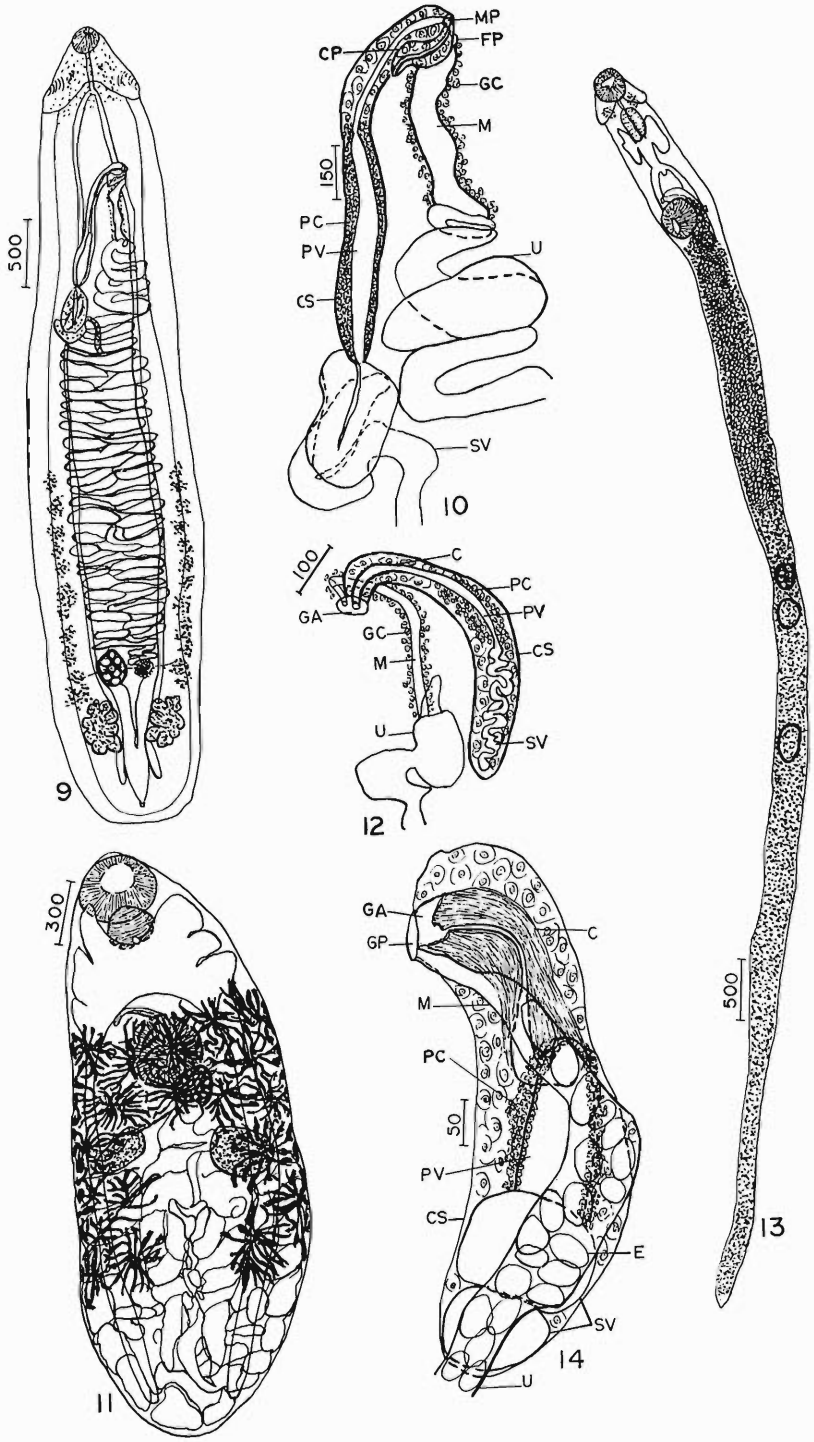
Pleurogonius puertoricensis sp. n. (Figs. 9, 10)

HOST: *Eretmochelys i. imbricata.*
HABITAT: Large intestine.
LOCALITY: Cabo Rojo, Puerto Rico.
DATES: 7, 11 October 1969.
SPECIMENS DEPOSITED: No. 73318 (holotype); No. 73319 (paratypes).

DESCRIPTION (based on one young adult with 45 eggs and one mature adult from one host, and one and two mature adults from two hosts; latter three worms measured): Pronocephalidae. Body elongate, narrow, aspinose, posterior extremity notched in two worms, rounded in two, lateral and posterior margins usually turning ventrally, 4,810–5,845 long by 1,030–1,210 wide. Head collar 525–590 by 700–710, ventral lappets well-developed or only slightly so, midventral incision reaching to oral sucker in one worm, more posteriorly in three, dorsal ridge absent. Eyespot pigment granules scattered mainly at level of collar. Oral sucker ventroterminal, large, 225–265 by 220–240. Esophagus 260–275 long, narrow, between lappets, surrounded by gland cells. Ceca narrow, extending posttesticular; postcecal space 205–475 long, distances 4–7 per cent of body length. Excretory vesicle V-shaped, arms uniting dorsal to esophagus; pore dorsal, 145–350 from posterior extremity, surrounded by gland cells.

Testes two, symmetrical, lobed, lateral to but overlapping ceca; right testis 380–485 by 250–330, left 375–435 by 290–325; posttesticular space 400–865 long, distances 8.3–12.7 per cent of body length. External seminal vesicle dextral, longitudinally coiled, proximal part only in uterine field. Cirrus sac 1,050–1,250 by 125–185, thick-walled, muscular, comma-shaped in dorsal view, dextral, overlapping right cecum, containing long prostatic vesicle (485–675 by 65–78) surrounded by dense mass of prostate cells, ejaculatory duct, and cirrus; latter protruded in all worms. Male genital pore usually intercecal, next to left cecum, occasionally overlapping latter, lying 890–1,125 from anterior extremity and 335–530 postbifurcal. Ovary 210–285 by 180–260, margins slightly wavy, slightly overlapping right cecum. Oviduct emerging from mesial margin of ovary. Mehlis' gland compact, sinistral, at same level as ovary, 150–160 by 165–185. Laurer's canal between ovary and Mehlis' gland, winding, opening dorsally. Vitelline follicles extracecal, in rosettes (right–left: 10–14, 13–15, 13–16), commencing 2,410–3,375 from anterior extremity, distances 49.6–56.0 per cent of body length. Vitelline reservoir small. Uterus in transverse coils overlapping ceca between ovarian level or just postovarian to level sinistral to posterior part of cirrus sac, overlapping level of latter 250–450. Metraterm 560–910 by 60–110, sinistral to and shorter than cirrus sac, walls very muscular and up to 35–50 thick, surrounded by gland cells. Female genital pore sinistral to male pore, ventral to left cecum. Eggs numerous, operculate, single thick filament at each end, 15 measuring 28–34 (30.3) (excluding filament) by 14–20 (17.1).

DISCUSSION: Our new species is closest to *P. longiusculus* Looss, 1901, *P. karachii* Mehra, 1939, and *P. grocotti* Caballero, 1954. They all differ in having a significantly smaller oral sucker. *P. karachii* is similar to our species in having the external seminal vesicle longitudinally coiled and dextral to the uterus, the Mehlis' gland at the same level as the ovary, and a coiled uterus sinistral to the posterior part of the cirrus sac; it differs further from our species, however, in having a very long esophagus extending posteriorly beyond the collar lappets, a short posttesticular space, a diagonally oriented, short, straight cirrus sac, a short metraterm, and smaller eggs lacking filaments. *P. grocotti* differs further in having a very sinuous cirrus sac, a nearly median ovary, a postovarian Mehlis' gland, and a metraterm as long as the cirrus sac, and a coiled uterus absent opposite the posterior part of the cirrus sac. *P. longiusculus* differ further in having a longer esophagus, the external seminal vesicle transversely coiled and filling the intercecal space at its level, and a median, postovarian Mehlis' gland, and in lacking a coiled



uterus opposite the posterior part of the cirrus sac.

Pachypsolus puertoricensis sp. n. (Figs. 11, 12)

HOST: *Eretmochelys i. imbricata.*
HABITAT: Stomach.
LOCALITY: Cabo Rojo, Puerto Rico.
DATE: 11 February 1970.
SPECIMEN DEPOSITED: No. 73320 (holotype).

DESCRIPTION (based on holotype only): Pachypsolidae. Body elongate, spined, 2,640 long by 1,075 wide just posttesticular. Forebody 830 long; hindbody 1,500 long; forebody–hindbody length ratio 1:1.8. Oral sucker ventroterminal, 315 by 360; acetabulum 310 by 375; sucker length ratio 1:0.98, width ratio 1:1.04. Prepharynx absent; pharynx overlapping oral sucker dorsally, 205 by 208; esophagus very short; bifurcation 135 preacetabular; ceca wide, extending to posterior extremity, anteriorly each cecum with three large diverticula extending laterally. Excretory vesicle wide, extending anteriorly to ovarian level, arms uniting dorsal to oral sucker, pore dorsoterminal.

Testes two, symmetrical, lightly lobed, transversely elongate, overlapping ceca ventrally; right testis 245 by 315, left 210 by 305; posttesticular space 1,110 long. Vas efferens emerging dorsally from anteromedian part of each testis, uniting and forming vas deferens. Cirrus sac 555 by 105, crescent-shaped, commencing 20 anterior to posterior margin of acetabulum, ascending dorsal to and terminating anterosinistral to latter. Seminal vesicle coiled in posterior part of cirrus sac. Prostatic vesicle tubular, surrounded by prostate cells. Cirrus short. Genital pore sinistral, next to left cecum. Ovary smooth, 200 by 245, anterior to and well separated from testes, dextromedian, overlapping acetabulum posterodorsally. Seminal receptacle posterodorsal to ovary, diameter 125. Laurer's canal dorsal to ovary. Vitellaria consisting of 17 rosettes of tubular follicles extending from just postbifurcal to near middle of hindbody (710 from posterior extremity), rosettes lateral and dorsal to ceca, confluent dorsally in two tiers of rosettes from anteriormost part to ovarian level, invading intercecal space posterior to latter. Uterus filling hindbody, ascending between testes, dorsal to acetabulum, and sinistral to ovary and cirrus sac. Metraterm thick-walled, muscular, commencing dorsal to acetabulum, shorter than cirrus sac. Eggs numerous, operculate, younger eggs yellow, becoming yellow-brown to brown farther along uterus, 10 measuring 52–59 (55.7) by 17–20 (18.6).

DISCUSSION: *P. puertoricensis* sp. n. differs significantly from all others in the genus in distribution of the vitelline rosettes. *P. ovalis* Linton, 1910, is closest to our species, but differs further in having considerably larger suckers. Linton (1910) notes that in a worm 2,760 long, which is close to ours in length, the oral sucker and acetabulum are 500 in diameter. In their description of *P. ovalis* Caballero et al. (1955) state that the oral sucker is larger than the acetabulum, but their measurements for length of the former as well as the ratio between the two suckers contradict this; from their figure 3 it is apparent that their statement is correct, but that the measurements and ratios are erroneously recorded. *P. ovalis* differs further in being widest at the acetabular or preacetabular level, and in having longitudinally elongated testes.

Calycodes caborojoensis sp. n. (Figs. 13, 14)

HOST: *Eretmochelys i. imbricata.*
HABITAT: Small intestine.
LOCALITY: Cabo Rojo, Puerto Rico.
DATE: 9 May 1970.
SPECIMEN DEPOSITED: No. 73321 (holotype).

DESCRIPTION (based on holotype only): Calycodidae. Body long, slender, widest from

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Figures 9–14. ***Pleurogonius puertoricensis*** **sp. n. 9. Whole mount, holotype, ventral view. 10. Terminal genitalia, holotype.** ***Pachypsolus puertoricensis*** **sp. n. 11. Whole mount, holotype, dorsal view. 12. Terminal genitalia, holotype.** ***Calycodes cabarojoensis*** **sp. n. 13. Whole mount, holotype, ventral view. 14. Terminal genitalia, holotype.**

anterior extremity to just postacetabular, 10,090 long by 460 wide just preacetabular and 210 wide at ovarian level, spined anteriorly. Anterior extremity with dorsal and ventral petaloid ridges continuous with each other laterally, 465 wide. Forebody 1,235 long; hindbody 8,510 long; forebody–hindbody length ratio 1:6.9. Eyespot pigment granules on each side at level of anterior part of pharynx. Oral sucker ventroterminal, diameter 255; acetabulum 305 by 275; sucker length ratio 1:1.20, width ratio 1:1.08. Prepharynx 87 long; pharynx 230 by 155; esophagus 180 by 105, anteriorly with pair of lateral diverticula, latter 255–290 long; ceca terminating at posterior extremity.

Testes two, tandem, smooth, 770 apart; anterior testis 210 by 155, posterior 260 by 175; posttesticular space 4,350 long (51 per cent of hindbody length). Cirrus sac 540 by 195, commencing 120 postacetabular, passing dorsal to acetabulum, terminating 115 preacetabular, containing bipartite seminal vesicle (posterior chamber 100 by 145, anterior 130 by 160), prostatic vesicle (195 by 78), prostate cells, and muscular cirrus. Genital pore median, 60 preacetabular. Ovary 210 by 130, smooth, 43 pretesticular and 2,660 postacetabular. Vitellaria commencing at posterior margin of acetabulum, follicles in lateral fields from latter to 325 preovarian and at levels of gonads, filling remainder of hindbody. Uterus preovarian; coils overlapping ceca, some extracecal. Metraterm thick-walled, muscular, sinistral to and shorter than cirrus sac. Eggs numerous, yellow-brown, operculate, 10 measuring 56–66 (62.2) by 41–45 (43.0).

Discussion: The genus contains only the type species, *C. anthos* (Braun, 1899) Looss, 1901. The latter differs from ours in having a forebody–hindbody length ratio of only about 1:3.0 from Braun's (1901) figure and 1:3.7 from Looss' (1902), much larger suckers (diameters in Looss' worm 10,750 long is 550 for the oral sucker and 760 for the acetabulum; diameter for both suckers in Braun's worm 12 mm long is 830), and a much wider body (1,400 in Braun's worm; 1,250 in Looss'), and the vitellaria commencing at the cecal bifurcation level and being in lateral fields from the latter to just posttesticular.

Previously Known Species

Hosts and localities of the species listed below are given by Yamaguti (1971). Subsequent data are noted herein where appropriate.

1. **Learedius orientalis* Mehra, 1939 (Spirorchiidae): one, two, and three worms were obtained from the heart of three hosts; collected 12 June 1970, 20 February 1971. Specimens deposited: No. 73322. We are identifying our worms as *L. orientalis* although the latter may be a synonym of *L. learedi* Price, 1934; both species have been found in *Chelone mydas* (L.). *L. learedi* was originally described by Price (1934) from a single specimen; a more detailed account based on 45 worms was given by Caballero, Zerecero and Grocott (1955). In light of the latter description most of the characteristics used by Mehra (1939) to differentiate *L. orientalis* (based on 24 worms) from *L. learedi* are invalidated as there is considerable overlapping. We have examined the holotype specimen of *L. learedi* (USNM Helm. Coll. No. 32567) and observed that the vitelline fields are united posteriorly and the egg is shaped as in *L. orientalis*. The only difference between the two species appears to be the relationship of the testes to one another. As illustrated by Price, and verified by us in the holotype worm, and by Caballero et al. the testes in *L. learedi* are more or less separated from one another and their margins are rounded, whereas in *L. orientalis* the testes have their margins flattened against one another without spaces between them. Our worms exhibit the latter arrangement.

2. **Hapalotrema synorchis* Luhman, 1935 (Spirorchiidae): one, one, three, and seven worms were recovered from the heart of four hosts; collected 11 October 1969, 9 May and 12 June 1970, 20 February 1971. Specimens deposited: No. 73323. Body spined, 2,755–3,585 long by 600–955 wide at anterior testicular mass level. Forebody 745–935 long; hindbody 1,670–2,345 long; forebody–hindbody length ratio 1:1.95–2.51. Eyespot pigment granules few, scattered around esophagus. Oral sucker 200–235 by 175–230; acetabulum fungiform, 285–340 by 275–355; sucker length ratio 1:1.30–1.46, width ratio 1:1.45–1.54. Esophagus sinuous, 800–1,150 by 70–115,

muscular bulb absent, surrounded by moderately thick layer of gland cells for most of length, followed by short aglandular region, prebifurcal part with very thick, more compact gland cell layer; cecal bifurcation anterodorsal to acetabulum or just preacetabular. Testes in two compact masses; anterior mass 640–910 by 235–300, with 20–34 testes, lying 195–400 postacetabular; posterior mass 395–600 by 200–275, with 12–29 testes; posttesticular space 115–235 long; individual testes varying considerably in shape and size, sides flattened against one another, sizes ranging from 39 by 65 and 44 by 55 to 133 by 203 and 157 by 110. External seminal vesicle 143–230 by 65–92. Cirrus sac sinistral, thick-walled, muscular, passing posteriorly dorsal to part of ovary, 72–145 by 36–56, containing ejaculatory duct and cirrus only. Genital atrium large. Genital pore sinistral, posteroventral to ovary, with well-developed circular muscle band around opening, radial muscles extending from circular muscle layer in all directions but lacking outer limiting membrane. Ovary multilobed, 200–300 by 220–375. Seminal receptacle dextral, 92–102 by 90–116. Laurer's canal opening medianly dorsal to anteriormost testes of posterior mass, opening surrounded by well-developed sucker (34–57 by 39–65). Vitellaria commencing at posterior margin of acetabulum or more posteriorly. Three worms with one egg each; one slightly collapsed egg about 225 (including filaments) by 28, one filament 93 long, other 58; another egg 285 (including filaments) by 35, one filament 127 long, other 80. Discussion: Our longest worm (with one egg) has most of the preacetabular body missing; the hindbody measures 3,065 by 990 and the acetabulum 330 by 340. We have examined the holotype specimen of *H. synorchis* (USNM Helm. Coll. No. 8909) and find that the genital "sucker" does not have an outer limiting membrane, and a definite sucker surrounds the opening of Laurer's canal, the esophagus has a short area lacking gland cells, and eyespot pigment granules are scattered around the esophagus. The only difference between our specimens and the holotype is that the vitellaria starts at the anterior margin of the acetabulum in the latter.

3. **Amphiorchis amphiorchis* Price, 1934 (Spirorchiidae): two worms, without eggs, were obtained from the blood vessels of the large intestine of one host; collected 9 May 1970. SPECIMENS DEPOSITED: No. 73337. The vitellaria in our worms is variable in distribution and different from Price's (1934) description of this species. In one worm the follicles are interrupted laterally only from the ovarian level to the seminal receptacle level on one side and just posterior to the vitelline reservoir on the other; the follicles do not invade the space between the acetabulum and anterior testis. In the second worm the follicles are interrupted laterally from the ovarian level to the posterior part of the vitelline reservoir on one side but are uninterrupted on the other side; they are confluent between the acetabulum and anterior testis. In both worms the lateral vitelline fields are continuous opposite each testis. We have examined some of Price's paratypes (USNM Helm. Coll. No. 32566) and find that our worms compare favorably with them. Apparently, the distribution of the vitellaria in this species is quite variable.

4. *Microscaphidium reticulare* (van Beneden, 1859) Looss, 1901 (Angiodictyidae): one worm was obtained from the large intestine; collected 16 May 1970. SPECIMEN DEPOSITED: No. 73324. A rosette of six diverticula surrounds the excretory pore.

5. **Octangium sagitta* (Looss, 1899) Looss, 1902 (Angiodictyidae): our collection contains 16, 19, 35, and 86 worms from the stomach and small and large intestines of four hosts; collected 11 October 1969; 26 February and April 1971. SPECIMENS DEPOSITED: No. 73325. (*Chelone mydas*, India.)

6. **Octangium travassosi* (Ruiz, 1943) Yamaguti, 1958: our collection consists of 19, 21, 28, 128, and 189 worms from the stomach and small and large intestines of five hosts; collected 9, 16 May 1970; 26 February and March 1971. SPECIMENS DEPOSITED: No. 73326. (*Chelone mydas*, Trinidad.)

7. **Schizamphistomum scleroporum* (Creplin, 1844) Looss, 1912 (Paramphistomidae): three worms were obtained from the stomach and one from the small intestine of one host; collected 11 October 1969. SPECIMENS DEPOSITED: No. 73338.

8. *Plesiochorus cymbiformis* (Rudolphi, 1819) Looss, 1901 (Gorgoderidae): two adult worms were recovered from the small intestine

of one host; collected 11 February 1970. SPECIMEN DEPOSITED: No. 73327. (*Eretmochelys imbricata*, *Chelone mydas*, India.)

9. *Cricocephalus albus* (Kuhl and van Hasselt, 1822) Looss, 1899 (Pronocephalidae): one adult worm was obtained from the stomach of one host and one from the small intestine of another; collected 9, 13 May 1970. SPECIMEN DEPOSITED: No. 73328. (*Chelone mydas*, India; *C. japonica*, Taiwan.)

10. **Cricocephalus megastomus* Looss, 1902: one adult worm was obtained from the stomach of one host and one from the small intestine of another; collected 23 April 1970; 26 February 1971. SPECIMENS DEPOSITED: No. 73329. (*Chelone mydas*, India; *C. japonica*, Taiwan.)

11. *Pleurogonius linearis* Looss, 1901 (Pronocephalidae): our collection contains 22 worms from one host; collected 9 May 1970. SPECIMENS DEPOSITED: No. 73339.

12. *Pleurogonius trigonocephalus* (Rudolphi, 1809) Looss, 1901: one worm was recovered from the large intestine; collected 9 May 1970. SPECIMEN DEPOSITED: No. 73340.

13. **Glyphicephalus lobatus* Looss, 1901 (Pronocephalidae): two and 14 adult worms were taken from the small intestine of two hosts; collected 11 February, 23 April 1970. SPECIMENS DEPOSITED: No. 73330.

14. **Pyelosomum posterorchis* Oguro, 1936 (Pronocephalidae): three worms were obtained from the small intestine of one host; collected 9 May 1970. SPECIMENS DEPOSITED: No. 73331.

15. *Diaschistorchis pandus* (Braun, 1901) Johnston, 1913 (Pronocephalidae): seven turtles harbored one, two (in three), three, six, and 11 worms in the stomach (mostly) and small intestine; collected 7, 11 October 1969; 10, 11 February, 9, 13 May 1970; 26 June 1971. SPECIMENS DEPOSITED: No. 73332. (*Eretmochelys imbricata*, India.)

16. **Metacetabulum invaginatum* Freitas and Lent, 1938 (Pronocephalidae): two and five worms were recovered from the stomach and small intestine of two hosts; collected 11 February 1970; 26 February 1971. SPECIMENS DEPOSITED: No. 73333. The eggs in our worms have an operculum on one end and a small tubercle on the other.

17. **Pachypsolus ovalis* Linton, 1910 (Pachypsolidae): four worms from the stomach of one host are in our collection; collected 13 May 1970. SPECIMENS DEPOSITED: No. 73341. Our worms readily fit the description of this species given by Caballero, Zerecero and Grocott (1955).

18. **Enodiotrema reductum* Looss, 1901 (Plagiorchiidae): one young adult from one host and one and three mature adult worms from two hosts were from the small intestine; collected 7 October 1969; 9 May 1970. SPECIMENS DEPOSITED: No. 73334.

19. *Styphlotrema solitarium* (Looss, 1899) Odhner, 1910 (Plagiorchiidae): one and 11 adult worms were obtained from the small intestine of two hosts; collected 11 October 1969; 11 February 1970. SPECIMENS DEPOSITED: No. 73335.

20. *Orchidasma amphiorchis* (Braun, 1899) Braun, 1901 (Telorchiidae): our collection consists of three adult worms from the stomach of one host; collected 13 May 1970. SPECIMENS DEPOSITED: No. 73460. The distribution of the vitellaria is different from those previously reported in that it extends to the posterior end of the body in one worm or 46 and 59 per cent of the posttesticular space in the other two. In prior descriptions of this species the posteriormost extent of the vitellaria is from a level significantly anterior to the posterior testis to the anterior margin of the latter. Apparently, the posterior extent of the vitellaria is variable in this species. (*Thalassochelys caretta*, Argentina.)

21. *Rhytidodes gelatinosus* (Rudolphi, 1819) Looss, 1901 (Rhytidodidae): one adult worm was obtained from the small intestine; collected 11 October 1969. SPECIMEN DEPOSITED: No. 73336. (*Chelone mydas*, Pakistan; *Caretta caretta*, French Mediterranean; *Eretmochelys squamosa*, India.)

