Volume 54 January 1987 Number 1

# PROCEEDINGS

of

# The Helminthological Society of Washington

*A semiannual journal of research devoted to Helminthology and all branches of Parasitology*

Supported in part by the
Brayton H. Ransom Memorial Trust Fund

CONTENTS



(*Continued on Outside Back Cover*)

# THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

*THE SOCIETY* meets once a month from October through May for the presentation and discussion of papers in any and all branches of parasitology or related sciences. All interested persons are invited to attend.

Persons interested in membership in the Helminthological Society of Washington may obtain application blanks in recent issues of THE PROCEEDINGS. A year's subscription to the Proceedings is included in the annual dues.

## OFFICERS OF THE SOCIETY FOR 1987

*President*: PATRICIA A. PILITT
*Vice President*: ROBIN N. HUETTEL
*Corresponding Secretary-Treasurer*: MICHAEL D. RUFF
*Assistant Corresponding Secretary-Treasurer*: DAVID J. CHITWOOD
*Recording Secretary*: JEFFREY D. BIER
*Archivist/Librarian*: DAVID R. LINCICOME
*Custodian of Back Issues*: GERHARD A. SCHAD
*Representative to the Washington Academy of Sciences*: KENDALL G. POWERS
*Representative to the American Society of Parasitologists*: WILLIS A. REID, JR.
*Executive Committee Members-at-Large*: J. KEVIN BAIRD, 1987
JOHN H. CROSS, 1987
ROBERT J. CHINNIS, 1988
DENNIS E. KYLE, 1988
*Immediate Past President*: RALPH P. ECKERLIN

# THE PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE PROCEEDINGS are published semiannually at Lawrence, Kansas by the Helminthological Society of Washington. Papers need not be presented at a meeting to be published in the Proceedings.

MANUSCRIPTS should be sent to the EDITOR, J. R. Lichtenfels, USDA, ARS, BARC-East No. 1180, Beltsville, MD 20705. Manuscripts must be typewritten, double spaced, and in finished form. The original and two copies are required. Photocopies of drawings may be submitted for review purposes but glossy prints of halftones are required; originals will be requested after acceptance of the manuscript. Papers are accepted with the understanding that they will be published only in the Proceedings.

REPRINTS may be ordered from the PRINTER at the same time the corrected proof is returned to the EDITOR.

AUTHORS' CONTRIBUTIONS to publication costs (currently $40/pg for members) will be billed by Allen Press and are payable to the SOCIETY.

BACK VOLUMES of the Proceedings are available. Inquiries concerning back volumes and current subscriptions should be directed to the business office.

BUSINESS OFFICE. The Society's business office is at Lawrence, Kansas. All inquiries concerning subscriptions or back issues and all payments for dues, subscriptions, and back issues should be addressed to: Helminthological Society of Washington, % Allen Press, Inc., 1041 New Hampshire St., Lawrence, Kansas 66044, U.S.A.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 1–14

# New Avian Filarioids (Nematoda: Splendidofilariinae): *Dessetfilaria guianensis* gen. n., sp. n., *Andersonfilaria africanus* gen. n., sp. n., and *Splendidofilaria chandenieri* sp. n.

CHERYL M. BARTLETT[1] AND ODILE BAIN[2]

[1] Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada and

[2] Laboratoire des Vers, associé au CNRS, Muséum National d'Histoire Naturelle, 61 rue de Buffon, 75231 Paris Cedex 05, France

ABSTRACT: The following new taxa are described: *Dessetfilaria guianensis* gen. n., sp. n. from a capsule along the outer wall of the aorta in a channel-billed toucan (*Ramphastos vitellinus* Lichtenstein [Ramphastidae]) collected near Cayenne, French Guiana; *Andersonfilaria africanus* gen. n., sp. n. from a fossa in the pelvic girdle of a common waxbill (*Estrilda astrild* (L.) [Estrildidae]) imported into France from Africa; and *Splendidofilaria chandenieri* sp. n. from subcutaneous tissues of the wing of the same common waxbill. Microfilariae occurred in the blood. *Dessetfilaria* is characterized by the presence of only two pairs of cephalic papillae and a distinctly divided esophagus. *Chandlerella braziliensis* Yeh, 1957, is transferred to *Dessetfilaria* as *D. braziliensis* (Yeh, 1957) comb. n. *Andersonfilaria* is characterized by the presence of four pairs of cephalic papillae and a poorly developed, undivided esophagus. *Splendidofilaria chandenieri* is distinguished from other bossate species from subcutaneous tissues by the absence of large preanal papillae.

KEY WORDS: Filarioidea, *Ramphastos vitellinus, Estrilda astrild,* bird parasites, microfilariae, nematode taxonomy, morphology, French Guiana, Africa, Paris, France.

The numerous reports of microfilariae in the blood of birds in the Neotropical and Ethiopian zoogeographic regions (Bennett et al., 1982) indicate that the resident avifauna is widely parasitized by filarioid nematodes. Few of the filarioid species present have been identified, however, largely because the requisite adult worms are often not looked for or, due to their cryptic locations, are overlooked. The present paper describes two new genera and three new species on the basis of material from a channel-billed toucan (*Ramphastos vitellinus*) from French Guiana and a common waxbill (*Estrilda astrild*) from Africa.

## Materials and Methods

The channel-billed toucan was obtained through the courtesy of Mr. Ferrere in the region of Cayenne, French Guiana, on 11 March 1983 and the common waxbill was obtained through the courtesy of Mr. Jacques Chandenier, who had purchased the bird at a pet store in Paris, France, in October 1985 after it had been imported from Africa. Both birds were examined for adult filarioids, and adults recovered were fixed in hot 70% alcohol, transferred to 70% alcohol/5% glycerin, and studied in glycerin. En face views were also studied in lactophenol. Transverse sections were prepared freehand using a mounted razor blade. Microfilariae from the blood or from the vagina were studied, and the specific techniques used accompany the descriptions of the microfilariae (blood smears were fixed in ethanol prior to staining).

## Results

### *Dessetfilaria* gen. n.

DIAGNOSIS: Spirurida, Filarioidea, Onchocercidae, Splendidofilariinae Chabaud and Choquet, 1953 (sensu Anderson and Bain, 1976). Cephalic extremity with 2 pairs of papillae. Cuticle transversely striated. Esophagus divided, anterior portion narrow and poorly developed, posterior portion broad and glandular. Posterior extremity of body bluntly rounded in both sexes. Caudal papillae in 2 ventrolateral rows. Spicules subequal. Vulva pre-esophageal. Microfilaria with rounded tail. Parasites of birds. Type species: *D. guianensis* sp. n.

### *Dessetfilaria guianensis* sp. n. (Figs. 1–27, 72)

GENERAL: Long, slender nematodes. Body width uniform over most of length, but tapering gradually toward bluntly rounded extremities. Cuticle thin, transverse striations delicate at both ends of body but becoming increasingly apparent and wider toward midbody. Cuticle in lateral fields slightly thicker than elsewhere (Figs. 10, 17, 22, 23) and not striated. Hemizonid readily visible, slightly protuberant. Triangular outline of underlying hypodermal tissue visible in en face view of female; not observed in male. Cephalic papillae tiny and difficult to discern, linearly arranged with 2 on either side of oral opening, inner 2 not protuberant, outer 2 slightly salient (Figs. 8, 16). Amphids slightly salient. Oral opening tiny, laterally compressed, and lacking circumoral ring. Pre-esophageal ring absent. Anterior extremity of esophagus not well defined (Figs. 2a, 4, 15a). Esophageal division distinct (Figs. 1a, 14), anterior portion devoid of transverse muscle fibers and much narrower than posterior glandular portion. Anterior portion continuing into glandular portion (Figs. 2a, 5, 15a), but becoming increasingly obscure toward posterior end of esophagus (Figs. 2d, 15d). Glandular portion markedly granular and containing numerous large nuclei (Figs. 2, 15). Cuticular lining of esophageal lumen distinct and frequently plicate in anterior portion (Fig. 4), obscure in posterior portion. Junction of esophageal and intestinal tissues distinct and oblique (Figs. 2e, 3, 15d, 21); junction of esophageal and intestinal lumens difficult to discern (Figs. 20a, b). Phasmids not observed.

MALE (3 specimens, measurements of holotype followed by paratypes): Length 29, 26, 23 mm. Maximum width 180, 160, 160 μm. Width at nerve ring 100, 100, 90 μm, at anus 50, 55, 55 μm. Approximate number of transverse cuticular striations over 100 μm at midbody 28, 29, 29. Nerve ring 140, 140, 120 μm from anterior extremity. Length of anterior portion only of esophagus 200, 145, 140 μm; length of glandular portion of esophagus 1.48, 0.85, 1.02 mm. Maximum width of anterior portion of esophagus 18, 25, 25 μm; maximum width of glandular portion of esophagus 75, 95, 95 μm. Caudal end in 2, 1.5, 1.5 coils. Spicules (Figs. 6, 7) dissimilar, uniformly cuticularized, left 94, 108, 102 μm long, right 82, 78, 80 μm long. Anus 80, 84, 85 μm from posterior extremity. Cuticular lips of anus thick (Fig. 9). Small semipedunculate pre-, ad-, and postanal papillae present in 2 rows on tail; asymmetric or symmetric in arrangement; 7 on left side and 8 on right side in holotype (Figs. 11, 12), 7 or 8 on either side in paratypes (Fig. 13).

FEMALE (3 gravid specimens, measurements of allotype followed by paratypes): Length 85, 82, 78 mm. Maximum width 300, 250, 250 μm. Width at nerve ring 130, 125, 120 μm, at vulva 180, 170, 190 μm, at anus 80, 80, 75 μm. Approximate number of transverse cuticular striations over 100 μm at midbody 14, 15, 15. Nerve ring 150, 125, 180 μm from anterior extremity. Length of anterior portion only of esophagus 230, 180, 280 μm; length of glandular portion of esophagus 1.28, 1.04, 1.35 mm. Maximum width of anterior portion of esophagus 20, 25, 22 μm; maximum width of glandular portion of esophagus 85, 82, 95 μm. Vulva 400, 350, 500 μm from anterior extremity. Cuticular lips of vulva thin, slightly salient (Fig. 24). Body not swollen in vulvar region (Fig. 14). Vagina directed posteriorly from vulva, not convoluted, 1.5, 2.3, 1.5 mm long. Didelphic and opisthodelphic. Uteri convoluted. Ovaries in posterior region of body. Anus visible as slightly salient delicate opening 95, 105, 140 μm from posterior extremity (Fig. 26). Posterior extremity with two inconspicuous papillae (Figs. 25, 26).

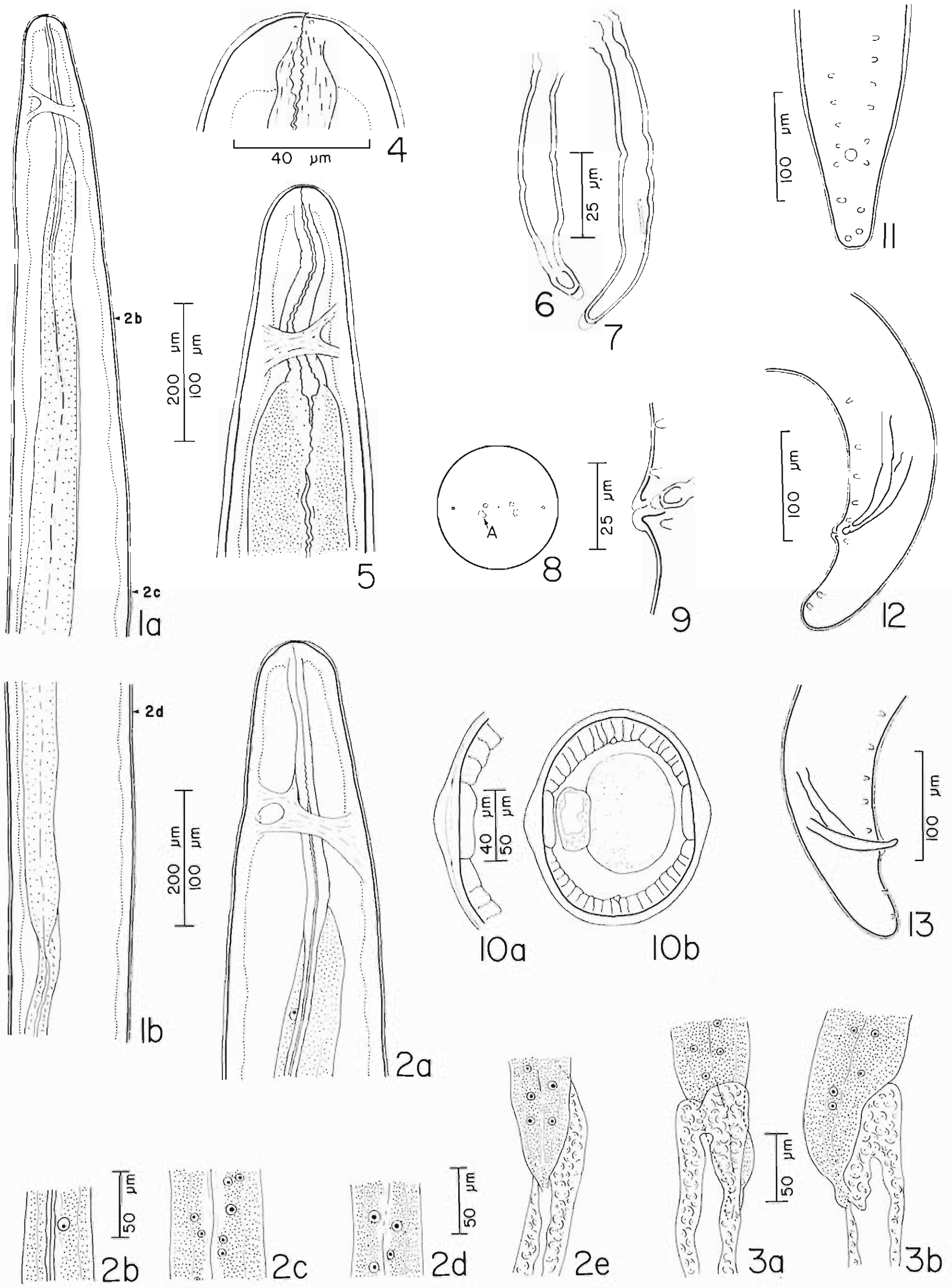
MICROFILARIA: Body short (Fig. 72) with transversely striated cuticle. Anterior extremity bluntly rounded. Cephalic cuticular structures not observed. Body width greatest at midbody, tapering slightly toward extremities, taper more pronounced in posterior region. Posterior extremity bluntly rounded. Posteriormost nucleus rounded, present at tail extremity (Fig. 72). Sheath absent. Small inner body visible in some specimens. Measurements (in micrometers) as follows:

1) 10 specimens from hematein-stained thin blood smears: length (range followed by mean) 37–62 (53); maximum width 6–7.
2) 3 specimens in which some fixed points were visible, from hematein-stained thin blood smears: length 58, 57, 55; maximum width 7, 6, 6; length of cephalic space 3, 3, 2; distance from anterior extremity to beginning of inner body 30, 31, 33; length of inner body 5, 2, 3; distance from anterior extremity to anal vesicle 48, 47, 46.
3) 10 specimens from the anterior vagina of a female worm: length (range followed by mean) 46–60 (54); maximum width 4–5.

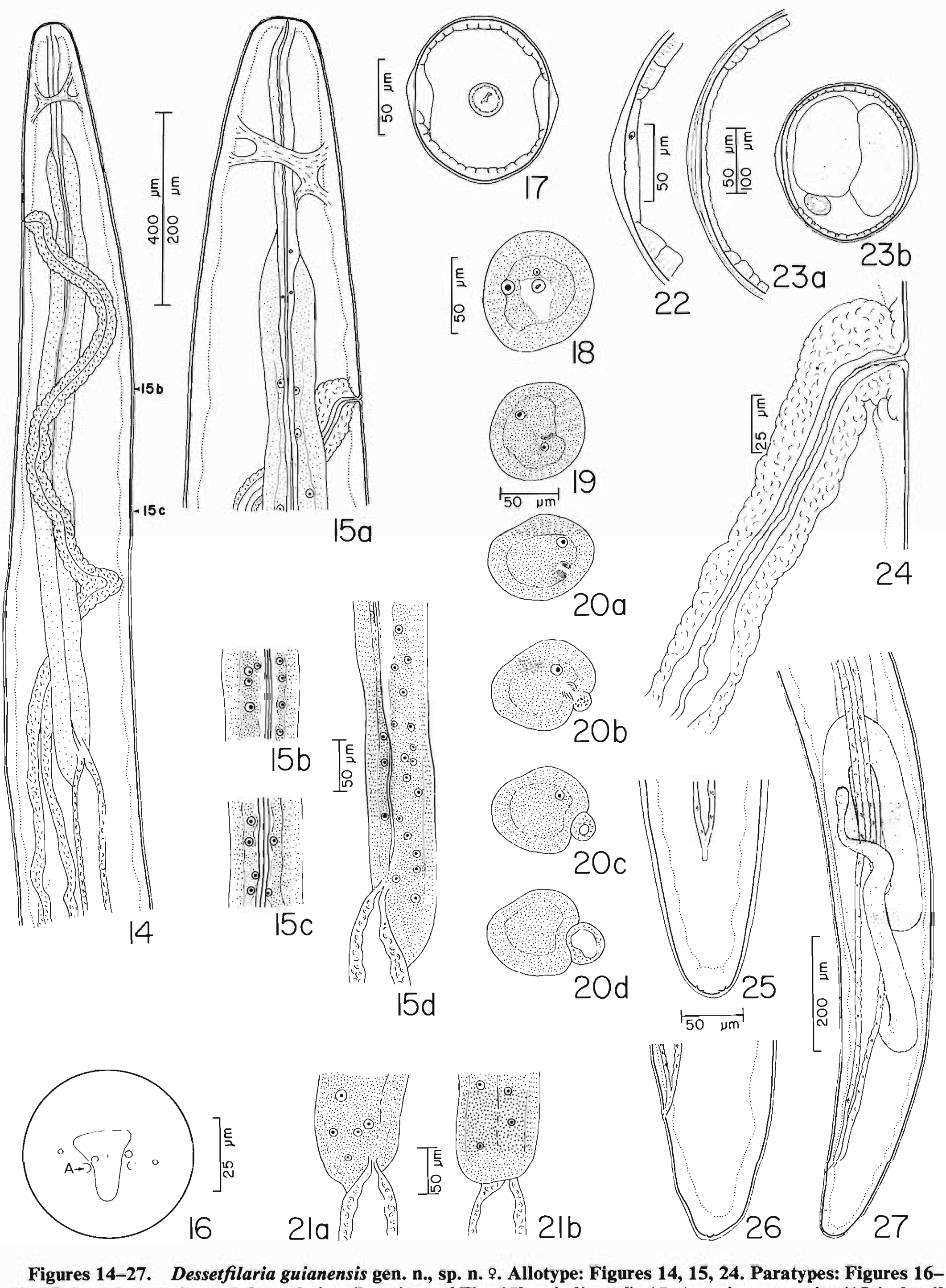
TYPE HOST: Channel-billed toucan, *Ramphastos vitellinus* Lichtenstein (Piciformes: Ramphastidae).

LOCATION IN HOST: Adults in delicate capsule along outer wall of aorta near junction with heart. Microfilariae in blood.

TYPE LOCALITY: Cayenne, French Guiana.




**Figures 1–13.** ***Dessetfilaria guianensis*** **gen. n., sp. n. ♂. Holotype: Figures 1, 2, 9, 11, 12. Paratypes: Figures 3–8, 10, 13. 1. Anterior end showing anteriormost region (1a) and esophageal–intestinal junction (1b); lateral view (locations of Fig. 2b–d indicated). 2. Anterior end (2a), detail of esophagus (2b–d), and detail of esophageal–intestinal junction (2e); lateral view. 3. Detail of esophageal–intestinal junction; views in different (3a, b) orientations. 4. Anterior extremity; lateral view. 5. Anterior end; lateral view. 6, 7. Spicules, right and left, respectively; lateral view. 8. En face view. A = amphid. 9. Detail of anal region; left lateral view. 10. Partial (10a) and whole (10b) transverse sections of midbody. 11–13. Posterior end; ventral, lateral, and lateral views, respectively.**




**Figures 14–27.** ***Dessetfilaria guianensis*** **gen. n., sp. n. ♀. Allotype: Figures 14, 15, 24. Paratypes: Figures 16–23, 25–27. 14. Anterior end; lateral view (locations of Fig. 15b, c indicated). 15. Anterior extremity (15a), detail of esophagus (15b, c), and detail of esophageal–intestinal junction (15d); lateral view. 16. En face view. A = amphid. 17. Transverse section of body immediately anterior to nerve ring. 18, 19. Transverse sections of glandular portion of esophagus; anterior and posterior regions, respectively. 20. Transverse sections of esophageal–intestinal junction at anteriormost (20a) and posteriormost (20b) regions. 21. Detail of esophageal–intestinal junction, views in different (21a, b) orientations. 22. Partial transverse section of body at esophageal–intestinal junction. 23.**

SPECIMENS: Muséum National d'Histoire Naturelle, Paris, France (MNHN). Holotype (♂), allotype (♀), and paratypes (♂ and ♀): MNHN No. 42 ED. Microfilariae: MNHN Nos. N VII 31–33.

TAXONOMIC COMMENTS: Within Splendidofilariinae (sensu Anderson and Bain, 1976) most genera have four pairs of cephalic papillae. *Dessetfilaria* gen. n., *Splendidofilaria* Skrjabin, 1923, and *Thamugadia* Seurat, 1917, have only two pairs, as might the monotypic and inadequately described genera *Pseudothamugadia* Lopez-Neyra, 1956, and *Onchocercella* Yorke, 1931 (sensu Anderson and Bain, 1976; not sensu Sonin, 1977). The papillae in *Dessetfilaria* and *Splendidofilaria* from birds differ from those in *Thamugadia* and *Pseudothamugadia* from reptiles, being generally small and asymmetrically arranged as opposed to large and symmetric; these four genera have smooth or transversely striated cuticles. The papillae in *Onchocercella* from mammals are small, and the cuticle has fusiform thickenings. In addition, in *Thamugadia* and *Pseudothamugadia* the entire esophagus is broad, whereas in *Dessetfilaria* and *Splendidofilaria* the anterior portion of the esophagus is narrow. *Dessetfilaria* has, however, a broad glandular posterior portion, whereas the entire esophagus in *Splendidofilaria* is devoid of glandular tissue (Anderson, 1961; Anderson and Bain, 1976) although occasionally abnormally dilated posteriorly (Bartlett and Anderson, 1985). The esophagus in *Dessetfilaria* is distinctly demarcated from the intestine but this is generally not the case in *Splendidofilaria.*

ETYMOLOGY: *Dessetfilaria* gen. n. is named in honor of Pierre Desset and Marie-Claude Durette-Desset of Paris, France; "*guianensis*" denotes the country where the infected bird was collected.

OTHER SPECIES: *Chandlerella braziliensis* Yeh, 1957, from a red breasted (=green billed) toucan (*Ramphastos dicolorus* L.) that died in the garden of the Zoological Society of London after having been imported from Brazil, is herein transferred to *Dessetfilaria* as *D. braziliensis* (Yeh, 1957) comb. n., based on the close resemblance of the spicules, pattern of caudal papillae, and major dimensions to *D. guianensis.* Yeh (1957) stated that "the digestive tract is not very well defined," but "the rather indistinct esophagus appears divided into muscular and glandular parts." This lack of observable detail was probably due to autolysis, as the specimens had come from a bird that had been dead for an unknown length of time. Nevertheless, Yeh did clearly illustrate a narrow anterior portion in the esophagus and a broad posterior portion. He did not describe the en face view.

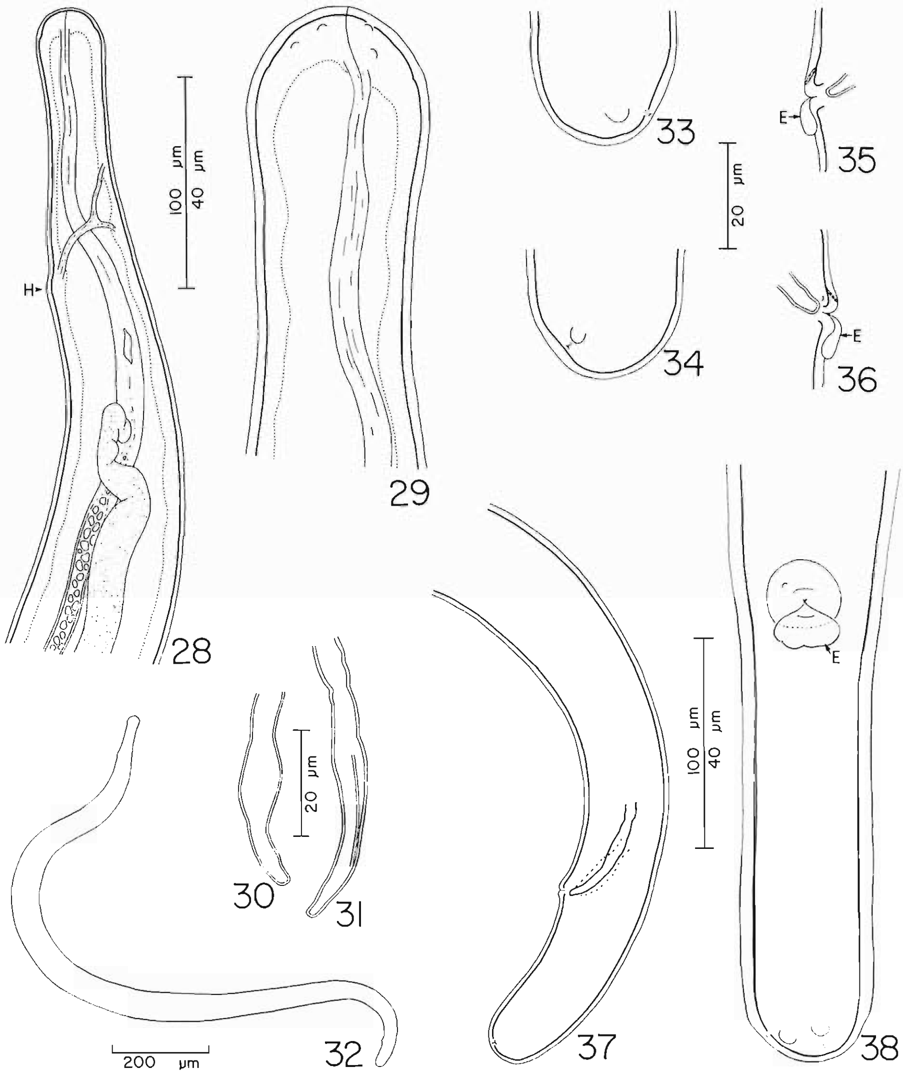
*Dessetfilaria guianensis* can be considered distinct from *D. braziliensis* because the former is narrower (160–180 vs. 200–250 μm in males, 250–300 vs. 380–460 μm in females) and has a longer glandular esophagus (850–1,480 vs. 500–540 μm in males, 1,040–1,350 vs. 770–840 μm in females). Inadvertent flattening of the specimens and intraspecific variation might account for these "differences," but because the type specimens of *D. braziliensis* are apparently lost (they are no longer present in the Helminthological Collection of the London School of Hygiene and Tropical Medicine [R. Muller, pers. comm.]), it seems best to consider the specimens from the channel-billed toucan as a new species (i.e., *D. guianensis*) and to base the description of *Dessetfilaria* on this material. The status of *D. braziliensis* and *D. guianensis* requires further evaluation, however, especially in view of the close ecologic and taxonomic relationship between their hosts. Haffer (1974) considered channel-billed and red-breasted toucans as a superspecies, noting that they are parapatric except in southeastern Brazil where they are locally sympatric and that they occupy the lower strata in lowland Neotropical forests.

### *Andersonfilaria* gen. n.

DIAGNOSIS: Spirurida, Filarioidea, Onchocercidae, Splendidofilariinae Chabaud and Choquet, 1953 (sensu Anderson and Bain, 1976). Cephalic extremity with 4 pairs of papillae. Cuticle transversely striated. Esophagus not divided, poorly developed. Esophageal–intestinal junction indistinct. Posterior extremity of body bluntly rounded in both sexes. Caudal papillae tiny and few in number. Spicules subequal. Vul-

←

**Partial (23a) and whole (23b) transverse sections of midbody. 24. Vulva and anterior vagina; lateral view. 25, 26. Posterior extremity; ventral and lateral views, respectively. 27. Posterior end, lateral view.**

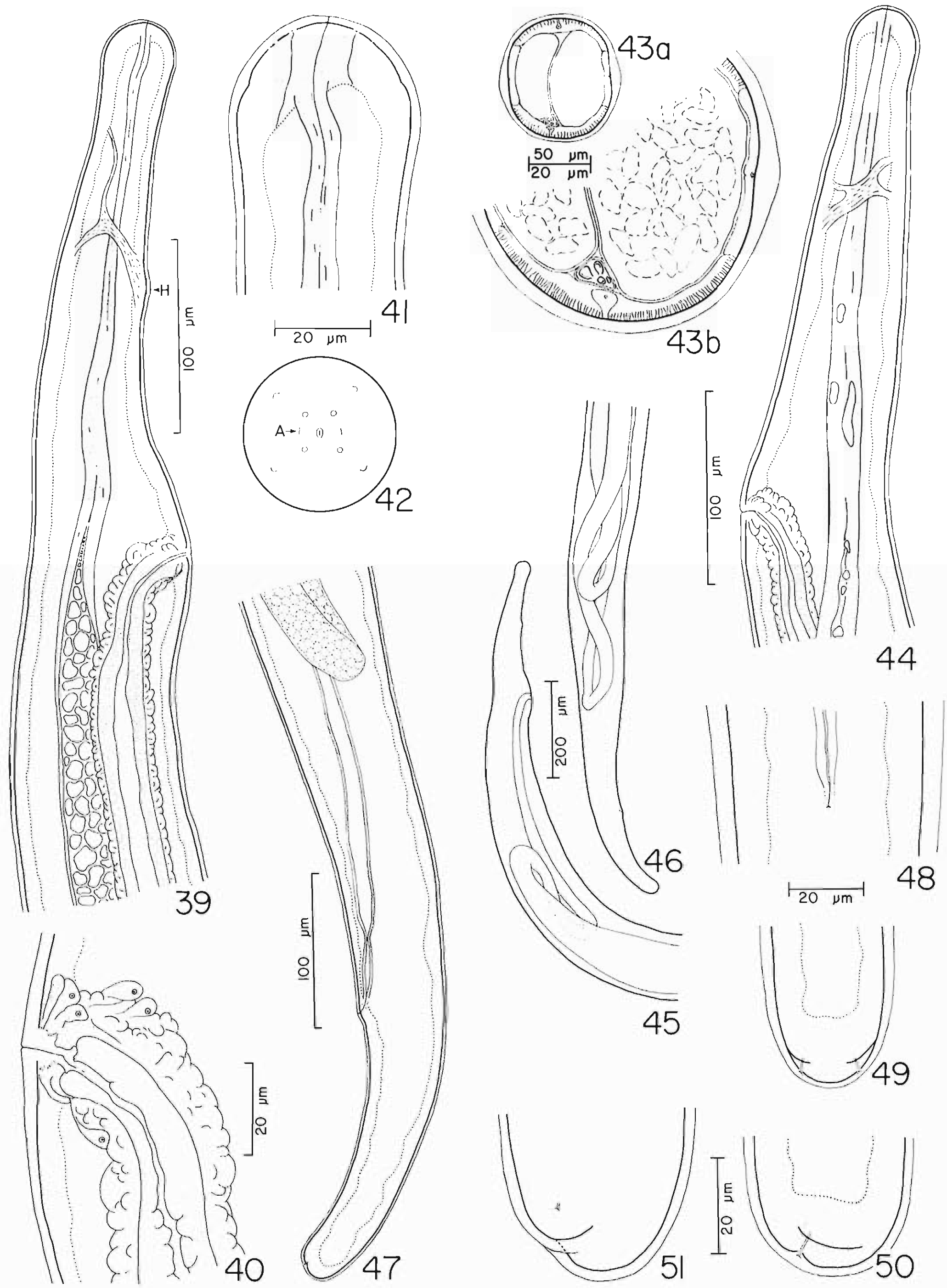



**Figures 28–38.** ***Andersonfilaria africanus*** **gen. n., sp. n. ♂, holotype. 28. Anterior end; lateral view. H = hemizonid. 29. Anterior extremity, dorsal–ventral view. 30, 31. Spicules, right and left, respectively; lateral view. 32. Outline of body; lateral view. 33, 34. Posterior extremity; right and left lateral views, respectively. 35, 36. Anal region; left and right lateral views, respectively. E = exudate. 37, 38. Posterior end; lateral and ventral views, respectively. E = exudate.**

va near esophageal–intestinal junction. Microfilaria with attenuated tail. Parasites of birds. Type species: *A. africanus* sp. n.

### *Andersonfilaria africanus* sp. n. (Figs. 28–51, 73–75)

GENERAL: Short, slender nematodes. Body width uniform over most of length but tapering gradually toward bluntly rounded extremities. Anterior end with constricted neck region and bulbous extremity. Cuticle thick, transverse striations delicate and closely spaced. Cuticle in lateral fields slightly thicker than elsewhere (Fig. 43) and not striated. Hemizonid readily visible, markedly protuberant in male and gravid female (Figs. 28, 39). Outline of underlying hypodermal tissue not observed in en face view of nongravid female (en face views of gravid female and male not examined). Cephalic papillae readily apparent, salient, symmetrically arranged in 2 circles, inner papillae slightly larger than outer (Fig. 42). Amphids not salient. Oral opening tiny, laterally




**Figures 39–51.** ***Andersonfilaria africanus*** **gen. n., sp. n. ♀. Allotype: Figures 39–41, 45–50. Paratypes: Figures 42–44, 51. 39. Anterior end; lateral view. H = hemizonid. 40. Vulva and anterior vagina; lateral view. 41. Anterior extremity; lateral view. 42. En face view. A = amphid. 43. Whole (43a) and partial (43b) transverse sections of midbody. 44. Anterior end; lateral view. 45, 46. Outlines of anterior and posterior ends, respectively; reproductive tract only shown. 47. Posterior end; lateral view. 48. Anal region, ventral view. 49–51. Posterior extremity; ventral, lateral, and lateral views, respectively.**

compressed, with delicate circumoral ring. Preesophageal ring absent. Anterior extremity of esophagus not well defined (Figs. 29, 41). Esophagus narrow in anterior region but increasing slightly in width posteriorly, neither muscular nor glandular tissue apparent (Figs. 28, 39), posterior region occasionally containing variable-sized vacuoles (Fig. 44). Cuticular lining of esophageal lumen difficult to discern. Gradual indistinct transition marking change from esophagus to intestine (Fig. 39). Phasmids subterminal.

MALE (1 specimen, holotype): Length 2.1 mm. Maximum width 85 μm. Width of head 35 μm, at constriction in neck region 31 μm, at nerve ring 43 μm, at anus 42 μm. Approximate number of transverse cuticular striations over 100 μm at midbody 130. Nerve ring 104 μm from anterior extremity. Approximate length of esophagus 210 μm. Maximum width of esophagus 12 μm. Posterior end of body in loose C-shaped ventral curve, not coiled or twisted. Spicules (Figs. 30, 31) dissimilar, uniformly cuticularized, left 55 μm long, right 37 μm long. Anus 88 μm from posterior extremity (note: a teardrop-shaped exudate extends posteriorly from the anal opening [Figs. 35, 36, 38]). Cuticular lips of anus thick, forming delicate circumanal ring (Fig. 38). Delicate nervelike strand of tissue present immediately anterior to anus, extending from hypodermis to cuticular surface (Figs. 35, 36). One (?2) minute adanal papilla(e) present on right side (Figs. 36, 38). No adanal papillae visible on left side (Fig. 35). Two small, sessile subterminal caudal papillae present (Fig. 38).

FEMALE (3 specimens, measurements of gravid allotype followed by 2 nongravid paratypes [second paratype damaged]): Length 9.7, 7.8, 6.7 mm. Maximum width 160, 114, 116 μm. Width of head 40, 38, 38 μm, at constriction in neck region 35, 35, 36 μm, at nerve ring 45, 50, — μm, at vulva 85, 80, 85 μm, at anus 58, 66, — μm. Approximate number of transverse cuticular striations over 100 μm at midbody 43, 56, 65. Nerve ring 110, 90, — μm from anterior extremity. Approximate length of esophagus 260, 280, 360 μm. Maximum width of esophagus 15, 18, 12 μm. Vulva 290, 260, 265 μm from anterior extremity. Lips of vulva same thickness as body cuticle, not protuberant (Fig. 40). Body swollen in vulvar region (Figs. 39, 45). Vagina directed posteriorly from vulva, occasionally looping, —, 930, 850 μm long. Didelphic and opisthodelphic. Uteri convoluted. Ovaries in posterior quarter of body. Anus visible as slightly salient delicate opening 188, 194, — μm from posterior extremity (Figs. 47, 48). Posterior extremity with slight bilateral swelling (Figs. 49–51).

MICROFILARIA: Body long (Fig. 74) with transversely striated cuticle. Anterior extremity bluntly rounded. V-shaped, cephalic cuticular structure present. Body width uniform over anterior ¾ of body, posterior region gradually tapering into attenuated tail with rounded extremity. Posteriormost nuclei elongate and linearly arranged, not extending to tail extremity (Fig. 73). Striated sheath present; tight around whole of body of microfilaria (Fig. 75) (note: sheath readily visible in Giemsa-stained thin blood smears and generally having become detached from body of microfilaria [Fig. 73], obscure in live specimens [Fig. 75], not visible in hematein-stained thin blood smears). Small inner body visible in some specimens. Measurements (in micrometers) as follows:

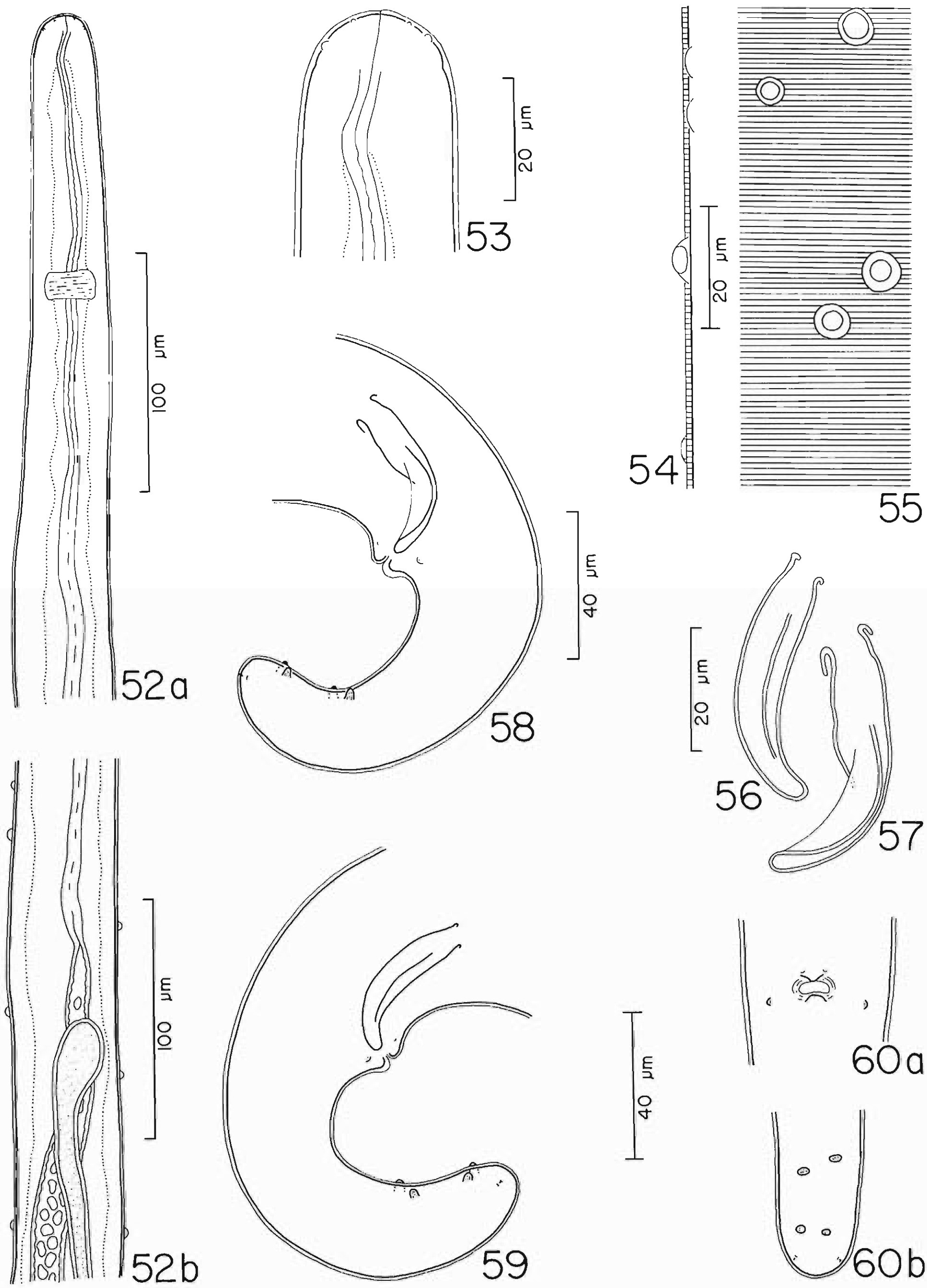
1) 10 specimens from each of 4 preparations, length (range with mean in parentheses) and maximum width:
   a) wet preparation (i.e., cover glass placed over drop of blood on slide), not stained (note: microfilariae were examined 24 hr after the preparation was made and at this time were moribund): 239–267 (255); 7.
   b) Giemsa-stained thin blood smear: 196–225 (209); 4.
   c) Giemsa-stained thick blood smear: 198–245 (214); 4.
   d) hematein-stained thin blood smear: 195–231 (213); 4–5.
2) 3 specimens in which some fixed points were visible, from Giemsa-stained thin blood smear: length 190, 215, 225; excretory pore 45, 45, 50; beginning of inner body 103, 118, 125; anal pore 140, 160, 170.

TYPE HOST: Common waxbill, *Estrilda astrild* (L.) (Passeriformes: Estrildidae).

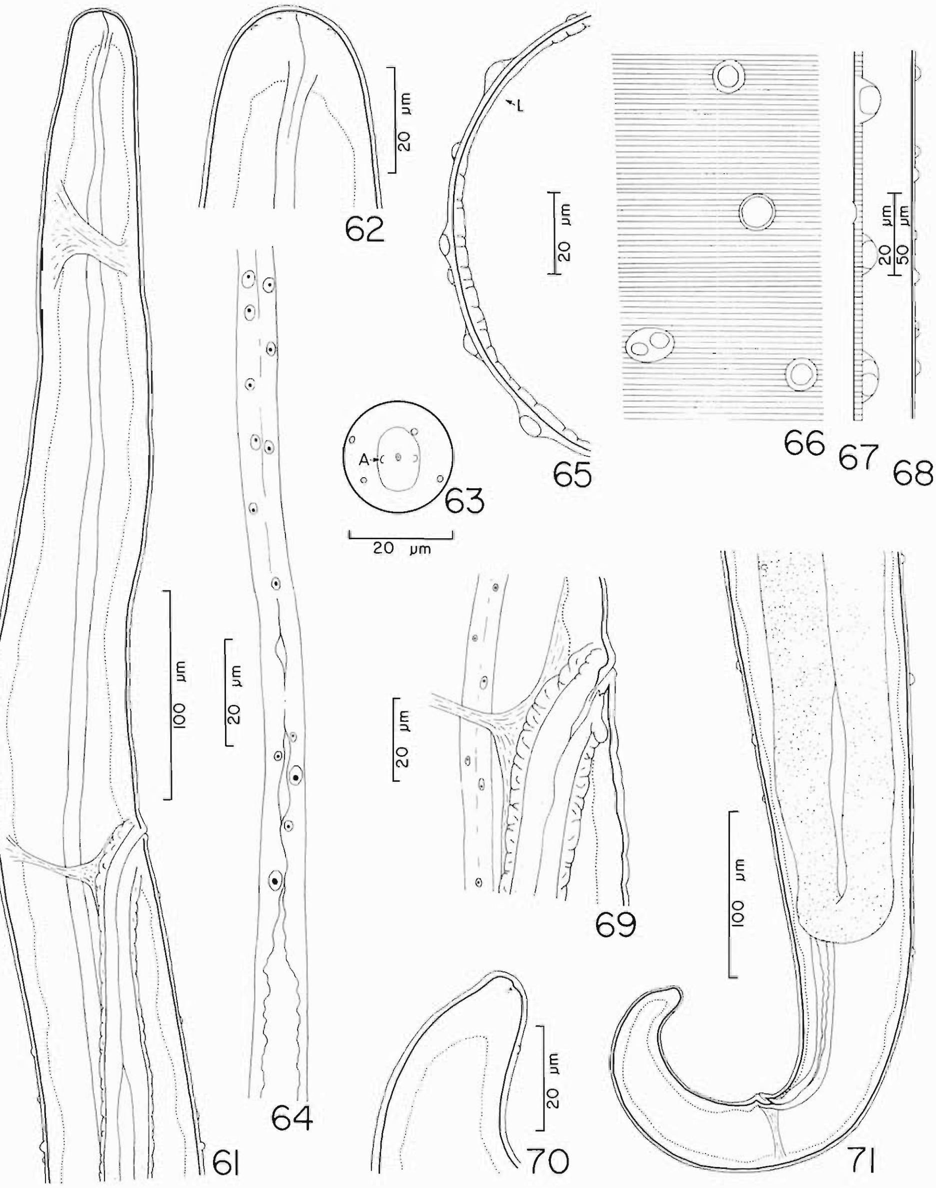
LOCATION IN HOST: Adults within fossa in dorsal wall of pelvic girdle underneath middle region of right kidney. Microfilariae in blood.

TYPE LOCALITY: Africa. Note: the bird was imported from Africa into Paris, France, where it was purchased at a pet store. The common waxbill is native to most of Africa south of the Sahara (Walters, 1980).

SPECIMENS: Muséum National d'Histoire



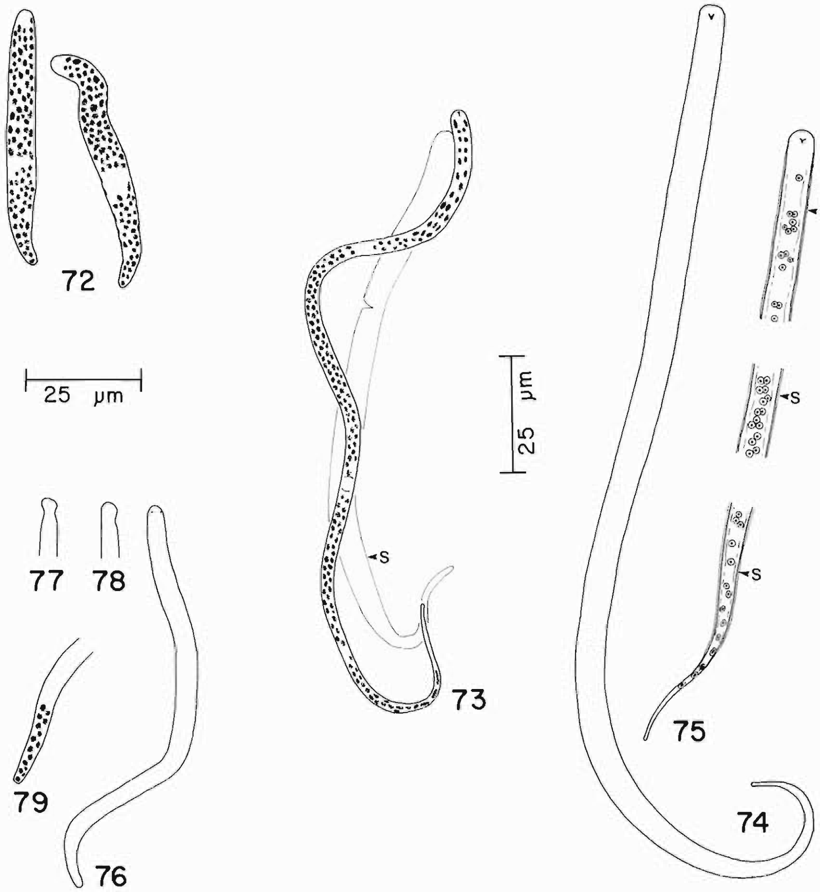

**Figures 52–60.** ***Splendidofilaria chandenieri*** **sp. n. ♂, holotype. 52. Anterior end, showing anteriormost region (52a) and esophageal–intestinal junction (52b); dorsal–ventral view. 53. Anterior extremity; dorsal–ventral view. 54, 55. Cuticle; lateral and surface views, respectively. 56, 57. Spicules, right and left, respectively; lateral view. 58, 59. Posterior end, lateral views. 60. Posterior end, showing anal region (60a) and extremity (60b); ventral view.**




**Figures 61–71.** ***Spendidofilaria chandenieri* sp. n. ♀, allotype. 61. Anterior end; lateral view. 62. Anterior extremity; dorsal–ventral view. 63. En face view. A = amphid. 64. Esophageal–intestinal junction; lateral view. 65. Partial transverse section of midbody. L = lateral field. 66–68. Cuticle; surface, lateral, and lateral views, respectively. 69. Vulva and anterior vagina; lateral view. 70. Posterior extremity; lateral view. 71. Posterior end; lateral view.**

Naturelle, Paris, France (MNHN). Holotype (♂), allotype (♀), and paratypes (♀): MNHN No. 85 DL. Microfilariae: MNHN Nos. N VII 28–30, 42, 43.

Taxonomic comments: Within Splendidiofilariinae, the four pairs of cephalic papillae and undivided, poorly developed esophagus in *Andersonfilaria* gen. n. from birds are also seen only in *Micipsella* Seurat, 1921, from lagomorphs and *Cardianema* Alicata, 1933, from turtles. However, *Andersonfilaria* and *Micipsella* have small, uniformly cuticularized spicules, whereas *Cardianema* has rather long spicules, the distal parts of which are membranous. *Andersonfilaria* has a




**Figures 72–79. Microfilariae (striations on body and/or on sheath not illustrated). 72. *Dessetfilaria guianensis* gen. n., sp. n.; from hematein-stained thin blood smear. 73. *Andersonfilaria africanus* gen. n., sp. n.; from Giemsa-stained thin blood smear; note sheath (S), which has detached from body of microfilaria. 74. *Andersonfilaria africanus,* outline of body; from unstained wet preparation. 75. *Andersonfilaria africanus,* detail of anterior end, midbody, and posterior end; note tight sheath (S); from unstained wet preparation. 76. *Splendidofilaria chandenieri* sp. n., outline of body; from unstained wet preparation. 77, 78. *Splendidofilaria chandenieri,* anterior end, showing "pinched" appearance; from unstained wet preparation. 79. *Splendidofilaria chandenieri,* detail of posterior end; from unstained wet preparation.**

smooth cuticle, whereas *Micipsella* has a bossate cuticle; moreover, the minute size (2.1 mm) of the male of *A. africanus* clearly contrasts with the large size (22–100 mm) of the males of *Micipsella* species. The long microfilaria with an attenuated tail and the reduced number of caudal papillae in *Andersonfilaria* are reminiscent of *Cardiofilaria* Strom, 1937, from birds. *Andersonfilaria* has, however, a poorly developed esophagus that is indistinctly demarcated from the intestine, whereas *Cardiofilaria* has a broad muscular esophagus, clearly demarcated from the intestine. *Cardiofilaria* also has a pre-esophageal ring, which *Andersonfilaria* lacks.

ETYMOLOGY: *Andersonfilaria* gen. n. is named in honor of Professor Roy C. Anderson of Guelph, Ontario, Canada; "*africanus*" denotes the continent from which the infected bird was imported.

### *Splendidofilaria chandenieri* sp. n. (Figs. 52–71, 76–79)

GENERAL: Spirurida, Filarioidea, Onchocercidae, Splendidofilariinae Chabaud and Choquet, 1953 (sensu Anderson and Bain, 1976), *Splendidofilaria* Skjrabin, 1923 (sensu Anderson and Bain, 1976). Long, slender nematodes. Body width uniform over most of length, but tapering gradually toward bluntly rounded extremities. Cuticle thin, transversely striated, with variable-sized oval to round bosses (Figs. 54, 55, 65–68). Bosses generally single, rarely double, not extending to extremities of body and not arranged in any discernible pattern; each boss with a round, more dense central portion. Cuticle in lateral fields not thicker than elsewhere (Fig. 65), striated. Oval outline of underlying hypodermal tissue visible in en face view of female (en face view of male not examined). Cephalic papillae readily apparent, asymmetrically arranged in broad circle around oral opening (Fig. 63). Amphids slightly salient. Oral opening tiny, laterally compressed, with delicate circumoral ring. Pre-esophageal ring absent. Anterior extremity of esophagus not well defined (Figs. 53, 62). Esophagus narrow, devoid of glandular tissue (Figs. 52, 61), posterior half with numerous large nuclei (Fig. 64). Cuticular lining of esophageal lumen difficult to discern. Junction of esophagus and intestine not well defined (Fig. 64). Caudal languettes not present. Phasmids terminal.

MALE (1 specimen, holotype): Length 17 mm. Maximum width 65 μm. Width of body at nerve ring 32 μm, at anus 42 μm. Number of transverse cuticular striations over 100 μm at midbody 90. Bosses commencing approximately ½ mm from anterior extremity, ending approximately 1 mm anterior to anus. Nerve ring 105 μm from anterior extremity. Approximate length of esophagus 545 μm. Maximum width of esophagus 9 μm. Posterior end of body in loose C-shaped ventral curve, not coiled or twisted. Spicules (Figs. 56, 57) slightly dissimilar, uniformly cuticularized, both 45 μm long. Anus 110 μm from posterior extremity. Cuticular lips of anus thickest posteriorly. Hypodermal swelling present immediately anterior to and posterior to anus (Fig. 60a). Preanal papillae absent. Sessile adanal papillae present, consisting of 2 obscure papillae at base of anterior hypodermal swelling and 2 larger papillae lateral to posterior hypodermal swelling (Fig. 60a). Semipedunculate postanal papillae present, consisting of 2 pairs on posterior half of tail (Figs. 58, 59, 60b).

FEMALE (1 gravid specimen, allotype): Length 38 mm. Maximum width 140 μm. Width at nerve ring 50 μm, at vulva 72 μm, at anus 45 μm. Number of transverse cuticular striations over 100 μm at midbody 75. Bosses commencing slightly posterior to vulva, ending approximately 130 μm anterior to anus. Nerve ring 110 μm from anterior extremity. Approximate length of esophagus 565 μm. Maximum width of esophagus 10 μm. Vulva 385 μm from anterior extremity. Cuticular lips of vulva thick, slightly salient (Fig. 69). Body not swollen in vulvar region (Fig. 61). Vagina directed posteriorly from vulva, not convoluted, 1.3 mm long. Didelphic and opisthodelphic. Ovaries in posterior 3 mm of body. Anus visible as salient, readily apparent opening 150 μm from posterior extremity (Fig. 71).

MICROFILARIA: Body short (Fig. 76) with transversely striated cuticle. Anterior extremity bluntly rounded, appearing "pinched" in some specimens (Figs. 77, 78). Two minute, cephalic cuticular structures present. Body width uniform over anterior ⅔ of body, posterior region tapering slightly toward rounded extremity. Posteriormost nucleus rounded, present at tail extremity (Fig. 79). Sheath absent. Small inner body present. Measurements (in micrometers) as follows:

1) 10 specimens from each of 4 preparations, length (range with mean in parentheses) and maximum width:
   a) Wet preparation (i.e., cover glass placed over drop of blood on slide), not stained (note: the microfilariae were examined 24 hr after the preparation was made and at this time were moribund): 96–113 (106); 4–6.
   b) Giemsa-stained thin blood smear: 47–68 (58); 3–4.
   c) Giemsa-stained thick blood smear: 45–75 (59); 3–4.
   d) hematein-stained thin blood smear: 43–50 (47); 3–4.

TYPE HOST: Common waxbill, *Estrilda astrild* (L.) (Passeriformes: Estrildidae).

LOCATION IN HOST: Adults in subcutaneous connective tissue near distal end of right humerus. Microfilariae in blood.

TYPE LOCALITY: Africa. Note: the bird was imported from Africa into Paris, France, where it was purchased at a pet store. The common waxbill is native to most of Africa south of the Sahara (Walters, 1980).

SPECIMENS: Muséum National d'Histoire Naturelle, Paris, France (MNHN). Holotype (♂) and allotype (♀): MNHN No. 85 DL. Microfilariae: MNHN Nos. N VII 28–30, 42, 43.

TAXONOMIC COMMENTS: Sixteen bossate species of *Splendidofilaria* have been previously described, 11 from the heart or body cavity of the host and five from the subcutaneous tissues. *Splendidofilaria chandenieri* sp. n. can be distinguished easily from other subcutaneous species, as *S. gedoelsti* Travassos, 1926, *S. gvozdevi* Sonin and Barus, 1978, *S. singhi* Sultana, 1962, *S. columbensis* Olsen and Braun, 1976, and *S. hibleri* Olsen and Braun, 1976, have large preanal papillae, which are lacking in the new species.

ETYMOLOGY: The new species is named in honor of Mr. Jacques Chandenier of Paris, France.

## Discussion

Significant progress has been made in avian filarioid systematics in the past 25 yr, yet, because of our limited knowledge of the filarioid fauna of birds in the equatorial regions and the Southern Hemisphere, undescribed taxa likely remain. The present study, in describing two new genera and three new species, places significant generic value on esophageal morphology and number of cephalic papillae, and thus follows earlier proposals by Anderson (1961, 1968) and Anderson and Bain (1976).

The poorly developed, undivided esophagus in *Splendidofilaria* and *Andersonfilaria* likely evolved from a well-developed, either divided or undivided, esophagus as in the presumed spirurid ancestors of the filarioids and in numerous extant filarioid genera. The esophagus in *Dessetfilaria guianensis* has no apparent muscular tissue in the reduced anterior portion, and thus may represent an intermediate stage in this evolution. The poorly developed, undivided esophagus appears to have arisen independently on a number of occasions, however, as it occurs in Onchocercinae and Lemdaninae as well as in Splendidofilariinae.

Anderson (1968) suggested that the cephalic papillae pattern observed in *Pseudofilaria* Sandground, 1935 (four submedian pairs plus six papillae in an inner circle), be regarded as the most primitive filarioid type, and he outlined the morphologic changes by which the "typical filarioid pattern" of only four submedian pairs (such as that in *Andersonfilaria* and the majority of genera) could be attained and eventually give rise to the highly specialized condition of only two, often asymmetric, pairs in *Splendidofilaria.* The extreme reduction in size and the linear arrangement of the two pairs in *Dessetfilaria* appears to be a further specialization of the *Splendidofilaria* condition.

The new taxa described in the present study were found in birds of the families Ramphastidae and Estrildidae. The former family is indigenous to the tropics of the New World, and 33 species are recognized, most being restricted to lowland forests, although there are a few exceptions (Haffer, 1974). Ramphastids are semigregarious, especially when feeding, and nest in tree cavities. In addition to *Dessetfilaria guianensis* and *D. braziliensis,* filarioids reported from the family include *Eulimdana micropenis* (Travassos, 1926) Bartlett, Wong, and Anderson, 1985, *Splendidofilaria gedoelsti* Travassos, 1926, and *Pelecitus helicinus* (Molin, 1860) Railliet and Henry, 1910.

The family Estrildidae (sometimes considered a subfamily of Ploceidae) contains about 125 species occurring in Africa, southeast Asia, and Australia (Walters, 1980). In general, estrildids occur in grasslands, scrublands, forest edges, forests, and clearings. A few occur in marshes. They are highly gregarious and many nest in huge colonies consisting of large domed nests. In addition to *Andersonfilaria africanus* and *Splendidofilaria chandenieri,* filarioids reported from Estrildidae include *Chandlerella sultana* (Sonin, 1966) Anderson and Freeman, 1969, and *Eufilaria mcintoshi* Anderson and Bennett, 1960.

## Acknowledgments

We thank Mr. Ferrere of Cayenne, French Guiana, and Mr. Chandenier of Paris, France, who provided the infected birds. This study was supported by the Muséum National d'Histoire Naturelle, Paris, France. C. M. Bartlett was the recipient of a Postdoctoral Fellowship from the Natural Sciences and Engineering Research Council of Canada.

## Literature Cited

**Anderson, R. C.** 1961. *Splendidofilaria wehri* n. sp. with a revision of *Splendidofilaria* and related species. Canadian Journal of Zoology 39:201–207.

———. 1968. The comparative morphology of cephalic structures in the superfamily Filarioidea (Nematoda). Canadian Journal of Zoology 46:181–199.

———**, and O. Bain.** 1976. Keys to the genera of the order Spirurida. Part 3. Diplotriaenoidea, Aproctoidea, and Filarioidea. Pages 59–116 *in* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 3. Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, England.

**Bartlett, C. M., and R. C. Anderson.** 1985. On the filarioid nematodes from the pulmonary arteries of birds. Canadian Journal of Zoology 63:2373–2377.

**Bennett, G. F., M. Whiteway, and C. Woodworth-Lynas.** 1982. A host–parasite catalogue of the avian haematozoa. Memorial University of Newfoundland, Occasional Papers in Biology, No. 5.

**Haffer, J.** 1974. Avian speciation in tropical South America. With a systematic survey of the toucans (Ramphastidae) and jacamars (Galbulidae). Publication of the Nuttall Ornithological Club, No. 14.

**Sonin, M. D.** 1977. Filariata of animals and man and diseases caused by them. *In* K. M. Ryzhikov, ed. Fundamentals of Nematology. Vol. 28, Part 4. Izdatel'stvo "Nauka," Moscow, U.S.S.R. (Translated from Russian for the United States Department of Agriculture by Amerind Publishing Co. Pvt. Ltd., 66 Janpath, New Delhi 110001, India, 1985.)

**Walters, M.** 1980. The Complete Birds of the World. David & Charles (Publishers) Limited, Newton Abbot.

**Yeh, L. S.** 1957. On *Chandlerella braziliensis* n. sp. from a green-billed toucan and a discussion on some related genera. Journal of Helminthology 31: 33–38.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 15–23

# Descriptions of Three Nematode Parasites of Salamanders (Plethodontidae: Desmognathinae) from the Southeastern United States

MICHAEL R. BAKER,[1] TIMOTHY M. GOATER,[2] AND GERALD W. ESCH[2]
[1] Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada and
[2] Department of Biology, Wake Forest University, Box 7325 Reynolda Station,
Winston-Salem, North Carolina 27109

ABSTRACT: Three species of parasitic nematodes (Ascaridida) are described from desmognathine salamanders of the Nantahala National Forest, Macon County, North Carolina. *Falcaustra plethodontis* sp. n. (Cosmocercoidea: Kathlaniidae: Kathlaniinae), from *Leurognathus marmorata* (type host), *Desmognathus quadramaculatus, D. monticola,* and *D. ochrophaeus,* is readily distinguished from all other *Falcaustra* species by the possession of three short, posteriorly directed cephalic cordons. It may also be distinguished from other North American species in lacking a caudal pseudosucker in males and in possessing markedly short, robust spicules. *Desmognathinema nantalaensis* gen. n., sp. n. (Seuratoidea: Quimperiidae: Quimperiinae), from *Desmognathus quadramaculatus* (type host) and *D. monticola,* represents the first Quimperiinae species reported in salamanders. It most closely resembles the monobasic genus *Quimperia* from African fish, but it differs in lacking well-developed lateral alae, in the distribution of caudal papillae in males, and in the male caudal musculature. *Omeia papillocauda* Rankin, 1937, is redescribed based on specimens from *Desmognathus quadramaculatus, D. monticola,* and *D. ochrophaeus. Omeia chickasawi* Walton, 1940, is shown to be a synonym of *O. papillocauda.*
KEY WORDS: *Desmognathinema nantahalaensis* gen. n., sp. n., *Falcaustra plethodontis* sp. n., *Omeia papillocauda, Desmognathus* spp., *Leurognathus marmorata,* taxonomy, morphology, redescription, North Carolina.

The three species of nematodes described herein were collected during a study of the parasite community ecology of sympatric desmognathine salamanders from southern Appalachian mountain streams of southwestern North Carolina (Goater, 1985).

## Materials and Methods

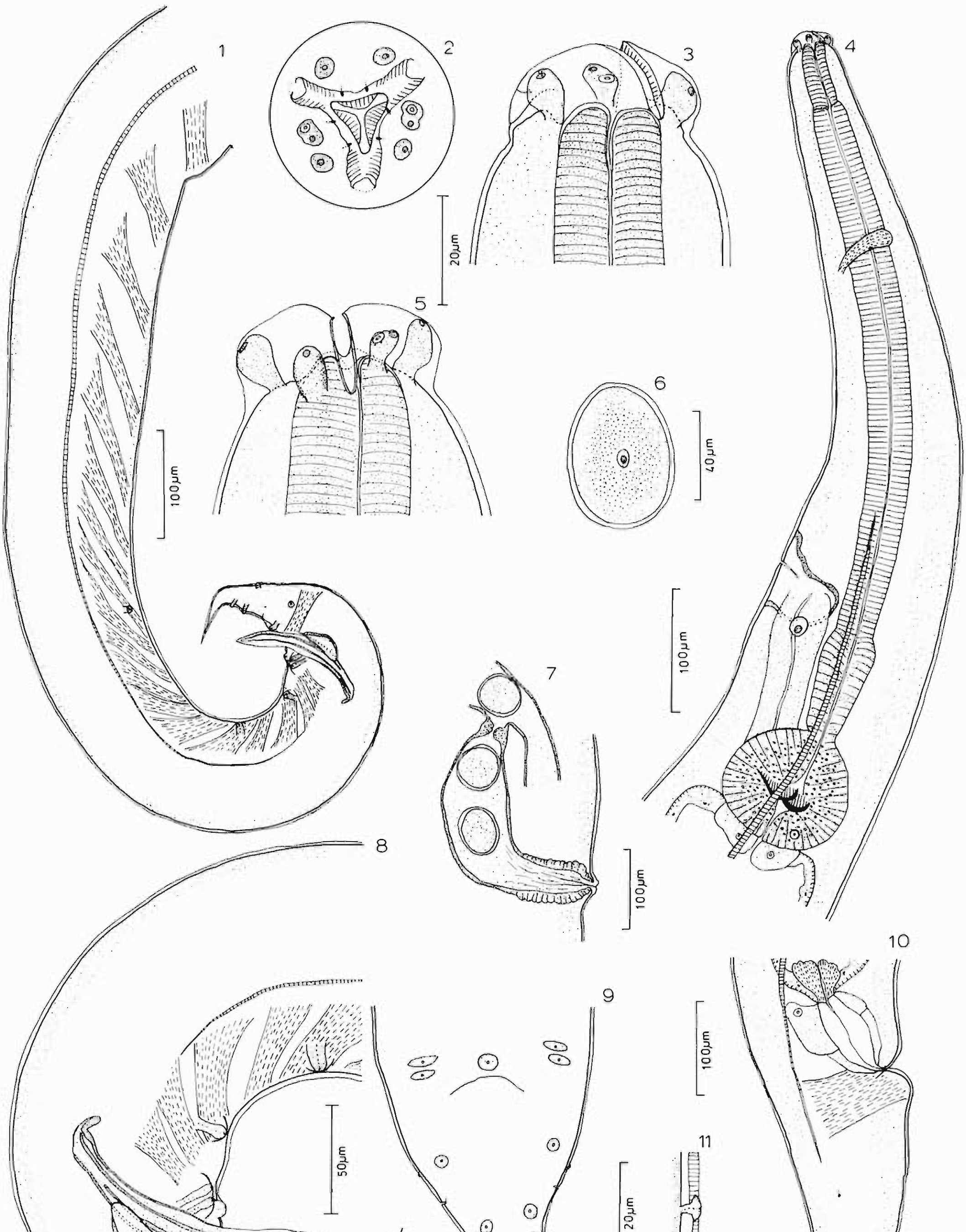
The specimens of the new species described herein were fixed and stored in 70% ethyl alcohol and cleared in lactophenol or glycerin jelly (for apical view of cephalic structures). For the new species, measurements given are for the holotype male and allotype female, followed in parentheses by range in measurements for paratype males and females. For *O. papillocauda,* measurements given are ranges of specimens examined. Measurements are given in micrometers unless otherwise specified.

Type specimens of *Omeia papillocauda* Rankin, 1937, from *Desmognathus quadramaculatus* of North Carolina (USNM Helm. Coll. No. 8980), and *O. chickasawi* Walton, 1940, from *Eurycea bislineata* of Tennessee (USNM Helm. Coll. No. 42084), were borrowed from the National Parasite Collection of the United States.

## *Falcaustra plethodontis* sp. n.
## (Figs. 1–11)

DESCRIPTION: Cosmocercoidea, Kathlaniidae, Kathlaniinae, *Falcaustra* Lane, 1915. Oral opening triangular, 3 small vesiculated lips present, 3 short groovelike cordons present between lips, extending posteriorly about 7. Cephalic papillae and amphids arising from 6 fleshy peduncles extending through vesiculated lips to edge of body cuticle: 6 small inner papillae arising from near base of peduncles and 6 outer papillae located at terminal end of peduncles, amphids associated with lateral peduncles. Lips lacking sclerotized cheilostomal support ring, but cuticle at 3 corners of oral opening thickened. Esophagus divided into short anterior pharyngeal portion, elongate posterior portion of corpus, slightly swollen isthmus, and spherical bulb with prominent valves. Narrow lateral alae present, extending from near excretory pore to about 70 anterior to anus in males and to anterior third of tail in females. Small lateral anterior deirids present near excretory pore. Excretory pore small, opening directly into large thick-walled vesicle with single large nucleus located posteriorly; 2 large posteriorly directed lateral canals arising from vesicle.

MALES (holotype, 4 paratypes): Total length 4.0 (3.9–4.3) mm. Length of esophagus 670 (633–684) (pharynx 64 [53–62], corpus 412 [391–430], isthmus 93 [79–97], bulb 101 [101–104]). Nerve ring 257 (214–265), excretory pore 441 (428–467) from anterior extremity. Tail 102 (110–113) long, conical, and sharply pointed. Caudal pa-




**Figures 1–11.** ***Falcaustra plethodontis* sp. n. 1. Caudal end of male, lateral view. 2, 3. Cephalic end of male, apical and lateral views. 4. Anterior end of male, lateral view. 5. Cephalic end of male, view between subventral**

pillae distributed as follows: preanal region with 1 unpaired papilla on the anterior lip of the cloaca and 5 pairs of subventral papillae, the posteriormost 2 pairs of which are located adjacent to each other; tail with 3 pairs of subventral papillae, 1 pair of subdorsal papillae, and 1 pair of lateral papillae. Phasmids located near middle of tail. Oblique caudal musculature present (28 pairs of muscles in holotype, 22–28 pairs in paratypes), not forming caudal pseudosucker. Spicules 131 (110–125) long, equal, alate and robust, with sharply pointed distal extremities. Gubernaculum 37 (37–39) long, lateral edges curved around spicules.

FEMALES (allotype, 4 paratypes): Total length 4.9 (4.2–5.8) mm. Length of esophagus 696 (638–761). Nerve ring 265 (265–294), excretory pore 464 (431–500), vulva 2.9 (2.7–3.5) mm from anterior extremity. Tail 240 (225–316) long, conical, and sharply pointed. Phasmids located near middle of tail. Vagina 300 long, curved anteriorly, muscular and thick-walled in proximal third, thin-walled in portion joining uteri. Uteri opposed, ovary of anterior uterus located anterior to vulva, ovary of posterior uterus located posterior to vulva. Eggs 59–67 long and 50–52 wide (based on 5 eggs), at 1-cell stage of development.

TYPE HOST: *Leurognathus marmorata* Moore, 1899 (Plethodontidae: Desmognathinae), shovel-nosed salamander.

LOCATION: Colon.

LOCALITY: Nantahala National Forest (approximately 900 m elevation), Macon County, North Carolina. The exact locality is not available. However, the type specimens were selected from salamanders collected at the following 2 sites: (1) Bear Pen and Curtis creeks, tributaries of the Nantahala River, 25 km SW of Franklin, North Carolina; (2) Abes and Overflow creeks, 15 km SW of Highlands, North Carolina.

SPECIMENS: USNM Helm. Coll. Nos. 79159 (holotype), 79160 (allotype), and 79161 (paratypes).

PREVALENCE: 22.0% of 50 salamanders sampled.

OTHER HOSTS: (1) *Desmognathus quadramaculatus* (Holbrook, 1840), black-bellied salamander. Same localities as type specimens. Prevalence was 0.9% of 115 salamanders sampled. (2) *Desmognathus monticola* Dunn, 1916, seal salamander. Same localities as type specimens. Prevalence was 8.0% of 125 salamanders sampled. (3) *Desmognathus ochrophaeus* Cope, 1859, mountain dusky salamander. Same localities as type specimens. Prevalence was 0.9% of 107 salamanders sampled.

COMMENTS: The possession of short cephalic cordons between the cephalic lips readily distinguishes *Falcaustra plethodontis* sp. n. from other species in the genus that lack these structures. In fact, in the Kathlaniinae cephalic cordons have been reported only in *Urodelnema* spp. from cryptobranchid salamanders of North America. This genus was distinguished from *Falcaustra* by the possession of three conspicuous cordons that arise from the three corners of the mouth and curve around the base of the cephalic lips (Baker, 1981). The cephalic cordons in *F. plethodontis* are possibly homologous with these highly specialized structures. However, the species has been placed in *Falcaustra* because the cordons are much less well developed and they do not curve around the cephalic lips. In addition, *F. plethodontis* is markedly different from *Urodelnema* species in male caudal morphology (spicules, gubernaculum, caudal musculature), suggesting it may not be closely related phylogenetically.

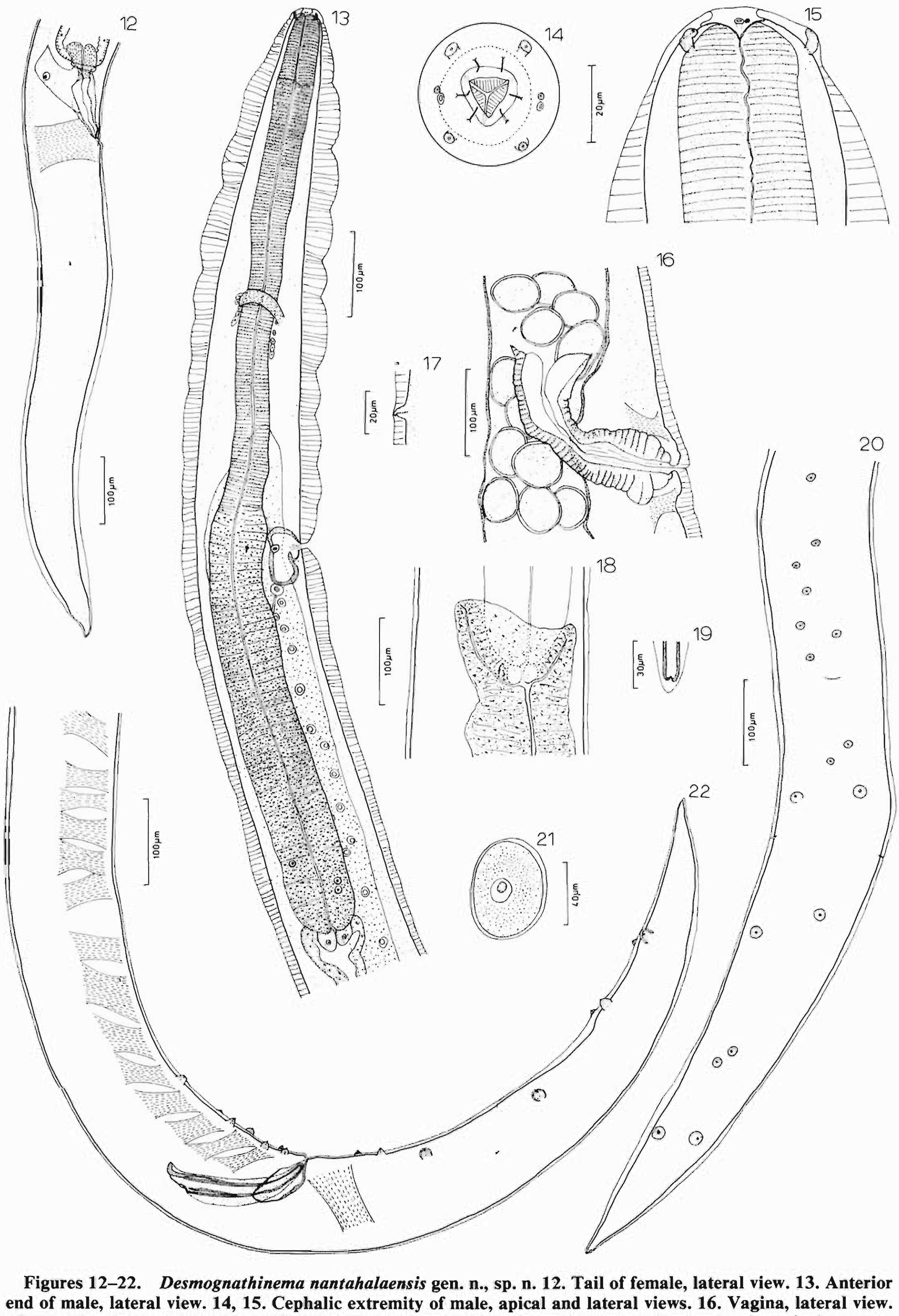
In morphology exclusive of the cephalic end, *F. plethodontis* is readily distinguished from all other *Falcaustra* species from North America in lacking a caudal pseudosucker in males and in possessing markedly short robust spicules.

### *Desmognathinema* gen. n.

DIAGNOSIS: Seuratoidea, Quimperiidae, Quimperiinae. Cephalic vesicle and cervical alae lacking, body cuticle irregularly thickened especially in anterior end, with prominent irregularly spaced transverse striations; mouth triangular, buccal capsule small and thin-walled; anterior extremity of esophagus lacking onchia; esophagus elongate and divided into glandular posterior portion and muscular anterior portion with distinct anterior pharyngeal part; caudal papillae in males all ventral or subventral in po-

←

**and dorsal lips to show cephalic cordon. 6. Egg from uterus. 7. Vagina, lateral view. 8, 9. Caudal end of male, lateral and ventral views. 10. Tail of female, lateral view. 11. Anterior deirid, dorsal view.**




**Figures 12–22.** ***Desmognathinema nantahalaensis*** **gen. n., sp. n. 12. Tail of female, lateral view. 13. Anterior end of male, lateral view. 14, 15. Cephalic extremity of male, apical and lateral views. 16. Vagina, lateral view. 17. Anterior deirid, lateral view. 18. Esophageal–intestinal junction of large female, showing intestinal caecum,**

sition; oblique muscle bands in preanal region of male present but not forming pseudosucker. Parasitic in the small intestine of plethodontid salamanders.

TYPE AND ONLY SPECIES: *Desmognathinema nantahalaensis* sp. n.

### *Desmognathinema nantahalaensis* sp. n. (Figs. 12–22)

DESCRIPTION: Oral opening triangular, lips lacking. Cephalic extremity with 6 small inner cephalic papillae, 4 large outer pedunculate cephalic papillae, and 2 outer sessile lateral papillae beside amphids. Cephalic capsule small, thin-walled. Anterior extremity of esophagus lacking onchia. Esophagus divided into posterior slightly swollen glandular portion and anterior muscular portion with relatively inconspicuous anterior pharyngeal portion. In small specimens intestine usually extending in straight line posterior to esophageal–intestinal junction; in large specimens intestine frequently extending forward over end of esophagus in form of an irregularly shaped intestinal diverticulum. Cephalic vesicle lacking. Body cuticle irregularly thickened especially in anterior end, with prominent irregularly spaced transverse striations. Lateral alae lacking. Blunt anterior deirids present, located near excretory pore. Excretory pore opening directly into conspicuous cuticle-lined vesicle; vesicle wall with single large terminal duct nucleus; vesicle surrounded by large mass of glandular tissue containing numerous nuclei and extending on both sides of body from point just anterior to excretory pore to posterior half of body.

MALES (holotype, 8 paratypes): Total length 8.5 (6.8–9.2) mm. Length of esophagus, 1,190 (925–1,080). Nerve ring 335 (310–333), excretory pore 580 (510–612) from anterior extremity. Tail 650 (635–655) long, conical, and sharply pointed. Caudal papillae distributed as follows: preanal papillae variable in location (all ventral to slightly subventral in position) and not clearly paired, 9 present in holotype and from 6 to 9 in paratypes; anterior third of tail with 1 relatively small ventral pair and 1 relatively large subventral pair; mid-region of tail with 1 relatively large subventral pair; posterior third of tail with 1 relatively small ventral pair and 1 relatively large subventral pair. Phasmids located in anterior third of tail. Oblique preanal caudal musculature present (21 pairs of muscle cells in holotype, 18–21 pairs in paratypes), not forming caudal pseudosucker. Spicules 170 (157–165) long, equal, alate with blunt distal extremities. Gubernaculum 75 (66–72) long, well sclerotized, lateral edges curved around spicules.

FEMALES (allotype, 10 paratypes): Total length 13.7 (7.9–17.4) mm. Length of esophagus 1,330 (960–1,280). Nerve ring 385 (340–420), excretory pore 730 (605–990), vulva 7.9 (4.9–11.1) mm from anterior extremity. Tail 1,120 (620–1,065) long, conical, and sharply pointed. Phasmids located in anterior third of tail. Vagina 200 long, curved slightly anteriorly. Uteri opposed, terminal point of ovary of posterior uterus located near posterior end of body, terminal point of ovary of anterior uterus variable in position, either located slightly anterior to vulva or slightly posterior to vulva. Eggs 61–67 long and 45–52 wide (based on 5 eggs), at 1-cell stage of development.

TYPE HOST: *Desmognathus quadramaculatus* (Holbrook, 1840) (Plethodontidae: Desmognathinae), black-bellied salamander.

LOCATION: Small intestine.

LOCALITY: Same as for *Falcaustra plethodontis* sp. n.

SPECIMENS: USNM Helm. Coll. Nos. 79156 (holotype), 79157 (allotype), and 79158 (paratypes).

PREVALENCE: 36.5% of 115 salamanders sampled.

OTHER HOST: *Desmognathus monticola* Dunn, 1916, seal salamander. Same localities as type specimens. Prevalence was 4.8% of 125 salamanders sampled.

COMMENTS: *Desmognathinema* gen. n. is the first Quimperiinae genus to be reported from salamanders. It most closely resembles the monospecific genus *Quimperia* Gendre, 1926, from African fish (see Vassiliades, 1971), in possessing an elongate divided esophagus that is slightly inflated posteriorly, and in cephalic morphology (mouth triangular, buccal capsule small and thin-walled, onchia absent). However, these two gen-

←

**lateral view. 19. Distal extremity of one spicule, lateral view. 20. Caudal end of male, ventral view. 21. Egg from uterus. 22. Caudal end of male, lateral view.**

era differ in the following: (1) lateral alae present in *Quimperia,* absent in *Desmognathinema*; (2) postanal caudal papillae all subventral in position in *Desmognathinema,* subventral, lateral, and subdorsal in *Quimperia*; and (3) preanal pseudosucker present in male *Quimperia,* absent in *Desmognathinema.*

*Desmognathinema* also resembles the monobasic genus *Pseudohaplonema* Wang, Zhao, and Chen, 1978, from freshwater turtles of China, in that both possess a divided esophagus and lack lateral alae, a cephalic vesicle, and a caudal pseudosucker in males. Unfortunately the cephalic and caudal structures of *Pseudohaplonema* were not described in enough detail to permit adequate comparison (Wang et al., 1978). Nevertheless, *Pseudohaplonema* and *Desmognathinema* may be distinguished by the following: (1) whereas the glandular portion of the esophagus in *Desmognathinema* is about the same length as the muscular portion, in *Pseudohaplonema* it is about three times the length of the muscular portion; and (2) the distribution of the caudal papillae in males is quite dissimilar in ventral view. In addition, as noted above, the host and geographical distributions are different.

The Quimperiinae are mainly parasitic in fish, with eight genera reported only in freshwater or catadromous fish throughout the world (*Quimperia* Gendre, 1928, *Paraseuratum* Johnston and Mawson, 1940, *Haplonema* Ward and Magath, 1917, *Paraquimperia* Baylis, 1934, *Paragendria* Baylis, 1939, *Ezonema* Boyce, 1971, *Pingus* Hsu, 1933, *Neoquimperia* Wang, Zhao, Wang, and Zhang, 1979, *Buckleynema* Ali and Singh, 1954), one in anuran amphibians of South America (*Subulascaris* Freitas and Dobbin, 1957), two in both fish and anurans of Africa and Indomalaysia (*Chabaudus* Inglis and Ogden, 1965, *Gendria* Baylis, 1930), and, as mentioned above, *Pseudohaplonema* in Chinese turtles and *Desmognathinema* in desmognathine salamanders of North America. The presence of isolated monobasic genera in hosts such as salamanders and freshwater turtles probably represents individual parasite "captures" by these hosts in an aquatic habitat. This "capture" phenomenon is of common occurrence in the evolutionary history of the nematodes of vertebrates (Chabaud, 1981).

