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New Subspecies of the Stomach Worm, *Obeliscoides cuniculi* (Graybill), of Lagomorphs

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ABSTRACT: Two new subspecies of *Obeliscoides cuniculi* are recognized. *Obeliscoides cuniculi cuniculi* (Graybill, 1923) Graybill, 1924 occurs in cottontail rabbits (*Sylvilagus floridanus*). *Obeliscoides cuniculi multistriatus* n. subsp. occurs in snowshoe hares (*Lepus americanus*). *Obeliscoides c. cuniculi* has 23–34 longitudinal cuticular ridges at midbody in males and 31–46 in females. *Obeliscoides c. multistriatus* has 53–76 longitudinal cuticular ridges at midbody in males and 72–127 in females. Ridges are larger and more prominent in *O. c. cuniculi* than in *O. c. multistriatus*. Dorsal and ventral ridges extend to the bursa in *O. c. cuniculi,* but only to the proximal end of spicules in *O. c. multistriatus*.

Lepus probably brought *Obeliscoides* to North America during the middle Pleistocene when the Bering Land Bridge was open to animal migration. Transfer of *O. cuniculi* from *Lepus americanus* to *Sylvilagus floridanus* and subsequent subspeciation could have occurred during the middle–late Pleistocene, when distribution of animals was disrupted by changes in temperature, moisture, and glacial activity.

Obeliscoides cuniculi (Graybill, 1923) Graybill, 1924 is a parasitic roundworm found in the stomach of cottontail rabbits (*Sylvilagus floridanus*), snowshoe hares (*Lepus americanus*), woodchucks (*Marmota monax*), and other hosts in North America (Ward, 1934; Maples and Jordan, 1966; Prestwood et al., 1973). However, it is essentially a parasite of lagomorphs. Graybill (1923, 1924) found his specimens in naturally infected domestic rabbits (*Oryctolagus cuniculus*), but indicated that the source of the infection was probably *Sylvilagus floridanus*.

In the present study, two new subspecies of *Obeliscoides cuniculi* are recognized and described. A possible origin of this subspeciation is also presented.

Materials and Methods

Specimens of *Obeliscoides cuniculi* were obtained from the stomachs of wild lagomorphs that were trapped, shot, or snared in Ontario and Alberta. Live animals were killed with Somnotol (MTC Pharmaceuticals, Hamilton, Ontario, Canada) or Nembutal (Abbott Laboratories, Montreal, Quebec, Canada).

Worms were examined live or fixed in 10% buffered formalin or hot glycerin alcohol (1 part glycerin to 9 parts 70% alcohol). Worms fixed in glycerin alcohol were cleared by allowing alcohol in the fixative to evaporate slowly, leaving worms in glycerin. The synlophe was studied by examining the surface of cleared whole mounts and by preparing cross sections of fixed and cleared worms. Cross sections were made at three locations, i.e., (a) in the region of the nerve ring, (b) midbody, and (c) near the distal extremity of spicules or near the anus in females. Cross sections were mounted in glycerin jelly, and longitudinal cuticular ridges were examined and counted using a microscope (400×). Cross sections of spicules were prepared similarly. Some worms were cleared in lactophenol or dissected to study spicules.

Worms examined using scanning electron microscopy were fixed in 10% buffered formalin and dehydrated through a series of increasing concentrations of

ethyl alcohol. Specimens were dried by critical-point drying using CO_2 substitution. Specimens were coated with a layer of gold palladium approximately 2×10^{-5} mm thick in a Technics Hummer 5 Sputter Coater, and were studied with an ETEC Autoscan scanning electron microscope (SEM) at 5–25 kV.

The following specimens from the United States Department of Agriculture, Beltsville, Maryland 20705, were examined:

USNM No.	Host	Locality
7736	*Oryctolagus cuniculus*	Texas
25470	*Oryctolagus cuniculus*	New Jersey
25471	*Oryctolagus cuniculus*	New Jersey
75434	*Sylvilagus floridanus*	So. Carolina
28292	*Sylvilagus* sp.	Middlefield (Conn.?)
42714	*Sylvilagus aquaticus*	Georgia
67096	*Marmota monax*	New York
66381	*Marmota monax*	Pennsylvania
65628	*Odocoileus virginianus*	Arkansas
58238-2	*Ovis canadensis*	Colorado

The following specimens from the National Museum of Natural Sciences, Ottawa, Ontario, K1A 0M8, were examined:

NMCIC(P) No.	Host	Locality
1979-261	*Lepus americanus*	Algonquin Park, Ontario
1978-1111	"rabbit"	Ontario
1978-1118	*Lepus* sp.	—
1978-1216	*Lepus americanus*	Algonquin Park, Ontario

In addition, specimens of *Obeliscoides cuniculi* from *Sylvilagus floridanus* collected in the southeastern United States were examined. Specimens of *O. cuniculi* that had been maintained in New Zealand white rabbits by continuous passage for 15 yr at the Ontario Veterinary College in Guelph were also examined. These latter specimens were originally obtained from snowshoe hare in Ontario.

Two lots of specimens of *O. cuniculi* maintained in domestic rabbits for a number of years in Montana were examined. One lot of specimens was originally obtained from wild cottontail rabbits (*Sylvilagus* sp.) collected in Ohio, and the other lot was originally obtained from wild woodchucks also collected in Ohio.

Results

Specimens of *O. cuniculi* from wild *Lepus americanus* and *Sylvilagus floridanus* can be distinguished by differences in the snylophe, and two subspecies of *O. cuniculi* are recognized. These two new subspecies are described below. The genus is redefined after examination of the type specimens and other material from lagomorphs in North America.

Obeliscoides Graybill, 1924

SYNONYM: *Obeliscus* Graybill, 1923.

Redefinition

Trichostrongyloidea, Trichostrongylidae (Leiper, 1908, subfam.) Leiper, 1912. Synlophe consisting of small, uniform, perpendicular cuticular ridges with internal cuticularized skeleton, and appearing as spines (arêtes[1]) in cross section. Inner circle of 6 large papillae and amphids peripheral to circumoral annulus. Amphids laterodorsal. Eight minute papillae present, occurring in pairs, lying in circle outside inner papillae. Buccal capsule absent. Deirids well developed, in form of hook. Prebursal papillae (Rays 1) present. Anteroventral and posteroventral rays (Rays 2 and 3) diverging at origin, then converging at distal extremity near edge of lobe. Externodorsal rays (Rays 8) not extending to edge of bursa. Dorsal lobe reduced. Dorsal ray bifurcating 2 or 3 times with paired lateral branches. Accessory bursal (dorsal) membrane present, containing small accessory dorsal ray. Spicules similar, short, equal in length. Gubernaculum absent.

Obeliscoides cuniculi cuniculi (Graybill, 1923) Graybill, 1924 (Figs. 2, 7–21, 23–33)

DESCRIPTION: Cuticle thin with weakly developed symmetrically arranged longitudinal ridges numbering at midbody 23–34 (N[2] = 44) in males and 31–46 (N = 45) in females. Ridges frequently discontinuous. Distance between ridges 15–32 μm (N = 5) in males and 15–22 μm (N = 5) in females. Anterior region of body lacking ridges, 69 μm in length in males and 125 μm in length in females. Ridges on lateral surfaces extending slightly anterior to those on dorsal and ventral surfaces. Transverse striations approximately 2 μm apart anterior to ridges. Transverse striations between ridges numbering approximately 1.5 per μm. Ridges extending to bursa in males and posterior to anus in females.

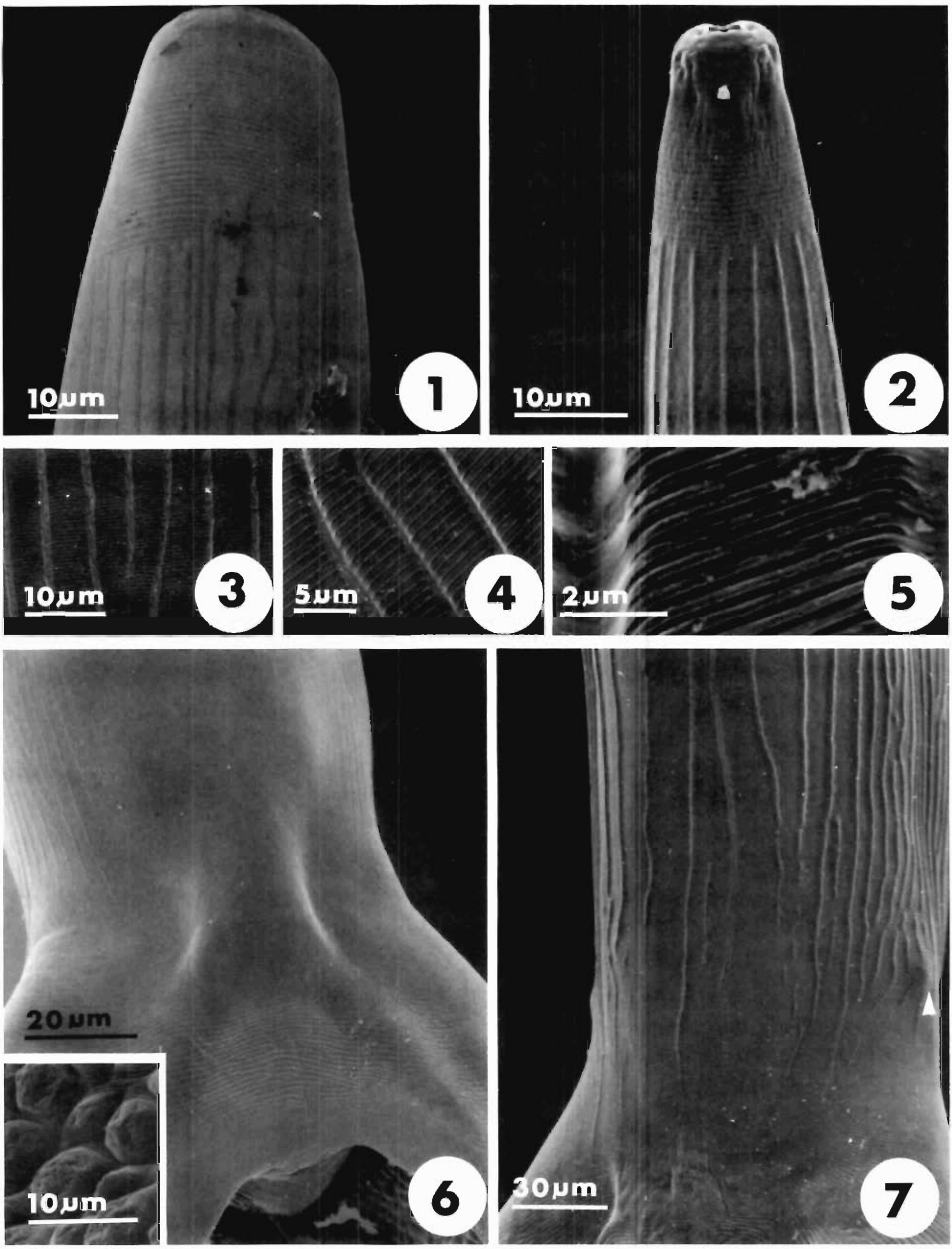
Oral opening triradiate with sclerotized circumoral annulus 3.5 μm in height in males and 5 μm in females. Excretory glands large with salient nuclei. Excretory glands extending posterior to esophageal–intestinal junction. Esophagus clavate; metacorpus indistinct. Esophageal gland nuclei prominent. Deirids usually at same level.

MALE (lectotype): Length 10.8 mm. Width of body at esophageal–intestinal junction 136 μm. Nerve ring 278 μm, excretory pore 498 μm, and deirids 553 μm from anterior extremity. Esophagus 812 μm in length.

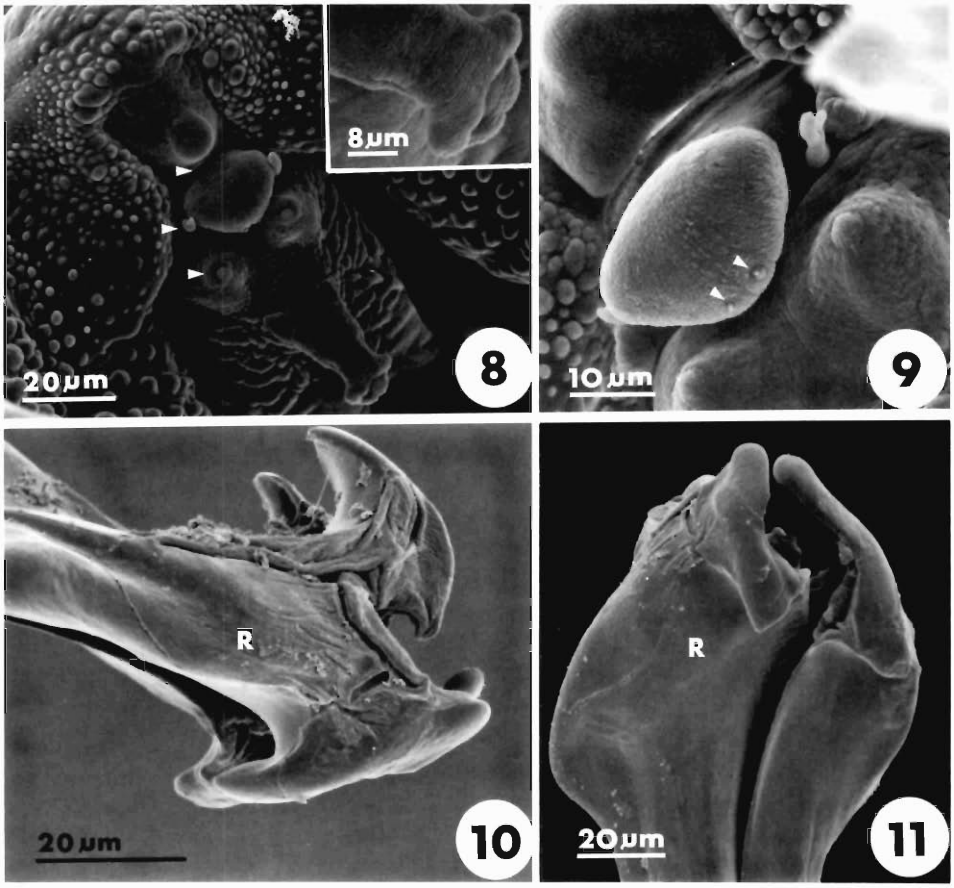
Prebursal papillae (Rays 1) conspicuous, lateral, pedunculated, 510 μm from posterior extremity. Bursa slightly longer than wide, consisting of 2 oval lateral lobes and small median dorsal lobe. Bosses prominent on inner surface of bursa except near margins. Fine radial striations present on outer surface of bursa. Anterolateral (Rays 4) and externodorsal rays (Rays 8) with distal extremity ending in papilla on dorsal surface of lateral lobes. Posterolateral rays (Rays 6) slightly longer than mediolateral rays (Rays 5), terminating closer to edge of lobe than

[1] Terminology of Durette-Desset (1978).

[2] N = number of specimens from Ontario examined.



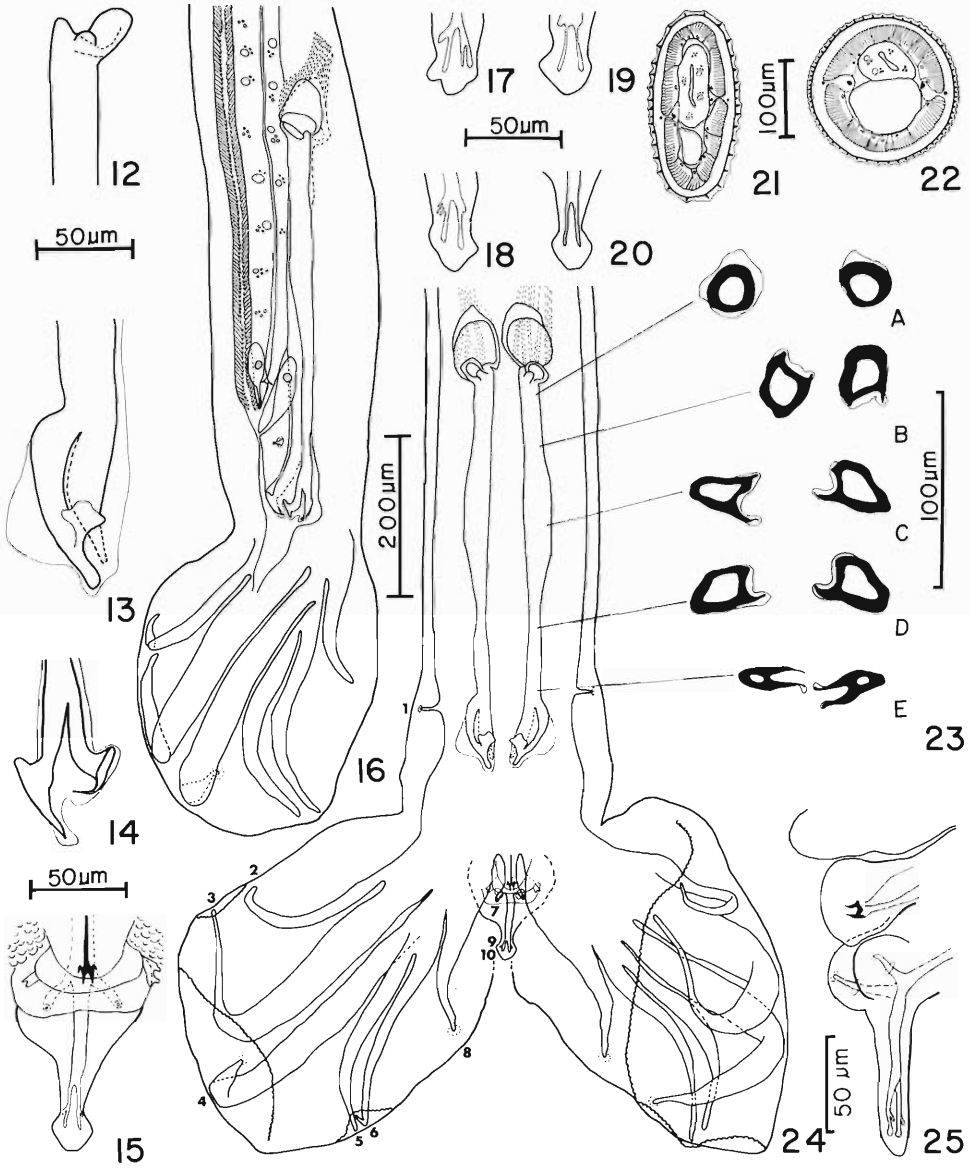
Figures 1–7. Scanning electron photomicrographs of *Obeliscoides cuniculi*. 1. Anterior extremity of adult female *O. cuniculi multistriatus*, lateral view. Note longitudinal ridges. 2. Anterior extremity of adult male *O. cuniculi cuniculi*, dorsal view. Note longitudinal ridges. 3. Longitudinal ridges on midbody of adult female of *O. cuniculi multistriatus*, lateral view. 4. Longitudinal ridges and transverse striations on midbody of adult male *O. cuniculi multistriatus*, lateral view. 5. Transverse striations on midbody of adult male *O. cuniculi multistriatus*, lateral view. 6. Bursa of adult male *O. cuniculi multistriatus*, dorsal view. Note that longitudinal ridges are absent dorsally. Inset shows bosses inside bursa, lateral view. 7. Prebursal region of adult male *O. cuniculi cuniculi*, ventral view. Arrow indicates prebursal papilla. Note that longitudinal ridges extend ventrally to bursa.



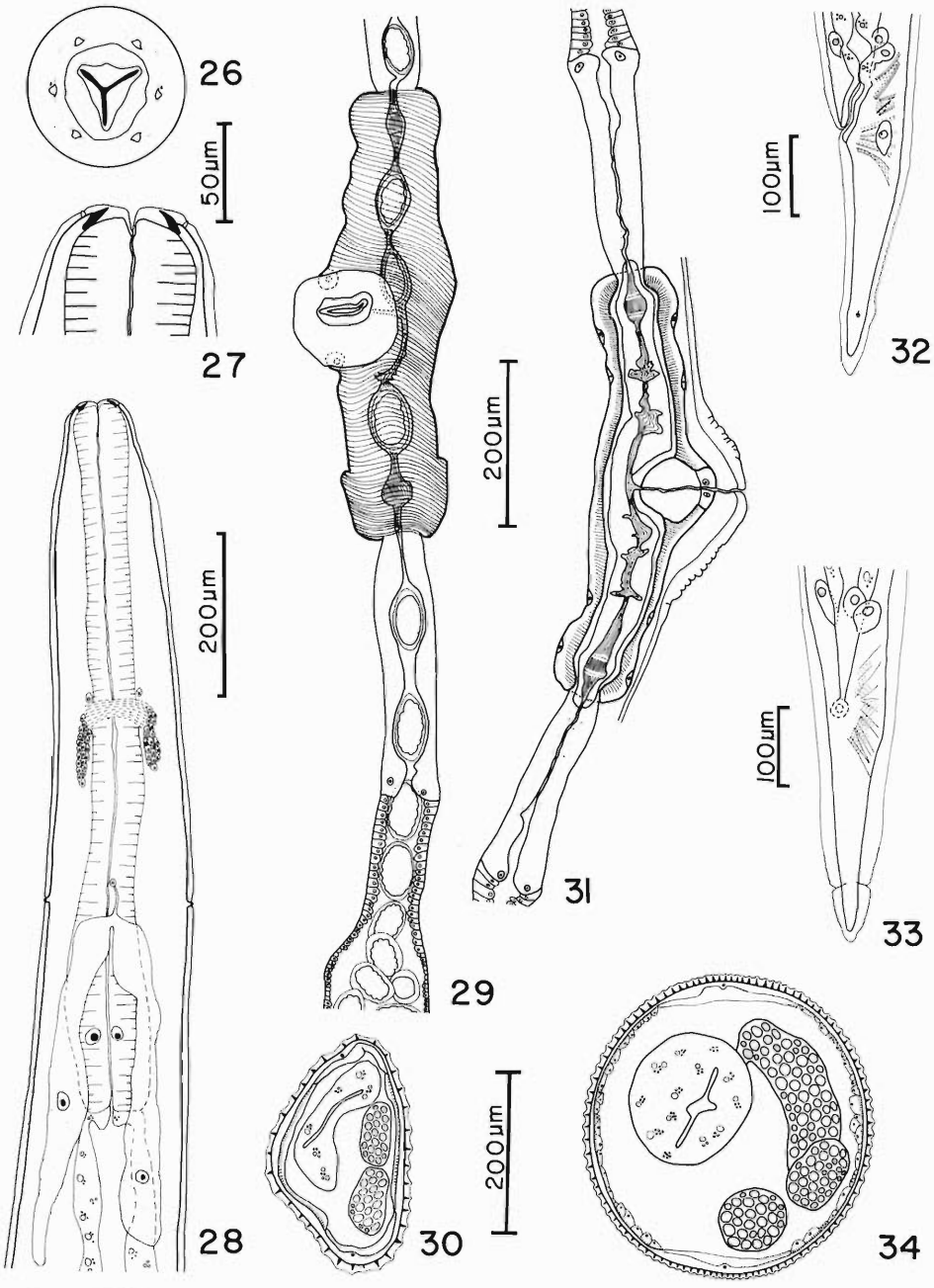
Figures 8–11. Scanning electron photomicrographs of adult male *Obeliscoides cuniculi*. **8.** Genital cone and dorsal ray, ventral view. Top arrow indicates location of anus; middle arrow indicates bifurcated structure lateral to accessory bursal (dorsal) membrane; bottom arrow indicates papillalike structures at base of dorsal lobe. Inset shows distal extremity of dorsal lobe, ventral view. **9.** Genital cone, ventral view. Arrows indicate papillae at posterior margin of accessory bursal (dorsal) membrane. **10.** Distal extremity of spicules, right lateral view. R = right spicule. **11.** Distal extremity of spicules, ventral view. R = right spicule.

mediolateral rays. Dorsal lobe lying within lateral lobes. Dorsal lobe short, filiform, with 1 terminal median and 2 lateral expansions at distal extremity. Dorsal ray 87 µm in length, bifurcating 3 times. Two lateral branches of dorsal ray present, 27 µm from distal extremity. Each lateral branch bifurcating into outer and inner branch. Outer branch shorter than inner branch. Third bifurcation of dorsal ray 49 µm from distal extremity. Lateral branches curving ventrally each through small opening and terminating in 2 large papillalike structures at base of dorsal lobe.

Genital cone with accessory bursal membrane containing small accessory dorsal ray with 4 terminal lightly sclerotized hooks. Accessory bursal membrane bearing 2 minute papillae on posterior margin. Two bifurcated fingerlike structures present lateral to accessory bursal membrane, lacking internal supporting apparatus.



Figures 12–25. Adult male *Obeliscoides cuniculi*. **12.** Proximal extremity of left spicule, lateral view. **13.** Distal extremity of right spicule, ventral view. **14.** Distal extremity of right spicule, lateral view. **15.** Genital cone and dorsal lobe, ventral view. Note minute hooklike extremity of accessory dorsal ray. **16.** Prebursal and bursal region, lateral view. Note spicules, ejaculatory duct and rays of bursa. **17–20.** Distal extremity of dorsal lobe, variations of dorsal ray, ventral view. **21.** Cross section of *O. cuniculi cuniculi* at midbody. Note number of longitudinal ridges. **22.** Cross section of *O. cuniculi multistriatus* at midbody. Note number of longitudinal ridges. **23A–E.** Cross sections of spicules made at indicated locations. **24.** Pre-bursal and bursal region, ventral view. **25.** Genital cone and dorsal lobe, lateral view. Note bifurcations of dorsal ray and minute hooklike extremity of accessory dorsal ray.



Figures 26–34. Adult female *Obeliscoides cuniculi*. **26.** Anterior extremity, apical view. **27.** Anterior extremity, lateral view. Note circumoral annulus. **28.** Anterior extremity, ventral view. **29.** Proximal region of reproductive tract, lateral view. Note vulva, vagina uterina, infundibulum, and proximal extremity of uterus. **30.** Cross section of *O. cuniculi cuniculi* at midbody. Note number of longitudinal ridges. **31.** Proximal region of reproductive tract, ventral view. Note vulva, vagina uterina, and infundibula. **32.** Posterior extremity, lateral view. **33.** Posterior extremity, ventral view. Note phasmids. **34.** Cross section of *O. cuniculi multistriatus* at midbody. Note number of longitudinal ridges.

Spicules equal, 500 μm in length. Capitulum of spicules with lateral swelling bearing circular transparent inflation 107 μm in diameter. Shaft of spicules slender, with small dorsal and ventral alae.

Distal extremity of spicules consisting of dorsal and ventral hooks, surrounded by transparent membrane. Ventral hook narrow, with anterior and posterior process. Dorsal hook broad, with dorsal and ventral process.

FEMALE: Length 14.8 mm. Width of body at esophageal–intestinal junction 185 μm. Nerve ring 428 μm, excretory pore 631 μm, and deirids 668 μm from anterior extremity. Esophagus 1,016 μm in length. Transverse slitlike vulva 11.9 mm from anterior extremity, lateral in position. Vulva with poorly developed lips. Vagina vera short with muscular walls. Vagina uterina 327 μm in length, with oval vestibule, 2 cylindrical sphincters, and long infundibula. Body posterior to vulva slightly flexed. Eggs in distal portion of uteri 61–81 μm long and 34–60 μm wide. Eggs in morula stage. Anus 197 μm and phasmids 67 μm from posterior extremity. Tail ending in blunt point.

TYPE HOST: *Oryctolagus cuniculus* Linnaeus.

SITE OF INFECTION: Stomach.

TYPE LOCALITY: Princeton, New Jersey, U.S.A.

OTHER HOSTS: *Sylvilagus floridanus* Allen, *Marmota monax* Linnaeus.

SPECIMENS: Specimens described above were from the National Parasite Collection, United States Department of Agriculture, USNM Helm. Coll. No. 25471, and were submitted by Graybill. He did not designate a holotype, and his specimens are considered syntypes. One male is designated as lectotype of *Obeliscoides cuniculi* according to the International Code of Zoological Nomenclature (Article 74(a)). All other specimens in Graybill's collection become paralectotypes (Article 74, Recommendation 74E of the International Code of Zoological Nomenclature). Specimens of *O. c. cuniculi* from *Sylvilagus floridanus* from Ontario have been deposited in the National Parasite Collection, United States Department of Agriculture (USNM Helm. Coll. No. 77092). Specimens have also been deposited in the Invertebrate Collection, National Museum of Natural Sciences, Ottawa, Canada (NMCIC(P) No. 1982-0672).

Obeliscoides cuniculi multistriatus n. subsp.
(Figs. 1, 3–6, 8–20, 22–29, 31–34)

DESCRIPTION: Longitudinal ridges numbering at midbody 53–76 ($N = 55$) in males and 72–127 ($N = 55$) in females. Ridges frequently discontinuous. Distance between ridges 3.5–6.0 μm ($N = 10$) in males and 4.8–9.0 μm ($N = 10$) in females. Anterior region of body lacking ridges, 85 μm in length in males and 104 μm in length in females. Ridges on lateral surfaces extending slightly anterior to those on dorsal and ventral surfaces. Transverse striations approximately 2 μm apart anterior to ridges. Transverse striations between ridges numbering approximately 4 per μm. Dorsal and ventral ridges extending to proximal end of spicules and slightly anterior to anus in females. Circumoral annulus 4 μm in height in males and 5 μm in females. Oral opening, excretory glands, esophagus, and deirids as in *O. c. cuniculi*.

HOLOTYPE: Length 7.8 mm. Width of body at esophageal–intestinal junction 81 μm. Nerve ring 263 μm, excretory pore 389 μm, and deirids 421 μm from anterior extremity. Esophagus 587 μm in length.

Table 1. Number of longitudinal ridges in various body regions of *Obeliscoides cuniculi* collected from wild lagomorphs in Canada.

Host (*N*)	Number of longitudinal ridges*					
	Males			Females		
	Anterior†	Posterior‡	Midbody	Anterior†	Posterior‡	Midbody
Sylvilagus floridanus (10)	21 ± 3 (20–26) [5]	43 ± 2 (41–46) [5]	28 ± 3 (23–34) [44]	27 ± 2 (25–29) [5]	42 ± 5 (36–50) [5]	38 ± 4 (31–46) [45]
Lepus americanus (5)	35 ± 3 (30–39) [5]	36 ± 3 (33–40) [5]	64 ± 6 (53–76) [55]	53 ± 8 (45–62) [5]	67 ± 7 (55–73) [5]	98 ± 10 (72–127) [55]

* Mean ± SD, range in parentheses, number of specimens in brackets.
† Cross section made in region of nerve ring.
‡ Cross section made near distal extremity of spicule or near anus in female.

Prebursal papillae similar to those in *O. c. cuniculi,* 390 μm from posterior extremity. Bursa and genital cone as in *O. c. cuniculi*. Dorsal ray 72 μm in length. Two lateral branches of dorsal ray 16 μm from distal extremity. Third bifurcation of dorsal ray 40 μm from distal extremity. Spicules similar in structure to those in *O. c. cuniculi,* 446 μm in length. Capitulum of spicules with small circular transparent inflation 67 μm in diameter.

ALLOTYPE: Length 13.8 mm. Width of body at esophageal–intestinal junction 153 μm. Nerve ring 458 μm, excretory pore 734 μm, and deirids 763 μm from anterior extremity. Esophagus 1,052 μm in length. Vulva as in *O. c. cuniculi,* 10.6 mm from anterior extremity. Vagina vera and vagina uterina as in *O. c. cuniculi*. Vagina uterina 377 μm in length. Eggs in distal portion of uteri 58–89 μm long and 37–51 μm wide. Eggs in morula stage. Anus 279 μm and phasmids 74 μm from posterior extremity. Tail as in *O. c. cuniculi*.

TYPE HOST: *Lepus americanus* Erxleben.

SITE OF INFECTION: Stomach.

TYPE LOCALITY: Lindsay, Ontario, Canada (44°21′N, 78°45′W).

ETYMOLOGY: The Latin "multi" meaning many, and the Latin "stria" meaning a ridge.

SPECIMENS: Holotype, allotype, and paratypes have been deposited in the National Parasite Collection, United States Department of Agriculture (USNM Helm. Coll. Nos. 77089, 77090, 77091). Specimens have also been deposited in the National Museum of Natural Sciences, Ottawa (NMCIC(P) No. 1982-0671).

DIAGNOSIS: In specimens collected from *Lepus americanus,* the longitudinal ridges on male and female worms at midbody are significantly greater in number than those in *O. c. cuniculi* ($P < 0.05$). The ridges are smaller and less prominent on specimens from *L. americanus*. In addition, specimens from *L. americanus* have dorsal and ventral ridges extending to the proximal end of spicules. Dorsal and ventral ridges extend to the bursa in specimens from *S. floridanus*. In *O. c. cuniculi* the number of longitudinal ridges increases anteriorly to posteriorly in males and females (Table 1). In *O. c. multistriatus* the number of longitudinal ridges is greatest at midbody in males and females (Table 1). The number of longitudinal ridges in anterior and posterior regions of males is similar. In *O. c.*

Table 2. Number of longitudinal ridges (midbody) on *Obeliscoides cuniculi cuniculi* (Graybill, 1923) Graybill, 1924 collected from wild *Sylvilagus floridanus* by locality.

Host (*N*)	Locality	Number of longitudinal ridges*	
		Males	Females
Sylvilagus floridanus (8)	Guelph, Ont.	29 ± 3 (25–34) [25]	40 ± 3 (31–46) [25]
S. floridanus (1)	Wellington Co., Ont.	29 ± 2 (27–32) [9]	37 ± 2 (33–40) [10]
S. floridanus (1)	Halton Co., Ont.	26 ± 2 (23–30) [10]	34 ± 3 (29–38) [10]

* Mean ± SD, range in parentheses, number of specimens in brackets.

multistriatus there are more ridges in the posterior region compared to the anterior region in females.

Other Specimens Studied

Most specimens examined from the United States National Museum were old and in poor condition. All specimens examined belonged to *Obeliscoides cuniculi* as labelled. Preparation of cross sections of museum material was not possible, and the approximate number of longitudinal ridges could only be determined from surface examination. All specimens except No. 58238-2 belonged to *O. c. cuniculi*. Specimen No. 58238-2 collected from *Ovis canadensis* appeared to have the number of ridges characteristic of *O. c. multistriatus*.

Specimens from the National Museum of Natural Sciences, Ottawa, were *O. c. multistriatus*, except No. 1978-1118 from *Lepus* sp., which was determined to be *Trichostrongylus calcaratus*. Specimens from *Sylvilagus floridanus* collected in the southeastern United States and specimens maintained in domestic rabbits in Montana belonged to *O. c. cuniculi*. Specimens from the Ontario Veterinary College belonged to *O. c. multistriatus*.

All specimens from *Sylvilagus floridanus* collected in Ontario belonged to *O. c. cuniculi* (Table 2). All specimens from *Lepus americanus* collected in Ontario and Alberta belonged to *O. c. multistriatus* (Table 3).

Discussion

Chandler (1924) described *O. cuniculi* from *Oryctolagus cuniculus* in Texas, and gave a detailed description of the dorsal lobe and genital cone. He reported 16–26 longitudinal ridges in males and 36–40 ridges in females, although he did not indicate the location on the body of the nematode where these were counted. A study of Chandler's specimens indicates they were *O. c. cuniculi*.

Alicata (1932) studied the development of *Obeliscoides cuniculi*, but from his description it is not apparent which subspecies he studied.

Durette-Desset (1978) described a male and a female *O. cuniculi* from *Marmota monax* collected in Pennsylvania. She reported 68 longitudinal ridges at midbody in the female, and observed that the ridges terminated 300 μm anterior to the

Table 3. Number of longitudinal ridges (midbody) on *Obeliscoides cuniculi multistriatus* n. subsp. collected from wild *Lepus americanus* by locality.

Host (*N*)	Locality	Number of longitudinal ridges*	
		Males	Females
Lepus americanus (1)	Belwood, Ont.	73 ± 3 (68–76) [10]	105 ± 8 (95–127) [10]
L. americanus (1)	Kirkland Lake, Ont.	65 ± 4 (57–72) [10]	104 ± 8 (95–127) [10]
L. americanus (1)	Sudbury, Ont.	66 ± 3 (61–69) [10]	99 ± 4 (92–106) [10]
L. americanus (1)	Lindsay, Ont.	58 ± 3 (53–64) [15]	89 ± 7 (77–101) [15]
L. americanus (1)	Edmonton, Alta.	63 ± 6 (56–73) [10]	95 ± 13 (72–110) [10]

* Mean ± SD, range in parentheses, number of specimens in brackets.

bursa. These specimens were also examined in the present study and shown to be *O. c. multistriatus*. Specimens examined from woodchucks in Guelph, Ontario, belonged to *O. c. cuniculi* (unpubl. data).

A pair of minute papillae on the accessory bursal membrane (=posterior lip of cloaca) has not been previously reported in *O. cuniculi,* although Andreeva (1958) noted the presence of sessile papillae on the accessory bursal membrane of some trichostrongyles. Chabaud et al. (1970) indicated that the two papillae on the posterior lip of the cloaca are the seventh cloacal papillae and do not represent a fusion of the seventh and eighth cloacal papillae as suggested by Osche (1958).

Chandler (1924) observed the accessory bursal membrane in *O. cuniculi* and noted a pair of minute rays that he termed accessory dorsal rays. These rays are lightly sclerotized, and Andreeva (1958) suggested that such rays support the accessory bursal membrane. The small fingerlike structures lateral to the accessory bursal membrane have not previously been reported in *O. cuniculi*. They probably serve a sensory function.

The dorsal ray of *O. cuniculi* was variable in specimens examined from snowshoe hares and cottontail rabbits. Generally the distal extremity of the dorsal ray was bifurcated twice. One bifurcation or an asymmetrical bifurcation was sometimes observed. Variations in the dorsal ray of other trichostrongyles have been observed by other authors (Travassos, 1937; Andreeva, 1958; Platt and Pence, 1981).

The oral opening of many trichostrongyles is surrounded by an inconspicuous circumoral membrane (Chitwood and Chitwood, 1950) termed a circumoral annulus or buccal ring by some authors (Wright, 1975; Durette-Desset, 1978). In *Obeliscoides cuniculi* this structure is lightly sclerotized. Wright (1975) studied the ultrastructure of the circumoral annulus and other cephalic structures in *Nip-*

postrongylus brasiliensis. No buccal capsule was visible. The cuticular lining of the esophagus joined the body cuticle at the oral opening of *N. brasiliensis*. Thus the cheilostome was absent. Wright (1975) describes *N. brasiliensis* as astomatous. Morphologically *O. cuniculi* is very similar, and ultrastructural studies would probably indicate that this nematode is also astomatous (as defined by Wright, 1975). Wright (1975) found that the annulus is composed of dense cuticle that resembled the dense component of the struts supporting the longitudinal cuticular ridges. He suggested the annulus may have similar rigidity. He further postulated that the circumoral annulus may restrict the extent to which the mouth opens during feeding. As most trichostrongyloids have a much reduced buccal capsule, the circumoral annulus may afford support in the oral region and aid feeding.

Two other species are included in the genus *Obeliscoides*. The first species, *Obeliscoides leporis* Schulz, 1931, was described from *Lepus timidus* Linnaeus collected on the island of Sakhalin, U.S.S.R. The male was incompletely described and the female is unknown (Skrjabin et al., 1954). Spicules are markedly different from those in *O. cuniculi*. The spicules of *O. leporis* have two crests. Each crest in the distal third of the spicule gives off one medial projection. The distal extremity of spicules terminate in a blunt projection.

The second species, *Obeliscoides travassosi* Liu and Wu, 1941, was described from *Caprolagus sinensis* Gray collected at Pehpei, Szechuan, China. The spicules terminate in a barb. Spicules also possess an inner slender pointed process. Liu and Wu (1941) described a median lobe of the bursa supported by a slender bifurcate ray beneath the dorsal lobe. They suggested that this median lobe could be an accessory bursal membrane.

The occurrence of two subspecies of *O. cuniculi,* each in a different lagomorph (namely *Lepus americanus* and *Sylvilagus floridanus*), raises the question of the origin and possible cause of this subspeciation. The fossil record suggests an Asian origin for lagomorphs, with the main course of evolution occurring in North America during the Oligocene and Miocene (Dawson, 1967). Fossils of *Lepus* and *Sylvilagus* in North America prior to the early Pleistocene are questionable (Dawson, 1958). Because *Lepus* fossils have been found in Europe in the Villafranchian and in China and North America in the middle Pleistocene, *Lepus* are probably not of North American origin (Repenning, 1967). *Caprolagus* fossils in Asia are known from the middle–late Pleistocene (Dawson, 1967).

Durette-Desset and Chabaud (1977) postulate an Eurasian origin for the trichostrongyloid evolutionary line that includes *Obeliscoides*. The occurrence of *Obeliscoides travassosi* in *Caprolagus sinensis* in China and *O. leporis* in *Lepus timidus* in the U.S.S.R. and the probability that *Lepus* originated in Eurasia further suggest that the genus *Obeliscoides* originated on that continent. Durette-Desset and Chabaud (1977) suggested that *Obeliscoides* entered the New World by the Bering Land Bridge. *Sylvilagus* is only found in North and South America. Therefore, *Lepus* probably introduced *Obeliscoides* to North America during the middle Pleistocene, when the Bering Land Bridge was open to animal migration.

Lepus americanus may have had primarily a northern distribution and *Sylvilagus floridanus* may have had primarily a southern distribution in North America (much as they do at present, but perhaps more pronounced) during periglacial time. Transfer of *O. cuniculi* from *Lepus americanus* to *Sylvilagus floridanus* and subsequent subspeciation could have occurred during the middle–late Pleis-

tocene, when distribution of animals was disrupted by changes in temperature, moisture, and glacial activity (Schultz, 1972).

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*** NEW EDITOR ***

Volume 50 marks the completion of my eighth year as editor of the Proceedings. It has been both an educational and an enjoyable experience and I am indebted to a great many people who made it so. I am very grateful to the many members of the Editorial Board for their unfailing efforts to render fair, constructive reviews, and to provide me with expert advice. I also appreciate the generous help and counsel of numerous other colleagues who have reviewed manuscripts for the Proceedings. The staff of Allen Press, Inc. deserve special thanks for their many kindnesses to me and for the excellence of their work in producing our journal. To the authors I owe a special debt for their strong support of the Proceedings, and for their courtesy, patience and constructive comments.

The new editor is Dr. J. Ralph Lichtenfels. He has been elected for a five year term beginning January 1, 1984. However, after June 1, 1983 all correspondence and manuscripts should be sent to Dr. Lichtenfels at the following address: Animal Parasitology Institute, Building 1180, BARC-East, Beltsville, Maryland 20705, U.S.A.

A. James Haley

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Development of Free-Living Stages of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983

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ABSTRACT: The free-living development of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983 was studied in a peptone-agar medium. Eggs released by gravid females were in the morula stage of development (non-larvated). Eggs at 22°C hatched and released first-stage larvae in 30–36 hr. First-stage larvae molted in 84 hr at 22°C. Second-stage larvae molted in 5–6 days at 22°C, but retained the old cuticle. Larvae were infective in 7–9 days at 22°C and were characterized by the presence of a buccal capsule, sheath, and filariform esophagus, and by the absence of lips. Lateral alae were observed in all free-living stages.

Eggs of *O. c. multistriatus* occurred throughout examined rabbit fecal pellets. Eggs and larvae did not remain viable in dried feces. Non-larvated eggs developed and hatched at 10°C but not at 4°C. Larvated eggs developed and hatched at 4°C. Eggs in feces did not survive freezing temperatures. Infective larvae survived freezing temperatures up to 4 wk.

Obeliscoides cuniculi multistriatus Measures and Anderson, 1983 is a parasitic roundworm found in the stomach of snowshoe hares (*Lepus americanus*) (Measures and Anderson, 1983). Alicata (1932) made preliminary studies of the free-living development of *Obeliscoides cuniculi*, but it is not apparent which subspecies he studied. He also examined effects of temperature on infective larvae, and found that some larvae could survive temperatures below freezing for various periods of time.

The present study examines the development of free-living stages of *O. c. multistriatus*. Larval stages are described in detail. The distribution of eggs in rabbit fecal pellets and the effects of temperature on eggs and free-living larvae are also examined.

Materials and Methods

Obeliscoides cuniculi multistriatus was originally obtained from snowshoe hares (*Lepus americanus*) in Ontario, and was maintained in New Zealand white rabbits (*Oryctolagus cuniculus*) by continuous passage.

Culture in peptone-agar medium

A peptone-agar medium was used to observe developing free-living stages. This medium consisted of 0.5% peptone (2.5 g Bacto-peptone [Difco Laboratories, Detroit, Michigan, U.S.A.] in 500 ml of water) poured over a thin layer of bacteriological agar (7.5 g bacteriological agar [Fisher Scientific Co. Ltd., 184 Railside Road, Don Mills, Ontario, Canada, M3A 1A9] in 500 ml of water) in sterile polystyrene Petri dishes (35 × 10 mm). Peptone and agar had been sterilized by autoclaving (20 min at 1.06 kg/cm^2 and 121°C). Medium in Petri dishes was not more than 1.5 cm in depth. Peptone was stored at 4°C until needed.

Eggs for inoculation into media were obtained from gravid adult female *O. c. multistriatus* from domestic rabbits. Worms were washed and uteri dissected in sterile distilled water. Eggs were liberated from uteri with fine dissecting needles

and washed twice in sterile distilled water. Eggs were then pipetted into Petri dishes containing the peptone-agar medium. As a source of food for larvae, *Escherichia coli lac* strain CSH4 was inoculated into the peptone-agar medium. *Escherichia coli* was maintained on nutrient agar plates by standard microbiological methods.

Study of free-living stages

Development of free-living stages was studied at 22°C using sterile distilled water for eggs and peptone-agar medium inoculated with *E. coli* for larval stages. At regular intervals, eggs and larvae were removed and studied live or fixed in hot glycerin alcohol (1 part glycerin to 9 parts 70% alcohol). Worms fixed in glycerin alcohol were cleared by allowing alcohol in the fixative to evaporate slowly, leaving worms in glycerin. Student's t test and Mann-Whitney U test were used to test significance of measurements of larvae.

Worms to be examined using scanning electron microscopy were fixed in cold 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH = 7.2). Worms were then treated with 1% osmium in 0.05 M phosphate buffer (pH = 7.2) and dehydrated through a series of increasing concentrations of ethyl alcohol. Specimens were dried by critical-point drying using CO_2 substitution. Specimens were coated with a layer of gold-palladium approximately 2×10^{-5} mm thick in a Technics Hummer 5 Sputter Coater, and were studied with an ETEC Autoscan scanning electron microscope (SEM) at 5–25 kV. Infective larvae were exsheathed in 1.3% sodium hypochlorite, washed three times in water, and then fixed for scanning electron microscopy.

Four experiments were conducted on free-living stages. The purpose of Experiment I was to determine the optimum temperature for development and hatching of eggs of *O. c. multistriatus*. For Experiment I, five sterile polystyrene Petri dishes (35 × 10 mm) each containing 50 eggs in the morula stage (non-larvated) in sterile water were placed at each of the following temperatures: 0, 4, 10, 15, 20, 25, and 30°C.

The purpose of Experiment II was to determine whether eggs containing first-stage larvae (larvated eggs) would hatch at temperatures lower than those at which non-larvated eggs would hatch. For this experiment, 15 similar Petri dishes each containing 50 eggs in the morula stage in sterile water were incubated at 22°C for 24 hr until eggs were larvated. Five dishes each were then placed at 15, 10, and 4°C.

Time of hatch and percent hatch were noted. Percent hatch was analyzed by one-way analysis of variance after arc sine transformation.

Fresh feces containing eggs of *O. c. multistriatus* for Experiments III and IV were collected from two New Zealand white rabbits with experimental infections of at least 1 mo duration. Feces were pooled and 2 g placed in plastic bags (12 × 30 mm). The temperatures in refrigerators used for Experiments III and IV were monitored daily.

The purpose of Experiment III was to determine whether eggs in feces could survive freezing temperatures. Five sealed bags of feces containing eggs of *O. c. multistriatus* (controls) were incubated at 22°C for 3 days. Forty sealed bags of feces containing eggs of *O. c. multistriatus* were stored for 1, 2, 3, or 4 wk at −6.4 ± 1.6°C or −13.4 ± 4.4°C (five bags in each treatment). At the end of each

period of storage, feces were incubated at 22°C for 3 days, placed in a Baermann apparatus containing distilled water for 24 hr, and the number of larvae present counted.

The purpose of Experiment IV was to determine whether infective larvae in feces could survive freezing temperatures. Feces containing eggs of *O. c. multistriatus* placed in bags were sprayed with water and sealed. Bags were incubated at 22°C for 10 days so that feces contained infective larvae. Larvae were recovered from five bags of feces by the Baermann technique prior to the start of the experiment. These served as controls. Forty sealed bags of feces were stored for 1, 2, 3, or 4 wk at −6.4 ± 1.6°C or −13.4 ± 4.4°C. At the end of each period of storage, feces were placed in Baermann apparatuses for 24 hr and the number of larvae present counted.

To determine the distribution of eggs in rabbit fecal pellets, feces were collected from two New Zealand white rabbits infected with *O. c. multistriatus*. These rabbits had patent infections of at least 1 mo duration. Feces were collected within 4 hr of being passed, softened in distilled water, and cut with a scalpel into 12 sections. Corresponding sections from 25 randomly chosen pellets were placed in separate Baermann apparatuses and incubated for 48 hr. Number of larvae in each section was counted using a dissecting microscope (40×).

Results

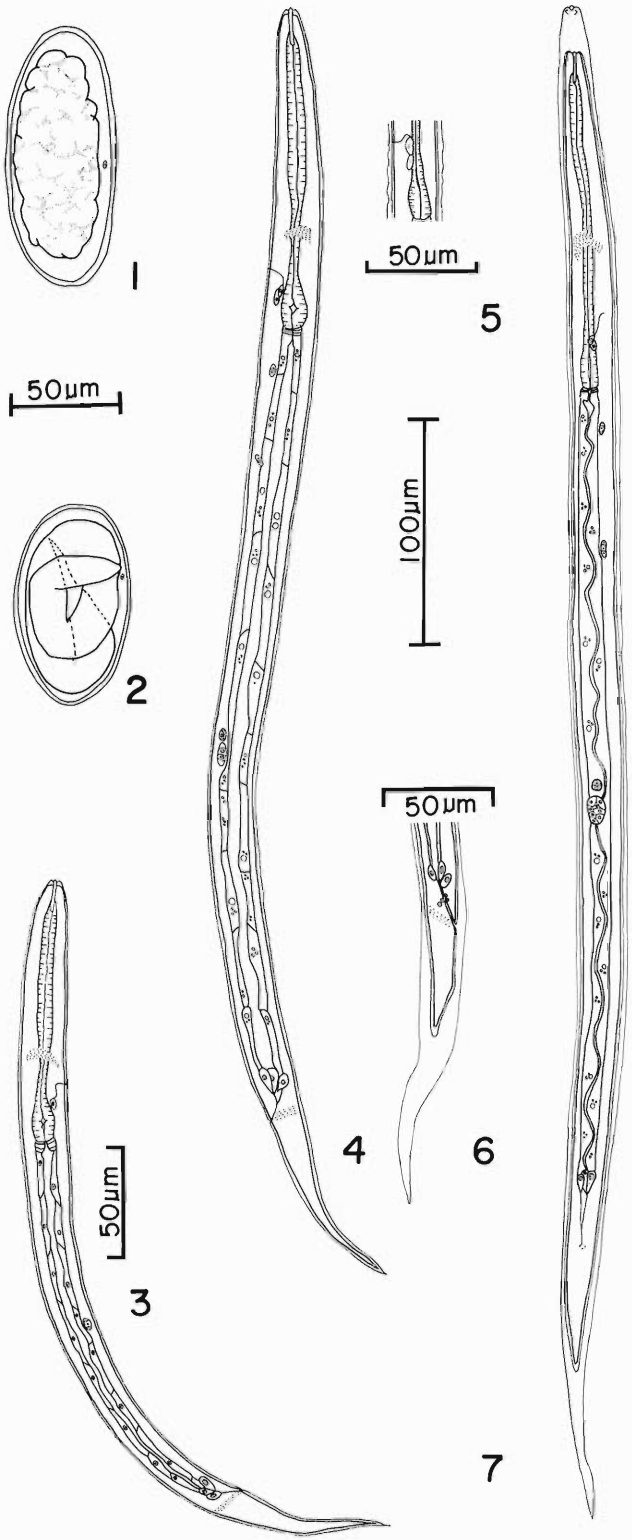
Free-living stages of *O. c. multistriatus*

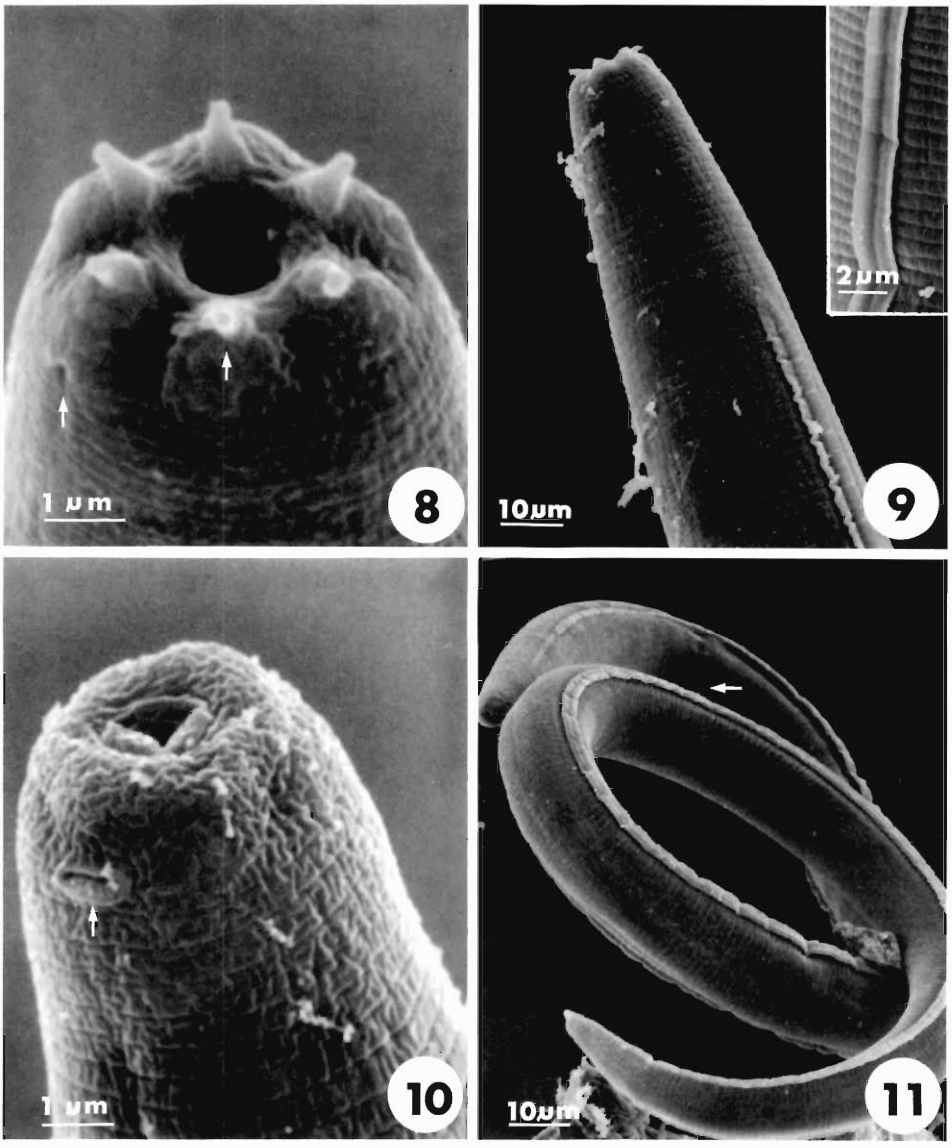
Gravid females placed in saline discharged eggs in the morula stage of development (Fig. 1). Eggs were oval or elliptical with a thin smooth shell. They were 102 ± 7 (91–111) μm long and 49 ± 4 (41–51) μm wide (N = 25). Eggs were fully larvated in 11 hr at 22°C (Fig. 2). These eggs hatched and released first-stage larvae in 30–36 hr.

First-stage larva (Fig. 3; Table 1) (N = 15, 31 hr): Cuticle thin with fine transverse striations. Lateral alae present, in form of flat cuticular thickening, with minute longitudinal striations. Alae extending from region of nerve ring to near tip of tail. Six inconspicuous lips present, each with minute apical process. Buccal cavity cylindrical, lined with thin cuticle. Esophagus rhabiditiform, metacorpus indistinct. Esophageal bulb prominent with valves. Excretory gland cells present near esophageal bulb. Excretory duct lined with thin cuticle. Minute excretory pore visible midway between nerve ring and esophageal bulb. Genital primordium oval, consisting of 1 large median and 2 small terminal cells. Intestine consisting of 16 cells. Three rectal glands present. Tail long and tapered, terminating in minute swelling.

First-stage larvae molted in 84 hr at 22°C. The old cuticle first separated from new cuticle near the anterior extremity and then in the tail region.

Second-stage larva (Figs. 4, 8, 9; Table 1) (N = 15, 5.5 days): Cuticle thin with fine transverse striations. Lateral alae present, in form of flat cuticular thickening, with minute longitudinal striations. Alae extending from region of nerve ring to near tip of tail. Six inconspicuous lips present, each with minute apical process. Buccal cavity cylindrical, lined with thin cuticle. Esophagus rhabiditiform, metacorpus indistinct. Esophageal bulb prominent with valves. Excretory gland cells present near esophageal bulb. Excretory duct lined with thin cuticle.





Figures 8–11. Scanning electron photomicrographs of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983. 8. Anterior extremity of second-stage larva, apical view. Arrows indicate amphid and apical process of lip. First-stage larvae also bear these structures. 9. Anterior extremity of second-stage larva, lateral view. Inset shows lateral alae. 10. Anterior extremity of exsheathed infective third-stage larva, apical view. Arrow indicates amphid. 11. Infective third-stage larva (exsheathed). Arrow indicates excretory pore.

←

Figures 1–7. Free-living stages of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983. 1. Egg in morula stage. 2. Larvated egg at 11 hr. 3. First-stage larva at 31 hr, lateral view. 4. Second-stage larva at 5.5 days, lateral view. 5. Excretory gland of third-stage larva (ensheathed) at 7.3 days, lateral view. 6. Posterior extremity of third-stage larva (ensheathed) at 7.3 days, lateral view. 7. Third-stage larva (ensheathed) at 7.3 days, ventral view.

Table 1. Major dimensions* of free-living stages of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983.

Stage	First stage	Molting first stage	Molting second stage	Ensheathed third stage
Age	31 hr	84 hr	6 days 9 hr	7 days 8 hr
Number	15	15	15	15
Length	380 ± 10 (369–399)	445 ± 16 (415–473)	558 ± 20 (529–589)	540 ± 23 (503–582)†
Width‡	21 ± 2 (19–27)	20 ± 2 (18–23)	22 ± 3 (17–27)	19 ± 3 (15–28)
Buccal capsule length	13 ± 1 (10–15)	12 ± 2 (8–15)	12 ± 2 (9–15)	13 ± 1 (11–14)
Nerve ring§	74 ± 4 (63–81)	77 ± 3 (69–84)	85 ± 7 (69–100)	85 ± 11 (78–102)
Excretory pore§	89 ± 6 (77–104)	95 ± 5 (85–102)	111 ± 12 (86–135)	108 ± 11 (82–124)
Esophagus length	114 ± 6 (102–125)	105 ± 4 (95–110)	131 ± 12 (114–157)	127 ± 12 (108–142)
Genital primordium§	201 ± 9 (183–214)	239 ± 11 (217–263)	311 ± 24 (268–347)	304 ± 13 (287–319)
Genital primordium length	8 ± 2 (4–12)	7 ± 2 (5–10)	11 ± 2 (9–14)	9 ± 2 (6–12)
Tail length	65 ± 8 (41–74)	72 ± 6 (62–83)	65 ± 12 (46–91)	47 ± 7 (29–54)

* Measurements in micrometers. Values are mean ± SD (range).
† Sheath not included in length.
‡ At esophageal–intestinal junction.
§ From anterior extremity.

Table 2. Effect of temperature on hatching of non-larvated and larvated eggs of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983.

	Temperature (°C)	Time to hatch	Percent hatch (±SD)
Non-larvated eggs	30	17–25 hr	91 ± 8
	25	26–33 hr	98 ± 9
	20	43–50 hr	98 ± 4
	15	73–88 hr	96 ± 7
	10	15–20 days	89 ± 7
	4	—	—
	0	—	—
Larvated eggs	15	75–95 hr	90 ± 7
	10	6–10 days	72 ± 15
	4	26–50 days	46 ± 8

Excretory pore visible midway between nerve ring and esophageal bulb. Genital primordium oval, consisting of 2 large median and 2 small terminal cells. Three oval pseudocoelomocytes present, 1 near esophageal–intestinal junction, 1 slightly anterior to genital primordium, and 1 midway between first and third pseudocoelomocyte. Three prominent rectal glands present. Tail long and tapered, terminating in minute swelling.

Second-stage larvae molted in 5–6 days at 22°C. The old cuticle was not shed.

THIRD-STAGE LARVA (Figs. 5–7, 10, 11; Table 1) (N = 15, 7.3 days): Larva enclosed in thin cuticular sheath (second-stage cuticle). Cuticle of larva thin with fine transverse striations. Lateral alae present, in form of flat cuticular thickening, with minute longitudinal striations. Alae extending from region of nerve ring to near tip of tail. Anterior extremity lacking lips. Buccal cavity cylindrical, lined with thin cuticle. Esophagus filariform with slight posterior swelling. Metacorpus indistinct. Esophageal bulb and valves absent. Excretory gland cells near posterior swelling of esophagus. Excretory duct lined with thin cuticle. Minute excretory pore visible midway between nerve ring and posterior swelling of esophagus. Genital primordium oval, consisting of approximately 8 cells. Three oval pseudocoelomocytes present. Two pseudocoelomocytes consisting of 2 or 3 cells, 1 located near esophageal–intestinal junction and 1 approximately ⅓ distance from distal extremity of esophagus to genital primordium. One pseudocoelomocyte, consisting of approximately 5 or 6 small cells, located slightly anterior to genital primordium. Intestine in living specimens dark and granular. Rectal glands prominent. Tail long, ending in conical tip.

Infective larvae were noticed in some Petri dishes as early as 5.5 days at 22°C. Most larvae, however, reached the infective stage in 7–9 days at this temperature.

Infective larvae at 7.3 days were significantly shorter than molting second-stage larvae at 6.3 days (Table 1) ($P < 0.05$). The tail of infective larvae was also significantly shorter than the tail of molting second-stage larvae ($P < 0.05$).

