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PROCEEDINGS OF THE
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Systematic Status of Proteocephalid Cestodes from Reptiles and Amphibians in North America with Descriptions of Three New Species[1]

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ABSTRACT: Based on collections of cestodes from reptiles and amphibians primarily in the southeastern United States, three new species of *Proteocephalus* are described and supplemental data for 16 additional species are presented. *Proteocephalus amphiumicola* sp. n. from *Amphiuma means* in southern Mississippi and southern Alabama most closely resembles *P. amphiumae* and *P. alternans* from which it differs in scolex morphology and number of uterine branches. It is unique in possessing spiraling rather than sinuous lateral longitudinal osmoregulatory ducts and has a vaginal "sphincter" similar to that in *P. alternans*. *Proteocephalus aberrans* sp. n. from *Siren lacertina* in northern Florida is unique among known members of the genus because it lacks lateral uterine branches. *Proteocephalus variabilis* sp. n. from *Natrix cyclopion cyclopion* in southern Louisiana and *N. rhombifer* in northern Louisiana resembles *P. perspicua* in size of scolex and number of uterine branches, but differs from it in average number of testes, position of genital pore and shape of ovary in gravid proglottids; it resembles *P. agkistrodontis* in the last three characteristics. New hosts for *P. perspicua* include *N. c. cyclopion*, *N. c. floridense*, and *N. fasciata confluens*; it also parasitized *N. rhombifer* and *N. sipedon*. The report of *P. perspicua* in northern and southern Florida, northern and southern Louisiana, and southern Mississippi represents new locality records. *Proteocephalus faranciae*, known previously from Texas in *Farancia abacura*, infected *F. abacura* in northern Florida and *N. c. floridense* in southern Florida. *Natrix c. cyclopion* and *N. c. floridense* represent new hosts for *P. grandis*, and both northern and southern Florida and Louisiana are new localities. *Agkistrodon piscivorus* hosted *P. marenzelleri* in northern and southern Louisiana and *P. agkistrodontis* in northern Louisiana, all new localities. *Proteocephalus sireni*, known previously from *Siren intermedia* in southern Illinois, was collected from the same host in southern Louisiana. *Proteocephalus testudo* from *Trionyx spiniferus* in Nebraska and Indiana and *Chrysemys scripta elegans* in Illinois, the latter two being new localities, more closely resembles *P. australis* from a teleost fish than any species from amphibians or reptiles. Circumstantial evidence suggests that *P. magnus*, *P. olor*, *P. saphenus*, and *P. gracilis*, all from aquatic frogs of the genus *Rana*, are geographical variants of a single species. The reports also includes results of examining type-material and deposited specimens of *P. agkistrodontis*, *P. perspicua*, *P. marenzelleri*, *P. grandis*, *P. olor*, *P. magnus*, *P. saphenus*, *P. cryptobranchi*, *P. filaroides*, *P. loennbergii*, *P. amphiumae*, and *P. alternans*. Since, on the basis of the new material, no distinctions exist among the genera *Ophiotaenia*, *Batrachotaenia*, and *Testudotaenia* from herptiles and *Proteocephalus* from teleost fishes other than host type, *Proteocephalus* is considered the senior synonym of all four, and appropriate new combinations are made. Analysis of the interspecific relationships of the 18 species parasitizing amphibians and snakes in North America reveals distinct groupings according to host category. A monophyletic origin of the species parasitizing caudate amphibians is postulated based on their morphological and geographical homogeneity and well-defined host–parasite relationships. The absence of *P. perspicua* in *N. erythrogaster flavigaster* may be explained on the basis of feeding and habitat preferences of the host species. A key distinguishes the 19 species of *Proteocephalus* parasitizing herptiles in North America.

A number of proteocephalid cestodes have been described as parasites of reptiles and amphibians in North America. All have medullary gonads and simple scolices, but for most

[1] This study was conducted in part by a grant entitled "Studies on helminths of the northern Gulf of Mexico" from the State of Mississippi to Robin M. Overstreet.

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of these species little descriptive information exists other than brief original descriptions. Because of this, considerable differences of opinion have resulted concerning their systematic positions. The present work describes three new species and presents supplemental data for 16 more; a diagnosis accompanies species for which new material was collected; salient features useful in deducing phylogenetic relationships are provided for other species. Based on that data, the study also examines the generic status of all proteocephalids with medullary gonads and simple scolices; it further considers the interspecific relationships of those species parasitizing reptiles and amphibians in North America.

Materials and Methods

Cestodes were relaxed in cold tap water or saline, fixed with AFA, and stored in 70% ethanol. After staining with Harris' hematoxylin, Mayer's hematoxylin, or Ehrlich's acid hematoxylin, worms were mounted in Histoclad. Unless otherwise stated, measurements of holdfast structures were based on examination of 25 scolices, and those of proglottids and their organs on 100 proglottids, since mean values for certain characteristics were found to be taxonomically useful. The cirrus of proteocephalids typically comprises a basal and distal portion. The thickness of the basal portion appears to be related to the length of the cirrus, but since it is difficult to obtain specimens with everted cirri, cirrus length has not proved to be useful taxonomically; I have, however, noted which examined species have thick- and thin-walled basal portions. Notations for deposited specimens are: USNM Helm. Coll. for United States National Museum Helminthological Collection, Beltsville, Maryland and HWML for Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska. Measurements are in micrometers unless otherwise noted, with averages in parentheses and previously known data in brackets; figures were drawn with the aid of a drawing tube.

Proteocephalus amphiumicola sp. n. (Figs. 1–6)

DESCRIPTION (based on 20 specimens): Strobila up to 300 mm long. Scolex aspinose, 309–390 (352) wide, with indistinct apical organ and four shallow, padlike suckers; suckers 222–297 (253) long by 117–155 (133) wide. Immature proglottids wider than long. Mature proglottids 662–1,576 (1,040) long by 394–820 (678) wide. Ratio of proglottid width to length 1:0.9–3.8 (1:1.9). Testes 63–136 (105), 7–18 (13) preporally, 25–53 (37) postporally, 31–70 (55) antiporally; 31–68 in diameter. Cirrus sac 110–205 (157) long by 63–95 (80) wide; ratio of cirrus sac length to proglottid width 1:2.5–4.2 (1:3.4); basal portion of cirrus thick-walled, up to 25 thick. Genital atrium without papillae. Genital pores alternating irregularly in anterior 12–28% (22%) of proglottids. Ovary bilobed with irregularly shaped lobes expanded anteriorly, 371–476 (425) wide; lobes 148–340 (250) long. Vagina anterior to and never crossing cirrus sac; muscles surrounding vagina near terminal end acting as sphincter; seminal receptacle present. Uterus preformed in mature proglottids. Vitelline follicles extending along entire length of proglottid; follicles not in single file; follicles and ovarian lobes proximate posteriorly. Gravid proglottids 820–3,814 (1,850) long by 394–820 (678) wide; ratio of proglottid width to length 1:2.5–5.0 (1:3.8). Uterus with 39–98 (59) lateral branches occupying 41–84% (65%) of proglottid width; preformed ventral uterine pores lacking. Eggs 49–62 in diameter, oncospheres 25–37. Excretory system composed of paired dorsal and ventral spiraling lateral longitudinal medullary ducts and cortical network of reticulate tubules; tubules anastomosing near posterior end of proglottid.

HOST: *Amphiuma means* Garden, two-toed conger eel.

SITE OF INFECTION: Upper small intestine.

LOCALITIES AND PREVALENCE: St. Andrew's, Jackson County, Mississippi (2/4 (type); Fowl River, Theodore, Mobile County, Alabama (1/1).

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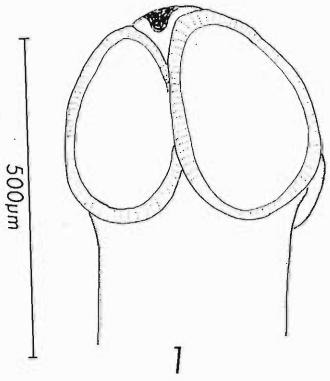
Figures 1–6. ***Proteocephalus amphiumicola.*** **1. Scolex. 2. Ootype region. 3. Terminal genitalia. 4. Immature proglottid. 5. Mature proglottid. 6. Gravid proglottid.**



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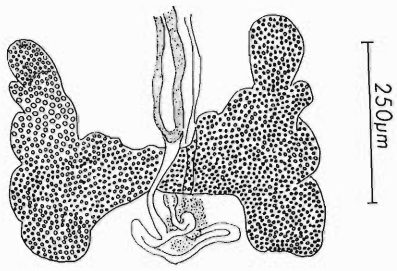

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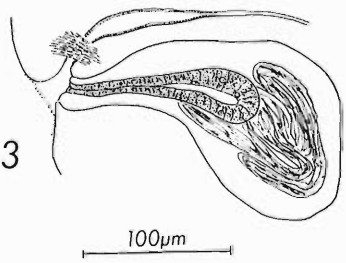

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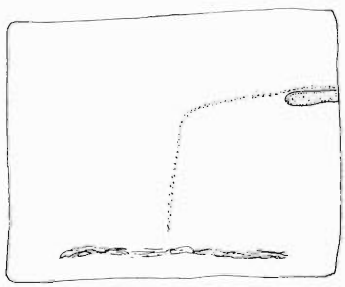

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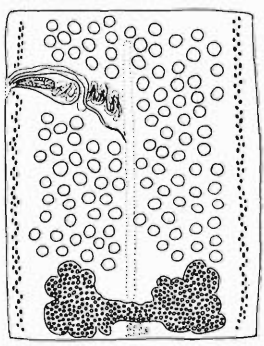

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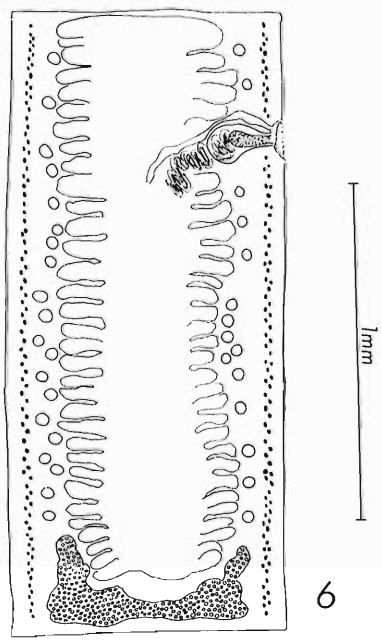

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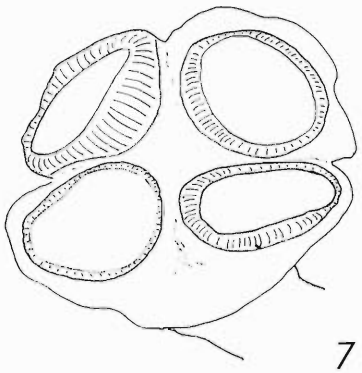

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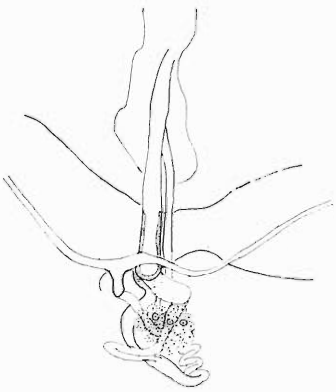

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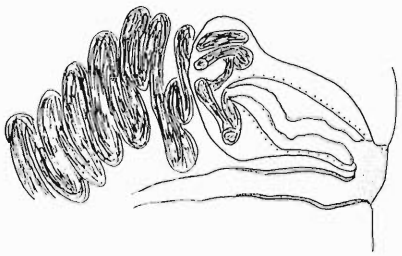

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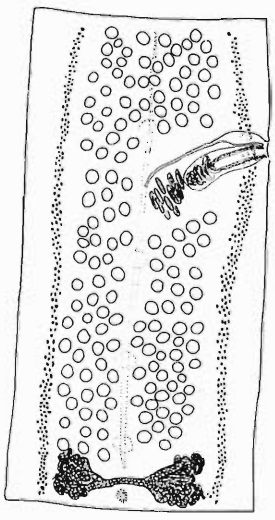

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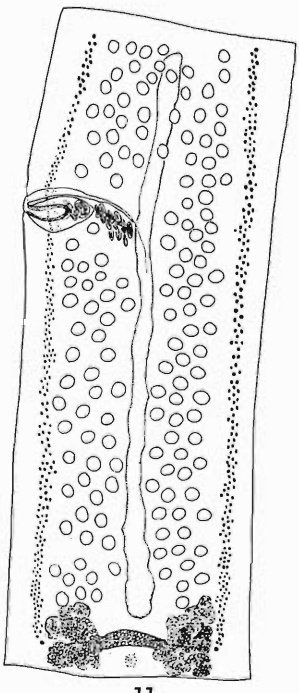

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HOLOTYPE: USNM Helm. Coll. No. 74330.
PARATYPES: USNM Helm. Coll. No. 74331; HMWL No. 20286 and 20324.

ETYMOLOGY: The specific name means "*Amphiuma*-inhabiting," and is derived from the Greek "amphi" meaning on both sides, plus "pneuma" meaning breath for the name of the host and "icola" inhabiting.

No other species of *Proteocephalus* from amphibians or snakes in North America possesses spiraling lateral longitudinal osmoregulatory ducts. *Proteocephalus amphiumicola* most closely resemble *P. amphiumae* (Zeliff, 1932) and *P. alternans* (Riser, 1942) from which it differs by having fewer lateral uterine branches and a more anteriorly placed genital pore; it further differs from *P. alternans* by having spherical rather than fusiform eggs and a vagina consistently anterior to the cirrus sac, and *P. amphiumae* in its overall smaller size and possession of a vaginal "sphincter." It resembles *P. sireni* (Brooks and Buckner, 1976) in shape of ovary and number of uterine branches, but has an average of 105 rather than 65 testes, a smaller scolex, and no preformed ventral uterine pores

Proteocephalus aberrans sp. n. (Figs. 7–11)

DESCRIPTION (based on three specimens): Strobila up to 300 mm long. Scolex aspinose, 860–2,580 wide, lacking apical organ, with four shallow suckers 344–783 long by 350–984 wide. Immature proglottids longer than wide. Mature proglottids 2.0–3.5 (2.4) mm long by 1.2–1.6 (1.4) mm wide. Ratio of proglottid width to length 1:1.3–2.6 (1:1.9). Testes 102–201 (145), 14–32 (22) preporally, 27–78 (48) postporally, 52–100 (75) antiporally; 40–120 in diameter. Cirrus sac 400–515 (460) long by 115–170 (143) wide; ratio of cirrus sac length to proglottid width 1:2.8–3.5 (1:3.1); basal portion of cirrus thick-walled, up to 35 thick. Genital atrium lacking papillae. Genital pores alternating irregularly in anterior 19–29% (25%) of proglottid. Ovary bilobed with irregularly shaped lobes expanded anteriorly, 532–798 wide; lobes 228–342 long. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles not in single file; follicles and ovarian lobes proximate posteriorly. Gravid proglottids 2.4–4.4 (3.3) mm long by 1.0–1.2 (1.1) mm wide. Ratio of proglottid width to length 1:2.2–3.8 (1:3.1). Genital pores in anterior 26–37% (29%) of proglottid. Ratio of cirrus sac length to proglottid width 1:2.3–3.3 (1:2.8). Uterus narrow, elongate, lacking lateral branches, occupying 7–22% of proglottid width. Preformed ventral uterine pores four. Eggs 23–34 in diameter, oncospheres 17–29. Excretory system composed of paired dorsal and ventral lateral longitudinal medullary ducts and associated reticulate cortical network of tubules; tubules anastomosing near posterior end of proglottid.

HOST: *Siren lacertina* L., greater siren.
SITE OF INFECTION: Upper small intestine.
LOCALITY AND PREVALENCE: Gainesville, Alachua County, Florida (1/1).
HOLOTYPE: USNM Helm. Coll. No. 73973.
PARATYPES: USNM Helm. Coll. No. 73974; HMWL No. 20285.

ETYMOLOGY: The specific name means "aberrant" and refers to the lack of lateral uterine branches.

No other species of *Proteocephalus* is known to lack lateral uterine branches. *Proteocephalus viperis* (Beddard, 1913) Woodland, 1925 was described as lacking lateral uterine branches, but Woodland (1925) redescribed the species showing that short lateral branches occurred.

By having a relatively large scolex with no apical organ, a bilobed ovary with lobes expanded anteriorly, and a genital pore in the anterior 25% of the proglottid, *P. aberrans* resembles *P. sireni* (Brooks and Buckner, 1976). However, *P. sireni* has a single ventral uterine pore rather than four, an average of 65 rather than 145 testes, and 42 to 97 lateral uterine branches.

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Figures 7–11. ***Proteocephalus aberrans.*** **7. Scolex. 8. Ootype region. 9. Terminal genitalia. 10. Mature proglottid. 11. Gravid proglottid.**

Proteocephalus sireni (Brooks and Buckner, 1976) comb. n.

SYNONYM: *Ophiotaenia sireni* Brooks and Buckner, 1976.

HOST: *Siren intermedia* LeConte, lesser siren.

SITE OF INFECTION: Upper small intestine.

LOCALITY AND PREVALENCE: Pearl River, Highway I-10, St. Tammany Parish, Louisiana, *new locality.*

SPECIMENS DEPOSITED: HWML No. 20321.

PREVIOUS REPORTS: Brooks and Buckner (1976) in Illinois from *Siren intermedia* (description).

SALIENT FEATURES: Strobila more than 250 mm long (1). Scolex with exceeding 1 mm (2). Average number of testes 65 (3). Ovarian lobes in mature proglottids expanded anteriorly (4). Average number of uterine branches 65 (5). Genital pores in anterior 20–30% of proglottid (6). Vagina always posterior to cirrus sac (7). Tegumental spines lacking (8). Gravid proglottids more than 2 times longer than wide (9). Preformed ventral uterine pores present (10). Vaginal "sphincter" lacking (11).

Discussion of new combinations occurs later in this paper.

Proteocephalus cryptobranchi (LaRue, 1914) comb. n.

SYNONYM: *Ophiotaenia cryptobranchi* LaRue, 1914.

HOST: *Cryptobranchus allegheniensis* (Daudin), hellbender.

SITE OF INFECTION: Small intestine.

LOCALITY: Meadville, Pennsylvania.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 48383.

PREVIOUS REPORTS: LaRue (1911) in Pennsylvania from *C. allegheniensis* (description); Rankin (1937) in North Carolina from *Desmognathus fuscus, D. ochrophaeus, D. monticola, Notopthalmus viridescens,* and *Plethodon jordani metcalfi*; Dyer and Brandon (1973) in Missouri from *C. allegheniensis*; Dyer and Peck (1975) in Alabama from *Eurycea lucifuga.*

SALIENT FEATURES: Strobila more than 250 mm long (1). Scolex width not exceeding 1 mm (2). Average number of testes 105 (3). Ovarian lobes in mature proglottids flat (4). Average number of uterine branches 35–50 (5). Genital pores in anterior 20–30% of proglottid (6). Vagina anterior or posterior to cirrus sac (7). Tegumental spines absent (8). Gravid proglottids more than 2 times longer than wide (9). Preformed ventral uterine pores lacking (10). Vaginal "sphincter" lacking (11). Preporal vitelline follicles lacking.

I examined the holotype of *P. cryptobranchi* (USNM Helm. Coll. No. 48383) and found it to consist of unmounted stained fragments. None of the proglottids possessed preporal vitelline follicles, although LaRue's (1911) figure depicts preporal follicles.

Proteocephalus amphiumae (Zeliff, 1932) comb. n.

SYNONYM: *Crepidobothrium amphiumae* Zeliff, 1932.

HOST: *Amphiuma tridactylum* Cuvier, three-toed conger eel.

SITE OF INFECTION: Small intestine.

LOCALITY: Baton Rouge, Louisiana.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 8118.

PREVIOUS REPORTS: Zeliff (1932) in Louisiana from *A. tridactylum* (description); Smith (1945) in Missouri from *Typhlotriton spelaous*; Odlaug (1954) in Ohio from *Desmognathus fuscus.*

SALIENT FEATURES: Strobila more than 250 mm long (1). Scolex width not exceeding 1 mm (2). Average number of testes 105 (3). Ovarian lobes in mature proglottids expanded anteriorly (4). Average number of uterine branches 100 (5). Genital pores in anterior 20–30% of proglottid (6). Vagina anterior to cirrus sac (7) Tegumental spines lacking (8). Gravid proglottids more than 2 times longer than wide (9). Preformed ventral uterine pores lacking (10). Vaginal "sphincter" lacking (11).

Proteocephalus alternans (Riser, 1942) comb. n.

SYNONYM: *Ophiotaenia alternans* Riser, 1942.

HOST: *Amphiuma tridactylum.*

SITE OF INFECTION: Small intestine.

LOCALITY: Reelfoot Lake, Tennessee.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 36818.

PREVIOUS REPORTS: Riser (1942) in Tennessee from *A. tridactylum* (description).

SALIENT FEATURES: Strobila less than 200 mm long (1). Scolex width not exceeding 1 mm (2). Average number of testes 100 (3). Ovarian lobes in mature proglottids expanded anteriorly (4). Average number of uterine branches 100 (5). Genital pores in anterior 20–30% of proglottid (6). Vagina anterior or posterior to cirrus sac (7). Tegumental spines lacking (8) Gravid proglottids more than 2 times longer than wide (9). Preformed ventral uterine pores lacking (10). Vaginal "sphincter" present (11).

Proteocephalus filaroides LaRue, 1909

HOSTS: See Brooks (1976).

SITE OF INFECTION: Small intestine.

LOCALITIES: See Brooks (1976).

SPECIMENS EXAMINED: HWML No. 20207, 20208.

PREVIOUS REPORTS: See Brooks (1976).

SALIENT FEATURES: Strobila less than 200 mm long (1). Scolex width not exceeding 1 mm (2). Average number of testes 70 (3). Ovarian lobes in mature proglottids expanded anteriorly (4). Average number of uterine branches 35 (5). Genital pores in anterior 20–30% of proglottid (6). Vagina anterior or posterior to cirrus sac (7). Tegumental spines lacking (8). Gravid proglottids more than 2 times longer than wide (9). Preformed ventral uterine pores lacking (10). Vaginal "sphincter" lacking (11).

Proteocephalus loennbergii (Fuhrmann, 1895) LaRue, 1909 (Figs. 12–14)

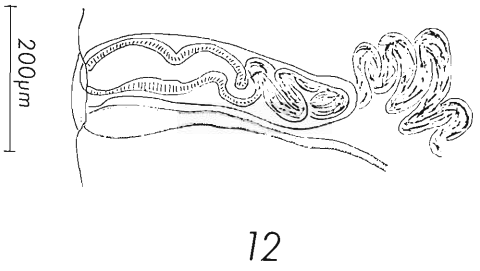
HOST: *Necturus maculosus* Rafinesque, mudpuppy.

SITE OF INFECTION: Small intestine.

LOCALITY: Carbondale, Illinois, *new locality*.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 49811, 60427, 32852.

PREVIOUS REPORTS: LaRue (1909) in Ohio



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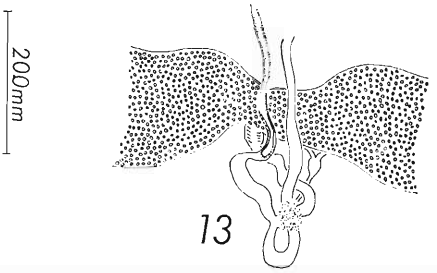

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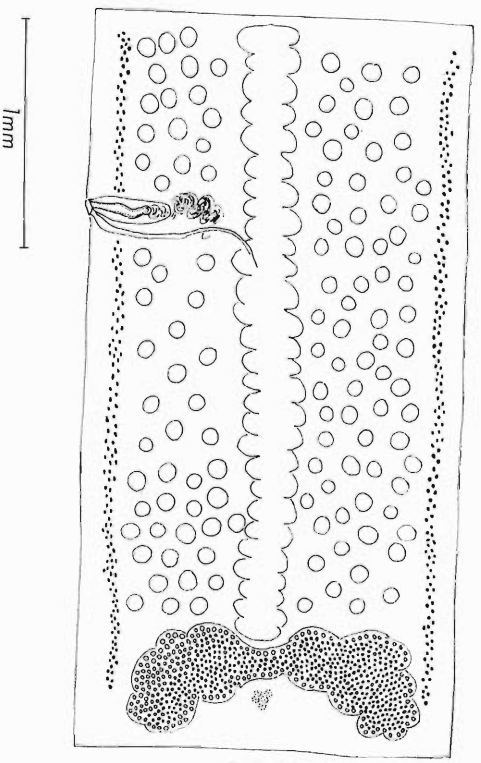

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Figures 12–14. *Proteocephalus loennbergii*. 12. Terminal genitalia. 13. Oötype region. 14. Subgravid proglottid.

and Indiana from *N. maculosus* (redescription); Odlaug (1954) in Ohio from *N. maculosus*. The original description by Fuhrmann (1895) contained no locality data.

DIAGNOSIS: Scolex 576–864 wide (500–600), aspinose; suckers 256–384 in diameter (240–250 long by 140–220 wide); apical organ lacking. Width: length ratios of mature proglottids 1:1.0–4.5. Testes 88–149 (90–160) averaging 125, 11–23 preporally, 28–44 postporally, 49–85 antiporally. Ratio of cirrus sac length: proglottid width 1:3.6–6.3; basal portion of cirrus. Inconspicuous genital atrium lacking papillae. Genital pores alternating irregularly in anterior 28–39%, averaging 30% of proglottids. Ovary bilobed with flat lobes in mature proglottids; lobes expanded posteriorly in gravid ones. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles tending to be in single file in gravid proglottids. Width: length ratios of gravid proglottids 1:1.5–5.0. Ratio of cirrus sac length: proglottid width 1:4.1–5.8. Genital pores in anterior 32–35%, averaging 33% of proglottid. Uterus with 35–60, averaging 50 lateral branches (25–40). Preformed ventral uterine pores lacking. Uterus occupying 15–20%, averaging 17% of proglottid width. Eggs not seen.

The original description by Fuhrmann (1895) and redescription by LaRue (1909) of this species, based on immature specimens, incorrectly listed the number of uterine branches and length of strobila, and failed to mention that the ovarian lobes in gravid proglottids expand posteriorly.

Proteocephalus magnus (Hannum, 1925) Harwood, 1932 nom. emend.

SYNONYM: *Proteocephalus magna* (Hannum, 1925) Harwood, 1932.

HOSTS: *Rana catesbeiana* Shaw, bullfrog and *R. clamitans* Latreille, green frog.

SITE OF INFECTION: Small intestine.

LOCALITIES: Greeley, Colorado and Nebraska (see Brooks, 1976 for exact Nebraska localities); Houston, Texas.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 30895; HWML No. 20205, 20206; collection of Dr. G. D. Schmidt.

PREVIOUS REPORTS: Buhler (1972) in Colorado from *R. catesbeiana* (postembryonic development; originally reported as *Ophiotaenia gracilis*); for all other reports see Brooks (1976).

I examined a specimen taken from a bullfrog in Greeley, Colorado and found it identical to specimens of *P. magnus* from Nebraska. It differed from *P. gracilis* in strobilar length, scolex width, and shape of ovarian lobes in gravid proglottids. Thus, I refer those specimens reported by Buhler (1972) from the same host and locality to *P. magnus*.

Proteocephalus saphenus (Osler, 1931) comb. n.

SYNONYM: *Ophiotaenia saphena* Osler, 1931.

HOST: *Rana clamitans*.

SITE OF INFECTION: Small intestine.

LOCALITY: Douglas Lake, Michigan.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 71490.

PREVIOUS REPORTS: Osler (1931) in Michigan from *R. clamitans* (description); Thomas (1934) in Michigan from *R. clamitans* (life cycle); Brandt (1936) in North Carolina from *R. catesbeiana*; Ulmer and James (1976) in Iowa from *R. pipiens* and *Bufo americanus*.

Proteocephalus olor (Ingles, 1936) comb. n.

SYNONYM: *Crepidobothrium olor* Ingles, 1936.

HOST: *Rana aurora* Baird and Girard, red-legged frog.

SITE OF INFECTION: Small intestine.

LOCALITY: Berkeley, Alameda County, California.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 8927.

PREVIOUS REPORTS: Ingles (1936) in California from *R. aurora* (description).

Proteocephalus gracilis (Jones, Cheng, and Gillespie, 1958) comb. n.

SYNONYM: *Ophiotaenia gracilis* Jones, Cheng, and Gillespie, 1958.

HOST: *Rana catesbeiana*.

SITE OF INFECTION: Small intestine.

LOCALITY: Mountain Lake, Giles County, Virginia.

SPECIMENS EXAMINED: None deposited.

PREVIOUS REPORTS: Jones, Cheng, and Gillespie (1958) in Virginia from *R. catesbeiana*.

The four nominal species of *Proteocephalus* parasitizing frogs in North America are highly similar ecologically as well as morphologically. In addition to occupying the same continent, they all parasitize aquatic frogs of the genus *Rana* and share the following nine salient features: scolex width not exceeding 1 mm (2); average number of testes 100 (3); average number of uterine branches 40 (5); genital pores in anterior 20–30% of proglottids (6); vagina anterior or posterior to cirrus sac (7); tegumental spines lacking (8); gravid proglottids less than 1.5 times longer than wide (9); preformed ventral uterine pores lacking (10); vaginal "sphincter" lacking (11).

Proceeding from east to west there is apparent geographic variation in size of scolex, strobilar length, and shape of ovarian lobes in gravid proglottids among these four taxa. *Proteocephalus gracilis* from Virginia has a scolex 350 μm wide, strobila 200 mm long, and dumbbell-shaped ovarian lobes in gravid proglottids; *P. saphenus* from North Carolina, Michigan, and Iowa has a scolex 270 to 320 μm wide, strobila 280 mm long, and dumbbell-shaped ovarian lobes in gravid proglottids; *P. magnus* from Oklahoma, Texas, Nebraska, Colorado, and Nevada has a scolex 450 to 550 μm wide, strobila up to 800 mm long, and flat ovarian lobes; and *P. olor* from California has a scolex 300 to 475 μm wide, strobila at least 500 mm long, and flat ovarian lobes. In the absence of unequivocal differences among these taxa other than size and minor differences in ovarian shape, it appears that each may represent a geographical variant of a single species but without adequate population studies subspecific designations are inappropriate at this time.

Proteocephalus variabilis sp. n. (Figs. 15–19)

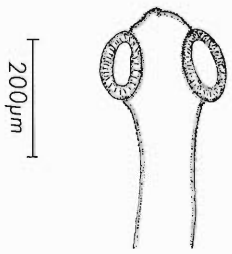
DESCRIPTION (based on 15 specimens): Strobila up to 300 mm long. Scolex aspinose, 170–200 (184) wide, lacking apical organ, with four suckers; suckers 102–160 (130) in diameter. Immature proglottids longer than wide. Mature proglottids 1.4–2.4 (2.0) mm long by 0.7–0.8 (0.8) mm wide. Ratios of proglottids length to width 1:1.8–3.0 (1:2.5). Testes 77–253 (130), 16–44 (20) preporally, 19–79 (40) postporally, 38–136 (70) antiporally; 31–93 in diameter. Cirrus sac 173–252 (215) long by 63–126 (95) wide; ratio of cirrus sac length to proglottid width 1:3.0–4.6 (1:3.9); basal portion of cirrus thick-walled, up to 15 thick. Inconspicuous genital atrium with indistinct papillae. Genital pores alternating irregularly in anterior 15–35% (30%) of proglottid. Ovary bilobed with flat lobes in mature proglottids, 389–509 wide; lobes dumbbell-shaped in gravid proglottids. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles tending to be in single file in gravid proglottids; follicles and ovarian lobes proximate posteriorly. Gravid proglottids 2.3–3.6 (3.0) mm long by 0.7–0.8 (0.8) mm wide. Ratio of width to length 1:2.9–4.8 (1:3.9). Ratio of cirrus sac length to proglottid width 1:2.9–4.0 (1:3.3). Genital pores in anterior 24–48% (32%) of proglottid. Uterus with 49–90 (66) lateral branches occupying 43–64% (54%) of proglottid width. Preformed ventral uterine pores lacking. Eggs 35–50 in diameter, oncospheres 30–40. Excretory system composed of paired dorsal and ventral lateral longitudinal medullary ducts and associated reticulate network of cortical tubules; tubules anastomosing near posterior end of proglottid.

HOSTS: *Natrix rhombifer* Hallowell, dia-

→

Figures 15–19. ***Proteocephalus variabilis.*** **15. Scolex. 16. Terminal genitalia. 17. Ootype region. 18. Mature proglottid. 19. Gravid proglottid.**

Figures 20–24. ***Proteocephalus perspicua.*** **20. Scolex. 21. Ootype region. 22. Terminal genitalia. 23. Mature proglottid. 24. Gravid proglottid.**



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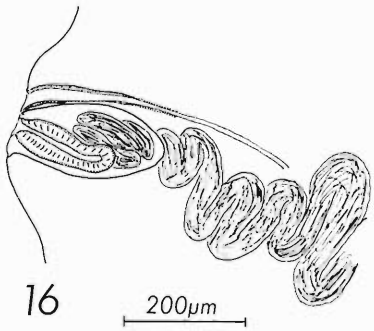

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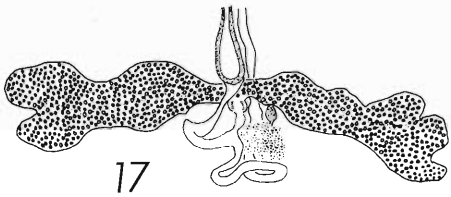

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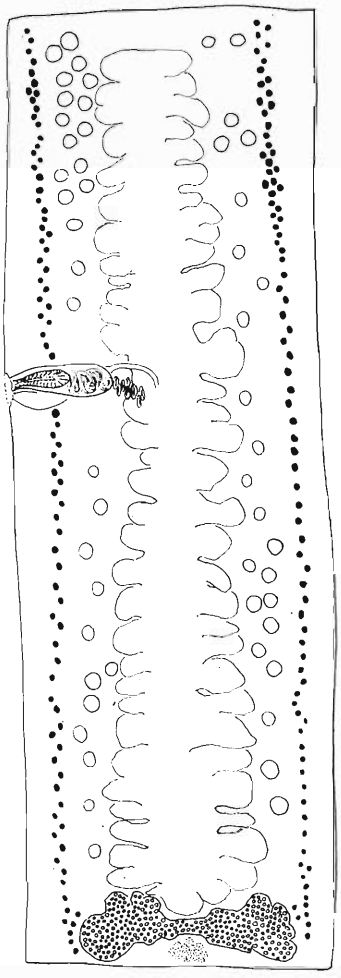

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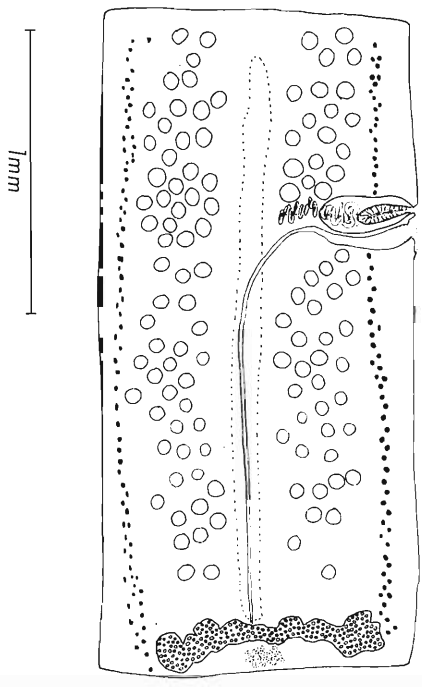

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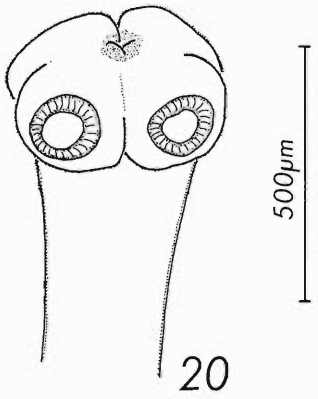

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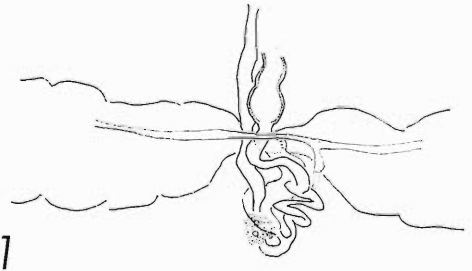

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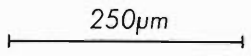

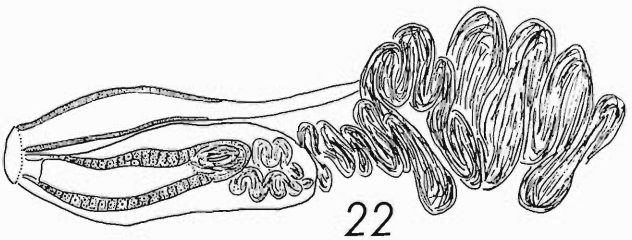

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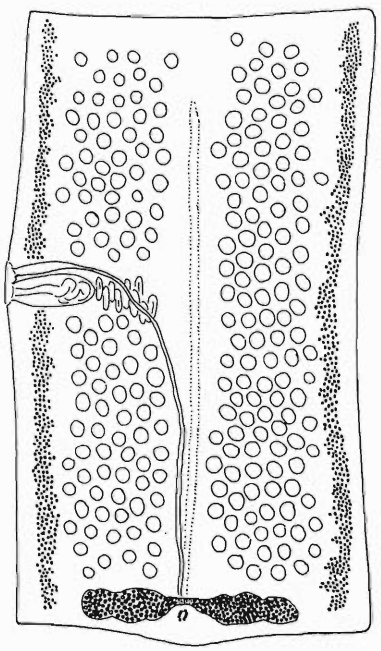

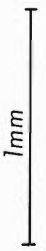

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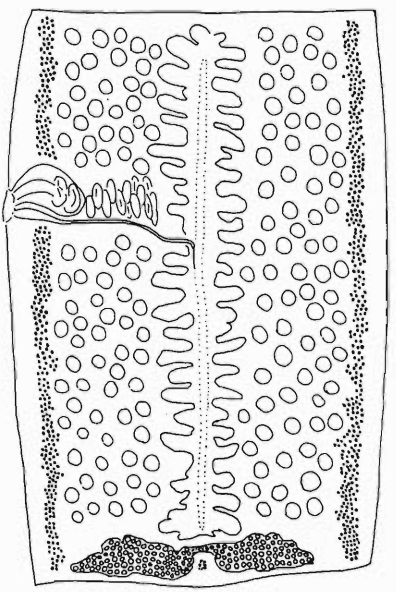

24

mond-backed water snake (type); *N. c. cyclopion* (Dumeril, Bibron, and Dumeril), green water snake.[3]

SITE OF INFECTION: Posterior half of intestine.

LOCALITIES AND PREVALENCE: Monroe, Ouachita Parish, Louisiana (type) (1/1); Rockefeller Wildlife Refuge, Cameron Parish, Louisiana (1/1).

HOLOTYPE: USNM Helm. Coll. No. 74328.

PARATYPES: USNM Helm. Coll. No. 74329, HWML No. 20325, 20326.

OTHER SPECIMEN: USNM Helm. Coll. No. 49813.

ETYMOLOGY: The specific name is derived from Latin meaning variable and refers to the variability in meristic characters exhibited by this species.

Proteocephalus variabilis resembles all other species parasitizing snakes in North America by having a spinose scolex. Among these species, *P. variabilis* is most similar to *P. agkistrodontis* in average number of testes (130 vs. 108), uterine branches (66 vs. 55), shape of ovary, and position of genital pore. *Proteocephalus agkistrodontis* has a scolex 850 to 950 μm wide while *P. variabilis* has a scolex 170 to 200 μm wide, and *P. variabilis* lacks preformed ventral uterine pores. The new species differs from *P. perspicua* in average number of testes, although the range of variation in testes number for each species is similar, in position of genital pore, and proglottid dimensions, but is similar in number of uterine branches and scolex size. *Proteocephalus grandis* and *P. faranciae* differ from *P. variabilis* in scolex size, average number of testes, average number of uterine branches, ovarian morphology, and position of genital pores, but *P. grandis* has similar proglottid dimensions. Among species possessing aspinose scolices, *P. variabilis* resembles *P. loennbergii* and *P. cryptobranchi* in average number of testes and position of genital pore, but differs in other previously mentioned characters.

Five additional species of *Proteocephalus* parasitize semiaquatic snakes in North America. Morphological data presented are designed to amplify the original descriptions; in certain cases, this is the first report of a specific characteristic for some species. Supplemental collections form the sole basis for data concerning four species; those for *P. agkistrodontis* are based also on examination of the holotype.

Proteocephalus perspicua (LaRue, 1911) Harwood, 1933 (Figs. 20–24)

HOSTS: *Natrix sipedon* L., banded water snake; *N. rhombifer*; *N. fasciata confluens* (Forster), broad-banded water snake, *new host*; *N. c. cyclopion*, *new host*; *N. c. floridense*, Florida green water snake, *new host*.

SITE OF INFECTION: Posterior half of intestine.

LOCALITIES AND PREVALENCE: Ocean Springs, Jackson County, Mississippi (1/1); Rockefeller Wildlife Refuge, Cameron Parish, Louisiana (1/1); Monroe, Ouachita Parish, Louisiana (2/4); Rockefeller Wildlife Refuge (1/1); Lake Okeechobee, Okeechobee County, Florida (1/1) and Payne's Prairie, Alachua County, Florida (1/1), respectively, *new localities*.

SPECIMENS DEPOSITED: HWML No. 20290, 20291.

PREVIOUS REPORTS: LaRue (1911) in Oklahoma and Illinois from *N. rhombifer* (description; genotype of *Ophiotaenia*); Anderson (1935) in Ohio from *N. sipedon* (as possible synonym of *Taenia lactea* Leidy and additional morphological information); Herde (1938) in Oklahoma from *N. sipedon* (early larval development); Thomas (1941) in Texas from *N. sipedon* (life cycle); Fantham and Porter (1954) in Montreal, Canada from *N. rhombifer*; Collins (1969) in North Carolina from *N. sipedon*, *N. taxispilota*, and *Agkistrodon piscivorus*; Gibson and Rabalais (1973) in Ohio from *N. sipedon* and *Thamnophis sirtalis*.

DIAGNOSIS: Scolex 184–528 wide (190–408), spinose; suckers 70–156 in diameter

[3] NOTE: Rossman and Eberle (1977, Copeia No. 2, p. 34–43) presented karyotypical, immunological, and morphological data supporting the separation of the species of snakes placed in the genus *Natrix* into the four following; Natrix (*s.s.*) Laurenti, 1768, composed of three species in Europe and northern Africa, *Sinonatrix* Rossman and Eberle, 1977, comprising five species in southeast Asia; *Afronatrix* Rossman and Eberle, 1977, with a single species in western Africa; and *Nerodia* Baird and Girard, 1953, containing eight North American species. They further related species of *Nerodia* more closely to other natricines in the New World than to any group of Old World natricines, placing the species of *Nerodia* in the tribe Thamnophiini, including the genus *Thamnophis*. Thus, without the single report of *Proteocephalus perspicua* from *Agkistrodon piscivorus* all hosts for *P. perspicua* are *Thamnophiini* (Table 2).

(88–170); apical protuberance lined with darkly-staining cells (rudimentary apical organ). Width: length ratios of mature proglottids 1:1.0–2.0. Testes 154–285 averaging 207 (150–215), 30–50 preporally, 36–75 postporally, 88–155 antiporally. Ratio of cirrus sac length: proglottid width 1:2.9–4.7 (1:2.6). Indistinct genital atrium with papillae. Genital pores alternating irregularly in anterior 27–44% averaging 40% of proglottid (17–50%). Ovary bilobed with flat lobes reaching nearly to vitelline fields. Vagina anterior or posterior to cirrus sac, crossing it or not. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles not tending to be in single file. Width: length ratios of gravid proglottids 1:1.6–2.7. Ratios of cirrus sac length: proglottid width 1:3.0–6.0. Genital pores in anterior 28–48% averaging 40% of proglottid. Uterus with 36–80 averaging 55 lateral branches (40–64). Preformed ventral uterine pores lacking. Uterus occupying 22–36% of proglottid width. Eggs 75–100 (45–100) in diameter, oncospheres 17–32 (18–21).

Specimens from *Natrix c. floridense* had larger scolices (420–528 μm wide vs. 184–400) and suckers (135–156 μm in diameter vs. 70–110) than specimens from the other hosts which were similar to those reported by LaRue (1911) and Anderson (1935). Flores-Barroeta (1953) reported this species from *Bothrops* sp. from Panama from fragmented specimens lacking scolices, but this identification should be confirmed based on better material. Deposited specimens of *P. perspicua* collected from *Lampropeltis getulus floridana* (USNM Helm. Coll. No. 56476 and 56479) and *Coluber constrictor foxi* (No. 56445) held in the collections of the Philadelphia Herpetological Society include no locality data. It is not known if the snakes may have acquired the parasites in captivity so they are not included in the host list.

Proteocephalus faranciae (MacCallum, 1921) Harwood, 1932 (Figs. 25–29)

HOSTS: *Farancia abacura* (Holbrook), mud snake; *Natrix c. cyclopion, new host.*

SITE OF INFECTION: Middle third of intestine.

LOCALITIES AND PREVALENCE: Payne's prairie, Alachua County, Florida (1/1); Lake Okeechobee, Okeechobee County, Florida (1/1); *new localities.*

SPECIMENS DEPOSITED: HWML No. 20288.

PREVIOUS REPORTS: Harwood (1932) in Texas from *F. abacura* (redescription). MacCallum (1921) based the original description on immature specimens from a captive host without locality data.

DIAGNOSIS: Scolex 402–600 wide (500), spinose; suckers 133–150 in diameter (200); inconspicuous apical protuberance lined with darkly staining cells (none reported). Width: length ratios of mature proglottids 1:1.0–1.9. Testes 374–489 (390–420) averaging 419; 71–102 preporally, 88–144 postporally, 200–263 antiporally. Ratio of cirrus sac length: proglottid width 1:4.4–5.8; basal portion of cirrus thin-walled, up to 10 thick. Inconspicuous genital atrium without papillae. Genital pores alternating irregularly in anterior 36–46% averaging 40% of proglottid. Ovary bilobed with flat lobes not reaching near vitelline fields. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles not in single file in gravid proglottids. Width: length ratios of gravid proglottids 1:1.7–2.4. Ratio of cirrus sac length: proglottid width 1:4.5–5.3. Genital pores in anterior 35–45% averaging 40% of proglottid. Uterus with 72–100 averaging 80 lateral branches (60–100). Preformed ventral uterine pores lacking. Uterus occupying 18–29% of proglottid width. Eggs 29–34 in diameter, oncospheres 17–23.

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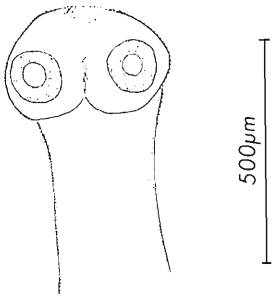
Figures 25–29. ***Proteocephalus faranciae.*** **25. Scolex. 26. Ootype region. 27. Terminal genitalia. 28. Mature proglottid. 29. Gravid proglottid.**

Figures 30–34. ***Proteocephalus grandis.*** **30. Scolex. 31. Ootype region. 32. Terminal genitalia. 33. Mature proglottid. 34. Gravid proglottid.**



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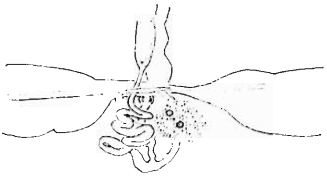

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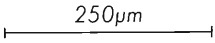

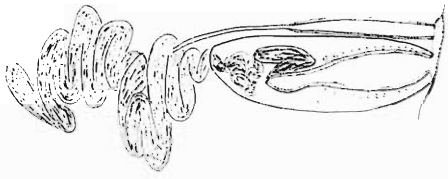

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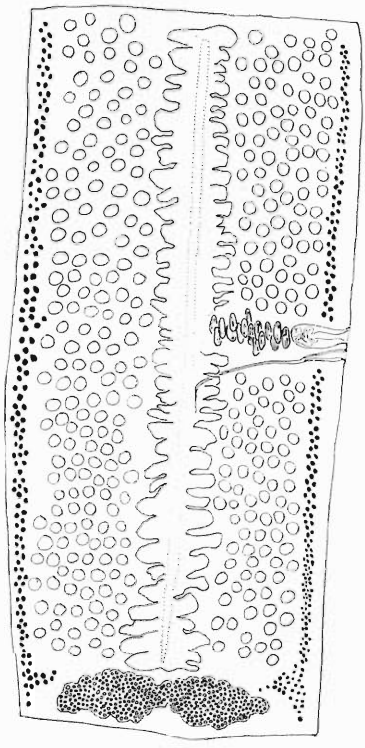

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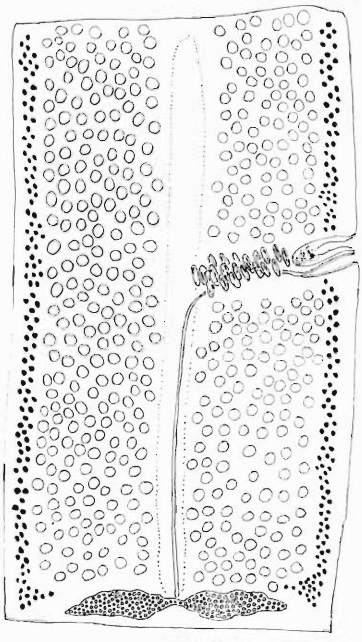

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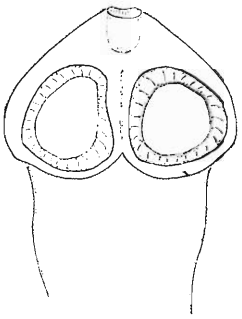
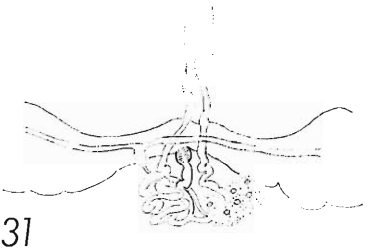


500µm

30



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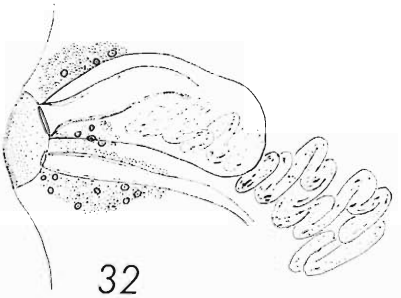
250µm



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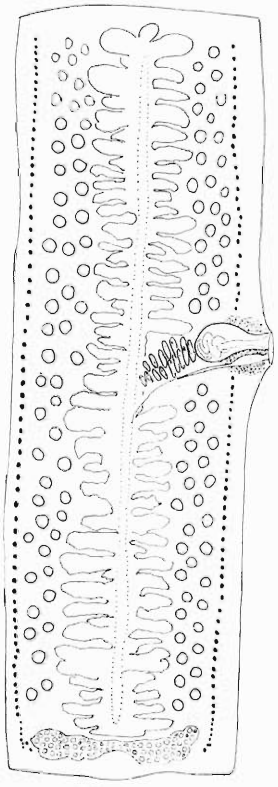
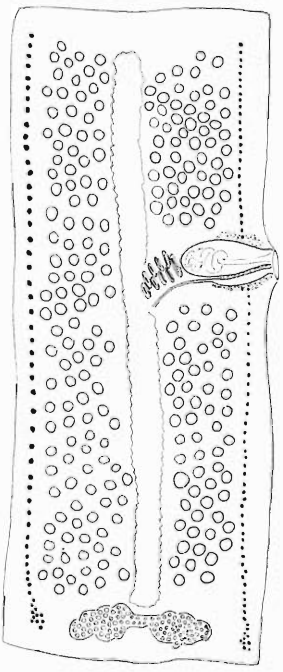


1mm

34



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Proteocephalus grandis (LaRue, 1911) Harwood, 1933 (Figs. 30–34)

HOSTS: *Natrix c. cyclopion*; *N. c. floridense*, *new hosts.*

SITE OF INFECTION: Anterior ⅔ of intestine.

LOCALITIES AND PREVALENCE: Rockefeller Wildlife Refuge, Cameron Parish, Louisiana (1/1); Payne's Prairie, Alachua County, Florida (1/1), and Lake Okeechobee, Okeechobee County, Florida (1/1), *new localities.*

SPECIMENS DEPOSITED: HWML No. 20289.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 14854.

PREVIOUS REPORTS: Fantham and Porter (1954) in Montreal, Canada from *Thamnophis sirtalis*, *T. ordinoides*, and *Agkistrodon piscivorus* (doubtful host identification); Roberts (1956) in Oklahoma from *A. piscivorus.* The original description by LaRue (1911) was based on specimens (USNM Helm. Coll. No. 14854) collected from an *A. piscivorus* which died at the National Zoo in Washington, D.C. and contained no locality data.

DIAGNOSIS: Scolex 624–750 wide (1.0–1.2 mm), spinose; suckers 250–310 in diameter (500–600); apical organ well developed (reported to be absent but present in holotype). Width: length ratios of mature proglottids 1: 1.8–2.6. Testes 141–287 averaging 220 (200–250); 32–62 preporally, 32–75 postporally, 77–156 antiporally. Ratio of cirrus sac length: proglottid width 1:2.8–3.3 (1:3–5); basal portion of cirrus thin-walled, up to 10 thick. Conspicuous genital atrium with papillae. Genital pores alternating irregularly in anterior 31–44% averaging 40% of proglottid. Ovary bilobed with flat lobes not reaching nearly to vitelline fields. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottids; follicles tending to be in single file in gravid proglottids. Width: length ratios of gravid proglottids 1:2.1–3.3. Ratio of cirrus sac length: proglottid width 1:3.1–3.5. Genital pores in anterior 41–46% averaging 43% of proglottid. Uterus with 66–97 averaging 80 lateral branches (80–120). Six preformed ventral uterine pores. Uterus occupying 23–55% of proglottid width. Eggs 52–76 in diameter (26–37), oncospheres 23–32 (15–16).

Specimens of *P. grandis* from both subspecies of *Natrix cyclopion* differed from those described by LaRue (1911) from *Agkistrodon piscivorus* in having smaller scolices, larger eggs, and slightly fewer uterine branches, but without evidence indicating that the variation is not caused by host influence or methods of fixation, I consider those specimens from water snakes conspecific with those from water moccasins.

Proteocephalus marenzelleri (Barrois, 1898) Railliet, 1899 (Figs. 35–39)

HOST: *Agkistrodon piscivorus* (Lacépède), water moccasin.

SITE OF INFECTION: Entire length of intestine.

LOCALITIES AND PREVALENCE: Rockefeller Wildlife Refuge, Cameron Parish, Louisiana (1/1); 16 kilometers east of Ruston, Lincoln Parish, Louisiana (1/1), *new localities.*

SPECIMENS DEPOSITED: HWML No. 20287, 20323.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 51179.

PREVIOUS REPORTS: Harwood (1933) from Texas in *A. piscivorus*; Fantham and Porter (1954) from Montreal, Canada in *A. piscivorus* (doubtful host identification); Collins (1969) from North Carolina in *A. piscivorus.* The original description by Barrois (1898) and redescriptions of the same material by Schwarz (1908) and Woodland (1925) presented no locality data.

DIAGNOSIS: Strobila composed of 90% immature proglottids. Scolex 1.5–2.3 mm wide (12–2.0 mm), spinose; suckers 590–970 in dimeter (600–700); apical organ 220–285 in diameter buried in scolex (none reported). Width: Length ratios of mature proglottids 1: 2.2–2.5. Testes 198–260 averaging 215 (150,

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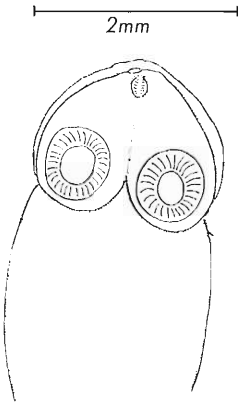
Figures 35–39. ***Proteocephalus marenzelleri.*** **35. Scolex. 36. Ootype region. 37. Terminal genitalia. 38. Mature proglottid. 39. Gravid proglottid.**



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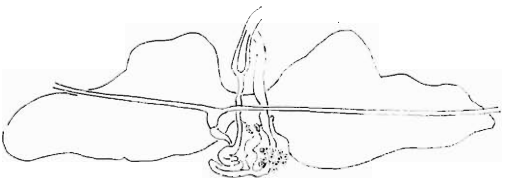

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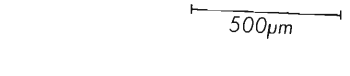

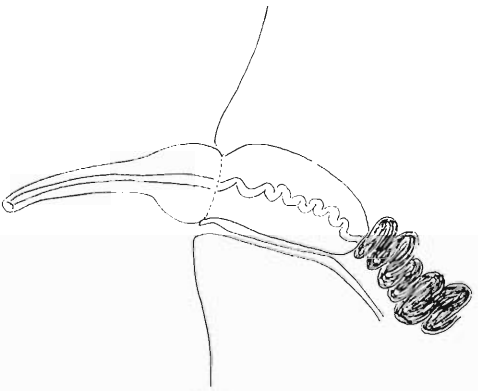

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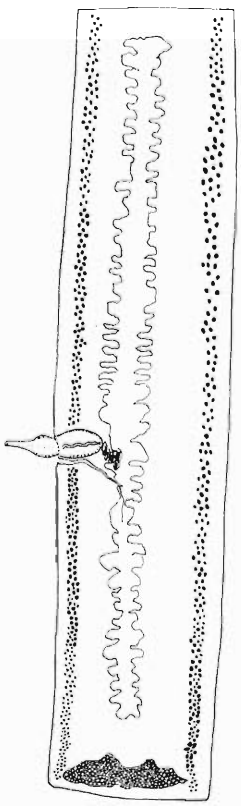

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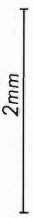

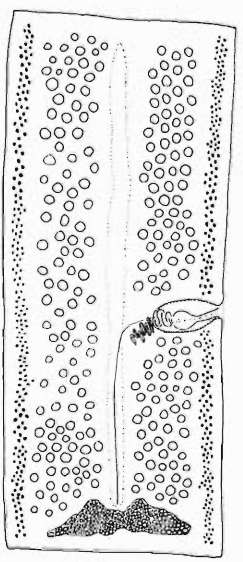

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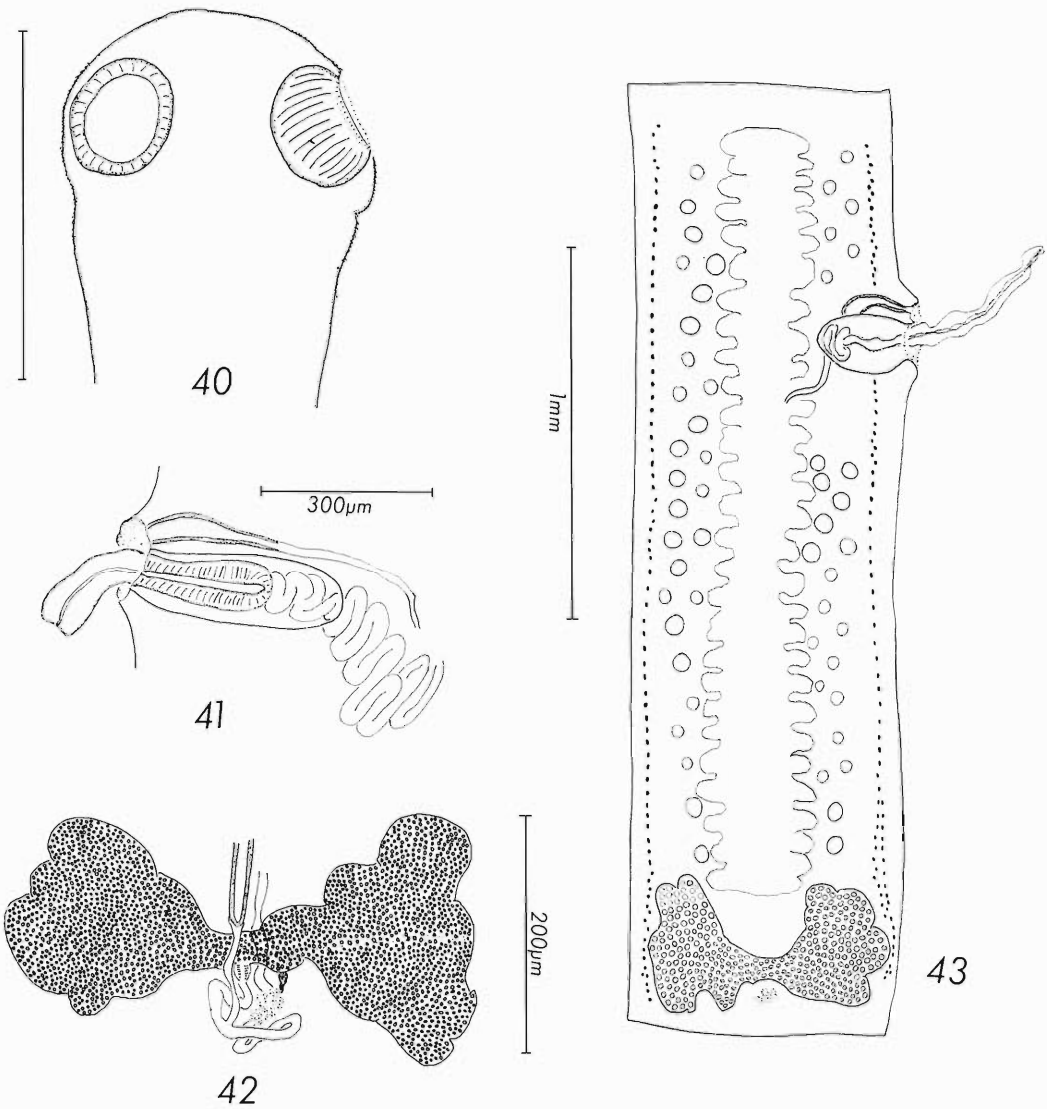


Figures 40–43. ***Proteocephalus agkistrodontis.*** **40. Scolex. 41. Terminal genitalia. 42. Ootype region. 43. Gravid proglottid.**

200–240, many); 52–90 preporally, 30–50 postporally, 100–130 antiporally. Ratio of cirrus sac length: proglottid width 1:3.5–4.0 (1:3). Inconspicuous genital atrium with papillae. Genital pores alternating irregularly in anterior 50–56% averaging 53% of proglottid. Ovary in mature proglottids bilobed with flat lobes not reaching nearly to vitelline fields; lobes expanded anteriorly in gravid proglottids reaching nearly to vitelline fields. Vagina anterior or posterior to cirrus sac, not crossing it; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles not in single file. Width: length ratios of gravid pro-

glottids 1:2.4–4.6. Ratio of cirrus sac length: proglottid width 1:2.9–3.9 Genital pores in anterior 42–58% averaging 50% of proglottid. Uterus with 46–80 averaging 65 lateral branches (40–50). Four preformed ventral uterine pores. Uterus occupying 20–38% of proglottid width. Eggs 29–40 in diameter, oncospheres 11–17.