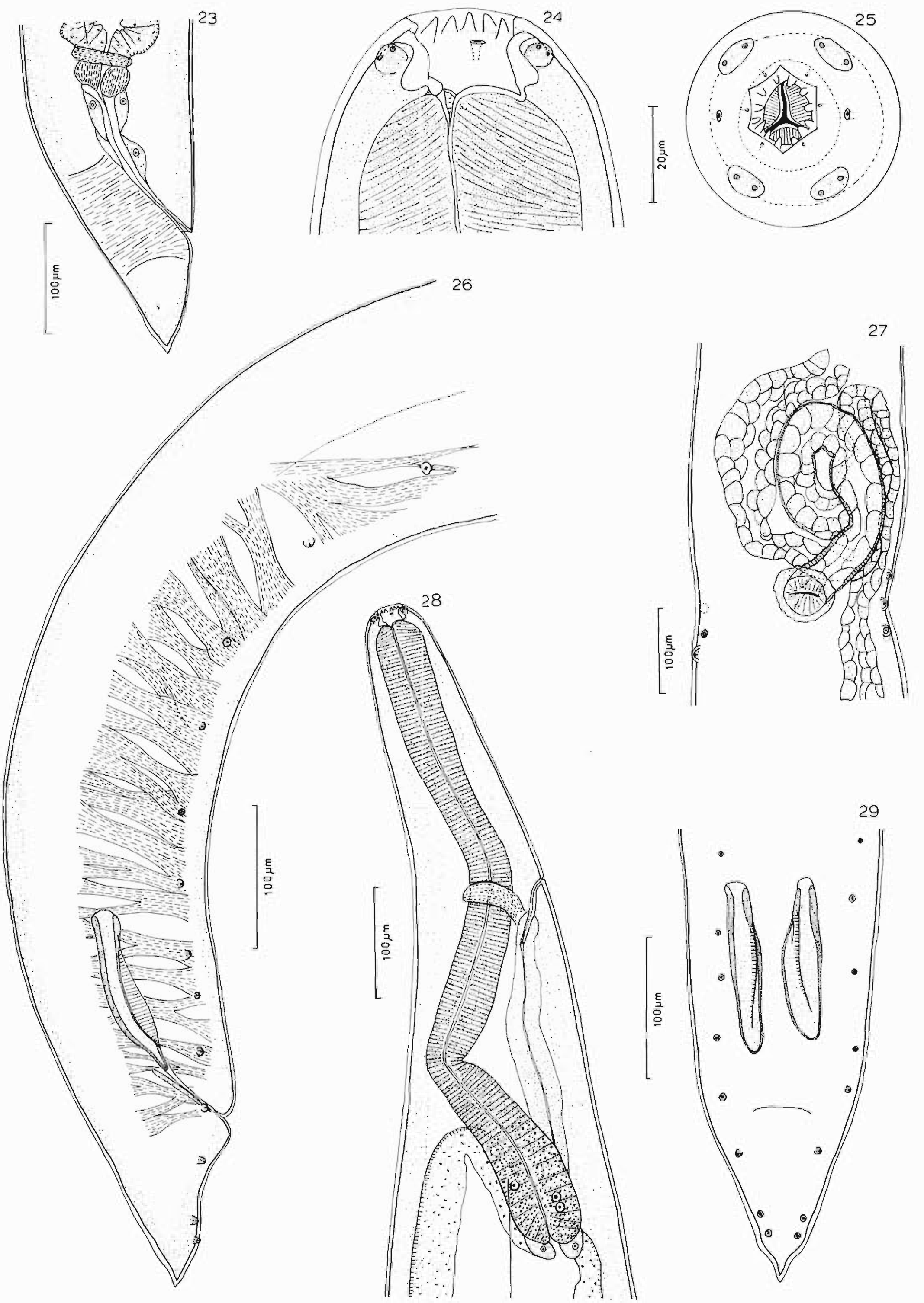
The presence of an intestinal diverticulum extending around the posterior portion of the esophagus in large specimens of *D. nantahalaensis* is not observed in other Quimperiinae. However, possession of a large intestinal caecum is one of the main diagnostic features of the subfamily Omeiinae (one genus, *Omeia* Hsu, 1933), which with the Quimperiinae constitutes the family Quimperiidae (see Chabaud, 1978). In addition, *D. nantahalaensis* and *Omeia papillocauda* Rankin, 1937, coexist in the same desmognathine salamander populations (Goater, 1985). These points may be construed as indicating a direct phylogenetic relationship between *Omeia* and *Desmognathinema.* However, several observations suggest otherwise. First, an intestinal caecum has apparently evolved independently several times in the order Ascaridida, i.e., in the Cruziinae of the superfamily Cosmocercoidea, in the Omeiinae (Quimperiidae) and Cucullanidae of the superfamily Seuratoidea, and in several groups in the superfamily Ascaridoidea. Second, the cephalic structures of *Omeia,* especially the presence of a robust, thick-walled buccal capsule, are significantly different from *Desmognathinema.*

The arrangement of caudal papillae in male *D. nantahalaensis* is unusual for the order Ascaridida. Whereas in other ascaridids the postanal papillae include pairs that are subventral, lateral, and subdorsal in position, in *Desmognathinema* all of the postanal papillae are subventral. Such a papillary pattern is typical of the order Spirurida (Chabaud and Petter, 1961). The appearance of spirurid-like characters in a group such as the Quimperiidae is not unexpected because the Spirurida are believed to have evolved from ascaridids such as the Seuratoidea and Cosmocercoidea (Chabaud, 1974).

### *Omeia papillocauda* Rankin, 1937
### (Figs. 23–29)

SYNONYM: *Omeia chickasawi* Walton, 1940.

DESCRIPTION: Seuratoidea, Quimperiidae, Omeiinae, *Omeia* Hsu, 1933. Oral opening hexagonal, 6 minute cephalic labia present, visible only in apical view. Six minute inner labial papillae on cephalic lips, 8 outer cephalic papillae grouped into 2 subdorsal and 2 subventral double papillae. Amphidial pores relatively small. Buccal capsule thick-walled, round in apical view, not enclosing anterior extremity of esophagus, with 12 or 13 prominent onchia on anterior border that are directed toward the oral opening. Anterior extremity of esophagus rounded, lacking onchia. Esophagus cylindrical and undivided, relatively slender. Single prominent intestinal




**Figures 23–29.** ***Omeia papillocauda*** **Rankin, 1937. 23. Tail of female, lateral view. 24, 25. Cephalic extremity of female, lateral and apical views. 26. Caudal end of male, lateral view. 27. Vagina, lateral view. 28. Anterior end of female, lateral view. 29. Caudal end of male, ventral view.**

caecum present at anterior end of intestine. Cuticle of body relatively thick, with inconspicuous transverse striations about 3.5 apart. Lateral alae present, markedly slender, extending from posterior half of esophagus to anterior portion of caudal musculature in males, and to end of intestine in females. Anterior deirids not observed. Excretory pore small, cuticle-lined terminal duct about 60 long, giving rise to 2 large posteriorly directed lateral canals.

MALES (3 specimens): Total length 2.9–10.1 mm. Length of esophagus 390–765. Nerve ring 117–218, excretory pore 111–211 from anterior extremity. Tail 90–125 long, conical, and sharply pointed. Caudal papillae distributed as follows: tail with 3 pairs of subventral papillae, preanal region with 7–10 pairs of subventral papillae, anterior lip of cloaca lacking unpaired papilla. Phasmids not observed. Oblique caudal musculature present, 15–22 muscle cells on each side of body, not forming caudal pseudosucker. Spicules 103–165 long, equal, robust with bluntly pointed distal extremities, mid-portion of shaft with prominent alate structure on ventral side. Gubernaculum 50–66 long, weakly sclerotized, not distinct in small specimens.

FEMALES (8 specimens): Total length 4.2–7.6 mm. Length of esophagus 584–630. Nerve ring 192–281, excretory pore 162–272, vulva 2.6–4.3 mm from anterior extremity. Tail 115–160 long, conical, and sharply pointed. Phasmids located in posterior third of tail. Vulva lateral in position, dorsal and ventral body cuticle at level of vulva with 3–5 large papillae. Vagina 400 long, directed anteriorly, either curved posteriorly in distal half connected to uteri, or straight. Uteri opposed, ovary of posterior uterus located anterior to vulva, ovary of anterior uterus located posterior to vulva. All specimens immature, lacking eggs in the uteri.

HOSTS: *Desmognathus monticola* Dunn, 1916; *D. quadramaculatus* (Holbrook, 1840); *D. ochrophaeus* Cope, 1859 (new host record); *Leurognathus marmorata* Moore, 1899 (new host record).

LOCATION: Stomach.

LOCALITY: Same as for *Falcaustra plethodontis* sp. n.

SPECIMENS: USNM Helm. Coll. No. 79162 (specimens from the various host species were pooled together before taxonomic study).

PREVALENCE: *Desmognathus monticola* (20.0% of 125 examined); *D. quadramaculatus* (13.0% of 115 examined); *D. ochrophaeus* (8.4% of 107 examined); *L. marmorata* (12.0% of 50 examined).

COMMENTS: Two *Omeia* species have been described from salamanders of the southeastern United States: *O. papillocauda* Rankin, 1937, and *O. chickasawi* Walton, 1940. *Omeia chickasawi* was described based only on one male specimen, and it was distinguished from *O. papillocauda* on the basis of size differences alone. Thus, Walton (1940) noted that his specimen was 8.7 mm long, whereas Rankin (1937) reported male *O. papillocauda* to be 3.95 mm long. In the present study, male specimens varied in size to a greater extent than the differences considered by Walton (1940) as sufficient to distinguish separate species. No morphological differences were noted in males of different sizes in the present study. Similarly, examination of the type specimens of *O. papillocauda* and *O. chickasawi* revealed no morphological differences between them and specimens collected for the present study. *Omeia chickasawi,* therefore, is synonymized with *O. papillocauda.*

In addition to *O. papillocauda,* three other species of *Omeia* are known. *Omeia hoepplii* Hsu, 1933, from *Rana tibetana* (Ranidae) of China, *O. ambocaeca* (Chabaud and Brygoo, 1957), from *Rhacophorus* sp. (Rhacophoridae) of Madagascar, and *O. vietnamensis* Moravec and Sey, 1985, from *Rana kuhlii* of N. Vietnam, are readily distinguished from *O. papillocauda* by the following: (1) presence of a relatively large and well-sclerotized gubernaculum (small and weakly sclerotized in *O. papillocauda*); (2) four pairs of caudal papillae on the posterior half of the tail in males (only two pairs in *O. papillocauda*); (3) the triangular shape of the buccal capsule when viewed apically (round in *O. papillocauda*); and (4) anterior border of buccal capsule with more than 60 minute onchia (12 or 13 relatively large onchia in *O. papillocauda*).

Rankin (1937) originally described *O. papillocauda* from *Desmognathus fuscus fuscus, D. monticola* (=*D. phoca*), *D. quadramaculatus,* and *Gyrinophilus porphyriticus danielsi* of North Carolina. Other host and locality reports (some under the name *O. chickasawi*) include the following: *Eurycea bislineata bislineata,* Tennessee (Walton, 1940); *Eurycea lucifuga,* Alabama (Dyer and Peck, 1975); *Eurycea bislineata, Desmognathus quadramaculatus, D. fuscus,* and *D. monticola,* Tennessee (Dunbar and Moore, 1979); *Gyrinophilus porphyriticus,* Ohio (Catalano et al., 1982).

**Acknowledgments**

Dr. J. R. Lichtenfels, curator of the U.S. National Museum Helminthological Collection, kindly lent specimens for study. This study was supported in part by a National Science and Engineering Research Council (Canada) operating grant to M.R.B. and a grant-in-aid of research from the Highlands Biological Station, Highlands, North Carolina, to T.M.G. Cam Goater and Al Bush assisted with initial collation and enumeration of nematode specimens.

**Literature Cited**

**Baker, M. R.** 1981. *Cordonema* n. g. (Cosmocercoidea: Kathlaniinae) from the salamander *Cryptobranchus alleghenienisis* (Cryptobranchidae) of North America. Systematic Parasitology 3:59–63.

**Catalano, P. A., A. M. White, and F. J. Etges.** 1982. Helminths of the salamanders *Gyrinophilus porphyriticus, Pseudotriton ruber,* and *Pseudotriton montanus* (Caudata: Plethodontidae) from Ohio. Ohio Journal of Science 82:120–128.

**Chabaud, A. G.** 1974. Keys to subclasses, orders and superfamilies. Pages 6–17 *in* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, England.

———. 1978. Keys to genera of the superfamilies Cosmocercoidea, Seuratoidea, Heterakoidea and Subuluroidea. 71 pages *in* R. C. Anderson, A. G. Chabaud, and S. Willmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 6. Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, England.

———. 1981. Host range and evolution of nematode parasites of vertebrates. Parasitology 82:169–170.

———**, and A. J. Petter.** 1961. Remarques sur l'evolution des papilles cloacales chez les nematodes phasmidiens parasites de vertebres. Parassitologia 3:51–70.

**Dunbar, J. R., and J. D. Moore.** 1979. Correlations of host specificity with host habitat in helminths parasitizing the plethodontids of Washington County, Tennessee. Journal of the Tennessee Academy of Science 54:106–109.

**Dyer, W. G., and S. B. Peck.** 1975. Gastrointestinal parasites of the cave salamander, *Eurycea lucifuga* Rafinesque, from the southeastern United States. Canadian Journal of Zoology 53:52–54.

**Goater, T. M.** 1985. Comparative ecology of helminth assemblages in sympatric salamanders (Desmognathinae). M.S. Thesis, Wake Forest University, North Carolina.

**Rankin, J. S.** 1937. New helminths from North Carolina salamanders. Journal of Parasitology 23:29–42.

**Vassiliades, G.** 1971. Redescription de *Quimperia lanceolata* Gendre, 1926 (Nematoda, Seuratoidea). Annales de Parasitologie Humaine et Comparée 46:61–67.

**Walton, A. C.** 1940. Some nematodes from Tennessee Amphibia. Journal of the Tennessee Academy of Science 15:402–404.

**Wang, P. Q., Y. R. Zhao, and C. C. Chen.** 1978. On some nematodes from vertebrates in South China. Fujian Shida Xuebao No. 2: 75–90. [In Chinese.]

# Erratum

In a recent issue of this journal, the following corrections should be made:

July 1986, 53(2):240, in the article by Nansen et al.:

In Figure 2, in the upper two graphs for *Cooperia oncophora* $L_1$ and $L_2$, the ranges for "Number of hyphal loops per mm$^2$" should be 20–100 rather than 200–1000.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 24–27

# Scanning Electron Microscopy of the Anterior and Posterior Ends of Adult Male *Pterygodermatites nycticebi* (Nematoda: Rictulariidae)[1]

C. H. GARDINER[2] AND G. D. IMES, JR.[3]

[2] Department of Parasitology, Naval Medical Research Institute Detachment, Lima, Peru, APO Miami 34031 and
[3] Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306

ABSTRACT: Scanning electron microscopy (SEM) was utilized to study the external cuticular structures of adult male *Pterygodermatites nycticebi.* Morphologic characteristics were as previously described with light microscopy, but SEM did allow an easier delineation of minute characters and a better understanding of the three-dimensional relationships among these structures.

KEY WORDS: cuticular ridges and combs, cephalic, adanal, and postanal papillae, *Callimico goeldii.*

The identification of rictularids from nonhuman primates is often difficult due to the small size of the several pairs of postanal papillae in adult males. In addition, the three-dimensional relationships of structures, when viewed in glycerin-cleared specimens with a compound microscope, are often not discernible. Scanning electron microscopy has been used in numerous studies to delineate minute characteristics of parasites, and thus was utilized here to examine the morphological characteristics of adult male *Pterygodermatites nycticebi* (Mönnig, 1920) Quentin, 1969.

## Materials and Methods

Adult *P. nycticebi* were recovered at autopsy of a Goeldi's marmoset (*Callimico goeldii*) from the Brookville Zoo, Chicago, Illinois. Twenty worms were received fixed in 70% ethanol. No estimation was given as to the number of worms in the host, but previous studies have shown that large numbers can be present. For information on the relationship between *P. nycticebi* and its nonhuman primate hosts the reader is referred to Montali et al. (1983). Five males were dehydrated in an ethanol series, critical point dried, coated with gold, and examined in a Philips 501B scanning electron microscope. The other male and female worms were dehydrated, cleared in glycerin, and studied with a compound microscope. Specimens are deposited in the U.S. Department of Agriculture Parasite Collection (USDA), Agricultural Research Service, Beltsville, Maryland, No. 69735.

## Results

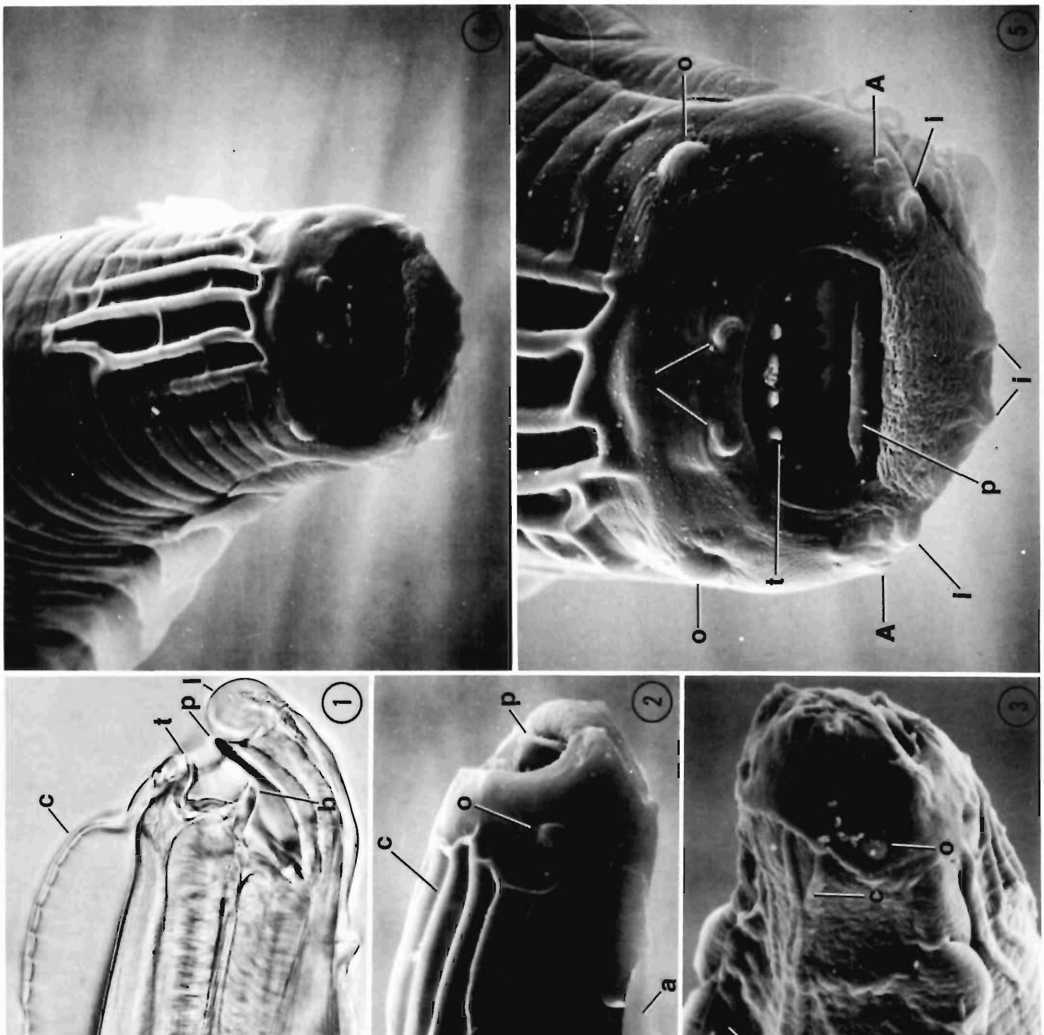
Figure 1 is a photomicrograph of what one would observe with the use of the compound microscope and a glycerin-cleared specimen. Figures 2–8 are compilation of photomicrographs of morphologic characteristics of the five males.

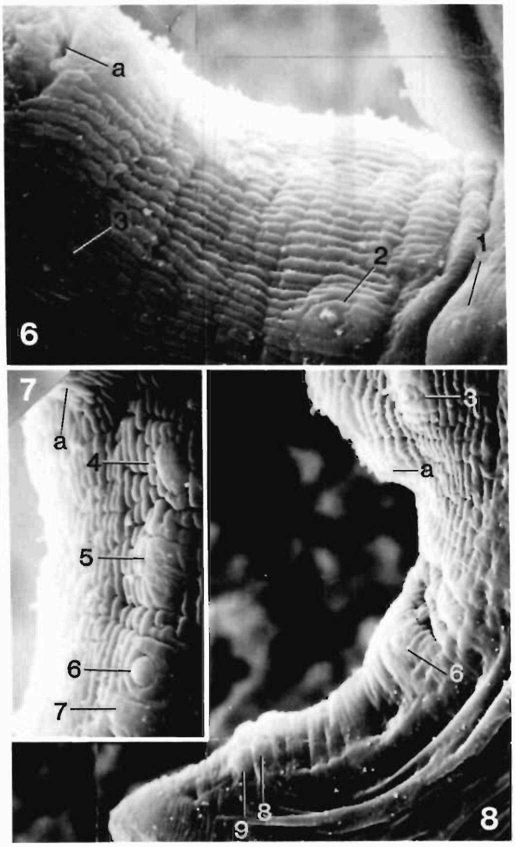
ANTERIOR END (Figs. 1–5): The mouth was inclined dorsally and somewhat hemispherical in shape. The ventral lip (l) was large and globular, and contained low cerebriform cuticular ridges. Ventrally the mouth was guarded by a cuticular plate (p), dorsally by a row of cuticular teeth (t). These teeth numbered eight to 10, and increased in size from the lateral areas. Three pairs of cervical papillae of the inner circle (i)

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy, the Department of the Army, or the Department of Defense. Dr. Gardiner's military designation is LCDR MSC USN. Dr. Imes's military designation is COL VC USA.

→

**Figures 1–5. Anterior end of adult male *Pterygodermatites nycticebi.* 1. Photomicrograph of anterior end cleared in glycerin. Buccal tooth (b), dorsal cuticular ridge (c), ventral lip (l), ventral plate (p), dorsal tooth (t). Optical view, ×400 (AFIP MIS #83-9251). 2. Lateral view. Subventral ala (a), dorsal cuticular ridges (c), ventral plate (p), paired dorsal papillae of outer circle (o). Scanning electron micrograph (SEM), ×640 (AFIP MIS #83-9437). 3. Ventral view. Subventral alae (a), ventral cuticular ridges (c), paired ventral papillae of the outer circle (o). SEM, ×640 (AFIP MIS #83-9355). 4. En face view. Note dorsal cuticular ridges. SEM, ×640 (AFIP MIS #83-9432). 5. En face view. Amphids (A), papillae of inner circle (i), ventral plate (p), papillae of outer circle (o), row of dorsal teeth (t). SEM, ×1,250 (AFIP MIS #83-9433).**






**Figures 6–8. Posterior end of adult male *Pterygodermatites nycticebi*. 6. Photomicrograph of area anterior to anus. Anus (a), preanal papillae (1–3). SEM, ×1,250 (AFIP MIS #83-9434). 7. Photomicrograph of area posterior to anus. Anus (a), postanal papillae (4–7). SEM, ×1,250 (AFIP MIS #83-9435). 8. Tip of tail. Anus (a), preanal papillae (3), large postanal papilla (6), couplet of paired postanal papillae (8, 9). SEM, ×1,250 (AFIP MIS #83-9436).**

and one pair of large amphids (A) were present. Four pairs of double papillae of the outer circle (o) were present, but each doublet often appeared as one large papilla. Both ends of worms had external cuticular cross striations. Middorsal longitudinal cuticular ridges (c) were present on the heads and numbered four to five, usually five. These ridges extended posteriad and often had tiny intercommunications, perhaps prominent cross striations. Two less prominent cuticular ridges were present in the midventral area (c). These did not remain in the midventral area, but extended laterally and then disappeared. Two very prominent ridges of alae (also called combs) were present subventrally (a).

POSTERIOR END (Figs. 6–8): The posterior end was curved and contained a prominent, conelike anus (a). Three large pairs of preanal papillae were evident; the last pair was situated almost adanally. Six pairs of postanal papillae were present. Four of these pairs were close to the anus. Pairs 4, 5, and 7 were much smaller than the large pair 6, and were the three pairs of papillae that were often difficult to demonstrate with the compound microscope. Pairs 8 and 9 were somewhat further from the other postanal papillae and were located near the tip of the tail in a couplet.

## Discussion

*Pterygodermatites nycticebi* (syn. *Rictularia nycticebi*) (Mönnig, 1920) Quentin, 1969, is a spirurid nematode that parasitizes several species on nonhuman primates (Montali et al., 1983). Two other species, *P. lemuri* and *P. alphi,* have also been reported from nonhuman primates (Lubimov, 1933; Chabaud and Brygoo, 1956). Often it is difficult to identify these rictularids to species because caudal papillae in the adult male are the distinguishing morphologic feature and these are difficult to visualize in glycerin-cleared worms (Yue et al., 1980).

The pattern of caudal papillae seen with scanning electron microscopy in these worms and reported herein agrees with that reported by Quentin and Krishnasamy (1979), i.e., three pairs of preanal, four pairs of adanal, and an additional two pairs of adanal papillae further posteriad. The arrangement of caudal papillae in *P. alphi* is somewhat uncertain. Lubimov (1933) described three pairs of preanal papillae and seven to eight pairs of postanal. One pair of postanal papillae is located immediately posterior to the anus. This pair is not found in *P. nycticebi.* The arrangement of caudal papillae in *P. lemuri* is not known because this species was described from juvenile females only (Chabaud and Brygoo, 1956).

## Literature Cited

**Chabaud, A. C., and E. R. Brygoo.** 1956. Description de *Rictularia lemuri* n. sp. (Nematoda: Thelaziidae). Mémoirs de l'Institut Scientifique de Madagascar, Series A, XI:43–49.

**Lubimov, M. P.** 1933. [Rictulariosis infection in monkeys of the Moscow Zoological Garden.] Zeitschrift für Infektionskrankheiten, Parasitäre Krankheiten und Hygiene der Haustiere 44:250–260.

**Montali, R. J., C. H. Gardiner, R. E. Evans, and M. Bush.** 1983. *Pterygodermatites nycticebi* (Nematoda: Spirurida) in golden lion tamarins. Laboratory Animal Science 33:194–197.

**Quentin, J. C., and M. Krishnasamy.** 1979. Sur la morphologie du Rictulaire de nycticebi *Pterygodermatites nycticebi* (Mönning, 1920) (Nematoda: Rictulariidae). Annales de Parasitologie Humaine et Comparée 54:527–532.

**Yue, M. Y., J. M. Jensen, and H. E. Jordan.** 1980. Spirurid infections (*Rictularia* sp.) in golden marmosets, *Leontopithecus rosalia* (syn. *Leontideus rosalia*) from the Oklahoma City Zoo. Journal of Zoo Animal Medicine 11:77–80.

## Fish and Wildlife Service Publications Available

The National Wildlife Health Center has available several publications of Dr. Malcolm McDonald. These publications are:

**McDonald, M.** 1969. Annotated Bibliography of Helminths of Waterfowl (Anatidae). USFWS Special Scientific Report, Wildlife 125. 333 pp. (128 copies)

**McDonald, M.** 1969. Catalogue of Helminths of Waterfowl (Anatidae). USFWS Special Scientific Report, Wildlife 126. 692 pp. (450 copies)

**McDonald, M.** 1984. Key to Trematodes Reported in Waterfowl. USFWS Resource Publication 142. 156 pp. (1,768 copies)

Interested persons should write to Louis N. Locke, DVM, National Wildlife Health Laboratory, 6006 Schroeder Road, Madison, Wisconsin 53711. Copies are available free of all charges.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 28–39

# Redescription of *Pulchrascaris chiloscyllii* (Johnston and Mawson, 1951) (Nematoda: Anisakidae), with Comments on Species in *Pulchrascaris* and *Terranova*

THOMAS L. DEARDORFF
Fishery Research Branch, U.S. Food and Drug Administration,
P.O. Box 158, Dauphin Island, Alabama 36528

ABSTRACT: A review of the genus *Pulchrascaris* Vicente and dos Santos (type species *P. caballeroi* Vicente and dos Santos) and a new diagnosis are provided. *Pulchrascaris* differs from the most closely related genus, *Terranova*, by possessing greatly reduced lips with four toothlike structures, two on the dorsal and one on each subventral lip, on the inner surface and by lacking dentigerous ridges. Three species belonging to the genus *Pulchrascaris* are recognized: *P. caballeroi, P. chiloscyllii,* and *P. secunda.* A redescription of *Pulchrascaris chiloscyllii,* based on the holotype and supplementary material collected from the lumen of the stomach or within gastric ulcers of the scalloped hammerhead, *Sphyrna lewini* (Griffith and Smith), showed the species has a single medial preanal papilla, 42–55 pairs of preanal and six pairs of postanal papillae, modified preanal annules, and cuticular plates on the ventral surface of males. Histological observations of the gastric nodules associated with these worms show broad areas where the host tissues had undergone coagulation necrosis. This report extends the geographical range of *P. chiloscyllii* into the waters offshore from the northern Gulf of Mexico and the Hawaiian Islands. The name *Pulchrascaris diazungriai* (Vado) is placed in the synonymy of *P. chiloscyllii. Pulchrascaris caballeroi* (Vicente and dos Santos) is considered a valid species in the genus by possessing only 26 preanal papillae. Five species in the genus *Terranova* that parasitize elasmobranchs are recognized and discussed: *T. antarctica, T. brevicapitata, T. nidifex, T. scoliodontis,* and *T. pristis.*

KEY WORDS: *Pulchrascaris caballeroi, P. secunda, Terranova antarctica, T. brevicapitata, T. nidifex* comb. n., *T. scoliodontis* comb. n., *T. pristis, Acanthocheilus, Pseudanisakis,* generic review, taxonomy, morphology, SEM, elasmobranch ascaridoids, gastric ulcers, *Sphyrna lewini,* sharks.

Mature nematodes were collected from the stomach of a scalloped hammerhead shark, *Sphyrna lewini* (Griffith and Smith), caught in the northern Gulf of Mexico and in waters near the Hawaiian Islands. Attempts to identify these materials, which are now considered in the genus *Pulchrascaris* Vicente and dos Santos, 1972, revealed that the literature concerning this genus contained numerous inaccuracies and omissions. Additionally, the generic diagnosis needed clarification and revision. The purposes of this paper are to distinguish *Pulchrascaris* from other genera of ascaridoid nematodes, redescribe a species belonging to the genus *Pulchrascaris* based on the holotype and supplemental material, discuss other material examined, and make appropriate synonyms and combinations.

## Materials and Methods

Worms were removed from the host, fixed in glacial acetic acid, stored in a solution of five parts glycerin and 95 parts 70% ethyl alcohol, and examined in glycerin after evaporation of the alcohol. For spicule ratios, the length of the left spicule was defined as one. All measurements are in micrometers unless otherwise stated. Figures were drawn with the aid of a drawing tube. Gastric ulcers were fixed in 10% phosphate-buffered formalin, processed, and stained by using standard procedures (Luna, 1968).

Specimens selected for scanning electron microscopy (SEM) were dehydrated, critical-point dried in liquid carbon dioxide, mounted on a specimen stub, coated with 200–300 Å of gold-palladium, and examined with a Cambridge Stereoscan 150 scanning electron microscope at 10 kV.

Abbreviations for repositories of examined nematodes are BMNH, British Museum (Natural History), London, England; OCI, Oswaldo Cruz Institute, Helminthology Collection, Rio de Janeiro, Brazil; TAM, The Australian Museum, Sydney, Australia; USNM, Helminthological Collection, United States National Museum, Beltsville, Maryland.

## Generic Diagnosis

### *Pulchrascaris* Vicente and dos Santos

*Pulchrascaris* Vicente and dos Santos, 1972 (type species *P. caballeroi*).

Body elongated, reaching greatest width near posterior third of body. Cuticle with annulations moderately defined. Cuticular alae distinct. Lips indistinct, greatly reduced, approximately equal in size; smooth, rounded, lacking cuticular flanges on lateral margins; internal pulp indistinct; dorsal lip with 2 lateral double papillae and 2 toothlike structures on inner dorsal surface between double papillae; subventral lips each with amphid, adjacent mediolateral double papilla, single lateral papilla, and single toothlike structure on the inner dorsal surface nearest double papilla. Dentigerous ridges absent. Interlabia absent. Deirids near nerve ring. Excretory pore between base of subventral lips; with excretory duct with nucleus near nerve ring and excretory filament, extending posteriad within left lateral cord past midbody, not present in posterior ¼ of worm. Ventriculus elongate. Intestinal cecum present. Spicules similar, alate, equal or slightly unequal in length. Gubernaculum absent. Cuticular plates present on males immediately posterior to anal opening. Modified annules present on ventral surface of cuticle immediately anterior to anus. Vulva anterior to midbody. Uterus didelphic, opisthodelphic. Tail conical; tip without ornamentation. Phasmids present. Parasites in the stomach of marine elasmobranchs and teleosts. Geographic distribution: amphitemperate and tropical seas.

COMPARISONS: By possessing an intestinal cecum and an excretory pore between the subventral lips and lacking a ventricular appendage and interlabia, the genera *Terranova* Leiper and Atkinson, 1914, and *Pulchrascaris* are most similar. In addition to the numerous morphological affinities between these genera, they both have a wide range of distribution and their subadults and adults principally parasitize elasmobranchs. These genera, however, are differentiated from each other based on the morphology of their lips: *Pulchrascaris* lacks distinct lips and dentigerous ridges and possesses toothlike structures. D. I. Gibson (pers. comm.) examined the female holotype of *Terranova antarctica* deposited in the BMNH and confirmed the presence of distinct lips and dentigerous ridges and the absence of toothlike structures.

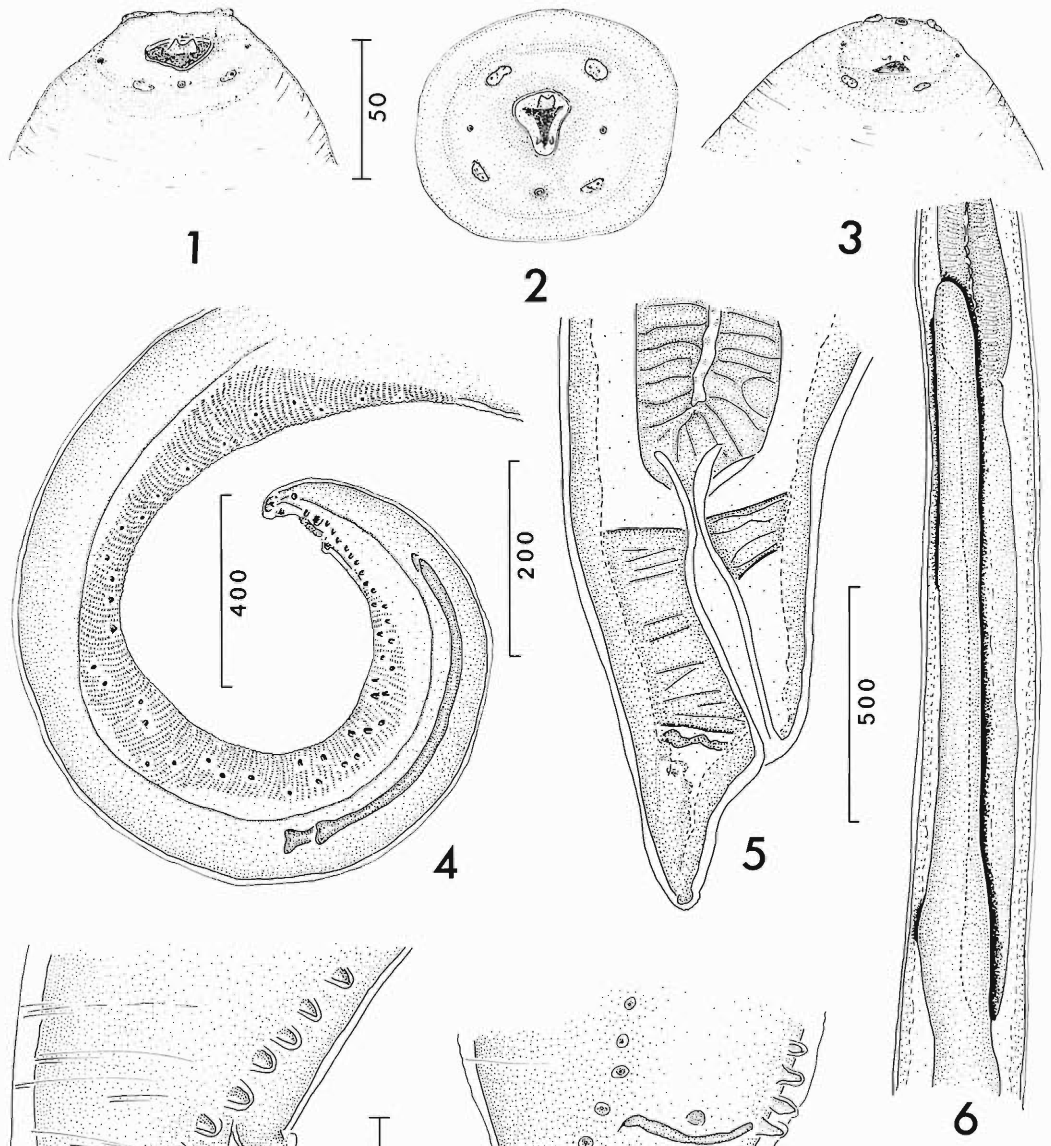
A toothlike projection similar to those on species of *Pulchrascaris* was found on a single species of *Terranova*. Sprent (1979) showed SEM photomicrographs of "aconical cusps" at the ventral end of the dentigerous ridge of each subventral lip of *T. caballeroi*, a parasite of snakes. No such structures, however, were present on the dorsal lip.

Considering all the genera recognized by Hartwich (1974) in his key to the genera of the Ascariodoidea, the lip morphology of *Acanthocheilus* Molin, 1858 (family Acanthocheilidae), bears a strong resemblance to *Pulchrascaris*. Like *Pulchrascaris*, species in the genus *Acanthocheilus* parasitize elasmobranchs. However, *Acanthocheilus*, by lacking an intestinal cecum, is easily differentiated from *Pulchrascaris*. A reexamination and reevaluation of the species belonging to the genus *Acanthocheilus* needs to be conducted to confirm the presence or absence of the intestinal cecum. It is possible that the intestinal cecum and/or other characters were not seen by researchers examining the worms, and the parasites were erroneously placed in *Acanthocheilus*. For example, I examined specimens purported to belong to *Acanthocheilus* that are deposited at the USNM Helminthological Collection. Of these, Nos. 6614 and 35535 (slide) definitely had an intestinal cecum, and both lots although in poor condition, had relatively pronounced lips; and No. 68650 (two vials) were third-stage larvae with enough lip formation under the sheath to suggest they were not in the genus. Numbers 34785 and 6534 were specimens of *Terranova* and are discussed later. Clearly, the type species should be critically reexamined.

Species belonging to the genus *Pseudanisakis* (Layman and Borovkova, 1926) Mozgovoi, 1951, infect elasmobranchs, and some of these species also have reduced lips when compared with other ascaridoids. These species, however, are easily differentiated from *Pulchrascaris* by having lips with one continuous dentigerous ridge.

REMARKS: The genus *Pulchrascaris* was erected by Vicente and dos Santos (1972) for the species *P. caballeroi*, which was collected from (?)*Squatina squatina*. The authors' reasons for erecting this genus, however, appeared to be insufficient. They differentiated the genus *Pulchrascaris* from the most closely related *Terranova* on the basis of "postanal chitinous apparatus (=cuticular plates) in the males, little teethlike structures on the inner face of the lips and a long glandular ventriculus." The first character was present in at least 12 species in the genus *Terranova*, the second in at least two species, and the third present in all species. Deardorff and Overstreet (1981) pointed out that the presence or absence and location of various characters in the genus *Pulchrascaris* needed to be determined.

The generic concept was upheld by Gibson and Colin (1982) for species of *Terranova* that lacked distinct lips. They transferred *T. chiloscyllii* Johnston and Mawson, 1951, *T. secundum*




**Figures 1–11.** ***Pulchrascaris chiloscyllii.*** **1. Dorsal view of lip. 2. Ventral view of lips. 3. En face view. 4. Posterior end of male, showing caudal papillae, spicules, and modified ventral annules, lateral view. 5. Posterior of female tail, lateral view. 6. Body at level of intestinal–ventricular junction. 7. Male tail, showing postanal and**

Chandler, 1935, and *T. diazungriai* Vado (as Vada), 1972, to *Pulchrascaris* and considered *P. caballeroi* as a junior synonym of *P. chiloscyllii.* Based on the type specimen of *P. chiloscyllii* (TAM W3551) deposited by Johnston and Mawson, as well as on supplemental material, the following redescription is provided.

### *Pulchrascaris chiloscyllii* (Johnston and Mawson) comb. n. (Figs. 1–17)

*Terranova chiloscyllii* Johnston and Mawson, 1951:291–292, figs. 1–4 (original description; type host *Chiloscyllium punctatum*; type locality Halfway Island, Central Queensland coast, Australia).

*Terranova diazungriai* Vado, 1972:487–489, fig. 5 (original description; type host *Sphyrna lewini*; type locality Isla de Margarita Juan Griego, Edo Nueva Esparrta, Venezuela).

*Pulchrascaris diazungria:* Gibson and Colin, 1982:xxxvi–xxxvii (new combination).

*Pulchrascaris chiloscyllii* Gibson and Colin, 1982: xxxvi–xxxvii (new combination; with *P. caballeroi* as junior synonym).

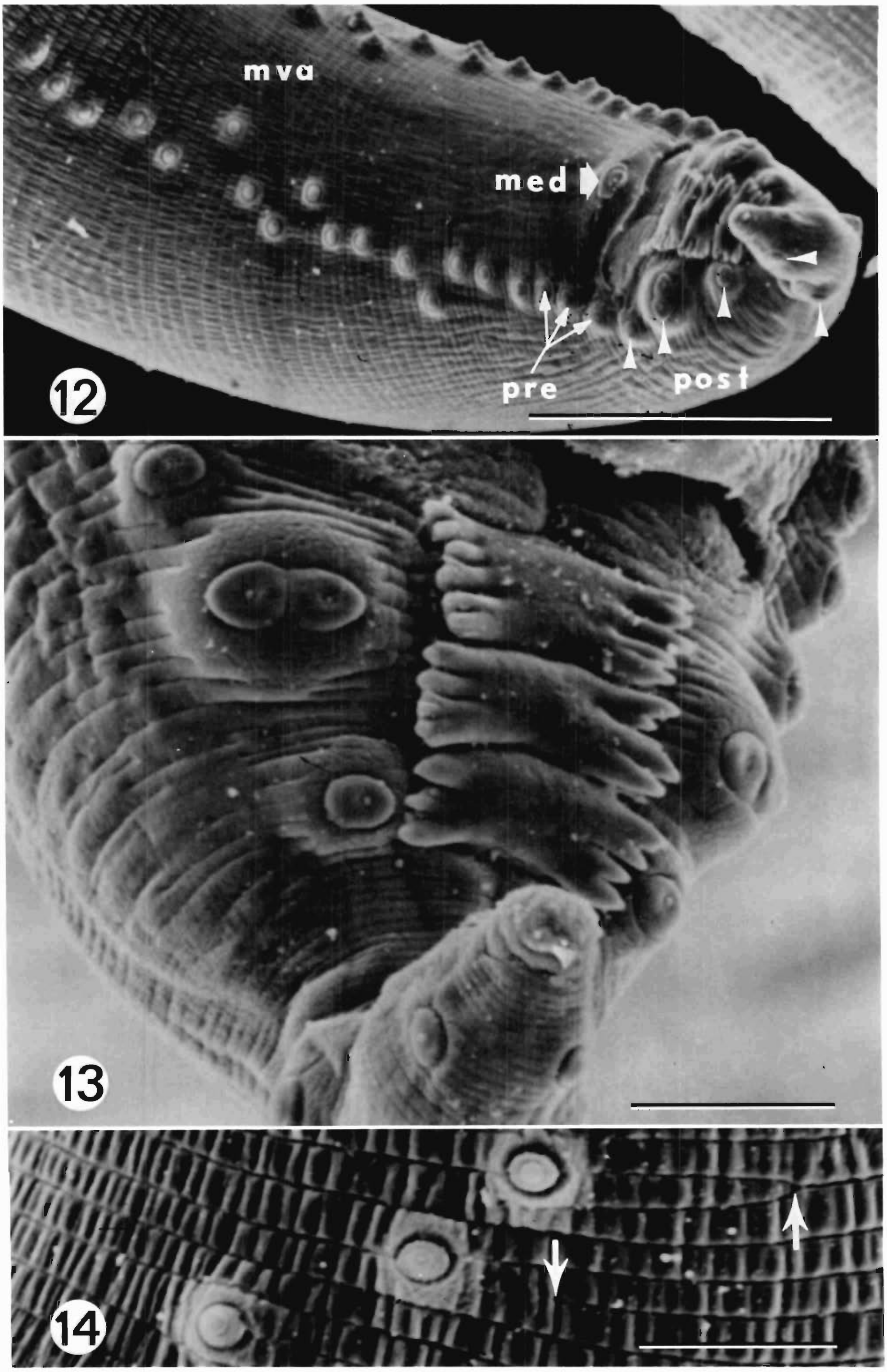
**Redescription**

GENERAL: Body reaching greatest width at posterior ¾ of worm. Cuticle with inconspicuous annulations. Lips approximately equal in size, widest at base, all wider than long; dorsal lip with lateral double papillae and 2 toothlike projections (Figs. 1, 16); subventral lips each with single mediolateral papilla, adjacent amphid, and mediolateral double papilla and 1 toothlike structure (Figs. 2, 3, 15); internal pulp round, indistinct. Interlabia absent. Dentigerous ridges absent. Esophagus 4.7–9.2% of body length. Ventriculus longer than wide. Intestinal cecum equal to or greater than ventriculus (Fig. 6) except in 2 specimens. Nerve ring located within anterior 18–25% of esophagus. Cervical papillar pair near level of nerve ring. Excretory pore opening between base of subventral lips; excretory canal single, extending posteriorly along left lateral cord beyond midbody; not present in posterior ¼ of worm. Lateral cords V-shaped and conspicuous in cross section (Figs. 9–11, 19). Tail conically shaped, curving slightly ventrad, terminating with bluntly rounded process (Figs. 5, 7); process slightly wrinkled.

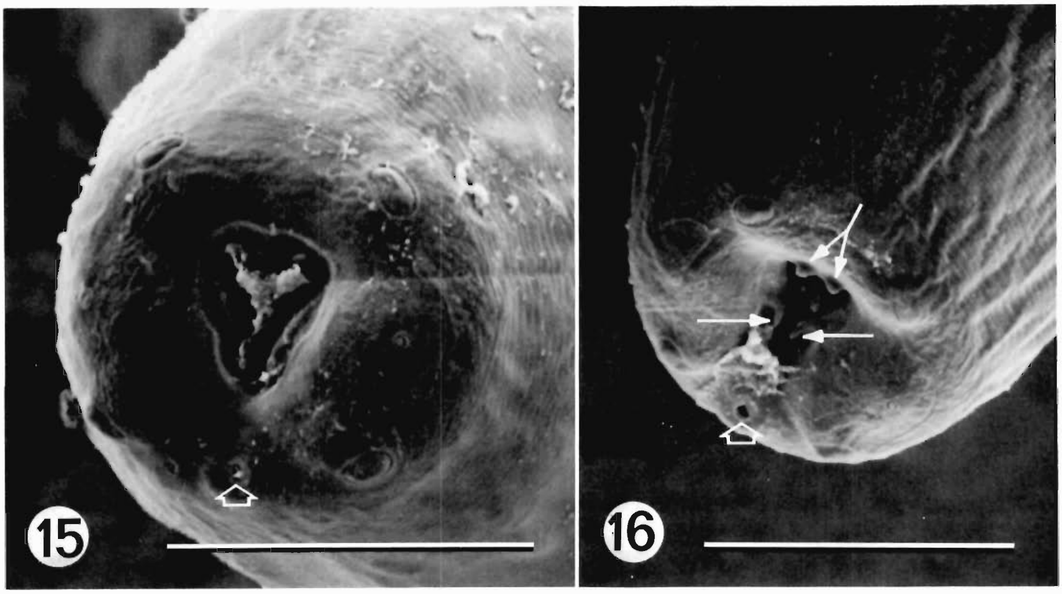
MALE (based on 12 specimens): Body 16–32 mm long by 203–350 wide at greatest width; ratio of greatest width to length 1:55–100. Lips 22–45 long by 45–55 wide. Nerve ring with center 290–350 from anterior extremity, 24–42 in breadth. Esophagus 1.3–1.7 mm long by 112–150 wide. Ventriculus 1.3–1.7 mm long by 90–150 wide; ratio of ventricular to esophageal lengths 1:0.9–1.1. Intestinal cecum 1.5–1.9 mm long by 81–150 wide; ratio of cecal to esophageal lengths 1:0.8–1.0; ratio of cecal to ventricular lengths 1:0.8–0.9. Spicules similar, alate, 2.4–4.4% of body length (Fig. 4), equal in length in 1 specimen; right spicule 470–910 long by 22–36 wide; left spicule 481–930 long by 22–36 wide, greater in length in 11 of 12 specimens; spicule to spicule ratio 1:0.9–1.0. Gubernaculum lacking. Caudal papillae 47–60 pairs; preanal pairs 42–55, 50 on average, becoming more lateral and irregularly spaced as progressing anteriad (Figs. 4, 12, 14); medial preanal papilla conspicuous, located immediately above anterior anal lip, 1 in number (Figs. 8, 12); postanal pairs 6, with pairs 2, 4, 5, and 6 from posterior located more ventral than pairs 1 and 3, and with pair 5 doubled and conspicuously larger than others (Figs. 7, 8, 13, 17); adanal papillae lacking. Cuticular plates 3, ventral, immediately posterior to anus, with serrated edges, each 11–22 long by 11–27 wide (Figs. 7, 8, 12, 13). Modified annules on ventral surface beginning near anus, extending anteriorly beyond preanal papillae (Figs. 4, 12, 14). Tail flexed ventrad, 123–180 long, with blunt process at posterior extremity; process 11–22 long.

FEMALE (based on 12 specimens): Body 27–30 mm long by 406–481 wide; ratio of greatest width to length 1:62–67. Lips 36–45 long by 56–67 wide. Nerve ring with center 340–370 from anterior extremity, 32–42 in breadth. Esophagus 1.7–2.0 mm long by 160–200 wide. Ventriculus 1.7–2.0 mm long by 130–150 wide; ratio of ventricular to esophageal lengths 1:1.0. Intestinal cecum 1.0–2.0 mm long by 140–260 wide; ratio of cecal to esophageal lengths 1:0.9–1.0; ratio of cecal to ventricular lengths 1:0.8–0.9. Vulva

←
**medial papillae, lateral view. 8. Male tail, showing postanal papillae and cuticular plates, ventral view. 9–11. Lateral ala at anterior (9), midbody (10), and posterior (11) of worm.**




**Figures 12–14. Scanning electron micrographs of *Pulchrascaris chiloscyllii*. Numbers in parentheses indicate scale lengths. 12. Posterior end of male, showing preanal papillae and medial (med) and modified ventral annules**




**Figures 15, 16. SEM micrographs of *Pulchrascaris chiloscyllii*. 15. En face view of male, showing papillae, amphids, and excretory pore (arrow). Bar = 100 μm. 16. En face view, showing toothlike structures (small arrows) and excretory pore (large arrow). Bar = 100 μm.**

opening 7.6–9.4 mm or 28–31% of body length from anterior extremity. Uterus didelphic, opisthodelphic. Eggs 33–42 in diameter. Phasmids located laterally. Tail 170–240 long.

HOSTS: *Sphyrna lewini* (Griffith and Smith), scalloped hammerhead; *S. zygaena* (Linnaeus), smooth hammerhead (Sphyrnidae).

SITES OF INFECTION: Free in lumen of stomach, within gastric ulcers.

LOCALITIES: Kaneohe Bay, Oahu, Hawaii (*S. lewini*); offshore from Alabama (*S. lewini*); South Africa (*S. lewini, S. zygaena,* and "shark").

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 79483 (pair); University of Nebraska State Museum No. 23632 (pair).

COMPARISONS: The primary distinguishing characteristic of *Pulchrascaris chiloscyllii* is the presence of 42–55 pairs of preanal papillae. This species is most similar to *P. caballeroi,* which has at least 26 papillae (see later discussion of *P. caballeroi*). *Pulchrascaris secunda,* the only other species belonging to this genus, is differentiated from *P. chiloscyllii* by lacking three cuticular plates on the ventral surface of the male.

## Remarks

In addition to adding new locality records, new synonyms, and new combinations, minor morphological variations, descriptions of previously unreported structures, and observations on pathology associated with this species are provided.

I have been unable to locate and examine specimens of *T. diazungriai* that were described by Vado (1972) from *Sphyrna lewini* caught in waters near Isla de Margarita Juan Griego, Venezuela. Based on the original description, however, these specimens are conspecific with *P. chiloscyllii*. *Pulchrascaris chiloscyllii,* then, is the senior synonym.

Two male and two female nematodes (BMNH Nos. 1982.2256–2260) from a "shark" and males (BMNH Nos. 1982.2261–2270) from *S. zygaena* that were all caught off South Africa were examined. They all appear to be *P. chiloscyllii*. One

←
**(mva) (100 μm). 13. Male tail, showing postanal papillae and three cuticular plates (20 μm). 14. Close-up of preanal papillae and modified ventral annules; note that some annules (arrows) do not completely encircle worm (20 μm).**

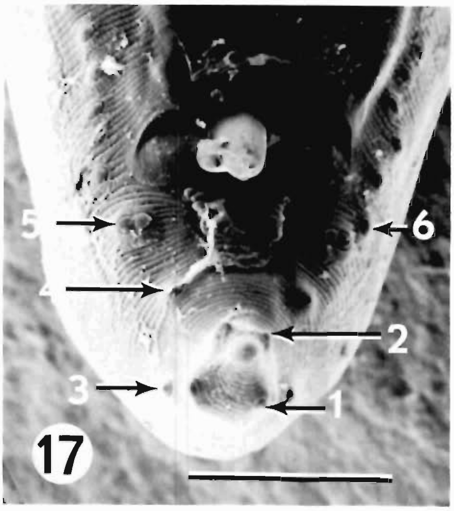



**Figure 17. Ventral view of tail of male *P. chiloscyllii*, showing pairs of postanal papillae. Bar = 100 μm.**

male and three female worms (BMNH Nos. 1976.2284–2299) that were removed from the mesentery of *S. lewini* also appeared to be *P. chiloscyllii,* but these specimens were not mature. A sheath over the anterior end suggests that these worms may be fourth-stage larvae. Apparently, this parasite occurs throughout the range of sphyrnids.

Not all worms identified as *Pulchrascaris chiloscyllii* and deposited in the BMNH can be referred to the genus *Pulchrascaris.* At least two mature males (BMNH Nos. 1982.2175–2177) that were collected from a black marlin, *Makaira indica* (Cuvier), near Ballito, South Africa, cannot. These specimens have salient lips with dentigerous ridges, and four ventral cuticular plates. They appear to belong to the genus *Terranova.* Based on the original description of Baylis (1931) and the paratypes (BMNH Nos. 1938.7.15.13–24), *T. scoliodontis* is the only species in the genus with four cuticular plates. The importance of cuticular plates as a taxonomic character is discussed later.

In addition to the 14 specimens of *P. chiloscyllii* found free in the lumen of the stomach of the infected shark caught near Hawaii, several specimens were firmly encysted within two fibrous ulcers in the gastric wall of the host. Each ulcer was open to the lumen of the stomach and nematodes within them were visible. Two male worms were dissected from one ulcer and, upon histological examination of the other ulcer, at least four adult worms were counted (Fig. 18). Aggregates of worms were surrounded by broad areas where the host tissues had undergone coagulation necrosis. The periphery of these zones of necrosis was heavily infiltrated with lymphocytes and mononuclear cells (Fig. 19). No nematode was attached to the host tissue.

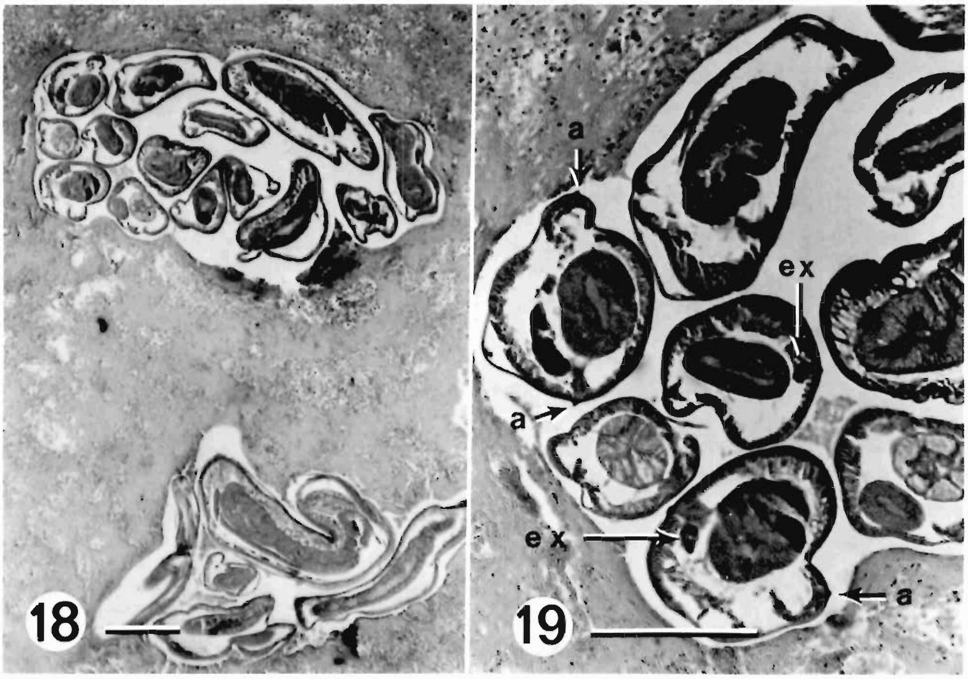
One other anisakine nematode, *T. nidifex,* has been reported within similar fibrous stomach nodules in a shark (see Linton, 1900, 1901, 1907, 1934). This gastric ulcer (USNM No. 6534) (Fig. 20) was 2.1 cm at its greatest width, 1.7 cm at its greatest opening, and approximately 0.5 cm in height. Whether *P. chiloscyllii* or *T. nidifex* caused the gastric ulcers or whether the ulcers were caused by some other source, in which case the worms lodged themselves within the ulcer and further aggravated it, is uncertain.

### *Pulchrascaris caballeroi* (Vicente and dos Santos)

*Pulchrascaris caballeroi* Vicente and dos Santos, 1972:17–19, figs. 1–6 (original description; type host *Squatina squatina*; type locality Macae, State of Rio de Janeiro, Brazil).

I examined the holotypes of *P. caballeroi* (OCI 30.649a, male; OCI 30.649b, female), which were mounted on glass slides, and found most structures, measurements, and ratios to be very similar to those reported by Vicente and dos Santos (1972). Additionally, I counted 26 preanal, one medial, and six postanal pairs of papillae, which differs from the 24 preanal, one adanal, and four postanal pairs reported by Vicente and dos Santos. Present on the male, but not reported or measured previously, were modified ventral annules, a 2.5-mm-long intestinal cecum, and lateral alae. *Pulchrascaris caballeroi* may possess more than 26 pairs of preanal papillae, but it is impossible to determine if more are present because the male holotype is permanently mounted on a slide. Based only on the original description of Vicente and dos Santos (Gibson, pers. comm.), Gibson and Colin (1982) considered *P. caballeroi* a junior synonym of *P. chiloscyllii.* However, although very similar, the holotype of *P. caballeroi* is best separated from its congener *P. chiloscyllii* by having 26 pairs of preanal papillae. It, therefore, must be considered a valid species.

Generally, the synonymy question for *P. caballeroi* could be answered by examining supplemental specimens collected from the type host and type locality; but, in this case, that would be




**Figures 18, 19. Sections through ulcer in stomach of *Sphyrna lewini*. 18. Specimens of *Pulchrascaris chiloscyllii* surrounded by necrotic host tissue. Bar = 400 μm. 19. Close-up of worms with nodule; note ala (a) and excretory canal (ex). Bar = 400 μm.**

difficult. Vicente and dos Santos reported the type host to be the angel shark *Squatina squatina* (Linnaeus, 1758). Their identification of the host is undoubtedly erroneous. According to Compagno (1984), *S. squatina* is only found in the eastern North Atlantic. Only the Argentine angel shark *S. argentina* (Marini, 1930) and the sand devil *S. dumeril* LeSueur, 1818, have been reported along the eastern coast of South America, but neither species has a geographical range near Macae, Brazil. One of these two species is probably the host examined by Vicente and dos Santos. The parasites of both *S. argentina* and *S. dumeril* should be critically examined.

***Pulchrascaris secunda* (Chandler)**

*Porrocaecum secundum* Chandler, 1935:145 (original description; type species *Trichiurus lepturus*; type locality Galveston Bay, Texas).

*Porrocaecum secundum:* Lent and Teixeira de Freitas, 1949:28–34 (description of adult and larval stages).

*Terranova secundum:* Olsen, 1952:188–189 (new combination).

*Pulchrascaris secunda:* Gibson and Colin, 1982: xxxvi–xxxvi (new combination; validation of genus).

Lent and Teixeira de Freitas (1949) collected and described what they believed to be mature adults of *Porrocaecum secundum* Chandler, 1935, at La Paloma, Uruguay, from the intestine of an Atlantic cutlassfish, *Trichiurus lepturus* Linnaeus. I examined a male (OCI 31.356a) and two females (OCI 31.656c, e) that were stained and permanently mounted on individual slides. The morphology of the lips differed slightly from the illustrations (figs. 45, 46) and description of Lent and Teixeira de Freitas. Each lip had the standard number and arrangement of papillae and amphids seen on other anisakine worms; thus, these characters contrast with the authors' description and illustration of only two papillae. Neither the toothlike structures illustrated in their figure 46 nor dentigerous ridges could be seen.

The cuticle appeared to be damaged or missing from portions of the male anteriad of the anus, making it difficult to determine the number of preanal papillae and the presence of modified ventral annules. Cuticular plates posterior to the

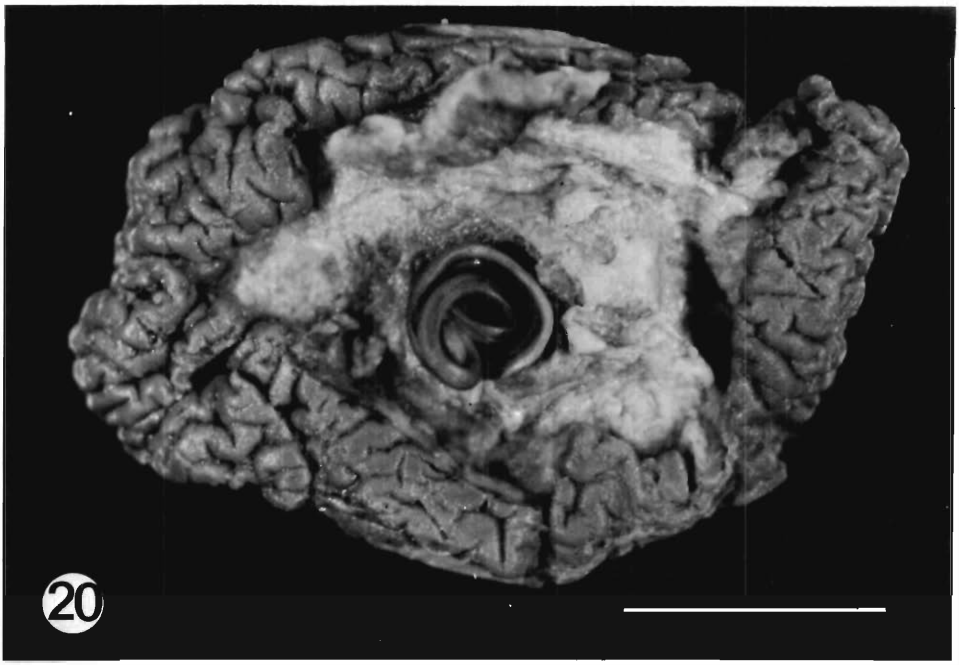



**Figure 20. Photograph of syntype of *Terranova nidifex* deposited in the National Museum (Helm. Coll. No. 6534) by Linton, showing nematode coiled within ulcer in stomach of *Galeocerdo tigrinus*; worm not attached to tissue. Bar = 2 cm.**

anal opening were lacking. Other material (OCI 16.602f, e) was all fragments of portions of female worms. These slides were badly yellowed and of little value. The third-stage larva (OCI 16.603) was as described by Lent and Teixeira de Freitas.

Because the specimens of Lent and Teixeira de Freitas possessed indistinct lips with what appeared in their illustrations to be toothlike projections, I consider them in the genus *Pulchrascaris,* as did Gibson and Colin (1982). *Pulchrascaris secunda* differs from the other two species in the genus by lacking cuticular plates on the male and by parasitizing a teleost.

Whether or not the third-stage larva reported by Chandler (1935) is actually the juvenile stage of the worm described by Lent and Teixeira de Freitas remains uncertain. Finding larval and adult stages, although similar, in the intestine of the same host does not necessarily suggest that they are the same species. Only life cycle studies can solve such problems.

Although species of *Pulchrascaris* may be differentiated from those of the genus *Terranova* on the basis of lip morphology—features not present on larval stages—separation of third-stage larvae between these genera is problematical. No larvae have yet been described as belonging to the genus *Pulchrascaris.* Rather, larvae of both genera apparently are considered as *Terranova* sensu lato. Based on the ratio between the lengths of the intestinal cecum and ventriculus, for example, *P. chiloscyllii* closely corresponds with the larval type designated as *Terranova* Hawaii type B (Deardorff et al., 1982, 1984). This larval type was generally found in the abdominal viscera of pelagic fishes such as carangids, lutjanids, scombrids, and serranids, and is probably the same as *T. secundum* (as *Porrocaecum*) of Chandler (1935) from *Trichiurus lepturus* in Galveston Bay, Texas, and *Terranova* type I of Cannon (1977) from many marine fishes offshore from southeastern Queensland. Based on similar relationships of the cecum and ventriculus, Cannon suggested the *Terranova* type I larva was most similar to *P. chiloscyllii* (as *Terranova chiloscyllii*). The finding of these larvae in the same localities as adult *P. chiloscyllii* further supports Cannon's speculations. Until further life cycle studies are conducted, however, I refrain from positively identifying *P. chiloscyllii* as the adult stage of *Terranova* Hawaii type B.

### *Terranova brevicapitata* (Linton)

*Ascaris brevicapitata* Linton, 1901:425, pl. III, figs. 19–22 (original description; type host *Galeocerdo tigrinus*; type locality Woods Hole, Massachusetts region).

*Porrocaecum brevicapitatum:* Baylis, 1931:97 (new combination).

*Terranova brevicapitata:* Mozgovoi, 1951:vol. II, p. 541 (new combination).

*Terranova brevicapitata:* Gibson and Colin, 1982: xxxvi–xxxvii (new combination; with *T. scoliodontis* as junior synonym).

Linton (1901) described *Terranova brevicapitata* (as *Ascaris brevicapitata*) based on four specimens that he collected from *Galeocerdo tigrinus* and one specimen "belonging to the National Museum." It appears that the single specimen Linton referred to was USNM No. 6382. Because no holotype was designated, I designate USNM No. 6382 as the lectotype of *T. brevicapitata* (Linton). Although the poor condition of the lectotype obstructed much of the internal structure, the lip morphology was not diagnostic of *Pulchrascaris.* No cuticular plates were seen. In 1920, MacCallum deposited additional material (USNM No. 34584) of *T. brevicapitata* (as *Ascaris brevicapitata*) that he collected from the same host and locality as the original specimens. Of the five specimens with visible lips, three were similar to *Pulchrascaris* and two were similar to *Terranova.* I counted 51 preanal and six postanal papillae and three cuticular plates on one of the male specimens lacking distinct lips. The two specimens belonging to *Terranova* were females. As it now stands, based on the original description, *T. brevicapitata* is distinguished from most other species in *Terranova* by lacking cuticular plates.

### *Terranova nidifex* (Linton) comb. n.

*Acanthocheilus nidifex* Linton, 1900:303, pl. 43, figs. 116–119 (original description; type host *Galeocerdo tigrinus*; type locality Woods Hole, Massachusetts, region).

*Porrocaecum nidifex:* Wulker, 1930:9 (new combination).

*Terranova nidifex:* Johnston and Mawson, 1945: 111 (first inference as belonging to *Terranova*; new combination not stated).

*Terranova nidifex* (as *Acanthocheilus nidifex*), which also was collected from the tiger shark, *Galeocerdo tigrinus,* from the Woods Hole region, was originally described by Linton (1900). Although in poor condition, the syntypes (USNM No. 6534) of *T. nidifex* deposited by Linton in 1900 possessed comparatively salient lips, an elongated ventriculus, and an intestinal cecum in one specimen. Dentigerous ridges may be present on the small, immature worm; however, the poor condition of the lips makes confirmation difficult. This material appears to belong in the genus *Terranova.* Owing to the incomplete description of *T. nidifex,* Olsen (1952), Gibson and Colin (1982), and others regarded it as species inquirenda. I agree. Corresponding males are necessary. Johnston and Mawson (1945) suggested that this species may belong to *Terranova* and that *T. galeocerdonis* may be a junior synonym. This species, however, was never formally placed in the genus, even though it is commonly referred to it. Therefore, *A. nidifex* (Linton) is transferred to the genus *Terranova,* as *T. nidifex* comb. n.

### *Terranova scoliodontis* (Baylis) comb. n.

*Porrocaecum scoliodontis* Baylis, 1931:95–97, figs. 1–3 (original description; type host *Scoliodon* sp.; type locality ?Cleveland Bay, Townsville, Australia).

*Terranova scoliodontis:* Johnston and Mawson, 1945:111 (new combination).

*Terranova brevicapitata:* Not of Linton. Gibson and Colin, 1982:xxxvi–xxxvii (in part; new combination with *T. scoliodontis* as junior synonym).

Although I agree with Gibson and Colin (1982) in their placement of *T. brevicapitata,* I do not believe that *T. scoliodontis* is a junior synonym of the former. The paratypes of *T. scoliodontis* (BMNH 1938.7.15.13–24) possess four ventral, cuticular plates, which distinguish this species from all other species in this genus. Further, within the genus, only *T. scoliodontis* has an excretory system that has both a right and left filament (Gibson, 1983). I consider *Terranova scoliodontis,* therefore, a valid species belonging to the genus *Terranova.*

## Discussion

I recognize three species belonging to *Pulchrascaris.* Both *Pulchrascaris caballeroi* and *P. chiloscyllii* parasitize elasmobranchs. If specimens of *P. caballeroi* from the type host and type locality are critically examined, this species may be found to be a synonym of *P. chiloscyllii.* The wide distribution of *P. chiloscyllii*, as well as the wide distribution of larvae with similar morphology, suggests that the intermediate hosts have a wide geographic range. It is reasonable to assume that these intermediate hosts are restricted to open-water fishes. Sharks probably acquire the infection throughout their geographical range. *Pulchrascaris secunda* is the only species in the genus known to parasitize teleosts.

This report recognizes five species belonging to the genus *Terranova* that infect elasmobranchs. *Terranova brevicapitata, T. scoliodontis,* and *T. nidifex* have been previously discussed. *Terranova antarctica* Leiper and Atkinson, the type species, was collected from the stomach of *Mustelus antarcticus* in Bay of Islands, New Zealand. This species is known from only female specimens. The fifth species, *T. pristis*, was described by Baylis and Daubney (1922) from the intestine of a sawfish, *Pristis perotteti*. I examined paratypes (BMNH 1982:2172–2174) and concur with the original description with the exception that the males have three cuticular plates immediately posterior to the cloacal opening and at least 54 pairs of preanal and six pairs of postanal papillae. Gibson and Colin (1982) synonymized *T. galeocerdonis* (Thwaite), *T. rochalimai* (Pereira), and *T. ginglymostomae* Olsen into *T. pristis*. I believe *T. cephaloscyllii*, which was found in the stomach of a cat shark, *Cephaloscyllium unbratile* Jordan and Fowler, from Nagasaki, should also be combined with *T. pristis* because, based on the description by Yamaguti (1941), these species cannot be differentiated. The holotype of *T. cephaloscyllii* could not be obtained to corroborate the original description. *Terranova pristis* is differentiated from all species in the genus by the males having three cuticular plates.

The presence of cuticular plates appears to be unique to these ascaridoids. Although the function of the cuticular plates on the males is uncertain, the presence or absence, number, and morphology of these plates on species belonging to both *Terranova* and *Pulchrascaris* may have evolutionary implications. Cuticular plates appear to be a primitive character, based on the fact that species that have similar cuticular plates generally infect primitive hosts (elasmobranchs). Hence, it is likely that the two genera are evolutionarily very close.

Further, the morphology of these cuticular plates is useful for identification to species, as evidenced in the genus *Terranova*. In addition to *T. pristis* and *T. scoliodontis*, which infect elasmobranchs, four other species—*T. crocodili* (Taylor), *T. lancellata* (Molin), *T. caballeroi* Barus and Coy Otero, and *T. draschei* (Stossich)—have been reported to have cuticular plates. The first three species are found in reptiles. The three cuticular plates on these species are semilunar in shape and directed posteriad. This contrasts sharply with the condition in the two species parasitizing elasmobranchs. The edges of each cuticular plate on *T. crocodili* and *T. lancellata* are serrated; those on *T. caballeroi* are smooth. *Terranova draschei* is the only species in the genus that has semilunar-shaped plates and does not parasitize a reptile; rather, it has been reported in the primitive fish *Arapaima gigas* from the Amazon (see Baylis, 1927; dos Santos et al., 1979). According to Sprent (1979), *T. caballeroi, T. crocodili,* and *T. lancellata* differ from *T. draschei* in the morphology of their spicules.

## Acknowledgments

I gratefully acknowledge David I. Gibson at the British Museum (Natural History), J. Ralph Lichtenfels at the U.S. National Museum Helminthological Collection, J. Julio Vicente and Delir Correa Gomes at the Oswaldo Cruz Institute, and Patricia Hutchings at The Australian Museum for loaning museum specimens.

## Literature Cited

**Baylis, H. A.** 1927. Some parasitic worms from *Arapaima gigas* (teleostean fish) with a description of *Philometra senticosa* n. sp. (Filarioidea). Parasitology 19:35–47.

———. 1931. On some Ascaridae from Queensland. Annals and Magazine of Natural History, series 10, 8:95–102.

———**, and R. Daubney.** 1922. Report on the parasitic nematodes in the collection of the Zoological Survey of India. Memoirs of the Indian Museum 7:263–347.

**Cannon, L. R. G.** 1977. Some larval ascaridoids from south-eastern Queensland marine fishes. International Journal of Parasitology 7:233–243.

**Chandler, A. C.** 1935. Parasites of fishes in Galveston Bay. Proceedings of the United States National Museum 83:123–157.

**Compagno, L. J. V.** 1984. FAO Species Catalogue. Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Part 1. Hexanchiformes to Lamniformes. FAO Fisheries Synopsis No. 125. Vol. 4. 249 pp.

**Deardorff, T. L., M. M. Kliks, M. E. Rosenfeld, R. A. Rychlinski, and R. S. Desowitz.** 1982. Larval ascaridoid nematodes from fishes near the Hawai-

ian Islands with comments on pathogenicity experiments. Pacific Science 36:187–201.
———, **and R. M. Overstreet.** 1981. Review of *Hysterothylacium* and *Iheringascaris* (both previously = *Thynnascaris*) (Nematoda: Anisakidae) from the northern Gulf of Mexico. Proceedings of the Biological Society of Washington (1980) 93:1035–1079.
———, **R. B. Raybourne, and R. S. Desowitz.** 1984. Description of a third-stage larva, *Terranova* type Hawaii A (Nematoda: Anisakinae), from Hawaiian fishes. Journal of Parasitology 70:829–831.
**dos Santos, E., J. J. Vicente, and C. R. Jardim.** 1979. Helmintos de peixes de Rios Amazonicos da colecao helmintologica do Instituto Oswaldo Cruz. II. Nematoda. Atas Sociedad Biologia de Rio de Janerio 20:11–19.
**Gibson, D. I.** 1983. The systematics of ascaridoid nematodes—a current assessment. *In* A. R. Stone, H. M. Platt, and L. F. Khalil, eds. Concepts in Nematode Systematics 22:321–338. Academic Press, London and New York.
———, **and J. A. Colin.** 1982. The *Terranova* enigma. Parasitology 85:xxxvi–xxxvii.
**Hartwich, G.** 1974. Keys to the genera of the Ascaridoidea. 15 pages *in* R. C. Anderson, A. G. Chabaud, and S. Wilmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 2. Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, England.
**Johnston, T. H., and P. M. Mawson.** 1945. Parasitic nematodes. B.A.N.Z. Antarctic Research Expedition 1929–1931 Reports, series B (Zoology and Botany) 5:73–160.
———, **and** ———. 1951. Report on some parasitic nematodes from the Australian Museum. Records of the Australian Museum, Sydney 22:289–297.
**Lent, H., and J. F. Teixeira de Freitas.** 1949. Uma colecao de nematodeos, parasitos de vertebrados, do Museu de Historia Natural de Montevideo. Memorias do Instituto Oswaldo Cruz 46:1–71.
**Linton, E.** 1900. Fish parasites collected at Woods Hole in 1898. Bulletin of the United States Fish Commission (1899) 19:267–304.
———. 1901. Parasites of fishes of the Woods Hole region. Bulletin of the United States Fish Commission (1899) 19:405–492.
———. 1907. Preliminary report on animal parasites collected at Tortugas, Florida, June 30 to July 18, 1906. Carnegie Institution of Washington, No. 5 (1906), Washington. 112–118 pp.
———. 1934. Some observations on the distribution of helminth Entozoa of fishes of the Woods Hole region (Massachusetts, U.S.A.). Transactions of the American Microscopical Society 52:121–131.
**Luna, L. G.,** ed. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. New York. The Blakiston Division, McGraw-Hill Book Co., 258 pp.
**Olsen, L. S.** 1952. Some nematodes parasitic in marine fishes. Publications of the Institute of Marine Science at the University of Texas 2:173–215.
**Sprent, J. F. A.** 1979. Ascaridoid nematodes of amphibians and reptiles: *Terranova.* Journal of Helminthology 53:265–282.
**Vado, E. Y.** 1972. Etude de huit nematodes parasites de vertebres du Venezuela et de la Colombie. Bulletin of the Museum of Natural History, Paris, series III, no. 41:476–498.
**Vicente, J. J., and E. dos Santos.** 1972. Sobre um nova genero da subfamilia *Filocapsulariinae* Yamaguti, 1961 (Nematoda, Ascaridoidea). Atas Sociedad Biologia de Rio de Janeiro 16:17–19.
**Yamaguti, S.** 1941. Studies on the helminth fauna of Japan. Part 33. Nematodes of fishes, II. Japanese Journal of Zoology 9:343–396 + pls. 4–7.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 40–47

# *Wanaristrongylus* gen. n. (Nematoda: Trichostrongyloidea) from Australian Lizards, with Descriptions of Three New Species

HUGH I. JONES
Zoology Department, University of Western Australia, Nedlands, Western Australia 6009

ABSTRACT: The genus *Wanaristrongylus* gen. n. (Herpetostrongylinae) is erected to accommodate three new species of trichostrongyloid nematodes from Australian reptiles. The genus is characterized by the possession of a synlophe comprising more than 10 relatively small, perpendicular cuticular ridges, monodelphy, a posteriorly situated vulva with pre- or paravulval alae, and female tail acutely flexed ventrally. *Wanaristrongylus pogonae* sp. n. from *Pogona minor mitchelli* (Agamidae), *Wanaristrongylus ctenoti* sp. n. from *Ctenotus grandis* (Scincidae), and *Wanaristrongylus papangawurpae* sp. n. from *Nephrurus laevissimus* (Gekkonidae) are distinguished from one another by the number and spacing of the ridges in the synlophe, the extent of the posterior alae in the females, and the size and character of the male spicules.

KEY WORDS: taxonomy, *Amphibiophilus, Austrostrongylus, Dessetostrongylus, Herpetostrongylus, Nasistrongylus, Paraustrostrongylus, Vaucherus, Wooleya, Antechinus, Ctenotus, Nephrurus, Pogona.*

The Trichostrongyloidea is the richest superfamily of parasitic nematodes, both in terms of the number of genera and the number of species (Durette-Desset, 1983), and their great structural similarity has resulted in many attempts at their classification. Earlier descriptions of this group did not include the examination of transverse sections of the worms, but recently the synlophe (the longitudinal or oblique cuticular ridges) has been accepted as being an important taxonomic character, resulting in a classification (Durette-Desset and Chabaud, 1977, 1981) based largely on this structure.

The Herpetostrongylidae parasitize Indian, Southeast Asian, and Australian reptiles and marsupials, and comprises the two subfamilies Globocephaloidinae and Herpetostrongylinae (Durette-Desset and Chabaud, 1981). Nine genera are referred to the Herpetostrongylinae; of these, *Vaucherus* and *Herpetostrongylus* are parasitic in reptiles and are considered to be phylogenetically distinct from those found in marsupials (Humphery-Smith, 1983). This paper describes three species from Australian lizards that are sufficiently distinct morphologically from both *Vaucherus* and *Herpetostrongylus* and from the genera parasitizing marsupials to necessitate the erection of a new genus.

## Materials and Methods

Worms were obtained from hosts preserved in the Western Australian Museum (WAM) and from the preserved stomachs of lizards collected by Dr. Eric Pianka. All hosts had been fixed in formalin and stored in 70% alcohol. Worms were cleaned, cleared in chlorolactophenol, examined using an Olympus BA microscope, and stored in 70% alcohol. Transverse sections of worms were cut near midbody by hand and by microtome, and permanent preparations of sections were stained with hematoxylin and eosin. All specimens have been deposited in the Western Australian Museum. Type material of *Amphibiophilus egerniae* was examined from the South Australian Museum.

Nomenclature of the bursal rays follows Chabaud et al. (1970); the notation 1-3-1 or 2-3 refers to the grouping of the lateral rays (Durette-Desset and Chabaud, 1981).

## Descriptions

### Trichostrongyloidea

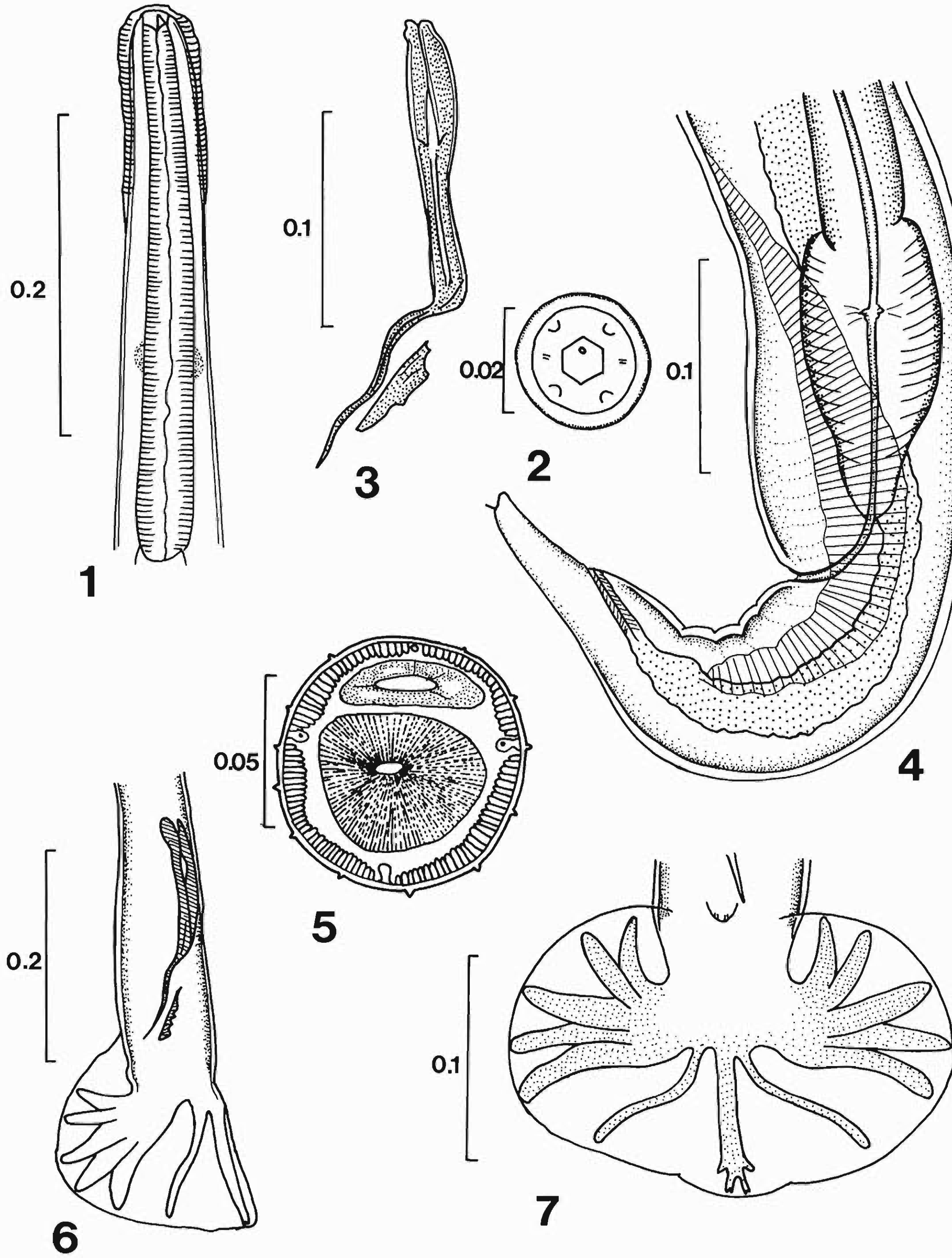
### Herpetostrongylidae Durette-Desset and Chabaud, 1981

### Herpetostrongylinae Skrjabin and Schulz, 1937

### *Wanaristrongylus* gen. n.

GENERIC DIAGNOSIS: Small thin worms frequently, but not invariably, in tightly coiled spiral. Buccal capsule hexagonal. Large dorsal esophageal tooth. Cephalic vesicle present. Synlophe with cuticular ridges small, perpendicular to body surface, and more than 10 in number. *Male:* Copulatory bursa symmetrical with small dorsal lobe. Lateral rays pattern 1:3:1 or 2:3, extending to bursal margin. Ray 8 thin, arising from base of dorsal ray, not extending to margin. Spicules variable; gubernaculum present. *Female:* Monodelphic, with vulva situated near tail. Tail flexed ventrally more than 90°. Ventral or ventrolateral alae or cuticular inflations anterior and lateral to vulva. Tail with 2 terminal knobs situated transversely and fine subterminal dorsal spike.

