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# PROCEEDINGS

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# Larval *Hysterothylacium* (=*Thynnascaris*) (Nematoda: Anisakidae) from Fishes and Invertebrates in the Gulf of Mexico

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ABSTRACT: Numerous fishes and invertebrates in the northern Gulf of Mexico contain larvae of four forms of *Hysterothylacium*: ?*H. reliquens* (=type MA, Mississippi A), type MB, *H. fortalezae* (=type MC), and type MD. The third-stage larvae (possible second-stage larva of type MB) of all are described and illustrated; furthermore, corresponding fourth-stage larvae of all but type MB are described. A key to all these inshore and nearshore larvae is included. Biological differences among related species are exemplified using *H. reliquens* and *Iheringascaris inquies*. The second-stage larva of *I. inquies* survives much longer in cold water than that of *H. reliquens*, and the fourth-stage larva of *I. inquies* is unique because it invades alimentary tissue of the final host.

Adult nematodes of the genus *Hysterothylacium* Ward and Magath, 1917 are restricted to the digestive tract, with one exception (Machida et al., 1978), of fishes, whereas their larvae parasitize various tissues of numerous fishes and invertebrates (e.g., Norris and Overstreet, 1976). Deardorff and Overstreet (1981) reviewed the genus, previously known as *Thynnascaris* Dollfus, 1933 or *Contracaecum* sensu lato, and examined adults of six species from the Gulf of Mexico.

In this paper, we describe the larvae of four forms of *Hysterothylacium* following the nomenclature initiated by Norris and Overstreet (1976) and provide a listing of hosts. The number of species in the inshore northern Gulf of Mexico may be greater because more than one species could have morphologically similar larvae. However, some known adults from the Gulf of Mexico are rare and not found in inshore northern regions where most larval specimens were collected. On the other hand, two forms may be different phases of the same species, and we may have included larvae of the related *Iheringascaris inquies* (Linton, 1901) Deardorff and Overstreet, 1981 which is not *Hysterothylacium* sensu stricto, even though placed in the genus *Thynnascaris* by most recent workers (see Deardorff and Overstreet, 1981).

Our studies of the ascaridoid fauna in the Gulf of Mexico have differentiated at least one species that is a potential public health hazard. Norris and Overstreet (1976) established that *Hysterothylacium* type MB rapidly penetrated the alimentary tract of white mice. That study has been expanded (Ebert and Norris, personal communication), and the worm also causes lesions and diffuse mucosal hemorrhaging in the rhesus monkey (Overstreet and Meyer, 1981). Petter (1969a, b) hypothesized that *H. aduncum* (Rudolphi, 1802) Deardorff and Overstreet, 1981 (as *Thynnascaris adunca*) from *Sardina pilchardus* (Walbaum) was the causative agent for several cases of anisakiasis in man; however, Vermeil et al. (1975) determined that the suspected nematode did not penetrate the rabbit stomach and questioned the hypothesis. Under stated experimental conditions, *Hysterothylacium* type MA did not penetrate tissues in mice (Norris and Overstreet, 1976).

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Nevertheless, species of *Hysterothylacium* other than type MB may be capable of penetrating the alimentary tract of mammals. In contrast, reviews on human anisakiasis (e.g., Jackson, 1975) consider only those forms that mature in marine mammals or birds, excluding those that mature in fishes.

## Materials and Methods

Fishes were eviscerated, and the viscera and remaining portions of each fish were examined separately. The somatic musculature was either examined by minute dissection under stereoscopic magnification, digested using a gastric juice solution maintained at 37°C for 3–4 hr in a shakerbath, or candled. To isolate larvae from viscera, we removed them individually or placed entire organs into aluminum screen cones set within saline-filled conical glasses for 8–15 hr at 23–26°C. Second-, third-, and fourth-stage larvae ($L_2$'s, $L_3$'s, and $L_4$'s) emerged from the viscera and were gathered from the bottom of the glass containers. Entire or portions of invertebrates were crushed and treated like viscera. Larvae were fixed in glacial acetic acid, stored in a solution of 5 parts glycerin plus 95 parts 70% ethyl alcohol, and later examined in lactic acid or in glycerin after evaporation of the alcohol from the storage solution. Selected worms and tissues were embedded in paraffin, sectioned, and stained with Harris' hematoxylin and eosin. Measurements locating the position of the nerve ring were taken from the anterior extremity of the worm to the center of the nerve ring. All measurements are in micrometers unless stated otherwise, and figures were drawn with the aid of a drawing tube. Most common names for fishes follow those of the American Fisheries Society's list (Bailey, 1970).

We investigated tolerances for combinations of two temperatures and three salinity concentrations by $L_2$'s of *Iheringascaris inquies* and *Hysterothylacium reliquens* (Norris and Overstreet, 1975) Deardorff and Overstreet, 1981. Within 5 hr of hatching, $L_2$'s from eggs recently laid in 15‰ seawater were placed into 55-mm glass stacking dishes containing 0, 15, or 30‰ artificial seawater (Instant Ocean® seasalts and artesian well water). For each species, one of these dishes containing about 1,000 larvae at each salinity level was placed into 4 ± 1°C or 28 ± 1°C temperature baths. Daily, about 40–50 larvae were pipetted from each stacking dish into stender dishes, and at least 20 were assessed for viability. If a larva moved after being probed, it was considered alive. Those larvae taken from 4°C were maintained at room temperature for up to 8 hr before being examined. Every second day, most dead larvae were removed from the primary stocks, and the water was exchanged with additional solutions at the same temperatures.

## Taxonomy

### *Hysterothylacium* Ward and Magath, 1917

Diagnosis of $L_3$: Body elongate, reaching greatest width near midbody. Cuticle with annules weakly defined (or not described). Cuticular alae present or absent. Boring tooth projecting anterio-ventrad; lips lacking. Ventriculus nearly spherical; ventricular appendage saclike or cylindrical; intestinal cecum usually shorter than ventricular appendage. Excretory pore located at or near level of nerve ring. Rectal glands present (or not described). Tail usually conical; tip of

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tail with or without ornamentation. Larvae encysted or free in body cavity, viscera, and musculature of finfishes and shellfishes.

DIAGNOSIS OF $L_4$: Same as $L_3$ except body larger; boring tooth absent; lips forming; reproductive system forming.

COMMON CHARACTERISTICS AMONG NORTHERN GULF OF MEXICO SPECIMENS: Body relatively wider with increased length of specimen. Cuticle with narrow annules; annules most visible at posterior end. Esophagus clavate; ventricular appendage cylindrical; intestinal cecum shorter than ventricular appendage. Ratios of lengths of ventricular appendage to esophagus, cecal to ventricular appendage, and cecal to esophagus not always related directly with length of worm. Excretory pore located at or near nerve ring. Rectal glands surrounding rectum. Tip of tail with or without ornamentation.

### ?*Hysterothylacium reliquens* (Norris and Overstreet, 1975) (Figs. 1, 5, 9, 13)

*Thynnascaris* type MA Norris and Overstreet, 1976.

DESCRIPTION OF $L_3$ (based on 30 specimens from *Micropogonias undulatus*): Body 4.0–9.0 mm long by 67–259 wide at greatest width; ratio of greatest width to length 1:26–61. Cuticle lacking lateral alae. Esophagus 0.4–1.0 mm long or 6–18% of body length. Ventriculus 26–50 long by 33–53 wide; ventricular appendage 278–599 long by 16–29 wide; ratio for lengths of ventricular appendage to esophagus 1:1.0–2.4. Intestinal cecum 120–550 long by 31–96 wide; ratio of cecal to ventricular appendage lengths 1:1.0–4.0; ratio of cecal to esophagus lengths 1:1.5–5.0. Nerve ring located within anterior 24–45% of esophagus, 14–29 in breadth. Excretory pore adjacent to or immediately posterior to nerve ring. Rectal glands nearly spherical, numbering 4. Tail 84–159 long or 1.0–4.0% of body length including spinous structure 12–29 long; spinous tip possessing numerous spines 1–2 long.

EARLY ENSHEATHED $L_4$ (based on 30 specimens from *M. undulatus*): Body 4.3–9.2 mm long by 80–173 wide at greatest width; ratio of greatest width to length 1:31–83. Cuticle lacking lateral alae. Esophagus 0.5–1.3 mm long or 8–15% of body length. Lips developing, longer than wide, 25–35 long. Ventriculus 31–82 long by 14–96; ventricular appendage 0.4–1.2 mm long by 12–94 wide; ratio for lengths of ventricular appendage to esophagus 1:1.1–2.4. Intestinal cecum 60–525 long by 24–92 wide; ratio of cecal to ventricular appendage lengths 1:2.0–6.0; ratio of cecal to esophagus lengths 1:2–9. Nerve ring located within anterior 15–26% of esophagus, 19–31 in breadth. Excretory pore immediately posterior to nerve ring. Rectal glands nearly spherical, numbering 4. Tail 77–173 long or 1–3% of body length including multispinous structure 17–50 long.

MALE $L_4$ (based on 4 specimens from mesentery of *M. undulatus*): Body 14–20 mm long by 253–441 at greatest width; ratio of greatest width to length 1:45–46. Lips longer than wide, 96–142 long by 31–120 wide. Nerve ring 376–482 from anterior extremity, 21–24 in breadth. Excretory pore immediately posterior to level of nerve ring. Esophagus 1.4–2.2 mm long. Ventriculus 84–148 long by 163–179 wide; ventricular appendage 0.7–1.2 mm long by 80–91 wide; ratio for lengths of ventricular appendage to esophagus 1:1.7–2.8. Intestinal cecum 197–315 long by 65–92 wide; ratio of cecal to ventricular appendage lengths 1:3–5; ratio of

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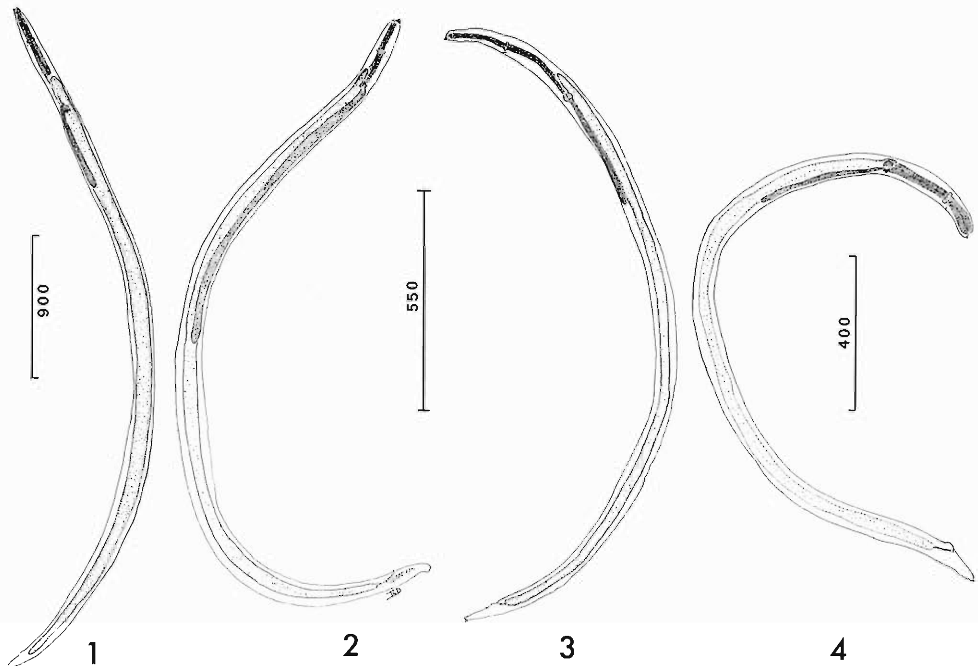


**Figures 1–4. Entire specimens of *Hysterothylacium* third-stage larvae showing intestinal cecum and ventricular appendage; lateral views. 1. ?*H. reliquens* (=type MA). 2. Type MB ($L_2$?). 3. *H. fortalezae* (type MC). 4. Type MD.**

cecal to esophageal lengths 1:4–7. Spicules similar, alate, 2.6–3.3% of body length, 420–560 long by 14–17 wide; unequal; right spicule slightly longer than left in all specimens. Gubernaculum absent. Caudal papillae not distinct. Tail conical, 137–173 long including multispinous structure 36–50 long.

FEMALE $L_4$ (based on 23 specimens from mesentery of *M. undulatus*): Body 8.7–22.5 mm long by 216–551 wide; ratio of greatest width to length 1:40–50. Lips longer than wide, 72–192 long by 70–120 wide. Nerve ring 321–506 from anterior extremity, 19–26 in breadth. Excretory pore immediately posterior to level of nerve ring. Esophagus 1.3–2.5 mm long. Ventriculus 68–185 long by 55–151 wide; ventricular appendage 0.8–1.2 mm long by 61–129 wide; ratio for lengths of ventricular appendage to esophagus 1:1.6–2.0. Intestinal cecum 216–315 long by 61–123 wide; ratio of cecal to ventricular appendage lengths 1:3–5; ratio of cecal to esophageal lengths 1:5.6–8.0. Vulva without salient lips, opening 8.2 mm or 36% of body length from anterior extremity in 1 select specimen (usually not apparent). Uterus didelphic, opisthodelphic. Ovaries not extending beyond anterior level of vulva. Tail conical, 132–364 long including multispinous structure 31–82 long.

HOSTS: Fishes: *Paralichthys albigutta* Jordan and Gilbert, Gulf flounder, *P. lethostigma* Jordan and Gilbert, southern flounder (Bothidae); *Caranx crysos* (Mitchill), blue runner, *Trachinotus carolinus* (Linnaeus), Florida pompano, *T. falcatus* (Linnaeus), permit (Carangidae); *Fundulus grandis* Baird and Girard, Gulf killifish, *F. heteroclitus* (Linnaeus), mummichog (Cyprinodontidae); *Dactyloscopus moorei* (Fowler), stargazer (Dactyloscopidae); *Anchoa hepsetus* (Lin-

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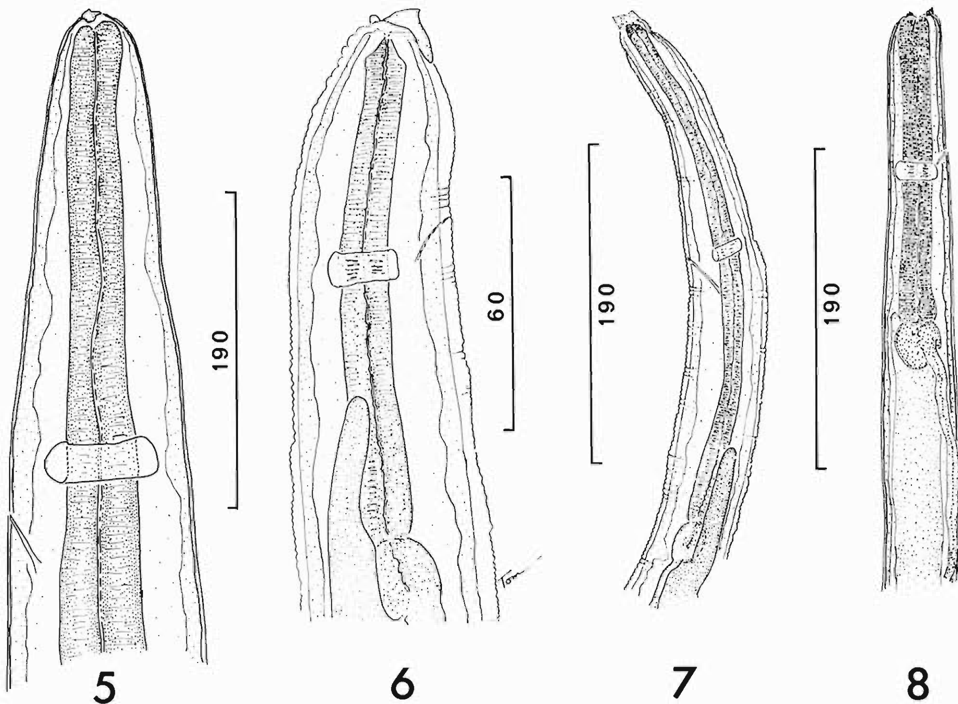


**Figures 5–8. Anterior extremities of *Hysterothylacium* third-stage larvae showing spacial relationship between excretory pore and nerve ring; lateral views. 5. ?*H. reliquens*. 6. Type MB ($L_2$?). 7. *H. fortalezae*. 8. Type MD.**

naeus), striped anchovy, *A. mitchilli* (Valenciennes), bay anchovy (Engraulidae); *Chaetodipterus faber* (Broussonet), Atlantic spadefish (Ephippidae); *Eucinostomus argenteus* Baird and Girard, spotfin mojarra (Gerreidae); *Lutjanus campechanus* (Poey), red snapper (Lutjanidae); *Mugil cephalus* (Linnaeus), striped mullet (Mugilidae); *Ophichthus gomesi* (Castelnau), shrimp eel (Ophichthidae); *Polydactylus octonemus* (Girard), Atlantic threadfin (Polynemidae); *Cynoscion arenarius* Ginsburg, sand seatrout, *C. nebulosus* (Cuvier), spotted seatrout, *Menticirrhus americanus* (Linnaeus), southern kingfish, *Micropogonias undulatus* (Linnaeus), Atlantic croaker, *Sciaenops ocellata* (Linnaeus), red drum, *Stellifer lanceolatus* (Holbrook), star drum (Sciaenidae); *Scomberomorus maculatus* (Mitchill), Spanish mackerel (Scombridae); *Centropristis philadelphica* (Linnaeus), rock sea bass, *Diplectrum formosum* (Linnaeus), sand perch, *Epinephalus morio* (Valenciennes), red grouper (Serranidae); *Archosargus probatocephalus* (Walbaum), sheepshead (Sparidae); *Peprilus alepidotus* (Linnaeus), harvestfish, *P. burti* Fowler, Gulf butterfish (Stromateidae); *Trichiurus lepturus* Linnaeus, Atlantic cutlassfish (Trichiuridae); *Prionotus scitulus* Jordan and Gilbert, leopard searobin (Triglidae). Invertebrates: *Cantharus cancellarius* (Conrad), cancellate cantharus (Gastropoda); *Lolliguncula brevis* (Blainville), brief thumbstall squid (Cephalopoda); *Callinectes sapidus* Rathbun, blue crab, *Clibanarius vittatus* (Bosc), striped hermit crab, *Penaeus aztecus* Ives, brown shrimp, *P. duorarum* Burkenroad, pink shrimp, *P. setiferus* (Linnaeus), white

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shrimp (Decapoda); *Luidia clathrata* (Say), starfish (Asteroidea); *Sagitta hispida* Conant, *S. tenuis* Conant, *Sagitta* sp., arrowworms (Chaetognatha).

SITES OF INFECTION: Mesentery of fishes, body cavity and digestive organ of invertebrates.

LOCALITIES: Sapelo Island, Georgia; Biscayne Bay, Florida (east coast); Tampa Bay, offshore from Tampa and Destin, Florida (west coast); Mississippi Sound and Gulf of Mexico, Mississippi; southeastern Louisiana; Galveston Bay, Texas.

**Remarks**

?*Hysterothylacium reliquens* (=*Hysterothylacium* type MA) differs from all other $L_3$'s studied from the Gulf of Mexico by having a tail tip covered with numerous, minute, spinous structures. It is a relatively large larva that lacks cuticular alae. Based on the spinous tail, it most closely corresponds with the prevalent adult *H. reliquens*. Type MA larva is the most prevalent one encountered in near-shore Gulf of Mexico animals. Additionally, the ratio between the lengths of the ventricular appendix and esophagus is about the same in $L_3$ and $L_4$ type MA as in adult *H. reliquens*. The value for the maximal relative difference between the lengths of the intestinal cecum and esophagus apparently increases with length of worm (1:2–5, 1:2–7, and 1:7–10 for $L_3$, $L_4$, and adult, respectively). No conspicuous differences occur between type MA and *H. reliquens*; however, they do not contain unique features in common either. Consequently, we refrain from positively identifying the larva as *H. reliquens*.

### *Hysterothylacium* type MB
### (Figs. 2, 6, 10, 14)

*Thynnascaris* type MB Norris and Overstreet, 1976.

DESCRIPTION (based on 30 specimens from *Paralichthys lethostigma*): Body 1.4–3.1 mm long by 31–58 wide at greatest width; ratio of greatest width to length 1:30–67. Cuticule lacking lateral alae. Esophagus 120–756 long or 4–13% of body length. Ventriculus 12–43 in diameter; ventricular appendage 457–772 long by 17–33 wide; ratio for lengths of ventricular appendage to esophagus 1:0.1–4.0. Intestine with obvious polygonal-shaped cells (in whole mounts) and with indistinct borders in cross section; intestinal cecum 14–193 long by 5–62 wide; ratio of cecal to ventricular appendage lengths 1:14–68; ratio of cecal to esophagus lengths 1:2–13. Nerve ring located within anterior 38–76% of esophageal, 14–21 in breadth. Excretory pore opening 20–40 anterior to nerve ring. Rectal glands oblong, numbering 4. Tail bluntly rounded, curving slightly dorsad, 48–166 long or 2–4% of body length, without spines.

HOSTS: Fishes: *Paralichthys lethostigma*, southern flounder (Bothidae); *Anchoa mitchilli*, bay anchovy (Engraulidae); *Mugil cephalus*, striped mullet (Mugilidae); *Cynoscion arenarius*, sand seatrout, *C. nebulosus*, spotted seatrout, *Menticirrhus americanus*, southern kingfish, *Micropogonias undulatus*, Atlantic croaker (Sciaenidae); *Scomberomorus maculatus*, Spanish mackerel (Scombridae); *Trichiurus lepturus*, Atlantic cutlassfish (Trichiuridae). Invertebrates: *Penaeus aztecus*, brown shrimp, *P. duorarum*, pink shrimp, *P. setiferus*, white shrimp (Penaeidae).

SITES OF INFECTION: Mesentery of fishes and hepatopancreas of shrimps.

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LOCALITIES: Tampa Bay, Florida; Mississippi Sound and Gulf of Mexico, Mississippi; Galveston Bay, Texas.

## Remarks

*Hysterothylacium* type MB is easily distinguished from all other larvae studied from the northern Gulf of Mexico by its lack of ornamentation on the tip of a bluntly rounded tail. We, however, have not ruled out the possibility that this common small nematode, often concurrent with the larger type MA, develops into that form or is an arrested stage of another form. It has a distinctive intestine because the outline of the basal portions of the epithelial cells are conspicuously polygonal when the entire worm is viewed (Fig. 2), but the cells appear rather indistinct or incompletely developed like those of an $L_2$ in cross section (Fig. 14). The consistent, poorly delineated, longitudinal muscles of type MB are reflected in the worm's more undulating swimming movement when compared to the whip like manner of type MA. Just as in type MA, no lateral alae are present. Also, no recognizable $L_4$'s were observed. A possible argument supporting separate status for type MB in addition to the tail's shape is the ratio of 1:14–68 for cecal to ventricular appendage lengths with the amount not dependent on length of worm. However, as shown below for type MD, the ratio of 1:12–25 for the $L_3$ of that species changes to 1:2–6 for the $L_4$.

If type MB represents a late $L_2$ or an early $L_3$ of type MA or another species, that might explain why type MB and not type MA invades the mammalian alimentary tract as mentioned in the introduction. If true, the similar stage of other species of *Hysterothylacium* may represent a public health hazard.

### *Hysterothylacium fortalezae* Klein, 1973
### (Figs. 3, 7, 11, 15)

=*Hysterothylacium* type MC.

DESCRIPTION OF $L_3$ (based on 10 specimens from *Scomberomorus maculatus*, *Peprilus alepidotus*, *P. burti*, and *Anchoa hepsetus*): Body 1.7–3.5 mm long by 47–80 wide at greatest width; ratio of greatest width to length 1:28–52. Cuticular alae extending length of worm, with T-shaped sclerotized supports; protruding portion of support pointed, extending through cuticle; base of support with each side about length of protruding portion. Esophagus 352–407 long or 11–18% of body length. Ventriculus 14–36 long by 17–31 wide; ventricular appendage 10–27 long by 5–10 wide; ratio for length of ventricular appendage to esophagus 1:0.7–1.3. Intestinal cecum 65–86 long by 12–33 wide; ratio of cecal to ventricular appendage lengths 1:3.8–4.4; ratio of cecal to esophagus lengths 1:3.0–5.0. Nerve ring located within anterior 18–22% of esophagus, 14–26 in breadth. Excretory pore adjacent to nerve ring. Rectal glands oblong, numbering 4. Tail 77–154 long or 3–5% of body length including tuft of approximately 6 spinous structures 9–12 long.

FEMALE $L_4$ (based on 14 specimens from mesentery and stomach of *Scomberomorus maculatus*): Body 5.0–13.5 mm long by 92–166 wide; ratio of greatest width to length 1:65–125. Cuticle with conspicuous annules. Lips 24–50 long by 24–48 wide. Esophagus 0.5–1.0 mm long by 31–55 wide or 7.0–8.6% of body length. Ventriculus 36–92 long by 36–53 wide; ventricular appendage 401–583

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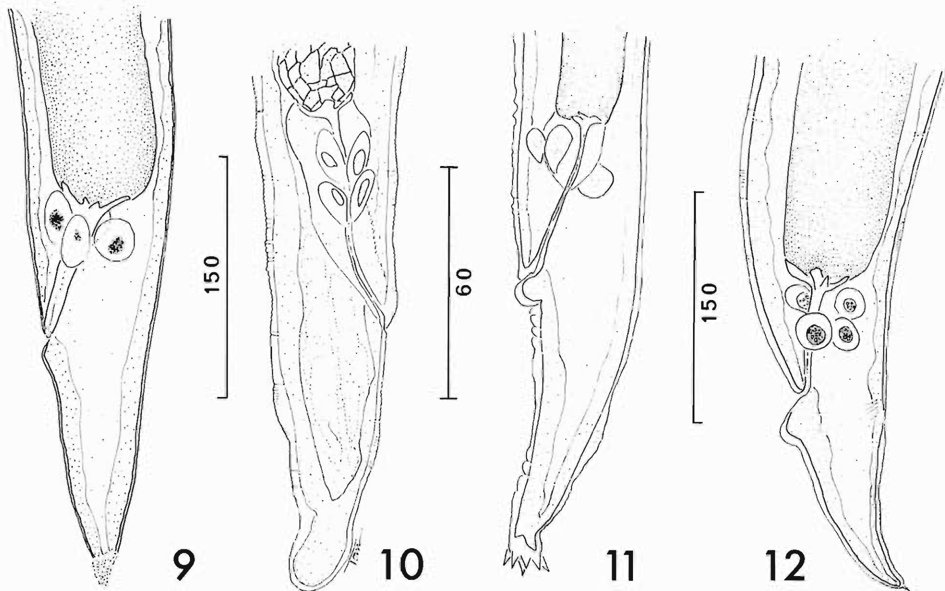


**Figures 9–12. Posterior extremities of *Hysterothylacium* third-stage larvae; lateral views. 9. Multispinous structure on ?*H. reliquens*. 10. Bluntly rounded tail of type MB ($L_2$?). 11. Tuft of spinouslike structures on *H. fortalezae*. 12. Conical tail with single spine on type MD.**

long by 24–49 wide; ratio for lengths of ventricular appendage to esophagus 1:1.1–2.7. Intestinal cecum 96–241 long by 24–48 wide; ratio of cecal to ventricular appendage lengths 1:2.1–4.5; ratio of cecal to esophageal lengths 1:3.2–6.7. Nerve ring located within anterior 30–41% of esophagus, 24–36 in breadth. Excretory pore immediately below posterior level of nerve ring. Rectal glands oblong, numbering 4. Vulva without salient lips, opening 4.0–4.6 mm or 35–40% of body length from anterior extremity. Uterus didelphic, opisthodelphic. Ovaries not extending beyond anterior level of vulva. Tail conical, 108–236 long including 12–14 stout spines from 7–12 long at posterior extremity.

HOSTS: Fishes: *Anchoa hepsetus*, striped anchovy, *A. lyolepis* (Evermann and Marsh), dusky anchovy, *A. mitchilli*, bay anchovy (Engraulidae); *Scomberomorus maculatus*, Spanish mackerel (Scombridae); *Centropristis melana* Ginsburg, southern sea bass (Serranidae); *Peprilus alepidotus*, harvestfish, *P. burti*, butterfish (Stromateidae); *Trichiurus lepturus*, Atlantic cutlassfish, (Trichiuridae). Invertebrate: *Penaeus setiferus*, white shrimp (Penaeidae).

SITES OF INFECTION: Mesentery of fishes and hepatopancreas of shrimp.

LOCALITIES: Biscayne Bay and offshore from Tampa, Florida; Mississippi Sound and Gulf of Mexico, Mississippi.

## Remarks

By possessing a tuft of about 6 to 12 spinelike projections distally on the tail, type MC larva differs from other larvae in this study. It has lateral alae like type MD, but the alar supports for type MC are T-shaped rather than wedge-shaped without flaring basal lateral projections. Also, the relative length of the ventricular appendage compared to the cecum is much less than for type MD.

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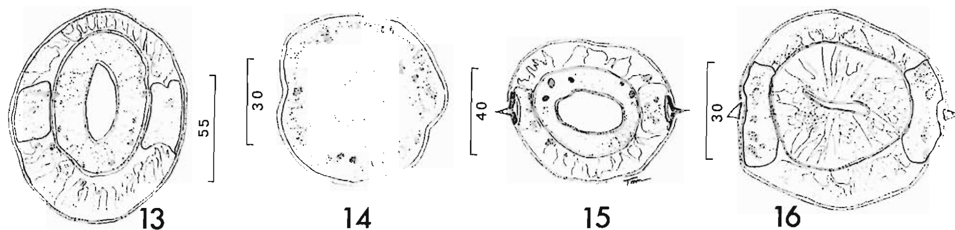


**Figures 13–16. Cross sections of *Hysterothylacium* third-stage larvae near middle of worm. 13. Lack of lateral alae on ?*H. reliquens*. 14. Indistinct borders of muscle and intestinal cells and no lateral alae on type MB ($L_2$?). 15. Lateral alae with T-shaped sclerotized supports on *H. fortalezae*. 16. Lateral alae with wedge-shaped sclerotized supports and high intestinal epithelium on type MD.**

We consider type MC to be *H. fortalezae*. Deardorff and Overstreet (1981) redescribed the species, and features of adult specimens show a natural progression with those of $L_4$'s and $L_3$'s in stomachs of individual Spanish mackerel. All stages have a similar spiny-tufted tail and similar ratios for lengths of cecum to ventricular appendage (1:2.1–5.4). In Biscayne Bay, Florida, R.M.O. observed *Anchoa lyolepis* infected with $L_3$'s and $L_4$'s being fed on by *Scomberomorus maculatus* and *Oligoplites saurus* (Bloch and Schneider), both of which had infections of adult *H. fortalezae*. Near Horn Island, Mississippi, we observed individuals of infected *S. maculatus* that were feeding heavily on *A. mitchilli*, *A. hepsetus*, and *Penaeus setiferus*, all infected with $L_3$'s. The mackerel also feeds on the other listed hosts.

### *Hysterothylacium* type MD
### (Figs. 4, 8, 12, 16)

DESCRIPTION (based on 5 specimens from *Scomberomorus maculatus*): Body 1.5–2.5 mm long by 48–62 wide at greatest width; ratio of greatest width to length 1:30–43. Cuticle with lateral alae extending length of worm; supports wedge-shaped, lacking basal extensions. Esophagus 212–289 long or 9–16% of body length. Ventriculus 24–36 in diameter; ventricular appendage 284–374 long by 19–27 wide; ratio for length of ventricular appendage to esophagus 1:0.7–0.8. Intestinal cecum 14–29 long by 9–17 wide, extending to or just anterior to forward margin of ventriculus; ratio of cecal to ventricular appendage lengths 1:12–25; ratio of cecal to esophageal lengths 1:10–20. Nerve ring located within anterior 38–53% of esophagus, 14–21 in breadth. Excretory pore opening 20–30 anterior to nerve ring. Rectal glands spherical, numbering 4. Tail conically shaped, curving slightly dorsad, 84–120 long or 4–6% of body length including single spinous structure 2–5 long.

GENERAL $L_4$ (based on 25 specimens from *Mugil cephalus*): Body 4.8–12.8 mm long by 201–348 wide at greatest width; ratio of greatest width to length 1:22–37. Cuticle with inconspicuous annules. Lips 23–55 long. Esophagus 0.5–1.0 mm long by 55–109 wide or 8–12% body length. Ventriculus 60–124 long by 69–126 wide; ventricular appendage 0.5–1.2 mm long by 49–184 wide; ratio for length of ventricular appendage to esophagus 1:0.4–1.3. Intestinal cecum 139–402 long by 27–109 wide; ratio of cecal to ventricular appendage 1:2–6; ratio of cecal to esophageal lengths 1:2–5. Nerve ring located within anterior 35–63% of esophagus, 23–46 in breadth. Excretory pore indistinct, possibly opening adjacent to nerve ring.

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Rectal glands oval, numbering 4. Tail 120–264 long or 1.0–3.5% of body length, including a single spine 2–5 long.

HOSTS: *Paralichthys albigutta*, Gulf flounder, *P. lethostigma*, southern flounder (Bothidae); *Seriola dumerili* (Risso), greater amberjack (Carangidae); *Chaetodipterus faber*, Atlantic spadefish (Ephippidae); *Mugil cephalus*, striped mullet (Mugilidae); *Cynoscion nebulosus*, spotted seatrout (Sciaenidae); *Scomberomorus maculatus*, Spanish mackerel (Scombridae); *Centropristis melana*, southern sea bass, *Epinephalus morio*, red grouper (Serranidae).

SITE OF INFECTION: Mesentery.

LOCALITIES: Tampa Bay and offshore from Tampa and Destin, Florida; Mississippi Sound and Gulf of Mexico, Mississippi; southeastern Louisiana; Galveston Bay, Texas.

### Remarks

Type MD is easily distinguished from all other larvae studied by having a conical tail tipped with a single spine. It exhibits many features, including the tail, in common with the larval *Thynnascaris* sp. (Type III) of Cannon (1977) from Queensland, Australia. No sympatric adult forms have been reported with a single terminal spinous structure; however, that structure could be lost during the worm's final molt. The intestinal epithelial cells, at least in the two sectioned specimens, are much deeper than those in the other studied larvae (Fig. 16).

### Key to Larvae of *Hysterothylacium* spp. Parasitizing Finfishes and Shellfishes from the Northern Gulf of Mexico

1. Boring tooth absent; lips and reproductive organs forming or formed (fourth-stage larva) ........ 5
   Boring tooth present; lips and reproductive organs lacking (second- or third-stage larva) ........ 2
2. Tip of tail with ornamentation ........ 3
   Tip of tail lacking ornamentation, bluntly rounded, curving slightly dorsad; ratio of cecal to ventricular appendage lengths 1:14–68; body length 1.4–3.1 mm; lateral cords and intestinal tract poorly delineated in cross section, lateral alae absent ........ type MB
3. Tip of tail with more than one minute spinous structure ........ 4
   Tip of tail with single spinous structure; ratio of cecal to ventricular appendage lengths 1:12–25; body length 1.5–2.5 mm; alar supports wedge-shaped ........ type MD
4. Tip of tail with about six spinouslike projections in tuft; ratio of cecal to ventricular appendage lengths 1:3.8–4.4; body length 1.7–3.5 mm; alar supports T-shaped ........ *H. fortalezae* (=type MC)
   Tip of tail with dense covering of numerous minute spinous structures not in tuft; ratio of cecal to ventricular appendage lengths 1:1.0–4.0; body length 4.0–9.0 mm; lateral cords prominent, intestinal tract distinct, and alae lacking in cross section ........ ?*H. reliquens* (=type MA)
5. Tip of tail with more than one minute spinous structure ........ 6
   Tip of tail with single spinous structure; tail slightly rounded; ratio of cecal to ventricular appendage lengths 1:2–6; body length 5–13 mm ........ type MD

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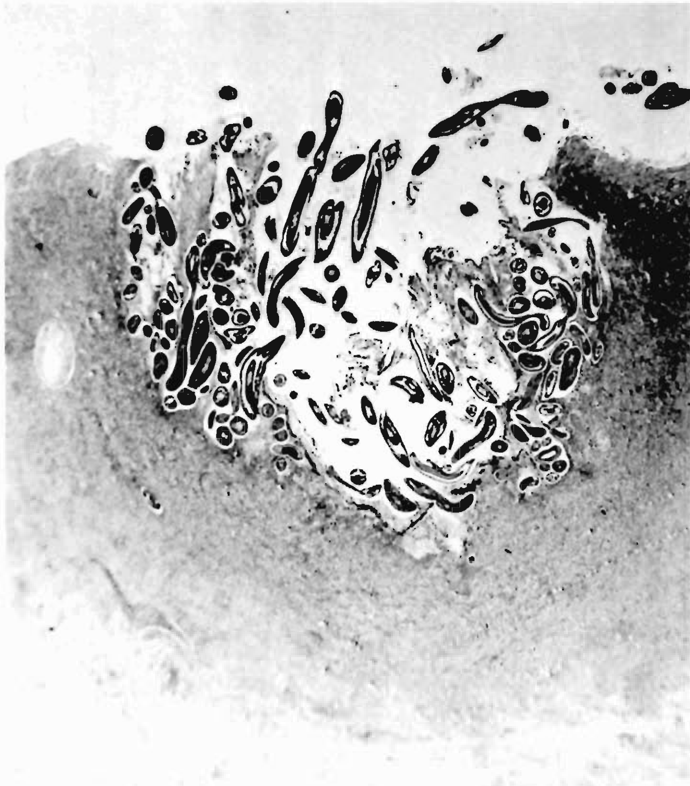

**Figure 17. Typical noninflamed, regenerating stomach tissue of *Rachycentron canadum* showing nest of $L_4$'s of *Iheringascaris inquies*, H and E.**

6. Tip of tail with 6–11 spinous structures; ratio of cecal to ventricular appendage lengths 1:2–5; body length 5–14 mm ........ *H. fortalezae* (=type MC)
   Tip of tail with multispinous structures; ratio of cecal to esophageal lengths 1:2–9; body length 4–23 mm ........ ?*H. reliquens* (=type MA)

## Biology

In addition to morphological differences, aspects of life histories of some larvae also differ. To show some of these differences, we use *Hysterothylacium reliquens*, occurring abundantly as both adults and presumably as larvae (type MA) in the northern Gulf of Mexico, and *Iheringascaris inquies*, a related species previously considered in the genera *Thynnascaris* and *Contracaecum* Railliet and Henry, 1912. As discussed by Deardorff and Overstreet (1981), adults of *H. reliquens* infect a large number of hosts, a few of which occasionally harbor several hundred specimens. In most definitive hosts, they occur as $L_4$'s or as adults in lumens of either the stomach or the intestine, even when present in large numbers. Never did we observe any of these worms invading a host's alimentary epithelium. Larval stages in the body cavity of fishes ($L_3$ and $L_4$)

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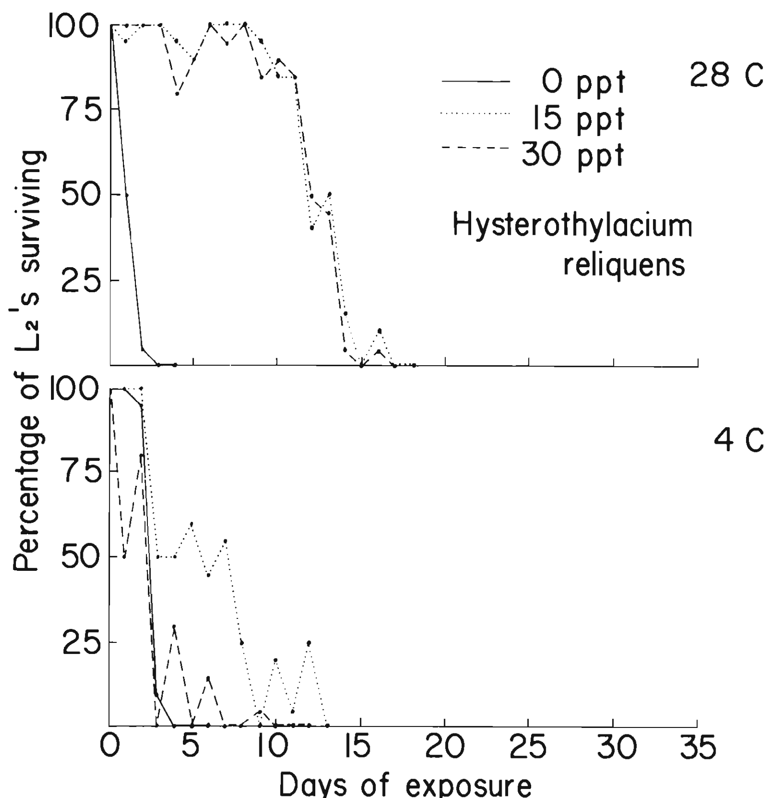


**Figure 18.** **Daily survival levels of second-stage larvae of *Hysterothylacium reliquens* at 4 ± 1 and 28 ± 1°C in 0, 15, and 30‰ salinity.**

occasionally penetrated visceral organs, but usually occurred free or encysted in the mesentery.

By contrast, *I. inquies* appears to be restricted as an adult to the cobia, *Rachycentron canadum* (Linnaeus). The $L_4$ occurs free in the lumen of the stomach or within stomach tissue of the cobia. Individuals or groups of the larva occasionally invade regenerating host tissue or occur in nests in such tissue (Fig. 17). We saw these $L_4$'s in nodules caused by the ascaridoid *Goezia pelagia* Deardorff and Overstreet, 1980 (see Deardorff and Overstreet, 1980) and presumably by embedded spines of prey. Adults of *I. inquies* infect both the lumen of the stomach and pyloric ceca, often in large numbers entwined among digesting prey.

Whether the $L_3$ of *I. inquies* has plicated annules like the $L_4$ and adult has not been established. Identification of larvae described by Kalyanker (1972) as *Thynnascaris inquies* from the gills of "sea-crabs" in Maharashtra, India, requires confirmation since Kalyankar's illustrations of the 4–9-mm-long worm show lips (not present on $L_3$ ascaridoids), an excretory pore 100 $\mu$m posterior to the nerve ring, and no cuticular plications. It was based on the worm's presence on "sea-crabs," common prey of infected cobia in the same locality. In any event, the presence of obvious plications and an excretory pore immediately posterior to the nerve ring on our $L_4$ material of *I. inquies* shows that it is not conspecific with type MA, MC, or MD larvae.

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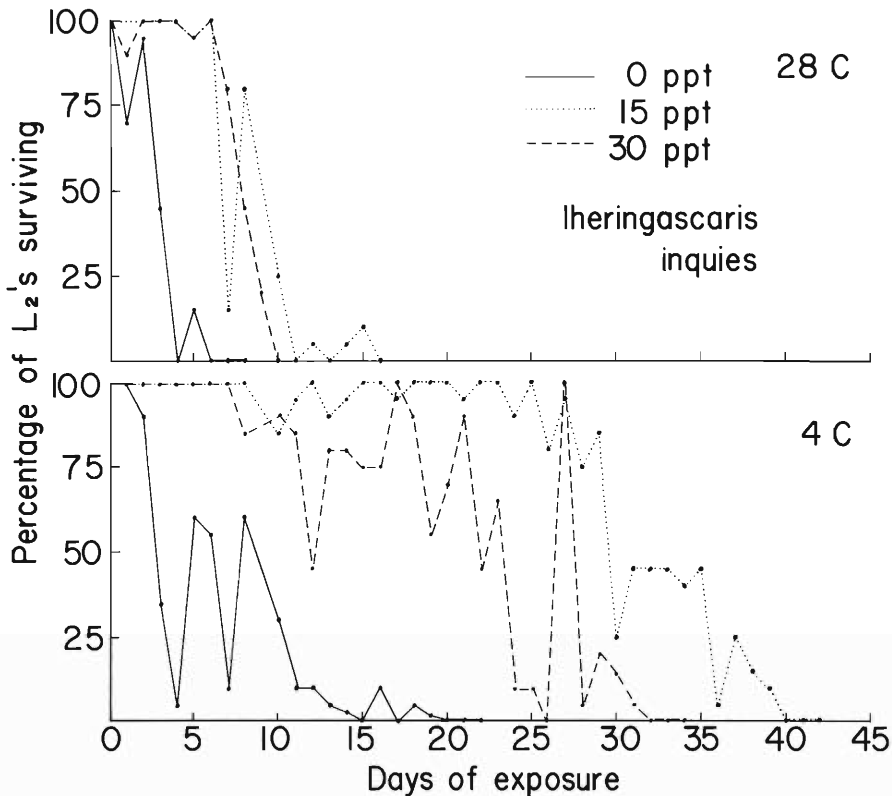


**Figure 19. Daily survival levels of second-stage larvae of *Iheringascaris inquies* at 4 ± 1 and 28 ± 1°C in 0, 15, and 30‰ salinity.**

The $L_2$'s of *H. reliquens* and *I. inquies* also tolerate various salinities and extreme temperatures differently (Figs. 18–19). At 4°C, one-half of the larvae of the former species die within 1–3 days at any salinity. At 15‰, however, about 25% survive for 12 days. About one-half of the larvae in both 15 and 30‰ survive for that period at 28°C, whereas those in freshwater die within 1–2 days. At 4°C, the $L_2$ of *I. inquies* survives over 30 days in 15 and 30‰ and also lives longer at that temperature than at 28°C. Individuals in freshwater live longer at 4° than at 28°C and live longer than those of *H. reliquens* at either temperature.

Probably larvae of both species in nature seldom encounter the extreme temperatures of the experiment. Still, the study shows that intermediate hosts of both species could acquire infections in either high salinity water or estuarine habitats where final hosts also occur. Larvae of *I. inquies* seem to survive a long period, especially in cold water. This feature accommodates the behavior of the cobia which can occur in open and deep water of the Gulf of Mexico and tropical regions of the Atlantic, Indian, and western Pacific oceans. The cobia visits the near-shore northern Gulf of Mexico primarily during warmer months. Intermediate and paratenic hosts for the $L_3$ have not been identified, and we do not known how the cobia becomes infected. On the other hand, since such a relatively large number of organisms host both the $L_3$ and adult of *H. reliquens* throughout

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the year, the free-living $L_2$ need not survive as long as that of *I. inquies* to assure completion of the cycle. Few fish in either low salinity bayous or offshore deep water of the Gulf harbor adult *H. reliquens*. Heavy adult infections are confined mostly to fish in relatively shallow and high-salinity water or those that recently fed in such waters. One could speculate that the $L_2$ of a species from the open-sea swordfish and billfishes, such as *H. incurvum* (Rudolphi, 1819) Deardorff and Overstreet, 1981, survives for long periods in cold water and short periods in 28°C like *I. inquies*, but for short periods in 15‰, unlike the $L_2$ of *I. inquies*.

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# Diurnal Migration of the Female of *Thelastoma bulhoesi* (Oxyurata: Thelastomida) in the American Cockroach, *Periplaneta americana*

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ABSTRACT: The average percentage of adult, female *T. bulhoesi* in each of three portions of the hindgut over a 24 hour period was determined. Worms were not found in the posterior portions of the hindgut between 4:00 p.m. and 2:00 p.m. but they were present in the rectal region between 8:00 a.m. and 12:00 noon. The number of males in the posterior half is greater than the number in the anterior half. The greatest number of eggs occurred in the rectum between 10:00 a.m. and 2:00 p.m. but were not found there after 8:00 p.m. *H. diesingi* was never found outside the anterior half of the hindgut nor were its eggs ever present in the rectum.

Mature females of *Enterobius vermicularis* migrate to the anal region of the host to deposit eggs. *Syphacia muris* and *S. obvelata,* found in rats and mice, respectively, also deposit eggs on the perianal skin of the host (Prince, 1950; Chan, 1952; Stahl, 1963). *Enterobius* females deposit eggs at night during the hosts quiescent period while *S. muris* deposits its eggs during the afternoon, a period of relative quiet for the rat host (van der Gulden, 1967). *Syphacia obvelata,* on the other hand, shows no periodicity in egg deposition (Chan, 1952).

Members of the family Thelastomatidae are entirely parasites of insects. Life cycles have been described for several species (e.g., Cali and Mai, 1965; Dobrovolny and Ackert, 1934) but the possibility of an anal migration has not been investigated. Guthrie and Tindall (1968) cite *Hammerschmidtiella diesingi* and *Thelastoma bulhoesi* as species common to the cockroach host *Periplaneta americana.* Both of these species are found in our colony at the University of Northern Colorado.

## Materials and Methods

The cockroaches used in this study were maintained in a 30-gallon glass aquarium with a glass top and cardboard egg cartons placed within for shelter and surface area. Water was provided in the bottom half of a petri dish as well as sprayed in mist form, to increase humidity, twice a week. Roaches were fed dry granulated dogfood ad libitum. The colony was kept in natural light conditions except that artificial light was used in the room until 10:00 p.m. most evenings.

The location of the nematode within the large intestine over a 24-hr period was determined by sacrificing 10 adult female cockroaches every hour for 24 hr, removing the colon and rectum and dividing it into three segments; the anterior half of the colon, the posterior half of the colon, and the rectum. The colon of the cockroach begins at the point where the ileum widens. It has a characteristic flexure at about the midpoint, and this was used to mark the division between the anterior and posterior halves. There is an obvious constriction demarcating the beginning of the rectal portion, and the rectum was excised at this point.

The above segments were isolated in separate containers of 0.75% NaCl, teased apart, and the number of adult female worms in each segment at each hour of the day were counted. In addition, the number of eggs visible in the rectal contents



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**Table 1. The mean percentage of adult female *T. bulhoesi* in various portions of the cockroach hindgut at different times of the day.**

| | Region of the large intestine | | |
|---|---|---|---|
| | Anterior ½ | Posterior ½ | Rectum |
| 8:00 p.m.–10:00 p.m. | 100 | 0 | 0 |
| 10:00 p.m.–12:00 a.m. | 100 | 0 | 0 |
| 12:00 a.m.–2:00 a.m. | 100 | 0 | 0 |
| 2:00 a.m.–4:00 a.m. | 80 | 20 | 0 |
| 4:00 a.m.–6:00 a.m. | 82 | 18 | 0 |
| 6:00 a.m.–8:00 a.m. | 80 | 20 | 0 |
| 8:00 a.m.–10:00 a.m. | 33 | 37 | 31 |
| 10:00 a.m.–12:00 p.m. | 77 | 8 | 15 |
| 12:00 p.m.–2:00 p.m. | 77 | 24 | 0 |
| 2:00 p.m.–4:00 p.m. | 92 | 8 | 0 |
| 4:00 p.m.–6:00 p.m. | 100 | 0 | 0 |
| 6:00 p.m.–8:00 p.m. | 100 | 0 | 0 |

were counted and the number and position of adult males in each segment was recorded. The above procedure was first conducted with five roaches per hour due to the time requirements of dissection. It was later replicated with similar results. The results in this paper are the combined data of these two trials and are based on 10 cockroaches.

## Results

Eighty-nine percent of the adult female cockroaches in our colony are infected with thelastomatid worms of two species. The mean number of *Thelastoma bulhoesi* per adult female host is 8.24, standard deviation 6.776 and the range was 0.37. The results in Table 1 represent, on the average, 160 worms for each 2-hr time period.

The average percentage of adult, female *T. bulhoesi* in each of three portions of the hindgut over a 24-hr period is detailed in Table 1. Note that worms were not found in the posterior portions of the hindgut between 4:00 p.m. and 2:00 p.m. and that they were present in the rectal region between 8:00 a.m. and 12:00 noon. In addition the number of males of *T. bulhoesi* in the posterior half of the large intestine is always greater than the number in the anterior half, as shown in Table 2. The greatest number of eggs of *T. bulhoesi* in the rectum occurred between 10:00 a.m. and 2:00 p.m. and then decreased sharply by 8:00 p.m. Eggs were not observed in the rectum between 8:00 p.m. and 10:00 a.m. However, *H. diesingi* was never found outside the anterior half of the hindgut nor were eggs of this species ever present in the rectum.

**Table 2. The percentage of males found in various regions of the large intestine.**

| Region of large intestine | % Males |
|---|---|
| Anterior ½ | 12.04 |
| Posterior ½ | 87.95 |
| Rectum | 0 |

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## Discussion

The two families Oxyuridae and Thelastomatidae are similar in anatomy and ecology. The oxyurids have received a considerable amount of attention as compared to the thelastomatids. It is not surprising, however, to observe in Table 1 that the females of *Thelastoma* appear to move toward the anus on a periodic basis. Their maximum numbers are in the rectum between 8:00 a.m. and noon. This corresponds to the normal period of quiescence of the host cockroach. This is similar to the behavior observed in *Enterobius* and *Syphacia* in vertebrate hosts.

In oxyurid nematodes of vertebrates this anal migration has been in preparation for subsequent egg deposition. This appears also to be the case for *Thelastoma*, in that large numbers of ova are present in the rectum between the hours of 10:00 a.m. and 2:00 p.m. Occasional eggs can be observed in the rectum during the afternoon hours but none was ever observed there between 8:00 p.m. and 10:00 a.m. There is a lag time of approximately 2 hr between the time the females can first be observed in the rectum and the time eggs are first observed.

An interesting sidelight of this observation is the large percentage of males (87.95%) found in the posterior half of the large intestine. Previous literature has always suggested that males of *T. bulhoesi* were rare. This was possibly due to their small size, and to their shorter life span. This report suggests that they may also occupy a different niche within the gut for part of the time. This could be advantageous in that it might minimize food and space competition between the sexes.

No evidence was seen that suggests migratory egg deposition occurs in *H. diesingi*, nor is there any periodicity of such movements. This may indicate different origins of the two genera. This is the first report of diurnal migration of the female in the family Thelastomatidae.

