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CONTENTS

Electron Scan of a Nematomorph Cuticle[1]

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ABSTRACT: The cuticular surfaces of male and female horse-hair worms, *Chordodes* sp. ?, from Australia are described using a Cambridge stereoscan electron microscope.

During a 1-year sabbatical leave (1970–71) spent in the Parasitology Department of the University of Queensland, Brisbane, Australia, some horse-hair worms were collected at the Mt. Crosby waterworks about 20 miles from Brisbane. Cylindrical water sieves are periodically lifted from their wells and cleaned. The two worms described here were clinging to one of these sieves.

Characteristics of the cuticular surface of nematomorpha have been used extensively in the taxonomy of this group. Much of the older literature on these and other aspects of horse-hair worms has been reviewed by Hyman (1951). Zapotosky (1971) has described the ultrastructure of the cuticle of *Paragordius varius* (Leidy, 1851) using the transmission electron microscope.

The characteristics of the cuticle reported here were determined with the use of the scanning electron microscope (Cambridge Stereoscan). The characters place these worms

in the family Chordodidae and possibly the genus *Chordodes*. Sciacchitano (1958, p. 98) lists *Chordodes annulatus* v. Linstow from Queensland, Australia. However, v. Linstow (1906) described this species as *Parachordodes annulatus*.

Materials and Methods

One worm of each sex was fixed in steaming hot 5% formalin. They were dehydrated with alcohol and methyl benzoate and held in xylene for several days to harden them so that they would not collapse when placed in vacuum. Each worm was cut into convenient lengths to be mounted on microscope chucks. The pieces were gold-coated and observed and photographed under the scanning microscope.

Observations

Male (Figs. 1–12)

The anterior part of the body tapers to a rounded head in the center of which is the circular mouth (Figs. 1, 2). The head appears

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Figures 1–6 (male). White bar applies to all figures. 1, Anterior end showing mouth. Bar = 50 μ. 2, Mouth enlarged, flat areola surrounded by (pores?), see arrow. Bar = 5 μ. 3, Cloaca with three broken spines. Bar = 5 μ. 4, Posterior showing cloaca, everted organ anterior to cloaca, spines, and weakly bilobed end. Bar = 50 μ. 5, Everted organ enlarged. Bar = 10 μ. 6, Everted organ further enlarged showing opening (arrow) and associated spines. Bar = 5 μ.

Figures 7–12 (male). 7, Areolae and what appear to be associated pores on anterior end. Bar = 2 μ. 8, Posteroventral view showing posterolateral spine patches. Bar = 50 μ. 9, Enlargement of spines. Bar = 14 μ. 10, Areolae near anterior end. Bar = 11 μ. 11, Areolae at posterior end. Bar = 10 μ. 12, Further enlargement of spines shown in Fig. 9. Bar = 5 μ.

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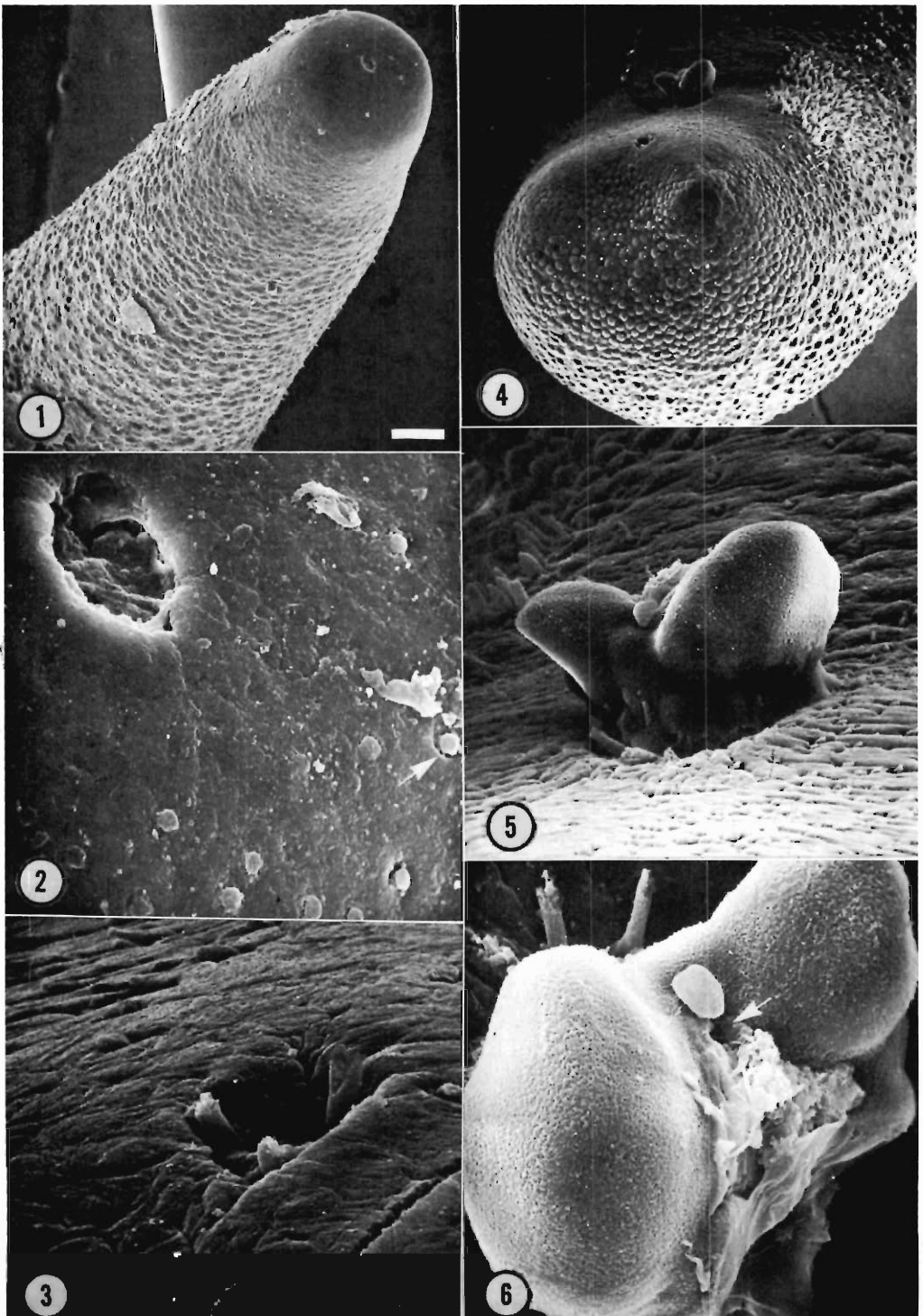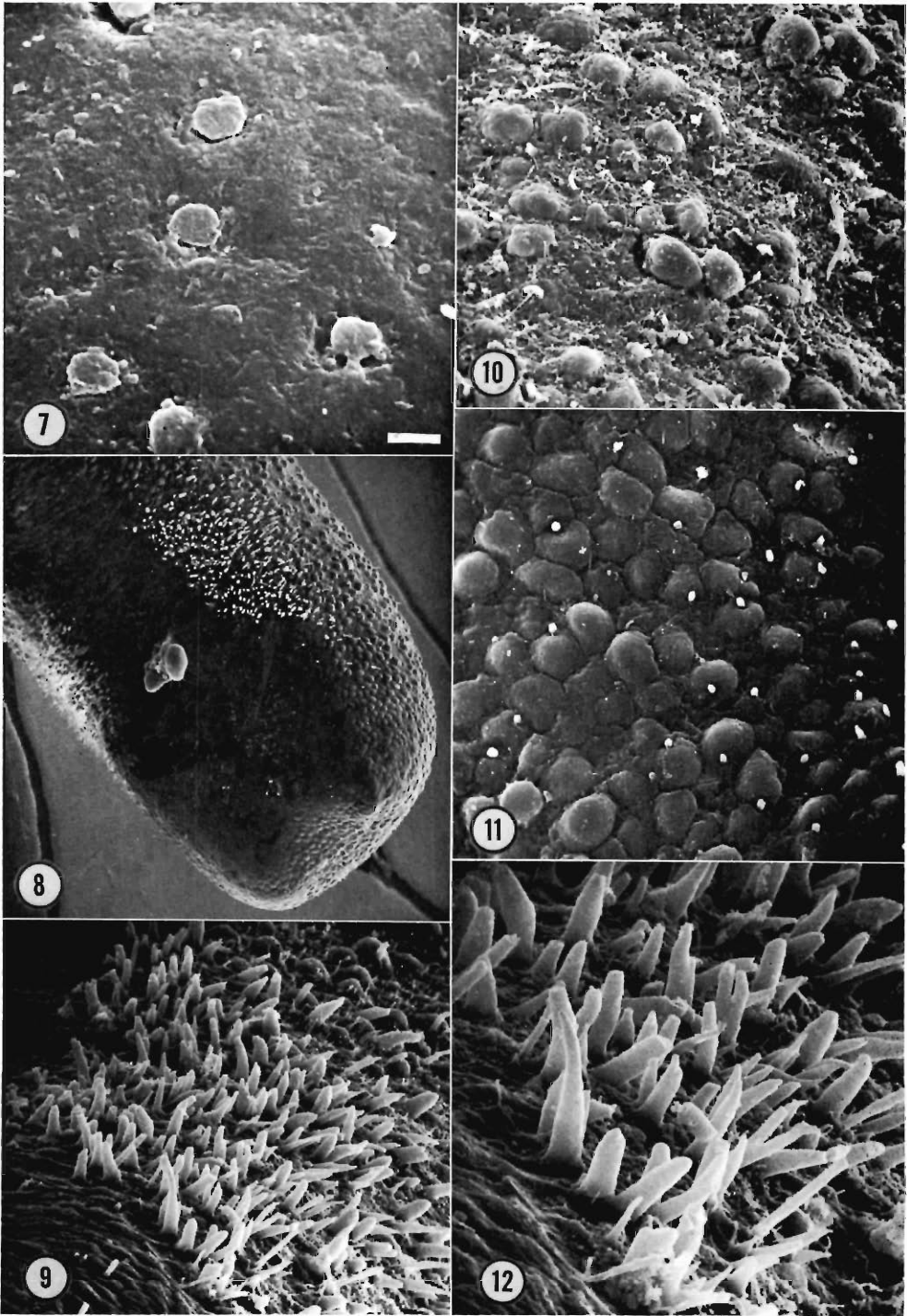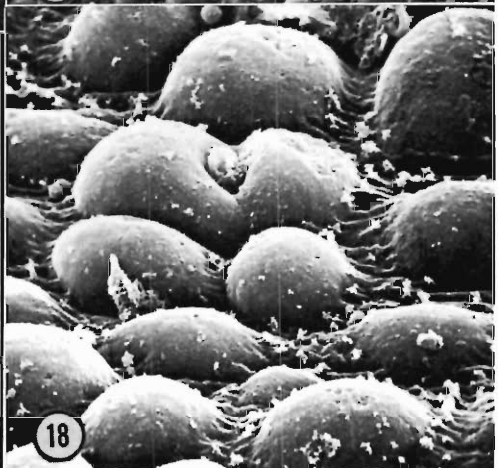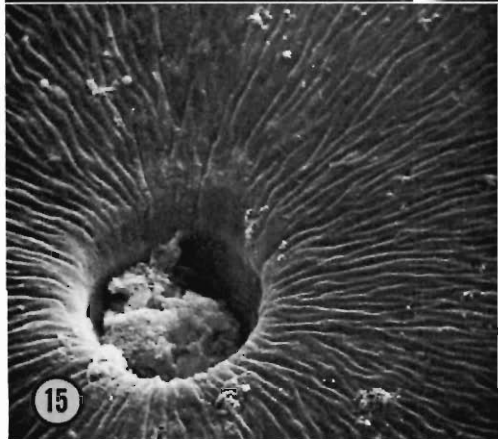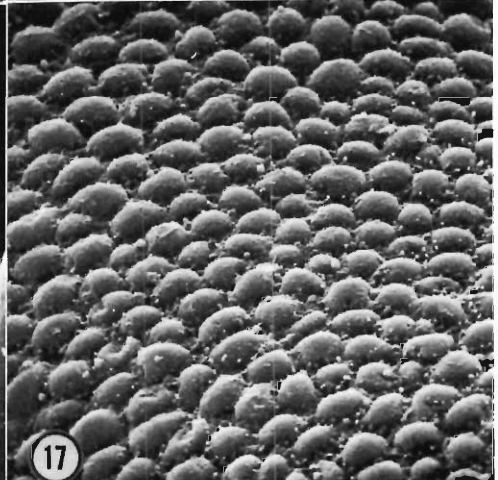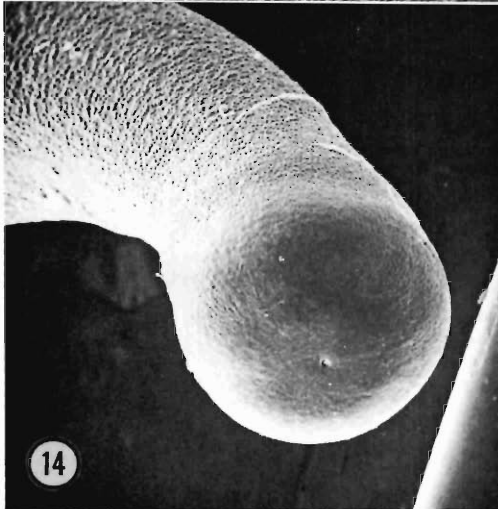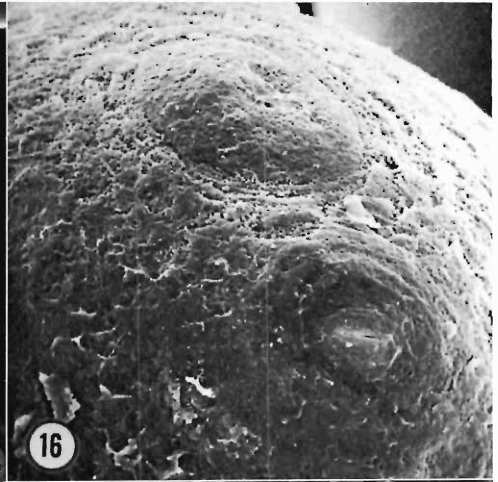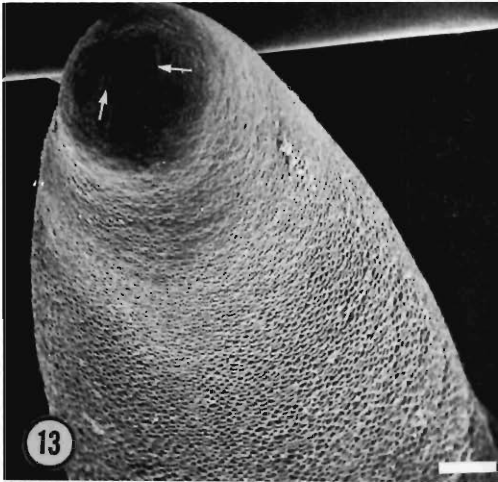
relatively smooth in Figure 1 but when more highly magnified nearly circular and flat areolae can be seen. Each areola seems to be ringed by minute pores (arrow, Fig. 2 and Fig. 7). Posterior to the head the areolae become more rounded and more closely packed, some showing multiple lobes (Fig. 10). Farther posteriad the areolae are even more closely packed and pores between them may extrude secretions (Fig. 11). The areolae continue to the posterior end with the exception of a posteroventral area in the region of the cloaca (Figs. 4, 8). The posterior end is bluntly rounded (Fig. 8). The cloaca is ventrally located near the posterior end and has a few spines around the opening (Figs. 3, 4, 8).

A short distance anterior to the cloaca there is a structure that probably is retractable since it apparently has never been described previously, and certainly could be seen with the light microscope (Figs. 4–6). Its base is associated with spines. It has two large lobes and a pore between (Fig. 6). From its position one would surmise that it has some as yet unknown function in reproduction. It evidently is not an exit for sperm because previous workers (Hyman, 1951; May, 1919) implicate the cloaca as the genital exit. Lateral to this organ and between it and the cloaca there are patches of spines (Figs. 8–10).

Female (Figs. 13–18)

Anteriorly the body tapers to a rounded end. The mouth is a transverse slit (left arrow, Figs. 13, 16). Above the mouth is a circular mound (Figs. 13, 16) whose function is unknown. Most of the body is covered with closely packed, rounded areolae (Figs. 17, 18). The posterior end is bluntly rounded with a terminal circular cloaca (Figs. 14, 15).

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Figures 13–18 (female). 13, Anterior end showing mouth (left arrow) and circular mound (right arrow). Bar = 45 μ. 14, Posterior end of female showing cloaca. Bar = 100 μ. 15, Cloaca. Bar = 5 μ. 16, Anterior end of female showing slit mouth and nearby circular mound. Bar = 9 μ. 17, Areolae of female body. Bar = 10 μ. 18, Greater enlargement of female areolae. Bar = 22 μ.

Behavior of Various Helminths in a Thermal Gradient[1]

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ABSTRACT: Various parasitic and free-living helminths were studied in a thermal gradient migration chamber. Contrary to a previous study the free-living oligochaete, *Enchytraeus*, was unable to seek a temperature preferendum. Single adults of *Leucochloridiomorpha constantiae* (Trematoda) migrated more rapidly than those in groups and only the former reached lethal temperature. Single *L. constantiae* metacercariae were positively thermotactic whereas those in groups showed no directional movement. Single *Posthodiplostomum minimum minimum* metacercariae migrated more rapidly than those in groups. Worm-mediated attraction apparently affected the positive thermotactic response of these trematodes. Negative thermotaxis was observed in the planarian, *Cura foremanii*, and in the leech, *Placobdella parasitica*, an ectoparasite of the painted turtle.

Various stages of parasitic helminths tested in a thermal gradient showed positive thermotaxis and an inability to select a temperature preferendum whereas the free-living oligochaete, *Enchytraeus*, when tested similarly, exhibited a temperature preferendum (McCue and Thorson, 1964). McCue and Thorson (1964) suggested that worm attractants may affect the rate of migration of parasitic helminths in a thermal gradient. The purpose of this study was to extend observations on the behavior of various parasitic and free-living helminths in a thermal gradient and to examine the possible effects of worm-mediated attraction on thermotaxis.

Materials and Methods

A thermal gradient migration chamber was constructed (McCue and Thorson, 1964) and filled with either Ringer's or filtered pond water to a level of 2.5 cm above the migration chamber floor. The chamber was operated in a constant temperature room (12 C) where the temperature gradient obtained in the chamber after 1.5 to 2 hr equilibration was 16 to 45 C (the latter temperature in the heating reservoir). The chamber was covered with a glass plate to prevent evaporation and the thermal gradient was maintained up to 24 hr by controlling the heater. A thermistor thermometer with three immersible probes was used with the probes touching the floor of the chamber for all measurements. All worms

tested migrated on the chamber floor. Illumination in the constant temperature room consisted of overhead diffuse light. Although some experiments were begun or continued in the evening, most were performed during the day. No attempt was made to examine possible differences in day–night migration rates (McCue and Thorson, 1965). *Leucochloridiomorpha constantiae* metacercariae and 7-day-old adults were obtained as previously described (Fried and Harris, 1971). *Posthodiplostomum minimum minimum* metacercariae were obtained following chemical or mechanical excystation of cysts (Fried, 1970). The leech, *Placobdella parasitica*, was obtained from the skin of naturally infected *Chrysemys picta belli* turtles purchased from J. F. Schettle Frog Farm (Stillwater, Minn.). Cultures of the black planarian, *Cura foremanii*, and the oligochaetous annelid, *Enchytraeus* sp., were purchased from Carolina Biological Supply Company (Burlington, N. C.). The chamber contained Ringer's for tests on trematodes and pond water for planarian and annelid experiments. Worms were briefly washed in the medium in which they were tested and used within 30 min of their removal from hosts, cysts, or cultures. Unless otherwise stated worms were placed in chambers at 33 C. Worms were tested either singly or in groups of five to determine the possible *effects* of worm-mediated attractants on thermotaxis. Temperature and position were recorded every 15 min for *Enchytraeus* sp. and periodically up to 24 hr for trematodes. The above measurements were recorded every 2 min for *C. foremanii* and *P. parasitica*.
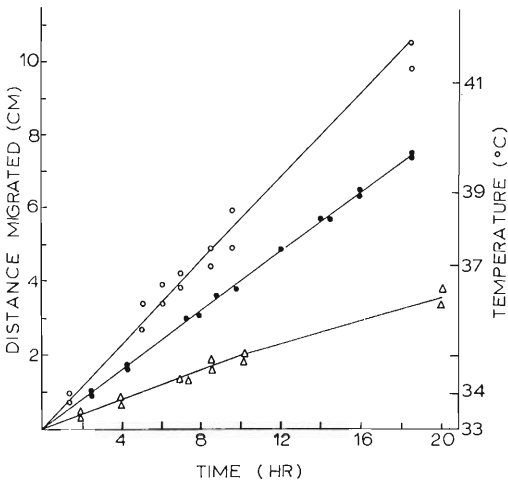
Figure 1. Migration in a thermal gradient of single metacercariae of *L. constantiae* (open points), single *P. minimum minimum* metacercariae (closed points), and *P. minimum minimum* metacercariae in groups (triangles). Each open point represents mean movement of 10 singles, each closed point mean movement of three or four singles, and each triangle represents mean movement of two or three groups.

Figure 2. Migration of *L. constantiae* adults singly or in groups in a thermal gradient. Each circle represents mean movement of five or six singles and each triangle mean movement of three or four groups. The square represents migration into the heating reservoir.

Results

Single metacercariae of *P. minimum minimum* migrated toward the heat source more rapidly than those in groups and within 18 to 20 hr at the termination of these experiments all worms were alive (Fig. 1, closed circles and triangles). The distance between any two metacercariae in group experiments never exceeded 0.8 cm. Single *L. constantiae* adults migrated more rapidly than those in groups and reached a lethal temperature of 45 C within 6 hr at which time the latter were alive at 41 C (Fig. 2). The distance between any two adults in group experiments never exceeded 1.5 cm. Single metacercariae of *L. constantiae* migrated toward the heating reservoir at the mean rate of 0.5 cm/hr and all were alive at the termination of these experiments after 18 hr (Fig. 1, open circles). Metacercariae in group experiments showed no directional movement within 24 hr and remained within 0.5 cm of each other. *Enchytraeus* sp. tested singly or in groups exhibited a positive thermotaxis and migrated toward lethality. Of

52 worms tested, 14 were killed at 35 C after 15 min, 27 at 38 C within 30 min, and the remaining 11 were dead within 45 min at 39 to 45 C. Eleven *C. foremanii* placed in the chamber at 29 or 30 C were studied singly. Eight showed negative thermotaxis and migrated to the cool reservoir (16 C) within 2 to 4 min whereas 3 showed an initial positive thermotaxis migrating to 31 or 32 C, prior to reversal of direction and movement to the cool reservoir within 2 to 5 min. Ten *P. parasitica* studied singly showed negative thermotaxis and migrated to the cool reservoir (16 C) within 10 min.

Discussion

Our findings based on 80% mortality of *Enchytraeus* between 35 and 38 C within 30 min are contrary to McCue and Thorson's (1964) report of a temperature preferendum

of about 40 C for this oligochaete. Since they did not state their source of *E. albidus* contradictory results may reflect strain or species differences. However, our evidence does not support in general the hypothesis of the "closed loop system" or negative feedback response they attributed to *Enchytraeus*. Single *L. constantiae* adults migrated to a lethal temperature supporting previous results first reported for various parasitic stages of helminths studied in a thermal gradient (McCue and Thorson, 1964). Positive thermotaxis was adversely affected when *P. minimum minimum* metacercariae or *L. constantiae* adults were in groups and was not apparent in group experiments on *L. constantiae* metacercariae. A previous study (Fried and Roberts, 1972) indicated that chemical attractants produced by worms mediated pairing of *L. constantiae* metacercariae or adults. Our results on trematodes singly and in groups substantiate McCue and Thorson's (1964) statement that attractants may affect the rate of migration of parasitic worms in a thermal gradient. Negative thermotaxis was clearly demonstrated in *C. foremanii* and *P. parasitica*. *P. parasitica* has been reported as an ectoparasite of painted turtles (Ernst, 1971). Our observations on placobdellid behavior appear to be the first report of negative thermotaxis of a parasitic helminth in a thermal gradient.

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Two New Species of *Gyrodactylus* (Trematoda: Monogenea) from the Georgia Coast

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ABSTRACT: *Gyrodactylus foxi* sp. n. from the body surface skin of *Fundulus heterochitus* and *G. mugelus* sp. n. from the body surface skin of *Mugel cephalus* are described from the estuary of Sapelo Island, Georgia. *G. foxi* differs from other species of *Gyrodactylus* in the shape of the marginal hooks and the presence of a peduncular bar. *G. mugelus* differs in the shape of the marginal hooks and anchors.

Host specimens were collected by cast net, seine, and trap during a study of development and seasonal abundance of striped mullet, *Mugil cephalus* L., and mummichogs, *Fundulus heteroclitus* L., conducted in the salt

marsh drainages of Sapelo Island, Georgia. The hosts were placed in a solution of 1:4,000 formalin (Putz and Hoffman, 1963) and 24 ppm sodium chloride. After 1 hr, enough formalin was added to make a 5% formalin solution. Parasites were later recovered from the solution in the laboratory and mounted in

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ammoniumpicrate–glycerin as described by Malmberg (1957) and glycerin jelly. Specimens were measured according to Malmberg (1970). Measurements are in microns; averages are followed by the range in parentheses. Illustrations were made with aid of a camera lucida and phase contrast photomicrographs.

Gyrodactylus foxi sp. n.

HOST AND LOCALITY: *Fundulus heteroclitus* (L.), mummichog, from a marsh pit in Dean's Creek drainage on Sapelo Island, Georgia.

LOCATION ON HOST: Body surface and gill filaments.

SPECIMENS STUDIED: 10 measured.

TYPE SPECIMENS: 2 syntypes, USNM Helm. Coll. No. 72576. Other syntypes in author's collection.

Description

Length 290 (257–337), greatest width 98 (80–119). Pharynx obovate, 34 (32–37) long by 32 (28–37) wide; haptor length 57 (40–66), width 73 (58–82); marginal hooks 16, total length 27 (24–29). Marginal hook shaft is open and only slightly closed near distal end. Anchors, total length 44 (39–49); ventral bar, length 19 (17–21), total width 27 (22–34); shield of ventral bar oblong. Dorsal bar present. Peduncular bar present just anterior and ventral to haptor; length 53 (45–58), width 11 (10–13). Peduncular bar made up of two connected lobes with elongate pits on the anterior margin and a slightly sclerotized posterior pair of lobes. Cirrus 7 (6–8) long by 6 (5–7) wide, with two spines, two large spinelets, and two small spinelets.

Remarks

G. trematoclithrus Rogers, 1967, and *G. prolongis* Hargis, 1955, are the only other species of *Gyrodactylus* reported to have a peduncular bar with elongate pits. *G. foxi* is much smaller than other species but the peduncular bar is longer than the reported length of the peduncular bar of *G. trematoclithrus* (Rogers, 1967). *G. foxi* also differs in the shape of the marginal hooks from *G. trematoclithrus* which has a more closed marginal hook shaft. The marginal hooks of *G. foxi* and *G. prolongis* are similar in shape although

these structures are smaller in *G. foxi*; the two species differ in the shape of the anchors.

It is significant that the three species of *Gyrodactylus* which have a peduncular bar with elongate pits occur on the same or closely related species. *G. foxi* and *G. prolongis* occurred on the same specimens of *Fundulus heteroclitus*. *G. trematoclithrus* was described from *Lucania goodei* Jordan (Rogers, 1967) and the host has been reported from Sapelo Island (Dahlberg and Scott, 1971).

The species was named in honor of Dr. Alfred C. Fox.

Gyrodactylus mugelus sp. n.

HOST AND LOCALITY: *Mugel cephalus* L., striped mullet, from the mouth of Big Hole Creek on Sapelo Island, Georgia.

SPECIMENS STUDIED: 10 measured.

TYPE SPECIMENS: 2 Syntypes, USNM Helm. Coll. No. 72577. Other syntypes in author's collection.

Description

Length 444 (406–515), greatest width 105 (99–119). Pharynx obovate, 38 (34–42) long by 40 (38–44) wide. Haptor length 70 (64–79), width 95 (90–100). Marginal hooks 16, total length 29 (26–31). Marginal hook shaft is a closed sickle shape with a heavy basal shaft area. Anchors stout, total length 54 (51–61). Ventral bar, length 31 (28–34), total width 43 (44–45); shield of ventral bar ovate. Dorsal bar present. Cirrus length 12 (11–13), width 10 (9–12) with one spine and four small spinelets.

Remarks

The marginal hooks of *G. mugelus* resemble those of *G. emembranatus* (Malmberg, 1970). *G. zhukovi* Mo-en, 1962, is the only member of the genus *Gyrodactylus* known to occur on *Mugil* sp. *G. mugelus* and *G. emembranatus* differ from other species of *Gyrodactylus* in the shape of the marginal hooks which are extremely closed sickle-shaped shafts with heavy basal shaft areas. *G. mugelus* differs from *G. emembranatus* because of the presence of a ventral bar shield in *G. mugelus*.

The species was named for the genus *Mugil*.
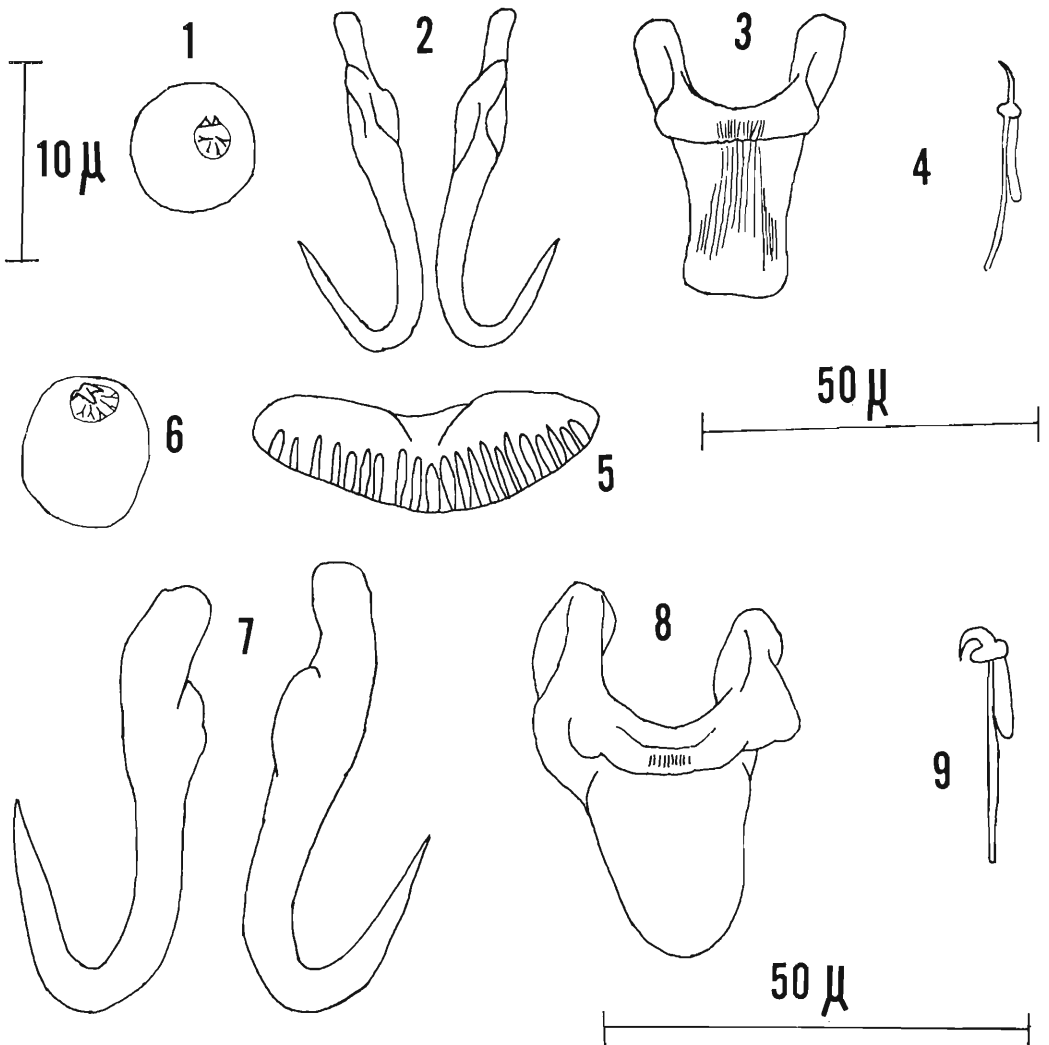
Figures 1–9. *Gyrodactylus foxi* from the mummichog (drawings 1–5) and *Gyrodactylus mugelus* from the striped mullet (drawings 6–9). 1, Cirrus. 2, Anchors. 3, Ventral bar. 4, Hooklet. 5, Penduncular bar. 6, Cirrus. 7, Anchors. 8, Anchors. 9, Hooklet.

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Incidence, Distribution, and Morphology of the Macroderoidid Trematode *Alloglossidium hirudicola* Schmidt and Chaloupka, 1969, from Leeches

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ABSTRACT: Four genera of leeches, *Haemopis, Macrobdella, Placobdella,* and *Erpobdella,* from six counties in Wisconsin were examined for *Alloglossidium hirudicola,* Schmidt and Chaloupka, 1969. *M. decora* (new host record) had an infection rate of 30.3% and *Haemopis* sp. 18.1%. Stained whole mounts, *in situ* sections, and scanning electron micrographs of *A. hirudicola* from *M. decora* revealed well-developed tegumental spines. Populations of *A. hirudicola* from *M. decora* differed in size and pattern of the tegumental spines from populations of *Haemopis* sp.

Alloglossidium hirudicola was recently named and described from the cecum of the leech *Haemopis* sp. from an unknown locality (Schmidt and Chaloupka, 1969). During the summers of 1970 and 1971, a total of 396 leeches belonging to four genera, *Haemopis, Erpobdella, Macrobdella,* and *Placobdella,* were collected and examined from six counties in Wisconsin. This paper will discuss the incidence, geographical distribution, and morphology of *A. hirudicola* found in these Wisconsin leeches.

Materials and Methods

Leeches were collected by searching under rocks or by baiting at night with raw meat. Trematodes recovered were fixed in FAA without coverslip pressure, stained in paracarmine, and counterstained with fast green. Intestines for *in situ* studies were fixed in Bouin's solution, sectioned at 8 μ, stained with Heidenhain's iron hematoxylin, and counterstained with eosin. Both preparations were mounted in Permount. For scanning electron micrographic studies, adult flukes were fixed in 10% formalin, dehydrated through 70% ethanol, air-dried, and mounted on brass pegs. Specimens were then placed in a vacuum and coated with gold.

Alloglossidium hirudicola from *Macrobdella decora* and *Haemopis* sp.

Measurements (Table 1) were made on gravid *A. hirudicola* from *M. decora* and *Haemopis* sp. and compared with those reported by Schmidt and Chaloupka (1969). Morphological characters considered diagnostic by them, including body and egg size, pharynx, oral sucker, testes, and ovary diameters, and extent of vitellaria were quite variable in *A. hirudicola* from the same and different hosts. However, one diagnostic morphological character, namely the tegumental spines, differed both in size and distribution in trematodes from the two hosts. *A. hirudicola* recovered from *Haemopis* sp. by Schmidt and
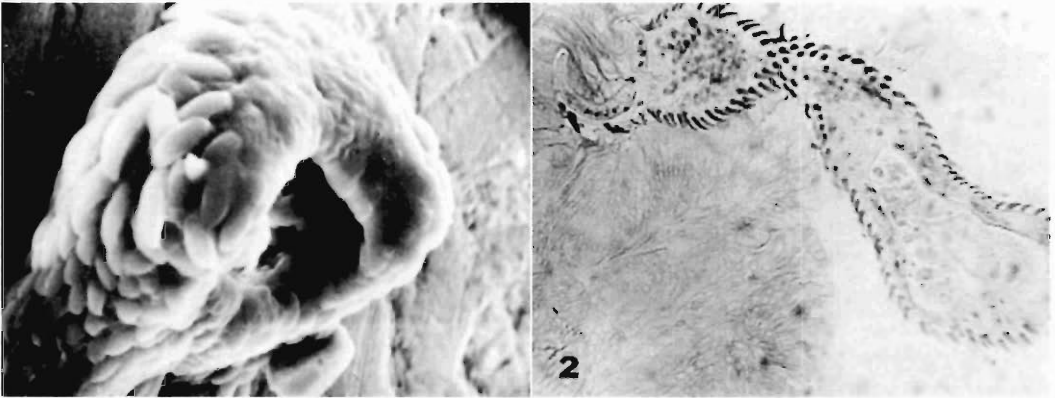
Figures 1, 2. *Alloglossidium hirudicola* from *Macrobdella decora*. 1, Scanning electron micrograph of tegumental spines. × 570. 2, *In situ* section. × 250.

Chaloupka and by us had minute spines that extended from the anterior end of the trematode to approximately the posterior margin of its oral sucker. However, *A. hirudicola* parasitizing *M. decora* had much larger spines and the pattern extended at least to the posterior margin of the acetabulum (Figs. 1, 2). Anterior to the dorsal lip of the oral sucker, an area

Table 1. Means and observed ranges of measurements of gravid *Alloglossidium hirudicola* from *Haemopis* sp. and *M. decora*.

	Haemopis sp.[1]		Haemopis sp. L. Metonga N[2] = 18 3,790	Chippewa L. N = 1 4,004	M. decora Pallen L. N = 21 3,560	Chippewa L. N = 28 4,040	L. Knockaby N = 10 3,780
Body length	2,100–2,600[3]		2,420–4,420		1,570–5,750	1,780–5,930	2,030–4,940
			487	624	294	223	213
Body width	450–500		298–649		165–330	143–374	132–275
Pharynx	80–100	length	85	138	70	64	73
	80–130	width	66–110		51–88	41–88	55–99
			97	153	73	70	75
			77–132		44–88	41–88	44–99
Oral sucker	160–200	length	170	147	99	91	144
	150–170	width	143–209		66–138	55–132	99–187
			167	142	97	94	144
			132–198		67–110	66–132	88–187
Acetabulum	160–200	length	183	137	100	102	168
	190–210	width	153–220		66–133	66–182	99–220
			192	153	98	92	174
			171–231		77–121	55–121	143–203
Anterior testis	200–280	length	238	214	279	321	303
	170–275	width	163–341		165–362	198–473	198–417
			188	138	182	203	177
			99–319		107–231	121–385	66–176
Posterior testis	240–320	length	264	204	350	390	343
	160–255	width	198–363		198–527	232–572	232–462
			179	138	187	210	118
			97–279		121–242	110–374	66–176
Ovary	200–280	length	360	296	185	198	243
	160–170	width	264–437		132–242	110–264	154–241
			227	188	139	134	110
			154–308		91–198	77–205	55–165
Eggs	24–28	length	30	28	33	34	25
	14–16	width	25–32		30–36	31–36	24–27
			18	13	23	23	16
			15–26		15–26	20–31	15–20

[1] Data from Schmidt and Chaloupka (1969), locality unknown.
[2] N = sample size of *A. hirudicola*.
[3] All measurements in microns.

Table 2. Geographical localities of leeches harboring *Alloglossidium hirudicola*.

Host	Year	Locality	No. examined	No. infected	Per cent infection
Haemopis sp.	1970	Lake Metonga Forest Co.	2	2	—
Haemopis sp.	1970	Chippewa Lake Vilas, Co.	6	1	16.6
Haemopis sp.	1971	Lake Metonga	1	1	—
Haemopis sp.	1971	Chippewa Lake	$\frac{13}{22}$	$\frac{0}{4}$	0.0
M. decora	1970	Chippewa Lake	25	23	92.0
M. decora	1971	Chippewa Lake	1	1	—
M. decora	1971	Farm pond SW ¼ of NW ¼ S13, T. 25N, R5E	57	0	0.0
M. decora	1971	Pallen Lake Portage Co.	208	50	24.0
M. decora	1971	Lake Knockaby Marinette, Co.	47	28	59.5
M. decora	1971	Mead Wildlife Refuge Marathon Co.	$\frac{2}{340}$	$\frac{1}{103}$	
E. puncta	1971	Temporary pond NW ¼ of SE ¼ S15, T. 24N, R8E	23	0	0.0
E. puncta	1971	Collins Lake Portage Co.	$\frac{8}{31}$	0	0.0
P. parasitica	1971	Cedarburg Bog Washington Co.	3	0	0.0

was devoid of spines. The largest spines were immediately posterior to this region. More posteriorly, the spines diminished in size and were deeply embedded in the integument. The spines level with the acetabulum barely protruded. These spines along with the well-developed oral sucker and acetabulum provided firm attachment to the host's tissue. Specimens of *A. hirudicola* were embedded as deeply as the connective and muscular tissue of the intestine (Fig. 2). Perforation of the intestine by one trematode was noted.

Geographical Records of the Hosts of *Alloglossidium hirudicola*

There are no recorded localities of occurrence of *A. hirudicola*. Therefore, the localities in Wisconsin provide information on the geographic distribution of this species. With the exception of leeches from Cedarburg Bog, Washington County, in southeastern Wisconsin, all others were collected north of Stevens Point, Wisconsin (Table 2). This parasite is common in certain areas, with a prevalance of 92% in one locality. The average number of helminths per parasitized leech was 2.1, and the largest number recovered from a single leech was 24.

Discussion

Schmidt and Chaloupka (1969) mentioned that trematodes were found in the ceca of the crop. *A. hirudicola* recovered by us occurred in the intestine of all hosts.

Percentages of infected leeches (Table 2) are probably correlated with relative abundance of snails. A farm pond sampled contained only *Sphaerium*, and none of the leeches was infected. Pallen Lake with an infection rate of 24.0% had very small populations of *Physa, Helisoma,* and *Ferrisia*. Bodies of water with a high incidence of infected leeches, such as Lake Metonga, Lake Knockaby, and Chippewa Lake all had large populations of *Physa, Helisoma,* and *Lymnaea*. Life cycle studies on species of *Alloglossidium* from fish by McCoy (1928) and McMullen (1936) showed *Helisoma* to be the first intermediate host. Life history studies in our laboratory are in progress.

Acknowledgments

many of the leeches, and to Dr. C. A. Long for critically reading the manuscript.

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A New Genus and Three New Species of Monogenetic Trematodes from North Carolina Centrarchids, with a Redescription of *Clavunculus okeechobiensis* (Mizelle and Seamster) comb. n.

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ABSTRACT: A new genus and three new species of Ancyrocephalinae (Trematoda: Monogenea) are described and *Clavunculus okeechobiensis* (Mizelle and Seamster) comb. n. is redescribed from North Carolina centrarchids. *Cleidodiscoides sulcata* gen. et sp. n. and *Urocleidus pomotis* sp. n. are described from the mud sunfish, *Acantharchus pomotis*. *Lyrodiscus lanceolatus* sp. n. is described from the redbreast sunfish, *Lepomis auritus*. *Clavunculus okeechobiensis* (Mizelle and Seamster) comb. n. is redescribed from the warmouth sunfish, *Lepomis gulosus*. *Cleidodiscoides* gen. n. most closely resembles *Cleidodiscus* Mueller, 1934, by possessing an accessory piece which is articulated with the cirrus and by having four anchors and two supporting bars. The morphology of the anchors, with the deep root modified into a saddlelike structure and a spur originating at the base of the anchor shaft and terminating near the point, and the basally articulated accessory piece, clearly distinguish *Cleidodiscoides* from other genera of Ancyrocephalinae.

Investigations of the monogenetic trematodes infesting centrarchids in North Carolina are sparse. Holl (1932) reported *Ancyrocephalus* spp. from the gills of *Lepomis gulosus, Lepomis gibbosus,* and *Enneacanthus gloriosus* obtained from the Cape Fear River near Pinehurst, North Carolina. *Urocleidus flieri* was described by Putz and Hoffman (1966) from *Centrarchus macropterus* collected in Columbus County, North Carolina. This is the first report of monogenetic trematodes from the mud sunfish, *Acantharchus pomotis*; and also reports a new species of *Lyrodiscus* from the redbreast sunfish, *Lepomis auritus*. Comparative studies indicated that *Actinocleidus okeechobiensis* Mizelle and Seamster, 1939,

should be *Clavunculus okeechobiensis* (Mizelle and Seamster) comb. n.

Materials and Methods

Hosts were brought to the laboratory where the gills and body were treated as prescribed by Rogers (1966). After 1 hr the parasites were recovered, preserved in 5% formalin, and either mounted unstained in glycerin gel or stained with Mayer's HCL carmine and mounted in a commercial resin. Measurements were made with a filar micrometer as suggested by Mizelle and Klucka (1953) and expressed in microns. Average measurements are followed by the ranges in parentheses. Illustrations were prepared with the use of a camera lucida.

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Results

Cleidodiscoides gen. n.

Generic diagnosis

Dactylogyridae, Ancyrocephalinae: Small forms with two pairs of eyespots; cephalic glands present. Gut bifurcate, confluent posteriorly. Haptor not well defined. Two pairs of similar anchors provided with deep roots which are differentiated into laterally projecting saddles facilitating bar and muscle attachment and prominent superficial roots. The anchors are provided with a spur originating at the base of the anchor shaft and joining the anchor point distally. Conspicuous double anchor wings present. Two dissimilar, nonarticulate, transverse bars and 14 hooks present. Ovary elongate in posterior ⅔ of body; testis smaller and posterior to ovary. Vagina not observed. Parasitic on gills of freshwater fish.

Remarks

This genus is closely related to *Cleidodiscus* Mueller, 1934, by possessing nonarticulate bars, four anchors, and a basally articulated accessory piece. The anchors possess spurs similar to those in the genus *Pterocleidus* Mueller, 1937. However, the unusual nature of the anchors (Plate I, Figs. 2 and 6) along with the basally articulated accessory piece distinguishes *Cleidodiscoides* from other genera of the Ancyrocephalinae. The generic name refers to the similarity to *Cleidodiscus* Mueller, 1934.

Cleidodiscoides sulcata gen. et sp. n.
(Plate I, Figs. 1–8)

HOST AND LOCALITY: *Acantharchus pomotis* (Baird), Black River, Harnett County, North Carolina.

LOCATION ON HOST: Gills.

SPECIMENS: USNM Helm. Coll. holotype No. 72152, paratype Univ. Nebraska State Mus. No. 20025 and in collection of authors.

Description

(Measurements based on 12 specimens.) Length 358 (298–470), width 88 (65–115). Two pairs of eyespots, members of anterior pair smaller and closer together than posterior pair. Cephalic glands present, indistinct.

Pharynx round, diameter 26 (23–30), gut bifurcated and joined posteriorly. Peduncle indistinct, haptor rectangular, length 67 (59–94), width 82 (67–93). Two pairs of similar anchors present. Each anchor composed of a saddlelike modification of the deep root and a prominent superficial root, short shaft, and an elongate point provided with an accessory structure originating at the base of the shaft and uniting with the point distally; anchor wings prominent. Ventral anchor length 33 (29–36), width 22 (19–24). Dorsal anchor slightly recurved distally, length 27 (19–30), width 15 (13–18). Two dissimilar, nonarticulated bars supporting anchors. Dorsal bar straight with knobbed ends, 34 (29–38) long. Ventral bar provided with a posteriorly directed protuberance, length 43 (36–49). Fourteen hooks present, each composed of a cylindrical base, a narrow shaft, sickle-shaped point, and a looped opposable piece, 16 (15–18) long. Ovary elongate, located in intercecal space in posterior ⅔ of body; vitellaria coextensive with intestine. Testis posterior to ovary; associated structures not visible. Copulatory complex composed of a cirrus and a basally articulated accessory piece. Cirrus a straight tube, length 20 (16–23); accessory piece bifurcates proximally, one part being straight, the other curving distally, length 14 (12–16). Vagina not observed.

Remarks

At present this is the only species in the genus. The specific name is derived from Latin (*sulca* = furrow) in reference to the shape of the anchors.

Urocleidus pomotis sp. n.
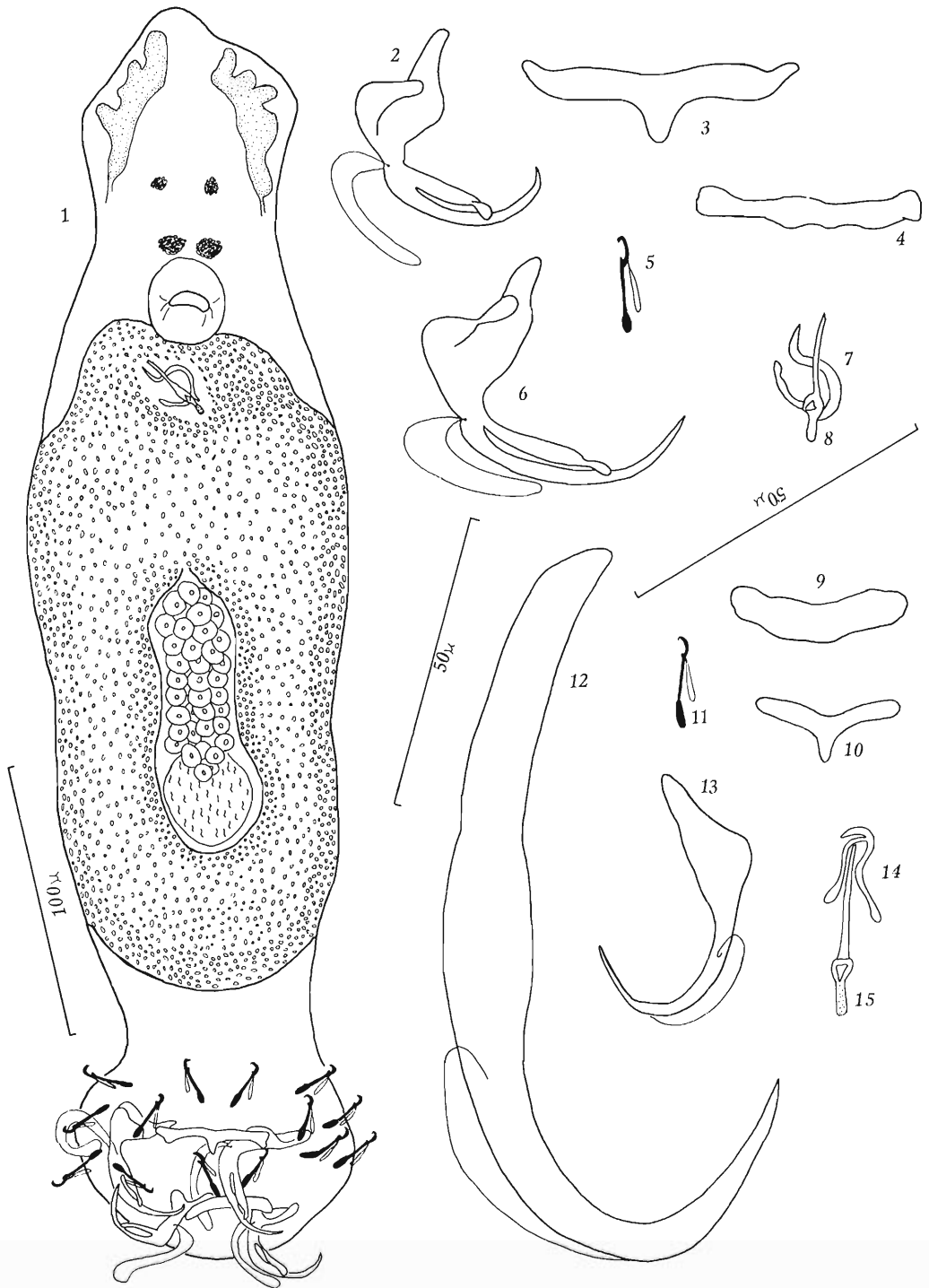### (Plate I, Figs. 9–15)

TYPE HOST AND LOCALITY: *Acantharchus pomotis* (Baird), Black River, Harnett County, North Carolina.

SPECIMENS: USNM Helm. Coll. holotype No. 72151, paratype Univ. Nebraska State Mus. No. 20,026.

LOCATION ON HOST: Gill filaments.