ETYMOLOGY: From *Wanari,* =mulga (*Acacia* spp.) in the language of the Western Desert ab-




**Figures 1–7.** ***Wanaristrongylus pogonae*** **sp. n. 1. Anterior end, lateral view, male paratype. 2. Anterior end, female paratype, en face. 3. Copulatory spicules and gubernaculum, male paratype, lateral view. 4. Posterior end, female paratype, lateral view. 5. Transverse section, midbody (dorsum uppermost). 6. Tail, male paratype, lateral view. 7. Tail, male paratype, ventral view.**

**Table 1. Measurements (in millimeters unless indicated otherwise) of *Wanaristrongylus pogonae* sp. n., *W. ctenoti* sp. n., and *W. papangawurpae* sp. n.**

| | *Wanaristrongylus pogonae* | | | | *Wanaristrongylus ctenoti* | | | | *Wanaristrongylus papangawurpae* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | | Male | | Female | |
| | Holotype | Paratypes (*N* = 8) | Allo-type | Paratypes (*N* = 5) | Holotype | Paratypes (*N* = 4) | Allo-type | Paratypes (*N* = 5) | Holotype | Paratypes (*N* = 6) | Allo-type | Paratypes (*N* = 5) |
| Length | 4.44 | 3.55–5.00 | 5.56 | 6.22–6.67 | 6.20 | 4.10–5.80 | 8.33 | 5.90–8.55 | 4.55 | 4.22–4.55 | 6.67 | 5.89–7.11 |
| Esophagus length | 0.36 | 0.33–0.36 | 0.40 | 0.34–0.39 | 0.32 | 0.31–0.35 | 0.35 | 0.33–0.37 | 0.33 | 0.31–0.34 | 0.40 | 0.41–0.42 |
| Esophagus width | 0.04 | 0.32–0.40 | 0.04 | 0.36–0.46 | 0.06 | 0.04–0.05 | 0.06 | 0.04–0.06 | 0.04 | 0.03–0.04 | 0.04 | 0.04–0.06 |
| Maximum width | 0.08 | 0.07–0.09 | 012 | 0.10–0.13 | 0.13 | 0.10–0.14 | 0.14 | 0.10–0.15 | 0.08 | 0.07–0.08 | 0.11 | 0.09–0.11 |
| Nerve ring* | — | — | — | — | 0.21 | 0.18–0.21 | 0.22 | 0.20–0.24 | — | 0.20–0.25 | 0.26 | 0.20 |
| Excretory pore* | 0.39 | 0.35–0.39 | 0.43 | 0.40–0.44 | — | — | — | — | 0.38 | 0.38–0.41 | 0.49 | 0.43–0.45 |
| Cephalic sheath | 0.10 | 0.08–0.10 | 0.10 | 0.10–0.11 | 0.10 | 0.10–0.11 | 0.11 | 0.10–0.11 | 0.08 | 0.09–0.10 | 0.10 | 0.10–0.11 |
| Spicules (μm) | 244 | 208–252 | — | — | 532 | 500–568 | — | — | 160 | 148–180 | — | — |
| Gubernaculum (μm) | 64 | 48–60 | — | — | 80 | 80–84 | — | — | 38 | 32–44 | — | — |
| Tail | — | — | 0.05 | 0.05–0.06 | — | — | 0.06 | 0.05–0.08 | — | — | 0.05 | 0.04–0.05 |
| Vulva† | — | — | 0.24 | 0.12–0.20 | — | — | 0.15 | 0.14–0.18 | — | — | 0.16 | 0.12–0.15 |
| Vagina | — | — | 0.03 | 0.03 | — | — | 0.02 | 0.02–0.04 | — | — | 0.04 | 0.04 |
| Vestibule | — | — | 0.10 | 0.08–0.10 | — | — | 0.26 | 0.21–0.28 | — | — | 0.06 | 0.06–0.08 |
| Sphincter | — | — | 0.03 | 0.02–0.03 | — | — | 0.05 | 0.04–0.05 | — | — | 0.04 | 0.03–0.05 |
| Infundibulum | — | — | 0.10 | 0.09–0.14 | — | — | 0.16 | 0.16–0.18 | — | — | 0.08 | 0.07–0.11 |
| Egg length (μm) | — | — | 68 | 60–69 | — | — | 72 | 68–72 | — | — | 72 | 68–72 |
| Egg width (μm) | — | — | 40 | 36–40 | — | — | 48 | 40–60 | — | — | 44 | 40–44 |

* Distance from anterior end.
† Distance from posterior end.

original people, referring to the predominant vegetation in two of the type localities.

### *Wanaristrongylus pogonae* sp. n. (Figs. 1–7; Table 1)

DESCRIPTION (based on approximately 20 males and 5 females): Small thin worms, coiled in tight spirals in some individuals, tapering anteriorly. Synlophe composed of 12 low longitudinal cuticular ridges, arranged perpendicularly to body wall, extending from near anterior end of worm to 0.2–0.3 mm anterior to vulva or bursa, smaller on dorsolateral surface, and absent middorsally. Cephalic vesicle at anterior end, with 30–35 conspicuous transverse striations. Four cephalic papillae and 2 amphids. Buccal cavity hexagonal in transverse section, with large triangular anteriorly directed tooth arising from anterior end of dorsal esophageal lobe; no teeth on subventral lobes. Esophagus surrounded about midlength by inconspicuous nerve-ring; excretory pore a short distance posterior to level of origin of intestine.

MALE: Bursa symmetrical, with small dorsal lobe. No prebursal lateral alae. Rays 2 and 3 directed anteriorly, and shorter than rays 4–6, which are larger and directed laterally. Ray 8 arises from base of dorsal ray, thin, extending almost to margin of bursa. Dorsal ray fairly thick, with terminal portion (papillae 10) divided. Spicules well sclerotized, with ventral flexion to about 45°, at or just past midlength. Distal portions fused, sinuous, terminating in single fine point. Gubernaculum small, well sclerotized, with smooth ventral and irregular dorsal surfaces.

FEMALE: Vulva situated near posterior end, with body flexed ventrally to approximately 90° at this level. Thick alae or cuticular inflations ventrolaterally, extending from anterior to vulva to midway between vulva and anus. Monodelphic. Tail with 2 small ventral knobs, and a very fine dorsally directed subterminal spike. Eggs large, thin-shelled, elongate, unembryonated.

TYPE HOST: *Pogona minor mitchelli* (Badham, 1976) (Agamidae).

LOCALITY: Pender Bay, Northwest Australia (16°45′S, 122°42′E).

LOCATION IN HOST: Small intestine.

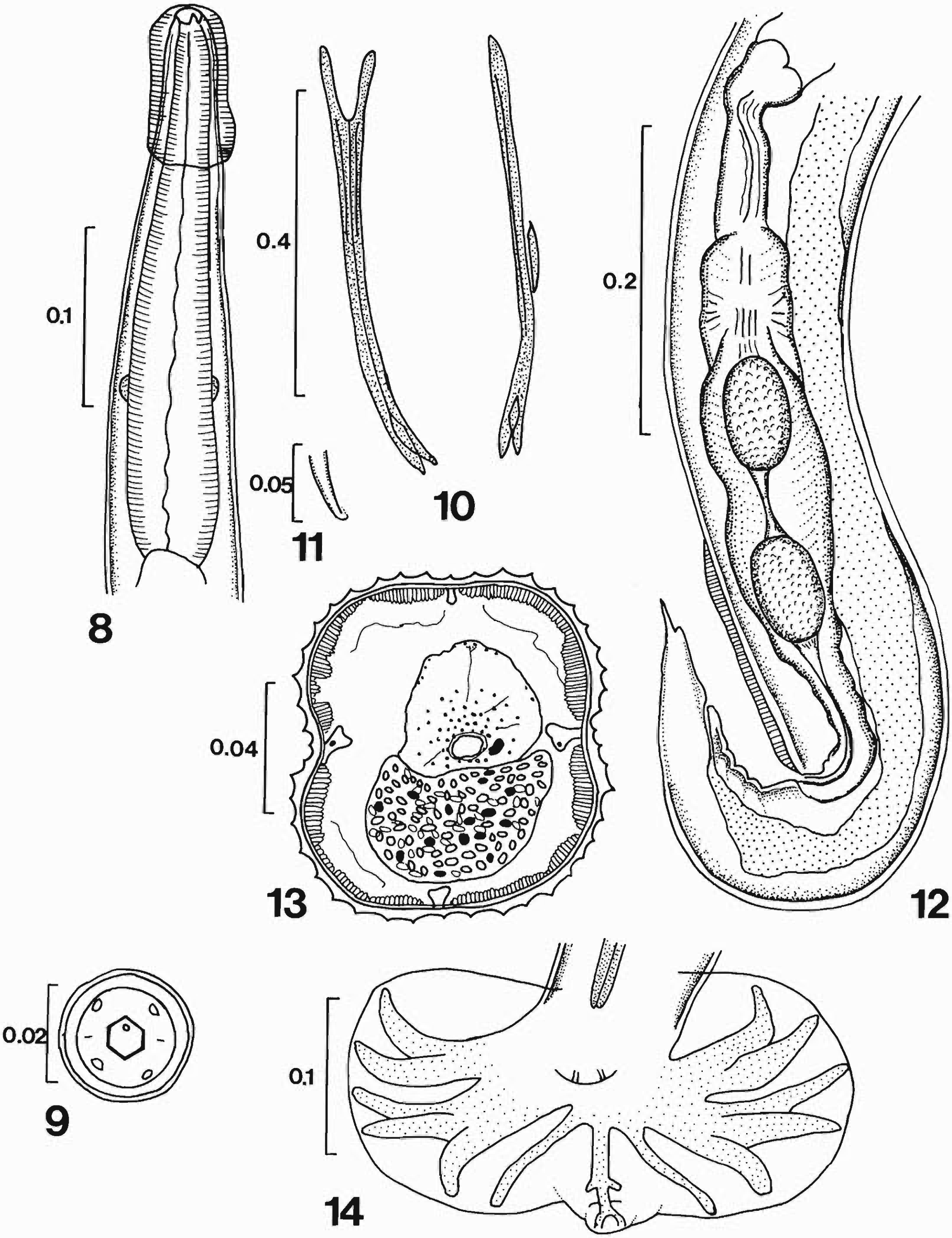
SPECIMENS: Male holotype (WAM 249-85), female allotype (WAM 250-85), and approximately 25 paratypes (WAM 251-85) collected 26 April 1977.

### *Wanaristrongylus ctenoti* sp. n. (Figs. 8–14; Table 1)

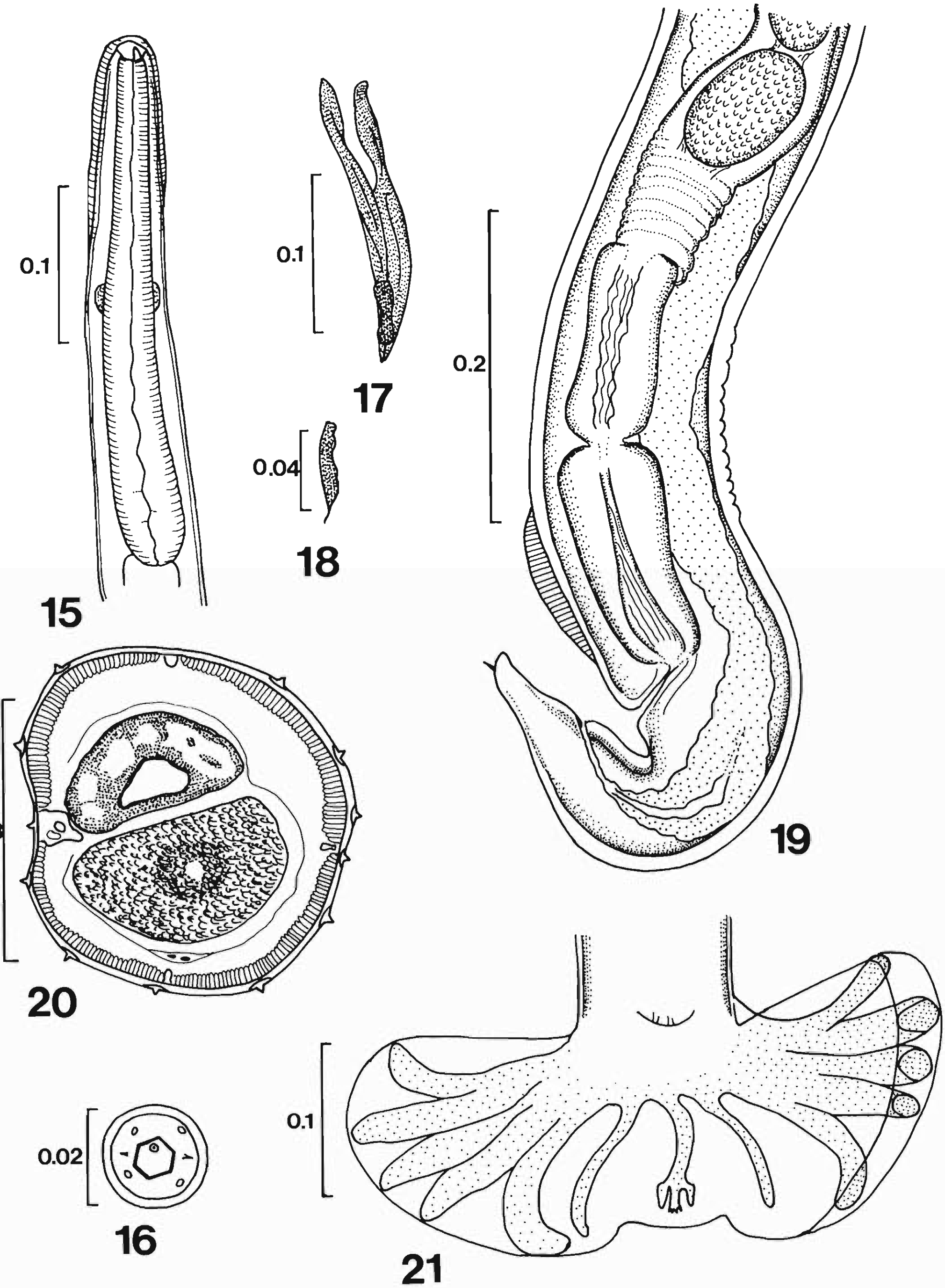
DESCRIPTION (based on approximately 20 males and 25 females): Small worms, tapering anteriorly, not tightly coiled, with very fine transverse cuticular striations. Synlophe comprised of between 40 and 55 small, equally spaced cuticular ridges, perpendicular to body wall. Ridges extend from posterior to buccal capsule to just anterior to cloaca in male; in female, ventral and lateral ridges terminate anterior to vulva, and dorsal ridges extend to level of anus. Cephalic vesicle at anterior end, bearing 25–30 conspicuous transverse striations. Four cephalic papillae and 2 amphids. Buccal capsule hexagonal in transverse section. Anterior end of dorsal esophageal lobe with large anteriorly directed triangular tooth, extending almost the depth of the buccal capsule; no teeth on subventral lobes. Esophagus increases in width posteriorly, surrounded near midlength by inconspicuous nerve-ring. Excretory pore a short distance posterior to level of origin of intestine.

MALE: Bursa symmetrical; dorsal lobe small. Ray 2 disposed anteriorly, fused in basal section with ray 3; rays 3–5 thick, approximately the same width, arising from a common trunk and extending to bursal margin. Ray 6 curved dorsally. Ray 8 arises from separate trunk, is considerably thinner than rays 2–6, and does not extend to bursal margin. Dorsal ray narrow, of uniform width, with pair of short lateral branches, disposed at right angles, at approximately ⅔ its length; posterior to these is a pair of very fine twiglike projections. Distally dorsal ray divides into 2 short branches, surrounded by small distinct lobe. Surface of bursa lined by close rows of very fine tubercles. No prebursal alae. Spicules long, similar, well sclerotized, with slight ventral curve. Separate at origin but in apposition and alate for most of their length, terminating in blunt points. Gubernaculum small with smooth convex dorsal surface.

FEMALE: Posterior end flexed to 180° just before tail in almost all individuals. Vulva an inconspicuous horizontal slit at point of flexion, short distance anterior to anus. Narrow short ventral alae terminate posteriorly at level of vulva. Monodelphic. Tip of tail with 2 small transversely placed knobs, beyond which extends a dorsal spike. Eggs large, thin-shelled, elongate, unembryonated.




**Figures 8–14.** ***Wanaristrongylus ctenoti*** **sp. n. 8. Anterior end, female paratype, lateral view. 9. Anterior end, female paratype, en face. 10. Copulatory spicules, male paratype, ventral and lateral views. 11. Tip of spicule, male paratype, lateral view. 12. Posterior end, female paratype, lateral view. 13. Transverse section, midbody (dorsum uppermost). 14. Tail, male paratype, ventral view.**




**Figures 15–21.** ***Wanaristrongylus papangawurpae*** **sp. n. 15. Anterior end, female paratype, lateral view. 16. Anterior end, female paratype, en face. 17. Copulatory spicules, male paratype, ventral view with gubernaculum superimposed. 18. Gubernaculum, lateral view. 19. Posterior end, female paratype, lateral view. 20. Transverse section, midbody (dorsum uppermost). 21. Tail, male paratype, ventral view.**

TYPE HOST: *Ctenotus grandis* Storr, 1969 (Scincidae).

LOCALITY: Approximately 45 km ENE of Laverton, Western Australia (28°28′S, 122°50′E).

LOCATION IN HOST: Stomach.

SPECIMENS: Male holotype (WAM 252-85), female allotype (WAM 253-85), and approximately 45 paratypes (WAM 254-85) from hosts collected by Dr. Eric Pianka, 14 February 1979.

### *Wanaristrongylus papangawurpae* sp. n. (Figs. 15–21; Table 1)

DESCRIPTION (based on approximately 15 worms): Small thin worms, not tightly coiled, tapering anteriorly, with very fine transverse cuticular striations. Synlophe consists of 12 symmetrically arranged small longitudinal cuticular ridges more spaced dorsally than laterally and absent middorsally, perpendicular to body surface. Ridges originate immediately posterior to buccal capsule and extend length of worm, terminating approximately 0.3 mm from tip of tail in male, and at approximately level of vulva in female. No alae. Cephalic vesicle at anterior end with 25–30 conspicuous transverse striations. Four cephalic papillae and 2 amphids. Buccal capsule hexagonal in transverse section. Large triangular tooth projects anteriorly from anterior end of dorsal esophageal lobe; no teeth on subventral lobes. Esophagus widens posteriorly, with ill-defined nerve-ring posterior to midlength. Excretory pore at level of, or slightly posterior to, esophago–intestinal junction.

MALE: Bursal lobes tightly flexed in most specimens. Two symmetrical lateral lobes and small dorsal lobe. Rays 2–6 arise from a common trunk, thick and of approximately equal width. Ray 2 directed anteriorly and partly united at base to ray 3; rays 3–5 extend laterally, and ray 6 directed posteriorly. Ray 8 arises from base of dorsal ray, considerably thinner than rays 2–6, of uniform width, not extending to bursal margin. Dorsal ray with pair of small posteriorly directed branches, narrow at their point of origin, at approximately ¾ its length; ray divides twice near its distal extremity, terminating some distance before edge of bursa. Bursa lined on ventral surface by rows of fine short transverse striations, which appear to be situated over margins of rays. No prebursal alae. Spicules similar, heavily sclerotized, short, alate, curved ventrally, twisted and in apposition for their posterior ⅔. Gubernaculum with smooth ventral surface, irregular dorsal surface, and fine posterior extension.

FEMALE: Posterior end flexed ventrally to 180° just anterior to tail; vulva posterior, with 2 narrow short ventral alae anterior to vulva. Monodelphic. Tail short, with 2 small terminal lobes and very fine subterminal dorsal spike, not extending as far as tip of tail. Eggs thin-shelled, large, elongate, unembryonated.

TYPE HOST: *Nephrurus laevissimus* Mertens, 1958 (Gekkonidae).

LOCALITY: Approximately 100 km ENE of Laverton, Western Australia (28°12′S, 123°36′E).

LOCATION IN HOST: Stomach.

SPECIMENS: Male holotype (WAM 255-85), female allotype (WAM 256-85), and approximately 15 paratypes (WAM 257-85) from hosts collected by Dr. Eric Pianka, 15 November 1978.

ETYMOLOGY: From *papangawurpa,* the Western Desert aboriginal name for a gecko.

## Discussion

The three species described in this paper have in common the features of monodelphy, a posteriorly situated vulva with pre- or paravulval alae, no somatic alae, female tail acutely flexed ventrally with a subterminal dorsal spike, and relatively small, perpendicularly arranged cuticular ridges. This combination of characters distinguishes the genus *Wanaristrongylus* from other genera in the subfamily Herpetostrongylinae. *Wanaristrongylus ctenoti* differs from *W. pogonae* and *W. papangawurpae* in possessing numerous longitudinal ridges, a more voluminous cephalic vesicle, longer, slightly curved spicules, and extradorsal rays. In the female the vestibule and infundibulum are longer, and the subterminal spike extends beyond the tip of the tail. *Wanaristrongylus pogonae* and *W. papangawurpae* are similar in many respects, but they are readily distinguished from one another by the longer, sharply flexed tapering spicules, larger gubernaculum, and more extensive paravulval alae in *W. pogonae.*

*Vaucherus* and *Herpetostrongylus,* both of which parasitize reptiles in Southeast Asia or Australia, possess either cuticular ridges with an oblique axis of orientation or cuticular swellings only (Durette-Desset, 1980; Humphrey-Smith, 1981). Monodelphy occurs in *Paraustrostrongylus* (which possesses cuticular inflations at the posterior end in both sexes; Mawson, 1973), one species of *Wooleya* (Mawson, 1973), *Dessetostrongylus,* and *Austrostrongylus,* all of which are found in Australian marsupials. The only other genus in this family that possesses perpendicular cuticular ridges is *Nasistrongylus,* described from the marsupial *Antechinus stuartii* by Durette-Desset and Beveridge (1982). Perpendicular ridges, and female tails with two terminal lobes and a spike, also occur in some members of the Nicollinidae, which parasitize amphibians, monotremes, and marsupials in Australia and Southeast Asia.

Three species of trichostrongyle nematode have been described previously from Australian reptiles. *Herpetostrongylus pythonis* appears to be restricted to northeastern tropical Queensland (Baylis, 1931; Jones, 1979). *Herpetostrongylus varani* also occurs in northeastern Queensland (Baylis, 1931), but has not been found in any *Varanus* lizards examined by me in Western Australia. Both of these species have been reexamined by Humphery-Smith (1981). Finally, *Amphibiophilus egerniae* was described from the skink *Egernia dahli* in the Musgrave Ranges in central Australia (Johnston and Mawson, 1947). Type specimens of *A. egerniae* have been reexamined; the male possesses spicules similar to those in *W. pogonae,* and the female is monodelphic, but the specimens differ from those described here in the absence of pre- or paravulval alae, and in the presence of very numerous closely set longitudinal cuticular ridges. However, the bursa of the only male specimen available is damaged, and the systematic position of this species cannot be ascertained until more specimens are available for study.

Nematodes from two other trichostrongyloid families have been recorded from reptiles in other parts of the world: *Mertensinema* (Dictyocaulidae), in amphibians and reptiles, and *Trichoskrjabinia, Oswaldocruzia,* and *Typhlopsia* (Molineidae) in chelonians, amphibians (principally), and typhlopsid snakes, respectively. Members of the genus *Wanaristrongylus* appear to be the only group so far recorded that have adapted to reptiles living in a hot and arid environment.


## Acknowledgments

I thank Dr. Eric Pianka from the University of Texas at Austin and Dr. Glen Storr and the staff of the Department of Ornithology and Herpetology at the Western Australian Museum for allowing me to examine material and for providing collection data, Dr. Ian Beveridge for reading and commenting on the manuscript, Mr. D. C. Lee for the loan of type material from the South Australian Museum, and Mr. Tom Stewart for histological preparations.


## Literature Cited


**Baylis, H. A.** 1931. Two more new trichostrongylid nematodes from Queensland. Annals and Magazine of Natural History, 7, series 10:500–507.

**Chabaud, A. G., F. Puylaert, O. Bain, A. J. Petter, and M.-C. Durette-Desset.** 1970. Remarques sur l'homologie entre les papilles cloacales des rhabditides et les côtes dorsales des Strongylida. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences 271:1771–1774.

**Durette-Desset, M.-C.** 1980. Nouveaux nématodes Trichostrongyloïdes parasites de sauriens en Malaisie et à Bornéo. Bulletin de Muséum National d'Histoire Naturelle, Paris, 4th series 2:697–706.

———. 1983. Keys to the genera of the superfamily Trichostrongyloidea. Pages 1–68 *in* R. C. Anderson, A. G. Chabaud, and S. Wilmott, eds. CIH Keys to the Nematode Parasites of Vertebrates. No. 10. Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, England.

———**, and I. Beveridge.** 1982. Deux genres aberrant de nématodes Trichostrongyloïdes parasites de Marsupiaux australiens: *Asymmetracantha* Mawson, 1960 et *Nasistrongylus* n. gen. Bulletin de Muséum National d'Histoire Naturelle, Paris, Zool. 3:1053–1059.

———**, and A. G. Chabaud.** 1977. Essai de classification des nématodes Trichostrongyloïdea. Annales de Parasitologie 52:539–558.

———**, and** ———. 1981. Nouvel essai de classification des nématodes Trichostrongyloïdea. Annales de Parasitologie 56:297–312.

**Humphery-Smith, I.** 1981. Compléments morphologiques au genre *Herpetostrongylus* Baylis, 1931 (Nematoda, Trichostrongyloidea). Bulletin de Muséum National d'Histoire Naturelle, Paris, 4th series 3:133–137.

———. 1983. An hypothesis on the evolution of Herpetostrongylinae (Trichostrongyloidea: Nematoda) in Australian marsupials, and their relationships with Viannaidae, parasites of South American marsupials. Australian Journal of Zoology 31:931–942.

**Johnston, T. H., and P. M. Mawson.** 1947. Some nematodes from Australian lizards. Transactions of the Royal Society of South Australia 71:22–27.

**Jones, H. I.** 1979. Gastrointestinal nematodes, including three new species, from Australian and Papua New Guinean pythons. Proceedings of the Helminthological Society of Washington 46:1–14.

**Mawson, P. M.** 1973. Amidostomatinae (Nematoda: Trichostrongyloidea) from Australian marsupials and monotremes. Transactions of the Royal Society of South Australia 97:257–279.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 48–52

# Survival of Third-stage Larvae of Washington Isolates of *Haemonchus contortus* and *Ostertagia circumcincta* Exposed to Cold Temperatures

DOUGLAS P. JASMER,[1,2] RICHARD B. WESCOTT,[3] AND JOHN W. CRANE[1]
Departments of [1]Zoology and [3]Veterinary Microbiology and Pathology,
Washington State University, Pullman, Washington 99164

ABSTRACT: The ability of third-stage larvae ($L_3$) of isolates of *Haemonchus contortus* and *Ostertagia circumcincta* from sheep in eastern Washington to survive cold stress was compared, and the role of the larval sheath in such survival was examined. Survival was estimated by determining the viability of $L_3$ subjected to temperatures from −10°C to −18°C on one or more occasions for varying lengths of time. Single exposure to −18°C for 5 hr decreased viability of *H. contortus* $L_3$ from >99% to <5%. Viability of similarly exposed *O. circumcincta* $L_3$ was 85%. Differences in survival of $L_3$ of these parasites were less pronounced when the larvae were subjected to temperatures of −10°C and −15°C. Exposure of *O. circumcincta* $L_3$ to freeze/thaw conditions decreased viability to 32% (−15°C/3°C) and 48% (−10°C/3°C) in 10 weeks. No *H. contortus* $L_3$ survived beyond 14 days when subjected to similar freeze/thaw conditions. Microscopic examination of ensheathed $L_3$ at freezing temperatures revealed that few (<11%) of either species froze at −25°C, whereas all exsheathed $L_3$ froze at 0°C. Supercooling in ensheathed $L_3$ was accompanied by an apparent loss of fluid from the larvae as the water surrounding them froze.

KEY WORDS: sheep, trichostrongylid nematodes, epizootiology.

The economic importance of trichostrongylid nematodes has prompted numerous studies directed at elucidating the effects of cold temperatures on free-living stages of these parasites (Furman, 1944; Marquardt et al., 1959; Anderson et al., 1966; Anderson and Levine, 1968; Todd et al., 1970, 1976; Waller and Donald, 1970; Pandey, 1972). These studies have demonstrated that the ability to withstand cold temperatures differs among genera of this group. For instance, *Ostertagia* spp. appear to be cold tolerant (Furman, 1944; Pandey, 1972), whereas cold is detrimental to the survival of *Haemonchus contortus* (Todd et al., 1976). However, factors responsible for these differences are not well understood and apparently vary within species (Le Jambre, 1981), so the survival characteristics of trichostrongylid nematodes are difficult to predict for most geographic locations. The purpose of the present study was to document the ability of third-stage larvae ($L_3$) of isolates of *O. circumcincta* and *H. contortus* from sheep in eastern Washington to survive exposure to cold temperatures, hypothesizing that this information would help explain the paucity of haemonchosis in that geographic area (Levine, 1968; Blanchard and Wescott, 1985). Survival of the egg stages of these parasites subjected to cold temperatures was reported previously (Jasmer et al., 1986).

## Materials and Methods

Feces from donor lambs monospecifically infected with eastern Washington isolates of *H. contortus* or *O. circumcincta* (Jasmer et al., 1986) were mixed in moist vermiculite, and incubated for 7–14 days at 25°C. The cultures then were wrapped in cheesecloth and placed in Baermann funnels filled with tap water. Infective $L_3$ were collected after 15 hr, diluted to desired concentrations in distilled water, and used immediately.

In experiments assessing survival, aproximately 100 $L_3$/ml of distilled water were placed in plastic petri dishes (10 × 34 mm) and exposed to various cold temperature regimens. Survival was measured by warming the dishes to 21°C for 24 hr and then determining the percentage of viable larvae they contained. $L_3$ demonstrating spontaneous movement while viewed for 15 sec with a dissecting microscope were counted as viable. Larvae that did not move during this observation period were counted as nonviable and were considered killed by the exposure.

The short-term effects of cold were evaluated by placing $L_3$ at −10, −15, or −18°C for 5, 10, and 15 hr. Mean percentage of $L_3$ surviving treatment was calculated from counts of viable and nonviable larvae in five 1-ml samples (five replicates) for each observation point. Controls included $L_3$ of both parasites that were stored at 21°C (room temperature) and 3°C (refrigerator temperature) for the duration of the experiment.

Long-term effects of cold were assessed by subjecting $L_3$ to alternating freezing and thawing conditions. This was accomplished by freezing at −10°C or −15°C for 15 hr and then thawing at 3°C for 9 hr. The freeze/

[2] Present address: Issaquah Health Research Institute, 1595 N.W. Gilman Blvd., Issaquah, Washington 98027.

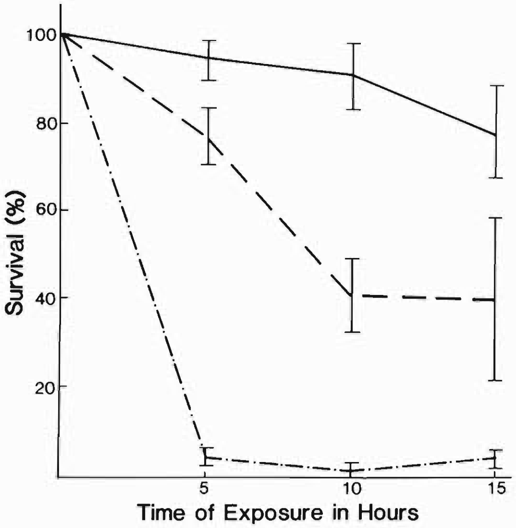



**Figure 1. Survival of $L_3$ of *Haemonchus contortus* as a function of length of exposure to $-10°C$ ——, $-15°C$ – – –, and $-18°C$ –·–·–. Each point represents the mean of five replicates; I = SD.**

thaw cycle was carried out for 5 consecutive days followed by maintenance at 3°C for 2 days and then repeated in subsequent weeks for a period of 10 wk. Survival was recorded on days 1, 3, 7, and 14 and every 2 wk thereafter for the duration of the experiment. Mean percentage of $L_3$ surviving treatment was calculated from counts of viable and nonviable larvae in 1-ml samples from three replicates for each temperature regimen. Controls included $L_3$ stored at 3°C for the 10-wk period. Significance of differences in numbers of $L_3$ surviving treatment were determined using two-way analysis of variance (Steel and Torrie, 1980) in both long-term and short-term experiments.

The role of the sheath in protection of $L_3$ from freezing was examined by exposing ensheathed and exsheathed $L_3$ of *H. contortus* and *O. circumcincta* to 0°C and $-25°C$ while viewing with a compound microscope fitted with a cold stage similar to that described by Sayre (1964). The percentage of $L_3$ freezing was determined by examining 100 ensheathed and 100

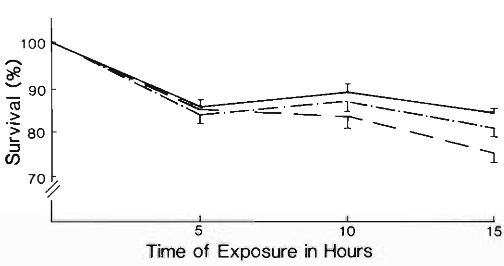



**Figure 2. Survival of $L_3$ *Ostertagia circumcincta* as a function of length of exposure to $-10°C$ ——, $-15°C$ – – –, and $-18°C$ –·–·–. Each point represents the mean of five replicates; I = SD.**

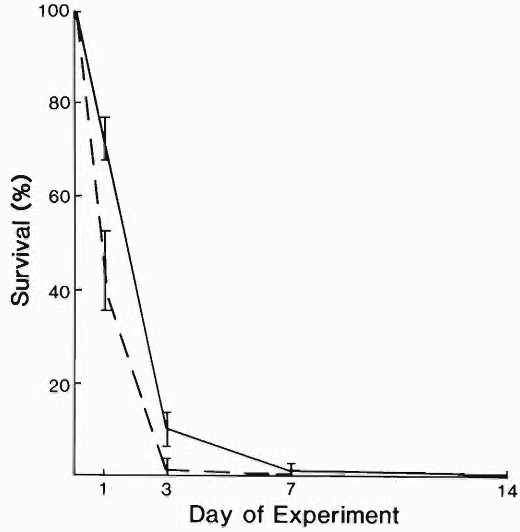



**Figure 3. Survival of $L_3$ of *Haemonchus contortus* subjected to a weekly freeze/thaw cycle at $-10°C/3°C$ —— or $-15°C/3°C$ – – –. Each point represents the mean of three replicates; I = SD.**

exsheathed $L_3$ exposed to these temperatures. Larvae that froze did so rapidly and were readily identified as they became opaque at the observation temperatures. Counts of frozen $L_3$ were made immediately after the cold stage reached 0°C and $-25°C$. Five replicates were performed with *H. contortus* and one with *O. circumcincta.* Exsheathed larvae were obtained by placing $L_3$ in 0.5% sodium hypochlorite (Davey and Rogers, 1982) for 1 min and then rinsing them twice with distilled water. Viability of exsheathed $L_3$ was determined by microscopic examination of controls maintained at 3°C for 3 days after treatment with hypochlorite.

## Results

The effects of short-term exposure to cold are shown in Figures 1 and 2. The percentage of

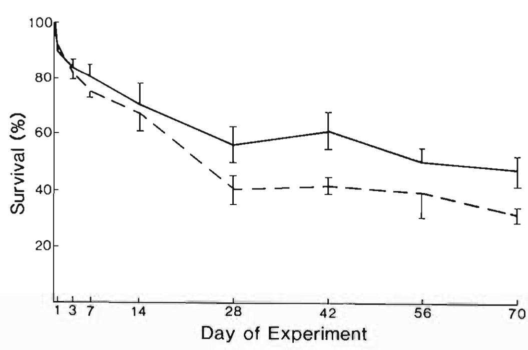
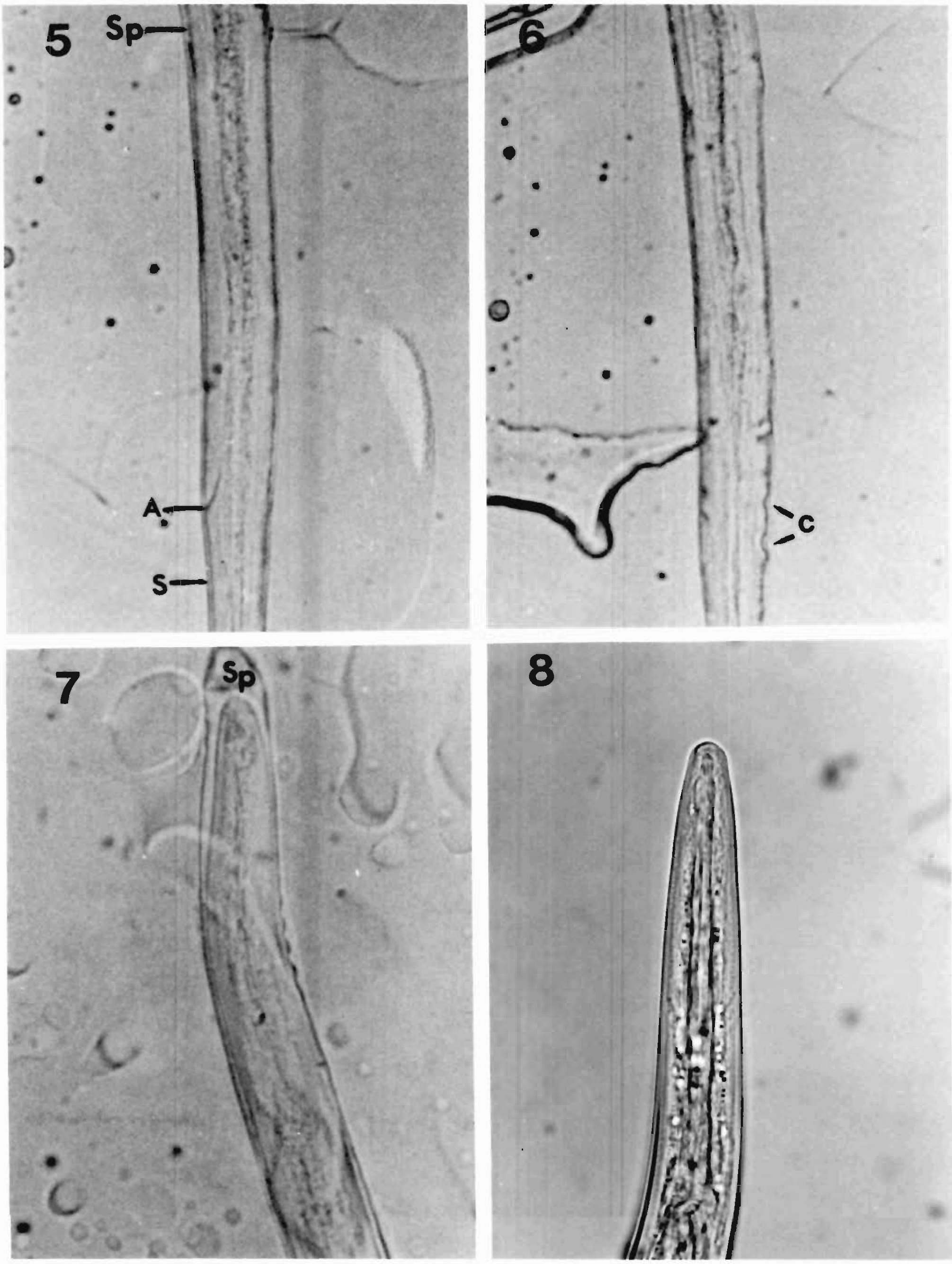



**Figure 4. Survival of $L_3$ of *Ostertagia circumcincta* subjected to a weekly freeze/thaw cycle at $-10°C/3°C$ —— or $-15°C/3°C$ – – –. Each point represents the mean of three replicates; I = SD.**




**Figures 5–8. Photomicrographs of *Haemonchus contortus* $L_3$. 5. Morphology of the sheath as ice forms in surrounding medium. Note perilarval space (Sp), anus (A), and sheath (S). 6. $L_3$ shown in Figure 5 immediately after completion of freezing in surrounding medium. Perilarval space is diminished and convolutions (C) are obvious in cuticle of larva. 7. Cephalic end as ice melts. Note the large space (Sp), normally occupied by the larva, within the sheath. 8. Cephalic end of fresh unfrozen $L_3$ with larva filling sheath.**

viable *H. contortus* $L_3$ decreased from >99% to <5% after exposure to −18°C for 5 hr, and differed significantly ($P < 0.05$) with temperature (−10, −15, and −18°C) at all observation times (Fig. 1). In addition, a significant ($P < 0.005$) interaction was observed between time and temperature for this species. Mean survival of *O. circumcincta* $L_3$ was greater than 79% at all observation points, and only minor differences were apparent among treatment groups (Fig. 2). Viability of $L_3$ of both species stored at 3°C and 21°C for the duration of the experiment was excellent (>99%).

The effects of long-term exposure to cold are shown in Figures 3 and 4. Under alternating freezing and thawing conditions, few *H. contortus* $L_3$ survived 7 days and none were viable after 14 days (Fig. 3). In contrast, 48% and 32% of *O. circumcincta* $L_3$ survived 10 wk of freezing and thawing at −10°C/3°C and −15°C/3°C, respectively. Survival of *O. circumcincta* $L_3$ also decreased significantly ($P < 0.005$) with time at both exposure temperatures. Viability of controls, $L_3$ of both parasites stored at 3°C, exceeded 90% at all observation points.

In trials examining the importance of the sheath in freezing, no ensheathed $L_3$ of *H. contortus* and *O. circumcincta* froze at 0°C and few (<11%) froze at −25°C. All exsheathed $L_3$ froze at 0°C. Ice formation began in the buccal cavity and moved rapidly to the tail in exsheathed $L_3$. The most notable change in ensheathed $L_3$ was that they shrank as the water surrounding them froze. Prior to cold exposure the space created by the sheath was loosely filled by the $L_3$. As ice formed around the sheath it appeared to be forced against the cuticle (Fig. 5) and convolutions appeared in the cuticle (Fig. 6). When the surrounding medium thawed, the sheath appeared flaccid initially but subsequently became turgid with the availability of water. After complete thawing, many $L_3$ failed to refill their sheaths (Figs. 7, 8). Viability of exsheathed $L_3$ stored at 3°C for 3 days exceeded 80%, indicating that hypochlorite treatment per se was not particularly damaging and probably had little effect on freezing.

## Discussion

Survival of $L_3$ of the Washington isolate of *H. contortus* exposed to cold temperatures in the present study was much like that reported for other isolates, in that they remained viable when stored at 3°C for over 10 wk (Boag and Thomas, 1985), but were killed by exposure to freezing temperatures (Todd et al., 1976). It is noteworthy that the Washington isolate was more resistant to very cold temperatures than was the Maryland isolate of *H. contortus* tested by Todd et al. (1976). The viability of the latter was reduced to 1% after 4 hr exposure to −10°C, whereas −18°C was required to produce similar changes with the former. The Washington isolate was extremely sensitive to freeze/thaw conditions (−10°C/3°C and −15°C/3°C), however, and $L_3$ of this parasite on pastures would not be expected to survive winters in much of the northwestern United States, which has temperature extremes more severe than those tested.

The $L_3$ of the Washington isolate of *O. circumcincta,* on the other hand, was extremely cold resistant. Most survived exposure to −18°C for 15 hr, and many survived freeze/thaw conditions for 70 days. Furthermore, comparison of results with those of Pandey (1972) indicates that $L_3$ of the Washington isolate again may be more tolerant to cold exposure than were $L_3$ of an isolate of *Ostertagia* from a warmer climate. In any case, the remarkable ability of $L_3$ of the Washington isolate to survive cold stress in vitro indicates that many would be expected to overwinter on pastures in the geographic area of their origin.

The epidemiology of *H. contortus* and *O. circumcincta* infections in sheep in the Northwest corresponds with observations of other investigators (Levine, 1968; Todd et al., 1976; Waller and Thomas, 1978) that cold resistance of the free-living stages, including egg stages (Jasmer et al., 1986), of these parasites is correlated directly to their prevalence. *Ostertagia circumcincta* has been recognized as the dominant gastrointestinal nematode of sheep in this area (Levine, 1968), and in that respect the situation resembles that in the north of England (Waller and Thomas, 1978). When haemonchosis occurs in the northwestern United States, the disease usually appears in high rainfall areas or is associated with the use of irrigated pastures (Wescott, unpubl.), suggesting that the condition is related to the humidity requirements of the $L_3$ in warm weather (Todd et al., 1970) as well as to cold tolerance.

The mechanism that protects free-living stages of nematodes from freezing was not determined in the present experiments, but the results indicate that the sheath either serves an insulating function or contains a cryoprotectant. The latter hypothesis is supported by the observations of loss in volume of ensheathed $L_3$ exposed to cold temperatures. The sheath apparently functions as a semipermeable membrane allowing water but not solutes to pass from $L_3$ during the freezing process, with the more concentrated solutes then exhibiting a depressed supercooling point. The fact that the sheaths of supercooled $L_3$ appeared flaccid but rapidly became turgid when surrounding ice thawed suggests that the solutes contained by the sheath produce a negative osmotic potential and the larvae rehydrate at temperatures over 0°C.

## Acknowledgments

We express our appreciation to Dr. Vincent Schultz for his critical reading of this manuscript.

This report is published as Scientific Paper No. 7434, College of Agriculture Research Center, Project No. 0114.

This work was supported in part by U.S. Department of Agriculture Cooperative Agreement No. 58-9AHZ-9-403, Biomedical Research Support Grant No. 2-S07-RR05465-18, and United States Agency for International Development SR-CRSP Subgrant No. 115-01.

## Literature Cited

**Anderson, F. L., and N. D. Levine.** 1968. Effect of desiccation on survival of the free-living stages of *Trichostrongylus colubriformis*. Journal of Parasitology 54:117–128.

———, **G. Wang, and N. D. Levine.** 1966. Effect of temperature on survival of the free-living stages of *Trichostrongylus colubriformis*. Journal of Parasitology 52:713–721.

**Blanchard, J. L., and R. B. Wescott.** 1985. Enhancement of resistance of lambs to *Haemonchus contortus* by previous infection with *Ostertagia circumcincta*. American Journal of Veterinary Research 46:2136–2140.

**Boag, B., and R. J. Thomas.** 1985. The effect of temperature on the survival of infective larvae of nematodes. Journal of Parasitology 71:383–384.

**Davey, K. G., and W. P. Rogers.** 1982. Changes in water content and volume accompanying exsheathment of *Haemonchus contortus*. International Journal for Parasitology 12:93–96.

**Furman, D. P.** 1944. Effects of environment upon the free-living stages of *Ostertagia circumcincta* (Stadelmann) Trichostrongylidae: I. Laboratory experiments. American Journal of Veterinary Research 5:79–86.

**Jasmer, D. P., R. B. Wescott, and J. W. Crane.** 1986. Influence of cold temperatures upon development and survival of eggs of Washington isolates of *Haemonchus contortus* and *Ostertagia circumcincta*. Proceedings of the Helminthological Society of Washington 53:244–247.

**Le Jambre, L. F.** 1981. Hybridization studies with Australian *Haemonchus placei* (Place, 1893), *Haemonchus contortus cayaguensis* (Das and Whitlock, 1960) and *Haemonchus contortus* (Rudolphi, 1803) from Louisiana. International Journal for Parasitology 11:323–330.

**Levine, N. D.** 1968. Nematode Parasites of Domestic Animals and of Man. Burgess Publishing Co., Minneapolis. Pages 218–234.

**Marquardt, W. C., D. H. Fritts, C. L. Senger, and L. Seghetti.** 1959. The effects of weather on the development and survival of the free-living stages of *Nematodirus spathiger* (Nematoda: Trichostrongylidae). Journal of Parasitology 45:431–439.

**Pandey, V. S.** 1972. Effect of temperature on survival of the free-living stages of *Ostertagia ostertagi*. Journal of Parasitology 58:1042–1046.

**Sayre, R. M.** 1964. Cold-hardiness of nematodes. I. Effects of rapid freezing on the eggs and larvae of *Meloidgyne incognita* and *M. hapla*. Nematologica 10:168–179.

**Steel, R. G. D., and J. H. Torrie.** 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill Book Company, New York. Pages 195–203.

**Todd, K. S., Jr., N. D. Levine, and P. A. Boatman.** 1976. Effect of temperature on survival of free-living stages of *Haemonchus contortus*. American Journal of Veterinary Research 37:991–992.

———, ———, **and C. C. Whiteside.** 1970. Moisture stress effects on survival of infective *Haemonchus contortus* larvae. Journal of Nematology 2:330–333.

**Waller, P. J., and A. D. Donald.** 1970. The response to desiccation of eggs of *Trichostrongylus colubriformis* and *Haemonchus contortus* (Nematoda: Trichostrongylidae). Parasitology 71:285–291.

———, **and R. J. Thomas.** 1978. Nematode parasitism in sheep in north-east England: the epidemiology of *Ostertagia* species. International Journal for Parasitology 8:275–283.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 53–59

# *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida), Parasitic in the Japanese Beetle, *Popillia japonica* (Scarabaeidae: Coleoptera), in Ohio

GEORGE O. POINAR, JR.,[1] TREVOR JACKSON,[2] AND MICHAEL KLEIN[3]

[1] Department of Entomological Sciences, University of California, Berkeley, California 94720
[2] Ministry of Agriculture and Fisheries, Lincoln, New Zealand and
[3] Horticultural Insects Research Laboratory, Wooster, Ohio 44691

ABSTRACT: *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida) is described from parasitized third-stage Japanese beetle larvae, *Popillia japonica* (Scarabaeidae: Coleoptera) collected in Ohio. Distinguishing morphological characters are the large infective-stage juveniles and the presence of a pseudopeloderan bursa. Infective-stage juveniles carry and release cells of the luminescent bacterium *Xenorhabdus luminescens* in the hemocoel of host insects. A high incidence of infection was noted in certain areas of the field.

KEY WORDS: nematode taxonomy, morphology, life cycle, *Xenorhabdus luminescens*, luminescent bacterium.

While digging for diseased Japanese beetle larvae in Jeromesville, Ohio, the latter two authors came across a field containing a number of dying, reddish-colored grubs. All of the third-stage larvae in a 1-m$^2$ clump of green grass had been killed. Upon closer examination, the grubs were discovered to have been attacked by a nematode belonging to the genus *Heterorhabditis.* The infective stages of this species recovered from field-collected hosts were used to reinfect Japanese beetle grubs and larvae of *Galleria mellonella* in the laboratory. Material sent to the senior author was determined to be a new species of *Heterorhabditis* and a description follows.

## Materials and Methods

The description presented here is based on specimens removed from *Galleria mellonella* larvae. Infected insects were maintained at 22°C and dissected on days 4 and 5 to recover the first-generation hermaphroditic females, and on days 7 and 8 to recover the males and the second-generation amphimictic females. Infective-stage juveniles were examined after they emerged from the host cadavers, approximately 14 days after initial exposure.

All nematodes were killed in hot (55°C) Ringer's, fixed in TAF, and processed to glycerin for measurements. Photographs were taken with a Nikon Optiphot microscope fitted for differential interference contrast.

The new species, together with *H. bacteriophora* and the American (NC) strain of *H. heliothidis,* were sent to John Curran for selection and comparison of restriction fragment length differences of genomic DNA. Digestion of genomic DNA from ground whole nematodes with restriction endonucleases generates a unique set of different-sized DNA restriction fragments dependent upon the base sequence of the genome. The size distribution of these restriction fragments is unique to the genotype and can be analyzed by agarose gel electrophoresis (Curran et al., 1985).

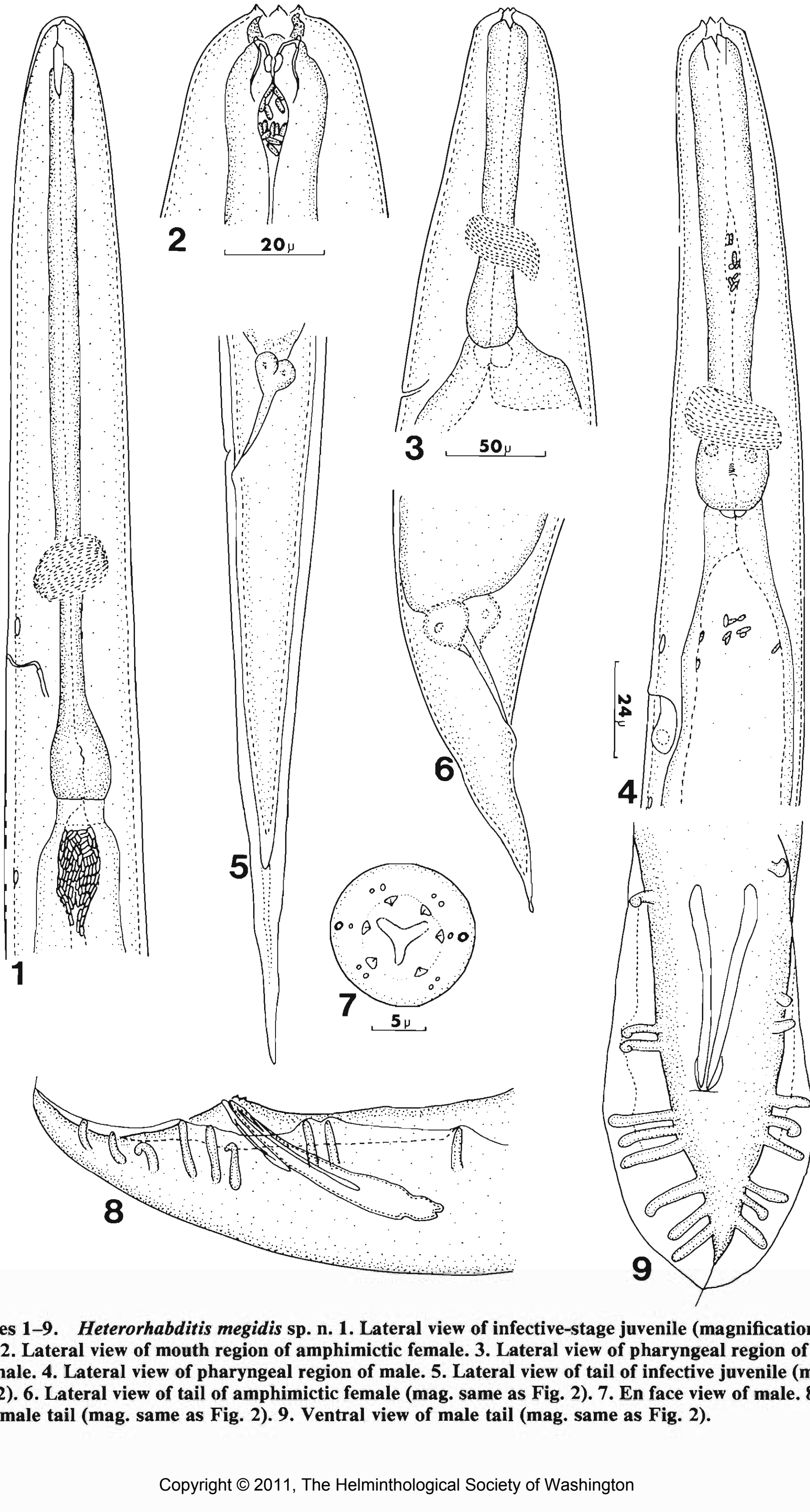
## Results

In the quantitative portion of the description, all figures are given in micrometers unless otherwise specified. The first number following the character is the average value and the numbers in parentheses indicate the range.

### *Heterorhabditis megidis* sp. n.

Heterorhabditidae Poinar, 1975; Rhabditoidea (Oerly).

DESCRIPTION: *Adults* (Figs. 2, 3, 4, 6–14): Head truncate or slightly rounded; 6 distinct protruding lips surrounding the mouth opening (in fixed mature hermaphroditic females, the lips are often withdrawn into the mouth opening); each lip bears a single labial papilla emerging at the tip; at the base of each submedial lip are 2 cephalic papillae. The lateral lips contain a single cephalic papilla and a circular amphidial opening. Cheilorhabdions represented as a refractile ring just below the lips and anterior to the pharynx. The remainder of the stoma is modified and could be interpreted as being telescoped on itself. The metarhabdions, each section bearing 1 or more fine teeth, are adjacent to the reduced pro- and mesorhabdions; telorhabdions are represented by fine elongate segments leading directly into the pharyngeal lumen. The anterior portion of pharynx encompasses all of the stoma except the cheilorhabdions. The pharynx lacks a metacorpus but contains an isthmus and pronounced basal bulb bearing some fine striations in the valve area, but not a distinct valve. Nerve ring distinct, located near the middle of the isthmus in the female, but usually on the anterior portion




**Figures 1–9.** ***Heterorhabditis megidis*** **sp. n. 1. Lateral view of infective-stage juvenile (magnification same as Fig. 2). 2. Lateral view of mouth region of amphimictic female. 3. Lateral view of pharyngeal region of amphimictic female. 4. Lateral view of pharyngeal region of male. 5. Lateral view of tail of infective juvenile (mag. same as Fig. 2). 6. Lateral view of tail of amphimictic female (mag. same as Fig. 2). 7. En face view of male. 8. Lateral view of male tail (mag. same as Fig. 2). 9. Ventral view of male tail (mag. same as Fig. 2).**

of the basal bulb in the male. A double-celled valve separates the pharynx from the single-cell-thick intestine. Four coelomocytes are especially noticeable in the males, where 1 pair occurs near the tip of the testis and another pair near the reflexed portion.

Females with paired, amphidelphic ovaries, the reflexed portion of which often extends past the vulvar opening. Hermaphroditic females with sperm occurring in the proximal portion of the ovotestis; amphimictic females with sperm collected in the proximal portion of the oviduct. Vulva of the hermaphroditic female with slightly protruding lips (Fig. 10), never with a copulation plug as occurs on all mated amphimictic females (Fig. 11) that possess a reduced vulva without protruding lips. Tail pointed, normally wavy in outline, usually with a postanal swelling; rectum distinct, usually expanded and filled with bacteria when living, 3 rectal glands surround the junction of intestine and rectum (Fig. 6).

Males with a single, reflexed testis; spicules paired and separate (Figs. 8, 9, 12), slightly curved, capitulum variable, but usually well set off from the shaft and longer than broad; shaft lacking a rostrum but with a single rib that usually divides at the distal portion; spicule tips pointed; gubernaculum flat, shorter than half the spicule length (Figs. 8, 12), with both the distal and proximal portions folded dorsally. Bursa open, usually pseudopeloderan with a fine tip extending beyond the bursal membrane; a semibursa that extends only partially up the bursal papillae is also present; bursa with 9 pairs of papillae, 3 pairs anterior to the cloacal opening and 6 pairs posterior. Numbering from anterior to posterior, 1 is isolated from 2 and 3 (occasionally 2 and 3 are fused); 4, 5, and 6 normally form a group, as do 7, 8, and 9. The fifth and eighth pair are usually bent outward (laterally), whereas the remainder are straight or bent inward (ventrally).

*Hermaphroditic females* (Fig. 10) ($N$ = 15): Length, 3.6 (2.4–4.9) mm; greatest width, 209 (120–333); length of stoma, 7 (5–10); width of stoma, 9 (8–11); distance from head to nerve ring, 162 (139–178); distance from head to excretory pore, 209 (193–270); length of pharynx, 229 (206–269); length of tail, 105 (95–124); body width at anus, 63 (38–86); percentage vulva, 48 (45–50); length of eggs in body, 60 (53–70); width of eggs in body, 40 (31–48); a = 17 (14–24); b = 15 (12–21); c = 34 (23–49).

*Amphimictic female* (Figs. 2, 3, 6, 11, 18) ($N$ = 15): Length, 2.1 (1.5–2.5) mm; greatest width, 123 (95–140); length of stoma, 5 (4–6); width of stoma, 7 (5–8); distance from head to nerve ring, 111 (105–120); distance from head to excretory pore, 178 (158–206); length of pharynx, 160 (155–168); length of tail, 86 (70–101); body width at anus, 31 (25–38); percentage vulva, 49 (47–51); length of eggs in body, 59 (48–70); width of eggs in body, 35 (25–41); a = 17 (15–19); b = 13 (10–16); c = 24 (18–32).

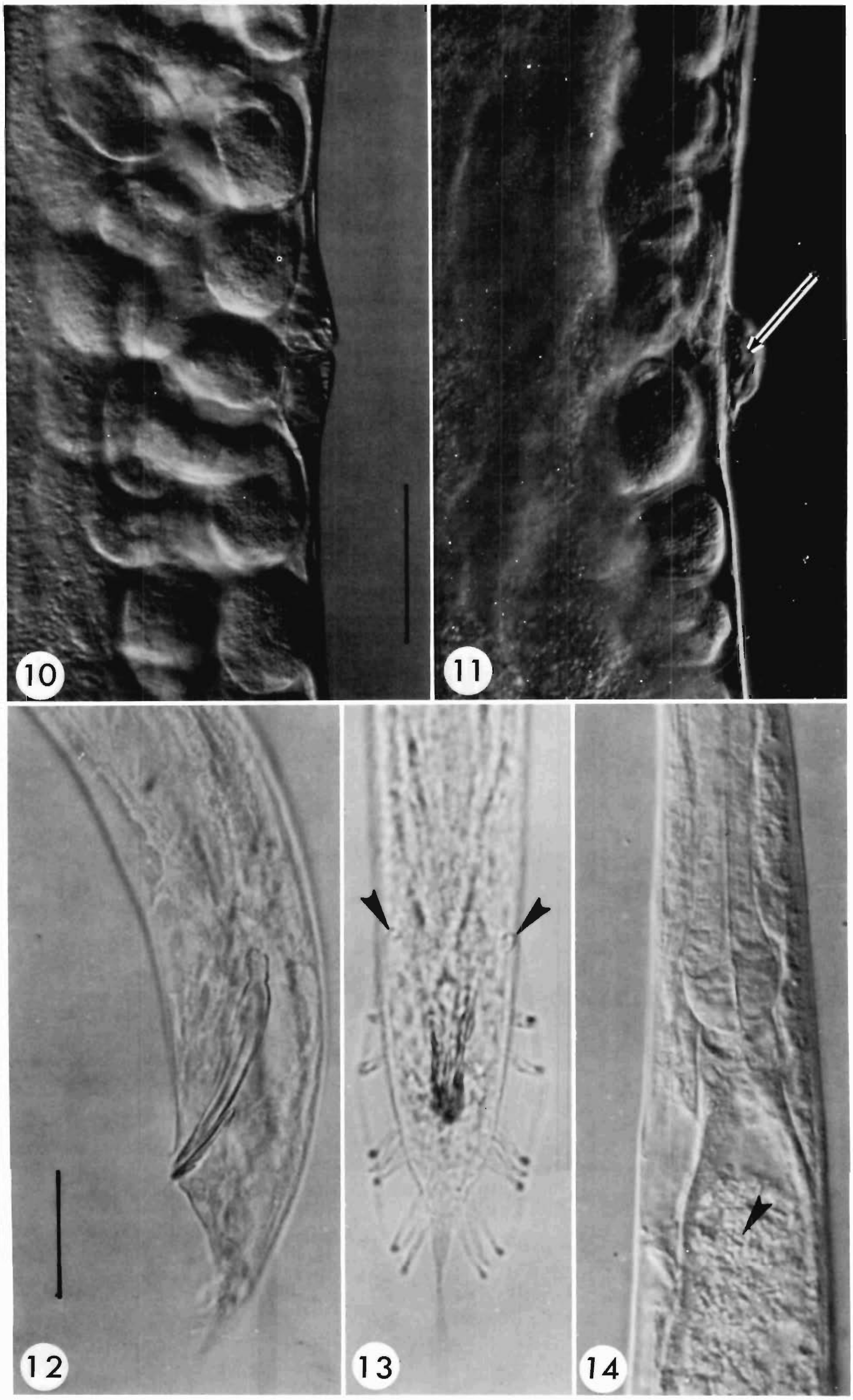
*Male* (Figs. 4, 7–9, 12–14) ($N$ = 15): Length 1.0 (0.8–1.1) mm; greatest width, 47 (44–50); length of stoma, 3 (2–4); width of stoma, 4 (3–6); distance from head to nerve ring, 104 (96–112); distance from head to excretory pore, 156 (139–176); length of pharynx, 128 (122–134); reflection of testis, 138 (117–230); length of tail, 39 (35–43); width at cloaca, 26 (22–31); length of spicules, 49 (46–54); width of spicules, 6 (5–8); length of gubernaculum, 21 (17–24); width of gubernaculum, 1.1 (0.3–1.6); a = 19 (18–22); b = 8 (7–9); c = 26 (23–31).

*Infective juveniles* (Figs. 1, 5, 15–19) ($N$ = 15):

→

**Figures 10–14. *Heterorhabditis megidis* sp. n. 10. Vulva of hermaphroditic female. Note slightly protruding lips. Bar represents 60 μm. 11. Vulva of amphimictic female. Note attached copulation plug (arrow) and absence of vulva lips (magnification same as Fig. 10). 12. Lateral view of male tail. Bar represents 24 μm. 13. Ventral view of male tail, showing nine pairs of papillae and pseudopeloderan bursa. Arrows show first pair of papillae (mag. same as Fig. 12). 14. Portion of pharynx and intestine of male. Note ingested bacteria (arrow) (mag. same as Fig. 12).**

**Figures 15–20. *Heterorhabditis megidis* sp. n. 15. Third-stage infective juvenile inside second-stage cuticle. Note dorsal hook on head of third-stage juvenile (arrow). E = excretory pore, N = nerve ring. Bar represents 24 μm. 16. Infective-stage juvenile pulled away from enclosing second-stage cuticle. Note dorsal head hook (arrow) (magnification same as Fig. 15). 17. Tail of third-stage infective juvenile (arrow) inside second-stage cuticle (mag. same as Fig. 15). 18. Infective-stage juveniles inside amphimictic female. Bar represents 120 μm. 19. Striations on second-stage cuticle enclosing third-stage infective juvenile. 20. Cells of *Xenorhabdus luminescens* released by infective juveniles in the hemolymph of a wax moth larva. Bar represents 12 μm.**


10
11
12
13
14

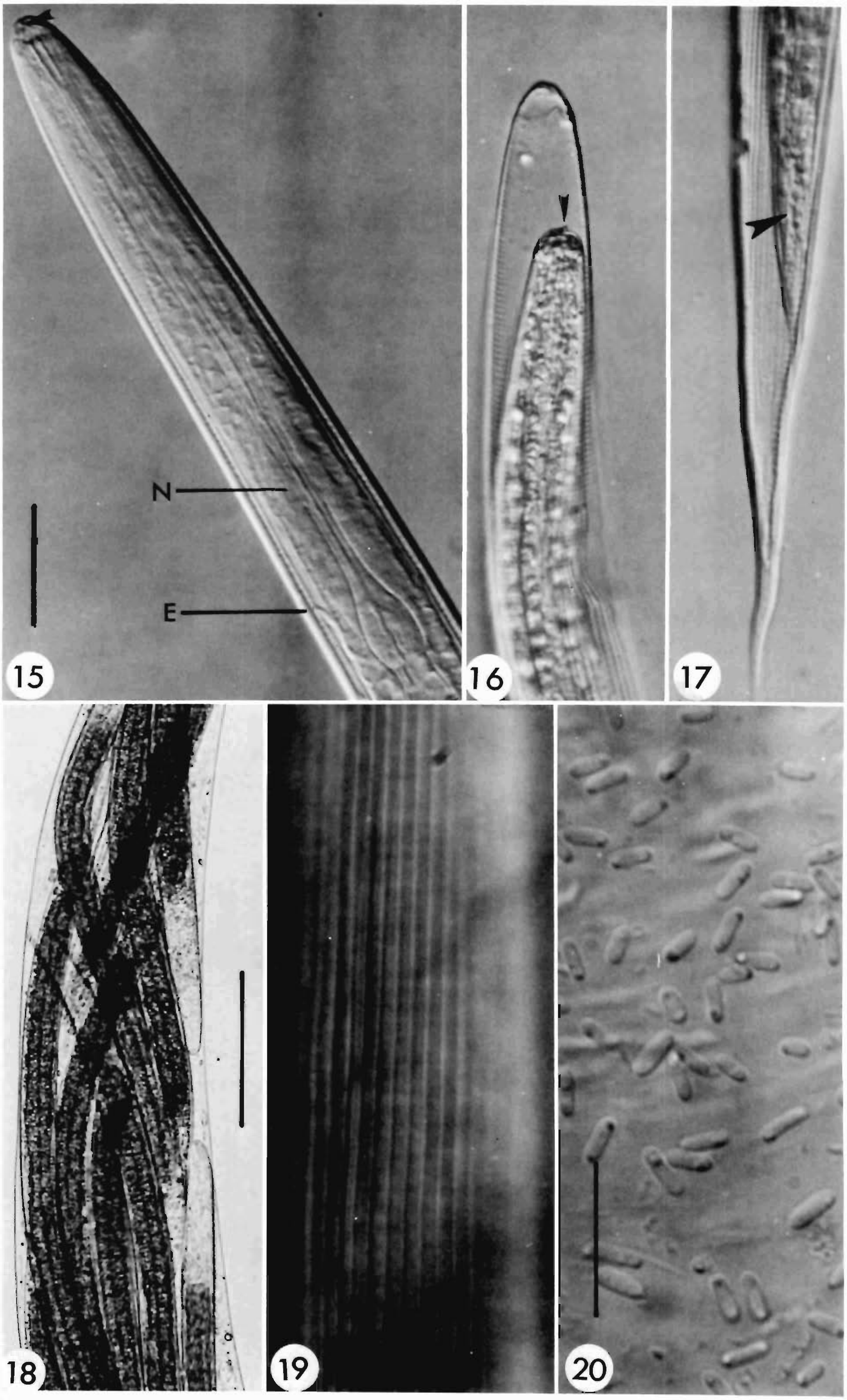

N
E
15
16
17
18
19
20

**Table 1. Comparison of the infective stages of *H. megidis* with other species and strains of *Heterorhabditis*.**

| Species/ strain | Body length | Distance from head to excretory pore | Length of pharynx | Reference |
|---|---|---|---|---|
| *H. megidis* | 768 (736–800) | 131 (123–142) | 155 (147–160) | Present study |
| *H. bacteriophora* | 570 (520–600) | 104 (94–109) | 125 (119–130) | Poinar (1975) |
| *H. heliothidis* | | | | |
| American (NC) | 644 (618–671) | | 133 (130–139) | Khan et al. (1976) |
| New Zealand | 685 (570–740) | 112 (94–123) | 140 (135–147) | Wouts (1979) |
| Cuban | 615 (540–700) | | 128 (109–152) | Hernandez and Mráček (1984) |

Length, 768 (736–800); greatest width, 29 (27–32); distance from head to nerve ring, 109 (104–115); distance from head to excretory pore, 131 (123–142); length of pharynx, 155 (147–160); length of tail, 119 (112–128); body width at anus, 19 (17–21); a = 26 (23–28); b = 5.0 (4.6–5.0); c = 6.5 (6.1–6.9). The infective stage is a third-stage juvenile inside the second-stage cuticle. The second-stage cuticle is strongly ribbed longitudinally, but also shows weak cross striations (Fig. 19) and is closely appressed to the third-stage juvenile. If the infectives that have just emerged from an insect are placed directly in 70% alcohol, the third-stage juvenile contracts and pulls away from the second-stage cuticle (Figs. 16, 17). The head of the third-stage juvenile bears a minute dorsal tooth (Figs. 1, 15, 16) that is probably used to rasp through tissue and aid entry into a host. In second-generation amphimictic females, the eggs hatch inside the females and develop to infective stages within the female body (Fig. 18).

*Symbiotic bacteria:* The infective-stage juveniles of *H. megidis* normally carry bacterial cells of *Xenorhabdus luminescens* in their intestines (Fig. 1). These cells are very characteristic in shape and can be found in the hemolymph of insects attacked by the nematodes (Fig. 20). They produce a red pigment and impart a red color to the infected host. They are also luminescent and freshly killed insects or agar plates with 1–2-day-old cultures are luminous in the dark.

TYPE LOCALITY: Mohican Hills Golf Course, Jeromesville, Ohio.

TYPE HOST: The Japanese beetle, *Popillia japonica* Newm. (Scarabaeidae: Coleoptera). Infection occurred in third-instar larvae on 17 and 18 October 1985.

TYPE SPECIMENS: Holotype (male) and allotype (hermaphroditic female) deposited in the Nematology Collection at the University of California, Davis, California.

DIAGNOSIS: There are only two adequately described species in the genus *Heterorhabditis*. One of these is the type species, *H. bacteriophora* Poinar, 1975, and the other is *H. heliothidis* (Khan, Brooks, and Hirschmann, 1976). The latter species is composed of American or NC (Khan et al., 1976), New Zealand (Wouts, 1979), and Cuban (Hernandez & Mráček, 1984) strains. *Heterorhabditis hoptha* (Turco, 1970) was described from specimens collected from the Japanese beetle in 1938 in Moorestown, New Jersey. However, because of the inadequate description, this and the other recorded species of *Heterorhabditis* listed by Poinar (1979) in his synopsis of the genus must remain nomina dubia.

Differences between the infective stages of *H. megidis, H. bacteriophora,* and the various strains of *H. heliothidis* are listed in Table 1. The average length of *H. megidis* infectives, as well as the average distance from the head to excretory pore and average length of the pharynx, separates this species from previously described species or strains of *Heterorhabditis*.

In the males, the ratio of the length of the gubernaculum to the length of the spicule distinguishes *H. megidis* (less than 0.5) from *H. bacteriophora* and the American strain of *H. heliothidis* (0.5 or greater). Also, the pseudopeloderan bursa, with the tail tip slightly protruding beyond the bursal rim, separates *H. megidis* from the other two species and strains of *Heterorhabditis*. The semibursa described here in *H. megidis* was not reported in the other two species, but could have been overlooked because of its delicate structure.

DNA ANALYSIS: Characterization by Dr. Curran of genomic DNA fragments of *H. heliothidis, H. bacteriophora,* and *N. megidis* resulted in bands of restriction fragments that showed a clear distinctness between *H. megidis* and the other two species. Because these bands represent multiple copies of respective DNA sequences and the restrictive fragment length differences between such bands can be used as diagnostic characters (Curran et al., 1985), these results support the conclusion that *H. megidis* is a distinct species.

## Discussion

The life history of *H. megidis* is similar to that reported for other species of the genus. Infective-stage juveniles enter the host and develop into hermaphroditic females, which then produce male and female progeny. These forms mate and the amphimictic females produce young, which normally develop into infectives inside their bodies. Contrary to Wouts's (1979) conclusion that the infectives of the New Zealand strain of *H. heliothidis* are second-stage juveniles, the infectives of *H. megidis* are clearly third-stage juveniles enclosed in a tightly fitting second-stage cuticle. This second-stage cuticle is closely appressed to the third-stage juvenile, thus making it appear that the infective is a second-stage juvenile. Wouts (1979) also assumed that the males of the New Zealand strain of *H. heliothidis* do not feed. However, the males of *H. megidis* ingest bacteria and have a normal alimentary tract (Figs. 4, 14).

## Literature Cited

**Curran, J., D. L. Baillie, and J. M. Webster.** 1985. Use of genomic DNA restriction fragment length differences to identify nematode species. Parasitology 90:137–144.

**Hernandez, E. M. A., and Z. Mráček.** 1984. *Heterorhabditis heliothidis,* a parasite of insect pests in Cuba. Folia Parasitologica (Praha) 31:11–17.

**Khan, A., W. B. Brooks, and H. Hirschmann.** 1976. *Chromonema heliothidis* n. gen., n. sp. (Steinernematidae, Nematoda) a parasite of *Heliothidis zea* (Noctuidae, Lepidoptera), and other insects. Journal of Nematology 8:159–168.

**Poinar, G. O., Jr.** 1975. Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida: Heterorhabditidae n. fam.). Nematologica 21:463–470.

———. 1979. Nematodes for Biological Control of Insects. CRC Press, Boca Raton, Florida. 277 pp.

**Turco, C. P.** 1970. *Neoaplectana hoptha,* sp. n. (Neoaplectanidae: Nematoda), a parasite of the Japanese beetle, *Popillia japonica* Newm. Proceedings of the Helminthological Society of Washington 37:119–121.

**Wouts, W. M.** 1979. The biology and life cycle of a New Zealand population of *Heterorhabditis heliothidis* (Heterorhabditidae). Nematologica 25: 191–202.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 60–67

# *Nemertinoides elongatus* gen. n., sp. n. (Turbellaria: Nemertodermatida) from Coarse Sand Beaches of the Western North Atlantic

NATHAN W. RISER
Marine Science Institute, Northeastern University, Nahant, Massachusetts 01908

ABSTRACT: *Nemertinoides elongatus* gen. n., sp. n. (Turbellaria: Nemertodermatida) is reported from intertidal coarse sand of beaches between New Brunswick and Massachusetts. This is the largest species described in the order. *Nemertinoides* differs from all other genera in having paired testes arising immediately behind the mouth, with the male ducts extending a short distance posteriorly to the dorsal male antrum in the anterior half of the body. The nervous system is epidermal, with two lateral nerves extending from the region of the statocyst to the posterior end of the body. These two trunks enlarge behind the statocyst and are connected in the epidermis by commissures. The two cords of *N. elongatus* are connected by additional commissures to a large ventral neural mass behind the statocyst.

KEY WORDS: taxonomy, morphology, differential diagnosis, *Nemertoderma, Meara, Flagellophora, Ototyphlonemertes,* Massachusetts, New Brunswick, Canada.

Dishes into which interstitial nemertineans (in meiofaunal sampling) are sorted from coarse sand beaches of New England and the Canadian maritime provinces by our group routinely contain individuals of a turbellarian species belonging to the order Nemertodermatida. The statocysts and the internal appearance of the anterior body region as it is viewed under high magnification with a dissecting microscope makes it possible to separate these turbellarians from nemertineans.

## Materials and Methods

Sediment was extracted with 7.5% $MgCl_2$ decanted through 153-$\mu$m screening, from which the animals were removed by jets of seawater from a squeeze bottle (Riser, 1985). Most of the animals were studied alive by interference contrast or transmitted light microscopy. Specimens for whole mounts or for sections were anesthetized with $MgCl_2$ or with xylocaine and fixed with Hollande's cupri-picri-formal-acetic. Whole mounts were stained with Mayer's alcoholic HCl carmine. Specimens were embedded in polyester wax for histological observations, and were stained with Heidenhain's azan procedure or with Heidenhain's iron alum hematoxylin and 0.2% azophloxin.

## Generic Diagnosis

### *Nemertinoides* gen. n.

Nemertodermatida Steinböck, 1930, with elongate body shape and relatively uniform body diameter; anterior and posterior ends bluntly rounded. Mouth opening ventral, anterior to male pore; pharynx simplex present; gut extends from anterior to posterior end of body. Testes behind mouth, dorsal, paired, elongate; leading posteriorly to seminal vesicle and male antrum located dorsally in anterior ⅖ of body. Female germinative zone dorsal, extended from male antrum to posterior end of body.

TYPE SPECIES: *Nemertinoides elongatus.*

ETYMOLOGY: *Nemertinoides,* nemertine-like.

## Species Description

### *Nemertinoides elongatus* sp. n.

MATERIAL: Numerous living specimens collected at all months between the years 1971 and 1985 from Barley Neck, Little Pleasant Bay, Orleans, Massachusetts (41°45′40″N, 69°57′38″W) to Pea Pt., Blacks Harbor, New Brunswick, Canada (45°02′N, 66°48′W). Serial longitudinal sections of anterior halves of 4 animals; serial cross sections of selected regions of 8 specimens.

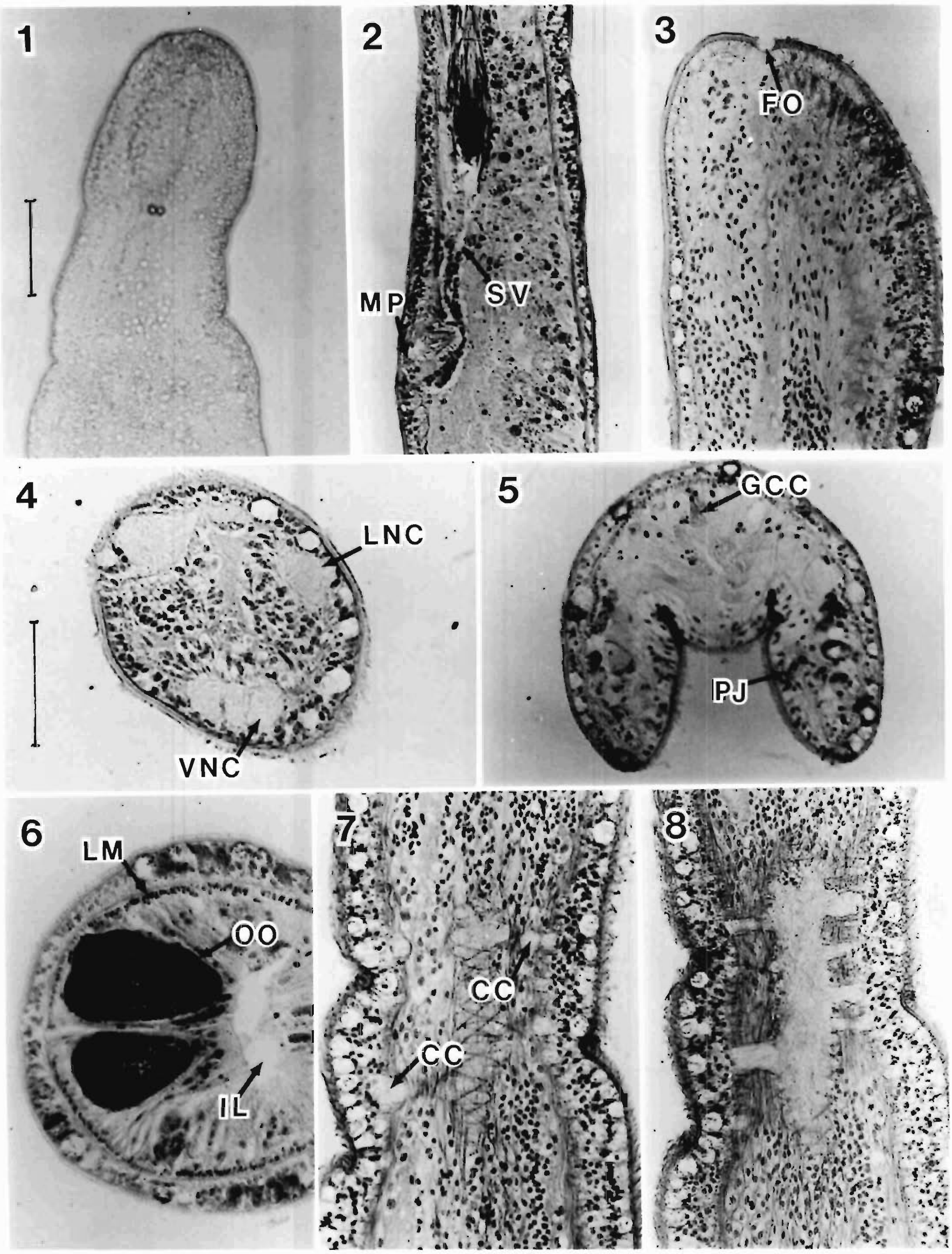
SPECIES DIAGNOSIS: Gliding sexually mature animals 6–10 mm long and 0.2–0.36 mm broad. Uniformly greyish white. Pattern of large amorphous gland cells in epidermis most obvious dorsally between statocyst and mouth. Statocyst 0.3–0.36 mm behind anterior end of body; oval, about 20 $\mu$m long and 0.30 $\mu$m wide; capsule with thicker walls anteriorly and posteriorly; 2 round multigranular statoliths to 13 $\mu$m in diameter.

MORPHOLOGY: The large amorphous epidermal gland cells visible in living animals appear as large vacuolated cells with azanophilous granules concentrated at the periphery of the vacuole (Figs. 5, 6). These granules appear to be concentrations of the vacuolar contents resulting from extraction of other components by fixation, dehydration, etc. These very large cells displace other cells, and thus account for much of the pseudostratified appearance of the epidermis. Gland cells of similar size and shape but densely packed with large basophilic (with hematoxylin) or cyanophilous (with azan) granules are abundant below the nerve cords behind the mouth. Mucous cells are sandwiched in among the other cells, and vary considerably in shape in sections but in general are quite thin. They are almost the only cells present around the male pore, accounting for its swollen appearance, and are abundant around the mouth and distal portion of the pharynx. Large insunk mucous cells also empty into the pharynx. The cell bodies of two ventral cords of insunk gland cells that discharge through the ventral epidermis (Fig. 3) anterior to the brain extend from the anterior margin of the mouth almost to the brain. They frequently occur in packets with their long axes in the dorsoventral plane. Processes from the cell bodies converge toward midventral line where they form a cyanophilous tract extending anteriorly to discharge through the ventral epidermis (Fig. 3) anterior to the brain. The cells are in various stages of activity; those closest to the mouth have a finely granular brownish cytoplasm and produce an azanophilous secretion which is readily dislodged during sectioning. In more anterior cells, the cytoplasm is mixed with the fine granular portion toward the apex and enclosing the nucleus, the remainder of the cell filled with secretion. Most of the cells in the anterior half of the cords are filled with secretion with little or no granular material present. The secretion is basophilic with hematoxylin, but ranges from azanophilous through cyanophilous, with various gradations within the cell bodies. The secretion in these cells of specimens anesthetized with xylocaine was more strongly azanophilous than in those anesthetized with $MgCl_2$, which, apparently from its solating effect, increased the cyanophilia. The secretion in the tracts is ropy and the necks of the cells carrying it dominate the epidermis at the tip of the body below the frontal organ (Fig. 3). In addition to the necks of these cells and a few of the giant azanophilous gland cells, there are a few widely scattered nuclei between the cell web and the remnants of the body-wall muscle fibers at the tip of the body (Fig. 3). The majority of the cell bodies in this region are insunk and cannot be traced. Two dorsolateral cords of nuclei appear along the lateral nerve cords a short distance behind the brain and extend almost to the anterior end of the body. A third cord of nuclei arises along the posterior projection of the ventral ganglion and extends foward to either side almost enclosing the ventral gland cell cords. A faint cyanophilous track extends from the region of these nuclei to the frontal organ (Fig. 3), implying that they belong to the cell bodies of that organ.