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# Observations of the Head and Tail Regions of Male *Physaloptera praeputialis* von Linstow, 1889, and *Physaloptera rara* Hall and Wigdor, 1918, Using Scanning Electron Microscopy

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ABSTRACT: The morphology of *Physaloptera praeputialis* and *Physaloptera rara* have been studied using light microscopy but details of taxonomic characteristics using SEM are limited. Based on observations by von Linstow (1889) and Hall and Wigdor (1918) using light microscopy, primary taxonomic characters for identification of these two species include the number, size, and location of posteroventral papillae. These characters as well as posteroventral microtopography, comparisons of the head and neck regions, and possible preparation artifacts are illustrated using SEM.

Morgan (1947) reviewed the Physalopterinae and listed characters for species determination. In male individuals Morgan considered the number and arrangement of male ventral papillae as well as the shape and length of spicules valid taxonomic characters. Based on the degree of variation within species Morgan (1947) stated the shape of the bursa and bursal markings cannot be used for species determination.

Generic characters for members of the family Physalopteridae (Railliet, 1893) Leiper, 1908 have recently been divided into genera by Chabaud (1975). Based on the keys for family, subfamily, and generic determination (Chabaud, 1975) cervical alae are not present as a generic or specific character. Specific characters for the genus *Physaloptera* (Ortlepp, 1922) do not include cervical alae. However, cervical alae have been reported by previous investigators (Marchiondo and Sawyer, 1978).

The present study was undertaken to (a) differentiate adult and fifth stage immature specimens of *Physaloptera praeputialis* and *Physaloptera rara* solely on the basis of posteroventral characteristics using SEM, (b) determine the validity of posteroventral microtopography as a taxonomic character, and (c) discuss possible artifacts associated with preparation. The head and neck regions are compared. Clarification of a previous report (Marchiondo and Sawyer, 1978) of cervical alae exhibited by *P. rara* is illustrated.

## Materials and Methods

Seventy-six bobcat (*Lynx rufus*) carcasses were collected from fur-buyers, taxidermists, trappers, and game biologists in Nebraska during the 1977 and 1978 trapping seasons. The carcasses had been frozen and thawed repeatedly between capture and examination. The condition of the worms varied from good to extremely poor. Adult specimens were identified as *Physaloptera praeputialis* and *P. rara*. Fifth-stage individuals were separated on the basis of size and placed in

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separate vials. All specimens were fixed in AFA and stored in a mixture of 70% ethanol and glycerine. Specimens prepared for light microscopy were cleared in lactophenol, further cleared in beechwood creosote, and mounted in Canada balsam.

For examination using SEM, the prepuce sheath of adult *P. praeputialis* and the superficial cuticle of immature *P. praeputialis* were removed. This step was not necessary for specimens of *P. rara*. Macrodebris was eliminated with the aid of microforceps. The neck region of both species was surgically removed below the excretory pore. To further eliminate any debris, the neck and tail sections of both species were placed in an ultrasonicator for 5 minutes. They were then dehydrated through 100% ethanol, critical point dried with a Sorvall $CO_2$ critical point drying system, coated with gold/palladium, and examined with a Cambridge stereoscan SEM at 20 keV.

## Results

Scanning electron micrographs (Figs. 1–4) illustrate the characteristic microtopographical features of the posteroventral region of adult and immature *P. praeputialis* and *P. rara*. Using light microscopy these taxonomic characters were described by von Linstow (1889) for *P. praeputialis* and Hall and Wigdor (1918) for *P. rara*.

*Physaloptera praeputialis* (Figs. 1, 3) possess four pairs of pedunculated papillae approximately equidistant from each other; two pairs of precloacal and two pairs postcloacal. Because of photographic limitations, not all these papillae are shown in Figure 1. However, these papillae are illustrated on the left ventrolateral side in Figure 3.

Three precloacal sessile papillae are situated in a transverse row, the median one being much larger than the other two; the external surface of the cloaca is divided into two marginal borders, the anterior border being much wider than the posterior border. In Figure 1, a single spicule is seen projecting from the cloacal orifice. Immediately posterior to the cloaca are two pairs of sessile papillae arranged almost transversely; a third pair directly follows; the remaining two pairs of posteroventral papillae are situated approximately between the cloaca and the end of the tail and near the posterior third of the tail. In Figure 3, there is an aberration from the normal number of posteroventral papillae (asterisk). A previous undescribed phasmid (Fig. 1) is situated approximately posteromedially between the third and fourth pairs of posteroventral papillae. Longitudinal ridges are present on either side of the cloaca which extend in a lateral direction supporting the pedunculated papillae. These ridges give rise immediately anteriorly and posteriorly to beadlike projections, bordered by lateral folds, and extend posteriorly to just short of the end of the tail. These beadlike projections are divided from their posterior extremity medially by a narrow strip of tegument, without ornamentation, and extend anteromedially to a thin line just below the third pair of posteroventral papillae. The beadlike projections surround the anterior portion of the precloacal papillae and the posterolateral margins of the two outer precloacal papillae. The large medial precloacal papilla is surrounded by its anterolateral margin while the inner margin touches the anterior marginal border of the cloaca. *Physaloptera rara* (Figs. 2, 4) possess four pairs of pedunculated papillae approximately equidistant from each other; two pairs are precloacal, and

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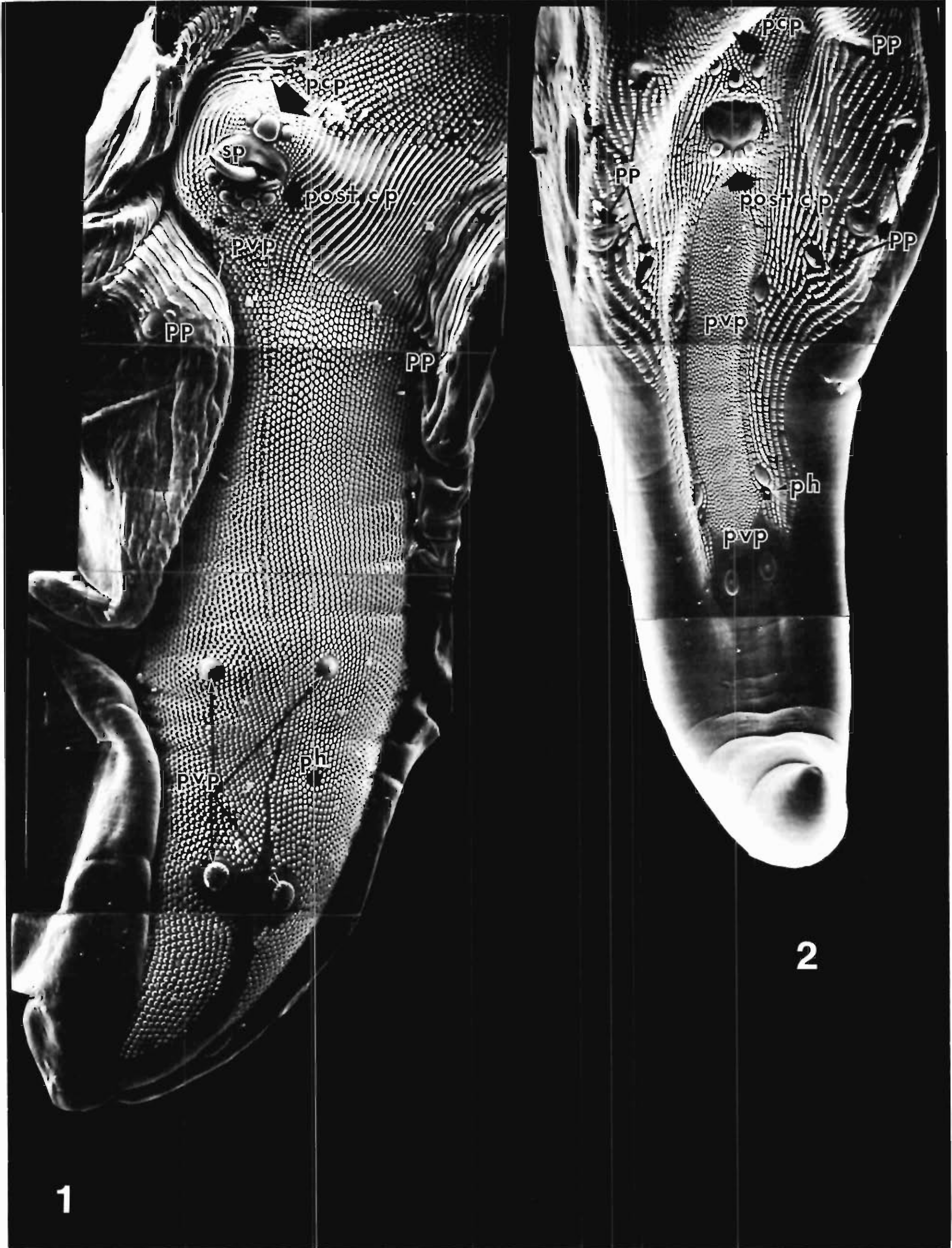


**Figures 1, 2.** Scanning electron photomicrographs of the posteroventral region of adult and immature specimens of *Physaloptera praeputialis* and *P. rara*. 1. Adult *P. praeputialis* illustrating posteroventral taxonomic characters and microtopography; precloacal papillae, arrow (pcp); pedunculated papillae (pp); spicule extending from the cloacal orifice (sp); postcloacal papillae, arrow (post c p); three pairs of posteroventral papillae, arrows (pvp); a single previously undescribed phasmid (ph); and the characteristic microtopography. ×120. 2. Adult *P. rara* illustrating posteroventral taxonomic characters and microtopography; three precloacal papillae (pcp); four pairs of pedunculated papillae, arrows (pp); two pairs of postcloacal papillae, arrow (post c p); three pairs of posteroventral papillae (pvp); and one pair of phasmids, arrow (ph). ×210.

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two pairs are postcloacal; three precloacal sessile papillae of equal size are arranged in a triangular row, the middle one being slightly closer to the cloacal region; the external surface of the cloaca is not divided as in *P. praeputialis*; a wide posterior marginal border is present but partly as an artifact due to critical point drying. In Figure 4, a single spicule is seen projecting from the cloacal orifice. Posterior to the cloaca (Fig. 2) are five pairs of sessile papillae and one pair of phasmids; the first two pairs are situated in a transverse row, the third and fourth pair equidistant from each other; one pair of phasmids is situated directly behind the fourth pair of sessile papillae. The fifth pair of papillae are approximately one fourth the distance from the end of the tail to the fourth pair of sessile papillae. Longitudinal rows of elongated projections extend from the anterior portion of the bursa, posteriorly to the fifth pair of sessile papillae. From the postcloacal papillae to just short of the fifth pair of sessile papillae, there is a strip of beadlike projections, tapered at either end, and limited to the inner margins between the third and fourth pair of sessile papillae. The lateral margins posterior to the bursa and the posterior third of the tail exhibit no ornamentation.

To differentiate *P. praeputialis* from *P. rara* on the basis of externolateral and internolateral teeth, several specimens of both species were examined using SEM (Figs. 7–10). There is a slight difference between the height of the single externolateral and the three internolateral teeth of *P. praeputialis* and *P. rara*. This minor difference is not large enough to support the use of this structure as a valid taxonomic character (Morgan, 1947).

Two porelike openings located below two of the three internolateral teeth (Marchiondo and Sawyer, 1978) were not discernible in either *P. praeputialis* or *P. rara* (Figs. 7–10). Spherical-shaped amphids located behind the large externolateral tooth and medial to the papillae (Marchiondo and Sawyer, 1978) were observed in the same location of *P. praeputialis* and *P. rara* (Figs. 7–10). The papillae on each pseudolabia are dome-shaped (Marchiondo and Sawyer, 1978) and exhibit the same arrangement as described by previous investigators. The neck region of *P. praeputialis* and *P. rara* exhibit the typical cylindrical shape (Figs. 5, 6). Some folding (Fig. 6) is present as an artifact due to critical point drying. The location of the excretory pore (Figs. 5, 6) shows no great differences and is of little taxonomic significance (Morgan, 1947).

## Discussion

Ortlepp (1922) stated that the arrangement of the caudal papillae of *P. praeputialis* differed considerably from those reported by von Linstow, 1889. Von Linstow (1889) stated that the arrangement of caudal papillae present on *P. praeputialis* may be different than he reported. He (1889) had only one male for examination, which was difficult to study. According to von Linstow, there are three pre-anal papillae; one immediately behind the anus; three papillae toward the tail; and an additional pair slightly in front of these three.

Morgan (1947) lists the number and arrangement of male ventral papillae as well as shape and length of the spicules as valid taxonomic characters. He (1947) rejected certain taxonomic characters because of the wide variation found within species. Among the characters listed are the posterior sheath, size and height of teeth, position of the excretory pore and cervical papillae, shape of bursa and bursal markings.

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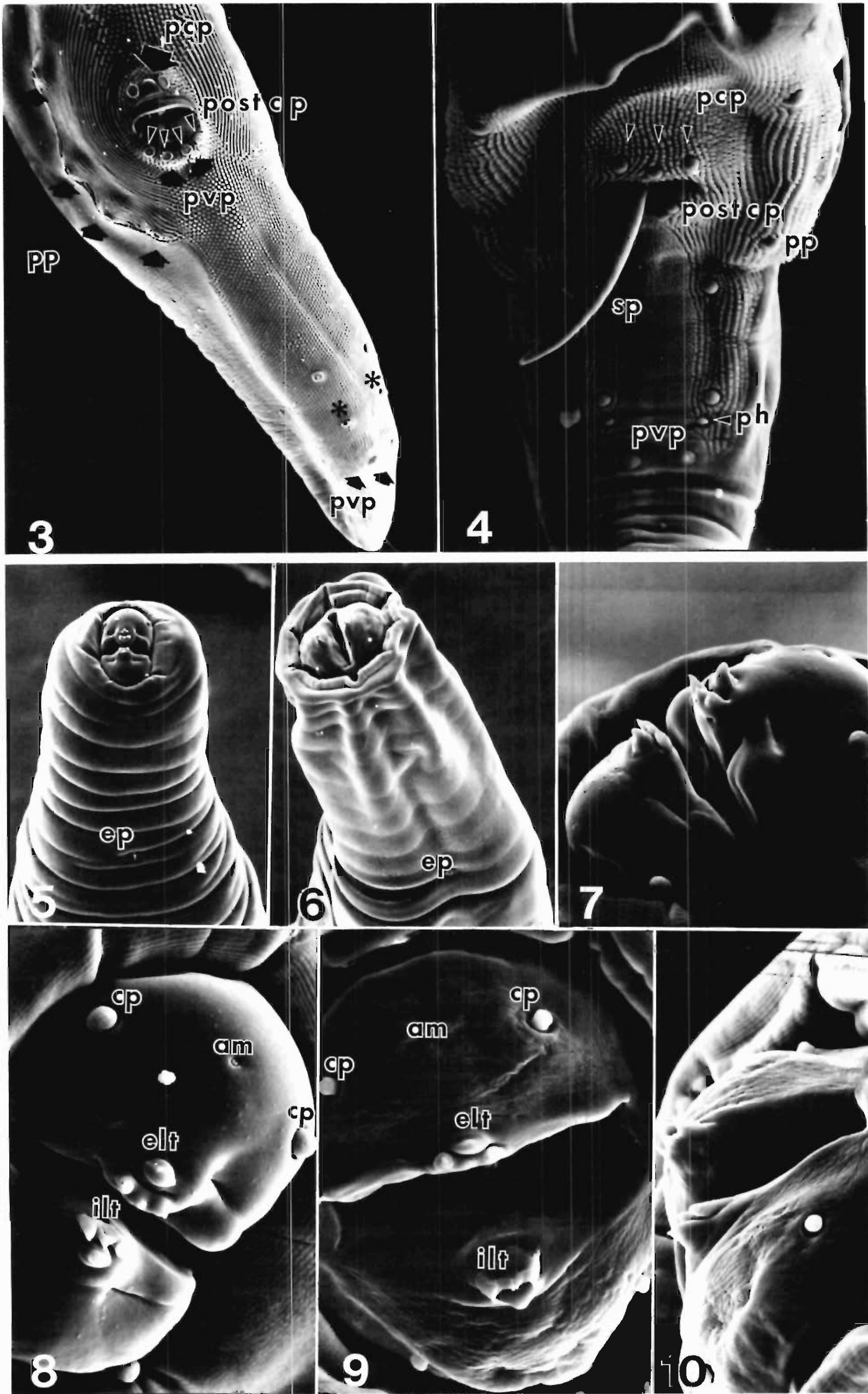


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However, upon examination of several hundred adult male *P. praeputialis* and *P. rara,* the prepuce sheath and shape of the bursa can be used for gross separation of *P. praeputialis* from *P. rara*. Aside from the number of posteroventral papillae, microtopography (bursal markings) can be used to differentiate these two species (Figs. 1–4). Posteroventral characters must be used to differentiate immature male individuals. The present study shows that in immature specimens of *P. praeputialis,* the rudimentary posteroventral papillae, which under light microscopy are difficult to differentiate, exhibit the same arrangement as in adult *P. praeputialis* (Figs. 1, 3). In Figure 3, there is an aberration from the normal number of posteroventral papillae in *P. praeputialis*. Out of a total of 484 specimens of *P. praeputialis* only five or approximately 1% of the immature individuals were found to exhibit an extra pair of papillae. In immature specimens of *P. rara* (Fig. 4) the posteroventral papillae exhibit the same arrangement as found in adult *P. rara* (Fig. 2).

As stated earlier, cervical alae are not characteristic of members of the family Physalopteridae. However, Marchiondo and Sawyer (1978) describe the anterolateral neck region of *Physaloptera felidis* (=*P. rara,* Morgan, 1946) as possessing four cervical alae forming a rectangular collarette when observed with SEM. In the present study, no cervical alae were observed on either *P. praeputialis* or *P. rara* (Figs. 5, 6). Differences in preparation for light microscopy as well as for SEM can lead to considerable variation in structural morphology and characters used in taxonomy. Variations in the cuticular region of the pseudolabia and the collapse of the neck region are considered artifacts caused during fixation, dehydration, and critical point drying (Figs. 6–10).

Primary taxonomic characters for identification of physalopteran males are the number, size, and location of posteroventral papillae and posteroventral microtopography. These characters, unlike the size and height of externolateral and internolateral teeth, the presence or absence of a posterior prepuce sheath in immature forms or cephalic collarette, remain constant during preparation for examination and throughout developmental and adult stages. Known generic and specific characters within a given genus, which are described using light microscopy, should be examined closely before an investigator can differentiate taxo-

←

**Figures 3–10. Scanning electron photomicrographs of *Physaloptera praeputialis* and *P. rara*. Figures 3–4. Posteroventral region. Figures 5–6. Head and neck regions. Figures 7–10. Head region of *P. praeputialis* and *P. rara* mounted en face. ×600. 3. Immature specimen of *P. praeputialis* showing: two of four pairs of pedunculated papillae, arrows (pp), one pair precloacal and one pair postcloacal; three precloacal papillae, arrow (pcp); two pairs of postcloacal papillae, arrows (post c p); three pairs of posteroventral papillae, arrows (pvp); and characteristic microtopography. An aberration marked by asterisk (*) is also illustrated. ×200. 4. Immature specimen of *P. rara* showing posteroventral region and microtopography as described in Figure 2 with addition of a spicule extending from the cloacal orifice. ×250. 5. *P. praeputialis* illustrating cylindrical shape with location of the excretory pore (ep). ×135. 6. *P. rara* illustrating cylindrical shape with collapse near the head region which is an artifact of preparation; location of excretory pore (ep). ×120. 7. Angular view of head region of *P. praeputialis*. 8. Same specimen in en face view illustrating cervical papillae (cp), amphid (am), externolateral tooth (elt), and internolateral teeth (ilt). 9. En face view of *P. rara* illustrating the characters described in Figure 8. 10. Same specimen of *P. rara* at different angle.**

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nomic characters from artifacts which may alter these characters or specimen's appearance (Tiekotter, 1980).

## Acknowledgments

The author wishes to express his appreciation to Ms. Carol Epperson Mahan for furnishing the bobcat carcasses for helminth examination and aid in necropsy, and to Professor Mary Hanson Pritchard for advice during the preparation of this paper.

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Proc. Helminthol. Soc. Wash.
48(2), 1981, pp. 137–147

# Three New Species of *Chapiniella* Yamaguti, 1961 (Nematoda: Strongyloidea) from Tortoises

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ABSTRACT: Three new species of *Chapiniella* are described and compared with three previously described species. The latter were all described from *Testudo denticulata* from South America. Two of the new species, *C. chitwoodae* sp. n. and *C. gallatii* sp. n., are from *Gopherus polyphemus* from North America, and the third new species, *C. jellisoni* sp. n., is from an unidentified tortoise from Southeast Asia. The two new species from *Gopherus* are smaller with shorter spicules (1.00 mm or shorter) and with a greater distance between the vulva and the anus than the other species. The new species from Asia has much longer spicules (3.4 mm) than any of the American species. *Chapiniella* is redefined based on a study of the new species and the type species, *C. variabilis*. New information in the generic redefinition includes (1) two leaf-crowns present; (2) prebursal papillae present; and (3) intestinal diverticula absent.

The only strongyloid nematodes known to parasitize reptiles are five species in two related genera (*Sauricola* Chapin, 1924 and *Chapiniella* Yamaguti, 1961), all parasitic in the South American tortoise, *Testudo denticulata* Linnaeus, 1766. One of us (TBS) has collected large numbers of undescribed strongyloid nematodes from the gopher tortoise, *Gopherus polyphemus* (Daudin, 1802) in Georgia, USA, including two new species of the genus *Chapiniella*. This report describes the two new species from the gopher tortoise and a third new species from a tortoise collected in Burma. A redefinition of *Chapiniella* is presented based on new information obtained in the study of the three new species and the type species *Chapiniella variabilis* (Chapin, 1924).

## Materials and Methods

### Hosts

Eight gopher tortoises were collected in Tift, Baker, and Colquitt counties of Georgia in 1969 and 1970. The tortoises were killed by decapitation. The digestive tracts were separated into stomach, small intestine, and large intestine, washed out in separate containers, and digested with pepsin and hydrochloric acid.

### Nematodes

All specimens were fixed in hot 70% ethanol, preserved in glycerine-alcohol or formalin, and cleared for study in temporary mounts in phenol-alcohol. En face preparations were mounted in glycerine jelly following the method of Buhrer (1949). Scanning electron micrographs were prepared from specimens processed according to the methods of Madden and Tromba (1976). Drawings were made with the aid of a camera lucida. All measurements are given as ranges followed by means in parentheses and are in micrometers unless indicated otherwise. Specimens are deposited in the U.S. National Museum Helminthological Collection (USNM Helm. Coll.), BARC-East, Beltsville, Maryland 20705, USA.



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## Results

The large intestines of all eight gopher tortoises were parasitized by Strongyloidea and Oxyuroidea and the small intestines of two by Trichostrongyloidea. *Chapiniella chitwoodae* sp. n. was found in four tortoises and *Chapiniella gallatii* sp. n. was collected from two tortoises. Two tortoises were parasitized by both *C. chitwoodae* and *C. gallatii*. Other undescribed Strongyloidea (Cyathostominae), the Oxyuroidea, and the Trichostrongyloidea will be described elsewhere.

### *Chapiniella* Yamaguti, 1961

REDEFINITION: Strongyloidea; Cyathostominae. Short, thin nematodes with inflated cuticle marked by coarse annulation. Buccal capsule shallow, ring-shaped, with long slender external leaf-crown elements and short inconspicuous internal leaf-crown elements inserted alternately at base of buccal capsule; border of oral opening divided into numerous extensions of external leaf-crown. Dorsal gutter absent. Spicules alate with slightly curved tips. Dorsal lobe of copulatory bursa longer than lateral lobes; dorsal lobe not separated from laterals; lateral lobes not completely separated ventrally. All rays of bursa reach edge of bursa except externolaterals and externodorsals. Ventroventral and lateroventral rays parallel, curve ventrally. Dorsal bursal ray with central bifurcate ramus and paired lateral rami that usually bifurcate near distal ends. Externodorsal rays originate from stem of dorsal ray. Prebursal papillae present. Female tail short; vulva near anus; ovejectors parallel, with thin muscles. Ova large, unembryonated. Intestinal parasites of tortoises.

#### Remarks

*Chapiniella* was established for *Deletrocephalus variabilis* Chapin, 1924, a species provisionally placed in the genus *Theileriana* Mönnig, 1924 by Yorke and Maplestone (1926). Subsequent study of the type of *C. variabilis*, the descriptions of *Chapiniella larensis* Diaz-Ungria and Gallardo, 1968 and *Chapiniella diazi* Chabaud and Tcheprakoff, 1977; and the study of three new species described herein provided sufficient new information to require a redefinition of the genus. The new information includes (1) two leaf-crowns present, (2) prebursal papillae present, and (3) intestinal diverticula apparently absent.

### *Chapiniella chitwoodae* sp. n.
### (Figs. 1–7, 17)

DESCRIPTION: Submedian cephalic papillae with short tips. Lateral papillae (amphids) slightly elevated. Buccal capsule short, wide and circular. Leaf-crowns of 19 elements each. Nerve ring at middle of esophagus. Excretory pore and

→

**Figures 1–7.** ***Chapiniella chitwoodae*** **sp. n., line drawings. Scale bars 50 $\mu$m unless indicated otherwise. 1. Anterior one-tenth of female, ventral view, showing cephalic papillae, buccal capsule, nerve ring, esophagus, cervical papillae, and excretory pore (ep). 2. En face view of female showing four submedian cephalic papillae, lateral papillae, internal leaf-crown elements (ilc) and external leaf-crown elements (elc), cuticular extensions of external leaf-crown (xt), and esophageal teeth (t). Scale bar 20**

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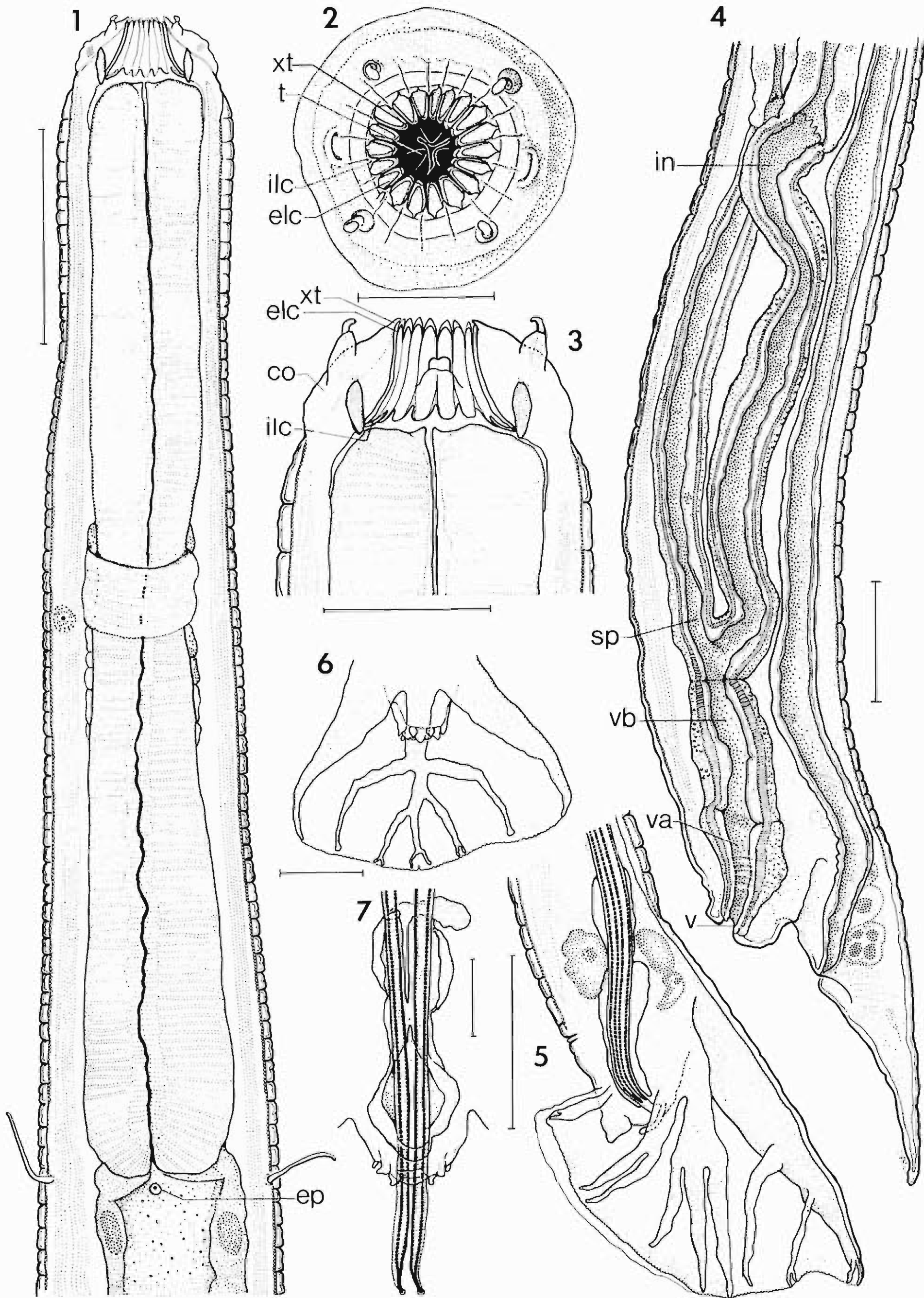


μm. 3. Anterior extremity of female, lateral view, showing inflated cephalic collar (co), two submedian cephalic papillae, lateral cephalic papilla, buccal capsule, internal leaf-crown (ilc), external leaf-crown (elc), and cuticular extensions of external leaf-crown (xt). Scale bar 20 μm. 4. Female tail, lateral view, showing vulva (v), vagina (va), vestibule (vb), thinly muscled paired sphincters (sp), and infundibula (in). 5. Male tail, lateral view, and proximal ends of spicules. 6. Dorsal bursal ray and dorsal view of genital cone. 7. Gubernaculum and genital cone. Scale bar 20 μm.

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cervical papillae near base of esophagus. Spicules 15–19% of body length. Gubernaculum trough-shaped with proximal, thick, spongy, lightly sclerotized portion and a distal, thin, densely sclerotized plate with lateral flanges; proximal portion with medial projection between spicules. Ovejectors short, sphincters and infundibula not markedly differentiated, together 5–7% of body length.

MALE (based on 10 specimens): Total length 2.81–3.62 (3.24) mm. Esophagus length 239–256 (246). Anterior end to nerve ring 113–146 (132); excretory pore 248–294 (272); cervical papillae 260–294 (273). Buccal capsule depth 6–8 (7); diameter 21–26 (23). Body width at base of cephalic collar 28–34 (31); base of esophagus 44–61 (54); midbody (63–96 (74). Width of cuticular annule at base of esophagus 4–5 (5); midbody 5–9 (6). Spicules 420–680 (514) long and 8–11 (10) wide at proximal ends. Gubernaculum length 62–70 (65). Genital cone length 18–40 (30): bears two digitiform dorsolateral papillae, two pear-shaped internodorsal papillae, and a ventral triangular projection with membranous skirt. Dorsal ray length 109–146 (120); externodorsal rays originate 55–79 (67) from distal end.

FEMALE (based on 10 specimens): Total length 3.28–4.07 (3.65) mm. Esophagus length 223–277 (256). Anterior end to nerve ring 125–156 (137); excretory pore 273–311 (286); cervical papillae 252–303 (281). Buccal capsule depth 6–8 (7); diameter 21–26 (23). Body width at base of cephalic collar 28–33 (31); base of esophagus 52–76 (59); at midbody 71–118 (86). Width of cuticular annule at base of esophagus 4–5 (5); at midbody 5–8 (6). Distance vulva to anus 49–78 (62). Vagina length 49–79 (64); vestibule length 59–109 (78); combined length of sphincter and infundibulum 194–285 (226). Egg length 94–97 (95); width 42–46 (44) (two specimens). Tail length 88–104 (96).

HOST: *Gopherus polyphemus*.

SITE OF INFECTION: Large intestine.

LOCALITY: Tift County (type) and Baker County, Georgia, USA.

HOLOTYPE: USNM Helm. Coll. 76380.

ALLOTYPE: USNM Helm. Coll. 76381.

PARATYPES: USNM Helm. Coll. 76382 (9 males, 9 females—one en face mount).

ETYMOLOGY: The species is named in honor of Dr. MayBelle H. Chitwood, USDA (retired), for her outstanding contributions to the study of nematodes.

## Remarks

*Chapiniella chitwoodae* sp. n. is the fourth species of the genus, the first known to parasitize a host other than *Testudo denticulata*. The new species is smaller than the previously known species and separated from them by its shorter spicules and by a greater distance between vulva and anus. The new species also differs (Table 1) from *C. variabilis* in diameter of the buccal capsule; from *C. diazi* in number of leaf-crown elements, position of the nerve ring, and egg size; and from *C. larensis* in diameter of the buccal capsule and position of the nerve ring (Table 1).

## *Chapiniella gallatii* sp. n.
**(Figs. 8–14, 18)**

DESCRIPTION: Submedian cephalic papillae with elongate digitiform tips. Lateral papillae (amphids) slightly elevated. Buccal capsule short, wide and circular.

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**Table 1. Comparative characteristics, hosts and distribution of species of *Chapiniella*.**

| Characteristics | Species of *Chapiniella* | | | | | |
|---|---|---|---|---|---|---|
| | *C. variabilis* (Chapin, 1924) | *C. larensis* Diaz-Ungria and Gallardo, 1968 | *C. diazi* Diaz-Ungria and Tcheprakoff, 1977 | *C. chitwoodae* sp. n. | *C. gallatii* sp. n. | *C. jellisoni* sp. n. |
| Host | *Testudo denticulata* | *Testudo denticulata* | *Testudo denticulata* | *Gopherus polyphemus* | *Gopherus polyphemus* | "turtle" |
| Geographic distribution | S.A. | S.A. | S.A. | N.A. | N.A. | Asia |
| Body length, males (mm) | 8.0 | 5.4 | 4.0 | 2.8–3.6 | 2.7–3.2 | 5.6 |
| Body length, females (mm) | — | 6.1 | 6.1 | 3.3–4.1 | 3.1–3.7 | — |
| Number of elements in leaf-crowns | 18 | 18 | 30 | 19 | 17 | 24 |
| Diameter of buccal capsule ($\mu$m) | 40 | 80 | 20–23 | 21–26 | 18–19 | 30 |
| Esophagus length/body length | 5% | 10% | 7% | 7–8% | 9–10% | 7% |
| Nerve ring to anterior end/esophagus length | 50% | 36% | 88% | 52–58% | 55–58% | 55% |
| Spicule length/body length | 19% | 46% | 33% | 15–19% | 27–34% | 61% |
| Vulva to anus/tail length | 37% | 20% | 12% | 58–75% | 79–91% | — |
| Ovejector length*/body length | 12% | — | 3% | 5–7% | 11–16% | — |

* Combined lengths of sphincter and infundibulum.

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Leaf-crowns of 17 elements each. Nerve ring slightly anterior to middle of esophagus. Excretory pore and cervical papillae near base of esophagus. Spicules 27–34% of body length. Gubernaculum trough-shaped with proximal, thick, spongy, lightly sclerotized portion and a distal, thin, densely sclerotized plate with lateral flanges; proximal portion with medial projection between spicules. Ovejectors long; sphincters and infundibula not markedly differentiated, together 11–16% of body length.

MALE (based on nine specimens): Total length 2.70–3.24 (3.00) mm. Esophagus length 262–278 (272). Anterior end to nerve ring 130–160 (144); excretory pore 246–292 (278); cervical papillae 262–313 (293). Buccal capsule depth 5 (5); diameter 18–19 (18). Body width at base of cephalic collar 25–30 (28); base of esophagus 50–64 (55); midbody 62–100 (83). Width of cuticular annule at base of esophagus 4–5 (5); midbody 6–7 (6). Spicules 800–1,000 (906) long; 7–11 (9) wide at proximal ends. Gubernaculum length 62–70 (65). Genital cone length 20–37 (29); bears two digitiform dorsolateral papillae and a triangular ventral papilla. Dorsal ray length 68–98 (82); externodorsal rays originate 42–86 (69) from distal end.

FEMALE (based on 10 specimens): Total length 3.12–3.73 (3.50) mm. Esophagus length 265–302 (285). Anterior end to nerve ring 126–143 (137); excretory pore 245–298 (283); cervical papillae 256–322 (299). Buccal capsule depth 5–7 (5); diameter 19–25 (21). Body width at base of cephalic collar 25–34 (30); base of esophagus 58–75 (66); midbody 87–142 (113). Width of cuticular annule at base of esophagus 5–6 (5); midbody 5–7 (6). Distance vulva to anus 80–147 (112). Vagina length 36–71 (59); vestibule length 44–96 (68); combined length of sphincter and infundibulum 416–580 (492). Egg length 86; width 47 (one specimen). Tail length 83–134 (96).

HOST: *Gopherus polyphemus*.

SITE OF INFECTION: Large intestine.

LOCALITY: Baker County (type) and Tift County, Georgia, USA.

HOLOTYPE: USNM Helm. Coll. 76383.

ALLOTYPE: USNM Helm. Coll. 76384.

PARATYPES: USNM Helm. Coll. 76385 (8 males, 9 females—including en face mount).

ETYMOLOGY: The species is named in honor of Prof. Walter W. Gallati, Indiana University of Pennsylvania, Indiana, Pennsylvania.

## Remarks

*Chapiniella gallatii* sp. n. is most similar to *C. chitwoodae* which is found in the same host species. Differences described between *C. chitwoodae* and the

→

**Figures 8–14.** ***Chapiniella gallatii* sp. n., line drawings. Scale bars 50 μm unless indicated otherwise. 8. Anterior one-tenth of female, lateral view. 9. En face view of female showing submedian cephalic papillae, lateral papillae, external leaf-crown elements, and cuticular extensions of external leaf-crown. Scale bar 20 μm. 10. Anterior extremity of male, ventral view. Scale bar 20 μm. 11. Female tail, lateral view showing elongate y-shaped ovejectors and distal ends of uteri. 12. Male tail, lateral view, and proximal ends of spicules. 13. Dorsal bursal ray and genital cone. 14. Gubernaculum and genital cone. Scale bar 20 μm.**

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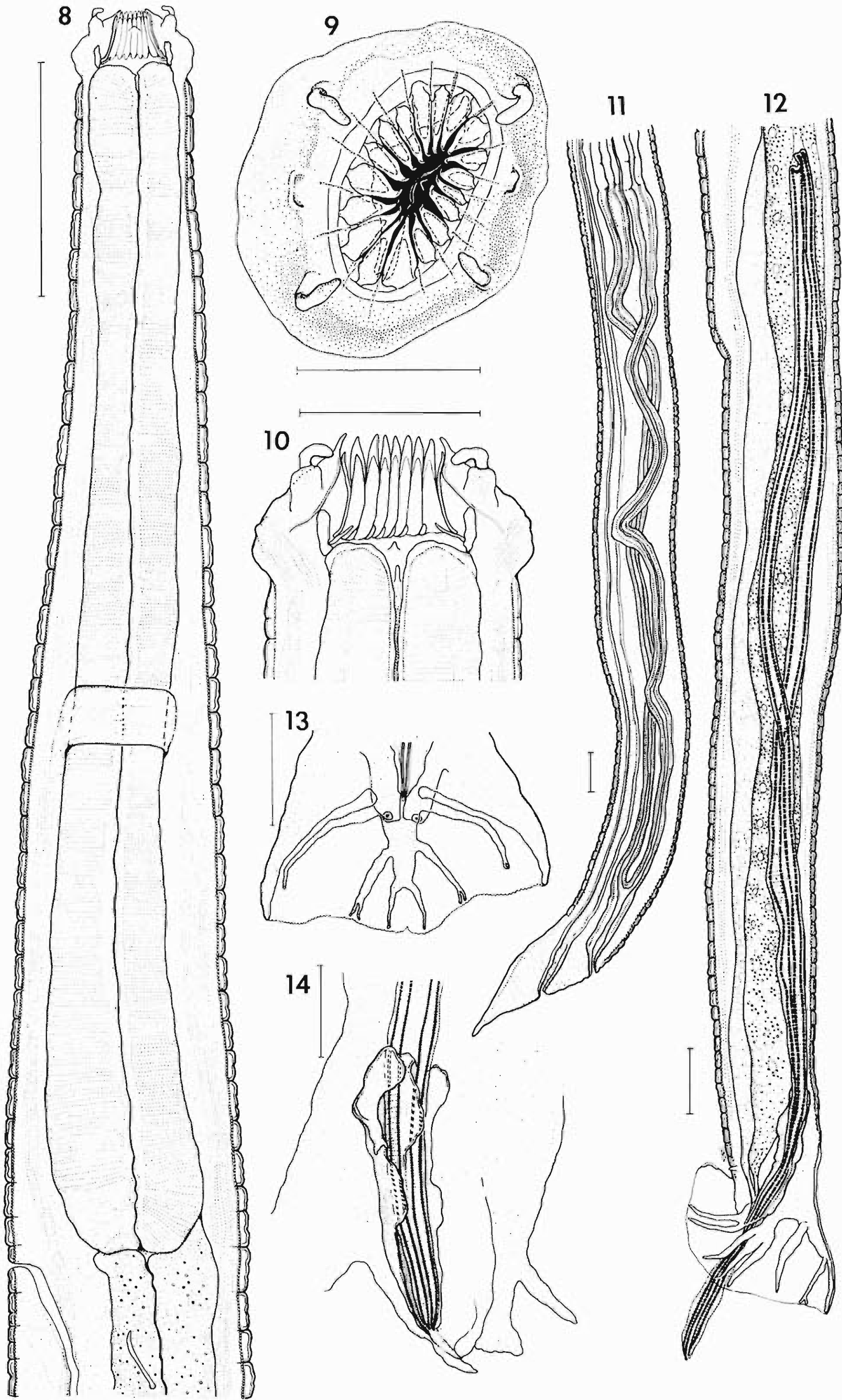


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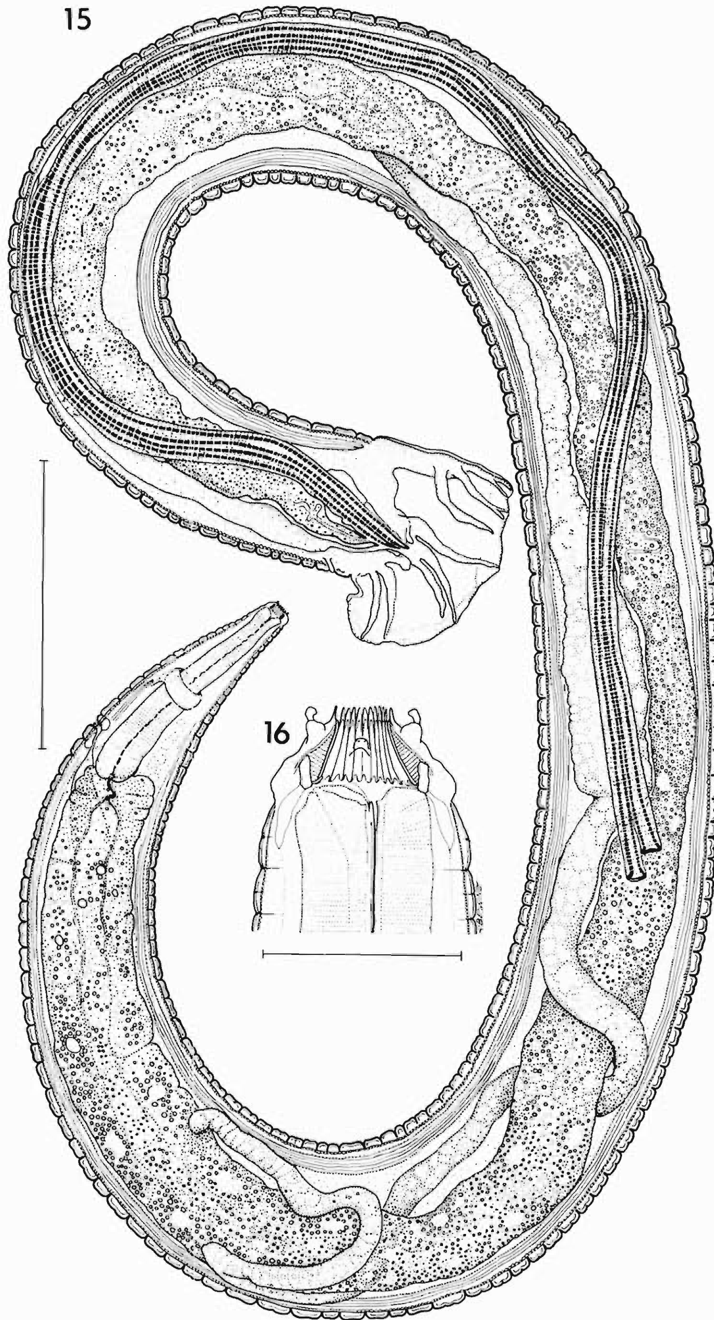


Figures 15, 16. *Chapiniella jellisoni* sp. n., line drawings. 15. Male, lateral view. Scale bar 500 $\mu$m. 16. Anterior extremity, lateral view. Scale bar 50 $\mu$m.

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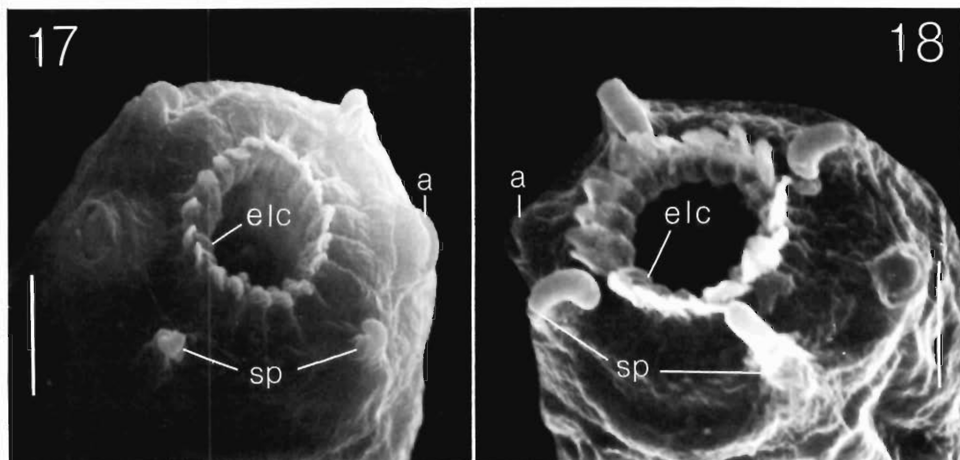


**Figures 17, 18. Scanning electron micrographs of *Chapiniella* spp., en face views showing four submedian papillae with digitiform tips (sp), two slightly elevated amphids with large C-shaped openings (a), and elements of the external leaf-crown (elc) and their overlying cuticular extensions. Scale bars 10 μm. 17. *Chapiniella chitwoodae* sp. n. 18. *Chapiniella gallatii* sp. n.**

three previously described species are the same differences found between *C. gallatii* and the three previously described species (Table 1). Nevertheless, sufficient differences exist between *C. gallatii* and *C. chitwoodae* to require that they be recognized as separate species. *Chapiniella gallatii* can be separated easily from *C. chitwoodae* by the much longer tips on the submedian cephalic papillae of the former. Although slightly smaller in size, *C. gallatii* has longer spicules, longer ovejectors, and greater vulva to anus distance than *C. chitwoodae*. The dorsal ray of *C. chitwoodae* is much longer than that of *C. gallatii* and the externodorsal rays of *C. gallatii* originate near the base of the dorsal ray compared to the middle of the dorsal ray of *C. chitwoodae*.

### *Chapiniella jellisoni* sp. n.
### (Figs. 15, 16)

DESCRIPTION: Submedian cephalic papillae with elongate bulbous tips. Lateral papillae not elevated. Buccal capsule short and wide. Leaf-crowns with 24 elements each. Nerve ring near middle of esophagus. Excretory pore and cervical papillae anterior to base of esophagus. Spicules 61% of body length. Gubernaculum with large spongy proximal portion and platelike flanged distal portion. Externolateral and mediolateral rays curve ventrally; posterolateral ray curves dorsally.

MALE (based on one specimen): Total length 5.60 mm. Esophagus length 370. Anterior end to nerve ring 202; excretory pore 323; cervical papillae 302. Buccal capsule depth 8; diameter 30. Body width at base of cephalic collar 46; base of esophagus 189; midbody 319. Width of cuticular annule at base of esophagus 5; midbody 6. Spicules 3.44 mm long; 38 wide at proximal ends. Gubernaculum length 164. Genital cone length 88. Dorsal ray length 231; externodorsal rays originate 134 from distal end.

FEMALE: Unknown.

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HOST: "turtle," *Cyclemys amboinensis.*
SITE OF INFECTION: Unknown.
LOCALITY: 8 miles North of Myitkyina, Burma.
HOLOTYPE: USNM Helm. Coll. 45589.
ETYMOLOGY: The species is named in honor of Dr. William L. Jellison who collected the specimen.

**Remarks**

The only known specimen of this species was discovered in the USNM Helminthological Collection where it was recorded as Cyathostominae collected from a "turtle" by Dr. William L. Jellison and Dr. Robert Traub on 21 October 1945. At my request Dr. Robert Traub recently searched old records to learn that a feral "turtle" they necropsied on that day was recorded as *Cyclemys amboinensis* (Daudin) the Amboina box-tortoise of Southeast Asia including Burma. The records also show, however, that a second "tortoise" was necropsied the same day. It is not certain that the second "tortoise" was conspecific with the "turtle." Neither is it certain that the nematode was collected from the latter although the notation "turtle" on the USNM accession record may so indicate. *Chapiniella jellisoni* sp. n. has the longest spicules of the six species. The most similar species appears to be *C. larensis* from which *C. jellisoni* differs in buccal capsule size and number of leaf-crown elements (Table 1).

## Discussion

The genus *Chapiniella* is of considerable interest because it is one of only two genera of Strongyloidea parasitic in the Reptilia. Previous workers (Chabaud and Tcheprakoff, 1977) regarded the strongyloid species in tortoises to be parasites of capture and concluded that the nearest relatives of the nematodes are among the Cyathostominae of mammals. Based on the new collections from *Gopherus polyphemus* and the discovery that *Chapiniella* occurs in North America and Asia as well as in South America, the Strongyloidea of tortoises is a much larger fauna than previously believed. This new information provides the first biogeographical evidence that *Chapiniella* may have evolved in tortoises prior to continental drift. Durette-Desset (1978) reached the same conclusion for *Trichoskrjabinia* Travassos, 1937 when she found species of that genus in tortoises of North America and Malaysia. Lichtenfels (1980) discussed morphological evidence that *Chapiniella* is among the most primitive members of the Strongyloidea. Further discussion of the evolution and classification of the Strongyloidea of tortoises will be deferred until we can complete the description of the new fauna from *G. polyphemus*.

Many of the differences described among the species of *Chapiniella* are morphometric. The morphometric differences between the two most similar species do not correlate with body length, however. They differ, therefore, from the morphometric differences that are caused by host effects. For example, although *C. gallatii* is slightly smaller than *C. chitwoodae* both its male and female reproductive organs are significantly larger. In addition, *C. gallatii* has significantly larger tips on its submedian cephalic papillae than *C. chitwoodae*. The relative size of submedian papillae tips was also found to be useful in separating Cyathostominae of horses (Lichtenfels, 1975). Furthermore, the differences between the

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two species cannot be attributed to host effects because both species were collected from the same individual host in two cases.

Descriptions of a new species from a single specimen should be avoided generally. However, the value of *C. jellisoni* in establishing the presence of *Chapiniella* in Asia made its description desirable. Fortunately, the unique morphology of *C. jellisoni,* with its small buccal capsule and exceptionally large spicules leaves little doubt of its specific distinctness.

## Acknowledgments

We thank Robert B. Ewing for preparing the drawings, Patricia A. Pilitt for the en face preparations, Philip A. Madden for the electronmicrographs, and Drs. J. A. Payne, G. T. Fincher, and Mr. Ricky Griffin for collecting some of the tortoises.

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Proc. Helminthol. Soc. Wash.
48(2), 1981, pp. 148–153

# *Molineus samueli* n. sp. (Nematoda: Trichostrongyloidea: Molineidae) from the Badger, *Taxidea taxus*

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ABSTRACT: *Molineus samueli* n. sp. is described from the small intestine of the badger, *Taxidea taxus*, from the central plains of North America. This is the fourth species of the genus *Molineus* reported from North American badgers. The new species is most similar to *M. patens* and *M. mustelae* but differs from the former in the shape of the gubernaculum and from the latter in the shape of the spicules and gubernaculum. The synlophe consists of interrupted crêtes and is consistent with previous reports for the genus.

Pence and Dowler (1979) reported the presence of an undescribed species of the trichostrongyloid genus *Molineus* from badgers, *Taxidea taxus*, in Kansas and West Texas. These specimens are described herein as a new species, *Molineus samueli*.

## Materials and Methods

Nematodes were collected, killed, and fixed as previously described (Pence and Dowler, 1979). Specimens were cleared and examined as wet mounts in either lactophenol or glycerine. En face mounts were prepared by the method of Anderson (1958).

Drawings were made with the aid of a drawing tube. All measurements were made with an ocular micrometer and are in micrometers ($\mu$m) unless otherwise stated. Measurements include the mean ± one standard deviation, followed by the range in parentheses.

## *Molineus samueli* n. sp.
## (Figs. 1–10)

Molineidae Durette-Desset and Chabaud, 1977; Molineinae Skrjabin and Schul'ts, 1937; *Molineus* Cameron, 1923.