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Nematotaenoides ranae gen. et sp. n. (Cyclophyllidea: Nematotaeniidae), from the Leopard Frog (*Rana pipiens*) in Iowa

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ABSTRACT: *Nematotaenoides ranae* gen. et sp. n. is described from the intestinal tract of the leopard frog, *Rana pipiens* Schreber, collected in Dickinson County in northwestern Iowa. The family Nematotaeniidae is emended to accommodate the new genus. Differing from other members of the family, the species possesses only one parauterine organ and a variable number (3–10) of testes. *N. ranae* is figured and its relationship within the family discussed. A modified key to the five genera of nematotaeniid cestodes is included.

Nematotaeniid cestodes comprise a relatively small but distinct group of cyclophyllidean cestodes parasitizing amphibians and reptiles. The taxonomy of nematotaeniids was reviewed most recently by Douglas (1958) and by Wardle, McLeod and Radinovsky (1974). These tapeworms have been assigned by various authors to such cyclophyllidean families as Taeniidae, Hymenolepididae, and Dilepididae. However, as indicated by Douglas (1958), they rightfully should be retained as an independent family within the order Cyclophyllidea. Wardle, McLeod and Radinovsky (1974) raised nematotaeniids to ordinal status, but presented no compelling evidence for such a change. Furthermore, these authors made no mention of Douglas' (1958) revision, nor of his subsequent publications dealing with these unusual cestodes. The Nematotaeniidae, established by Lühe (1910) include tapeworms characterized by (1) the presence of parauterine organs developing anterior to the ovary, (2) a cylindrical body form, (3) a small number of testes, and (4) definitive hosts limited to amphibians and reptiles. Four genera and 12 species were listed by Douglas (1958) as comprising the family. Previous keys to genera have been presented by Lawler (1939), Wardle and McLeod (1952), Yamaguti (1959) and by Wardle, McLeod and Radinovsky (1974). Additional species descriptions which have appeared in recent years now bring the total number to more than twenty, distributed among the following genera: *Baerietta* Hsü, 1935; *Cylindrotaenia* Jewell, 1916; *Distoichometra* Dickey, 1921; *Nematotaenia* Lühe, 1899; and a new genus and species, *Nematotaenoides ranae*, described here.

In a survey (1953–1974) of cestodes of 706 amphibians, collected from various regions near Iowa Lakeside Laboratory in northwest Iowa, an adult leopard frog (*Rana pipiens* Schreber) was infected with 20 adult tapeworms of a new genus of nematotaeniid. The host was collected July 8, 1971 at Kettleson Hogsback near Marble Lake, Dickinson County, Iowa. Although additional *R. pipiens* were collected from the same area on subsequent occasions, no additional specimens infected with this species have been recovered to date. Examination of the adult cestodes indicates them to be markedly different from related nematotaeniids and the name *Nematotaenoides ranae* gen. et sp. n. is hereby proposed for them.

The diagnosis of *N. ranae* is based upon the study of twelve whole mounts and two sets of transverse serial sections through entire worms, as well as on additional sagittal serial sections of individual gravid proglottids. Specimens for whole mounts were fixed in A.F.A., stained in either paracarmine or Harris' haematoxylin. Paraffin embedded sections of strobilae cut at 10μ were stained in Delafield's haematoxylin and counterstained in eosin Y. All measurements are indicated in microns, unless otherwise specified.

Nematotaeniidae Lühe, 1910

EMENDATION: In order to accommodate the new genus and species herein described, it is necessary to emend the family diagnosis from that presented by Yamaguti (1959) and Wardle and McLeod (1952), as follows (major changes italicized).

Cyclophyllidea, small worms, apolytic. Scolex nonrostellate, unarmed, lacking apical organ, with four weakly muscularized suckers. Strobila cylindrical *to oval* in cross section; segmentation *absent or weak anteriorly*, becoming distinct in posterior of strobila; longitudinal parenchymal muscles weakly developed to form a circular boundary between medulla and cortex. Excretory stems within medulla, rarely between cortex and medulla. Testes medullary, *ranging in number from 1 to 10.* Cirrus pouch present. Genital pores lateral, alternating irregularly. Ovary and vitellaria compact, medullary. Gravid *uterus with one or more parauterine organs* containing egg capsules, *each with one to many eggs.* Vagina usually opening posterior to male pore. Adults in amphibians and reptiles. Type genus: *Nematotaenia* Lühe, 1899. Other previously described genera: *Baerietta* Hsü, 1935; *Cylindrotaenia* Jewell, 1919; and *Distoichometra* Dickey, 1921. New genus.

Nematotaenoides gen. n.

DIAGNOSIS: With characteristics of the family Nematotaeniidae (as emended). Testes 3 to 10 (usually 8) dorsal, medullary. Ovary median, posteroventral to testes. Parauterine organ single, containing approximately 20–40 eggs. Parasitic in intestines of frogs. Type and only species.

Nematotaenoides ranae sp. n. (Figs. 1–7)

DESCRIPTION: With characteristics of the genus. Total body length of gravid, apolytic worms, 3–4 cm. Scolex unarmed, 353 (320 to 380) in diameter, not clearly differentiated from strobila; 4 circular, weakly muscularized suckers, diameter 155 (138 to 175). Neck short, cylindrical, diameter 300 (220 to 430); external segmentation indistinct anteriorly but appearing approximately 8 mm from anterior end; mature segments appearing approximately 3 mm posterior to scolex; segmentation pronounced posteriorly. Body diameter varies from narrow neck region 300 (220 to 430) to 682 (640 to 720) in mature segments, then narrows perceptibly 290 (270 to 300) in region of gravid proglottids. Terminal proglottids longer than broad, 520 × 270; mature proglottids craspedote, broader than long; early gravid proglottids appearing approximately 10 mm posterior to scolex. Parenchymal muscles weakly developed.

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Plate I

***Nematotaenoides ranae*, gen. et sp. n.**

(All figures drawn to scale shown in Fig. 1.)

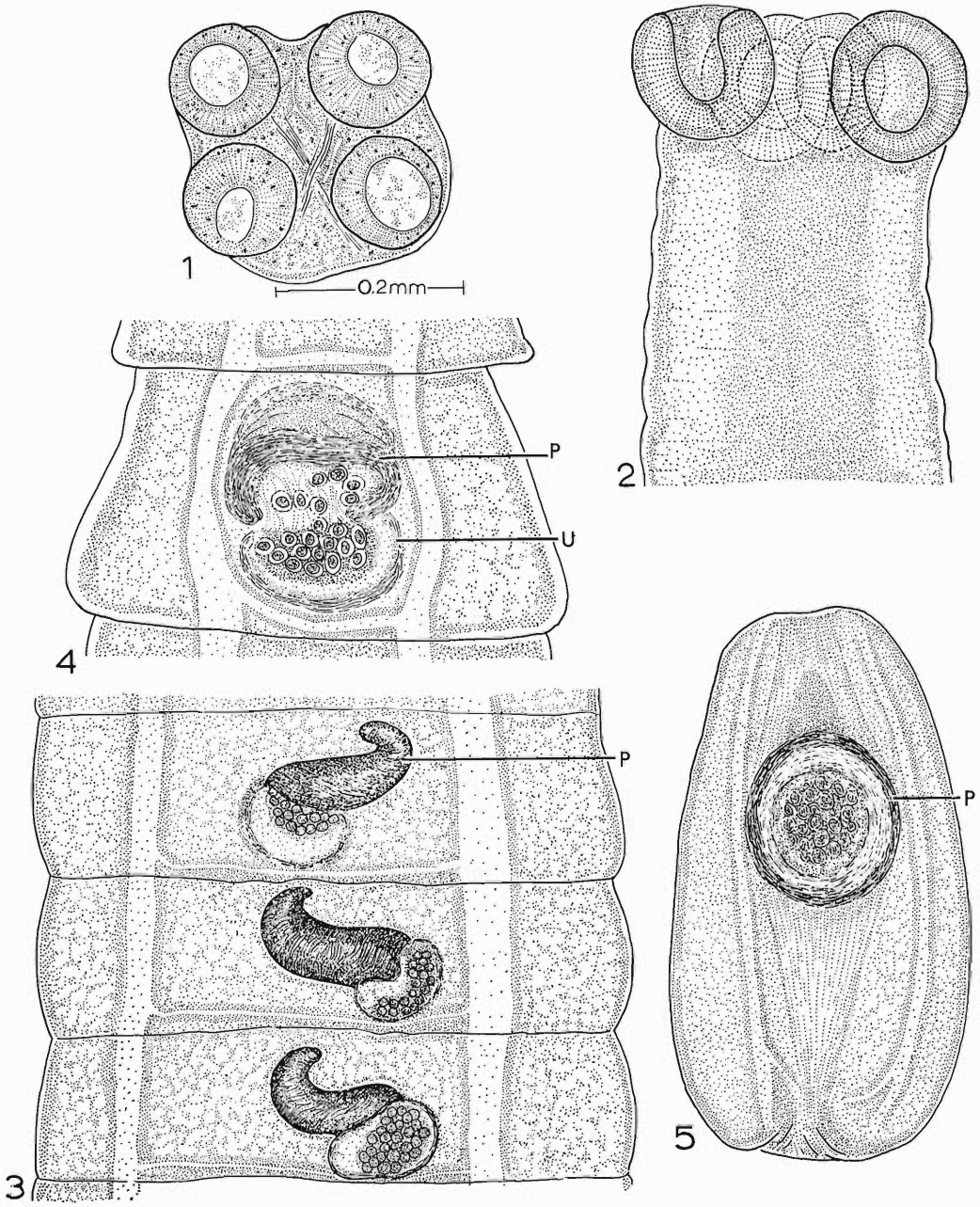

Figure 1. Scolex, transverse section (composite of two adjacent sections).
Figure 2. Scolex, lateral view.
Figure 3. Parauterine organ, early stage of formation.
Figure 4. Later stage of parauterine organ formation.
Figure 5. Gravid proglottid, showing fully developed parauterine organ.
Abbreviations: P—parauterine organ. U—uterus.

Genital pores alternating irregularly; reproductive organs medullary. Testes varying in number from 3 to 10 in mature proglottids but more frequently 8, subspherical to oval 40.8 (37.5 to 50) × 30.8 (27.5 to 37.5), frequently compressed by crowding. Cirrus pouch oval, well developed, 60 (50 to 62.5) × 20 (17.5 to 20) extending to, or slightly into, medullary region. Ovary single, midventral, posteroventral to testes, essentially globate 55.5 (50 to 62.5). Vitelline gland single, saccate-spheroidal, measuring 31.5 (27.5 to 32.5), lying at level of ovary between ovary and uterus (or parauterine organ). Uterus tubular, central. Parauterine organ single, subspheroidal to ovoid 146 (130 to 162) × 181 (175 to 187). Approximately 20–40 eggs within well-developed parauterine organ; eggs (measurements based on 20 eggs within parauterine organ in serial sections) 15.5 (12.5–17) × 11.5 (10–13.5) with oncospheres 9.5 (8–11.5) × 7 (5.5–9); membranes surrounding eggs not clearly distinguishable in sectioned material or whole mounts. Vagina opening posterior to cirrus.

TYPE HOST: *Rana pipiens* Schreber (leopard frog).

SITE: small intestine.

LOCALITY: Kettleson Hogsback, Dickinson County, Iowa U.S.A.

HOLOTYPE: USNM Helm. Coll. No. 73480. Paratype No. 73481.

Remarks

The strobila of *N. ranae*, unlike that of other members of the genus, appears more oval than round in cross section and is segmented in all but the short neck region. Segmentation, although anteriorly weak, is not limited to the posterior region as in other described genera within the family. Because of the apolytic nature of the nematotaeniids, the total length of a gravid *N. ranae* or the total number of its proglottids cannot be determined with certainty.

The reproductive system of a mature proglottid is seen only with difficulty in whole mounts. Details are best observed in transverse serial sections (Figs. 6, 7). The male system (Fig. 7) includes delicate vasa efferentia as well as a single, prominent vas deferens. Occasionally, a single mature proglottid may contain a double set of terminal male genitalia and two genital pores, one on each of the lateral body surfaces. In such anomalies, one set appears less developed and non-functional.

In the female system (Fig. 6), the vagina opens into the genital pore posterior to the male orifice and parallels the cirrus pouch. Frequently an expanded seminal receptacle-like swelling may be observed. Near the testes, the vagina meets a duct from the vitelline gland and ovary and then continues as a uterine duct to the uterus. After fertilization, the uterine duct becomes greatly coiled before proceeding medially to the short uterus, surrounded by darkly staining cells of the developing parauterine organ. The uterus-parauterine organ complex forms a cornucopia-like structure (Fig. 3) until the eggs, initially in the terminal part of the uterus, are shunted into the parauterine organ (Figs. 3, 4, 5). The single parauterine organ, fully developed in gravid, terminal proglottids (Fig. 5), is ovoid to spherical, heavy-walled and contains approximately 20–40 eggs. It was not determined if eggs of *N. ranae* possess three membranes as do those in other genera of this family.

Discussion

The two most striking differences between *Nematotaenoides ranae* and other nematotaeniid cestodes include varying number of testes (3 to 10) and the presence of a large, single parauterine organ. These characteristic differences necessitate not only modification of the familial diagnosis but the erection of a new genus as well. The extreme range of testis number (3–10) within single specimens of *N. ranae* suggests variability similar to that characteristic of another cyclophyllidean family, the Mesocestoididae (James, 1969). Because specimens of *Nematotaenoides ranae* were all recovered from a single host, it seems unlikely that such variation is host-induced.

Comparison of the various genera assigned to the Nematotaeniidae indicates a sequential series relative to the number of parauterine organs or number of testes. Thus, as illustrated in Fig. 8, parauterine organs vary from one (*Nematotaenoides*) to many (*Nematotaenia*). Number of testes per proglottid varies from one (*Cylindrotaenia*) to as many as 10 (*Nematotaenoides*).

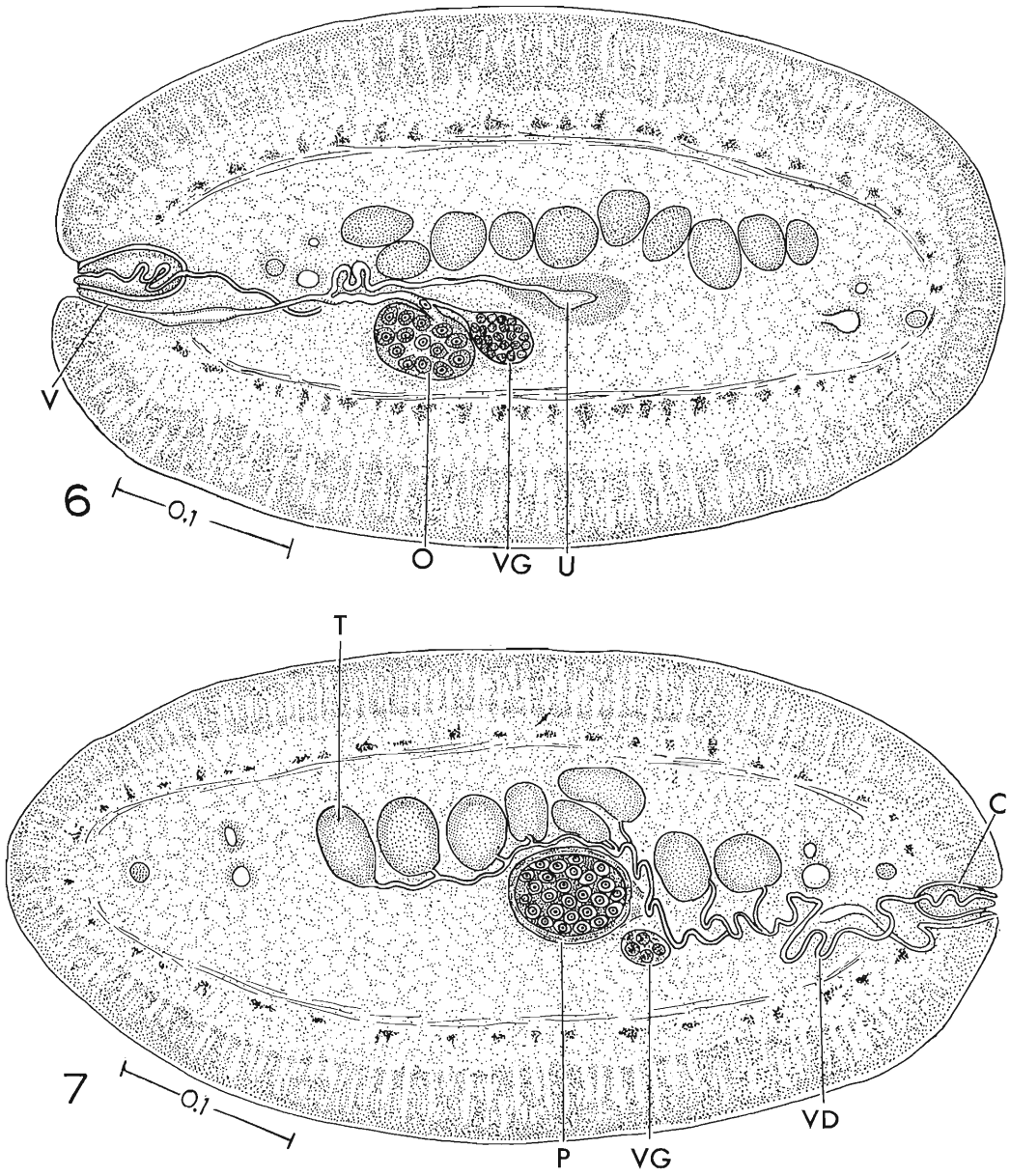

Plate II

Figure 6. Cross-section, showing relationships of female reproductive system (diagrammatic).

Figure 7. Composite of three adjacent cross-sections, showing relationships of male reproductive system (diagrammatic).

Abbreviations: C—cirrus pouch. O—ovary. P—parauterine organ. U—uterus. VD—vas deferens. VG—vitelline gland.

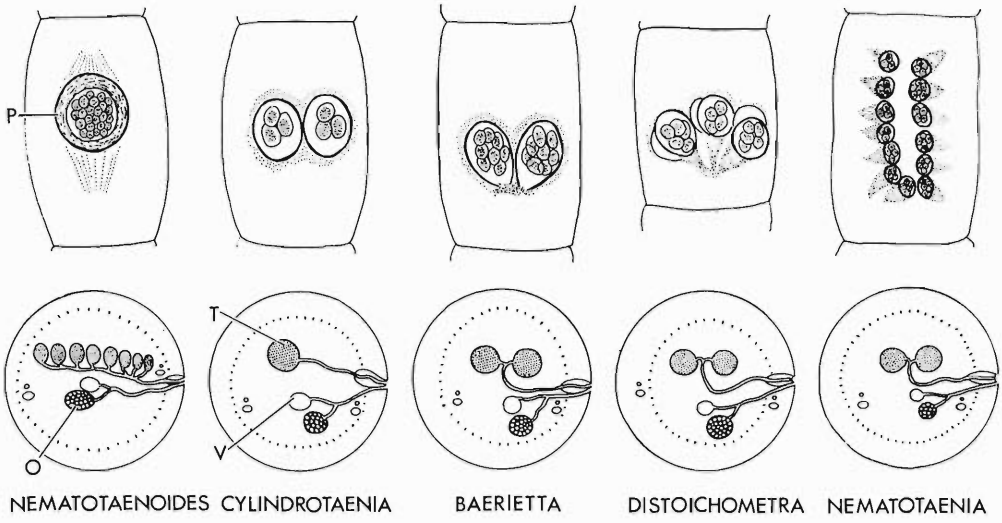

Plate III

Figure 8. Comparison of characteristic features of proglottids from the five genera of Nematotaeniidae (diagrammatic). Upper row: gravid proglottids showing parauterine organ(s) (P). Lower row: transverse sections of mature proglottids showing ovary (O), testis (T), and vitellaria (V).

The phylogenetic position of *Nematotaenoides* among the varied nematotaeniid genera is uncertain, but will probably depend upon the significance ascribed to the testes or parauterine structures in establishing relationships within the family. It is probable that the single parauterine organ characteristic of *Nematotaenoides*, a genus found only in amphibian hosts, represents a more primitive condition than the multiple parauterine organs typical of *Nematotaenia* whose members are found in reptiles as well as in amphibians. The discovery of *Nematotaenoides* increases the number of genera within the Nematotaeniidae to five; the obvious differences between these genera emphasize the need for a comprehensive, systematic review of these cestodes as already suggested by Wardle, McLeod and Radinovsky (1974).

We feel that the latter authors in their revision of cestode taxonomy do not provide sufficient evidence for elevating the Nematotaeniidae to ordinal status and thus prefer to retain this family within the order Cyclophyllidea.

The following key to the genera of Nematotaeniidae is based in part on previously published keys by Yamaguti (1959) and Wardle, McLeod and Radinovsky (1974), and is emended to include *Nematotaenoides* gen. n.

Key to the Genera of Nematotaeniidae

1. One parauterine organ per segment ..... *Nematotaenoides*
   Two or more parauterine organs per segment 2
2. Two parauterine organs per segment; testes single or double 3
   More than two parauterine organs per segment; testes double 4
3. Testes single ..... *Cylindrotaenia*
   Testes double ..... *Baerietta*
4. Egg capsules clustered together in parauterine organs having fused bases ..... *Distoichometra*
   Egg capsules within parauterine organs scattered throughout parenchyma ..... *Nematotaenia*

Acknowledgment

We wish to express our thanks to Dr. Philip T. LoVerde and Mr. Darwin Wittrock for use of specimens of *N. ranae* from their personal collections.

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Studies on the Helminth Fauna of Iowa II. Cestodes of Amphibians

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ABSTRACT: A survey of 706 amphibians representing 8 species, collected principally during the summers of 1953–74, reveals an incidence of 13.6% infection with cestodes, infected hosts having been collected from 24 areas of northwest Iowa. Almost all infections represent new locality records. Hosts examined include *Ambystoma tigrinum* (Green), *Bufo americanus* Holbrook, *Bufo cognatus* Say, *Acris crepitans* Baird, *Hyla versicolor* LeConte, *Pseudacris triseriata* (Wied), *Rana catesbeiana* Shaw, and *Rana pipiens* Schreber.

Three species of adult cestodes are represented in the collection, namely: *Cylindrotaenia americana* Jewell, 1916; *Ophiotaenia saphena* Osler, 1931; and *Nematotaenoides ranae* Ulmer and James, 1976, the latter from *Rana pipiens*.

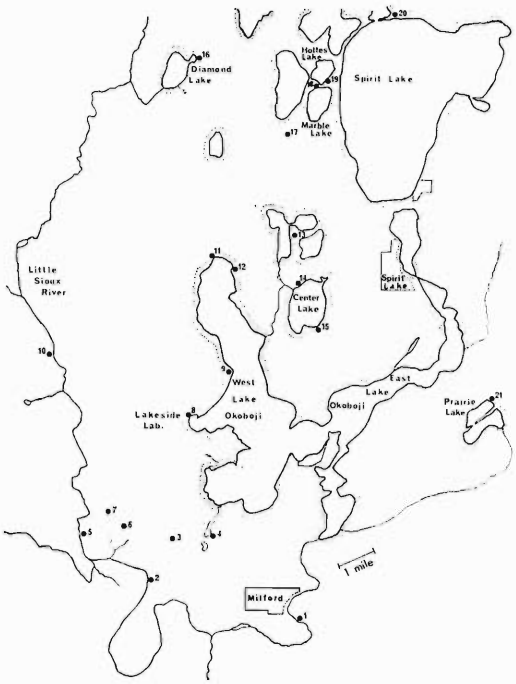
Two types of larval cestodes occur: tetrathyridia of *Mesocestoides*, and proteocephalan plerocercoids. *Rana pipiens*, the most abundant host in the region, harbors all five species of cestodes recovered. Each of seven hosts (5 *R. pipiens* and 2 *B. americanus*) harbored more than one type of tapeworm infection.

This study, the second in a series of continuing investigations on the helminth fauna of Iowa (Ulmer, 1970), is based on collections of amphibians from the northwest region of the state.

Seven hundred and six amphibians were examined; 96 harbored cestodes. Infected hosts were collected from 24 localities (Map 1) representing four counties (Clay, Dickinson, Palo Alto, and Woodbury). Most specimens were collected in the vicinity of the Iowa Lakeside Laboratory on West Lake Okoboji, Dickinson County, during the summers of 1953–74. Data on host species examined and number of those harboring cestodes appear in Table 1. Adult and larval cestodes are indicated in Table 2. Names of hosts are in accordance with the listings by Conant (1958).