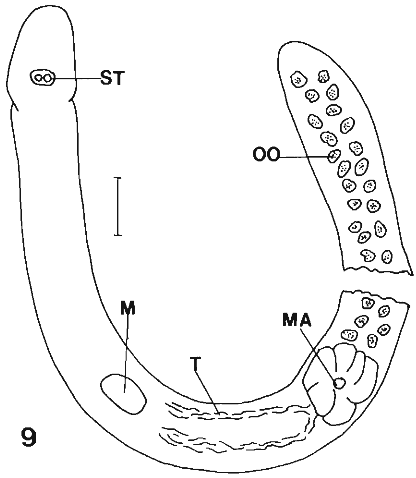
The circular muscle layer of the body wall consists of thin fibers that appear to branch at various levels so that the spaces between circular fibers may be traversed by diagonal branches. In contrast, the longitudinal layer consists of unbranched, closely packed large fibers forming a single layer (Fig. 6). The characteristic cell bodies of the longitudinal fibers project inward toward the gut. The muscle layers are thinner anterior to the brain and lose their integrity at the anterior end of the body, where the epidermal cells become insunk. The circular muscle fibers develop a diagonally cross-hatched pattern beneath the three neuropilar swellings of the brain (Fig. 7). The pharynx is attached dorsally and laterally to the body wall by isolated muscle cells. The statocyst is suspended by a mesh of myofibrillae that arise from lateral body-wall muscle cells.

Contraction associated with fixation results in a round cross section in which the lateral nerve cords, which are located external to the body-wall muscles, are carried somewhat dorsolaterally. There are two primary trunks that increase in size for about 0.1 mm prior to their incorporation in the nerve ring. A very large ventral neural mass appears in the epidermis in this same general region. Posteriorly this mass is divided into three large tracts or lobes (Fig. 3) separated by muscle fibrillae. Anteriorly these condense into two and farther anteriorly to one neuropilar mass. Commissures are present between the ventral and the two lateral cords (Figs. 7, 8) and the latter are also connected by commissures (Figs. 4, 10). Two small nerve fibers extend anteriorly from the lateral cords. The number, relative size in relation to one another, and distribution of the commissures could not be ascertained to the satisfaction of the author.

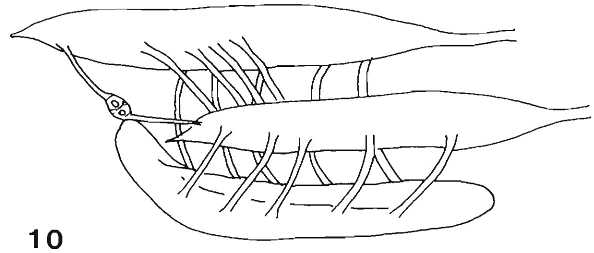
Branches from the longitudinal muscles insert around the mouth. Their contraction during fixation may account for the very large oral aperture of fixed animals. Nerves from the lateral cords extend toward the margins of the mouth, but neither a pharyngeal nerve ring nor a postoral commissure are evident. No oral sphincter is present, and the mouth opens directly into the pharynx (Fig. 5). The juncture is clearly demarcated by the change in epithelium and absence




**Figures 1–8.** *Nemertinoides elongatus.* **1. Anterior end of living specimen. Scale = 0.2 mm. 2. Longitudinal section through posterior portion of testis and male reproductive ducts and male antrum. 3. Longitudinal section (ventral to right) of body anterior to statocyst through frontal organ. 4. Transverse section through brain region: two dorsolateral trunks connected by commissure, ventral neural mass divided into three neuropilar tracks. 5. Transverse section through mouth and pharynx simplex near postulated opening into intestine. 6. Transverse section through posterior body region: two oocytes undergoing vitellogenesis, intestinal lacuna open. 7, 8. Sequential longitudinal sections through left lateral neural expansion and commissures: Figure 7, against muscle layers; Figure 8, more epidermal. Figures 2–8 all same scale (0.1 mm). Abbreviations: CC, commissures to lateral**




9



10

**Figure 9. Habitus sketch of *Nemertinoides elongatus*. Scale bar = 0.5 mm. Abbreviations: M, mouth; MA, male antrum; OO, oocyte; ST, statocyst; T, testes.**

**Figure 10. Schematic diagram of central nervous system from sections; anterior to left.**

of musculature beneath the pharyngeal cells. The epithelial nature of the intestine is most obvious (Fig. 5) above the pharynx, but the opening between the two organs was not observed. The characteristic granular club cells (Fig. 5) ("Körnerkolben" auctorem) are abundant in the dorsal wall of the gut immediately above the pharynx and a few are present anterior to the mouth but are rarely encountered further anteriorly. They are located primarily ventrally behind the mouth and sporadically occur above the level of the nerve cords behind the male pore. The granules are packed in the inflated apical end of these teardrop-shaped cells, and the densely staining nucleus sits like a plug in the attenuated basal region of the cell. The thin basal region ends in fibrils intermingled with the body-wall musculature.

The testes occur as two dorsal cords (Fig. 9) extending from just behind the pharynx almost to the male pore. They arise just dorsal to the nerve cords and extend in a rather straight line to the middle of the body, dividing the gut into a larger ventral and smaller dorsal portion connected by a narrow isthmus. Each testis becomes rounder in cross section posteriorly and the space between them becomes wider. Spermatogonia extend from the body wall along the ventral side of the testes for almost the entire length of the organ. Spermatocytes undergoing maturation occur in packets surrounded by a membrane that appears to be derived from the membrane enclosing the entire organ. All cells within a packet are in the same stage of development. Packets undergoing maturation lie dorsal to the gonial bands, with spermatids undergoing spermiogenesis as well as clumps of sperm occupying the uppermost portion of the organ. The membranes enclosing mature sperm disappear so that strands of sperm fill most of the space above the gonial area of each testis. The cavities containing the sperm fuse to form a seminal vesicle. Epithelial cells that are somewhat cuboidal occur in the walls of the two cavities near where they unite and continue as a layer if the seminal vesicle is empty (Fig. 2), or pull apart and occur as isolated cells when the vesicle is full of sperm. A few attach the seminal vesicle to the dorsal body wall to either side of the middorsal line, and form a cul-de-sac attaching to the middorsal body wall immediately behind the ejaculatory duct. The attachment indicates that the cul-de-sac is not involved with sperm storage in the seminal vesicle, but rather with the ejaculatory duct and its

←

**nerve above, to ventral neural mass lower side; FO, frontal organ; GCC, granular club cell; IL, intestine lumen; LM, longitudinal muscle layer; LNC, lateral nerve cord; MP, male pore; OO, oocyte; PJ, oral/pharyngeal junction; SV, seminal vesicle; VNC, ventral neuropilar cord.**

eversion. At the juncture of the seminal vesicle and ejaculatory duct, the epithelium becomes simple ciliated columnar (Fig. 2), with cells comparable to those present in the epidermis. The ejaculatory duct passes through the body-wall musculature to the male pore, which is surrounded by mucous cells and the necks of mucous cells whose bodies lie in the surrounding epithelium. The absence of an obvious cell web is unique to this area of the epidermis.

The ovary is dorsal, and lies close behind the male pore. Some oocytes beginning vitellogenesis occur in the ovary, but most vitellogenic oocytes are present in two dorsal rows extending to the posterior end of the body. Most are accompanied by a few gonial and/or previtellogenic oocytes. Vitellogenic oocytes tend to occur as pairs of cells in approximately the same stage of development (Fig. 6) in each row. Intestinal cells in the vicinity of oocytes actively accumulating yolk contain individual and membrane-bound packets of granules, indicating the possibility of heterosynthetic yolk formation. Oocytes 0.07–0.1 mm in diameter are the largest observed and are abundant in the holotype. No female ducts or pores are present. Two to three packets of sperm 0.04 mm in diameter have been encountered far posteriorly in several specimens, but a seminal receptacle is not present.

HOLOTYPE: Totomount from upper tide zone, coarse sand, Ellisville Beach, Ellisville, Massachusetts, 41°49′N, 70°33′W, 10 September 1984, American Museum of Natural History (AMNH) No. 1238.

PARATYPE: Serial ls., ant. end., from HT zone, coarse sand, Pagan Pt., St. Andrews, New Brunswick, Canada, 45°05′N, 67°03′W, 14 February 1980, Naturhistoriska Riksmuseet (Stockholm) Sektionen for Evertebratzoology (SMNH1) No. 3591.

## Discussion

The order Nemertodermatida contains a single family, Nemertodermatidae, comprised of the genera *Nemertoderma, Meara,* and *Flagellophora.* The anterior dorsal location of the male pore and the topography of the gonads distinguishes *Nemertinoides* from the previously described genera. Sterrer (1966, fig. 5) reported, without description, a nemertodermatid species "in littoral sand from Kristineberg and the Adriatic" that looks as if it could be allied to *Nemertinoides.* The four genera are quite distinct, but in the absence of hard parts other than the statoliths, few species have been named. Several undescribed species of *Nemertoderma* have been reported by Riedl (1960), Tyler and Rieger (1977), and Riser (1985). These species have been recognized as different on the basis of the appearances of living individuals, while Tyler and Rieger (1977) have recorded distinctive ultrastructural characteristics of the species to which they refer. Dörjes (1968) described and figured a species of *Nemertoderma* from Helgoland that he considered to be very similar to one described by Westblad (1937), but he carefully avoided the use of a specific name. The difficulty in identifying species is long-standing. Westblad (1937) stated that the species of *Nemertoderma* that he was describing was probably *N. bathycola* Steinböck, 1930 (in spite of advice from Steinböck that it was a different species), but elected to use only the generic name in his description. Steinböck (1938) immediately named the species *Nemertoderma westbladi* and, in 1966, continued to stress the distinction between the two species. The peculiar cellularity of the members of the order has made the use of photomicrographs essential to all descriptions of species. Much of the literature on the Nemertodermatida has concentrated on a controversy over the cellular vs. syncytial vs. plasmodial nature of the gut and the status of the lumen of that organ. The controversy was resolved to a great extent by Karling (1967), utilizing Westblad's slides, but the ultrastructural finding that the intestinal epithelium consisted of large interdigitating cells by Tyler and Rieger (1977) was necessary to explain the reasons for the various interpretations.

Discrete cells in the gastrodermis occur in the ventral wall of the intestine immediately above the pharynx in *Nemertinoides elongatus.* A lumen, variable in size, also is present here. Other regions of the intestine may show cavities (lacuna, auctorem) (Fig. 6), especially in individuals in which the gastrodermal cells are filled with granules or large food globules. Failure to observe cilia projecting into the cavities indicates that, if they are present, they are widely scattered. *Nemertinoides elongatus* does not clarify any of the nemertodermatid morphological problems at the light microscope level.

Westblad (1937) interpreted fine fibrils attaching to the cell web in the epidermis to be muscle fibers. One cannot distinguish between cell membranes and extracellular fibers in the distorted epidermis of these worms. Tonofilament bundles have been figured and described in the epidermis of one species of *Nemertoderma* by Tyler and Rieger (1977) using transmission electron microscopy (TEM). They considered these bundles to be structurally supportive.

The large azanophilous epidermal gland cells are comparable to those in the photographs of sections of *Nemertoderma* sp. by Karling (1967, figs. 3, 4), Riedl (1960, figs. 3, 5), and Westblad (1937, figs. 11, 12). In all of these photographs, the extraction and concentration of the contents at the periphery of the vacuoles is consistent. The large gland cell in the dorsal epidermis of *Nemertoderma* sp. D of Tyler and Rieger (1977, fig. 2) could be one of these cells with intact secretory product. Particles extruded from these cells and trapped in the glycocalyx and cilia could be mistaken for rhabdites; however, rhabdites are absent.

The simple thickening of the nerve net at the anterior end of the body in *Meara* and *Nemertoderma* has been compared with that of certain acoelous turbellarians, and referred to as the central nervous system. Hyman (1951, p. 83) discussed the problem of the use of the term "brain" in this primitive organization of the nervous tissue. The species of *Nemertoderma* described from four different geographical areas by Riedl (1960) were reported each to have specific minor swellings in the thickened nerve cap. He considered these differences to be insignificant because of the condition of the sections of the animals. *Flagellophora apelti* Faubel and Dörjes, 1978, was described and figured with a medullary brain behind the statocyst; however, these authors did not utilize this unique character in their generic diagnosis. The enlargement of the dorsolateral nerve cords as well as the ventral nerve mass and the commissures connecting these nerve centers (Fig. 10) constitute an epidermal brain in *Nemertinoides elongatus.* This extensive development occurs behind the statocyst and may be a generic character. Faubel (1976), in the type description of *Nemertoderma rubra,* described a nerve ring internal to the body-wall musculature at the level of the statocyst. His figure is somewhat reminiscent of Westblad's (1937, fig. 7b) figure of the "brain" of *N. westbladi.* In both sets of figures, the angle at which the sections were cut is such that they could also be interpreted as showing nerves extending from the nerve ring to the statocyst rather than the statocyst lying in a "brain." A nerve accompanied by a few muscle cells passes from the anterior end of each of the dorsolateral nerve trunks to the statocyst of *Nemertinoides elongatus.* The anterior end of the ventral nerve mass almost touches the statocyst, but no nerve fibers (other than the commissures) have been found associated with this mass. The bulk and density of the nervous tissue probably account for the indentation near the statocysts and collar-like bulge behind it in living animals and influence the length and shape of the prestatocyst region. The prestatocyst region in Figure 1 is almost fully extended. Contraction of the longitudinal muscles can draw the anterior tip of the body almost back to the statocyst.

Statocysts are approximately the same size and shape in all nemertodermatid species described to date. *Nemertinoides elongatus* is the first member of the order to be described with polygranular statoliths. Sketches of an undescribed nemertodermatid from Rovinj, Yugoslavia, furnished by W. Sterrer, appear to belong to a *Nemertinoides* species also with polygranular statoliths. Kirsteuer (1977) observed that the granules in statoliths of species of the nemertinean genus *Ototyphlonemertes* could only be counted satisfactorily in specimens that had been adequately squeezed to separate the granules. Riedl (1960) and Sterrer (1966) recorded polylithophorous (with more than one statolith in a statocyst) nemertodermatid specimens, and the latter author figured a species that morphologically does not belong to *Nemertinoides,* but has polygranular statoliths. The two statoliths of *N. elongatus* are separated from one another in the statocyst by the cells that produce them. They appear to be intracellular concretions and the two statocytes are distinctly different cytologically from the statocyst wall. Supernumerary statoliths have not been observed in any statocytes.

There is no evidence of parenchyma between the body wall musculature and the gut of *N. elongatus.* Processes extending from the distal ends of the granular club cells to or toward the body-wall musculature make it difficult at the light microscope level to determine whether they belong to the gut or are parenchymal cells pushing into the gut. Westblad described similar processes from the club cells of *Nemertoderma* (1937) and *Meara stichopi* (1949). There is very little morphological or topological similarity between nemertodermatid club cells and the granular club cells surrounding the initial portion of the intestine of proxenetid rhabdocoels figured and described by Luther (1943). The homology of the granular club cells of acoels, nemertodermatids, and triclads remains a question. Ax (1961) also noted that the function of the granular club cells was controversial, although Jennings (1962) demonstrated that these cells in triclads are secretory and produce at least one digestive enzyme.

The random observation of allochthonous sperm packets indicates that sperm are transferred in groups, but does not explain how the cluster enters the individual. Spermatophores have not been observed. Allochthonous sperm are present in all tissues of the body of sexual individuals, indicating that the sperm wander considerably from the site of packet entrance. Tyler and Rieger (1975) in their description of the ultrastructure of the sperm of a species of *Nemertoderma* noted that the sperm were modified for internal fertilization.

The four nemertodermatid genera can be distinguished easily, and distinct species can be sorted in the living condition, but, as noted by Riedl (1960) and Dörjes (1968), species characters are unclear partly because of the absence of hard parts. However, TEM studies of undescribed species have been used to resolve a number of the problems that the peculiarities of the cellularity of the order have posed. Specific differences have been reported, but these do not presently help in identifying species at the light microscope level.

Karling (1940) utilized a simple mouth opening, epithelial nervous system, and male opening at the posterior tip of the body among the diagnostic characters for the Nemertodermatida. The definition was based upon the three known species, *Nemertoderma bathycola, N. westbladi,* and *Meara stichopi* Westblad, 1949. Since then, Faubel (1976) has described *Nemertoderma rubra,* which has a pharynx simplex, a character present in *Nemertinoides elongatus. Flagellophora apelti* has a medullary brain, and *N. elongatus* has a ventral epidermal brain connected by commissures to two dorsolateral ganglia from which the lateral nerve cords extend posteriorly. It is also characterized by the male pore lying in the anterior half of the body. The statocyst containing two statoliths as the only remaining character separating Nemertodermatida from the Acoela was considered to be autapomorphic by Ehlers (1984). When coupled with uniflagellate sperm (a characteristic unique to the Nemertodermatida among Platyhelminthes), as recorded by Tyler and Rieger (1975) and Hendelberg (1977), it takes on added significance. Tyler and Rieger (1977) and Smith (1981) considered the Acoela and Nemertodermatida to be closely allied sister-groups on the basis of ultrastructural morphology, and not one derived from the other.

### Acknowledgments

The helpful suggestions and critical editing of an earlier version of this manuscript by Dr. J. P. S. Smith, and the copies of drawings of the undescribed species from Rovinj, voluntarily sent by Dr. W. Sterrer, are gratefully acknowledged. The assistance of Dr. M. P. Morse in assembling the plate of figures and inking the line drawings was indispensable.

This is Contribution Number 141 from the Marine Science Institute, Northeastern University.

### Literature Cited

**Ax, P.** 1961. Verwandschaftsbeziehungen und Phylogenie der Turbellarien. Ergebnisse der Biologie 24:1–68.

**Dörjes, J.** 1968. Die Acoela (Turbellaria) der Deutschen Nordseekuste und ein neues System der Ordnung. Zeitschrift für Zoologische Systematik und Evolutionsforschung 6:56–452.

**Ehlers, U.** 1984. Phylogenetisches System der Plathelminthes. Verhandlungen des Naturwissenschaftliche Verein Hamburg (NF) 24:291–294.

**Faubel, A.** 1976. Interstitielle Acoela (Turbellaria) aus dem Litoral der nordfriesischen Inseln Sylt und Amrum (Nordsee). Mitteilungen aus dem Hamburgischen Museum und Institut 37:17–56.

———**, and J. Dörjes.** 1978. *Flagellophora apelti* gen. n. sp. n.: a remarkable representative of the order Nemertodermatida (Turbellaria: Archoophora). Senckenbergiana Maritima 10:1–13.

**Hendelberg, J.** 1977. Comparative morphology of turbellarian spermatozoa studied by electron microscopy. Acta Zoologica Fennica 154:149–162.

**Hyman, L. H.** 1951. The Invertebrates. Vol. II: Platyhelminthes and Rhynchocoela. The Acoelomate Bilateria. McGraw-Hill, New York. 550 pp.

**Jennings, J. B.** 1962. Further studies on feeding and digestion in triclad Turbellaria. Biological Bulletin 123:571–581.

**Karling, T. G.** 1940. Zur Morphologie und Systematik der Alloeocoela Cumulata und Rhabdocoela Lecithophora (Turbellaria). Acta Zoologica Fennica 26:1–260.

———. 1967. Zur Frage von dem systematischen Wert der Kategorien *Archoophora* und *Neoophora* (*Turbellaria*). Commentationes Biologicae Societas Scientiarum Fennica 30:1–11.

**Kirsteuer, E.** 1977. Remarks on taxonomy and geographic distribution of the genus *Ototyphlonemertes* Diesing (Nemertina, Monostilifera). Microfauna Meeresboden 61:167–181.

**Luther, A.** 1943. Untersuchungen an Rhabdocoelen Turbellarien: IV. Ueber einige Reprasentanten der Familie Proxenetidae. Acta Zoologica Fennica 38: 1–95.

**Riedl, R.** 1960. Uber einige nordatlantische und mediterrane *Nemertoderma*-Funde. Zoologischer Anzeiger 165:232–248.

**Riser, N. W.** 1985. General observations on the intertidal interstitial fauna of New Zealand. Tane 30(1984):239–250.

**Smith, J. P. S.** 1981. Fine-structural observations on the central parenchyma in *Convoluta* sp. Hydrobiologia 84:259–265.

**Steinböck, O.** 1938. Uber die Stellung der Gattung *Nemertoderma* Steinbock in System der Turbellarien. Acta Societatis pro Fauna et Flora Fennica 62:1–26.

———. 1966. Die Hofsteniiden (Turbellaria acoela) Grundsatzliches zur Evolution der Turbellarien. Zeitschrift fur Zoologische Systematik und Evolutionsforschung 4:58–195.

**Sterrer, W.** 1966. New polylithophorous marine Turbellaria. Nature 210:436.

**Tyler, S., and R. M. Rieger.** 1975. Uniflagellate spermatozoa in *Nemertoderma* (Turbellaria) and their phylogenetic significance. Science 188:730–732.

———, **and** ———. 1977. Ultrastructural evidence for the systematic position of the Nemertodermatida (Turbellaria). Acta Zoologica Fennica 154: 193–207.

**Westblad, E.** 1937. Die Turbellarien-Gattung *Nemertoderma* Steinbock. Acta Societatis pro Fauna et Flora Fennica 60:45–89.

———. 1949. On *Meara stichopi* (Bock) Westblad, a new representative of Turbellaria archoophora. Arkiv for Zoologi 1:43–57.

## New Book on Turbellaria

*Turbellaria of the World. A Guide to Families and Genera,* by L. R. G. Cannon, 1986, is available from Queensland Museum, Queensland Cultural Centre, P.O. Box 300, South Brisbane, Queensland 4101, Australia. Cost: $A14.00 includes postage and handling.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 68–72

# *Aototrema dorsogenitalis* gen. et sp. n. (Trematoda: Lecithodendriidae) and Other Helminths from the Peruvian Red-necked Owl Monkey, *Aotus nancymai*

DAVID E. BEAN-KNUDSEN,[1] LESLIE S. UHAZY,[2] AND JOSEPH E. WAGNER[1]

[1] Research Animal Diagnostic and Investigative Laboratory, Department of Veterinary Pathology, University of Missouri–Columbia, Columbia, Missouri 65211

[2] Division of Biological Sciences, University of Missouri–Columbia, Columbia, Missouri 65211

ABSTRACT: *Aototrema dorsogenitalis* gen. et sp. n. (Trematoda: Digenea, Lecithodendriidae) is described from the Peruvian red-necked owl monkey, *Aotus nancymai* (karyotype I, 2N = 54). *Aototrema* differs from other Neotropical lecithodendriids by its large acetabulum, dorsolateral position of the genital opening, body shape, and morphology of the cirrus sac. Nineteen of 28 fecal samples (67.9%) from cohort *A. nancymai* contained ova consistent with *Aototrema*. Ova similar to those of *Controrchis* and an oxyurid nematode were also identified.

KEY WORDS: taxonomy, morphology, histopathology, *Controrchis, Oryzomytrema, Phaneropsolus,* nematode.

The owl monkey has recently been reclassified into several separate species (Hershkovitz, 1983) based on coloration, presence of marking glands, karyotype, geographic distribution, and ecological pattern. The Peruvian red-necked owl monkey, *Aotus nancymai* Hershkovitz, 1983, is a relatively restricted species, found only in northeastern Peru and adjacent areas of Colombia and Brazil north of the Amazon River. This primate inhabits the open rain forest canopy, rarely contacts the ground, and is omnivorous, subsisting mainly on fruits, nuts, and insects. Wild-caught *A. nancymai* are transported to a regional research and quarantine center at Iquitos, Peru, where some individuals are selected for use in malarial research conducted by the Agency for International Development in the United States and elsewhere. The colony of *A. nancymai* presently at our institution is being held for quarantine and conditioning for this agency.

During the routine necropsy of a dead, wild-caught *A. nancymai* accessioned to our laboratory, trematode cross sections were identified microscopically within the lumen of the proximal duodenum. After washing whole flukes from formalin-preserved tissue and examination of whole-mounted specimens, the trematode was classified as belonging to the family Lecithodendriidae Odhner, 1910, based on size and the presence of a pharynx, bilaterally symmetrical testes, short digestive ceca, follicular vitellaria, and a seminal receptacle (Yamaguti, 1958). Histopathological studies were also done to observe the parasite in situ and the host's response to infection.

The animal's death was attributed to acute renal failure. Other degenerative lesions of the viscera were noted, although gross and microscopic examination of the intestinal tract revealed no abnormalities. Premortem hematological findings included a microfilaremia, although no adult filarial nematodes were found during necropsy to account for this observation. Cross sections of adult oxyurids were noted during the microscopic examination of the cecum. No fecal examination was done prior to death.

Lecithodendriid trematodes are mainly found in insectivorous birds and bats (Martin, 1969; Marshall and Miller, 1979), though they do occur in other insect-eating mammals, such as rats, marsupials, and procyonids (Thatcher, 1982) in the Neotropics. *Phaneropsolus orbicularis* Brown, 1901, the only trematode of this family to occur in New World primates, has been reported by Cosgrove (1966) in *Cebus, Saimiri,* and *Tamarinus* as well as in *Aotus griseimembra* from Colombia by Cosgrove (1966) and Thatcher and Porter (1968). Prosimian primates in the Oriental region, notably *Tupaia glis* and *Nycticebus coucang,* have been shown to harbor the lecithodendriid trematodes *Novetrema nycticebi, Odeningotrema bivesicularis,* and *O. apidion,* reported by Dunn (1964).

Non-lecithodendriid trematodes previously identified in Neotropical primates include three genera of Dicrocoeliidae: (1) *Controrchis biliophilus,* in *Ateles,* and (2) *Athesmia foxi,* in *Tamarinus,* both reported by Yamaguti (1958), in *Cebus, Saimirus,* and *Oedipomidas,* reported by Faust (1967), and in *Callicebus,* by Travassos (1944), and (3) *Platynosomum* sp., in *Callimico* and *Tamarinus,* by Cosgrove (1966). Additionally, one trematode of the family Diplostomidae,

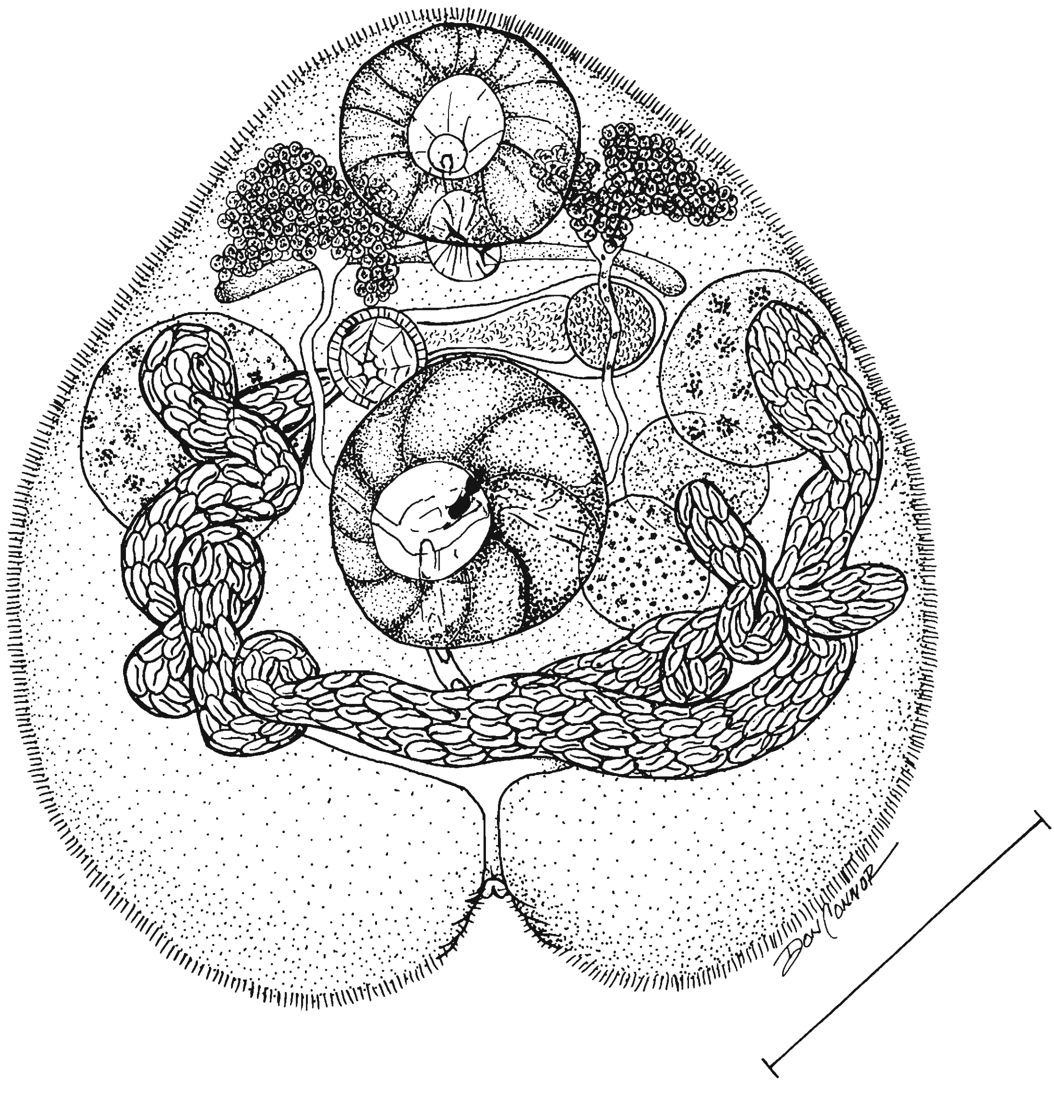



**Figure 1.** ***Aototrema dorsogenitalis*** **gen. et sp. nov. Holotype. Bar = 500 $\mu$m.**

*Neodiplostomum* sp., has been reported by Dubois (1966) in *Tamarinus.*

Herein we describe a new genus and species of Lecithodendriidae from the Peruvian red-necked owl monkey, *A. nancymai,* and report on other helminths observed.

## Materials and Methods

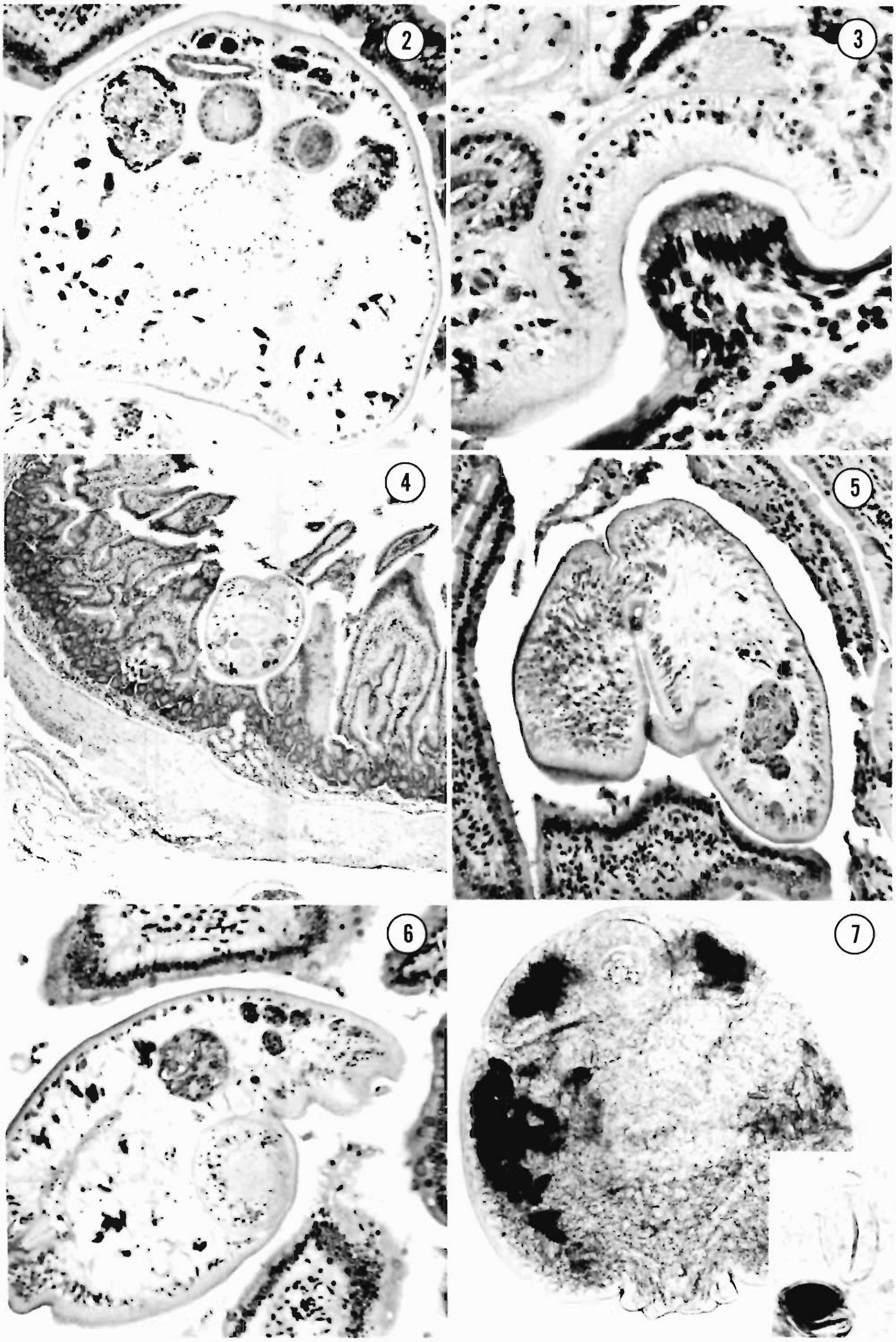
Trematodes were first observed at low magnification in 6-$\mu$m sections, with hematoxylin and eosin staining, of the proximal duodenum. Subsequently, individual flukes were washed from formalin-fixed mucosa with saline and placed into 70% ethanol for storage. Morphological characteristics were observed from whole-mount specimens stained with Semichon's acetocarmine (Pritchard and Kruse, 1982) and from unstained specimens under interference contrast microscopy. Drawings and measurements were made with the aid of a drawing tube. All measurements are in micrometers, with means followed by ranges in parentheses.

Fecal samples from 28 living *A. nancymai* were preserved in neutral buffered formalin and examined qualitatively for the presence of parasite ova by flotation in Sheather's sugar solution (Levine et al., 1960).

## Description

### *Aototrema* gen. n.

DIAGNOSIS: Lecithodendriidae. Body round to reniform, flattened, tegument spinous. Oral sucker subterminal and well developed; pre-




Figures 2–7. *Aototrema dorsogenitalis.* 2. Transverse section in situ. Plane of section is dorsal to the acetabulum. Hematoxylin and eosin, ×410. 3. Sagittal section in situ. Attachment of the oral sucker to adjacent host duodenal mucosa. Hematoxylin and eosin, ×1,025. 4. Transverse section in situ. Minimal pathology is associated with the presence of the parasite. Hematoxylin and eosin, ×102.5. 5. Partial sagittal section in situ. Prolapsed cirrus sac engaged in self-fertilization. Hematoxylin and eosin, ×410. 6. Sagittal section in situ. Caudal folds

pharynx long; pharynx moderate; esophagus absent; digestive ceca lateral, anterior to testes. Acetabulum large, equatorial to pre-equatorial. Cirrus sac lateral and elongate, preacetabular and postcecal; contains seminal vesicle, prostatic cells, and cirrus. Genital opening dorsolateral, between cecum and acetabulum, and opposite to body of cirrus sac; metraterm round and muscular. Testes large, ovoid, opposite, lateral; preacetabular. Ovary ovoid, slightly lateral, acetabular, opposite to genital pore. Seminal receptacle large, ovoid; between ovary and testis. Vitellaria prominent, follicular, clustered; prececal. Uterus tubular; much folded, with descending, transverse, and ascending limbs. Eggs large, numerous; operculate. Excretory bladder Y-shaped, with terminal pore surrounded by accessory folds and appendages. Intestinal parasites of primates.

TYPE SPECIES: *A. dorsogenitalis* sp. n.

### *Aototrema dorsogenitalis* sp. n. (Figs. 1–7)

DESCRIPTION (17 specimens studied, 13 measured): With characteristics of genus. Body reniform, 940 (795–1,140) long by 977 (840–1,235) wide. Oral sucker large, subterminal, 230 (175–300) long by 243 (170–330) wide. Prepharynx 66 (40–105) long; pharynx round, 94 (65–120) long by 99 (75–120) wide; digestive ceca short, extending laterally, pretesticular, 226 (180–300) long by a maximum of 45 wide. Acetabulum large, pre-equatorial, 298 (240–375) long by 336 (280–410) wide. Cirrus sac long, horizontal, 218 (180–380) in length and 121 (90–160) wide at level of seminal vesicle. Genital opening dorsolateral, positioned between digestive cecum and acetabulum, surrounded by glandular tissue and metraterm, 41 (30–50) long by 19 (10–30) wide. Metraterm round, muscular, 131 (95–180) long by 123 (100–170) wide. Testes lateral, opposite, ovoid, preacetabular, 228 (180–305) long by 178 (140–260) wide. Ovary slightly lateral and acetabular, opposite to genital opening, ovoid, 158 (120–190) long by 133 (120–165) wide. Ootype bipartite, dorsal to acetabulum, medial, preovarian, 63 (55–75) long by 42 (35–50) wide. Seminal receptacle ovoid, between ovary and testis, 154 (105–185) long by 114 (75–145) wide. Vitellaria prominent, follicular, prececal to cecal; vitelline ducts lead from vitellaria to ootype. Uterus tubular with descending, transverse and ascending limbs. Eggs large, numerous, operculate, 63 (55–75) long by 42 (35–50) wide. Excretory bladder Y-shaped, body of bladder 129 (100–135) long by 107 (60–165) wide at fundus. Excretory pore terminal, surrounded by accessory folds.

HOST: *Aotus nancymai* (karyotype I, 2N = 54), red-necked owl monkey.

LOCATION: Duodenum.

LOCALITY: Iquitos, Department of Loreto, Peru.

HOLOTYPE: USNM Helm. Coll. No. 79133.

PARATYPES: University of Nebraska State Museum HWML No. 23101; USNM Helm. Coll. No. 79134.

ETYMOLOGY: The generic name is derived from that of the host. The specific name refers to the position of the genital opening, as *dors(um)* = the back + *genitalis* = belonging to birth.

## Remarks

*Aototrema* gen. n. differs from other Neotropical lecithodendriids by virtue of its large oral sucker and acetabulum, the dorsolateral position of the genital opening, body shape, and the morphology of the cirrus sac. The presence of a muscular metraterm and other features suggest some similarities to *Oryzomytrema* (Thatcher, 1982), though the degree of muscularity and the configuration of the cirrus and genital pore differ. Further discoveries of a broader range of Lecithodendriidae in this region may provide insight to the proper position of *Aototrema* within the family.

Through a series of 6-$\mu$m sections, the host response and appearance of the parasite in situ were investigated. The fluke inhabited only the segment of the proximal duodenum between the pyloris and the common pancreatic duct. No flukes were found within either the pancreatic ducts or the biliary apparatus. In confirmation of observations made on whole-mounted specimens, the genital opening was seen in section dorsal to the plane of the acetabulum and oral sucker (Fig. 2). Attachment of the oral sucker or

←

**associated with excretory pore. Hematoxylin and eosin, ×410. 7. Paratype. Numerous caudal folds associated with the excretory pore. Acetocarmine, ×410. Inset: Ripened egg at the genital opening. Acetocarmine, ×1,640.**

acetabulum, with actual feeding by the former (Fig. 3), onto adjacent intestinal mucosa elicited a minimal inflammatory response. This was characterized mainly by a mild diffuse chronic infiltration of lymphocytes and macrophages into the lamina propria (Fig. 4). One section revealed the cirrus process extended out of the genital opening, presumably to engage in self-fertilization (Fig. 5). The fluke was also seen in sagittal section, showing the extent of the caudal folds and appendages associated with the excretory pore (Fig. 6). These caudal folds were also noted in whole-mounted specimens (Fig. 7).

One specimen was radically different from all other individuals collected, in that the genital opening was ventral, medial, and adjacent to the pharynx, and the egg was smooth and quite a bit smaller. Placement of this individual may be made tentatively into the genus *Phaneropsolus* Looss, 1899, but more specimens would have to be identified from cohort hosts to make any formal description.

A survey of fecal specimens from 28 other *Aotus nancymai* (karyotype I, 2N = 54) received by our institution as a cohort of the previously described host was done to determine incidence of *Aototrema* in the colony and to estimate the presence of any other helminth parasites. Nineteen samples (67.8%) contained ova with mean measurements of 65 $\mu$m long and 43 $\mu$m wide. These ova were consistent in both size and morphology with mature eggs of *Aototrema* in whole-mounted specimens (Fig. 7, inset). In both fecal specimens and mounted specimens, ova were characterized by a sigmoid, longitudinal fissure. This could have been shrinkage artifact introduced by formalin fixation, though ova from other species showed no such fissure. Additionally, 10 samples (35.7%) had dicrocoeliid ova, with mean measurements of 37 $\mu$m long by 23 $\mu$m wide, consistent with Travassos's (1944) measurements of *Controrchis,* but not with those of *Athesmia* reported by Faust (1967). Five samples (17.8%) contained characteristic oxyurid ova, possibly *Enterobius* or *Trypanoxyuris,* as reported by Sandosham (1950) and Inglis (1960). Eight samples contained two or more of the ova types.

## Acknowledgments

We are indebted to Dr. Stephen Kelley for his advice on host biology and for providing red-necked owl monkey fecal samples, and to Donald Conner and John Fischer for their assistance with illustrations and photomicrographs, respectively.

This work was supported in part by DHHS NIH grants RR00471 and RR07004, and USAID contract DPE-0453-C00-3058-00.

## Literature Cited

**Cosgrove, G. E.** 1966. The trematodes of laboratory primates. Laboratory Animal Care 16:23–39.

**Dubois, G.** 1966. Un neodiplostome (Trematoda: Diplostomidae) chez le tamarin, *Leontocebus nigricollis* (Spix). Revue Suisse Zoologique 73:37–42.

**Dunn, F. L.** 1964. *Odeningotrema apidon* n. sp. (Trematoda: Lecithodendriidae) from a Malayan primitive primate. Proceedings of the Helminthological Society of Washington 31:21–25.

**Faust, E. C.** 1967. *Athesmia* (Trematoda: Dicrocoeliidae) Odhner, 1911 liver fluke of monkeys from Colombia, South America, and other mammalian hosts. Transactions of the American Microscopical Society 86:113–119.

**Hershkovitz, P.** 1983. Two new species of night monkeys, genus *Aotus* (Cebidae, Platyrrhini): a preliminary report on *Aotus* taxonomy. American Journal of Primatology 4:209–243.

**Inglis, W. G.** 1960. The oxyurid parasites (Nematoda) of primates. Proceedings of the Zoological Society of London 136:103–122.

**Levine, N. D., K. N. Merhra, D. T. Clark, and I. J. Aves.** 1960. A comparison of nematode egg counting techniques for cattle and sheep feces. American Journal of Veterinary Research 21:511–515.

**Marshall, M. E., and G. C. Miller.** 1979. Some digenetic trematodes from Ecuadorian bats including five new species and one new genus. Journal of Parasitology 65:909–917.

**Martin, D. R.** 1969. Lecithodendriid trematodes from the bat *Peropteryx kappleri* in Colombia, including discussions of allometric growth and significance of ecological isolation. Proceedings of the Helminthological Society of Washington 36:250–260.

**Pritchard, M. H., and G. O. W. Kruse.** 1982. The Collection and Preservation of Animal Parasites. University of Nebraska Press, Lincoln. 141 pp.

**Sandosham, A. A.** 1950. A species of *Enterobius* from the feline douroucouli (*Aotus felineus*). Transactions of the Royal Society of Tropical Medicine and Hygiene 44:5–6.

**Thatcher, V. E.** 1982. Five new neotropical species of Lecithodendriidae (Trematoda: Digenea) including three new genera, all from Panamanian and Colombian mammals. Proceedings of the Helminthological Society of Washington 49:45–55.

**———, and J. A. Porter.** 1968. Some helminth parasites of Panamanian primates. Transactions of the American Microscopical Society 87:186–196.

**Travassos, L.** 1944. Revisão da familia Dicrocoeliidae (Odhner, 1910). Monagrafias do Instituto Oswaldo Cruz. Vol. 2.

**Yamaguti, S.** 1958. Systema Helminthum. Vol. I. Pts. 1 and 2, The Digenetic Trematodes of Vertebrates. Interscience Publ. Inc., New York. 1575 pp.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 73–77

# Description and Growth Pattern of *Eurytrema pancreaticum* from *Bos indicus* from East Java[1]

WINAWATI WIRORENO,[2] W. PATRICK CARNEY,[3,5] AND M. ANSORI[4]
[2] National Biological Institute, Indonesian Institute of Sciences, Bogor, Indonesia
[3] U.S. Naval Medical Research Unit No. 2 (NAMRU-2), Jakarta, Indonesia and
[4] Department of Statistics and Computation, Bogor Agricultural University, Bogor, Indonesia

ABSTRACT: *Eurytrema pancreaticum* (Janson, 1889) (Trematoda: Dicrocoeliidae) is described from *Bos indicus* from East Java, Indonesia. Select measurable characters, correlated with body size, revealed the greatest variability in the acetabulum, pharynx, and left testis, and the smallest variability in the length of the fore and hind body. Allometric exponents for all organs and body measurements (except the testes and hind body) were negative.

KEY WORDS: intraspecific variation, allometric growth.

*Eurytrema pancreaticum* (Janson, 1889) (Trematoda: Dicrocoeliidae) was first reported from the Indonesian Archipelago in 1907 by DeDoes, who found this fluke in the pancreatic ducts of cattle from Java (Kraneveld and Mansjoer, 1948). Burggraaf (1933, 1935) later isolated this parasite from the pancreatic ducts of cattle, water buffalo, and goats in Sumatra, and in 1948, 26% of a sample of cattle from Central Java were said to be infected (Kraneveld and Mansjoer, 1948). Further documentation of the occurrence and distribution of *E. pancreaticum* is lacking.

In March 1971, a collection of *E. pancreaticum* was made from cattle, *Bos indicus,* raised in East Java. Five of nine cattle were infected with worm burdens of ±250, 1,000, 3,000, 3,000, and 3,000, respectively. The gravid specimens showed considerable variation in body size (Fig. 1) and correspondingly, in the relative sizes of common diagnostic features. Because large populations were available for study and because many helminth descriptions are based on relative sizes of organs and body proportions, this offered an opportunity not only to describe the intraspecific variation of the Indonesian strain of *E. pancreaticum* and report its enzooticity to East Java, but also to study apparent growth patterns of this trematode as evidenced in large natural populations.

## Materials and Methods

One hundred gravid specimens representing the wide range of body sizes in the populations were selected for study. They were killed in hot water (50°C), fixed in 10% formalin, stained with Gower's carmine, and mounted in Permount® under slight coverslip pressure. The analyses of allometric growth patterns were done according to the allometric formula $y = b \cdot x^{\alpha}$, where y = organ size, x = body size (average diameter = [length + maximum width]/2), $\alpha$ = allometric exponent, and b = constant. This formula can be converted into $\log y = \log b + \alpha \cdot \log x$, which corresponds graphically to a straight line in a double-logarithmic system of coordinates (e.g., Rohde, 1966). Organ sizes were plotted against body size in a system of double logarithmic coordinates in order to obtain a linear relationship. Data obtained were computerized and regression analyses and scattergrams produced using MEDPAK programs developed by Mr. Richard See, NAMRU-2. All measurements are expressed in millimeters unless otherwise stated, and were made as illustrated in Figure 1. Drawings were made with a Bausch and Lomb tri-simplex projector.

## Results

### Description of *Eurytrema pancreaticum* from Indonesia based on 100 specimens ($N$ = 100 for each variable) (Fig. 1)

*Eurytrema* Looss, 1907 (Trematoda: Dicrocoeliidae). Body flattened, oval, fusiform, or lanceolate, distinct caudal appendage lacking. Length 7.06 ± 1.92 (3.30–10.80); width 3.30 ± 0.99 (1.2–5.4). Tegument aspinose, without protuberances. Oral sucker subterminal, 0.72 ± 0.18 (0.33–1.07) in diameter; prepharynx absent, pharynx oval, 0.28 ± 0.06 (0.13–0.42) in length and 0.22 ± 0.04 (0.14–0.32) in width. Esophagus 0.12 ± 0.04 (0.07–0.24) in length. Intestinal bi-

[1] Contribution from the National Biological Institute of the Indonesian Institute of Sciences, Bogor, Indonesia, and U.S. Naval Medical Research Unit No. 2 (NAMRU-2), Jakarta, Indonesia. The opinions and assertions contained herein are those of the authors, and are not to be construed as official or reflecting the views of the Indonesian Institute of Sciences, the Bogor Agricultural University, or the U.S. Navy Department.

[5] Present address: Naval Biosciences Laboratory, Naval Supply Center, Oakland, California 94625-5015.

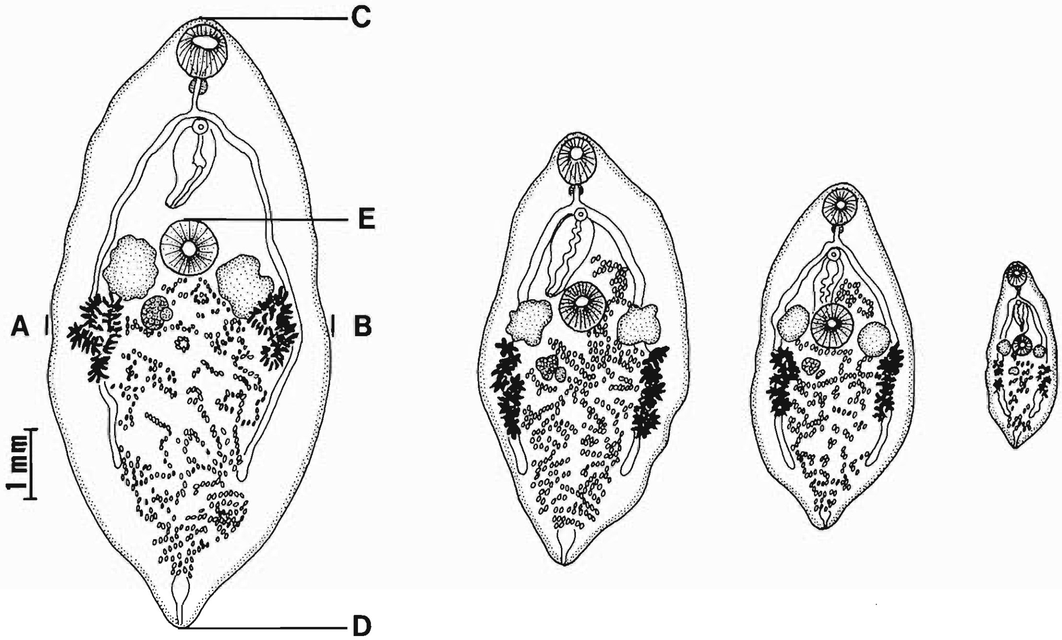



**Figure 1.** ***Eurytrema pancreaticum*, illustrating how measurements were made, extreme variation in size of gravid specimens, and topography of morphological characters in large, medium, and small specimens. A–B, body width; C–D, body length; C–E, fore body length; E–D, hind body length; other organs, (length + maximum width)/2.**

furcation closer to oral sucker than to acetabulum; ceca terminate 0.97 ± 0.41 (0.42–2.17) from posterior end. Acetabulum 0.80 ± 0.18 (0.38–1.12) in diameter. Testes paired, lateral or short distance posterior to acetabulum, margin smooth or slightly lobed; right testis 0.70 ± 0.25 (0.21–1.30) in diameter; left testis 0.69 ± 0.23 (0.23–1.24). Cirrus sac obliquely aligned in some cases toward the right side and in the other cases toward the left, 1.00 ± 0.26 (0.45–1.57) in length and 0.24 ± 0.10 (0.18–0.70) in maximum width. Cirrus sac containing coiled seminal vesicle followed by a short pars prostatica surrounded by unicellular prostatic glands. Ovary margin smooth or faintly lobed, 0.27 ± 0.08 (0.13–0.42) in diameter, smaller than testes, usually situated slightly to left of median line. Ovary, seminal receptacle and Mehlis' gland located close together, usually partly overlapping. Seminal receptacle 0.12 ± 0.03 (0.08–0.19) in diameter; Mehlis' gland 0.18 ± 0.04 (0.12–0.28) in diameter. Uterus strongly convoluted, occupying most of posttesticular region. Eggs 0.29 ± 0.01 (0.28–0.33) in diameter. Vitellaria paired, follicular, posterior to testes on each side of body ventral to intestinal ceca. Excretory vesicle tubular; other features of excretory system not determined.

HOST: *Bos indicus.*

HABITAT: Pancreatic ducts.

LOCALITY: East Java, Indonesia.

DATE: 3 March 1971.

SPECIMENS DEPOSITED: Museum Zoologicum Bogoriense No. 201; USNM Helm. Coll. No. 74494; remaining specimens in Helm. Coll. NAMRU-2, Jakarta, WPC: 19–23.

**Growth patterns of *Eurytrema pancreaticum***

Figure 2 demonstrates that measurements of organs and body portions are linear in relation to body size. Correlation coefficients for all measurements were very high ($R = 0.86$–$0.96$). The greatest variability was seen in the size of the acetabulum ($R = 0.87$), the pharynx ($R = 0.86$) and the left testis ($R = 0.86$). The smallest variability was seen in the length of the fore body ($R = 0.96$) and in the length of the hind body ($R = 0.95$).

The allometric exponents for the various organs and body portions differed considerably (Fig. 2). All organs, except the testes, showed negative allometric growth, i.e., they grew more slowly

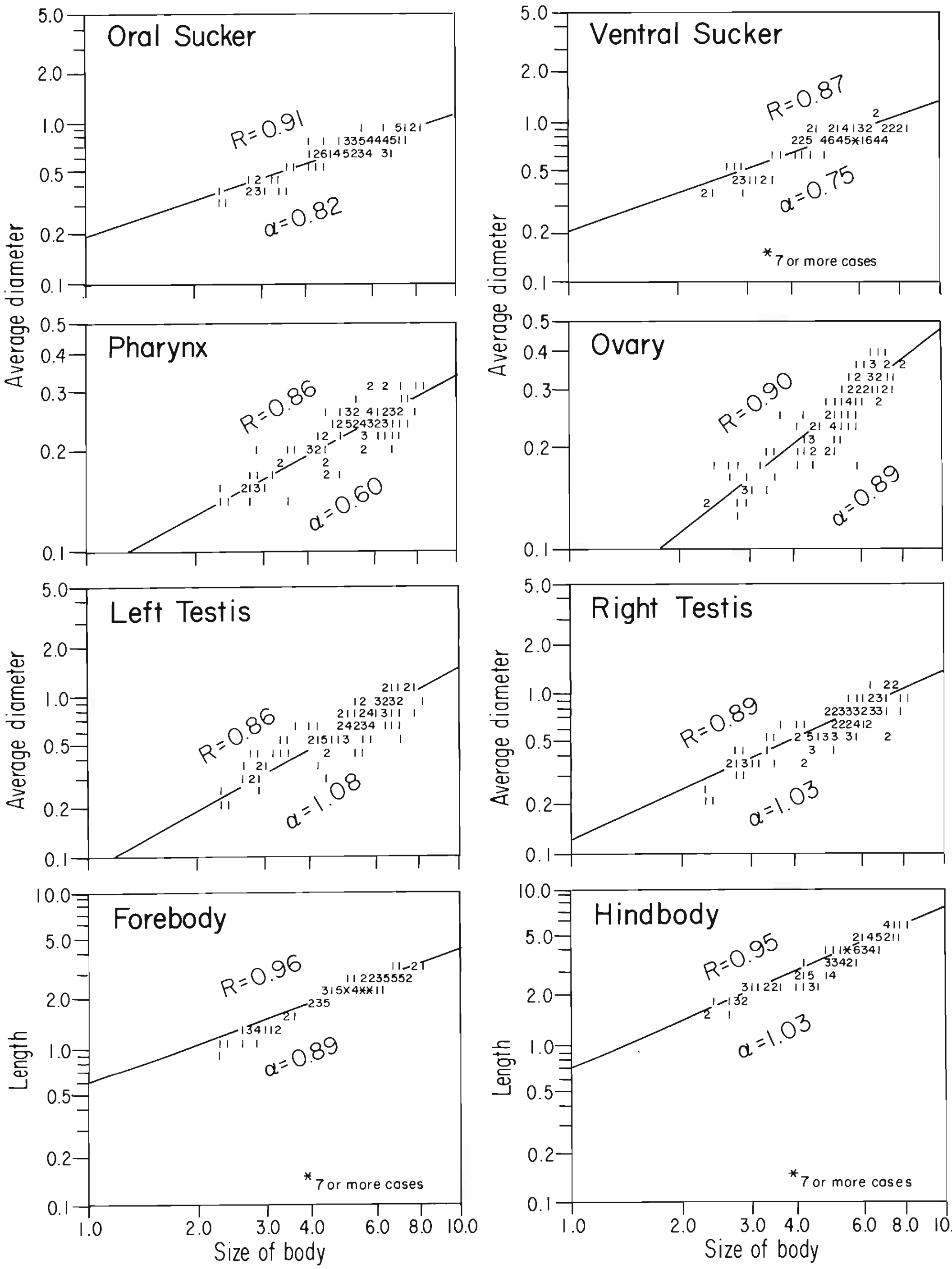



**Figure 2.** Growth patterns of selected organs and body portions of *Eurytrema pancreaticum*. Size of body = (length + maximum width)/2. Measurements in mm. *R* = correlation coefficient; α = allometric exponent; 1–6 = number of cases; * = seven or more cases.

than the whole body (allometric exponent smaller than 1). The greatest negative allometry was shown by the pharynx (0.60). Both testes showed positive allometric growth patterns (allometric exponent greater than 1; 1.08 for the left testis and 1.03 for the right testis). The fore body length (0.89) showed a negative allometric growth, which contrasted with the positive allometric growth of the hind body (1.03).

## Discussion

Although *E. pancreaticum* is a common trematode parasite of ruminants in continental and insular Asia (Basch, 1965), the morphology of many insular strains has not been described. Ovigerous Indonesian specimens of *E. pancreaticum* were intermediate or small in size when compared to previous descriptions of this species summarized and discussed by Travassos (1944), Bhalerao (1936), and Yamaguti (1971). Eggs of the Indonesian strain were consistently smaller and rounder than those reported elsewhere (Yamaguti, 1971).

Yamaguti (1971) listed eight species in the genus *Eurytrema* in addition to *E. pancreaticum,* and noted that five of them, all of which are parasites of ruminants, have been considered junior synonyms of *E. pancreaticum* by various taxonomists. Pryadko (1962) synonymized *Eurytrema coelomaticum* (Giard and Billet, 1892) and *Eurytrema medium* Cherttova, 1957, with *E. pancreaticum. Eurytrema dajii,* 1924, was synonymized with *E. pancreaticum* by Chatterji (1938), and Bhalerao (1936) synonymized *Eurytrema ovis* Tubangui, 1925, with *E. pancreaticum.* Yamaguti (1971) considered *Eurytrema parvum* Senoo, 1907, as an immature form of *E. pancreaticum.* Thus, with the exception of *Eurytrema tonkinense* Gilliard and Dany-van Ngu, 1941, which was reported from *Bos taurus* in Tonkin, Vietnam, all eurytremids from ruminants in Asia have been considered varieties of *E. pancreaticum* (Yamaguti, 1971).

The linear relationship between respective measurements and size of body as presented in Figure 2 indicates that these specimens, even though they vary considerably, belong to one population. Ovigerous specimens of *E. pancreaticum* vary considerably in size and relative proportions of commonly used diagnostic features in the same host species. These differences presumably are due to differences in worm age. Kinsella (1971), using a rodent–*Quinqueserialis quinqueserialis* model, demonstrated that trematodes continue to grow considerably after reaching sexual maturity. Kinsella's study supported Stunkard's (1957) observation that continuing growth after sexual maturity may be a major difficulty in the delineating species of parasitic flatworms. Host reactions in cattle already or previously infected with *E. pancreaticum* may also play a role in stunting the growth of some specimens in this host–parasite system. In a cotton rat–filarid worm (*Litomosoides carinii*) model, Macdonald and Scott (1953) showed that growth and development of this filaria in cotton rats with one or more previous infections was significantly lower than that in parallel infections in previously uninfected controls. Likewise, crowding may have simply depleted food reserves essential for development to maximum size. However, in hosts the size of cattle, even worm burdens of ±3,000 are not likely to deplete essential food reserves significantly. Recent studies have shown that allometric growth of body parts and organs of trematodes can also be influenced by host species (Fischthal et al., 1980), by intensity of infection in the same species (Fischthal et al., 1982b), and by site of infection in a host (Fischthal et al., 1982a). The allometric growth patterns described herein, however, reflect variations in a worm population in one site of a single host species.

A study of allometric growth patterns of another dicrocoeliid, *Anchitrema sanguineum* (Sonisino, 1894), showed that all organs had strongly negative allometric growth, but that the hind body had a positive allometric growth pattern (Rohde, 1966). Betterton and Lim (1977) recently discussed allometric growth of two rodent dicrocoeliids, *Skrjabinus* in *Rattus* spp. and *Zonorchis* in *Callosciurus* spp. They noted that the sucker size of *Zonorchis* sp. increased slightly with increasing body length, whereas there was no detectable change in the sucker size of *Skrjabinus.* In both cases, if an allometric exponent had been calculated it would have been negative. Likewise, the testes and vitelline fields of *Skrjabinus* and *Zonorchis* proportionately decreased with increasing length, indicating a negative allometric pattern. Similarly, Martin's (1969) study of lecithodendriid trematodes (*Castroia amplicata* and *C. silvai*) demonstrated that the growth rates of the ovary and testes were less than that of the hind body. In *E. pancreaticum,* the ovary grew slower than the whole body as a whole ($\alpha = 0.89$);

however, the testes both had positive allometric exponents ($\alpha = 1.03$ for the right testis and $\alpha = 1.08$ for the left testis). The hind body of *E. pancreaticum* also demonstrated a positive allometric growth pattern.

## Literature Cited

**Basch, P. F.** 1965. Completion of the life cycle of *Eurytrema pancreaticum* (Trematoda: Dicrocoeliidae). Journal of Parasitology 51:350–355.

**Betterton, C., and B. L. Lim.** 1977. Patterns in the morphological variation of *Zonorchis* and *Skrjabinus* (Trematoda: Dicrocoeliidae) from small mammals of Malaysia. International Journal of Parasitology 7:73–82.

**Bhalerao, G. D.** 1936. Studies on the helminths of India. Trematoda. Indian Journal of Helminthology 14:163–180.

**Burggraaf, H.** 1933. Bijdrage tot de kennis der pancreasdistomatose bij het rund, veroorzaakt door *Eurytrema pancreaticum* (Janson, 1889) Looss, 1907. Tijdschrift voor Diergeneeskunde 60:1277–1282.

———. 1935. Pancreas distomatose. Tijdschrift voor Diergeneeskunde 62:407–469.

**Chatterji, R. C.** 1938. Annotated list of the helminths recorded from domesticated animals of Burma. Part I. Trematoda. Proceedings of the National Academy of Sciences, India 8:93–104.

**Fischthal, J. H., D. O. Carson, and R. S. Vaught.** 1982a. Comparative allometry of size of the digenetic trematode *Bucephalus gorgon* (Linton, 1905) Eckmann, 1932 (Bucephalidae) in two sites of infection in the marine fish *Seriola dumerili* (Risso). Journal of Parasitology 68:173–174.

———, ———, and ———. 1982b. Comparative size allometry of the digenetic trematode *Lissorchis attenuatus* Monorchiidae at four intensities of infection in the white sucker. Journal of Parasitology 68:314–318.

———, **B. L. Fish, and R. S. Vaught.** 1980. Comparative allometric growth of the digenetic trematode *Metadena globosa* (Linton 1910) Manter 1947 (Cryptogonimidae) in three species of Caribbean fishes. Journal of Parasitology 66:642–644.

**Kinsella, J. M.** 1971. Growth, development and intraspecific variation of *Quinqueserialis quinqueserialis* (Trematoda: Notocotylidae) in rodent hosts. Journal of Parasitology 57:62–70.

**Kraneveld, F. C., and M. Mansjoer.** 1948. Infectie met *Eurytrema pancreaticum* (Janson, 1889) by runderen op Java. Veterinaire Snapshots No. 44. Nederlandsch Indiesch Blad voor Diergeneeskunde 55:2266.

**Macdonald, E. M., and J. A. Scott.** 1953. Experiments on immunity in the cotton rat to the filarial worm, *Litomosoides carinii*. Experimental Parasitology 2:174–184.

**Martin, D. R.** 1969. Lecithodendriid trematodes from the bat, *Peropteryx kappleri* in Colombia, including discussions of allometric growth and significance of ecological isolation. Proceedings of the Helminthological Society of Washington 36:250–259.

**Pryadko, E. I.** 1962. The identification of various species of *Eurytrema*. Trudy Institute of Zoology, Alma-Ata 16:52–56.

**Rohde, K.** 1966. On the trematode genera *Lutztrema* Travassos, 1941 and *Anchitrema* Looss, 1899 from Malayan bats, with a discussion of allometric growth in helminths. Proceedings of the Helminthological Society of Washington 33:184–199.

**Stunkard, H. W.** 1957. Intraspecific variation in parasitic flatworms. Systematic Zoology 6:7–18.

**Travassos, L.** 1944. Revisao da familia Dicrocoeliidae Odhner, 1910. Monografias do Instituto Oswaldo Cruz, Rio de Janeiro, No. 2. 357 pp.

**Yamaguti, S.** 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vol. I. Keigaku Publishing Company, Japan. 1074 pp.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 78–83

# *Dactylogyrus* (Monogenea: Dactylogyridae) from *Hybopsis* and *Notropis* (*Cyprinella*) (Pisces: Cyprinidae) from the Tennessee River Drainage, with Descriptions of Three New Species and Remarks on Host Relationships

DONALD G. CLOUTMAN
Duke Power Company, Production Environmental Services, Rt. 4, Box 531, Huntersville, North Carolina 28078

ABSTRACT: Three previously described and three new species of *Dactylogyrus* are reported from *Hybopsis* and *Notropis* (*Cyprinella*) from the Tennessee River drainage: *D. amblops* Mueller, 1938, and *D. plegadus* Rogers, 1967, were found on *H. amblops*; *D. moorei* Monaco and Mizelle, 1955, occurred on *H. monacha, N. galacturus,* and *N. spilopterus*; *D. beckeri* sp. n. and *D. dissimili* sp. n. are described from *N. galacturus* and *H. dissimilis,* respectively; *D. nuntius* sp. n. is described from *H. monacha* and *N. galacturus.* No *Dactylogyrus* species were found on *H. cahni* and *H. insignis.* The presence of *D. moorei* and *D. nuntius* on *H. monacha* and members of *Notropis* (*Cyprinella*) corroborates recent ichthyological evidence that *H. monacha* is more closely related to certain species of *Notropis* (*Cyprinella*) than to *Hybopsis.*

KEY WORDS: *Dactylogyrus beckeri* sp. n., *D. dissimili* sp. n., *D. nuntius* sp. n., fish, taxonomy, morphology, new localities.

From host–parasite lists provided by Mizelle and McDougal (1970) and Kritsky et al. (1977), it is evident that most species of North American *Dactylogyrus* Diesing, 1850, parasitize either one species or groups of closely related hosts. Those species of *Dactylogyrus* that parasitize more than one host species should offer supporting evidence for species relationships determined from traditional ichthyological studies. Burkhead and Bauer (1983) and Jenkins and Burkhead (1984) provided evidence based on morphology and reproductive behavior that *Hybopsis monacha* (Cope) is allied closely with *Notropis* (*Cyprinella*), rather than with *Hybopsis* (*Erimystax*) where it is currently placed. I examined one species of *Hybopsis* (*Hybopsis*), four species of *Hybopsis* (*Erimystax*) (including *H. monacha*), and two species of *Notropis* (*Cyprinella*) from the Tennessee River drainage to determine if the *Dactylogyrus* infesting these hosts indicate the same host relationships suggested by the ichthyological studies mentioned above. Three previously described and three new species of *Dactylogyrus* are reported herein, and evolutionary relationships of hosts based on infesting *Dactylogyrus* species are discussed.

## Materials and Methods

The species and numbers of hosts examined are listed in Table 1. Immediately after capture, some hosts were placed in jars containing a 1:4,000 formalin solution; after approximately 1 hr, enough formalin was added to make a 10% solution (Putz and Hoffman, 1963). Museum specimens of some hosts loaned by R. E. Jenkins, Roanoke College, were also examined.

All parasites, collected from the gills of their hosts, were mounted in glycerin jelly, and observations were made with a phase contrast microscope. Drawings were made with the aid of an ocular grid and graph paper (Mayr, 1969). Measurements, in micrometers, were made as presented by Mizelle and Klucka (1953); means are followed by ranges in parentheses. All type specimens of new species and representative specimens of previously described species were deposited in the helminthological collection of the National Museum of Natural History, Smithsonian Institution (USNM). Other nontype material was retained in the author's collection. For comparative purposes, all original descriptions and redescriptions of North American *Dactylogyrus* species and type specimens of the following species were examined: *D. amblops* Mueller, 1938, seven syntypes (USNM 71453); *D. confusus* Mueller, 1938, five syntypes (USNM 71447); *D. plegadus* Rogers, 1967, holotype (USNM 61604) and one paratype (USNM 61605).

### *Dactylogyrus amblops* Mueller, 1938

HOST: *Hybopsis amblops* (Rafinesque), bigeye chub.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 79300, 1 specimen); Lewis Co., Buffalo River at the mouth of Grinders Creek (USNM 79299, 3 specimens). Virginia: Washington Co., North Fork Holston River at "Peatail Island."

REMARKS: This is the first report of *Dactylogyrus amblops* since its original description from *Hybopsis amblops* in New York (Mueller, 1938).

*Dactylogyrus amblops* was found only on *H. amblops,* where it occurred in mixed infestations with *D. plegadus.*

### *Dactylogyrus moorei* Monaco and Mizelle, 1955

HOSTS AND LOCALITIES: *Hybopsis monacha* (Cope)—Virginia: Washington Co., North Fork Holston River off Co. Rt. 615 at island, 0.7 air km S of jct. of Co. Rts. 614 and 615 (USNM 79303, 1 specimen); North Fork Holston River, Rt. 614 bridge at Mendota. *Notropis galacturus* (Cope)—Tennessee: Blount Co., Little River near Waland; Monroe Co., Citico Creek (USNM 79301, 2 specimens). *Notropis spilopterus* (Cope)—Tennessee: Blount Co., Little River near Waland; Hancock Co., Clinch River at Frost Ford (USNM 79302, 1 specimen).

REMARKS: *Dactylogyrus moorei* was described from *Notropis deliciosus missuriensis* (=*N. stramineus* (Cope)) and *N.* (*Cyprinella*) *lutrensis* (Baird and Girard) from Oklahoma (Monaco and Mizelle, 1955), and has been reported subsequently from five other species of *Notropis* (*Cyprinella*) from the central and eastern United States (Rogers, 1967; Cloutman, 1974; Kritsky et al., 1977). I suspect that the single report of *D. moorei* on *N. stramineus* was the result of either transposed data or an "accidental" infestation due to close association with *N. lutrensis,* and that *D. moorei* normally infests only members of *Notropis* (*Cyprinella*). I have found *D. moorei* on *N. lutrensis* from the Smoky Hill River, Ellis Co., Kansas (Cloutman, 1974), and Rattlesnake Creek, Stafford Co., Kansas (unpubl. data), but lacking on syntopic *N. stramineus.*

### *Dactylogyrus plegadus* Rogers, 1967

HOST: *Hybopsis amblops* (Rafinesque), bigeye chub.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 79305, 2 specimens); Lewis Co., Buffalo River at the mouth of Grinders Creek (USNM 79304, 3 specimens). Virginia: Washington Co., North Fork Holston River at "Peatail Island."

REMARKS: This is the first report of *Dactylogyrus plegadus* since its original description from *Hybopsis amblops* and *H. winchelli* (Girard) (reported as *H. amblops* by Rogers [1967] before Clemmer [1980] resurrected *H. winchelli* to species status). *Dactylogyrus plegadus* appears to parasitize only species of *Hybopsis* (*Hybopsis*).

### *Dactylogyrus beckeri* sp. n. (Figs. 1–8)

TYPE HOST: *Notropis galacturus* (Cope), whitetail shiner.

TYPE LOCALITY: Tennessee: Monroe Co., Citico Creek.

TYPE SPECIMENS: Holotype, USNM 79289; 7 paratypes, USNM 79290 (6 specimens) and USNM 79291 (1 specimen).

OTHER LOCALITY: Tennessee: Blount Co., Little River near Waland.

DESCRIPTION: With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; length 446 (338–518), greatest width 147 (122–187). Two pairs of eyes approximately equal in size, anterior pair farther apart than posterior pair. Peduncle lacking. Haptor 54 (43–65) long, 65 (58–72) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, solid shaft, and recurved point. Anchor length 25 (23–26), greatest width of base 16 (14–19). Dorsal bar length 22 (20–24). Vestigial ventral bar length 15 (14–17). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 15 (13–15); 2, 15 (14–16); 3, 16 (15–17); 4, 16 (14–17); 4A, 6 (5–6); 5, 15 (15–16); 6, 15 (14–15); 7, 15 (14–17). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus with enlarged robust base bearing straight process and curved tubular shaft that is attenuated to a point. Cirrus length 58 (46–65). Process length (measured from base of cirrus shaft to distal tip of process) 13 (11–15). Shaft length 37 (34–41). Accessory piece bifurcate, distal ramus curved and attenuated to a point; medial ramus recurved, attenuated to a point. Accessory piece length 27 (26–28). Vagina sclerotized, irregular in shape, opening dextroventrally posterior to cirrus. Vitellaria moderate to heavy, usually distributed from pharynx to haptor. Egg elliptical, 61 (60–61) long and 43 (42–44) wide.

REMARKS: *Dactylogyrus beckeri* most closely resembles *D. confusus* by possessing similar-shaped anchors and cirrus, but comparison of the syntypes taken from *Clinostomus elongatus* (Kirtland) and original description of *D. confusus* (Mueller, 1938) reveals some trenchant differences: *D. beckeri* possesses smaller hooks, a larger cirrus, and longer cirrus process. The cirrus process of *D. beckeri* is tapered distally, whereas that of *D. confusus* is enlarged distally.

ETYMOLOGY: *Dactylogyrus beckeri* is named in honor of the late Dr. David A. Becker, my mentor at the University of Arkansas.

### *Dactylogyrus dissimili* sp. n. (Figs. 9–16)

TYPE HOST: *Hybopsis dissimilis* (Kirtland), streamline chub.

TYPE LOCALITY: Tennessee: Hancock Co., Clinch River at Frost Ford.

TYPE SPECIMENS: Holotype, USNM 79292; 10 paratypes, USNM 79293 (9 specimens) and USNM 79294 (1 specimen).

OTHER LOCALITY: Virginia: Washington Co., North Fork Holston River at "Peatail Island."

DESCRIPTION: With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; length 210 (187–252), greatest width 75 (58–86). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, anterior pair farther apart than posterior pair. Pharynx circular to ovate (dorsal view), transverse diameter 20 (16–23), gut not observed. Peduncle lacking. Haptor 47 (43–50) long, 54 (43–72) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, and solid shaft that curves to a sharp point. Anchor length 43 (42–45), greatest width of base 20 (19–21). Dorsal bar length 26 (23–31). Vestigial ventral bar length 23 (21–26). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 20 (18–22); 2, 21 (20–22); 3, 28 (22–33); 4, 24 (22–26); 4A, 7; 5, 23 (19–27); 6, 21 (18–22); 7, 19 (18–20). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus with small base bearing straight process and curving tubular shaft that is bent near distal end and attenuated to a point, length 23 (18–30). Accessory piece Y-shaped, distal ramus with club-shaped terminus and medial ramus with rounded terminus. Accessory piece length 20 (14–25). Vagina not observed. Vitellaria moderate, distributed from pharynx to haptor.

REMARKS: The closest apparent relative of *Dactylogyrus dissimili* is *D. nuntius* sp. n., but *D. dissimili* is easily distinguished by the presence of a process on the cirrus base and a club-shaped terminus on the distal ramus of the accessory piece.

ETYMOLOGY: *Dactylogyrus dissimili* is named after its host.

### *Dactylogyrus nuntius* sp. n. (Figs. 17–24)

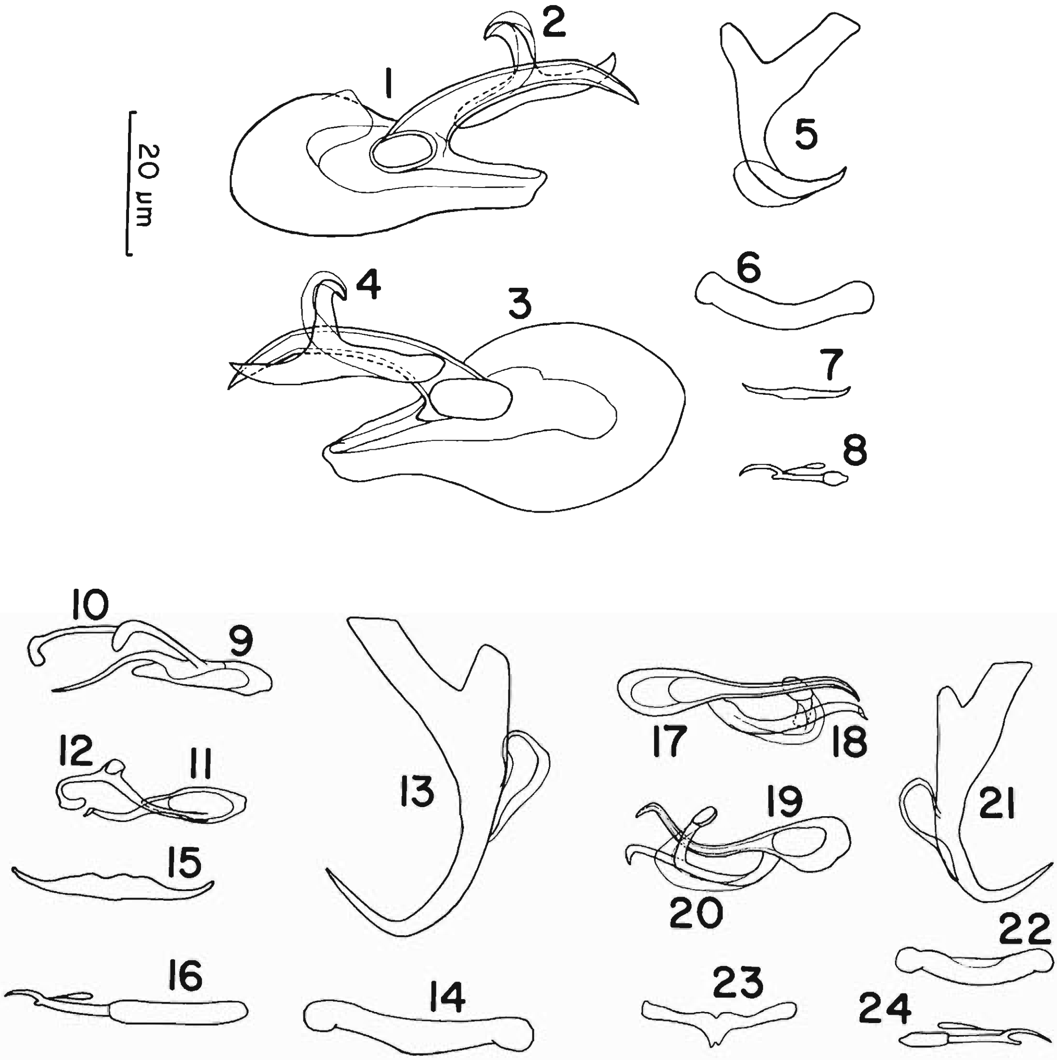
TYPE HOST: *Notropis galacturus* (Cope), whitetail shiner.

TYPE LOCALITY: Tennessee: Hancock Co., Clinch River at Frost Ford.

TYPE SPECIMENS: Holotype, USNM 79295; 13 paratypes, USNM 79296 (7 specimens) and USNM 79297 (6 specimens).

OTHER HOSTS AND LOCALITIES: *Notropis galacturus* (Cope)—Tennessee: Blount Co., Little River near Waland; Monroe Co., Citico Creek. *Hybopsis monacha* (Cope)—Virginia: Washington Co., Middle Fork Holston River below ford on Co. Rt. 707, 9.3 air km ESE of Abingdon; North Fork Holston River, Fleenor Mill Road; North Fork Holston River off Co. Rt. 615 at island, 0.7 air km S of jct. of Co. Rts. 614 and 615; North Fork Holston River at Hobbs Ford off Co. Rt. 614, 1.6 air km E of Mendota; North Fork Holston River, Rt. 614 bridge at Mendota (USNM 79298, 2 specimens).

DESCRIPTION: With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; length 409 (288–504), greatest width 118 (72–137). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, anterior pair ranges from closer to farther apart than posterior pair. Pharynx circular to ovate (dorsal view), transverse diameter 28 (25–29); gut not observed. Peduncle lacking. Haptor 48 (43–72) long, 73 (58–86) wide. Single pair of dorsal anchors; each having solid base with short deep root and elongate superficial root, solid, slightly inflated shaft, and sharp point. Anchor length 33 (30–35), greatest width of base 14 (11–16). Dorsal bar length 20 (15–23). Vestigial ventral bar length 19 (17–22). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Miz-




**Figures 1–24. 1–8. *Dactylogyrus beckeri.* 1, 3. Cirrus. 2, 4. Accessory piece. 5. Anchor. 6. Dorsal bar. 7. Ventral bar. 8. Hook. 9–16. *Dactylogyrus dissimili.* 9, 11. Cirrus. 10, 12. Accessory piece. 13. Anchor. 14. Dorsal bar. 15. Ventral bar. 16. Hook. 17–24. *Dactylogyrus nuntius.* 17, 19. Cirrus. 18, 20. Accessory piece. 21. Anchor. 22. Dorsal bar. 23. Ventral bar. 24. Hook.**

elle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 17 (15–18); 2, 18 (15–20); 3, 20 (17–22); 4, 19 (17–22); 4A, 4; 5, 19 (18–21); 6, 17 (14–19); 7, 18 (15–20). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus with rounded base, bearing a curving tubular shaft bent near distal end and attenuated to a point, length 31 (28–35). Accessory piece Y-shaped, distal ramus recurved and pointed, medial ramus with knoblike terminus. Accessory piece length 20 (18–22). Vagina not observed. Vitellaria moderate to heavy, usually distributed from pharynx to haptor.

**Table 1. Prevalence (% infestation), range, and relative density (total number of parasites/total number of hosts) of *Dactylogyrus* infesting species of *Hybopsis* and *Notropis* (*Cyprinella*) from the Tennessee River drainage. Numbers in parentheses represent the number of hosts.**

| | Prevalence | Range | Relative density |
|---|---|---|---|
| *Hybopsis* (*Hybopsis*) *amblops* (Rafinesque), bigeye chub (10) | | | |
| *Dactylogyrus amblops* Mueller, 1938 | 100.0 | 1–6 | 3.7 |
| *Dactylogyrus plegadus* Rogers, 1967 | 90.0 | 0–16 | 5.9 |
| *Hybopsis* (*Erimystax*) *cahni* Hubbs and Crowe, slender chub (10) | | | |
| *Hybopsis* (*Erimystax*) *dissimilis* (Kirtland), streamline chub (7) | | | |
| *Dactylogyrus dissimili* sp. n. | 100.0 | 7–21 | 15.7 |
| *Hybopsis* (*Erimystax*) *insignis* Hubbs and Crowe, blotched chub (11) | | | |
| *Hybopsis* (*Erimystax*) *monacha* (Cope), spotfin chub (14) | | | |
| *Dactylogyrus moorei* Monaco and Mizelle, 1955 | 14.3 | 0–1 | 0.1 |
| *Dactylogyrus nuntius* sp. n. | 92.9 | 0–5 | 2.4 |
| *Notropis* (*Cyprinella*) *galacturus* (Cope), whitetail shiner (10) | | | |
| *Dactylogyrus beckeri* sp. n. | 80.0 | 0–15 | 3.9 |
| *Dactylogyrus moorei* Monaco and Mizelle, 1955 | 30.0 | 0–13 | 2.1 |
| *Dactylogyrus nuntius* sp. n. | 40.0 | 0–15 | 3.1 |
| *Notropis* (*Cyprinella*) *spilopterus* (Cope), spotfin shiner (3) | | | |
| *Dactylogyrus moorei* Monaco and Mizelle, 1955 | 66.7 | 0–1 | 0.7 |

REMARKS: The closest apparent relative of *Dactylogyrus nuntius* is *D. dissimili* (see remarks for *D. dissimili*).

ETYMOLOGY: The specific name is Latin (*nuntius* = a messenger), referring to the parasite providing evidence that its two hosts are closely related (see discussion for details).

## Discussion

The apparent close relationship of *Dactylogyrus dissimili* and *D. nuntius* may indicate that their hosts (Table 1) are phyletically linked. This hypothesis supports the traditional placement of *Hybopsis monacha* with *H. dissimilis* in *Hybopsis* (*Erimystax*), but fails to explain the presence of *D. nuntius* on *Notropis galacturus*. The presence of *D. nuntius* on *H. monacha* and *N. galacturus* and its absence from other fishes (Table 1) indicate a close relationship between these two hosts. This interpretation is further strengthened by the presence of *D. moorei* on *H. monacha, N. galacturus, N. spilopterus* (Table 1), and other species of *Notropis* (*Cyprinella*), and its absence from other hosts (see remarks for *D. moorei*). *Dactylogyrus moorei* and *D. nuntius* provide strong evidence corroborating the findings of Burkhead and Bauer (1983) and Jenkins and Burkhead (1984) that *H. monacha* is most closely allied with *Notropis* (*Cyprinella*), although some relationship between *H. monacha* and *Hybopsis* (*Erimystax*) cannot be ruled out because of the apparent close relationship between *D. dissimili* and *D. nuntius.*

## Acknowledgments

I thank Dr. Robert E. Jenkins, Roanoke College, and Robert Wallus, TVA, for providing some of the host specimens. Dr. J. Ralph Lichtenfels loaned type specimens of *Dactylogyrus* from the USNM.