Description

(Measurements based on three specimens; ranges in parentheses.) Length 472 (390–559), width 113 (93–132). Two pairs of eye-

spots, members of anterior pair smaller and closer together than posterior pair. Cephalic glands present. Pharynx round, diameter 28 (26–33). Intestine bifurcate, rejoins posteriorly. Haptor poorly defined, length 112 (108–118), width 79 (69–94). Dorsal anchor larger than ventral anchor; prominent superficial root present, deep root lacking, length 100 (99–101), width 21 (20–22). Ventral anchor 31 (30–33) long by 13 wide; superficial root present, deep root lacking. Two bars present, nonarticulate, dissimilar in size and shape. Dorsal bar robust, length 26 (25–27). Ventral bar slender with a knob projecting posteriorly, length 19 (18–20). Haptoral hooks 14, normal in arrangement. Each hook composed of an oval base, a slender shaft, and a sickle-shaped point with an opposable piece. Hooks, one to four, and six and seven, subequal in length, 16 (15–17), pair number five, 11 (9–13). Ovary ovoid and pretesticular. Vitellaria well developed, extending from the level of the pharynx posteriorly along intestine. Vagina not observed. Copulatory complex composed of a cirrus and an accessory piece. Cirrus a straight tube, length 26 (25–27); accessory piece is composed of a distal sickle-shaped structure which is attached to a bifurcated unit with the rami extending toward the base of the cirrus, length 15 (14–16). Testis postovarian. Vas deferens not observed.

Remarks

Urocleidus pomotis sp. n. is most closely related to *U. circumcirrus* Allison and Rogers, 1970. The anchors and bars are similar. However, the ratio of the dorsal anchor to the ventral anchor is 3:1 for *U. pomotis* and 2:1 for *U. circumcirrus*. Also the bars of *U. pomotis* are not as robust as those of *U. circumcirrus*. The copulatory complex of *U. pomotis* is similar to that of *U. circumcirrus*, *U. udicola* Allison and Rogers, 1970, *U. carolinensis*

Mayes (1973), and *U. adsimulatus* Mayes (1973). The relationship of these parasites to one another and the implications of this relationship is discussed by Mayes (1973).

This species is named from the host, *Acantharchus pomotis* (Baird).

Clavunculus okeechobiensis comb. n. (Plate II, Figs. 1–8)

SYNONYM: *Actinocleidus okeechobiensis* Mizelle and Seamster, 1939.

HOST AND LOCALITY: *Lepomis gulosus*, Fincrest Pond, Wake County, North Carolina.

LOCATION ON HOST: Gills.

SPECIMENS: USNM Helm. Coll. No. 72154.

Redescription

(Measurements based on 10 specimens.) Length 750 (420–1,170), width 112 (54–145). Haptor distinct, umbrellalike with scalloped margin, 70 (60–86) long by 113 (82–144) wide. Bars, which are located on a protuberance in the center of the haptor, dissimilar in shape, articulated; posterior bar 28 (24–33) long, anterior bar 35 (30–37) long. Ventral and dorsal anchors similar in shape, bases slightly bifurcate. Length of anterior anchors 36 (32–38); length of posterior anchors 31 (25–34). Each hook composed of a small ovate base, a solid shaft, a sickle-shaped point, and an opposable piece. Hook arrangement modified; pairs six and seven are located on posterior dorsal border of haptor. Pair number five located between the bars on the central protuberance of the haptor. Hooks one to four and six and seven 13 (11–16) long; pair number five 11 (10–12). Copulatory complex composed of a cirrus and a basally articulated accessory piece. Cirrus is a cuticularized tube which tapers and curves distally, 36 (31–46) long. Accessory piece is a cuticularized sleevelike structure 29 (26–33) long. Testis posterior and dorsal to ovary, diameter

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Plate I

Figures 1–8. *Cleidodiscoides sulcata* gen. et sp. n. 1, Whole mount. 2, Dorsal anchor. 3, Ventral bar. 4, Dorsal bar. 5, Hook. 6, Ventral anchor. 7, Accessory piece. 8, Cirrus.

Figures 9–15. *Urocleidus pomotis* sp. n. 9, Dorsal bar. 10, Ventral bar. 11, Hook. 12, Dorsal anchor. 13, Ventral anchor. 14, Accessory piece. 15, Cirrus.

92 (89–96). Vas deferens departs from the dorsal aspect of testis, loops around the left arm of the gut, and leads to a large seminal vesicle, large prostate present, each structure with a tube leading into the cirrus. Ovary 65 (61–67) in diameter. Uterus extends medio-anteriorly from the ovary and opens posterior to copulatory complex. Vagina sinistral, cuticularized, and connected to the seminal receptacle. Vitellaria well developed, originating at or near the pharynx, and extending posteriorly alongside the gut which is confluent posteriorly. Two pairs of eyespots and cephalic glands present.

Remarks

This species was tranferred to the genus *Clavunculus* based on the characteristics of the genus as described by Mizelle et al. (1956).

Lyrodiscus lanceolatus sp. n.
(Plate II, Figs. 9–15)

TYPE HOST AND LOCALITY: *Lepomis auritus* (Linnaeus), Dutchman's Creek, Wake County, North Carolina.

LOCATION ON HOST: Gills; possibly skin.

SPECIMENS: USNM Helm. Coll. holotype No. 72153, paratype Univ. Nebraska State Mus. No. 20,027.

Description

(Measurements based on three specimens.) Large-sized dactylogyrid, length 1,378 (857–1,670), width 235 (109–303). Two pairs of eyespots; members of anterior pair smaller and closer together than posterior pair. Cephalic glands present, poorly defined. Pharynx round, diameter 64 (48—73); gut bifurcates and rejoins posteriorly. Haptor well defined, lyre-shaped, length 212 (160–244), width 237 (206–270). Two pairs of dissimilar anchors supported by two dissimilar, nonarticulate bars. Ventral anchor composed of an elongate base without roots, a tapering shaft, and a short
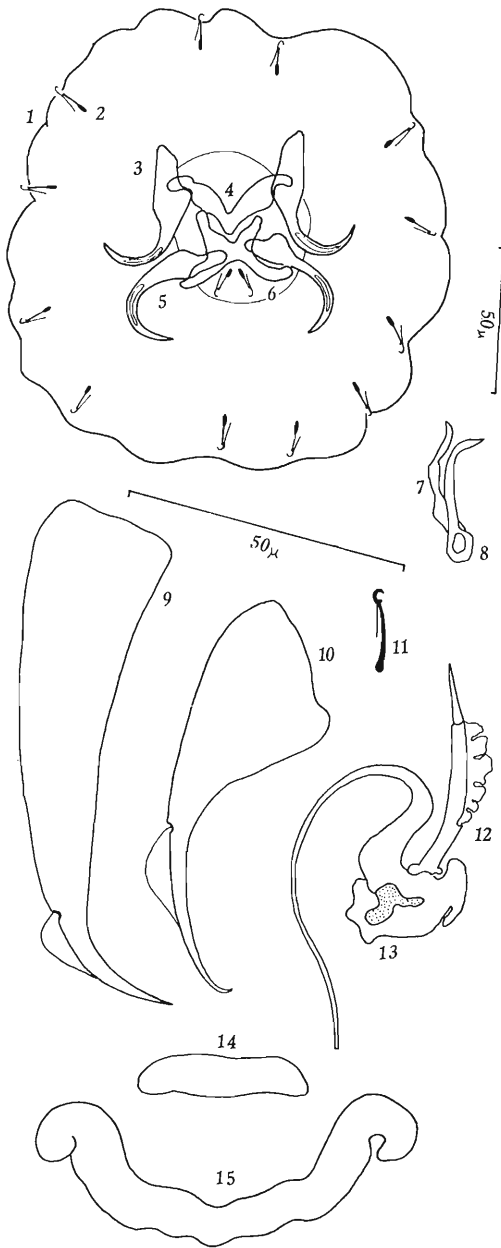

Plate II

Figures 1–8. *Clavunculus okeechobiensis* (Mizelle and Seamster) comb. n. 1, Haptor. 2, Hook. 3, Anterior anchor. 4, Anterior bar. 5, Posterior anchor. 6, Posterior bar. 7, Accessory piece. 8, Cirrus.

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Figures 9–15. *Lyrodiscus lanceolatus* sp. n. 9, Ventral anchor. 10, Dorsal anchor. 11, Hook. 12, Accessory piece. 13, Cirrus. 14, Ventral bar. 15, Dorsal bar.

attenuated point. Dorsal anchor composed of a broad base without roots, an elongate solid shaft, and a short point. Each anchor with distinct anchor wings arising from a notch in the anchor shaft. Ventral anchor length 157 (145–176), width 43 (38–48). Dorsal anchor length 125 (116–130), width 49 (47–51). Dorsal bar robust and yoke-shaped, the expanded ends appear to wrap around anchors, length 129 (120–137). Ventral bar stout, with rough lateral edges, length 65 (62–69). Seven pairs of hooks present, similar in shape, variable in size. Each hook composed of an elongate base, a slender shaft, and a sickle-shaped point provided with an opposable piece. Length: No. 1, 26 (22–29); 2, 28 (27–29); 3, 30 (28–32); 4, 28 (26–31); 5, 34 (28–37); 6, 30 (29–33); 7, 28 (26–28). Testis and ovary within intercecal space. Testis postovarian, vas deferens not observed. Large seminal vesicle present. Ovary small, partially overlapped by larger testis. Copulatory complex composed of a cirrus and a basally articulated accessory piece. Cirrus a long tapering tube with an inflated base, length 147 (122–162). Accessory piece a solid shaft with a serrate process arising at midlength. Accessory piece length 77 (73–80). Vagina sinistral, duct running medially where it enters the seminal receptacle through a heavily cuticularized process. Vitellaria poorly developed, coextensive with intestine.

Remarks

The copulatory complex and haptoral bars of *Lyrodiscus lanceolatus* sp. n. resemble those of *L. seminolinsis* Rogers, 1967. However, the base of the ventral anchor of *L. lanceolatus* is not as robust and the anchor point is much shorter than that of *L. seminolinsis*. The anchors of *L. muricatus* Rogers, 1967, resemble those of *L. lanceolatus*; however, the copulatory complex with the lancelike accessory piece distinguishes *L. lanceolatus* from *L. muricatus*. The proposed name was derived from the lancelike accessory piece.

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In Memoriam

Alan Collins Pipkin
May 2, 1911–April 5, 1973
Member since 1959
Vice-president, 1968
President, 1969

Ivan Pratt
September 18, 1908–April 14, 1973
Member since 1964

A New Tapeworm, *Schizorchodes dipodomi* gen. et sp. n. (Cestoda: Anoplocephalidae), from the Merriam Kangaroo Rat, *Dipodomys merriami vulcani*

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ABSTRACT: *Schizorchodes dipodomi* gen. et sp. n. in the small intestine of the Merriam kangaroo rat, *Dipodomys merriami vulcani* Benson, from southwestern Utah is described. The general morphological organization of *Schizorchodes* appears to be similar to the genus *Schizorchis*. However, *Schizorchodes* is separated from *Schizorchis* by the absence of a neck, the possession of a bilobed ovary, and two posterolateral fields of testes. In addition, the eggs of *Schizorchodes* lack a pyriform apparatus.

As a result of a study on the parasitism of the Merriam kangaroo rat, *Dipodomys merriami vulcani* Benson, conducted at Dixie State Park, Washington County, Utah, several specimens of an apparently new tapeworm were obtained. Examination of the specimens indicated that they did not readily fit into existing genera, but were closest to *Schizorchis* (Hansen, 1948) in structural arrangements of organs. The eggs lack a pyriform apparatus, but the scolex is anoplocephaline in structure. Consultation with Dr. Malcolm MacDonald, U. S. Fish and Wildlife Service, Dr. Robert Rausch (author of description of two species of *Schizorchis*), Arctic Health Research Center, and Dr. Gerald Schmidt, University of Northern Colorado, did not resolve the taxonomy. Suggestions were made by these workers favoring the erection of a new genus in either the family Anoplocephalidae or Dilepididae. Comparative morphological studies indicate the former family to be preferable. The habitat of the Merriam kangaroo rat in the study area appears to provide for an extremely high incidence of cestodes for desert rodents.

Materials and Methods

Two specimens were obtained from hosts, which had been preserved in formaldehyde. The tapeworms were made more pliable by soaking in 3% carbolic acid as described by Kirby (1950). Later dissections of hosts sacrificed in the laboratory produce one additional specimen that was relaxed in distilled water and fixed in A.F.A. stained with carmine and mounted in Eukitt. All measurements in microns unless otherwise noted.

Schizorchodes gen. n.
(Figs. 1–4)

HOST: *Dipodomys merriami vulcani* Benson.
LOCATION: Small intestine.
TYPE LOCALITY: Dixie State Park, Washington County, Utah.
HOLOTYPE: USNM Helm. Coll. (No. 72587).

Unarmed scolex well developed, rostellum absent. Neck absent. Craspedote segments. Single set of reproductive organs per segment, genital pores alternating irregularly, cirrus unarmed, external seminal vesicle present. Testes numerus, arranged in two distinct lateral groups in posterior half of segment. Ovary bilobed, median in position. Vitelline gland postovarian. Vaginal pore ventral to cirrus. Uterus transverse, saclike. Eggs: pyriform apparatus lacking.

Schizorchodes dipodomi sp. n.

Unarmed scolex strongly developed, 300 by 191, suckers quite large, 91 by 118. Strobila 70 mm long with as many as 181 segments, maximum width 1.1 mm in gravid segments. Strobila widens gradually from anterior to posterior end, but the major part fairly uniform in width. All segments wider than long. Elongated cirrus sac, positioned in anterior portion of segment, occupying ⅛ of width of proglottid measuring 91 by 46. Well-developed external seminal vesicle. Testes 27 by 30 situated in two separate posterolateral
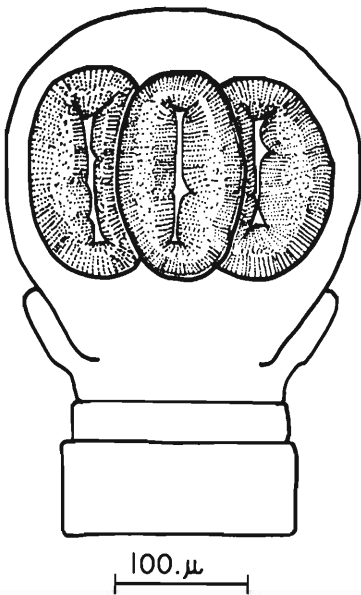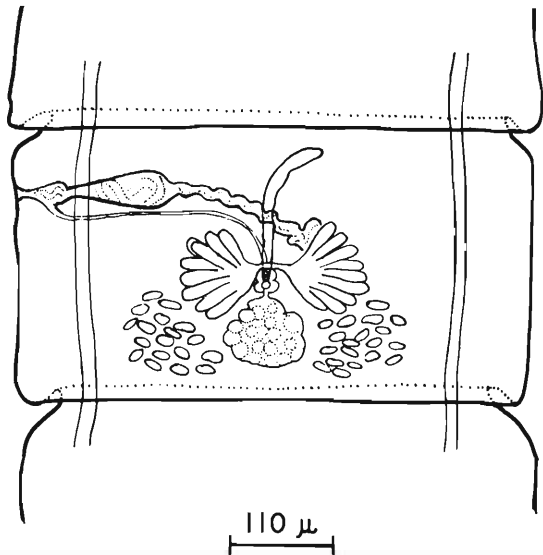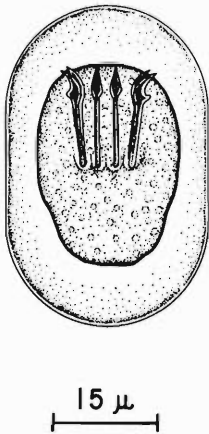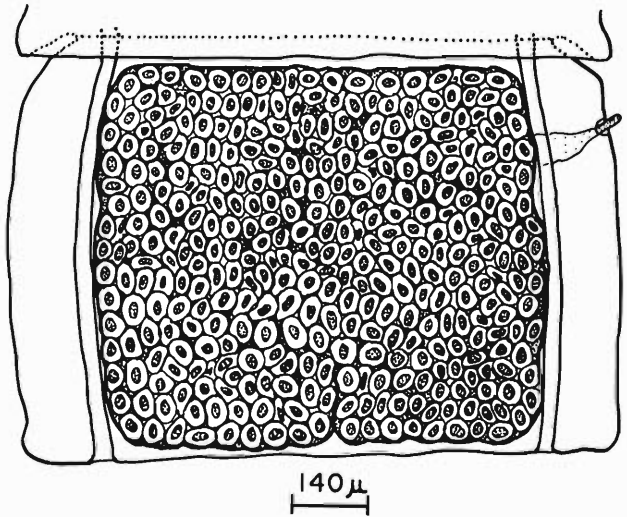
Figures 1–4.   *Schizorchodes dipodomi* gen. et sp. n.   1. Scolex;   2. Mature proglottid;   3. Egg;   4. Gravid proglottid.

fields, 14 to 22 testes on each side (see Fig. 2). Anlagen of testes appearing at 35th segment, the two groups separated, occasionally one to two testes median and connecting groups. Laterally positioned, testes not extending to excretory canals. Anlagen of ovary appearing at 10th segment, measuring 137 by 100. Bilobed ovary occupying about ¼ of width of mature segment. Genital pore opening into genital atrium in anterior one-third region of proglottid. Uterus transverse, saclike. Gravid uterus not extending beyond ventral longitudinal excretory canal (see Fig. 4). Eggs 36 by 46.

Discussion

Comparison of the new genus with *Schizorchis*, the genus that appears to be most closely allied, showed that the arrangements of the genital organs, although similar, have several notable differences. The testes of both occur in two lateral fields, but *Schizorchodes* has far fewer testes and a bilobed ovary. The scolex of *Schizorchodes* is prominent and more strongly developed and lacks the distinct neck region found in *Schizorchis*. The eggs of the new genus also lack a pyriform apparatus and are distinctly smaller in size.

Hansen (1948) described the genus *Schizorchis* from the pika in Colorado. Studies by the authors of parasitism in *Ochotona* show that a high phylogenetic host specificity exists and that other mammals such as *Marmota*, *Eutamias*, *Peromyscus*, etc., that occupy the same localities and habitat as pika do not exchange parasites with them. Some confusion as to host definition exists in the literature. Spassky (1951) reported that *Schizorchis altaica* (Gvozdev) was taken from hares at Lake Baikal. In table IV, page 145 of this work, he states that *Schizorchis* was found in rodents. However, further analysis of his writings showed that he not only considers pika to be in the same subfamily as the hares, but places the hares in the order Rodentia. However, Walker (1968) states, "Lagomorpha was regarded as a suborder of the Rodentia for many years, but few modern zoologists now treat it as such. The origin of these animals is uncertain. Blood tests indicate the phylogenetic distinction of lagomorphs and show no relationship between lagomorphs and

rodents. There are some similarities to the various groups of hoofed animals." As it now stands, all species of *Schizorchis* have been described from pika and the genus appears to be host-specific.

Because of the unusually high number of cestode genera recovered from the 190 hosts examined, a short discussion of the type locality is warranted. Genera recovered in order of prevalence were: *Mathevotaenia*, *Andrya*, *Catenotaenia*, and *Schizorchodes*. The type locality is in the transitional zone between the cold desert habitat of the Great Basin Faunal Area and the warm Mojave Desert Faunal Area to the south with about two-thirds of the plant species representing the latter. The type locality is also at the point where the Virgin River Province of the Colorado Plateau Faunal Area reaches its western limits and the Middle Rocky Mountain Faunal Area, as represented by the chain of mountain ranges extending north and south through Utah, reaches its southern and western limits. The host, subspecies, *D. m. vulcani*, is restricted to this epicenter encompassing only a few square miles of the region around St. George, Utah, where a suitable sand habitat exists. While considerable parasitological work has been completed in the Great Basin and Middle Rocky Mountain Faunal Areas to the north, little has been done in the northern Mojave and nothing to the authors' knowledge in the Colorado Plateau Faunal Area. Designation of faunal areas is based upon the Biotic Provinces of Dice (1943) as modified by Durrant (1952).

Colonies of the pika, *Ochotona princeps*, containing *Schizorchis ochotonae* Hansen, are present in the mountains about 40 miles from the type locality. The pika is almost always restricted to talus slopes in the alpine tundra. The habitat conditions existing between the pika colonies and the type locality of the Merriam kangaroo rat, a warm desert form at the northern limits of its range in Utah, constitute a formidable barrier at the present time.

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Studies on Monogenea of Pakistan. II. Polyopisthocotyleans from the Gills of *Pellona elongata* (Bennett)

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ABSTRACT: Three new species are described from the marine fish, *Pellona elongata* (Bennett), from Korangi Creek, Karachi, Pakistan: *Paramazocraes tripathii*, *Choricotyle pellonae*, and *Pellonicola lanceolatum*. The genus *Pellonicola* Unnithan, 1967, is emended to include *P. lanceolatum* sp. n.

This study is based on collections made by the second author as part of a continuing investigation of the helminth parasites of fishes from Pakistan. Previously, *Pseudochauhanea elongatus* (Gastrocotylidae) was described from the gills of the freshwater host, *Labeo rohita* (Ham.), from Kalri Lake, Sind, Pakistan (Kritsky et al., 1972). In the present study, three new species of Polyopisthocotylea, *Paramazocraes tripathii* (Mazocraeidae), *Choricotyle pellonae* (Diclidophoridae), and *Pellonicola lanceolatum* (Gastrocotylidae), are described from the gills of the marine fish, *Pellona elongata* (Bennett), from Korangi Creek, Karachi, Pakistan.

Methods used in the collection, preservation, and preparation of the Monogenea for study

are the same as those described by Kritsky et al. (1972), except that Mayer's acid carmalum was used to differentiate internal anatomy. Measurements, in microns unless otherwise indicated, were made according to the procedures of Dillon and Hargis (1965). The camera lucida and microprojector were used in the preparation of the plates. Holotypes are in the USNM Helminthological Collection, and paratypes are in the collection of the senior author.

Paramazocraes tripathii sp. n.
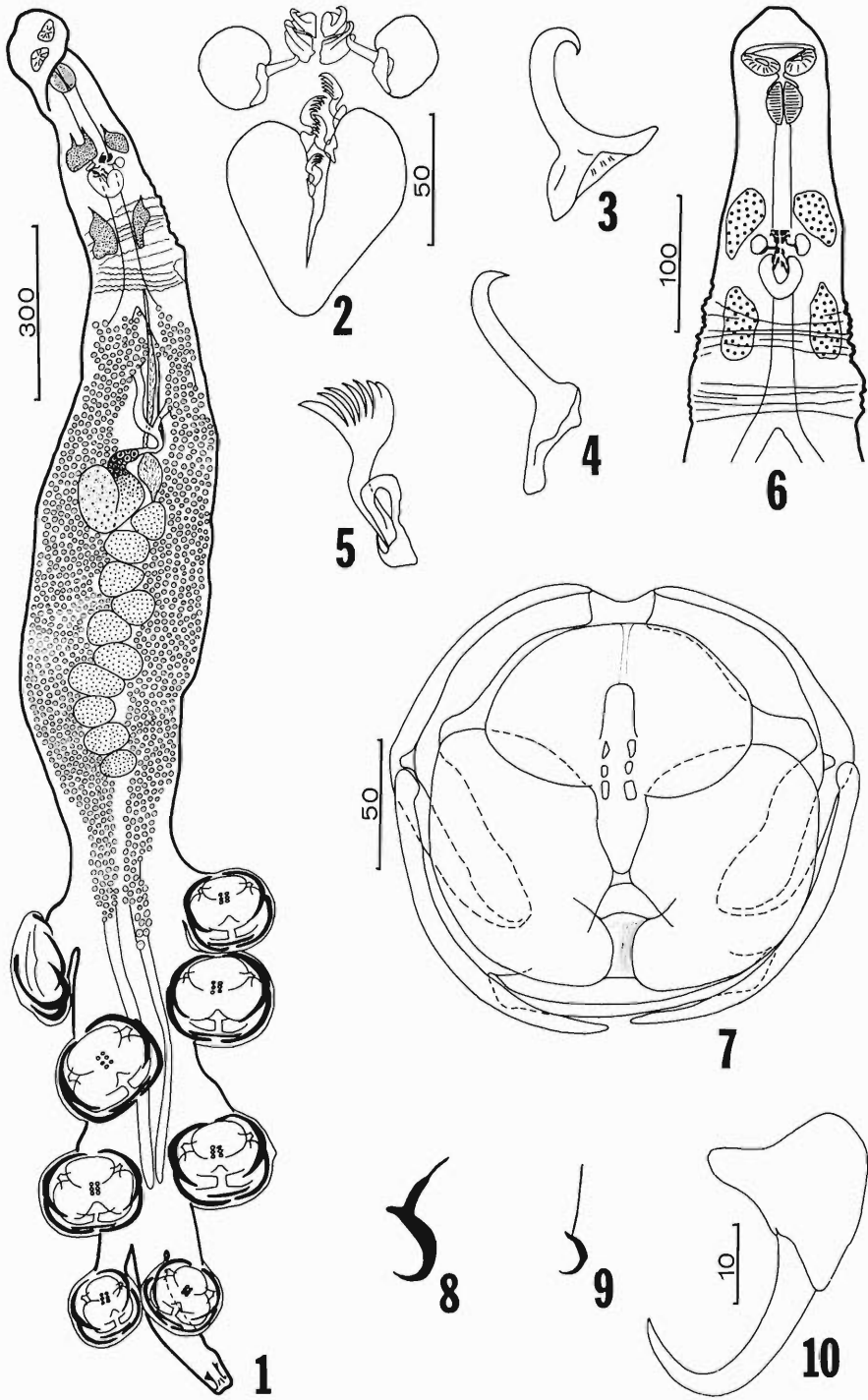### (Figs. 1–10)

SPECIMENS STUDIED: 10.
HOLOTYPE: USNM Helm. Coll. No. 72431.

Description

Mazocraeidae, Mazocraeinae. Body fusiform, with short broad peduncle; length 2.54 mm

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(1.59 to 3.87), greatest width 367 (252 to 567) near midlength. Cuticle thin, smooth. Dorsal surface of trunk anterior to level of bifurcation of gut with several sclerotized transverse ridges. Prohaptor a pair of subovate suckers, 53 (45 to 62) by 55 (45 to 65), situated in posterolateral wall of buccal funnel. Opisthaptor 1,001 (630 to 1,525) long by 592 (454 to 806) wide, symmetrical, with elongate terminal lappet and four pairs of pedunculate clamps. Clamps similar in shape: pr. 1—162 (146 to 172), 2—187 (143 to 218), 3—183 (156 to 215), 4—140 (126 to 169) wide. Structure of clamp as follows: Center piece with six perforations, lightly sclerotized; dorsal sclerite with flattened internal margin; ventral sclerite rod-shaped. Anchors ventral in lappet; lateral anchor 45 (42 to 49) long, with recurved point and poorly developed roots, base 23 (19 to 26) wide. Outermost medial anchor 14 (13 to 15) long, with elongate shank on base; hooklet 5 long. Innermost anchor 17 (15 to 19) long, with elongate deep root and short superficial root.

Mouth subterminal, ventral. Pharynx subspherical, immediately posterior to buccal funnel; diameter 52 (45 to 62). Esophagus elongate, without diverticulae, bifurcates at level of anterior margin of vitellaria. Crura simple, lack diverticulae, terminate in haptor at level of third pair of clamps.

Six to 11 testes, intercecal, subovate, postovarian; length 88 (71 to 114). Seminal vesicle at level of ovary, vas deferens straight. Genital atrium consisting of an anterior cluster of four hooks with large bases, 18 (17 to 20) long, two bilateral muscular plates each with hook, 29 (26 to 33) long, and posterior V-shaped muscular piece with two pairs of palmate hooks, 27 (22 to 31) long.

Ovary intercecal, folded, 227 (176 to 265) long. Oviduct narrow, united anteriorly with vitelline reservoir. Genitointestinal canal, vagina, seminal receptacle not observed. Uterus a narrow tube extending along vas deferens. Vitellaria dense, coextensive with gut except absent in distal haptor. Transverse vitelline ducts join medially to form vitelline reservoir, dextral duct larger. Egg not observed.

Two pairs of conspicuous glands situated near genital atrium. Excretory ducts and nervous system indistinct; eyes absent.

Remarks

Paramazocraes tripathii sp. n. differs from *P. thrissocles* Tripathi, 1957, the type species and its closest relative, by (1) a more restricted distribution of vitellaria, (2) anterior haptoral clamps noticeably smaller than clamps of second and third pairs, (3) poorly differentiated roots on the largest anchor of the lappet, (4) several cuticularized ridges on the dorsal body surface at the level anterior to the bifurcation of the gut, and (5) the host (Figs. 1–10; Tripathi, 1957, fig. 42). This species is named in honor of Dr. Y. R. Tripathi, author of the genus.

Choricotyle pellonae sp. n.
(Figs. 11–16)

Specimens studied: 12.
Holotype: USNM Helm. Coll. No. 72429.

Description

Diclidophoridae, Choricotylinae. Body elongate, with long narrow peduncle; length 5.87 mm (3.50 to 8.90), greatest width 380 (252 to 529) in anterior half. Cuticle thin, smooth. Prohaptoral suckers 112 (97 to 133) by 84 (66 to 98), muscular, subovate, situated in posterior wall of buccal funnel. Opisthaptor palmate, symmetrical, with four pairs of clamps and short terminal lappet; haptor 1.30 mm (1.05 to 1.63) long, greatest width 1.37 mm (1.13 to 1.63). Clamps similar in shape, subequal, asymmetrical; prs. 1, 2, 3—236 (195 to 290), pr. 4—212 (176 to 252) in diameter. Each clamp with eight sclerites and well-developed proximal sucker; sclerotized ridges in distal quadrants. Terminal lappet of adult with pair of anchors and pair of dart-shaped sclerites. Anchor 24 (22 to 26) long, with

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Figures 1–10. *Paramazocraes tripathii* sp. n. 1, Ventral view of holotype. 2, Genital corona. 3–5, Hooks of genital corona. 6, Cephalic region and anterior trunk of paratype (ventral view). 7, Clamp (ventral view). 8, Innermost anchor. 9, Outermost medial anchor. 10, Lateral anchor. Figures 3, 4, 5, 8, 9, 10, were drawn to the same scale (10 μ).
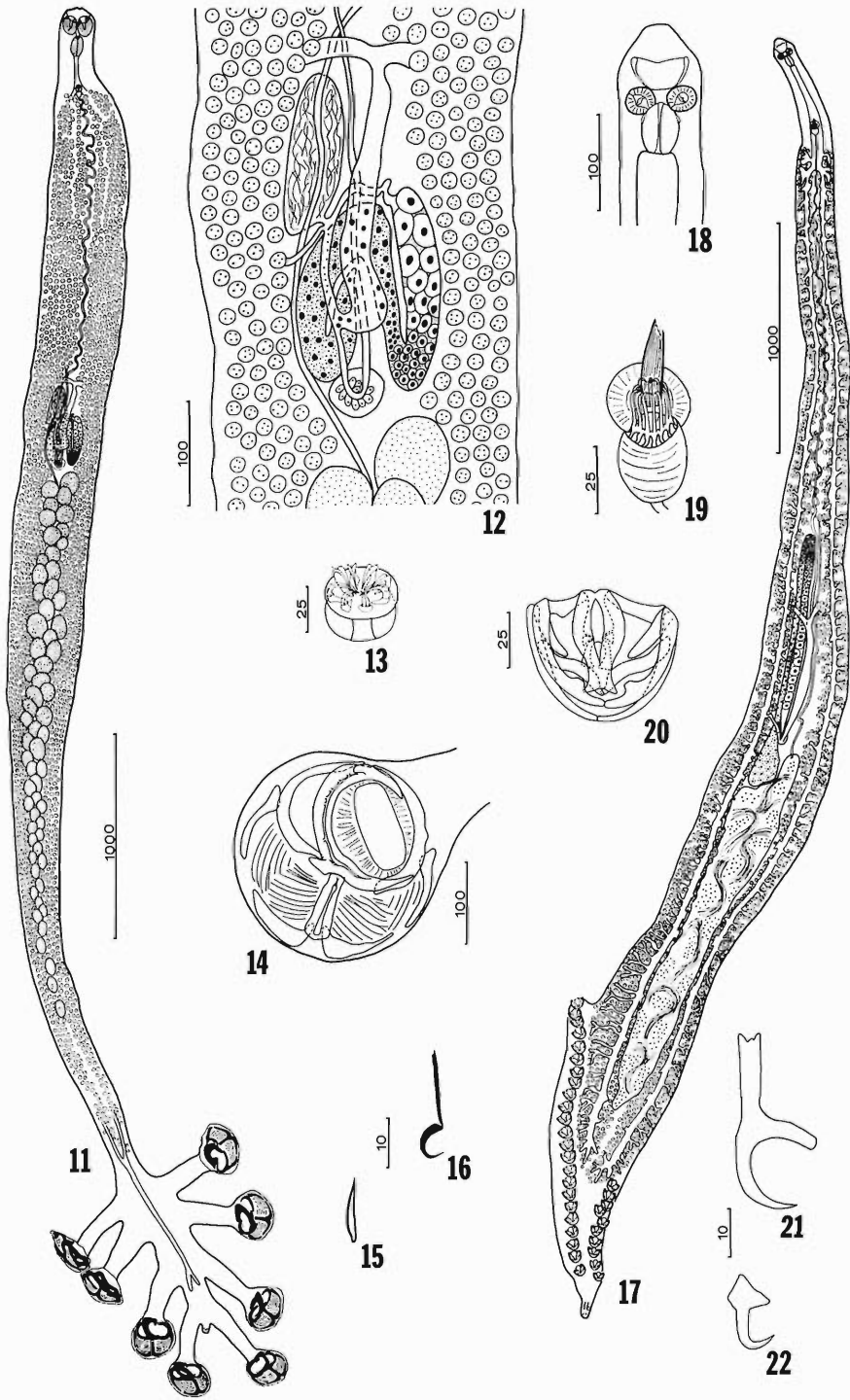
elongate shank and recurved point. Immature specimens occasionally with two pairs of anchors. Dart-shaped sclerite 14 (11 to 15) long, situated medial to anchor.

Mouth subterminal, ventral. Pharynx elongate ovate, 69 (49 to 81) wide, situated immediately posterior to buccal funnel. Esophagus short, without diverticulae, bifurcates at level of genital atrium. Crura with lateral and medial diverticulae, united in peduncle and extend into haptor as blind, variably branched cecum.

Testes postovarian, subovate, intercecal, approximately 60 in number; length 55 (39 to 81). Vas deferens coiled; seminal vesicle bulbous, immediately posterior to genital atrium. Genital atrium 40 (35 to 46) wide, armed with eight hooks; hook length 11 (9 to 12).

Ovary in anterior half of body, folded, 248 (162 to 351) long. Oviduct coiled; seminal receptacle large; genitointestinal canal short; ootype conspicuous, postovarian, surrounded by numerous unicellular glands. Uterus delicate, midventral. Vitellaria dense, coextensive with crura except absent in distal peduncle and haptor. Vitelline reservoir T-shaped, branches subequal. Egg not observed.

Excretory ducts and nervous system indistinct; eyes absent.

Remarks

The closest relative of *Choricotyle pellonae* sp. n. appears to be *C. multaetesticulae* (Chauhan, 1945) Sproston, 1946, as indicated by the general morphology of the body, gut, and haptor. *C. pellonae* differs from this species primarily by possessing fewer testes, a restricted distribution of vitellaria, and a terminal lappet (Figs. 11–16; Chauhan, 1945, figs. 11–13). This species is named after the host.

Pellonicola lanceolatum sp. n.
(Figs. 17–22)

SPECIMENS STUDIED: 29.
HOLOTYPE: USNM Helm. Coll. No. 72430.

Description

Body lanceolate, lacking peduncle; length 4.06 mm (3.00 to 5.70), greatest width 384 (214 to 630) usually at level of ovary. Cuticle thin, smooth. Prohaptoral suckers subspherical, septate, 32 (26 to 36) in diameter, situated in posterolateral wall of buccal funnel; lip of buccal funnel highly developed. Opisthaptor asymmetrical, with elongate terminal lappet and two unequal, lateral rows of similar *Gastrocotyle*-like clamps; haptor 1,147 (844 to 1,501) long, greatest width 490 (390 to 604) at anterior margin. Short row of clamps dextral or sinistral, with eight to 11 members; long row with 20 to 25. Clamp sclerites robust; ventral loop element with median expansion, dorsal loop elements articulated medially, center piece perforated, accessory sclerites present; clamps 51 (42 to 55) wide, 62 (50 to 72) long. Two pairs of dissimilar anchors ventral in lappet. Anterior anchor with well-developed roots and recurved shaft and point, anchor 41 (34 to 44) long, base 16 (13 to 20) wide. Posterior anchor without differentiated roots, 16 (14 to 17) long, base 9 (6 to 10) wide.

Mouth subterminal, ventral. Pharynx elongate ovate, 37 (32 to 52) wide. Esophagus elongate, with few diverticulae near proximal end, bifurcates at level of anterior margin of vitellaria. Crura blind, with medial and lateral diverticulae, terminate near level of anterior clamp of short row.

Testis a variably developed mass posterior to ovary, intercecal, 1,046 (756 to 1,588) by 196 (126 to 290). Vas deferens loosely coiled. Genital atrium 29 (27 to 33) in diameter, armed with 12 hooks; cirrus eversible, spined. Genital pore midventral anterior to bifurcation of gut.

Ovary 925 (630 to 1,260) long, intercecal, folded, with ascending and descending limbs; limbs usually not crossed. Oviduct straight; genitointestinal canal frequently filled with vitelline material, unites with right intestinal crus;

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Figures 11–22. Polyopisthocotylea. 11–16, *Choricotyle pellonae* sp. n. 11, Holotype (ventral view). 12, Enlargement of worm at level of reproductive systems (ventral view). 13, Genital corona. 14, Clamp (ventral view). 15, Dart-shaped sclerite. 16, Anchor. 17–22, *Pellonicola lanceolatum* sp. n. 17, Ventral view of holotype. 18, Cephalic region. 19, Genital corona. 20, Clamp (ventral view). 21, Anterior anchor. 22, Posterior anchor.

glands of ootype poorly developed, vitelline reservoir Y-shaped, ventral; uterus a fine, relatively straight duct. Vitellaria scattered, coextensive with intestinal crura. Vaginae not observed.

Several small unicellular glandlike cells situated in anterior portion of cephalic region. Excretory and nervous system indistinct; eyes absent.

Remarks

Pellonicola lanceolatum sp. n. differs from *P. elongata* Unnithan, 1967, by possessing (1) diverticulae of the intestinal crura, (2) a large testicular mass rather than numerous discrete testes, (3) a spined cirrus, and (4) in the comparative morphology of the clamps, anchors, cephalic region, and hooks of the genital atrium (Figs. 17–22; Unnithan, 1967, figs. 26–33). The specific name is from Latin (*lanceolatus* = lance-shaped) and refers to the shape of the body.

Pellonicola Unnithan, 1967

Emended generic diagnosis

Gastrocotylidae, Gastrocotylinae: Body divisible into cephalic region, trunk, and opisthaptor. Opisthaptor composed of two unequal bilateral frills of sessile, *Gastrocotyle*-like clamps, and a terminal lappet with two pairs of dissimilar anchors. Anterior one-half of long frill of clamps extends along lateral margin of posterior trunk. Prohaptoral suckers muscular, usually septate; pharynx small; esophagus short, with or without diverticulae; crura simple, blind, with or without diverticulae, extend to level of anterior clamp of short frill. Testes para- and postovarian, single or many. Vas deferens loosely coiled; seminal vesicle an indistinct dilation of vas deferens; cirrus usually spined, protrusible; genital atrium with 12 hooks; genital pore medioventral, at level of esophagus. Ovary near midlength, dextral, with ascending and descending portions. Vagi-

nae unarmed, dorsal, submarginal. Eggs with filament at each pole. Vitellaria coextensive with intestinal crura. Parasites of gills of marine fishes.

TYPE SPECIES: *P. elongata* Unnithan, 1967, from *Pellona* (*Ilisha*) *brachysoma* (Blkr.), Trivandrum.

Remarks

This emended generic diagnosis was made in order to accommodate *Pellonicola lanceolatum* sp. n., which possessed characters differing from those reported for the type species. Principal points involved were the presence of (1) diverticulae of the gut, (2) a single large testicular mass rather than discrete and numerous testes, (3) a spined cirrus, and (4) septate prohaptoral suckers.

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Cinclotaenia filamentosa gen. et sp. n. (Cestoda: Dilepididae) from the Dipper in Oregon

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ABSTRACT: A new genus and species of cestode, *Cinclotaenia filamentosa*, from the dipper in Oregon is described. *Cinclotaenia* differs from *Sacciuterina* in having two circles of hooks and from *Choanotaenia* in that it has several eggs in each egg capsule and the genital ducts are dorsal to the osmoregulatory canals. It differs from other described dilepidids in having very long filaments extending from the oncosphere coats of the egg packets.

In a survey of the parasites of the dipper, *Cinclus mexicanus unicolor* Bonaparte, six to 15 birds collected along streams of the lower Columbia River Gorge of western Oregon contained from two to five specimens each of a dilepidid cestode belonging to a new genus and species herein described.

Specimens were removed from the birds within several hours, fixed in Gilson's fluid under slight coverglass pressure, and stained in Kornhauser's hematein. Measurements are in microns unless otherwise stated and were made from the seven best specimens. Averages in most cases are followed by minima and maxima in parentheses.

Cinclotaenia gen. n.

Diagnosis

Dilepidinae Fuhrmann, 1907. Small worms. Scolex with two circles of hooks. Proglottids craspedote. Genitalia single in each proglottid. Genital pores irregularly alternating. Genital ducts dorsal to osmoregulatory canals. Testes numerous; testicular field mainly in posterior half of proglottid. Cirrus unarmed. Cirrus sac lacking seminal vesicle. Ovary wide, in anterior half of proglottid. Uterus sacciform, deeply lobed. Several eggs in each egg capsule. With long filaments extending from oncosphere coats of the egg packets.

TYPE SPECIES: *Cinclotaenia filamentosa* sp. n.

Cinclotaenia differs from *Sacciuterina* in having two circles of hooks and from *Choanotaenia* in that it has several eggs in each egg capsule and the genital ducts are dorsal to the osmoregulatory canals.

Cinclotaenia filamentosa sp. n.
(Figs. 1–10)

Worms 4.3 to 13 mm long, proglottids craspedote, wider than long, genital pores irregularly alternating. Scolex (Fig. 1) subglobular, 0.52 mm (0.44 to 0.62) wide by 0.49 mm (0.38 to 0.59) long, with short rostellum bearing a crown of hooks (Fig. 8) slightly alternating in position. Rostellar sac present. Hooks 20 to 21 in number (Figs. 2, 10); average 15 long; handle long, curved ventrad, blade short, blunt, shorter than handle, guard shorter than blade. Suckers large, protruding, 248 (236 to 260) in diameter. Neck narrow, 232 (119 to 310) long by 260 (190 to 320) wide. Strobilae have from 20 to 29 immature proglottids, 5 to 13 mature proglottids, and from two to four gravid proglottids.

Mature proglottids (Fig. 7) tend to be craspedote and measure 1.36 mm (1.25 to 1.65) wide by 0.46 mm (0.30 to 0.59) long. Testes 20 to 27 in number, each 68 (60 to 84) in diameter, and each consisting of a core of large, dark cells, irregularly arranged and surrounded by whorls of sperm cells. Testicular field posterior to ovary and about as wide as ovary. Cirrus sac 186 (150 to 225) long by 43 (36 to 45) wide, lacking seminal vesicle but containing a somewhat coiled ejaculatory duct, protrusible, unarmed cirrus, and scattered cells. Ovary two-winged, in anterior half of proglottid, 1.03 mm (0.92 to 1.09) wide by 0.10 (0.06 to 0.14) long, midpart typically narrowed. Vitelline mass 231 (186 to 273) wide by 48 (42 to 60) long, immediately posterior to ovary. Vagina extending from area of Mehlis' gland and reaching genital atrium just posterior to cirrus sac. Central part of vagina
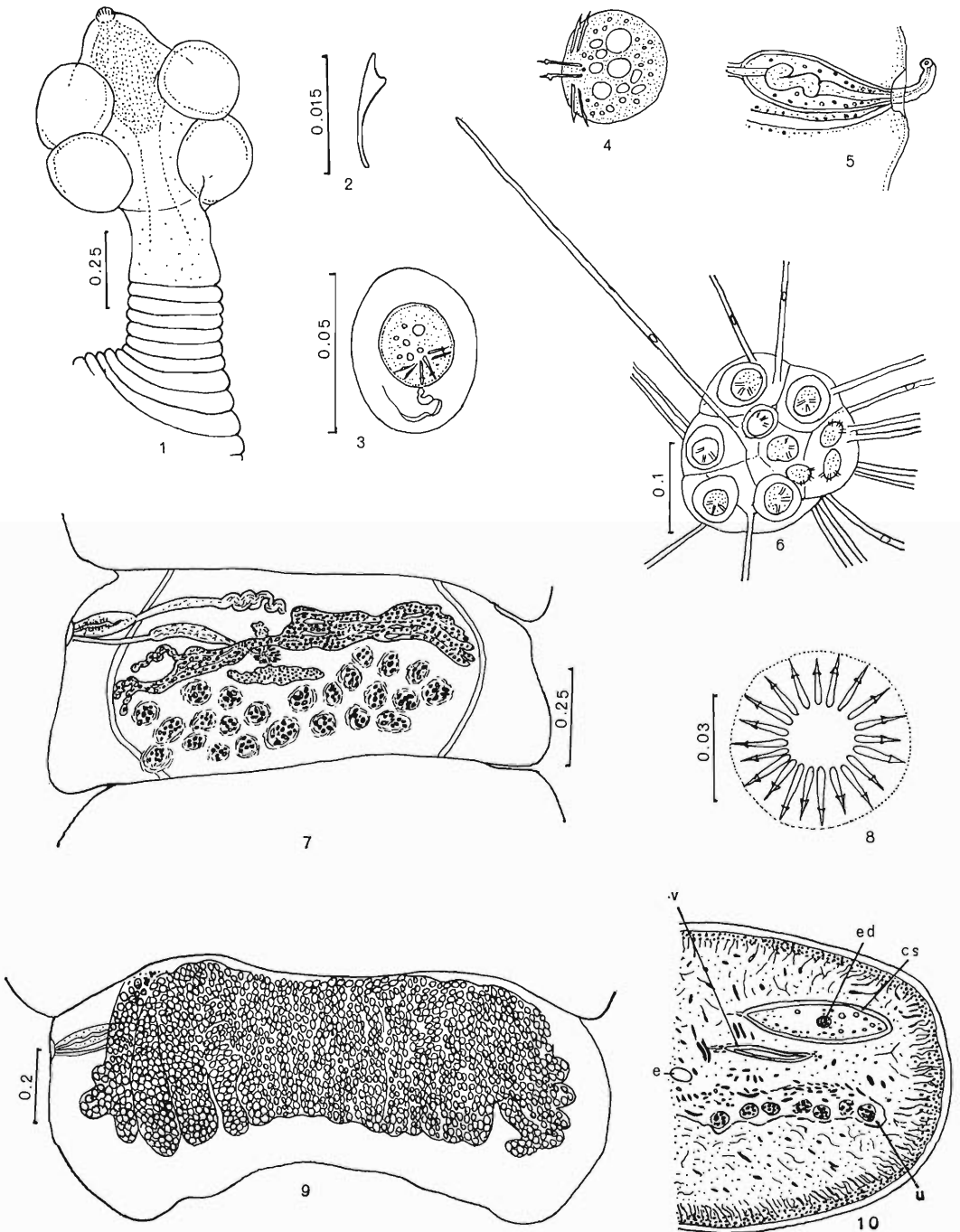
expanded into a seminal receptacle 166 (150 to 210) long by 28 (21 to 33) wide.

Gravid proglottids (Fig. 9) 1.55 mm (1.37 to 1.65) wide by 0.51 (0.39 to 0.63) long. Uterus filling larger part of proglottid, partially divided into compartments by thin membranes. In transverse sections (Fig. 10) the uterus appears narrow and irregular, extending nearly the full width of the proglottid and containing a large number of eggs. Egg packets (Fig. 6) 189 to 201 in diameter, containing eight to 12 eggs, each with an outer membrane. Each live packet with some active oncospheres devoid of oncosphere coats. Live eggs (Fig. 3) average 46 wide by 54 long, oval, with thin shell; polar filament in egg arising from one end of oncosphere coat. Hatched, live oncospheres average 27 wide by 33 long, with six hooklets each 11 to 12 long. Egg packet with about 16 long, slender filaments 378 (354 to 401) long by 10 to 12 wide. A cell nucleus seen in some filaments.

HOST: *Cinclus mexicanus unicolor* Bonaparte.

HABITAT: Small intestine.

TYPE LOCALITY: Streams of the Columbia River Gorge, Multnomah County, Oregon.

TYPE SPECIMENS: Holotype No. 71112 and paratype No. 71113 deposited in the USNM Helm. Coll.

Discussion

Belopolskaya (1958) has cited some instances of cestode oncospheres with filaments, including those *Anomotaenia platyrhyncha* (Krabbe) in which each oncosphere coat of a small chain is provided with two filaments, and small clusters of oncospheres of *A. paramicrorhyncha* Dubinina with one filament for each oncosphere coat. Eggs of *A. stentorea* (Froel.), *Dilepis glarcola* Dubinina, and *Diorchis nyrocae* Yamaguti each have two long polar filaments. Prestwood and Reid (1966) described and figured numerous hairlike filaments on coats of individual oncospheres of *Drepanidotaenia watsoni* from wild turkeys. A number of filaments at the poles of the elongate outer shell of the eggs of *Sacciuterina mathevossiani* were described by Schmidt and Neiland (1971). Such filaments tend to allow the eggs or egg packets to float in water and this would facilitate contact with cladocera or other plankton invertebrates which might serve as intermediate hosts. Egg packets remained suspended at different levels in refrigerated water for at least a week.

Filaments on the egg packets of *Cinclotaenia filamentosa* were evident only when dissected from fresh, unpreserved, ripe proglottids. It is therefore possible that filaments on some other species of dilepidid cestodes described from preserved specimens, even if present, may not have been observed.

Jarecka (1958) found the larval stage of *Sacciuterina ciliata* (Fuhr., 1913) in the cladoceran *Simocephalus expinosus* collected in Lake Gotdapiwo, Poland, which suggests that the larval stage of *C. filamentosa* may occur in a related crustacean.

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Figures 1–10. *Cinclotaenia filamentosa* gen. et sp. n. 1, Scolex. 2, Rostellar hook. 3, Egg. 4, Oncosphere. 5, Region of genital pore showing protruding cirrus. 6, Egg packet showing one complete filament and bases of others. 7, Mature proglottid. 8, Crown of rostellar hooks, *en face* view. 9, Terminal gravid proglottid. 10, Lateral part of gravid proglottid, transverse section. Figures 3, 4, and 6 drawn from live material. Osmoregulatory canal, e; ejaculatory duct, ed; cirrus sac, cs; uterus, u; vagina, v.

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Host–Parasite Relationships of *Fessisentis necturorum* (Acanthocephala: Fessisentidae)

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ABSTRACT: Species of the acanthocephalan genus *Fessisentis* occur in piscine and amphibian hosts of North America, but *F. necturorum* has been reported from aquatic stages of amphibians only. In northeastern Georgia larvae of *Ambystoma opacum* and *Pseudotriton montanus* are heavily parasitized by *F. necturorum*. In this region larval salamanders begin acquiring infections of *F. necturorum* during winter months and the parasite prevalence increases to a peak in early spring. When metamorphosis begins in late April, acanthocephalan infections decline until they are apparently absent in fully metamorphosed *A. opacum* and *Pseudotriton montanus*.

Near Athens, Georgia, isopods, *Asellus scrupulosus*, are naturally infected with cystacanths of *F. necturorum* but attempts to infect them in the laboratory failed. Collecting data suggest that environmental conditions for infection may be rigid and that study of subtle environmental differences at collecting sites may be necessary before laboratory confirmation of the *F. necturorum* life cycle is possible.

Fifty-seven of 77 larval *Ambystoma opacum*, 24 of 25 larval *Pseudotriton montanus*, 3 of 8 larval *Eurycea* (*E. bislineata* and/or *E. longicauda*), and 1 of 6 adult *Notophthalmus viridescens* collected from Sandy Creek near Athens, Georgia, were parasitized by *F. necturorum*. None of 2 adult *A. opacum*, 15 adult *P. montanus*, 5 *Ictalurus natalis*, or 6 *Lepomis macrochirus* collected from the same site was parasitized by acanthocephalans.

Recoveries of *F. necturorum* from *Asellus scrupulosus*, *Eurycea* sp., *N. viridescens*, and *P. montanus* constitute new host records.