Representative slides of cestodes collected during the course of this investigation have been deposited in the helminthological collection of Iowa State University at Ames.

Cestodes were fixed in AFA, 10% formalin or Ristroph's fluid, and whole mounts were stained either in Mayer's paracarmine, Mayer's HCl carmine, Delafield's or Harris' hematoxylin. Fast green (0.1% in 95% ethanol) was frequently used as a counterstain for carmine-stained preparations.



Map 1

Lakes region of northwest Iowa (Dickinson County), indicating collecting sites of amphibians.

1. **Milford Creek**
2. **Little Sioux River**
3. **Crossroads pond**
4. **Garlach Slough**
5. **Little Sioux River**
6. **Fairy shrimp pond**
7. **Kettlehole**
8. **Lakeside Laboratory**
9. **Manhattan Slough**
10. **Little Sioux River**
11. **Triboji Slough**
12. **Methodist camp**
13. **Jemmerson Slough**
14. **Center Lake (North)**
15. **Center Lake (South)**
16. **Diamond Lake**
17. **Kettleson Hogsback**
18. **Marble Lake**
19. **Hottes Lake**
20. **Spirit Lake**
21. **Prairie Lake**

Three additional collecting areas, not shown on map, include: Trumbull Lake (Clay County), Virgin Lake (Palo Alto County), and the Big Sioux River near Stone Park (Woodbury County).

Drawings were made with the aid of a Leitz microprojector.

Many of the specimens obtained for this study were provided by graduate students at Iowa Lakeside Laboratory, to whom grateful acknowledgment is made for their contributions to the helminthological collection.

Table 1. Amphibian hosts examined 1953–74.

Hosts	No. examined	No. infected with cestodes	% infection
Order Caudata			
Family Ambystomatidae			
Ambystoma tigrinum (Green) (Tiger Salamander)	54	0	0
Order Salientia			
Family Bufonidae			
Bufo americanus Holbrook (American Toad)	101	7	6.9
Bufo cognatus Say (Plains Toad)	4	0	0
Family Hylidae			
Acris crepitans Baird (Cricket Frog)	26	4	15.4
Hyla versicolor LeConte (Common Tree Frog)	1	0	0
Pseudacris triseriata (Wied) (Western Chorus Frog)	8	0	0
Family Ranidae			
Rana catesbeiana Shaw (Bullfrog)	21	0	0
Rana pipiens Schreber (Leopard Frog)	491	85*	7.3
Total	706	96	13.6

* Includes 11 hosts whose cestode parasites were not available for study.

Support for this study was provided in part by grants from the Iowa State University Alumni Research Foundation and by National Science Foundation grants G-9022, G-23597, GB-2384 and GB-5465X.

Table 2. Adult and larval cestodes recovered 1953–74.

Cestodes recovered	No. infected hosts: *Acris crepitans*	*Bufo americanus*	*Rana pipiens*
Adult cestodes			
Family Nematotaeniidae			
Cylindrotaenia americana	4	1	1
Nematotaenoides ranae			1
Family Proteocephalidae			
Ophiotaenia saphena		1	26
Larval cestodes			
Family Mesocestoididae			
Mesocestoides tetrathyridia		4	10
Family Proteocephalidae			
Proteocephalan plerocercoids		1	36
Total	4	7	74

Adult Cestodes
Order Cyclophyllidea
Family Nematotaeniidae

1. *Cylindrotaenia americana* Jewell, 1916 (Figs. 6–9)

HOSTS: *Rana pipiens* Schreber (leopard frog), *Bufo americanus* Holbrook (American toad), *Acris crepitans* Baird (northern cricket frog).

HABITAT: Intestine.

This cylindroid species, originally described by Jewell (1916) from the intestines of various anurans including the southern cricket frog (*Acris gryllus*), is represented in our collection by specimens from 4 *Rana pipiens*, 1 *Bufo americanus*, and a single *Acris crepitans* taken in the Okoboji region of northwest Iowa. *C. americana* is easily recognizable from other genera within the family Nematotaeniidae by the presence of two parauterine organs per segment, and by the relatively few eggs within each.

The formation of the parauterine organs within a given proglottid was described in considerable detail by Jewell (1916) and involves the production of a pair of conspicuous truncated cones, one dorsal and one ventral, each of which consists of two portions: a smaller, basal and a larger, bulbular, apical portion containing the oncospheres (Fig. 7).

The life cycle of *C. americana*, as reported by Joyeux (1924), is said to be direct. His studies, however, did not involve experimental infections. Because Douglas (1958) questioned the validity of *R. pipiens* as a host for *C. americana*, it will be necessary that experimental studies be undertaken to determine its relationship to a closely related species, *C. quadrijugosa* described by Lawler (1939) from this species of anuran. Reference to Lawler's account, particularly with reference to the parauterine organ, indicates that specimens in our collection are *C. americana*.

2. *Nematotaenoides ranae* Ulmer and James, 1976 (Figs. 16–18)

HOST: *Rana pipiens* Schreber (leopard frog).

HABITAT: Intestine.

A single *Rana pipiens*, collected July 8, 1971 at Kettleson Hogsback, near Marble Lake (Dickinson County), was infected with *Nematotaenoides ranae* Ulmer and James 1976, 20 specimens having been recovered from the intestine. Attempts to find additional specimens in frogs of the Okoboji region in subsequent years have been unsuccessful. Characteristic of gravid proglottids in this species is the presence of a single parauterine organ, certain developmental stages of which are shown in Figures 17–18. All other described species of nematotaeniids in genera currently ascribed to this family (i.e., *Baerietta* Hsü 1935, *Cylindrotaenia* Jewell 1916, *Distoichometra* Dickey 1921, and *Nematotaenia* Lühe 1899) are characterized by the presence of two or more parauterine organs. A detailed morphological description of adult *N. ranae* was presented by the authors (1976).

The creation of a new order, Nematotaeniidea by Wardle, McLeod and Radinovsky (1974), appears unjustified in our opinion, and hence we prefer to retain the family Nematotaeniidae in the order Cyclophyllidea.

Order Proteocephala
Family Proteocephalidae LaRue, 1911

3. *Ophiotaenia saphena* Osler, 1931 (Figs. 1–5, 10, 11)

HOSTS: *Rana pipiens* Schreber (leopard frog), *Bufo americanus* Holbrook (American toad).

HABITAT: Intestine.

This is the most commonly encountered tapeworm of amphibians of northwest Iowa, almost all examples having been recovered from leopard frogs (*R. pipiens*), and only once in a toad (*B. americanus*). The species was originally described by Osler (1931) from specimens found in *Rana clamitans* in Michigan. Thomas (1931, p. 191), referring to Fortner's (1923) statement that only 1% of 177 *R. pipiens* in the Douglas Lake region of Michigan harbored tapeworms, recorded the presence of proteocephalan tapeworms in this amphibian, but did not identify the species involved.

Ophiotaeniid cestodes follow a typical proteocephalan life cycle, involving a procercoid larva containing a cercomer and devel-

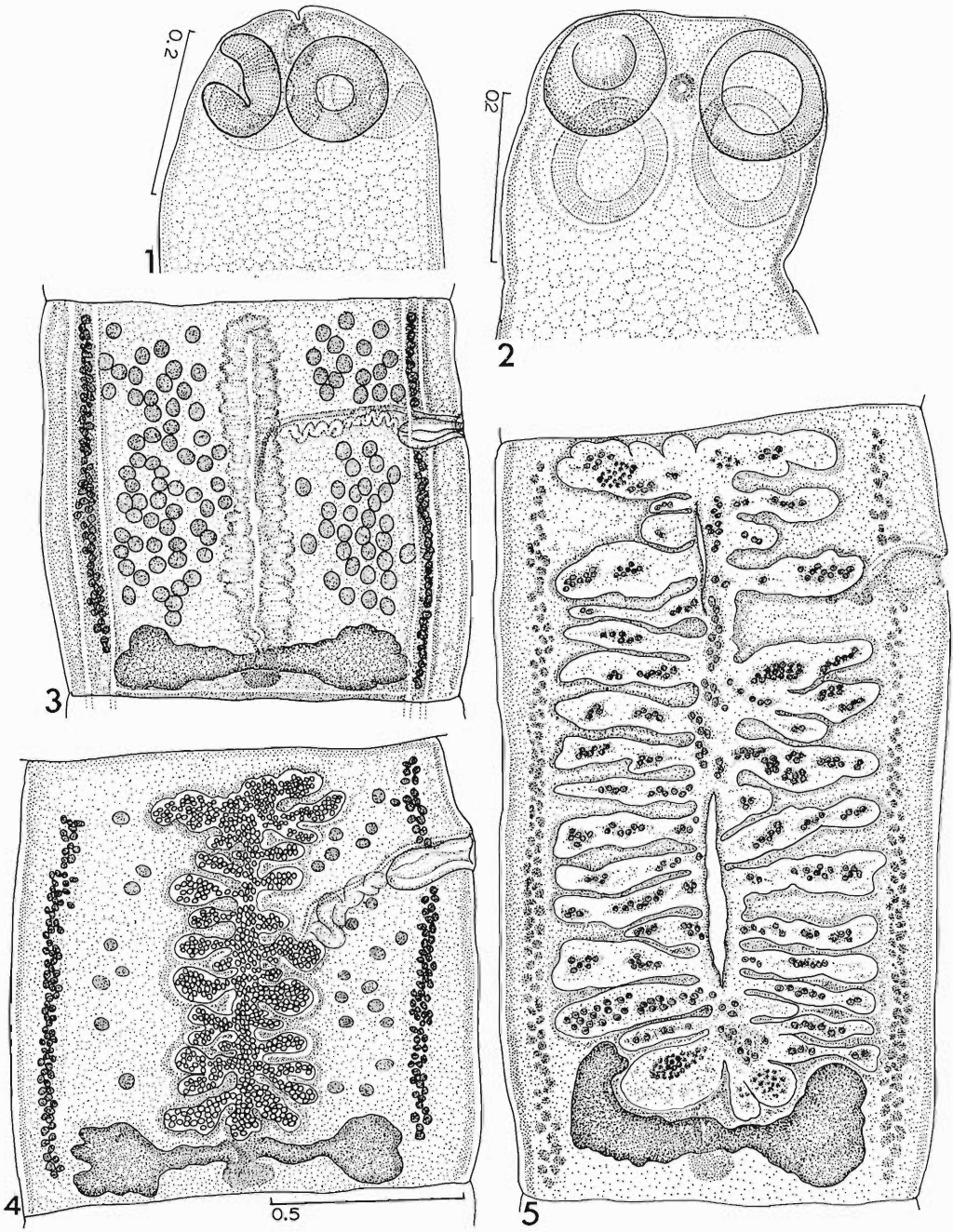


Plate 1

Figures 1–5. *Ophiotaenia saphena* Osler, 1931 (all specimens from *R. pipiens*). 1, 2. Scolex. 3. Mature proglottid. 4. Gravid proglottid. 5. Spent proglottid. Note uterine clefts.

oping within the haemocoel of a copepod host. The life cycle of *O. saphena* was elucidated by Thomas (1931, 1934a), and involves the copepods *Cyclops vulgaris* var. *brevispinosus* and *Mesocyclops obsoletus*, in which mature procercoids develop in 12–14 days following ingestion of eggs. Thomas observed that the youngest adults recovered from the intestines of *Rana clamitans* resembled in all respects but size the well-developed procercoids found in the copepod intermediate host, and suggested the possibility of direct infection by frogs through accidental ingestion of infected *Cyclops*. More recent studies on the life cycles of proteocephalans, however (e.g., Fisher and Freeman, 1969), have shown that in a related species (*P. ambloplites*) in smallmouth bass, the parenteral plerocercoids are capable of leaving the viscera and penetrating the gut of the same bass host, and that a well-developed end organ is involved in such penetration. Specimens recovered from *R. pipiens* and *B. americanus* in this study agree in all respects with the species description provided by Osler (1931). Unidentified plerocercoid larvae with well-developed apical organs are also found in the same species of hosts harboring the adult worm, and occasionally larvae and adults appear concurrently in a single host. Thomas (1931) referred to the hypertrophy of the apical or end organ in the plerocercoid stage, followed by its atrophy and vestigial condition in adult worms.

Two instances of anomalies involving supernumerary genitalia were found in specimens of *O. saphena* recovered in this survey. In one, a single mature proglottid contained a double set of male and female terminal genitalia (Fig. 10) and in another, a gravid proglottid was provided with a double ovary (Fig. 11).

This study constitutes the first report of adult *O. saphena* from *Rana pipiens*, previous accounts having indicated that *R. clamitans* and *R. catesbeiana* serve as definitive hosts for this ophiotaeniid. Freze (1965) placed all ophiotaeniid tapeworms from amphibians in the genus *Batrachotaenia* Rudin 1917 and referred to this species as *B. saphena* (Osler, 1931). Although Freze cited *R. pipiens* as a host, no references included in his monograph list this amphibian host as harboring adult *O. saphena*. Apparently Freze misinterpreted data presented by Thomas (1931) regarding hosts of this species.

Larval Cestodes
Order Proteocephala
Family Proteocephalidae LaRue, 1911

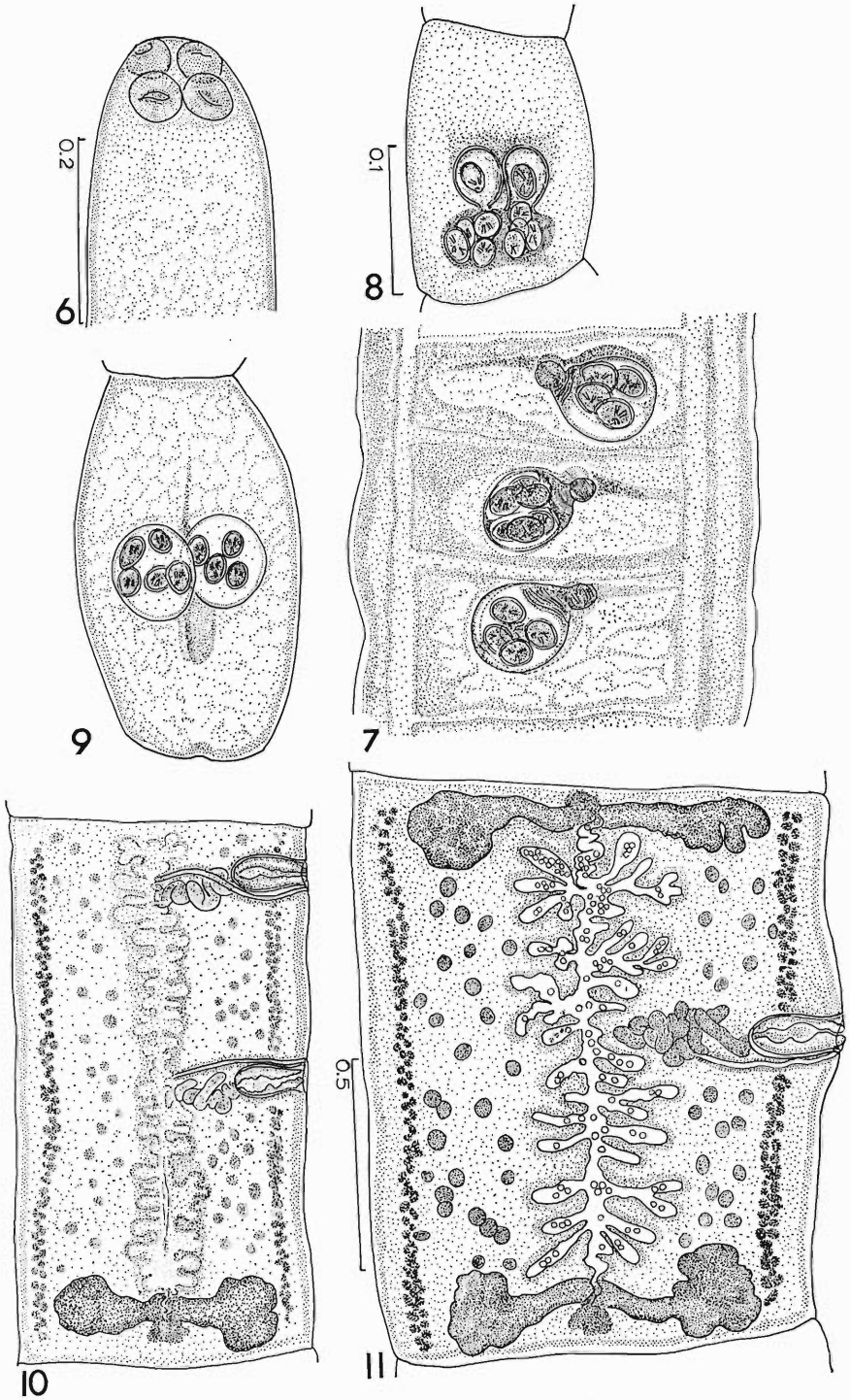
4. Proteocephalan plerocercoids (Figs. 12–14)

HABITAT: Liver, mesenteries, coelomic cavity.

Encysted and non-encysted proteocephalan plerocercoids of varying size (0.3 to 30+ mm) were recovered from 36 *Rana pipiens* and a single *B. americanus* on various occasions between June and October. Larger plerocercoids show evidence of immature proglottids posteriorly. All plerocercoids recovered were apparently of similar type, characterized by the presence on the scolex of a well-developed apical organ. Several investigators, including Wood (1965) and Fisher and Freeman (1969) indicate this apical or end organ in proteocephalans to be an exocrine gland, used in lysing host tissue.

Although such plerocercoids have not been identified with certainty, they may represent immature stages of *Ophiotaenia perspicua*, a cestode of garter snakes and water snakes whose life cycle was determined by Thomas (1934b, 1941) and by Herde (1938). Thomas (1941), however, distinguished plerocercoids of *O. perspicua* from those of *O. saphena* by the presence of minute scale-like spines in the tegument of the former species. Our specimens show no evidence of such tegumental structures.

Thomas (1941, p. 77) suggested that plerocercoids of *O. perspicua* may require a sojourn within the tissues of a second intermediate host before becoming infective and establishing themselves within the intestine. More recently, Mead and Olsen (1971) in a study of *O. filaroides*, whose adults parasitize salamanders, indicated that development to the mature adult within the definitive host is dependent upon the degree of development of plerocercoids when ingested. If fully developed, they rapidly attain a strobilate condition in the intestine; if, however, copepod intermediates are ingested before the metacestode is well-developed, plerocercoids undergo a tissue (par-



enteral) phase of varying length, ultimately returning to the intestinal lumen by penetration or by being ingested by another suitable definitive host by cannibalism. Such variations in life cycles of ophiotaeniids apparently are of rather common occurrence among those species whose development has been experimentally studied. Fisher and Freeman (1969) described the penetration of such parenteral plerocercoids of *Proteocephalus ambloplitis* (Leidy) into the gut lumen of smallmouth bass, reporting the phenomenon as a seasonal one. Prolonged sojourns of plerocercoids in definitive hosts may also provide an effective means for survival of the species over winter, as suggested by Mead and Olsen (1971) for *O. filaroides*.

The identification of proteocephalan plerocercoids of amphibian hosts in our collection is difficult, and experimental studies are needed to establish their precise taxonomic status.

Order Cyclophyllidea
Family Mesocestoididae

5. *Mesocestoides* tetrathyridia (Fig. 15)

HOSTS: *Rana pipiens* Schreber (leopard frog), *Bufo americanus* (American toad).

HABITAT: Mesenteries, connective tissues of brachial region, embedded in mesonephros, liver, and muscular layers of intestinal wall.

Tetrathyridia of *Mesocestoides* were recovered from four *B. americanus* and 10 *R. pipiens*. Such larvae lie scattered in varying numbers, but occur most frequently embedded within the intestinal wall, in liver and mesonephric tissue, and are also associated with mesenteries of the brachial region. Both single and multiple cysts were recovered, enclosed in thin cyst walls of host origin.

Specific identification of these larvae was not attempted, but unpublished studies by James provide evidence that the genus is monotypic, all described species probably being *M. lineatus* (Goeze, 1782). The first report of North American amphibians harboring tetrathyridia was that of James and Ulmer (1967) who reported their presence in northwest Iowa in the two host species indicated above.

Multiple Infections

Multiple infections by helminths within a single amphibian host have been reported frequently and were observed often in this study. However, infections involving more than a single species of cestode within an individual amphibian are relatively infrequent. Brandt (1936), for example, in a study of more than 350 specimens of six species of salientians from North Carolina, found only a single frog (*R. catesbeiana*) harboring two different species of cestodes.

During the course of the present investigation, seven instances of multiple cestode infections were encountered, five involving *R. pipiens* and two, *B. americanus*. Only one of these (a *R. pipiens* collected 8 July 1971) involved two species of adult cestodes (*Ophiotaenia saphena* and *Nematotaenoides ranae*, Ulmer and James, 1976). Three instances of concurrent infection involved *Mesocestoides* tetrathyridia and proteocephalan plerocercoids, two of such double infections having been encountered in *R. pipiens*, one in *B. americanus*. Additionally, two examples of *R. pipiens* harboring both *Ophiotaenia saphena* adults and unidentified proteocephalan plerocercoids, and a single occurrence of tetrathyridia and adult *O. saphena* in *B. americanus* were also recorded.

Discussion

Cestodes of North American amphibians are singularly few when compared with trematodes reported from these vertebrates. Leidy (1851) was apparently the first North American investigator to have indicated the presence of tapeworms when he reported "*Taenia*

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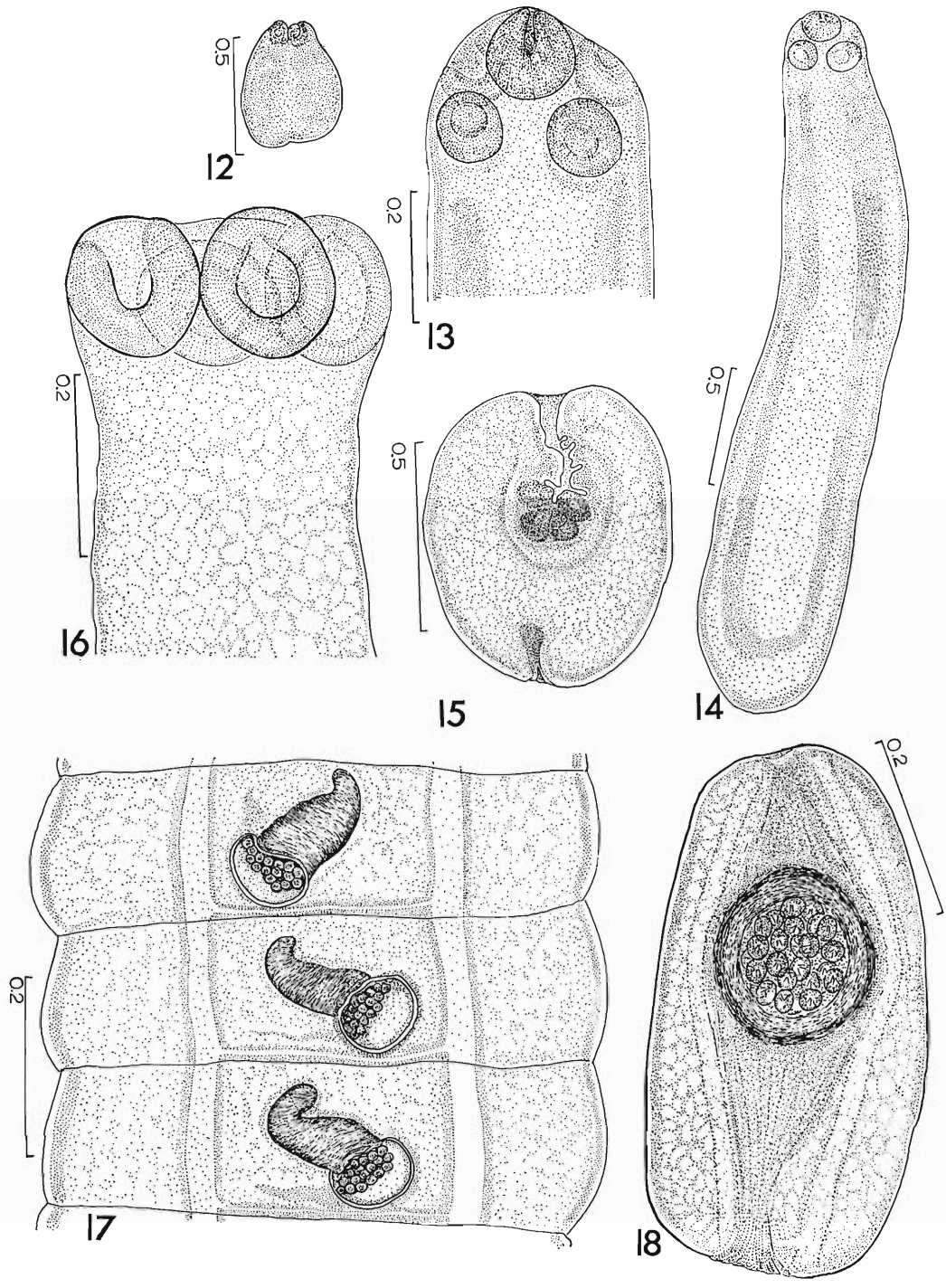
Plate 2

Figures 6–9. *Cylindrotaenia americana* Jewell, 1916. 6. Scolex (Host: *Acris crepitans*). 7, 8. Developing parauterine organs (Host: *Bufo americanus*). 9. Terminal proglottid, showing dorsal and ventral parauterine organs (Host: *B. americanus*).