GENERAL: Medium-sized worms, tapering toward extremities. Cephalic inflation present, pronounced anteriorly, tapering posteriad terminating in a faint, transverse groove (Figs. 1, 2). Lips absent, 6 externolabial and 4 cephalic papillae present, internolabial papillae not observed. Amphids small, circular (Fig. 3). Deirids not observed. Excretory pore opens ventrally into cervical groove, nerve ring slightly anterior to cervical groove (Fig. 1). Cervical groove complete. Cuticle with fine, transverse striations. Synlophe composed of interrupted crêtes (Figs. 4, 5). Synlophe composed of 22 to 52 crêtes; 22 in anterior region (posterior to cervical groove, increasing to 52 in the midregion of body. Anterior region: 1 pair on either side of lateral hypodermal cord directed at a 45° angle toward dorsal and ventral surfaces. Remaining 18 crêtes at 90° from body surface, 9 in each field (dorsal and ventral), 4 in each quadrant, a single crête adjacent to dorsal and ventral hypodermal cords. Midregion: 52 crêtes aligned at 90° from body surface, 8 enlarged crêtes (4 + 4) on either side of lateral hypodermal cords. 18 crêtes in dorsal and ventral field slightly reduced. Crêtes terminate anterior to the anus.



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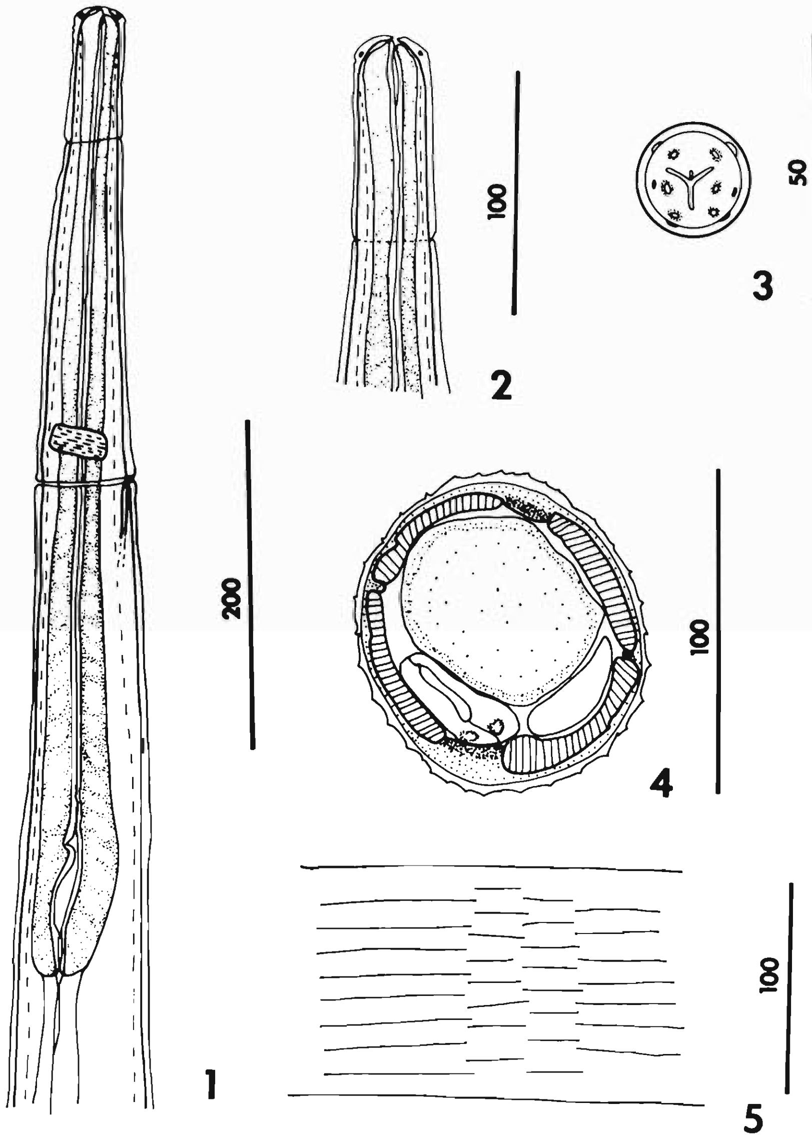


**Figures 1–5.** ***Molineus samueli*** **n. sp. 1. Allotype anterior end. 2. Allotype anterior end. 3. Female en face. 4. Female synlophe—xs. 5. Allotype—synlophe.**

MALE (based on 7 specimens): 9.5 ± 1.1 mm (8–11.8 mm) long, 107 ± 11.5 (99–134) wide (maximum). Cephalic bulb 27 ± 2.73 (25–29) wide at anterior tip, by 68 ± 5.87 (59–75) long. Distance from anterior end to cervical groove-excretory pore 268 ± 29.8 (232–310) and to nerve ring 220 ± 22.6 (190–253). Esophagus 512 ± 61.5 (462–655) long by 38 ± 3.1 (34–44) in maximum width. Copulatory bursa (Fig. 7) fully developed, covered internally by minute spinelike structures, which decrease in number dorsal to the externodorsal ray and ventral to the ventroventral ray. Ventroventral and lateroventral rays separate near base, run parallel, reach bursal margin. Ventrolateral ray diverges at base from other lateral

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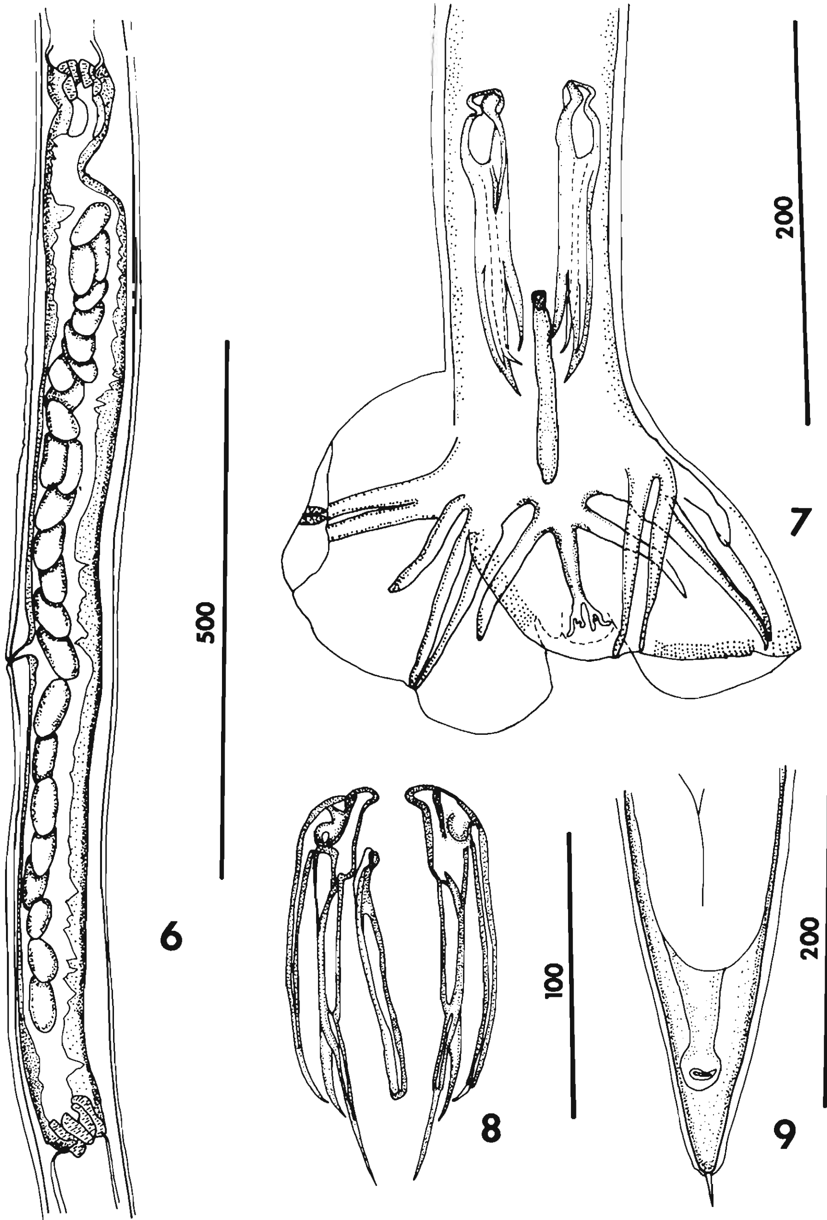


**Figures 6–9.** ***Molineus samueli*** **n. sp. 6. Allotype vulval region. 7. Holotype spicules, gubernaculum, and copulatory bursa (ventral). 8. Spicules and gubernaculum dissected from the surrounding tissue. 9. Allotype posterior end.**

rays, deflected ventrad, approximately ½ length of other lateral rays. Laterolateral and laterodorsal rays diverge close to base, are parallel, and reach bursal margin. Externodrosal ray originates approximately 1/5 distance from base of dorsal ray, directed dorsolaterally, not reaching bursal margin. Dorsal ray slender, branching approximately ¼ distance from distal tip. Each branch bifurcate,

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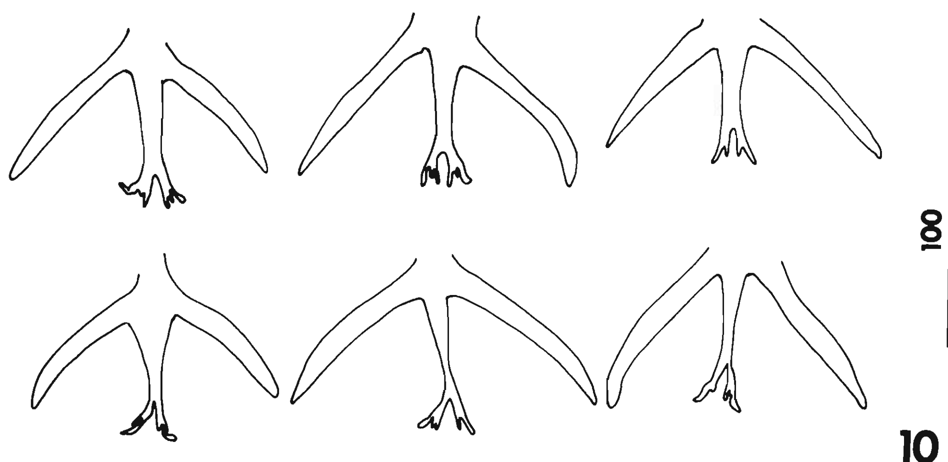


**Figure 10. Variability in the dorsal ray of *Molineus samueli* n. sp.**

internal branch may divide again, distinctly cheliform (Fig. 10). External branch longer, curving laterad. Spicules complex, equal 153 ± 5.6 (147–169) long (Figs. 6, 7), divided approximately 1/5 distance from proximal end into ventral and lateral processes. Ventral process slender to moderate thickness. Dorsal process bifurcates ⅓ distance from distal tip; dorsal projection long, terminating in a point; ventral projection terminates in a point at the level of ventral process. Gubernaculum 95 ± 4.03 (88–100) long by 12 ± 2.26 (9–16) wide; coarse, curving ventrad, with ventral projection and slight anterior protrusion (Figs. 6, 7). Distal end terminates bluntly.

FEMALE (based on 10 specimens): Tapering at both ends, 12.7 ± 0.45 mm (12.1–13.6 mm) long by 138 ± 9.5 (121–152) in maximum width, at the vulva. Cephalic bulb 34 ± 5.6 (29–46) wide by 67 ± 5 (60–74) long. Distance to nerve ring and cephalic groove-excretory pore from anterior end, 246 ± 34 (241–320) and 282 ± 25 (251–339), respectively. Esophagus 620 ± 49 (546–702) long by 47 ± 5.5 (38–60) in maximum width. Anal lips slightly salient 98 ± 23 (74–147) from posterior end. Body terminates in a sharp spine (Fig. 9) 10 ± 6 (3–19) long (n = 9, absent in one specimen). Opisthodelphic; vulva in posterior ¼ of body, 2.29 ± 0.16 mm (2.0–2.55 mm) from posterior end. Anterior ovejector 679 ± 46.3 (606–720) long (vestibule, 604 ± 47.5 (606–720); infundibulum, 60 ± 11.6 (44–74); sphincter, 55 ± 5.7 (47–66)); posterior ovejector 611 ± 58 (546–726) long (vestibule, 538 ± 74.2 (456–690); infundibulum 62 ± 7.3 (51–70); sphincter, 59 ± 9.5 (46–71)). Eggs (in utero, n = 50) embryonated, elliptical 71 ± 6.35 (51–82) long by 35 ± 4.65 (26–50) wide, forming a single row in the uterus (Fig. 6). One pair minute ventrolateral caudal papillae, near posterior extremity (Fig. 9).

TYPE HOST: *Taxidea taxus* (badger).

LOCATION: Small intestine.

LOCALITY: Western Kansas.

OTHER LOCALITIES: King County, Texas; Chaves County, New Mexico.

SPECIMENS DEPOSITED: Holotype (♂) USNM Helm. Coll. 75760 and allotype (♀) USNM Helm. Coll. 75761; additional specimens (6 ♂, 9 ♀) USNM Helm. Coll. 75762.

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**Table 1. The genus *Molineus* in badgers from North America.**

| Species | Locale | Author(s) |
|---|---|---|
| *Molineus felineus* Cameron, 1923 | Utah | Grundmann, 1957 |
| *Molineus mustelae* Schmidt, 1965 | Wyoming | Keppner, 1969 |
| *Molineus patens* (Dujardin, 1845) | Minnesota | Erickson, 1946 |
| | North Dakota | Leiby et al., 1971 |
| *Molineus samueli* | Texas, Kansas | Present study |
| | Alberta (Canada) | Platt (pers. obser.) |
| | Iowa* | Present study† |

* Originally reported as *Molineus mustelae* by Wittrock and Ulmer (1974).
† Personal observation.

ETYMOLOGY: This species is named in honor of Dr. William M. Samuel, University of Alberta, in honor of his contributions to wildlife parasitology.

## Discussion

*Molineus samueli* is the fourth species of the genus reported from badgers in North America (Table 1). It most closely resembles *M. patens* (Dujardin, 1845) and *M. mustelae* Schmidt, 1965. The new species differs from *M. patens* in the shape of the gubernaculum. Although the gubernaculum of *M. patens* was not mentioned in the original description (Dujardin, 1845), subsequent descriptions of this species (Petrov, 1928; Leiper, 1936; Kozlov, 1977) have reported this structure as ventrally curved, tapering to a point at both ends. This form was also observed by one of us (T.R.P.) in a specimen of *M. patens* collected from *Ursus arctos* in Siberia by Rausch et al. (1979). The gubernaculum of *M. samueli* has a distinct ventral projection at the proximal end (neither mentioned nor illustrated in previous descriptions of *M. patens*) and is bluntly rounded distally (Figs. 6, 7). The caudal papillae of the female *M. samueli* differ considerably from those described for *M. patens* by Leiper (1936). The papillae of the new species are minute and situated in an almost lateral position near the posterior extremity, while Leiper (1936) figured the papillae of *M. patens* as large and immediately posterior to the anus, well removed from the posterior extremity.

*Molineus samueli* differs from *M. mustelae* in the shape of the spicules and gubernaculum. The proximal end of the gubernaculum is similar in these species, although the anterior projection found in *M. mustelae* is absent or poorly developed in *M. samueli*. The distal tip of the gubernaculum of *M. mustelae* is similar to that of *M. patens*. The spicules of the two species are divided into distinct dorsal and ventral processes. The ventral process has a sublateral spur. The spur fuses with the main ventral shaft in *M. mustelae* but remains separate in the new species (Figs. 6, 7). This character is, however, difficult to observe in intact specimens. The new species further differs from *M. mustelae* in the size of the ovejector. Schmidt (1965) reported the combined length of the ovejectors in *M. mustelae* as 253 to 341 ($\bar{x}$ = 305) while this structure is 4 to 5 times longer in *M. samueli*.

The dorsal ray of *M. samueli* is quite variable (Fig. 10). The dorsal ray bifurcates twice giving rise to a minimum of four terminal rays. Either or both of the internal rays may divide internally, yielding a distinct cheliform structure similar

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to that described by Schmidt (1965) for *M. mustelae*. The extreme variability of this structure renders it useless for species level taxonomy.

The synlophe consists of interrupted crêtes (Figs. 4, 5), ridges containing no supporting element (Durette-Desset, 1971). Durette-Desset (1973) reported the synlophe of eight species of *Molineus* from insectivores in Madagascar, all of which consisted of crêtes. She did not mention, however, if the crêtes were continuous or interrupted. The segments of the crêtes were variable in length and no discernible pattern was observed. This is the first report of the morphology of the synlophe of the genus *Molineus* from a carnivore.

## Acknowledgments

The authors wish to thank Drs. E. J. Keppner and D. D. Wittrock for the loan of specimens of *Molineus* from badgers; Dr. J. Ralph Lichtenfels for the loan of the type specimens of *M. mustelae* and *M. barbatus*, as well as specimens of *M. patens*, all from the U.S.N.M.; and Dr. W. R. Tenney for assistance in preparation of the plates.

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48(2), 1981, pp. 154–158

# *Andersonstrongylus milksi* (Whitlock, 1956) n. comb. (Metastrongyloidea: Angiostrongylidae) with a Discussion of Related Species in North American Canids and Mustelids

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Abstract: Specimens of *Filaroides milksi* Whitlock, 1956 removed from the same lung tissue as were the type specimens were re-examined. Because of morphological features similar to those found in the genus *Andersonstrongylus* Webster, 1978 and inconsistent with those in *Filaroides* van Beneden, 1850, *F. milksi* is removed from the latter and placed in *Andersonstrongylus* as *A. milksi* (Whitlock, 1956). All available specimens of *A. milksi* from North American and Belgian canids and mustelids were re-examined. The only valid report of *A. milksi* is that of Whitlock (1956) in dogs; other reports being either presumptive, misidentified or the material is no longer available. The filaroidid and angiostrongylid nematodes of North American skunks are either respectively *Filaroides mephitis* Webster, 1967 or *Andersonstrongylus captivensis* Webster, 1978.

The members of the genus *Filaroides* van Beneden, 1858 are all small fragile nematodes and it is only on rare occasions that complete specimens can be teased from the lung tissue in which they are embedded. Because of this, the morphological features necessary for identification are often destroyed. When present, they are so small that they may be overlooked or misinterpreted. This has led to some confusion in the systematics of this genus and its relatives.

Recently there has arisen some uncertainty over the identity of certain filaroidid and angiostrongylid nematodes in North American carnivores, in particular the occurrence of *Filaroides milksi* Whitlock, 1956 in various host species. A restudy of specimens teased from the same lung tissue from which Whitlock's types were obtained revealed morphological characters inconsistent with the other members of the genus *Filaroides* but similar to those of *Andersonstrongylus* Webster, 1978. Accordingly, *F. milksi* is transferred to *Andersonstrongylus* as *A. milksi* n. comb.

Representative specimens from all known infections of *A. milksi* in North America were requested. Nematodes were cleared in a phenol-alcohol solution which was replaced with glycerin. Drawings were made with the aid of a Zeiss drawing tube.

## *Andersonstrongylus milksi* (Whitlock, 1956) n. comb. (Figs. 1, 4a)

*Filaroides milksi* Whitlock 1956.

Description: As in Whitlock (1956) with the exception of the caudal end of the male. Tail of male usually curved ventrally. Bursa present. Bursal rays short but distinct; ventral rays divided approximately ½ their length; lateral rays arising from a common stalk and separated at the ends; dorsal ray reduced to a single pair of minute sessile papillae.

Type host and locality: *Canis familiaris*, Geneva, N.Y., USA.

Specimens examined: USNM Helm. Coll. 74734, NMCIC(P) 1980-137.



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## Discussion

One of the main diagnostic characters used by Anderson (1978) to separate the two families Angiostrongylidae and Filaroididae is the presence (in the former) or absence (in the latter) of a bursa. Unfortunately, this exceedingly delicate structure can be difficult to detect in preserved material although it is generally easily seen in fresh specimens. Neither Georgi (1979) nor this study could demonstrate with any certainty, the presence of such a structure in the specimens of *A. milksi* obtained from the type lung tissue. However, Whitlock (1956) was quite definite in his finding of a bursa ". . . and it showed more distinctly with phase microscopy." The morphology and disposition of the bursal rays are similar to those of *Andersonstrongylus captivensis* Webster, 1978 (Fig. 2). Both the ventral and lateral ray groups are small but distinct. The dorsal ray is represented by a single pair of small papillae. It is upon these morphological features and Whitlock's statement that a bursa was present that *A. milksi* is removed from *Filaroides* (Filaroididae) and placed in *Andersonstrongylus* (Angiostrongylidae). The genus *Filaroides* contains only those abursate species in which the rays (when present) are relatively large, bulbous, undifferentiated papillae.

Since the original description by Whitlock (1956), *A. milksi* has been reported on numerous occasions as occurring mainly in dogs or skunks.

Mills and Nielsen (1966) and Greenway and Stockdale (1970) reported *A. milksi* from dogs, but the diagnoses were made from the examination of histological sections. Corwin et al. (1974) reported *A. milksi* in a dog from Michigan. No diagrams were presented and the only material now available from that infection are tissue sections. To date, there are no accurate diagnostic criteria to differentiate between the species in *Filaroides* and/or *Andersonstrongylus* on histological examination. Peckham et al. (1960) described *A. milksi* from an Iowa dog as having the male bursa ". . . reduced to 4 or 5 closely grouped papillae-like lobes." These specimens are no longer available. Cremers et al. (1978) reported *A. milksi* in a dog from Belgium. The author has examined these and has found that they are *F. hirthi* Georgi and Anderson, 1975.

Pence (1978) described nematodes from the hog-nosed skunk *Conepatus mesoleucus* from Texas. He noted that the male had a ". . . very rudimentary bursa bearing two pairs of large pedunculate, postanal papillae" and that the spicules were 54–77 (65) μm in length. After comparing the types of *Filaroides mephitis* Webster, 1967 with his specimens, he concluded that they were the same and that *F. mephitis* should therefore become a synonym of *A. milksi*. Pence apparently did not examine the types of *A. milksi*. However, specimens from *Conepatus mesoleucus* (Mus. Texas Tech. Univ. 1007, 1010) have been examined by the author and are in fact *Andersonstrongylus captivensis*, a species quite distinct from *Filaroides mephitis* (Fig. 3). Pence and Dowler (1979) recorded but did not describe *A. milksi* from Kansas badgers. Georgi (1979) described *A. milksi* from a striped skunk from New York. These also have been examined and are *A. captivensis* as is material from a second skunk from New York (courtesy of Dr. Georgi). Levine et al. (1965) recovered nematodes from the lungs of a skunk *Mephitis mephitis* from Iowa, USA which they identified as *A. milksi*. Although these specimens are apparently no longer available, based upon their description, the accompanying photograph, and the original designation, it can be assumed that they were *Andersonstrongylus* sp.

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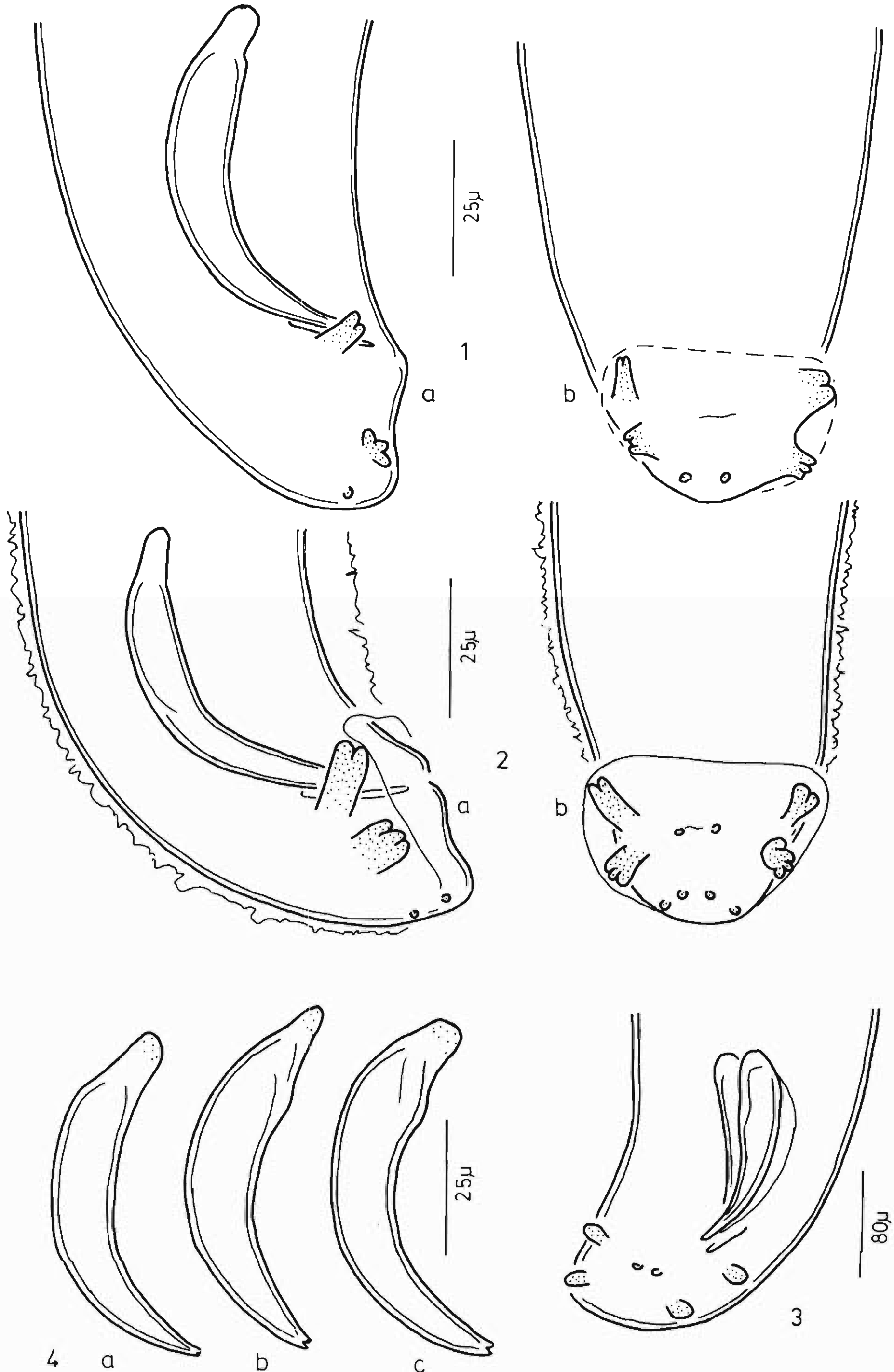


**Figures 1–4.** 1. ***Andersonstrongylus milksi*** **n. comb. Lateral (a) and ventral (b) views of male tail NMCIC(P) 1980-137. Dotted line indicates bursa described by Whitlock (1956). 2.** ***Andersonstrongylus captivensis*****. Lateral (a) and ventral (b) views of male tail NMCIC(P) 1977-258. 3.** ***Filaroides mephitis*****.**

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In summary, of all the reports of *A. milksi* in dogs, the only one which can be validated is the original by Whitlock (1956). All others are either presumptive (based upon tissue sections), misidentified, or the material is no longer available. It is quite possible that the common filaroidid nematode of dogs in North America is *F. hirthi* (see Georgi, 1979). Of the reports of *A. milksi* in wild carnivores, those of Pence (1978) and Georgi (1979; unpublished) are in fact of *A. captivensis*. The specimens reported upon by Levine et al. (1965) and by Pence and Dowler (1979) are no longer available for study.

Two species appear to infect skunk lungs. *Filaroides mephitis* is a common filaroidid nematode in various regions of North America (Webster, 1967; Dyer, 1979). *Andersonstrongylus captivensis*, originally found in ranch-raised striped skunks (Webster, 1978), has now been shown to occur in wild skunks as well in Texas and the eastern USA. These two species are quite distinct morphologically (Figs. 2, 3). When considering the size and the conformation of the caudal end of the males, there is no difficulty in distinguishing between the two. In addition, *F. mephitis* tends to be found in nests around the bronchioles while *A. captivensis* is widely distributed throughout the lung tissue.

*Andersonstrongylus milksi* and *A. captivensis* are very closely related morphologically. The spicules, although similar in morphology are slightly smaller in the former (Figs. 4a, b, c) and only one pair of the minute dorsal papillae could be found in the type material of *A. milksi* as opposed to two pairs in *A. captivensis*. It has been shown (Webster, 1980) that *A. captivensis* has a direct life cycle in striped skunks; i.e., without the usual metastrongyloid requirement of an intermediate host. Although this species is capable of readily producing patent infections lasting at least 3 months in experimentally infected skunks, only transient patent infections could be produced in mink (unpublished) or in dogs (Georgi, personal communication). To date there have been no reports on life cycle studies for *A. milksi*.

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**Ventral view of male tail from *Mephitis mephitis*. 4. Spicules of various species. a. *A. milksi* NMCIC(P) 1980-137 from dog. b. *A. captivensis* NMCIC(P) 1977-258 from *Mephitis mephitis*. c. *A. captivensis* Mus. Texas Tech. Univ. 1007, 1010 from *Conepatus mesoleucus*.**

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Proc. Helminthol. Soc. Wash.
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# Redescription of *Pneumonema tiliquae* Johnston, 1916 (Nematoda: Rhabdiasidae) from an Australian Skink

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ABSTRACT: *Pneumonema tiliquae* Johnston, 1916, parasitic in the lung of *Tiliqua scincoides* (Shaw) from Australia, is redescribed. The spines and cervical alae on the body cuticle are specializations not observed in other rhabdiasoids. Nevertheless the cephalic morphology of this monotypic genus closely resembles that observed in the large genus *Rhabdias* Stiles and Hassall, 1905, and *Pneumonema* is interpreted as having evolved from *Rhabdias*.

The monotypic genus *Pneumonema* Johnston, 1916 (Rhabdiasidae) is known from two inadequate descriptions (Breinl, 1913; Yorke and Maplestone, 1926). In the present study *P. tiliquae* is redescribed and shown to be closely related to *Rhabdias* Stiles and Hassall, 1905.

## *Pneumonema tiliquae* Johnston, 1916
## (Figs. 1–10)

DESCRIPTION (based on 7 specimens): Total length 7.6–10.6 mm. Body cuticle smooth and relatively thin (1–2 μm) over most of body surface, slightly inflated on posterior half of tail. Thick pair of lateral cervical alae present just posterior to head. Cervical alae 122–163 μm long, oriented laterodorsally, divided into thick anterior portion and less thick posterior portion. Cuticle on anterior third of body with numerous sublateral, posteriorly directed spines variable in shape, number, size and distribution (Figs. 4–6). Spines most numerous in region of esophagus, decreasing in number and size posteriorly, with tendency to be clumped in groups. On each sublateral surface spines occur in two rows, one on dorsal and the other on ventral side. Largest spines 130–140 μm long, smallest about 4 μm. In five specimens examined spines numbered from 67 to 82. Prominent anterior deirids present 141–181 μm from anterior extremity. Cephalic extremity anterior to esophagus slightly flattened dorsoventrally. Oral opening circular, lined by slender cuticular ring. Two prominent lateral pseudolabia present beside oral opening, each with large dorsal and ventral protuberance on inner margin. Each pseudolabium with three small digitiform papillae on inner edge. Amphidial pores small, on lateral edge of each pseudolabium. Buccal cavity circular in apical view, lined with thick cuticle. Opening to lumen of esophagus surrounded by ring of cuticle. Esophagus 566–688 μm long (5.5–8.8% of body length), club-shaped, with slightly inflated corpus. Nerve ring 153–184 μm and excretory pore 169–203 μm from anterior extremity. Excretory pore minute, renette cells not observed. Subventral esophageal gland duct openings near nerve ring, dorsal duct opening into buccal cavity. Vulva in form of small slitlike opening 4.1–5.2 mm from anterior extremity (45–54% of body length). Vagina reduced to short cuticle lined duct lacking muscular walls. Amphidelphic. Uteri containing numerous thin-shelled eggs 75–88 μm long and 44–53 μm wide (based on 5 spec-



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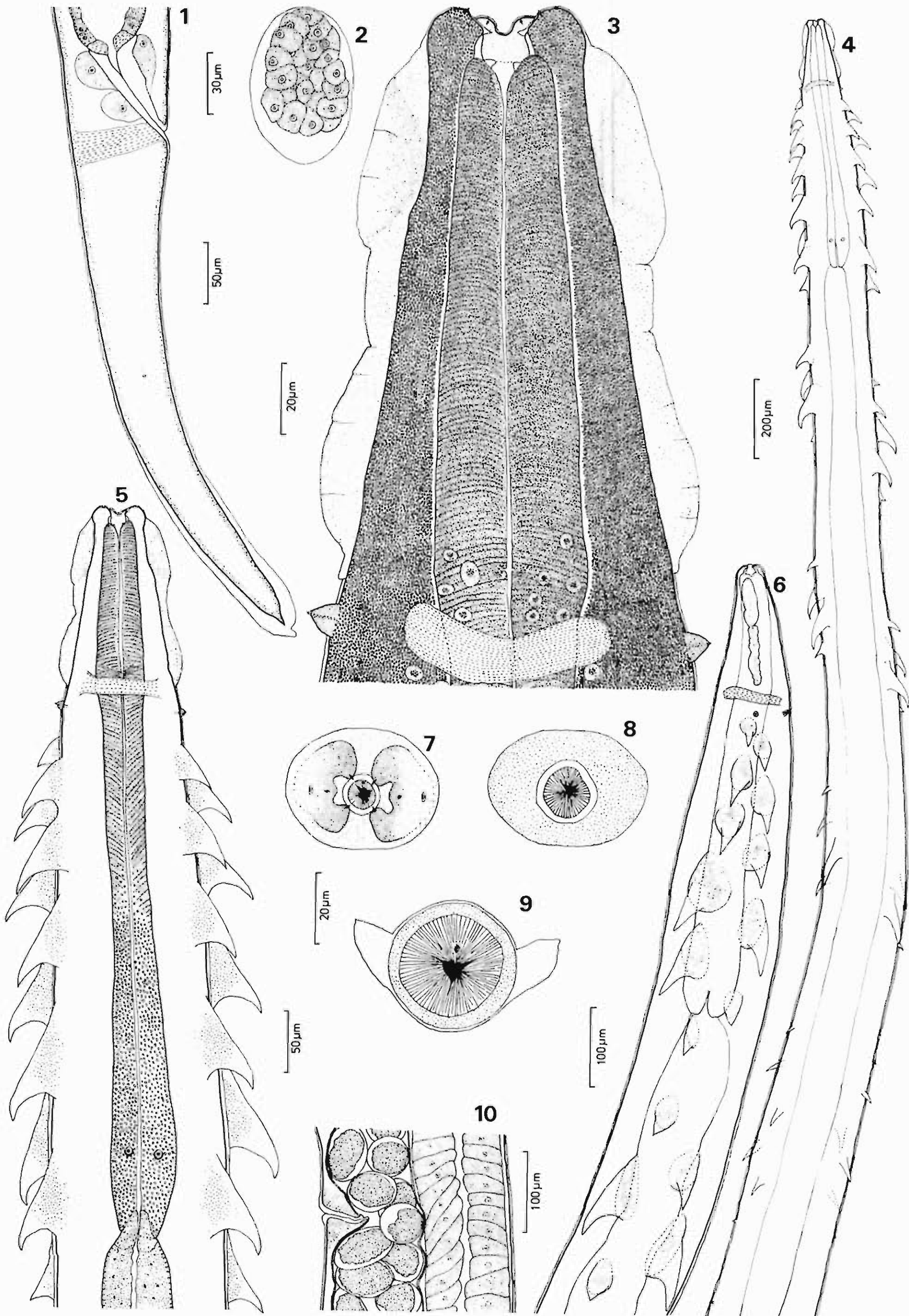


**Figures 1–10.** ***Pneumonema tiliquae*** **Johnston, 1916. 1. Tail, lateral view. 2. Egg from uterus near vulva. 3. Cephalic end, ventral view. 4. Anterior end showing spines on body cuticle, ventral view. 5. Cephalic end, ventral view. 6. Cephalic end, lateral view. 7. Cephalic extremity, apical view. 8. Cephalic extremity, optical section through buccal cavity. 9. Cephalic extremity, optical section through anterior end of esophagus. 10. Vulva, lateral view.**

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imens). Eggs not observed beyond gastrula stage of development. Tail 394–569 μm long (4.6–5.5% of body length), slender, phasmids present at midregion.

FREE-LIVING GENERATION: Not observed.

HOST: Blue-tongued lizard, *Tiliqua scincoides* (Shaw, 1790) (Scincidae).

LOCATION: Lung.

LOCALITY: The specimens studied herein were from a zoo animal. *Pneumonema tiliquae* has been reported in *T. scincoides* of Queensland (Breinl, 1913; Johnston, 1916) and New South Wales (Johnston, 1916). *Tiliqua scincoides* is restricted in distribution to Australia.

SPECIMENS: Muséum national d'Histoire naturelle, Paris, 1122BA.

## Remarks

Breinl (1913) described nematodes occurring in the lung of the skink *Tiliqua scincoides* from Queensland, Australia. He did not name the species, but he suggested it might belong in the Gnathostomidae (Spirurida). Johnston (1916) examined specimens of Breinl's species from the same host and locality, and although he did not publish a description, he proposed the name *Pneumonema tiliquae* for it. The new genus *Pneumonema* was not classified to family. Yorke and Maplestone (1926) gave a brief redescription of *P. tiliquae* and they classified *Pneumonema* in the family Rictulariidae (Spirurida). Finally Ballantyne and Pearson (1963) showed that *P. tiliquae* is not a spirurid but rather belongs in the family Rhabdiasidae (Rhabditida). Unfortunately they did not provide a redescription.

The present study supports the classification of *Pneumonema* within the Rhabdiasidae. In cephalic and buccal capsule morphology *Pneumonema* most closely resembles *Rhabdias*. For example, the buccal cavity and pseudolabia of gravid specimens of *Rhabdias americana* (see Baker, 1978, 1979) are markedly similar to *Pneumonema*. Since the rhabdiasoid genera *Acanthorhabdias* Pereira, 1927, *Entomelas* Travassos, 1930, and *Rhabdias* are distinguished mainly by differences in cephalic structures, it is clear that *Pneumonema* and *Rhabdias* are closely related. Differences in the appearance of the body cuticle provide the only distinction between *Pneumonema* and *Rhabdias*. In *Rhabdias* many species have a markedly inflated body cuticle, but it is never modified into the spines and cervical alae characteristic of *Pneumonema*. It is most likely that *Pneumonema* evolved as an aberrant form from a *Rhabdias*-like ancestor. All Rhabdiasidae which have been studied have a monoecious parasitic generation (hermaphrodite or parthenogenic female) alternating with a dioecious free-living generation.

An emended diagnosis of *Pneumonema* is given below.

### Emended diagnosis of *Pneumonema* Johnston, 1916

Rhabditida, Rhabditoidea, Rhabdiasidae. Cuticle of body with pair of thick lateral cervical alae and numerous sublateral spines in anterior third of body. Oral opening circular, two large pseudolabia present. Buccal cavity relatively small, with thick cuticular wall. Amphidelphic.

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# Phylogenetic and Biogeographic Hypotheses in Leptonchidae (Nematoda: Dorylaimida) and a New Classification

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ABSTRACT: A phylogeny of Leptonchidae derived by numerical cladistic methods suggests a new family classification as follows: subfamily Leptonchinae with *Funaria*, *Leptonchus*, and *Bertzuckermania*; subfamily Tyleptinae with *Tyleptus*, *Basirotyleptus*, and *Sclerostylus*; subfamily Xiphinemellinae with *Meylis*, *Xiphinemella*, *Loncharionema*, *Proleptonchus*, and *Proleptonchoides*. A testable biogeographic hypothesis (based on cladistic analyses) is that the leptonchids are descended from a Pangaean ancestor with subsequent radiation primarily in areas formerly part of the Gondwanian supercontinent.

In a series of generic revision in Leptonchidae (Goseco et al., 1975a, b; Ferris et al., 1979) we grouped the genera by traditional methods, based on overall similarity, into four subfamilies, viz. Leptonchinae, Tyleptinae, Belonenchinae, and Xiphinemellinae. We have since examined phylogenetic relationships within the family Leptonchidae using the phylogenetic (or cladistic) approach of Hennig (1966) and Brundin (1968). This approach is concerned with genealogy, i.e., with evolutionary branching sequences among taxonomic units without regard to phenetic similarities (Camin and Sokal, 1965). Hennig's (1966) technique is based on the principle that monophyletic sister groups are detected by the common possession of derived homologous character states (termed synapomorphies). Shared ancestral homologous character states (called symplesiomorphies) tell us nothing about genealogical relationships. Data for deciding whether a character state is plesiomorphic or apomorphic come largely from outgroup comparisons for nematodes, as for other groups which leave no fossil sequences. Ferris (1979) discussed the use of cladistics in nematode systematics.

## Materials and Methods

To analyze the family Leptonchidae we prepared a data matrix of genera, with coded character states of all the characters that we hypothesized to be homologous and for which a reasonable hypothesis could be made regarding the direction of evolution. Both qualitative and quantitative characters were used. For coding, the character states were arrayed in what seemed to be logical order (from an evolutionary standpoint) and coded in linear sequence. Hypothesized primitive states were coded zero and derived states positive or negative according to our assumptions of the direction of evolutionary trends (Camin and Sokal, 1965; Kluge, 1976; Brooks, 1977; Ferris, 1979). We compared several different methods to derive phylogenies from the matrix. The first method was to join genera and groups of genera on the basis of synapomorphies in the manner of Hennig (1966), Brundin (1968), and Ross (1974). In addition, we used the numerical phyletic method of Camin and Sokal (1965) and the Wiss and Wagner tree algorithms (Hennig Program, version 2/3/77 and Wagner 78) developed by J. S. Farris (Farris, 1970; Farris et al., 1970). For the latter a hypothesized most primitive ancestor with all states scored as plesiomorphic was included in the data set as a means of rooting the trees. The Camin-Sokal technique and the Wiss algorithm permit



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**Table 1. Characters and character state polarities, with codings, used to prepare cladogram of genera of Leptonchidae, Figure 1.**

| Character number | Name of character | Plesiomorphic character state | Apomorphic character state |
|---|---|---|---|
| 1 | esophagus | no constriction (0) | constriction (1) |
| 2 | esophageal bulb | long, cylindrical (0) | short, pyriform (1) |
| 3 | male supplements | numerous and closely spaced (0) | few (1) or fewer (2) and widely spaced |
| 4 | female gonads | didelphic (0) | opisthodelphic (−1) or prodelphic (+1) |
| 5 | odontostyle | slender, short (0) | widened (−1), needlelike (1), very long (2), secondarily reduced in length (3) |
| 6 | odontophore | smooth (0) | flanges developed (1) or flanges secondarily lost (2) |
| 7 | lip region | smooth (0) | liplets or disc (1) or liplets or disc secondarily lost (2) |
| 8 | cardia | large (0) | reduced (1) |

no evolutionary reversals, whereas the Wagner algorithm makes no such assumptions. In nematodes (as in other organisms) derived character states may not be unique, but may have arisen repeatedly in different branches of the group (Camin and Sokal, 1965). The numerical phyletic algorithms all generate the most parsimonious trees and minimize the changes which must be considered to result from convergence or parallel evolution.

## Results and Discussion

### Phylogeny of Leptonchidae

The characters used for the leptonchid genera and the hypothesized character state polarities are given in Table 1. The most parsimonious trees obtained from all the numerical techniques agreed closely with the tree obtained by joining genera on the basis of synapomorphies after inspection of the data matrix. The cladogram based on the Camin-Sokal solution for the best pattern (with the most compatibilities and the fewest extra steps) is given in Figure 1. We had considerable difficulty coding the character states of the odontostyle and odontophore and in coding modifications of the lip region, such as liplets or disc. Analysis of the initial compatibility matrix indicated a high probability of miscoding in these characters, suggesting that in some instances absence of a disc or flange was a plesiomorphic character state and in others an apomorphic state resulting from a secondary disappearance. These states were recoded (as they now appear in Table 1) to improve the pattern and fit of these characters in the matrix. Camin and Sokal (1965) recommend excluding such "poor" characters from the matrix and fitting them later to the cladogram obtained from the other characters. (We omitted characters 5 and 6 from the data matrix used to derive our Wiss and Wagner trees.)

The cladogram (Fig. 1) shows a division into two groups, based on presence or absence of a constriction in the esophagus. In the cladogram, those genera that possess fewer synapomorphies are at the center, and those that possess more

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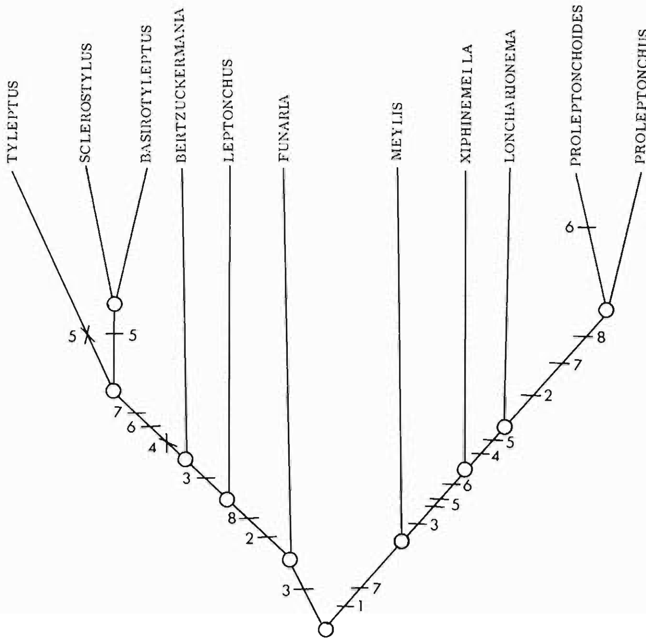


**Figure 1. Cladogram for Leptonichidae based on characters of Table 1. An evolutionary step increasing a character state code by one is indicated as a short line accompanied by the number of the character. A step which decreases the state code by one is indicated by an X. The state code at the base of the cladogram is assumed to be 0 for each character. The circles at nodes represent hypothetical ancestors and the steps along a stem are cumulative.**

synapomorphies are at either extremity. Within each group, as one progresses from the center outwards, several trends are evident as follows: The plesiomorphic long cylindrical esophageal bulb becomes shortened and finally attains the pyriform shape (Fig. 2). The plesiomorphic didelphic female gonads become monodelphic. In one group (on the left in Fig. 1) opisthodelphy predominates, whereas in the other group (on the right) the females become prodelphic. The male supplements change from the plesiomorphic state of being numerous and closely spaced to being fewer in number and more widely spaced (Fig. 3). Various modifications of the lip region and spears occur as discussed above. Our present interpretation is that the odontostyle of the common (hypothetical) ancestor of *Loncharionema, Proleptonchus,* and *Proleptonchoides* became secondarily reduced in length; that the flanges of the odontophore of *Proleptonchus* were secondarily lost; and that the ancestor of *Proleptonchus* and *Proleptonchoides* secondarily lost the labial disc. Likewise, we consider the wide odontostyle of *Tyleptus* species to have resulted from an apomorphic widening of the basic leptonchid narrow odontostyle. Flanges on the odontophore secondarily disappeared in some species of *Tyleptus* and *Basirotyleptus* (not shown in Fig. 1).

## Classification of Leptonchidae

We agree with Gaffney (1979) that a classification that mirrors a phylogenetic hypothesis (however transitory) is the most useful and our new phylogeny provides an improved basis for classification of the Leptonchidae. Our former clas-

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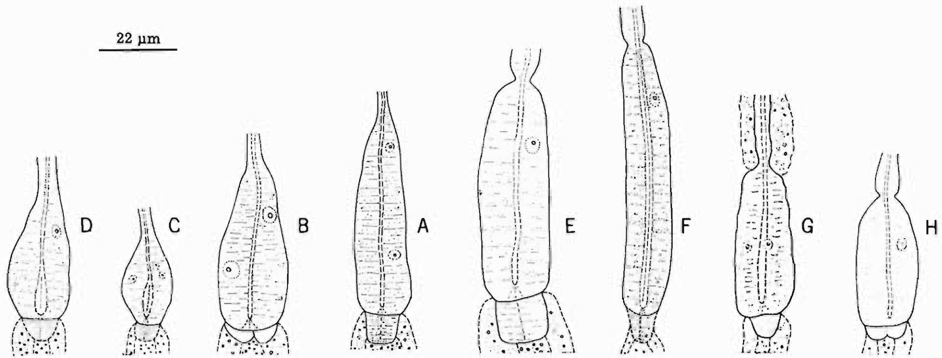


**Figure 2. Typical esophagi in several genera of Leptonchidae, with genera arranged as in cladogram, Figure 1. A = *Funaria*, B = *Leptonchus*, C = *Basirotyleptus*, D = *Tyleptus*, E = *Meylis*, F = *Loncharionema*, G = *Proleptonchoides*, H = *Proleptonchus*. See text for discussion of trends.**

sification was based partly on tradition and partly on overall phenetic similarity. Thus, we grouped *Funaria* and *Meylis* together because both exhibit the long cylindrical basal esophageal bulb (Fig. 2) and we felt they were "close." We now believe that the long bulbs represent a symplesiomorphy and therefore can tell us nothing about genealogical relationships. We divide the genera on the left of Figure 1 into two subfamilies, Leptonchinae and Tyleptinae. For the present we group those on the right of Figure 1 under Xiphinemellinae, although future data may suggest that the *Proleptonchus*, *Proleptonchoides* group should be under a separate subfamily. The classifications (old and new) are summarized below as follows:

| Old Classification (Goseco et al., 1975a, b) | New Classification (based on Fig. 1, this paper) |
|---|---|
| Leptonchidae Thorne, 1935 | Leptonchidae |
| Leptonchinae Thorne, 1935 | Leptonchinae |

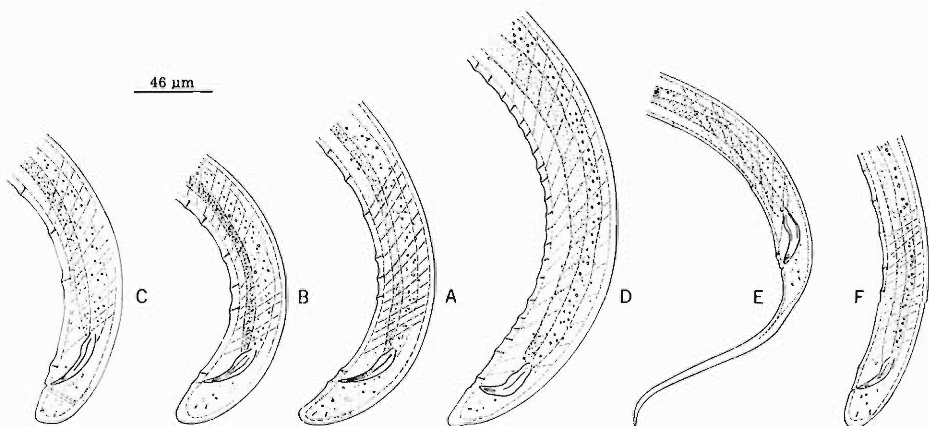


**Figure 3. Posterior portions of males in several genera of Leptonchidae, with genera arranged as in cladogram, Figure 1. A = *Funaria*, B = *Leptonchus*, C = *Tyleptus*, D = *Meylis*, E = *Loncharionema*, F = *Proleptonchus*. See text for discussion of trends.**

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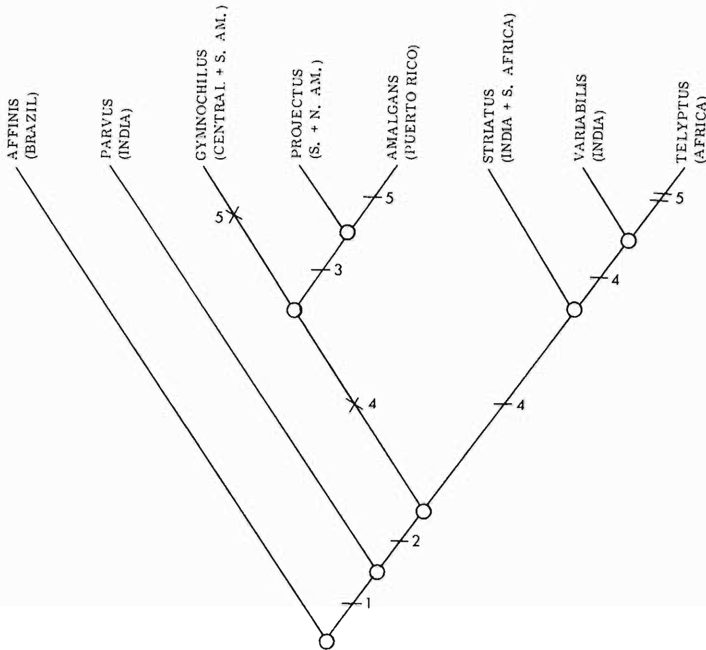


**Figure 4. Cladogram for *Tyleptus* based on characters of Table 2. See legend of Figure 1 for explanation of markings.**

| | |
|---|---|
| *Funaria* van der Linde, 1938 | *Funaria* |
| *Meylis* Goseco, Ferris et Ferris, 1975 | |
| *Leptonchus* Cobb, 1920 | *Leptonchus* |
| *Proleptonchus* Lordello, 1955 | *Bertzuckermania* |
| *Bertzuckermania* Khera, 1970 | |
| Tyleptinae Jairajpuri, 1964 | Tyleptinae |
| *Tyleptus* Thorne, 1939 | *Tyleptus* |
| | *Athernema*[1] Ahmad and Jairajpuri, 1978 |
| | *Basirotyleptus* |
| | *Sclerostylus* Goseco, Ferris et Ferris, 1980 |
| Belonenchinae Thorne, 1964 | |
| *Basirotyleptus* Jairajpuri, 1964 | |
| Xiphinemellinae Jairajpuri, 1964 | Xiphinemellinae |

[1] This monotypic genus was described in Nematologica 24(4):445–448, which we did not receive until 1979, after our study was completed. We have not examined specimens and did not include *Athernema* in our numerical analysis, but we place it in Tyleptinae.