## Literature Cited

**Burkhead, N. M., and B. H. Bauer.** 1983. An intergeneric cyprinid hybrid, *Hybopsis monacha* × *Notropis galacturus,* from the Tennessee River drainage. Copeia 1983:1074–1077.

**Clemmer, G. H.** 1980. *Hybopsis winchelli* (Girard), clear chub. Page 195 *in* D. S. Lee et al. Atlas of North American Freshwater Fishes. North Carolina State Museum of Natural History, Raleigh. 854 pp.

**Cloutman, D. G.** 1974. Monogenean and copepod parasites of fishes from the Smoky Hill River, Ellis County, Kansas. Transactions of the Kansas Academy of Science 77:225–229.

**Jenkins, R. E., and N. M. Burkhead.** 1984. Description, biology and distribution of the spotfin chub, *Hybopsis monacha,* a threatened cyprinid fish of the Tennessee River drainage. Bulletin of the Alabama Museum of Natural History 8:1–30.

**Kritsky, D. C., R. J. Kayton, and P. D. Leiby.** 1977. *Dactylogyrus unguiformis* sp. n. (Monogenea) from the mottled sculpin, *Cottus bairdi* Girard, in Idaho, with some taxonomic considerations in the genus *Dactylogyrus.* Proceedings of the Helminthological Society of Washington 44:142–147.

**Mayr, E.** 1969. Principles of Systematic Zoology. McGraw-Hill, Inc., New York. 428 pp.

**Mizelle, J. D., and J. W. Crane.** 1964. Studies on monogenetic trematodes, XXIII. Gill parasites of *Micropterus salmoides* (Lacepede) from a California pond. Transactions of the American Microscopical Society 83:343–348.

———**, and A. R. Klucka.** 1953. Studies on monogenetic trematodes. XIV. Dactylogyridae from Wisconsin fishes. American Midland Naturalist 49:720–737.

———**, and H. D. McDougal.** 1970. *Dactylogyrus* in North America. Key to species, host–parasite and parasite–host lists, localities, emendations, and description of *D. kritskyi* sp. n. American Midland Naturalist 84:444–462.

**Monaco, L. H., and J. D. Mizelle.** 1955. Studies on monogenetic trematodes. XVII. The genus *Dactylogyrus.* American Midland Naturalist 53:455–477.

**Mueller, J. F.** 1938. Additional species of North American Gyrodactyloidea (Trematoda). American Midland Naturalist 19:220–235.

**Putz, R. E., and G. L. Hoffman.** 1963. Two new *Gyrodactylus* (Trematoda: Monogenea) from cyprinid fishes with synopsis of those found on North American fishes. Journal of Parasitology 49:559–566.

**Rogers, W. A.** 1967. Studies of Dactylogyrinae (Monogenea) with descriptions of 24 new species of *Dactylogyrus,* 5 new species of *Pellucidhaptor,* and the proposal of *Aplodiscus* gen. n. Journal of Parasitology 53:501–524.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 84–90

# Phylogenetic Relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and Their Ictalurid (Siluriformes) Hosts: An Hypothesis

G. J. KLASSEN AND M. BEVERLEY-BURTON
Department of Zoology, College of Biological Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT: An hypothesis is proposed for the phylogeny of *Ligictaluridus* species (Monogenea: Ancyrocephalidae) based on a cladistic analysis primarily involving the morphology of the male copulatory apparatus. Comparison of host (ictalurid) and parasite phylogenies in terms of Fahrenholz's Rule indicates two cases of host transfer, one of which is not congruent with the historical component of host–parasite coevolution. Alternate hypotheses are considered, and it is concluded that the interspecific parasite phylogeny appears strongly indicative of ecological association between hosts.

KEY WORDS: cladistic analysis, host relationships, *Ictalurus* (*Ictalurus*), *Ictalurus* (*Amiurus*), *Noturus* (*Noturus*), *Noturus* (*Rhabdias*), *Noturus* (*Schilbeodes*), *Pytodictis,* fishes.

Brooks (1979, 1981, 1985) and Mitter and Brooks (1983) developed the partitioning of host–parasite coevolution into historical and ecological associations, with the historical component considered a subset of the ecological component. It is the historical component that can be directly tested by comparing host and parasite phylogenies. Based on this premise, an attempt is made to test the hypothesis that these parasites and their hosts coevolved in terms of Fahrenholz's Rule, which states that "parasite phylogeny mirrors host phylogeny" (Brooks, 1979; see also Eichler, 1948; Wardle et al., 1974; Price, 1980; Noble and Noble, 1982), by comparing parasite and host phylogenies as outlined by Brooks (1979).

## Materials and Methods

Data used in the phylogenetic (cladistic) analysis for *Ligictaluridus* spp. were obtained from Klassen and Beverley-Burton (1985) and Klassen et al. (1985). With one exception, only characters synapomorphic for two or more of the ingroup species were used. Five of the six known *Ligictaluridus* spp. were used in the interspecific analysis (see Klassen and Beverley-Burton [1985] for discussion of the inadequacy of the description of *L. bychowskyi* (Price and Mura, 1969)). Multistate characters were handled according to the procedure for additive binary coding presented by Farris (1970) and outlined by Brooks et al. (1984). The techniques used in present study conform to the theoretical framework for phylogenetic analyses presented by Hennig (1966) and Wiley (1981). The cladograms were first constructed by hand, then compared to results from the programs MIX, PENNY, and DOLLO of the PHYLIP (Phylogenetic Inference Programs) package available from Dr. J. Felsenstein (Department of Genetics SK-50, University of Washington, Seattle, Washington 98195) on an IBM Personal Computer. The choice of outgroup (*Urocleidus aculeatus*) for the interspecific analysis was based on the results of a preliminary intergeneric analysis of 17 genera of North American Ancyrocephalidae (Klassen, 1985, unpubl. M.Sc. Thesis). Analyses using other potential sister groups as outgroups for the interspecific cladogram showed no effect on internal parsimony.

## Results

### Characters

Each character used in the cladistic analysis is listed below with character states polarized using the outgroup. Transformation series for each of the multistate characters are presented in Figure 1.

1) PENIS CURVATURE (Fig. 1a): Plesiomorphic state: lightly bowed penis shaft with inflated base, distal aperture oriented such that distal and proximal apertures are more or less parallel (Fig. 1a: 0). Apomorphic state: strongly curved penis with distal aperture oriented on same plane as proximal (Fig. 1a: 1).

2) PENIS DIAMETER (Fig. 1b): Plesiomorphic state: penis shaft slender (Fig. 1b: 0). Apomorphic states: diameter of entire penis shaft enlarged (Fig. 1b: 1); addition of distally flaring, funnel-like opening surrounding distal aperture (Fig. 1b: 2).

3) BASAL HANDLE OF PENIS (Fig. 1c): Plesiomorphic state: basal handle elongate, attached to lateral surface of the penis base and directed away from distal aperture (Fig. 1c: 0). Apomorphic states: handle reduced (Fig. 1c: 1); addition of second handle attached to opposite surface of

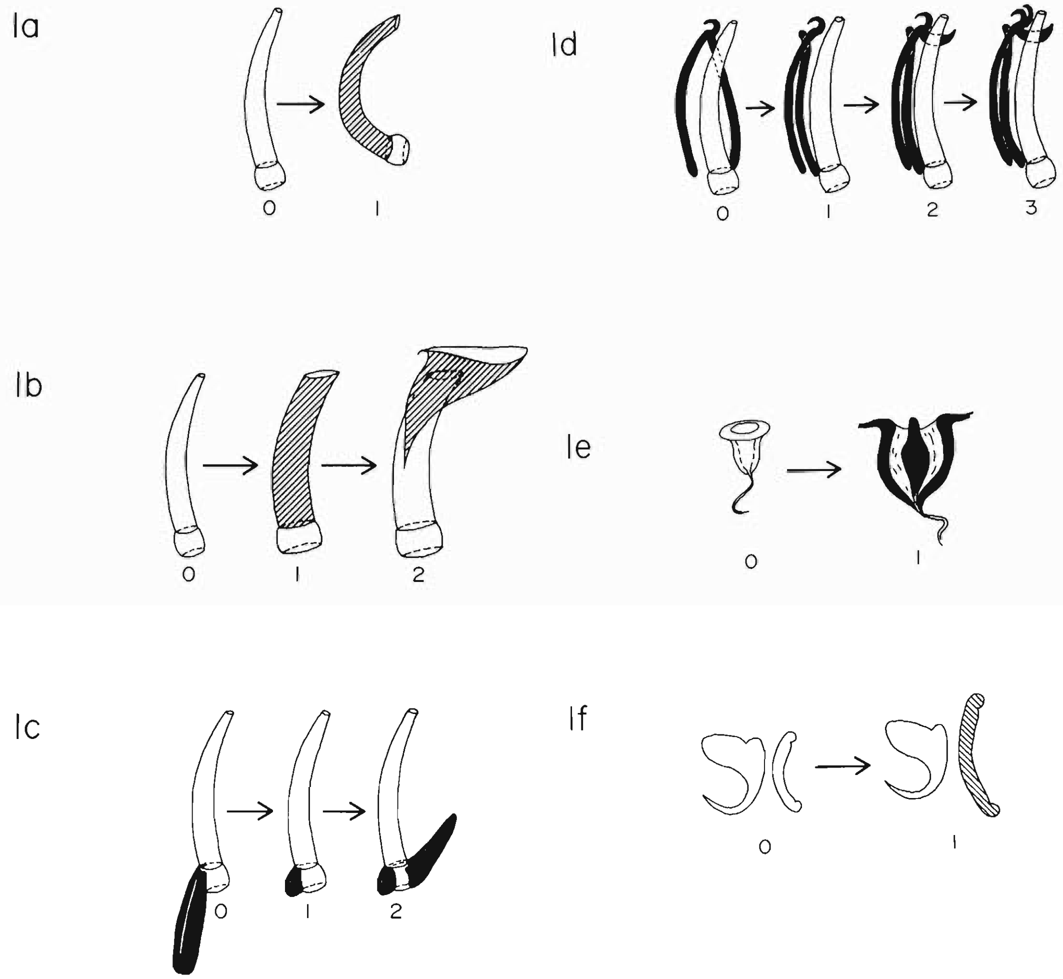



**Figure 1. Character states for interspecific cladogram relating to *Ligictaluridus* spp. a. Penis curvature. b. Penis diameter c. Basal handle. d. Accessory piece. e. Vagina. f. Transverse bar. Numbers below figures indicate polarity (0—plesiomorphic; positive numbers—apomorphic); arrows indicate direction of character evolution.**

penis base and directed toward distal penis aperture (Fig. 1c: 2).

4) ACCESSORY PIECE (Fig. 1d): Plesiomorphic state: accessory piece consisting of 2 rami, of which at least 1 is basally attached, rami distally fused with single terminal hook (Fig. 1d: 0). Apomorphic states: 2 rami distally separated and aligned parallel, on one side of penis (Fig. 1d: 1); lightly sclerotized, leaflike projection added to ramus retaining hook (Fig. 1d: 2); addition of second hook in tandem with first (Fig. 1d: 3).

5) VAGINA (Fig. 1e): Plesiomorphic state: vagina unsclerotized and inconspicuous (Fig. 1e: 0). Apomorphic state: vaginal walls heavily sclerotized with centrally located conical projection (Fig. 1e: 1).

6) TRANSVERSE BARS OF HAPTOR (Fig. 1f): Plesiomorphic state: transverse bars approximately equal in length to associated hamuli (Fig. 1f: 0). Apomorphic state: bars greatly exceed hamuli in length (Fig. 1f: 1).

## Parasite phylogeny

The resultant cladogram represents the most parsimonious hypothesis for the phylogeny of the species studied based on known morphological data (Fig. 2).

Three distinct groups, or clades, are recognized within *Ligictaluridus*. Synapomorphic for the entire genus are a reduced basal handle (Fig. 1c: 1) and distally separated accessory piece (Fig. 1d: 1). Synapomorphic for the first clade is the

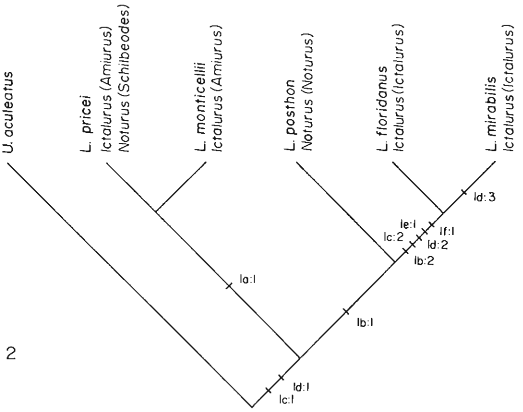



**Figure 2. Interspecific cladogram for *Ligictaluridus* spp. Names of the parasite species and their host subgenera are labeled at the terminal nodes. Apomorphies are labeled according to character state as shown in Figure 1.**

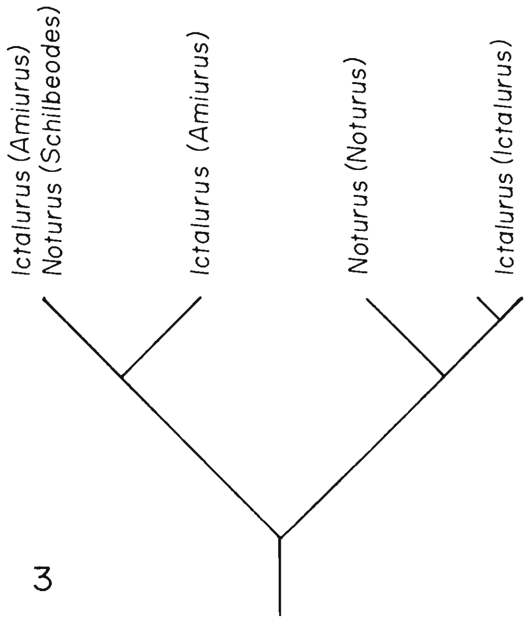



**Figure 3. Modified interspecific cladogram for *Ligictaluridus* spp. Names of the ictalurid host subgenera are inserted at the terminal nodes.**

strongly curved penis (Fig. 1a: 1). The members of this clade are *Ligictaluridus pricei* and *L. monticellii*. Synapomorphic for the rest of the species is the increased diameter of the penis shaft (Fig. 1b: 1). *Ligictaluridus posthon* is split off, lacking further modifications.

Synapomorphic for *Ligictaluridus floridanus* and *L. mirabilis* are the funnel-shaped distal opening of the penis (Fig. 1b: 2), the additional basal handle (Fig. 1c: 2), the leaflike projection on the accessory piece (Fig. 1d: 2), sclerotized vagina (Fig. 1e: 1), and enlarged transverse bars (Fig. 1f: 1). *Ligictaluridus mirabilis* is further distinguished by a second hook on the accessory piece (Fig. 1d: 3).

### Host relationships

Fish from three genera of ictalurid hosts (*Ictalurus, Noturus,* and *Pylodictis*) are known to be parasitized by *Ligictaluridus* spp. (see Klassen and Beverley-Burton [1985] and Klassen et al. [1985] for a complete list of recorded host species). Lundberg (1970) recognized two groups of *Ictalurus* species: the subgenus *Amiurus* (represented by *Ictalurus* (*A.*) *brunneus* (Jordan), *I.* (*A.*) *catus* (Linnaeus), *I.* (*A.*) *melas* (Rafinesque), *I.* (*A.*) *natalis* (Lesueur), *I.* (*A.*) *nebulosus* (Lesueur), *I.* (*A.*) *platycephalus* (Girard), and *I.* (*A.*) *serracanthus* Yerger and Relyea) and the subgenus *Ictalurus* (represented by *I.* (*I.*) *balsanus* (Jordan and Snyder), *I.* (*I.*) *furcatus* (Lesueur), and *I.* (*I.*) *punctatus* (Rafinesque)). In addition, three subgenera of *Noturus* Rafinesque are currently recognized (Taylor, 1969): *N.* (*Rhabdias*) Jordan and Evermann, *N.* (*Schilbeodes*) Bleeker, and *N.* (*Noturus*) Rafinesque.

In the present study, the following ictalurid hosts were examined for ancyrocephalids: subgenus *Amiurus—Ictalurus* (*A.*) *melas, I.* (*A.*) *natalis,* and *I.* (*A.*) *nebulosus*; subgenus *Ictalurus—I.* (*I.*) *punctatus*; subgenus *Schilbeodes—Noturus* (*S.*) *gyrinus* and *N.* (*S.*) *exilis*; and subgenus *Noturus—N.* (*N.*) *flavus*. Although a variety of ictalurids have been recorded as hosts for *Ligictaluridus* spp. (Klassen and Beverley-Burton, 1985), all the available original material was considered and many of the records were found to be doubtful, thus only material examined by the present authors is considered in the present context.

*Ligictaluridus pricei* (Mueller, 1936) Klassen and Beverley-Burton, 1985, was found only on *Ictalurus* (*Amiurus*) and *Noturus* (*Schilbeodes*). *Ictalurus* (*Amiurus*) *nebulosus* was confirmed as the host for *L. monticellii* (Cognetti de Martiis, 1924) Klassen and Beverley-Burton, 1985. *Noturus* (*Noturus*) *flavus* is the only host recorded for the new species *Ligictaluridus posthon* Klassen, Beverley-Burton, and Dechtiar, 1985. *Ictalurus* (*Ictalurus*) *punctatus* was confirmed as the host for *Ligictaluridus floridanus* (Mueller,

4

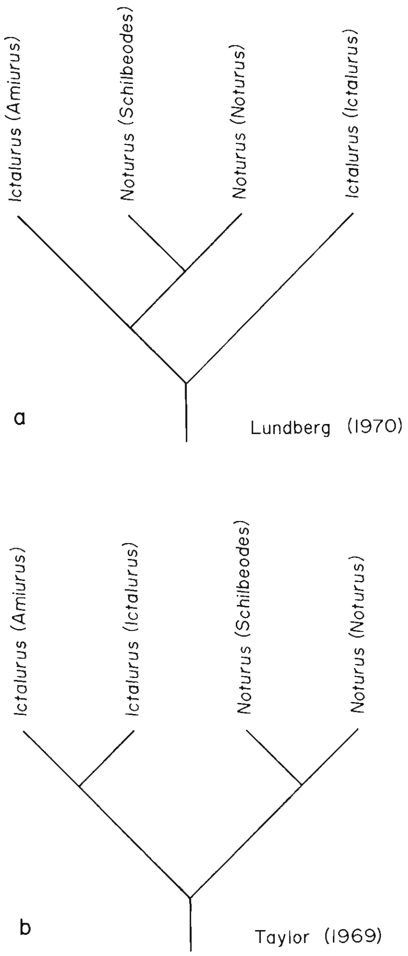



**Figure 4. Host cladograms for the four subgenera of the family Ictaluridae used in the present study. The cladograms represent two competing hypotheses on the phylogenetic relationships between four subgenera: a, proposed by Lundberg (1970); b, proposed by Taylor (1969) (modified from Lundberg, 1970).**

5

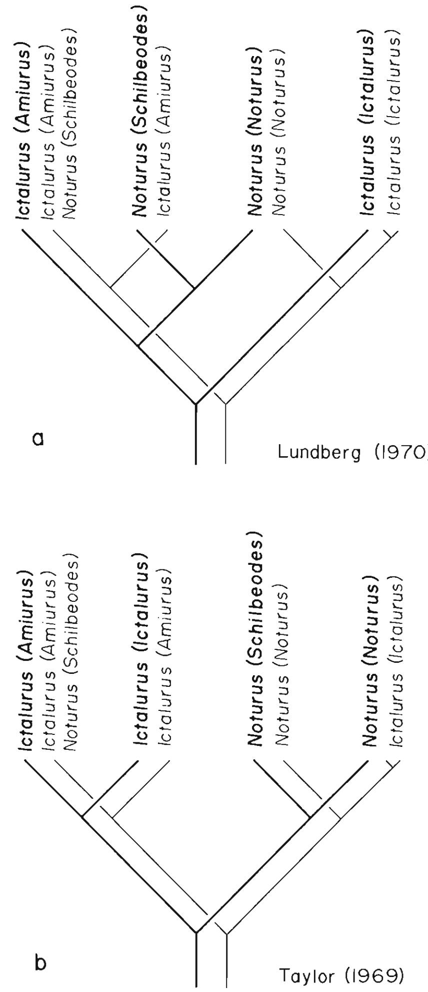
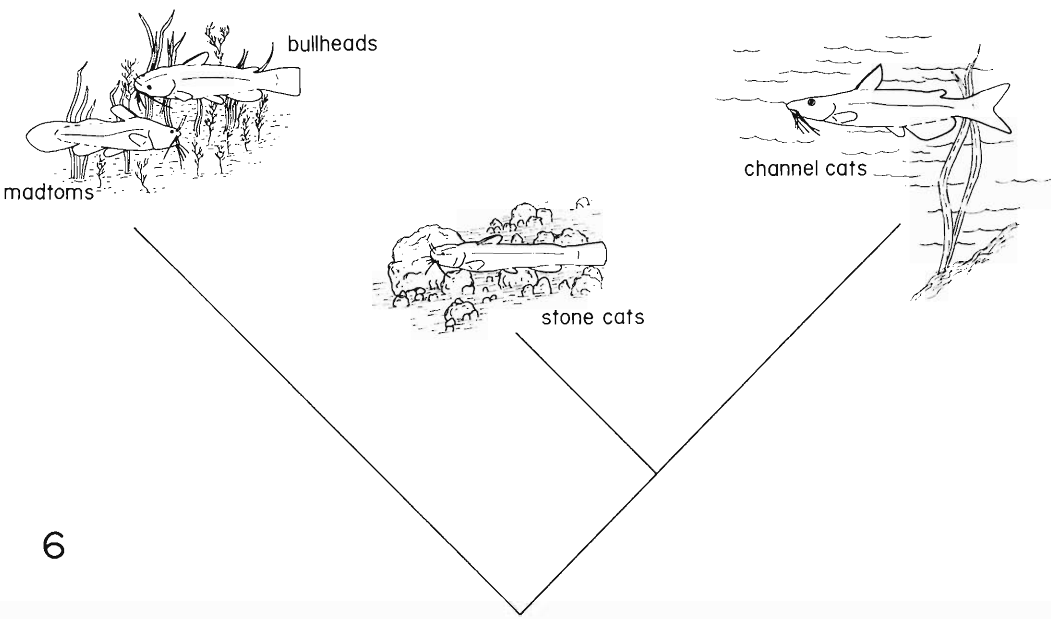



**Figure 5. Comparison of parasite (*Ligictaluridus* spp.) and host (four ictalurid subgenera) cladograms. The parasite cladogram (thin) is superimposed on each of the competing host cladograms (thick). Host subgeneric names (thin for parasite cladogram and thick for host cladogram) are labeled at each terminal node.**

1936) Klassen and Beverley-Burton, 1985, as well as *L. mirabilis* (Mueller, 1937) Klassen and Beverley-Burton, 1985 (Fig. 2).

Figure 3 represents a parasite cladogram modified such that only confirmed host subgenera are labelled at the terminal nodes. Lundberg (1970) presented cladograms of several ictalurid phylogenies, and simplified versions of two of these competing hypotheses are shown in Figure 4 (a, b), where only the phylogenetic relationships between *Ictalurus* (*Ictalurus*), *Ictalurus* (*Amiurus*), *Noturus* (*Noturus*), and *Noturus* (*Schilbeodes*) are included. The first (Lundberg, 1970) states that *Ictalurus* (*Amiurus*) is more closely related to the *Noturus* subgenera than to *Ictalurus* (*Ictalurus*); the second (Taylor, 1969) shows that *Ictalurus* (*Amiurus*) and *Ictalurus* (*Ictalurus*) are more closely related to one another than either is to the *Noturus* subgenera. Comparison of the parasite cladogram with the host cladograms (Fig. 5) shows that the parasite cladogram does not perfectly match either of the host cladograms.




6

**Figure 6. Simplified species-level cladogram for *Ligictaluridus* spp. A graphic representation of how the parasite phylogeny reflects host environment for the subgenera *Amiurus* (bullheads), *Schilbeodes* (madtoms), *Noturus* (stone cats), and *Ictalurus* (channel cats).**

## Discussion

Interspecific phylogeny of *Ligictaluridus* spp. is most strongly demonstrated by the character evolution of the male copulatory apparatus. The observed haptoral characters serve as supporting evidence.

The fact that the parasite cladogram does not perfectly match either of the host cladograms (Fig. 5) may indicate either misinterpretation of the cladograms (parasite and/or host) or that some factor other than the historical component of coevolution is responsible for the relationship between parasites and hosts. These alternatives will now be considered.

In the present context, it is assumed that the parasite cladogram is optimal. Lundberg (1970) proposed a phylogenetic reconstruction for ictalurids based on 121 characters derived from the study of 22 extant species, and compared it with three alternate cladograms, including one generated by a reanalysis of data provided by Taylor (1969). Lundberg (1970) showed his cladogram to be most parsimonious and proposed that the genus *Ictalurus* may be a paraphyletic assemblage. This proposal was subsequently confirmed by Lundberg (1982). Lundberg's cladogram (Fig. 4a) is supported by the present study, inasmuch as *L. pricei* (found on *Ictalurus* (*Amiurus*)) is not sister species to *L. floridanus* and *L. mirabilis* (found on *Ictalurus* (*Ictalurus*)).

Still, a complete match is not apparent, as *L. pricei* and *L. posthon,* which are not one another's sister species (Fig. 2), are found to parasitize *Noturus* (*Schilbeodes*) (albeit not exclusively) and *Notorus* (*Noturus*), respectively, which are one another's sister species (Fig. 5a, b). This may indicate a misinterpretation of one or the other of the cladograms; however, as noted above, the parasite cladogram is considered the best available, and there is convincing evidence for the monophyly of *Noturus* presented by Taylor (1969) and supported by Lundberg (1970, 1982). Thus, an historical association between host and parasite may explain only two-thirds of the observed host–parasite relationships, where *L. posthon* is an obvious exception to historical association, and the transfer of *L. pricei* to *Notorus* (*Schilbeodes*), although not necessarily an exception to historical association, cannot be predicted by it.

If the present-day ecology of the hosts is considered, an alternative explanation for these relationships may be proposed (Fig. 6). Bullheads (subgenus *Amiurus*) and madtoms (subgenus *Schilbeodes*) prefer a shallow-water habitat with muddy bottom and large amounts of vegetation.

In contrast, stonecats (subgenus *Noturus*) are found in rapid-flowing, deep streams and rivers, with rocky bottoms, and channel cats (subgenus *Ictalurus*) prefer a predominantly pelagic existence in deep rivers and lakes (Taylor, 1969; Lundberg, 1970; Scott and Crossman, 1979; Christie, pers. comm.). It is hypothesized that ancyrocephalids of the genus *Ligictaluridus* (possibly a progenitor) transferred once (i.e., a single evolutionary event) by ecological association from a centrarchid to an ictalurid host. This hypothesis is supported by Gusev (1978), who stated that "entering an area rich in the local group of Centrarchidae, the predecessors of the Ictaluridae might have picked up the Ancyrocephalinae [sic; =Ancyrocephalidae sensu Murith and Beverley-Burton, 1984] from these fish." Furthermore, it is suggested that the ancyrocephalid parasites found on the subgenera *Ictalurus* (*Ictalurus*) and *Noturus* (*Noturus*) underwent allopatric speciation as their hosts established themselves in new habitats and that the interspecific phylogeny of *Ligictaluridus* reflects host adaptation to new environments rather than host taxonomy.

Further research regarding the phylogeny of Monogenea, as well as other ectoparasites, will allow a better understanding not only of the phylogenetic relationships among parasite taxa, but also of the ecological associations among their hosts and how these may have changed over (geologic) time.


## Acknowledgments

We thank Dr. S. A. Marshall, Department of Environmental Biology, University of Guelph, and Dr. D. R. Brooks, Department of Zoology, University of British Colombia, Vancouver, British Columbia, for their helpful comments and constructive criticisms, and Mr. J. Christie, Ontario Ministry of Natural Resources, Fisheries Station, Glenora, Ontario, for supplying information on channel cat habitats. Financial support was provided from a Natural Sciences and Engineering Research Council operating grant to M. Beverley-Burton (grant No. 801-81).


## Literature Cited


**Brooks, D. R.** 1979. Testing the context and extent of host–parasite coevolution. Systematic Zoology 28:299–307.

———. 1981. Hennig's parasitological method: a proposed solution. Systematic Zoology 30:229–249.

———. 1985. Historical ecology: a new approach to studying the evolution of ecological associations. Annals of the Missouri Botanical Garden 72:660–680.

**———, J. N. Caira, T. R. Platt, and M. H. Pritchard.** 1984. Principles and methods of phylogenetic systematics: a cladistic workbook. Special Publication Number 12, Museum of Natural History, University of Kansas.

**Eichler, W.** 1948. Some rules in ectoparasitism. Annals and Magazine of Natural History 1:588–598.

**Farris, J. S.** 1970. Methods for computing Wagner trees. Systematic Zoology 19:83–92.

**Gusev, A. V.** 1978. Monogenoidea of freshwater fishes: principles of systematics, analysis of world fauna and its evolution. Zoologic Institute, USSR Academy of Science, Parazitologicheskii Sbornik XXVIII, Leningrad, Nauka Press.

**Hennig, W.** 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

**Klassen, G. J., and M. Beverley-Burton.** 1985. *Ligictaluridus* Beverley-Burton, 1984 (Monogenea: Ancyrocephalidae) from catfishes (Siluriformes: Ictaluridae) in North America with redescriptions of the type species, *Ligictaluridus pricei* (Mueller, 1936) and three others. Canadian Journal of Zoology 63:715–727.

**———, ———, and A. O. Dechtiar.** 1985. *Ligictaluridus posthon* n. sp. (Monogenea: Ancyrocephalidae) from *Noturus flavus* Rafinesque (Siluriformes: Ictaluridae) in Ontario, Canada. Canadian Journal of Zoology 63:2071–2073.

**Lundberg, J. G.** 1970. The evolutionary history of North American catfishes, family Ictaluridae. Ph.D. Dissertation, The University of Michigan, Ann Arbor.

———. 1982. The comparative anatomy of the toothless blindcat, *Trogloglanis pattersoni* Eigenmann, with a phylogenetic analysis of the ictalurid catfishes. Miscellaneous Publications, Museum of Zoology, University of Michigan, No. 163.

**Mitter, C., and D. R. Brooks.** 1983. Phylogenetic aspects of coevolution. Pages 65–98 *in* D. J. Futuyama and M. Slatkin, eds. Coevolution. Sinauer Associates, New York.

**Murith, D., and M. Beverley-Burton.** 1984. *Tetracleidus banghami* Mueller, 1936 (Monogenea: Ancyrocephalidae) from *Micropterus dolomieui* Lacepede (Pisces: Centrarchidae) in Ontario, Canada: anatomy, systematic position, and emended familial and generic diagnoses. Canadian Journal Zoology 62:992–997.

**Noble, E. R., and G. A. Noble.** 1982. Parasitology: The Biology of Animal Parasites, 5th ed. Lea & Febiger, Philadelphia.

**Price, P. W.** 1980. Evolutionary Biology of Parasites. Princeton University, Princeton, New Jersey.

**Scott, W. B., and E. J. Crossman.** 1979. Freshwater fishes of Canada. Bulletin of the Fisheries Research Board of Canada 184.

**Taylor, W. R.** 1969. A revision of the catfish genus *Noturus* Rafinesque with an analysis of higher groups in the Ictaluridae. United States National Museum Bulletin 282:1–315.

**Wardle, R. A., J.A. McLeod, and S. Radinovsky.** 1974.

Advances in the Zoology of Tapeworms, 1950–1970. University of Minnesota Press, Minneapolis.

**Wiley, E. O.** 1981. Phylogenetics: The Theory and Practice of Phylogenetic Systematics. Wiley-Interscience, New York.

## Fourth International Immunoparasitology Symposium

The Fourth International Immunoparasitology Symposium will be held 29–31 July 1987 in Lincoln, Nebraska. This will be the fourth in a series of symposia designed to present new and novel techniques and approaches in immunoparasitological research. Among the topics covered will be immunodiagnosis, antigen isolation and purification, parasite-induced modulation of host immune systems, basic host–parasite immune interactions, vaccine development, and related topics. The three-day symposium will be comprised of a series of speakers as well as a poster session. Although the speakers have been selected, individuals interested in presenting posters are encouraged to contact the symposium chair.

Because this meeting will be held just prior to the annual meeting of the American Society of Parasitologists, also in Lincoln, Nebraska, it affords the opportunity of attending two meetings at the same location with no additional travel costs.

For additional information and registration forms, contact:

Dr. Gary L. Zimmerman
Chair, Fourth International Immunoparasitology Symposium
College of Veterinary Medicine
Oregon State University
Corvallis, Oregon 97331
Telephone (503) 754-2927

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 91–95

# *Microcotyle hiatulae* Goto, 1900 (Monogenea), a Senior Synonym of *M. furcata* Linton, 1940, with a Redescription and Comments on Postlarval Development

DENNIS A. THONEY AND THOMAS A. MUNROE
Virginia Institute of Marine Science, School of Marine Science,
The College of William and Mary, Gloucester Point, Virginia 23062

ABSTRACT: *Microcotyle hiatulae* Goto, 1900, is redescribed on the basis of new material, representing over 150 specimens collected throughout the entire known range of the parasite. No appreciable geographic variation was observed in the characters studied. *Microcotyle furcata* Linton, 1940, was found to be a junior subjective synonym of *M. hiatulae*. Postlarval development of *M. hiatulae* is described for the first time (based on 43 specimens). Postlarval development consists mostly of the acquisition of clamps, elongation of the body, and development of genitalia and gut. Clamp number is highly correlated ($r^2 = 0.97$) with length, and can be used as an indicator of growth. Developmental stages are compared when possible with postlarval stages reported for other *Microcotyle* species.

KEY WORDS: taxonomy, *Tautoga*, Atlantic, parasitology, fish.

Goto (1900) described *Microcotyle hiatulae* from the gills of the labrid *Tautoga onitis* (Linnaeus) (tautog) collected near Newport, Rhode Island. Forty years later Linton (1940) described a second species of *Microcotyle*, *M. furcata*, from the same host species collected near Woods Hole, Massachusetts. The same host species and similarities in descriptions suggested that these two species of *Microcotyle* might be identical. Because Linton did not mention *M. hiatulae* in his discussion of *M. furcata*, it is likely he was unaware of Goto's earlier published description of *M. hiatulae*.

Our examinations of *Microcotyle* specimens taken from *T. onitis* at Sakonnet Point, Rhode Island, Woods Hole, Massachusetts, and several locations at the mouth of Chesapeake Bay indicate that worms collected from all three locations are indistinguishable morphologically from *M. hiatulae* Goto. Comparison of both meristic and morphometric characters of the holotype of *M. furcata* further reveals that this specimen also belongs to *M. hiatulae*. It is apparent from these observations that a single species of *Microcotyle*, *M. hiatulae* Goto, parasitizes the gills of the tautog, and that *Microcotyle furcata* Linton, 1940, should therefore be considered a junior subjective synonym of *M. hiatulae* Goto, 1900.

The purpose of this paper is to provide a redescription of *M. hiatulae* based on all available material. The absence of data on intraspecific variation and some important taxonomic characters in the original description, and the lack of detail in the original figures, prompted this redescription and refiguring. Holotype and additional material were examined to resolve this problem. Additionally, the collection of a wide size range of *M. hiatulae* permitted a description of postlarval development. This description is particularly valuable, because postlarval development has been studied little in the Monogenea, but has been studied and reviewed (Thoney, 1986) in the genus *Microcotyle*.

## Methods and Materials

Fish were collected by angling and fish traps from several nearshore habitats at the mouth of Chesapeake Bay, Virginia (36°59′N, 76°09′W), in July 1982 and from coastal waters of Sakonnet Point, Rhode Island (41°31′N, 71°15′W) in July 1983. Gills were removed from fishes, then fixed and stored in 10% neutral buffered formalin. Monogeneans removed from gills were stained with Semichon's acetocarmine or VanCleave's hematoxylin and mounted on slides in Piccolyte. Measurements were made with a calibrated ocular reticle, and drawings were made with the aid of a camera lucida.

Redescription of *M. hiatulae* is based on counts and measurements of 44 mature individuals from samples representing the known parasite range (Table 1). An additional 63 mature individuals collected during this study from Virginia and Rhode Island were also examined for specific characters. The holotype of *M. hiatulae* could not be located at the Meguro Parasitological Museum, Tokyo, Japan (pers. comm., S. Kamegai), the University of Tokyo (pers. comm., I. Tomoda), or the USNM Helminthological Collection (pers. comm., J. R. Lichtenfels). Values for the holotype of *M. hiatulae* appearing in Table 1 are taken from Goto's (1900) original description. Comparative material collected by Cooper and MacCallum as well as the holotype of *M. furcata* from Newport, Rhode Island, were borrowed from the USNM Helminthological Collection.

**Table 1. Meristics and morphometrics of *Microcotyle* collected from *Tautoga onitis* (mean with range in parentheses, in mm).**

| | *Microcotyle furcata* holotype USNM 8164 | *Microcotyle hiatulae* USNM 51711 | *Microcotyle hiatulae* USNM 36481 USNM 36484 | Rhode Island *M. hiatulae* | Virginia *M. hiatulae* | *Microcotyle hiatulae* Goto, 1900 holotype* |
|---|---|---|---|---|---|---|
| Total length | 3.488 | 2.70 (2.38–2.88) | 4.51 (3.84–5.12) | 3.24 (2.10–4.38) | 4.41 (3.55–5.05) | 3.5 |
| Atrium % from anterior | 9.7% | 9.2% (7.4–10.8%) | 7.7% (6.6–8.8%) | 10.2% (7.6–12.4% | 9.9% (7.3–11.6%) | |
| Vagina % from anterior | — | — | — | 24.1% (18.3–29.4%) | 20.7% (19.1–24.5%) | |
| Ovary % from anterior | 41.3% | 45.5% (35.9–51.3%) | 43.9% (34.6–50.0%) | 40.4% (36.2–45.9%) | 46.4% (40.9–55.9%) | |
| % of total of testes | 22.9% | 20.6% (18.0–26.0%) | 17.5% (14.0–19.7%) | 20.5% (14.6–25.0%) | 19.5% (15.8–22.6%) | |
| No. testes | 24 | 17 (8–22) | 16 (11–20) | 16 (13–20) | 17 (8–24) | 15 |
| % body of opisthaptor | 33.8% | 34.4% (31.1–38.2%) | 40.1% (29.4–48.1%) | 31.8% (27.0–37.8%) | 29.4% (23.9–33.3%) | |
| No. clamps | 48 | 44 (32–50) | 47 (36–53) | 44 (36–52) | 48 (38–56) | 46 |
| Width at ovary | 0.600 | 0.62 (0.55–0.68) | 0.51 (0.51–0.65) | 0.65 (0.39–0.85) | 1.00 (0.84–1.18) | |
| Prohaptor sucker diameter | 0.060 | 0.070 (0.057–0.072) | 0.065 (0.060–0.075) | 0.063 (0.044–0.076) | 0.091 (0.076–0.112) | |
| Pharynx diameter | 0.065 | 0.052 (0.047–0.057) | 0.056 (0.045–0.065) | 0.047 (0.036–0.056) | 0.073 (0.060–0.084) | |
| Genital atrium length | 0.14 | 0.110 (0.094–0.120) | 0.152 (0.125–0.195) | 0.094 (0.060–0.140) | 0.167 (0.122–0.244) | |
| Genital atrium width | 0.13 | 0.135 (0.104–0.192) | 0.145 (0.103–0.165) | 0.110 (0.056–0.148) | 0.195 (0.164–0.224) | |
| Largest clamp width | 0.085 | 0.090 (0.083–0.094) | 0.083 (0.075–0.090) | 0.083 (0.068–0.096) | 0.110 (0.100–0.120) | |
| Post clamp width | 0.060 | 0.060 (0.057–0.065) | 0.054 (0.050–0.055) | 0.057 (0.036–0.068) | 0.069 (0.062–0.076) | |
| Egg (length or length × width | — | 0.139 (0.122–0.156) | 0.200 | | 0.253 × 0.091 (0.148–0.308 × 0.052–0.116) | |
| *N* = | 1 | 8 | 6 | 15 | 15 | |
| Location | Woods Hole, Mass. | unknown | NY Aquarium | Rhode Island | Virginia | |
| Collector | Linton | Cooper | MacCallum | This study | This study | Goto |

* Measurements from Goto (1900).

Description of postlarval development is based on 43 specimens removed from gills of tautog collected from both Rhode Island and Virginia.

## *Microcotyle hiatulae* Goto, 1900
### (Figs. 1–5; Table 1)

*Microcotyle hiatulae* (see MacCallum and MacCallum, 1913) listed.

*Microcotyle hiatulae* (see Meserve, 1938) listed.

*Microcotyle furcata* Linton, 1940, original description.

REDESCRIPTION (measurements in mm): Body bilaterally symmetrical, lanceolate, 2.10–5.10 long by 0.39–1.18 wide at level of ovary, tapering anteriorly to a blunt point. Cuticle thin, lacking spines or scales. Opisthaptor symmetrical, bearing 32–56 clamps in 2 bilateral rows; 23.9–48.1% of total body length; opisthaptor extends a short distance anteriorly on ventral side of body. Number of clamps on one side may exceed other by 1 or 2 in adults. Clamp structure typical of the Microcotylidae (Yamaguti, 1963); clamps generally increase in size anteriorly, posterior clamp 0.036–0.076, largest clamp 0.068–0.120 usually 14 or 15 clamp-pairs from posterior. Hooks and hamuli absent in adults. Prohaptor consisting of 2 biloculate suckers 0.044–0.112 in width, located ventrolaterally in buccal funnel. Mouth subterminal; prepharynx short; pharynx spherical 0.036–0.084 in diameter. Highly ramified intestine bifurcates anterior to genital atrium, branches rejoin anterior to opisthaptor into which the intestine extends. Ovary pretesticular, dorsal to vitelline reservoir, variable in shape; 34–56% of the body from anterior end; elongate, convoluted portion of the ovary containing developing ova extends anteriorly dorsal to uterus. Ovary narrows to form oviduct, which is joined first by short duct from seminal receptacle; seminal receptacle just anterior to proximal portion of ovary. Ootype surrounded by Mehlis' gland and emerges as uterus that extends anteriorly ventral to vas deferens and dorsal to vitelline ducts to genital atrium. Genital–intestinal canal proceeds from right crus and appears to join with vitelline duct medially. Genital atrium 0.060–0.244 long by 0.056–0.224 wide, size depends on state of contraction, armed with curved spines, located 6–12% of body from anterior end; atrial spines 0.007–0.018 long. Vitellaria follicular, extending in bilateral bands from level of intestinal bifurcation posteriorly to opisthaptor, uniting posterior to testes. Y-shaped vitelline ducts originate from each side behind genital atrium and unite posteriorly into a common medial duct or reservoir ventral to ovary. Unarmed vaginal pore located medially on dorsal side 18.3–29.4% of body from anterior end. Testes ovoid, variable in size, 8–24 in number, occupying 14–26% of body length behind ovary, anterior to opisthaptor, intercecal. Vas deferens sinuous, extending anteriorly dorsal to uterus and exiting via genital atrium. Eggs 0.122–0.308 long by 0.052–0.116 wide, with short stout posterior filament and extremely long, twisted anterior filament 10–30 times the length of egg; uterus containing up to 12 eggs.

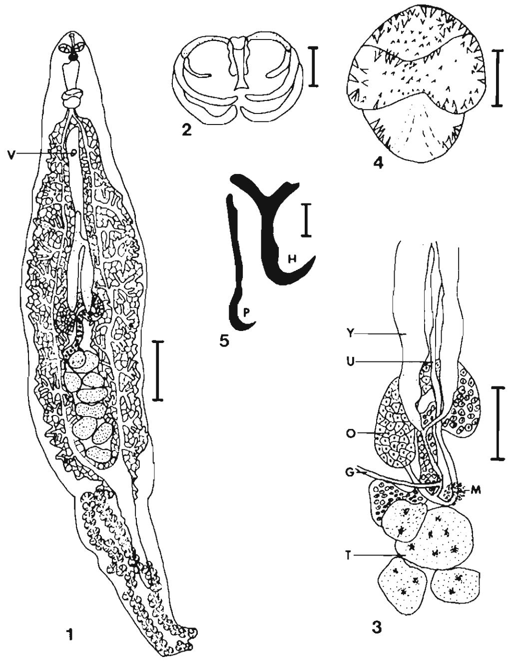
Type host: *Tautoga onitis* (Linnaeus), tautog.

Host range: Atlantic coast of North America from Nova Scotia to South Carolina.

Site: Gill filaments.

Known parasite range: Woods Hole, Massachusetts, to Chesapeake Bay, Virginia.

Type locality: Newport, Rhode Island.




**Figures 1–5. Camera lucida drawings of *Microcotyle hiatulae*. 1. Ventral view of whole worm; bar = 0.5 mm. 2. Ventral view of clamp; bar = 0.03 mm. 3. Ventral view of genitalia; bar = 0.25 mm. 4. Genital atrium; bar = 0.06 mm. 5. Hamulus (H) and posterior hook (P) taken from postlarva with two pairs of clamps; bar = 0.01 mm. G, genital–intestinal canal; M, Mehlis' gland; O, ovary; T, testis; U, uterus; V, vagina; Y, yolk (vitelline) ducts.**

Specimens deposited (15 adults, 15 juveniles): USNM Helm. Coll. Nos. 79409, 79410, 79411.

### Postlarval Development

Postlarval development consists mostly of the acquisition of clamps, elongation of the body, development of genitalia, and expansion of the gut. Clamps are acquired in succession, with new ones developing anterior to existing ones, as in other members of the order Mazocraeidea (Frankland, 1955; Bychowsky, 1957; Llewellyn, 1963; Thoney, 1986).

The most posterior pair of clamps in juveniles ranged from 0.052 to 0.068 mm in width, which is within the size range found in adults. Hence, the posterior clamps of *M. hiatulae* do not increase in size after development. This is similar to the pattern observed by Thoney (1986) in *M. sebastis*.

The number of clamps is highly correlated with length, and can be used as an indicator of growth.

Thoney (1986) successfully used this method to predict growth in *M. sebastis.* Clamp number is related to length by the equation $Y = 0.318 + 0.028X$, where $Y$ is the natural logarithm of (length [mm] + 1) and $X$ is the number of clamps ($r^2 = 0.97$).

The larval opisthaptor (languette) of *M. hiatulae* bears one pair each of posterior hooks, hamuli, and posterolateral hooks. Early stages with one to four pairs of clamps did not possess any of the four pairs of lateral hooks typically present in the oncomiracidia of *Microcotyle* species. This further suggests that the first four pairs of clamps do not replace the lateral hooks in sequence in *M. hiatulae,* but that they merely occupy similar positions, as was suggested by Thoney (1986) for *M. sebastis.* This differs from the pattern reported for other polyopisthocotylids studied by Frankland (1955), Bychowsky (1957), and Llewellyn (1963), where the first four pairs of clamps replace the lateral hooks.

Within the genus *Microcotyle,* a flexible joint occurs between the blade and handle of the posterior hooks in oncomiracidia, which later thickens and fuses during postlarval development (Thoney, 1986). Posterior hooks of postlarval *M. hiatulae* are also solid without joints (Fig. 5). Their joints probably fused during early postlarval development. The larval opisthaptor is lost between the four- and 26-clamp stages (Thoney, 1986) in all described species of *Microcotyle,* including *M. hiatulae,* which loses its larval opisthaptor following the acquisition of 10 clamps.

The adult form of the prohaptor, which consists of two biloculate suckers, is present in juveniles of *M. hiatulae* with only two clamps. Juveniles of *M. gotoi* and *M. chrysophrii* with two clamps also possess two buccal suckers (Bychowsky, 1957; Euzet, 1958, respectively), as do the four-clamp juveniles of *M. mormyri* and *M. sebastis* reported by Ktari (1971) and Thoney (1986), respectively.

Early in postlarval development, the gut bifurcates as the genitalia initiate development. With further development, the caeca become highly ramified in the lateral areas. Testes development was apparent between the acquisition of 18 and 20 clamps, and continued through the 24–28-clamp stages, when spermatozoa became evident. The genital atrium first formed as a shallow depression between the 20- and 22-clamp stages; minute spines appeared soon after, and full development occurred by the 30-clamp stage. The ovary, vitellaria, and associated ducts began development between the 24- and 28-clamp stages. Maturation, as indicated by presence of eggs, occurred between the 30- and 40-clamp stages. *Microcotyle hiatulae* is protandrous, as are other Monogenea (see Llewellyn, 1963; Thoney, 1986). The different components of the genitalia described above develop in the same sequence as those reported by Ktari (1971) for *M. mormyri* and by Thoney (1986) for *M. sebastis.*

## Discussion

Our examinations of specimens of *Microcotyle* recovered from the gills of the labrid *Tautoga onitis* collected from throughout most of the known host range, including the type locality of *M. hiatulae* in Rhode Island, the type locality of Linton's *M. furcata* at Woods Hole, Massachusetts, and from several locations in Chesapeake Bay, Virginia, indicate that only a single species of *Microcotyle* infects the gills of this host. Both meristic and morphometric characters of Linton's holotype of *M. furcata* are within the range for *M. hiatulae. Microcotyle hiatulae* Goto, 1900, has priority, and *M. furcata* Linton, 1940, is regarded as a junior subjective synonym.

Eighteen other species of *Microcotyle* have previously been described from 15 host species that live sympatrically with *T. onitis* along the Atlantic coast of the United States. Attempts to compare *M. hiatulae* to these species were difficult. Examination of published descriptions reveals that many of the 18 species are morphologically similar. In fact, it is extremely difficult, if possible at all, to identify an individual worm to species without knowing the host species.

Considerable intraspecific variation has been observed by Thoney (1986, unpubl. data) in *M. sebastis, M. pomatomi* (unpubl. data), and *M. hiatulae* (this study). This variation is especially evident in the number of testes and clamps, and in shapes of the ovary and vitelline ducts. These characters also vary ontogenetically. Furthermore, the effects that host size may have on worm size and development are unknown; however, in *M. sebastis,* host size strongly influences worm size (Thoney, 1986). In addition, artifacts from fixation are seen in shape and orientation of the body, opisthaptor, genitalia, genital atrium, and most other soft structures. Unfortunately, these same labile characters are the very ones that have previously been employed to distinguish species within *Microcotyle.* These limitations, coupled with the fact that most *Microcotyle* species found on hosts along the Atlantic seaboard have been described from only one or a few specimens, limit any meaningful comparisons among the various species.

It is very likely that further synonymization within this group of species will be necessary. However, resolution based entirely on morphological comparisons may not be possible for the reasons outlined above. Biochemical techniques or experiments involving cross-host-infestation(s) may be the only sure way to clarify species determinations in *Microcotyle.*

### Acknowledgments

Dr. J. Ralph Lichtenfels of the USNM Helminthological Collection provided the holotype and other specimens for comparison with our study material. Drs. Eugene M. Burreson and William J. Hargis, Jr. of the Virginia Institute of Marine Science provided critical reviews of an earlier draft of this manuscript. Finally, we extend our appreciation to L. Donna Munroe, who generously provided financial support from the family budget to purchase fish, bankroll local fishing trips, and provided travel funds to support a collecting trip to Rhode Island. VIMS Contribution No. 1340.

### Literature Cited

**Bychowsky, B. E.** 1957. Monogenetic trematodes, their systematics and phylogeny. Akademiya Nauk U.S.S.R. (English translation by A.I.B.S., Washington, D.C. William J. Hargis, ed. 1961. Virginia Institute of Marine Science Translation Series Number 1. 509 pp.)

**Euzet, L.** 1958. Sur le développement post-larvaire des Microcotylidae (Monogenoidea, Polyopisthocotylea). Bulletin de la Société Neuchateloise des Sciences Naturelles 81:79–84.

**Frankland, H. M. T.** 1955. The life history and bionomics of *Diclidophora denticulata* (Trematoda: Monogenea). Parasitology 45:313–351.

**Goto, S.** 1900. Notes on some exotic species of ectoparasitic trematodes. Journal College of Science Imperial University of Tokyo 12:263–295.

**Ktari, M. H.** 1971. Recherches sur la reproduction et le développement de poissons marins. Ph.D. Thesis, Academie de Montpellier, Université des Sciences et Techniques du Languedoc. 327 pp.

**Linton, E.** 1940. Trematodes from fishes, mainly from the Woods Hole region, Massachusetts. Proceedings of the United States Natural History Museum 88:1–172.

**Llewellyn, J.** 1963. Larvae and larval development of monogeneans. Advances in Parasitology 1:287–306.

**MacCallum, G. A., and W. G. MacCallum.** 1913. Four species of *Microcotyle, M. pyragaphorus, macroura, euides,* and *acanthophallus.* Zoologische Jahrbuecher Abteilung fuer Systematik Oekologie und Geographie de Tiere, East Germany 34:223–244.

**Meserve, F. G.** 1938. Some monogenetic trematodes from the Galapagos Islands and the neighboring Pacific. Reports Obtained in the Allan Hancock Pacific Expeditions (1932–1937) 2:31–89.

**Thoney, D. A.** 1986. Post-larval growth of *Microcotyle sebastis* (Platyhelminthes: Monogenea), a gill parasite of the black rockfish. Transactions of the American Microscopical Society 105:170–181.

**Yamaguti, S.** 1963. Systema Helminthum. Vol. IV. Monogenea and Aspidocotylea. Intersciences Division, John Wiley and Sons, Inc., New York. 699 pp.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 96–104

# Morphology and Development of the Adult and Cotylocidium of *Multicalyx cristata* (Aspidocotylea), a Gall Bladder Parasite of Elasmobranchs

DENNIS A. THONEY AND EUGENE M. BURRESON
Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062

ABSTRACT: Scanning electron microscopy of mature worms revealed an unarmed buccal funnel, an unarmed conical cirrus, a single row of up to 1,072 ventral alveoli delineated by transverse and lateral ridges, marginal organs at each junction of the ridges, and a terminal papilla. Marginal organs are glandular and may function as accessory suckers. Growth after maturity consists of addition of alveoli at the posterior end of the worm, resulting in a gradual anterior shift in position of the testis from its original terminal location at maturity. Cotylocidia, which lacked cilia and ocelli, were fully developed at egg deposition, but could not be induced to hatch.

KEY WORDS: SEM, *Rhinoptera, Myliobatis,* bullnose rays, histology, larvae, eastern USA.

*Multicalyx cristata* Faust and Tang, 1936 (order Stichocotylida), was originally described from a single preserved specimen collected from the spiral valve of the cownose ray, *Rhinoptera bonasus* (Mitchill). The lack of live material affected Faust and Tang's interpretation of the holdfast morphology. They stated that the holdfast consisted of a short anterior sucking disc divided into eight sucking cups and a long posterior acetabular complex with elevated luglike crests provided with transverse ridges. Both Manter (1954) and Bray (1984), who studied additional material, indicated that the luglike crests were temporary elevations of the muscular holdfast. Dollfus (1958a) and Stunkard (1962) redescribed *M. cristata* and also elaborated on the structure of the holdfast.

Faust and Tang (1936) and Stunkard (1962) described the internal anatomy of *M. cristata,* but did not include the marginal organs (marginal bodies) located laterally on the transverse ridges of the holdfast. Hendrix and Overstreet (1977) briefly mentioned these organs, but did not comment on their function. In an abstract, Burt (1968) briefly described the marginal organs of the closely related *Taeniocotyle elegans* (Olsson, 1869) Stunkard, 1962, but did not mention whether the organs were primarily sensory or glandular. In Rohde's (1972) review of the Aspidocotylea, he mentioned that some authors consider these organs glandular, whereas others consider them sensory in function. Rohde concluded that the marginal organs in *Multicotyle purvisi* Dawes, 1941, were primarily glandular.

Larval development of aspidocotyleans has been studied in only a few species (for review see Rohde, 1972). Larval development and morphology of *M. cristata* have not been examined, although Brinkmann (1957) briefly described larvae of *T. elegans.* In this study, light and scanning electron microscopy are used to examine the adult and larval morphology of *M. cristata.* Growth of *M. cristata* is also discussed.

## Methods and Materials

Specimens of *Multicalyx cristata* were collected from gall bladders of bullnose rays, *Myliobatis freminvillei* Lesueur, obtained from continental shelf waters of the eastern USA from Cape Fear, North Carolina, to Long Island, New York, while the authors participated in the National Marine Fisheries Service Ground Fish Surveys (1982–1984) (Thoney and Burreson, 1986). Worms were relaxed in a saturated chlorobutanol-seawater mixture (Hargis, 1953) and fixed in 10% formalin and seawater or placed in elasmobranch physiological saline (as formulated by Bapkin et al., 1933), which was then chilled on ice for transport back to the Virginia Institute of Marine Science (VIMS). Worms placed in saline remained alive for at least 2 wk with daily saline changes. Formalin-fixed specimens and eggs to be used in scanning electron microscopy (SEM) were washed for 24 hr in water, postfixed for 2 hr in 1% osmium tetroxide in 0.1 M sodium cacodylate and 0.19 M NaCl, dehydrated in ethanol and acetone, then critical-point dried. Specimens were coated with gold-palladium by vacuum evaporation. Eggs were sticky with mucus and difficult to clean. Paraffin sections (6 μm) of *M. cristata* were stained with the Alcian blue–PAS method for mucosubstances (Luna, 1968) or with hematoxylin and eosin. Nipp's solution (Corrington, 1941) was used to detect the presence of hematin in gut contents of worms. Specimens used in temporary whole mounts were cleared in glycerin. Cleared eggs and larvae were examined using brightfield and Nomarski microscopy. To follow embryonic development, eggs obtained from live specimens were placed in watch glasses with filtered seawater and maintained at 23°C. Water was changed daily. Specimens of *M. cristata* examined by Manter (1931) and Hendrix and Overstreet (1977) were also obtained for comparison from the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska and the USNM Helminthological Collection, USDA, Agricultural Research Service, Beltsville, Maryland, respectively. Thirty-five-millimeter slides of *M. cristata* from *R. bonasus* were obtained from Ronald A. Campbell, Southeastern Massachusetts University, North Dartmouth, Massachusetts.

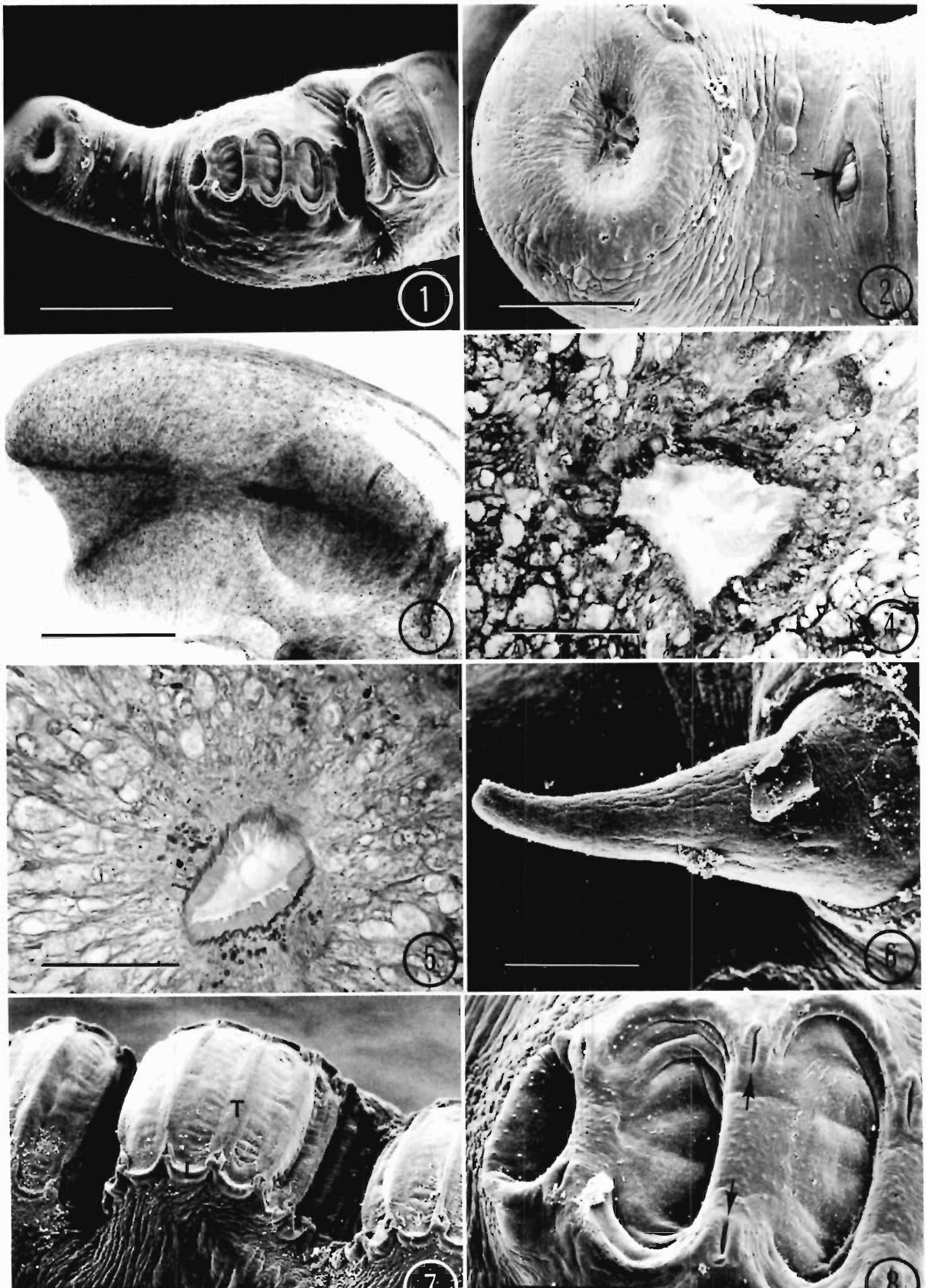
Three specimens of *M. cristata* have been deposited at the USNM Helminthological Collection, Beltsville, Maryland (Nos. 79412, 79413).

## Results and Discussion

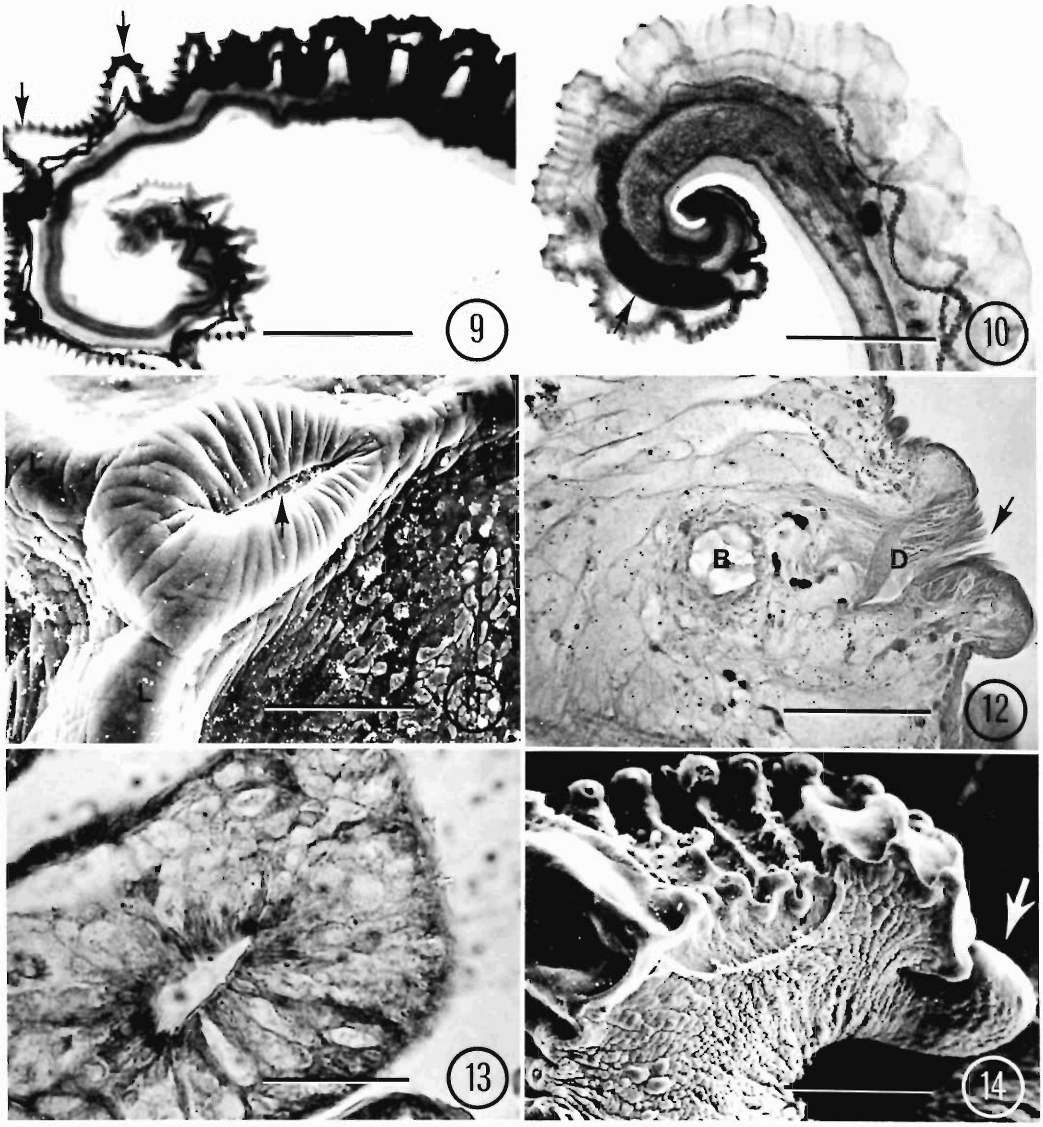
The elongate muscularized body of *M. cristata* was strongly coiled in its natural state within gall bladders of elasmobranchs. The mouth was located terminally within a simple, unarmed buccal funnel at the anterior end (Figs. 1–3). The mouth funnel (Halton, 1972) of *Aspidogaster conchicola* Baer, 1827, is similarly structured, except an infolding of the tegument delimits the lips. Even though the mouth was unarmed, it evidently erodes the gall bladder wall sufficiently to allow feeding on blood, as suggested by the presence of hematin in the cecal contents. *Fellodistomum fellis* (Olsson, 1868) Nicoll, 1909, a gall bladder-inhabiting digenean of *Anarhichas lupus* Linnaeus, also feeds on host blood (Halton, 1982). Evidence of glands could not be seen externally (Fig. 1), but gland cells were scattered in the tissue surrounding the prepharynx and to a lesser extent surrounding the pharynx (Figs. 4, 5, respectively). These unicellular glands probably help with external digestion of host tissue. Anticoagulants, proteases with elastolytic and keratotylic properties, and collagenases have been demonstrated in several digeneans and nematodes (Barrett, 1981). Unicellular glands similar in structure to those of the tegument were also found surrounding the lumen of the prepharynx of *A. conchicola* by Halton (1972). Halton stated that the location of these cells suggests a feeding function. The gland cells of *M. cristata* stained light blue at pH 2.5, suggesting the presence of acidic mucosubstances (Luna, 1968) that may have one or more of these previously mentioned properties. Halton and Hendrix (1978) also found that the tegument and gastrodermis of *Lobatostoma ringens* (Linton, 1907) Eckmann, 1932, were reactive for mucopolysaccharides. *Multicalyx cristata* most likely feeds on abraded epithelial tissue, as does the digenean *Fasciola hepatica* Linnaeus, 1758 (see Dawes, 1963). The bile of infected elasmobranchs was dark in color and gelatinous with small granular inclusions. This appearance may have been the result of blood leaking into the bile from abraded areas of the gall bladder or from hematin discharged by *M. cristata* after digestion, as has been described in the bile-inhabiting *F. hepatica* by Boray (1969) and Ross et al. (1966, 1967). Infiltration of blood cells and probable leakage of bile into surrounding tissue gave the liver a dark gray appearance. Healthy livers are red-brown in color. Aspidocotyleans have not previously been reported to initiate pathological responses in vertebrate hosts.

An unarmed, cone-shaped cirrus was located posterior to the mouth, just anterior to the alveoli (Fig. 6), and was usually inverted in live worms (Fig. 2). Other aspidocotyleans have a similarly located cirrus.

The holdfast consisted of a single row of longitudinally arranged alveoli that were delineated by transverse and lateral ridges (Fig. 7). The anterior eight to 10 alveoli were usually smaller, more rounded, and deeper than those more posterior (Figs. 1, 8; cf. Fig. 7), and resembled those of juvenile specimens collected from teleosts by Manter (1931) and Hendrix and Overstreet (1977). These alveoli are probably retained from the juvenile condition. They were continuous with more posterior alveoli, hence they do not constitute a separate holdfast organ as described by Faust and Tang (1936) and Parukhin and Tkachuk (1980). Bray (1984) also noted that the anterior alveoli do not constitute a separate holdfast, but in his specimens the anterior alveoli were larger than those more posterior. Live worms held in dishes of elasmobranch saline passed waves down the highly muscularized holdfast in an undulating motion that was independent from the dorsal portion of the body, and resulted in various states of folding after fixation (Fig. 9). Hence, the irregular crests with transverse ridges as described by Faust and Tang (1936) are indeed temporary folds, as suggested by Manter (1954) and Bray (1984). Live worms were extremely distensible and could stretch up to 200 mm or more. Worms relaxed prior to fixation ranged from 72 to 129 mm in length, and number of alveoli ranged from 551 to 1,072. The number of alveoli is similar to those reported for *M. cristata* by Faust and Tang (1936) and Manter (1954).




**Figures 1–8. Morphology of *Multicalyx cristata*. 1. Scanning electron micrograph of the ventral side of the anterior end. Bar = 0.57 mm. 2. Scanning electron micrograph of the anterior end, showing mouth and inverted**




**Figures 9–14. Morphology of *Multicalyx cristata*. 9. Glycerin-cleared posterior end, showing relationship of alveoli (arrows) to the dorsal portion of the worm. Bar = 3.0 mm. 10. Posterior end of worm from *Rhinoptera bonasus*, showing position of testis (arrow) at maturity. Bar = 1.50 mm. Photo by R. A. Campbell. 11. Scanning electron micrograph of a marginal organ, showing lateral ridge (L), transverse ridge (T), and pore (arrow). Bar = 0.030 mm. 12. Longitudinal section of marginal organ, showing lumen at base (B), duct (D), and pore (arrow). Bar = 0.023 mm. 13. Cross section of marginal organ, showing mucous cells. Bar = 0.014 mm. 14. Scanning electron micrograph of posterior end, showing posterior terminus and position of excretory papilla (arrow). Bar = 0.030 mm.**

---

←

**cirrus (arrow). Bar = 0.160 mm. 3. Lateral view of anterior end, showing buccal funnel and pharynx. Bar = 0.140 mm. 3. Transverse section through prepharynx, showing mucous cells and mucus within lumen. Bar = 0.074 mm. 5. Transverse section through pharynx, showing mucous cells. Bar = 0.074 mm. 6. Scanning electron micrograph of everted cirrus. Bar = 0.095 mm. 7. Scanning electron micrograph of alveoli from midsection, showing lateral (L) and transverse (T) ridges. Bar = 0.430 mm. 8. Scanning electron micrograph of anterior alveoli and marginal organs (arrows). Bar = 0.140 mm.**

Bray (1984) found over 1,500 alveoli in a specimen from a scalloped hammerhead, *Sphyrna lewini* (Griffith and Smith).

The gradual decrease in size of alveoli near the posterior end suggests that alveoli develop terminally. In juvenile worms, alveoli apparently develop just anterior to the posteriorly located rudimentary testis, by apposition and stretching, because at maturity the testis is located at the posterior extremity (Fig. 10). Further growth involves the addition of alveoli posterior to the testis, as reported by Stunkard (1962), and overall increase in girth. Hence, after maturation, the genital organs appear to shift forward gradually with increased growth, and in large specimens are located in the anterior half of the body. Comparison of several specimens indicates that the number of alveoli anterior to the testis is highly variable (197–268 alveoli). In addition, growth of worms is apparently dependent on host size. George W. Benz (pers. comm., University of British Columbia, Vancouver, British Columbia, Canada) has found specimens up to 600 mm long in *S. lewini*; these are much larger than any of our specimens from bullnose rays. Infection experiments would allow a more detailed study of maturation and growth.

A marginal organ was located at each junction of the lateral and transverse ridges of each alveolus (Fig. 11). Transverse sections through this organ revealed a bulbous structure with a lumen at the base and a duct that extended ventrally (Fig. 12), opening externally through the center of the marginal organ (Fig. 11). The raised lip of the marginal organ and the area surrounding the duct were strongly muscularized. Gland cells surrounding the base stained magenta at pH 2.5, suggesting the presence of neutral mucosubstances or hexoses and deoxyhexoses with vicinal groups (Luna, 1968) (Fig. 13). Apparently mucus is secreted into the lumen, and exits via the duct and pore. Thus, the marginal organs of *M. cristata* are glandular, as was found in *Multicotyle purvisi* by Rohde (1972). Burt (1968) also found the marginal organs of *T. elegans* to be surrounded by unicellular glands. No longitudinal or transverse ducts were present connecting adjacent marginal organs as was described in *M. purvisi* by Rohde (1972). The musculature of the marginal organ surrounding the duct may function in regulating mucus flow as suggested by Rohde (1972), but may have another function. The marginal organs in *M. cristata* are sucker-like and can be protruded actively. Their position at the lateral corners of the alveoli, their structure, and their ability to secrete mucus would permit them to act as accessory suckers; however, it is difficult to determine biological roles of structures without observing the animals in situ.

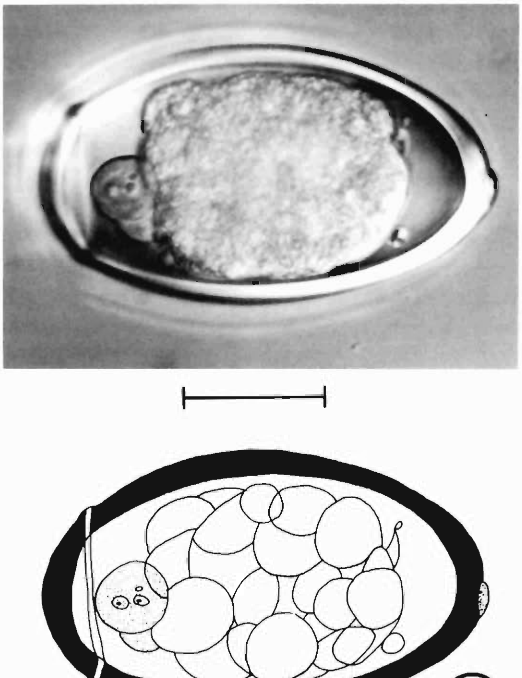
The excretory system exits through a papilla (Fig. 14) located at the posterior terminus dorsal to the most posterior alveoli. Excretory pores of other described species are located similarly.

The operculated ectolecithal eggs were oval and similar to other aspidocotylean eggs reviewed by Rohde (1972), except for the presence of a small knob at the aboperculate end (Figs. 15–18). Internal dimensions of eggs measured 0.095–0.103 mm in length and 0.055–0.063 mm in width. Egg size is slightly smaller than those described by Faust and Tang (1936), Manter (1954), Dollfus (1958a), Stunkard (1962), and Hendrix and Overstreet (1977).

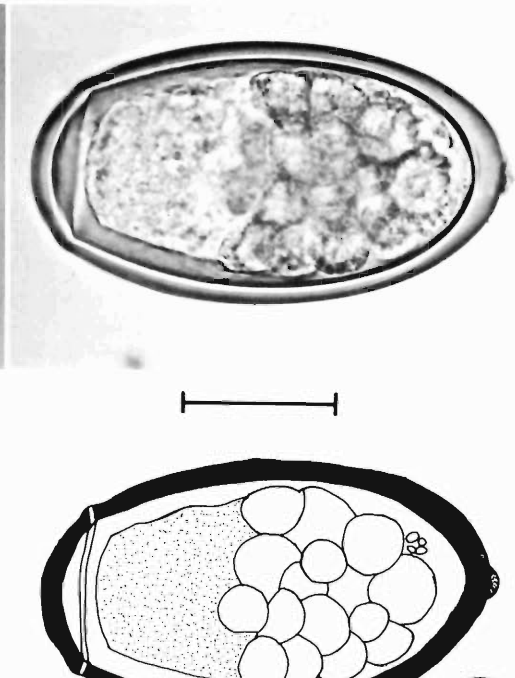
The ovary of *M. cristata* was located approximately halfway between the anterior end of the worm and the testis. The uterus extended posteriorly from the ovary to the testis, where it turned and extended anteriorly in a highly coiled manner to the genital pore. Eggs collected at the proximal end of the uterus had lightly tanned egg capsules and embryos consisting of two or three cells (Fig. 15). Vitelline cells filled the remainder of the egg. Eggs collected at the level of the testis were well tanned, and the embryos consisted of an ovoid mass of cells at the opercular end of the egg (Fig. 16). Reduced members of vitelline cells filled the remainder of the egg. The embryo of *M. cristata* develops at the opercular end of the egg (Figs. 15, 16), as has been described in the closely related *Taeniocotyle elegans* by Brinkmann (1957) and in *Cotylogaster michaelis*

→

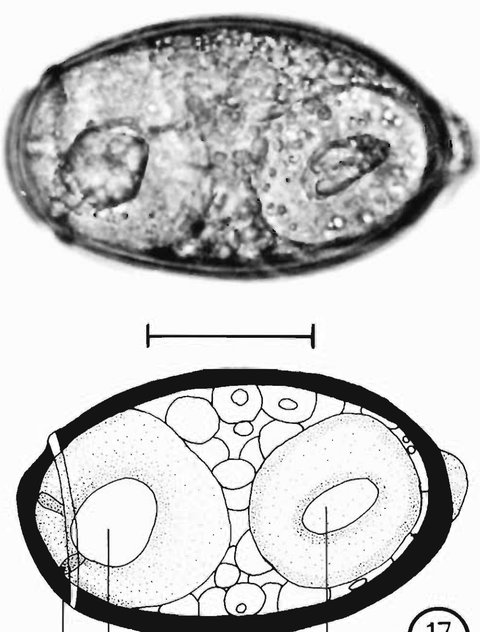
**Figures 15–18. Matching line drawings and micrographs of eggs of *M. cristata*. Embryos are stippled; vitelline cells are clear circles. CG, cephalic glands; M, mouth; PVS, posterior ventral sucker. Bar = 0.050 mm. 15. Two-cell stage. 16. Undifferentiated multicellular stage filling half of egg. 17. Embryo with mouth and posterior ventral sucker. 18. Fully developed, folded cotylocidium.**



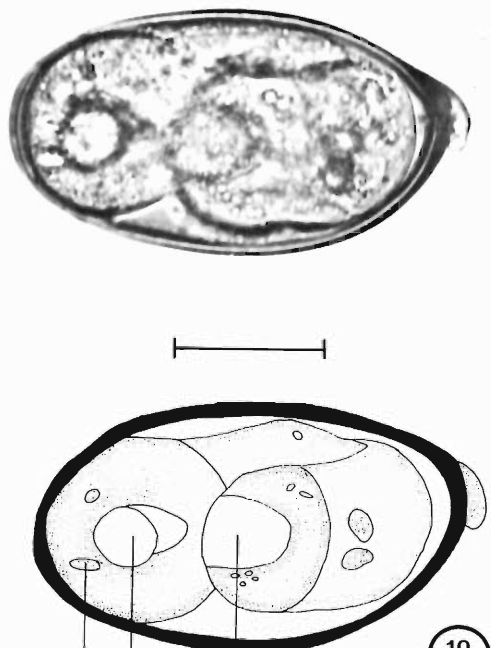
15



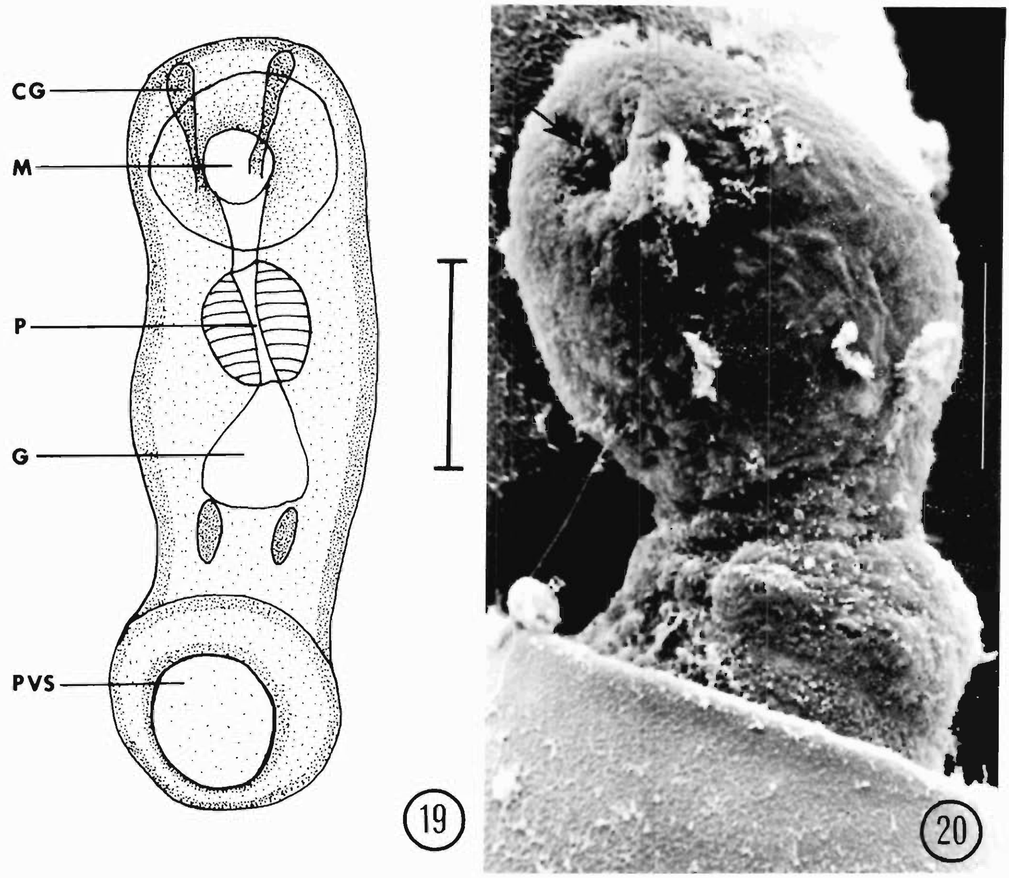
16




17




18




**Figures 19, 20. Cotylocidium of *M. cristata*. 19. Composite line drawing based on preserved specimens. CG, cephalic glands; G, gut; M, mouth; P, pharynx; PVS, posterior ventral sucker. Bar = 0.050 mm. 20. Scanning electron micrograph of cotylocidium emerging from egg. Arrow = mouth. Bar = 0.017 mm.**

Monticelli, 1892 by Dollfus (1958b). In contrast, *Cotylaspis insignis* Leidy, 1857, and *M. purvisi* develop in the center of the egg, surrounded by vitelline cells (Rohde, 1972). The significance of the embryonic position within eggs is unknown. At the level of the ovary in the anterior ascending section of the uterus, the embryos had developed a posterior sucker, head glands, and a mouth (Fig. 17). Just a few vitelline cells surrounded the embryo. Embryos collected at the distal end of the uterus were fully developed and folded on themselves within the egg (Fig. 18). Only remnants of vitelline cells remained. Thus, in this study, eggs were fully developed at deposition, as they were in specimens collected by Dollfus (1958a) and Hendrix and Overstreet (1977). Larvae of *Stichocotyle nephropis* Cunningham, 1884, *Lophotaspis vallei* Faust and Tang, 1936, *Cotylogaster occidentalis* Nickerson, 1902, *Aspidogaster indica* Dayal, 1943, *A. conchicola,* and *Rugogaster hydrolagi* Schell, 1973 (see Odhner, 1910; Manter, 1932; Dickerman, 1948; Rai, 1964; Bakker and Davids, 1973; Schell, 1973, respectively), were also fully developed at deposition. In contrast, Faust and Tang (1936) reported that eggs from *M. cristata* were in early stages of development at deposition, as they were in *Cotylaspis sinensis* Faust and Tang, 1936, and *L. orientalis* Faust and Tang, 1936. *Multicotyle purvisi* also deposits eggs in early stages of development (Rohde, 1972).

The fully developed larvae or cotylocidia of *M. cristata* (Fig. 19) were fusiform, and 10 larvae ranged from 0.114 to 0.142 mm in length and from 0.032 to 0.046 mm in width. They had a well-developed posterior sucker, mouth, prepharynx, pharynx, and gut, as in all other described cotylocidia. They were also in the size range of other species (0.1–0.2 mm). The larval mouth was simple and resembled that of the adult (Fig. 20). The micrograph (Fig. 20) does not suggest the presence of an oral sucker, but larvae (Figs. 17, 18) appear to have an oral sucker, which may be an artifact of their mouths being compressed against the sides of the egg capsules. Larvae removed from egg capsules following fixation (Fig. 19) had only a slight suggestion of an oral sucker. However, oral suckers have been diagrammed in other larvae, including *A. conchicola, L. manteri* Rohde, 1973, and *R. hydrolagi* (see Dollfus, 1958b; Rohde, 1973; Schell, 1973, respectively). Cephalic gland ducts were prominent features in *M. cristata* (Fig. 19), as they were in *A. conchicola* (see Dollfus, 1958b); however, the structure of gland cells could not be distinguished clearly. In *M. purvisi,* gland cells were scattered and did not exit through common ducts (Rohde, 1972). *Multicalyx cristata* did not possess cilia or ocelli, and thus was similar to *A. conchicola, T. elegans, A. indica, L. manteri,* and *R. hydrolagi* (see Williams, 1942; Brinkman, 1957; Rai, 1964; Rohde, 1973; Schell, 1973, respectively). Other species have a variety of ciliary tufts (Rohde, 1972).