Figures 10, 11. *Ophiotaenia saphena*, anomalies (from *R. pipiens*). 10. Mature proglottid with double set of male and female terminal genitalia. 11. Gravid proglottid with double ovary.



pulchella" from *Bufo americanus*, indicating only that his specimens consisted of "immature forms of uncertain classification." In Yamaguti's (1959) *Systema Helminthum*, Vol. II (The Cestodes of Vertebrates) only eight pages are devoted to cestodes of amphibians, far fewer than to cestodes of any other vertebrate group.

Surveys of amphibian cestodes often refer to the paucity of these helminths. Early workers (LaRue, 1909, 1911, 1914, 1914a; Dickey, 1921; Woodland, 1925; and Hannum, 1925) for example, frequently noted this, as have investigators in more recent years when extensive surveys have been undertaken. Thus, Ingles (1936) in a study of 264 California amphibia reported cestodes as "very rare"; Rankin (1945) reported a single infection in each of two species of adult cestodes (*Bothriocephalus rarus* Thomas, 1937 and *Cylindrotaenia americana* Jewell, 1916) and single infections of larval tapeworms in three hosts; Bouchard (1951) found but a single infection of cestode (*Cylindrotaenia americana*) in 195 amphibians collected in Maine; Odlaug (1954) in a survey of helminths of 14 species of Ohio amphibians recorded but a single cestode (*Distoichometra bufonis* Dickey, 1921). Lehmann (1960) examined 178 California amphibians and recorded only a single infection of pseudophyllidean (*Bothriocephalus rarus*) in a newt and *Cylindrotaenia* in 10 specimens of salamanders. Waitz (1961) recorded the presence of only one undetermined species of *Baerietta* in 10 specimens of salamanders (*Plethodon*) in a survey of 167 amphibians from Idaho. In marked contrast to these findings, Brandt (1936), in an extensive survey of 368 North Carolina amphibians, observed cestode infections ranging from 0–51% in six species of hosts. He also reported that larger hosts harbored more adult cestodes than did younger ones, and that larval cestodes were far more abundant than adults in a given host. Our results confirm his finding that larval cestodes are considerably more abundant than are adults in amphibian hosts (Table 2). His study is apparently the only one indicating a higher percentage of cestode infections in amphibians than the 13.6% infection in 706 hosts representing eight species of amphibians reported here.

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Plate 3

Figures 12–14. Proteocephalan plerocercoids from *R. pipiens*. 12. Young plerocercoid. 13. Anterior end of plerocercoid showing well-developed apical gland. 14. Large plerocercoid with apical gland.

Figure 15. Tetrathyridium of *Mesocestoides*, from *R. pipiens*.

Figures 16–18. *Nematotaenoides ranae*, Ulmer and James 1976 from *R. pipiens*. 16. Scolex. 17. Developing parauterine organ. 18. Terminal proglottid showing single parauterine organ.

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Artificial Infection of Sweet Corn Seedlings with *Anguina tritici* Steinbuch (1799) Chitwood, 1935

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ABSTRACT: Potted sweet corn and sorghum seedlings were inoculated with second stage larvae of *Anguina tritici* Steinbuch (1799) Chitwood, 1935. The larvae could not penetrate, or could rarely penetrate, the stem below the first node. When more soil was added to the pots after the first node had formed, which occurs at the soil surface, and the inoculum was then applied, heavy invasion could result in the new leaf tissue above the node.

The wheat nematode, *Anguina tritici* Steinbuch (1799) Chitwood, 1935, has been reported as a pest of six or more cereals other than wheat. Filipjev and Stekhoven (1941), apparently referring to Marcinowski's work, stated that "the larvae seem to miss the capacity of selecting the proper host. They attack the first plant they meet." The ease with which the larvae from refrigerated galls can be used for experimental work, as shown by Limber (1973), suggested a study of their action on unreported hosts. Tests on two such plants, sorghum and sweet corn, are reported below.

Materials and Methods

Sorghum vulgare Pers. was used only once in a preliminary experiment and was of an unknown variety. The sweet corn, *Zea mays* L., was of the varieties Golden Bantam and Golden Cross Bantam. The nematode larvae were from wheat galls of the North Carolina collection of 1948 which is in the U.S. Department of Agriculture Nematode Collections at Beltsville, Md.

The seeds were germinated in wet paper toweling placed in a plastic container to retain the moisture. When the plumules and radicals appeared, the seedlings were covered with ½ to 1 inch of soil in clay pots. Usually the contents of a single gall, containing 10,000 to 25,000 second stage larvae, were used to inoculate the 6 to 9 plants in a pot. About 2,000 to 4,000 active larvae, suspended in water, were applied closely around each plant with a medicine dropper. There were from 5 to 20% of dead larvae in most of the galls.

After the first test indicated that the tissue below the node was resistant to invasion, the method was amended as follows: when the plumules appeared they were allowed exposure to daylight for 12 hr, then 1 to 1½ inches more soil was added before they were inoculated. Thus the first node and the leaves arising from it were beneath the surface of the soil.

Dissection of the inoculated plants began after 8 days and continued until all of the plants in a pot were examined, usually between the 8th and 11th days but up to the 15th day.

Sections were made to determine whether morphology might explain the resistance below, and susceptibility above, the first node.

Results and Discussion

The first test yielded little invasion of either the sorghum or the sweet corn. It was noticed that of the two invaded sorghum plants, the one with the most larvae (3), was a plant which had grown through the soil in a prostrate manner before it emerged. The first node had formed beneath the surface of the soil. Since the first leaves of sorghum and sweet corn arise at the first node, some leaf tissue of this plant was under the soil. The larvae had entered through leaf tissue. Therefore in the tests which followed, the amended method, outlined above, was used.

One hundred and twelve sweet corn seedlings were inoculated over a period of 11 months. Seventy-five plants were invaded. From one to 171 second stage larvae were found in an invaded plant. Out of 1,301 larvae

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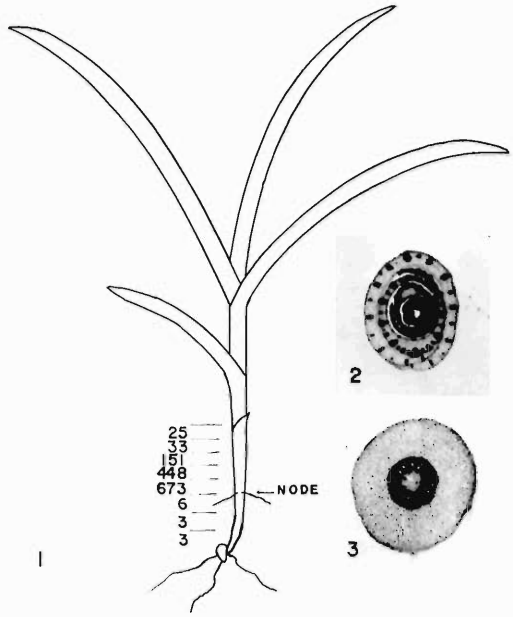

Figures 1–3. 1. A schematic drawing showing the distribution of 1,301 larvae of *A. tritici* from 112 sweet corn plants in the period of 8 to 15 days after inoculation. The stem sections above the node are approximately 4 mm in length. The stem below the node was divided into three parts. 2. Section of a sweet corn stem just above the first node. 3. Section of the stem below the first node.

recovered from the plants, only 12 were found in the stem below the node. These results indicate that the tissues below the node are resistant or at least unfavorable for invasion.

Figure 1 shows the distribution of the 1,301 larvae which invaded the 112 plants. The groupings shown are those found in the first 15 days after inoculation. It will be seen that most of the larvae enter the seedlings in the first centimeter above the node. Probably upward movement in the host depends more on the upward growth of the leaves and meristem than on independent movement of the larvae.

The presence of a few larvae below the node in an occasional plant suggested that injuries might permit entry. Such injuries might be caused by insects, fungi, or the emergence of rootlets. Therefore the effect of wounds made by needle punctures and by scratches on the stems below the nodes was tested 12 times. Sixty-three plants were wounded. Of these, 32 plants were invaded and a total of 230 larvae were recovered. This is approximately 50% invasion of the stems which were injured below the nodes. Of the 112 plants in the preceding experiments, reported above, only 12 were invaded below the first nodes, or about 10.7%. This suggests that uninjured stems are rarely invaded below the first node.

In some wounded stems the larvae were closely associated with the wounds, but in many they had moved, usually downward, and were found several millimeters from the wounds.

Morphology and Invasion

Sections of the stem below the first node (Fig. 3) show dense cellular tissue with xylum tubes at the center. The cortex is comparatively firm. Above the node (Fig. 2), at this stage of development, there is a short conical bit of meristem at the center surrounded by developing leaves which wrap around each other about 1½ times. There are open spaces between some of the leaves and above the meristem. The leaf tissue is quite soft. Thus penetration by the larvae and movement within and between the leaves is comparatively easy.

Up to the present, attempts to follow the course of invasion have met with limited success. One larvae, nearly adult, was found associated with the developing tassel still in the lower stem. This specimen was lost in transit when it was mailed for identification. So it is not certain that it was *A. tritici*. With the possible exception of this larvae, no evidence of growth of the invading larvae was apparent. Four larvae which were recovered from rolled leaves 14 cm above the soil after 52 days, and 43 larvae found in a directly inoculated, immature kernel after 22 days showed no evidence of growth. Critical measurements were not made. The infested kernel was quite black.

Seven plants were grown to maturity in pots and formed ears. With the exception of the directly inoculated kernel, noted above, no galls or larvae were found in these. However, since invasion of a plant cannot be known until it is dissected, there may never have been any larvae in these plants.

The populations of different galls were shown by Limber (1973) to revive very differently.

This was most apparent in the percentage of revival but there were also differences in the time required before activity appeared. A similar variation was found in the present work with regard to the ability of different gall populations to invade sweet corn. These variations were between the populations of galls which had been stored in the same container. Therefore the differences must have resulted from causes acting during the development of the galls or possibly from genetic differences.

The evidence presented in this paper supports the record that sweet corn has not been reported as a host of *A. tritici*, or at least the nematode is not one of its pests. However it seems possible that under unusual conditions sweet corn could be invaded in the field. The natural protection, which is the formation of the leaf tissues above the first node at the soil surface, could be lost if sweet corn, planted in infested soil, is cultivated when it is still small, in such a way that wet soil is thrown against the leaves. The same effect might be produced by infested, wet soil being washed over young plants by heavy rains.

Acknowledgments

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A Histochemical Study of Egg Shell Formation in the Monogenetic Trematode *Octomacrum lanceatum* Mueller, 1934[1]

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ABSTRACT: The origin of shell precursors, their chemical nature, and the formation of egg shells were studied in the monogenetic trematode *Octomacrum lanceatum* by histochemistry. Shell precursors were identified as basic proteins, phenolic substances, and phenolases all found within vitelline cell globules. The presence of these compounds indicate the egg shell is a highly stable, quinone tanned protein. The egg shell is formed in the ootype and proximal uterus following coalescence of shell globules released from vitelline cells. Developing ova, the walls of the oviduct, ootype, and proximal uterus as well as the Mehlis' glands did not appear to add precursor components to the shell.

Most histochemical studies of egg shell formation in trematodes have dealt with digenetic trematodes (Stephenson, 1947; Johri and Smyth, 1956; Hanumantha-Rao, 1959; Smyth and Clegg, 1959; Burton, 1963; Coil, 1965, 1966, 1969; Coil and Reid, 1965; Madhavi, 1966, 1968; Wilson, 1967; and Nollen, 1971). Gerzeli (1968) studied the process in *Aspidogaster conchicola*, and, according to Smyth and Clegg (1959), Rennison investigated egg shell formation in the monogenetic trematode *Diclidophora merlangi*. Regarding other Monogenea, egg shell formation has been studied

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in *Pricea multae* (Ramalingam, 1971, 1972) and *Rajanchocotyle batis* (Rigby and Marx, 1962).

Results of these studies indicate that egg shells of many trematodes are sclerotin, a quinone tanned and inelastic protein formed through the action of phenolases on precursors having their origin from shell globules in vitelline cells. The chemistry of quinone tanning has been reviewed by Smyth and Clegg (1959) and Smyth (1966). However, it appears that not all trematodes possess a quinone tanning system (Smyth and Clegg, 1959; Madhavi, 1966, 1968; Nollen, 1971).

Since so few studies on egg shell formation have focused on monogenetic trematodes, such an investigation appeared desirable. The present study gives evidence for the existence of a quinone tanning system in the monogenetic trematode *Octomacrum lanceatum* Mueller, 1934.

Materials and Methods

Long nose suckers, *Catostomus catostomus* Girard, were collected by seine from Trout Creek immediately below Lake Manitou, Teller Co., Colorado. The trematodes were removed immediately from the gills and fixed in either 70% ethyl alcohol, phosphate buffered formalin (pH 7.3) or Carnoy's fluid. Stains employed, following the methods of Johri and Smyth (1956), were: 0.1% aqueous catechol for detection of phenolases, 1.0% aqueous fast red B for detection of phenolic compounds (fixation in 70% ethyl alcohol) 1.0% aqueous bromphenol blue, and 0.5% aqueous malachite green as tests for basic proteins (fixation in buffered formalin). Treatment with aqueous catechol and fast red B were carried out prior to embedding in paraplast. Serial sections were cut at 5 to 10 μ. The PAS reaction was employed for the detection of polysaccharides, and control sections were treated with fresh saliva for glycogen determination. Flattened specimens were stained with Delafield's hemotoxylin and borax carmine for details of gross anatomical features.

Observations

The results of the various histochemical tests appear in Table 1. Vitelline cells contained numerous shell globules which stained intensely with bromphenol blue and malachite green indicating the presence of basic proteins. The same globules exhibited a strong positive reaction to fast red B and catechol, demonstrating the presence of phenolic compounds and phenolases, respectively. The vitelline cell cytoplasm was rich in glycogen, but shell globules were negative.

Table 1. Results of histochemical tests on the female reproductive system of *Octomacrum lanceatum*.

	Catechol	Fast red B	Bromphenol blue	Malachite green	PAS	PAS and saliva
Vitelline globules	++	++	++	++	−	−
Vitelline cell cytoplasm	−	−	−	−	+	−
Vitelline cells in eggs	−	−	−	−	+	−
Egg shell (newly formed)	++	+	+	+	−	−
Mehlis' gland mucous cells	−	−	−	−	+	+
Mehlis' gland serous cells	−	−	−	−	+	−
Proximal uterine wall	−	−	−	−	+	−
Ootype wall	−	−	−	−	+	−
Developing ova in ovary and oviduct	−	−	−	−	−	−

Fully formed eggs were oval with a long, anteriorly directed polar filament. Usually only one was present in the uterus at a given time. Shells of newly formed eggs showed positive reactions to catechol, fast red B, bromphenol blue, and malachite green. However, these reactions were weaker in shells of older eggs (those located more distal to the ootype).

Mehlis' glands consisted of both mucous and serous cells. The latter were smaller and positioned very close to the ootype while mucous cells extended out into the adjacent parenchyma and were joined to the ootype by long, slender ducts. Mucous cells exhibited a strong PAS reaction before and after treatment with saliva, but both serous and mucous cells were negative to fast red B, catechol, bromphenol blue, and malachite green.

Small secretion granules were observed in serous cells along with some glycogen. Glycogen was also detected in the musculature of the ootype and proximal uterus. Maturing ova

within the ovary and proximal oviduct were negative for all histochemical tests used.

Discussion

Egg shell formation in *O. lanceatum* parallels the process as demonstrated in digenetic trematodes having a quinone tanning system (Smyth and Clegg, 1959). The presence of basic proteins, phenolic compounds, and phenolases within the shell globules of vitelline cells coupled with the amber color of the shell strongly suggests a quinone tanning system. Further supporting evidence comes from the observation that older egg shells exhibited a decreased affinity for fast red B, malachite green, and bromphenol blue. Presumably this is because the phenols have been oxidized to quinones through the action of phenolases, and the free amino groups of the basic proteins have become bonded to quinones.

Though it is apparent that Mehlis' gland cells do not contribute any of the shell precursors, we are not able to add any new knowledge as to their function. Several plausible ideas have been put forth by previous workers. Hanumantha-Rao (1959) suggested Mehlis' gland secretions in *Fasciola hepatica* act on vitelline cells in such a way as to effect release of the shell precursors. More recently Ramalingam (1970, 1971) has shown in *Pricea multae* that the phenolic compounds existed in a masked state joined to acid mucopolysaccharides, and that the phenolases existed as proenzymes. Secretions from Mehlis' glands are thought to free the phenols from acid mucopolysaccharides as well as to activate phenolases thereby allowing oxidation of phenols to quinones only at the proper time.

All of the necessary components of the egg shell (basic proteins, phenolic substances, and phenolases) are contained within the shell globules of vitelline cells of *O. lanceatum*. Developing ova, the walls of the oviduct, ootype, and proximal uterus as well as the Mehlis' gland do not appear to contribute any of these basic components to the egg shell.

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Occurrence of Carbonic Anhydrase and its Relation to Ammonia Produced and Attraction of Both Sexes of *Pelodera strongyloides*

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ABSTRACT: Histochemical methods for carbonic anhydrase, which were inhibited with 0.001 M Diamox, detected fine black granules in the intestine of both male and female *Pelodera strongyloides* cultured on nutrient agar only. When 0.001 M Diamox was incorporated into the nutrient agar, fewer fine black granules were detected so that the worms' intestines appeared unstained or lightly stained, and the ability of males and females to find one another was significantly reduced. In addition, NH_3 gas variably produced by both male and female worms was inhibited with 0.001 M Diamox.

Stringfellow (1974) reported that males of *Pelodera strongyloides* were attracted to the alkaline pH produced by aggregates of males and females. The present studies were undertaken to determine how these worms affect their microenvironment based on data of: (1) Scott and Whittaker (1970) who showed that NH_3 was highly concentrated in the medium used to culture *P. strongyloides*; (2) Dr. Eder Hanson (pers. comm.) who inquired whether NH_3 was involved in altering the worms' microenvironment; and (3) Carter (1972) who reviewed the little understood relationship between ammonium ion secretion and carbonic anhydrase.

Materials and Methods

The following experiment was run to determine the presence and distribution of the enzyme carbonic anhydrase in *P. strongyloides* as seen by the presence of fine black granules when suitable histochemical methods are used. Pellets of male and female *P. strongyloides* picked off nutrient agar with size No. 1 insect pins and calf pancreas fixed in cold acetone were stained with Haüsler's variant of Kurata's method for carbonic anhydrase as given in Lillie (1965). Pancreatic and worm tissues, incubated with Diamox* (2-acetamino-1,3,4-thiadiazole-5-sulfonamide), a specific inhibitor of carbonic anhydrase, or incubated without the substrate sodium bicarbonate, were used as controls.

An experiment was run to determine whether the enzyme carbonic anhydrase was qualitatively affected when male and female *P. strongyloides* were cultured for 24 hr on nutrient agar with and without 0.001 M Diamox incorporated into it. Male and female *P. strongyloides* from these cultures and from calf pancreas were stained as for carbonic anhydrase, described above.

Methods described by Stringfellow (1974) were used to determine whether 0.001 M Diamox affected the migration of male and female *P. strongyloides* under restrained conditions. Diamox at 0.001 M was considered

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* Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may be suitable.

Table 1. Milligrams of ammonia nitrogen produced by male and female *Pelodera strongyloides*.

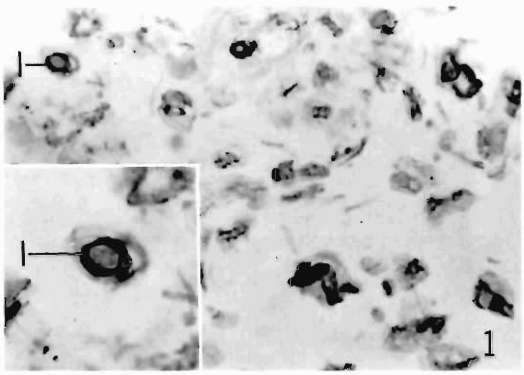
Design	Without 0.001 M Diamox		With 0.001 M Diamox	
	mg NH_3N** 600 worms	Range	mg NH_3N** 600 worms	Range
A*	0.04	0.00–0.08	0.03	0.00–0.06
A+B	0.05	0.00–0.08	0.06	0.00–0.11
A+B+M	0.18	0.14–0.25	0.08	0.00–0.11
A+B+F	0.22	0.06–0.28	0.09	0.03–0.14
A+B+M+F	0.11	0.00–0.24	0.05	0.00–0.11

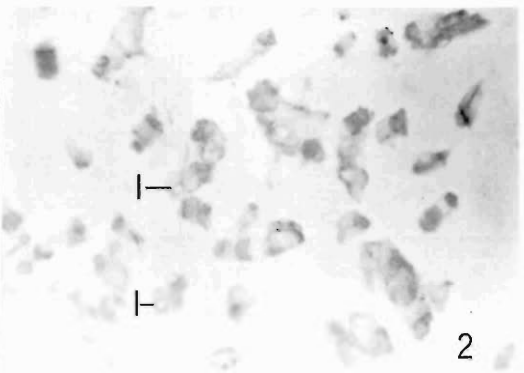
* A = agar; B = bacteria; M = male; F = female.
** Based on 6 repetitions, range of accuracy of the test = 0.05–3 mg.

optimal because the worms had cultured and reproduced normally, and the Diamox apparently did not alter the nutrient agar gel because the worms traveled over it extensively. With the basic design, 50 worms of each sex were tested in each of 4 groups. Group 1 consisted of one viable male and a virgin female nematode placed separately in isolation at posts 1 and 2; whereas, no nematodes were placed at posts 3 and 4, but were inoculated with their associated unidentified bacteria that were grown separately from the worms and did not appear to influence the results. A single worm of a selected sex was placed at the center of the nutrient agar plate and allowed to migrate freely. Groups 2 and 3 were like the first group except that 0.001 M Diamox was incorporated into the nutrient agar petri plates. Group 4 was like groups 2 and 3 except that only isolation apparatuses were at posts 1–4. The freely migrating worms from groups 1, 2, and 4 had no prior exposure to 0.001 M Diamox whereas worms from group 3 had 12 to 24 hr exposure. The data were tested with the Chi square test at 3 degrees of freedom.

Measurement with a Warburg respirometer indicated that, in addition to CO_2, another gas was produced by *P. strongyloides*. A consultation of the literature indicated that the gas most likely was NH_3; so the quantity and pattern of NH_3 produced by male and female *P. strongyloides* were measured with a modified Conway cell (Oser, 1965) with the design given in Table 1. The Conway cell was a petri dish 10 cm in diameter and 1.5 cm deep. A petri dish top 4 cm in diameter served as the center well to absorb NH_3, and two plastic caps 1.5 cm in diameter were placed on opposite sides of the center well to absorb CO_2. A mat of agar containing 0.1% glucose and 0.025% of an antibiotic mixture (Streptomycin: 1 gm; Penicillin: 1×10^6 units; Mycostatin: 5×10^5 units; Fungizone: 50 mg in 110 ml of distilled water) was added to the agar in the petri plate. Five ml of 2% boric acid bromcresol green in distilled water was added to the center well, and 0.4 ml of 20% KOH was added to each of the plastic caps. The bottom half of each petri dish, previously inoculated with 600 males, 600 females, or 300 males: 300 females, was mated and sealed with petrolatum to each top half and incubated inverted at room temperature (22 C). Parallel groups of agar plates, one containing 0.001 M Diamox and one without it, were run concurrently for 3 days when the contents of the center well were titrated with 0.02N of H_2SO_4 until the characteristic yellow color indicated that the starting pH was achieved. Results are reported, corrected with blank determinations, as mg of NH_3 nitrogen per 600 worms where 1 ml of 0.02 N acid equals 0.28 mg nitrogen. An NH_4Cl standard was run to determine the accuracy and suitability of the method; and these data were tested with Student's t test at 5 degrees of freedom. This culture system allowed growth and reproduction of the worms; the associated bacterial flora was used as a food source and contained no protein substrate in the agar which the bacteria could degrade to produce NH_3 nitrogen. The system also inhibited bacterial and fungal growth, absorbed CO_2 which interfered with NH_3 nitrogen measurements, and allowed me to work with a contaminated system because performing these experiments in a sterile manner was not technically feasible.