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**Table 2. Characters and character state polarities, with codings, used to prepare cladogram of species of *Tyleptus*, Figure 4.**

| Character number | Name of character | Plesiomorphic character state | Apomorphic character state |
|---|---|---|---|
| 1 | shape of tail | conoid (0) | hemispherical (1) |
| 2 | esophagus | relatively long (0) | relatively short (1) |
| 3 | subcuticle | coarse striations (0) | fine striations (1) |
| 4 | odontophore | about same length as odontostyle, narrow, plain (0) | longer than odontostyle (−1), base widened (1), or flanged (2) |
| 5 | inner liplets | projecting (0) | disappeared (−1), fused (1), sunken (2) |

*Xiphinemella* Loos, 1950
*Loncharionema* Goseco, Ferris et Ferris, 1975

*Meylis*
*Xiphinemella*
*Loncharionema*
*Proleptonchus*
*Proleptonchoides* Ferris, Goseco et Kumar, 1979

## Species cladograms

Development of cladograms at the species level in Leptonchidae is difficult because of our present inability to discover unique derived character states to delimit sister groups unequivocally. Also, it is almost imperative that the researcher looking for such character states be able to examine specimens of every nominal species. For genera with large numbers of described species (some with lost type materials) this can be nearly impossible. We have derived a phylogeny (Fig. 4) for species of the genus *Tyleptus*, which has many derived character states including lip ornamentations and a short pyriform esophageal bulb with internal thickenings. *Tyleptus* has been known and studied since 1939 and seems to be an evolutionarily inert genus with only eight nominal species (Goseco et al., 1975b). It is interesting to note that after intensive and extensive collecting over 12 years we have been able to find only one species in North America.

We have grouped the species of *Tyleptus* (Fig. 4) on the basis of six characters for which we hypothesize plesiomorphic and apomorphic character states (Table 2). In Figure 4 the species closest to our hypothesized primitive ancestor is *T. affinis* Monteiro, 1970, at the left. *Tyleptus telyptus* Andrássy, 1969, and *T. variabilis* Jairajpuri and Loof, 1966, share a number of synapomorphies, as do *T. gymnochilus* Loof, 1964, *T. projectus* Thorne, 1939, and *T. amalgans* Thorne, 1964.

Most *Basirotyleptus* are opisthodelphic as are *Tyleptus* (Fig. 1). A small group of six species, however, shows a progressive sequence toward prodelphism (the Leptonchinae are all didelphic, which we believe to be the plesiomorphic state). Figure 5 is a diagram of a possible phylogeny, based largely on female gonads (Table 3), for this small group of *Basirotyleptus*. *Basirotyleptus bunocephalus*

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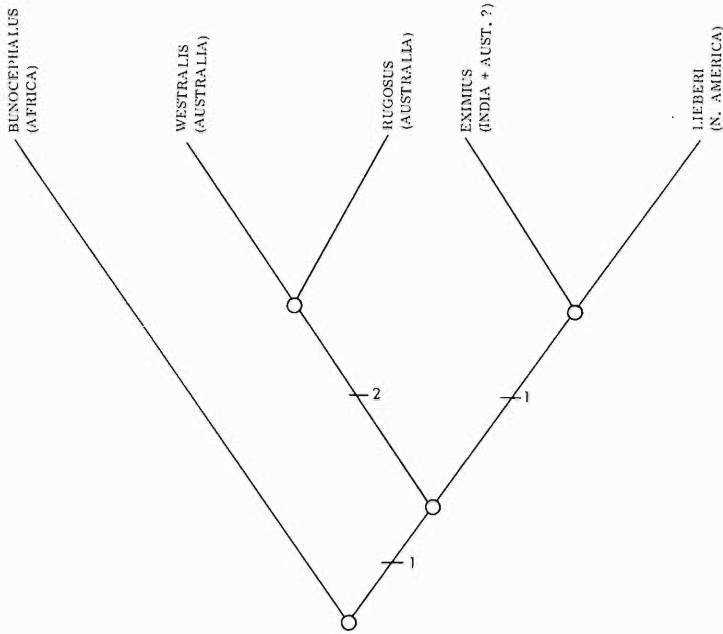


**Figure 5. Cladogram for prodelphic group of *Basirotyleptus* based on characters of Table 3. See legend of Figure 1 for explanation of markings.**

Siddiqi, 1970, is the only known species of *Basirotyleptus* in which the female gonads are didelphic symmetric, a condition we hypothesize as plesiomorphic. *Basirotyleptus westralis* Siddiqi, 1970, and *B. rugosus* Sauer, 1966, are didelphic with a shortened posterior gonad. (In addition, both have exceptionally long odontostyles relative to other species of *Basirotyleptus*.) *Basirotyleptus eximius* Siddiqi and Khan, 1965, and *B. lieberi* Goseco, Ferris and Ferris, 1975, are the only known prodelphic species of *Basirotyleptus*.

## Biogeography of Leptonchidae

Cladistic analysis makes possible biogeographic analysis (Nelson, 1969). We have previously suggested vicariance interpretations to explain present-day distributions in several nematode groups (Ferris et al., 1976, 1978; Ferris, 1977, 1979;

**Table 3. *Basirotyleptus* characters and character state polarities, with codings, used to prepare cladogram of *Basirotyleptus* species, Figure 5.**

| Character number | Name of character | Plesiomorphic character state | Apomorphic character state |
|---|---|---|---|
| 1 | female gonads | didelphic symmetric (0) | didelphic asymmetric (1), prodelphic (2) |
| 2 | odontostyle | short (0) | long (1) |

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Ferris et al., 1980). The reported distribution of *Tyleptus* species as indicated on the cladogram of Figure 4 suggests that the ancestral form was distributed in Gondwanaland as far as India. Extant species are restricted to Gondwana areas plus Puerto Rico, except for one species in North America (*T. projectus*) which is also in Venezuela, and which probably entered North America from South America across the Central American land bridge. If *Tyleptus striatus* Heyns, 1963, is truly in South Africa and India, then it must have persisted in the two areas for a very long time. *Tyleptus gymnochilus, T. projectus*, and *T. amalgans* must have had a South American ancestor. *Tyleptus affinis*, which we hypothesize (Fig. 4) to be most similar to the ancestral form is found in Brazil.

Similar evidence for a Gondwana history may be seen from the distributions of the prodelphic group of *Basirotyleptus* (Fig. 5). *Basirotyleptus bunocephalus*, with didelphic symmetric female gonads is in Africa. *Basirotyleptus westralis* and *B. rugosus*, both with didelphic asymmetric gonads, are in Australia. One prodelphic species was described from India (with a similar form reported from Australia) and the other is in North America. We suggest that this small group of *Basirotyleptus* was distributed in Gondwana prior to 110 million years ago to account for the presence in Australia prior to the splitting off of Australia and its northward journey to the present-day position. Again, we hypothesize that the North American species arrived late from South America via the Central American land bridge. It is likely that prodelphic species will be found in South or Central America.

The known opisthodelphic *Basirotyleptus* can be arranged along a cline with progressive shortening of the anterior gonad. All are in Gondwana areas plus North America, and, to our knowledge, none has been reported from Europe or Asia, other than the subcontinent of India. We recently reported the occurrence of four *Basirotyleptus* species in Central America (Goseco et al., 1981) with affinities to species in India (two), Puerto Rico (one), and North America (one).

For the family Leptonchidae, the genus *Funaria* is the only one that seems to have radiated primarily in Laurasian areas (principally North America). The rest of the genera seem to have radiated primarily in Gondwana areas, as did *Tyleptus* and *Basirotyleptus*. We postulate that the ancestor of the group was in Pangaea, as was *Funaria*, but that most of the other genera originated and radiated in Gondwana subsequent to the breakup of Pangaea about 180 million years ago. All new data on leptonchid species and their distributions can be used to falsify or refine the phylogenetic and biogeographic hypotheses we have suggested.

## Acknowledgments

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# Evaluation of Actual and Relative Measurements Used in the Description of *Metorchis conjunctus* (Cobbold, 1860) Looss, 1899 (Trematoda: Opisthorchiidae)

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ABSTRACT: Significant changes in measurements of *Metorchis conjunctus* have been observed subsequent to dehydration of the adult worms. Actual measurements changed 14.62% to 22.78%. Ratios computed from actual measurements reduced the effects of dehydration such that no significant differences could be detected by the Student's *t* test. Linear regression analysis indicated that relationships between components of various ratios were significant enough to warrant use in the taxonomy of this and possibly other Digenea.

Uniformity in the method of treatment and fixation of platyhelminths has been lacking. While some authors heat-inactivate their worms (Fried and Guy, 1974; Dronen and Guidry, 1977; Bakke, 1978; Fischthal, 1978), others relax their worms in the cold before fixation. Treatment of specimens prior to examination also has varied between using live, stained unflattened, or stained flattened flukes for growth and comparative morphological studies (Fried and Guy, 1974).

Because of these inconsistencies, measurements of digeneans that appear in the literature must be used with caution when comparing species. Basic digenean taxonomy has relied almost entirely on actual dimensions which are sometimes supported by morphological variations. The value of actual measurements of worms has to be questioned in the light of severe shrinkage as reported for *Ochetosoma ellipticum* (Pratt, 1903) and *O. aniarum* (Leidy, 1891) by Dronen and Guidry (1977).

The use of morphometric ratios in growth and taxonomy has recently received critical evaluation (Atchley et al., 1976; Corruccini, 1977; Atchley, 1978; Dodson, 1978). The problem is one of analysis and interpretation. Excessive abstractions from morphometric analysis have shown that ratios have a number of faults that must be recognized by the biologist (see the discussion by Atchley et al., 1976). Care must be taken when ratio data are examined statistically.

The use of ratios in platyhelminth taxonomy was examined by Wardle and McLeod (1968) and more recently in digenean taxonomy by Fried and Guy (1974) and Dronen and Guidry (1977). These and other researchers failed to realize the possibility that the ratios they have used could create spurious correlations. Often the effect of a worm's size is not removed from the analysis when ratios are used. The appropriate analysis to demonstrate the correlation of value in a ratio is a linear regression. In this way consistency between two variables can be demonstrated. This implies that the line plotted from the linear regression must pass through the origin and that the regression coefficient is significant. If these two criteria are met, the ratio becomes simplified eliminating the variation caused by an intercept that does not intersect the origin.

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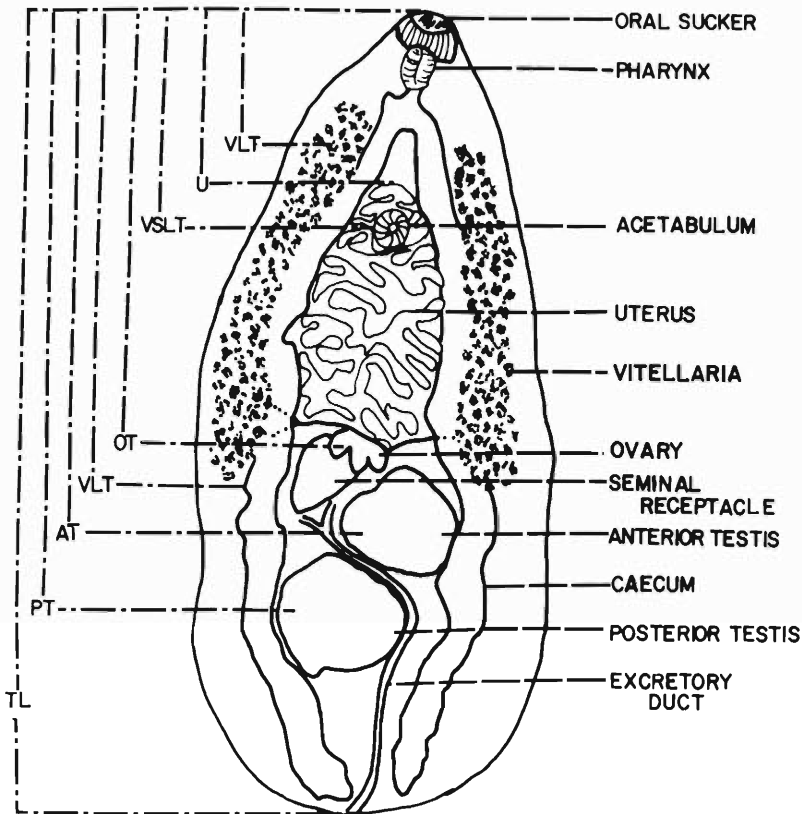


**Figure 1. Schematic diagram illustrating the morphology and some morphometric measurements taken on *Metorchis conjunctus* (×25).**

Examination and measurement of digeneans usually require that they be stained and mounted. A review of the literature indicated that this seems to have been the case with most members of the Metorchiinae described to date. More often than not, procedures taken in killing, fixing, and staining specimens have been inconsistent. Often specimens have been removed from hosts that have been dead, frozen, cooled, or preserved for some time. It is generally conceded that killing helminths in hot media tends to increase tissue swelling and fixation in the absence of acetic acid increases shrinkage of specimens. The present experiment was designed to: (1) identify variations of measurements of adults of *Metorchis conjunctus* (Cobbold, 1860) Loss, 1899 following procedures which normally cause substantial swelling and shrinkage, and (2) determine the applicability of ratios for standardization in the descriptive systematics of this particular fluke.

## Materials and Methods

To evaluate swelling, 28 adult *M. conjunctus* were removed from the bile ducts of a cat that had maintained a single experimental infection for 33 months. The worms were washed in sodium phosphate buffered saline (PBS) at 22–24°C and immediately killed in PBS at 70–75°C. Each worm was placed on a microscope

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**Table 1. Alterations of *Metorchis conjunctus* measurements taken after killing in hot saline (70–75°C) and dehydration in absolute alcohol.**

| Variable* | Heat killed† | Dehydrated | df | $t$ | Shrinkage (%) | Coefficient of variation: Heat killed | Coefficient of variation: Dehydrated |
|---|---|---|---|---|---|---|---|
| OSW | 0.345 ± 0.034 | 0.281 ± 0.031 | 42 | 6.2429‡ | −18.50 | 10.04 | 11.10 |
| VSW | 0.335 ± 0.037 | 0.296 ± 0.045 | 42 | 2.9807‡ | −14.62 | 11.01 | 15.11 |
| VSW/OSW | 0.973 ± 0.072 | 1.053 ± 0.127 | 42 | −2.3047 | +8.22 | 7.44 | 12.11 |
| ATW | 0.584 ± 0.065 | 0.451 ± 0.064 | 42 | 6.5277‡ | −22.78 | 11.20 | 14.24 |
| PTW | 0.581 ± 0.081 | 0.475 ± 0.097 | 42 | 3.7030 | −18.26 | 13.90 | 20.42 |
| ATW/PTW | 1.012 ± 0.108 | 0.971 ± 0.135 | 42 | 1.0481 | −4.05 | 10.70 | 13.90 |
| VLT | 2.014 ± 0.302 | 1.648 ± 0.220 | 54 | 5.1713‡ | −18.16 | 15.02 | 13.36 |
| U | 1.066 ± 0.123 | 0.823 ± 0.111 | 54 | 7.7558‡ | −22.79 | 11.57 | 13.45 |
| VL | 2.780 ± 0.287 | 2.271 ± 0.276 | 54 | 6.7532‡ | −18.34 | 10.32 | 12.18 |
| VSLT | 1.434 ± 0.143 | 1.166 ± 0.122 | 54 | 7.5693‡ | −18.69 | 9.95 | 10.47 |
| OT | 2.931 ± 0.257 | 2.366 ± 0.268 | 54 | 8.0644‡ | −19.28 | 8.76 | 11.33 |
| AT | 3.354 ± 0.321 | 2.697 ± 0.331 | 54 | 7.5387‡ | −19.59 | 9.56 | 12.29 |
| PT | 3.977 ± 0.391 | 3.220 ± 0.392 | 54 | 7.2369‡ | −19.03 | 9.83 | 12.16 |
| TL | 5.454 ± 0.664 | 4.366 ± 0.632 | 54 | 6.2820‡ | −19.95 | 12.17 | 14.48 |
| TW | 1.891 ± 0.192 | 1.523 ± 0.231 | 54 | 6.4864‡ | −19.46 | 10.18 | 15.16 |
| TL/TW | 2.893 ± 0.307 | 2.891 ± 0.373 | 54 | 0.0179 | 0 | 10.61 | 12.90 |
| VL/TL | 0.512 ± 0.037 | 0.523 ± 0.040 | 54 | −1.0931 | +4.19 | 7.27 | 7.71 |
| U/VSLT | 0.743 ± 0.045 | 0.706 ± 0.062 | 54 | 2.5612 | −4.98 | 6.10 | 8.76 |
| OT/TL | 0.541 ± 0.042 | 0.545 ± 0.037 | 54 | −0.4118 | +0.73 | 7.80 | 6.88 |
| VSLT/TL | 0.265 ± 0.024 | 0.269 ± 0.024 | 54 | −0.7385 | +1.49 | 9.07 | 8.92 |
| VL/OT | 0.948 ± 0.053 | 0.960 ± 0.045 | 54 | 0.0179 | +1.26 | 5.61 | 4.65 |
| AT/TL | 0.618 ± 0.040 | 0.621 ± 0.041 | 54 | −0.2832 | +0.48 | 6.51 | 6.60 |
| PT/TL | 0.732 ± 0.038 | 0.741 ± 0.034 | 54 | 0.8736 | +1.21 | 5.27 | 4.65 |
| VLT/TL | 0.370 ± 0.043 | 0.379 ± 0.032 | 54 | −0.8921 | +2.42 | 11.76 | 8.57 |
| (VL − VLT)/U | 0.723 ± 0.153 | 0.758 ± 0.093 | 54 | −1.0297 | +4.60 | 21.21 | 12.31 |
| (OT − U)TL | 0.344 ± 0.030 | 0.355 ± 0.026 | 54 | −1.4926 | +3.13 | 8.73 | 7.21 |
| TL × TW | 10.384 ± 2.031 | 6.719 ± 1.677 | 54 | 7.3640‡ | −34.87 | 19.56 | 24.96 |

* See text.
† Mean ± 1 standard deviation.
‡ Student's $t$-value significant at $P < 0.001$.

slide in PBS under a 20 × 20 mm coverslip and measured with an ocular micrometer in a dissecting microscope. Measurements recorded (Fig. 1) were: body length (TL), maximum body width (TW), oral sucker width (OSW), ventral sucker width (VSW), anterior testis width (ATW), posterior testis width (PTW), oral sucker to ventral sucker center (VSLT), oral sucker to ovary (OT), oral sucker to midpoint of anterior testis (AT), oral sucker to midpoint of posterior testis (PT), oral sucker to anterior extent of uterus (U), oral sucker to posterior extent of vitellaria (VL), oral sucker to anteriormost tip of vitellaria (VLT).

The flukes were then removed from the slides, fixed in sodium phosphate buffered 10% formalin for 48 hr to maximize shrinkage, and transferred to 70% ethanol for 24 hr. The worms were hydrated, stained in acetocarmine for 12 to 24 hr, dehydrated between two glass microscope slides in ethanol, cleared in xylene, and mounted in Permount®. Each fluke was then measured as before. The data for the treatments were compared by Student's $t$ test (Sokal and Rohlf, 1969).

Worms measured to examine the applicability of ratios in morphometrics were recovered from experimentally infected cotton rats. The specimens were prepared

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**Table 2. Linear regression analysis between some morphometric variables commonly used for the taxonomy of *Metorchis conjunctus*.**

| Variables* | Degrees of freedom | Intercept | Slope | Regression coefficient | Coefficient of variation | Student's *t*-value |
|---|---|---|---|---|---|---|
| VSW/OSW | 1 & 159 | 0.025 | 0.797 | 0.8687 | 16.66 | 22.120† |
| OSW/OSL | 1 & 220 | 0.020 | 1.054 | 0.8341 | 18.16 | 22.432† |
| VSW/VSL | 1 & 182 | 0.007 | 1.037 | 0.9606 | 9.33 | 46.628† |
| PW/OSW | 1 & 212 | −0.001 | 0.666 | 0.8043 | 16.72 | 27.581† |
| PW/PL | 1 & 212 | 0.042 | 0.568 | 0.6726 | 26.51 | 13.234† |

* See text.
† Significant at $P < 0.01$.

as previously reported. Linear regressions were determined on an IBM 370 Computer using the Statistical Analysis System (SAS) (Barr et al., 1976).

## Results and Discussion

All absolute measurements were significantly different between treatments, however, no significant differences were observed in the relative measurements or ratios (Table 1).

The most variable characteristics of the killed worms were VLT, U, PTW, and TL. The measurements of dehydrated worms were all more variable than their heat-killed counterparts. The only exception to this was VLT in which the coefficient of variation (CV) declined by 1.66%. The most striking increase in the CV was in PTW which changed by 6.52%.

The CV tended to be lower in the relative data for both killed and dehydrated worms, however, as a general rule there was less variability in the fixed flukes than in the killed worms. The most marked change was a drop of 8.90% in the CV in (VL − VLT)/U. Variability was increased in U/VSLT (2.66%), TL/TW (2.29%), (TL × TW) (5.40%), VSW/OSW (4.67%), and ATW/PTW (3.20%).

After dehydration reduction in the absolute dimensions varied from 14.62% to 22.78% (Table 1). Ratios tended to reduce the effects of dehydration, however, only in the ratio TL/TW was the effect completely eliminated. Increased variability in TL/TW undoubtedly arises from the dramatic increase in the variability of TW. Dehydration definitely increased the variability within the characteristics that were measured. Caution must be exercised in comparing characteristics that show both a marked increase in the CV and a high degree of shrinkage. This would tend to eliminate all absolute body measurements from morphometric comparisons. However, it is clear that all ratios have reduced the effect of shrinkage and could have substantial value in taxonomy of *M. conjunctus* and probably other flukes. Only U/VSLT, and possibly VL/TL, are unstable ratios which are unreliable for use in comparative taxonomy.

Regression data used to examine certain ratios most often used in systematics of Digenea are presented in Table 2. In all cases the regression lines effectively passed through the origin. All regression coefficients were significant. With these criteria satisfied the ratios generated by these variables can be adopted.

In conclusion, morphometric ratios can be used as a taxonomic tool in describing *M. conjunctus*. For comparative purposes this could be usefully extended to encompass all members of the Metorchiinae as well as other digeneans.

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# *Neascus pyriformis* Chandler, 1951 (Trematoda: Diplostomatidae), Redescription and Incidence in Fishes from Brule Creek, South Dakota

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ABSTRACT: Of 2,354 fishes from Brule Creek, South Dakota examined for black spot in the fall of 1978, 883 (37.4%) belonging to seven species and two families were infected. The black spot trematode was identified as *Neascus pyriformis* Chandler, 1951. A redescription of the larva is given along with the prevalence of infection in the hosts. This is the first report of *N. pyriformis* from fishes in South Dakota and six of the hosts are new host records. An attempt to experimentally infect four 1-day-old unfed chicks with larvae was unsuccessful.

Although Hugghins (1959) reported the black spot trematode, *Uvulifer ambloplitis* (Hughes, 1927), from fishes in South Dakota, this is the first report of *Neascus pyriformis* Chandler, 1951. A redescription of *N. pyriformis* is given herein as well as the host range, prevalence, and mean intensity of infection in the fish hosts.

## Materials and Methods

Fish were collected from Brule Creek, Union County, South Dakota in September and October 1978 using a knot mesh minnow seine, 2.95 m × 1.2 m with a mesh size of 6.3 mm, bar measure. On two occasions, September 19 and October 9, all fish collected were preserved in 10% formalin for determining the prevalence of black spot infection.

Cysts used for morphological studies and measurements were removed with needles from the skin and fins of both recently killed and preserved fish. Host cyst measurements from unfixed material were made without pressure after removing the melanocyte portion of the cyst. Parasite cyst measurements from unfixed and preserved material were made without pressure after forcing them from the host cyst with a slight coverslip pressure. Measurements are in micrometers; averages are followed by ranges in parentheses.

Excysted metacercariae were obtained by pretreating cysts in Locke's balanced salt solution (BSS) (pH 2.0) for 15 min at 41°C and then transferring them to an excystment medium (0.5% trypsin and 0.5% sodium cholate in Locke's BSS) at 41°C. The trypsin (1-300 hog pancreas) and sodium cholate (sodium salt of cholic acid) were purchased from ICN Pharmaceutical Inc. The excystment medium was adjusted to pH 7.4 with 7.5% sodium bicarbonate. Larvae started excysting after about 15 min in the medium.

Larvae used for measurements were fixed without pressure in hot 10% formalin and cleared in glycerine. Whole-mount specimens were fixed in hot formalin, stained in borax carmine, cleared in cedarwood oil, and mounted in Permount.

The reserve excretory system was studied in living larvae using light microscopy. Other morphological features were studied by phase-contrast microscopy of living larvae or in glycerine-cleared or stained whole mounts.



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**Table 1. Incidence of *Neascus pyriformis* in fishes from Brule Creek.**

| Host species | No. examined | No. (%) infected | Mean/host (SD) | Range | Mean host length (SD) (cm) |
|---|---|---|---|---|---|
| Fathead Minnow | 1,193 | 545 (45.7) | 1.77 (3.95) | 1–47 | 5.15 (0.82) |
| Creek Chub | 274 | 212 (77.4) | 4.47 (7.11) | 1–57 | 7.58 (3.96) |
| Plains Minnow | 159 | 76 (47.8) | 1.09 (1.64) | 1–10 | 7.26 (0.44) |
| White Sucker | 46 | 25 (54.3) | 0.87 (1.07) | 1–5 | 11.35 (4.26) |
| Stone Roller | 22 | 15 (68.2) | 1.27 (1.32) | 1–15 | 6.84 (0.63) |
| Common Shiner | 15 | 3 (20.0) | 0.04 (0.91) | 1–3 | 6.60 (1.14) |
| Red Shiner | 9 | 6 (66.7) | 0.78 (0.67) | 1–2 | 4.54 (0.83) |

Since the chick has a relatively fast intestinal emptying time (Macy et al., 1968), only treated cysts were used for experimental infections. Two 1-day-old unfed chicks were force-fed 70 and 140 cysts, respectively, which had been treated for 1 hr at 41°C in the excystment medium. Two 1-day-old unfed chicks were each force-fed 70 cysts which had been pretreated for 15 min in Locke's BSS, pH 2.0, at 41°C and then treated for 10 min in the excystment medium at 41°C.

The Statistical Analysis System (SAS) (Barr et al., 1979) was used for all statistical procedures. All computations were carried out on the IBM 3031-3 computer at The University of South Dakota.

## Results

Of 2,354 fishes of 10 species and three families collected in the fall of 1978, 882 (37.4%) belonging to seven species and two families were infected with black spot (Table 1). The prevalence of infection for each species was: fathead minnow (*Pimephales promelas*), 545 of 1,193 (45.7%); creek chub (*Semotilus atromaculatus*), 212 of 274 (77.4%); plains minnow (*Hybognathus placitus*), 76 of 159 (47.8%); white sucker (*Catostomus commersoni*), 25 of 46 (54.3%); stone roller (*Campostoma anomalum*), 15 of 22 (68.2%); common shiner (*Notropis cornutus*), 3 of 15 (20.0%); and red shiner (*Notropis lutrensis*), 6 of 9 (66.7%). No infections occurred in 244 sand shiner (*Notropis stramineus*), 393 bigmouth shiner (*Notropis dorsalis*), or 6 johnny darter (*Etheostoma nigrum*). The mean intensity and range of infection was higher in fathead minnows ($\bar{x}$ = 1.77, 1–47 cysts) and creek chubs ($\bar{x}$ = 4.47, 1–57 cysts) than in other hosts (Table 1).

The cysts were present in the skin and fins of the hosts and also in the mouth of more heavily infected fish. The cyst has a thick outer cellular layer and a thin inner noncellular layer (Fig. 2). The outer layer in cysts of this type is considered of host origin (host cyst) and the inner layer of parasite origin (parasite cyst) (Hoffman and Putz, 1965). The outer portion of the host cyst is made up of a melanocyte layer which can be removed with needles (Fig. 2). The material in the space between host and parasite cyst layers appeared colorless with light microscopy, but as a homogeneous granular material with phase-contrast microscopy (Fig. 2).

Twenty host cysts with the melanocyte layer removed measured 380 (360–405) by 313 (270–375). The parasite cysts were pear-shaped with 18 unfixed cysts measuring 225 (195–255) by 125 (105–150) (Fig. 3). The parasite cysts were easily forced from the host cysts with slight coverslip pressure (Fig. 1), whereas it was

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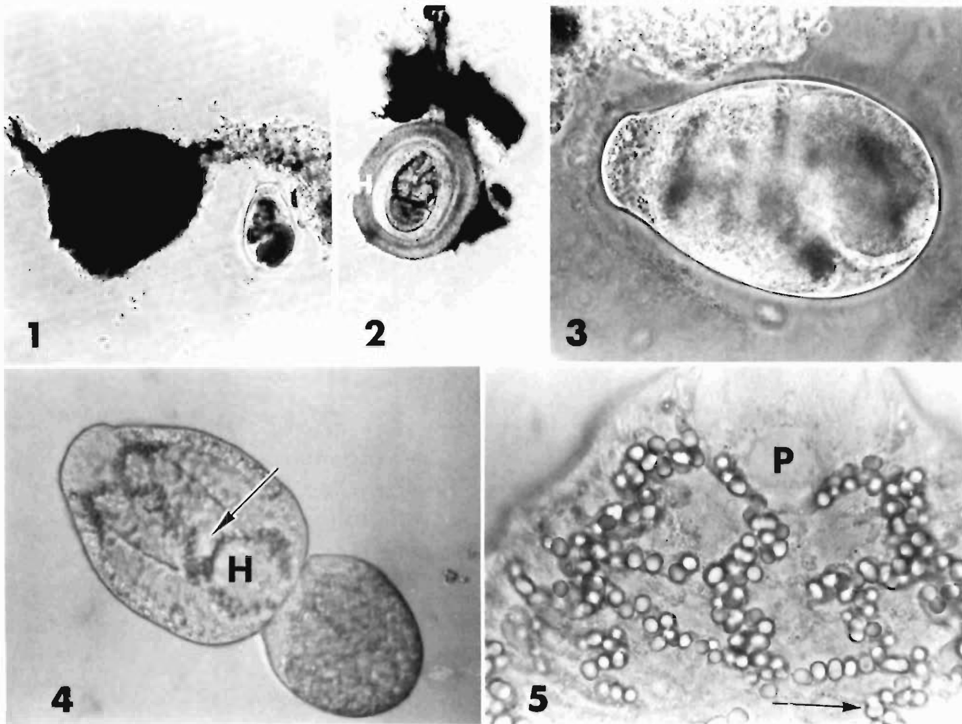


**Figures 1–5.** *Neascus pyriformis.* **Figures 1–3 phase-contrast microscopy; Figures 4–5 light microscopy. 1. Pigmented cyst removed from fish host with parasite cyst forced out of host cyst. ×80. 2. Cyst with melanocyte portion removed, showing rest of host cyst (H), inner parasite cyst, and homogeneous material between cyst layers. ×80. 3. Pear-shaped parasite cyst, showing thin cyst wall and larva inside the cyst. ×325. 4. Excysted larva, showing median dorsal vessel passing on one side of ventral sucker (arrow) and encircling holdfast organ (H). ×250. 5. Anterior end of excysted larva (P = pharynx), showing three transverse commissural vessels on each side from median dorsal vessel and intralateral vessel (arrow) extending from most posterior commissural vessel. ×940.**

essentially impossible to force larvae out of the parasite cysts or dissect them out without damage. The larva with the forebody folded back on itself completely filled the parasite cyst (Fig. 3).

The size of 10 preserved parasite cysts from each of the seven host species were: fathead minnow, 228 (210–255) by 122 (105–135); creek chub, 221 (195–240) by 122 (105–150); common shiner, 234 (210–240) by 126 (105–135); stone roller, 221 (195–255) by 122 (105–135); red shiner, 228 (210–255) by 122 (105–135); plains minnow, 225 (210–240) by 123 (105–135); and white sucker, 225 (195–240) by 129 (105–150). Differences among the length and width means were evaluated using Duncan's multiple range test at the 0.05 level. There was no significant difference in cyst length ($F = 1.21$, $P = 0.31$, df = 6, 63) nor in cyst width ($F = 0.53$, $P = 0.78$, df = 6, 63) among the seven host species. The overall measurements of the 70 parasite cysts were 226 (195–255) by 123 (105–150).

No worms were found in the small intestine of the four experimentally infected chicks which were killed 4 days, 5 days, 6 days, and 7 days, respectively, after infection.

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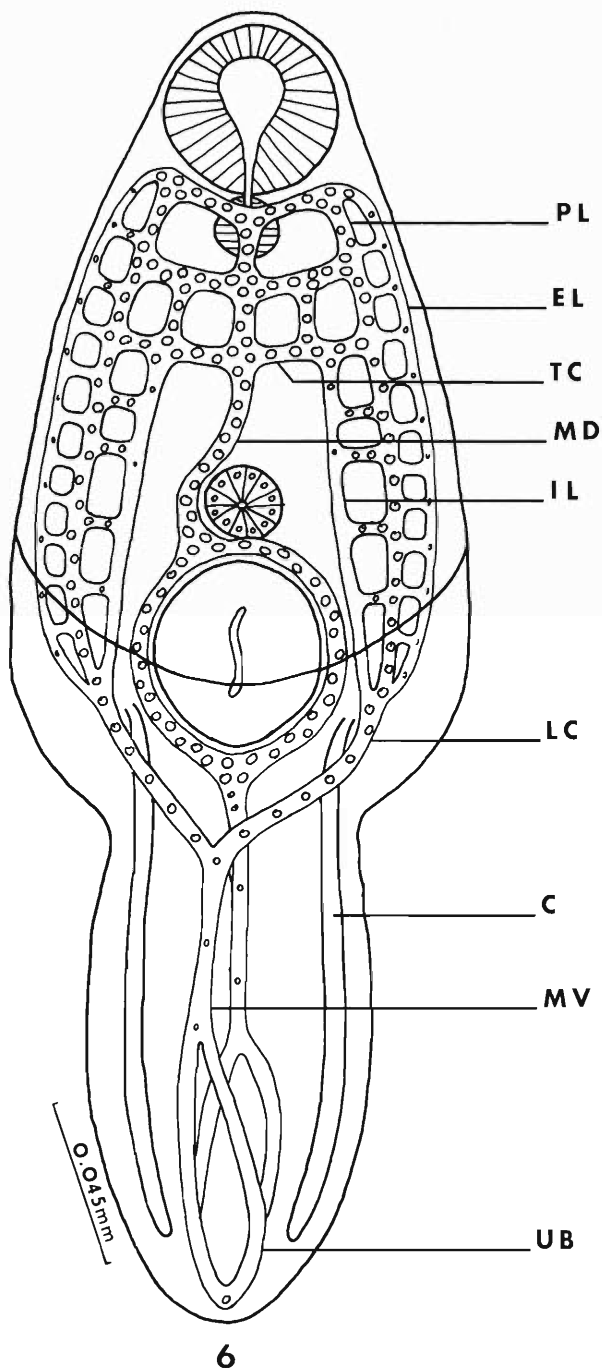


Figure 6. *Neascus pyriformis*. Ventral view of whole mount, drawn freehand to scale. Ceca shown only in posterior half of body. Abbreviations: c, cecum; el, extralateral vessel; il, intralateral vessel; lc, lateral collecting vessel; md, median dorsal vessel; mv, median ventral vessel; pl, primary lateral vessel; tc, transverse commissural vessel; ub, urinary bladder.

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**Table 2. Comparative measurements of *Neascus pyriformis* and *Uvulifer ambloplitis* in micrometers.**

| | | *N. pyriformis* (present study) | *N. pyriformis* (after Chandler, 1951) | *U. ambloplitis* (after Hughes, 1927) | *U. ambloplitis* (after Hunter, 1928) |
|---|---|---|---|---|---|
| Host cyst* | length | 380 (360–405) | (420–550) | | (510–850) |
| | width | 313 (270–375) | (300–500) | | |
| Parasite cyst | length | 225 (195–255) | (225–319) | 377 (360–390)† | (300–340) |
| | width | 125 (105–150) | (130–195) | 200 (172–236) | |
| Larva‡ | length | 371 (315–420) | | | (378–817) |
| Forebody | length | 233 (195–278) | (435–465) | (255–675) | (196–473) |
| | width | 117 (105–135) | (240–255) | (150–232) | (95–351) |
| Hindbody | length | 138 (120–165) | (150–250) | (187–675) | (115–419) |
| | width | 65 (60–75) | (125–175) | (75–187) | (41–236) |
| Oral sucker | length | 41 (37–44) | 70 | (60–90) | (40–79) |
| | width | 44 (34–48) | 45 | (diameter) | (diameter) |
| Ventral sucker | length | 21 (17–24) | (38–40) | (30–80) | (22–43) |
| | width | 23 (20–24) | (38–45) | (diameter) | (diameter) |
| Pharynx | length | 18 (15–24) | (21–23) | 22 | (16–20) |
| | width | 16 (14–17) | (17–18) | (diameter) | (diameter) |
| Holdfast organ | length | 47 (41–58) | (85–105) | (52–60) | (40–79) |
| | width | 43 (37–48) | (80–95) | (diameter) | (diameter) |

* Hunter's measurements apparently included melanin pigment layer; present study and Chandler's did not.
† Chandler (1951). Parasite cysts 340–420 long by 200–225 wide in *Ambloplites rupestris*.
‡ Chandler's and Hughes' measurements from living larvae; present study and Hunter's from preserved.

## Metacercaria
## (Figs. 4–6)

Because of the difficulty of freeing larvae from the parasite cysts, Chandler's (1951) original description was based only on a few live worms. A redescription is given here with measurements from preserved worms.

DESCRIPTION (measurements from 10 metacercariae): Typical neascus in type. Total body length, 371 (315–420); forebody 233 (195–278) long by 117 (105–135) wide, hindbody 138 (120–165) by 65 (60–75). Oral sucker 41 (37–44) long by 44 (34–48) wide; ventral sucker 21 (17–24) by 23 (20–24); pharynx 18 (15–24) by 16 (14–17); holdfast organ 47 (41–58) by 43 (37–48). Ceca extending nearly to posterior end of hindbody. Genital primordium not evident.

Median dorsal vessel extends from oral sucker to middle of hindbody, then bifurcates with posterior ends entering anterior ends of urinary bladders; median vessel passes on one side of acetabulum but encircles holdfast organ. Median vessel in forebody usually gives rise to 3 but sometimes 4 or 5 transverse commissural vessels on each side connecting to a primary lateral vessel. Each primary vessel gives rise to about 10 commissures connecting to an extralateral vessel. Only 1 extralateral vessel apparent on each side. Intralateral vessel extends posterior from most posterior transverse commissural vessel on each side, with about 4 commissures connecting to primary lateral vessel. Lateral to holdfast organ, lateral vessels from each side merge forming a lateral collecting vessel. The 2 collecting vessels merge at posterior end of forebody to form a median ventral vessel, which bifurcates in middle of hindbody and near end of hindbody converge

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to form the urinary bladders. Fusion of bladders near excretory pore not clearly seen. Excretory granules larger and more numerous in vessels in forebody than in hindbody.

## Discussion

Hoffman (1967) listed five valid species of neascus with pigmented cysts from North American freshwater fish. The black spot trematode studied here in fishes from Brule Creek, South Dakota was identified as *Neascus pyriformis* Chandler, 1951, because the host cyst was oval, the parasite cyst pear-shaped, the larva filled the parasite cyst, the reserve excretory system and other morphological features were similar, and the host and parasite cyst measurements were in the range given by Chandler (Table 2). Larval measurements were not similar since Chandler used living larvae rather than preserved as in this study.

*Neascus pyriformis* is most closely related to the neascus of *Uvulifer ambloplitis* (Hughes, 1927), although the host cyst, parasite cyst, and larvae of the latter species are larger (Table 2) and the parasite cysts not distinctly pyriform. In the fall of 1978, we examined black spot larvae identified as *U. ambloplitis* in fishes from Perry Creek in Northeastern Iowa. The difference between the reserve excretory system of this species and *N. pyriformis* was immediately evident, with the pattern of the main vessels more easily determined in *U. ambloplitis*.

Although multiple infections with black spot trematodes are known to occur (Chandler, 1951; Evans and Mackiewicz, 1958; Voth and Larson, 1968), *N. pyriformis* was the only species identified in fishes from Brule Creek. This conclusion is supported by the fact that no significant differences were found among the length and width means of preserved parasite cysts from the seven host species.

This is the first report of *N. pyriformis* from fishes in South Dakota and, except for the fathead minnow, all hosts (creek chub, plains minnow, stone roller, common shiner, red shiner, and white sucker) are new host records. Except for the white sucker (Catostomidae), all host species are cyprinids. With these seven hosts and those reported by Chandler (1951), Larson (1966), and Voth and Larson (1968), *N. pyriformis* is known from 13 host species of five families, indicating a wide host range.

Nothing is known of the life history of *N. pyriformis*. Chandler (1951) speculated that the adult may be *Uvulifer semicircumcisus* Dubois and Rausch, 1950, originally described from the kingfisher, *Megaceryle alcyon,* in Michigan. He found a young kingfisher infected in Itasca Park, Minnesota and kingfishers were known to feed on the hosts in that area. Dubois (1969) also reported *U. semicircumcisus* from the kingfisher in Itasca Park.

The unsuccessful experimental infection of four 1-day-old chicks in this study may indicate that *N. pyriformis* is quite host specific in the adult stage. There are apparently no successful infections of chicks with *Uvulifer* spp., although infections of this host with neasci of other strigeoids has been reported (Hoffman, 1954, 1958).

## Acknowledgment

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# ERRATUM

*Nematospiroides dubius* of Bergstrom and Werner (1981) a misidentification for *Citellinema bifurcatum* Hall, 1916.

Nematodes reported as *Nematospiroides dubius* Baylis, 1926 (Syn., *Heligmosomoides polygyrus* (Duj., 1845) Railliet et Henry, 1909) from *Spermophilus armatus* by Bergstrom and Werner (1981, Proc. Helminthol. Soc. Wash. 48:13–16) were misidentified. The nematodes are *Citellinema bifurcatum* Hall, 1916 (Syn., *Citellinema sleggsi* Manter, 1930) which has been reported previously from *S. armatus* (Syn., *Citellus armatus*) by Dikmans (1938, Proc. Helminthol. Soc. Wash. 5:55–58).

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# In Vitro Excystment of the Black Spot Trematode *Neascus pyriformis* Chandler, 1951 (Trematoda: Diplostomatidae)

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ABSTRACT: Maximum in vitro excystment with *Neascus pyriformis* metacercariae required two treatments: (1) a low pH pretreatment [acidified pepsin or acidified Locke's balanced salt solution (BSS)], and (2) a trypsin-sodium cholate excystment medium at pH 7.4. The excystment rate, however, was higher with acidified Locke's BSS than with acidified pepsin. No enhancement or excystment was observed with a 0.03% sodium dithionite pretreatment between acidified Locke's BSS and the excystment medium. Excystment occurred in trypsin alone and in all trypsin-containing media with or without pretreatment, but not in Locke's BSS, bile salts, or sodium cholate alone. Excystment of *N. pyriformis* was primarily an active process with the larva breaching the narrow end of the parasite cyst, although digestion of the host cyst and some wrinkling of the parasite cyst occurred in excystment media.

There are no detailed studies on excystment of black spot trematodes of North American freshwater fishes, although some workers developed an excystment procedure in studies on larval identification and morphology (Hughes, 1927, 1928; Hoffman, 1955; Hoffman and Putz, 1965; Evans and Mackiewicz, 1958). The purpose of this study was to investigate in vitro excystment of the black spot trematode *Neascus pyriformis* Chandler, 1951. This metacercaria was reported by Duru et al. (1981) from fishes in Brule Creek in southeastern South Dakota.

## Materials and Methods

Cysts were obtained from naturally infected fathead minnows (*Pimephales promelas*), creek chubs (*Semotilus atromaculatus*), and common shiners (*Notropis cornutus*) from Brule Creek, South Dakota. Fish were collected using a knot mesh minnow seine, 2.95 m × 1.2 m with a mesh size of 6.3 mm, bar measure. They were maintained in aerated aquaria and fed crushed 'Shrimp-El-Etts' fish food (Carolina Biological).

Cysts (8–10/test) removed from the skin and fins of fish were either transferred directly to the test media or to Locke's balanced salt solution (BSS) (Hoar and Hickman, 1967). Watch glasses were used for pretreatment media and covered petri dishes (35 × 10 mm) for excystment media. Cysts pretreated were rinsed twice in Locke's BSS before being pipetted to another medium. All experiments were carried out in triplicate (25–30 cysts total) in an incubator at 41°C. Counts of excysted metacercariae were made every 30 min up to 120 min in excystment media.

The excystment media used in experiments were: (1) Locke's BSS, pH 7.4; (2) 0.5 trypsin (1-300 hog pancreas), pH 7.4; (3) 0.5% bile salts (ox bile extract), pH 7.4; (4) 0.5% sodium cholate (sodium salt of cholic acid), pH 7.4; (5) 0.5% trypsin and 0.5% bile salts, pH 7.4; (6) 0.5% trypsin and 0.5% bile salts, pH 8.1; (7) 0.5% trypsin and 0.5% sodium cholate, pH 7.4; and (8) 0.5% trypsin and 0.5% sodium cholate, pH 8.1. The pretreatment media were: (1) Locke's BSS, pH 2.0 (acidified Locke's); (2) 1% pepsin (1-15,000 hog stomach mucosa), pH 2.0; (3)



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**Table 1. Excystment of *N. pyriformis* metacercariae in various excystment media. Abbreviations: 0 = no pretreatment; A = acidified pepsin 1% pH 2.0 (15 min); B = acidified Locke's BSS pH 2.0 (15 min); C = acidified Locke's BSS pH 2.0 (15 min) and $Na_2S_2O_4$ 0.03%, time as indicated; D = acidified pepsin 1% pH 2.0 (15 min) and $Na_2S_2O_4$ 0.05% (15 min).**

| Pretreat | Time (min) | pH | Excystment medium | Excystment % by 30 min intervals | | | | SD* |
|---|---|---|---|---|---|---|---|---|
| | | | | 30 | 60 | 90 | 120 | |
| 0 | — | 8.1 | Trypsin | 0 | 10 | 10 | 10 | (0.0) |
| 0 | — | 8.1 | Trypsin + BS† | 0 | 10 | 10 | 10 | (0.0) |
| 0 | — | 8.1 | Trypsin + SC‡ | 10 | 13 | 20 | 27 | (5.8) |
| 0 | — | 7.4 | Trypsin | 0 | 27 | 47 | 60 | (36.1) |
| 0 | — | 7.4 | Trypsin + BS | 0 | 10 | 10 | 27 | (20.8) |
| 0 | — | 7.4 | Trypsin + SC | 0 | 17 | 27 | 67 | (15.3) |
| A | — | 7.4 | Trypsin | 20 | 27 | 47 | 50 | (10.0) |
| A | — | 7.4 | Trypsin + BS | 20 | 33 | 43 | 67 | (15.3) |
| A | — | 7.4 | Trypsin + SC | 30 | 47 | 60 | 87 | (5.8) |
| B | — | 7.4 | Trypsin + SC | 73 | 83 | 87 | 87 | (5.5) |
| C | 4 | 7.4 | Trypsin + SC | 56 | 60 | 76 | 84 | (5.6) |
| C | 8 | 7.4 | Trypsin + SC | 79 | 79 | 83 | 83 | (7.2) |
| C | 10 | 7.4 | Trypsin + SC | 67 | 70 | 81 | 81 | (6.4) |
| C | 12 | 7.4 | Trypsin + SC | 79 | 79 | 79 | 90 | (0.6) |
| C | 14 | 7.4 | Trypsin + SC | 68 | 71 | 82 | 82 | (10.9) |
| C | 16 | 7.4 | Trypsin + SC | 68 | 71 | 86 | 93 | (6.1) |
| C | 20 | 7.4 | Trypsin + SC | 59 | 67 | 74 | 78 | (2.5) |
| D | — | 7.4 | Trypsin + BS | 0 | 0 | 0 | 0 | |
| D | — | 7.4 | Trypsin + SC | 0 | 0 | 0 | 0 | |

* SD = standard deviation based on total percent excystment (120 min).
† Trypsin + BS = trypsin + bile salts.
‡ Trypsin + SC = trypsin + sodium cholate.

0.03% sodium dithionite ($Na_2S_2O_4$), pH 7.4; and (4) 0.05% sodium dithionite, pH 7.4. Cysts were treated for 15 min in acidified Locke's, pepsin and 0.05% sodium dithionite and for times given in Table 1 for 0.03% sodium dithionite. The sodium dithionite was purchased from Sigma Chemical and all other chemicals from ICN Pharmaceutical Inc. All chemicals were dissolved in Locke's BSS and pH adjustments made with either 6 N HCl or 7.5% sodium bicarbonate.

## Results

Because the cyst of *N. pyriformis* has been described (Chandler, 1951; Duru et al., 1981), only a brief description will be given here. The cyst consists of a thick outer cellular layer (host cyst) and a thin inner noncellular layer (parasite cyst) (Figs. 2, 4). The outer portion of the host cyst is composed of melanocytes (Fig. 1) which can be removed with needles (Fig. 2). The metacercaria completely fills the parasite cyst with the folded forebody near the narrowed end (Fig. 4). The parasite cyst is easily forced out of the host cyst with slight cover-glass pressure, but it is not possible to force the larva out of the parasite cyst without injury.

### Effects of excystment media (Table 1)

Maximum excystment percentages of metacercariae occurred with acidified Locke's pretreatment, pepsin pretreatment and acidified Locke's and 0.03% so-

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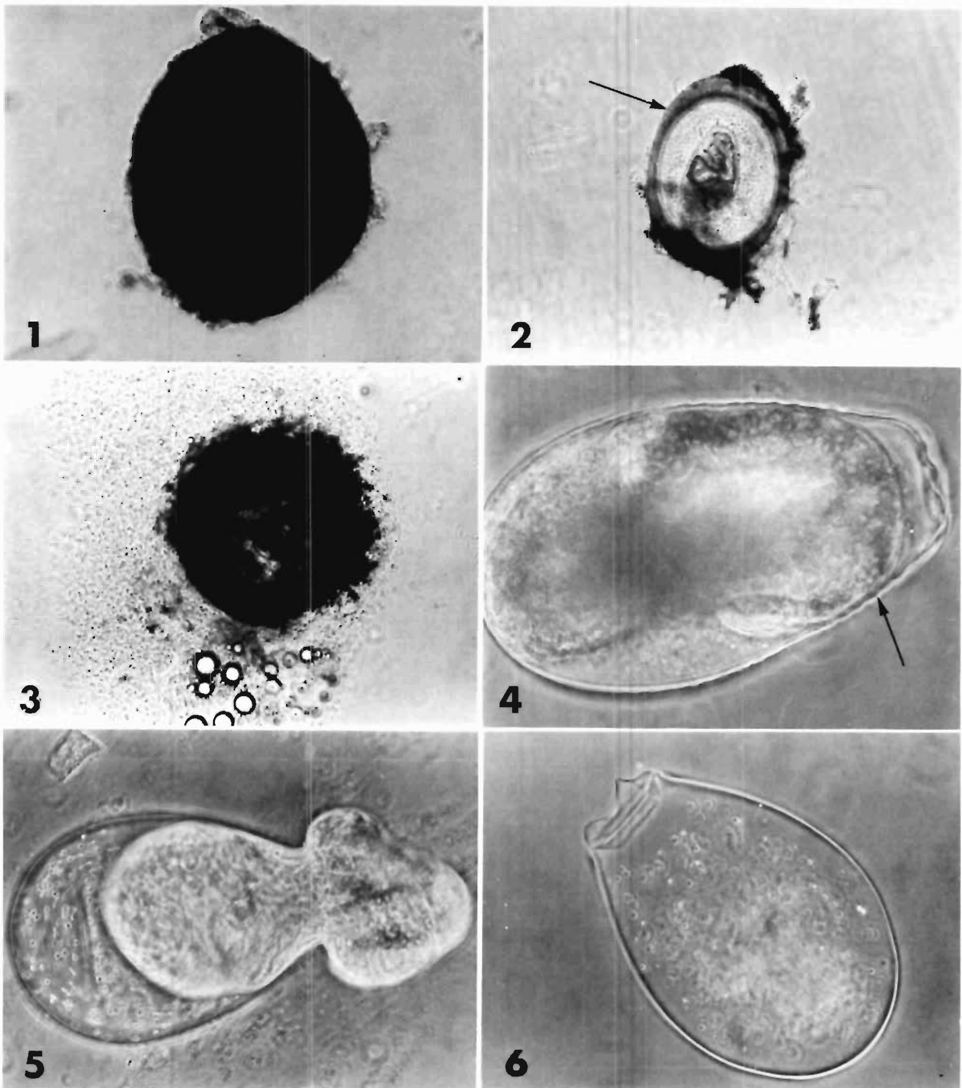


**Figures 1–6.** *Neascus pyriformis.* **Phase-contrast microscopy. 1. Untreated pigmented cyst removed from fish host. ×150. 2. Cyst with melanocyte portion removed, showing rest of host cyst (arrow) and inner parasite cyst. ×85. 3. Cyst after 10 min exposure to trypsin-sodium cholate. Note parasite cyst still within partially digested host cyst. ×70. 4. Parasite cyst in trypsin-sodium cholate showing wrinkling (arrow) of cyst wall in narrow half of cyst. ×445. 5. Metacercaria escaping from narrow end of parasite cyst. ×300. 6. Empty parasite cyst showing breached narrow end. ×345.**

dium dithionite pretreatments following in all three cases by incubation in trypsin-sodium cholate at pH 7.4. However, the excystment rate was lower with pepsin than with the other two pretreatments. No distinct differences in excystment percentages and rates were evident among the different pretreatment times with 0.03% sodium dithionite. In contrast to the results with 0.03% sodium dithionite, no excystment was observed and larval movement ceased after 1 hr incubation following pretreatment with 0.05% sodium dithionite.