Agkistrodon piscivorus apparently does not range farther north than southern Illinois (Conant, 1958); therefore, Fantham and Porter's (1954) report may have been in error.

Proteocephalus agkistrodontis Harwood, 1933 (Figs. 40–43)

HOST: *Agkistrodon piscivorus.*

SITE OF INFECTION: Anterior ¼ of intestine.

LOCALITIES AND PREVALENCE: Houston, Texas; Corney Lake, Union Parish, Louisiana (1/1), *new locality.*

SPECIMENS DEPOSITED: HWML No. 20322.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 8584.

PREVIOUS REPORTS: Harwood (1933) from Texas in *A. piscivorus* (description); Roberts (1956) from Oklahoma in *A. piscivorus* (tentatively identified from immature specimens).

DIAGNOSIS: Scolex 850–950 wide, spinose; suckers 200–300 in diameter; apical organ lacking. Testes 85–142 averaging 108 (90–110); 7–19 preporally, 33–49 postporally, 41–79 antiporally. Ratio of cirrus sac length: proglottid width 1:2.4–3.3. Inconspicuous genital atrium with papillae (none reported). Genital pores alternating irregularly in anterior 27–30% averaging 29% of proglottid. Ovary bilobed with lobes expanded anteriorly in mature proglottids reaching nearly to vitelline fields. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter absent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria extending nearly entire length of proglottid; follicles tending to be in single file in gravid proglottids. Width: length ratios of gravid proglottids 1:3.2–6.4. Ratio of cirrus sac length: proglottid width 1:2.0–2.5. Genital pores in anterior 16–28% averaging 22% of proglottid. Uterus with 40–70 averaging 55 lateral branches (50–60). Four preformed ventral uterine pores. Uterus occupying 40–70% of proglottid width. Eggs 40–57 in diameter, oncospheres 17–34.

Harwood (1933) figured only a mature proglottid in describing *P. agkistrodontis*; my figures 40–43 illustrate the holotype. Even though neither Harwood nor I show preformed uterine pores on the holotype, they are evident on the Louisiana specimens.

Proteocephalus testudo (Magath, 1924) Hughes, Baker, and Dawson, 1941 (Figs. 44–45)

HOSTS: *Trionyx spiniferus* (LeSueur), spiny softshell turtle; *Pseudemys elegans* (Wied-Neuwied) (= *Chrysemys scripta elegans*).

SITE OF INFECTION: Middle third of intestine.

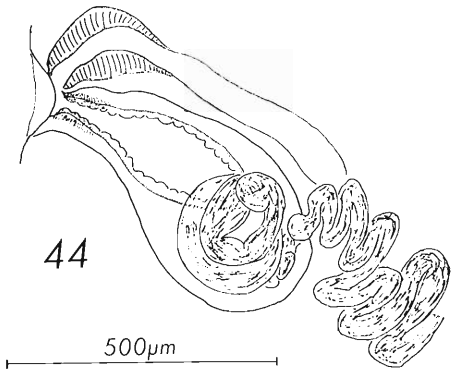
LOCALITIES AND PREVALENCE: Missouri River, 2.5 kilometers south of Brownville, Nebraska (2/5) and Lake of the Woods, Marshall County, Bremen, Indiana (2/2), *new locality*; Meredosia, Illinois, *new locality.*

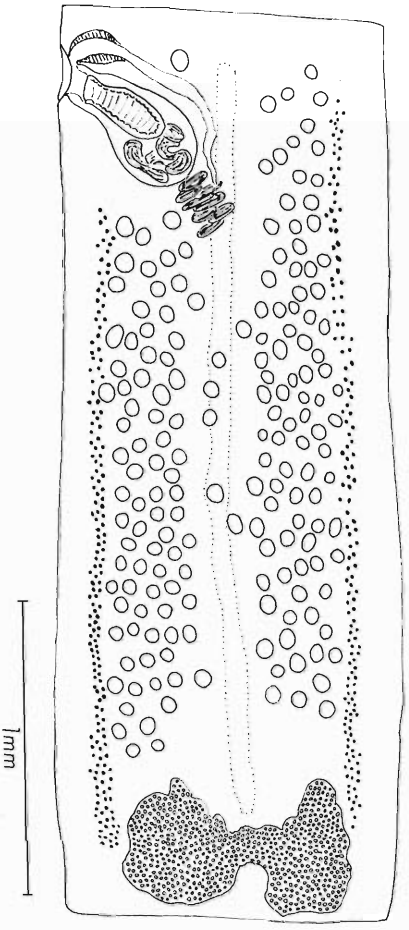
SPECIMENS DEPOSITED: HWML No. 20451.

SPECIMEN EXAMINED: USNM Helm. Coll. No. 51180.

PREVIOUS REPORTS: Magath (1924) from Minnesota in *T. spiniferus* (description); Magath (1929) from Minnesota in *T. spiniferus* (larval development); McKnight (in Acholonu, 1970) from Oklahoma in *Graptemys pseudogeographica* Grey and *Pseudemys* (= *Chrysemys*) *scripta elegans*; Acholonu (1970) from Louisiana in *T. spiniferus*; Brooks and Mayes (1975) from Nebraska in *T. spiniferus.*

DIAGNOSIS: Scolex 310–600 wide (630), aspinose; suckers 105–139 in diameter (100); prominent apical protuberance lacking glandular cells. Width: length ratios of mature proglottids 1:1.3–2.9. Testes 120–229 averaging 181 (125–200), 0–6 preporally, 72–112 postporally, 69–111 antiporally. Ratio of cirrus sac length: proglottid width 1:2.3–3.6. Inconspicuous genital atrium lacking papillae. Genital pores alternating irregularly in anterior 10–14% averaging 11% of proglottid. Ovary in mature proglottids bilobed with lobes expanded anteriorly reaching nearly to vitelline fields. Vagina anterior or posterior to cirrus sac, crossing it or not; vaginal sphincter prominent, seminal receptacle present. Uterus preformed in mature proglottids. Vitellaria





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lacking preporally, follicles not in single file. Width: length ratios of gravid proglottids 1: 2.0–3.3. Ratio of cirrus sac length: proglottid width 1: 1.7–2.4. Genital pores in anterior 11–17% averaging 14% of proglottid. Uterus with 24–54 averaging 40 lateral branches (30–40). Preformed ventral uterine pores lacking. Uterus occupying 47–60% of proglottid width. Eggs 29–37 in diameter (29), oncospheres 18–27 (19–21).

Proteocephalus testudo most closely resembles *P. australis* Chandler, 1935 from *Lepisosteus osseus* L., a teleost fish from Texas. The two species share the following characteristics: no preporal vitelline follicles, prominent vaginal sphincter, aspinose scolex with rudimentary apical protuberance, ovarian lobes expanded anteriorly, and an average of 40 lateral uterine branches. *Proteocephalus australis* differs by having a shorter strobila with wider and longer proglottids, a subglobose scolex not sharply set off from the strobila, a shorter neck, a smaller cirrus sac, and fewer testes. Lonnberg (1894) described *Tetrabothrium trionychinum*, generally considered a proteocephalid from *Trionyx ferox* in Florida, but Magath (1924) and Yamaguti (1959) considered his description too general for adequate differentiation from other proteocephalids. McKnight (see Acholonu, 1970) collected proteocephalids from *Trionyx spiniferus spiniferus*, *T. s. emoryi* (reported as *T. ferox emoryi*), *Graptemys pseudogeographica*, and *Pseudemys* (= *Chrysemys*) *scripta elegans* in southern Oklahoma. Those specimens he collected from *Trionyx* he referred to as *Proteocephalus trionychinum* (Lonnberg, 1894) and those from other turtles as *P. testudo*. The characteristics which he cited as distinguishing the two species constitute those which distinguish *P. testudo* from *P. australis*. Because of the similarity between McKnight's specimens from *Trionyx* and *P. australis*, and because no collections have been made from *Trionyx ferox* in Florida, I consider the identity of *Tetrabothrium trionychinum* and of *P. trionychinum sensu* McKnight unresolved; with *P. australis* and *P. testudo* they may form a complex of closely related species.

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Figures 44–45. ***Proteocephalus testudo.*** **44. Terminal genitalia. 45. Mature proglottid.**

Discussion

The proteocephalids of reptiles and amphibians in North America have been discussed in detail primarily by LaRue (1914) and Freze (1965).[4] Freze summarized previous taxonomic opinions relating to supraspecific categories and divided the proteocephalids parasitizing these hosts and possessing medullary gonads and simple scolices into the three genera *Ophiotaenia*, *Testudotaenia*, and *Batrachotaenia* based on the five characters discussed below.

1. Host

Freze *à priorally* based his entire classification scheme on the unsubstantiated principle that the outstanding feature of the taxonomic and systematic status of the proteocephalids was their pronounced host specificity. Accordingly, he placed similar cestodes of teleost fishes (*Proteocephalus*) in one family and those of snakes (*Ophiotaenia*), amphibians (*Batrachotaenia*), and turtles (*Testudotaenia*) in another. The degree of confidence one may place in Freze's scheme depends on the degree to which other characters, such as morphological and ontogenetic ones, also support the scheme. Differences in host are by themselves not adequate means of delineating phylogenetic relationships.

2. Relative size of Mehlis' gland

Freze did not present means of quantifying the relative size of this organ, but did state that species of *Batrachotaenia* possessed prominent Mehlis' glands and those of *Ophiotaenia* did not; his diagnosis of *Testudotaenia* failed to mention the character. Since the species *grandis* and *variabilis* from snakes each has a prominent Mehlis' gland and *aberrans* and *loennbergii* from amphibians have small ones, there is no reason to suspect that differences in the relative size of this organ are indicative of more than specific differences; also, original features of *Proteocephalus tigrinus* Woodland, 1925 and *P. niuginii* Schmidt, 1975 suggest that those two species from frogs in Asia also lack prominent Mehlis' glands.

[4] The English translation of Freze's monograph (see Lit. Cited) reverses the terms "mature" and "gravid" consistently throughout the monograph.

3. Presence or absence of tegumental spines

Freze stated that species of *Ophiotaenia* have tegumental spines, but those of *Batrachotaenia* and *Testudotaenia* do not. All six species of proteocephalids from snakes in North America have tegumental spines; those from amphibians and *P. testudo* do not. However, *P. trigrinus* *P. niuginii* possess such spines. Even though this character holds true for North American species, it is an unreliable generic character.

4. Extent of vitellaria

The two species Freze placed in *Testudotaenia* reportedly have vitelline follicles which do not extend near the anterior end of the proglottid, although one of them, *Ophiotaenia cohospes* Coredo, 1946, has preporal vitelline follicles. *Proteocephalus testudo*, *P. australis*, and *P. cryptobranchi* parasitizing a turtle, teleost fish, and amphibian, respectively, all lack preporal vitelline follicles, but this is not due to a compacting of the follicles posteriorly as suggested by Freze. I have already stated reasons for considering *P. testudo* closely related to *P. australis*, and the occurrence of restricted vitelline follicles in four species from three different host groups does not, in my opinion, warrant generic recognition.

5. Relative width of proglottids

Species of *Ophiotaenia sensu* Freze purportedly possess proglottids much longer than wide, but those of *Batrachotaenia* have proglottids which are not significantly longer than wide; again, this character was not mentioned in the generic diagnosis of *Testudotaenia*. The width: length ratios of proglottids for six species of *Batrachotaenia* and six of *Ophiotaenia* as defined by Freze, which I have examined, are presented below to show overlap in these ratios among the members of this group of cestodes.

Batrachotaenia		*Ophiotaenia*	
magnus	1 : 0.9–1.1	*faranciae*	1 : 1.0–2.6
sireni	1 : 1.7–3.3	*perspicua*	1 : 1.0–2.7
filaroides	1 : 2.4–3.4	*grandis*	1 : 1.8–3.3
aberrans	1 : 1.3–3.8	*variabilis*	1 : 1.8–4.8
loennbergii	1 : 1.0–5.0	*agkistrodontis*	1 : 1.9–6.4
amphiumicola	1 : 0.9–5.4	*marenzelleri*	1 : 2.2–4.6

Since the dichotomies among these proteocephalids proposed by Freze have no universal significance on the basis of the above five

characters, I consider *Ophiotaenia* LaRue, 1914 the senior synonym of *Batrachotaenia* and *Testudotaenia*.

Critical examination of Freze's familial distinctions revelas that the genera *Proteocephalus* and *Ophiotaenia* (*s. l.*) differ in the following manner: *Proteocephalus* species parasitize teleost fish and lack preformed uteri in mature proglottids, and *Ophiotaenia* species parasitize herptiles and have preformed uteri in mature proglottids (which makes the testes appear to lie in two separate fields), characters which previous authors also considered to be of generic significance. Freze cited the presence or absence of preformed uteri as strong corroboration of his assignment of the two genera to different families, ignoring three shared characterstics: identical scolex morphology, identical internal arrangement of gonads (all medullary), and identical life cycle and development patterns. Even the phenomenon of autoinfection by *Proteocephalus ambloplitis* in centrarchid fishes is duplicated by *P. filaroides* in ambystomatid salamanders (Mead and Olsen, 1971). To preserve his classification scheme, Freze considered *Ophiotaenia fragile* Essex, 1929 from an ictalurid fish a species of *Proteocephalus sensu lato*. I examined specimens of *Proteocephalus ambloplitis* (Leidy, 1887) Benedict, 1900, *P. buplanensis* Mayes, 1976, *P. pinguis* LaRue, 1911, *P. pearsei* LaRue, 1919, and *P. singularis* LaRue, 1911 as well as *O. fragile*. Of these piscine species, only *P. buplanensis* lacks preformed uteri. Freze mentioned that many piscine species of *Proteocephalus* possessed vaginal sphincters, but did not attribute to that fact any taxomonic significance. All piscine species examined in this study, as well as *P. testudo* and *P. calmettei* (Barrois, 1898) Railliet, 1899 (USNM Helm. Coll. No. 30939 from a snake on Martinique), have vaginal sphincters. *Proteocephalus amphiumicola* and *P. alternans* have parenchymal muscles surrounding the terminal end of the vagina which presumably function as sphincters (Fig. 3); otherwise, no species from herptiles in North America possess vaginal sphincters. Unfortunately, the status of this character is lacking for many species and until such time as a consistent character other than host is documented, I consider *Ophiotaenia* a junior synonym of *Proteocephalus*.

As a result of this study, I am designating the following new combinations (some also noted in text): *Crepidobothrium olor* becomes *Proteocephalus olor* (Ingles, 1836) comb. n.; *C. amphiumae* becomes *P. amphiumae* (Zeliff, 1932) comb. n.; *Ophiotaenia alternans* becomes *P. alternans* (Riser, 1942) comb. n.; *O. cryptobranchi* becomes *P. cryptobranchi* (LaRue, 1911) comb. n.; *O. saphena* becomes *P. saphenus* (Osler, 1931) comb. n.; *O. gracilis* become *P. gracilis* (Jones, Cheng, and Gillespie, 1958) comb. n.; *O. sireni* becomes *P. sireni* (Brooks and Buckner, 1977) comb. n.; *O. fragile* becomes *P. fragile* (Essex, 1929) comb. n.

This report brings to 19 the known nominal species of *Proteocephalus* parasitizing herptiles in North America. I attempted to gain additional insight into their host–parasite relationships using the method of cladistical analysis proposed by Camin and Sokal (1965) based on a suite of 11 morphological characters. I have already expressed the opinion that *P. testudo* is more closely related to *P. australis* than to the other species from herptiles; a great deal of ambiguity in initial results was eliminated by removing *P. testudo* from the analysis and basing it instead on the 18 species from snakes, frogs, and salamanders. Coding of characters which form a continuous variable, such as scolex morphology, proglottid dimensions, or relative uterine width, or for which the derived character-state occurs in only one species were not used. Similarly, sucker size was not used, being redundant with scolex width.

The characters and their coded states:

1. Length of strobila. 0: more than 300 mm long; 1: less than 280 mm long.

2. Scolex width. Two states. 0: does not exceed 1 mm; 1: exceeds 1 mm.

3. Average number of testes. Four states. 0: 100–150; –1: 65–80; 1: 175–225; 2: over 400.

4. Shape of ovarian lobes in mature proglottids. Two states. 0: flat; 1: expanded anteriorly.

5. Average number of uterine branches. Four states. 0: 55–80; 1: 35–50; 2: none; –1: 100.

6. Position of genital pores. Two states. 0: in anterior 15–35% of proglottid; 1: in anterior 40–60% of proglottid.

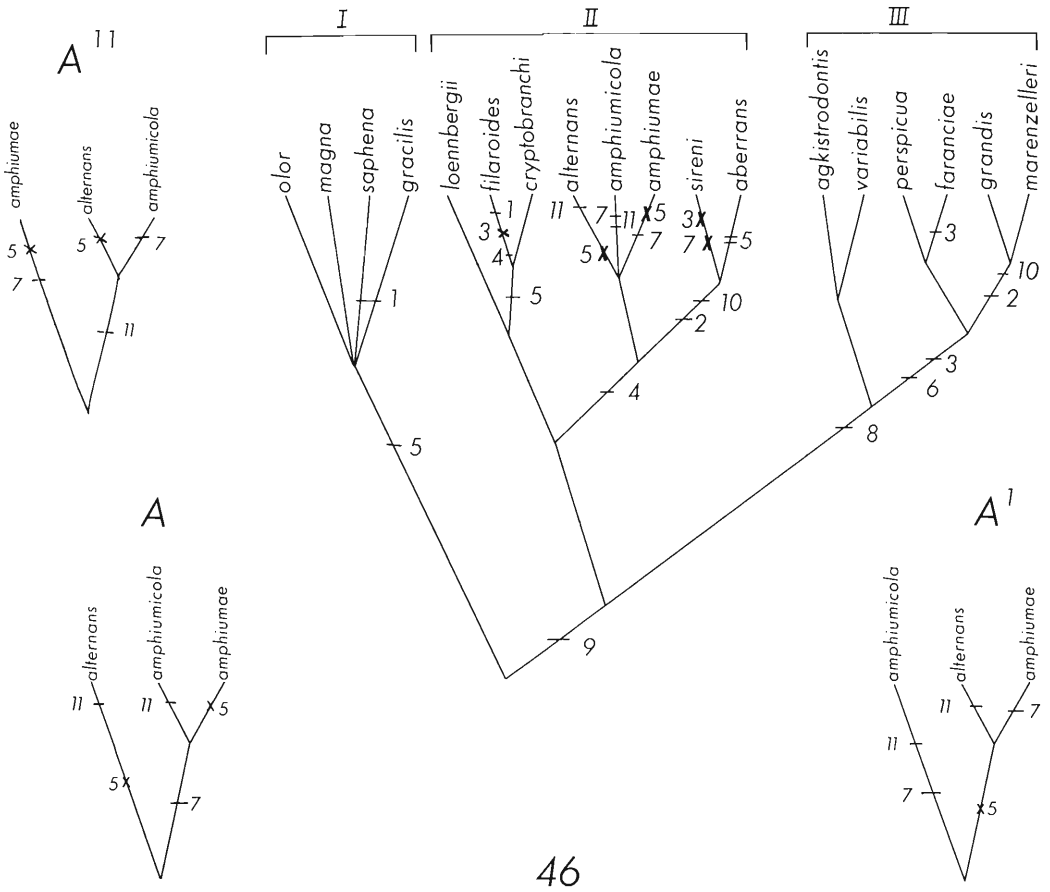

Figure 46. Cladogram depicting interspecific relationships of proteocephalid cestodes parasitizing salamanders, snakes, and frogs in North America. A, A′, and A″ represent subcladograms showing equally parsimonious arrangement of species *Proteocephalus amphiumae*, *P. alternans*, and *P. amphiumicola*.

7. Relative position of vagina. Three states. 0: either anterior or posterior to cirrus sac; −1: posterior only; 1: anterior only.

8. Presence or absence of tegumental spines. Two states. 0: absent; 1: present.

9. Width: length ratios of gravid proglottids. Two states. 0: never more than 1.5 times longer than wide; 1: more than 2 times longer than wide.

10. Presence or absence of preformed ventral uterine pores. Two states. 0: absent; 1: present.

11. Presence or absence of vaginal "sphincter." Two states. 0: absent; 1: present.

Parsimonius arrangement of species based on shared derived morphological characteristics (synapomorphies of Hennig, 1966) (Fig. 46) places the 18 species in lineages I to III. Those in I and II parasitize anurans and caudate amphibians, respectively, and would be placed by Freze in *Batrachotaenia*, while those in III occur in snakes and would be placed in *Ophiotaenia*. As shown earlier, however, the distinctions cited by Freze have no universal significance, and the lineages of the North American species are important relative only to each other. Since their host group is heterogeneous, and since the parasites themselves

Table 1. Parasite–host list of proteocephalid cestodes parasitizing North American salamanders.

Parasite	Hosts (family in parentheses)
Proteocephalus cryptobranchi	*Cryptobranchus alleghaniensis* (Cryptobranchidae) *Desmognathus fuscus* *D. ochrophaeus* *D. monticola* *Eurycea lucifuga* *Notopthalmus viridescens* *Plethodon jordani metcalfi* (Plethodontidae)
Proteocephalus loennbergii	*Necturus maculosus* (Proteidae)
Proteocephalus filaroides	*Ambystoma tigrinum* *A. macrodactylum* *A. maculatum* (Ambystomatidae)
Proteocephalus amphiumae	*Amphiuma tridactylum* (Amphiumidae) *Desmognathus fuscus* (Plethodontidae) *Typhlotriton spelaous* (Salamandridae)
Proteocephalus alternans	*Amphiuma tridactylum* (Amphiumidae)
Proteocephalus amphiumicola	*Amphiuma means* (Amphiumidae)
Proteocephalus sireni	*Siren intermedia* (Sirenidae)
Proteocephalus aberrans	*Siren lacertina* (Sirenidae)

exhibit a marked degree of host specificity (Table 1), the host–parasite relationships of members of lineage II have been examined in greater detail. The subcladogram for them provides three equally parsimonious arrangements for the species *amphiumicola*, *amphiumae*, and *alternans* (A, A′, and A″). Arrangement A indicates closer relationship between two geographically proximate species parasitizing different host species, A′ closer relationship between two geographically disparate species parasitizing the same host species, and A″ between two geographically disparate species parasitizing different host species. Personal bias suggests that a combination of both host and geography often determine speciation-events; thus, although requiring an extra evolutionary step (therefore becoming less parsimonious), my preference for the arrangement of the above three species is that shown in the cladogram—the consensus of A, A′, and A″ (cf. method of Adams, 1972). Regardless of which arrangement one prefers, there is a marked degree of concordance between the phylogenetic relationships of the cestodes and the phylogeny of their host families (Fig. 47), strongly suggesting that these cestodes evolved in a manner paralleling that of their hosts. Because they are geographically proximate, are morphologically homogeneous, and have coevolved with their hosts, I submit that the members of lineage II constitute a monophyletic species-group within the genus.

Eight species and subspecies of snakes harbor species of *Proteocephalus* in North America (Table 2). All are semiaquatic or are commonly found in aquatic habitats. Reported hosts of *P. perspicua* include six species and subspecies of water snakes in the genus *Natrix* Laurenti. Of those water snakes examined in this study, only *N. erythrogaster flavigaster* failed to host *P. perspicua*. Recent studies by Hebrard and Mushinskie (1976) and Mushinskie and Hebrard (1976) show that *N. e. flavigaster* feeds primarily on frogs and toads, and *N. fasciata*, *N. cyclopion*, and *N. rhombifer* feed primarily on fish; also, *N. e. flavigaster* spends relatively less time in contact with aquatic habitats than the others. Since *P. perspicua* utilizes small fish as intermediate hosts (Thomas, 1941), the absence of the parasite in *N. e. flavigaster* apparently results from ecological specificity rather than any other parameter.

Four species of *Proteocephalus* have been described from reptiles and amphibians in North America since Freze's monograph. On the basis of specimens of the four new species (*sireni*, *amphiumicola*, *aberrans*, and *variabilis*), specimens of 14 of the 15 previously described species, and all pertinent literature, the following key separates all recognized species from North American reptiles and amphibians.

Key to Species of *Proteocephalus* Parasitizing Reptiles and Amphibians in North America

1a. Gravid proglottids never more than 1.5 times longer than wide, parasites of aquatic frogs 2

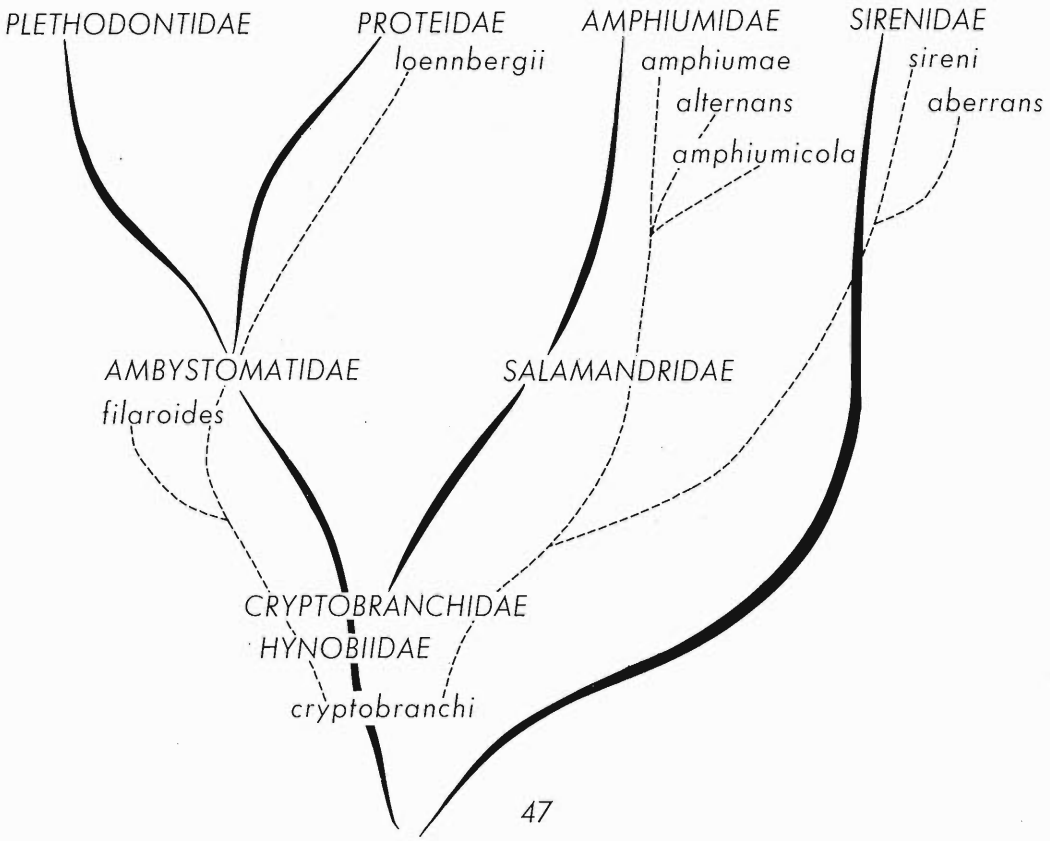

Figure 47. Proposed evolutionary development of proteocephalid cestodes parasitizing salamanders in North America compared with the phylogenetic relationships of their host families.

1b. Gravid proglottids more than 2 times longer than wide, parasites of salamanders, semiaquatic snakes, or turtles 5
2a. Ovarian lobes in gravid proglottids flat, strobila up to 800 mm long 3
2b. Ovarian lobes in gravid proglottids dumbbell-shaped, strobila up to 280 mm long 4
3a. Scolex 300–475 μm wide, parasite of red-legged frogs in California ..... *olor*
3b. Scolex 450–550 μm wide, parasites of bullfrogs and green frogs ..... *magnus*
4a. Scolex 270–320 μm wide ..... *saphenus*
4b. Scolex 350 μm wide ..... *gracilis*
5a. Preporal vitelline follicles absent 6
5b. Preporal vitelline follicles present 7
6a. Vagina always anterior to cirrus sac,

Table 2. Host–parasite list for proteocephalids parasitizing snakes in North America. Numbers in parentheses indicate number of specimens examined in present study; *indicates new host record reported in this study; †indicates parasite collected from host in this study and reported previously.

Agkistrodon piscivorus (3)
Proteocephalus perspicua
P. marenzelleri†
P. grandis
P. agkistrodontis†
Natrix fasciata confluens (4)
*Proteocephalus perspicua**
Natrix cyclopion cyclopion (5)
*Proteocephalus perspicua**
*P. grandis**
*P. variabilis**
Natrix erythrogaster flavigaster (3)
negative
Thamnophis sirtalis
Proteocephalus perspicua
P. grandis
Natrix sipedon (4)
Proteocephalus perspicua†
*P. variabilis**
Natrix rhombifer (4)
Proteocephalus perspicua†
Natrix taxispilota
Proteocephalus perspicua
Natrix cyclopion floridense (3)
*Proteocephalus perspicua**
*P. grandis**
*P. faranciae**
Farancia abacura (2)
Proteocephalus faranciae
Thamnophis ordinoides
Proteocephalus grandis

vaginal sphincter present, testes averaging 180 in number ..... *testudo*

6b. Vagina anterior or posterior to cirrus sac, vaginal sphincter lacking, testes averaging 100 in number ..... *cryptobranchi*

7a. Genital pores in anterior 20–35% of proglottid 8

7b. Genital pores in anterior 40–60% of proglottids 16

8a. Lateral uterine branches absent ..... *aberrans*

8b. Lateral uterine branches present 9

9a. Scolex averaging more than 900 μm wide 10

9b. Scolex averaging less than 650 μm wide 11

10a. Vagina always posterior to cirrus sac, testes averaging 65 in number ..... *sireni*

10b. Vagina anterior or posterior to cirrus sac, testes averaging 108 in number ..... *agkistrodontis*

11a. Ovarian lobes in mature proglottids flat 12

11b. Ovarian lobes in mature proglottids expanded anteriorly 13

12a. Scolex spinose, averaging less than 300 μm wide, uterine branches averaging 66 in number, ovarian lobes in gravid proglottids dumbbell-shaped ..... *variabilis*

12b. Scolex aspinose, averaging 650 μm wide, uterine branches averaging 50 in number, ovarian lobes in gravid proglottids expanded posteriorly ..... *loennbergii*

13a. Uterine branches averaging 35 in number, testes averaging 70 in number ..... *filaroides*

13b. Uterine branches averaging more than 60 in number, testes averaging 100–115 in number 14

14a. Vagina anterior or posterior to cirrus sac, vaginal "sphincter" present, fusiform, uterine branches averaging 100 in number ..... *alternans*

14b. Vagina anterior to cirrus sac, eggs spherical 15

15a. Vaginal "sphincter" present, uterine branches averaging 66 in number ..... *amphiumicola*

15b. Vaginal "sphincter" lacking, uterine branches averaging 100 in number ..... *amphiumae*

16a. Scolex averaging 1.5 mm wide, genital pores in average proglottids 50% of proglottid length from anterior end, 90% of proglottids immature ..... *marenzelleri*

16b. Scolex averaging less than 1 mm wide, genital pores in average proglottids 40% of proglottid length from anterior end 17

17a. Testes averaging more than 400 in number ..... *faranciae*

17b. Testes averaging less than 250 18

18a. Uterine branches averaging 55 in number, scolex averaging 300 μm wide ..... *perspicua*

18b. Uterine branches averaging 80 in number, scolex averaging 650 μm wide (may exceed 1 mm) ..... *grandis*

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Duplication of Reproductive Systems in Monozoic Cestodes (Caryophyllidea)

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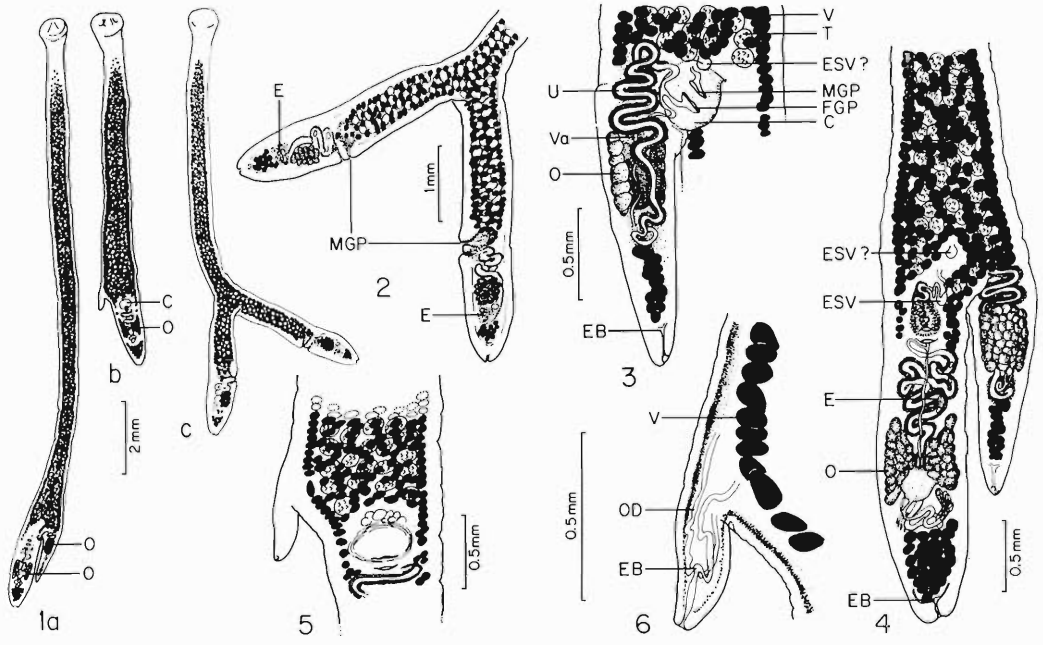
ABSTRACT: Five anomalies involving the duplication of reproductive systems are described and illustrated from *Glaridacris catostomi* Cooper, 1920, and an undescribed species of *Penarchigetes* Mackiewicz, 1969. A lateral swelling from *Promonobothrium minytremi* Mackiewicz, 1968 is also reported. One of the examples each from *G. catostomi* and *Penarchigetes* had a complete set of reproductive organs and contained eggs. Important questions of the orientation, symmetry, cytodifferentiation, development, and evolution of caryophyllid cestodes and of the cestoda are discussed, and it is concluded that it is highly unlikely that strobilization arose through secondary branching from a monozoic ancestor.

Morphological anomalies involving the duplication of gonopores and reproductive systems of polyzoic tapeworms are well known (Clapham, 1939). On the other hand, similar anomalies among monozoic tapeworms, namely the Caryophyllidea, are unknown (Mackiewicz, 1972). Such anomalies in the Caryophyllidea are of particular interest because they may offer clues to the evolution or origin of the polyzoic morphology from monozoic ancestors, assuming caryophyllideans are primitively monozoic. This paper, the first of its kind, reports of the duplication of the reproductive systems of *Glaridacris catostomi* Cooper, 1920, from *Catostomus commersoni* and *Penarchigetes* sp. from *Erimyzon sucetta*.

Appreciation and thanks are extended to: Mr. P. Muzzall, University of New Hampshire, for the specimen shown in Figure 1c; Mr. L. Grimes, University of North Carolina, for the specimen shown in Figure 8; Dr. E. Williams, University of Puerto Rico, for the specimens shown in Figures 11–13; and to Mr. Anthony Grey, for the specimen shown in Figure 1b and for assistance in collecting hosts.

Specimens were stained with Semichon's carmine, and except for *Example* 2 of *G. catostomi*, all were studied only as whole mounts. The following orientation is used in the descriptions: ventral, surface on which the gonopores open; left side (actually the worm's right) corresponding to the observer's left when the ventral surface is facing the observer and, using the face of a clock for orientation, the anterior end (scolex) is at 12:00 o'clock. Drawings were made with the aid of a microprojector.



Figures 1a–6. ***Glaridacris catostomi*** **Cooper, 1920. 1a, b, c. Whole worms showing secondary branches. 2. Detail of Figure 1c. 3. Dorsal view of Figure 1a. 4. Ventral view and detail of Figure 1a. 5. Enlargement of branch of Figure 1b showing its relationship to cirrus of parent worm. 6. Internal structures of branch of Figure 1b. Abbreviations (Figs. 1a–13): cirrus (C), egg (E), excretory bladder (EB), excretory pore (EP), external seminal vesicle (ESV), female gonopore (FGP), gonopore (GP), male gonopore (MGP), ovary (O), osmoregulatory duct (OD), scolex (S), testis (T), uterus (U), uterine glands (UG), vitelline follicle (V), and vagina (Va).**

Glaridacris catostomi Cooper, 1920 (Figs. 1–6)

Example 1 (Figs. 1b, 5, 6)

LOCALITY: New York, Albany Co., Bozenkill Creek, a small tributary of Watervliet Reservoir on the Normanskill River.

This example consists of a slightly contracted, mature worm (9.5 mm long) with a small fingerlike projection or branch on the left side, *slightly* anterior of the cirrus. This lateral projection is 0.37 mm long on its shortest (inner) longitudinal axis and contains an excretory pore, reduced excretory bladder (Fig. 6) and three osmoregulatory ducts; anlage of other structures are absent. As seen from Figures 1b and 5, the greatest width of the worm is in the region of the projection. As the preovarian vitelline field passes anteriorly past the cirrus (Fig. 5), it is deflected laterally thus keeping the cortical parenchyma a uniform thickness in the region of the projection. The subtegumentary layer is continuous over the branch and retains a uniform thickness throughout (Fig. 6). Because of the lack of internal structures within the branch, it is impossible to distinguish which side is ventral.

Example 2 (Figs. 1a, 3, 4)

This example, from the same locality as the first, consists of a well extended mature worm (17.3 mm long) with a large secondary branch on the right side just above the cirrus of the main or mother worm (Fig. 1a). This branch is 1.7 mm long on its shortest longitudinal axis and takes its origin from the dorsolateral surface of the main worm. Attempts to make serial sections of the posterior end were unsuccessful, and hence it was not possible to

elucidate more precisely the relationship of internal structures, i.e., vas deferens, of each worm to each other.

The secondary branch is in a different state of maturity from the mother worm since it lacks all traces of ova. As far as could be determined, it has a complete set of reproductive organs, and an excretory bladder is present (Fig. 3). There are differences from the normal morphology, however. For example, the uterus is more anterior with respect to the cirrus than normally found in this species (compare Figs. 3 and 4); furthermore, the ovary does not have well-developed arms but appears as a broad band. It is difficult to establish whether or not the secondary branch has its own external seminal vesicle, although it appears that way from the distance separating the two cirri and the presence of what appears to be a second external seminal vesicle (Fig. 4).

The orientation of the secondary branch is difficult to interpret because part of its cirrus is incorporated into the dorsal side of the parent worm. But from Figures 3 and 4 one can see that posterior to the cirrus the orientation is like that of the main worm with the ovarian commissure also facing the ventral side. While there may have been a slight amount of twisting in the process of mounting the worm, it is my impression that it is too slight to account for a complete rotation of the ovary. It would thus appear that the secondary branch is basically oriented the same way as the main worm but that the cirrus has been rotated dorsally because of its position at the junction of the two worms.

Example 3 (Figs. 1c, 2)

LOCALITY: New Hampshire, Stratford Co., Oyster River. This example consists of a mature worm (12 mm long) with a prominent bifurcation two thirds of the way from the scolex (Fig. 1c). Both branches are about the same size (3.5 mm). The normal attitude of the specimen is assumed by rotating the scolex 90° to the left.

Each branch contains a full complement of reproductive organs, including testes, and is in the same stage of development with the left branch containing 22 eggs and the right one, 6. Internal organs of both branches are arranged in the pattern characteristic for *G. catostomi*.

It is difficult to distinguish which branch is the secondary one because both are so similar in size and development. It is my interpretation that the branch on the right in Figures 1c and 2 is the main one because it is larger and its dorsal surface is the longest one without any conspicuous angle in its length. On the other hand, the left branch is slightly smaller, and when one rotates the worm, it joins the worm at an acute angle. If this interpretation is correct then we have the following arrangement of the two branches: both share a continuous ventral side that extends from the excretory bladder of one, inside the inverted "V" of Figure 1c, to the excretory bladder of the other; the secondary branch has a dorsal surface that becomes the *ventral* surface of the main worm. Unfortunately it was not possible to trace the longitudinal muscles or the osmoregulatory ducts from the secondary branch to the main worm.

Penarchigetes sp. (Figs. 7–11)

This material consists of an immature and mature worm with a posterior bifurcation. The species is undescribed.

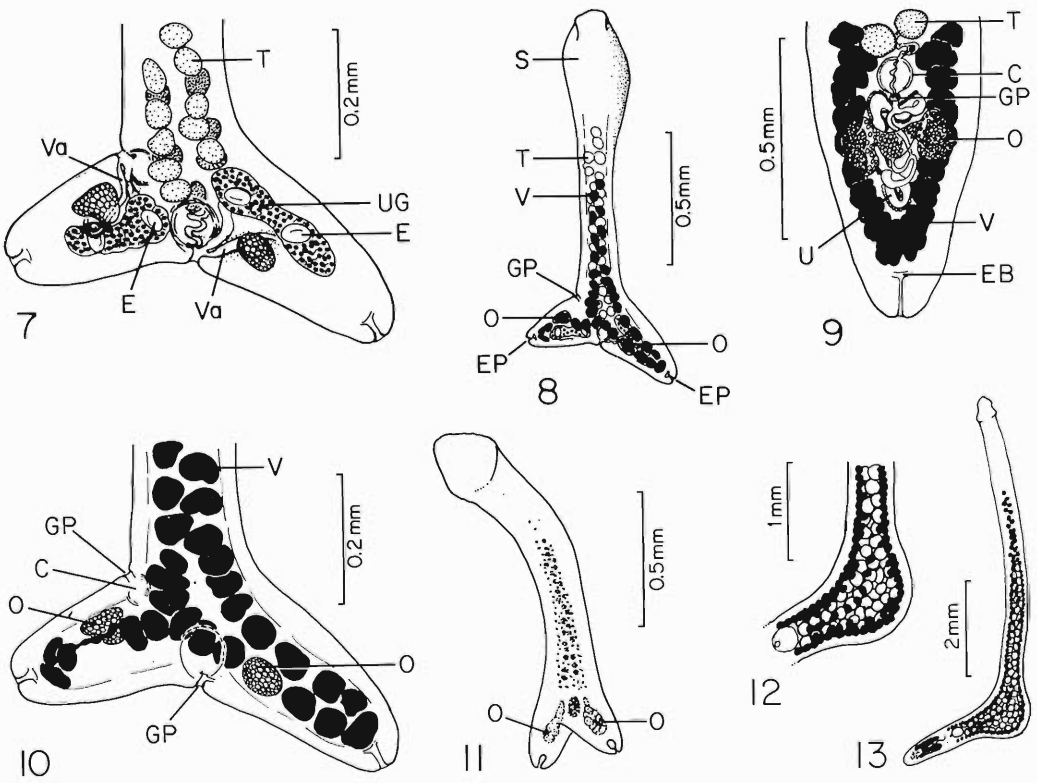
Example 1 (Fig. 11)

LOCALITY: Alabama, Macon Co., Uphapee Creek. Because the worm is immature and the gonopores not yet developed it is difficult to know what is the full extent of the bifurcation. From Figure 11 one can see that the bifurcation results in equal branches, each having an ovary and excretory bladder. The equal development of each branch makes it difficult to determine which branch is the main worm.

Example 2 (Figs. 7, 8, 10)

LOCALITY: North Carolina, Wake Co., Lake Raleigh. This single mature worm has a posterior bifurcation (Fig. 8) that arises on the ventral surface of the parent worm. The normal morphology of this species is shown in Figure 9.

The mature specimen was mounted on its side (Fig. 7), thus exposing the internal struc-



Figures 7–11. ***Penarchigetes*** **sp. 7. Lateral view and detail of Figure 8, vitellaria not drawn in. 8. Lateral view of whole worm showing bifurcation, parent worm at right. 9. Morphology of normal worm. 10. Lateral view and detail of Figure 8, testes not drawn in. 11. Immature worm. Figures 12–13.** ***Promonobothrium minytremi*** **Mackiewicz, 1968. 12. Enlargement of swelling. 13. Whole worm showing enlargement.**

tures of both branches. The parent worm has the longer branch and contains testes reaching the cirrus (Fig. 7) and has preovarian vitellaria continuous with postovarian vitellaria (Figs. 7, 10). Sperm are present in the testes.

The smaller, secondary posterior end contains a full complement of reproductive structures, but there is evidence that the development of some is less than that of the main worm. For example, the cirrus is smaller and less muscular and the postovarian vitellaria are not as numerous. Similar eggs containing vitelline cells are present in both branches.

Orientation of this secondary branch is quite different from any thus far observed. It would appear that both branches have the cirrus on the ventral side, resulting in an unusual arrangement of the dorsal and ventral surfaces. Using Figure 8 for reference, the ventral surface extends from the excretory pore to the gonopore of the main worm and from the excretory pore to gonopore (of secondary branch) to scolex apex (of main worm); the dorsal surface is from the excretory pore to scolex apex of main worm and from the excretory pore to a point near but not including gonopore (of main worm) of the secondary branch. I was unable to trace the osmoregulatory ducts or discover the fate of the dorsal and ventral musculature in adjoining dorsal and ventral sides between the two branches. Testes (N = 29) do not extend into

the smaller branch (Fig. 7) as evidenced by the uninterrupted dorsal and ventral rows.

Promonobothrium minytremi Mackiewicz, 1968 (Figs. 12–13)

This single, mature worm from *Minytrema melanops* in an unnamed tributary of the Chattahoochee River near Southwest Lanett, Alabama (Chambers Co.) is included here because it represents a type of duplication of only a portion of the reproduction system. From Figures 12 and 13 one can see the conspicuous bulge containing additional testes yet there is no specialization of the subcuticula into anlage of an excretory bladder or other structure. The worm was otherwise normal.

Discussion

Though differing in size, these secondary branches resemble each other in being a short distance anterior of the cirrus of the main worm, and when large, having a full set of reproductive organs (Figs. 1a, c; 8); an osmoregulatory system terminating in an excretory bladder is evident even in the smallest branch (Figs. 1b, 5, 6). A basic dissimilarity is the different orientation of the branches from that of the parent worm with two (Figs. 4, 8), having it in the same dorsal-ventral orientation as the main worm, while one (Fig. 2) has it oriented in an opposite direction. From these few examples it appears that the orientation of secondary branches in caryophyllid cestodes is not under the same genetic control as that of the parent worm.

Regardless of the orientation there appear to be changes in the polarity of certain sides. For example, the dorsal side of the secondary branch in Figure 2 (left one) is continuous with the ventral side of the parent worm; and in Figure 10 we have dorsal and ventral sides continuous with each other between the bifurcation. Without knowing the disposition of the longitudinal muscles of the branches and the parent worm, it is difficult to interpret the preparations; however, it seems unlikely that there are two separate muscle systems, i.e., dorsal and ventral, along the same side between the excretory pores of Figure 10 because of the absence of prominent constrictions or other evidence of additional muscle insertions. The presence of a single continuous muscle system from the branch to the parent worm would seem to indicate that the dorsal-ventral plane of the worm may be reversible and that dorsal-ventral polarity is determined by the position of the ovary and gonopores, not the sides anterior the cirrus or even the orientation of the scolex. If this is true then the body of the worm anterior of the cirrus is nonpolar with respect to the dorsal-ventral plane, thus explaining the occurrence of branches with an orientation "different" from that of the main worm. The implication of such an interpretation is that the designations "dorsal" and "ventral" have little meaning when describing the scolex or any part (e.g., muscles) of an adult tapeworm anterior the gonopores or ovary and that the *position* of the ovary or gonopores, with respect to a worm surface, is independent of scolex and body orientation. Within the cestoda the gonopores occupy different positions in the same genus, as in *Raillietina* Fuhrmann (Cyclophyllidea) where they may be on opposite lateral sides of adjacent proglottids. Within the order Spathebothridea we even find the gonopores alternating on "dorsal" and "ventral" sides as in *Spathebothrium* Linton and *Cyathocephalus* Kessler. It would thus appear that the great variation in gonopore position in cestodes is not a relatively recent evolutionary event, beginning with the advent of strobilization, but constitutes a basic developmental feature of the monozoic ancestor that is still evident in caryophyllidean cestodes.

The presence of eggs in two of the most developed stages (Figs. 2, 7) suggests that the complex processes involved in egg assembly have been achieved; however, there was no way to determine if these eggs were fertile, although they appeared similar to those of the parent worm. From the morphology of the secondary branch and presence of eggs, it would appear that the branches have reached a high level of development, which in the case of example 3 of *G. catostomi* (Fig. 1c), is similar to that of the posterior end of the parent worm. In this last case more eggs (22) were found in the branch than in the parent worm (6). If the eggs of the branches are fertile, and there is no reduction in egg output of the parent worm, the branch would

then function to increase the biotic potential of the worm, much as a proglottid does in polyzoic tapeworms. That this method (i.e., branching) of increasing biotic potential has not been selected for is evident by its absence in mature cestodes, although one does find some forms, such as *Cathetocephalus thatcheri* Dailey and Overstreet, 1973 (Tetraphyllidea), with multiple strobilae and a single scolex. While the increase in reproductive potential by nonlinear additions in caryophyllid cestodes is functionally like strobilization of polyzoic tapeworms, its morphology and development is so different that it is highly unlikely that the type of branching observed here was the evolutionary progenitor of the first strobilate tapeworms.

Perhaps the most important question is: how did the secondary branches develop? One possibility is that an injury to the tegument might have stimulated secondary growth. Unfortunately we know nothing of regeneration phenomena in these cestodes, but such an explanation has merit on theoretical grounds because these cestodes lack the well developed growth zone (Mackiewicz, 1972) found in the neck of polyzoic cestodes and therefore may not have regenerative capabilities restricted to one part of the body, if they are present at all. Another possibility is that germinal cells, acting as stem cells for the differentiation of various tissues (Gustafsson, 1976a, b), gave rise to the branch under the influence of some unknown stimulus. Little is known of the histology of mature caryophyllids but germinal cells have been identified near the tegument of immature *Archigetes* (Wiśniewski, 1930). On the other hand Gustafsson (1976a) has found that germinal cells lose their ability to divide once they have begun to differentiate. Judging from the size of two of the branches (Figs. 1a, b), it would appear that they may have formed after the worm had reached a large size and the reproductive system was well differentiated. The presence of the branches would thus provide indirect evidence of germinal cells in the parenchyma of large worms. That germinal cells are present in vitelline follicles of mature worms has already been demonstrated (Swiderski and Mackiewicz, 1976). It would appear that both of these possibilities involve the differentiation of cells in the cortical parenchyma, and perhaps interaction between germinal and tegumental cells, rather than in the medullary parenchyma where differentiation is apparently confined to a duplication of testes and vitellaria, as seen in *Promonobothrium* (Fig. 13). On the other hand, if one assumes that all germinal cells in the parenchyma have differentiated or at least begun to differentiate once the worms have reached the size shown in Figures 1a and b, then it is quite possible that the branches occurred early in the growth of the worm but have grown at a much slower rate.

The discovery and analysis of these anomalies have raised many important questions concerning the orientation, symmetry, cytodifferentiation, development, and evolution of caryophyllid cestodes and the cestoda as a whole; it has also shown that it is highly unlikely that strobilization arose through secondary branching from a monozoic ancestor.

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Pronocephalid Trematodes from a Malaysian Turtle Including a New Species of *Renigonius* Mehra, 1939[1]

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ABSTRACT: *Diaschistorchis multitesticularis*, *Parapleurogonius brevicaecum*, and *Renigonius cuorensis* sp. n. infected the Malaysian box turtle *Cuora amboinensis*. This represents a new host record for *P. brevicaecum*. *Renigonius cuorensis* differs from the only other species in the genus, *R. orientalis*, by having a relatively more slender and elongate body, more deeply lobate and less elongate testes, a lobate ovary, and unbranched ceca; eggs possess a single polar filament each, not previously reported for the genus.

The second author collected the digeneans reported in this study from a Malaysian box turtle, *Cuora amboinensis* (Daudin), captured in Telok Anson, Malaysia. Digeneans were removed from hosts, flattened with minimal coverslip pressure, fixed with AFA, and stored in 70% ethanol. After staining with acetocarmine and counterstaining with Fast Green, the worms were mounted in Canada balsam for study as whole mounts. All measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube.

Diaschistorchis multitesticularis Rohde, 1962

Rhode (1962) described *D. multitesticularis* from the turtle *Hieremys annandalei* (Boulenger) from southern Malaysia. The species has subsequently been reported from *C. amboinensis* (see Yamaguti, 1971). We deposited two specimens in the University of Nebraska State Museum, Manter Laboratory, as HWML No. 20864.

Parapleurogonius brevicaecum Sullivan, 1977

This species, described by Sullivan (1977) from *Kachuga trivittata* (Boulenger) from Malaysia, differs from species of *Pleurogonius* by exhibiting ceca terminating immediately pretesticularly rather than extending posterolateral to the testes. *Cuora amboinensis* is a new host for the species. One specimen is deposited as HWML No. 20865.

Renigonius cuorensis sp. n. (Figs. 1–3)

DESCRIPTION (based on 4 mature and 19 immature specimens, only mature specimens measured): Body 2.5–2.8 mm long by 0.8–1.2 mm wide at midbody. Tegument aspinose. Head collar continuous dorsally and ventrally, 630–780 wide. Oral sucker 113–135 long by 130–163 wide. Esophagus 315–450 long (12.6–16.8% of total body length). Cecal bifurcation 20.1–23.4% of total body length from anterior end. Ceca lined with epithelium, esophagus not lined; ceca extending to within 14.1–16.8% of total body length from posterior end.

Testes symmetrical, immediately postcecal, deeply lobate; posttesticular space 4.8–6.3% of total body length. Left testis 209–254 long by 141–209 wide; right testis 197–265 long by 169–214 wide. External seminal vesicle coiled in anterior portion of intercecal space, extending posteriorly to midbody. Cirrus sac 79–131 long by 21–37 wide, ventrolateral to ceca, anterior to level of seminal vesicle, containing internal seminal vesicle, prostatic cells, and eversible unarmed cirrus.

Ovary pretesticular, intercecal, submedian, slightly lobate, 124–141 long by 101–130 wide. Seminal receptacle lacking. Mehlis' gland postovarian; Laurer's canal dorsolateral to ovary. Vitellaria composed of paired extracecal longitudinal rows extending from level

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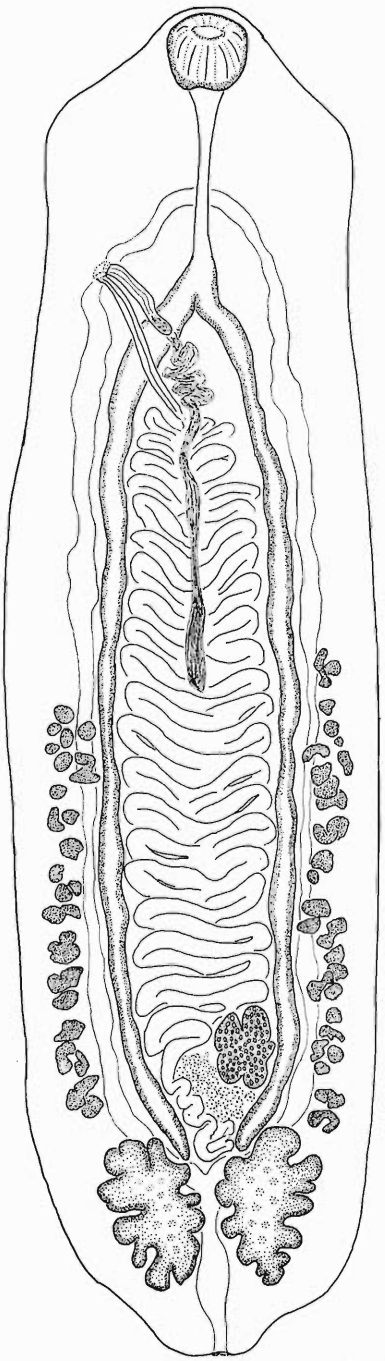

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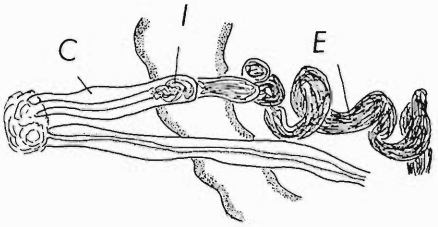

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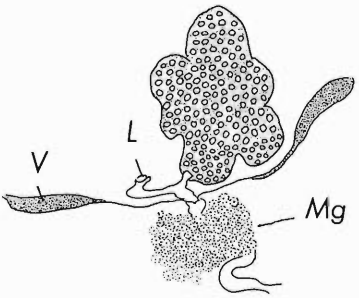

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Figures 1–3. ***Renigonius cuorensis.*** **1. Ventral view of holotype. 2. Ootype region. 3. Terminal genitalia. Abbreviations: C = cirrus sac; E = external seminal vesicle; I = internal seminal vesicle; L = Laurer's canal; Mg = Mehlis' gland; Mt = metraterm; V = vitelline duct.**

of posterior margin of ovary (16.3–19.0% of total body length from posterior end) to middle of ceca (48.9–54.4% of total body length from anterior end). Uterus composed of tightly coiled lateral intercecal loops extending from cecal tips to level of coiled portion of external seminal vesicle; uterine space 48.9–57.5% of total body length. Metraterm thick-walled, extending laterally posterior to cirrus sac, 450–645 long. Genital pore 16.8–20.1% of total body length from anterior end. Eggs 24–26 long by 10–11 wide, each with polar filament 4–6 times longer than eggs.

Excretory vesicle Y-shaped with arms uniting 8.2–11.0% of total body length from anterior end; pore terminal.

HOST: *Cuora amboinensis* (Daudin), Malaysian box turtle.

SITE OF INFECTION: Small intestine.

LOCALITY: Telok Anson, Malaysia.

HOLOTYPE: USNM Helm. Coll. No. 73058.

PARATYPES: USNM Helm. Coll. No. 73059; HWML No. 20867, and in collections of authors.

ETYMOLOGY: The specific name of the new species is derived from the specific name of the host.

Renigonius cuorensis represents the second known species of the genus. It resembles the other species, *R. orientalis* Mehra, 1939, from *Kachuga dhongoka* in India by having a lateral genital pore and relatively small cirrus sac and by possessing pretesticular ceca, characters which Mehra (1939) considered of generic significance. The new species differs, however, by having a more elongate body, more deeply lobate and less elongate testes, a lobate rather than spherical ovary, and unbranched ceca. The host for *R. cuorensis*, while different than that of *R. orientalis*, nonetheless occurs in India as well as Malaysia. Mehra (1939) reported *R. orientalis* as lacking polar egg filaments, a statement which Yamaguti (1971) questioned. Eggs of *R. cuorensis*, at least, possess such filaments.