Effects of temperature on free-living stages

EXPERIMENT I: There was no significant difference in mean percent hatch of eggs held at 30, 25, 20, 15, and 10°C ($P < 0.05$). Eggs took longer to hatch as temperature decreased (Table 2). Eggs placed at 0°C and 4°C developed to the

Table 3. Number of larvae (±SD) of *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983 recovered from feces (2 g) held at different temperatures.

Weeks		Temperature	
		−6.4 ± 1.6°C	−13.4 ± 4.4°C
0*	324 ± 85		
1		202 ± 91	194 ± 109
2		226 ± 100	28 ± 11
3		149 ± 36	48 ± 15
4		74 ± 42	52 ± 17

* Control samples.

early tadpole stage, but did not develop further after storage 79 and 66 days, respectively; when transferred gradually to 20°C (5°C per day), these eggs did not develop and appeared dead.

EXPERIMENT II: Eggs hatched at 15, 10, and 4°C (Table 2). There was no significant difference between mean percent hatch of larvated eggs incubated at 15°C and non-larvated eggs incubated at 15°C. Similarly, there was no significant difference between mean percent hatch of larvated eggs incubated at 10°C and non-larvated eggs incubated at 10°C. Non-larvated eggs incubated at 10°C took twice as long to hatch as larvated eggs incubated at 10°C (Table 2). Larvated eggs incubated at 15°C took slightly longer to hatch than non-larvated eggs incubated at 15°C.

EXPERIMENT III: Feces from the five control bags maintained at 22°C contained a mean of 456 ± 40 larvae. Larvae were not found in any of the samples that had been kept at −6.4°C and −13.4°C.

EXPERIMENT IV: Larvae were recovered from all samples (Table 3). There were no significant differences ($P < 0.05$) in number of larvae recovered at −6.4°C and at −13.4°C compared to the control except at the 4-wk and 2-wk periods of storage, respectively. At 4 wk storage at −6.4°C there was a significant decrease in number of larvae recovered. At 2 wk storage at −13.4°C there was significant decrease in number of larvae recovered. Larvae were active after storage at −6.4°C, whereas larvae after storage at −13.4°C were inactive and sluggish.

Infective larvae cultured in feces at 22°C, collected using a Baermann apparatus and stored in distilled water at 4°C for 1 yr, remained infective to rabbits.

Distribution of eggs in rabbit fecal pellets

Larvae of *O. c. multistriatus* were found in all sections of feces examined and were distributed fairly uniformly throughout fecal pellets.

Discussion

Alicata (1932) found that eggs of *O. cuniculi* hatched in tap water in about 30 hr. He observed second-stage larvae in 65 hr at 20–24°C. He did not state when molting occurred. In contrast, first-stage larvae molted in 84 hr at 22°C in the present study. The lengths of second- and third-stage larvae given by Alicata were greater than those found in the present study. He did not indicate whether specimens were measured after fixation as in the present study.

Alicata (1932) reported the presence of six lips and a cylindrical buccal capsule in first- and second-stage larvae. In the present study, lips on first- and second-stage larvae were large and each bore an apical process that was probably a labial papilla. First- and second-stage larvae are microbivorous, and these lips may function in feeding. The amphids were oval and large compared to those seen in adults. Lips were absent in third-stage larvae. The lips were lost during the second molt and remnants of them could be seen on the second-stage cuticle enclosing the third-stage larva. They were not seen on exsheathed third-stage larvae, which were not observed to feed.

A buccal capsule was present in infective larvae of *O. c. multistriatus*. Alicata (1932) did not recognize a buccal capsule and indicated that the oral opening was not patent.

Infective larvae were significantly shorter than molting second-stage larvae. This is attributable to the significantly shorter tail in infective larvae compared to second-stage larvae. Alicata (1932) also noted that infective larvae were shorter and had a shorter tail than second-stage larvae.

Alicata (1932) did not note lateral alae in free-living stages. These alae are difficult to see with normal light microscopy. They are readily apparent with the aid of scanning electron microscopy (SEM). Alae in some larvae of Strongylida have only recently been studied using SEM. Scanning electron photomicrographs of sheathed infective larvae of various species of *Ancylostoma, Nippostrongylus brasiliensis,* and *Necator americanus* (Lee, 1969; Sakumoto et al., 1971; Setasuban, 1974; Yoshida, 1974; Yoshida et al., 1974) are similar to those seen on exsheathed *O. c. multistriatus* infective larvae.

The presence of lateral alae on free-living stages may be related to locomotion in water films. Nematodes move on their lateral surfaces (Lee and Atkinson, 1976). Lateral alae and thickenings of the cuticle with longitudinal striations may provide traction on a substrate and reduce side-slip during locomotion.

Eggs of *O. c. multistriatus* occurred throughout rabbit fecal pellets. Silverman and Campbell (1959) observed variations in the rate of drying at different sites within sheep pellets containing eggs of *Haemonchus contortus*. In the present study, experiments to determine whether *O. c. multistriatus* can tolerate desiccation were not performed. However, in some preliminary experiments it was noted that eggs and larvae did not remain viable in dried feces. Alicata (1932) showed that most infective larvae died when allowed to dry under laboratory conditions at 23°C for 1 hr.

Eggs of *O. c. multistriatus* in rabbit feces did not survive freezing temperatures. Non-larvated eggs kept in water at 4°C did not develop. In contrast, larvated eggs kept in water at 4°C developed and hatched. Similarly, larvated eggs of *Haemonchus contortus* and *Trichostrongylus columbriformis* survived temperatures of 7°C and 4°C, respectively, but non-larvated eggs of these species did not (Silverman and Campbell, 1959; Andersen et al., 1966).

Infective larvae of *O. c. multistriatus* in rabbit feces survived freezing temperatures for periods up to 4 wk. Alicata (1932) subjected infective larvae of *O. cuniculi* in moist charcoal to temperatures as low as −18°C for varying periods of time. He reported that larvae survived 30 days at temperatures of 2°C to −4°C, but did not survive −18°C for more than 72 hr. Infective larvae of *O. c. multi-*

striatus remained infective to rabbits after storage for 1 yr in water at 4°C. Hutchinson et al. (1972) also noted that infective larvae of *O. cuniculi* survived and remained infective when stored 146 days in water at 5°C.

Dorney (1963) observed developing ova and live larvae of *O. cuniculi* in overwintering fecal pellets of cottontail rabbits collected in April in Wisconsin. Temperatures during the periods when the study was conducted were not reported. Dorney indicated, however, that freezing temperatures were not lethal to all ova and that some surviving in feces over winter were a possible source of infection for cottontail rabbits in spring and summer. He also suggested that snow may aid survival by providing insulation against sudden temperature change. The present study indicates that larvated eggs or infective larvae can survive low or freezing temperatures.

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Occurrence and Pathogenicity of *Heligmosomoides* spp. (Nematoda: Heligmosomidae) Associated with Cecal Villi in Arvicolid Rodents

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ABSTRACT: The host response to the nematodes *Heligmosomoides hudsoni* (Cameron, 1937) and *H. johnsoni* Rausch and Rausch, 1973 (Heligmosomidae) was investigated in their natural hosts, varying lemmings, *Dicrostonyx* spp., and heather voles, *Phenacomys intermedius* Merriam (Rodentia: Arvicolidae), respectively. The nematodes occupy the cecum of the host, where they coil tightly around the long cecal villi. In individual animals, the comparatively few villi occupied became much enlarged, exhibiting severe hyperplasia of the mucosa and other microscopic changes, as described. The mechanism of pathogenesis involved three factors: strangulation of the villi, pressure atrophy, and chronic irritation by the crests of the synlophe. In lemmings, the presence of abundant plasma cells in affected villi indicated that *H. hudsoni* evokes a strong immune response. The distribution and zoogeography of the nematodes and their hosts are discussed.

Nematodes of the genus *Heligmosomoides* Hall, 1916 (Heligmosomidae) characteristically inhabit the small intestine of rodents of the family Arvicolidae in the Holarctic (Durette-Desset, 1971). Two species, *H. hudsoni* (Cameron, 1937) and *H. johnsoni* Rausch and Rausch, 1973, are known only from varying lemmings, *Dicrostonyx* spp., and the heather vole, *Phenacomys intermedius* Merriam, respectively, in which they are found coiled tightly around long villi arising from the cecal wall. The presence of villi in the cecum of varying lemmings apparently had not been reported before they were noted by Cameron (1937) in connection with observations on *Heligmosomoides hudsoni*. Voge and Bern (1949) described cecal villi in the arboreal vole, *Phenacomys* (=*Arborimus*) *longicaudus* True, and confirmed their presence in *P. intermedius* and *A. albipes* (Merriam); later, these authors (1955) discussed the villi in varying lemmings from northern Alaska, mainly with respect to the previous findings in *A. longicaudus*.

We observed that the presence of the nematodes caused marked enlargement and other changes in the affected villi. The present paper describes the lesions produced by *H. hudsoni* and *H. johnsoni* in their hosts, and considers some zoogeographic characteristics of these nematodes.

Materials and Methods

Cecal tissues studied in detail were obtained from eight lemmings and three heather voles. Two infected lemmings were collected at Point Barrow and near the shore of Beaufort Lagoon, on the Arctic coast of Alaska. Origins of six noninfected animals were as follows: Beaufort Lagoon, 1; laboratory-reared, from stock captured at Beaufort Lagoon, 1; Bathurst Island, Canadian Arctic Archipelago, 2; laboratory-reared, from stock captured on Bathurst Island, 2. Two infected heather voles were trapped on the Olympic Peninsula, and one in Pierce County, Washington.

The ceca removed from the rodents were opened, usually washed free of contents, and preserved in a 10% solution of formalin. Portions of ceca with villi attached were sectioned by the paraffin-embedding method and stained in hematoxylin-eosin, Mallory's aniline blue collagen stain, and by the periodic acid-Schiff method. Serial transverse and sagittal sections were prepared of single villi, both normal and with nematodes in situ. The anatomic details of the cecum were determined mainly from a series of laboratory-reared lemmings, *Dicrostonyx* cf. *groenlandicus* (Traill) (stock from Bathurst Island), and from a specimen of heather vole preserved intact.

Results

The biotope of *Heligmosomoides hudsoni* and *H. johnsoni* cannot be completely defined at present, but a specialized function of the cecum is indicated by the greatly increased absorptive surface resulting from the presence of the long villi. (Assuming it to be a cylinder with a smooth surface, a villus 20 mm long with a diameter of 0.5 mm would have an area of approximately 31.4 mm^2, or more than 150 times greater than the 0.2-mm^2 area of its base.) In *Dicrostonyx*, we observed that the cecum has an abundant flora and fauna. Protozoa were numerous in sections of the intact cecum of a wild lemming, and we found trichomonads, *Tritrichomonas* sp., in great numbers in laboratory-reared animals (stock from Bathurst Island). Also in the cecal content were many bacteria, consisting, in order of abundance, of very long Gram-negative rods, Gram-positive rods, and Gram-positive cocci. A detailed description of the structure of the cecum in *Dicrostonyx* spp. and *Phenacomys intermedius* was required in order to assess the pathologic changes induced by *Heligmosomoides* spp. In the following accounts, measurements are given in micrometers unless otherwise stated.

Cecal villi in *Dicrostonyx* spp. (Figs. 1–3)

The gross (external) characteristics of the cecum of *Dicrostonyx* have been described by Vorontsov (1967; cf. Fig. 136) and will not be considered here. The organ is situated posteriorly in the peritoneal cavity, with the long axis directed more or less transversely, and with the lesser curvature dorsal. The post-cecal spiral, consisting of three to five loops in our material, was situated ventrally on the left, and the apex (blind end) of the organ on the right. The ascending colon ran mediad and then dorsad, paralleling the terminal portion of the ileum. On the surface of the cecum, along the lesser curvature (dorsal midline), large vessels enclosed by the serosa extended for the full length of the organ. The ileum joined the cecum dorsally, just anterior to the midline, and about a third of the length of the organ from the ceco-colic orifice.

Filamentous villi (Fig. 1) were distributed in two dorsal fields that extended nearly the full length of the cecum. None was present on the mucosa of the ventral half of the organ, although a few scattered nodules of mucosa were observed in this area. An aggregation of long villi surrounded the ileo-cecal orifice. From the latter orifice nearly to the blind end of the organ, a series of about 20 uniform, parallel ridges, 1.5–3 mm apart, extended ventrad from the dorsal midline along both the anterior and posterior surfaces of the cecum. Usually, eight to 11 long villi arose from each ridge bilaterally. Dorsally, the mucosa between the ridges exhibited a series of low, longitudinal ridges, from which occasional villi of small-

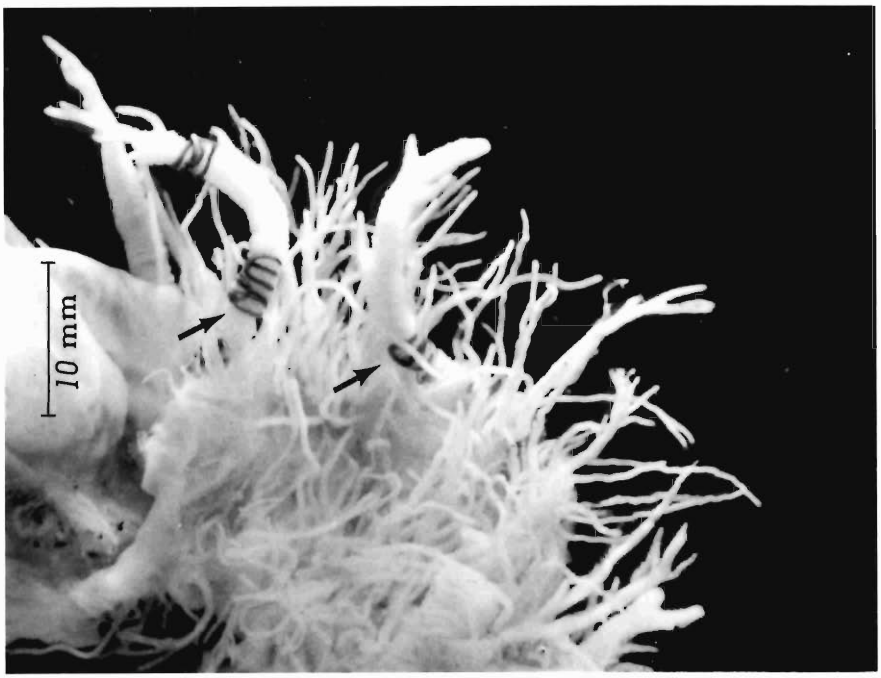
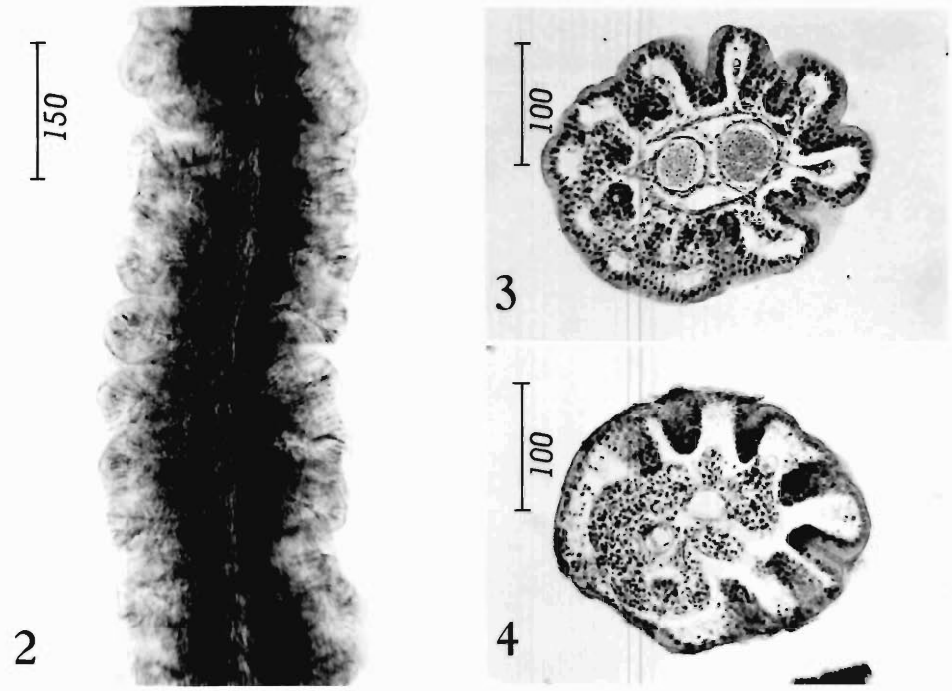


Figure 1. Part of the mucosal surface of the cecum of a varying lemming (Beaufort Lagoon, 21 July 1970), with normal villi and villi occupied by *Heligmosomoides hudsoni* (arrows).

er size arose in an irregular pattern. The distal ends of the large, vertical ridges subdivided, forming several divergent lower ridges that extended ventrad over that part of the cecal surface lacking villi. Villi at the apex of the cecum were short, and their length decreased progressively also toward the ceco-colic orifice, where they disappeared. Prominent folds of mucosa, which presumably function as valves, extended through the orifice into the first loop of the post-cecal spiral. The blood supply to the villi was provided by the large artery extending along the dorsal surface of the cecum. From it, a single branch extended into each of the parallel ridges; this artery in turn gave off a branch to each villus. The same course, in reverse, was taken by the veins. Vessels extended as well through the less prominent ridges on the mucosa ventrally in the cecum.

The filamentous villi in *Dicrostonyx* were slightly attenuated distad, usually somewhat flattened, and ranged in length from only 2–3 mm along the margins of the dorsal fields to at least 22 mm; in transverse section, they were about 300–630 in greatest diameter by about 300–400. At low magnification, the mucosa of the villi as well as that of the ridges from which they arose had a uniform, wrinkled appearance, imparted by the presence of the numerous crypts of Lieberkühn (Fig. 2). The ridges and villi were covered by a simple columnar epithelium, 20–24 in height, with nuclei 5–6 in diameter situated near the basal membrane. Goblet cells were few in the epithelium of normal villi. The epithelium extended into the crypts of Lieberkühn, where the cells were of low columnar type. Goblet cells were numerous in the crypts, but cells of Paneth were not observed. The contents of the goblet cells were PAS positive. In transverse section, the crypts were 50–70 in diameter.



Figures 2–4. Normal structure of cecal villi. Hematoxylin-eosin (HE). 2. Median sagittal section of a cecal villus from a varying lemming. 3. Transverse section of cecal villus from a varying lemming. 4. Transverse section of cecal villus from a heather vole.

The presence of the numerous crypts caused the villi to have an irregular outline in transverse section (Fig. 3). The lamina propria, consisting of loose connective tissue between the epithelium and the muscularis mucosae, contained numerous lymphocytes and histiocytes, and sections of capillaries, 5–7 in diameter, often were discernible. The muscularis mucosae was an extension of that in the vertical ridges, and formed a layer 5–7 in thickness around the central area containing the central artery and vein. (It was evident that the villi were capable of considerable contraction, based on the observation of tissues fixed in formalin immediately following the death of the animal.) The lumen of the central vein ranged from about 41–53 in greatest diameter; that of the artery, 17–34. The wall of the latter ranged from 4–5 in thickness. The central lymphatic vessel, or lacteal, was situated somewhat laterally between the vein and artery; its diameter was usually less than that of the vein.

Cecal villi in *Phenacomys intermedius* (Fig. 4)

The gross relationships of the cecum in situ were not precisely determined in the heather vole. The internal characteristics of the organ, however, appeared to be identical with those described above for *Dicrostonyx,* involving the same arrangement of vertical ridges from which the long villi arose. In *Arborimus albipes,* of which we studied a single specimen, the internal structure of the cecum differed mainly in the less regular arrangement of the vertical ridges.

In *P. intermedius,* the cecal villi ranged in length from 3 to at least 12 mm, and

appeared to be more cylindrical than those in *Dicrostonyx*; in transverse section, they measured 310–400 in greatest diameter, by 260–350. As compared with the cecal villi in *Dicrostonyx,* crypts of Lieberkühn were considerably fewer. In structure, the villi of the heather vole seem closely to resemble those of *Arborimus longicaudus,* as described by Voge and Bern (1949).

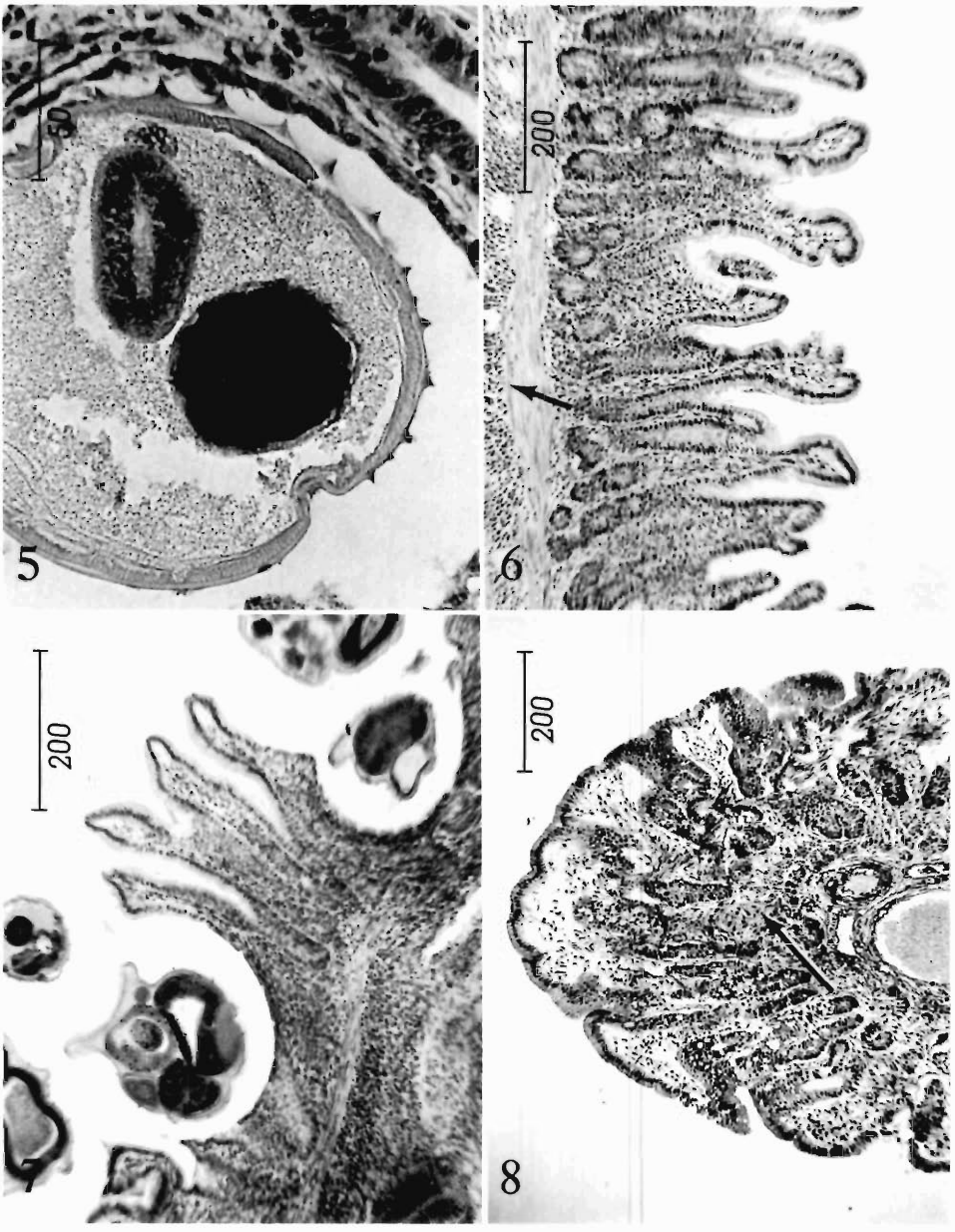
The villi were covered by columnar epithelium 14–17 in height, with nuclei 5–7 in diameter. Goblet cells, not observed by Voge and Bern (1949) in *A. longicaudus,* were present. The crypts of Lieberkühn, 53–61 in diameter, were lined by low-columnar or cuboidal epithelium. Goblet cells were numerous in the crypts. As seen in transverse section (Fig. 4), the structure of the villi differed somewhat from that in *Dicrostonyx*. Peripherally, the lamina propria consisted of loose fibers with few cells, but centrally an aggregation of cells with granular nuclei surrounded the central vessels. The muscularis mucosae, up to 10 thick, closely surrounded the central vein and artery. The lumen of the vein ranged from about 29 to 49 in greater diameter; that of the artery, 17 to 34. The central lymphatic vessel was not prominent, and often could not be identified. Capillaries were observed in the lamina propria.

Changes in villi occupied by *Heligmosomoides* spp. (Figs. 5–8)

Macroscopically, the nematodes were found tightly coiled around the affected villi, their red color contrasting with the white host tissue (Fig. 1). Their bodies, including the cephalic and caudal ends, lay within grooves in the hyperplastic mucosa, and occasionally were almost enclosed. In infected rodents, both male and female nematodes were present; all nematodes were mature, and the females contained abundant eggs.

The mode of attachment of heligmosomids to the intestinal villi of the mammalian host has been described in detail by Durette-Desset (1971). Such nematodes coil sinistrally around the villus, with the cephalic end toward its base. The synlophe of heligmosomids has an important function in attachment, in that the crests or arêtes of the ventral or left-ventral surface of the nematode are embedded in the epithelium (cf. Durette-Desset, 1971, Fig. 1). In transverse section, the crests of the nematodes in *Dicrostonyx* and *Phenacomys* were seen to be sharply pointed (Fig. 5). In nematodes of both species, the crests arose from the second (interior) layer of cuticle and protruded through the external layer (cf. Bogoiavlenskii, 1973, Fig. 5). In transverse sections of specimens in situ, the numbers of crests ranged from 10 to 12 in *H. hudsoni*. They emerged from the cuticle at an angle; the largest, 15 in height, were on the left-ventral surface of the body of the nematode. In *H. johnsoni,* the crests were about 10 in maximum height, and were broader at the base; numbers of crests visible in nematodes sectioned in situ ranged from 12 to 16. The ventral crests emerged vertically from the cuticle; those situated laterally emerged at an angle, with their distal edges directed dorsad. The crests of both species were strongly PAS positive.

In both the varying lemming and the heather vole, the nematodes occupied only a small proportion of the total number of cecal villi in individual animals. In 13 infected lemmings for which counts were made, numbers of *H. hudsoni* ranged from one to 122, but only two (with 33 and 122) had more than 11 specimens. The maximum number of *H. johnsoni* recorded was 25, in a heather vole captured near Heart Lake in the Olympic Mountains. In rodents of both genera,



Figures 5–8. Changes in cecal villi induced by *Heligmosomoides hudsoni* and *H. johnsoni*. 5. Section of *H. johnsoni* in situ, showing crests of the synlophe. PAS. 6. Affected villus of a varying lemming, showing characteristic hyperplasia of the mucosa induced by *H. hudsoni*. Plasma cells (arrow) are abundant medial to the muscularis mucosae. HE. 7. Appearance of the mucosa adjacent to the body of *H. hudsoni* in a varying lemming. HE. 8. Transverse section of villus occupied by *H. johnsoni* in a heather vole, showing hyperplasia of the mucosa and extensive fibrosis of the central area (arrow). Mallory's aniline blue collagen stain.

the nematodes usually occurred singly on the villi, but occasionally two were present (Fig. 1). After the cecal contents had been removed by washing, the affected villi were immediately obvious macroscopically, because of their greatly increased diameter. The nematodes, red in color when living, usually also were obvious, but they frequently were embedded deeply in the hyperplastic mucosa. In occasional lemmings, whether or not infected, degenerate, shrunken villi were observed, made distinctive by a characteristic brownish color.

In varying lemmings, the affected villi attained a diameter of as much as 4 mm, or more than six times the diameter of normal villi. Macroscopically, they appeared opaque, with an apparently smooth surface. In sections, marked hyperplasia of the epithelium was apparent, with a corresponding proliferation of the crypts of Lieberkühn (Fig. 6). Except for the mucosa in direct contact with the body of the nematode, proliferation of the epithelium had resulted in the formation of many secondary, villuslike projections 20–50 long over the entire surface. These were covered by columnar epithelium as much as 30 in height. Goblet cells were numerous in the columnar epithelium on the surface, and abundant in the crypts. Numbers of mitoses had increased significantly in the epithelium of the surface as well as in that of the crypts. Secretion by goblet cells had increased greatly in quantity, as evident in sections stained by the PAS method.

Internally, the organization of tissues had been modified. The muscularis mucosae, usually about 36–40 in thickness (as seen in transverse sections), had been displaced laterad, essentially obliterating the lamina propria. The central area (submucosa), enclosed by the muscularis mucosae, was densely packed with leukocytes, of which plasma cells made up the majority. Scattered Russell's bodies were present, and eosinophils were numerous. The central vessels were much enlarged. The lumina of veins ranged from 146 to 180 in greater diameter; of arteries, 49 to 61. As seen in sections, the walls of arteries had increased in thickness, ranging from 17 to 36. The lymphatic vessels also were enlarged, but only to about the same degree as the veins and arteries. The mucosa exhibited increased vascularity.

Within the spiral groove produced and occupied by the nematode, the mucosa was flattened and ranged from only 130 to 340 in thickness. As observed in sagittal sections, the tissue in contact with the ventral surface (and crests) of the nematode lacked epithelium, and the underlying cells were much compressed (Fig. 7); leukocytic infiltration was not evident.

Sections of the brown, degenerated villi occasionally found in the lemmings exhibited the same hyperplastic changes observed in villi with nematodes in situ. Sections of these stained poorly, in a manner typical of necrotic tissue.

The findings in the infected heather voles appeared fundamentally to be like those in *Dicrostonyx,* but some qualitative and quantitative differences were evident. The affected villi, attaining a diameter of about 2 mm, macroscopically resembled those in lemmings. The mucosa was less hyperplastic, its thickness ranging from only about 350 to 400, and the superficial protrusions characteristic of the mucosa of affected villi in *Dicrostonyx* were not observed. The epithelium was hyperplastic, with columnar cells as much as 19–24 in height. Numbers of crypts had increased moderately, and mitoses were more numerous than in normal villi. Goblet cells were abundant. The most striking difference as compared with findings in the lemmings was the generalized fibrosis of the lamina propria

and submucosa (Fig. 8). The muscularis mucosae was thickened and displaced laterad. Central vessels were enlarged; the lumina of veins ranged from 146 to 220 in greater diameter, and of the arteries, from about 60 to 73. The walls of both were somewhat thickened. The lymphatic vessels were not disproportionately enlarged. Peripherally, the lamina propria exhibited increased vascularity. The extent of leukocytic infiltration was somewhat variable, and sometimes quite diffuse. The infiltrating cells consisted of neutrophils, lymphocytes, and histiocytes; plasma cells were not evident. Scattered, small aggregations of eosinophils were present in the mucosa.

Within the grooves produced in the mucosa by the nematodes, the epithelium was flattened, or only nuclei were discernible in the compressed tissue. Around the crests of the nematodes, the epithelium was frequently lacking. Adjacent to the nematodes, the arrangement of the crypts of Lieberkühn often was distorted. Focal infiltration by leukocytes in the vicinity of the crests was not observed. A layer of homogeneous, PAS-positive material, possibly the accumulated secretions of the crypts, was sometimes present between the body of the nematode and the host tissue.

Voucher specimens

Slides containing sagittal sections of nematodes in situ have been deposited in the USNM Helminthological Collection: *Heligmosomoides hudsoni,* from a varying lemming collected on the Arctic coast of Alaska (Beaufort Lagoon) on 21 July 1970, No. 77074; *H. johnsoni,* from a heather vole collected at Corral Pass, Pierce County, Washington, on 16 September 1979, No. 77075.

Discussion

As noted by Durette-Desset (1971), nematodes of the family Heligmosomidae characteristically coil around one or several villi, typically in the duodenum of the mammalian host. Because of the slight tissue reaction usually evoked by such nematodes, she considered that they might be mobile, not remaining long at one locus. Spurlock (1943) found that *Heligmosomoides polygyrus* (Dujardin, 1845) (syn.: *Nematospiroides dubius* Baylis, 1926) in experimentally infected mice, *Mus musculus* L., caused severe erosion of the intestinal mucosa, with local hemorrhage. The lesions produced by *Nippostrongylus brasiliensis* (Travassos, 1914) (Heligmosomidae: Nippostrongylinae) in the jejunum of experimentally infected rats, *Rattus norvegicus* (Berkenhout), in some respects resembled those caused by the two species considered here (Taliaferro and Sarles, 1939; Symons and Fairbairn, 1963). *Nippostrongylus brasiliensis,* however, remains in the host for only a short time (ca. 2 wk), and the lesions produced by it are not chronic in character.

The lesions caused by *H. hudsoni* and *H. johnsoni* appear consequently not to be comparable with those associated with congeners or related nematodes inhabiting the small intestine of the host. In both varying lemmings and the heather vole, the tissue response is limited to individual, isolated villi, with no indication that adjacent cecal tissues are affected. The mechanism of pathogenesis appears to involve three factors: strangulation of the villus, pressure atrophy in the vicinity of the nematode, and chronic irritation by the crests. The extent of the chronic lesions produced seems to indicate that the nematodes are site-tenacious

and comparatively long-lived. The presence of abundant plasma cells in affected villi of *Dicrostonyx* indicates a strong immunogenic stimulation and a comparable response by the host. In lemmings, the nematodes seem ultimately to cause necrosis of the entire villus. The life span of the nematodes is unknown, but a single specimen of *H. hudsoni* was present in a varying lemming maintained in the laboratory for approximately 8.5 mo following its capture in western Alaska. In our captive colonies of *Dicrostonyx* spp., we have no evidence that the cycle of this nematode may be completed under such conditions. As is characteristic of rodents of other genera in the family Arvicolidae, the life span of varying lemmings usually is not much more than a year under natural conditions.

The two species considered here appear to be highly evolved members of the genus *Heligmosomoides,* which Durette-Desset (1971) considered to have arisen in the western Palaearctic during the late Pliocene. Their hosts, placed in different tribes in the family Arvicolidae, are not closely related, and we consider that the similar structural organization of the cecum and the presence of cecal villi in varying lemmings and in *Phenacomys* s.l. are attributable to convergence. The morphologic similarity of the two species of *Heligmosomoides* (i.e., loss of crests dorsally) as well may be attributable to convergence, through a process of adaptation to the highly specialized habitat unique to their hosts.

The genus *Phenacomys* s.l. is of Palaearctic origin, having appeared in the Nearctic more than a half-million years ago (Repenning, 1980). Little is known concerning the geographic distribution of *H. johnsoni,* because only three heather voles, all collected within the state of Washington, have been found to be infected. In that region, *P. intermedius* is characteristically found at high elevations (our specimens were trapped at elevations from about 1,370 to 1,675 m). The two mountain masses from which the animals were obtained are separated by lowlands more than 160 km in extent, unsuitable as habitat for the heather vole. It thus appears that *H. johnsoni* became widely dispersed in this vole prior to the end of the last (Würm) glacial period, more than 10,000 years ago.

Varying lemmings have an extensive distribution in the Holarctic zone of tundra, including many of the Arctic islands. They compose a superspecies (Artenkreis), as defined by Mayr (1963). The allopatric species are distinguished by a diversity in diploid numbers of chromosomes (Rausch, 1977). *Dicrostonyx* has a long fossil record in the Palaearctic and was considered by Zazhigin (1976) to have spread into Beringia more than a million years ago. Its dispersal into the Nearctic apparently dates only from the late Pleistocene, ca. 175,000 years ago (Repenning, 1980).

In addition to Cameron's (1937) records of *H. hudsoni* from the Ungava Peninsula and Baffin Island, we have obtained this nematode from varying lemmings at widely separated localities in the Nearctic (Table 1). In Eurasia, *H. hudsoni* has been reported from only two localities, the Iamal Peninsula in western Siberia (Luzhkov, 1964) and Wrangel Island in the Soviet Far East (within the former limits of Beringia) (Nadtochii, 1970). Luzhkov's specimens, however, were found in the duodenum of brown lemmings, *Lemmus sibiricus* (Kerr), narrow-skulled voles, *Microtus gregalis* (Pallas), as well as in varying lemmings. The occurrence of the nematodes in the small intestine of the host and their lack of host specificity make questionable Luzhkov's determination of the species. Nadtochii's report of *H. hudsoni* from six of 10 varying lemmings examined did not include further

Table 1. Records of occurrence of *Heligmosomoides hudsoni* in *Dicrostonyx* spp. in the Nearctic.

Geographic locality	Number of animals examined	Number of animals infected
Alaska		
Umnak Island	19	11
Cape Peirce	1	1
Becharof	1	—
St. Lawrence Island	9	—
Nome	1	—
Central Brooks Range	12	1
Wainwright	1	—
Point Barrow	18	—
Meade River	1	—
Beaufort Lagoon	2	1
Lake Schrader	5	—
Canada		
Banks Island	1	—
Victoria Island	1	—
Prince Patrick Island	5	—
Churchill	3	—
Rankin Inlet	6	6
Bathurst Island	5	—
Devon Island	3	—
Chimo	4	—
Greenland		
Smith Sound	3	—

information. The geographic distribution of *H. hudsoni* suggests that it is of Beringian origin and that it became widely dispersed in *Dicrostonyx* during the late Pleistocene. Its presence on Wrangel Island is compatible with this concept, because the low diploid number of chromosomes in the varying lemming there ($2n = 28$) suggests affinities with Recent Alaskan species, rather than with those on the Eurasian continent.

The parasite–host assemblages involving the species of *Heligmosomoides* in *Dicrostonyx* spp. and *Phenacomys intermedius* are relatively ancient. The comparatively small proportion of cecal villi occupied by the nematodes even in the most massive infections observed would seem to have no adverse effect on the host.

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C. Kelley assisted with photography. These contributions are gratefully acknowledged.

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Scanning Electron Microscopy of the Eggs of *Baylisascaris procyonis, B. transfuga,* and *Parascaris equorum,* and Their Comparison with *Toxocara canis* and *Ascaris suum*

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ABSTRACT: The surface structure of the eggs of *Baylisascaris procyonis, B. transfuga,* and *Parascaris equorum* was studied using light and scanning electron microscopy, and found to be almost identical. By light microscopy, the egg shells of these species appear to be of three layers, and the surface is finely granulated or particulate. By scanning EM, the surface of these eggs is irregularly granular; by higher magnification, the many granules are part of a fine three-dimensional reticular framework or lattice of fibrils. There is no evidence of openings or sutures in these eggs, and the eggs are not mammillate or pitted as described by others. A fine reticular substructure is also seen in the surface of *Toxocara canis* eggs by high-magnification SEM, but not in *Ascaris suum* eggs. The operculumlike region described at one pole of *A. suum* eggs is confirmed.

The surface morphology of nematode eggs is a useful characteristic for identification as well as for assessing taxonomic relationships (Hartwich, 1962). Scanning electron microscopy (SEM) has been used successfully in studying and comparing the fine-structural morphology of various nematode eggs (Ishii, 1972; Ishii and Habe, 1973; Specian et al., 1973; Ubelaker and Allison, 1975; Baruš et al., 1979; Wharton, 1979).

Baylisascaris procyonis and *B. columnaris,* the common ascarids of raccoons and skunks, respectively, have surfaced as important causes of visceral larva migrans and fatal cerebrospinal nematodiasis in animals (see Kazacos et al., 1981, and Reed et al., 1981, for references). Because both species also possess marked zoonotic potential (Kazacos, 1981; Kazacos et al., 1981), it is important to recognize properly *Baylisascaris* eggs from feces or soil. Few descriptions of *Baylisascaris* eggs exist; most are based on light microscopic observations, and are inconsistent and oftentimes contradictory (Goodey and Cameron, 1923; McClure, 1933; McIntosh, 1939; Stefański and Żarnowski, 1951; Sprent, 1953, 1968, 1970; Okoshi et al., 1962; Kikuchi et al., 1979; Uni and Takada, 1981). By light microscopy, the eggs of *Baylisascaris* spp. resemble those of *Parascaris equorum,* although lighter in color (pers. obs.).

In this paper we report on the surface structure of *Baylisascaris procyonis, B. transfuga,* and *Parascaris equorum* eggs, as determined by light and scanning electron microscopy. Comparisons are made to the eggs of *Toxocara canis* and *Ascaris suum,* and new information is presented on the fine structure of these eggs.

Materials and Methods

Adult female *Baylisascaris procyonis* were collected from the intestines of raccoons live-trapped in West Lafayette, Indiana. Eggs were removed from the terminal uteri of the worms and fixed in cold 3% glutaraldehyde in phosphate buffer (pH 7.4). Eggs of *B. transfuga* were collected from formalin-fixed worms

previously collected from black bears at a local zoo. *Parascaris equorum, Toxocara canis,* and *Ascaris suum* adults were collected locally from horses, dogs, and swine, respectively, and the eggs fixed as described for *B. procyonis.*

Following fixation, the eggs were processed by routine methods. They were rinsed in 0.1 M phosphate buffer (pH 7.4), postfixed for 1 hr in 1% osmium tetroxide in phosphate buffer (pH 7.4), and rinsed again in buffer. They were then dehydrated through a graded series of ethanol, critical-point dried with carbon dioxide as the transition fluid, sputter coated with gold, and examined in an ISI Super MINI-SEM.

Results

The eggs of *Baylisascaris procyonis, B. transfuga,* and *Parascaris equorum* are rounded–oval in shape. By light microscopy the shell appears to be of three layers: an outer surface layer, a middle shell, and an inner lipid layer (Fig. 1). By light microscopy the surface of the eggs of *B. procyonis, B. transfuga,* and *P. equorum* is finely granulated or particulate, the numerous granules or particles being irregular in size and shape and with no apparent pattern (Fig. 2).

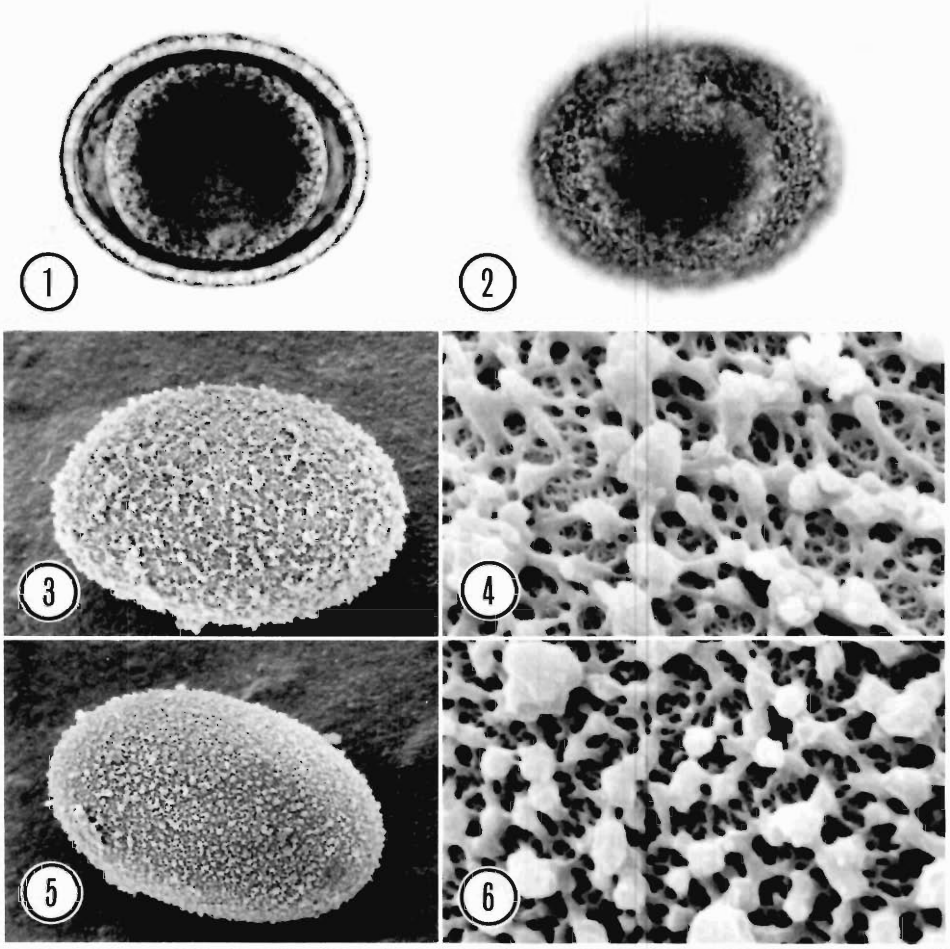
This surface morphology is confirmed and further elucidated with SEM, which reveals that the surface of the eggs of these three species is almost identical (Figs. 3–8), and very different from that of *T. canis* and *A. suum* eggs (Figs. 9–12). By lower magnification SEM, the surface of *Baylisascaris* and *Parascaris* eggs is covered by many irregularly shaped granules or particles, which give the impression of being debris adherent to the eggs (Figs. 3, 5, 7). The granules are readily apparent at higher magnification, and are found to be part of the egg shell and to consist of one to many smaller granules or particulate subunits (Figs. 4, 6, 8). Also apparent at this magnification, the surface consists of a fine three-dimensional reticular framework or lattice of fibrils, which interconnect and branch with each other and with the granules, which are a part of the structure. The entire surface has this arrangement, and there is no evidence of any openings or sutures in the eggs of these species.

By SEM, the eggs of *Toxocara canis* (Figs. 9, 10) are basically similar to what was seen by Ishii and Habe (1973) and Ubelaker and Allison (1975), except that the surface network of ridges and depressions is not nearly as irregular in pattern or distribution as that depicted and described by those workers. Also, under high magnification, a fine reticular substructure is seen in both the ridge and depression areas (Fig. 10).

The eggs of *Ascaris suum* (Figs. 11, 12) are essentially as depicted previously (Ishii and Habe, 1973; Ubelaker and Allison, 1975), with a surface of large ridges and depressions. The operculumlike area seen by Ubelaker and Allison (1975) at one pole of these eggs was also observed (Fig. 11). Under high magnification, the fine reticular substructure seen in the *T. canis* eggs is not seen in the *A. suum* eggs (Fig. 12).

Discussion

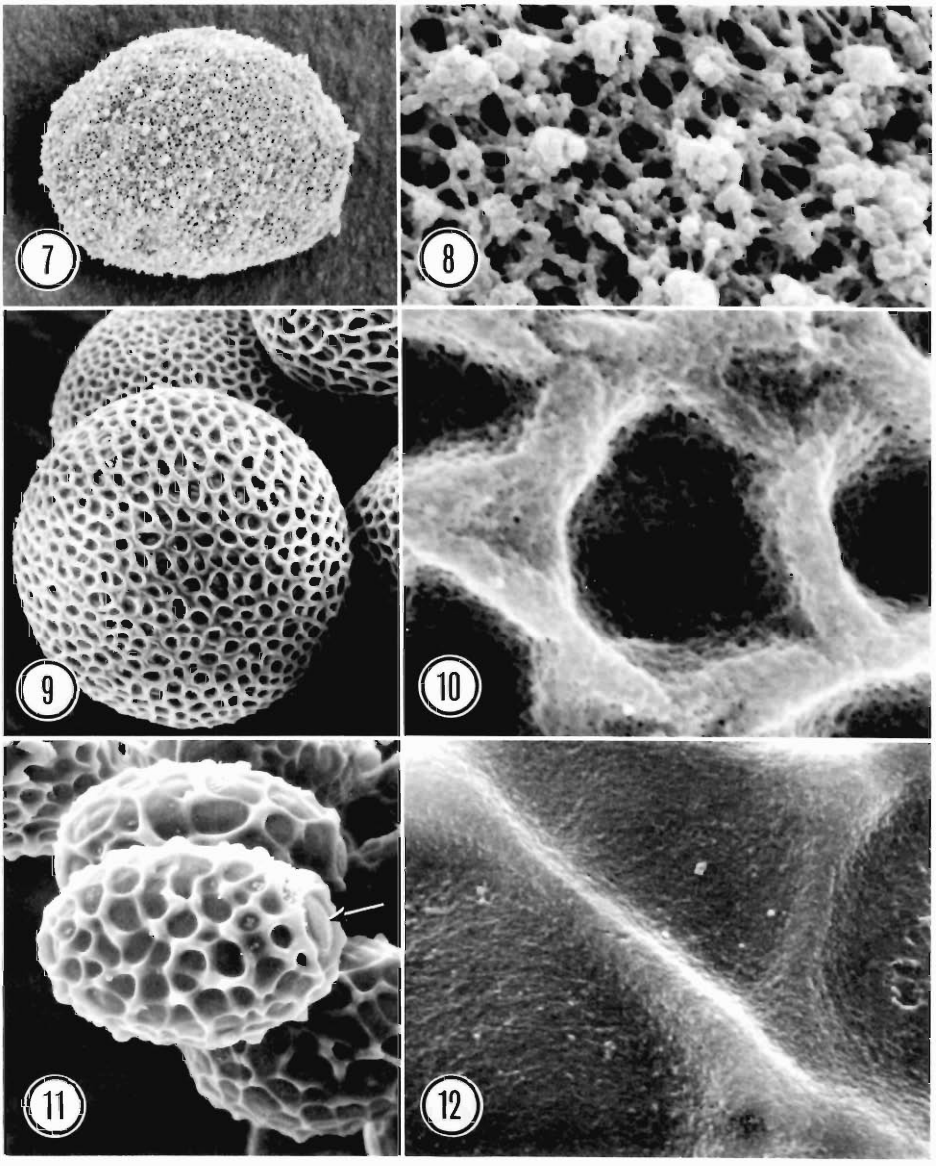
The eggs of *Baylisascaris* spp. nematodes have been variously described by different investigators. McIntosh (1939) felt that the eggs of *B. schroederi* were mammillated, whereas Goodey and Cameron (1923) and McClure (1933) felt that those of *B. columnaris* and *B. procyonis,* respectively, were finely mammillated.



Figures 1–6. Light and scanning electron micrographs of *Baylisascaris procyonis* and *B. transfuga* eggs. 1, 2. *Baylisascaris procyonis,* light microscopy. Figure 2 shows surface detail. ×800. 3, 4. *Baylisascaris procyonis,* SEM. Note irregularly granular surface and reticular framework substructure of granules and fibrils. ×925 and ×9,100, respectively. 5, 6. *Baylisascaris transfuga,* SEM. Structure similar to Figures 3 and 4. ×950 and ×9,100, respectively.

Sprent (1953) described the eggs of *B. devosi* as covered with minute pits and elevations, but "evidently sticky as the eggs usually become coated with particulate matter." In his description of the genus *Baylisascaris,* Sprent (1968) stated that the eggs were finely pitted; this is also stated in the key of Hartwich (1974). Sprent (1970), however, indicated that the eggs of *B. tasmaniensis* were smooth and without pits, but with faint corrugations on their surface. Okoshi et al. (1962) stated that the egg of *B. transfuga* "resembles that of *Ascaris lumbricoides,*" which is not really supported by their light-microscopic photographs, our work, or the work of others (Uni and Takada, 1981).

Our light and SEM studies indicate that the surface of the eggs of *B. procyonis, B. transfuga,* and *P. equorum* is finely granulated, as it is comprised of many irregular granules on a lattice substructure (Figs. 1–8). The surface is not mam-



Figures 7–12. Scanning electron micrographs of *Parascaris equorum, Toxocara canis* and *Ascaris suum* eggs. 7, 8. *Parascaris equorum,* SEM. Structure similar to Figures 3–6. ×950 and ×8,925, respectively. 9, 10. *Toxocara canis,* SEM. Note regular surface patterning and fine reticular substructure. ×925 and ×13,725, respectively. 11, 12. *Ascaris suum,* SEM. Note surface patterning, operculumlike area (arrow), and lack of visible reticular substructure. ×975 and ×9,200, respectively.

millate or pitted as in the eggs of *Ascaris* and *Toxocara,* respectively (Webster's Third New International Dictionary, 1971; Figs. 9–12), as described by others. Our results are in agreement with Stefański and Żarnowski (1951), who by light microscopy found the eggs of *B. procyonis* to be oval "à coque irregulierement granuleuse." An irregularly fine granular outer shell on *B. procyonis* eggs was

also noted by Hartwich (1962), although in his key he described *Baylisascaris* eggs as being finely pitted (Hartwich, 1974). Using SEM, Kikuchi et al. (1979) described the eggs of *B. schroederi* as covered by a proteinaceous coat upon which minute protuberances have formed (translation). Uni and Takada's (1981) excellent SEM photographs of *B. transfuga* eggs agree exactly with ours, but they described the eggs as being uneven and crepuscular (?). It is probable that the coating of *B. devosi* eggs with particulate matter that was noted by Sprent (1953) was actually a reflection of the irregularly granular outer surface itself, rather than adherent foreign material. However, some variation in egg structure may exist among *Baylisascaris* species, such as for *B. tasmaniensis* (Sprent, 1970) versus those reported here, because intrageneric variation in eggs is known for both *Toxocara* and *Ascaris* (Ubelaker and Allison, 1975).

The operculumlike area seen at the pole of *Ascaris* eggs by Ubelaker and Allison (1975) was confirmed by Uni and Takada (1981) and in this study. No such area was seen by Ubelaker and Allison (1975) in *Toxocara canis* or *T. felis* eggs, nor has one been seen by others, including us in this study. Regarding *Baylisascaris* eggs, Uni and Takada (1981) indicated that "under light microscopy we believe the operculum-like area was probably there, as seen in one pole of the egg shell in some eggs." However, no SEM evidence of such a structure on *Baylisascaris* eggs was presented by them, nor was one seen by us in any of the *Baylisascaris* or *Parascaris* eggs examined by light microscopy or SEM. We therefore doubt the existence of such a structure on these eggs. With regard to the differences we saw in the regularity and structure of the ridges and depressions on *T. canis* eggs as compared to what was seen by other workers (Specian et al., 1973; Ubelaker and Allison, 1975), we attribute these differences to artifacts in their eggs, which showed marked shrinkage, distortion, and wrinkling. For the preparation of helminth eggs for our SEM studies, routine methods worked well, and the special glycerol preparative procedures, etc. advocated by Specian et al. (1973) apparently are not necessary.

Based on the transmission EM studies by Foor (1967), coupled with their own SEM studies, Ubelaker and Allison (1975) suggested that the surface sculpturing present on many ascarid eggs (*Ascaris, Toxocara,* etc.) was due to outfolding and modification of the chitinous layer of the eggs, rather than directly due to uterine secretions (Monné and Hönig, 1954) or to surface tension properties of coating proteins (Christenson et al., 1950). This was strongly supported by Wharton (1979), who found that the *Toxocara*-like surface of *Porrocaecum ensicaudatum* eggs was formed by variations in the thickness of the chitinous layer. Wharton (1979) suggested that this sculpturing would increase the structural strength of the shell, thus increasing the protection afforded to the embryo or larva within. From our studies, it can be seen that the surface structure of *Baylisascaris* and *Parascaris* eggs is very different from that of *Ascaris* or *Toxocara*. It is not known whether the eggs' chitinous layer contributes to the granular surface or reticular lattice in *Baylisascaris* or *Parascaris,* or whether the structure of these eggs is simply a reflection of the external uterine layer, and the result of a congealing or condensation of uterine protein secretions, somewhat along the lines of what Christenson et al. (1950) had in mind.

With regard to *Baylisascaris* and *Parascaris* eggs, this question of shell makeup and contribution of the chitinous layer should be resolved by transmission EM studies. If the surface morphology of eggs is a valid characteristic in the assess-

ment of taxonomic relationships, then *Baylisascaris* and *Parascaris* would appear perhaps to have some historical affinities. However, based upon their major differences in definitive hosts and life cycles, especially the utilization of intermediate hosts by most species of *Baylisascaris* (Sprent, 1953, 1954, 1973; Tiner, 1953a, b), their close similarities in eggshell surface structure are probably the result of parallel evolution in eggshell formation.

Acknowledgments

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Ultrastructure of the Stomatal Region of the Juvenile Stage of the Soybean Cyst Nematode, *Heterodera glycines*

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ABSTRACT: A study of the ultrastructural morphology of the infective juveniles of the soybean cyst nematode reveals an extensive cephalic framework, stylet, musculature, and cuticular support that are required for host penetration and feeding. The cephalic framework and portions of the stomatal wall have a fibrillar matrix that merges with and supports the surface cuticle. The flat broad base of the dorsal and ventral cephalic framework blades imparts a bilateral orientation to the framework to which stylet protractor and somatic muscles are attached by hemidesmosomes. Protractor muscles consist of ten muscle elements that extend posteriorly and make lateral hemidesmosomal contacts with the stomatal wall and adjacent somatic muscles. The ten muscle elements merge to form three distinct muscle groups that attach to the stylet knobs. The protractor muscles are attached to the stylet knobs by extensive hemidesmosomes. Interaction between the stylet knobs and sarcoplasm is provided by the extensive hemidesmosomes and interhemidesmosomal membrane evaginations that extend from the stylet knob surfaces into the protractor muscle cells.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, is one of the most destructive nematodes of soybeans. Previous histological studies have established that the second stage infective larva of *H. glycines* penetrates the roots and establishes a feeding site, where a specialized multinucleate host cell is induced. This host–parasite interaction results in the maturation and reproduction of the nematode at the expense of the host plant (Endo, 1964). Recent observations on the ultrastructural anatomy of the anterior sensory organs of a root-knot nematode species (Wergin and Endo, 1976; Endo and Wergin, 1977) and of the soybean cyst nematode (Endo, 1980) illustrate the complexity of the sensory organs in the second-stage larvae of these species. Other stages of plant-parasitic nematodes, as well as animal-parasitic and free-living species of nematodes, also exhibit similar complexity (Yuen, 1967; Baldwin and Hirschmann, 1973, 1975a; McLaren, 1974, 1976; Wright, 1974, 1980; Ward et al., 1975; Ware et al., 1975; De Grisse, 1977; De Grisse et al., 1979; Natasasmita, 1979). Sensory perception is ultimately allied to host penetration and feeding. The following observations of the juvenile stage of *H. glycines* provide an ultrastructural evaluation of the stomatal region, which is involved in cellular penetration and the initiation of the infection process. The ultrastructural relationship between the cephalic framework and the stylet, the stoma, and related anterior protractor and somatic muscles is emphasized.

Materials and Methods

Juvenile stages of *Heterodera glycines* Ichinohe, 1952 were prepared for electron-microscopic examination by using published procedures (Endo and Wergin, 1973; Wergin and Endo, 1976). Briefly, nematodes in a suspension of water were mixed with warm liquefied 2% water-agar. The mixture was poured into small grooves in agar-filled petri dishes. The solidified agar, containing the nematodes, was diced into 2–3-mm blocks that were transferred to glass vials containing 3%

glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 22°C for chemical fixation of the larvae. Subsequent rinsing and postfixation in osmium tetroxide were also carried out in 0.05 M phosphate buffer (pH 6.8). The glutaraldehyde fixation (for 1.5 hr) was followed by washing in six changes of buffer over a period of 1 hr. Then agar blocks were postfixed in 2% osmium tetroxide for 2 hr at 22°C, dehydrated in an acetone series, and infiltrated with a low-viscosity embedding medium (Spurr, 1969). Silver–gray sections of selected nematodes were cut with a diamond knife and mounted on uncoated 75 × 300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 electron microscope that was operated at 60 kV with a 20-μm aperture.[1]

Results

CEPHALIC FRAMEWORK: The cephalic framework of the second-stage larva of the soybean cyst nematode consists of a cuticular fibrillar material that is shaped into six blades that radiate from a central cylinder that is continuous with the stomatal wall (Fig. 1). Each of the anteriorly curved blades widens posteriorly as it merges with the basal plate of the cephalic framework (Figs. 2, 7). The sublateral blades have arch-shaped underbridges through which cephalic receptors traverse (Figs. 3, 6). The sublateral blades differ slightly from the dorsal and ventral blades (Fig. 4), which do not have curved underbridges but broaden to form extensive delta-shaped bases (Fig. 8) that merge with the basal plate, and thereby provide a broad attachment region for the somatic and stylet protractor muscles (Figs. 4, 9).