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No excystment occurred when Locke's BSS, bile salts, or sodium cholate were used alone. Excystment was observed in trypsin alone at both pH 7.4 and 8.1, although at a low percentage at the latter pH and with variable results at the former. The initial excystment rate was higher and results more consistent at pH 7.4 when pepsin pretreatment preceded trypsin incubation, but the total excystment percentage was higher without pretreatment.

There was no difference in the excystment results between incubation in trypsin and trypsin-bile salts at pH 8.1, whereas at pH 7.4 the results were better with trypsin alone. However, with pepsin pretreatment the excystment percentage and rate at pH 7.4 was higher with trypsin-bile salts.

The excystment percentages with incubation in trypsin-sodium cholate were higher in all cases than with trypsin alone or with trypsin-bile salts and, with the exception of trypsin alone at pH 7.4, the rates were also higher.

**Process of excystment**

Pepsin pretreatment resulted in a breakdown of the host cyst which was manifested by melanocyte loss in some areas and the frequent appearance of the parasite cyst freed from the host cyst. In the process of rinsing and transferring cyst to excystment media, complete dissolution of the host cyst usually occurred. In trypsin-containing media without pepsin pretreatment, digestion of the host cyst was evident after 10–30 min (Fig. 3) with complete dissolution after 60–90 min. No change in the host cyst was seen with acidified Locke's pretreatment.

A morphological change in the parasite cyst was only observed in incubation media containing trypsin. The first noticeable change was evident after 10 min and was seen as a wrinkling of the wall in the narrow half of the cyst (Fig. 4). This wrinkling became more apparent especially at the narrowed end as incubation continued.

Larval activation did not occur during pepsin pretreatment but was displayed in trypsin-containing media after 10–30 min incubation. During activation, low levels of movement alternated with periods of vigorous movement in which the oral sucker was thrust repeatedly against the narrow end of the cyst. Larvae eventually breached the narrowed end of the cyst and escaped after as little as 10 min (Figs. 5, 6) in trypsin-sodium cholate medium following acidified Locke's pretreatment.

## Discussion

Chandler (1951) stated that no excystment occurred with *Neascus pyriformis* metacercariae using either trypsin or artificial gastric juice; no procedural details were given. In the closely related black spot trematode, *Uvulifer ambloplitis*, Hughes (1927) observed excystment with a warm trypsin-sodium bicarbonate solution and Hoffman and Putz (1965) with a pepsin HCl medium at 40°C. The latter authors found no additional excystment after transfer to alkaline trypsin. Hughes (1928) reported excystment of the black spot trematode, *Crassiphiala bulboglossa*, using the same method employed for *U. ambloplitis*. Evans and Mackiewicz (1958) achieved excystment of black spot metacercariae with acidified pepsin followed by incubation in trypsin.

The results of this study showed that excystment occurred in trypsin alone, but maximum excystment percentages required two treatments: (1) a low pH pre-

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treatment (acidified pepsin or acidified Locke's), and (2) a trypsin-sodium cholate excystment medium at pH 7.4 (Table 1). However, the excystment rate was higher with acidified Locke's pretreatment, even though breakdown of the host cyst resulted with acidified pepsin.

Excystment of *N. pyriformis* is predominately an active process, although a morphological change in the parasite cyst was seen in media containing trypsin. It is not known if exogenous enzymes or lytic substances released by the larva were responsible for this change. The escape of the activated larva from the narrow end of the parasite cyst was similar to that described by Hughes (1927) and Hoffman and Putz (1965) for *U. ambloplitis* and Hughes (1928) for *C. bulboglossa*.

A number of workers have reported increased excystment with an acidified pepsin pretreatment but without comparison to an acidified balanced salt solution (BSS) pretreatment (McDaniel, 1966; Macy et al., 1968; Fried and Roth, 1974; Asanji and Williams, 1975; Fried et al., 1978). In some studies when a comparison was made, higher percentages and/or rates were found with acidified pepsin (Erasmus and Bennett, 1965; Dixon, 1966; Howell, 1970), whereas in another study a higher percentage and rate occurred with an acidified BSS (Kirschner and Bacha, 1980). The excystment rate in this study was higher with an acidified BSS but the final percentages were similar.

The enhancement of metacercarial excystment with reducing agents (sodium dithionite or cysteine) is well established (Howell, 1970; Mohandas and Nadakal, 1978; Fried et al., 1978; Kirschner and Bacha, 1980). These agents have been used primarily as a separate pretreatment between pepsin pretreatment and the excystment medium, although they have proven effective as a part of the excystment medium (Dixon, 1966; McDaniel, 1966; Asanji and Williams, 1975; Fried and Butler, 1979). In this study there was no distinct excystment enhancement with an acidified BSS and 0.03% sodium dithionite pretreatments compared to acidified BSS alone.

The adverse effects observed here after 15 min pretreatment with 0.05% sodium dithionite was probably due to excessive exposure time. Fried et al. (1978) and Kirschner and Bacha (1980) found sodium dithionite to be effective for 1 to 10 min but longer exposure times resulted in reduced excystment and larval death. No detrimental effects were observed here with 0.03% sodium dithionite even up to 20 min pretreatment.

The use of a trypsin-bile salts medium with or without a low pH pretreatment has been used successfully to induce metacercarial excystment with a number of digenetic trematodes (Erasmus and Bennett, 1965; McDaniel, 1966; Howell, 1970; Fried and Butler, 1978; Fried et al., 1978; Mohandas and Nadakal, 1978; Kirschner and Bacha, 1980). With few exceptions, trypsin-bile salts is better yhan trypsin alone (Macy et al., 1968) or fresh bile or bile salts alone (Dixon, 1966; Fried and Butler, 1979). In this study higher percentages and rates were found with a trypsin-sodium cholate excystment medium compared to trypsin alone when preceded by a low pH pretreatment (Table 1). In contrast, results at pH 7.4 were better with trypsin alone compared to trypsin-bile salts (ox bile extract) without any pretreatment and results were the same at pH 8.1. Although nothing definite is known on the synergistic effects of trypsin and bile salts, Dixon (1966) suggested these possible roles: (1) stimulating larval movement, (2) enhancing the

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effects of larval released enzymes, (3) enhancing the effect of host enzymes, and (4) producing cyst wall lysis which increases permeability of cyst membranes.

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# *Pseudogymnophallus alcae* gen. n. et sp. n. (Trematoda: Gymnophallidae) from Alcids (Charadriiformes) in Subarctic Seas

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ABSTRACT: *Pseudogymnophallus alcae* gen. n. et sp. n. is described for trematodes from alcids (Charadriiformes: Alcidae) from the North Pacific region (Gulf of Alaska, Bering Sea, and Chukchi Sea): horned puffin, *Fratercula corniculata*; tufted puffin, *Lunda cirrhata*; parakeet auklet, *Cyclorrhynchus psittacula*; and least auklet, *Aethia pusilla*; and from the western North Atlantic Ocean, (Gulf of St. Lawrence): razorbill, *Alca torda*. *Pseudogymnophallus* is distinguished from other genera of Gymnophallidae by the intertesticular position of the ovary; a cylindrical, undivided seminal vesicle; and by an intestinal diverticulum dorsal to the arch of each cecum.

A trematode species representing a previously unrecognized genus of Gymnophallidae has been collected from puffins, *Fratercula corniculata* and *Lunda cirrhata*, in the Gulf of Alaska, Bering Sea, and Chukchi Sea. The species was recovered less frequently from the parakeet auklet, *Cyclorrhynchus psittacula* and the least auklet, *Aethia pusilla* from St. Lawrence Island and St. Paul Island, Alaska, and the razorbill, *Alca torda* from St. Mary's Islands, Quebec, in the western North Atlantic. The only previous record of gymnophallids from an alcid host is that of *Gymnophallus deliciosus* (Olsson, 1893), a common parasite of gulls (Laridae); Belopol'skaia (1952) reported that species from puffins, *Fratercula arctica* in the Barents Sea.

Trematodes from puffins examined in the field at Ugaiushak and Kodiak Islands, Gulf of Alaska, were fixed in buffered 10% formalin at 85°C and prepared as whole mounts stained in Semichon's acetic carmine. Colleagues provided additional mounted and preserved specimens from puffins and auklets in Alaska and razorbills in Quebec; 10 from puffins at Cape Thompson, Alaska, were prepared as sagittal sections. All measurements are in micrometers and are given as ranges. Only those for eggs are followed by mean values with standard errors. The following description is based on measurements from 75 trematodes (16 from Cape Thompson; 23 from St. Lawrence Island; and 36 from Ugaiushak Island). In some, the egg-filled uterus made it impossible to measure all organs. Specimens from *Alca torda* and *Aethia pusilla* were not used in preparing the description due to their poor state of preservation.

## *Pseudogymnophallus* gen. n.

DIAGNOSIS: Gymnophallidae: Gymnophallinae. Body small, oval to pyriform, spinose. Oral sucker subterminal, without lateral papillae. Prepharynx absent. Pharynx small, esophagus short. Ceca long, extending into posterior third of body; arch of each cecum with dorsal diverticulum. Acetabulum in middle third of body; smaller than oral sucker. Testes smooth, symmetrical or slightly diagonal, lateral to acetabulum. Cirrus pouch absent. Seminal vesicle cylindrical to weakly bipartite. Pars prostatica followed by very short ejaculatory duct. Genital pore median, on anterior margin of acetabulum. Ventral pit absent. Ovary



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smooth, submedian, intertesticular, in middle third of body posterior to acetabulum. Seminal receptacle absent. Laurer's canal present. Vitelline follicles in a pair of submedian groups anterolateral to acetabulum. Uterus voluminous, sometimes filling body posterior to pharynx. Excretory vesicle Y-shaped, with arms reaching pharyngeal level. Parasitic in gall bladder and intestine of birds (Charadriiformes: Alcidae).

TYPE AND ONLY SPECIES: *Pseudogymnophallus alcae* sp. n.

### *Pseudogymnophallus alcae* sp. n.
### (Figs. 1–5)

DESCRIPTION: With characteristics of the genus. Body, 967–2,780 long by 642–1,611 in maximum width. Entire tegument with alternating transverse rows of scalelike spines, 7–32 long by 1–9 wide at base, generally larger toward posterior end of body. Oral sucker, 236–389 in diameter; acetabulum 153–295. Ratio of transverse diameter of oral sucker to acetabulum 1:0.58–0.95. Pharynx cylindrical, 61–139 long by 58–124 wide. Esophagus 58–145 long, often convoluted. From inflated arches, ceca narrow to turn ventromediad, pass between testes and terminate near their posterior margins. Ceca lined with single layer of large glandular cells from level of bifurcation. Diverticulum of each cecum 159–203 long by 116–117 wide, extending dorsomediad from arch nearly to bifurcation. Genital atrium shallow, wider than pore. Testes ovoid, 247–778 long by 177–554 wide; near side of body in middle third. Prostatic cells and vesicle conspicuous, their combined mass 128–283 long by 122–232 wide. Seminal vesicle 272–551 long by 101–130 in diameter curving dorsad to dorsoposteriad from prostatic vesicle. Ovary ovoid, 141–342 long by 118–307 wide; dorsal, intertesticular, sinistral, dextral, or seldom median; slightly posterior to or overlapping acetabulum and median margin of testis. Mehlis' gland median, ventral to ovary, 145–227 long by 87–145 wide. Vitellaria with 13–30 follicles in each group, dorsal and anterolateral to acetabulum. Vitelline ducts extending posteroventrad to unite and join ootype ventrally. Oviduct leaves ovary lateroventrally. Laurer's canal often convoluted extending posterodorsad from oviduct to open middorsally. Uterus extending anteriad from ootype in a slightly convoluted loop as far as cecal bifurcation, then posteriad in descending and ascending loops filling posterior ⅔ of body. Metraterm 15–43 in diameter, approaching genital atrium sinistrally or dextrally. Eggs 23–32 long by 15–23 wide ($\bar{x}$ = 26 ± 0.18 by 17 ± 0.10). Excretory vesicle with prominent sphincter near terminus of stem; arms terminating anteriorly in transverse dilatations ventral to ceca and flanking esophagus.

HOSTS: Horned puffin, *Fratercula corniculata* (Naumann) (type host); tufted puffin, *Lunda cirrhata* (Pallas); parakeet auklet, *Cyclorrhynchus psittacula* (Pallas); least auklet, *Aethia pusilla* (Pallas); and razorbill, *Alca torda* Linnaeus.

SITE: Gall bladder and intestine.

LOCALITIES: Cape Thompson (lat. 68°06′N; long. 165°46′W) (type locality), Ugaiushak, Kodiak, St. Paul and St. Lawrence Islands, Alaska, and St. Mary's Islands, Quebec.

TYPE SPECIMENS: Holotype USNM Helm. Coll. 75935 and Paratype 1, 75936 from a horned puffin collected by L. G. Swartz; Paratypes 2 and 3, 75937 and 75938, from horned and tufted puffin, respectively at Ugaiushak Island; Paratypes

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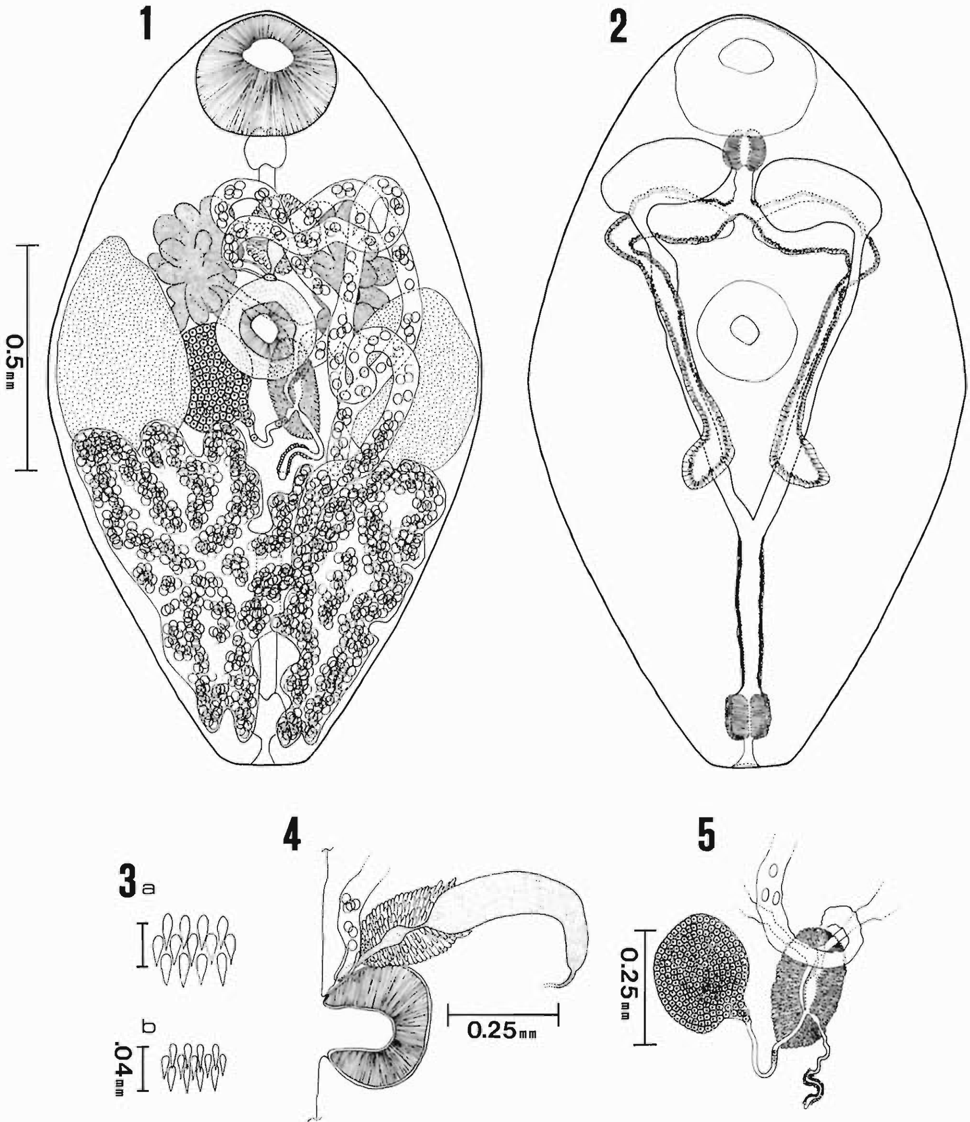


**Figures 1–5.** ***Pseudogymnophallus alcae.*** **1. Ventral view showing all organs except the excretory system and ceca. 2. Ventral view showing relationship of the ceca and excretory systems. 3. (a) Tegumental spines of specimens from Ugaiushak Island; (b) tegumental spines of specimens from Cape Thompson. 4. Lateral view of the genital pore, showing metraterm, and position of prostatic and seminal vesicles. 5. Ventral view of female genital system: ovary, Mehlis' gland, and associated ducts including the ovarian duct, vitelline ducts, Laurer's canal, and ascending uterus.**

4 and 5, 75939 from a parakeet auklet at St. Lawrence Island. In addition a voucher specimen, 76213, from a razorbill was deposited to substantiate the host record. The remaining specimens are retained in the collection of the author.

ETYMOLOGY: The specific name *alcae* is derived from the Swedish and Icelandic *alka* signifying auk, a general name for members of the family Alcidae.

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## Discussion

*Pseudogymnophallus* gen. n. differs from all other genera of Gymnophallidae in having relatively long ceca with diverticula, an intertesticular ovary (pretesticular in other genera), and a cylindrical seminal vesicle (bipartite or club-shaped). It is most similar to *Gymnophallus* Odhner, 1900, in general body size, position of the genital pore, arrangement of vitelline follicles, and extent of uterus. It can be distinguished further from *Lacunovermis* Ching, 1965, and *Gymnophalloides* Fujita by Dollfus, 1925, by the absence of a ventral pit anterior to the acetabulum, and from *Parvatrema* Cable, 1953, and *Meiogymnophallus* Ching, 1965, by the absence of lateral papillae on the oral sucker. In addition it differs from *Paragymnophallus* Ching, 1973, in the position and size of the genital pore. *Pseudogymnophallus* appears to be restricted to birds of the family Alcidae, whereas other genera of Gymnophallidae are known from other groups of Charadriiformes, or from Anseriformes.

The position of the ovary (i.e., relative to the testes and acetabulum) and other morphological attributes which characterize *P. alcae* were constant. The normal habitat of *P. alcae* appears to be the gall bladder, although numerous specimens were found in the intestines of puffins at Ugaiushak. Such specimens were characterized by a patchy loss of spination, and probably represent individuals which had migrated from the gall bladder to the intestine and were in the process of being eliminated from the host. *G. deliciosus* has also been reported from both the intestine and gall bladder of its hosts, which was presumed by Ellis and Williams (1973) to indicate its ability to travel from the gall bladder to the intestine through the bile duct. Although the present specimens were alive when collected, loss of spination suggests that the intestine is not viable habitat for *P. alcae*.

The pattern of tegumental spination was identical for trematodes from different host species. However, spines on specimens from Ugaiushak (Fig. 3a) were broader and more massive than those on specimens from Cape Thompson (Fig. 3b) and St. Lawrence Island (spines had been lost on the specimens from Quebec and consequently could not be compared). This could be an indication of some degree of endemism associated with the Gulf of Alaska and the northern Bering Sea, respectively.

Of life cycles thus far elucidated for gymnophallids, the majority includes two intermediate hosts, both of which are usually intertidal lamellibranchs (Loos-Frank, 1971). Benthic polychaetes have been reported as second intermediate hosts for several species (Loos-Frank, 1969; Margolis, 1971, 1973; Popova and Nikitina, 1978). Most final hosts of these trematodes are species of Charadriiformes, including larids, and Anseriformes (primarily sea ducks) whose foraging occurs in relatively shallow marine littoral areas. A major component of their diets consists of intertidal lamellibranchs, gastropods, and benthic polychaetes.

In contrast the hosts of *P. alcae* are predominately planktonic foragers which exploit macrozooplankton (*Aethia pusilla* and *Cyclorrhynchus psittacula*), a mixture of macro- and megazooplankton and nekton, including various species of fishes and squids, and polychaetes (*Lunda cirrhata* and *Fratercula corniculata*) or almost exclusively fishes (*Alca torda*) (Bédard, 1969a, b; Belopol'skii, 1957; Sanger et al., 1978; Swartz, 1966; Wehle, 1976). The prey types expected to serve as intermediate hosts for *P. alcae* either occur rarely or have not been recorded

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in the diets of its known final hosts. However, Bédard (1969b) pointed out that zooplanktonic foragers may be particularly limited by prey availability in the winter when the bulk of the zooplankton biomass is beyond the depth range of feeding, thus auklets and puffins may be forced to exploit alternate prey species which could serve as intermediate hosts for *P. alcae*.

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# Trematodes of Marine Fishes from South Australia. 3. *Lepocreadium angelae* sp. n. (Lepocreadiidae)

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ABSTRACT: *Lepocreadium angelae* sp. n. (Lepocreadiidae) is described from *Scorpis georgianus* Cuvier and Valenciennes from Pt. Willunga, South Australia. It is similar to *L. floridanum* Sogandares-Bernal and Hutton, 1959, from Florida, but differs in having a narrower body, not being spined in posterior half, a larger sucker ratio, a shorter posttesticular space, a prostatic vesicle followed by an anterior portion of the seminal vesicle, vitellaria not extending to the pharynx, and a long prepharynx.

The trematodes described below were received from the Australian Helminthological Collection (see Kruse, 1978), University of Adelaide, South Australia, as fixed specimens stored in glycerin alcohol. The glycerin was removed by placing them in several changes of 70% EtOH over a period of time. Whole mounts were stained in Mayer's hematoxylin, dehydrated in EtOH, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a camera lucida; measurements are in micrometers with averages in parentheses.

## *Lepocreadium angelae* sp. n.
**(Figs. 1, 2)**

DESCRIPTION (based on 7 specimens): One specimen is in excellent condition, on all others the tegument is partially macerated. The measurements below include those from 2 submature adults, resulting in wider measurement ranges, but affecting the averages only little.

Body elongate, tapering only gradually from near midbody; posterior end more or less truncate. Length 392 to 834 (624); greatest width 138 to 324 (200). Body spines absent in posterior half of hindbody. Forebody 304 to 400 (328) long; with scattered pigment granules. Oral sucker 22 to 36 (26) long by 22 to 30 (25) wide; acetabulum 34 to 74 (60) long by 38 to 72 (52) wide; sucker width ratio 1:2.4. Prepharynx 50 to 110 (87) long; pharynx 24 to 44 (34) long by 12 to 32 (20) wide; esophagus 19 to 44 (32) long; ceca narrow, near sides of body, ending blindly near posterior end of body.

Genital pore anterosinistral to acetabulum. Testes rounded, anterior testis 44 to 120 (87) long by 44 to 132 (90) wide, posterior testis 54 to 80 (67) by 40 to 132 (89) wide, tandem, close together in posterior half of hindbody; posttesticular space 34 to 90 (70) long. Cirrus sac elongate, slightly curved, from genital pore dorsal to left half of acetabulum, extending almost to ovary; 76 to 260 (205) long by 22 to 60 (45) wide; containing small rounded posterior portion of internal seminal vesicle, large, ovate prostatic vesicle, tubular anterior portion of internal seminal vesicle, and thick-walled, unspined cirrus. External seminal vesicle between cirrus sac and ovary.

Ovary ovoid, 24 to 72 (47) long by 24 to 64 (43) wide, slightly to right, separated from anterior testis only by yolk reservoir. Seminal receptacle flask-shaped, posterior to ovary, dorsal to anterior testis. Vitellaria extending from a little posterior



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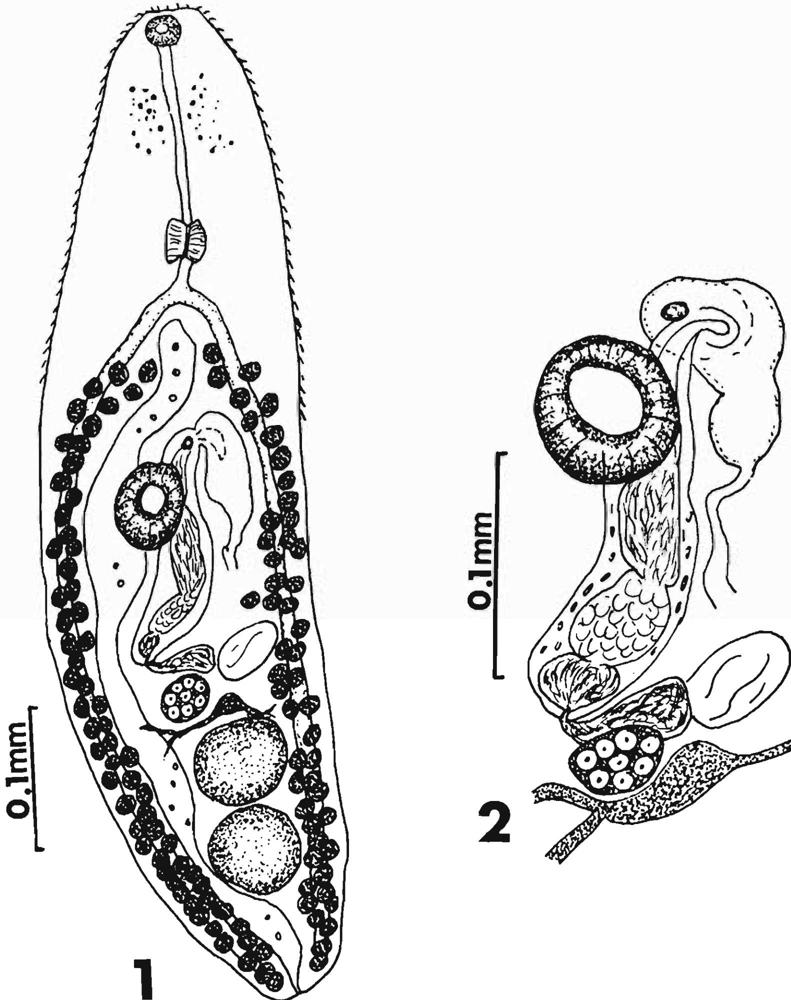


**Figures 1, 2.** ***Lepocreadium angelae*** **sp. n. from** ***Scorpis georgianus*** **in South Australia. 1. Paratype, ventral view. 2. Paratype, terminal genitalia, ventral view.**

to bifurcation to near posterior end of body; mostly ventral, lateral, and dorsal to ceca. Uterus short, to left of ovary and cirrus sac. Metraterm conspicuous, ovoid, 80 long by 26 to 40 (33) wide, unspined, to left of cirrus sac. Genital atrium moderately deep. Eggs 50 to 56 (53) long by 28 to 30 (28) wide.

Excretory pore terminal; vesicle wide, I-shaped, ascending to right of gonads and between acetabulum and right cecum to end just posterior to cecal bifurcation; containing concretions.

The name *angelae* is in honor of L. Madeline Angel, Curator of the Australian Helminth Collection.

Hosts: *Scorpis georgianus* Cuvier and Valenciennes; Scorpidae; Banded Sweep.

Location: Intestine.

Locality: Pt. Willunga, South Australia, 1934.

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TYPES: Due to the condition of the specimens all types are deposited together in the South Australian Museum; Coll. No. V2753–V2764.

## Discussion

This species is very similar to *Lepocreadium floridanum* Sogandares-Bernal and Hutton, 1959, from *Lagodon rhomboides* (Linn.) in Florida. However, the body is not completely spined and is narrower, the esophagus is shorter, the posttesticular space is smaller, and the oral sucker is much smaller. No prostatic vesicle was described for *L. floridanum*, but checking paratypes in the Harold W. Manter Laboratory verified its presence in this species. A prostatic vesicle is described for several other species of *Lepocreadium* but in no case is it followed by an anterior portion of the seminal vesicle as it is in *L. angelae*.

Extent of the excretory vesicle seems to be an important and constant character in species of this genus. When it extends anterior to the acetabulum it is usually conspicuous, especially if it crosses a cecum and extends into the forebody.

Various authors (e.g., Stunkard, 1969) have noted the confusion in species of *Lepocreadium*. *Lepocreadium pyriforme* (Linton, 1900) Linton, 1940, has been especially confusing because Linton included several species in it and did not designate a holotype specimen. Linton (1900) named the species from specimens from *Palinurichthys perciformis* (Mitchell), the rudderfish, at Woods Hole, Massachusetts. While he may have had more than one species in his collection, Stunkard (1969) believes such characters as the extent of the excretory vesicle and the spined cirrus are evident, and he redescribes the species in a new genus *Neopechona pyriforme* (Linton, 1900) Stunkard, 1969. I believe the main justification for the genus *Neopechona* is the union of the ceca and excretory vesicle to form a uroproct. Other characters include a cellular esophagus which is not shown in Stunkard's figure and does not seem comparable to the bipartite esophagus and pseudoesophagus of *Opechona*. Spines in the cirrus and in the metraterm were inconspicuous and visible in mounted specimens, and were not mentioned in the generic diagnosis. The excretory vesicle extends in the right side of the body only to the anterior border of the acetabulum, not to the pharynx as stated in the generic diagnosis. It is evident that most reports of "*Lepocreadium pyriforme*" are not the same species as *Neopechona pyriforme*. In most cases the ceca do not even approach the excretory vesicle.

## Acknowledgments

The author wishes to express his appreciation to L. Madeline Angel, Curator of the Australian Helminthological Collection, for providing the specimens and to Prof. Mary H. Pritchard under whose direction this research was undertaken.

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# *Plagioporus gyrinophili* sp. n. (Trematoda: Opecoelidae) from *Gyrinophilus porphyriticus duryi* and *Pseudotriton ruber* (Caudata: Plethodontidae)

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Abstract: Based on 33 specimens from *Gyrinophilus porphyriticus duryi* (Weller) and *Pseudotriton ruber* (Latreille) from Ohio, a new species, *Plagioporus gyrinophili* sp. n., is described and distinguished from related forms. A species of *Plagioporus* has not previously been reported from amphibian hosts.

In a survey of helminth parasites of Ohio salamanders, an undescribed trematode species of the genus *Plagioporus* Stafford, 1904 was found in the small intestine of both the spring salamander, *Gyrinophilus porphyriticus duryi* (Weller), and red salamander, *Pseudotriton ruber* (Latreille). Description of the species is based on 33 mature specimens for which the name *Plagioporus gyrinophili* sp. n. is proposed.

Salamanders were obtained by hand or dipnet and kept alive under refrigeration until autopsied, usually within 1 day. The worms were observed alive and then fixed in AFA or neutral buffered 10% formalin under light cover-glass pressure. Whole mounts were stained with Semichon's carmine or a one-step Mallory-Heidenhain stain (Cason, 1950) and mounted in gum damar. Four specimens sectioned at 8 μm were stained with Harris' hematoxylin and eosin or Mallory-Heidenhain stain. Measurements are in micrometers with the mean followed by the range in parentheses.

## *Plagioporus gyrinophili* sp. n.
## (Figs. 1–4)

Description: Body fusiform with thin aspinous tegument, white to pinkish in life, 1,560 (780–2,425) long × 551 (308–875) wide at acetabulum. Oral sucker subterminal, 139 (77–210) long × 171 (84–250) wide; acetabulum anterior in middle third of body, 248 (180–420) long × 254 (170–380) wide; oral sucker to acetabulum ratio 0.60 (0.49–0.72):1.0. Prepharynx very short, pharynx 62 (38–95) long × 82 (49–160) wide; esophagus 167 (100–290) long; intestinal bifurcation preacetabular; intestinal crura short, reaching only to level of ovary or anterior testis. Excretory bladder saccate, ending dorsal to anterior testis; flame cell formula $2[(2+2)+(2+2)] = 16$. Testes in posterior ⅓ of body, spherical to ovoid, usually oblique but tandem in some specimens; anterior testis sinistral, 144 (50–240) × 159 (60–240); posterior testis median 144 (50–257) × 182 (70–320); vasa efferentia join short vas deferens close to cirrus sac which lies diagonally anterior to the acetabulum. Cirrus sac 269 (155–520) long × 63 (40–83) wide, containing saccate seminal vesicle, poorly developed pars prostatica, and unarmed cirrus. Genital pore sinistral just anterior to level of intestinal bifurcation. Ovary immediately pretesticular or opposite anterior testis, 113 (30–190) × 123 (50–230); Laurer's canal and seminal receptacle present. Uterus fills space between testes and acetabulum and may have a single loop which extends to the posterior ex-



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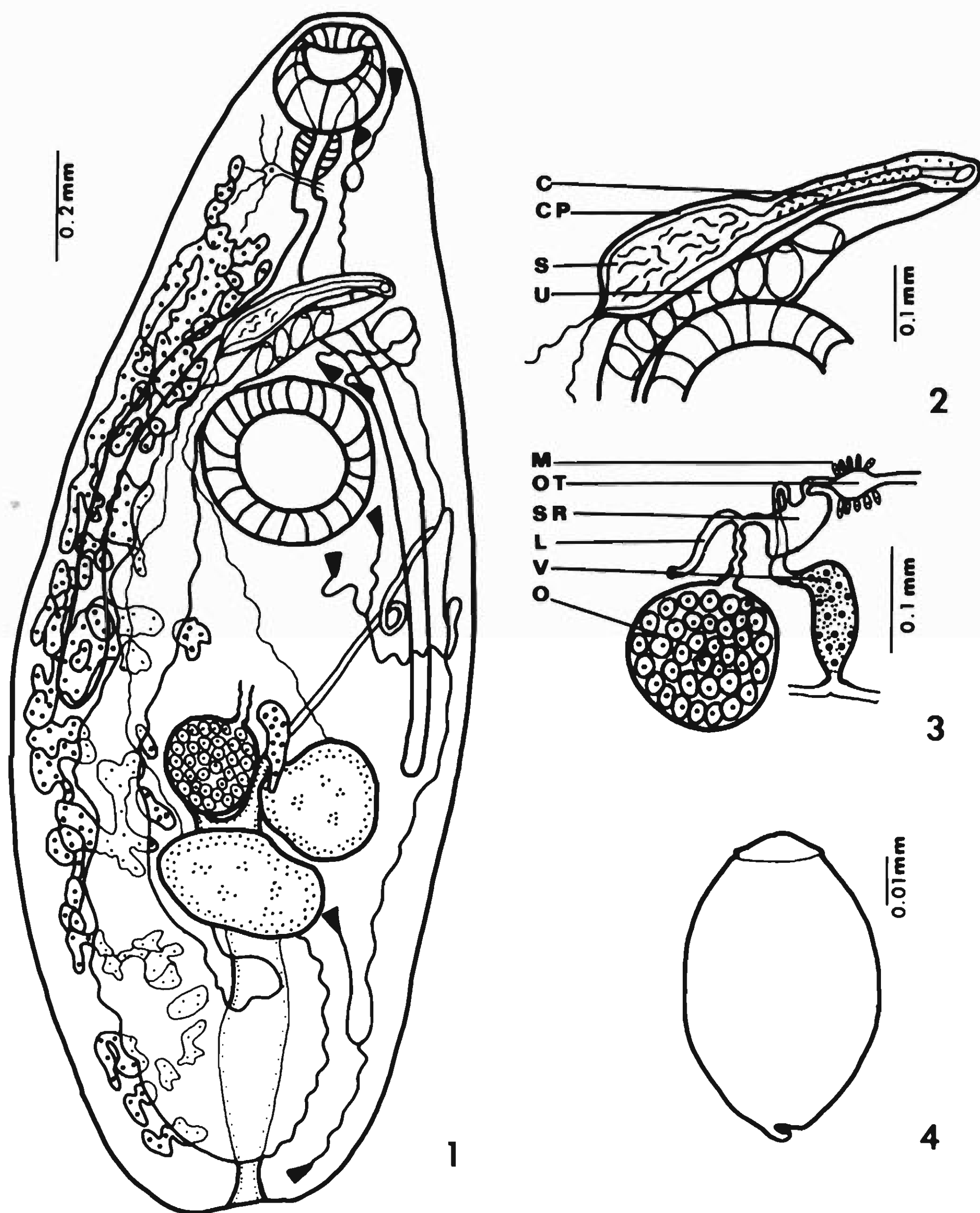


**Figures 1–4.** ***Plagioporus gyrinophili*** **sp. n. 1. Adult, ventral view of holotype drawn from projected photomicrographs; excretory system a composite from live and stained material. 2. Terminal genitalia showing cirrus pouch (CP), seminal vesicle (S), cirrus (C), and uterus (U). 3. Female genitalia showing ovary (O), seminal receptacle (SR), vitelline reservoir (V), ootype (OT), Mehlis' gland (M), and Laurer's canal (L). 4. Egg showing typical anopercular boss.**

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tremity and contains 100–300 eggs in mature worms. Eggs operculate, thick shelled, bright yellow, with anopercular boss; uterine eggs in whole mounts 60 (55–69) × 38 (41–46) (N = 45); unfixed eggs 60.5 (55–65) × 42 (39–46) (N = 25). Vitelline follicles large, numerous, in lateral fields extending from level of pharynx usually to hindtestis but sometimes almost to posterior extremity.

Hosts: *Gyrinophilus porphyriticus duryi* (Weller); *Pseudotriton ruber* (Latreille).

Location in host: Anterior quarter of small intestine.

Localities: (1) Tributary of Turkey Creek, Scioto Co., Ohio; (2) McBride Run, Scioto Co., Ohio; and (3) tributary of Longlick Run, Adams Co., Ohio.

Prevalence: *G. p. duryi*, 80% (4/5) adults (locality 1); 54% (14/26) larvae (locality 1); 100% (1/1) larva (locality 2); and *P. ruber*, 100% (1/1) adult (locality 3).

Holotype: USNM Helm. Coll. 76317.

Paratypes: USNM Helm. Coll. 76318, 76319.

## Discussion

Adult trematodes of the families Allocreadiidae (Looss, 1902) Stossich, 1903 and Opecoelidae Ozaki, 1925 are often difficult to distinguish from one another. Consequently, larval characteristics and excretory system are usually considered more reliable for determining their family (Manter, 1947; Cable, 1956). Though the life history of *Plagioporus gyrinophili* is unknown, the small number of flame cells, lack of eyespot pigment, and position of the genital pore place it in the Opecoelidae. The new species is assigned to the genus *Plagioporus* Stafford, 1904 because of the characterisitic form and position of the cirrus sac, position of the genital pore, extent of the vitellaria, saccate excretory vesicle, morphology of the gonads, and the $2[(2+2)+(2+2)] = 16$ flame cell pattern. The short intestinal ceca and extensive uterus are unusual, and its occurrence in an amphibian host is unique in the genus.

The similarity of *Plagioporus* to two other opecoelid genera, *Podocotyle* (Dujardin, 1854) and *Hamacreadium* Linton, 1910 was noted by Manter (1947, 1954) and Bravo-Hollis and Manter (1957). The unlobed ovary and anterior extent of the vitellaria distinguish *P. gyrinophili* from *Podocotyle*; while the shorter cirrus sac, unlobed ovary, and position of the genital pore differentiate it from *Hamacreadium*.

*Plagioporus gyrinophili* is distinguished from all other congeneric species by its relatively short cecal pouches, the extent of the uterus to the posterior extremity, and its presence in an amphibian definitive host. Of the species of *Plagioporus* which have been described from North American freshwater fish, *P. gyrinophili* resembles the species which Manter (1954) included in the subgenus *Caudotestis* Isaitschikow, 1928, *P. sinitsini* Mueller, 1934, and *P. cooperi* (Hunter and Bangham, 1932) Price, 1934, in having ceca which do not extend posterior to the testes. However, it differs in that its genitalia are not as posteriorly situated and the ceca are relatively shorter. In addition, *P. gyrinophili* differs from *P. sinitsini* in its intestinal rather than gall bladder location in the host and clearly differs from *P. cooperi* in extent of vitellaria, smaller egg size, and uncoiled seminal vesicle.

The six other North American species were placed in the subgenus *Plagioporus*

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by Manter (1954), Hendrix (1973), and Schell (1975). In the key presented by Manter (1954), *P. gyrinophili* keys out to *P. macrouterinus* Haderlie, 1953. However, it differs markedly from this species in the smaller sizes of the oral sucker, pharynx, and eggs, and in that the vitellaria are not confluent posteriorly. *Plagioporus gyrinophili* is distinguished from *P. angusticollis* (Hausmann, 1896) Dobrovolny, 1939 and *P. shawi* (McIntosh, 1939) Schell, 1975 by its shorter cirrus sac. It differs from *P. hypentelii* Hendrix (1973) in its larger body, oral sucker, acetabulum, and larger number of eggs (>50 vs. <20); and from *P. siliculus* Sinitsin, 1931 in its smaller body size, oral sucker, pharynx, ovary, testes, and anterior extent of the vitellaria. *Plagioporus gyrinophili* differs from *P. serotinus* Stafford, 1904 in having a straight rather than s-shaped cirrus sac and smaller eggs.

*Plagioporus serratus* Miller, 1940 was also described from North American freshwater fish. Yamaguti (1971) and Hendrix (1973) included it in *Plagioporus*, however, we agree with Manter (1947, 1954) that the spined body excludes it and suggest allocation to the Lepocreadiidae. To our knowledge, placement in another genus has not been proposed, so its status remains uncertain.

## Acknowledgments

The authors thank Dr. Sherman S. Hendrix for his examination of specimens and his critical suggestions and Dr. Andrew M. White for providing the specimen from *Pseudotriton ruber*.

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Proc. Helminthol. Soc. Wash.
48(2), 1981, pp. 202–208

# The Circumoval Precipitate and Cercarienhüllen Reaktion of *Austrobilharzia variglandis*

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ABSTRACT: The Circumoval Precipitin Reaction (COP) and the Cercarienhüllen Reaktion (CHR) were characterized for the marine avian schistosome, *Austrobilharzia variglandis*, utilizing sera from infected and artificially immunized chickens.

Circumoval precipitins were present in chicken sera 25 days following infection. No positive COP reactions were observed for eggs incubated in sera from animals artificially immunized with cercariae or adults. Complement was not essential in the development of positive COP reactions.

A positive CHR was observed in chicken serum collected 25 days following exposure to cercariae, but no reaction occurred in 14-, 32-, and 39-day sera. Sera from chickens artificially immunized with cercarial or adult homogenates elicited a positive CHR.

Normal chicken serum and normal guinea pig serum were cercaricidal. The role of complement in cercaricidal action was verified, but its participation in CHR formation is uncertain.

The Circumoval Precipitate (COP), first described for *Schistosoma mansoni* by Oliver-Gonzalez (1954), is typified by the appearance of a precipitate contiguous with the eggshell following incubation in immune serum. Newsome (1958), Yogore et al. (1968), and others have described the COP for other mammalian schistosomes but it has never been reported for avian forms.

The Cercarienhüllen Reaktion (CHR) of Vogel and Minning (1949) is characterized by the appearance of a sheath external to the cercarial tegument following incubation in immune serum. The CHR is well documented for mammalian schistosomes (Stirewalt and Evans, 1955; Ratanaret-Brokelman, 1972, and others). It has also been reported for three freshwater avian schistosome cercariae, *Trichobilharzia ulvae*, *T. physellae*, and *T. stagnicola* (Hendricks and Cort, 1956) and for an unknown marine avian form (Leflore and Martin, 1972).

In the present study, the COP and CHR were characterized for the marine avian schistosome, *Austrobilharzia variglandis* (Miller and Northup, 1926; Penner, 1953), using sera from infected and artificially immunized birds.

## Materials and Methods

To obtain cercariae for infections, naturally infected *Nassarius obsoletus* were isolated in dishes of seawater the afternoon of the day preceding exposure of chicks. Twelve-day-old white leghorn chicks were collectively exposed to approximately 4,000–5,000 cercariae by wading (5 birds/battery jar) for 90 min.

Adult worms were obtained from chicks infected 4 weeks or longer. Organs from these birds were teased apart and refrigerated overnight in normal saline. Worms obtained were washed six times in saline followed by three times in distilled water, frozen with Cryokwik (Damon/TEC Division, Needham Hts., Massachusetts), lyophilized, and stored at 4°C in capped vials.

[1] Taken from a thesis submitted to the Graduate School of Rutgers University in partial fulfillment of the requirements for the degree of Master of Science.



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**Table 1. Immunization schedule for artificially immunized chickens.**

| | Day 1 | Day 14 | Day 21 |
|---|---|---|---|
| Controls (2 birds) | *FCA, intramuscular, 0.2 ml/thigh | FCA, subcutaneous, 0.1 ml amounts in 4 different locations | |
| Cercarial immunization (2 birds) | Cercarial homogenate in FCA, 1:1 (v/v), intramuscular, 0.2 ml/thigh | Cercarial homogenate in FCA, 1:1 (v/v), subcutaneous, 0.1 ml in 4 different locations | Cercarial homogenate only, intravenous, 0.4 ml |
| Adult worm immunization (2 birds) | Adult worm homogenate in FCA, 1:1 (v/v), intramuscular, 0.2 ml/thigh | Adult worm homogenate in FCA, 1:1 (v/v), subcutaneous, 0.1 ml in 4 different locations | Adult worm homogenate only, intravenous, 0.4 ml |

* FCA = Freund's complete adjuvant.

Eggs were isolated from infected chicken livers using the method of James and Colley (1974). To prevent hatching, however, 0.85% rather than 1.7% saline was used. Eggs were stored in the dark in saline at 4°C and used within 3 days.

Seawater containing cercariae was filtered through four layers of cheesecloth and then through a Millipore apparatus fitted with a 47 mm, 8 $\mu$m filter. The filter with adherent cercariae was weighed, placed in a petri dish, frozen with Cryo-kwik, and stored at −20°C until needed. Approximately 30 mg (wet weight) defrosted cercariae were flushed from the filter paper with 1 ml ice cold 0.85% saline and homogenized with a Kontes hand driven tissue grinder. This suspension was used as antigen for artificial immunization. Adult whole worm antigen was similarly prepared using 10 mg lyophilized worms.

Cercariae used for the CHR were collected as above but were not frozen. When seawater was added to a dish containing a processed filter, cercariae were released and removed from solution with a pipette.

Experimentally infected chickens were bled by cardiac puncture at 14 (7 birds), 25 (11 birds), 32 (6 birds), and 39 days (5 birds) postinfection. Eight uninfected birds of the same age as the 39-day postinfection group were also bled. Six 14-day-old white leghorn chicks were immunized as shown in Table 1. All artificially immunized chickens were bled 1 week following their last injection and controls were bled 2 weeks after their last injection. Sera from similarly treated animals were pooled, preserved with sodium azide (0.10%) and stored in small aliquots at −20°C.

All COP and CHR tests were done in triplicate on covered 3 × 1 inch glass slides using a drop of serum handled in each of the following ways: (1) untreated, (2) inactivated (incubated at 56°C for 30 min), and (3) inactivated plus complement (one drop guinea pig serum). COP preparations were examined at 24 and 72 hr. They were scored as weak if the length of the precipitate chain was less than half the diameter of the egg and strong if longer. CHR preparations were observed at 1, 4, and 24 hr. A strong positive reaction was always indicated by a thick, sharply defined envelope surrounding the entire cercaria, whereas a weak positive reaction resulted in a thinner, more delicate appearing envelope that sometimes was confined to the tail only.

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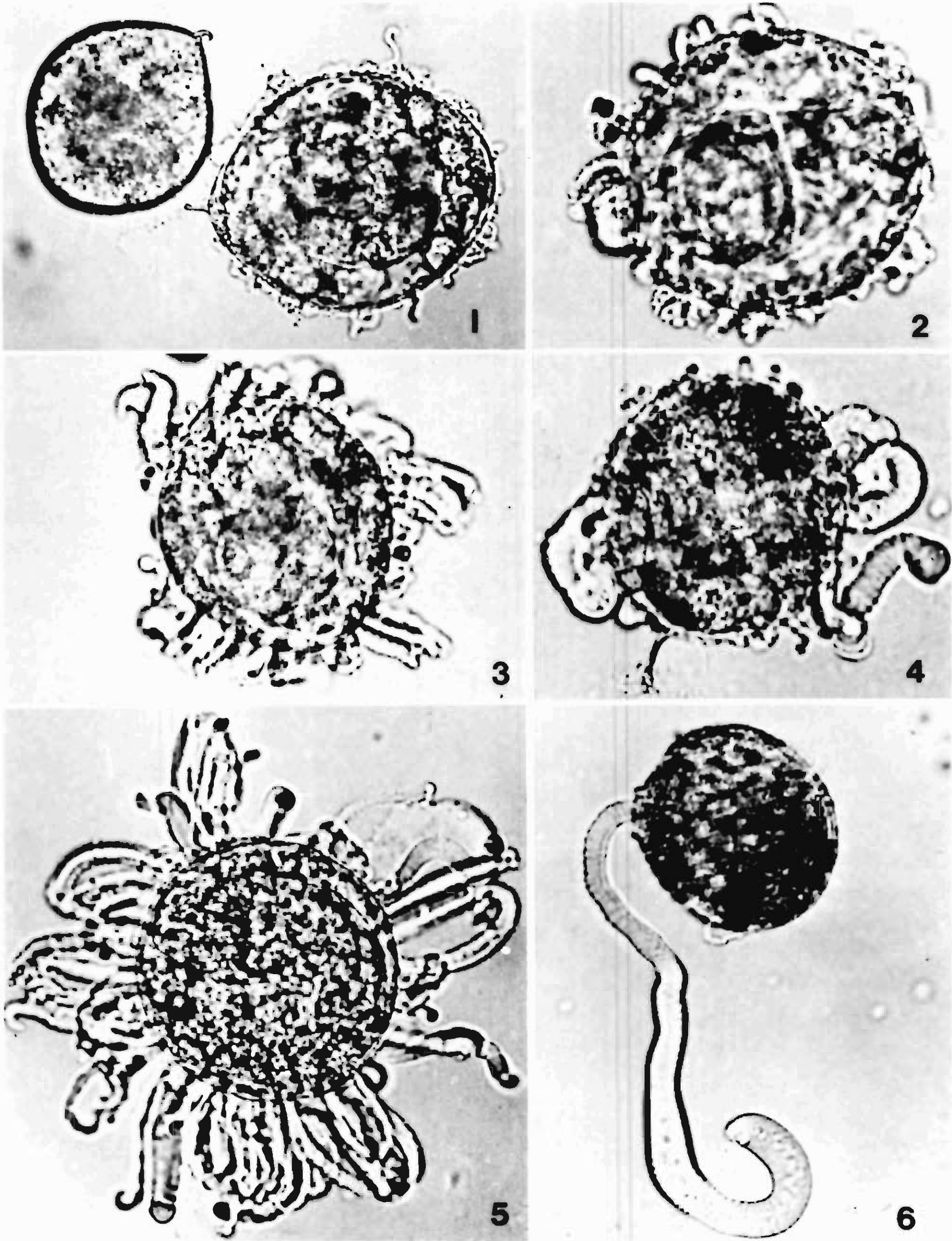


Figures 1–6. Photomicrographs of COP responses of *A. variglandis* eggs. 1. Eggs incubated in 39-day postinfection chicken serum for 72 hr. Note that smaller immature egg shows no COP. ×420. 2. Egg incubated in 25-day postinfection chicken serum for 72 hr. Weak positive reaction. ×490. 3. Egg incubated in 25-day postinfection inactivated chicken serum for 72 hr. Weak positive reaction. ×420. 4. Egg incubated in 32-day postinfection chicken serum for 72 hr. Strong positive reaction; note segmentation. ×420. 5. Egg incubated in 32-day postinfection chicken serum for 72 hr. Strong positive reaction; note outline of developing miracidium. ×490. 6. Egg incubated in 39-day postinfection chicken serum for 72 hr. Strong positive reaction. ×380.

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## Results

### COP

No positive COP response occurred in sera from control animals or from those immunized with cercarial or adult homogenates. Dead, empty, vacuolated, or immature eggs rarely showed a reaction whereas those containing a well-formed miracidium usually did (Fig. 1). Positive reactions occurred in sera from experimentally infected chickens harboring worms for 25, 32, and 39 days (Figs. 2–6) but serum obtained 14 days postinfection was negative. Most precipitates exhibited a typically segmented appearance. The percentage of eggs showing a positive reaction increased with the age of the infection. In 25-, 32-, and 39-day postinfection sera the mean percent positives were 14.0, 24.6, and 26.7, respectively. Inactivation of these sera reduced the percentages to 7.9, 12.1, and 11.7. Addition of complement did not restore full activity.

### CHR

Positive CHR activity was never observed in serum from control animals. Although most cercariae incubated in control serum from uninfected birds were not affected after 24 hr (Fig. 7), a few became motionless, and developed oral deposits and curled furcae within 1 hr. When inactivated, this serum was harmless to cercariae but became strongly cercaricidal when complement was added, suggesting a cercaricidal effect of guinea pig serum. Serum taken from experimentally infected chickens at 14, 32, and 39 days was also cercaricidal. Massive agglutination and positive CHR activity occurred within 24 hr when cercariae were incubated in 25-day postinfection serum (Fig. 8) but these effects were not observed at 1 or 4 hr. Agglutination and CHR activity were lost after inactivation and were not restored by adding complement. No activity was observed with 32- or 39-day sera. Artificially immunized control (FCA only) chicken serum was cercaricidal. In such serum most cercariae became spastic within 1 hr and a sticky deposit appeared at the oral end. A fine granular precipitate then appeared around the body, furcae curled, and motion ceased (Fig. 9). This effect did not occur in inactivated serum, but was restored by adding complement. Cercariae incubated in serum from chickens artificially immunized with cercarial antigen became very "sticky" within the first hour of incubation and at 24 hr all displayed definite sheaths and tended to agglutinate (Fig. 10). Inactivation of such serum resulted in the appearance of a flocculent precipitate that adhered to cercariae and hindered observation. Weak envelopes, however, were often evident (Fig. 11). Addition of complement to such serum did not restore original activity but an even more copious precipitate developed. Weak envelopes occurred occasionally in chicken anti-adult serum (Fig. 12). Envelopes were not observed with anti-adult inactivated plus complement sera, but massive precipitates occurred at the oral end (Fig. 13).