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The Cephalic Glands and Associated Structures in *Gyrodactylus eucaliae* Ikezaki and Hoffman, 1957 (Monogenea: Gyrodactylidae)

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ABSTRACT: The cephalic glands of *Gyrodactylus eucaliae* comprise three distinct types based on the morphology and stain affinities of their secretions. Three bilaterally paired groups of dorsal glands produce elongate acidophilic secretion units; a single paired group of anteroventral glands lying anterior to the pharynx produce a basophilic secretion resembling that of mucoid glands of turbellarians; the posteroventral glands lie immediately posterior to the pharynx and secrete a granular, acidophilic unit. Cephalic glands are thought to have a primary function of adhesion. The differential occurrence of microtubules in the walls of the ducts of the cephalic glands and their presence in the formation of the rod-shaped secretion granule of the dorsal gland supports present concepts concerning their function as the support organelle of the cell. The structure of the cephalic lobe and head organ is described.

Monogenetic trematodes of the suborder Monopisthocotylea characteristically possess several bilateral groups of unicellular glands in their cephalic and anterior trunk regions. Termed cephalic glands, they empty via individual ducts either directly at the cephalic margin or into cavities of cephalic lobes. In some species the ducts enter head organs prior to discharging at the surface. Functions of extra corporeal digestion and adhesion for attachment and locomotion have been suggested for their secretions (Fuhrmann, 1928; Bychowsky, 1957).

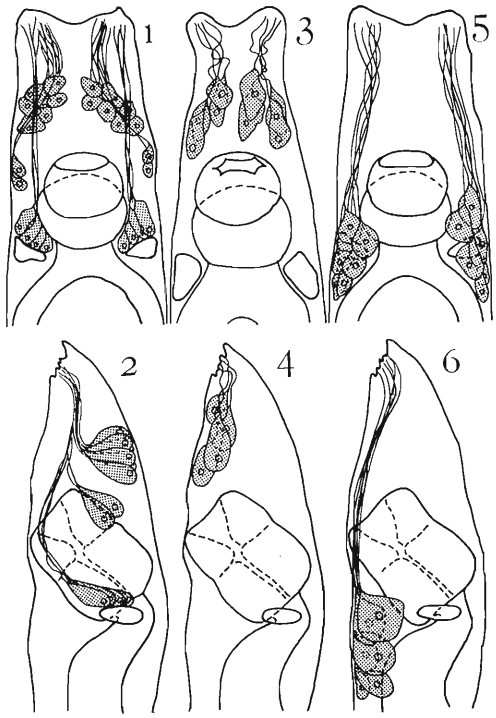
Wagener (1860) first reported in detail the distribution of cephalic glands in gyrodactylids. He, and later Katheriner (1894), found bilaterally paired groups of prepharyngeal, pharyngeal, and postpharyngeal glands in the dorsal half of the cephalic and anterior trunk regions in species of *Gyrodactylus*. Wagener (loc. cit.) also reported twelve to fifteen cells similar to "Pflasterepithel" located "dicht hinter dem unteren grösseren Zellenhaufen (postpharyngeal group of glands) unter der Rückseite des Thieres," which he believed represented stem cells from which the postpharyngeal group of cephalic glands developed. However, Kritsky (1971) demonstrated that they were associated with the digestive system and designated them esophageal glands. In the present study three types of cephalic glands, each differing in the morphology of the secretory product, were found in *G. eucaliae* Ikezaki & Hoffman 1957. A discussion on the position and fine structure of each gland, the head organ, and the cephalic lobe is presented.

Materials and methods for the preparation of helminths for electron microscopy were presented previously (Kritsky and Kruidenier, 1976). Gross structural relations of the cephalic glands and their associated ducts were determined from specimens stained by the PAS method or Delafield's haematoxylin with eosin counterstain.

Observations

Cephalic lobe

The two terminal cephalic lobes are each provided with a cuplike cavity which opens broadly on the respective anteroventral margin and contains the emergent portion of a head organ. The wall of the lobe supports a well-developed musculature and sense organs which Lyons (1969a, b) designated as spike sensilla and tangoreceptors. The somatic tegument covers the superficial surface of the lobe, recurves over the rim, and extends approximately half the distance to the floor of the cavity where a septate desmosome binds it to a distinct visceral (lobal) tegument (Fig. 7). The lobal tegument (0.04–0.36 μ thick) lines the lower walls and floor of the cavity and coats the partly emergent head organ. An irregular distribution of dense, rod-shaped



Figures 1–6. Cephalic glands and associated ducts of *Gyrodactylus eucaliae* in relation to the pharynx and excretory vesicle. 1, 2. Ventral and lateral views of the dorsal glands, respectively. 3, 4. Same of anteroventral glands. 5, 6. Same of posteroventral glands.

granules (0.1 μ by 0.3 μ) in the finely reticulate ground substance distinguishes the lobal from the somatic tegument. Irregular mitochondria are also present. The 73 Å tripartite membranes of the basal and superficial surfaces of the lobal tegument are continuous in the septate desmosome. The basal membrane rests on a fibrous basal lamina which is confluent with adjacent intercellular matrices (Fig. 7).

The visceral tegument of each lobe is connected by short processes to a syncytium located in the respective ventrolateral cephalic region (Fig. 8). These "subtegumental" syncytia are characterized by a light cytoplasm containing numerous secretions, mitochondria, and Golgi complexes. The cytoplasm is enclosed by a 70 Å surface membrane frequently infolded deeply into the syncytial mass. Each spherical to subovate nucleus possesses a usually eccentric nucleolus and relatively few areas of peripheral chromatin (Fig. 8).

Head organ

The head organ comprises an assemblage of terminations of ducts through which cephalic glands discharge on their respective sides. Individual ducts from these glands converge into several groups at the base of their respective head organ but do not become confluent. The groups vary in the number of constituent ducts and in the combinations of glands involved (Fig. 9). As they course through the head organ, each group is bound together by a relatively heavy basal lamina. Approximately the anterior half of each unit of ducts protrudes into the cavity of the cephalic lobe to form a small papilla, at the apex of which the ducts discharge individually (Fig. 7). The lobal tegument and basal lamina extend almost to the tip of the papilla, where the former connects to the membranous wall of each duct by septate desmosomes. Here the tegument flares anterolaterally into a narrow ledge from whose margins a halo of regularly spaced microvilli (0.1 μ in diameter) arises (Figs. 7, 9). Longitudinal muscle fibers, apparently functional in papillary retraction, and one or

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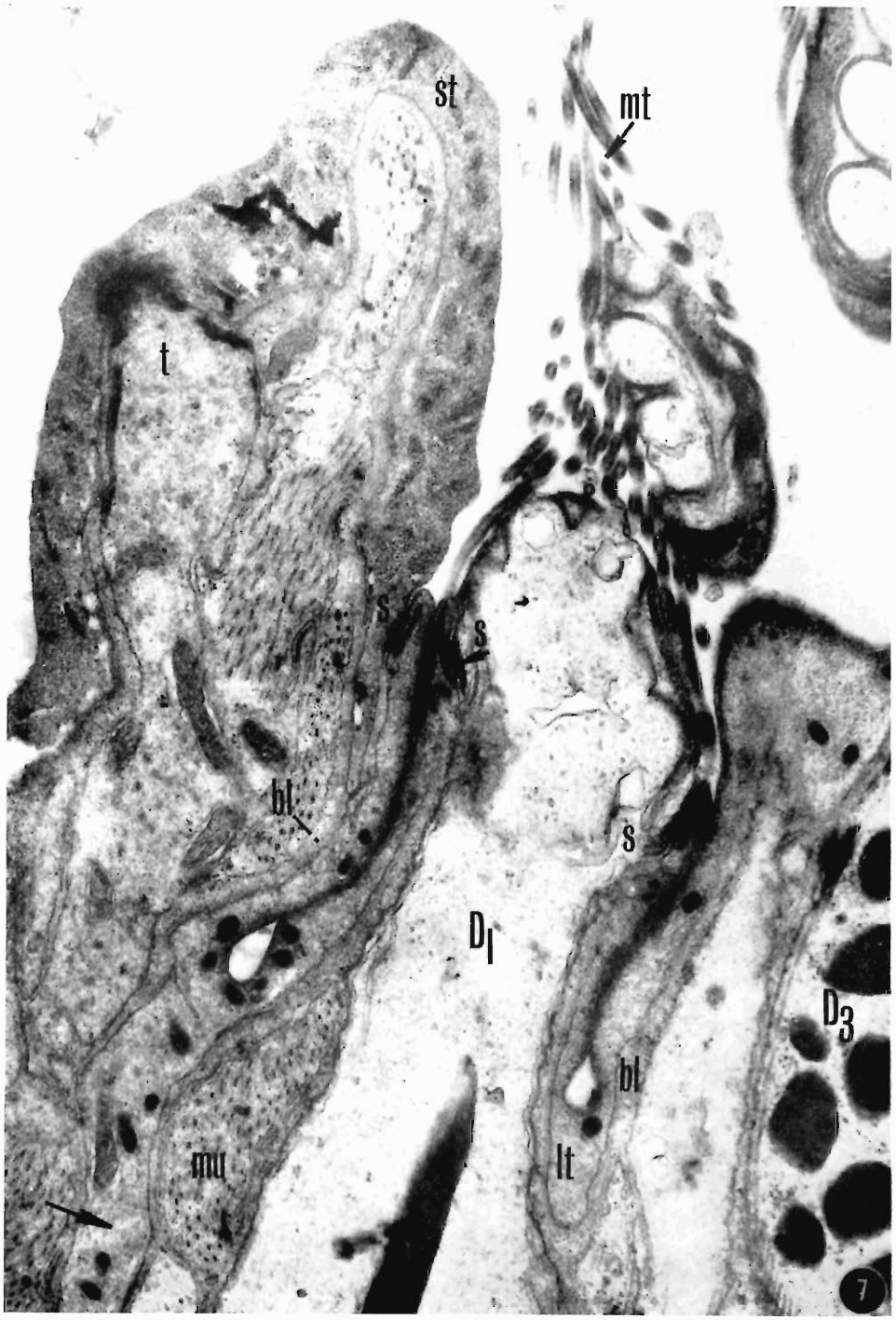
Figure 7–17. Electron micrographs of the cephalic glands and associated structures of *Gyrodactylus eucaliae*. Abbreviations: bl, basal lamina; c, unit membrane; D_1, duct (dorsal gland); D_2, duct (anteroventral gland); D_3, duct (posteroventral gland); Ev, excretory vesicle; f, fibers; G, *Golgi complex*; lt, lobal tegument; m, mitochondrion; Mi, microtubule; mt, microvilli; mu, myofiber; N, nucleus; RER, rough endoplasmic reticulum; s, septate desmosome, st, somatic tegument; t, tangoreceptor; V_1, secretory granule (immature); V_1, secretory granule (dorsal gland); V_2, secretory granule (anteroventral gland).

Figure 7. Electron micrograph of longitudinal section of cephalic lobe and head organ papillae. Large arrow indicates a cytoplasmic process connecting the lobal tegument (lt) to subtegumental syncytia. × 25,000.



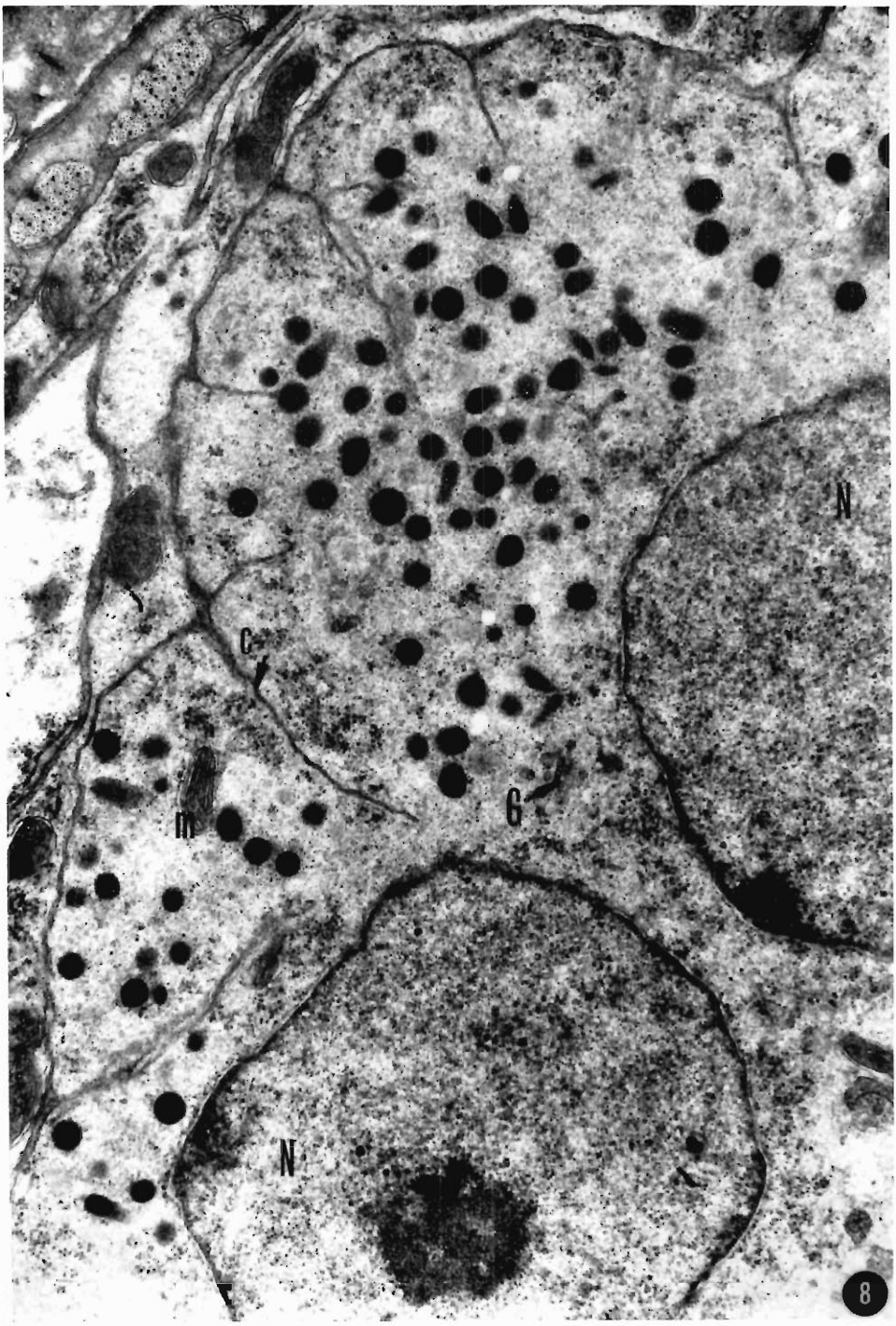


Figure 8. Subtegumental syncytium of lobal tegument. × 24,000.

more tangoreceptors usually accompany each group of ducts (Fig. 9).

Cephalic glands

DORSAL GLANDS: The three paired groups of dorsal glands of Wagener are discussed as a unit because of their similar fine structure and high acidophilia. Their position in relation to the pharynx and excretory vesicle, and the paths of their ducts in the cephalic region are presented in Figures 1, 2. The anterior pair normally consists of 6–8 unicellular glands in each group; each middle group has 2–3 cells; and the posterior groups vary from 3–5 cells each. Cells of the latter become progressively smaller toward the midline of the parasite.

The long axes of the pear-shaped cell bodies are oriented dorsoventrally. Each cell is approximately 15 μ long by 9 μ wide in lateral view and narrows ventrally to form a fine duct. A large, round–polygonal nucleus with a finely granular nucleoplasm, an eccentric nucleolus, and several zones of peripheral chromatin occurs near the dorsal pole of the cell (Fig. 11). The external surface of the outer component of the porous, double nucleolemma is lined with ribosomes and continuous at many locations with the rough endoplasmic reticulum.

The plasmalemma, continuous with that of the duct, is free of ribosomes, about 70 Å thick, and irregularly involuted into the cell body. Irregular mitochondria with tubular or lamellar cristae abut the inner surface of this membrane but also frequent the remaining cell volume (Fig. 12). Rough endoplasmic reticulum with cisternae dilated by a moderately dense material is well-developed throughout the cell body and peripherally in the cell neck. Smooth endoplasmic reticulum is absent except as small tubules or vesicles in the region of the large, centrally located Golgi complex (Fig. 12).

Greatly elongate, rod-shaped secretions are produced in the dorsal glands. Rarely bent, they are moderately rigid and contain a dense fine-grained substance enclosed by a membrane about 106 Å thick (Fig. 12). Developing secretion granules are usually closely encircled by a single ring of microtubules. Free microtubules are common in the Golgi region (Fig. 13).

The structure of the proximal duct resembles that of the neck of the gland. The rough endoplasmic reticulum is restricted to a thin layer near the periphery but decreases in amount and finally disappears some distance from the cell. Mitochondria are sparse but still occur far anterior into the head organ. Secretion granules are in the central portion of the duct. Immature granules frequent the proximal duct but were not observed distally (Fig. 13). The wall of the duct is thin and possesses a few supporting microtubules at the level of the proximal portion of the head organ. It consists of the rarely infolded plasmalemma until the level of the pore complex is attained, where a doubled and thickened membrane rests on a heavy basal lamina. Apically, multiple involutions of the double membrane subdivide the terminal pore of the duct; thus, it superficially resembles the cap of a salt shaker (Figs. 7, 9).

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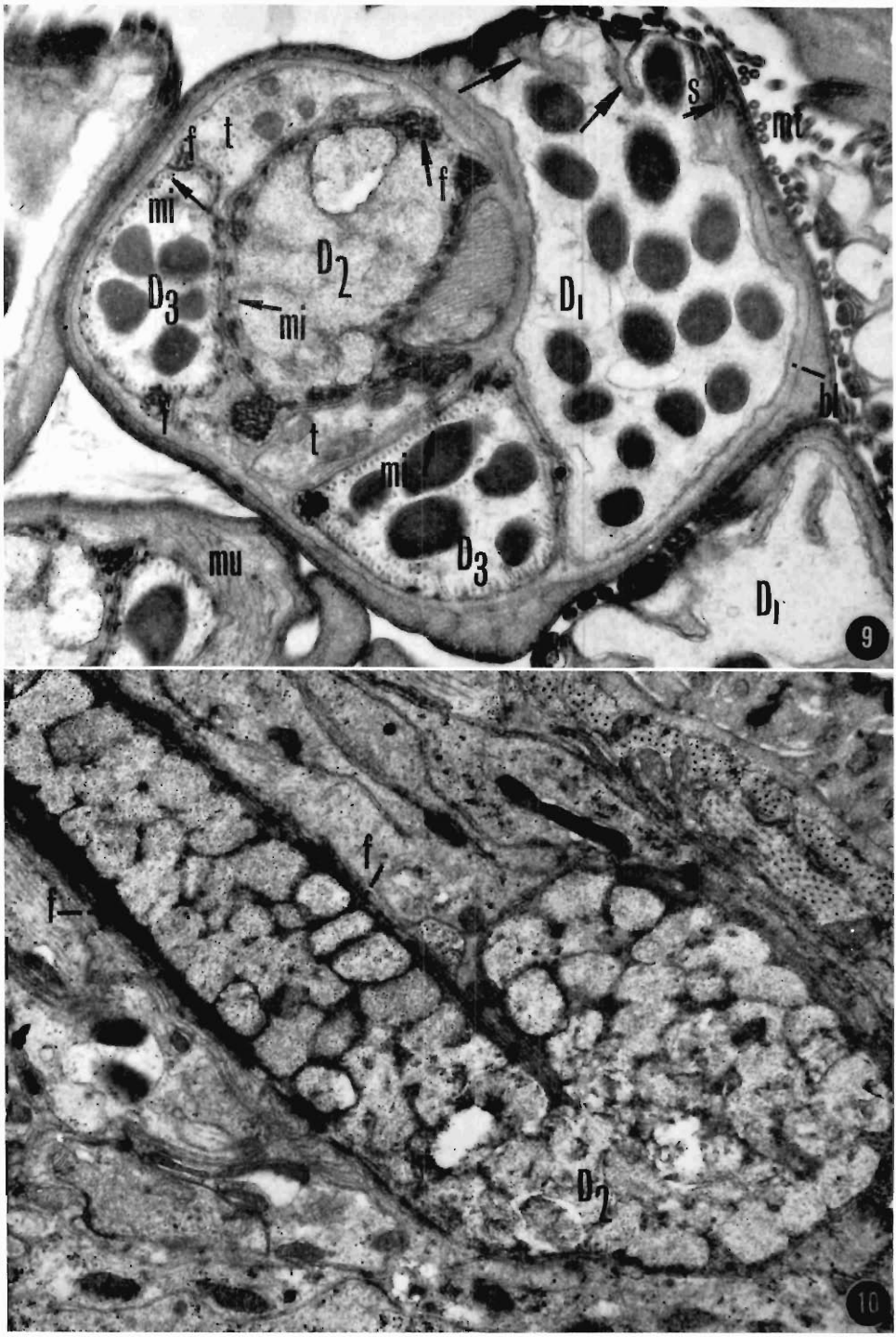
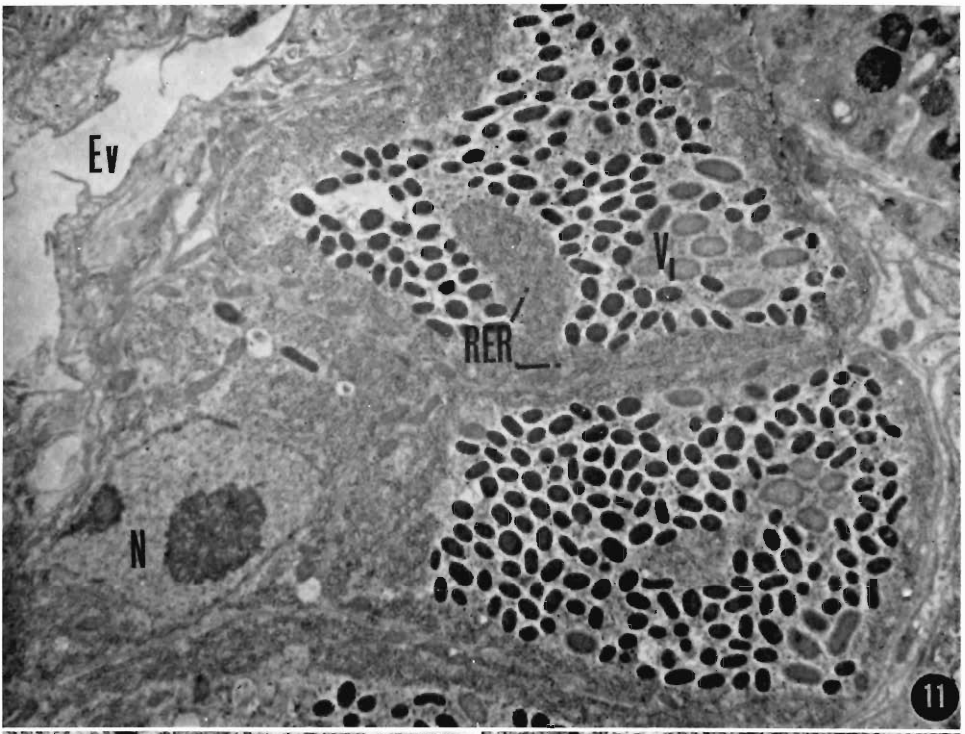
Figure 9. Transverse section of head-organ papillae showing ducts from cephalic glands. Large arrows indicate terminal involutions of dorsal-gland duct which imparts the salt-shaker effect. × 20,000. Figure 10. Longitudinal section of a duct from an anteroventral gland (D_2) as it enters the head organ. Note the fibrous condition of the duct wall in the head organ. × 15,400.

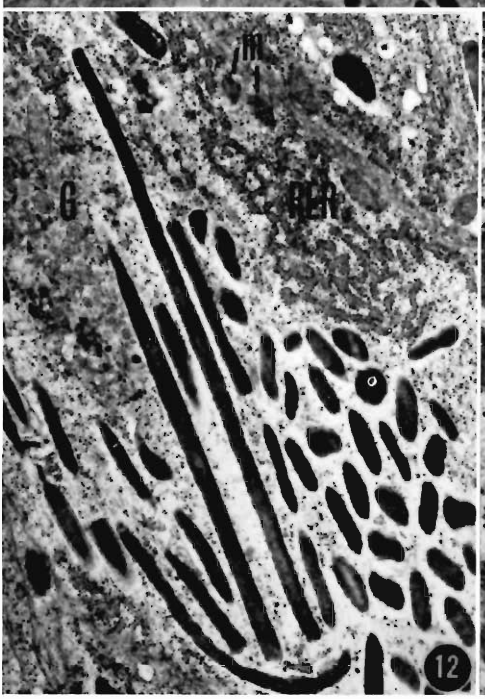
Figure 11. Micrograph showing profiles of two dorsal gland cells located near the excretory vesicle (Ev). × 7,400. Figure 12. The secretory product of dorsal gland. × 9,800. Figure 13. Transverse sections of developing secretory products of dorsal gland showing microtubules free in cytoplasm and encircling the immature granules (arrows). × 26,000.

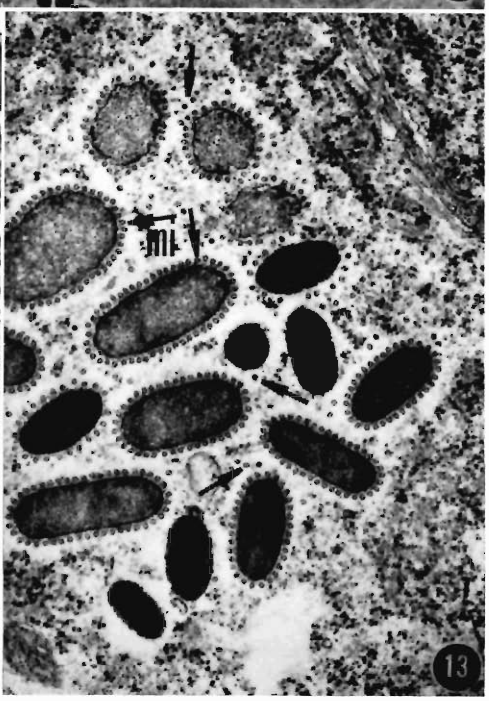
Figure 14. Electron micrograph of cell periphery of anteroventral gland showing well-developed rough endoplasmic reticulum and coalesced secretion units. × 33,500. Figure 15. Profile of Golgi complexes, secretion units, and nucleus of anteroventral gland. Arrow indicates presumed fusion of Golgi vacuole with secretion units. × 23,000.

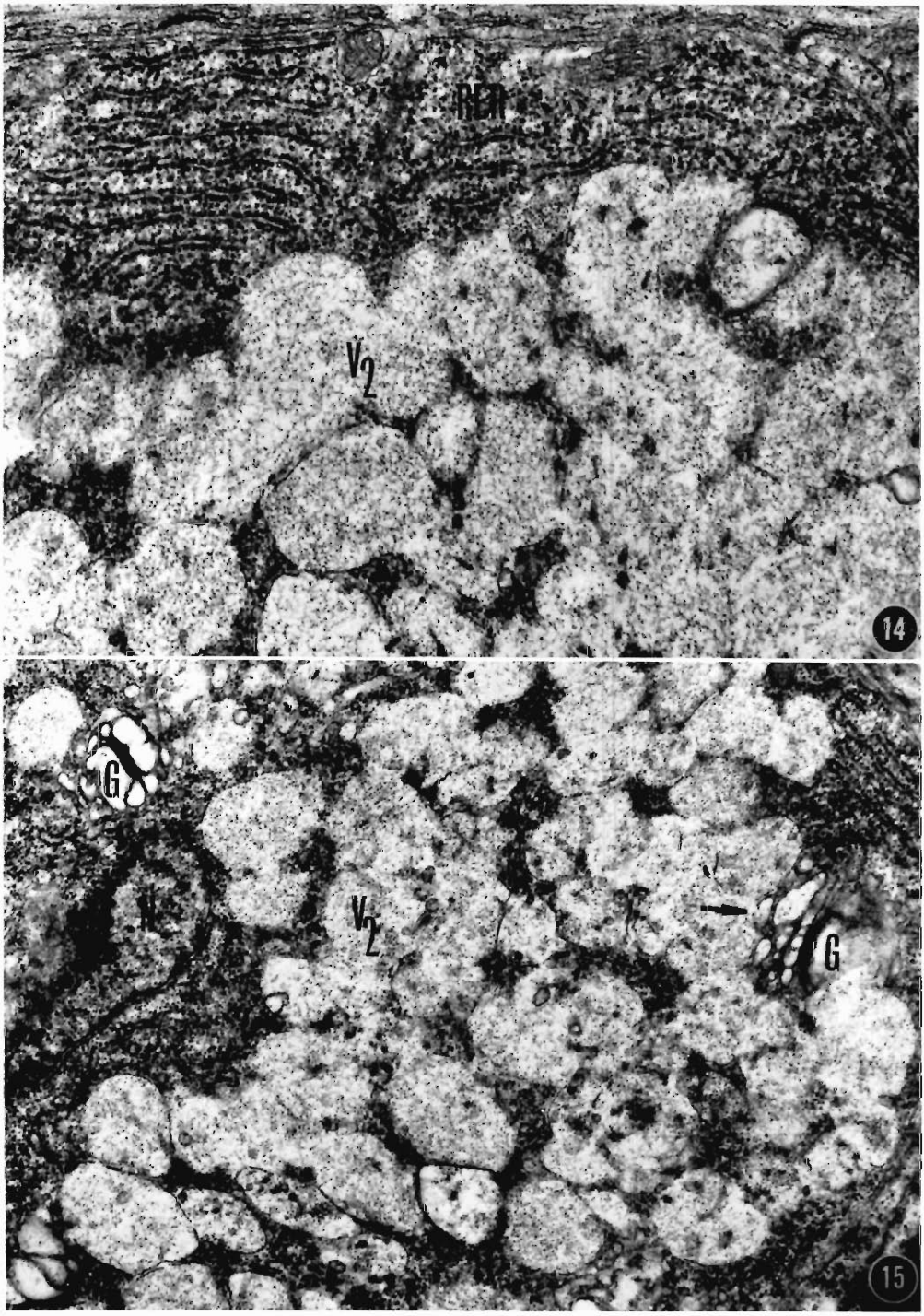
Figure 16. Transverse section of proximal duct of posteroventral gland. Arrow indicates longitudinally oriented microtubules. × 16,300. Figure 17. Profile of a posteroventral gland. × 11,500.

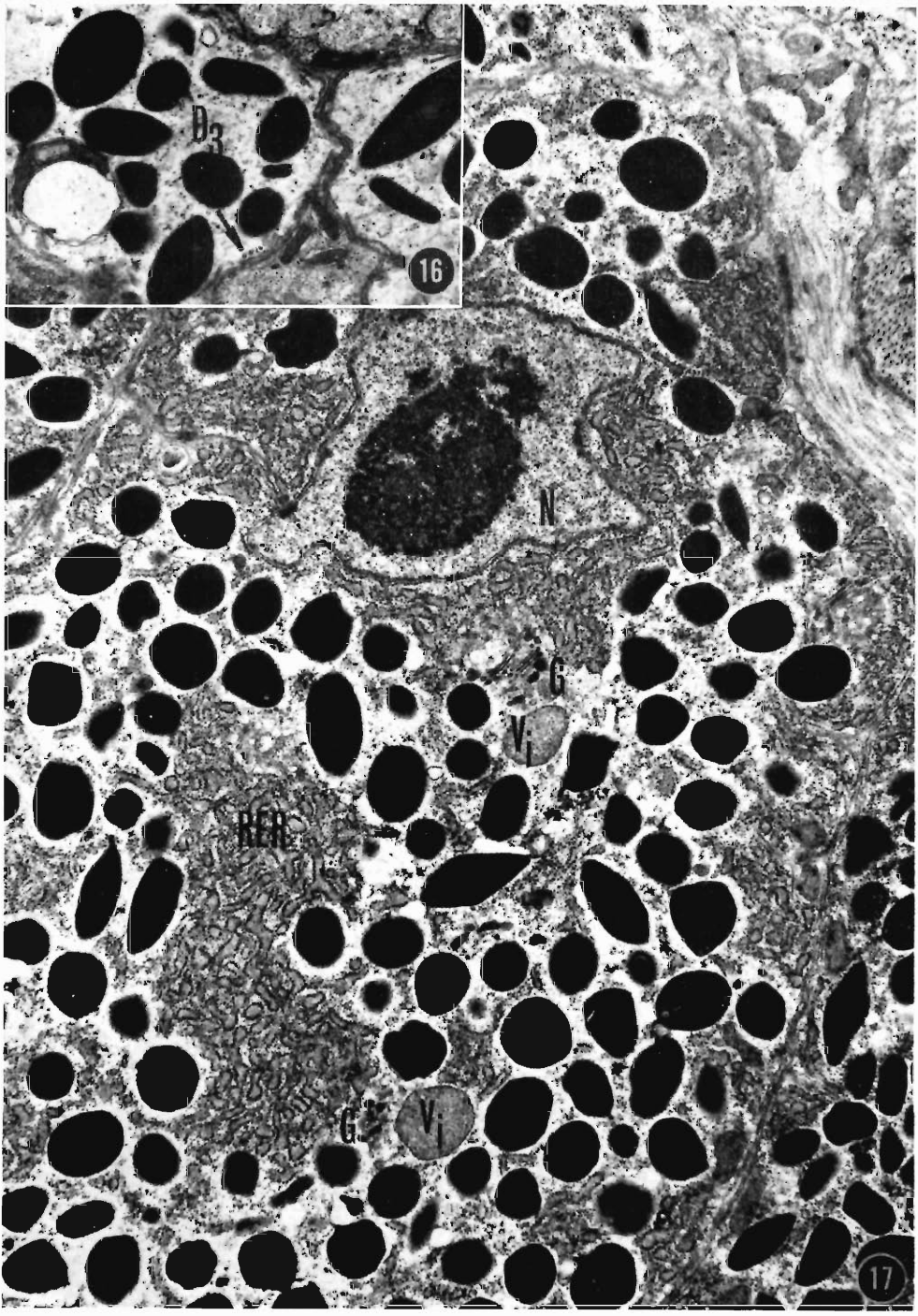












ANTEROVENTRAL GLANDS: The two bilateral groups of anteroventral glands are situated in the ventral portion of the cephalic region immediately anterior to the pharynx (Figs. 3, 4). Each group is comprised of four unicellular glands, two on either side of the closely associated ducts of the posteroventral and dorsal glands, and with one member of each pair dorsal to the other. A single duct filled with a highly basophilic secretion arises from the anterior margin of each cell and winds anteriorly toward the base of the respective head organ.

Cell bodies are spherical to pear shaped and measure 8 μ by 13 μ (lateral view). An irregular nucleus is situated near the cell margin and contains a large, eccentric nucleolus and several smaller zones of peripheral chromatin. The nucleolemma is porous, double, with the outer surface of its external component associated with ribosomes and the rough endoplasmic reticulum (Fig. 15). Rough endoplasmic reticulum frequently occurs in irregular concentric layers near the cell periphery, with small amounts scattered among the secretion units (Fig. 14). Cisternae are uniform and contain a moderate to light material. Mitochondria with tubular cristae are usually near the infolded plasmalemma and in the perinuclear region. The central portion of the cell is closely packed with secretion units, between which free ribosomes and small Golgi complexes occur. Golgi vesicles, sacs and vacuoles contain materials of a comparable light density, with vacuoles appearing to fuse with nearby secretory units (Fig. 15).

The secretion units are polygonal when closely packed. Each is 0.5–0.6 μ in diameter, bound by a discontinuous membrane, with neighboring units appearing to coalesce. Secretions are filled with a fine particulate to reticulate matrix with irregular dense areas (Figs. 10, 14, 15).

The ducts are usually closely packed with coalesced secretion (Fig. 10). Proximally, their thin walls consist of a simple extension of the plasmalemma whose frequent infoldings impart a multi-layered appearance. In the head organ, the peripheral cytoplasm of the duct is supported by a ring of longitudinal, irregularly spaced microtubules of 240 Å diameter each and dense longitudinal fibrous masses external to the ring of microtubules (Figs. 9, 10). Secretions are released into the cavity of the cephalic lobe through a rupture of the plasmalemma at the papilla tip.

POSTEROVENTRAL GLANDS: The posteroventral glands constitute the largest single mass of cephalic glands. The six to eight cells of each group are progressively larger anteriorly and normally located ventrolateral to the esophagus and posterior pharyngeal bulb (Figs. 5, 6). They may be shifted from the immediate ventrum in relaxed specimens to the more dorsal level of the excretory vesicle in specimens which are contracted or contain a near-term embryo. Nevertheless, they are easily distinguished from the dorsal glands by a lower acidophilia, which is probably due to a more diffuse distribution of secretion in the gland cell (the secretion packed ducts stain intensely with Eosin Y). The ducts extend lateral to the pharynx, slightly ventrolateral to those of the postpharyngeal dorsal glands. Each enters the respective head organ and empties independently in the cephalic lobe cavity.

The gland cells are subspherical and vary from 6 to 11 μ in diameter. Each possesses a relatively large, irregular nucleus provided with an eccentric nucleolus and scattered zones of chromatin. Ribosomes occur on the external component of the double nucleolemma which is frequently continuous with the rough endoplasmic reticulum. Nuclear pores are numerous. The cytoplasm is richly supplied with rough endoplasmic reticulum near the cell periphery and in the perinuclear region. Cisternae are locally dilated by a moderately dense, granular substance. Sparse mitochondria with tubular cristae occur near the rough endoplasmic reticulum and margins of the cell. The plasmalemma is free of ribosomes and frequently infolded (Fig. 17).

Secretion granules occur throughout the cytoplasm but tend to accumulate near the cell center. Each usually appears as a flattened oval (1.5 μ by 9 μ) enclosed by a tripartite membrane 145 Å thick (Figs. 16, 17). Developing secretion units occur near small Golgi complexes and consist of an irregularly shaped vacuole containing a reticular, electron-light matrix (Fig. 17).

Typical Golgi complexes usually occur in the perinuclear region. Golgi vesicles and sacs

appear to form in relation to rough endoplasmic reticulum and contain a moderately dense material, intermediate between that of the developing and mature granules. Golgi vacuoles with a density similar to that of the developing granule frequent the margins of the Golgi complex (Fig. 17).

The wall of the proximal duct consists of a single membrane with few to many infoldings (Fig. 16). Prior to the entry of the duct into the head organ, longitudinal microtubules with relatively dense centers accumulate in the peripheral cytoplasm. They become more numerous anteriorly until an irregularly spaced row completely lines the inner wall of the duct as it traverses the head organ (Fig. 9). In the head organ, the wall is also provided with groups of dense longitudinal fibers. Ducts contain an electron-light ground substance surrounding secretion units, free ribosomes, and occasional mitochondria. Developing secretion units were not observed in the ducts.

Discussion

The cephalic and anterior trunk regions of *Gyrodactylus eucaliae* are abundantly supplied with secretory units which empty via individual ducts into the cavities of the cephalic lobes. Based on ultrastructural evidence and stain affinities, three types of glands disposed into distinct and homogeneous groups can be identified. Although speculative at present, these differences suggest that the cephalic glands of *G. eucaliae* have more than one function. The basophilic secretion of the anteroventral glands morphologically resembles that found in mucous glands of acoel turbellarians by Pedersen (1965), where the secretion is thought to assist in adhesion during rest (Jennings, 1957). Jennings also indicates that acidophilic secretions from surface glands in triclads are especially sticky. This suggests that the dorsal and posteroventral glands in *G. eucaliae* which produce acidophilic secretions may also have a primary function of adhesion especially in instances of need of a relatively strong bind to the substrate (i.e., during locomotion and feeding). The combined structure of the cephalic lobe and head organ supports this proposed primary function. Presumably, the well-developed musculature in the wall of the cephalic lobe facilitates this structure in acting as a "suction cup," while the head organ delivers binding secretory products to the attachment site. The numerous sense organs functioning as both chemo- and tangoreceptors (Lyons, 1969a, b) would allow for orientation of the parasite during feeding and locomotion.

The somatic tegument of monogeneans is considered to be continuous and without internal membrane boundaries except in regions where it is penetrated by sensory endings and gland ducts (Lyons, 1973). Lyons (1970) reported that septate desomosomes join the somatic tegument with a distinct tegument surrounding the ducts of the cephalic glands in *Entobdella soleae*. A similar condition occurs in the cavity of the cephalic lobe in *G. eucaliae*. The lobal tegument of the latter species differs from its general body covering most conspicuously by containing numerous distinct secretion granules in the surface layer and uniting via ducts with syncytial subtegumental areas which apparently produce these secretion units. The functional significance of the lobal tegument is unknown, but it could be related to the proposed adhesive function of the cephalic lobes and glands. It seems likely that the contents of the secretory inclusions may also have adhesive properties which function upon release from the surface.

Present micrographs clearly show the secretory nature of the cephalic glands. All possess a well-developed rough endoplasmic reticulum, frequently in close proximity to a substantial Golgi system and a large nucleus containing a prominent nucleolus. The finding of developing granules in the regions of the Golgi complexes of the posteroventral and dorsal glands, and the frequent profiles of the Golgi region suggesting coalescence of Golgi vacuoles with secretion units in the anteroventral glands (Fig. 15, arrow), support the idea that this organelle is involved in granule formation. Similar relationships of the Golgi complex serving as the site of assembly of secretory granules have been reported in other organisms (Kurosumi, 1961; Hand and Oliver, 1975).

In a variety of cells, microtubules are believed to have supportive functions as well as being involved in the process of intracellular organelle movement (Halton and Dermott, 1967; Oschman, 1967; Kemp and Powell,

1970; Dorsey 1974, 1975; Borgers et al., 1975; Warren and Burnside, 1975; and others). Present findings support these concepts. Microtubules are rare or absent in the ducts of the dorsal glands which produce a greatly elongated and moderately ridged secretion granule. Supposedly these granules, which generally fill the interior of the duct, provide the necessary support for the duct wall. Conversely, ducts of the anteroventral and posteroventral glands are usually richly supplied with longitudinally oriented microtubules in the wall, where apparently the granular secretory products lend little support.

That microtubules have a supportive function is further substantiated by the occurrence of microtubules closely encircling the developing rod-shaped granule of the dorsal glands. Apparently "immature" granules are not sufficiently ridged to maintain their shape, and the microtubules serve as a developmental mold.

All three kinds of cephalic glands are of a merocrine type, in that the individual gland cells survive the cycle of secretory activity. The mode of extrusion of the secretory product of the dorsal glands (and probably that of the posteroventral and anteroventral glands as well) is apparently through the loss of the secretory product only (eccrine secretion), as indicated by the terminal structure of the duct. Here, a salt-shaker appearance is obtained with individual pores of the approximate diameter of a secretion granule. Each pore is occluded by a unit membrane. Although not visualized with the electron microscope, the content of the rod-shaped granule is apparently released by union of its limiting membrane with the pore membrane and the eventual development of a small terminal portal which allows only the release of the granule contents. This is based in part on the fact that formed granules were never observed in the cavity of the cephalic lobe.

The structure of the head organ in monogenetic trematodes has been variously described based on observations with the light microscope. Brown (1953) considered each head organ as a thickened elliptical tube connected at its proximal end to a duct from the posterior cephalic glands. Others have labeled them as glandular bodies (Jain, 1958; Bychowsky, 1957) or as concentrated groups of cephalic-gland ducts (Mizelle, 1938; and others). Present electron micrographs demonstrate these structures in *G. eucaliae* to comprise an assemblage of terminations of ducts which are supported by a thickened basal lamina and longitudinally oriented microtubules and fibers. The distal end of the head organ protrudes into the cephalic lobe cavity as small retractable papillae which deliver the secretory products of the cephalic glands into the cavity of the cephalic lobe.

Acknowledgments

I would like to thank Dr. F. J. Kruidenier for many useful comments, reviews and consultations concerning the present study; Dr. R. P. Hathaway for considerable field assistance and consultations; and the director of the Central Electron Microscope Laboratory at the University of Illinois for allowing me to use facilities during the initial part of this study. Drs. Larry Farrell and Robert Anderson graciously provided equipment from their laboratories at Idaho State University.

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Protancyrocephaloides liopsettae gen. et sp. n. (Monopisthocotylea: Dactylogyridae) from Smooth Flounder, *Liopsetta putnami* (Gill)

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ABSTRACT: *Protancyrocephaloides liopsettae* gen. et sp. n. (Ancyrocephalinae) is described from the gills of smooth flounder, *Liopsetta putnami* (Gill), from New Hampshire. The species is most similar to *Protancyrocephalus* Bychowsky, 1957, from which it is separated by the presence of a ventral bar. The affinities of the two genera are examined.

Smooth flounder, *Liopsetta putnami* (Gill), were seined for a survey of their parasite fauna from the Great Bay estuary, New Hampshire, and their gills were examined microscopically for trematodes. Some parasites were examined alive; others were fixed in AFA, mounted in Turtox CMCP aqueous medium, or stained according to Lynch's precipitated borax-carmine method (Galigher and Kozloff, 1971) and mounted in Canada balsam. Some specimens were embedded in Paraplast and sec-

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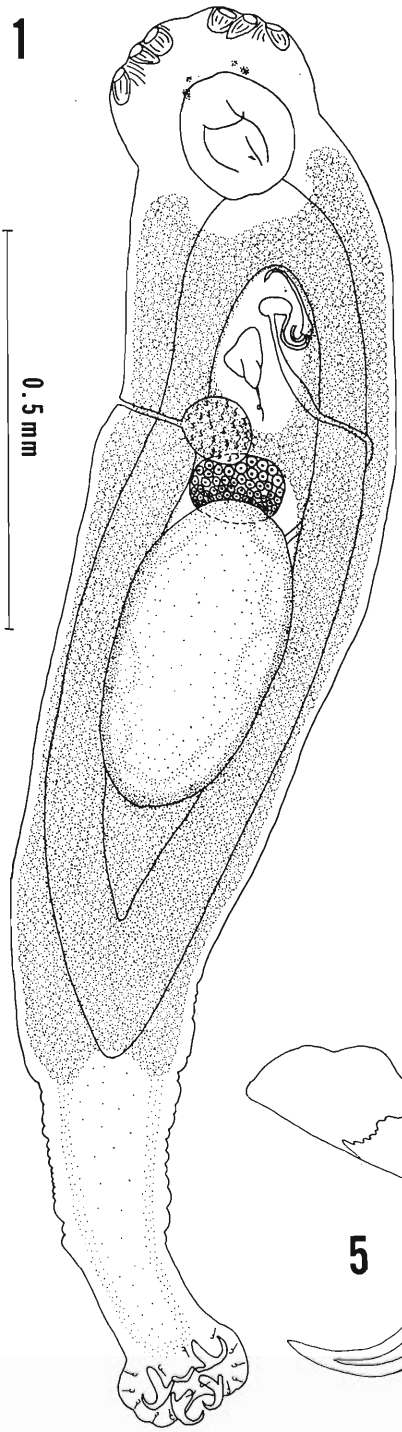

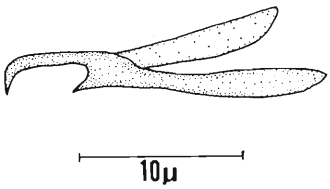

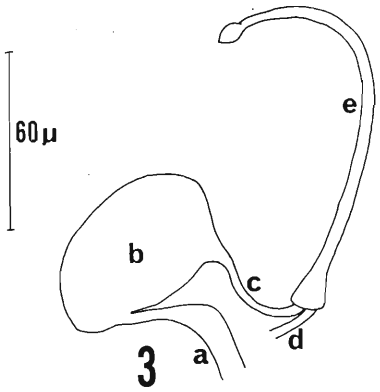

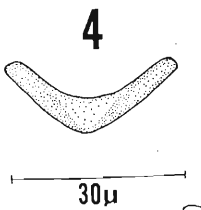

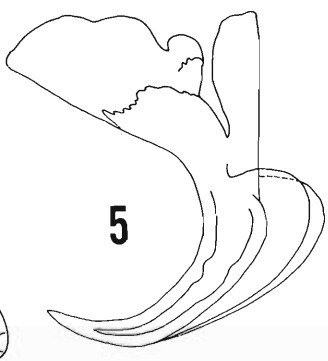

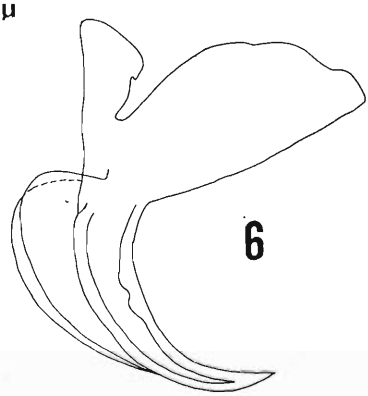

tioned at seven microns. Measurements (as described by Mizelle, 1962) were made with an ocular micrometer and are in microns unless otherwise stated. Averages are followed by ranges in parentheses. Illustrations were prepared with the aid of a camera lucida.

Protancyrocephaloides gen. n.

DIAGNOSIS: Dactylogyridae, Ancyrocephalinae. Bilobed opisthaptor distinct; two pairs of similar anchors, ventral pair connected by delicate bar; fourteen marginal hooks, distribution ancyrocephaline (Mizelle, 1936). Four eyes; six head organs. Pharynx prominent. Intestinal crura simple, confluent posteriorly. Testis ovoid, postovarian. Vas deferens looping around the left intestinal cirrus; seminal vesicle present. Simple tubular cirrus, accessory piece absent; genital pore immediately postbifurcal. Ovary ovoid, eggs with polar filament. Vitellaria well developed, extending from level of pharynx to beyond posterior confluence of intestine. Vagina dextroventral, leading to seminal receptacle. Parasitic on gills of marine (estuarine) teleosts. Type and only species.

Protancyrocephaloides liopsettae sp. n. Figures 1–6

HOST AND LOCALITY: *Liopsetta putnami* (Gill), Great Bay estuary, New Hampshire.

LOCATION ON HOST: Gills.

SPECIMENS STUDIED: 78 (20 measured).

TYPE SPECIMENS: USNM Helm. Coll. Holotype No. 74608, paratypes No. 74609. Additional paratypes in the author's collection.

DESCRIPTION: With characteristics of genus. Length 1.62 mm (1.25–2.10), width 340 (250–340). Anterior eyes smaller and closer together. Cephalic glands present; three pairs of indistinctly separated head organs. Pharynx length 144 (118–178), width 129 (108–173). Haptor 86 (73–100) long by 116 (90–140) wide. Dorsal anchor length 45 (35–50), width 35 (30–40); ventral anchor length 50 (45–56), width 40 (36–48). Anchor filaments extending from anchor shafts. Ventral bar poorly developed, length 29 (25–30). Hook length 19 (18–20); hook filaments present. Testis much larger than ovary; margin of the testis often appearing somewhat thickened. Vas deferens expanded as it extends from under the left intestinal limb, distinct seminal vesicle immediately postbifurcal. Ejaculatory and prostatic ducts entering simple tubular cirrus. Cirrus length 96 (75–113). No prostatic reservoir. Vitellaria confluent anteriad to genital pore, posteriad to testis, and ventrally just anteriad to the ovary. No vitelline reservoir.

Discussion

Protancyrocephaloides occupies a morphological position intermediate between *Haliotrema* Johnson and Tiegs 1922, and *Protancyrocephalus* Bychowsky, 1957, which possess two and zero haptoral bars, respectively, and are otherwise very similar. Of other ancyrocephalid genera having a single bar, only *Parancyrocephaloides* Yamaguti, 1938, is comparable to *Protancyrocephaloides*. The two are easily separated, however, since the vas deferens of *Parancyrocephaloides* does not loop around an intestinal limb, and its testis is conspicuously folded.

The relationship of *Protancyrocephaloides* and *Protancyrocephalus* is worthy of further mention. The latter genus is known only from Figure 46 (whole animal) and scattered text references and figures of *Protancyrocephalus strelkowi* in Bychowsky's (1957) monograph. The specimens on which this description was based were collected from *Limanda aspera* and "several" other members of the Pleuronectidae from South Sakhalin (region of Yablochnoii) and the Island of Shikotan, both on the east coast of the Soviet Union. On the basis of Bychowsky's figure, Yamaguti (1963) gave a diagnosis for *Protancyrocephalus*. Although Yamaguti stated that "Bychowsky's detailed original description was not available," there is no indication in the literature that such a description was ever published. According to Lebedev (1976, personal communication), there is no known formal description and there

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Figures 1–6. ***Protancyrocephaloides liopsettae*** **gen. et sp. n. 1. Mature animal, ventral view. 2. Marginal hook. 3. Male copulatory apparatus, ventral view. A. Vas deferens. B. Seminal vesicle. C. Ejaculatory duct. D. Prostatic duct. E. Cirrus. 4. Ventral bar. 5. Dorsal anchor. 6. Ventral anchor.**

are no type specimens at the Zoological Institute in Leningrad, where Bychowsky's collection is kept. The limited material in the Bychowsky monograph, however, does constitute a valid species description according to the International Rules of Nomenclature, although it precludes any detailed comparison with other species.

The only definite differences between *Protancyrocephalus* and *Protancyrocephaloides* are those of specific significance (overall size, relative size of testis, extent of vitellaria), the number of head organs, plus the presence in *Protancyrocephaloides* of the ventral bar. This bar, moreover, is difficult to discern in balsam mounted specimens, and fades completely from view in certain aqueous media. The two genera are the only members of the subfamily found parasitic on fishes from other than the orders Perciformes and Cypriniformes (Bychowsky, 1957), and are in fact both specific for the Pleuronectidae.

Morphological and host occurrence data indicate that *Protancyrocephalus* and *Protancyrocephaloides* are closely allied. On the basis of current knowledge, the genera are best considered to be distinct. Reexamination of *Protancyrocephalus* is desirable, however, both to extend our limited knowledge of the genus and to facilitate taxonomic comparisons.

Acknowledgments

I wish to thank Dr. Wilbur Bullock of the Department of Zoology and Dr. Richard Strout of the Department of Animal Science, University of New Hampshire, for their constructive criticisms of this manuscript. Special thanks are also due Dr. B. Iv. Lebedev of the Institute of Biology and Pedology, USSR Academy of Sciences, Vladivostok, who provided valuable information and advice concerning the status of *Protancyrocephalus strelkowi.*

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Errata

Kingston, N. and J. Crum. 1977. *Trypanosoma cervi* Kingston and Morton, 1975 in white-tailed deer, *Odocoileus virginianus*, in the southeastern United States. Proc. Helm. Soc. Wash. 44: 179–184.

1) p. 180 Table 1 column BL, 1st line should be 4.54.

2) p. 182 column 2, line 1 should be July 1975.

3) p. 184 column 1, line 13 should be (> 1:5).

4) p. 184 delete reference to Krinsky, W. L. and L. L. Pechuman.

Description of *Acanthostomum quaesitum* (Nicoll, 1918) Hughes, Higginbotham, and Clary, 1942 (Digenea: Cryptogonimidae) in *Crocodylus johnsoni* Krefft from Australia

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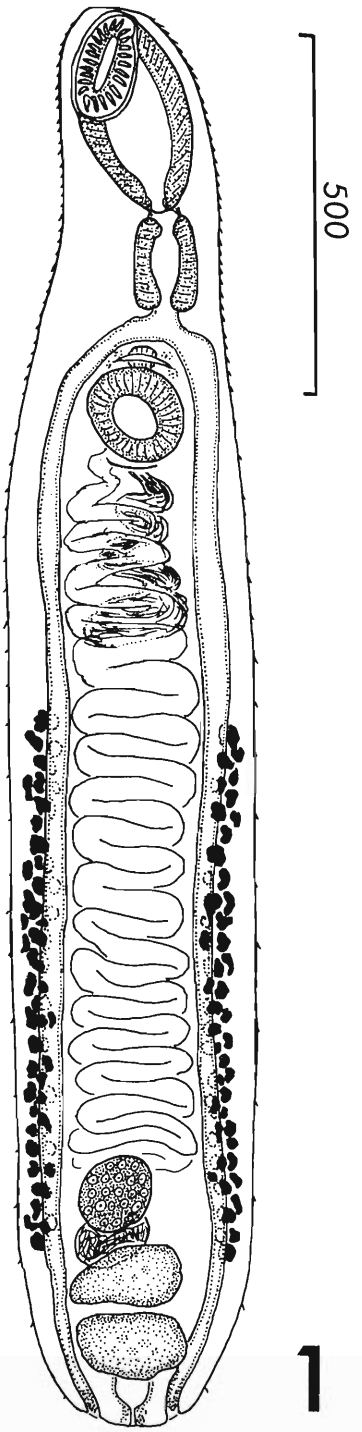
ABSTRACT: *Acanthostomum quaesitum*, formerly a *nomen nudum* and *species inquirenda*, is described from specimens collected in northern Australia from *Crocodylus johnsoni*. The species most closely resembles *A. atae*, *A. elongatum*, and *A. crocodili* but differs by having vitelline follicles not reaching the posterior margin of the seminal vesicle and a prepharynx shorter than the pharynx, and by having uniformly 24 oral spines.

Nicoll (1918) reported collecting acanthostome digeneans from *Crocodylus johnsoni* Krefft in northern Australia, stating that the worms differed from any other known species but were in such poor condition that no description could be made. He did, however, name the species *Acanthochasmus quaesitus* sp. inq. Hughes, Higginbotham, and Clary (1942) transferred the species to *Acanthostomum* because *Acanthochasmus* was a junior synonym of that genus. Until the present report the species has remained a *species inquirendum* and *nomen nudum*. During a study of the acanthostomes, the first author borrowed and examined Nicoll's specimens and found them to consist of macerated, unmounted, unidentifiable fragments. Subsequently, the second author obtained specimens of acanthostomes from *C. johnsoni* in northern Australia near the locality of Nicoll's material. The somewhat contracted specimens are distinct from any other known species, and are similar in shape and size to those collected by Nicoll. We therefore assign our specimens to that species and present the following description to validate the *nomen nudum*. We assign the species to the family Cryptogonimidae following the suggestions of Cable and Hunninen (1942).

Worms were collected from intestinal scrapings of a preserved male *Crocodylus johnsoni* 101.5 cm in total length and 53.9 cm in snout–vent length. Some specimens were mounted in Canada balsam or Histoclad after staining with acetocarmine or Mayer's hematoxylin for study as whole mounts, and others were cut into serial sagittal sections at 8 μm and stained with hematoxylin-eosin. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube.

Acanthostomum quaesitum (Nicoll, 1918) Hughes, Higginbotham, and Clary, 1941 (Figs. 1, 2)

DESCRIPTION (based on 9 whole mounts and sectioned material): Body elongate with somewhat truncated posterior end, 1.79 to 1.85 mm long by 0.30 to 0.37 mm wide; widest point variable in hindbody; ratio of body width to length 1:4.5 to 6.0. Tegument with numerous spines up to 5 long anteriorly; spines sparse posteriorly. Eyespot pigment dispersed. Oral sucker cup-shaped, terminal, 169 to 253 long by 169 to 229 wide, surrounded by single uninterrupted row of 24 spines; spines 36 to 41 long by 7 to 10 wide. Acetabulum 135 to 181 long by 137 to 186 wide. Forebody 15 to 23% (18%) of total body length. Ratio of oral sucker width to acetabular width 1: 0.75 to 0.95 (1:0.85). Prepharynx 20 to 30 long, thin-walled. Pharynx barrel-shaped, 108 to 181 long by 60 to 121 wide. Ratio of oral sucker width to pharyngeal width 1:0.35 to 0.44 (1:0.39); ratio of acetabular width to pharyngeal width 1:0.41 to 0.51 (1:0.46). Esophagus up to 25 long, lined with thin epithelium. Cecal bifurcation less than 5% of



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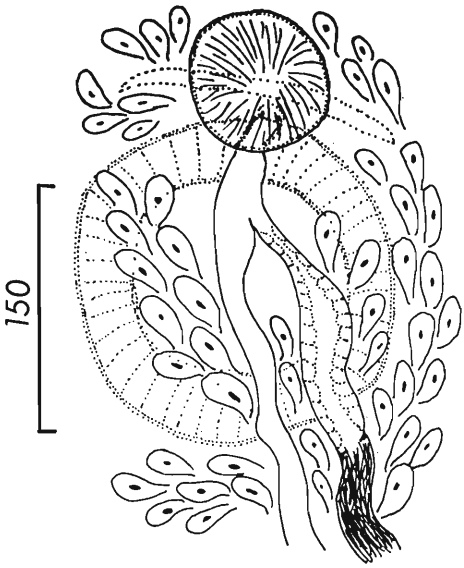

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total body length preacetabular; ceca lined with epithelium, opening separately at posterior end of body; ceca not atrophied.