Species of the acanthocephalan genus *Fessisentis* occur in piscine and amphibian hosts of North America. *F. friedi* Nickol, 1972 (= *F. vancleavei* Haley and Bullock, 1953) occurs in piscine hosts while *F. necturorum* Nickol, 1967, and *F. vancleavei* (Hughes and Moore, 1943) occur in amphibians. *F. fessus* Van Cleave, 1931, has been reported from both host groups (Nickol, 1972). Although in certain localities fishes are frequently parasitized by members of *Fessisentis*, there is no report of females with fully formed eggs having been

taken from a piscine host. With one possible exception, individuals from amphibians have been reported only from aquatic forms. Malewitz (1956) reported *Acanthocephalus vancleavei* (= *F. vancleavei*) from *Eurycea multiplicata* without noting whether the hosts had metamorphosed. Adults of this species, however, are also aquatic. In a survey of Louisiana Caudata, Nickol (1969) found 10 of 11 waterdogs, *Necturus beyeri*, infected with *F. necturorum*, but none of 176 metamorphosed salamanders representing nine species harbored this acanthocephalan. The sample included 23 adult specimens of *Ambystoma*

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Table 1. *Fessisentis necturorum* of larval salamanders from Sandy Creek east of highway.

Date 1968	Develop. stage	Host body length (cm)	No. Exam.	No. Inf.	No./Inf. host		No. *Fessisentis*		
					Range	Mean	♂	♀	Total
			Host:	*Ambystoma opacum*					
22 Jan.	✳	2.8–5.4	25	22	1–7	3.2	32	39	71
6 March	✳	5.0–5.9	10	9	3–22	6.8	25	36	61
27 March	✳	6.0–7.0	10	9	1–5	3.3	10	20	30
18 April	✳✳	7.4–8.4	4	3	1–6	3.3	2	8	10
9 May	✳✳✳	ca. 8	4	3	1–5	2.7	1	7	8
		2.8–8.4	53	46	1–22	3.9	70	110	180
		Host:	*Pseudotriton montanus*						
21 Jan.	✳	6.8–8.2	20	19	1–7	2.9	37	19	56
3 March	✳	7.6–8.3	5	5	1–13	5.0	12	13	25
		6.8–8.3	25	24	1–13	3.4	49	32	81

✳ Larval.
✳✳ Near metamorphosis.
✳✳✳ Metamorphosis nearly completed.

opacum. Since in northeastern Georgia larvae of *A. opacum* and *Pseudotriton montanus* are heavily parasitized by *F. necturorum*, an ecological study of the *F. necturorum* host–parasite relationship was initiated.

Methods

Sandy Creek, Clarke County, Georgia, was selected as the study site. About 2 miles north of Athens, U. S. Highway 441 crosses the creek which in this area flows through a mixed hardwood swamp. From January through mid-May water stands in shallow pools from ½ to 2 ft deep. During these months in 1968 *Ambystoma opacum* larvae in progressive stages of development were collected from pools and examined for *Fessisentis necturorum*. Larvae of other salamanders were studied when available. Metamorphosed salamanders, *Notophthalmus viridescens* and *Pseudotriton montanus*, were collected from the region and examined. Yellow bullhead, *Ictalurus natalis*, and blue gills, *Lepomis macrochirus*, were also sampled. Those arthropod species which occurred abundantly in the pools were examined in an effort to discover the intermediate host of *F. necturorum*. Initially separate collections were made on each side of the highway, but after January collecting west of the highway was discontinued due to the paucity of infected hosts. Surveying was terminated on the east side as well after the second week of May because salamanders had metamorphosed and acanthocephalans had nearly disappeared from the population.

In an effort to demonstrate the life cycle of *F. necturorum* in the laboratory, small laboratory-reared isopods (*Asellus obtusus* and *A. scrupulosus*), amphipods (*Crangonyx serratus*), and ostracods were isolated in culture dishes of spring water and exposed to thousands of eggs dissected from gravid female worms. After 3 weeks at room temperature 20 specimens of each species were dissected and examined for developing acanthocephalan larvae.

Results

Table 1 reports results of surveys for *Fessisentis necturorum* in larval *Ambystoma opacum* and *Pseudotriton montanus* taken from Sandy Creek east of U. S. 441. Throughout the study period the percentage of salamanders infected remained relatively uniform, 75% or higher, but infection intensity and parasite sex distribution varied. The average number of acanthocephalans in each infected host increased from late January to a peak in early March. As the year progressed, female acanthocephalans constituted an increasing proportion of the parasite population.

Acanthocephalan frequency and intensity were less in specimens of *A. opacum* collected from west of the highway than in specimens from east of it. Eleven of 24 salamanders collected from west of the highway on 27 January were infected with from 1 to 4 (mean, 1.9) specimens of *F. necturorum*. However, as in January infections from the east side, male acanthocephalans constituted a large pro-

portion (13 males, 8 females) of the parasites recovered (cf. Table 1).

Of the other salamanders examined from east of the highway, none of 15 adult specimens of *P. montanus* was infected with *F. necturorum*; 1 of 6 adult *Notophthalmus viridescens* hosted one specimen; and 3 of 8 larval *Eurycea* (*E. bislineata* and/or *E. longicauda*) harbored 1, 1, and 2. None of 5 yellow bullheads, *Ictalurus natalis*, or 6 blue gills, *Lepomis macrochirus*, was parasitized by acanthocephalans.

Four of 90 male and 2 of 65 female specimens of *Asellus scrupulosus* from the same pools in which infected salamanders occurred were parasitized by one *F. necturorum* cystacanth each (2 males and 4 females were recovered). Cystacanths were in the hemocoelom lying adjacent to digestive glands with proboscides fully inverted. The trunk of each cystacanth was greatly contracted with the anterior end directed posteriorly in the host.

Fessisentis necturorum develops nearly to maturity within the intermediate host. Proboscis armature does not differ from that described by Nickol (1967) for the adult, and trunk length, 6.5 mm for males and 7.0 mm for females, is as great as that attained by some mature worms. Vasa deferentia and seminal vesicles of male cystacanths stained very darkly indicating possible presence of semen. Each cystacanth ovary had fragmented filling the pseudocoelom with masses of ovarian balls. There was, however, no evidence of further development.

Attempts to infect a variety of crustaceans, including *Asellus scrupulosus*, in the laboratory were unsuccessful.

Discussion

Recoveries of *Fessisentis necturorum* from *Asellus scrupulosus*, *Eurycea* sp., *Notophthalmus viridescens*, and *Pseudotriton montanus* constitute new host records. No arthropod has previously been reported parasitized by this acanthocephalan species. Haley and Bullock (1953) reported *F. friedi* from *Lepomis gibbosus* and subsequently it has been reported from other piscine species (Nickol, 1972). *F. fessus* is also known to occur in a fish host (Van Cleave, 1931). *F. necturorum*, however, did not occur in any specimen of *Lepomis macrochirus* or *Ictalurus natalis* examined from Sandy Creek.

Since not all acanthocephalans in a stage infective to vertebrates encyst, some workers find use of the term cystacanth objectionable when applied indiscriminately to this stage. *F. necturorum* is among those species which do not encyst but for the sake of uniformity the term cystacanth is retained.

Although twice as many female as male (4:2) cystacanths of *F. necturorum* were recovered from *A. scrupulosus*, sampling was not extensive enough to determine the sex ratio. In adequately sampled intermediate hosts, acanthocephalans occur in a 1:1 sex ratio (Amante, Fresi, and Laneri, 1967; Crompton and Whitfield, 1968; Parenti, Antoniotti and Beccio, 1965). Occurrence of *F. necturorum* in nearly a 1:1 ratio during early infections of *Ambystoma opacum* (Table 1) suggests that the ratio in *Asellus scrupulosus* is also nearly 1:1. As the year progressed, however, male *F. necturorum* became relatively fewer in definitive hosts (Table 1). The overall ratio of male:female worms in *Ambystoma opacum* was 0.64. Avery (1971) reported a 0.94 male:female ratio of *Acanthocephalus anthuris* in the newts *Triturus helveticus* and *T. vulgaris*. Other workers, including Awachie (1966), Chubb (1964), and Pennycuick (1971), have also found female acanthocephalans more numerous than males in definitive hosts. Crompton and Whitfield (1968) have shown that a shift in the sex ratio of *Polymorphus minutus* is explained by greater longevity of females. Similarly Awachie (1966) found that female *Echinorhynchus truttae* persist longer than males in *Salmo trutta*. These studies indicate that comparisons of overall sex ratios in definitive hosts may be meaningful only when courses of infection are considered.

It is likely that *F. necturorum* requires only one intermediate host in its life cycle, but laboratory study is necessary to verify the role of *Asellus scrupulosus* in its life cycle. One possible explanation of failure to produce laboratory infections of *F. necturorum* in *A. scrupulosus* is that environmental conditions required for infection and development may be very rigid. Circumstantial evidence of this comes from studies on both sides of the high-

way at Sandy Creek and from study of other similar swamps. Eighty-seven per cent of larval *Ambystoma opacum* collected from Sandy Creek east of the highway were parasitized by *F. necturorum*. Infected salamanders averaged 3.9 acanthocephalans each. Only 45% of larval *A. opacum* from west of the highway harbored *F. necturorum* and infected hosts averaged 1.9 worms each. These two collecting areas are less than 100 yards apart and superficially appear ecologically identical.

Fifty larval *A. opacum* (3 to 4 cm long) from shallow pools in Riedy Creek swamp (March, 1968), 100 miles S Athens, and 25 from a similar swampy area on Long Creek (February, 1968), 25 miles E Athens, were all negative for *F. necturorum*. These two areas and Sandy Creek had basically identical invertebrate faunas (i.e., the same species of *Asellus*, sphaeriid clams, may fly larvae, and amphipods) and appeared otherwise ecologically equivalent. All three collecting areas were mixed hardwood with very little understory vegetation. Pools from which salamander larvae were collected were ½ to 2 ft deep with clay–silt (some sand) bottoms. Pool bottoms were always covered with dead leaves in various stages of decay. Large numbers of invertebrates were associated with leafy bottoms of the pools. In a study of helminth populations in newts and tadpoles of England, Avery (1971) was unable to explain great differences in infection levels of *Acanthocephalus anthuris* from different ponds. A study of subtle environmental differences at collecting sites may be necessary before laboratory confirmation of the *F. necturorum* life cycle is possible. Such a study is also needed to understand factors influencing distribution and population densities of *F. necturorum* and perhaps other acanthocephalan species.

It appears that *F. necturorum* parasitizes only aquatic stages of salamander hosts. It occurs in *Necturus beyeri*, and larvae of *Pseudotriton montanus*, *Eurycea* sp., and *Ambystoma opacum*. With the exception of a single specimen from an adult *Notophthalmus viridescens* from Sandy Creek, *F. necturorum* has not been recovered from a metamorphosed amphibian. Table 1 shows that while the percentage of infected *A. opacum* larvae re-

mained high (75%) as metamorphosis began, the intensity of parasitism decreased as it continued. Acanthocephalans from specimens collected in early May were yellowish in color, inactive, unattached, and often in the lower gut. Those from younger salamanders were only slightly more active, but were white in color and attached in a narrow zone immediately posteriad to the stomach. Two completely metamorphosed *A. opacum* collected from the Sandy Creek area were not parasitized by *F. necturorum*.

In contrast to results of this study, Rankin (1937) found *Acanthocephalus acutulus* parasitizing both larval and adult specimens of salamanders in North Carolina. Likewise Avery (1971) recovered *A. anthuris* from larval and adult specimens of *Triturus helveticus* in England. An explanation may be the fact that adult *Ambystoma opacum* are found in water much less frequently than adults of many other salamander species. Adult *A. opacum* do not enter standing water to breed. Rather, eggs are laid in moist leaf litter which will later be inundated. At Sandy Creek, pools begin to form in late fall and the eggs hatch. *A. opacum* larvae develop throughout winter months when during heavy rains the swamp floor may be covered with more than 5 ft of water. During this time they begin acquiring infections of *F. necturorum*. Parasitism is light in midwinter but increases to a peak in early spring (Table 1) as water levels begin falling. Salamanders begin metamorphosis in late April and complete it in early May. Intensity of acanthocephalan infections declines accompanying metamorphosis. By late spring most pools have dried up. They remain dry, though leafy bottoms remain wet, until late fall.

Fessisentis necturorum develops nearly to maturity within the intermediate host. Adult size is reached, there is evidence of semen production, and ovaries fragment forming masses of large ovarian balls. Schmidt and Olsen (1964) reported that *Prosthorhynchus formosus* was the only acanthocephalan known to develop sufficiently for the ovary to fragment while still in the intermediate host. Merritt and Pratt (1964) added *Neoechinorhynchus rutili* to the list and now *F. necturorum* is the third species in which such early fragmentation in known to occur. Since it appears

that *F. necturorum* parasitizes only aquatic hosts, and is lost from *Ambystoma opacum* populations with metamorphosis, *A. opacum* must become infected and the worms must mature and pass eggs during the relatively short period that salamanders exist as larvae. The advanced stage of development achieved by *F. necturorum* within an intermediate host seems to be an adaptation facilitating rapid maturation and egg production after a definitive host is reached.

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Some Morphological and Functional Observations on *Fessisentis fessus* Van Cleave (Acanthocephala) from the Dwarf Salamander, *Siren intermedia* Le Conte[1]

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ABSTRACT: *Fessisentis fessus* Van Cleave (1931) was recently reported from *Siren intermedia* by Nickol (1972). This paper provides additional information concerning the unique morphology of the hooks, the form and function of the female reproductive system, and the structure of the male reproductive system.

Published records of the occurrence of acanthocephalan parasites in amphibians are rare. Van Cleave (1915) in a report on Acanthocephala from North American amphibia indicated that at that time only a single record existed. He later (1931) stated that his identification of *Acanthocephalus ranae* from *Diemictylus viridescens* was based on "rather unsatisfactory material." It is important to note that at that time Van Cleave had also examined the collections of H. B. Ward and George LaRue which between them involved about 300 hosts from the Midwest and Southwest. No acanthocephalan parasites from salamanders existed in these collections. The absence of acanthocephalan material from amphibians was further emphasized by the work of Rankin (1937a), who failed to find them in 1,000 specimens from 19 different species of North Carolina salamanders, and by Panitz (1969), who observed no Acanthocephala in salamanders examined from Oregon. Nevertheless, some amphibia have been reported to contain these parasites (Table 1).

In 1971 Miller and Dunagan reported but did not further describe a neoechinorhynchid from *Siren intermedia*. In 1972, Nickol reported *Fessisentis fessus* from the same host. *Fessisentis fessus* is very common in this area and has been observed in sirens by herpetologists and parasitologists for better than a decade (Landewe, 1963; Nickol, 1972).

During the past 3 years the writers have collected dwarf salamanders from a roadside ditch along Illinois Route 3 near Fountain Bluff, Illinois. One hundred per cent of the 24 animals collected have been infected with *Fessisentis fessus*. Although these collections have been made only during April and May of each year, the female parasites have been fully developed and filled with mature eggs.

This report concerns additional observations of the morphology and physiology of the acanthocephalan *Fessisentis fessus*.

Materials and Methods

Sirens (*Siren intermedia*) were collected in each of the years 1970, 1971, and 1972 during April and May along Illinois Route 3 near the Fountain Bluff area of Jackson County where they are plentiful until the ditches dry up during the summer months. The salamanders were brought to the laboratory and placed in well water in a cold room at 50 F until used. At this temperature they retained their worm burden until sacrificed which was never longer than 5 weeks. However, if kept at 72 F and fed a diet of liver, the parasites appeared in the feces within 1 or 2 days. Dr. Ronald Brandon, who identified the salamanders in this study, indicated that they were immature specimens (12 to 16 inches long).

Acanthocephala were initially cleaned in a physiological saline solution, osmotically adjusted with 0.18 M sucrose. A small camel's-hair brush was used to remove the larger debris. The proboscis was then irrigated by a pressurized stream of Ascaris Ringer's solution containing 0.005 M EGTA (Miller and Dunagan, 1971). The latter step was important for the removal of closely adhering mucus

Table 1. Acanthocephala reported in the literature as occurring in North American salamanders.

Acanthocephalan	Host	Author
Acanthocephalus acutulus	*Notophthalmus viridescens*	Van Cleave, 1931
	Notophthalmus viridescens	
	Ambystoma opacum	
	*Desmognathus fuscus fuscus	
	Plethodon glutinosus	Rankin, 1937b
	*D. quadramaculatus	
Acanthocephalus ranae	*Notophthalmus viridescens viridescens* (*Diemictylus*)	Rankin, 1937b
Pomphorhynchus bulbicolli	*Notophthalmus viridescens viridescens*	Rankin, 1937b
Fessisentis vancleavei	*Eurycea tynerensis*	Hughes & Moore, 1943
	E. multiplacata	Malewitz, 1956
Fessisentis necturorum	*Ambystoma opacum*	Nickol, 1967
	Necturus beyeri	
Neoechinorhynchus sp.	*Siren intermedia*	Miller & Dunagan, 1971
Fessisentis fessus	*Siren intermedia*	Nickol, 1972

* Only cysts observed.

material which was not completely successful even with this treatment (Fig. 1–D). For scanning electron microscopy, the proboscis was then removed at the level of the praesoma and fixed in cold AFA osmotically adjusted with sucrose. Following fixation, the proboscis was dehydrated through an alcohol series into 100% methanol over a 12-hr period. Air-dried specimens subsequently attached by DAG Type 215 conductive cement to specimen holders. Although this technique does not completely eliminate the effects of dehydration, it is very good for routine work. If distortion from dehydration is apparent the techniques of Small and Marszalek (1969) or Cleveland and Schneider (1969) work well. The scanning electron photomicrographs in this study are from specimens using the latter technique in which the tissues have been infiltrated with Epon 812 and subsequently hardened.

After mounting the specimen holders were placed in a Denton vacuum evaporator and a thin film of carbon followed by equal parts of gold and palladium evaporated onto the specimens in a high vacuum (5×10^{-6} torr). During the evaporation the specimens were rotated and tilted rapidly to insure metal deposition on all parts to a depth of approximately 100 Å. Samples were then stored in a chemical desiccator until examined in a Cambridge Stereoscan IIA Microscope. The oscilloscope image was recorded on Polaroid PN-55 film.

For light microscopy, the worms were studied alive for most structures but, where sections were necessary, the worms were fixed in 0.15% formalin containing 0.03 M bromoacetate and 0.3 M sucrose for 12 hr. They were then moved into a glycerol–water series to 50% glycerol overnight. The specimens were then dehydrated, embedded in 56 C wax, sectioned, stained with hematoxylin–eosin, and examined.

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Figure 1, A–F. *Fessisentis fessus.* A—Scanning electron microscope view of the apical portion of the proboscis hook armature. B—Light microscopy of living specimen showing quincunxial hook arrangement, cephalic ganglion, and club shape of the proboscis. C—Light microscopy of living eggs showing egg membranes and some acanthor structure. Arrows indicate hooks on embryo. Body spines are faintly visible along lateral margins of acanthor. D—Lateral view of proboscis using scanning microscope which indicates absence of hook grooves. Some mucus material still visible on hooks. E—Light microscopy of hooks from living specimen. Arrows point to notches, protuberances, etc., a part of the unique root architecture. F—Light microscopy of hooks from living material. Notice the enormous differences in the blades and roots of hooks adjacent to one another. The arrows point to anterior root extensions. Open arrows indicate hooks with no posterior root extension.
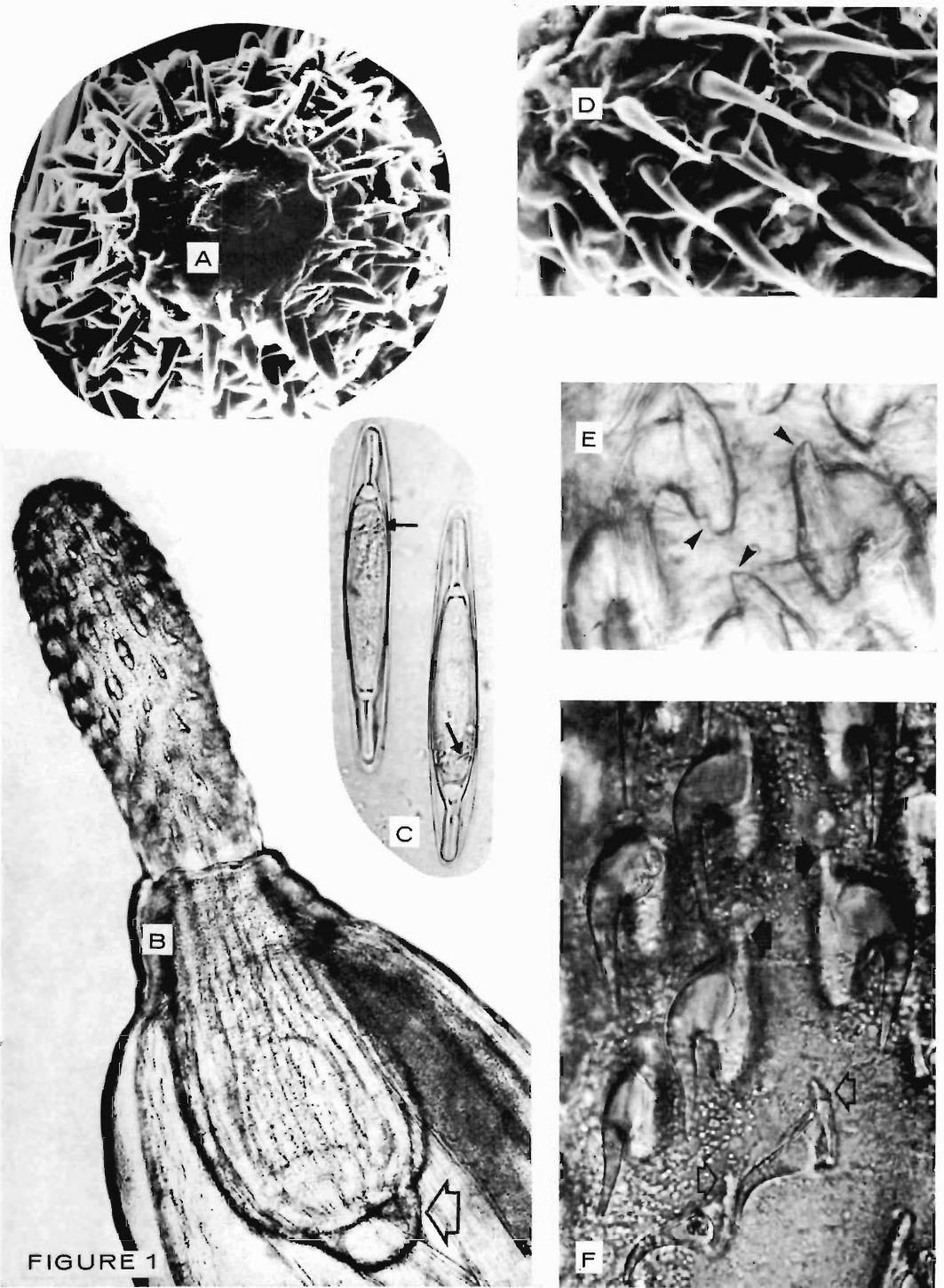
FIGURE 1

Results and Discussion

Figure 1–A and D are scanning electron microscope photographs showing surface features of the hooks. Both polar and lateral views clearly indicate that the outer curvature of the blade does not have grooves or other morphological modifications similar to those we reported (Miller and Dunagan, 1971) for *Macracanthorhynchus hirudinaceus.* Neither was there an intense host reaction with subsequent nodule formation. Occasionally a mild inflammation was observed with an individual worm but this was the exception rather than the rule.

Figure 1–B depicts the overall appearance of the anterior region of the parasite. As shown, the proboscis in the living material in this study had the greatest diameter between row 6 and 8 of the hooks; however, occasionally the terminal portion of the proboscis would be expanded in a "knoblike" form. The typical palaeacanthocephalan hook pattern is clearly visible as is the cephalic ganglion, receptacle retractor muscles, and retinaculum. The arrow indicates the nuclear pouch at the posterior extremity of the outer wall of the proboscis receptacle. It is obvious that this structure is a prominent feature of the proboscis. However, in fixed specimens viewed as whole mounts this structure is more difficult to see.

Nickol (1972) mentions that the large hooks have a prominent root but neither he nor Van Cleave (1931) show the variability in hook root architecture. This is understandable because each hook seems to differ from its neighbor and there is no gradation from the anterior to the posterior part of the proboscis.

Therefore, adjacent hooks may differ considerably in morphology and regional differences are very pronounced. Figure 1–F points out the magnitude of these changes. Notice that adjacent hooks may have roots with anterior and posterior elongations or no posterior elongation. Notice also that hooks with posterior root elongations have blades that curve sharply posteriorly following their emergence from the proboscis. The axis of these blades may thus be parallel with the root axis or form an acute angle with it. Hooks of this type are also more massive than the others. Hooks without posterior root elongations are much smaller in size and have considerably more variation in the appearance of the projecting blade. Frequently the blade projects at right angles from the root and curves only at the tip whereas at other times it resembles hooks with posterior root elongations.

The morphology of the hook root must play an important role in the movement of the hook as the various elongations and protuberances provide ample attachment for muscles. Figure 1–E indicates smaller irregularities on the surface of the root. These appear in the form of notches and small elevations. The functional role of these surface irregularities is unclear but the fact that they occur in living worms is an indication that they are not artifacts of preparation.

The structure of the egg (shelled acanthor) is typical of those belonging to the palaeacanthocephala; namely, the acanthor is enclosed by a shell containing four layers. Figure 1–C is a photograph of live eggs which shows these four layers. Notice also that the acanthor occupies most of the area surrounded by the

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Figure 2, A–E. *Fessisentis fessus.* A—Anterior part of uterus showing uterine bell complex. A-1, outside surface of the uterine bell is rugose reflecting the distribution of muscles in bell wall. A-2, one of two bell pockets. A-3, anterior part of uterus attached to muscular uterine duct of bell complex. B—Bursa of male reproductive system. B-1, common sperm duct which empties via conical penis. B-2 is the thick muscular wall of the bursa. B-3, penis. B-4, tube from Saefftigen's pouch. B-5 and 6, secretory type cells around mouth of muscular bursa. C—Posterior part of female reproductive system showing vagina musculature. C-1, posterior part of uterus showing attachment to thick muscular egg guide. C-2, highly muscular vaginal sphincter. C-3, small sphincter attached to posterior margin of vaginal sphincter and opening into a short duct leading to the outside of the animal. D—Male reproductive system. D-1, posterior testis. D-2, sperm duct from anterior testis. D-3, enlargement in sperm duct. D-4, cement glands. D-5, Saefftigen's pouch. D-6, seminal vesicle. D-7, muscular portion of bursa. D-8, secretory cells. E—Different stages in contraction of the uterine bell.
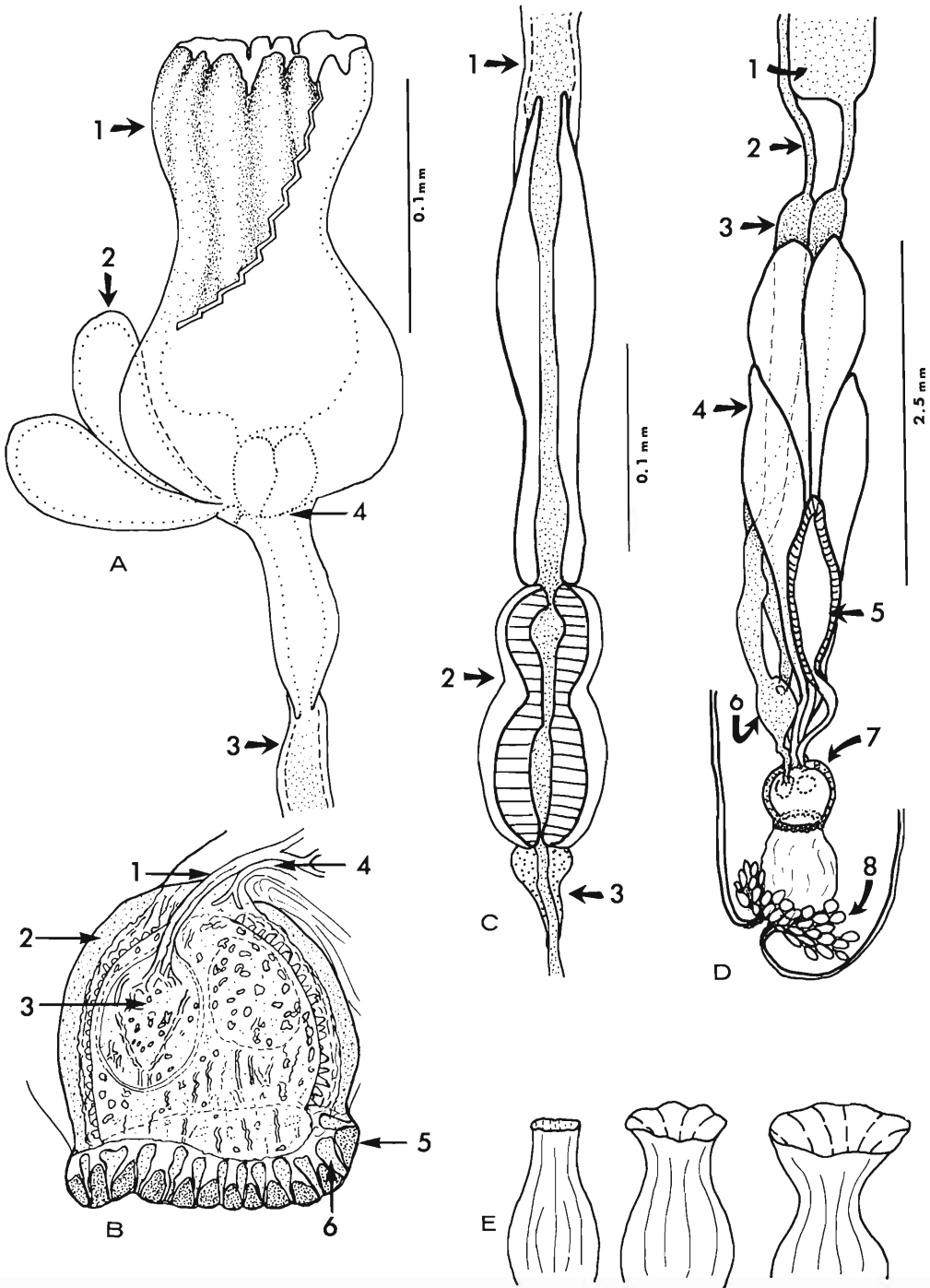
FIGURE 2

innermost layer and contains a large number of nuclei. The arrows point out the anterior hooks of the rostellar apparatus. Spines which cover the body surface are also visible along the outer margin of the acanthor larva.

The female reproductive system in *Polymorphus minutus* has been described by Whitfield (1968) as being composed of syncytial components of some modest complexity. This is certainly the case with *F. fessus*. In *Fessisentis*, the reproductive system consists of a uterine duct opening anteriorly into the pseudocoelom by a complex uterine bell and posteriorly to the outside via a somewhat simpler vagina. Eggs, however, do not of necessity exit to the outside once they have entered the uterine bell. The anterior edge of this bell may open quite wide as depicted in Figure 2–E. This action creates a suction which may or may not be coupled with contraction of the body wall musculature. Eggs are thus pulled via suction created by the opening of the bell musculature and pushed by contraction of the general body musculature into the uterine bell. The anterior uterine bell opening then begins to close in peristaltic fashion forcing the eggs from the anterior part of the chamber toward the posterior which contains a complex series of passages associated with the posterior bell chamber commonly called the egg selector apparatus. Even after long exposure to slide conditions one rarely sees an egg escape through the opening by which it entered the uterine bell. Eggs generally move through the basal opening connecting the anterior chamber with the lumen of the lateral pocket syncytium and into the pockets themselves. Indeed eggs may be shuttled several times into and out of these pockets before they leave this area of the system. Two choices of exit are evident, and each involves the posterior bell chamber musculature whose upper portions protrude slightly into the anterior bell chamber. The first and most frequently observed is exit of the eggs directly through the anterior wall of the posterior bell musculature and into the general body cavity. This opening is visible in living worms only when eggs are in the process of exiting via this route and is located immediately below and between the two diverticula of the lateral pocket syncytium.

Occasionally one observes movement of eggs from the anterior chamber directly through this opening without entering the lumen of the lateral pocket syncytium. Unfortunately, information at hand is inadequate to identify the presence of additional grooves and ducts in this area.

The second pathway is exit of eggs into the uterine duct and thence into the uterus. Several eggs may enter the uterus and in some instances this structure may become considerably distended before eggs are moved through the series of muscles that make up the vagina complex and thence to the outside. The first component in this system is a thickened tube that acts as an egg guide insuring that eggs enter the remaining vagina musculature one at a time and with the proper orientation. The anteriormost margin of this tube is not attached to the uterus (Fig. 2–C) but is free in the uterine cavity. Immediately posterior to the thick-walled egg guide is a highly muscular vagina sphincter (Fig. 2–C–2) followed by a smaller sphincter leading to a short duct which opens directly to the outside. Considerable coordination is observed in this series of muscles as eggs move from one part of the system to another.

It is unclear to us what function the uterus plays in egg release. Perhaps its primary role is associated with fertilization. It would also appear that the vagina sphincter musculature is much too thick to act merely as a valve in regulating flow of eggs to the outside. It seems more likely that it is used to withstand considerable stress such as during copulation where it may attach to the penis.

We were unable to verify the presence of separate median cell grooves and uterine ducts for the unilateral movement of eggs below the lateral pocket syncytium as has previously been reported by Whitfield (1968) for *Polymorphus minutus*. The two pockets of the lateral pocket syncytium protrude ventro-laterally from the posterior part of the anterior chamber (Fig. 2–A–2). Their actual position varies considerably from their point of attachment depending on internal movements and the number of eggs they contain. However, their elasticity was not great and the wall musculature never seemed to stretch to the point of thinness.

The anterior portion of the uterine bell is a most dynamic structure. Figure 2–E illustrates the sequence involved in moving eggs from the general body cavity into the lumen of the uterine bell. The entire process is largely controlled by circular muscles and the length of the bell changes little if any during muscle movement. The mouth of the chamber rapidly widens until it is funnel-shaped and then begins to progressively contract beginning first with the mouth opening while simultaneously there is a progresive expansion of the lower part of the bell chamber. In this way peristaltic waves at the rate of one every 3 seconds move eggs into the uterine bell. Although these waves of contraction undoubtedly put pressure on the musculature of the remainder of the uterine bell system, it is clear that coordination is required to move eggs from the anterior chamber through the basal opening into the area of the lateral pocket syncytium.

The serrations seen in the general appearance of the opening of the uterine bell depicted in Figure 2–A vary considerably between specimens and in the same specimen with time. We have not observed this variation in fixed material to the same degree as in living samples. It is possible that these represent lateral undulations in the surface which are exaggerated along the anterior margin.

Van Cleave (1931) presents a lateral view of the male reproductive system of *F. fessus*. That presented here (Fig. 2–D) is a ventral view of a specimen whose body is slightly twisted at the posterior terminus causing the opening to be off-center. Immediately surrounding this opening is a group of glands (Fig. 2–D–8) that are more numerous on the dorsal surface than on the ventral. They are clearly seen only on young worms. Mature or old worms generally do not demonstrate these thin wall structures.

The sperm duct from the anterior testis has two or three spermiducal vesicles positioned along its length. In the vicinity of the claviform cement glands both sperm ducts enlarge several diameters and continue dorsal and lateral to the cement glands to the posterior margin of Saefftigen's pouch. Here, the two ducts unite to form an enlarged seminal vesicle whose common sperm duct penetrates the muscular bursa to terminate in a short conical penis. The common sperm duct receives another duct formed first by the union of each of two cement gland ducts which further unite to form a single duct prior to joining the common sperm duct immediately before entering the muscular bursa (Fig. 2–B–1). Saefftigen's pouch empties into the bursa via a separate duct (Fig. 2–B–4).

An interesting feature of the bursa is the series of glandlike cells (Fig. 2–B–5,6) along the peripheral margin of the muscular opening. There seem to be two types of these: one is reniform with homogenous contents whereas the other is spatulate with nonhomogenous granular-looking contents. Each numbers approximately 30 and they alternate along the periphery. We were unable to establish an opening for these structures. However, they are ideally located to perform a catalytic function in the formation of the cap on the posterior extremity of the female worm.

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Ultrastructure of the Cuticle of *Bunonema* spp. (Nematoda: Bunonematidae)

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ABSTRACT: The ultrastructure of the cuticle of *Bunonema richtersi* and *B. reticulatum* were studied by electron microscopy, including details of their unusual asymmetry. The right side has a cuticular network with numerous "palisade rods" which appear to be proteinaceous in nature.

In the course of a review of *Criconema* spp. soil and root samples were obtained from the type locality of *Criconema cobbi* Micoletzky. This was possible through the kind cooperation of Dr. K. Lindhardt who collected the samples from sphagnum near a small lake at Gribsee—Moor just north of Hillerød in Denmark.

The samples contained specimens of *C. cobbi* and among other species also present were females and juveniles of four species of *Bunonema*. It was remarkable that four species were identified from this single locality, two of which, *B. reticulatum* Richters and *B. ditlevseni* Micoletzky, were reported from this area by Micoletzky (1925). The other species found in the present samples were *B. richtersi* Jägerskjöld and *B. penardi* Stefanski. A single female which appeared to be *B. multipapillatum* Stefanski also was present but this identity could not be confirmed.

The great variability of wartlike structures on the cuticle of *Bunonema* (*Rhodolaimus*) *voulliemei* as reported by Rühm (1962) suggests more detailed studies are needed to confirm the validity of species in that subgenus and in subgenus (*Bunonema*) as well. However, based on present knowledge the above identifications are believed to be quite reliable.

The unusual morphology of the paired tubercles and the asymmetry of these nematodes suggested further detailed study would be of special interest.

Materials and Methods

Both fresh and formalin-preserved specimens were collected from the Danish samples. Some specimens were preserved for permanent mounts in glycerin, others prepared for sectioning and study by electron microscopy. *En face* and cross sections were also made in glycerin-jelly mounts from hand-cut sections.

Selected adult nematodes were transferred to tap water and chilled. After drawing down the tap water a cacodylate-buffered solution of 3% glutaraldehyde was slowly added. This
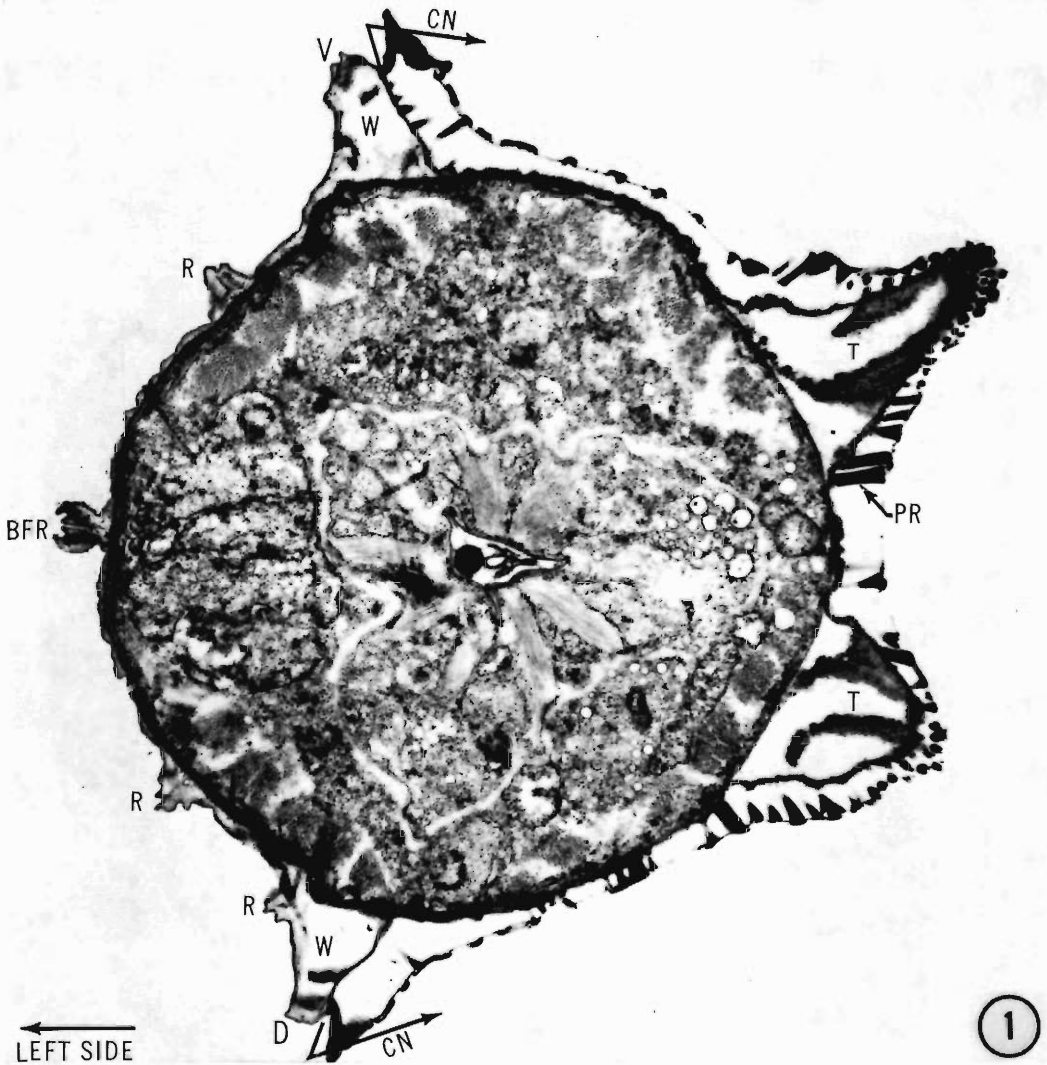
Abbreviations for all plates: **D**, dorsal; **V**, ventral; **BFR**, bifurcate ridge; **C**, canal or tubule; **CN**, cuticular network; **PR**, palisade rod; **R**, ridge; **T**, tubercle; **W**, wing.

Figure 1. Transverse section of *B. richtersi* at anterior end of posterior bulb. Left lateral side is to the left, and the dorsoventral wings at top and bottom. Left lateral side shows the bifurcate mid-left ridge and three of the four other small left ridges. On the right side is a pair of tubercles. × 8,500.

was necessary in order to maintain the conformity of the nematodes. The uncut specimens were fixed for 16 to 36 hr then rinsed for 1 hr in the buffered solution. This was followed by 2 to 3 hr in 2% osmium tetroxide in phosphate buffer containing 4.9% sucrose and a 1-hr rinse in the phosphate buffer. Dehydration was done in cold ethanol concentrations of 35, 50, 70, and 95% for 15-min periods, and cold absolute ethanol for 30 min. Following this were two 15-min immersions in propylene oxide prior to embedment in either
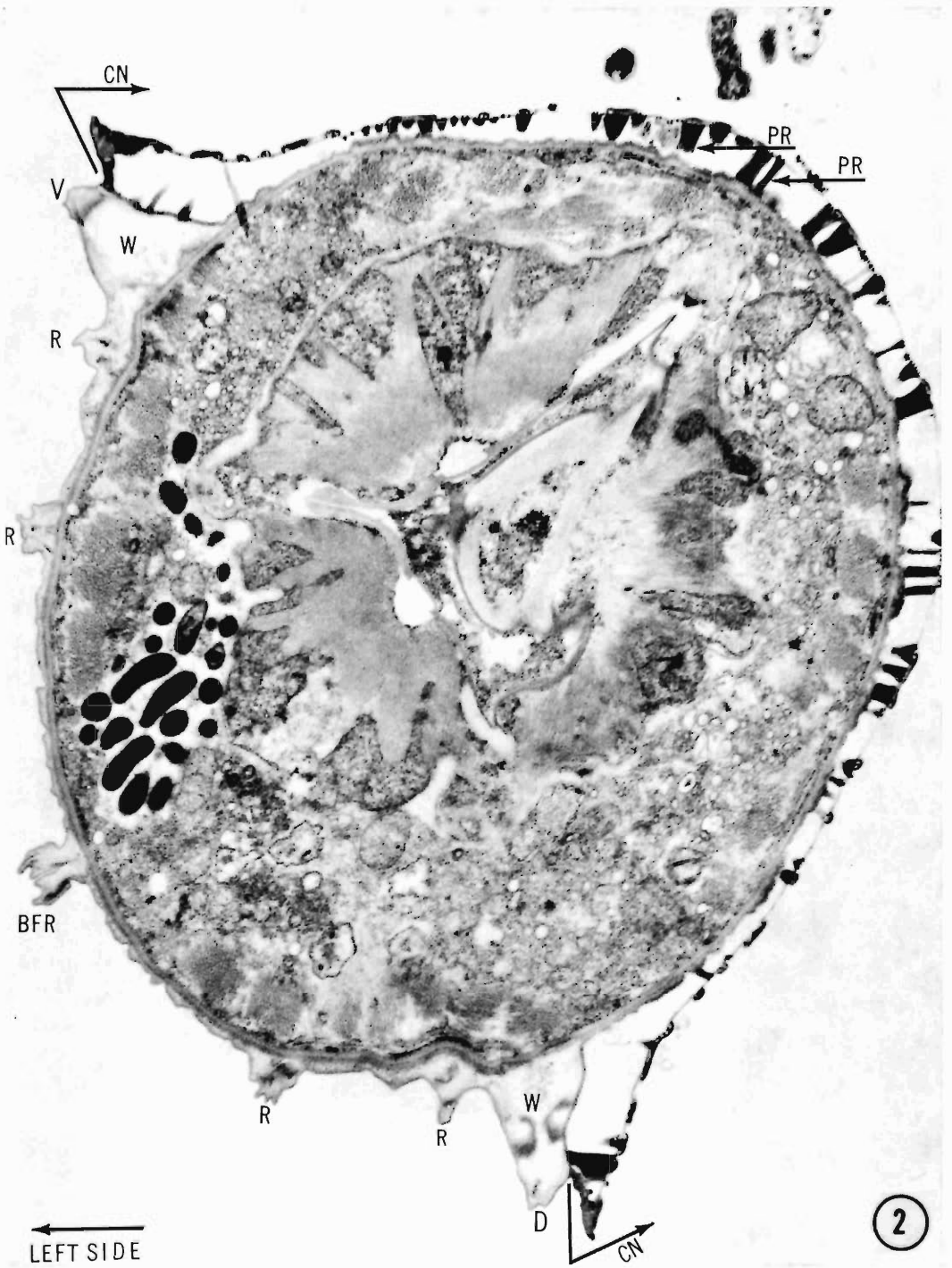
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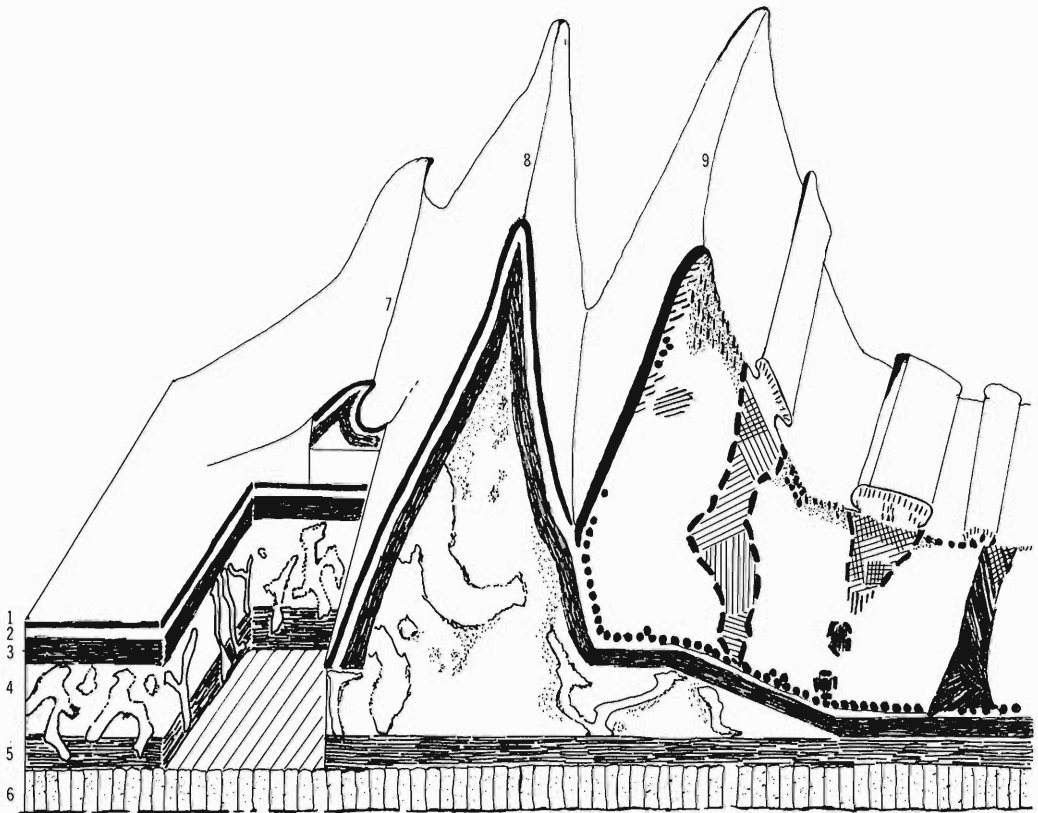
Figure 3. Schematic drawing representing the cuticle of *Bunonema*. 1–6, Layers of the cuticle; 7, small, left-side, longitudinal ridge adjacent to 8, left dorsal (or ventral) longitudinal wing; 9, cuticular network.

ERL-4206 or an Epon–Araldite mixture. The fixation and dehydration were carried out at 4 C with the specimens being brought to room temperature in the propylene oxide.

Because of the rarity of these nematodes a few were recovered from mass collection storage. These specimens had been killed by gently heating in water prior to a 5-month room temperature storage in 2.5% formalin. Selected specimens from the collection were rinsed in several changes at room temperature of a pH 7.2 phosphate buffer containing 4.9 sucrose over a 24-hr period. They were then stored at 4 C for 6 days in 3% glutaraldehyde

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Figure 2. Transverse section at level of posterior bulb of the same worm shown in Figure 1. The section was made at a level between pairs of tubercles and shows extracuticular network is continuous on the right side. Left lateral side shows the bifurcate mid-left ridge and all four other small left ridges plus both wings. × 12,900.

in sodium cacodylate-buffered solution. After a number of cold buffer rinses specimens were processed through the osmium tetroxide schedule outlined above. Some good material was obtained from these specimens.

Thin sections were cut from the above specimens using diamond knives on an LKB Ultratome. Sections were mounted on Formvarcoated copper grids. They were then stained with magnesium uranyl acetate and lead citrate or these stains following aqueous phosphotungstic acid. Examination was done on an RCA EMU-3G electron microscope operating at 50 kv with a 50-μ aperature.

Specimens for whole-mount preparations for study by light microscopy were gently heated to kill prior to fixation in 3% glutaraldehyde or 5% formalin for 24 hr or more. They were then passed through an alcohol–glycerin dehydration series before mounting in dehydrated glycerin. Hand-cut cross sections were made of some specimens and mounted in glycerin jelly as described by Cobb (1920).

Results

There are two unusual features of the cuticle of *Bunonema*. One is the asymmetry in the left–right orientation. The other is the elaborate cuticular network on the right lateral side of the nematode. Three species were prepared for thin sectioning, *B. richtersi*, *B. reticulatum*, and *B. penardi*. These are distinguished from each other by the presence of paired tubercles on the right side of the first two; tubercles are lacking in *B. penardi*. *B. reticulatum* differs from *B. richtersi* in the greater number of tubercles (30 to 35 pairs plus 3 singles posteriad vs. 19 to 21 pairs plus 0 to 2 singles posteriad for *richtersi*). *B. reticulatum* also has especially prominent refractive elements in the network of the right side lacking in the other two species. Details of the network and cuticle (except for the presence of tubercles) was found to be similar in the three species except for the dense refractive elements of *reticulatum*. Most of the observations reported here were made on *B. richtersi*.

A. Cuticle

The cuticle, exclusive of the network, swells or evaginates on the left lateral side to form seven protrusions when seen in cross sections (Figs. 1–2). These form longitudinal ridges which extend along most of the length of the body. Two ridges (here referred to as dorsoventral wings) are larger than the others and are conoid projections in approximately dorsoventral position.

Midway between the dorsoventral wings on the left side is a single bifurcate (mid-left) ridge. At midbody there are four more longitudinal ridges about the size of the mid-left and spaced nearly evenly, two on each side between the mid-left and the dorsoventral wings. Those two nearer the dorsoventral wings approach and merge with the wings anteriorly and posteriorly approximately 50 to 70 μ from each extremity.

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Figure 4. Transverse section through a tubercle of *B. richtersi*. × 36,300. Inset is a longitudinal section through palisade rod showing dense elements surrounding the rods (arrows). × 112,000.