## Discussion

Uninfected control serum presumably contains no specific antibody (circumoval precipitin) against eggs, and negative results can be expected. Only after eggs begin to collect in tissues of infected birds can COP positive serum be anticipated. Our results indicate that the formation of a response occurs between

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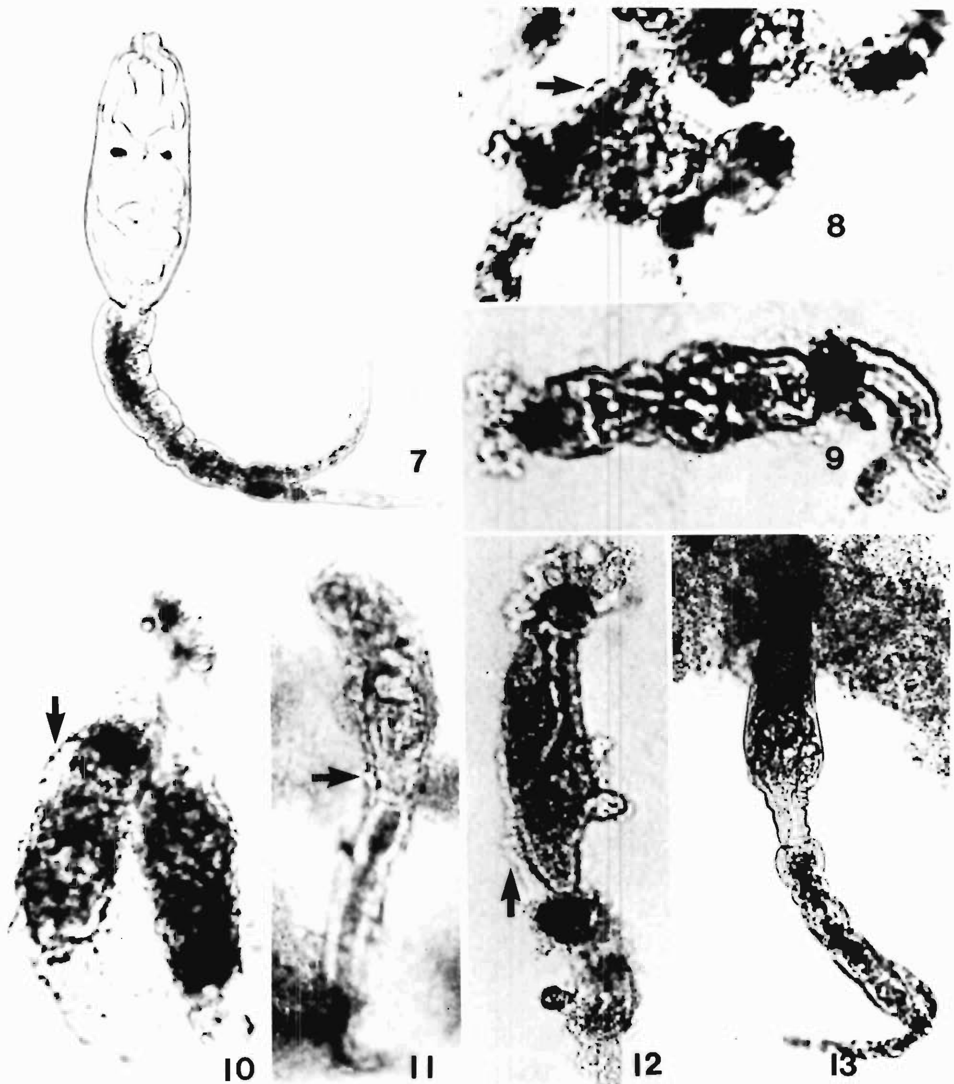


**Figures 7–13.** Photomicrographs of CHR responses of *A. variglandis*. 7. Normal *A. variglandis* cercaria, wet mount in seawater. ×135. 8. Cercariae incubated in 25-day postinfection chicken serum for 24 hr. Note envelope (arrow). ×110. 9. Cercaria incubated in serum from artificially immunized chicken (control-FCA only) for 24 hr. Note oral deposit and fine precipitate covering body. ×135. 10. Cercariae incubated in artificially immunized chicken serum (anticercarial) for 24 hr. Note typical CHR (arrow). ×110. 11. Cercaria incubated in artificially immunized chicken serum (anticercarial, inactivated) for 24 hr. Note envelope (arrow). ×110. 12. Cercaria incubated in artificially immunized chicken serum (anti-adult) for 24 hr. Note envelope (arrow). ×110. 13. Cercaria incubated in artificially immunized chicken serum (anti-adult, inactivated) for 24 hr. Note precipitate at oral end. ×110.

14 and 25 days postinfection. Our observation that all eggs showing a positive COP contained a live miracidium is consistent with previous findings (Oliver-Gonzalez, 1954; Newsome and Robinson, 1956; Newsome, 1958).

When COP positive sera utilized in this study were inactivated, the percentage

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of positive reactions was about half that seen for noninactivated serum. The addition of complement to inactivated serum did not, however, restore original activity. This suggests that another heat labile factor is involved but is weakened by heating.

The failure of sera from any artificially immunized chicken to elicit a COP response indicates that circumoval precipitin is evoked exclusively by egg antigens. This conclusion agrees with the findings of Oliver-Gonzalez (1954) that eggs absorb circumoval precipitins but adult or cercarial tissue does not.

The cercaricidal effects of all control chicken sera and all inactivated control sera (guinea pig serum added) are consistent with the findings of Kagan and Levine (1956) and Standen (1952). Inactivated control sera were not cercaricidal, suggesting a role for complement. Observations by Standen (1952) and Machado et al. (1975) support this view.

Serum dilution delays CHR envelope formation and enhances agglutination (Stirewalt and Evans, 1955). Hendricks and Cort (1956) and LeFlore and Martin (1972) also observed retarded sheath formation in diluted antisera. *Austrobilharzia variglandis* cercariae agglutinated strongly and formed envelopes slowly in 25-day chicken serum thereby suggesting a low CHR antibody titer.

The role of complement in anticercarial and anti-adult serum is unclear as addition of it to inactivated serum did not restore agglutinating or CHR activity.

A positive CHR response with chicken anti-adult serum was not surprising as schistosome adults and cercariae are known to share antigens (Kemp et al., 1974).

The lack of any CHR or agglutinating activity in 32- and 39-day sera is believed to be indicative of a decreased titer.

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# Helminths from the Short-tail Shrew, *Blarina brevicauda*, in Connecticut with Reference to the Histopathology of *Capillaria*

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ABSTRACT: Three species of trematodes, three species of cestodes, seven species of nematodes, and one species of acanthocephalan were collected from 143 short-tail shrews from Connecticut. Necropsy reports showed that 82.5% of the shrews were infected with some kind of helminth. These included Trematoda: *Brachylaima thompsoni* (9.7%), *B. rhomboideus* (53.1%), *Panopistus pricei* (21.6%); Cestoda: *Hymenolepis anthocephalus* (14.6%), *Pseudodiorchis reynoldsi* (0.6%), *Protogynella blarinae* (8.3%); Nematoda: *Porrocaecum americanum* (13.9%), *P. encapsulatum* (4.8%), *Capillaria* sp. (liver, 6.2%), *Capillaria* sp. (spleen, 0.6%), *Capillaria blarinae* (40.5%), *C. urinicola* (4.8%), *Spirura talpae* (0.6%); and Acanthocephala: *Prosthorhynchus formosus* (2.0%). The relationship of habitat type with specific helminth infections is presented. The histopathology associated with *Capillaria* infections is presented. A new host record is reported for *Spirura talpae*.

*Blarina brevicauda*, the short-tail shrew, is an abundant insectivore in Connecticut but its helminth fauna has not been documented except by Bray (1954) who studied the capillary worms from this host. Shrew helminth literature in the continental United States has dealt primarily with descriptions of new species. Surveys on short-tail shrew helminths have been reported from central Ohio by Oswald (1958), by Miller et al. (1974) in North Carolina, and by Wittrock and Hendrickson (1979) in Iowa.

## Materials and Methods

This study was conducted from October 1974 to March 1975. One hundred forty-three shrews from Connecticut were examined to determine helminth species present, severity of infection, histopathology associated with such infections, correlation of infection with food habits, and correlation of infection with habitat types. The study areas encompassed four habitat types: grass monoculture, forest, freshwater swamp, and salt marshes.

Shrews were captured using small Victor snap traps and Sherman small animal live traps. Standard collection techniques for helminths were employed. Helminths were stained with Mayer's HCl carmine, dehydrated in an alcohol series to toluene and mounted in Permount for identification. Nematodes were also mounted in glycerin jelly for study.

Tissues were fixed in 10% formalin, dehydrated in an alcohol series, embedded in paraffin, sectioned at 6 $\mu$m, and stained with hematoxylin and eosin.

Specimens were deposited in the National Parasite Collection, Beltsville, Maryland.

## Results

Seventy-two female and 71 male shrews were trapped. Necropsy reports showed that 82.5% of the shrews were infected with some species of helminth.

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**Table 1. Helminths of *Blarina brevicauda* in Connecticut, prevalence of infection, range of intensity and anatomic location.**

| Parasite | Prevalence of infection (%) (n = 143) | Range of intensity | Anatomic location |
|---|---|---|---|
| Trematoda | | | |
| *Brachylaima thompsoni* | 9.7 | 1–91 | small intestine |
| *Brachylaima rhomboideus* | 53.1 | 1–32 | small intestine |
| *Panopistus pricei* | 21.6 | 1–47 | large intestine |
| Cestoda | | | |
| *Hymenolepis anthocephalus* | 14.6 | 1–4 | small intestine |
| *Pseudodiorchis reynoldsi* | 0.6 | 2 | small intestine |
| *Protogynella blarinae* | 8.3 | 2–6 | small intestine |
| Nematoda | | | |
| *Porrocaecum americanum* | 13.9 | 4–8 | cysts in intestinal mesentery |
| *Porrocaecum encapsulatum* | 4.8 | 2–19 | subcutaneous |
| *Capillaria blarinae* | 40.5 | 3–6 | esophageal epithelium |
| *Capillaria urinicola* | 4.8 | 5–11 | urinary bladder |
| *Capillaria* sp. (liver) | 6.2 | 1 | liver |
| *Capillaria* sp. (spleen) | 0.6 | 1 | spleen |
| *Spirura talpae* | 0.6 | 3 | stomach |
| Acanthocephala | | | |
| *Prosthorhynchus formosus* | 2.0 | 1–4 | small intestine |

Three species of trematodes, three species of cestodes, seven species of nematodes, and one species of acanthocephalan were found. Table 1 lists the helminth species collected, severity of infection, and anatomic location. No seasonal variation in parasite burden was noted. Males and females were parasitized in equal intensity. Table 2 depicts the relationship of habitat type with specific helminth infections. The grass monoculture habitat yielded the greatest number of shrews for study and had the greatest variety of parasite species. The parasite variety for shrews by habitat type followed the order of grass monoculture (12), forest (10), salt marsh (8), and swamp (4).

The presence of adult *Capillaria blarinae* in the stratified squamous portion of the esophageal epithelium was noted in 40.5% of the shrews examined. The worms produced a parakeratosis in the epithelium. The dominant inflammatory cells in the basal layer of the stratum germinativum were eosinophils. Lymphocytes were occasionally found. The adults occupied wave shaped tunnels in the epithelium, usually with part of the body looped into the esophageal lumen. The *Capillaria blarinae* eggs, 60 by 20 $\mu$m, were found in the tissues and free in the lumen of the esophagus.

Eggs of *Capillaria* sp. were noted in the livers of 6.2% of the shrews. There was no consistency in the location within a liver lobule. The mean dimensions of the eggs measured 61.8 by 28.1 $\mu$m. One partial worm was removed from the liver and there was no grossly obvious reaction to the worm. Characteristic hepatic lesions were macroscopically visible in one of the shrews examined. Sec-

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**Table 2. Relationship of habitat type with specific helminth infections.**

| Parasite | Percent infected by habitat type | | | | |
|---|---|---|---|---|---|
| | Forest | Grass monoculture | Swamp | Salt marsh | Total |
| Trematoda | | | | | |
| *Brachylaima thompsoni* | 6.8 (2)* | 9.6 (9) | — | 25.0 (3) | 9.7 |
| *Brachylaima rhomboideus* | 44.8 (13) | 57.5 (57) | 66.2 (2) | 33.3 (4) | 53.1 |
| *Panopistus pricei* | 3.4 (1) | 22.2 (22) | — | 16.6 (2) | 21.6 |
| Cestoda | | | | | |
| *Protogynella blarinae* | — | 11.1 (11) | 33.3 (1) | — | 8.3 |
| *Hymenolepis anthocephalus* | 13.7 (4) | 17.1 (17) | — | — | 14.6 |
| *Pseudodiorchis reynoldsi* | — | 1.1 (1) | — | — | 0.6 |
| Nematoda | | | | | |
| *Porrocaecum encapsulatum* | 10.3 (3) | 2.1 (2) | 33.3 (1) | 8.3 (1) | 4.8 |
| *Porrocaecum americanum* | 6.8 (2) | 15.0 (14) | — | 33.3 (4) | 13.9 |
| *Capillaria blarinae* | 3.4 (1) | 60.2 (56) | — | 8.3 (1) | 40.5 |
| *Capillaria urinicola* | 6.8 (2) | 5.0 (5) | — | — | 4.8 |
| *Capillaria* sp. (liver) | 10.3 (3) | 5.0 (5) | — | 8.3 (1) | 6.2 |
| *Capillaria* sp. (spleen) | 3.4 (1) | — | — | — | 0.6 |
| *Spirura talpae* | — | — | 33.3 (1) | — | 0.6 |
| Acanthocephala | | | | | |
| *Prosthorhynchus formosus* | — | 1.1 (1) | — | 16.2 (2) | 2.0 |
| No. of shrews examined | 29 | 99 | 3 | 12 | 143 |

* Values in parentheses represent the number of animals infected.

tions disclosed discrete, scattered foci that were comprised of eggs. Most of the hepatic tissue was normal in appearance.

The spleen of one shrew was found to contain eggs of *Capillaria* sp. and portions of one worm. These eggs were in the white pulp of the spleen. A few eosinophils were observed in the area where the eggs were present. The mean dimensions of six bipolar operculate eggs measured in situ in the spleen were 67 by 27 $\mu$m.

## Discussion

The results presented in this study emphasize the prevalence of infection with one or more helminth species in *Blarina brevicauda*. One of these helminths, *Spirura talpae*, has not been reported previously from this host in the continental United States.

From the four areas sampled, similar parasite fauna was collected so that a variation in the parasite burden as the result of habitat type was not detected. The parasite burdens in the shrew reflect the food habits of this animal. Shull (1907) found that many beetles and their larvae, other insects and their pupae, earthworms and sowbugs, snails of the genus *Polygyra* and meadow mice are taken as food. Snails in the genus *Lymnaea* when available are taken by the short-tail shrew. The metacercariae of the flukes recovered from shrews in this study occur in a number of land snails and slugs. Consequently, infections were probably contracted by eating infected mollusks. Nineteen double infections with

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brachylaimid trematodes were noted in this study. Multiple infections of brachylaimids are quite common in land snails according to Villella (1954), who observed *Panopistus pricei* associated with other species of brachylaimids within the same snail host.

The snail hosts *Ventridens ligera* and *V. suppressus* which have been listed for *Brachylaima rhomboideus* (Yamaguti, 1971) are not reported from Connecticut (Burch, 1962). The prevalence of *B. rhomboideus* was 53.1% which indicates that either the snail host has a new distribution or that another intermediate host is present in sufficient numbers to allow for such a high prevalence of infection.

Since 68.5% of the shrews examined were infected with trematodes, it appears that snails and slugs are a preferred food source for the shrew.

No life cycles are known for cestodes which parasitize *Blarina brevicauda*, although van Gundy (1935) found very immature specimens of *Hymenolepis anthocephalus* associated with larval elaterid beetles in the stomach of shrews. The hymenolepidids very frequently utilize an arthropod as an intermediate host which probably explains the large number of species of hymenolepidids which are found in insectivorous animals such as shrews. *Hymenolepis anthocephalus* was not found in shrews from the swamp habitat, an environment which may be unsuitable for the beetle intermediate host.

The method by which the host becomes infected with *Porrocaecum encapsulatum* is not known. Oswald (1958) suggested that the eggs are picked up first by some invertebrate such as an earthworm and that the shrew becomes infected by ingesting the eggs or very early developmental stages in the invertebrate animal.

The life cycle of *Capillaria blarinae* is not known. Some species in this genus require an earthworm as an intermediate host (Hyman, 1951). In view of the high prevalence 40.5% of infection of shrews in this study and the low reported (Hamilton, 1930) prevalence of earthworm consumption, two alternative hypotheses may be proposed: (1) Earthworms consituted a greater portion of the diet of shrews in this study than that of Hamilton (1930); and (2) *Capillaria blarinae* may utilize an intermediate host other than the earthworm. The histopathology in the shrew esophagus agrees with the description by Ogren (1953). Ogren (1953) observed no abnormal tissue proliferation as was the case for the shrews from Connecticut.

*Capillaria urinicola* was collected from the urinary bladder of 4.8% of the shrews examined, no reactions were noted to the presence of the parasite. It has been reported only once before by Bray (1954). *Capillaria plica* has been reported by Miller et al. (1974) from shrews in North Carolina.

The mean dimensions of 50 formalin-fixed eggs collected from the liver of the shrews in this study measured 61.8 by 28.1 μm which is longer and not as wide as the measurements given by Bancroft (1893) for live *Capillaria hepatica* eggs (55 by 30 μm). The discrepancy may be due to the fixation process.

The dimensions (67 by 27 μm) of the eggs of *Capillaria* sp. collected from the spleen of one shrew were comparable to those (64 by 26 μm) of unidentified eggs recovered from the spleen of a rat (*Rattus rattus*) by Mackerras (1957).

*Spirura talpae* was collected from the stomach in one shrew from a swamp habitat. Larvae encyst in the abdomen of *Blatta orientalis* according to Seurat (1911). Sprehn (1932) reported this parasite in *Talpa europea* in Europe. The

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report of *Spirura talpae* from the short-tail shrew in Connecticut is a new host record.

Juvenile acanthocephalans of *Prosthorhynchus formosus* which were collected from two shrews suggests that the short-tail shrew can serve as a paratenic host of this acanthocephalan if these parasites become encysted viscerally. Nickol and Oetinger (1968) recovered 45 cystacanths of *P. formosus* from the mesenteries of a short-tail shrew in New York. The shrews in this study probably acquired the parasites by eating infected isopods. Although *P. formosus* has not been reported from a bird of prey, it is possible that parasitizations do occur, since shrews are consumed by these predators.

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# Surface Ultrastructure of *Eimeria tenella, E. dispersa,* and *E. meleagrimitis* Motile Forms

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ABSTRACT: Three previously undescribed surface structures were detected when *Eimeria tenella, E. dispersa,* and *E. meleagrimitis* sporozoites were examined by high-resolution scanning electron microscopy. There were apical projections that varied in number from two to five, depending on the species examined. These projections may connect with the rosette-rhoptry complex and aid in host-cell penetration. Secondly, there were papillalike structures arranged in longitudinal rows extending from the apex of the sporozoite to its midpoint; on merozoites of *E. tenella* the papillae were found the entire length of the organism. Additionally, longitudinal ridges are described and are suggested to be surface manifestations of the internal microtubules.

Sporozoites, the infective motile forms of bovine coccidia (*Eimeria*), flex and glide when penetrating host cells (Fayer and Hammond, 1967). This type of movement suggests a complex ultrastructure, various aspects of which have been reported by numerous workers and reviewed by Scholtyseck (1979). D'Hase et al. (1977) described subpellicular microtubules that extended from the anterior polar ring to the midpoint of *Eimeria* sporozoites. Although Porchet-Hennere' and Ponchel (1974) and Mehlhorn and Heydorn (1978) reported superficial longitudinal striations or riblike structures on the pellicular surface of *Sarcocystis tenella* merozoites, such structures have not been reported on the sporozoites of poultry coccidia.

The penetration of host cells is essential to the survival of the parasite, yet very little is known about the actual mechanisms of penetration. Jensen and Edgar (1976) suggested that the saclike rhoptries in bovine coccidia probably functioned to secrete a substance that aided in penetration. Dubremetz and Torpier (1978) observed an "apical rosette" lying anterior to the rhoptries but below the surface of the sporozoite pellicle. With scanning electron microscopy (SEM), Vetterling et al. (1971) demonstrated that the conoidal complex of *Eimeria tenella*, a poultry coccidium, protruded in some sporozoites.

The present study was designed to examine sporozoites of poultry coccidia and use high resolution SEM to locate surface structures that may aid movement and penetration.

## Materials and Methods

Oocysts of *Eimeria dispersa, E. tenella,* and *E. meleagrimitis* were collected from experimentally infected birds and sporulated and excysted as described by Doran and Vetterling (1967). Excysted sporozoites were purified by the glass-bead column method of Doran (1970). Sporozoites were fixed in 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.3, for 3 hr, washed 3× in buffer (0.05 M cacodylate with 0.2 M sucrose, pH 7.3) for 15 min, and postfixed for 1 hr (1% $OsO_4$ in 0.05 M cacodylate with 0.15 M sucrose). The temperature of all solutions throughout the processing was 4°C. The fixed sporozoites were washed 3× in distilled $H_2O$, suspended in 25% ethanol, and placed in a 10-ml syringe with an



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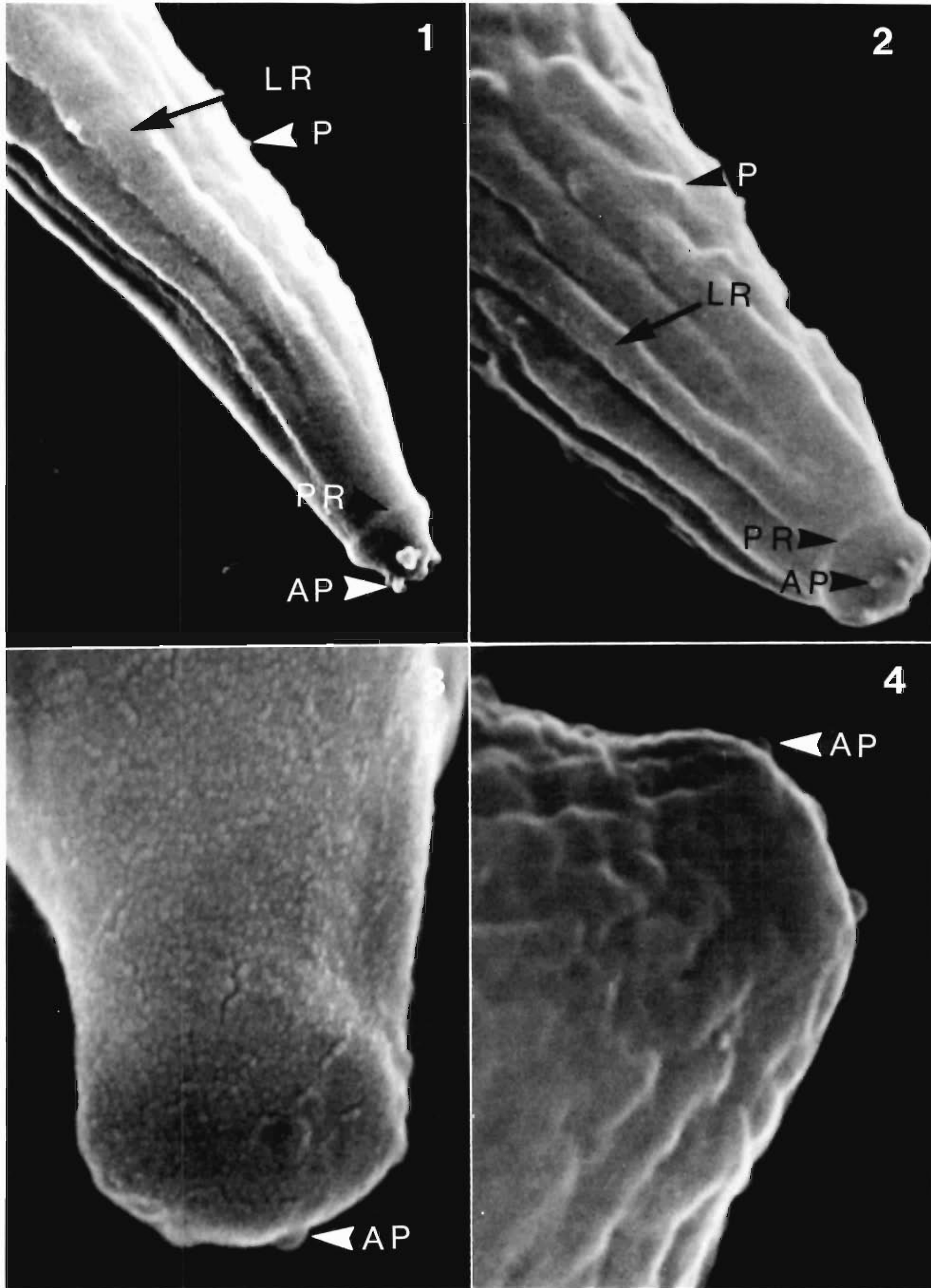


Figures 1–4. Scanning electron micrographs of three species of *Eimeria* sporozoites and *E. tenella* merozoites. PR = polar ring, LR = longitudinal ridges, P = papilla, AP = apical projections, AG = anterior refractile granule, PG = posterior refractile granule, RBC = erythrocyte. 1. *Eimeria dispersa*. Note apical projections on sporozoite with protruded conoid, longitudinal ridges, and papillae (×33,000). 2. *Eimeria dispersa*. Note apical projections, polar ring, longitudinal ridges, and papillae; conoid not protruded (×42,000). 3. *Eimeria meleagrimitis*. Note apical projections. (×110,000). 4. *Eimeria tenella*. Note apical projections (×130,000).

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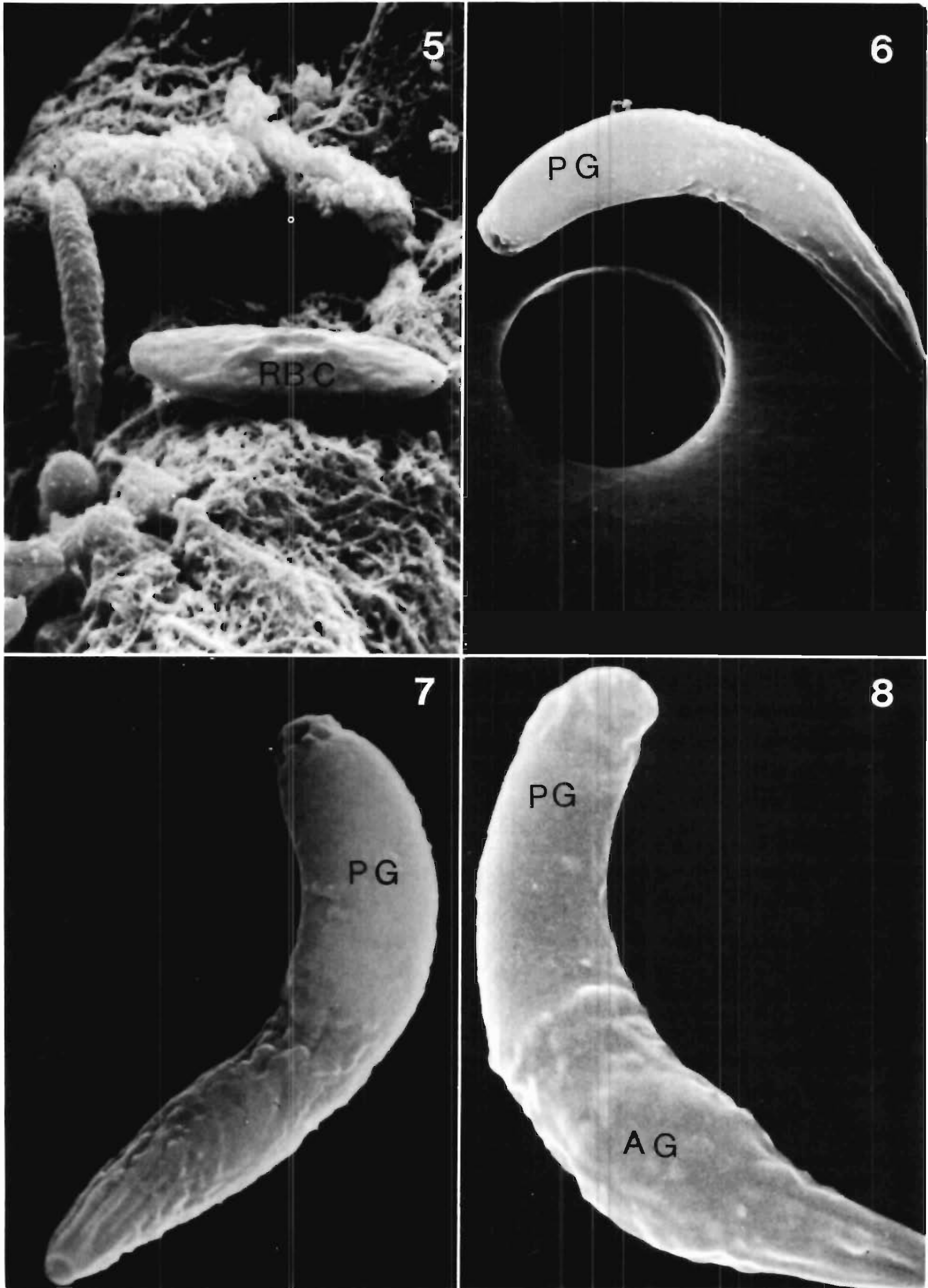


Figures 5–8. Scanning electron micrographs of *Eimeria tenella* and *E. dispersa*. Abbreviations as in Figures 1–4. 5. *Eimeria tenella* merozoite. Note cucumber appearance from parallel rows of papillae; erythrocyte (×6,200). 6, 7. *Eimeria dispersa*. Note rough anterior half, smooth swollen posterior half, blunt posterior, and uniformity of the entire sporozoite (×8,000 and ×11,500, respectively). 8. *Eimeria dispersa*. Note swollen rough anterior and smooth posterior (×11,000)

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attached 3-μm-pore polycarbonate membrane. A graded series of ethanols (25, 50, 75, 95, 100%) was passed over the retained sporozoites and was followed by four passages of 100% ethanol at room temperature to complete the dehydration. The membrane with the attached sporozoites was dried in a critical-point drying apparatus (Madden and Tromba, 1976) and a piece was mounted on a specimen stub with cellulose adhesive. The specimens were coated with 20 nm of 60/40 Pd-Au alloy in an ion sputtering device.

Merozoites of *E. tenella* only, were prepared by removing cecal tissue from infected birds and examining the cut surface as described by Madden and Vetterling (1977).

The specimens were examined at 100 kV accelerating voltage in a JEOL 100-C[1] transmission electron microscope with scanning coils.

## Observations and Discussion

When examined by high-resolution SEM, sporozoites of *E. dispersa, E. meleagrimitis,* and *E. tenella,* had several common morphological structures on the pellicular surface. These structures, however, projections and papillae, and longitudinal ridges, have not been previously reported. The projections were on the apex of most of the sporozoites examined. On the apex of *E. dispersa* there was an evenly spaced, circular arrangement of five projections. These could be observed on apices of both protruded (Fig. 1) and nonprotruded (Fig. 2) conoids. Apical projections on sporozoites with protruded conoids were 60 nm long and jutted out prominently from the apex. Sporozoites with nonprotruded conoids were 18 nm long and appeared blisterlike. The whole sporozoites of *E. meleagrimitis* were blunter than those of *E. dispersa* and had three apical projections that were not as distinct as those of *E. dispersa* (Fig. 3). The sporozoites of *E. tenella* were the bluntest of the three species examined, and the number of projections varied from two to three (Fig. 4).

Dubremetz and Torpier (1978) using freeze-etch techniques reported apical rosettes in the Pe fracture face (Branton et al., 1975) of *Eimeria nieschulzi* sporozoites, which lies below the pellicular surface of the plasmalemma and above a small vesicle surrounded by an irregular circle of openings that might be rhoptry ducts. These ducts are believed to secrete substances that aid the sporozoite in the penetration of host cells (Jensen and Edgar, 1976).

The presence of the apical projections immediately above the reported location of the apical rosettes suggest that these projections may be extensions of the rhoptry-rosette complex and as such may be involved in penetration. These projections may serve as surface conduits or ducts for the hypothesized secretory product of the rhoptry. The projections themselves could also be the secretory product adhering to the surface of the sporozoite. Because the projections were not observed on all sporozoites, it is possible that their presence is transitional, or that the projections may have been removed during preparation of the material for SEM. Raised areas or small papillae were also observed on the pellicular

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surface of sporozoites and merozoites (Figs. 1, 2, 5). These were arranged in parallel rows running longitudinally from the anterior to the midpoint of the sporozoite. On merozoites, the papillae appeared more uniformly spaced and extended nearly the entire length of the merozoite, giving it the appearance of a cucumber (Fig. 5). The presence of the normally formed erythrocyte observed with this specimen suggests that these papillae are not artifactual (Fig. 5). These papillae have not been previously described for the genus *Eimeria* but they may be a common feature for the apicomplexa, since they are discernible in micrographs of *S. tenella* (Porchet-Hennere' and Ponchel, 1974).

Longitudinal ridges extending from the polar ring to a point about midway on the pellicle were observed regularly on sporozites (Figs. 6, 7). These ridges appear to be analogous to those found on merozoites of *S. tenella* and called superficial striations (Porchet-Hennere' and Ponchel, 1974) or riblike structures (Mehlhorn and Heydorn, 1978). D'Hase et al. (1977) have shown that the subpellicular microtubules in *Eimeria* sporozoites extend from the polar ring to midpoint. The longitudinal ridges may be surface manifestations of the internal microtubular organization.

The posterior half of the sporozoites examined, except for the caudal end, appeared smooth and was swollen, probably because of support from the underlying refractile granule (Figs. 6–8). However, some sporozoites swollen from an anterior refractile granule still had the rough surface (Fig. 8). Figures 6 and 7 are lower-magnification micrographs of the sporozoites in Figures 1 and 2 and show that the morphology of sporozoites is uniform.

The posterior end of the sporozoites in all three species was blunt compared with the anterior end. As an example, in *E. dispersa* sporozoites (Figs. 6–8) the posterior end, measuring 1 μm in diameter just distal to the posterior refractile granule, was twice as blunt as the anterior end, which measured 0.5 μm at the polar ring and 0.35 μm at the protruded conoid.

We have elucidated the surface morphology of some motile forms of *Eimeria*, and have attempted to correlate the anterior projections with the rhoptries and apical rosettes which are believed to aid in host cell penetration. Further investigation is needed to determine whether the number of apical projections varies between other species of coccidia. Additionally, serial sectioning and transmission electron microscopy might enable us to determine further the true nature of the projections.

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# *Verutus volvingentis* n. gen., n. sp. (Heteroderidae: Tylenchida) in Verutinae n. subf., a Phytoparasitic Nematode Infesting Buttonweed in Florida

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Abstract: *Verutus volvingentis* n. gen., n. sp. is described from *Diodia virginiana* L. from Florida, USA. Females differ from females in all subfamilies of Heteroderidae in having a sausage or reniform-shaped body with an uncommonly large vulva in a subequatorial position. Semi-endoparasitic females do not retain eggs or form a cyst. Eggs are deposited naked in the substrate. Males have a truncated tail, tubus, and an untwisted body. There is a close resemblance between *V. volvingentis* males and males of several genera in the Heteroderidae. Larvae closely resemble larvae of most genera in the Heteroderidae.

Larvae and males of *V. volvingentis* differ from all other larvae in the Heteroderidae in lacking a detectable phasmid.

In March 1969, Mr. Wayne W. Smith, Agricultural Products Specialist with the Florida Department of Agriculture and Consumer Services, submitted 14 soil samples from *Diodia virginiana* L. (buttonweed) from a field near Apopka, Florida, for regulatory analysis. Four of the samples were infested with larvae that resembled *Heterodera* sp. A search of the sample material for *Heterodera* cysts revealed females that did not fit the generic concept of any described phytoparasitic nematode genus.

## Taxonomy of the New Genus

### Measurement and preparation methods

Specimens to be measured were placed in water within a "Zut" ring on a glass microscope slide (Esser, 1973b), and a cover slip placed on the zut. The nematodes ceased moving in 3 to 5 min, after which measurements were taken (Esser, 1971), and camera lucida drawings made. The specimens were then transferred to a BPI watch glass for permanent fixation in lactophenol (Esser, 1973a). Specimens used were reared on buttonweed in the greenhouse inoculated with specimens of the new genus from the type locality.

## Verutinae n. subf.

Diagnosis: Heteroderidae (Filipjev & Schuurmans Stekhoven, 1941) Skarbilovich, 1947.

Female (Fig. 1A): Mature female saccate, sausage to reniform-shaped (Fig. 2), vulva uncommonly large, subequatorial in position, vulval lips strongly protuberant, ovaries reflexed, anus subterminal, cyst stage absent, body striae present, phasmid obscure, strong sexual dimorphism present.

Male (Fig. 3A): Body vermiform, caudal alae absent, tail bluntly rounded, sometimes truncate, body untwisted, one testis present.

Type genus: *Verutus* n. gen. (from the Latin "armed with a dart").



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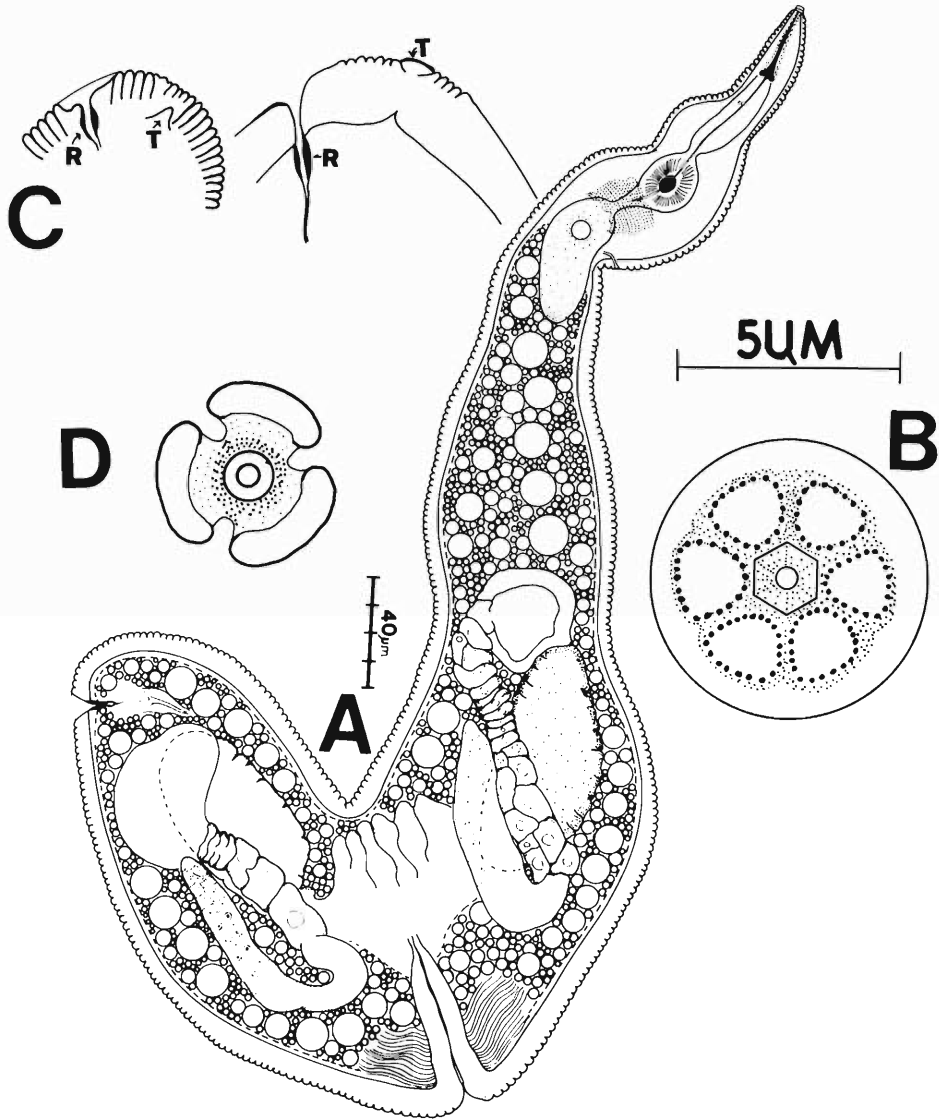


**Figure 1.** **A.** ***Verutus volvingentis*** **mature female. B. En face. C. Lateral view of female tails, showing vestigial larva tail tip (T), and rectum (R). D. Telorhabdions, posterior view.**

### *Verutus* n. gen.

DIAGNOSIS: Verutinae, with characters of the subfamily. Mature female (Fig. 1A): Body swollen, reniform or sausage-shaped (Fig. 2). Cephalic framework moderately sclerotized, lips striated, set off, amphids obscure, oral disc hexagonal (Fig. 1B). Body striated, lateral lines irregular, sometimes indistinct. Crystalline layer present (Fig. 15). Stylet tylenchoid, dorsal gland orifice near telorhabdion base. Uncommonly large protuberant postequatorial vulva. Gonads didelphic and

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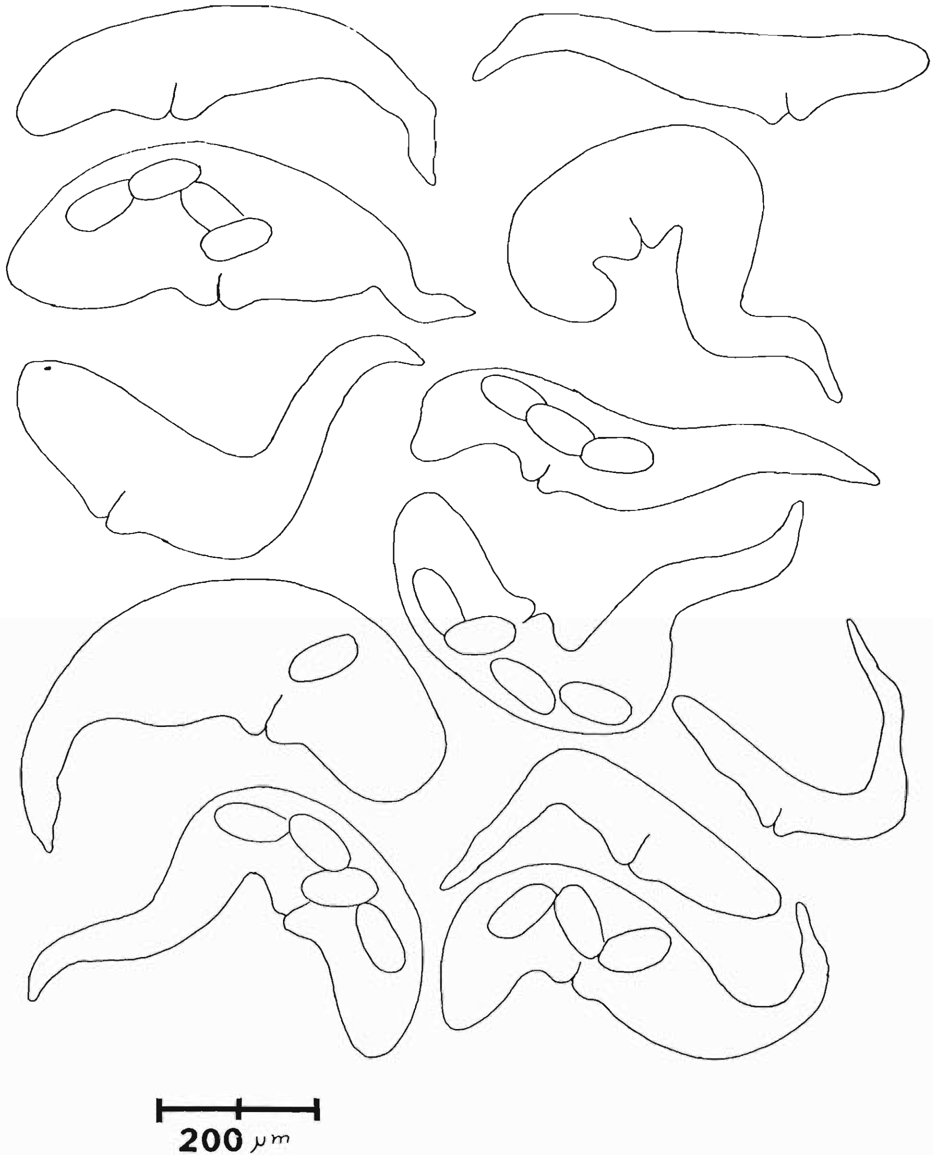


**Figure 2.** ***Verutus volvingentis* n. gen., n. sp., female body shapes.**

amphidelphic. Ovaries reflexed. Anus subterminal forming small depression (Fig. 1C). Tail vestigial (Fig. 1C) or absent.

FEMALE: Females differ from all other females in the Heteroderidae in possessing a reniform or sausage-shaped body, with an uncommonly large vulva in a postequatorial position with strongly protuberant lips. Esophagus typically tylenchoid, procorpus moderately expanded, metacorpus moderate in size. Isthmus narrower than procorpus, esophageal gland a single lobe moderately overlapping the intestine. Deirids and phasmids not observed.

MALE (Fig. 3A): Body vermiform, monodelphic, lips striated; not set off, oral

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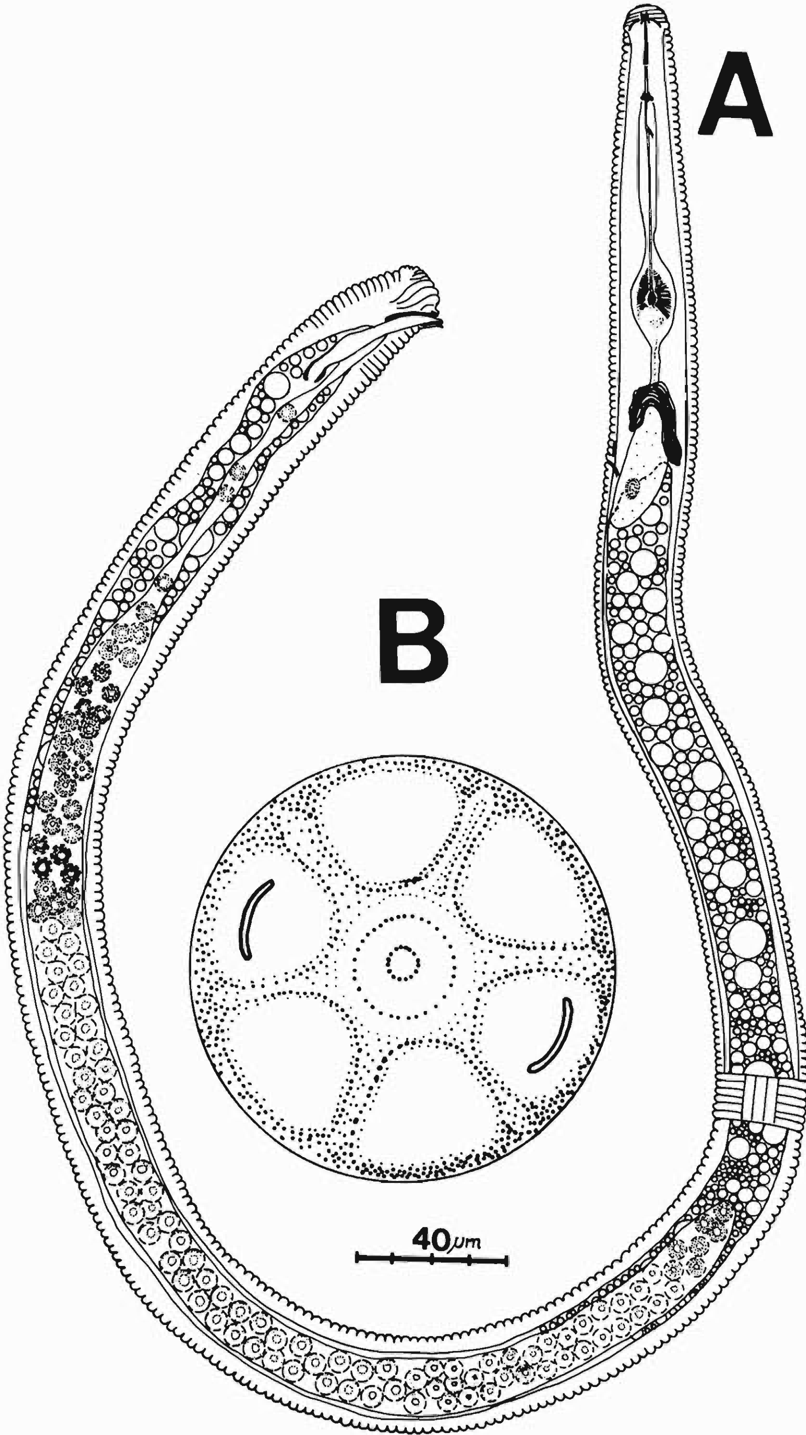


**Figure 3. A. Mature male of *Verutus volvingentis*. B. En face.**

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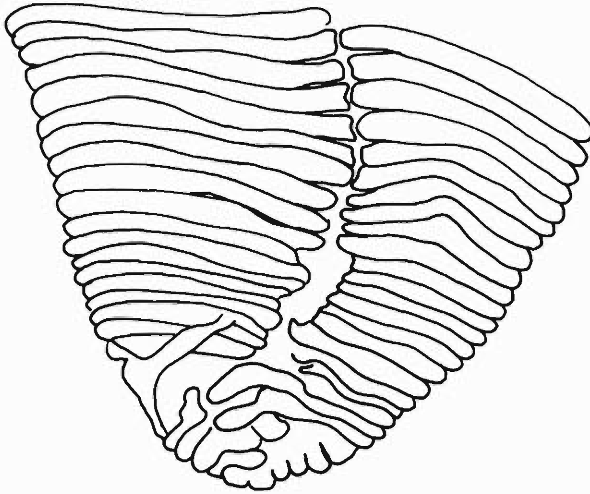

**Figure 4. Female tail area showing lateral field irregularities.**

disc circular (Fig. 3B), amphidial openings elliptical on lateral lips. Body untwisted. Spicules and gubernaculum tylenchoid. Caudal alae absent, tail terminus angular truncate (Fig. 23B). Phasmids or deirids not detected.

TYPE AND ONLY SPECIES: *Verutus volvingentis* n. gen., n. sp.

### *Verutus volvingentis*[1] n. sp.

FEMALE (35 specimens): Total length = 662.7 (500–930) μm; width = 141.4 (94–207) μm; tail = 10.2 (3.9–15) μm; esophagus = 188.3 (150–290) μm; a = 4.7 (3.0–6.5); c = 69.4 (34–155); total stylet length = 26.1 (23.5–29.4) μm; vulva % = 67.5 (50–75); excretory pore = 139.8 (122–183) μm from anterior end.

FEMALE HOLOTYPE: Total length = 540 μm; width = 118 μm; tail = 15 μm; esophagus = 148 μm; a = 4.6; b = 3.6; c = 36; stylet = 27.2 μm; vulva % = 71.2; excretory pore = 114 μm from anterior end.

FEMALE DESCRIPTION (Fig. 1A): Body pearly white, reniform or sausage-shaped (Fig. 2), anterior part of body sometimes twisted upward lying in a different plane than the posterior swollen portion. Head and neck occasionally reflexed across the posterior body. Six equidistant lips surround a hexagon-shaped oral disc (Fig. 1B). Amphid apertures or lip papillae not observed. Lips set off, comprised of 2 annules. Cuticle 9–10 μm thick, evenly striated, striae about 2.5 μm apart. The occurrence and appearance of lateral lines are variable: lines may proceed for a short distance beyond anus and fade out, or appear as midline cuticular interruptions or irregularities extending slightly past the vulva area. They appear as 1 or 2 lines of irregular blocks in the cervical area. In the tail area they appear as a mass of irregularities in the tail tip area with sometimes a wide separation (5 μm) of the striae (Fig. 4). Lateral lines were not observed in a few females. Stylet tylenchoid, telorhabdions (Fig. 1A, D), 4–5 μm wide by 1–2 μm long, directed posteriad. Prorhabdions 14 μm. Two stylets with tips pro-

[1] volv = vulva, ingen = remarkable size.

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truding from the body measured 25.5 and 25.6 μm. The dorsal gland orifice appears 7–11 μm posterior to the telorhabdion base. A moderately swollen orifice appears 7–11 μm posterior to the telorhabdion base. A moderately swollen procorpus narrows prior to the well-developed metacorpus. A clearly defined metacorpal valve is present. A short narrow isthmus leads from the metacorpus followed by a single distinct esophageal gland situated ventrally over the intestine. The intestine extends from beneath the mid-area of the esophageal gland to the rectal intestinal valve. The sclerotized portion of the rectum is 12 μm long in lateral view. The rectum dilates anteriorly, extending 30 μm beyond the sclerotized portion (Fig. 1C) as a finely sclerotized tube (15 μm long) which joins the intestine. The oval anus lies in a depression (Fig. 1C). The tail is usually absent, occasionally vestigial (Fig. 1C). Differences in orientation of the body do not permit an accurate tail annule count or anal body diameter measurement. One female was noted (Fig. 5) with a granular mass over the anus, assumed to be excreta. The excretory pore lies at the level of the esophageal gland 134.4 (113–183) μm from the oral opening. The nerve ring appears as a mass of tissue surrounding the isthmus.