Testes spherical to subspherical, smooth, tandem, contiguous; anterior testis 84 to 128 long by 145 to 181 wide, posterior testis 132 to 145 long by 108 to 176 wide; posttesticular space 2.0 to 6.6% (3.4%) of total body length. Seminal vesicle bipartite, sinuous, median, intercecal, extending 1.5 to 2.5 times acetabular length postacetabular. Prostatic duct surrounded by few prostatic cells free in parenchyma. Preacetabular pit lined with tegumental spines, with transverse aperture 48 to 106 wide, containing solid-muscular gonotyl 43 to 67 long by 69 to 84 wide; pit surrounded by gland cells free in parenchyma. Genital pore immediately preacetabular, not opening through preacetabular pit. Postacetabular pit a transverse slit 72 to 84 wide.

Ovary less than ovarian diameter pretesicular, not contiguous with anterior testis, spherical, 84 to 132 long by 108 to 145 wide. Seminal receptacle posterodorsal to ovary, 55 to 120 long by 120 to 200 wide. Ootype region not clearly seen. Uterus wound in ascending intercecal loops between ovary and acetabulum; loops occupying 50 to 65% (56%) of total body length; short muscular metraterm joining hermaphroditic duct dorsal to acetabulum. Vitellaria follicular; follicles in two longitudinal rows dorsal and lateral to ceca, extending from level of middle of ovary or of anterior testis to 21 to 23% of total body length postacetabular; follicles not reaching anteriorly to level of posterior margin of seminal vesicle, 13 to 24 long by 10 to 17 wide. Eggs 29 to 33 long by 10 to 12 wide.

Excretory vesicle Y-shaped; bifurcation posterodorsal to acetabulum; arms reaching posterior margin of oral sucker; pore terminal with muscular sphincter surrounded by gland cells.

HOST: *Crocodylus johnsoni* Krefft.

SITE OF INFECTION: Intestine.

LOCALITY: Lynd River, Amber Station, Mount Surprise, North Queensland, Australia.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74504. Other specimens in collections of North Queensland Museum and South Australian Museum.

By having a solid-muscular gonotyl, esophagus shorter than the pharynx, preovarian uterine loops, and postovarian seminal receptacle, *Acanthostomum quaesitum* resembles *A. coronarium* (Cobbold, 1861) Looss, 1899; *A. productum* (Odhner, 1902) Stossich, 1905; *A. vicinum* (Odhner, 1902) Stossich, 1905; *A. gonotyl* (Dollfus, 1950) Morozov, 1955; *A. atae* Tubangui and Masiluñgan, 1936; *A. elongatum* Tubangui and Masiluñgan, 1936; and *A. crocodili* Yamaguti, 1954. It differs from the first four and resembles the latter three by having anal openings at the posterior end of the body rather than laterally, and possessing a prepharynx less than two times longer than the pharynx. *Acanthostomum quaesitum* differs from *A. atae*, *A. elongatum*, and *A. crocodili* by having vitelline follicles not reaching the level of the posterior margin of the seminal vesicle, and exhibiting a prepharynx much shorter rather than slightly longer than the pharynx. *Acanthostomum atae* reportedly possesses 25 to 26 oral spines, *A. quaesitum* has 24, *A. crocodili* 23, and *A. elongatum* 21 to 22, but we do not rely heavily on those differences because of the reported variation in number of oral spines among other species of acanthostomes.

Acknowledgments

We express appreciation to Miss L. Madeline Angel, South Australian Museum, for the loan of original material of *A. quaesitum*, to Mr. Colin Limpus, Queensland National Parks and Wildlife Service, for the opportunity to examine the specimen of *Crocodylus johnsoni*, and to Roswitha Buxton for technical assistance.

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Figures 1, 2. ***Acanthostomum quaesitum.*** **1. Ventral view of whole mount. 2. Terminal genitalia, dorsal view.**

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The African Baboon (*Papio cynocephalus* Linnaeus 1766) as an Experimental Host for *Schistosoma mattheei* Veglia and Le Roux 1929[1]

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ABSTRACT: The African baboon (*Papio cynocephalus*) is highly compatible to infection by *Schistosoma mattheei*, a member of the *S. haematobium* complex. Very minor pathology was exhibited despite a moderate number of eggs deposited in tissues of the major visceral organs. There were no eggs and no pathologic involvement of the urinary bladder. Egg passage with feces was low and erratic, and no eggs were detected in the urine.

Schistosoma haematobium and *S. mansoni* are recognized as the two most important schistosomes of man in Africa and the Middle East. The former, long suspected of causing bladder cancer in man, has attracted increasing attention in the past few years since investigations (Kuntz et al., 1972) demonstrated that moderate to heavy *S. haematobium* infections in some nonhuman primates induce carcinoma of the urinary system. Allied studies in our laboratory have shown *S. intercalatum*, a close relative to *S. haematobium*, induces bladder lesions interpreted as superficially infiltrating undifferentiated carcinoma (Cheever et al., 1976) in cynomolgus monkeys (*Macaca fascicularis*). Variable pathologic involvement of the urogenital system with macroscopic lesions in the bladder have been reported for cattle infected with *S. bovis* (Condy, 1960) and *S. mattheei* (Lawrence and McKenzie, 1972; McCully and Kruger, 1969).

Since members of the terminal spine egg complex other than *S. haematobium*, i.e., *S. bovis*, *S. intercalatum*, and *S. mattheei*, have been shown to induce bladder pathology, it is only natural that members of this schistosome group should receive further study in an effort to gain a better understanding of their basic biology and determine whether they possess a potential for inducing bladder carcinoma. This is one of a series of publications in which the definitive host–parasite relationships of different combinations of schistosomes in nonhuman primates have been investigated.

Materials and Methods

Six young adult Kenya baboons (*Papio cynocephalus*) were obtained from an animal importer (Primate Imports, Port Washington, New York) and held in quarantine for routine microbiologic and parasitologic monitoring. Subsequently, baboons were lightly anesthetized with Ketaset (ketamine hydrochloride), the abdominal hair clipped, and the skin cleansed with water. Each host was exposed to 500 *S. mattheei* (South Africa) cercariae pooled from 23 *Bulinus africanus* and counted in drops of water on glass coverslips.

Fecal samples from three of six hosts collected from pans under individual cages were examined at weekly intervals beginning ap-

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Table 1. ***Schistosoma mattheei*** **(South Africa) infection in African baboons (*Papio cynocephalus*) 13 months postexposure to 500 cercariae.**

Location (organs)	Hosts					
	1-X 630 ♀	1-X 631 ♂	1-X 632 ♂	1-X 633 ♀	1-X 634 ♂	1-X 635 ♀
	A. Worm recovery and distribution					
Lungs	0	0	0	0	0	0
Liver	5 pr	3 pr 7 ♂	1 pr	3 pr	4 pr	6 pr
Hepatic portal	18 pr 4 ♂	3 pr 3 ♂	0	5 pr	0	7 pr
Small int.	35 pr 3 ♂	29 pr 2 ♂	8 pr	57 pr	27 pr	64 pr
Large int.	72 pr 3 ♂	66 pr 4 ♂	84 pr	113 pr	85 pr	68 pr
Urogenital	0	0	0	0	0	0
Total parasite recovery	270 130 pr 10 ♂	218 101 pr 16 ♂	186 93 pr	356 178 pr	232 116 pr	290 145 pr
Percentage recovery	54.0	43.6	37.2	71.2	46.4	58.0
	B. Eggs (eggs per organ$^{10.3}$) distribution					
Lungs	0.7	0	0	0	0	0
Liver	182.0	138.4	128.8	150.5	71.3	64.4
Stomach	0.8	0	0	0	0	0
Small int.	50.6	55.6	15.3	211.1	65.6	15.4
Large int.	42.7	294.8	174.7	345.7	182.8	9.2
Cecum	7.4	46.4	116.8	82.8	26.9	9.0
Pancreas	0	0.3	0	0	0	0
Mesentery s. int.	0	0	2.5	1.6	1.2	0.9
Mesentery l. int.	0.3	0.9	7.9	0.5	0.7	0.2
Genitals	0	0	0	0	0	0
Ureters	0	0	0	0	0	0
Urinary bladder	0	0	0	0	0	0
Total body egg count	284.5	536.4	446.0	792.2	348.5	99.1
Eggs per worm pair	2.2	5.3	4.8	4.5	3.0	0.7

proximately 12 weeks postinfection. Urine samples collected in pediatric urine bags were examined for eggs and cellular elements at irregular intervals. Twenty-four hour stool collections were processed by the Stoll technic (1923). Urine was sedimented or centrifuged prior to examination. At necropsy, visceral organs were removed and examined for adult schistosomes and gross lesions. Parasites were removed from viscera by perfusion and with small forceps under a dissecting microscope.

Fresh tissue crushes were done to determine egg distribution and viability. Eggs in tissues were counted following digestion in 2.5% KOH (Cheever, 1970). Adult female schistosomes were examined prior to fixation and intrauterine eggs counted. The urinary bladder was fixed by injection of modified Millonig's phosphate buffer formalin. Selected tissue samples for histopathologic evaluation were also fixed in Millonig's fluid, embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin.

Results

Host–parasite relationships and distribution of eggs in African baboons examined 13 months postexposure to S. *mattheei* cercariae are given in Table 1.

Of the cercariae inoculated, 37.2 to 71.2% were recovered as adults indicating that *P. cynocephalus* is a satisfactory host for S. *mattheei.*

Adult schistosomes were not demonstrated in the lungs or urogenital system, and only a few were present in the liver. Most schistosomes were located in the mesenteric venous circulation, principally that of the large intestine. The total body egg count/baboon ranged from 99,100 to 792,200. Egg distribution, as determined by fresh tissue crushes and tissue digestion, was variable. In two baboons (1-X 630, 1-X 635), the majority of eggs was deposited in the liver, whereas the other hosts had eggs principally in the intestinal tissues, with the greater number in the large intestine. A few eggs were found in the

pancreas of one host, but none were discovered in the urogenital system of any host.

Tissue egg loads/worm pair ranged from 700 to 2,200, the former recorded in a baboon which harbored 145 pairs of schistosomes. Examination of fresh tissue samples taken at random revealed eggs in all the major viscera and viability ranged from 0 to 100%.

For three animals, fecal egg counts initiated the 12th week postinfection revealed a marked weekly fluctuation in the number of eggs passed with stools. Peak production, i.e., 510,600 and 770 EPG, occurred in the 20th week. No eggs or indication of pathology were present in the urogenital system, based upon cytological examination of urine specimens. All baboons were asymptomatic throughout the study and all showed substantial weight gains.

Intrauterine eggs were counted in female worms from different organs. Abnormally small female schistosomes were isolated from liver. Most of these females contained no intrauterine eggs. A maximum of 107 eggs was counted in a female removed from a vein in the wall of the small intestine. An average of 8 to 48 intrauterine eggs was present in females from veins of the intestinal tract.

Histopathology

Early granulomatous lesions composed principally of lymphocytes and eosinophils were present in the periportal tissue of the liver of all six baboons. In general, 1–4 schistosome eggs in early stages of development were located within these lesions. Occasional lesions were infiltrated by histiocytes and showed evidence of organization. Schistosome egg granulomas were demonstrated in the mucosa and submucosa of the distal small intestine and the entire large intestine of the six animals. These lesions typically contained 5–10 eggs and were well organized with macrophages being the predominant cell type. A few of these lesions were undergoing central necrosis. In addition, a mixed inflammatory cell infiltrate was diffusely distributed throughout the lamina propria of the small and large intestine. Eosinophils were frequently numerous. Goblet cell hyperplasia was marked in the small intestine. A strong lymphoid response was suggested by a prominence of large germinal centers within the intestinal submucosa, spleen, and visceral lymph nodes. Lesions attributable to the schistosome infection were not observed in other visceral organs.

Discussion

Schistosoma mattheei presents a plethora of unanswered biological questions even though it has long been recognized as a parasite of lower mammals and man in Africa. Taxonomically, it is considered a member of the *S. haematobium* or terminal spine egg complex. In man it coexists with *S. haematobium* and *S. mansoni*. A similarity in the eggs of *S. mattheei* and *S. haematobium* has led to questionable reports of its prevalence. Its biology is variable, especially with reference to definitive host–parasite relationships regarding its longevity, egg producing capabilities, and its potential for pathology.

The present study on definitive host–parasite relationships agrees with previous work by Taylor et al. (1973) in which it was demonstrated that *S. mattheei* is highly infective to baboons. These investigators exposed two baboons to 2,000 cercariae each and suggested that partial self-cure had occurred by the time necropsy was performed 28 weeks following infection. Twenty-seven and 59% of the cercariae developed into adult parasites. By contrast, our baboons exposed to 500 cercariae each maintained their infections well. At 52 weeks postinfection, 37.2 to 71.2% of cercariae were retrieved as adult worms. Earlier, Newsome (1956), working with hamadryad baboons (*Papio hamadryas*), found that *S. mattheei* were eliminated within 6 months following infection. It is not surprising that the baboons used by Taylor et al. (1973) possessed much greater total tissue egg loads than ours which harbored fewer adult schistosomes. However, egg passage in the feces was low in both studies. Taylor et al. (1973) noted intense small intestine pathology in their baboons but found more than 1% of tissue eggs in urinary bladder. We failed to find eggs in the bladder and detected only minor pathologic involvement of the visceral organs in spite of rather wide egg distribution.

Pitchford and Visser (1975) followed *S. mattheei* infections, particularly egg passage with excreta, in man and in naturally infected

baboons and cattle in South Africa. Based upon observations and compiled information, they learned that infections wane in baboons and concluded that the parasite probably could not maintain itself in this host under natural conditions without an input from other infected definitive hosts. They also concluded that *S. mattheei* infection dies out in man but apparently not as rapidly as in the baboon. It is important to note also that they showed that school children infected with *S. mattheei* passed eggs with their urine.

The present investigation indicates that the African baboon (*P. cynocephalus*) is a satisfactory host for general studies of definitive host–*S. mattheei* relationships.

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Authors' Note

In the last issue of the Proceedings of the Helminthological Society of Washington, Kritsky et al. (p. 141–147) assigned Kimpel's (1939) names of *Dactylogyrus* spp., which were declared ***nomina nuda*** by Yamaguti (1963), to available (*sensu* the International Code of Zoological Nomenclature) names. These assignments were based in part on the examination of 5 of Kimpel's original slides which were on loan from Dr. F. J. Kruidenier, University of Illinois. After the paper was published, the authors received permission from Dr. Kruidenier to deposit these slides in the USNM Helminthological Collection. The accession numbers are as follows:

D. phenacobius (Kimpel, 1939) (= *D. seamsteri* Price, 1967)	11111
D. whipplius (Kimpel, 1939) (= *D. moorei* Monaco and Mizelle, 1955)	11111
D. superficialis (Kimpel, 1939) (= *D. bullosus* Mizelle and Donahue, 1944)	11111
D. semotilus (Kimpel, 1939) (= *D. lineatus* Mizelle and Klucka, 1953)	11111
D. umbratilus (Kimpel, 1939) (= *D. attenuatus* Mizelle and Klucka, 1953)	11111

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Observations on Strigeoid Trematodes from the Eyes of Southeastern Wyoming Fish. I. *Diplostomulum spathaceum* (Rudolphi, 1819)[1]

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ABSTRACT: Metacercariae of *Diplostomum spathaceum* were found in a wide variety of fish species and habitats in southeastern Wyoming. Fish hosts included brook trout (*Salvelinus fontinalis*), rainbow trout (*Salmo gairdneri*), brown trout (*S. trutta*), longnose sucker (*Catostomus catostomus*), white sucker (*C. commersoni*), brassy minnow (*Hybognathus hankinsoni*), fathead minnow (*Pimephales promelas*), creek chub (*Semotilus atromaculatus*), and yellow perch (*Perca flavescens*). Levels of infection increased in white suckers of increasing lengths and in rainbow trout following stocking. *Lymnaea palustris*, *L. stagnalis*, and *Physa gyrina* served as first intermediate hosts in nature. The seasonal emergence of cercariae of *D. spathaceum* from *L. palustris* peaked on or near September 1. Recommendations regarding management of infected sport fisheries are made and discussed.

Metacercariae of *Diplostomum spathaceum* (Rudolphi, 1819) occur in the eyes of a wide variety of fish species throughout the Nearctic and Holarctic regions (Skrjabin, 1960; Hoffman, 1967). Although inhabiting primarily the lens (Skrjabin, 1960; Hoffman, 1967), metacercariae have also been reported from the retina (Davies et al., 1973) and vitreous humor (Wooten, 1974). *Diplostomulum spathaceum* is highly pathogenic in some hosts, often manifesting itself in blindness and more rarely death. Blindness presumably makes the fish more susceptible to predation by gulls and other piscivorous birds serving as definitive hosts. Despite the lack of quantitative data regarding the effect of blindness in fish on fishing success, Davies et al. (1973) reported blindness to be widespread in both game and rough fish in North Park, Colorado, and suggested that the quality of fly and lure fishing had declined as a result.

The life cycle was determined for this species by van Haitsma (1930), who considered it to be *D. flexicaudum* (Cort and Brooks, 1928) van Haitsma, 1930. Later workers (Dubois, 1953; Hoffman, 1967) have considered *D. flexicaudum* to be a synonym of the European species *D. spathaceum* (Rudolphi, 1819). Dubois (1970), however, considered *D. spathaceum* to be made up of four distinct subspecies: *D. s. spathaceum* in Europe and Asia, *D. s. murrayense* in Australia, and *D. s. indistinctum* and *D. s. huronense* in North America.

This study was initiated to determine the prevalence and distribution of this parasite in the fish of southeastern Wyoming, and to aid in better management practices.

Materials and Methods

Fish and/or heads of fish were obtained by gill netting, electrofishing, and from anglers. Preservation and examination of fish was carried out using the methods of Davies et al. (1973).

Snail infections were determined by isolation using the methods of Hendrickson and Kingston (1974). If cercariae were not observed within 6 days, snails were discarded and recorded as uninfected. In order to confirm the diagnosis of the cercaria of *D. spathaceum*, four laboratory reared rainbow trout fingerlings (1–2 inches in length) were exposed to cercariae. A single fish died 24 hr postexposure. No metacercariae were recovered. Hoffman and Dunbar (1963) and others have also observed the death of fishes due to cercarial penetration. The remaining fish were necropsied at 15 days postexposure. All har-

[1] This study was supported in part by Federal Aid in Fish Restoration Project, Wyoming FW-3-R and is a portion of a thesis submitted to the Graduate College, University of Wyoming, in partial fultillment of the degree of Master of Science.
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Table 1. Levels of infection of southeastern Wyoming fish with metacercariae of *D. spathaceum*. Number infected/number examined (percent infected) over average number of metacercariae per infected fish (range).

	Basin or plains type habitats	Montane or subalpine type habitats
Catostomus catostomus (Forster)	38/40 (95.0) 16.3 (1–60)	4/13 (30.8) 48.3 (1–105)
Catostomus commersoni (Lacépède)	184/193 (95.3) 87.2 (1–433)	0/1 (0)
Hybognathus hankinsoni Hubbs	2/5 (40.0) 2.5 (1–4)	none examined
Perca flavescens (Mitchill)	3/33 (9.1) 1.0 (1–1)	none examined
Pimephales promelas Rafinesque	2/2 (100) 3.5 (2–5)	none examined
Rhinichthys cataractae (Valenciennes)	none examined	0/9 (0)
Salmo gairdneri Richardson	33/226 (14.6) 1.91 (1–11)	30/35 (85.7) 13.9 (1–126)
Salmo trutta Linnaeus	1/22 (4.5) 1.0 (1–1)	0/51 (0)
Salvelinus fontinalis (Mitchill)	2/3 (66.7) 3.5 (2–5)	71/111 (64.0) 8.1 (1–38)
Semotilus atromaculatus (Mitchill)	14/14 (100) 11.1 (1–43)	none examined

bored metacercariae of *D. spathaceum* in the lenses of the eyes.

Study Sites

Collections were made in the vicinity of Laramie, Wyoming and in the Snowy Range Division of the Medicine Bow National Forest about 40 miles west-southwest of Laramie. Nine habitats representing eutrophic plains environments (Alsop, Gelatt, Hattie, Meeboer, Porter, and West Carroll Lakes; Roach and Susan's Ponds; and the Big Laramie River at Laramie, Wyoming); and eight habitats representing oligotrophic subalpine or montane habitats were examined (Owen, Rob Roy, and Towner Lakes; Middle Crow, North Branch of Middle Fork of Pole, Cottonwood, and Rock Creeks; and the Big Laramie River at Wood's Landing, Wyoming).

Results

Ten species of fish were examined during the study (Table 1). All but the longnose dace harbored metacercariae of *D. spathaceum*. Metacercariae were recovered from the lenses of the nine other species examined, and from the retinas, choroids, and vitreous humors of brook and rainbow trout.

Many of the habitats represent sport fisheries maintained by stocking programs. Some samples were collected after annual stocking was completed. Rates of infection among resident fish populations, therefore, may be higher than those reported here.

Table 2. Levels of infection of white suckers from the Big Laramie River at Laramie, Wyoming, with metacercariae of *D. spathaceum* according to length of fish. Number infected/number examined (percent infected) over average number of metacercariae per infected fish (range).

Length (cm)	Infection levels
0–4.9	2/6 (33.3) 10.5 (8–13)
5.0–9.9	11/15 (73.3) 10.5 (1–27)
10.0–14.9	9/9 (100) 21.1 (5–39)
15.0–19.9	7/7 (100) 25.9 (9–39)
20.0–24.9	13/13 (100) 40.4 (15–81)
25.0–29.9	12/12 (100) 48.5 (25–97)
30.0–34.9	14/14 (100) 131.9(62–302)
35.0–39.9	5/5 (100) 175.8(76–433)

The relationship between levels of infection and length of fish was investigated for white suckers from the Big Laramie River at Laramie, Wyoming (Table 2). The trend is for increased prevalence of infection (as percent of fish infected) and intensity of infection (as average number of metacercariae per infected fish) with increasing size. This relationship between level of infection and length of fish probably reflects a similar relationship between level of infection and age of fish, es-

Table 3. Levels of infection of rainbow trout following planting in West Carroll Lake according to sampling dates. Number infected/number examined (percent infected) over average number of metacercariae per infected fish (range).

Date	Infection levels
July 22, 1973	0/9 (0)
July 29	0/29 (0)
August 12	0/11 (0)
August 25	1/19 (5.2) 1.0 (1–1)
September 8	2/21 (9.5) 1.5 (1–2)
September 15	3/23 (13.0) 1.7 (1–2)
October 6	4/14 (28.6) 1.8 (1–3)

Table 4. The emission of cercariae of *D. spathaceum* from Lake Owen *Lymnaea palustris* according to sampling dates. Number infected/number examined (percent infected).

Date	Cercaria of *D. spathaceum*	Any species of cercaria
June 26, 1973	3/155 (1.9)	10/155 (6.5)
July 10	4/169 (2.4)	11/169 (6.5)
August 5	11/308 (3.6)	24/308 (7.8)
September 1	12/219 (5.5)	21/219 (9.6)
September 29	7/186 (3.8)	16/186 (8.6)
October 14	5/147 (3.4)	10/147 (6.8)

pecially for the smaller size classes. The metacercariae are quite long lived (3 years; see discussion), and a continual buildup in their numbers occurs throughout the life of the fish.

The course of infection in rainbow trout following planting was observed in West Carroll Lake (Table 3). Throughout the study period, levels of infection remained low compared with other habitats examined. Fish were first observed infected on August 25, and showed a trend toward increasing levels of infection with time until the end of the study period.

During this study, 2,432 snails were examined for emerging cercariae. Cercariae of *D. spathaceum* were identified from *Lymnaea palustris* (Müller) (Lake Owen: 42/1,339 [3.1%], Susan's Pond: 2/15 [13.3%]), *Lymnaea stagnalis* Say (Susan's Pond: 1/4 [25%]), and from *Physa gyrina* Say (Susan's Pond: 2/225 [0.9%]).

The seasonal release of cercariae of *D. spathaceum* by *L. palustris* in Lake Owen was observed (Table 4). Infections with cercariae of *D. spathaceum* comprised nearly 50% of the total number of infections with any species of cercariae. The rate of infection with *D. spathaceum* rose steadily from 1.9% on June 26, to 5.5% on September 1, and then declined to 3.4% on October 14.

The levels of infection of rainbow trout increased with time (Table 3) immediately following the peak period of cercarial infections in snails (Table 4). Although two different habitats are under consideration, these two variables seem closely correlated.

Discussion

In all cases, trout were less heavily infected with metacercariae of *D. spathaceum* than were suckers from the same habitat. This may in part be due to the management program of stocking and removal which may, to some extent, alleviate the eye trematode problem in game fish. Trout showed an apparent partial loss of sight, as indicated by lens opacity, at much lower levels of infection than did suckers. Although no measurements of degree of blindness were made, a conservative estimate would be that 25–40% of the brook and rainbow trout examined from Lake Owen exhibited opacity in one or both eyes. Many of the white and longnose suckers examined exhibited this condition and often both eyes were affected. This may be due to the localization of metacercariae in the posteromedial portion of the lens (Fig. 1). In this location they tend to cause maximum blockage of incident light with minimum number of metacercariae. Ashton et al. (1969) observed that metacercariae of the European form of *D. spathaceum* had a tendency for anteromedial localization in the guppy.

Brown trout were rarely infected regardless of habitat. Betterton (1974) found this also true in an English trout farm. She reported that brown trout harbored one tenth as many metacercariae as did rainbow trout. Experimental infections using both species demonstrated that brown trout were indeed less susceptible to infection. Brown trout, therefore, are the "species of choice" for stocking programs in habitats where *D. spathaceum* is known to be a problem.

Skrjabin (1960) stated that, "The life span of metacercariae within the eye, according to a number of authors, is at least 8 months." During the course of this study a case was reported to me by Douglas L. Mitchum, Wyoming Game and Fish Commission Research Laboratory, Laramie, of a single grayling (*Thymallus arcticus* [Pallas]) harboring active metacercariae within the eye lens after having been held in a research aquarium for just over 3 years. The aquarium was supplied with water from an underground well and the fish was fed on pelleted food, eliminating any possibility of reinfection during this period. This, in addition to the continual buildup of metacercariae in suckers of increasing length (age), may indicate that the longevity of the metacercariae may approach that of the fish host. Hoffman (pers. comm.) observed a

Figure 1. ***Diplostomulum spathaceum*** **localized in the posteromedial portion of the lens of** ***Semotilus atromaculatus.*** **× 49. Abbreviations L, lens; R, retina.**

similar life span for this metacercaria in catfish raised at Leetown, West Virginia.

This longevity of metacercariae suggests that wild trout should exhibit an increase in the levels of infection with increasing length (age), as was the case with white suckers in this study. If small trout were stocked in problem habitats, the course of infection could approach that in the wild population. If large fish were stocked, they would be of catchable size before significant infection could occur. Thus, stocking larger fish where native fish are heavily infected would tend to reduce the detrimental effects of the trematode on fish.

Increased rates of infection, seen in rainbow trout following planting in West Carroll Lake, are to be expected in light of the above. If an age III+ fish is to have more metacercariae than an age II+ fish, it must acquire these additional metacercariae during its third year. Fish become infected only during the warmer months at a time when snail hosts emit cercariae.

The seasonal release of the cercariae of *D. spathaceum* provides information regarding the best time for stocking fish. The incidence of cercarial release from *L. palustris* reaches a peak on September 1. Thus, subcatchable fish should not be stocked in problem lakes between August 1 and October 1. The shock of stocking combined with cercarial penetration could result in significant mortality.

Certain generalizations can be made regarding the best time for stocking fish of various size categories. Catchable and larger fish should be stocked as early in the spring as possible, allowing fish to recover from the shock of stocking prior to peak periods of cercarial release, and still making them available to fishermen for the coming summer. A similar program should be followed for stocking subcatchable fish in low nutrient lakes. If subcatchable fish are to be stocked in high nutrient lakes where growth continues throughout the winter (e.g. West Carroll and other basin lakes), stocking should take place as late in the fall as possible, when concentrations of cercariae are reduced, thus avoiding an additional exposure of fish to cercarial penetration during the summer.

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Observations on Strigeoid Trematodes from the Eyes of Southeastern Wyoming Fish. II. *Diplostomulum scheuringi* Hughes, 1929; *Neascus ptychocheilus* (Faust, 1917); and Other Types[1]

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ABSTRACT: Four types of metacercariae, in addition to *Diplostomulum spathaceum*, were recovered from the eyes of southeastern Wyoming fish. *Diplostomulum scheuringi* was recovered from the vitreous humor of brown trout (*Salmo trutta*) and yellow perch (*Perca flavescens*). Metacercariae of *Ornithodiplostomum ptychocheilus* were recovered from the eyes of the brassy minnow (*Hybognathus hankinsoni*) and creek chub (*Semotilus atromaculatus*). Two additional types thought to be of the larval genus *Neascus* were recovered from the eyes of the creek chub, brassy minnow, and fathead minnow (*Pimephales promelas*).

Diplostomulum scheuringi Hughes, 1929 is restricted in distribution to the Nearctic (Hoffman, 1967). It has been reported from 11 families of fish, but it is primarily a parasite of Centrarchidae, Cyprinidae, and Percidae (Hoffman, 1967; and others). It occurs most often in the humors of the eye (Hughes, 1929; Hoffman, 1967) but also in the brain (Etges, 1961) and coelom (Haderlie, 1953). Etges (1961) described its intramolluscan stages from *Helisoma anceps*. He found cercariae penetrate and develop to metacercariae in either fish or newts. He postulated that the life cycle of *D. scheuringi* may involve four hosts, basing this hypothesis on the ability of metacercariae to encyst in mice and to resist digestion in chicks and mice. Holliman (pers. comm.) has indicated that *D. scheuringi* is the metacercaria of *Diplostomum trituri* with *Chelydra serpentina* serving as its definitive host.

The metacercaria of *Ornithodiplostomum ptychocheilus* was described by Faust (1917) as *Cercaria ptychocheilus*. Hughes and Piszczek (1928) redescribed this metacercaria including it in the larval genus *Neascus*. *Neascus ptychocheilus* has been reported mainly from cyprinids, principally the genera *Notropis*, *Pimephales*, and *Semotilus* (Hughes and Piszczek, 1928; Hoffman, 1953, 1954, 1958; Molnar et al., 1974; and others) but also from *Fundulus* by Wiles (1975), *Perca*

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and *Etheostoma* by Hoffman (1958) and Larson (1966), and from two species of *Catostomus* by Amin (1969). Metacercariae localize in the mesenteries of the body cavity and viscera (Hughes and Piszczek, 1928; Hoffman, 1954, 1958) but in certain fish species they occur commonly on the surface of the brain (Hoffman, 1954, 1958). In heavy experimental infections, Hoffman (1958) recovered metacercariae from the orbit and vitreous chamber of the eye (occasionally subcorneal) and from the body musculature. The geographical distribution of *N. ptychocheilus* includes most of North America, having been reported from Arizona (Amin, 1969); Connecticut (Hunter, 1942); Michigan (Hughes and Piszczek, 1928); Minnesota (Larson, 1966); Montana (Faust, 1917); North Dakota (Hoffman, 1954, 1958); Nova Scotia (Wiles, 1975); Ontario (Molnar et al., 1974); Wisconsin (Amin, 1975); and Wyoming (this paper).

Diplostomulum scheuringi and *D. spathaceum* are the most commonly encountered species of trematodes occurring in the eyes of North American freshwater fish. A number of other strigeoid metacercariae including those of *O. ptychocheilus* occasionally occur in the eyes of fish, resulting from very heavy infections or perhaps employment of an abnormal host.

Materials and Methods

Fish were collected and examined according to the methods of Hendrickson (1978). Samples were also collected by means of minnow traps baited with raw beef liver.

To confirm the diagnosis of *N. ptychocheilus*, newly hatched chicks and laboratory mice were force-fed large numbers of metacercariae by means of a stomach tube. Hosts were maintained in the laboratory for 24–144 hours. Experimental chicks received no food during this period. At necropsy, 10 of 13 chicks and 1 of 7 mice harbored adult *O. ptychocheilus*.

Metacercariae and adult worms were fixed in hot 10% formalin (70°C). Fish and/or eyes for sectioning were fixed in Bouin's fixative at room temperature. Further processing was according to routine histological procedure.

Study Sites

Fish infected with *D. scheuringi* were collected from Lake Hattie. Minnows infected with the other metacercariae were collected from the Big Laramie River and its associated runoff ponds within the city limits of Laramie, Wyoming. Brief descriptions of these study sites are given by Hendrickson (1978).

Results

In addition to *D. spathaceum*, as reported by Hendrickson (1978), four types of strigeoid metacercariae were found in the eyes of southeastern Wyoming fish. *Diplostomulum scheuringi* and *N. ptychocheilus* were identified, the latter by rearing experiments. Two additional types were found but were not identified since only metacercariae were available. These were not studied in sufficient detail to permit specific diagnosis.

Diplostomulum scheuringi Hughes, 1929

Diplostomulum scheuringi was recovered from the vitreous humor of brown trout (*Salmo trutta* Linnaeus) and yellow perch (*Perca flavescens* [Mitchill]) collected from Lake Hattie. Thirteen brown trout were examined, four (31%) being infected with an average of 3.0 (1–4) metacercariae per infected fish. Fourteen yellow perch were examined, 12 (86%) being infected with an average of 4.4 (1–11) metacercariae per infected fish.

Brown trout and yellow perch from other habitats were not infected with *D. scheuringi* but were infected with *D. spathaceum*; *D. spathaceum* was recovered from other species of Lake Hattie fish. However, a double infection involving *D. scheuringi* and *D. spathaceum* was never observed.

Infection levels were low in both fish species and it is believed that the effect on the vision of the fish was minimal. No indication of pathology was observed. Because of its occurrence in the humor, *D. scheuringi* may be less pathogenic than *D. spathaceum*.

Neascus ptychocheilus (Faust, 1917)

Metacercariae of *Ornithodiplostomum ptychocheilus* were recovered from the retina, choroid, and sclera of the creek chub (*Semo-*

tilus atromaculatus [Mitchill]) and from the choroid of the brassy minnow (*Hybognathus hankinsoni* Hubbs). In many instances, metacercarial cysts were recovered near the region in which the optic nerve passes through the choroid layer (Fig. 1). Three of five (60%) brassy minnows were infected with an average of 2.7 (1–3) metacercariae per infected fish and 10 of 14 creek chubs, with an average of 2.5 (1–8) metacercariae per infected fish.

During the initial stages of this investigation only the eyes were examined, but later more thorough examinations were conducted. *Ornithodiplostomum ptychocheilus* metacercariae were abundant in the mesenteries of the body cavity of creek chubs and within the cranial cavity of fathead minnows (*Pimephales promelas* Rafinesque). In the fathead, the brain was generally coated dorsally and laterally with a single layer of parasite cysts. A few cysts were also observed within the brain ventricles (Fig. 2). No eye involvement was observed in fathead minnows.

Neascus I

Immature metacercariae of the larval genus *Neascus* were recovered from the retina, choroid, and sclera of the creek chub, the retina and sclera of the brassy minnow, and from the choroid and sclera of the fathead minnow. Seven of 14 (50%) creek chubs were infected with an average of 2.4 (1–3) metacercariae per infected fish and two of five (40%) brassy minnows, with an average of 1.5 (1–2) metacercariae per infected fish. One of two fathead minnows was infected with two metacercariae. The localization of *Neascus* I within the eye is seen in Figure 3.

Neascus I was recovered from preserved cyprinids collected during the fall of 1973, and from living specimens collected during the summer of 1974. Observations made on living *Neascus* I suggest that this metacercaria is a stage in the life cycle of *O. ptychocheilus* or a variant resulting from crowding or localization in an abnormal site. Specimens were often encapsulated by material apparently of parasite origin (Fig. 4). This material, presumably a precursor to the cyst of parasite origin, indicates an affinity with the *Neascus* metacercariae.

Neascus II

Another unidentified *Neascus* species was recovered from the retina, sclera, and choroid of the brassy minnow, the sclera of the fathead minnow, and the choroid of the creek chub. Four of five (80%) brassy minnows, one of two (50%) fathead minnows, and one of 14 (7.1%) creek chubs were infected with one metacercaria each.

Neascus II was found in very fragile cysts generally broken upon dissection of the host eye. *Neascus* II resembles the metacercaria of *Posthodiplostomum minimum minimum*, common in the body cavities of the cyprinids examined, but because living specimens were not observed and a sufficient number of fixed specimens were not available, specific diagnosis was not possible. Hoffman and Hutcheson (1970) previously reported *P. m. centrarchi* from the orbit of the eye of *Roccus saxatilis*.

Discussion

The four strigeoid metacercariae (exclusive of *D. spathaceum*) occurred at low levels within the eye, and it is likely that they had little, if any, effect on the host's vision. *Neascus ptychocheilus* may have some deleterious effects due to its "normal" site of localization on the brain but this was not investigated.

Multiple infections involving *Neascus* I, *Neascus* II, *N. ptychocheilus*, and *D. spathaceum* were common in the eyes of the creek chub and brassy minnow, both of which harbored all four types. Of 14 creek chubs ex-

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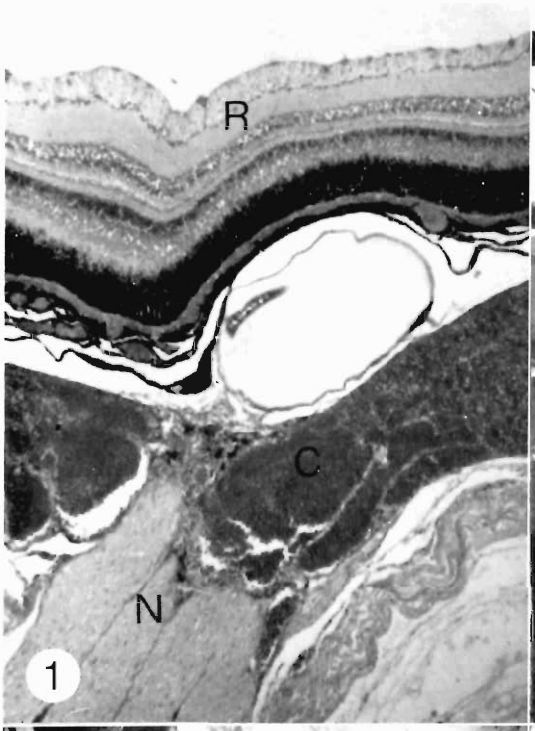
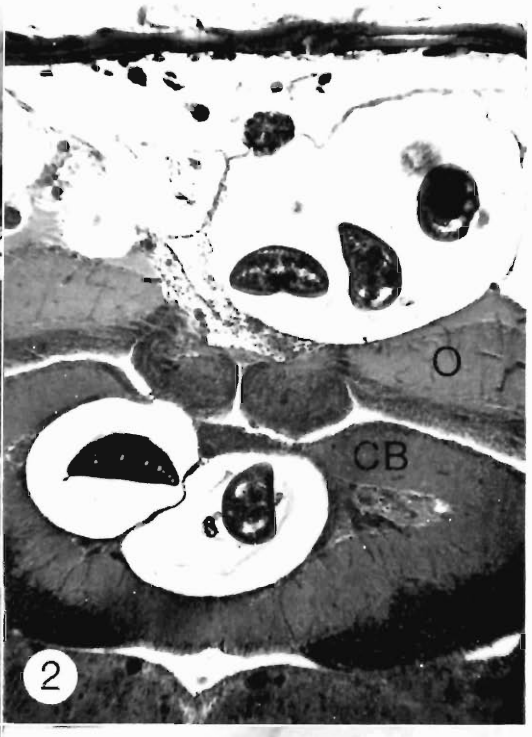
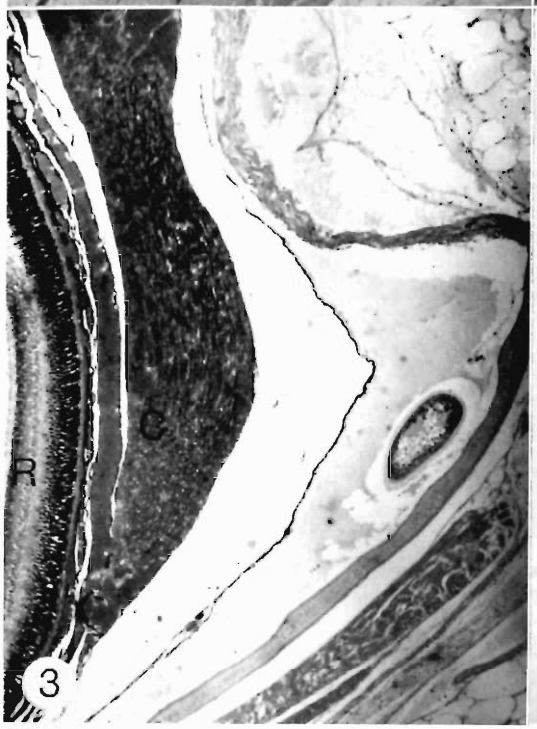
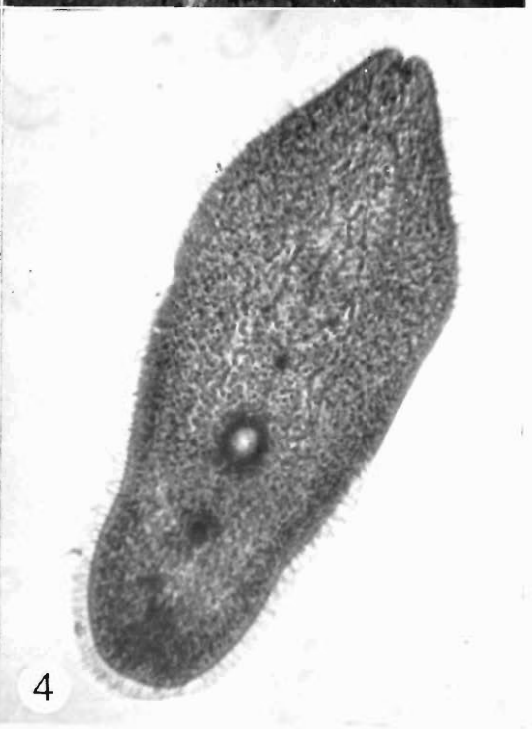
Figure 1. ***Neascus ptychocheilus*** **localized within the eye of** ***Semotilus atromaculatus.*** **× 61.**
Figure 2. ***Neascus ptychocheilus*** **localized upon and within the brain of** ***Pimephales promelas.*** **× 72.**
Figure 3. ***Neascus*** **I localized within the eye of** ***Semotilus atromaculatus.*** **× 48.**
Figure 4. ***Neascus*** **I whole mount from the vitreous humor of** ***Semotilus atromaculatus.*** **Note encapsulation. × 125. Abbreviations: C, choroid layer; CB, cerebellum; N, optic nerve; O, optic tectum; R, retina.**









amined 2 single, 5 double, and 7 triple infections were noted.

Neascus I may be a new species, an undescribed stage in the life cycle of *O. ptychocheilus* or other strigeoid, or a variant resulting from crowding or localization in an abnormal site. Because live specimens were not examined in sufficient detail, descriptions were not attempted.

Neascus II was not studied in sufficient detail to permit specific diagnosis. It resembles the metacercaria of *P. m. minimum* and its occurrence in the eye may have resulted from very heavy infections in the body cavities of the minnows examined.

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Muscles of the Reproductive System of Male *Moniliformis moniliformis* (Acanthocephala)[1]

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ABSTRACT: The copulatory act in Acanthocephala requires a complicated set of responses on the part of the male worm. The muscles associated with these responses include a set of bursal protrusor muscles plus a large bursal depressor muscle which participates in the eversion of the bursa. The anterior and posterior bursal accessory muscles are associated with the bursal depressor muscle and are described for the first time. The bursa itself consists of a unique pattern of radial muscles surrounding a bursal lining or sheath and is in turn surrounded by a loose arrangement of circular muscles. Saefftigen's pouch penetrates the outer covering and empties into the ventral anterior margin of the bursa. This pouch is enclosed by the genital sheath for much of its length. The genital sheath also serves as the site for the origin or insertion for most muscles associated with the reproductive system. An analysis of the histology of the muscles associated with this system indicates that they possess the unique tubelike construction observed throughout this group.

The anatomy of Acanthocephala was of interest in the middle to late 19th century to only a few German scientists and may have been the outgrowth of discriminating taxonomic work begun around 1850. Present-day detailed information on the unique internal construction of the "Kratzer" was the result of research by Schneider (1868), Leuckart (1876), Saefftigen (1884), Kaiser (1893) and other early workers. This work has been summarized in reviews by Rauther (1930) and Meyer (1933). More recent work has emphasized other aspects of their biology although an occasional paper such as Whitfield's (1968) has been published on anatomy. General reviews that are more recent are those of Hyman (1951), Baer (1961) and Nicholas (1973). Nevertheless, much remains to be done in the area of general descriptive anatomy of most acanthocephalan species.

Most of the original work on the detailed anatomy of Acanthocephala was written in German, and although this work does not describe *Moniliformis moniliformis*, Kaiser (1893) did compare certain features of *M. moniliformis* with *Macracanthorhynchus hirudinaceus*. We attempted the present study because little had been done on the reproductive anatomy of male *M. moniliformis*. Since most of the available literature was published in the 19th century in German publications, which are difficult to obtain, we have included German terms where appropriate for reference purposes. This study is based on muscles observed in association with the genital sheath and bursa and does not include muscles anterior to the junction of the ligament sac with the genital sheath. Nomenclature for previously described muscles has been taken from Kaiser (1893) for *M. hirudinaceus* and Kilian (1932) for *Oligacanthorhynchus microcephala* (*Hamanniella microcephala*).

Materials and Methods

Moniliformis moniliformis (*M. dubius*) was provided through the courtesy of Dr. Frank Fisher, Jr., Rice University. After cleaning, male worms measuring 60–80 mm long were fixed in AFA or in a solution consisting of 0.4 ml formaldehyde, 100 ml water, 0.03 M bromoacetate and 0.18 M sucrose at 0°C as previously described by Dunagan and Miller (1970). Routine paraffin embedding in 56°C wax followed a brief wash of the fixed tissue in physiological saline solution. Sectioning at 8–10 μ and mounting of serial sections was done in the usual manner. Staining was accomplished by the usual methods for H & E, toluidine blue, cresylecht violet and PAS-hematoxylin. The stained sections were photographed on a Leitz microscope. Outline tracings were made and three-dimensional models constructed.

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Table 1.

Abbreviation	Term
AA	Anterior bursal accessory muscle
B	Bursal cap muscle
BD	Bursal depressor muscle
C	Cement gland ducts
CM	Circular muscle of body wall
DLC	Dorsal lacunar channel
E	Ejaculatory duct muscle
G	Bursal ganglion
GG	Genital ganglion
GS	Genital sheath
L	Bursal lining
LM	Longitudinal muscle of body wall
N	Posterior body wall nerve
P	Bursal protrusor muscle
PA	Posterior bursal accessory muscle
S	Bursal sac
SP	Saefftigen's pouch
T	Tegument
VD	Vas deferens
VE	Vas efferens
X	Penis

For scanning electron microscope (SEM) studies, the worms were fixed in osmium tetroxide and dehydrated in a Pearse-Edwards vacuum dryer. After mounting the holders were placed in a Denton vacuum evaporator for metal coating. Specimens were examined in a Cambridge Stereoscan IIA microscope.

Results

Figures 1–28 are a collection of illustrations of cross sections of a portion of the reproductive apparatus beginning with the genital sheath (Fig. 1) and extending to the genital opening (Fig. 28). No part of the body wall appears in Figures 1–15, but Figures 16–28 contain the circular muscles and all structures medial thereto. Components of the nervous system are included beginning with the genital ganglia (Figs. 8–11). Major posterior nerves most of which insert into the bursa itself are also shown. It should be noted that one of these nerves extends through the pseudocoel to the posterior extremity of the worm where it disappears into the longitudinal musculature along the ventral body wall surface (Fig. 25). Figure 6 shows a portion of the bursal ganglion which innervates the bursal depressor muscle via its attachment to the body wall.

The genital sheath (GS) (Figs. 2–3) encloses the eight cement gland ducts, sperm ducts, Saefftigen's pouch, and associated muscles which together are also sometimes called the ejaculatory duct. Posteriorly, the genital sheath terminates on the msucular cap of the bursa while anteriorly it is continuous with the ligament sac. The thickness of the sheath varies considerably from a thick muscular wall at the anterior extremity to a very thin wall at the level of the genital ganglion. Saefftigen's pouch and associated muscle penetrate the posterior ventral surface (Fig. 7), and the anterior bursal accessory muscle penetrates the middorsal surface (Fig. 5).

The genital sheath as a very interesting architectural design. When viewed in whole mounts of living worms, it appears as a feltwork of anastomosing units that encircles the reproductive elements. This unique pattern is not seen in cross sections of this area. Fixation and subsequent dehydration sufficiently modify this mantle so as to make the proper interpretation of its appearance difficult. Whole mount and SEM studies are therefore particularly rewarding.

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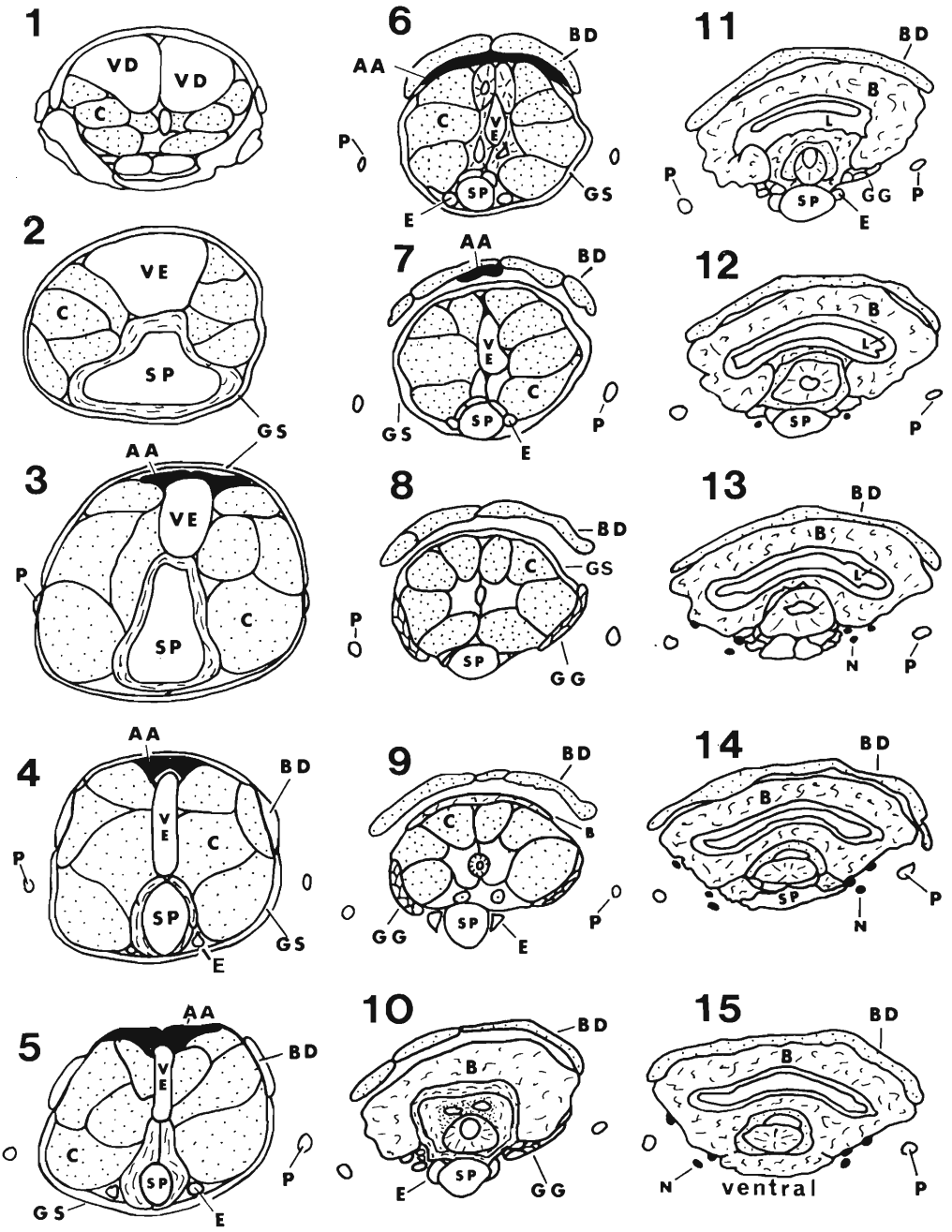
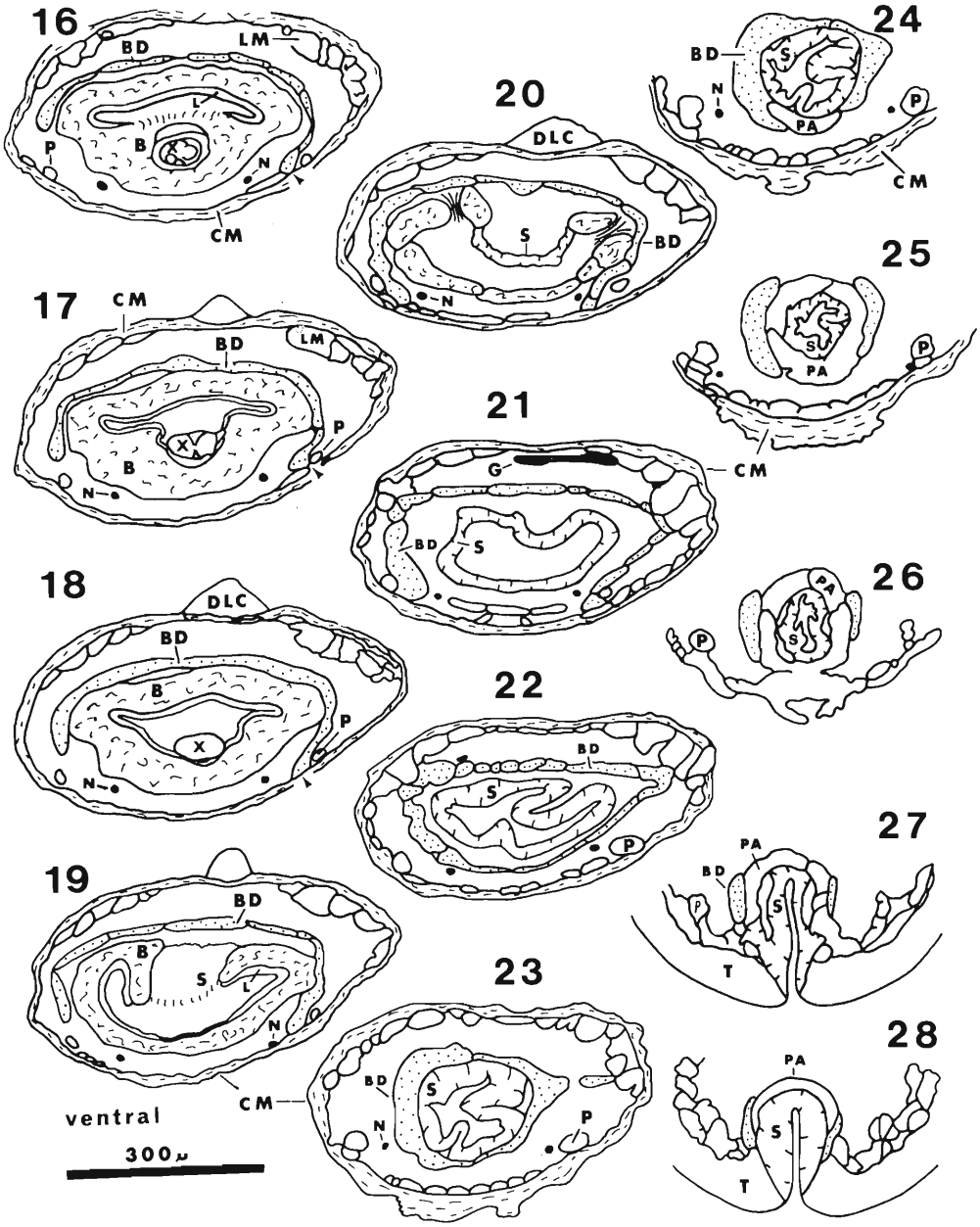
Figures 1–15. Illustrations of cross sections of the reproductive system of male *Moniliformis moniliformis*. Body wall has been deleted. Measurements have only relative value and are from posterior terminus of worm. The scale below Figure 19 also applies to these illustrations. 1. Anterior margin of genital sheath prior to vas deferens (VD), forming common sperm duct. 2. Saefftigen's pouch (SP) with thick circular muscle coat is evident as is a common sperm duct (VE). Distance 3.2 mm. 3. Origin of bursal protrusor muscle (P) and anterior bursal accessory muscle (AA). Distance 2.7 mm. 4. Origin of bursal depressor muscle (BD) and longitudinal ejaculatory duct muscle (E). Distance 2.1 mm. 5. Penetration of anterior bursal accessory muscle (AA) through genital sheath (GS). Distance 1.21 mm. 6. Dorsal movement of bursal depressor muscle (BD). 7. Insertion of anterior bursal accessory muscle (AA) on bursal depressor muscle (BD). Distance 0.80 mm. Saefftigen's pouch (SP) penetrates ventral surface of genital sheath. 8. Genital ganglion (GG) located along ventral lateral surface of genital sheath. 9. Bursal cap muscle (B) appears along dorsal surface of genital sheath (GS). Distance 0.58 mm. 10. Enlargement of bursal cap muscle (B). Distance 0.53 mm. 11. Appearance of bursal lining (L). Posterior margin of genital ganglion (GG). Distance 1.7 mm. 12, 13. Ventral



movement of bursal cap muscle (B) forming completed cap. Distance 1.65 mm and 1.6 mm, respectively. 14. Union of Saefftigen's pouch (SP) wih anterior ventral surface of bursal cap muscle (B). Distance 1.53 mm. 15. Saefftigen's pouch is no longer distinct from the bursal cap muscle (B). Distance 1.51 mm.



Figures 16–28. Illustrations of cross sections of reproductive system of male *Moniliformis moniliformis*. Circular muscle of body wall has been included. Measurements have only relative value and are made from posterior terminus of worm. 16–18. Progressive penetration of bursal muscle by penis (X) and insertion (see arrow) of one side of bursal depressor muscle (BD) onto circular muscle of body wall. Distance 1.49, 1.45, and 1.43 mm, respectively. 19, 20. Disappearance of dorsal bursal muscle and appearance of bursal sac (S). Attachment of remaining free edge of bursal depressor muscle. Dis-

The bursal protrusor muscles (P) are a pair of small longitudinal muscles that originate on the ventral body wall at the posterior extremity of the worm adjacent to the genital pore (Fig. 27). Each of the two muscles inserts on the respective lateral outside surface of the genital sheath shortly posterior to the appearance of the ligament sac (Fig. 3). These two muscles are visible in the pseudocoel throughout their length and are frequently seen in association with a nerve (Fig. 22–25) from the posterior margin of the genital ganglion. Contraction of these muscles everts the bursa; however, because of their small size, it is likely that they are used largely for fine control and precise position adjustments in the everted bursa.

The anterior bursal accessory muscles (AA) originate on the inside dorsal surface of the genital sheath at about the same level as the insertion of the bursal protrusor muscle (Fig. 3). They are a pair of small longitudinal muscles alongside the dorsal lateral surface of the vas efferens. They penetrate the overlying genital sheath prior to formation of a continuous dorsal muscle sheet by the bursal depressor muscle (Fig. 5). It is at this point that they reach their greatest size. More posteriorly they become smaller before inserting on the medial ventral surface of the bursal depressor muscle (Fig. 7). At the point of their insertion the much larger bursal protrusor muscle has separated from the genital sheath. Immediately prior to this separation, each anterior bursal accessory muscle is supplied with nerves from that half of the genital ganglion located on the same side as the muscle. The function of these two muscles apart from the general role of the bursal protrusor muscle is unknown.

Circular muscle of Saefftigen's pouch (Ringmuskelmantel des Bursalmarkbeutels) covers the surface of Saefftigen's pouch while it is within the genital sheath (Figs. 2–5). This circular muscle is frequently thick and evenly distributed around the anterior margin of that pouch. However, immediately posterior to the level where the bursal protrusor muscle inserts on the genital sheath (Fig. 4), the circular muscle is much thicker on the dorsal and lateral surface than on the ventral surface. Moreover, this muscle has completely disappeared at the level of the posterior margin of the genital ganglion. One suspects that this disappearance is associated with the passage of Saefftigen's pouch through and to the outside of the genital sheath. The function of this muscle is purported to be to shift fluid from the pouch to bursal cap and associated muscle. This pressure change may facilitate the gripping or holding characteristics of the everted bursa. We do not believe it plays an important function in the eversion of the bursa.