The symmetry of the basal plate of the cephalic framework is directly related to the orientation of the anteriormost terminals of the somatic and stylet protractor muscles. The dorsal and ventral elements of the protractor muscles make direct contact with the central part of the dorsal and ventral delta-shaped basal plates, whereas the sublateral elements of the protractor muscles are limited to

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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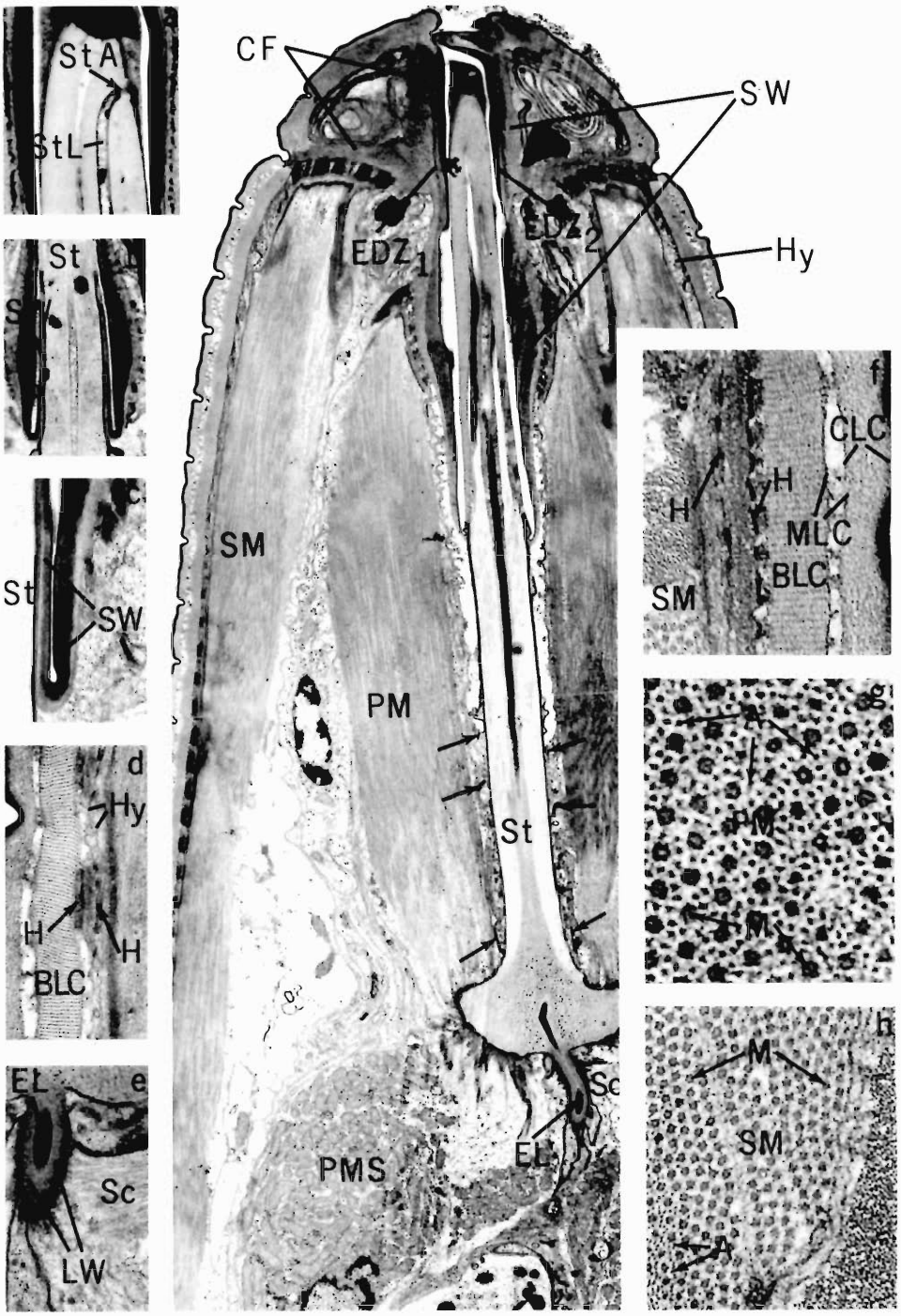
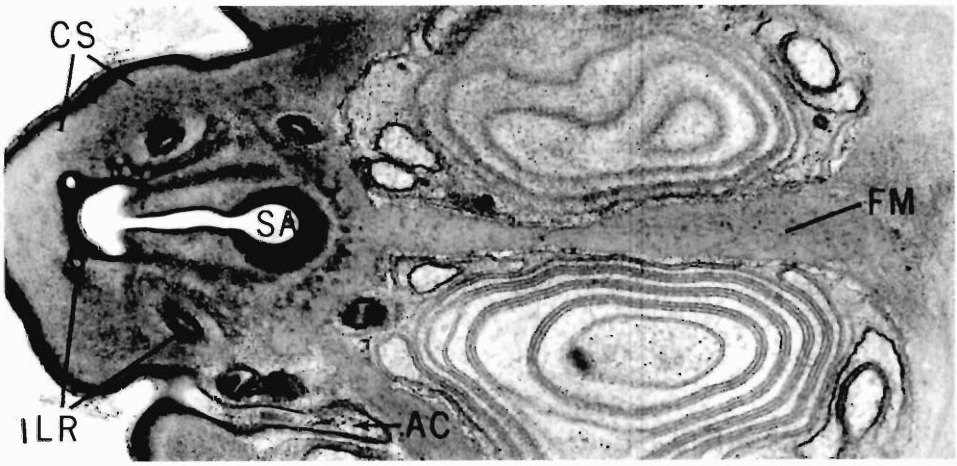
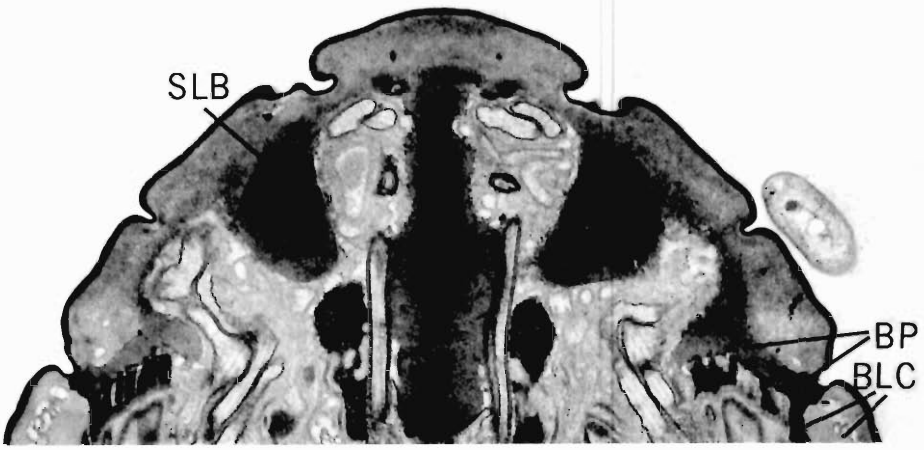
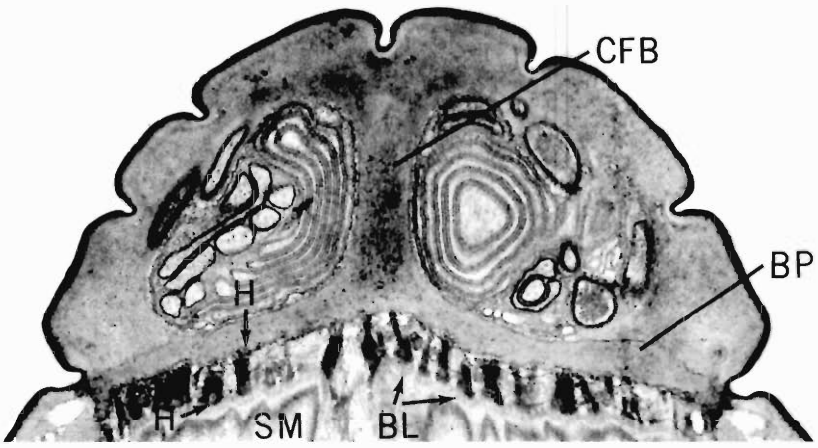
Figure 1. Lateral longitudinal section through the anterior region of a second-stage larva of *Heterodera glycines*. The cephalic framework (CF), stomatal wall (SW), and protractor muscles (PM) provide the guidance required for stylet protraction. Nematode salivation and food ingestion occur through the stylet lumen (StL), which has a ventrally located aperture (StA) (Fig. 1a). The posterior portion of stomatal wall (SW) is invaginated during stylet (St) protraction (Fig. 1b, c). Figure 1b and c represents larvae that invaded soybean roots and were sampled 2 and 7 days after inoculation, respectively. Figure 1d is a longitudinal section of a nematode showing hemidesmosomes (H) within the hypodermis (Hy) that interact with the striated basal layer of the cuticle (BLC) and with the basal lamina of the somatic muscle. Figure 1e is an oblique view of a secondary muscle (Sc) attachment to the esophageal lumen wall (LW). Figure 1f shows a cross section of a nematode with a linear orientation of hemidesmosomes (H) that show the interaction between the cuticle and somatic muscle (SM) basal lamina with the hypodermis as an intermediary. A, actin; Arrows, membrane junctions of tissue related to stylet shaft; CLC, cortical layer of cuticle; EDZ_1, particulate electron-dense zone; EDZ_2, amorphous electron-dense zone; EL, esophageal lumen; M, myosin; MLC, median layer of cuticle; PMS, protractor muscle sarcoplasm. Figure 1, ×6,900; 1a, ×14,650; 1b, ×7,800; 1c, ×16,800; 1d, ×33,900; 1e, ×39,850; 1f, ×41,120; 1g, ×161,630; 1h, ×66,250.









the inner subterminal boundaries of the somatic muscles (Fig. 11) that attach to the narrow region of the base of the cephalic framework (Fig. 9).

STOMATAL WALL: All cephalic blades are continuous with the anterior stomatal wall, where they intermesh with the fibrillar matrix that extends posteriorly into the narrowed portion of the stomatal wall (Fig. 1).

Within the fibrillar matrix of the stomatal wall, an electron-opaque particulate zone is prominent in the region anterior to the base of the cephalic framework (Figs. 1, 5, 8, 9). Extending posteriorly, this zone merges with a moderately electron-opaque region in the stomatal wall that widens just below the cephalic framework (Figs. 1, 10, 11) and later narrows to a thin and flexible wall (Figs. 1, 1b, 1c, 12) that terminates as an attachment to the shaft of the stylet (Fig. 1). The inner surface of the stomatal wall has longitudinally oriented invaginations through the thickened region (Fig. 1). In cross section these infoldings interrupt the inner circular contour of the stomatal wall (Figs. 10, 11). The flexibility of the posterior portion of the stomatal wall is shown in sections through the stylet regions of advanced larval stages of *H. glycines* during its feeding on syncytia induced in host tissues (Fig. 1b, c).

In addition to the electron-opaque particulate zone and moderately dense layer of the stomatal wall, there is an electron-opaque cylinder that extends from just below the stoma opening to the base of the cephalic framework. This cylinder, shown in insets Figure 8a and 8b, consists of closely packed radial filaments. The inner surface of this dense matrix is bounded by a trilaminar layer (Figs. 1, 8, 9).

The outer edge of the fibrillar basal plate of the cephalic framework joins the edge of the striated basal layer of the body cuticle (Figs. 1, 3–7). Anterior to this junction, the striated layer of the cuticle is absent and is replaced by the homogeneous matrix of cuticle that merges with and forms the outer outline of the head and lip regions of the nematode. Surface invaginations of the cuticle provide openings for the amphids and inner labial receptors (Figs. 2, 5). Although the exocuticle with its homogeneous granular appearance differs from the fibrillar structure of the cephalic framework and portions of the stomatal wall, there is close interfacing of these two structural components (Figs. 2, 6–8).

MUSCLE ATTACHMENTS: Somatic and stylet protractor muscles attach to the basal plate of the cephalic framework by irregularly arranged groups of hemidesmosomes with associated electron-dense microfilaments. Coinciding with the del-

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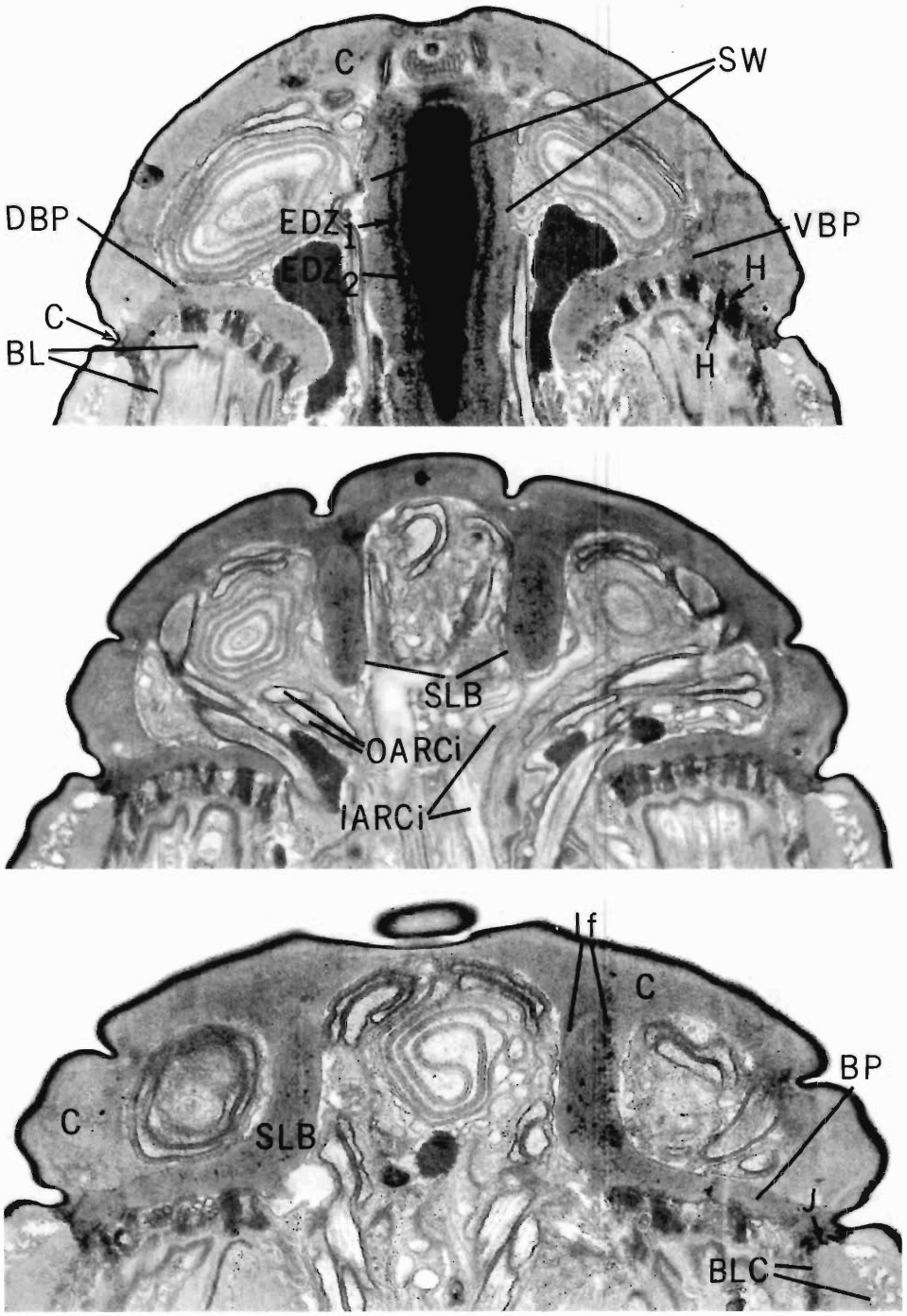
Figure 2. Tangential section through a dorsoventral sector of cephalic framework that shows the interfacing of the fibrillar matrix (FM) of the framework with the cuticular surface (CS). This view also shows the relationship of the cuticularized stomatal aperture (SA) surrounded by apertures of the inner labial receptors (ILR) and portions of an amphidial canal (AC). ×28,050.

Figure 3. Submedian longitudinal section through the cephalic framework that shows a portion of the anterior part of the stomatal wall and portions of two sublateral blades (SLB) of the cephalic framework. The narrow lateral base of the cephalic framework (BP) lies adjacent to the striated basal layer (BLC) of the nematode body. ×15,750.

Figure 4. Section through a dorsal or ventral sector of the cephalic framework that shows a relatively thick blade (CFB) that is continuous with the basal plate (BP). Hemidesmosomes (H) provide contact points for bundles of tonofilaments that traverse the hypodermis and that lie between the basal plate (BP) of the cephalic framework and the subjacent termini of somatic (SM) and protractor muscles. BL, basal lamina. ×18,950.



ta-shaped base of the dorsal and ventral blades of the framework, the muscle attachment region is more extensive in these sectors than in adjoining lateral sectors (Fig. 9). Transverse sections near the anterior termini of the stylet protractor and somatic muscles show the irregular pattern of the basal lamina of muscles to which hemidesmosomes attach.

Hemidesmosomes of irregular arrangement also occur between the somatic muscles and the striated basal layer of the cuticle underlying the first body annulation (Figs. 1, 9) and between the stylet protractor muscles and some surface areas of the spindle-shaped stomatal wall (Fig. 11). In contrast to the irregularly arranged hemidesmosomes, most of the hemidesmosomes between the cuticle and somatic muscles have a linear orientation.

The linearly arranged hemidesmosomes are characterized by a pair of adjacent hemidesmosomes within the hypodermal tissue. One hemidesmosome extends out to contact the membrane of the body cuticle or stomatal wall, whereas the other contacts the basal lamina of the adjacent muscle cells (Figs. 1, 1d, 1f, 11). In cross sections of nematodes, these hemidesmosomes appear as two bands under each cuticle annulation, and encircle the nematode where somatic muscles lie adjacent to the cuticle. Similarly, some hemidesmosomes are arranged linearly along the stylet protractor muscles, where lateral attachments are made to the stomatal wall (Fig. 1).

SOMATIC MUSCLE MORPHOLOGY: Coinciding with the extensive hemidesmosomal region in the subdorsal and subventral sectors of the cephalic framework, cross sections show that the attached subdorsal and subventral somatic muscles occupy twice as much area as the adjacent sublateral somatic muscles within each quadrant (Fig. 10). As the somatic muscles extend posteriorly, they are joined by two additional pairs of somatic muscles at the dorsal and ventral positions at a level where the stomatal wall becomes narrow, but before the stomatal wall attaches to the stylet shaft (Fig. 12). A third set of somatic muscles appears between the subdorsal, dorso-sublateral muscles and the subventral, ventro-sublateral muscles at a level adjacent to the shaft portion of the retracted stylet and just below the basal body region of the amphidial receptor cilia (Fig. 13).

PROTRACTOR MUSCLES: The stylet protractor muscles are an integral part of

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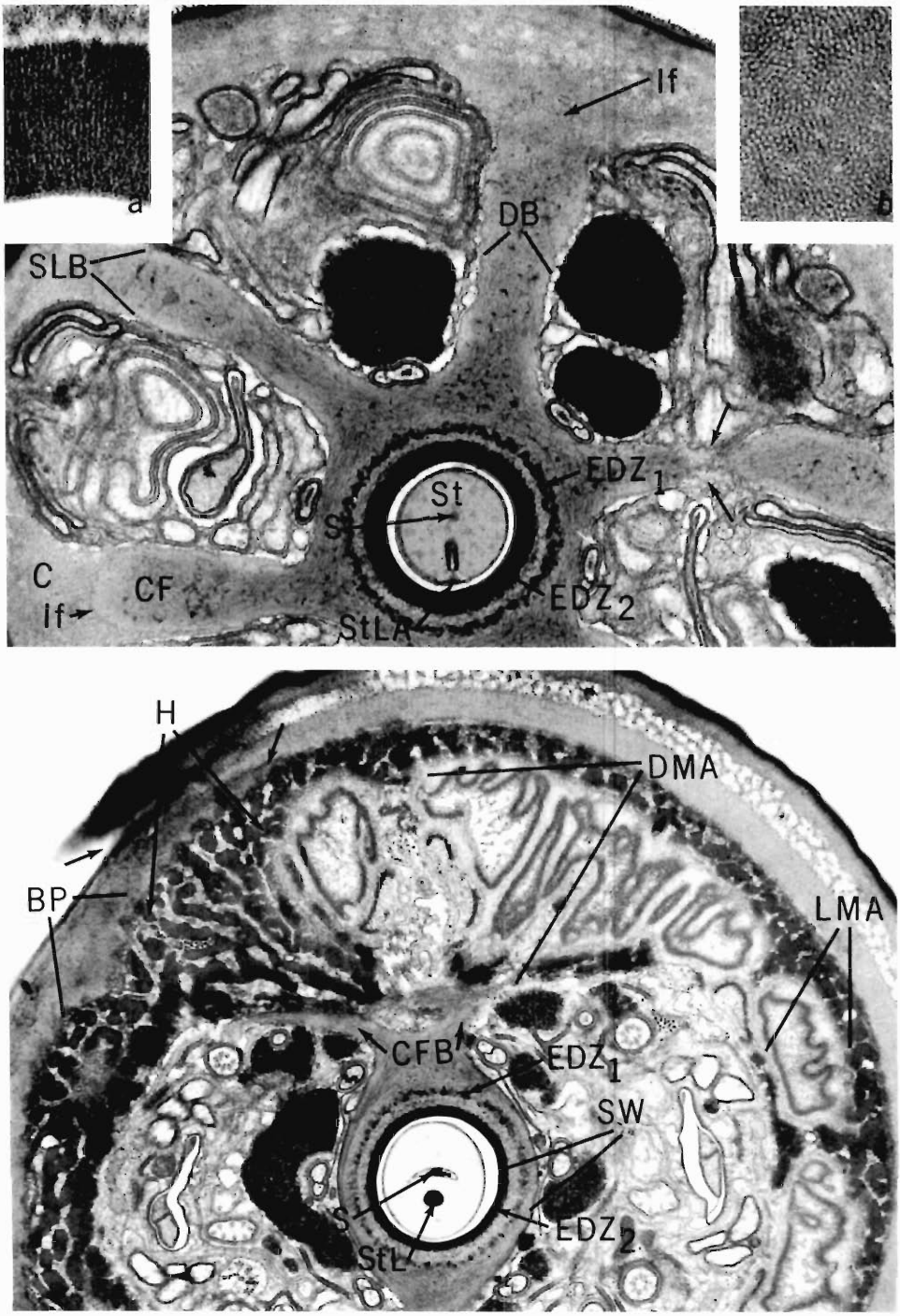
Figures 5–7. Lateral sections through the cephalic framework to show the relationship of stomatal wall, blades, and basal plate.

Figure 5. Lateral, slightly submedian section through cephalic framework that shows the fibrillar stomatal wall (SW) with electron-dense particulate zone (EDZ_1) and the centrally located electron-dense filamentous zone (EDZ_2). Cross section through the dorsal (DBP) and ventral (VBP) regions of the basal plate shows hemidesmosomes (H) between the basal plates and the basal lamina (BL) of somatic muscles. The cuticle (C) that covers the basal plate is especially thin at the last head annulation. ×15,400.

Figure 6. Submedian section of cephalic framework. The two portions of sublateral framework blades (SLB) illustrate the curved underbridge region of these blades through which accessory cilia traverse. Both inner (IARCi) and outer (OARCi) accessory receptor cilia are shown in their transition from the lateral to the ventral and dorsal positions within the cephalic framework. ×18,000.

Figure 7. A lateral section, slightly into somatic musculature, that shows the sublateral blades (SLB) as they extend outward and merge with the basal plate (BP). Note the interface (If) between the framework and the anterior cuticle (C) and the distinct junction (J) of the base of the cuticle with the terminus of the striated basal layer of the cuticle (BLC). ×22,500.



the anterior musculature of the nematode. The anterior termini of the protractor muscles are located at the dorsal, ventral, subdorsal, and subventral regions of the cephalic framework (Fig. 10). Intermuscular contacts between protractor muscles and somatic muscles are extensive (Figs. 10, 11). Electron-opaque hemidesmosomes indicated the interaction among the various protractor muscles and adjacent somatic muscles. Each of the protractor muscles of the latero-subdorsal radii is subdivided at the anteriormost portion of the muscle (Figs. 10, 11). This division continues throughout the length of the muscle and eventually separates to form the boundaries of the three sets of protractor muscles that attach to the dorsal and two subventral knobs of the stylet (Fig. 14). Thus, the upper half of each latero-subdorsal protractor muscle joins with the two dorsal protractor muscle elements and extends posteriorly to form the muscle that attaches to the dorsal knob of the stylet. The lower half of the latero-subdorsal muscle element merges with each ventral protractor muscle that attaches to the subventral knobs of the stylet (Figs. 10–14). In this manner, the bilateral symmetry of the anteriormost protractor muscles, having 10 muscle elements, changes to the triradiate symmetry of combined muscle elements that attach to each of three stylet knobs.

The extensive intermuscular junctions between protractor and somatic muscles are reduced to apposed membrane contacts as the protractor muscles extend below the spindle-shaped region of the stomatal wall (Figs. 11–13). Subsequently, protractor muscles are separated from the adjacent somatic musculature, thereby providing freedom of lateral movement of the stylet base during stylet thrusts.

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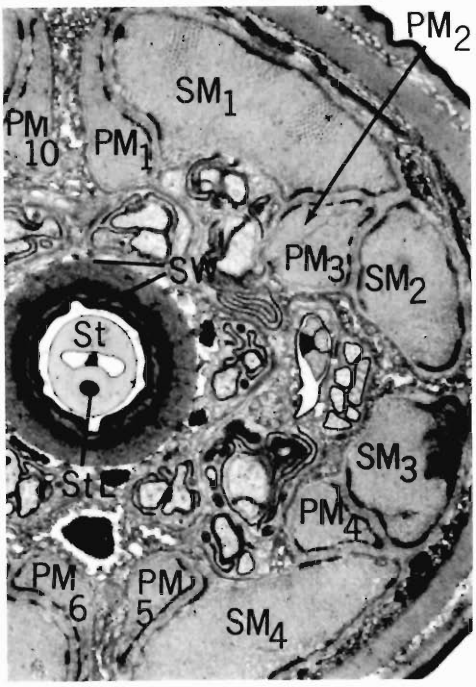
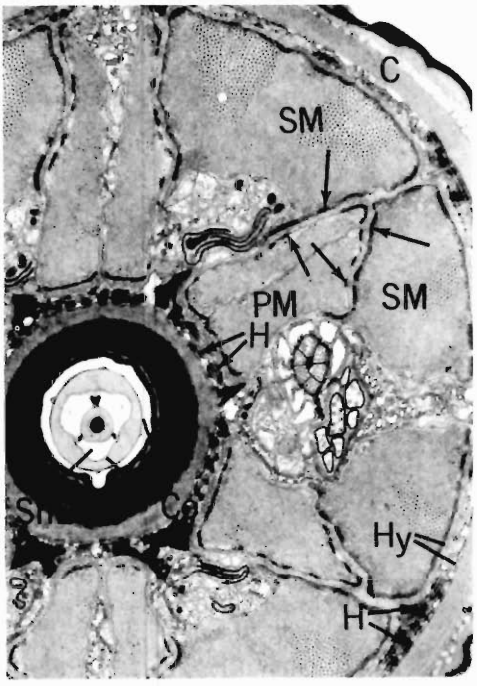
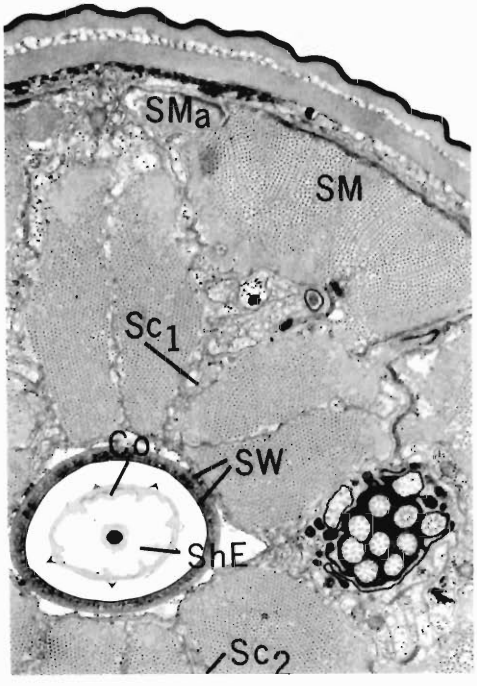
Figures 8, 9. Cross sections through two levels of the nematode framework that show the fibrillar region of the cephalic framework and the muscle attachment zone between the basal plate and the anterior basal lamina of stylet protractor and somatic muscles.

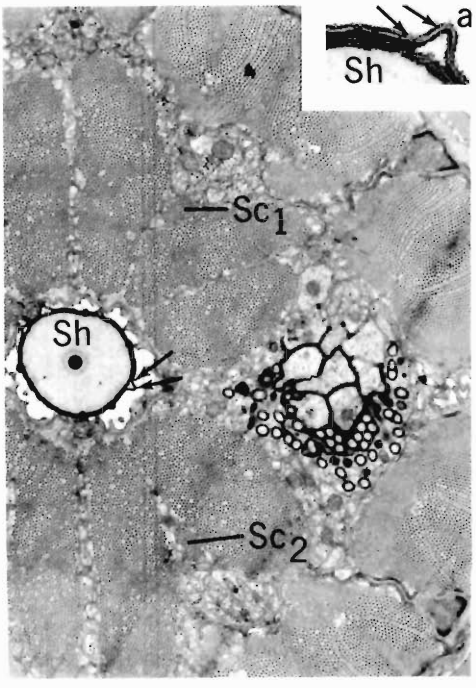
Figure 8. Cross section through the cephalic framework (CF) just anterior to the basal plate. The dorsal blade (DB) is slightly wider than three of the four sublateral blades (SLB). Although the cephalic framework is highly compartmentalized anterior to this section, interaction between the dorsal and lateral sectors is provided by the curved underbridge of the sublateral blades (arrows). The stylet (St) is shown in cross section with the anteriorly oriented lumen aperture (StLA) and the terminal part of the sinus (S). An electron-dense particulate zone (EDZ_1) lies in the fibrillar region of the stomatal wall. The highly electron-dense cylinder of closely packed radial filaments of the stomatal wall (EDZ_2) has an irregular margin that interacts with the outer layer of the stomatal wall. The internal surface appears trilaminar. An enlargement of a similar stomatal wall region is shown in cross section in Figure 8a and in tangential-longitudinal section in Figure 8b. The tangential section through the cephalic framework blades shows the interface (If) between the fibrillar component of the framework (CF) and outer head cuticle (C). Figure 8, ×25,250; 8a, ×187,000; 8b, ×390,000.

Figure 9. Cross section through hemidesmosomes (H) and bundles of tonofilaments that traverse the hypodermis. This section is between the basal plate (BP) and anteriormost portions of the protractor and somatic muscles that are associated with the cephalic framework. The section through the trough of the first body annulation (arrows) shows the fibrillar cuticular zone of the basal plate and its junction with the striated basal layer of the cuticle. Tangential and cross-sectional views of hemidesmosomes show the highly electron-dense irregular pattern of this junctional complex that lies between the cephalic framework and the basal lamina of muscles. Note the broad dorsal muscle attachment (DMA) area versus the narrow lateral (LMA) areas. Posterior to its ventrally located aperture, the stylet lumen (StL) is circular in cross section and the sinus (S) is crescent-shaped. At this level the stomatal wall (SW) support is reduced to flanged portions of the dorsally and ventrally extended cephalic framework blades (CFB). EDZ_1, electron-dense zone, particulate; EDZ_2, electron-dense zone, amorphous stomatal wall. ×48,750.









Centripetally, these same protractor muscles make linearly arranged hemidesmosomal attachments to the widest region of the spindle-shaped stomatal wall (Figs. 1, 11).

Among the contractile portions of the stylet protractor muscle elements, thin myofilaments (actin) predominate in the region between muscle attachment to the cephalic framework and the level where centripetal attachment is made to the stomatal wall (Figs. 10, 11). Extending posteriorly, the region occupied by thin myofilaments is reduced, and the central region of each muscle element is comprised of a combination of thick (myosin) and thin (actin) myofilaments (Figs. 1g, 12). At the level of the basal region of the amphidial receptors, thick myofilaments are predominant in the protractor muscle sarcomere (Fig. 13). Descending toward the stylet knobs, adjacent to the nerve process, the protractor muscle sarcomeres again have combinations of thick and thin myofilaments (Fig. 1g). Just before stylet knob attachment, protractor muscles consist of thin myofilaments (Fig. 14).

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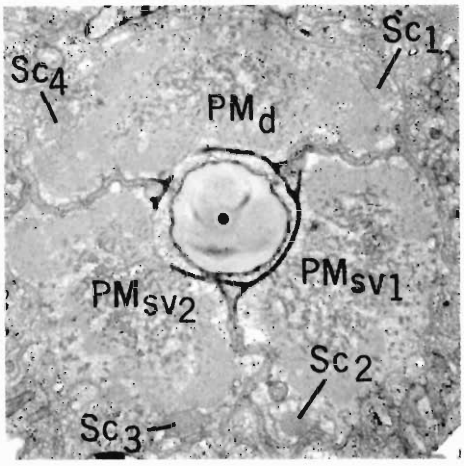
Figures 10–13. Series of cross sections through the protractor muscles, stomatal wall, and stylet.

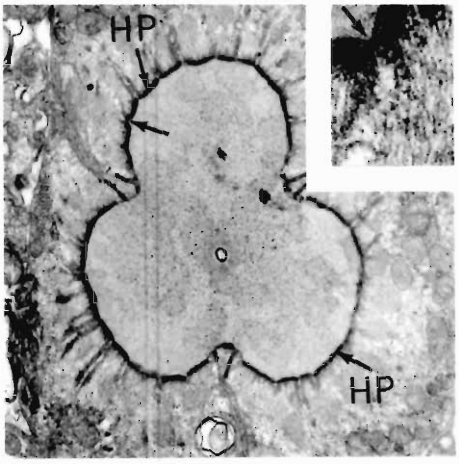
Figure 10. Cross section through the stomatal wall slightly below the cephalic framework and its juncture with the somatic (SM) and protractor (PM) muscles. The inner stomatal wall (SW) shows the outline of longitudinally oriented evaginations that relate to the flexibility of the wall during stylet movement. The stylet protractor muscles PM_2 and PM_3 (plus PM_8 and PM_9), although paired anteriorly, separate and merge with each of the three stylet knob muscles posteriorly (Fig. 4). Thus, muscle elements PM_9, PM_{10}, PM_1, and PM_2 form the dorsal knob muscle, and PM_3, PM_4, and PM_5 plus PM_6, PM_7, and PM_8 form the ventro-sublateral stylet knob muscles. (Note: PM_7, PM_8, and PM_9 are not illustrated.) Both the somatic and protractor muscles show an increase in girth as they extend posteriorly. When Figure 10 is compared with Figure 11, the protractor muscles show enlargement centripetally to make contact with the outer surface of the stomatal wall. Thin myofilaments occur throughout the protractor muscles at this level of sectioning. The cone portion of the stylet (St) has a flattened sinus and a tubular support for the stylet lumen (StL), which is embedded in the cone matrix. ×21,550.

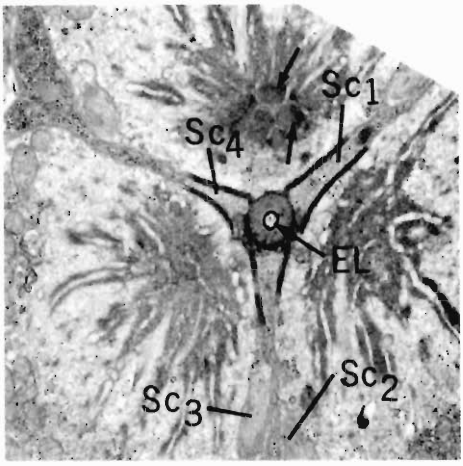
Figure 11. A cross section through the widest part of the stomatal wall (Fig. 1) with two electron-dense inner layers surrounded by the fibrillar matrix that is continuous with the cephalic framework. Hemidesmosomes (H) provide contact between the stomatal wall and the centripetal surfaces of the protractor muscles through the bundles of tonofilaments of the intermediate hypodermis. Hemidesmosomes between stylet protractor (PM) and adjacent somatic muscles (SM) may provide stability for stylet protraction. Most of the hemidesmosomes between the stomatal wall and protractor muscles appear similar to those found between the somatic muscles and the cuticle (C), where a linear orientation appears predominant. The stylet cone (Co) is reduced in thickness and encloses a triradiate extension of the stylet shaft (ShE). The stylet lumen and surrounding support are centrally located. Hypodermis. ×14,850.

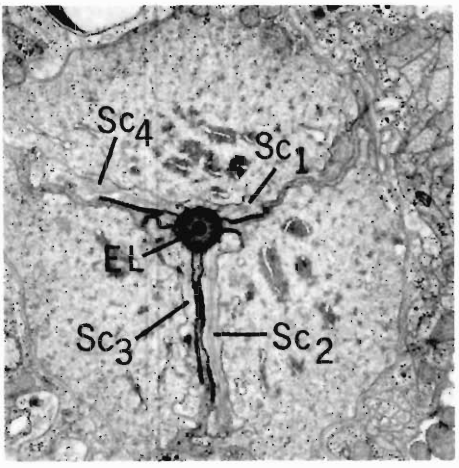
Figure 12. Cross section through the posteriorly extended thin stomatal wall near its attachment to the stylet shaft (Fig. 1). Stomatal wall (SW) separation from the stylet protractor muscles is recognized by the absence of hemidesmosomes and the space formed between these components. Concurrently, general hemidesmosomal attachments between the stylet protractor muscles and the somatic muscles are greatly reduced. The terminus of a different set of somatic muscles (SMa) appears in the extreme dorsal position. Secondary muscles (Sc_1 and Sc_2) are shown between the dorso- and the ventro-sublateral protractor muscles. The cone (Co) portion of the stylet is reduced to a thin outer cone that is supported by extensions of the shaft (ShE). The flexibility of this thin portion of the stomatal wall was illustrated in Figure 1b and 1c. SM, somatic muscle. ×33,200.

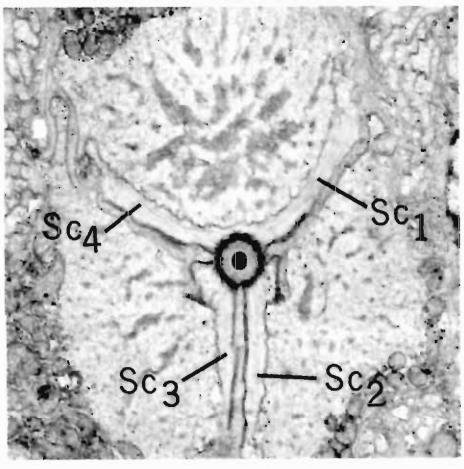
Figure 13. A section through the retracted stylet that shows the stylet shaft (Sh) with a relatively uniform electron-lucent matrix and a moderately electron-dense region surrounding the stylet lumen. The wall of the hypodermis that extends posteriorly from the juncture of the stomatal wall and stylet is appressed to the stylet shaft (Sh) (See inset 13a). Arrows in Figure 13 and Figure 13a show identical sites. Electron-dense deposits occur between the stylet shaft surface and hypodermal membrane. Sc, secondary muscles. Figure 13, ×14,150; 13a, ×62,150.

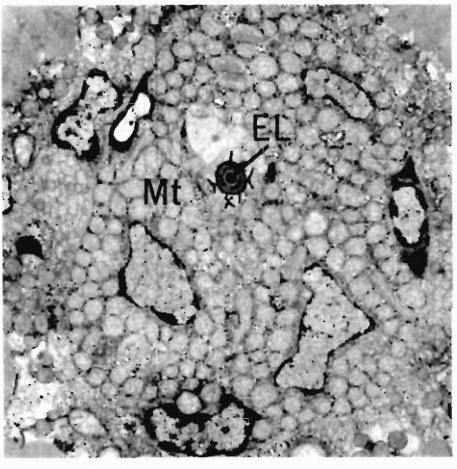












In the A zone of the sarcomere, the ratio of the actin : myosin configuration is near 10:1, with wide variations at the peripheral zones of the filament types (Fig. 1g).

The anterior termini of each of the four secondary protractor muscles are recognizable at the subdorsal and subventral nerve bundle regions, where they appose the plasma membrane of stylet protractor muscles and the adjacent nerve bundles (Figs. 12, 13). The pair of subdorsal secondary muscles shifts to the outer border of the dorsal protractor muscle (Fig. 14) and they later join at their terminals as a common band posterior to the stylet knobs (Figs. 14, 16, 17), with attachment to the outer wall of the esophageal lumen (Figs. 1, 1e, 14–18). Similarly, the two secondary muscles of the subventral sectors traverse posteriorly to the subventral knobs and extend parallel and centripetally to terminate with junctions on the outer wall of the esophageal lumen (Figs. 14–18).

The noncontractile portions of the stylet protractor muscles, posterior to the stylet knobs, appear as electron-lucent fibrils that attach to their respective stylet knobs and merge with the sarcoplasm of the supporting muscle cells. The sarcoplasm of the protractor muscle cells has high populations of mitochondria (Figs. 1, 19).

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Figures 14–19. Cross sections through the basal part of the stylet shaft, the stylet knob, and stylet protractor muscle sarcoplasm.

Figure 14. A section through the stylet protractor muscles slightly anterior to the stylet knobs. Membrane junctions show the tripartite muscle arrangement of the once bilateral (10-muscle-element) symmetry of these stylet protractor muscles. Muscle elements PM_9, PM_{10}, PM_1, and PM_2 combine to form the dorsal stylet knob protractor muscle (PM_d). Similarly, muscle elements PM_{3-5} and PM_{6-8} combine to form the two subventral stylet knob muscles (PM_{sv1} and PM_{sv2}). Secondary muscles (Sc) appear as discrete elements. ×26,100.

Figure 15. A cross section through the stylet knobs. The stylet knobs make anterior and lateral contact with the surrounding myofilaments of the protractor muscles via electron-dense hemidesmosomal plates (HP). The outer supporting membrane of the stylet knob evaginates into the surrounding muscle cells (Fig. 15, 15a) to form an extensive membrane surface network between the stylet and the supporting muscle cells. Arrows of Figure 15 and Figure 15a point to identical sites. The outer surface of the evaginated membranes has electron-dense accumulations. Figure 15, ×13,100; 15a, ×77,330.

Figure 16. Section slightly posterior to the stylet knobs shows the esophageal lumen (EL), its wall, and supporting membranes. Secondary muscles (Sc 1–4) converge toward the lumen wall. Electron-dense material accumulates on the outer surface of the cisternae formed from the interhemidesmosomal membrane evaginations that originate from the stylet knob surfaces (Figs. 1, 15). The section through the dorsal knob reveals the plateletlike units of hemidesmosomes with spaces formed by the evaginated stylet knob membranes. These membrane-supported spaces are continuous with the cisternae that extend into the noncontractile part of the stylet protractor muscles (Fig. 1). ×15,900.

Figure 17. A section into the noncontractile part of the protractor muscle shows the stylet knob-associated cisternae and electron-dense accumulations. Ventral and subdorsal secondary muscles (Sc 1–4) make terminal attachments to the wall of the esophageal lumen (EL). ×18,400.

Figure 18. A slightly oblique section of the esophagus just below the stylet shows the firm attachment of the secondary muscles to the wall of the esophageal lumen. The two dorso-sublateral muscle elements (Sc_1 and Sc_4) merge at the attachment zone. The ventro-sublateral secondary muscles (Sc_2 and Sc_3) lie adjacent to each other and extend centripetally to form a similar junction with the lumen wall, but each muscle retains its identity. A longitudinal view of this junction is shown in Figure 1e. ×14,950.

Figure 19. Section through the protractor muscle sarcoplasm that shows the esophageal lumen (EL) with its supporting wall and associated esophageal cells. These cells are characterized by an abundance of mitochondria (Mt) that are an integral part of the stylet protractor muscles. ×9,700.

STYLET: Anatomically, the stylet of the soybean cyst nematode larvae consists of two components: the anterior part or cone, which is sloughed off during molt and remains attached to the cuticle and cephalic framework, and the shaft portion with its stylet knobs. The stylet has a rounded chisellike terminus. When viewed laterally, the anterior terminus of the stylet is rounded (Fig. 1, 1a), but when viewed dorsoventrally, it is conical (Endo, 1980). The lumen of the stylet terminates as a narrow ventrally oriented orifice near the anterior terminus of the stylet cone (Fig. 1a). Cross sections through the anterior part of the stylet cone show the circular lumen and crescent-shaped sinus (Fig. 9). Proceeding posteriorly, the electron-lucent sinus enlarges, changes to a triangular, and then to an irregular, circular cross section before it merges with the similar electron-lucent components of the stylet shaft. At the juncture of the cone and the shaft, the cone wall becomes narrow and has an irregular inner surface (Fig. 12). Thus, it appears that the electron-lucent shaft components extend into the cone portion of the stylet. This transition zone between the cone and shaft is also the point of attachment between the posterior terminus of the stomatal wall and the stylet. The electron-lucent part of the shaft is altered by a slightly electron-dense zone that surrounds the stylet lumen and widens into the stylet knobs (Figs. 1, 10–13).

The stylet knobs are attached to the protractor muscles by hemidesmosomes (Figs. 1, 15). Muscle attachment by hemidesmosomes occurs over the anterior and lateral surface of the stylet knobs; however, hemidesmosomal contacts to the posterior surface of stylet knobs arise primarily from noncontractile components of these muscle cells. Further contact between the stylet and the sarcoplasm occurs through the evaginations of the interhemidesmosomal membranes that extend from the stylet surface into the sarcoplasm. Plateletlike morphology of hemidesmosomes (Fig. 16) is revealed by a section along the posterior surface of a dorsal stylet knob. In cross section, evaginations appear as flattened cisternae bordered by electron-dense accumulations (Figs. 1, 15–18).

Similar cytoplasmic contact with the stylet appears to occur along the basal portion of the shaft, where membrane contacts are present between the adjacent supporting cells and the shaft structure (Figs. 1, 1c, 13). There are three sets of cell junctions that appear along the shaft (Fig. 1). The cell junction at the base of the shaft has a circular orientation (Fig. 14).

Discussion

Although the cephalic framework of the male of *Heterodera glycines* has been described as radially symmetrical (Baldwin and Hirschmann, 1976), the larval stage of this species has a framework that is better described as bilaterally symmetrical. The six blades of the framework are radially spaced anteriorly; however, bilateral symmetry is accentuated by the broadened basal region of the dorsal and ventral blades, which are distinctive from the sublateral blades that are narrower and have a curved understructure. The blades have a fibrillar structure that is continuous with the base of the cephalic framework. The dorsal and ventral cephalic sectors form broad extensive junctures with the subjacent somatic and protractor muscles, whereas comparable junctures in the lateral sectors are limited. The lateral sectors of the cephalic framework are relatively open for the initial entry of anterior sensory organs and their supporting cells. This bilateral symmetry of the cephalic framework of the tylenchids was reported in ultrastruc-

tural studies of males of *Meloidogyne incognita* (Baldwin and Hirschmann, 1976), females of *Rotylenchus robustus* (De Grisse et al., 1974), and various stages of *Tylenchulus semipenetrans* (Natasasmita, 1979).

Furthermore, bilateral symmetry of the cephalic region of larvae of *H. glycines* is further reflected in the arrangement of the anterior region of the protractor muscles. The radial orientation of the dorsal and ventral protractor muscle elements corresponds to the broad basal plate of the cephalic framework and region they occupy between the spindle-shaped stomatal wall and the interchordal hypodermis. A similar outward intrusion of the sublateral protractor muscles is restricted by somatic muscles that lie between the protractor muscles and body cuticle (Figs. 10, 11).

The moderately dense cuticular component of the labial framework of *Tylenchorhynchus dubius* (Byers and Anderson, 1972) is similar to the fibrillar portion of the cephalic framework observed in *H. glycines*. In *T. dubius* this fibrillar component of the cuticle extends radially for a short distance along each of the six blades of the framework, but in *H. glycines* the cuticular component extends to the outer margins of the cephalic framework and includes a major part of the stomatal wall. Although the cephalic framework of the male of *H. glycines* (Baldwin and Hirschmann, 1976) is described as having dense radial blades demarcated from the cuticle of the basal ring, additional sectioning through this region may show continuity of the blades with the fibrillar component of the basal ring and the flanged muscle attachment region, as found in larvae of *H. glycines*. Furthermore, the fibrillar cephalic basal ring of *H. glycines* larvae is apposed to the striated, somatic basal layer of the cuticle and reveals only a narrow cuticular layer at the annulation that forms the junction between the somatic and lip regions (Fig. 6).

Wisse and Daems (1968) have reported that hemidesmosomes attach the striated basal layer of the cuticle to the basement membrane of somatic muscles in larvae of *Heterodera rostochiensis*. Similarly, an extensive regular array of hemidesmosomes was described for *Tylenchulus semipenetrans* (Natasasmita, 1979). Hemidesmosomes usually occur between apposing muscle cells, as in *T. semipenetrans* (Natasasmita, 1979), males of *H. glycines* (Baldwin and Hirschmann, 1976), and larvae of *H. glycines*. When cross sections were made through hemidesmosomes, definitive light to dark bands appeared as part of the muscle attachment system. In tangential sections, however, these same hemidesmosomes may appear as microfibrils of electron-dense units in the hypodermis (Baldwin and Hirschmann, 1975b). Some hemidesmosomes appear as regularly aligned bands when longitudinal and cross sections of larvae of *H. glycines* are compared. Thus, linearly arranged hemidesmosomes occur in the hypodermis between the somatic muscle elements and the body cuticle, as well as between protractor muscle elements and the spindle-shaped stomatal wall surface. The linearly arranged hemidesmosomes that followed the contour of the annulations of the cuticle were absent when the striated basal layer of the cuticle was discontinuous. Except for the somewhat irregular arrangement of hemidesmosomes near the extreme anterior of the larva, the pair of ribbons of hemidesmosomes under each annulation appears to provide optimal support and mobility for nematode movement. In contrast, the irregular dense matrix of hemidesmosomes, which is present at the base of the cephalic framework and at parts of the stomatal wall,

provides strong muscle attachments that are required for stylet protraction during host penetration and feeding.

It appears that the extensive contact between the protractor muscles, the stomatal wall, and somatic muscles provides the nematode with maximum mobility and coordination for stylet extension and side thrusts that are exhibited by this and other plant-parasitic species during root-cell penetration (Wyss, 1973; Seymour, 1975). Hemidesmosome contacts between and among protractor and somatic muscles provide protractor muscles with indirect contact to the nematode cuticle. In addition, the dorsal and ventral protractor muscles have direct contact with the surface cuticle because of their centripetal orientation. Similar protractor muscle contact with the nematode body wall was reported by Natasasmita (1979), who showed that protractor muscles of *Tylenchulus semipenetrans* made contact with the body wall from the level of the base of the stoma up to the cephalic framework.

Thus, it appears that stylet guidance and stability of *H. glycines* larvae and other tylenchids are established through hemidesmosomal contacts between protractor and somatic muscles, the stomatal wall, the cephalic framework, and the cuticle. Cuticular contact with the protractor muscles may be direct or indirect depending on their position and relation to the stylet.

The arrangement of the stylet protractor muscles of *H. glycines* larvae is similar to that described for other tylenchid species (Byers and Anderson, 1972; Baldwin and Hirschmann, 1976; De Grisse, 1977; Natasasmita, 1979). The three main protractor muscles of males of *H. glycines* have posteriad attachments to stylet knobs, and collectively branch anteriorly into 10 muscle elements. These muscle elements were shown to attach via basal lamina to the cephalic framework (Baldwin and Hirschmann, 1976). The stylet protractor muscle arrangement of *H. glycines* larvae is similar to that reported for the males, but the muscle attachment of the anterior terminus not only involves the stomatal wall and body wall, but makes direct attachment to the entire basal surface of the cephalic framework. Adult female specimens of *Tylenchorhynchus dubius* have similar protractor muscle arrangements, where three main muscles attach to the stylet knobs and divide anteriorly into 10 discrete muscle elements that have insertions to the body wall at the lip constriction (Byers and Anderson, 1972). The protractor muscle elements of *Tylenchulus semipenetrans* larvae, males and females, terminate in the various sectors of the head and attach by hemidesmosomes to the head wall and blades of the cephalic framework (Natasasmita, 1979). Observations made of these attachments to the base of the cephalic blades coincide with the observations made on *H. glycines* larvae. Evidence for direct contact between the protractor muscle terminus with the head cuticle of *T. semipenetrans* was not clearly shown. However, this may be due partially to the relatively thin blades of the cephalic framework of the species. From reports of *T. semipenetrans* and observations of *H. glycines,* there is general agreement on the presence of extensive basal lamina and muscle attachment sites on the basal region of the cephalic framework. In contrast, Yuen reported (1967) that the stylet protractor muscles of *Ditylenchus dipsaci* terminate at the body wall, just below the base of the head skeleton. Furthermore, no lateral protractor muscle attachments were made to the guiding apparatus (stomatal wall). This observation on *D. dipsaci* differed from earlier light-microscopic observations by Coomans (1962) on another tylen-

chid species. Coomans reported that spear protractor muscles of *Rotylenchus goodeyi* have attachments partly on the vestibule extension and partly on the basal plate. Similar conclusions were drawn by Hirschmann (1959) from histological observations of *H. glycines* males. In an ultrastructural study of the male of the same species, however, this facet of the protractor muscle attachment to the cephalic framework was not described (Baldwin and Hirschmann, 1976). It is apparent from the present study that stylet protractor muscles of *H. glycines* larvae have similarities to other tylenchid species, and that *H. glycines* larvae have extensive protractor muscles with strong hemidesmosomal contact with various structural components.

Baldwin and Hirschmann (1976) reported that secondary muscles of the protractor muscle system have their origin near the level of the base of the vestibule extension or stomatal wall. The relationship of these secondary muscles to the esophageal lumen wall was described for males of *M. incognita,* but was not reported for males of *H. glycines*. It is apparent that, for larvae of *H. glycines,* secondary muscle attachments to the wall of the esophageal lumen are quite extensive. The secondary muscles are readily traceable because they appear as separate elements adjacent to the primary protractor muscles anteriorly and converge centripetally toward the esophageal lumen just below the stylet knobs. The general arrangement and positions of secondary muscles in relation to the protractor muscles appear the same among several tylenchid species, including *Tylenchulus semipenetrans* (Natasasmita, 1979), *Meloidogyne incognita* (Baldwin and Hirschmann, 1976), and *Tylenchorhynchus dubius* (Byers and Anderson, 1972). The secondary protractor muscles of *H. glycines* and possibly other tylenchid species apparently support the esophageal canal during penetration of the host and feeding. Not only is there extensive cell-to-cell contact of the secondary protractor muscles to the main protractor elements, but the terminals of these secondary muscles have broad attachment points along the wall of the esophageal canal.

The stylet of *H. glycines* consists of two components, the cone and the shaft, with the knobs as an integral part of the shaft. This agrees with descriptions of other tylenchid species (Wen and Chen, 1972; De Grisse, 1977; Natasasmita, 1979). Ultrastructural observations on larvae of *H. glycines* (unpubl. data) show that the cone portion of the stylet is sloughed off during molt. The fate of the stylet shaft and knobs during and after molt is not yet clear for this species. The stylet of the tylenchid *Criconemoides curvatus* has a tooth, shaft, and knobs, but the tooth appears more cuticularized than the other parts. The shaft begins at the basal knobs and extends toward the tip of the stylet. Six ducts within the shaft are connected to the cytoplasm outside the stylet, and extend from the base to the tip of the shaft (Wen and Chen, 1972). A similar two-part structure of stylets was reported for *Pratylenchus penetrans*. It was proposed that the shaft and knobs are living tissues, and that the tooth is a product of sclerotized secretions (Chen and Wen, 1972).

In larvae of *H. glycines,* there appears to be cellular contact to the shaft through membranes that tightly appose the shaft surface. The multilayered cellular support is revealed by the three levels of circular membrane junctions in the cells that surround the stylet shaft. At the level of the knobs, extensive cytoplasmic contact is made through cisternalike membrane evaginations from the stylet knob

surface into the various protractor muscles. This extensive interaction between the stylet knob surfaces and the noncontractile portion of the protractor muscles provides further support for the esophagus-related stylet ontogeny that was suggested for males of this species (Baldwin and Hirschmann, 1976).

The soybean cyst nematode larva, like other plant-parasitic species (Bird, 1971), utilizes its stylet for emergence from the egg, penetration of the host tissue, and the initiation and feeding on a syncytium (Endo, 1964). The contraction of the protractor muscles that extend from the base of the cephalic framework to the stylet knobs thrusts the stylet forward and through the stomatal aperture. During stylet extension, the anterior part of the stylet, the cone, and portions of the extended stylet shaft are guided by the stomatal wall and the supporting cephalic framework.

Clearly, larvae of the soybean cyst nematode have robust stylets that are supported by an extensive network of protractor muscles. These muscles, in turn, are attached to the stylet, the cephalic framework, and body cuticle through a network of irregular and linearly oriented hemidesmosomes. The extensive stylet protractor muscle system observed in *H. glycines* larvae supports studies conducted on the mechanics and design of tylenchid stylets (Seymour, 1975). Because of the anatomical structure of the stomatal region of its larvae, the soybean cyst nematode is well adapted to penetrate soybean roots and establish a feeding site that can cause irreparable damage to the host plant.

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Juvenoid Effects on *Nippostrongylus brasiliensis* and *Heterodera glycines* (Nematoda)

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ABSTRACT: Treatment of third-stage larvae of *Nippostrongylus brasiliensis* with several natural and synthetic juvenile hormones (JH) caused a subsequent reduction in egg production/female. Juvenoids also inhibited the development of *N. brasiliensis* and *Heterodera glycines* (Race 3) from eggs. Farnesol had strong activity against both species, although the other juvenoids varied in their effect. Extraction and chromatographic separation of endogenous JH from *N. brasiliensis* larvae and adults revealed only insignificant activity by insect bioassay. However, the exogenous juvenoids apparently blocked pheromone production by female *N. brasiliensis* and diminished male responsiveness.

A number of investigations have examined the influences of exogenous juvenile hormones (JH) and their analogues on various nematodes. Meerovitch (1965) initially reported the effect of JH on nematodes, and Shanta and Meerovitch (1970) found farnesol and farnesyl methyl ether inhibited in vitro molting of *Trichinella spiralis* at 10^{-4} M. Low concentrations of JH produced a specific developmental inhibition of the male copulatory apparatus in *T. spiralis,* and treatment of the phytoparasite *Heterodera schachtii* caused hyperplastic development of the male gonads (Johnson and Viglierchio, 1970). Also, JH reduced the number of *Nematospiroides dubius* that molted, but more interestingly, increased oviposition by females (Dennis, 1976). The sensitivity of the gonadotrophic effect was suggested by Dennis to preclude a response to environmental stress. However, Dennis (1977) found no effect of JH on the polyribosomal profile of *Panagrellus redivivus*. Larval arrestment and lethality were found also in *Haemonchus contortus* after treatment with a number of JH derivatives (Boisvenue et al., 1977). The greatest activity was present in farnesol and its analogues.

Other studies partially contradict the above reports. Hansen and Buecher (1970) found developmental changes and reduced viability in some species, whereas other nematodes were not influenced by JH treatment. Davey (1971) proposed that JH activity on *Phocanema decipiens* resulted from neuroendocrine stimulation that caused an inappropriate molt as a nonspecific, stressful reaction to hormonal exposure.

Additional reports imply the presence of an endogenous JH in nematodes. Dropkin et al. (1971) found that exogenous JH inhibited the growth of *Caenorhabditis elegans* and that chromatographic fractions of lipids from *Panagrellus redivivus* and *Nematospiroides dubius* inhibited reproduction in *C. elegans*.

Hitcho and Thorson (1971) reported that the Williams and Law mixture of JH's increased the length of larval *T. spiralis,* but had no effect on molting. Lipid fractions of larval *T. spiralis* also increased the length of exposed larvae, but gave variable molting.

Rogers (1973) demonstrated that JH compounds inhibited egg hatching of *H. contortus,* but not of *Ascaris lumbricoides*. The development of *H. contortus* and *P. redivivus* was inhibited also, but exsheathment of *H. contortus* was not altered. Thin-layer chromatography of extracts of *H. contortus* and *P. redivivus*

revealed a substance with juvenoid activity by insect bioassay and that moved with farnesyl methyl ether.

Based on the above, preliminary studies were conducted on the effects of juvenoids and similar compounds on the rodent hookworm, *Nippostrongylus brasiliensis*, and the soybean cyst nematode, *Heterodera glycines* Race 3. This investigation sought to examine any influences on development and reproduction in these worms as potential means of hormonal control.

Materials and Methods

Mouse-adapted *Nippostrongylus brasiliensis* were maintained as previously reported (Bone et al., 1978). Animal inoculation and helminth recovery were similar to previous methods unless otherwise indicated.

Third-stage larvae of *N. brasiliensis* were collected from vermiculite-fecal cultures and exposed to various concentrations of JH for 3 hr prior to host inoculation. The synthetic JH's ZR-512 (ethyl 2E,4E-3,7,11-trimethyl-2,4-dodecadienoate, Zoecon), ZR-515 (isopropyl-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, Zoecon), and MV-678 (2-methoxy-9-4(isopropylphenyl)2,6-dimethylnone, Stauffer) were tested. The natural juvenoids JH I, JH II, JH III, and farnesol (Sigma) were used also. At least five concentrations were tested for each compound over a 1,000-fold range. Juvenoids were dissolved and placed in agitated suspensions of larvae with 5% methanol. Controls consisted of a 3-hr exposure of larvae to 5% methanol. Then, groups of three mice were inoculated with 750 larvae each for each treatment or control.

Total egg production in feces was determined from the sixth through tenth days postinfection for each treatment according to MacInnis and Voge (1970). Then, animals were sacrificed for recovery of female *N. brasiliensis* to evaluate quantitative aspects of reproduction.

Juvenoid effects on egg hatching and larval development of *N. brasiliensis* were examined also. Host feces were collected on the eighth day postinfection. Twelve-hr-old eggs were recovered by gauze straining of a fecal suspension. The juvenoids ZR-512, ZR-515, MV-678, JH III, and farnesol were dissolved and added to the egg suspension in 5% methanol. Then, 1-ml aliquots were placed in covered culture dishes in triplicate. Egg hatching and subsequent larval molting to the second stage were determined after 3 days.

Additionally, the possible effect of juvenoid hormone on pheromone communication in *N. brasiliensis* was investigated. After treatment of larvae with JH-512 at 2.5 mM for 3 hr, animals were inoculated for recovery of adults after 7 days. Initially, females were incubated in Tyrode's solution to prepare pheromone for the bioassay response of normal males (Bone et al., 1978). Six dosages from 100 to 500 female-hr of pheromone were tested, with at least 30 replicates per dosage. Juvenoid-treated males were obtained similarly to examine their responses to pheromone incubate from normal females. Multiple dosages and replicates were done as above.

The presence of any juvenoid activity in third-stage larvae of *N. brasiliensis* was examined also. Ten million larvae were extracted according to Folch et al. (1957), for recovery of 101.7 mg total lipid. Preparative TLC plates (20 × 20 × 0.05 cm) were used to fractionate 75 mg of extract with benzene as a solvent. Bands were visualized by UV or iodination for R_f determinations prior to removal

Table 1. Percent reduction in egg production/female of *N. brasiliensis* after treatment of larvae at 2×10^{-4} M.

Juvenoid	% Egg reduction
ZR-512	60.1
JH I	53.2
Farnesol	52.9
JH II	39.1
ZR-515	38.2
MV-678	26.6
JH III	0

of the gel and methanol extraction for recovery of the compounds. Approximately 100-μg dosages of eight areas were applied topically to synchronized pupae of *Tenebrio molitor* (Bowers and Thompson, 1968). Also, 7-day-old adults (5×10^4) were prepared and tested similarly for JH activity by insect bioassay. Dosages of 45–145 μg/pupa were used, depending on recovery. Additionally, the effects of larval and adult extracts (0.05 and 0.07%, respectively) on egg hatching and larval development of *N. brasiliensis* were investigated through previous procedures.

Heterodera glycines (Race 3) was maintained as previously reported (Rende et al., 1982). Eggs were obtained from mature cysts by manual crushing and exposed to various concentrations of juvenoids according to the above procedures. Tested juvenoids were farnesol, ZR-512, ZR-515, and MV-678. Additionally, possible JH precursors and related compounds were examined. These substances included oleic methyl ester, oleic acid, linolenic acid, mevalonic acid, docosahexaenoic acid, and squalene (Sigma). The percentage of egg hatching was determined at 2-day intervals for a period of 2 wk.

Data were evaluated by linear regression. The 0.05 probability level was considered significant. For comparison for the multiple lines from dosagewise assay, results are presented as the percent reduction of development or fecundity for a single dosage (predicted by regression analysis) versus the control for each trial. This selected dosage approximated the regression mean among the various compounds.

Results

The effects of juvenoid treatment of larvae on subsequent egg production by individual females of *N. brasiliensis* are given in Table 1. Comparison of the dosage-response lines showed that all compounds, except JH III, reduced egg production at 2×10^{-4} M. These reductions were significantly different ($P \leq 0.02$) from the controls. However, the range of activity among the seven compounds was greater than twofold. None of the tested compounds prevented egg production even at a 33 mM concentration. Although significant activity was not found for JH III at 2×10^{-4} M, a 50% reduction of egg output did occur at 3.4 mM, which indicates less activity than the other juvenoids.

Treatment with the juvenoid ZR-512 at 2.5 mM apparently eliminated the production or release of pheromone by female *N. brasiliensis*. The response of normal males was not dosage-dependent, and none of the responses by males were different from zero. JH-treated males were influenced also, according to their

Table 2. Percent reduction in larval development of *N. brasiliensis* after juvenoid treatment at 5.6×10^{-4} M.