Eggs placed in watch glasses at the two- or three-cell stage (Fig. 15) were fully developed following 16 days of incubation at 23°C. *Multicotyle purvisi* has a slightly longer developmental period (25 days) at warmer temperatures (28–29°C) (Rohde, 1972). Those species that lay embryonated eggs usually hatch within 1 or 2 days. Fully developed eggs of *M. cristata* could not be induced to hatch in this study by using agitation or light stimulus, and began dying by 23 days. The absence of cilia and ocelli may indicate that the eggs have to be eaten by an intermediate host before hatching. Rohde (1973) found that eggs of *L. manteri* had to be eaten before hatching would occur. However, three other morphologically similar larvae, *A. conchicola, A. indica,* and *R. hydrolagi,* hatch soon after deposition (Bakker and Davids, 1973; Rai, 1964; Schell, 1973, respectively). The intermediate host of *M. cristata* is presently unknown (Thoney and Burreson, 1986). Gibson and Chinabut (1984) suggested that the order Stichocotylida may use crustaceans as intermediate hosts. When this host is found, infection experiments will provide additional information on the biology of *M. cristata.*

**Acknowledgments**

We thank scientists aboard *RV Albatross IV* during the NMFS spring and fall groundfish surveys for assisting with sample collection, Ronald A. Campbell for slides of *M. cristata,* Mary Hansen Pritchard and J. Ralph Lichtenfels for loan of specimens of *M. cristata,* and George W. Benz for information on aspidocotyleans from scalloped hammerhead sharks. This is VIMS Contribution No. 1341.

## Literature Cited

**Bakker, K. E., and C. Davids.** 1973. Notes on the life history of *Aspidogaster conchicola* Baer 1827 (Trematoda; Aspidogastridae). Journal of Helminthology 47:269–276.

**Bapkin, B. P., D. J. Bowie, and J. V. V. Nicholls.** 1933. Structure and reactions to stimuli of arteries (and conus) in the elasmobranch genus *Raja.* Contributions Canadian Biology and Fisheries n.s. 8:207–225.

**Barrett, J.** 1981. Biochemistry of Parasitic Helminths. University Park Press, Baltimore. 308 pp.

**Boray, J. C.** 1969. Experimental fascioliasis in Australia. Advances in Parasitology 7:95–210.

**Bray, R. A.** 1984. Some helminth parasites of marine fishes and cephalopods of South Africa: Aspidogastrea and the digenean families Bucephalidae, Haplosplanchnidae, Mesometridae and Fellodistomidae. Journal of Natural History 18:271–292.

**Brinkmann, A., Jr.** 1957. Fish trematodes from Norwegian waters. IIa. The Norwegian species of the orders Aspidogastrea and Digenea (Gasterostomata). Universitetet I Bergen Arbok 1957, Naturvitenskapelig Rekke 4:1–31.

**Burt, D. R. R.** 1968. On the adhesive organ of *Macraspis elegans* Olsson, 1869 (Aspidogastrea). Parasitology 58 (Proceedings of the Society of Parasitology, pages 13–14) (abstract).

**Corrington, J. D.** 1941. Working with the Microscope. McGraw-Hill Book Co., New York. 418 pp.

**Dawes, B.** 1963. Some observations of *Fasciola hepatica* L. during feeding operations in the hepatic parenchyma of the mouse, with notes on the nature of liver damage in this host. Parasitology 53: 135–143.

**Dickerman, E. E.** 1948. On the life cycle and systematic position of the aspidogastrid trematode, *Cotylogaster occidentalis* Nickerson, 1902. Journal of Parasitology 34:164.

**Dollfus, R. Ph.** 1958a. Sur *Macraspis cristata* (E.-C. Faust et C.-C. Tang 1936) H. W. Manter 1936 et sur une emendation nécessaire a ma definition de la famille des Aspidogastridae (Trematoda). Annales de Parasitologie Humaine et Comparée 33: 227–231.

———. 1958b. Cours d'helminthologie. I. Trematodes, sous-classe Aspidogastrea. Annales de Parasitologie Humaine et Comparée 31:11–13.

**Gibson, D. I., and S. Chinabut.** 1984. *Rohdella si-*

*amensis* gen. et sp. nov. (Aspidogastridae: Rohdellinae subfam. nov.) from freshwater fishes in Thailand, with a reorganization of the classification of the subclass Aspidogastrea. Parasitology 88:383–393.

**Faust, E. C., and C.-C. Tang.** 1936. Notes on new aspidogastrid species, with a consideration of the phylogeny of the group. Parasitology 28:487–501.

**Halton, D. W.** 1972. Ultrastructure of the alimentary tract of *Aspidogaster conchicola* (Trematoda: Aspidogastrea). Journal of Parasitology 58:455–467.

———. 1982. An unusual structural organization to the gut of a digenetic trematode, *Fellodistomum fellis.* Parasitology 85:53–60.

**———, and S. S. Hendrix.** 1978. Chemical composition and histochemistry of *Lobatostoma ringens* (Trematoda: Aspidogastrea). Zeitschrift fuer Parasitenkunde 57:237–241.

**Hargis, W. J., Jr.** 1953. Chlorotone as a trematode relaxer, and its use in mass-collecting techniques. Journal of Parasitology 39:224–225.

**Hendrix, S. S., and R. M. Overstreet.** 1977. Marine aspidogastrids (Trematoda) from fishes in the northern Gulf of Mexico. Journal of Parasitology 63:810–817.

**Luna, L. G.** 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Company, New York. 258 pp.

**Manter, H. W.** 1931. Some digenetic trematodes of marine fishes of Beaufort, North Carolina. Parasitology 23:396–411.

———. 1932. Continued studies on trematodes of Tortugas. Carnegie Institution of Washington Year Book 31:287–288.

———. 1954. Some digenetic trematodes from fishes of New Zealand. Transactions of the Royal Society of New Zealand 82:475–568.

**Odhner, T.** 1910. *Stichocotyle nephropis.* J. T. Cunningham, ein aberranter Trematode der Digenenfamilie Aspidogastridae. Kungliga Svenska Vetenskapsakademiens Handlingar 45:3–16.

**Parukhin, A. M., and L. P. Tkachuk.** 1980. New trematode species from the Indian Ocean fishes. Biologicheskie Nauki 6:41–44.

**Rai, S. L.** 1964. Morphology and life history of *Aspidogaster indicum* Dayal, 1943 (Trematoda: Aspidogastridae). Indian Journal of Helminthology 16:100–141.

**Rohde, K.** 1972. The Aspidogastrea, especially *Multicotyle purvisi* Dawes, 1941. Advances in Parasitology 10:78–151.

———. 1973. Structure and development of *Lobatostoma manteri* sp. nov. (Trematoda: Aspidogastrea) from the Great Barrier Reef, Australia. Parasitology 66:63–83.

**Ross, J. G., C. Dow, and J. R. Todd.** 1967. The pathology of *Fasciola hepatica* infection in pigs: comparison of the infection in pigs and other hosts. British Veterinary Journal 123:317–322.

**———, J. R. Todd, and C. Dow.** 1966. Single infections of calves with the liver fluke *Fasciola hepatica* (Linnaeus, 1758). Journal of Comparative Pathology 76:67–81.

**Schell, S. C.** 1973. *Rugogaster hydrolagi* gen. et sp. n. (Trematoda: Aspidobothrea: Rugogastridae fam. n.) from the ratfish, *Hydrolagus colliei* (Lay and Bennett, 1839). Journal of Parasitology 59:803–805.

**Stunkard, H. W.** 1962. *Taeniocotyle* nom. nov. for *Macraspis* Olsson, 1869, preoccupied, and systematic position of the Aspidobothrea. Biological Bulletin, Marine Biological Laboratory, Woods Hole 122:137–148.

**Thoney, D. A., and E. M. Burreson.** 1986. Ecological aspects of *Multicalyx cristata* (Aspidocotylea) infections in northwest Atlantic elasmobranchs. Proceedings of the Helminthological Society of Washington 53:162–165.

**Williams, C. O.** 1942. Observations on the life history and taxonomic relationship of the trematode *Aspidogaster conchicola.* Journal of Parasitology 28:467–475.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 105–110

# Parasites of the Emerald Shiner, *Notropis atherinoides,* from Two Localities in the St. Marys River, Michigan, with Emphasis on Larval Trematodes

PATRICK M. MUZZALL AND C. ROBERT PEEBLES
Department of Natural Science, North Kedzie Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT: Emerald shiners from Lake Munuscong and Raber Bay in the St. Marys River were examined for parasites. All 100 emerald shiners collected from Lake Munuscong in June and August 1983, and 96 of 100 from Raber Bay in July 1984, were infected with parasites. The parasite fauna consisted of *Gyrodactylus* sp., *Centrovarium* sp., *Diplostomum spathaceum, Diplostomum* sp. (brain), *Neascus* sp., *Neochasmus* sp., *Posthodiplostomum* sp., *Bothriocephalus* sp., *Eubothrium* sp., *Raphidascaris* sp., *Spinitectus* sp., *Epistylis* sp., *Trichodina* sp., and *Trichophyra* sp. The helminth communities of emerald shiners from both localities were dominated numerically by *Neochasmus* sp., primarily found in the muscle, followed by *D. spathaceum,* found in the lens of the eye. The intensities of *Neochasmus* sp. and *D. spathaceum* infections had significant positive correlations with host length.

KEY WORDS: *Diplostomum spathaceum, Neochasmus, Gyrodactylus, Centrovarium, Neascus, Posthodiplostomum, Bothriocephalus, Eubothrium, Raphidascaris, Spinitectus, Epistylis, Trichodina, Trichophyra,* ecology, prevalence, fishes.

The emerald shiner, *Notropis atherinoides* Rafinesque, commonly inhabits large open lakes and rivers of North America (Campbell and MacCrimmon, 1970). Although Cooper (1915), Bangham and Hunter (1939), and Dechtiar (1972) examined emerald shiners for parasites, little is known about the parasite fauna of this fish species. The present study reports on parasites of emerald shiners from two localities in the St. Marys River, Michigan, with emphasis on larval trematodes.

## Materials and Methods

Emerald shiners, *Notropis atherinoides,* were collected with small mesh trap nets from the St. Marys River, the only outflow of Lake Superior to the lower Great Lakes. Liston et al. (1980) and Thomas (1985) described the physical, chemical, and biological features of the St. Marys River. Emerald shiners sampled from Raber Bay in June and August 1983 and from Lake Munuscong in July 1984 were preserved in 10% formalin. The two collection localities were approximately 15 km apart (Muzzall, 1984). One hundred shiners from Lake Munuscong and 100 from Raber Bay were selected to fill arbitrarily established classes based on total length, so that each class had approximately 20 individuals. Sex and total length to the nearest millimeter were recorded when the entire fish was necropsied. Helminths found in preserved fish were processed using conventional parasitological techniques.

The terms prevalence and mean intensity follow the definitions of Margolis et al. (1982). The value following a mean is the standard deviation. Chi-square analysis was used to determine if differences existed in the prevalence of parasites between localities and between female and male fish. Student's $t$-test and analysis of variance followed by 95% confidence intervals were used to determine if the mean infection intensity of *Neochasmus* sp. and *Diplostomum spathaceum* differed between fish sexes and among length classes.

Voucher specimens of the following helminths (USNM Helminthological Collection No.) have been deposited in the USNM Helminthological Collection: *Diplostomum spathaceum* (79247), *Diplostomum* sp. (brain) (79248), *Neochasmus* sp. (79249), *Raphidascaris* sp. (79250), *Spinitectus* sp. (79251), and *Centrovarium* sp. (79259). Specimens of the other helminth species were not retained by the authors and therefore were not deposited.

## Results

### Fish population

Emerald shiners examined from Lake Munuscong had a significantly larger mean total length (75.1 mm ± 15.2) than shiners from Raber Bay (58.5 mm ± 18.9) ($t = 46.9$, $P < 0.001$). The total length ranges of shiners from Lake Munuscong and Raber Bay were 50–103 mm and 23–89 mm, respectively. Emerald shiners from Lake Munuscong over 90 mm in length were females; shiners from Raber Bay under 50 mm were males. Female shiners (81.8 mm ± 13.2) from Lake Munuscong were significantly larger than males (62.2 mm ± 9.4) ($t = 59.0$, $P < 0.001$). There was no significant difference in mean total length of female (58.2 mm ± 19.4) and male (61.5 mm ± 16.9) shiners from Raber Bay.

**Table 1. Prevalence, total, and mean intensity of parasites found in 100 *Notropis atherinoides* from Lake Munuscong and 100 *N. atherinoides* from Raber Bay.**

| | Lake Munuscong | | | Raber Bay | | | |
|---|---|---|---|---|---|---|---|
| Parasite | No. infected | No. found (% of comm.) | Mean intensity ± 1 SD (range) | No. infected | No. found (% of comm.) | Mean intensity ± 1 SD (range) | Location in host |
| Monogenea | | | | | | | |
| *Gyrodactylus* sp.* | 19 | | | 7 | | | Gills |
| Digenea | | | | | | | |
| *Centrovarium* sp.† | 2 | 2 (0.1) | 1.0 | 0 | | | Muscle |
| *Diplostomum spathaceum*† (Rudolphi, 1819) | 92 | 635 (36) | 6.9 ± 7.1 (1–35) | 67 | 451 (38.7) | 6.7 ± 4.5 (1–21) | Lens |
| *Diplostomum* sp.† | 12 | 26 (1.4) | 2.2 ± 2.6 (1–10) | 22 | 52 (4.4) | 2.4 ± 1.7 (1–4) | Brain |
| *Neascus* sp.† | 0 | | | 2 | 2 (0.2) | 1.0 | Integument |
| *Neochasmus* sp.† | 84 | 1,076 (62) | 12.8 ± 11.8 (1–53) | 70 | 650 (55.8) | 9.3 ± 11.3 (1–56) | Muscle, eye orbit, gills |
| *Posthodiplostomum* sp.† | 2 | 2 (0.1) | 1.0 | 4 | 6 (0.5) | 1.5 ± 0.6 (1–2) | Mesenteries |
| Cestoda | | | | | | | |
| *Bothriocephalus* sp.‡ | 4 | 5 (0.2) | 1.3 ± 0.5 (1–2) | 2 | 2 (0.2) | 1.0 | Intestine |
| *Eubothrium* sp.‡ | 2 | 2 (0.1) | 1.0 | 0 | | | Intestine |
| Nematoda | | | | | | | |
| *Raphidascaris* sp.‡ | 0 | | | 2 | 2 (0.2) | 1.0 | Intestine |
| *Spinitectus* sp.‡ | 1 | 1 (0.1) | 1.0 | 0 | | | Intestine |
| Protozoa | | | | | | | |
| *Epistylis* sp.* | 3 | | | 2 | | | Gills |
| *Trichodina* sp.* | 3 | | | 1 | | | Gills |
| *Trichophyra* sp.* | 4 | | | 0 | | | Gills |

* Adult parasites.
† Larval stages.
‡ Immature parasites.

**Parasites—general**

All emerald shiners examined from Lake Munuscong and 96 from Raber Bay were infected with at least one species of parasite. The uninfected shiners had a mean total length of only 27 mm ± 2.7. Fourteen species of parasites belonging to five taxonomic groups (one Monogenea, six Digenea, two Cestoda, two Nematoda, three Protozoa) were found in emerald shiners (Table 1). *Gyrodactylus* sp., *Centrovarium* sp., *Neascus* sp., *Neochasmus* sp., *Eubothrium* sp., *Raphidascaris* sp., *Spinitectus* sp., *Epistylis* sp., *Trichodina* sp., and *Trichophyra* sp. are reported for the first time from emerald shiners. Eight parasite species are common to shiners from both localities. *Centrovarium* sp., *Eubothrium* sp., *Spinitectus* sp., and *Trichophyra* sp. infected only Lake Munuscong shiners. *Neascus* sp. and *Raphidascaris* sp. occurred only in Raber Bay shiners. The helminth communities of emerald shiners from both localities were dominated numerically by *Neochasmus* sp., followed by *Diplostomum spathaceum.* No significant differences existed in the prevalence and mean intensity of *D. spathaceum* and *Neochasmus* sp. in shiners between localities, or between sexes from either locality. *Gyrodactylus* sp. infected significantly more emerald shiners from Lake Munuscong than from Raber Bay ($\chi^2 = 4.7$, $P < 0.05$).

***Neochasmus* sp.**

*Neochasmus* sp. represented 62% and 56% of the helminth communities in emerald shiners from Lake Munuscong and Raber Bay, respec-

**Table 2. Prevalence and mean intensity of *Neochasmus* sp. in selected length classes of emerald shiners from two localities of the St. Marys River.**

| Length class (mm) | Lake Munuscong | | Raber Bay | |
|---|---|---|---|---|
| | Prevalence | Mean intensity ± 1 SD (95% conf. inter.) | Prevalence | Mean intensity ± 1 SD (95% conf. inter.) |
| ≤39 | – | – | 8/25 (32) | 1.5 ± 0.8 (0.9–2.1) |
| 40–49 | – | – | 4/10 (40) | 1.8 ± 1.5 (−0.6–4.1) |
| 50–59 | 12/22 (55)* | 3.6 ± 2.6 (1.9–5.3) | 7/7 (100) | 3.7 ± 3.4 (−0.6–6.8) |
| 60–69 | 17/19 (89) | 11.0 ± 12.8 (4.4–17.6) | 19/22 (86) | 6.7 ± 7.5 (3.1–10.4) |
| 70–79 | 16/17 (94) | 11.3 ± 5.6 (8.3–14.2) | 15/20 (75) | 7.9 ± 7.1 (3.8–12.0) |
| 80–89 | 18/20 (90) | 12.3 ± 11.0 (6.8–17.8) | 15/16 (94) | 23.6 ± 13.9 (15.9–31.3) |
| ≥90 | 21/22 (96) | 20.9 ± 13.9 (14.6–27.3) | – | – |

* No. infected/no. examined (%).

tively, and had the highest mean intensities from these localities. It was the most prevalent parasite in shiners from Raber Bay. The mean intensities of *Neochasmus* sp. in emerald shiners between localities were not significantly different at the 0.05 level; at the 0.10 level, however, Lake Munuscong hosts had a higher mean intensity than did Raber Bay hosts ($t = 3.59$, $P < 0.10$). There were no significant differences in mean intensities of *Neochasmus* sp. between infected Lake Munuscong males (10.2 ± 11.0, $N = 20$) and females (13.9 ± 12.1, $N = 58$), or between Raber Bay males (8.1 ± 9.3, $N = 43$) and females (11.3 ± 14.0, $N = 27$).

The prevalence of *Neochasmus* sp. was high in Lake Munuscong emerald shiners longer than 59 mm and in Raber Bay shiners longer than 49 mm (Table 2). Distinct relationships between prevalences and length classes were not present. The mean intensity of *Neochasmus* sp. at both localities increased with host length. The intensity of *Neochasmus* sp. was positively correlated with host length in Lake Munuscong ($r = 0.40$, $P < 0.01$) and Raber Bay ($r = 0.60$, $P < 0.01$).

### *Diplostomum spathaceum*

*Diplostomum spathaceum* occurred unencysted in the lens of 92 Lake Munuscong emerald shiners and represented 36% of the helminth community. It infected 67 Raber Bay shiners and represented 38% of the helminth community. The mean intensities of *D. spathaceum* were similar in hosts from both localities.

*Diplostomum spathaceum* infected emerald shiners in all length classes (Table 3). The mean intensity and generally the prevalence of *D. spathaceum* increased at both localities as shiners increased in length. Correlation coefficients between host length and intensity of *D. spathaceum* were significant (Lake Munuscong, $r = 0.71$, $P < 0.01$; Raber Bay, $r = 0.70$, $P < 0.01$). Infected female shiners (8.6 ± 7.7, $N = 64$) from Lake Munuscong had a significantly higher mean intensity of *D. spathaceum* than did males (3.1 ± 3.2, $N = 28$) ($t = 13.4$, $P < 0.001$); infected females from Lake Munuscong also were significantly larger (82.0 mm ± 13.2) than males (63.1 mm ± 9.5) ($t = 59.0$, $P < 0.001$). No significant difference in mean intensity of *D. spathaceum* was found between infected females (7.6 ± 4.3, $N = 22$) and males (6.4 ± 1.6, $N = 44$) from Raber Bay. Mean total lengths of infected females (67.9 mm ± 12.6) and males (72.5 mm ± 11.2) were not significantly different. No significant differences in mean intensity of *D. spathaceum* between sexes in each length class were found.

Comparison of the mean intensity of *D. spathaceum* between right and left lenses showed no significant differences for infected emerald shiners. Mean intensities of *D. spathaceum* in the right and left lenses of Lake Munuscong shiners were 4.1 ± 3.9 ($N = 81$) and 3.8 ± 3.7 ($N = 81$), respectively. For Raber Bay shiners, mean intensity in the right lens was 3.9 ± 2.7 ($N = 61$); mean intensity in the left lens was 3.6 ± 2.1 ($N = 58$). Two percent of Lake Munuscong shiners and 5% of Raber Bay shiners showed significant asymmetry between lenses when numbers of *D. spathaceum* were compared.

### *Diplostomum* sp. (brain)

*Diplostomum* sp. was found unencysted in the brains of 12 emerald shiners from Lake Munuscong and 22 from Raber Bay. There was no significant difference in mean intensity of *Diplo-*

**Table 3. Prevalence and mean intensity of *Diplostomum spathaceum* in selected length classes of emerald shiners from two localities of the St. Marys River.**

| Length class (mm) | Lake Munuscong | | Raber Bay | |
|---|---|---|---|---|
| | Prevalence | Mean intensity ± 1 SD (95% conf. inter.) | Prevalence | Mean intensity ± 1 SD (95% conf. inter.) |
| ≤39 | – | – | 3/25 (12) | 1.3 ± 0.6 (−0.1–2.8) |
| 40–49 | – | – | 2/10 (20) | 1.5 ± 0.7 (−4.9–7.9) |
| 50–59 | 18/22 (82)* | 1.6 ± 0.7 (1.2–1.9) | 5/7 (71) | 3.2 ± 2.7 (−0.1–6.5) |
| 60–69 | 16/19 (84) | 3.1 ± 1.9 (2.1–4.2) | 21/22 (96) | 4.9 ± 2.3 (3.8–5.9) |
| 70–79 | 17/17 (100) | 3.9 ± 2.2 (2.8–5.0) | 20/20 (100) | 7.0 ± 4.4 (4.9–9.0) |
| 80–89 | 19/20 (95) | 7.9 ± 5.1 (5.5–10.4) | 16/16 (100) | 11.6 ± 3.5 (9.7–13.4) |
| ≥90 | 22/22 (100) | 15.4 ± 8.3 (11.7–19.1) | – | – |

* No. infected/no. examined (%).

*stomum* sp. in shiners between Lake Munuscong (2.2 ± 2.6) and Raber Bay (2.4 ± 1.6). The mean total lengths of hosts from Lake Munuscong and Raber Bay were 80.1 mm ± 17.5 and 72.7 mm ± 8.1, respectively. The correlation coefficient (−0.36) between the intensity of *Diplostomum* sp. and host length from Raber Bay was not significant. Measurements in micrometers of 15 *Diplostomum* sp. fixed in situ from nine emerald shiners were forebody length 221–357; maximum width 133–200; hindbody length 48–86; oral sucker 30–42 by 31–45; pharynx 23–33 by 13–16; ventral sucker 30–44 by 35–50; holdfast 48–71 by 50–94.

## Discussion

Muzzall (1984) found 32 species of helminths in the digestive tracts of 233 fishes (representing 30 species) from the St. Marys River. Additional species found in emerald shiners in the present study were *Gyrodactylus* sp., *Diplostomum spathaceum, Diplostomum* sp. (brain), *Neascus* sp., *Neochasmus* sp., *Posthodiplostomum* sp., *Epistylis* sp., *Trichodina* sp., and *Trichophyra* sp. These parasites occurred mainly in or on organs that were not examined in 1984. The parasite faunas of emerald shiners from Lake Munuscong and Raber Bay, as well as between 1983 and 1984, were similar taxonomically and in number of species. Six of the 11 helminth species in emerald shiners were larval digenetic trematodes; the helminth fauna was dominated numerically by *Neochasmus* sp. occurring in muscle, followed by *D. spathaceum* in the lens.

In the months when emerald shiners were examined in our survey, the helminths with indirect life cycles were immature or occurred infrequently. The digenetic trematode species most likely infect emerald shiners by penetration of cercariae. Emerald shiners acquire the cestode and nematode species by feeding on invertebrate intermediate hosts. The infrequent infections of cestodes and nematodes probably are not related to the availability of intermediate hosts, as the guts of numerous emerald shiners were full of invertebrates. Thomas (1985) examined 429 emerald shiners from the St. Marys River and identified over 20 taxa of food items; insects were common in May and early June, whereas zooplankton were common in June and July.

Flittner (1964), Fuchs (1967), Campbell and MacCrimmon (1970), and Parsons (1971) reported that emerald shiners are a common food item for game fishes. Courtney and Blokpoel (1980) found that emerald shiners are important food for the common tern, *Sterna hirundo,* on Lake Erie and Lake Michigan. Langlois (1954) and Courtney and Blokpoel (1980) observed that emerald shiners exhibit near-surface swimming, which facilitates avian predation. The near-surface swimming of emerald shiners may increase the transmission of *Diplostomum, Neascus,* and *Posthodiplostomum* to their avian definitive hosts, which probably include the common tern and black tern, *Chlidonias niger,* on the St. Marys River.

Although the parasite fauna of forage fishes is well documented (Hoffman, 1967; Margolis and Arthur, 1979), little is known about larval trematodes occurring in muscle. Peters, working in Michigan in 1959, found progenetic *Neochasmus* in the muscle of fish (pers. comm. *in* Hoffman, 1967). *Neochasmus* specimens in the present study exhibit extreme variation in size and shape, which may be due to fixation. Many specimens appear to be of different ages and a few are almost adult. Adult *Neochasmus umbellus* has been reported from smallmouth bass, *Micropterus dolomieui,* by Bangham and Hunter (1939) and Dechtiar (1972). Muzzall (1984), however, did not find *Neochasmus* in 17 smallmouth bass or in other fishes examined from the St. Marys River.

The ecology, host–parasite relationships, and pathology of *D. spathaceum* in the eyes of fishes have been studied in North America by Palmieri et al. (1977), Heckmann (1983), and Holloway and Leno (1983). Hendrickson (1978) found that prevalence and intensity of *D. spathaceum* increased as white suckers, *Catostomus commersoni,* increased in length. Ching (1985), in a study of *D. baeri bucculentum* from the retina, found that six of nine fish species, where sample sizes were large, showed significant correlations of increasing intensity with increasing length. The present study found that intensity of *D. spathaceum* in the lens significantly increased as emerald shiners increased in length. The significant difference in mean intensity of *D. spathaceum* between female and male shiners from Lake Munuscong in 1984 is related to their respective lengths; because more large females were examined and found to be infected, more *D. spathaceum* were found. Male and female emerald shiners from Raber Bay in 1983 were not significantly different in length, and no significant difference in mean intensity of *D. spathaceum* was found.

Additional observations on *D. spathaceum* infecting emerald shiners are as follows. Generally, when three or more *D. spathaceum* occurred in the lens, they were clumped in the same area. In some lenses, pockets that apparently contained *D. spathaceum* were observed. Upon dissection, however, parasites often were not found in these pockets. Herniations of emerald shiner lenses infected with *D. spathaceum* were not observed. LaRue et al. (1926) working in Michigan and Larson (1965) in North Dakota demonstrated herniations of lenses associated with *Diplostomum* infections in bullheads, *Ictalurus* spp. The asymmetry percentages for *D. spathaceum* in the lens of emerald shiners are comparable to those found for *Diplostomum* sp. in the lens of whitefish, *Coregonus clupeaformis,* by Rau et al. (1979).

*Diplostomum baeri eucaliae* was synonymized with *D. scudderi* by Dubois (1968), and is the only species of *Diplostomum* reported from the brain of fish in North America. Dechtiar (1972) found *D. baeri eucaliae* in the brain of brook stickleback, *Culaea inconstans.* Lester (1974) reported *D. scudderi* in the area between the retina and choroid of the three-spined stickleback, *Gasterosteus aculeatus.* Measurements of *Diplostomum* sp. found unencysted in the brain of emerald shiners from the St. Marys River were at the low end of measurements of *D. baeri eucaliae* given by Hoffman and Hundley (1957). The variability in body measurements, relationships of body regions, and contraction of our specimens make specific identification difficult.


## Acknowledgments

We thank Dr. Charles R. Liston and Michael V. Thomas, Department of Fisheries and Wildlife, Michigan State University, who provided the emerald shiners. We also thank Dr. Glenn L. Hoffman for confirming identification of *Neochasmus* sp. and Dr. Richard A. Heckmann for his correspondence on *Diplostomum* spp.


## Literature Cited


**Bangham, R. V., and G. W. Hunter III.** 1939. Studies on fish parasites of Lake Erie. Distribution studies. Zoologica; New York Zoological Society 24:385–448.

**Campbell, J. S., and H. R. MacCrimmon.** 1970. Biology of the emerald shiner *Notropis atherinoides* in Lake Simcoe, Canada. Journal of Fish Biology 2:259–273.

**Ching, H. L.** 1985. Occurrence of the eyefluke, *Diplostomum (Diplostomum) baeri bucculentum* Dubois et Rausch, 1948, in salmonid fishes of northern British Columbia. Canadian Journal of Zoology 63:396–399.

**Cooper, A. R.** 1915. Trematodes from marine and freshwater fishes, including one species of ectoparasite turbellarian. Transactions of the Royal Society of Canada 9:181–205.

**Courtney, P. A., and H. Blokpoel.** 1980. Food and indicators of food availability for common terns on the lower Great Lakes. Canadian Journal of Zoology 58:1318–1323.

**Dechtiar, A. O.** 1972. Parasites of fish from Lake of the Woods, Ontario. Journal of the Fisheries Research Board of Canada 29:275–283.

**Dubois, G.** 1968. Synopsis des Strigeidae et des Diplostomatidae. Memoires de la Societe Neuchateloise des Sciences Naturelles 10:1–723 (2 vols.).

**Flittner, G. A.** 1964. Morphometry and life history of the emerald shiner, *Notropis atherinoides* Rafinesque. Ph.D. Thesis, University of Michigan, Ann Arbor, Michigan. 208 pp.

**Fuchs, E. H.** 1967. Life history of the emerald shiner, *Notropis atherinoides,* in Lewis and Clark Lake, South Dakota. Transactions of the American Fisheries Society 96:247–256.

**Heckmann, R.** 1983. Eye fluke (*Diplostomum spa-*

*thaceum*) of fishes from the upper Salmon River near Obsidian, Idaho. Great Basin Naturalist 43: 675–683.

**Hendrickson, G. L.** 1978. Observations on strigeoid trematodes from the eyes of southeastern Wyoming fish. I. *Diplostomulum spathaceum* (Rudolphi, 1819). Proceedings of the Helminthological Society of Washington 45:60–64.

**Hoffman, G. L.** 1967. Parasites of North American Freshwater Fishes. University of California Press, Berkeley and Los Angeles. 486 pp.

**———, and J. B. Hundley.** 1957. The life-cycle of *Diplostomum baeri eucaliae* n. subsp. (Trematoda: Strigeida). Journal of Parasitology 43:613–627.

**Holloway, H. L., Jr., and G. H. Leno.** 1983. *Diplostomum spathaceum* in ecologically different fish. Proceedings of the North Dakota Academy of Science 37:84.

**Langlois, T. H.** 1954. The Western End of Lake Erie and Its Ecology. J. W. Edwards, Ann Arbor, Michigan. 479 pp.

**Larson, O. R.** 1965. *Diplostomum* (Trematoda: Strigeoidea) associated with herniations of bullhead lenses. Journal of Parasitology 51:224–279.

**LaRue, G. R., E. P. Butler, and P. G. Berkhout.** 1926. Studies on the trematode family Strigeidae (Holostomidae). No. IV. The eyes of fishes, an important habitat for larval Strigeidae. Transactions of the American Microscopical Society 45:282–288.

**Lester, R. J. G.** 1974. Parasites of *Gasterosteus aculeatus* near Vancouver, British Columbia. Syesis 7:195–200.

**Liston, C. R., W. G. Duffy, D. E. Ashton, C. D. McNabb, and F. E. Koehler.** 1980. Environmental baseline and evaluation of the St. Marys River dredging. U.S. Fisheries and Wildlife Service Report FWS/OBS-80/62.

**Margolis, L., and J. R. Arthur.** 1979. Synopsis of the parasites of fishes of Canada. Bulletin of the Fisheries Research Board of Canada No. 199.

**———, G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad.** 1982. The use of ecological terms in parasitology. Journal of Parasitology 68:131–133.

**Muzzall, P. M.** 1984. Helminths of fishes from the St. Marys River, Michigan. Canadian Journal of Zoology 62:516–519.

**Palmieri, J. R., R. A. Heckmann, and R. S. Evans.** 1977. Life history and habitat analysis of the eye fluke *Diplostomum spathaceum* (Trematoda: Diplostomatidae) in Utah. Journal of Parasitology 63: 427–429.

**Parsons, J. W.** 1971. Selective food preferences of walleyes of the 1959 year class in Lake Erie. Transactions of the American Fisheries Society 100:474–485.

**Rau, M. E., D. M. Gordon, and M. A. Curtis.** 1979. Bilateral asymmetry of *Diplostomum* infections in the eyes of lake whitefish *Coregonus clupeaformis* (Mitchill) and a computer simulation of the observed metacercarial distribution. Journal of Fish Disease 2:291–297.

**Thomas, M. V.** 1985. Distribution patterns of the emerald shiner, *Notropis atherinoides*, in the lower St. Marys River, Michigan, and related environmental and behavioral factors. M.S. Thesis, Michigan State University, East Lansing, Michigan. 60 pp.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 111–114

# Parasites of Dover Sole, *Microstomus pacificus* (Lockington), from Northern California

GARY L. HENDRICKSON AND WIPAWAN YINDEEPOL
Department of Fisheries and Telonicher Marine Laboratory,
Humboldt State University, Arcata, California 95521

ABSTRACT: A total of 232 Dover sole (*Microstomus pacificus*) from northern California were examined for parasites. Of these, 226 (97.4%) were infected with at least one kind of parasite. Seventeen species of parasites were recovered. Dover sole are new host records for *Kudoa clupeidae, Triglicola* sp., *Brachyphallus crenatus, Deretrema* sp., *Derogenes varicus, Otodistomum veliporum* metacercaria, *Phyllobothrium* sp. plerocercoid, trypanorhynch plerocercoid, *Cucullanus annulatus* adult and juvenile, *Anisakis simplex* juvenile, and *Corynosoma strumosum* juvenile. *Triglicola* sp., *Cucullanus annulatus* adult and juvenile, *Echinorhynchus gadi,* and *Acanthochondria margolisi* are reported for the first time from California waters.
KEY WORDS: survey, prevalence, new host and new locality records.

The Dover sole, *Microstomus pacificus* (Lockington), is a fairly large flatfish belonging to the family Pleuronectidae. Commercially important stocks of Dover sole range from British Columbia, Canada, to Santa Barbara, California (Mearns and Allen, 1976), but the best fishing grounds are near Eureka and Fort Bragg, California. Eureka is the most important port of landing, accounting for 32% of the total Dover sole landings in California (California Department of Finance, 1984).

Despite the commercial importance of Dover sole, little is known about their parasites. To date, only 14 species of parasites have been reported from Dover sole (Table 1). This study was conducted to provide information about parasites of northern California Dover sole.

## Materials and Methods

Fish were obtained whole or as filleted carcasses. Whole fish were obtained from the commercial draggers *F/V Donna Mae* and *F/V Anna W.* out of Eureka, California. Filleted carcasses were obtained off the fillet line at Eureka Fisheries Inc., Fields Landing, California, or Tom Lazio Company, Eureka, California.

Fish were transported to the laboratory and were refrigerated or placed on ice. All specimens were necropsied within 48 hr. The external surface (if whole fish), mouth, nasal cavities, gills, eyes, musculature (if whole fish), viscera, and mesenteries were examined for parasites. Parasites were prepared for identification following standard helminthological techniques. Voucher specimens of most parasites have been deposited in the United States National Museum Helminthological Collection (79574–79585).

## Results

The 232 Dover sole examined harbored 17 species of parasites (Table 2). A total of 226 (97.4%) of these fish were infected with at least one kind of parasite. The acanthocephalan *Echinorhynchus gadi* was the most common parasite encountered. Juveniles of the acanthocephalan *Corynosoma strumosum* and of the nematode *Anisakis simplex* were also common. Several larval helminths could not be specifically identified due to their rarity and (or) lack of definitive morphological features.

## Discussion

This is the first comprehensive survey of parasites of Dover sole. Dover sole are new host records for *Kudoa clupeidae, Triglicola* sp., *Brachyphallus crenatus, Deretrema* sp., *Derogenes varicus, Otodistomum veliporum* metacercaria, *Phyllobothrium* sp. plerocercoid, trypanorhynch plerocercoid, *Cucullanus annulatus* adult and juvenile, *Anisakis simplex* juvenile, and *Corynosoma strumosum* juvenile. *Triglicola* sp., *Cucullanus annulatus* adult and juvenile, *Echinorhynchus gadi,* and *Acanthochondria margolisi* are reported for the first time from California waters.

The monogenean *Triglicola* sp. and the digenean *Deretrema* sp. appear to be new species. However, good specimens are not available in sufficient quantity to describe them here. *Triglicola* contains only three species described from *Lepidotrigla, Trigla,* and *Pterigotrigla* from the Indian Ocean, South China Sea, and Tasman Sea (Mamaev and Parukhin, 1972; Mamaev, 1976). *Deretrema* contains about 18 species of mainly gall bladder-inhabiting trematodes (Yamaguti, 1971). Only two of these species have previously been reported from North America.

Although 17 species of parasites were re-

**Table 1. Parasites found in the Dover sole, *Microstomus pacificus*.**

| Parasite | Anatomic location | Geographic location | Reference |
|---|---|---|---|
| Protozoa | | | |
| *Ceratomyxa hopkinsi* | Gall bladder | California | Jameson (1929) |
| *Kudoa* sp. | Muscle | Washington | Patashnik and Groninger (1964) |
| *Trypanosoma* sp. | Blood | Oregon | Love and Moser (1983) |
| Digenea | | | |
| *Eurycreadium vitellosum* | Intestine | Washington | Ching (1961) |
| *Fellodistomum brevum* | Intestine | Washington | Ching (1960) |
| *Lepidapedon calli* | Intestine | Washington | Acena (1947), Ching (1961) |
| *Zoogonus dextrocirrus* | Intestine | Washington | Aldrich (1961) |
| *Zoogonoides viviparus* | Intestine | Washington | Ching (1960) |
| Cestoda | | | |
| *Nybelinia surmenicola* (post-larva) | Stomach wall | British Columbia | Margolis (1952) |
| Pseudophyllidean plerocercoid | Body cavity | British Columbia | Margolis (1952) |
| Acanthocephala | | | |
| *Echinorhynchus gadi* | Intestine | Oregon | Miller (1977) |
| Nematoda | | | |
| *Anisakis* sp. | Muscle<br>Mesenteries | British Columbia | Margolis (1952) |
| Copepoda | | | |
| *Acanthochondria margolisi* | Gills | British Columbia | Kabata (1984) |

covered, the parasite fauna of Dover sole is not a particularly rich one. Most parasites are characterized by low prevalences and low mean intensities. Only *E. gadi, A. simplex* juvenile, and *C. strumosum* juvenile could be classified as common. Infection levels observed for adult Digenea are somewhat misleading. About 75% of *Zoogonis dextrocirrus* and 50% of *D. varicus* recovered were immature (nongravid), suggesting that both species reach maturity in Dover sole only with difficulty.

Most parasites of Dover sole are relatively non-host-specific. Most notable of these are the various juvenile parasites, *K. clupeidae, B. crenatus, D. varicus, Z. dextrocirrus,* and *E. gadi,* which have been reported from a variety of fish hosts (Margolis and Arthur, 1979; Love and Moser, 1983). Notable exceptions include *Triglicola* sp., *Deretrema* sp., and *A. margolisi* restricted to Dover sole and *C. annulatus* restricted to pleuronectid flatfish.

*Anisakis* juveniles and *C. strumosum* juveniles are capable of infecting man (Myers, 1975; Schmidt, 1971). Infections of humans by either helminth are acquired by eating uncooked or insufficiently cooked fish flesh. Human infections acquired from Dover sole would seem unlikely. Prevalence of both helminths in the muscle of Dover sole is low, even several days after death of the fish host. In addition, Dover sole is a high-water-content fish requiring a reasonable amount of cooking to make it acceptable to most consumers. This cooking probably exceeds the 60°C for 1 min established by Bier (1976) as minimum cooking required to kill anisakine juveniles.

## Acknowledgments

We express our appreciation to Mr. Jerry Thomas (Eureka Fisheries Inc.), Mr. Vince Thomas (Tom Lazio Company), and the crews of the *F/V Donna Mae* and *F/V Anna W.* for aid in obtaining fish and fish carcasses. We also thank Dr. Jeffrey W. Bier (U.S. Food and Drug Administration), Dr. Mike Moser (Long Marine Laboratory, University of California, Santa Cruz), and Mr. Dennis Thoney (Virginia Institute of Marine Science) for help in identifying and commenting on several parasites.

This work is a result of research sponsored in part by NOAA, National Sea Grant College Program, Department of Commerce, under grant number NA80AA-D-00120, project number R/F-89, through the California Sea Grant College Program, and in part by the California State

**Table 2. Prevalences, mean intensities, ranges, and anatomic locations of parasites in 232 Dover sole, *Microstomus pacificus*, collected in the vicinity of Eureka, California.**

| Parasite | No. of fish infected (%) | Mean intensity | Range in nos. per infection | Location of parasites |
|---|---|---|---|---|
| Protozoa | | | | |
| *Kudoa clupeidae** | 4 (0.4)† | ‡ | ‡ | Musculature |
| Unidentified myxozoan | 23 (9.9) | ‡ | ‡ | Gall bladder |
| Monogenea | | | | |
| *Triglicola* sp.*·§ | 8 (3.5) | 14.1 | 2–88 | Gills |
| Digenea | | | | |
| *Brachyphallus crenatus** | 4 (1.7) | 2.5 | 1–5 | Stomach, intestine |
| *Deretrema* sp.* | 2 (0.6)‖ | 11.0 | 3–19 | Gall bladder, bile ducts |
| *Derogenes varicus** | 11 (4.7) | 1.7 | 1–5 | Stomach, intestine |
| *Zoogonus dextrocirrus* | 11 (4.7) | 12.1 | 1–51 | Intestine |
| *Otodistomum veliporum* (metacercaria)* | 1 (0.4) | 1.0 | 1 | Stomach wall |
| Unidentified metacercaria | 32 (13.8) | ‡ | 1–100's | Stomach wall, intestinal wall, mesenteries of body cavity |
| Cestoda | | | | |
| *Phyllobothrium* sp. plerocercoid* | 3 (0.9)‖ | 1.3 | 1–2 | Gall bladder, ovary |
| Trypanorhynch plerocercoid* | 2 (0.9) | 1.0 | 1 | Ovary, stomach wall |
| Unidentified plerocercoid | 13 (5.6) | 3.3 | 1–7 | Intestine, intestinal wall, stomach wall, liver, mesenteries of body cavity |
| Nematoda | | | | |
| *Cucullanus annulatus**·§ | 30 (12.9) | 1.9 | 1–6 | Stomach, intestine, pyloric caeca |
| *Cucullanus annulatus* juvenile*·§ | 1 (0.4) | 2.0 | 2 | Stomach wall |
| *Anisakis simplex* juvenile* | 119 (51.3) | 3.19 | 1–68 | Intestine, stomach wall, surface of internal organs, mesenteries of body cavity, musculature |
| Acanthocephala | | | | |
| *Echinorhynchus gadi*§ | 164 (70.7) | 5.1 | 1–30 | Intestine (especially posteriorly) |
| *Corynosoma strumosum* juvenile* | 116 (50) | ‡ | 1–100's | Surface of internal organs, mesenteries of body cavity, musculature |
| Copepoda | | | | |
| *Acanthochondria margolisi*§ | 20 (8.6) | 1.5 | 1–10 | Gills |

* New host record.
† Examined in 1,000 fish.
‡ Value not calculated.
§ New state record.
‖ Examined in 332 fish.

Resources Agency. The U.S. Government is authorized to reproduce and distribute reprints for governmental purposes.

## Literature Cited

**Acena, S. P.** 1947. New trematodes from Puget Sound fishes. Transactions of the American Microscopical Society 66:127–139.

**Aldrich, L. E., Jr.** 1961. Two new digenetic trematodes from marine fishes of Puget Sound, Washington. Journal of Parasitology 47:77–80.

**Bier, J. W.** 1976. Experimental anisakiasis: cultivation and temperature tolerance determinations. Journal of Milk and Food Technology 39:132–137.

**California Department of Finance.** 1984. California Statistical Abstract. California Department of Finance, Sacramento, California. 230 pp.

**Ching, H. L.** 1960. Some digenetic trematodes of fishes of Friday Harbor, Washington. Journal of Parasitology 46:241–250.

———. 1961. Redescription of the digenetic trematodes *Lepidapedon calli* and *L. pugetensis* Acena, and new host records for *L. calli* and *Eurycreadium vitellosum* Manter from fishes of Washington state. Canadian Journal of Zoology 39:615–621.

**Jameson, A. P.** 1929. Myxosporidia from California fishes. Journal of Parasitology 16:59–68.

**Kabata, Z.** 1984. A contribution to the knowledge of Chondracanthidae (Copepoda: Poecilostomatoida) parasitic on fishes of British Columbia. Canadian Journal of Zoology 62:1703–1713.

**Love, M. S., and M. Moser.** 1983. A checklist of parasites of California, Oregon, and Washington marine and estuarine fishes. National Oceanic and Atmospheric Administration Technical Report NMFS SSRF-777. 576 pp.

**Mamaev, Iu. L.** 1976. *Triglicola australis* sp. n. (Monogenea: Plectanocotylidae), a parasite from fishes of the Tasman Sea [in Russian, English summary]. Biologiia Moria, Vladivostok 2(6):52–54 (For English translation see Soviet Journal of Marine Biology 2(6):388–390.)

———**, and A. M. Parukhin.** 1972. Monogeneans of the family Plectanocotylidae Poche, 1926, new representatives of the subfamily Plectanocotylinae [in Russian, English summary]. Parazitologiia 6: 65–74.

**Margolis, L.** 1952. Studies on parasites and diseases of marine and anadromous fish from the Canadian Pacific coast. Ph.D. Dissertation, McGill University, Montreal, Canada. 235 pp.

———**, and J. R. Arthur.** 1979. Synopsis of the parasites of fishes of Canada. Fisheries Research Board of Canada Bulletin 199. 269 pp.

**Mearns, A. J., and M. J. Allen.** 1976. Life history of the Dover sole. Southern California Coastal Water Research Project El Segundo (USA). Annual Report of the Southern California Water Resources Project for 1976:223–228.

**Miller, R. L.** 1977. The biology of two species of *Echinorhynchus* (Acanthocephala) from marine fishes in Oregon. Ph.D. Dissertation, Oregon State University, Corvallis, Oregon. 118 pp.

**Myers, B. J.** 1975. The nematodes that cause anisakiasis. Journal of Milk and Food Technology 38:774–782.

**Patashnik, M., and H. S. Groninger, Jr.** 1964. Observations on the milky condition in some Pacific coast fishes. Journal of the Fisheries Research Board of Canada 21:335–346.

**Schmidt, G. D.** 1971. Acanthocephalan infections of man, with two new records. Journal of Parasitology 57:582–584.

**Yamaguti, S.** 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vols. I and II. Keigaku Publishing Company, Tokyo, Japan. 1074 pp., 1796 figs.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 115–117

# Scolex Structural Homologies and the Systematic Position of the Genus *Spiniloculus* (Cestoda: Tetraphyllidea)

J. N. CAIRA
Ecology and Evolutionary Biology, University of Connecticut, U-43, Room 312,
Storrs, Connecticut 06268

ABSTRACT: The current interpretation of homologies between scolex structures of *Spiniloculus* and members of other onchobothriid genera is discussed. An alternative interpretation of these structural homologies is suggested based on the hypothesis that the anterior loculus of *Spiniloculus* is homologous with the muscular pad of other onchobothriids. The alternative interpretation is endorsed because it is consistent with positional, structural, and developmental data. Under this interpretation *Spiniloculus* fits readily into the family Onchobothriidae.

KEY WORDS: positional and developmental data, classification, morphology, *Onchobothrium, Acanthobothrium.*

The genus *Spiniloculus* Southwell, 1925, currently contains a single species, *S. mavensis* Southwell, 1925, which was originally collected from *Mustelus* sp. in Moreton Bay, Brisbane, Australia. Two additional records of this species exist. Williams (1964) described specimens from *Hemiscyllium punctatum* (Müller and Henle) (?=*Chiloscyllium punctatum* Müller and Henle) in Australian waters that he provisionally identified as *Spiniloculus mavensis,* and Subhapradha (1955) reported specimens of "*Spiniloculus mavensis*" from *Chiloscyllium griseum* Müller and Henle off the Madras Coast of India.

Because of the "peculiar" nature of the scolex, there has been some disagreement as to the systematic position of the genus *Spiniloculus.* Southwell (1925) considered it to be closely related to *Ceratobothrium* Monticelli, 1892, and *Onchobothrium* Blainville, 1828, both of which he recognized as members of the family Onchobothriidae. Baer and Euzet (1962), however, argued that the scolex of *S. mavensis* was so distinctive that the position of this species among the onchobothriids was very difficult to establish.

It is my primary objective here to suggest that *Spiniloculus* appears peculiar (unusual), and thus difficult to place systematically, because homologies between the scolex structures of *Spiniloculus* and all other onchobothriid species have been misinterpreted. An alternative interpretation of the scolex of *Spiniloculus* is suggested, and, in view of the developmental data presented by Hamilton and Byram (1974), acceptance of this alternative interpretation is endorsed.

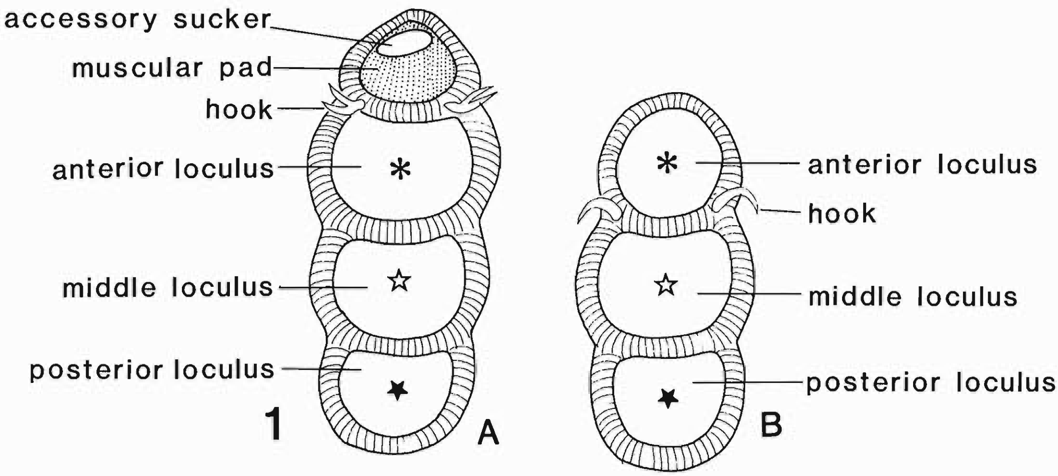
### Current Interpretation

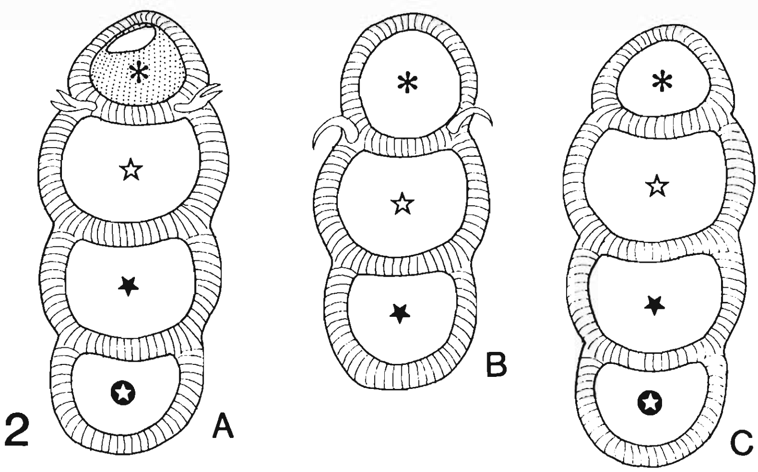
The bothridial structure of *Spiniloculus* appears to be unique among the onchobothriids, presumably because the hooks are not anterior to the anteriormost loculus but, rather, apparently positioned on the posterolateral borders of the anteriormost loculus (thus the name *Spiniloculus*). This interpretation is consistent with the descriptions of Southwell (1925) and Williams (1964). Schmidt (1986, p. 136), using this interpretation, distinguished *Spiniloculus* from *Onchobothrium* on the basis of the couplet: "a. Hooks anterior of front loculi [vs.] b. Hooks lateroposterior of front loculi."

The assumption inherent in this interpretation is that the anterior loculus on each bothridium of *Spiniloculus* is homologous with the "anterior loculus" on each bothridium in other Onchobothriidae, such as *Acanthobothrium* van Beneden, 1849, and *Onchobothrium* etc., and homologies of the subsequent posterior loculi follow (see Fig. 1). Under this interpretation, the bothridial structure of *Spiniloculus* is also unique in that the muscular pad (and accessory sucker) or its homologue must be considered to be entirely lacking.

### Alternative Interpretation

An alternative interpretation of the bothridial morphology of *Spiniloculus* is possible. This interpretation is based on the hypothesis that the anterior loculus on each bothridium of *Spiniloculus* is homologous with the muscular pad (and accessory sucker when present) in other Oncho-




**Figures 1, 2. Schematic representations of single bothridia showing interpretations of homologous structures. Structures thought to be homologous, within each figure, are indicated with similar symbols. 1. Current interpretation of homologous structures. A. Bothridium of *Acanthobothrium* (adult). B. Bothridium of *Spiniloculus* (adult). 2. Alternative interpretation of homologous structures. A. Bothridium of *Acanthobothrium* (adult). B. Bothridium of *Spiniloculus* (adult). C. Bothridium of *Acanthobothrium* from plerocercoid (after Hamilton and Byram, 1974).**

bothriidae, rather than with the first post-hook loculus (Fig. 2A, B). Under this interpretation, the position of the hooks in *Spiniloculus* is not unique, and the homologue of the muscular pad (and accessory sucker) is not lacking, but is instead represented by the anterior loculus in *Spiniloculus*.

### Choosing Between the Two Interpretations

In the absence of developmental data, two structures are generally considered to be homologous if they are positionally and structurally similar. The difficulty with *Spiniloculus* is that positional and structural evidence appears to be contradictory.

Under the current interpretation, structural evidence is implicitly considered to be superior to positional evidence, i.e., the anterior depression in *Spiniloculus* looks much more like a loculus than it does a muscular pad and thus is considered to be homologous to the first loculus-like structure on the bothridia of other onchobothriids. This is in spite of the fact that the position of this structure with respect to the hooks must then be considered to differ between genera.

Under the alternative interpretation, positional evidence is given more weight than structural evidence. Despite the fact that the anterior depression in *Spiniloculus* does not resemble a muscular pad, it is hypothesized to be homologous with the structure anterior to the hooks in other onchobothriid genera, the muscular pad.

Without developmental data, this dilemma would be difficult to resolve. However, in this instance helpful developmental evidence is available. Hamilton and Byram (1974) documented the development of the scolex of an onchobothriid species of the genus *Acanthobothrium.* These authors discovered that plerocercoids of this species possess *four* loculi per bothridium (Fig. 2C). As the scolex matures, the anteriormost (accessory) loculus thickens into the muscular pad and an anterior depression in the accessory loculus becomes the accessory sucker. At the same time, hooks develop at the posterolateral margins of the anterior accessory loculus. Thus, both the muscular pad and its accessory sucker develop as modifications of an anteriormost loculus.

These data now contribute structural evidence in an ontogenetic context that corroborates the alternative interpretation based on positional evidence given above. That is, the anterior depression in *Spiniloculus* structurally resembles the precursor of a muscular pad. Thus, the alternative interpretation is consistent with more data and is preferable to the current interpretation of bothridial morphology. Under the alternative interpretation, *Spiniloculus* is readily recognizable as a member of the family Onchobothriidae, and the bothridial structure of members of this genus can be considered to differ from other onchobothriids primarily in terms of the number of post-hook loculi, a feature already known to vary somewhat among other onchobothriid genera (e.g., *Uncibilocularis* Southwell, 1925, has two post-hook loculi, *Potamotrygonocestus* Brooks and Thorson, 1976, has one post-hook loculus, etc.).

It should be noted that using data on the development of the plerocercoid of a species of *Acanthobothrium* to interpret the morphology of *Spiniloculus* requires the assumption that development of the plerocercoid of *Spiniloculus* is similar to that of *Acanthobothrium.* In fact, based on this assumption it is predicted that each of the bothridia on the plerocercoid of *Spiniloculus* has only three loculi, and the hooks develop between the anterior two loculi.

Williams (1964) noted that the specimens of *Spiniloculus* reported by Subhapradha (1955) possess a total of only two loculi per bothridium, one anterior and one posterior to the hooks. If this is actually the case, these specimens are not *Spiniloculus mavensis* and may represent an undescribed genus. Study of specimens is necessary before the status of that taxon can be verified.

## Literature Cited

**Baer, J. G., and L. Euzet.** 1961. Revision critique des cestodes tetraphyllides décrits par T. Southwell. Bulletin de la Societé Neuchâteloise des Sciences Naturelles 85:143–172.

**Hamilton, K. A., and J. E. Byram.** 1974. Tapeworm development: the effects of urea on a larval tetraphyllidean. Journal of Parasitology 60:20–25.

**Schmidt, G. D.** 1986. Handbook of Tapeworm Identification. CRC Press, Boca Raton, Florida.

**Southwell, T.** 1925. A monograph on the Tetraphyllidea with notes on related cestodes. Liverpool School of Tropical Medicine Memoirs 2.

**Subhapradha, C. K.** 1955. Cestode parasites of fishes of Madras Coast. Indian Journal of Helminthology 7:41–132.

**Williams, H. H.** 1964. Some new and little known cestodes from Australian elasmobranchs with a brief discussion on their possible use in problems of host taxonomy. Parasitology 54:737–748.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 118–121

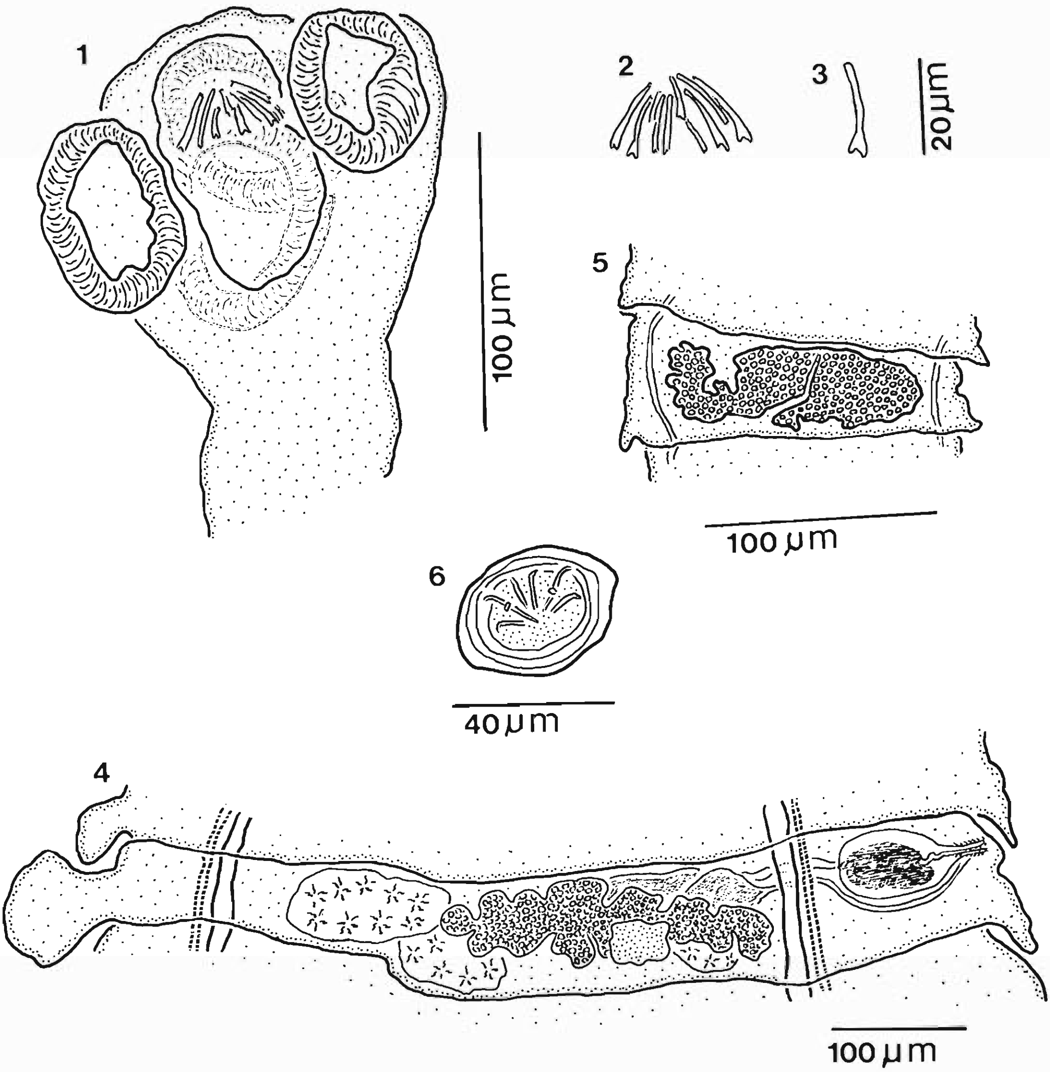
# *Passerilepis minor* sp. n. (Cestoda: Hymenolepididae) from the Blue Magpie, *Cyanocorax chrysops*, in Paraguay

P. ILLESCAS-GOMEZ,[1] V. GOMEZ-GARCIA,[1] AND F. JIMENEZ-MILLAN[2]

[1] Institute of Parasitology "Lopez-Neyra" C.S.I.C. Ventanilla, 11 18001 Granada, Spain and
[2] Department of Zoology, University of Granada, Spain

ABSTRACT: *Passerilepis minor* sp. n. is described, and distinguished from its congeners mainly on the basis of the size (19.1–19.4 μm long) and shape of rostellar hooks and dimensions of the cirrus sac (55–134 by 41–63 μm). The helminths of the blue magpie, *Cyanocorax chrysops* L., have not been previously investigated in Paraguay.

KEY WORDS: taxonomy, morphology, species review, birds.




**Figures 1–6.** ***Passerilepis minor*** **sp. n. 1. Scolex. 2. Crown of rostellar hooks. 3. Rostellar hook. 4. Mature segment, ventral view. 5. Gravid segment. 6. Egg.**

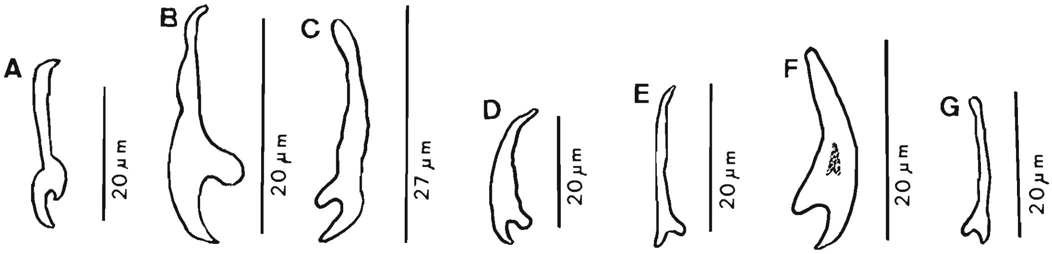



**Figure 7. Diagrams comparing different rostellar hook shapes. A. *Passerilepis ababili*. B. *Passerilepis crenata*. C. *Passerilepis passeris*. D. *Passerilepis schmidti*. E. *Passerilepis petrocinclae*. F. *Passerilepis spasskii*. G. *Passerilepis minor* sp. n. According to: Singh, 1952 (A); Spasskaya, 1966 (B, C, F); Deardorff and Brooks, 1978 (D); Krabbe, 1879 (F).**

Helminths of birds in Paraguay were investigated by members of an expedition from the Doñana Biological Research Station C.S.I.C. The parasites collected were fixed in situ and sent to the "Lopez-Neyra" Institute of Parasitology. One of three blue magpies, *Cyanocorax chrysops* L., harbored six specimens of cestodes belonging to the genus *Passerilepis* Spassky and Spasskaya, 1954, which represent a new species.

## Materials and Methods

Cestodes were fixed and stored in 70% ethanol, stained in Semichon's acetic carmine, cleared in methyl benzoate, and mounted in balsam. Figures were prepared with the use of a camera lucida. Measurements are in micrometers unless otherwise stated.

## Description of Species

### *Passerilepis minor* sp. n. (Figs. 1–6)

Strobila 350–400 mm long by 1.5 mm wide near posterior end. Segments craspedote. Mature segments 122 long by 860 wide; gravid segments 550 long by 1.5 mm wide. Ventral osmoregulatory canals situated laterally, 14–33 in diameter; dorsal canals, usually overlapping ventral canals, 2–16 in diameter. Scolex 134 long by 134 in maximum width. Rostellum retracted in all specimens. Rostellar sac 97 long by 47 in diameter, extending posteriad to level of suckers. Rostellar hooks 10 in number, 19.1–19.4 long. Suckers unarmed, 55–69 long by 47–58 wide. Neck well developed. Reproductive system protandrous. Genital pores unilateral, situated near middle of segmental margin. Genital ducts extending dorsally across osmoregulatory canals. Cirrus 19 long by 11 wide, with spines. Cirrus sac 55–134 long by 41–63 wide in mature segments. External seminal vesicle 110–182 long by 27–61 in diameter. Testes 3, one poral and 2 aporal, 48–134 long by 41–61 wide. Vagina situated posteroventral to male genital duct. Seminal receptacle anterior to poral lobe of ovary, and extending anteriad to margin of segment when distended, 55–91 long by 27–55 in diameter. Ovary transverse, weakly lobed, situated somewhat porally in middle field of segment, 22–85 long by 158–365 wide. Vitelline gland rounded, situated ventrally with respect to dorsal testes and porally near the posterior margin of the ovary, 41–61 long by 49–134 wide. Uterus separated into 2 sacs, filling most of the gravid segment between the osmoregulatory canals. Egg subspherical, 36–47 in greatest diameter by 25–36; second membrane 30–39 by 25–27; inner membrane 30–33 by 19–25. Oncosphere subspherical, 22–28 at greatest diameter by 16–25. Oncospheral hooks 14–16 long.

HOST: Blue magpie, *Cyanocorax chrysops* L.

LOCATION IN HOST: Small intestine.

LOCALITY: Department of Nueva Asunción, Paraguay.

HOLOTYPE: Deposited in the Helminthological Collection of the "Lopez-Neyra" Institute of Parasitology, Granada, Spain, No. 105851.

PARATYPES: Deposited as holotype, No. 105852.

## Discussion

According to the taxonomic criteria provided by Spasskaya (1966), species of *Passerilepis* vary primarily in the size and shape of the rostellar hooks and in the size of the cirrus sac. Based on these characters, *Passerilepis minor* sp. n. most closely resembles the following six species (see Fig. 7A–G): *P. ababili* (Singh, 1952) Spassky, 1963, *P. crenata* (Goeze, 1782) Sultanov and Spasskaya, 1959, *P. passeris* (Gmelin, 1790) Spassky and Spasskaya, 1954, *P. petrocinclae*

**Table 1. Morphometrics of seven species of *Passerilepis* (all measurements in μm unless otherwise indicated).**

| | *P. ababili* | *P. crenata* | *P. passeris* | *P. schmidti* | *P. petrocinclae* | *P. spasskii* | *P. minor* sp. n. |
|---|---|---|---|---|---|---|---|
| Length of strobila (cm) | 6–14.8 | — | — | 4.5–5.5 | 3.5 | 8.1 | 3.5–4 |
| Width of strobila (mm) | 1.1–1.2 | — | — | 1.9 | — | — | 1.5 |
| Scolex | 110 × 152 | 176–182 | — | 265–332 × 242–270 | — | 234 × 144 | 134 × 134 |
| Rostellar sac | 78 | — | — | 213–216 × 87–124 | — | — | 97 × 47 |
| Rostellar hooks | 25–27 | 20–25 | 21–26 | 21–26 | 18 | 21 | 19.1–19.4 |
| Suckers | 62 × 47–53 | 79 × 83 | — | 107–130 | — | 84 × 114 | 55–69 × 47–58 |
| Cirrus pouch | 171–217 × 44–56 | 140–180 | 140–180 | 173–231 × 42–89 | — | 146–148 | 55–134 × 41–63 |
| Sem. receptacle | — | — | — | 110–166 × 200–236 | — | — | 55–91 × 27–55 |
| Ext. sem. ves. | — | — | — | 18–94 | — | — | 110–182 × 27–61 |
| Ovary | 264–285 | — | — | 113–270 × 169–440 | — | 36–39 × 61–64 | 158–365 × 22–85 |
| Vitellaria | 93–99 | — | — | 73–141 × 68–141 | — | 24–27 × 33–36 | 49–134 × 41–61 |
| Testes | 93–110 | 160 × 90 | — | 113–186 × 125–225 | — | — | 73–134 × 36–73 |
| Egg | | | | | | | |
| 1° env. | 44 | 85 × 98 | 95 × 67 | 45–68 | 94 × 65 | — | 36–47 × 24–36 |
| 2° env. | 37 | — | — | — | — | — | 30–33 × 25–27 |
| 3° env. | — | — | — | — | — | — | 30–33 × 19–25 |
| Oncosphere | 28 | 57 × 41 | — | 21–50 | 33 × 35 | 47 × 50–53 | 22–27 × 16–24 |
| Hooks | 13 | 22–25 | — | 16–24 | 14 | — | 14–16 |

(Krabbe, 1879) Spassky and Spasskaya, 1954, *P. schmidti* Deardorff and Brooks, 1978, and *P. spasskii* (Sudarikov, 1950) Spassky and Spasskaya, 1954 (see Table 1). The hook lengths range from 18 to 28 $\mu$m for these species. The hooks of *P. minor* differ in shape from all except *P. petrocinclae* (see Fig. 7E, G). However, *P. minor* has smaller eggs (36–47 by 24–36 $\mu$m) than *P. petrocinclae* (94 by 65 $\mu$m). Furthermore, all six of the above species have a larger cirrus sac than *P. minor*. In some species the sac may cross the poral half of the mature segment. The measurements of these seven species are summarized in Table 1.

## Acknowledgments

We should like to express our gratitude to all the staff of Doñana Biological Station C.S.I.C., Sevilla (Spain), and its Director, Dr. Castroviejo, for his cooperation in supplying the material examined, without which it would not have been possible to prepare this paper. Likewise, we wish to thank Ms. Concepción Rodríguez-Gallego, librarian at the "Lopez-Neyra" Institute of Parasitology for her help in translating the Russian references.

## Literature Cited

**Deardorff, T. L., and D. R. Brooks.** 1978. *Passerilepis schmidti* sp. n. (Cestoidea: Hymenolepididae) from the blue jay *Cyanocitta cristata* L. in Nebraska. Proceedings of the Helminthological Society of Washington 45(2):190–192.

**Krabbe, E.** 1879. Cestodes collected in Turkestan by A. A. Fedchenko (Fedchenko's travels in Turkestan 3. Vermes Pt. 1). Izvestiya Imperatorskovo Obshestra Lyubitelei Estestuosnaniya, Antropologii i Etnografia, Moscow 34:1–23.

**Singh, K. S.** 1952. Cestode parasites of birds. Indian Journal of Helminthology IV(1):1–72.

**Spasskaya, L. P.** 1966. Cestodes of Birds. Hymenolepids. Akademya Nauk SSSR, Institut Zoologii. 698 pp.

**Spassky, A. A., and L. P. Spasskaya.** 1954. Systematic structure of the hymenolepids parasitic in birds. Trudy Gel'mintologicheskoi Laboratorii Akademya Nauk SSSR 7:55–119.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 122–125

# Attraction and Behavior of *Heterodera glycines*, the Soybean Cyst Nematode, to Some Biological and Inorganic Compounds[1]

ROBIN N. HUETTEL[2] AND HOWARD JAFFE[3]

[2] Nematology Laboratory and [3] Livestock Insects Laboratory, U.S. Department of Agriculture, ARS, Beltsville Agricultural Research Center, Beltsville, Maryland 20705

ABSTRACT: Positive movement to some ionic solutes and biological compounds was exhibited by male soybean cyst nematodes, *Heterodera glycines.* Males were highly attracted to different concentrations of glycerol, moderately attracted to KOH, and repelled by HCl. Males were highly attracted to some amino acids, adenosine 3′:5′-cyclic monophosphate, and adenosine 5′-monophosphate. The amino acids that were most attractive were histidine, isoleucine, methionine, proline, serine, tyrosine, and tryptophan. The males were tested for precopulatory and premating behavior in response to all solutions that were attractive, but none of them caused the coiling behavior previously observed due to sex pheromones. Males appeared to be stimulated by many chemical cues by exhibiting positive movement to the source. The lack of coiling behavior observed with each of the tested solutions indicates that coiling is a specific behavior associated only with sex pheromones.

KEY WORDS: Nematoda, chemotaxis.

Chemotaxis is recognized as an important aspect of nematode behavior (Zuckerman and Jansson, 1984, for review). Nematodes are able to perceive many attractive and repellent chemical cues in their environment. These cues enable them to complete their feeding, reproduction, and survival strategies (see Huettel, 1986, for review). However, many studies on nematode behavior have utilized bioassays based only on positive movement to a source to determine attraction to stimulants, such as in sex attraction studies (Green, 1980; Rende et al., 1982; Huettel and Rebois, 1986). No consideration has been given to the differences between chemotaxis involving attraction to a stimulant, such as a food source, and attraction to a specific signal that is related to a behavior, such as mate finding.

Pheromones and allelochemics are two types of chemical cues that promote host and mate findings in many organisms (Nordlund et al., 1981). The behavioral response of a nematode to an allelochemical or to a pheromone, however, might require different external cues but result in the same behavior, i.e., positive movement to the source. For instance, *Nippostrongylus brasiliensis* was significantly attracted to adenosine 3′:5′-cyclic monophosphate in in vitro bioassays. However, this compound was not found in the incubate from females, which presumably contains sex pheromones and also elicits positive movement in males (Ward et al., 1984).

Recently, Huettel and Rebois (1986) demonstrated a specific coiling behavior that appeared to occur only when male soybean cyst nematodes, *Heterodera glycines,* were in direct contact with females or near agar discs where females had been placed. Because other cues could possibly elicit this response, further studies were necessary to determine if this behavior could be induced by other substrates. The present study reports the behavioral responses of soybean cyst males to various chemical and biological compounds, some of which are known to be attractive to other nematode species.

## Materials and Methods

STOCK CULTURES: *Heterodera glycines* Ichinohe, race 3, was obtained from previously established cultures of monoxenic root explant cultures (Lauritis et al., 1982). Nematodes were maintained on excised root tips of *Glycine max* (L.) Merr. cv. Kent grown on Gamborg's B-5 medium (Gamborg et al., 1976; Huettel and Rebois, 1985).

BIOASSAYS: Test solutes were modified from those described by Ward et al. (1984) for the ionic and biological solutions. The following concentrations of solutions were used: 0.001 N HCl, 0.01 N HCl, 0.001 N KOH, 0.01 N KOH, 0.001 N NaOH, 0.01 N NaOH, 0.01 M $Na_2HPO_4$ (pH 8.6), 50%, 10%, and 1% glycerol, and deionized water. The following were made as 0.001 M solutions in deionized water (however, some were first dissolved in 1 ml 95% ethanol before being placed in 99 ml deionized water): guanosine 5′-triphosphate, adenosine 3′:5′-cyclic monophosphate, adenosine 5′-monophosphate, serine, proline, tyrosine, tryptophan, isoleucine, histidine, methionine, threonine, phenylalanine, leucine, alanine, and valine. L-isomers of the amino acids were used.

[1] Mention of a trademark, proprietary product, or pesticide does not constitute a recommendation for its use by the U.S. Department of Agriculture to the exclusion of other suitable products nor does it imply registration under FIFRA as amended.

**Table 1. Response of male *Heterodera glycines* to some ionic solutes and glycerol after 6 hr.**

| Compound | Tracks* | Percent response† |
|---|---|---|
| 0.001 N HCl | – | 0 |
| 0.01 N HCl | – | 0 |
| 0.001 N KOH | + | 0 |
| 0.01 N KOH | + | 0 |
| 0.001 N NaOH | ++ | 0 |
| 0.01 N NaOH | + | 0 |
| 0.01 M $Na_2HPO_4$ | +/– | 0 |
| 50% glycerol | +++ | 50 |
| 10% glycerol | +++ | 30 |
| 1% glycerol | + | 10 |
| $H_2O$ | +/– | 0 |

* –, repelled; +/–, no attraction, random movement; +, positive movement, 1 male or tracks only to disc; ++, positive movement, 3 or more males at disc; +++, positive movement, 5 or more males at disc.

† Percentage of males recovered at disc in four replicates ($N = 40$ males).

**Table 2. Response of male *Heterodera glycines* to some biological compounds after 6 hr.**

| Compound | Number of males at disc* | Percent response |
|---|---|---|
| Adenosine 3′:5′-cyclic monophosphate | 15 | 50 |
| Adenosine 5′-monophosphate | 9 | 30 |
| Alanine | 4 | 13 |
| Guanosine 5′-triphosphate | 0 | 0 |
| Histidine | 15 | 50 |
| Isoleucine | 15 | 50 |
| Leucine | 0 | 0 |
| Methionine | 15 | 50 |
| Phenylalanine | 3 | 10 |
| Proline | 0 | 0 |
| Serine | 9 | 30 |
| Threonine | 0 | 0 |
| Tyrosine | 9 | 30 |
| Tryptophan | 15 | 50 |
| Valine | 0 | 0 |

* Total number of males at disc in three replicates ($N = 30$ males).

The test conditions were modified from those of Papademetriou and Bone (1983). A 7-mm (No. 1 Whatman) filter paper disc (cut with a standard hole punch) was placed on the surface of a petri plate (15 × 60 mm) containing 5 ml of sterile 1.5% AgarNoble. A drop of each solution (ca. 0.01 ml) was placed on the disc. Male nematodes were extracted from root explant plates by a modified Baermann funnel, hand picked into clean deionized water, and aerated for 2 hr to remove any traces of attractants from the culture plates. Ten males were then placed ca. 15 mm from the disc and incubated for 6 and 24 hr at 28°C. Each test solution was replicated a minimum of three times. The test plates were incubated in a moisture chamber to prevent desiccation.

Attractance to or repulsion from a source was determined by presence of the males in or on the discs and tracks on the agar to the disc. After the disc was lifted from the plate, it was soaked in deionized $H_2O$ for 2 hr to allow any entrapped males to move out of the filter paper. The degree of attractiveness of a source was used based on the following ratings: if five or more males were at the disc, the attractiveness was rated positive "+++"; if three or more males were at the disc, positive "++"; if one male was at the disc, positive "+". Negative movement or repellency was considered to have occurred if no males were located at the disc and no tracks to the source were present; negative movement was labelled "–". If tracks were at the source but no males remained at the disc, the movement was considered random "+/–".

All solutions that elicited positive movement were also tested for coiling behavior. Males, extracted as above, were placed in ca. 0.01 ml of all positive test solutions with a dental canal pick. After 30 sec, they were removed and observed (with a Nikon Stereomicroscope) for coiling.

Data were analyzed by chi-square analysis.

## Results

The results of experiments with movement of males to the ionic solutes and to the solutions of glycerol are listed in Table 1. The males appeared to be repelled by both concentrations of HCl. The males exhibited random movement to the other solutes, with tracks to the discs but no males remaining at the source. The glycerol solution was highly attractive to the males, with up to 50% of the males tested exhibiting positive movement to the highest concentration of glycerol. The movement of males to deionized water was not any different from the response to the high-pH buffer tested, resulting in random movement on the petri plate.

The responses of the males to solutions of various amino acids and nucleotides are listed in Table 2. The males were highly attracted to cyclic adenosine monophosphate and some amino acids. The most attractive compounds were adenosine 3′:5′-cyclic monophosphate, histidine, isoleucine, methionine, and tryptophan. The nonattractive amino acids were leucine, threonine, and valine.

The comparisons of 6-hr and 24-hr bioassays showed no significant ($P < 0.001$) differences in the number of males that moved to and remained at the disc. However, there appeared to be more tracks to some discs during the 24-hr bioassays, because the males had a longer period of time to move. Also, it was more difficult to locate all the males after 24 hr, because some had moved downward into the agar instead of staying on the surface.

The following biological compounds that promoted positive movement were tested for induction of coiling behavior: adenosine 3′:5′-cyclic monophosphate, adenosine 5′-monophosphate, histidine, isoleucine, methionine, proline, serine, tyrosine, and tryptophan. None of these solutions resulted in the male coiling behavior previously observed in response to the presumed sex pheromone of *H. glycines*.

## Discussion

This study confirms that other types of chemotaxis aside from those related only to sex pheromones occur in male *H. glycines*. The attractancy and repellency of nematodes to ionic solutes has been demonstrated previously in *Caenorhabditis elegans* by Dusenbery (1980). *Rotylenchulus reniformis, Anguina agrostis,* and *Meloidogyne javanica* exhibited different responses to salts (Riddle and Bird, 1985). The male soybean cyst nematodes in this study appeared to be repelled by acids and moderately attracted to some bases. The role of recognition of these compounds and the subsequent behavioral responses of males are not known at this time. It has been speculated, however, by Riddle and Bird (1985) that the differences in responses of plant-parasitic nematodes may play a role in their distribution in a natural environment. The use of chemotaxis-defective mutants of free-living nematodes, such as *C. elegans,* may further answer questions on recognition of these compounds by nematodes and the role they may play in host and mate finding (Dusenbery, 1980; Zuckerman and Jansson, 1984).

The osmotic potential of the glycerol solutions was not taken into consideration as a possible cause of positive movement in male nematodes. Epps (1963) found that sucrose-induced water potentials over $-3.5 \times 10^5$ Pa are not effective in reduction of hatch of eggs of *Heterodera glycines,* but Clarke et al. (1978) showed them to reduce hatch in other closely related species. No studies have been conducted to determine this effect on males at different water potentials, however.

The soybean cyst nematode males were highly attracted to adenosine 3′:5′-cyclic monophosphate, as males of other species were in previous studies (Ward, 1973; Ward et al., 1984). Larvae of *Neoaplectana carpocapsae* were attracted to some cyclic compounds (Pye and Burman, 1981), as were the plant-parasitic nematode species tested by Riddle and Bird (1985). However, the specific function of this cyclic nucleotide on nematode behavior is unknown at this time.

Ward et al. (1984) demonstrated that *N. brasiliensis* was attracted to several amino acids and other compounds, as were males of *H. glycines*. Many investigators have examined increased metabolic activities in nematode-infected roots. Sidhu and Webster (1977) demonstrated an increase in several amino acids in *M. incognita*-infected roots as compared to uninfected roots. Of these, threonine, histidine, proline, and methionine were found to increase in tomato roots infected with root-knot nematodes. Some of these amino acids were found to be highly attractive to soybean cyst nematode males. It is possible that infected roots themselves are slightly attractive to males even though the males are not thought to feed. This attraction, along with sex pheromones, might help to orient males to sites of females. In the rhizosphere, many chemical cues are being released at any one time, and multiple cues could only help to assure that mate finding is successful. Based on these tests, positive movement to a source could result from one or many chemical compounds found in the soil environment.

The precopulatory and coiling behaviors previously observed in soybean cyst nematode males appear to be associated only with females and their direct pheromonal secretions (Huettel and Rebois, 1986). None of the tested compounds elicited a similar behavioral response. Based on these observations, the rapid bioassays based on coiling appear to be valid.

Further research is needed to understand how chemical communication occurs between nematode individuals and between host and nematode. An understanding of these chemical cues in plant-parasitic nematodes may provide further management strategies through disruption of their life cycles by modifying behavior.

## Literature Cited

**Clarke, A. J., R. N. Perry, and J. Hennessy.** 1978. Osmotic stress and the hatching of *Globodera rostochiensis.* Nematologica 24:385–392.

**Dusenbery, D. B.** 1980. Responses of the nematode *Caenorhabditis elegans* to controlled chemical stimulation. Journal of Comparative Physiology 136:327–331.

**Epps, J. M.** 1963. Effects of sugar treatments on the viability of eggs and larvae in *Heterodera glycines* cysts, and larvae and adults of other nematode species. Plant Disease Reporter 47:180–182.

**Gamborg, O. L., L. Murashige, T. A. Thorpe, and I. K. Vasil.** 1976. Plant tissue culture media. In Vitro 12:473–478.

**Green, C. D.** 1980. Nematode sex attractants. Helminthological Abstracts Series B, Plant Nematology 49:81–94.

**Huettel, R. N.** 1986. Chemical communicators in nematodes. Journal of Nematology 18:3–8.

**———, and R. V. Rebois.** 1985. Culturing nematodes—culturing plant parasitic nematodes using root explants. Pages 155–158 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds., Plant Nematology Laboratory Manual. University of Massachusetts Agricultural Experiment Station, Amherst.

**———, and ———.** 1986. Bioassay comparisons for pheromone detection in *Heterodera glycines,* the soybean cyst nematode. Proceedings of the Helminthological Society of Washington 53:63–68.