The ejaculatory duct muscles (E) (Längsmuskeln des Ductus ejaculatorius) are a pair of longitudinal muscles associated with Saefftigen's pouch throughout most of its length. These muscles originate on the inside ventral surface of the genital sheath at about the same level as the insertion of the bursal protrusor muscles (Fig. 3). They may be observed adjacent to the genital sheath and between Saefftigen's pouch and a cement gland duct until the genital ganglion where Saefftigen's pouch penetrates the sheath. Here they follow the exit of the pouch from the ejaculatory duct and are located adjacent to each lateral

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tance 1.32 mm and 1.21 mm, respectively. 21. Bursal cap muscle no longer evident but bursal sac (S) has been completed. Bursal ganglion (G) is evident at this level. Distance 1.05 mm. Notice four separate attachments of bursal depressor muscle to longitudinal muscle of body wall. Components of ventral musculature of body wall extend into pseudocoel and are incorporated into bursal depressor muscle (Section B), forming its ventral margin. 22. Insertion of bursal protrusor muscle (P) onto body wall. Distance 0.8 mm. 23. Detachment of bursal depressor muscle from body wall. Distance 0.74 mm. 24. Appearance of posterior bursal accessory muscle (PA) along ventral surface of bursal sac (S). Bursal depressor muscle (BD) becomes divided into two separate muscle groups. Distance 0.58 mm. 25. Posterior bursal accessory muscle completely encloses (PA) bursal sac. Bursal depressor appears as two separate lateral muscle groups. Distance 0.53 mm. 26. Posterior bursal accessory muscle fuses with circular muscle of body wall. Distance 0.46 mm. 27, 28. Genital opening formed through tegument (T). Insertion of bursal depressor muscle (BD) onto body wall. Distance 0.31 mm and 0.29 mm, respectively.

surface (Figs. 9–11). The size of the muscle increases significantly on leaving the duct and may occupy the ventral was well as lateral surfaces of Saefftigen's pouch. The ejaculatory duct muscles insert on the anterior ventral surface of the bursal cap (Fig. 12). This pair of muscles is believed to participate in the evacuation of Saefftigen's pouch.

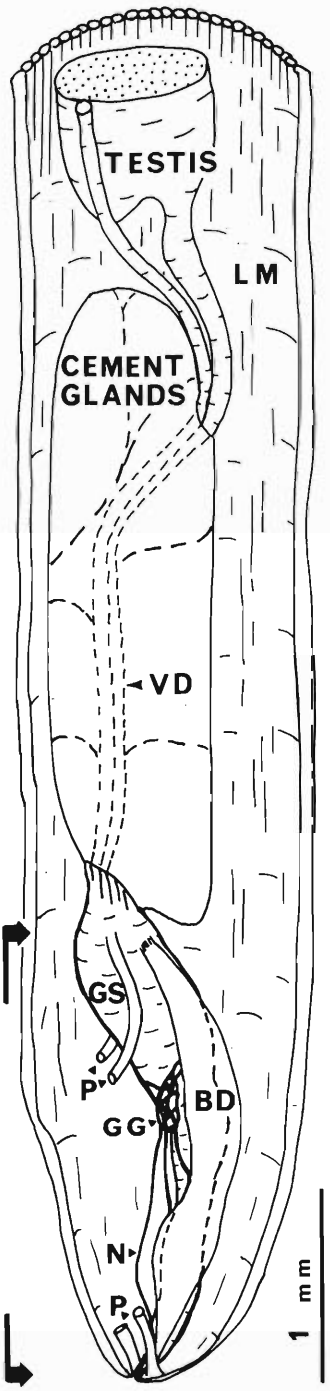
The bursal depressor muscle (BD) is one of the most conspicuous muscles associated with the ejaculatory duct. This longitudinal muscle originates as a pair of muscles attached to the body wall adjacent to the genital opening (Fig. 28) and in association with the posterior bursal accessory muscle. It quickly forms two large prominent muscles along the lateral margins of the "Bursalschlauch" which unite ventrally then dorsally to completely enclose the bursal tube (Fig. 22), at which time the bursal depressor muscle attaches to the body wall in the vicinity of the medial longitudinal canal along each lateral surface. This attachment is prominent as are the longitudinal muscles of the body wall in this area (Figs. 21–22), whereas the longitudinal muscles along the dorsal and ventral body wall surfaces are very sparse and small. This attached muscle tube continues anteriorly a short distance before the bundles of longitudinal muscle, which are located along its ventral surface, separate and attach to the body wall (Fig. 21). The free margins of the remaining sheet of bursal depressor muscle then attach to the ventral lateral margin of the body wall (Figs. 18–21). At this point the bursal depressor muscle is attached to the body wall at two points on each lateral surface. Interestingly, the free margins attach ventral but adjacent to the bursal protrusor muscle. The most lateral of these attachments is then lost while the other remains (Fig. 20) until the anterior margin of the bursal cap where they too separate from the body wall (Fig. 15). At this point the bursal depressor muscle forms a dorsal cap to the ejaculatory duct and continues as such until the anterior bursal accessory muscle penetrates the genital sheath. Here, the bursal depressor muscle divides (Fig. 6) and then somewhat more anteriorly inserts on the dorsal lateral surface of the genital sheath (Fig. 4). The function of this large sheet of muscle is to help evert the bursa.

The bursa consists of large bundles of radially arranged muscle (Radialmuskelfasern des Bursalmuskel) covered on the outside by a dense mantle of fibers (Bursalmuskelschlauches) which has scattered small bundles of circular muscles (Ringfibrillen) on its external surface. These muscles are collectively referred to as the bursal cap which is covered on its inside surface with a distinctive lining (Sarkelemmaauskleidung). Another lining which in some specimens appears as an extension of the bursal lining but in others as a separate structure (Bursalschlauch) extends to the genital opening. After leaving the bursal cap this extended lining or bursal sac (Fig. 21, S) is partially enclosed by the bursal depressor muscle and at the posterior extremity temporarily completely enclosed by this muscle (Fig. 22). Throughout, the bursal sac is always independent of the bursal depressor muscle. Prior to its attachment to the body wall the bursal sac is enclosed by the posterior bursal accessory muscle (Fig. 25). The unique structure and staining characteristics of this tube enable it to be easily separated from associated muscles. The combination of the bursal cap, bursal lining, and bursal sac is called the bursa; however, the reader may occasionally see the term bursa applied to any one of them.

The anterior ventral surface of the bursa is continuous with Saefftigen's pouch (Figs. 15–16). The contents of the pouch have direct access to the tubular muscles of the bursal cap without an intervening sphincter. The degree of contraction of the circular muscles of Saefftigen's pouch would be the determiner of the distribution of its contents. It is also through the ventral surface that the vas efferens empties. This structure is accompained by a thin coat of circular muscle which probably acts as a sphincter to keep the duct closed as well as a means to expel the sperm.

The origin of the bursa occurs along the dorsal surface of the genital sheath and ventral to the bursal depressor muscle (Fig. 9). The bursa very rapidly enlarges, however, and at the posterior margin of the genital ganglion occupies most of the pseudocoel.

Figure 29 is a diagrammatic illustration of the posterior part of the male reproductive system. It shows the relative position (arrows) of the worm represented in the cross sections of Figures 1–28. Note that this does



not present: (1) any of the unique architectural features of the genital sheath, (2) features of the body wall, (3) all of the muscles or their origins and insertions. Moreover, the two bursa protrusor muscles (P) have had their center portion removed for clarity. However, Figure 29 indicates the complexity of the male reproductive system. A complexity which apparently requires additional ganglia to properly control.

Discussion

Kaiser (1893) presented a review of previous literature concerned with the genital apparatus of male acanthocephalans. He began with the work of O. F. Mueller and concluded with that of R. Koehler in 1887. The work of 20 different investigators was discussed by him but *M. moniliformis* was not mentioned in this survey which covered about 100 years. Since then, little has been said about the musculature of the male genital apparatus that was not available in 1900. However, the studies of Bieler (1913, 1914) and Kilian (1932) are exceptions to that statement. Bieler (1914) studied the male genital apparatus of two species of *Corynosoma* and one species of *Arhythmorhynchus*. He also presented a number of figures of cross sections through the male genital apparatus depicting many of the same features described by Kaiser (1893) but like Kaiser did not illustrate anatomical features outside the genital sheath nor identify all those within. More general reviews of this group had to rely largely on the work of Kaiser (1893) and/or his predecessors. While it is true that a lot of excellent taxonomic work has been done since then, this work has not included an examination of muscles of the reproductive system and has frequently discussed the reproductive appara-

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Figure 29. Diagrammatic illustration of the posterior portion of the reproductive system of *M. moniliformis*. Many features of the body wall have been omitted but the longitudinal muscles (LM) are shown. The arrows enclose that area represented as cross sections in Figures 1–28. Abbreviations: BD, bursal depressor muscle; GG, genital ganglion; GS, genital sheath; P, bursal protrusor muscle; N, nerve; VD, vas deferens.

tus only in general terms. Thus, little information is currently available to compare with this data.

Yamaguti (1963, p. 8) lists four muscles (taken from Kilian, 1932) which he states "Under harmonious mechanism of these muscles the copulatory act begins. . . ." However, an examination of Kilian's work indicates eight, not four different muscles as being associated with the male reproductive system of *H. microcephala*. All of these muscles except the bursal retractor muscle were studied in *M. moniliformis*. Kilian illustrated the bursal depressor muscle as similar in construction to the bursal protrusors. In *M. moniliformis* the bursal depressor is a much larger muscle than the bursal protrusors and its interactions with other muscles is more complicated.

The ejaculatory duct is surrounded by a thin muscle layer throughout its length. This muscle is a continuation of the muscle layer associated with the posterior end of the genital sheath. The organizational features of this muscle have not been depicted in previous studies. Harada (1931), however, did mention its presence in *Bolbosoma turbinella*. Most previous authors call this muscle mantle the genital sheath and illustrate it as being uniform in shape, size, and structure. Some few investigators (i.e., Kilian) have indicated that this covering has a nonuniform thickness when viewed in cross section. Kilian (1932) has pictured this muscle layer as extending over the muscular cap of the bursa whereas Bieler (1913, 1914) showed this muscle layer as ending at the anterior margins of the bursa. Hyman (1951) stated that this muscular tube, the genital sheath, terminated on the muscle cap of the bursa. It may be that the genital sheath may terminate at different places, but we think this unlikely and that Kilian's experimental material should be reexamined.

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The Host–Parasite Relationships and Seasonal Occurrence of *Fessisentis friedi* (Acanthocephala: Fessisentidae) in the Isopod (*Caecidotea communis*)

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ABSTRACT: A total of 2,996 isopods (*Caecidotea communis*) from the Old Reservior, Durham, New Hampshire were examined during the period March 1975 through August 1976. One hundred and sixty-eight (5.6%) were infected with larval stages of *Fessisentis friedi*. Prevalence was high in isopods between 4.0–12.9 mm in length. Mean intensity was highest in medium size isopods between 8.0–11.9 mm. Most of the cystacanths found were facing posteriad in the isopods. Cystacanths occupied the left and right sides of the hemocoel more frequently than the median area. Cystacanths of *F. friedi* were precocious in their development in isopods.

Although larval acanthocephalans were recovered throughout the sampling period, *F. friedi* exhibited a definite seasonal prevalence in isopods. Prevalence reached its highest values in April through July 1975. It then declined and remained low in August through March 1976. Although prevalence was again high in April through July 1976, the number of isopods infected was significantly lower than the number infected in the same months of 1975. Recruitment of acanthellae of *F. friedi* into the isopod population occurred in late May through July 1975 and April through July 1976. Factors believed to be involved in the maintenance of this seasonal occurrence are discussed.

Haley and Bullock (1953) described *Fessisentis vancleavi* from sunfish of the "Old Reservoir" at Durham, New Hampshire. Fried, Kitchen, and Koplin (1965) investigated the seasonal periodicity of *F. vancleavi* in *Catostomus commersoni* in Pennsylvania. Fried and Koplin (1967) examined the morphological variability of *F. vancleavi*. Nickol (1972), who revised the genus *Fessisentis*, renamed this species *F. friedi*.

Miller (1954), in an unpublished Master's Thesis (University of New Hampshire), partially investigated the host–parasite relationships of larval *F. friedi* in *Asellus militaris* (shown to be *Caecidotea communis* by Bowman, 1975). Nickol and Heard (1973) examined the relationships of *F. necturorum* infecting *A. scrupulosus*.

The present study was undertaken to elucidate some aspects of the host–parasite relationships and seasonal occurrence of larval *F. friedi* in its intermediate host (*Caecidotea communis*).

Materials and Methods

The area of study was the "Old Reservoir," located in Durham, New Hampshire. It is a long, narrow body of water that is divided by a culvert. Isopods were collected by removing them individually from leaf litter with the aid of forceps in that region of the reservoir west of the culvert during the months of March 1975 through August 1976. This area is shallow, with large amounts of vegetation and leaf litter. There are many overhanging bushes, elm and pine trees. The leaf litter consists of elm leaves and pine needles.

Isopods were brought to the laboratory alive. They were sexed and measured by a substage micrometer. The length was measured from the anterior margin of the cephalothorax to the posterior margin of the abdomen. The live isopods were dissected within 36 hr of collection. Any acanthocephalans present were removed and relaxed in distilled water, and their number and sex recorded where possible. They were then assigned to stages of acanthella or cystacanth. These stages were invariably found in the hemocoel. The areas occupied were: left, right, and median. Cystacanths were oriented with the presoma either facing anteriad or posteriad.

Divisions of the year, based on ambient water temperatures, were established as follows: April–July 1975; August–November; December–March 1976; and April–July 1976.

Prevalence is the percent of infected hosts in a given sample. Mean intensity is the num-

Table 1. Prevalence and mean intensity of *F. friedi* recovered from 2,996 *C. communis* of various size classes (mm), examined during March 1975 through August 1976.

Isopod size	No. isopods examined	No. infected and prevalence	Mean intensity	Standard deviation
2.0–2.9	153	0 (0.00)	0.00	0.00
3.0–3.9	191	3 (1.57)	1.00	0.12
4.0–4.9	452	22 (4.86)	1.13	0.46
5.0–5.9	209	10 (4.78)	1.00	0.21
6.0–6.9	83	7 (8.43)	1.00	0.28
7.0–7.9	123	7 (5.69)	1.00	0.23
8.0–8.9	145	20 (13.79)	1.40	0.68
9.0–9.9	311	23 (7.39)	1.26	0.68
10.0–10.9	396	29 (7.32)	1.17	0.60
11.0–11.9	315	29 (9.20)	1.27	0.52
12.0–12.9	171	11 (6.43)	1.00	0.25
13.0–13.9	129	3 (2.30)	1.00	0.15
14.0–14.9	122	3 (2.45)	1.00	0.16
15.0–15.9	90	0 (0.00)	0.00	0.00
16.0–16.9	86	1 (1.16)	1.00	0.11
17.0–17.9	20	0 (0.00)	0.00	0.00
Total	2,996	168 (5.60)		

ber of worms per infected host. Statistical procedures used were from Sokal and Rohlf (1969).

Specimens of *Fessisentis friedi* from the isopods have been deposited at the Manter Parasitology Lab of the University of Nebraska State Museum.

Results

Host–parasite relationships

A total of 2,996 isopods were examined in the period from March 1975 through August 1976. One hundred and sixty-eight (5.6%) were infected with larval stages of *Fessisentis friedi*. There was no significant difference between the number of infected male (79) and female (89) isopods. Of the 168, 145 (86.3%) had single infections and 23 (13.7%) had multiple infections. There was no significant difference between the number of multiple infections in female (15) and male (8) isopods. The ranges and mean lengths of infected male and female isopods were 3.0–16.0 (9.15 mm) and 3.0–12.0 (8.37 mm), respectively. The number of acanthocephalan larvae was not related to the size of infected isopods.

Isopods smaller than 3.9 mm and larger 13.0 mm were seldom infected (Table 1). Prevalence increased in isopods over 4.0 mm, and was generally stable in isopods up to 12.9 mm, except for those 8.0–8.9 mm in length, where prevalence was maximum (13.79%). Generally, mean intensity was low in all isopod size-classes. However, it reached its highest values in medium sized isopods between 8.0–11.9 mm.

The total number of acanthocephalans recovered from the 168 infected isopods was 198. The maximum intensity was four. The sex distribution of cystacanths was close to the theoretical value of 1 : 1, 86 male and 82 female.

One hundred and one (60.1%) cystacanths were directed posteriorly and 67 (39.8%) directed anteriorly. There was a significant difference between the number of cystacanths directed posteriorly (46) and anteriorly (26) in male isopods ($\chi^2 = 5.54$, $P < 0.025$), but not in female isopods.

The number of larval acanthocephalans found in each position in the hemocoel was as follows: 86 (50.2%) occupied the left side; 68 (39.7%) occupied the right side; and 17 (9.9%) occupied a median position. More cystacanths occupied the left and right hemocoel positions than the median position ($\chi^2 = 46.2$, $P < 0.005$; $\chi^2 = 30.6$, $P < 0.005$, respectively). They were not found in the gill (opercular) area. Five female and three male cystacanths were bent and reflected on themselves.

Seasonal occurrence

Isopods were infected with cystacanths of *F. friedi* in every month sampled (Table 2). *Fessisentis friedi* exhibited a distinct seasonal prevalence. Following a March 1975 low, prevalence increased sharply in April to a peak in June, and decreased in July. Prevalence remained low and similar in August through March 1976. It then increased in April 1976, peaked again in June, and decreased during July through August. Mean intensity was low throughout the sampling period, with highest intensity values occurring during April through June 1975 and July 1976, corresponding to the high prevalences during these times.

A significantly larger number of infected isopods was collected during April through July 1975 ($\chi^2 = 106.68$, $P < 0.005$) and in the same months of 1976 ($\chi^2 = 32.64$, $P < 0.005$) when compared to the other established

Table 2. Prevalence and mean intensity of *F. friedi* in 2,996 *C. communis* examined during March 1975 through August 1976.

Month	No. isopods examined	No. infected and prevalence	Mean intensity	Standard deviation	Mean length of infected isopod	Standard deviation
Mar. (75)	205	5 (2.4)	1.0	.15	11.70	2.19
Apr.	176	16 (9.1)	1.3	.38	10.00	1.66
May	298	51(17.1)	1.3	.57	9.93	1.67
June	96	20(20.8)	1.4	.66	10.14	1.28
July	210	11 (5.2)	1.0	.22	4.18	.60
Aug.	221	3 (1.4)	1.0	.11	4.33	.57
Sep.	162	3 (1.9)	1.0	.13	4.00	0.00
Oct.	173	4 (2.3)	1.0	.15	5.50	.58
Nov.	178	3 (1.7)	1.0	.12	6.66	1.33
Dec.	198	6 (3.0)	1.0	.17	9.50	3.01
Jan. (76)	173	1 (0.6)	1.0	.07	9.00	0.00
Feb.	186	2 (1.1)	1.0	.10	11.00	1.41
Mar.	112	2 (1.8)	1.0	.13	12.00	2.82
Apr.	114	6 (5.3)	1.0	.22	9.83	1.72
May	112	7 (6.3)	1.0	.24	10.94	.83
June	112	13(11.6)	1.0	.32	9.30	2.47
July	123	12 (9.8)	1.3	.41	4.58	2.10
Aug.	147	3 (2.0)	1.0	.14	5.16	.57
Total	2,996	168 (5.6)				

divisions of the year (RXC test of independence; analysis completed by A posteriori simultaneous test procedure). Prevalence, however, was higher in April through July 1975 than in these months in 1976.

Discussion

Host–parasite relationships

The cystacanths of *Fessisentis friedi* are free and unencysted in the hemocoel of isopods. The proboscides of all cystacanths were fully inverted, and in a few instances, the resulting vestibule was covered by a small plug of yellow material. Female and male cystacanths were similar in size and were impossible to differentiate without the aid of a microscope.

Fessisentis friedi is precocious in its development in isopods. The testes, seminal vesicles, and cement glands of male cystacanths stain darkly, indicating possible presence of semen. The ovaries of female cystacanths were fragmented, forming masses of ovarian balls. Similar fragmentation was observed for *Prosthorhynchus formosus*, *Neoechinorhynchus rutili*, *F. necturorum*, and *Acanthocephalus jacksoni* (Schmidt and Olsen, 1964; Merrit and Pratt, 1964; Nickol and Heard, 1973; Muzzall and Rabalais, 1975b).

The sex ratio of *F. friedi* of 1:1 infecting isopods is similar to that found by other authors working with larval acanthocephalans, most notably Parenti, Antoniotti, and Beccio (1965); Crompton and Whitfield (1968); Amante, Fresi, and Laneri (1967); and Muzzall and Rabalais (1975b).

Although more cystacanths of *F. friedi* occupied the left side (86) than the right side (68) of the hemocoel, the difference was not significant. A larger number of cystacanths were directed posteriorly (101) than anteriorly (67); however, this difference was only significant in male isopods. Similar results have been reported by Muzzall and Rabalais (1975b) for *A. jacksoni* infecting *Lirceus lineatus*, and by Nickol and Heard (1973) for *F. necturorum*, which faced posteriad in *Asellus scrupulosus*.

The overall prevalence (5.6%) of *F. friedi* infecting isopods is quite low when compared to the results of Seidenberg (1973), Hine and Kennedy (1974), and Muzzall and Rabalais (1975b).

A few authors (Munro, 1953; Hynes, 1955; Hynes and Nicholas, 1963) demonstrated that larval acanthocephalans interfered with the development and attainment of sexual maturity of amphipod and isopod intermediate hosts. Muzzall and Rabalais (1975b) never observed female isopods infected with *A. jacksoni* carrying eggs in the field or laboratory.

The following observations of isopods infected with *F. friedi* were noted in the present study: one male and six females were in precopula, four females carried eggs, three females had live young in the brood pouch, and two females had empty brood pouches. These

results suggest that a small number of female isopods infected with *F. friedi* appear to undergo "normal" development and mating behavior to sexual maturity. It is not known if the number of eggs or larvae carried by infected female isopods is smaller than those carried by noninfected females. Similar results were reported by Spaeth (1951), who found that female *Hyalella azteca* infected with *Leptorhynchoides thecatus* developed ova and bore young.

Pigmentation differences between infected and noninfected isopods were not observed.

Seasonal occurrence

Several authors (Hynes and Nicholas, 1957, 1963; Awachie, 1965; Seidenberg, 1973; Spencer, 1974; Muzzall and Rabalais, 1975a) demonstrated seasonal occurrence of larval acanthocephalans infecting their respective intermediate hosts. On the other hand, Hine and Kennedy (1974) did not observe a seasonal occurrence of *Pomphorhynchus laevis* infecting *Gammarus pulex*.

Fessisentis friedi exhibits a seasonal occurrence in prevalence and, to a lesser degree, in mean intensity in its isopod intermediate host (Table 2). Two periods of high prevalence were observed. One occurred in the months of April through July 1975. Prevalence was again high in the same months of 1976, constituting the second period. The difference in magnitude of these two periods cannot be accounted for.

It is difficult to explain the seasonal occurrence of a parasite in its intermediate host without knowing the seasonal aspects of the definitive host–parasite system. Fried et al. (1965) found that white suckers (*Catostomus commersoni*) were infected with *F. friedi* in October through December and not in May. The investigation of Miller (1954) in the Old Reservoir may help to explain the seasonal occurrence of *F. friedi* in isopods from the same locality. He found that pickerel (*Esox americanus*) averaging 9.4 inches in length harbored adults of *F. friedi* in February, March, and May. In February and March fish were caught in water 5 feet in depth or more. Pickerel may have become infected during the previous spring while spawning and/or in the winter, since Miller found isopods to be a common food item at this time. He seined pickerel in shallow water in the spring and summer; they were not trapped in deep water. As pickerel move into shallow areas of ponds and lakes ("Old Reservoir") to spawn in the spring (Scarola, 1973), eggs of *F. friedi* may be released into the environment.

Following a low prevalence in March 1975, more isopods became infected and prevalence increased during April through June 1975 as water temperature increased (Table 2). Most of the isopods infected in April through June were of medium size between 8.0–11.9 mm. An abrupt decrease in prevalence occurred in July and August due to the natural mortality of large, infected individuals from the isopod population in June. The ranges and mean lengths of infected and noninfected isopods decreased from 7.0–14.0 (10.30 mm) in June to 2.0–7.0 (3.71 mm) in July. This decrease in prevalence also resulted from the addition to the population of many small, uninfected isopods. In late May through early June, large numbers of female isopods carried eggs and larvae. Small isopods between 2.0–5.0 mm were prevalent in July through September. Prevalence remained low and generally uniform in August 1975 through March 1976. At the end of September, new leaves, which provide a suitable substrate for isopods, entered this host–parasite system. Stark (1965) and Seidenberg (1973) noted a similar "dilution" of infected individuals of the old generation with a new uninfected generation of *Gammarus zaddachi* infected with *Diplocotyle*, and *Asellus intermedius* infected with *Acanthocephalus dirus*, respectively.

Although cystacanths were recovered in all months sampled in 1975, it is believed that late May through July 1975 was the period when most isopods became infected, since 12 acanthellae, representing the recruitment portion of the population, were recovered during this period. The range and mean length of these infected isopods was 6.5–14.0 (9.18 mm). During the fall of 1975 and winter of 1976, the size of infected (Table 2) and noninfected isopods increased. One acanthella was recovered in December 1975. Seventeen acanthellae were recovered in April through July, constituting the recruitment period in 1976. The range and mean length of isopods infected with acanthellae during this time was

3.0–12.0 (5.76 mm). The prevalence of cystacanths of *F. friedi* in isopods increased in April through June 1976 as water temperature increased, dropped slightly in June, and declined in August when water temperature was maximum (30°C).

The seasonal occurrence of *F. friedi* in isopods may be related to the spawning habits of pickerel which harbor adults of *F. friedi*; the close timing between the release of eggs into the environment and the appearance of a new generation of isopods; and changes in the composition of the isopod population.

Acknowledgments

Acknowledgment is due to Drs. Wilbur L. Bullock and Robert A. Croker, Zoology Department, University of New Hampshire for their review of the early manuscript and to Dr. Thomas E. Bowman, Department of Invertebrate Zoology, Smithsonian Institution, who kindly identified the isopod species.

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Observations on the Subfamily Aetholaiminae Jairajpuri, 1965 (Nygolaimidae: Nematoda)

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ABSTRACT: *Mylodiscus nanus* Thorne, 1939 is redescribed from specimens collected in the State of Bahia, Brazil. It has an axial odontostyle and therefore does not belong with *Aetholaimus* Williams, 1962 to the Nygolaimidae, subfamily Aetholaiminae. The abrupt esophageal expansion, location of dorsal esophageal gland orifice, and presence of oval organs in the lateral chord indicate that it belongs to Discolaimidae. *Dorylaimus rotundicauda* de Man, 1880, currently placed under *Carcharolaimus* Thorne, 1939, is redescribed from specimens collected in the Netherlands and Switzerland. The presence of a mural tooth and of three "cardiac glands" show that it belongs to the Nygolaimidae; it is transferred to *Aetholaimus*. The latter genus now contains four species and is briefly reviewed.

In 1939 Thorne described a new genus and species *Mylodiscus nanus* from a single female collected on the Island of Sumatra, Indonesia. He placed this genus in the subfamily Actinolaiminae of the family Dorylaimidae because of the presence of sclerotization in the stomatal region. He could not decide whether it had an axial stylet or a mural tooth.

In 1962 Williams described a new genus and species *Aetholaimus bucculentus* from the Island of Mauritius, which he placed in the family Nygolaimidae because it possessed a mural tooth instead of an axial odontostyle. He noted, however, that this species also possessed a character reminiscent of the Actinolaiminae, viz. sclerotization in the stomatal region; and he noted certain resemblances between *Aetholaimus* and *Mylodiscus*. In 1965 Jairajpuri united these two genera into the new subfamily Aetholaiminae—recently raised to family rank by Andrássy (1976); again he noted that the position of *Mylodiscus* required further clarification.

During a survey of plant parasitic nematodes in the State of Bahia, Brazil, carried out in cooperation with Dr. R. D. Sharma, the second author collected several females and juveniles of *Mylodiscus*, so that redescription is possible and its taxonomic position can be clarified. Paratypes of *Aetholaimus bucculentus* were kindly put at our disposal by Mr. D. J. Hooper, Rothamsted, England, and Dr. M. S. Jairajpuri lent a specimen from India, identified by him as *Ae. indicus*.

Before dealing with these species, however, we want to discuss the taxonomic value of stomatal sclerotization in the Dorylaimoidea. In our opinion this value has been strongly overrated. Thorne (1939) united all dorylaims which possess such sclerotization, viz. the genera *Actinolaimus* Cobb, 1913 (in its old wide sense), *Antholaimus* Cobb, 1913, *Carcharolaimus* Thorne, 1939, *Mylodiscus* Thorne, 1939 and *Trachypleura* Thorne, 1939, to a subfamily Actinolaiminae of the Dorylaimidae. Meyl (1957) raised the group to family rank; Thorne (1967) made it a superfamily Actinolaimoidea with six families: Actinolaimidae, Neoactinolaimidae, Paractinolaimidae, Trachypleurosidae, Carcharolaimidae and Mylodiscidae. Leaving aside the Trachypleurosidae—based upon two insufficiently known species—we can say that the first three families contain species with long-tailed females and short-tailed males (*Brittonema* Thorne, 1967 was defined as having long-tailed males, but Thorne's drawing suggests that the long male tail of *B. sulcatum* is an artifact, and males are unknown in all other species; the genus may be identical with *Actinca* Andrássy, 1964. Thorne also reported a long-tailed male of the genus *Practinocephalus* Andrássy, 1973 [= *Actinocephalus* Thorne, 1967 nec Stein, 1848] but did not describe or illustrate it), whereas the Carcharolaimidae and Mylodiscidae have short tails, generally rounded (except in a few species of *Carcharolaimus*), and no sexual dimorphism. The first three families stand, in general morphology, close to certain genera of the Dorylaimidae *sensu stricto* such as *Dorylaimus*, *Laimydorus* and *Ischiodorylaimus*: the cuticle may possess longitudinal ridges; the esophagus widens more or less gradually, and the orifice of the dorsal gland lies in the expansion region; in some groups the supplements are concentrated into a few clusters; the lip region is usually almost continuous. The Carcharolaimidae, on the other hand, have the lip region strongly offset by constriction; the lateral chord contains a series of large, conspicuous oval organs, each connected with the body surface by a large pore; the esophagus widens very abruptly and the orifice of the dorsal gland lies well behind the expansion zone. In all these characters the Carcharolaimidae agree with *Discolaimus*. Following a suggestion of Loof and Coomans (1970), Krnjaić and Loof (1976) placed *Carcharolaimus* in the family Discolaimidae.

It thus appears that the superfamily Actinolaimoidea as conceived by Thorne is an artificial taxon. The families Actinolaimidae, Neoactinolaimidae and Paractinolaimidae have such close connections with Dorylaimidae that we think it best to place them as a single family beside the Dorylaimidae (see Baqri et al., 1975). Stomatal sclerotization may evidently develop independently in several groups of dorylaims, and the presence of such sclerotization in *Aetholaimus* should not a priori be interpreted as evidence of actinolaimid relationship.

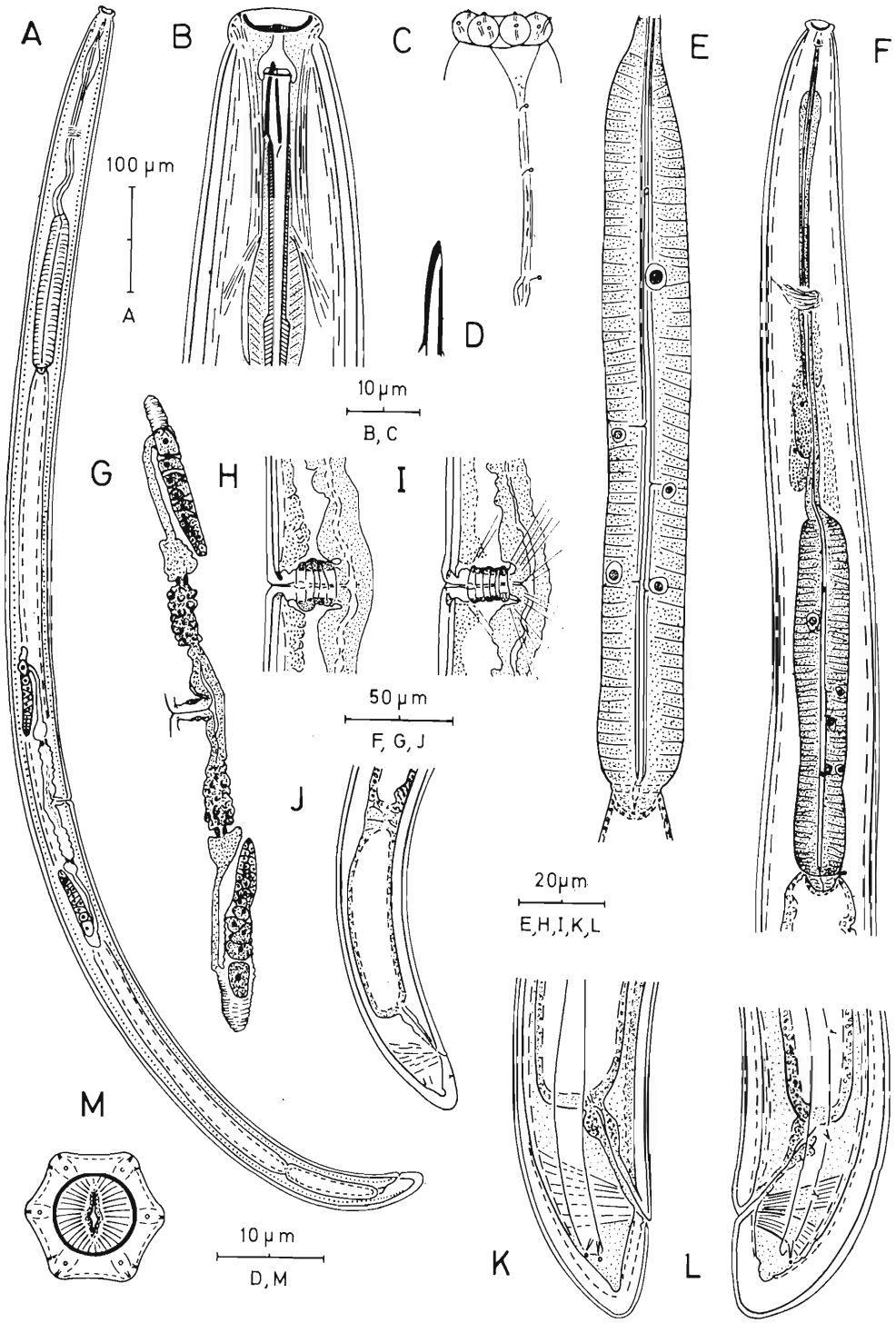
Mylodiscus nanus Thorne, 1939 (Fig. 1; Diagram 1)

DIMENSIONS (based on 13 females): L = 1.09–1.78 mm; width = 36–53 μm; a = 24–38; b = 3.1–4.1; c = 56–72; V = $^{9-17}56-59^{9-18}$; odontostyle = 10–12 μm; odontophore = 23–26 μm; T/ABW = 0.7–0.9.

Body ventrally curved after fixation; cylindrical throughout the greater part of its length but markedly narrowing near the anterior end. Cuticle 2–3 μm thick in midbody, increasing to 6–9 μm on tail; with fine but distinct transverse striae. Lateral chord 3–6 μm wide or ⅛–½ of body width at midbody. Lateral body pores in single line along the dorsal side of the chords; in one specimen there were 35: 11 in the neck region, 23 between esophagus base and tail, and one on the tail.

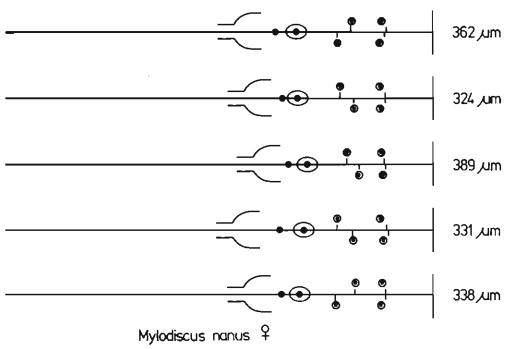
Lip region 12–15 μm wide or two fifths to one third as wide as the base of the neck; disclike with inner bowl-shaped sclerotized lining, the bottom of which is thickened and has radiating ridges. Lips clearly demarcated, with the usual set of six inner and ten outer papillae. Amphids with triangular pouch, 5–8 μm wide or about three fifths the width of the base of the lip region. Sensillae situated opposite the base of the odontophore, 34–40 μm behind head end. Oral opening a dorsoventral slit. Odontostyle slender and conical, about 0.80× as long as lip region width; with thickened tip and small aperture. Odontophore linear, 23–26 μm long. Guiding ring "double," fixed ring 8.5–12 μm or slightly less than one lip region width from head end.

The esophagus measures 326–395 μm; an-



terior part a narrow tube except for the odontophore region, which is wider and spindle-shaped. At 53–58% of the neck length the esophagus expands suddenly, forming the heavily muscular posterior part. Dorsal gland outlet 26–35 μm behind the expansion or 8–9% of neck length. Dorsal esophageal gland nucleus oval, measuring 6–8 × 4–5 μm, with rounded nucleolus 3 μm in diameter; located 11–19 μm behind the outlet of the gland. First pair of ventrosublateral gland nuclei about 15 μm apart; the nuclei are rather small (3.0–3.5 μm, nucleolus 1.5 μm), oval to rounded to somewhat triangular. Second pair of ventrosublateral gland nuclei at about the same level; measuring 3.5–5.5 μm, nucleolus 2–2.5 μm; rounded.

LOCATIONS: (gland nuclei and orifices of 5 females): DO 63–67%; DN 68–71%; DO–DN 3.3–5.8%; S_1N_1 77–80%; S_1N_2 81–83%; dist. 2.8–4.6%; S_2N 87–88%; S_2O 89–90%; K = 65–77; K′ = 73–81.



The nerve ring encircles the slender anterior part of the esophagus and is located 102–128 μm or 30–35% of neck length from head end. Behind the nerve ring and apart from the ganglia, there is a pair of apparently unicellular subdorsal bodies packed with brownish granules. Esophago-intestinal valve 5–9 × 9–15 μm; dome-shaped, 1.3–2.5× as wide as long, its width one third to one fourth of corresponding body width. Intestine thin-walled, separated from the prerectum by high columnar cells. Prerectum 67–96 μm or 2.1–3.5 anal body diameters long; rectum 22–36 μm or about equal to anal body width.

Female reproductive system didelphic, amphidelphic; each branch composed of a reflexed ovary, well-developed ovarial sac, distally slender and proximally enlarged oviduct, sphincter, and uterus consisting of two about equally long parts: a distal glandular one and a proximal one that connects with the vagina. The vagina extends about halfway into the body and is surrounded by a well-developed sphincter consisting of five muscle bands. Vulva a transverse slit, with very faintly sclerotized lips. One intrauterine egg measures 109 × 41 μm.

Tail 20–25 μm long; dorsally convex-conoid with broadly rounded terminus; usually two caudal pores present on each side, in some specimens only one.

LOCALITIES AND HABITATS: The specimens studied were collected by Dr. R. D. Sharma from soil around roots of coconut and oil palms, Bahia; *Syringa* sp. and *Eugenia caryophyllata* Thunb., Ituberá; and coffee, Itabuna and Maraú; all localities in the State of Bahia, Brazil.

Remarks

The specimens are considered to belong to the only species described so far, although there are some minor differences with the holotype described by Thorne (1939), viz. the more broadly rounded tail and slightly shorter odontophore in the latter.

Discussion

Although the odontostyle of *Mylodiscus* is small and narrow, it is axial. Furthermore the S_1N lie at different levels; cardiac glands are absent; the esophagus widens abruptly. All these characters indicate that the genus does not belong to Nygolaimidae (and *a fortiori* not to Aetholaiminae) but to the Dorylaimoidea. The distinctly offset lip region, abrupt esophageal expansion, positions of outlet of

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Figure 1. ***Mylodiscus nanus.*** **A: entire female; B: anterior end; C: lip region and amphid; D: odontostyle; E: basal portion of esophagus; F: esophageal region; G: female reproductive system; H–I: vulva region; J: posterior body end; K–L: tail; M: en face view.**

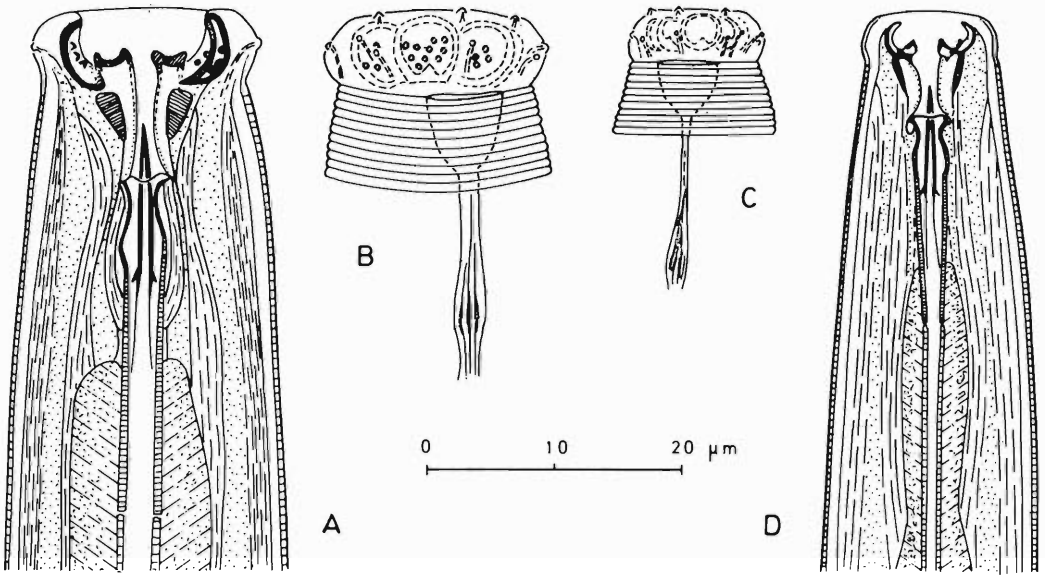


Figure 2. ***Aetholaimus*** **spp. A–B:** ***Ae. indicus.*** **A: anterior end; B: lip region and amphid. C–D.** ***Ae. bucculentus.*** **C: lip region and amphid; D: anterior end of same specimen.**

dorsal esophageal gland, lateral pores arranged into a single row, and short rounded tail indicate that *Mylodiscus* belongs in the family Discolaimidae. Within this family it shows characters intermediate between those of *Discolaimus* and of *Carcharolaimus*, mainly in the structure of the lip region and in the development of the esophageal gland nuclei. In *Discolaimus* both S_1N are well developed and distinct, in *Carcharolaimus* they are very small and obscure (cf. Loof and Coomans, 1970); in *Mylodiscus* they are distinct and intermediate in size.

Aetholaimus bucculentus Williams, 1962 (Fig. 2, C–D; Diagram 2)

Dimensions (based on 7 female paratypes): L = 1.35–1.71 mm; a = 43–60; b = 3.9–4.7; c = 71–96; V = $^{6-9}41–45^{7-11}$; tooth = 8–9 µm.

Little need be added to Williams' (1962) and Heyns' (1968) adequate descriptions and illustrations. The width of the lip region is 10–11 µm. Radial sclerotizations fine. The esophagus widens gradually at 43–46% and attains its full width at 48–51% of its length from head end. DO lies 22–32 µm behind the latter level. DN was visible in one female only, located 29 µm behind DO. The S_1O lie at about one and the same level.

Locations: DO 56–58%; DN 65%; DO–DN 8.3%; S_1O 82%; S_2O 93%.

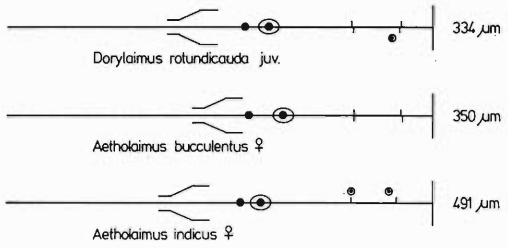
The sinuous body attitude after fixation, thin cuticle, mural tooth, distinct cardiac glands indicate that this genus belongs in the Nygolaimidae. Also the general shape of the esophagus, which narrows a short distance from the base, thereby suggesting that the posterior pair of ventrosublateral glands is less strongly developed than the anterior pair, is in agreement (see Loof and Coomans, 1970). Because of the presence of stomatal sclerotization, *Aetholaimus* may be placed in a separate subfamily Aetholaiminae Jairajpuri, 1965, but it is the only genus in this subfamily. Under the present circumstances we prefer not to raise this taxon to family level.

Aetholaimus indicus Jairajpuri, 1965 (Fig. 2, A–B; Diagram 2)

Dimensions (based on 1 female): L = 1.63 mm; a= 42; b = 3.3; c = 71; V = $^{7}50^{8}$; tooth = 13 µm.

The width of the lip region is 17 μm. A peculiar character of this species, not described before, is the presence of sclerotized knobs on the cheilorhabdia. The radial sclerotizations are heavy. The esophagus begins to widen at 38% and attains its full width at 44% of its length from head end. DO lies 54 μm behind the latter level, DN lies 23 μm behind DO. DN measures 8.5 × 5 μm, nucleolus 2.5 μm.

LOCATIONS: DO 55%; DN 60%; DO–DN 4.7%; S_1N_1 81% (right one); S_2N 90% (right one); S_2O 92% (right one).



In connection with the genus *Aetholaimus*, another species must be discussed, viz. *Dorylaimus rotundicauda* de Man, 1880, which also has stomatal sclerotization. For this reason Steiner (1916) transferred it to *Actinolaimus*, whereas Thorne (1939) brought it to *Carcharolaimus* because of the short, round female tail. In the latter genus it has remained to this day; it does, however, not fit there very well. The female tail is asymmetrical, the esophagus widens more gradually, the odontostyle was described by de Man (1880, 1884) as rather slender, whereas *Carcharolaimus* species have robust odontostyles. The type specimens of *D. rotundicauda* are lost (Loof, 1961). The type locality is the sand dune region near Scheveningen, The Netherlands, but this region is now largely occupied by the city of The Hague. Repeated collecting in the remaining part of it and the adjoining sand dune region failed to yield nematodes which could be this species. In other parts of the sand dune region we had more success: near Heemskerk we found some juveniles, on the Island of Terschelling a female. Moreover, in soil samples from Airolo, Switzerland, we found a species, the females of which agree with the Terschelling one and with de Man's description, and the juveniles agree with those found at Heemskerk. So we may assume that this species is the true *rotundicauda*.

Redescription
(Figs. 3, 4; Diagram 2)

DIMENSIONS (based on 17 females): L = 1.14–1.54 mm; width = 26–41 μm; a = 36–44; b = 2.9–4.1; c = 52–71; V = $^{5-9}41-48^{4-14}$; tooth = 10–12 μm.

de Man gave: L = 1.7 mm; a = 37–43; b = 3.7; c = 65–70; V = slightly pre-equatorial.

Body ventrally curved to spiral after fixation; cylindrical throughout the greater part of its length, but narrowing slightly towards both ends. Cuticle 2–3 μm thick, marked with fine transverse striae which are wider and more distinct near head end and on the tail. Lateral chords 7–14 μm or about one-third body width at midbody. Lateral body pores obscure.

Lip region 14–16 μm wide or about half as wide as neck base; disclike with inner bowl-shaped sclerotized lining. Lip region offset by constriction; lips provided with the usual set of six inner and ten outer papillae. Amphid apertures occupying only 5.5–6 μm or two fifths of the corresponding head width, usually difficult to observe. Cheilostome consisting of two parts: a wide anterior part with bowl-shaped, sclerotized lining ending anteriorly in curved ridges and at its base with six well-developed radial differentiations, each opposite the center of a lip. The outer part of these radial differentiations is connected with the cheilostomatal lining, and together with it, they apparently form the cheilorhabdia. Each radial differentiation has two lateral projections. The innermost parts of the differentiations project posteriorly around the small hexagonal lumen of the second part of the cheilostome. The latter is, in lateral view, somewhat conical with six elongate triangular to oval sclerotizations in its anterior half, and with narrow walls in its posterior half. The sclerotizations are round in cross section and interlabial in position, thus alternating with the radial differentiations of the anterior part of the cheilostome. Guiding ring not sclerotized, about two-third lip-region width from the anterior end. Stoma armed with a tooth about three-quarter lip-region width long, narrow and pointed, hollow to

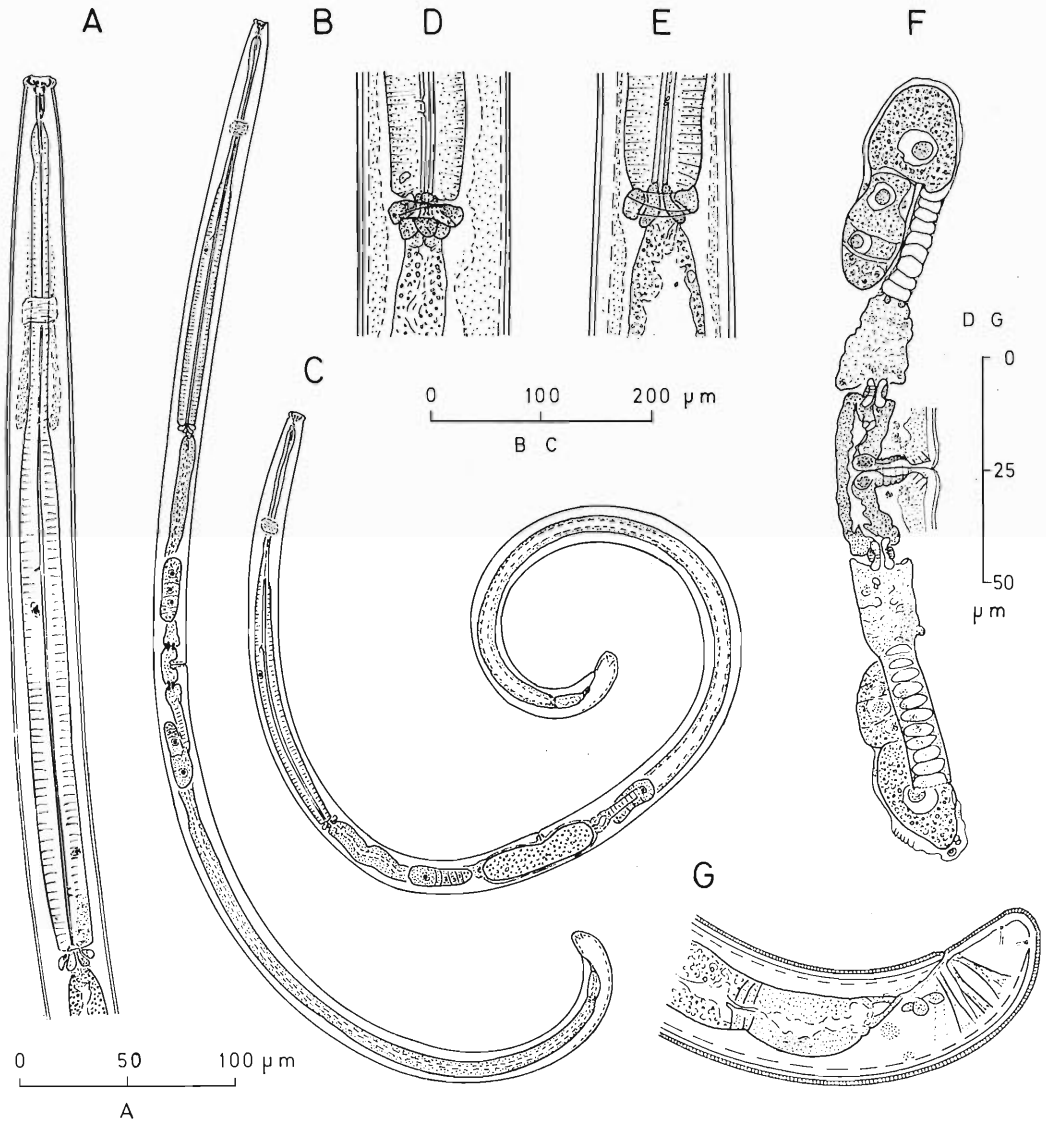


Figure 3. *Aetholaimus rotundicauda.* A: esophageal region; B–C: entire female; D–E: Esophagointestinal junction in left ventrosublateral (D) and right ventrosublateral (E) position; F: female reproductive system; G: tail region.

→

Figure 4. *Aetholaimus rotundicauda.* A: anterior region of J_2; B: same of J_3 in J_2 cuticle; C: same of J_3; D: same of J_4 in very early stage of molting; E–G: anterior end of females with tooth in different positions; H: en face view; I: optical cross section through second part of cheilostome; J: head end in surface view (same specimen as G). *i.l.s.* = interlabial sclerotization; *r.d.* = radial differentiation.

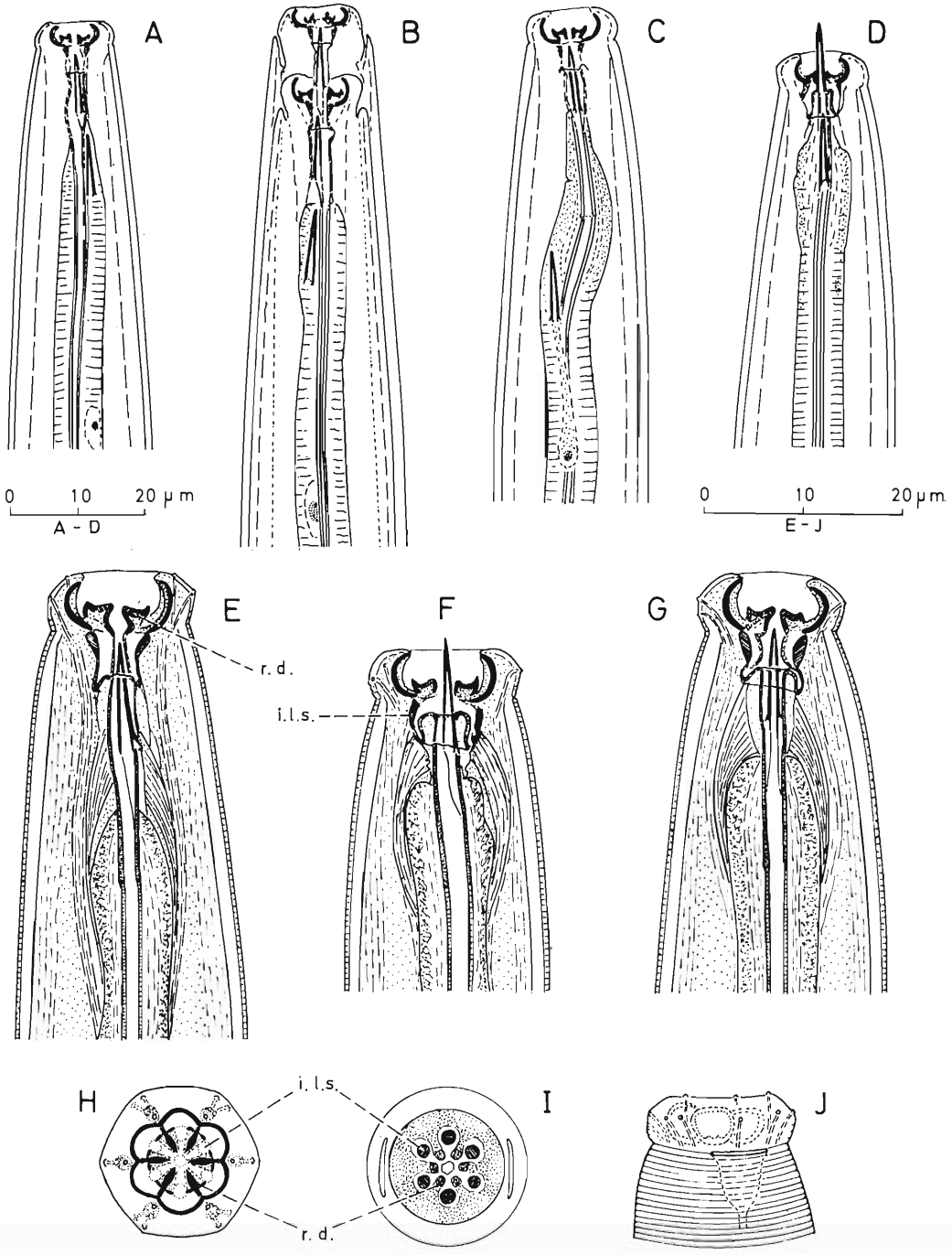


Table 1. Dimensions of juveniles of *Aetholaimus rotundicauda*.

	J_2	J_{2-3}	J_3	J_4	J_4–ad.
L	0.87 mm	0.91 mm	1.06 mm	1.14 mm	1.15 mm
a	34	35	39	47	37
b	3.0	2.9	3.2	3.5	3.5
c	51	49	52	63	55
Lip region width (μm)	12	12.5	13.5	13.5	13.5
Odontostyle-1 (μm)	9	9	10	10	10
Odontostyle-2 (μm)	9.5	10	10.5	–	11
Odontostyle-3 (μm)	–	11	–	–	–
Esophagus (μm)	293	313	334	322	333
Anterior part (μm)	139	139	135	138.5	140
Posterior part (μm)	154	174	199	183.5	193
DO (%)	60	56.5	56.8	58	55.8
DN (%)	65.3	62.8	62.5	63.7	61.5
Prerectum (μm)	–	22.5	27	22	23.5
Prerectum/AD	–	1.23	1.45	1.18	1.16
Rectum/AD	–	1.24	1.1	1.18	1.1
Rectum (μm)	–	20.5	20.5	22	22.5
Tail (μm)	17	18.5	20.5	18	21
Tail/AD	0.95	1.1	1.1	0.97	1.04
Gonad primordium (μm)	13.5	43	30.5	62	93
Gonad primordium (%)	49.5	53.5	51.5	47.5	45.6

its apex. Odontophore 11–15 μm long, only slightly longer than the tooth, faintly demarcated from the esophageal lining.

The esophagus is 348–424 μm long and consists of a slender anterior portion, which is narrowest where is passes through the nerve ring; posteriorly it widens gradually to the basal part which occupies 59% (56–61) of total esophagus length. The outlet of the dorsal gland lies 58 μm (49–65) behind the expansion. Dorsal gland nucleus prominent, oval in shape, measuring 6–13 × 4–6.5 μm, with rounded nucleolus 2.5–3.5 μm in diameter; located 21 μm (18–25) behind the outlet of the gland. Ventrosublateral gland nuclei seen only in some juveniles; outlets of first pair 0–3.5 μm, of second pair 0–3 μm apart. Esophago-intestinal junction with three knoblike glandular bodies (one dorsal, two ventrosublateral), the base of which is surrounded by a muscular ring. Lumen of intestine usually narrow in its anterior end, becoming wider posteriorly, separated from prerectum by columnar cells. Prerectum 14–33 μm long or 0.65–1.45× anal body width; rectum 20–32 μm or 0.9–1.3× anal body width.

Female reproductive system didelphic, amphidelphic (only in one apparently abnormal specimen, the posterior branch is reduced to a small sac of 18.5 μm or 1.5% of body length). Each branch is composed of a reflexed ovary, very extensible ovarial sac, distally slender and proximally enlarged oviduct, sphincter, and short, undifferentiated uterus. The slender portion of the oviduct is about half as wide as the enlarged part. The uteri of both branches join above the vagina. Both uteri together measure 29–37 μm (N = 5) or 0.88–1.23× corresponding body width when empty, but uterus is greatly extended when containing an egg. The only intrauterine egg observed measures 112 × 28 μm. Vagina narrow, extending halfway or slightly more into the body. Vulva a transverse slit 16 μm long (N = 1) without sclerotization. Tail dorsally convex-conoid with broadly rounded terminus; its length 18–25 μm or about equal to anal body diameter. Two caudal pores on each side.

Males were not found and the female reproductive system did not contain sperm.

JUVENILES: The five juveniles studied apparently belong to the following stages: J_2; J_{2-3}; J_3; J_4 and J_4–adult. The molting J_{2-3} is near the end of the molting process, hence it can be considered as a J_3 still covered by the J_2 cuticle. The other J_3 specimen has the replacement tooth about 21 μm behind the functional one, whereas this distance is only 2.5 μm in the J_2. The J_4 specimen possesses only an abortive replacement tooth, recognizable as two minute rods some 25 μm from the anterior end. The other J_4 specimen is about to molt; ecdysis has not yet started, but the replacement tooth has already moved forward. In the molting and premolt specimens two esophageal nuclei were observed at 90.4% of neck length, more than 7% behind S_1O, 4.3% anterior to S_2O, so they probably

represent the S_2N. Tail broadly rounded in all juveniles.

Discussion

The above description shows that this species belongs to the Nygolaimidae: it has a mural tooth, cardiac glands, sinuous body attitude after fixation. The structure of the stoma, as well as the location of DO and DN, is identical to those of *Aetholaimus bucculentus*. Hence *Dorylaimus rotundicauda* is herewith transferred to *Aetholaimus*, becoming *Aetholaimus rotundicauda* (de Man, 1880) nov. comb.