The ability of *P. strongyloides* to alter the pH of its microenvironment with and without 0.001 M Diamox incorporated into the nutrient agar was measured with the Fisher Model 520 pH meter with Markson microprobe electrodes.





Figures 1–2. 1. Low power of pellet stained with methods for carbonic anhydrase of *P. strongyloides*, showing black intestinal granules (× 60); inset (× 125). 2. Low power of pellet of *P. strongyloides* stained with methods for carbonic anhydrase after inhibition with 0.001 M Diamox, showing the absence of black intestinal granules (× 60).

The procedure of Stringfellow (1974) was used except that no buffer was incorporated into the nutrient agar.

Results

In addition to granules commonly found in the intestine of *P. strongyloides*, fine black granular deposits were detected with methods for carbonic anhydrase (Fig. 1). These deposits were generally distributed throughout the intestine of both males and females posterior to the esophageal bulb. It was not technically feasible to dissect out the worms' intestine and measure quantitatively the carbonic anhydrase activity because of its small size. Few to none of these deposits were detected when reactions of the worm sections were inhibited with 0.001 M Diamox (Fig. 2), and no deposits were detected when the substrate (sodium bicarbonate) was left out of the incubating medium. Pancreatic acinar cells became stained with methods for carbonic anhydrase but did not become stained when inhibited with 0.001 M Diamox or when the substrate was absent from the medium.

The fewer fine black granules detected in the intestines of those worms grown on 0.001 M Diamox indicated possible inhibition of carbonic anhydrase. The overall effect was one of variable staining. Control tissues were stained as mentioned above.

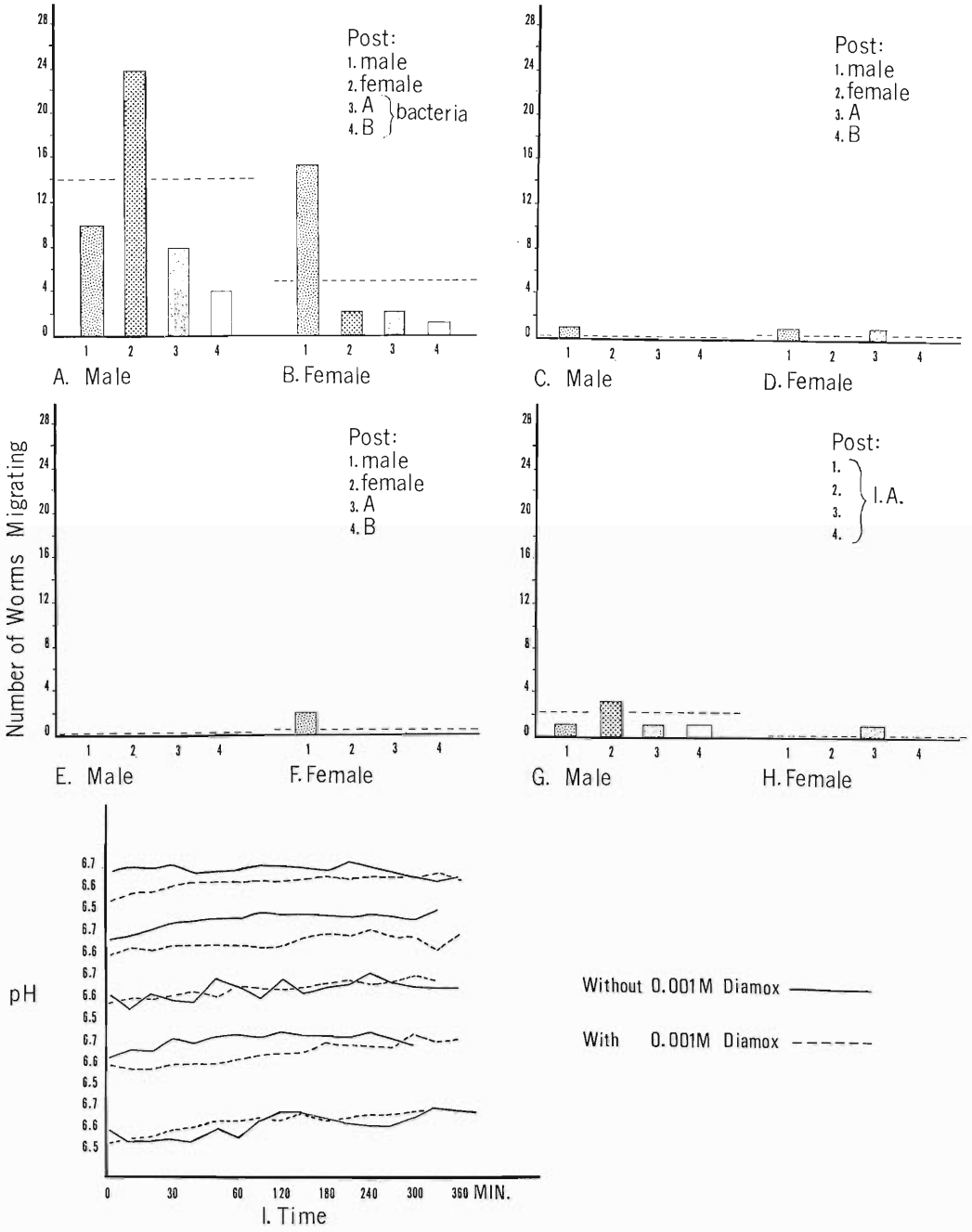
The migration of males and females as influenced by 0.001 M Diamox was as follows:

GROUP 1: When no 0.001 M Diamox was incorporated into the nutrient agar, 46 (90%) of 50 males migrated to all posts (Fig. 3A). The males migrated more times to the female and male than to posts 3 and 4. Twenty-one (42%) of the females migrated to all posts (3B). The females migrated significantly more times to the males than to posts 3 and 4.

GROUP 2: When 0.001 M Diamox was incorporated into the agar with no prior exposure of the worms, 1 (2%) of 50 male worms migrated to all posts (3C). Two (4%) females migrated to all posts (Fig. 3D).

→

Figure 3. A–I. Group 1: A, The male attracted to the male and female as opposed to *attraction* to bacterial controls. B, The female attracted to the male rather than to the female and bacterial controls. Group 2: C, D, Effect of 0.001 M Diamox on decreasing the ability of the male to find both male and female and female to find the male (no prior exposure to 0.001 M Diamox). Group 3: E, F, Effect of 0.001 M Diamox on decreasing the ability of the male to find both male and female and female to find the male (12–24 hr prior exposure to 0.001 M Diamox). Group 4: G, H, Male and female worms equally attracted to all uninoculated isolation apparatus with 0.001 M Diamox incorporated into the nutrient agar (no prior exposure to 0.001 M Diamox). I, pH measurements in microenvironment of worms (16–24



hr prior exposure) with and without 0.001 M Diamox incorporated into the nutrient agar. Top to bottom: nutrient agar blank, bacterial control, 200 males, 200 females, 100 males: 100 females.

Abbreviations: I, intestine; IA, isolation apparatus.

GROUP 3: When the freely migrating worms were given 12–24 hr prior exposure to 0.001 M Diamox, 0 (0%) of 50 male worms migrated to all posts (Fig. 3E). Two (4%) of 50 female worms migrated to all posts (Fig. 3F).

GROUP 4: When 0.001 M Diamox was incorporated into the nutrient agar and only uninoculated isolation apparatuses were placed at each post, 6 (12%) of 50 males migrated to all posts (Fig. 3G); whereas, 1 (2%) of 50 females migrated to all posts (Fig. 3H). The data of groups 2, 3, and 4 reflected nothing more than random migration.

The number of milligrams of NH_3 nitrogen produced by both male and female *P. strongyloides* is summarized in Table 1. Ammonia was variably produced between groups of worms, but those worms cultured with 0.001 M Diamox consistently produced less NH_3 than those cultured without Diamox incorporated into the agar. The bacterial associates did not appreciably produce NH_3; and when males and females were incubated together, they produced significantly less NH_3 than when they were incubated separately.

Measurements of pH in the microenvironment of *P. strongyloides* showed that: (1) Differences were not obvious between nutrient agar with and without bacterial controls and with and without 0.001 M Diamox; (2) male worms not exposed to 0.001 M Diamox periodically altered the pH of their microenvironment but not male worms exposed to Diamox; female worms not exposed and exposed to 0.001 M Diamox did not obviously alter the pH of their microenvironment; (3) male: female worms not exposed to 0.001 M Diamox obviously altered the pH of their microenvironment but not the exposed male: female worms (Fig. 3I).

Discussion

The data show the following relationships: (1) carbonic anhydrase visualized as fine black deposits detected histochemically in the intestines of both male and female *P. strongyloides* was inhibited with 0.001 M Diamox (Figs. 1–2); (2) in general, few fine black intestinal granules were detected in the worms' intestines when males and females were cultured for 12–24 hr on nutrient agar containing 0.001 M Diamox; (3) the ability of both male and female worms to find one another was radically inhibited when 0.001 M Diamox was incorporated into the nutrient agar (Figs. 3A–H); and (4) the quantity of NH_3 variably produced by both male and female worms was reduced when 0.001 M Diamox was incorporated into the agar (Table 1). The data show less clearly that 0.001 M Diamox inhibited the ability of female worm aggregates to alter the pH of their microenvironment (Fig. 3I). The fact that the female produced NH_3 (Table 1) but did not produce definite changes in pH (Fig. 3I) results from the necessity of reporting data sets; other unpublished data clearly show pH changes in the microenvironment of aggregates of females. These relationships indicate that NH_3, a strong nucleophile variably produced by aggregates of both male and female *P. strongyloides*, contributed to alter the pH of their microenvironment to the alkaline side even though it was not technically feasible to determine if NH_3 and the alkaline pH were produced concurrently. Ward (1973) and Stringfellow (1974) showed that *Caenorhabditis elegans* and the males of *P. strongyloides* were sensitive to and attracted to an alkaline pH. Both on agar and in nature, *P. strongyloides* lives in an aqueous medium that serves well to remove NH_3, a toxic end product of nitrogen metabolism. These data do not exclude the possibility that compounds other than NH_3 produced by the worms might alter their microenvironment; the data also sheds little or no light about the female other than that aggregates variably produce NH_3 producing an alkaline microenvironment.

These data indicate that carbonic anhydrase influenced the production of NH_3 by this worm. Certainly, results in the present study indicate its presence in the intestine of *P. strongyloides* (Figs. 1–2) but its function remains as yet unknown in nematodes. Scott and Whittaker (1970) suggested that NH_3 was the only product of purine degradation in *P. strongyloides* which has nothing to do with carbonic anhydrase. The action of Diamox may affect other metabolic pathways known to be directly or indirectly involved in NH_3 production (Carter, 1972).

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Endoparasites of Selected Populations of Gray Squirrels (*Sciurus carolinensis*) in the Southeastern United States[1]

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ABSTRACT: Examination of 270 gray squirrels (*Sciurus carolinensis*) from 18 localities in the southeastern United States revealed 29 species of endoparasites, including 5 protozoans, 2 trematodes, 4 cestodes, 1 acanthocephalan, and 17 nematodes. Five of these represented new host records, and one new species of filarial worm was recovered. Data are presented on the prevalence and intensity of infection with each species along with information on geographical distribution. Seven parasites (*Hepatozoon griseisciuri*, *Taenia* sp., *Baylisascaris procyonis*, *Dirofilariaeformia pulmoni*, *Heligmodendrium hassalli*, *Physocephalus sexalatus*, and *Strongyloides robustus*) were observed to produce lesions in the host. Numbers of some intestinal nematodes differed significantly between seasons, age classes, potential natural vegetative types, and localities.

Gray squirrels, *Sciurus carolinensis*, harbor numerous species of endoparasites, but little information is available on the distribution, prevalence, or intensity of infection (Clarke, 1959; Parker, 1971). Similarly, the pathogenicity of most endoparasites and their role as mortality factors in gray squirrel populations remain largely speculative.

Parasitism has been considered a possible factor initiating emigrations of gray squirrels. Although Flyger (1969) did not attribute much significance to parasitism of squirrels emigrating during 1968, Parker and Holliman (1971) emphasized the need for additional parasitologic data during ordinary conditions to serve as a basis for evaluating parasitism during emigrations.

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In an effort to obtain information on endoparasitism in gray squirrels of the southeastern United States, a study was undertaken to (1) identify the endoparasitic fauna; (2) ascertain the prevalence and intensity of infections; (3) evaluate differences attributable to season, age, sex, vegetative type, or collection site; and (4) evaluate the pathogenicity of each species.

Materials and Methods

Three collection sites were sampled in each of the six major potential natural vegetative types (Küchler, 1964) of the southeastern United States: Appalachian oak, oak-hickory, oak-hickory-pine, southern floodplain, southern mixed, and mixed mesophytic (Fig. 1). Five gray squirrels were collected from each site by shooting within the periods of 1–24 May 1972, 15 August–9 September 1972, and 2 January–8 February 1973.

Squirrels were placed in individual plastic bags, kept on ice, and necropsied within 4 hr

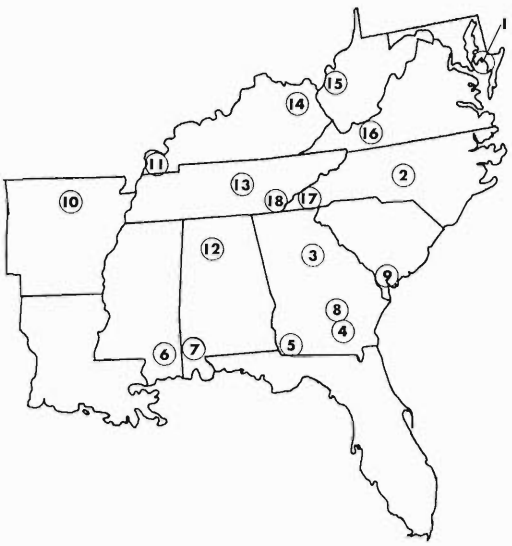

Figure 1. Distribution of collection sites within the southeastern United States. Location of sites within potential natural vegetative types are as follows: (1) Dorchester Co., (2) Montgomery Co., (3) Oglethorpe Co.—Oak–hickory–pine; (4) Jeff Davis Co., (5) Decatur Co., (6) Forrest Co.—Southern mixed; (7) Clarke Co., (8) Jeff Davis Co., (9) Hampton Co.—Southern floodplain; (10) Stone Co., (11) Trigg Co., (12) Lawrence Co.—Oak–hickory; (13) Cumberland Co., (14) Rowan Co., (15) Mason Co.—Mixed mesophytic; and (16) Montgomery Co., (17) Buncombe Co., (18) Monroe Co.—Appalachian oak.

after collection. After skinning, major organs were removed and examined for helminth parasites. The gastrointestinal tract was opened, scraped, the contents washed separately through 100 mesh screens, and the retained material preserved in formalin (5%)-acetic acid (2.5%) solution. Examination for intestinal protozoans was made by direct saline smears. When large numbers of coccidian oocysts were observed, a portion of the intestinal contents was collected in 2.5% potassium dichromate solution, refrigerated, and the oocysts subsequently sporulated at room temperature. Methods of examination for blood parasites and histologic techniques were described elsewhere (Davidson and Calpin, 1975).

Complete parasite counts were made. Parasite numbers and other pertinent data were coded and placed on computer cards. Data on helminth parasites which occurred in more than 25% of the squirrels were analyzed by an analysis of variance test (Service, 1972) for differences related to season, age, sex, vegetative type, or collection site. Prior to analysis, parasite numbers were changed to approximate a normal distribution by the log of n + 1 transformation.

Results and Discussion

Examination of 270 gray squirrels revealed 5 protozoans, 2 trematodes, 4 cestodes, 1 acanthocephalan, and 17 nematodes (Table 1). One species of nematode was previously undescribed, and the gray squirrel was established as a new host for five species. Parasites are discussed in order of prevalence within each phylogenetic group.

Protozoa

Coccidian oocysts similar morphologically to *Eimeria ascotensis*, *E. lancasterensis*, *E. moelleri*, and *E. neosciuri* were observed. Since oocyst morphology often is not adequate for differentiating species, all thin-walled coccidia were considered as *Eimeria* spp. Two species with unique thick-walled oocysts, *E. confusa* and *E. ontarioensis*, were easily distinguished in intestinal smears but were detected rarely. Joseph (1971, 1972) reported that two successive infections with *E. confusa* conferred immunity to subsequent infections. Acquired immunity may account for the low prevalence of infection with this species. Squirrels from the oak-hickory vegetative type had a significantly lower ($P < 0.01$) prevalence of *Eimeria* spp. Joseph (1973, 1975) demonstrated that most eimerians described from gray squirrels occur naturally in or are transmissible to fox squirrels (*S. niger*); thus the epizootiology of these sciurid coccidia apparently involves two hosts.

The pathogenicity of coccidia in gray squirrels apparently varies among species. Webster (1960) noted significant lesions due to *E. neosciuri* infection whereas Joseph (1972) considered *E. confusa* and *E. lancasterensis* as non-pathogenic. Although massive infections with any species probably would be detrimental, the absence of significant lesions due to coccidiosis during this study sug-

Table 1. Endoparasites recovered from 270 gray squirrels collected from the southeastern United States.

Parasite	Percent prevalence	Number per infection		Distribution
		Mean	Range	
PROTOZOA				
Eimeria spp. (1)*	81	–**	–	1–18†
Eimeria confusa (1)	1	–	–	5, 17
Eimeria ontarioensis (1)	1	–	–	8, 16, 17
Hepatozoon griseisciuri (6, 7)	41	–	–	1–12, 14–18
Sarcocystis sp. (5)	8	–	–	1, 2, 4, 5, 7–9, 12, 16, 18
TREMATODA				
Brachylaima sp. (1)	<1	2	1–2	1, 17
Nudacotyle norvica (2)	<1	2	2	7
CESTODA				
Catenotaenia dendritica (1)	<1	2	1–2	11, 18
Hymenolepis diminuta (1)	2	2	1–4	11, 14, 15
Raillietina bakeri (1)	6	4	1–19	5–7, 12
Taenia sp. (7, 8)	3	1	1–3	2, 5, 8, 10, 12
ACANTHOCEPHALA				
Moniliformis clarki (1)	1	1	1	7, 9
NEMATODA				
Ascaris sp. (1)	<1	1	1	18
Baylisascaris procyonis (11)	<1	11	11	1
Bohmiella wilsoni (2)	29	6	1–48	2–10, 12
Capillaria americana (1)	14	3	1–12	1, 4–6, 8–11, 14–16
Citellinema bifurcatum (1)	37	14	1–108	2, 3, 5, 7, 9–18
Dipetalonema interstitium (9)	5	5	1–50	3–5, 9, 13
Dirofilariaeformia pulmoni (10)	2	–	–	1, 11
Enterobius sciuri (3)	26	11	1–152	1–18
Gongylonema pulchrum (4)	5	3	1–29	2, 4, 5, 7–9, 13, 16, 18
Heligmodendrium hassalli (1)	89	124	1–964	1–18
Microfilariae (6)††	31	–	–	1–10, 12–14, 16
Physocephalus sexalatus (2)	<1	41	41	3
Physaloptera sp. (2)	<1	1	1	15
Pterygodermatites parkeri (1)	3	1	1–3	2, 11, 13, 14
Strongyloides robustus (1)	86	51	1–568	1–18
Syphacia thompsoni (3)	5	5	1–34	2, 6, 9, 10, 12, 14, 15, 17
Trichostrongylus affinis (3)	3	2	1–6	3, 5–8
Trichostrongylus calcaratus (3)	16	3	1–36	1–9, 12, 13, 15, 17

* The numbers in parentheses indicate locations in the host: (1) small intestine, (2) stomach, (3) cecum and large intestine, (4) esophagus, (5) muscle, (6) blood, (7) lung, (8) liver, (9) subcutaneous, (10) pulmonary arteries, and (11) thoracic cavity.
** Value not determined.
† Map number.
†† Morphologically similar to *D. interstitium microfilariae.*

gests that coccidiosis is not a major problem in squirrels in the Southeast.

Information on *Hepatozoon griseisciuri* infection in these animals has been presented in detail (Davidson and Calpin, 1976). These authors emphasized that the lungs were a major site of schizogonic development, discussed the probable chronologic epizootiology, and postulated that *H. griseisciuri* infection might be related to mid-winter mortality in gray squirrels.

The prevalence of *Sarcocystis* sp. must be considered minimal since examination of additional muscle sections undoubtedly would have revealed more infected hosts. All sporozoan cysts detected in skeletal muscle tentatively were designated as *Sarcocystis* sp. Recent reports (Frenkel, 1974; Frenkel and Dubey, 1975) suggest that transmission studies are required to differentiate *Sarcocystis* from *Hammondia.* Although the status of the gray squirrel in the life cycle of *Sarcocystis* sp. is unknown, it presumably is similar to that of other intermediate hosts (Frenkel, 1974), with wild carnivores as final hosts. The gray squirrel is established as a new host for *Sarcocystis* sp.

Trematoda

Flukes were encountered rarely, and their sporadic occurrence apparently is related to the dietary habits of squirrels resulting in infrequent ingestion of suitable intermediate hosts.

Brachylaima sp. was recovered from only two squirrels. Although specimens resembled *B. virginiana*, the limited number of specimens precluded identification to species. The gray squirrel is recorded as a new host for *Brachylaima* sp.

Nudacotyle norvica, a common parasite of muskrats, was recovered from a single squirrel. Welborn (1975) recently found *N. norvica* in gray squirrels of western Tennessee, and Olexik et al. (1969) reported *Nudacotyle* sp., probably *N. norvica*, from the same locality.

Cestoda

Raillietina bakeri occurred frequently and with high intensity in several southern sites but did not occur in northern sites. Previous reports of this cestode in other hosts also have been from southern areas (Chandler, 1942; Harkema, 1946; Huggins, 1951), suggesting that the distribution of *R. bakeri* may be related to the distribution of suitable intermediate hosts. The gray squirrel is established as a new host for *R. bakeri*.

Hymenolepis diminuta and *Catenotaenia dendritica* occurred infrequently and in low numbers. Although both species have been reported previously from gray squirrels (Rausch and Tiner, 1948; Parker, 1971; Welborn, 1975), the low prevalence of infection in these reports and the present study suggest that the gray squirrel is an accidental host to both helminths.

Cysticerci of *Taenia* sp. conformed to the morphologic description of *T. pisiformis*; however, three viable cysticerci failed to develop when fed to a young, cestode-free dog. The specific identity of these cestodes therefore is uncertain. Cysticerci were found only in subadult or adult squirrels. Minor lesions associated with *Taenia* sp. infections were focal fibrotic hepatitis.

Acanthocephala

Moniliformis clarki occurred with a prevalence and intensity of infection similar to previous reports in gray squirrels (Parker, 1971; Welborn, 1975). Although none of the squirrels examined had lesions attributable to *M. clarki*, Moore (1946) reported fatal peritonitis in flying squirrels (*Glaucomys volans*) due to heavy *M. clarki* infections. Because of its large size, *M. clarki* also might occlude the intestinal lumen if present in high numbers. The pathogenic potential of *M. clarki* is offset by its infrequent and localized occurrence (Chandler, 1947; Parker, 1971; Welborn, 1975).

Nematoda

Heligmodendrium hassalli was the most frequently encountered parasite and was recovered from each collection site. Harkema (1936), Chandler (1942), and Welborn (1975) also found *H. hassalli* to be the predominant parasite of gray squirrels.

Juvenile squirrels had significantly lower ($P < 0.01$) numbers of *H. hassalli* than subadults, and this difference was attributed to less exposure of the juveniles to infective larvae.