**Lauritis, J. A., R. V. Rebois, and L. S. Graney.** 1982. Technique for gnotobiotic cultivation of *Heterodera glycines* Ichinohe on *Glycine max* (L.) Merr. Journal of Nematology 14:422–424.

**Nordlund, D. A., R. J. Jones, and W. J. Lewis.** 1981. Semiochemicals: Their Role in Pest Control. John Wiley and Sons, New York.

**Papademetriou, M. K., and L. W. Bone.** 1983. Chemotaxis of larval soybean cyst nematode, *Heterodera glycines* race 3, to root leachates and ions. Journal of Chemical Ecology 9:387–396.

**Pye, A. E., and M. Burman.** 1981. *Neoplectana carpocapsae*: nematode accumulations on chemical and bacterial gradients. Experimental Parasitology 51:13–20.

**Rende, J. F., P. M. Tefft, and L. W. Bone.** 1982. Pheromone attraction in the soybean cyst nematode, *Heterodera glycines* race 3. Journal of Chemical Ecology 8:981–991.

**Riddle, D. L., and A. F. Bird.** 1985. Responses of the plant parasitic nematodes *Rotylenchulus reniformis, Anguina agrostis* and *Meloidogyne javanica* to chemical attractants. Parasitology 91:185–195.

**Sidhu, G. S., and J. M. Webster.** 1977. The use of amino acid fungal auxotrophs to study predisposition phenomena in root-knot-wilt complex of tomato. Physiological Plant Pathology 11:17–127.

**Ward, J. B., R. M. Nordstrom, and L. W. Bone.** 1984. Chemotaxis of male *Nippostrongylus brasiliensis* (Nematoda) to some biological compounds. Proceedings of the Helminthological Society of Washington 51:73–77.

**Ward, S.** 1973. Chemotaxis of the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by the use of mutants. Proceedings of the National Academy of Sciences of the United States of America 70:817–821.

**Zuckerman, B. M., and H. B. Jansson.** 1984. Nematode chemotaxis and possible mechanisms of host/prey recognition. Annual Review of Phytopathology 22:95–113.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 126–132

# *Ochoterenella caballeroi* sp. n. and *O. nanolarvata* sp. n. (Nematoda: Filarioidea) from the Toad *Bufo marinus*

J. H. ESSLINGER
Tulane Medical Center, 1430 Tulane Avenue, New Orleans, Louisiana 70112

ABSTRACT: *Ochoterenella caballeroi* sp. n. and *O. nanolarvata* sp. n. are described from specimens collected by E. Caballero from the giant toad *Bufo marinus* in Mexico and other localities in Central America. Although the males are not known for either species, the female of *O. caballeroi* is readily distinguished from most of the other members of the genus by the size (all measurements in micrometers) and disposition of the midbody cuticular bosses (approximately 13 long, 31 between bosses, 48 between bands). This species most closely resembles *O. royi* and *O. oumari*, but its microfilaria (82 from vagina, 74 in blood film) is only about half the size of that of the former, and the filiform tail contrasts with the abruptly attenuated, rounded tail of the latter. The combination of features that distinguishes *O. nanolarvata* from the other species of *Ochoterenella* includes the tail (abruptly attenuated with digitiform tip), the flexed muscular portion of the esophagus, the dimensions and arrangement of the midbody cuticular bosses (approximately 11 long, 33 between bosses, 39 between bands), and the minute size (60 from vagina, 37 in blood film) and characteristic shape (robust, abruptly attenuated digitiform caudal extremity) of the microfilariae. With the addition of *O. caballeroi* sp. n. and *O. nanolarvata* sp. n., the total number of species in the genus *Ochoterenella* is brought to 11, all known from bufonids and leptodactylids in the Neotropical region.

KEY WORDS: taxonomy, morphology, microfilariae, blood, toad, Mexico, Costa Rica, Guatemala.

The genus *Ochoterenella* was erected by Caballero (1944) to accommodate certain filarial worms recovered from the body cavity of *Bufo marinus* in Chiapas, Mexico. He subsequently collected several lots of specimens from this host in different regions of Mexico and Central America, and considered them all to belong to the type species, *O. digiticauda*.

During the course of redescribing *O. digiticauda* and redefining the genus (Esslinger, 1986), it was determined that several distinct species were represented in Caballero's collections. Two of these, based on gravid female worms and the microfilarial stage, are herein described as *Ochoterenella caballeroi* sp. n. and *O. nanolarvata* sp. n.

## Materials and Methods

The specimens herein described as new species of *Ochoterenella* were selected after examining several lots of worms obtained from the Helminth Collection of the Instituto de Biología, Universidad Nacional Autónoma de México, México, Distrito Federal, Mexico. These were all labeled as *Ochoterenella digiticauda*, and are listed elsewhere (Esslinger, 1986).

The description of *O. caballeroi* sp. n. is based on two gravid females from IBUNM 106-3 (Caballero's 24 female paratypes of *O. digiticauda* collected in 1943, Huixtla, Chiapas, Mexico) and one gravid female (anterior portion damaged) from IBUNM 144-5 (eight females, one posterior fragment of male collected in 1953, San José, Costa Rica). Additionally, microfilariae from the vagina vera and vagina uterina of the holotype specimen were examined unstained and stained with Azure II in glycerin. A blood film (IBUNM II-64) corresponding to IBUNM 106-3 also provided microfilariae, although this sample also contained *O. digiticauda*.

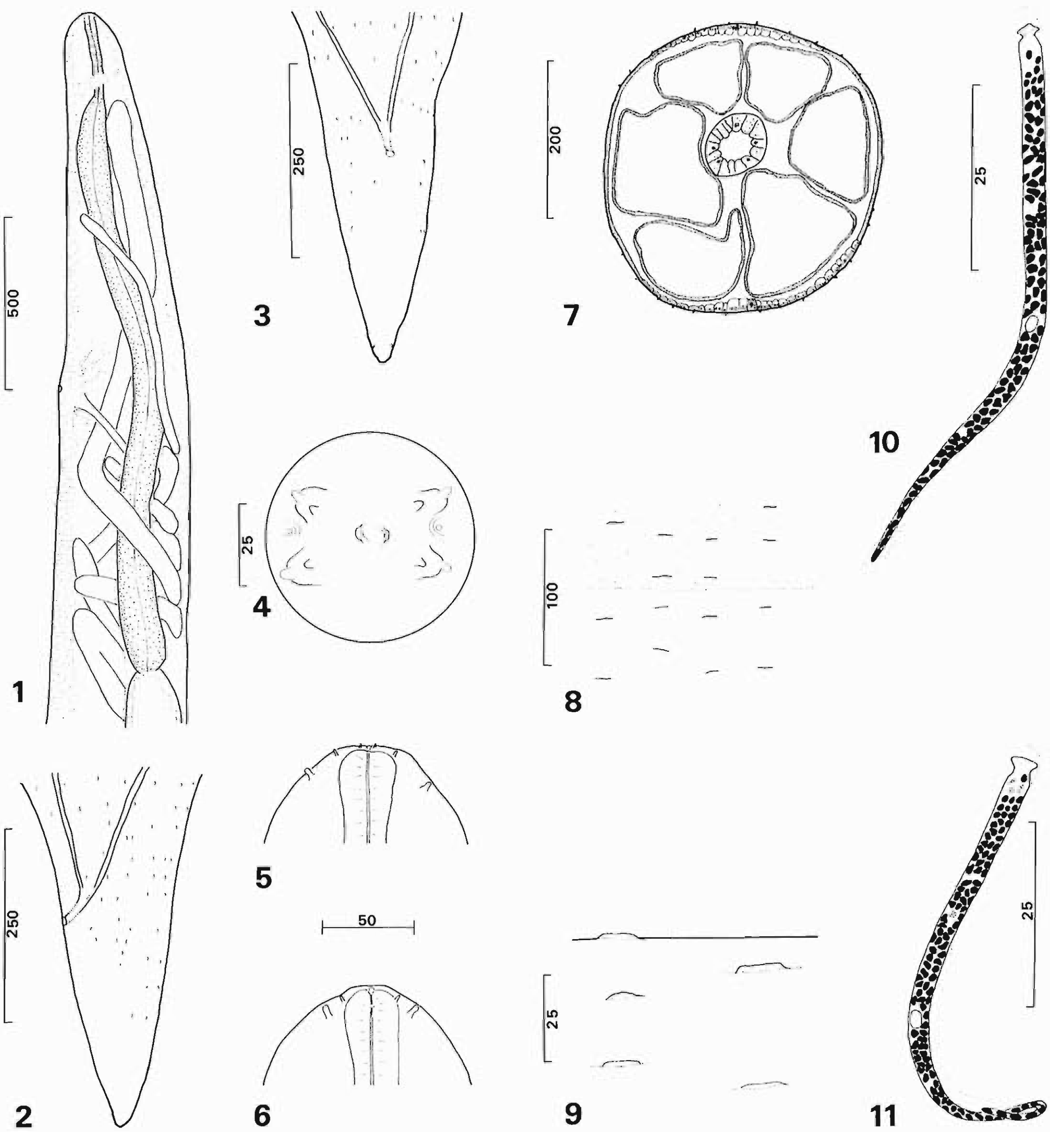
The description of *O. nanolarvata* sp. n. is based on 14 gravid females from IBUNM 128-5 (28 females, two males collected 1947 by Caballero in Tuxtepec, Oaxaca, Mexico). Microfilariae from the vagina and uterus of a broken specimen were examined unstained and stained with Azure II in glycerin. Although two blood films (IBUNM II-72) corresponding to IBUNM 128-5 were examined, only microfilariae of *O. digiticauda* were present. A blood film from *Bufo marinus* in Guatemala provided by Dr. H. Figueroa of the Ministerio de Salud Pública y Asistencia Social in Guatemala, Guatemala, had microfilariae of *O. nanolarvata*, and these are used in the present description.

Adult worms were examined in pure glycerin following slow evaporation from 70% ethanol containing 5% glycerin. Illustrations were made with the aid of a Wild drawing apparatus, and measurements were made with an ocular micrometer. In the following descriptions, all measurements are in micrometers unless otherwise indicated. Dimensions given for individual bosses, distances between bosses, and distances between bands of bosses are based on 30–60 measurements of each feature for each worm. Locations of structures in the microfilariae are given as the distance from the anterior extremity, and are expressed as ranges with the means in parentheses.

## *Ochoterenella caballeroi* sp. n.
## (Figs. 1–11)

### Description

GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae




**Figures 1–11.** ***Ochoterenella caballeroi*** **sp. n. 1. Anterior of female, lateral view. 2. Posterior of female, lateral view. 3. Posterior of female, ventral view. 4. Cephalic extremity of female, en face view. 5. Cephalic extremity of female, dorsal view. 6. Cephalic extremity of female, lateral view. 7. Transverse section in midbody region of female; contents of uterus not shown. Note cuticular bosses on dorsal and ventral surfaces. 8. Bands of cuticular bosses on female, midbody in region of lateral cord (shaded). 9. Detail of midbody bosses of female, lateral view. 10. Microfilaria ex vagina vera, holotype specimen. 11. Microfilaria, blood film from** ***Bufo marinus,*** **IBUNM II-64A.**

Bain and Prod'hon, 1974; *Ochoterenella* Caballero, 1944 (sensu Esslinger, 1986). Male unknown. Female and microfilariae with characters of the genus.

FEMALE (based on 3 gravid specimens; measurements given: holotype followed by paratype, with those of the Costa Rican specimen [when available] enclosed with brackets; Figs. 1–9): Body distinctly attenuated at both extremities (Figs. 1–3); widest posterior to esophago–intestinal junction. Cephalic extremity rounded; cephalic plate not markedly salient; parastomal structures (Figs. 4–6) broad, low, approximately 5–6 wide, 2–3 high. Cuticle with annular bands of low, longitudinally oriented, bacillary bosses (Figs. 8, 9); bands extending over dorsal and ventral margins of lateral cords, discontinuous near midlateral line. Esophagus (Fig. 1) clearly divided; posterior glandular portion approximately 3 times width of anterior muscular part. Body length 49, 44, [58] mm, maximum width 436, 416, [535]. Width of body at nerve ring 209, 168; at junction of muscular and glandular portions of esophagus 216, 187; at esophago–intestinal junction 366, 371; at vulva 366, 358. Cephalic plate 28 by 48, 34 by 58, [38 by 58]. Esophagus total length 1,931, 1,832; muscular portion 226, 267 long by 30, 29 wide; glandular portion 1,705, 1,565 long by 100, 98 wide; ratio of length glandular to muscular 7.54, 5.86. Nerve ring 168, 192, [240] from anterior extremity. Vulva (Fig. 1) not salient, 1,406, 1,104 from anterior extremity, just posterior to middle of glandular portion of esophagus. Tail (Figs. 2, 3) 259, 370, [310] long, slightly constricted subterminally; dorsoventral thickness at level of anus 141, 178, [144]; ratio of tail length to thickness at anus 1.83, 2.08, [2.15]. Cuticular bosses on tail sparse, poorly developed. Midbody bosses as illustrated (Figs. 8, 9). Individual bosses (ranges with means in parentheses) 9–15 (13), 9–15 (12), [9–16 (12)]; distance between bosses 15–48 (31), 17–43 (29), [15–46 (31)]; between bands 38–62 (48), 36–67 (48), [44–69 (56)]. In transverse section at midbody (Fig. 7), cuticle thin, with bosses evident on dorsal and ventral surfaces; lateral cords broad, low; dorsal and ventral cords inconspicuous; musculature low, confined to slightly less than dorsal and ventral quadrants; intestine ⅕–¼ body diameter; uterine loops occupying most of body cavity.

MICROFILARIAE, A, FROM VAGINA VERA AND VAGINA UTERINA OF HOLOTYPE (40 specimens, stained with Azure II in glycerin; Fig. 10): Body slender, cylindrical, with gradual attenuation of posterior ¼; tail distinctly filiform, often reflexed near tip. Sheath present. Cephalic extremity usually set off by subterminal constriction, with expanded, flattened appearance. Cephalic hook difficult to distinguish from terminal expansions. Somatic nuclei ovoid to irregular, column 2 or 3 nuclei wide. Cephalic space often with distinct isolated anterior nucleus, 2 or 3 other nuclei occupying more posterior position. Caudal nuclei elongate, extending nearly to tip, usually posteriormost 5–7 in single file. Body length 76–88 (82), maximum width 3.4–4.1 (3.8). Cephalic space 3.8–5.7 (4.7); nerve ring seldom discerned; excretory space 24–29 (27); Innenkörper ovoid, hyaline, 40–55 (47); anal space 55–69 (62).

MICROFILARIAE, B, IN BLOOD FILM (8 specimens from IBUNM II-64, hematoxylin; Fig. 11): Body slender, posterior ¼ attenuated, tail filiform. Sheath present. Cephalic extremity set off by constriction, expanded, with flattened appearance. Cephalic hook not observed. Somatic nuclei ovoid to irregular, column 2 or 3 nuclei wide. Cephalic space with anterior, darkly staining, isolated nucleus; 2 or 3 more posterior, lightly staining nuclei. Caudal nuclei elongate, 5–8 in single file, extending nearly to tip. Body attitude curved to looped; tail usually sharply flexed 5–9 from tip, nucleus at flexure strongly compressed, often threadlike. Body length 71–78 (74); maximum width at nerve ring 3.2–4.0 (3.4); cephalic space 5.0–6.8 (5.6); nerve ring 17–19 (19); excretory space 23–28 (26); Innenkörper ovoid, hyaline, 37–42 (40); anal space 56–63 (59).

TYPE HOST: *Bufo marinus* Linnaeus, 1758, giant toad.

SITE OF INFECTION: Body cavity.

TYPE LOCALITY: Mexico, Chiapas, Huixtla.

OTHER LOCALITY: Costa Rica, San José.

SPECIMENS DEPOSITED: All specimens are deposited in the Helminth Collection of the Instituto de Biología, Universidad Nacional Autónoma de México, México, Distrito Federal, Mexico. Museum numbers assigned to them have been augmented to distinguish them from the original lots. IBUNM 106-3A-1, holotype female; IBUNM 106-3A-2, paratype female; IBUNM II-64A, slide with blood containing microfilariae (mixed infection); IBUNM 144-5A, additional female from San José, Costa Rica.

ETYMOLOGY: The species is named for Dr. Eduardo Caballero y Caballero.

## Remarks

The lengths of the midbody bosses of *O. caballeroi* (ave. 12–13) readily distinguish it from most of the other species. Those of *O. digiticauda* (ave. 8.7), *O. dufourae* (4–7), and *O. guyanensis* (ave. 5) are shorter, and those of *O. albareti* (20–25) are longer. *Ochoterenella caballeroi* most closely resembles *O. royi* and *O. oumari* in general dimensions and in the measurements and disposition of the midbody bosses. The microfilariae of *O. caballeroi* (77–88 ex utero) are, however, distinctly shorter than those of *O. royi* (130–163 ex utero). The microfilariae of *O. oumari* have an abruptly attenuated tail rounded at the tip, which contrasts with the filiform appearance of the tail of *O. caballeroi.*

Although a detailed comparison with the three species briefly described by Travassos (1929) is not feasible, *O. convoluta* is a smaller worm and the vulva is located at the esophago–intestinal junction. Both *O. scalaris* and *O. vellardi* have longer bosses (20 and 26, respectively), and the latter species has a much longer tail. The worm described as "*O. digiticauda*" from *Bufo paracnemis* in Paraguay by Lent et al. (1946) likewise differs from *O. caballeroi* in that the cuticular bosses as well as the bands are much closer together in the former.

### *Ochoterenella nanolarvata* sp. n.
### (Figs. 12–25)

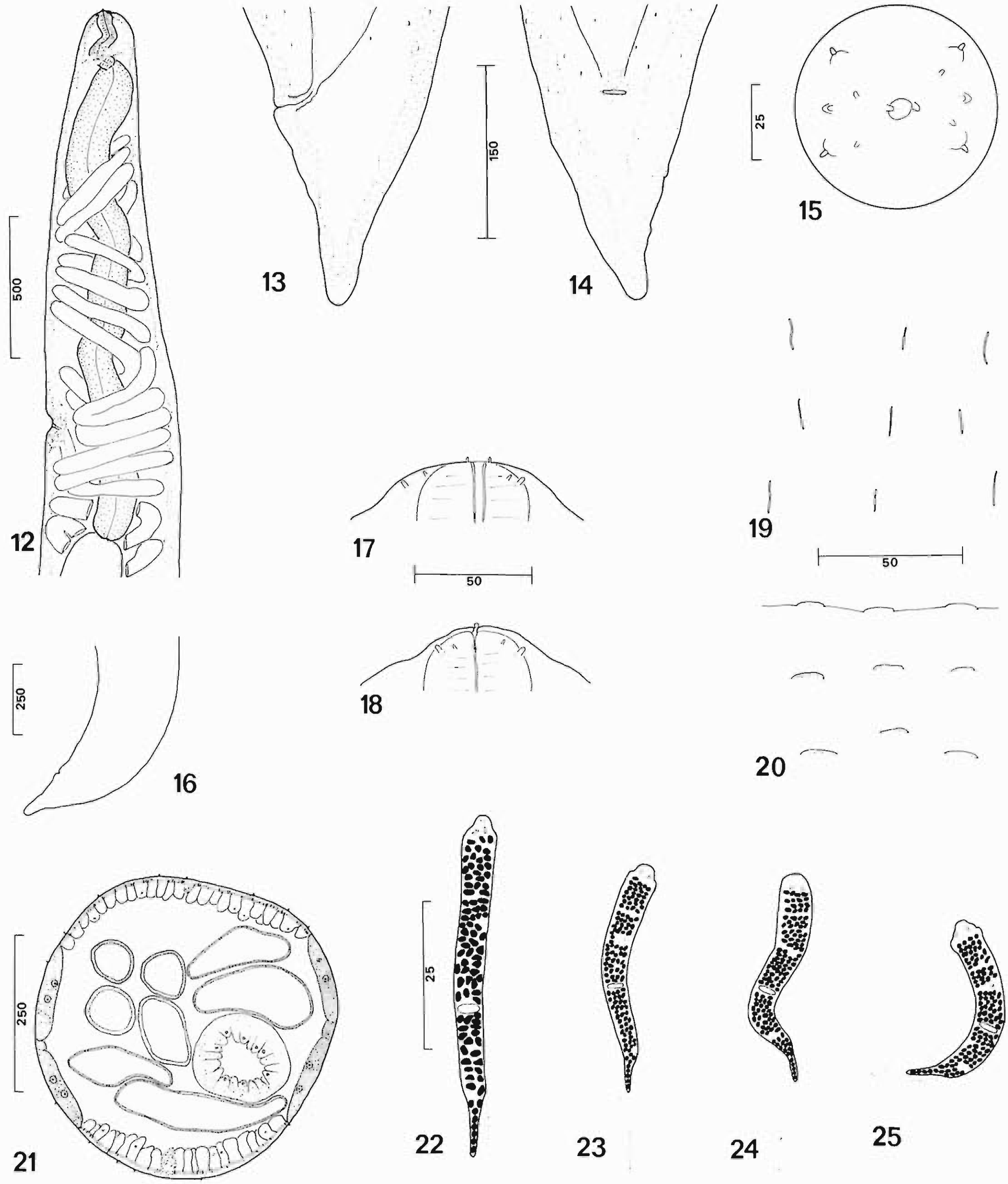
### Description

GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974; *Ochoterenella* Caballero, 1944 (sensu Esslinger, 1986). Male unknown. Female and microfilariae with characters of the genus.

FEMALE (based on 14 gravid specimens; Figs. 12–25): Body robust throughout anterior ⅔, posterior ⅓ gradually attenuated; body widest immediately posterior to esophago–intestinal junction. Both extremities (Figs. 12, 16) attenuated, posterior markedly so. Cephalic extremity (Figs. 15, 17, 18) rounded; cephalic plate only slightly salient; parastomal structures small, low, approximately 2 wide, 2–3 high, 5 apart. Cuticle with annular bands of low, slender, longitudinally oriented bacillary bosses (Figs. 19, 20); bands extending over dorsal and ventral margins of lateral cords, discontinuous near midlateral line. Esophagus (Fig. 12) clearly divided; anterior muscular portion flexed in all specimens; posterior glandular portion 3–5 times width of muscular. Body length 38.8–47.9 (43.1) mm, maximum width 485–594 (528). Width of body at nerve ring 192–247 (214); at junction of muscular and glandular portions of esophagus 213–282 (241); at esophago–intestinal junction 406–505 (454); at vulva 386–446 (410). Cephalic plate 31–45 (36) by 58–67 (62). Esophagus total length 1,724–2,316 (1,927); muscular portion 216–336 (263) long, 34–53 (45) wide; glandular portion 1,436–1,851 (1,665) long, 132–183 (162) wide; ratio of length glandular to muscular 5.00–8.57 (6.44). Nerve ring 144–216 (175) from anterior extremity. Vulva (Fig. 12) in depressed or retracted area of body wall, usually just anterior to esophago–intestinal junction (slightly posterior in 1 specimen), 1,197–1,960 (1,511) from anterior extremity. Tail (Figs. 13, 14, 16) 144–320 (211) long, abruptly attenuated with digitiform tip; cuticular bosses lacking or rarely occurring posterior to anus; anal region slightly salient, dorsoventral thickness of body at level of anus 114–201 (145); ratio of tail length to thickness at anus 1.17–1.67 (1.43). Midbody cuticular bosses as illustrated (Figs. 19, 20). Individual bosses 8–15 with averages for each worm ranging from 9.7 to 11.5 (overall mean for 14 worms 10.7); distance between bosses with range of averages 28–37 (overall mean 33); distance between bands (center to center) with range of averages 35–44 (overall mean 39). In transverse section at anterior ⅓ (Fig. 21), cuticle thin, with bosses evident on dorsal and ventral surfaces; lateral cords well developed, broad, low, with conspicuous nuclei, demarcated median portion at level of lateral line; dorsal and ventral cords inconspicuous; musculature moderately well developed, confined to dorsal and ventral quadrants; intestine approximately ⅓ body diameter; uterine loops occupying most of body cavity.

MICROFILARIAE, A, FROM VAGINA OF FIXED WORM, IBUNM 128-5 (80 specimens; Fig. 22): Body robust, anterior extremity often narrowed in region of cephalic space giving shouldered appearance; diameter gradually decreasing posteriorly; caudal extremity abruptly attenuated, resulting in short (5–8), digitiform tail with narrow, rounded tip. Sheath present, extending slightly beyond extremities. Cephalic hook inconspicuous. Somatic nuclei ovoid to irregular, column 3 or 4 nuclei wide. Cephalic space with pair of large nuclei. Caudal nuclei spheroid to ovoid, the posteriormost 6 or 7 within narrow portion of tail in single file, nearly reaching tip. Body length 51–67 (60), maximum width just behind cephalic space 5.1–6.2 (5.7). Cephalic space 1.8–4.0 (2.9); nerve ring often obscure, 12–19 (15); excretory space 20–31 (23). Innenkörper ovoid, hyaline, with long axis usually transverse, 29–38 (33). Anal space 42–56 (50), just anterior to caudal attenuation.

MICROFILARIAE, B, IN BLOOD FILM, FROM *BUFO MARINUS* IN GUATEMALA (40 specimens, hematoxylin and eosin; Figs. 23–25): In general appearance, remarkably small and stubby. Shape of anterior extremity varies with state of con-




**Figures 12–25.** ***Ochoterenella nanolarvata*** **sp. n. 12. Anterior of female, lateral view. 13. Caudal extremity of female, lateral view. 14. Caudal extremity of female, ventral view. 15. Cephalic extremity of female, en face view. 16. Posterior of female, illustrating profile of tail region. 17. Cephalic extremity of female, dorsal view. 18. Cephalic extremity of female, lateral view. 19. Midbody cuticular bosses of female, surficial view. 20. Midbody cuticular bosses of female, lateral view. 21. Transverse section of female at anterior one-third; contents of uterine loops not shown. 22. Microfilaria from vagina of fixed specimen (IBUNM 128-5B). 23–25. Microfilariae from blood film of** ***Bufo marinus*** **in Guatemala. Note variation in appearance of anterior extremity resulting from contraction.**

traction; most specimens similar to those from vagina of fixed worm with cephalic space constricted and distinct shoulders present (Fig. 23), some lack constriction (Fig. 24), others with constriction and collar-like expansion of body at posterior margin of cephalic space (Fig. 25). Body gradually attenuated posteriorly with caudal extremity abruptly attenuated and digitiform, 5–7 long; tip narrow, rounded; tail often flexed at angle (up to 90°) to longitudinal axis of body. Sheath present, approximately 60 long, extending well beyond extremities. Cephalic hook inconspicuous in most specimens. Cephalic space with pair of isolated, ovoid nuclei. Somatic nuclei spheroid to ovoid, densely packed, column 3–5 nuclei wide; caudal region with 5–7 nuclei in single file, nearly reaching tip. Body length 34–43 (37), maximum width just behind cephalic space 5.2–6.0 (5.6). Cephalic space 1.9–3.4 (2.6); nerve ring 8–11 (10); excretory space 11–14 (13); Innenkörper distinct, ovoid, with long axis transverse, 18–23 (20); anal space 27–33 (29).

TYPE HOST: *Bufo marinus* Linnaeus, 1758, giant toad.

SITE OF INFECTION: Body cavity.

TYPE LOCALITY: Mexico, Oaxaca, Tuxtepec.

OTHER LOCALITY: Guatemala, Guatemala (based on microfilaria only).

SPECIMENS DEPOSITED: All specimens are deposited in the Helminth Collection, Instituto de Biología, Universidad Nacional Autónoma de México, México, Distrito Federal, Mexico. Museum numbers assigned to them have been augmented to distinguish them from the original lots. IBUNM 128-5B-1, holotype female; IBUNM 128-5B-2.01 to 128-5B-2.12, 12 paratype females; IBUNM 128-5B-3, microfilariae in blood film from *Bufo marinus* in Guatemala.

ETYMOLOGY: The specific name *nanolarvata* (dwarf + larva) refers to the exceptionally short, stubby microfilariae of this species.

## Remarks

The combination of features that distinguishes the female of *O. nanolarvata* from those of the other known species of *Ochoterenella* includes the tail (abruptly attenuated with digitiform tip, absence of cuticular bosses posterior to anus), the muscular portion of the esophagus (flexed), the body in the vicinity of the vulva (depressed), and the dimensions and arrangement of the midbody cuticular bosses (approximately 11 long, 33 between bosses, 39 between bands). The size and shape of the microfilarial stage (approximately 60 from vagina, 37 in blood films; robust, abruptly attenuated, digitiform caudal extremity) likewise separate *O. nanolarvata* from the other species.

The small size of the microfilariae of the present species is approached only by those of *O. albareti* (62–68 from uterus) described by Bain et al. (1979), and *O. caballeroi* sp. n. (77–88 from vagina; 71–78 in blood film). Compared to *O. nanolarvata,* however, the female of the former species has longer cuticular bosses (20–25) with the bands closer together (18–25), and the tail lacks the digitiform tip and is provided with prominent bosses. The microfilariae of *O. caballeroi* are notably more slender than those of *O. nanolarvata* (width approximately 3–4 vs. 5–6), and have a gradually attenuated, filiform caudal extremity; the tail of the adult female has distinct, although sparse, bosses.

Although the microfilariae are unknown for *O. convoluta* and *O. scalaris* from *Leptodactylus* and *O. vellardi* from *Bufo marinus* collected in Brazil (Travassos, 1929), the midbody bosses of these species are longer (approaching 15–20) than those of *O. nanolarvata.*

None of Caballero's male worms examined corresponded to the females of either *O. caballeroi* sp. n. or *O. nanolarvata* sp. n. on the basis of the lengths of the body bosses. This criterion appears to be reliable, as noted in previous observations (Bain and Prod'hon, 1974; Bain et al., 1979; Esslinger, 1986). Further collections in the type localities will be necessary to obtain and describe the males of these species.

The present report brings the total number of species in the genus to 11, all described from anuran hosts in the Neotropical region. *Ochoterenella digiticauda* (type species), *O. vellardi, O. guyanensis, O. royi, O. oumari, O. dufourae, O. albareti, O. caballeroi* sp. n., and *O. nanolarvata* sp. n. have been recovered from *Bufo marinus.* Two species, *O. convoluta* and *O. scalaris,* were found in the frogs *Leptodactylus pentadactylus* and *L. ocellatus,* respectively.

**Acknowledgments**

I express my appreciation to M. en C. Rafael Lamothe-Argumedo and Dr. Alejandro Cruz-Reyes of the Instituto de Biología, Universidad Nacional Autónoma de México, Dr. H. Figueroa-M. of the Ministerio de Salud Pública y Asistencia Social, S.N.E.M., Laboratorio de Investigacion Cientifica "Dr. Isao Tada," Guatemala, Guatemala, and Drs. A. G. Chabaud and O. Bain, Muséum d'Histoire Naturelle, Paris, for generously providing the specimens involved in this study.

## Literature Cited

**Bain, O., D. C. Kim, and G. Petit.** 1979. Diversité spécifique des filaires du genre *Waltonella* coexistant chez *Bufo marinus.* Bulletin du Muséum National d'Histoire Naturelle, Paris (4th series) 1(section A):199–212.

———**, and J. Prod'hon.** 1974. Homogenéité des filaires de batracien des genres *Waltonella, Ochoterenella* et *Madochotera*: création des Waltonellinae n. subfam. Annales de Parasitologie Humaine et Comparée 49:721–739.

**Caballero, E.** 1944. Estudios helmintologicos de la region oncocercosa de Mexico y de la Republica de Guatemala. Nematoda: 1a parte. Filarioidea. I. Anales del Instituto de Biología, México 15:87–108.

**Esslinger, J. H.** 1986. Redescription of *Ochoterenella digiticauda* Caballero, 1944 (Nematoda: Filarioidea) from the toad *Bufo marinus,* with a redefinition of the genus *Ochoterenella* Caballero, 1944. Proceedings of the Helminthological Society of Washington 53:210–217.

**Lent, H., J. F. T. de Freitas, and M. C. Proença.** 1946. Alguns helmintos de batráquios colecionados no Paragui. Mémorias do Instituto Oswaldo Cruz 44: 195–214.

**Travassos, L.** 1929. Filarides des batraciens du Brésil. Comptes Rendus des Séances de la Société de Biologie, Paris 100:967–968.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 133–140

# *Dirofilaria immitis*: Fine Structure of Cuticle During Development in Dogs

J. R. LICHTENFELS,[1] P. A. PILITT,[1] AND W. P. WERGIN[2]
[1] Biosystematic Parasitology Laboratory, Animal Parasitology Institute and
[2] Plant Stress Laboratory, Plant Physiology Institute,
Agricultural Research Service, USDA, Beltsville, Maryland 20705

ABSTRACT: Larval nematodes, *Dirofilaria immitis* (Leidy, 1856), collected from mosquitoes 14 or 15 days after inoculation (DAI) and from dogs 3, 9, 12, 15, 21, 30, 41, 50, and 58 DAI were examined with a transmission electron microscope to document evidence of nematode molts during development in dogs. The cuticle of the infective larva was less than 1 μm thick and consisted of three layers; no evidence was seen of the cuticle of the next stage. At 3 DAI the surface of the larval cuticle was deeply annulated and appeared similar to fourth-stage cuticle described previously by others in *D. immitis* in the third molt in vitro. Beginning 9 DAI and continuing through 21 DAI, an electron-dense fibrous layer and then an electron-translucent layer formed next to the hypodermis. At 30–50 DAI annulations believed to be the surface membrane of the new fifth-stage cuticle were formed in the electron-dense fibrous layer. At 58 DAI the fifth-stage cuticle had an annulated surface layer, a thin electron-dense layer, and a thick electron-translucent internal layer consisting of three sublayers. These results support the conclusions of a previous study of the morphogenesis of *D. immitis,* which indicated that the third molt occurs just prior to 3 DAI and the fourth molt about 58 DAI in dogs.

KEY WORDS: heartworm, nematode, molting larvae, *Canis familiaris,* transmission electron microscopy.

An earlier report described the morphogenesis of *Dirofilaria immitis* (Leidy, 1856) in the dog (Lichtenfels et al., 1985). That study and the present one used developmental stages of *D. immitis* recovered in a study by Kotani and Powers (1982). The series of specimens collected by Kotani and Powers (1982) is one of the most complete series of developmental stages of *D. immitis* ever collected. The availability of these specimens provided an opportunity to obtain additional information on the morphogenesis of the heartworm of dogs through a study of the fine structure of the cuticle.

The study of the morphogenesis of *D. immitis* by Lichtenfels et al. (1985) provided evidence that the third molt occurs prior to 3 days after inoculation (DAI) in dogs rather than 9–12 DAI as reported by Orihel (1961). The present report describes the fine structure of the cuticle of *D. immitis* infective larvae from mosquitoes and developmental stages collected from dogs 3–58 DAI. The results provide additional evidence on the time of the molts in the dog.

## Materials and Methods

Specimens of the heartworm, *D. immitis,* were collected from beagle dogs, *Canis familiaris* L., in a previous project reported by Kotani and Powers (1982) and were used in an earlier study of morphogenesis reported by Lichtenfels et al. (1985). Specimens selected for this study were collected from dogs 3, 9, 12, 15, 21, 30, 41, 50, and 58 DAI and from the mosquito, *Aedes aegypti* (L.) (Liverpool strain), 14 or 15 DAI (Kotani and Powers, 1982). The available specimens had been fixed in hot (60°C) 5% neutral buffered formalin.

For transmission electron microscopy (TEM), two specimens from each collection period were postfixed for 2 hr in 2% osmium tetroxide and dehydrated and embedded using the methods of Wergin and Endo (1976). All sections were longitudinal or sagittal from the middle region of the nematodes. Terminology for stage of development follows that of Douvres et al. (1969).

There is no accepted terminology for describing the various layers of nematode cuticles (Bird, 1971), although Chitwood and Chitwood (1950), Maggenti (1979), and Bird (1980) have proposed standard terminologies for this purpose. This report avoids naming the layers, which are minimal in larvae, and instead describes changes occurring in (1) the hypodermis, (2) the external or older cuticle of the stage being described, and (3) the new cuticle. The layers of the cuticle are described, but the determination of the proper terminology for the layers is avoided until a sufficient number of nematodes from various groups has been studied to allow meaningful comparisons to be made.

## Results

INFECTIVE LARVA (third stage, Fig. 1): Infective larvae collected from mosquitoes had a hypodermis of uniform thickness, about 0.25 μm. The cuticle consisted of three layers: an amorphous inner layer about 0.5 μm thick, a middle layer consisting of a thin electron-dense band, and an external surface layer of uneven thickness (10–20 μm) due to a low, flat surface annulation.

1

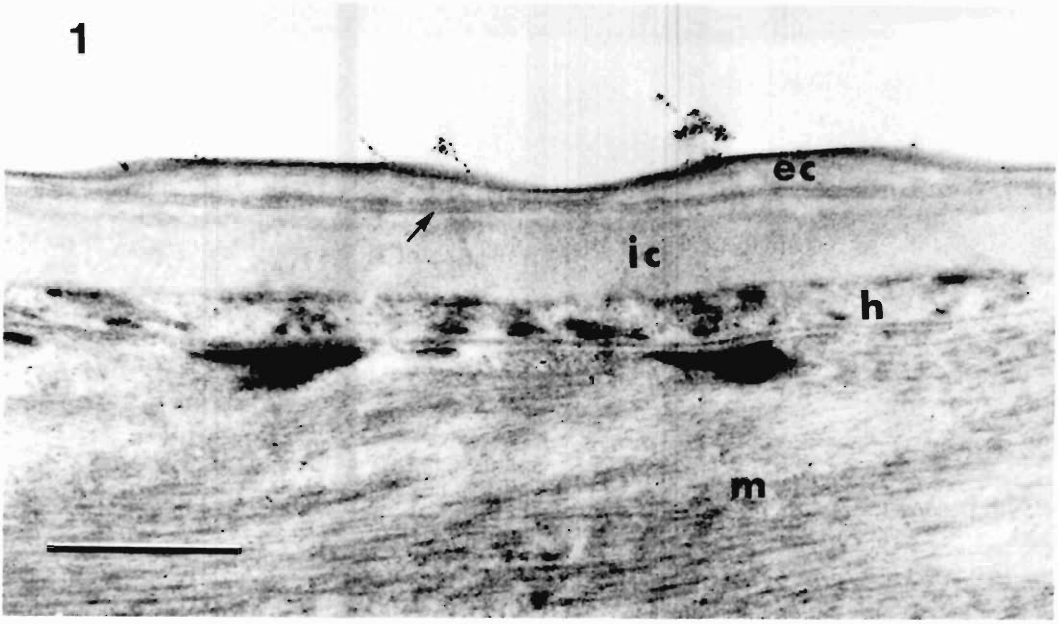



**Figure 1. Electron micrograph of cuticle of infective larva of *Dirofilaria immitis* collected from mosquito mouthparts 14 DAI. Longitudinal section near midbody, showing layer of somatic muscle (m) adjacent to the hypodermis (h), and a cuticle consisting of three layers including a thick internal layer (ic), a thin electron-dense layer (arrow), and an external surface layer (ec) of uneven thickness. The external surface of the external layer (ec) has a thin electron-dense external surface. Scale bar = 1 $\mu$m.**

The external surface layer includes an amorphous internal layer and a thin electron-dense external layer. There was no evidence of the presence of more than one cuticle at this stage of development.

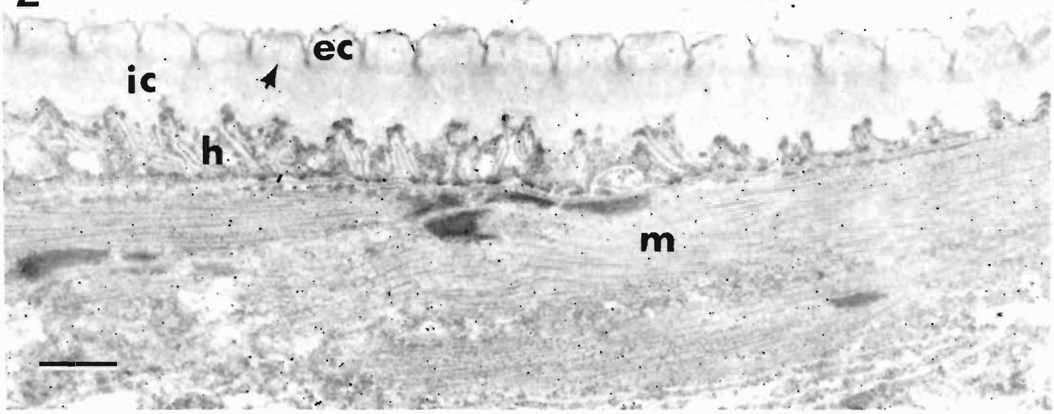
EARLY FOURTH STAGE (Fig. 2): At 3 DAI the hypodermis showed regular extensions (plicae) ending in electron-dense beads that extended into the cuticle. The cuticle continued to exhibit three layers: an internal amorphous layer, a thin electron-dense band, and an external layer with regular convolutions or annuli. The number of plicae present was directly proportional in a 1:1 relationship to the number of annuli present on the surface of the cuticle. The surface annuli were a feature of the external cuticle only, and did not extend into or below the thin electron-dense band.

MID-FOURTH STAGE (Figs. 3–7): At 9–15 DAI an electron-dense fibrous layer had appeared near the base of the internal layer of cuticle and an electron-translucent layer had developed between the fibrous layer and the hypodermis (Figs. 3, 5). The hypodermis in this stage was marked by irregular electron-dense banding. The annulation on the surface of the cuticle was usually low and regular, but was somewhat variable in different specimens and different parts of the same specimen, perhaps due to uneven fixation or contraction of the body wall. The main differences between the mid-fourth stage at 9–15 DAI and the earlier fourth stages were the lower, wider annulations in the external layer of the cuticle and the more regular thickness of the hypodermis in the mid-fourth larvae. Both of these changes could result from stretching a contracted body wall. At 21 DAI the electron-translucent layer was present separating the fibrous layer from the hypodermis (Fig. 6). At 30 DAI the electron-

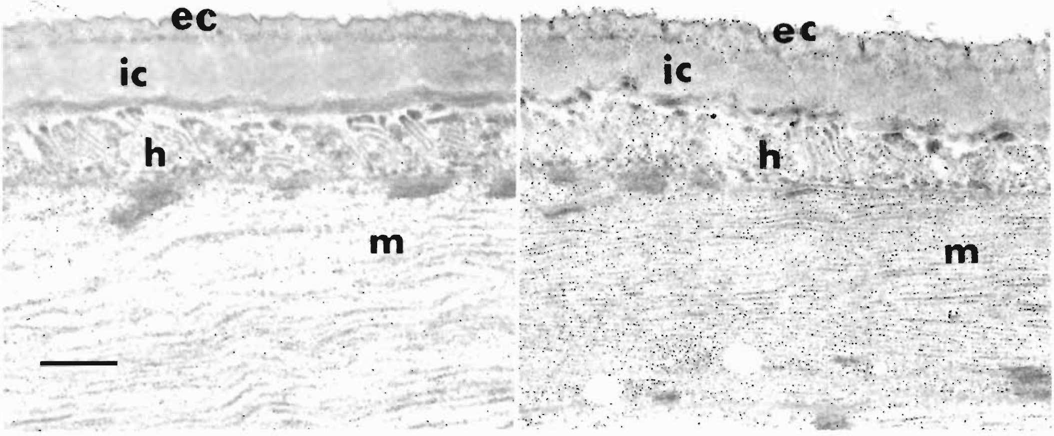
⟶

**Figures 2–4. Electron micrographs of cuticles of larval *Dirofilaria immitis* from dogs 3–12 DAI. Scale bars = 1 $\mu$m. Abbreviations: muscle (m), hypodermis (h), internal cuticle (ic), external cuticle (ec). 2. Early fourth-stage larva, 3 DAI, showing somatic muscles, hypodermis with numerous electron-dense bands, and plicae or folds that project into the internal cuticle and correspond with tall annules on the surface of the cuticle. The**

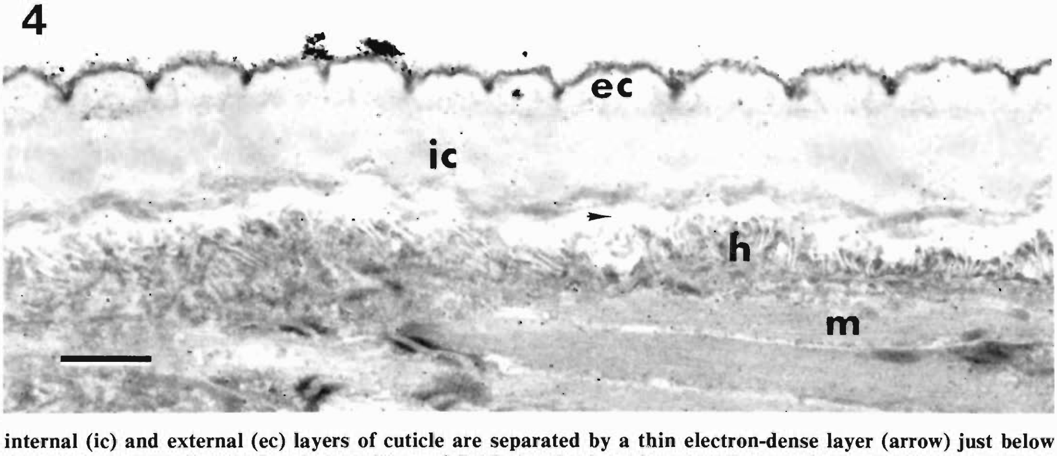
2



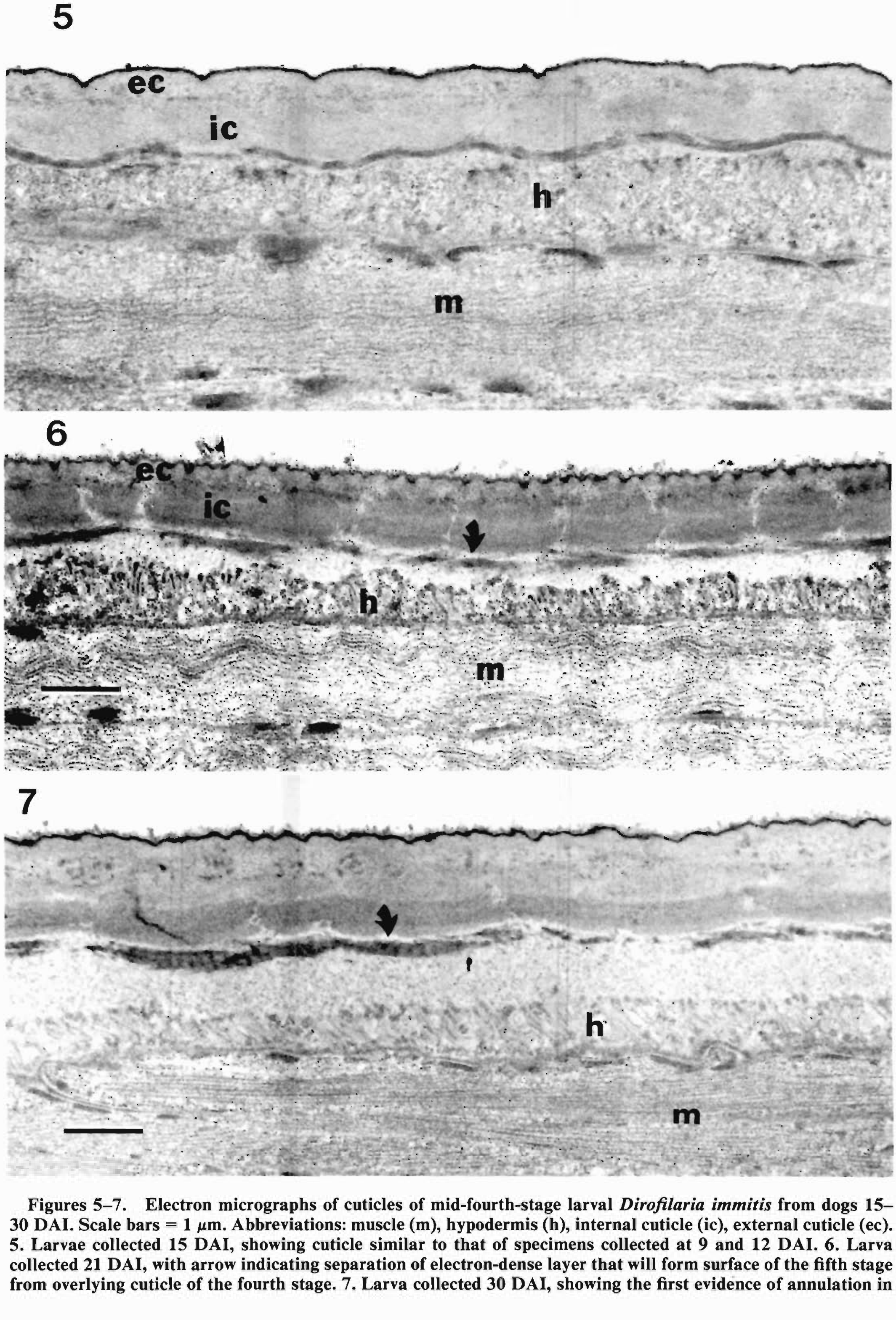

3a 3b




4




**internal (ic) and external (ec) layers of cuticle are separated by a thin electron-dense layer (arrow) just below the annulation. 3A, B. Mid-fourth-stage larvae, 9 DAI, showing hypodermis without projections but with numerous electron-dense bands, an electron-dense fibrous layer above and adjacent to the hypodermis, and a cuticle with short surface annules (A and B are different specimens). 4. Mid-fourth-stage larva, 12 DAI, similar to larvae at 9 DAI but with a new electron-translucent layer (arrow) adjacent to hypodermis (h). The hypodermis is of irregular thickness and has electron-dense bands. The external surface layer of the cuticle (ec) is more markedly annulated than in the 9-DAI specimens.**




**Figures 5–7.** Electron micrographs of cuticles of mid-fourth-stage larval *Dirofilaria immitis* from dogs 15–30 DAI. Scale bars = 1 μm. Abbreviations: muscle (m), hypodermis (h), internal cuticle (ic), external cuticle (ec). 5. Larvae collected 15 DAI, showing cuticle similar to that of specimens collected at 9 and 12 DAI. 6. Larva collected 21 DAI, with arrow indicating separation of electron-dense layer that will form surface of the fifth stage from overlying cuticle of the fourth stage. 7. Larva collected 30 DAI, showing the first evidence of annulation in

translucent layer adjacent to the hypodermis was much thicker and the fibrous layer had changed from a layer of longitudinal fibers to form a palisade-like line of convolutions (annulations), each with a rounded extremity oriented away from the hypodermis and toward the external surface of the cuticle (Fig. 7).

LATE FOURTH STAGE (Figs. 8, 9): At 41 and 50 DAI the palisade-like layer was present, and although similar at 41 DAI (Fig. 8) to earlier stages, by 50 DAI the palisade-like annulations were taller and closer together (Fig. 9). The electron-translucent layer was present next to the hypodermis, which had large vesicles (Fig. 8).

FOURTH MOLT (Fig. 10): At 58 DAI the cuticle of the fourth stage was separated from the underlying annulated cuticle of the fifth stage. The cuticle of the fourth stage at 58 DAI appeared to be thicker than at 50 DAI (Fig. 9) because of the apparent dispersion of part of the fibrous layer (below arrow, Fig. 10) that formed the annulated surface of the fifth-stage cuticle. An electron-translucent granular layer (Fig. 10) was present between the remnants of the fibrous layer and the annulated surface of the cuticle of the fifth stage. The fifth-stage cuticle consisted of three layers: a deeply annulated external surface layer, a thin electron-dense layer, and a thick electron-translucent layer composed of three sublayers (Fig. 10).

## Discussion

Infective larvae from mosquitoes had a cuticle with three layers, but no evidence of the cuticle of the fourth stage was present. Devaney (1985) reported identical findings in infective larvae of *D. immitis,* but he did not provide an electron micrograph of the infective larva. The cuticle of the infective larva of *Wuchereria bancrofti* (Cobbold, 1877) was described by Weber (1984) as thick and composed of three layers similar to those described here for *D. immitis.* The transverse surface striation we described in *D. immitis* infective larvae was observed by Hendrix et al. (1984) with scanning electron microscopy.

The appearance of the larval cuticle at 3 DAI was consistent with that of a specimen recently molted. The cuticle, with one hypodermal plica per annule, was similar to the fourth-stage cuticle illustrated by Devaney (1985) in a molting specimen of *D. immitis* after 60 hr in vitro.

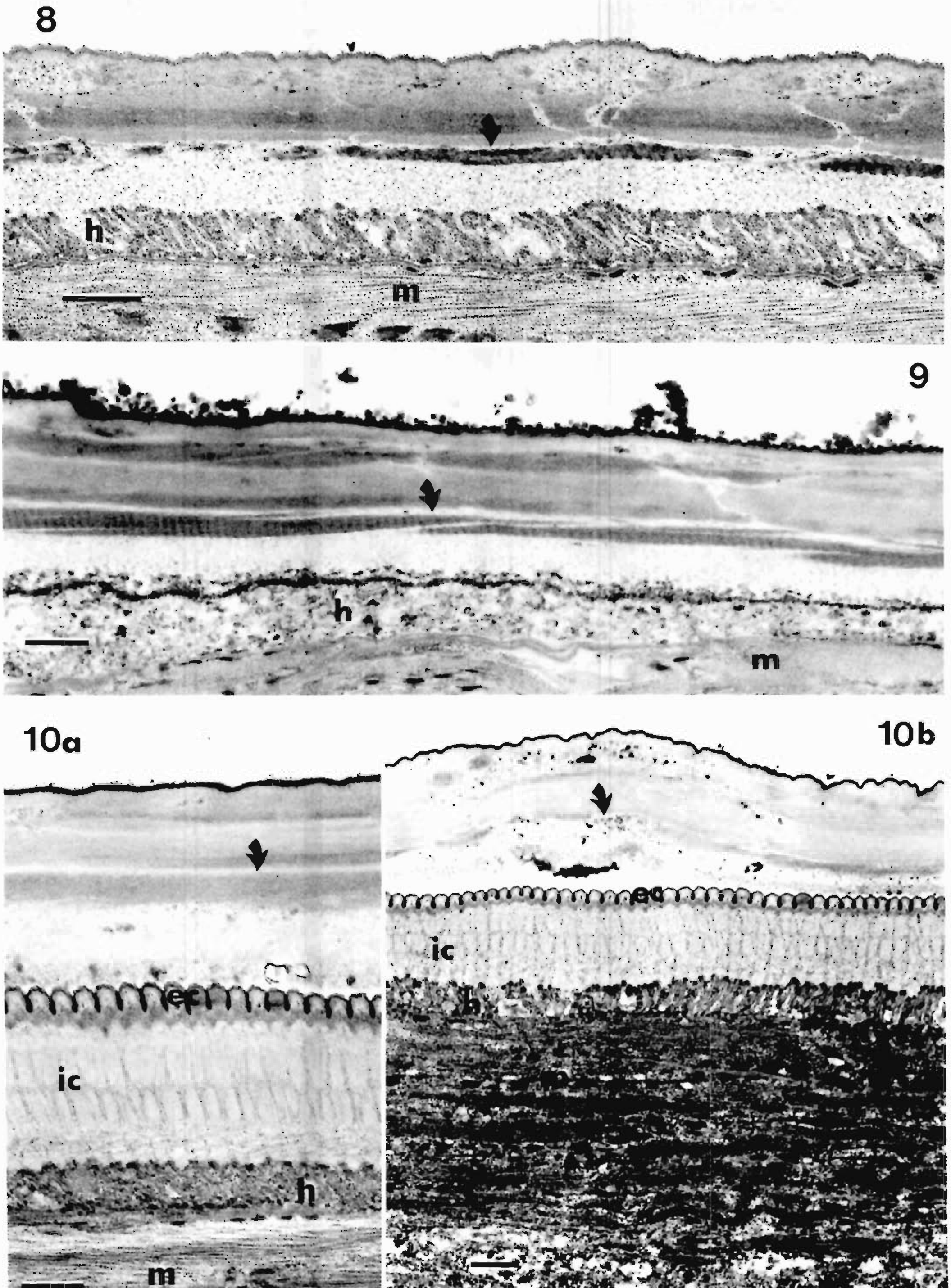
The lack of any evidence of the fourth-stage cuticle in infective larvae in dogs reported here and by Devaney (1985) in in vitro-grown larvae and the evidence that the third molt occurs prior to 3 DAI in dogs (Lichtenfels et al., 1985) and by 3 to 4 DAI in vitro (Sawyer, 1965; Devaney, 1985) indicates a rapid process of cuticle formation in this period. Further investigation of cuticular changes in this period should provide interesting information.

By 9 and 12 DAI the tall annuli seen at 3 DAI had stretched out to show longer and flatter annuli, and the first phases of formation of the fifth-stage cuticle had begun—the formation of an electron-dense fibrous layer. The deposition of this fibrous layer by the hypodermis may be equivalent to the formation and deposition of "peg-like processes" by the hypodermis of *Syphacia obvelata* (Rudolphi, 1802) larvae in the early stages of the formation of a new cuticle (Dick and Wright, 1973). More recently, Weber (1984) showed a similar deposition of fibers by the hypodermis in first- and second-stage larvae of *W. bancrofti.* Kozek (1971) described the first stage of formation of a new cuticle in *Trichinella spiralis* (Owen, 1835) larvae as an accumulation of dense material on the surface of the hypodermis. Also, it appears that the first evidence of the formation of a new cuticle in third- and fourth-stage larvae of *Brugia pahangi* (Buckley and Edeson, 1956) was the appearance of an electron-dense fibrous layer adjacent to the hypodermis (Howells and Blainey, 1983).

The second phase in the formation of the fifth-stage cuticle was the formation of an annulated surface membrane, first seen at 30 DAI, in the electron-dense fibrous layer. The electron-dense fibrous layer was also the site of the formation of the annulated surface membrane of the new cuticle in *B. pahangi* as described by Howells and Blainey (1983). Similarly, the annulated surface membranes of *S. obvelata* and *W. bancrofti* were formed at the site of, and following the

←

**the electron-dense layer (arrow) and a much thicker electron-translucent layer adjacent to the hypodermis than seen in earlier phases of development.**




**Figures 8–10. Electron micrographs of cuticles of *Dirofilaria immitis* from dogs 41–58 DAI. Scale bars = 1 μm. Abbreviations: muscle (m), hypodermis (h), internal cuticle (ic), external cuticle (ec). 8. Late fourth-stage larva, 41 DAI, showing the hypodermis with large vesicles, and annulations of the developing surface membrane**

deposition of, "peg-like processes" to form a fibrous layer (Dick and Wright, 1973; Weber, 1984).

The cuticle of the fifth stage consisted of several layers at 58 DAI that were not present at 50 DAI. The origin of the thin electron-dense layer and the thick internal layer with its three sublayers and the time of their formation could not be determined from a comparison of specimens collected at 50 and 58 DAI. Unfortunately, specimens were not collected between 50 and 58 DAI. The three sublayers in the thick internal cuticle appear to be equivalent to the three layers of oblique fibers described in the cuticle of adult *D. immitis* and other nematodes by Chitwood and Chitwood (1950).

The changes observed in the fine structure of the cuticle provide additional evidence on the times of the molts of *D. immitis* occurring in the dog. The appearance of the cuticle of infective larvae from mosquitoes and the cuticle of larvae collected from dogs at 3 DAI indicates the third molt had occurred in dogs prior to 3 DAI. No evidence of the fourth molt was observed until 50–58 DAI. Thus, the results of the present study agree with the results of numerous in vitro studies (including, but not limited to Sawyer (1965) and Devaney (1985)), a study of *D. immitis* development in a ferret (pers. comm., P. Supakorndej, J. J. Jun, and J. W. McCall, College of Veterinary Medicine, University of Georgia, Athens, Georgia), and a study of the morphogenesis of *D. immitis* in dogs by Lichtenfels et al. (1985), who all concluded the third molt occurs about 3 days after inoculation into a definitive host or host substitute.

## Acknowledgments

We thank Gretchen Kaminski, EM Laboratory, Beltsville Agricultural Research Center, for processing and photographing specimens for electron microscopy. We thank Dr. Kendall G. Powers, Bureau of Veterinary Medicine, Food and Drug Administration, Beltsville, Maryland (presently, National Cancer Institute, Bethesda, Maryland), and Dr. T. Kotani, University of Osaka, Osaka, Japan, for making available their specimens collected from dogs; and Catherine A. Palmer, University of Wisconsin–Eau Claire, for the opportunity to examine electron micrographs of developing stages of *D. immitis* in the mosquito. Peer review of this paper was handled by Dr. David R. Lincicome, Emeritus Editor of *Experimental Parasitology* and currently Editor of *International Goat and Sheep Research,* as a courtesy to protect the confidentiality of the review process and to avoid any appearance of conflict of interest because the first two authors edit the *Proceedings.*

## Literature Cited

**Bird, A. F.** 1971. The Structure of Nematodes. Academic Press, New York. 318 pp.

———. 1980. The nematode cuticle and its surface. *In* B. M. Zuckerman, ed. Nematodes as Biological Models. Vol. 2. Academic Press, New York. 306 pp.

**Chitwood, B. G., and M. B. Chitwood.** 1950. The external cuticle and hypodermis. Pages 28–42 *in* B. G. Chitwood and M. B. Chitwood. An Introduction to Nematology. Monumental Printing Company, Baltimore. [Reprinted 1974, University Park Press, Baltimore.]

**Devaney, E.** 1985. *Dirofilaria immitis*: the moulting of the infective larva in vitro. Journal of Helminthology 59:47–50.

**Dick, T. A., and K. A. Wright.** 1973. The ultrastructure of the cuticle of the nematode *Syphacia obvelata* (Rudolphi, 1802). I. The body cuticle of larvae, males, and females, and observations on its development. Canadian Journal of Zoology 51: 187–196.

**Douvres, F. W., F. G. Tromba, and G. M. Malakatis.** 1969. Morphogenesis and migration of *Ascaris suum* larvae developing to fourth stage in swine. Journal of Parasitology 55:689–712.

**Hendrix, C. M., M. J. Wagner, W. J. Bemrick, J. C. Schlotthauer, and B. E. Stromberg.** 1984. A scanning electron microscope study of third-stage larvae of *Dirofilaria immitis.* Journal of Parasitology 70: 149–151.

**Howells, R. E., and L. J. Blainey.** 1983. The moulting process and the phenomenon of intermoult growth

←

**of the fifth-stage cuticle (below arrow) in electron-dense layer. 9. Late fourth-stage larva, 50 DAI, showing taller annulations in the electron-dense layer (below arrow). 10A, B. Specimens in fourth molt, 58 DAI, showing the fifth-stage cuticle with tall annulations in the surface layer, a thin electron-dense layer, and a thick electron-translucent internal layer with three sublayers. The cuticle of the fourth stage is separated from the new cuticle by a clear space and a broad band of granular debris. The electron-dense layer (below arrow), apparently the remnants of the layer in which the surface of the new fifth-stage cuticle was formed, has been added to the cuticle to be shed with the fourth molt (A and B are different specimens).**

in the filarial nematode *Brugia pahangi*. Parasitology 87:493–505.

**Kotani, T., and K. G. Powers.** 1982. Developmental stages of *Dirofilaria immitis* in the dog. American Journal of Veterinary Research 43:2199–2206.

**Kozek, W. J.** 1971. The molting pattern in *Trichinella spiralis*. II. An electron microscope study. Journal of Parasitology 57:1029–1038.

**Lichtenfels, J. R., P. A. Pilitt, T. Kotani, and K. G. Powers.** 1985. Morphogenesis of developmental stages of *Dirofilaria immitis* (Nematoda) in the dog. Proceedings of the Helminthological Society of Washington 52:98–113.

**Maggenti, A. R.** 1979. The role of cuticular strata nomenclature in the systematics of Nemata. Journal of Nematology 11:94–98.

**Orihel, T. C.** 1961. Morphology of the larval stages of *Dirofilaria immitis* in the dog. Journal of Parasitology 47:251–262.

**Sawyer, T. K.** 1965. Moulting and exsheathment in vitro of third stage *Dirofilaria immitis*. Journal of Parasitology 51:1016–1017.

**Weber, P.** 1984. Electron microscope study on the developmental stages of *Wuchereria bancrofti* in the intermediate host: structure of the body wall. Tropenmedizin und Parasitologie 35:221–230.

**Wergin, W. P., and B. Y. Endo.** 1976. Ultrastructure of a neurosensory organ in a root-knot nematode. Journal of Ultrastructure Research 56:258–276.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 141–145

# Contaminative Potential, Egg Prevalence, and Intensity of *Baylisascaris procyonis*-Infected Raccoons (*Procyon lotor*) from Illinois, with a Comparison to Worm Intensity

DANIEL E. SNYDER AND PAUL R. FITZGERALD[1]
Department of Veterinary Pathobiology, University of Illinois,
2001 S. Lincoln Avenue, Urbana, Illinois 61801

ABSTRACT: The gastrointestinal tracts and corresponding rectal fecal samples of 100 raccoons (*Procyon lotor*) were collected in November and December 1980 in Illinois and examined for the presence of *Baylisascaris procyonis* and its eggs. The raccoons were classified as either juveniles (animals less than 1 yr old) ($N = 72$) or adults ($N = 28$). The prevalence of *B. procyonis* for all the raccoons examined was 86%, and the mean parasite intensity was 51.6 (±5.3). Juvenile raccoons had a significantly higher ($P < 0.005$) worm prevalence (93%) and intensity (62.4 ± 6.1) than did adults (67%; 13.3 ± 2.7). The egg prevalence for all the raccoons examined was 73%; the mean egg intensity was 26,215 (±4,486) eggs per gram (epg) feces. Juvenile raccoons had a significantly higher ($P < 0.005$) egg prevalence (86%) and intensity (29,719 ± 5,147 epg feces) than did adults (39%; 6,454 ± 2,168 epg feces). In the other comparisons, no significant differences ($P < 0.05$) in either prevalence or intensity were found.

KEY WORDS: Nematoda, parasite, eggs per gram feces.

The ascarid of raccoons, *Baylisascaris procyonis,* has been implicated or shown to be the cause of cerebrospinal, ocular, and visceral larva migrans (VLM) in a number of animal species (Snyder, 1983; Kazacos and Kazacos, 1984; Kazacos et al., 1984). More recently this ascarid has been linked to two fatal cases of VLM in children, in which eosinophilic meningoencephalitis was the cause of death (Huff et al., 1984; Fox et al., 1985). There is little information in the literature describing the interrelationship between *B. procyonis* and the raccoon (Snyder and Fitzgerald, 1985). Due to the potential health risks to humans and animals exposed to embryonated eggs of this parasite, the purpose of the present report is to describe and contrast the prevalence and intensity of eggs of this ascarid from 100 Illinois raccoons of known age and sex. The corresponding gastrointestinal tracts were also examined to determine the worm intensity.

## Materials and Methods

The gastrointestinal tracts of 100 steel-trapped or hunter-shot raccoons were obtained in November and December 1980 from Perardi Brothers Fur and Wool, Inc., Farmington, Fulton County, Illinois. The raccoons came from within approximately a 161-km radius of Farmington. A specific location for each animal was not obtainable. At the time of collection, each carcass was weighed and measured, and sex and age were determined. The viscera were placed in individual plastic bags with identification numbers and stored at −25°C until examined. Prior to placing in individual bags, rectal fecal samples were obtained from each animal, placed in individual containers, and refrigerated until examined for the eggs of *B. procyonis.*

The raccoons were classified as either juveniles ($N = 72$) or adults ($N = 28$) based on the work of Sanderson (1961). Because most raccoons in Illinois are born in April, those examined were either 8–9 mo of age (juvenile) or 20–21 mo of age or older (adult).

At the time of examination, the esophagus, stomach, and intestine were dissected longitudinally, the mucosa was scraped, and the contents examined for immature and mature *B. procyonis.* Worms found were categorized according to sex and counted, and representative specimens were deposited in the U.S. national parasite collection at Beltsville, Maryland (accession number 77699). The presence of *B. procyonis* eggs in the fecal samples was determined with the aid of a quantitative flotation technique (McMaster method), utilizing a sucrose solution with a specific gravity of approximately 1.2 (Thienpont et al., 1979). Two counts were made on each sample, and the mean rate expressed as eggs per gram (epg) of feces. Fecal samples that were negative by the McMaster technique were further subjected to a simple flotation using a sucrose solution as described above.

A Student's *t*-test was used to compare mean egg and parasite intensities, and the chi-square test was used to compare prevalence of eggs and worms, in relation to host sex and age. A scatter plot was prepared comparing worm intensity versus eggs per gram of feces (Fig. 1).

## Results and Discussion

Data on the prevalence and intensity of eggs and worms of *B. procyonis* are presented in Tables 1 and 2, respectively. Jacobson et al. (1976),

[1] Present address: 625 E. Canyon Drive, Springville, Utah 84663.

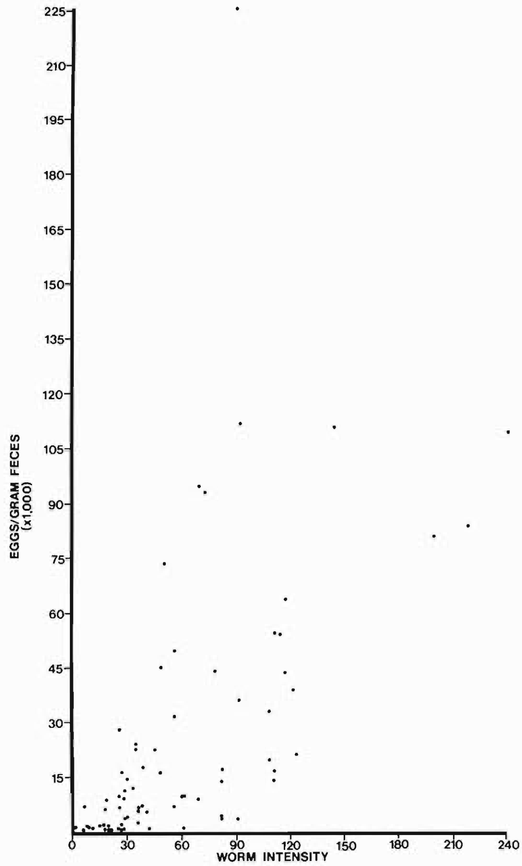



**Figure 1. Scatter plot comparing worm intensity versus eggs per gram of feces in 73 raccoons infected with *Baylisascaris procyonis*.**

in studying the epizootiology of an outbreak of *B. procyonis*-induced cerebrospinal nematodiasis in cottontail rabbits (*Sylvilagus floridanus*) and woodchucks (*Marmota monax*), reported a wild raccoon shedding 25,750 (±3,912) epg feces. Jacobson et al. (1982) found eggs of *B. procyonis* in 62 (28.9%) of 218 raccoon scats examined in Indiana. These same authors also live-trapped 95 raccoons and examined individual rectal fecal samples for the presence of eggs of *B. procyonis* and found a similar prevalence (20%) with the aid of this sampling technique. Kazacos (1982) found that three young pet raccoons linked to an outbreak of *B. procyonis*-induced central nervous system (CNS) disease in bobwhite quail (*Colinus virginianus*) were shedding 1,300–5,400 epg feces. Kazacos (1983) examined fecal samples from 200 wild raccoons in Indiana, and found that an "average" animal was shedding approximately 19,850 epg feces. The overall prevalence and intensity of eggs of *B. procyonis* as reported in Table 1 are significantly higher than those reported by Jacobson et al. (1982) and Kazacos (1982). The intensity of eggs of *B. procyonis* (Table 1) is similar to or higher than those reported by Jacobson et al. (1976) and Kazacos (1983), respectively. Previous studies have not compared the effects of host age and sex on the prevalence and intensity of the eggs of *B. procyonis*. In analyzing relationships of host age and sex, it was found that juvenile raccoons had a significantly higher ($P < 0.005$) prevalence and mean intensity of eggs than did adults (Table 1). In the other comparisons, no significant differences ($P < 0.05$) in either egg prevalence or mean egg intensity were found (Table 1).

Raccoons are a common and ubiquitous mammal found throughout the continental United States. They are adapted to both rural and urban environments (Hoffman and Gottschang, 1977), and are found commonly in zoos and wildlife parks and often kept as pets. The contaminative ability of raccoons infected with *B. procyonis*, particularly juveniles, may be high. The contaminative potential is compounded when these animals are concentrated artificially, as often occurs when they are maintained in cages in zoos, or as pets, or as might occur in natural areas that have a high population density of infected raccoons. Raccoons will frequently use the same area for defecation and urination (latrines). Under these situations, large accumulations of feces with *B. procyonis* eggs can occur in hay and straw mows, feed storage bins, barns, attics, abandoned buildings, downed timber, and cages, and thus may further increase the potential for transmission of infective eggs to other animals and humans. Animals and man may be exposed to embryonated eggs of *B. procyonis* from contaminated soil, water, food, hands, fomites, or raccoon scats. Once in the environment, embryonated eggs of this ascarid can probably persist for a number of years under natural environmental conditions. Kazacos (1982) reported on the contaminative ability of three young pet raccoons infected with *B. procyonis* that were incriminated in an outbreak of cerebrospinal nematodiasis that killed 85 bobwhites. Kazacos calculated the approximate number of eggs passed per day in each of the infected raccoons in this outbreak. Using the same calculations, a twice-daily defecation rate, and each stool approximating 100 g, the 73

**Table 1. Age/sex relationships in prevalence and intensity of eggs of *Baylisascaris procyonis* from raccoons collected in Illinois during November and December 1980.**

| Host age/sex class | Number and percent of hosts with eggs in feces | Mean egg intensity per gram feces ± SE | Range in number of eggs per gram feces |
|---|---|---|---|
| All animals (adult, juvenile, male, and female) ($N$ = 100) | 73 (73) | 26,215 ± 4,486 | 200–228,000 |
| Juvenile female ($N$ = 36) | 31 (86.1) | 32,032 ± 8,621 | 600–228,000 |
| Juvenile male ($N$ = 36) | 31 (86.1) | 27,406 ± 5,750 | 200–113,200 |
| Juvenile (male and female) ($N$ = 72) | 62 (86.1)* | 29,719 ± 5,147* | 200–228,000 |
| Adult (male and female) ($N$ = 28) | 11 (39.3)* | 6,454 ± 2,168* | 200–23,600 |
| Adult female ($N$ = 12) | 4 (33.3) | 4,150 ± 3,489 | 200–14,600 |
| Adult male ($N$ = 16) | 7 (43.8) | 7,771 ± 2,834 | 1,200–23,600 |
| Male (juvenile and adult) ($N$ = 52) | 38 (73.1) | 23,789 ± 4,866 | 200–113,200 |
| Female (juvenile and adult) ($N$ = 48) | 35 (72.9) | 28,846 ± 7,780 | 200–228,000 |

* Significant difference ($P < 0.005$) between juvenile and adult egg prevalence and mean egg intensity.

infected raccoons with a mean egg intensity of 26,215 (±4,486) epg feces (Table 1) would each be shedding approximately 5,243,000 (±897,000) eggs per day. The 62 juvenile raccoons with *B. procyonis* eggs in their feces were calculated to each be shedding approximately 5,943,000 (±1,029,000) eggs per day, and the 11 infected adults were each shedding approximately 1,200,000 (±433,000) eggs per day. One juvenile raccoon was shedding 228,000 epg feces and, using the same calculations, this animal would be contaminating the environment with approximately 45,600,000 eggs per day. This is a tremendous number of eggs being shed into the environment that could potentially embryonate and infect other animals, producing visceral larva migrans. If this animal had been kept in an enclosed area for a 3-mo period, as occurred in the outbreak described by Kazacos (1982), approximately $4.1 \times 10^9$ potentially infective eggs would have contaminated the enclosure. As Kazacos (1982) noted, all these calculations were based on the assumption that daily egg production was constant, which is probably not true, as shown by Olsen et al. (1958).

The prevalence and intensity of *B. procyonis* for all the raccoons examined (Table 2) represent the highest prevalence reported for raccoons in the United States when compared to other moderate to large samples. The prevalence and intensity reported in Table 2 represent a portion of a larger study in Illinois, and the values are similar to those previously reported (Snyder and Fitzgerald, 1985). In comparing the prevalence of eggs and worms of *B. procyonis,* it was found that 13% of the raccoons were infected with this ascarid but were not passing eggs or eggs were not present in sufficient numbers to be detected by the McMaster technique. Five of the 13 negative fecal samples contained only male worms, thus partially explaining this difference. In the eight fecal samples that were negative and had female worms in the intestine, a direct flotation yielded no additional positive samples. This information indicates that the monitoring of raccoon scats for the presence of the eggs of *B. procyonis* may not accurately represent the true prevalence of this ascarid in infected animals. Many reports in the literature have not attempted to evaluate the effects of host sex and age on the prevalence and intensity of *B. procyonis* (see Snyder and Fitzgerald, 1985). In analyzing relationships of host age and sex, it was found that juvenile raccoons had a significantly higher ($P < 0.005$) prevalence and parasite intensity than did adults (Table 2). In the other comparisons, no significant differences ($P < 0.05$) in either prevalence or intensity were found (Table 2).

The mean number of male and female worms per host was determined, and there was a male : female ratio of approximately 1:1 (Table 2). Kazacos (1982) found a male:female ratio of 1:1.5. Jones and McGinnes (1983) found a male : female ratio of 1:2 in 19 infected raccoons. The mean fecundity of one *B. procyonis* female in the juvenile raccoons was calculated and found to be approximately 178,000 eggs per day. Kazacos (1982) calculated the mean fecundity of individual *B. procyonis* females to be 115,000 eggs per day. The juvenile raccoon that was shedding 228,000 epg feces had 52 female worms, yielding a mean fecundity per female of approximately

**Table 2. Age/sex relationships of *Baylisascaris procyonis*-infected raccoons collected in Illinois during November and December 1980.**

| Host age/sex class | Number and percent of hosts infected* | Mean parasite intensity ± SE* | Range in number of parasites | Mean number female worms ± SE | Range in number of female worms | Mean number male worms ± SE | Range in number of male worms |
|---|---|---|---|---|---|---|---|
| All animals (adult, juvenile, male, and female) ($N = 100$) | 86 (86) | 51.6 ± 5.3 | 1–241 | 27.8 ± 3.1 | 1–160 | 26.7 ± 2.5 | 1–109 |
| Juvenile female ($N = 36$) | 34 (94.4) | 59.4 ± 9.7 | 3–241 | 31.1 ± 5.8 | 4–160 | 29.2 ± 4.3 | 3–109 |
| Juvenile male ($N = 36$) | 33 (91.7) | 65.4 ± 7.5 | 1–201 | 35.6 ± 4.0 | 3–110 | 32.3 ± 3.7 | 1–91 |
| Juvenile (male and female) ($N = 72$) | 67 (93.0)† | 62.4 ± 6.1† | 1–241 | 33.3 ± 3.6 | 3–160 | 30.7 ± 2.8 | 1–109 |
| Adult (male and female) ($N = 28$) | 19 (67.9)† | 13.3 ± 2.7† | 1–37 | 7.2 ± 1.4 | 1–20 | 9.6 ± 1.7 | 2–19 |
| Adult female ($N = 12$) | 8 (66.7) | 9.9 ± 3.9 | 1–30 | 4.6 ± 1.5 | 1–11 | 9.4 ± 3.1 | 2–19 |
| Adult male ($N = 16$) | 11 (68.8) | 15.7 ± 3.7 | 1–37 | 9.3 ± 2.1 | 1–20 | 8.9 ± 1.8 | 2–19 |
| Male (juvenile and adult) ($N = 52$) | 44 (84.6) | 53.0 ± 6.5 | 1–201 | 29.7 ± 3.6 | 1–110 | 26.8 ± 3.2 | 1–91 |
| Female (juvenile and adult ($N = 48$) | 42 (87.5) | 50.0 ± 8.4 | 1–241 | 26.4 ± 5.1 | 1–160 | 26.7 ± 3.9 | 2–109 |

* Represents partial data from Snyder and Fitzgerald (1985).
† Significant difference ($P < 0.005$) between juvenile and adult prevalence and parasite intensity.

877,000 eggs per day. The mean fecundity of one *B. procyonis* female in the adult raccoons was approximately 179,000 eggs per day. This number probably should be higher, because immature worms were included when calculating the mean number of female worms per infected adult raccoon. This indicates that in adult raccoons, those adult females present are able to produce approximately the same number of eggs per female per day as compared to juveniles who have a significantly higher intensity. As Olsen et al. (1958) pointed out while examining the fecundity of *Ascaris suum,* the daily egg production of individual female ascarids, like that of other nematodes, is probably influenced by the age of the worms, the number of worms present, and the physiological condition of the host. A great deal of variability was noted when comparing the number of eggs per gram of feces and the intensity in individually infected animals (Fig. 1). The above data do indicate that infected raccoons, particularly juveniles, can shed large numbers of potentially infective *B. procyonis* eggs into the environment.

The embryonated eggs of this ascarid have been shown to be highly pathogenic in a number of mammalian and avian species (Snyder, 1983; Kazacos and Kazacos, 1984). The eggs of *B. procyonis* collected in this study were shown to migrate extensively and kill when inoculated into outbred laboratory mice, chickens (*Gallus domesticus*), and domestic dogs (*Canis familiaris*) (Snyder, 1983). Sheep (*Ovis aries*) inoculated with these embryonated eggs were refractory to extensive visceral migration, and the larvae were walled off, forming small granulomata on the serosal surface of the gastrointestinal tract (Snyder, 1983). It has also been shown that it takes relatively few larvae in the CNS of small mammals to cause severe CNS dysfunction (Tiner, 1953; Dubey, 1982). This marked pathogenicity is directly related to the rapid growth and large size of the larvae, and to their propensity to enter and migrate in the CNS (Kazacos et al., 1981). This ascarid has been linked to two fatal cases of VLM in children, in which eosinophilic meningoencephalitis was determined to be the cause of death (Huff et al., 1984; Fox et al., 1985).

The information reported above on the prevalence and intensity of *B. procyonis* and its eggs may aid public health officials, veterinarians, epidemiologists, and others in assessing the potential human and animal health hazards associated with environmental contamination by eggs of this ascarid. It seems advisable that if raccoons, particularly juveniles, are kept in captivity for whatever reason, they should be systematically and routinely treated with an appropriate anthelminthic to remove adult worms from the intestine. Also, contaminated fecal matter should be removed routinely and discarded properly. Kazacos et al. (1983) described methods for decontaminating areas contaminated with *B. procyonis* eggs.

## Acknowledgments

We thank Dr. Glen C. Sanderson, Natural History Survey, Champaign, Illinois, who determined the sex and age of the raccoons examined, and Dr. K. R. Kazacos, Department of Veterinary Microbiology, Purdue University, West Lafayette, Indiana, for reading and commenting on the manuscript. We also thank the staff of the Word Processing Center, College of Veterinary Medicine, University of Illinois, for typing the manuscript.

## Literature Cited

**Dubey, J. P.** 1982. *Baylisascaris procyonis* and eimerian infections in raccoons. Journal of the American Veterinary Medical Association 181(11): 1292–1294.

**Fox, A. S., K. R. Kazacos, N. S. Gould, P. T. Heydemann, C. Thomas, and K. M. Bayer.** 1985. Fatal eosinophilic meningoencephalitis and visceral larva migrans caused by the raccoon ascarid *Baylisascaris procyonis.* New England Journal of Medicine 312:1619–1623.

**Hoffman, C. O., and J. L. Gottschang.** 1977. Numbers, distribution, and movements of a raccoon population in a suburban residential community. Journal of Mammalogy 58:623–626.

**Huff, D. S., R. C. Neafie, M. J. Binder, G. A. DeLeon, L. W. Brown, and K. R. Kazacos.** 1984. The first fatal *Baylisascaris* infection in humans: an infant with eosinophilic meningoencephalitis. Pediatric Pathology 2:345–362.

**Jacobson, H. A., P. F. Scanlon, V. R. Nettles, and W. R. Davidson.** 1976. Epizootiology of an outbreak of cerebrospinal nematodiasis in cottontail rabbits and woodchucks. Journal of Wildlife Diseases 12: 357–360.

**Jacobson, J. E., K. R. Kazacos, and R. H. Montague, Jr.** 1982. Prevalence of eggs of *Baylisascaris procyonis* (Nematoda: Ascaroidea) in raccoon scats from an urban and a rural community. Journal of Wildlife Diseases 18:461–464.

**Jones, E. J., and B. S. McGinnes.** 1983. Distribution of adult *Baylisascaris procyonis* in raccoons from Virginia. Journal of Parasitology 69:653.

**Kazacos, K. R.** 1982. Contaminative ability of *Baylisascaris procyonis* infected raccoons in an outbreak of cerebrospinal nematodiasis. Proceedings of the Helminthological Society of Washington 49:155–157.

———. 1983. Raccoon roundworms (*Baylisascaris procyonis*). A cause of animal and human disease. Purdue University Agricultural Experiment Station Bulletin No. 442, pp. 1–25.

———**, and E. A. Kazacos.** 1984. Experimental infection of domestic swine with *Baylisascaris procyonis* from raccoons. American Journal of Veterinary Research 45:1114–1121.

———**, W. M. Reed, E. A. Kazacos, and H. L. Thacker.** 1983. Fatal cerebrospinal disease caused by *Baylisascaris procyonis* in domestic rabbits. Journal of the American Veterinary Medical Association 183:967–971.

———**, W. A. Vestre, and E. A. Kazacos.** 1984. Raccoon ascarid larvae (*Baylisascaris procyonis*) as a cause of ocular larva migrans. Investigative Ophthalmology and Visual Science 25:1177–1183.

———**, W. L. Wirtz, P. B. Burger, and C. S. Christmas.** 1981. Raccoon ascarid larvae as a cause of fatal central nervous system disease in subhuman primates. Journal of the American Veterinary Medical Association 179:1089–1094.

**Olsen, L. S., G. W. Kelley, and H. G. Sen.** 1958. Longevity and egg-production of *Ascaris suum.* Transactions of the American Microscopical Society 77:380–383.

**Sanderson, G. C.** 1961. Techniques for determining age of raccoons. Illinois Natural History Survey, Biological Notes 45:1–16.

**Snyder, D. E.** 1983. The prevalence, cross-transmissibility to domestic animals and adult structure of *Baylisascaris procyonis* (Nematoda) from Illinois raccoons (*Procyon lotor*). Ph.D. Dissertation, University of Illinois, Urbana, Illinois. 233 pp.