There are two further reports of *Carcharolaimus rotundicauda* in the literature. Tulaganov (1949) mentioned the species from Uzbekistan; he did not describe his specimens, but merely repeated de Man's description, so that the identity of his specimens remains uncertain. Andrássy (1963) reported it from Argentina, but his description and illustrations clearly show that he had a true *Carcharolaimus* before him. Thus *Aetholaimus rotundicauda* is known with certainty only from the Netherlands and from Switzerland.

In 1974 Thorne described *Ae. gracilis* from the U.S.A. He gave dimensions of one specimen only. The species seems very close to *Ae. rotundicauda*, from which it may differ by equatorial vulva and slightly shorter esophagus, but variability is unknown. The tail appears to be slightly different in shape.

The four species of *Aetholaimus* may be distinguished as follows.

1. Cheilorhabdia with sclerotized knobs; vulva equatorial to post-equatorial; lip region 17 μm wide; radial sclerotizations heavy ..... *indicus*
   Cheilorhabdia without sclerotized knobs; vulva equatorial to pre-equatorial 2
2. Tail clavate; body slender (a = 43–60); lip region 10–11 μm wide; radial sclerotizations of cheilostome fine ..... *bucculentus*
   Tail not clavate; body less slender (a = 36–44); lip region 14–16 μm wide; radial sclerotizations well developed 3
3. Vulva pre-equatorial; b = 2.9–4.1; tail asymmetrical ..... *rotundicauda*
   Vulva equatorial; b = 4.3; tail symmetrical ..... *gracilis*

Résumé

Observations sur la sousfamille Aetholaiminae Jairajpuri, 1965 (Nygolaimidae)

Mylodiscus nanus Thorne, 1939 est décrit de nouveau d'après des exemplaires récoltés dans l'état de Bahia au Brésil. Possédant un odontostyle axial, il ne peut pas appartenir, tel que *Aetholaimus* Williams, 1962, au Nygolaimidae, sousfamille Aetholaiminae. L'expansion oesophagienne abrupte, la position de l'orifice de la glande dorsale ainsi que la présence d'organes latéraux indiquent qu'il appartient à la famille des Discolaimidae.

Dorylaimus rotundicauda de Man, 1880, couramment placé sous *Carcharolaimus* Thorne, 1939, est décrit de nouveau d'après des exemplaires récoltés aux Pays-Bas et en Suisse. La présence d'une dent murale et de trois "glandes cardiales" démontre qu'il appartient au Nygolaimidae et l'espèce est transférée au genre *Aetholaimus*. Ce genre contient actuellement quatre espèces et est révisé brièvement.

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Thelastoma endoscolicum sp. n. (Oxyurida: Nematoda) a Parasite of Earthworms (Oligochaeta: Annelida)

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ABSTRACT: *Thelastoma endoscolicum* sp. n. (Oxyurida: Nematoda) is described from the intestine of the earthworm, *Eudrilus eugeniae* Kinberg (Oligochaeta: Annelida) in Africa. The new species can be separated from existing members of the genus *Thelastoma* by the position of the excretory pore, absence of lateral alae in the male, the nonoffset lip cone and female tail spike, tail length and relatively large size of the eggs. This is apparently the first example of a representative of the Oxyurida occurring in a member of the Annelida. Considering the evolutionary primitive status of the annelids and nonspecialized characters of *T. endoscolicum*, it is suggested that this nematode is one of the most primitive members of the family Thelastomatidae and one of the earliest known animal parasitic nematodes.

During an investigation of the nematodes associated with African earthworms, a member of the genus *Thelastoma* was discovered living in the intestine of *Eudrilus eugeniae* Kinberg. This apparently is the first time an annelid has been found serving as host to an oxyurid nematode. The invertebrate parasitic members of this order normally occur in the intestinal tract of insects and millipedes.

The nematodes found in the intestine of *E. eugeniae* were studied and found to be undescribed. The present paper describes this species, compares its morphology with other members of the genus, and discusses its evolutionary position.

Materials and Methods

Specimens of *Eudrilus eugeniae* Kinberg collected from a ditch outside the laboratory at Bouaké, Ivory Coast, West Africa were maintained in a container of soil and periodically dissected in a 1.0% NaCl solution. Nematodes removed from the intestine were heat-killed, fixed in TAF (triethanolamine, formalin, and water) and processed to glycerin.

Results

Nematodes removed from the intestine of *E. eugeniae* were found to be a new species in the genus *Thelastoma* and are described

below. In the quantitative portion of the description, the first figure after the character represents the average value for that character, while the numbers in parenthesis represent the range. All measurements are in microns unless otherwise specified.

Thelastoma endoscolicum sp. n. (Thelastomatidae: Thelastomatoidea: Oxyurida)

ADULTS: Head with eight labial papillae and paired amphids; lips united into a mouth cone which is not set off from the rest of the body; stoma reduced; pharynx lacking median bulb; basal valvated bulb present; vulva located slightly anterior to middle of body; ovaries paired; spicule present; male with four pairs of genital papillae; lateral alae absent.

FEMALE (N = 10) (Figs. 1, 3, 4): Body covered with cuticular annulations approximately 7.5 (6.5–9.1) microns apart; total length 2.39 (2.00–2.54) mm; width near vulva 132 (120–140); lip cone small, not set off from remainder of head; height of lip cone 5.3 (5.0–6.0); width of lip cone 19.6 (18.2–31.0); lip cone bearing eight papillae and two lateral amphids; length of stoma 6.9 (6.5–7.8); width of stoma 7.0 (6.5–7.8); length of pharynx 378 (323–450); distance from tip of head to nerve ring 154 (138–175); distance from tip of head to excretory pore 312 (269–362); percent vulva 41 (30–46) from head; length of vagina 149 (133–170); length of tail 514 (440–640); tail gradually tapering to a fine point; vagina directed anteriorly; amphidelphic; both ovaries directed posteriorly; eggs 77 (71–92) by 50 (46–58); phasmids inconspicuous.

MALE (N = 4) (Figs. 4, 5, 6): Total length 620 (560–680); greatest width 41 (39–49); length stoma 3.0 (2.6–3.9); width stoma 2.6; length of pharynx 131 (124–149); distance from tip of head to nerve ring 76 (71–87); distance from tip of head to excretory pore 162 (150–171); length of genital cone, 9.3 (6.2–12.4); length single spicule 27 (26–29); testis single, reflexed; tail with four pairs of papillae, three pairs on genital cone and one pair located further down on the tail; length of tail 68 (65–74); length of tail spine 67 (65–74); distance from base of spine to fourth pair of papillae 22 (21–34); distance from fourth pair of papillae to tip of tail 43 (37–55); lateral alae absent; phasmids inconspicuous.

TYPE HOST: *Eudrilus eugeniae* Kinberg (Eudrilidae: Oligochaeta) (intestine).

TYPE LOCALITY: Bouaké, Ivory Coast, West Africa.

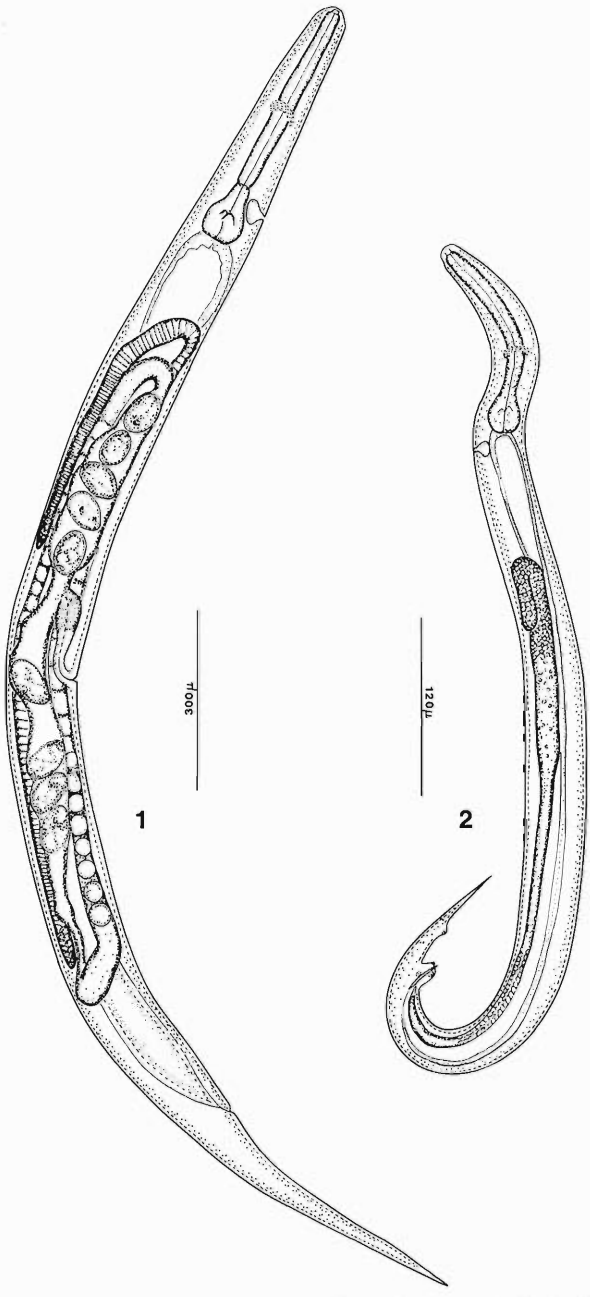
TYPE SPECIMENS: Deposited in the Nematology Collection at the Division of Nematology, University of California, Davis, California.

DIAGNOSIS: There are approximately 40 species in the genus *Thelastoma*. This genus has been placed in the subfamily Thelastomatinae, family Thelastomatidae, and superfamily Thelastomatoidea by Skrjabin et al. (1966). Diagnostic generic characters for *Thelastoma* are two ovaries, vulva in middle portion of body, male tail longer than anal body width and the absence of metarhabdial teeth, culticular alae in the female, cuticular scales and egg filaments. *Thelastoma endoscolicum* can be separated from most other species in the genus by the slightly anterior position of the vulva, the lip cone and female tail spike not offset from the rest of the body, the length of the tail in both sexes, the absence of lateral alae in the male, the relatively large size of the eggs, and the anterior position of the excretory pore in the female. The descrepancy in the position of the excretory pore in male and female specimens is commonly found in this group.

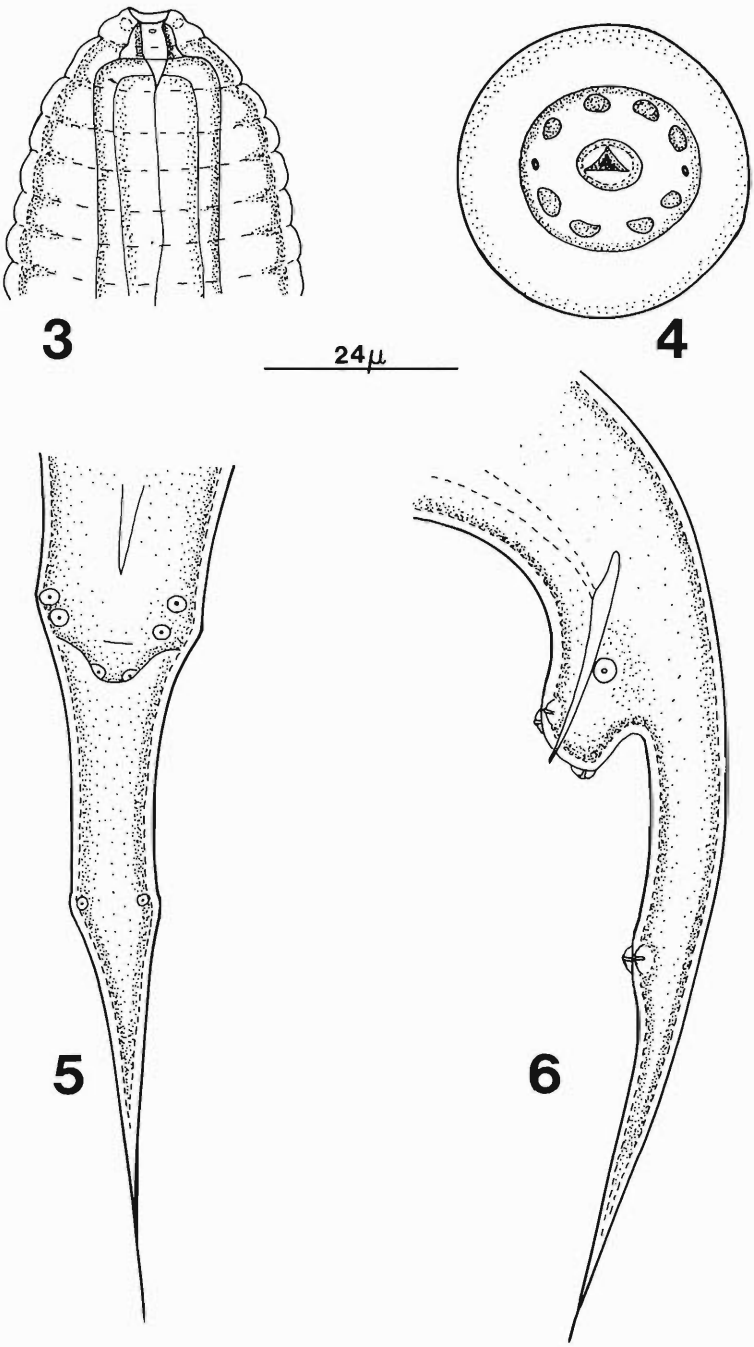
The species of *Thelastoma* most similar to *T. endoscolicum* are *T. palmettum* Chitwood and Chitwood (1933), which possesses a wide bowl-shaped stoma not characteristic of the present species; *T. platyrhaci* (Parona, 1896), which lacks a genital cone and the female possesses a distinct isthmus portion to the pharynx; and *T. rovinjense* Leibersperger (1960), whose genital papillae differ from those of *T. endoscolicum* by having the second pair adjacent to the anal opening and the third pair fused.

BIOLOGICAL OBSERVATIONS: Out of a sample of 10 specimens of *E. eugeniae*, five harbored specimens of *T. endoscolicum* in their intestines. Up to five nematodes were recovered from a single earthworm.

The nematode life cycle is probably similar to that of other oxyurid parasites of invertebrates, i.e., eggs passed out of parasitized hosts



Figures 1, 2. *Thelastoma endoscolicum* sp. n. 1. Lateral view of adult female. 2. Lateral view of adult male.



Figures 3–6. ***Thelastoma endoscolicum*** **sp. n. 3. Lateral view of female head. 4. "En face" view of female. 5. Ventral view of male tail. 6. Lateral view of male tail.**

serve as the infective stage for healthy earthworms. It is surprising that the relatively large eggs are so thin-walled, however. How this would affect their survival in the soil is not known.

Thelostoma endoscolicum shares its location in the intestine of *E. eugeniae* with another nematode belonging to the Drilonematoidea. The description and life cycle of this latter nematode will be presented separately.

Discussion

Chabaud (1974) raised the Oxyurida to ordinal level and Skrjabin et al. (1966) listed four superfamilies whose members occur in arthropods. To the author's knowledge, the present species is the first example of a member of the Oxyurida occurring in a member of the phylum Annelida. All other invertebrate parasitic species have been found in millipedes and insects.

The Oxyurida represent one of the most ancient groups of animal parasitic nematodes, and the invertebrate parasitic members undoubtedly were the first to arise from terrestrial Rhabditida. Inglis (1965) considers the Thelastomatidae as the most primitive member of the Oxyurida. Since the annelids are the most primitive coelomates known (Raymond, 1950), with fossil remains in the lower Cambrian, whereas insects and millipedes represent the higher arthropods that appeared later in the Devonian, it is possible that *T. endoscolicum* represents one of the most primitive members of the Oxyurida and thus one of the earliest known animal parasites. Unfortunately, the separation of the Oxyurida from the Rhabditida seems to be very ancient with no known transitional forms. Primitive and advanced characters are difficult to judge in the Thelastomatidae, whose members show little variation. However, the absence of lateral alae, the separation of the third pair of genital papillae, and the absence of an offset lip cone might be regarded as primitive, nonspecialized characters which are not commonly found in other thelastomatids. This would support the contention that *T. endoscolicum* is a very primitive member of the thelastomatids.

Acknowledgments

The author wishes to thank Dr. Bernard Philippon for providing laboratory space in Bouaké, Ivory Coast, West Africa for this study.

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Mesidionema praecomasculatis gen. et sp. n.; Mesidionematidae fam. n. (Drilonematoidea: Rhabditida), a Nematode Parasite of Earthworms

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ABSTRACT: *Mesidionema praecomasculatis* gen. et sp. n.; Mesidionematidae fam. n. (Drilonematoidea: Rhabditida) is described from earthworms in West Africa. The new family is characterized by the presence of well-developed lips, an oval nonvalvated muscular pharynx, paired ovaries and a single spicule. Morphological and biological characters of the new species places it in an intermediate position between the Drilonematoidea and Thelastomatoidea. On the basis of data collected during this study, it is proposed that the Drilonematoidea are a specialized group which evolved from primitive members of the Thelastomatoidea.

Members of the superfamily Drilonematoidea occur in earthworms and are considered one of the most ancient groups of nematode parasites (Timm, 1964). They possess a number of specialized characters and very little is known of their bionomics or phylogeny.

The present paper describes a member of the Drilonematoidea which occurs in earthworms in West Africa. This nematode has morphological and biological characteristics showing distinct relationships with the Thelastomatoidea and suggests that the Drilonematoidea may have evolved from a primitive member of the Thelastomatoidea.

Materials and Methods

Two hundred specimens of the earthworm, *Eudrilus eugeniae* Kinberg (Eudrilidae: Oligochaeta), were collected in a ditch adjacent to the laboratory in Bouaké, Ivory Coast, West Africa. The above earthworms were maintained in a container of soil and periodically dissected in a 1.0% NaCl solution. Both the coelom and intestine of the earthworms were carefully examined for nematodes. All nematodes collected were first observed alive, then killed in hot 1.0% NaCl solution and processed to glycerin.

Results

Nematodes of the superfamily Drilonematoidea removed from the coelom and intestine of *E. eugeniae* were considered new to science and a description follows below. In the quantitative portion of the description, all measurements are in microns, unless otherwise specified.

Mesidionematidae fam. n. (Drilonematoidea: Rhabditida)

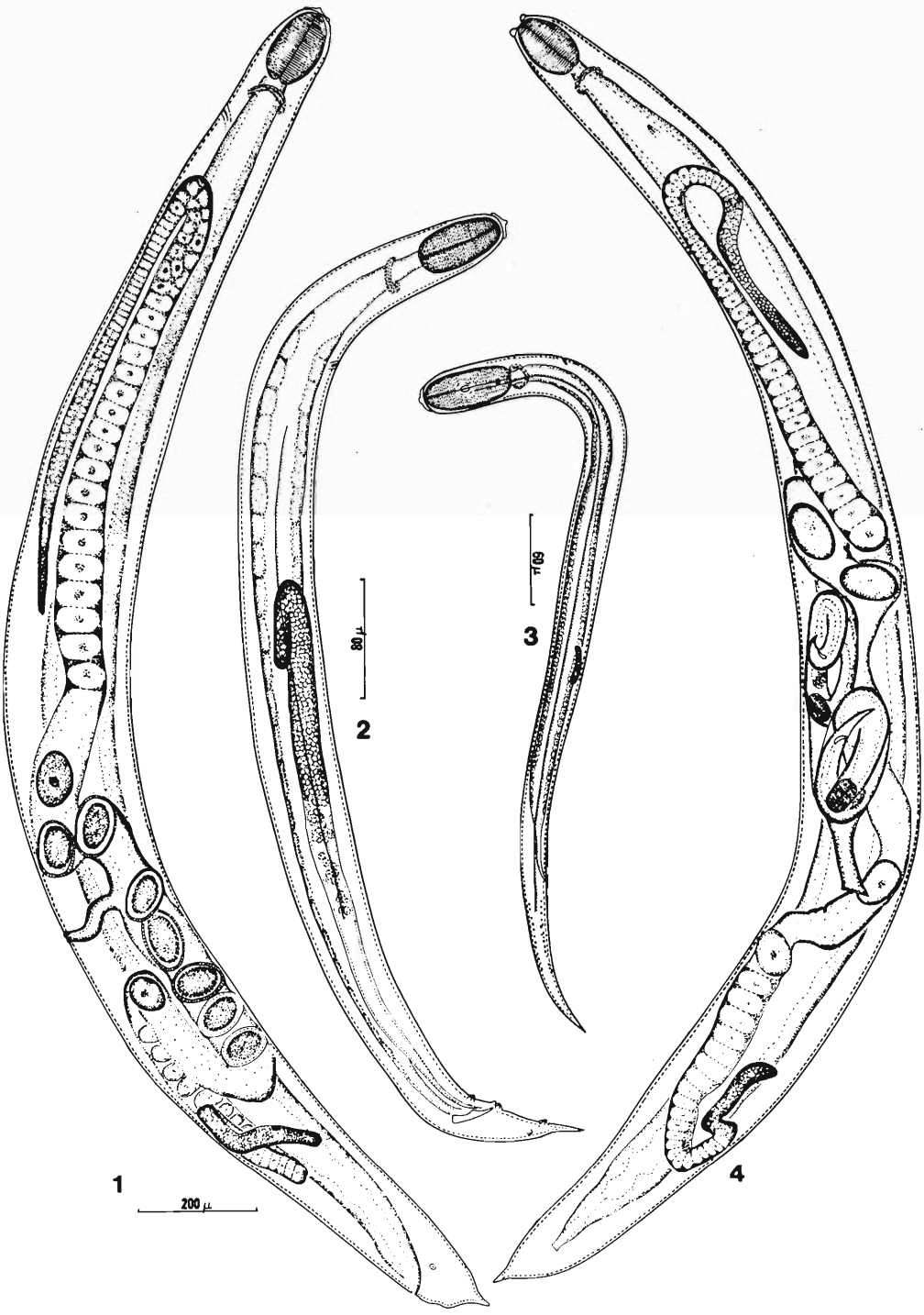
Stoma absent or greatly reduced; mouth hooks absent; lips well developed; pharynx oval, muscular, without corpus, isthmus or valvated bulb; ovaries paired; spicule single; phasmids not enlarged; found in the coelom and intestine of earthworms.

Mesidionema n. gen.

Head bearing two lateral lip regions; each lip region subdivided into three lips, a lateral and two submedial. Amphids slightly raised on tip of lateral lips; stoma absent; pharynx oval, muscular; nerve ring posterior to pharynx; ovaries outstretched or reflexed; phasmids porelike.

Mesidionema praecomasculatis sp. n.

Cuticle smooth, lateral alae present in males and young females; mouth terminal; head bearing two pronounced lateral lip regions, each composed of one lateral and two submedial (one subdorsal and one subventral) partially fused lips. Each submedial lip contains a pair of papillae, one located on the outer edge (representing an outer labial papilla) and one



extended into a filament which is directed inwards toward the mouth of the nematode (representing an inner labial papilla). Each lateral lip contains an inner small papilla (representing part of the inner labial papillary group) and a conspicuous peglike amphid. This results in six inner labial papillae, four outer labial papillae and two amphids. The stoma is absent. The pharynx is oval and muscular, without a valve; it is extremely complex and the anterior portion is capable of opening widely to grasp host tissue. When opened, each of the three sections of the pharynx bear six retrorse, anteriorly placed teeth. Young juvenile females possess ridges lining the walls of the basal portion of the pharynx (pseudovalve) and thickenings in the median portion of the pharynx. These characters, which are best seen in living specimens, are not visible in older specimens. Nerve ring encircles the anterior portion of intestine; excretory pore located posterior to the nerve ring; intestine composed of a single row of cells; vulva slitlike, vagina muscular, ovaries paired, flexed or extended at tip; two types of eggs produced; a thick-walled egg (wall composed of three major layers) which does not develop in the uterus and a larger thin-walled egg that develops "in uteri" into males; anus distinct, functional; tail conical, with a short acute tip; phasmids porelike and located opposite anus in female, inconspicuous in male; testis single, flexed at tip; spicule single; gubernaculum and bursa absent; four pairs of genital papillae present.

FEMALE (N = 10) (Figs. 1, 3, 4, 5, 6, 8, 9): Length, 2,030 (1,150–2,640); width at vulva, 174 (100–220); distance from tip of head to base of pharynx, 111 (85–123); distance from tip of head to nerve ring, 125 (92–146); distance from tip of head to excretory pore, 202 (123–239); percent vulva, 62 (58–65); length of tail, 113 (91–124); dimensions of thick-walled eggs, 55–65 × 85–97; dimensions of thin-walled eggs, 108 × 220. Narrow lateral alae in juvenile and young adult females (2–6) extends from the pharynx to just beyond the anus (Figs. 2, 3).

MALE (N = 10) (Figs. 2, 7, 10): Length, 696 (620–800); greatest width, 45 (39–64); distance from tip of head to base of pharynx, 50 (40–59); distance from tip of head to nerve ring, 73 (62–84); distance from tip of head to excretory pore, 109 (99–153); length of tail, 64 (59–74); length of spicule, 27 (22–33); reflexion of testis, 48 (25–93); diameter of spherical sperm cells, 4; lateral alae narrow (about 6 μ wide), extending from the excretory pore about to the middle of the vas deferens; tail with four pairs of anal papillae, one pair preanal, and three pairs postanal.

TYPE SPECIMENS: Holotype and allotype deposited in the collection of the Nematology Department, University of California, Davis, California.

TYPE HOST: *Eudrilus eugeniae* Kinberg (Eudrilidae: Oligochaeta).

TYPE HABITAT: Coelom and intestine.

TYPE LOCALITY: Bouaké, Ivory Coast, West Africa.

DIAGNOSIS: The present nematode shares characters found in both the Drilonematoidea and Thelastomatoidea and is unique among both superfamilies in having a simple, oval nonvalvated pharynx and six lips separated into paired lateral lip regions.

The present species is very similar to *Pharyngonema pheretimae* Timm (1959); however, the original description of *Pharyngonema* Pierantoni (1923) is very different from *P. pheretimae* Timm and the present species. Pierantoni (1923) described the genus *Pharyngonema* as possessing a swollen region in the anterior portion of the body, bristlelike papillae and a single ovary. The description is very brief, but the above characters clearly show that the *Pharyngonema* of Pierantoni is different from *M. praecomasculatus* and *P. pheretimae* Timm.

The nominal species *P. pheretimae* Timm agrees with all characters of the new genus *Mesidionema* and is hereby transferred to this

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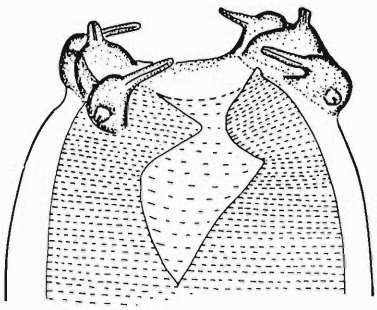
Figures 1–4. ***Mesidionema praecomasculatis*** **gen. et sp. n. 1. Lateral view of a mature female with thick-walled eggs. 2. Lateral view of a male. 3. Lateral view of a newly hatched female. 4. Ventral view of a mature female with thin-walled eggs (same mag. as Fig. 1).**



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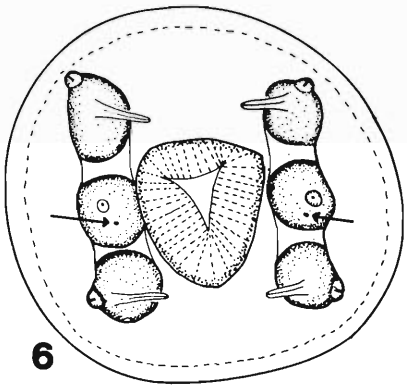

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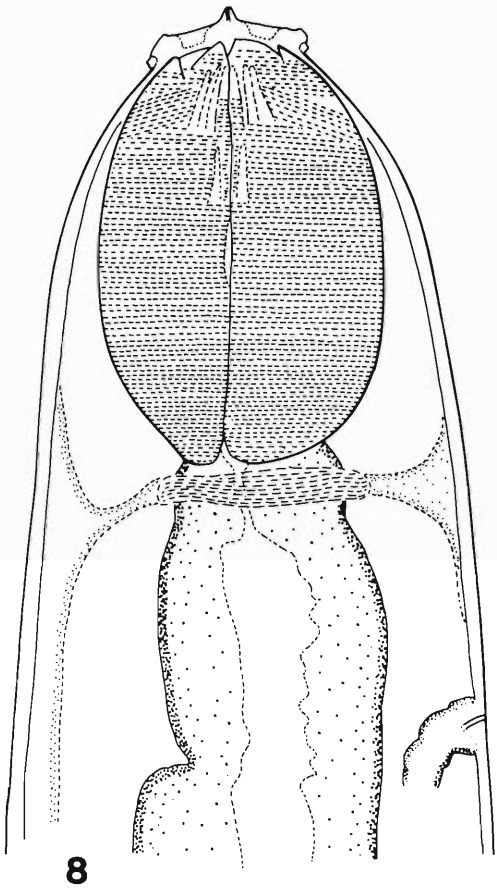

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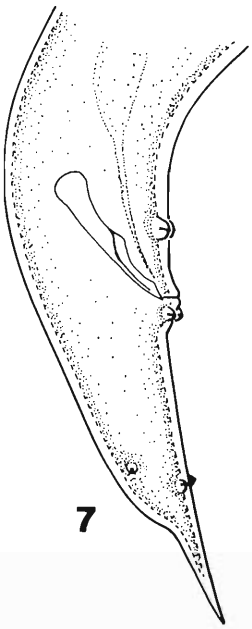

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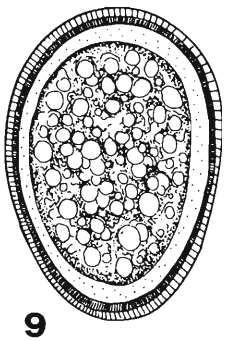

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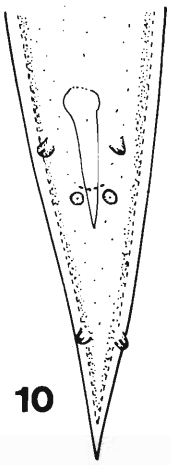

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genus. It can be easily separated from *M. praecomasculatis* by the shape of the pharynx (panduriform rather than oval) and tail (significantly longer). The lips of *M. pheretimae* as figured by Timm are withdrawn into the oral cavity, which also occurred during fixation in about half of the specimens of *M. praecomasculatis*. Living specimens of *M. pheretimae* probably would have shown a lip arrangement similar to that of *M. praecomasculatis*. Timm (1959) did not possess mature males or young juveniles of *M. pheretimae*.

BIOLOGICAL NOTES: Approximately 50% of all earthworms examined contained one or more specimens of *M. praecomasculatis*. Adult nematodes of both sexes as well as juvenile females were collected from the coelom of the host, whereas only adult and very young juvenile females were found in the earthworm's intestine. In the coelom, the nematodes were either moving freely or were anchored by their pharynx to various tissues of the host. The nematodes probably ingest material released from the damaged cells. Most of the females found in the body cavity contained thin-walled eggs in various stages of development. Some contained both thick-walled and thin-walled eggs. In contrast, nearly all the females found in the earthworm's intestine contained thick-walled eggs.

Males of *M. praecomasculatis* develop to maturity inside the thin-walled eggs in the uterus of the coelomic females. No juvenile males were collected in the soil or intestine of the host during this investigation. However, various stages of juvenile females were collected from the coelom of the earthworm. Young juvenile females were very active and thrashed about similar to free-living rhabditids. Soil containing infected earthworms was analyzed for nematodes but no free-living motile stages were found. From the above observations, a probable life cycle can be surmised. Female nematodes containing thick-walled eggs enter the intestine of the host and oviposit. The eggs pass out of the earthworm, develop in the soil, and serve as the infective stage. The eggs hatch after being ingested by another earthworm, and the emerging juvenile females immediately enter the host's coelom. In this location, they grow and produce thin-walled eggs (either by parthenogenesis or hermaphroditism) that develop into males "in uteri." The males enter the host's coelom and mate (this is supposition since mating was never observed, but sperm identical to that found in the male occurred in the female oviducts), and this union produces thick-walled eggs. After producing a number of thick-walled eggs, the female again passes through the intestinal wall into the intestinal lumen for oviposition.

Discussion

It is now clear that *M. praecomasculatis* possesses characters of both the Drilonematoidea and Thelastomatoidea. With the former superfamily it shares a valveless pharynx, greatly reduced stoma and association with earthworms (coelom). With the latter superfamily, it shares paired ovaries, a single spicule, four pairs of genital papillae, lateral alae, and its association in the intestine (at least during part of its life) of the host.

Finding a "pseudovalve" in the basal portion of the pharynx of newly hatched juvenile females of *M. praecomasculatis* indicates that the nonvalvated pharynx characteristic of the adults is a secondary character. It is known that other rhabditids which have become parasitic and changed to a semiliquid diet (e.g., *Heterorhabditis, Neoaplectana*) have lost a distinct valve in the basal bulb which is so characteristic of microphagous forms. Since the Drilonematoidea probably obtain most, if not all, of their nourishment (semiliquid) from the coelom of earthworms, the basal valve so typical of the Thelastomatoidea has become vestigial.

The discovery that adult females of *M. praecomasculatis* enter the intestine of the

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Figures 5–10. ***Mesidionema praecomasculatis*** **gen. et sp. n. 5. Ventral view of female head 6. En-face view of female (arrows indicate lateral inner labial papillae) (same mag. as Fig. 5). 7. Lateral view of male tail (same mag. as Fig. 5). 8. Lateral view of female head region. 9. Thick-walled egg. 10. Ventral view of male tail (same mag. as Fig. 5).**

earthworm (presumably to deposit their eggs) shows a close biological tie with the Thelastomatoidea. It is interesting that a member of the genus *Thelastoma* also occurred in the intestine of *Eudrilus eugeniae*, along with *M. praecomasculatis*, showing for the first time that earthworms also serve as hosts for thelastomatoid nematodes. Primitive thelastomatoids living in the gut of earthworms could have entered the coelom and evolved forms which adapted to this niche. Once in this relatively unexploited habitat, further development could proceed rapidly, although certain specialized characters found in drilonematoids (e.g., mouth hooks, enlarged amphids and phasmids) may indicate a polyphyletic origin for this superfamily, whose members only share a valveless pharynx and common host group. The oval, muscular pharynx of *Mesidionema* is undoubtedly a further specialized adaptation for attachment to host tissue. On the basis of this study, the author proposes that the Drilonematoidea are a specialized group which evolved (at least in part) from primitive Thelastomatoidea. Many of the specialized morphological characters of the Drilonematoidea could have been formed after this group split off from the thelastomatoids. Both the Thelastomatoidea and Drilonematoidea undoubtedly arose relatively early in the evolutionary scale.

Acknowledgments

The author is grateful to Dr. Bernard Philippon for providing space in his laboratory in Bouaké, Ivory Coast, West Africa for undertaking the present study.

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ANNOUNCEMENT: HONORARY MEMBER

At the 509th Meeting of the Society Horace W. Stunkard was elected to Honorary Membership.

Notes on Two Species of *Filaroides* (Nematoda: Filaroididae) from Carnivores in Texas

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ABSTRACT: The pathology and host–parasite relationships of two species of the genus *Filaroides* from west Texas carnivores are discussed. These are *Filaroides milksi* Whitlock, 1956 from the lungs of the hog-nosed skunk, *Conepatus mesoleucus*, and *Filaroides osleri* (Cobbold, 1879) Skrjabin, 1933 from the trachea and bronchi of the coyote, *Canis latrans*. The lungs of the hog-nosed skunk infected with *F. milksi* were extremely congested, and there was a severe interstitial pneumonia surrounding numerous granulomatous foci containing adult and larval nematodes. This species is briefly redescribed from the hog-nosed skunk, which represents a new host record. *Filaroides mephitis* Webster, 1966 is considered a synonym of *F. milksi*. Also, the validity of *Filaroides hirthi* Georgi and and Anderson, 1975 is suspect. *Filaroides osleri* in the coyote presented as a mild to severe verminous bronchitis. Infections ranged from a small pinpoint nodule at the tracheal bifurcation to many large nodular lesions extending from the proximal ⅓ of the trachea into the anterior bronchi. Cellular reactions consisting of epithelioid cells, histiocytes, and a few eosinophils surrounded the entwined nematodes. A mild interstitial pneumonia and, in one instance, small parenchymal granulomas surrounding immature worms were observed in the lungs of some infected animals.

Studies on the helminth fauna of west Texas carnivorous mammals revealed infections in the coyote, *Canis latrans*, and hog-nosed skunk, *Conepatus mesoleucus*, with *Filaroides osleri* (Cobbold, 1879) Skrjabin, 1933 and *Filaroides milksi* Whitlock, 1956, respectively. The pathology, host–parasite relationships, and taxonomy of these species from their respective hosts are discussed.

Materials and Methods

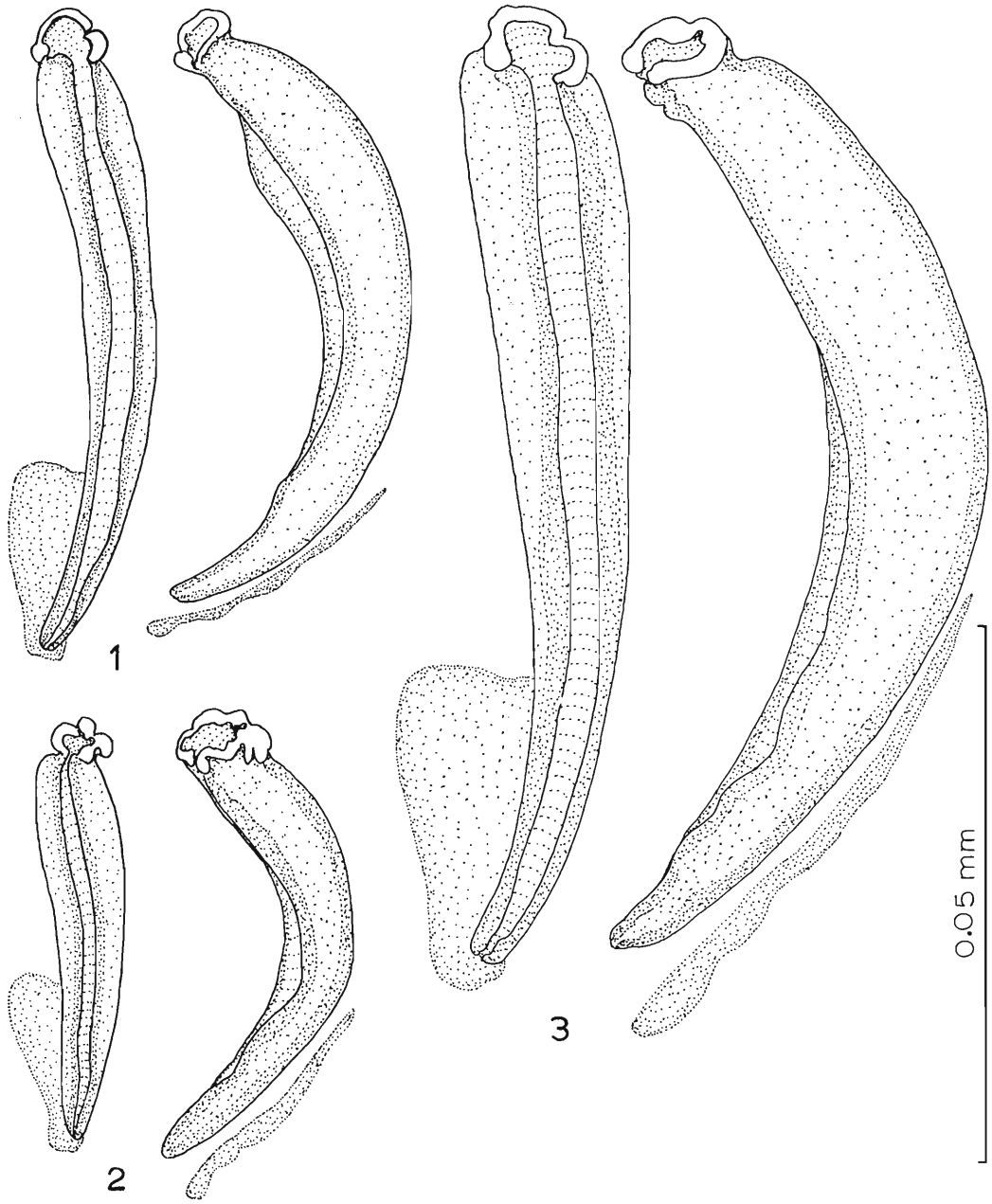
Animals were routinely necropsied; nematodes removed and fixed in glacial acetic acid, and preserved in a mixture of 70% ethyl alcohol and 5% glycerine by volume. One cm square portions of lung parenchyma and small pieces of trachea from infected hosts were fixed in 10% buffered formalin. Sections were cut at 4–6 μ and stained with hematoxylin and eosin or Giemsa. In the following description all measurements are in microns unless otherwise indicated. Drawings were made with the aid of a Leitz drawing tube.

Filaroides milksi Whitlock, 1956

NEW SYNONYMY: *Filaroides mephitis* Webster, 1966 (Can. J. Zool. 45: 145).

DESCRIPTION (based on fragments of numerous ♂♂ and ♀♀ specimens): Filaroididae Schultz, 1951; *Filaroides* Van Beneden, 1858: long, slender, filiform nematodes. Cuticle smooth, without striations, very delicate, often detached from hypodermis. Buccal capsule very shallow, indistinct, not sclerotized. Four large papillae in outer circle, four smaller papillae in inner circle, amphids lateral. Esophagus muscular, simple. Excretory pore slightly posteriad to nerve ring. Posterior extremity of female with blunt tail, vulva close to anus without conspicuous vulvar inflation. Vagina muscular. Uteri paired, ovoviviparous, with hatched larvae. Male with very rudimentary bursa bearing two pairs large pedunculate, postanal papillae. Spicules similar, subequal, scimitar-shaped, without transverse striations, blunt-tipped (Fig. 1). Gubernaculum lightly sclerotized, ⅓ length of spicules. Eggs thin-shelled, with well-developed larvae.

FEMALE (based on 10 specimens): One fragmented female 10.6 mm long, remaining specimens broken, total length undetermined. 82–94 (89) wide (maximum). Esophagus 122–140 (133) long. Nerve ring and excretory pore 65–88 (74) and 94–129 (109) from anterior extremity, respectively. Vulva and anus 76–82 (77) and 20–26 (23) from posterior extremity. Larvae 234–280 (254) long.



Figures 1–3. Lateral and ventral views of spicule and gubernaculum from three described *Filaroides* species. 1. *Filaroides milksi* from *Conepatus mesoleucus* in Texas. 2. *F. hirthi* Georgi and Anderson, 1975 from beagle dog (Paratype, USNM Helm. Coll. No. 79243). 3. *F. mephitis* Webster, 1966 from *Mephitis mephitis* in Canada (USNM Helm. Coll. No. 61679).

MALE (based on 10 specimens): One broken male 7.5 mm long, remainder of specimens fragmented, total length undetermined. 70–85 (79) wide (maximum). Esophagus 140–180 (153) long. Nerve ring and excretory pore 70–76 (73) and 94–123 (107) from anterior extremity, respectively. Cloacal opening 15–18 (17) from posterior extremity. Spicules 54–77 (65) long. Gubernaculum 18–28 (23) long.

HOSTS: *Conepatus mesoleucus*, hog-nosed skunk. One animal infected of six examined.

LOCATION: Small bronchioles and parenchyma of lungs.

LOCALITY: Six mi N, 16 mi W of Eden, Concho Co., Texas. Collected 11 November 1975 by M. Baird.

DISPOSITION OF SPECIMENS: The Museum of Texas Tech University, Department of Medical Zoology, No. 1001 to 1025.

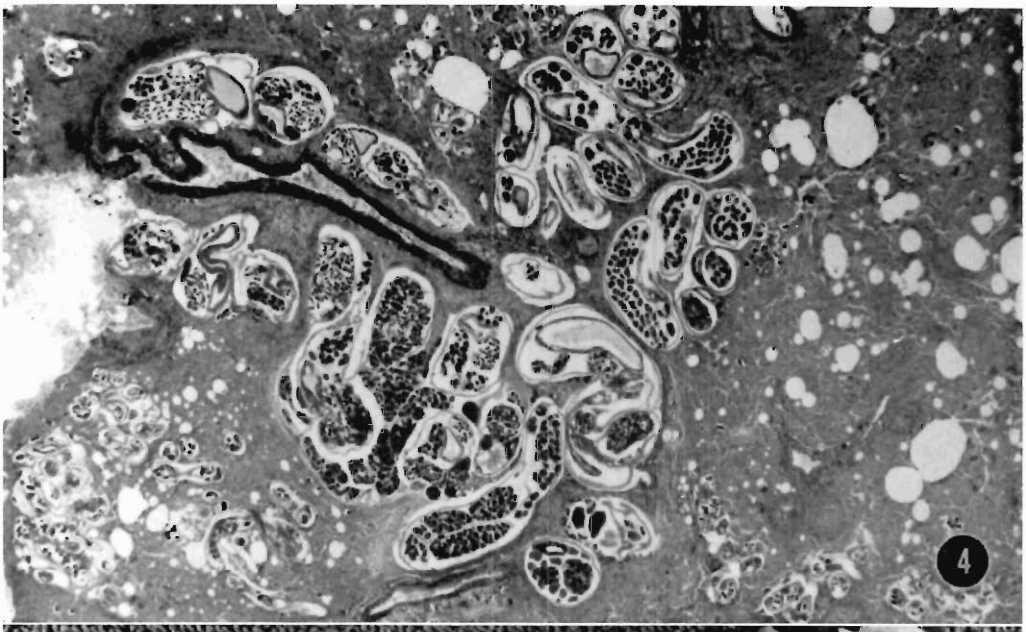
PATHOLOGY: On necropsy, lungs were edematus, congested, not collapsed, and a mild pleuritis was evident. Hard, yellowish subpleural foci were evident in all lobes of the lungs. Blood and edema oozed from cut sections.

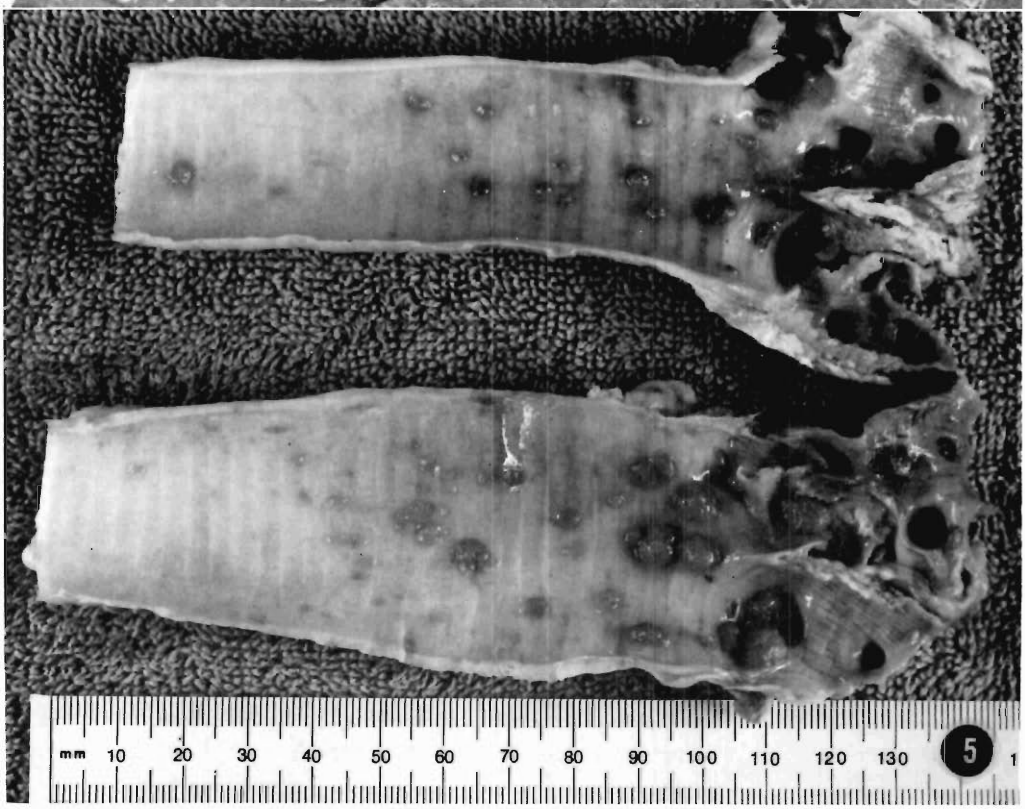
Histologically, the entire parenchyma on all sections was extremely congested, edematous, and there was a severe interstitial pneumonia surrounding numerous granulomatous foci containing nematodes (Fig. 4). There was a perivasculitis of arterioles and veins and a perilymphangitis adjacent to these granulomas. This consisted predominately of lymphocytes and a few eosinophils. Adult and larval nematodes were predominantly localized in alveolar spaces and septa. Occasionally, they were found in terminal and secondary bronchioles. The granulomatous reaction consisted of epithelioid cells, plasma cells, lymphocytes, eosinophils, fibroblasts, and a few foreign body giant cells. There was only mild fibroplasia surrounding masses of *F. milksi*. Often this was absent in the vicinity of lesions with single or only a few nematodes. Terminal and secondary bronchioles presented with an eosinophilic peribronchiolitis, a bronchiolar exudate of epithelioid cells and lymphocytes, and hyperplasia of smooth muscle fibers. Nematodes in varying stages of maturity were observed ranging from gravid females to larvae. Larvae were most frequently observed in the parenchyma, but occasionally found in bronchioles. There was little or no cellular reaction associated directly with larvae.

Remarks

Filaroides milksi has been previously reported from dogs in New York (Whitlock, 1956; Judd, 1960), Iowa (Peckham et al., 1960), Connecticut (Mills and Nieldsen, 1966), and Canada (Greenway and Stockdale, 1970), and the striped skunk, *Mephitis mephitis*, from Iowa (Levine et al., 1965). *Filaroides mephitis* was described as a distinct species by Webster (1966) from *Mephitis mephitis* in eastern Canada. Dyer (1970) also reported this species from the same host in North Dakota. This species was differentiated on the basis of larger size of the male (up to 24 mm versus 3.4–4.4 mm for *F. milksi*), larger spicules (48–60 in *F. milksi* versus 85.5–89.3 in *F. mephitis*), and presence of a prominent vulvar inflation in *F. milksi*. Specimens from the hog-nosed skunk in the present study appear intermediate in size (a single broken male measuring 7.5 mm), spicules 59–78 long, and females with only a slight to no inflation in the vulvar region. Examination of the type specimens of *F. mephitis* (USNM Helm. Coll. No. 61679 and 61680) revealed no morphological differences by which these species could be distinguished. Spicules of *F. mephitis*, although larger, appeared identical to those recovered from the hog-nosed skunk (Figs. 1, 3). Therefore, *Filaroides mephitis* Webster, 1966 is considered a synonym of *F. milksi*.

Examination of type specimens of *Filaroides hirthi* Georgi and Anderson, 1975 revealed few morphological differences between specimens collected in the present study and this species. This species supposedly differed from *F. milksi* by (1) smaller size and (2) slightly stouter spicules with broader knobs for attachment of retractor muscles. Paratypes of *F. hirthi* (USNM Helm. Coll. No. 72943 and 72944) appear to be immature specimens. The spicules of the species reported as *F. hirthi* appear very similar to those observed herein from the hog-nosed skunk and those reported as *F. mephitis* by Webster (1966) (Figs. 1–3). Additionally, Hirth and Hottendorf (1973) in the original description of the pathology of this nematode stated the male was up to 6.0





mm long and 90 wide, while the female approached 10.0 mm long and 185 wide. This information was not included in the new species description of *F. hirthi* by Georgi and Anderson (1975) who described living specimens as 2.3–3.2 mm long and 35–43 wide in the male and 6.6–13.0 mm long and 58–102 wide in the female. Thus, both the spicules and range of measurements are probably within the range of intraspecific variation of *F. milksi*.

Filaroides milksi is differentiated from *F. martis* (Werner, 1782) Dougherty, 1943 and *F. osleri* principally by the smaller size of the spicules which are 46–89 as compared to 174–183 and 99–133 in the latter two species, respectively (Levine et al., 1976). Additionally, the spicules of *F. osleri* have prominent transverse ridges, the tail of the female is rounded and the anus and vulva are very close. *Filaroides milksi* differs from *F. canadensis* Anderson, 1963 in that the distal ends of the spicules are not split and the gubernaculum is considerably larger (Anderson, 1963).

Filaroides osleri (Cobbold, 1879) Skrjabin, 1933

HOST RECORD: *Canis latrans*, coyote. Twenty four animals infected of 94 examined.

LOCALITY: Benjamin, Knox Co., Texas; Ross and Adams Ranches, King Co., Texas; Beggs and Pitchford Ranches, Dickens Co., Texas. Collected by the author from September 1973 to November 1976.

LOCATION: Trachea and bronchi forming nodular lesions under tracheal epithelium.

DISPOSITION OF SPECIMENS: The Museum of Texas Tech University, Department of Medical Zoology, No. 1594–1673.

PATHOLOGY: Animals less than 1 year of age were rarely infected (3/24) while the majority of cases were noted in animals over 2 years of age (15/24). The extent of pathological manifestations in the 24 infected coyotes varied considerably. Usually the anterior ⅔ of the trachea was not involved. The middle one third occasionally had a few nodules measuring 1–3 mm in diameter. In the posterior one third of the trachea at the bifurcation of the bronchi and sometimes into the bronchi themselves, the lesions became larger and more numerous. Grossly, the lesions appeared as white to pink polypoid or sessile nodules just under the mucosa, 1–12 mm in diameter (Fig. 5). Often the anterior and posterior extremities of numerous nematodes protruded from the lesion. In more severe infections the majority of the lesions were located in the tracheal bifurcation with the larger adjacent lesions sometimes becoming confluent. They were confined to the ventral and ventrolateral aspects of the trachea and were raised as much as 5 mm above the mucosal surface. In no instance were the lesions so numerous as to occlude the lumen of the trachea or bronchi. The lungs of infected animals showed no signs of gross pathological changes.

Histologically, the size of the nodules corresponded to the number of adult nematodes present in the lesion. Smaller lesions contained only a few nematodes and in some instances only a single worm. Occasionally, the ciliated columnar epithelium of the trachea was continuous over the lesion. Often this appeared to be absent with the surface of the lesion covered by a layer of connective tissue (Fig. 6). In all lesions the host reaction was substantial. Basically, the nodule consisted of masses of entwined adult and larval nematodes surrounded by plasma cells, lymphocytes, collagen, fibroblasts, neocapillaries, eosinophils, and a few histiocytes (Fig. 7). Usually the lesions did not progress beyond the level of the perichondrium. At the base of the nodules the mucous alveoli and tracheal glands were destroyed, and occasionally a few lymphocytes, plasma cells, and eosinophils were seen in tracheal tissue adjacent to the lesions. Histiologic sections of trachea and bronchi occasionally revealed first-stage larvae, and immature adults in the dilated lymphatics of the bronchial and tracheal walls (Fig. 8).

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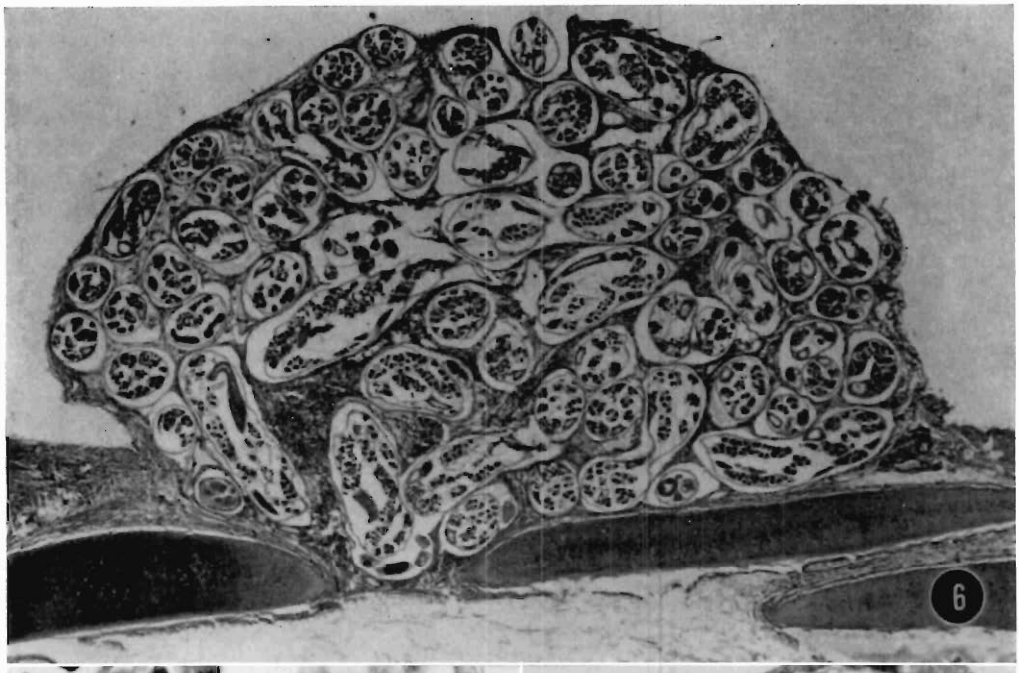
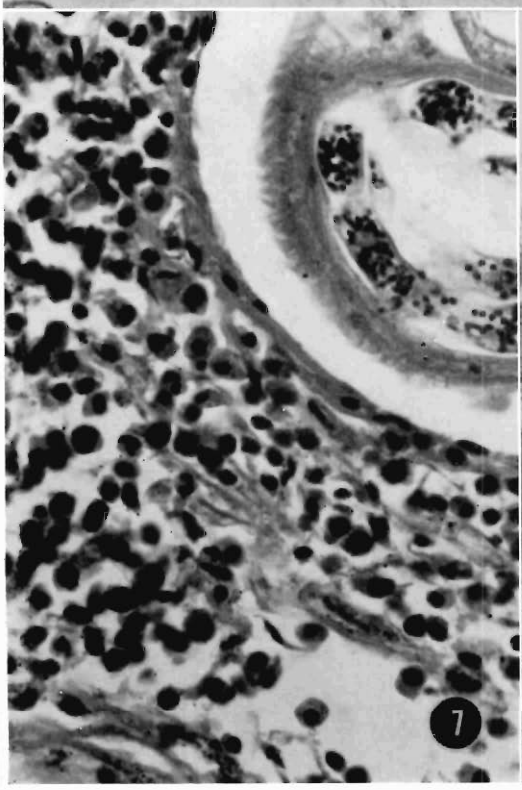
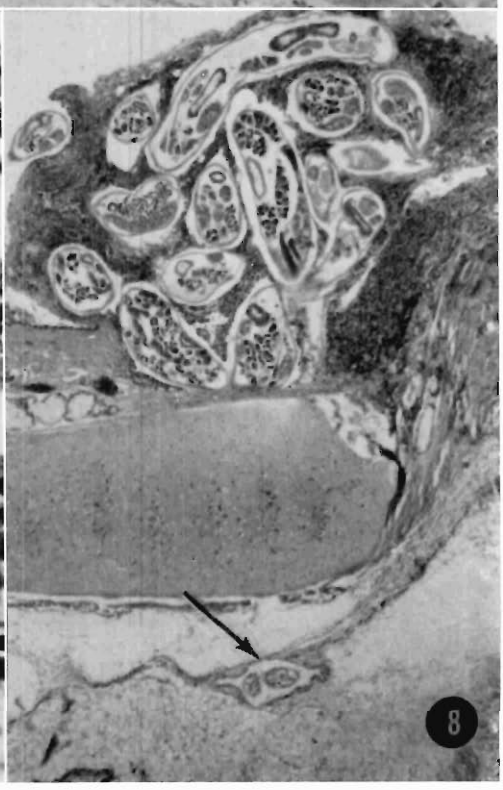
Figures 4–5. Pathology of *Filaroides* species. 4. Histologic section of *F. milksi* in *Conepatus mesoleucus*. Note numerous adult and larval nematodes, peribronchiolar infiltration, hyperplasia of smooth muscle, and interstitial pneumonia. Hematoxylin and eosin. × 26. 5. Gross lesions of *F. osleri* in trachea of *Canis latrans*.







These were sometimes surrounded by lymphocytes, plasma cells, and a few eosinophils. Occasionally, there was a mild hyperplasia of lymph nodes adjacent to these lesions.

The lung parenchyma of infected animals in most instances was normal. Several of the animals had a mild interstitial pneumonia, probably unrelated to the *F. osleri* infection. In the most severe case of *F. osleri* observed in the coyote, there was a moderate degree of insterstitial pneumonia with congestion and edema most severe near the hilus of the lung. A few granulomatous lesions were scattered throughout the parenchyma. These contained larval nematodes, presumably *F. osleri*, usually surrounded by an intense reaction of lymphocytes, plasma cells, eosinophils, and a few histiocytes.