Juvenoid	% Larval reduction
Farnesol	95.9
MV-678	65.6
ZR-515	50.8
ZR-514	50
JH III	44.6

locomotor response to a pheromone incubate from normal females. The 400 and 500 female-hr dosages of pheromone yielded significant responses by JH-treated males of +0.3 and +0.65 cm, respectively. However, these responses are less than 50% of the expected results according to previous dosage-response analysis (Bone et al., 1978). Lower dosages of pheromone caused insignificant responses by treated males. Therefore, the hormonal effects were more pronounced on pheromone production/release by female *N. brasiliensis* than in sensory reception and movement by the male.

Selected juvenoids influenced also the development of larvae from *N. brasiliensis* eggs (Table 2). When compared to the controls, the tested compounds at 5.6×10^{-4} M prevented development of approximately 45–96% of the larvae after hatching. Again, the tested compounds revealed a twofold range of activity. Farnesol had the most significant effect on larvae, although its activity on adults was similar to other synthetic and natural JH's. Higher concentrations of all examined juvenoids arrested development and were lethal to most larvae at 1.25–2 mM levels. As expected, larval stages were more sensitive to exogenous juvenoids than were adults.

Additionally, *H. glycines* larvae were affected by juvenoid treatment (Table 3). Farnesol had the most pronounced influence and arrested most larval development. In contrast to results in *N. brasiliensis*, little activity was obtained with ZR-512 and MV-678 at the tested concentrations. Selected fatty acids and their related derivatives showed pronounced influences on *H. glycines* larvae (Table 4). Their influences were comparable to or exceeded the inhibitory action of the tested synthetic JH's at identical concentrations. The juvenile hormone and steroid precursors mevalonic acid and squalene, respectively, had no demonstrable effect at the tested levels.

Studies were conducted to obtain JH activity from extraction of third-stage larvae and adults of *N. brasiliensis*. Insect bioassay of a 75-μg dosage from extracted larvae at R_f 0.4 gave an insignificant *Tenebrio* score of 1.5. Other re-

Table 3. Percent reduction in larval development rate of *H. glycines* after juvenoid treatment at 3.5×10^{-4} M.

Juvenoid	% Larval reduction
Farnesol	98.2
ZR-515	66.5
MV-678	9.9
ZR-512	0

Table 4. Percent reduction in larval development rate of *H. glycines* after treatment with the indicated compounds at 3.5×10^{-4} M.

Compound	% Larval reduction
Oleic methyl ester	100
Docosahexaenoic acid	74.6
Oleic acid	71.4
Linolenic acid	58
Mevalonic acid	0
Squalene	0

gions gave normal *Tenebrio* scores of 1.0. Insignificant activity was suggested also from adult *N. brasiliensis* by a 45-μg dosage from R_f 0.7, which gave a 1.2 *Tenebrio* score. Solvent extracts from adult and third-stage larvae did not inhibit hatching or larval development of exposed *N. brasiliensis* eggs, based on development rates of 96 and 98%, respectively.

Discussion

The specificities of the natural juvenile hormones of insects have been discussed by Peter et al. (1981). These four JH's (0, I, II, III) differ in various species and stages of the same species. Thus, the level of any individual component has been suggested as important for hormonal regulation, in addition to total JH concentration at any developmental stage.

If the above considerations are applicable also to nematodes, then many of the literature conflicts could be reconciled along similar lines. Thus, the JH effects on *Nematospiroides dubius* that were reported by Dennis (1976) at 3.3×10^{-11} M may not be comparable to the influences seen at $3–4 \times 10^{-3}$ M in *Haemonchus contortus* or the helminths in this study, due to hormonal and organismal differences. Therefore, generalizations are tenuous, because few in-depth studies have been conducted; however, a consensus that the less specialized juvenoids, such as farnesol and its derivatives, are more active does appear evident. Any general versus specific effects of fatty acids could also be clarified by further study of organ development.

Our inability to extract endogenous JH activity from *N. brasiliensis* larvae is perplexing. However, Rogers (1973) used about five times more material than we did and reported variability in results. Different nematode species might be expected to yield different results, if one considers the status of insect endocrinology. Also, some effects may be attributed to other types of lipoidal compounds, as reported by Dropkin et al. (1971). Similar results may account for the inhibition of *H. glycines* that was seen in this study.

The probable interference of the juvenoids on the pheromone system of *N. brasiliensis* cannot be clarified until an understanding of the modulation of chemical communication is achieved. However, Ramaswamy and Gupta (1981) have discussed the few studies of insects that indicate that JH treatment causes retention of nymphal sensilla and, thus, blocks pheromone perception. However, precocious development is found in other insects. If pheromone reception in nematodes is disrupted morphologically, then reduced fecundity may result.

Juvenile hormone influences ovarian maturation in insects (Koeppe et al., 1981)

and JH mimics cause necrosis in crab oocytes (Hinsch, 1981). Ovarian alterations were not examined in this study, although Cheng and Samoiloff (1972) have suggested that the ovaries of *Panagrellus silusiae* may be involved, directly or indirectly, in pheromone production. If so, then ovarian changes could account for the absence of pheromone production in treated nematodes and reduced fecundity.

Acknowledgments

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A New Species of Thelastomatid (Nematoda: Thelastomatidae) from the Desert Millipede, *Orthoporus ornatus* (Diplopoda: Spirostreptidae)

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ABSTRACT: *Thelastoma collare* sp. n. (Nematoda: Thelastomatidae) is described from the anterior hindgut of the desert millipede, *Orthoporus ornatus* (Diplopoda: Spirostreptidae). This nematode differs from other members of the genus by the position of the excretory pore, the presence of lateral alae in males, the offset lip cone in females, and because the 10–13(12) most anterior annules in the females are modified to form short, sharply projecting, posteriorly pointed rings. A list of all members from the genus *Thelastoma* and synonyms is provided for clarification, and the ecological and evolutionary relationship between parasite and host is discussed.

Thelastomatid nematodes inhabit the alimentary tracts of many terrestrial arthropods. Although the nature of this symbiotic relationship remains unclear, the fact that these pinworms tend to occupy specific areas where ingested materials are temporarily stored following digestion in the midgut suggests something less than a strong parasitic relationship.

In this paper we describe a new species of *Thelastoma* inhabiting the anterior hindgut of the desert millipede, *Orthoporus ornatus* (Girard, 1853) Causey, 1954. This diplopod is widely distributed throughout the Chihuahuan and Sonoran deserts of North America (Causey, 1975), and, for a millipede, is unusually well adapted to a xeric environment (Crawford, 1972, 1978; Wooten et al., 1975). The specific population from which the *Thelastoma* species was taken inhabits a mid-Pleistocene volcanic escarpment just west of Albuquerque, New Mexico, USA. This escarpment is approximately 0.19 million years old (Kudo and Kelley, 1977). Judging from recent estimates of the age of desert scrub communities in the northern Chihuahuan desert (Van Devender and Spaulding, 1979), the host population has probably been isolated from conspecific assemblages for at least 4,000 yr. Subsequent examination of *O. ornatus* from three other populations (Cochiti Dam, 64 km NE of Albuquerque; Tomé hill formation, 40 km S of Albuquerque; and Malaga, 16 km SE of Carlsbad, New Mexico) has revealed the same new species (unpubl. data).

Following the taxonomic description, we summarize the known species and synonyms of *Thelastoma,* and comment on the ecological and evolutionary relationship between these organisms and their invertebrate hosts.

Materials and Methods

Adult female and male nematodes were recovered between June and October 1979 from the anterior hindgut of 18 desert millipedes ranging in midsegment width from 2.4 to 7.7 mm. Nematodes were fixed in a warm mixture of 70%

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ethanol (90%) and glycerine (10%). After the ethanol had evaporated, worms were kept in pure glycerine until later mounted in glycerine jelly for light and phase-contrast microscopy. All measurements are in micrometers (μm), except total body lengths and body widths, which are in millimeters (mm), with the mean followed by the standard error and then the range in parentheses.

Nematodes used for scanning electron microscopy were fixed in 2.5% glutaraldehyde, dehydrated with ethanol, and critical-point dried using liquid CO_2. They were then sputter coated with gold-palladium and photographed with an ETEC autoscan SEM.

Results

All nematodes removed from the anterior hindgut of four isolated populations of *O. ornatus* represent a previously undescribed species. Thirty-five of 39 (90%) millipedes randomly examined harbored this species, with a mean of 11 (0–139) worms per millipede. Several stages of nematode were found, with sexually mature adults more confined than larvae to the portion of anterior hindgut directly behind the pylorus. Ratios of adult female to male nematodes averaged about 10:1; however, extremes of 134 females to five males in one millipede and 57 males to zero females in another were recorded.

Description

FAMILY: Thelastomatidae (=Lepidonemidae Travassos, 1920) Travassos, 1929.
GENUS: *Thelastoma* Leidy, 1849.

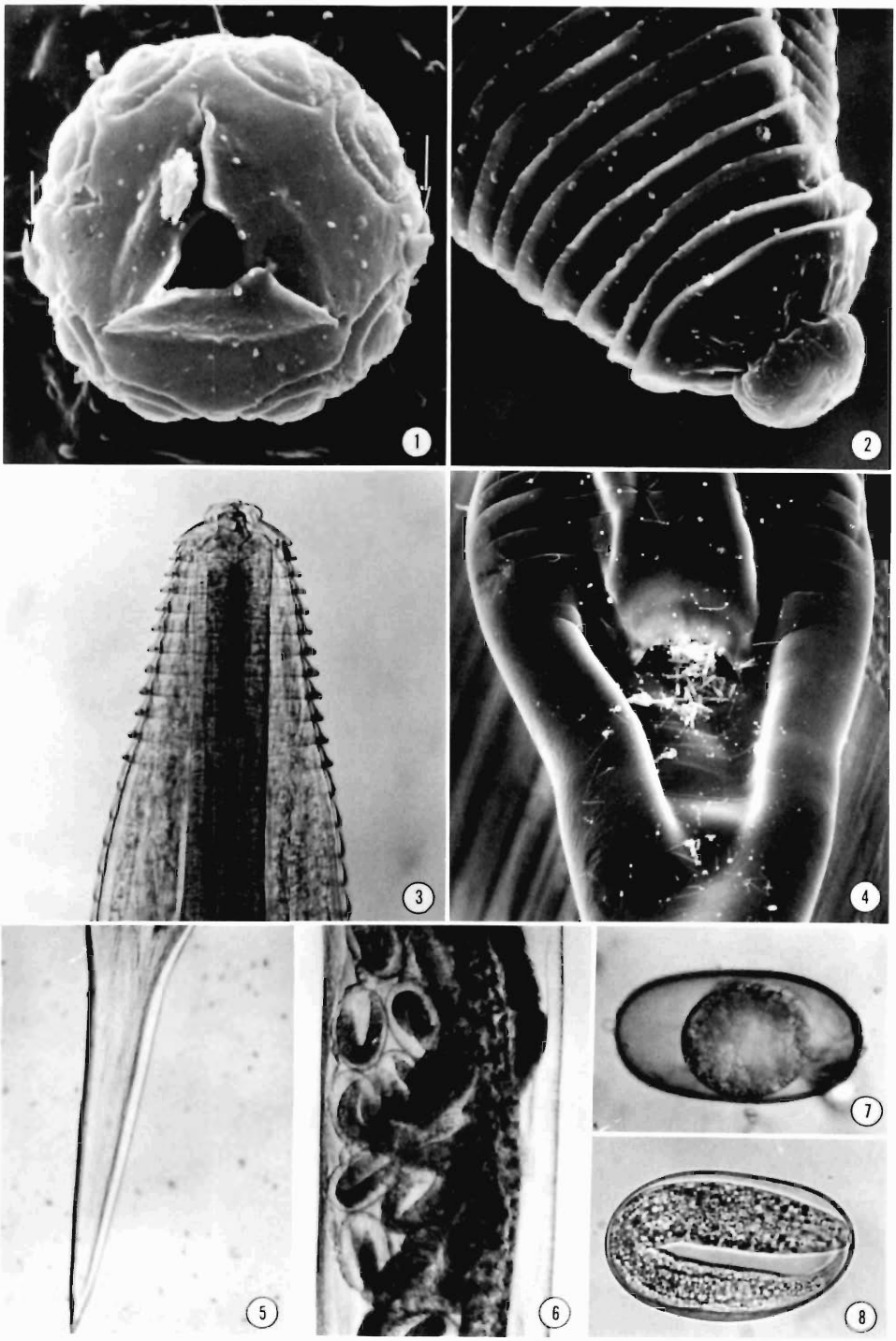
Small nematodes with annulated cuticle, body cylindrical. Lateral alae may or may not be present. Tail tapering rapidly, genital cone present in males. Mouth surrounded by 3 lips in females, triangularly arranged and failing to meet in the middle. Dentition absent. Eight labial papillae on lip cone, 2 pairs subdorsal, 2 pairs sublateral. Two lateral amphids. Stoma small, esophagus cylindrical, with a narrowing isthmus widening into a pyriform bulb. Ovaries paired, gonoducts opposed. Males with or without spicules.

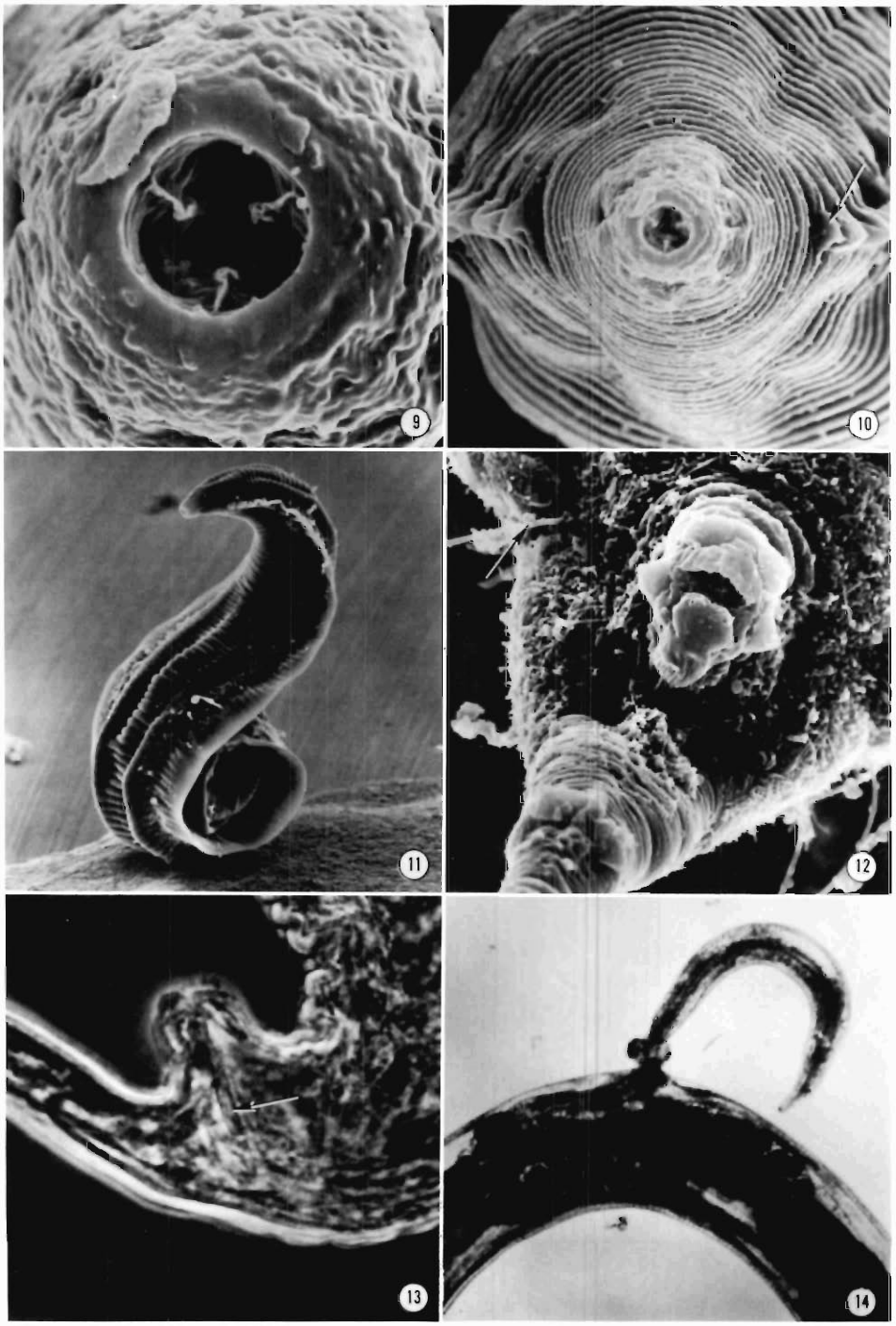
Thelastoma collare sp. n.
(Thelastomatidae: Oxyuroidea)
(Figs. 1–20)

FEMALE (N = 20) (Figs. 1–8, 15, 17): Cuticle annulate to tail, with the 10–13(12) most anterior annules projecting sharply posteriorly. Lip cone offset from remainder of head, and excretory pore opening adjacent to basal bulb. Lateral alae absent. Ovaries paired, gonoducts opposed. Vulva slightly posterior to middle of body, with the vagina vera projecting anteriorly. Rectal glands present. Caudal papillae and phasmids absent. Tail cylindrical and smooth. Body length

→

Figures 1–8. Female. 1. SEM, en face view of lip cone. Note absence of dentition, arrangement of sensory papillae, and lateral amphids (arrows). ×5,300. 2, 3. SEM and light microscopy photograph (LM), lateral views of anterior portion of worm showing posteriorly projecting annules. ×2,625 and ×510, respectively. 4. SEM of anal region showing absence of anal papillae and presence of annulations to aperture. ×650. 5. LM of tail. ×240. 6. LM of developing embryos within female. ×175. 7, 8. LM of developmental stages of eggs. ×500.





Figures 9–14. Male. 9. SEM en face view showing absence of lip cone or oral papillae. ×5,710. 10. SEM en face view showing beginning of lateral alae (arrow). ×1,300. 11. SEM of entire male. ×320. 12. SEM of anal aperture and sensory papillae upon genital cone. Note intense bacterial and fungal associations (arrow) much like that demonstrated by Wright (1979). ×2,650. 13. Phase-contrast photograph of male genital cone showing spicule (arrow). ×860. 14. LM of male and female in copula. ×120.

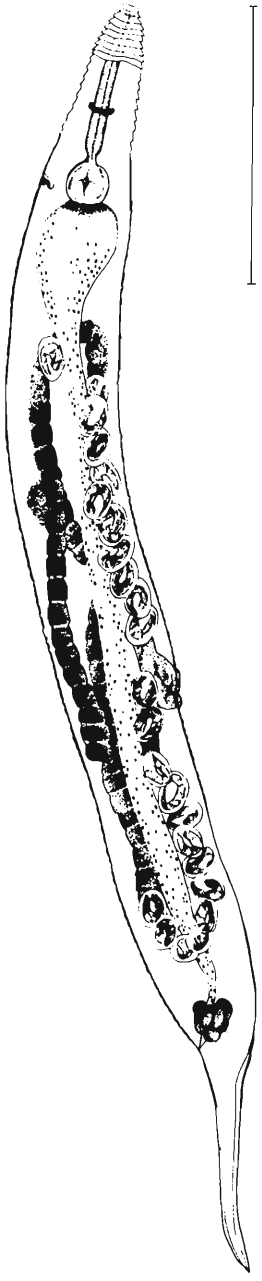


FIGURE 15

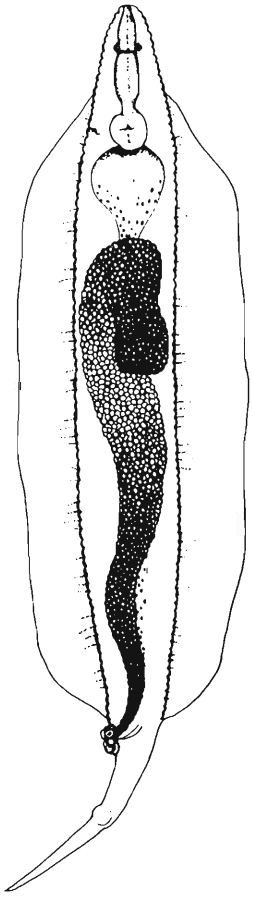


FIGURE 16

Figures 15, 16. Diagrammatic representations of entire female (Fig. 15) and male (Fig. 16).

2.2 ± 0.14 mm (1.4–2.7 mm); body width at base of esophagus 1.6 ± 0.01 mm (1.2–2.1 mm); body width near vulva 1.7 ± 0.01 mm (1.1–2.4 mm); total length of esophagus 341 ± 5.25 (287–381); length of stoma 8.8 ± 0.3 (6–13); length of corpus 251 ± 4.3 (208–281); length of isthmus 20.3 ± 1.1 (14–32); bulb length 70.1 ± 1.3 (62–82); bulb width 70.1 ± 1.2 (63–82); distance from anterior end to nerve ring 149 ± 2.5 (134–173); width of esophagus at nerve ring 24 ± 0.3 (21–27); distance from anterior end to excretory pore 279 ± 8.9 (244–336); distance

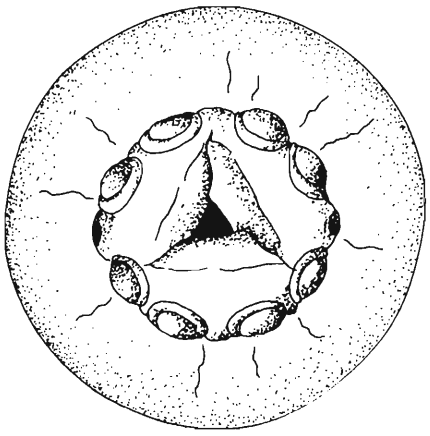


FIGURE 17

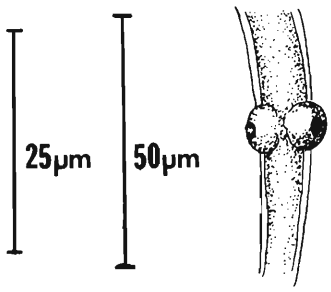


FIGURE 18

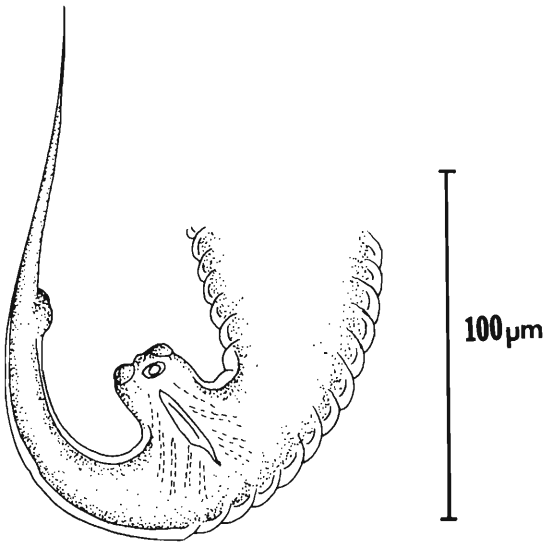


FIGURE 19

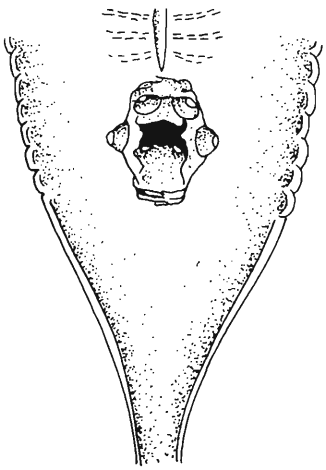


FIGURE 20

Figures 17–20. Diagrammatic representations of *Thelastoma collare* sp. n. 17. Female lip cone. 18. Ventral view of fourth pair of male sensory papillae. 19, 20. Lateral (Fig. 19) and ventral (Fig. 20) views of male genital cone.

from anterior end to vulva 1,214 ± 47.5 (892–1,513): distance from anal aperture to tip of tail 357 ± 17.2 (229–474). Eggs (N = 11) 75.1 ± 0.004 × 46.7 ± 0.002 (61.5–96.1 × 39.4–52.2), smooth and slightly flattened on 1 side; shell bilayered.

MALE (N = 12) (Figs. 9–14, 16, 18–20): Cuticle annulate to tail. Oral papillae and amphids absent. Lateral alae present, extending from the region slightly posterior of nerve ring to genital cone. Excretory pore opening adjacent to bulb. Spicule present, single. Tail containing 4 pairs of papillae, 3 pairs on genital cone

and 1 pair near middle of tail. Testes single and reflexed. Body length 0.78 ± 0.013 mm (0.71–0.86 mm); midbody width 0.07 ± 0.002 mm (0.06–0.09 mm); midbody width including lateral alae 0.16 ± 0.002 mm (0.16–0.18 mm); distance from anterior end to beginning of lateral alae 65.3 ± 4.5 (54–79); total length of esophagus 125.3 ± 1.5 (114–134); length of corpus 85 ± 1.2 (82–90); length of isthmus 8.6 ± 0.5 (6–11); bulb length 32 ± 0.4 (28–35); bulb width 31 ± 0.4 (28–33); distance from anterior end to nerve ring 54 ± 0.9 (47–58); distance from anterior end to excretory pore 105 ± 2.0 (99–110); distance from anal aperture to tip of tail 142 ± 3.4 (126–158); length of spicule 31 ± 0.6 (28–33); distance from fourth pair of papillae to tip of tail 90 ± 2.6 (80–107).

TYPE HOST: *Orthoporus ornatus* (Girard, 1853) Causey, 1954 (Diplopoda: Spirostreptidae).

LOCATION IN HOST: Anterior hindgut (ileum).

TYPE LOCALITY: Mid-Pleistocene volcanic escarpments on the western outskirts of Albuquerque, New Mexico, USA.

TYPE SPECIMENS: Holotype female USNM Helm. Coll. No. 76619. Paratype females and males USNM Helm. Coll. No. 76620. Deposited at the National Parasite Research Laboratory, Beltsville, Maryland.

Discussion

Members of the genus *Thelastoma* resemble members of three closely related genera within the family Thelastomatidae. These include *Johnstonia* Basir, 1956, *Severianoia* Schwenk, 1926, and *Cephalobellus* Cobb, 1920. *Thelastoma* spp. may be differentiated from *Johnstonia* spp. by the possession of paired ovaries rather than a single ovary. They differ from *Severianoia* spp. by having eggs without longitudinal striations. Differences between *Thelastoma* and *Cephalobellus* include males having a genital cone with sensory papillae in *Thelastoma* and *Thelastoma* females having a relatively filiform tail (Jarry and Jarry, 1968).

Basir (1956), Leibersperger (1960), Kloss (1965), and Skrjabin et al. (from Poinar, 1978; original not seen) provided lists of the known species of Thelastomatidae with keys to their identification. Since their reports, many new species have been reported. Table 1 summarizes the known species and synonyms of the genus *Thelastoma*.

Thelastoma collare most closely resembles *T. alii* Farooqui, 1970, *T. indica* Rao, 1958, *T. macramphidum macramphidum* (Christie, 1931) Basir, 1956, *T. ornata* Singh, 1965, *T. pteroton* Dollfus, 1952, and *T. riveroi* Chitwood, 1932. Similar features among males include lateral alae and a spicule, and among females include a vulva slightly posterior to the middle of the body and an excretory pore adjacent to the esophageal bulb. Additionally, there are seven species in which females are as above but males have not been described. These include *T. depressum* (Hammerschmidt, 1838) Leidy, 1853, *T. dollfusi* Osche, 1960, *T. labiatum* Leidy, 1850, *T. madecassa* van Waerebeke, 1969, *T. palmatum* Chitwood, 1933, and two unnamed species that Dollfus (1964) designated sp. A and sp. B.

There are a number of ways in which each congeneric species differs from *T. collare*. *Thelastoma alii* is considerably larger than *T. collare* and possesses a more filiform tail and rounder eggs. Males of *T. indica* are much larger than those of *T. collare* and the females of *T. indica* possess a more filiform tail and lack a lip cone. Males of *T. m. macramphidum* are also larger, possess a lip cone (unlike

Table 1. ***Thelastoma*** **nematodes associated with alimentary tracts of terrestrial detritivores.**

Genus/species/synonym	Host	Host locality	Reference
Thelastoma (=*Oxyuris* Rud, 1903) (in part); *Bulhõesia* Schwenk, 1926 (in part); *Schwenkiella* (Basir, 1956) Leidy, 1849.			
alii Farooqui, 1970	*Spirostreptus* sp.*	India	Farooqui, 1970
attenuatum Leidy, 1849 *Aorurus attenuatum* Leidy, 1849 *Thelastomum attenuatum* Leidy, 1853 *Anguillula attenuatus* (Leidy, 1849) Diesing, 1861 *Aorurus attenuatus* (Leidy, 1849) Walton, 1927	*Narceus annularis*†	USA	Basir, 1956
aurangabadense (Farooqui, 1970) comb. n.	*Periplaneta americana*	India	Farooqui, 1970
basiri Farooqui, 1970	*Periplaneta americana*	India	Farooqui, 1970
blabericola Leibersperger, 1960	*Blaberus cranifer, Blaberus dubia*	Germany	Leibersperger, 1960
collare sp. n.	*Orthoporus ornatus*	New Mexico, USA	this study
delphyhystera Dollfus, 1964	*Plagiodesmus tuberosus*	Congo	Dollfus, 1964
depressum (Hammerschmidt, 1838) Leidy, 1853‡ *Oxyuris depressa* Ham, 1838 *Oxyuris dialatata* Ham, 1847 *Oxyuris laticollis* Ham, 1847 *Thelastoma dialatatum* (Ham, 1847) Leidy, 1851 *Thelastoma laticolle* (Ham, 1847) Leidy, 1851 *Aorurus laticollis* (Ham, 1847) Walton, 1927 *Anguillula laticollis* (Ham, 1847) Diesing, 1851 *Anguillula depressa* (Ham, 1838) Diesing, 1851 *Thelastomum depressum* (Ham, 1838) Leidy, 1853 *Anguillula laticolle* (Ham, 1847) Diesing, 1861 *Anguillula depressus* (Ham, 1838) Walton, 1927	*Pachnotosia marmorata* (larva), *Oxythyrea funesta* (larva)	Germany	Basir, 1956
dollfusi Osche, 1960	a diplopod from Spirostreptinae	E. Africa	Osche, 1960
endoscholicum Poinar, 1978	*Eudrilus eugeniae*§	W. Africa	Poinar, 1978

Table 1. Continued.

Genus/species/synonym	Host	Host locality	Reference
figuli van Waerebeke, 1970	*Figulus sublaevis*	Madagascar	van Waerebeke, 1970a
icemi (Schwenk, 1926) Travassos, 1929 *Bulhõesia icemi* Schwenk, 1926 *Thelastoma aligarhica* Basir, 1940 *Schwenkiella icemi* (Schwenk, 1926) Basir, 1956	*Barata selvagem, Periplaneta americana, Periplaneta brunneau*	Brazil, USA	Basir, 1956
indica Rao, 1958	*Spirostreptus* sp.*	India	Rao, 1958
labiatum Leidy, 1850 *Aorurus labiatum* Leidy, 1851 *Thelastomum labiatum* (Leidy, 1850) Leidy, 1856 *Anguillula labiatum* (Leidy, 1850) Diesing, 1861 *Aorurus labiatus* (Leidy, 1850) Walton, 1927 *Thelastoma myolabiatum* Cobb, 1929	uncertain Xystodesmidae ‖	USA	Basir, 1956
longicaudata (Meyer, 1896) Travassos, 1929 *Oxyuris longicaudata* Meyer, 1896 *Thelastomum longicaudatum* (Meyer, 1896) Skrjabin, 1923 *Aorurus longicaudata* (Meyer, 1896) Travassos, 1929 *Schwenkiella longicaudata* (Meyer, 1896) Basir, 1956	Harpagophoridae, gen. et sp. uncertain#	Ceylon	Basir, 1956
macramphidum (Christie, 1931) Basir, 1956 subsp. *gallicum* Théodoridés, 1955¶	*Oryctes nasicornis* (larva), *Cetonia* sp., *Potosia cuprea, Potosia* sp.	France	Théodoridés, 1955; Leibersperger, 1960; Jarry, 1964
macramphidum macramphidum (Christie, 1931) Basir, 1956 *Thelastoma papilliferum* Christie, 1931	larva of *Osmoderma*	Michigan, USA	Basir, 1956
madecassa van Waerebeke, 1969	*Elliptoblatta madecassa*	Madagascar	van Waerebeke, 1969
malaysiense, Khairul and Paran, 1977	*Periplaneta americana*	Malaysia	Khairul and Paran, 1977

Table 1. Continued.

Genus/species/synonym	Host	Host locality	Reference
mamba van Waerebeke, 1973	*Oryctes boas*	Madagascar	van Waerebeke, 1973
meadsi Clark, 1978	*Procyliosoma tuberculatum*	New Zealand	Clark, 1978
nasuta Kloss, 1965	*Heterostreptus coeruleopes*	Brazil	Kloss, 1965
ornata Singh, 1965	*Thyroglutus malayus***	India	Singh, 1965
pachyjuli (Parona, 1896) Travassos, 1929 *Oxyuris pachyjuli* Parona, 1896 *Oxyuris bulhõesi* Magalhães, 1900 *Bulhõesia bulhõesi* (Magalhães, 1900) Schwenk, 1926 *Aorurus bulhõesi* (Magalhães, 1900) Walton, 1927 *Aorurus pachyjuli* (Parona, 1896) Walton, 1927 *Thelastoma bulhõesi* (Magalhães, 1900) Travassos, 1929	*Periplaneta americana*, *Gymnostreptus* sp., *Pachyiulus* sp.††	N. and S. America	Basir, 1956; Leibersperger, 1960
pachyjuli (Parona, 1896) Travassos, 1929 infrasubsp. *tampoketsii* van Waerebeke, 1969¶	*Blatta* sp.	Madagascar	van Waerebeke, 1969
palmatum Chitwood, 1933	*Panesthia javanica*	Philippines	Basir, 1956
paronai Kloss, 1965	*Dicranostreptus restingae*	Brazil	Kloss, 1965
patellae van Waerebeke, 1970	*Hexodon patella*, *Hexodon latissimum*	Madagascar	van Waerebeke, 1970b
periplaneticola Leibersperger, 1960	*Periplaneta americana*	Czechoslovakia	Leibersperger, 1960
pteroton Dollfus, 1952	*Julus* sp.	France	Dollfus, 1952; Basir, 1965
pterygoton Poinar, 1973	*Oryctes monoceros*	W. Africa	Poinar, 1973
pyrrhus van Waerebeke, 1973	*Oryctes pyrrhus*	Madagascar	van Waerebeke, 1973
ritteri van Waerebeke, 1973	*Oryctes politus*	Madagascar	van Waerebeke, 1973
riveroi Chitwood, 1932	*Periplaneta* sp.	Havana, Cuba	Basir, 1956
robustum (Leidy, 1850) Travassos, 1929 *Aorurus robustum* Leidy, 1853 *Thelastomum robustum* Leidy, 1853 *Anguillula robusta* (Leidy, 1850) Diesing, 1861 *Aorurus robustus* (Leidy, 1850) Walton, 1927 *Schwenkiella robustum* (Leidy, 1850) Basir, 1956	*Osmoderma scabra* (larva), *Xyloreyetes satyrus* (larva)	USA	Basir, 1956

Table 1. Continued.

Genus/species/synonym	Host	Host locality	Reference
rovinjense Leibersperger, 1960	*Pachyiulus fascipes*	Yugoslavia	Leibersperger, 1960
spicatum Cobb, 1929	*Spirobolus marginatus*‡‡	USA	Basir, 1956
toxi van Waerebeke, 1970	*Figulus sublaevis, Prosopocoelus serricornis*	Madagascar	van Waerebeke, 1970a
wechi (Farooqui, 1968) comb. n.	*Spirostreptus* sp.*	India	Farooqui, 1968
sp. A Dollfus, 1964	*Rhamphidarpe aloysiisabaudiae*	Congo	Dollfus, 1964
sp. B Dollfus, 1964	*Rhamphidarpe aloysiisabaudiae*	Congo	Dollfus, 1964

T. brevicaudatum Leidy, 1951 is now *Cephalobellus brevicaudatus* (Leidy, 1851) Christie, 1933.
T. brumpti Théodoridés, 1952 is now *Cephalobellus galliardi* (Dollfus, 1952) Basir, 1956.
T. cuspidatum (Rudolph, 1814) Théodoridés, 1955 is now *Cephalobellus papillifer* Cobb, 1920.
T. galliardi Dollfus, 1952 is now *Cephalobellus galliardi* (Dollfus, 1952) Basir, 1956.
T. glomericola Dollfus, 1952 is now *Severianoia glomericola* (Dollfus, 1952) Basir, 1956.
T. glomeridis (v. Linstow, 1885) Travassos, 1929 is now *Severianoia glomeridis* (Linstow, 1885) Basir, 1956.
T. gracile (Ham, 1838) Leidy, 1851 is now *Severianoia gracilis* (Hammerschmidt, 1838) Basir, 1956.
T. heterogaminae (Galeb, 1878) Travassos, 1929 is considered species inquirendum by Basir (1956), and is kept in the category under *Oxyuris heterogaminae* Galeb, 1878.
T. indiana Basir, 1940 is now *Cephalobellus brevicaudatus* (Leidy, 1851) Christie, 1933.
T. lanceolata (Linstow, 1883) Travassos, 1929 is now *Linstowiella lanceolata* (Linstow, 1883) Basir, 1956.
T. magalhãesi (Schwenk, 1926) Travassos, 1929 is now *Cephalobellus magalhãesi* (Schwenk, 1926) Basir, 1956.
T. ovocostata (v. Linstow, 1885) Leidy, 1851 is now *Severianoia glomeridis* (Linstow, 1885) Basir, 1956.
T. panesthiae (Galeb, 1878) Travassos, 1929 is now *Leidynemella pansthiae* (Galeb, 1878) Chitwood and Chitwood, 1933.
T. skrjabini Sergiev, 1923 is now *Gryllophila skrjabini* (Sergiev, 1923) Basir, 1956.
T. socialis (Leidy, 1850) Travassos, 1929 is now *Leidynemella socialis* (Leidy, 1850) Basir, 1956.

* Although the original description lists *Spirostreptus* sp. as the host, no spirostreptids are found in India. This is surely from Harpagophoridae.
† Basir lists the hosts as *Julus marginatus* and *Sporobolus marginatus*. Leidy's type material was certainly *Narceus annularis* (Rafinesque), Spirobolidae.
‡ Basir lists this species as species inquirenda.
§ *Eudrilus eugeniae* (Oligochaeta) is the only non-arthropod host of *Thelastoma*.
‖ Basir lists the species as *Polydesmus virginiensis* and *Fontaria marginata*; however, these species are unknown to us.
Basir lists *Julus* sp.; however, this is uncertain to us.
¶ It is doubtful anyone will successfully show the existence of subspecies or infrasubspecies of thelastomatid nematodes for some time. We have, however, included these in the manner presented by the original author. Varieties have either been changed to subspecies or infrasubspecies in accordance with the International Code of Zoological Nomenclature, Art. 45e(i.ii).
** This is probably *Gonoplectus malayus*.
†† Although this species was not listed in Basir's article, the name implies *Pachyiulus* is also a host.
‡‡ This may be either *Narceus annularis* or *Narceus americanus*, depending upon where Cobb's specimens came from. *Spirobolus* is now a Chinese genus with four species; all other "*Spirobolus*" belong to other genera and families.

T. collare), and the nerve ring in females is more posterior. Both males and females of *T. ornata* are larger than those of *T. collare,* and the anterior cuticle of males is raised into numerous small papillae. Females of *T. pteroton* possess an extremely long, filiform tail and the excretory pore is anterior to the basal bulb. Males possess a long, expanded lip cone, unlike *T. collare.* Females of *T. riveroi* have a much longer esophagus, a filiform tail, and appear much larger.

Thelastoma dollfusi is slightly smaller, has a more filiform tail, an excretory pore more anterior to the bulb than *T. collare,* and also has accentuated rings slightly anterior to the anus. *Thelastoma madecassa* is considerably more robust than *T. collare,* and the eggs of the former are spherical instead of oval. *Thelastoma depressum, T. labiatum, T. palmatum,* and sp. A are much too small to be *T. collare,* and *T. palmatum* has three palm leaf-like structures in the buccal cavity. All seem to have considerably longer and more filiform tails than *T. collare.* Dollfus (1964) described only one female of sp. B; its bulb diameter is one-fourth larger and the vulva more posterior to the midportion of the body than in *T. collare.*

Of the nearly 40 recognized species of *Thelastoma,* only *T. malaysiense* (Khairul and Paran, 1977) is shown as having the posteriorly projecting annules described for *T. collare.* However, females of *T. malaysiense* are larger and have a more filiform tail than those of *T. collare,* and possess two papillae rather than one on each of the two median labiopapillae. Additionally, males of *T. malaysiense* are larger and lack lateral alae, unlike *T. collare.* Because of the posteriorly projecting annules on the anterior end of the females of our new species, we propose the specific epithet "*collare.*"

Prior to molting, *O. ornatus* defecates most of the material kept in its gut during its months of subterranean dormancy (Crawford, unpubl. data). Simultaneously, we have noted that pinworm densities appear to decrease dramatically (90%) within the millipede population and their eggs become numerous in the premolt feces. When *O. ornatus* resumes feeding on plant litter and soil (mainly after the onset of summer rains), larval and then adult nematodes reappear in the hindgut. A resumption of feeding on detritus, and possibly the ingestion of part of the exuvium or entry via rectal imbibition, seems critical to reinfection. The millipede in question is not coprophagous except as a juvenile in its fecal egg pellet.

We observed spatial distributions of the nematodes within the millipede hindgut similar to those observed by Peregrine (1974) in *Periplaneta americana.* As numbers increase, the nematodes tend to occupy an increasing portion of the hindgut, with the males and larvae showing a greater linear distribution than females. Adult female nematodes appear much smaller when crowded than when only a few worms are contained in one millipede (pers. obs.).

Although the incidence of the nematode is high within *O. ornatus,* we randomly sampled 16 sympatric atopetholid millipedes from June to August 1979 and found none to be infected with pinworms. This suggests that the nematodes may be very host specific and that the traditional lumping of similar morphological types of the thelastomatids into the same species may be premature. Another possibility is that the atopetholid millipedes somehow failed to ingest infective stages.

A wide variety of arthropods appears to be infected by thelastomatid pinworms (Table 1; a similar table pertaining to *Cephalobellus, Severianoia,* and *Johnstonia* nematodes is available upon request from the authors). Diplopod hosts, for ex-

ample, range from closely related groups (e.g., spirostreptids and julids) to decidedly disjunct taxa (e.g., polydesmoids, glomerids, and sphaerotheriids). Thus, although instances of host specificity may occur, thelastomatid genera show no obvious host-association patterns. *Thelastoma endoscolicum* even appears in an oligochaete (Poinar, 1978).

There is, however, one ecological pattern that all of the host species seem to have in common. They are all detritivores. Apparently, then, the scavenging habit of the host is essential for life-cycle completion by the nematode. If this hypothesis is indeed correct, the prediction follows that thelastomatids will continue to be found only in detritivorous terrestrial invertebrates.

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Review of the Lecithodendriidae (Trematoda) from *Eptesicus fuscus* in Wisconsin and Minnesota

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ABSTRACT: Fifteen species of Lecithodendriidae have been collected from *Eptesicus fuscus* in Wisconsin and Minnesota: *Acanthatrium eptesici, A. microcanthum, A. oligacanthum, A. pipistrelli, Allassogonoporus marginalis, Glyptoporus noctophilus, Ochoterenatrema breckenridgei, O. diminutum, O. travassosi, Paralecithodendrium chilostomum, P. macnabi, P. naviculum, P. nokomis, P. swansoni,* and *P. transversum*.

The name *Paralecithodendrium* has priority over the name *Prosthodendrium* at the rank of genus. *Acanthatrium lunatum* is a junior synonym of *A. pipistrelli*. *Ototrema schildti* is a junior synonym of *Glyptoporus noctophilus*. *Lecithodendrium breckenridgei* and *Paralecithodendrium travassosi,* which possess a pseudogonotyl to the left of the acetabulum, are transferred to the genus *Ochoterenatrema*. *Ochoterenatrema pricei* is a junior synonym of *O. breckenridgei*. *Paralecithodendrium lucifugi* is distinct from *P. nokomis*. *Paralecithodendrium emollidum* is distinct from *P. swansoni*. *Paralecithodendrium aranhai* and *P. volaticum* are junior synonyms of *P. transversum*.

Seven species of Lecithodendriidae have been reported from *Eptesicus fuscus* (big brown bat) in Minnesota (Macy, 1936a, 1937, 1938, 1940a) and none from Wisconsin. Over the past 2 yr we have recovered 15 species of Lecithodendriidae from 113 *E. fuscus* collected in Eau Claire, Wisconsin, or St. Peter, Minnesota. Herein we review the taxonomic status of these species and tabulate their New World host and locality records (Table 1).

Trematodes were examined alive, fixed in formalin, and stained with Van Cleave's hematoxylin (whole mounts) or hematoxylin and eosin (paraffin sections). All measurements are in micrometers. USNM Helm. Coll. refers to the United States National Museum Helminthological Collection, Beltsville, Maryland.

Lecithodendriidae Odhner, 1911

Acanthatrium Faust, 1919

TYPE SPECIES: *Acanthatrium nycteridis* Faust, 1919.

Acanthatrium eptesici Alicata, 1932 (Figs. 3, 5)

SPECIMENS EXAMINED: USNM Helm. Coll. No. 30136 (paratype).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76770.
SITE OF INFECTION: Anterior third of small intestine.

Acanthatrium microcanthum Macy, 1940 (in part) (Figs. 4, 6)

SPECIMENS EXAMINED: USNM Helm. Coll. No. 36669 (holotype).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76769.
SITE OF INFECTION: Posterior third of small intestine.

Table 1. Host and locality records of Lecithodendriidae from *Eptesicus fuscus* in Wisconsin and Minnesota.

Species	Host	Locality	Reference
Acanthatrium eptesici	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Ramsey Co.	Macy, 1940a
		Washington, D.C.*	Alicata, 1932
		Virginia: Albermarle Co.	Cheng, 1959a
		Canada: British Columbia	Webster and Casey, 1973
	Lasiurus borealis	USA: Iowa	Blankespoor and Ulmer, 1970
	Tadarida brasiliensis	USA: Oklahoma	Rogers, 1965 (in Martin, 1976)
A. microcanthum	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Ramsey Co.*	Macy, 1940a
A. oligacanthum	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Ramsey Co.	Macy, 1940a
		Virginia: Russell Co.*	Cheng, 1957
	Myotis lucifugus	USA: Virginia: Giles Co.	Cheng, 1959a
A. pipistrelli	*Eptesicus fuscus*	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Nicollet Co.*	Present study
		Iowa	Blankespoor and Ulmer, 1970
		Ohio: Franklin Co.	Williams, 1960
		Kentucky: Carter Co.	Williams, 1960
	"bat"	USA: Virginia: Albermarle Co.	Cheng, 1959b
	Lasiurus borealis	USA: Iowa	Blankespoor and Ulmer, 1970
	Myotis lucifugus	USA: Iowa	Blankespoor and Ulmer, 1970
	*Pipistrellus subflavus**	USA: Minnesota: Nicollet Co.*	Macy, 1940a
Allassogonoporus marginalis	*Eptesicus fuscus*	USA: Wisconsin: Eau Claire Co.	Present study
		Oregon: Benton Co.	Knight and Pratt, 1955
	Myotis californicus	USA: Oregon: Yamhill Co.	Macy, 1940b
	M. grisescens	USA: Kansas: Crawford Co.	Ubelaker, 1966
	M. lucifugus	USA: Minnesota: Hennepin Co.	Macy, 1940c
		Iowa	Blankespoor and Ulmer, 1970
		Oregon: Linn Co.	Knight and Pratt, 1955

Table 1. Continued.

Species	Host	Locality	Reference
	M. sodalis	USA: Kentucky: Carter Co.	Williams, 1962
	M. velifer	USA: Kansas: Comanche Co.	Ubelaker, 1966
	*Ondatra zibethicus**	USA: Michigan: Muskegon Co.*	Olivier, 1938
	Tadarida brasiliensis	USA: Louisiana: Orleans Par.	Martin, 1976
Glyptoporus noctophilus	*Eptesicus fuscus*	USA: Minnesota: Nicollet Co.	Present study
	*Myotis lucifugus**	USA: Wisconsin: Eau Claire Co. Minnesota: Nicollet Co.*	Font, 1978 Macy, 1936b
	M. keenii	USA: Wisconsin: Dodge Co.	Coggins et al., 1981
Ochoterenatrema breckenridgei	*Eptesicus fuscus*	USA: Minnesota: Nicollet Co.* Cuba: Las Villas Prov.	Present study Groschaft and Valle, 1969
	Artebius jamaicensis	Cuba	Perez Vigueras, 1940 (in Dubois, 1960)
	Molossus major	Cuba	Zdzitowiecki and Rutkowska, 1980
	M. molossus	Cuba: Santa Clara Prov.	Odening, 1969
	Mormoops blainvillei	Cuba: Santa Clara Prov.	Odening, 1969
	Natalus lepidus	Cuba	Zdzitowiecki and Rutkowska, 1980
	Phyllonycteris poeyi	Cuba: Matanza Prov.	Groschaft and Valle, 1969
	*Pipistrellus subflavus**	USA: Minnesota: Nicollet Co.*	Macy, 1936c
	Tadarida laticaudata	Cuba	Zdzitowiecki and Rutkowska, 1980
	T. minuta	Cuba: Santa Clara Prov. Las Villas Prov.	Odening, 1969 Groschaft and Valle, 1969
O. diminutum	*Eptesicus fuscus*	USA: Wisconsin: Eau Claire Co. Cuba	Present study Odening, 1969
	Chilonycteris fuliginosa	Cuba	Zdzitowiecki and Rutkowska, 1980
	Lasiurus intermedius	USA: Mississippi: Oktibbeha Co.	Byrd and Macy, 1942
	Molossops planirostris	Brasil: Rio de Janeiro	Freitas, 1957
	Molossus major	Cuba: Oriente Prov.	Groschaft and Valle, 1969
	M. molossus	Cuba: Santa Clara Prov.	Odening, 1969

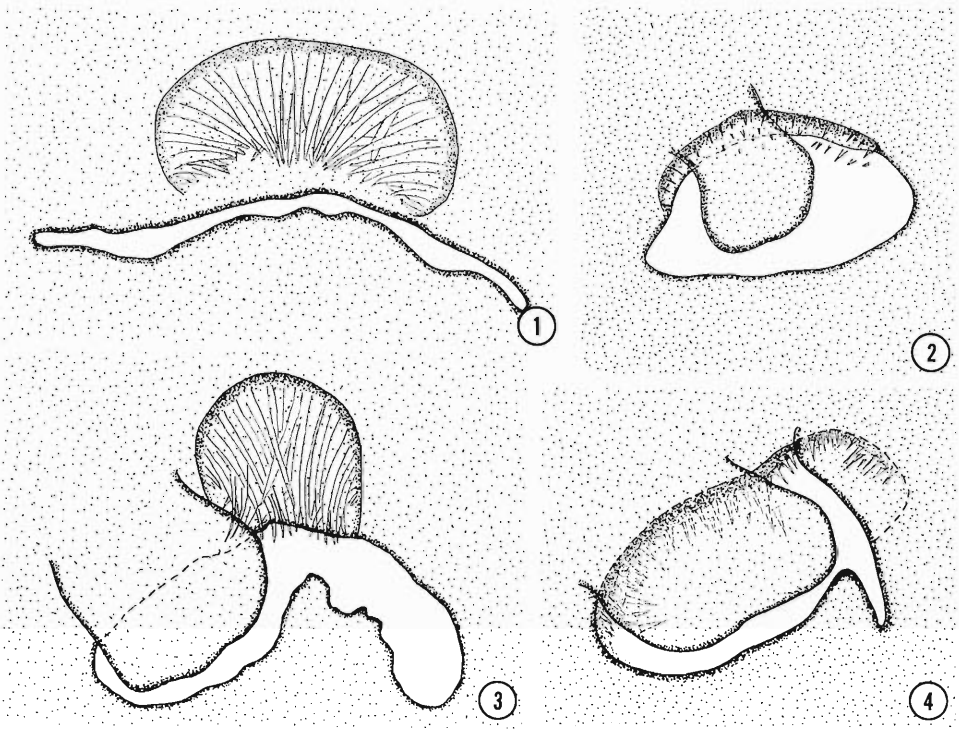
Table 1. Continued.

Species	Host	Locality	Reference
	Mormoops blainvillei	Cuba	Zdzitowiecki and Rutkowska, 1980
	Myotis sodalis	USA: Kentucky: Carter Co.	Williams, 1962
	*Nycticeius humeralis**	USA: Texas: Harris Co.*	Chandler, 1938
		Iowa	Ubelaker and Kunz, 1971
(metacercaria)	*Orconectes rusticus*	USA: Indiana: Tippecanoe Co.	Williams, 1967
	Procyon lotor	USA: Ohio	Williams, 1961
	Tadarida brasiliensis	Cuba: Havana Prov.	Odening, 1969
	T. laticaudata	Cuba	Zdzitowiecki and Rutkowska, 1980
	T. minuta	Cuba: Havana Prov.	Odening, 1969
O. travassosi	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Ramsey Co.*	Macy, 1938
Paralecithodendrium chilostomum	*Eptesicus fuscus*	USA: Wisconsin: Eau Claire Co.	Present study
	Pipistrellus subflavus	USA: Nebraska: Cass Co.	Manter and Debus, 1945 (see Jones, 1957)
P. macnabi	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Nicollet Co.	Present study
		Minnesota: Ramsey Co.*	Macy, 1936a
		Indiana: St. Joseph Co.	Seamster and Stevens, 1948
		Canada: British Columbia	Webster and Casey, 1973
	Myotis lucifugus	USA: Alaska: Juneau	Neiland, 1962
	Lasionycteris noctivagans	Canada: British Columbia	Webster and Casey, 1973
	Lasiurus cinereus	Mexico: Distrito Federal	Caballero and Zerecero, 1951
P. naviculum	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Nicollet Co.	Present study
		Minnesota: Ramsey Co.*	Macy, 1936a
	Balantiopteryx ochoterenai	Mexico: Puebla	Caballero, 1943b
	Lasiurus cinereus	Mexico: Distrito Federal	Caballero and Zerecero, 1951
	Mustela vison	USA: Michigan: Emmet Co.	Lang and Dronen, 1972
	Myotis volans	Canada: British Columbia	Webster and Casey, 1973

Table 1. Continued.

Species	Host	Locality	Reference
	Procyon lotor	USA: Georgia	Harkema and Miller, 1964
	Tadarida brasiliensis	Mexico: Distrito Federal	Caballero, 1940
P. nokomis	*Eptesicus fuscus**	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Ramsey Co.*	Macy, 1937
	Lasiurus borealis	USA: Minnesota: Ramsey Co.*	Macy, 1937
		Iowa	Blankespoor and Ulmer, 1970
P. swansoni	*Eptesicus fuscus*	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Nicollet Co.*	Present study
		Iowa	Blankespoor and Ulmer, 1970
	Lasiurus borealis	USA: Iowa	Blankespoor and Ulmer, 1970
	Myotis grisescens	USA: Kansas: Crawford Co.	Nickel and Hansen, 1967
	*M. lucifugus**	USA: Minnesota: Nicollet Co.*	Macy, 1936a
		Iowa	Blankespoor and Ulmer, 1970
		Canada: British Columbia	Webster and Casey, 1973
	Tadarida brasiliensis	Jamaica	Webster, 1971
P. transversum	*Eptesicus fuscus*	USA: Wisconsin: Eau Claire Co.	Present study
		Minnesota: Nicollet Co.	Present study
		Iowa	Blankespoor and Ulmer, 1972
		Cuba: Pinar del Rio Prov.	Zdzitowiecki and Rutkowska, 1980
	*Lasiurus borealis**	USA: Tennessee: Lake Co.*	Byrd and Macy, 1942
		Iowa: Boone Co.	Kunz, 1968
	Molossus crassicaudatus	Paraguay: Assunção	Lent et al., 1945
		Brasil	Freitas and Dobbin, 1960 (in Dubois, 1962)
	Myotis keenii	USA: Wisconsin: Dodge Co.	Coggins et al., 1981
		Iowa: Jones Co.	Blankespoor and Szymusiak, 1974
	M. sodalis	USA: Kentucky: Carter Co.	Williams, 1962
	Tadarida laticaudata	Paraguay: Assunção	Lent et al., 1945

* Type host or type locality.



Figures 1–4. Genital atria of four species of *Acanthatrium*. Ventral view. 1. *A. pipistrelli*. 2. *A. oligacanthum*. 3. *A. eptesici*. 4. *A. microcanthum*.

Acanthatrium oligacanthum Cheng, 1957
(Figs. 2, 7)

Acanthatrium microcanthum Macy, 1940 (in part).
Acanthatrium beuschleini Cheng, 1959 (fide Dubois, 1961).

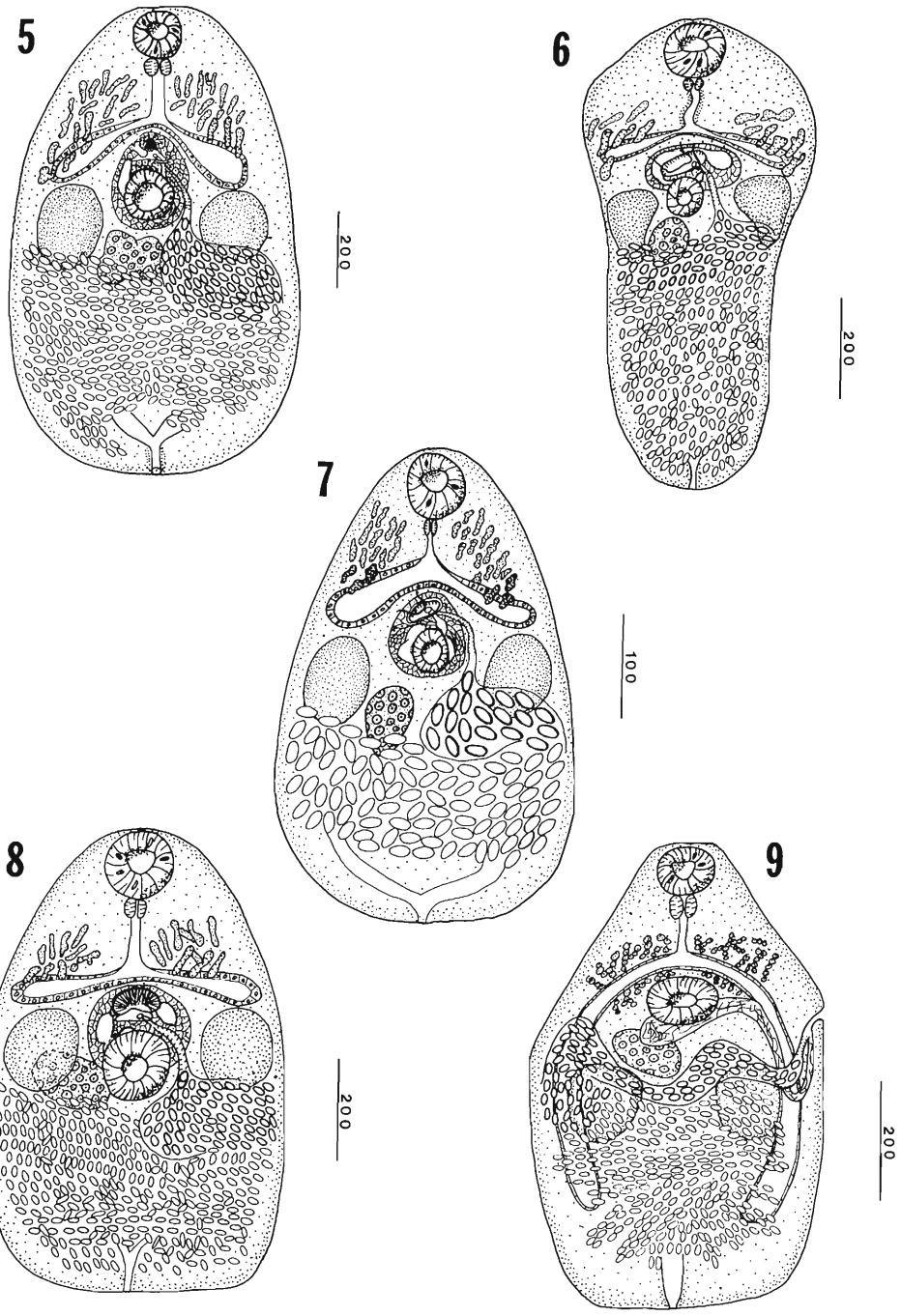
SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 38174 (holotype) and 38388 (holotype of *A. beuschleini*).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76771.

SITE OF INFECTION: Primarily anterior half of small intestine.

We agree with the synonymy of *A. beuschleini* and *A. oligacanthum* (Dubois, 1961). In addition, Dubois (1961) suggested that Figure 8 in Macy (1940a) represents *A. oligacanthum* and not *A. microcanthum* as labeled. We have examined both *A. oligacanthum* and *A. microcanthum* and agree with Dubois (1961). In addition, we consider Figure 7 of Macy (1940a) to represent *A. oligacanthum*. Macy (1940a) illustrates the holotype of *A. microcanthum* in Figure 10. His Figure 11, drawn from the holotype of *A. microcanthum*, depicts atrial spines completely surrounding the genital atrium. However, our observations of the holotype and of our material indicate that spines are present only on the anterior half of the genital atrium (Fig. 4, this paper).

The two species can be distinguished in that *Acanthatrium oligacanthum* possesses a short esophagus and numerous small vitelline follicles extending from



Figures 5–9. Adult specimens of Lecithodendriidae. 5. *Acanthatrium eptesici*. 6. *A. microcanthum*. 7. *A. oligacanthum*. 8. *A. pipistrelli*. 9. *Allassogonoporus marginalis*.

the anterior margin of the testes to the level of the pharynx, and *Acanthatrium microcanthum* possesses a long esophagus and vitellaria not reaching the level of the pharynx. Differences also exist in the genital atria of the two species (Figs. 2, 4). The blunt papilla overlying the genital pore of both species is not always present.

Acanthatrium pipistrelli Macy, 1940
(Figs. 1, 8)

Acanthatrium lunatum Williams, 1960, new synonym.

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 36670 (holotype) and 38890 (holotype and paratype of *A. lunatum*).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76772.

SITE OF INFECTION: Primarily anterior half of small intestine.