———**, and P. R. Fitzgerald.** 1985. The relationship of *Baylisascaris procyonis* to Illinois raccoons (*Procyon lotor*). Journal of Parasitology 71:596–598.

**Thienpont, D., R. Rochette, and O. F. J. Vanparijs.** 1979. Diagnosing Helminthiasis through Coprological Examination. Janssen Research Foundation, Beerse, Belgium. 187 pp.

**Tiner, J. D.** 1953. Fatalities in rodents caused by larval *Ascaris* in the central nervous system. Journal of Mammalogy 34:153–167.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 146–149

# *Neoechinorhynchus lingulatus* sp. n. (Acanthocephala: Neoechinorhynchidae) from *Pseudemys nelsoni* (Reptilia: Emydidae) of Florida

BRENT B. NICKOL[1] AND CARL H. ERNST[2]

[1] School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588-0118
[2] Department of Biology, George Mason University, 4400 University Drive, Fairfax, Virginia 22030

ABSTRACT: During a 2-mo illness that ended in death, a female Florida red-bellied turtle (*Pseudemys nelsoni*) passed more than 500 specimens of *Neoechinorhynchus lingulatus* sp. n. The new species resembles *N. chelonos* and *N. magnapapillatus,* also parasites of turtles, in possessing a prominent process at the posterior end of females. *Neoechinorhynchus lingulatus* sp. n. differs from these species in the shape of the posterior papilla, in possessing a larger proboscis with longer hooks, and in having spindle-shaped eggs with polar elongations of the fertilization membrane. The eggs of *N. lingulatus* sp. n. are similar to those of *N. chrysemydis*; however, *N. chrysemydis* possesses only a small, spherical knob at the posterior end of females. This report constitutes the first record of an acanthocephalan from *P. nelsoni* and from a turtle caught in Florida.

KEY WORDS: taxonomy, morphology, Acanthocephala of turtles, species review.

In December 1981 a female Florida red-bellied turtle (*Pseudemys nelsoni*), captured north of Tampa, Florida, the previous May, stopped eating and appeared sick. Its condition steadily declined untl it died in February. During the 2-mo illness, approximately 500 acanthocephalans were passed, many individually, but others in boluses of as many as 25 worms.

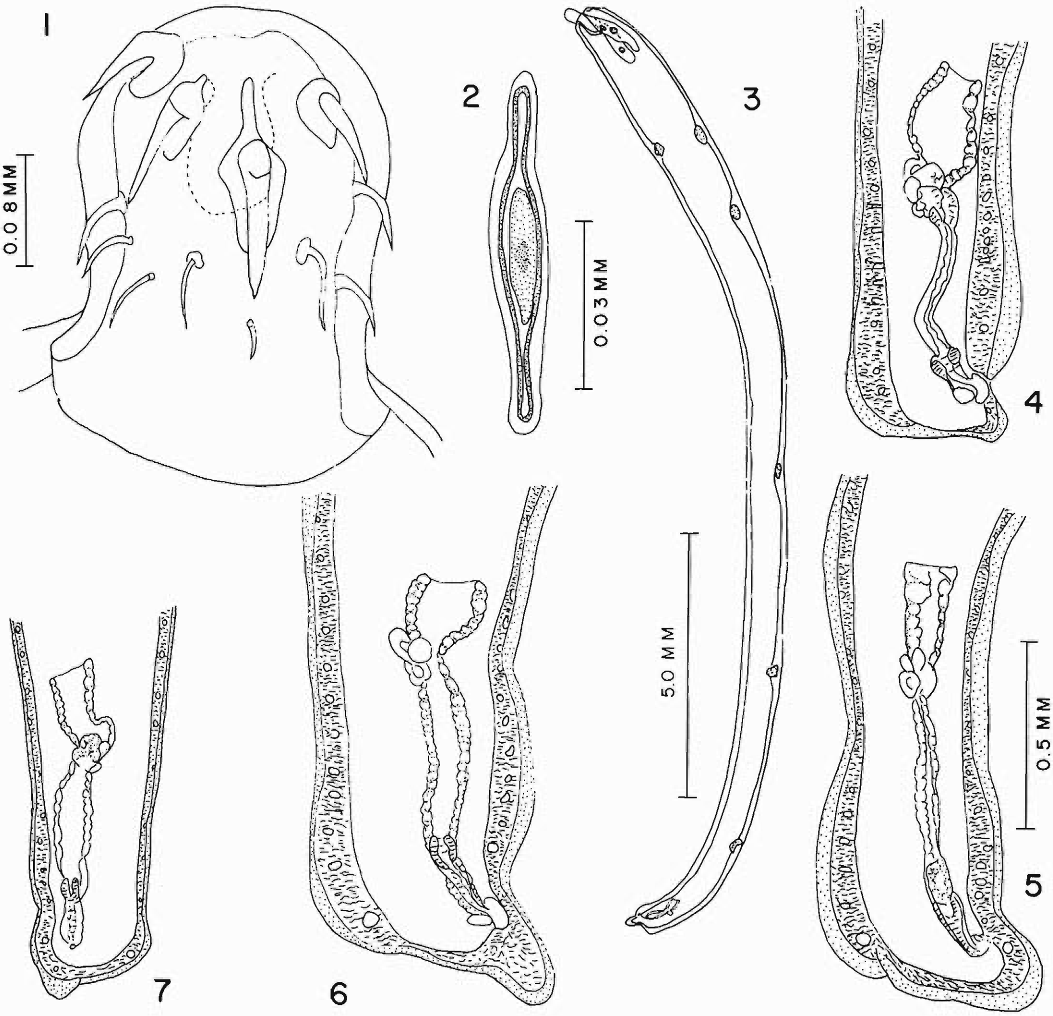
The passed worms represent an undescribed species of *Neoechinorhynchus.* Until Cable and Hopp (1954) recognized *N. chrysemydis* and *N. pseudemydis,* all acanthocephalans of turtles were thought to be a single species, *N. emydis* (Leidy, 1851) Van Cleave, 1916. Fisher (1960) added *N. emyditoides* from turtles. *Neoechinorhynchus stunkardi* was described by Cable and Fisher (1961), *N. constrictus* by Little and Hopkins (1968), and *N. magnapapillatus* by Johnson (1969). Schmidt et al. (1970) added *N. chelonos,* and convincingly considered *N. constrictus* to be synonymous with *N. pseudemydis.* Addition of *N. lingulatus* sp. n. from *Pseudemys nelsoni* results in eight species being recognized from turtles.

## Description of Species

Measurements, in micrometers unless noted, are of 50 gravid females and 50 mature males. Numbers in parentheses are means.

### *Neoechinorhynchus lingulatus* sp. n. (Figs. 1–6)

With the characteristics of the genus. Trunk slightly curved ventrally; 19.3–26.1 mm (21.7 mm) long and 0.979–1.690 mm (1.287 mm) wide in females, 15.5–20.7 mm (17.2 mm) long and 0.979–1.661 mm (1.138 mm) wide in males. Trunk of female terminating in a large, tongue-shaped papilla 340–557 (471) long dorsally and 288–326 (304) long ventrally. No consistent sexual dimorphism in proboscis size or proboscis hook dimensions. Proboscis slightly wider than long; 192–240 (220) long by 197–254 (229) at widest point. The lateral hooks of anterior circle, 106–125 (116) long, distinctly posterior to other 4 hooks, 82–110 (95) long; hooks of middle circle 53–70 (60) long; hooks of basal circle 48–58 (51) long. Neck wider than long; 106–163 (123) long by 240–312 (274) wide. Lemnisci 1.229–1.968 mm (1.526 mm) long; not appreciably different in length. Proboscis receptacle 451 long when contracted longitudinally to 720 long when constricted; 144–278 wide. Male reproductive system 4.4–8.4 mm (6.4 mm) long; occupies posterior 36% of trunk. One testis, but not always the same, usually larger than other. Anterior testis 0.912–2.736 mm (1.466 mm) long by 288–470 (377) wide; posterior testis 1.056–2.852 mm (1.676 mm) long by 240–461 (364) wide. Cement gland elongate; 1.171–2.861 mm (1.829 mm) long. Cement reservoir spherical to elongate; 336–653 (538) in largest dimension. Everted bursa very large; 1.152–1.728 mm (1.368 mm) long by 480–883 (690) across. Female system 0.960–1.286 mm (1.048 mm) from mouth of uterine bell to genital pore at proximal base of caudal papilla. Uterine bell, exclusive of selector apparatus, 336–480 (422) long by 106–192 (142)




**Figures 1–7. Camera lucida drawings. 1–6. *Neoechinorhynchus lingulatus* sp. n. 1. Proboscis. 2. Egg. 3. Outline of entire female. 4. Posterior end of immature female. The specimen was 16.4 mm long and contained ovarian balls but no eggs. 5. Posterior end of nongravid female. The specimen was 18.7 mm long and contained ovarian balls, many eggs without fully developed membranes, and a few fully formed eggs. (The projection beside Figure 5 applies equally to Figures 4–7.) 6. Posterior end of gravid female. The specimen was 19.4 mm long and contained only fully formed eggs. 7. Posterior end of a female *Neoechinorhynchus chrysemydis*. The specimen was 15.6 mm long and contained only fully formed eggs.**

wide. Uterus 307–547 (413) long; vagina 221–317 (255) long. Preserved, fully formed eggs removed from body cavity spindle-shaped with polar elongations of fertilization membrane; 48–67 (58) long by 8–12 (10) wide.

TYPE HOST: *Pseudemys nelsoni,* Florida red-bellied turtle.

TYPE LOCALITY: Florida, Hillsborough Co., Hillsborough River north of Tampa.

SPECIMENS: USNM Helm. Coll. Nos. 79261 (holotype), 79262 (allotype), 79263 (paratype); University of Nebraska State Museum, Manter Laboratory of Parasitology, Lincoln, Nebraska, No. 23106 (3 paratypes); other paratypes in collections of authors.

ETYMOLOGY: *Lingulatus* is Latin, meaning tongue-shaped, and refers to the papilla at the posterior end of females.

## Remarks

The eight species of *Neoechinorhynchus* described from turtles are very similar in most morphological features. They are readily distinguished, however, by the contour of the posterior end of females and by the shape of eggs when fully formed. *Neoechinorhynchus lingulatus* sp. n. most closely resembles *N. chelonos* and *N. magnapapillatus* in the shape of the posterior end of females, and it is most like *N. chrysemydis* in the shape of eggs.

Among the species described from turtles, only *N. lingulatus* sp. n. and *N. chrysemydis* have spindle-shaped eggs with polar elongations of the fertilization membrane (terminology of West, 1964) (Fig. 2). Eggs of both species are of about the same length, but those of *N. lingulatus* sp. n. seem to be narrower (8–11 μm compared to 19–22 μm). The difference might be due to the fact that dimensions for *N. lingulatus* sp. n. are based on preserved eggs, whereas those of *N. chrysemydis* are from living eggs.

The large, prominent papilla at the posterior end of female *N. lingulatus* sp. n. distinguishes it from all species of the genus except *N. chelonos* and *N. magnapapillatus,* also parasites of turtles. The papilla of *N. lingulatus* sp. n. is tongue-shaped rather than digitiform as in *N. chelonos* and *N. magnapapillatus. Neoechinorhynchus chrysemydis* and *N. stunkardi* also have processes at the posterior end of females; that of *N. chrysemydis* is rounded and that of *N. stunkardi* is conical. Both are significantly smaller than that of *N. lingulatus* sp. n.

The posterior process of *N. lingulatus* sp. n. begins as an outgrowth of the ventral body wall. Initially in young females (Fig. 4) it bears a resemblance to that of gravid *N. chrysemydis* (Fig. 7). By the time worms are mature, however, the process of *N. lingulatus* sp. n. is much larger and of a distinctive shape (Fig. 6).

Because the most reliable characters for distinguishing the species of *Neoechinorhynchus* found in turtles are features of females, identification of males is frequently difficult or impossible. The males of *N. lingulatus* sp. n., however, are readily recognized because the proboscis and comparable hooks are appreciably larger than those of the other species from turtles, except they are only slightly larger than in *N. constrictus* as described by Little and Hopkins (1968), or *N. pseudemydis* if the synonymy proposed by Schmidt et al. (1970) is accepted. Males of *N. lingulatus* sp. n. differ from those of this species by being wider (absolutely and proportionally). The maximum width of male *N. pseudemydis* is 830 μm (Cable and Hopp, 1954), *N. constrictus* 800 μm (Little and Hopkins, 1968), and *N. lingulatus* sp. n. 1.296 mm. The width : length ratio of male *N. pseudemydis* as described by Cable and Hopp (1954) is 0.031, *N. constrictus* as described by Little and Hopkins (1968) is 0.042, and *N. lingulatus* sp. n. is 0.069.

## Discussion

Because the terminal process of young, immature female *N. lingulatus* sp. n. bears a resemblance to that of gravid *N. chrysemydis,* and because Cable and Hopp (1954) had only one female for their description, a series of 15 *N. chrysemydis* collected from *Trachemys scripta elegans* (red-eared turtle) in Louisiana was studied for comparison. No appreciable difference was found between Louisiana specimens and the original description. As a consequence of the original species description being based on a single male and a single female and largely repeated in an expanded description by Fisher (1960), study of additional specimens permits a better understanding of the variation of *N. chrysemydis.* The following measurements are from Louisiana specimens, with the dimensions reported by Cable and Hopp (1954) following in parentheses. Measurements are in micrometers unless noted. The trunk of mature males was 11.1–12.3 mm long (12.9 mm) by 758–768 wide (680), and in gravid females it was 13.8–15.7 mm (13.7 mm) by 720–912 (680). The proboscis was 163–197 long (132–170) by 187–196 wide (200–207). Lateral hooks in the apical circle were 94–97 long (80–140), and the others of that circle were 82–84 long (48–82). Hooks of the middle circle were 46–50 long (44–61), and those of the basal circle were 34–43 long (24–48). The male reproductive system occupied the posterior 49% of the trunk (48%). Preserved eggs were shaped as illustrated in the original description and measured 46–50 long (55–60, living) by 7–12 wide (19–22). The terminal papilla of females (Fig. 7) was as illustrated by Cable and Hopp (1954).

It is unknown whether the demise of the Florida red-bellied turtle of this study was related to the presence of acanthocephalans. Certainly numbers greater than 500 constitute a heavy infection; however, infections of more than 100 worms are relatively common in apparently healthy turtles of other species, and the turtle was a large female (27.3-cm carapace).

There have been several attempts to interpret the geographical distribution of acanthocephalans in turtles. Occurrence seems to depend on feeding preference of hosts and upon geographical distribution of the parasites (Fisher, 1960; Acholonu, 1969). Although some species parasitic in turtles are widely distributed, large differences in prevalence seem to indicate a degree of geographical isolation. Present information is too limited to assess the biogeographical significance of the new species from Florida. This report of *N. lingulatus* sp. n. from *Pseudemys nelsoni,* of which no subspecies is recognized (Ernst and Barbour, 1972), constitutes a new record of an acanthocephalan from this species and the first report of an acanthocephalan from a Florida turtle.

## Literature Cited

**Acholonu, A. D.** 1969. Acanthocephala of Louisiana turtles with a redescription of *Neoechinorhynchus stunkardi* Cable and Fisher, 1961. Proceedings of the Helminthological Society of Washington 36: 177–183.

**Cable, R. M., and F. M. Fisher, Jr.** 1961. A fifth species of *Neoechinorhynchus* (Acanthocephala) in turtles. Journal of Parasitology 47:666–668.

**———, and R. M. Hopp.** 1954. Acanthocephalan parasites of the genus *Neoechinorhynchus* in North American turtles with the descriptions of two new species. Journal of Parasitology 40:674–680.

**Ernst, C. H., and R. W. Barbour.** 1972. Turtles of the United States. University of Kentucky Press, Lexington. 347 pp.

**Fisher, F. M., Jr.** 1960. On Acanthocephala of turtles, with the description of *Neoechinorhynchus emyditoides* n. sp. Journal of Parasitology 46:257–266.

**Johnson, C. A., III.** 1969. *Neoechinorhynchus magnapapillatus* sp. n. (Acanthocephala) from *Pseudemys scripta scripta* (Chelonia). Proceedings of the Helminthological Society of Washington 36:277–280.

**Little, J. W., and S. H. Hopkins.** 1968. *Neoechinorhynchus constrictus* sp. n., an acanthocephalan from Texas turtles. Proceedings of the Helminthological Society of Washington 35:46–49.

**Schmidt, G. D., G. W. Esch, and J. W. Gibbons.** 1970. *Neoechinorhynchus chelonos,* a new species of acanthocephalan parasite of turtles. Proceedings of the Helminthological Society of Washington 37: 172–174.

**West, A. J.** 1964. The acanthor membranes of two species of Acanthocephala. Journal of Parasitology 50:731–734.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 150–153

*Research Note*

# Detection of Ensheathed Third-stage Larvae of *Haemonchus contortus* (Trichostrongylidae) in Sheep: Delayed Exsheathment

ERIC P. HOBERG AND GARY L. ZIMMERMAN
College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331

KEY WORDS: variation in larval tail and sheath length, inhibition of exsheathment.

During a routine anthelmintic trial, ensheathed third-stage larvae ($L_3$) of *Haemonchus contortus* (Rudolphi, 1803) were found in the abomasa of 10 of 30 sheep, *Ovis aries* L., that were maintained in a parasite-free environment (see Richards et al., in press, Veterinary Parasitology). Larvae of trichostrongyloid nematodes morphologically identical to those in the infective third stage have not previously been reported from hosts under similar conditions. Observation of ensheathed $L_3$ *H. contortus* in hosts not recently exposed to infection suggests that exsheathment was delayed or inhibited and development was discontinuous prior to completion of the second larval molt.

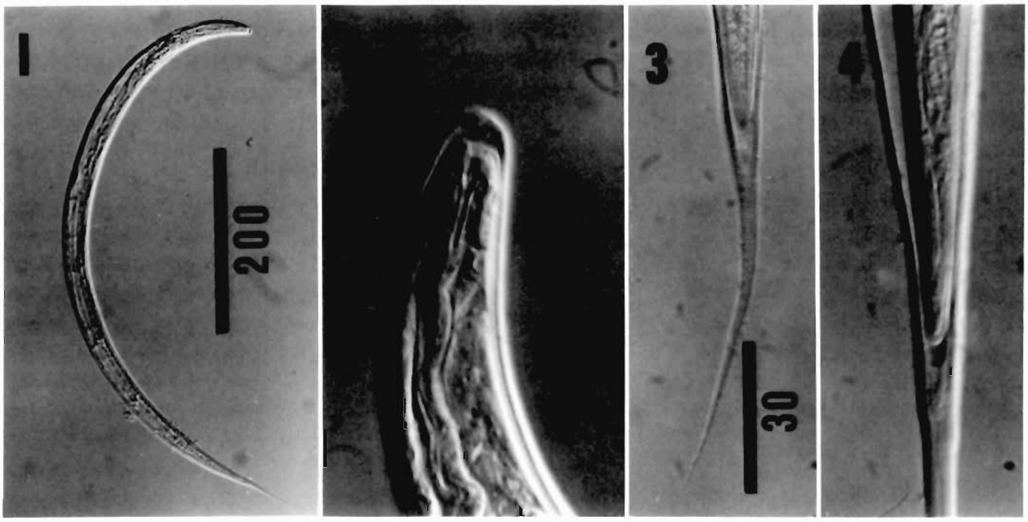
CONDITIONS OF THE ANTHELMINTIC TRIAL: Thirty sheep purchased from a local producer in Philomath, Oregon, on 14 June 1984 were maintained on pastures of the Veterinary Medical Animal Isolation Laboratory (VMAIL) at Oregon State University until 5 October 1984, when they were transferred to an indoor isolation barn with concrete stalls (walls and floors) for the remainder of the study. While in the isolation facility, animals were maintained in two equal groups in rooms that were pressure-washed daily. Dry pelleted feed was provided in plastic containers also cleaned on a daily basis. Spillage was minimal, and feed was not made available on the floor. Animals were isolated in these conditions for a period of 39–46 days prior to necropsy. On 5 November 1984, 20 of these sheep were treated with Netobimin (SCH 32481, Schering Corporation) (10 at 7.5 mg/kg of body weight and 10 at 20 mg/kg) and 10 untreated animals were used as controls. Necropsies were conducted on 12 and 19 November, when equal numbers of animals (control and treated) were examined.

Equipment (sieves, buckets, etc.) and instruments used during the necropsies had been thoroughly washed, rinsed, and dried prior to use. Sieves had been disinfected and air-dried in a hot-air circulator and stored at room temperature for 6 mo prior to the study. Storage containers were new, and none of the equipment had ever been used in studies dealing with infective larvae of *Haemonchus* spp. or other trichostrongyles. These factors, in conjunction with the experimental protocol and long-term isolation of the sheep, precluded the possibility of contamination of equipment and instruments or reinfection during the course of the trial. As stalls were cleaned on a daily basis, there was insufficient time to allow the hatching of eggs and development of larvae to the infective third stage (Silverman and Campbell, 1959, Parasitology 47: 23–38; Gibson and Everett, 1976, British Veterinary Journal 132:50–59).

At necropsy, the abomasum, small intestine, large intestine, and cecum were ligated in situ and later processed separately. The abomasum of each animal was opened longitudinally and the mucosa rubbed under running water. All washings and contents were then brought to a known volume, from which two 5% aliquots were saved. Aliquots were sieved through a 400-mesh (37.5-$\mu$m) screen, and all material retained was preserved with 70% ethanol/iodine. Each abomasum was then incubated in tap water at room temperature for 24 hr. Following incubation, the abomasum was scraped and washed; material and rinse water were sieved and preserved as specified.

Aliquots of abomasal contents and samples of abomasal incubates were examined for helminths; all larval and adult nematodes were removed and stored in 70% ethanol until final identification. Details of larval morphology (cephalic and caudal structure) were assessed in a random subsample of 250 individuals; 120 larvae were measured with an opisometer to aid in identification.

LARVAL MORPHOLOGY: Morphologically, $L_3$ *H. contortus* found in the present study were




**Figures 1–4.** ***Haemonchus contortus,*** **ensheathed $L_3$. 1. Entire larva. 2. Cephalic end, showing buccal capsule and associated structures. 3. Tail of sheath. 4. Tail of larva within sheath (scale as Fig. 2). All scale lines in micrometers.**

identical to those described elsewhere from sheep (Veglia, 1915, Reports of the Director of Veterinary Research, Onderstepoort, Pretoria, South Africa, Nos. 3 and 4:349–500; Monnig, 1931, Annual Report of the Director of Veterinary Services, Onderstepoort, Pretoria, South Africa, 255–264; Dikmans and Andrews, 1933, Transactions of the American Microscopical Society 42:1–25)

**Table 1. Comparison of $L_3$ *Haemonchus* spp. All measurements are in micrometers.**

| Authority | Host | Total length* | Tail of sheath† | Sheath extension‡ | Larval tail§ |
|---|---|---|---|---|---|
| Present study | Sheep | | | | |
| Range | | 672–856 | 86–166 | 44–82 | 32–96 |
| $\bar{x}$ | | 769 | 130 | 66 | 65 |
| Veglia (1915)‖ | Sheep | | | | |
| $\bar{x}$ | | 715 | 130 | – | 60 |
| Monnig (1931)‖ | Sheep | | | | |
| Range | | 694–772 | 145–165 | – | 63–71 |
| $\bar{x}$ | | 733 | 149 | – | 67 |
| Dikmans and Andrews (1933)‖ | Sheep | | | | |
| Range | | 650–751 | 119–146 | – | 54–68 |
| $\bar{x}$ | | 693 | 134 | – | 61 |
| Keith (1953)‖ | Cattle | | | | |
| Range | | 750–850 | 160–190 | 90–110 | – |
| Hansen and Shivnani (1956)‖ | Cattle | | | | |
| Range | | 682–780 | – | 55–82 | – |
| $\bar{x}$ | | 739 | – | 66 | – |
| Range | | 749–866 | – | 87–119 | – |
| Borgsteede and Hendriks (1974)‖ | Cattle | | | | |
| Range | | 633–764 | 115–139 | 59–83 | – |
| $\bar{x}$ | | 724 | 129 | 68 | – |

* Length of sheath.
† Distance from anus of larva to tip of sheath.
‡ Distance from tip of larval tail to tip of sheath.
§ Distance from anus to tip of larval tail.
‖ Consult text for citations.

**Table 2. Data for infections of larval and adult *H. contortus* in individual animals.**

| | Numbers of worms in individual hosts* | | | |
|---|---|---|---|---|
| Group | $L_3$ | Early $L_4$ | Late $L_4$ | Adult |
| Control† | 0 | 0 | 0 | 196 |
| Control | 4 | 144 | 0 | 120 |
| Control | 520 | 160 | 40 | 200 |
| Control | 4 | 52 | 0 | 4 |
| Control | 0 | 0 | 0 | 52 |
| Control | 0 | 32 | 0 | 12 |
| Control | 0 | 88 | 0 | 24 |
| Control | 0 | 0 | 0 | 4 |
| Control | 920 | 0 | 0 | 80 |
| 7.5‡ | 128 | 0 | 0 | 0 |
| 7.5 | 4 | 0 | 0 | 0 |
| 7.5 | 144 | 12 | 0 | 0 |
| 7.5 | 0 | 64 | 0 | 40 |
| 7.5 | 0 | 12 | 0 | 0 |
| 7.5 | 9,180 | 68 | 0 | 0 |
| 7.5 | 136 | 0 | 0 | 0 |
| 20.0 | 0 | 4 | 0 | 0 |
| 20.0 | 1,140 | 0 | 0 | 0 |

* Counts from abomasal incubates and contents combined.
† Animals received no drug.
‡ Dosage of Netobimin in mg/kg of body weight.

**Table 3. Distribution of larval and adult *Haemonchus contortus* in the abomasum.**

| | Number of sheep infected | Prevalence (%) | Intensity of infection (range)* | Intensity of infection ($\bar{x} \pm$ SD)* |
|---|---|---|---|---|
| Control group ($N$ = 10) | | | | |
| $L_3$ | 4 | 40 | 4–920 | 362 ± 444.47 |
| Early $L_4$ | 5 | 50 | 32–160 | 95 ± 55.88 |
| Late $L_4$ | 1 | 10 | 0–40 | –† |
| Adults | 9 | 90 | 4–200 | 77 ± 78.63 |
| 7.5-mg treatment ($N$ = 10)‡ | | | | |
| $L_3$ | 5 | 50 | 4–9,180 | 1,918 ± 4,059.76 |
| Early $L_4$ | 4 | 40 | 12–68 | 39 ± 31.22 |
| Late $L_4$ | 0 | 0 | 0 | –† |
| Adults | 1 | 10 | 0–40 | –† |
| 20.0-mg treatment ($N$ = 10)‡ | | | | |
| $L_3$ | 1 | 10 | 0–1,140 | –† |
| Early $L_4$ | 1 | 10 | 0–4 | –† |
| Late $L_4$ | 0 | 0 | 0 | –† |
| Adults | 0 | 0 | 0 | –† |

* Numbers of worms per infected host.
† Means not given for $N \leq 2$.
‡ Dosage of Netobimin in mg/kg of body weight.

and cattle (Keith, 1953, Australian Journal of Zoology 1:223–235; Hansen and Shivnani, 1956, Transactions of the American Microscopical Society 75:91–102; Borgsteede and Hendriks, 1974, Tijdschrift voor Diergeneeskunde 99:103–113). Measurements of the larval sheath and associated structures were generally in agreement with those reported previously (Table 1). However, a broad range in variation in the length of the larval tail and sheath was noted. Larvae with short tails superficially resembled those of *Trichostrongylus* spp. (see Borgsteede and Hendriks, 1974, loc. cit.), but were referred to *Haemonchus* based on cephalic morphology and structure of the buccal capsule and tail (Douvres, 1957, American Journal of Veterinary Research 66:81–85; Sommerville, 1966, Journal of Parasitology 52:127–136; Mapes, 1969, Parasitology 59:215–231) (Figs. 1–4).

The coiled posture and small size ($\bar{x}$ = 769 μm) of $L_3$ *H. contortus* reported here made them particularly cryptic and difficult to detect in the abomasal samples. It is notable that inhibited, exsheathed $L_3$ *Trichostrongylus* spp. with similar dimensions (750–850 μm in length) were only recently recorded in several studies of ruminant parasites (Eysker, 1978, Veterinary Parasitology 4:29–33; Ogunsusi and Eysker, 1979, Research in Veterinary Science 26:108–110; Waller and Dobson, 1981, Research in Veterinary Science 30:213–216). Thus, it is possible that such minute larval trichostrongyles may not have been recovered or detected during differential counts in earlier studies.

Distribution of larvae: Ensheathed $L_3$'s, fourth-stage larvae ($L_4$), and adults of *H. contortus* were found in the abomasal contents and incubates of sheep from each study group (Tables 2, 3). Only 50% of infected animals had both $L_3$'s and early $L_4$'s in the abomasum. These data suggest the possibility of arrested development of larvae at the early fourth stage. However, there are no unequivocal patterns evident in the relative abundances of $L_3$'s and $L_4$'s to imply an association between populations of these larvae in individual hosts indicative of continuing development.

Observations of $L_3$ *H. contortus* in incubated samples, although of equivocal interpretation, could indicate that these larvae were viable, resident in the host mucosa at the time of collection and not transient in the abomasum. Larvae apparently had been present in individual hosts longer than the period required for exsheathment to the parasitic third stage and development of the fourth stage. Inactivity of Netobimin against these stages was shown by the presence of larvae in all treatment groups.

Exsheathment should typically occur in the rumen or at a point anterior to the abomasum (Soulsby, 1982, Helminths, Arthropods and Protozoa of Domesticated Animals, Lea and Febiger, Philadelphia). That such did not occur suggests the proper stimuli inducing exsheathment were not encountered or that the larvae were not capable of responding. Stewart (1958, Proceedings of the Helminthological Society of Washington 25:131–132) reported inhibition of exsheathment in massive experimental infections of *Cooperia punctata* (von Linstow, 1906) in cattle. He concluded that acquired resistance to *C. punctata* limited the development of the third-stage larvae. It is not known what factors may have influenced delayed exsheathment of $L_3$ *H. contortus* in the present study.

Arrested development of trichostrongyloid nematodes in sheep has been well documented (Michel, 1974, Advances in Parasitology 12:279–366; Schad, 1977, pages 111–166 *in* G. W. Esch, ed., Regulation of Parasite Populations, Academic Press, New York). These reviews indicated that inhibition of development occurred generally at the early fourth stage in some species and genera of Trichostrongylidae in ruminants. Michel (1952, Nature 169:933–934) reported inhibition of exsheathed $L_3$ *Trichostrongylus retortaeformis* (Zeder, 1800) in rabbits, and only recently has this become a recognized phenomenon among other *Trichostrongylus* spp. in sheep (Waller and Dobson, 1981, loc. cit.). Apparent inhibition at the time of exsheathment of the $L_3$ has not been previously reported, although there may be a parallel with the incomplete second ecdysis of dauer larvae among some free-living nematodes (Rogers and Sommerville, 1963, Advances in Parasitology 1:109–177; Schmidt and Roberts, 1981, Foundations of Parasitology, C. V. Mosby, St. Louis). Actual inhibition could only be indicated by determining whether or not these larvae were capable of completing ecdysis and resuming development. Consequently, we interpret the present observations as delayed exsheathment of the $L_3$. Whether the observations reported here represent a phenomenon related to arrested development (in the accepted definition according to Michel, 1974, loc. cit.) remains to be determined.

We acknowledge the assistance of L. G. Rickard during the preparation of this manuscript.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 154–155

*Research Note*

# Larval Philometrid Nematodes (Philometridae) from the Uterus of a Sandbar Shark, *Carcharhinus plumbeus*

George W. Benz,[1] Harold L. Pratt, Jr.,[2] and Martin L. Adamson[1]
[1] Department of Zoology, The University of British Columbia,
Vancouver, British Columbia V6T 2A9, Canada and
[2] Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA,
Narragansett, Rhode Island 02882

KEY WORDS: morphology, morphometrics, life cycle, tumor, New York.

On 14 July 1973, during reproductive system examinations of sharks landed at Shinnecock, New York, one of us (HLP) noticed a small, tumor-like growth in the left uterus of a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827). The shark (169 cm fork length, 57.3 kg) was mature and had large, flaccid, and scarred uteri, indicating parturition probably a month or so prior to capture.

The tumor was located within the proximal lateral uterine wall about midway between the isthmus and vagina. It was approximately 2 cm in diameter, firm, and colored as the surrounding tissues. The tumor and a portion of one oviducal gland were preserved in Bouin's fixative. In the laboratory, these tissues were dehydrated through a graded ethanol series, embedded in paraffin, and sectioned (10 $\mu$m) using a rotary microtome. Staining was in Mallory's trichrome stain. Measurements are reported as $\bar{x} \pm 1$ SD ($N = 10$).

Microscopically, the tumor consisted of a dense aggregation of coiled nematode larvae embedded in granulation tissue (Fig. 1). Individual larvae were surrounded by a thin membranous capsule (? =egg membrane), and macrophages (large cells with eccentric nuclei and a high cytoplasm-to-nucleus ratio) were present inside and outside the capsule. Some macrophages adhered to the external surface of the capsule. The tumor itself was not delimited.

The nematode larvae (Fig. 2) were similar to larval philometrids collected from sharks by others (Steiner, 1921, Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten I. Abt. Originale 86:591–595; Johnston and Mawson, 1943, Transactions of the Royal Society of South Australia 67:187–190; de Ruyck and Chabaud, 1960, Vie et Milieu 11:386–389; Mudry and Dailey, 1969, Proceedings of the Helminthological Society of Washington 36:280–284; Rosa-Molinar et al., 1983, Journal of Wildlife Diseases 19:275–277). Larvae were 348.6 $\pm$ 9.5 $\mu$m long, 17.7 $\pm$ 0.7 $\mu$m wide, and were characteristically coiled in about one and one-half coils. Cuticle with tiny transverse striations over entire length. Anterior extremity with tiny toothlike projection. Esophageal lumen staining intensely and with characteristic shape: arrowhead-shaped anteriorly, thereafter becoming narrow (approximately 2 $\mu$m wide) and cylindrical, broadening slightly (4 $\mu$m) about 25 $\mu$m posterior to anterior extremity and abruptly constricted at esphago–intestinal junction. Esophagus proper roughly cylindrical, 76.0 $\pm$ 3.3 $\mu$m long, with sphincter-like formation at junction with intestine. Nerve ring encircling esophagus about 50 $\mu$m from anterior extremity. Excretory system indistinct, with long tubular terminal duct leading to excretory pore about 65 $\mu$m from anterior extremity. Intestine 177.8 $\pm$ 7.8 $\mu$m long, lumen staining intensely. Genital primordium consisting of 2 cells on ventral side of body about 70 $\mu$m anterior to anus. Six rectal gland cells: 1 dorsal, 2 subventral large, 1 ventral, 2 subdorsal small. Tail whiplike, 59 $\pm$ 4.0 $\mu$m long. A voucher slide containing larvae has been deposited in The National Parasite Collection as USNM Helminthological Collection Number 79284. No parasites were found in sections of the oviducal gland.

Identification of these nematodes to species was impossible due to their immature condition, however they did possess all the characteristics of larvae of Philometridae. To date, *Phlyctainophora* Steiner, 1921, is the only philometrid genus reported from sharks. Gravid female *Phlyctainophora* are generally found superficially embedded below the epidermis, their presence often betrayed externally by a small bump (Steiner, 1921, loc. cit.; Mudry and Dailey, 1969, loc. cit.). Free larvae thought to be *Phlyctainophora*

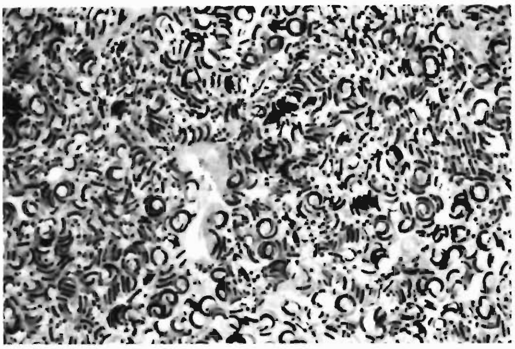


**Figure 1.** **Massive *Phlyctainophora*-like philometrid larva burden within uterus of *Carcharhinus plumbeus*.**

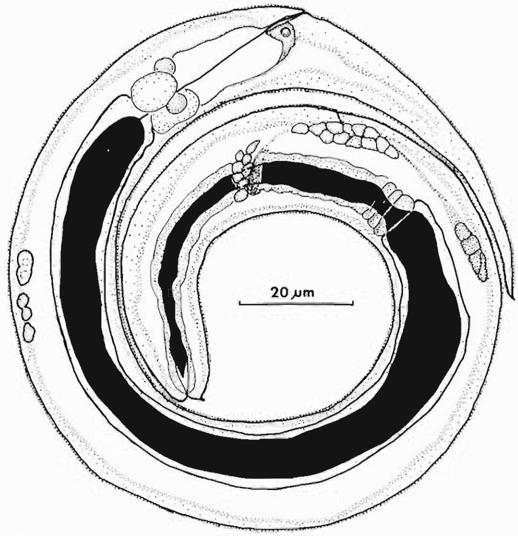



**Figure 2.** ***Phlyctainophora*-like philometrid larva from uterus of *Carcharhinus plumbeus*. Scale bar = 20 µm.**

have been reported on several occasions from sharks (Johnston and Mawson, 1943, loc. cit.; de Ruyck and Chabaud, 1960, loc. cit.; Rosa-Molinar et al., 1983, loc. cit.). Whereas the first two reports recorded larvae from superficial body tissues, the latter documented ovarian granulomas. The present report is the first record of philometrid *Phlyctainophora*-like larvae from the uterus of a shark, and is also a new host record for the family Philometridae.

Typical philometrid life cycles involve females releasing larvae into the water via some breach in the host's epidermis, and the subsequent development of larvae in an intermediate copepod host (Platzer and Adams, 1967, Canadian Journal of Zoology 45:31–43; Uhazy, 1977a, Canadian Journal of Zoology 55:265–273; Uhazy, 1977b, Canadian Journal of Zoology 55:1430–1441). The presence of these larvae deep within the body is somewhat problematic. They may represent the remains of a gravid female, although there was no sign of a female within the tumor. The condition reported herein probably is not widespread, as one of us (HLP) has thoroughly examined the reproductive systems of over 200 sandbar sharks and has only this once seen evidence of these worms.

We thank Mr. L. Schaefer and others involved with the Shinnecock Shark Fishing Tournament for allowing HLP to examine landed sharks, Mr. J. G. Casey (NOAA) for field assistance, Dr. J. R. Lichtenfels (USDA, ARS, Animal Parasitology Institute) for loaning specimens, Drs. L. R. Penner (University of Connecticut) and D. R. Brooks (University of British Columbia) for laboratory facilities used in examining specimens, and The University of British Columbia for fellowship support to GWB during the final phases of the study.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 156–157

*Research Note*

# Mermithidae (Nematoda) Infection of the Aquatic Stages of *Simulium* (*Edwardsellum*) *damnosum* from Nine River Systems in Kenya

YEMANE MEBRAHTU,[1] C. P. M. KHAMALA,[2] LARRY D. HENDRICKS,[1] AND RAYMOND F. BEACH[1]

[1] Walter Reed Project, Biomedical Sciences Research Centre, P.O. Box 30137, Nairobi, Kenya and
[2] University of Nairobi, Department of Zoology, P.O. Box 30197, Nairobi, Kenya

KEY WORDS: *Gastromermis* spp., *Isomermis* spp., *Mesomermis ethiopica*, infection rates, blackflies.

The most important genera of Mermithidae parasitizing blackflies belong to *Isomermis, Gastromermis,* and *Mesomermis* (=*Neomesomermis*); to a lesser extent, the genera *Limnomermis* and *Hydromermis* also parasitize blackflies (Poinar, 1981, pages 159–170 *in* M. Laird, ed., Blackflies, Academic Press, London). Poinar (1981, loc. cit.) pointed out that a collective genus *Agamomermis* Stiles was created to include all postparasitic juvenile Mermithidae that could not be identified to genus. On the other hand, Rubtsov (1981, pages 171–180 *in* M. Laird, ed., Blackflies, Academic Press, London) contended that juveniles have more diagnostic criteria than adults, and hence are more practical for diagnosis. *Simulium damnosum* Theobald has been reported to harbor several species of mermithids, namely *Gastromermis leberrei* Mondet, Poinar, and Bernadou, 1977, *Gastromermis philipponi* Mondet, Poinar, and Bernadou, 1977, *Isomermis lairdi* Mondet, Poinar, and Bernadou, 1977, *Isomermis tansaniensis* Rubtsov, and *Mesomermis ethiopica* Rubtsov (Poinar, 1981, loc. cit.).

This study is based on postparasitic juvenile mermithid larvae collected from *Simulium damnosum* s.l. in Kenya and kept in 80% alcohol preservative since 1981. Personal communication by Dr. L. D. Hendricks with Dr. G. O. Poinar revealed that fresh material of both adults and postparasites that are heat-killed (in hot water—60°C) and placed in 3% formalin immediately after collection is needed for identification to species. Because of this requirement, and because our mermithid collections were not heat-killed, but were directly immersed in 80% alcohol, we are placing these mermithid juveniles in the genus *Agamomermis* Stiles until we collect fresh materials for a proper identification to species.

Carlsson (1970, World Health Organization WHO/ONCHO/70.81:1–16) reported that the infection rate of *Simulium* species by mermithid nematodes from Kenyan rivers varied considerably from locality to locality, even in the same river system. Based on this earlier information, nine river systems (the Tsavo, Kibwezi, Thiba, Nyamindi, Mutonga, Yala, Lusumu, Isiukhu, and Nzoia) were searched for their mermithid parasitemia percentage rate.

Studies conducted from 1981 to April 1983 on the infection rate of simuliids by mermithids from the above rivers revealed varying rates of infection. The highest percentage of mermithid infections reported by Carlsson (1970, loc. cit.) was 15% in the Isiukhu River, where all sixth-instar *S. damnosum* s.l. larvae were infected. In the present study, it was found that these same *Simulium* had an infection rate of 17% in the same river, but at a different upstream site from that reported by Carlsson (1970, loc. cit.).

In both the Isiukhu and Lusumu rivers, only *S. damnosum* s.l. larvae were infected. Larvae from the Lusumu River had an infection rate of 13%; the Lusumu River is reported here for the first time to be a suitable breeding place for *S. damnosum* s.l. In the Nzoia and the Yala rivers, infection rates were 6% and 4%, respectively, pooled from *S. damnosum* s.l. and *S. medusaeforme* larval infections. Carlsson (1970, loc. cit.) did not report finding *S. damnosum* s.l. from the Nzoia River, and hence no mermithid infection rate was given; however, he did record between 6 and 10% filarial worm infection of the aquatic instars at the Yala River. The present study revealed that *S. damnosum* s.l. and *S. medusaeforme* were found infected with mermithid nematodes in both the Nzoia and the Yala rivers.

*Simulium damnosum* s.l. from the Thiba and the Nyamindi rivers had very low infection rates of 2% for each river system. Unlike their condition in rivers in Western and Nyanza provinces (the Yala, Lusumu, Isiukhu, and Nzoia), the aquatic instars of *S. damnosum* s.l. had aquatic mites attached to them, especially the pupae. Whether these mites were really parasites or were inadvertently collected from the flora and fauna of the river systems and later attached to these aquatic stages in the collecting troughs needs further investigation.

The mermithid nematode larvae recorded during this study generally left their *Simulium* hosts while in the collecting tubes. Free-living specimens were not seen in the different river systems, but many *Simulium* larvae with parasites breaking out of their bodies were observed during field spot-identifications. Large mermithid larvae may easily be seen inside *Simulium damnosum* s.l. or *S. medusaeforme. Simulium* larvae were not dissected to look for Mermithidae, as the worms could be easily seen through the integument of the abdomen using a stereomicroscope or even with the naked eye. Unlike nonparasitized *Simulium* larvae, all parasitized larvae had quite distended abdomens, and the mermithid worms inside showed a bright green coloration. The color is so distinct and the contour of the parasite so clear from the internal organs of the larvae that dissection was found unnecessary.

Percentage infection rates given here are based on the large nematode worms inside the *Simulium* larvae plus the worms that had bored their way out through the abdomens of the larvae. As a general rule, mermithids damage their blackfly hosts during the last stage of development in the hemocoel and kill them in the process of emergence. Therefore, percentages of parasitism given in the literature are good measures of insect mortality resulting from mermithid infection (Welch, 1965, Annual Review of Entomology 10:275–302).

A host of reviewers have concluded that mermithid nematodes offer a promising alternative or adjunct to existing methods for controlling simuliids (Welch, 1964, Bulletin of the World Health Organization 31:857–863; Gordon et al., 1973, Experimental Parasitology 33:226–238; Roberts and Castillo, 1980, Bulletin of the World Health Organization 58:1–197; Poinar, 1981, loc. cit.; etc.). These nematodes kill and sterilize their hosts and, under natural conditions, appear only to infect simuliids (Gordon, 1984, pages 821–848 *in* W. R. Nickle, ed., Plant and Insect Nematodes, Marcel Dekker, Inc., New York). In addition, Garris and Noble (1975, Journal of Medical Entomology 12:481–482) found that the larvicide Abate® has little or no effect on Mermithidae. Based on previous findings (Carlsson, 1970, loc. cit.) and the present available data on the infection rate of *Simulium damnosum* s.l. in Kenya and elsewhere, there is a potential for the mermithid worms as biocontrol agents.

This study was undertaken through the World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases (TDR), which is gratefully acknowledged. We also acknowledge Dr. R. Whitmire, Director, U.S. Army Medical Research Unit–Kenya, for his support and for approving this paper for publication.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 158–160

***Research Note***

# *Nematotaenoides ranae* (Cestoda: Nematotaeniidae) Transferred to the Genus *Anonchotaenia* (Paruterininae)

MALCOLM K. JONES[1]
Department of Parasitology, University of Queensland, St. Lucia, Queensland 4067, Australia

KEY WORDS: *Anonchotaenia ranae* (Ulmer and James, 1976) comb. n., morphology, paruterine organs, amphibian host.

Douglas (1958, Journal of Parasitology 44:261–273) listed four characters that he considered were consistently displayed by cestodes of the family Nematotaeniidae Lühe (Cyclophyllidea): paruterine organs that develop on the anterior face of the uterus, a small but definite number of testes, a cylindrical body, and parasitism in amphibians and reptiles. According to the original description (Ulmer and James, 1976a, Proceedings of the Helminthological Society of Washington 43:185–190), *Nematotaenoides ranae* possesses a single paruterine organ developing at the anterior end of the uterus (Fig. 1), from three to 10 testes per segment, parasitism in a frog, *Rana pipiens* Schreber, and an ovoid cross section. Ulmer and James (1976a, loc. cit.) thus placed *N. ranae* within the family Nematotaeniidae, but proposed distinct generic status for it as it has only one paruterine organ and from three to 10 testes per segment.

The description of *N. ranae* is excellent and is not repeated here. However, after examining the only paratype (from *Rana pipiens*, Iowa, USNM 73481), I am convinced that this species has been mistakenly identified as a nematotaeniid. *Nematotaenoides ranae* and true nematotaeniids differ markedly in the way that their paruterine organs are formed.

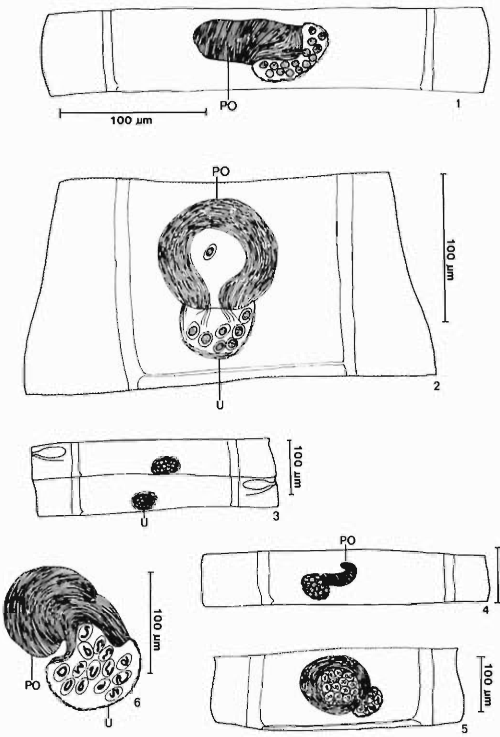
In *N. ranae*, the uterine duct is coiled (Ulmer and James, 1976a, loc. cit.) and passes to a saccate uterus. The dense, fibrous, and, at times, cornuate paruterine organ appears anterolateral to the uterus (Ulmer and James, 1976a, loc. cit.) (Fig. 1). Internally, the fibers of the paruterine organ hollow (Fig. 2) to form a space for the eggs. The fully developed paruterine organ has thick, multilayered walls (Fig. 2). A narrow opening remains between the uterus and paruterine organ (Fig. 2).

Such a sequence of paruterine organ formation is unknown for true nematotaeniids. In members of the Nematotaeniidae, paired or multiple paruterine organs develop usually anterior to the uterus (Jones, 1985, Journal of Parasitology 71: 4–9). Thin-walled capsules, called paruterine capsules, develop from specialized precursors at the apex of the paruterine organs and form initially as saccular extensions of the paruterine organs. Hence, in nematotaeniids, the paruterine capsules grow anterior to the original paruterine organs rather than from within as seen in *N. ranae*. The paruterine capsule wall in nematotaeniids is thin and apparently single-layered when viewed with the light microscope, and not thick and multilayered as in *N. ranae*. Therefore, *N. ranae* cannot be considered a member of the Nematotaeniidae.

Four other cestode groups possess paruterine organs: the Mesocestoididae Perrier, the Idiogeninae Fuhrmann (Davaineidae), the Thysanosomatinae Skrjabin (Anoplocephalidae), and the Paruterininae Fuhrmann (Dilepididae). Among these groups, one genus, namely *Anonchotaenia* Cohn of the Paruterininae, has an anatomy similar to that of *N. ranae*.

The descriptions of paruterine organ formation given for *A. globata* (Linstow), *A. quisquali* Rausch and Morgan, and *Anonchotaenia* spp. (Rausch and Morgan, 1947, Proceedings of the American Microscopical Society 66:203–211; Saxena and Baugh, 1978, Angewandte Parasitologie 19:85–106; Voge and Davis, 1953, University of California Publications in Zoology 59: 1–29) are almost identical with that given for *N. ranae*. In members of *Anonchotaenia*, for example *A. quisquali* (paratypes of *A. quisquali* from *Quisqualis quisquala* (Linnaeus), Ohio, USNM 71425), the uterus is saccate (Fig. 3). The paruterine tissue develops anterolateral and slightly aporal to the uterus as a small cone (Saxena and

[1] Present address: Queensland Institute of Medical Research, Bramston Terrace, Herston, Queensland 4006, Australia.




**Figures 1–6. 1. Pregravid segment of *A. ranae.* 2. Gravid segment of *A. ranae.* 3. Uterus of *A. quisquali.* 4. Pregravid segment of *A. quisquali.* 5. Early gravid segment of *A. quisquali.* 6. Early paruterine organ formation in *A. quisquali.* PO, paruterine organ; U, uterus. (All drawings original.)**

Baugh, 1978, loc. cit.). The single paruterine organ may become elongate and cornuate in *A. quisquali* (Fig. 4), and later hollows and receives the eggs (Figs. 5, 6). The walls of the fully formed paruterine organ in *Anonchotaenia* consist of multiple layers of fibers (Fig. 6) (Rausch and Morgan, 1947, loc. cit.; Saxena and Baugh, 1978, loc. cit.).

*Nematotaenoides ranae* has from three to 10 testes per segment and *Anonchotaenia* spp. have a similarly variable and small number of testes (Saxena and Baugh, 1978, loc. cit.; Voge and Davis, 1953, loc. cit.). Furthermore, both *N. ranae* and *Anonchotaenia* spp. have a simple aspinous cirrus and cirrus pouch, a preformed seminal receptacle, a spherical to oval ovary, and a compact vitellarium in each mature segment (Ulmer and James, 1976a, loc. cit.; Saxena and Baugh, 1978, loc. cit.). In *N. ranae* and *A. mexicana,* the vitelline duct connects with the oviduct before the entrance of the copulation canal (Ulmer and James, 1976a, loc. cit.; Voge and Davis, 1953, loc. cit.). In nematotaeniids, the oviduct unites with the copulation canal before it joins the vitelline duct. The anterior segments of both *N. ranae* and *A. globata* are acraspedote, whereas gravid segments of both species are craspedote (Ulmer and James, 1976a, loc. cit.; Saxena and Baugh, 1978, loc. cit.). Some species of *Anonchotaenia* are known to have vermiform oncospheres (Figs. 5, 6), although this is apparently not a feature of all species of *Anonchotaenia* or of closely related genera (Voge and Davis, 1953, loc. cit.). The oncospheres of *N. ranae* were not observed clearly, and are depicted in the accompanying illustrations as oval bodies.

It is most certain that *N. ranae* belongs in the genus *Anonchotaenia.* Comparisons with other species in the genus are not yet practicable because specific characters have not been precisely defined. Rausch and Morgan (1947, loc. cit.) distinguished species by the number of testes in mature segments. The number of testes given for *N. ranae* overlaps that of many species. Publications since Rausch and Morgan (1947, loc. cit.) also report variability in numbers of testes (Voge and Davis, 1953, loc. cit.; Saxena and Baugh, 1978, loc. cit.). The most recent revision of the genus (Matevosian, 1969, *in* Skriabin, ed., Principles of Cestodology, 7, Nauka, Movska, Izdatel'stvo Akademii Nauk 7, 303 pp.) provides no better information. Thus, it is difficult to determine whether *N. ranae* is a synonym of an already described species until further specific characters are defined. For this reason, *N. ranae* is transferred to *Anonchotaenia* but is retained as *Anonchotaenia ranae* (Ulmer and James) comb. n. *Nematotaenoides* Ulmer and James, 1976, becomes a junior synonym of *Anonchotaenia.*

It remains to consider why a member of a genus normally parasitic in birds is found in an amphibian. It is possible that the infection in *Rana pipiens* was accidental. This is supported by the low prevalence of the cestode in *R. pipiens*; Ulmer and James (1976b, Proceedings of the Helminthological Society of Washington 43:191–200) recorded *N. ranae* in only one of 491 frogs examined. The large number of cestodes (20) present in that one host may suggest that it had eaten either an infected fledgling or an intermediate host bearing a multiple infection.

This is the second record of a paruterinine from an amphibian. Macías-Palacios and Flores-Barroeta (1967, Revista Iberica de Parasitologia 27:43–62) described *Hexaparuterina mexicana* from *Rana montezumae* Baird in Mexico. Spasskii (1977, Izvestiya Akademii Nauk Moldarvskoi SSR 5:65–70) regarded *H. mexicana* as a species of *Metroliasthes.* Macías-Palacios and Flores-Barroetta (1967, loc. cit.) recorded *H. mexicana* from only one frog, which also suggests accidental infection.

I thank Dr. J. R. Lichtenfels and Ms. P. Pilitt for lending me specimens from the collection of the United States National Museum, and Professor C. Dobson and Dr. J. C. Pearson for reading drafts of the manuscript. This work was performed while I was in receipt of an Australian Commonwealth Postgraduate Research Award.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 160–161

***Research Note***

# Recovery of Third-stage Larvae of *Ostertagia ostertagi* from the Abomasa of Experimentally Inoculated Calves by Prolonged Saline Incubation

LOUIS C. GASBARRE
Helminthic Diseases Laboratory, Animal Parasitology Institute,
Agricultural Research Service, BARC-East, Beltsville, Maryland 20705

KEY WORDS: Nematoda, pepsin–HCl digestion vs. saline digestion, methodology, *Bos taurus.*

The recovery of larval *Ostertagia ostertagi* from the abomasal tissues is important not only in epidemiological studies but also in studies aimed at delineation of the effects of drug treatment or immunization. The most commonly used methods for the recovery of larval *O. ostertagi* are pepsin–hydrochloric acid digestion of infected abomasa (Herlich, 1956, Proceedings of the Helminthological Society of Washington 23:102–103) or incubation of the abomasal tissue in saline or tap water (Williams et al., 1978, American Journal of Veterinary Research 40:1087–1090; Williams et al., 1981, Veterinary Record 108: 228–230; Downey, 1981, pages 69–73 *in* Mansen et al., eds., Epidemiology and Control of Nematodiasis in Cattle). Recent work with *O. circumcincta* in sheep has indicated that incubation of infected abomasa in saline for a time interval comparable to that of the digestion procedure yields similar numbers of recovered larvae (Jackson et al., 1984, Research in Veterinary Science 36:380–381). In fact, room-temperature incubation in tap water has been judged to be a superior method for the recovery of *O. ostertagi* when the integrity of the recovered larvae is important (Snider et al., 1985, Veterinary Record 116:69–72). In contrast, Kingsly and Gerber (1984, Veterinary Record 115:334) reported that a 4–6-hr incubation of abomasa in saline left a majority of *O. ostertagi* larvae in the abomasal tissues.

To test the efficacy of digestion versus saline incubation for the recovery of parasitic third-stage larvae of *O. ostertagi,* 4–12-wk-old Holstein-Freisan steers that had been raised on concrete since 1 day of age were inoculated orally with $2 \times 10^5$ infective third-stage larvae of *O. ostertagi,* which have been maintained as a laboratory strain for over 25 yr and do not exhibit arrested development (Herlich et al., 1984, Veterinary Parasitology 16:253–260). The calves were killed 4 days after infection, a time at which worms have not yet undergone the molt to the

**Table 1.** ***Ostertagia ostertagi*** **larvae recovered from the abomasa of calves 4 days after experimental infection with $2 \times 10^5$ infective larvae.**

| Calf number | Incubation conditions | | |
|---|---|---|---|
| | 4 hr pepsin–HCl | 4 hr saline | 24 hr saline |
| 30 | 8,100 | – | 20,390 |
| 31 | 9,290 | – | 26,630 |
| 35 | 10,540 | 7,160 | – |
| 36 | 14,240 | 9,840 | – |

fourth-stage larvae, and the abomasa were removed and the exteriors carefully trimmed of fat and connective tissue. The abomasa were cut open longitudinally along the midline of the lesser curvature of the stomach. The contents were removed and the mucosa thoroughly but gently washed under a stream of tap water. The cleaned abomasa were then longitudinally cut into halves. One half was placed in 1% pepsin–1% HCl for a 4-hr incubation at 37°C. The other half of each abomasum was placed in Dulbecco's phosphate buffered saline (D-PBS) and incubated at 37°C for either 4 or 24 hr. At the end of the incubation period, the mucosal surface of each abomasal half was gently stripped of mucus and adhering material by pulling the abomasum between the thumb and forefingers. The strippings were then added back to the incubation solution and the entire material was fixed by the addition of formalin to a final concentration of 5%. For larval counts, 20% of the fixed incubation fluid from each abomasal half was taken and a small amount of Lugol's iodine was added to facilitate identification of the larvae. The total larvae in each 20% aliquot was determined under a dissecting microscope at 10–30×.

Incubation in saline for 4 hr was found to recover slightly fewer third-stage larvae than pepsin–HCl digestion for a comparable time period (Table 1). The two abomasa so treated had recoveries that were 68 and 69% of the recoveries observed after digestion (Table 1). In contrast, saline incubation for 24 hr was found to result in substantially higher larval recoveries as compared to a 4-hr pepsin–HCl digestion (Table 1). Larval recoveries after this prolonged incubation were 252 and 287% of those seen in the corresponding abomasal halves after digestion (Table 1).

These results indicate that a prolonged incubation in saline is superior to pepsin–HCl digestion for the recovery of third-stage larvae of *O. ostertagi* under the conditions tested. Although short-term (4 hr) saline incubation yielded slightly fewer larvae, an incubation of 24 hr resulted in the recovery of almost three times as many larvae as the standard 4-hr digestion procedure. In addition, as previously noted (Snider et al., 1985, loc. cit.), the larvae recovered after tap water incubation were in better condition as compared to those usually seen after digestion.

Also worth noting is the fact that when expressed as larvae recovered per abomasum, the percentages of the total inoculum recovered after saline incubation were 20 and 27%. These percentages are similar to the percentage of inoculum usually recovered in this laboratory when adult worm recoveries are performed 4–5 wk postinfection (data not shown). In contrast, the recovery of larvae after digestion is lower than would be expected if the infection were allowed to go to patency.

Although these results are in general agreement with those of Downey (1981, loc. cit.), who found similar numbers of fourth-stage larvae recovered for each half of abomasa taken from five calves infected naturally, care should be taken in extrapolating these results beyond a laboratory situation. The procedure described in this report relies on the ability of the larvae to migrate from the decomposing abomasal tissue. Conditions that would result in substantial mucosal damage or scarring, such as that seen in chronic ostertagiasis under field conditions, might interfere with this migration. Also, this study does not address the migratory ability of hypobiotic larvae, although Williams et al. (1978, 1981, loc. cit.) have reported the recovery of large numbers of arrested larvae by tap water incubation. As such, 24-hr saline incubation appears to be the method of choice in recovering larvae from the experimental infection of naive calves.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 162–165

***Research Note***

# Analysis of *Schistosoma mansoni* Tegumental Proteins by Hydrophobic Chromatography[1]

EUGENE G. HAYUNGA, INGRID MÖLLEGÅRD, AND MARY P. SUMNER
Division of Tropical Public Health, Department of Preventive Medicine and Biometrics,
F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences,
4301 Jones Bridge Road, Bethesda, Maryland 20814-4799

KEY WORDS: Trematoda, antigens, membranes, electrophoresis, alkylagarose, amino alkylagarose, protein isolation/purification.

The characterization of surface antigens of parasites has received considerable attention. Logically, it is the surface antigens that are first seen by the host and are most likely to encounter the molecules and cells of the immune system. Thus, knowledge of parasite surface chemistry may provide an understanding of how these organisms evade the immune response of the host. Immunochemical analyses may also lead to direct practical applications in antigen purification for improved serodiagnosis or vaccine development.

For the most part, efforts to isolate antigenic fractions of *Schistosoma mansoni* have involved hydrophilic interactions of soluble proteins or glycoproteins, with separation obtained on the basis of differences in charge or molecular weight, or by affinity for immobilized antibodies or lectins. However, the redundant surface membrane and lipid-rich tegument of this parasite both suggest that hydrophobic proteins may be important components of the surface plasmalemma, and there is evidence that intrinsic membrane proteins may escape detection by more conventional means. For example, certain lipophilic membrane proteins of lymphocytes have been termed "cryptic" components because their demonstration required the use of special radiolabeling methods (Sidman, 1981, The Journal of Immunology 127:1454–1458). Recently, a tegumental alkaline phosphatase of *S. mansoni* was shown to be such a cryptic component, as it appeared to be buried within the plasmalemma (Payares et al., 1984, Molecular and Biochemical Parasitology 13:343–360). In the present study, we have attempted to identify hydrophobic membrane proteins in the tegument of *S. mansoni* adult worms, following a modification of the method originally described by Shaltiel (1974, Methods in Enzymology 34:126–140).

Hydrophobic chromatography is a form of affinity chromatography using alkanes or ω-amino alkanes bound to agarose. Binding of proteins is based upon interactions between the nonpolar side chains affixed to the agarose and exposed hydrophobic patches on the protein molecules. Proteins bound to these columns are removed by increasing the hydrophobicity of the eluting solvent, which is accomplished either by increasing the salt concentration or by adding ethylene glycol to the buffer (Hofstee, 1973, Biochemical and Biophysical Research Communications 50: 751–757). Although the presence of a polar $NH_2$ group on ω-amino alkylagarose and the use of saline for elution suggest that separation of proteins may be the result of ion exchange, it has been shown that chromatography using both alkylagarose and ω-amino alkylagarose columns primarily involves hydrophobic rather than ionic interactions (Halperin et al., 1981, The Journal of Chromatography 215:211–228). Because the eluted fractions remain in aqueous solution, they may subsequently be analyzed by conventional methods such as immunoprecipitation, SDS-PAGE, or Western blotting.

Freeze–thaw (AFT) and detergent-extracted (NP40) antigen preparations were obtained from adult worms as described previously (Hayunga

[1] Portions of this paper were presented at the joint meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists, December 1983, San Antonio, Texas.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Animal Resources, National Research Council, DHEW Pub. No. (NIH) 78-23.

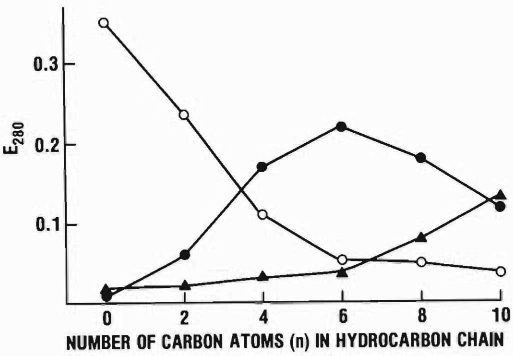



**Figure 1. Adsorption and elution of *S. mansoni* freeze–thaw antigen (AFT) aliquots from ω-amino alkylagarose columns of different carbon chain lengths (n). Three fractions were collected from each column: ○——○ the nonadsorbed or "fall through" fraction; ●——● the 1 M NaCl eluate; and ▲——▲ the 50% ethylene glycol eluate.**

and Sumner, 1986, The Journal of Parasitology 72:283–291). The yield from 4,000 worms was approximately 50 mg AFT at a concentration of 5 mg/ml, or 4 mg NP40 at a concentration of 1 mg/ml. Alkylagarose and ω-amino alkylagarose were obtained commercially from Miles Laboratories (Elkhart, Indiana). Analytical scale columns, containing approximately 1 ml gel, were equilibrated with 0.05 M Tris-HCl, pH 8.0; antigen preparations were dialyzed against Tris-HCl prior to column application. Aliquots of approximately 1 mg antigen were applied to each of the alkylagarose and ω-amino alkylagarose columns, as well as the agarose control columns, and allowed to enter the gel bed at a flow rate of approximately 0.2 ml/min (by gravity). The nonadsorbed or "fall through" peak was obtained by applying 2 ml Tris-HCl to the column and collecting the entire fraction. The column was then washed with approximately 20–25 ml Tris-HCl. Adsorbed proteins were collected by elution with 2 ml 0.05 M Tris-HCl, pH 8.0, containing 1 M NaCl, followed by elution with 2 ml Tris-HCl containing 1 M NaCl and 50% ethylene glycol following the method of Hofstee (1973, loc. cit.). The fractions were dialyzed against several changes of deionized water, lyophilized, and analyzed by SDS-PAGE, using 5–15% gradient slab gels.

Aliquots of the freeze–thaw antigen preparation from *S. mansoni* adult worms (AFT) were applied to six ω-amino alkylagarose columns, with carbon chains ranging in length from 0 (control) to 10 (ω-amino decylagarose). Three fractions were collected from each column: the nonadsorbed or "fall through" fraction, the 1 M NaCl eluate, and the 50% ethylene glycol eluate. As shown in Figure 1, increasing the length of the carbon chain (n) results in increased binding of material to the column. Quantitatively, more than two-thirds of the antigen is adsorbed by columns with carbon chain length as low as n = 4. The greatest amount of bound material is recovered from the 1 M NaCl eluate from the ω-amino hexylagarose column (n = 6). In the case of the longer carbon chains (n = 8 or 10), it appears that material is tightly bound; yields from the 1 M NaCl eluate are reduced and elution with ethylene glycol is necessary to remove the protein. This indicates significant hydrophobic affinity in the *S. mansoni* freeze–thaw preparation. Similar results were obtained using alkylagarose columns and detergent-extracted tegumental proteins (data not shown).

Eluates from each of the hydrophobic columns were next analyzed by SDS-PAGE. Equal aliquots of eluate in terms of volume were applied to each gel lane so that both quantitative and qualitative differences would be apparent. Comparison of the 1 M NaCl eluates from the control (n = 0), ethyl- (n = 2), butyl- (n = 4), and hexyl- (n = 6) agarose columns reveals the separation of distinctly different classes of molecules based upon their hydrophobic interactions (Fig. 2, upper gel). Comparisons between the eluates from alkylagarose and ω-amino alkylagarose, or between columns with different carbon chain lengths, reveal the sensitivity of this method in detecting relatively small differences in hydrophobic affinities. For example, a 47,000 molecular weight protein in the AFT preparation has an affinity for ω-amino decylagarose but is not adsorbed by ω-amino octylagarose (Fig. 2, arrows; second gel). A possible role for hydrophobic chromatography in protein purification is suggested by the last gel in Figure 2, where the n = 10 fraction, if not homogeneous, is certainly enriched with regard to the 30,000 molecular weight protein (arrow).

Previously, phenyl Sepharose chromatography was used to separate *S. japonicum* soluble egg antigen (SEA) into an enriched hydrophobic fraction of immunogenic glycoproteins and a hydrophilic fraction with lesser activity (Long et al., 1981, Infection and Immunity 34:397–406). More recently, hydrophobic chromatography us-

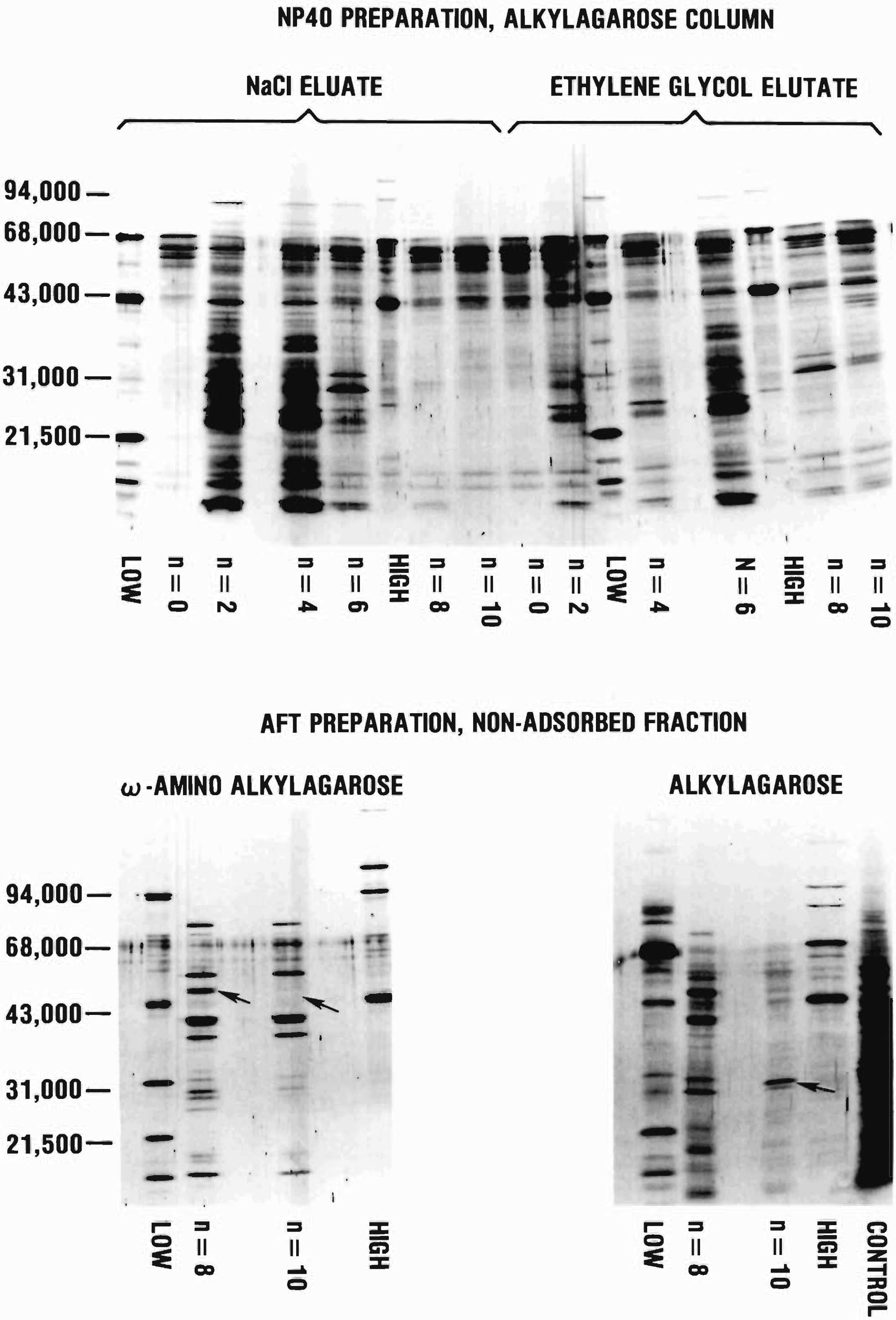


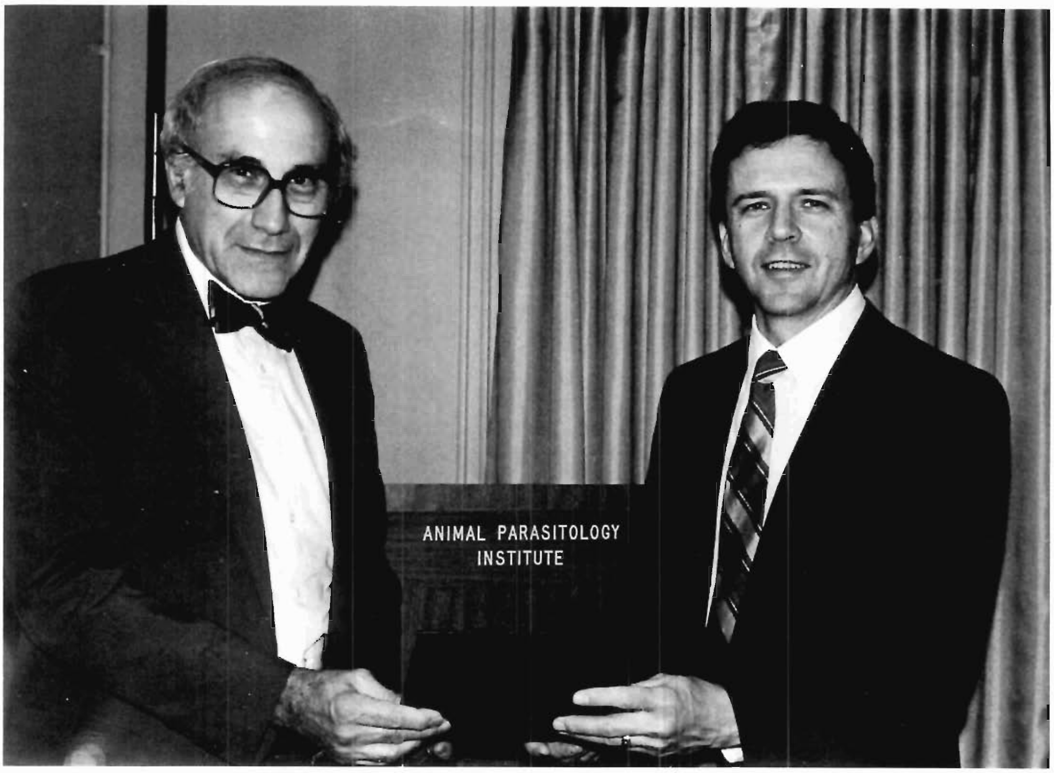

**Figure 2.** SDS-PAGE of aliquots of *S. mansoni* tegumental extracts fractionated by hydrophobic chromatography. Silver-stained, 5–15% gradient gel.

ing ω-amino octylagarose and ω-amino decylagarose facilitated the isolation of a cercarial antigen that was subsequently used to develop a circulating antigen assay for the early detection of schistosomiasis (Hayunga et al., 1986, The Lancet II:716–718). Although the actual role of hydrophobic proteins in schistosomiasis is not known, these findings, together with the observation that the delayed-type hypersensitivity (DTH) response to peptide antigens is enhanced by lipophilic carriers (Coon and Hunter, 1973, The Journal of Immunology 110:183–190), suggest that hydrophobic components may constitute an important source of "relevant" antigens.

This work was supported in part by Grant No. 18361 from the National Institute of Allergy and Infectious Diseases. Live material was provided by Drs. M. Stirewalt and F. Lewis, Biomedical Research Institute, Rockville, Maryland. We are grateful to J. Duncan, C. DeiSanti, and S. Mammino for technical support. We also thank F. Langley for illustrations and B. Holland and D. Boyle for editorial assistance.

Proc. Helminthol. Soc. Wash.
54(1), 1987, pp. 166–167

# PRESENTATION OF THE 1986 ANNIVERSARY AWARD TO LOUIS STANLEY DIAMOND




**Dr. Redington (right) presenting Anniversary Award to Dr. Diamond.**

Mr. President, members of the Helminthological Society of Washington, and guests: It gives me great pleasure to announce that the Awards Committee has selected Dr. Louis Stanley Diamond as the Helminthological Society of Washington's Anniversary Award recipient for 1986.

This award, as specified in the Bylaws of our Society, is bestowed on Dr. Diamond based on his outstanding contributions to parasitology, which have brought honor and credit to the Society, and also on his outstanding service to the Society.

Dr. Diamond, or Buddy as he has come to be known, was born on February 6, 1920, in Philadelphia, Pennsylvania. After graduation from a Philadelphia high school at age 16, he enrolled at the University of Pennsylvania, from which he graduated four years later. Equipped with a B.A. degree, Buddy then traveled west to Ann Arbor, Michigan, where a year later at age 21, he received a Master of Science degree.

It was 17 years before Buddy would receive his Ph.D., as his educational pursuits were interrupted by World War II. After serving as an Ordnance Inspector in Detroit for two years, he enlisted in the military in 1943 to serve as a Medical Laboratory Technician. This enlistment began a 37-year association with the military, as he subsequently served as a 2nd Lieutenant in the Army Sanitary Corps followed by a long career in the Active Reserves as a Medical Service Corps Officer. Finally, he entered the Retired Reserve as a Colonel for five years until his separation in 1980.

During his active duty assignment at the 4th Service Command Medical Laboratory at Fort McPherson, Georgia, toward the end of WWII, Buddy met two active duty parasitologists who were to have profound effects on his life, Franklin Wallace and Leon Jacobs.

Around the time that Dr. Diamond entered the Active Reserves in 1946, he found himself back in graduate school as a student of Franklin Wallace at the University of Minnesota. Although his dissertation addressed trypanosomes in amphibians, his interest in *Entamoeba histolytica* had been kindled by Leon Jacobs, who had returned to NIH following his active duty service to resume work on this organism.

In 1951, Buddy commenced what was to become a long and distinguished career as a government civil servant, serving initially as a Wildlife Disease Biologist at the Patuxent Research Center in Laurel, Maryland, under Carlton Herman. After two years in this assignment, Buddy crossed over the Baltimore–Washington Parkway to become a parasitologist at the Animal Disease and Parasite Research Division at Beltsville. It was while serving in this position that Buddy was awarded the Ph.D. degree in parasitology from the University of Minnesota in 1958.

In 1959, Dr. Diamond began his present assignment at the invitation of Leon Jacobs. Since then, he has served as a Research Zoologist in the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases at NIH. Since 1971, he has been Head of the Parasite Growth and Differentiation Section in the Laboratory of Parasitic Diseases.

Dr. Diamond has been a faithful supporter of the Helminthological Society of Washington and has made many notable contributions since joining the Society in 1951. In June 1961, when the American Society of Parasitologists met jointly with our Society in Washington, D.C., Buddy served as the Local Arrangements Chairman. He later served for two years on the Society's Executive Committee from 1965 to 1966. Later, he spent five years on the Anniversary Awards Committee. In 1981, he was elected Vice President of our Society and served as its President in 1982.

In addition to his membership in this Society, Buddy has been active in numerous other societies, including the Society of Protozoologists, the Society for Cryobiology, the American Society of Parasitologists, the American Microscopical Society, the American Society of Tropical Medicine and Hygiene, and the Wildlife Disease Association. Dr. Diamond recently served as President of the Society of Protozoologists in 1984.

Buddy has published extensively for over 30 years, with his greatest love being the world of protozoa as evidenced as far back as his first publication in 1945. The title of that initial paper was "A new rapid stain technic for intestinal protozoa using tergitol-hematoxylin." This interest actually surfaced a number of years earlier, when as a high school freshman, Buddy bought his first microscope and began cultivating *Paramecium* at his home. Since his initial scientific report, Buddy has published around 80 papers and has served on numerous and diverse panels, workshops, and symposia too numerous to delineate. In conjunction with these activities, he has devoted much time and energy in collaborating with fellow scientists in other countries, most notably Israel and Mexico.

Dr. Diamond joined the staff of Dr. Jacobs at the Laboratory of Parasitic Diseases, NIH, in 1959, and shortly thereafter in 1961 reported findings for which he is most noted, that is, the axenic cultivation of *E. histolytica*. In other words, he had accomplished in vitro growth in an environment free of any other metabolizing cells. This first successful culture medium was called TTY-S-CEEM25. His methodologies, which led up to this landmark discovery, were those of the classic research scientist, as he thoughtfully incorporated the theories and results of others who labored before him into his own studies.

In the intervening years, Buddy has made numerous improvements to the medium, which currently is used by scientists worldwide. He has made monumentous strides as evidenced by the following comparison. When his first successful medium (TTY-S-CEEM25) was inoculated with 10,000 amoebae per ml of overlay, it yielded a threefold increase in the number of cells after 72 hours' incubation. At present, when the "new improved" TYI-S-33 is inoculated with 500 amoebae per ml of medium, a 400-fold increase is attained in the same incubation period. Buddy now pursues an even higher goal, which is cultivation of *E. histolytica* in a completely chemically defined medium.

As Dr. Diamond pursues further discoveries, we salute him on this occasion. On behalf of the Helminthological Society of Washington and the members of the Awards Committee (Margaret Stirewalt and Leon Jacobs), I am pleased and honored to present the 1986 Anniversary Award to Dr. Louis Stanley Diamond.

Bryce C. Redington
Chairman
Awards Committee

## The Helminthological Society of Washington

### *Application for Membership*

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

The Recording Secretary
Helminthological Society of Washington
Post Office Box 368
Lawrence, Kansas 66044

Print name: ______________________________ Date of birth: ________

Mailing address: ______________________________
______________________________
______________________________

Degree and year received: ______________________________

Present position: ______________________________

Field of interest: ______________________________
______________________________

Signature of applicant: ______________________________ Date: ________

Signature of sponsor: ______________________________
(a member)

## ANNIVERSARY AWARD RECIPIENTS

| | | | |
|---|---|---|---|
| Edna M. Buhrer | 1960 | E. J. Lawson Soulsby | 1974 |
| Mildred A. Doss | 1961 | David R. Lincicome | 1975 |
| * Allen McIntosh | 1962 | Margaret A. Stirewalt | 1975 |
| * Jesse R. Christie | 1964 | * Leo A. Jachowski, Jr. | 1976 |
| Gilbert F. Otto | 1965 | Horace W. Stunkard | 1977 |
| * George R. LaRue | 1966 | Kenneth C. Kates | 1978 |
| * William W. Cort | 1966 | * Everett E. Wehr | 1979 |
| * Gerard Dikmans | 1967 | O. Wilford Olsen | 1980 |
| * Benjamin Schwartz | 1969 | Frank D. Enzie | 1981 |
| * Willard H. Wright | 1969 | Lloyd E. Rozeboom | 1982 |
| Aurel O. Foster | 1970 | Leon Jacobs | 1983 |
| Carlton M. Herman | 1971 | Harley G. Sheffield | 1984 |
| May Belle Chitwood | 1972 | A. Morgan Golden | 1985 |
| * Elvio H. Sadun | 1973 | Louis S. Diamond | 1986 |

## HONORARY MEMBERS

| | | | |
|---|---|---|---|
| * George R. LaRue | 1959 | Justus F. Mueller | 1978 |
| Vladimir S. Ershov | 1962 | John F. A. Sprent | 1979 |
| * Norman R. Stoll | 1976 | Bernard Bezubik | 1980 |
| Horace W. Stunkard | 1977 | Hugh M. Gordon | 1981 |

## CHARTER MEMBERS 1910

| | | | |
|---|---|---|---|
| * W. E. Chambers | * Philip E. Garrison | * Maurice C. Hall | * Charles A. Pfender |
| * Nathan A. Cobb | * Joseph Goldberger | * Albert Hassall | * Brayton H. Ransom |
| * Howard Crawley | * Henry W. Graybill | * George F. Leonard | * Charles W. Stiles |
| * Winthrop D. Foster | | | |

## LIFE MEMBERS

| | | | |
|---|---|---|---|
| * Maurice C. Hall | 1931 | Carlton M. Herman | 1975 |
| * Albert Hassall | 1931 | Lloyd E. Rozeboom | 1975 |
| * Charles W. Stiles | 1931 | Albert L. Taylor | 1975 |
| * Paul Bartsch | 1937 | David R. Lincicome | 1976 |
| * Henry E. Ewing | 1945 | Margaret A. Stirewalt | 1976 |
| * William W. Cort | 1952 | * Willard H. Wright | 1976 |
| * Gerard Dikmans | 1953 | * Benjamin Schwartz | 1976 |
| * Jesse R. Christie | 1956 | Mildred A. Doss | 1977 |
| * Gotthold Steiner | 1956 | * Everett E. Wehr | 1977 |
| * Emmett W. Price | 1956 | Marion M. Farr | 1979 |
| * Eloise B. Cram | 1956 | John T. Lucker, Jr. | 1979 |
| * Gerald Thorne | 1961 | George W. Luttermoser | 1979 |
| * Allen McIntosh | 1963 | John S. Andrews | 1980 |
| Edna M. Buhrer | 1963 | * Leo A. Jachowski, Jr. | 1981 |
| * Benjamin G. Chitwood | 1968 | Kenneth C. Kates | 1981 |
| Aurel O. Foster | 1972 | Francis G. Tromba | 1983 |
| Gilbert F. Otto | 1972 | A. James Haley | 1984 |
| * Theodor von Brand | 1975 | Paul C. Beaver | 1986 |
| May Belle Chitwood | 1975 | Raymond M. Cable | 1986 |

* Deceased.

CONTENTS

(*Continued from Front Cover*)



RESEARCH NOTES



ANNOUNCEMENTS



Date of publication, 6 April 1987

* * *

PRINTED BY ALLEN PRESS, INC., LAWRENCE, KANSAS 66044, U.S.A.