Figure 5. Longitudinal-oblique section of cuticle of *B. richtersi* showing palisade rods in extracuticular network which overlays cortical layer of wing with interconnecting canals or tubules. Periodicity of basal layer appears in mid-left of photo (small arrow); lattice effect of this layer is in lower right of photo (large arrow). × 81,900.

Figure 6. Longitudinal section of a pair of canals in inner cortical cuticular layer (*B. richtersi*). Base of canal seems to penetrate median layer to basal layer while apex extends to cortical layer but not through it. × 127,200.
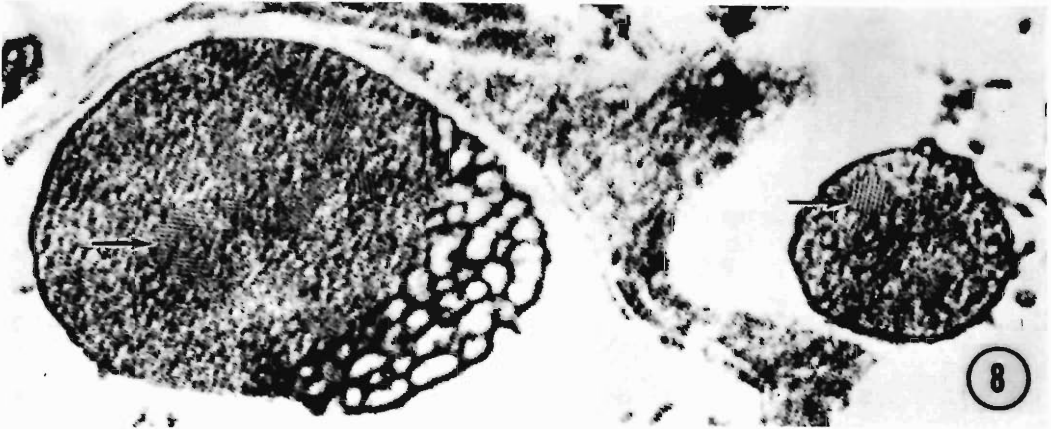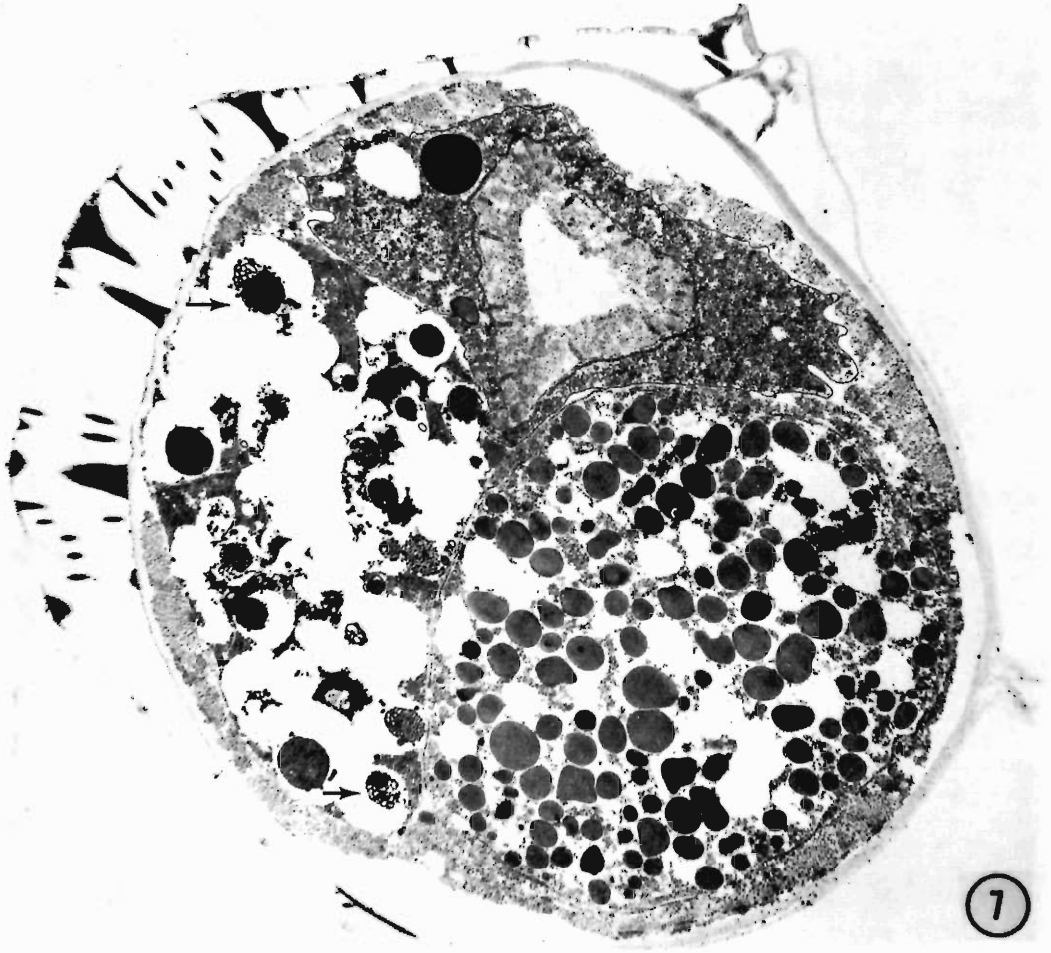
Figure 7. Transverse section of posterior end of *B. richtersi* showing right lateral location of fractionating organelles (arrows). × 6,350.

Figure 8. Enlargement of two of the cells (Fig. 7) showing proteinlike units inside these cells (arrows). × 66,300.
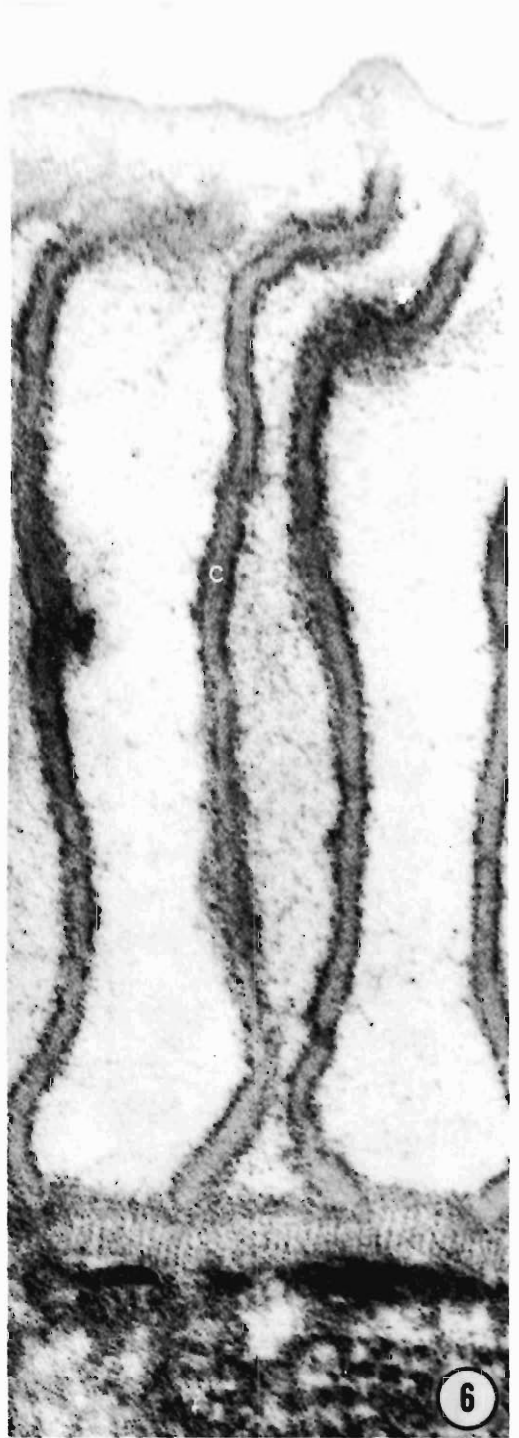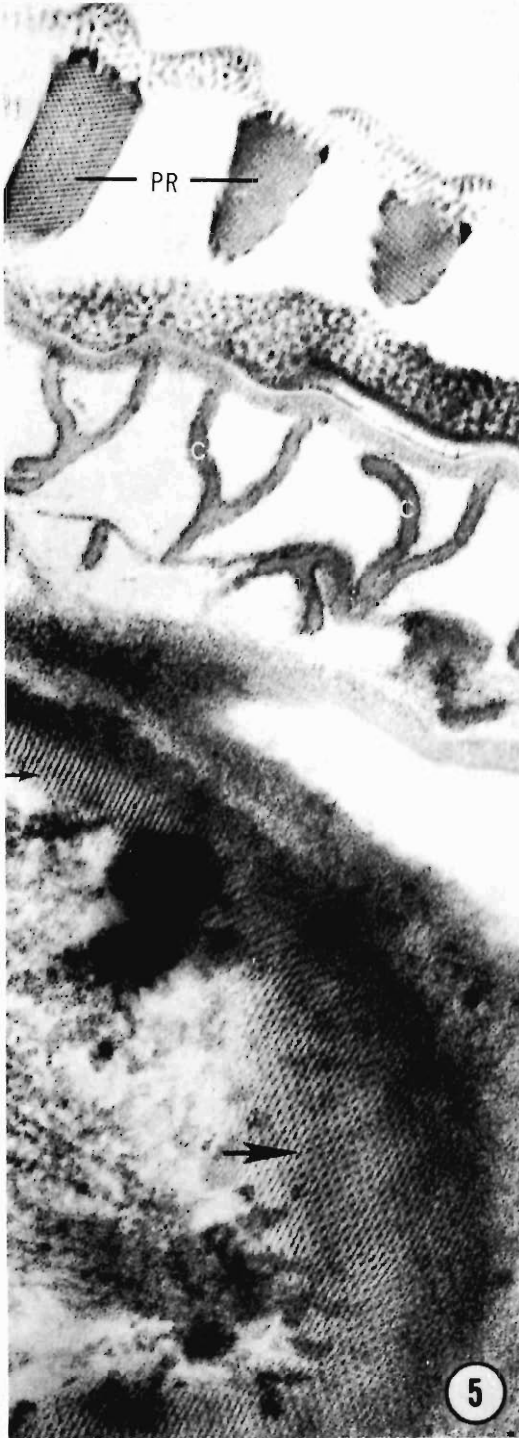
Other protrusions of the cuticle referred to as tubercles or warts occur on the right side usually in pairs. Occasionally the most anteriad tubercle is single with the other half of the pair missing. The remaining single tubercle is located in its normal position. In some cases the most posteriad two, three or four tubercles are single and are located centrally on the right side.

Layers of the cuticle (Fig. 3)
1. Cortical

a. OUTER CORTICAL LAYER: The outermost osmiophilic triple layer described in most nematodes was not definitely seen but there is an outer dark layer (1) about 8 mμ thick with a light layer (2) under it of about the same thickness. Underlying these is another layer fairly dense and darkly stained (3) which appears to be about 30 mμ thick and is more sharply delimited on the outer side than it is on the inner.

b. INNER CORTICAL LAYER (4): This has a lighter granular texture in appearance, is variable in thickness, and is the layer that is evaginated to form the five ridges of the left side, the dorsoventral wings and the tubercles on the right side. There are structures inside this layer that appear to be interconnecting canals or tubules which are most highly developed in the tubercles and dorsoventral wings. These canals extend from the inner edge of the outer cortical layer through the median layer and are in close contact with the basal layer. In the dorsoventral wings the canals appear in one direction to be paired (Fig. 6) but divide and connect (Fig. 5). In the tubercles the canals are variable from gross and simple (Fig. 4) to complex and ramifying (Fig. 3) depending on direction and location of the sections. Whether these canals are similar or serve the same function in all these locations is not known. The tubules are present in the tubercles, dorsoventral wings, and all five longitudinal ridges on the left side.

2. Median layer (5)

This appears as a simple dark, granular layer fairly uniform in thickness (about 40 mμ) next to the basal layer.

3. Basal layer (6)

This appears as a single fiber layer 60 mμ thick with fibers spaced at 20 mμ. In an oblique view these appear as a type of lattice structure (Fig. 5).

4. Basement membrane

This layer was not definitely observed.

The above layers were evident around the entire body except for the outermost thin dark layer (1) which may be the osmiophilic triple layer. This was seen on the left side and extended to both of the laterad junctures where the cuticular network starts on the right side. The next outermost, thin, light layer (2) obviously continued on in its usual location proximal to or under the network. It is not clear whether layer (1) separates to cover the network or remains close to the inner light-colored layer (2) over the entire circumference of the body.

B. Cuticular network

This is a complex organization of the body surface on the right side of the nematode (Figs. 1, 2) which seems to be basically similar in all the nematodes studied. It begins on the right side of the dorsoventral wings slightly proximal to the apex of these wings. From its point of origin on the wing, the network projects outward giving another conical outline in cross section even taller than the wing itself. It is continuous over the right side to the opposite wing (Figs. 1, 2) varying in thickness but fairly uniform over most of the right lateral area becoming notably thinner where it covers the tubercles when present.

The predominant structures in the network are the palisade rods scattered throughout

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Figure 9. Longitudinal section of *B. reticulatum* showing large dense palisade rods packed between two tubercles. × 15,000.

Figure 10. One rod (arrow) of Figure 9 showing detail. × 69,000.
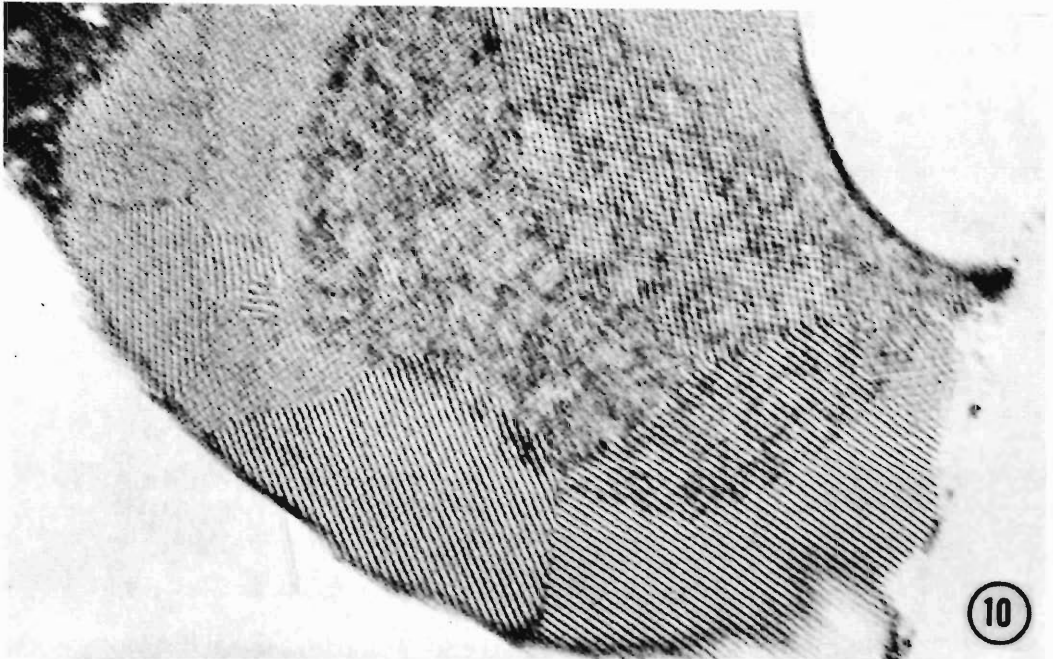
(Figs. 1, 2, 5, 6). The different sizes of these palisade rods and their distribution when seen in whole mounts on the light microscope form patterns which are distinctive and quite constant and are used in identification of species. The interspaces between the rods take no stain and appear white except at the surface where closely spaced individual elements appear as a line of dots in cross section. These elements vary in size and may be present as a single or as a multiple layer. Similar elements line the inner part of the network next to the cortical layer of the cuticle proper. The palisade rods are oriented mostly perpendicular to the body (Figs. 1, 2) beginning next to the cuticle proper though some may be underlaid with the elements. Distad they reach mainly to the surface but are usually covered by the elements in one or more layers. Often there is also a mushroomlike swelling of these outer packs of elements. Covering these swellings and discontinuous, irregularly between is a layer about 5 mμ in thickness. It seems to have a very fine, dark outer line and inner line but is not quite the same as the outer layer (1) (Fig. 3) of the left-side cuticle.

1. Network tubules

As reported above these appear as simple elements but are more likely continuous strands or tubules which is suggested in the cross sections near the apex of the junction with the dorsoventral wings (Fig. 4). There seem to be extremely fine refractive elements in these tubules too small to illustrate. These are often irregular in size and distribution but were seen to arrange sometimes so as to have a hollow core or center.

2. Palisade rods

Internally these rods are crosshatched in appearance, usually uniform throughout but occasionally show diverging directions and orientation along definite lines setting off areas within the rods (Fig. 4). Closely surrounding the palisade rods are denser strandlike elements also perpendicular to the body and distinct from each other (Fig. 4, inset). The palisade rods are in tissue which takes no osmium stain.

Only one species shows a different palisade rod development. *B. reticulatum* has very large refractive elements especially dense and packed into the area between the tubercles when seen in whole mounts. Some were seen to have an inner core of differently oriented tissue surrounded by the usual crosshatched material found in the other palisade rods. The palisade rods of *B. reticulatum* also have a much darker covering of irregular thickness on the external surface (Figs. 9, 10).

3. Fractionating organelle

In the posterior region under the surface of the right side of the body is a cell containing many individual units referred to here as fractionating organelles (Figs. 7, 8). These vary in size up to about 0.5 to 1.3 μ in diameter and on one end is a ramifying reticulate protrusion. Internally are small proteinlike units similar to those found in the palisade rods but the dense lines are spaced 82 to 97 Å apart. They may be the site of origin of the basic substance of the palisade rods. Assuming this is true there is no evidence of the means by which the material is brought to the network. One possibility could be by deposition at time of molting and these fractionating organelles have simply carried over and persist but are nonfunctional in the adult female.

Discussion

The unusual cuticular structures on both left and right sides result in a symmetry oriented dorsoventrally in contrast to the bilateral symmetry of most nematodes. It is possible the complex network and palisade rods evolved for a peculiar or specialized form of locomotion and the tubercles are an aid to such movements.

The palisade rods seem to be proteinaceous in composition by their considerable similarity to the protein platelets described by Poinar (1970) in *Hydromermis*, to the yolk proteid body reported by Roth and Porter (1964) in the mosquito, *Aedes aegypti*, and to the oocytes in *Rana pipiens* (Ward, 1962). The internal highly organized pattern of dense lines are evenly spaced at 134 to 146 Å in the palisade rods of *Bunonema* whereas Poinar reports 270 Å in *Hydromermis*, and Ward reports 71 to 83 Å in *R. pipiens*. Even admitting the proteid nature of the rods, there is no clue as to their function.

The tubules or canals found in the tubercles of the right side and in the wings and ridges of the left side have similarities to the cuticular canals reported by Nicholas (1972), who suggested the canals in *Heterotylenchus* may be involved in assimilation. In *Heterotylenchus* the canals appear to connect the hypodermis to the exterior. The canals of *Bunonema* differ in that they are limited to the inner cortical layer except proximally they penetrate the median layer to the boundary of the basal, fiber layer.

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Three New Species of Digenetic Trematodes from Puget Sound Fishes

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ABSTRACT: Three new species of digenetic trematodes are described from marine fishes collected in the vicinity of the San Juan Islands, Washington. The species are *Helicometra pugetensis* in the family Opecoelidae Ozaki, 1925; *Hexagrammia longitestis* and *Faustula gasterostei* in the family Fellodistomatidae Nicoll, 1913.

Three new species of digenetic trematodes were found in marine fishes collected in the vicinity of the San Juan Islands, Washington. Wholemounts were stained with Gower's carmine and serial sections with Harris' hematoxylin. Measurements are in microns unless indicated otherwise. Drawings are largely from the holotypes.

Family Opecoelidae Ozaki, 1925
Helicometra pugetensis sp. n.
(Fig. 1)

Body elongate, nonspinous, flat, 2.2 to 3.2 mm long by 0.6 to 0.8 mm wide. Oral sucker terminal, funnel-shaped, 546 to 670 by 436 to 590. Ventral sucker immediately preequatorial, 280 to 343 in diameter. Pharynx 234 to 265 by 156 to 187; short prepharynx visible in frontal sections; esophagus about half as long as pharynx; ceca extend to posterior end of body. Cirrus sac spindle-shaped, anterodorsal to ventral sucker, contains folded seminal vesicle, small prostatic complex, and small nonspinous cirrus. Genital pore to left of pharynx. Testes tandem, lobed, intercecal, postequatorial, 124 to 171 by 218 to 265. Ovary lobed, median, pretesticular, about same size as testes. Seminal receptacle to left of
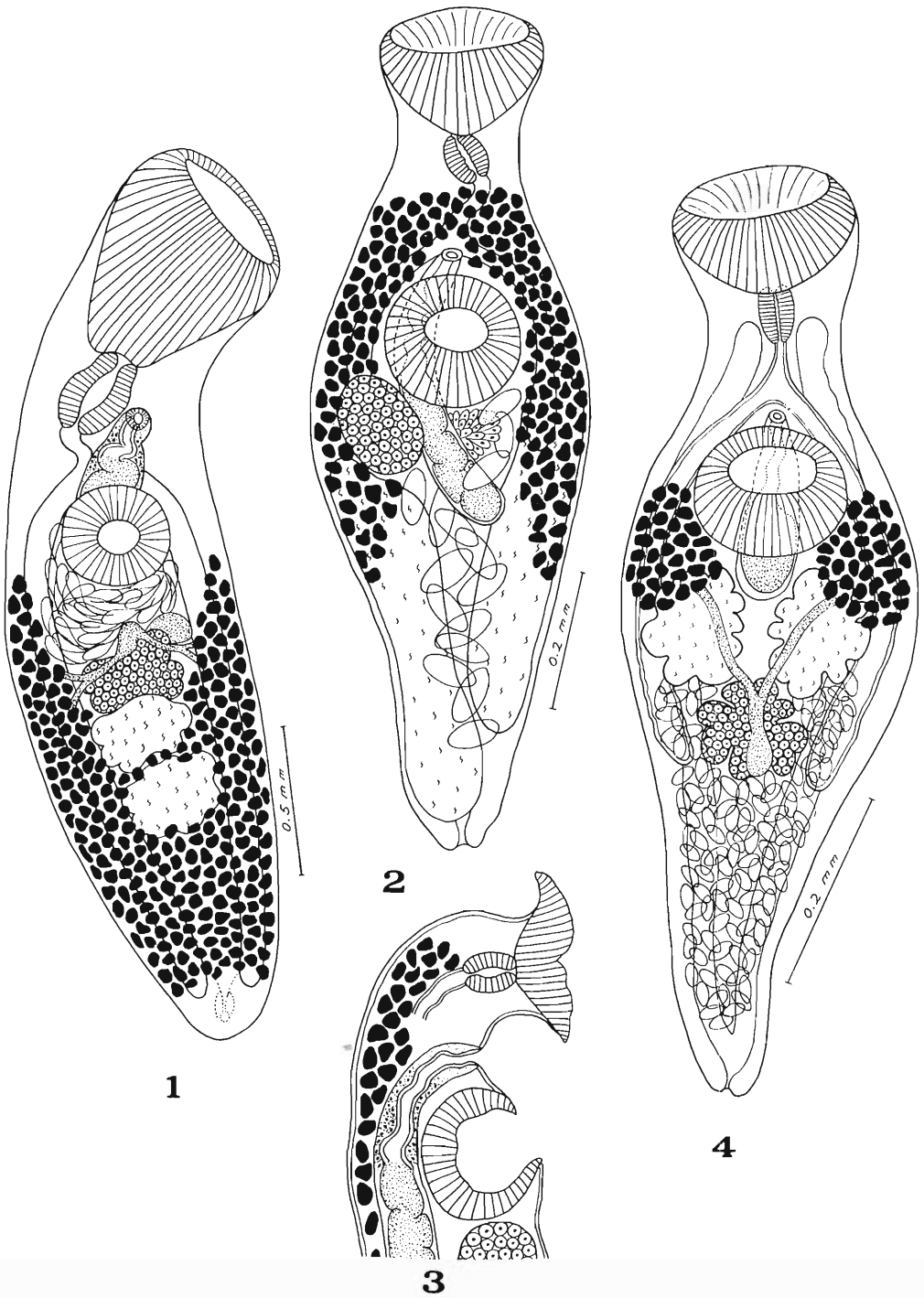
ovary, pyriform. Vitellaria circumcecal, extend from posterior margin of ventral sucker to posterior end of ceca, confluent posterior to testes. Vitelline reservoir immediately preovarian, median and ventral to Mehlis' gland which is also preovarian. Uterus fills area between ovary and ventral sucker, spirally coiled. Eggs pale amber, have unipolar filament, 70 to 72 by 27 to 28 excluding filament. Excretory vesicle large, sac-shaped, occupies intercecal space dorsal to testes and more posterior vitellaria. Excretory pore opens on dorsal surface near posterior end of body.

HOST: Kelp greenling, *Hexagrammos decagrammus* (Pallas, 1810).

HABITAT: Intestine.

TYPE LOCALITY: Puget Sound in vicinity of San Juan Islands, Washington.

TYPE SPECIMENS: Deposited in USNM Helm. Coll. Holotype No. 72447, Paratypes No. 72448.

Remarks

This species is described from seven stained wholemounts and two serial frontal sections. It differs from all other species except *H. insolita* Polyansky, 1955, in having a terminal, funnel-shaped oral sucker. In the latter species the suckers are of equal size, but in *H. pugetensis* the ventral sucker is about 40% smaller than the oral sucker. *H. insolita* differs further in being larger (3.0 to 4.5 mm long by 0.87 to 1.0 mm wide). The reported hosts for *H. insolita* are primarily sculpins and pricklebacks collected from the Barents Sea.

Family Fellodistomatidae Nicoll, 1913
Hexagrammia longitestis sp. n.
(Figs. 2, 3)

Body elongate, nonspinous, 0.8 to 1.3 mm long by 0.4 to 0.5 mm wide at level of ovary, tapering gradually toward posterior end. Oral sucker terminal, directed forward, 109 to 140 by 234 to 310, wider than forebody. Ventral sucker immediately preequatorial, diameter 170 to 230. Pharynx 78 to 93 by 47 to 62;

prepharynx very short; esophagus slightly longer than pharynx; ceca extend short distance posterior to ventral sucker. Cirrus sac 343 to 395 by 45 to 52, extends some distance posterior to ventral sucker and contains folded seminal vesicle, prostatic complex, and nonspinous cirrus. Testes sausage-shaped, 390 to 502 by 78 to 109, occupying much of hindbody, opposite to slightly oblique, left one usually slightly more anterior than the right. Ovary ovoid, dextral, 93 to 109 by 62 to 78, lateral or posterolateral to ventral sucker, immediately anterior to right testis. Mehlis' gland immediately posterior to ventral sucker. Vitellaria extend from posterior end of pharynx to about middle of testes on both sides of body, confluent anterior to ventral sucker. Vitelline reservoir ventral to Mehlis' gland. Uterus occupies intertesticular region of hindbody. Eggs large, pale amber, 78 to 85 by 35 to 39. Metraterm weakly developed, passes dorsal to ventral sucker, either to right or left of cirrus sac. Genital pore median, opens into depression a short distance anterior to ventral sucker. Excretory vesicle possibly Y-shaped, with long median stem, arms not observed.

HOST: Kelp greenling, *Hexagrammos decagrammus* (Pallas, 1810).

HABITAT: Gastric ceca.

TYPE LOCALITY: Puget Sound in vicinity of San Juan Islands, Washington.

TYPE SPECIMENS: Deposited in USNM Helm. Coll. Holotype No. 72443, Paratypes No. 72444.

Remarks

This species is described from 14 stained wholemounts and three serial sagittal sections. The only other species in the genus is *H. zhukovi* Baeva, 1965, a parasite of the atka mackerel, *Pleurogrammus monopterygius* (Pallas, 1810) from Abachinski Bay in the Far East. *H. zhukovi* differs in having a larger body (2.22 by 0.61), larger eggs (91 to 99 by 50 to 60), vitellaria confined to the preovarian region on the right side but extending

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Figures 1–4. 1, *Helicometra pugetensis* sp. n., ventral view. 2, *Hexagrammia longitestis* sp. n., ventral view. 3, Median sagittal section of *H. longitestis*, showing cirrus sac and depressed genital pore. 4, *Faustula gasterostei* sp. n., ventral view.

to the posterior end of the uterus on the left side of the body, the cirrus sac does not extend posterior to the ventral sucker, and the genital pore is located some distance from the ventral sucker.

Faustula gasterostei sp. n.
(Fig. 4)

Body small, 702 to 910 long by 187 to 202 wide at level of vitellaria, nonspinous, tapering gradually toward posterior end. Oral sucker terminal, bowl-shaped, directed forward, 67 to 75 by 149 to 180, wider than forebody. Ventral sucker preequatorial, 111 to 135 in diameter. Pharynx 64 to 78 by 30 to 35; prepharynx absent; esophagus about half as long as pharynx; ceca extend to posterior margin of ovary. Cirrus sac passes dorsal to ventral sucker and extends short distance posterior to it; contains seminal vesicle, few prostate cells, and nonspinous cirrus. Testes opposite, multilobed, immediately posterior to ventral sucker, 85 to 102 in diameter. Ovary posttesticular, median, lobed, 67 to 85 in diameter. Genital pore median, close to anterior margin of ventral sucker. Vitellaria in compact clusters posterolateral to ventral sucker. Vitelline ducts pass obliquely posteriad and merge at vitelline reservoir which is ventral to ovary. Gravid uterus fills area posterior to gonads. Metraterm weakly developed, passes dorsal to ventral sucker, either to right or left of cirrus sac. Eggs pale amber, 28 to 32 by 14 to 16. Excretory vesicle V-shaped, arms extend to level of pharynx. Laurer's canal and seminal receptacle not observed.

HOST: Threespine stickleback, *Gasterosteus aculeatus* L., 1758.

HABITAT: Upper part of intestine.

TYPE LOCALITY: Vicinity of San Juan Islands, Washington.

TYPE SPECIMENS: Deposited in USNM Helm. Coll. Holotype No. 72445, Paratypes No. 72446.

Remarks

This species is described from 15 wholemounts and three serial frontal sections. The gonads are best observed in younger specimens in which the uterus is not filled with eggs. In shape of body, distribution of vitellaria, and contents of cirrus sac this species is similar to *F. sayori* (Yamaguti, 1942), but differs from that species and all other species in the genus in having lobed testes.

Acknowledgment

The author is grateful for the use of facilities at the Friday Harbor Laboratories where the work was done.

Paratylenchoides gen. n. and Two New Species (Nematoda: Paratylenchidae)

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ABSTRACT: *Paratylenchoides* gen. n. is proposed in the family Paratylenchidae with two new species, *P. sheri* and *P. israelensis*. The new genus is diagnosed principally on its unique head region with heavy sclerotization.

Nematode specimens assembled in the course of a review of the genus *Paratylenchus* Micoletzky, 1922, included some tentatively identified as belonging to that genus. Further study showed differences which are believed to represent generic rank. So far they are known only from the Mediterranean area, the genotype from France and another species from Sicily and two localities in Israel. Descriptions of these species follow:

Paratylenchoides gen. n.

DIAGNOSIS: Family Paratylenchidae. Head sclerotization strong, less so in males. Females with large stylet, lacking in males. Head of female narrower dorsoventrally; lateral lips larger than dorsal or ventral lips. Heads of males and females protrude near oral aperture giving a distinctive outline projecting forward at anterior end of conical lip region. Similar stylet and head structures also present in juveniles. Four lines in lateral field.

The name *Paratylenchoides* is derived from Gr. *para*, beside or near; Gr. *tylos*, a knot or callus; and *oides*, a contraction of Gr. *o + eidos*, denoting likeness of form to indicate close relationship to the genus *Paratylenchus*.

TYPE SPECIES: *Paratylenchoides sheri* sp. n.

OTHER SPECIES: *Paratylenchoides israelensis* sp. n.

This genus is most closely related to *Paratylenchus* from which it differs in the strong sclerotization of the head which is conical, smooth, and bears a narrow rounded projection of the anterior end. The juveniles, including the fourth stage or preadult, of *Paratylenchoides*, also bear a strong stylet.

Paratylenchoides sheri sp. n.
(Fig. 1, A–F, J–L)

31 ♀♀: L = 0.42 mm (0.33–0.51); a = 24 (19–28); b = 4.2 (3.5–5.3); c = 11 (10–12); V = 80 (78–82); stylet = 22 μ (20–23).

6 ♂♂: L = 0.41 mm (0.35–0.49); a = 33 (30–35); b = 4.2 (3.2–4.8); c = 10 (10–11); spicules = 25 μ (23–28); gubernaculum = 5 μ (4–6).

HOLOTYPE (♀): L = 0.51 mm; a = 26; b = 4.4; c = 14; V = 81; stylet = 23 μ. Head with strong sclerotization, conical in outline to the protruding lips surrounding the oral aperture. Stylet robust with backwardly directed knobs. Dorsal gland orifice 6 μ from knobs. Excretory pore 105 μ (77–99 μ in paratypes) from anterior end, located on posterior edge of hemizonid (in some paratypes may be at level of or posterior to hemizonid). Gonad outstretched with large spermatheca on left lateral side. Tail tapers moderately and gradually to a bluntly rounded terminus. Body annules average 1.7 μ in width. Lateral field with four lines about 1 μ apart, the inner two less distinct than the outer two. In cross

section the lateral field protrudes markedly (Fig. 1, D).

ALLOTYPE (♂): L = 0.47 mm; a = 37; b = 4.6; c = 11; spicules = 27 μ; gubernaculum = 4 μ. Head with moderate sclerotization; with conical taper to a rounded protrusion around oral aperture. Esophagus not developed, only simple outline barely distinguishable. Excretory pore 86 μ (81–87 μ in paratypes) from anterior end, located anterior to hemizonid. Annules at midbody about 1.5 μ wide; lateral field with four lines about 1 μ apart, the two center ones less distinct than the two outer ones. Tail tapers slightly posteriorly to cloaca, then more abruptly near terminus to a bluntly rounded tip.

JUVENILES (SECOND STAGE): L = 0.26 mm (0.20–0.30); a = 22 (18–26); b = 3.1 (2.6–3.8); stylet = 16 μ (15–18). Head similar in shape and sclerotization to adults. Stylet slender but prominent. Genital primordium 9–16 μ in length (avg 14 μ), located 42–64 μ (avg 55 μ) from terminus. Body tapers very gradually posteriad giving shape almost cylindrical with bluntly rounded, almost hemispherical, tail.

JUVENILES (FOURTH STAGE, ♀): L = 0.34 mm (0.31–0.38); a = 25 (23–30); b = 3.6 (3.3–3.9); stylet = 17 μ (17–18). Similar to second-stage juvenile differing in larger genital primordium, 48–73 μ (avg = 63), located 57–69 μ (avg = 64) from terminus.

JUVENILES (FOURTH STAGE, ♂): L = 0.34 mm (0.33–0.36); a = 25; b = 3.8 (3.6–4.1); stylet = 17 μ. Similar to female juvenile differing in slightly less developed stylet and esophagus. Also genital primordium 48 μ long is located more posteriorly, 27 μ from terminus.

HOLOTYPE: Female collected 6 October 1963 by S. A. Sher, slide number 1305 deposited at University of California Nematode Survey Collection, Davis, California.

ALLOTYPE: Male, same data as holotype, slide number 1306, University of California Nematode Survey Collection, Davis, California.

PARATYPES: 57 ♀♀, 6 ♂♂, 27 juveniles, same data as holotype deposited as follows: 3 ♀♀, 1 ♂ at United States Department of Agriculture Nematode Collection, Beltsville, Maryland; 3 ♀♀, 1 ♂ at Plantenziektenkundige Dienst, Wageningen, The Netherlands; 3 ♀♀, 1 ♂ at National Nematode Collection, Indian
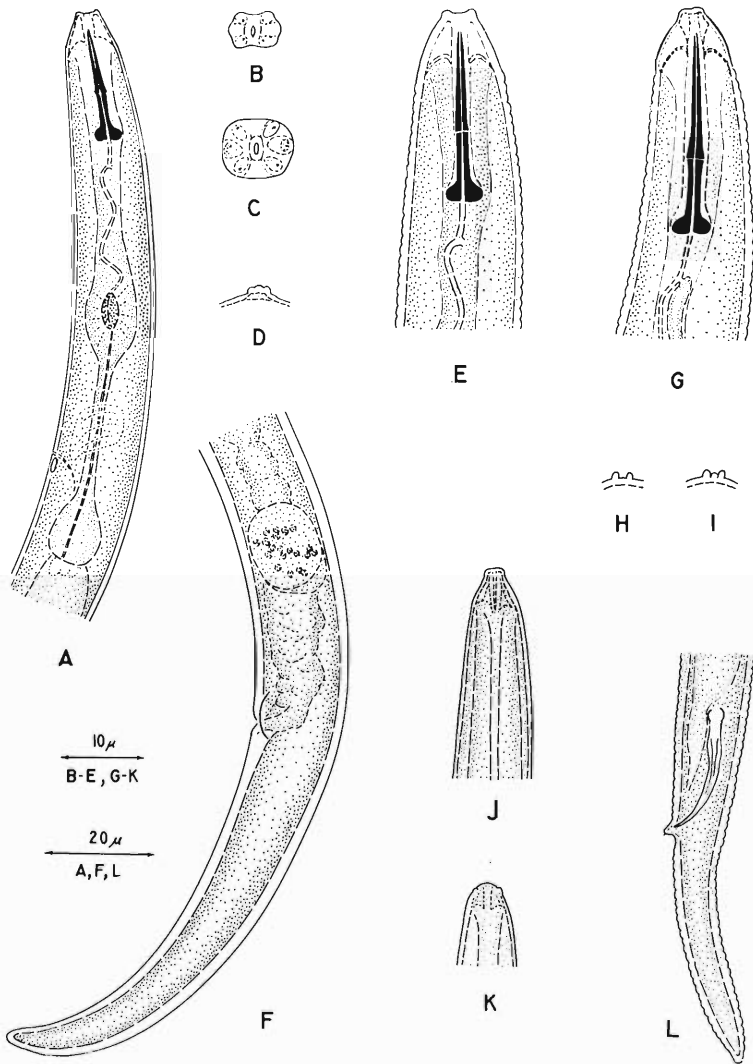
Figure 1. *Paratylenchoides sheri* sp. n. A–F Female: A—Esophageal region; B—En face section; C—Cross section posterior to en face; D—Head region; E—Cross section of lateral field; F—Posterior region. J–L Male: J–K—Head regions; L—Posterior region. *Paratylenchoides israelensis* sp. n. Female: G—Head region; H–I—Cross sections of lateral field.

Agricultural Research Institute, New Delhi, India; 3 ♀♀, 1 ♂, 3 juveniles at Nematology Department, Rothamsted Experimental Station, Harpenden, England; 45 ♀♀, 2 ♂♂, 24 juveniles, slides numbered 1307–1319 at University of California Nematode Survey Collection, Davis, California.

TYPE HOST: Grass and weed soil.

TYPE LOCALITY: Two miles south of Digne, France.

DIAGNOSIS: This species is closely related to *Paratylenchoides israelensis* from which it differs in the shorter, less robust stylet, lesser sclerotization of the head than in *P. israelensis*,

and in the uniform outline of the lateral field as seen in cross section.

Paratylenchoides israelensis sp. n.
(Fig. 1, G–I)

11 ♀ ♀: L = 0.45 mm (0.39–0.50); a = 22 (18–24); b = 4.3 (3.7–4.8); c = 11; V = 79 (78–81); stylet = 26 μ (24–28).

HOLOTYPE (♀): L = 0.42 mm; a = 21; b = 3.9; c = 9; V = 79; stylet = 27 μ. Head with very strong sclerotization, conical in outline to protruding lips surrounding the oral aperture. Stylet robust with backwardly directed knobs. Prorhabdion 17 μ (15–18 μ in paratypes). Excretory pore 88 μ from anterior end (74–93 μ in paratypes). Gonad outstretched, spermatheca elongate, ovoid, and without sperm. Tail tapers gradually to a bluntly rounded terminus. Lateral field shows four lines about 1 μ apart, the inner two less distinct than the outer two. In cross section these show as three bands, the outer two protrude more markedly than the inner one (Fig. 1, I). Some sections show the inner band does not protrude but forms a contour level with the rest of the body outline (Fig. 1, H).

MALE: Unknown.

JUVENILES (FOURTH STAGE ♀): L = 0.39 mm (0.37–0.43); a = 20 (17–23); b = 4.4 (4.2–4.7); stylet = 20 μ (18–21). Similar to adult, stylet slightly less robust. Excretory pore 74 μ (68–80 μ) from anterior end. Tail bluntly rounded, almost hemispherical.

HOLOTYPE: Female collected in December 1970, slide number 1320 deposited at University of California Nematode Survey Collection, Davis, California.

PARATYPES: 9 ♀ ♀, 16 juveniles, same data as holotype deposited as follows: 1 ♂, 5 juveniles at United States Department of Agriculture Nematode Collection, Beltsville, Mary-land; 2 ♀ ♀ at Plantenziektenkundige Dienst, Wageningen, The Netherlands; 1 ♂, 1 juvenile at National Nematode Collection, Indian Agricultural Research Institute, New Delhi, India; 1 ♂, 8 juveniles at Nematology Department, Rothamsted Experimental Station, Harpenden, England; 4 ♀ ♀, 2 juveniles, slides numbered 1321–1327 at University of California Nematode Survey Collection, Davis, California.

TYPE HOST: Soil about the roots of plum.

TYPE LOCALITY: Shiller, Israel.

DIAGNOSIS: This species is closely related to *P. sheri* differing in its longer more robust stylet, stronger sclerotization of the head, and different outline of lateral field in cross section.

OTHER COLLECTIONS: 2 ♀ ♀ about the roots of citrus, Rambam, Israel; 3 ♀ ♀, 12 juveniles from soil about the roots of Italia and Regina grape varieties in the government nursery near Palermo, Sicily. These five females conform to the type specimens in most characteristics but extend slightly the range of some. One female from Rambam, Israel, has a length of 0.53 mm; a = 23; c = 13; and the excretory pore varies from 94–101 μ from the anterior end. For the three females from Sicily a = 26–30 and c = 9–12. These are considered infraspecific variations.

Remarks

The characteristic sclerotization of the lip region of these specimens are the most divergent among the Paratylenchidae found to date. It is possible this divergence might be useful as an indication of relationship to other tylenchs but the particular head structure of these species is not clearly similar to any other groups of nematodes. More collections and discoveries of additional related species would be very helpful in trying to establish the significance of *Paratylenchoides* species in such relationships.

A Description of the Male and Redescription of the Female of *Camallanus oxycephalus* Ward and Magath, 1916 (Nematoda: Camallanidae)[1]

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ABSTRACT: The male of the species is described for the first time and the female description is emended to include a more posterior position for the vulva. Both sexes are differentiated from *Camallanus ancylodirus* Ward and Magath, 1916, and two European species which have been reported in North America. Four new host records are reported for *C. oxycephalus* Ward and Magath, 1916, from Lake Erie fishes.

Camallanus oxycephalus Ward and Magath, 1916, is a common and widely distributed nematode of fishes in eastern North America. It occurs from Massachusetts (Sindermann, 1953) westward through the Great Lakes drainage to South Dakota (Hugghins, 1959) and south to Oklahoma (McDaniel, 1963). It has not been reported west of the Mississippi drainage nor farther north than northwest Ontario. The original description is based on female specimens taken from *Pomoxis nigromaculatus* and *Morone chrysops* at Fairport, Iowa. Since that time, *C. oxycephalus* has been reported from 48 species of fishes which are distributed over 32 genera and 17 families. In addition to those fishes listed as hosts for this nematode by Hoffman (1967), we add the following four species from western Lake Erie: *Dorosoma cepedianum, Alosa pseudoharengus, Osmerus mordax,* and *Notropis spilopterus*.

Although *C. oxycephalus* has been collected and identified frequently in eastern North America since its description, the male has never been described. This species has been separated from *C. ancylodirus* Ward and Magath, 1916, the only other indigenous species of *Camallanus* described from North American freshwater fishes, by the orientation of the buccal capsule and esophageal dimensions of the female. Ward (1918) constructed a key using the head orientation and vulvar position to discriminate between these two

species and this has been the basis for identification of *C. oxycephalus* since that time.

The nematodes used in this study were killed in hot AFA and fixed in AFA for 24 hr. They were preserved in 10% glycerin alcohol, cleared, and studied in glycerin. Several en face views were studied in a mixture of glycerin jelly and agar. All measurements are in microns unless otherwise stated.

Camallanus oxycephalus Ward and Magath, 1916
(Figs. 1–6)

Description

Camallanidae Raillet and Henry, 1915. Slender nematodes, widest in middle third of body and tapering slightly toward tail. Living worms red in color. Head straight, not bent ventrally. Oral opening elongate. Buccal capsule divided into two sclerotized lateral valves with smooth longitudinal riblike thickenings internally; inflation of valves forms sclerotized ring at junction of buccal capsule and esophagus. Two trident-shaped processes present, one dorsal, one ventral, at junction of valves. Three pairs of simple circumoral papillae in outer circle; two pairs in inner circle located at dorsal and ventral ends of oral opening. Esophagus divided into anterior club-shaped muscular portion and posterior cylindrical glandular portion. Intestine straight. Anal lips slightly salient.

MALE (10 specimens): Length 4.43–5.20 mm (avg 4.57), width 120–184 (152). Cuticle with striations barely perceptible. Buccal cap-
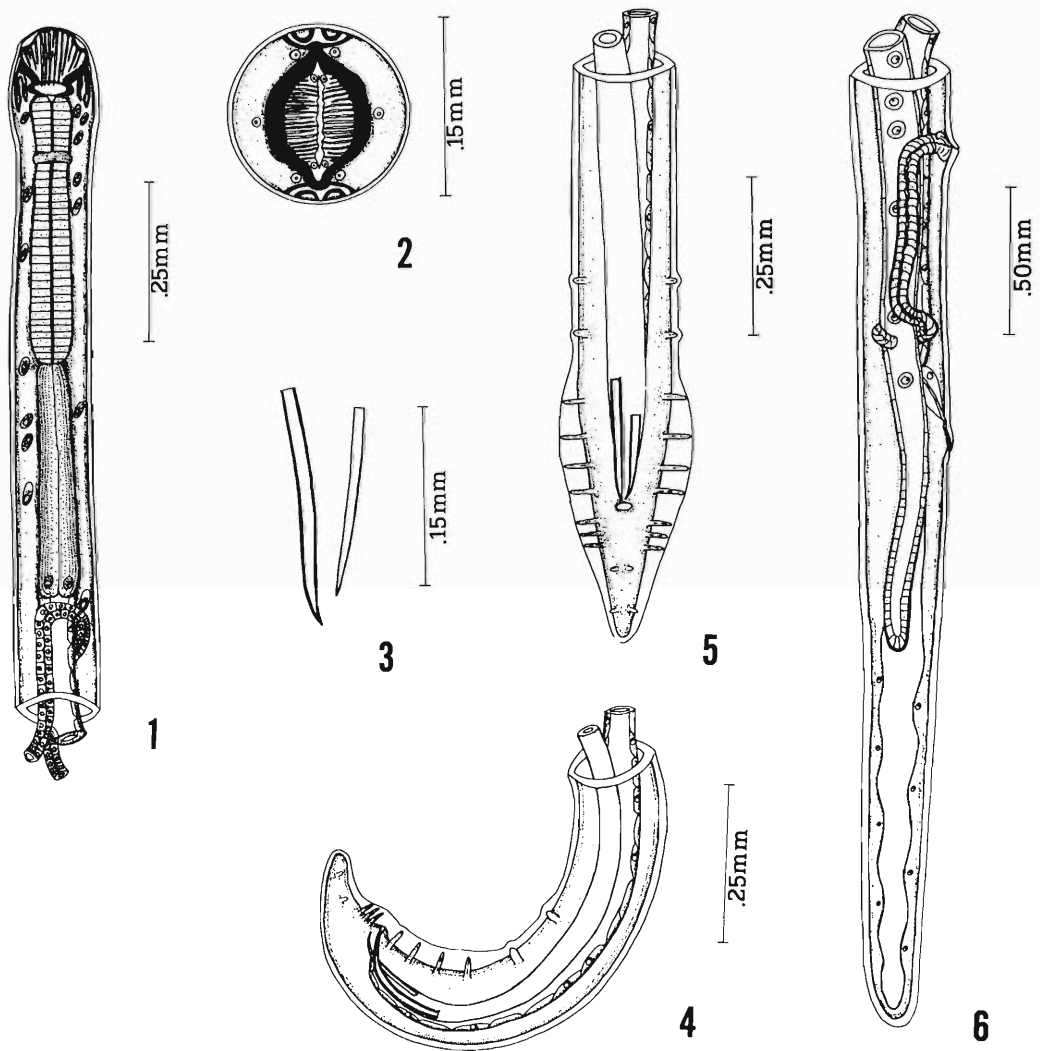
Figures 1–6. *Camallanus oxycephalus.* 1, Male anterior end, lateral view. 2, En face view. 3, Spicules. 4, Male tail, lateral view. 5, Male tail, ventral view. 6, Female tail, lateral view.

sule 96–112 (103) by 96–107 (100); tridents 86–104 (94) in length. Muscular esophagus 360–422 (390) long; glandular esophagus 428–530 (461) long. Nerve ring 168–210 (189) from anterior end. One testis reaching almost to glandular esophagus, then reflexed; reproductive tract with several swollen portions separated by constrictions. Tail 109–136 (121) long, rolled ventrally in mature specimens, ending bluntly without mucrones. Thin caudal

alae supported by papillae. Eleven pairs of pedunculate papillae, six pairs preanal, five pairs postanal; first three pairs of postanal papillae arranged close together. Two spicules, unequal but similar; left spicule weakly sclerotized, right spicule heavily sclerotized, 146–166 (154) in length. No gubernaculum.

FEMALE (10 specimens): Length 15.93–25.05 mm (18.18); width 206–282 (245). Cuticle with striations barely perceptible. Buc-

cal capsule 128–142 (137) by 136–165 (151); tridents 134–144 (138) in length. Muscular esophagus 483–666 (569) long; glandular esophagus 558–748 (652) long. Nerve ring 222–300 (262) from anterior end. Vulva 2.24–3.11 mm (2.69) from tip of tail, lips slightly salient. Vagina very muscular, directed posteriorly. One ovary reaching to level of muccular esophagus then reflexing; posterior branch of uterus reaching end of tail, ending blindly in highly muscular sac. Tail 1.53–2.21 mm (1.87) long, bluntly rounded without mucrones. Ovoviviparous; larvae 629–645 (635) long.

HOST: *Morone chrysops.*

LOCATION: Large intestine, rectum.

LOCALITY: Lake Erie off Buckeye Point, South Bass Island, Put-in-Bay Twp., Ottawa Co., Ohio.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 72457.

Discussion

The description of the female of *C. oxycephalus* by Ward and Magath (1916) is incomplete. Only the body length and vulvar position as given by Ward and Magath are useful for comparison. The vulva was described as being "located at the anterior margin of the middle third of the body." Our investigation of this character shows that the vulva is situated much farther posterior, close to the anus (714–918 from the anus). This discrepancy, which Tornquist (1931) was unable to resolve in his review, cannot be merely ascribed to allometric growth. We examined immature female specimens and the vulva is in approximately the same position, proportionally, as it is in gravid adults. This position is consistent in the 1,200 female worms examined. Ward and Magath indicated that females reached 25 mm in length, but failed to mention if these worms contained larvae or embryos. Our measurements are based upon gravid females containing embryos and larvae and the maximum is consistent with the original description. Despite the difference in vulvar position, which is an important taxonomic character in this group, we elect to retain our material in the species *C. oxycephalus.* The absence of a male description and the few good female characters given by Ward and Magath seem to justify this.

Camallanus oxycephalus can easily be separated from *C. ancylodirus* Ward and Magath, 1916, although a complete description of the latter is lacking. The head is bent ventrally in *C. ancylodirus* and the males reach a length of 15 mm, which is three times the maximum male length of *C. oxycephalus.* Two European species of *Camallanus* have been reported just once from North American fishes (Meyer, 1954; Maine). *Camallanus oxycephalus* can be differentiated from both of these species by the number and arrangement of the papillae on the male tails. *Camallanus lacustris* (Zoega, 1776) males possess 13 pairs of caudal papillae and reach a maximum length of 2.28 mm (Moravec, 1969). Females of *C. lacustris* reach a maximum length of 7.08 mm and posses a vulva with two highly elevated lips situated in the middle of the body. The males of *C. truncatus* (Rudolphi, 1814) possess 12 pairs of caudal papillae which are arranged differently than on *C. oxycephalus.* In addition, the tridents on both males and females of *C. truncatus* are very long, reaching more than halfway to the nerve ring.