Dubois (1961) considered *A. pipistrelli* to be a synonym of *A. eptesici*. He noted differences in size of the genital atria and the ratio of suckers to body size, but thought these differences resulted from variation in fixation. We do not accept Dubois's synonymy. *Acanthatrium eptesici* consistently possesses a blunt papilla to the right of the genital atrium. The papilla is clearly figured by Alicata (1932), and is present on the holotype and on all of our material (Fig. 3). *Acanthatrium pipistrelli* lacks a papilla (Fig. 1). The atrial spines of *A. eptesici* are limited to a small pouch off the anterior wall of the genital atrium. The atrial spines of *A. pipistrelli* are not limited to a pouch, but line the entire anterior wall of the genital atrium.

No differences exist between *A. pipistrelli* and the type material of *A. lunatum*. Williams (1960) distinguished *A. lunatum* from *A. pipistrelli* by the length of the esophagus (longer in *A. lunatum*), the number of atrial spines (greater in *A. lunatum*), and the spined tegument of *A. lunatum*. The contracted state of the *A. pipistrelli* holotype accounts for the seemingly short esophagus. *Acanthatrium pipistrelli* has approximately the same number of atrial spines as the type material of *A. lunatum*. Exact counts were not obtainable because of the clumped spinal distribution in all specimens examined. The holotype of *A. pipistrelli* has a spined tegument.

Allassogonoporus Olivier, 1938

Myotitrema Macy, 1940.
Moedlingeria Yamaguti, 1958.

TYPE SPECIES: *Allassogonoporus marginalis* Olivier, 1938.

Allassogonoporus marginalis Olivier, 1938
(Fig. 9)

Allassogonoporus vespertilionis Macy, 1940 (fide Gilford, 1955).
Myotitrema asymmetrica Macy, 1940 (fide Gilford, 1955).

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 9194 (holotype), 36672 (paratype of *A. vespertilionis*), and 36673 (holotype of *Myotitrema asymmetrica*).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76773.

SITE OF INFECTION: Anterior third of small intestine.

Glyptoporus Macy, 1936

Ototrema Font, 1978.

TYPE SPECIES: *Glyptoporus noctophilus* Macy, 1936.

Glyptoporus noctophilus Macy, 1936

Ototrema schildti Font, 1978, new synonym.

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 8947 (holotype), 73098 (holotype of *O. schildti*), and 73099 (paratype of *O. schildti*).

SPECIMENS DEPOSITED: None.

SITE OF INFECTION: Anterior third of small intestine.

We have recovered three immature specimens of *G. noctophilus* from one *Eptesicus fuscus* collected in St. Peter, and consider its occurrence to be accidental.

We have also recovered numerous adult specimens of *G. noctophilus* from *Myotis lucifugus* (type host) in Eau Claire and St. Peter (type locality). We consider *Ototrema schildti* to be a synonym of *Glyptoporus noctophilus*. Macy (1936b) did not mention oral papillae in his original description of *G. noctophilus*; nevertheless, his holotype bears papillae, as does our material.

Ochoterenatrema Caballero, 1943

TYPE SPECIES: *Ochoterenatrema labda* Caballero, 1943.

Ochoterenatrema breckenridgei (Macy, 1936) comb. n. (Fig. 10)

Lecithodendrium breckenridgei Macy, 1936.
Lecithodendrium pricei Perez Vigueras, 1940, new synonym.
Ochoterenatrema pricei: Odening, 1969.
Lecithodendrium vivianae Groschaft and Valle, 1969 (fide Odening, 1973).

SPECIMENS EXAMINED: USNM Helm. Coll. No. 8949 (holotype).

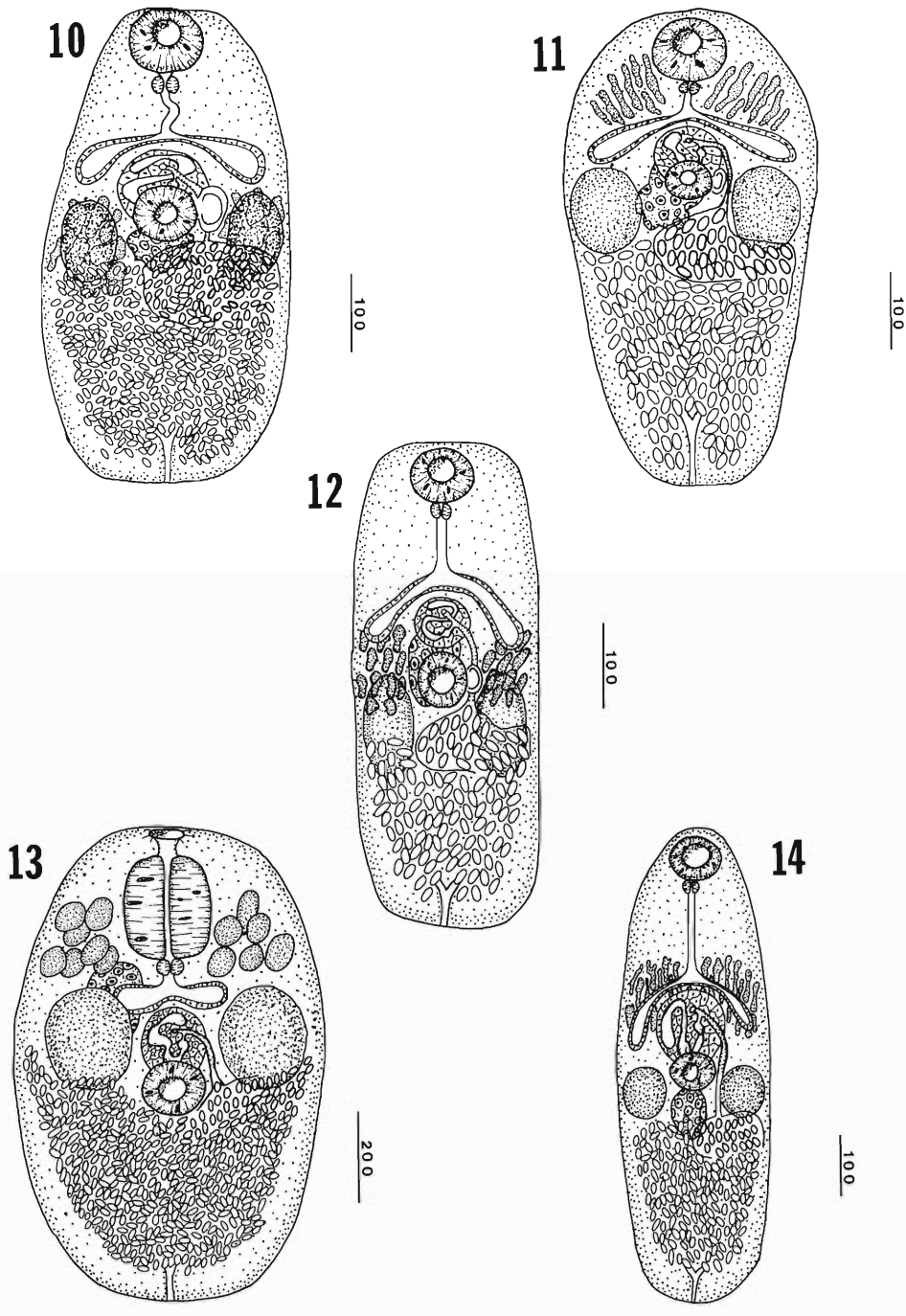
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76776.

SITE OF INFECTION: Anterior half of small intestine.

Macy (1936c) described *Lecithodendrium breckenridgei* from *Pipistrellus subflavus* captured in St. Peter, Minnesota. We have recovered *L. breckenridgei* from *P. subflavus* as well as *Eptesicus fuscus* taken at St. Peter. Most of our specimens exhibit a well-developed papilla to the left of the ventral sucker; we consider this papilla to be a pseudogonotyl (sensu Cain, 1966). Because of the presence of a pseudogonotyl we transfer *L. breckenridgei* to the genus *Ochoterenatrema*. The eversible pseudogonotyl is not evident on Macy's holotype; however, his specimen is identical to our material in all other respects.

Odening (1969) recovered *Lecithodendrium pricei* from the type locality (Cuba) and transferred it to the genus *Ochoterenatrema* because it possessed a pseudogonotyl. Measurements of *O. pricei* (Odening, 1969; Zdzitowiecki and Rutkowska, 1980) correspond to the measurements of *O. breckenridgei* that we obtained from our specimens and the holotype. We consider *O. pricei* to be a synonym of *O. breckenridgei*.

In the original description of *O. breckenridgei* Macy (1936c) reported that the



Figures 10–14. Adult specimens of Lecithodendriidae. **10.** *Ochoterenatrema breckenridgei*. **11.** *O. diminutum*. **12.** *O. travassosi*. **13.** *Paralecithodendrium chilostomum*. **14.** *P. macnabi*.

acetabulum was slightly larger than the oral sucker. However, we found the acetabulum to be slightly smaller than the oral sucker on the holotype and on our material.

Ochoterenatrema diminutum (Chandler, 1938) (Fig. 11)

Limatulum diminutum Chandler, 1938.
Prosthodendrium naviculum: Byrd and Macy, 1942 (misidentification of *Ochoterenatrema diminutum*).
Ochoterenatrema caballeroi Freitas, 1957 (fide Cain, 1966).
Prosthodendrium naviculum: Williams, 1961, 1962, 1967 (misidentification of *Ochoterenatrema diminutum*).
Ochoterenatrema diminutum: Dubois, 1960.
Paralecithodendrium nokomis: Groschaft and Valle, 1969 (misidentification of *Ochoterenatrema diminutum* fide Odening, 1973).
Prosthodendrium buongerminii: Groschaft and Valle, 1969 (misidentification of *Ochoterenatrema diminutum* fide Odening, 1973).

SPECIMENS EXAMINED: Instituto Oswaldo Cruz Helminthological Collection No. 22.023-d (paratype of *O. caballeroi*). USNM Helm. Coll. Nos. 39072 (voucher specimen, Williams, 1961) and 61794 (voucher specimen, Williams, 1967). Rice University Helminthology Collection specimen of *O. diminutum* sectioned and identified by Asa Chandler.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76775.

SITE OF INFECTION: Anterior half of small intestine.

The original description of *Prosthodendrium naviculum* from *Eptesicus fuscus* in Minnesota reported small eggs, 19–21 long by 12 wide (Macy, 1936a). Byrd and Macy (1942) identified *P. naviculum* with large eggs, 25–34 by 13–18, from *Lasiurus intermedius* in Mississippi. In spite of the difference in egg size, Byrd and Macy thought that the large-egged form was conspecific with the small-egged form. Subsequently, Williams (1961) reported large-egged forms from *Procyon lotor* in Ohio. He also reared large-egged forms in *Mus musculus* from metacercariae recovered from *Orconectes rusticus* in Indiana (Williams, 1967). Initially, we identified the large-egged specimens (eggs 25–30 by 12–15) from the anterior half of the small intestine of our bats as *P. naviculum*. However, upon examination of living specimens without coverslip pressure we noted the presence of a pseudogonotyl to the left of the acetabulum. We observed that some of these same specimens no longer displayed the pseudogonotyl when stained. Because of these observations, we now consider our specimens to be in the genus *Ochoterenatrema*. Although a pseudogonotyl is not evident on Williams's voucher specimens that we have examined, they are otherwise identical to our material. We suspect that the large-egged *P. naviculum* found by Byrd and Macy (1942) is also conspecific with our specimens.

We have compared our specimens with either type or voucher specimens of all species of *Ochoterenatrema* (*O. labda,* voucher specimens from Cain; *O. fraternum,* Instituto Oswaldo Cruz Helminthological Collection No. 29.167-b and 29.168-c paratypes; and specimens listed in this paper). Our specimens differ from all examined material, and on this basis we cannot assign them to any of these

species. We were unsuccessful in our attempts to obtain whole mounts of *O. diminutum* from Chandler's collection; however, we were able to examine a sectioned specimen. Because our specimens cannot be excluded from *O. diminutum* on the basis of Chandler's description (the examination of sections was inconclusive), other than in possessing larger eggs, we assign our specimens to *O. diminutum* until Chandler's type material can be examined.

Ochoterenatrema travassosi (Macy, 1938) comb. n. (Fig. 12)

Prosthodendrium travassosi Macy, 1938.
Pycnoporus travassosi: Yamaguti, 1958.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 36674 (holotype).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76774.
SITE OF INFECTION: Anterior half of small intestine.

The holotype and our material possess an indistinct pseudogonotyl to the left of the acetabulum. Our material is smaller and less heavily gravid than the type specimen; otherwise our specimens correspond to the species description (Macy, 1938) and relative measurements of the holotype.

Paralecithodendrium (Odhner, 1911) Travassos, 1921

Prosthodendrium Dollfus, 1931.
Chiroptodendrium Skarbilovich, 1943.
Skrjabinodendrium Skarbilovich, 1943.
Travassodendrium Skarbilovich, 1943.
Longitrema Cheng, 1954.

TYPE SPECIES: *Paralecithodendrium anticum* (Stafford, 1905) Travassos, 1921.

Odhner (1911, cited in Dubois, 1962) erected the subgenus *Paralecithodendrium* for *Lecithodendrium glandulosum* (Looss, 1896), *L. spaerula* (Looss, 1896), *L. obtusum* (Looss, 1896), and *L. anticum* Stafford, 1905. He characterized the taxon as possessing a lobed or branched ovary situated anterior to the acetabulum, but failed to designate a type species. Travassos (1921) elevated *Paralecithodendrium* to the rank of genus, characterized the genus by pretesticular vitellaria, and designated *L. anticum* as the type species. Subsequently, Dollfus (1931) erected the genus *Prosthodendrium,* characterized it by pretesticular vitellaria, and designated *Lecithodendrium dinanatum* Bhalerao, 1926 as the type species. Later, Dollfus (1937) considered the genus *Paralecithodendrium* (Odhner, 1911) Travassos, 1921 to be inappropriate because Travassos (1921) had changed Odhner's definition of *Paralecithodendrium.* Dollfus (1937) included the subgenera *Paralecithodendrium* (with a lobed ovary) and *Prosthodendrium* (with an entire ovary) in the genus *Prosthodendrium* (with pretesticular vitellaria). Cain (1966) was the first to recognize that Dollfus's scheme was nomenclaturally incorrect; i.e., a change in diagnosis has no bearing on nomenclatural matters—only the characters of the type species define the genus. Although Cain (1966) preferred to recognize *Paralecithodendrium* and *Prosthodendrium* as distinct genera, we accept Dollfus's relative ranking of taxa. Application of the "law of priority," however, necessitates that *Prosthodendrium* Dollfus, 1931 be relegated

to a subgenus in *Paralecithodendrium* (Odhner, 1911) Travassos, 1921. Therefore, the genus *Paralecithodendrium* sensu Travassos (1921) contains the subgenera *Paralecithodendrium* sensu Odhner (1911) and *Prosthodendrium* sensu Dollfus (1937).

There have been three type species designated for *Paralecithodendrium*. Travassos (1921) designated *Lecithodendrium anticum,* Stiles and Nolan (1931) designated *L. obtusum,* and Dollfus (1937) designated *L. glandulosum*. Travassos's designation has priority.

Paralecithodendrium chilostomum (Mehlis, 1831) (Fig. 13)

Distoma chilostoma Mehlis, 1831.
Distoma ascidioides Van Beneden, 1873 (fide Braun, 1900, cited in Dollfus, 1937).
Paralecithodendrium chilostomum: Joyeux and Isobe, 1925 (cited in Dubois, 1960).
Lecithodendrium cordiforme laxmii Bhalerao, 1926 (fide Dubois, 1955).
Prosthodendrium piriforme Yamaguti, 1939 (fide Dubois, 1955).
Prosthodendrium oligolecithum Manter and Debus, 1945 (fide Dubois, 1960).
Travassodendrium rhinolophi Rysavy, 1956 (fide Dubois, 1960).
Travassodendrium raabei Soltys, 1959 (fide Dubois, 1963).

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 36924 (holotype of *P. oligolecithum*) and 36925 (paratype of *P. oligolecithum*).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76777.
SITE OF INFECTION: Anterior third of small intestine.

Paralecithodendrium macnabi (Macy, 1936) comb. n. (Fig. 14)

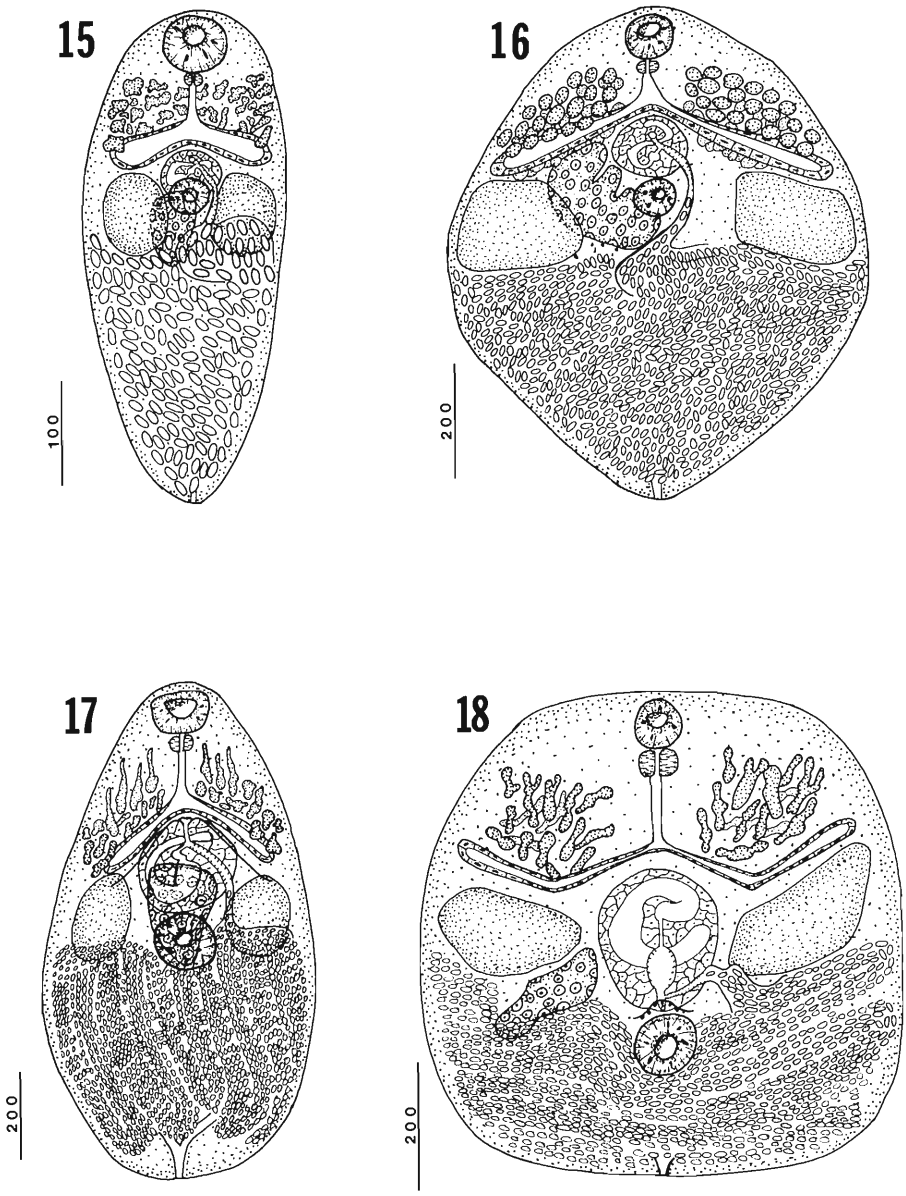
Prosthodendrium macnabi Macy, 1936.
Prosthodendrium mizellei Seamster and Stevens, 1948 (fide Dubois, 1955).
Prosthodendrium duboisi Neiland, 1962 (fide Webster and Casey, 1973).

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 8951 (holotype), 8952 (paratype), and 39097 (holotype of *P. duboisi*).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76782.
SITE OF INFECTION: Anterior three-fourths of small intestine.

Paralecithodendrium naviculum (Macy, 1936) comb. n. (Fig. 15)

Prosthodendrium naviculum Macy, 1936.
Limatulum scabrum Caballero, 1940 (fide Dubois, 1955).
Prosthodendrium scabrum: Caballero, 1943a.
Prosthodendrium tetralobulatum Caballero, 1943 (fide Dubois, 1962).
Prosthodendrium paeminosum Caballero, 1943 (fide Dubois, 1955).
Prosthodendrium ascidia naviculum: Dubois, 1955 (fide Dubois, 1960).

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 8953 (holotype) and 8954 (paratype).
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76781.
SITE OF INFECTION: Posterior half of small intestine.



Figures 15–18. Adult specimens of Lecithodendriidae. 15. *Paralecithodendrium naviculum*. 16. *P. nokomis*. 17. *P. swansoni*. 18. *P. transversum*.

Paralecithodendrium nokomis (Macy, 1937) comb. n. (Fig. 16)

Prosthodendrium nokomis Macy, 1937.

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 8955 (holotype) and 8956 (paratype).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76780.

SITE OF INFECTION: Central third of small intestine.

Dubois (1962)synonymized *Paralecithodendrium lucifugi* (Macy, 1937) comb. n. with *P. nokomis*. We have recovered *P. nokomis* from *Eptesicus fuscus* and *P. lucifugi* from *Myotis lucifugus,* and have examined the holotype of *P. lucifugi* (USNM Helm. Coll. No. 8950). We consider *P. lucifugi* to be distinct from *P. nokomis*. *Paralecithodendrium nokomis* differs from *P. lucifugi* in body size (*P. nokomis* = 940–990 long by 793–830 wide; *P. lucifugi* = 442–520 by 319–397) and in oral sucker : body length ratio (*P. nokomis* = 0.08–0.09; *P. lucifugi* = 0.17–0.21).

Paralecithodendrium swansoni (Macy, 1936) comb. n. (Fig. 17)

Prosthodendrium swansoni Macy, 1936.

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 8957 (holotype) and 8958 (paratype).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76779.

SITE OF INFECTION: Anterior half of small intestine.

Dubois (1960) synonymized *Paralecithodendrium emollidum* (Caballero, 1943) comb. n. with *P. swansoni*. We consider *P. emollidum* to be valid. The oral sucker : ventral sucker ratio of *P. swansoni* is close to 1.0 (pers. obs.), but the sucker ratio of *P. emollidum* is about 1.5 (Caballero, 1943a; pers. obs.).

Our observations of *P. emollidum* are from a specimen that was incorrectly labeled as a paratype of *Ochoterenatrema caballeroi* (Instituto Oswaldo Cruz Helminthological Collection No. 22.023-i). The measurements of this paratype do not correspond to those given by Freitas (1957) for *O. caballeroi* or to those of another *O. caballeroi* paratype (No. 22.023-d) that we examined. The prostatic mass of the misidentified paratype is considerably larger than that of *O. caballeroi,* and the specimen has an oral sucker : ventral sucker ratio of 1.5. For these reasons we consider the specimen to be *P. emollidum*. *Paralecithodendrium emollidum* differs from *P. swansoni* in larger sucker ratio and the smaller vitelline follicles.

Byrd and Macy (1942) reported *P. swansoni* from *Tadarida brasiliensis* taken in New Orleans, Louisiana. Their Figure 1 appears to be *Ochoterenatrema labda* Caballero, 1943, not *P. swansoni*. A structure figured to the left of the acetabulum may be an inverted pseudogonotyl. Also, the distribution of the vitellaria corresponds more closely to *O. labda* than to *P. swansoni*. Our conclusion that the specimens identified by Byrd and Macy (1942) as *P. swansoni* are, in fact, *O. labda* is further substantiated by the findings of Martin (1976). He reported six species of trematodes from *Tadarida brasiliensis* collected monthly in New Orleans over a period of a year. *Ochoterenatrema labda* was the predominant helminth; *P. swansoni* was not recovered.

Paralecithodendrium transversum (Byrd and Macy, 1942) comb. n. (Fig. 18)

Prosthodendrium transversum Byrd and Macy, 1942.
Paralecithodendrium aranhai Lent, Freitas, and Proenca, 1945, new synonym.
Paralecithodendrium brachycolon Freitas and Dobbin, 1960 (fide Dubois, 1962).
Prosthodendrium volaticum Blankespoor and Ulmer, 1972, new synonym.

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 44998 (holotype), 44999 (paratype), and 70517 (holotype of *P. volaticum*).

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 76778.

SITE OF INFECTION: Anterior third of small intestine.

Blankespoor and Ulmer (1972) distinguished *P. volaticum* from all other species of the genus by the recurved ceca; however, *P. transversum* also exhibits recurved ceca. The original description of *P. transversum* reported measurements that indicated the oral sucker is larger than the ventral sucker (Byrd and Macy, 1942). Our examination of a paratype reveals the reverse is true; i.e., the oral sucker is 87 in diameter, and the ventral sucker is 97 long by 92 wide. The oral sucker of the holotype is missing. Our series of specimens exhibits a body shape that varies from wider than long as described by Byrd and Macy (1942) to longer than wide as described by Blankespoor and Ulmer (1972).

Blankespoor and Ulmer (1972) distinguished *P. volaticum* from *P. aranhai* by the recurved ceca, the uterine configuration, the position of the ovary, and the body shape. The description and figure of *P. aranhai* by Lent et al. (1945) exhibit no differences in the first three features. The difference in body shape is within the variation we have observed in our specimens of *P. transversum*.

Key to the Species of Lecithodendriidae of *Eptesicus fuscus* in Minnesota and Wisconsin

1. Genital pore lateral; prostatic glands and pseudocirrus sac (sac surrounding prostatic glands and seminal vesicle) absent ..... *Allassogonoporus marginalis*
   Genital pore medial; prostatic glands and pseudocirrus sac present 2
2. Genital atrium and atrial spines present 3
   Genital atrium and atrial spines absent 6
3. Atrial spines shorter than 10 μm 4
   Atrial spines longer than 15 μm 5
4. Vitellaria extend from ceca to pharynx ..... *Acanthatrium oligacanthum*
   Vitellaria do not reach pharynx ..... *Acanthatrium microcanthum*
5. Atrial spines contained in small diverticulum off anterior wall of genital atrium; papilla present to right of genital pore ..... *Acanthatrium eptesici*
   Atrial spines not contained in diverticulum, but line whole anterior wall of genital atrium; papilla absent ..... *Acanthatrium pipistrelli*
6. Papilla (pseudogonotyl) present to left of ventral sucker 7
   Papilla absent to left of ventral sucker 9
7. Vitellaria overlap testes 8
   Vitellaria do not overlap testes ..... *Ochoterenatrema diminutum*
8. Vitellaria overlap ceca ..... *Ochoterenatrema travassosi*
   Vitellaria do not overlap ceca ..... *Ochoterenatrema breckenridgei*
9. Fewer than 10 vitelline follicles per side; follicles larger than 95 μm ..... *Paralecithodendrium chilostomum*
   More than 10 vitelline follicles per side; follicles smaller than 90 μm 10
10. Ventral sucker in posterior half of body; ceca often recurved ..... *Paralecithodendrium transversum*
    Ventral sucker in anterior half or middle of body; ceca not recurved 11

11. Vitellaria extend anteriorly to level of pharynx or oral sucker 14
    Vitellaria do not extend anteriorly to level of pharynx 12
12. Vitellaria overlap testes 13
    Vitellaria do not overlap testes ..... *Paralecithodendrium macnabi*
13. Vitellaria overlap ceca ..... *Ochoterenatrema travassosi*
    Vitellaria do not overlap ceca ..... *Ochoterenatrema breckenridgei*
14. Oral sucker and ventral sucker equal in size ..... *Paralecithodendrium swansoni*
    Oral sucker distinctly larger than ventral sucker 15
15. Body length greater than 800 μm; vitelline follicles round, numerous, and compacted ..... *Paralecithodendrium nokomis*
    Body length less than 800 μm; vitelline follicles with irregular margins, fewer in number and less compacted 16
16. Eggs less than 22 μm long; tiny papillae surround genital pore ..... *Paralecithodendrium naviculum*
    Eggs greater than 22 μm long; tiny papillae surrounding genital pore absent 17
17. Vitellaria extend from ceca to pharynx 18
    Vitellaria do not reach pharynx ..... *Acanthatrium microcanthum*
18. Posterior margin of uterus without cleft ..... *Acanthatrium oligacanthum*
    Posterior margin of uterus with cleft surrounding excretory bladder ..... *Ochoterenatrema diminutum*

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HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1982–83 MEETING SCHEDULE

15 October	Uniformed Services University of the Health Sciences Bethesda, Maryland
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14 January	National Institutes of Health Bethesda, Maryland
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14 May	New Bolton Center, University of Pennsylvania Kennett Square, Pennsylvania

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Euclinostomum heterostomum Metacercariae (Trematoda: Clinostomatidae) from the Aquarium Ram, *Apistogramma ramirezi* (Pisces: Cichlidae)

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ABSTRACT: *Euclinostomum heterostomum* metacercariae were present in conspicuous swellings on two aquarium rams, *Apistogramma ramirezi*, imported from Hong Kong. The larvae were individually encapsulated in the striated muscles, with no evident inflammatory response. This is a new host record for the genus and species and the first report from aquarium fishes.

There are few reports of trematode metacercariae infecting small aquarium fishes, and in most instances the larvae were not identified (Gratzek et al., 1978; Leibovitz et al., 1980). This is true even though millions of aquarium fishes are imported and exported each year from all over the world (Gratzek et al., 1978) and metacercariae are common in many fish species (Hoffman, 1970). The present report describes natural infections of rams (*Apistogramma ramirezi*) with *Euclinostomum heterostomum* metacercariae.

Case History and Methods

Two live rams showing conspicuous swellings were submitted to the Animal Disease Diagnostic Laboratory, Purdue University, for pre- and postmortem examination. The owner had purchased several of these fish from a local pet shop, which had obtained them from a Hong Kong supplier.

The fish were observed for 2 hr in a 40-liter aquarium tank at 27°C. One fish was killed and examined for helminths and Wright's-stained blood smears examined for protozoans. The single metacercaria recovered was fixed in warm AFA and stained with Semichon's carmine. The other fish was fixed in neutral buffered 10% formalin, after incising the abdominal wall along the ventral midline. Whole coronal slices (3–4 mm) were embedded in paraffin and 6-μm sections stained with hematoxylin and eosin.

Results and Discussion

One fish had a large (4 mm), gray, conspicuous swelling on the left side of the trunk adjacent to the dorsal fin. When examined later for helminths, a single living metacercaria (Fig. 1) was recovered from this swelling; no other lesions or parasites were seen. The other fish, later fixed in formalin, had three similar swellings (3–5 mm) in the head and nape regions. The nodules seemed to have an irritating effect, as both fish occasionally rubbed themselves against aquarium rocks.

Sections of the swellings revealed that each contained one metacercaria encapsulated in striated muscle (Fig. 2). The three slightly oval cysts in cross section measured 1,558 (1,137–1,958) μm long by 1,305 (1,010–1,516) μm wide. The cyst wall was composed of a thin layer of connective tissue, with no inflammatory

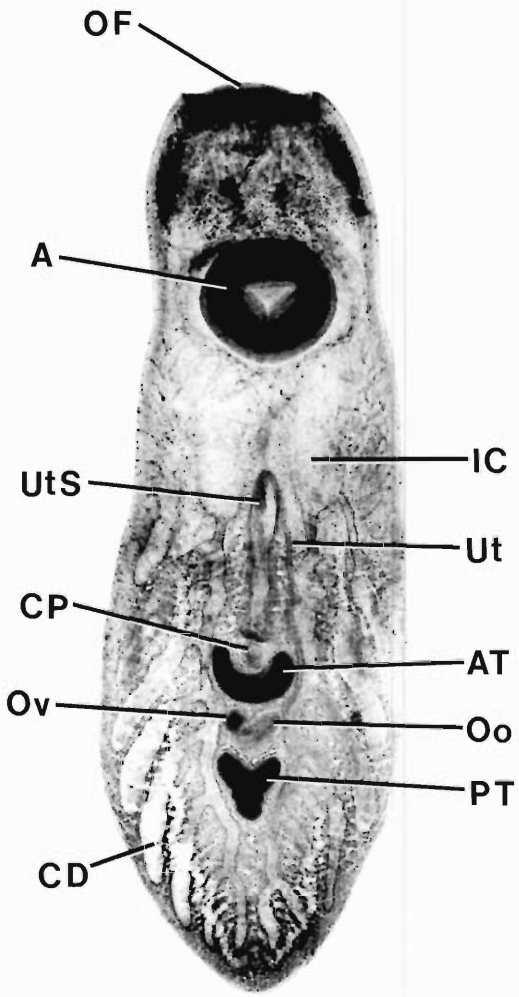

Figure 1. Whole mount of *Euclinostomum heterostomum* metacercaria from *Apistogramma ramirezi*. A, acetabulum; AT, anterior testis; CD, cecal diverticulum; CP, cirrus pouch; IC, intestinal cecum; OF, oral field; Oo, ootype; Ov, ovary; PT, posterior testis; UT, uterus; UtS, uterine sac. Semichon's carmine, ×21.

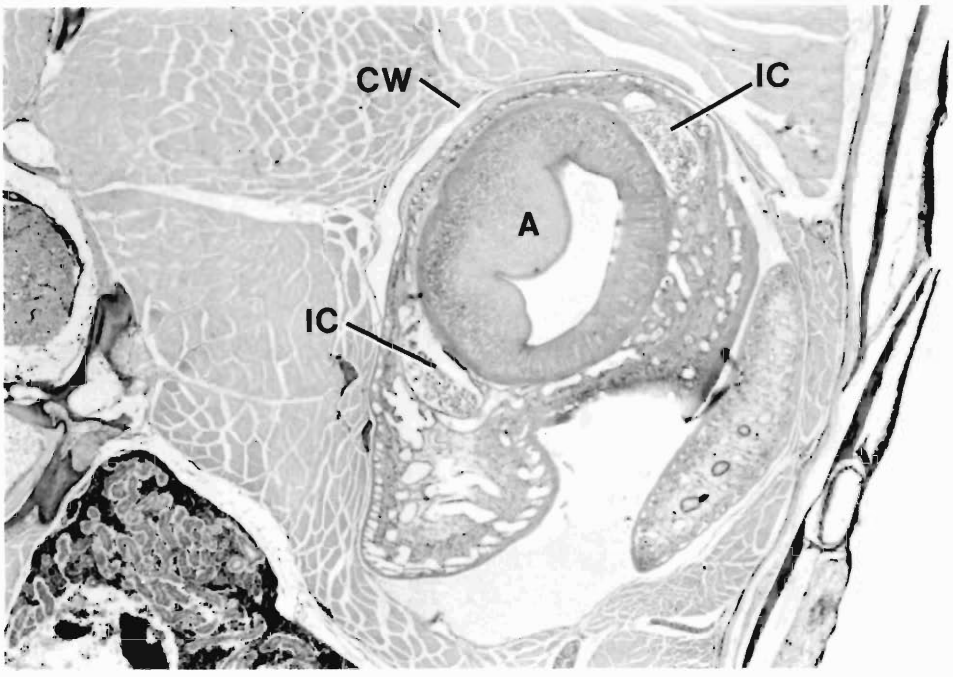

Figure 2. Cross section through acetabular region of *E. heterostomum* metacercaria encapsulated in musculature of *A. ramirezi*. Note thin connective tissue wall, mild compression of surrounding muscle tissues, and lack of host inflammatory response. A, acetabulum; CW, cyst wall; IC, intestinal cecum. H&E, ×41.

response present. Except for some compression of surrounding muscle fibers by the cysts, no other histopathologic lesions were seen.

The whole-mount specimen was readily identified as *E. heterostomum* (Rudolphi, 1809) Travassos, 1928 from previous descriptions of this species (Travassos, 1928; Baer, 1933; Fischthal and Kuntz, 1963; Ukoli, 1966; Dennis and Sharp, 1973). The larva measured 5.73 mm long by 1.97 mm wide, with measurements of other structures falling into the ranges given for this species (see Dennis and Sharp, 1973). *Euclinostomum heterostomum* metacercariae are progenetic, and various sizes and stages of development may be found for individual specimens (Ukoli, 1966; Dennis and Sharp, 1973). The metacercariae of *E. africanum* (Stossich, 1906) Dollfus, 1932, *E. clarias* Dubois, 1930, and *E. indicum* Bhalerao, 1942 are regarded as synonyms of *E. heterostomum* (Van der Kuyp, 1953; Ukoli, 1966; Dennis and Sharp, 1973).

Euclinostomum adults are parasites of the oral cavity and esophagus of fish-eating birds, primarily herons and egrets. Although several *Euclinostomum* spp. have been described, Ukoli (1966) and Dennis and Sharp (1973) regard only *E. heterostomum* and *E. multicaecum* Tubangui and Masilungan, 1935 as valid species. Adult *E. heterostomum* have been reported from *Ardea cinerea, A. purpurea, Ardeola goliath, A. ralloides, Bulbicus ibis, Egretta garzetta, Nycticorax nycticorax, Scopus umbretta,* and experimentally from *Anhinga ruffa* and *Phalacrocorax africanus* (Dollfus, 1932, 1950; Baer, 1933; Ukoli, 1966; Dennis and Sharp, 1973; Prudhoe and Hussey, 1977).

The metacercariae of *E. heterostomum* exhibit little host specificity, as they have been reported from at least nine fish hosts belonging to five families: Anabantidae (*Anabas scandens, A. testudineus*), Channidae (*Channa* (*Ophicephalus*) *punctatus*), Cichlidae (*Apistogramma ramirezi, Tilapia heudeloti, T. nilotica, T. zilli*), Clariidae (*Clarias angolensis*), and Cyprinidae (*Barbus canis*) (Joyeux and Houdemer, 1928; Dubois, 1930; Dollfus, 1932; Bhalerao, 1942; Srivastava, 1950; Van der Kuyp, 1953; Adiwinata, 1955; Fischthal and Kuntz, 1963; Paperna, 1964; Ukoli, 1966; Rai, 1970; Dennis and Sharp, 1973; present study). This report of *E. heterostomum* from *Apistogramma ramirezi* is a new host record and the first for this genus and species from aquarium fishes. Because the distribution of the intermediate and definitive hosts includes Africa, Asia, Europe, and South America, it is likely that *E. heterostomum* will be found in the future in aquarium fish from these areas.

The apparent irritation to the fish by *E. heterostomum* may have been caused by movements of the encapsulated larvae, because there was little host reaction to the metacercariae. The disfigurement produced by the larvae was apparently a reflection of the small size of this host as compared to other fish hosts. No other reports were found of *E. heterostomum* producing conspicuous swellings in fish hosts, and Ukoli (1966) stated that "metacercariae of *Euclinostomum* are usually encysted in the body cavity of fish." There are reports of *E. heterostomum* in the body wall and musculature of *Anabas scandens, Channa* (*Ophicephalus*) *punctatus, Tilapia heudeloti,* and *T. zilli,* but no reference to any disfigurement (Joyeux and Houdemer, 1928; Srivastava, 1950; Ukoli, 1966; Rai, 1970). As compared to *E. heterostomum, E. multicaecum* has been seen encysted in the muscles and unencysted lying beneath the visceral peritoneum of *Channa* (*Ophicephalus*) *striatus* (Tubangui and Masilungan, 1935; Velasquez, 1960).

Metacercariae are more common in tropical fish from South America than in those from the Far East (Gratzek et al., 1978). This is probably a reflection of husbandry practices, as fish originating from the Far East are usually aquarium raised and thus not exposed to snail hosts. The case reported here concerns a native Venezuelan fish imported from Hong Kong; it is not known where the fish were actually raised.

The whole-mount specimen has been deposited in the National Parasite Collection, Beltsville, Maryland, as USNM Helm. Coll. No. 77069.

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Age Resistance of Sheep to *Fasciola hepatica*

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ABSTRACT: Two experiments in succeeding years demonstrated that sheep 18 mo and older were more resistant to experimental *Fasciola hepatica* infections than lambs 6 mo old, when all experimental animals were inoculated with 350 *F. hepatica* cysts. At necropsy, numbers of flukes averaged 92 and 43 ($P > 0.025$) in Experiment 1 and 90 and 41 ($P > 0.01$) in Experiment 2 for the lambs and sheep, respectively; egg counts averaged 582 and 310 epg (eggs per gram) (not significantly different) and 426 and 187 epg ($P > 0.01$) for the same respective groups and experiments. Gamma glutamyl transpeptidase (GGT) activity levels peaked in both groups at 8 wk in Experiment 1 and 12 wk in Experiment 2. No significant differences occurred in GGT levels except in Experiment 2, when the sheep had significantly higher ($P > 0.05$) levels than the lambs at 12 wk PI; however, at necropsy only half as many flukes were found in the sheep.

Greater resistance to parasitic nematode infections with increased age of host has been reported for a variety of host–parasite relationships (e.g., Ackert et al., 1935; Stewart and Gordon, 1953; Herlich, 1960; Brunsdon, 1962; Gibson and Parfitt, 1972; Gallie, 1973; Knight and Rodgers, 1974). Little information is available for such relationships with fluke infections. Boray (1967a) found that cattle 17 and 24 mo old had about $^1/_2$ and $^1/_5$, respectively, the number of *Fasciola hepatica* recovered from cattle 6.5–8.5 mo old. Rajasekariah and Howell (1977) reported that rats 10 wk old were more resistant to experimental infection with *F. hepatica* than rats 5 wk old. Although sheep are unable to develop resistance to challenge infection of *F. hepatica* through repeated inoculations of cysts (Knight, 1980), and sheep 4–5 yr old can be infected (Boray, 1967b), no information is available concerning the relationship of age of sheep and susceptibility to *F. hepatica* infections. Two experiments were designed to determine whether an age resistance to experimental infection with *F. hepatica* could be demonstrated in sheep, and the results are reported here.

Materials and Methods

Two experiments were conducted in succeeding years using Polled Dorset lambs and sheep. In each year two experimental groups were set up as follows. Experiment 1—Group 1 comprised 13 lambs 6 mo old and Group 2 comprised 10 sheep: seven were 18 mo old, two were 66 mo old, and one was 141 mo old. Prior to infection all sheep had been raised and maintained in stilt pens indoors, except for three sheep older than 18 mo that had been pastured as part of the breeding flock. None had ever been exposed to *F. hepatica*. Experiment 2—Group 1 comprised 14 lambs 6 mo old and Group 2 comprised 15 sheep 18 mo old. All lambs and sheep in Experiment 2 were raised and maintained indoors in stilt pens prior to the experiment, precluding exposure to *F. hepatica*.

All lambs and sheep in both experiments were inoculated with 350 *F. hepatica*

Table 1. Average numbers of *Fasciola hepatica* and average egg counts (epg) at necropsy of lambs and sheep with experimental infection.

Animals	Average no. flukes (range)	Average epg
Experiment 1		
Group 1, lambs	92* (20–153)	582
Group 2, sheep	43 (0–84)	310
Experiment 2		
Group 1, lambs	90† (26–158)	426†
Group 2, sheep	41 (13–90)	187

* Significant ($P > 0.025$).
† Significant ($P > 0.01$).

cysts (Baldwin Enterprises,[1] Monmouth, Oregon) each on filter paper in gelatin capsules on the same day. Prior to inoculation the cysts were examined under the microscope for viability, and only clear transparent cysts with sharply defined internal structures were used. Postinoculation (PI) the lambs and sheep were placed together in a field pen for the continuance of the experiment and were fed alfalfa hay ad lib.

Blood samples were collected in 10-ml vacuum tubes monthly for analysis of gamma glutamyl transpeptidase (GGT) activity levels to monitor the infection. Blood was allowed to clot at room temperature and centrifuged at 3,000 *g* for 20 min and the serum was assayed spectrophotometrically (Clinicard Analyzer, Model 368, Instrumentation Laboratory, Inc.,[1] Lexington, Massachusetts). At 12 and 16 wk PI fecal samples were collected for egg counts (eggs per gram, epg). All lambs and sheep were necropsied 16 wk PI, when livers were removed, examined grossly, and sliced to recover flukes for counting.

Data were subjected to analysis of variance for significance.

Results and Discussion

Worms counts and average egg counts (epg) at necropsy are presented in Table 1.

The average numbers of flukes recovered from the two groups of lambs in the two experiments were nearly equal, as were the average numbers of flukes from the sheep; however, the sheep harbored about half the number of flukes found in the lambs. Average epg at necropsy for both experiments was higher in the lambs than in the sheep, though the difference was significant only in Experiment 2.

Levels of GGT for both groups peaked 8 wk PI in Experiment 1 and 12 wk PI in Experiment 2 (Table 2). The sheep had a significantly higher peak than the lambs only in Experiment 2 at 12 wk PI, but by the time of necropsy there was no difference between groups in either experiment. Despite the fact that the sheep had half as many worms as the lambs at necropsy in both experiments, GGT values for the sheep were equal to or higher than values for the lambs. If GGT

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

Table 2. Levels of gamma glutamyl transpeptidase (GGT) activity in lambs and sheep with experimental infections of *Fasciola hepatica*.

Animals	Weeks postinoculation			
	0	8	12	16
Experiment 1				
Group 1, lambs	105	304	291	172
Group 2, sheep	101	174	157	141
Experiment 2				
Group 1, lambs	73	170	186	164
Group 2, sheep	139	226	312*	239

* Significant difference ($P > 0.05$).

levels are related to liver damage, this would seem to indicate either a greater tissue response to the infections in the sheep caused by fewer flukes or an elimination of immature flukes after entry into the bile ducts. There was no evidence of greater gross liver damage in the sheep than in the lambs at necropsy, but this was 4–8 wk after the GGT peak. Those animals with the greatest numbers of flukes had the greatest damage, correlating damage with level of infection.

These experiments indicate that sheep more than 18 mo of age are more resistant to the development of *F. hepatica* infections than lambs 6 mo old. This is in agreement with other host–parasite relationships in animals. There was no significant difference in numbers of worms among the sheep: 3–83 in the 18-mo-old sheep, 59 and 84 in the 66-mo-old sheep, and 31 in the 141-mo-old sheep, suggesting that age resistance does not increase after 18 mo.

Inasmuch as sheep 4–5 yr old are unable to develop immunity to *F. hepatica* challenge infections (Boray, 1967b), it would appear that the greater resistance to infection of the sheep is due to factors other than immunological ones.

Two possible explanations are suggested for the lower infection levels in the sheep: (1) a barrier effect that hinders migration through the liver parenchyma and entry into the bile ducts because of a greater tissue response to the migrating flukes (Boray, 1967a) in the sheep than in the lambs, but produced by half as many flukes, or (2) the newly excysted worms, after entering the peritoneal cavity from the intestine, may have greater difficulty finding the liver due to the larger body volume or greater fat deposits of the sheep.

The age resistance demonstrated in this report seems unlikely to be protective, because the sheep in this study became infected, as did the 4- and 5-yr-old sheep that Boray (1967b) reported on, and liver damage can be produced by low numbers of flukes.

It would be expected that the pathology caused by *F. hepatica* infections could be tolerated better by sheep because they have a larger liver mass and therefore a greater liver reserve to compensate for the liver damage.

Under field conditions or natural infections, sheep are continually exposed to small numbers of cysts being ingested over a period of time rather than to a single inoculation with a large number of cysts. An accumulation of flukes thereby occurs in the bile ducts, and liver damage would be produced more gradually without massive tissue response.

Age resistance in sheep to *F. hepatica* infection underlines the importance of

using young sheep of the same age for experimental studies to maximize the numbers of flukes established following inoculation and to reduce variation resulting from dissimilar host responses of different-aged sheep.

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Penarchigetes macrorchis sp. n. (Cestoidea: Caryophyllaeidae) from the Lake Chubsucker, *Erimyzon sucetta* (Lacépède), in Western Kentucky

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ABSTRACT: *Penarchigetes macrorchis* sp. n. is described from the intestine of the lake chubsucker, *Erimyzon sucetta* (Lacépède, 1803), from Murphy's Pond, Kentucky. *Penarchigetes macrorchis* differs from *P. oklensis* Mackiewicz, 1969 and *P. fessus* Williams, 1979 in both number and size of testes. Testes average 129 by 139 μm in *P. macrorchis*, compared with 97 by 89 and less than 50 μm in *P. fessus* and *P. oklensis*, respectively. Fifty-five percent of *P. macrorchis* examined lacked testes, and 31, 9, and 5% contained one, two, and three testes, respectively.

The genus *Penarchigetes* was erected by Mackiewicz (1969) when he described *P. oklensis* from the spotted sucker, *Minytrema melanops* (Rafinesque, 1820), in Oklahoma. Recently Williams (1979) described a second species, *P. fessus*, from the lake chubsucker, *Erimyzon sucetta* (Lacépède, 1803), in Alabama. This paper describes a third species of *Penarchigetes* from *E. sucetta* in western Kentucky.

Materials and Methods

Two *E. sucetta* were collected incidentally from baited turtle traps in Murphy's Pond, Hickman County, Kentucky, during July 1981. Both fish were necropsied the same day they were collected. Cestodes used for whole mounts were pipetted directly into AFA and stained with Mayer's paracarmine. Some specimens were fixed in Bouin's fluid, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin. Figures were drawn with the aid of an Olympus drawing tube. Measurements are in micrometers unless otherwise stated, and are given as range followed by the mean in parentheses; dimensions of organs are stated as length by width. Comparative material included: *P. oklensis*, paratype, USNM Helm. Coll. No. 71263, one whole mount and three slides of serial sections; *P. fessus*, paratypes, USNM Helm. Coll. Nos. 72544 and 74354, five whole mounts. 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.

Penarchigetes macrorchis sp. n. (Figs. 1–9)

DESCRIPTION (based on 11 mature specimens): Mature adults 1.47–2.11 mm (1.70 mm) long and 0.25–0.37 mm (0.32 mm) wide at gonopore. Scolex 0.30–0.50 mm (0.42 mm) wide. Neck distinct. Outer longitudinal muscles poorly developed; inner longitudinal muscles consisting of 2 ventral and 2 dorsal fascicles and 2 smaller pairs of lateral fascicles in anterior half of body.

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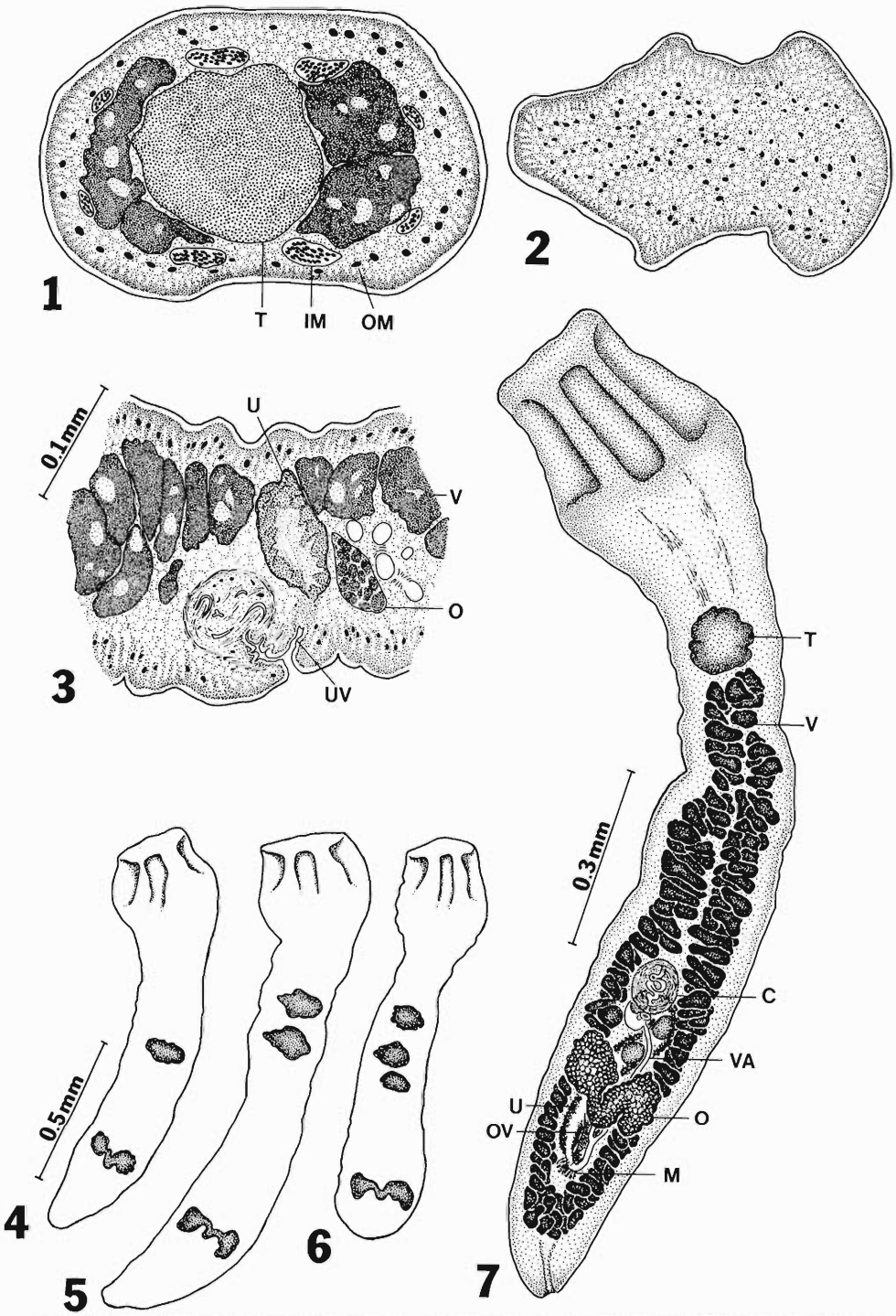
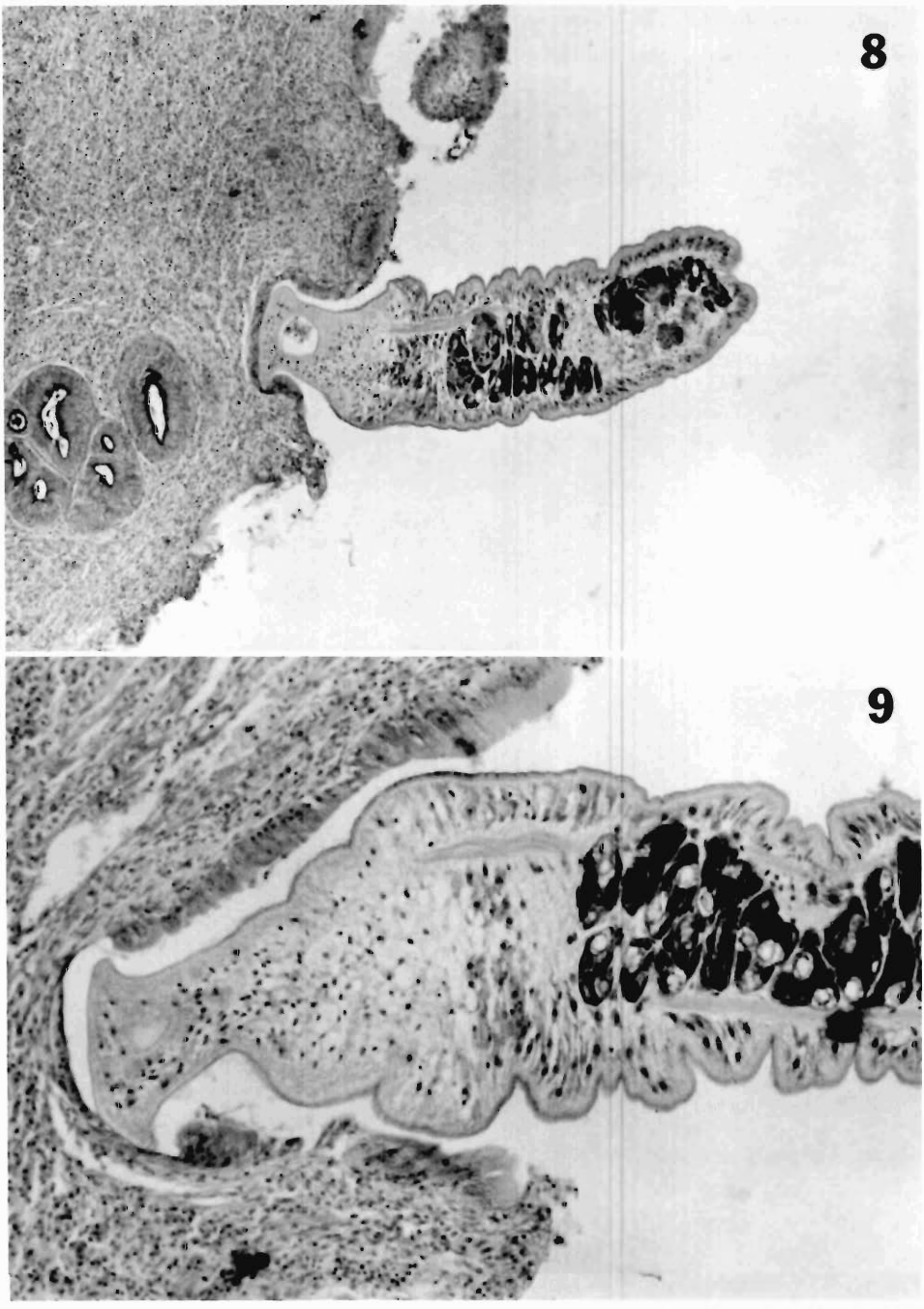


Figures 1–7. *Penarchigetes macrorchis* **sp. n. 1. Cross section through testis. 2. Cross section through midregion of scolex. 3. Sagittal section through gonopore. 4–6. Mature specimens showing different locations and numbers of testes. 7. Ventral view of holotype. Figures 1–3 drawn to same scale. Abbreviations: C, cirrus; IM, inner longitudinal muscle; M, Mehlis' gland; O, ovary; OV, oviduct; T, testis; U, uterus; UV, utero-vaginal canal; V, vitelline gland; VA, vagina.**



Figures 8, 9. Sections of *Penarchigetes macrorchis* sp. n. in situ, *Erimyzon sucetta* intestine. Note compression of surface epithelium.

Testes number 0–3, randomly arranged, 91–156 (122) by 85–174 (139) (N = 8). Cirrus sac round 83–99 (92). Genital aperture 287–616 (460) from posterior end. Seminal vesicle absent.

Vitelline follicles medullary, in 2 lateral bands; preovarian vitellaria 38–63 (47) by 71–123 (95) beginning 501–799 (623) from tip of scolex, continuous with postovarian vitellaria over dorsal part of ovary. Postovarian vitelline field longer than ovary. Ovary dumbbell-shaped, ovarian commissure forms on open "V"; ovarian commissure 150–371 (289) from posterior end. Wings of ovary 84–140 (105) long. Seminal receptacle absent. Eggs 52–64 (56) by 37–44 (41) (N = 8), measured in utero. Presence or absence of operculum not determined.

TYPE HOST: *Erimyzon sucetta* (Lacépède, 1803).

TYPE LOCALITY: Murphy's Pond, Hickman County, Kentucky.

SITE: Anterior intestine, firmly attached.

HOLOTYPE: USNM Helm. Coll. 77119.

PARATYPES: USNM Helm. Coll. 77120 (4); HWML 21375 (3).

ETYMOLOGY: The Greek *macro* and *orchis* refer to the large testes.

Discussion

Penarchigetes macrorchis is markedly different from *P. oklensis* and *P. fessus* in both the size and number of testes. Testes number 11–21 and 20–26 in *P. oklensis* and *P. fessus,* respectively, compared with zero to three in *P. macrorchis*. Testes average 97 by 89 in *P. fessus,* less than 50 in *P. oklensis,* and 129 by 139 in *P. macrorchis*.

The majority of *P. macrorchis* recovered totally lacked testes. Of 99 cestodes examined, 54 (55%) lacked testes, and 31 (31%), nine (9%), and five (5%) contained one, two, and three testes, respectively. Many of those without testes were gravid specimens. Although meiotic figures were noted in the testes of sectioned material, the presence of fully formed "normal" spermatozoa could not be verified. The reduced number or total lack of testes seen in *P. macrorchis* is similar to what is seen in *Glaridacris oligorchis* Haderlie, 1953.

It is possible that *P. macrorchis* is a parthenogenetic species similar to another caryophyllaeid cestode, *Atractolytocestus huronensis* Anthony, 1958 (Jones and Mackiewicz, 1969). Studies are currently in progress in an attempt to answer this and other complex questions concerning spermatogenesis, parthenogenesis, and progenesis in the Caryophyllidea.

The scolex of *P. macrorchis* is somewhat different from that of the genotype or *P. fessus*. The scolex is not expanded, and has a larger terminal disc and longer median bothria than seen in either *P. fessus* or *P. oklensis*.

Murphy's Pond is a shallow, relict cypress swamp in northeastern Hickman County that is periodically confluent with Obion Creek. It is not an open expanse of water, but is divided into four major pool areas by dense vegetation; therefore, this habitat is very similar to the collecting sites described for both *P. oklensis* and *P. fessus*. Recently, however, we have recovered two *P. oklensis* from one of 95 spotted suckers collected from Kentucky Lake and Lake Barkley in western Kentucky. These lakes are large reservoirs quite unlike the habitat previously described for *P. oklensis*. A determination of the intermediate host(s) for this genus would help explain their somewhat limited distribution.

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A Survey of Cestodes From Borneo, Palawan, and Taiwan, With Special Reference to Three New Species[1]

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ABSTRACT: Fifty-three distinct species of adults and metacestodes were collected from 78 species of vertebrates from Borneo, Palawan, and Taiwan. *Ophiotaenia anderseni* sp. n. of *Trimeresurus stejnegeri* from Taiwan is described. The smaller overall size, the scolex with an apical organ, 42–116 testes, and a mature segment with a 5:1 ratio of length to width distinguish this species from all other ophiotaeniids. *Oochoristica chinensis* sp. n. of *Japalura swinhonis* from Taiwan is described. The 12–25 testes per segment, the semicircular distribution of the testes around the vitellarium and posterior portion of the ovary, the cirrus sac extending 30–47% the width of the mature segment, and embryonic hooks of 12–14 μm characterize this species of *Oochoristica*. *Parvitaenia heckmanni* sp. n. of *Spilornis cheela* from Palawan is unique in having 32 rostellar hooks. *Paraprogynotaenia charadrii* comb. n. of *Charadrius alexandrinus* and *C. dominicus* from Taiwan is reassigned because of its single circle of rostellar hooks, irregular alternating genital pores, testes in two lateral fields, and cirrus sac passing between the osmoregulatory canals. *Paronia* sp. of *Amaurornis phoenicurus* from Taiwan is described in detail.

From 1957 to 1962 the third author was engaged in epidemiologic and epizootiologic investigations that involved the examination of vertebrates from different geographic regions for parasites. The present taxonomic study is based upon an examination of specimens taken by Dr. R. E. Kuntz and associates of the United States Naval Medical Research Unit No. 2, Taipei, Taiwan. Field studies were also conducted in outlying areas, including North Borneo (Malaysia) and Palawan Island (Republic of the Philippines).

Materials and Methods

Hosts were obtained by various means (e.g., hand capture, seining, traps, shooting, etc.), and examined for parasites as soon as possible after procurement. Amphibians and lizards were pithed or anesthetized with ether, snakes were drowned, and birds and mammals were anesthetized with ether.