Squirrels from the oak-hickory-pine vegetative type had significantly higher ($P < 0.01$) numbers of *H. hassalli* than squirrels from all other vegetative types. Although lower than in squirrels from oak-hickory areas, squirrels from southern mixed and southern floodplain vegetative types had significantly higher ($P < 0.01$) numbers of *H. hassalli* than did squirrels from the three remaining vegetative types. These differences were attributed to two factors. First, squirrel population densities were generally higher on sites representative of these vegetative types. Welborn (1975) found a positive correlation between nematode infection intensities and squirrel density as estimated by hunter success indices. Second, these sites were in milder climatic regions where survival of eggs and larvae probably would be higher.

Gross and microscopic lesions associated with intense *H. hassalli* infections were mucosal hyperemia and hemorrhagic enteritis. These lesions were found in less than 10% of the squirrels and only in those harboring greater than 300 worms.

Strongyloides robustus had a prevalence of infection and distribution very similar to *H. hassalli*. Although always lower, average numbers of *S. robustus* in squirrels from each site paralleled average number of *H. hassalli* (correlation coefficient = 0.7043). Similar trends also were noted for infections within different age classes and vegetative types. Factors

responsible presumably are the same as postulated for *H. hasalli*.

Strongyloides robustus was the most pathogenic helminth found with substantial frequency. Infections of greater than 150 worms frequently caused a severe hemorrhagic enteritis. Parker (1971) considered *S. robustus* to be the most pathogenic nematode in squirrels of southwestern Virginia. Consistent with the findings of Parker (1971), lesions associated with *S. robustus* were confined to the duodenum.

Citellinema bifurcatum had a prevalence and intensity of infection similar to previous reports (Katz, 1938; Olexik et al. 1969; Parker, 1971; Welborn, 1975). Although *C. bifurcatum* occurred in squirrels from all vegetative types, infections were significantly lower ($P < 0.05$) in squirrels from the southern floodplain and southern mixed vegetative types. In this respect, *C. bifurcatum* apparently is influenced more by environmental conditions than host densities.

Bohmiella wilsoni was recovered frequently from squirrels in all southern collection sites but was absent in northern areas. Although *B. wilsoni* has been reported from more northern areas (Lucker, 1943; Rausch and Tiner, 1948; Parker, 1971; Welborn, 1975), data from this study indicate that *B. wilsoni* is found more frequently in gray squirrels from southern portions of their range.

Enterobius sciuri, which was only recently detected in gray squirrels in North America (Parker, 1971; Parker and Holliman, 1971; Welborn, 1975), had a much higher prevalence than previously reported. This helminth occurred in squirrels from each collection site and was present in significantly higher ($P < 0.01$) numbers during the winter than in other seasons.

Prevalence and intensity of infection by *Capillaria americana* were similar to the findings of Parker (1971) and Welborn (1975). Although this helminth occurred infrequently, it was widely distributed in southeastern gray squirrels.

Dipetalonema interstitium is reported for the second time, having been originally described from gray squirrels in Maryland (Price, 1962). Infection intensities usually were similar to those reported by Price (1962); however, one squirrel harbored a much larger number of worms (50) than had been reported. The high prevalence and wide distribution of microfilariae suggest that *D. interstitium* occurs throughout the non-mountainous areas of the Southeast.

The prevalence and intensity of *Pterygodermatites parkeri* was similar to previous reports (Parker, 1971; Welborn, 1975). According to Parker (1971), worms of the genus *Rictularia* previously reported from gray squirrels (Katz, 1938; Rausch and Tiner, 1948; Parker 1968; Olexik et al. 1969) should be considered *Pterygodermatites*, and in all probability *P. parkeri*.

A description of and information on *Dirofilariaeformia pulmoni* was presented by Davidson (1975). Circulatory lesions associated with *D. pulmoni* were considered severe and conceivably could result in occasional mortality.

Based on the low prevalence of infection and infectivity for other hosts, the gray squirrel probably is an accidental host for *Trichostrongylus calcaratus*, *T. affinis*, *Syphacia thompsoni*, *Gongylonema pulchrum*, *Physocephalus sexulatus*, *Physaloptera* sp., *Ascaris* sp., and *B. procyonis*. The occurrences of *T. affinis* and *P. sexulatus* in the gray squirrel constitute new hosts records.

Although infections apparently were accidental, the pathologic consequences of *P. sexulatus* and *B. procyonis* warrant consideration. Ulcerative gastritis associated with *P. sexulatus*, the swine stomach worm, was the most severe lesion encountered, and such infections would undoubtedly result in occasional mortality. Domestic swine frequented the site from which the squirrel originated, and similar infections could be expected on areas cohabited by squirrels and wild or feral swine.

The ability of *B. procyonis* (= *Ascaris columnaris* of Tiner 1949) larvae to produce fatal neurologic disease in gray squirrels has been demonstrated experimentally (Tiner, 1949). Four of six experimentally-infected squirrels died, and the two survivors had larvae encysted in the myocardium, pericardium, caval veins, lungs, and pulmonary pleura. Larvae found during this study were in similar locations. Considering that infected hosts which develop neurologic disease are more susceptible to predation (Tiner, 1949) and that recently an

epizootic of this disease has occurred within the Southeast (Nettles et al. 1975), the low prevalence of *B. procyonis* may not be indicative of the significance of this helminth for gray squirrel populations.

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Development of *Hammondia hammondi* in Cell Cultures

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ABSTRACT: Artificially excysted sporozoites of *Hammondia hammondi* invade and multiply within primary cell cultures of monkey kidney or mouse embryo or a cell line of human diploid fibroblasts (WI-38). Division was observed at approximately daily intervals through 8 days. Endodyogeny was the only type of division seen. Continuous multiplication was not achieved in long term culture although organisms were present in culture fluids for 4–6 weeks. *H. hammondi* organisms had typical coccidian ultrastructure when observed after penetration and during division. A double membrane pellicle with underlying microtubules, a conoid, micronemes, rhoptries, and a micropore, all resembling those of *Toxoplasma gondii*, were present. A significant difference is that *H. hammondi* organisms contain storage granules which appear to be depleted following cell invasion. Ultrastructure, cultivation, and division of *H. hammondi* are indicative of its close relation to *Toxoplasma gondii*.

Coccidian oocysts resembling those of *Toxoplasma gondii* were recovered from the feces of a cat in Hawaii by Wallace (1973). When these oocysts were fed, after sporulation, to mice toxoplasma-like cysts were found in skeletal muscle and occasionally in the central nervous system. Immunological studies and transmission experiments between cats and between mice indicated that the parasite was not *T. gondii*. Wallace designated the organism WC1170 and presumed it to be *Sarcocystis muris*. Further study allowed differentiation between the latter two parasites (Wallace, 1975). An organism having the same characteristics as WC1170 was isolated from a cat in Kansas by Frenkel (1974) and named *Hammondia hammondi* gen. n. et sp. n.

Hammondia hammondi has similarities to *Toxoplasma*, *Sarcocystis*, and *Besnoitia* (Frenkel, 1974; Wallace, 1975; Wallace and Frenkel, 1975). The oocyst, an isosporan type which morphologically resembles that of *Toxoplasma*, is passed in the unsporulated state and sporulates within 72 hr. When oocysts are fed to mice cysts are produced in the skeletal muscle; bradyzoites within the cysts resemble those of *Toxoplasma*. Schizonts and gametocytes produced in the intestinal epithelium of cats are similar to those of *Toxoplasma*. *H. hammondi* resembles members of the genus *Sarcocystis* by producing cysts in skeletal muscle and, more importantly, by requiring an intermediate host for transmission.

The ultrastructure of the sporozoite of *H. hammondi* closely resembles that of *T. gondii* (Sheffield and Melton, 1974) and the ultrastructural characteristics of *T. gondii* development in cultured cells are known (Sheffield

and Melton, 1968). Therefore, an *in vitro* study of *H. hammondi* was undertaken to further differentiate these two organisms.

Materials and Methods

Oocysts of the WC1170 strain of *Hammondia hammondi* isolated from a cat in Hawaii by Wallace (1973) were fed to laboratory mice. Cyst-containing skeletal muscle of the mice was fed to cats to provide material for this study. Oocysts collected from the experimentally infected cats were sporulated at room temperature in 1% sulfuric acid then stored at 4 C until use. Prior to excystation, sporulated oocysts were rinsed, then treated with 2.5% sodium hypochlorite to facilitate rupture of the oocyst wall. Following removal of the sodium hypochlorite by washing with water, the oocysts were suspended in Eagle's medium and sonicated for 40–60 sec. The sporocysts freed by sonication were then incubated in excystation fluid consisting of 0.75% sodium taurocholate and 0.25% trypsin in Eagle's medium for 1 hr at 37 C to obtain sporozoites for culture inoculation.

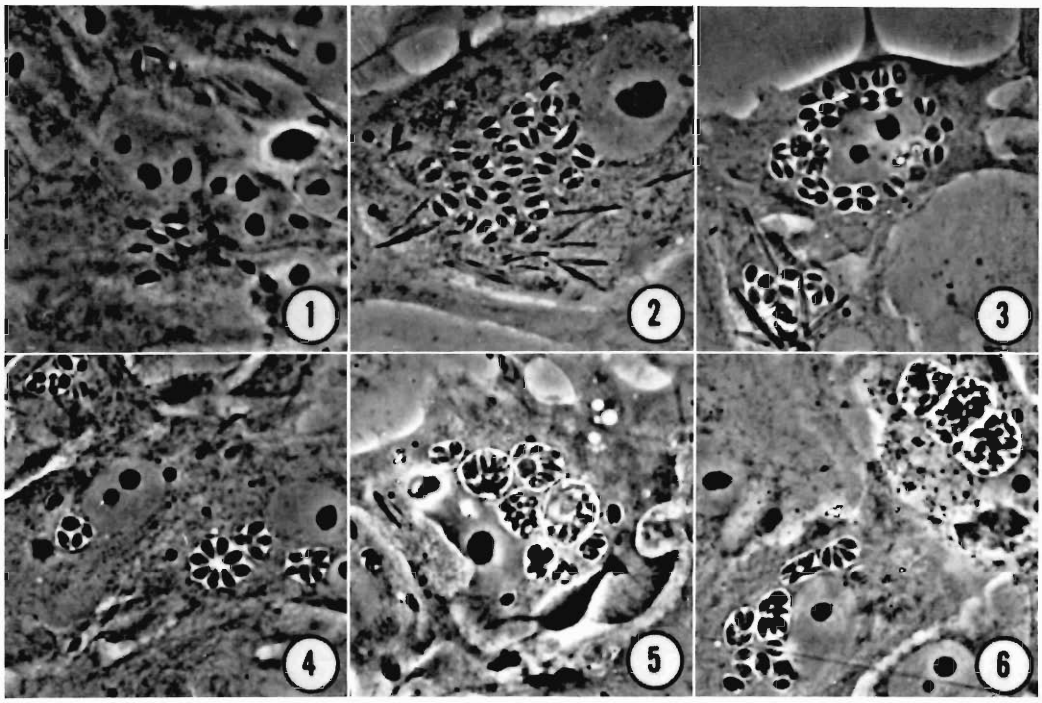
Primary rhesus monkey kidney cells or WI-38 human diploid fibroblasts (19–25th passage) grown in small plastic culture dishes or on glass or plastic coverslips in Leighton tubes were inoculated with one half to three million excysted sporozoites suspended in Eagle's medium. One half to one hour later cultures were renewed with fresh medium. Infected cultures were fixed in 5% glutaraldehyde in phosphate buffer, pH 7.3, at 24 hr intervals for 8 days after inoculation. Some cultures were rinsed with water after fixation and mounted with glycerin jelly for examination by phase microscopy. Others were postfixed with OsO_4, dehydrated and embedded *in situ* on coverslips or in culture dishes. Thin sections were cut and stained with uranyl acetate and lead citrate for observation in the electron microscope.

For long term cultivation studies (1 to 18 weeks) primary cell cultures of monkey kidney and whole mouse embryo as well as WI-38 human diploid fibroblasts were used. Cultures were maintained at 37 C in either stationary plastic flasks with 75 cm^2 area or 2×15 cm culture tubes in a roller drum. Eagle's medium with 5% heated fetal bovine serum, glutamine, and penicillin and streptomycin was used for all cultures. Cultures were infected with varying numbers of excysted sporozoites. Multiplication of organisms was determined by microscopic observation of living cultures, or stained cultures on coverslips and by counting free organisms in the culture medium. Cells for transfer or subculture were obtained by typsinization or scraping.

Results

Within 24 hr after introduction into cultures, sporozoites of *Hammondia hammondi* penetrated into the culture cells and were situated in a parasitophorous vacuole (Fig. 1). Penetration appeared to be random with some cells containing a single parasite whereas others contained many. By 48 hr division occurred resulting in two organisms lying side by side in the parasitophorous vacuole as is typical in division by endodyogeny (Fig. 2). Vacuoles commonly contained four parasites by the third day although pairs and single organisms were present (Fig. 3). Rosettes composed of eight parasites apparently resulted from three sequential divisions and were found four days after inoculation of the cultures (Fig. 4). On days 6 and 8 the parasitophorous vacuoles were large and contained numerous parasites (Figs. 5 and 6). Smaller vacuoles were also common and contained organisms formed by one to several divisions. In some vacuoles the posterior ends of the parasites appeared to be attached to a residual mass.

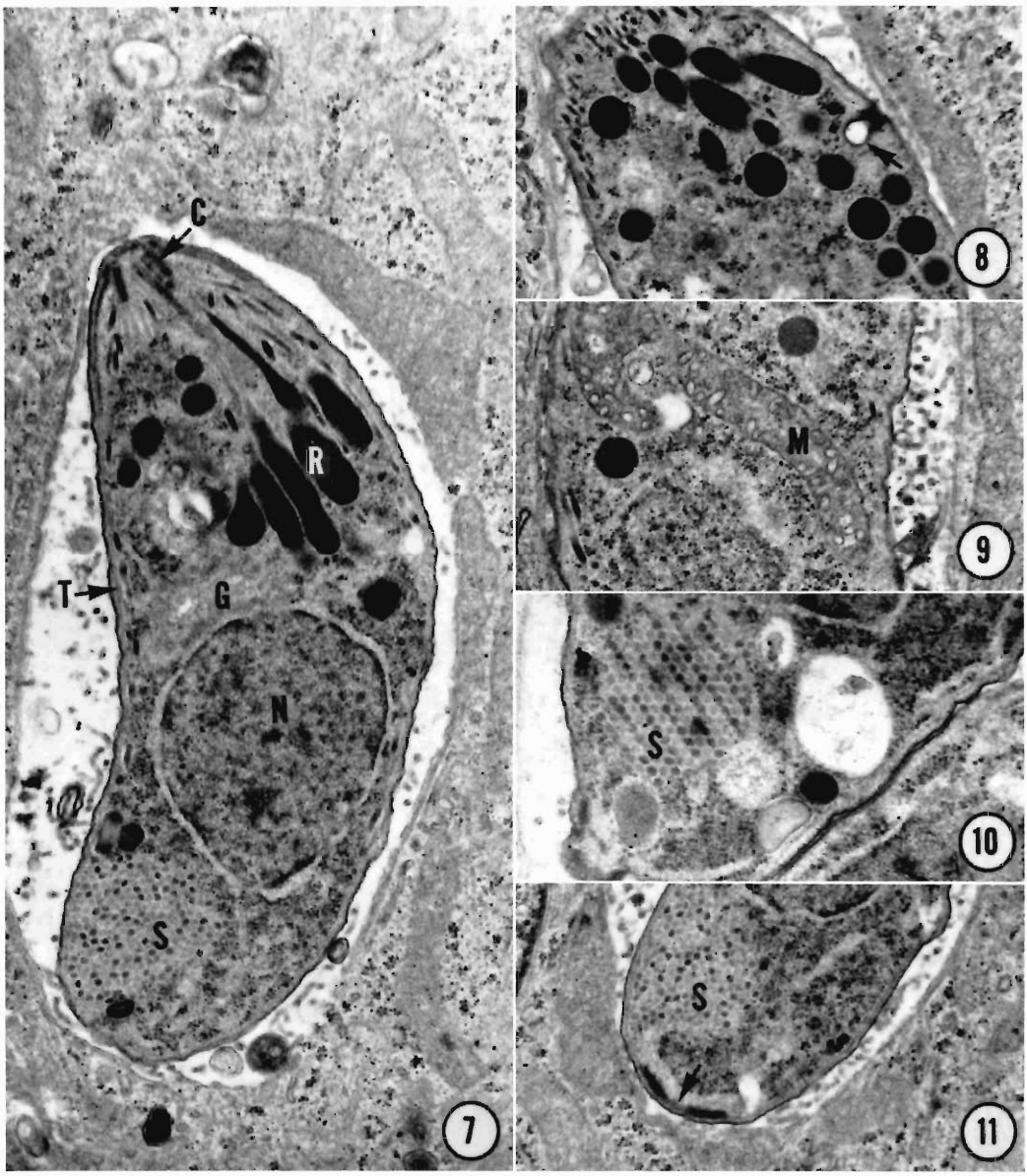
A longitudinal section of a typical *H. hammondi* is shown in Figure 7. The organism is somewhat crescent-shaped and is surrounded by a plasma membrane which is more electron dense than the membrane of the parasitophorous vacuole in which the parasite is situated. Numerous stereocilia are also present within the vacuole. The pellicular complex of the organism consists of the plasma membrane, a less dense inner membrane and a series of microtubules. The inner membrane lies subjacent to the outer or plasma membrane and is continuous except for openings at the anterior end, posterior end, and micropore. A thick area of dense material is present on the cytoplasmic side of the inner membrane surrounding the posterior opening (Fig. 11). The microtubules extend longitudinally from the



Figures 1–6. Light micrographs of *Hammondia hammondi* in monkey kidney cell cultures. Phase contrast. 1. Fixed 1 day after infection. 2. Two days. 3. Three days. 4. Four days. 5. Six days. 6. Eight days.

polar ring, which is a thickening of the anterior portion of the inner membrane, to the level of the nucleus or beyond. A typical conoid is present at the anterior end of the parasite. Associated with it and filling most of the anterior end are numerous micronemes and rhoptries. The latter are very electron dense in the bulbous portion and have thin necks which extend to the anterior tip of the organism through the conoid. The micropore, an invagination of the plasma membrane through a collar formed by the inner membrane, is laterally situated in the anterior one third of the parasite (Fig. 8). Vacuoles containing particulate material are sometimes present near the micropore and may result from the micropore activity. One or more mitochondria, or perhaps a single branched one, lie in the area posterior to the rhoptries (Fig. 9). Occasionally a mitochondrion extends alongside the nucleus and to the posterior end. The mitochondria have numerous bulbous or tubular cristae. The nucleus is centrally located and contains much heterochromatin at its periphery. A large nucleolus has been observed in some specimens. The Golgi complex is situated at the anterior edge of the nucleus (Fig. 7). Organisms which have recently penetrated the host cell and have not yet divided usually contain an accumulation of small granules, about 400 Å in diameter, in the region between the nucleus and the posterior end (Fig. 7). In opportune sections the granules can be seen in a hexagonal arrangement (Fig. 10) and appear to constitute a structure termed the crystalloid body by earlier workers (see Discussion). In parasites that have divided this structure is usually absent (Figs. 12–14) and larger, more dense granules are often present (Fig. 13). An occasional amylopectin granule has been observed in the posterior region (Fig. 7).

Examination of cultures over an 8 day period revealed that *H. hammondi* divides, in cultured cells, exclusively by endodyogeny. By 48 hr



Figures 7–11. Electron micrographs of *Hammondia hammondi*. 7. Longitudinal section of organism in parasitophorous vacuole. C, conoid; G, Golgi; N, nucleus; R, rhoptry; S, storage granules; T, subpellicular microtubules. × 20,250. 8. Anterior end of parasite containing dense rhoptries, micronemes (N), and a micropore (arrow). × 20,150. 9. Mid-region of parasite showing mitochondrion (M) with tubular cristae. × 19,700. 10. Crystalline-like arrangement of storage granules (S) in posterior end. × 29,250. 11. Posterior end of parasite with thickening of inner membrane (arrow). × 20,100.

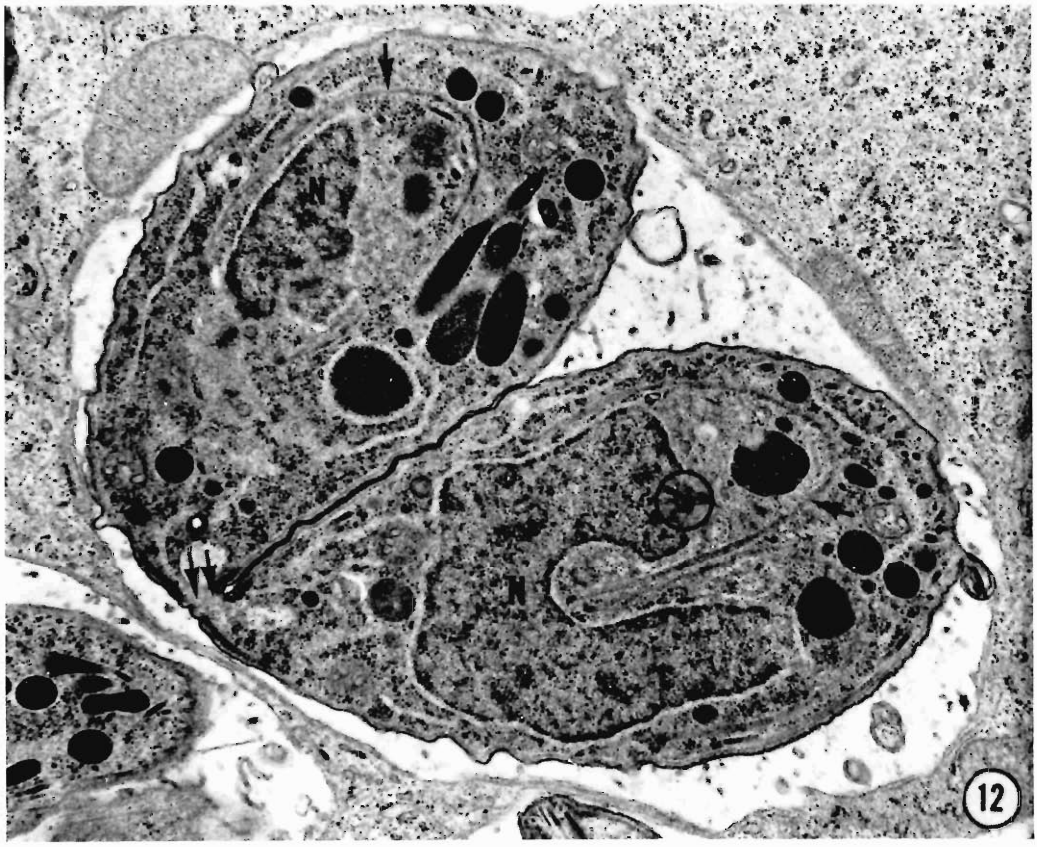


Figure 12. Electron micrograph of dividing *Hammondia hammondi*. Both organisms contain new inner membrane (arrow) of daughter cell partially encircling nucleus (N). Even though second division is occurring the progeny of the first division are still connected at their posterior ends (double arrow) where thickened termination of inner membranes is present. In the lower parasite the nucleus is horseshoe-shaped and the polar spindle is visible at one end (circle). Centrioles are also visible between the ends of the nucleus. × 22,000.

the organism has finished one division and 2 offspring are contained within the same parasitophorous vacuole (Fig. 13). Often organisms initiated a second division before they completely separated after the first division (Fig. 12). In the earliest stages of endodyogeny observed the anterior portion of the daughter cell's inner membrane had formed anterior to the nucleus. A conoid, a large dense granule which presumably is the anlagen of the rhoptries and pellicular microtubules were present within the developing inner membrane. In organisms seen in a later stage of division the daughter membranes were extended posteriorly and the nucleus had become horseshoe-shaped. A polar spindle was present on the anterior surface of the nucleus and a centriole was located near the spindle in each daughter cell (Fig. 12). Sequential divisions result in rosette forms or groups of organisms contained within single vacuoles (Figs. 4 and 14).

In long term cultivation studies it was not possible to obtain continuous multiplication of *H. hammondi* in cell cultures. In four separate trials, using sporozoites excysted from oocysts of two different cats, continuous multiplication of the organism could not be established.