Gonad amphidelphic, anterior branch 136–147 μm long, posterior branch 117–130 μm long. The vulva appears as a transverse slit (Fig. 6) about 62 μm wide. In some females the vulval lips protrude markedly. The vulval striae do not form a distinctive pattern but surround the vulva rather uniformly (Fig. 6). In some females, prolapse of the vaginal walls causes the vulva to widen and the vaginal lining prolapses externally (Fig. 7). Wide muscle bands, the *dilator vulvae,* appear at either end of the vulva underlying the cuticle (Fig. 6). Vulval epitygma were not observed. The vagina extends 42 to 70.5 μm into the body where it joins the well-developed *vagina uterina* (58–70 μm; Fig. 1A). A constriction appears at the junction of the *vagina uterina* and the uterus. The uterus is a large muscular sac (70 × 35 μm) that joins directly with the spermatheca (Fig. 9). It comprises 4 to 5 rows of large cells with a furrow in the center for expansion. The spermatheca is a roughly circular thick-walled chamber 35 to 40 μm in diameter. The oviduct, a thickened area comprised of small cells, lies between the spermatheca and the maturation zone of the ovaries. The ovary is reflexed at the spermatheca and once or twice in the maturation zone area. The cap cell and germinal zone cells are rarely delineated in live or fixed specimens.

MALES (24 specimens, Fig. 3A): Body length = 830.8 (650–1,020) μm; width = 28.9 (25.5–35.5) μm; tail = 9.5 (5–12.7) μm; esophagus = 153.5 (122–188) μm; a = 28.7 (24.3–32.8); b = 5.4 (4.5–6.6); c = 99.2 (59.1–178.6); total stylet length = 25.3 (21.5–27.4) μm; dorsal gland orifice = 4.6 (2–6.8) μm behind the base of the telorhabdions; spicules = 40.4 (36.2–46.6) μm; gubernaculum = 16.2 (14.7–18.6) μm.

ALLOTYPE: Total body length 790 μm; width = 25 μm; tail = 6 μm; esophagus = 140 μm; a = 31.6; b = 5.6; c = 131.7; total stylet length = 22 μm; dorsal gland orifice = 6 μm; spicules = 40 μm; gubernaculum = 15 μm.

MALE DESCRIPTION: Body vermiform, untwisted (Fig. 3A), 6 equidistant lips (Fig. 3B), surround a circular oral disc that stands out clearly in profile. Crescent-shaped amphids appear indistinctly on posterior margin of lateral lips. Labium moderately sclerotized, comprising 4 to 7 labial annules, counting from first reduced annule at onset of cephalic sclerotization. Labium rounded, not set off,

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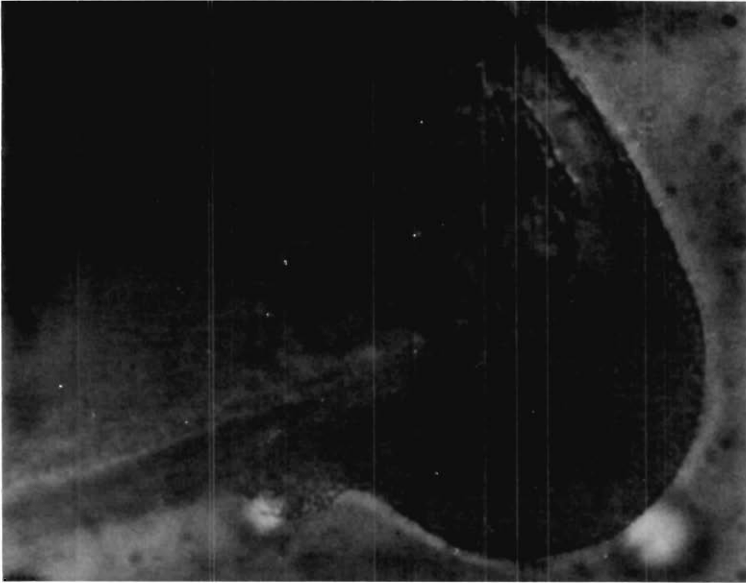

**Figure 5. Excreta exuded from the anus of a mature female.**

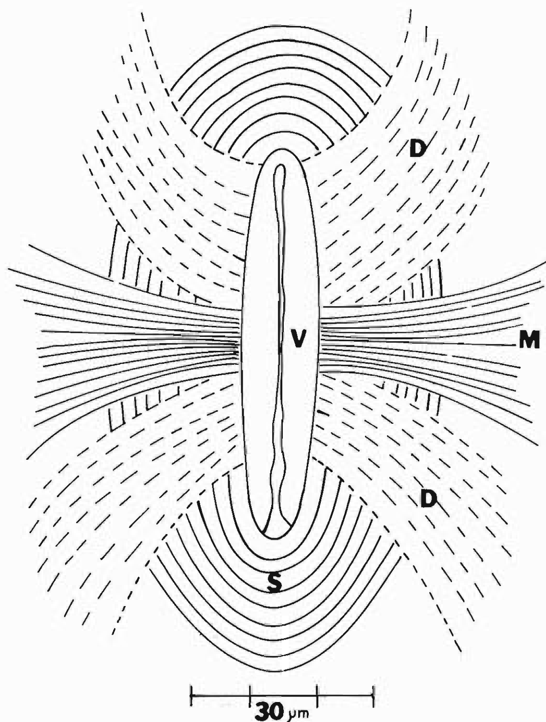


**Figure 6. Vulva and vulva muscles of a mature female. V, vulva lips; S, striae surrounding vulva; D, *dilator vulvae* muscles; M, median *dilator vulvae* muscles (striae cut away to show underlying muscles).**

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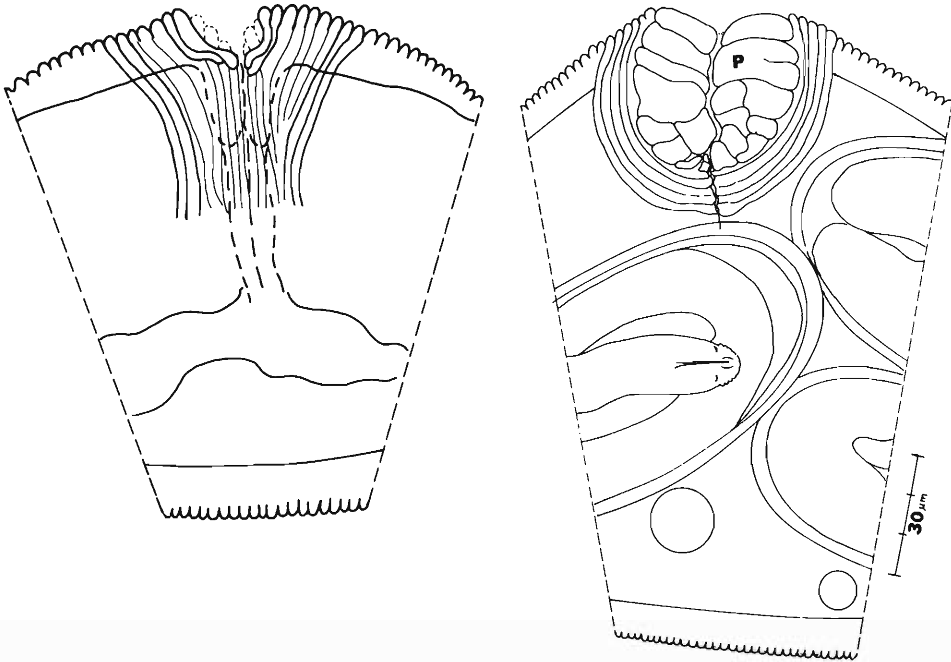


**Figure 7. Lateral views of an unprolapsed (left) and prolapsed (right) vagina of mature females. P, prolapsed vaginal tissue.**

papillae not observed. Body striae about 2 $\mu$m wide, sometimes ending irregularly at the terminus (Fig. 3A). Lateral fields unareolated with 4 lines present, extending from region of corpus to cloacal area. Phasmid not observed. Excretory pore lying in posterior esophageal gland area, 103–146 $\mu$m from oral disc (mean = 124 $\mu$m). Hemizonid 4 $\mu$m long, located just posterior to execretory pore. Stylet typically tylenchoid. Telorhabdions sloping posteriorly. Cheilorhabdions extending through the second to fourth lip annule. Esophagus comprising a moderately swollen procorpus that dilates just prior to oval, distinct metacorpus containing a valve slightly smaller than that of female. A narrow isthmus precedes a single ventral esophageal gland with single nucleus. Cardia not observed. Nerve ring appearing as an irregular band of tissue overlapping isthmus and extending past the esophageal gland about ⅓ of its length (Fig. 3A). Intestine overlapping about ½ of esophageal gland and extending uniformly to cloaca. Tail bluntly hemispherical to truncate; tail terminus annulated. Anal lips in form of tubus (Fig. 23B). Caudal alae absent. Spicules equal and slightly arcuate when seen in lateral view. Capitulum moderately swollen, followed by slight constriction and moderately swollen calomus. Lamina wide at the center tapering at both extremities. Sclerotized piece arising at junction of lamina and calomus, and projecting along ventral wall of calomus. The gubernaculum with teeth on lateral sides of cuneus seen in ventral view when cuneus is situated between spicules. Male gonaduct originating from ventral face of the cloaca. Narrow vas deferens (Fig. 3A) about 90 $\mu$m long is followed by rather long seminal vesicle, usually filled with sperm. Germinal and growth zones sometimes indistinguishable. Testes have been ob-

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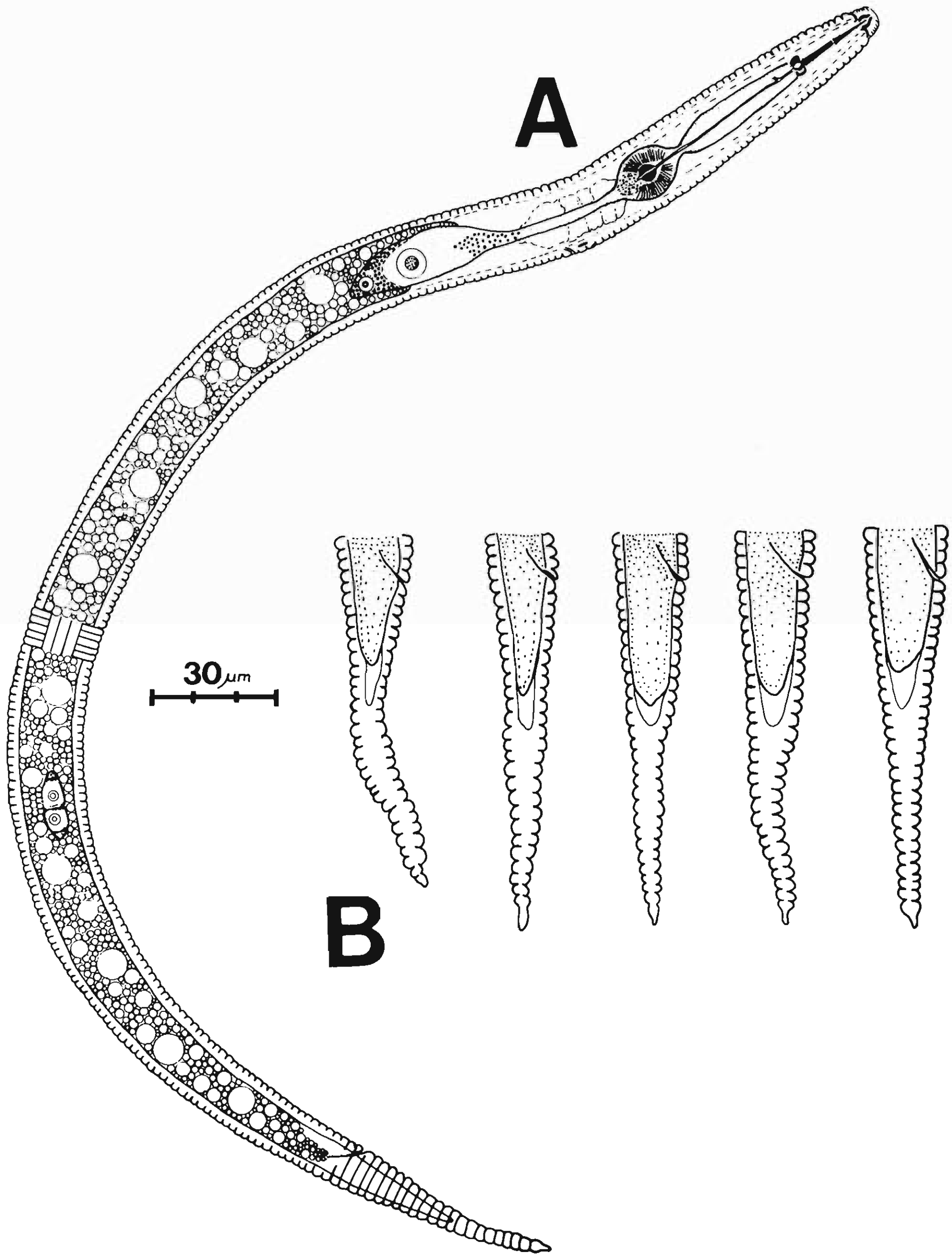


**Figure 8. A. First-stage larva of *Verutus volvingentis* n. gen., n. sp. B. Larval tail shapes.**

served in which the entire tube was filled with sperm and an observable germinal and growth zone was not present. Cephalids and deirids not observed.

Larval description (49 first-stage larvae; Fig. 8A): Length = 492 (430–540) µm; width = 18.5 (16–20.2) µm; tail = 53.6 (46–64) µm; esophagus = 163 (132–190) µm; a = 26.5 (22–30); b = 3.2 (2.6–3.7); c = 9.1 (6.7–10.5); anal body diameter = 4.5 (4.0–5.2) µm; stylet = 23.1 (21.5–24.5) µm; dorsal gland orifice 6.4

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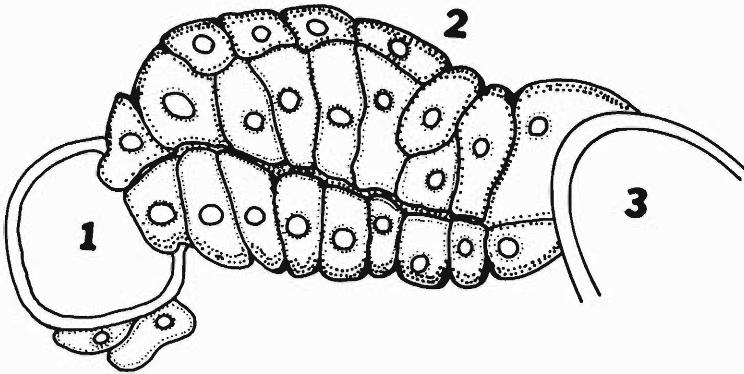


**Figure 9. Uterus of a mature female. 1, spermatheca; 2, uterus; 3, egg.**

(4–8.9) μm posterior to telorhabdions; excretory pore 93.8 (79–103) μm from oral disc.

Body vermiform, labium rounded, cephalic framework consisting of 16 C-shaped sclerotized pieces lying 4 μm below oral disc. Head bearing 6 annules. Four lateral incisures beginning as single line, 45 μm posterior to oral disc forming 4 lines at median procorpus. The 4 lines resolve into a single line just posterior to anus (30 μm from the tail tip). Width of lateral incisures at midbody is 5–7 μm. Excretory pore located in mid-isthmus area. Hemizonid located 1 annule anterior to excretory pore. Stylet well developed, prorhabdion 10.8–12 μm long. Rounded telorhabdions usually lying in an even plane, occasionally sloping posteriorly. Procorpus (38 μm long by 6–7 μm wide), moderately swollen, narrowing just prior to the well-developed metacorpus (15 μm long by 12 μm wide). Meta-

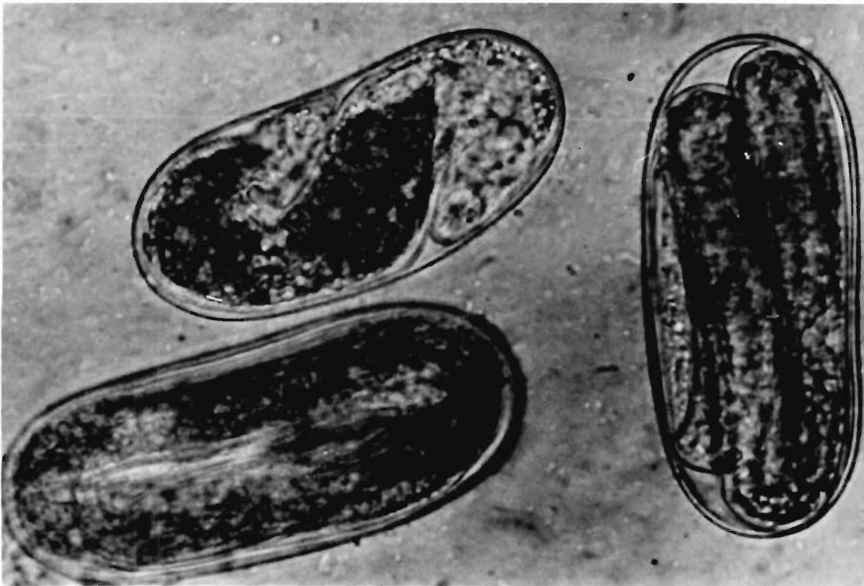

**Figure 10. Ova of *V. volvingentis*.**

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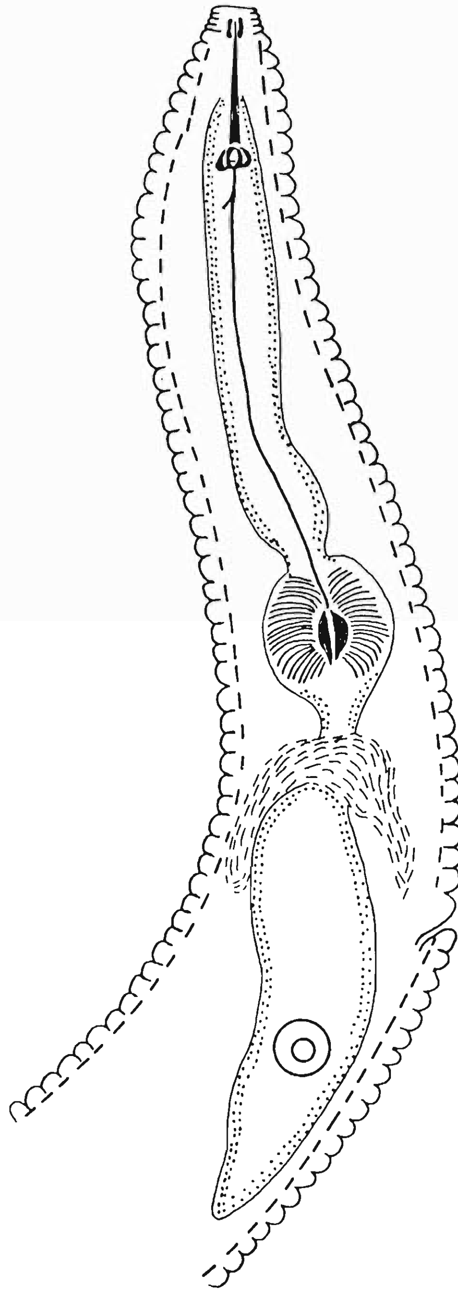

**Figure 11. Anterior portion of third-stage larva.**

corpus valve a wide oval shape. Isthmus narrow 25 μm long. Esophageal glands about 35 μm long, lying on ventral side of body. Posterior lobe sometimes filled with coarse granules (digestive fluid). Coarse granules also appearing in anterior end of anterior lobe, in isthmus and in a large vesicle in posterior part of metacorpus (Fig. 8A). A single large nucleus present in the posterior esophageal gland

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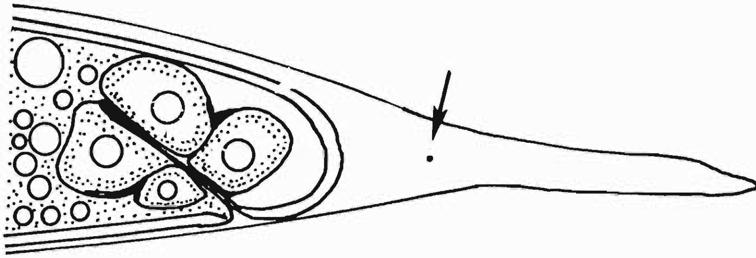

**Figure 12. Spicular primordia cells in cloacal area of an early second-stage male. Phasmid is shown (arrow) on the first-stage exuvia.**

lobe. Anterior esophageal gland lobe not strongly set off. It is delineated by a weak line of demarcation on posterior lobe and has a very large nucleus surrounded by a large clear area (Fig. 8A). Esophageal glands overlapping intestine by about ½ of their length, the intestine extending as a straight tube to undilated rectum. Anus oval, 1 to 2 μm wide. Nerve ring appearing either as group of nerve cells or as fine band of tissue surrounding isthmus (Fig. 8A). Genital primordia appearing about 160 μm anterior to tail tip (Fig. 8A). Tail conoid (Fig. 8B) with 26–29 annules. Tail tip usually awl-shaped. Hyaline area of tail 25.4 (23.5–31.3) μm long. Examination of 400 larvae within eggs and 400 larvae pressed out of eggs failed to reveal any indication of molting. Deirids, phasmids, and cardia not observed.

OVA (Fig. 10): Eggs broadly oval 50 × 100 μm. No markings observed on shell.

THIRD-STAGE LARVAE (10 specimens): Length = 572 (500–677) μm; width = 31.3 (27.4–34.3) μm; tail = 11.1 (9.8–13.7) μm; a = 19.5 (17.3–22.6); c = 54.5 (46.7–63.2); dorsal gland orifice = 3.9 (2.9–6.8) μm; excretory pore = 115.9 (109–122) μm. Body slightly swollen, tail rounded, head and esophagus similar to that of first stage larvae (Fig. 11).

TYPE SPECIMENS: Holotype collected May 1969 by Wayne W. Smith. Collection number B5018; Allotype same data as holotype. Holotype slide deposited in the USDA Nematode Collection, Slide T-339-t, Beltsville, Maryland, USA. Paratype slides in Bureau of Nematology, Division of Plant Industry Nematode Collection, Gainesville, Florida, USA.

TYPE HABITAT: Soil about roots, and roots of *Diodia virginiana* growing near bodies of water.

TYPE LOCALITY: Irrigation ditch bank bordering State Road 50, 4 miles west of U.S. Highway 27 near Clermont, Florida. (Type locality is now commercially developed.)

## Anatomy

PHASMID: Lateral views of over 100 each of males, females, and first-stage larvae were examined for phasmids with negative results. A few specimens of each stage were also examined in ventral views without detecting the phasmid. The phasmid and its lining were only detected on the cast integument of first-stage larvae early in the first molt (Fig. 12). The phasmid was located between the 14th and 18th annules from the tail tip. When its location was known based on molted exuviae fixed and living first-stage larvae were examined to see if the phasmid was detectable; in no case was it observed.

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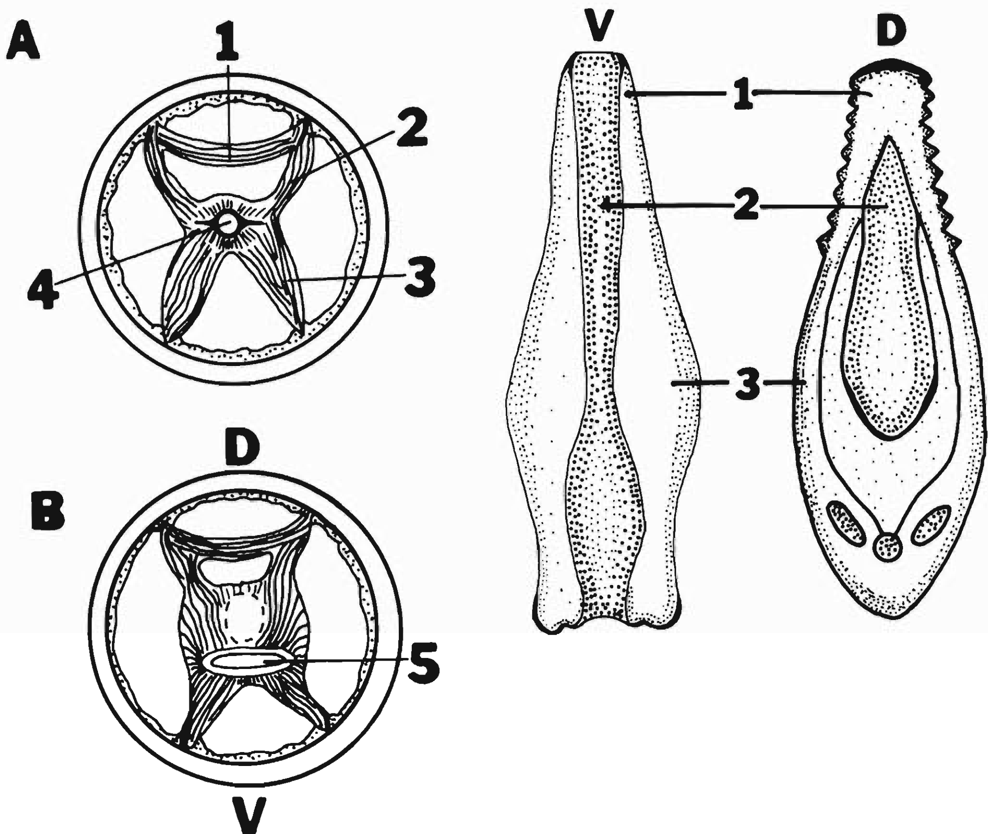


**Figures 13, 14. 13 (left). Rectal musculature. A. Rectal-intestinal valve area: 1, sarcoplasm; 2, *depressor ani*; 3, *dilator ani*; 4, rectal intestinal valve. B. Rectal area: 5, midrectum. D. Dorsal side. V. Ventral side. 14. (right). Gubernaculum (top is anterior). V. Ventral face (4 μm wide by 11 μm long). D. Dorsal face (5 μm wide by 16 μm long). 1, cuneus; 2, corpus; 3, crura.**

## Muscles

VULVA: The *dilator vulvae* musculature are well developed in broad bands in mature females, extending from vaginal epithelium to a ventrolateral insertion in the hypodermis (Fig. 6). A band of muscle also attaches to vagina on either side of median part of vulva, herein called "median *dilator vulvae*" (Fig. 6).

RECTAL MUSCLES: Rectal musculature was observed in a third-stage female (Fig. 13). The H-shaped muscle surrounds the rectum or rectal intestinal valve. The *depressor ani* extends into the dorsal hypodermis, and the *dilator ani* is inserted in ventral hypodermis. A sarcoplasm band nucleus as described by Chitwood and Chitwood (1937) was not observed.

## Nervous system

In males and females stained with chlorazol black-E the circumesophageal commisure appears as a flat band of tissue that surrounds the posterior part of isthmus (Figs. 1A, 3A) and proceeds posteriorly a short distance past the anterior part of the basal bulb as a ventral ganglion. Anterior and posterior nerve cords

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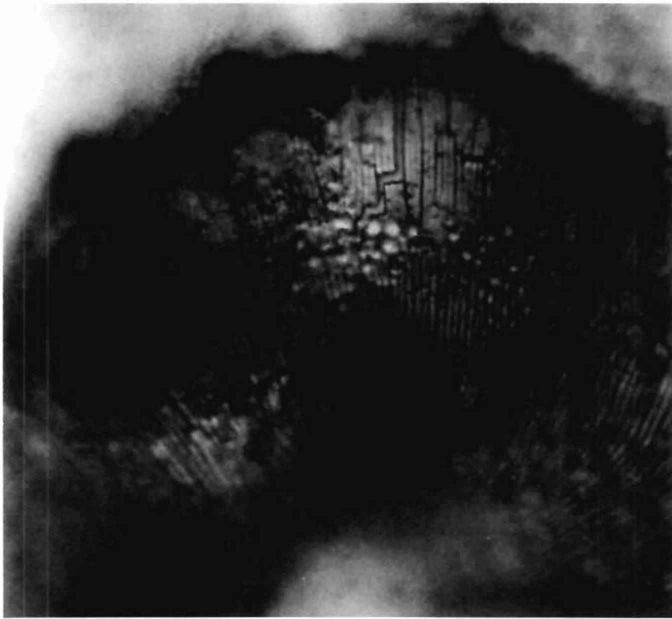

**Figure 15. Crystalline layer at midbody of mature female.**

were not seen. In first-stage larvae stained with chlorazol black-E, the circumesophageal commissure appears looped around the isthmus, either as a flat band of tissue (Fig. 8A) or as an accumulation of nerve cells (Fig. 16). The ventral ganglion proceeds posteriorly a short distance, branching dorsally and ventrally. The dorsal nerve arises from the dorsal portion of the ventral ganglion and becomes indistinguishable a short distance posterior to the esophageal gland. The ventral nerve arises from the ventral portion of the ventral ganglion and proceeds as a chain of ganglia (92 in 1 specimen) in the hypodermis (Fig. 17). The ganglial chain forms rectal commissures that surround the rectum with three dorsal and five ventral ganglia (Fig. 18). A dorsal rectal ganglion (Fig. 18), is present where rectal commissures rejoin postrectally in the dorsal position. Three ganglia are present in the medial caudal nerve (Fig. 18). Anteriorly, a large ganglion arises from the dorsal portion of the nerve ring and one from the ventral side (Fig. 16). The two nerve cords extend around either side of the metacorpus, forming a small mass of nerve cells just anterior to the metacorpus. Dorsal and ventral nerves proceed from this ganglion to sclerotized area of the labium. Cephalic nerves appear as elongate, spindle-shaped processes. Lateral and papillary nerves were not detected.

## Gubernaculum

A gubernaculum was isolated from surrounding tissue for observation of the dorsal and ventral faces (Fig. 14V, D). The dorsal face is longer, measuring 16 $\mu$m, and shows serrated margins on the cuneus (Fig. 14D). The ventral face is shorter (11 $\mu$m), ventrally grooved, and serrations not observed in the focal plane (Fig. 14V).

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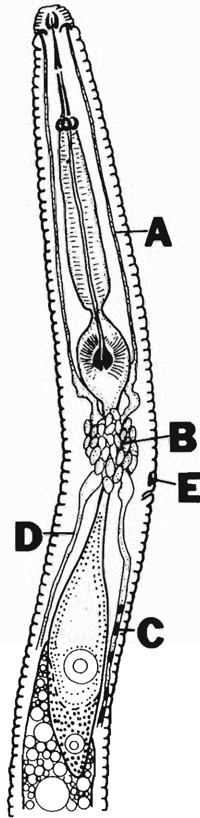


**Figure 16. Anterior nervous system in a first-stage larva. A, anterior ventral nerve cord; B, circum-oral commissure; C, ventral nerve; D, dorsal nerve; e, hemizonid.**

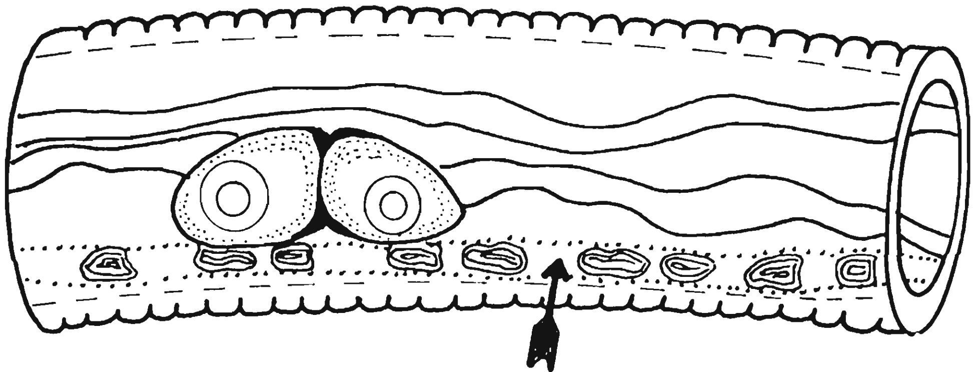

**Figure 17. Ventral nerve cord (arrow) in the area of the genital primordia.**

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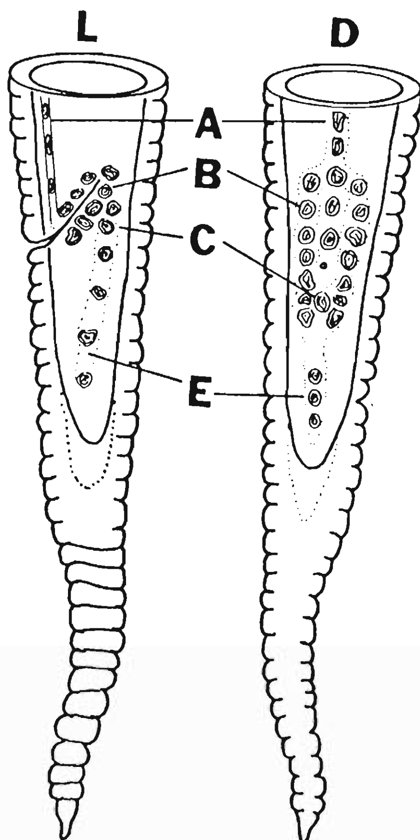


**Figure 18. Nerves in the tail of a first-stage larva. A, ventral ganglion; B, rectal commissure; C, dorsal rectal ganglion; D, dorsal view; E, medial caudal nerve; L, lateral view.**

## Taxonomic Position of the New Genus

### Affinities with subfamilies in the family Heteroderidae

FEMALES: *Verutus* n. gen. differs from known members of the Heteroderinae (Stone, 1977; Wouts, 1973; Wouts and Sher, 1971) and Meloidoderita (Golden, 1976; Poghossian, 1966) in the Meloidoderinae (Wouts, 1973), in lacking a cyst stage and a terminal vulva. It differs from members of the Ataloderinae (Robbins, 1978; Wouts, 1972) and Meloidogyninae (Stone, 1977; Wouts, 1972) in lacking a terminal or subterminal vulva, and a spheroid body (Fig. 19). *Verutus* is most closely related to Meloidoderinae, members of which possess a subequatorial vulva (Fig. 20) and a spheroid or subspheroid body shape. *Verutus* eggs are deposited as they mature (Fig. 21) and are not retained in large numbers within the female as in females of Meloidoderinae (Fig. 20). The body is completely annulated in the subfamilies Meloidoderinae, Meloidogyninae, and Verutinae n. subf. Members of Ataloderinae and Heteroderinae possess an irregular body pattern or lack body markings. In some members of these two subfamilies, annulation may be present on the cervical area or about the vulva but not on the body.

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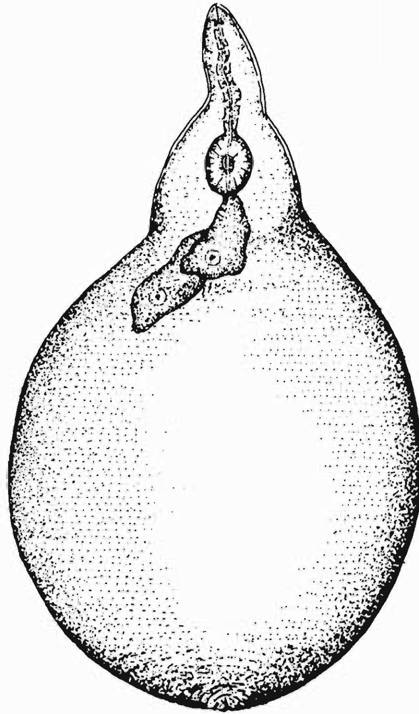

**Figure 19.** ***Meloidogyne*** **sp., showing spheroid shape and terminal vulva of a mature female.**

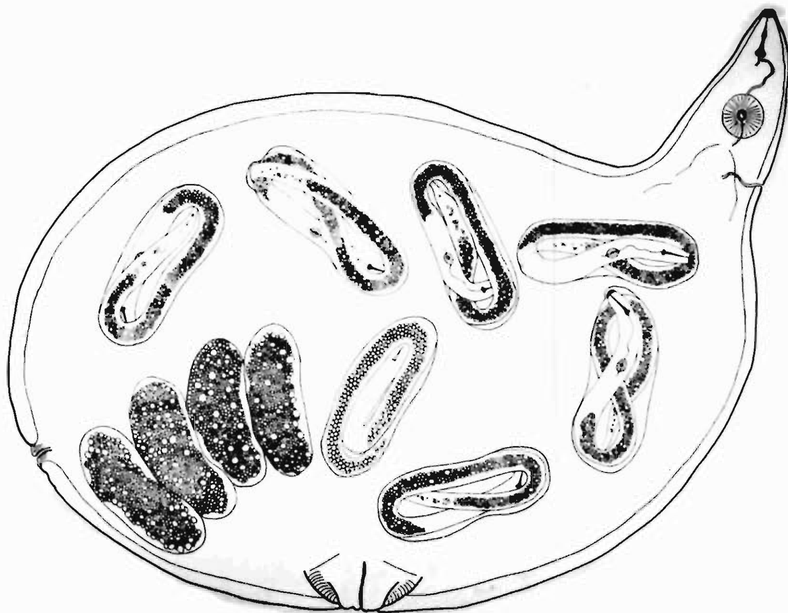

**Figure 20.** ***Meloidodera floridensis*** **Chitwood, Hannon, and Esser, 1956, showing spheroid shape and equatorial vulva position.**

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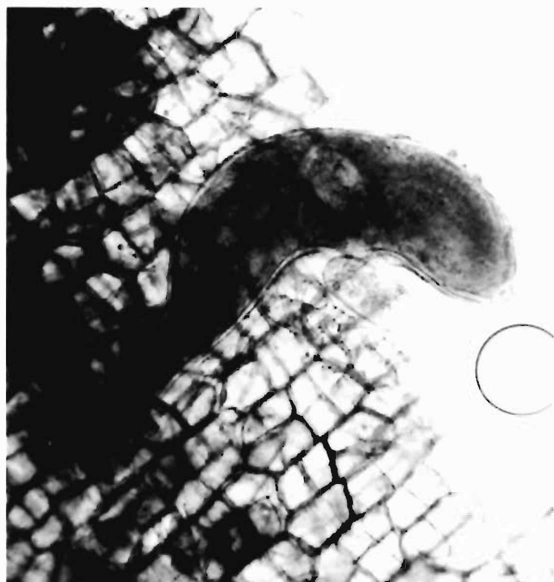

**Figure 21.** ***Verutus volvingentis*** **female in root tissue with a single deposited egg.**

The vulva is widely separated from the anus in the Verutinae (Fig. 1A) and in the genus *Meloidodera* (Chitwood et al., 1956) (Fig. 20) in the Meloidoderinae. In all other subfamilies the vulva is located in the perineal area or near the anus. In some members of Ataloderinae and Heteroderinae the vulva is situated on a papule (Fig. 22). Genera contained in Meloidoderinae and Verutinae lack papules. A labial disc (Fig. 1B) is described only in Ataloderinae, Meloidoderinae, and Verutinae.

The genus *Hylonema* (Luc et al., 1978) was not placed in any subfamily of the

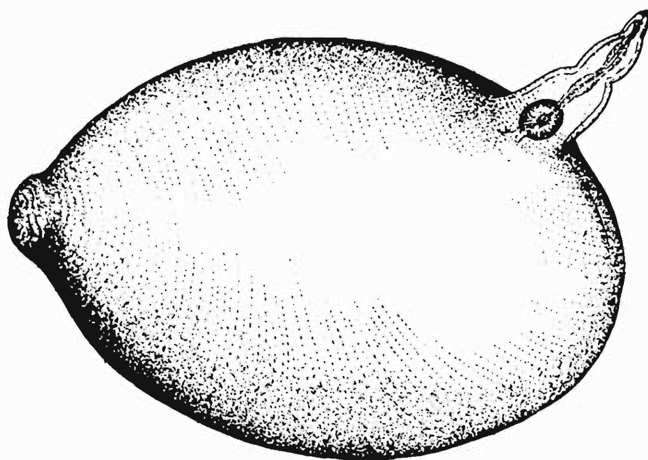

**Figure 22.** ***Hypsoperine graminus*** **Sledge and Golden, 1964. A mature female showing a vulva situated on a papule.**

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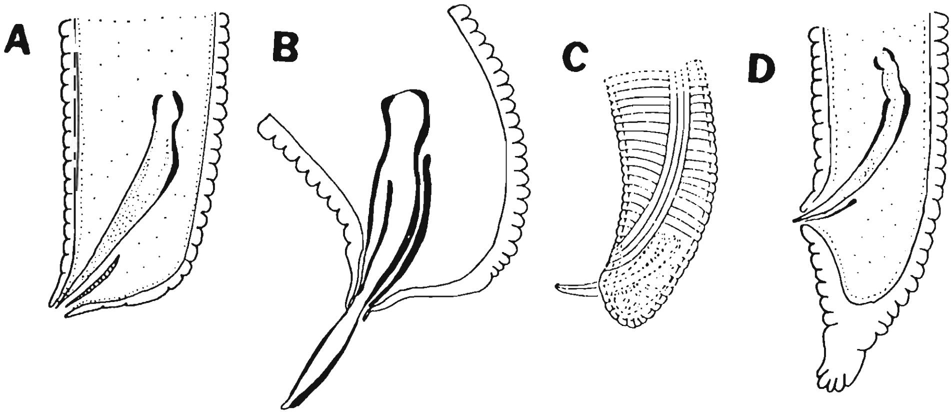


**Figure 23. Heteroderidae male tail types; A, B, truncate with tubus. A, *Sherodera* (redrawn from Wouts and Sher 1973); B, *Verutus*; C, rounded, twisted type. *Meloidodera* (after Hopper 1960); D, blunt conoid. *Meloinema* (redrawn from Choi and Geraert 1973).**

Heteroderidae because the authors felt the genus did not fit comfortably in any existing subfamily and lacked sufficient definitive characters to establish a new subfamily. The body shape of *Hylonema* and *Verutus* is similar. *Hylonema* females differ from *Verutus* in possessing a posterior vulva and an underbridge, and in lacking total body annulation. An attempt was made to utilize the presence of a gelatinous matrix or a subcrystalline layer (Brown et al., 1971) (Fig. 15) in the diagnosis. This was not possible due to lack of data concerning these criteria in many species descriptions. Genera of the Heteroderidae were also compared to *Verutus* on the basis of the alpha ratio (length/greatest body width). This was found to be infeasible due to omitted data and differences in the measurement criteria used by some authors. Some measure the total body length, others (Mulvey and Stone, 1976) exclude the neck and head from the measurement.

MALES: Few definitive differences exist between males in the Heteroderidae. Only the subfamilies Ataloderinae and Verutinae and the genus, *Hylonema,* contain members without a twist in the male body (Fig. 23C). Males of both subfamilies and *Hylonema* also possess a truncate tail terminus, similar spicules and gubernaculum, and a tubus (Fig. 23A, B). Stylet and body length measurements also overlap in both subfamilies. Except for the striated gubernaculum of the genus *Sherodera* (Wouts, 1973) it would be difficult to differentiate between males of Ataloderinae and Verutinae. The only other genus with a truncate tail terminus and tubus is *Sarisodera* in the subfamily Heteroderinae. The *Sarisodera* and *Hylonema* males can be separated from the males of Ataloderinae and Verutinae by the long stylet (38–46 μm). *Meloidoderita* possesses a sharp conoid tail and is the only male in the Heteroderidae with caudal alae. All other genera not included in the aforementioned have rounded or bluntly conoid tails (Fig. 23C) with or without a twist. The only small males have been described in *Meloidoderita* (350–432 μm) and *Meloidodera* (457–505 μm) (Hopper, 1960). Males in the genus *Meloinema* (Choi and Geraert, 1973) stand apart from all other males in possessing a distinct subacutely conoid tail (Fig. 23D).

LARVAE: The larvae of the new genus (Fig. 8A) closely resemble larvae in the

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genus *Hylonema* and the subfamilies Atalodorinae, Meloidoderinae, and all of the larvae in the genera of Heteroderinae (except *Meloidoderella*) (Husain and Kahn, 1967; Kahn, 1972), which has a short stylet (11–16 μm) and reduced telorhabdions. Larvae of the Meloidogyninae differ from *Verutus* for the most part, in having a small stylet, small telorhabdions, and fine body striae. The principal character peculiar to first-stage larvae of the new genus is the absence of a detectable phasmid.

**Affinities with the subfamily Rotylenchulinae Husain & Kahn, 1967 (Husain and Kahn, 1967)**

The females of *Verutus* closely resemble females in Rotylenchulinae. *Verutus* females differ by the absence of a well-defined tail tip, a dorsal gland orifice that originates less than one stylet length from the base of the telorhabdions, possession of a large muscular uterus, and, principally, by the absence of a vermiform, vulvate juvenile female stage.

Verutinae males differ markedly in general appearance from Rotylenchulinae males which have a tapering conoid tail, are usually less than 500 μm long, and assume a C-shaped body position. Rotylenchulinae males possess an elongate, truncate cephalic framework in constrast to the slightly convex, shallow cephalic framework of Verutinae males.

DISCUSSION: The new subfamily Verutinae does not fit within the concepts of the subfamilies of the family, Heteroderidae, or the subfamily Rotylenchulinae. It is therefore proposed as a new subfamily. Subfamilies proposed by Husain (1976) but not included in the analysis are Meloineminae and Meloidoderellinae.

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## CALL FOR PAPERS

The Proceedings needs papers. There is no backlog and publication time for good quality well-written manuscripts is relatively short. Manuscripts in any and all areas of parasitology are welcomed. Material in final form for the January issue is sent to Allen Press in mid-October and that for the July issue in mid-April.

The Editor

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Proc. Helminthol. Soc. Wash.
48(2), 1981, pp. 241–243

*Research Note*

# A Rapid Method for Hatching *Ascaris suum* Eggs In Vitro

Methods available in the literature for hatching eggs of *Ascaris suum* were tested and found to be lengthy or inadequate in providing larvae suitable for cultivation in vitro. A hatching procedure was developed that can rapidly yield aseptic inocula containing large numbers of viable second-stage larvae (L2) without the use of special equipment. The procedure is outlined in Figure 1 and the steps are numbered to correspond to the following descriptive details: (1) Fertilized eggs were isolated and decoated as described by Costello (1961, J. Parasitol. 47:24). Briefly, the anterior 2–3 cm of the uteri of adult females obtained at a local abattoir from freshly killed swine were removed and homogenized in 0.5 N NaOH using a glass Dounce tissue grinder. (2) The resulting suspension of fertilized eggs was washed twice by centrifugation at 200 *g* for 5 min with 0.5 N NaOH, and 5 times in distilled water; this removed the proteinaceous coat. (3) The eggs were embryonated to infective larvae by the methods of Costello et al. (1963, Arch. Biochem. Biophys. 103:345–351), as follows: eggs were suspended in 0.1% formalin, placed in a separatory funnel with continuous aeration from a small pump, and maintained at approximately 25°C for 28–30 days. Larvae pressed free of the eggs between microscope slide and coverslip were identified as first-molt or second-stage from the descriptions of Alicata (Tech. Bull. 489, USDA, Dec. 1935, p. 46). (3a) Infective eggs were collected and stored in 0.1% formalin at 4°C until use. (4) For hatching, the infective eggs were washed 5 times by centrifugation of a 40-ml suspension in 0.85% NaCl at 200 *g* for 1 min with no braking of the rotor. (A packed egg volume of 0.5 to 1.0 ml yields approximately $5 \times 10^5$ to $1 \times 10^6$ viable larvae at the end of the hatching procedure.) The pellet of eggs was resuspended in 20 ml of a solution of 5.25% sodium hypochlorite (undiluted commercial Clorox[1]) at 37°C for 3 min followed by 10 washes in 0.85% NaCl. (4a) This procedure, adapted from Haskins and Weinstein (1957, J. Parasitol. 43:28–32) and Magat et al. (1972, Exp. Parasitol. 32:102–108), removed most of the chitinous layer from the eggs. (5) The hypochlorite-treated eggs were suspended aseptically in 4–8 ml of Earle's balanced salt solution without phenol red (EBSS) and then transferred to a flat-bottom 125-ml flask containing a layer of glass beads (4–6 mm diameter) and a spinner bar (Cleeland and Laurence, 1962, J. Parasitol. 48:35–38; Stromberg and Soulsby, 1976, Vet. Parasitol. 2:197–208). The flask was maintained in an incubator at 37°C over a magnetic stirrer which slowly moved the beads for 30–45 min. The extent of hatching was determined microscopically. Generally, larvae hatched from 80–90% of the eggs and only a small percentage of these larvae were damaged. (6) The hatched larval suspension was then transferred to a centrifuge tube and washed (as previously described) 3 times in EBSS at 37°C to remove the egg debris. (7) The larval

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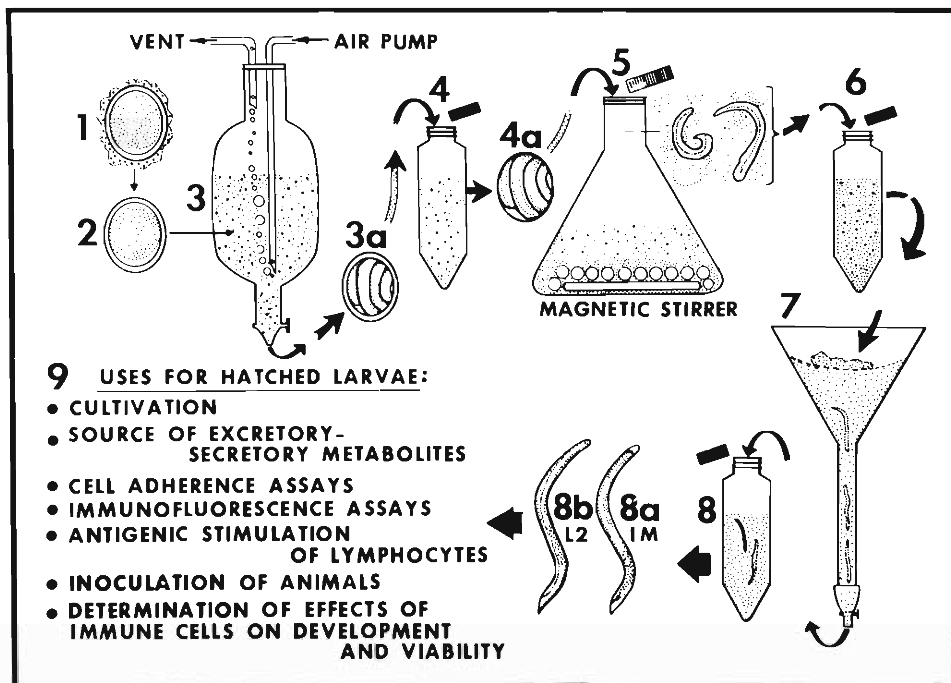


**Figure 1. Scheme for development of infective *Ascaris suum* eggs and hatching procedure: (1) fertilized egg, (2) proteinaceous coat removed; (3) embryonation of eggs in aerated vessel; (3a) infective egg; (4) hypochlorite treatment and washing method; (4a) chitinous layer removed; (5) hatching method using glass beads and magnetic stirrer; (6) removal of egg debris; (7) filtration through cotton; (8) aseptic washing procedure; (8a) first-molt larvae (1M); (8b) second-stage larvae (L2); (9) list of uses for larvae.**

suspension was layered over a pad of cotton gauze (dry weight of 2.5 g) immersed in EBSS in a 10-cm covered funnel, and maintained for 1.5–2 hr at 37°C in an incubator (Magat et al., 1972, loc. cit.). (8) Larvae which settled in the neck of the funnel were collected aseptically in a 5-ml aliquot, and washed 10 times with warm sterile Dulbecco's phosphate buffered saline. (8b) The washed larval suspension consisted of approximately 85–90% L2-larvae; (8a) the remainder were first-molt larvae. (9) More than 95% of the larvae obtained by this procedure were intact and motile, and suitable for a variety of uses in vitro or in vivo.

In our laboratories, larvae from this source have been used as inocula for large scale cultivation in a roller-bottle system (more than 40,000 larvae per bottle) and in stationary multi-well plates containing host immune cells to study both larval development and the in vitro effects of immune cells on development. The larvae stimulate a specific blastogenic response in immune lymphocytes obtained from *A. suum* infected swine in vitro (unpublished observation). The larvae have been used in immunofluorescence assays, in cell adherence assays, as a source of excretory-secretory products or extracted products, and for inoculation of swine and laboratory animals.

This procedure consistently provides large numbers of viable larvae, free of microbial contamination, in less than 4 hr. Antimycotics are not required in the

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culture media, and only prophylactic levels of antibiotics (penicillin 100 units/ml and streptomycin 100 $\mu$g/ml) are added during cultivation.

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*Research Note*

# Intraspecific and Interspecific Pairing of *Echinostoma revolutum* (Trematoda) and *Zygocotyle lunata* (Trematoda) Adults In Vitro

Intraspecific pairing of trematodes in vitro has been reported for three species: *Echinostoma revolutum* (Fried et al., 1980, J. Parasitol. 66:1014–1018), *Leucochloridiomorpha constantiae* (Fried and Roberts, 1972, J. Parasitol. 58:88–91), and *Schistosoma mansoni* (Imperia et al., 1980, J. Parasitol. 66:682–684). Interspecific studies were reported by Fried and Jacobs (1980, Proc. Helminthol. Soc. Wash. 47:136–138) who examined pairing between *E. revolutum* and *Zygocotyle lunata* or *L. constantiae*. Observations on intraspecific and interspecific pairing in the aforementioned studies were made at 0.5- to 1-hr intervals up to 24 hr. The present study examines intraspecific and interspecific pairing of *E. revolutum* and *Z. lunata* at 5-min intervals up to 1 hr.