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Isorchis manteri sp. n. from Australian Mullet and a Key to Haploporid Trematode Genera with Two Testes[1]

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ABSTRACT: A new species of Isorchis, I. manteri, is reported from the small intestine of Mugil cephalus collected in the Brisbane River, Queensland, Australia. The haploporid trematode genus Allomegasolena Siddiqui and Cable, 1960, may be a synonym of Vitellibaculum Montgomery, 1957.

During sabbatical leave (1970–71) spent at the University of Queensland, Brisbane, Australia, a study of haploporid trematodes from mullet was made. Seven specimens representing the genus Isorchis Durio and Manter, 1969, were recovered from Mugil cephalus L. and are described as a new species in this paper.

The worms were fixed without pressure in hot 5% formalin, stained with Mayer's paracarmine, cleared in methyl benzoate, and mounted in Canada balsam.

All measurements are in microns, averages in parentheses.

Isorchis manteri sp. n.
(Figs. 1–3)

Specific diagnosis

With the characters of the genus. Measurements based on seven specimens. Tegument relatively thick, with spines in quincunx. Remnants of eyespots in pharyngeal region. Many tubular glands in neck region. Body fusiform 910–1,512 (1,178) long, 364–742 (484) wide. Oral sucker 106–128 (113) long, 127–143 (137) wide; acetabulum 124–162 (145) long, 152–171 (162) wide; sucker ratio 1:1.2. Prepharynx 31–47 (38) long; pharynx 84–96 (89) long, 75–96 (83) wide; esophagus about 170 long, bifurcating at acetabular level; ceca voluminous, with fairly thick walls, reaching near to posterior end of body. Vitellaria follicular to branched, from acetabular level to near ends of ceca. Ovary oval, near midbody, immediately anterior to testes, 62–171 (105) long, 47–134 (97) wide. Uterine seminal receptacle present, Laurer's canal not observed. Uterus from midlevel of testis to that of hermaphroditic pouch. Eggs with thin yellow shells, operculated, 40–59 (54) long, 34–37 (35) wide, none with fully developed miracidia. Testes two, nearly transverse, in anterior part of hindbody, 103–218 (171) long, 87–165 (123) wide. Vas deferens joins external seminal vesicle which may bend on itself. Hermaphroditic sac oval, between pharynx and acetabulum, contains internal seminal vesicle, prostatic bulb, a few prostate cells, terminal portion of uterus, and hermaphroditic duct surrounded distally by a muscular bulb. Genital pore midventral between pharynx and acetabulum. Excretory bladder with long stem extending to testes and short crura.

[1] Supported in part by NSF GB6962.

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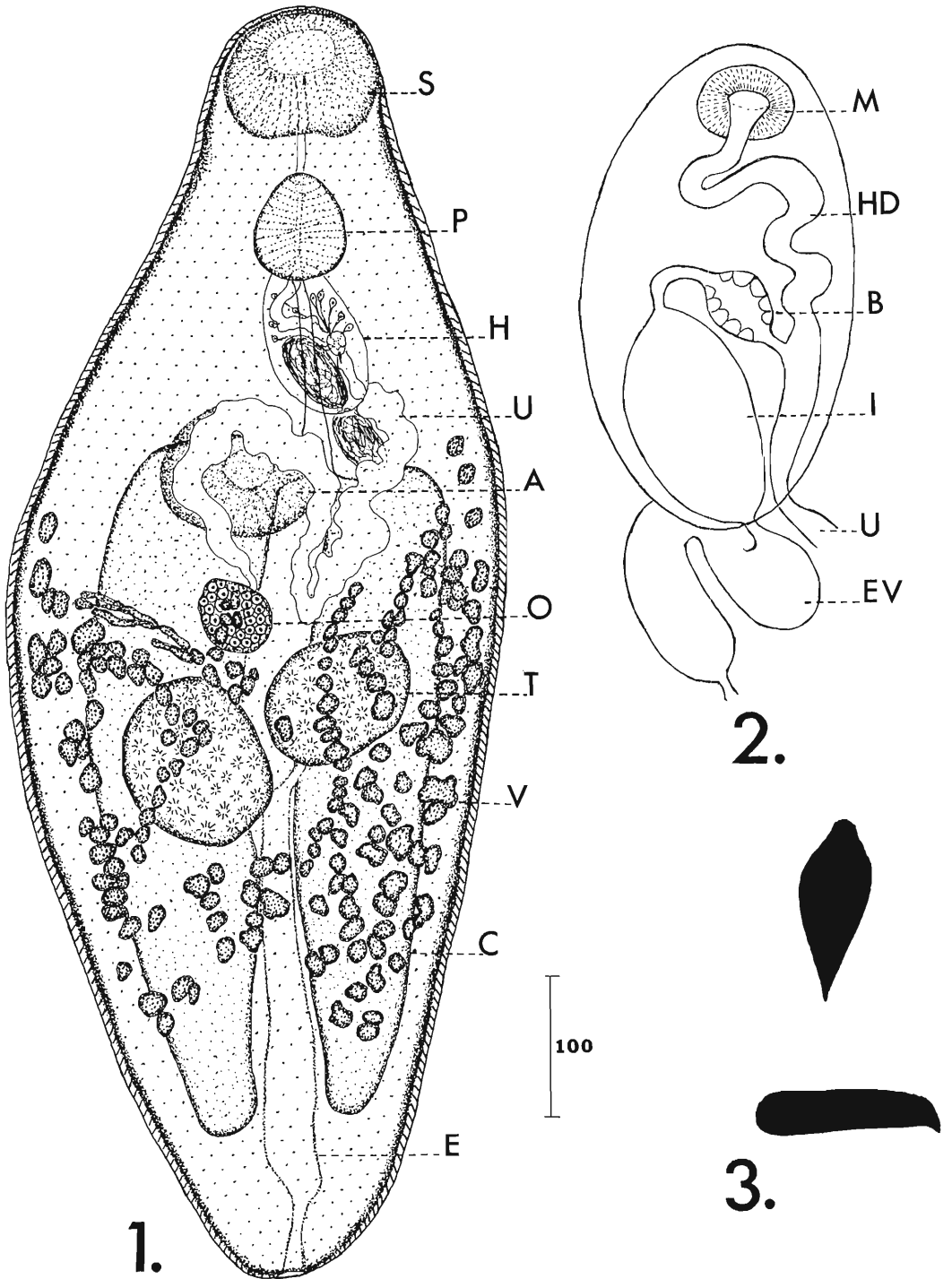
HOST: *Mugil cephalus* L., sea mullet.

HABITAT: Small intestine.

LOCALITY: Brisbane River, Queensland, Australia.

Holotype deposited as No. 7111 Hancock Parasitology Collection, University of Southern California.

Discussion

Durio and Manter (1969) erected the genus *Isorchis* with *I. parvus* as type for specimens found in the intestine of a New Caledonia marine fish, *Choanos choanos* (Forskål). *Isorchis manteri* differs from *I. parvus* in the extent of the ceca posterior to the testes; the vitellaria extending anteriorly to mid-hermaphroditic pouch level rather than to the pharynx; the ovary anterior to the testes instead of between them; smaller eggs; and the acetabulum larger than the oral sucker instead of equal suckers. Durio and Manter mentioned an undescribed species of *Isorchis* in Australia. Perhaps this is *I. manteri*. *I. manteri* does not agree with the generic diagnosis in the level of the intestinal bifurcation which is anterior to the acetabulum in *I. parvus* and at the acetabular level in *I. manteri*. The "slight" pressure used in fixing *I. parvus* probably accounts for that difference.

Vitellaria range from follicular in smaller specimens to partly branched in larger (presumably older) *I. manteri*. Two individuals had vitellaria extending slightly posterior to cecal terminations. One individual had the uterus extend to near the posterior testis margin.

Isorchis is one of eight haploporid genera possessing two testes instead of one in other genera of the family. The diorchid genera can be separated as follows:

1. a) Testes preacetabular ....
    .... *Atractotrema* Goto and Ozaki, 1929
    b) Testes postacetabular 2
2. a) Testes transverse ....
    .... *Isorchis* Durio and Manter, 1969
    b) Testes tandem 3
3. a) Hermaphroditic sac absent ....
    Neomegasolena Siddiqi and Cable, 1960
    b) Hermaphroditic sac present 4
4. a) Acetabulum papillate ....
    .... *Scorpidicola* Montgomery, 1957
    b) Acetabulum nonpapillate 5
5. a) Ceca not extending posterior to testes
    .... *Metamegasolena* Yamaguti, 1970
    b) Ceca extending posterior to testes 6
6. a) Without genital sucker or muscular bulb around distal end of hermaphroditic duct .... *Megasolena* Linton, 1910
    b) With genital sucker or muscular bulb around distal end of hermaphroditic duct 7
7. a) With two pairs of lymphatic vessels *Allomegasolena* Siddiqi and Cable, 1960
    b) With three pairs of lymphatic vessels .... *Vitellibaculum* Montgomery, 1957

Allomegasolena is quite similar to and may be a synonym of *Vitellibaculum*. The former has a genital sucker but the latter has a muscular bulb around the distal end of the hermaphroditic duct. If the muscular bulb is everted it would appear as a sucker. Manter and Pritchard (1961), Yamaguti (1970), and Martin (1973) have observed haploporids evert part of the hermaphroditic duct through the genital pore. Martin suggested that the everted hermaphroditic duct may function as a male copulatory organ. *Allomegasolena* has two pairs of lymphatic vessels and *Vitellibaculum* three pairs. Excretory tubes in haploporids are frequently enlarged, convoluted, and bent back on themselves. They may be confused with lymphatic vessels. For example, Manter and Pritchard (1961) described *Hapladena spinosa* with two pairs of well-developed

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Figure 1. Dorsal view of *Isorchis manteri*. A, acetabulum; C, cecum; E, excretory vesicle; H, hermaphroditic sac; O, ovary; P, pharynx; S, oral sucker; T, testis; U, uterus; V, vitellaria. Magnification scale applies to Fig. 1.

Figure 2. Diagram of terminal genitalia. B, prostatic bulb; EV, external seminal vesicle; HD, hermaphroditic duct; I, internal seminal vesicle; M, muscle bulb; U, uterus.

Figure 3. Upper, longitudinal view of spine; lower, side view of spine.

lymph vessels, yet Yamaguti (1970) in re-describing this species stated that these vessels were actually excretory tubes and that no true lymphatic system was present. He also stated that Siddiqi and Cable (1960) concurred with this interpretation in their description of *Hapladena acanthuri* but I cannot find such a statement in their report. In fact they described *H. acanthuri* as having two pairs of longitudinal lymphatic vessels. Montgomery (1957) could have counted one pair of excretory tubes as lymphatic.

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Helminth Parasites of the American Coot, *Fulica americana americana,* on Its Winter Range in Florida[1]

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ABSTRACT: Seventeen species of helminths were found in 60 coots, *Fulica americana americana,* from six localities in Florida. These included eight species of trematodes, two species of cestodes, six species of nematodes, and one species of acanthocephalan. Thirteen of the 17 species appear to be common parasites of the coot and the rest accidental parasites acquired on the winter range. The helminth fauna of the coot in Florida appears to be reduced in comparison to that reported on its summer range. The fauna of the coot shows a close relationship to that of the common gallinule (*Gallinula chloropus cachinnans*) found in the same habitats.

The American coot, *Fulica americana americana* Gmelin, winters throughout the southern United States from California to the Atlantic Coast (Ryder, 1963). In Florida, the coot occurs as an abundant winter resident in all parts of the state, and breeds only sporadically in small numbers in the summer (Howell, 1932). Migrating coots arrive in Florida in November and leave in April. In order to compare the helminths of wintering coots with those found in coots from their summer range in Alberta by Colbo (1965), 60 birds were

collected in Florida in the winters of 1970–71 and 1971–72.

Materials and Methods

Most birds were collected by shotgun; a few were found dead on roads from collisions with wires or automobiles. All birds were collected in the months of November through March, with the majority falling in December and January. Areas sampled were Lake Alice, Paynes Prairie, Orange Lake, and Lake Lochloosa, Alachua Co.; Johnson Pond, Marion Co.; and Backwater Lake, Citrus Co. A few birds were examined while still fresh, but most were frozen and examined at a later date. Necropsy methods and treatment of worms were the same as those outlined by Kinsella and Forrester (1972). Nasal sinuses and subcutaneous tissues were not examined.

Results and Discussion

Seventeen species of helminths were recovered from the 60 coots. Only one bird was free of helminths. These included eight species of trematodes, two species of cestodes, six species of nematodes, and one species of acanthocephalan (Table 1). *Conspicuum icteridorum*, *Hystrichis tricolor*, and *Capillaria* sp. have not been previously recorded from the coot.

A species of *Strongyloides* commonly found here in the coot closely resembles the *Strongyloides* sp. found by Kinsella, Hon, and Reed (1973) in the common gallinule, *Gallinula chloropus cachinnans*, and the purple gallinule, *Porphyrula martinica*. *Strongyloides avium* Cram, 1929, was reported from the coot by Cram (1930) and Colbo (1965). However, in light of the high incidence of infection found in the two species of gallinules and in the Florida duck by Kinsella and Forrester (1972), species identification of *Strongyloides* in these waterfowl has been deferred pending further studies on life cycles and host specificity.

In an extensive study of the helminths of 371 coots on their summer breeding grounds in northern Alberta, Colbo (1965) found 36 species of worms (including one leech). The coot was classified as the main host of 14 species and an auxiliary host of four species, these categories corresponding roughly to the "nor-

Table 1. Helminths of 60 American coots (*Fulica americana americana*) in Florida.

	No. in-fected	No. of worms	
		Mean	(Range)
Trematoda			
Notocotylus pacifer (Noble, 1933) (4)[1]	55	18	(1–250)
Echinostoma attenuatum Lumsden and Zischke, 1963 (3)	17	3	(1– 9)
Cyclocoelum mutabile (Zeder, 1800) (7)	15	5	(1– 26)
Cyclocoelum oculeum Kossack, 1911 (7, 8)	5	2	(1– 3)
Leucochloridium problematicum Magath, 1920 (5)	4	17	(1– 55)
Tanaisia atra (Nezlobinski, 1926) (6)	2	1	(1)
Prosthogonimus ovatus (Rudolphi, 1803) (5)	2	1	(1)
Conspicuum icteridorum Denton and Byrd, 1951 (9)	1	1	(1)
Cestoda			
Diorchis ransomi Schulz, 1940 (3)	14	6	(1– 50)
Diorchis americana Ransom, 1909 (3)	11	10	(1– 50)
Nematoda			
Amidostomum fulicae (Rudolphi, 1819) (2)	50	10	(1– 30)
Tetrameres globosa (Linstow, 1879) (1)	40	4	(1– 21)
Strongyloides sp. (3)	36	12	(1–137)
Capillaria fulicae (Pavlov and Borgarenko, 1959) (4)	14	4	(1– 19)
Hystrichis tricolor Dujardin, 1845 (1)	2	1	(1)
Capillaria sp. (2)	2	1	(1)
Acanthocephala			
Polymorphus trochus Van Cleave, 1945 (3)	17	7	(1– 30)

[1] Location in host: (1) proventriculus, (2) under gizzard lining, (3) small intestine, (4) ceca, (5) cloaca, (6) kidneys, (7) air sacs, (8) trachea, (9) liver.

mal" and "secondary" host categories of Dogiel (1966). The remaining 18 species were classified as accidental parasites of the coot.

The helminth fauna of the coot on its winter range appears to be greatly reduced in comparison to its fauna on the summer range. However, of the 14 species classified by Colbo as normal coot parasites, 12 were present in Florida. Although the coot was classified by Colbo as an auxiliary host of *Strongyloides avium* Cram, 1929, the present study indicates that *Strongyloides* is a common parasite in coots. The remaining four species, *Prosthogonimus ovatus*, *Conspicuum icteridorum*, *Hystrichis tricolor*, and *Capillaria* sp., were not found by Colbo and appear to be accidental parasites which the coot picks up during migration or on its winter range.

The common gallinule and the purple gallinule, two rallids of comparable size and habits to the coot, occur with coots in winter. Comparison with a study of the helminths of these two species by Kinsella et al. (1973) shows that the coot shares 10 species of helminths with the common gallinule and eight with the purple gallinule. Incidence and intensity of infections indicate a closer ecological relationship between the coot and common gallinule than between either of these species and the purple gallinule.

Acknowledgments

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Immunosuppressive Activity of Azathioprine in Experimental Infection of the Guinea Pig with *Trichinella spiralis*[1]

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ABSTRACT: Experiments were conducted in guinea pigs to compare the number of adult *Trichinella spiralis* recovered from the intestine and larvae from skeletal muscle in control and azathioprine-treated animals. Azathioprine suppressed the delayed (cellular) hypersensitivity response and allowed significantly ($P < 0.01$) more adults to remain in the intestine. There was no significant difference ($P > 0.10$) in the number of muscle larvae in azathioprine-treated guinea pigs as compared to controls. These results suggest that the immunologic responses operating in guinea pigs are similar to those operating in mice and rats.

Guinea pigs infected with *Trichinella spiralis* characteristically expel the majority of adult worms from the intestine during the second week of the initial infection (Roth, 1939). Evidence indicates that this expulsion is due to delayed (cellular) hypersensitivity (Kim, 1966; Larsh, 1967; Cypess et al., 1971). This "spontaneous cure" (Mulligan, 1968) has been suppressed in hamsters with 6-mercaptopurine, methotrexate, and cortisone (Ritterson, 1959, 1968); and in mice with cortisone (Coker, 1955) and antilymphocytic serum (DiNetta et al., 1972). These immunosuppressive compounds suppressed the "spontaneous cure" phenomenon and thus prolonged the duration

of parasitosis. As a result, the objectives of this study were to demonstrate the expected (but unreported) efficacy of another immunosuppressive compound (Azathioprine) in another host (the guinea pig) and to provide more evidence that immunity to the intestinal stages of *T. spiralis* in the guinea pig is due to delayed (cellular) hypersensitivity.

Materials and Methods

Male albino guinea pigs (400 to 500 g) were randomly selected and allocated to four groups, each group containing 10 animals. All animals were infected at the same time with 5,000 ± 250 excysted *T. spiralis* larvae obtained from stock guinea pigs according to the methods of Castro and Olson (1967). Determination of the number of adults in the intestine on day 20 postinfection (PI) and the number of larvae in the musculature on day 30 PI was according to the methods of Larsh and Kent (1949). Day 20 PI was selected to insure that maximum expulsion had taken place in the intestine; day 30 PI was selected since this is the time when the majority of "migratory" larvae are in/around muscle fibers and can be most accurately counted.

Twenty guinea pigs served as controls and received only *T. spiralis* larvae. Ten of these animals were killed on day 20 PI and the number of adults in the intestine counted. The other 10 were killed on day 30 PI and the number of muscle larvae in the entire animal determined.

Twenty guinea pigs served as experimental animals and received in addition to *T. spiralis*, Azathioprine (Imuran[R], Wellcome Research Laboratories). The dose was 5 mg/kg/day beginning 2 days preinfection and continuing daily through day 20 PI. Ten of these animals were killed on day 20 PI and the number of adults in the intestine counted. The other 10 were killed on day 30 PI and the number of muscle larvae in the entire animal determined.

Five control rats were infected with 3,000 ± 100 *T. spiralis* larvae (from the same lot given to the guinea pigs) to insure that larvae administered to guinea pigs were infective and capable of exceeding certain reproductive potential limits as described by Harley and Gallicchio (1971).

Student's *t* test (Snedecor, 1956) for paired data was used to determine any significance of the observed differences between control and experimental animals. A probability of 0.05 or less was considered significant.

Results and Discussion

Rat controls on the viability of larvae used to inoculate the guinea pigs were well above the minimum infection for the given inoculum as set forth by Harley and Gallicchio (1971); thus, the larvae administered to the guinea pigs were infective and viable.

The data obtained are reported in Table 1. On day 20 PI, control guinea pigs had a significantly ($P < 0.01$) lower ($\overline{X} = 140$) worm burden as compared to azathioprine-treated guinea pigs ($\overline{X} = 239$). From this, it can be concluded that the guinea pig develops a marked resistance to a primary infection with *T. spiralis* which results in a "spontaneous cure." Administration of azathioprine suppressed the delayed (cellular) hypersensitivity response and thus prevented the expulsion of adult worms from the gut. This suppression of delayed hypersensitivity to specific antigens by azathioprine is in agreement with Hoyer et al. (1962) findings using mercaptopurine (the parent compound of azathioprine) in guinea pigs.

On day 30 PI, there was no significant ($P > 0.10$) difference in the number of muscle larvae recovered from azathioprine-treated vs. control guinea pigs (Table 1). Superficially, it would appear that if adults were remaining in the intestine for a longer period of time, more first-stage migratory larvae would be produced which in turn would increase the number of first-stage muscle larvae. However, as shown in this study, the above was not true. Instead [as shown by Harley and Gallicchio (1971) in the rat], once deposition of larvae begins, it is a very rapid and constant process, with major deposition occurring only over a period of 11 days. Thus, in the guinea pig, major larvae deposition had probably occurred in both azathioprine and control animals at the same time. The adults that remained in azathioprine-treated animals produced only a few more larvae as indicated by a slightly higher (not significant) number of muscle larvae (115,000 vs. 87,000).

Table 1. Effect of azathioprine on the number of adult *T. spiralis* and muscle larvae recovered from guinea pigs.

Group	Adults from intestine[1]			Encysted muscle larvae[3]		
	N	Range	Mean ± SE	N	Range	Mean ± SE
Control	10	77–240	140 ± 55[2]	10	30,000–96,000	87,000 ± 31,000[4]
Azathioprine	10	121–362	239 ± 64[2]	10	82,000–152,000	115,000 ± 28,000[4]

[1] On day 20 postinfection.
[2] Statistical significance between the means ($t = 3.84$; $P < 0.01$).
[3] On day 30 postinfection.
[4] Statistical significance between the means ($t = 1.55$; $P > 0.10$).

This present study thus provides further evidence that immunity in guinea pigs infected with *T. spiralis* is primarily due to delayed (cellular) hypersensitivity as postulated by Larsh (1967). However, Campbell et al. (1963) have shown that biogenic amines (i.e., 5-hydroxytryptamine and histamine) also play a role in the "spontaneous cure" mechanism and must be taken into consideration when evaluating hypersensitivity reactions. With this in mind, azathioprine is an antimetabolite and interferes at the enzyme level with purine metabolism. Thus, it has has no affect (either antagonistically or inhibitory) in respect to biogenic amines. With these amines still operable in azathioprine-treated guinea pigs, again the final immunological response on the *T. spiralis* adults in the intestine appears to be the final effector mechanism for parasite expulsion.

Finally, this described host–parasite model system can be a useful adjunct to evaluate the immunosuppressive action of other experimental drugs that are designed to inhibit the hypersensitivity phenomena and/or antibody formation.

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Additional Digenetic Trematodes of Birds from North Borneo (Malaysia)[1]

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ABSTRACT: Ten digenetic trematodes of birds are reported from North Borneo (Malaysia). New species described are: Diplostomatidae, *Diplostomum (Dolichorchis) sabahense*; Lecithodendriidae, *Phaneropsolus borneoensis, Pseudocryptotropa malaysiae*; Microphallidae, *Maritrema borneoense, Odhneria sabahensis*; Prosthogonimidae, *Prosthogonimus malaysiae*; Schistosomatidae, *Pseudobilharziella lonchurae*. Previously described diplostomatids reported are *Diplostomum ketupanense* Vidyarthi, 1937, and *Uvulifer denticulatus* (Rudolphi, 1819) Dubois, 1937. *Parorchis* sp. (Philophthalmidae) is described and illustrated but not allocated to species as it is based on an immature specimen; it differs from all others in the genus in having a bipartite esophagus.

The trematodes of this paper are part of a collection made by the junior author while a member of the U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan. Parasites were washed in saline, killed in hot water, and transferred immediately to FAA fixative; after 4 to 8 hours they were stored in 70% alcohol plus 2% glycerin. Staining was in Mayer's carmalum, and all were mounted in permount. Host names recorded herein are those listed by Kuntz (1969). Specimens of each trematode species reported have been deposited in the U. S. National Museum Helminthological Collection as noted. All measurements are in microns.

Family Diplostomatidae
Diplostomum (Dolichorchis) ketupanense Vidyarthi, 1937

HOST: *Ketupa ketupa ketupa* (Horsfield), owl (Strigiformes: Strigidae).

HABITAT: Small intestine.

LOCALITY: Inanam.

DATE: 28 September 1960.

SPECIMEN DEPOSITED: No. 72494.

DISCUSSION: Our single worm, lacking eggs, closely fits the characteristics of the two recorded subspecies from the same host genus from India and North Vietnam and from a ciconiiform (Ardeidae) bird from India. The chief difference appears to be the location of the ovary 140 posterior to the junction of the anterior and posterior body segments rather than at or near this junction.

Diplostomum (Dolichorchis) sabahense sp. n.
(Figs. 1, 2)

HOST: *Pelargopsis capensis javana* (Boddaert), stork-billed kingfisher (Coraciiformes: Alcedinidae).

HABITAT: Small intestine.

LOCALITY: Tuaran.

DATE: 19 September 1960.

SPECIMENS DEPOSITED: No. 72495 (holotype); No. 72496 (paratypes).

DIAGNOSIS (based on 23 slightly macerated worms from one bird; six measured): Body elongate, spinose, distinctly two-segmented, 1,030–1,135 long; anterior segment 380–415 long, widest (172–252) at level of tribocytic organ, narrowing considerably to anterior ex-

tremity, 74–110 wide just posterior to pseudo-suckers; posterior segment with sides nearly parallel, 640–755 by 152–208; length ratio of anterior to posterior segment 1:1.61–1.99. Oral sucker subterminal ventral, round or nearly so, 42–44 by 36–44; acetabulum muscular, transversely elongate, 40–50 by 50–57, lying 205–247 from anterior extremity, distance 18–23% of anterior segment length; sucker length ratio 1:0.91–1.14, width ratio 1:1.28–1.47. Pseudosuckers well developed, appearing segmented like peeled orange, usually opening laterally, overlapping level of posterior part of oral sucker and anterior part of pharynx, almost filling body width at their level, round or nearly so, longitudinal extent 34–44, transverse extent 29–44. Tribocytic organ with median slit, spined, protrusible, lying short distance postacetabular, when protruded overlapping acetabulum partially to extending preacetabular, 120–145 by 90–145. Proteolytic gland at posteriormost part of anterior segment, transversely elongate, more or less dumbbell-shaped, 27–36 by 61–98. Large gland cells in parenchyma between proteolytic gland and anterior testis. Prepharynx 5–25 long; pharynx 34–42 by 19–29; esophagus 12–31 long; ceca narrow, posterior extent not discernible.

Testes two, tandem, contiguous; anterior testis asymmetrical, usually sinistromedian, occasionally dextromedian, 135–172 by 110–148; posterior testis filling body width at its level, usually slightly concave anteromedianly, slightly more and always concave posteromedianly, shorter in longitudinal extent on side where contiguous with seminal vesicle, 145–230 by 120–177; posttesticular space 165–242 long, distance 26–33% of posterior segment length. Seminal vesicle posttesticular, dextral, sinistral or median, somewhat coiled, large. Ejaculatory duct joining uterus to form hermaphroditic duct within genital cone. Latter muscular, diagonally oriented from side opposite seminal vesicle to midline, projecting into genital atrium, protrusible, 97–196 by 55–85. Bursa copulatrix 97–145 wide, occasionally slightly constricted from remainder of posterior segment. Genital pore subterminal dorsal, surrounded by thick muscular sphincter.

Ovary smooth, median to slightly submedian, round to transversely elongate, contiguous with anterior testis, occasionally overlapping latter, 61–104 by 63–104, lying 70–132 posterior to anterior–posterior segment junction, distance 9–20% of posterior segment length. Vitellaria extending from acetabular level to near posterior extremity, absent from tribocytic organ, in separate lateral fields in anterior segment from proteolytic gland anteriorly, variably confluent medianly in posterior segment. Uterus with single ascending and descending loop, extending to anterior–posterior segment junction, descending ventral to testes on side opposite seminal vesicle. Eggs few (12 worms without eggs, remainder with 1–11), operculate, nine uncollapsed eggs measuring 78–94 (86.1) by 46–60 (53.2).

DISCUSSION: Our species differs significantly from all others in the subgenus *Dolichorchis* Dubois, 1961, in the shape of its anterior segment. In the keys given by Dubois (1970) our specimens keyed to a choice between *Diplostomum* (*Dolichorchis*) *ketupanense* Vidyarthi, 1937, from strigiform (Strigidae) birds from India, Vietnam, and North Borneo and *D.* (*D.*) *heronei* Srivastava, 1954, from a ciconiiform (Ardeidae) bird from India; some authors consider these species synonymous.

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Figures 1–17. *Diplostomum* (*Dolichorchis*) *sabahense* sp. n. Abbreviations: BW, body wall; C, cirrus; CS, cirrus sac; FP, female genital pore; GA, genital atrium; GC, gland cells; GP, genital pore; LC, Laurer's canal; M, metraterm; OD, oviduct; OV, ovary; PC, prostate cells; PP, pars prostatica; SR, seminal receptacle; SV, seminal vesicle; U, uterus; V, vitelline follicles; VD, vitelline duct. 1. Whole mount, holotype, ventral view. 2. Anterior end of body showing narrow forebody and pseudosuckers, paratype, ventral view. *Phaneropsolus borneoensis* sp. n. 3. Whole mount, holotype, ventral view. 4. Terminal genitalia, holotype. *Pseudocryptotropa malaysiae* sp. n. 5. Whole mount, holotype, ventral view. 6. Terminal genitalia, holotype. *Maritrema borneoense* sp. n. 7. Whole mount, holotype, dorsal view. 8. Terminal genitalia, paratype, ventral view. *Odhneria sabahensis* sp. n. 9. Whole mount, syntype, ventral view. 10. Terminal genitalia, syntype, ventral view (based on broken, partial specimen exposing entire terminal genitalia). *Parorchis* sp. 11. Whole mount, ventral view. *Prosthogonimus malaysiae* sp. n. 12. Whole mount, holo-

type, ventral view. 13. Terminal genitalia, holotype. *Pseudobilharziella lonchurae* sp. n. 14. Whole mount of entire female, holotype, sinistrolateral view. 15. Seminal receptacle–Laurer's canal region of female, holotype. 16. Whole mount of longest male fragment with anterior part of body complete, paratype, partial dextrolateral view. 17. Posterior end of body of male fragment showing testes and excretory pore, paratype, lateral view.
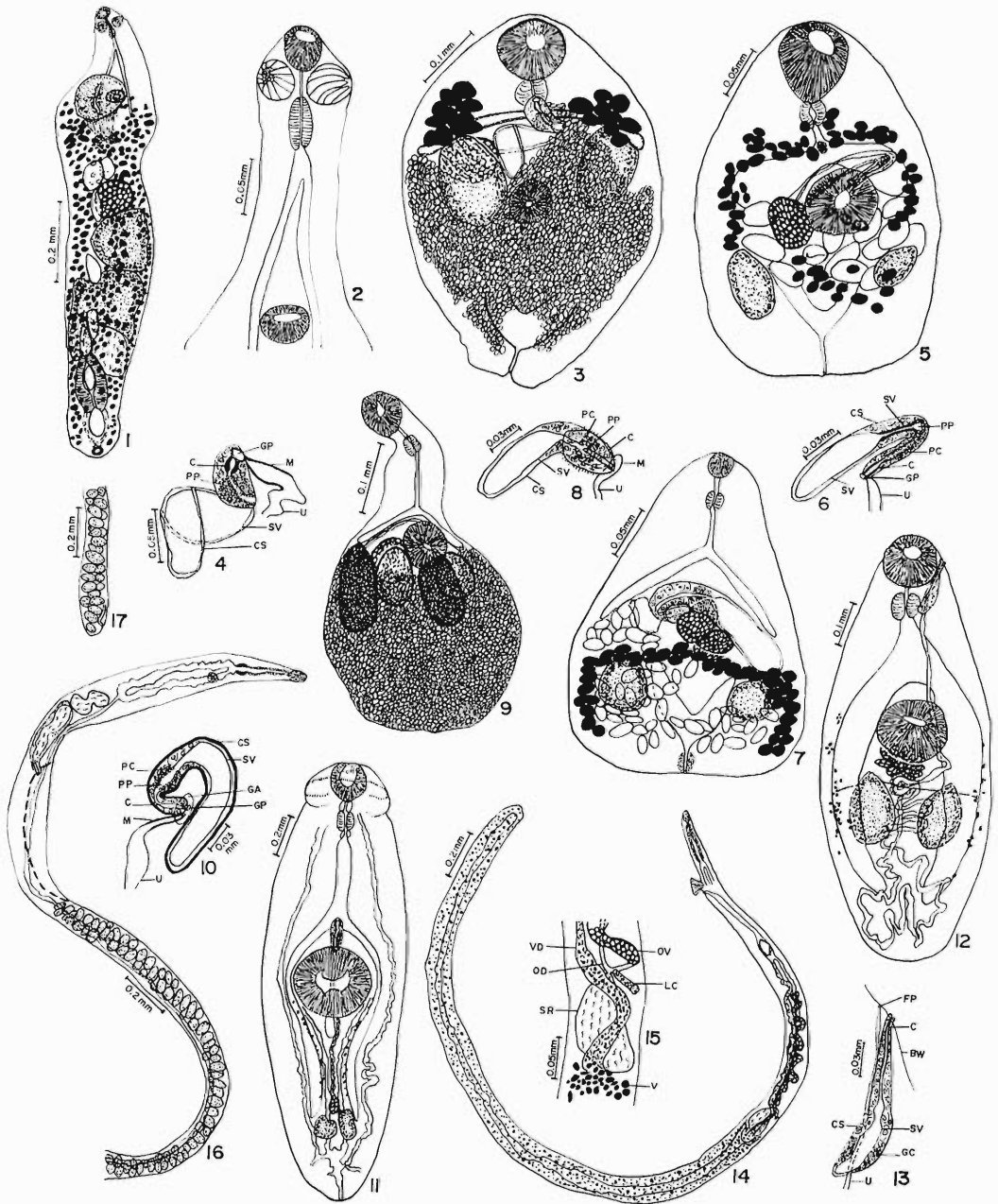
Our species differs further from the latter two species in the shape and structure of the pseudo-suckers, shape of the posterior testis, and presence of a thick muscular sphincter around the genital pore.

Uvulifer denticulatus (Rudolphi, 1819) Dubois, 1937

Host: *Alcedo meninting verreauxii* de la Berge, deep blue kingfisher (Coraciiformes: Alcedinidae).

Habitat: Small intestine.

Locality: Kasiqui.

Date: 30 August 1960.

Specimens deposited: No. 72497.

Discussion: In the keys given by Dubois (1970) our specimens keyed to this species from the same host genus from Europe and Asia (Tadjikistan, eastern Siberia, Vietnam) and are similar to it in every respect. Our collection contains 28 adult worms from one kingfisher.

Family Lecithodendriidae *Phaneropsolus borneoensis* sp. n. (Figs. 3, 4)

Hosts: Type, *Pycnonotus goiavier gourdini* Gray, yellow-vented bulbul (Passeriformes: Pycnonotidae); *Pycnonotus plumosus hutzi* Stresemann, large olive bulbul; *Hemipus hirudinaceus* (Temminck), black-winged flycatcher-shrike (Passeriformes: Campephagidae).

Habitat: Small intestine.

Localities: Kasiqui, Bukit Padang, Tuaran, Petergas.

Dates: 30 August, 2, 3, 6, 9, 10, 14, 18, 22 September 1960.

Specimens deposited: No. 72498 (holotype, from *P. goiavier*); No. 72499 (paratypes, *P. goiavier*); No. 72500 (paratypes, *P. plumosus*); No. 72501 (paratype, *H. hirudinaceus*).

Diagnosis (based on 104 adult worms from 12 *P. goiavier* harboring one to 39 specimens, three from one *P. plumosus*, and two from one *H. hirudinaceus*; 10 measured): Body elongate, oval to pyriform, entirely spined to spines absent at anteriormost and posteriormost tips of body, 402–573 long by 350–430 wide. Forebody longer than hindbody in only four worms measured, 169–255 long; hindbody 136–290 long; forebody–hindbody length ratio 1:0.64–1.47. Oral sucker subterminal ventral, some-

what wider than long, 68–100 by 73–110, with conspicuous circular muscle layer within margins, muscle layer thicker anteriorly and anterolaterally; preoral space 3–10 long. Acetabulum round or nearly so, 53–73 by 51–73. Sucker length ratio 1:0.64–0.78, width ratio 1:0.59–0.70. Prepharynx absent; pharynx round or nearly so, usually overlapping oral sucker dorsally, 32–43 by 35–50; esophagus very short; ceca short, widespread, straight, tubular, conspicuously cell-lined, extending more laterally than posteriorly, preacetabular, terminating near lateral body margins.

Testes two, symmetrical, at acetabular level, contiguous with ceca, occasionally overlapping latter, smooth, round to longitudinally or transversely elongate; right testis 80–170 by 102–170, left testis 90–163 by 117–165. Cirrus sac elongate, much winding preacetabular and intergonadally but occasionally overlapping latter slightly; seminal vesicle winding; pars prostatica distinct; cirrus short, muscular; genital pore usually slightly submedian to median, ventral to pharynx.

Ovary smooth to partly slightly wavy, round to longitudinally or transversely elongate, usually lying dorsal or anterodorsal to median part of right testis, occasionally in similar position relative to left testis, 63–116 by 86–116. Seminal receptacle large, posteromedian to ovary. Vitelline follicles large, fields lying anterolaterally anterior to ceca, occasionally overlapping latter as well as gonads, fields usually separated but median extent considerably variable from compact independent lateral masses to being confluent. Uterine coils extensive, extending to posterior extremity, laterally to body margins, with anterolateral loops reaching posterior part of testes, occasionally also lateral to testes, coiling and ascending preacetabularly on side opposite ovary and more or less between cirrus sac and vitelline field. Metraterm short, thick-walled. Eggs numerous, yellow-brown, operculate, 40 measuring 22–28 (24.7) by 13–16 (14.2).

Excretory bladder Y-shaped, stem very short, ductlike, thick-walled, arms widespread, somewhat U-shaped, much dilated, extending to posterior margin of testes, pore terminal.

Discussion: The only species of this genus from birds is *P. sigmoideus* Looss, 1899, from passeriform (Ploceidae) and caprimulgiform

(Caprimulgidae) birds from Egypt and a passeriform (Dicruridae) bird from eastern Siberia. It differs from our species in having its relatively smaller testes well separated from the ceca, the ovary median to the testis, the genital pore postpharyngeal, and smaller eggs (19 by 8).

Pseudocryptotropa malaysiae sp. n.
(Figs. 5, 6)

HOST: *Phaenicophaeus curvirostris microrhinus* Berlepsch, chestnut-breasted malcoha (Cuculiformes: Cuculidae).
HABITAT: Small intestine.
LOCALITY: Ranau.
DATE: 22 September 1960.
SPECIMEN DEPOSITED: No. 72502 (holotype).
DIAGNOSIS (based on one good and two somewhat macerated worms from one bird; all measured): Body elongate oval, anterior extremity rounded, posterior truncated, tegument entirely spined except at anterior tip of body, 343–369 long by 230–235 wide. Forebody 135–170 long, hindbody 128–155 long, forebody–hindbody length ratio 1:0.80–1.11. Oral sucker subterminal ventral, nearly round to transversely elongate, 71–80 by 76–88. Acetabulum round to slightly transversely elongate, 58–80 by 58–87. Sucker length ratio 1:0.80–1.00, width ratio 1:0.66–1.14. Prepharynx absent; pharynx 32–33 by 27–39; esophagus 17–44 long; ceca short, narrow to inflated, terminating near body margins at acetabular level.

Testes two, smooth, symmetrical, widely separated, nearly round to longitudinally elongate; right testis 67–117 by 60–83, left testis 57–105 by 60–72. Cirrus sac J-shaped, thin-walled, narrow, transversely oriented, 110 in overall length by 30 (in holotype), commencing posterior to proximal part of right cecum and dorsal to acetabulum, extending sinistrally ventral to left cecum, then curving dorsomedian short distance. Seminal vesicle bipartite; proximal part somewhat saccular, thin-walled, 80 by 28 (holotype); distal part tubular, thick-walled, muscular, 30 by 14, surrounded by few prostate cells. Prostatic vesicle extending dorsally from seminal vesicle, then dorsomedianly, latter part lying posterior to seminal vesicle, surrounded by more compact mass of prostate cells. Ejaculatory duct short,

continuing dorsomedianly. Genital atrium shallow. Genital pore opening sinistrally over anterodorsal part of acetabulum.

Ovary median to submedian (right), overlapping acetabulum, smooth, 53–65 by 48–96. Seminal receptacle large, postovarian. Vitellaria more or less in form of broad, inverted U; transverse arm continuous and just prececal, some follicles extending anteriorly to pharyngeal level; lateral arms extending posteriorly to testes; separate central mass of follicles lying postovarian, inter- and posttesticular, occasionally overlapping testes; in one worm left lateral vitellarian arm continuous with posterior central mass while right one separated. Uterus extending from postovarian anterodextrally to cecum or transverse vitellarian arm, then looping posteromedianly and more or less paralleling ascending part, finally ascending anterosinistrally to acetabular level; presence or absence of muscular uterine sphincter not ascertainable. Eggs relatively few (14, 19, 25), shell brown, thick, clear, operculate, most with collar, some with small anopercular knob, 16 measuring 31–44 (37.3) by 22–30 (25.4).

Excretory bladder Y-shaped, stem short, arms extending to intertesticular level; pore terminal.

DISCUSSION: Two species are allocated to the genus: *P. macrotestis* (Belopolskaja, 1954) Yamaguti, 1958, from a caprimulgiform (Caprimulgidae) bird from the maritime region of eastern Siberia; *P. nycticebi* (Rohde, 1962) Khotenovskii, 1965, from a lemuroid (Lorisidae) primate from Malaya. The latter species differs from ours in being a larger worm, in having a mammalian host, in the vitellaria lying in separate fields anteriorly, and in the eggs averaging larger (43 by 32) and the shell appearing granular. *P. macrotestis* differs in being a larger worm, in having a crescent-shaped cirrus sac, and extremely large testes filling the space between the acetabulum and posterior extremity of the body.

Family Microphallidae
Maritrema borneoense sp. n.
(Figs. 7, 8)

HOST: *Charadrius leschenaultii* Lesson, large sand plover (Charadriiformes: Charadriidae).
HABITAT: Small intestine.
LOCALITY: Tanjong Aru beach.

DATE: 22 September 1960.

SPECIMENS DEPOSITED: No. 72503–4 (holotype and paratypes).

DIAGNOSIS (based on 90 adult worms from one bird, 10 measured): Body elongate, oval to pyriform, posterior extremity truncated, anterior rounded, spined to testicular level, 225–424 (309) long by 172–275 (210) wide at testicular level. Forebody 97–189 long, hindbody 105–189 long, forebody–hindbody length ratio 1:0.77–1.08. Oral sucker subterminal ventral, usually transversely elongate, occasionally round to longitudinally elongate, 17–39 by 19–43. Acetabulum usually longitudinally elongate, occasionally round, 23–46 by 19–41. Sucker length ratio 1:1.15–1.52, width ratio 1:0.95–1.27. Prepharynx narrow, 10–31 long; pharynx round or nearly so, usually slightly longer than prepharynx, occasionally same length or shorter, 14–31 by 15–26; esophagus narrow, usually considerably longer than either prepharynx or pharynx, rarely shorter, 16–41 long; cecal bifurcation 28–56 preacetabular; ceca cell-lined, narrow, extending posterolaterally, terminating near body margins at acetabular level.

Testes two, smooth, symmetrical, widely separated, usually transversely elongate, occasionally round to slightly longitudinally elongate, margins or all of testes almost always obscured by eggs; right testis 36–50 by 40–62, left testis 40–50 by 42–62. Cirrus sac J-shaped, thin-walled, 90–172 in overall length by 28–53, commencing dextrally, arching anterior to acetabulum, opening into shallow genital atrium near sinistrolateral margin of acetabulum. Seminal vesicle saccular, 43–120 by 24–44. Pars prostatica tubular, straight to sinuous, long, surrounded by large, densely packed prostate cells. Cirrus thick-walled, muscular, inverted within cirrus sac or protruding from genital pore as large papillalike projection (37–62 by 31–46 in six worms) or entirely protruded as typical elongate structure (150 by 27 in one worm), covered with spines 3–7 long on about distal half.

Ovary median to submedian, overlapping acetabulum, deeply three-lobed, transversely elongate, 34–62 by 46–90. Ootype complex posterior to ovary. Vitellaria inverted U- to M-shaped; transverse arm confluent or slightly separated in midline, lying between ovary and testes, dorsal to uterus; lateral arms gradually passing posteroventrally lateral to testes, terminating at posterolateral margins of body, curving very slightly medianly or not. Uterus filling most of hindbody; dextrally with loop to proximal part of cirrus sac or ovarian level, occasionally extending more anteriorly to acetabular level or slightly more posteriorly to level of transverse vitellarian arm; sinistrally with loop to acetabular level at distal end of left cecum. Metraterm thick-walled, muscular, unspined, sinuous, much shorter than cirrus sac, sinistral to ovary and acetabulum. Eggs yellow-brown, operculate, 30 measuring 20–31 (26.1) by 13–18 (15).

Excretory bladder nearly Y-shaped, posterior portion of stem very narrow, surrounded by gland cells, remainder of stem and arms dilated, arms intertesticular and slightly overlapping testes, extending anteriorly to transverse vitellarian arm; pore terminal.

DISCUSSION: In the key to the genus given by Deblock and Combes (1965) and by Deblock (1971) our species keyed to a choice between *M. patulus* Coil, 1955, from charadriiform (Charadriidae) birds from Mexico and Puerto Rico, *M. kitanense* Shibue, 1953, obtained experimentally from albino rats fed metacercariae occurring in freshwater shrimp from Japan, and *M. urayasense* Ogata, 1951, obtained experimentally from mice fed metacercariae occurring in decapods from Japan. The latter species differs from ours in being very much larger (1,240 by 790 as a metacercaria), and in having a completely spined tegument, smaller eggs (17–18 by 10–12), and the stem of the V-shaped excretory bladder completely dilated. *M. patulus* differs from our species in having a completely spined tegument, a thin-walled metraterm, and shorter eggs (12–21 long). *M. kitanense* differs from our species in being larger (450–560 by 332–392 as a metacercaria, 550 by 362 as an adult) and entirely spined, in having the uterine loops extending more anteriorly on the side of the body containing the proximal part of the cirrus sac rather than the genital pore side, in the metraterm being spined (shown in the illustration but not so stated in the text), in the eggs averaging smaller (20–24 by 10–14), and the stem of the V-shaped excretory bladder being entirely dilated.

Odhneria sabahensis sp. n.
(Figs. 9, 10)

HOSTS: Type, *Alcedo meninting verreauxii* de la Berge, deep blue kingfisher (Coraciiformes: Alcedinidae); *Copsychus saularis* (L.), magpie robin (Passeriformes: Muscicapidae: Turdinae).

HABITAT: Small intestine.

LOCALITIES: Kasiqui, Petergas.

DATES: 31 August, 16 September 1960.

SPECIMENS DEPOSITED: No. 72505 (syntypes, from *Alcedo*); No. 72506 (syntype, *Copsychus*).

DIAGNOSIS (based on 47 and 420 adult worms from two kingfishers and one adult from a robin; measurements are composite of many worms): Body elongate, club- to wash bottle-shaped, extremities rounded, occasionally posterior extremity truncated, 495–978 long. Forebody dorsoventrally flattened, much narrower than hindbody, 200–510 by 58–85; hindbody wide, broadly rounded, often bulging ventrally when distended with eggs, 240–395 by 220–295; forebody–hindbody length ratio 1:0.77–1.39. Oral sucker subterminal ventral, round to slightly longitudinally or transversely elongate, 50–88 by 50–92. Acetabulum round or nearly so, at beginning of enlarged part of body, 50–73 by 46–65. Sucker length ratio 1:0.83–1.06, width ratio 1:0.71–1.19. Prepharynx 36–68 long; pharynx 30–41 by 21–26; esophagus longer than length of prepharynx and pharynx combined, 88–280 long; ceca narrow, extending posterolaterally to near body margins, terminating at acetabular level. Excretory bladder not observable; pore terminal.

Testes completely obscured by eggs, observed in few crushed worms (unsuitable for measurement) as two in number, smooth, symmetrical or nearly so, widely separated, posterolateral to acetabulum. Cirrus sac very long, 225–344 by 42–68, with muscular walls 3–7 thick, inverted U-shaped, nearly filling body width at its level, proximal end commencing posterolateral to acetabulum on ovarian side, passing dorsal to acetabulum, distal end terminating lateral to acetabulum on side opposite ovary but slightly more anteriorly than proximal end. Seminal vesicle saccular, thin-walled, very long, filling more than half of cirrus sac length, 145–194 by 34–53. Pars prostatica short, surrounded by prostate cells. Ejaculatory duct long, surrounded by few prostate cells. Cirrus unspined, protruding into genital atrium as muscular papilla. Genital pore posterosinistral to acetabulum.

Ovary smooth, median to lateral or posterolateral (right or left) to acetabulum, may overlap latter, overlapping anterior part of right or left testis, usually longitudinally elongate, 58–92 by 50–85. Vitelline follicles in two short lateral fields at ovariotesticular level, fields 92–218 long. Uterus completely filling hindbody and obscuring all other anatomical features. Metraterm short, muscular, sinistral or dextral, opening into genital atrium posterodorsally at base of papilliform cirrus. Eggs numerous, yellow-brown, operculate, with small opercular collar, 30 measuring 16–19 (17.7) by 10–12 (11).

DISCUSSION: In spite of the large number of specimens in our collection no single one shows a sufficiency of details to be designated as the holotype. Therefore, the above description is based on a large series of worms which have been designated as syntypes. All the worms were macerated to some degree so that tegumental spines were not present. In the keys given by Belopolskaja (1963) and by Yamaguti (1971) our species keyed to the genus *Pseudospelotrema* Yamaguti, 1939. Deblock (1971) considered the latter a synonym of *Odhneria* Travassos, 1921. In having a thick-walled, muscular cirrus sac our species differs from all others listed by Yamaguti (1971) in *Pseudospelotrema*, except *P. uriae* Yamaguti, 1939, from an alciform (Alcidae) bird from Japan and *P. ammospizae* Hunter and Vernberg, 1953, from a passeriform (Fringillidae) bird from the United States. Deblock (1971) considered *P. ammospizae* a brevivitellate species of *Maritrema* Nicoll, 1907. The latter species differs from ours in body shape, in having the prepharynx and esophagus much shorter, the cirrus sac lying in a transverse plane, a spined cirrus, and the genital pore sinistrolateral to the acetabulum. *O. uriae* differs from ours in the shape of the hindbody, in having a larger acetabulum, the crescent-shaped cirrus sac commencing anterodextral to the acetabulum and curving anterior to it, and the eggs being larger (21–24 by 12–13).

A single worm 365 long, which undoubtedly is this species, was obtained from a golden-

backed three-toed woodpecker, *Dinopium javanense borneoense* (Dubois) (Piciformes: Picidae) collected at Petergas on 13 September 1960; comparatively few eggs are present and all are abnormal, suggesting that this host is an unnatural one.

Family Philophthalmidae
Parorchis sp.
(Fig. 11)

HOST: *Calidris ruficollis* (Pallas), little stint (Charadriiformes: Scolopacidae).

HABITAT: Small intestine.

LOCALITY: Petergas.

DATE: 16 September 1960.

SPECIMEN DEPOSITED: No. 72507.