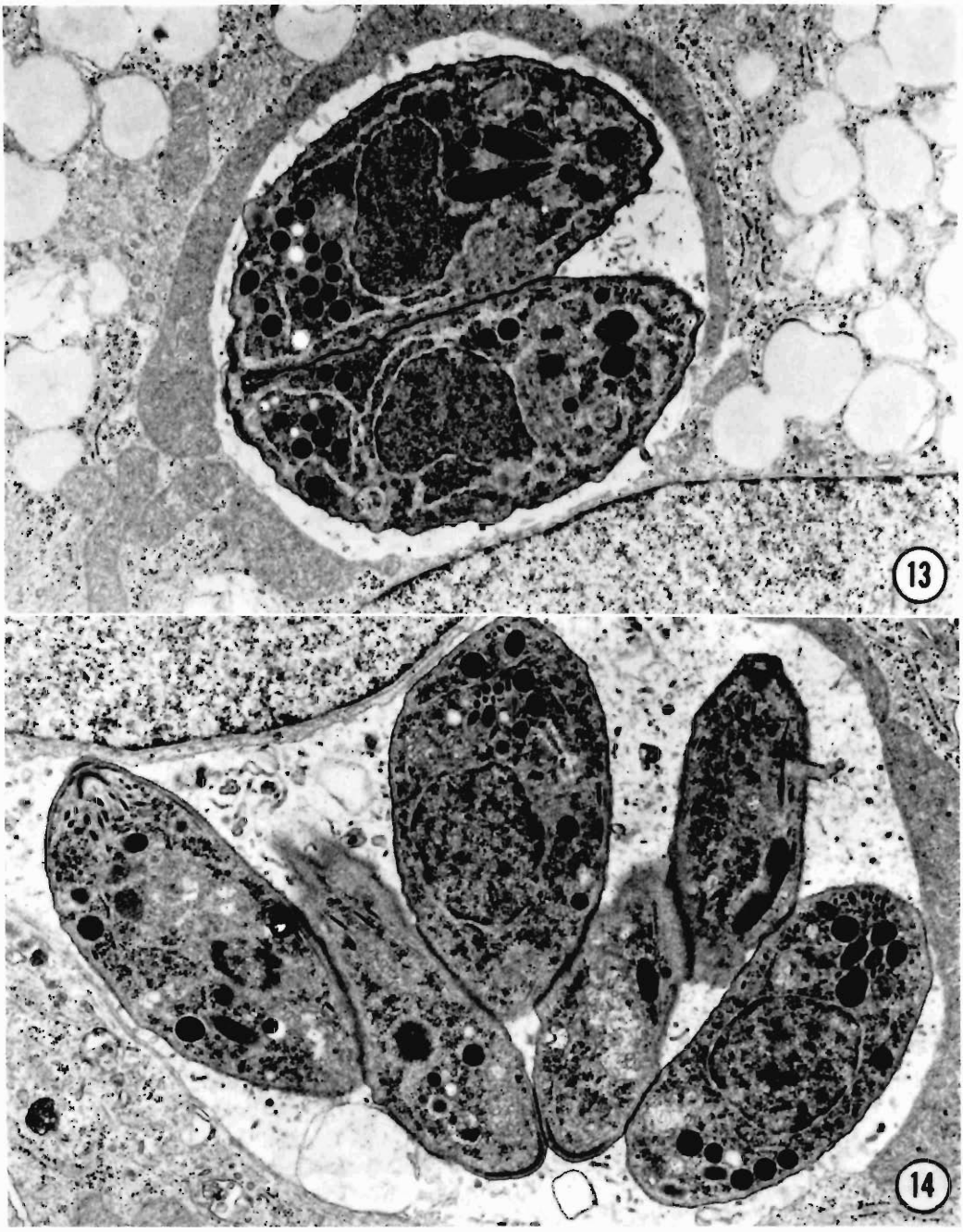


Figure 13–14. Electron micrographs of *Hammondia hammondi*. 13. Recently divided parasite with daughter cells joined by narrow region at posterior ends. Note absence of storage granules and presence of several dense spherical bodies. × 11,500. 14. Parasitophorous vacuole containing, probably, 8 parasites (6 visible) in this section. Note absence of storage granules. × 12,350.

Table 1. Progressive decline in organisms from monkey kidney cultures inoculated with *H. hammondi*.

	Day of harvest		
	21	27	30
Average number of motile organisms per culture*	40,500	9,000	2,500

* Fluids from 2 to 4 cell cultures pooled, centrifuged and motile zoites (? sporozoite or trophozoites) counted.

Inocula consisted of 20,000 to 184,000 motile sporozoites per culture vessel. In primary cell cultures of monkey kidney or mouse embryo or a cell line of human diploid fibroblasts (WI-38) there was evidence for initial infection of cells and multiplication of the parasites as judged by direct observation of intracellular organisms in cultures, by microscopic examination of stained coverslips removed from infected cultures, and the finding of motile organisms (? sporozoites or trophozoites) in the centrifuged sediments of culture fluids for several weeks after inoculation. In one representative experiment (Table 1), the numbers of recognizable parasites from infected cultures progressively decreased. Organisms were no longer found in centrifuged fluids from cultures 4 to 6 weeks after inoculation.

Attempts to transfer or subculture organisms to a second culture also failed to establish continuous growth of the parasite. In one series of experiments four serial passages were carried out; the first into mouse embryo cells for 44 days, the second into mouse and beef embryo, monkey kidney and WI-38 human fibroblasts for 40 to 112 days, the third passage to WI-38 cells for 33 days, and a final passage to WI-38 cells for 125 days. Recognizable organisms could only be seen in material used for the second passage (day 44 fluids of first passage) and were not seen thereafter.

In some instances cell cultures which had previously been inoculated with sporozoites and failed to show evidence of their multiplication were "challenged" with trophozoites of *Toxoplasma gondii* (RH strain from peritoneal cavity of infected mouse). The toxoplasma challenge for such cultures and appropriate controls was applied more than 2 months after inoculation with sporozoites. Toxoplasma infection readily developed and destroyed inoculated cultures with no indication that they were resistant to challenge.

Discussion

Hammondia hammondi sporozoites readily penetrate cultured rhesus monkey kidney or WI-38 cells. Once intracellular, the parasite proceeds to divide by endodyogeny. Under the conditions of the present study and the strain used, *H. hammondi* completed a division approximately once every 24 hr. Penetration and multiplication of *H. hammondi*, as seen by light microscopy, are very similar to those of *Toxoplasma gondii* described by Sheffield and Melton (1970) who inoculated cell cultures with excysted sporozoites. Development of *T. gondii* in cultures inoculated with other forms of the parasite (for review, see Doran, 1973) also compare favorably with *H. hammondi* with the exception of "schizonts" occurring in cultures inoculated with tachy—and bradyzoites of *T. gondii* (Azab and Beverley, 1974). Schizonts were not seen in cultures of *H. hammondi*. The only other coccidians reported to divide by endodyogeny when sporozoites were introduced into cell cultures are *Isospora canis* (Fayer and Mahrt, 1972), *I. rivolta* (Fayer, 1972), *Hepatozoon griseisciuri* (Hendricks and Fayer, 1973), and *I. felis* (Fayer and Thompson, 1974).

The ultrastructural characteristics of *H. hammondi* shortly after penetration of the host cell by the sporozoite and during endodyogeny closely resemble those of *T. gondii* as reported by Sheffield and Melton (1968). All of the organelles commonly associated with the coccidia (Scholtyseck, 1973) such as the conoid, pellicular complex, micropore, rhoptries, and micronemes are present in *H. hammondi*.

The occurrence of a crystalloid body in sporozoites of *H. hammondi* (Sheffield and Melton, 1974) and in early intracellular stages as reported here distinguishes the parasite from *Toxoplasma*. The crystalloid body was first described by Garnham, Bird, and Baker (1962) who found it in ookinetes of *Plasmodium cynomolgi*. Its structure resembled a virus inclusion and was so described by Terzakis (1969). Desser and Trefiak (1971) found similar inclusions in *Leucocytozoon simondi*. After histochemical tests indicated that neither DNA or RNA was present they concluded that the

crystalline material was not virus and gave evidence for its proteolipid nature. Presence of a crystalloid body has previously been reported in *Eucoccidium dinophili* (Bardele, 1966), *Aggregata eberthi* (Porchet-Henneré and Richard, 1971), *Isospora canis* (Roberts, Mahrt and Hammond, 1972), and *Sarcocystis fusiformis* (Sheffield and Fayer, 1976). In the latter two organisms large accumulations of granules which comprise the crystalloid body were found both anterior and posterior to the nucleus.

While the function of the crystalloid body is not known it has been suggested by Desser and Wright (1968) that it serves as a reserve energy source. Porchet-Henneré and Richard (1971) thought that the crystalloid body was homologous with the refractile body of the sporozoites of *Eimeria* spp. which also has been reported to have a storage function (Roberts and Hammond, 1970). This hypothesis is consistent with the data presented here. Intracellular *H. hammondi* sporozoites have a posteriorly located crystalloid body. The structure is seen occasionally after the parasite has undergone endodyogeny and rarely after 2–3 cycles of endodyogeny. The large dense granules seen in the posterior portion of older parasites may represent a product derived from the crystalloid body material.

The ultrastructural and cultural characteristics of *H. hammondi* augment the findings of Frenkel (1974) and Wallace (1975) that *H. hammondi* is very closely related to *T. gondii*. Using morphological criteria alone the organisms can only be distinguished by the presence of the crystalloid body. It has not been determined that the latter is visible by light microscopy.

Failure of *H. hammondi* to maintain an infection in cell cultures beyond several weeks may indicate differences in its growth requirements from those of *Toxoplasma*. Chernin and Weller (1954) were successful in maintaining *T. gondii* in roller tube cultures for 81 days. However, the yield of organisms from cell cultures is low and many are nonviable (Jacobs, 1956). The fact that cultures inoculated with *H. hammondi* were not resistant to subsequent challenge with *Toxoplasma* suggests that failure of continuous replication was not due to an interferon-like substance.

Considering the similarities between *H. hammondi* and *T. gondii* comparative studies both *in vivo* and *in vitro* may prove useful in determining some of the parameters concerning the wide host range and virulence of *Toxoplasma* in man and animals.

Acknowledgments

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Announcement

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Report on the Brayton H. Ransom Memorial Trust Fund

Balance on hand, 1 January, 1975	$3,712.59
Receipts: Interest received in 1975	253.52
	$3,966.11
Disbursements: Grant to Helminthological Society of Washington	$ 10.00
On hand, 31 December, 1975	$3,956.11

A. MORGAN GOLDEN
Secretary-Treasurer

Research Note

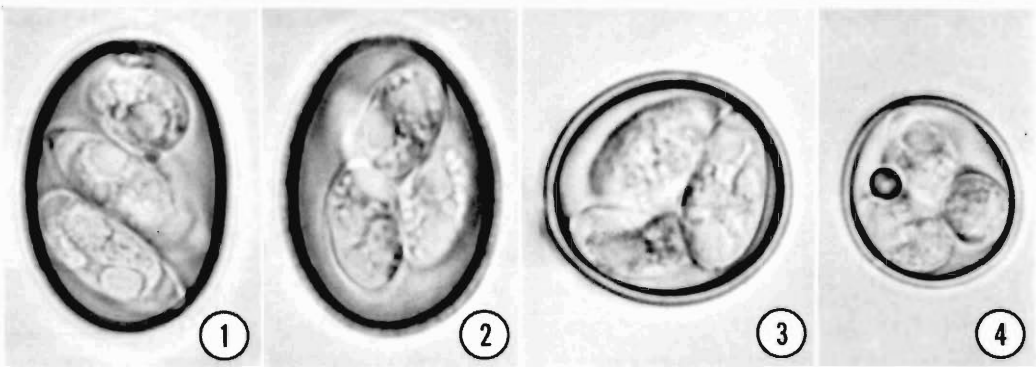
Coccidian Parasites from Game-Farm Reared Pheasants, *Phasianus colchicus*, in Iowa

From 21 January through 3 June 1973, fecal samples from a mixed flock of 150 male and 300 female ring-necked pheasants, *Phasianus colchicus*, belonging to a private game farm in Jasper Co., Iowa, were examined for coccidian oocysts. Oocysts of four species of *Eimeria* and one of *Isospora* were found to be present. For the eimerian species, oocyst production and prevalence rates are reported, and photographs of the sporulated oocysts are documented, apparently for the first time.

The pheasants sampled ranged in age from 9 to 11 months at the beginning of the survey period, and were confined for the entire time to a compound completely enclosed by wire except for the floor, which was of exposed soil. The compound was 25 m long, 5 m wide and 2 m high. The birds were maintained on a nonmedicated diet consisting of a mixture of ground corn, oats, soybean meal, meat, and bone meal. The feed was spread on the compound floor at daily intervals and water was provided *ad libitum* in several uncovered metal troughs placed at various locations inside the compound.

Fecal samples were collected over a single 48-hr period each week during the 20 week survey by suspending 8 porcelain coated metal pans at random locations under the roosts. The pans, each having a surface area of 1,125 sq cm, were covered with wire in order to prevent their contents from becoming scattered. At the end of each collection period, a representative sample of feces was removed from each pan, and the oocysts contained therein were prepared for examination as previously reported (Wacha, 1973, Proc. Helm. Soc. Wash. 40: 56–58). For each sample, the frequency with which oocysts of the different *Eimeria* spp. occurred (in %) was determined by differential counting, and the number of oocysts/gm of feces produced was determined by using a modified McMaster's technique (Whitlock, 1948. J. Counc. Sci. Ind. Res., Austral. 21: 177–180; Nyberg et al. 1967. Proc. Helm. Soc. Wash. 34: 13–14). These data, which are expressed in greater detail elsewhere (Fisher, 1973. M.A. Thesis, Drake Univ.), are combined to provide the overall values for prevalence and oocyst production for the entire survey period. The photographs were taken with the aid of a Zeiss photomicroscope equipped with neofluar objectives.

The eimerian oocysts found were those of *Eimeria phasiani* Tyzzer, 1929 (Fig. 1), *Eimeria pacifica* Ormsbee, 1939 (Fig. 2), *Eimeria duodenalis* Norton, 1967 (Fig. 3) and *Eimeria tetartooimia* Wacha, 1973 (Fig. 4).



Figures 1–4. Sporulated oocysts of *Eimeria* spp. from ring-necked pheasants in Iowa. ×2,000. 1. *E. phasiani*. 2. *E. pacifica*. 3. *E. duodenalis*. 4. *E. tetartooimia* (note spherical polar granule).

Oocysts of those species occurring with the greatest frequency in the samples studied were *E. phasiani*, which had a mean differential count value of 42 (12–78)% for the entire survey, and *E. pacifica*, also with a mean differential count value of 42 (3–69)%. Those species having the lowest frequency of occurrence were *E. duodenalis* and *E. tetartooimia*, with mean differential count values of 5 (0–8) and 11 (3–25)%, respectively. For all species, the mean number of oocysts produced per gram of feces for the entire study was 477 (157–2,410).

Some fecal samples contained a small number of coccidian oocysts resembling those of *Isospora lacazei*. Twenty of these oocysts had length-width dimensions averaging 23.4 (21.0–26.4) × 21.6 (20.0–26.0) μm; L/W ratio 1.1 (1.0–1.1). A Stieda body and a finely-granular sporocyst residuum were present; some sporocysts contained a conspicuous, inverted, nipple-like structure which extended into the matrix of the substieda body. This structure was similar to one shown for *I. lacazei* by Levine and Mohan (1960. J. Parasit. 46: 733–741). The prevalence of this isosporan was not determined but it was thought to be a contaminant from one or more species of passeriform birds which roosted in close proximity to the pheasants. Both starlings (*Sturnus vulgaris*) and English sparrows (*Passer domesticus*) occasionally were seen perching in trees overhanging the compound in which the pheasants were housed. However, no isosporan species were ever detected in fecal samples taken from juvenile pheasants which were housed under roofed shelters, although these young pheasants did pass oocysts of all four of the eimerian species observed in mature pheasants.

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Research Note

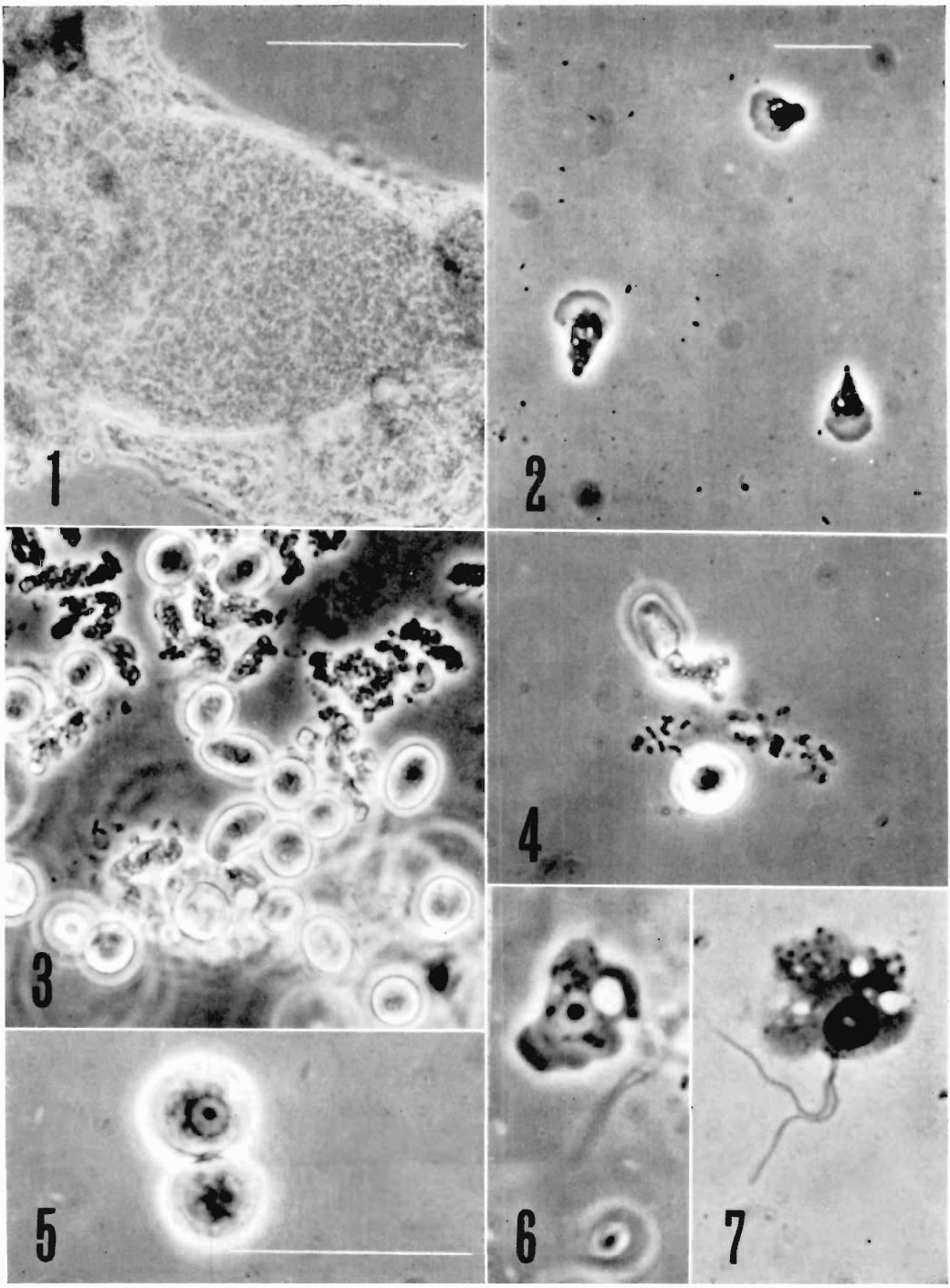
Naegleria sp., an Amoebo-Flagellate from *Physa gyrina* Say in a High Mountain Lake in Wyoming[1]

Species of the genus *Naegleria*, normally considered soil or freshwater forms, have been implicated as causative agents of primary amoebic meningoencephalitis in humans (Carter, 1972, Trans. Roy. Soc. Trop. Med. Hyg. 66: 193–213). Experimental work with mice and monkeys has demonstrated the pathogenicity of members of this genus for other homeotherms (Culbertson, 1971, Ann. Rev. Microbiol. 25: 231–254).

In the fall of 1975, snails (*Physa gyrina* Say) from Bear Lake, a high mountain lake in Wyoming, which were isolated for cercarial studies, passed "pseudocysts" (Fig. 1) in their feces. When ruptured, pseudocysts released large numbers of trophic, monopodal amoebae (Fig. 2) mixed with other amoebae, bacteria, diatom frustrules, and other debris. Some of these amoebae became rounded and extended flagella (2) (Fig. 6) and then rapidly swam away. The presence of a single broad pseudopodium, a nucleus with a prominent endosome (Figs. 5, 6, 7), transformation of the amoeboid form into a flagellated form (Fig. 6) and excystment (Fig. 4) through pores place these forms in the genus *Naegleria* Alexeieff, 1912 emend. Calkins, 1913. The species of *Naegleria* recovered from *Physa* is at present unidentified.

Amoebas from fecal pseudocysts multiplied rapidly when placed on agar on which *Aero-*

[1] Publication approved by Director, Agriculture Experiment Station, University of Wyoming, Laramie, Wyoming 82071 as JA 837.



bacter sp. was grown. Cyst formation (Fig. 3) followed the trophic phase. When cysts were placed in distilled water, excystment occurred. A single amoeba emerged from the intact cyst wall which was left behind as the amoeba crawled away (Fig. 4). Transformation of amoebae in dilute solutions occurred frequently resulting in large numbers of small rounded, rapidly swimming forms being present. Reversion to the amoeboid form occurred when the fluid in which the flagellates were swimming (under a cover glass) was partially evaporated. Under slight coverslip pressure flagellated forms extended their pseudopodia once more; using amoeboid movement they moved across the slide trailing the flagella (Fig. 7). Active amoebas measured 12 to 14 μm. Flagella were approximately equal in length and measured about 20 μm; they seem to arise from a pedicle or short stalk before bifurcating. Cysts measured from 10 to 12 μm. The pseudocyst is ovoid with twisted or trailing ends and measured 310 $\times$ 140 to 250 μm.

Studies on the effects of this amoeba on homeotherms are underway; understanding the effects on the snail host and on other poikilotherms would be highly desirable. The only known reference to *Naegleria* in snails is that of Richards (1970, J. Parasit. 56: 4: Sec. II Pt. 1. 282) who recovered it from intestinal contents of laboratory-reared freshwater mollusks. He did not mention pseudocyst formation. The pseudocysts observed in feces of *P. gyrina* contained large numbers of bacteria and appeared to function as an "incubator" for the growth, multiplication, and concentration of these amoebae.

Bear Lake is located, at an elevation of 3,079.2 m, 75.6 km from Laramie, 6.4 km southeast of Highway 130, in Albany County (R79, T16, S27, NE ¼) Wyoming. Thus it is situated in the Snowy Range of the Medicine Bow Mountains and is a part of the Libby Creek drainage. The lake is 5.3 hectares in area and has maximum depth of 12.8 m, the total volume is not known; the bottom consists of detritus and large boulders; it is considered an oligotrophic alpine lake.

The finding of this possibly pathogenic amoebo-flagellate in a high mountain lake would suggest that it be sought in the snail host in other localities, and that other snails be examined to determine host distribution of the amoebo-flagellates. In addition various chemicals used in water treatment should be evaluated to define the parameters of viability of these amoebae.

We would like to acknowledge the help of Gene Stagner, Department of Zoology and Physiology, University of Wyoming, who provided the original snails studied and for topographic and limnological data concerning Bear Lake.

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←

Figures 1–7. ***Naegleria*** **sp. from the snail,** ***Physa gyrina.*** **1. Pseudocyst from snail feces. 2. Amoeboid forms from pseudocyst. 3. Cyst from agar-*** ***Aerobacter*** **culture at 37 C. 4. Excystation. 5. Cyst with a single nucleus with a prominent endosome. 6. Amoeboid form with paired flagella, endosome prominent. 7. Giemsa stained amoebo-flagellate, endosome prominent.**

Figures 1–6. Live material.

Figure 7. Fixed with methanol and stained with Giemsa.

Scale: Figure 1 = 0.1 mm. Figures 2–7 = 0.02 mm. All photographs phase contrast.

Research Note

A New Device for Rapid Picking-Up of *Heterodera* Cysts

A new device for efficient picking-up of *Heterodera* cysts was developed, the construction and working details of which are described herein.