The majority of hosts was fixed and/or prepared as museum skin specimens with host–parasite number and information attached. Host identifications in some instances were made in the field by recognized experts, but most host specimens were forwarded to the Field Museum of Natural History, Chicago, U.S. National

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Museum, Washington, D.C., or to other institutions for taxonomic evaluations. Host identifications are given by Kuntz (1969a, b) and Kuntz and Ming (1970).

Parasites were removed from visceral organs by teasing individual organs in petri dishes under a dissecting microscope. Helminths were washed and relaxed in normal saline, then killed in hot tap water, fixed in FAA for 4–12 hr, and transferred to 70% ethyl alcohol plus 5% glycerine for storage. Worms were stained in Semichon's carmine, dehydrated in alcohol, cleared in xylene, cut to convenient lengths, mounted in Canada balsam, and allowed to dry at room temperature. Characters (e.g., ovary, testes, seminal receptacle, etc.) of late mature and early gravid segments were measured for length and width. The results are given as range, with means and standard deviations in parentheses, and are in micrometers unless otherwise stated.

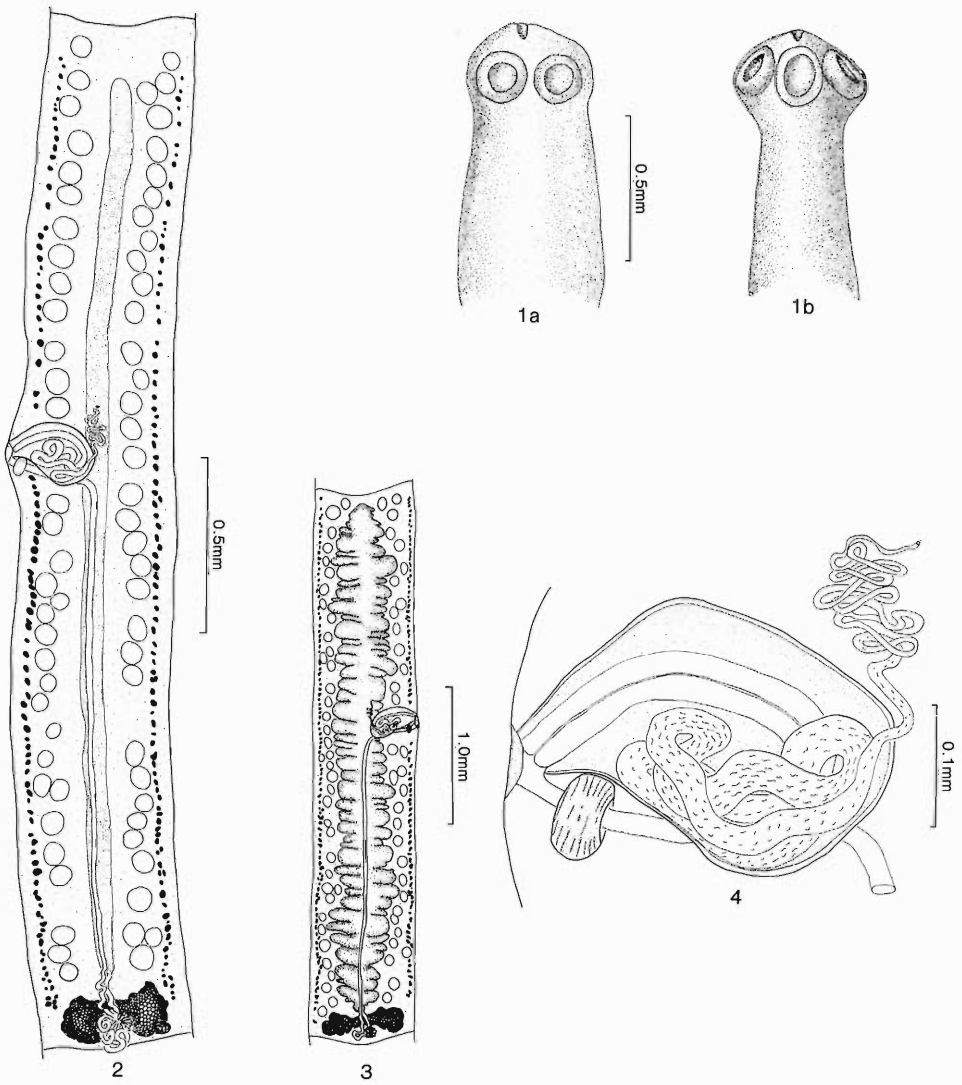
Results

Fifty-three species of adults and metacestodes were collected from 78 species of vertebrates (Table 1).

Ophiotaenia anderseni sp. n. (Figs. 1–4)

Numerous specimens of *Ophiotaenia* La Rue, 1911 were obtained from eight bamboo vipers, *Trimeresurus stejnegeri* Schmidt, 1925, of Tai-chung and Hualien, Taiwan, from March 22 to July 6, 1960. The material consisted of six immature worms and dozens of fragments of varying lengths. Many stages of development were represented.

DESCRIPTION: Whole, gravid specimens not available. Estimated length of 1 composite exceeds 130 mm with a width of 0.7 mm, and another incomplete specimen approximates 170 mm by 1.0 mm. Complete but immature worms (N = 6) 12.5–28 mm by 0.4–0.5 mm ($\bar{x}$ = 20.4 ± 5.8 by 0.5 ± 0.06). Metamerism distinct, acraspedote, prominent genital atrium alternating irregularly. Scolex (N = 35) 0.22–0.45 mm by 0.26–0.51 mm ($\bar{x}$ = 0.32 ± 0.07 by 0.41 ± 0.01), apical organ present, scolex without spines (Fig. 1a, b). Apical organ (N = 25) 18–35 by 24–48 ($\bar{x}$ = 27.3 ± 4.9 by 32.6 ± 5.5), oval to pyriform. Suckers (N = 57) 150–220 by 134–202 ($\bar{x}$ = 178.8 ± 17.8 by 162.3 ± 17.8), round to oval. Neck (N = 19) 3.1–6.7 mm by 0.34–0.55 mm ($\bar{x}$ = 4.4 ± 1.0 by 0.5 ± 0.06), constricted slightly behind scolex. Mature segments (N = 83) 2.5–4.6 mm by 0.4–0.8 mm ($\bar{x}$ = 3.6 ± 0.47 by 0.7 ± 0.09), markedly longer than wide, average ratio of length to width is 5:1. Gravid segments (N = 4), 3.7–4.2 mm by 0.6–1.0 mm. Testes (N = 200), 44–95 by 42–88 ($\bar{x}$ = 66.8 ± 10.2 by 57.2 ± 8.3), oval to round, in two lateral groups (Figs. 2, 3), generally more numerous on aporal side. Number of testes per segment (66 segments) 42–116 ($\bar{x}$ = 74.0 ± 18.1). Vas deferens convoluted, directly dorsal to developing uterus in mature segments. Cirrus sac (N = 30) 220–354 by 106–177 ($\bar{x}$ = 293.0 ± 34.7 by 156.2 ± 26.5), expanded proximally to accommodate highly coiled ejaculatory duct (Figs. 2, 4), length extends into nearly ½ width of segment when cirrus is retracted, anterior and dorsal to vagina (Fig. 2). Vaginal sphincter present. Vagina with copulatory portion nearly at right angle to lateral margin, bending sharply caudad upon reaching uterus, becoming sinuous near ovarian commissure, joining convoluted oviduct behind ovary (Fig. 2). Ovary



Figures 1–4. ***Ophiotaenia anderseni*** **sp. n. of the bamboo viper from Taiwan. 1a, 1b. Scolex. 2. Late mature segment, dorsal view. 3. Gravid segment, dorsal view. 4. Cirrus sac and terminal genital ducts, dorsal view.**

(N = 76) 309–587 wide by 153–380 long ($\bar{x}$ = 409.8 ± 65.8 by 217.6 ± 44.2), markedly bilobed in mature and gravid segments. Vitelline follicles (N = 200) 13–44 by 9–40 ($\bar{x}$ = 23.6 ± 6.1 by 18.4 ± 5.1), oval or irregular, forming 2 lateral bands 35 to 81 from margin. Uterus with approximately 100 diverticula (Fig. 3). Eggs (N = 21) 26–35 by 23–31 ($\bar{x}$ = 31.6 ± 2.5 by 27.5 ± 2.2), round to oval. Onchosphere (N = 20) 16–26 by 16–24 ($\bar{x}$ = 21.6 ± 2.6 by 19.6 ± 2.5), round to oval.

TYPE HOST: Bamboo viper, *Trimeresurus stejnegeri* (Crotalidae).

SITE OF INFECTION: Small intestine.

TYPE LOCALITY: Tai-chung, Taiwan, Republic of China.

Table 1. Some adult and larval cestodes of vertebrates from Borneo, Palawan, and Taiwan.

Cestode	Host	Site of infection*	Locality	USNM Helm. Coll. No.
Caryophyllidea	Fishes			
Caryophyllaeidae				
Lytocestus sp.	*Clarias batrachus*	I	Taiwan	69415
Tetraphyllidea				
Oncobothriidae				
Scolex pleuronectis, uniloculate form	*Muraenesox cinereus*	G, S	Taiwan	†
Scolex pleuronectis, biloculate form	*Muraenesox cinereus*	G, S	Taiwan	†
Trypanorhyncha				
Pterobothriidae				
Pterobothrium hira (plerocercus) Yamaguti, 1952	*Gazza minuta*	B	Palawan	69382
Pterobothrium sp. (plerocercus)	*Caranx affinis*	B	Palawan	†
Pseudophyllidea				
Diphyllobothriidae	Amphibians			
Diphyllobothrium sp. (sparganum)	*Rana tigrina*	L	Taiwan	†
	Rhacophorus eiffingeri	B		†
Proteocephalidea				
Proteocephalidae	Reptiles			
Ophiotaenia anderseni sp. n. (Figs. 1–4)	*Trimeresurus stejnegeri*	I	Taiwan	77165, 77166
Ophiotaenia japonensis Yamaguti, 1935	*Bungarus multicinctus*	I	Taiwan	69397
	Trimeresurus mucrosquamatas			†
Proteocephalidae gen. sp. (plerocercoid) (Fig. 10)	*Ptyas mucosus*	I	Taiwan	†
Cyclophyllidea				
Anoplocephalidae				
Oochoristica chinensis sp. n. (Figs. 5–9)	*Japalura swinhonis*	I	Taiwan	77167, 77168
Oochoristica hainanensis Hsü, 1933	*Japalura swinhonis*	I	Taiwan	†
Oochoristica sp.	*Elaphe carinata*	I	Taiwan	†
	Eumeces chinensis			†
	Hemidactylus frenatus			†
	Tackydromus septentrionalis			†

Table 1. Continued.

Cestode	Host	Site of infection*	Locality	USNM Helm. Coll. No.
Tetraphyllidea				
Oncobothriidae				
Scolex pleuronectis, uniloculate form (Fig. 11)	*Pelamis platurus*	I	Taiwan	69419
Trypanorhyncha				
Dasyrhynchidae				
Callitetrarhynchus gracilis (plerocercus) (Rudolphi, 1819) Pinter, 1931	*Cerberus rhynchops*	B	Palawan	69418
Pseudophyllidea				
Diphyllobothriidae (sparganum) (Fig. 12)	*Natrix chrysaga*	B	Borneo	†
	Bungarus multicinctus		Taiwan	†
	Dinodon rufozonatum			†
	Elaphe carinata			†
	Naja naja			†
	Natrix stolata			77129
	Ptyas korros			†
	Ptyas mucosus			77130
	Trimeresurus mucrosquamatus			†
	Trimeresurus stejnegeri			†
	Vipera russelli			†
	Zaocys dhumnades			†
	Eumeces chinensis			†
Cyclophyllidea	Birds			
Dilepididae				
Parvitaenia heckmanni sp. n. (Figs. 13–17)	*Spilornis holospilus*	I	Palawan	77169, 77170
Parvitaenia macropeos (Wedl, 1855) Baer and Bona, 1960	*Nycticorax nycticorax*	I	Taiwan	69380
Dilepis undula (Schrank, 1788) Weinland, 1858	*Zoothera dauma*	I	Taiwan	69403
Paradilepis scolecina (Rudolphi, 1819) Hsü, 1935	*Apus pacificus*	I	Taiwan	69407
Neoliga diplacantha Singh, 1952	*Apus pacificus*	I	Taiwan	69381
Dictymetica nymphaea (Schrank, 1970) Spasskaya and Spassky, 1971	*Numenius minuta*	I	Taiwan	69408

Table 1. Continued.

Cestode	Host	Site of infection*	Locality	USNM Helm. Coll. No.
Amoebotaenia vanelli Fuhrmann, 1907	*Charadrius alexandrinus*	I	Taiwan	†
Choanotaenia sp.	*Turdus pallidus*	I	Taiwan	69413
Vitta rustica (Neslobinsky, 1911) Baer, 1959	*Hirundo rustica*	I	Taiwan	†
Vitta sp.	*Hirundo daurica*	I	Taiwan	†
Trichocephaloides megalocephala (Krabbe, 1869) Clerc, 1902	*Calidris alpina*	I	Taiwan	69409
Anoncotaenia globata (Linstow, 1879) Cohn, 1900	*Anthus hodgsoni*	I	Taiwan	†
Anoncotaenia sp.	*Garrulax canorus*	I	Taiwan	†
	Heterophasia auricularis			†
	Liochichla steeri			†
	Prunella collaris			†
Ptilotolepis reductorhyncha (Spasskaya, 1957) Spasskaya and Spassky, 1977	*Parus monticolus*	I	Taiwan	69402
Cladotaenia globifera (Batsch, 1786) Cohn, 1901	*Accipiter trivirgatus*	I	Taiwan	†
	Falco tinnunculus			69396
Hymenolepididae				
Aploparaxis sp.	*Anas falcata*	I	Taiwan	†
Colymbilepis multistrata (Rudolphi, 1810) Spasskaya, 1966	*Podiceps ruficollis*	I	Taiwan	69406
Diorchis sp.	*Anas platyrhynchos*	I	Taiwan	†
Drepanidolepis anatina (Krabbe, 1869) Spassky, 1963	*Anas platyrhynchos*	I	Taiwan	69401
Microsomacanthus styloides (Fuhrmann, 1906) Spassky and Spasskaya, 1954	*Rostratula bengalensis*	I	Taiwan	69412
Sobolevicanthus krabbeella (Hughes, 1940) Ryjikov, 1956	*Anas crecca*	I	Taiwan	69374, 69375
Davaineidae				
Raillietina (Paroniella) culiauna Tubangui and Masiluñgan, 1937	*Oriolus chinensis*	I	Taiwan	69392
Raillietina (Raillietina) echinobothrida (Megnin, 1880) Fuhrmann, 1924	*Gallus gallus*	I	Taiwan	77124
Raillietina (Raillietina) korkei Joyeux and Houdemer, 1927	*Columba livia*	I	Taiwan	69389

Table 1. Continued.

Cestode	Host	Site of infection*	Locality	USNM Helm. Coll. No.
Raillietina (Raillietina) paucitesticulata (Fuhrmann, 1909) Fuhrmann, 1924	*Streptopelia chinensis*	I	Borneo	†
	Anas platyrhynchos		Taiwan	69394
	Streptopelia traquebarica			69393
Raillietina (Raillietina) taiwanensis Yamaguti, 1938	*Sphenurus sieboldii*	I	Taiwan	69390
Raillietina (Raillietina) tetragona (Molin, 1858) Fuhrmann, 1924	*Gallus gallus*	I	Taiwan	69391
Raillietina (Raillietina) sp.	*Chalcophaps indica*	I	Taiwan	69387
	Turnix suscitator			69384
Raillietina sp.	*Corvus levaillantii*	I	Taiwan	†
	Hypsipetes madagascariensis			†
	Lophura swinhoii			69388
	Megalaema oorti			†
	Parus monticolus			†
	Pycnonotus goiavier		Borneo	69385, 69386
	Spizixos semitorques		Taiwan	†
Cotugnia meggitti Yamaguti, 1935	*Streptopelia chinensis*	I	Taiwan	69379
Cotugnia sp.	*Anas platyrhynchos*	I	Taiwan	†
Amabiliidae				
Tatria acanthorhynchus (Wedl, 1855) Kowalewski, 1904	*Podiceps ruficollis*	I	Taiwan	69395
Dioecocestidae				
Gyrocoelia perversa Fuhrmann, 1899	*Charadrius alexandrinus*	I	Taiwan	69399, 69400
	Charadrius dubius			†
Dioecocestus sp.	*Podiceps ruficollis*	I	Taiwan	69410, 69411
Progynotaeniidae				
Paraprogynotaenia charadrii (Yamaguti, 1956) comb. n. (Figs. 18–22)	*Charadrius alexandrinus*	I	Taiwan	69405
	Charadrius dominicus			69404
Anoplocephalidae				
Paronia pycnonoti Yamaguti, 1935	*Pycnonotus sinensis*	I	Taiwan	69417
Paronia sp. (Figs. 23–26)	*Amaurornis phoenicurus*	I	Taiwan	77185

Table 1. Continued.

Cestode	Host	Site of infection*	Locality	USNM Helm. Coll. No.
Pseudophyllidea				
Diphyllobothriidae				
Diphyllobothrium sp. (sparganum)	*Gallus gallus*	B	Taiwan	†
Cyclophyllidea	Mammals			
Dilepididae				
Dipylidium caninum (Linnaeus, 1758) Leuckart, 1863	*Canis familiaris*	I	Taiwan	†
Taeniidae				
Taenia taeniaeformis (Batsch, 1786)	*Felis domesticus*	I	Taiwan	†
Taenia taeniaeformis (strobilocercus)	*Rattus losea*	L	Taiwan	†
	Rattus norvegicus			†
	Rattus rattus			†
Hymenolepididae				
Hymenolepis diminuta (Rudolphi, 1819) Weinland, 1858	*Bandicota nemorrvaga*	I	Taiwan	77122
	Callosciurus erythraeus			77126
	Rattus coxinga			†
	Rattus losea			†
	Rattus norvegicus			77128
	Rattus rattus			69376, 77127
Staphylocystis (*Staphylocystis*) *suncusensis* Olsen and Kuntz, 1978	*Suncus murinus*	I	Taiwan	69377, 69378
Staphylocystis (*Staphylocystis*) *sindensis* Nama, 1976	*Suncus murinus*	I	Taiwan	†
Vampirolepis nana (Siebold, 1952) Spassky, 1954	*Mus formosanus*	I	Taiwan	77125
	Rattus rattus			†

Table 1. Continued.

Cestode	Host	Site of infection*	Locality	USNM Helm. Coll. No.
Davaineidae				
Diorchiraillietina contorta (Zschokke, 1895) Yamaguti, 1959	*Manis javanica*	I	Borneo	69416
Raillietina (Fuhrmannetta) bandicotensis (Olsen and Kuntz, 1979) comb. n.	*Bandicota indica*	I	Taiwan	77123
Raillietina (Raillietina) celebensis (Janicki, 1902) Fuhrmann, 1920	*Lepus sinensis*	I	Taiwan	†
	Rattus coxinga			†
	Rattus losea			†
	Rattus norvegicus			69383
	Rattus rattus			†
Mesocestoididae				
Mesocestoides sp. (tetrathyridia)	*Paguma larvata*	B	Taiwan	†
Pseudophyllidea				
Diphyllobothriidae				
Diphyllobothrium erinacei Faust, Campbell, and Kellogg, 1929	*Felis domesticus*	I	Taiwan	77121
	Viverricula indica			69414
Diphyllobothrium sp. (preadult)	*Rattus losea*	I	Taiwan	†

* Abbreviations: B, body cavity; G, gall bladder; I, intestine, L, liver; S, stomach.
† Specimens retained in the collection of Dr. Gerald D. Schmidt for further study.

ETYMOLOGY: Patronymic in honor of Dr. Ferron Andersen, Professor of Zoology, Brigham Young University.

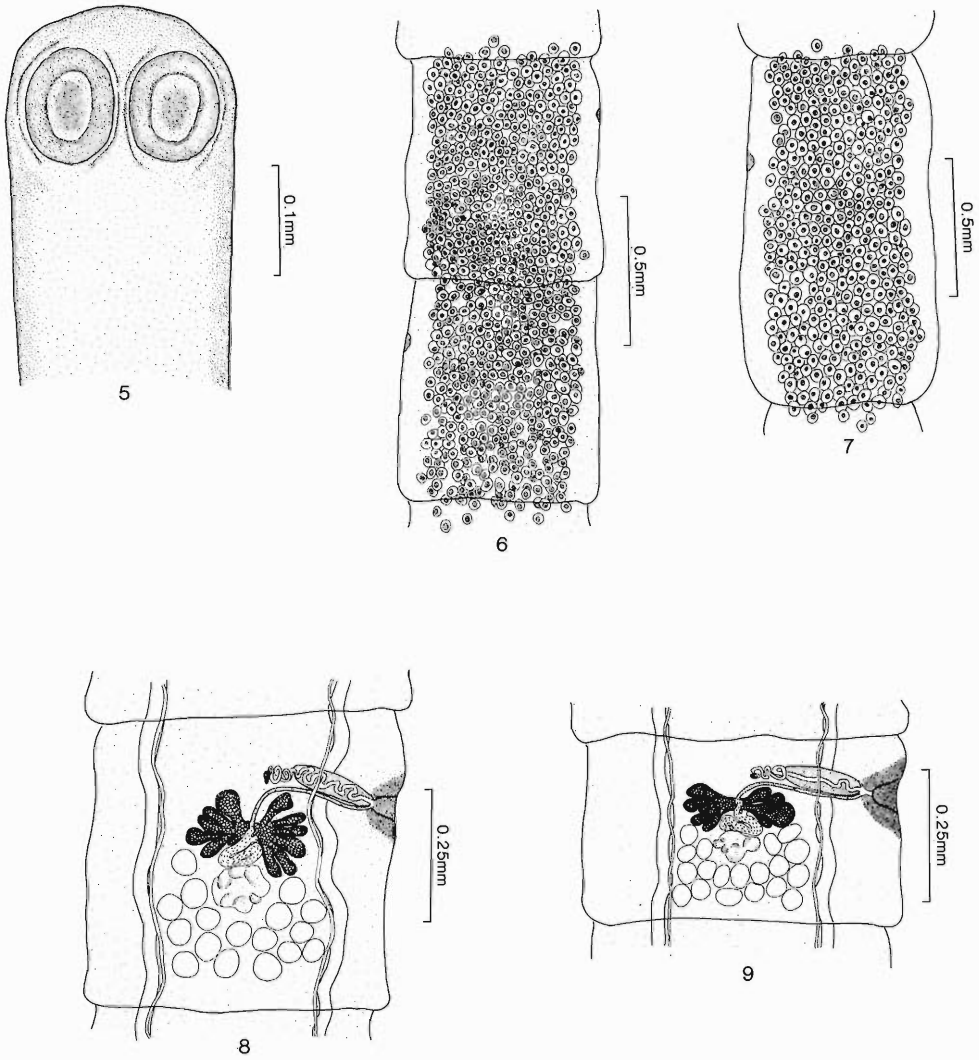
TYPE SPECIMENS: Holotype USNM Helm. Coll. No. 77165; paratypes USNM Helm. Coll. No. 77166.

REMARKS: This species is similar to *O. phillipsi* Burt, 1937 in the general shape of the mature and gravid proglottids, genital arrangement, and size of the eggs. In addition, both species infect snakes of the genus *Trimeresurus*. Nevertheless, *O. anderseni* sp. n. is differentiated from *O. phillipsi* because of the smaller size of the strobila (approximately 170 by 0.70 mm vs. 400–920 mm by 1.5–2.1 mm), a smaller scolex (0.22–0.45 mm by 0.26–0.51 mm vs. 0.67 mm in diameter), an apical organ on the scolex, and fewer testes (42–116 vs. 170–230). Of the described ophiotaeniids possessing an apical organ, none has mature proglottids with a 5:1 ratio of length to width.

Oochoristica chinensis sp. n.
(Figs. 5–9)

A new species of *Oochoristica* Lühe, 1898 was found in five lizards, *Japalura swinhonis* (Guenther, 1864), from Taiwan, September 28 to October 13, 1960. The following description is based on 10 complete specimens and several fragments.

DESCRIPTION: Worms (N = 12) 21–71 mm by 0.11–0.92 mm ($\bar{x}$ = 37.0 ± 16.4 by 0.57 ± 0.20), 65–128 ($\bar{x}$ = 88.8 ± 17.4) segments. Metamerism distinct, acraspedote, lateral margins parallel, genital ducts passing between dorsal and ventral osmoregulatory canals. Scolex (N = 14) 0.18–0.27 ($\bar{x}$ = 0.22 ± 0.03) wide, not distinctly separated from neck (Fig. 5). Suckers (10 worms, N = 20) 84–132 by 62–106 ($\bar{x}$ = 112.4 ± 9.5 by 88.3 ± 10.2), oval. Neck (N = 15) 0.77–1.87 mm ($\bar{x}$ = 1.10 ± 0.27) long, not constricted behind scolex. Mature segments (15 worms, N = 117), 0.25–0.61 mm by 0.32–0.82 mm ($\bar{x}$ = 0.4 ± 0.09 by 0.56 ± 0.11), wider than long (Fig. 9) or longer than wide (Fig. 8), 6–21 segments completely mature per worm. Gravid segments (10 worms, N = 119) 0.4–1.68 mm by 0.26–0.64 mm ($\bar{x}$ = 0.85 ± 0.25 by 0.54 ± 0.13), markedly longer than wide, 12–62 segments partially or fully gravid (Figs. 6, 7). Dorsal and ventral osmoregulatory canals sinuous, ventral canal larger. Testes (11 worms, N = 110) 32–62 by 26–62 ($\bar{x}$ = 44.5 ± 6.1 by 39.8 ± 6.4), round to oval, medullary, proximal to osmoregulatory canals, forming semicircle around vitellarium and posterior portion of ovary (Figs. 8, 9), occasionally divided into 2 zones. Testes per segment (15 worms, 142 segments) 12–25 ($\bar{x}$ = 18.5 ± 2.4). Vas deferens irregularly looped. Ejaculatory duct serpentine. Cirrus sac (11 worms, N = 110) 108–220 by 32–58 ($\bar{x}$ = 155.4 ± 18.4 by 44.8 ± 4.9), oval. Extent of cirrus sac in mature segment (N = 34) 30–47% ($\bar{x}$ = 37.6 ± 0.04). Genital atrium irregularly alternating, approximately in anterior ⅓ of mature segment, does not protrude. Vagina posterior to cirrus sac, becoming sinuous and slightly expanded at level of ovary. Oviduct highly convoluted, swollen, filled with sperm. Ovary (17 worms, N = 85, measured in 5 most mature segments) 136–339 by 62–218 ($\bar{x}$ = 218.8 ± 48.4 by 130.2 ± 31.8), bilobed, medullary, in anterior half of segment. Vitellarium (15 worms, N = 110), 32–148 by 37–124 ($\bar{x}$ = 89.2 ± 23.3 by 71.5 ± 18.1), irregular and lobate, posterior to isthmus of ovary. Uterus recognizable in only 2 or 3 segments, replaced with egg capsules. Eggs (11 worms, N = 10, outer shell measured) 33–60 by 28–



Figures 5–9. ***Oochoristica chinensis*** **sp. n. of a lizard from Taiwan. 5. Scolex, dorsal view. 6. Early gravid segments. 7. Gravid segment near end of worm. 8. Longer-than-wide mature segment, dorsal view. 9. Wider-than-long mature segment, dorsal view.**

50 ($\bar{x}$ = 40.2 ± 5.7 by 35 ± 4.9), round to oval. Onchosphere (11 worms, N = 110) 18–33 by 16–32 ($\bar{x}$ = 25.9 ± 3.2 by 22.6 ± 2.4), round to oval. Length of embryonic hooks (11 worms, N = 110) 12–16 ($\bar{x}$ = 14 ± 1).

TYPE HOST: Lizard, *Japalura swinhonis* (Agamidae).

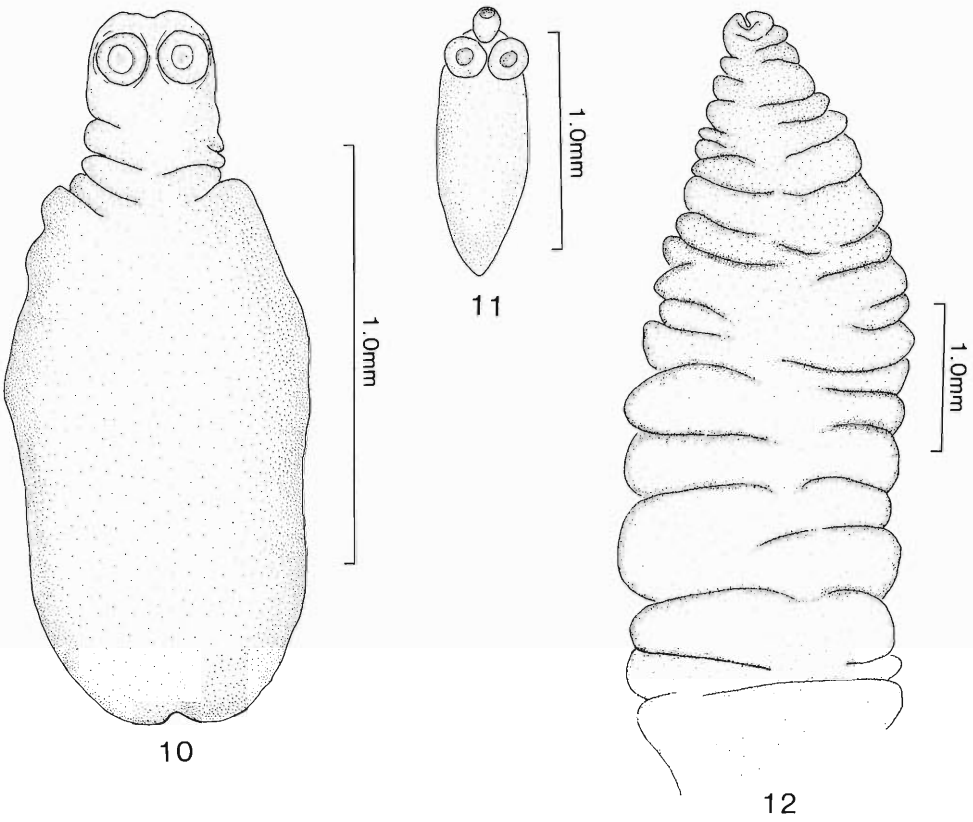
SITE OF INFECTION: Small intestine.

TYPE LOCALITY: Ping-tung, Taiwan, Republic of China.

ETYMOLOGY: Refers to the type locality.

TYPE SPECIMENS: Holotype USNM Helm. Coll. No. 77167; paratypes USNM Helm. Coll. No. 77168.

REMARKS: *Oochoristica chinensis* sp. n. is most similar to *O. lygosomae* Burt, 1933 and *O. lygosomatis* Skinker, 1935 in the size of the cirrus sac and the number



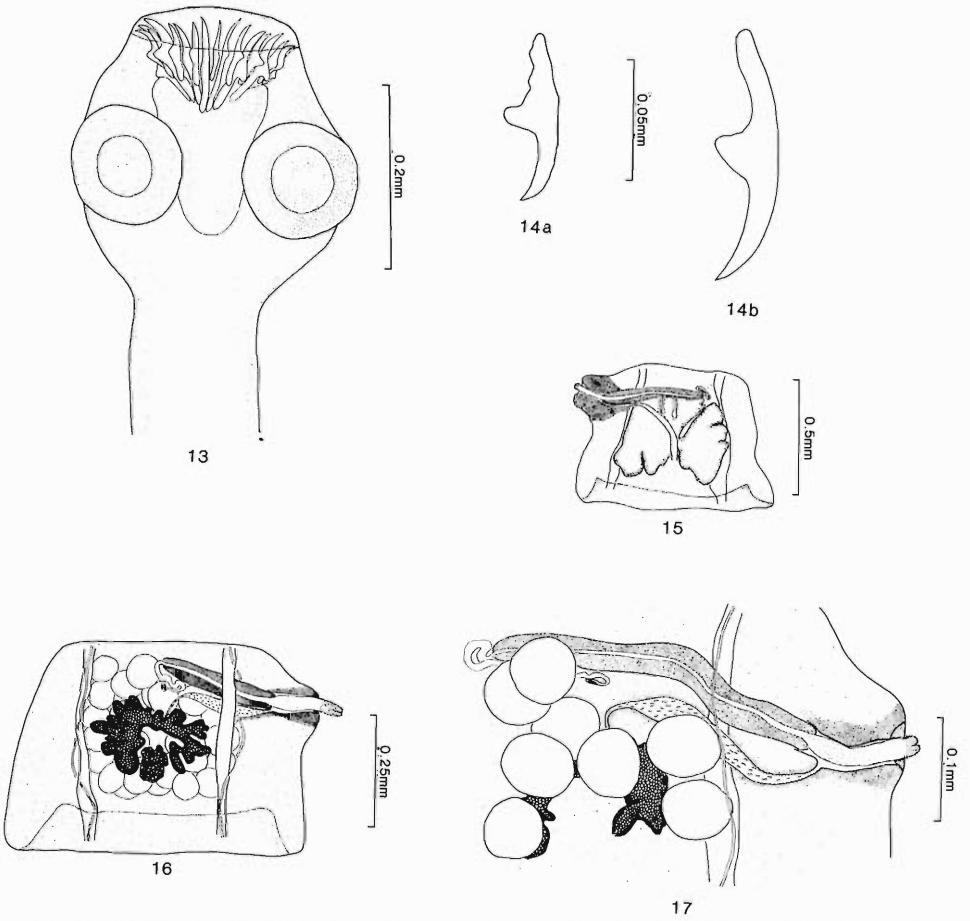
Figures 10–12. Metacestodes of snakes from Taiwan. 10. Plerocercoid from *Ptyas mucosus*. 11. *Scolex pleuronectis*, uniloculate form from *Pelamis platurus*. 12. Anterior end of sparganum from *Natrix piscator*.

of testes. Distinguishing features of *O. chinensis* from *O. lygosomae* and *O. lygosomatis* include the larger strobila (21–71 mm by 0.11–0.92 mm vs. 8–15 by 0.6 mm and 8–11 by 0.3–0.35 mm), the greater number of segments (65–128 vs. 35–45 and 45), the cirrus sac not reaching the midline of the strobila, and larger embryonic hooks (12–16 vs. 11 and 12–13). In *O. chinensis* the testes are arranged in a semicircle around the vitellarium and the posterior portion of the ovary, whereas in *O. lygosomae* the testes are entirely behind the vitellarium in 1 uninterrupted field. *Oochoristica chinensis* is further separated from *O. lygosomatis* by the greater number of mature segments (6–21 vs. 4) and the greater number of gravid segments (12–62 vs. 9). It differs from *O. hainanensis* Hsü, 1935, also from China, in the length of the strobila (21–70 vs. 134) and in possessing fewer testes (12–25 vs. 40–50).

Parvitaenia heckmanni sp. n.
(Figs. 13–17)

A serpent eagle, *Spilornis cheela* Latham, 1790, from Palawan yielded eight tapeworms of the genus *Parvitaenia* Burt, 1940.

DESCRIPTION: One complete worm measures 85 mm by 0.93 mm; 3 worms without scolices or necks measure 35–59 mm by 0.49–0.86 mm. Metamerism



Figures 13–17. ***Parvitaenia heckmanni*** **sp. n. of a serpent eagle from Palawan. 13. Scolex, dorsoventral view. 14a. Small hook. 14b. Large hook. 15. Gravid segment, dorsal view. 16. Mature segment, ventral view. 17. Terminal genital ducts in mature segment, dorsal view.**

distinct, markedly craspedote, hyperapolytic. Genital ducts pass between dorsal and ventral osmoregulatory canals. Scolex (N = 1) 0.32 by 0.28, armed with double crown of 32 large and small falciform hooks (Fig. 13). Large hooks (1 worm, N = 3) 100–105, blade and handle approximately equal in length, guard prominent (Fig. 14b). Small hooks (1 worm, N = 8) 65–70 ($\bar{x}$ = 66.6 ± 1.9), blade and handle approximately equal in length, guard prominent (Fig. 14a). Rostellar sac (N = 1) 175 by 126, pyriform. Suckers (N = 2) 121 by 117–123. Neck (N = 1) 544 by 153. Mature segments (5 worms, N = 50) 0.29–0.51 mm by 0.50–0.78 mm ($\bar{x}$ = 0.40 ± 0.06 by 0.65 ± 0.08), wider than long, may be slightly campanulate (Fig. 16). Gravid segments (5 worms, N = 50) 0.36–0.70 mm by 0.63–0.99 mm ($\bar{x}$ = 0.50 ± 0.07 by 0.82 ± 0.09), wider than long. Testes (5 worms, N = 50) 65–94 by 56–84 ($\bar{x}$ = 76.1 ± 6.5 by 67.3 ± 5.9), round to oval, medullary, dorsal to ovary, mainly medial to osmoregulatory canals, may overlap one another in more mature segments (Fig. 17). Number of testes per segment (8 worms, 75 segments) 22–32 ($\bar{x}$ = 25.9 ± 2.5). Convoluted vas deferens medial and caudal to

cirrus sac. Cirrus sac (5 worms, $N = 50$) 220–356 by 31–53 ($\bar{x} = 296 \pm 37.6$ by 40 ± 5.1), elongated and slightly sinuous, may extend to midline or beyond in mature segments, distal portion nearly touching anterior margin of segment. Cirrus may be extended, diameter (5 worms, $N = 50$) 21–31 ($\bar{x} = 25.4 \pm 2.1$). Self copulation common. Genital atrium prominent, deeply recessed, in anterior ¼ to ⅓ of segment, alternating irregularly. Vagina posterior to cirrus sac, widening almost immediately into conspicuous elongate seminal receptacle (Fig. 17). Ovary (3 worms, $N = 20$) 75–246 by 150–348 ($\bar{x} = 161 \pm 44.4$ by 250 ± 63.6), bilobed, medullary, medial. Vitellarium (5 worms, $N = 50$) 53–154 by 53–167 ($\bar{x} = 101 \pm 22.5$ by 105 ± 27.2), round to lobate, posterior to isthmus and medial to lobes of ovary (Fig. 16). Uterus saccular (Fig. 15). Eggs not fully developed.

TYPE HOST: Serpent eagle, *Spilornis cheela* (Falconiformes).

SITE OF INFECTION: Small intestine.

TYPE LOCALITY: Puerto Princella, Palawan Island, Philippines.

ETYMOLOGY: Patronymic after Dr. Richard Heckmann, Professor of Zoology, Brigham Young University.

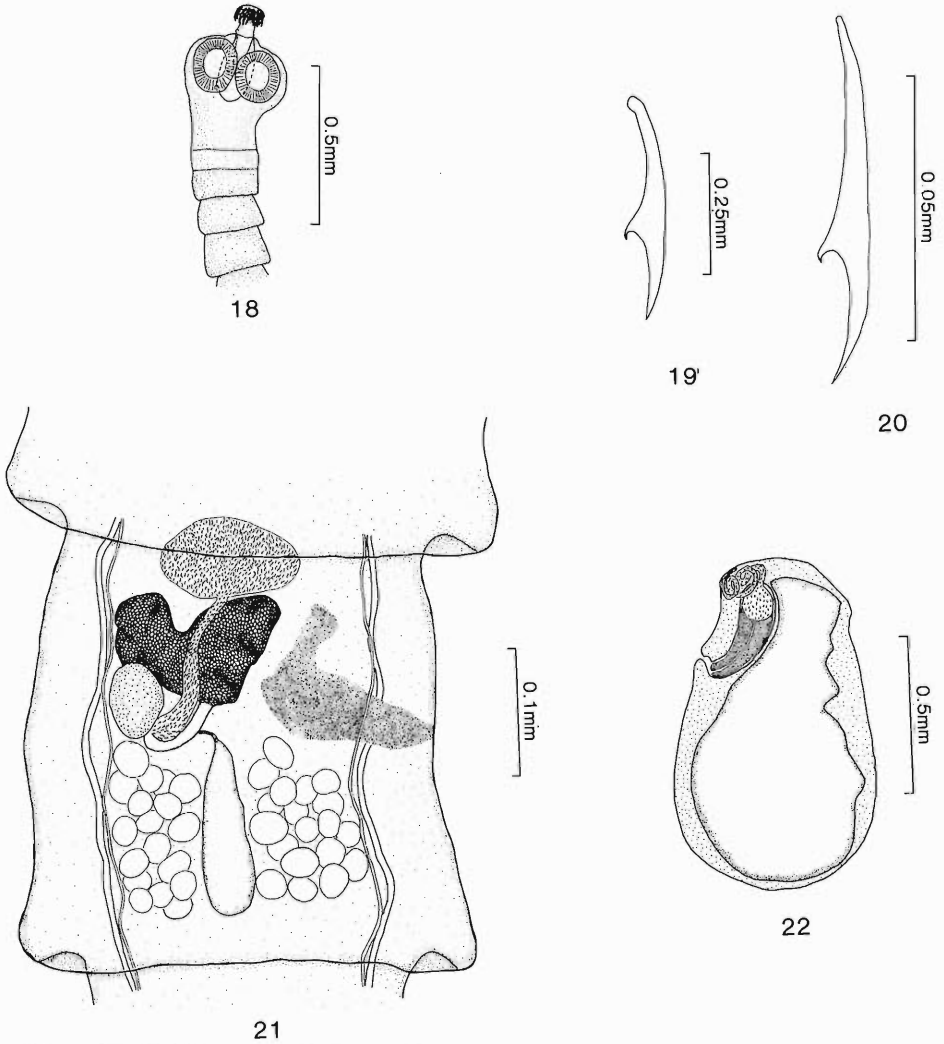
TYPE SPECIMENS: Holotype USNM Helm. Coll. No. 77169; paratypes USNM Helm. Coll. No. 77170.

REMARKS: *Parvitaenia heckmanni* sp. n. differs dramatically from the known species of *Parvitaenia* in the number of hooks (32 vs. 20). Indeed, a crown of 32 large and small alternating hooks is inconsistent with the usual number of 20 (Bona, 1975), and the presence of *Parvitaenia* sp. in an eagle is unusual. Consequently, this peculiar dilepidid may deserve new generic status. On the other hand, the shape of the hooks and genital arrangement are characters typical of the genus. Therefore, until more specimens become available, an allocation to the genus *Parvitaenia* seems preferable.

Paraprogynotaenia charadrii (Yamaguti, 1956) comb. n. (Figs. 18–22)

From April 6, 1960 to October 12, 1961, numerous specimens of Progynotaeniidae were obtained from the small intestines of two species of plovers, *Charadrius alexandrinus* Linnaeus, 1758 from Sha-kang, and Ma-kung, Peng-hu Island, and Ping-tung, and *C. dominicus* Muller, 1776 from Ma-kung, Peng-hu Island, Taiwan. The species is identical to the original description of *Proteogynotaenia charadrii* Yamaguti, 1956 in *C. alexandrinus* from Japan. However, a taxonomic realignment now appears desirable, because the single circle of hooks is consistent with the genus *Paraprogynotaenia* Rysavy, 1966 and not *Proteogynotaenia* Fuhrmann, 1911.

DESCRIPTION: Worms ($N = 20$) 3.3–6.3 mm by 0.34–0.68 mm ($\bar{x} = 5 \pm 1.0$ by 0.47 ± 0.1). Total number of segments ($N = 23$) 16–29 ($\bar{x} = 22.7 \pm 3.3$). Metamerism distinct, craspedote, strobila fragments easily, protogynous. Scolex ($N = 86$) 0.19–0.37 mm ($\bar{x} = 0.27 \pm 0.05$) wide, armed with single circle of 16 hooks (Fig. 18). Hooks cauducous, handle longer than blade, with prominent guard (Figs. 19, 20). Two hooks that are positioned laterally are significantly shorter than other hooks (Fig. 19). Large hooks (17 worms, $N = 55$) 62–76 ($\bar{x} = 70.2 \pm 4$). Lateral small hooks (7 worms, $N = 9$) 40–57 ($\bar{x} = 49.8 \pm 6.5$). Rostellum ($N = 30$) 79–246 by 62–119 ($\bar{x} = 155.6 \pm 55.4$ by 80.3 ± 16.8), bilobed. Rostellar sac ($N = 46$) 125–237 by 66–127 ($\bar{x} = 186.7 \pm 28.8$ by 94.1 ± 12.2), extending to level



Figures 18–22. ***Paraprogynotaenia charadrii*** **comb. n. of plovers from Taiwan. 18. Scolex, dorsal view. 19. Small hook. 20. Large hook. 21. Mature segment, dorsal view. 22. Gravid terminal segment, dorsal view.**

of posterior margin of sucker or just beyond (Fig. 18). Suckers (55 worms, N = 100) 92–202 by 75–172 ($\bar{x}$ = 145.7 ± 23.8 by 120.7 ± 21.5), oval. Neck short. Mature segments campanulate, wider than long to slightly longer than wide (Fig. 21). Terminal gravid segments (N = 11) 510–996 by 227–595 ($\bar{x}$ = 736.5 ± 132.1 by 447.5 ± 109.1), longer than wide (Fig. 22). Testes (10 worms, N = 100) 35–92 by 31–79 ($\bar{x}$ = 56.6 ± 11.3 by 48.3 ± 9.5), round to oval, medullary, proximal to osmoregulatory canals, in 2 groups, lateral to uterus, generally more numerous on aporal side. Number of testes per segment (7 worms, 10 segments) 33–42 ($\bar{x}$ = 38 ± 2.9). Vas deferens convoluted, anterior to cirrus sac. Internal seminal vesicle (9 worms, N = 9) 88–189 by 84–128 ($\bar{x}$ = 140.7 ± 39.5 by 106.1 ± 15.3), oval, in proximal portion of cirrus sac. Cirrus 31–41 wide, armed. Cirrus sac club-

shaped, varying greatly in size, passing between osmoregulatory canals. Prominent genital atrium may protrude in mature and gravid segments, irregularly alternating. No vagina. Seminal receptacle (20 worms, $N = 26$) 101–229 by 97–202 ($\bar{x} = 164.4 \pm 23.3$ by 135.2 ± 29.6), round to oval, anterior to ovary. Accessory duct filled with sperm, extends posteriad and joins oviduct near vitellarium (Fig. 21). Ovary (11 worms, $N = 13$) 92–185 by 48–114 ($\bar{x} = 134.4 \pm 25.3$ by 84.7 ± 21.9), lobate, antiporal. Vitellarium (10 worms, $N = 13$) 44–92 by 37–66 ($\bar{x} = 63.7 \pm 14.5$ by 50.3 ± 8.6), oval, mainly posterior to ovary, usually aporal. Uterus saccular. Eggs ($N = 30$) 44–66 by 35–64 ($\bar{x} = 56.1 \pm 5.7$ by 48.1 ± 6.3), round to oval. Onchosphere ($N = 30$) 24–46 by 22–34 ($\bar{x} = 33.4 \pm 4.6$ by 29.8 ± 3.2), round to oval.

DEFINITIVE HOSTS: Eastern kentish plover, *Charadrius alexandrinus,* and eastern golden plover, *C. dominicus* (Charadriiformes).

SITE OF INFECTION: Small intestine.

LOCALITY: Taiwan, Republic of China.

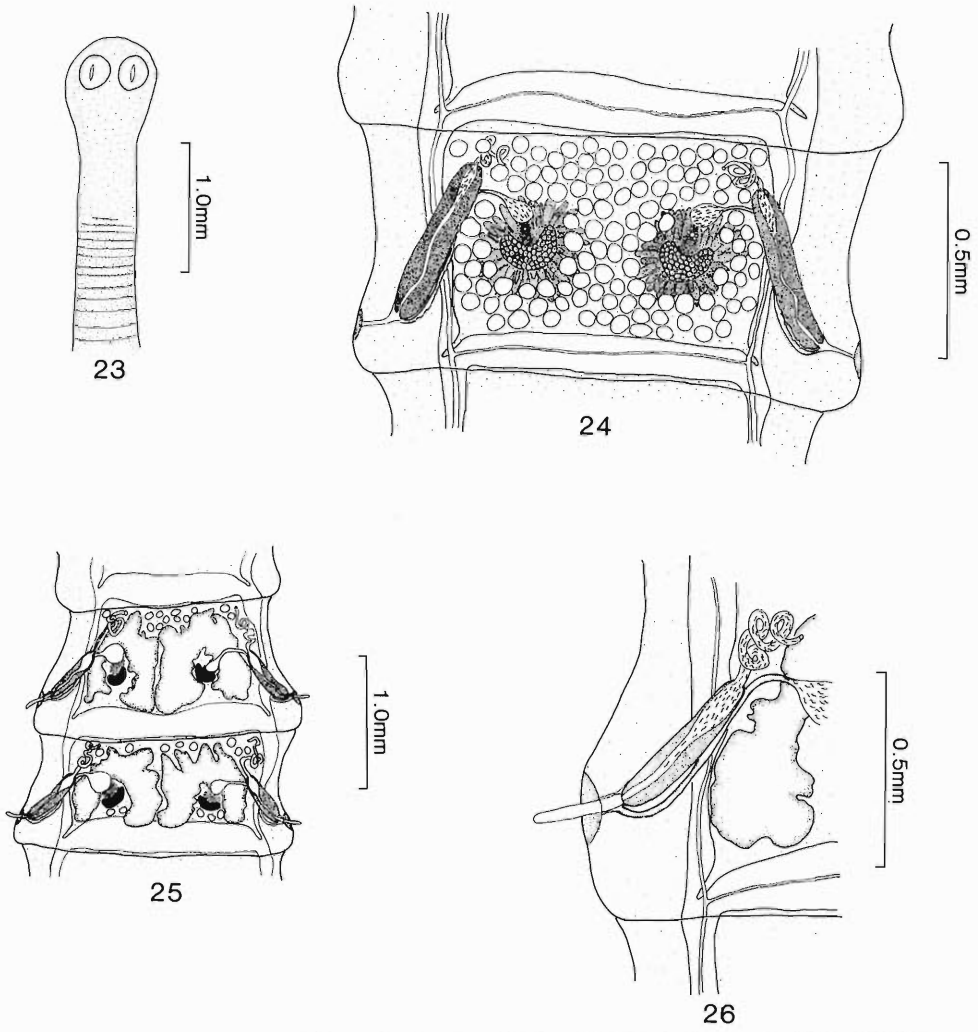
REPRESENTATIVE SPECIMENS: USNM Helm. Coll. Nos. 69404 and 69405.

REMARKS: The single circle of hooks, irregularly alternating genital atria, and testes in two lateral fields are characteristics of the genus *Paraprogynotaenia* Rysavy, 1966. Yamaguti (1956) referred this cestode to the genus *Proteogynotaenia* even though the crown of hooks was uncharacteristic for that genus. *Paraprogynotaenia* was established for progynotaeniids with a single circle of hooks, irregularly alternating genital pores, testes in two lateral fields, and the cirrus sac passing between the osmoregulatory canals (Rysavy, 1966). Therefore, this cestode is appropriately placed in *Paraprogynotaenia*.

Paronia sp.
(Figs. 23–26)

A single worm was collected from the Chinese white-breasted water hen, *Amaurornis phoenicurus* (Pennant, 1769). The bird was trapped March 21, 1958 at the edge of a sugar cane field near Ping-tung, Taiwan. The cestode is similar to *Paronia pycnonoti* Yamaguti, 1935. However, the hosts are in different orders, and conspecificity of the worms must therefore be questioned.

DESCRIPTION: Worm ($N = 1$) 50 mm by 2.10 mm, 101 segments. Metamerism distinct, craspedote, dual set of reproductive organs per segment. Genital ducts passing dorsal to osmoregulatory canals. Scolex ($N = 1$) 0.65 mm by 0.79 mm, with 4 suckers ($N = 4$), 220–250 by 220–240 ($\bar{x} = 240$ by 230), round to oval, unarmed (Fig. 23). Neck ($N = 1$) 0.59 mm by 0.51 mm. Osmoregulatory canals slightly sinuous, both dorsal and ventral canals with distinct lateral branching (Fig. 24). Ventral canals with characteristic flaps at junction of transverse anastomoses (Figs. 24, 26), transverse anatomoses dilate to 306 wide in gravid segments. Immature segments wider than long. Mature segments ($N = 6$) 1.10–1.70 mm by 0.65–0.82 mm, wider than long (Fig. 24). Gravid segments ($N = 18$) 1.70–2.12 mm by 0.80–1.22 mm ($\bar{x} = 1.78 \pm 0.16$ by 0.93 ± 0.11), wider than long (Fig. 25). Testes ($N = 100$) 48–101 by 35–84 ($\bar{x} = 73.6 \pm 10.9$ by 62.2 ± 9.4), oval, medullary, in single, confluent layer, proximal to osmoregulatory canals, dorsal to ovary. Number of testes per segment (13 segments) 104–140 ($\bar{x} = 125.6 \pm 11.9$). Vas deferens convoluted, anterior to cirrus sac. Internal seminal vesicle ($N = 20$) 84–289 by 33–62 ($\bar{x} = 155 \pm 47.8$ by 46.5 ± 7.1), at base of cirrus sac.



Figures 23–26. ***Paronia*** **sp. of the water hen from Taiwan. 23. Scolex, dorsoventral view. 24. Mature segment, dorsal view. 25. Gravid segment, dorsal view. 26. Terminal genital ducts, dorsal view.**

Ejaculatory duct serpentine. Cirrus (N = 20) 26–44 ($\bar{x}$ = 34.7 ± 4.5) wide, unarmed, frequently protruding. Cirrus sac (N = 30) 299–510 by 66–97 ($\bar{x}$ = 411.2 ± 51.3 by 80.3 ± 9), oval, obliquely and anteriorly situated, extending beyond osmoregulatory canals. Genital atrium deeply recessed, in posterior ⅓ to ¼ of segment. Vagina initially posterior to cirrus sac, extending beneath cirrus sac at level of ventral osmoregulatory canals, eventually dilating into seminal receptacle. Seminal receptacle (N = 36) 84–210 by 75–177 ($\bar{x}$ = 146.7 ± 35.9 by 115.4 ± 24.3), oval to pyriform, dorsal to ovary. Ovary (N = 13) 391–573 by 340–510 ($\bar{x}$ = 505.1 ± 65.3 by 412.6 ± 60.4), rosette-shaped, medullary. Vitellarium (N = 30) 154–227 by 62–172 ($\bar{x}$ = 192.6 ± 23 by 121.4 ± 24.5), directly dorsal to ovary, bending around Mehlis' gland, concavity directed anteriolaterally. Uterus horseshoe- or stirrup-shaped, with numerous diverticula. Eggs hardly discernible.

DEFINITIVE HOST: Chinese white-breasted water hen, *Amaurornis phoenicurus* (Gruiformes).

SITE OF INFECTION: Small intestine.

LOCALITY: Taiwan, Republic of China.

REPRESENTATIVE SPECIMEN: USNM Helm. Coll. No. 77185.

REMARKS: This species of *Paronia* Diamare, 1900 does not differ significantly from *P. pycnonoti,* also from Taiwan (Yamaguti, 1935). Nevertheless, a complete identification is reserved until more specimens become available. This is the first report of *Paronia* sp. in the Chinese white-breasted water hen and the order Gruiformes. The host specificity of *P. pycnonoti* needs further investigation.

Acknowledgments

Success in collection of materials is attributed to the faithful support of the technical staff of NAMRU No. 2 and to Captain Robert A. Phillips, MC USN (deceased), Commanding Officer, who recognized the importance of basic biomedical endeavors. Thanks are due to Dr. D. R. R. Burt of Gatty Marine Laboratory in Great Britain for his invaluable correspondence regarding some of the avian cestodes. We are also grateful to Dr. John S. Mackiewicz of State University of New York for his assistance regarding the caryophyllidean genus *Lytocestus*.

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Vampirolepis schmidti sp. n. (Cestoidea: Hymenolepididae) from *Triaenops persicus* (Hipposideridae) of Tanzania

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ABSTRACT: *Vampirolepis schmidti* sp. n. is described from the triple leaf-nose bat, *Triaenops persicus*, of Tanzania. The number, length, and characteristic shape of the rostellar hooks distinguish this cestode from all other species in the genus. The tapeworm was observed in 10.1% of 109 bats.

Specimens of Cestoidea representing a species of *Vampirolepis* Spassky, 1954 were found in the triple leaf-nosed bat, *Triaenops persicus* Dobson, 1871, of Tanzania. To date there have been no published accounts of *Vampirolepis* in *T. persicus*; nevertheless, members of this genus have been reported in other species of African bats. Baer (1933) described *V. sandgroundi* (Baer, 1933) from *Pipistrellus nanus* of Zimbabwe, Mahon (1954) described *V. aelleni* Mahon, 1954 from *Epomophorus wahlbergi* of Zaire, and Edungbola (1981) reported *V. kerivoulae* (Hübscher, 1937) from *Hipposideros caffer* and *Nycteris gambiensis* of Nigeria.

From May 1975 to October 1981, 109 bats were collected from an abandoned kaolin mine in Kisarawe District, Tanzania, and were examined for tapeworms. The worms were relaxed in tap water, fixed in 10% formalin, cleared in xylene, and mounted in Canada balsam. Measurements are given as the range, with the mean in parentheses, and are in micrometers unless otherwise stated.

Vampirolepis schmidti sp. n.
(Figs. 1–4)

DESCRIPTION: Worms (N = 5) 15–33 mm by 1.3–2.0 mm ($\bar{x}$ = 25.6 by 1.6). Metamerism distinct, craspedote, margins slightly serrate, genital ducts dorsal to osmoregulatory canals. Scolex (N = 6) 238–286 ($\bar{x}$ = 260) in width, not distinctly set off from neck (Fig. 1), armed with single circle of 16 hooks. Hooks (8 worms, N = 50) 16–21 ($\bar{x}$ = 19), handle, guard, and blade approximately equal in length (Fig. 2), or guard may be slightly shorter than handle and blade. Rostellar sac (N = 8) 112–151 by 100–125 ($\bar{x}$ = 138 by 114), muscular, pyriform, extending posteriad to suckers. Rostellum (N = 7) 51–68 by 49–69 ($\bar{x}$ = 58 by 60), retractable. Suckers (6 worms, N = 10) 70–100 by 75–91 ($\bar{x}$ = 85 by 82), muscular. Neck (N = 8) 70–388 ($\bar{x}$ = 226). Well-developed longitudinal muscles. Mature segments (6 worms, N = 60) 0.1–0.2 mm by 0.7–1.1 mm ($\bar{x}$ = 0.1 by 0.9), markedly wider than long (Fig. 3). Gravid segment (8 worms, N = 8) 0.2–0.5 mm by 1.0–1.8 mm ($\bar{x}$ = 0.3 by 1.4), markedly wider than long (Fig. 4). Dorsal and ventral osmoregulatory canals sinuous. Dorsal canal situated directly above ventral canal. Testes (3 worms, N = 30) 75–133 by 62–99 ($\bar{x}$ = 107 by 80), 3 per segment, round to oval, medullary, dorsal, forming transverse row in posterior half of segment. External seminal vesicle elongate, directly dorsal to seminal receptacle, situated in anterior half of segment. Internal seminal vesicle gradually enlarges until it fills proximal portion of cirrus sac. Cirrus sac (3 worms, N = 25) 148–211 by 23–39 ($\bar{x}$ = 170 by 30), positioned anteromediad from genital atrium, extending

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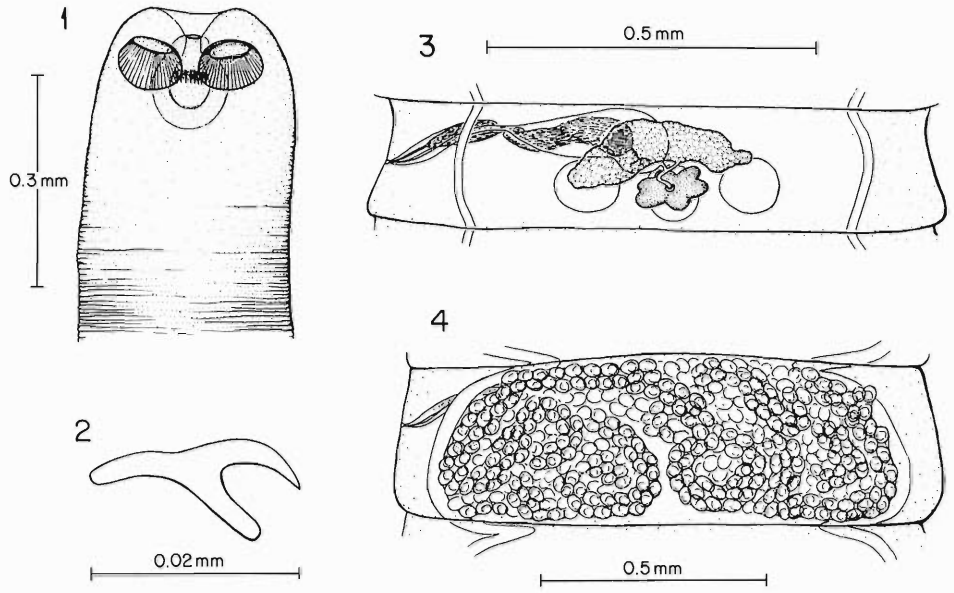

Figures 1–4. ***Vampirolepis schmidti*** **sp. n. from** ***Triaenops persicus*****. 1. Scolex, dorsoventral view. 2. Rostellar hook. 3. Mature segment, ventral view. 4. Gravid segment, ventral view.**

beyond osmoregulatory canals. Genital atria unilateral, situated at midline or in anterior half of segment, do not protrude. Vagina initially posterior to cirrus sac, passes beneath cirrus sac just prior to crossing osmoregulatory canals, gradually expands into voluminous seminal receptacle. Ovary (5 worms, $N = 45$) 143–439 by 20–112 ($\bar{x} = 266$ by 55), medial, ventral to testes, transversely elongate, frequently irregularly lobate, at midline or in anterior half of segment. Vitellarium (5 worms, $N = 50$) 78–166 by 23–122 ($\bar{x} = 111$ by 48), lobate, medial, posterior to ovary. Uterus saccular, occupies area between osmoregulatory canals in gravid segment. Eggs (6 worms, $N = 60$) 30–49 by 26–42 ($\bar{x} = 41$ by 33), oval. Onchosphere (4 worms, $N = 40$) 27–36 by 24–32 ($\bar{x} = 31$ by 27), round to oval. Length of embryonic hooks (4 worms, 31 eggs, $N = 31$) 13–18 ($\bar{x} = 14$).

DEFINITIVE HOST: *Triaenops persicus,* the triple leaf-nosed bat.

LOCATION: Small intestine.

LOCALITY: Kisarawe District, Tanzania.

PREVALENCE: Found in 10.1% of 109 bats examined from May 1975 to October 1981.

WORM BURDEN: One to two per host.

ETYMOLOGY: Patronymic after Dr. Gerald D. Schmidt, Parasitologist and Professor of Zoology, University of Northern Colorado.