DESCRIPTION (based on one immature worm): Body elongate, 2,005 long by 665 wide at acetabular level, extremities rounded, entirely spined except for head collar, spines scalelike and more numerous on forebody; head collar 415 wide, with poorly defined ventral prominence on each side of oral sucker, bearing single row of about 72 spines measuring 11–13 by 7–10, spines similar to those on ventral part of forebody; forebody 845 long, hindbody 820 long, length ratio 1:0.97; oral sucker subterminal ventral, 195 by 198; acetabulum 340 by 355; sucker length ratio 1:1.74, width ratio 1:1.79; prepharynx 63 by 41, lumen narrow, with muscular walls 23–30 thick; pharynx 97 by 95; esophagus bipartite, anterior part 34 by 44, lumen narrow, lining continuous with that of prepharynx and pharynx, with muscular walls 14–17 thick, posterior part 350 by 85, lumen wide, with thin walls lined with same cells as ceca; cecal bifurcation 90 preacetabular; ceca narrow, extending to lateral side of testes; postcecal space 345 long; testes with slight lobing, right testis 128 by 124, left testis 165 by 95; posttesticular space 265 long; external seminal vesicle 405 by 55, commencing 87 postacetabular, proximal postacetabular part saccular, part dorsal to acetabulum tubular; cirrus sac oval, 145 by 60, overlapping anterior margin of acetabulum 20, terminating ventral to cecal bifurcation, containing tubular prostatic vesicle (72 by 17) surrounded by prostate cells in proximal half and muscular cirrus in distal half; genital pore bifurcal; ovary median, 86 by 58, smooth, contiguous with left testis and overlapping its level, lying 340 post-

acetabular; vitellaria extending along median part of ceca from ovarian to acetabular level; uterus median, sinuous, without eggs; metraterm thick-walled, muscular, dextrodorsal to cirrus sac; excretory bladder inverted cone-shaped with lateral diverticula, with two median and two lateral collecting ducts, median ducts embracing ovary; excretory pore subterminal dorsal.

DISCUSSION: This worm differs from all species in the genus in the bipartite nature of the esophagus. Inasmuch as our collection contains only one immature specimen we are not naming it as a new species, but must await additional specimens.

Family Prosthogonimidae
Prosthogonimus malaysiae sp. n.
(Figs. 12, 13)

HOST: *Phaenicophaeus curvirostris microrhinus* Berlepsch, chestnut-breasted malcoha (Cuculiformes: Cuculidae).

HABITAT: Small intestine.

LOCALITY: Ranau.

DATE: 22 September 1960.

SPECIMEN DEPOSITED: No. 72508 (holotype).

DIAGNOSIS (based on one adult worm): Body elongate, oval, narrower anteriorly, extremities rounded, spined, 982 long by 400 wide at ovarian level. Forebody 390 long, hindbody 425 long, forebody–hindbody length ratio 1:1.1. Oral sucker subterminal ventral, 124 by 108; acetabulum nearly equatorial, 167 by 172; sucker length ratio 1:1.35, width ratio 1:1.59. Prepharynx distinct, 17 long; pharynx 77 by 80; esophagus short, wide, 70 by 64; cecal bifurcation 75 preacetabular; ceca wide, extending posttesticularly to within 120 of posterior extremity. Excretory bladder Y-shaped, pore terminal.

Testes two, smooth, oval, symmetrical, overlapping ceca dorsally; right testis 172 by 98, overlapping level of acetabulum 7; left testis 158 by 109, lying 22 postacetabular; posttesticular space 255 long. Cirrus sac elongate, narrow, 145 by 30 at proximal end, commencing sinistral to pharynx, overlapping latter ventrally. Seminal vesicle tubular, sinuous. Cirrus elongate, protruded slightly through genital pore. Latter sinistral to oral sucker, 45 from anterior extremity.

Ovary somewhat round, 122 by 122, con-

sisting of 14 closely clustered lobes, median, overlapping acetabular dorsum (70) and testicular level. Seminal receptacle posterodextral to ovary, 56 by 41. Mehlis' gland well developed, median, postovarian. Vitelline follicles few, small, right field extending from 55 posterior to anterior margin of acetabulum to 31 posterior to right testis, left field extending from 80 posterior to anterior margin of acetabulum to 45 posterior to posterior margin of left testis; transverse vitelline ducts crossing anterior part of testes dorsally, uniting ventral to Mehlis' gland at posterior margin of ovary. Uterus coiled mostly inter- and posttesticular, undulating anteriorly from left side of ovary, passing ventral to proximal end of cirrus sac, lying between latter and oral sucker. Female genital pore anterior to male genital pore, 34 from anterior extremity. Eggs relatively few, shape variable, shell yellowish, operculate, 10 measuring 19–27 (22.4) by 13–16 (13.9), usually with small anopercular knob or very short spinelike projection as for *Prosthogonimus macrorochis* Macy, 1934.

DISCUSSION: Our specimen is a young adult as relatively few eggs are present. The tegument is somewhat macerated and although spines were observed their distribution was not ascertainable. *P. malaysiae* sp. n. differs from all others in the genus in having the cirrus sac commencing far anteriorly at the level of the pharynx. In the key to the species of the genus (modified from Jaiswal) given by Skrjabin (1961) our worm, based on the sucker length ratio, keyed to *P. pellucidus* (von Linstow, 1873) Lühe, 1899, and, based on the sucker width ratio, to *P. dollfusi* Jaiswal, 1957. The latter species differs further from ours in being a considerably larger worm, in lacking a prepharynx, and in having a sucker ratio of about 1:2.5. *P. pellucidus* differs further from our species in being considerably larger even as a young adult, and the eggs averaging longer (length range 26–33). Species described since Skrjabin (1961) are not closely related to ours.

Family Schistosomatidae
Pseudobilharziella lonchurae sp. n.
(Figs. 14–17)

HOST: *Lonchura fuscans* (Cassin), dusky munia (Passeriformes: Ploceidae).

HABITAT: Mesenteric veins.

LOCALITY: Ranau.

DATE: 15 September 1960.

SPECIMENS DEPOSITED: No. 72509 (holotype, complete female); No. 72510 (paratypes, one female and several male fragments).

DIAGNOSIS (based on fragments of five and 17 mature males from two hosts, eight measured; and one complete mature female and one mature fragment from host with five males, both measured): Body filamentous, flattened, extremities rounded, posterior end of body same width as more anteriorly and not expanded fanlike, tegument spined. Oral sucker terminal, opening ventrally, longer than wide. Acetabulum usually larger than oral sucker, stalked, appearing mushroom-shaped, only one of five worms showing slight cup, spined, longer than wide. Esophagus bipartite; long anterior part wide, with thick layer of gland cells externally, usually most expanded posteriorly; short posterior part in form of thick-walled, muscular bulb. Cecal bifurcation preacetabular; cecal union at different levels in male and female; common cecum undulating, terminating near posterior extremity. Excretory pore subterminal ventral.

MALE: Forebody 307–425 long by 87–120 wide at cecal bifurcation. Hindbody of unknown length, 102–180 wide at anteriormost margin of external seminal vesicle, 85–190 wide at gynaecophoric canal, 85–130 wide in testicular region. Oral sucker 75–86 long by 61–76 deep (in three worms); acetabulum 44–110 long by 39–75 deep (in seven); sucker length ratio 1:1.18–1.38 (in three with oral sucker), depth ratio 1:0.81–1.23. Esophagus 206–232 long; glandular anterior part 143–167 by 44–48, muscular part 53–65 by 24–51. Cecal bifurcation 77–120 preacetabular; separated ceca extending posteriorly 375–540; cecal union 8–172 anterior to external seminal vesicle in seven worms, union overlapping latter extracecally by 24 in one. Testes usually in two compact rows but occasionally some in single compact row in same worm, numerous, usually transversely elongate, occasionally round, 36–75 by 41–106. Vas deferens undulating, union with external seminal vesicle not observed. External seminal vesicle saclike, undulating, widest anteriorly, 123–208 by 56–102. Cirrus sac oval, thin-walled, widest anteriorly, 232–317 by 85–90, contiguous with external semi-

nal vesicle. Internal seminal vesicle saclike, undulating, widest anteriorly, 155–241 by 60–78. Pars prostatica thick-walled, muscular, bulbous anteriorly, 56–75 by 18–34, surrounded by few prostate cells. Ejaculatory duct thin-walled, partly within cirrus sac, partly within muscular genital papilla, occasionally short part in parenchyma between cirrus sac and genital papilla. Latter projecting into gynaecophoric canal near anteriormost margin. Gynaecophoric canal very weakly muscular throughout length, appearing nonmuscular, spined, 565–640 long. Excretory pore 34 from posterior extremity in one small fragment.

FEMALE: Holotype (mounted in sinistrolateral view so that all measurements are length by depth). Body 4,060 long. Forebody 275 long, narrowing gradually from acetabulum anteriorly, 275 deep at cecal bifurcation. Hindbody 3,725 long, 80–100 deep. Forebody–hindbody length ratio 1:13.2. Oral sucker 51 by 44; acetabulum 60 by 51; sucker length ratio 1:1.20, width ratio 1:1.16. Esophagus 198 long; glandular anterior part 160 by 35, muscular part 46 by 24. Cecal bifurcation 45 preacetabular; separated ceca extending posteriorly 1,270; cecal union 68 posterior to seminal receptacle. Ovary considerably coiled, longitudinal extent 437 by 25–44, anteriormost extent 605 postacetabular. Oviduct emerging from posteriormost end of ovary, passing ventrally and receiving seminal receptacle and Laurer's canal, then extending anteriorly in undulating manner. Seminal receptacle oval, 125 by 61, lying between ovary and anteriormost margin of vitellaria. Laurer's canal thick-walled, muscular, arising near junction of oviduct and seminal receptacle, passing to dorsal surface. Vitellaria follicular, filling space around cecal union and common cecum between posterior margin of seminal receptacle and posterior extremity. Common vitelline duct passing anteriorly with much undulation, uniting with oviduct 170 preovarian. Distance from latter union to genital pore 387. Ootype short distance anterior to union, containing one egg. Uterus thick-walled, muscular, undulating slightly, commencing immediately anterior to ootype, 286 long. Uterine pore ventral, median, just postacetabular. Egg oval, thin-shelled, somewhat collapsed, 85 by 40,

bearing very small spine at posterior end. Excretory pore 15 from posterior extremity.

FEMALE: Paratype (sinistrolaterally mounted; anterior part from just postacetabular missing, also posterior part within vitellarian field). Similar to comparable part of holotype. Hindbody 75–100 deep; cecal union 44 posterior to seminal receptacle; ovary 415 by 26–42; seminal receptacle 112 by 52; oviduct–common vitelline duct union 143 preovarian; single collapsed egg 78 by 31, bearing spine.

DISCUSSION: This species keyed to the subfamily Bilharziellinae and genus *Pseudobilharziella* Ejsmont, 1929, in the keys to the Schistosomatidae of birds given by Yamaguti (1971). Our species is closest to *P. brantae* (Farr and Blankemeyer, 1956) Yamaguti, 1971, and *P. corvi* Yamaguti, 1941 (both sexes known for both species). They have the testes in more than one row, and the eggs are oval with a small spine at the posterior end. All species in the genus, except *P. brantae*, lack the bipartite esophagus. *P. brantae* differs from our species in having the posterior end of the body blunt and fanlike, the gynaecophoric canal consisting of a relatively nonmuscular anterior part and a muscular posterior part rather than all appearing nonmuscular, the pars prostatica and ejaculatory duct only enclosed in the cirrus sac, and Laurer's canal coming off the oviduct some distance from where the seminal receptacle opens rather than near the latter. *P. corvi* differs further from our species in having the tegument and acetabulum unspined, and a gynaecophoric canal with well-developed circular muscles. In having the tegument unspined, a short gynaecophoric canal, and the testes in a single row, *P. burnetti* Brackett, 1942 (male only known), *P. horiconensis* Brackett, 1942 (male only known), *P. kegonsensis* Brackett, 1942 (male only known), and *P. waubesensis* Brackett, 1942 (both sexes known) differ further from our species. *P. kowalewskii* Ejsmont, 1929 (male only known) and all but *P. burnetti* of Brackett's species have the external seminal vesicle commencing close to the acetabulum. *P. kowalewskii*, *P. horiconensis*, and *P. waubesensis* differ further in having some or all of the testes scattered with spaces between them rather than in compact rows. In *P. horiconensis* and *P. kegonsensis* the posterior end of the body is blunt and fan-

like. The egg of *P. waubesensis* is pointed at both ends.

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Halocercus monoceris sp. n. (Nematoda: Metastrongyloidea) from the Narwhal, *Monodon monoceros*

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ABSTRACT: *Halocercus monoceris* sp. n. (Nematoda: Metastrongyloidea) obtained from the lungs of two narwhals, *Monodon monoceros* L., caught in the eastern Arctic is described and figured. Comparison is made with other members of the genus.

During the summer of 1970 Dr. M. Newman, Director of the Vancouver Public Aquarium, captured six specimens of the narwhal, *Monodon monoceros* L. One was obtained from Grise Fiord on the south coast of Ellesmere Island, N.W.T., and five from the area of Milne Inlet on the northeast coast of Baffin Island, N.W.T. All narwhals were successfully transferred to Vancouver, but within the next 4 months became ill and died.

Numerous specimens of a nematode of the genus *Halocercus* Baylis and Daubney, 1925, were recovered from the bronchioles of two of these animals. Comparison with other members of the genus show that they represent a heretofore undescribed species.

All specimens, removed from formalinized lung tissue, were cleared and examined in phenol-alcohol. Diagrams were made with the aid of a Zeiss drawing tube. Unless otherwise stated, measurements are in microns.

Halocercus monoceris sp. n.
(Figs. 1–5)

Pseudaliidae Railliet, 1916; Halocercinae Delyamure, 1952; *Halocercus* Baylis and Daub-

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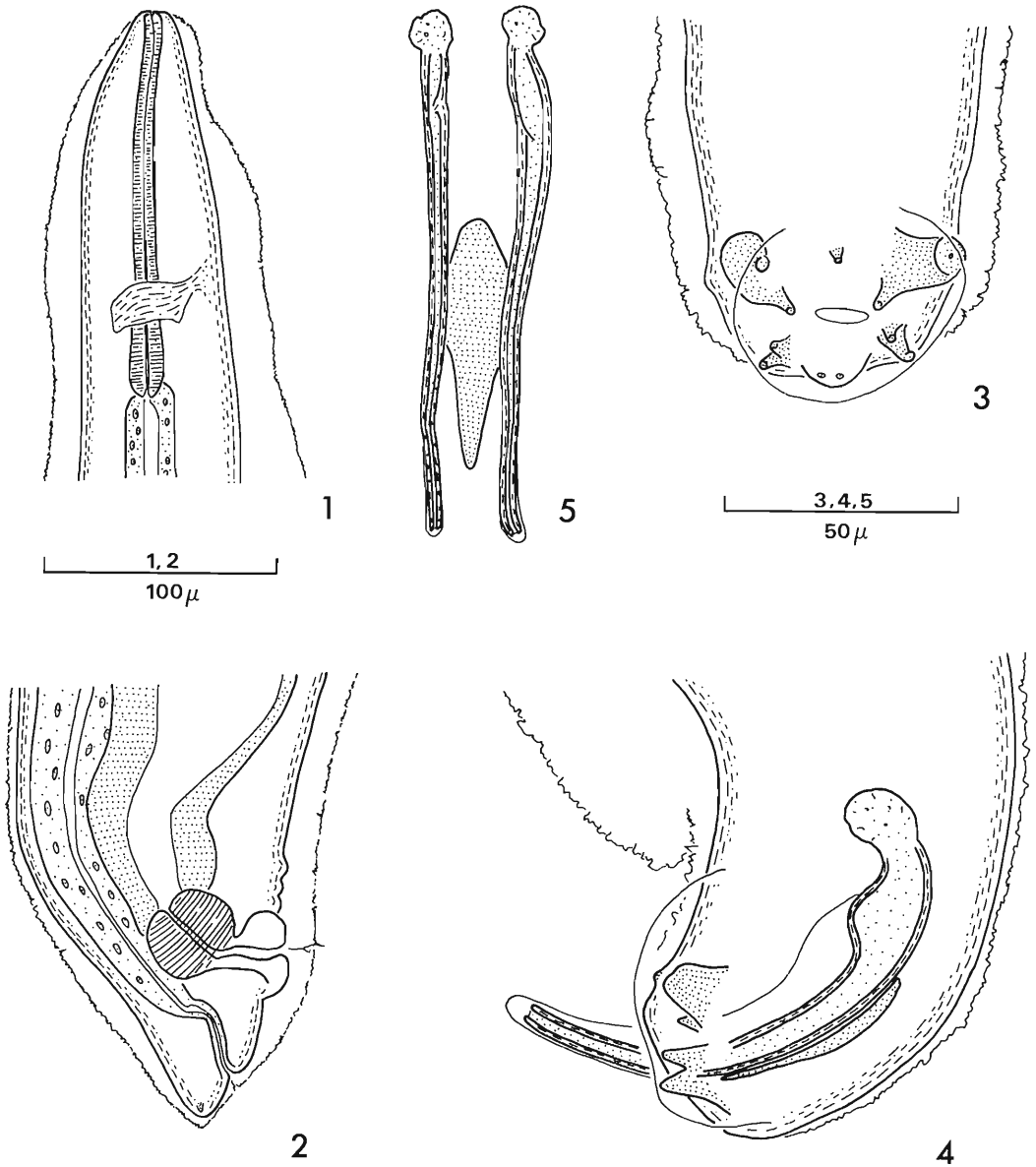
Figures 1–5. *Halocercus monoceris* sp. n. 1, Anterior portion, female. 2, Posterior portion, female. 3, Posterior portion, male, ventral view. 4, Posterior portion, male, lateral view. 5, Spicules and gubernaculum, ventral view.

ney, 1925. Delicate, filiform nematodes. Buccal cavity absent. Lips absent. Cephalic papillae very difficult to discern. Cuticle without alae or striations. Teguminal sheath present.

MALE (based on two complete specimens and numerous fragments): Length 7.5 and 8.2 mm; maximum width excluding teguminal sheath 115–150. Esophagus simple, 155–165
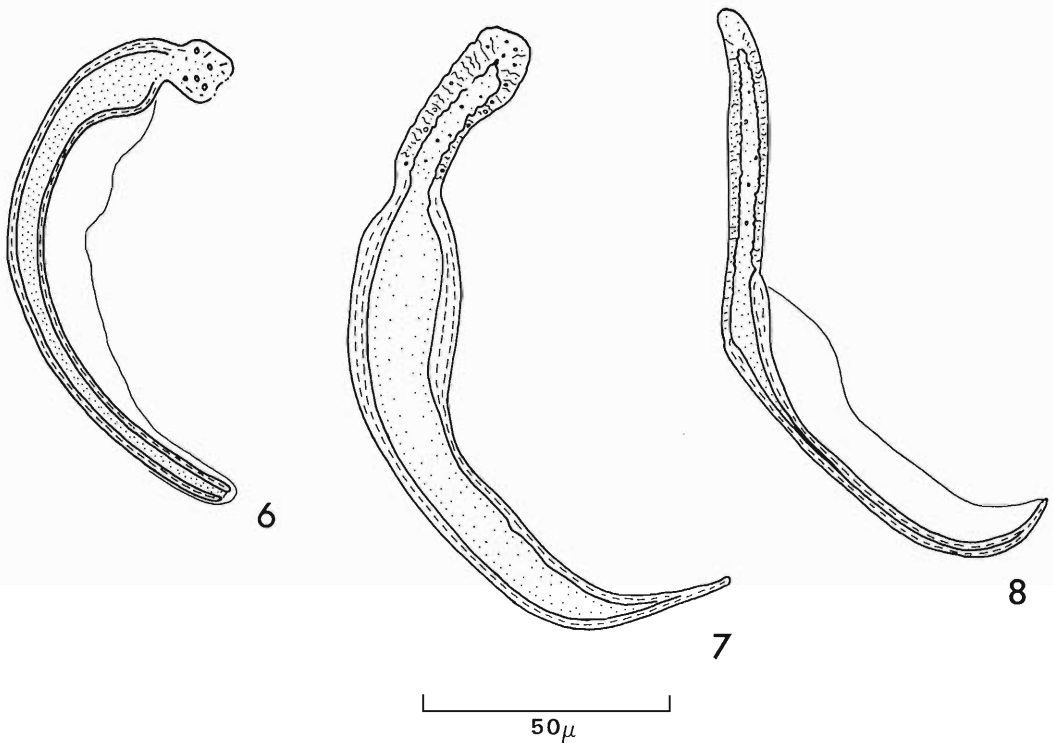
Figures 6–8. Spicules of some *Halocercus* spp. 6, *H. monoceris* sp. n. 7, *H. invaginatus* (USNM Helm. Coll. No. 36876). 8, *H. kirbyi* (USNM Helm. Coll. No. 36867).

long. Nerve ring 70–75 from the anterior extremity. Tail curled ventrally. Bursa distinct but small. Bursal rays reduced to little more than papillae. Ventral rays represented by one large papilla and a smaller pedunculate papilla both on a bulbous base. Lateral rays represented by two small pedunculate papillae. Dorsal papillae double, very small. A single median, stalked, preanal papilla present. Spicules short, 115–135 long, broadly curved with large membranous ventral alae. Gubernaculum 45–60 long, well chitinized.

FEMALE (based on five complete specimens and numerous fragments): Length 11–15 mm; maximum width excluding teguminal sheath 120–150. Esophagus 140–165 long. Nerve ring 70–85 from the anterior extremity. Tail conical, phasmids prominent. Anus 25–45 from the posterior extremity. Vulva salient, 70–100 from the posterior extremity, bounded usually both anteriorly and posteriorly by well-developed labia. Vagina vera short, 35–45 long; muscular ejector short, 25–45 long; uteri double. Larvae (in utero) approximately 320 long and 15 wide; tail simple, without spine.

HOST: *Monodon monoceros* L.—narwhal.

HABITAT: Bronchioles.

LOCALITY: Vancouver Public Aquarium.

SPECIMENS: Holotype male, allotype female USNM Helm. Coll. 72129. Paratypes ADRI Parasite Coll. 912.

Discussion

There are, to date, 11 species of the genus *Halocercus* Baylis and Daubney, 1925, all parasitic in the lungs of cetaceans. Of the various species described, those characters necessary for species determination are all found associated with the posterior region of the male.

H. brasiliensis Almeida, 1933; *H. dalli* Yamaguti, 1951; *H. delphini* Baylis and Daubney,

1925; *H. kleinenbergi* Delyamure, 1951; *H. lagenorhynchi* Baylis and Daubney, 1925; and *H. pingi* Wu, 1929, are all much larger species with spicules greater than 500 in length (see Delyamure, 1955; Yamaguti, 1951).

Of those species with spicules less than 250 in length, *H. monoceris* sp. n. is distinguished from *H. ponticus* Delyamure, 1946, and *H. taurica* Delyamure in Skrjabin, 1942, by the lack of three well-defined lateral papillae, the presence of two ventral papillae, and by the morphology and size of the spicules; the spicules of the latter species without a knob-like capitulum and considerably longer.

H. sunameri Yamaguti, 1951, has spicules approximately twice the length of those of *H. monoceris*. There is only a single papillar termination representing the ventral ray. Yamaguti (1951) considered the ventral ray as one of the laterals; ". . . rudimentary bursa supported by two pairs of lateral rays and an unpaired posterior ray. The anterior lateral ray lying on a level with the cloacal aperture has a broad base and a minute papillary termination, whereas the posterior lateral ray . . . has a double termination." It is evident from the diagrams that Yamaguti erred in his interpretation and that the "anterior lateral" with the broad base and minute papillary termination is in reality the ventral ray.

The spicules of *H. invaginatus* (Quekett, 1841) Dougherty, 1943, are slightly longer than those of *H. monoceris* and have a well-defined elongate capitulum while the spicules of *H. kirbyi* Dougherty, 1944, although of similar size as those of *H. monoceris*, have an elongate capitulum and a conspicuous alar expansion in the middle of each spicule (Figs.

6–8). Both *H. invaginatus* and *H. kirbyi* have ventral rays with a single termination and lateral rays with three papillary terminations.

The presence or absence of cuticular ridges is usually considered to be a character at the generic level. Migaki et al. (1971) present a figure in which they note cuticular ridges and spines. Examination of cross sections of *H. monoceris* has failed to reveal any indications of longitudinal cuticular ridges.

Acknowledgments

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Interaction of Gastrointestinal Nematodes Established in Calves by Two Spaced Inoculations

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ABSTRACT: In some cases a primary infection, established in calves by a single inoculation with about 150,000 gastrointestinal nematode larvae, interfered to some extent with the establishment or development of larvae of a second inoculation with about 150,000 larvae given 10 to 123 days later. Apparently, the primary infection was not affected by the second inoculation.

This study was one in a series dealing with internal population dynamics of gastrointestinal nematodes of cattle (Goldberg, 1973). The studies are intended to define the conditions of exposure to larvae which result in serious worm burdens so that they may be avoided or properly counteracted. There have been few studies on the relationship of two spaced inoculations to worm burden in ruminants. These have concerned infections with a single species (Roberts et al., 1962; Ross, 1963; Ross and Dow, 1964; Sinha, 1967) or two species (Ross et al., 1968). The purpose of this study was to determine if a primary infection with one to four species established by a single inoculation would affect the establishment and development of larvae of a second inoculation with two to four species, and, conversely, if the latter would affect the primary population.

Materials and Methods

Thirty Holstein steers, raised worm-free, and maintained in individual concrete stalls under conditions preventing extraneous infection, were used. Principals and controls averaged 5.4 months of age at the time of initial inoculation.

The infective larvae were obtained from sphagnum-cultured feces of calves artificially infected with one or more species of gastrointestinal nematodes, namely *Ostertagia ostertagi, Trichostrongylus axei, T. colubriformis, Cooperia oncophora,* and *C. punctata.* The number of larvae in a batch was determined by dilution counts in triplicate. The larvae were administered in a single dose to the experimental calves in about 10 ml of water.

In a series of four trials (Table 1), eight calves were simultaneously inoculated with 150,000 larvae of four species from the same thoroughly agitated batch of larvae. Four of them were reinfected with 165,000 larvae and four were kept as initial infection controls. The intervals between the first and second inoculations were 10, 20, 32, or 46 days. The composition of the inoculum is given in Table 1.

In three additional trials (Table 2) six calves were inoculated with 150,000 or 165,000 larvae of two or four species. Three were reinfected with 150,000 or 165,000 larvae of two or three species and three were kept as initial infection controls. The intervals between the first and second inoculations were 35, 84, or 123 days. The composition of the inocula is given in Table 2.

Finally, in four trials (Table 3), six calves were inoculated with 76,000 to 178,000 larvae of a single species. Four were reinfected with 150,000 larvae of two or four species 30 to 36 days later and two were kept as initial infection controls. The composition of the inocula is given in Table 3.

In all trials, whenever a calf was reinfected, a worm-free calf was similarly and simultaneously inoculated to act as a reinfection control.

As an indication of the effect of a second inoculation on a primary population of worms, and for correlation of egg output with worm burden, differential egg counts were made on fecal samples collected weekly from each calf. The eggs were recovered by centrifugal flotation in saturated NaCl solution, and the number in 1 g of feces/sample was counted, or the number in ⅓ to ½ g when the number of eggs/g (EPG) exceeded 1,000.

In all trials, 13 to 16 days after each calf was reinfected it and its reinfection and initial

Table 1. Establishment and development of gastrointestinal nematodes resulting from two spaced inoculations: Data from first series of trials.[1]

	Initial infection control	Rein-fected calf	Rein-fection control	Initial infection control	Rein-fected calf	Rein-fection control
Calf number	247	239	240	248	238	243
No. days 1st to 2nd dose	—	10	—	—	20	—
No. days 1st dose to necropsy	27	26	16	34	33	13
No. days 2nd dose to necropsy	—	16	—	—	13	—
Ostertagia ostertagi						
Total recovered	5,591	9,623	5,385	5,204	6,855	3,756
% mature adults	100	59.3	0	99.8	86.0	0
% immature 5th stage	0	39.6	98.1	0.2	5.6	66.5
% 4th stage	0	1.1	1.9	0	8.4	33.5
Trichostrongylus axei						
Total recovered	657	427	130	1,116	1,266	123
% mature adults	89.2	94.6	0	94.4	85.2	0
% immature 5th stage	10.8	5.4	100	5.6	14.8	51.2
% 4th stage	0	0	0	0	0	48.8
Cooperia punctata						
Total recovered	38,200	67,400	29,800	41,375	43,000	19,200
% mature adults	100	98.2	22.1	100	89.8	51.0
% immature 5th stage	0	1.8	77.9	0	10.2	49.0
Cooperia oncophora						
Total recovered	33,600	56,000	31,400	10,250	32,400	35,600
% mature adults	100	67.5	0	97.6	97.5	0
% immature 5th stage	0	32.5	94.3	2.4	0	98.9
% developing 4th stage	0	0	5.7	0	2.5	1.1
Cooperia larvae						
Total recovered[2]	800	3,200	4,000	750	9,200	4,200
Peak egg count (EPG)[3]	—	—		2,544	1,832	
No. days 1st dose to peak	—	—		19	28	
Average egg count	2,418	2,235		1,604	1,543	
Count at necropsy	2,952	3,144		404	1,328	
Calf number	246	244	250	249	245	251
No. days 1st to 2nd dose	—	32	—	—	46	—
No. days 1st dose to necropsy	48	47	15	61	62	16
No. days 2nd dose to necropsy	—	15	—	—	16	—
Ostertagia ostertagi						
Total recovered	5,781	5,016	2,536	6,866	4,892	626
% mature adults	99.9	100	0	100	96.6	0
% immature 5th stage	0.1	0	99.7	0	3.1	85.9
% 4th stage	0	0	0.3	0	0.3	14.1
Trichostrongylus axei						
Total recovered	1,666	916	300	1,861	1,073	0
% mature adults	91.0	100	0	100	86.0	0
% immature 5th stage	9.0	0	100	0	14.0	0
% 4th stage	0	0	0	0	0	0
Cooperia punctata						
Total recovered	14,251	32,600	7,600	56,351	9,100	1,600
% mature adults	100	100	97.4	100	100	93.8
% immature 5th stage	0	0	2.6	0	0	6.2
Cooperia oncophora						
Total recovered	125	1,400	21,800	46,813	6,600	17,100
% mature adults	100	85.7	0	100	9.1	0.6
% immature 5th stage	0	0	99.1	0	90.9	98.8
% developing 4th stage	0	14.3	0.9	0	0	0.6
Cooperia larvae						
Total recovered[2]	125	9,000	3,200	438	4,600	2,200
Peak egg count (EPG)[3]	648	2,212		3,476	502	
No. days 1st dose to peak	19	19		35	19	
Average egg count	343	611		2,142	272	
Count at necropsy	423	99		1,172	309	

[1] First inoculation, 150,000 larvae of four species; second inoculation, 165,000 larvae from the same batch 10, 20, 32, or 46 days later. Composition of inocula: *O. ostertagi* 26%, *T. axei* 31%, and *Cooperia* spp. 43%.
[2] All inhibited fourth stage.
[3] Apparently, peak was not yet attained in calves 247 and 239; final count was highest.

Table 2. Establishment and development of gastrointestinal nematodes in calves resulting from two spaced inoculations: Data from second group of trials.[1]

	Initial infection control	Rein- fected calf	Rein- fection control	Initial infection control	Rein- fected calf	Rein- fection control	Initial infection control	Rein- fected calf	Rein- fection control
Calf number	296	295	297	282	281	293	294	298	307
No. days 1st to 2nd dose	—	35	—	—	84	—	—	123	—
No. days 1st dose to necropsy	50	49	14	99	98	14	138	137	14
No. days 2nd dose to necropsy	—	14	—	—	14	—	—	14	—
Ostertagia ostertagi									
Total recovered	0	6,750	11,800	808	15,152	30,251	2,935	3,995	1,860
% mature adults	0	0	0	97.5	2.9	0	100	77.5	0
% immature 5th stage	0	68.5	77.5	1.2	51.7	96.4	0	0	66.4
% 4th stage	0	31.5	22.5	1.2	45.4	3.6	0	22.5	33.6
Trichostrongylus axei									
Total recovered	0	0	0	353	351	0	10	170	0
% mature adults	0	0	0	100	100	0	100	100	0
% immature 5th stage	0	0	0	0	0	0	0	0	0
% 4th stage	0	0	0	0	0	0	0	0	0
Cooperia punctata									
Total recovered	0	75	1,875	900	150	0	0	0	0
% mature adults	0	0	0	100	100	0	0	0	0
% immature 5th stage	0	100	100	0	0	0	0	0	0
Cooperia oncophora									
Total recovered	375	8,775	83,625	4,425	8,100	7,650	3,500	400	15,838
% mature adults	100	5.1	0	98.3	8.3	0	92.8	100	0
% immature 5th stage	0	0	99.1	1.7	89.8	99.0	3.6	0	87.8
% developing 4th stage	0	94.9	0.9	0	1.9	1.0	3.6	0	12.2
Cooperia larvae									
Total recovered[2]	0	44,325	1,875	375	600	75	0	200	613
Trichostrongylus colubriformis									
Total recovered	0[3]	450[4]	0	0	0	0	0	0	0
Peak egg count (EPG)	55	85		511[5]	745		1,244	257	
No. days 1st dose to peak	23	23		27	50		28	87	
Average egg count	20	39		360	244		260	113	
Count at necropsy	13	4		500	130		179	16	

[1] First inoculation, 150,000 or 160,00 larvae of two or four species; second inoculation, 150,000 or 165,000 larvae of two or three species 35, 84, or 123 days later. Composition of inocula: Calves 296, 295 (1st)—*T. colubriformis* 99%, *C. oncophora* 1%; 295 (2nd), 297—*O. ostertagi* 40%, *Cooperia* spp. 60%; 282, 281 (1st)—*O. ostertagi* 48%, *T. axei* 6%, *Cooperia* spp. 46%; 281 (2nd), 293, 294, 298 (1st)—*O. ostertagi* 72%, *T. axei* 4%, *C. oncophora* 24%; 298 (2nd), 307—*O. ostertagi* 36%, *C. oncophora* 64%.
[2] All inhibited fourth stage.
[3] *Trichostrongylus* eggs had been present in the feces.
[4] All mature adults.
[5] Second peak of 500 EPG on day 99.

infection controls were necropsied. This interval between reinfection and necropsy was selected to facilitate distinguishing the first from the second population of worms since, except for *C. punctata* and very few *C. oncophora*, the species used in this study do not become fertile in that period.

Each gastrointestinal organ was examined separately for worms. Duplicate 0.6 to 1.0% aliquots were taken from its combined contents and washings. When the number of worms was great, smaller aliquots were examined. Nevertheless, in such cases usually 100 to 200 worms were present per sample. The abomasum was digested after thorough washing, and duplicate 5% aliquots were examined for worms.

Nongravid fifth-stage females not obviously senescent and fifth-stage males with spicules not yet darkened were counted as immature fifth-stage worms. Fourth-stage larvae which remained in the newly molted condition, despite elapse of sufficient time for growth, were counted as inhibited fourth-stage larvae.

Results

In initial infections with two to four species, generally fewer *O. ostertagi*, *C. oncophora*, and *C. punctata* larvae of the second inoculation became established in reinfected calves than in reinfection controls (Tables 1, 2). This decrease was evident even in calf 239, reinfected only 10 days after the primary in-

Table 3. Establishment and development of gastrointestinal nematodes in calves resulting from 2 spaced inoculations: Data from 3rd group of trials.[1]

	Initial infection control	Rein-fected calf	Rein-fection control	Initial infection control	Rein-fected calf	Rein fection control	Rein-fected calf	Rein-fected calf	Rein-fection control
Calf number	285	284	288	287	286	289	338	339	337
Species in 1st dose	*O. ostertagi*		—	*T. axei*		—	*T. axei*		—
No. larvae in 1st dose	165,000	165,000	—	165,000	165,000	—	76,000	178,000	—
No. days 1st to 2nd dose	—	36	—	—	36	—	33	30	—
No. days 1st dose to necropsy	49	50	14	54	50	14	47	45	14
No. days 2nd dose to necropsy	—	14	—	—	14	—	14	15	—
Ostertagia ostertagi									
Total recovered	11,590	50,625	27,990	0	14,350	44,989	905	1,720	1,725
% mature adults	98.0	56.3	0.6	0	0	0	0	0	0
% immature 5th stage	0.6	16.0	92.3	0	51.0	94.5	87.3	98.3	86.7
% late 4th stage	0.1	20.9	6.7	0	47.0	5.5	12.7	1.7	13.3
% inhibited 4th stage	1.3	6.7	0.3	0	1.9	0	0	0	0
Trichostrongylus axei									
Total recovered	0	0	0	63,490	84,350	175[2]	22,425	51,240	0
% mature adults	0	0	0	99.7	99.6	100	100	100	0
% immature 5th stage	0	0	0	0.3	0	0	0	0	0
% 4th stage	0	0	0	0	0.4	0	0	0	0
Cooperia punctata									
Total recovered	0	225	150	0	0	375	0	0	0
% mature adults	0	33.3	100	0	0	20.0	0	0	0
% immature 5th stage	0	66.7	0	0	0	80.0	0	0	0
Cooperia oncophora									
Total recovered	0	6,825	6,600	0	375	9,525	12,100	12,500	16,275
% mature adults	0	0	0	0	0	0	0	0	0
% immature 5th stage	0	98.9	98.9	0	20.0	100	98.3	98.4	98.1
% developing 4th stage	0	1.1	1.1	0	80.0	0	1.7	1.6	1.9
Cooperia larvae									
Total recovered[3]	0	2,025	225	0	8,175	600	2,300	3,600	2,730
Peak egg count	225	—[4]		768	441		134	264	
No. days 1st dose to peak	29	—		35	50		32	37	
Average egg count	111	249		611	338		99	169	
Count at necropsy	179	421		569	441		62	160	

[1] First inoculation, 76,000 to 178,000 larvae of one species; second inoculation, 150,000 larvae of two or four species 30 to 36 days later. Composition of inocula: Calves 284 (2nd), 288, 286 (2nd), 289—*O. ostertagi* 72%, *T. axei* 4%, *Cooperia* spp. 24%; 338 (2nd), 339 (2nd), 337—*O. ostertagi* 31%, *C. oncophora* 69%.
[2] Possibly these had just become mature; eggs were not recovered from the feces.
[3] All inhibited fourth stage.
[4] Apparently, peak was not yet attained; final count was highest.

fection. However, except for *C. oncophora*, total worm burden of each species in a reinfected calf was usually greater than that in its reinfection control. Frequently, development of many *Cooperia* of the second inoculation was arrested in early fourth stage in reinfected calves and reinfection controls, but generally fewer in the latter. In calves 238 and 281, development of the second *O. ostertagi* population was delayed. *T. axei* was absent from the second inoculation or present in numbers to small for a valid comparison.

The egg counts and numbers of worms recovered at necropsy evidenced individual difference in susceptibility to infection with *C. oncophora*. Of four calves simultaneously administered 150,000 larvae from the same thoroughly agitated batch (Table 1, calves 246 to 249), the number of worms of this species recovered 1 to 2 months later ranged from 125 to 46,813 and the peak EPG from 648 to 3,476. The calf (246) apparently resistant to this species had the lowest peak egg count and lowest *C. oncophora* worm burden at necropsy, but it had as many *T. axei* and *O. ostertagi* worms as the others.

In the initial infections with a single species (Table 3), infection of calf 284 with *O. ostertagi* did not interfere with establishment of *O. ostertagi* and *Cooperia* of the second inoculation. However, development of the *O. ostertagi* population resulting from reinfection was not as advanced as that of the reinfection control. Of three calves initially infected with

T. axei, retarded development of many *O. ostertagi* of the second inoculation, in late fourth stage, and inhibited development of many *Cooperia* larvae, in early fourth stage, occurred in only one (286). Also, in that calf, *T. axei* may have interfered with establishment of *O. ostertagi* of the second inoculation.

Discussion

Since almost the entire *O. ostertagi* population of the calves simultaneously inoculated with the same number of larvae (Table 1, calves 246 to 249) and necropsied between 27 and 61 days after infection consisted of mature adults and was similar in number in each calf, apparently there was little loss of worms between 1 and 2 months after single inoculation. The same generally applied for *T. axei*.

Establishment of the second infection

Roberts et al. (1962) administered a second *O. radiatum* inoculation of 1,000 to 100,000 larvae to calves 9 to 41 weeks after the first. Necropsies at intervals after the second inoculation indicated that a considerable number of larvae reached the fourth stage, but most were eliminated in 2 to 3 weeks, and rarely a few worms became adult. Ross (1963) inoculated calves with 2,000 or 100,000 *O. ostertagi* and 3 weeks later reinoculated them with a second dose of 100,000 larvae. Primary infection with 2,000 larvae did not interfere with establishment of the larvae of the second inoculation, but primary infection from 100,000 may have interfered. Sinha (1967) inoculated lambs of two groups with 20,000 *T. axei* each and reinoculated those of one with 100,000 larvae 8 weeks later. At the same time he inoculated reinfection controls with 100,000 larvae. At necropsy 13 weeks after the initial inoculation, the considerably greater number of worms recovered from the reinfection controls compared with reinfected lambs indicated either that the primary infection interfered with establishment of larvae of the second inoculation, or that the host had developed some resistance to reinfection, or both.

In the present study, calves were initially inoculated with 39,000 to 108,000 *O. ostertagi* and 36,000 to 69,000 *Cooperia* spp. They were reinoculated with 42,900 to 59,400 *O. ostertagi* and 36,000 to 105,600 *Cooperia* spp.

Generally fewer *O. ostertagi*, *C. oncophora*, and *C. punctata* of the second inoculation became established in reinfected calves than in reinfection controls. This decrease was evident even in a calf reinfected 10 days after the first inoculation. Nevertheless, considerable numbers of *O. ostertagi* and *C. oncophora* of a second inoculation became established in a calf inoculated with these species 12 weeks earlier.

Development of the second infection

Roberts et al. (1962) reported that the second inoculation rarely resulted in a rise in egg count. Sinha (1967) found that the second inoculation did not increase the egg output. The average count of the reinfection controls was 10 times that of the reinfected lambs. Ross (1963) reported that apparently there was some increase in the egg output resulting from a second *O. ostertagi* inoculation administered 3 weeks after the first. However, he and Ross and Dow (1964) found that the primary infection could delay development of larvae of the second inoculation. The calves were necropsied 7 to 9 or 13.4 to 15 weeks after the initial inoculation. The present author found that a primary infection with species could delay development of the worms in a calf reinoculated at 5 weeks and necropsied at 7 weeks after the initial inoculation. In infections with two to four species, development of many *Cooperia* of the second inoculation was frequently arrested in early fourth stage in reinfected calves and in reinfection controls, but generally fewer in the latter. Arrested development of a considerable percentage of a *C. oncophora* population, resulting from a single primary inoculation, has been reported by Herlich (1965) and has occurred in some of my other studies.

Effect of second inoculation on primary infection

Roberts et al. (1962) considered that a second *O. radiatum* inoculation did not influence the rate at which adult worms were eliminated. Ross (1963) considered that as a result of reinfecting calves with 100,000 *O. ostertagi* 3 weeks after an initial inoculation with 100,000, some worms of the primary infection may have been eliminated. Sinha (1967) compared the

number of worms recovered from initial infection controls with that from lambs reinfected with 100,000 *T. axei* 8 weeks after an initial inoculation with 20,000 larvae, and reported that reinfection had resulted in elimination of some worms of the primary infection. In the present study, since calves reinoculated with *O. ostertagi*, *T. axei*, and *Cooperia* spp., and necropsied 2 weeks later, had approximately the same number of mature adult *O. ostertagi* as initial infection controls, and since there was no evidence that reinfection caused a decline in the egg output of the primary population, the primary *O. ostertagi* population was not affected by the second inoculation. Reinfection may not have affected the primary *T. axei* and *Cooperia* populations either, but the evidence from worm burdens was not as strong.

Interaction of different species

Ross et al. (1968) found that a primary *O. ostertagi* population did not interfere with establishment or development of *T. axei* of a second inoculation, but they considered that a primary *T. axei* population may have interfered with establishment of *O. ostertagi* of a second inoculation. They found no evidence to suggest that a primary infection with one species retarded development of a subsequent infection with the other species.

In the present study, primary infection with *O. ostertagi* did not interfere with establishment of *Cooperia* of the second inoculation, but development of many was inhibited. Of three calves initially infected with *T. axei*, the establishment of *O. ostertagi* of the second inoculation may have been hindered in one calf and the development of many of them was retarded. Also, in that calf, development of many *Cooperia* was inhibited.

Although a primary infection, established from a single inoculation, might interfere with establishment or development of larvae ingested later, the interference did not appear to be consistent enough or great enough to justify use of a limited artificial primary infection to attempt to prevent a serious infection later.

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Free-Living Marine Nematodes from Biscayne Bay, Florida. VI. Ceramonematidae: Systematics of *Pselionema annulatum* var. *beauforti* Chitwood, 1936, and a Note on the Production and Transport of an Egg Capsule[1]

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ABSTRACT: *Pselionema annulatum* var. *beauforti* Chitwood, 1936, is redescribed from specimens collected from Card Sound and Biscayne Bay, Florida. Females carry their eggs in sacs attached externally at the vulva with embryogenesis occurring within the egg sac. *P. hexalatum* Chitwood, 1936, and *P. rigidum* Chitwood, 1936, are synonymized with *P. annulatum* var. *beauforti*. *Pselionema ornatum* (Timm, 1961) comb. n. is established for *Pterygonema ornatum*. *Pterygonema alatum* Gerlach, 1954, is regarded as *genus et species inquirendum*. Taxonomic significance is suggested for the number and distribution of caudal setae on the male tail.

Among the many marine nematode species occurring in collections from sediments in and around Biscayne Bay, Florida, numerous specimens of *Pselionema annulatum* var. *beauforti* Chitwood, 1936, were found in samples collected from Card Sound and South Biscayne Bay. The following account redescribes and figures the mature adults of this species and records the production and transport of an egg capsule. The systematic relationships of Chitwood's (1936) three taxa, *P. annulatum* var. *beauforti*, *P. hexalatum*, and *P. rigidum*, are considered from an examination of the type slides made available through Dr. W. Duane Hope from the U. S. National Museum.

Pselionema annulatum var. *beauforti* Chitwood, 1936
(Figs. 1–14)

SYNONYMS: *Pselionema rigidum* Chitwood, 1936, new synonymy; *P. hexalatum* Chitwood, 1936, new synonymy.

Body of male of nearly uniform diameter throughout (Fig. 12), fusiform in female (Fig. 13); with eight longitudinal ridges extending from posterior portion of head annule to terminal annule. Distance between sublateral ridges in female greater than that in male (cf. Figs. 1, 2). Additional longitudinal ridge, ventral in position, starting on annule bearing excretory pore (Fig. 3) and stopping at anal/

cloacal opening. Head with abbreviated cephalic capsule, with prominent fenestrae and incisions along posterior edge associated with each cephalic seta; position of labial and cephalic papillae (pitlike?) indicated by presence of porelike canals. Sexual dimorphism also occurring in shape of amphids and in annular tiling (Figs. 1, 2, 10, 11). Male amphids with unequal arms, ventral arm extending to posterior limit of head annule (helmet) (Fig. 1); female amphid with arms nearly equal (Fig. 2); with a common base in one aberrant example (Fig. 8). Amphid of embryonic juvenile a single, double-looped spiral (Fig. 7). Amphidial duct leading nearly perpendicular into body cavity and becoming closely associated with surface of esophagus (Fig. 6).

Male 700–780 μm long, with 88–95 annules; maximum width 24–26 μm; esophagus 137–152 μm long, spanning an interval of 16–18 somatic annules; tail 123–133 μm long with 19–20 annules. Annule width about 7 μm near head, 8–9 μm at midbody, 4.5–7 μm on tail; cloacal annule 10–12 μm, terminal annule 15–23 μm. Excretory pore on body annule 13 or 14. Cloaca opening through body annule 69–74. Cloacal body diameter 23–25 μm anterior to aperture, 20–21 μm posteriorly.

Head with six labial papillae, six cephalic papillae, and four cephalic setae, 9–12 μm in length. Head diameter 20–22 μm at midhelmet. Helmet, 29–33 μm long. Amphid with arms of unequal length, ventral arm 11–12 μm long, dorsal arm 8–9 μm long; amphid width
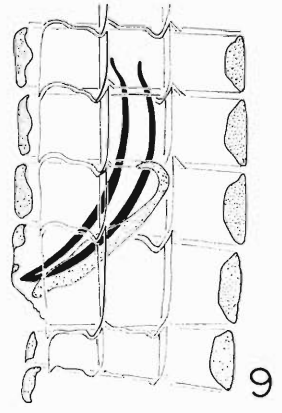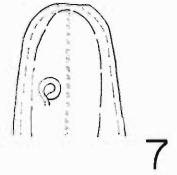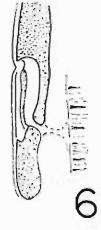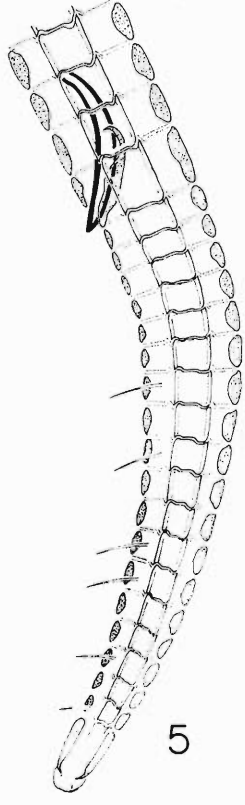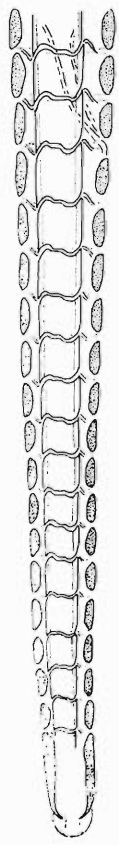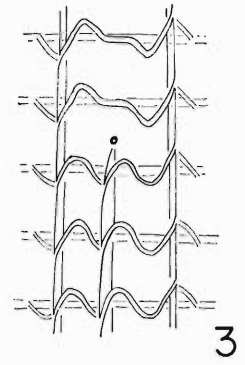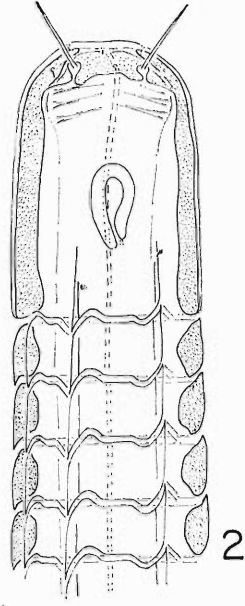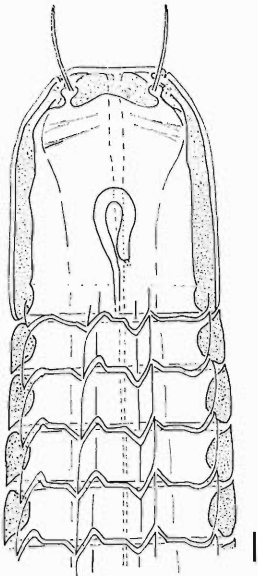
Table 1.   Distribution of caudal setae on tails of male *Pselionema* species (+ = seta present on that annule. A = aperture of cloaca).

Species	Side	Number of caudal setae	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
P. annulatum var. *beauforti* Chit.	L.	(6)	+		+		+		+			+			+							A		
	R.	(7)	+		+		+		+		+		+		+							A		
#2	L.	(8)	+	+		+	+		+		+		+		+							A		
	R.	(6)	+		+		+			+			+		+							A		
#3	L.	(7)	+	+		+		+			+		+		+							A		
	R.	(7)	+	+		+			+		+		+			+						A		
#4	L.	(7)	+	+		+			+	+			+		+							A		
	R.	(8)	+	+		+	+		+		+	+			+							A		
#5	L.	(7)	+	+		+		+		+		+			+							A		
	R.	(7)	+	+		+		+		+		+		+								A		
Chitwood, 1936	L.	(6)	+	+		+			+			+			+							A		
	R.	(6)	+	+		+		+			+				+							A		
P. annulatum of Gerlach, 1950	L.	(6)	+		+		+		+			+								A				
P. parasimplex Vitiello, 1970	L.	(4)	+		+			+			+					A								
P. richardi de Coninck, 1942	R.	(4)	+			+			+		+													A
P. simile de Coninck, 1942	R.	(7)	+			+		+		+			+			+					+	A		
P. simplex de Coninck, 1942	L.	(3)		+				+			+											A		
#2	L.	(4)			+		+		+		+							A						

5.3–6 μm; anterior edge of amphid 13–16 μm posterior to cephalic extremity.

Spicules 34–36.5 μm long, chord 30.7–32 μm; gubernaculum 17.6–18.9 μm long, chord 17.2–18.1 μm. Tail with 6–8 pairs of subventral setae, 8.3 μm long anteriorly, decreasing in length gradually to 6.9 μm on the terminal annule. Distribution of caudal setae on tail given in Table 1. Three to four caudal annules are narrower ventrally than dorsally, viz. 4.5 μm vs. 6 μm, which imparts a slight ventral curvature to the tail.