*Echinostoma revolutum* and *Z. lunata* adults, 14 to 21 days old, obtained from experimentally infected domestic chicks (Fried and Weaver, 1969, Proc. Helminthol. Soc. Wash. 36:153–155; Fried, 1970, J. Parasitol. 56:44–47) were washed in three changes of sterile Locke's solution, and used for behavior studies within 0.5 hr. Petri dishes, 3.5 cm diameter, with an agar substrate and a Locke's overlay (Fried and Roberts, 1972, loc. cit.) were used to study pairing, and two worms were placed 1 cm apart in each dish. Behavior was studied for up to 1 hr at 5-min intervals by plotting worm movements on graph paper. Measurements were made in millimeters from the miduterus of *E. revolutum* to the center of the body of *Z. lunata,* and worms within 7.5 mm of each other were considered paired, since worms within this range showed increased body movements compared with worms maintained singly or nonpaired worms. The following in vitro pairing experiments were done: *E. revolutum* vs. *E. revolutum* (26 trials); *Z. lunata* vs. *Z. lunata* (25 trials); and *E. revolutum* vs. *Z. lunata* (52 trials). Trials were

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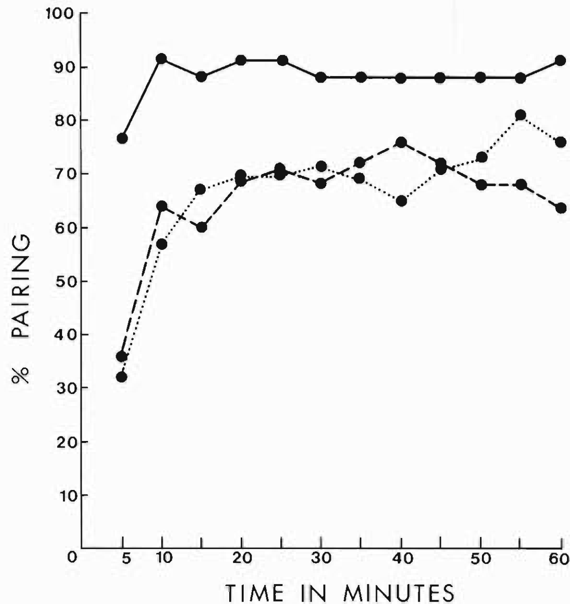


**Figure 1.** **Summary of pairing data. Solid line = *Echinostoma revolutum* intraspecific studies; broken line = *Zygocotyle lunata* intraspecific studies; dotted line = interspecific studies.**

performed in a controlled environment room at 37.5 ± 1°C under subdued light and all worms were alive at the end of experiments.

Worms showed no tendency to pair with dead worms or inert objects placed in petri dishes as discussed previously (Fried and Roberts, 1972, loc. cit.). Moreover, based on computer simulation, the probability that two randomly selected points in the petri dish were within 7.5 mm of each other is approximately 0.15 (Wagner, 1975, Principles of Operations Research, Prentice Hall, New Jersey). Results of the pairing experiments at 5-min intervals are presented in Figure 1. Chi square tests showed that by 10 min pairing was highly significant ($P < 0.002$) in all three protocols. In intraspecific studies, *E. revolutum* paired more rapidly than *Z. lunata*. For example, 77% of the *E. revolutum* paired within 5 min, compared to only 36% for *Z. lunata* (Fig. 1). The frequency of intraspecific pairing of *E. revolutum* within 1 hr (96%) was greater than that of *Z. lunata* (84%). Of those *E. revolutum* that paired, only 8% separated within the hour, compared to 38% for *Z. lunata*.

Interspecific pairing occurred at a rate similar to that for intraspecific pairing of *Z. lunata* (Fig. 1). The frequency of interspecific pairing (90%) was equal to the average of the two intraspecific frequencies. Worms that paired interspecifically, later separated more frequently (26%) than *E. revolutum* pairs (8%), but less frequently than *Z. lunata* pairs (38%).

The results of this study indicate that *E. revolutum* and *Z. lunata* are capable of interspecific pairing. Free sterols are involved in intraspecific pairing of *E. revolutum* (Fried et al., 1980, loc. cit.). Unpublished studies in our laboratory indicate that free sterols are the major lipophilic excretory-secretory products of

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*Z. lunata* adults. Free sterols may serve as chemoattractants for interspecific pairing of *Z. lunata* and *E. revolutum*.

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***Research Note***

## Prevalence of *Tanaisia zarudnyi* (Trematoda: Eucotylidae) from Ruffed Grouse (*Bonasa umbellus*) in Two Wisconsin Counties

From 1970 through the 1980 grouse hunting season 146 ruffed grouse from a southern county (Richland) and a central county (Portage) in Wisconsin, were examined for the presence of *Tanaisia zarudnyi*.

Grouse kidneys and ureters were removed, placed in avian saline, and dissected under a binocular microscope. *Tanaisia zarudnyi* obtained were flattened using coverslip pressure, fixed in AFA, stained with paracarmine with fast green as a counterstain and mounted in permount.

One of 65 birds from Richland Co. harbored seven gravid *T. zarudnyi*; 21 of 81 birds from Portage Co. were infected with from one to 30 gravid *T. zarudnyi*. There was no significant difference in the worm burdens between males and females.

Kingston (1965, Can. J. Zool. 43:953–969) found 19 of 184 grouse, collected over a 7-year period from Michigan and Ontario, parasitized with *T. zarudnyi*. Over an 8-year period Davidson, Doster, Pursglove, and Prestwood (1977, Proc. Helminthol. Soc. Wash. 44:156–161), in a survey of ruffed grouse parasites from six states (Georgia, Kentucky, Maine, Michigan, New York, and West Virginia), found three of 80 birds infected from two northern Michigan counties.

One possible reason for the lower prevalence in Richland Co. than in Portage Co. might involve intermediate host distribution. Kingston (1965, op. cit.) in his life cycle study of *T. zarudnyi* from ruffed grouse, was able to infect *Angiuspira alternata* and *Succinea ovalis* with miracidia experimentally. Though both species have been reported from Wisconsin by Levi and Levi (1950, The Nautilus, 63:131–138) it is possible that the upland ridges characteristic of Richland Co., where birds were collected, are less suitable land snail habitat than the lowlands and marsh edges where the Portage Co. collections were made. Mueller (1941, Proc. Helminthol. Soc. Wash. 8:14–15), in his survey of ruffed grouse helminths from New Hamshire, stated that grouse in swampy or lowland habitats tend to have more trematodes than grouse from drier localities.

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Representative specimens of three gravid *T. zarudnyi* have been deposited in the National Parasite Collection, Beltsville, Maryland, as USNM Helm. Coll. 763555.

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***Research Note***

## The Influence of Immunosuppressants and Antagonists of Biogenic Amines on the Development of *Ancylostoma caninum* in Mice

Efforts to develop *Ancylostoma caninum* in mice and other laboratory hosts have been mostly unsuccessful (Soh, 1958, J. Parasitol. 44:515–519; Sen, Joshi, and Seth, 1965, Trans. R. Soc. Trop. Med. Hyg. 59:684–689). However, the development of other species of hookworms such as *Ancylostoma ceylanicum* has been achieved in mice following the administration of hydrocortisone acetate (Ray, Bhopale, and Srivastava, 1975, Parasitology 71:193–197). But, *A. caninum* could not be established in mice even after the administration of large doses of cortisone (Sen, Joshi, and Seth, 1965, loc. cit.). In the present experiment the development of *A. caninum* in mice treated with immunosuppressants and antagonists of biogenic amines has been achieved.

*Ancylostoma caninum* larvae were obtained from fecal cultures of infected dogs and each mouse was infected with 500 third-stage infective larvae per os. Eighty albino mice (Swiss strain), weighing 20–24 g, were divided into four groups of 20 each and treated as follows: group I (cyprohepatadine), group II (mepyramine maleate), group III (mepyramine maleate and prednisolone), and group IV (untreated infected control). The schedule, dosage and frequency of administration of the drugs used are given in Table 1. The first dose of the drug was given on the day of infection and subsequently at biweekly intervals until the end of the experiment. Control animals were given an equal volume of saline through the same route. The number of worms recovered from the small intestine of mice and the growth rate at different intervals (7, 14, 21, and 28 days postinfection) is shown in Tables 1 and 2. Significantly more worms were recovered from the group which had received a combination of drugs. The worms reached sexual maturity by day 21. The buccal capsule, oesophagus, and male and female reproductive organs were well developed. An interesting observation was the presence of fully developed eggs in the female worms which were, however, not fertile. Eggs were first detected in the feces of infected mice on days 21 and 28 after infection but the counts in general were low.

It is clear from this study that the administration of immunosuppressants and antagonists of biogenic amines significantly increased the retention of the worms

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**Table 1. Recovery of *A. caninum* from the small intestine of mice in treated and control group at different intervals after infection with 500 larvae.**

| Group | Drug | Dose/route | | Mean worm counts, Days after infection: 7 | 14 | 21 | 28 |
|---|---|---|---|---|---|---|---|
| I | Cyprohepatadine | 10 mg/kg (oral) | Biweekly | 189.00 ± 7.74 | 58.25 ± 5.79 | 48.50 ± 2.45 | 28.00 ± 1.18 |
| II | Mepyramine maleate | 15 mg/kg (s/c) | Biweekly | 169.00 ± 486 | 39.80 ± 2.31 | 26.00 ± 2.12 | 16.00 ± 2.21 |
| III | Mepyramine maleate and prednisolone | 15 mg/kg (s/c)<br>75 mg/kg (I/M) | Biweekly<br>Biweekly | 251.16 ± 7.42 | 68.40 ± 2.70 | 43.80 ± 2.77 | 36.40 ± 1.72 |
| IV | Untreated control | — | — | 0 | 0 | 0 | 0 |

in mice. Mepyramine maleate is a potent antihistaminic agent (Bovet, Horelois, and Ialtnert, 1944, C.R. Soc. Biol. 138:99–110) and cyprohepatadine is a powerful antagonist of both histamine and serotonin (Steve, Wenger, Ludden, Stavorski, and Ross, 1961, J. Pharm. Exp. Ther. 131:73–74). It is likely that hookworm larvae when given orally may produce local tissue damage to intestinal mucosa and enterochromaffin cells which contain a high level of histamine and serotonin thus resulting in the release of these two amines. A significant increase in the intestinal mast cells and intestinal histamine levels corresponds to the disappearance of the worms from the small intestine of mice (Vardhani and Johri, 1978, Indian J. Parasitol. 2:55–59). Administration of the drug causes the suppression of rejection of worms possibly by antagonizing the biogenic amines which are released during this process. Cyprohepatadine inhibited the migration more effectively than mepyramine maleate. Since this drug has both antihistaminic and antiserotonic effects, it is likely that both these amines are involved in the rejection of the worms. Similarly in the group treated with mepyramine maleate and prednisolone, the retention of the worms was significantly prolonged. The mech-

**Table 2. The growth rate of *A. caninum* in mice infected with 500 larvae each and treated with immunosuppressants and antagonists of biogenic amines.**

| Age of worms (days) | Cyprohepatadine Range (mean) (mm) Male | Female | Mepyramine maleate Range (mean) (mm) Male | Female | Mepyramine maleate and prednisolone Range (mean) (mm) Male | Female | Untreated infected control Range (mean) (mm) Male | Female |
|---|---|---|---|---|---|---|---|---|
| 7 | 1.50–2.20 (1.80) | | 1.4–2.2 (1.7) | | 1.40–2.30 (1.9) | | 0 | |
| 14 | 1.60–2.5 (2.40) | 1.9–2.8 (2.3) | 1.65–2.56 (2.20) | 1.85–2.7 (2.2) | 1.7–2.4 (2.2) | 1.8–2.75) (2.4) | 0 | |
| 21 | 2.2–2.6 (2.40) | 2.8–3.3 (3.00) | 2.2–2.50 (2.45) | 2.8–3.1 (2.9) | 2.24–2.65 (2.46) | 2.7–3.30 (3.46) | 0 | |
| 28 | 2.3–2.7 (2.45) | 3.2–3.7 (3.50) | 2.45–2.85 (2.56) | 3.0–3.5 (3.4) | 2.4–2.45 (2.60) | 3.0–3.70 (3.47) | 0 | |

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anism of action of prednisolone is not clear but the administration of the drug may have reduced inflammation and the tissue reaction and thus reduced the host resistance.

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*Research Note*

## Inactivity of Phenacetin as a Fasciolicide in Sheep

Unavailability of an effective fasciolicide in the United States is assuming greater importance as outbreaks and chronic infections of fascioliasis occur increasingly in cattle and sheep. Albendazole alone is available, and only on an investigational new animal drug (INAD) basis.

Recent work in the USSR by Kolesnikov (1977, Sib. Nauchno Issled. Vet. Inst. 28:143–146) and Dol'nikov et al. (1980, Veterinariya 57:34–35) which reported >90% efficacy of phenacetin (dose rate 2 × 75 mg/kg) against *Fasciola hepatica* in sheep with natural and experimental infections is of immediate interest. However, Harfenist (1973, Pestic. Sci. 4:871–882) found phenacetin to be ineffective against *F. gigantica* in mice. Since this drug is widely available and because the published reports are at variance, a study of the efficacy, toxicity, and pharmacokinetic properties of phenacetin as a fasciolicide under an experimental protocol as described by Dol'nikov et al., was considered desirable. The results of this study are reported here.

Thirty Polled Dorset lambs (6 to 8 months old) were allocated on the basis of body weights into four groups comprised as follows: Group 1, 10 lambs; Group 2, 10 lambs; Group 3, 3 lambs; and Group 4, 7 lambs. The lambs were kept in raised pens indoors and fed pelleted feed throughout the experiment. Each lamb was inoculated with 100 *F. hepatica* cysts on filter paper in gelatin capsules. Viability of the cysts was determined by microscopic examination for movement and flickering of flame cells. All lambs were weighed and 10-ml blood samples collected every 2 weeks. Packed cell volumes (PCV) were determined by heparinized microhematocrit tubes. Blood was allowed to clot at 25°C for 1 hr, and centrifuged at 3,000 × *g* for 15 min (4°C). Serum was immediately assayed for gamma-glutamyl transpeptidase (GGT) activity spectrophotometrically.[1] Blood was collected at 1- or 2-hr intervals for 8 hr after treatment. Serum was filtered and frozen for later extraction and liquid chromatographic analysis for phenacetin

[1] Clinicard Analyzer, Model 368, Instrumentation Laboratory, Inc., Lexington, Massachusetts.

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**Table 1. Experimental protocol and average numbers (SD) of *Fasciola hepatica* recovered from lambs posttreatment with phenacetin.**

| Group no. | No. lambs/ group | Dose rate mg/kg | Treatment time (weeks PI) | Ave. no. (SD) of flukes |
|---|---|---|---|---|
| 1 | 10 | 2 × 75 | 3 | 40 ± 18 |
| 2 | 10 | 2 × 75 | 12 | 45 ± 12 |
| 3 | 3 | 1 × 200 | 14 | 38 ± 5 |
| 4 | 7 | None | — | 41 ± 19 |

concentration as previously described for diamfenetide, except diamfenetide was used as the internal standard (Rew et al., 1980, Int. Goat Sheep Res. 1:96–103).

Phenacetin[2] was given in gelatin capsules. Groups 1 and 2 were treated 3 and 12 weeks postinoculation (PI), respectively, at a dose rate of 2 × 75 mg/kg, administered on subsequent days. Group 3 was treated with a single dose at a rate of 200 mg/kg 14 weeks PI. Group 4 comprised untreated controls.

Twenty-seven lambs (Groups 1, 2, and 4) were necropsied 13 weeks PI and 3 lambs (Group 3) were necropsied 15 weeks PI. Liver damage was assessed by gross examination only. Bile ducts were slit and livers sliced to recover flukes which were placed in saline and counted.

Numbers of recovered flukes are shown in Table 1. No differences were found in averages of numbers of flukes, lamb weight gains, PCV, or serum GGT levels among the groups.

The toxicity of a single oral dose of phenacetin at a dose rate of 300 mg/kg was examined in a single sheep in a separate test. A marked torpor was seen within 2 hr of treatment which lasted for several hours, and disappeared within 12 hr. This sheep began showing signs of wool and weight loss, light sensitivity, and inflammation around mucous membranes approximately 2 weeks later. Serum GGT, serum aspartate aminotransferase (SGOT) and serum bilirubin was assayed[1] to assess liver function and were found to be elevated above levels of a matched control sheep: GGT elevated 9×, SGOT elevated 5×, and bilirubin elevated 16×. The sheep was necropsied 1 week later (3 weeks posttreatment) and formalin-fixed sections of internal organs were examined for pathological changes. The most striking pathological changes were seen in the liver and spleen. Large accumulations of iron pigments (probably hemosiderin) supposedly resulting from methoglobin or "sulfhemoglobin" formation (The Pharmacological Basis of Therapeutics, Macmillan Co., pp. 332–335) were seen in the spleen and liver. A marked extramedullary hematopoiesis was also present in the spleen, apparently as a compensatory response.

Phenacetin was extracted from filtered serum, injected onto a μBondapak C-18 column with 30% acetonitrile as the mobile phase and detected at 254 nm in a uv detector. The $K'_1$ for phenacetin was 4.75, $K'_2$ for the internal standard diamfenetide was 5.68 and $\alpha$ was 1.20 ($K'_1 = (V_1 - V_0)/V_0$ and $K'_2 = (V_2 - V_0)/V_0$

[2] Phenacetin (Acetophenetidine), Sigma Chemical Co., St. Louis, Missouri. Mention of a trademark, proprietary products, or vendor 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 or vendors that may also be suitable.

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where $V_0$ = void volume; $V_1$ = volume to move peak 1 through column; $V_2$ = volume to move peak 2 through column; $\alpha = K'_1/K'_2$).

Serum phenacetin concentration was determined at 1, 2, 4, 6, and 8 hr following treatment with 75 mg/kg. Serum phenacetin levels increased to 100 nmoles/ml at 1 hr, peaked at 170 nmoles/ml at 2 hr, dropped to 100 nmoles/ml by 4 hr and 30 nmoles/ml by 6 hr. Eight hours following treatment phenacetin levels were barely detectable.

In a screening program for fasciolicides effective against immature flukes conducted at Burroughs-Wellcome (Harfenist, 1973, loc. cit.), a series of additions and substitutions of phenacetin were shown to be highly effective, culminating in the development of diamfenetide. Interestingly, phenacetin itself showed no activity against *F. gigantica* in mice. Subsequently, Kolesnikov (1977, loc. cit.) found that phenacetin was not effective against *F. hepatica* in rabbits, but continued trials in sheep because the drug was tasteless and odorless, characteristics he desired in a feed additive. Kolesnikov (1977, loc. cit.) and Dol'nikov et al. (1980, loc. cit.) reported phenacetin to be highly effective against *F. hepatica* in sheep.

Attempts to repeat the results of Kolesnikov and Dol'nikov et al. in our laboratory have been unsuccessful even though we attempted to repeat their protocols as closely as possible. Our results indicate that phenacetin is completely ineffective as a fasciolicide in sheep at 2 × 75 mg/kg at 3 or 12 weeks PI or 200 mg/kg at 14 weeks PI. An additional discrepancy arises between our work and that of Dol'nikov et al. (1980, loc. cit.) in that we found toxicity at a level of 300 mg/kg, whereas they reported toxicity only at levels exceeding 600 mg/kg.

Recently, we have accumulated evidence that length of time of exposure to a drug is very important in the fasciolicidal activity of various compounds (Rew et al., 1980, loc. cit.; Rew and Knight, 1980, J. Am. Vet. Med. Assoc. 179:1353–1354). The rapid turnover of serum phenacetin as compared to serum diamfenetide (Rew et al., 1980, loc. cit.) may partially explain the difference in efficacy of these two structurally related compounds.

In an attempt to explain the differences in efficacy and toxicity found between this study and previous studies referred to, we should consider that though the breeds of sheep were not given in the other paper and presumably would be different from Polled Dorset, and the strain of parasite is different and could be affected differently, the study herein suggests the possibility that a drug other than phenacetin was used in the test by Dol'nikov et al.

We would like to thank Dr. R. Fetterer and Mr. Herb Haines for assistance throughout the study. We would like to thank Dr. John Strandberg for evaluation of the fixed tissues.

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*Research Note*

# Pheromonal Response of *Echinostoma revolutum* in the Absence of Worm-Tactile Behavior

Pheromonal attraction in the absence of worm-tactile behavior has been described for three species of trematodes: *Leucochloridiomorpha constantiae* (Fried and Gioscia, 1976, J. Parasitol. 62:326–327), *Echinostoma revolutum* (Fried et al., 1980, J. Parasitol. 66:1014–1018), and *Schistosoma mansoni* (Imperia et al., 1980, J. Parasitol. 66:682–684). Little information is available on pheromonal response in trematodes, but Imperia et al. (1980, loc. cit.) found that one male of *S. mansoni* was more effective in attracting a female in vitro than three males. The present study examines pheromonal response of *E. revolutum* adults in the absence of worm-tactile behavior. The design used in this study is a modification of that presented by Imperia et al. (1980, loc. cit).

Adult *E. revolutum*, 2–3 weeks old, were removed from experimentally infected domestic chicks, rinsed in three changes of sterile Locke's nonnutrient salt solution and used within 0.5 hr. Agar-Locke's petri dish cultures (Fried and Roberts, 1972, J. Parasitol. 58:88–91) with cylindrical stainless steel screen chimneys to eliminate worm-tactile contact were used to study worm behavior.

Steel screen (40-mesh) was cut into 2 cm × 1.5 cm rectangles and bent into cylinders 1.5 cm in height. Two chimneys were placed in agar at either end of a 3.5 cm petri dish (Fig. 1). A sterile Locke's overlay was added then, filling the dish. In one chimney 2, 4, or 6 *E. revolutum* adults were placed and the other

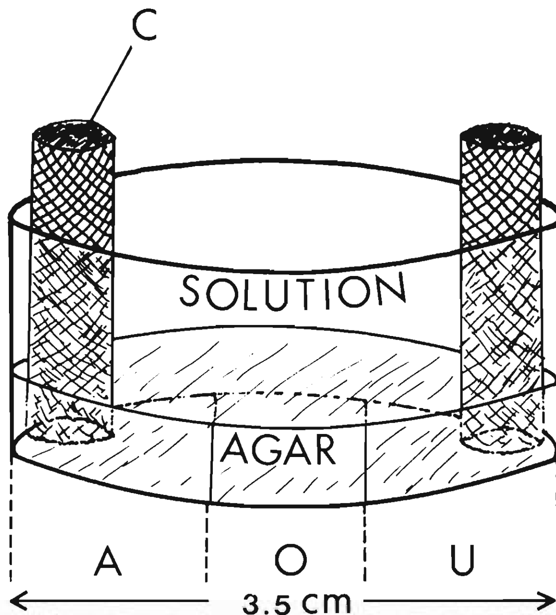


**Figure 1.** **Petri dish chamber used in pheromonal studies. C = chimney; A = attraction zone; O = neutral zone; U = unattraction zone.**



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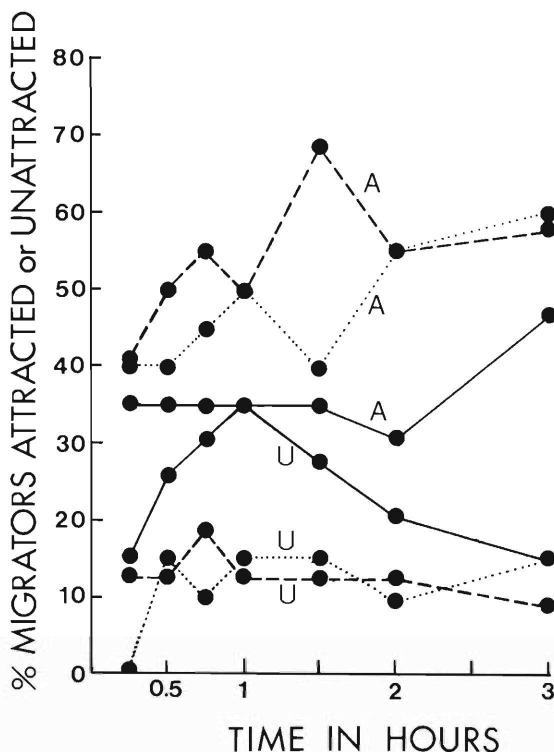


**Figure 2. Summary of migrators attracted (A) or unattracted (U) to releasers in chimney. Solid line = two releasers in chimney; broken line = four releasers in chimney; and dotted line = six releasers in chimney.**

left empty as a control. Then one adult *E. revolutum* was placed midway between the two chimneys after 5 min to allow for chemical diffusion. The worms within the barrier were designated "releasers" and those outside the barrier, "migrators" (Belosevic and Dick, 1980, J. Parasitol. 66:88–93). All studies were performed in a controlled environment room at 37.5 ± 1°C under subdued light. Movements of the migrator worms were recorded at 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 hr. A 1-cm neutral zone (O) was drawn midway between the two chimneys (Fig. 1) and worms at either side were considered attracted (A) or unattracted (U). Worms in the neutral zone were considered neither attracted nor unattracted. All worms were alive at the end of each trial.

Results of this study are shown in Figure 2. The number of trials for 2, 4, and 6 releasers were 19, 22, and 20, respectively. Chi-square tests indicate that *E. revolutum* migrators were significantly attracted to four releasers at 0.75, 1.5, 2, and 3 hr ($P < 0.05$), and to six releasers at 0.25, 2, and 3 hr ($P < 0.05$). Migrators were less significantly attracted to two releasers at all time periods ($P > 0.1$).

Our results indicate that *E. revolutum* adults emit pheromone that attracts other adults of the same species. Fried et al. (1980, loc. cit.) found that *E. revolutum* adults were significantly attracted to the free sterol fraction of excretory-secretory products of adult *E. revolutum*. Since free sterols are only slightly soluble in aqueous solutions (Haberland and Reynolds, 1973, Proc. Natl. Acad.

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Sci. 70:2313–2316), it is advantageous to have the largest pore size possible in barrier designs. Unpublished studies in this laboratory show that barriers of filter paper, dialysis tubing and small mesh steel screen (100-mesh) were less effective in facilitating attraction in *E. revolutum* than the larger (40-mesh) screen used in this study.

Increasing the pheromonal dosage beyond an optimum level can cause a decreased attraction rate. Bone et al. (1977, J. Parasitol. 63:364–367), Balakanich and Samoiloff (1974, Can. J. Zool. 52:835–845) and Imperia et al. (1980, loc. cit.) observed this variable dosage response in nematode and trematode studies. No conclusions are drawn at present concerning the optimum number of *E. revolutum* releasers using our protocol, but four and six are more effective than two.

We acknowledge the assistance of Dr. Randy Stonesifer, Department of Mathematics, Lafayette College, Easton, Pennsylvania for aid with statistical analyses.

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***Research Note***

## *Khawia sinensis* (Caryophyllidea: Lytocestidae) from *Cyprinus carpio* in North America

While conducting a study of the caryophyllidean cestodes of fish and oligochaetes in Oregon, 11 nongravid (Figs. 1–3) and two gravid (Figs. 4–5) *Khawia* were obtained from the intestine of a *Cyprinus carpio* collected from the southern shore of Fern Ridge Reservoir, Lane County, Oregon in April 1975. Twenty-nine *C. carpio* collected from the same habitat in June 1977 and 1979 and September 1979 were not parasitized by *Khawia* but contained *Atractolylocestus huronensis* Anthony, 1958.

The gravid specimens agree with the description and illustrations of the Eurasian lytocestid, *K. sinensis* Hsü, 1935, in all respects except that the posterior ovarian arms appear to be united. As described and figured by Hsü (1935, Rev. Suisse Zool. 42:487–492), *K. sinensis* has an H-shaped ovary. Thus, regarding ovary shape, it is possible that these *Khawia* from Oregon *C. carpio* represent an undescribed species. However, from the literature, reports of variation in ovary shape are not uncommon among carophyllideans. Until additional specimens of *Khawia* from the above locality are studied regarding ovary variation, we believe these *Khawia* should be reported as a variant of *K. sinensis*. This report is the first record of *K. sinensis* in North America.

One specimen (No. 75492) was submitted to the USNM Helm. Coll., Beltsville, Maryland.

Of the five caryophyllidean species known to parasitize *C. carpio* in the Nearc-

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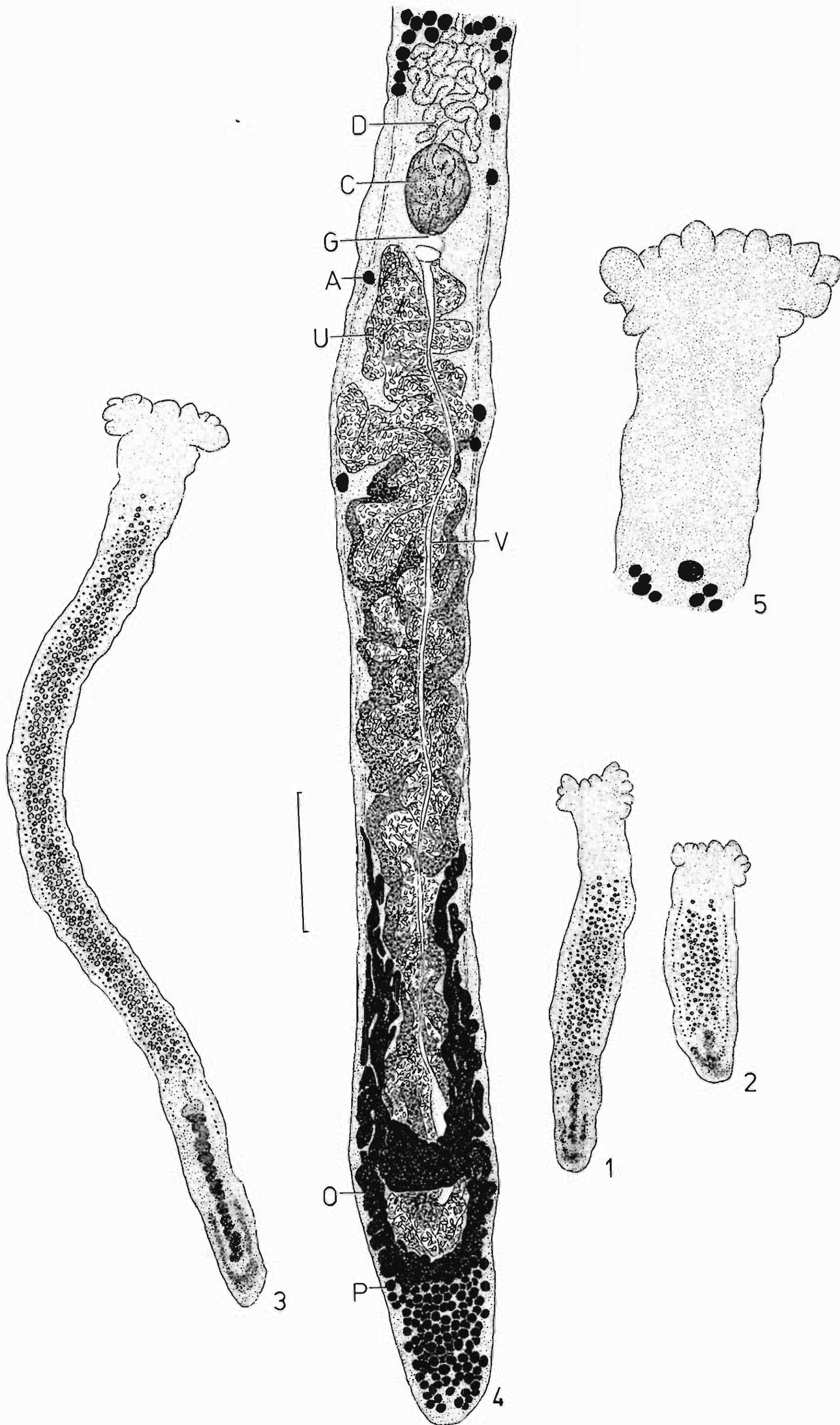


**Figures 1–5.** ***Khawia sinensis*** **from** ***C. carpio*****, Fern Ridge Reservoir, Oregon. 1–3. Nongravid cestodes. 4. Posterior of gravid cestode. 5. Scolex. All figures drawn to same scale. Scale equals 1 mm. Abbreviations: A, anterior vitellarium; C, cirrus sac; D, vas deferens; G, genital atrium; O, ovary; P, posterior vitellarium; U, uterus; V, vagina.**

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**Table 1. Comparison of gravid *K. iowensis* and *K. sinensis* from *C. carpio* (measurements in mm).**

| | *K. iowensis* | *K. iowensis* | *K. sinensis* | *K. sinensis* |
|---|---|---|---|---|
| Length | 11–26 | 14–48 | 55–170 | 62, 69 |
| Width | 1.0–2.1 | 1.0–3.2 | 1.1–5.0 | 1.1, 1.2 |
| Number of testes | 328–490 | n = 19 235–513 | not given | 467, 494 |
| Cirrus sac | 0.66 × 0.61 spherical | 0.60–0.69 × 0.60–0.69 spherical | 0.563 × 0.806 elliptical | 0.46, 0.51 × 0.65, 0.68 elliptical |
| Posterior extent of preovarian vitellaria | adjacent to ovary | adjacent to ovary, often extending posteriorly to postovarian vitellaria | anterior to ovary | anterior to ovary |
| Extent of postovarian vitellaria | does not obscure postovarian arms | does not obscure postovarian arms | partially obscures postovarian arms | partially obscures postovarian arms |
| Number of specimens studied | not given | 136 | not given | 2 |
| Reference and location | Calentine and Ulmer, 1961, J. Parasitol. 47:795–805. Iowa | Wis., Iowa, Neb. (Localities given in Williams, 1980, Rep. Fauna Flora Wis., Univ. Wis.–Stevens Point 17:1–14. | Hsü, 1935, loc. cit. Asia | this paper |

tic (*Archigetes iowensis* Calentine, 1962, *A. sieboldi* Leuckart, 1878, *A. huronensis, K. iowensis* Calentine and Ulmer, 1961, and *K. sinensis*), only *A. sieboldi* and *K. sinensis* are also found in Eurasia (comparison of *K. sinensis* and *K. iowensis* is given in Table 1).

Appreciation is expressed to Mr. Don G. Schmidt, Eugene, Oregon, Frau R. H. Schulze-Wencke, Hildrizhausen, Bundesrepublik Deutschland, and Dr. Stephen J. Taft, University of Wisconsin–Stevens Point, for assistance; to Dr. Robert L. Calentine, University of Wisconsin–River Falls and Dr. Martin J. Ulmer, Iowa State University of Science and Technology, Ames, for their comments.

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*Research Note*

# Simple and Inexpensive Computer Cataloging for Parasite Collections Using SAS

Curators of collections have been looking for ways to catalog specimen information with the aid of computers. The tremendous costs involved in purchasing or leasing the necessary equipment and the difficulty of understanding and communicating with the computer have discouraged many curators from using the more efficient computer cataloging.

Data handling (changing, rearranging, and combining data values) is a major part of any computer project. SELGEM is one program used to handle and catalog museum specimen data, but it has been shown over the years to be quite immobile, difficult to edit, too slow, and therefore too expensive. Koeppl (1981, Association of Systematics Collections Newsletter 9:19–21) proposed a simpler, more efficient computerized data management system based on SELGEM, introducing compact files which are more easily edited.

As scientists often find, similar questions are asked in various branches of research and, therefore, similar techniques are employed. Our paper discusses a quick and inexpensive way to computerize museum catalog records, but we had no input in writing the program; we developed only its application.

In reviewing a statistical program package, we dicovered that a curator of a parasite collection will ask of his specimens essentially the same questions that a statistician who is working on biometric problems will ask of his data. The program we are now using at the Harold W. Manter Laboratory is the Statistical Analysis System (SAS) which is a computer system for data analysis readily available at most universities in this country and at many larger companies overseas using IBM equipment.

A computer system is a group of computer programs that work together. SAS is a computer system which was developed several years ago for statistical analyses in business. Since then it has been widely used and tested so that the present SAS is well refined.

Helwig and Council (1979, SAS User's Guide, SAS Institute, Cary, North Carolina) explained that because SAS is a system, one does not have to prepare various computer jobs to plot data, to print special reports or to perform regressions; it can all be done with one SAS job. This makes SAS inexpensive in regard to computer time.

SAS is useful to all who need to analyze data; it reads data values (alphanumeric) in virtually any form from cards, disk, or tape; then organizes the values into a SAS data set. SAS data sets are automatically self-documenting, because they contain both the data values and their descriptions. When data values need to be modified, a complete set of SAS statements and functions is available. Just as SAS reads data in almost any form, it can write data in almost any form including graphs and charts. In addition to the preformatted reports produced by SAS procedure, the user can design and produce his own.

We are now applying SAS to collection data for parasite specimens deposited in our museum. The only problem the authors encountered was to code the collecting data (Fig. 1) appropriately so that it may be read and used by the



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Harold W. Manter Laboratory — Division of Parasitology — University of Nebraska State Museum
Collection No 20838 — Storage No
Parasite: Fessisentis vancleavei (Hughes and Moore, 1943) Nickol, 1972
Host: Eurycea sp. (larval)
Body location: anterior portion of small intestine
Locality: Fleming Creek, 4.3mi. SW of Saint Paul, Madison Co., Arkansas
Collected by: D.R. Brooks and M.A. Mayes — Date: 22 March 1975
Identified by: R.L. Buckner — Date:
No. slides: (1) ♂ — Type designation: voucher
Remarks:
Donor: R.L. Buckner

Harold W. Manter Laboratory — Division of Parasitology — University of Nebraska State Museum
Collection No 20980 — Storage No.
Parasite: Monostephanostomum manteri Kruse
Host: Arripis georgeanus (Cuvier and Valenciennes)
Body location: Intestine
Locality: Gulf St. Vincent, South Australia
Collected by: (Johnston Collection) — Date: 1932
Identified by: G.O.W. Kruse — Date: 17 October 1979
No. slides: one — Type designation: 3. Paratype
Remarks: published in J.P. Dec. 1979

Harold W. Manter Laboratory — Division of Parasitology — University of Nebraska State Museum
Collection No. 21336 — Storage No.
Parasite: Metastrongylus apri (Ebel, 1777) Vostokov, 1905
Host: Eisenia foetida Savegny, 1826
Body location: Calciferous gland and Pseudo hearts
Locality: Lincoln, Nebraska (University of Nebraska Campus)
Collected by: G.O.W. Kruse — Date: May 1979
Identified by: G.O.W. Kruse — Date: April 1981
No. slides: 30 with serial sections — Type designation: Voucher
Remarks: Publ.: Kruse and Ferguson, 1980. Vet. Med. Nachr. 2/80: 113–130

1

HAROLD W. MANTER LABORATORY
Division of Parasitology
University of Nebraska State Museum

CATALOGUE DATA SHEET

| COLL NO | Par Typ | Life-C stage | Par sex | PARASITE GENUS | PARASITE SPECIES | Host type | Adlt/Non | Host/sex | Expt/Nat | HOST GENUS | HOST SPECIES | Locat | LOCALITY country-state | Habitat | Slide/Wet | No in lot | Type design | Donor Collector Identifier | OCI | | Last |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 2 3 4 5 6 7 | 8 | 9 10 11 | 12 | 13–22 | 23–32 | 33 34 | 35 | 36 | 37 | 38–46 | 47–56 | 57 58 | 59–63 | 64 | 65 | 66 67 68 | 69 | 70 71 72 73 | 74 | 75 76 77 78 79 | 80 |
| 20838 | 5 | 1 | 2 | Fessisenti | vancleavei | 1 | 2 | | | Eurycea | | 15 | USAAR | 4 | 1 | 1 | 9 | 74 | D | | |
| 20980 | 6 | 1 | 3 | Monostepha | manteri | 72 | 1 | | | Arripis | georgeanus | 15 | AUS57 | 1 | 1 | 1 | 2 | 66 | D | | |
| 21336 | 5 | 9 | | Metastrong | apri | 61 | 1 | 3 | 1 | Eisenia | foetida | 5 | USANE | 6 | 2 | 30 | 9 | 66 | D | | X |

2

**Figure 1. Accession forms for the three lots represented in the coding information in Figure 2.**

**Figure 2. Catalog data sheet showing coding information for three lots of specimens.**

computer. The fact that a parasite was collected from a certain host at a certain locality is explicit information while the fact that a number of other specimens share the same attributes is normally implicit information. Explicit facts may be directly read while implicit information can be obtained by counting, sorting or otherwise manipulating elements of the data base. To minimize and automate those manipulations is the best reason for recording data in machine-readable form. Potential access to implicit information is gained by the transcription of specimen data.

Vance (1974, Museum Data Bank Research Report I, Museum Data Bank Committee, Rochester, New York) explained that an index of propositions may carry the entire information content of the index data base as a "file inversion"; i.e., originally explicit information becomes implicit while much that was implicit becomes explicit. Indexing and identification of propositions among data rely on a data organization called coding. The coding used in this paper is an alphanumeric representation of a proposition. Following the recommendations of the Association of Systematics Collections Council on Standards for Systematics Collections (Black et al., 1975, Association of Systematics Collections Newsletter 3, Insert) and personal experience, we designed a coding scheme with a catalog data sheet (Fig. 2) appropriate for parasite collections.

The computer reads a line of information as it is entered in designated columns. Thus, appropriate numbers of columns are assigned to various items of data. SAS has the capability of reading numbers and letters as values. Coding then becomes a matter of ascribing values to the various states of information (e.g., "2" in column 12 equals "male"). When the information is recalled, SAS will automatically substitute the appropriate word for the code symbol and "2" will appear as "male" in the printed report.

## Coding System for a Parasitological Catalog Using an 80-Symbol Line

IBM cards and terminal screens accommodate an 80-space line. Designing our coding system for the same 80-space line facilitates reading and editing. Should users wish to expand the data or coding, additional line(s) per specimen may be

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used but the fact must be indicated in the input statement. Regardless of the number of lines used, data bases from different institutions are compatible as long as they are based on SAS.

Column

1 (Blank)
This column may be used to identify data bases generated by other institutions and/or collections. Institutions using the same system can exchange computer tapes from which the data can be read directly into their files. A symbol in column 1 will identify the originating institution and distinguish it from their own collection.

2– 7 (Coll. no.)
Specimens deposited in our collection are assigned an accession number. If this were the only reference to the specimen, it would be difficult and time consuming to search the records in order to respond to inquiries concerning specific genera or species of parasites or hosts.

8– 9 (Parasite type)
Used as describer (number coded) for basic animal groups (e.g., Crustacea = 6, Digenea = 65).

10–11 (Life-cycle stage)
In conjunction with columns 8–9, we can here identify which stage of the life cycle has been deposited. Column 10 designates the life-cycle stage (e.g., miracidium, sporocyst, redia, etc.) while column 11 may be used for information from experimental studies (e.g., subadults at 1 day, 2 days, etc.).

12 (Parasite sex)
Numerical coding for the sex of the parasite deposited (e.g., hermaphrodite = 3).

13–22 (Parasite genus)
23–32 (Parasite species)
The scientific name of the specimen using up to 10 letters for each word. Though the names may be incomplete, the 20 letters will identify the species. This information is critical and the spelling should be double checked in each data set because it is at this point that a specimen will become "lost" in the data base if the name is not spelled correctly.

33–34 (Host type)
See columns 8–9.

35 (Adult/non)
In this column we code the maturity of the host.

36 (Host sex)
See column 12.

37 (Expt./non)
A marker (=1) in this column indicates voucher specimen derived from

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experimental infection. A blank in this space symbolizes a natural infection.

38–46 (Host genus)
47–56 (Host species)
See columns 13–32.

57–58 (Location)
The site of infection expressed by a numerical value derived from a list of major locations (microhabitats) found in/on invertebrates and vertebrates.

59–63 (Locality: country, state)
The locality from which the host was collected. Geographic coordinates are the most specific form of location identifier, but it is almost impossible to use them to retrieve faunal lists for generalized areas (e.g., North Sea, Nebraska, etc.).

The coding of the locations is done by alphanumeric symbols. The English Olympic National Abbreviations are used in columns 59–61 for coding the country (e.g., INA = Indonesia, PAN = Panama, USA = United States of America).

If necessary, the countries may be subdivided into states, provinces, departments, etc., for which a two-letter symbol is used in columns 62–63 (for the USA the two-letter state abbreviations published by the U.S. Postal Service are used (e.g., MO = Missouri, NE = Nebraska).

A problem arose with marine collecting sites, but this was solved by employing the *FAO Classification of Major Fishing Areas for Statistical Purposes* (see *FAO Fisheries Circular No. 420,* Rome, Dec. 1972) in columns 62–63. If the hosts were collected at open sea, columns 59–61 are left blank; if the host is coastal, those same columns are filled with the appropriate country code in conjunction with the fishing area code (e.g., coast of South Australia = AUS52).

64 (Habitat)
The major ecosystems from which hosts are collected are numerically coded. Retrieval must be made in conjunction with the Locality code (columns 59–63) to identify specific habitats.

65 (Slide/wet)
Various forms of specimen preparation are coded numerically (e.g., whole mount on slide = 1, section on slide = 2, specimens in fluid = 6).

66–68 (No. in lot)
This is curatorial information. In conjunction with other data, one may ask statistical questions about specimens (e.g., how many specimens were deposited by Yamaguti in our collection? Or how many specimens do we have of *Derogenes varicus*?).

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69 (Type designation)
The major taxonomic type designations are numerically coded (e.g., holotype = 1, lectotype = 4, neotype = 7).

70–73 (Donor, collector, identifier)
74 (DCI)
The name of each donor, collector, and identifier of specimens in our collection has been entered in an alphabetical file and on a consecutively numbered list. The number of one of these corresponding with the name on the list is entered in columns 70–73.

The letter D, C, or I in column 74 describes the capacity of the individual indicated in columns 70–73.

75–79 (Blank)
Reserved for expansion of the program (e.g., additional categories or expansion of existing categories).

80 (Loan)
A flag (=X) to designate specimens on loan; removed when the loan is returned.

It should be pointed out that any of the above categories may be expanded to accommodate whatever information needs to be stored.

The type of program discussed above permits one to ask direct questions (e.g., how many specimens of species X are in the collection?) or string questions (e.g., how many males of species X from host Y in locality Z do we have in the collection?). With the same data base one can then generate various statistical analyses and have these printed in report form.

Curators of collections interested in using our SAS based catalog are invited to write to the authors for more specific information on the coding procedures.

The authors wish to thank Roger Deaton, Statistical Consultant and Manager of Data Processing, the University of Nebraska Institute of Agriculture and Natural Resources Biometrics and Information Center, for reviewing our coding and procedure.

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# Report on the Brayton H. Ransom Memorial Trust Fund

| | |
|---|---|
| Balance on hand, 1 January 1980 | $5132.80 |
| Receipts: Net interest received in 1980 | 472.13 |
| | $5604.93 |
| Disbursements: Grant to The Helminthological Society of Washington for 1979 | 10.00 |
| Grant to The Helminthological Society of Washington for 1980 (made on February 3, 1981) $50.00 | — |
| On hand, 31 December, 1980 | $5594.93 |

HARLEY G. SHEFFIELD
*Secretary-Treasurer*

**Trustees of the Brayton H. Ransom Memorial Trust Fund**

A. Morgan Golden, *President*
Harley G. Sheffield, *Secretary-Treasurer*
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# MINUTES

## Five Hundred Thirty-Third Through Five Hundred Fortieth Meetings

*533rd Meeting:* Seventieth Anniversary Dinner, Holiday Inn, College Park Maryland, 8 October 1980. The 1980 Anniversary Award was presented to Dr. O. Wilford Olsen by Dr. L. S. Diamond. Dr. Bernard Bezubik received Honorary membership in the Society. Life membership was presented to Dr. John S. Andrews by Dr. Harry Herlich. Dr. Gilbert Otto was given a Special Service Award presented by Dr. A. James Haley in recognition of his 43 years of service on the Editorial Committee and completion of 48 years service to the Proceedings. Edna Buhrer was the recipient of a Special Service Award for a long history of service to the Society. In absentia the award was accepted on her behalf by Dr. A. O. Foster. Life members present at the Anniversary Dinner were: J. S. Andrews, M. H. Chitwood, M. A. Doss, M. M. Farr, A. O. Foster, C. H. Herman, D. R. Lincicome, G. F. Otto, L. E. Rozeboom, and M. A. Stirewalt. Dr. Harley G. Sheffield presided as master of ceremonies for the Anniversary Dinner program. The following slate of officers was presented: Nancy D. Pacheco (President), Louis S. Diamond (Vice President), Sherman S. Hendrix (Corresponding Secretary-Treasurer), Milford N. Lunde (Recording Secretary).

*534th Meeting:* Animal Parasitology Institute, USDA, Beltsville, Maryland, 14 November 1980. The slate of officers presented at the 533rd meeting was elected. Dr. David Stiller presided over the following papers: "Specific T Lymphocyte Response to *Trypanosoma brucei* in the Mouse," Louis C. Gasbarre; "An Experimental Trial Using a Non-viable *Babesia bovis* Vaccine," Kenneth L. Kuttler; "Strongyloid Nematode Fauna Discovered in Gopher Turtles," J. Ralph Lichtenfels, "Index Catalogue of Medical and Veterinary Zoology, New and Easier-to-Use Format," (Poster presentation), Shirley Edwards.

*535th Meeting:* Plant Protection Institute, USDA, and US Food and Drug Administration, Bureau of Veterinary Medicine, Beltsville, Maryland, 5 December 1980. Dr. A. M. Golden presented the Resolution for Incorporation which was drafted by the Ad Hoc Resolutions Committee consisting of himself, H. Herlich, and H. G. Sheffield. Officers elected at the 534th meeting were installed. The following papers were presented, Dr. Raymond V. Rebois presiding: "Prokaryotic Pathogens of Plant Parasitic Nematodes," R. M. Sayer; "On the Current Status of the Pine Killing Nematode in the United States," W. R. Nickle; "Non Water-borne Transmission of *Giardia lamblia,*" Robert J. Barnard; "Developmental Stages of Heart Worm Disease in the Dog," T. Kotani; "The Ultrastructure of the Anterior Neurosensory Organs of the Larvae of the Soybean Cyst Nematode," B. Y. Endo (Poster presentation).

*536th Meeting:* National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, 9 January 1981. The Resolution for Incorporation presented at the 535th meeting was passed. President N. D. Pacheo announced the following executive appointments: Duane G. Erickson and Thom-



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as W. Simpson (Executive Committee), Raymond V. Rebois (Assistant Corresponding Secretary), Patricia A. Pilitt and Michael D. Ruff (Auditors), Harley G. Sheffield (Awards Committee), and A. J. Haley (Business Advisory Committee). Dr. Franklin A. Neva presided over the following papers: "Attachment of *Plasmodium falciparum* Infected Erythrocytes to Endothelial Cells *in vitro,*" I. Udeniya; "A Phenotypic Change in a Malaria Parasite Dependent on the Spleen," J. W. Barnwell; "Immune Responses to *Strongyloides* Infection in Rats and Men," R. M. Genta; "Influence on Interferon Induction on Innate Resistance to Acute *Trypanosoma cruzi* Infection in Mice," S. L. James; "Human Schistosomiasis mekongi: Clinical, Immunologic, and Therapeutic Studies," M. Hofstetter.

*537th Meeting:* Naval Medical Research Institute, Bethesda, Maryland (Co-sponsored by Oxford Laboratory, NOAA), 13 February 1981. The audited financial report for the year ending 31 December 1980 was presented by S. S. Hendrix, Secretary-Treasurer, and was approved. Dr. Aaron Rosenfield presided over the following papers contributed by NOAA: "Microscopic Observation of Epiphytic Organisms on the Gills of *Cancer irraratus,*" Joel E. Bodammer; "The Occurrence of *Eustrongylides* sp. in Chesapeake Bay Eel," Martin W. Newman. Dr. Wilton E. Vannier then presided over the following papers from the NMRI: "Resistance to *Schistosoma mansoni* Infection in Parabiotic Mice," David A. Dean; "Filariasis in Indonesia," James R. Palmieri.

*538th Meeting:* Walter Reed Army Institute of Research, Washington, D.C., 13 March 1981. The death of Earl H. Fife was announced. Col. David Davidson presided over the following papers: "Pharmacology of Leishmaniasis *in vitro,*" Jonathan Berman; "Visceral Leishmaniasis in Kenya: Diagnosis, Clinical Manifestations and Response to Therapy," Wayne Hockmeyer; "Interaction of *Leishmania tropica* and Mouse Macrophages *in vitro,*" Carol Nacy.

*539th Meeting:* Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, 10 April 1981. Dr. Everett Schiller presided over the following papers: "Secretion of a Lysosomal Enzyme by *Leishmania donovani,*" Michael Gottlieb; "Immune Mechanisms in Chagas' Disease," Thomas Trischmann; "Immunochemistry of *Onchocerca volvulus* Antigens and Associated Circulating Immune Complexes," Kamorudeen Ojodu; "Mode of Action in a Virus," Christopher Taylor; "Neurochemistry of *Schistosoma mansoni*: Cholinergic Parameters," Christopher Molineaux.

*540th Meeting:* The University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, 9 May 1981. This was a joint meeting with the New Jersey Society for Parasitologists. Proposed changes in the Constitution and Bylaws necessitated by the incorporation of the Society, were presented to the membership by H. G. Sheffield. The title of the Symposium for the meeting was "Contemporary Ecology: Its Relevance for Parasitology and Tropical Medicine." Dr. Gerhard S. Schad presided. The following papers were presented: "Population Biology of Parasitic Diseases," Robert May; "Ecological Aspects of Multiply Infected Hosts," Joel Cohen; "Parallels Among Parasites of Plants and Animals," Daniel Janzen.

The following 34 new members were elected at the meetings indicated: *533rd:*

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Michael R. Baker, George A. Conder, Ted Drouin, Jose Gonzalez, Jon A. Lowrance, Steven A. Martinez, James Palmieri, Guillermo O. Vilchez, Thomas G. Watson, and Karl A. Western. *534th:* Robert J. Barnard, Edward P. Caswell, David B. Conn, Brian P. Hayden, Gary S. Paffenberger, Thomas M. Rice, David M. Rutherford, and Jane A. Starling. *535th:* Dominic A. Strohlein. *536th:* Robert W. Poindexter. *537th:* Howard C. Goodman, Rosanne L. Hearn, Joan E. Decker Jackson, Peter R. Jackson, Susan G. Langreth, and Sharon Ann Long. *538th:* Robert L. Callentine and Richard A. Heckman. *539th:* Patrick B. McGreevy, Michael Riggs, Wesley L. Shoop, and Peter J. Weekes. *540th:* John C. Mergo, Jr. and Ellen J. Winchell.

MILFORD LUNDE
*Recording Secretary*

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