TYPE SPECIMENS: Holotype USNM Helm. Coll. No. 77087; paratypes USNM Helm. Coll. No. 77088; paratypes British Museum (Natural History) Nos. 1982.4.16.1–2.

REMARKS: *Vampirolepis schmidti* sp. n. most resembles *V. hipposideri* (Prudhoe and Manger, 1969) comb. n. and *V. sandgroundi* in the number of rostellar hooks and the size of the eggs, and *V. bidentalus* Zdzitowiecki and Rutkowska,

Table 1. A comparison of the species of *Vampirolepis* Spassky, 1954 from bats in Africa.

Cestode	Size of strobila (mm)	No. of rostellar hooks	Length of rostellar hooks	Arrangement of testes	Host	Locality
V. aelleni	17 by 0.8	40	26–30	Triangular to longitudinal row	*Epomophorus wahlbergi*	Zaire
V. kerivoulae	20–25 by 0.32	20–22	22–23	Triangular	*Hipposideros caffer, Nycteris gambiensis*	Nigeria
V. sandgroundi	15 by 0.72	16–18	24	Triangular	*Pipistrellus nanus*	Zimbabwe
V. schmidti sp. n.	15–33 by 1.3–2	16	16–21	Longitudinal row	*Triaenops persicus*	Tanzania

1980 in the length of the rostellar hooks. The rostellar hooks of *V. schmidti*, however, are characteristically different, because the handle and blade are approximately equal in length. *Vampirolepis schmidti* can also be separated from *V. bidentalus* in that it possesses fewer rostellar hooks (16 vs. 18–22), a longer strobila (15–33 mm vs. 5–10 mm), and larger eggs (30–49 by 26–42 vs. 26–36 in diameter). It differs from *V. hipposideri* and *V. sandgroundi* in the length of the rostellar hooks (16–21 vs. 22–24, and 24) and in the arrangement of the testes (transverse row vs. triangular distribution). A comparison of some important taxonomic features for the described species of *Vampirolepis* in African bats is given in Table 1.

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Redescription of the Piscicolid Leech *Trulliobdella capitis* Brinkmann

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ABSTRACT: The Antarctic leech *Trulliobdella capitis* Brinkmann, 1947 is redescribed based on recently collected specimens and subsequent re-examination of the type material. The leech possesses three pairs of tentacles on the oral sucker, up to five pairs of eyes on both oral sucker and nuchal region, up to 14 ocelli on the caudal sucker, 11 pairs of pulsatile vesicles, esophageal diverticula, six pairs of cleft crop ceca, postceca with four pairs of cleft lateral ceca, and five pairs of testisacs.

The genus *Trulliobdella* Brinkmann, 1947 was established for a leech infesting *Parachaenichthys georgianus* (Fisher) and *Chaenocephalus aceratus* (Lönnberg) (as *Chaenocephalus bouvetensis* Nybelin), from South Georgia and Bouvet islands, respectively. This leech, *Trulliobdella capitis* Brinkmann, 1947, was taken by the Norwegian Expeditions to the southern oceans, 1927–1928, but was not studied until 20 yr later (Brinkmann, 1947, 1948).

A study of relatively fresh, mature and immature, engorged and unfed specimens shows that affinities of the genus have been obscured by the omission of certain anatomic characteristics from the original description, undoubtedly due to the poor condition of the 20-yr-old specimens. The present paper, based on the new material and a re-examination of Brinkmann's type material, attempts to clarify the anatomy of this leech and emends accordingly the description of *Trulliobdella*.

Material Examined

Syntypes, Zoologisk Museum, Universitetet, Bergen, Norway, No. 41817, 8 specimens in Bouin's, 2 whole mounts, 1 complete set each sagittal, frontal, and transverse sections. The following material was received from the Smithsonian Oceanographic Sorting Center: *Islas Orcadas* cruise 575, May 1975, Sta. 55, 57°47.2′S, 26°22.5′W, 64–88 m, 2 mounted specimens; Sta. 56, 57°47.2′S, 26°22.2′W, 90 m, 84 specimens, 10 mounted; Sta. 57, 57°43.9′S, 26°24.1′W, 37–55 m, 1 mounted specimen. *Hero* cruise 702, March 1970, Sta. 459, 62°58.3′S, 60°47.2′W, 110–165 m, 8 specimens, 3 mounted; Sta. 509, 64°49.9′S, 63°33.0′W, 46 m, 2 specimens. *Eltanin* cruise 6, January 1963, Sta. 435, 63°14′S, 58°40′W, 73 m, 4 specimens, 1 mounted, from chaenichthyid; Sta. 437, 62°50′S, 60°35′W, 267–311 m, 5 specimens, 1 mounted, from chaenichthyid; Sta. 445, 62°02′S, 59°05′W, 101 m, 5 specimens, 1 mounted. *Eltanin* cruise 9, August 1963, Sta. 671, 54°41′S, 38°31′W, 220–320 m, 5 specimens, 2 mounted, 1 sectioned. Eight specimens (4 mounted) collected at South Georgia from *Chaenocephalus aceratus* on 2 April 1978 were received from V. Siegel, Institut für Seefischerei, Hamburg, Germany. Twenty-seven specimens from *Champsocephalus gunneri* Lönnberg: *Islas Orcadas* cruise 876, 16 February 1976, Sta. UMO 108, 60°25.9′S, 46°23.6′W to 60°25.4′S, 46°24.4′W; and 26 specimens from *Chaenocephalus aceratus*: *Islas Orcadas* cruise 876, 20 February 1976, Sta. UMO 117, 62°11.3′S, 42°43.3′W to

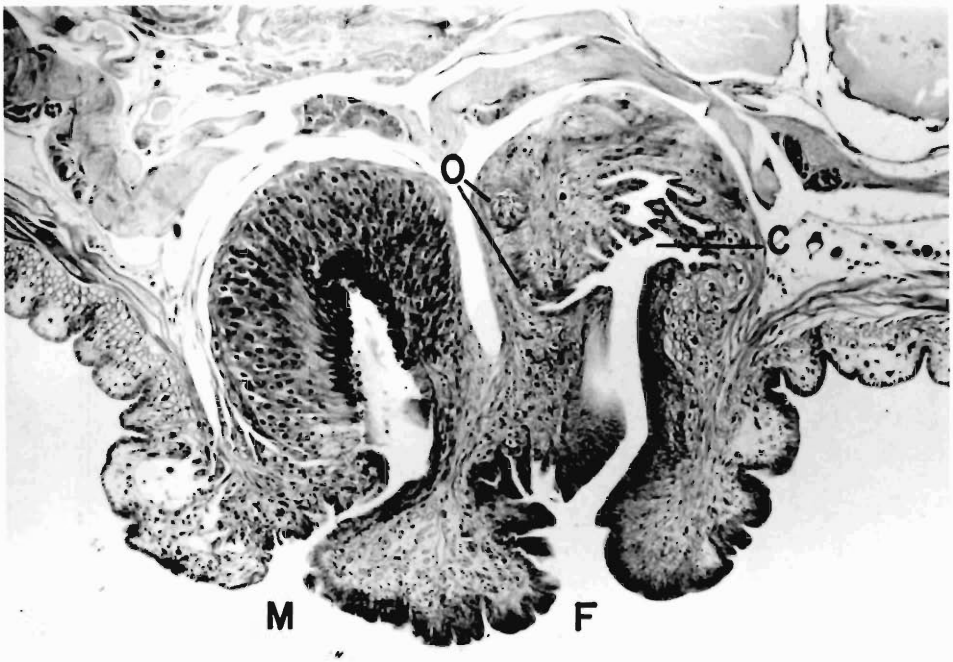


Figure 1. ***Trulliobdella capitis,*** **sagittal section through terminal genitalia. C, invaginated pouch; F, female gonopore; M, male gonopore; O, common oviduct.**

62°11.0′S, 42°43.9′W were gifts of Bruce Daniels. One whole mount and one complete series each of frontal, sagittal, and transverse sections were made from 4 specimens from *C. aceratus*.

Descriptive measurements include the mean followed by the range in parentheses. Length measurements include both suckers, are based on 10 or more readings, and are in millimeters unless otherwise indicated.

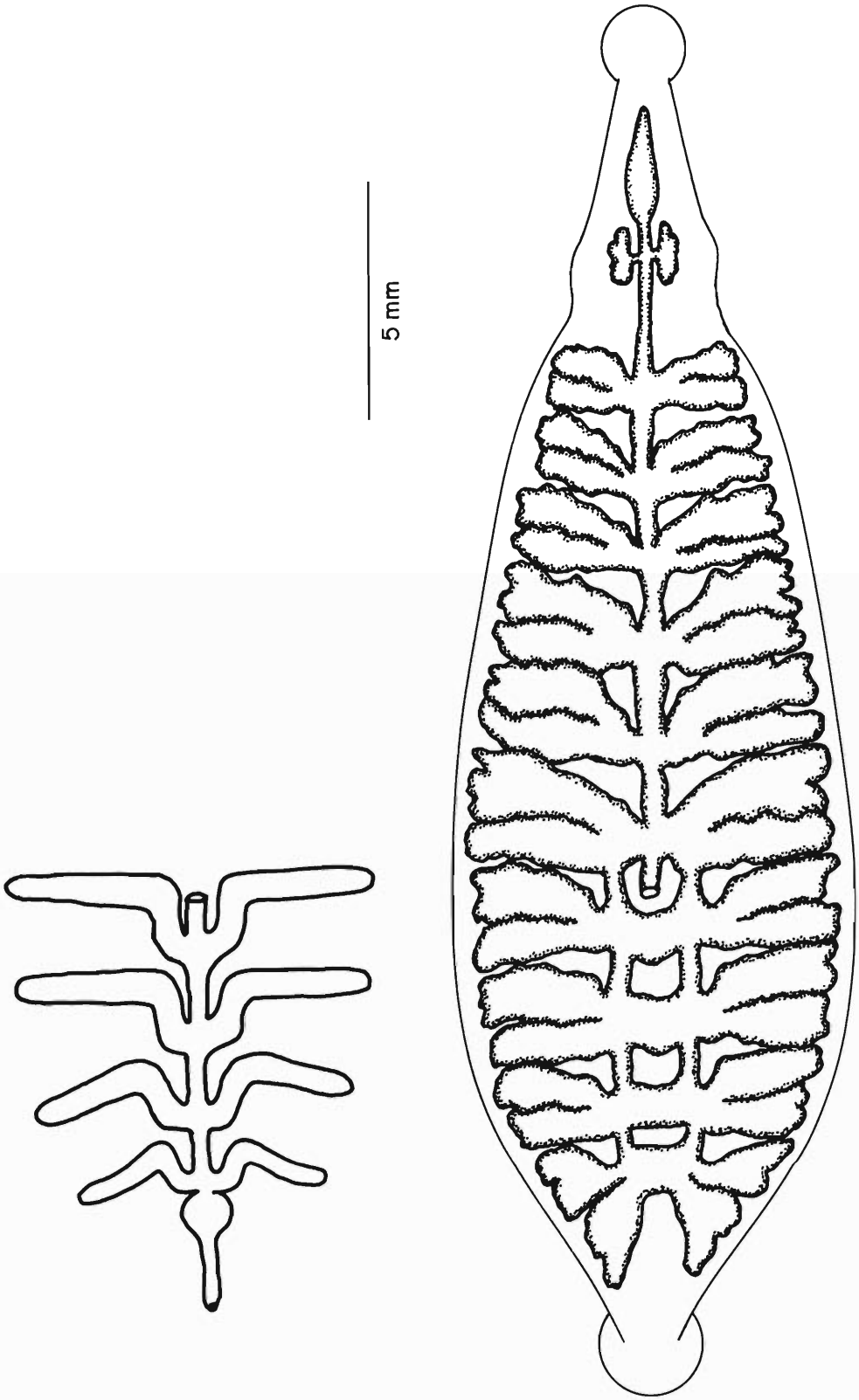
Trulliobdella Brinkmann, 1947

DIAGNOSIS: Body dorsoventrally flattened, with short, subcylindrical trachelosome; urosome longer and wider than trachelosome; body surface smooth, without tubercles, papillae, or gills; pulsatile vesicles present; tentacles and eyes on oral sucker, eyes on nuchal region; complete segments 3-annulate, each annulus further but less distinctly divided; metameric ocelli on a2, paired dorsally and ventrally; ocelli on posterior sucker; esophageal ceca and postceca present; common oviduct opens into large invaginated pouch; testisacs 5 pairs.

TYPE AND ONLY SPECIES: *Trulliobdella capitis* Brinkmann, 1947.

Trulliobdella capitis Brinkmann, 1947 (Figs. 1, 2)

DIAGNOSIS: Size up to 40 long and 15.1 wide; oral sucker with 3 pairs of tentacles and up to 5 pairs of eyes; first 2 nuchal annuli with up to 5 pairs of eyes; urosome with 11 pairs of pulsatile vesicles and up to 10 pairs of metameric



ocelli; caudal sucker with up to 14 ocelli; crop with 6 pairs of cleft lateral ceca; postceca fused, with 4 pairs of cleft lateral ceca.

EXTERNAL FEATURES: Body usually divided into short, subcylindrical trachelosome and much longer and wider, dorsoventrally flattened urosome, resembling Glossiphoniidae, 28.4 (11.9–40.0) long × 10.0 (3.4–15.1) wide. Immature and some unfed specimens not sharply divided into trachelosome and urosome. Oral sucker 2.2 (1.0–3.1) in diameter, eccentrically attached, with 3 pairs of wart-shaped elevations or small "tentacles" and up to 5 pairs of crescent-shaped eyes. Concavities of eyes facing posterolaterally. First 2 nuchal annuli of trachelosome with up to 5 pairs of eyes, concavities facing anterolaterally. Four eyes not uncommon in either region, on one or both sides, occasionally 3 or 6 eyes. Urosome margins subparallel, but converging posteriorly. Body surface smooth, lacking tubercles, papillae, or gills. Complete segments 3-annulate with each primary annulus further, but less distinctly, divided. Segmental ocelli present, paired dorsally and ventrally; mature specimens possess up to 10 pairs, immature specimens may possess 11 to 12 pairs. Male and female gonopores large and conspicuous, separated by 2 annuli. Anus 2 annuli anterior to centrally attached caudal sucker, 2.8 (1.0–3.5) in diameter. Caudal sucker with up to 4 marginal ocelli.

REPRODUCTIVE SYSTEM: Testisacs 5 pairs, at XIV through XVII, alternating with crop ceca. Vasa deferentia pass forward to about IV, where they widen and become coiled. Anterior limbs widen further, forming seminal vesicles, situated dorsal and lateral to atrial cornu. Seminal vesicles continuing anteriorly to X, then bending ventromedially caudad and entering apex of atrial cornu. Cornua, in XI, large, bulbous, muscular, and unite to form male atrium. Female gonopore, in XII, actually opening to large invaginated pouch (Fig. 1). Common oviduct opens into anterior margin of pouch. Oviduct bending dorsally from pouch opening and bifurcating into 2 ovisacs that bend posterolaterally and extend, dorsal to pouch, to ganglion in XIV. Ovisacs become convoluted, commonly winding anteriorly to level of female gonopore.

DIGESTIVE SYSTEM: Mouth central within small raised area in oral sucker. Esophageal ceca emerging from esophagus in anterior portion of XI and extending anteriorly into X. Crop with 6 pairs of deeply cleft lateral ceca (Fig. 2), branching into postceca and intestine at XIX. Postceca fused, with 4 pairs of cleft lateral ceca; intestine with 4 pairs of simple ceca, decreasing in size posteriorly. In mature specimens first 2 pairs of intestinal ceca directed anterolaterally, then bending sharply laterally; second 2 pairs either may be directed anterolaterally, then bending laterally or posterolaterally, or may be directed only laterally. In immature specimens all intestinal ceca are directed only laterally. Posterior to last cecum, intestine opens into so-called "folded-organ," followed by large rectum.

KNOWN HOSTS: Syntypes from *Parachaenichthys georgianus* and *Chaenocephalus aceratus* (as *C. bouvetensis*). Other hosts include *Pseudochaenichthys georgianus* Norman, *Champsocephalus gunnari,* and *Chionodraco* sp.

←

Figure 2. ***Trulliobdella capitis,*** **digestive system, with intestine displaced laterally to show postceca and fenestrae, dorsal view.**

KNOWN LOCALITIES: Syntypes from South Georgia and Bouvet islands. Other localities include South Sandwich Islands, South Orkney Islands, and South Shetland Islands.

Discussion

Trulliobdella capitis was described from a series of specimens that had been fixed unrelaxed and preserved for 20 yr. Leeches often suffer some deformation in the course of haphazard fixation and long storage, so that many anatomical structures may not be discernible. This is especially true of empty, collapsed alimentary tract ceca, which may be difficult or impossible to detect even in serial sections. In addition, eyes and ocelli are often not discernible, even in recently preserved specimens after clearing, let alone in 20-yr-old specimens. The present redescription elucidates structures omitted from the original description for reasons mentioned above, and more clearly defines the range of variations, thus making the species easier to identify.

The invaginated pouch of the female reproductive system in *T. capitis* is remarkably similar to the so-called seminal receptacle in *Mysidobdella borealis* described and figured by Burreson and Allen (1978). In *M. borealis* the ovisacs, after passing dorsal to the large, invaginated "seminal receptacle," bend ventrally and fuse into a common oviduct that opens through the ventral body wall as the true female gonopore anterior to the invaginated pouch opening. In *T. capitis*, however, the common oviduct opens into the anterior face of the invaginated pouch and the pouch opening becomes the female gonopore.

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Ecology of Helminth Parasitism of Mourning Doves in Florida[1]

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ABSTRACT: Between 1973 and 1978, examination of 455 mourning doves (*Zenaida macroura*) from eight localities in Florida revealed 14 species of helminths, including seven nematodes, two trematodes, and five cestodes. The number of species per infected host varied from one to five (mean 1.5), with 61 doves free of helminths. The total number of helminths per infected bird ranged from one to 163 (mean 14.0). Four of the 14 species of helminths were considered as major components of the helminth fauna, and included *Ornithostrongylus* spp., *Dispharynx nasuta*, *Ascaridia columbae*, and *Aproctella stoddardi*. *Ascaridia columbae* occurred at higher intensities in adult doves than in juvenile doves. No other parasites showed sex or age effects. *Ornithostrongylus*, *Dispharynx*, and *Aproctella* exhibited peaks in prevalence and intensity in the spring and summer months, whereas *Ascaridia* showed higher prevalences in the winter months and a relatively stable intensity during the rest of the year. *Ornithostrongylus* spp. made up the largest proportion of helminth parasitism (72%) and were common in all localities, especially in southern Florida. Simpson's index was moderately low (0.34), which indicated a fairly diverse fauna statewide; however, doves from southern Florida had less diverse faunas than doves from northern Florida. High indices of similarity existed in the helminth faunas of the doves within the state. The helminth fauna of doves in Florida was compared to those of doves in nine other southeastern states and found to be most similar to those from Alabama and Mississippi.

In 1973 a study was initiated to gather information on the parasites of mourning doves (*Zenaida macroura*) in Florida. Reports on the interrelationships of parasites of mourning doves and white-winged doves (*Z. asiatica*) and on the haematozoan parasites of mourning doves have been published (Shamis and Forrester, 1977; Conti and Forrester, 1981). The present paper is concerned with the prevalence and intensity of helminth parasitism of mourning doves in Florida and certain ecological aspects relating to the distribution of these parasites.

Materials and Methods

A total of 455 doves was collected from eight counties in Florida (Fig. 1) between October 1973 and August 1978. The birds were live-trapped in modified Stoddard box traps or were killed with shotguns. Ages were determined by analysis of feather development (Swank, 1955). The mourning dove population in Florida is composed of migratory and nonmigratory components. A recent study (Marion et al., 1981) showed that 57% of the doves harvested during the fall and winter in Florida originated from out of state, whereas 99% of the doves banded in Florida during the summer remained within the state. From that study it can

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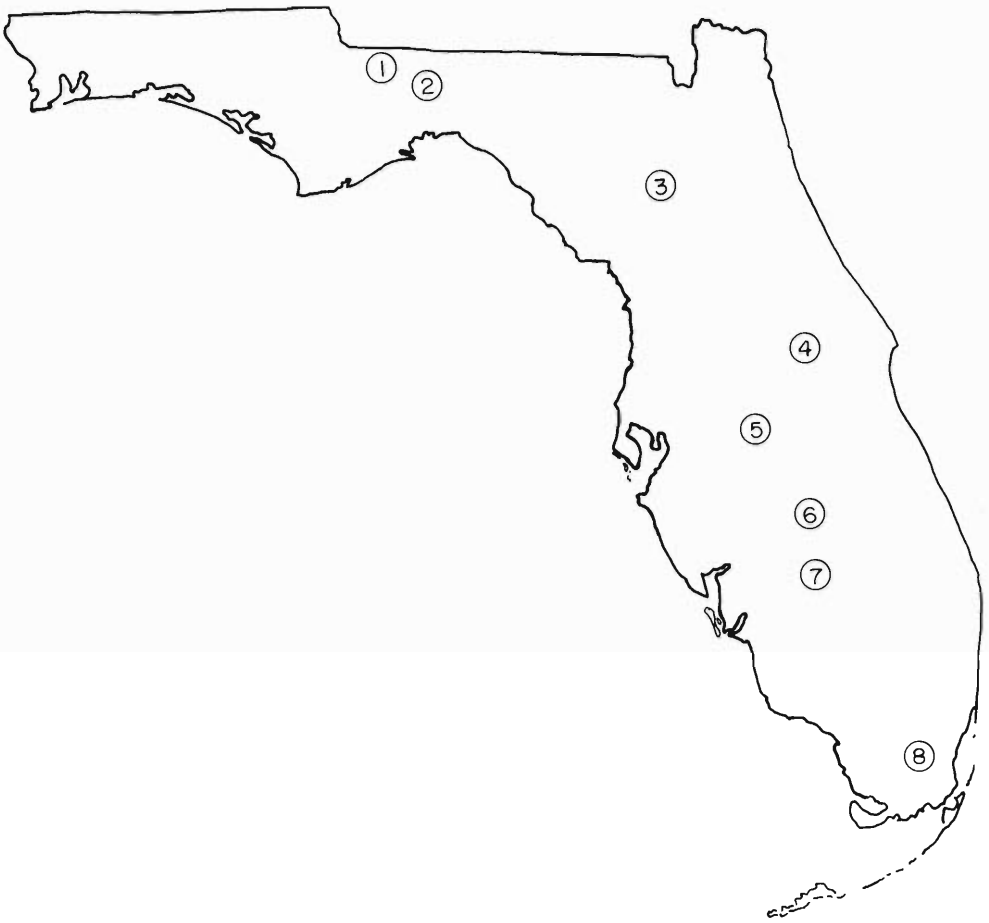


Figure 1. Collection areas for mourning doves in Florida. Numbers of birds collected from each area are as follows: (1) Gadsden Co., $N = 15$; (2) Leon Co., $N = 15$; (3) Alachua Co., $N = 52$; (4) Orange Co., $N = 191$; (5) Polk Co., $N = 90$; (6) Highlands Co., $N = 15$; (7) Glades Co., $N = 30$; (8) Dade Co., $N = 47$.

be assumed that virtually all of the doves collected for our work from April to September were resident birds and those collected during other times of the year were a mixture of resident and nonresident doves.

Techniques for recovering (including the use of fine-mesh screens), fixing, preserving, and staining helminths followed those described by Kinsella and Forrester (1972). Representative specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland) as USNM Helm. Coll. Nos. 74639–74644 and 75574–75578. Data on some of the helminths from doves in Alachua and Dade counties have been discussed elsewhere (Conti and Forrester, 1981) in a different context.

Prevalence data were analyzed as a linear model using FUNCAT from the Statistical Analysis System (SAS) (Helwig and Council, 1979). This was a multiway contingency analysis incorporating tests of age, sex, and county of origin

Table 1. Helminths of mourning doves in Florida.

Parasite	Site*	Prevalence (%)	Intensity†	
			Mean	Range
Nematoda				
Ornithostrongylus spp.‡	SI	67.3	13.1	1–160
Ascaridia columbae	SI	30.5	3.7	1–43
Dispharynx nasuta	PR	16.0	10.9	1–144
Aproctella stoddardi	BC	10.3	6.0	1–34
Tetrameres columbicola	PR	4.2	1.6	1–28
Capillaria obsignata	SI	0.7	5.7	1–15
Trematoda				
Tanaisia bragai	KI	0.2	12.0	12
Brachylaima sp.	SI	0.2	1.0	1
Cestoda				
Hymenolepididae§	SI	1.3	1.0	1
Killigrewia delafondi	SI	0.7	1.0	1
Raillietina spp.§	SI	0.2	2.0	2

* SI = small intestine; PR = proventriculus; BC = body cavity; KI = kidneys.
† Number of worms/infected dove.
‡ A complex of two species, *O. quadriradiatus* and *O. theringi* in a ratio of 14:1 based on males only.
§ A complex of at least two species.

simultaneously for all doves, and tests of age, sex, and season for a series of doves (N = 191) collected in Orange County from April 1974 through February 1975. Square-root transformations were performed on intensity data and analyzed by ANOVA and Duncan's Multiple Range tests. Indices of similarity and diversity were prepared according to Holmes and Podesta (1968). Statements of statistical significance refer to $P < 0.05$.

Results and Discussion

Fourteen species of helminths (seven nematodes, two trematodes, and five cestodes) were found. In Table 1 the site, prevalence, and intensity (means and range) of each species are presented.

With the exception of five species (*Tanaisia bragai, Brachylaima* sp., *Tetrameres columbicola, Ornithostrongylus iheringi* and *Capillaria obsignata*), all of the helminths found in the present study have been reported commonly from mourning doves (Barrows, 1975; Barrows and Hayes, 1977; Conti and Forrester, 1981). The five species in question are found in a variety of other columbiform hosts (Byrd and Denton, 1950; Yamaguti, 1961, 1971; Mollhagen, 1976; Levine, 1980), and may represent accidental infections in mourning doves.

The number of helminth species per infected host varied from one to five (mean, 1.5), with 61 doves free of helminths; the number was highest in Orange County in central Florida (1.7) and lowest at the two geographical extremes (Gadsden, 1.3; Dade, 1.1). The total number of helminths per infected bird ranged from one to 163 (mean 14.0). Four of the 14 species of helminths were considered as major components of the helminth fauna. These included *Ornithostrongylus* spp., *Dispharynx nasuta, Ascaridia columbae,* and *Aproctella stoddardi.* Of the 5,518 specimens obtained from all the doves, 99.6% were nematodes. *Ornithostron-*

Table 2. Helminths of mourning doves from different localities in Florida.

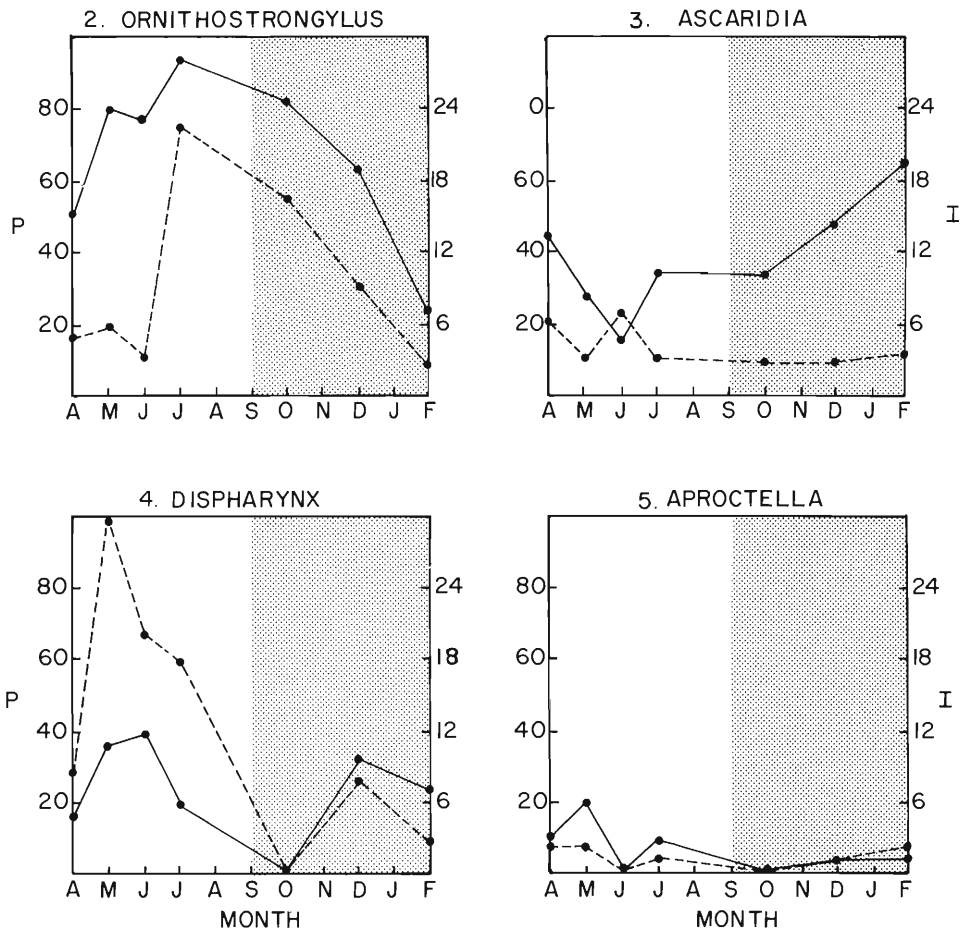
	Ga*	Le	Al	Or	Po	Hi	Gl	Da
Sample size	15	15	52	191	90	15	30	47
Simpson's index	0.34	0.53	0.23	0.30	0.4	0.45	0.62	0.74
% Hosts infected with								
Ornithostrongylus spp.	40	100	31	64	70	73	90	98
Ascaridia columbae	0	7	37	41	23	40	30	11
Dispharynx nasuta	7	13	35	23	7	0	0	4
Aproctella stoddardi	33	13	19	8	14	13	0	0
Tetrameres columbicola	0	0	2	9	0	0	0	2
Capillaria obsignata	0	0	0	2	0	0	0	0
Tanaisia bragai	0	0	2	0	0	0	0	0
Brachylaima sp.	0	0	2	0	0	0	0	0
Hymenolepididae	13	7	0	0	3	0	0	0
Killigrewia delafondi	0	0	2	0	2	0	0	0
Raillietina spp.	0	0	2	0	0	0	0	0

* Ga = Gadsden Co., Le = Leon Co., Al = Alachua Co., Or = Orange Co., Po = Polk Co., Hi = Highlands Co., Gl = Glades Co., Da = Dade Co.

gylus spp. made up the bulk (72%) of helminth parasitism and were common in doves from all counties, with statistically higher prevalences in doves from the southern counties such as Glades (90%) and Dade (98%) (Table 2). Although doves from one of the northern sites (Leon County) had a higher sampled prevalence than doves from the southern sites (Table 2), this was not statistically significant due to the small sample size. The prevalences of the other three species of helminths varied significantly among doves from some counties, but there was no apparent pattern to these differences. There was no effect of sex or age of the doves on the prevalences of helminths. Likewise, there was no significant interaction of sex, age, and county of origin on helminth prevalences.

The intensity of infection of all parasites combined varied significantly by collection site, with doves from the southern areas (Dade and Glades counties) having higher intensities than doves from sites farther north. This finding was influenced primarily by *Ornithostrongylus,* which was the most numerous parasite in the doves. The intensity of *Ascaridia* was significantly higher in adult doves than in juveniles, but this age effect was not true for any of the other helminths. There was no relationship between the sex of the doves and intensity of helminth infections.

Seasonal variations in the prevalence and intensity of infection were examined for the four major components of the helminth fauna in doves from Orange County during a single year (Figs. 2–5). *Ornithostrongylus, Dispharynx,* and *Aproctella* exhibited peaks in prevalence and intensity in the spring and summer months, which may be related to rainfall levels at that time of year (Fig. 6). The differences for only *Ornithostrongylus* were statistically significant, however. *Ascaridia* showed higher prevalences in the winter months and a relatively stable intensity throughout the year. With the exception of *Ascaridia,* the peaks of prevalence and intensity occurred during the time of year when the dove populations were composed mostly of birds that were hatched in Florida. As pointed out above, *Ascaridia* infections were more intense in adult doves, and because there was an



Figures 2–5. Seasonal variation in prevalence (P, solid line) and mean intensity (I, broken line) for doves in Orange County 2. *Ornithostrongylus* spp. 3. *Ascaridia columbae*. 4. *Dispharynx nasuta*. 5. *Aproctella stoddardi*. The darkened areas refer to data on mixed populations of migratory and nonmigratory doves; clear areas refer to data on nonmigratory doves.

increase in prevalence during the winter months, it is likely that migrating doves brought this parasite into the state in significant numbers rather than acquiring infections in Florida.

These results on seasonal variation can be compared with prevalence information obtained from a study of the helminths of wild turkeys (*Meleagris gallopavo*) in southern Florida (Hon et al., 1978). In that study the prevalence of *Dispharynx nasuta* also peaked during the summer months and was lowest during the winter. *Ascaridia dissimilis* showed distinct summer-winter peaks in wild turkeys, as opposed to the single winter peak of *A. columbae* seen in the doves examined in the present study. The trichostrongyles of turkeys (*Trichostrongylus tenuis*) showed a rise in prevalence during the winter, whereas the trichostrongyles of doves (*Ornithostrongylus* spp.) exhibited a summer rise. The differences may be related to effects of weather, especially rainfall, because the two studies

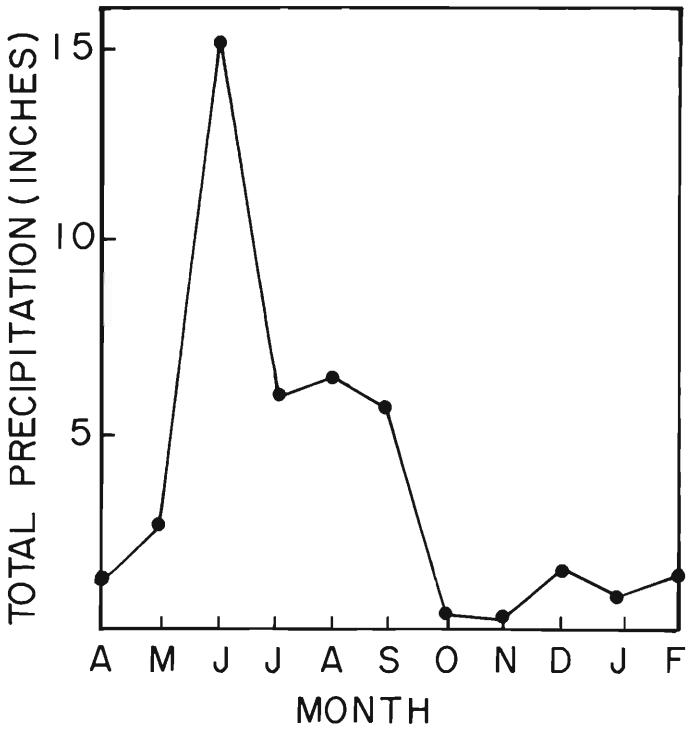


Figure 6. Monthly precipitation for April 1974 through February 1975 in Orlando, Florida, near collection area no. 4 (see Fig. 1) in Orange Co. (U.S. Department of Commerce, 1974–1975).

were conducted during different years. It is also possible that the nature of the dove population in Orange County (i.e., a mixture of migratory and nonmigratory doves during September–February) may have contributed to the observed variation. Influxes of migratory doves might have created a bias resulting in lower or higher prevalences of parasites than would be seen without this effect. Differential densities of the dove populations as suggested by Barrows (1975) could be another reason for some variation in helminth prevalence.

Simpson's index was moderately low (0.34), indicating a fairly diverse fauna statewide, but when this index was calculated for doves on a county basis it became evident that doves from the southern part of the state had less diverse faunas than those from the northern part (Table 2). Indices of similarity were computed comparing each county with every other county and a trellis diagram was constructed (Fig. 7). The least similarity (38) existed between doves from Alachua and Dade counties, but most values were high, indicating similarities in the helminth faunas of doves within the state.

Indices of similarity were computed for doves from Florida and compared to data on helminths of doves from other southeastern states given by Barrows (1975). A trellis diagram was constructed (Fig. 8) that showed that the helminth fauna of doves from Florida was fairly similar (68–69) to those of doves from Alabama and Mississippi, moderately similar (52–64) to those of doves from Louisiana, Arkansas, Tennessee, South Carolina, North Carolina, and Virginia, but

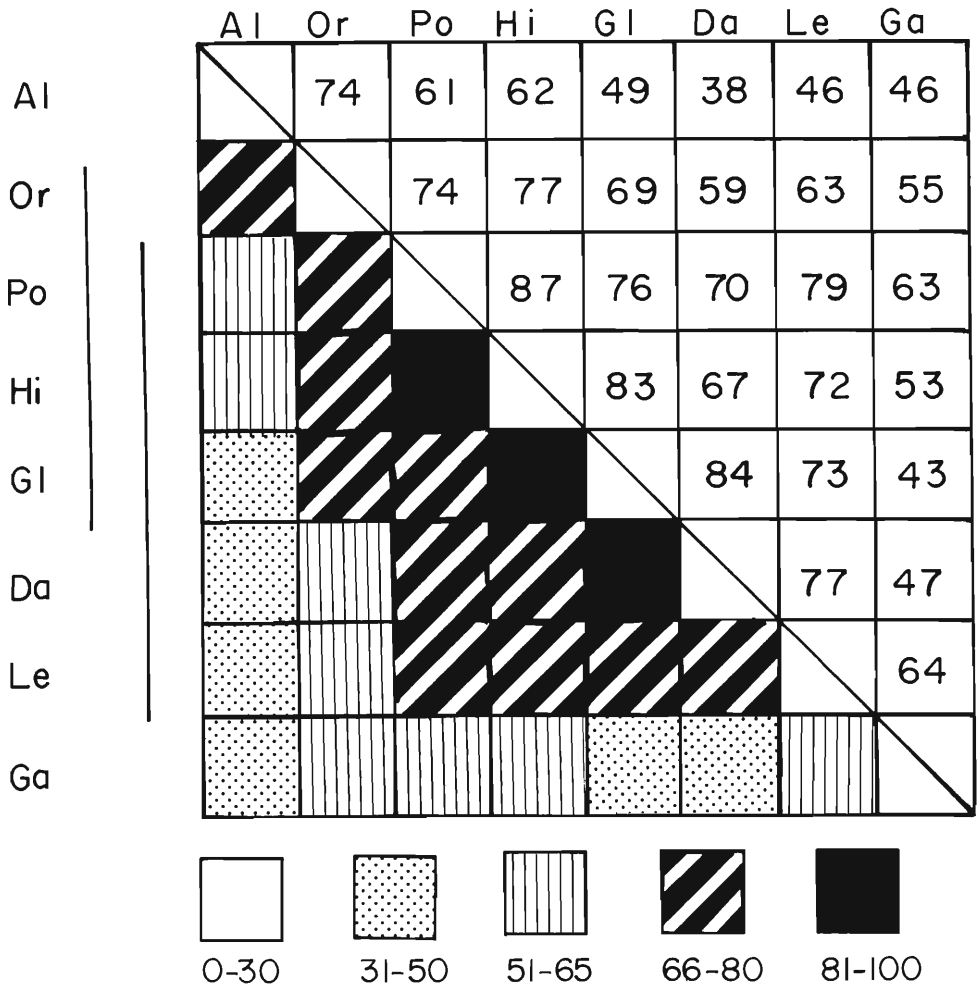


Figure 7. Trellis diagram of indices of similarity for helminth faunas of mourning doves from eight localities in Florida. Abbreviations refer to county names as given in Table 2. Vertical bars on ordinate indicate clusters of closely related indices.

dissimilar (22) to that of doves from Georgia. Figure 8 also shows the indices of similarity among doves from each of the 10 southeastern states. Doves from several states (such as Virginia and Alabama) showed high similarities in helminth faunas to a number of other states. These results may be related to migration patterns and common sources of helminth infective stages.

The pathogenic effects of the helminths of mourning doves are understood incompletely. The present study was not designed to investigate the impact of helminths on dove populations, but several comments on nematode infections are in order. *Ornithostrongylus* was the most prevalent parasite and occurred in the highest numbers. It occurred in 67% of the doves in Florida, with a mean of 13.1 per infected dove. Barrows and Hayes (1977) found it in 51% of 255 mourning doves from 25 localities in 12 southeastern states, including Florida. Their doves

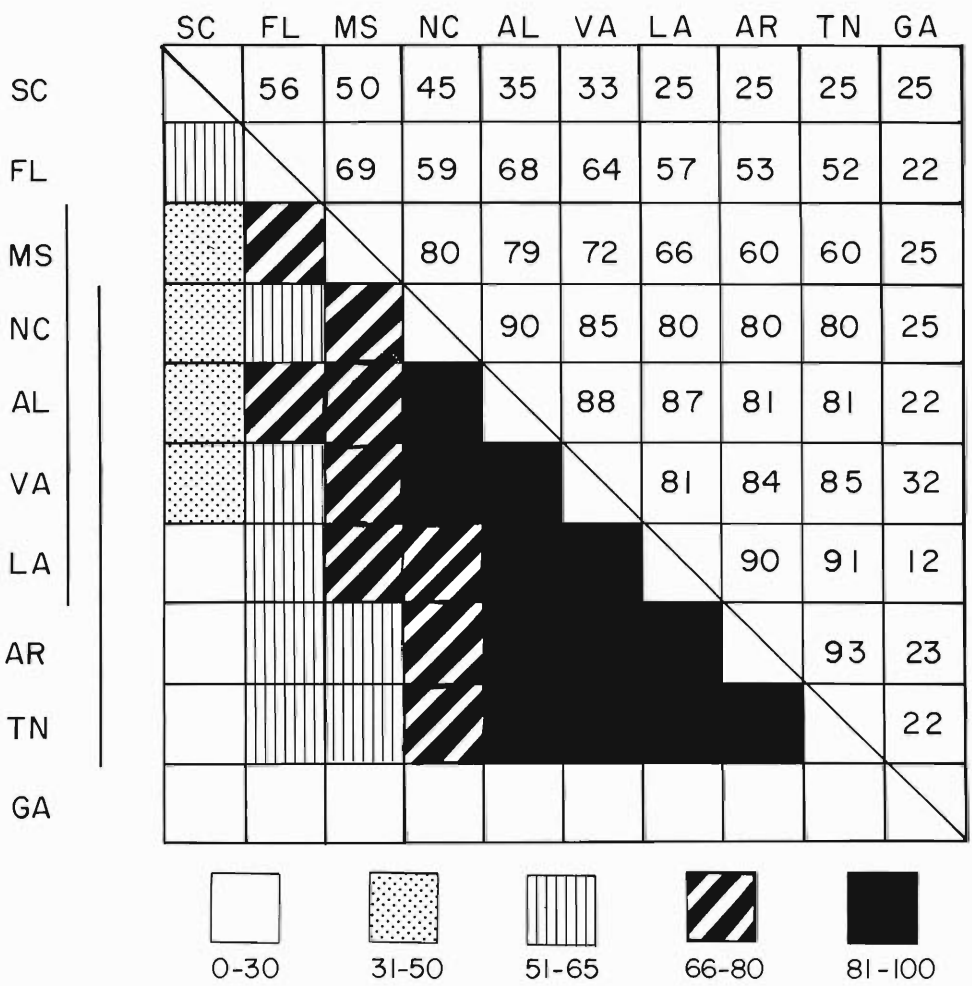


Figure 8. Trellis diagram of indices of similarity for helminth faunas of mourning doves from 10 southeastern states. FL = Florida; GA = Georgia; AL = Alabama; MS = Mississippi; LA = Louisiana; AR = Arkansas; TN = Tennessee; SC = South Carolina; NC = North Carolina; VA = Virginia. Vertical bars on ordinate indicate clusters of closely related indices.

had a mean of nine per infected bird. Large numbers of this nematode have caused catarrhal enteritis and anemia in domestic pigeons (Cuvillier, 1937), and could cause similar effects in doves. Several other nematodes have been associated with pathogenic effects in doves and/or pigeons. *Ascaridia columbae* infections in pigeons have been found to result in perforation of the intestine, peritonitis, and death (Mozgovoi, 1953). Barrows and Hayes (1977) reported distention of the duodenum and localized hyperemia of the mucosa in infected mourning doves. *Dispharynx nasuta* has been associated with pathologic changes and/or death in ruffed grouse, *Bonasa umbellus* (Goble and Kutz, 1945), blue grouse, *Dendragapus obscurus fuliginosus* (Bendell, 1955), wild turkeys (Hon et al., 1975), and domestic pigeons (Hwang et al., 1961). No lesions were reported in mourning doves infected with this nematode by Barrows and Hayes (1977). *Aproctella*

stoddardi has been associated with granulomatous pericarditis and adhesions of the liver and small intestine to the body wall (Barrows and Hayes, 1977). *Tetrameres columbicola* has been found to cause proventriculitis and death in domestic pigeons (Ewing et al., 1967; Flatt and Nelson, 1969). Further studies on the life cycles and pathogenicity of these helminths are needed before the significance of parasitism to mourning dove populations will be understood adequately.

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Sarcocystis ferovis sp. n. from the Bighorn Sheep (*Ovis canadensis*) and Coyote (*Canis latrans*)

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ABSTRACT: A new species of *Sarcocystis*, *Sarcocystis ferovis*, is reported from the bighorn sheep (*Ovis canadensis*) and coyote (*Canis latrans*). Sarcocysts are microscopic, septate, thin walled (0.6 μm thick), and contain 10–14 × 3–3.5-μm bradyzoites. A coyote (*Canis latrans*) fed infected meat shed sporocysts and oocysts 9 days later. Sporulated oocysts were 19.6 × 14 μm, and sporocysts were 13.6 × 9.7 μm. The structure of the sarcocyst wall was different from that of sarcocysts in sheep, goats, and oxen. *Sarcocystis ferovis* was not transmissible to goats, sheep, and oxen.

Sarcocysts infection is common in sheep, cattle, and goats. *Sarcocystis* species are generally host specific for their intermediate hosts (for reviews see Mehlhorn and Heydorn, 1978; Dubey, 1980a; Levine and Ivens, 1981). This report describes a new species of *Sarcocystis* from the bighorn sheep (*Ovis canadensis*).

Materials and Methods

A 6-yr-old ram was shot on 31 December 1981 near Gardner, Montana, because of blindness unrelated to sarcocystosis. This ram was part of a protected herd maintained in the Yellowstone National Park. The carcass was transported to the Veterinary Research Laboratory, Montana State University, and necropsied within 4 hr of death. No macroscopic sarcocysts were found in the tongue, heart, esophagus, diaphragm, or other muscles of the body. After finding microscopic sarcocysts in heart muscle in squash unstained preparations, pieces of muscles from the legs, abdomen, diaphragm, heart, eye, tongue, and esophagus were fixed in 10% Millonig's buffered formalin (MBF), Bouin's, and Helly's fixatives. Paraffin-embedded sections were cut at 5 μm. Pieces of heart fixed in Bouin's fluid were embedded in glycol-methacrylate and sectioned at 3 μm. Sections were stained with hematoxylin and eosin, periodic acid Schiff's hematoxylin, and iron hematoxylin. Pieces of heart fixed in MBF were processed for transmission electron microscopy and examined under a JEOL 100CX electron microscope to visualize the structure of the sarcocyst wall.

Portions of heart, tongue, esophagus, diaphragm, and thigh muscles were ground and stored at 4°C. Beginning the next day, ground muscles were fed over a period of 3 days to a laboratory-raised, female, 8-mo-old coyote. The coyote had been in the laboratory since 1 wk of age and had never eaten raw meat before the experiment (Dubey, 1980b). Feces of the coyote were examined for coccidian oocysts daily by the sugar flotation method from the day the coyote first ingested infected meat of the bighorn sheep. The coyote was killed on day 12, and portions of its small intestine were fixed in MBF, Helly's, and Bouin's fixatives. Paraffin- and plastic-embedded sections were cut at 5 and 3 μm, respectively. Oocysts and sporocysts were obtained from intestinal scrapings and stored in a balanced salt-antibiotic mixture as described by Dubey (1980b, 1981).

Table 1. Experimental inoculation of sheep, goats, and an ox with *S. ferovis* sporocysts.

Animal species and no.	Days old	No. of sporocysts fed (× millions)	Necropsy day postinoculation
Ox 1	2	10	70
Sheep 1	3	25	70
Sheep 2	3	25	17
Sheep 3	2	25	21
Sheep 4	13	2.5	31
Sheep 5	13	2.5	45
Sheep 6	1	none	21
Goat 1	8	1	70
Goat 2	9	1	17
Goat 3	8	none	17
Goat 4	9	none	70

Experimental inoculation of sheep, goats, and an ox with *S. ferovis* sporocysts

Two goats (*Capra hircus*), five sheep (*Ovis aureus*), and an ox (*Bos taurus*) were fed in milk 1–25 million sporocysts of *S. ferovis* and necropsied between 17 and 70 days later (Table 1). The sporocysts were collected from the coyote fed naturally infected bighorn sheep and had been stored in a balanced salt-antibiotic mixture for 18–65 days at 4°C. The sheep, goats, and ox were fed cow's milk until necropsy and housed in an enclosed building to avoid exposure to carnivores. At necropsy, portions of bone marrow, eyes, pituitary, salivary and adrenal glands, thymus, lungs, heart, diaphragm, spleen, kidneys, liver, gallbladder, urinary bladder, omentum, rumen, reticulum, omasum, abomasum, small and large intestines, esophagus, skeletal muscle from abdomen and thigh, lymph nodes (superficial cervical, mandibular, retropharyngeal, mediastinal, hepatic, gastric, mesenteric, subiliac), cerebrum, cerebellum, pons, medulla, spinal cord, and tongue were fixed in MBF. Paraffin-embedded sections were cut at 5 μm and examined after staining with hematoxylin and eosin.

Results

Microscopic sarcocysts with septa were found in the muscles of the tongue, esophagus, heart, diaphragm, eye, and limbs; most of them were in the heart (Fig. 1). They were 173 × 57 μm (30–780 × 30–100 μm; $N = 31$); sarcocysts in the heart were smaller than those in other muscles. Under the light microscope, the sarcocyst wall was thin (<1 μm) and smooth and contained numerous bradyzoites that were 10–14 × 3–3.5 μm ($N = 10$).

One sarcocyst was examined ultrastructurally. The sarcocyst wall consisted of a 600-nm (250–840 nm; $N = 11$)-thick wall, excluding evaginations. The primary cyst wall consisted of single unit membrane (parasitophorous vacuolar membrane [PVM]) and an electron-dense ground substance (Gr) located just beneath the PVM (Fig. 2). The PVM membrane had minute undulations at uneven distances apart and mushroomlike evaginations that were 1,157 × 160 nm (630–1,800 × 115–250 nm; $N = 13$). The electron-dense layer extended into these evaginations on the PVM and in the septa. Sarcocysts contained numerous bradyzoites, a few

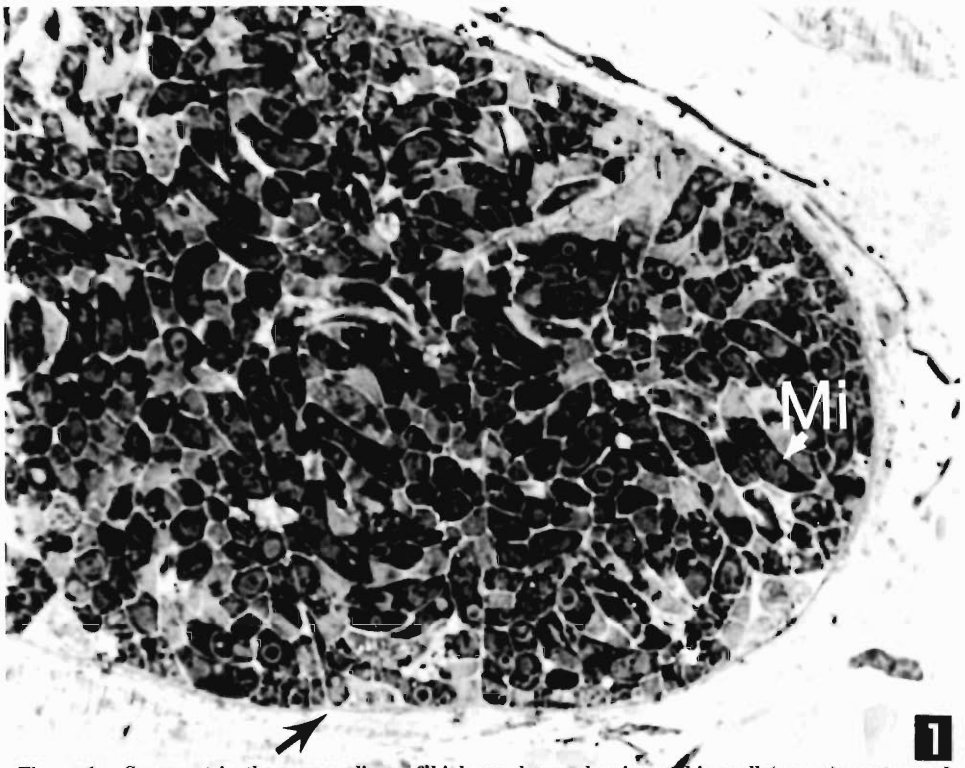

Figure 1. Sarcocyst in the myocardium of bighorn sheep, showing a thin wall (arrow), septa, and numerous bradyzoites. Bradyzoites have large mitochondria (Mn). One-μm section, toluidine blue, ×1,000.

metrocytes, and degenerate organelles appearing as myelinlike bodies (Fig. 2). Bradyzoites were 3.11 μm thick (2.6–3.50 μm; $N = 14$) and contained all organelles typically found in Sarcocystidae (Mehlhorn and Heydorn, 1978). Metrocytes were few and were located beneath the cyst wall. They were 7.3 × 4.2 μm (5.0–9.8 × 3.5–5.5 μm; $N = 5$).

The coyote shed sporocysts and oocysts 9 days after ingesting infected meat. Sporulated and unsporulated oocysts were found in sections of small intestine and were located just below the epithelium (Fig. 3). Occasionally, the lamina propria of infected villi was infiltrated with neutrophils (Fig. 3). Sporulated oocysts from intestinal scrapings were 19.6 × 14 μm (17.5–21 × 14–15 μm; $N = 14$) and contained two sporocysts that were 13.6 × 9.7 μm (13–15 × 9–11 μm; $N = 52$). Each sporocyst contained four sporozoites and a compact residual body. A Stieda body was absent. Ten living sporozoites were 7–8.5 × 2 μm.

Sarcocystis ferovis was not found in any of the inoculated domestic sheep, goats, or the ox. A few immature thick-walled *S. tenella* sarcocysts were found in the tongue of sheep 1.

Discussion

The structure of the sarcocyst wall is a useful criterion for differentiating species of *Sarcocystis* within a given host (Mehlhorn et al., 1976). The structure of the

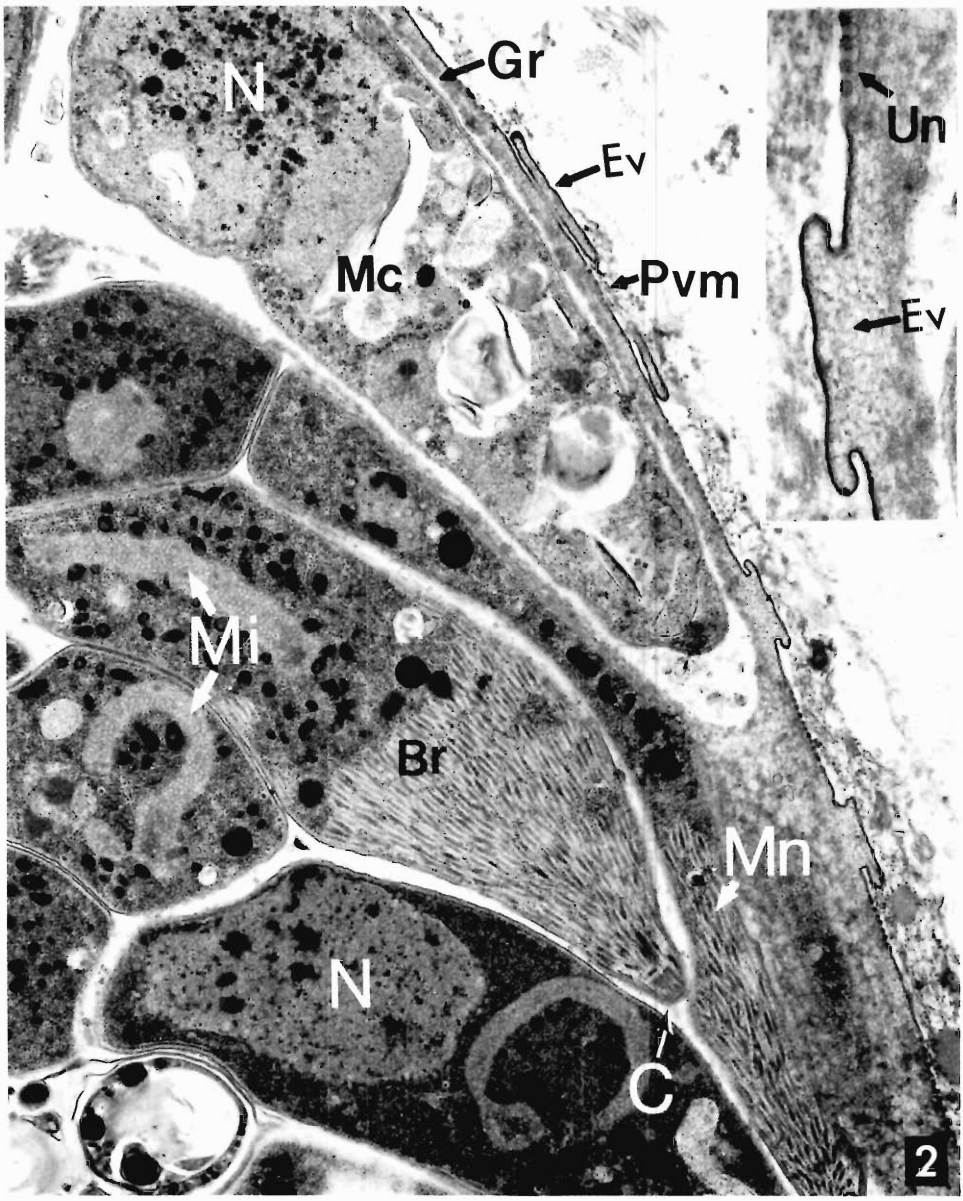

Figure 2. Transmission electron micrograph through the periphery of the sarcocyst shown in Figure 1. Note parasitophorous vacuolar membrane (PVM), minute undulations (Un) on PVM, evaginations (Ev) on PVM, ground substance (Gr) in the wall, a metrocyte (Mc) lying just beneath the sarcocyst wall, and several bradyzoites (Br). Note large coiled mitochondrion (Mi), numerous micronemes *(Mn)*, a conoid (C), and nucleus (N) in each bradyzoite. A degenerate organelle appearing as a myelinlike body is shown at the extreme left corner. ×10,000. Inset shows a mushroomlike evagination (Ev) and undulations (Un) on the cyst wall. × 33,000.

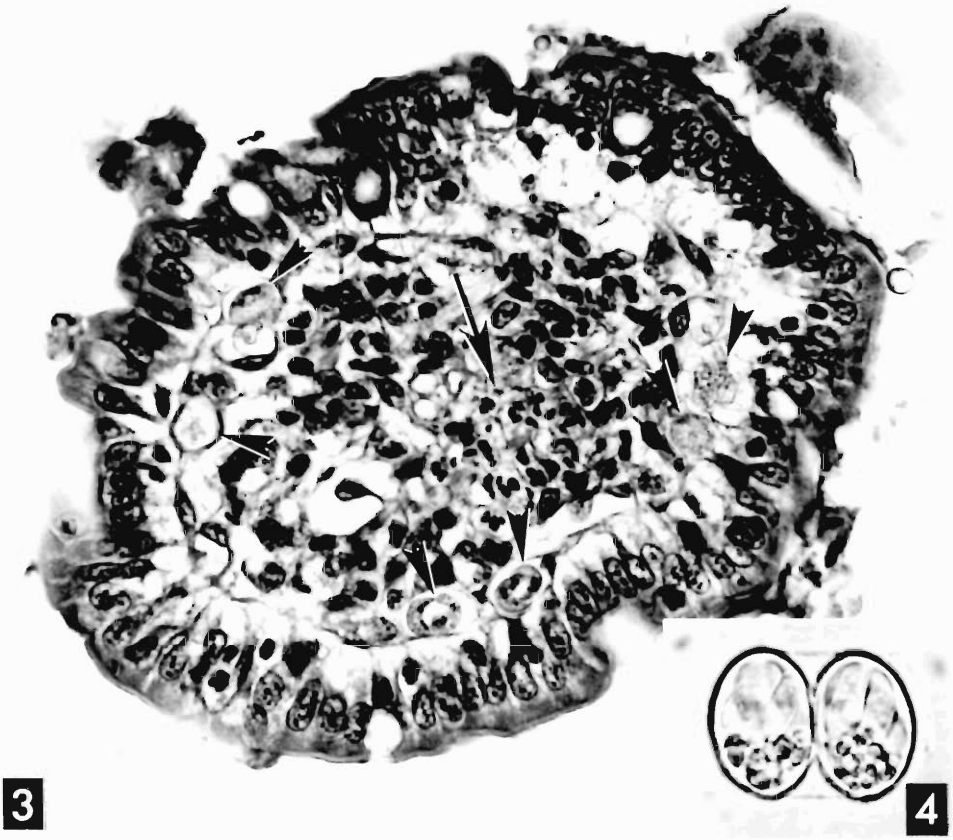

Figure 3. Cross section of villus in small intestine of the coyote fed infected meat. Note several oocysts (arrowheads) lying in the lamina propria, just beneath the basement membrane. Arrow points to neutrophils. H&E, ×400.

Figure 4. Sporulated oocyst from the intestinal scrapings of coyote. Note the thin oocyst wall, two sporocysts, sporozoites, and sporocystic residual bodies. Unstained, ×1,000.

sarcocyst wall from the bighorn sheep is different from that of sarcocysts in sheep, goats, oxen, and any of the other species of *Sarcocystis* studied so far (Mehlhorn et al., 1976). In addition, *S. ferovis* is not transmissible to domestic sheep, goats, and oxen. Three species of *Sarcocystis* are currently known to occur in domestic sheep: *S. tenella* (canid cycle) and *S. gigantea* and *S. medusiformis* (felid cycles). *Sarcocystis tenella* sarcocyst has a cross-striated 3.5-μm-thick wall with pallisade protrusions (Mehlhorn et al., 1975). *Sarcocystis gigantea* and *S. medusiformis* sarcocysts are macroscopic and are distinguished by protrusions on the primary cyst wall. In *S. gigantea* the protrusions are cauliflowerlike, whereas in *S. medusiformis* the villi are rounded and blisterlike invaginations confined to their bases. In addition, the secondary cyst wall present in *S. gigantea* is absent in *S. medusiformis* (Collins et al., 1979). The sarcocyst of the bighorn sheep is thin-walled and lacks the villar protrusions of the three species in the domestic sheep. Therefore, the new name *Sarcocystis ferovis* is proposed for the sarcocyst in the bighorn sheep.