Construction details (Fig. 1): Two glass tubes (C,D) of 0.5 cm diameter were inserted into the neck of a conical flask (E) of 25 ml capacity through an airtight rubber cork. The tube 'D' (10 cm length) was bent at a 135° angle and tapered into a jet at the other end. The tube 'C' (5 cm length) was bent at a 90° angle. One end which was inside the flask was covered with a nylon net (60 meshes/linear inch). The other end of the tube 'C' was connected to an aspirator 'B' (Syn. filter pump) through a rubber tubing (0.5 cm diameter) of desirable length. The aspirator was connected to a water tap 'A.'

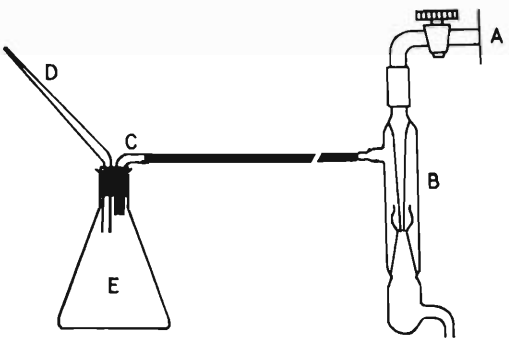

Figure 1. Diagram of the apparatus used for picking-up of *Heterodera* cysts. A: water tap, B: aspirator, C: glass tube bent at 90° angle and having nylon net around its mouth, D: glass tube bent at 135° angle and having jet at outer end, and E: conical flask.

Working details: The cysts were isolated by passing the soil-water suspension through a set of sieves of 60 meshes. The "catch" was later poured over a funnel having No. 1 filter paper. When water was drained off, the filter paper with the "catch" was dried and placed under focus of a stereoscopic microscope. Before picking the cysts, the water tap 'A' was opened which started sucking the air into the aspirator causing a partial vacuum in the conical flask 'E.' The flask 'E' was held in the one hand and the jet 'D' was introduced near the cyst which eventually gained entry into the flask. This operation was repeated with other cysts. The nylon net around the mouth of tube 'C' checked the entry of cysts into the aspirator. The suction pressure was adjusted by regulating the flow of water through the aspirator. Later the cysts, collected in flask 'E,' were removed with the help of fine camel-hair-brush after closing the water tap 'A.'

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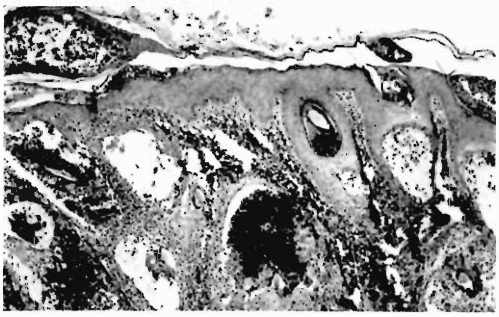
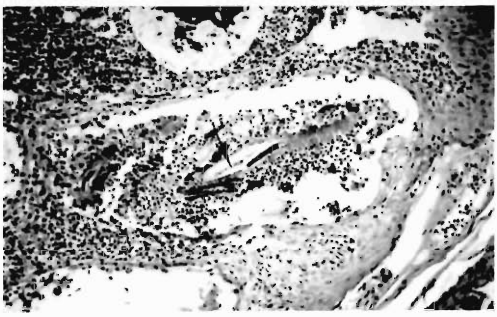
Research Note

Dermatitis in Sheep Caused by *Pelodera strongyloides*

Pelodera strongyloides is a free-living nematode that is found in decaying organic material or moist soil, but may invade the skin of some domestic animals. *P. strongyloides* has previously been found in the skin of cattle and dogs and in the orbits of the eyes of several species of rodents (Levine, 1968, Nematode Parasites of Domestic Animals and of Man, Burgess Publishing Co.). The present communication reports a new host for this nematode.

In March 1972, it was observed that sheep in a flock of 35 crossbred ewes in northern





Figures 1–2. Photomicrographs of skin from the abdomen of a ewe with dermatitis due to *Pelodera strongyloides*. H & E stain. 1. Degeneration of the hair follicles and cellular accumulation in the dermis. × 46. 2. Larva within an inflamed wool follicle. × 115.

Illinois were losing patches of wool. The ewes were confined to a three acre muddy lot and had access to an open-front shed that was clean and well-bedded with old hay. Multiple foci of moist dermatitis 10 to 20 cm in diameter developed on the ventral abdomen and thorax of about 25% of the ewes. There was almost complete loss of wool in the affected areas of the skin, but there was no evidence of pruritis. No lesions were found on several lambs nursing ewes with active dermatitis.

Numerous nematode larvae were found by direct microscopic examination of scrapings of the skin lesions. Part of the tissue from one sheep was examined by microdissection and larvae and the posterior end of one adult male were recovered and identified as *P. strongyloides*. An affected area of one ewe was biopsied and the tissue was prepared for histologic examination. Study of the sections revealed extensive inflammation of the skin (Fig. 1). There was mild, irregular acanthosis with collections of fibrin and granulocytes in superficial parakeratotic foci. Numerous eosinophils were scattered throughout the dermis. A severe folliculitis was characterized by cystic degeneration of the follicles with accumulations of granulocytes, macrophages, and profiles of nematodes in the follicular lumens (Fig. 2).

It was concluded that invasion of the follicles by *P. strongyloides* caused the intense dermatitis and loss of wool. Unusually rainy weather and the muddy condition of the lot probably provided a favorable environment for the nematodes to multiply and invade the skin of the ewes. The lesions regressed soon after treatment with a topical spray[1] and an anthelmintic.[2] Coincident with treatment were less rainy weather and drying of the soil.

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[1] Neoblu Aerosol—Affiliated Laboratories, Myerstown, Pennsylvania.
[2] Thiabendazole—Merck and Company, Rahway, New Jersey.

Research Note

Some Parasites of the White Perch, *Morone americana* (Gmelin), in Chesapeake Bay

The white perch, *Morone americana*, is distributed along the Atlantic Coast from Nova Scotia to South Carolina in coastal marine, estuarine and fresh waters (Mansueti, 1961, Chesapeake Sci. 2: 142–205). It occurs also in the lower Great Lakes (Scott and Christie, 1963. J. Fish. Res. Bd. Canada 20: 1189–1195). The parasite fauna of *M. americana* in fresh water has been fairly well documented (Hoffman, 1967. Univ. California Press, Berkeley and Los Angeles, 486 p.; Tedla and Fernando, 1969, J. Parasit. 55: 1063–1066), but literature on its marine parasites appears to be limited to six species of digenetic trematodes recorded at Woods Hole, Massachusetts by Linton (1940, Proc. U. S. Nat. Mus. 88: 1–172). This report presents the results of a study in Chesapeake Bay, where the white perch is a valued commercial and sport fish.

Collections were made from May to October 1970, from the following five localities in Chesapeake Bay: Lane Memorial Bridge, Patuxent River, Potomac River, Tilghman's Island, and Hooper's Island. Fish were captured by seine, otter trawl, gill net and hook and line and placed on ice for transport to the laboratory at College Park. The body and organs of each fish were examined with the aid of a dissection microscope and all parasites found were killed in hot water, fixed in AFA, stored in 70% alcohol, and later prepared for identification by standard techniques. In addition, the fish were measured for standard length and their ages were determined by using scales according to the procedure of Mansueti (loc. cit.).

During the course of the study 213 white perch, ranging from 6–26 cm in total length and from 1–10 years in age, were examined. The species of parasites recovered are shown in Table 1.

Among the nematodes, *Rhabdochona* sp. represents a new host record. *Dacnitoides cotylophora*, which is a common parasite of white perch in fresh water, is reported for the first time in estuarine or marine populations of *M. americana*. *Contracaecum* sp. also is reported for the first time in estuarine white perch. Tedla and Fernando (loc. cit.) found larval *Contracaecum spiculigerum* (Rudolphi, 1809) in *M. americana* from fresh water. The three species of digenetic trematodes identified by us were seen previously in white perch and several other hosts at Woods Hole by Linton (loc. cit.). The unidentified Didymozoidae also represents a new host record. It was

Table 1. Parasites recovered from the white perch, *Morone americana* in Chesapeake Bay.

Parasite	Site of infection	Per cent infected	No./infected fish	
			Mean	Range
Nematoda				
**Contracaecum* sp.	mesenteries	19.2	2.2	1–5
Dacnitoides cotylophora Ward & Magath, 1916	intestine	41.7	3.1	1–17
Rhabdochona sp.	stomach	0.5	2.0	1
Trematoda				
Brachyphallus crenatus (Rudolphi, 1802)	intestine	0.5	1.0	1
Homalometron pallidum Stafford, 1904	intestine	1.4	1.0	1
Lepocreadium trullaforme Linton, 1940	intestine	0.9	1.0	1
unidentified Didymozoidae	body cavity	22.1	1.9	1–2
*Cestoidea	liver	1.4	1.6	1–3
Acanthocephala	intestine	6.6	2.2	1–8
Isopoda				
Livoneca ovalis (Say, 1818)	gills	15.1	1.2	1–2

* Larval forms.

assigned to this family with the assistance of Dr. Allen McIntosh. These curious, long, slender flukes occurred in tightly bound pairs, with only one pair being found in each of 46 infected hosts. One fish had a single worm. They were difficult to study due to being filled with innumerable small dark eggs but the following average measurements (in mm) were obtained from 10 specimens: length 4.49, width 0.65, oral sucker, 0.18, pharynx 0.15, acetabulum 0.55, eggs 0.015×0.011. The Acanthocephala were members of the families Echinorhynchidae, Pomphorhynchidae and Rhadinorhynchidae, and were found only in fish collected from Hooper's Island. Unfortunately, due to faulty preparation they would not be identified further with certainty.

The authors wish to thank Mrs. May Belle Chitwood and Dr. Thomas Bowen, respectively, for confirming the identification of the nematodes and isopods.

We also acknowledge the financial assistance received from the Chesapeake Bay Funds of the University of Maryland.

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Research Note

Helminth Parasites of the Herring Gull, *Larus argentatus*, from the Bass Island Region of Western Lake Erie

The number of studies of helminth parasitism in herring gulls, *Larus argentatus*, in the Great Lakes are limited. Thomas (1947, J. Parasit. 33: 107–117) studied the life cycle of *Diphyllobothrium oblongatum* from this species of gull in Lake Michigan and Jensen (1966, Proc. Ist Internat. Cong. Parasit. 539–540) studied the epidemiology of *Cotylurus platycephalus* infections in these birds from Lake Erie and Lake Ontario. As part of an ongoing series of studies seeking to determine the distribution and abundance of parasite populations with established nidi of infection in the Bass Island region of Lake Erie, a number of gulls were examined to determine the nature and extent of their helminth fauna. The study sample was drawn from a population nesting on Ballast, Gibraltar, Green, Middle Bass, and Rattlesnake islands during the summer months. Birds taken during the fall months probably included migrants from other areas.

Herring gulls in the Lake Erie area commence fall migration in mid-September. During June and July of our study, herring gulls were the most abundant of the Laridae in the Island region. With termination of the nesting season in August and during September, the local herring gull population diminished in size.

Gulls were killed with a twelve gauge shotgun. The total number examined was determined by the limit established by a U. S. Fish and Wildlife Service scientific collection permit. Birds were necropsied immediately upon return to the laboratory or were frozen for later examination. The brain, nasal and body cavities, orbits of eyes, trachea, lungs, liver, gall bladder, esophagus, proventriculus, gizzard, intestine, and cloaca were examined using standard procedures. Helminths removed were relaxed in heated Ringer's 'Warm' solution, fixed in AFA (alcohol-formaldehyde-acetic acid mixture) and preserved in 70% alcohol. Nematodes were cleared in glycerine-alcohol and studied in glycerine. Trematodes and cestodes were stained with Semichon's carmine and mounted on glass slides in piccolyte medium.

Thirty-three birds were examined between 17 June and 5 November 1974. Examinations

Table 1. The prevalence and intensity of helminths in 33 herring gulls from the Bass Island region of western Lake Erie.

Helminths	Prevalence %	No. per infected host		No. birds infected	
		Mean	Range	Adults (N = 18)	Juveniles (N = 15)
TREMATODA					
Apophallus brevis Ransom, 1920	67	45.5	1–181	9	13
Cotylurus platycephalus (Creplin, 1825)	12	3.0	1–5	1	3
Diplostomum spathaceum (Rudolphi, 1819)	93	60.8	1–442	18	13
Echinochasmus cohensi Rao, 1951	18	29.0	1–70	1	5
Mesoophorodiplostomum pricei (Krull, 1934)	36	10.5	1–63	5	7
Stephanoprora denticulata (Rudolphi, 1802)	73	5.2	1–18	14	10
CESTODA					
Anomotaenia sp.	3	1.0	1	0	1
Diphyllobothrium dendriticum (Nitzsch, 1824)	3	9.0	9	1	0
NEMATODA					
Capillaria contorta (Creplin, 1839)	21	3.1	1–10	1	6
Capillaria sp.	6	1.5	1–2	1	1
Contracaecum spiculigerum (Rudolphi, 1819)	6	1.0	1	0	2
Cosmocephalus obvelatus (Creplin, 1825)	60	5.2	1–35	12	8
Eustrongylides sp.	39	2.7	1–7	8	5
Tetrameres crami Swales, 1933	6	13.5	2–25	1	1

included 18 adults, 13 fledglings (recently fledged from the nest) and 2 nestlings (unfledged). All of these were infected with one or more species of helminth parasites. Fourteen species of helminths were identified: 6 species of trematodes, 2 cestodes, and 6 nematodes. The prevalence and intensity of each are reported in Table 1. *Eustrongylides* sp. has been tentatively identified as *E. tubifex* (Nitzsch, 1819). A redescription of this species based upon material collected in the Bass Island region is in preparation. Helminth species occurring in nestling and fledgling gulls were most likely acquired in the study area. The cestode parasites encountered in these birds apparently were not acquired in the study area. Cestodes were absent from collections until late October, at which time they were found in birds that most likely were migrants. Only one juvenile bird was examined after the commencement of the fall migration.

The authors are indebted to Dr. Paul C. Stromberg and Mr. John C. Baker for field assistance and Dr. C. E. Herdendorf, Director, Center for Lake Erie Area Research, The Ohio State University for providing support facilities.

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Research Note

Psammomermis sp. (Nematoda: Mermithidae): A New Nematode Parasite of the Japanese Beetle, *Popillia japonica* (Coleoptera: Scarabaeidae)

Although biological control of the Japanese beetle, *Popillia japonica* Newman, has been investigated since 1920, there have been no published reports of mermithid parasites associated with this beetle (Fleming, 1968, USDA Tech. Bull. 1383, 78 pp.). However, immature mermithids recovered from Japanese beetle larvae in 1930, 1941, and 1942 are in the Steiner Mermithid Collection at Beltsville, Maryland. Unpublished monthly reports of the Japanese Beetle Laboratory, formerly at Moorestown, New Jersey, indicate that the parasites from 1941 to 1942 were present in 3 to 4 percent of the larvae collected at Kirkwood, Delaware for use in milky disease inoculation work. This paper reports the first identification of a mermithid from the Japanese beetle.

In the last few years, parasitized Japanese beetle grubs were collected from golf courses at Schenectady, Beacon, Kingston, and Saratoga Springs, New York as well as from the towngreen at Brattleboro, Vermont. About 60% of the Japanese beetle grubs from one area of light sandy soil containing 17 grubs/ft^2 at Brattleboro were infected with the mermithid. In addition, infected grubs were collected at the following three widely separated locations in Connecticut: Wallingford Country Club where 15.8% of 39 grubs collected from an area with 15.4 grubs/ft^2 were infected; and Mixville Park, Cheshire and East Rock Park, New Haven, where 4.5% of 22 and 23 grubs, respectively, collected from areas with 3.4 grubs/ft^2 contained mermithids.

Adults of the mermithid were reared from material from all three states and were identified as *Psammomermis* sp. closely resembling the species *korsakowi* (Nickle, 1972, J. Nematol. 4: 113–146). This genus of mermithids has, up to the current discovery in the Northeastern part of the USA, only been known from the Soviet Union. There it was reported to kill 60% of the May beetle grubs, *Melolontha hippocastani* Fabr. (Pologentsev, 1941 Bashkirskoi Sci. Res. Veterinary Sta. Proc. 111: 301–441).

Japanese beetle larvae became infected in late summer. Mermithids emerged in March from larvae collected in October and held in cold storage until January. The parasites emerged in mid-May from larvae collected in April when held at 27 C. The mermithids, about 20.3 cm long, could easily be observed coiled inside the larvae, extending from the thoracic region to the penultimate abdominal segment (Fig. 1). The pressure of the rectal sacs against the integument kept the nematode out of the last segment while the grub was alive

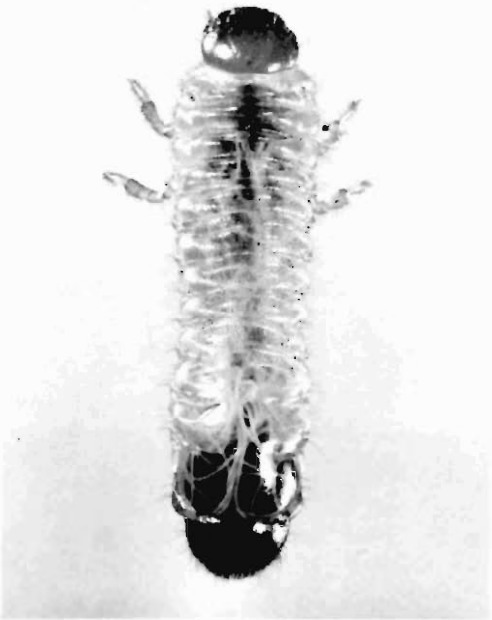

Figure 1. Dorsal view of Japanese beetle larva containing mermithid.

Figure 2. Emergence of mermithid from Japanese beetle larva.

and active. Most of the nematode was found dorsally, although several strands were visible on the ventral side.

At the time of emergence of the mermithid, the Japanese beetle grub had ceased to move and the only signs of life were the contractions of the dorsal vessel and feeble movements of the mouth parts. In contrast to the situation reported from *M. hippocastani*, the Japanese beetle larval body did not rupture to permit emergence of the parasite. Rather, the mermithids emerged either through the intersegmental membrane of the prothoracic coxa (Fig. 2), or through the mouth of the larva. A single mermithid normally emerged from each grub, although as many as three parasites were recovered from one host. All of the host larvae were in the third instar when the mermithids emerged with the exception of one. In that case, the mermithid that emerged from a second instar host died within 48 hr. After emergence, the mermithids entered the soil and remained one–two months before becoming mature.

This mermithid nematode parasite may be an important compliment to the milky disease in the suppression of populations of the Japanese beetle grubs in the Northeast.

Acknowledgment

We acknowledge the assistance of David Chittick, Fairfax Biological Laboratory, Clinton Corners, New York, for furnishing the infected larvae from New York. We also thank Glenn Berkey, Ohio Agricultural Research and Development Center, for taking the photographs. This study was conducted in cooperation with the Ohio Agricultural Research and Development Center. Approved for publication as Journal Article No. 117-75 of the Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

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MINUTES

Four Hundred Ninety-third Through Five Hundredth Meetings

493rd Meeting: University of Maryland, College Park, Maryland, October 17, 1976. President R. S. Isenstein announced that the Society's Anniversary Award would be presented at a later meeting. Slate of officers for 1976 presented: A. Morgan Golden (President); Kendall G. Powers (Vice President); William R. Nickle (Corresponding Secretary-Treasurer); J. Ralph Lichtenfels (Recording Secretary). Papers presented: "Effects of concurrent infections with *Nippostrongylus brasiliensis* upon *Hymenolepis diminuta* in the small intestine of rats," Sherman Hendrix; "Some parasitic diseases of marine fish and shellfish," Thomas K. Sawyer; "Anisakid nematodes—problems in their classification," J. Ralph Lichtenfels.

494th Meeting: Cosmos Club, Washington, D. C. (Sponsored by the Animal Parasitology Institute, U. S. Department of Agriculture, Beltsville, Maryland; joint meeting with the Washington Academy of Sciences), November 20, 1975. The slate of officers presented at the previous meeting was elected by ballots distributed at the meeting. Paper presented: "Role of research in agriculture, as it relates to the American farmer, the American consumer, and the world food crisis," J. Phil Campbell, Under Secretary of Agriculture.

495th Meeting: Animal Parasitology Institute, Beltsville, Maryland. (Sponsored by the Biological Laboratory, National Marine Fisheries Service, Oxford, Maryland.) December 19, 1975. Anniversary Awards were presented to Drs. Margaret A. Stirewalt and David R. Lincicome by J. M. Vetterling. Papers presented: "Pathogenic free-living amoebae—past and present," Joe L. Griffin; "A decade of progress in marine disease research," Aaron Rosenfield.

496th Meeting: Laboratory of Parasitic Diseases, National Institute of Allergic and Infectious Diseases, Bethesda, Maryland, January 16, 1976. President A. Morgan Golden led attending members in expressing special thanks to Harley G. Sheffield for his five year term as Editor of the Proceedings of the Society. Papers presented: "Receptors for crythrocytic invasion by malaria parasites," S. J. Mason; "Gamete emergence in malaria," M. Nijhout; "Investigations of the immune response in Bancroftian filariasis," E. A. Ottesen; "Problems in parasitic disease control programs," L. J. Olivier; "The future of research on parasites," J. R. Seal.

497th Meeting: Nematology Laboratory, Plant Protection Institute, U. S. Department of Agriculture, Beltsville, Maryland, February 20, 1976. W. R. Nickle presented the Annual Financial Report indicating that no increase in dues or page charges will be necessary this year. Papers presented: "Viroids—the smallest known causal agents of plant disease," T. O. Diener; "Fine structures of the amphids of root-knot nematode, *Meloidogyne incognita*, larvae. A Poster Presentation," W. P. Wergin and B. Y. Endo.

498th Meeting: Walter Reed Army Institute of Research, Washington, D. C., March 19, 1976. Papers presented: "The Sternberg Legacy: The overseas research programs of the Army Medical Service, past and present," BG (Ret.) William D. Tigertt.

499th Meeting: Naval Medical Research Institute, Bethesda, Maryland, April 23, 1976. Papers presented: "A nostalgic evening with Navy Parasitology," Malcolm S. Ferguson and Margaret Stirewalt.

500th Meeting: University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, May 15, 1976. President A. Morgan Golden announced that four members had been elected to Life Membership in the Society: David R. Lincicome, Margaret A. Stirewalt, Willard H. Wright, and Benjamin Schwartz. President Golden then announced that the Society had chosen to honor one member by making him an Honorary Member: Norman R. Stoll. Dr. Stoll responded with brief remarks and received a standing ovation. Papers presented: "Control of Parasitic Infections—

Role of the parasite," T. C. Cheng; "Role of drugs," W. C. Campbell; "Role of the host," E. J. L. Soulsby.

The following 47 new members were elected at the meetings indicated: *493rd*: G. Caballero, P. Kumar, J. Ahmad, E. H. Williams, Jr., E. W. Cake, Jr., D. R. Brooks, M. L. Eberhard, R. L. Buckner, T. R. Meyers, J. R. Dooley, W. R. Davidson, A. W. Cheever. *495th*: S. A. MacLean, H. B. Tanowitz, W. A. Krissinger, D. G. Huffman, J. Vargas-Mena, D. R. Sutherland, W. E. Carr, Jr., R. H. Cypress, R. C. Neafie, E. J. Kuzia. *496th*: D. Stiller, M. N. Novilla, H. W. Armstrong, W. M. Frerichs. *497th*: J. L. Gibb, D. J. Ebert, H. E. Jordan, J. W. Bier, D. M. Miller, R. P. Hobbs, J. Watertor. *498th*: E. L. Jarroll, Jr., L. Lightner, G. R. Brown, R. S. Rew, N. O. Dronen, Jr., S. D. Folz, W. J. Foreyt. *499th*: L. A. Jensen, W. C. Campbell, M. McCullough. *500th*: R. Holder, Jr., L. Grisi, A. J. Grey, P. V. Arambulo III.

J. RALPH LICHTENFELS
Recording Secretary

Anniversary Awards of the Helminthological Society of Washington for 1975

In recognition of their many contributions to parasitology and for their distinguished service to the Helminthological Society of Washington, Anniversary Awards were presented to Dr. David R. Lincicome and Dr. Margaret A. Stirewalt at the 495th Meeting of the Society. The presentation was made by the Chairman of the Awards' Committee, Dr. John M. Vetterling.

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