Female 744–806 μm long, with 82–93 annules; maximum width 30–37 μm; esophagus 140–146 μm long, with 16–18 annules; tail 127–152 μm long with 14–18 annules. Annule width about 7 μm near head, 7.6–9 μm at base of esophagus, 9–9.7 μm at midbody, 8.3–9.7 μm at anal region, 6.1–7 μm near tail tip; terminal annule 18–19 μm. Excretory pore on body annule 12–14. Anal aperture between body annules 68 and 69–75 and 76. Anal body diameter, 17–19 μm.

Head with six labial papillae, six cephalic papillae, and four cephalic setae, 9–10.7 μm in length. Head diameter 22–25 μm at midhelmet. Helmet, 30–34 μm long. Amphid with arms of subequal length, ventral arm 9–11 μm, dorsal arm 7–9.5 μm long; amphid width 5–6 μm; anterior edge of amphid 15–17 μm posterior to cephalic extremity.

Vulva position at 49.5–51.6% of body length, located between body annules 41 and 42–46 and 47. Egg sac (capsule and attachment stalk) 103–145 μm long, capsule 75–90 μm long, stalk 21–55 μm long.

Figures 1–9.   *Pselionema annulatum* var. *beauforti.* 1, Male head. 2, Female head. 3, Ventral view of excretory pore and beginning of the midventral longitudinal ridge. 4, Female tail. 5, Male tail. 6, Amphid, dorsoventral view showing connection to esophageal wall. 7, Juvenile head, showing spiral amphid. 8, Aberrant amphid with dorsal and ventral arms united. 9, Male, cloacal area, showing left spiculum and gubernaculum.
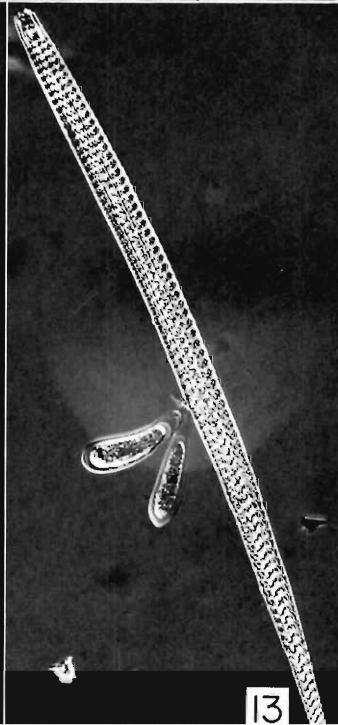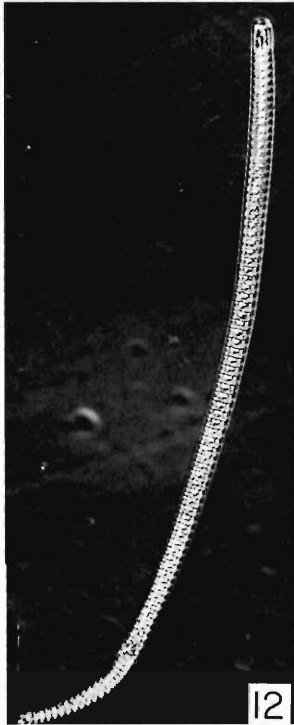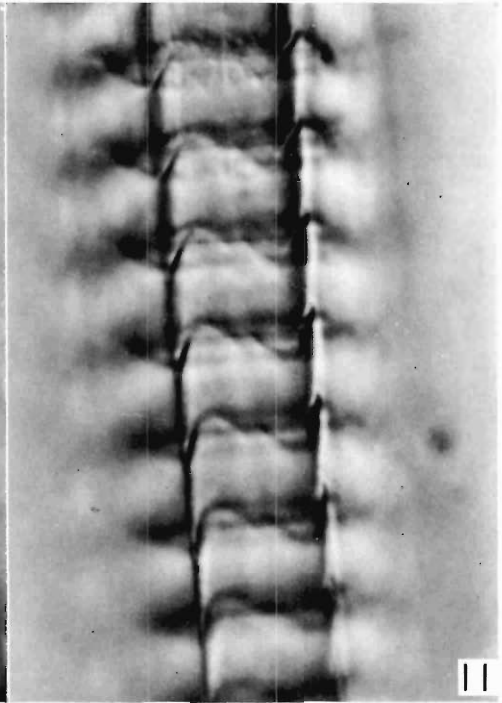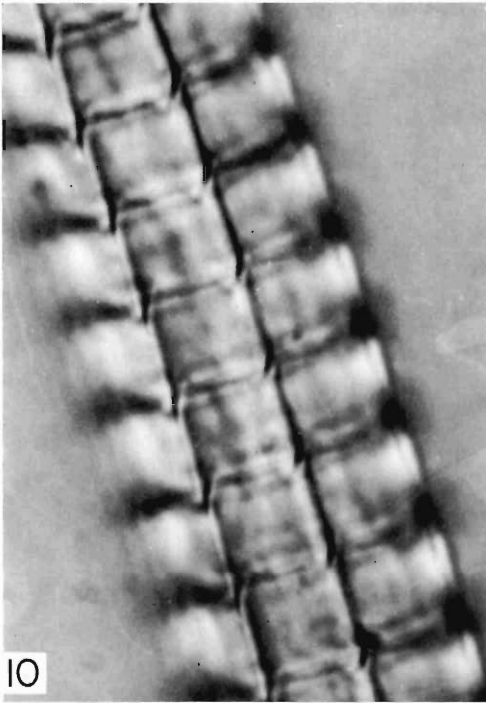
LOCALITY AND COLLECTION DATA: The majority of the specimens incorporated in this study were extracted from surface sediments within the sparse growth of a bed of turtle grass, *Thalassia testudinum* König, located in Card Sound approximately 2 miles northeast from the mouth of the Model Land Co. canal and 1 mile from the mainland shoreland; five males and 10 females collected 11 November 1970, by I. M. Masters and S. Newell. The species has also been collected from Biscayne Bay in the vicinity of the Florida Power and Light Co. generating plant located at Turkey Point, Florida.

Remarks

Prominent saclike structures, resembling suctorian commensals, often found associated with specific members of the Desmodorodae, i.e., *Spirinia parasitifera* (Bastian), were associated with each female individual taken in samples from Card Sound and South Biscayne Bay. Upon closer inspection, these appendages were found to contain developing eggs and embryos and were attached to the female body at the vulva.

These structures, herein termed "egg sacs," vary in length from 103–145 μm and are divisible into two parts: an egg capsule which contains the developing embryo and an attachment stalk. The egg capsule is 75–90 μm long by 28–30 μm wide and has walls which range in thickness, in most examples, from 2–5 μm. However, distally, the thickness can be as much as 10 μm on capsules containing eggs either unsegmented or in early cleavage.

Each of the 10 female specimens available from Card Sound had either one or two egg sacs present. Females with a single egg sac showed evidence of there once having been two. In all likelihood, a single egg is contributed from each of the two ovaries. No specimen was seen to exhibit evidence of there having been more than two egg sacs present.

This is a most unusual modification from the normal procedure of depositing eggs freely into the environment and/or attaching them to various substrates via sticky secretions. Within the marine Nematoda, only a few members of the Desmoscolecidae are known to have eggs undergoing embryogenesis in intimate external contact with the body (Timm, 1970). In these cases, however, the eggs are fastened to modified somatic setae and are not enclosed within specialized structures presumably arising from the female reproductive system.

Redescription of Chitwood's (1936) Type Material and their Systematic Relationships

Pselionema annulatum var. *beauforti* Chitwood, 1936

Male 648 μm long, with 90 annules; maximum width 21.2 μm; esophagus 132 μm long, spanning an interval of 19 somatic annules; tail 113 μm long, with 19 annules. Annule width at midbody, 7.6–8 μm; cloacal annule wider than others, 9.7 μm, immediate postcloacal annules smaller than precloacal annules, 5.5 vs. 8.3 μm, terminal annule 17 μm long. Excretory pore on body annule 13. Cloaca opening through body annule 71. Cloacal body diameter 21 μm anterior to aperture, 18.2 μm posteriorly.

Head with four cephalic setae, about 8 μm in length. Head diameter, 19 μm at midhelmet. Helmet 26 μm long. Amphid with arms of unequal length, ventral arm 10 μm long, dorsal arm 8 μm; anterior edge of amphid 12 μm posterior to cephalic extremity.

Spicules 32.3 μm long, chord 30.2 μm; gubernaculum 17.6 μm long. Tail with six pairs of subventral setae, 8 μm long anteriorly, decreasing in length gradually to 7 μm on the terminal annule. Distribution of caudal setae given in Table 1. Terminal annule with a pair of setae located 10 μm from anterior edge.

LOCALITY AND COLLECTION DATA: USNM No. 33946. Marine below low tide mark. Beaufort, N. C. Coll. by B. G. Chitwood. Summer 1934. Glycerin mount.

←

Figures 10–14. *Pselionema annulatum* var. *beauforti*. 10, Male showing cuticular tiling pattern. 11, Female showing cuticular tiling pattern. 12, Male, entire body. 13, Female, entire body. 14, Egg sac with encapsulated embryo.

Pselionema rigidum Chitwood, 1936

Female 657 μm long, with 92 annules; maximum width 29.5 μm; esophagus 138 μm long, spanning an interval of 19 somatic annules; tail 103 μm long, with 16 annules. Head with four cephalic setae, about 7 μm in length. Head diameter, 20 μm at midhelmet. Helmet 32 μm long. Amphid with arms of subequal length, ventral arm 9 μm long, dorsal arm 7.5 μm; anterior edge of amphid 14 μm posterior to cephalic extremity. Excretory pore on body annule 15. Vulva position at 49% of body length, located between body annules 46 and 47. Egg sac (capsule and attachment stalk) 117 μm long, capsule 79 μm long, stalk 38 μm long. Terminal annule about 16 μm long, with a pair of 3-μm-long setae about 3 μm from anterior edge.

Remarks

This specimen shows evidence of the remains of two egg sacs protruding from the vulva. A complete egg sac is detached from the body and is to be seen floating on this slide, USNM No. 33947.

LOCALITY AND COLLECTION DATA: Cotype, USNM No. 33947. Marine below low tide mark. Beaufort, N. C. Coll. by B. G. Chitwood. Summer 1934. Glycerin mount.

Pselionema hexalatum Chitwood, 1936

Female 647 μm long, with 95 annules; maximum width 27 μm; esophagus 127 μm long, spanning an interval of 18 somatic annules; tail 117 μm long, with 17 annules. Head with four cephalic setae, about 5 μm in length (distorted). Head diameter, 20 μm at midhelmet. Helmet 27 μm long. Amphid with arms of unequal length, ventral arm 9 μm long, dorsal 6 μm; anterior edge of amphid 14 μm posterior to cephalic extremity. Excretory pore on body annule 15. Vulva position at 51% of body length, located between body annules 45 and 46. Terminal tail annule 18 μm long with a single 4–5-μm-long seta on left side, 6 μm from anterior edge.

Remarks

No evidence of egg sacs was detected in this specimen.

LOCALITY AND COLLECTION DATA: Holotype:

USNM No. 33948. Marine shallows. Bogue Sound, N. C. Coll. by B. G. Chitwood. Summer 1934. Glycerin mount.

Discussion

The original distinction between Chitwood's variety and *P. rigidum*, based on differences in the form of the amphids and in the cuticular tiling, is attributable to sexual dimorphism. The occurrence of a prominent egg sac on the slide bearing the cotype specimen of *P. rigidum* is further evidence of the cospecificity of the two taxa, as is the fact that, on the basis of the original recorded collection data, they apparently were taken from the same sample.

P. hexalatum, which bears eight longitudinal ridges, plus the shorter midventral ridge, was originally separated from *P. annulatum* var. *beauforti* by the form of the tilings, the minute setae, and the shape of the amphids. The form of the tilings and the amphid shape are attributable to sexual dimorphism. The shorter cephalic setae appear to be distorted on the single specimen available from the U. S. National Museum, and are considered to be fixation artifacts.

Comparison of Chitwood's three *Pselionema* species to those collected from Card Sound and South Biscayne Bay show that Chitwood's materials represent a single species. Based on positional priority on page 3 of the original publication, *Pselionema annulatum* var. *beauforti* is to be considered the valid name of this taxon.

The relationship of *P. annulatum* var. *beauforti* Chitwood, 1936, with the type species of *Pselionema*, *P. annulatum* (Filipjev, 1922) Chitwood, 1936 (synonym *Steineria annulata* Filipjev, 1922), is uncertain. The original description is too insufficient to be of much assistance in determining the relationship between it and its variety. Among the data not furnished by Filipjev are: (1) the total number of body annules, (2) the excretory pore placement, and (3) the distribution of the caudal setae on the male tail.

Gerlach's (1950) redescription of specimens regarded to be Filipjev's *P. annulatum* provides more details. The number of body annules is given as 91 to 95, sexual dimorphism of the amphids is recorded, and the dimorphism in

the cuticular tilings is suggested in the illustrations. However, the male is drawn without the wide cloacal annule. This feature was expressly pointed out by Filipjev: "L'anneau anal est plus large que ses voisins." (The anal annule is wider than its neighbors.) This point, on the Kiel Bay specimens, needs clarification.

In view of the insufficient knowledge of *P. annulatum* (Filipjev), it is herein proposed that the varietal name "beauforti" be retained for the North American east coast specimens until such time that specimens of Filipjev's species become available for comparative study.

Taxonomic Importance of Male Caudal Setae

The taxonomic value of the numbers and the distribution of the setae on the male tail needs to be explored. In the current study, based on the six males available, five from Card Sound, Florida, and Chitwood's type specimen, a definite setal pattern begins to emerge for *P. annulatum* var. *beauforti* when specimens are compared among themselves and with the information available in the literature for other species (Table 1). Prior to a discussion of comparisons based on information given in the literature, it must be recognized that such data may be inaccurate or invalid due to the lack of precision in determining the presence and the numbers and annular placement of the caudal setae. For example, numbered consecutively from the terminal annule anteriorly, Chitwood originally figured the left side of the male tail of *P. annulatum* var. *beauforti* with three subventral and one subdorsal setae. Examination of his original specimen shows that, indeed, there are setae on annules 1, 10, and 13. However, setae also occur on annules 2, 4, and 7; and there is no subdorsal seta.

In the present study of *P. annulatum* var. *beauforti*, setae were found to occur most commonly on annules 1, 2, 4, 7, and 13, with the cloacal aperture on annule 20 (20–21). Being mindful of the potential inaccuracies, this pattern is sufficient to distinguish this taxon from all others in which males are known. With one exception, no other species

has setae beyond annule 11. The singular exception, *P. simile* de Coninck, 1942, has a similar total number of caudal setae, 7 vs. 6–8, but has these extended to the 19th annule. The cloacal aperture placement is also similar, occurring on annule 21.

Males of other species with caudal setae are recorded as having no more than 3 or 4. *P. annulatum* (Filipjev, 1922), *P. longissimum* Gerlach, 1952, and *P. ornatum* (Timm, 1961) comb. n., are figured without caudal setae. (Note: Timm depicts a single terminal seta in Fig. 45c for his species.)

Systematic Status of *Pterygonema* Gerlach, 1954, and its Species

Pterygonema Gerlach, 1954 (type species *P. alatum*), was based on a single juvenile specimen collected from the shores of Tunisia near Tunis. Juvenile specimens are known to exhibit characteristics which differ from those to be found in adult individuals. The existence of such possible differences preclude reliable comparison with other ceramonematid taxa and it is herein proposed to regard *Pterygonema* and *P. alatum* as *genus et species inquirendae*.

In view of the extreme similarity between *Pterygonema ornatum* Timm, 1961, and species of *Pselionema*, Timm's taxon is herein transferred to *Pselionema* and becomes *Pselionema ornatum* (Timm, 1961) comb. n. (Syn. *Pterygonema ornatum* Timm, 1961).

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Notes on the Longevity of *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936, and Its Ability to Invade Wheat Seedlings after Thirty-Two Years of Dormancy

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ABSTRACT: *Anguina tritici*, second-stage larvae, in wheat galls which were stored in sealed glass tubes under conditions of low, constant humidity, and others stored in a refrigerator at about 5 C, were able to resume activity after 32 years. Larvae from the refrigerated galls were able to invade wheat seedlings readily. Larvae stored at low constant humidity were not tested. The reactivated larvae in tap water retained ability to move for as long as 408 days.

This work is a continuation of the dormancy test reported by Limber (1962) on the wheat nematode, *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936. In addition, through the kindness of Dr. A. Morgan Golden (Plant Science Research Division, ARS—USDA, Beltsville, Md. 20705), the writer received more galls of the same collection from which Dr. G. Steiner had provided the material for the earlier experiments. These galls had been stored in a refrigerator since 1939 and 1948, respectively, at a temperature of about 5 C.

The wheat galls sealed in glass tubes, which remained from the earlier work, had been stored from 1961 until 1970 at room temperatures, as previously, except for 3 days in September 1970 when they were in an attic where the temperature reached 107 F for a few hours each day.

The percentage of viable larvae was determined as follows. The galls were wrapped in wet paper toweling and placed in a 25-ml plastic vial for 48 hr. The softened galls were opened with dissecting needles without injury to the larvae and placed, individually, in vials containing 2 ml of water. After shaking, the suspension was diluted until 10 or 12 drops contained from 100 to 150 larvae. Drops were then placed in rows on a microscope slide and the larvae counted, using a 14× hand lens or a microscope with the 25× lens combination.

Since thousands of larvae were to be counted, criteria which would permit a rapid count were necessary. Therefore activity was chosen. But since Fielding (1951) has shown that some inactive larvae may still be alive, some larvae were judged by appearance. Inactive larvae which lie in coils or in smooth curves will nearly always be seen to move if observed long enough. Any errors in judgment are not believed to be significant for our purposes.

Distilled water, tap water, rainwater, and leachings from pots of growing wheat seedlings were tested for reactivation of larvae. Since all gave about the same results, distilled water and tap water were used for most of the tests because there was less difficulty from infusorians and fungi.

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Table 1. Revival of *Anguina tritici* (second-stage larvae) stored for 32 years under constant humidity, and 32 and 23 years under refrigeration at about 5 C.

Treatment	Time	Maximum activity	Populations	Average
Constant humidity, undried galls	32 yr	26%	3	26%[1]
Constant humidity, galls dried 5 min at 76 C	32 yr	96%	6	91%[2]
Refrigerated galls	32 yr	100%[3]	48	74%
Refrigerated galls	23 yr	100%[3]	8	97%

[1] Average of two living populations.
[2] Average of two living populations.
[3] Since only a 100-larvae sample was examined each day, the presence of a few dead larvae in the population is not excluded.

Figure 1. The rise and decline of activity in *A. tritici* populations, each stored differently, over a period of time. The dotted line, 40 A, shows the percentage of active larvae of a population stored under constant humidity for 32 years; the solid line, 40 A CaCl₂, represents the pattern of activity of a population dried for 5 min at 76 C then stored in a constant humidity tube which contained a few flakes of CaCl₂ separated from the galls by cotton; the broken line, #60, shows the pattern of activity of a population refrigerated for 32 years at about 5 C.

Larvae under constant humidity in sealed tubes

Three galls of a lot sealed in a glass tube without drying [second item listed in Table 1 (Limber, 1962)] were soaked on 19 December 1970, and were opened on 22 December. The highest percentage of revival, 26%, was reached on 26 January, 31 days after the first observed movement.

Six galls of a lot dried for 5 min at 76 C, then sealed in a glass tube containing a few flakes of CaCl₂ covered by a loose cotton plug, were soaked and opened after 48 hr. Four of these galls contained no living larvae. The larvae in the fifth gall showed a maximum revival of 96% after 6 days and activity remained high for 40 days. The population of the sixth gall showed maximum activity of 84% after 35 days. The last active larvae were found on the 167th day.

Thus, in both of these constant humidity experiments, many larvae were alive after 32 years.

Refrigerated galls stored for 32 years

These galls, collected in 1939, were stored in a refrigerator at a temperature of about 5 C. Forty-eight gall populations were examined by the same methods as used for the galls stored in sealed glass tubes.

Of these 48, two populations contained no living larvae, but 20 populations gave counts of 100% active larvae in from 1 to 20 days. The other populations varied greatly, from 1

to 98%; but most of them were above 69%. One population reached its maximum in 48 hr. The others required from 7 to 41 days.

Refrigerated galls stored for 23 years

These galls were from the North Carolina collection of 1948 and were stored in the same manner as those of the 1939 collection above. Eight gall populations were studied by the same methods. These larvae revived quite uniformly in contrast to those stored for 32 years. They averaged 97% revival (average of the 10 best counts of each population).

Summary of Revival Tests

The summary is given in tabular form in Table 1.

The graph (Fig. 1) shows three revival patterns. Due to the great variation in the revival of the larvae in different galls after long stor-

age, these are only examples and cannot be considered as representative of every population in their groups.

Inoculation Tests

Since so many populations of the refrigerated galls were strongly active after 32 years, the populations of two galls were combined and tested for ability to invade wheat seedlings. Eighteen wheat seeds were moistened for 48 hr in wet paper and placed on unsterilized soil in a clay pot. They were inoculated by placing several thousand (estimate) active larvae directly on the seeds with a dropper before the seeds were covered. The larvae used had been active for 19 days; 95% of the larvae were active.

Examinations were begun after 18 days and were continued for 20 days. Four infested plants were found but in only one were there more than two larvae. That plant contained four.

The experiment was repeated using freshly activated larvae and soil partly sterilized with boiling water. Fifteen plants were examined. Nine were infested. Infestation ranged from six to 500 larvae but usually was less than 50 per plant. The larvae were nearly all in the basal inch of the stem in the period of examination which was less than 20 days.

Since these tests differ in two ways, six more tests were run using freshly activated larvae in unsterile soil and in partly sterile soil in order to determine the effect of the different soil treatment. When the results for these six tests were averaged there was no significant difference in the amount of infestation. This seems to eliminate the difference in soil as a cause. The variation is probably the result of chance, or that after 19 days the larvae lose some of their ability to invade seedlings.

Discussion

The experiments on low constant humidity storage were suggested by the writer's observation, in 1938, that the galls of *Anguina tritici* varied in weight, from day to day, directly with the atmospheric humidity.

Revival of second-stage larvae of *A. tritici* occurred after 32 years in storage by each method, i.e., under low constant humidity and under refrigeration at about 5 C. This is an interval of 4 years longer than Fielding (1951) reported for this nematode.

Low respiration and low metabolism, with resulting low use of stored fats in larvae, have been reported by von Brand (1960) to vary directly with the temperature. Von Brand (1960) stated that Pigon and Weglarska found the respiration rate of dehydrated *Macrobiotus hufelandii* 600 times slower than active ones. Thus it seems probable that the long life of *A. tritici* in both types of storage is the result of reduced respiration and metabolism which either low humidity or low temperature can produce.

Golden and Shafer (1960) found that the larvae of *Heterodera schactii* remained active in plain tap water for 6 months, but all were apparently dead at the end of 7 months (approximately 214 days). Larvae of many populations of *A. tritici*, stored for 32 years, contained a few active larvae after 240 days. The longest observed period of activity among the populations studied is 414 days. This population still contains a few larvae which make slow movements separated by relatively long intervals of inactivity.

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Free-Living Marine Nematodes from Biscayne Bay, Florida. VII. Enoplidae: *Enoplus* Species in Biscayne Bay with Observations on the Culture and Bionomics of *E. paralittoralis* Wieser, 1953[1]

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ABSTRACT: *Enoplus geminivelatus* sp. n. and *E. paralittoralis* Wieser, 1953, are described from specimens collected from Biscayne Bay, Florida. The term "triad" is introduced for a group of three cervical setae which are of constant occurrence on the lateral line at a short distance behind the cephalic capsule. At 24 C and 15‰ salinity, the life cycle of *E. paralittoralis* is completed in 19 to 24 days.

The following paper describes two species of the genus *Enoplus* from Biscayne Bay, Florida. The description of *E. paralittoralis* Wieser is based on specimens cultured in the laboratory. A brief account of the culture procedure and some biological observations follow the taxonomic descriptions.

Enoplus geminivelatus sp. n.
(Figs. 1–7)

Cuticle externally smooth, with densely and closely aligned transverse rows of minute intracuticular punctations. Maximum body diameter 113–127 μm in male, 117–134 μm in female. Head 45–50 μm wide, with three thin lip flaps about 2–3 μm in height, six prominent labial papillae, and 10 (6 + 4) cephalic setae, 12–14 μm + 10–12.4 μm long. Lateral cervical triad,* 36—56 μm behind cephalic suture; setae 6.4–8.8 μm long, arranged in a forward-pointing triangle. Circle of four submedian cervical setae 3.2–4 μm long, located between cephalic capsule and lateral cervical triad. Second group of cervical setae of about 5 μm in length, located posterior to lateral cervical triad. Additional cervical-somatic setae 3–4 μm long sparsely distributed throughout body along dorsal and ventral margins of lateral hypodermal chords. Female without modified (lengthened) dorsal and ventral somatic setae in vulval region.

Amphid aperture transversely oval, 2–4.2 μm long by 4–6 μm wide; amphidial pouch 6.8–9.2 μm long, traversed by cephalic suture. Cephalic capsule 18–25 μm long (measured from anterior extremity of head); cephalic suture nearly straight, with slight posterior bulge in region of amphidial aperture (Fig. 1). Mandibles 15.5–17.6 μm long by 6–7.2 μm wide, measured across anterior extremity. Esophagus 372–424 μm long in male, 384–512 μm long in female. Nerve ring encircling esophagus 176–220 μm from anterior extremity. Excretory pore 152–187 μm posterior to cephalic extremity. Pigmented accumulations ("pigment spots") diffuse and variable in form; lenslike structures absent. Anterior margin of pigmented zone 43–48 μm from anterior extremity. Tail length in male 204–234 μm, in female 208–244 μm. Anal body diameter 72–84 μm. Spinneret glands confined to tail.

Male 2.85–3.56 mm long. Spicules symmetrical, 117–133 μm long (chord 103–118 μm), each with single semicircular plate. Gubernaculum 36–51 μm long, with caudally directed apophyses, 12–17 μm in length. Precloacal supplement, narrow, tubular, 46–55 μm long, located 134–162 μm anterior to cloacal aperture. Subventral setae in precloacal region 10–12 μm long. A prominent, ventral pedunculated papilla occurs immediately post-cloacally.

Female 2.70–3.68 mm long; V = 56.5–59.1%. Maximum number of eggs observed per uterus, 2; egg size 98–138 μm long by 76–88 μm wide.

HOLOTYPE: Male, on slide "ENOPLUS #3
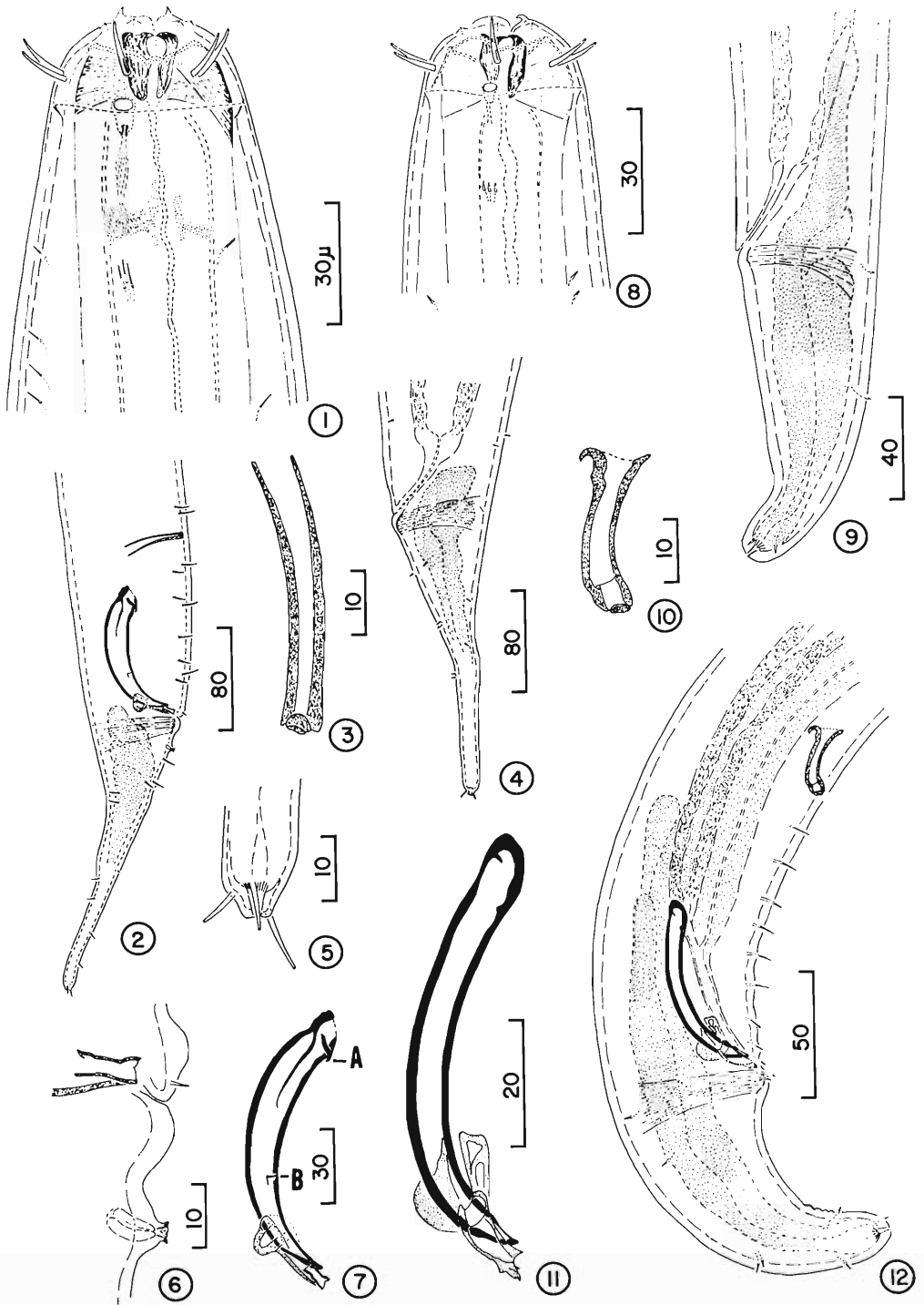
slide #3" on deposit in authors' personal collection.

ALLOTYPE: Female on slide "ENOPLUS #3, slide #3," held in authors' personal collection.

PARATYPES: Four males and four females on slides "ENOPLUS #3, slides #1, #2 and #3." All held in authors' personal collection.

TYPE LOCALITY AND COLLECTION DATA: Two males and one female recovered from sediment within a bed of turtle grass, *Thalassia testudinum* König, located offshore from Matheson Hammock, Florida (Site D of Hopper and Meyers, 1967. Collected 1 January 1967 by B. E. Hopper). An additional male and female were collected from the type locality on 12 February 1968 by B. E. Hopper and R. C. Cefalu.

OTHER LOCALITIES: In sediment washed from *Halimeda* sp. from the northwest shore of Key Biscayne (two males and three females collected by B. E. Hopper, 24 February 1965).

DIFFERENTIAL DIAGNOSIS: *Enoplus geminivelatus* sp. n. is most closely related to *E. velatus* Wieser, 1959, and is distinguished from it by having the cephalic suture crossing over the amphidial pouch at the latter's midpoint, while in *E. velatus* the cephalic suture lies immediately posterior to the amphidial pouch. In addition to the smaller dimensions of *E. geminivelatus*, the male differs in the shape and size of the spicula plus the presence of a single semicircular plate on each spiculum. Proximally, the spicula are provided with a prominent S-shaped, hooklike structure which appears to be missing from Wieser's species. The spicules, while having shorter absolute dimensions, are relatively wider than those depicted for *E. velatus*.

Remarks

Noteworthy for the two species *E. geminivelatus* sp. n. and *E. velatus* is the presence of the large papilla immediately posterior to the cloacal aperture. *E. geminivelatus* lacks the second "tubular papilla" located ventrally midway down the tail as described and figured for *E. velatus* although a much smaller papilla does occur in this same relative position.

The two species are regarded as an example of "geminate species" or "twin species," terms introduced by D. S. Jordan (1908) to suggest evolution from a common ancestor.

Enoplus paralittoralis Wieser, 1953 (Figs. 8–12)

Cuticle externally smooth, with densely and closely aligned transverse rows of minute intracuticular punctations. Maximum body diameter 70–80 μm in male, 80–83 μm in female. Head 37–40 μm, with three thin lip flaps about 2 μm in height, six prominent labial papillae, and 10 (6 + 4) cephalic setae, 9.2–11.2 μm + 8.4–8.8 μm long. Lateral cervical triad 15–34 μm posterior to cephalic suture; setae 2.4 μm long and aligned in an approximate transverse row. Circle of four individual cervical setae, 2 μm long, located 14–24 μm posterior to lateral cervical triad (no cervical setae anterior to triad). Additional cervical-somatic setae of about 2 μm length sparsely distributed along body at dorsal and ventral margins of lateral hypodermal chords. Female without modified (lengthened) dorsal and ventral somatic setae in vulval region.

Amphid aperture transversely oval, 3.4 μm long by 5.2 μm wide; amphidial pouch 7.2 μm long, traversed by cephalic suture. Cephalic capsule 16–20 μm long (measured from anterior extremity of head); cephalic suture undulating posteriorly in region of amphidial aperture (Fig. 8). Mandibles 14.0–15.6 μm long by 5.4–6.0 μm wide, measured across anterior extremity. Esophagus 394–427 μm in male, 402–440 μm in female. Nerve ring encircling esophagus 200–234 μm posterior to cephalic extremity. Pigmented accumulations absent. Tail length in male 116–126 μm, in female 114–139 μm. Anal body diameter 62–72 μm in male, 58–60 μm in female. Spinneret

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Figures 1–7. *Enoplus geminivelatus* sp. n. 1, Male head. 2, Male tail. 3, Precloacal supplement. 4, Female tail. 5, Tail terminus. 6, Male, region of cloacal aperture showing postcloacal papilla. 7, Right spiculum and gubernaculum complex: a, S-shaped, hooklike structure; b, semicircular plate.

Figures 8–12. *Enoplus paralittoralis* Wieser, 1953. 8, Male head. 9, Female tail. 10, Precloacal supplement. 11, Right spiculum and gubernaculum complex. 12, Male tail.

glands extending into preanal area in both sexes.

Male 3.29–3.58 mm long. Spicules symmetrical, 75–80 μm long (chord 66–70 μm), without semicircular plates. Gubernaculum 20–24 μm long, with caudally directed apophyses about 5 μm in length. Precloacal supplement tubular, bent, 26–29 μm long, and located 98–111 μm anterior to cloacal aperture. Subventral setae in precloacal area 7–10 μm long. Two pairs of subventral setae occur immediately postcloacally.

Female 3.15–3.69 mm long; V = 51.6–52.2%. Maximum number of eggs observed per uterus, 3. Egg size 77–112 μm long by 44–58 μm wide.

LOCALITY AND COLLECTION DATA: Laboratory culture. Source: Originally extracted from decaying mangrove leaves (*Rhizophora mangle*) gathered from the mangrove forests fringing the shores of Matheson Hammock, Florida, collected by Ingrid Hunter and Steve Newell, 5 January 1972.

GEOGRAPHICAL DISTRIBUTION: Chile (Strait of Magellan) Wieser, 1953; Puget Sound (Golden Gardens and Richmond Beach) Wieser, 1959, and Biscayne Bay, Florida (Matheson Hammock), present record. While the present record of this species from Florida waters removes it from confinement to cold temperate regions (Wieser, 1959), it remains restricted to the upper intertidal zones.

Remarks

The present description of *Enoplus paralittoralis* is very close to those given previously by Wieser (1953, 1959) except for the smaller overall dimensions which one would expect of more tropical representatives when compared to their cold-watered counterparts following Bergmann's Rule (cf. Ray, 1960). The dorsal hooklike structure found on the proximal extremity of the precloacal supplement appears to be at variance with the earlier descriptions. Likewise, the number of subventral setae present between the cloacal region and the supplement are less numerous in the Florida specimens than originally reported, 9 vs. 18. Wieser shows 13 to occur in this region in his 1959 figure. We do not feel that these two deviations are sufficient to warrant specific status for the Florida representatives.

The occurrence and taxonomic value of the lateral cervical triad

While examining live specimens of *Enoplus paralittoralis* under the compound research microscope, the presence of a group of three cervical setae was detected on the lateral line a short distance behind the cephalic capsule. Observations of additional specimens revealed not only that the presence of these setae was constant, but also they varied little in their size and arrangement. Examination of specimens representative of five additional *Enoplus* species similarly showed intraspecific constancy in number, form, and arrangement of these setae. The term "triad" is herein introduced for this group of setae.

Based on their common occurrence within the material examined, the triad appears to represent a generic characteristic.

A review of past literature revealed that only two authors had previously detected these setae. De Man (1886) was first to note their presence. Writing first of *E. communis*, de Man states "Gleich hinter den Augenflecken . . . stehen, gerade in der Laterallinien, drei kurze Börstchen in einer Gruppe dicht bei einander." For *E. brevis*, de Man writes "An jeder Seite des vorderen Körperendes, ein wenig hinter der Kopflinie, bemerkt man bei beiden Geschlechtern, wie auch beim *communis*, drei kurze Börstchen, welche, in einer Gruppe dicht bei einander, genau in der Laterallinie stehen und zwar bei beiden Geschlechtern an derselben Körperstelle."

Our examination of specimens of these two species confirms de Man's observations of their presence on these species as well as the fact that the triads occur in both sexes.

In describing what was considered to be an example of *E. meridionalis* Steiner, 1921, now regarded as *E. stekhoveni* Wieser, 1953, Schuurmans-Stekhoven (1950) recorded the presence of "a couple of large setae" immediately following the pigment spot. He also commented that "the other setae of the same region are however minute." That only a "couple" of setae occur on this taxon is doubtful, as a number of specimens need to be closely examined before the number, size, and arrangement can be determined. Such setae, when appressed against the body surface, become extremely difficult to detect. Their

presence is more easily noticed in specimens resting slightly on the oblique from the lateral aspect.

The following data give (1) the length of the triadal setae, (2) their distance posterior to the cephalic capsule (cephalic suture), and (3) their arrangement. The following terms, with their definitions, are used to describe setal arrangement: "triangle"—where the middle seta is prominently anterior to the outer setae; "reverse triangle"—where the middle seta is prominently posterior to the outer setae; "transverse"—where all three setae are approximately on the same transverse level; and "erratic"—where no clear pattern is discernible.

E. communis Bastian, 1865: (1) 8–10 μm, (2) 47.4 (40–56) μm, (3) reverse triangle.

E. brevis Bastian, 1865: (1) 9.6–10.8 μm, (2) 41.3 (24–62) μm, (3) triangle.

E. geminivelatus n. sp.: (1) 6.4–8.8 μm, (2) 41.1 (34–56) μm, (3) triangle.

E. paralittoralis Wieser, 1953: (1) 2.4 μm, (2) 24.8 (15–34) μm, (3) transverse.

E. anisospiculus Nelson, Hopper, and Webster, 1972: (1) 10.8–13.2 μm, (2) 51.7 (44–63) μm, (3) reverse triangle.

Enoplus sp.: (1) 6.8 μm, (2) 42.5 (33–51) μm, (3) erratic.

As can be seen, little taxonomic significance can be ascribed to the length of the triadal setae. With the exception of *E. paralittoralis*, the same appears to be true for the position of the triad posterior to the cephalic capsule. However, the arrangement of the three setae does appear to have taxonomic merit. This newly rediscovered feature is valuable as it occurs in adult specimens of both sexes. Juveniles have a lesser number of setae, the number present being dependent upon age. One- to 2-day-old juveniles of *E. paralittoralis* have only a single lateral seta, while 9-day-old specimens have two. Preadult specimens of an age of 23 days have the groups of three setae but these vary in their arrangement from a "reverse triangle" to nearly the "transverse" pattern normally seen in adults.

When information is available for additional species, a new key to *Enoplus* species can be devised based on such characters as: presence or absence of pigmented accumulations ("eye-spots"), arrangement and size of triadal setae,

and the relation of the cephalic suture to the amphidial pouch. Only after these characteristics have been fully utilized need the male characteristics be employed in species separation. A study of the correlation between the above characters and the shape of the pre-cloacal supplement and presence or absence of semicircular plates on the spicula might be instrumental in uncovering new groupings of species of phylogenetic significance.

Biological observations on *E. paralittoralis*

Laboratory cultures of *E. paralittoralis* were established by placing decaying mangrove leaves (*Rhizophora mangle*) on the surface of a cornmeal–seawater–agar medium (Difco Corn Meal Agar, 8.5 g/l in filtered seawater of 15 ‰ salinity) into which the nematodes migrated, grew, and reproduced. Microorganisms coexisting in the mangrove leaves with the nematodes also reproduced on the cornmeal–agar and appeared to serve as a primary food source for the nematodes. Monospecific stock cultures were established by the transfer of selected specimens to new agar plates.

Life cycle studies were initiated by the transfer of freshly deposited eggs in small pieces of agar from stock cultures to new plates. Standard laboratory incubators were employed to maintain various test temperatures used in these trials. Generation time was recorded as the time elapsed between egg deposition of two successive generations.

On media of 15 ‰ salinity, the average generation periods and ranges were recorded for three different temperatures as: 21 C, 40 days (range 27–59); 24 C, 23 days (range 19–24); and 28 C, 22 days (range 19–24 days). Each life cycle average is based on five to nine separate experiments.

E. communis Bastian, 1865, is the only other *Enoplus* species for which life cycle data is known (Wieser, 1959a; Wieser and Kanwisher, 1960; McCloskey, 1970). These authors report that under natural field conditions, this species has an annual life cycle. The results of our study show that for another member of the genus, life cycle duration in laboratory culture is considerably shorter. However, we do not feel that the life cycle results for these two species are directly comparable due to the many possible divergent factors existing be-

tween field and laboratory studies. It would be of interest to determine the life cycle pattern of *E. communis* under laboratory conditions similar to those used to reveal the generation time of *E. paralittoralis*.

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On the Synonymy of *Basiroides* Thorne and Malek, 1968 with *Basiria* Siddiqi, 1959 (Nematoda: Tylenchidae), with a Note on *Neopsilenchus* Thorne and Malek, 1968

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ABSTRACT: The genus *Basiroides* Thorne and Malek, 1968, is discussed. The location of the median esophageal bulb (MB value) is discussed in various species of *Basiroides* and *Basiria* and is not considered to be a character of sufficient taxonomic importance to separate this genus from *Basiria*. The genus *Basiroides* is accordingly suppressed as a synonym of the genus *Basiria*, raising the total number of species of *Basiria* to 17. *B. indica* (Chawla et al., 1968) Khan and Nanjappa, 1971, is withdrawn to its former generic position.

Psilenchus magnidens is treated as a species of *Basiria* and the genus *Neopsilenchus* Thorne and Malek, 1968, is considered as a synonym of *Basiria*. *Psilenchus tumidus* Colbran, 1960, is regarded as a species of the genus *Clavilenchus* (Jairajpuri, 1966) Thorne and Malek, 1968.

The genus *Basiria* was proposed by Siddiqi (1959) but Goodey (1963) considered it as a synonym of the subgenus *Filenchus* Andrassy, 1954, of the genus *Tylenchus* Bastian, 1865.

Siddiqi (1963) published a new definition of the genus *Basiria*. He regarded *Basiria*, *Psilenchus*, and *Macrotrophurus* as forming a group within the Tylenchidae characterized by

slitlike amphidial openings behind the bases of lateral lips, distinct deirids and phasmids, elevated domelike head, and with corpus more than half the esophageal length. *Basiria graminophila* was designated the type species and *Psilenchus aberrans* Thorne, 1949, and *P. gracilis* Thorne, 1949, were also placed in the genus *Basiria*.

Jairajpuri (1966) proposed the retention in the genus *Psilenchus* of only the didelphic species and placed the monodelphic species in the genus *Tylenchus*. He reverted *Basiria* to synonymy with *Tylenchus* (*Filenchus*) as was proposed by Goodey (1963). The same plan was followed by Geraert (1965).

However, Geraert (1968), while reviewing *Basiria*, came to the conclusion that this was a valid genus. By attaching a very great importance to the form of the amphidial openings but not to the position of dorsal esophageal gland nor to the position of the median esophageal bulb and tail form, he listed 11 species as belonging to this genus. He transferred *Psilenchus tumidus* Colbran, 1960, *P. duplexa* Hagemeyer and Allen, 1952, and *P. magnidens* Thorne, 1949, to the genus *Basiria*. The additional species listed in the genus were: *B. aberrans* (Thorne, 1949) Siddiqi, 1963; *B. flandriensis* Geraert, 1968; *B. gracilis* (Thorne, 1949) Siddiqi, 1963; *B. graminophila* Siddiqi, 1959; *B. kashmirensis* Jairajpuri, 1965; *B. minor* Geraert, 1968; *B. noctiscripta* Andrassy, 1962; and *B. pravamphidia* Andrassy, 1963.

At almost the same time, Thorne and Malek (1968) described two new genera, *Basiroides* and *Neopsilenchus*, in the Psilenchinae and upgraded the subgenus *Clavilenchus* Jairajpuri, 1966, of the genus *Tylenchus* to a generic rank. Geraert (pers. comm.) was unaware of this work at the time he was reviewing the genus *Basiria*. Thorne and Malek (1968) proposed *Neopsilenchus* for the accommodation of a single species, *Psilenchus magnidens* Thorne, 1949, as this species is characterized by the possession of a single ovary as against the presence of two ovaries in the genus *Psilenchus*.

However, *Psilenchus magnidens* fits easily into the genus *Basiria* on the basis of its amphidial structure and tail shape. The present authors support Geraert (1968) in treating this as a *Basiria* species. The genus *Neopsilenchus* is accordingly suppressed here as a synonym of the genus *Basiria*.

Thorne and Malek (1968) are justified in placing *Psilenchus tumidus* Colbran, 1960, in the genus *Clavilenchus* because of its clavate tail as the *Basiria* species hitherto known have filiform tails only.

The genus *Basiroides* Thorne and Malek, 1968, was proposed to accommodate those nematodes that had the general characters of *Basiria* but possessed a median esophageal bulb which was located anteriorly (MB less than 50%). The two species described were *Basiroides obliquus* and *B. conurus*. Later on, Elmiligy (1971) added *B. nortoni*.

Khan and Nanjappa (1971) transferred *Trophurus indicus* Chawla et al., 1968, to the genus *Basiria*. However, because of its clavate tail, the present authors suggest the exclusion of this species from *Basiria*, relegating it to its former generic position.

Of the 14 species considered by the authors as belonging to the genus *Basiria*, six, viz., *B. duplexa* (Hagemeyer and Allen, 1952) Geraert, 1968, *B. flandriensis* Geraert, 1968, *B. magnidens* (Thorne, 1949) Geraert, 1968, *B. minor* Geraert, 1968, *B. obscura* Szczygiel, 1969, and *B. dolichura* Loof, 1971, have an MB value of 50% or less while six, viz., *B. aberrans* (Thorne, 1949) Siddiqi, 1963, *B. graminophila* Siddiqi, 1959, *B. noctiscripta* Andrassy, 1962, *B. gracilis* (Thorne, 1949) Siddiqi, 1963, *B. incita* Szczygiel, 1969, and *B. affinis* Thorne and Malek, 1968, have an MB value of 50% or more. In the remaining two species, *B. kashmirensis* Jairajpuri, 1965, and *B. pravamphidia* Andrassy, 1963, the MB value varies between 47 and 50% in the former and 47 and 52% in the latter species.

In all other characters listed by Thorne and Malek (1968) such as slitlike amphids, stylet with small to large basal knobs, anterior ovary outstretched, posterior uterine branch shorter than body diameter, and tail uniformly slender conoid to subacute terminus, *Basiroides* resembles the genus *Basiria*. The location of the median esophageal bulb can thus at best be considered to be of specific importance only. The genus *Basiroides* is accordingly suppressed here as a synonym of the genus *Basiria*. The three species described under *Basiroides* adjust well as species of the genus *Basiria*, raising the total number of species in the genus to 17.

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Gonofilaria rudnicki gen. et sp. n. (Nematoda: Filarioidea) from Malaysian Lizards[1]

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ABSTRACT: *Gonofilaria rudnicki* gen. n., sp. n. (Nematoda: Filarioidea) is described from the Malaysian lizards, *Gonocephalus borneensis* and *Acantosaura armata*. The worm belongs in the subfamily Oswaldofilariinae and is characterized by its stout appearance, markedly divided esophagus, and posterior vulva. A single immature female worm was recovered from the Malayan giant frog, *Rana macrodon*. Microfilariae are unsheathed and appear in the peripheral blood.

During routine blood collections for arbovirus studies in Selangor State, West Malaysia, microfilariae were found circulating in the peripheral blood of several specimens of the lizards *Acanthosaura armata* (no common name) and *Gonocephalus borneensis* (anglehead lizard). Adult worms were later recovered from the subcutaneous tissues of both species, principally in the thoracic and abdominal regions. Only one or two worms were obtainable from most of the hosts autopsied and a gravid worm in only one instance. A single nongravid female worm was also recovered from *Rana macrodon* (Malayan giant frog).

The species is named in honor of Dr. A.

Figures 1–8. Line drawings of *Gonofilaria rudnicki* sp. n. 1. Anterior extremity, *en face* view (not to scale), showing amphids (solid) and cephalic papillae. 2. Anterior extremity of female. 3. Posterior extremity of female, showing position of vulvar opening. 4. Microfilaria stained with Giemsa (NR, nerve ring; IK, Innenkörper; AP, anal pore). 5. Left spicule (left lateral view). 6. Right spicule (right lateral view). 7, 8. Ventral views of male tails showing papillar patterns.

Rudnick, G. W. Hooper Foundation, University of California, San Francisco, who collected the original specimens.

Materials and Methods

Lizards were anesthetized with chloroform, and blood was collected via cardiac puncture. Semi-thick films, made thin enough so that the nuclei of the host's red blood cells did not obscure the microfilariae, were stained with dilute Giemsa after air-drying.

Adult worms could easily be seen and collected when the skin was peeled away from the carcass. They were killed and preserved in 70% alcohol and 5% glycerin. The worms were studied in pure glycerin.

Measurements and drawings were made with the aid of a camera lucida and ocular micrometer. All measurements are in millimeters unless otherwise noted, with averages in parentheses.

Description

Gonofilaria gen. n.

Filarioidea: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Oswaldofilariinae Chabaud and Choquet, 1953. Thick, stout worms. Body of uniform diameter with little tapering toward blunt anterior extremity. Cuticle smooth, head smooth and rounded. Oral opening simple, surrounded by four pairs of cephalic papillae and two amphids. Esophagus moderately long, composed of a short,

narrow anterior muscular portion and a longer wider posterior glandular portion. Vulva in posterior part of body. Tail in both sexes short and bluntly rounded. Male with numerous papillae encircling anus and four to seven caudal papillae near tip of tail. Spicules short, similar, unequal. Microfilariae unsheathed, found in peripheral blood. Parasites of lizards and frogs. Type species *Gonofilaria rudnicki* sp. n.

Gonofilaria rudnicki sp. n.

With characters of the genus (description based on four males and three adult females, only one of which was gravid):

MALE: Body length 23–28 (25); greatest width 0.510–0.875 (0.718). Muscular esophagus 0.262–0.292 (0.272) long, glandular esophagus 2.2–3.0 (2.5). Nerve ring just anterior to muscular-glandular junction. Caudal papillae sessile, consisting of 21 or 22 perianal papillae arranged circumferentially around anus, and four to seven postanal papillae near tip of tail. Spicules short, stout, and similar; length of left spicule 0.300–0.320 (0.310), right spicule 0.220–0.260 (0.240). Tail 0.330–0.390 (0.365) long; posterior extremity usually coiled 1½ times.

FEMALE: Body length 49–55.5 (52); maximum width 1.25–1.46 (1.37). Length muscular esophagus 0.285–0.325 (0.310), glandular portion 2.26–3.64 (2.70). Vulva 1.80–3.85 (3.10) from posterior end. Muscular vagina approximately 1.5 long, dividing into two uterine branches that run anteriorly. Anus 0.206–0.364 (0.307) from posterior end of body.

MICROFILARIAE: Found circulating in blood. Head blunt. Cephalic space short. Last six body nuclei in single row extending to tip of tail. Body nuclei few in region of Innenkörper. Length Innenkörper 6–10 μ. Total length 0.148–0.167 (0.156). Fixed points expressed as percentages of mean body length: nerve ring, 19.2%; excretory pore, 24.4%; beginning of Innenkörper, 37.2%; G1, 67.3%; R2, 77.0%; R3 and R4, 83.5%; anal pore, 89.8%.

TYPE HOST: *Gonocephalus borneensis.*

LOCATION: Subcutaneous tissue.

ADDITIONAL HOSTS: *Acanthosaura armata* and *Rana macrodon*, both from type locality.