Acknowledgments

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Amoebae in Tennessee and Cumberland River Drainages, with Special Reference to Thermophilic *Naegleria*

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ABSTRACT: Water samples from a variety of aquatic habitats were analyzed for the presence of amoebae. All water samples harbored limax amoebae. Thermophilic *Naegleria* were isolated only from reservoir habitats and most commonly from thermally enriched areas in reservoirs. These *Naegleria* were nonpathogenic in laboratory mice.

The occurrence and distribution of small free-living amoebae, loosely termed "limax amoebae," have in years past received only nominal interest in the scientific community. More recently these amoebae, particularly those of the genus *Naegleria,* have become of considerable interest to biologists and physicians. *Naegleria fowleri,* a small freshwater amoeba, is the most common causative agent of primary amoebic meningoencephalitis (PAM). It is a usually fatal disease of the central nervous system and is acquired via amoebic invasion of the nares during vigorous aquatic activity (Carter, 1970). Amoebae migrate from the nasal mucosa along the olfactory nerve to the brain, with death typically ensuing 3–5 days after onset of symptoms (Chang, 1979). To date over 150 cases of PAM have been reported worldwide and from various regions of the United States including California, Virginia, Florida, and Texas.

Of particular interest is the seasonal incidence of PAM. Nearly all documented infections have occurred in the warm summer months. This is a time of greatly increased aquatic activity for many persons, but other factors may play an as yet undetermined etiological role. It has been established that most victims contracted PAM while swimming or diving in warm, often polluted water. It is believed that discharge of organic matter and/or heat into these waters enhances the growth of *Naegleria* species (Carter, 1978). In addition, the possibility that an organically and/or thermally polluted environment selects for and adapts amoebae for growth and pathogenicity in a warm-blooded host cannot be excluded.

A prerequisite for pathogenicity is the ability to multiply at 37°C. Amoebae capable of growth at 37°C or higher temperatures are considered thermophilic. Pathogenic *Naegleria* usually can multiply at 42–45°C, but not all thermophilic amoebae are pathogenic. Potential pathogenicity among thermophiles can be determined by using mice as experimental hosts because the course of PAM in mice is almost identical to that observed in humans (Martinez et al., 1971).

The purpose of this investigation was to (1) determine the presence or absence of amoebae in both unpolluted and polluted (municipal, industrial, and thermal pollution, as defined by Tennessee Valley Authority criteria) aquatic habitats in selected areas of Tennessee and Cumberland river drainages during the warm weather months; (2) determine if amoebae were thermophilic (capable of growth

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Table 1. Recovery of amoebae from water samples.

Water quality type and location	Collection month(s)	River mile	Water temperature (°C)	Growth at 25°C	Growth at 42–45°C	*Naegleria* species
Unpolluted						
Big Bear Creek	Aug.	27.3	24.5	+	–	ND*
Toccoa River	July	52.8	14.0	+	–	ND
	Sept.	52.8	20.0	+	–	ND
South Fork Holston	July	49.8	8.3	+	–	ND
Municipally polluted						
French Broad River	July	145.8	21.7	+	–	ND
	Sept.	145.8	18.5	+	–	ND
Duck River	July	132.8	24.4	+	–	ND
	Sept.	132.8	22.0	+	–	ND
	Jan. (mud sample)	133.9	8.9	+	+	no
Industrially polluted						
Tennessee River	Aug.	274.9	27.0	+	–	ND
	Oct.	274.9	17.0	+	–	ND
Holston River	Aug.	118.4	18.9	+	–	ND
	Oct.	118.4	16.7	+	–	ND
Reservoir (nonthermal)						
Tennessee River	July	245.0	27.5	+	–	ND
Tennessee River	July	100.4	27.0	+	+	no
Cumberland River	Aug.	108.0	25.5	+	+	no
Clinch River	July	4.0	20.0	+	+	yes
Cumberland River	July	108.5	27.3	+	+	no
Reservoir (thermal)						
Tennessee River	July	99.2	32.8	+	+	yes
Cumberland River	July	242.5	29.0	+	–	ND
	Aug.	242.5	27.0	+	+	yes
Tennessee River (cooled water from Brown's Ferry)	Aug.	294.0	28.0	+	+	yes
Tennessee River (heated water from Brown's Ferry)	Aug.	294.0	37.5	+	+	yes
Clinch River	July	2.6	28.0	+	+	yes
Holston River	July	106.2	26.1	+	+	no
Cumberland River	Sept.	102.8	ND	+	–	ND

* ND = not determined.

at 42–45°C); (3) identify thermophilic *Naegleria* (to genus); and (4) determine if thermophiles were pathogenic in laboratory mice.

Materials and Methods

Water samples were collected during summer and early autumn of 1979 and in January 1980 from a variety of aquatic habitats in Tennessee, Georgia, Alabama, and North Carolina. Site location was indicated by river mile (Table 1). Water samples (500 ml) and their temperatures were taken from near surface waters.

Water samples were analyzed by filtering a 250-ml portion of the sample (0.45-

μm Gelman membrane filter) to a volume of 10 ml. Water remaining on the filter was picked up with a pipet and used to rinse off material trapped on the filter. A volume of 0.1–0.4 ml of the suspension was added to a thick suspension of heat-killed *Escherichia coli* that had been concentrated by centrifugation before killing. Penicillin and streptomycin (one drop from a stock solution containing 250 μg/ml each) were added to inhibit bacterial contamination. These antibiotics did not appear to inhibit amoebic growth.

To determine the presence of amoebae capable of growth at 25 and 42–45°C, a plaque assay was used (Fulton, 1970). The suspension containing amoebae from the water sample, heat-killed *E. coli,* and antibiotics (total volume 0.5–1.0 ml) was spread evenly over a 100 × 15-mm Petri dish containing nonnutrient agar. This procedure was done in duplicate for each sample. After absorption of fluid, plates were inverted and incubated at 25 and 42–45°C (humid conditions had to be maintained in the incubator). After 48 hr, amoebic growth was observed by the formation of plaques. As individual amoebae ingested bacteria and multiplied, areas devoid of bacteria were formed. To identify thermophilic *Naegleria* a small agar plug was cut from plaques formed at 42–45°C. This plug was suspended in 1 ml of distilled water or Page amoeba saline (PAS) (Page, 1967) and incubated at 37 or 42–45°C. At 15-min intervals an aliquot was placed on a slide and amoebae were observed (200×) for formation of flagella, a taxonomic criterion used to separate *Naegleria* from other genera of the family Vahlkampfiidae. Thermophilic isolates were recultured (37 or 42–45°C) on agar plates with heat-killed *E. coli* and antibiotics. After 24–48 hr thermophilic amoebae were harvested for inoculation into mice. Amoebae were scraped from the agar surface with a scapel after adding 1 or 2 ml of PAS. The number of amoebae/ml in the inoculum was determined by hemocytometer counts. Mice (Flow Laboratories strain ICR) were held in a supine position after ether anesthetization for inoculation from a Pasteur or micropipet. A 0.05–0.1-ml suspension of PAS containing 12,000–80,000 amoebae of each thermophilic isolate was delivered into the nares of each mouse. A minimum of five mice was used for each thermophile tested. The same protocol was followed to experimentally infect control mice with the Lee strain of pathogenic *Naegleria fowleri.* Control mice showed typical PAM symptoms of hyperexcitability, loss of appetite, and ruffling of neck hair. These were followed by convulsions and death. PAM was confirmed by observation and reisolation of amoebae from morbid brain tissue.

Results

The presence or absence of amoebae from a variety of aquatic habitats was determined (Table 1). When water samples were incubated at 25°C, amoebae were readily cultured from both pristine and polluted waters. Thermophiles were not recovered from unpolluted waters. With one exception, no thermophiles were cultured from municipally or industrially polluted waters. This exception was recovered from a bottom mud sample collected in January 1980 from a municipally polluted area of the Duck River. Thermophilic isolates were readily recovered, however, from two types of reservoir habitats. One group of reservoirs received thermal pollution at or near the collection site, whereas no thermal pollution was directly associated with the second type of reservoir. Morphologic criteria and positive tests for flagellation revealed the presence of thermophilic

Naegleria in both types of reservoirs. However, *Naegleria* species were more readily isolated from thermally enriched reservoirs. All thermophilic isolates, including the *Naegleria* species, were nonpathogenic when inoculated into mice.

Discussion

In 1966 Butt coined the term primary amoebic meningoencephalitis (PAM) to describe cerebral infections caused by normally free-living amoebae. By 1968, 31 fatal cases of PAM had been reported worldwide, including in the United States (Carter, 1978). The early 1970's saw the recognition of a cause-and-effect relationship between vigorous aquatic activity in warm water of high organic content and PAM (Neva, 1970). Since that time investigators have attempted to understand the role of the aquatic habitat and its predisposition for harboring pathogenic limax amoebae.

According to Page (1976) the genus *Naegleria* is comprised of *N. gruberi, N. thorntoni, N. jadini,* and *N. fowleri.* The latter is considered to be the principal causative agent of PAM. More recently *N. australiensis* and *N. lovaniensis* (Stevens et al., 1980) have been added to this genus. *Naegleria* amoebae have been described from various soil types and from water habitats such as rivers, lakes, swamps, and even tap water (Fulton, 1970). *Naegleria* is clearly ubiquitous in the environment and the relatively few documented cases of PAM are probably not a true reflection of human contact with it. In light of this fact, much time and effort have been expended to identify and characterize the different species of *Naegleria,* with particular emphasis on the differentiation of pathogenic and nonpathogenic forms. This determination has been hampered because differentiation based on morphologic characteristics is sometimes difficult (De Jonckheere and van de Voorde, 1977b). Also, some species are weakly pathogenic when introduced intranasally, but are acutely pathogenic when administered intracranially, and some highly pathogenic strains can lose their virulence when maintained in laboratory culture (De Jonckheere, 1979).

De Jonckheere et al. (1975) investigated the distribution of pathogenic amoebae in Belgium and reported the isolation of pathogenic *Naegleria* only from thermally polluted water. Subsequently they suggested that thermally polluted but biologically healthy water is the primary site for *Naegleria fowleri* proliferation (De Jonckheere and van de Voorde, 1977a).

There has been some conjecture on the role of thermal pollution in the distribution of thermophilic pathogens in the southern United States. Willaert and Stevens (1976) isolated pathogenic *Naegleria* in Florida only from thermally polluted waters. Wellings et al. (1977) isolated pathogenic *Naegleria* from non-thermally associated waters in Florida, and they concluded that thermal pollution played little if any role in the maintenance of pathogenic *Naegleria* in that semitropical area. Obviously, increased water temperature, whether from thermal pollution, sunlight, or other sources, provides the best environment for proliferation of thermophilic pathogens.

In our investigation, no thermophilic amoebae were recovered from unpolluted waters. With one exception, no thermophiles were cultured from municipally or industrially polluted habitats. This exception (a non-*Naegleria* species) was recovered from a bottom mud sample taken from a municipally polluted river in January when the water temperature was 8.9°C. According to Wellings et al.

(1977), *Naegleria* survive the winter in bottom sediments. They also observed that water samples from some lakes in Florida were negative for pathogenic *Naegleria,* but pathogens were isolated from bottom mud samples. Our isolation of thermophilic amoebae from a bottom sample complements their observations and shows that future surveys should include mud and sediment samples in addition to surface samples.

We were able, however, to isolate thermophilic amoebae routinely from reservoir habitats. An association with thermal enrichment was not a prerequisite for isolation of these thermophiles. However, reservoirs had higher water temperatures than the other aquatic habitats, and most of the thermophilic *Naegleria* were isolated from thermally enriched reservoirs. This suggests that thermal enrichment enhances the growth of *Naegleria* in the southern United States.

Our investigation provides additional insight on *Naegleria* amoebae and underscores the apparent environmental effect on their distribution. Thermally enriched waters promoted the incidence of thermophilic *Naegleria,* indicating a potential in these waters for harboring pathogens. This observation is significant because pathogenic *Naegleria* share a common environment with nonpathogenic variants; it has been postulated that such variants may be precursors of highly virulent *N. fowleri* (De Jonckheere and van de Voorde, 1977b). That no pathogenic strains were found in our investigations was encouraging; however, the environmental influence of thermally enriched waters should not be disregarded. Further investigations on the ecological niches associated with pathogenic and nonpathogenic *Naegleria,* and the role of the aquatic habitat on amoeba distribution are needed.

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Observations on *Theileria cervi* and *Trypanosoma cervi* in White-tailed Deer (*Odocoileus virginianus*) from the Southeastern United States

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ABSTRACT: Thin blood films from 240 white-tailed deer (*Odocoileus virginianus*) from 43 locations in 10 southeastern states were examined for hematotropic protozoans. *Theileria cervi* and *Trypanosoma cervi* both occurred in 49% of the animals. The occurrence of *Th. cervi* in various deer herds was associated ($P < 0.01$) with the presence of its optimum vector, *Amblyomma americanum*. Other species of ticks, *A. maculatum* and *Ixodes scapularis*, were not associated ($P > 0.05$) with the presence of *Th. cervi*. The prevalence of *Th. cervi* was seasonally stable; however, *Tr. cervi* had a distinct seasonal peak in prevalence between July and September that was attributable to an abundance of epimastigote stages. Neither *Th. cervi* nor *Tr. cervi* produced specific pathologic conditions.

White-tailed deer (*Odocoileus virginianus*) have been reported as natural hosts for four hematotropic protozoans, viz., *Babesia odocoilei, Plasmodium odocoilei, Theileria cervi,* and *Trypanosoma cervi* (Garnham and Kuttler, 1980; Kingston, 1981). Information on the distribution, prevalence, and ecology of these protozoans is incomplete. This report presents information on hematotropic protozoans in white-tailed deer in the southeastern United States that was obtained incidental to other studies.

Materials and Methods

White-tailed deer examined during this study were collected from throughout the southeastern United States for herd health assessments or other research purposes by the Southeastern Cooperative Wildlife Disease Study (SCWDS). Included in this report are 240 animals collected between May 1976 and September 1981. Groups of five or 10 deer were obtained from 43 locations in 39 counties and subjected to detailed necropsy examinations (Nettles, 1981).

Whole blood was collected via cardiac puncture in evacuated tubes containing EDTA. Blood films were made, fixed in 100% methanol, stained with Giemsa, and examined (100×–1,000×) for blood protozoans. Trypanosomes were classified as to morphologic form (trypomastigote or epimastigote) whenever possible. Each deer was searched for ticks, and infestations were rated as to intensity (none, light, moderate, or heavy). Representative specimens of ticks were collected from infested deer and preserved in 70% ethyl alcohol. Tick identifications were performed by National Veterinary Services Laboratory, APHIS, USDA, Ames, Iowa.

Data were examined for geographic and seasonal variations. The association between potential tick vectors and *Th. cervi* infection was examined on both an individual animal and herd basis by use of the chi-square test.

Table 1. Prevalence of *Trypanosoma cervi* and *Theileria cervi* in 240 white-tailed deer in the southeastern United States.

State	*N*	*Trypanosoma cervi*	*Theileria cervi*
Alabama	25	52%	60%
Arkansas	30	23%	77%
Florida	55	42%	53%
Georgia	55	73%	55%
Louisiana	15	60%	0
Maryland	5	60%	100%
South Carolina	5	0	100%
Tennessee	10	50%	0
Virginia	15	67%	67%
West Virginia	25	52%	0
Total	240	49%	49%

Results

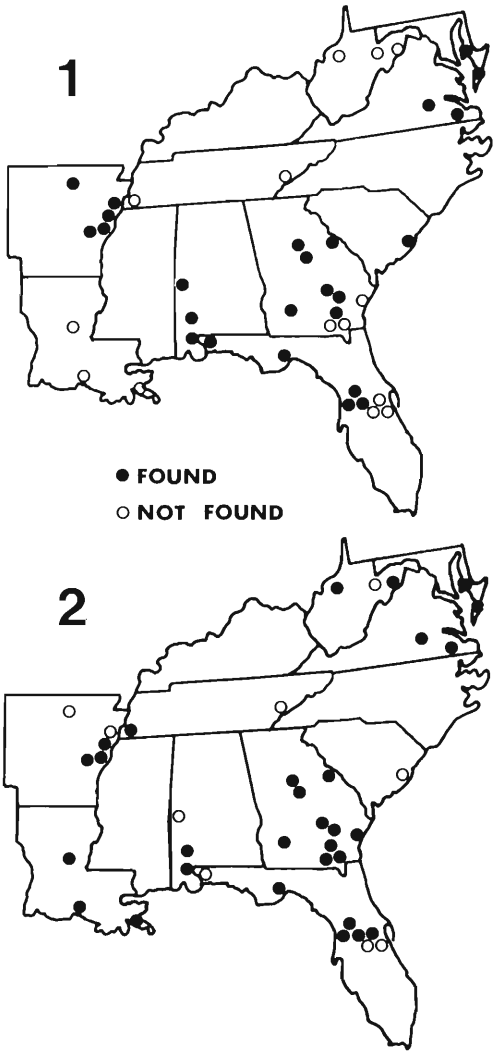
Two species of blood protozoans (*Th. cervi* and *Tr. cervi*) were widely distributed in southeastern white-tailed deer (Table 1). *Theileria cervi* was present in deer from 25 (64%) counties (Fig. 1) and *Tr. cervi* occurred in 33 (77%) counties (Fig. 2). *Theileria cervi* did not exhibit seasonal trends in prevalence; however, *Tr. cervi* had a distinct peak in prevalence in late summer (Table 2). In addition, a clear seasonal difference was apparent in the occurrence of the two morphologic forms of *Tr. cervi*. The prevalence of trypomastigotes remained relatively stable throughout the year. The prevalence of epimastigotes, however, was much higher during the late summer (Table 2). Ninety-seven percent of all deer with epimastigotes were collected during late summer. Seven deer collected in July and August had both epimastigotes and trypomastigotes.

Three species of ticks (*Amblyomma americanum, A. maculatum,* and *Ixodes scapularis*) were recovered. Efforts were not made to quantify infection intensities for each species of tick; however, *A. americanum* occurred on 117 (49%) deer from 31 locations and was by far the most abundant species. *Ixodes scapularis* occurred on 23 (10%) deer from nine locations, and *A. maculatum* occurred on 10 (4%) deer from five locations. Infestations by any of these species of ticks were not associated with infections of *Th. cervi* in individual deer ($P > 0.05$). On a herd basis, however, infestations of *A. americanum* and infection by *Th. cervi*

Table 2. Seasonal prevalence of *Theileria cervi* and *Trypanosoma cervi* in 240 white-tailed deer in the southeastern United States.

		*Trypanosoma cervi**			
Period	*N*	Trypomastigotes	Epimastigotes	Total	*Theileria cervi*
Jan.–Mar.	45	18%	2%	20%	62%
Apr.–June	20	30%	0%	30%	35%
July–Sept.	155	31%	19%	61%	49%
Oct.–Dec.	20	5%	0%	30%	30%

* Morphologic classification was not possible in all cases; seven deer had both trypomastigotes and epimastigotes.



Figures 1, 2. Collection sites and distribution of *Theileria cervi* (Fig. 1) and *Trypanosoma cervi* (Fig. 2). STATE: counties or parishes—ALABAMA: Baldwin, Clarke, Sumter; ARKANSAS: Arkansas, Crittenden, Lee, Phillips, Stone; FLORIDA: Brevard, Citrus, Escambia, Lake, Marion, Orange, Osceola, Wakulla; GEORGIA: Charlton, Clinch, Dougherty, Jasper, Jeff Davis, Jones, McIntosh, Richmond, Telfair, Ware; LOUISIANA: Iberia, LaSalle, Plaquemines; MARYLAND: Dorchester; SOUTH CAROLINA: *Georgetown*; TENNESSEE: Blount, Shelby; VIRGINIA: Nansemond, Nottoway; WEST VIRGINIA: Hardy, Tucker, Wirt.

were associated ($P \leq 0.01$). Infestations of *A. maculatum* and *I. scapularis* in herds were not associated with *Th. cervi* infections ($P \geq 0.05$).

Discussion

The methods used during this study were for obtaining standard hematologic data and were not designed to specifically detect all hematotropic protozoans. Thus, information presented herein must be considered to represent minimal

infection values. The absence of *B. odocoilei* and *P. odocoilei* in white-tailed deer in the Southeast thus may be artifactual, although neither species has been found east of Texas (Garnham and Kuttler, 1980; Kingston, 1981). The standardized techniques, however, did afford data on *Th. cervi* and *Tr. cervi* from which comparisons could be made relative to seasonal and geographic variables.

This report extends the known range of *Th. cervi* in white-tailed deer to include Alabama, Arkansas, Florida, Georgia, Maryland, South Carolina, and Virginia. The prevalence of *Th. cervi* infection in the Southeast was very similar to the prevalence (57%) found in Texas (Kingston, 1981). The lone star tick, *A. americanum,* has been considered the most important vector of *Th. cervi* (Kingston, 1981) and has been demonstrated to be capable of transtadial transmission (Kuttler et al., 1967). Present data provide additional evidence regarding the demographic association of *A. americanum* and *Th. cervi* (Kuttler et al., 1967; Samuel and Trainer, 1970) and substantiate Kingston's (1981) assumption that the distribution of *Th. cervi* probably corresponds to that of *A. americanum,* its optimum vector.

Trypanosoma cervi is widely distributed in North America and occurs in several native cervids including white-tailed deer, mule deer (*O. hemonius*), and elk (*Cervus canadensis*) (Kingston, 1981). This report extends the known distribution of *Tr. cervi* to include Arkansas, Maryland, Tennessee, and West Virginia. During this study, a much higher (117/240) prevalence of *Tr. cervi* was detected utilizing conventional thin blood films than that reported earlier (0/500) for thin blood films examined from southeastern white-tailed deer (Kistner and Hanson, 1969). This difference is attributable to an artifact of technique in that *Tr. cervi* was found almost exclusively in the feathered edge of blood films during the present study. Because the feathered edges of blood films were not examined during routine hematologic examinations conducted by Kistner and Hanson (1969), in all probability, this factor accounts for the difference in detection rates.

Theileria cervi has been considered to be a contributing factor in a syndrome of malnutrition and parasitism in overpopulated deer herds. In contrast, deer that are otherwise healthy harbor *Th. cervi* without any apparent effects (Kingston, 1981). During the present study, specific effects attributable to *Th. cervi* were not encountered; however, *Th. cervi* was present in deer herds with, and possibly contributed to, a general syndrome of severe parasitism and malnutrition characterized by anemia and poor physical condition. *Trypanosoma cervi* has been considered essentially nonpathogenic (Kingston, 1981), and effects attributable to this parasite were not noted in the present study.

Acknowledgments

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Research Note

Taxonomy and Life Histories of Two North American Species of "Carneophallus" (=Microphallus) (Digenea: Microphallidae)

Digeneans from North American coastal waters referred to as *Carneophallus* spp. summon attention because they infect commercially important crustaceans, constitute potential public health hazards, and present taxonomic problems.

The nomenclature and taxonomy of worms placed into the genus *Carneophallus* Cable and Kuns, 1951 are not clearly defined. Bridgman (1969, Tulane Stud. Zool. Bot. 15:81–105) described the two species to which we direct most of our concern as *C. basodactylophallus* Bridgman, 1969 and *C. choanophallus* Bridgman, 1969. The type locality for both is the Mississippi River estuary. Later, Deblock (1971, Bull. Mus. Nat. Hist. Nat. 3^{e} Ser., Zool. 7:353–468) considered the former species as *Microphallus basodactylophallus* and the latter as a junior synonym of *M. turgidus* (Leigh, 1958). Even though he and Capron, Deblock, and Biguet (1957, Bull. Soc. Zool. Fr. 82:378–392) considered *Carneophallus* a junior synonym of *Microphallus* Ward, 1901, we regard the name valid for at least the type species, *C. trilobatus* Cable and Kuns, 1951, on the basis of the copulatory papilla being deeply lobed, partially banded by small papillae, and penetrated by the male duct, which opens through one of the lobes. Perhaps some or all of the several remaining species that have been placed in *Carneophallus* should be transferred to *Spelotrema* Jägerskiöld, 1901, with *Spelophallus* Jägerskiöld, 1908 as a junior synonym, rather than to *Microphallus* which, in fact, may be monotypic for *M. opacus* (Ward, 1894). No agreement concerning generic position of those species has been reached.

The range of *Microphallus basodactylophallus* extends at least from the coastal marshes of the Chesapeake Bay area to Louisiana and Texas. We have examined specimens of *M. basodactylophallus* from the Chesapeake Bay area, apparently conspecific with those referred to as *Megalophallus* sp. by Perkins (1971, J. Parasitol. 57:9–23) and Couch (1974, J. Invertebr. Pathol. 23:389–396), as well as specimens from Galveston, Texas, presumably conspecific with those reported as "similar to *Spelotrema nicolli*" by More (1969, Tex. Parks Wildl. Dep. Tech. Ser. 1:1–31). Actually, the species may extend to Costa Rica or further south and prove to be a junior synonym of *M. skrjabini* (Caballero, 1958).

Microphallus turgidus is known from New Jersey (Heard, 1970, Proc. Helminthol. Soc. Wash. 37:147–153) to Louisiana, and probably has a range similar to that of *M. basodactylophallus*. We follow Deblock (1971, loc. cit.) in considering *M. choanophallus* a junior synonym of *M. turgidus* because (1) the anatomy is similar for both taxa, (2) *M. choanophallus* uses several hydrobiid hosts, including *Litterodinops monroensis* (Frauenfeld), in common to both Gulf and Atlantic coasts, (3) *Palaemonetes pugio* is the principal crustacean hosts for both taxa, and (4) migratory birds serve as final hosts for both. Still, experimental cross-infection studies among and between Atlantic and Gulf of Mexico forms should be conducted to see if physiological races or sibling species occur.

Life histories of both digeneans include many more hosts than noted by Bridgman (1969, loc. cit.). He reported the cercaria of *M. basodactylophallus* to de-

velop in a spined form of *Lyrodes parvulus* Guilding (=*Pygophorus p.*), penetrate the blue crab, *Callinectes sapidus* Rathbun, and become a metacercaria infective to the raccoon, *Procyon lotor* (Linnaeus), and laboratory mice and rats. Heard (1967, M.S. Thesis, Univ. Georgia) noted *Hydrobia* sp. and *C. sapidus* as hosts for *Carneophallus* sp. (=*M. basodactylophallus*) in coastal Georgia, and Kinsella (1974, Am. Mus. Novit. No. 2540. 12 pp.) listed *Uca* spp. as hosts from Cedar Key, Florida. We infected the blue crab with cercariae of *M. basodactylophallus* from *Litterodinops palustris* Thompson from Jackson County, Mississippi, and Cedar Key in Levy County, Florida; from *Onobops jacksoni* (Bartsch) from Jackson County, Mississippi; and from two undescribed species of *Heleobops,* one from Horn Island, Mississippi, Little Dauphin, Alabama, and St. Marks Wildlife Refuge in Wakulla County, Florida, and the other from the same locality in Florida. Near Grand Isle, Louisiana, *Litterodinops monroensis* was infected. We additionally observed the presumed metacercariae in the digestive gland of *Uca longisignalis* Salmon and Atsaides and *U. minax* (Le Conte) from Mississippi, Alabama, and Florida and of *U. minax* and *U. pugnax* (Smith) from Georgia. The marsh rice rat, *Oryzomys palustris* (Harlan), was as good a natural definitive host as the raccoon for *M. basodactylophallus*. We successfully used the outbred albino mouse (COBS® CD®—1 stock) as the experimental host throughout our study.

Bridgman reported the cercaria of *C. choanophallus* (=*M. turgidus*) from the "unspined form of *Lyrodes parvula*" (=*Pygophorus p.*) to infect *Palaemonetes pugio* Holthuis and *Macrobrachium ohione* (Smith), which in turn infected the raccoon, Norwegian rat, and six experimental mammals. Previously, Heard (1967, loc. cit.) reported *Hydrobia* sp., *P. pugio, Rallus longirostris* Boddaert, and unnamed birds as hosts in Georgia. We observed developmental stages in *Litterodinops palustris* in Jackson County, Mississippi, Little Dauphin Island, Alabama, and Cedar Key, Florida; *L. monroensis* on Horn Island, Mississippi; and *L. tenuipes* (Couper) and *L. monroensis* from North Carolina to southeastern coasts of Florida. We observed the metacercaria in the abdominal musculature of *Palaemonetes pugio, P. vulgaris* (Say), *P. paludosus* (Gibbes), *Macrobrachium ohione,* and *Penaeus setiferus* (Linnaeus). Moreover, we experimentally infected *P. setiferus* with cercariae from *L. palustris* and *P. pugio* with cercariae from that snail and *L. monroensis*. Rather than mammals being the primary definitive hosts, our observations suggest that aquatic birds may be more important, supporting a hypothesis advanced by Yamaguti (1975, A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates. Keigaku Publ. Co., Tokyo. 1029 pp.). In Mississippi, we found the digenean infecting the red-breasted merganser, *Mergus serrator* Linnaeus; bufflehead, *Bucephala albeola* (Linnaeus); common goldeneye, *B. clangula americana* (Bonaparte); eastern green heron, *Butorides virescens virescens* (Linnaeus); little blue heron, *Florida caerulea caerulea* (Linnaeus); willet, *Catoptrophorus semipalmatus* (Gmelin); and clapper rail, and it probably infects many other birds. It often occurs in large numbers; we found over 2,000 adults of *M. turgidus* in one merganser.

Flame cells and cephalic glands are often difficult to observe. Whereas Bridgman (1969, loc. cit.) reported a flame cell formula of $2[(1+1) + (1+1)] = 8$ and two pairs of "penetration" glands in cercariae of both *M. basodactylophallus* and *M. turgidus,* we observed a formula of $2[(2+2) + (2+2)] = 16$ and four pairs

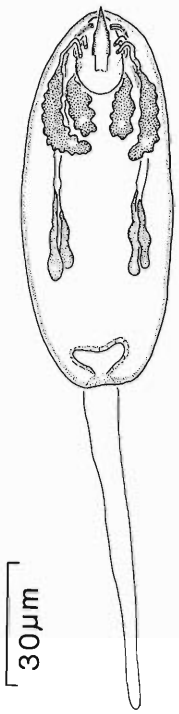
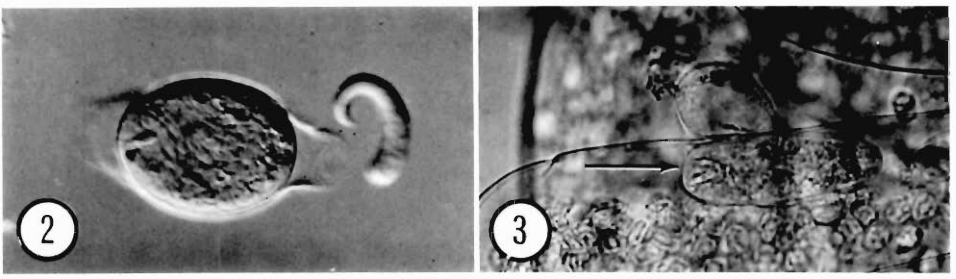


Figure 1. Heat-killed cercaria of *Microphallus basodactylophallus* from *Onobops jacksoni* in Jackson County, Mississippi, ventral view.

of glands of two types differing in size and location, as has become generally accepted as typical of microphallid cercariae (Fig. 1). Two pairs of larger glands posterolateral to the oral sucker narrow anteriorly as ducts that turn inward at about midlevel of the oral sucker and open at well-separated ventrolateral pores. Weak staining of these glands after forming a penetration cyst indicates their functioning in that process. Cercariae of both species form penetration cysts on the gills immediately after they attach and shed their tails (Figs. 2–3). If properly positioned, the penetration cyst allows rapid entrance into the lamellar sinus by the cercaria. Probably incorporated into the life history of many related microphallids, it is rarely reported (e.g., called pseudocyst by Prevot [1974, D.S. Thesis, Univ. Droit, Marseille] and penetration cyst by Helluy [1982, Ann. Parasitol. 57:263–270]). The other two pairs of glands lie slightly posterior to the midbody level, are smaller than the anterior glands, and take a much lighter stain with neutral red. Their ducts are narrower and extend anteriorly farther than those of the larger glands and open close together nearer the stylet. Because the posterior glands remain unchanged until encystment begins, they probably contribute to that process.

Our attempts to obtain *P. parvulus* from near the mouth of the Mississippi River where Bridgman collected snails proved unsuccessful, not allowing us to compare cercariae from those snails with the cercariae we describe. We, however, did collect infected *L. monroensis* and *L. palustris* from that area.

Behavior of the two species differs. The cercaria of *M. turgidus* swims faster



Figures 2–3. Penetration cysts. 2. Cysts of *Microphallus basodactylophallus* attached to a glass slide, showing encased larva and detached cercarial tail. 3. Cyst of *M. turgidus* on gill lamella of *Palaemonetes pugio* with underlying cercaria (arrow).

and more erratically than the larva of *M. basodactylophallus,* which stops periodically. Both species, however, stop if disturbed, such as when encountering respiratory currents of potential hosts, thereby allowing them to be swept into the branchial chamber of the host to penetrate the gills.

In heat-killed cercariae of *M. basodactylophallus* from *Litterodinops monroensis* near Grand Isle, Louisiana, the body measured 86–92 μm long by 38–40 μm wide, the tail 80–92 μm long, and the stylet 17 μm long. The entire stylet is easy to see in living specimens, but only the more heavily sclerotized portion 15 μm long is distinct in heat-killed specimens. The thin-walled metacercarial cysts averaged 233 (220–245) μm across at both 40 and 67 days. The metacercariae became infective at 25–30 days. Specimens in the blue crab for 40 days developed in mice and started discharging eggs within 48 hr.

In heat-killed cercariae of *M. turgidus* shed by *L. palustris* from Ocean Springs, Mississippi, the body measured 76–80 μm by 38–44 μm, the tail measured 80–90 μm long, and the stylet length appeared 15 μm in fixed material and 18 μm in fresh specimens. The tail detached when the cercaria attached to the gill lamella. and produced the penetration cysts. The cyst averaged 62(53–75) μm long by 41(39–43) μm wide by 36(34–40) μm high. After entering the circulatory system, the body could be seen rotating irregularly along the major vessels. By 18 hr postexposure, most of the worms lodged in the hepatopancreas and abdominal musculature and started developing a cyst wall. By 21 days, the metacercaria was infective, and after 30 days, its vitelline follicles became more compact and its cyst wall thicker. A 30-day-old metacercaria placed in 0.85% saline at 39–40°C began to produce eggs within 8 hr. In grass shrimps, the cyst had a thick, laminated, outer layer of host origin. The cyst from *Palaemonetes pugio* measured 278–420 μm long by 222–333 μm wide including that outer layer; without it, the cyst measured 247–290 μm by 203–222 μm. The relatively thin-walled cyst in the white shrimp at 46 days measured 320 by 220 μm.

Limited data on blue crab infections with *M. basodactylophallus* suggest that the cercaria from *Litterodinops palustris* encysts only in the thoracic ganglion. The cercaria of *M. basodactylophallus* from other snails encysts mostly in the hepatopancreas, but it often passes through the circulatory system and lodges within the somatic musculature. Because of the potential harm to the blue crab fishery from neurological infections, we recognize the need to see if different forms exist by infecting a variety of snails with eggs from adults originating both

from *L. palustris* and crab nervous tissue and from other snails and crab hepatopancreas. In any event, a heavy infection with metacercariae of either species from any source can kill the crab or shrimp hosts.

In addition to possibly harming the crustacean host, either digenean might also be able to harm man. *Microphallus brevicaeca* (Africa and Garcia, 1935) (syn. *Carneophallus b.*) has been implicated in adverse and fatal involvement of the heart, spinal cord, and other vital organs of people in the Philippines (e.g., Africa, Leon, and Garcia, 1937, Philipp. J. Sci. 62:393–399). Even though crustaceans have not been eaten raw historically in the United States as they have in the Philippines, recent innovations in cuisines give Americans the opportunity to eat more raw or minimally cooked shrimps and crabs.

We thank Ralph Lichtenfels for the opportunity to examine specimens of *C. trilobatus* and other microphallids from the U.S. National Museum Helminthological Collection. Tom Mattis collected the naturally infected white shrimp. The study was conducted in cooperation with the U.S. Department of Commerce, NOAA, NMFS, under PL 88-309, projects 2-325-R and 2-382-R.

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Research Note

The Geographical Distribution of an Adult Trematode, *Proctoeces maculatus*, in the Gastropod *Nucella lapillus* from New England

The gastropod *Nucella lapillus* (L.) (Prosobranchia: Muricidae) is a common intertidal predator found along the rocky coasts of North America and Europe. A study of the parasites of this snail revealed the presence of adult *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 (Digenea: Fellodistomidae). The present study reports the geographical distribution of this parasite from New England populations of *N. lapillus*.

Snails were collected from eight sites along the New England coast (Eastport, Maine; New Harbor, Maine; Pemaquid Pt., Maine; Manomet, Massachusetts; Block Is., Rhode Island; Pt. Judith I, Rhode Island; Pt. Judith II, Rhode Island; Avery Pt., Connecticut) from May 1980–Aug. 1981. They were measured, crushed, sexed, and then examined for parasites. The BMDP-3F program (Dixon and Brown, 1979, Biomedical Computer Programs, University of California Press, Los Angeles), which uses multiple contingency tables, was used to examine whether parasitism was independent of a snail's size and sex.

None of the snails examined from sites north of Cape Cod, Massachusetts harbored adult *P. maculatus*, while to the south, almost all infections were recorded from Pt. Judith II (Table 1). Eighty-two adults were found in the kidneysof

54 snails ($\bar{x}$ = 1.5 ± 1.5 [Range 1–9] parasites per infected snail), of which 55% were ovigerous, 30.5% were nonovigerous mature forms, and 11.0% immatures and metacercariae. Ovigerous adults were found throughout the year, whereas other stages were more prevalent from late fall to early winter. Statistical analysis revealed that parasitism was independent of both snail size and sex (Pearson χ^2 = 5.58, P = 0.59, 7 df).

Proctoeces maculatus, a fish parasite in the warmer waters of its range, can mature in a variety of molluscs in temperate seas (Bray and Gibson, 1980, Bull. Br. Mus. Nat. Hist. (Zool.) 37:199–293). Infections have been reported from the gastropods *Nucella lapillus* and *Buccinum undatum* from the English Channel, Great Britain (Bray and Gibson, 1980, op. cit.), and *Rissoa splendida* from the Black Sea, USSR (Dolgikh, 1967, Parazitologiya 1:219–221). Additional gastropod hosts would be added to these if Bray and Gibson (1980, loc. cit.) are correct in synonymizing all species of *Proctoeces* with *P. maculatus*. Until the present study the mussel *Mytilus edulis* was the only reported invertebrate host for ovigerous adults of *P. maculatus* in North America (Stunkard and Uzmann, 1959, Biol. Bull. 116:184–193). However, precocious metacercariae (nonovigerous) of *P. maculatus* were reported in the mussels *Ischadium recurvum* and *Mytilopsis leucopheata* from Texas (Wardle, 1980, Bull. Mar. Sci. 30:737–747) and those of *Proctoeces* sp. (=?*P. maculatus*) in *Crassostrea virginica* from estuaries along the Gulf of Mexico in Mississippi, Alabama, and Florida (Winstead and Couch, 1981, Trans. Am. Microsc. Soc. 100:296–305). Larval stages of *P. maculatus* have been reported from North America in *I. recurvum* from the Gulf of Mexico (Hopkins, 1954, J. Parasitol. 40:29–31; Wardle, 1980, op. cit.) and in *Mytilus edulis* from the Atlantic Coast (Uzmann, 1953, J. Parasitol. 39:445–451; Lang and Dennis, 1976, Ophelia 15:65–75). Populations of *Mytilus edulis* north of Cape Cod were negative for both larval and adult *P. maculatus* (Uzmann, 1953, op. cit.; Pondick, unpubl. obs.).

Proctoeces maculatus shows a marked seasonal cycle in *M. edulis* from coastal New Jersey, where the prevalence of adults peaks in early winter and drops off to zero by mid-spring (Lang and Dennis, 1976, op. cit.). I have observed a similar pattern for mussels from Rhode Island and Connecticut, but did not observe such a pattern in the snails of this report. This suggests that adult *P. maculatus* naturally infecting *N. lapillus* might live considerably longer than the 30-day maximum life span of adult *Proctoeces* reported for experimentally infected fish (Freeman, 1962, J. Mar. Biol. Assoc. U.K. 43:113–123; Sakaguchi et al., 1970, Bull. Natl. Pearl Res. Lab. 15:1939–1947). Supporting evidence is provided by field and experimental observations (Shimura, 1980, Mar. Ecol. Prog. Ser. 3:145–149) showing that adult *P. ichiharai* can live at least 14 mo in the top shell, *Batillus cornutus*. It is not possible to determine whether this situation is typical because there are no published data concerning the life span of adult *P. maculatus* in their natural fish hosts.

The restriction of both adult and larval *P. maculatus* to populations of *N. lapillus* and *M. edulis* south of Cape Cod suggests that the colder waters north of the Cape might be an effective barrier to this parasite. Furthermore, rather specific ecological conditions may be required for infection of this snail, as illustrated by the differences in infections found between the Pt. Judith sites (Table 1).

Table 1. Geographical distribution and prevalence of adult *Proctoeces maculatus* in *Nucella lapillus*.

Site	*N*	% Parasitized
Northern		
Eastport, Maine	961	0
Pemaquid Pt., Maine	1,262	0
New Harbor, Maine	225	0
Manomet, Massachusetts	942	0
Southern		
Block Is., Rhode Island	464	0.2
Pt. Judith I, Rhode Island*	196	0
Pt. Judith II, Rhode Island*	1,117	4.7
Avery Pt., Connecticut	312	0.3
Total	5,479	1.0

* Pt. Judith sites I and II, an exposed rock-boulder beach and the exposed side of a jetty, respectively, were less than ¼ mi apart.

I would like to thank L. R. Penner, C. W. Schaefer, and G. W. Benz for reading the manuscript, C. Katz and U. Koehn for statistical advice, and R. A. Bray for providing a copy of his paper. Computer facilities were provided by the University of Connecticut Computer Center.

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Proc. Helminthol. Soc. Wash.
50(1), 1983, pp. 176–178

Research Note

Occurrence of Two Species of *Neoechinorhynchus* (Acanthocephala) in Golden Shiners of Alabama and Mississippi

During February, March, and April of 1981, golden shiners, *Notemigonus crysoleucas* (Mitchell), were collected in west Alabama and central Mississippi and examined for acanthocephalans. Two species of *Neoechinorhynchus* not previously known from this area were collected. All measurements are in micrometers with averages in parentheses unless otherwise stated.

Fifty-seven specimens of *Neoechinorhynchus notemigoni* Dechtiar, 1967 were obtained from 10 of 30 golden shiners collected in tributaries of the Tombigbee River in Choctaw County, Alabama, and tributaries of the Tallahatchie and Quiver rivers in LeFlore County, Mississippi. *Neoechinorhynchus notemigoni* was previously known only from its type locality, Lake Ontario (A. Dechtiar, 1967, Can. J. Zool. 45:155–159).

Neoechinorhynchus notemigoni is distinguished (Dechtiar, 1967, loc. cit.) by its cylindrical proboscis, possession of lemnisci of unequal lengths, and small

Table 1. Measurements, in micrometers, of selected features of *Neoechinorhynchus notemigoni* Dechtiar, 1967 as originally described from Canada and as present in specimens from the southeastern United States.

	Trunk length	Proboscis length	Proboscis hook length			Egg dimensions
			Ant.	Mid.	Bas.	
Males						
Canada	2,830–3,700	79–99	24–28	18–31	15–23	—
US	2,060–4,500	71–92	24–28	19–26	19–21	—
Females						
Canada	3,300–6,200	89–99	26–39	20–29	17–23	10–22 × 10–17
US	3,180–7,500	71–104	28–36	21–26	17–24	19–21 × 14–17

trunk, proboscis, proboscis hooks, and eggs. Mature specimens from the present collection (13 males with spermatozoa and 17 females with eggs) exhibit only minor deviations from those specimens originally described (see Table 1). Although the largest eggs observed were about equal to the maximum egg dimensions originally reported, no mature eggs as small as the reported minimum dimensions were found. This difference may be due, in part, to the difficulty in distinguishing mature from immature eggs. Females have subterminal genital pores. However, females were originally described as having terminal genital pores. Examination of the allotype (USNM No. 62879) and a paratype (USNM No. 62880) revealed the genital pore to be subterminal. The greatest deviation from the original description observed in the present specimens is the length of the lemnisci. Uninucleate lemnisci are 525–1,200 long in males and 825–1,763 long in females. Binucleate lemnisci are 1,013–1,763 long in males and 1,500–3,188 long in females. These lengths exceed those reported originally.

Specimens have been deposited in the USNM Helminth Collection (No. 77017).

The second species is tentatively identified as *Neoechinorhynchus rutili* (Mueller, 1780) Hamann, 1892. Five males and two females were obtained from two of eight golden shiners collected in tributaries of the Tallahatchie and Quiver rivers in LeFlore County, Mississippi. Only one specimen, a male with a trunk length of 4.2 mm, is mature. One female (3.2 mm long) has an unfragmented ovary and the other female (5.3 mm long) contains numerous ovarian balls. All specimens possess a short, globular proboscis, 88–95 (91) long by 88–100 (94) wide in males and 107, 111 long by 119, 123 wide in females. Lengths of proboscis hooks are apical, middle, and basal rows 59; 40–45 (42); and 19 in males and 64, 66; 38, 50; and 21, 24 in females.

Praesomal characters and trunk lengths of these specimens are consistent with the description of *N. rutili* provided by Van Cleave and Lynch (1950, Trans. Am. Microsc. Soc. 69:156–171). Specimens were compared to a series of *N. rutili* from Ohio and Nebraska made available by Dr. Brent Nickol (The University of Nebraska–Lincoln). Except for having slightly larger mid-proboscis hooks, these specimens are in agreement with that series of *N. rutili*.

Specimens are close to *Neoechinorhynchus saginatus* Van Cleave and Bangham, 1949. However, they differ in that they possess a smaller proboscis and longer mid-proboscis hooks. Also, these specimens are not as robust as *N. saginatus*. Enlargement of the trunk where it joins the praesoma, as described for

N. saginatus, is lacking. Instead, the trunk tapers gradually from the first dorsal hypodermal nucleus to its junction with the praesoma.

There are numerous reports of the occurrence of *N. rutili* in various areas throughout the Northern Hemisphere. In North America most reports of *N. rutili* are from areas in Canada and the northern portion of the United States. However, it has been reported as far south as central Missouri (J. Y. Niederkorn, 1974, Trans. Mo. Acad. Sci. 7–8:160–163).

Specimens have been deposited in the USNM Helminth Collection (No. 77018).

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Research Note

New Host Records for Some Nematode Parasites of Fishes from Alabama and Adjacent Waters

More than 5,000 specimens representing 252 species of fishes were examined for parasites in Alabama and adjacent waters (Williams, 1974, Diss. Abstr. 35: 1461; Williams and Gaines, 1974, J. Mar. Sci. Ala. 2:135–148). Only nematodes with new host records were considered in this report. Host fishes were collected between March 1969 and May 1974 with 3-, 4.6-, and 15.2-m seines, monofilament gill and trammel nets, hook and line, gig, dip net, rotenone, trawl, speargun, crab-trap, boat and back pack shockers, and lift net. They were either examined immediately or held alive and examined within 24 hr of capture. The alimentary system, coelomic cavity, gills, swim bladder, and urinary bladder of all fishes were examined, as well as the kidney, liver, spleen, heart, brain and anterior spinal cord, and muscle of some. Nematodes were fixed in hot 5% formalin, prepared by the standard alcohol/glycerine method, and examined in glycerine, glycerine jelly, or lactophenol. Specimens of all nematode species are deposited in the United States National Museum Helminth Collection (USNM). Host species, numbers examined, and size ranges for marine fishes were listed by Williams and Gaines (1974, loc. cit.); all species of fishes examined were listed by Williams (1974, loc. cit.). All localities are in Alabama unless otherwise noted. All infections occurred in the intestine of the host. Twenty new host records are reported for seven species of adult nematodes (Table 1).

Family Anisakidae

Hysterothylacium reliquens (Norris and Overstreet)

REMARKS: Norris and Overstreet (1975, J. Parasitol. 61:330–336) recovered specimens of *H. reliquens* in the slippery dick, *Halichoeres bivittatus* (Bloch.).

No *H. reliquens* were recovered from four adult specimens of *H. bivittatus* examined from Perdido Bay during the 1972 and 1973 collections. The striped burrfish, *Chilomycterus schoepfi* Walbaum, and the sheepshead, *Archosargus probatocephalus* (Walbaun), were also parasitized by this nematode.

Family Hedruridae

Hedruris sp. of Sullivan

REMARKS: This nematode is a new species that is being described by Dr. Joseph R. Sullivan (pers. comm.) from another species of host (Sullivan, 1977, Dissertation, Auburn Univ.).

Family Cystidicolidae

Spinitectus carolini Holl

REMARKS: The bluegill, *Lepomis macrochirus* Rafinesque; redear sunfish, *L. microlophus* (Gunther); and largemouth bass, *Micropterus salmoides* (Lacepede), were also parasitized by this nematode.

Spinitectus gracilis Ward and Magath

REMARKS: One specimen examined in the present study was longer (17.4 mm) than the longest measurement given for *Spinitectus gracilis* by Van Cleave and Mueller (1932, Roosevelt Wildl. Ann. 3:79–138). The redbreast sunfish, *Lepomis auritus* (Linnaeus); green sunfish, *L. cyanellus* Rafinesque; *L. macrochirus*; and *Micropterus salmoides* were parasitized by this nematode.

Spinitectus micracanthus Christian

REMARKS: The majority of the specimens of *Spinitectus micracanthus* possessed an esophagus beginning at the first spine row, as described by Christian (1972, Proc. Helminthol. Soc. Wash. 39:51–54); however, in a few specimens the esophagus began between the first and second spine row, and in some at the second spine row. These differences cannot be explained by any differences in fixation or handling in the material. *Lepomis macrochirus* was also parasitized by this nematode.

Family Camallanidae

Camallanus oxycephalus Ward and Magath

REMARKS: The silverjaw minnow, *Ericymba buccata* Cope, was also parasitized by this nematode.

Spirocamallanus cricotus Fusco and Overstreet

REMARKS: The new host records add five families of hosts (Ariidae, Blennidae, Carangidae, and Poeciliidae) to the nine previously reported by Fusco and Overstreet (1978, J. Parasitol. 64:239–244). The silver perch, *Bairdiella chrysura* (Lacepede); banded drum, *Larimus fasciatus* Holbrook; spot, *Leiostomus xanthurus* Lacepede; southern kingfish, *Menticirrhus americanus* (Linnaeus); Atlantic croaker, *Micropogonias undulatus* (Linnaeus); southern flounder, *Paralichthys lethostigma* Jordan and Gilbert; Atlantic threadfin, *Polydactylus octonemus* (Girard); blackcheek tonguefish, *Symphurus plagiusa* (Linnaeus); and hogchoker,

Table 1. New host records for nematode parasites of some Alabama and Florida fishes.

Parasite	Host	Locality, date	Number of hosts examined/number infected/average number of worms per infected host	USNM Helm. Coll. No.
Hysterothylacium reliquens (Norris and Overstreet)	*Diplodus argenteus* (Valenciennes) (silver porgy)	Mouth of Perdido Bay, 31 September 1972	1/1/3	76495
	Urophycis floridanus (Bean and Dresel) (southern hake)	Pensacola Bay, Florida, 21 March 1969	3/2/2	76496
Hedruris sp. of Sullivan	*Dorosoma petenense* (Gunther) (threadfin shad)	Auburn Experimental Station Ponds, Lee County, 10 April 1970	5/2/3.5	76501
	Esox americanus Gmelin (redfin pickerel)	Uphapee Creek, northeast of Tuskegee, Macon County, 28 March 1970	1/1/3	76502
Spinitectus carolini Holl	*Percina nigrofasciata* (Agassiz) (blackbanded darter)	Halawakee Creek, covered bridge, Lee County, 20 February 1969	3/3/1.7	76503
Spinitectus micracanthus Christian	*Lepomis cyanellus* Rafinesque (green sunfish)	Calabee Creek, south of Tuskegee, Macon County, 19 July 1971	1/1/5	76505
	Lepomis microlophus (Gunther) (redear sunfish)	Saughatchee Creek, Tallapoosa County, 22 February 1970	5/4/3.8	76507
	Micropterus punctulatus (Rafinesque) (spotted bass)	Cahaba River, Perry County, August 1968	1/1/2	76506
Camallanus oxycephalus Ward and Magath	*Minytrema melanops* (Rafinesque) (spotted sucker)	Wehaokee Creek, Chambers County, at Alabama-Georgia state line, 25 August 1972	1/1/3	76499
Spirocamallanus cricotus Fusco and Overstreet	*Porichthys poroissimus* (Valenciennes) (Atlantic midshipman)	Mississippi Sound north of Dauphin Island, 11 August 1969	5/1/1	76492
	Hypsoblennius ionthas (Jordan and Gilbert) (freckled blenny)	Mouth of Perdido Bay, 22 September 1972	1/1/1	76487
	Etropus crossotus (Jordan and Gilbert) (fringed flounder)	Gulf of Mexico south of Dauphin Island, and mouth of Mobile Bay, August 1969, 4 June 1970	10/7/3.6	76486

Table 1. Continued.

Parasite	Host	Locality, date	Number of hosts examined/number infected/average number of worms per infected host	USNM Helm. Coll. No.
	Bagre marinus (Mitchill) (Gafftopsail catfish)	Mississippi Sound north of Dauphin Island, 15 July 1969	24/1/1	76485
	Lutjanus griseus (Linnaeus) (gray snapper) (juvenile)	Boat slip, Dept. Conservation and Natural Resources, eastern end of Dauphin Island, 21 August 1969	1/1/1	76489
	Menticirrhus saxatilis (Bloch and Schneider) (northern kingfish) (juveniles)	Ship channel, Mobile Bay and Mississippi Sound north of Dauphin Island, 16 July, 11 August 1969	5/1/1	—
		South of Dauphin Island, 12 August 1969	3/2/2.5	76490
	Trachinotus falcatus (Linnaeus) (permit) (juveniles)	East end of Dauphin Island, 9 June 1970	4/3/1.7	76494
	Trachinotus carolinus (Linnaeus) (pompano) (juveniles)	South beaches of Dauphin Island, 1969–1972	200/22/12	76493
	Poecilia latipinna (Lesueur) (sailfin molly)	Pools on eastern end of Dauphin Island, July 1969	2/2/1.5	—
		Pools on eastern end of Dauphin Island, 3 June 1970	12/1/1	76491
	Lutjanus apodus (Walbaum) (schoolmaster)	Mouth of Perdido Bay, 31 September 1972	10/2/1	76488
Dacnitoides cotylophora Ward and Magath	*Ictalurus catus* (Linnaeus) (white catfish)	Lake Okeechobee, Okeechobee County, Okeechobee, Florida, 25 October 1972	11/8/4	76500

Trinectes maculatus (Bloch and Schneider), were also parasitized by this nematode.

Family Cucullanidae

***Dacnitoides cotylophora* Ward and Magath**

REMARKS: The channel catfish, *Ictalurus punctatus* (Rafinesque), was also parasitized by this nematode in the Florida location.

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PRESENTATION

1982 Anniversary Award of the Helminthological Society of Washington

Mr. President, Guests, and Members of the Helminthological Society of Washington:

Tonight we recognize a fellow member who has, over the years, not only made significant contributions to the field of parasitology, but has supported, nurtured, and furthered this society. It is my pleasure to present the 1982 Anniversary Award to Dr. Lloyd Eugene Rozeboom.

Dr. Rozeboom was born October 17, 1908 in Orange City, Iowa—the capital of the U.S. Rozeboom clan. Despite his departure, the Rozeboom name still dominates any issue of the Orange City Chronicle. In the fall of 1927, he entered Morningside College at Sioux City, Iowa. After 2 years he transferred to Iowa State University where he graduated in 1931 with a B.S. in entomology and with a Phi Kappa Phi Key. He began his post graduate education that fall as a teaching assistant in Medical Entomology under the late Francis Metcalf Root. His graduation with the Sc.D. in 1934 marked the beginning of a long and brilliant career as a medical entomologist.

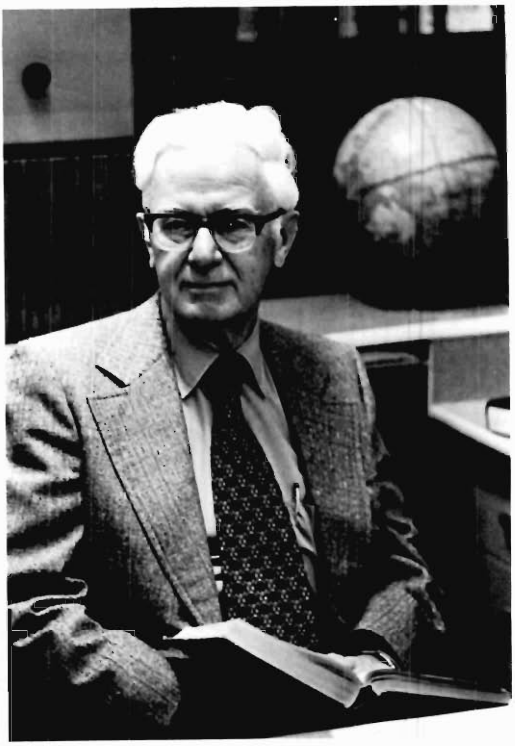
He first went to the Gorgas Memorial Institute in Panama (Canal Zone) as the Medical Entomologist in a team of three young scientists which included Aurel O. Foster (Helminthologist) and Carl M. Johnson (Protozoologist). He spent three years (1934–37) helping to develop solutions to the anopheline-malaria control problem.

This was followed by two years (1937–39) as Associate Professor of Medical Entomology at Oklahoma A & M University (Stillwater). There he deliniated the anopheline-malaria-vector problem and inquired into the transmission of bovine anaplasmosis. While at Oklahoma A & M, he also met Mae Thompson, a clothing specialist in the University's extension service. Tired of his own cooking, he courted and married Mae on August 4, 1939. It has been suggested by some of his long time colleagues that this is why he always looks so complacent and well fed.

Except for his World War II service in the Navy, he has remained at the Johns Hopkins University School of Hygiene and Public Health as Associate Professor (1939–58), Professor (1958–74), and Emeritus Professor of Medical Entomology (and consultant and lecturer, 1974–date).

In 1941, he went to Trinidad for the Army and unraveled the enigma of *Anopheles belletor*, arborial bromeliades, immortelle trees, cocoa plants, and malaria. In the process he acquired malaria which was not a scheduled part of the research design. He had to take time out from his chills and fever to identify the "strange schizonts" in his own blood. They were schizonts of malignant tertian malaria (*P. falciparum*).

In 1944 he was commissioned in the Navy and with Kenneth Knight and Jean Laffoon conducted extensive studies of the mosquitoes in the Pacific Islands. After the war he remained in the reserves, retiring as Captain and commanding officer of his unit in Baltimore.



Dr. Lloyd E. Rozeboom

Dr. Rozeboom's research and that of his graduate students has been mainly on the biology, ecology, and taxonomy of mosquitoes as disease vectors. This has included experimental studies on the influence of genetics and environmental factors on the susceptibility or resistance of mosquito strains and populations to malaria and filariae. These studies have provided the basis for numerous mosquito control programs. Although not heavily involved in control himself, Dr. Rozeboom did experimentally evaluate a possible competitor-predator mosquito for control of a prime vector of filariasis on a South Pacific Island.

That mosquito control *per se* is not his prime interest is illustrated by 2 Christmas cards his team sent from the South Pacific during the war. All members of the team, except Lloyd, were armed with either a fly swatter or a spray gun. Rozie had his protecting arm around an overgrown mosquito, naturally a female, while his other hand held a do not touch sign.

In 1976, Dr. Rozeboom went back to Orange City to receive an honorary Sc.D. from Northwestern College in that city. Other honors include the Bailey K. Ashford award by the American Society of Tropical Medicine and Hygiene in 1941, election to Phi Beta Kappa in 1971, and the John M. Belkin Memorial Award by the American Mosquito Control Association in 1982.

Dr. Rozeboom has held numerous offices in professional societies including Treasurer, 1941–43, and Vice President, 1963, of the American Society of Parasitologists; Treasurer, 1970, of the Second International Congress of Parasitology; and President, 1974, of the American Society of Tropical Medicine and Hygiene. In 1940, he presented the first of seven papers given at Society

meetings and became a member in 1951. In 1975 he was elected a Life Member. He has served the Society as a member of the Executive Committee, 1954; Vice President, 1958; President, 1961; and Assistant Treasurer, 1969–71. In 1969, he delivered the Anniversary Address. Dr. Rozeboom has not only served as a member and chairman of the Awards Committee, but also presented the First Anniversary Award to Miss Edna Buhier, thus it seems particularly fitting that he should now be on the receiving end.

On behalf of the Society's membership and the other members of the Awards Committee (Dr. R. L. Beaudoin and Dr. H. G. Sheffield) is my pleasure and honor to present this award to Dr. Rozeboom.

M. D. Ruff, Chairman, Anniversary Award Chairman

Acceptance of the 1982 Anniversary Award

Let me assure you that I was not a little surprised when informed that I had been selected as the recipient of the 1982 Anniversary Award of the Helminthological Society of Washington. It is a cherished honor and I am most grateful to Dr. Ruff and his committee. I should like to think that this also is a recognition of the long association between our department at the Johns Hopkins School of Hygiene and Public Health and the Helminthological Society.

The Department of Protozoology and Medical Zoology began its operations on October 1, 1918, under Dr. Robert Hegner's direction, and by the following year the members of the staff, which by then included Drs. W. W. Cort and F. M. Root, had been elected to membership in the Society. From that time on, with few exceptions, the Society has held one of its meetings each year at the School of Hygiene and Public Health. Hegner organized his department to include what he considered to be the three basic categories of public health parasitology: protozoology, helminthology, and medical entomology. Throughout these many years, the close association between our department and the Helminthological Society, which has been so valuable to both our students and faculty, underscores the broad parasitological expertise that is so characteristic of this Society, for it is in our meetings and in the Proceedings that protozoologists and even medical entomologists join with the helminthologists in furthering the progress of the science of parasitology. I think this is reemphasized by your selection of me, a medical entomologist, as the recipient of this year's Award.

Again, thank you very much.

Sincerely,
LLOYD E. ROZEBOOM

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Proceedings is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues are payable on notification of election. Send this completed form to:

The Recording Secretary
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