Remarks

Specimens from the coyote collected in this study conform in all respects to previous descriptions of *F. osleri* infections in dogs, coyotes, and wolves. *Filaroides pararostratus* described from nodules in the trachea of the dog in Mexico City by Flores-Barroeta (1955) is probably a synonym of *F. osleri*. Spicules of this species were only 88 long compared to 99–113 for *F. osleri*, as reported by Olsen and Bracken (1959). Specimens collected from the coyote in the present study had spicules measuring 91–115 (mean 94; 30 specimens; 10 worms from 3 animals, respectively). The prominent transverse ridges on the blade of the spicules as noted by Olsen and Bracken (1959) should serve to differentiate this species from others of the genus. Clarification of the status of *F. pararostratus* must await careful comparison with *F. osleri*.

Discussion

The taxonomy, host–parasite relationships, and distribution of the genus *Filaroides* in North American carnivores are poorly understood. Their small size, location, and few good taxonomic characteristics for species differentiation adds to the confusion. Additionally, both the species reported herein demonstrate considerable meristic variability in key characters used in differentiation. Thus, *F. mephitis* Webster is regarded as a synonym of *F. milksi*, while the validity of *F. hirthi* Georgi and Anderson and *F. pararostratus* Flores-Barroeta is in question.

Filaroides milksi is reported from dogs in several areas of southern Canada and the eastern United States; *Mephitis mephitis* in southern Canada, Iowa, and North Dakota; and *Conepatus mesoleucus* in west Texas. Examination of numerous specimens of other skunks from the same locality, including *Spilogale gracilis*, *Mephitis mephitis*, and *Mephitis macroura*, in west Texas failed to reveal infections with this nematode. Additionally, numerous other carnivores examined from the same area were not infected.

Filaroides osleri is apparently cosmopolitan in the dog (Levine, 1968). Coyotes and wolves are reported infected with this species in Alberta (Holmes and Podesta, 1970) and Minnesota (Erickson, 1944). Thornton et al. (1974) previously reported this species from south Texas coyotes.

The lesions produced by *F. milksi* in the hog-nosed skunk appeared similar to those described by Levine et al. (1965) in the eastern striped skunk and by Judd (1960), Peckham et al. (1960), Mills and Nielsen (1966), Greenway and Stockdale (1970), and Hirth and Hottendorf (1973) from the dog. The differences were less granulomatous response surrounding the worms, more severe peribronchiolar infiltration and hyperplasia of smooth muscle, and a more severe interstitial pneumonia with congestion and edema in the parenchyma. Although Babero (1960) noted that *Filaroides* sp. in Louisiana skunks was highly pathogenic, he failed to describe the lesions. Likewise, lesions attributable to *F. mephitis* (= *F. milksi*) were not described by Webster (1966) or Dyer (1970). Judging

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Figures 6–8. Pathology of *Filaroides osleri* in *Canis latrans*. 6. Histological section of *F. osleri* nodule on trachea. Note numerous adult nematodes surrounded by granulation tissue. Hematoxylin and eosin. ×26. 7. Cellular reaction consisting of histiocytes, eosinophils, fibroblasts, plasma cells, and lymphocytes from tracheal nodule. Hematoxylin and eosin. ×320. 8. Section of trachea with small lesion on mucosa containing *F. osleri* and immature adult in lymphatics (arrow). Hematoxylin and eosin. ×26.

from the severe reactions and heavy infections in the hog-nosed skunk in Texas and the case described from the striped skunk in Iowa by Levine et al. (1966), this nematode is highly pathogenic to skunks. The effect of this species on wild skunk populations remains to be determined.

Apparently *F. osleri* is not highly pathogenic to coyotes. Although the lesions are similar to those reported in the dog (Mills and Nielsen, 1966; Mills, 1967; Dorrington, 1968) and dingo (Dunsmore and Burt, 1972), the infection apparently does not reach the intensity nor produce the severe effects as reported in the latter species. In no instance was an infection in the coyote noted in this study or by Thornton et al. (1974) in which there were sufficient nodular lesions to block the trachea or bronchi, thus suffocating the animal. Although a mild interstitial pneumonia was noted in several coyotes, in only one instance was this probably attributable to *F. osleri* infection in the trachea. Histologically, the only difference in lesions from the coyote as compared to those in the dog was the abundance of eosinophils. The effects of this species on the morbidity and mortality of coyote populations is apparently insignificant.

Acknowledgments

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A New Species of *Spirocamallanus* Olsen, 1952 (Nematoda: Camallanidae) from *Trachycorystes insignis* (Steindachner) (Pisces: Doradidae) in Colombia[1]

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ABSTRACT: *Spirocamallanus penneri* sp. n. parasitizing the doradid catfish *Trachycorystes insignis* in the Rio Atrato of Colombia is characterized by 12 to 20 spiral bands in the buccal capsule, equal to subequal but dissimilar spicules 227–317 and 251–325 long, three preanal and three postanal pairs of papillae, and vulva in the anterior half of the body. It most closely resembles species of *Spirocamallanus* parasitizing freshwater fish in South America, but those previously described South American species more closely resemble each other than any resembles S. *penneri* in having small, similar spicules.

Pinto et al. (1975a, b, 1976) and Pinto and Noronha (1972) have recently reexamined species of *Spirocamallanus* [*Procamallanus* (*Spirocammallanus*) of Pinto] endemic to Brazil. They concluded that 13 valid species exist, 12 of which infect freshwater fishes and one infecting a marine fish. One additional species from South America, *S. krameri*, was described by Petter (1974) from a freshwater fish in Guayana. The species described herein is the first report of the genus from Colombia.

Worms were removed from the hosts' intestines and fixed with 10% formalin. They were stored in 90 parts 70% ethanol plus 10 parts glycerine. For study, worms were cleared in glycerine and studied as wet mounts. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube.

Spirocamallanus penneri sp. n. (Figs. 1–7)

DESCRIPTION: Body with region of greatest width slightly anterior to midpoint. Lips lacking. Cephalic papillae in three rings of four, 45° from dorsal-ventral axis; amphids lateral. Mouth dorsoventrally elongated. Cuticle with fine transverse striations 2–4 apart. Buccal capsule striated with between 12 and 20 chitinous spiral bands. Esophagus with anterior muscular portion slightly club-shaped and posterior glandular portion elongated and ending in two bilobed valves. Nerve ring at level of anterior ½–⅓ of muscular esophagus. Excretory pore located posterior to nerve ring opposite posterior ½ of muscular esophagus. Tail with single spine.

MALE (based on three mature specimens): Body 9.4–11.2 mm long by 195–287 wide at junction of muscular and glandular portions of esophagus, increasing posteriorly to between 322–385 at level of greatest width, 28–31 times longer than wide. Buccal capsule 98–116 long by 87–107 wide, striated with 15–20, averaging 17.0 spiral bands; that of two specimens containing 1–3 bifurcating spiral bands. Esophagus 976–1,138 long 9–10% of total body length; muscular portion 419–500 long by 98–115 at widest point, comprising 43–46% of entire esophagus; glandular portion 540–638 long by 92–103 wide. Nerve ring 267–310 from cephalic end, 26–32 in height. Excretory pore located 431–580 from anterior end, 167–270 posterior to nerve ring. Testis moderately sinuous, 900–1,032 from cephalic end in region of glandular esophagus. Spicules dissimilar, equal to subequal in length; left spicule weakly sclerotized, thin, tapering to a point distally, 227–317 long; right spicule thicker, strongly sclerotized, of approximately equal thickness along its length, truncated distally, 251–325 long; spicule ratio 1:1–1.1. Gubernaculum absent. Caudal alae united ventrally, 546–598 long, ending 40–46 from posterior end, supported by six elongated, symmetrical pairs of papillae; precloacal pairs

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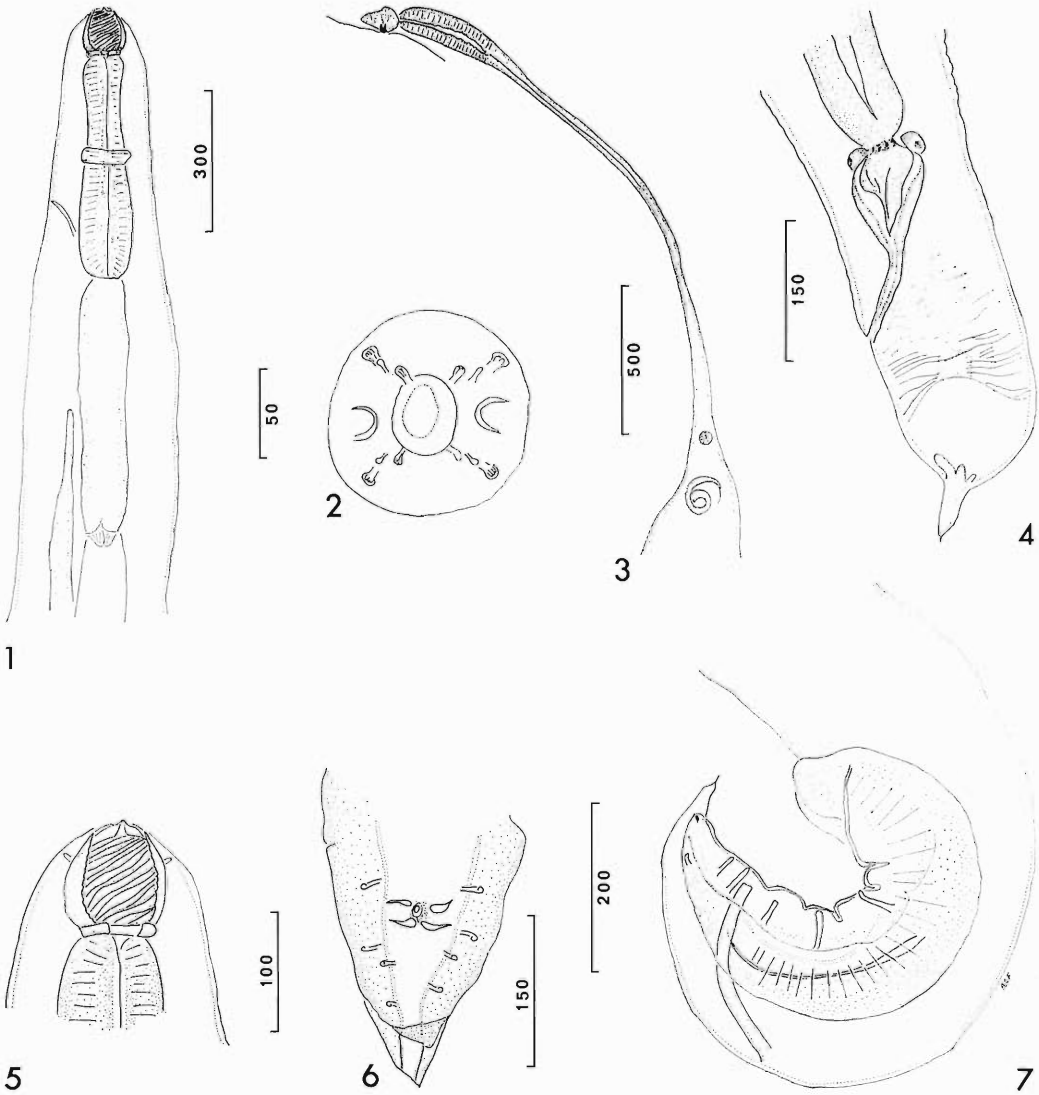

Figures 1–7. ***Spirocamallanus penneri.*** **Measurements are in micrometers. 1. Anterior portion of male holotype, lateral view. 2.** ***En face*** **view, stippled papilla indicates presumed position. 3. Female reproductive system showing vulva, vagina vera, vagina uterina, and a portion of the uterus, drawn from a dissected specimen. 4. Tail of female allotype, lateral view. 5. Cephalic end of male holotype, ventral view. 6. Broken tail region (broken above third pair preanal papillae and missing extreme posterior region) of male paratype to illustrate adanal papillaelike structures not visible in lateral view. 7. Tail region of male holotype, lateral view.**

three, of equal length; postcloacal pairs three, the first two pairs equal in length and located along ventral surface of alae, third pair located laterally; sessile adanal papillae-like structures two pairs at cloacal opening. Phasmids paired at posterior tip of alae. Ventral prominent muscular bands in anal region extending anteriorly from cloaca to slightly posterior to

Table 1. Comparison of selected morphological characters for certain South American species of *Spirocamallanus*.

	S. penneri	*S. barroslimai*	*S. cearensis*	*S. inopinatus*	*S. hilarii*	*S. krameri*	*S. wrighti*	*S. pexatus*
Length of body in mm								
Male	9.4–11.2	3.1	4.06	3.9–8.7	6	7	3.86–4.6	3.72–4.26
Female	11.4–16.2	—	10.34–14.03	11.7–30	14	14–20	8.2–10.6	12.8–20.5
Length × width of buccal capsule								
Male	98–116 × 87–107	30 × 40	41 × 45	90–120 × 40–120	57 × 57	50 × 90		53–59 × 46–59
Female	104–122 × 90–99	—	45–49 × 49–53	90–150 × 85–150	62 × 62	100 × 100		66 × 53–66
Number of spiral bands	12–20	20	18±	15–20	16	16–20	12–20	3–7
Length of spicules	227–317 251–325	60 50	57	80–120	82 62	80 75	45	90–110
Spicules	dissimilar	similar	similar	similar	similar	similar	similar	similar
Spicules	equal–subequal	subequal	equal	equal	subequal	subequal	equal	equal
Preanal papillae	3	3	4	3–4	4	4	4	4
Postanal papillae	3	3	3	6	4	5	4	3
Length of vulva as % of body length from anterior extremity	42–48	—	51–55	36–52	50±	42	37–42	53–64

beginning of alae. Tail flexed ventrally, 194–226 long.

FEMALE (based on 10 mature specimens): Body 11.4–16.2 mm long by 201–348 wide at junction of muscular and glandular portions of esophagus, increasing posteriorly to between 402–684 at level of greatest width, 20–38 times longer than wide. Buccal capsule 104–122 long by 90–99 wide, striated with 12–17, averaging 14.8 spiral bands; that of four specimens containing 1–2 bifurcating spiral bands. Esophagus 1,051–1,248 long, 7–10% of body length; muscular portion 413–494 long by 86–115 at widest point, 37–45% of entire esophagus; glandular portion 580–754 long by 92–116 wide. Nerve ring 270–322 from cephalic end, 40–46 in height. Excretory pore located 402–459 from anterior end, 106–189 posterior to nerve ring. Vulva situated anterior to midpoint, 5.0–7.6 mm, 42–48% of total body length from anterior end. Vagina vera 311–434 long, extending posteriorly from vulva, 49–82 at widest point; vagina uterina 697–1,025 long, 2–4 times longer than vagina vera; uterus J-shaped, ending in blind sac posteriorly; oviduct coiled forming indistinct seminal receptacle; ovary cylindrical, elongated, directed posteriad, usually extending beyond vulva; posterior ovary absent. Tail 212–277 long including finger-like digit; digit 28–45% of tail length. Rectum 154–218 long, surrounded by two rectal glands anteriorly. Larvae 250–372 long by 17–23 wide tapering to a fine pointed tail.

HOST: *Trachycorystes insignis* (Steindachner) (Nematognathii: Doradidae).

SITE OF INFECTION: Intestine.

LOCALITY: Rio Atrato, vic. Quibdo Chocó, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 74583.

ALLOTYPE: USNM Helm. Coll. No. 74584.

PARATYPE: (2 males, 2 females): Coleçao Helmintologica do Instituto Oswaldo Cruz.

ETYMOLOGY: The species is named after Dr. Lawrence R. Penner of the University of Connecticut for his support and encouragement of the first author in pursuing a career in parasitology.

Of the 14 previously reported South American species of *Spirocamallanus* six have between 12 and 20 spiral bands in the buccal capsule and equal or subequal spicules: *S. barroslimai* (Pererira, 1935) Olsen, 1952; *S. cearensis* (Pereira, Dias, and Azevedo, 1936) Olsen, 1952; *S. inopinatus* (Travassos, Atrigas, and Pereira, 1935) Olsen, 1952; *S. hilarii* (Vaz and Pereira, 1934) Olsen, 1952; *S. krameri* Petter, 1974; and *S. wrighti* (Pereira, 1935) Olsen, 1952. Pertinent differences among the above species and *S. penneri* are summarized

in Table 1. These species can easily be distinguished from *S. penneri* by possessing smaller and similar rather than dissimilar spicules. In addition, *S. hilarii* and *S. wrighti* have eight and *S. krameri* and *S. inopinatus* have nine pairs of caudal papillae, whereas *S. penneri* has six pairs. The number of caudal papillae in *S. barroslimai* and *S. cearensis* is similar to that of *S. penneri*, but their buccal capsules are smaller than the new species. Further, the vulva of *S. cearensis* is located in the posterior rather than anterior half of the body. *Spirocamallanus pexatus* Pinto et al., 1976 has equal spicules, but they are smaller than those of *S. penneri* and similar rather than dissimilar in shape. *Spirocamallanus pexatus* also exhibits seven or fewer spiral bands in the buccal capsule which is smaller than that of *S. penneri*, and a post-equatorial vulva.

Gery (1969) stated that the ichthyofauna of the region of South America including the Rio Atrato (Gery's "transandean region") comprises approximately 26% endemic species, 60% species common to the eastern slope of the Andes as well as the western, and the rest possessing marine or Central American affinities. The uplifting of the Andes, which them selves became an effective isolating barrier, pinched off the transandean fauna from the eastern fauna. It is therefore no surprise that *Spirocamallanus penneri* most closely resembles species from freshwater fish in Brazil and Guayana, but that the Brazilian and Guayanese species more closely resemble each other than any resembles *S. penneri* (Table 1). *Trachycorystes insignis*, host for *S. penneri*, also occurs in the Magdalena River of the eastern slope of the Andes; future studies may reveal a sister-species of *S. penneri* in the Magdalenean *T. insignis*.

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Effect of Temperature on Vector–Parasite Relationships of *Aedes trivittatus* and *Dirofilaria immitis*[1]

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ABSTRACT: The effects of six different developmental temperatures on the vector–parasite relationships of *Aedes trivittatus* and *Dirofilaria immitis* were investigated. Complete parasite development occurred at 18.5°, 22.5°, 26.5°, 30.5°, and 34.5°C, but no development was noted at 14.5°. Rate of development was directly related to temperature, and the maximum rate occurred by 30.5°. Parasites survived in mosquitoes at 14.5° for 30 days and completed development in nine days when transferred to 26.5°. Greater infection rates and parasite burdens were observed in mosquitoes maintained at 18.5–26.5°. A smaller percentage of mosquitoes was infected, and individual mosquitoes harbored fewer parasites at the temperature extremes. It was concluded that the optimum developmental temperature for *D. immitis* in *Ae. trivittatus* was 22.5–26.5°.

Aedes trivittatus (Coquillett, 1902) is an effective laboratory vector of *Dirofilaria immitis* (Leidy, 1850) and has been reported as a natural vector in central Iowa (Christensen, 1977). This mosquito can tolerate heavy parasite burdens in the laboratory (Christensen, 1978) and in nature (Christensen and Andrews, 1976). We therefore considered it appropriate to use this mosquito to determine what effects different temperatures might have on mosquito–*D. immitis* relationships.

Otto (1972) stressed the need for additional, more critical studies of temperature as it relates to the development of dog heartworm in mosquitoes. Kutz and Dobson (1974) subsequently showed that the rate of *D. immitis* development in a laboratory colony of *Anopheles quadrimaculatus* increased at higher temperatures. Their findings were similar to those obtained by Beam (1967) in his work with *Aedes sollicitans*.

The studies by Kutz and Dobson (1974) and Beam (1967) seem to be the only published accounts dealing with temperature as it effects *D. immitis* in the mosquito. These reports, however, concern only the rate of parasite development. They did not determine what effect different temperatures might have on the intensity or rate of infection of dog heartworm in the mosquito. A knowledge of these effects would contribute significantly to our understanding of the epidemiology of canine dirofilariasis.

This study therefore was designed to determine the effect of different temperatures on the developmental rate, intensity of infection, and infection rate of *D. immitis* in *Ae. trivittatus*.

Materials and Methods

Aedes trivittatus used in this study were hatched from eggs obtained from field-collected mosquitoes. Methods used for rearing and exposing mosquitoes to infection were the same as previously described (Christensen, 1977).

Mosquitoes were exposed to a dog (02)[3] with a microfilaremia of 82–90 microfilariae/25 μl. Microfilaremias were determined by drawing two 25-μl blood samples immediately before feeding mosquitoes. Two feedings were necessary to obtain enough mosquitoes for these experiments, and microfilaremias were determined to be 82 and 89 microfilariae/25 μl in the first, and 89 and 90 microfilariae/25 μl in the second feeding. Exposure took place at 26.5 ± 1°C. Blood-fed mosquitoes were separated after feeding and immediately placed in environmental chambers at 14.5 ± 1°, 18.5 ± 1°, 22.5 ± 1°, 26.5 ± 1°, 30.5 ± 1°, or 34.5 ± 1°C. Relative humidity was maintained at 80–90% for all temperatures.

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[3] The dog was furnished by John W. McCall (School of Veterinary Medicine, University of Georgia) through a program supported by the U.S.–Japan Cooperative Medical Sciences Program-NIAID.

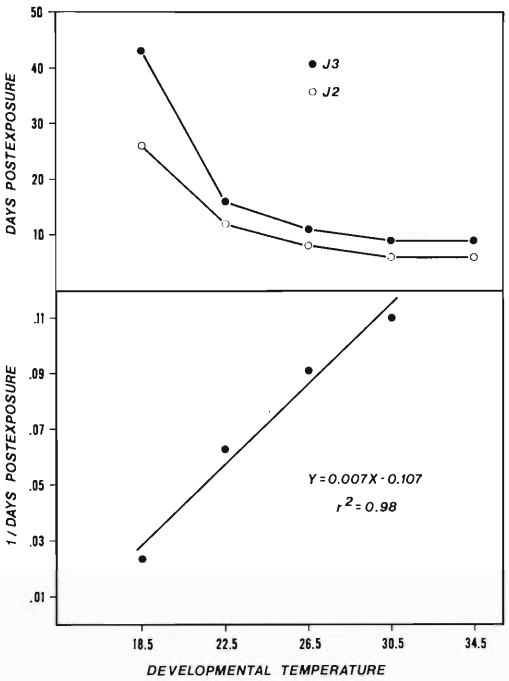


Figure 1. Rate of development for *Dirofilaria immitis* in *Aedes trivittatus* at different developmental temperatures. Points indicate first appearance of developmental stages. Y, minimal time required for development; 0.007, slope; –0.107, intercept; X, developmental temperature.

Mosquitoes were dissected at various times postexposure, depending upon the rate of parasite development observed during the course of the study for each developmental temperature. A total of 70, 79, 80, 76, 77, and 48 mosquitoes were dissected from 14.5°, 18.5°, 22.5°, 26.5°, 30.5°, and 34.5°, respectively. Location, number, and developmental stage of juveniles recovered were recorded for each dissection.

Infection rate in this study refers to the number of mosquitoes with *D. immitis* in the Malpighian tubules in relation to the number that blood fed on a microfilaremic dog. Likewise, parasite burden or intensity of infection refers to the number of juveniles in the Malpighian tubules of a single mosquito. The average number of microfilariae ingested per mosquito was not determined because this differs markedly from the number that actually reach the tubules and begin developing. In *Ae. trivittatus*, nearly an equal number of microfilariae are retained within the midgut as reach the Malpighian tubules (Christensen, 1977).

Table 1. Percentage of time required for the development of first- and second-stage juvenile *Dirofilaria immitis* in *Aedes trivittatus* at different developmental temperatures.

	Days to 1st appearance		Percentage of time in each stage	
Temperature	J2	J3	J1	J2
14.5°	—	—	100	—
18.5°	26	43	60	40
22.5°	12	16	75	25
26.5°	8	11	73	27
30.5°	6	9	67	33
34.5°	6	9	67	33

Results and Discussion

Rate of development was directly related to temperature (Fig. 1). Development beyond the first-stage juvenile did not occur at 14.5°, and the maximum rate of development to second- and third-stage juveniles occurred at 30.5°. This rate did not increase at 34.5°. The proportion of time that parasites existed in the first two developmental stages was quite similar for each temperature studied (Table 1).

By expressing days as a reciprocal, a linear regression was determined for the relationship between temperature (18.5°–30.5°) and the minimum time required for complete development of *D. immitis* (Fig. 1). This seems to be a good predictor for the minimal time required for complete development of dog heartworm in *Ae. trivittatus* at constant temperatures. How closely this could predict developmental times in the field where temperatures fluctuate cannot be stated. It would, however, give one a reasonable approximation when average weekly temperatures are known.

Kutz and Dobson (1974) recorded somewhat faster rates of development at similar temperatures with *An. quadrimaculatus*. At 32.2°C, second- and third-stage *D. immitis* were first recovered at 5 and 8 days postexposure, respectively. Development at 26.7°C was about 2 days faster in *An. quadrimaculatus*

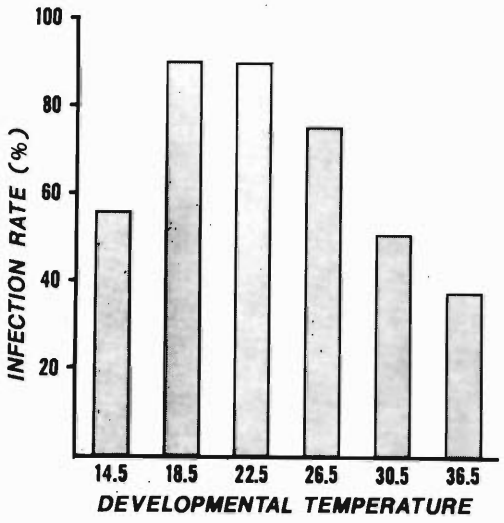
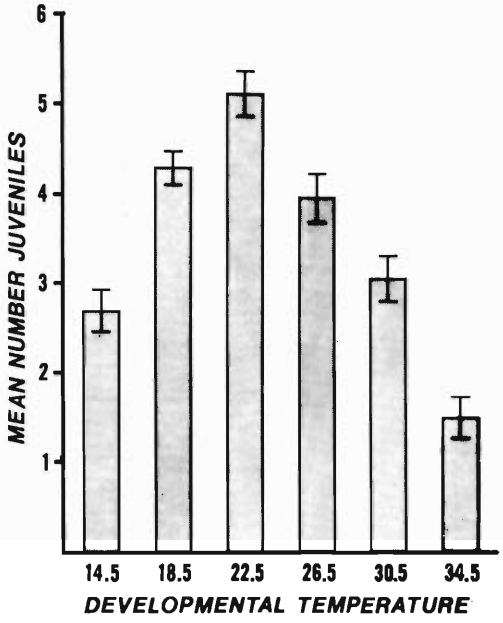


Figure 2. Infection rate of *Dirofilaria immitis* in *Aedes trivittatus* at different developmental temperatures.



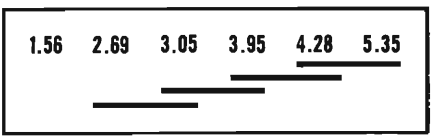


Figure 3. Mean parasite burdens ± standard error of *Dirofilaria immitis* in *Aedes trivittatus* at different developmental temperatures. Newman-Keuls' comparison of means, where any means not underlined by the same line are significantly different ($P > 0.05$).

as compared with our results for *Ae. trivittatus* at 26.5°. *Dirofilaria immitis* evidently is able to develop faster in *An. quadrimaculatus* than in *Ae. trivittatus* because of some unknown factor.

Mosquitoes held at 14.5° were transferred to 26.5° at 30 days postexposure. At this time, developmental stages were still microfilaria-like. After transfer to 26.5°, juveniles molted from first- to second- and second- to third-stage juveniles at 6 and 9 days after transfer, respectively. These data are similar to those obtained for *An. quadrimaculatus* by Kutz and Dobson (1974) and suggest that *D. immitis* can remain viable in *Ae. trivittatus* for long periods of unfavorable temperatures. This is especially relevant in northern climates where long periods of cool weather commonly occur after the first emergence of floodwater mosquitoes.

Percentage of mosquitoes infected varied considerably depending on developmental temperature (Fig. 2). Infection rates were reduced at the 14.5°, 30.5°, and 34.5° developmental temperatures. Direct evidence to explain this phenomenon was not obtained in this study, but it seems likely that the extreme temperatures had a detrimental effect on the microfilariae ingested, thereby inhibiting their ability to migrate to the Malpighian tubules. Whether this is a direct or indirect effect of temperature remains to be determined.

There was a similar pattern in the mean parasite burden of mosquitoes dissected at various temperatures (Fig. 3). A Newman-Keuls' test of these means showed that the differences were significant ($P > 0.05$) (Fig. 3). The optimal temperature for maximum parasite burden (22.5°) is the mean temperature for June, July, and August for a 30-yr period in the area around Ames, in central Iowa (U.S. Weather Bureau, 1955, 1964).

A reduced frequency distribution in the

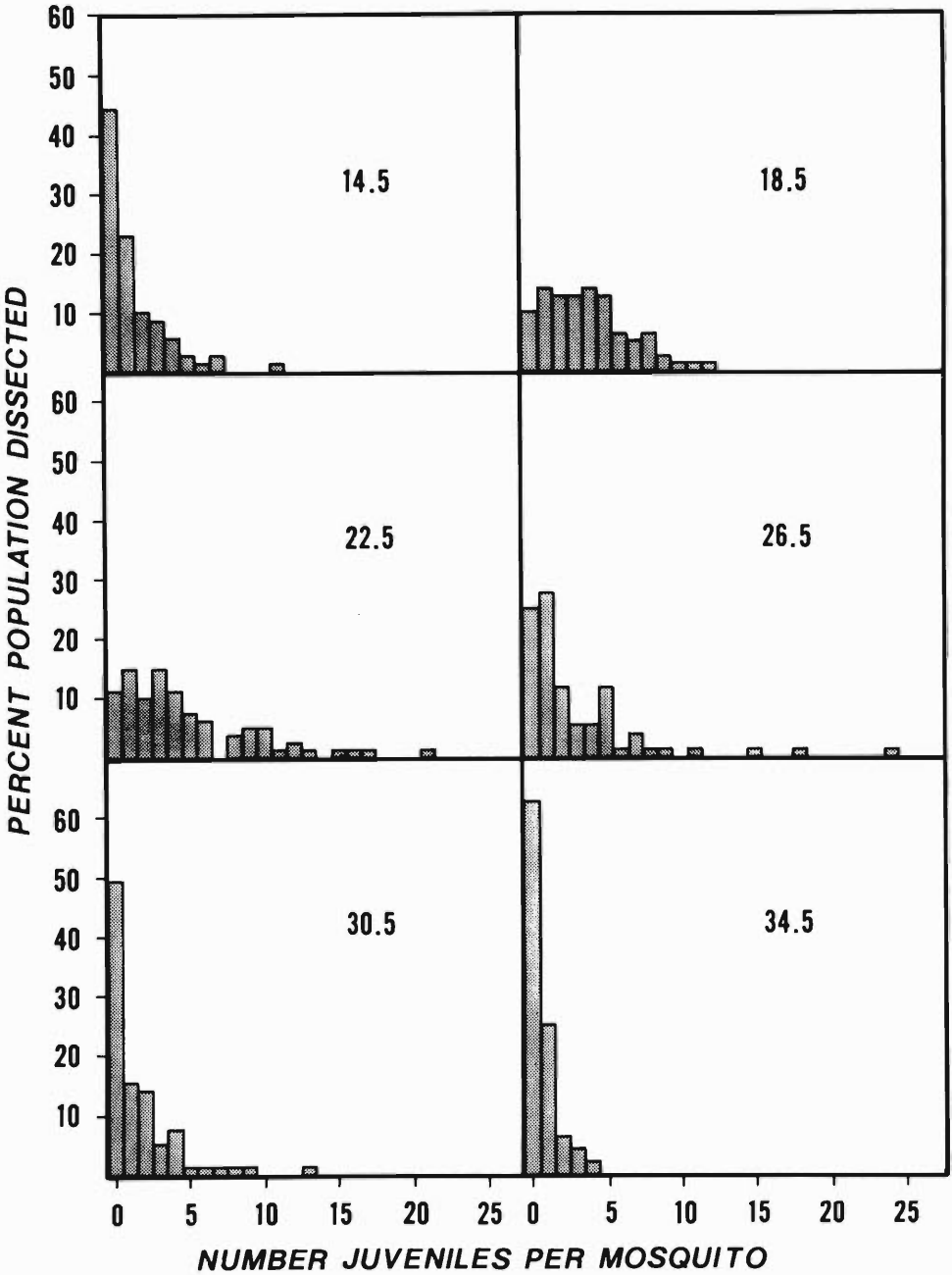


Figure 4. Frequency distributions of the number of *Dirofilaria immitis* recovered from *Aedes trivittatus* at different developmental temperatures.

number of juveniles obtained from dissected mosquitoes was evident at the temperature extremes (Fig. 4). All mosquitoes at 34.5°, and 94% of the mosquitoes at 30.5° and 14.5°, contained fewer than six juveniles. A much wider distribution was noted at the other developmental temperatures (Fig. 4).

It is evident from these data that the temperature at which the maximum rate of development occurs is not necessarily the optimal temperature for this vector–parasite system. The reduced infection rate and parasite burden seem to overshadow the advantages that might be obtained from an increased rate of development. In addition, higher temperatures also are known to decrease the life span of both infected and uninfected mosquitoes (Kutz and Dobson, 1974), thus reducing an infected mosquito's chances of survival for a sufficient time to transmit the infection. The opposite seems true for *Ae. trivittatus* infected with *D. immitis* and maintained at 18.5°. Although the infection rate and parasite burden were favorable to the maintenance of the disease, the time required for the complete development of the parasite precludes the chances of survival of the vector for that time in nature; therefore, a temperature range of about 22.5°–26.5° seems optimal for the development of *D. immitis* in *Ae. trivittatus*.

The question arises, however, concerning the adaptability of a laboratory strain of *D. immitis* obtained from Georgia and an Iowa strain of *Ae. trivittatus*. It cannot be determined from this study if these reflect the relationship between mosquito and parasite from the same geographic area. Although different strains of mosquitoes are known to vary markedly in their ability to support parasite development, this has not been shown to be the case with different strains of *D. immitis*. Intermill (1973) found no difference between three geographical strains of *D. immitis* in their ability to develop in one strain of *Ae. triseriatus*.

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Prevalence of Fish Parasitism in Raritan Bay, New Jersey

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ABSTRACT: A baseline study was made in southern Raritan Bay, New Jersey from March through August 1974 to inventory the parasites of fish in that area. Approximately 200 fish representing 16 species were examined and the prevalence and intensity of each parasite species was recorded. Parasites identified represented 6 genera of nematodes, 8 of cestodes, 9 of monogenetic trematodes, 8 of digenetic trematodes, 4 of acanthocephalans, 5 of copepods, and 1 genus each of annelids, isopods, and protozoans. Results of the survey are discussed with respect to parasite prevalence and intensity, observed gross pathology in several fish species, and probable reasons for the abundance of certain parasites.

A survey of 16 species of marine fish and their parasites was made in Raritan Bay, New Jersey during the months of March through August 1974. The parasitological study was part of a major assessment of the Bay conducted by Environmental Concern, Inc., St. Michaels, Maryland for the Jersey Central Power and Light Company. Studies were designed to provide a baseline record of biological data prior to the construction of a proposed fossil fuel generating plant in the town of Union Beach, New Jersey. The parasite survey was made for the following reasons: (1) to estimate the prevalence of parasites known to be potentially harmful to commercial and sport fish species, (2) to record the diversity and prevalence of parasite species in the bay prior to the potential impact of major construction and operation of the generating plant. A knowledge of some of the host–parasite relationships in the Raritan Bay ecosystem prior to significant environmental change was considered to be essential for future studies on potential modifications of biological activity.

Raritan Bay is recognized to be polluted with biological and industrial wastes (Federal Water Pollution Control Administration Proceedings, 1967; Mahoney et al., 1973; Pararas-Carayannis, 1973; Ziskowski and Murchelano, 1975). Pararas-Carayannis (1973) found a 7 to 32 percent prevalence of an unidentified tapeworm in the yellowtail flounder, *Limanda ferruginea*, from the adajacent New York Bight, and Ziskowski and Murchelano (1975) have reported on the etiology and prevalence of fin rot disease in the same area. However, very little work has been done on host–parasite relationships, and nothing is known about the effects of pollution on aquatic animals indigenous to the Raritan Bay–New York Bight system. The present report summarizes the data collected from an intensified study on the principal parasites of fish residing in Raritan Bay during spring and summer months, and provides an historical account which may be used as a reference for future parasite surveys.

Methods

Adult fish were sampled by otter trawl from four separate stations offshore of the proposed power plant site. The trawl net had a head rope 25 ft in length and a cod-end stretch-mesh netting of 1¼ in. Bottom time for each trawl was approximately 15 to 20 min and towing speed averaged from 1 to 2 knots. When possible, trawling samples were taken twice a month from March through August 1974. Sampled fish were bagged on ice immediately after capture for transportation to the laboratory where subsamples were either frozen or examined fresh under a dissecting microscope for parasites. Time did not permit examination of the blood, nervous systems, and cranial cavities for that minority of parasites which may inhabit those areas. Recovered parasites, excepting the nematodes and protozoa, were stained in Delafield's hematoxylin and prepared as whole mounts for identification. Permanent smears of protozoa were fixed in methanol and stained in a 1/20 dilution of stock Giemsa for 10–15 min. Nema-

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Table 1. Summary list of fish species from Raritan Bay, New Jersey, examined during March–August 1974.

Name	No. exam.	Length/mm (Range)	Length/mm Mean	Length/mm SD*	Weight/g (Range)	Weight/g Mean	Weight/g SD*
Pseudopleuronectes americanus (Winter flounder)	44	(143–328)	231	39.7	(62–415)	158	82.9
Paralichthys dentatus (Summer flounder)	31	(308–532)	384	50.9	(272–1,000)	608	236.9
Scophthalmus aquosus (Windowpane flounder)	16	(185–287)	231	31.7	(48–348)	158	90.5
Pomatomus saltatrix (Bluefish)	54	(295–425)	340	29.2	(272–729)	394	100.0
Cynoscion regalis (Weakfish)	10	(575–710)	636	38.5	(1,498–3,632)	2,170	615.1
Merluccius bilinearis (Silver hake)	30	(285–476)	342	43.1	(158–605)	299	115.9
Urophycis chuss (Squirrel hake)	1	241	—	—	128	—	—
Peprilus triacanthus (Butterfish)	9	(175–236)	213	19.9	(70–189)	134	42.2
Acipenser oxyrhynchus (Atlantic sturgeon)	2	(576–637)	607	43.1	(736–926)	831	134.3
Prionotus strigatus (Red-winged sea robin)	1	190	—	—	70	—	—
Myoxocephalus octodecemspinosus (Longhorn sculpin)	1	263	—	—	182	—	—
Brevoortia tyrannus (Menhaden)	7	(248–375)	289	43.2	(164–614)	283	161.9
Alosa pseudoharengus (Alewife)	8	(205–300)	264	36.1	(78–357)	228	86.9
Clupea harengus (Atlantic herring)	4	(318–356)	331	17.3	(205–353)	293	63.3
Alosa sapidissima (American shad)	1	295	—	—	224	—	—
Mustelus canis (Smooth dogfish)	4	(590–700)	658	47.9	(681–1,135)	965	197.4

* SD—standard deviation.

todes were left unstained and cleared in lactophenol after fixation. Taxonomic identifications were based on characters presented in Yamaguti (1934, 1958, 1959, 1961, 1963), Dawes (1968), Wardle and McLeod (1968), and York and Maplestone (1926). Parasite prevalences, intensities and locations were recorded for all fish species examined. In cases where small parasites such as protozoans or cestode larvae were too numerous to count, intensities were expressed as heavy or light (reflecting the extent of encystment) and greater than 100 organisms per fish.

All fish taken from April through June were frozen for subsequent necropsy. Most fish taken from July through August were refrigerated and examined fresh, but additional specimens were frozen to avoid decomposition prior to necropsy. Fish collected in March were sent to the National Marine Fisheries Service Laboratories at Sandy Hook, New Jersey for evaluation of fin rot disease, and were not available for necropsy. Fish were identified according to the recommendation published by the American Fisheries Society (1970).

Small bait and young-of-the-year fish were beach-seined twice a month from two stations near the west shore of Conaskonk Point. Time did not permit complete necropsies of these fish, but records were kept on the prevalence and intensities of conspicuous ectoparasites.

Results

Parasite examinations of trawled fish

Fish caught in the otter trawl from March through August 1974 are listed by species in Table 1. The scientific names will not appear in the text hereafter when listed in Table 1. A total of 223 fish, representing 16 species, yielded six genera of nematodes, eight of cestodes, nine of monogenetic trematodes, eight of digenetic trematodes, four of acantho-

Table 2. Prevalence of parasites found in adult fish from Raritan Bay, New Jersey from April to August 1974.

Host (No. examined) / Parasite	No. hosts infected	No. per host	Parasite location
Winter flounder (44)			
Nematoda			
Contracaecum sp. Railliet and Henry, 1912 (larvae)	3	1–2	I, M
Capillaria sp. Zeder, 1800	1	1	G (regurgitated)
Cestoda			
Bothriocephalus scorpii Mueller, 1776	3	1–2	I, P
Unidentified cysts	2	1–3	Iw
Acanthocephala	1	1	I
Echinorhynchus gadi Zoega in Müller, 1776			
Summer flounder (31)			
Nematoda			
Anisakis sp. Dujardin, 1845 (Type 1 larva)	1	1	I
Contracaecum sp. (larvae)	6	1–2	I, M, Pc
Porrocaecum sp. Railliet and Henry, 1912 (larva)	1	1	M
Nematode fragments	1	3	Ba
Philometra sp. Costa, 1845	1	1	Mo
Dichelyne cylindricus Chandler, 1935	1	7	P
Cestoda			
Dasyrhynchus sp. Pintner, 1928 (encysted plerocercoid)	20	1–>50	Sw, Iw, P, M
Nybelinia sp. Poche, 1926 (encysted plerocercoid)	1	2	Sw
Scolex pleuronectis Mueller, 1788	28	>100	I, P, B, S
Pseudophyllidean plerocercoid (unid.)	1	1	Iw
Trematoda			
Neoheterobothrium affine Linton, 1898	2	1–2	G, Mo
Diclidophoropsis sp. Gallien, 1937	2	1–2	G, Ga
Peracreadium sp. Nicoll, 1909	1	1	P
Stephanostomum dentatum Linton, 1900	1	5	I
Stephanostomum sp. Looss, 1899 (metacercariae)	1	>50	G, H
Synaptobothrium sp. Linstow, 1904	1	2	S
cysts (unid.)	11	>10	G
Acanthocephala			
Southwellina hispida Van Cleave, 1925 (juvenile)	3	1–3	M
Serrasentis socialis Leidy, 1851	2	2	M
Pomphorhynchus rocci Cordonnier and Ward, 1967	1	1	M
Echinorhynchus gadi	1	1	I
Copepoda			
Acanthochondria galerita Wilson, 1932	2	1	Mo
Lepeophtheirus edwardsi Wilson, 1935	1	1	Sk
Windowpane flounder (16)			
Cestoda			
Bothriocephalus scorpii	6	2–12	I
Dasyrhynchus sp. (encysted plerocercoid)	1	1	Sw
Trematoda			
Bothitrema bothi MacCallum, 1913	11	2–18	G
Bluefish (54)			
Nematoda			
Contracaecum sp. (larvae)	3	1–2	M
Porrocaecum sp. (larvae)	7	1–2	M
Philometra sp.	8	1–6	Pc, R, O, Sv, Pr, P
Cestoda			
Dasyrhynchus sp. (encysted plerocercoid)	15	1–>100	Sw, P, Pr, M
Nybelinia sp. (encysted plerocercoid)	3	1–3	Pc, M, G
Callitetrarhynchus gracilis Rudolphi, 1819 (encysted plerocercoid)	3	1–>100	P, M
Scolex pleuronectis	50	>100	I, P
Pseudophyllidean plerocercoid (unid.)	1	1	Iw
Trematoda			
Microcotyle pomatomi Goto, 1899	53	3–32	G
Synaptobothrium sp.	9	1–5	S
Aponurus sp. Looss, 1907	1	1	G (regurgitated)
Acanthocephala			
Southwellina hispida (juvenile)	2	1	M, O
Serrasentis socialis	3	1	M, O
Copepoda			
Lernanthropus pomatomi Rathbun, 1887	28	1–5	G
Lernaeenicus longiventris Wilson, 1917	8	1–2	Ab, Op, A, i, D, C
Lernaeenicus radiatus Le Seuer, 1824	2	1–2	Op, A
Isopoda			
Lironeca ovalis Say, 1818	2	1–2	Oc

Table 2. ***Continued.***

Host (No. examined)	Parasite	No. hosts infected	No. per host	Parasite location
	Protozoa			
	Henneguya sp. Thelohan, 1892	8	medium to heavy encyst.	Sv
	cysts (questionable origin)	24	medium to heavy encyst.	Pr
Weakfish (10)				
	Nematoda			
	Contracaecum sp. (larvae)	9	1–15	Pn, M
	Cestoda			
	Nybelinia sp. (encysted plerocercoid)	1	1	Pr
	Scolex pleuronectis	10	>100	I, S, B
	cysts (unid.)	1	>10	Iw
	Trematoda			
	Microcotyle sp. Beneden and Hesse, 1863	2	1	G
	Acanthocephala			
	Southwellina hispida (juvenile)	1	1	M
	Protozoa			
	Henneguya sp.	1	light encyst.	V, A, C, T
Silver hake (30)				
	Nematoda			
	Contracaecum sp. (larvae)	18	1–9	M, I, Pn, g, P, L, Sw
	Anisakis sp. (Type 1 larvae)	2	1	Pn, L
	Capillaria sp.	3	1–9	I
	Cestoda			
	Clestobothrium crassiceps Rudolphi, 1808	19	1–7	I, P, R
	Grillotia sp. Guiart, 1927 (encysted plerocercoid)	7	1	Iw, L, M, Sw
	Scolex pleuronectis	8	1 > 100	P, I
	plerocercoids (unid.)	2	1	I, G
	Trematoda			
	Anthocotyle merluccii Van Beneden et Hesse, 1863	1	1	G
	Hemiurus levinseni Odhner, 1905	2	1–7	S
	Peracreadium sp.	1	1	I
	Acanthocephala			
	Pomphorhynchus rocci	1	10	Pn, M
	Annelida			
	Carcinobdella sp. Oka, 1910	1	1	Ga
Squirrel hake (1)				
	Trematoda			
	Diclidophoroides maccallumi Price, 1943	1	2	G
Butterfish (9)				
	Nematoda			
	Contracaecum sp. (larvae)	9	1–14	I, M
	Philometra sp.	1	4	O
	Cestoda			
	Scolex pleuronectis	3	>100	I
	Trematoda			
	Microcotyle poronoti MacCallum, 1915	7	1–7	G
	Lepidapedon elongatum Nicoll, 1915	6	1 > 100	I
Atlantic sturgeon (2)				
	Nematoda			
	Capillaria sp.	1	4	Iw
	Trematoda			
	Deropristis hispida Abildgaard, in Rudolphi, 1819	1	7	I
Red-winged sea robin(1)				
	Nematoda			
	Contracaecum sp. (larva)	1	1	Pn
	Trematoda			
	Diclidophoropsis sp.	1	3	G
Longhorn sculpin (1)				
	Nematoda			
	Anisakis sp. (Type 1 larva)	1	1	Pn
	Cestoda			
	Scolex pleuronectis	1	>100	I

Table 2. ***Continued.***

Host (No. examined)	Parasite	No. hosts infected	No. per host	Parasite location
Menhaden (7)				
	Cestoda			
	Scolex pleuronectis	2	>100	I, P
	Trematoda			
	Clupeocotyle brevoortia Hargis, 1955	5	1–15	G
	Aphanurus sp. Looss, 1907	1	1	S
	Copepoda			
	Lernaeenicus radiatus	1	2	b
	Miscellaneous			
	cysts (unid.)	1	>10	G
Alewife (8)				
	Nematoda			
	Contracaecum sp. (larvae)	5	3–30	Pn, M, I
	Anisakis sp. (Type 1 larva)	1	1	g
	Trematoda			
	Mazocraeoides georgei Price, 1936	3	1–5	G
	Hemiurus sp. *Rudolphi*, 1809	2	1–4	S
	Copepoda			
	Alella sp. Leigh-Sharpe, 1925	1	6	Op
	Annelida			
	Carcinobdella sp.	1	3	G
Atlantic herring (4)				
	Nematoda			
	Anisakis sp. (Type 1 larvae)	1	3	Pn
American shad (1)				
	Nematoda			
	Contracaecum sp. (larva)	1	1	I
Smooth dogfish (4)				
	Cestoda			
	Calliobothrium verticillatum Rudolphi, 1819	4	1–3	I
	Calliobothrium eschrichtii Beneden, 1849	1	1	I
	Trematoda			
	Hexabothrium sp. Nordmann, 1840	2	1–2	G

cephalans, five of copepods, and one genus each of annelids, isopods and protozoans (Table 2). Abbreviations for the location from which parasites were isolated are listed below. However, further discussion will only include those parasites having high rates of infection (arbitrarily determined as ⩾20%) or the potential for causing significant pathological changes in the host.

Location in the host

A—anal fin; Ab—abdomen; B—common and cystic bile ducts; Ba—branchial artery; b—back, near dorsal fin; C—caudal fin; D—dorsal fin; G—gill filaments; Ga—gill arch; g—gonad; H—heart ventricle; I—intestine; Iw—intestinal wall; i—isthmus (jugular position); L—liver surface; M—mesenteries; Mo—mouth cavity; O—ovary; Oc—opercular cavity; Op—operculum, P—pyloric caeca; Pc—pericardial cavity; Pn—peritoneal cavity; Pr—pericardium; R—rectum; S—stomach; Sk—epidermal surface; Sv—bulbus arteriosus; Sw stomach wall; T—pectoral fin; V—pelvic fin.

Rate of infestation

It was not possible to make any assumptions concerning trends in the prevalence of parasitism on a monthly basis due to insufficient sample size of any one fish species for more than two consecutive months. In fish species where larger sample sizes were available for two successive months, the prevalence of parasitism did not differ very much. The relative occurrence of parasites did not appear to differ between frozen and fresh fish; however, precise numbers recovered per fish may have differed since dead parasites were more difficult to find during necropsy.

The parasites most frequently observed were

Table 3. Distribution of parasites occurring in more than one host species.

Parasite	Host
I. Nematoda	
Contracaecum sp. (larvae)	*Prionotus strigatus, Alosa pseudoharengus, Alosa sapidissima, Pseudopleuronectes americanus, Paralichthys dentatus, Pomatomus saltatrix, Cynoscion regalis, Merluccius bilinearis, Peprilus triacanthus*
Porrocaecum sp. (larvae)	*Paralichthys dentatus, Pomatomus saltatrix*
Anisakis sp. (Type 1 larvae)	*Paralichthys dentatus, Merluccius bilinearis, Myoxocephalus octodecemspinosus, Clupea harengus, Alosa pseudoharengus*
Capillaria sp.	*Pseudopleuronectes americanus, Merluccius bilinearis, Acipenser oxyrhynchus*
Philometra sp.	*Paralichthys dentatus, Pomatomus saltatrix, Peprilus triacanthus*
II. Cestoda	
Bothriocephalus scorpii	*Pseudopleuronectes americanus, Scophthalmus aquosus*
Dasyrhynchus sp. (larvae)	*Paralichthys dentatus, Scophthalmus aquosus, Pomatomus saltatrix*
Nybelinia sp. (larvae)	*Paralichthys dentatus, Pomatomus saltatrix, Cynoscion regalis*
Scolex pleuronectis	*Paralichhys dentatus, Pomatomus saltatrix, Cynoscion regalis, Brevoortia tyrannus, Merluccius bilinearis, Peprilus triacanthus, Myoxocephalus octodecemspinosus*
Pseudophyllidean plerocercoid	*Paralichthys dentatus, Pomatomus saltatrix*
III. Trematoda	
Diclidophoropsis sp.	*Paralichthys dentatus, Prionotus strigatus*
Peracreadium sp.	*Paralichthys dentatus, Merluccius bilinearis*
Synaptobothrium sp.	*Paralichthys dentatus, Pomatomus saltatrix*
Hemiurus sp. (*levinseni*)	*Merluccius bilinearis, Alosa pseudoharengus*
IV. Acanthocephala	
Southwellina hispida	*Paralichthys dentatus, Pomatomus saltatrix, Cynoscion regalis*
Pomphorhynchus rocci	*Paralichthys dentatus, Merluccius bilinearis*
Serrasentis socialis	*Paralichthys dentatus, Pomatomus saltatrix*
V. Annelida	
Carcinobdella sp.	*Merluccius bilinearis, Alosa pseudoharengus*
VI. Copepoda	
Lernaeenicus radiatus	*Pomatomus saltatrix, Brevoortia tyrannus*
VII. Protozoa	
Henneguya sp.	*Pomatomus saltatrix, Cynoscion regalis*

nematodes of the genera *Contracaecum* and *Anisakis* and a larval tetraphyllidean cestode, *Scolex pleuronectis*. At least two different tetraphyllidean plerocercoids of the *S. pleuronectis* type were observed indicating the possibility of more than one species being represented. Of the 16 fish species examined, 56%, 31%, and 44%, respectively, were found to harbor the above three parasites (Table 3).

Adult tapeworms identified as *Bothriocephalus scorpii* and *Clestobothrium crassiceps* were recovered from 38% of windowpane flounder (16 examined) and 63% of silver hake (30 examined), respectively. Additionally, high prevalences of 64% (31 examined) and 28% (54 examined) were found for the encysted trypanorhynch larvae of *Dasyrhynchus* sp. in summer flounder and bluefish, respectively. *Grillotia* sp., another trypanorhynch larva, occurred in 23% of the silver hake examined. The only selachians sampled were four smooth dogfish each having infections of adult tetraphyllidean cestodes, *Calliobothrium verticillatum*, in the intestine.

Generally, digenetic trematodes were found in low prevalences among the fish species examined. However, 67% of the butterfish (9 examined) had intestinal infections of *Lepidapedon elongatum* and 25% of the alewives (8 examined) harbored *Hemiurus* sp.

Conversely, monogenetic flukes were present in considerably higher prevalences in many of the fish species: 98% of 54 bluefish were infected with *Microcotyle pomatomi*; 69% of the windowpane flounder (16 examined) had gill infections of *Bothitrema bothi*; 7 of the 9 butterfish were parasitized by *Microcotyle poronoti*; 5 of 7 menhaden carried gill infestations of *Clupeocotyle brevoortia*; 3 of 8 alewives had *Mazocraeoides georgei*; 2 of 4 smooth dogfish harbored *Hexabothrium* sp. Only one side of the gill chamber in each dogfish was examined.

Parasitic copepods also were found in low prevalences except in the bluefish where 52%

(54 examined) carried *Lernanthropus pomatomi* on the gill filaments.

Observed gross pathology

Pathology was observed in very few instances. In a butterfish infected with three nematodes, *Philometra* sp., one of the ovaries showed a marked swelling. All of the 10 weakfish examined and 90% of the 31 summer flounder had heavy intestinal infections of perhaps several hundred of the larval cestode *Scolex pleuronectis*. In many of the fresh specimens, the common and cystic bile ducts were dilated and quite transparent with many large larvae visible through the duct walls.

Large numbers of *Dasyrhynchus* sp. were visible as many small white foci within the walls of the gastrointestinal tracts of summer flounder and bluefish. Fewer foci were observed on the pericardium in some of the bluefish. The stomach wall was often the most commonly infected organ with many larvae (>100) appearing to encyst in the submucosal or mucosal tissues. Similar small white lesions representing encystment of *Callitetrarhynchus gracilis* were observed on the surfaces of pyloric ceca and in the mesenteries of certain bluefish.

Encysted *Stephanostomum* sp. metacercariae were visible as small white nodular foci in the gill filaments and throughout the cardiac tissue of a single summer flounder. Histological sections of the latter tissue indicated that most of the cysts were in the myocardium. Typically, each cyst contained a larva surrounded by a laminalike sheath of trematode origin and a thick outer capsule of host fibrous connective tissue in concentric layers. The apparent health of this fish was remarkable considering the heavy parasitism of the heart.

Two species of parasitic copepods, *Lernaeenicus radiatus* and *L. longiventris*, were found associated with significant degrees of tissue damage depending upon their locations in the host. Most frequently, they were found imbedded in muscle tissue near the dorsal fin or in the opercular flaps. At the point of parasite entry the host tissue was ulcerated and hemorrhagic. In one of the bluefish a single *L. longiventris* was deeply imbedded in the isthmus and appeared to penetrate the ventral aorta. In another bluefish the head and cephalothorax regions of the same copepod species were recovered from the intestinal lumen. The remainder of the body must have been dislodged and was not seen.

Parasitic isopods were represented by one species, *Lironeca ovalis*, which occurred on the inside wall of the gill chamber in each of two bluefish. The two parasitized fish showed erosion of gill filaments and exhibited flared opercula on the infected sides due to the bulk of the parasites. Irregular, small yellow cysts (1–5 mm) containing myxosporidan spores of *Henneguya* sp. were found deep within and on the surface of the bulbus arteriosus of 15% of the 54 bluefish examined. In some cases these cysts protruded markedly into the lumen of the organ. In spite of this, the condition of the infected fish appeared normal. A similar appearing spore-forming species was found in several small white cysts scattered within the pelvic, anal, pectoral and caudal fin epithelia of a single weakfish.

Parasite examination of seined fish

The parasitic copepod, *L. radiatus*, was found infecting the bay anchovy during July and August. Prevalence and intensity figures from the two seining stations are summarized below:

First station: July 11, 0 parasites found in 184 fish examined; July 22, 22/145 infected, 1–3 per host; July 31, 63/92, 1–4 per host; no fish examined in August.

Second station: July 11, 0/115; July 22, 16/63, 1–2 per host; August 16, 17/91, 1–2 per host.

Despite gaps in these data, caused by the disappearance of the anchovy during certain sampling trips, the rate of infection appeared to increase from 15% to 69% at the second station in late July followed by a decrease to 19% at the first station by early August. No other fish species seined were observed to have this parasite. The gross pathology observed was the same as that described for adult fish infected with this same copepod species.

Discussion

As can be seen, some of the parasite prevalence figures were based on undesirably small fish samples. This was especially true of the clupeid fishes because their condition often

deteriorated considerably before and during transportation back to the laboratory thus making necropsy impossible in several cases. In other instances, certain fish species were only available in small numbers due either to our collection methods or the time of year.

It should also be noted that when the systematics of *Echinorhynchus* acanthocephalans is sorted out, the specimens reported here as *E. gadi* may prove to be another previously undescribed species (Dr. Wilbur Bullock, Department of Zoology, University of New Hampshire, personal communication, February 19, 1975).

The fish parasites encountered from Raritan Bay included a relatively diverse group, some having high rates of infection. The extreme eutrophic condition of this estuarine area may, in part, contribute to such high incidences. Eutrophication tends to promote greater densities of organisms but less species diversity. This axiom was certainly apparent in our other surveys concerning phytoplankton, zooplankton, epibenthic crustacea and baitfish, all of which are important food organisms for fish species in the study area (Garbisch, 1975). Obviously, endoparasitic diversity and prevalences within a fish population are directly influenced by the food species available which may act as intermediate parasite hosts. Thus, a greater number as well as diversity of such food organisms could conceivably increase the chance occurrence of parasitism in predator fish.

Many larval cestode parasites were found having the highest prevalences and intensities and were observed the most frequently among the fish species examined, indicating rather low host specificity. Pseudophyllidean and trypanorhynchan cestodes such as *Clestobothrium* and *Gillotia* are known to use copepod crustacea as first intermediate hosts. Tetraphyllidean life cycles are little known, but it is believed they utilize some invertebrates as first intermediate hosts (Wardle and McLeod, 1968). In Raritan Bay, those fish species, excepting menhaden, which had high prevalences of tetraphyllidean plerocercoids were those which fed primarily on *Crangon* shrimp (Garbisch, 1975). The implication here is that the sand shrimp may be a potential first intermediate host for certain tetraphyllidean cestodes. Menhaden may have become infested via planktonic shrimp larvae. For unknown reasons, windowpane flounder did not harbor these plerocercoids despite their heavy diet of shrimp. The selection of the sand shrimp by predator fish as a major food item was not surprising since it appeared to be the most abundant food species during every month of the study. Shrimp were observed in large colonies covering the substrate at most of the collecting stations by our scuba diver (Bruce Harke, Environmental Concern, Inc., St. Michaels, MD, personal communication, February 1975).