TYPE LOCALITY: Tanjong Rabok, Kuala Langat Forest Preserve, Selangor, West Malaysia.

SPECIMENS: Holotype (male) USNM Helm. Coll. No. 72625, Paratypes 72626.

Discussion

The production of microfilarioid embryos and the position of the vulva in the posterior portion of the body place this worm in the family Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959, and the subfamily Oswaldofilariinae Chabaud and Choquet, 1953. Four genera have been described in the subfamily Oswaldofilariinae (Chabaud et al., 1959; Yamaguti, 1961). *Conspiculum* Pandit, Pandit, and Iyer, 1929, has a longitudinally striated cuticle, equal spicules, and several pairs of large subventral caudal papillae in the male. *Piratuba* Freitas and Lent, 1947, also has a longitudinally striated cuticle; however, in the male, there are 10 or 11 pairs of caudal papillae. *Oswaldofilaria* Travassos, 1933, has seven pairs of caudal papillae. Both *Conspiculum* and *Piratuba* have an equatorial vulva and in the genus *Oswaldofilaria* the vulva is anterior to midbody. *Solafilaria* Chabaud, Anderson, and Brygoo, 1959, has a posterior vulva; however, other characters such as the two longitudinal lateral bands, markedly unequal spicules, thick spicular sheath, and tubercles on the tail of the male differentiate it from *Gonofilaria*. Because it lacks affinity with existing members of the subfamily Oswaldofilariinae, I propose *Gonofilaria* as a new genus.

According to Dunn and Ramachandran (1969) records of filarial parasites in Southeast Asian reptiles and amphibians are few. Two species of the genus *Icosiella* have been reported from amphibians in Vietnam, Singapore, and Malaysia, and *Hastospiculum macrophallos* from the monitor lizard is the only species recorded from reptiles in the region.

Since no extensive surveys were made, the host and geographic range of *G. rudnicki* are unknown. The parasite was found fairly often in the two species of lizards (*Acanthosaura armata* and *Gonocephalus borneensis*) in the Tanjong Rabok peat swamp forest, but in only one frog (*Rana macrodon*), and the single specimen recovered from it was an immature female. Whether frogs are a suitable host to maintain this parasite is unknown.

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Helminth Fauna of Nicaragua. V. *Cardiofilaria stepheni* sp. n. (Onchocercidae) and Other Nematodes of Birds

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ABSTRACT: Eight species of nematodes are reported from birds, all new records for Nicaragua. *Cardiofilaria stepheni* sp. n., from *Cymbilaimus lineatus*, has spicules 90 to 95 and 118 to 122 μ long. It differs from *C. andersoni* and *C. chabaudi* in possessing a well-defined preesophageal ring, and in having more caudal papillae.

This is the fifth of a series of reports based on a collection of helminth parasites of vertebrates made by the second author at the Agricultural Experiment Station, El Recreo, Zelaya, Nicaragua, on the Rio Escondido. Previous papers were published by Neiland (1955, 1961) and Schmidt and Neiland (1966, 1971). One hundred and sixty-seven birds representing 84 genera and 35 families were examined in June and July 1954. Data on the bird hosts were published by Howell (1957).

Specimens were fixed in hot alcohol and cleared by glycerin–alcohol dehydration. All measurements are in microns unless otherwise stated.

Eight species of nematodes could be identified (Table 1). All are new records for Nicaragua, and most are new host records. One new species is described herein.

Cardiofilaria stepheni sp. n.
(Figs. 1–6)

Two males and five females found in the mesenteries of the liver, gizzard, and intestine of a fasciated antshrike, *Cymbilaimus lineatus*, represent a new species and form the basis of the following description. The species is named in honor of Stephen Schmidt, who has made many contributions to the laboratory of the senior author.

Description

Medium-sized worms, rounded at both ends, widest at about middle. Cuticle smooth, lacking bosses or conspicuous striae. Mouth (Fig. 1) round or dorsoventrally elongated. Peribuccal ring absent. Four papillae in outer circle, four in inner circle. Amphidial pores conspicuous. Alae absent. Buccal capsule

Table 1. Nematodes from Nicaraguan birds.

Species	Host	Host order and family	USNM No.
Allodapa (A.) travassosi (Barreto, 1919) Quentin, 1969.	*Notharcus macrorhynchus* *Trogon massena	Piciformes, Bucconidae Trogoniformes, Trogonidae	72362 72363
Ascaridia hermaphrodita (Froelich, 1789) Railliet et Henry, 1914	*Amazona autumnalis	Psittaciformes, Psittacidae	72366
Cardiofilaria stepheni sp. n.	*Cymbilaimus lineatus	Passeriformes, Formicariidae	
Cyrnea (C.) semilunaris (Molin, 1860) Seurat, 1914	Piaya cayana	Cuculliformes, Cuculidae	72365
Diplotriaena agelaius (Walton, 1927) Anderson, 1959	*Gymnostinops montezuma	Passeriformes, Icteridae	72364
Porrocaecum ardeae (Froelich, 1802) Baylis, 1936	Florida caerulea	Ardeiformes, Ardeidae	72361
Subulura (S.) carlosi Barreto, 1919	*Nyctidromus albicollis	Caprimulgiformes, Caprimulgidae	72368
Thelazia digitata Travassos, 1918	*Trogon massena	Trogoniformes, Trogonidae	72367

* New host record.

(Fig. 2) small, with thick cuticular wall (pre-esophageal ring). Esophagus (Fig. 3) not divided, muscular throughout. Nerve ring at about anterior one-third of esophagus. Excretory pore at level of nerve ring or posterior to it. Deirids absent.

MALE: 11.0 to 11.5 mm long, 145 greatest width. Buccal cavity 8 deep, 8 to 10 wide. Esophagus 440 to 480 long. Nerve ring 150 to 170, excretory pore 170 to 225 from anterior end. Tail (Figs. 4, 5) rounded, 50 to 55 long. Spicules unequal, dissimilar; right 90 to 95 long, with rounded tip, left 118 to 122 long with pointed tip. Gubernaculum absent. Caudal papillae (Fig. 5) arranged as follows: 10 small in circle around anus, one large pair behind these, followed by two pairs near end of tail.

FEMALE: 20.0 to 23.5 mm long, 195 to 200 greatest width. Buccal cavity 8 to 10 wide, 8 to 10 deep. Esophagus 480 to 512 long. Nerve ring 140 to 170, excretory pore 170 to 230 from anterior end. Tail (Fig. 6) bluntly rounded; anus atrophied, 50 to 90 from posterior end. Vulva posterior to esophagus, slightly salient, 1.8 to 2.0 mm from anterior end. Microfilaria 100 to 110 long, with blunt head and sharp tail.

TYPE HOST: Fasciated antshrike, *Cymbilaimus lineatus fasciatus* (Passeriformes, Formicariidae).

LOCATION: Mesenteries of liver, gizzard, and intestine.

TYPE LOCALITY: El Recreo, Zelaya, Nicaragua.

TYPE SPECIMENS: USNM Helm. Coll. Holotype male no. 72491, allotype female no. 72492, paratype females no. 72493.

Remarks

Cardiofilaria Strom, 1937, was reviewed by Anderson and Freeman (1969), who included 10 species. *C. pyrrhurae* (Freitas et Mendonça, 1952) Sonin, 1968, was tentatively assigned to *Sarconema* Wehr, 1939, by Anderson and Prestwood (1969); the short tail and arrangement of caudal papillae exclude this species from *Cardiofilaria*. Similarly, Anderson and Prestwood (1969) placed *C. micropenis* (Travassos, 1926) Sonin, 1963, into the genus *Eufilaria* Seurat, 1921. The short tail of this species excludes it from *Cardiofilaria*.

The species with spicules most similar in size to those of *C. stepheni* sp. n. are *C. andersoni* (Chabaud, Brygoo et Richard, 1964) Dissanaike et Fernando, 1965, which has spicules 83 and 102 long; and *C. chabaudi* Dissanaike et Fernando, 1965, which has spicules 75 and 100 long. Both are parasites of birds of Madagascar. Both differ from *C. stepheni* in lacking a sclerotized preesophageal ring, and in number of caudal papillae. The genus has not previously been reported from Central or South America, or from this host.

Dissanaike and Fernando (1965) suggest that the characteristically shaped distal end of the right spicule might be a constant generic character for this group. The presence of this character in *C. stepheni* supports this premise.
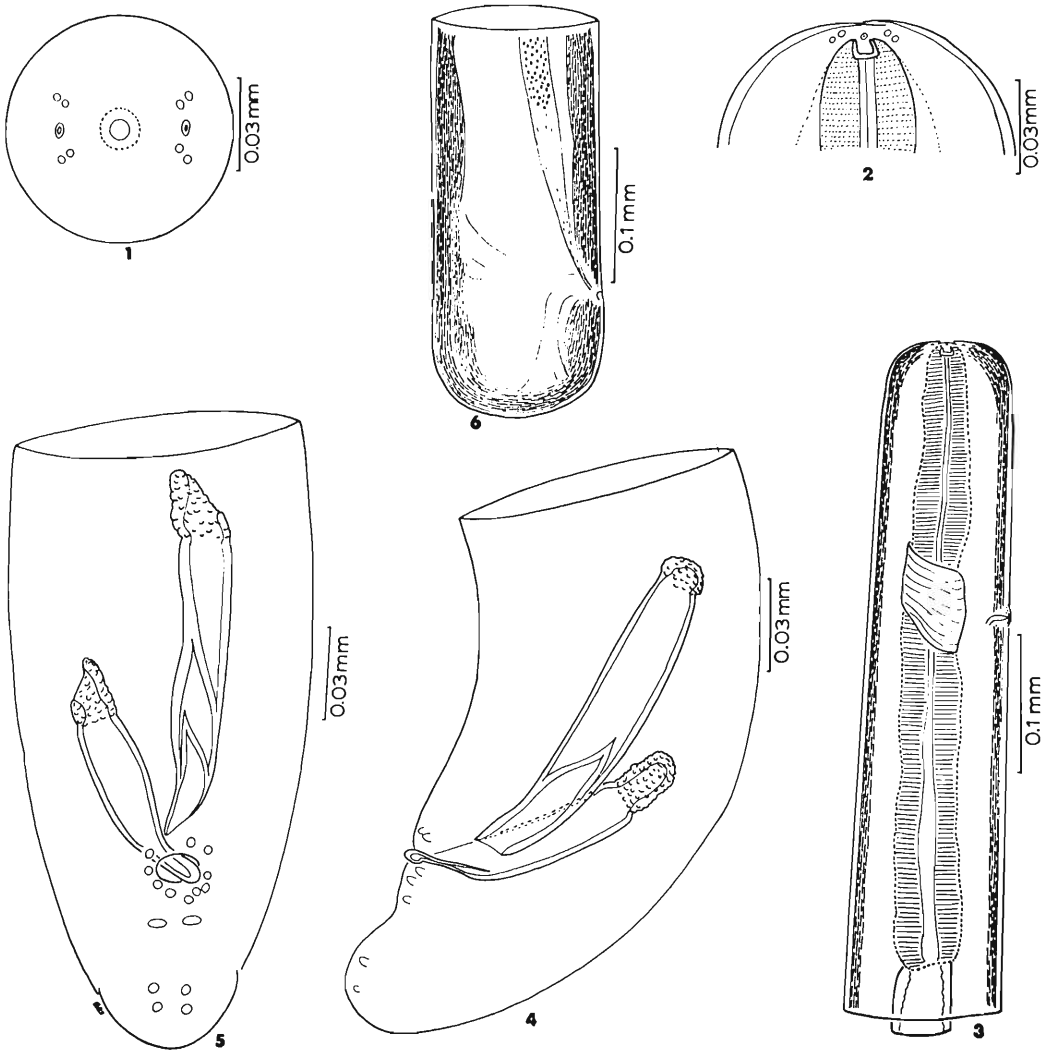
Figures 1-6. *Cardiofilaria stepheni* sp. n. from an antshrike in Nicaragua. 1. En face. 2. Anterior end, lateral view. 3. Anterior end of female, lateral view. 4. Tail of male, lateral view. 5. Tail of male, ventral view. 6. Tail of female, lateral view.

The right spicule appears to function as a gubernaculum for the left spicule in this genus; the shape of its tip is quite typical of the gubernaculum of many nematodes.

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Relationship of Spaced Administration of Larvae to Worm Burden in Calves

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ABSTRACT: This experiment tested the effect of spaced administration of larvae on establishment, maturation, and persistence of worms, and on egg output. Calves in three groups of four each were inoculated with *O. ostertagi, T. axei, C. oncophora, T. colubriformis,* and *O. radiatum* as follows: group 1—1 dose of 200,000; group 2—10 doses of 20,000 in 13 days; group 3—four weekly doses of 50,000 in 21 days. Average eggs/g of feces (EPG) was equal in the three groups up to the time the population age averaged about 1 month; however, at about 2 months, the EPG of group 3 was lower than the others. At 1 month, recovery of *T. axei, C. oncophora, O. radiatum,* and generally *O. ostertagi* did not differ between groups. The poorest take occurred with *T. colubriformis;* more of this species were recovered after single than spaced exposure. Rate of maturation of the worm populations was equal between groups. It varied markedly between calves for *C. oncophora;* the rate was slower for *C. oncophora* and *O. radiatum* than for others. There was no significant loss of *T. axei, C. oncophora,* and *O. radiatum* between 1 and 2 months in any group; however, there was considerable loss of *O. ostertagi* and *T. colubriformis.*

This study was one in a series on the relationship of rate and period of administration of larvae to worm burden (Goldberg, 1973). Specifically, the main purpose of the present study was to test the effect of spaced administration of larvae on the establishment, development, and persistence of worms, and on egg output.

Dineen et al. (1965) inoculated sheep with 100 *Haemonchus contortus* larvae/day for 30 consecutive days. Considering the time ordinarily taken for the species to attain the fifth stage, the time of occurrence of peak egg production, and the degree of maturation of nematode populations when the sheep were necropsied, they reasoned that it seemed likely that infective larvae given after day 9 or 10 failed to develop beyond the fourth stage. Under natural grazing conditions, Durie and Elek (1966) considered that larvae of *Oesophagostomum radiatum* ingested by calves after the first week did not contribute significantly

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to the adult population found at necropsy. However, despite the inability of the larvae to complete their development, pathogenic effects of the continuous intake continued until the 20th week. However, Michel (1970), who inoculated calves with 200 to 1,600 *Ostertagia ostertagi* daily, considered that there was continuous turnover in the adults so that a new population of them was present about every 5 weeks. The present study provided evidence relevant to this problem.

Materials and Methods

The experiment used 12 Holstein steers, 4.6 months old at the time of single or initial inoculation, raised worm-free, and maintained in individual concrete stalls under conditions preventing extraneous infection.

Infective larvae were obtained from sphagnum-cultured calf feces (lamb feces were used for *Trichostrongylus colubriformis*). The source animals had been monospecifically infected. Each calf was given 44,000 *O. ostertagi*, 73,000 *T. axei*, 44,000 *T. colubriformis*, 36,000 *Cooperia oncophora*, and 3,000 *O. radiatum*. The larvae were administered orally (10 ml of water/dose) to calves in three groups of four each. Those of group 1 were given a single dose. Those of group 2 were given 10 doses of 20,000 larvae in 13 days on consecutive workdays. Those of group 3 were given four weekly doses of 50,000 larvae in 21 days. Calves of groups 2 and 3 were given their first dose at the time those of group 1 were inoculated. It was considered that the number of larvae of each species administered would result in infections commonly occurring under field conditions.

For comparison of egg output between groups, egg counts were made on rectal fecal samples collected weekly from each calf. The eggs were recovered by centrifugal flotation in saturated NaCl solution, and the number in 1 g of feces/sample was counted, or the number in ⅓ to ½ g when the EPG exceeded 1,000.

To estimate the extent of spontaneous loss of worms, 75- to 100-g fecal samples were collected weekly from each calf, pooled by groups, and screened. Two aliquots/screening, amounting to 1.3 to 4.0% of the material retained by the screens (23.6 meshes/cm), were examined for worms.

Six calves (two from each group) were necropsied for recovery of parasites when the worm population age averaged 32 to 36.5 days and six when it averaged 61 to 64.5 days. Duplicate 0.5 to 1% aliquots were taken from the combined contents and washings of stomach, of small intestine, and of cecum and colon. When the number of worms was large, smaller aliquots were examined. Nevertheless, in such cases, each sample contained about 100 worms. Since there were fewer *O. radiatum* than the other species, a 6.3 to 10% aliquot of each large intestine collection was screened and examined for that species. The *O. radiatum* nodules in the intestines were dissected to determine their worm content. The abomasum was digested after thorough washing, and duplicate 5% aliquots were examined for worms.

Nongravid fifth-stage females, not obviously senescent, and fifth-stage males, before darkening of the spicules, were counted as immature fifth-stage worms. Fourth-stage larvae that remained in the newly molted condition, despite elapse of sufficient time for growth, were counted as inhibited fourth-stage larvae.

Results and Discussion

Average EPG up to the time the population age averaged about 1 month was 328, 315, and 303 in groups 1 to 3, respectively. Peak EPG of groups 1 to 3 averaged 689, 1,242+ and 698+, respectively, and occurred 33, 47+, and 45+ days atfer the first dose, respectively. The averages for groups 2 and 3 are not exact because apparently the peak had not yet been attained in one calf of group 2 and two calves of group 3, since the count was highest at the time of necropsy. Its occurrence in groups 2 and 3 at least 12 to 14 days after that in group 1 perhaps indicated that in spaced administration of larvae, establishment and development to maturity can occur for at least that period, somewhat longer than the period suggested by Dineen et al. (1965) and Durie and Elek (1966). EPG averaged 668, 837, and 351 in groups 1, 2, and 3, respectively, in calves necropsied when worm population age averaged 2 months. The average final count, when the population age averaged 2 months, was 825, 1,213, and 159 in groups 1, 2, and 3, respectively.

Table 1. Worms recovered at necropsy at averages of about 1 and 2 months after single or spaced administration of larvae.

Larvae administered	1 dose of 200,000				10 doses of 20,000 in 13 days				4 weekly doses of 50,000 in 21 days			
Calf number	524	526	523	525	531	534	532	533	528	529	527	530
Age of worms (days)	32	32	61	61	35.3 (29–42)	35.3 (29–42)	63.3 (57–70)	63.3 (57–70)	36.5 (26–47)	36.5 (26–47)	64.5 (54–75)	64.5 (54–75)
O. ostertagi												
Mature adults	27,320	5,410	12,795	12,190	31,963	31,400	12,410	5,020	29,056	29,100	2,120	5,865
Immature 5th	40	10	100	10	60	330	30	30	1,620	1,030	0	100
Developing 4th	0	0	0	0	280	50	0	0	40	0	0	100
Inhibited 4th	20	10	20	10	130	100	30	30	290	310	20	290
T. axei												
Mature adults	28,510	19,590	22,920	25,340	22,540	19,910	19,825	19,600	22,790	14,360	13,890	15,410
Immature 5th	0	520	0	0	510	20	0	0	0	0	0	0
C. oncophora												
Mature adults	13,825	300	22,062	22,300	2,313[1]	24,750	25,730	26,600	10,763	6,900	14,925	19,350
Immature 5th	350	3,225	525	500	8,375[1]	150	175	600	3,763	3,075	1,125	1,400
Developing 4th	0	0	0	200	63	300	0	600	1,226	1,125	225	0
Inhibited 4th	2,100	2,550	350	100	188	1,200	0	600	1,400	1,125	600	100
T. colubriformis												
Mature adults	18,525	2,875	700	300	2,088	750	525	0	175	150	175	0
Immature 5th	175	0	0	0	0	0	0	0	0	0	0	0
Developing 4th	175	0	0	0	0	0	0	0	0	0	0	0
O. radiatum												
Mature adults	855	690	1,230	1,344	642	612	1,456	1,204	696	690	770	372
Immature 5th	630	1,220	0	0	876	516	32	0	372	228	0	12
Developed 4th[2]	2	0	6	4	2	6	2	8	31	50	4	2
3rd stage[2]	19	21	51	23	37	21	15	1	22	31	3	16

[1] Most were abnormal, showing disproportionate growth, irregular swelling, and vacuolation.
[2] All recovered from nodules except 24 and 48 developed fourth stage from calves 528 and 529, respectively.

This study indicated that worms are generally as readily established by multiple doses administered during a period of 13 or 21 days as by a single equivalent dose. Numbers of worms recovered at necropsy are given in Table 1. At 1 month, recovery of *T. axei*, *C. oncophora*, *O. radiatum*, and generally *O. ostertagi* did not differ appreciably between groups. The percentage of *T. colubriformis* that became established was almost invariably less than that of the other species, perhaps partly because the source animal was a lamb. More of this species were recovered after single exposure than after equivalent, spaced, multiple exposure.

As in some of my previous studies (Goldberg, 1973), individual difference in susceptibility to *C. oncophora* was greater than the effect of rate and period of administration of larvae on worm burden. The worms of this species in calf 531 were abnormal, showing disproportionate growth, irregular swelling, and vacuolation. The condition is rare but its cause may be worth investigating. If it is due to an agent that can be cultivated and fed to cattle without ill effects on them, it would be a possible means of controlling the parasite. It seems to act specifically against *C. oncophora* because the other species were not affected.

The stage of development of the worms is

given in Table 1. Rate of maturation of the worm populations was about equal among groups. It varied markedly between animals for *C. oncophora*. It was slower for *C. oncophora* and *O. radiatum* than for the other species, because development of part of the population of the former was inhibited, and because of the normally longer maturation period of the latter. At an average of 32 to 36.5 days, an average of 66% of the *C. oncophora* and 51% of the *O. radiatum* were mature adults, whereas 97 to 99% of the other species were mature. At an average of 61 to 64.5 days, 95 to 100% of the worms of all species were mature.

As determined from the worms recovered antemortem and at necropsy, there was no significant loss of *T. axei*, *C. oncophora*, and *O. radiatum* between 1 and 2 months in any group. The maximum recovery of these species from the feces was 40, 56, and 3 worms/1,000 g, respectively, between 7 and 8 weeks after the single or initial dose. There was considerable loss of *O. ostertagi* and *T. colubriformis*, but least for *O. ostertagi* in group 1. The maximum recovery of *O. ostertagi* from the feces was 378 and 413 worms/1,000 g for groups 2 and 3, respectively, 6 weeks after the initial dose, and 81/1,000 g for group 1, 8 weeks after inoculation. Maximum recovery of *T. colubriformis* was 237/1,000 g, 5 weeks after inoculation in group 1, and 24 and 61 in groups 2 and 3, respectively, between 3 and 8 weeks after the initial dose. All worms recovered antemortem were mature adults.

The average percentage of worm recovery (ratio of larvae administered to worms recovered, in %) at necropsy for *T. axei* at 1 and 2 months was 33 and 33, 29 and 27, and 25 and 20 for groups 1 to 3, respectively. For *O. radiatum* it was 57 and 44, 45 and 45, and 35 and 20 for groups 1 to 3, respectively. For *C. oncophora* it was 31 and 63, 73 and 75, and 40 and 52 for groups 1 to 3, respectively. The *C. oncophora* recovery from the calf with abnormal worms of this species was not considered in the computation. For *T. colubriformis*, it was 25 and 1, 3 and 1, and 0.4 and 0.2 for groups 1 to 3, respectively. For *O. ostertagi*, it was 38 and 29, 73 and 20, and 70 and 10 for groups 1 to 3, respectively. In this study, rapid turnover in adult *O. ostertagi*, suggested by Michel (1970) for the conditions of his study, did not occur since many of those passed were not replaced.

Acknowledgment

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A. O. FOSTER
Secretary-Treasurer

Research Note

The Use of Ducks as Biological Control Agents of *Fasciola hepatica*[1]

A search of the literature indicates sporadic interest in the use of waterfowl as control agents in molluscan-borne diseases dating back to 1919 (Elgood and Cherry, 1919, Lancet 197: 636–637; Cawston, 1920, J. Trop. Med. Hyg. 23: 274–276; and others). This interest was revived in 1969 by several "Letters to the Editor" published in The Veterinary Record. Some correspondents believed that waterfowl would effectively reduce the population of snail intermediate hosts of helminth diseases (Hancock, 1969, Vet. Rec. 84: 228; Locke, 1969, Vet. Rec. 84: 260; Campbell, 1969, Vet. Rec. 84: 444; Gordon and Boray, 1970, Vet. Rec. 86: 288–289; Cooke, 1970, Vet. Rec. 86: 707). Others felt that these birds would be of little value because of the high reproductive capacity of the snails or the selective feeding habits of the birds, particularly geese (Fennell, 1969, Vet. Rec. 84: 260; Boutflower, 1969, Vet. Rec. 84: 351; Allonby, 1969, Vet. Rec. 84: 351–352; Heppleston, 1969, Vet. Rec. 84: 469). Despite the considerable interest generated by this controversy, only a few practical tests have been made of the effectiveness of this type of control (Karmatskaya, 1955, Veterinariya 32: 42–43; Shiff and Mowbray, 1970, Rhodesia Agr. J. 67: 28–29).

During part of the late summer months of 1969, 1970, and 1971, we evaluated the possible effectiveness of 9-week-old Rouen ducks as biological control agents to reduce a natural population of *Lymnaea* (*Stagnicola*) *palustris*, within an endemic liver fluke area near Springerville, Arizona. Although this aquatic snail does not appear to be one of the principal intermediate hosts of the common liver fluke, *Fasciola hepatica*, in other areas of the world, it is our principal laboratory host (Wilson and Samson, 1971, Proc. Helm. Soc. Wash. 38: 52–56) and has been experimentally infected

by others (Kendall, 1949, Vet. Rec. 61: 462; Krull, 1934, N. Am. Vet. 15: 13–17; Berghen, 1964, Exp. Parasit. 15: 118–124). Circumstantial evidence also indicated that it was responsible for an outbreak of fascioliasis in California (Hjerpe, Tennant, Crenshaw, and Baker, 1971, J. Am. Vet. Med. As. 159: 1266–1271). It also appears to be the chief intermediate host within the area where our tests were made.

The ducks were used in small-scale controlled tests for 4 days during either the months of August or September, depending upon the amount of moisture and the snail population of the area. We confined 6 ducks in a 10-by 10-ft portable screened pen for 23 hours and then moved the pen to a new location within four selected plots on boggy and flooded pastures. The ducks seemed to prefer the snails but readily ate other small invertebrates and plant material. No supplemental feed was provided.

The snail population was estimated before and after introduction of the ducks by counting the snails in comparable template samples 1 foot square. Since the snails were not evenly distributed over the entire plot, the samples did not necessarily represent $\frac{1}{100}$ of the population but were made as representative as possible. All vegetation and mud to a depth of 2 inches were removed and carefully screened to recover all the snails in each sample area. The snails were floating on the water, attached to the vegetation, and on or embedded in the mud of the samples. Although snails representing the genera *Lymnaea*, *Physa*, and *Gyraulus* were found on some plots, only the counts of the *Lymnaea* species (the potential hosts of *Fasciola*) are given in Table 1. The results of each year's test are tabulated separately. Other snail species were readily eaten, but no estimate of population reductions were made since these were not vectors of *Fasciola*. Because of the moisture conditions of the general area and the reproductive potential of the different snail species, the counts

within the experimental plots varied from year to year and from plot to plot. During our tests in 1970 and 1971, cold and inclement weather appeared to reduce the feeding activity of the ducks; in 1971, one duck died the third night.

The ducks appreciably reduced the population of lymnaeid snails within the plots and, weather permitting, might have reduced the population even more. They were observed actively eating the snails on the surface of the mud and floating in the water. However, their stomachs were not examined for snails or snail shells at the termination of each year's test.

Similar tests were conducted in southern Colorado within an enzootic fluke area near Durango in 1968 using white Chinese geese. The geese fed primarily on grass and aquatic plant material and did not noticeably reduce the natural snail population. We agree with Boutflower (1969) that geese are primarily vegetarian in their eating habits.

Our observations indicate that Rouen ducks might effectively reduce snail populations within small wet enzootic pastures such as are found in Arizona, Colorado, and New Mexico provided they are confined on pastures during spring and summer. However, they might not have practical use in large areas of bog similar to those along the Gulf Coast.

We wish to thank Dr. Joseph P. E. Morrison, Associate Curator, Division of Mollusks, National Museum of Natural History, Washing-

Table 1. Reduction of snail populations by Rouen ducks in 100-square-foot plots; six ducks per plot.

Plot No.	Lymnaeid snails per square-foot sample		Per cent lymnaeids destroyed
	Before ducks	After ducks	
	1969		
1	163	10	94.0
2	63	6	90.5
3	224	10	95.5
4	145	13	91.0
	595	39	93.4
	1970		
1	63	4	93.7
2	67	22	67.2
3	77[1]	104	0.0
4	16	3	81.3
	223	133	40.4
	1971		
1	23	0	100.0
2	28	1	96.4
3	26[1]	11	57.7
4	75[2]	29	61.3
	152	41	73.0

[1] Inclement weather.
[2] Only five ducks were used.

ton, D. C., and Dr. Richard H. Russell, Department of Biological Sciences, University of Arizona, Tucson, for their help in identifying the snails.

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Research Note

Amphimerus vallecaucensis nom. n., a Replacement for A. minimus Thatcher, 1970 (Trematoda: Opisthorchiidae) Preoccupied

Amphimerus minimus Thatcher, 1970 (Proc. Helm. Soc. Wash. 37: 207–211) from a Colombian opossum has recently been found to be a junior homonym of *A. minimus* Chertkova, 1963, from a bird in the Soviet Union (Chertkova, A. N., 1963, in Gelmint. Chelovieka, Zhivot. Rasteb. i Borba nimi).

To replace the name *A. minimus* Thatcher,

1970, *A. vallecaucensis* is hereby proposed. The new name makes reference to the area in which the type locality of the parasite is located.

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Research Note

Excystation of *Sarcocystis fusiformis* Sporocysts from Dogs

Sarcocystis was demonstrated to be a coccidian parasite when organisms liberated from intramuscular cysts entered cultured cells and developed to gametes and a stage resembling an oocyst (Fayer, 1970, Science 168: 1104–1105; 1972, Ibid. 175: 65–67). Heydorn and Rommel (1972, Berl. Munch. Tierarztl. Woch. 85: 121–123) found that dogs, when fed bovine gullets infected with S. *fusiformis*, passed sporocysts in their feces 9 or 10 days later. The present study was undertaken to elucidate the factors necessary for excystation of sporozoites from such sporocysts obtained by feeding infected bovine heart to dogs.

Fecal samples from 11 4-month-old beagle puppies were examined for 25 consecutive days without finding any coccidia. Six puppies were then fed approximately 1 pound of minced raw cattle heart that contained zoites of S. *fusiformis*. Five control puppies were fed dry dog food. Feces from all 11 puppies were examined daily thereafter for 36 days.

Pairs of sporocysts as well as single sporocysts were first observed in the feces of each of the six puppies 13 days after their having eaten the infected heart. Each sporocyst contained four sporozoites. Since a distinct wall surrounding the paired sporocysts could not be seen, it could not be determined if these pairs were isosporan oocysts. Only single sporocysts were found during the following 23 days that feces were collected. No coccidia were observed in the feces from the five control dogs.

The sporocysts were cleaned of fecal debris as described by Hammond et al. (1968, J. Parasit. 54: 550–558) before incubation at 37 C in test solutions. All solutions containing trypsin, bile, or both were at pH 7.4 to 7.8. Each type of bile was pooled from three or more animals.

Sporozoites failed to excyst when sporocysts were incubated for 3½ hr in the following solutions: (1) 0.25% trypsin and 5% chicken bile in Ringer's solution; (2) 0.4% trypsin and 8% bovine bile in Ringer's solution; and (3) 0.4% trypsin and 8% bovine bile in 0.2 M Tris maleate aqueous solution. Sporozoites also failed to excyst when sporocysts were incubated 20 hr in Ringer's solution followed by an additional 20 hr incubation in solution 1. These findings were unexpected since Farr and Doran (1962, J. Prot. 9: 403–407) successfully used trypsin–bile to induce excystation of sporozoites from sporocysts of four species of *Eimeria* and many subsequent investigators have excysted other *Eimeria* and *Isospora* species from released sporocysts with similar mixtures.

Jackson (1962, Nature 194: 847–849) found that, before treatment with trypsin and bile, pretreatment with carbon dioxide was essential for excystation of sporozoites from intact oocysts from sheep and that its action was enhanced by cysteine and other reducing agents. In the present study, sporocysts suspended in 5 ml of 0.02 M cysteine hydrochloride were incubated for 1 to 18 hr in a 50% CO_2–50% air atmosphere or for 18 hr in air alone. The suspension was then centrifuged at 290 *g* for 5 min, the supernatant decanted, and the sporocysts resuspended in mixtures of trypsin, bile, or both in either Ringer's solution or in 0.2 M Tris maleate solution (Table 1). Samples were examined at ½-hr intervals until either excystation had occurred or a period of 3½ hr had elapsed. Certain relationships were evident: (1) excystation occurred whenever bovine bile was used, even in the absence of trypsin, and after sporocysts had been preincubated in cysteine with an air atmosphere; (2) no excystation occurred when canine bile was used or when trypsin was used without bile; (3) as incubation time in 50% CO_2–50% air increased from 1 to 5 hr, the time required for excystation in trypsin–bovine bile solution decreased (Table 1). Although the greatest amount of excystation (90%) occurred after 18 hr pretreatment and 90 min in trypsin–bile solution, 70% occurred after 5 hr pretreatment and 30 min in trypsin–bile solution, and 65 to 75% occurred after 2 to 4 hr pretreatment and 120 min in trypsin–

Table 1. Excystation of *Sarcocystis fusiformis* sporozoites.

Incubation in cysteine												
Atmosphere	50% CO_2–50% Air											100% Air
Time (hr)	1	2	3	4	5	18	18	18	18	18	18	18
Incubation in trypsin–bile												
% trypsin	0.4	0.4	0.4	0.4	0.4	0	0.4	0.4	0.4	0.4	0.4	0.4
% bile	8	8	8	8	8	8	0	8	8	8	8	8
Type of bile	B	B	B	B	B	B	0	B	B	C	C	B
Medium	R	R	R	R	R	R	R	R	T	R	T	R
Excystation first observed Time (min)	120	60	60	60	30	30	—[1]	90[2]	90[2]	—[1]	—[1]	120

B = Bovine bile.
C = Canine bile.
R = Ringer's solution.
T = Tris maleate.
[1] Samples observed for 3½ hr; no excystation.
[2] Samples not observed before this time.

bile. Percentage of excystation was calculated from the number of empty sporocysts in the first 50 sporocysts observed at each time interval.

Sporocysts examined within 24 hr after passage in the feces averaged 10.7 by 16.4 μ ($N = 30$); they had no Stieda body, and contained four indistinct sporozoites and a compact granular residuum (Fig. 1). Sporocysts suspended in trypsin–bile solution after incubation in cysteine with a 50% CO_2–50%

air atmosphere appeared larger and the sporozoites and residuum within were separated from one another and appeared quite distinct (Fig. 2). Immediately before excystation, the sluggish twisting movements of the sporozoites became more rapid and the shape of the sporocyst changed from ovoid to asymmetrical as a transverse break occurred in the wall (Fig. 3). Granules from the residuum appeared to be forceably expelled through the broken wall and, within 50 to 70 sec, the four sporozoites
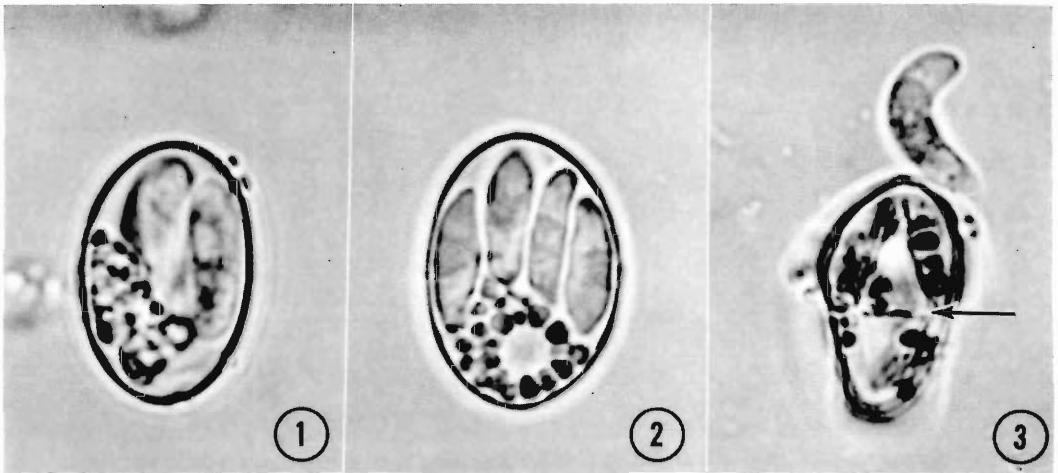
Figures 1–3. Photomicrographs of *Sarcocystis fusiformis* sporocysts. × 2,000. 1, Untreated sporocyst, typical of those observed in the daily fecal flotations. 2, Sporocyst incubated in cysteine hydrochloride with 50% CO_2–50% air atmosphere for 2 hr at 37 C. Sporocyst appears larger, showing separated sporozoites and residuum. 3, Excysted sporocyst; note transverse split (arrow), and free sporozoite.

usually departed at the same location with short gliding movements.

In the present study, excystation of sporozoites from sporocysts resembles excystation of cimerian sporozoites from intact oocysts in that either a CO_2 or reducing agent stimulus or both are required prior to incubation in trypsin–bile solution. The fact that canine bile failed to excyst sporozoites is unexplainable.

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Research Note

A Rapid Lactophenol–Glycerin Method for Preserving Nematodes[1]

There are several methods using lactophenol as an intermediate solution to facilitate passage of nematodes into glycerin. Baker (1953, Can. Entomol. 85: 77–78) was one of the first to develop such a method for nematodes while Tarjan (1967, Nematologica 13: 153–154) proposed a variation of that technique. These methods adhere to the logical premise that specimens must be passed gradually through a series of solutions each with an increasing concentration of glycerin. As is often the way with discoveries, a classroom demonstration to show the impossibility of transferring nematodes directly from pure lactophenol to pure glycerin provided a surprise for all concerned. A new method was developed which, after a year of testing, was taught to technical assistants in Central America. Those technicians prepared well over 200 slides in 1969 and 1971. The mounted specimens are frequently examined and, with very few exceptions, are in good condition.

There are no anatomical parts of the nematodes that are distorted or obscured any more by this method than by other preservative methods. The outlet of the dorsal esophageal gland is just as recognizable as it would be if processed by the usual glycerin methods. In several cases, the granular nature of the gland immediately adjacent to the outlet of the duct becomes and remains quite visible. Lateral incisures, gonad structure, spicular detail,

various ducts, and tail annules are wholly discernible by this technique.

One shortcoming of the procedure is that improperly fixed specimens will collapse, or in some cases develop swollen cuticular areas which remain as permanent distortions of the specimen. I am unable to say definitely what "improper fixation" is (assuming that a proper fixative was used) except possibly the result of specimens being kept in the fixative for too short a time (possibly less than 24 hr). Yet, some specimens kept several days in fixative will distort in this and other lactophenol procedures. Troublesome genera, with reference to fixability, are Criconemoides, Trichodorus, and Belonolaimus. Quite often, specimens belonging to these genera will pass into glycerin without any cuticular distortions, suggesting that they had been properly fixed. Other times it is possible to restore partially collapsed specimens by allowing them to remain in hot glycerin (72 C) for various lengths of time during which they regain their normal shapes.

The method is simple. One should have on hand a rheostat-controlled "hot plate" or warming table which has been calibrated to 72 C. This is done by placing on it a 3-mm-thick glass depression slide with the cavity filled to excess with glycerin. By vigilantly rotating the bulb of a thermometer in the glycerin one can determine the proper temperature. Ordinary lactophenol can be used. The formula which I employ is: liquid phenol, 209 ml; lactic acid, 177 ml; glycerin, 354

ml; and distilled water, 230 ml. With this lactophenol, prepare a small amount of a 1% cotton blue dye stock solution. The nematode staining solution is prepared by adding 6 drops of the stock 1% cotton blue lactophenol to 50 ml of clear lactophenol.

With the slide containing the staining solution at the proper temperature of 72 C on the hot plate, fixed specimens are transferred directly into the solution. After completing the transfer, the slide should be taken from the hot plate and momentarily examined to insure that the nematodes are well immersed in the staining liquid. I have found that a 1- to 3-minute immersion of the nematodes in the lactophenol stain solution will suffice. The nematodes can then be transferred directly to a depression slide containing glycerin heated to the same temperature. The specimens should be heated in glycerin for at least 5 minutes after which the nematodes may be mounted in glycerin on slides or the depression slide with the nematodes can be stored in a desiccator for the complete withdrawal of any remaining water.

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Research Note

An Examination of the Australian Blind Cavefish, *Milyeringa veritas* Whitley, 1945, for Helminth Parasites

In the summer of 1971, 26 specimens of the rare troglobitic species of blind gudgeon, *Milyeringa veritas* Whitley, 1945, were kindly loaned to us from the Western Australian Museum at Perth by Mr. R. J. McKay. The fish were collected on a number of occasions by different persons from Milyering Well. The well is relatively shallow (2.5 m) and is located in coral rock on Yardie Creek Station, 20 miles southwest of Vlaming Head, North West Cape Peninsula. Systematics of the fish and a description of its habitat are presented by Mees (1962, J. Roy. Soc. West. Austral. 45: 24–32).

The gills on one side and the viscera of each specimen were removed and carefully examined. In addition, the body cavity, body surface, and mouth were thoroughly checked for the presence of any cysts or other parasitic stages.

It is interesting that the only helminth parasite recovered was a single specimen of a nematode, a juvenile *Acuaria* sp. from the body cavity of one of the fish. According to Dr. G. D. Schmidt of the University of Northern Colorado, who identified the worm, nematodes of this genus normally occur as adults in the gizzards of terrestrial birds. The fish probably acquired the worm from eating an infected arthropod which fell into the well. Mees (loc. cit.) states that numerous isopods live under the covers of Milyering and other wells in the area. The occurrence of the juvenile nematode in the body cavity of the fish undoubtedly indicates that the latter is an unnatural host.

Since very little is known about the factors influencing host–parasite relationships in cave environments, it cannot be said why the other specimens of *M. veritas* were devoid of helminth parasites, especially adult forms.

This report constitutes the first record of an examination of *Milyeringa veritas* for helminth parasites.

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Research Note

Parasites and Commensals of Chimpanzees Captured in Sierra Leone, West Africa

Rather extensive use of nonhuman primates in the many disciplines of biomedical research in the past few years has stimulated an interest in the parasitology of these mammals. In endemic areas, the parasites of primates have been studied for varied purposes. At times these have been related to specific parasites and parasitic diseases. Attention also has been aroused as studies on larger numbers of animals in different geographic areas have indicated that these mammals are parasitized by species closely related to those found in man, and in some instances nonhuman primates have been incriminated as factors in parasitic disease zoonoses. Surveys of the parasites of non-human primates at the time of their capture in the field are limited. Extensive checklists of commensals and parasites of several species of nonhuman primates—baboons (Myers and Kuntz, 1965, Primates 6: 137–195), Taiwan macaque (Kuntz and Myers, 1969, Primates 10: 71–80), and chimpanzees (Myers and Kuntz, 1972, Primates 13:433–471)—have been compiled but much of the available information has come from investigators and institutions employing only a few hosts of a given species for varied research purposes, and most of the host–parasite records have emanated from animals examined after considerable periods in captivity. Much information has come from zoos and other unnatural situations where chimpanzees and other nonhuman primates have had considerable contact with man and other mammals.

A checklist of commensals and parasites recorded for the chimpanzee (Myers and Kuntz, 1972, Primates 13:433–471) indicates their susceptibility to infection by a long list of intestinal and blood protozoa, helminths, and arthropods. Parasitological data on chimpanzees in the field, however, are essentially lacking even though there have been several reports on limited numbers of animals shortly after importation into the United States (Kuntz and Myers, 1969, Proc. 2nd Internatl. Cong. Primat. 3: 184–190; van Riper et al., 1966,

Lab. Animal Care 16: 360–363). The present brief report is based upon an examination of feces and several ectoparasites obtained by one of us (J.A.K.) from 110 chimpanzees shortly after capture in Sierra Leone. Fecal samples were collected from cages after hosts were brought to a central processing facility subsequent to capture by indigenous collectors.

Table 1 indicates organisms recorded after study of formalin fixed fecal samples (direct/concentration) shipped to San Antonio. Since experience in primatology has shown identification of commensals and parasites in such material to the species level to be difficult, if not impossible, entries are made at the generic level (Myers, 1970, Lab. Animal Care 20: 342–344). This is especially true for the conglomeration of amebae as well as other intestinal protozoa, and for the strongyle nematodes for which there is considerable overlap in egg measurements and in other egg characteristics. The presence of *Ascaris*

Table 1. Parasites and commensals in chimpanzees from Sierra Leone.

Number of animals examined	110
Intestinal	
PROTOZOA:	
Amebae	
Entamoeba sp.	16*
Ciliates	
Balantidium sp.	7
Troglodytella sp.	70
Fecal samples without protozoa	20
HELMINTHS:	
Cestodes	
Bertiella sp.	4
Nematodes	
Ascaris sp.	1
Enterobius sp.	3
Hookworm (rhabditiform larvae)	13
Physaloptera sp.	1
Strongyles (mixed)	47
Strongyloides (rhabditiform larvae)	19
Trichostrongylus sp.	2
Fecal samples without helminths	6
Ectoparasites	
ARTHROPODS:	
Ticks	
Amblyomma sp. (scrotum)	1
Fleas	
Tunga penetrans (footpads)	1

* Sixteen of 110 animals examined were infected.

constitutes an unusual record. The chigoe flea, *Tunga penetrans*, parasitizes a number of vertebrates, but this is the first record for a non-human primate.

Several species of worms were removed from chimpanzees not included in the present study but examined shortly after death by one of us (J.A.K.). These included adults of *Enterobius anthropopitheci, Primasubulura distans, Oesophagostomum dentigerum, Bertiella* sp., and *Schistosoma mansoni*, immature female *Ascaris* sp., and larvae of *Strongyloides*.

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The Harold W. Manter Laboratory of Parasitology

The Harold W. Manter Laboratory of Parasitology was established in March, 1971 to serve the profession of parasitology by promoting knowledge of systematics of animal parasites through conserving specimens, gathering literature and continuing original research. It is a part of the Division of Parasitology, University of Nebraska State Museum and was organized around the nucleus of Professor Manter's 45-year collection of specimens and literature. Contributions of specimens, older papers and duplicate reprints are welcomed.

For additional information contact Dr. Mary Hansen Pritchard, Curator, The Harold W. Manter Laboratory of Parasitology, W-529 Nebraska Hall West, University of Nebraska—Lincoln 68508.

MINUTES

Four Hundred Sixty-Ninth Through
Four Hundred Seventy-Sixth Meetings

469th Meeting: University of Maryland, Zoology Department, College Park, Maryland, October 20, 1972. Dr. Sheffield, Editor, announced that after September 22, those members publishing in the Proceedings will be charged approximately one-third of the cost of page charges. Slate of officers for 1973 presented: H. Herlich (President); K. G. Powers (Vice-President); A. M. Golden (Recording Secretary); R. S. Isenstein (Corresponding Secretary-Treasurer). Papers presented: "Classification of the microsporidia," V. Sprague; "Scanning electronmicroscopy of division of the rumen ciliate, *Ophryoscolex* sp.," Jeanette Esser and E. B. Small; "Canine filariasis in Maryland," G. F. Otto.

470th Meeting: Conference House, Animal Parasitology Institute, Beltsville, Maryland, November 17, 1972. After Dr. A. O. Foster presented a brief outline of the contributions made by Dr. G. F. Otto to the Society, Dr. Otto was unanimously elected a life member. Dr. A. O. Foster reviewed the professional history and accomplishments of Mrs. May Belle Chitwood and then presented her with the Society's Anniversary Award. The slate of officers presented at the previous meeting were elected by acclamation. Papers presented: "Non-enzymic histochemistry of bovine abomasal tissue infected with *Ostertagia ostertagi*," Frank Stringfellow; "Hydrolytic enzymes of the 'excretory gland cells' of *Stephanurus dentatus*," Robert D. Romanowski and Marcia L. Rhoads; "Clinical study of *Babesia caballi* infections of Equidae," Patricia C. Allen.

471st Meeting: Conference Room, Bioscience Building, Beltsville, Maryland, December 15, 1972. Papers presented: "Microbial control agents of insects," A. M. Heimpel; "Microecology of soil borne pathogens," G. C. Papavizas; "Current research on plant parasitic and insect parasitic nematodes," J. M. Good. Newly elected officers were installed. Members and guests visited Nematology and other laboratories in the new Bioscience Building.

472nd Meeting: National Institutes of Health, Bethesda, Maryland, January 19, 1973. Papers presented: "Calcium in the liver of mice infected with *Trypanosoma cruzi*," T. I. Mercado; "Superinfection of jirds with *Brugia malayi*," G. Pacheco; "Quantitative features of the intracellular reproductive cycle of *Trypanosoma cruzi* in vitro," N. A. Luban and J. A. Dvorak; "Post-embryonic developmental mutants of *Caenorhabditis elegans*," R. Pertel. In addition, two movies were presented, one by Joyce Conroy and the other by Ruth Pertel.

473rd Meeting: Walter Reed Army Institute of Research, Washington, D.C., February 19, 1973. The report of the Corresponding Secretary-Treasurer for the period January 1–December 31, 1972 was accepted unanimously. Attention was called by Dr. H. G. Sheffield to the recently published Special Issue of the Proceedings, entitled "Basic Research in Malaria," edited by E. H. Sadun. Papers presented: "The pathology of *Trypanosoma congolense* in dogs," P. K. Hildebrandt; "Glomerulonephritis in experimental African trypanosomiasis," R. B. Nagle; "Humoral and cell mediated immunity to *Schistosoma mansoni* in inbred rats," S. M. Phillips; "Parasitic diseases of the Amazon," L. K. Martin.

474th Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, March 16, 1973. Papers presented: "Sporogony of *Leucocytozoon simondi* in the black fly *Cnephia ornithophilia*," Barry Tarshis; "Studies on mitosis of the giant amoebae," Dennis Goode; "Disease problems related to oyster importation," Aaron Rosenfield; "Three extracellular blood parasites of the blue crab," Martin Newman.

475th Meeting: National Institutes of Health, Bethesda, Maryland, April 20, 1973 (Hosted by Naval Medical Research Institute, National Naval Medical Center). Members and guests stood for a minute of silence in memory and respect of Dr. A. C. Pipkin and Dr. Irving Pratt, both of whom passed away recently. Papers presented: "IgM antibodies

to red cells and autoimmune anemia in patients with malaria," G. T. Strickland; "Protective antibodies in malaria," D. M. Stetson; "NAMRU-3 Research Detachment, Ethiopia: An overview," T. T. Palmer; "Observations on the distribution of some acanthocephala," A. E. Harrises and L. W. Allen.

476th Meeting: Alumni House, New Bolton Center, Kennett Square, Pennsylvania, June 2, 1973. Papers presented: "Cuticular differences detected in early parasitic stages of *Ascaris suum*," Ruth Leventhal; "Progression of lymphoid cell responsiveness at the local site during *Ascaris* infection," Philip B. Khoury; "Passive immunization to a larval cestode," Sheelagh Lloyd; "Occurrence of antibodies during granuloma formation to *Capillaria hepatica*," Richard B. Raybourne and Gene B. Solomon; "A cryptosporidial parasite causing clinical disease in cattle," Alan M. Kelly and E. J. L. Soulsby.

Following the scientific presentations a most congenial hospitality session and dinner were enjoyed by the members and guests.

The following 36 persons were elected to membership at the meetings as indicated: *469th*: W. B. Ahern; D. M. Burkhart; M. M. Joshi; C. D. Minchew; S. K. Minckley; A. C. Olson, Jr.; A. W. Phillips. *470th*: W. S. Bailey; E. M. Ernst; D. P. Limber; D. J. Moncol; W. Threlfall. *471st*: J. R. Arthur; R. V. Rebois; B. T. Ridgeway; W. Wanson. *472nd*: Norman L. Levin. *473rd*: R. J. Glaudel; D. W. Heacock; J. L. Olsen. *474th*: Patricia C. Allen; Kristina Archambault; R. L. Cordell; M. D. Little; T. R. Platt; R. D. Specian. *475th*: A. E. Harrises; Jane E. Huffman; A. A. Ilemobade; Joan R. Lally. *476th*: G. M. Davis; K. R. Kazacos; Eileen H. Pike; V. D. Schinski; T. W. Simpson; D. D. Wittrock.

A. MORGAN GOLDEN
Recording Secretary

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