Larval anisakine nematodes, particularly *Contracaecum* sp., seemed to have little host specificity, often occurring at high intensities among many of the fish species from the study area. Larval *Porrocaecum* sp. and *Anisakis* sp. type 1 larvae also occurred in more than one host species, but to a lesser extent. According to Chitwood (1970), all of these nematodes require at least one or more transport hosts. However, these hosts have not been thoroughly identified. Polyanskii (1955) claimed that the first intermediate host of *Contracaecum aduncum* may include copepods and other invertebrates, which might account for its wide distribution in fish which feed on them. Fish which act as transport hosts tend to harbor the parasites in the body cavity, on the liver surface, and in the mesenteries, as opposed to the stomach and intestines in definitive hosts (Chitwood, 1970). These parasites are important not only for their debilitating effects on the fishery but also for their potential harm to public health. Human anisakiasis involving gastric or intestinal lesions can result from consumption of improperly cooked or pickled fish which are infected with these nematode larvae (Arean, 1971).

Fish species with strong predatory habits tended to be parasitized frequently with an often diverse parasite fauna. This is not surprising in view of the previous discussion concerning the direct relationship between the quantity and variety of prey consumed and parasite prevalence and diversity. Bluefish, weakfish, silver hake and especially summer flounder were predators having both the highest prevalences and generally the highest intensities of various parasites. This trend did not occur among winter flounder which were relatively free of parasitism in comparison with

other fish species. The most striking possibility which may account for this difference is the winter flounder diet of clam necks and polychaete worms, contrasted with the shrimp diet of the other predator fish (Garbisch, 1975). The latter prey organisms, at least in Raritan Bay, may play host to larger numbers of parasites than clams or worms. Such speculation can only be assessed by more intense investigations including feeding experiments and closer examination of adjacent waters to compare fish diets and parasite prevalences with those of Raritan Bay.

Acknowledgments

I wish to thank the following people for their kind help: Dr. Wilbur L. Bullock of the Department of Zoology at the University of New Hampshire, Durham for his assistance in identifying some of the Acanthocephala; Dr. J. Ralph Lichtenfels of the USDA Animal Parasitology Institute, Beltsville, Maryland for identifying some of the Nematoda; Dr. Jacob H. Fischthal of the Department of Biological Sciences at the State University of New York, Binghamton for his identification of the *Stephanostomum* metacercariae; Dr. Paul Meglitsch of the Department of Biology at Drake University, Des Moines, Iowa for confirming my identification of the myxosporidan spores as *Henneguya*; Dr. Sifford Pearre of Environmental Concern, Inc., St. Michaels, Maryland, and his wife, Ona, for reviewing the manuscript; Mrs. Lang, librarian at the NMFS Oxford Laboratory, Oxford, Maryland for her patience and help in the library. I wish to express very special thanks to Dr. Thomas K. Sawyer of the NMFS Oxford Laboratory whose advice and help in preparing the final manuscript went beyond measure.

The Oxford Laboratory also processed the histological preparations of the *Stephanostomum* metacercariae and have incorporated 25 mounted parasite specimens from this collection into their Registry of Marine Pathology. Twenty-three mounted and vialed specimens from this collection may also be found in the parasite registry of the USDA Animal Parasitology Institute at Beltsville.

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Anthelmintic Activity of Oxibendazole Against Gastrointestinal Parasites of Cattle

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ABSTRACT: The effect of oxibendazole feed premix (Smith Kline Animal Health Products, West Chester, Pa. 19380) in removing naturally acquired gastrointestinal parasites in cattle was studied in a controlled test with 12 treated and 12 control animals. The drug was administered as a feed premix (mixed with 0.45 kg of a commercial ration) at a dosage of 15 mg/kg. No signs of toxicity were associated with administration of the drug. Percentage efficacies were as follows: *Bunostomum phlebotomum, Oesophagostomum radiatum, Haemonchus placei, Trichostrongylus axei, Cooperia* spp., and *Trichostrongylus colubriformis*: >99%; *Ostertagia ostertagi*: >96%. Efficacy against *Trichuris* was >80%, and *Moniezia* >71%. Efficacy against immature nematodes in the abomasum and small intestine was high, >95% and >91%, respectively, but total numbers of worms were not great. Efficacy against immature worms in the cecum-large intestine was >58%.

In preliminary field testing of the anthelmintic oxibendazole, Theodorides et al. (1973) and Theodorides and Chang (1974) demonstrated a high level of efficacy against adult stages of the common gastrointestinal nematodes of cattle and sheep. In a critical test of oxibendazole at dosages of 5 and 10 mg/kg in cattle, Herlich (1975) reported efficacy of 85 to 100% against adults of the common gastrointestinal nematodes. Efficacy against *Haemonchus contortus* and *Cooperia oncophora* was a little less at the lower dosage. In a controlled test at a dosage of 10 mg/kg against 3- and 7-day-old worm infections, Herlich (1975) reported an efficacy above 90% against *H. contortus, Trichostrongylus colubriformis,* 76 and 87% against *Trichostrongylus axei,* 34% against *Ostertagia ostertagi,* and no activity against *Oesophagostomum radiatum.* Crowley et al. (1976) presented results of a controlled test in which cattle infected with artificially induced and naturally acquired infections were treated at 3, 7, and 42 days after infection, with oxibendazole feed premix at a dosage of 15 mg/kg. Efficacy against adults of the common gastrointestinal nematodes exceeded 98%; efficacy against immature stages was highly effective except for *O. radiatum* (0%).

The purpose of the present work was to determine the efficacy of oxibendazole feed premix in cattle at 15 mg/kg in a controlled test against naturally acquired gastrointestinal nematodes and the tapeworm *Moniezia.*

Materials and Methods

Twenty-four head of newly weaned calves from a group of 44 acquired were used in the study and all were purchased from a single farm near Baton Rouge, Louisiana. The calves were predominantly of the Angus breed or Angus crossed with Hereford or Brahman, both steers and heifers. They were selected for a weight range of 160 to 200 kg. Initial fecal examination indicated high egg counts and a good distribution of worm genera common to the region; however, numbers of *Trichostrongylus axei* were minimal and *T. colubriformis* is not common in cattle in Louisiana. During an 8-day acclimatization period on a small drylot, all calves were experimentally inoculated with two successive daily doses of 2,000 *T. axei* and 2,000 T. *colubriformis* infective larvae. Grass hay only was fed to the calves during the acclimatization period. Quantitative fecal examinations (centrifugation-flotation with saturated sucrose solution) were made on all 44 calves on a weekly basis prior to selection of the 24 test calves and treatment. To provide time for maturation of the superimposed *Trichostrongylus* spp. infections and acquisition of additional pasture infection, all calves were grazed for 4 weeks on a 1-ha

[1] Ralston Purina, Checkerboard Square, St. Louis, Missouri 63188.
[2] Oxibendazole, Smith Kline Animal Health Products, West Chester, Pa. 19380.

Table 1. Results of quantitative fecal examinations on 44 cattle prior to selection of 24 for anthelmintic testing.

	Sample date				
	9/10	9/16	9/27	10/7	10/14
Avg. E.P.G.	1,056.2	1,036.5	500.5	497.8	534.2
Bunostomum present	29/44	36/44	31/43	31/44	32/44
Moniezia present	10/44	5/44	5/44	6/44	10/44

pasture of Coastal Bermuda grass. Grass hay was continuously accessible to supplement grazing.

After the 4-week grazing period 24 calves were selected for the experiment, based on fecal egg counts and body weights and divided into two groups of 12. On several occasions prior to time of treatment all 24 calves were given access to small amounts of feed. The feed used for this purpose and also for mixing with oxibendazole at treatment was Purina Omolene.[1] Calves designated to receive feed with the anthelmintic or feed alone as controls were withheld from any feed source in small pens for 24 hours prior to treatment. All calves were weighed 24 hours prior to treatment. Twelve untreated controls were given 0.45 kg of Omolene without anthelmintic; the 12 treated calves were given oxibendazole[2] feed premix at a dosage of 15 mg/kg mixed in 0.45 kg of Omolene. Following treatment all calves were maintained on a small pasture and all were killed at 7 days after treatment. Treatment and subsequent slaughter of all treated and control calves was staggered over several days; equal numbers of treated and control calves were processed on a given day.

At necropsy the abomasum, small intestine, and cecum-large intestine were stripped from the visceral mass and each segment was tied off at both ends. Each segment was cut open and thoroughly washed and rubbed in the following volumes of tap water, abomasum in 20 liters, small intestine in 30 liters, cecum-large intestine in 30 liters. A well-mixed 10% aliquot was removed for each gut segment and preserved. The mucosa of the abomasum and small intestine was thoroughly scraped and digested in acidified pepsin (1 liter volume) to facilitate recovery of immature stages in tissue. Twenty percent aliquots of the digests were examined for worm counts. Bulk washings of the contents of the small intestine and cecum-large intestine, excluding the 10% aliquots, were poured over a screen of 24 meshes per linear cm for gross recovery of *Moniezia*. Most tapeworms were recovered in this manner. All worm counts were made with a dissecting microscope. In cases where total numbers from an aliquot were less than 100, all were identified. With greater numbers, at least 200 were identified.

Results and Discussion

Results of quantitative fecal examination on the calves initially and for 4 subsequent weeks prior to grouping and treatment indicated that all the common nematode genera were consistently present and the general level of infection was suitable for testing the anthelmintic (Table 1). However, the presence of *Bunostomum phlebotomum* and *Moniezia* was variable for different calves over the 5-week observation period. *Bunostomum phlebotomum* eggs were found in feces of nearly all calves in at least one examination.

All calves designated for the treatment and control groups were positive for *B. phlebotomum* in the fecal examination immediately before treatment. While only 10 calves were positive for *Moniezia* immediately before treatment, half of the 44 calves under observation had been positive for *Moniezia* in one or more of the previous fecal examinations. Based on these observations it was assumed that a majority of the calves were infected with *Moniezia*. At necropsy, *B. phlebotomum* was recovered from 11 of 12 control calves; no worms were recovered from treated calves. *Moniezia* was recovered from 10 of 12 controls and 6 of 12 treated calves.

The parasites recovered from the treated and control calves and the percentage efficacy of oxibendazole against the various genera is shown (Table 2). The efficacy of oxibendazole was in excess of 96% against adult worms of all genera except for *Moniezia* and *Trichuris* sp. Although the generic composition of immature worms in the different gut segments was not defined in Table 2, good activity against immature stages in the abomasum and small intestine was demonstrated.

Table 2. Effect of oxibendazole feed premix on naturally acquired gastrointestinal nematode infections of cattle at a dosage level of 15 mg/kg.

Parasites	Worms recovered at necropsy						% Efficacy
	Controls (N = 12)			Oxibendazole (N = 12)			
	Range	Mean	SE	Range	Mean	SE	
Abomasum							
Haemonchus placei	150–6,983	2,298	662	0–50	7	5	>99
Ostertagia ostertagi	200–12,793	2,975	1,211	5–335	89	31	>96
Trichostrongylus axei	752–2,822	1,818	184	0–11	1	1	>99
Immature	0–1,676	799	172	0–122	34	12	>95
Small Intestine							
Cooperia spp.	1,856–38,435	15,956	3,283	0–50	4	4	>99
Trichostrongylus colubriformis	0–2,815	1,033	244	0–50	8	5	>99
Bunostomum phlebotomum	0–568	155	46	0	0	0	>99
Immature	56–4,785	1,945	524	0–650	157	47	>91
Moniezia sp.	0–13	4	1	0–8	1	1	>71
Cecum and Large Intestine							
Oesophagostomum radiatum	31–1,650	434	142	0	0	0	>99
Trichuris sp.	0–133	35	10	0–50	7	4	>80
Immature	0–300	70	30	0–100	29	9	>58
Mean total							
Worm burden		27,564			321		

The majority of immature worms recovered from the abomasum of treated and control calves were *O. ostertagi*; immature worms in the small intestine were *Cooperia* spp., *B. phlebotomum*, and *O. radiatum*. Immature worms recovered from the cecum-large intestine were primarily *Trichuris* sp., but included small numbers of *O. radiatum*. The percentage efficacy against these forms was poor.

Results obtained in the present work are comparable to those reported by Herlich (1975) and Crowley et al. (1976) for corresponding genera and stages of development. The efficacy of oxibendazole against *Trichuris* sp. adults in the present study (80%) was less than that (98%) reported by Crowley et al. (1976).

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ANNOUNCEMENT—HONORARY DEGREE

MayBelle Chitwood was awarded the Honorary Degree of Doctor of Science by Northern Michigan University on August 6, 1977.

Phyllobothrium kingae sp. n., a Tetraphyllidean Cestode from a Yellow-spotted Stingray in Jamaica[1]

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ABSTRACT: *Phyllobothrim kingae* sp. n. is described from *Urolophus jamaicensis* from Discovery Bay, Jamaica. It is most similar in size of strobila and number of testes to *P. biacetabulum* Yamaguti, 1960, but differs from that species in having bilaterally expanded bothridia, each with about 60 loculi and a single accessory sucker, compared with rounded bothridia having about 40 loculi and a double accessory sucker. This is the first species of *Phyllobothrium* from the Urolophidae.

This report is based upon four specimens recovered from the spiral valve of a yellow-spotted stingray, *Urolophus jamaicensis*, which I speared at Discovery Bay, Jamaica, in March 1977. They represent a new species and are the basis for the following measurements and description. All measurements are in micrometers unless otherwise stated.

Phyllobothrium kingae sp. n. (Figs. 1–4)

DESCRIPTION: Scolex (Fig. 1) with four stalked bothridia, 0.88 to 1.32 mm long by 0.88 to 1.38 mm wide, measured from extremities of bothridia. Each bothridium (Fig. 2) bilaterally expanded, with about 60 weak, marginal loculi and a single, median, proximal accessory sucker which is 70 to 80 by 65 to 90. Bothridia 400 to 450 long by 0.88 to 1.12 mm wide. Peduncle of scolex 160 to 200 long by 170 by 200 wide. Neck absent.

Strobila delicate, about 10 mm long, consisting of 30 to 35 proglottids. Proglottids acraspedote, apolytic. Mature proglottids (Fig. 3) 1.15 to 1.75 mm long, 150 to 200 greatest width. Gravid proglottid (Fig. 4) (attached to strobila) 2 mm long, 440 to 460 greatest width. Reproductive systems protandrous. Genital atrium absent. Genital pores post-equatorial, one fourth to one third from posterior end, irregularly alternating.

MALE GENITALIA: 26 to 32 testes in two longitudinal rows, 12 to 16 aporal, 14 to 16 poral, none located posterior to cirrus pouch. Each testis 50 to 70 wide in mature segment. Cirrus pouch ovoid, thick-walled, 100 to 120 long, 80 to 90 wide, containing a short ejaculatory duct. Vas deferens posterior to cirrus pouch. Cirrus short, apparently unarmed but spines may have been lost. Cirrus pouch and cirrus present only in mature and gravid segments.

FEMALE GENITALIA: Ovary near posterior end of segment, U-shaped with two equal, anteriorly directed lobes that nearly reach level of cirrus pouch, 320 to 480 long, 95 to 150 across tips of both anterior lobes. Vitelline follicles few, small, lateral. Distal end of vagina anterior to cirrus pouch, with thick lining and muscular wall. Proximal portion of vagina thin-walled. Seminal receptacle small. Uterus a median, longitudinal tube, becoming a thin-walled sac filled with eggs anterior to cirrus pouch (Fig. 4). Eggs collapsed during preparation for slides so could not be measured.

TYPE HOST: Yellow-spotted stingray, *Urolophus jamaicensis* (Cuvier, 1817).

LOCATION: Spiral valve.

TYPE LOCALITY: Discovery Bay, Jamaica.

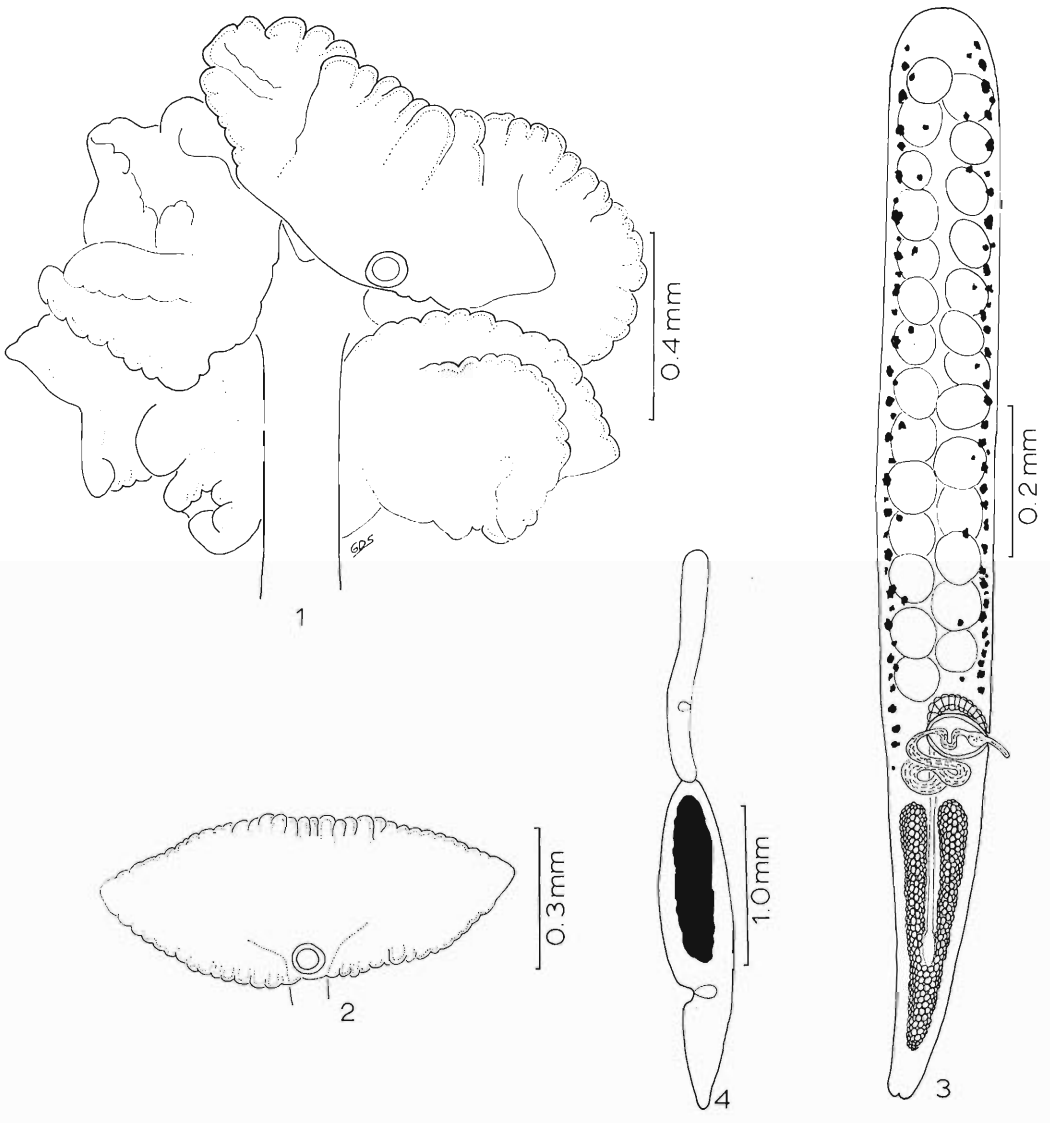
TYPE SPECIMENS: USNM Helm. Coll. holotype no. 74636; 3 paratypes no. 74637.

ETYMOLOGY: The species is named in honor of Ms. Kinga Kovacs, my diving buddy during two expeditions to Jamaica.

Remarks

Phyllobothrium was reviewed by Williams (1968) who retained 22 species as provisionally valid. The only species added since that monograph is *P. piriei* Williams, 1968. The present species was compared with descriptions of all of these, as well as those of *Crosso-*

[1] Contribution No. 148, Discovery Bay Marine Laboratory, Jamaica, W.I.



Figures 1–4. ***Phyllobothrium kingae*** **sp. n. from a Jamaican stingray. 1. Scolex. 2. Bothridium. 3. Mature proglottid. 4. Gravid proglottid (bottom) attached to a nearly mature proglottid (top), showing disparity of size. The black area illustrates the gravid utreus.**

bothrium Linton, 1889, which are similar to *Phyllobothrium*.

The size and shape of the bothridia of *P. kingae* are most similar to those of *P. lactuca* Beneden, 1849, a common parasite of elasmobranchs, with apparently world-wide distribution. That species, however, has a strobila up to 150 mm long, and about 200 testes, compared with a strobila only 10 mm long, and 26 to 32 testes for *P. kingae*.

In size of strobila and number of testes, *P. kingae* is most similar to *P. biacetabulum* Yama-

guti, 1960 from *Rhinobatus schlejeli* from the Inland Sea, Japan. The strobila of that species is 3.5 to 8.5 mm long and it has 18 to 30 testes. However, its bothridia are nearly circular, and each has about 40 marginal loculi, compared with 60 in *P. kingae*. Further, there are two fused accessory suckers, one behind the other, compared with a single, simple sucker in the case of *P. kingae*.

It is therefore clear that the specimens from *Urolophus jamaicensis* represent a species new to science. The fact that this is the first species in the genus to be described from any member of the Urolophidae supports this conclusion as there is very high host specificity in *Phyllobothrium* (Williams, 1968).

Acknowledgments

I wish to thank Dr. Jeremy Woodley, Acting Head, and Ms. Eileen Graham, Administrative Assistant, of the Discovery Bay Marine Laboratory, University of the West Indies for encouragement and the use of laboratory facilities during the initial stages of this study.

Literature Cited

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OBITUARY NOTICES

PRESTON M. BAUMAN
Nov. 8, 1914–Aug. 16, 1977

ALLEN MCINTOSH
May 3, 1893–Oct. 22, 1977

DALE A. PORTER
Nov. 4, 1909–Dec. 10, 1977

BENJAMIN SCHWARTZ
Nov. 25, 1889–Oct. 20, 1976

Research Note

Infectivity of *Sarcocystis* in Beef and Beef Products from a Retail Food Store

Cysts of *Sarcocystis cruzi* (previously referred to as *S. fusiformis* and *S. bovicanis*) containing viable bradyzoites were present in freshly ground beef purchased from a grocery store and a supermarket in the Maryland suburbs of Washington, D.C. (Fayer 1975, Proc. Helminthol. Soc. Wash. 42: 138–140). Raw and rare hamburger patties prepared from this ground beef were infectious for dogs. Medium, well-done, and raw but previously frozen patties were not infectious. The present investigation was undertaken to substantiate and extend results of the previous study. Three experiments were conducted. In the first experiment luncheon meats (beef bologna and beef frankfurters), frozen beef (flaked sandwich steaks and hamburger patties), fresh beef (round steak and chuck roast), and cooked beef were purchased from a different supermarket in the Maryland suburbs of Washington, D.C. Beef bologna, beef frankfurters, frozen flaked sandwich steaks, and frozen hamburger patties were purchased at one time at the beginning of the experiment, and each was then appropriately stored until it was used. Fresh chuck roast and round steak and cooked rare roast beef were each purchased 3 times during the experiment. Each of the seven types of beef was cut into small pieces, trimmed of excess fat and fed to two dogs; 227 g was fed to each dog daily for 5 consecutive days. Two control dogs were not fed beef (Table 1). Dogs obtained from the Food and Drug Administration Sprawl Laboratory were 13- to 25-week old beagles that had never eaten meat. Except for the days on which they were fed the test meat, they were maintained on a ration of commercial pelleted dry feed. They were housed in separate cages in an isolated building. Feces were collected daily for 20 consecutive days beginning on the day of the first meat feeding (day 0) and were examined microscopically for the presence of *S. cruzi* sporocysts. Sporocysts were not found in feces from any dogs fed frozen beef (dogs 7, 8, 9, 10) or luncheon meat (dogs 11, 12, 13, 14) or from the controls (dogs 15, 16). A few *S. cruzi* sporocysts were found sporadically in feces from both dogs fed chuck roast (dogs 1, 2) beginning on day 13; from both dogs fed round steak (dogs 3, 4) beginning on day 15; and from both dogs fed rare roast beef (dogs 5, 6) beginning on day 11. All dogs were killed on day 20. The intestine from each was removed, washed of fecal contents, and weighed. Random segments totaling 25% of the total weight of the intestine were macerated in an electric blender containing a 5% solution of sodium hypochlorite to digest the intestinal tissue (J. P. Dubey, personal communication). The mixture was stirred for a minimum of 30 minutes at room temperature on a magnetic stirrer, strained through cheesecloth, diluted with 3 volumes of tap water, and centrifuged at approximately 225 *g* for 10 minutes. The supernatant fluid was discarded, the residue was resuspended in tap water. A drop of this suspension was placed on a hemocytometer, and any sporocysts present were counted (Table 1). All dogs that had *S. cruzi* sporocysts in their feces also had sporocysts in the intestinal digest, and dogs that had no sporocysts in their feces had no sporocysts in the intestinal digest (Table 1). The absence of infections in dogs fed the two frozen products substantiates previous findings that frozen hamburger was not infectious (Fayer, loc. cit.) (Gestrich and Heydorn, 1974, Berl. Münch. Tierärztl. Wschr. 87: 475–486). Processed meats such as beef bologna and beef frankfurters are required to attain a temperature of 148°F (64.4°C). However, most manufacturers/processors cook meat at temperatures as high as 160°F (71.1°C) in order to prolong the shelf life of the product.

In experiment 2 (Table 1), two dogs and one of the authors (R. F.) each ate 227 g of rare roast beef daily for 5 days. All fecal examinations were negative for sporocysts for 20 days beginning on the day of the first feeding. The two dogs were killed 21 DAI and the intestine minced and digested with sodium

Table 1. Recovery of *Sarcocystis cruzi* sporocysts from dogs and attempted recovery of *Sarcocystis* sporocysts from a human volunteer fed retail purchased beef.

Exp. No.			Type of beef fed and host No.*	Sporocysts in feces	Prepatent period (days)	No. of *Sarcocystis* sporocysts calculated†
1	D	1	Chuck roast	Positive	13	330
	D	2	" "	"	13	312
	D	3	Round steak	"	15	1,488
	D	4	" "	"	18	2,080
	D	5	Roast—rare	"	14	576
	D	6	" "	"	11	744
	D	7	Frozen minute steak	Negative	Negative	0
	D	8	" "	"	"	0
	D	9	Frozen hamburger patties	"	"	0
	D	10	" "	"	"	0
	D	11	Beef bologna	"	"	0
	D	12	" "	"	"	0
	D	13	Frankfurters	"	"	0
	D	14	"	"	"	0
	D	15	(Control)	"	"	0
	D	16	"	"	"	0
2	D	17	Roast—rare	Negative	Negative	44
	D	18	" "	"	"	60
	H	1	" "	"	"	Not done
3	D	19	Ground beef	Positive	22	672
	D	20	" "	"	12	1,872
	H	1	" "	Negative	Negative	Not done

* Abbreviations D = dogs; H = human.
† Thousands in intestine; experiment 1, day 20; experiment 2, day 21; experiment 3, day 23.

hypochlorite as described above. Although sporocysts had not been found in the fecal floats, sporocysts were recovered from the intestinal digest (Table 1).

In experiment 3 (Table 1), two dogs and one of the authors (R. F.) each ate 227 g of ground beef daily for 3 days. Feces were examined for 22 days beginning on the day of the first feeding. Both dogs passed sporocysts (day 20 and days 12, 14, 22) but the human did not (Table 1).

In three recent reports, human volunteers who consumed raw beef became infected with *Sarcocystis hominis* and shed sporocysts in their feces (Rommel and Heydorn, 1972, Berl. Münch. Tierärztl. Wschr. 85: 143–145; and Aryeety and Piekarski, 1976, Z. Parasitenkd. 50: 109–124; Heydorn, 1977, Arch. Lebensmittelhyg. 28: 27–31). The public health implications suggest illness directly attributed to *Sarcocystis* as reported for a human volunteer who consumed raw beef (Heydorn, loc. cit.). The human in the present study neither shed sporocysts nor had any signs of illness in either experiment 2 or 3. No conclusions can be drawn, however, regarding prevalence of *S. hominis* in beef in the United States from such a small sampling.

The present finding, that *S. cruzi* in chuck roast, round steak, and rare roast beef is infectious for dogs, extends the previous finding that *S. cruzi* is present and infectious in retail beef and suggests that *S. hominis*, when present, could probably also survive in retail beef.

Acknowledgment

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Research Note

Neoechinorhynchus cylindratus (Acanthocephala) from the Troglodytic Fish, *Amblyopsis spelaea*, in Kentucky

Despite the fact that scientific interest in caves began in 17th Century Europe and early biological studies emphasized descriptions and faunal surveys (Poulson and White, 1969, Science 165: 971–981), no acanthocephalan is previously known from a troglodytic (obligate cave form) fish. It was surprising, therefore, when one of 50 specimens of the troglo-

dyte, *Amblyopsis spelaea*, was discovered to harbor four specimens of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) Van Cleave, 1919, a widely distributed parasite of numerous piscine species. The infected fish was captured in "Under the Road Cave" in Breckenridge County, Kentucky. Other specimens examined were from that cave and several private, little known caves of the immediate vicinity. No helminth has been previously reported from *A. spelaea*.

The acanthocephalans were intestinal but immature. Three males were 3.4, 4.0, and 4.3 mm in length. The single female, 4.1 mm long, contained ovarian balls but no eggs. Specimens from the cave fish did not differ in any detail from the original description of *Neoechinorhynchus cylindratus* by Van Cleave (1913, Zool. Anz. 43: 177–190) or the expanded redescription by Ward (1940, Trans. Am. Microsc. Soc. 59: 327–347).

Amblyopsis spelaea is typical of troglodytes in being blind and lacking pigment and in possessing numerous neuromasts and elaborated lateral lines (Poulson, 1963, Am. Midl. Nat. 70: 257–290). According to Poulson (1963, loc. cit.), a sequence in adaptation of members of the Amblyopsidae to caves is shown in order by the epigean (surface) fish *Chologaster cornuta*, the troglophilic (facultative cave form) *C. agassizii*, and the troglodytic *A. spelaea*. It is possible that *Neoechinorhynchus cylindratus* is distributed from the surface to deep within caves through parasitism, although not yet discovered, of such a series of fishes. It is more likely, however, that epigean ostracod intermediate hosts washed into caves during floods or through sinkholes are the source of infection. The known range of *A. spelaea* is from Mammoth Cave (Ky.) northward into caves of unglaciated regions of Indiana (Clay, 1975, Ky. Dept. Fish Wildl. Resour., Frankfort, 416 p.). The entire area is karst limestone riddled with a multitude of interconnecting subterranean streams forming an enormous subterranean network. This network of caves is an open system with indirect connections to large rivers, such as the Cumberland, Green, and Ohio, and to the surface through sinkholes characteristic of the region's plains. Dyes introduced into sinkholes streams indicate that surface water flows from these streams through the caves and finally drains into major rivers (Mohr and Poulson, 1966, McGraw-Hill, N.Y., 232 p.). Influx from rivers, streams, and sinkholes during periods of heavy rain and high water brings organic matter and small organisms into the cave environment and even results in plankton blooms within the hypogean system (Poulson and Smith, 1969, Proc. 4th Int. Congr. Speleol. 4: 197–201). Such flow could readily carry acanthocephalan infected ostracods into the cave system. *Amblyopsis spelaea* forages along walls and ledges, on the substrate, and sometimes in midwater of its cave environment. In the laboratory, copepods and amphipods are readily located and consumed (Poulson, 1963, loc. cit.).

The inability of troglodytic fauna to survive in natural surface waters provides a barrier whereby certain parasites could be isolated in the hypogean environment. Although there is no evidence that a population of *Neoechinorhynchus cylindratus* is isolated in this manner, such occurrences, if detected, could provide models for study of speciation. Specimens of *N. cylindratus* collected from *Amblyopsis spelaea* are morphologically identical to those from surface fishes, and no sign of divergence was detected. This incident does, however, reflect the dynamic nature of the cave environment and illustrates its potential as a study system for helminthologists.

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Research Note

The Passage of Cysts of *Fasciola hepatica* in the Feces of the Snail Intermediate Host

Campbell and Todd (1956, Trans. Am. Microsc. Soc. 75: 241–243) and Taylor and Parfitt (1957, Trans. Am. Microsc. Soc. 76: 327–328) noted the passage of cysts of *Fasciola hepatica* in the feces of the snail intermediate host of this parasite. The former conducted an infectivity test on a ewe using fecal cysts but failed to establish infection; the latter showed such cysts were infective in mice. The origin of fecal cysts is not fully understood, and there appear to be two possibilities. First, they may be ingested by snails; secondly, cercariae in infected snails may gain access to the alimentary tract and encyst there. Thus, fecal passage may be a "natural" form of release. If both phenomena occurred, an explanation for the conflicting results in previous work would be apparent. This report describes some investigations carried out in relation to these questions.

A laboratory strain of *Lymnaea tomentosa*, the intermediate host of *F. hepatica* in Australia, was utilized in this study; the snails were experimentally infected each with 20 miracidia of *F. hepatica*. Both infected and uninfected snails were used for observations on the occurrence of cysts in the feces. Cysts were isolated from feces under a dissection microscope and washed thoroughly in distilled water. Some cysts had lost their outer walls; the outer cyst walls were mechanically removed from the remainder. The viability of metacercariae in their inner cysts was assessed by the presence of refractile excretory granules (Boray, 1969, Adv. Parasitol. 7: 95–210). Viable cysts were used to infect five-week-old male Wistar rats, a suitable age group for infection (Rajasekariah and Howell, 1977, Int. J. Parasitol. 7: 119–121).

The presence of cysts in fecal strands of *L. tomentosa* is shown in Figure 1. The appearance of fecal cysts and its relationship to the presence or absence of metacercariae in the environment is shown in Table 1. It is clear that if snails, whether infected or not, have access to cysts, they ingest them. When snails are placed in a cyst-free environment, infected specimens eventually pass cysts in their feces, suggesting that the environment becomes contaminated with cysts and these are ingested. After this experiment, 18 infected snails were kept in individual petri dishes, and the release of fecal cysts more closely observed. Twelve snails (67 percent) passed cysts in their feces, but only after cercariae had been discharged and encysted. This observation indicated that the passing of fecal cysts was closely linked with the appearance of cysts in the environment, but, nevertheless, it did not exclude the possibility that fecal passage of cysts was a a "natural" form of release. An attempt was made to clarify this question. Infected snails (9 in each group) were placed either on wire mesh, partially immersed in water so that the snails could not gain access to cysts, or left free in the dish. After 24 hours the base of the dish containing snails on wire mesh was studded with numerous cysts but none were seen in the feces. The dish containing the other group of snails contained only a few cysts, but cysts were frequently seen in the snails' feces. These results suggest that the passage of cysts in the feces is not a natural form of

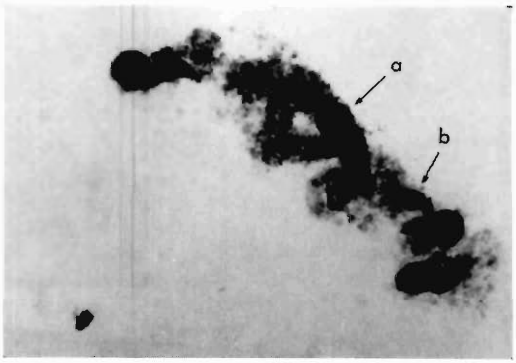

Figure 1. Cysts of *Fasciola hepatica* in a fecal strand of *Lymnaea tomentosa*. Arrows show (a) intact cyst and (b) cyst without its outer wall.

Table 1. Cysts of *Fasciola hepatica* in the feces of infected snails, 24 hours after snails were set up in petri dishes.

L. tomentosa	Number used	Number of cysts in each petri dish initially	Presence of cysts in fecal material	Number of cysts ingested by snails
Infected	8	300	Positive	240
Noninfected	8	275	Positive	230
Infected	7	—	Positive	Cysts seen in petri dish
Noninfected	7	—	Negative	—

emission; rather the snails ingest cysts present in the environment and these pass in the feces.

The viability of fecal cysts and their infectivity to rats is shown in Table 2. Thirteen percent of fecal cysts were viable, compared to 78 percent of cysts which were formed on the base of the petri dish. Significantly more ($P < 0.01$) worms were recovered from rats which received naturally occurring cysts than from those which received fecal cysts. Fecal cysts showed 93.4 percent loss of infectivity.

The above results indicate that *L. tomentosa* releases cercariae of *Fasciola hepatica* which encyst on the surface of petri dishes. Snails which have access to the cysts ingest them. The cysts pass in the feces, some of them

Table 2. Infectivity of fecal cysts of *Fasciola hepatica* to the rat.

Rats	Source of cysts	Percentage viability	Viable metacercariae administered	Worm recovery Mean ± SD
6	Feces	13	10	0.66 ± 0.8^a
6	Naturally formed	78	10	3.16 ± 1.7^b

a < b, $P < 0.01$; Mann Whitney U-test.

lacking the outer cyst wall (Dixon, 1966, Parasitology 56: 431–456). There seems to be no indication as suggested by Campbell and Todd (1956, loc. cit.) that cercariae enter the snail gut, encyst, and pass in the feces.

The apparent viability (presence of refractile excretory granules) of fecal cysts is not a reliable indicator of their infectivity to rats. However, it is possible that metacercariae derived from such cysts retain some of their antigenic potential, perhaps in a manner similar to those attenuated by X-irradiation (Sokolic, 1968, Isotopes and Radiation in Parasitology 1: 93–105), and are capable of immunizing the host against reinfection.

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Research Note

Helminths of Sympatric Barbary Sheep and Mule Deer in the Texas Panhandle

The Texas Parks and Wildlife Department released 268 mule deer, *Odocoileus hemionus*, in Palo Duro Canyon from 1949 through 1951 to augment a small remnant population (DeArment, 1971, Fed. Aid Rept., Texas Parks Wildl. Dept., Austin, 20 p.). Forty-four Barbary sheep, *Ammotragus lervia*, were introduced into the canyon in 1957–58. Palo Duro Canyon is a deep irregular gorge formed by erosion in the otherwise flat featureless topography of the Texas Panhandle. It is about 80.5 km long by 17.5 km wide (maximum). Canyon walls form steep bluffs overlooking the floor 244 m below. Erosion resistant formations are preserved as mesas, ledges, benches, or ridge fingers. The vegetation reflects this topographic diversity and includes mesic species along stream bottoms as well as typical prairie and semiarid plants. The broken topography and good browse afford suitable

Table 1. Helminths of Barbary sheep and mule deer from Palo Duro Canyon, Texas.

Host Parasite	No. of hosts infected	No. of parasites recovered (range)
Barbary sheep (N = 5)		
Monezia expansa	1	1
Skrjabinema caprae	2	1–88
Haemonchus contortus	2	2–16
Mule deer (N = 5)		
Dictyocalus viviparus	1	1
Haemonchus contortus	3	2–24

habitat for increasing populations of both large herbivores. This study was conducted to compare the helminth faunas of sympatric Barbary sheep and mule deer from this area.

The study area is located along the northern canyon rim 38.6 km Southeast of Amarillo, Armstrong County, Texas. Viscera were collected from five Barbary sheep and five deer harvested during the hunting seasons in November, 1976. This sample size is consistent with that reported for similar studies by Prestwood et al. (1975, J. Am. Vet. Med. Assoc. 166: 787–789; 1976, J. Wildl. Dis. 12: 380–385). Necropsy and parasitologic techniques were similar to those described by Prestwood et al. (1973, J. Am. Vet. Med. Assoc. 163: 556–561). Simpson's diversity index (Holmes and Podesta, 1968, Can. J. Zool. 46: 1193–1204) was calculated to quantify the degree to which helminth species were equitably concentrated within each of the ungulate hosts. An index of similarity (Holmes and Podesta, 1968, loc. cit.) indicated the extent to which the helminth fauna differs between Barbary sheep and mule deer. Representative specimens of parasite species recovered are deposited in the USNM Helm. Coll. (Nos. 74538–74542).

Two nematodes and one cestode, and two nematodes were recovered from Barbary sheep and mule deer, respectively, but worm burdens were very light (Table 1). Two Barbary sheep and two mule deer were not infected with helminths. Simpson's index was 0.36 for Barbary sheep and 0.63 for mule deer. The index of similarity was 40.

Allen et al. (1956, J. Parasitol. 42: 19) reported 14 helminth species from two New Mexico Barbary sheep. Seventeen species of helminths were recovered from seven Barbary sheep hosts, also collected in New Mexico (Allen, 1960, Desert Bighorn Council, Trans. 4: 17–22). The species reported herein were also recovered in those studies. Although *Haemonchus contortus* has been found in Texas white-tailed deer, *Odocoileus virginianus* (Robinson et al., 1967, J. Wildl. Manage. 31:455–459; Emerson, 1969, Bull. Wildl. Dis. Assoc. 5: 137–139), this is the first record of this species in Texas Mule deer. Apparently this is the first record of *Dictyocalus viviparus* in mule deer from the Southwest.

As Levine (1968, Nematode Parasites of Domestic Animals and Man, Burgess Publ. Co., p. 27) indicated, the critical phase of the nematode life cycle is transmission from host to host. The range of environmental conditions which tends to promote optimum pasture transmission of several common ruminant trichostrongyles (Levine, 1968, loc. cit., p. 31) ordinarily limits the possibility of transmission to spring and summer months (May to September) in the Texas Panhandle. High evapotranspiration potentials reduce soil moisture during much of this potential transmission period (June through August) further limiting possibilities for trichostrongyle larval development and survival. These factors probably account for low *Haemonchus contortus* infection levels and the absence of other trichostrongylids such as species of *Ostertagia* and *Trichostrongylus* in this area.

The relatively high diversity indexes, particularly that for mule deer, confirm the concentration of dominance in a few helminth species. The low index of similarity indicates that the helminth faunas of these two sympatric ungulates are separate and distinct. With the possible exception of *H. contortus*, neither ungulate is a reservoir for the other. Considering the paucity of helminth species and the low levels of infection, neither Barbary sheep nor mule deer can be considered an important helminth reservoir for domestic livestock in this area.

The authors are grateful to Messrs. Terrill and Tom Christian, Ed Harrell, and Roy and Don Ransom for permission to collect viscera from animals shot on the Christian, Harrell, and Ransom Ranches. Mr. James Standridge was helpful in providing many of the viscera examined. Dr. Kenneth L. Stromborg, De-

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Research Note

Helminth Parasites of Some Anurans of Northwestern Ohio

One hundred thirty anurans of six species: *Rana pipiens* Shreber, 1782, *R. catesbeiana* Shaw, 1802, *Acris crepitans* Baird, 1854, *Hyla crucifer* Weid, 1838, *Pseudacris triseriata* (Weid, 1838) Stjneger and Barbour, 1917, and *Bufo americanus* Holbrook, 1836, were collected from Fulton, Hancock, Lucas, Ottawa and Wood counties in N.W. Ohio. Specimens were collected by hand or with the aid of a hand net, transported to the laboratory, killed with a blow to the head and dissected. All internal organs were removed, placed in a petri dish with 0.85% saline and examined. The body cavity also was thoroughly examined. Trematodes were flattened and fixed in Lavdowsky's FAA, stained in Mayer's hemalum, dehydrated, cleared and mounted in Canada balsam and identified to species. Nematodes were fixed in glacial acetic acid, stored in a solution of 5% glycerin in 70% ethanol, and transferred to 100% glycerin for examination and species identification.

Four species of trematodes and eight species of nematodes were recovered from infected amphibians (Table 1). All of the trematodes have previously been reported from Ohio (Koeppe, 1941, M.A. Thesis, KSU, Kent, Ohio), although *Pseudacris triseriata* is reported as a new host for *Glypthelmins quieta* (Stafford, 1900) Stafford, 1905 in Ohio. Ulmer (1970, Am. Midl. Nat. 83: 38–64) reported *P. triseriata* as a host in Iowa. Other

Table 1. Parasites of six species of anurans from northwestern Ohio.

Host	No. examined	No. infected	% infected
Acris crepitans	30		
Nematodes			
Physaloptera ranae (larval forms)		3	10.0
Rhabdias ranae		9	30.0
Bufo americanus	34		
Nematodes			
Cosmocercoides dukae		4	11.8
Oswaldocruzia leidyi		1	2.9
Physaloptera ranae (larval forms)		2	5.9
Rhabdias bufonis		17	50.0
Hyla crucifer	12		
Trematodes			
Glypthelmins quieta		10	83.3
Pseudacris triseriata	24		
Nematodes			
Cosmocercoides dukae		6	25.0
Oswaldocruzia leidyi		3	12.5
Trematodes			
Glypthelmins quieta		15	62.5
Rana catesbeiana	24		
Nematodes			
Foleyella americana		1	4.2
Physaloptera ranae (larval forms)		2	8.3
Rhabdias ranae		1	4.2
Spironura catesbeiana		5	20.8
Trematodes			
Glypthelmins quieta		7	29.2
Gorgodera amplicava		15	62.5
Haematoloechus longiplexus		14	58.3
Megalodiscus temperatus		1	4.2
Rana pipiens	6		
Nematodes			
Cosmocercoides dukae		1	16.7
Oswaldocruzia leidyi		1	16.7
Physaloptera ranae (larval forms)		2	33.3
Rhabdias ranae		3	50.0
Spironura ranae		1	16.7

new host records in Ohio include *Acris crepitans* for *Physaloptera ranae* Walton, 1931, and *Rhabdias ranae* Walton, 1929: *Bufo americanus* for *Oswaldocruzia leidyi* Travassos, 1917, *Rhabdias bufonis* (Schrank, 1788), and *P. ranae*: *Pseudacris triseriata* for *O. leidyi*; *Rana catesbeiana* for *Foleyella americana* Walton, 1929, *Spironura catesbeiana* (Walton, 1929), *P. ranae* and *R. ranae*; and *Rana pipiens* for *Spironura ranae* Walton, 1941, *O. leidyi* and *P. ranae*.

Oswaldocruzia leidyi from *P. triseriata*, *P. ranae* from *A. crepitans* and *S. ranae* from *R. pipiens* constitute new host records. Nematodes reported for the first time in Ohio include *F. americana*, *O. leidyi*, *R. bufonis*, *S. catesbeiana* and *S. ranae*.

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Research Note

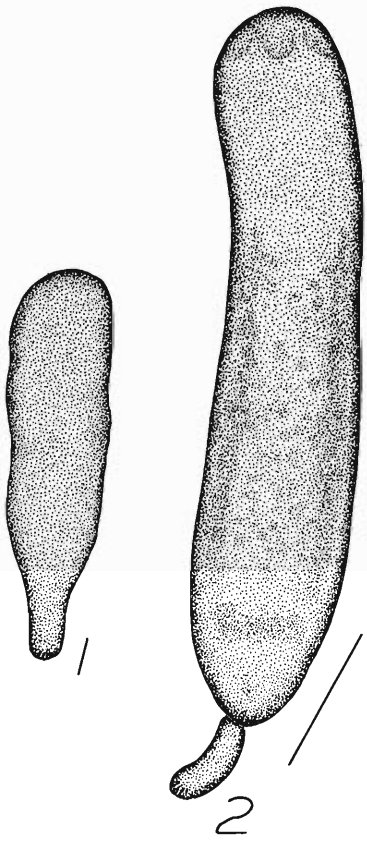
Larval Development of *Glaridacris vogei* (Cestoda: Caryophyllaeidae)

Six species comprise the genus *Glaridacris* Hunter. The larval development of three species, *G. catostomi* Cooper, *G. confusa* Hunter, and *G. oligorchis* Haderlie, was reported by Calentine (1967, Proc. Iowa Acad. Sci. 72: 418–424), Calentine and Williams (J. Parasitol. 53: 692–693) and McCrae (Diss. Abstracts 21: 2835–2836), respectively. This report presents data on the experimental larval development of a fourth species, *G. vogei* Mackiewicz.

Gravid *G. vogei* were obtained from large-scale suckers, *Catostomus macrocheilus* Girard, from Lane Co. Oregon (Fern Ridge Reservoir) and kept in distilled water until eggs were shed. All experiments were conducted at room temperature (19 to 23°C). Oligochaetes, obtained from a commercial scientific supply company or from various areas in Lane Co., were maintained in the laboratory for 42 days or more and examined at 40 × magnification prior to use. Operculate eggs, after 18 days embryonation, were placed in sterilized mud and oligochaete annelids were exposed to this medium. After exposure for 1–2 days, the oligochaetes were maintained in sterilized lake water under constant aeration. Cestodes and oligochaetes for morphological studies were fixed in cold and hot formalin, respectively.

Four feeding experiments involving five species of oligochaetes were conducted. The results were (number exposed–number infected): (Tubificidae) *Limnodrilus claparedeianus* Ratzel (198–89); *L. hoffmeisteri* Claparède (122–39); *Tubifex templetoni* Southern (69–23); (Naididae) *Dero digitata* (Muller) (67–4); *Stylaria lacustris* (L.) (74–0). Oligochaetes were examined at five-day intervals postinfection. Infection percentage was determined upon examination at 10 days postinfection. Procercoids developed in three species of Tubificidae, but the four individuals in *D. digitata* observed at 10 days were not present at 15 days postinfection. Undeveloped embryonated eggs were, however, observed in the digestive system of both naid species. Procercoid development required 38 to 70 days. By 55 days postinfection, fully developed procercoids (Fig. 1) from tubificids averaged 1.5 mm (1.3–1.9) in body length with a cercomer length of 0.63 mm (0.58–0.70).

Of oligochaetes collected from Fern Ridge Reservoir during a four-month period (September–December 1975), *G. vogei* procercoids parasitized 0.8% of 2,046 *L. claparedeianus* but none of *D. digitata*. The identification of these procercoids was based upon comparing morphology with *G. vogei* from experimental infections. Also, the only other caryophyllaeids that I have found in Fern Ridge Reservoir



Figures 1, 2. ***Glaridacris vogei*** **procercoids from** ***Limnodrilus claparedeianus.*** **1. Procercoid at 23 days. 2. Procercoid at 44 days. Scale equals 0.3 mm.**

possess a nonloculate scolex: *Khawia* spp. Calentine and Ulmer, *Hunterella nodulosa* Mackiewicz and McCrae, and *Atractolytocestus huronensis* Anthony. The results of this study indicate that tubificids are the probable oligochaete host and, experimentally, that eggs of *G. vogei* may be ingested by oligochaetes of the family Naididae, but procercoids do not develop.

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THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON CONSTITUTION

ARTICLE 1

Name

The name of the Society shall be the Helminthological Society of Washington.

ARTICLE 2

Object

The object of the Society shall be to provide for the association of persons interested in parasitology and related sciences for the presentation and discussion of items of interest pertaining to those sciences.

ARTICLE 3

Membership

Section 1. There shall be three classes of members, namely, regular, life, and honorary.

Section 2. Any person interested in parasitology or related sciences may be elected to membership in the Society. The privileges and responsibilities of members are set forth in the By-Laws.

Section 3. Any person who has rendered conspicuous and continuous service as a member of the Society for a period of not less than 15 years, and has reached the age of retirement, may be elected to life membership. Life members shall have all the privileges of regular members but shall be exempted from payment of dues. The number of life members shall not exceed 5% of the membership at the time of election.

Section 4. Any person who has attained eminent distinction in parasitology or related sciences may be elected to honorary membership. An honorary member shall have all the privileges of membership except voting, holding office, or having any interest in the real or personal property of the Society. He shall be exempted from the payment of dues. The number of honorary members shall not exceed 10 at any one time and not more than one honorary member shall be elected in any one year.

ARTICLE 4

Officers

Section 1. The officers of the Society shall be a President, a Vice-President, a Recording Secretary, a Corresponding Secretary-Treasurer, and such other officers as the Society may deem necessary. Only members in good standing and whose dues are not in arrears shall be eligible for election to office. Terms of office shall be 1 year.

Section 2. The President shall preside over all meetings, appoint all committees except the Executive Committee, and perform such other duties as may properly devolve upon a presiding officer. The President may appoint an Archivist, a Librarian, a Custodian of Back Issues, and an Assistant Corresponding Secretary-Treasurer as needed.

Section 3. The Vice-President shall preside in the absence of the President, and when so acting shall perform such duties as would otherwise devolve upon the President. The Vice-President shall serve as Program Officer.

Section 4. In the absence of both President and Vice-President, the member, among those present, who last held the office of President shall be the presiding officer. Under other circumstances, members may elect a presiding officer but business action taken shall be reviewed by the Executive Committee.

Section 5. The Recording Secretary shall record the proceedings of all meetings and shall present at each meeting a written report of the transactions of the preceding meeting, shall keep an accurate and complete record of the business transacted by the Society in its meetings, and shall notify the Corresponding Secretary-Treasurer of the election of new members. He shall prepare for publication in the Proceedings an annual digest of scientific meetings and business transacted, including elections of officers and new members.

Section 6. The Corresponding Secretary-Treasurer shall be responsible for all funds, collections, payment of bills, and maintenance of financial records. At the beginning of each year, he shall present to the Society an itemized statement of the receipts and expenditures of the previous year; this statement shall be audited by a least two members of the Society.

Article 5

Executive Committee

Section 1. There shall be an Executive Committee which shall be the administrative body of the Society.

Section 2. The number of members of the Executive Committee, their duties, terms of office, the method of selecting them, and of filling vacancies shall be provided in the By-Laws.

Article 6

Awards Committee

Section 1. There shall be an Awards Committee to select individuals for special commendaton.

Section 2. The number of its members, their duties, and terms of office shall be provided in the By-Laws.

Article 7

Editorial Board

Section 1. There shall be an Editorial Board for the Society's publications, which shall include The Proceedings of the Helminthological Society of Washington.

Section 2. The number of its members, their duties, terms of office, the method of selecting them and of filling vacancies shall be provided in the By-Laws.

Article 8

Publication

The publications of the Society shall be issued at such times and in such form as the Society through its Editorial Board may determine.

Article 9

Meetings

Section 1. Meetings of the Society shall be held monthly during January, February, March, April, May, October, November, and December, the time and place to be determined by the officers of the Society.

Section 2. The October meeting shall be known as the Anniversary Meeting and the Anniversary Award, when made, ordinarily shall be presented at this meeting.

Article 10

Amendments to the Constitution

Any amendment to the Constitution shall be presented in writing at a regular meeting. It shall not be acted upon until the following meeting. A two-thirds vote of the members in attendance shall be required for the adoption of any proposed amendment.

BY-LAWS

Article 1

Procedure

The rules contained in Robert's *Rules of Order*, Revised, shall govern the Society in all cases to which they are applicable, and in which they are not inconsistent with the By-Laws or the special rules of order of this Society.

Article 2

Order of Business

Call to order.
Reading of minutes of previous meeting.
Election of new members.
Reports of committees.
Unfinished business.
New business.
Presentation of notes and papers.

Article 3

Election of Members

Section 1. Candidates for election to regular membership may be sponsored and proposed only by members in good standing. The candidate shall submit a duly executed and signed application to the Recording Secretary, who in

turn shall submit the application to the Executive Committee. The Committee shall review the application and submit its findings to the Society. Voting may be either by voice or by ballot. The Corresponding Secretary-Treasurer shall inform the candidate of the action of the Society.

Section 2. Payment of dues shall be considered as evidence of acceptance of membership in the Society. Election to membership shall be void if the person elected does not pay dues within 3 months after the date of notification of election.

Section 3. Nominations for Honorary and Life Membership, approved by the Executive Committee, shall be submitted to the membership for election at a regular meeting.

Article 4

Nomination and Election of Officers

Section 1. The Executive Committee, acting as the nominating committee of the Society, shall prepare a slate of officers and present this to the Society at the October meeting of each year. Independent nominations may be made in writing by any five members. In order to receive consideration, such nominations must be in the hands of the Recording Secretary at the time of the election at the November meeting.

Section 2. The election of officers shall be held prior to the presentation of notes and papers at the November meeting. Voting may be either by voice or by ballot.

Section 3. The last order of business at the December meeting shall be the installation of officers, and the naming of necessary appointees.

Article 5

Meetings

Notice of the time and place of meetings shall be given by the Recording Secretary at least 10 days before the date of the meeting.

Article 6

Quorum

The members in attendance at any regular meeting shall constitute a quorum.

Article 7

Dues and Debts Owed to the Society

Section 1. Annual dues shall be fixed by the Executive Committee, subject to ratification by the Society. Spouses of members who do not wish independent subscriptions to the Proceedings may be admitted to full membership upon payment of one dollar annual dues.

Section 2. The fiscal year for payment of dues and for all other business purposes shall be the same as the calendar year, that is, from 1 January to 31 December, and dues shall be payable on or before 1 January. The dues of a newly elected member paid prior to 1 July of the year of his election shall be credited to that year; if paid after 1 July, they shall be credited either to the current fiscal year or to the following one, at the option of the new member. The dues shall include subscription to the Society's publication; only those members whose dues are paid shall receive the publication.

Section 3. All other obligations owed to the Society by members or nonmembers shall be due and payable 30 days after bills are rendered; the further extension of credit to those whose obligations are in arrears shall be a matter for decision by the Executive Committee.

Article 8

Suspension and Reinstatement

Any member whose dues are in arrears for 2 years shall be dropped from membership. Members who have been dropped for nonpayment of dues may be reinstated automatically upon payment of the dues in arrears and the dues for the current year, or may be otherwise reinstated by action of the Executive Committee.

Article 9

Editorial Board

Section 1. The Editorial Board shall consist of an Editor and other members in good standing, representing to the fullest practicable degree the varied scientific interests and the employment-group affiliations of the Society's membership.

Section 2. The Editor shall be elected by the Society for a term of 5 years on nomination by the Executive Committee.

Section 3. Other members of the Editorial Board shall be appointed for terms of 3 years.

Section 4. The Editor, after consultation with the Editorial Board, shall appoint new members, formulate publication policies, and make all decisions with respect to format and content of the Society's publications. The Editor shall operate within financial limitations determined by the Executive Committee.

ARTICLE 10

Executive Committee

Section 1. The Executive Committee shall consist of 10 members in good standing as follows: The President, Vice-President, Recording Secretary, Corresponding Secretary-Treasurer, Editor, Immediate Past-President, and four members-at-large. The Committee shall represent to the fullest practicable degree the varied scientific interests of the Society's membership and the local distribution of its members.

Section 2. The President shall serve as chairman of the Executive Committee.

Section. 3. Members-at-large shall serve for a term of 2 years. Two members-at-large shall be appointed each year in November by the President-elect for the prescribed term of 2 years.

Section 4. Vacancies occurring on the Executive Committee for any reason shall be filled by appointment by the President, except as otherwise provided, the appointee to serve for the remainder of the unexpired term.

Section 5. The Executive Committee shall carry out the provisions of the Constitution and By-Laws and shall make decisions on all matters of general and financial policy not otherwise set forth in the Constitution and By-Laws and shall report its actions to the Society annually at the last regular meeting.

Section 6. The Executive Committee shall approve the selection of a depository for the current funds, direct the investment of the permanent funds, and act as the administrative body of the Society on all matters involving finance. It shall prepare and present to the Society at the beginning of each calendar year a budget based on the estimated receipts and expenditures of the coming year with such recommendations as may seem desirable.

Section 7. With the presentation of the annual budget the Executive Committee shall present to the Society, if feasible, the estimated cost for publication to be charged to contributors to the Society's publication for that year.

Section 8. Costs of publication, in excess of amounts borne by the Society, shall be borne by authors in accordance with guidelines established by the Executive Committee.

Section 9. The Executive Committee shall pass on all nominations for membership and on the reinstatement of deliquent members, except as otherwise provided, and shall make its recommendations to the Society.

ARTICLE 11

Awards Committee

Section 1. The Awards Committee shall consist of three members.

Section 2. Members shall serve for a term of 3 years with appointments staggered so that one new member is added each year. The senior member of the Committee shall serve as Chairman.

Section 3. The Awards Committee shall be charged with the duty of recommending candidates for the Anniversary Award which may be given annually or less frequently at the discretion of the Committee.

Section 4. The recipient of the Anniversary Award shall be or have been a Society member who is honored for one or more achievements of the following nature: (a) Outstanding contributions to parasitology or related sciences that bring honor and credit to the society, (b) an exceptional paper read at a meeting of the Society or published in its Proceedings, (c) outstanding service to the Society, and (d) other achievement or contribution of distinction that warrants highest and special recognition by the Society.

Section 5. The individual recommended shall be subject to approval by the Executive Committee.

ARTICLE 12

Provision for Dissolution of Funds

In the event the Society is disbanded, all monies shall be presented to the Trustees of the Brayton Howard Ransom Memorial Trust Fund for such purposes as that continuing body may deem advisable.

ARTICLE 13

Amendments to the By-Laws

Any amendment to these By-Laws shall be presented in writing at a regular meeting. It shall not be acted upon until the following meeting. A two-thirds vote of the members in attendance shall be required for adoption.

MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

(Alabama through Mississippi; remainder of list will appear in July issue)

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* Life Member
** Honorary Member
† Spouse Member
‡ Life Member deceased

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Date of publication, 8 February 1978

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